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Biochar-assisted phytoextraction of Cd and Zn by *Noccaea caerulescens* on a contaminated soil: a four-year lysimeter study

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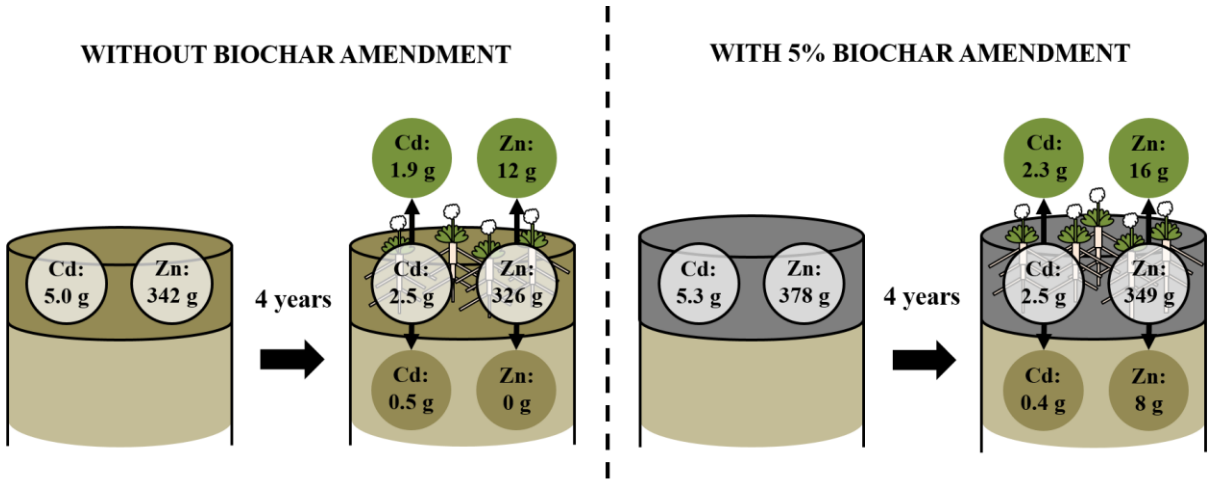
HIGHLIGHTS

- 40% of soil Cd had been phytoextracted by *N. caerulescens* after four years
- Biochar amendment favored seed germination and plant survival
- Biochar amendment did not affect shoot Cd and Zn concentrations
- Phytoextraction efficiency decreased over successive harvests

GRAPHICAL ABSTRACT



ABSTRACT

Amendments of biochar, the residual solid of biomass pyrolysis, have been shown to enhance metal phytoextraction from contaminated soils with hyperaccumulating plants in specific situations. In order to investigate this phenomenon over successive harvests in field conditions, two identical undisturbed soil cylinders (1-m^2 section x 1.85-m height) were excavated from a contaminated agricultural plot and monitored with instrumented lysimeters. Wood-derived biochar was added at a rate of 5% (w/w) in the first 30 cm of one of the two lysimeters. The Cd/Zn-hyperaccumulator *Noccaea caerulescens* was then grown for the next four years on both lysimeters. Our results showed that the hyperaccumulating plant was able to remove about 2 g m^{-2} of Cd and 12-16 g m^{-2} of Zn within four years, representing about 40% and 4% of the initial Cd and Zn soil contamination, respectively. Biochar amendment improved plant germination and survival and increased root surface density. However, no significant effect of biochar on shoot metal content of *N. caerulescens* was observed. Mass balances suggested that up to 10% the metal contamination moved from the disturbed Ap horizon to the deeper horizons, particularly in the biochar-amended soil profile. Furthermore, shoot Cd and Zn concentration generally decreased over the successive harvests, together with soil metal availability. Depending on the way to account for this progressive decrease in efficiency, our estimations of the time necessary to remove the excess of metals in the topsoil in these conditions ranged from 11 to 111 years for Cd and from 97 years to an infinite time for Zn. In conclusion, the simultaneous use of *N. caerulescens* and biochar amendment can lead to a significant removal of specific metallic elements from the topsoil, but the risk of metal movement down the soil profile and the observed decrease in phytoextraction efficiency over time deserve further investigations.

KEYWORDS: phytoremediation, heavy metal, hyperaccumulating plant, leaching

INTRODUCTION

The improvement of phytoremediation techniques has been seen as a necessity to reclaim vast areas of metal-contaminated soils. It has been estimated that more than 6% of the total agricultural land in the European Union, *i.e.* 137,000 km^2, need remediation because of soil concentrations in heavy metals being higher than the recommended threshold value - *e.g.* 1, 60 and 200 mg kg^{-1} for Cd, Pb and Zn according to the Finish Ministry of Environment (Tóth et al., 2016). In China, more than 17% of land is considered to be moderately to extremely contaminated with Cd (Chen et al., 2015). When metals are concentrated in a small volume of soil, the disposal of the contaminated soil as hazardous waste is feasible. However, for large areas with diffuse contamination, the cost of soil excavation and disposal become prohibitive. In these cases, phytoremediation strategies may represent the best alternative, *e.g.* phytoextraction, which consists in removing the metals from soil by favoring their uptake by roots and their translocation to the aerial parts of the plants. With successive harvests, soil contamination may therefore be progressively removed. Plants that can tolerate high metal contamination and accumulate large amounts of metals in their shoots have been targeted for implementing this technique. In particular, hyperaccumulators correspond to plants which are able to reach very high shoot metal concentrations, typically 100 mg kg^{-1} for Cd, 1000 mg kg^{-1} for Ni or Pb and 10,000 mg kg^{-1} for Zn (Baker and Brooks, 1989), although alternative thresholds can be defined (van der Ent et al., 2013). The use of hyperaccumulators has been tested in field conditions with various success rates (Hammer and Keller, 2003; Jacobs et al., 2018; McGrath et al., 2006). One of the main limitations of phytoextraction is the time needed for reaching acceptable soil metal concentrations, which can range from 4 to more than 100 years (Hammer and Keller, 2003; Mahar et al., 2016). For this reason, some have suggested that phytoextraction of metals with hyperaccumulators may be more suited to a strategy of bioavailable contaminant stripping, aiming to significantly decrease the size of plant-available pool of metals (Jacobs et al., 2018). Whether phytoextraction can be sustained over repeated harvests is also uncertain, as metal phytoavailability may decrease over time and thus continuously reduce the amount of metal removed from the soil.

In order to improve the efficiency of phytoextraction techniques, soil amendments may be used to increase either plant growth or plant metal uptake, or to decrease the risk of metal dissemination by leaching or soil erosion. Biochars, *i.e.* the solid products of the pyrolysis of plant-derived materials, organic wastes or any other type of biomass, have been intensively investigated over the last decade as a soil amendment with objectives as diverse as building a stable soil carbon stock, improving soil productivity, or restoring degraded and contaminated lands (Beesley et al., 2011; Lehmann et al., 2006). Contrasted results of biochar amendments on soil metal availability and plant metal uptake have been obtained, depending on the investigated plant species, the type of soils and contamination, and the nature and amount of biochar. Biochars have been suggested to have the capacity to bind metals (*e.g.* Cd, Cr, Cu, Ni, Pb, Zn) by electrostatic interactions,

complexation with organic functional groups, interactions with π electronic systems from C=C bonds, or by precipitation, *e.g.* with phosphate and carbonate (Li et al., 2017; Wu et al., 2017). Biochar amendments can also indirectly decrease the mobility of most metallic elements in soils by modifying soil redox potential and by increasing soil pH (Beesley et al., 2011; Chen et al., 2018; Houben et al., 2013; Rees et al., 2014). Consequently, a decrease in plant metal uptake following biochar amendments has been reported in most cases (e.g. Chen et al., 2018; O'Connor et al., 2018; Rizwan et al., 2016).

However, in the cases of metal-accumulating or hyperaccumulating plants, the influence of biochar amendments on shoot metal concentrations remains unclear. A 50-85% decrease in shoot Cd concentrations amended with 3% (w:w) biochars derived from poultry litter or eucalyptus wood was reported with the metal accumulator *Amaranthus tricolor* grown for 2 months on an agricultural topsoil sampled nearby a waste landfill (Lu et al., 2014). Similarly, Fellet et al. (2014) reported a decrease in shoot Cd concentrations of the metal accumulators *Anthyllis vulneraria* and *Noccaea rotundifolium* grown on mine tailings amended with 1.5% or 3% (w:w) biochars derived from pruning residues or from manure, but they also observed an increase ranging from 40% to more than 2-fold in the shoot Cd concentrations of these plants with their wood-derived biochar. With the Ni-hyperaccumulator *Alyssum murale*, a 25% decrease in shoot Ni concentration was observed with 3% or 5% (w:w) amendments of a wood-derived biochar in an ultramafic soil, while no decrease was detected on a Technosol (Rue et al., 2019). No effect of biochar amendments at 2.5% (w:w) was observed on Cd concentration in the shoots of the Cd-hyperaccumulator *Sedum alfredii* grown for 2.5 months on an urban agricultural soil together with upland kangkong (*Ipomoea aquatica*), despite a significant decrease in Cd uptake by the leguminous plant (Hu et al., 2014). Similarly, no effect of corncob-derived biochar amendment at 1% or 5% (w/w) was seen on the shoot Cd concentration of the hyperaccumulator *Solanum nigrum* in an artificially contaminated soil (Li et al., 2019). With the Cd/Zn hyperaccumulator *Sedum plumbizincicola*, a decrease of 20-50% in shoot Cd concentrations was observed with 1% or 5% (w:w) additions of biochars derived from bamboo or rice straw after a 3-month growth on a soil contaminated by smelter activities (Lu et al., 2014), while no effect of 2% (w/w) biochar derived from maize straw was seen on the shoot Cd and Zn concentrations of this hyperaccumulator in four contaminated soils (Fan et al., 2019). However, a 25-50% increase in shoot Cd and Zn concentrations was also reported in the case of *Sedum plumbizincicola* plants grown for 2.5 months on a spiked soil amended with 5% corn-straw biochar (Li et al., 2018). Our previous works showed an increase of 30%-40% in Cd and Zn concentrations in the shoots of the Cd- and Zn-hyperaccumulator *Noccaea caerulescens* grown in smelter-contaminated soils with 5% (w:w) additions of a wood-derived biochar pyrolyzed at 450°C (Rees et al., 2016, 2015). We previously explained this increase in plant metal uptake by i) a decrease in the competition between Cd^{2+}, Zn^{2+} and the major cations in solutions (*e.g.* Ca^{2+}) for the symplastic transport across root cell membranes (Rees et al., 2015)

and ii) an increase in the total surface developed by roots in the presence of biochar amendments (Rees et al., 2016).

The present study was designed to verify whether this increase in plant Cd and Zn uptake by *N. caerulescens* with biochar amendments would also be observed in conditions close to the field and over successive harvests. In order to evaluate all possible advantages and limitations of biochar-assisted phytoextraction, we chose to test again the effect of a high dose of biochar (5%) on the growth and metal uptake of *N. caerulescens*, but this time over four years and using two large intact soil monoliths excavated from a contaminated site and monitored with instrumented lysimeters. To our knowledge, this is the first time that such a monitoring system has been used to investigate the effect of biochar on metal uptake by hyperaccumulating plants. We hypothesized that i) the successive harvests of *N. caerulescens* will enable to remove a significant fraction of metal contamination from the topsoil and decrease soil metal mobility, and that ii) biochar amendments will favor plant growth, decrease soil metal availability and increase Cd and Zn hyperaccumulation potential by *N. caerulescens*.

MATERIAL & METHODS

Materials

Biochar was produced by Carbon Terra GmbH by pyrolysing a mix of 80% softwood and 20% hardwood at 450 °C for 36 h. Biochar was dried in ambient conditions for one week, crushed and sieved to 2 mm. Its main properties are presented in Table 1. Additional biochar properties can be found in our previous works (Rees et al., 2017, 2016, 2015, 2014).

The investigated soil belonged to an agricultural plot planted with poplar trees for more than a decade, and located nearby a lead and zinc smelter in the Northern part of France in Evin-Malmaison (N 50° 26' 10.7'', E 03° 00' 57.1''). Mean annual temperature in this area is 10.8 °C and mean annual total precipitation is 740 mm. Soil was classified as a Cambisol (FAO and IUSS, 2015). Soil initial properties of the top 30-cm are indicated in Table 1. As a consequence of atmospheric deposition from the smelter, high concentrations of Cd, Pb and Zn were present in the first 30 cm of the soil (Sterckeman et al., 2000). The topsoil was characterized as a silt loam and was slightly alkaline (pH 8.1) because of repeated liming operations by farmers.

In June 2009, two identical, undisturbed cylindrical monoliths of soil (1 m^2, 1.85-m deep) were sampled and transported to the lysimeter experimental station of the French Scientific Interest Group for Industrial Wasteland (GISFI) in Homécourt, France. Climatic conditions there are similar as in the sampling site, with a mean annual temperature of 9.8 °C and a mean total annual precipitation of 750 mm.

Lysimeter monitoring

The two undisturbed soil columns were installed outdoor in instrumented stainless steel lysimeters (Umwelt Geräte Technik GmbH, Müncheberg, Germany) for a long-term monitoring of water and metal fluxes in natural environmental conditions. The total weight of each soil column was measured hourly by three 3510 shearbeam load cells (SOEMER Messtechnik GmbH, Lennestadt, Germany), with a precision of 0.1 kg. The flux of percolated water at 185-cm depth was monitored every hour with a 100-ml tip counter. Samples of percolates were collected every 10 to 20 L, as well as soil pore water samples extracted through suction cups under vacuum at 50 cm, 100 cm and 150 cm depth. Water samples were kept at 4°C until further analysis.

Biochar amendment

From June 2009 to March 2013, the grass cover present at the surface of each column was left unmanaged. The most abundant species was *Arrhenatherum elatius*. In March 2013, all plant shoots were harvested, four cylindrical soil cores were taken in the 0-10 cm surface horizon to measure the initial soil bulk density and water retention curves, and the top 30 cm of each soil

column was excavated. The corresponding soil was air-dried inside the station building for a few days to facilitate its processing. Representative soil samples were taken from each excavated pile for further analysis. Biochar was then thoroughly mixed to the excavated topsoil of one of the column, while the other one was left unamended. Intimate mixing between soil and biochar was achieved by manually mixing 20-kg subsamples. A similar manipulation was done on the unamended excavated topsoil to avoid any bias. A total of 340 kg of moist topsoil, equivalent to 290 kg of dry mass, were then reintroduced at the surface of each soil column, with one of the column containing additional 15.3 kg of biochar, corresponding to an amendment dose of +5% (w/w) or 153 t ha^{-1}. Soil was compacted after each addition of a 5-cm layer with the help of a hand compactor. Due to the addition of biochar, soil surface in the amended soil column became 4.5 cm higher than the one in the control soil column.

Plant cultivation and sampling

From 2013 (Year 1) to 2016 (Year 4), the hyperaccumulator *Noccaea caerulescens* from Ganges population was grown at the surface of each lysimeter at a target density of 124 plants per m^2. Plants were either transplanted as 3-week seedlings or directly sown at the surface of the soil, sometimes several times a year due to unsuccessful plant growth (see details in Table S1 in the Supplementary Material). The annual period of growth therefore ranged from 4 months to 7 months, depending on the annual variations of climate and other environmental factors. In the cases where seedlings were grown in controlled conditions for 3 weeks before transplantation, subsamples from control or biochar-amended topsoil were used as the growing media, and germination rate was recorded over the first two weeks. A fertilizer solution of $CaSO_4$ and $Ca(NO_3)_2$ (4 gN m^{-2} and 1.5 gS m^{-2}) was added at the surface of each lysimeter in July of Year 2 in order to optimize the growth of *N. caerulescens*. A molluscicide (Naturasol, 1% ferric phosphate) was also added every year at a dose of 5 g m^{-2} to prevent any slug invasion.

The surface of each lysimeter was divided in four quadrants, and distinct soil and plant samples were taken from each quadrant (Fig. S1). Every year in early October, all shoots of *N. caerulescens* were harvested after counting the individuals on each lysimeter. Five soil samples were taken from the 0-25 cm disturbed horizon with a gouge and homogenized to get one representative soil sample from each quadrant (Fig. S1). Roots samples were collected by digging 20-cm cubes of soil and sieving them to collect roots. The sieved soil was then reintroduced in the lysimeters. After taking all samples, the topsoil with all remaining roots was homogenized with a shovel, and new seeds of *N. caerulescens* were sown at its surface.

Besides the above-mentioned procedure, additional samples were taken. In July of Year 1, samples of both shoots and roots from 5-10 plants per quadrant were collected. In October of Year 1, 2, and 3, shoots of weed plants that had spontaneously grown together with the hyperaccumulator were collected separately. In Year 4, no other plant species than *N. caerulescens* was allowed to

grow on the lysimeters, and weed plants were regularly uprooted by hand and left at the surface of the soil. In October of Year 1, three 10-cm cylindrical soil cores were taken at the surface of each lysimeter for characterizing water retention and hydraulic conductivity curves. Four additional soil cores were also taken in each lysimeter in October of Year 1 in the 5-10 cm horizon for preparing thin sections (Watteau et al., 2018). In October of Year 3, soil samples were taken in deeper horizons (30-35 cm, 40-45 cm, 60-65 cm and 105-110 cm) in each lysimeter with the help of an auger.

Analyses

Soil samples were air-dried, sieved to 2 mm and analyzed by the *Laboratory for Soil Analyses* of INRA-Arras (France) using standard techniques (AFNOR, 2013): soil particle size distribution (NF X 31-107), pH in water (1:5 ratio (v/v), NF ISO 10390), total organic C (NF ISO 10694), total N (NF ISO 13878), available P (Olsen method, NF ISO 11263), CEC (Metson method, NF ISO 23470), total trace elements (extraction with HF-$HClO_4$, NF ISO 14869-1) and exchangeable trace elements with 0.01 M $CaCl_2$ (1:10 (w/v), NEN 5704).

Root samples were washed with deionized water, scanned using an Epson 10000 XL at 800 dpi and analyzed for total root length, total root surface and mean diameter using Winrhizo software (V. 2005c, Regent Instruments). Roots were then sonicated and washed again several times with deionized water. Shoots were rinsed two times with deionized water. Roots and shoots were then dried for 72 h at 40 °C, weighed and ground. The content of C and N was measured by dry combustion. Subsamples of roots and shoots were mineralized according to the following procedure: 500 mg were placed in a polypropylene tube (DigiTUBE, SCP Science, Québec, Canada) with 8 mL of HNO_3 65% and 4 mL of H_2O_2 30%, left overnight at ambient temperature and then heated for 180 min at a final temperature of 95 °C in a heating block (DigiPREP, SCP Science). Elemental concentrations in digests were measured with ICP-AES (aiCAP6300 Duo, ThermoScientific, Waltham, USA).

Soil cores taken at the surface of the lysimeters in March 2013 (Year 1, before disturbance) and in October 2013 (Year 1, after the first harvest) were used to determine soil bulk density as well as the water retention and hydraulic conductivity curves by Schindler's method (Schindler and Müller, 2006) with the help of a ku-pF analyzer (Umwelt Geräte Technik GmbH).

Water samples collected at -50 cm, -100 cm and -150 cm and water samples collected at the bottom of the lysimeters (-180 cm) were analyzed for pH and electrical conductivity on the same day of collection, and later for major and trace elements concentrations by ICP-AES (limits of quantification are provided in Table S2) and NO_3^-, SO_4^{2-} and Cl^- concentrations by ionic chromatography (Dionex IC25, ThermoScientific). Water samples from Year 4 were not analyzed by ionic chromatography because of technical issues with the instrument.

Calculations

Mass balances were calculated on each lysimeter for the 0-30 cm disturbed topsoil and for some of the underlying horizons. Soil metal stocks were calculated from the measured mass concentrations and the bulk density (1.3, 1.1 and 1.4 t m^{-3} for the topsoil of the control lysimeter, the topsoil of the biochar-amended lysimeter and the underlying horizons, respectively). The mass of phytoextracted metals was calculated for each lysimeter quadrant from the measured shoot biomass and shoot metal concentration. Water evapotranspiration (mm day^{-1}) on each lysimeter was calculated by mass balance according to the following equation:

$$E(\Delta t) = R(\Delta t) - P(\Delta t) - \Delta m$$

where $E(\Delta t)$ is the calculated evapotranspiration over the period Δt, $R(\Delta t)$ the amount of precipitation, $P(\Delta t)$ the amount of percolated water at the bottom of the lysimeter and Δm the variation of the total weight of the lysimeter over the same period.

Mean individual biomass was estimated by dividing the total shoot biomass measured on each quadrant by the corresponding number of plants. Root biomass (g cm^{-3}) and root surface (cm^2 cm^{-3}) were expressed as a density relative to the volume of soil in which roots were sampled. Metal translocation factors were calculated as the ratio of shoot over root concentrations. Mean values and standard errors of *N. caerulescens* individual shoot biomass, root biomass and surface density, elemental concentration and metal translocation factors were calculated from the four values obtained from each lysimeter quadrant, which were considered to reflect the variability of plant parameters. The significance of difference of these parameters obtained between the control and the biochar-amended lysimeter and over time was assessed with a two-way analysis of variance (ANOVA). The Newman-Keuls test was used to compare the mean values, which were considered statistically different at the significance level $p = 0.05$. Calculations were made using R (2.13.0) software (R Development Core Team, 2008).

RESULTS

Soil analyses

Soil properties of the topsoil sampled every year in the control and biochar-amended lysimeters are indicated in Table S3. The total concentration of Cd decreased over the successive years, particularly at the end of the first annual harvest of *N. caerulescens*. Comparatively, the concentrations of Zn and Pb did not significantly diminish. 0.01 M $CaCl_2$ extractable metals (Cd, Cu, Ni, Pb and Zn) generally decreased over time, particularly in the case of Cd, where this fraction represented 1.8% of the total soil Cd at the beginning of Year 1 and only 0.6% at the end of Year 4. Total soil organic C content of the biochar-amended soil was three times higher compared to the control, and did not diminish over the four successive years. Soil pH remained similar between the control and the biochar-amended soil and over the different years of sampling. Cation exchange capacity increased over time, particularly in the biochar-amended soil.

Water dynamics and composition

Following the introduction of biochar in the amended lysimeter, differences in water dynamics were observed when compared with the control lysimeter. From Year 1 to Year 3, the cumulated volume of water collected at the bottom of the biochar-amended lysimeter was lower than on the control lysimeter (Fig. S2), while no differences were observed between both lysimeters prior to the amendment. Evapotranspiration was generally higher at the surface of the biochar-amended lysimeter in hot summer days (Fig. S23 and S4). Water retention curves, calculated on the topsoil of both lysimeters before and after the amendment in Year 1, showed that water retention was increased for low water tension values after the disturbance of the topsoil on each lysimeter (Fig. S5). The biochar-amended topsoil had a higher water retention capacity compared to the control topsoil (Fig. S5), and a higher available volumetric water content (30% versus 18%). No clear differences in unsaturated hydraulic conductivity curves were observed before and after the disturbance of topsoil or between both lysimeters (Fig. S6).

The analysis of water samples collected between Year 1 and Year 4 in the percolates at the bottom of the lysimeter and in the suction cups at different depth showed that Cd and Pb were rarely detected, with concentrations lower than 5 µg l^{-1}, while Zn concentrations measured remained below 100 µg l^{-1} (data not shown). Higher concentration of nitrate were measured in water samples collected at -50 cm in Year 1 and Year 2 and in percolates in Year 2 and Year 3 in the control lysimeter compared to the biochar-amended one (Fig. S7 and S8). No other major differences in water composition were observed between both lysimeters from Year 1 to Year 4 (data not shown).

Plant growth

Results of annual shoot biomass production of *N. caerulescens* and weed plants are presented in Fig. 1. A mean annual yield of 0.41 ± 0.04 kg m^{-2} was observed for the hyperaccumulator on both lysimeters, with the exception of the control lysimeter in Year 3 where only 0.05 kg m^{-2} was recorded. This low yield was probably linked to the particularly dry climatic conditions in the spring of Year 3 (Fig. S9a and S9b), as only 7 plants grew over the summer on the control lysimeter compared to 127 plants on the biochar-amended lysimeter (Fig. 1). The benefit of biochar amendment at germination stage was confirmed in controlled conditions, when seedlings of *N. caerulescens* were grown in germination plates (Fig. S10). More generally, a slightly higher number of *N. caerulescens* individuals was observed every year on the biochar-amended lysimeter compared to the control (Fig. 1). The mean shoot biomass of each plant did not vary over the years or between the two lysimeters, with the exception of Year 3, where the few individuals grown on the control lysimeter had a higher weight compared to other years and compared to the numerous individuals grown on the biochar-amended lysimeter (Fig. S11). Interestingly, the number of *N. caerulescens* individuals having reached the flowering stage (Fig. S12a,b) at October harvest decreased over the successive years up to the point of observing no flowers in Year 3 and Year 4.

Besides *N. caerulescens*, other plant species (hereafter referred to as "weeds") spontaneously grew at the surface of lysimeters, mainly leguminous plants such as *Vicia sativa* and *Trifolium repens* (Fig. S12d). No obvious differences in terms of plant communities and weed biomass production were observed between the control and the biochar-amended lysimeter (Fig. 1 and Fig. S13).

Annual root biomass density was similar between both lysimeters except in Year 3 (Fig. S14). However, root surface density was increased in the presence of biochar amendment (Fig. S15). The increase in root surface was consistent for each class of root diameter (Fig. S16-18).

Plant elemental composition

The elemental composition of shoots and roots of *N. caerulescens* harvested every year in October is indicated in Table 2. Concentrations of Cd and Zn in the shoots of *N. caerulescens* generally decreased over the successive annual harvests (Fig. 2a,b), except in the case of Year 3. Translocation factors, defined as the ratio of shoot concentration over root concentration, were significantly lower in Year 4 compared to Year 1 (Fig. S19 and S20). The comparison of Cd and Zn concentrations in *N. caerulescens* shoots in July (age: ~3 months) and October (age: ~5.5 months) of Year 1 suggested that Zn concentrations increased with plant age while Cd concentrations remained identical (Fig. S21 and S22). However, no correlation was found throughout the four years of monitoring between the age of *N. caerulescens* plants at October harvest and their shoot Cd or Zn concentrations (data not shown). In general, no significant differences were observed between the control and the biochar-amended lysimeter in terms of root or shoot concentrations of Cd and Zn in *N. caerulescens*, with the exceptions of higher root Cd concentrations in the biochar-amended soil in Year 2 and Year 3 (Table 2). The translocation factor

of Cd was consequently significantly lower in these years on the biochar-amended soil compared to the control soil (Fig. S19).

In Year 1, significantly lower concentrations of Ni and Pb were measured in *N. caerulescens* shoots grown on the biochar-amended lysimeter compared to the plants grown on the control soil (Table 2). Such differences were not observed in the subsequent years. In general, no significant differences of Cd, Cu, Ni, Pb and Zn concentrations in weed plants were observed between the control and the biochar-amended lysimeter (data not shown).

Concentrations of N and P were lower in the shoots of *N. caerulescens* grown on the biochar-amended soil in Year 2 and 3, while no difference was observed between both lysimeters in Year 1 and Year 4 (Table 2). Concentrations of Ca in the shoots of *N. caerulescens* followed the same trend as Cd and Zn concentrations, with the exception of Year 3 on the control lysimeter where the few plants that grew had a concentration of Ca unusually high compared to other years. When eliminating this point, shoot Ca concentration was shown to be highly correlated to Zn and, to a lower extent, Cd concentrations (Fig. S23 and S24). No such correlation could be observed with other cationic elements such as K, Mg or Na (data not shown).

Mass balance

The overall mass balance of Cd, Pb and Zn in the topsoil of each lysimeter over the four successive years of growth of *N. caerulescens* is summarized in Table 3. The amount of phytoextracted Cd after four years represented about 40% of the mass of Cd initially present at the beginning of Year 1. Comparatively, the amount of phytoextracted Zn only accounted for 3-4% of the initial amount present in the soil, and phytoextracted Pb corresponded to less than 0.1% of the initial soil Pb. The cumulated mass of Cd and Zn phytoextracted by *N. caerulescens* over the four years was 20-30% higher on the biochar-amended lysimeter than on the control one. The amount of Cd phytoextracted by *N. caerulescens* decreased over the successive annual harvests on both lysimeters (Table S4). The amount of phytoextracted Zn also decreased after Year 2 (Table S4). The mass of Cd and Zn phytoextracted by the shoots of weed plants represented less than 0.2% of the mass phytoextracted by the shoots of the hyperaccumulator (data not shown).

In most cases, a significant fraction of the amount of metals removed from the topsoil could not be accounted by phytoextraction, or by the sampling of soil and roots over the different years (Table 3). We hypothesized that this unexplained loss of metals was due to the leaching of metals under dissolved or colloidal forms in the deeper soil layers following the disturbance of the topsoil in both soil columns at the beginning of Year 1. The maximal amount of dissolved metals that could have been lost in the percolates collected at 1.85 m depth or through the sampling of pore water was estimated from all the concentration values detected by ICP-AES, and was shown to be insignificant compared to the unexplained losses of metals from the first 30 cm (Table S5). The

analysis of soil samples taken at higher depth at the end of Year 3 showed an accumulation of Cd, Pb and Zn between 30 and 45 cm compared to the initial soil profile analyzed 7 years before (Table S6). Rough estimations of the amount of metals accumulated in these horizons in Year 3 compared to the initial soil profiles were of the same order of magnitude as the unexplained loss of metals from the topsoil horizon (Table S7). This estimated migration of metals from the topsoil to the underlying horizons was higher in the biochar-amended lysimeter than in the control one.

Leaching of nitrate-N, P and K from the bottom of biochar-amended lysimeter was considerably lower than that of the control lysimeter (Table S5). In particular, the estimated amount of nitrate collected in the percolates over the first 3 years was equivalent to a nitrogen loss of 6.5 g N m^{-2} for the control lysimeter and 1.5 g N m^{-2} for the biochar-amended lysimeter.

DISCUSSION

Efficiency of phytoextraction over time

The efficiency of phytoextraction can be defined either in terms of soil decontamination, by examining the fraction of the initial soil metal concentration that has been removed by the plant, or in terms of agromining, by looking at the concentrations of metal in the harvestable parts of the plants and the corresponding total amount of metal that can recovered through phytoextraction. In the perspective of soil remediation, the strategy of growing *N. caerulescens* and harvesting its aerial parts every year led to a significant removal of Cd from the contaminated topsoil after 4 years, representing up to 40 % of the initial contamination. However, the amount of Zn recovered from the shoots represented only 4% of the initial pollution. In the perspective of agromining, the concentrations of Cd and Zn in the shoots of *N. caerulescens* recorded in Year 1 (3000 mg Cd kg^{-1} and 13,000 mg Zn kg^{-1}) were high. Comparatively, concentrations in the range of 10-800 mg kg^{-1} for Cd and 300-30,000 mg kg^{-1} for Zn have been reported by for 60 different populations of *N. caerulescens* grown on a spiked soil containing 9 mg Cd kg^{-1} and 1000 mg Zn kg^{-1} (Sterckeman et al., 2017). Such high concentrations in the aerial parts of the plants are favorable to agromining strategies. The selective recovery of Cd and Zn from the shoots of the plants harvested in Year 1 was indeed proven to be feasible using a hydrometallurgical process (Hazotte et al., 2017), paving the way to the development of a Cd and Zn agromining chain.

However, the efficiency of phytoextraction for Cd and Zn decreased over successive harvests during the 4-year monitoring period, whether considering the fraction of metal removed from the soil or the concentration of metal in the harvested shoots. By defining an annual index of removal efficiency as the ratio between the amount of metals in the shoots at harvest and the total amount of metals initially in the soil at the beginning of the year, we calculated that the efficiency of removal for Cd was 24% in Year 1, 18% in Year 2, and only 6% in Year 4. For Zn, the removal efficiency represented 1.5% in both Year 1 and 2, but only 0.5% in Year 4. This decrease in phytoextraction efficiency resulted in the concentrations of Cd and Zn in shoots to be respectively 7 times lower and 3 times lower in Year 4 compared to Year 1. A decrease of up to 50% in shoot Cd and Zn concentrations of *N. caerulescens* over three successive harvests within the same year had also previously been reported for plants which were transplanted on the field, but not for plants directly sown on the soil (Hammer and Keller, 2003). With the hyperaccumulator *Sedum plumbizincicola*, a decreasing trend in shoot Cd and Zn concentrations over successive harvests was also reported in the case of acidic soils - but not calcareous soils - and was concomitant with a decrease in soil $CaCl_2$-extractable metals (Fan et al., 2019; Li et al., 2014; Zhou et al., 2018).

The drop in shoot metal concentrations observed here over successive harvests may be linked to a decrease in the size of the pool of soil metal available to the plant. A previous sequential extraction of metals on the soil used in our study showed that most of the Cd (78-96%) was present in the

acid-soluble and reducible fractions, and was therefore mostly considered as non-exchangeable (Gommy, 1997). In our study, the correlation between the initial amount of soil $CaCl_2$-extractable Cd and the shoot metal concentration in *N. caerulescens* at the end of the year (Fig. S25) or the total amount of Cd phytoextracted (Fig. S27) supports the hypothesis of a limitation of Cd phytoextraction by the availability of Cd in the soil. Even if the pool of metals extractable by $CaCl_2$, which represented less than 2% of the total Cd in our work, only shows a snapshot of the plant-extractable pool at a given time, the amount of metals extracted from a given soil by 0.01M $CaCl_2$ has been shown to correlate well with the corresponding concentrations of metals in the different organs of several plant species (Lebourg et al., 1996; Menzies et al., 2007). However, plant metal uptake cannot be predicted solely from $CaCl_2$-extractable pool, as seen in the present work by the absence of such correlations for Zn, and as observed in our previous work, where an increase in Cd uptake by *N. caerulescens* was measured despite a decrease in $CaCl_2$-extractable Cd (Rees et al., 2015). The decrease in phytoextraction efficiency over the years might also be explained by less favorable growth conditions over successive harvests, as seen by the decreasing number of plants reaching the flowering stage. Considering that changes in the total root surface developed by the plant can also affect plant metal uptake (Rees et al., 2016), the fact that the highest root surface density was recorded in Year 1 (Fig. S15) also suggests that *N. caerulescens* may have decreased its ability to extract metals over successive harvests because of a smaller or thicker root system.

One might also hypothesize that competition for light, soil nutrients or soil metals could decrease the phytoextraction potential of *N. caerulescens*. During the first 3 years, weeds spontaneously grew together with the hyperaccumulator. The amount of metals extracted by weeds represented less than 1% of that extracted by *N. caerulescens*, and the shoot biomass of weeds less than 5% of the biomass of the hyperaccumulator over the first two years, suggesting that the presence of weeds did not significantly alter the growth and metal uptake of *N. caerulescens*. In Year 3 however, the production of weeds was higher on the biochar-amended soil and the mean individual shoot biomass of *N. caerulescens*, lower, suggesting a possible competition effect. Considering that most of weed plants corresponded to nitrogen-fixing species, it is unlikely that the weed plants represented a serious competition for the acquisition of nitrogen by the hyperaccumulator. However, the growth of *N. caerulescens* could also have been limited by the high density of plants (124 individuals per m^2) in the first two years. In Year 3, the poor germination rate on the control lysimeter led to a very low number of *N. caerulescens* large plants, which produced in average 60% more shoot biomass and had a nitrogen content more than two times higher than individuals grown in other years. This shows that the high plant density chosen in this study was indeed limiting the individual growth of *N. caerulescens*. Jacobs et al. (2018) recently reported a similar growth limitation of *N. caerulescens* by high plant density (100 plant m^{-2} compared to 50 plant m^{-2}), but also showed that the higher density led to a better removal of Cd and Zn from the soil because of the higher biomass production.

It remains difficult to assess how long phytoextraction should be operated in order to reach an acceptable level of soil contamination. Based on our results, we estimated the time necessary to conduct successive harvests in order to decrease soil metal concentrations up to the mean concentrations of uncontaminated loess soils from the same region as the soil investigated in this study, *i.e.* 0.42 mg Cd kg^{-1} and 73.7 mg Zn kg^{-1} (Sterckeman et al., 2002). The theoretical time for reaching such a target was calculated to be 4 years when considering a linear model based on the first year of study only, 11 years by considering a linear model based on the four years of study, and 111 years by using a logarithmic model on the four successive years, *i.e.* by assuming that the phytoextraction efficiency would keep decreasing over years (see details in Supplementary material, Fig. S29 and S30). For Zn, these estimations were respectively 69 years, 97 years or an infinite time. This shows that phytoextraction of metals from such contaminated soil by hyperaccumulating plants can be considered as a strategy to remove a significant fraction of some contaminant, but should not be seen as a definite solution to eliminate the whole soil contamination.

Another concern lies in the transfer of metals down the soil profile. Our mass balance suggests that 10% of the Cd contamination initially present within the first 30-cm migrated toward deeper horizons, especially during the first year of investigation. The excavation, partial air-drying and manual homogenization of the topsoil at the beginning of Year 1 prior to the transplantation of *N. caerulescens* probably caused a large disturbance for soil aggregates and the metals that may have been associated to them. Such heavy operations would not be feasible on the field, but they were done here to decrease the spatial heterogeneity of topsoil within each soil monolith. As a result, a fraction of metals may have been mobilized, either as soluble or colloidal form, and transferred to the underlying horizons once the topsoil was reintroduced in the lysimeter. The continuous increase in nitrate concentrations in percolated water, which started in Year 1 a few months after the reintroduction of the topsoil, also suggests that these disturbances caused an additional mineralization of soil organic matter and/or nitrification of ammonium ions previously retained by the soil, which could also have affected the mobility of metals. Soil sampling at the end of Year 3 showed an accumulation of metals in the 30-45 cm layer, but not in deeper layers. The soil horizons between 35 cm and 50 cm, which had a high clay content, probably acted as a barrier to this transport. This may explain why no significant amount of Cd or Zn was detected in the soil pore water collected at 50 cm and why the amount of Cd, Pb and Zn leached from the bottom of the lysimeters was very low. Thus, the unexpected movement of metal down the soil profile could not result in any contamination of groundwater.

Benefits and limits of biochar-assisted phytoextraction

The amount of phytoextracted Cd from the biochar-amended soil over the four years was 20% higher compared to the control soil. This increase was mainly due to the higher number of plants in Year 3, as only 7 plants were harvested on the control soil due to harsh climatic conditions. This

beneficial effect of biochar on plant growth may have come from a better germination rate (Rees et al., 2016; Rue et al., 2019), which was confirmed in this work during the preparation of the seedlings (Fig. S10), and/or from a higher survival rate when facing water stress, as suggested by the higher water retention measured in the biochar-amended soil.

Despite our expectations, no significant increase in Cd or Zn concentrations in the shoots of *N. caerulescens* was observed when biochar was present, at least when the number of plants was similar between the two lysimeters. The translocation factor of Cd from roots to shoots was even significantly lower in Year 2 and 3 when biochar was present (Fig. S19). Previous experiments in pots (Rees et al., 2015) or in rhizoboxes (Rees et al., 2016) had shown that Cd and Zn concentrations in the aerial parts of the same population of *N. caerulescens* could be increased by more than 30% on a similar alkaline soil amended with 5% of the same biochar, despite a decrease in soil metal extractability. This increase in plant metal uptake was first interpreted as the result of a decrease in competition between Cd or Zn and major cations (*e.g.* Ca^{2+}) in the soil solution, caused by the drop of Ca^{2+} concentrations in the soil solution with an increase in soil pH (Rees et al., 2015). Such increase in soil pH have regularly been reported by various authors, *e.g.* in the context of metal-contaminated soils (Beesley et al., 2011; Houben et al., 2013). Here, no significant increase in soil pH was seen after the introduction of the biochar, which could be due to the already high pH (8.1) of the contaminated soil. The strong positive correlation between the concentrations of Ca and Zn or Cd in the shoots of *N. caerulescens* observed in this work (Fig. S23 and S24) may suggest that the uptake of the target metallic elements occurred through the same transporter system as for Ca, although biochar amendments did not favor this time the uptake of Cd or Zn to the detriment of Ca. Sterckeman et al. (2017) similarly found a positive correlation between Ca and Zn, but a weak negative correlation between Ca and Cd, when comparing different populations of *N. caerulescens* growing in soils rich in Cd, Pb and Zn. We also previously interpreted the increase in Cd and Zn uptake by plants by an increase in root surface density in the presence of biochar (Rees et al., 2016). An increase in root surface density was observed here in the biochar-amended topsoil in specific years. However, this increase in the surface of exchange between the soil and the plant was apparently not strong enough to induce a significant effect on metal uptake. Nonetheless, it should be stressed that Cd and Zn concentrations in the shoots of *N. caerulescens* did not decrease when biochar was present, contrary to the concentrations of Pb in Year 1 and Ni in Year 1 and Year 2. Thus, the potential of Cd and Zn hyperaccumulation by *N. caerulescens* was not impaired by biochar amendments.

The risk of metal mobilization is another criterion to evaluate the pros and cons of any *in situ* soil remediation technique. As noted earlier, the 0.01 M $CaCl_2$ extractable amount of Cd sharply decreased over the first year of growth of the hyperaccumulator, and, to a lower extent, that of other investigated metals (Cu, Ni, Pb, Zn) (Table S2). The comparison between the biochar-amended soil and the control soil suggests that Cu and Ni extractability decreased in the presence of biochar, why no significant effect was seen with Cd and Pb. Intriguingly, Zn extractability

appeared to be 25-33% higher in the biochar-amended soil in Year 3 and Year 4, why no difference could be seen at the end of Year 1. Although we do not have clear explanations to interpret this observation, this suggests that biochar could eventually have increased the mobility of Zn compared to the unamended soil. The mass balance calculated over the four years also suggested that a higher amount of Zn, Pb and Cu was lost from the topsoil when biochar was present (Table 3 and Table S2), which could be interpreted as a migration of the pollution down the soil profile. Even if such process may largely result from soil handling operations at the beginning of Year 1 as discussed above, biochar amendments might have exacerbated this effect by releasing dissolved organic compounds able to complex metals (Beesley et al., 2014; Park et al., 2011), by increasing water conductivity in the topsoil (Lim et al., 2016), or by acting itself as colloidal carrier able to move the metals down the soil profile. The observation of thin sections from the soil samples taken at the end of Year 1 suggested that small particles of biochar were able to move and accumulate around roots and soil aggregates (Watteau et al., 2018). It has also been previously demonstrated that physical weathering can lead to a loss of up to 40% of the initial carbon by leaching of particles or dissolved compounds (Naisse et al., 2014). Although no formal proof has been made here about the possibility of metal dissemination by a transport mediated by biochar particles, we recommend to investigate further this hypothetical mechanism to evaluate this risk.

Eventually, the use of biochar for enhancing phytoextraction of metals with hyperaccumulators can be questioned based on the related costs and benefits. Adding an amount of biochar equivalent to 5% of the topsoil on 30 cm corresponded to an average input of 150 t ha^{-1}, which represents a considerable investment considering the current market price of biochar. The 20%-amelioration of the amount of phytoextracted metal after 4 years may therefore seem insufficient to justify such a large input of biochar. While the dose of biochar should probably be lowered to a more reasonable value, *e.g.* 1%, when applied on the field, it should also be pointed out that additional benefits can be associated to the amendment besides the increase in phytoextraction. In particular, biochar addition resulted in a 3-fold increase in soil organic carbon stock, which was maintained over the four years of monitoring. This efficient way of storing carbon from photosynthetic origins over the long-term adds to the list of biochar-enhanced soil functions and related ecosystem services, *e.g.* the higher soil water retention and the lower risk of eutrophication by N, P, and K leaching observed in this work.

CONCLUSION

This work aimed to assess the potential of Cd and Zn phytoextraction from a contaminated soil by *Noccaea caerulescens*, and to evaluate how biochar amendments may affect it. Our results showed that the hyperaccumulating plant was able to remove over 40% of the initial Cd contamination from the topsoil within four years, and to decrease the plant-available fraction of metals. However, the low fraction of initial Zn that was removed and the decrease in the efficiency of Cd and Zn phytoextraction over successive harvests suggest that a very long time may be needed to remove soil contamination in these conditions. Biochar amendments did not significantly affect shoot Cd or Zn concentrations, but favored plant installation and root development, thus increasing by 20% the total amount of phytoextracted Cd. Furthermore, biochar amendments increased water retention in the topsoil, and helped to limit the losses of N by leaching. Biochar amendments might however have increased the transfer of metals from topsoil to deeper horizons, although this phenomenon may have originally been caused by topsoil excavation and disaggregation prior to the first year of growth. Further research is needed to determine whether maintaining a high soil metal phytoavailability over successive harvests of hyperaccumulating plants can be achieved, and to which costs.

ASSOCIATED CONTENT

Additional data is available online in a Supplementary Material file (6 tables, 30 figures), containing details about: i) plant growth management, ii) soil sampling procedure, iii) evolution of soil properties, iv) water dynamics and nitrate leaching from the lysimeters, v) wheather data, vi) germination rate and shoot growth, vii) root production, viii) translocation factors, ix) correlation between shoot Ca, Cd and Zn concentrations, x) mass balance over the whole soil profile, xi) correlations between soil metal availability and plant metal uptake, and xii) estimations of the time required for removing all soil metal contamination.

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LIST OF TABLES

Table 1: Initial properties of topsoil and biochar at the beginning of Year 1. CEC: Cation exchange capacity. NA: Not available

Measure	Topsoil	Biochar
Sand (50-2000 µm) /%(w/w)	21	NA
Silt (2-50 µm) /%(w/w)	63	NA
Clay (0-2 µm) /%(w/w)	16	NA
pH /-	8.1	9.2
Organic C /%(w/w)	1.9	75
Inorganic C /%(w/w)	0.09	0.21
Total N /%(w/w)	0.11	0.26
Available P (Olsen) /mg kg^{-1}	27	20
CEC /$cmol^{+}$ kg^{-1}	10.5	5
Total Cd /mg kg^{-1}	17.2	0.4
Total Cu / mg kg^{-1}	36.3	9.8
Total Ni / mg kg^{-1}	15.6	5.2
Total Pb / mg kg^{-1}	870	7.2
Total Zn / mg kg^{-1}	1180	112

Table 2: Elemental concentrations measured in October from Year 1 to Year 4 on the shoots and the roots of *N. caerulescens* grown at the surface of the unamended lysimeter ("Control") and the lysimeter amended with 5% biochar on the first 30 cm ("Amended"). Results are presented as Mean ± Standard error. For a given lysimeter, different letters indicate statistical differences between harvest years; for a given year, an asterisk indicates statistical difference between the two lysimeters ($p<0.05$). NA: Not available

		Shoot compartment		**Root compartment**	
		Control	*Amended*	*Control*	*Amended*
Ca content / *mg kg*$^{-1}$	*Year 1*	16020 ± 306 a	16766 ± 369 a	6899 ± 443 a	7397 ± 215 a
	Year 2	13885 ± 565 b	12621 ± 255 b	9169 ± 1226 b	8646 ± 950 a
	Year 3	22647 ± 793 c	12974 ± 563 b*	10712 ± 349 b	7583 ± 316 a*
	Year 4	8932 ± 347 d	9556 ± 378 c	6116 ± 345 a	6671 ± 340 a
Cd content / *mg kg*$^{-1}$	*Year 1*	3057 ± 55 a	3084 ± 78 a	1278 ± 160 a	1044 ± 123 ab
	Year 2	1058 ± 19 b	1150 ± 34 b	573 ± 36 b	1156 ± 162 a*
	Year 3	852 ± 50 c	1058 ± 56 b*	308 ± 2 b	764 ± 73 b*
	Year 4	435 ± 52 d	442 ± 19 c	384 ± 12 b	355 ± 28 c
Cu content / *mg kg*$^{-1}$	*Year 1*	7.1 ± 0.7 a	6.5 ± 0.2 a	35 ± 8 a	41 ± 11 a
	Year 2	5.6 ± 0.3 b	4.6 ± 0.3 b	19 ± 1 a	15 ± 2 a
	Year 3	10.8 ± 0.6 c	8.9 ± 0.6 c*	47 ± 9 a	78 ± 21 a
	Year 4	4.3 ± 0.4 b	3.6 ± 0.2 b	168 ± 19 b	233 ± 50 b*
Fe content / *mg kg*$^{-1}$	*Year 1*	322 ± 47 a	118 ± 13 a*	490 ± 154 a	403 ± 38 a
	Year 2	117 ± 7 b	110 ± 14 a	1238 ± 168 b	648 ± 102 ab*
	Year 3	339 ± 33 a	283 ± 27 b	1418 ± 126 b	1605 ± 193 c
	Year 4	234 ± 5 c	235 ± 17 b	1320 ± 94 b	998 ± 69 b
K content / *mg kg*$^{-1}$	*Year 1*	34609 ± 850 a	34120 ± 398 a	12141 ± 2162 a	10007 ± 584 a
	Year 2	18635 ± 260 b	20842 ± 799 b	3115 ± 750 b	8143 ± 1657 a
	Year 3	46291 ± 3435 c	34049 ± 1078 a*	2192 ± 815 b	18403 ± 1546 b*
	Year 4	21124 ± 1244 b	19430 ± 810 b	10493 ± 2641 a	11520 ± 2021 a
Mg content / *mg kg*$^{-1}$	*Year 1*	1829 ± 42 a	1698 ± 66 a	1758 ± 393 a	1570 ± 106 a
	Year 2	1369 ± 54 b	962 ± 46 b*	1106 ± 191 a	1581 ± 115 a
	Year 3	3140 ± 185 c	1585 ± 38 a*	1261 ± 3 a	1437 ± 64 a
	Year 4	1024 ± 77 d	1145 ± 43 b	1261 ± 105 a	1463 ± 53 a
N content / *mg kg*$^{-1}$	*Year 1*	19720 ± 688 a	17581 ± 345 a	NA	NA
	Year 2	20224 ± 842 a	16904 ± 647 a*	17078 ± 865 a	17379 ± 306 a
	Year 3	44704 ± 1011 b	19868 ± 1122 a*	NA	17967 ± 739 a
	Year 4	15256 ± 1368 c	16707 ± 866 a	16365 ± 438 a	18757 ± 252 a
Na content / *mg kg*$^{-1}$	*Year 1*	81 ± 10 a	61 ± 5 a	280 ± 135 a	549 ± 471 a
	Year 2	27 ± 1 b	24 ± 3 b	68 ± 39 a	22 ± 1 a
	Year 3	90 ± 13 a	58 ± 5 a*	112 ± 23 a	166 ± 20 a
	Year 4	86 ± 12 a	67 ± 6 a	251 ± 26 a	361 ± 40 a

		Shoot compartment		**Root compartment**	
		Control	*Amended*	*Control*	*Amended*
Ni content / mg kg^{-1}	*Year 1*	48 ± 2 a	25 ± 2 a*	5.1 ± 1.4 a	3.4 ± 0.2 a
	Year 2	47 ± 5 a	37 ± 5 a*	6.3 ± 0.8 a	4.2 ± 0.6 a*
	Year 3	9 ± 1 b	17 ± 1 a*	4.9 ± 0.4 a	4.3 ± 0.4 a
	Year 4	14 ± 5 b	12 ± 1 a	3.9 ± 0.6 a	3.5 ± 0.3 a
P content / mg kg^{-1}	***Year 1***	**3024 ± 87 a**	**2708 ± 88 a**	**5683 ± 748 a**	**4954 ± 383 a**
	Year 2	2633 ± 46 b	2107 ± 125 b*	2498 ± 304 b	3815 ± 495 a
	Year 3	4419 ± 164 c	3062 ± 115 a*	2183 ± 307 b	5625 ± 475 a*
	Year 4	2090 ± 138 d	2319 ± 163 b	3409 ± 812 b	4728 ± 552 a
Pb content / mg kg^{-1}	*Year 1*	33 ± 3 a	24 ± 1 a*	224 ± 24 ab	220 ± 8 a
	Year 2	12 ± 0 b	11 ± 1 b	172 ± 13 a	142 ± 7 bc
	Year 3	23 ± 2 c	24 ± 2 a	254 ± 11 b	181 ± 12 ab*
	Year 4	16 ± 0 b	15 ± 1 b	196 ± 20 ab	127 ± 9 c*
S content / mg kg^{-1}	*Year 1*	6463 ± 141 a	5974 ± 156 a	5995 ± 858 a	4929 ± 352 a
	Year 2	NA	NA	NA	NA
	Year 3	7688 ± 242 b	6238 ± 196 a*	3932 ± 553 a	8115 ± 466 b*
	Year 4	3882 ± 219 c	3896 ± 219 b	5910 ± 1043 a	6988 ± 347 b
Zn content / mg kg^{-1}	*Year 1*	13003 ± 356 a	14045 ± 374 a	2884 ± 567 a	2551 ± 189 a
	Year 2	9427 ± 665 b	9198 ± 596 b	1958 ± 269 a	1625 ± 247 a
	Year 3	10508 ± 526 b	11694 ± 421 c	2450 ± 301 a	1646 ± 147 a
	Year 4	4536 ± 403 c	4451 ± 172 d	1719 ± 132 a	1951 ± 202 a

Table 3: Mass balance of the metals present in the 0-30 cm topsoil at the initial state (beginning of Year 1) and final state (end of Year 4) in the unamended lysimeter ("Control") and the lysimeter amended with 5% biochar on the first 30 cm ("Amended"). Known exports of metals from the 0-30 cm over the four successive years corresponded to phytoextraction and to soil and root sampling. The net balance between the initial and final state and the calculated known exports led to the calculation of unexplained losses.

	Cd (g m^{-2})		**Pb (g m^{-2})**		**Zn (g m^{-2})**	
	Control	*Amended*	*Control*	*Amended*	*Control*	*Amended*
Initial state	***5.0***	***5.3***	***252***	***281***	***342***	***378***
Phytoextraction	-1.9	-2.3	-0.0	-0.0	-11.8	-16.0
Soil and root sampling	-0.1	-0.1	-3	-3	-5	-5
Unexplained loss	-0.5	-0.4	-1	-14	-0	-8
Final state	***2.5***	***2.5***	***250***	***263***	***326***	***349***

LIST OF FIGURES

Fig. 1: Photos of the unamended lysimeter ("Control") and the lysimeter amended with 5% biochar on the first 30 cm ("Amended") taken in October of each year before harvest, with indication of the number of *N. caerulescens* individuals, the corresponding dry shoot biomass production, and the dry shoot biomass of weed plants per lysimeter (surface: 1 m^2)

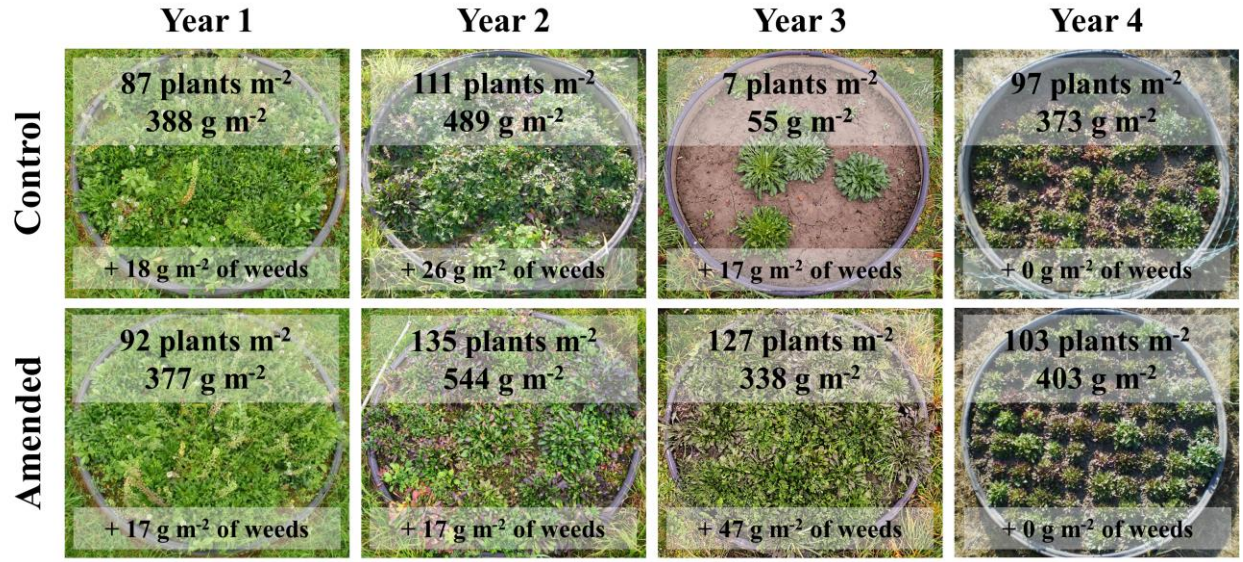
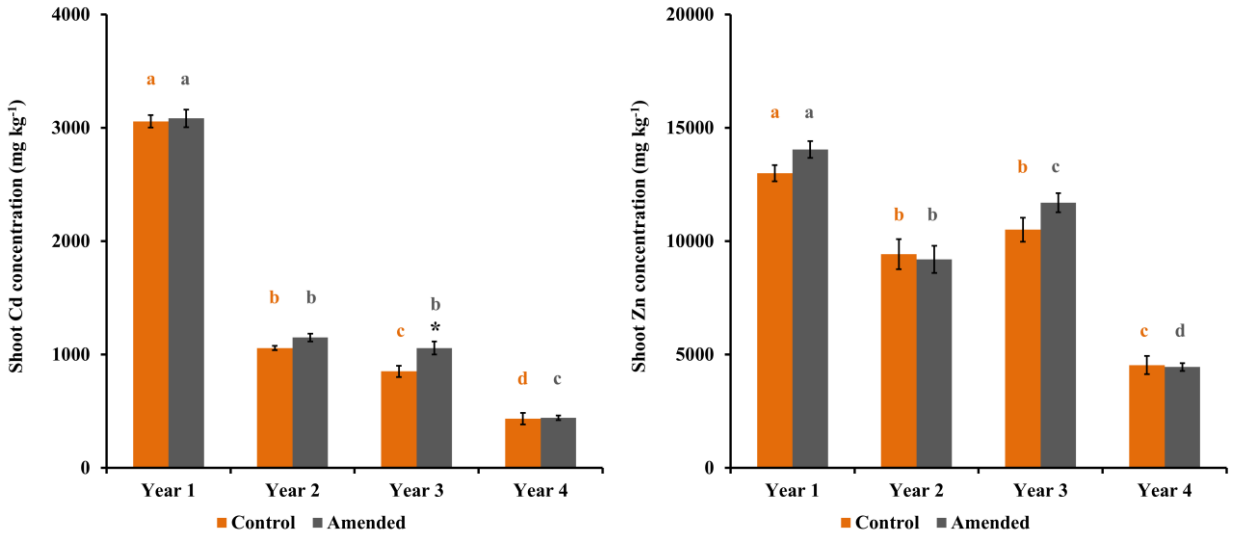


Fig. 2: Mean Cd (a) and Zn (b) concentration in shoots of *N. caerulescens* harvested in October of each year on the unamended lysimeter ("Control") and the lysimeter amended with 5% biochar on the first 30 cm ("Amended"). Error bars represent standard error calculated from the four quadrants of each lysimeter. For a given lysimeter, different letters indicate statistical differences between the years. For a given year, an asterisk indicates a statistical difference between the two lysimeters.



SUPPLEMENTARY MATERIAL

Biochar-assisted phytoextraction of Cd and Zn by *Noccaea caerulescens* on a contaminated soil: a four-year lysimeter study

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Number of tables: 7
Number of figures: 31

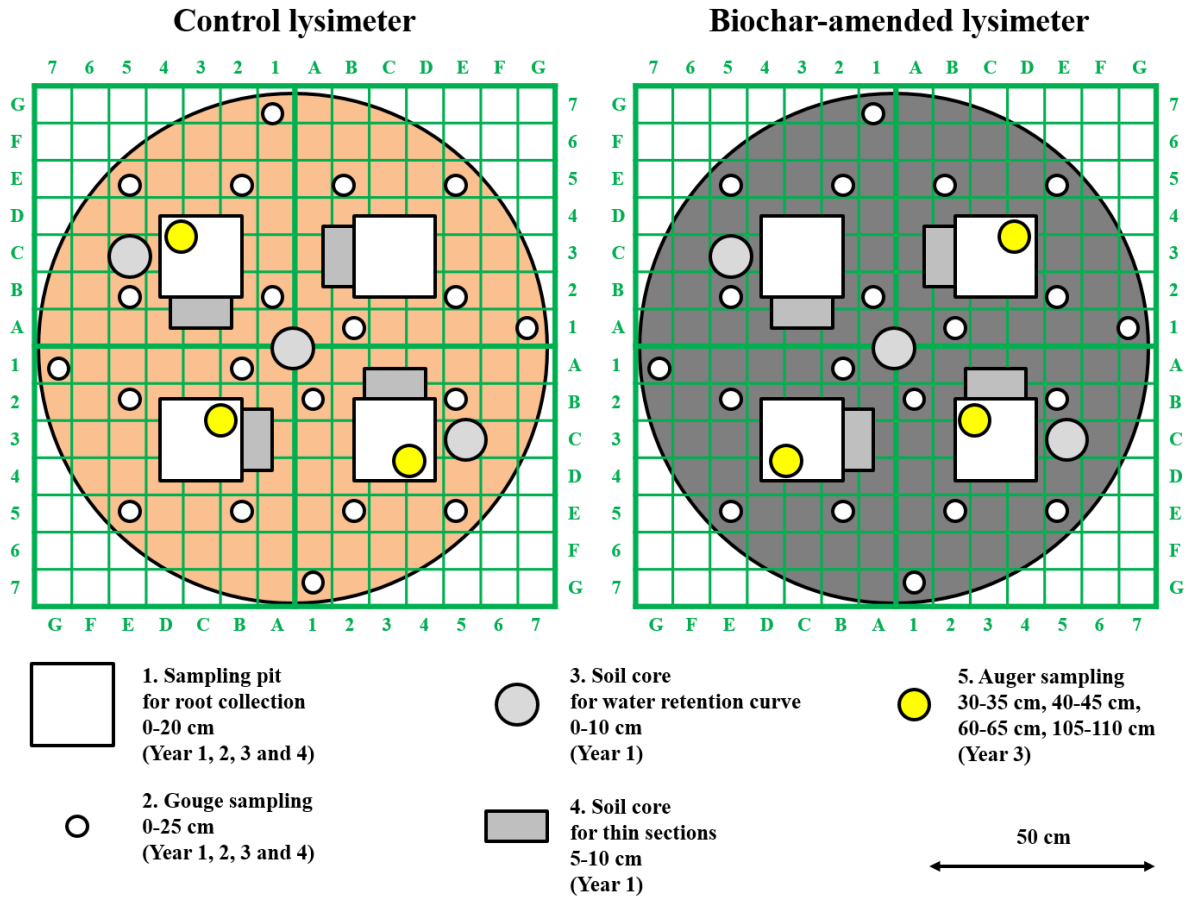
Plant growth management from Year 1 to Year 4

Table S1: Details of the management of *Noccaea caerulescens* growth at the surface of both lysimeters from 2013 (Year 1) to 2016 (Year 4)

Year	Month	Day	Operations
Year 1	4	5	Introduction of 2* 150 seeds of *Noccaea caerulescens* in germination plates
	5	6	Transplantation of 124 seedlings at the surface of each lysimeter
	7	23	Partial sampling of *N. caerulescens* (shoots and roots)
	10	8	**Harvest of all shoots - Estimated age of *N. caerulescens*: 5.5 months**
		25	Introduction of 2* 124 seeds on each lysimeter
Year 2	3	20	Introduction of 2* 124 seeds on each lysimeter
	7	1	Fertilizer input: 4 gN m^{-2} and 1.5 gS m^{-2}
	10	20	**Harvest of all shoots - Estimated age of *N. caerulescens*: 7 months**
		21	Introduction of 2* 124 seeds on each lysimeter
Year 3	5	5	Harvest of all existing shoots and introduction of 2* 124 seeds on each lysimeter
	6	17	Introduction of 2* 124 seeds on each lysimeter
	10	19	**Harvest of all shoots - Estimated age of *N. caerulescens*: 3-4 months**
Year 4	4	20	Introduction of 2* 150 seeds in germination plates
	6	7	Transplantation of 124 seedlings at the surface of each lysimeter
	10	4	**Harvest of all shoots - Estimated age of *N. caerulescens*: 5 months**

Soil sampling procedure

Fig. S1: Scheme of the soil and root sampling protocol after October's harvest from Year 1 to Year 4.



Limits of quantification for metal concentrations

Typical limits of quantification for the elemental analysis by ICP-AES are presented in Table S2. These limits were calculated from the analytical signal obtained on blank water samples.

Table S2: Limits of quantification of elemental concentrations measured by ICP-AES (aiCAP6300 Duo, ThermoScientific, Waltham, USA) calculated from the values obtained from control water samples

Al	4.6	$\mu g\ l^{-1}$
As	7.5	$\mu g\ l^{-1}$
Ca	59	$\mu g\ l^{-1}$
Cd	0.4	$\mu g\ l^{-1}$
Co	1.5	$\mu g\ l^{-1}$
Cr	1.2	$\mu g\ l^{-1}$
Cu	2.1	$\mu g\ l^{-1}$
Fe	2.4	$\mu g\ l^{-1}$
K	56	$\mu g\ l^{-1}$
Mg	11	$\mu g\ l^{-1}$
Mn	0.4	$\mu g\ l^{-1}$
Mo	21	$\mu g\ l^{-1}$
Na	39	$\mu g\ l^{-1}$
Ni	1.2	$\mu g\ l^{-1}$
P	5.3	$\mu g\ l^{-1}$
Pb	3.0	$\mu g\ l^{-1}$
S	31	$\mu g\ l^{-1}$
Zn	0.6	$\mu g\ l^{-1}$

Evolution of soil properties

Table S3: Soil properties measured in the 0-30 cm topsoil of the unamended lysimeter ("Control") and the lysimeter amended with 5% biochar on the first 30 cm ("Amended"). Soil samples were taken in March of Year 1 prior to the amendment and in October of Year 1, 2, 3, and 4 after plant harvest. CEC: Cation exchange capacity. ***Please note that in Year 2, the soil in the Control lysimeter may have been sampled over 35 cm instead of 25 cm – the analysis should therefore be used with caution!***

			Initial	Year 1	Year 2	Year 3	Year 4
Main properties	pH (-)	Control	8.1	8.1	8.2	8.2	8.1
		Amended	8.1	8.2	8.1	8.0	8.0
	C_{org} (g kg^{-1})	Control	19	16	12	16	18
		Amended	17	48	45	52	51
	N (g kg^{-1})	Control	1.2	1.1	0.9	1.1	1.3
		Amended	1.1	1.3	1.3	1.3	1.5
	P_{Olsen} (mg kg^{-1})	Control	31	29	23	30	32
		Amended	32	32	32	34	32
	CEC (cmol+ kg^{-1})	Control	10.5	10.4	10.4	10.6	11.0
		Amended	10.1	10.6	11.1	11.9	11.9
Total concentration (HF)	Cd (mg kg^{-1})	Control	17.2	10.2	5.8	8.8	8.8
		Amended	17.4	12.2	9.9	8.9	8.2
	Cu (mg kg^{-1})	Control	36.3	32.3	26.9	35.2	36.3
		Amended	38.1	36.5	36.0	35.7	36.2
	Ni (mg kg^{-1})	Control	15.6	15.9	15.7	16.8	17.2
		Amended	15.4	15.8	16.3	15.7	15.7
	Pb (mg kg^{-1})	Control	870	775	492	836	874
		Amended	920	859	813	884	874
	Zn (mg kg^{-1})	Control	1180	1030	732	1098	1140
		Amended	1240	1160	1095	1160	1160
Exchangeable concentration (0.01M $CaCl_2$)	Cd (µg kg^{-1})	Control	311	95	41	64	51
		Amended	295	99	72	49	50
	Cu (µg kg^{-1})	Control	172	130	79	84	108
		Amended	170	86	66	71	73
	Ni (µg kg^{-1})	Control	32	25	21	< 15	16
		Amended	23	18	16	< 15	< 15
	Pb (µg kg^{-1})	Control	60	45	19	46	39
		Amended	61	42	35	46	27
	Zn (µg kg^{-1})	Control	2140	1480	556	1178	1220
		Amended	2100	1490	1260	1568	1520

Water dynamics in each lysimeter

Fig. S2: Cumulated amount of water collected every year from the unamended lysimeter ("Control") and the lysimeter amended with 5% biochar on the first 30 cm ("Amended"), from 2012 (prior to the amendment) to 2016 (Year 4). Collected water corresponds to the sum of percolated water and the water extracted from the suction cups.

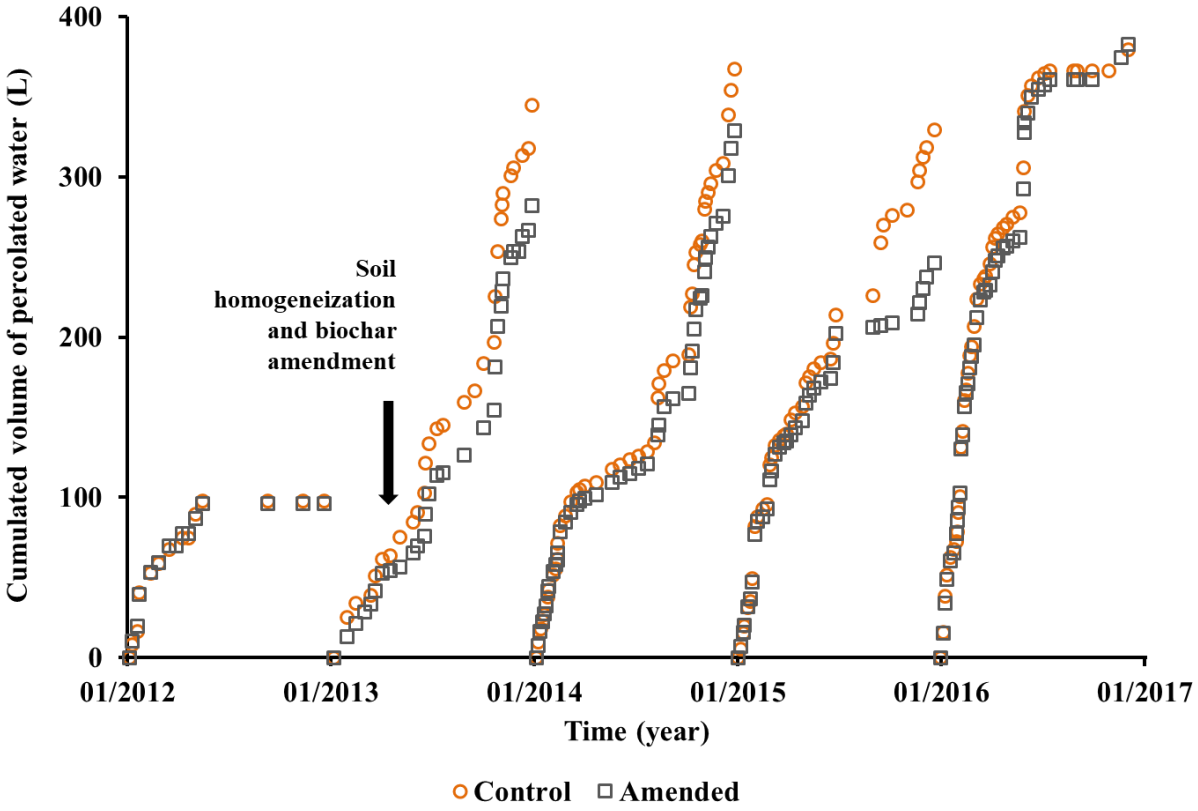


Fig. S3: Daily water evapotranspiration at the surface of the unamended lysimeter ("Control") and the lysimeter amended with 5% biochar on the first 30 cm ("Amended"), for 2012 (prior to the amendment), 2014 (Year 2) and 2016 (Year 4).

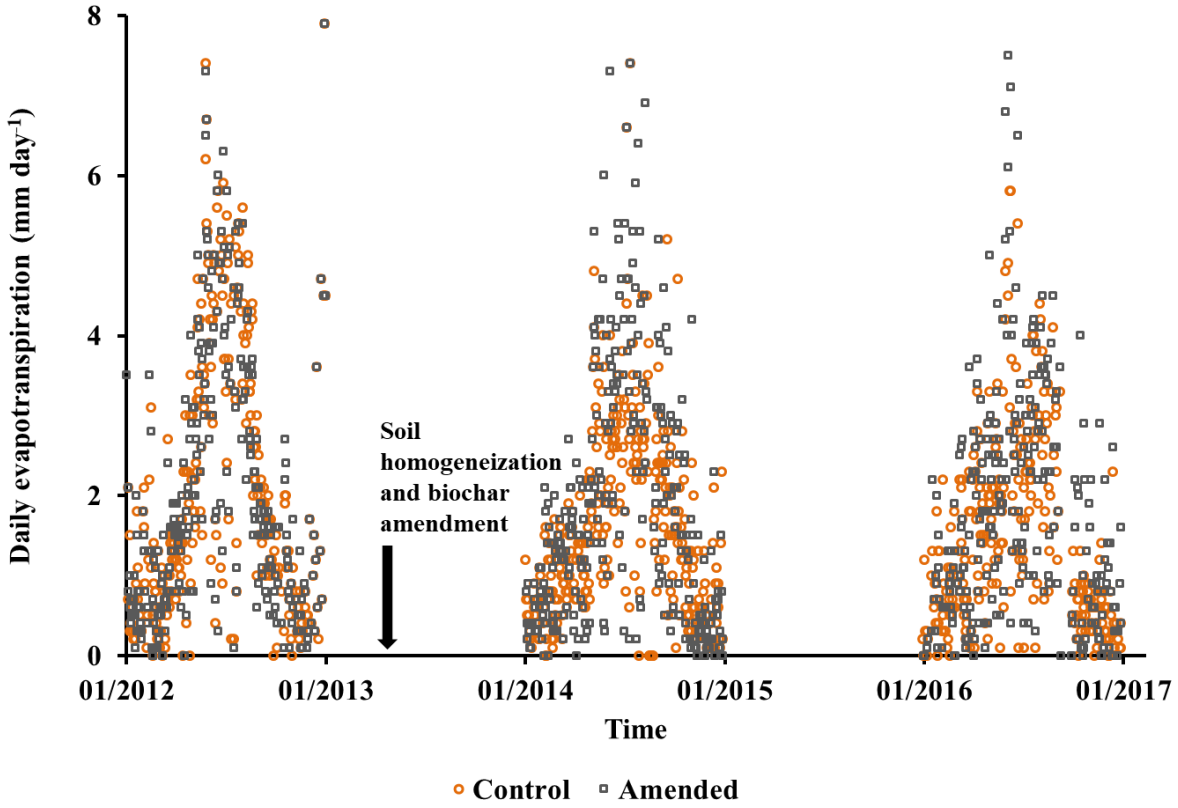


Fig. S4: Daily amount of water evapotranspirated at the surface of the unamended lysimeter ("Control") and the lysimeter amended with 5% biochar on the first 30 cm ("Amended") in Year 2.

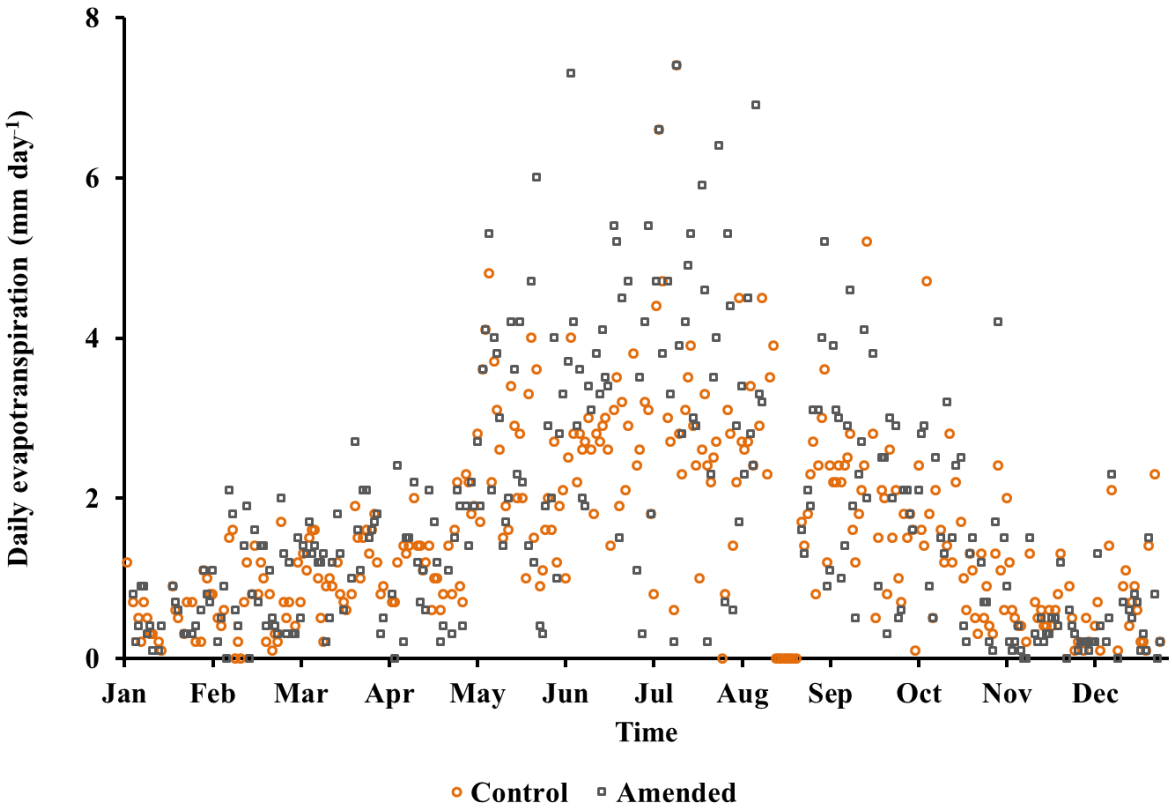


Fig. S5: Water retention curves obtained by Schindler's method and modelled with van Genuchten equation for soil cores taken on both lysimeters prior to biochar amendment ("Initial") and at the end of Year 1 on the unamended lysimeter ("Control") and the lysimeter amended with 5% biochar on the first 30 cm ("Amended").

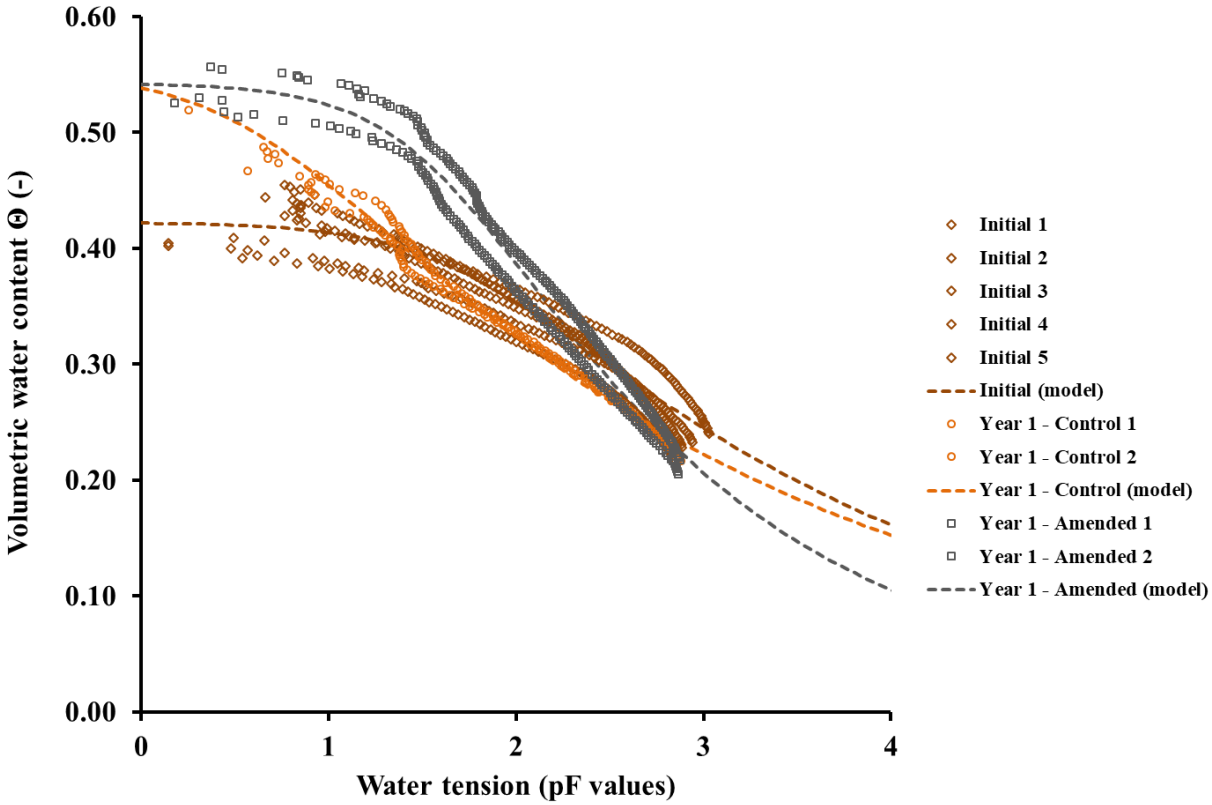
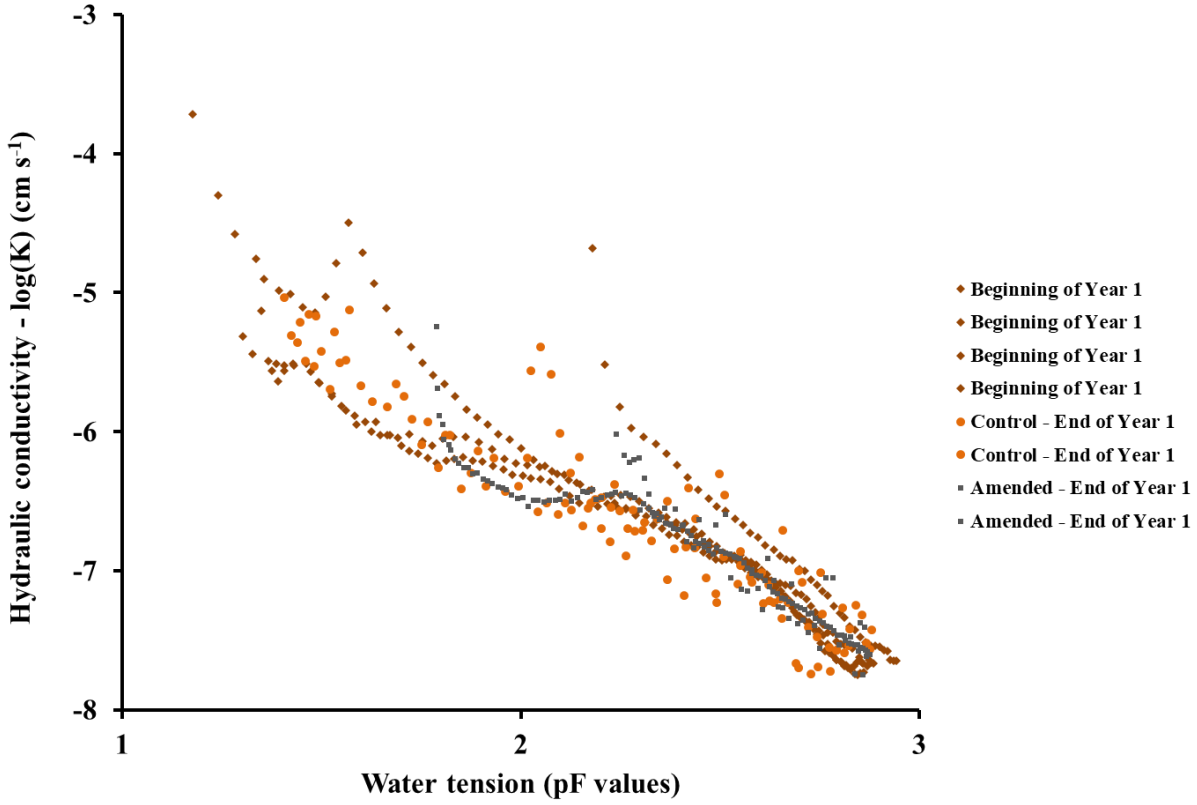


Fig. S6: Unsaturated hydraulic conductivity curves obtained by Schindler's method with soil cores taken on both lysimeters prior to biochar amendment at the beginning of Year 1 and at the end of Year 1 on the unamended lysimeter ("Control") and the lysimeter amended with 5% biochar on the first 30 cm ("Amended"). Because of technical limitations inherent to the method, reliable data on hydraulic conductivity could not be obtained for pF values lower than 1.2.



Nitrate concentration in water samples

Fig. S7: Concentration of nitrate measured in soil solutions samples collected at -50 cm in the unamended lysimeter ("Control") and the lysimeter amended with 5% biochar on the first 30 cm ("Amended"), from 2012 (prior to the amendment) to 2015 (Year 3).

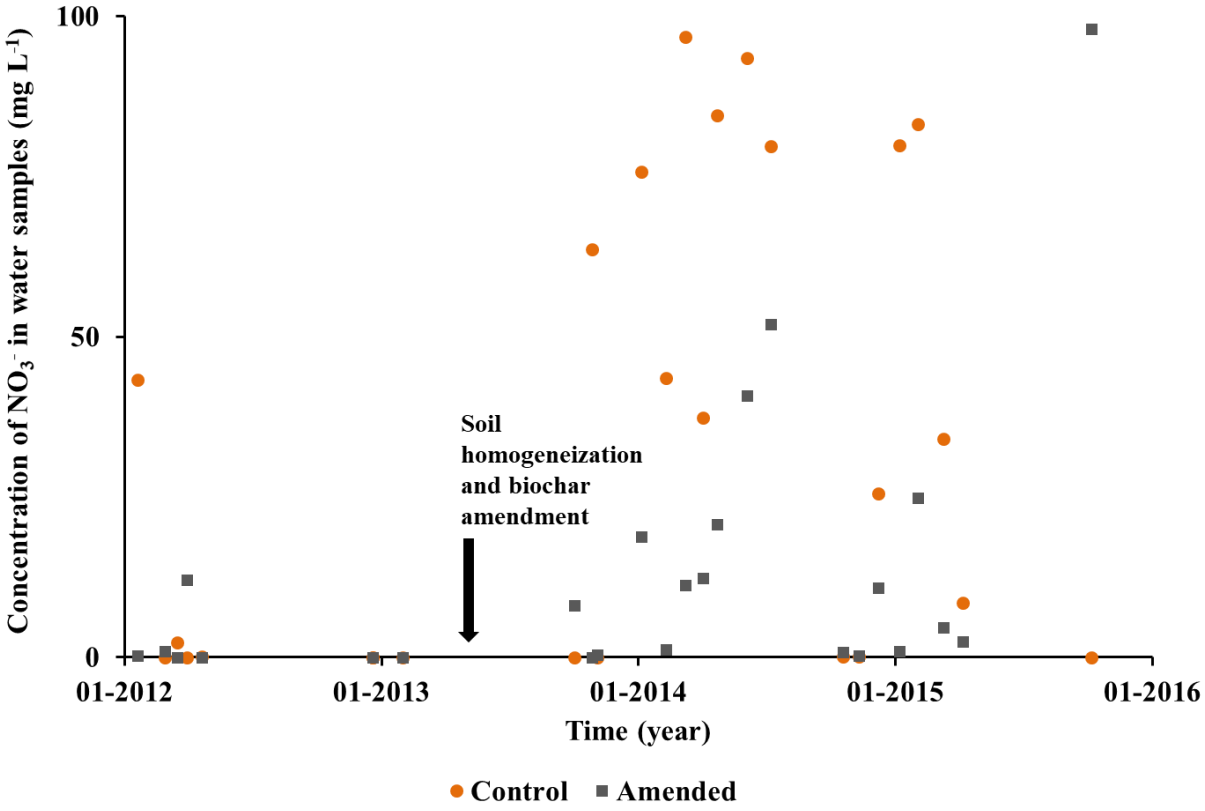
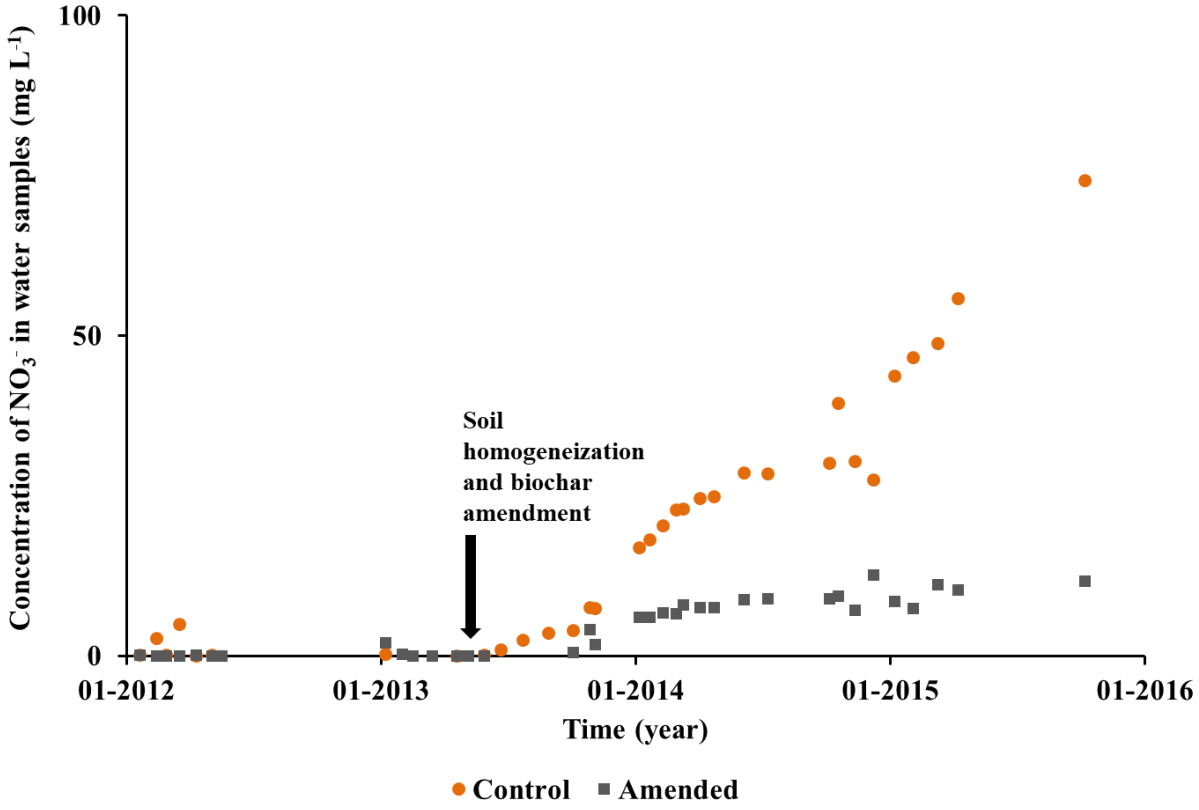


Fig. S8: Concentration of nitrate measured in percolates collected at -180 cm in the unamended lysimeter ("Control") and the lysimeter amended with 5% biochar on the first 30 cm ("Amended"), from 2012 (prior to the amendment) to 2015 (Year 3).



Weather data

Fig. S9a shows the pluviometry cumulated over the month and the average monthly temperature calculated from the data obtained from the weather station installed in Homécourt, 100 meter away from the two lysimeters. As instrumental failure occurred at different periods, similar data obtained from the weather station of Metz, located 30 km away from Homécourt, is displayed in Fig. S9b for comparison. As can be seen from this data, spring in 2015 (Year 3) was unusually hot and dry, which probably resulted in the poor germination rate observed on the control lysimeter.

Fig. S9a: Cumulative rainfall and average air temperature calculated for each month from January 2011 to December 2016 from the data obtained from Homécourt weather station, located 100 meter away from the two lysimeters. Year 1 to 4 correspond to 2013-2016. Gaps in pluviometry data are indicated with red color; gaps in temperature data are indicated by line breaking.

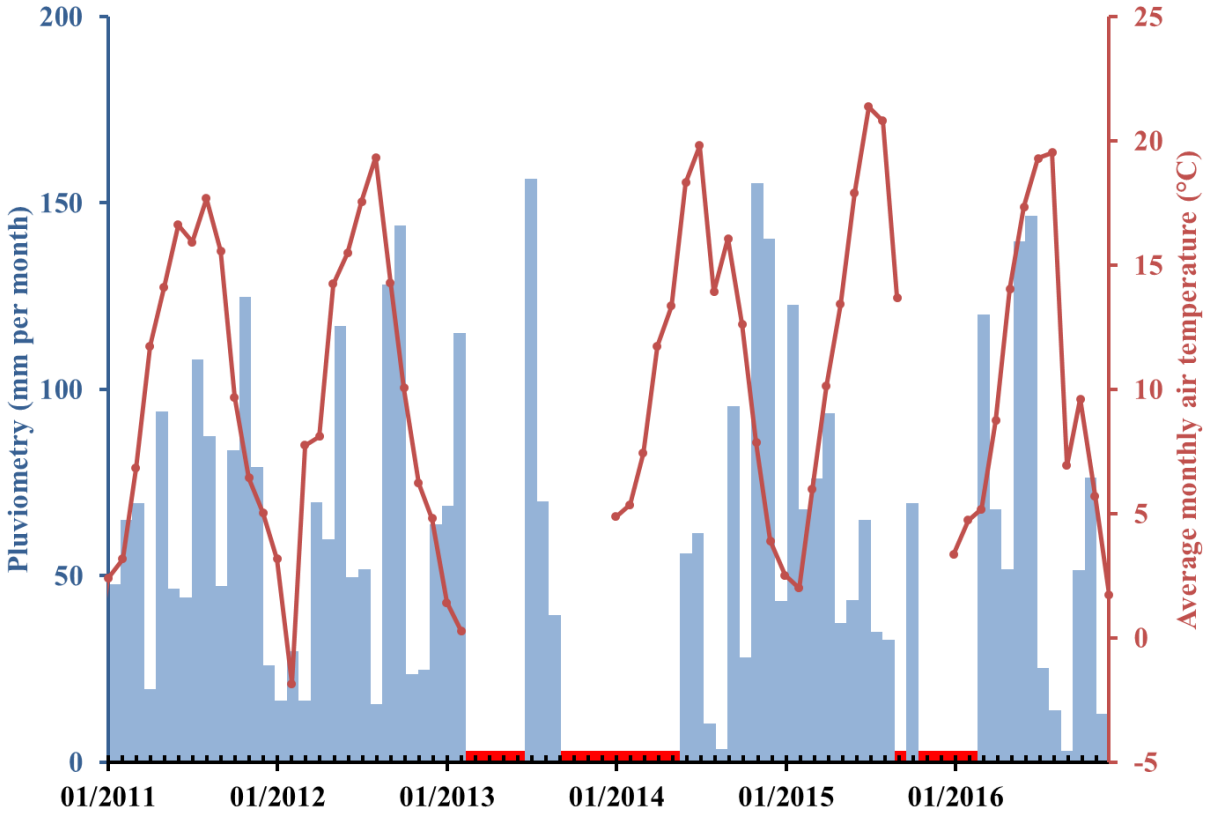
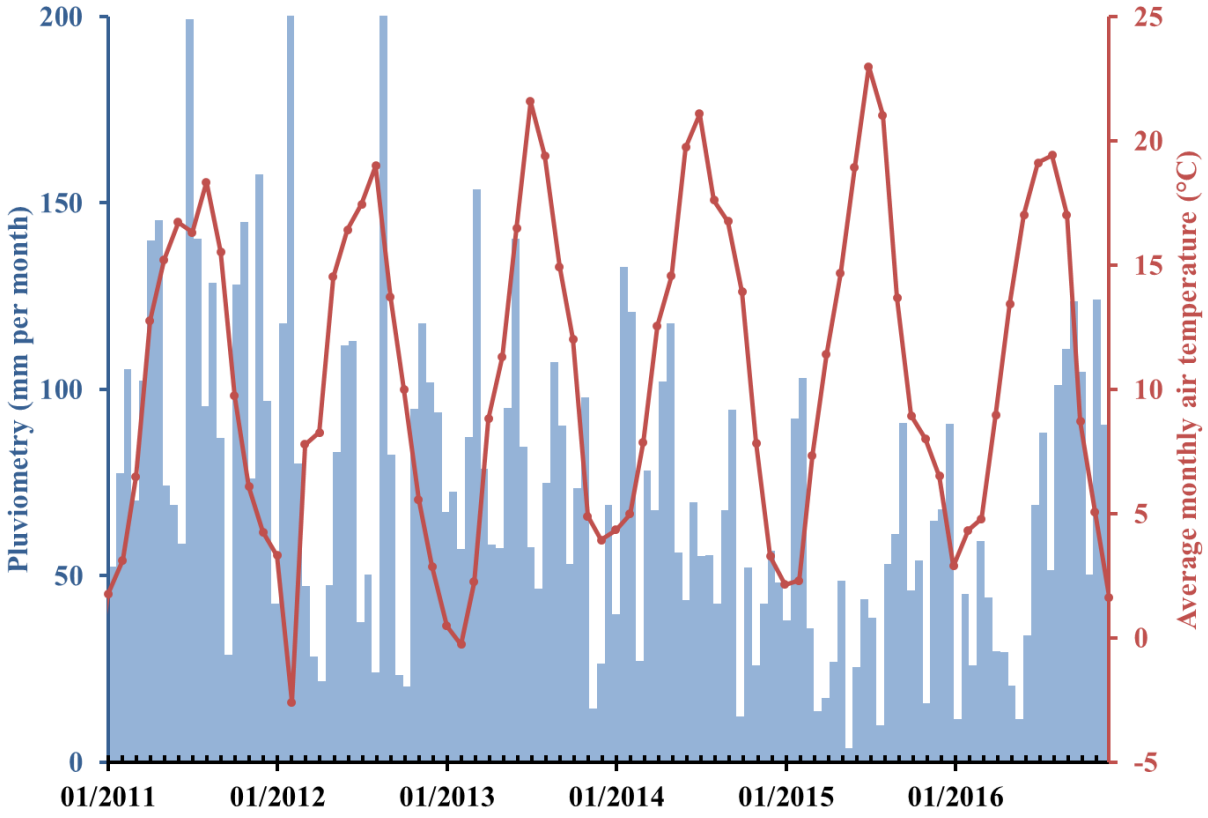
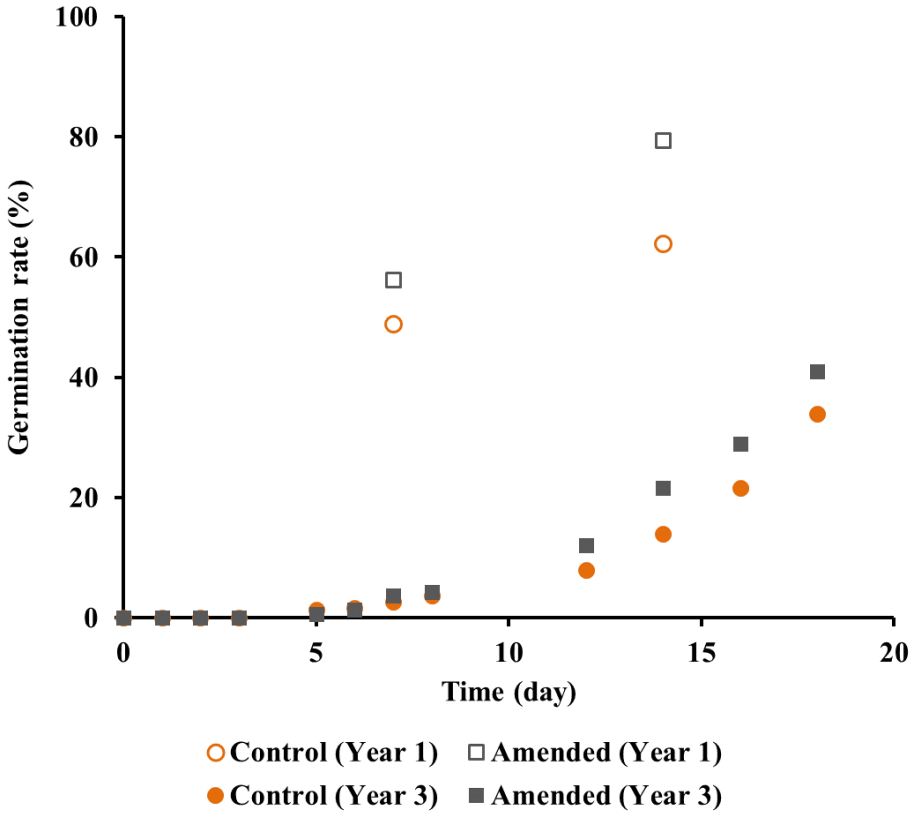


Fig. S9b: Cumulative rainfall and average air temperature calculated for each month from January 2011 to December 2016 from the data obtained from Metz, 30 km away from the two lysimeters (downloaded from www.historique-meteo.net/france/lorraine/metz/, accessed on 2019-10-31). Year 1 to 4 correspond to 2013-2016.



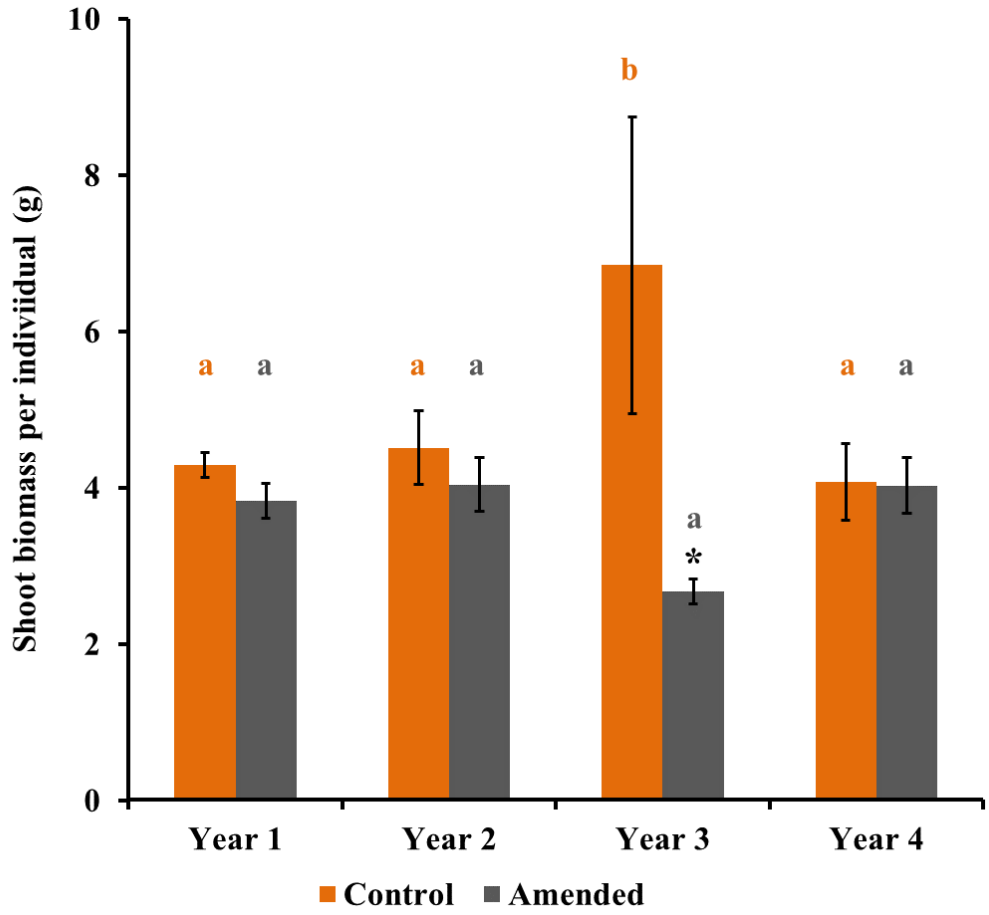
Germination rate of *N. caerulescens*

Fig. S10: Evolution of the germination rate of *N. caerulescens* in germination plates for the preparation of seedlings in controlled conditions in Year 1 and Year 3. Soil from the unamended lysimeter ("Control") and the lysimeter amended with 5% biochar on the first 30 cm ("Amended") were used as the growth medium. 300 seeds were used per germination plate. The differences of germination rate between Year 1 and Year 3 reflect the difficulty in maintaining adequate water supply to the germination plate wells.



Individual shoot biomass of *N. caerulescens*

Fig. S11: Average shoot biomass per individual of *N. caerulescens* at October's harvest measured on the unamended lysimeter ("Control") and the lysimeter amended with 5% biochar on the first 30 cm ("Amended"). Error bars represent standard error calculated from the four quadrants of each lysimeter. For a given lysimeter, different letters indicate statistical differences between the years. For a given year, an asterisk indicates a statistical difference between the two lysimeters.



Details on plant features and composition

Fig. S12: Pictures taken at the surface of the lysimeters, showing examples of *N. caerulescens* flowers (a,b), nutrient-deficiency symptoms (c) and weed plants (d)

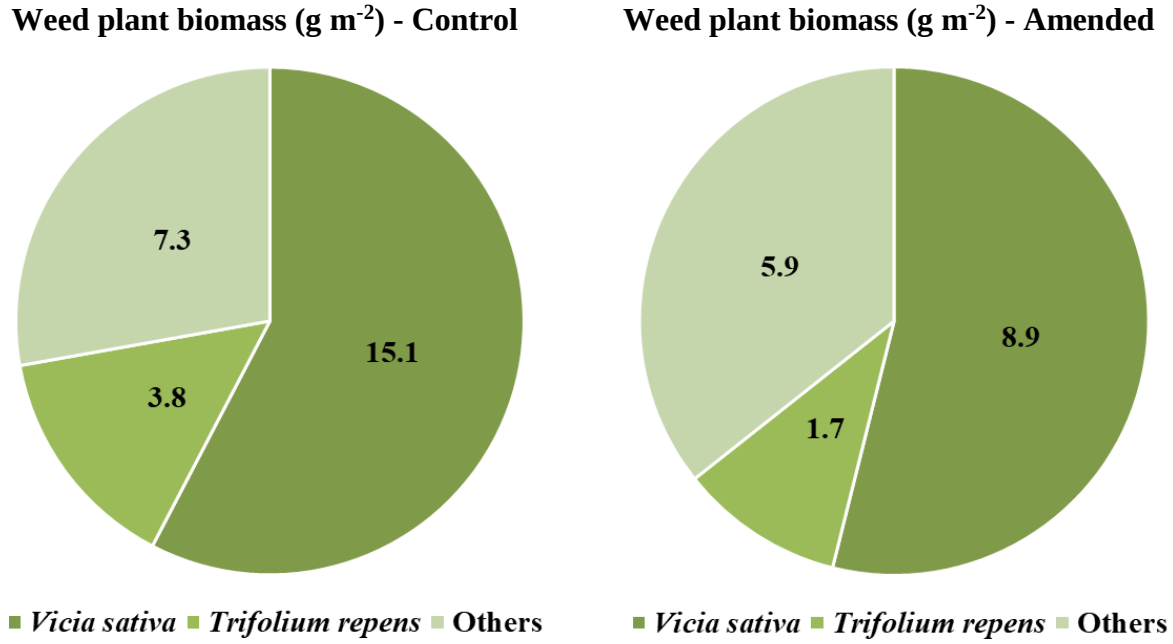
a. Year 1 (July) – Exemple of *N. caerulescens* at flowering stage

b. Year 1 (July) – Exemple of *N. caerulescens* at flowering stage

c. Year 2 (October) – Nutrient deficiency symptoms observed on *N. caerulescens*

d. Year 2 (October) – Example of weed plants grown together with *N. caerulescens*

Fig. S13: Composition of weed plants harvested in October of Year 2 on the unamended lysimeter ("Control") and the lysimeter amended with 5% biochar on the first 30 cm ("Amended"). Numbers on the graphs indicate the corresponding dry shoot biomass (g) for each plant group.



Root parameters

Fig. S14: Average root biomass density per volume of soil measured on the first 20 cm of the unamended lysimeter ("Control") and the lysimeter amended with 5% biochar on the first 30 cm ("Amended"). Error bars represent standard error calculated from the four quadrants of each lysimeter. For a given lysimeter, different letters indicate statistical differences between the years. For a given year, an asterisk indicates a statistical difference between the two lysimeters.

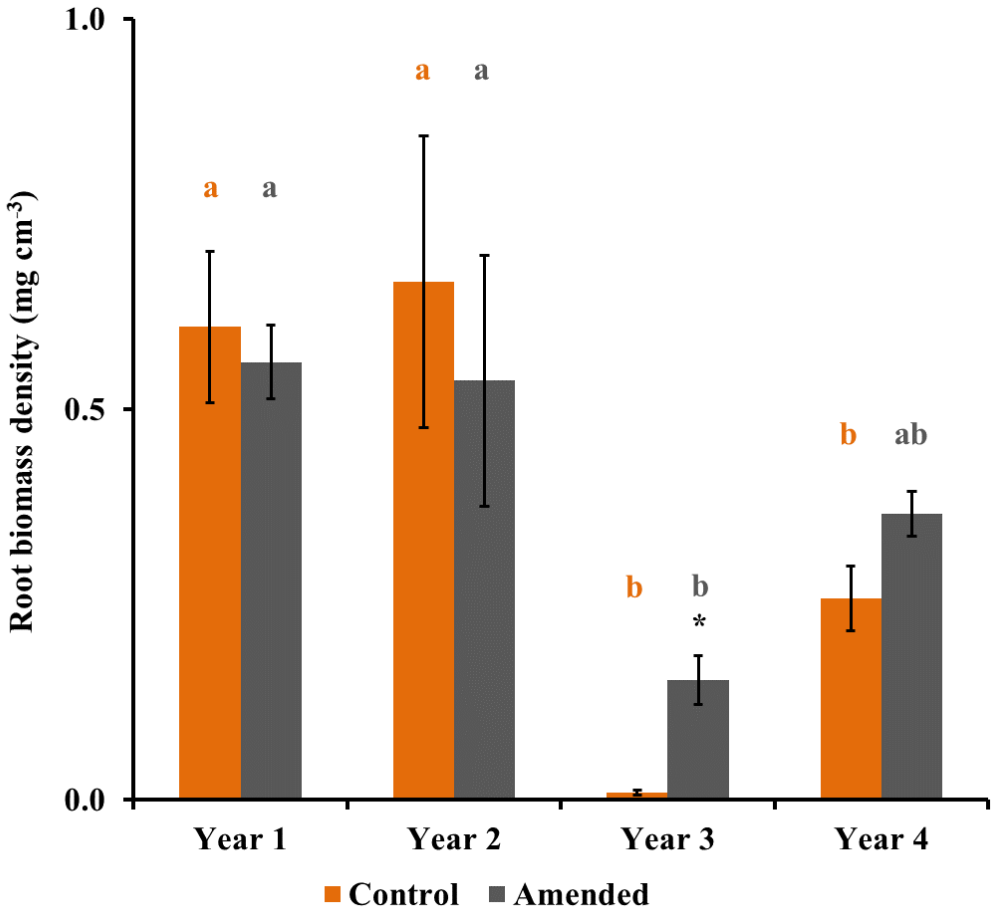


Fig. S15: Average root surface density per volume of soil measured on the first 20 cm of the unamended lysimeter ("Control") and the lysimeter amended with 5% biochar on the first 30 cm ("Amended"). Error bars represent standard error calculated from the four quadrants of each lysimeter. For a given lysimeter, different letters indicate statistical differences between the years. For a given year, an asterisk indicates a statistical difference between the two lysimeters. *NA*: Not available.

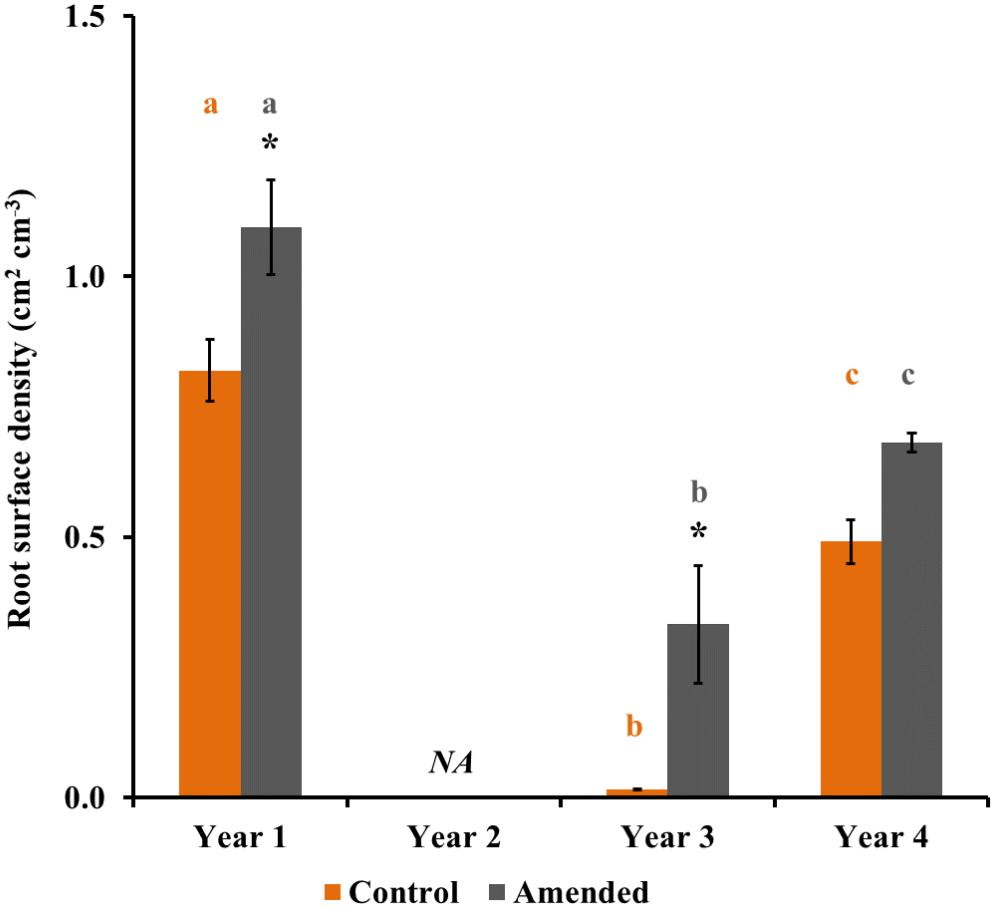


Fig. S16: Distribution of average root surface density per volume of soil for each class of root diameter measured on the first 20 cm of the unamended lysimeter ("Control") and the lysimeter amended with 5% biochar on the first 30 cm ("Amended") in Year 1. Error bars represent standard error calculated from the four quadrants of each lysimeter.

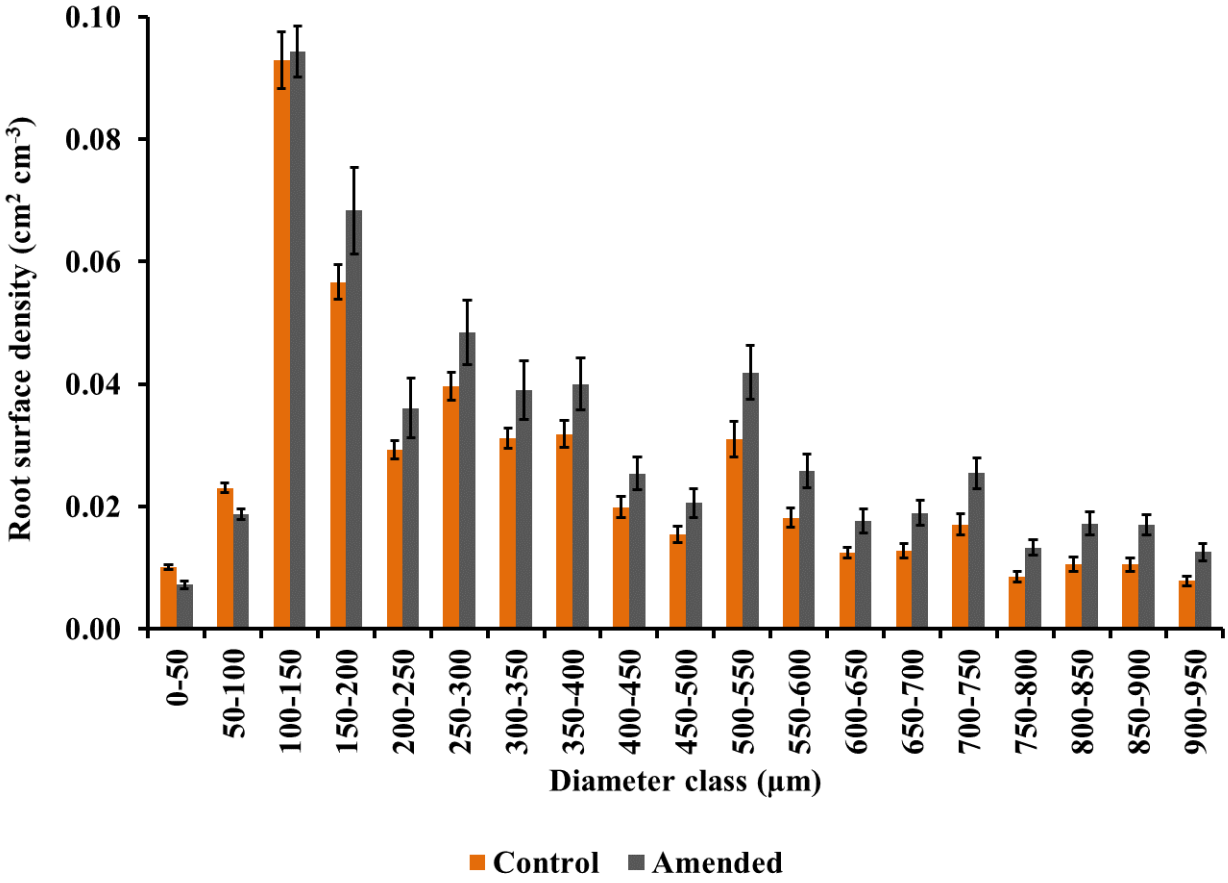


Fig. S17: Distribution of root surface density per volume of soil for each class of root diameter measured on the first 20 cm of the unamended lysimeter ("Control") and the lysimeter amended with 5% biochar on the first 30 cm ("Amended") in Year 3. Error bars represent standard error calculated from the four quadrants of each lysimeter.

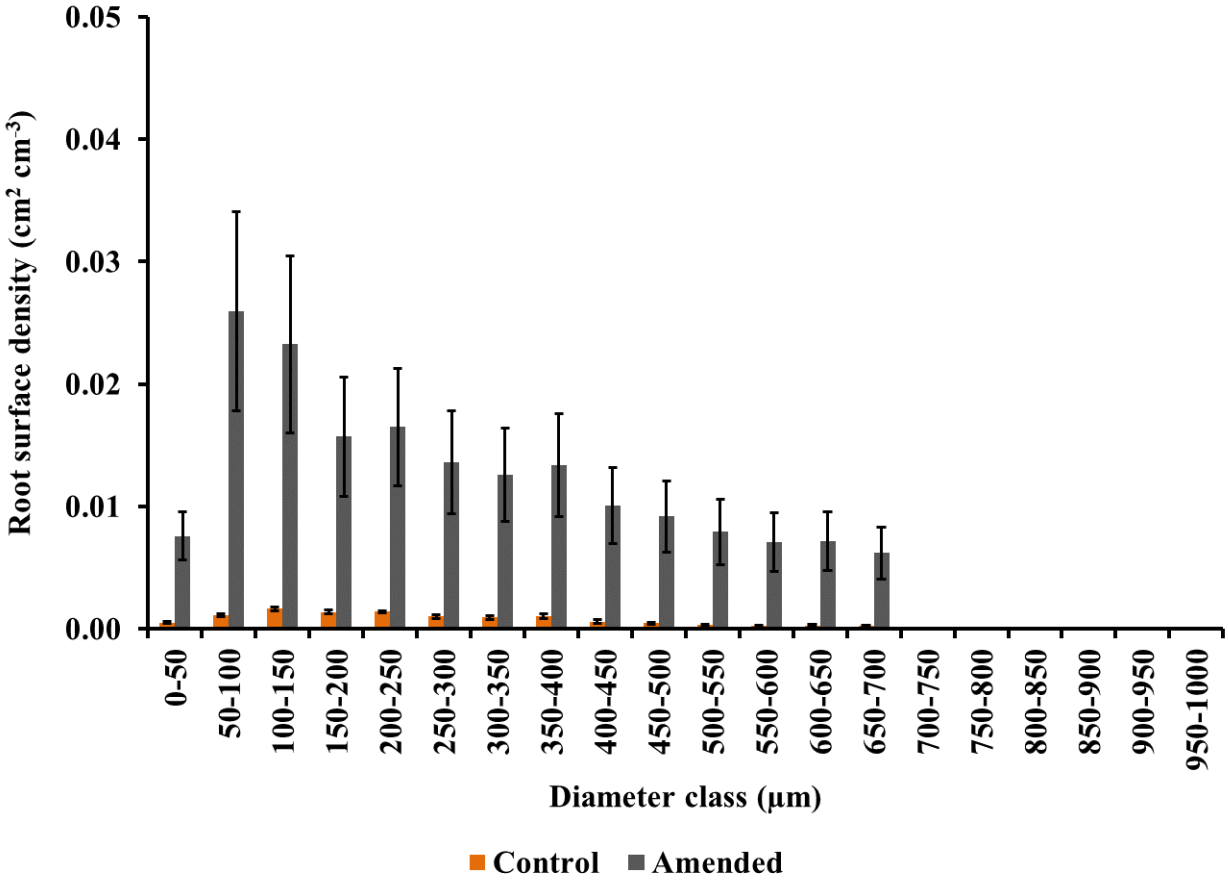
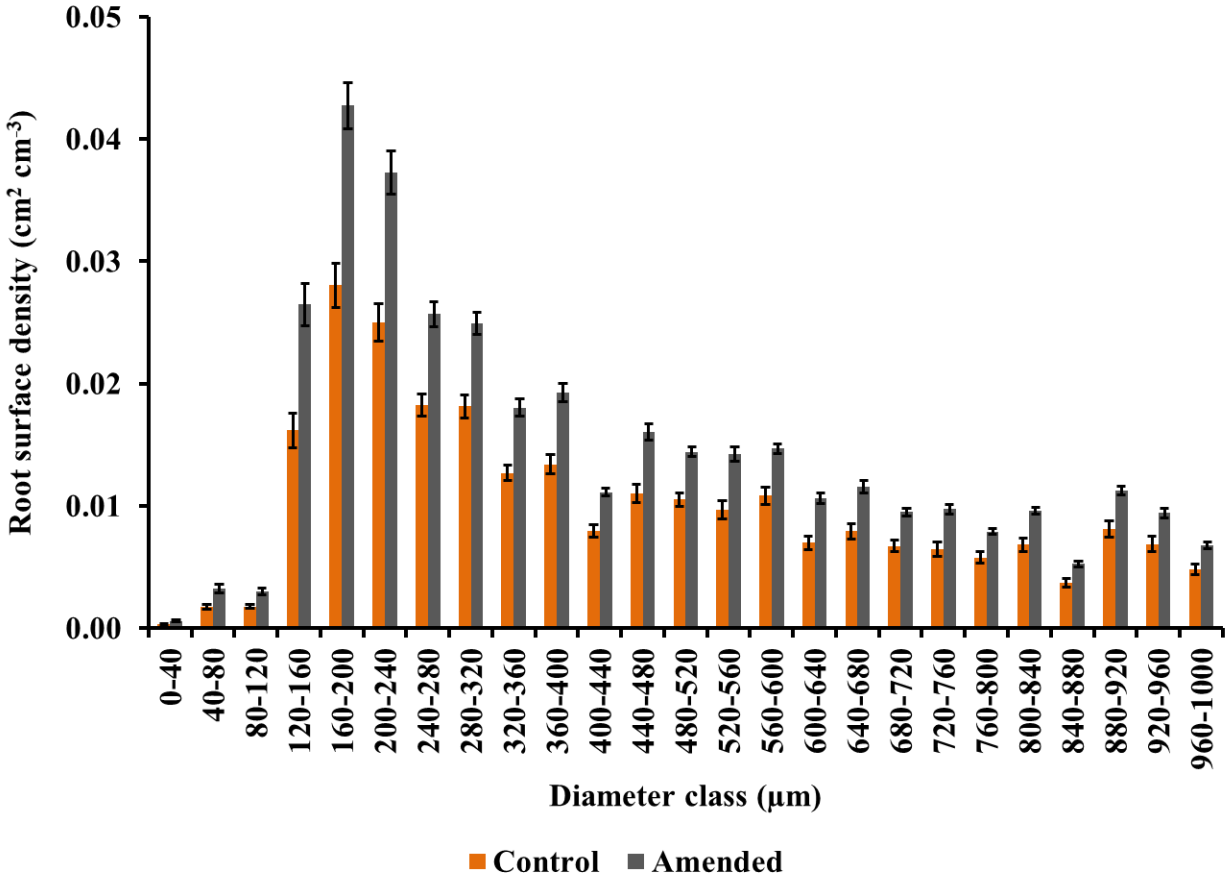


Fig. S18: Distribution of root surface density per volume of soil for each class of root diameter measured on the first 20 cm of the unamended lysimeter ("Control") and the lysimeter amended with 5% biochar on the first 30 cm ("Amended") in Year 4. Error bars represent standard error calculated from the four quadrants of each lysimeter.



Metal translocation factors

Fig. S19: Average shoot:root ratio of concentrations of Cd in *N. caerulescens* harvested in October of each year on the unamended lysimeter ("Control") and the lysimeter amended with 5% biochar on the first 30 cm ("Amended"). Error bars represent standard error calculated from the four quadrants of each lysimeter. For a given lysimeter, different letters indicate statistical differences between the years. For a given year, an asterisk indicates a statistical difference between the two lysimeters.

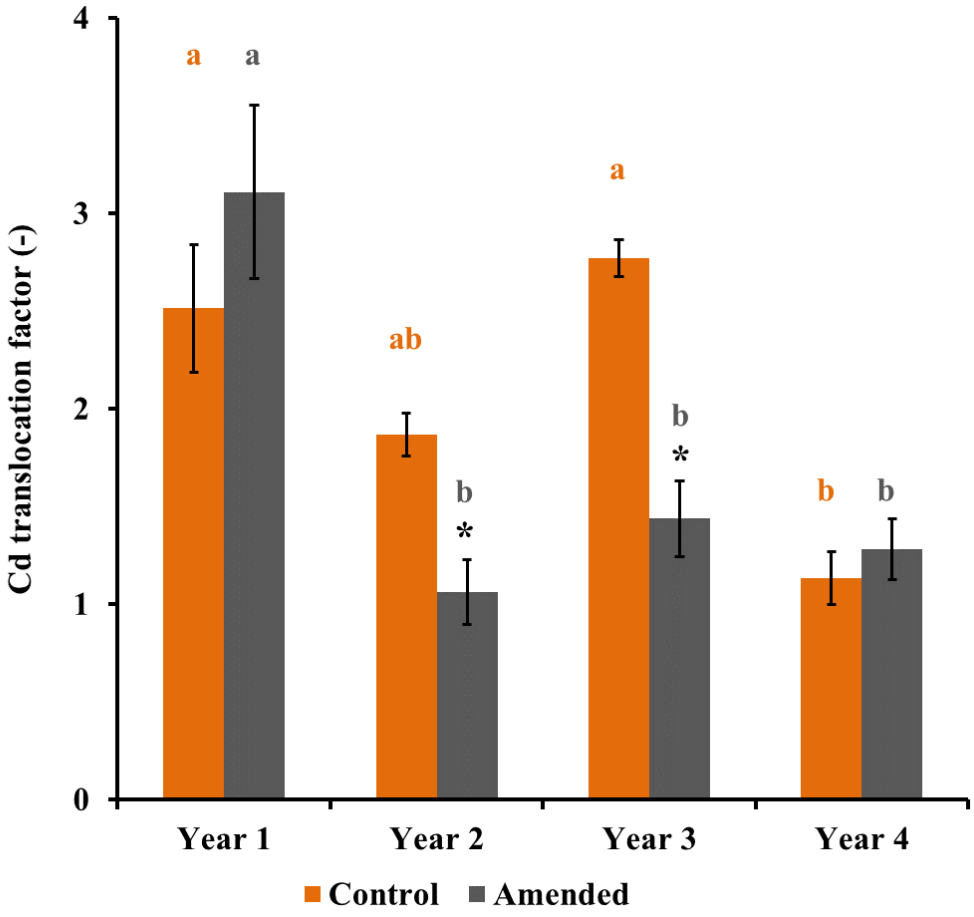
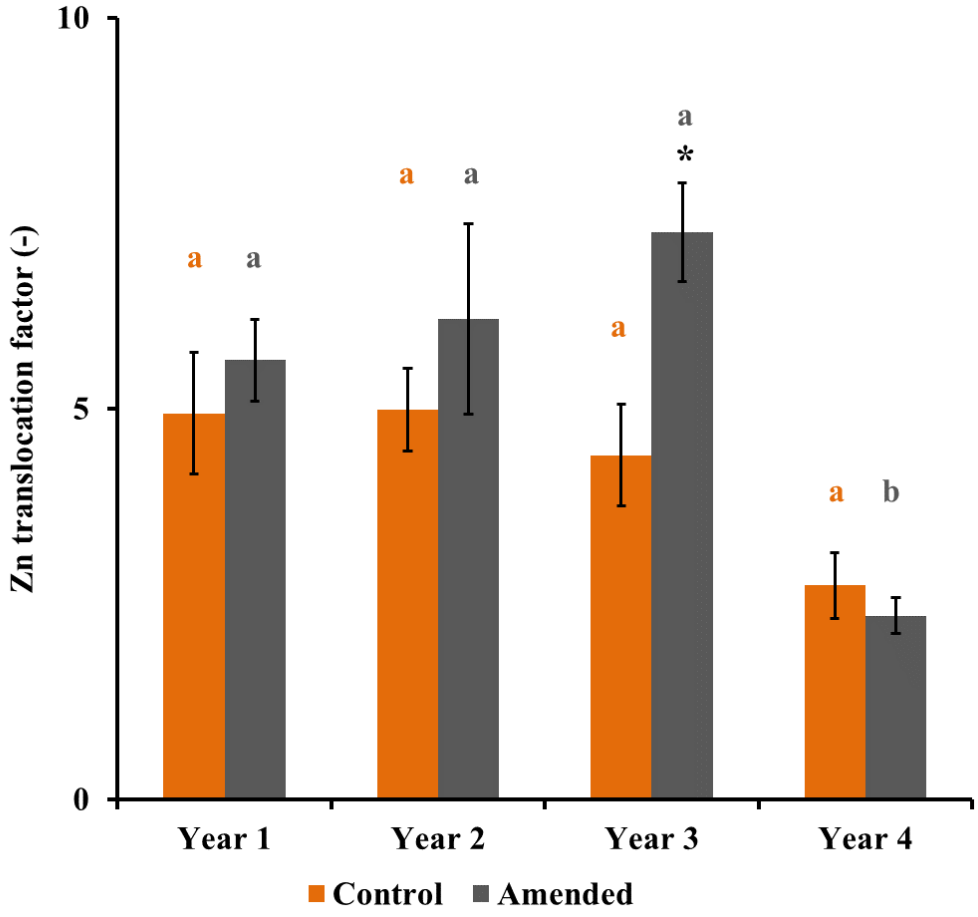


Fig. S20: Average shoot:root ratio of concentrations of Zn in *N. caerulescens* harvested in October of each year on the unamended lysimeter ("Control") and the lysimeter amended with 5% biochar on the first 30 cm ("Amended"). Error bars represent standard error calculated from the four quadrants of each lysimeter. For a given lysimeter, different letters indicate statistical differences between the years. For a given year, an asterisk indicates a statistical difference between the two lysimeters.



Evolution of metal accumulation with plant age

Fig. S21: Evolution of the average Cd concentration in shoots of *N. caerulescens* with the age of the plant in Year 1 on the unamended lysimeter ("Control") and the lysimeter amended with 5% biochar on the first 30 cm ("Amended"). Error bars represent standard error calculated from the four quadrants of each lysimeter. For a given lysimeter, different letters indicate statistical differences between the years. For a given year, an asterisk indicates a statistical difference between the two lysimeters.

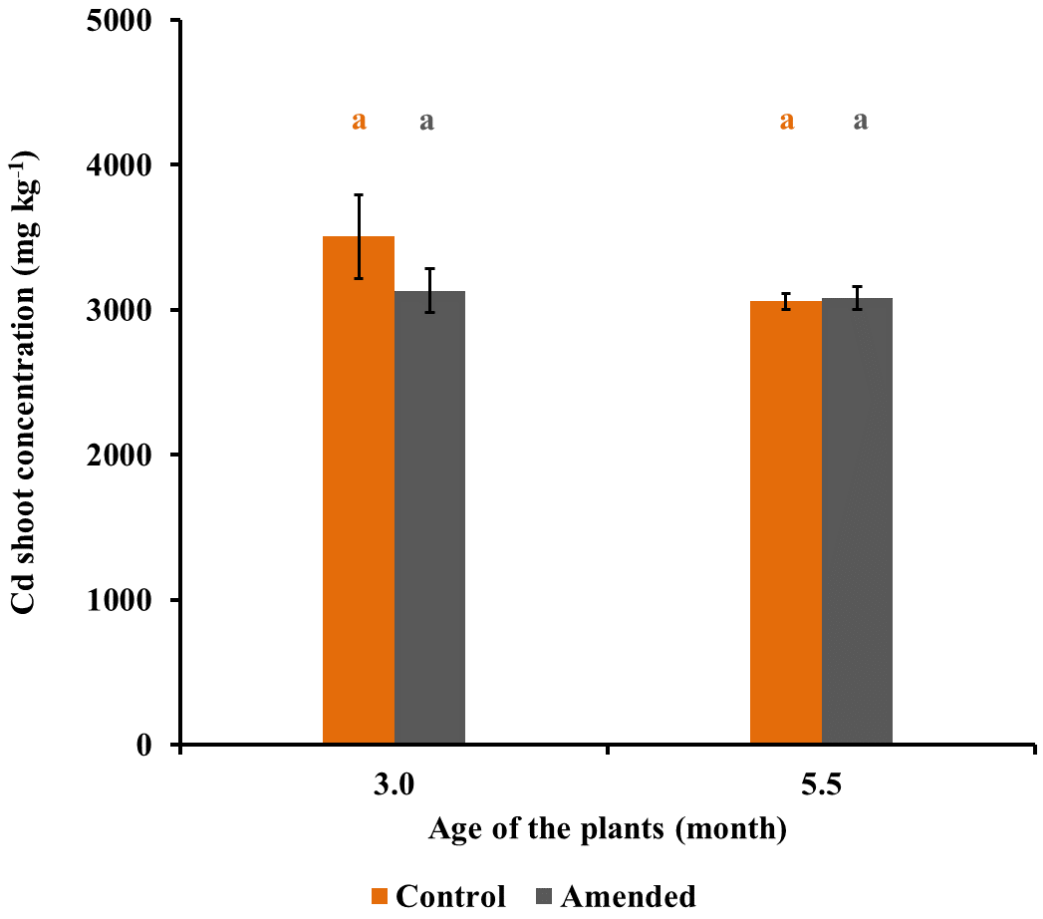
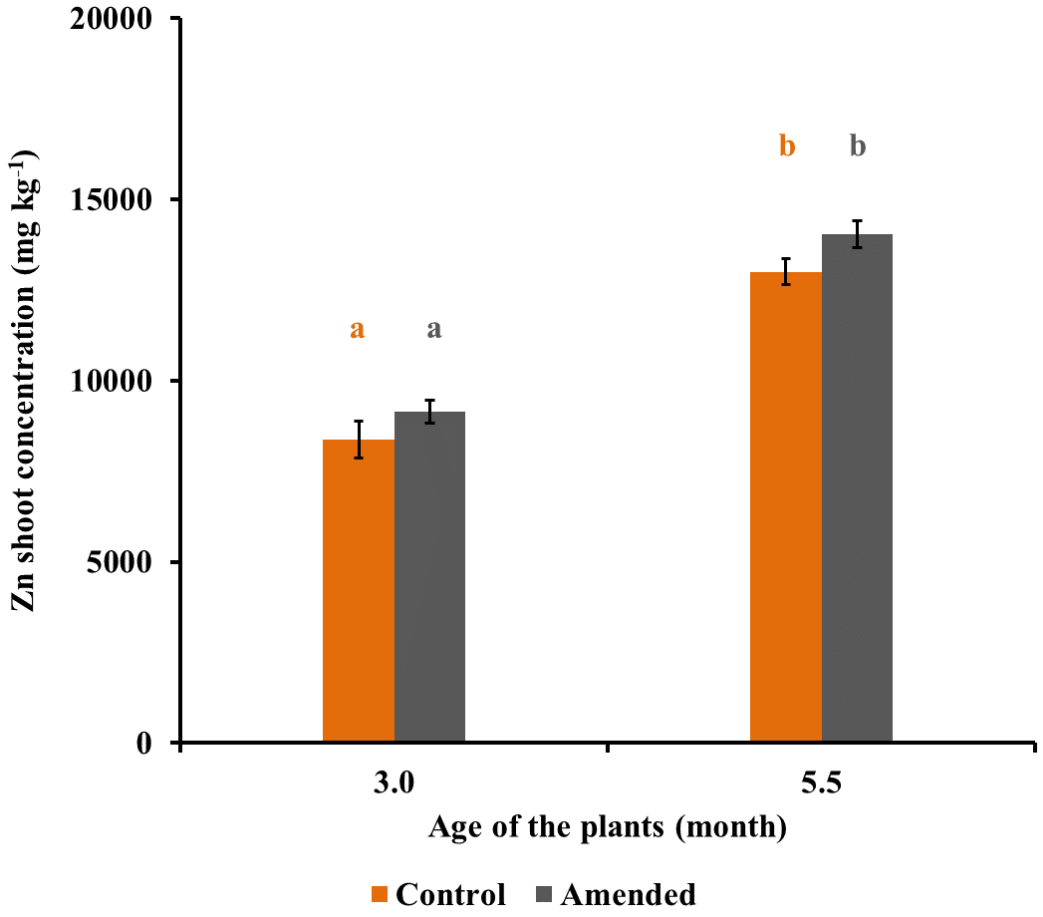


Fig. S22: Evolution of the average Zn concentration in shoots of *N. caerulescens* with the age of the plant in Year 1 on the unamended lysimeter ("Control") and the lysimeter amended with 5% biochar on the first 30 cm ("Amended"). Error bars represent standard error calculated from the four quadrants of each lysimeter. For a given lysimeter, different letters indicate statistical differences between the years. For a given year, an asterisk indicates a statistical difference between the two lysimeters.



Correlation between Ca, Cd and Zn concentrations in the shoots of *N. caerulescens*

Fig. S23: Relationship between Cd and Ca concentrations in the shoots of *N. caerulescens*. A linear regression was used on the data from both lysimeters at October's harvest of Year 1, Year 2 and Year 4. Data from Year 3 and from July of Year 1 is also indicated on the graph, but was not taken into account in the linear regression.

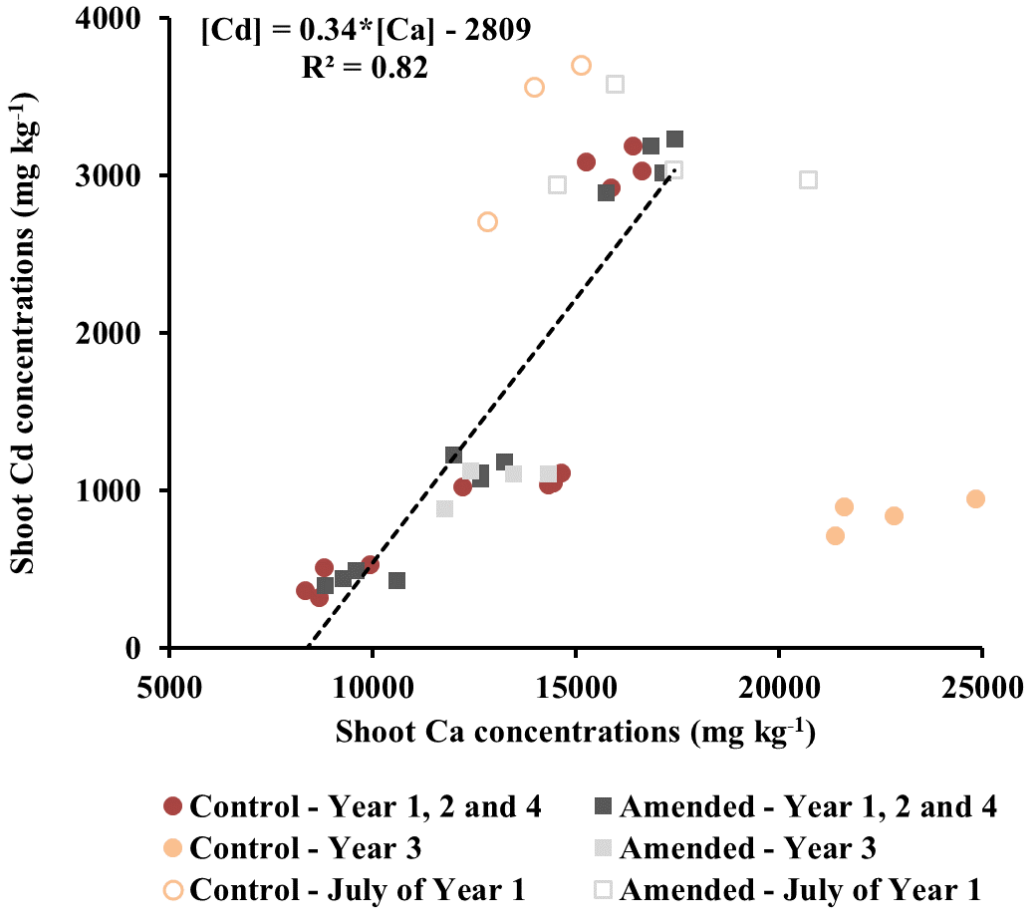
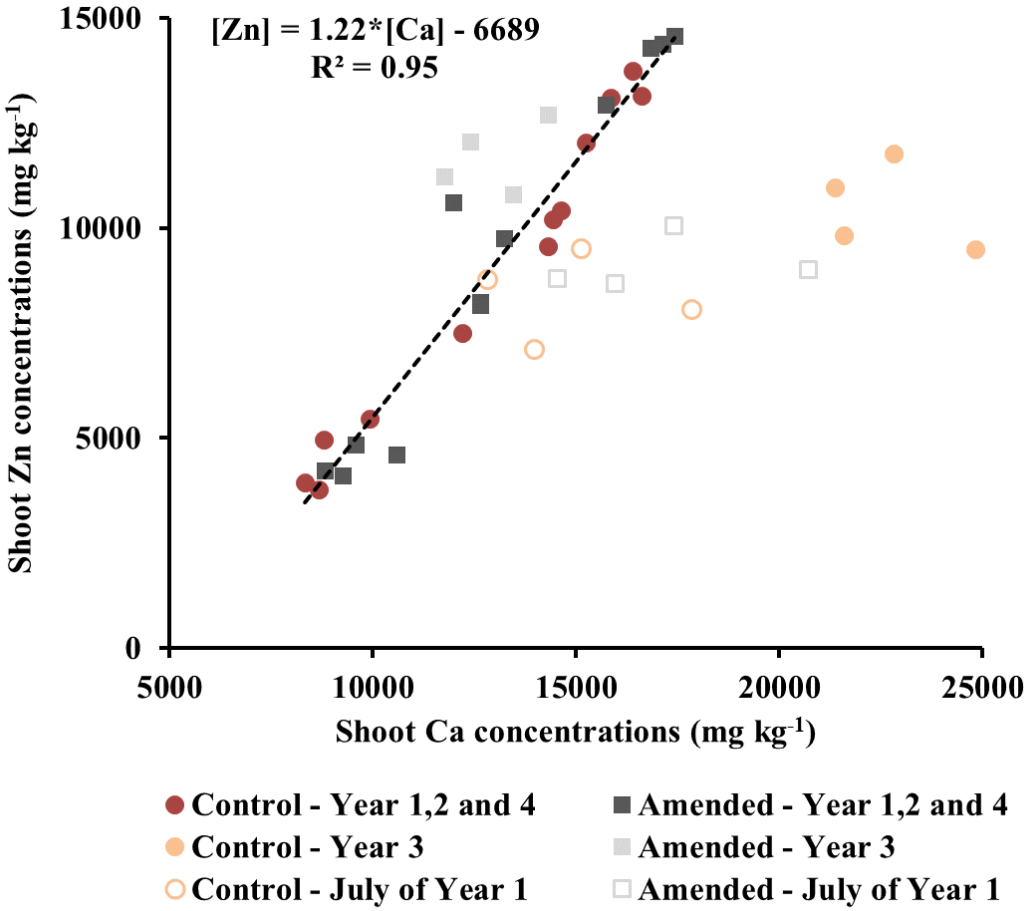


Fig. S24: Relationship between Zn and Ca concentrations in the shoots of *N. caerulescens*. A linear regression was used on the data from both lysimeters at October's harvest of Year 1, Year 2 and Year 4. Data from Year 3 and from July of Year 1 is also indicated on the graph, but was not taken into account in the linear regression.



Mass balance in the topsoil and the underlying soil horizons

Table S4: Estimated mass of metals present in the 0-30 cm at the initial state (beginning of Year 1) and at the end of each year in the unamended lysimeter ("Control") and the lysimeter amended with 5% biochar on the first 30 cm ("Amended"), with amounts of metals removed from the topsoil by phytoextraction, by soil and root sampling or by other unexplained processes over the four successive years. Unexplained losses may correspond to leaching processes, and were calculated based on a mass balance assuming a perfectly homogenized soil system. The removal of metals from the topsoil is expressed here as a negative value, and positive values observed in the "unexplained losses" might be due to the presence of hotspots of metal concentrations in one year compared to the previous one.

		Cd (g)		Pb (g)		Zn (g)	
		Control	***Amended***	***Control***	***Amended***	***Control***	***Amended***
	Initial state	***5.0***	***5.3***	***252***	***281***	***342***	***378***
Year 1	Phytoextraction	*-1.2*	*-1.2*	*0.0*	*0.0*	*-5.0*	*-5.2*
	Soil and root sampling	*0.0*	*0.0*	*-0.8*	*-0.9*	*-1.0*	*-1.2*
	Unexplained loss	*-0.8*	*-0.4*	*-28*	*-19*	*-39*	*-19*
	Final state	***2.9***	***3.7***	***224***	***261***	***298***	***353***
Year 2	Phytoextraction	*-0.5*	*-0.6*	*0.0*	*0.0*	*-4.6*	*-5.1*
	Soil and root sampling	*0.0*	*0.0*	*-0.8*	*-0.8*	*-1.2*	*-1.1*
	Unexplained loss	*0.2*	*-0.1*	*3*	*-14*	*39*	*-15*
	Final state	***2.7***	***3.0***	***226***	***246***	***331***	***332***
Year 3	Phytoextraction	*0.0*	*-0.4*	*0.0*	*0.0*	*-0.6*	*-3.9*
	Soil and root sampling	*0.0*	*0.0*	*-0.8*	*-0.9*	*-1.1*	*-1.2*
	Unexplained loss	*-0.1*	*0.1*	*15*	*22*	*-14*	*24*
	Final state	***2.5***	***2.7***	***240***	***267***	***315***	***350***
Year 4	Phytoextraction	*-0.2*	*-0.2*	*0.0*	*0.0*	*-1.7*	*-1.8*
	Soil and root sampling	*0.0*	*0.0*	*-0.9*	*-0.9*	*-1.2*	*-1.2*
	Unexplained loss	*0.1*	*0.0*	*11*	*-3*	*14*	*2*
	Final state	***2.5***	***2.5***	***250***	***263***	***326***	***349***
From Year 1 to Year 4	***Initial state***	***5.0***	***5.3***	***252***	***281***	***342***	***378***
	Phytoextraction	**-1.9**	**-2.3**	**0.0**	**0.0**	**-11.8**	**-16.0**
	Soil and root sampling	**-0.1**	**-0.1**	**-3.3**	**-3.4**	**-4.5**	**-4.6**
	Unexplained loss	**-0.5**	**-0.4**	**1.0**	**-14.1**	**0.2**	**-8.4**
	Final state	***2.5***	***2.5***	***250***	***263***	***326***	***349***

Table S5: Estimation of the amount of water or elements/compounds lost in percolates or removed through pore water sampling from March 2013 to December 2016 *. NA: Data not available

			Percolates	Pore water sampling		
Type	**Unit**	**Lysimeter**	**At -185 cm**	**At -50 cm**	**At -100 cm**	**At -150 cm**
Water	*liter m^{-2}*	Control	1430	20	3	4
		Amended	1249	30	11	10
Al	*mg m^{-2}*	Control	2.471	0.065	0.018	0.010
		Amended	2.167	0.076	0.010	0.057
As	*mg m^{-2}*	Control	5.656	0.022	0.011	0.002
		Amended	6.459	0.016	0.001	0.013
Ca	*mg m^{-2}*	Control	111222	1330	223	239
		Amended	92148	1533	172	194
Cd	*mg m^{-2}*	Control	0.010	0.000	0.004	0.000
		Amended	0.006	0.002	0.000	0.000
Cr	*mg m^{-2}*	Control	5.022	0.013	0.006	0.001
		Amended	5.405	0.016	0.000	0.001
Cu	*mg m^{-2}*	Control	1.702	0.030	0.013	0.022
		Amended	1.007	0.039	0.028	0.004
Fe	*mg m^{-2}*	Control	16.349	0.452	0.027	0.002
		Amended	7.557	0.440	0.018	0.009
K	*mg m^{-2}*	Control	16125	195	37	38
		Amended	6133	391	47	24
Mg	*mg m^{-2}*	Control	9205	145	15	20
		Amended	12655	209	24	39
Mn	*mg m^{-2}*	Control	0.620	0.001	0.008	0.000
		Amended	0.137	0.000	0.017	0.001
Na	*mg m^{-2}*	Control	12261	89	22	33
		Amended	25660	134	42	80
Ni	*mg m^{-2}*	Control	4.453	0.031	0.015	0.012
		Amended	5.136	0.042	0.012	0.011
P	*mg m^{-2}*	Control	47.893	0.017	0.140	0.001
		Amended	9.780	0.331	0.396	0.116
Pb	*mg m^{-2}*	Control	3.302	0.008	0.010	0.008
		Amended	2.122	0.029	0.004	0.079
Zn	*mg m^{-2}*	Control	18.225	0.128	0.031	0.052
		Amended	2.381	0.327	0.077	0.023
Chloride *	*mg m^{-2}*	Control	10651	173	NA	NA
		Amended	12019	354	NA	NA
Sulfate *	*mg m^{-2}*	Control	24496	414	NA	NA
		Amended	42817	465	NA	NA
Nitrate *	*mg m^{-2}*	Control	29109	748	NA	NA
		Amended	6730	245	NA	NA

**** Estimations of anions lost between March 2013 and December 2015 (no measurements could be made in 2016)***

Table S6: Total concentration of Cd, Pb and Zn in different horizons of the unamended lysimeter ("Control") and the lysimeter amended with 5% biochar on the first 30 cm ("Amended"), four years before and three years after the amendment. Red color indicate high concentrations; green color indicate low concentrations.

	Year -4						Year 3					
	Cd (mg kg^{-1})		Pb (mg kg^{-1})		Zn (mg kg^{-1})		Cd (mg kg^{-1})		Pb (mg kg^{-1})		Zn (mg kg^{-1})	
*Horizon**	*Control*	*Amended*	*Control*	*Amended*	*Control*	*Amended*	*Control*	*Amended*	*Control*	*Amended*	*Control*	*Amended*
0-5 cm	24.6	29.9	1010	1160	1390	1570		8.9		884		1160
5-10 cm							8.8		836		1098	
10-15 cm	17.0	18.1	808	866	1290	1360						
15-20 cm												
20-25 cm												
25-30 cm												
30-35 cm	0.4	0.5	20	22	54	62		1.4		52		131
35-40 cm							0.7		52		107	
40-45 cm								1.6		89		179
45-50 cm	0.2	0.5	16	21	62	72	0.4		28		79	
50-55 cm												
55-60 cm												
60-65 cm								0.7		26		85
65-70 cm							0.2		19		62	
70-75 cm	0.2	0.2	16	16	57	58						
75-80 cm												
80-85 cm												
85-90 cm												
90-95 cm												
95-100 cm												
100-105 cm	0.1	0.1	14	15	52	53						
105-110 cm								0.1		16		55
110-115 cm							0.2		19		57	
115-120 cm												
120-125 cm	0.1	0.1	12	14	50	53						
125-130 cm												
130-135 cm												
135-140 cm												
140-145 cm												
145-150 cm												
150-155 cm	0.1	0.1	9	9	35	35						
155-160 cm												
160-165 cm												
165-170 cm												
170-175 cm	0.1	0.1	7	7	25	24						

**** Horizons refer to the initial soil profile prior to disturbance in Year 1. As a fraction of the excavated soil was not reintroduced and as biochar was added on the amended soil, the thickness of the disturbed topsoil slightly varied between both lysimeters after Year 1.***

Table S7: Estimated mass (g) of Cd, Pb and Zn present in the horizon 0-30 cm and 30-70 cm of the unamended lysimeter ("Control") and the lysimeter amended with 5% biochar on the first 30 cm ("Amended"), four years before and three years after the operations of homogenization and soil amendment. Calculations are based on the measurement of soil metal concentrations. For a given element, red color indicate high concentrations; green color indicate low concentrations.

	Year -4						Year 3					
	Cd (g m^{-2})		Pb (g m^{-2})		Zn (g m^{-2})		Cd (g m^{-2})		Pb (g m^{-2})		Zn (g m^{-2})	
Horizon*	***Control***	***Amended***	***Control***	***Amended***	***Control***	***Amended***	***Control***	***Amended***	***Control***	***Amended***	***Control***	***Amended***
0-30 cm	6.7	7.4	307	334	476	509	2.6	2.7	242	269	318	353
30-70 cm	0.1	0.2	9.4	11.5	33	37	0.2	0.7	17	31	45	74

Correlations between soil metal availability and plant metal uptake

We tested relationships between the initial concentration of 0.01M-$CaCl_2$ extractable Cd or Zn in the topsoil and the uptake of Cd and Zn by *N. caerulescens*, expressed either as the concentration of metal in the shoots, or as the total amount of metal phytoextracted over a year. The initial fraction of extractable metal for a given year was highly correlated to the concentration of Cd in the shoots (Fig. S25), and, even more, to the total amount of metal phytoextracted by the shoots of *N. caerulescens* (Fig. S27). Such correlations were much weaker for Zn (Fig. S26, Fig. S28).

Fig. S25: Relationship between the initial soil $CaCl_2$-extractable pool of Cd (µg kg^{-1}) and the average concentration of Cd (mg kg^{-1}) in the shoots of *N. caerulescens* harvested in October from Year 1 to Year 4 on the unamended lysimeter ("Control") and the lysimeter amended with 5% biochar on the first 30 cm ("Amended")

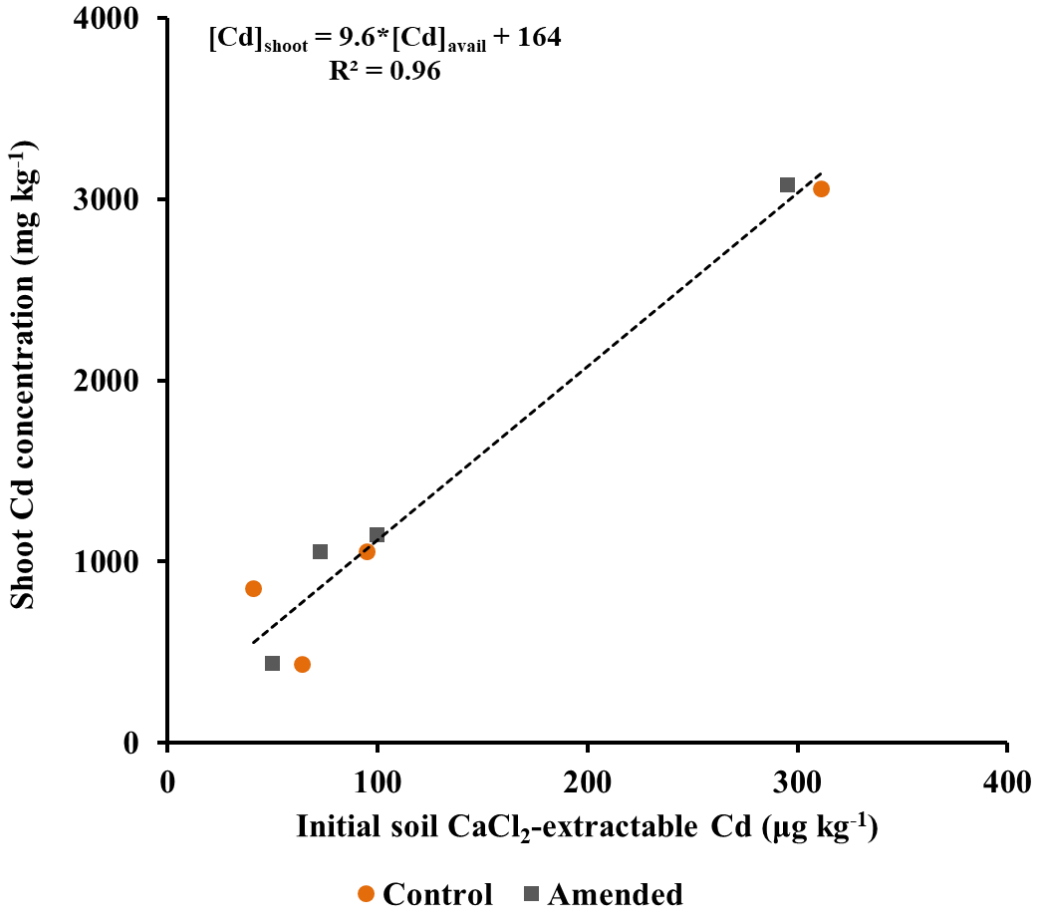


Fig. S26: Relationship between the initial soil $CaCl_2$-extractable pool of Zn (µg kg^{-1}) and the average concentration of Zn (mg kg^{-1}) in the shoots of *N. caerulescens* harvested in October from Year 1 to Year 4 on the unamended lysimeter ("Control") and the lysimeter amended with 5% biochar on the first 30 cm ("Amended")

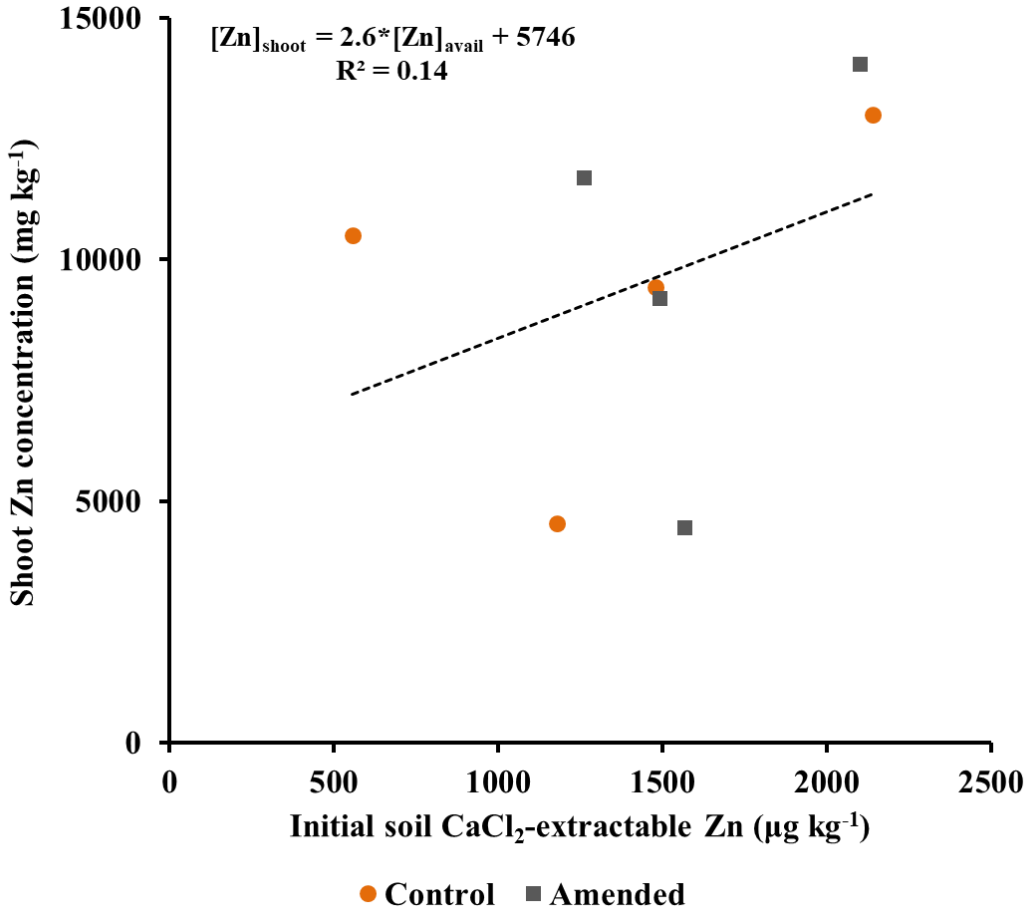


Fig. S27: Relationship between the initial soil $CaCl_2$-extractable pool of Cd (µg kg^{-1}) and the total annual amount of Cd (mg kg^{-1}) extracted by the shoots of *N. caerulescens* from Year 1 to Year 4 on the unamended lysimeter ("Control") and the lysimeter amended with 5% biochar on the first 30 cm ("Amended")

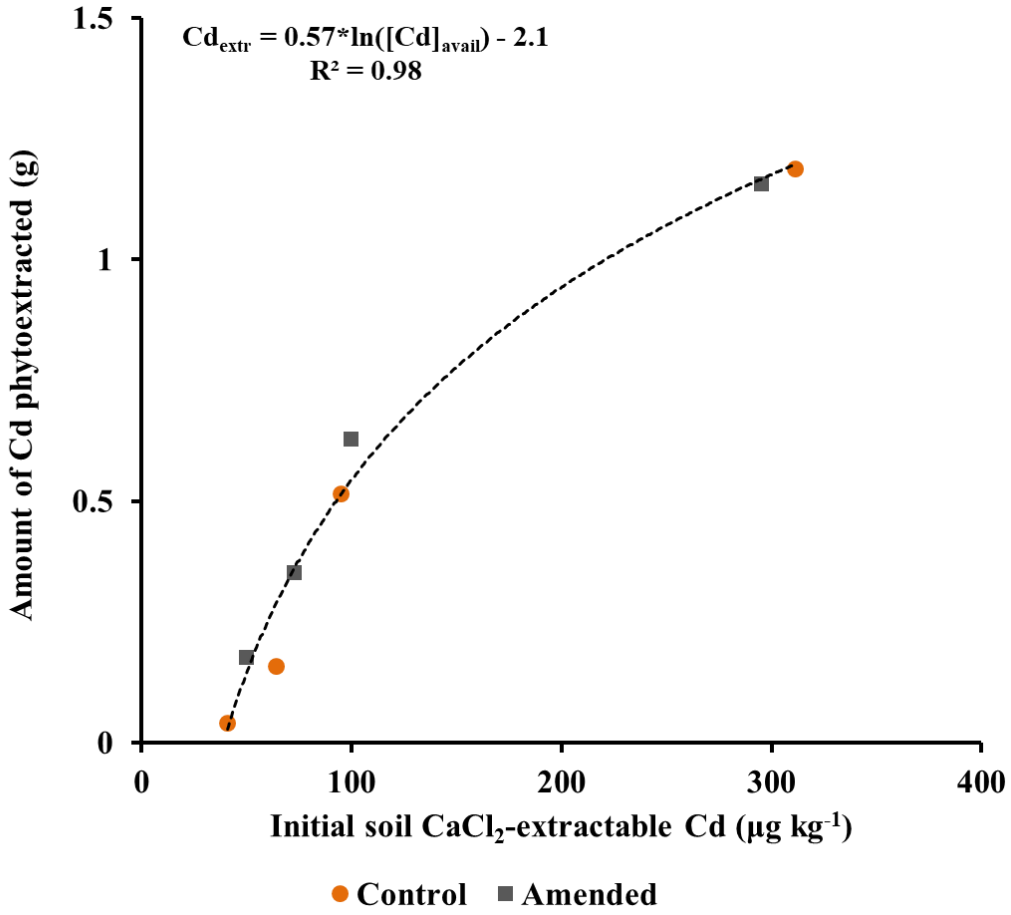
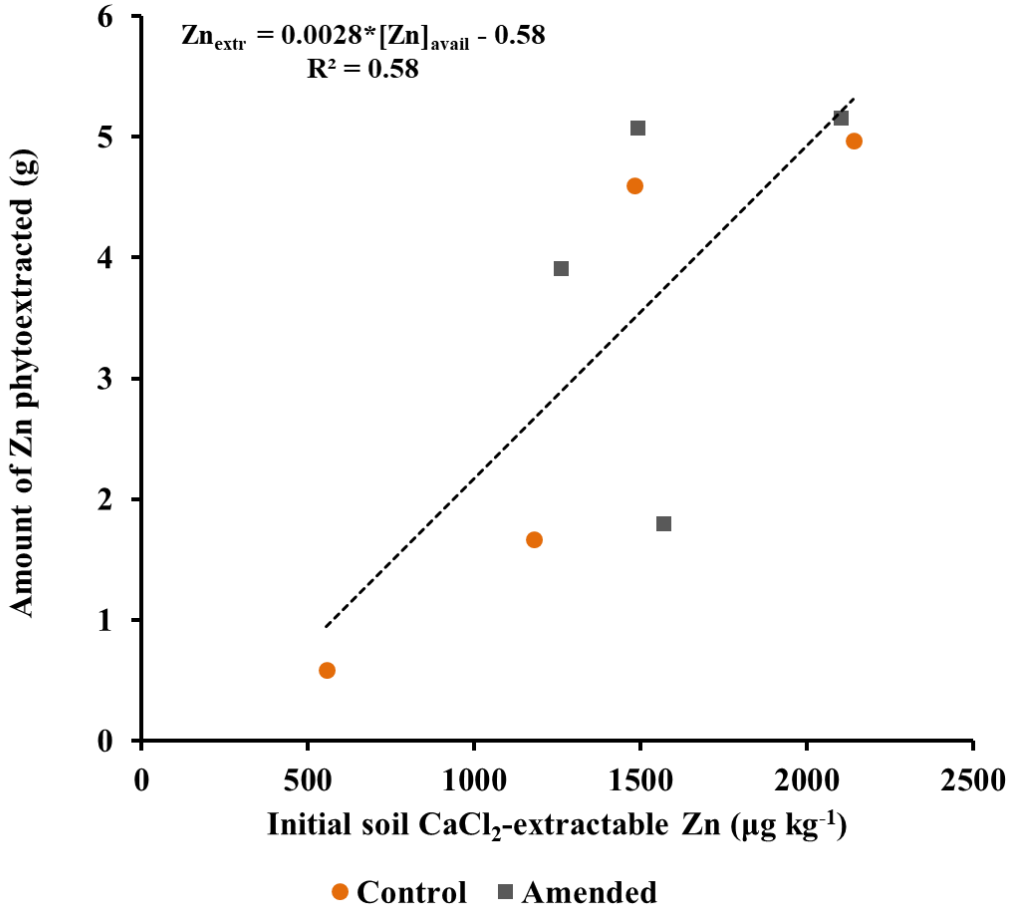


Fig. S28: Relationship between the initial soil $CaCl_2$-extractable pool of Zn (µg kg^{-1}) and the total annual amount of Zn (mg kg^{-1}) extracted by the shoots of *N. caerulescens* from Year 1 to Year 4 on the unamended lysimeter ("Control") and the lysimeter amended with 5% biochar on the first 30 cm ("Amended")



Estimations of the time required for removing soil metal contamination

We calculated the time necessary to remove all Cd and Zn from the investigated topsoil with empirical models based on the extrapolation of the removal results obtained between Year 1 and Year 4. We considered the cumulated amount of phytoextracted metal over time from the biochar-amended topsoil only, as the removal on the control lysimeter was very poor in Year 3 due to the unexpected low plant growth. We estimated the time necessary to remove all Cd and Zn using: i) a linear model based on the data of Year 1 only, ii) a linear model based on the data from Year 1 to Year 4, iii) a logarithmic model based on the data from Year 1 to Year 4. While the first two models consider that phytoextraction efficiency will remain constant over time, the third one assumes that phytoextraction efficiency actually decreased over time based on a log evolution. We did not try to model phytoextraction based on the amount of $CaCl_2$-extractable pool. Our results showed large variations in the predicted complete removal time based on the hypotheses made (Fig. S29 and S30). The theoretical time for reaching a total removal of Cd was calculated to be 5 years, 12 years or 129 years, using the first the second or the third model, respectively. For Zn, these estimations were 74 years, 104 years or an infinite time, respectively.

Fig. S29: Cumulative fraction of the Cd phytoextracted over years relative to the total initial Cd in the biochar-amended topsoil. Experimental data is shown with diamonds, while lines represent three models based on this data. Model 1 is a linear model based on the data of Year 1 only, Model 2 is a linear model based on the data from Year 1 to Year 4; Model 3 is a logarithmic model based on the data from Year 1 to Year 4.

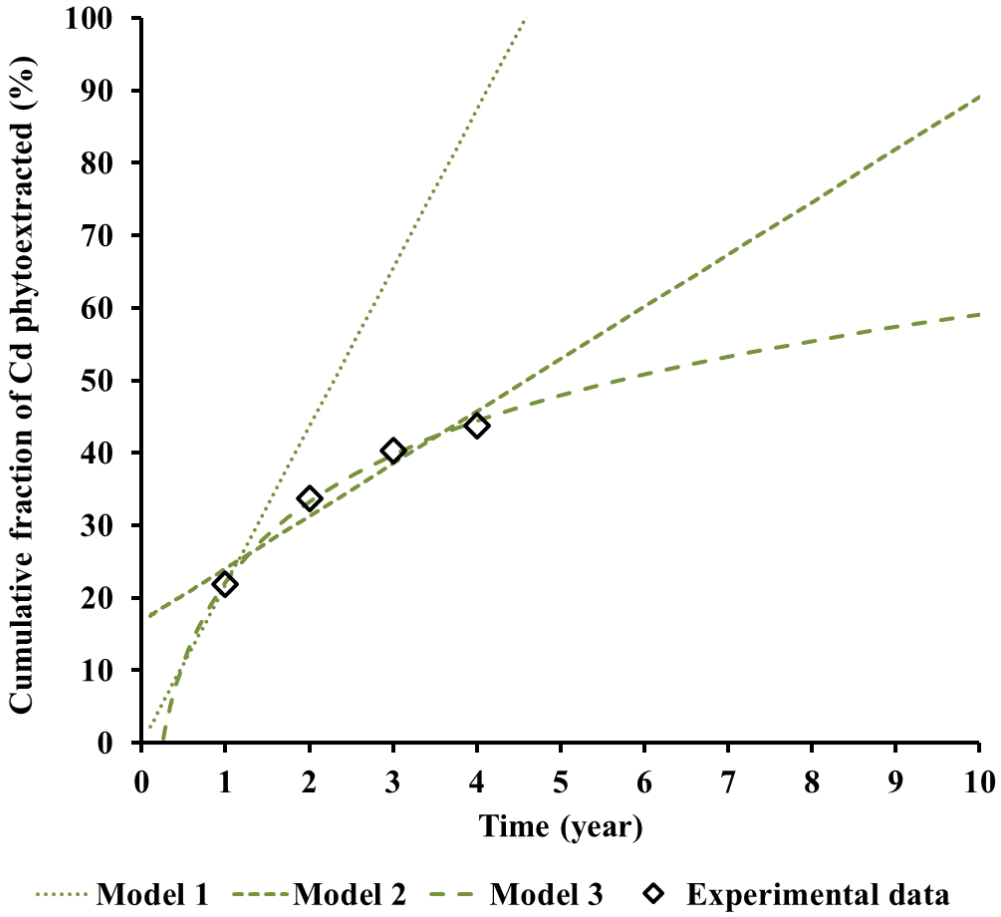


Fig. S30: Cumulative fraction of the Zn phytoextracted over years relative to the total initial Zn in the biochar-amended topsoil. Experimental data is shown with diamonds, while lines represent three models based on this data. Model 1 is a linear model based on the data of Year 1 only; Model 2 is a linear model based on the data from Year 1 to Year 4; Model 3 is a logarithmic model based on the data from Year 1 to Year 4.