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Biochar and urban solid refuse ameliorate the inhospitality of acidic mine tailings and foster effective spontaneous plant colonization under semiarid climate

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ABSTRACT

Phytomanagement is considered a suitable option in line with nature-based solutions to reduce environmental risks associated to metal(loid) mine tailings. We aimed at assessing the effectiveness of biochar from pruning trees combined with compost from urban solid refuse (USR) to ameliorate the conditions of barren acidic (pH ~5.5) metal(loid) mine tailing soils (total concentrations in mg kg^{-1}: As ~220, Cd ~40, Mn ~1800, Pb ~5300 and Zn ~8600) from Mediterranean semiarid areas and promote spontaneous plant colonization. Two months after amendment addition were enough to observe improvements in chemical and physico-chemical tailing soil properties (reduced acidity, salinity and water-soluble metals and increased organic carbon and nutrients content), which resulted in lowered ecotoxicity for the soil invertebrate *Enchytraeus crypticus*. Recalcitrant organic carbon provided by biochar remained in soil whereas labile organic compounds provided by USR were consumed over time. These improvements were consistent for at least one year and led to lower bulk density, higher water retention capacity and higher scores for microbial/functional-related parameters in the amended tailing soil. Spontaneous growth of native vegetation was favored with amendment addition, but adult plants of remarkable size were only found after three years. This highlights the existence of a time-lag between the positive effects of the amendment on tailing soil properties being observed and these improvements being translated into effective spontaneous plant colonization.

1. Introduction

The vast amount of ore-processing wastes produced by metal mining activities is a serious environmental problem worldwide (Lottermoser, 2010). Particularly worrying are the so-called mine tailings (open-air piles that store muddy residues). Tailing wastes often show adverse characteristics including high metal(loid) and salt levels, extreme pH values (from acid to basic), low organic matter and nutrients content, and lack of soil structure. These inhospitable conditions hamper soil biological activity and plant colonization (Xie et al., 2016; Fazekaš et al., 2019), which puts mine tailings at high risk of erosion, exacerbating their role as major source of metal(loid) pollution for surrounding areas (Conesa and Jiménez-Cárceles, 2007; Mendez and Maier, 2008). Different remediation technologies have been described to manage mine tailings. Conventional techniques (e.g., removal operations, soil washing, dam building, on-site isolation by sealing) require high economic investment and are often technically difficult to implement (Tordoff et al., 2000; Mendez and Maier, 2008; Conesa and Schulin, 2010). On the contrary, phytomanagement describes a set of technologies that include the manipulation of the soil-plant system to control the fluxes of pollutants in the environment (Robinson et al., 2009). It comprises a set of cheaper and environmentally friendly alternative options that can be carried out without a great use of heavy machinery (Mendez and Maier, 2008; Robinson et al., 2009; Conesa and Schulin, 2010; Navarro-Cano et al., 2018; Wei et al., 2021). Within phytomanagement, phytoextraction through plants is not efficient in

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environments with huge amounts of metal(loid)s such as mine tailings (Ali et al., 2013; Oreja et al., 2020). In turn, aided phytostabilization (afforestation following soil amendments application) is especially recommended for metal(loid) mine tailings areas where an option may be to immobilize metal(loid)s on site (Zornoza et al., 2015; Oustriere et al., 2016). Aided phytostabilization seeks to minimize erosion problems with the maintenance of a permanent vegetation cover and to reduce pollutant mobility and availability. Passive restoration, based on the capacity of native plants to grow spontaneously in disturbed sites (Prach and Tolvanen, 2016), is another option particularly interesting when mine tailings are embedded in vegetated areas that can spread propagules and seeds to tailings (Navarro-Cano et al., 2018). Although there are numerous experiences that combine soil amendments and direct afforestation (Mendez and Maier, 2008; González-Alcaraz and Álvarez-Rogel, 2013; Pardo et al., 2017), improving tailing conditions just to facilitate spontaneous colonization of native vegetation is not as common (Oreja et al., 2020).

Some studies have identified that spontaneous colonization of barren metal(loid) mine tailings by pioneer plant species ameliorates microclimate conditions through shading and promotes soil improvement (e.g., increasing organic carbon and nutrients cycling, microbial activity) and the creation of dense vegetation patches (fertility islands) that facilitates the colonization of less tolerant species (Parraga-Aguado et al., 2013; Navarro-Cano et al., 2018; Oreja et al., 2020). This colonization process is sometimes favored by the existence of specific edaphic niches with more hospitable conditions such as lower salinity or softer structure that facilitate roots penetration (Párraga-Aguado et al., 2013). Hence, the amelioration of tailings soil conditions to facilitate the growth of primo-colonizers and the formation of fertility islands can be an interesting option when economic resources and/or the implementation of other technologies are more limited. Due to the difficulties for the establishment and survival of afforested plants in these extreme environments (Huang et al., 2012), favoring the natural recruitment of native species might help to create nucleation spots and fertility islands to trigger vegetation expansion within mine tailings.

Among the organic amendments applied to improve metal(loid)-affected soils biochar is one of the most promising (Chen et al., 2018). It consists of a product of organic materials pyrolysis in the absence of oxygen (Sohi et al., 2010) that favors pH increase, water retention, carbon sequestration, nutrient replenishment, and microbial activity (Lehmann and Joseph, 2012). Particularly, biochar has been proposed as an effective amendment to improve mine tailings soils by immobilizing metals, reducing their mobility and availability (Beesley et al., 2011; Wu et al., 2017; Nie et al., 2018; Palansooriya et al., 2020). However, because biochar contains high levels of recalcitrant organic matter, it is poor in labile organic carbon and readily available nutrients and its use at high dose in large areas is not always feasible given its high economic cost (Rodríguez-Vila et al., 2014; Ghosh et al., 2015; Wu et al., 2016, 2017). Among others, these limitations can be solved by mixing biochar with composted raw organic materials that are richer in more labile organic matter. In particular, mixing biochar with composted urban solid refuse (USR) has shown good results when applied to metal(loid)-polluted soils (Karami et al., 2011; Wu et al., 2017). Moreover, the generation of USR has increased significantly in the last decades and its management and possible recycling is one of the priorities within the European Union's environmental policy as part of the circular economy strategy (European Commission, 2008).

This study aimed at assessing the effectiveness of an organic amendment composed of biochar from pruning trees and compost from USR to ameliorate the conditions of barren metal(loid) mine tailings soils from Mediterranean semiarid areas. The specific objectives were: 1) assess the effects of the organic amendment on tailings soil conditions and if these effects persist seasonally over a year; 2) assess whether the organic amendment favors spontaneous plant colonization. The initial hypotheses were: 1) the organic amendment would improve tailings soil conditions and these effects would be modulated by the time lasted from the application and the season of the year; 2) the organic amendment would favor the spontaneous colonization of tailing soils by pioneer native plants from surrounding areas.

2. Material and methods

2.1. Study area

The former metal mining district of La Unión-Sierra de Cartagena is located in southeast Spain (Cartagena, Murcia Region; Figure S1, Supplementary material). It presents a Mediterranean semiarid climate (mean annual precipitation ~200–300 mm, mean annual temperature ~17 °C, and mean annual evapotranspiration rate ~850 mm). The mining district has been exploited since Roman times. Nevertheless, it was during the second half of the 20th century when mining reached its peak. The main minerals extracted were pyrite (FeS_2), blend (ZnS), and galena (PbS). Currently, 89 tailings occupying ~2.18 km^2 and that store ~23 Mm^3 of mine wastes remain abandoned in the area, becoming a source of pollution for the surroundings territories (Conesa and Schulin, 2010). Although some tailing areas have been spontaneously colonized by native vegetation (Oreja et al., 2020) large surfaces remain bare. The present study focused on one of these mine tailings that was abandoned ~40 years ago (El Lirio; 37°36′33″N, 0°49′2″W; Figure S1, Supplementary material). The tailing (surface ~64,000 m^2) was built by mid-60's to store wastes from sphalerite-galena ore exploitations (IGME, 2002). Most of the tailing soils are acidic and lack of vegetation, except in some disperse zones with a neutral-slightly basic pH that has been spontaneously colonized by native plant species (Conesa et al., 2006; Álvarez-Rogel et al., 2021).

2.2. Organic amendment characteristics and field application

The organic amendment consisted of a 3:1 mixture of biochar and composted USR based on data reported by other authors (e.g., Wu et al., 2016 and references cited therein). The biochar used was a commercial biochar manufactured by the company Proininso S.A. (Málaga, Spain) from the pyrolysis of oak (forest woody biomass pyrolyzed by reactor/gasifier at >900 °C and 0% oxygen content). The company is involved in the International Biochar Initiative and its biochar has previously been applied to restore mining soils (Rodríguez-Vila et al., 2014). The composted USR was provided by the municipal solid waste treatment plant of the Cartagena city (Spain). The main characteristics of both organic materials are given in Table S1 (Supplementary material). The biochar was alkaline, nearly decarbonated and poorly saline, with K^+ as the most abundant soluble ion. The USR was neutral, carbonated and saline, with high concentrations of soluble Cl^-, SO_4^{2-}, Na^+ and Ca^{2+}. The biochar had higher content of total organic carbon (TOC) but lower of total nitrogen (TN) than the USR (~4-fold higher and ~3-fold lower, respectively). The TOC:TN ratio was ~118 and ~9 in the biochar and USR, respectively. Water-soluble organic carbon (WSOC) and nitrogen (WSN) concentrations were higher in the USR (32-fold and 437-fold, respectively). These characteristics indicated much more recalcitrant organic matter in the biochar and higher content of labile organic compounds in the USR. Total metal(loid) concentrations were higher in the USR except for Mn (in mg kg^{-1}).

Four plots of 4 m × 2 m were located in acidic barren areas at El Lirio tailing in April 2017. Each plot was manually ploughed in the upper ~15 cm and divided into two paired subplots (2 m × 2 m) (Figure S2a, Supplementary material). One of the subplots was amended at 3% dry weight with the amendment (38.7 and 13.0 ton ha^{-1}, respectively; amended treatment –A–, n = 4) (Figures S2b and S2c, Supplementary material). No amendment was applied to the other subplot (not amended treatment –NA–, n = 4). Details on the selection of experimental field plots and organic amendment dose are available in the Supplementary material.

2.3. Soil sampling and field monitoring

Two months after ploughing and amendment application, five soil aliquots (upper ~10 cm) were randomly collected from each subplot and placed in the same plastic bag to constitute a representative sample per subplot for an initial evaluation (Table S2, Supplementary material). Samples were extracted with a shovel and taken to the laboratory where they were air-dried, sieved (2 mm mesh) and stored at room temperature prior to laboratory analyses. Particle size distribution, total $CaCO_3$, cation exchange capacity (CEC), TN and total metal(loid)s (As_{tot}, Cd_{tot}, Fe_{tot}, Mn_{tot}, Pb_{tot}, and Zn_{tot}) were determined (section 2.4). Ecotoxicity bioassays were also performed (section 2.6).

After that, a seasonal monitoring program including soil sampling and analysis and *in situ* collection of soil and vegetation data were carried out (Table S2, Supplementary material). In June 2017 (summer 2017), November 2017 (autumn 2017), January 2018 (winter 2018) and April 2018 (spring 2018) new composite soil samples were extracted with sterilized spoons (upper ~10 cm), put in sterile Falcon tubes and taken to the laboratory on ice inside a portable cooler. Material in Falcon tubes was not sieved or dried. Some tubes were stored at −20 °C to measure TOC, microbial biomass carbon (MBC) and dehydrogenase activity (DH), and to perform soil:water suspensions to analyze pH, EC, water-soluble salts (Cl^-, SO_4^{2-}, Na^+, K^+, Ca^{2+}, and Mg^{2+}), water-soluble metal(loid)s (As_{Ws}, Cd_{Ws}, Fe_{Ws}, Mn_{Ws}, Pb_{Ws}, and Zn_{Ws}) and WSOC (section 2.4). The rest of the material was frozen with liquid nitrogen and stored at −80 °C to evaluate the community-level physiological profile (section 2.5).

In parallel, the decomposition of organic matter and the feeding activity of soil dwelling organisms were evaluated seasonally *in situ* (Tables S2, Supplementary material) Organic matter decomposition was estimated by the tea bag index (TBI) method (Keuskamp et al., 2013). Lipton rooibos tea (EAN: 82 22,700 18,843 8) and Lipton green tea Sencha exclusive collection (EAN: 87 14,100 77,054 2) were used. Tea bags were buried seasonally at ~10 cm depth (Figure S3a, Supplementary material) and regularly collected during ~100 d to determine the remaining mass and calculate the TBI (http://www.teatime4science.org/publications/). Additionally, at each tea bag sampling time, soil samples were collected to determine the moisture content by drying at 65 °C and surface soil temperature was measured *in situ* with a portable thermometer. Faunal feeding activity was estimated with the bait-lamina method (Kratz, 1998; ISO, 2016). Baited sticks 10 cm long (Terra Protecta® GmbH, Berlin, Germany) were vertically buried at each season (Figure S3a, Supplementary material). Stick holes contained a mixture of cellulose (70%) and bran powder (30%) and a small amount of activated carbon. Partially and fully empty holes were recorded after 20 d buried (Figure S3b, Supplementary material).

After finishing the described seasonal monitoring in spring 2018, new composite soil samples were collected to carry out new ecotoxicity bioassays (Table S2, Supplementary material). Undisturbed soil cores (98 cm^3) were also collected to measure bulk density and water retention capacity (section 2.4). Additionally, soil CO_2 emission (soil respiration) was measured *in situ* with an SRC-1 Respiration System CIRAS-2 (Figure S3c, Supplementary material). Soil bulk density and water retention capacity were not measured at the beginning because the soil was recently ploughed. Soil CO_2 emissions could not be measured before spring 2018 due to operational problems with the device.

Plant species colonizing the plots were recorded throughout the study period. The number of individuals and the cover percentage (visual estimation) from each species were annotated at each seasonal sampling time. Additionally, vegetation data were newly recorded two years after finishing the seasonal sampling program (spring 2020).

2.4. Physical, chemical and physico-chemical soil analyses

Particle size distribution was determined by the Bouyoucos's densimeter method (Gee and Bauder, 1986) and CEC with 1 N CH_3COONH_4 (Chapman, 1965) in air-dried, sieved samples. Aliquots of these samples were grounded in an Agatha mortar for measuring total $CaCO_3$, TOC and TN with an elemental analyzer (LECO CHN628, LECO Corporation, The Netherlands) (ISO, 1995), and total metal(loid)s by X-ray fluorescence (Bruker S4 Pioneer, Bruker Corporation, Germany). X-ray fluorescence has previously been applied successfully to determine total metal(loid) concentrations in El Lirio tailing and others with similar mine wastes (Alcolea et al., 2012; Pellegrini et al., 2016). The recorded spectra were evaluated by the fundamental parameters' method using the SPECTRAplus software (EVA 1.7, Pioneer Hill Software, USA). A standard-less method was used owing to the lack of satisfactory certified reference materials with metal(loid) concentrations in similar ranges to those of the analyzed samples (Rousseau, 2001).

Soil:water suspensions (1:2.5 w:v, soil:water) were performed with aliquots of the samples stored at −20 °C, after thawing at room temperature. Extracts were filtered through nylon membrane syringe filters (0.45 μm, WICOM Germany GmbH). pH and EC were measured with a Crison Basic 20 pH-meter and a Crison Basic 30 conductivity-meter, respectively (Crison-Hach, Spain). The concentration of WSOC (TOC analyzer, TOC-VCSH Shimadzu, Shimadzu Corporation, Japan), soluble salts (ion chromatograph, Metrohm 861, Metrohm AG, Switzerland) and metal(loid)s (ICP-Ms, Agilent 7500 A, Agilent Technologies, Inc., USA; detection limit 0.002 mg L^{-1}) were analyzed.

Undisturbed soil cores were dried at 65 °C until constant weight to calculate the soil bulk density as the weight:volume ratio (Burke et al., 1986). After dried, the cores were used to calculate the water retention capacity at 33 kPa (field capacity) in a 15-bar ceramic plate extractor (Soilmoisture Equipment Corp., USA).

2.5. Microbiological soil analyses

Aliquots of soil samples stored at −20 °C were thawed at room temperature before the analysis of MBC and DH. MBC was estimated by the fumigation-extraction method (Vance et al., 1987; Wu et al., 1990). DH was determined by the iodonitrotetrazolium formazan (INT) method (García et al., 1993). Community-level physiological profile (CLPP) was determined in aliquots of the samples stored at −80 °C from the summer, autumn and spring samplings, after thawing at room temperature. CLPP was evaluated by the Biolog EcoPlate system (BIOLOG Inc., CA, USA) according to Preston-Mafham et al. (2002) and Samarajeewa et al. (2017). Inoculated EcoPlates were incubated at 20 °C in the dark for 192 h and optical densities at 590 nm were recorded every 24 h with a spectrophotometer (Biolog MicroStation System, Hayward, CA, USA). Substrates were classified within six carbon source groups according to Sala et al. (2006): amines and amides, amino acids, carbohydrates, carboxylic acids, phenolic acids, and polymers. Microbial catabolic activity was calculated as the average well-color development (AWCD) and substrates consumption preference as the substrate average well-color development (SAWCD) (Garland, 1997; Sofo et al., 2010). The following ecological indices were also calculated: substrate richness, S (number of substrates consumed); Shannon-Weaver index, H' (diversity of substrates consumed); Pielou index, J' (equitability/dominance of activities across all substrates consumed) (Garland, 1997; Sofo et al., 2010; Gryta et al., 2014).

2.6. Soil ecotoxicity bioassays

The model invertebrate species *Enchytraeus crypticus* (phylum *Annelida*, class *Oligochaeta*, family *Enchytraeidae*) was selected to perform the ecotoxicity bioassays due to its key role in soil functioning processes (e.g., organic matter decomposition, nutrient cycling, bioturbation) and its use as bioindicator of soil quality conditions (Didden and Römbke, 2001). Besides, due to its highly permeable skin (soft-bodied oligochaete with significant dermal uptake route), it lives in close contact with the soil solution and therefore with the metal(loid)s present in the system, being a good bioindicator of the biological impact of metal(loid)s (Didden and Römbke, 2001; Castro-Ferreira et al., 2012). Bioassays followed the ISO and OECD guidelines (ISO, 2004; OECD, 2004). Adult organisms were incubated in soil moistened at 50% of its maximum water retention capacity for 21 d inside an acclimatized room at 20 °C and 16 h:8 h light:dark photoperiod (n = 4). Lufa 2.2 soil (Speyer, Germany) was used as control soil. Soil moisture and food (oat flakes) availability were regularly checked during the incubation period and replenished when necessary. After 21 d the surviving adults and juveniles produced were counted (González-Alcaraz et al., 2015).

2.7. Statistical analyses

Statistical analyses were performed with IBM SPSS Statistics 24 (SPSS, 2020) and differences were considered significant at $p < 0.05$. Data were transformed when they failed to pass the Shapiro-Wilk's test (normal distribution) and/or Levene's test (homogeneity of variance). Student's *t*-test was used to check for differences between treatments (NA vs. A). Repeated-measures ANOVA followed by Bonferroni post-hoc test was used to compare how parameters evolved over seasons and between treatments. The factors included in the analysis were: an intra-subject factor, the season - repeated factor, with four levels (summer 2017, autumn 2017, winter 2018 and spring 2018); an inter-subject factor, the treatment, with two levels (NA and A). A significant effect of season indicates that the parameter evolves significantly over the study period. A significant effect of treatment indicates that the average parameter value differed among treatments. A significant effect of season × treatment interaction indicates that the evolution of the parameter over the study period differed among treatments. Person's rank correlations were performed to analyze the relationships between parameters.

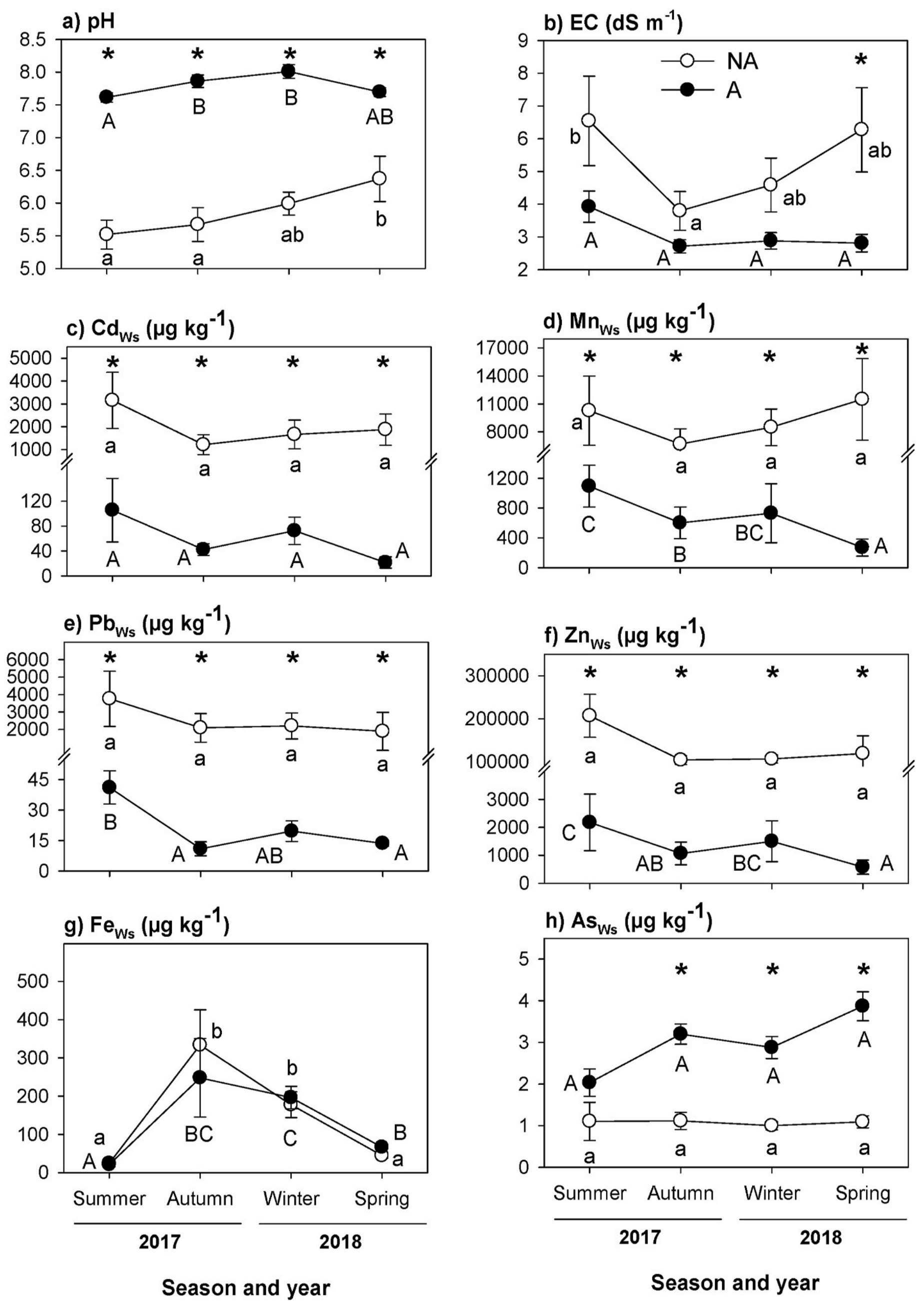

Fig. 1. Seasonal evolution of pH (a), electrical conductivity (EC) (b) and water-soluble metal(loid) concentrations (Me_{Ws}) (c–h) during the study period in both soil treatments: NA (not amended) and A (amended). Dots represent average values and bars SE (n = 4). Asterisks (*) indicate significant differences between treatments per season (Student's *t*-test, $p < 0.05$). Different letters (lowercase for NA, uppercase for A) indicate significant differences among seasons (repeated-measures ANOVA followed by Bonferroni post-hoc test, $p < 0.05$).

3. Results

3.1. Particle size distribution, total $CaCO_3$, CEC, TN and total metal(loid)s

Soils were sandy loam in both treatments with ~71–75% sand, ~20–22% silt and scarce clay content (~5–7%) (Table S3, Supplementary material). Two months after the application of the amendment the contents of total $CaCO_3$ (~14-fold higher, significant) and TN (~1.8-fold higher) had increased, but no differences were found for CEC values between treatments (~4–5 $cmol_c\ kg^{-1}$) (Table S3, Supplementary material). Amendment application slightly decreased total metal(loid) concentrations (~1.2–1.7-fold lower), probably due to a dilution effect, but differences between treatments were only significant for Pb (Table S3, Supplementary material).

3.2. Evolution of pH, EC, water-soluble salts and water-soluble metal(loid)s

Seasonal monitoring showed that the amendment significantly affected soil pH (season, treatment, and season × treatment interaction) and EC (season and treatment) (Fig. 1a and b). Soil pH had increased two months after the application of the amendment (from ~5.5 in NA to ~7.6 in A in summer 2017) and differences between treatments were maintained over the study period (Fig. 1a). In treatment NA soil pH increased over time, with significant higher values in spring 2018 (~6.4). On the contrary, lower pH variations were observed in treatment A (~7.6–8.0). Soil EC decreased with the application of the amendment (from ~6.6 dS m^{-1} in NA to ~3.9 dS m^{-1} in A in summer 2017), although differences between treatments were only significant in spring 2018 (Fig. 1b). Treatment NA showed marked seasonal changes in EC, which was significantly lower in autumn 2017 (~3.8 dS m^{-1}) compared to summer 2017 (~6.5 dS m^{-1}), and tended to increase again in winter (~4.6 dS m^{-1}) and spring 2018 (~6.3 dS m^{-1}). However, treatment A showed no significant variations in EC over the study period (~2.7–3.9 dS m^{-1}). The Cl^-, SO_4^{2-}, Na^+ and Mg^{2+} were more abundant in NA, while Ca^{2+} and K^+ reached higher concentrations in treatment A (Figure S4, Supplementary material).

Water-soluble Cd, Mn, Pb and Zn concentrations were significantly affected by the amendment (season and treatment). Concentrations were extremely high in treatment NA and significantly decreased two months after amendment application (Cd_{Ws}, Pb_{Ws} and Zn_{Ws} ~100-fold lower and Mn_{Ws} ~10-fold lower in summer 2017) (Fig. 1c–f). These differences remained of similar magnitude over the study period. In treatment NA no significant seasonal variations were found for these four metals. On the contrary, in treatment A, a general tendency to decrease from summer 2017 to spring 2018 was observed. Water-soluble Fe concentrations were not affected by the amendment and similar seasonal variations were observed for both treatments, with higher concentrations in autumn 2017 and winter 2018 and lower in summer 2017 and spring 2018 (Fig. 1g). Amendment application led to a progressive increase of As_{Ws} in treatment A, with significant higher concentrations than treatment NA in autumn 2017, winter 2018 and spring 2018 (~2.6–3.5-fold) (Fig. 1h).

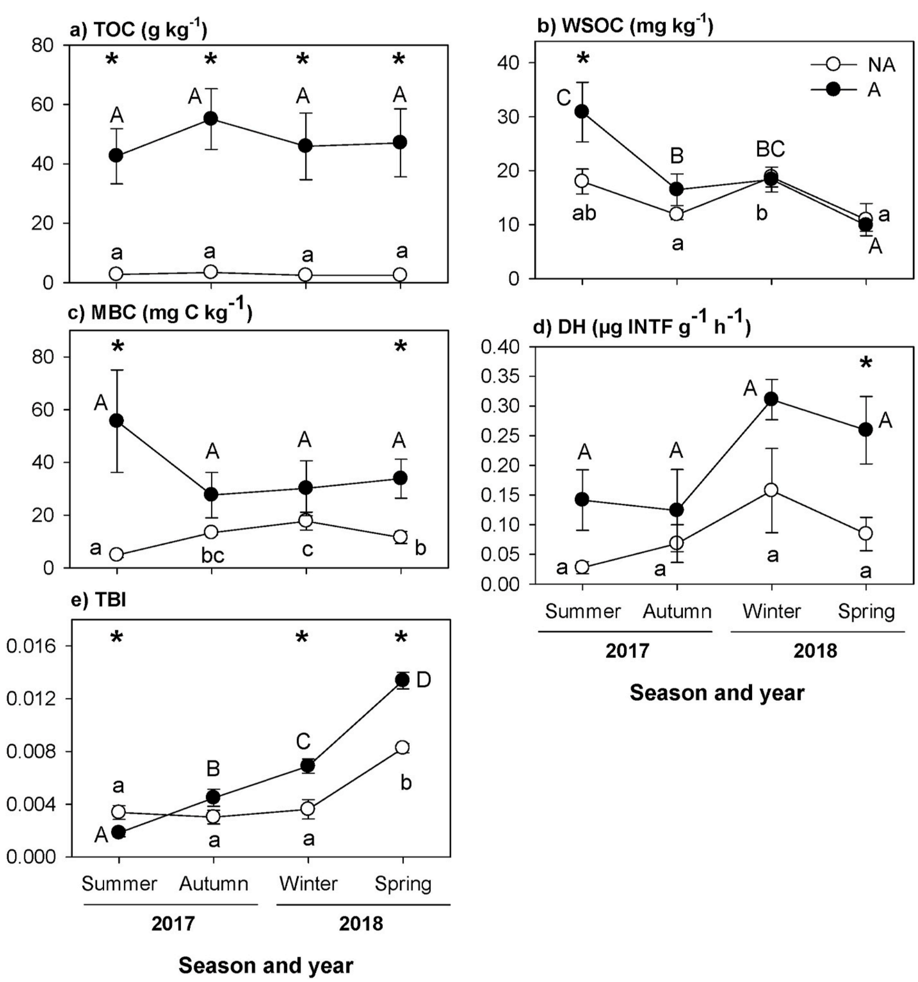

Fig. 2. Seasonal evolution of total organic carbon (TOC) (a), water-soluble organic carbon (WSOC) (b), microbial biomass carbon (MBC) (c), dehydrogenase activity (DH) (d) and tea bag index (TBI) (e) during the study period in both soil treatments: NA (not amended) and A (amended). Dots represent average values and bars SE (n = 4). Asterisks (*) indicate significant differences between treatments per season (Student's *t*-test, $p < 0.05$). Different letters (lowercase for NA, uppercase for A) indicate significant differences among seasons (repeated-measures ANOVA followed by Bonferroni post-hoc test, $p < 0.05$).

3.3. Evolution of TOC, WSOC, MBC, DH, CLPP, TBI, temperature, moisture and feeding activity

Two months after the application of the amendment (summer 2017) TOC, WSOC, MBC and DH had increased significantly (~16-, ~1.7-, ~12- and ~4.7-fold higher, respectively) (Fig. 2a–d). For TOC, the differences between treatments were maintained during the study period and no seasonal variations were found (significant effect of treatment) (Fig. 2a). For WSOC and MBC, both parameters decreased in autumn 2017, without differences between treatments (WSOC ~12–17 mg kg^{-1}; MBC ~13–28 mg C kg^{-1}). WSOC continued to decrease throughout the study period and reached the lowest concentrations in spring 2018 (significant effect of season) (Fig. 2b). However, MBC remained stable from autumn 2017 onwards and it was significantly higher in treatment A in spring 2018 (significant effect of season × treatment interaction) (Fig. 2c). For DH, it tended to increase in both treatments throughout the study period and was significantly higher in treatment A in spring 2018 (Fig. 2d).

Amendment application favored higher soil microbial catabolic activity throughout the study period (Fig. 3a). In both treatments, AWCD values were almost negligible during the first 96 h of incubation. From this time on and for all the study seasons, AWCD tended to increase in treatment A and reached the highest values upon 192 h of incubation (~0.09 in summer 2017, ~0.06 in autumn 2017 and ~0.04 in spring 2018). This was not the case of treatment NA that showed a very low consumption of carbon substrates in all the study seasons. Regarding substrates consumption pattern (SAWCD) (Fig. 3b), the consumption of amino acids (from ~18% to ~10%), carbohydrates (from ~30% to ~26%) and phenolic acids (from ~7% to ~2%) tended to decrease from summer 2017 to spring 2018, while that of amines/amides (from ~5% to ~8%) and carboxylic acids (from ~15% to ~24%) to increase. Polymer's consumption tended to increase from summer 2017 (~25%) to autumn 2017 (~35%) and decreased again in spring 2018 (~30%). SAWCD was not calculated for NA treatment due to the low absorbance values registered. Regarding the ecological diversity indices derived from CLPP analysis in treatment A, S and H′ showed higher values in summer 2017 and tended to decrease towards spring 2018 (S from ~8.5 to ~4.8; H′ from ~1.8 to ~1.5) (Table S4, Supplementary material). On the contrary, J′ tended to increase throughout the study period in treatment A (from ~0.9 in summer 2017 to ~0.9 in spring 2018) (Table S4, Supplementary material). Diversity indices could not be calculated for treatment NA.

Opposite to the previous parameters described, TBI was negatively affected by the amendment two months after its application (~1.9-fold lower) (Fig. 2e). However, in treatment A, it increased significantly throughout the study period and reached significant higher values than treatment NA in winter and spring 2018 (~1.6–1.9-fold higher) (significant effect of season and treatment).

Soil temperature and moisture significantly varied during the study period (effect of season), but without effects of the treatment and treatment × season interaction (Figure S5, Supplementary material). The highest soil temperatures were registered in summer 2017 (~24 to ~31 °C) and the lowest in winter 2018 (~6 to ~11 °C). Soil moisture was always <10% and slightly higher in treatment NA than in A. Soils were drier in summer 2017 and wetter in winter 2018.

Feeding activity of soil dwelling organisms was scarce and widely variable (Figure S6, Supplementary material). No clear tendencies were found in relation to the application of the amendment, except in spring 2018 when the feeding activity seemed to be favored in treatment A (~6.3-fold higher).

3.4. Bulk density, water retention capacity and CO_2 emission

One year after starting the experiment (spring 2018), the amendment had significantly decreased soil bulk density (~1.1-fold lower) and increased water retention capacity (~1.4-fold higher) (Fig. 4a and b). Soil CO_2 emissions had also increased significantly in treatment A in spring 2018 (~4.5-fold higher) (Fig. 4c).

3.5. Soil ecotoxicity

The survival of *E. crypticus* was not affected by the organic

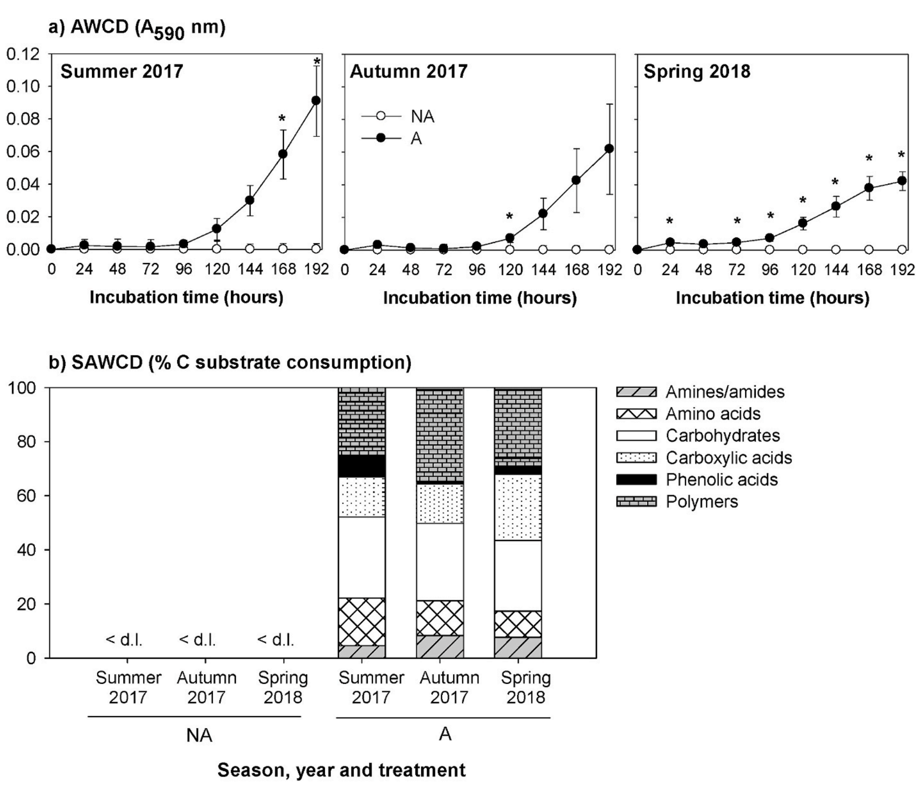

Fig. 3. a) Average well color development (AWCD) at 590 nm during the study period. Dots represent average values and bars SE (n = 4). Asterisks (*) indicate significant differences between treatments per incubation time (Student's t-test, p < 0.05). b) Substrate average well color development (SAWCD) during the study period expressed as percentage of substrate consumption (n = 4). d.l. (detection limit: absorbance values at 590 nm < 0.06). Soil treatments: NA (not amended); A (amended).

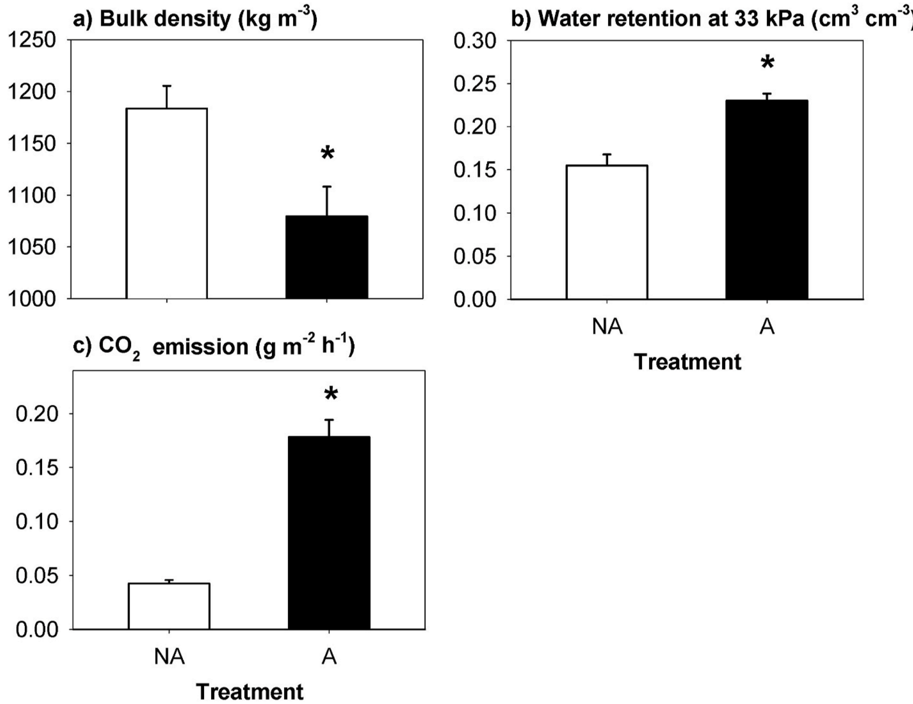

Fig. 4. Bulk density (a), water retention capacity at 33 kPa (b) and CO_2 emission (c) in spring 2018 in both soil treatments: NA (not amended) and A (amended). Columns represent average values and bars on columns standard error (n = 4). Asterisks (*) indicate significant differences between treatments (Student's *t*-test, $p < 0.05$).

amendment, both in summer 2017 (~60–80%) and spring 2018 (~66–74%) (Fig. 5a). However, *E. crypticus* reproduction was significantly stimulated two months after the application of the amendment (~4 juveniles in NA vs. ~32 juveniles in A in summer 2017) and this effect was maintained one year later (~1 juvenile vs. ~40 juveniles in spring 2018) (Fig. 5b).

3.6. Plant colonization

The application of the amendment significantly influenced the spontaneous plant colonization of the study plots (season, treatment, and season × treatment interaction). *Zygophyllum fabago* L. (Zygophylaceae) was the only plant species that appeared in the study plots between summer 2017 and spring 2018 (Figure S7a, Supplementary material). The plots of treatment NA remained bare or very low covered throughout the study period (~1 individual in autumn 2017 and ~5 individuals in spring 2018) (Fig. 6a). However, the plots of treatment A had significantly higher number of individuals from autumn 2017 onwards, reaching a maximum average value in spring 2018 with ~41 individuals (Fig. 6a). Between summer 2017 and spring 2018, the *Zygophyllum* seedlings that colonized the plots were very small (height <~2.5 cm) and, therefore, plant cover was very low (<1%), even in treatment A (Fig. 6b). However, in spring 2020, in the plots of treatment A, *Z. fabago* had reached ~25% average cover with plants up to ~50–60 cm high. Furthermore, it was also observed the presence of the perennial herb *Piptatherum miliaceum* (L.) Cosson (Gramineae) with an average cover ~2% (Figure S7d, Supplementary material).

4. Discussion

The organic amendment applied (mixture of biochar from pruning trees and composted USR) improved the unfavorable chemical and physico-chemical conditions of the acidic mine tailing soil from the beginning of its application. This improvement, which, for some parameters, was maintained over time, resulted in a decrease in the ecotoxicity of the tailing soil and, consequently, in an increase in the scores

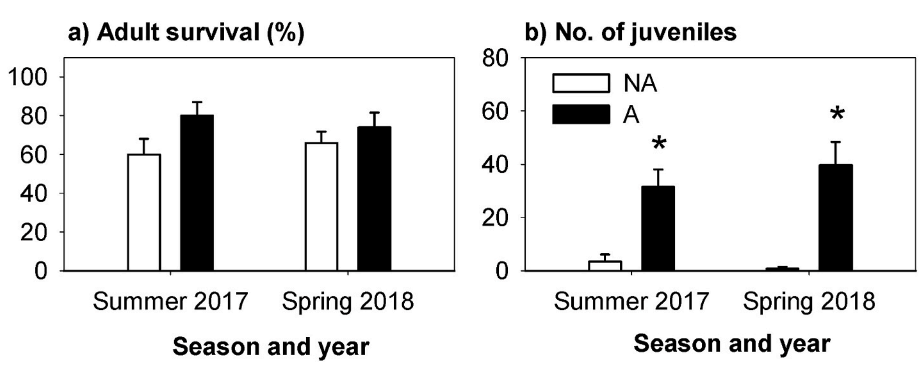

Fig. 5. Adult survival (a) and reproduction (number of juveniles produced) (b) of the soil invertebrate species *Enchytraeus crypticus* in summer 2017 and spring 2018 in both soil treatments: NA (not amended) and A (amended). Columns represent average values and bars on columns standard error (n = 4). Asterisks (*) indicate significant differences between treatments per season (Student's *t*-test, $p < 0.05$).

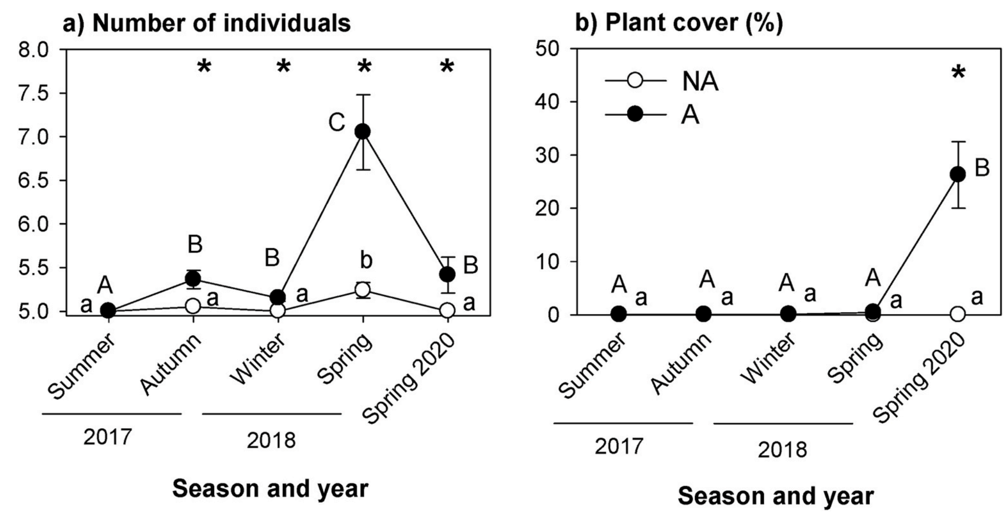

Fig. 6. Evolution of the number of individuals (a) and plant cover (b) during the study period in both soil treatments: NA (not amended) and A (amended). Dots represent average values and bars SE ($n = 4$). Asterisks (*) indicate significant differences between treatments per season (Student's *t*-test, $p < 0.05$). Different letters (lowercase for NA, uppercase for A) indicate significant differences among seasons (repeated-measures ANOVA followed by Bonferroni post-hoc test, $p < 0.05$).

of parameters related to the microbiology and functionality of the edaphic system. Besides, relief from the stressful tailing soil conditions allowed some plants to germinate and grow, covering a significant part of the amended plots three years later. All this highlighted the suitability of the organic amendment used for the transition of a barren mine tailing towards a self-sustainable soil-plant system.

4.1. *Effects of the organic amendment on soil pH, salinity, water-soluble metal(loid)s and ecotoxicity*

The alkalinity of the biochar and the high content of $CaCO_3$ of the USR contributed to rising the pH of the mine tailing soil, and this effect was sustained over the study seasons (Fig. 1a; Table S1, Supplementary material). Soil ploughing at the beginning of the experiment could boost the oxidation of sulfides such as pyrite (a common geochemical process in these types of mine wastes when they are aerated) (Huang et al., 2012; Pellegrini et al., 2016), which was reflected by the lower pH in summer 2017. Re-settling of soil material with time must have slowed down this process and the pH tended to rise. This was not observed in treatment A due to the alkalizing and buffer effect of the amendment applied. Despite the high salinity of the USR, its combination with biochar led to a decrease in soil salinity in treatment A (Fig. 1b; Table S1, Supplementary material). This could be related to the adsorption capacity of biochar (Ahmad et al., 2014), but also to other inherent properties of this type of organic amendments such as the high pH values. In fact, a significant negative correlation between pH and EC was found ($r = -0.540$, $p < 0.001$), which indicated that the increased pH led to a decrease of some dissolved elements like metals by precipitation or co-precipitation mechanisms (as discussed later). In addition, the calcium added with the organic amendment directly as dissolved Ca^{2+} or in the form of $CaCO_3$ could react with SO_4^{2-} by forming gypsum ($CaSO_4{\cdot}2H_2O$) and, therefore, lowered the concentration of soluble SO_4^{2-} (Figure S4b, Supplementary material) (Zornoza et al., 2015). It should be also noted that the concentration of K^+ in soil was 6-fold higher in treatment A (Figure S4d, Supplementary material), which could favor plants establishment due to its role as macronutrient (Marschner, 1995).

Soil salinity strongly varied over the study period. It increased in summer 2017 and spring 2018, the warmer and drier seasons, while decreased in autumn 2017 and winter 2018, the wetter seasons (Fig. 1b; Figure S5, Supplementary material). Seasonal salinity changes in the upper soil centimeters have been previously described in saline soils (González-Alcaraz et al., 2014). They are mainly caused by the upward capillary movement of soluble ions during drier and warmer periods and their leaching to deeper layers during wetter seasons. This phenomenon affected to a greater extent to the more mobile ions, such as Cl^- and Na^+, and was more evident in treatment A (Figure S4, Supplementary material). Most likely the latter was related to the higher porosity induced by the organic amendment, as shown by the lower soil bulk density in treatment A (Fig. 4a). Interestingly, the increase in K^+ concentration in treatment A was maintained throughout the study period and hardly changed over seasons (Figure S4d, Supplementary material).

The higher soil pH in treatment A was accompanied by a decrease in the concentration of water-soluble Cd, Mn, Pb and Zn (significant negative correlation between pH and Cd_{WS}, Mn_{WS}, Pb_{WS} and Zn_{WS}; $r \leq -0.615$, $p \leq 0.002$) (Fig. 1). Precipitation or co-precipitation is considered the major mechanism involved in reducing metal solubility when pH increases after liming acidic soils (Simón et al., 2018). Nevertheless, biochar addition implies that other mechanisms can play relevant roles such as electrostatic interactions between metal cations and negatively charged biochar surfaces, cation exchange and/or ligand complexation with biochar surface functional groups (Kołodyńska et al., 2017; Wu et al., 2017). Houben and Sonnet (2015) found that the alkalinizing effect of biochar lowered the concentration of soluble Cd, Pb and Zn in an acidic contaminated soil, and that these metals were mainly sequestered in carbonates. Zornoza et al. (2016) found that Cd was mainly immobilized in carbonates and Fe/Mn oxides in acidic mine soils amended with biochar. Simón et al. (2018) stated that the fate of Cu, Pb and Zn on biochar implies the formation of metal complexes with functional groups of this carbonaceous material. Even though numerous studies have reported the effectiveness of organic amendments, mainly biochar, to reduce metal solubility, this matter is still under debate since the effects may change with aging (Chen et al., 2018; Manzano et al., 2020). For example, in a soil incubation experiment, He et al. (2019) stated that different biochar types had contrasting impacts on metal speciation and lability upon 2-years aging. The use of USR in mine tailing soils has also been discussed (Párraga-Aguado et al., 2017). The addition of organic amendments with labile fractions can effectively reduce metals mobility at the short-term, but metals could be released at the long-term due to organic matter mineralization (Schwab et al., 2007). According to Bian et al. (2014), in our study, the effect of the organic amendment was consistent over the study period and its effectiveness did not decrease by the time elapsed since the application was done. Nevertheless, if the presence of USR favored the release of some metals with aging the presence of biochar probably counteracted that effect.

Unlike the aforementioned metals, the organic amendment did not affect the concentration of Fe_{Ws} and the seasonal variation of this element was similar in both treatments (Fig. 1g). Wetter soil conditions in autumn 2017 and winter 2018 (Figure S5b, Supplementary material) could have led to lower redox potential values that provoked the dissolution of Fe oxy-hydroxides (González-Alcaraz and Álvarez-Rogel, 2013) and, therefore, higher Fe_{Ws} concentrations during these seasons. In the case of As, the amendment applied was not only ineffective for reducing its solubility, but also progressively favored the increase of As_{Ws} concentration (Fig. 1h). The higher content of labile organic matter in treatment A, as shown by the greater concentration of WSOC and MBC (Fig. 2b and c), could facilitate the mobilization of As (Simón et al., 2014). In addition, a significant positive correlation between pH and As_{Ws} was found ($r = 0.732$, $p < 0.001$). This agreed with Simón et al. (2010, 2014) who showed that As fixation is hindered under high pH and $CaCO_3$ conditions due to the predominance of $HAsO_4^{2-}$, which shows low adsorption capacity on solid surfaces (González et al., 2012; Simón et al., 2014). These authors stated that Fe-oxides are highly efficient in reducing the mobility and availability of As in the absence of $CaCO_3$. Hence, in treatment NA, lower As_{Ws} concentration could be attributable to the immobilization of this metalloid by binding onto amorphous and crystalline Fe-oxides that are abundant in this type of mine wastes (Parraga-Aguado et al., 2015).

Amelioration of mine tailing soil acidity, salinity, and solubility of some metals (Cd, Mn, Pb and Zn), following amendment application, was translated into decreasing toxic effects to the soil invertebrate *E. crypticus* (Fig. 5). The possible ecotoxicological effect of biochar, recently reported by Godlewska et al. (2021), was clearly overweighted by its beneficial effects in the mine tailing soil. Ecotoxicity bioassays showed that invertebrates' survival was not affected neither by the organic amendment nor by the time elapsed since its application (adult survival ~60–80% in summer 2017 and spring 2018 in both treatments) (Fig. 5a). However, reproduction, a parameter more sensitive to changes occurring in soil conditions (Castro-Ferreira et al., 2012; González-Alcaraz and van Gestel, 2015; Zhang and Van Gestel, 2019), showed a clear improvement with the amendment. The number of juveniles produced in treatment A was 8-fold higher two months after the application of the amendment (summer 2017) and 40-fold higher one year later (spring 2018) (Fig. 5b), which evidenced the reduced ecotoxicity of the amended tailing soil. These results are in accordance with Beesley et al. (2014) who found decreasing ecotoxicity risks of a heavily contaminated metal(loid) mine soil after being amended with a mixture of olive mill waste compost and biochar from orchard pruning residues.

4.2. Effects of the organic amendment on soil organic carbon and microbial, functional-related and physical parameters

As expected, amendment addition was accompanied by an increase in tailing soil organic carbon. The contrasting composition of biochar and USR determined the way in which both materials contributed to the organic carbon pool in treatment A (Wu et al., 2016). Biochar provided stable organic compounds (TOC:TN ratio ~118) and USR more labile organic matter (TOC:TN ratio ~9) (Table S1, Supplementary material). Hence, the consisted higher TOC content in treatment A along the study period was mainly attributable to biochar while USR was responsible for the initial boost of WSOC concentration, which tended to decrease to similar levels to treatment NA from autumn 2017 onwards (Fig. 2a and b). The sharp increase in WSOC concentration followed by a fast decline after the addition of an amendment enriched in labile organic matter has been previously described, whereas organic carbon from biochar may persist in soils for longer periods (Moreno-Barriga et al., 2017a).

Since biochar provided a limited amount of easily available organic carbon for microorganisms, the initial increase in MBC observed in summer 2017 in treatment A was mainly attributable to the extra energy source provided by the WSOC contained in USR (significant positive correlation; $r = 0.533$; $p < 0.005$) (Fig. 2b and c). Furthermore, increasing pH together with decreasing salinity and water-soluble concentrations of Cd, Mn, Pb and Zn had to facilitate the growth of tailing soil microorganisms. In fact, MBC was significantly positively correlated with pH ($r = 0.537$, $p = 0.002$) and negatively with Cd_{Ws}, Mn_{Ws}, Pb_{Ws} and Zn_{Ws} ($r \geq -0.386$, $p \leq 0.029$). Microorganisms colonizing the USR could also have contributed to the initial increase in MBC of treatment A (Huang et al., 2012). This was probably not the case for biochar which probably had a very low microbial load (if any) as it was a commercially manufactured product. However, biochar could provide shelter for microorganisms and so facilitate their resistance against metal(loid) pollution as suggested by Tu et al. (2020). All this was reflected in a greater rate of substrate utilization (AWCD, i.e., catabolic potential of the microbial community) and a greater functional diversity (ecological diversity indices derived from CLPP analysis) in treatment A, especially in summer 2017 (Fig. 3a; Table S4, Supplementary material).

The concomitant decline in MBC and WSOC from summer to autumn 2017 in treatment A (Fig. 2b and c) pointed out that the initial boost of the microbial population tended to disappear after the consumption of the initial load of labile organic matter. However, even though WSOC continued to decline, MBC stabilized or even increased slightly from autumn 2017 onwards. This could be related to the progressive disappearance of the exogenous microorganisms introduced with the organic amendment in favor of the proliferation of communities better adapted to local conditions (Huang et al., 2012). Zornoza et al. (2015, 2016) found changes in microbial communities' structure of amended acidic mine tailing soils and related these results to the increase in pH and organic matter content and reduced metal availability with amendment addition. Furthermore, these authors found that the microbial communities of the amended tailings were closer to those of the surrounding native forest soils than to the original mine waste microbial communities. In our case, the increasing soil functionality of the amended plots over time, reflected by the better scores for parameters such as DH, TBI and CO_2 emission (Fig. 2d, e and 5c), supported the possible shift of microbial populations. Moreover, the changes observed in the consumption pattern of the different carbon sources groups (SAWCD, i.e., metabolic fingerprint) towards spring 2018 (Fig. 3b) could be a consequence of the possible transition from exogenous to native microorganism species over time.

The higher content of soil organic carbon along with the improved microbial activity in the amended plots contributed to the decrease in bulk density and the increase in water retention capacity of the mine tailing soil (Fig. 4a and b), both parameters associated with higher soil porosity. The latter indicated soil structure development in treatment A, which is of utmost importance for improving soil functioning and deliver ecosystem services since pores and channels harbor life underground and allow the growth of plant roots (Morgado et al., 2017; Rabot et al., 2018). Moreover, soil structure development is also paramount for air and water storage and solute movement including salts and metal(loid) lixiviation (Pellegrini et al., 2016).

4.3. Plant colonization

The hostile conditions that plants must cope with in metal(loid) mine tailings hinder the colonization of these environments, even after the application of soil treatments (Oreja et al., 2020). Our findings highlight that while amendments can be effective to modify certain chemical and physico-chemical soil conditions in the short time, a more prolonged time-lag might be necessary to reach an effective plant colonization. In this sense, although one year (until spring 2018) was enough to improve tailing soil conditions and a significant higher number of seedlings were observed in treatment A, adult plants did not proliferate (Fig. 6a; Figure S7, Supplementary material). It was in spring 2020, about three years after soil ploughing and application of the organic amendment, when a consistent plant cover with the presence of two pioneer species (*Z. fabago* and *P. miliaceum*) was observed in the amended plots (Fig. 6b; Figure S7, Supplementary material). Moreno-Barriga et al. (2017b) also observed a delay in the response of *P. miliaceum* to the addition of biochar at a dose of 4.8% to an acidic mine tailing soil of similar characteristics in a pot experiment that lasted ten months. The authors found that biochar was ineffective in stimulating plant growth for the first five months after amendment addition probably due to the immobilization of nutrients by biochar, but that this effect did not persist after ten months.

Z. fabago (Syrian bean-caper) is a perennial xerohalophyte shrub widely spread worldwide and that tolerates drought conditions (Menzel and Lieth, 2003). This species is one of the main primo colonizers of barren metal(loid) mine tailings growing in some of the most unfavorable edaphic niches (Párraga-Aguado et al., 2013). It has been shown that *Z. fabago* enhances soil microbial activity within the rhizospheric environment and that it can accumulate a wide range of metal(loid)s in the leaves but at concentrations lower than phytotoxic levels (Párraga-Aguado et al., 2016). Since belowground organs of this species are persistent, we cannot be sure whether some of these plants were survivors from previous years or new individuals. Anyway, it was only in spring 2020 that adult plants with remarkable size were observed in the amended plots, and hence three years were necessary to reach a consistent plant cover.

P. miliaceum (smilograss) is a perennial grass worldwide distributed in nitrified lands, roadsides, slopes, and other types of altered environments (Párraga-Aguado et al., 2013). It is characterized by presenting a dense well-developed root system, including rhizomes, that favors microbial activity (Moreno-Barriga et al., 2017a) and protects soil against erosion (De Baets et al., 2007). Results from field (Conesa et al., 2006; Parraga-Aguado et al., 2015) and pot (Párraga et al., 2015; Moreno-Barriga et al., 2017a) experiments revealed that this species is suitable for phytoremediation as it shows limited translocation of metals from roots to shoots. *P. miliaceum* is also considered a pioneer colonizer of metal(loid) mine tailings. However, its role may be controversial. Navarro-Cano et al. (2018), based on field works, classified this species as a nurse plant (plants with high capacity to cope with multi-stressed conditions that facilitate the further colonization by other less stress-tolerant species). However, Martinez-Oró et al. (2017), based on a pot experiment, stated that *P. miliaceum* can behave as an opportunistic competitor when growing together with the pine tree *P. halepensis*. The fact that *P. miliaceum* was only observed in the experimental field plots in spring 2020 further supports that three years after organic amendment application were necessary to reach a step forward in system sustainability.

5. Conclusions

The results obtained highlight the suitability of combining biochar from pruning trees and composted USR to ameliorate the hostile conditions of barren acidic metal(loid) mine tailings soils in Mediterranean semiarid areas and effectively favor spontaneous plant colonization. Improvements in chemical and physico-chemical soil properties were observed just two months after amendment addition (e.g., increase in pH and TOC and decrease in salinity, water-soluble metals and ecotoxicity). The recalcitrant organic carbon provided by biochar remained in the soil system for at least one year. The more labile organic compounds provided by USR triggered an initial boost of microbial growth, although they were consumed over time. These improvements were consistent for at least one year and led to lower bulk density, higher water retention capacity and better functionality of the tailing soil as shown by the higher scores for microbial/functional-related parameters (e.g., MBC, microbial catabolic activity and CO_2 emission). Despite the overall improvement described, increased pH following amendment application led to higher water-soluble As concentration. The stabilization (or slightly increase) of MBC from about seven months after amendment addition, despite the WSOC drop, and the changes occurring in the metabolic fingerprint, suggested a progressive shift from a microbial population dominated by microorganisms introduced with the amendment to one dominated by native species better adapted to tailing soil conditions. Although vegetation was able to spontaneously colonize the amended tailing soil, one year was not enough to reach a consistent plant cover with adult plants, which was only observed three years after soil treatment. This highlights the slowness of the recovery processes in these extreme environments and the existence of a time-lag between the positive effects of the amendment on soil properties being observed and these improvements being translated into effective spontaneous plant colonization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jenvman.2021.112824.

Credit author statement

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