



Response of field-grown native plant species to a combined amendment for phytomanaging metal(loid)-mine tailings

Héctor M. Conesa · José-Antonio Franco · Matías Ceacero-Moreno ·
Juan-José Martínez-Sánchez · M. Nazaret González-Alcaraz ·
José Álvarez-Rogel

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Abstract

Background and aims Phytostabilization of semi-arid mine tailings involves establishing vegetation to reduce metal(loid) fluxes. Beyond biomass production and metal(loid) uptake, assessing ecophysiological traits is critical for selecting suitable species. This study examined six semiarid species (four shrubs, two conifers) in an 18 months field-scale tailing phytomanagement project using an organic amendment combining biochar and municipal organic waste.

Methods Plant responses were evaluated in amended and unamended plots by measuring biomass (above- and belowground), foliar metal(loid) concentrations

(As, Cd, Cu, Mn, Pb, Zn), nutrient content (N, P, K), and isotopic signatures ($\delta^{13}\text{C}$, $\delta^{15}\text{N}$, $\delta^{18}\text{O}$).

Results Amended plots showed improved soil fertility, including a threefold increase in total N and tenfold increase in organic carbon. Up to five species in amended plots exhibited higher biomass. Foliar metal(loid) concentrations varied by species and element. While P deficiency was alleviated, foliar N concentrations did not increase despite higher soil N. Enhanced P uptake was linked to higher $\delta^{13}\text{C}$ values, suggesting improved water-use efficiency. Among C3 species, isotopic indicators revealed contrasting water-use strategies. The conifers (*Pinus halepensis* and *Tetraclinis articulata*) showed conservative traits, with lower stomatal conductance and higher WUE. *Tetraclinis articulata* in amended plots exhibited tighter water regulation, reflected by reduced foliar Ca and elevated $\delta^{18}\text{O}$. In contrast, shrubs (*Dorycnium pentaphyllum*, *Maytenus senegalensis*, *Rhamnus*

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H. M. Conesa (✉) · J.-A. Franco (✉) ·
M. Ceacero-Moreno · J.-J. Martínez-Sánchez ·
M. N. González-Alcaraz · J. Álvarez-Rogel
Escuela Técnica Superior de Ingeniería Agronómica,
Departamento de Ingeniería Agronómica, Universidad
Politécnica de Cartagena, Paseo Alfonso XIII, 48,
Cartagena 30203, Spain
e-mail: hector.conesa@upct.es

J.-A. Franco
e-mail: josea.franco@upct.es

M. Ceacero-Moreno
e-mail: matias.ceacero@upct.es

J.-J. Martínez-Sánchez
e-mail: juan.martinez@upct.es

M. N. González-Alcaraz
e-mail: nazaret.gonzalez@upct.es

J. Álvarez-Rogel
e-mail: jose.alvarez@upct.es

J.-A. Franco
Instituto de Biotecnología Vegetal, Edificio I+D+I, Plaza
del Hospital s/n, Universidad Politécnica de Cartagena,
Cartagena 30202, Spain

lycioides) displayed more spendthrift strategies, characterized by higher transpiration and foliar N content. **Conclusions** The contrasting water and nutrient acquisition strategies suggests that not all species respond equally to amendments, emphasizing the need for species-specific approaches in successful phytostabilization.

Keywords Phytostabilisation · Stable isotope · Soil amendments · Water use efficiency · *Pinus halepensis* · *Tetradlea articulata* · *Atriplex halimus*

Introduction

Phytomanagement through phytostabilisation of semiarid abandoned mine tailings consists of the implementation of a vegetation cover to reduce the environmental fluxes of metal(loid)s from tailing surfaces (Robinson et al. 2009; Fellet et al. 2011; Álvarez-Rogel et al. 2021). Abandoned mine tailings piles come from the left-over of mineral concentration processes and typically form trapezoidal structures. Their unfavourable edaphic properties, such as extreme pHs levels, salinity, poor fertility and/or high metal(loid) concentrations, create a challenging environment for plant establishment (Mendez and Maier 2008; Párraga-Aguado et al. 2013). As a consequence, their untreated surfaces remain bare and exposed to erosion, which increases the spread of metal(loid) enriched particles through erosive processes, causing deleterious impacts on environmental health (Conesa and Schulin 2010).

The key to achieving feasible phytomanagement of tailings lies in the prior selection of suitable plant species, which must include those tolerant to the aforementioned edaphic constraints of these materials (Navarro-Cano et al. 2018). In arid and semiarid areas, plants must also cope with drought and high evapotranspiration rates (Mendez and Maier 2008; Atika et al. 2024). The search for a feasible plant list for phytomanaging semiarid tailings may benefit from local population of colonists developing on abandoned tailing surfaces at a given region, because they have already acclimated to its particular bioclimatic conditions (Sánchez-López et al. 2015; Navarro-Cano et al. 2019). During this selection process, aspects such as low metal(loid) uptake capacity (to diminish metal entry into the food chain),

extension of the root system, capacity for providing perennial vegetation cover or xerophytic character (in case of semiarid areas), are key determinants in making a selection, while other critical ecophysiological constraints that may condition plant adaptation to these environments -and thus determine the final success of the phytomanagement at a specific mining site- are often overlooked (Martínez-Oró et al. 2019; Conesa et al. 2024). Therefore, the main challenge in the implementation of phytomanagement in semiarid tailings lies in overcoming the limiting factors affecting plant growth, as these may delay ecological successional processes and, in turn, hinder the establishment of a stable plant cover within an acceptable timescale (Álvarez-Rogel et al. 2021; Ramírez-Zamora et al. 2022). To accelerate these processes, the use of soil conditioners or amendments has been often suggested (Constantinescu et al. 2019; Peñalver-Alcalá et al. 2021; Al-Lami et al. 2022; Conesa et al. 2024). Organic amendments derived from urban and agricultural wastes have been widely used in the remediation of contaminated soils due to their availability and proven benefits in enhancing soil fertility, and consequently, enhancing plant performance (Clemente et al. 2015). Soil amendments pairing organic biosolids and biochar are effective in promoting sustainable plant growth in metalliferous mine tailings piles (Rodríguez-Vila et al. 2016, 2017; Forján et al. 2018; Peñalver-Alcalá et al. 2021; Al-Lami et al. 2022). Biosolids and/or composts derived from urban and agricultural wastes provide a readily available source of nitrogen (Clemente et al. 2012, 2015), thereby addressing plant nutrient deficiencies in mine tailings (Conesa et al. 2024). The use of these by-products aligns with the environmental framework established by EU regulators to promote a circular economy, offering sustainable alternatives for waste management (European Commission 2008). Although there is a general acceptance of the benefits associated to the addition of these biosolids in tailings, recent works consider necessary to carry out a specific assessment to avoid any undesirable effects such as increases of metal(loid) available pools (Pardo et al. 2014; Venegas et al. 2015; Forján et al. 2018). An interesting option to overcome the potential constraints posed by urban and agricultural biosolids waste materials lays on their use in combination with biochar (Bogusz and Oleszczuk 2018; Forján et al. 2018). Biochar, an organic amendment

produced through the pyrolysis of organic materials, can show, among other properties, a high capacity for metal complexation, attributable to its large specific surface area and numerous sorption sites, as well as high stability due to its elevated recalcitrant organic carbon content (Fellet et al. 2014; Rodríguez-Vila et al. 2017). Moreover, biochar can contribute to the long-term improvement of soil physical properties by supplying stable carbon and decreasing metal(loid) mobility (Rodríguez-Vila et al. 2016, 2017; Bogusz and Oleszczuk 2018; Peñalver-Alcalá et al. 2021).

Although there is extensive research focused on mine tailings and the effects of amendments on plant biomass or metal uptake in field conditions (Clemente et al. 2012; Gil-Loaiza et al. 2016; Acosta et al. 2018; Constantinescu et al. 2019) or under controlled conditions (Romero-Estonillo et al. 2023; Lacalle et al. 2023), more studies on field conditions are necessary to assess the ecophysiological response of plants following the application of amendments to mine tailings. Understanding plant resource-use strategies is essential for assessing plant responses to soil resource availability and environmental stresses, as well as for evaluating their potential for the phytostabilisation of semiarid tailings, where water stress represents an additional constraint (Párraga-Aguado et al. 2014a; Navarro-Cano et al. 2018). The use of ecophysiological indicators at individual plant level is considered an effective approach for characterising plant growth strategies (Zhao et al. 2006). For example, foliar $\delta^{13}\text{C}$ values indicate the balance between net photosynthetic rate and stomatal conductance, offering a time-integrated estimate of Water Use Efficiency (WUE) over the growing season (Farquhar et al. 1989; Seibt et al. 2008; Cernusak et al. 2013). Additionally, foliar $\delta^{18}\text{O}$ concentrations can provide a time-integrated measure of plant stomatal conductance when other sources of variation (mainly $\delta^{18}\text{O}$ of the water source) are negligible (Barbour 2007). Given that foliar $\delta^{18}\text{O}$ values fluctuate with stomatal conductance but remain unaffected by carbon assimilation rates, the simultaneous assessment of $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ in foliar tissues may help disentangle the independent effects of carbon fixation and stomatal conductance (Scheidegger et al. 2000; Prieto et al. 2018). Finally, foliar $\delta^{15}\text{N}$ values reflect the net effect of various processes, including the isotopic signature of the soil N sources used by the plant, mycorrhizal associations, soil fertility levels, temporal and spatial variation in

N availability, and changes in plant demand (Craine et al. 2009; Ruiz-Navarro et al. 2016).

The goal of this study was to evaluate the ecophysiological responses (assessed through the coupled interpretation of stable isotopes and ionic measurements) of six semiarid plant species (four shrubs and two coniferous tree species) in a field scale phytomanagement project, which included the addition of an organic amendment containing a combination of biochar and municipal organic solid waste. The selection of plant species included xerophyte plant species which were already listed among spontaneous vegetation in local tailings (e.g. Párraga-Aguado et al. 2013, 2014a; Navarro-Cano et al. 2018; Oná et al. 2021). After 18 months of experimentation, the effects of the amendment on plants were assessed by evaluating biomass (aboveground and belowground), foliar metal(loid) (As, Cd, Cu, Mn, Pb and Zn) uptake, foliar nutrient (N, P, K, Ca, Mg and Na) and isotope ($\delta^{13}\text{C}$, $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$) concentrations. We hypothesised that although these plant species are able to spontaneously colonize local tailings with no aid, the use of amendments would provide more robust plant establishment (faster growth) and an improved foliar nutritional status (through higher uptake of N and P) together with higher water use efficiency compared to the not amended conditions. This study presents a novel, context-specific evaluation of combined biochar and biosolid amendments on semiarid mine tailings under Mediterranean field conditions -a setting where metal contamination and water scarcity co-occur. By incorporating a multi-species approach with six native plants, assessing ecophysiological responses rather than just biomass or metal uptake, and integrating $\delta^{13}\text{C}$ as a proxy for water use efficiency, we offer new mechanistic and valuable insights into nutrient-physiology interactions in semiarid mining degraded systems.

Materials and methods

Studied area and mine tailings pile

The study was conducted on a mine tailing abandoned about 40 years ago without any restoration measures. The site is located in the former mining district of Cartagena-La Unión (southeast Spain, 110–0 m, a.s.l.; 37°37'20'' N, 0°50'55'' W– 37°40'03'' N,

0°48'12'' W, Figure SM1). The local climate is Mediterranean, with an average annual temperature of 18°C and annual precipitation about 250–300 mm. Historical mining activity (currently discontinued) was focused on metallic sulphide minerals, including galena, pyrite and sphalerite. Further details on the environmental impacts of mining in this region have been reviewed by Conesa and Schulin (2010).

The experimental plot (~2000 m²) was established in a barren area on the upper surface of the tailing (Figure SM1). The tailings topsoil consists of a sandy-loam material (~65% sand) with a calcium carbonate content of ~3%, neutral pH (~7), and moderate salinity (electrical conductivity ~2.4 dS m⁻¹) (Table SM1). Total metal(loid) concentrations (*e.g.* ~11,000 mg Zn kg⁻¹, ~7400 mg kg⁻¹ Pb and Mn; ~390 mg As kg⁻¹; Table SM1) were comparable to other mine tailings in the area (Párraga-Aguado et al. 2013; Álvarez-Rogel et al. 2021). Neutral pH of the tailings, indicative of a non-pyritic mine waste, is frequent in some of the

tailings piles in the region (Conesa and Schulin 2010; Párraga-Aguado et al. 2013).

Characteristics of the amendments, application method, plant species selection and planting

Two amendments were used in the experiment (Table 1): a commercial biochar (*LivingChar*) derived from agricultural and forestry residues from pruning (provided by *CarbonVivo* Barcelona, Spain, www.livingchar.com) and a Municipal Organic Solid Waste (MOSW, from now on) provided by *Lhicarsa* from the Cartagena city recycling plant (Cartagena, Spain). The biochar was made of wood from *P. halepensis* and pruning of *O. europaea* through pyrolysis at 700°C during approx. 10 min. The MOSW consisted of homogenised material derived from the organic fraction of the city garbage, stabilized for two months.

The characterisation of the biochar and the MOSW is shown in the Table 1. Total Cu, Pb and

Table 1 Characteristics of the biochar and the Municipal Organic Solid Waste (MOSW) employed in the experiment

1:10 corresponds to parameters measured in the 1:10 amendment:water extracts: pH, EC Electrical Conductivity, WSOC Water Soluble Organic Carbon, WSN Water Soluble Nitrogen, water extractable macronutrients and water extractable metal(loid)s. Total metal(loid)s and total phosphorus (Total P) were determined according to USEPA Method 3051a (USEPA 2007). All data correspond to the analyses of bulk biochar (0.05–30 mm size) and bulk MOSW except TOC (Total Organic Carbon), TN (Total Nitrogen) and $\delta^{15}\text{N}$, which corresponded to ground homogenised material. Data are average $\pm$ standard error of 4 replicates

Parameter	Biochar	MOSW
pH (1:10)	9.69 $\pm$ 0.04	6.42 $\pm$ 0.01
EC (1:10) (dS m ⁻¹)	1.55 $\pm$ 0.11	7.50 $\pm$ 0.09
TOC (%)	62.41 $\pm$ 2.86	31.58 $\pm$ 1.52
WSOC (1:10) (mg kg ⁻¹)	167 $\pm$ 21	23,400 $\pm$ 333
TN (%)	0.39 $\pm$ 0.03	1.72 $\pm$ 0.02
WSN (1:10) (mg kg ⁻¹)	8.248 $\pm$ 0.85	2574 $\pm$ 41
$\delta^{15}\text{N}$ (‰)	-0.45 $\pm$ 0.26	7.06 $\pm$ 0.03
Total P (mg kg ⁻¹)	1728 $\pm$ 98	4527 $\pm$ 460
C/N ratio	160 $\pm$ 4.16	18.41 $\pm$ 1.16
Water extractable nutrients (1:10) (mg kg ⁻¹)	K	4357 $\pm$ 384
	Ca	150 $\pm$ 37
	Mg	88 $\pm$ 5
	Na	364 $\pm$ 30
		4507 $\pm$ 62
Total metal(loid)s (mg kg ⁻¹)	Cd	< 5
	Cu	9.8 $\pm$ 1.1
	Mn	49.7 $\pm$ 4.8
	Pb	8.4 $\pm$ 3.0
	Zn	18.1 $\pm$ 5.0
	As	< 5
		130 $\pm$ 23
		157 $\pm$ 8
Water extractable metal(loid)s (1:10) (mg kg ⁻¹)	Cd	0.175 $\pm$ 0.001
	Cu	0.264 $\pm$ 0.025
	Mn	0.268 $\pm$ 0.079
	Pb	0.072 $\pm$ 0.004
	Zn	38.45 $\pm$ 1.04
	As	0.232 $\pm$ 0.004
		1.38 $\pm$ 0.07

Zn concentrations in the MOSW were at least ten-fold higher than in the biochar, but neither of the two amendments exceeded the European Union regulatory thresholds for total metal concentrations in organic fertilizers ($<300 \text{ mg Cu kg}^{-1}$; $<120 \text{ mg Pb kg}^{-1}$; $<800 \text{ mg Zn kg}^{-1}$) (European Union 2019).

The tailings surface was tilled in strips separated 1 m and to a depth of 20 cm (Figure SM1). A mixture of both amendments, in a ratio of 1.8 kg biochar per 1 kg of MOSW, was manually incorporated into the tilled strips at a rate of 4.4 kg m^{-1} ($\sim 44 \text{ t ha}^{-1}$), except in 6 designated control plots of $4 \text{ m} \times 3 \text{ m}$, which remained unamended. Six paired monitoring plots of $4 \text{ m} \times 3 \text{ m}$ were established within the amended strips next to the aforementioned control plots (Figure SM1). The higher biochar proportion compared to MOSW aimed to modulate the potential water-extractable metal pool provided by the MOSW. The selected biochar:MOSW ratio was based on the adjustment of previously employed ratios in similar tailing materials (e.g. Peñalver-Alcalá et al. 2021; Conesa and Párraga-Aguado 2022). The application rate of the combined amendment ($\sim 44 \text{ t ha}^{-1}$) was much lower than those employed in similar field studies carried out in tailings (e.g. Clemente et al. 2012; Kabas et al. 2012; Gil-Loaiza et al. 2016). We deliberately applied a low amendment dose in order to promote soil improvement without inducing excessive plant growth that could lead to disproportionate competitive advantages for certain species (Martínez-Oró et al. 2019). By using a conservative amendment approach, our aim was to enhance key soil functions while maintaining balanced interspecific interactions and allowing for a more equitable evaluation of species performance under semi-arid field conditions.

A selection of plant species previously reported in field surveys of the Cartagena-La Unión Mining District were planted (e.g. Párraga-Aguado et al. 2013, 2014a; Navarro-Cano et al. 2018; Oná et al. 2021). We selected different size and family plant species to promote higher biodiversity and ecological complementarity (Párraga-Aguado et al. 2014a). In addition, these plant species are classified as xerophytes by flora reviews (Ruiz de la Torre 2006) and therefore they are adapted to local semiarid conditions. The selected plant species included the shrubs *Atriplex halimus* L. (Chenopodiaceae), *Dorycnium pentaphyllum* Scop. (Fabaceae), *Maytenus senegalensis* (Lam.) Exell. (Celastraceae) and *Rhamnus lycioides*

L. (Rhamnaceae), as well as the trees *Pinus halepensis* Miller (Pinaceae) and *Tetraclinis articulata* (Vahl) Mast. (Cupressaceae). Seedlings were provided by *Viveros Muzalé* (Abanilla, Spain). Plants were arranged along the tilled strips at 50 cm intervals and planted in December 2021, each with a cardboard tree protector (Figure SM1). Each plot (both amended monitoring and unamended control plots) contained 6 individuals of each tree species and 3 individuals of each shrub species. An initial irrigation when planting and an additional one after a month were applied by addition approx. 10 L per plant. Planting was done in December to take advantage of winter and spring rains typical of these semiarid climate conditions. Some climatic data during the period of our field trial are shown in the Table SM2.

Soil and plant sampling

Two months after amendment application, a composite soil sample was collected from the 0–20 cm depth layer at each $4 \text{ m} \times 3 \text{ m}$ plot. Each composite sample was made from six randomly selected points per plot. This resulted in six replicate samples per treatment (six from unamended control plots and six from amended monitoring plots). Soil sampling after 2 months was intended to capture the chemical changes in the substrate resulting from amendment application. This time should be enough to allow the contact amendment-tailing and stabilize some soil parameters. In May 2023 (18 months after planting, Figure SM2), one individual of each plant species was randomly selected and harvested at each plot giving six individuals of each plant species per treatment (amended and unamended plots). Plant sampling at 18 months allowed to evaluate long-term effects on plant establishment, and nutrient uptake, ensuring sufficient biomass development for meaningful analysis. The harvested plants included both belowground and aboveground parts, for further analysis. In the amended plots cumulative mortality after 18 months since planting was 0% for *P. halepensis* and *A. halimus*, 2% for *T. articulata*, 11% for *M. senegalensis*, 33% for *D. pentaphyllum* and 11% for *R. lycioides*; in unamended plots, mortality rates were 0% for *P. halepensis* and *R. lycioides*, 2% for *T. articulata*, 5% for *A. halimus*, 17% for *M. senegalensis*, 50% for *D. pentaphyllum*. For the species *D. pentaphyllum* the unamended treatment only provided individuals

in four plots. Although there are no established criteria regarding an acceptable failure rate for afforestation projects in semiarid areas (as this depends on technical and economic considerations), a 50% plant survival rate may be considered a valid threshold for determining the suitability of our study.

Soil analytical procedures

Soil samples were air-dried in the laboratory and subsequently, sieved through a 2 mm mesh. Soil/water (1:2.5) ratio extracts were obtained after two hours of shaking process on a rotatory shaker. Previous studies have demonstrated the relevance of water-soluble metal(loid) extraction in risk assessment contexts because it provides an operationally simple and reproducible approach that reflects the mobility of soluble elements under aqueous conditions (Li et al. 2023). The extracts were filtered using syringe filters (pore size 0.45 μm) and analysed for pH, electrical conductivity (EC), and water extractable macronutrients, including K^+ (W-K), Ca^{2+} (W-Ca), Mg^{2+} (W-Mg) and Na^+ (W-Na), using a Metrohm 861 Ion Chromatographer (Metrohm AG, Switzerland). Additionally, water-soluble organic carbon (WSOC) and water-soluble nitrogen (WSN) were determined using a TOC-VCSH Shimadzu (Shimadzu corporation, Japan), while water extractable arsenic (W-As) and metals (W-Cd, W-Cu, W-Mn, W-Pb, W-Zn) were measured using an ICP-MS Agilent 7500 A (Agilent Technologies, Inc., USA). These water extractable metal(loid) concentrations were intended to characterise the water-soluble and potentially leachable fractions—not the phytoavailable pools (Li et al. 2023). Aliquots of each tailings sample were finely ground with an agate mortar for the determination of total organic carbon (TOC) and total nitrogen (TN) using an elemental analyzer multi N/C 3100 analyzer (Inycom-Nunsys, Spain).

Soil $\delta^{15}\text{N}$ analyses were done using a Flash IRMS analyzer, coupled via a Conflo IV interface to a Delta V Advantage isotope ratio mass spectrometer (ThermoScientific, USA) at the *Unidade de Técnicas Instrumentais de Análise* (UTIA) of the *Universidade da Coruña*. The $\delta^{15}\text{N}$ results were expressed in ‰ relative to atmospheric air. In each analytical sequence, the following standards were used as secondary standards for $\delta^{15}\text{N}$: USGS 40 (−4.52‰), USGS 41a (+47.55‰), IAEA-N-1 (+0.4‰), IAEA-N-2 (+20.3‰), and USGS-25 (−30.4‰).

Plant analytical procedures

The harvested plants were separated into roots (belowground biomass) and shoots (aboveground biomass) and then they were weighed separately. Plant rhizospheres were unearth carefully using hoes. Then they were placed on a plastic surface and roots were manually collected. Leaves (needles in the case of *P. halepensis* and squamiform leaves for *T. articulata*) were detached from the shoots, rinsed with deionised water, oven dried at 65°C for 72 h, and then ground for analysis. An ~0.5 g aliquot of each ground foliar sample was digested using microwave assisted acid digestion. The acid combination was 3:1 HNO_3 (63%): HCl (37%), following the USEPA Method 3051a (USEPA 2007). Foliar As, Cu, Cd and Pb concentrations were determined using an ICP-MS Agilent 7900 (Agilent Technologies, Inc., USA) whereas foliar Ca, K, Mn, Mg, P, Na and Zn concentrations were measured by an ICP-OES PerkinElmer optima 8300-DV (PerkinElmer.U.S. LLC, USA). Recoveries for total elemental composition were 96–115% (certified reference ryegrass ERM®- CD281).

Stable isotope analyses of C, N and O were conducted at the *Unidade de Técnicas Instrumentais de Análise* (UTIA) of the *Universidade da Coruña*. Foliar C, $\delta^{13}\text{C}$, N and $\delta^{15}\text{N}$ analyses were measured using a Flash IRMS analyser, coupled via a Conflo IV interface to a Delta V Advantage isotope ratio mass spectrometer (ThermoScientific, USA). The $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ results were expressed in ‰ relative to atmospheric air and VPDB (Vienna Pee Dee Belemnite), respectively. In each analytical sequence, the following were used as secondary standards for $\delta^{15}\text{N}$: USGS 40 (−4.52‰), USGS 41a (+47.55‰), IAEA-N-1 (+0.4‰), IAEA-N-2 (+20.3‰), and USGS-25 (−30.4‰). For $\delta^{13}\text{C}$, the standards used were: USGS 40 (−26.39‰), USGS 41a (+36.55‰), NBS 22 (−30.031‰), and USGS 24 (−16.049‰). Foliar $\delta^{18}\text{O}$ analyses were performed using a TC/EA pyrolyser (ThermoFinnigan, USA) connected to a MAT253 isotope ratio mass spectrometer (ThermoFinnigan, USA). The final delta values are expressed relative to international standard V-SMOW (Vienna Standard Mean Ocean Water). The $\delta^{18}\text{O}$ measurements were referenced using the standards IAEA-601(+23.32‰ vs. VSMOW), IAEA-602 (+71.4‰ vs. VSMOW), USGS 34 (−27.9‰ vs. VSMOW) and USGS 35 (+57.5‰ vs. VSMOW).

Statistics

Statistical analyses were conducted using IBM SPSS Statistics 24. Comparisons of soil and plant parameters between unamended and amended plots for each plant species were performed using t-test ($p < 0.05$). Given the distinct ecophysiological characteristics of *A. halimus*, a C4 halophytic plant species, compared to the rest of plant species, which were C3 glycophytes, we evaluated that species separately from the rest. Although the experimental design included blocks, preliminary mixed-model analyses showed that the variance associated with blocks was zero or redundant. Therefore, blocks were not included as a random effect in the final analyses, and all statistical comparisons focus on the treatment effect. Multivariate Analysis of Variance (MANOVA) for the factors *Species*, *Amendment* and their interaction were carried out grouping variables according to their biological dimension: Biomass (belowground biomass and aboveground biomass), macronutrients (C, N, P, K, Ca, Mg, Na), Metal(loid) (As, Cu, Cd, Mn, Pb, Zn), stable isotopes ($\delta^{13}\text{C}$, $\delta^{15}\text{N}$, $\delta^{18}\text{O}$) and nutrient ratios (C/N, C/P and N/P). Since these factors proved to be significant (Table SM4, 6 Pillai's, $p < 0.05$) we ran general linear models (GLM) procedures to conduct analysis of variance (ANOVA) for the selected plant parameters. Comparisons of plant parameters within

each treatment (unamended and amended) were conducted using one-way ANOVA with Tukey's post hoc test ($p < 0.05$). Pearson's correlations ($p < 0.01$ and $p < 0.05$) were used to assess relationships between plant parameters. When necessary, data were log transformed to achieve normal distribution and homogeneity of variance (evaluated using Levene's test).

Results

Effects of amendments on soil properties

Amendments markedly altered several soil properties. Compared with unamended control plots, amended soils exhibited significantly higher pH, TN (~threefold increase), $\delta^{15}\text{N}$ (~1.5-fold increase), TOC (~tenfold increase) and WSOC concentrations (~twofold increase) (Table 2; $p < 0.05$). No significant differences were observed for water-extractable Ca, Mg and Na ($p > 0.05$) while a slight increase in water-extractable potassium (W-K) concentrations, undetectable in the unamended plots ($< 0.5 \text{ mg kg}^{-1}$), was recorded in the amended plots. Regarding trace metals, amendments promoted a ~fourfold increase in water-extractable Mn, but reduced cadmium and zinc concentrations by about 50% compared to the unamended control plots. Detectable levels of

Table 2 Characterisation of soil samples from unamended and amended plots

1:2.5 means data from the 1:2.5 soil:water extract. Data are average $\pm$ standard error of 6 repetitions. * between columns indicates significant differences between unamended and amended treatments (t test, $p < 0.05$). EC Electrical conductivity, TN Total nitrogen, WSN Water soluble nitrogen, TOC Total organic carbon, WSOC Water soluble organic carbon

Parameter	Unamended plots	Amended plots
pH (1:2.5)	7.11 $\pm$ 0.09*	7.45 $\pm$ 0.02
EC (1:2.5) (dS m ⁻¹)	2.49 $\pm$ 0.28	2.93 $\pm$ 0.08
TN (%)	0.025 $\pm$ 0.001*	0.073 $\pm$ 0.005
WSN (1:2.5) (mg kg ⁻¹)	1.60 $\pm$ 0.59	3.38 $\pm$ 0.58
$\delta^{15}\text{N}$ (‰)	3.83 $\pm$ 0.33*	5.52 $\pm$ 0.25
TOC (%)	0.09 $\pm$ 0.03*	0.88 $\pm$ 0.08
WSOC (1:2.5) (mg kg ⁻¹)	10.3 $\pm$ 4.4*	23.4 $\pm$ 3.4
Water extractable nutrients (1:2.5) (mg kg ⁻¹)	K	< 0.5
	Ca	1358 $\pm$ 36
	Mg	209 $\pm$ 114
	Na	207 $\pm$ 145
	As	178 $\pm$ 57
Water extractable As and metals (1:2.5) ($\mu\text{g kg}^{-1}$)	Cd	35.7 $\pm$ 6.70*
	Cu	< 10
	Mn	23.5 $\pm$ 10.7*
	Pb	< 10
	Zn	2288 $\pm$ 352*
	As	< 10
		14.3 $\pm$ 3.5

water-extractable As, Cu and Pb were also present in amended soils, whereas these remained below detection limits ($< 10 \mu\text{g kg}^{-1}$) in unamended conditions.

Plant biomass

Biomass production increased substantially in the amended plots for all species except *M. senegalensis* (belowground biomass), with gains of up to ~33-fold ($p < 0.05$; Table SM3). No visual symptoms of phytotoxicity were observed after 18 months (Figure SM2). Under unamended conditions, no significant inter-specific differences among C3 species were found in either above- or belowground biomass ($p > 0.05$). However, the C4 species, *A. halimus* exhibited more than threefold greater aboveground biomass than the rest of species. In the amended plots, *D. pentaphyllum* displayed markedly greater biomass (3- and 26-fold higher) than the other C3 plant species. Variance analyses confirmed that both *Species* and *Amendment* factors significantly influenced biomass, with *Amendment* showing a stronger effect (Table SM5; $F \sim 114$ for aboveground, $F \sim 92$ for belowground) than *Species* ($F \sim 22$ for aboveground, $F \sim 3.5$ for belowground).

Foliar ionome concentrations

Most foliar C, N, K, and Mg concentrations (Tables 3, SM6) did not differ significantly between amended and unamended conditions ($p > 0.05$). Nevertheless, P concentrations increased up to two-fold in *M. senegalensis*, *R. lycioides*, *P. halepensis* and *T. articulata* under amended conditions (Table 3). In contrast, *T. articulata* exhibited a ~70% reduction in foliar Ca and Na concentrations in the amended plots (Table SM6, $p < 0.05$). *Dorycnium pentaphyllum* consistently had the highest foliar N concentration ($\sim 21 \text{ g kg}^{-1}$) while *M. senegalensis* showed the highest foliar K concentrations ($\sim 10 \text{ g kg}^{-1}$) (Table 3). The C4 species *A. halimus* showed higher foliar Ca (~1.5-fold; $p < 0.05$) in the amended plots compared to the unamended plots (Table SM6). Analysis of variance further indicated that *Species* had a significant effect on the foliar concentrations of all nutrients, except for foliar P, where both *Species* and *Amendment* had significant effects (Tables SM7, SM8).

Nutrient ratios further differentiated species responses. *Pinus halepensis* and *T. articulata* consistently displayed higher C/N ratios ($\geq$ twofold

Table 3 Foliar elemental and isotopic concentrations

Plant Species	C %	N g kg ⁻¹	P g kg ⁻¹	K g kg ⁻¹	$\delta^{13}\text{C}$ ‰	$\delta^{18}\text{O}$ ‰	$\delta^{15}\text{N}$ ‰
No amendment (C3 plants)							
<i>D. pentaphyllum</i>	46.2±0.81a	21.3±1.05d	0.477±0.086ab	6.5±0.20a	-30.2±0.54ab	28.4±0.87ab	-2.12±0.17b
<i>M. senegalensis</i>	42.1±0.66b	15.0±1.82c	0.716±0.060b	10.8±0.95b	-30.1±0.28ab	26.1±0.62a	-1.98±0.50b
<i>R. lycioides</i>	46.7±0.77bc	13.2±1.06bc	0.403±0.054a	5.68±0.38a	-30.6±0.13a	26.8±0.57a	-0.82±1.09b
<i>P. halepensis</i>	48.8±0.43c	9.67±1.30ab	0.604±0.082ab	6.46±0.66a	-29.3±0.23b	26.8±0.38a	-5.52±0.70a
<i>T. articulata</i>	46.5±0.27bc	6.64±0.44a	0.477±0.078ab	5.93±0.75a	-29.6±0.15ab	30.0±0.33b	-2.68±0.59b
Amendment (C3 plants)							
<i>D. pentaphyllum</i>	48.2±0.83B	19.7±0.62D	0.508±0.046A	5.46±0.54A	-31.0±0.17A	26.7±0.72A	-1.28±0.53A
<i>M. senegalensis</i>	44.3±0.73A	13.3±1.16C	1.085±0.145C*	10.5±0.89B	-30.3±0.21A	26.8±0.26A	2.63±0.58C*
<i>R. lycioides</i>	47.7±0.48B	13.4±1.41C	0.651±0.095AB*	5.75±0.77A	-30.7±0.25A	26.3±0.29A	1.97±0.39BC
<i>P. halepensis</i>	48.7±0.27B	9.56±0.80B	1.173±0.059C*	7.73±0.49AB	-28.9±0.25B	26.8±0.29A	-0.99±0.31A*
<i>T. articulata</i>	46.4±0.31AB	5.89±0.50A	0.895±0.112BC*	5.81±0.30A	-29.0±0.21B	31.4±0.40B*	0.77±0.24B*
<i>A. halimus</i> (C4 plant)							
No amendment	33.2±0.86	18.6±1.6	1.015±0.100	60.2±6.88	-16.3±0.28	31.6±0.31	3.63±1.09
Amendment	33.7±0.98	17.3±1.0	1.188±0.059	53.5±4.68	-16.3±0.22	31.2±0.31	6.21±0.31

Data are average ± standard error. * and bold lettering indicate significant differences between unamended and amended treatments for each specific plant species. Different lowercase letters among plant species within the unamended treatment indicate significant differences (ANOVA, Tukey's test, $p < 0.05$); different capital letters among plant species within the amended treatment indicate significant differences (ANOVA, Tukey's test, $p < 0.05$)

greater) than the other C3 species and were unaffected by amendment (Table SM9; $p > 0.05$). Both tree species, however, exhibited ~50% lower C/P ratios under amendment (Table SM9; $p < 0.05$). Likewise, *M. senegalensis* and *T. articulata* showed ~50% lower N/P ratios in amendment conditions ($p < 0.05$), while *D. pentaphyllum* consistently exhibited the highest N/P ratios in both treatments.

Variance analysis confirmed strong *Species* effects for all foliar nutrient ratios, with *Amendment* contributing significantly to C/P and N/P (Table SM10).

Foliar metal(loid)s concentrations

In general, foliar concentrations of most metal(loid)s did not differ significantly between amended and unamended treatments and zinc consistently showed the highest levels in both treatments (Table 4). Among C3 species, *M. senegalensis* accumulated the highest concentrations of Cd (~12 mg kg⁻¹), Cu (~8–9 mg kg⁻¹) and Zn (~393 mg kg⁻¹). Amendment reduced the foliar concentration of specific elements in some species: Cu in *D. pentaphyllum*,

Pb and As in *T. articulata*, and Zn in *M. senegalensis* (~50% reduction; $p < 0.05$). Variance (Table SM11) analyses indicated that both *Species* and *Amendment* factors shaped foliar metal(loid) profiles. *Species* effects were particularly significant for Cd, Cu, Mn and Zn, while *Amendment* significantly influenced As and Pb. *Atriplex halimus* (C4) clearly stood out, with much higher Cu (~11–14 mg kg⁻¹) and Zn (~1200 mg kg⁻¹) concentrations than C3 species, independent of amendment.

Foliar isotopic concentrations

Foliar $\delta^{13}\text{C}$ values fell within the expected ranges for C3 (–32.0‰ to –22.0‰) and C4 plants (–16.6‰ to –10.4‰), with *A. halimus* (C4 plant) clearly separated from the C3 species (Table 3). Significant interspecific differences were observed, with *P. halepensis* and *T. articulata* exhibiting higher $\delta^{13}\text{C}$ than the C3 shrubs under amended conditions ($p < 0.05$). Foliar $\delta^{18}\text{O}$ also varied significantly across species, with *T. articulata* consistently showing higher values than the other C3

Table 4 Foliar metal(loid)s concentrations in mg kg⁻¹

Plant Species	Cd	Cu	Mn	Pb	Zn	As
No amendment (C3 plants)						
<i>D. pentaphyllum</i>	2.68±0.79ab	6.03±0.742cd	240±63.2b	84.8±33.2	213±59.5ab	5.9±2.00
<i>M. senegalensis</i>	11.7±3.20c	9.58±1.666d	19±34.0b	59.3±24.2	393±62.1b	4.1±1.40
<i>R. lycioides</i>	0.55±0.10a	2.45±0.315ab	88.6±21.0a	36.4±3.89	125±23.3a	2.3±0.24
<i>P. halepensis</i>	2.10±0.16b	3.81±0.341bc	307±63.0b	39.0±7.24	216±28.8ab	2.9±0.51
<i>T. articulata</i>	4.42±1.41bc	2.11±0.260a	149±19.9ab	77.3±10.5	253±49.6ab	4.1±0.58
Amendment (C3 plants)						
<i>D. pentaphyllum</i>	2.96±0.82B	3.54±0.604BC *	197±35.3AB	40.5±4.86	181±16.6	2.2±0.28
<i>M. senegalensis</i>	12.3±1.60C	8.64±1.850D	149±29.2AB	38.0±10.1	211±24.4*	2.1±0.56
<i>R. lycioides</i>	0.76±0.12A	2.19±0.165AB	112±9.82A	33.6±7.35	143±18.3	2.2±0.47
<i>P. halepensis</i>	2.15±0.15B	4.27±0.329C	245±22.9B	25.2±2.11	181±13.6	2.1±0.26
<i>T. articulata</i>	2.64±0.29B	1.95±0.067A	133±32.4A	37.1±7.06 *	179±30.6	2.3±0.48 *
<i>A. halimus</i> (C4 plant)						
No amendment	7.07±1.49	13.9±2.791	256±40.2	41.9±8.64	1155±226	2.8±1.11
Amendment	6.07±1.67	11.4±1.234	248±49.5	38.7±2.69	1240±190	2.3±0.44
Normal ranges in plants ^a	0.05–0.2	5–30	30–300	5–10	27–150	1.0–1.7
Range for phytotoxicity ^a	5–30	20–100	400–1000	30–300	100–400	5–20

^a (Kabata-Pendias 2001)

Data are average ± standard error. * and bold lettering indicate significant differences between unamended and amended treatments for each specific plant species. Different lowercase letters among plant species within the unamended treatment indicate significant differences (ANOVA, Tukey's test, $p < 0.05$); different capital letters among plant species within the amended treatment indicate significant differences (ANOVA, Tukey's test, $p < 0.05$)

plants (Table 3). Foliar $\delta^{15}\text{N}$ responded strongly to amendment: *Maytenus senegalensis*, *P. halepensis* and *T. articulata* showed significant increases in $\delta^{15}\text{N}$ under amended conditions (Table 3), while *D. pentaphyllum* displayed the lowest overall variation between treatments.

Variance analyses confirmed that the *Species* factor significantly influenced $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ (Table SM7). The *Species* $\times$ *Amendment* interaction significantly affected $\delta^{18}\text{O}$ and was marginally significant for $\delta^{13}\text{C}$ ($p=0.06$). Foliar $\delta^{15}\text{N}$ was influenced by both factors and their interaction ($p<0.05$), with *Amendment* exerting a markedly stronger effect ($F=72.96$) than *Species* ($F=14.25$).

Across all species, $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ values were positively correlated (Fig. 1). Moreover, both isotopes were negatively correlated with foliar N concentrations ($p<0.01$; Fig. 2A, B). A significant positive correlation was observed between $\delta^{13}\text{C}$ and foliar phosphorus concentrations ($p<0.01$; Fig. 2C).

Discussion

Effects of the amendment on soil fertility and water-extractable metal(loid)s

The combined application of biochar and municipal organic solid waste (MOSW) markedly improved soil conditions in the mine tailings, particularly the parameters linked to soil organic matter (TN, TOC, WSOC; Table 2), resulting in significantly greater above- and below-ground biomass in the amended plots (Table SM3). The significant increase in soil $\delta^{15}\text{N}$ values of the amended plots (Table 2) can be attributed to the isotopically enriched MOSW input (Table 1). The amendment also altered the water extractability of several metal(loid)s. The increase in water-extractable Cu, Mn, Pb and As largely reflects the higher soluble fractions in MOSW compared with biochar. However, the absolute concentrations of water-extractable metals remained very

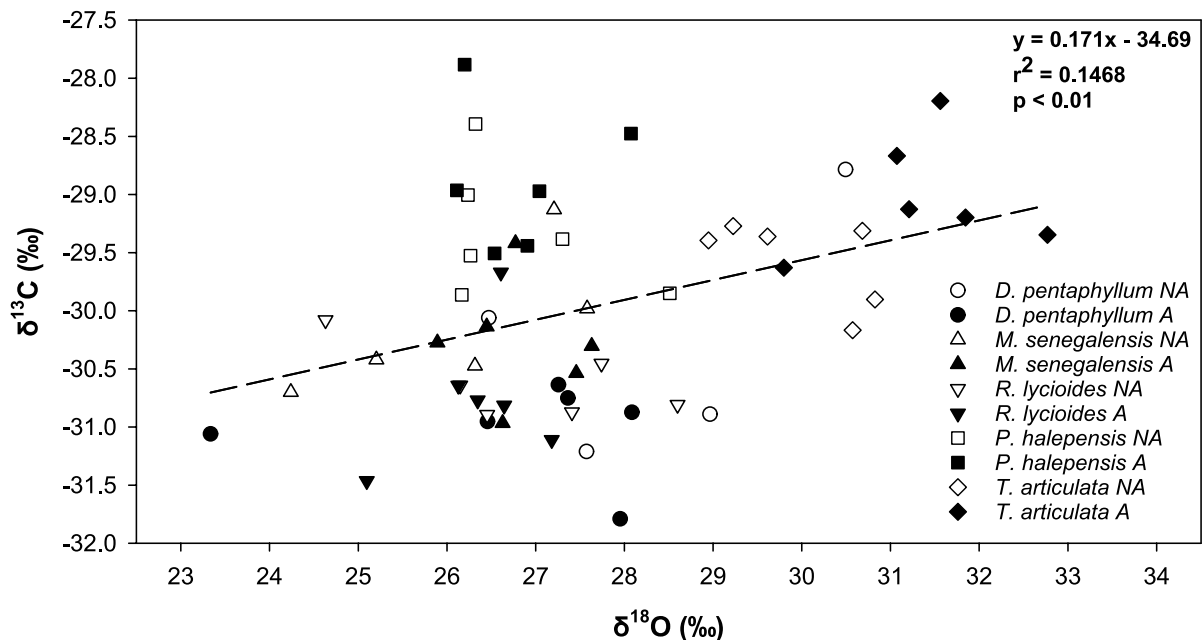


Fig. 1 Foliar $\delta^{13}\text{C}$ vs. foliar $\delta^{18}\text{O}$ for each plant species. White symbols represent plants in unamended plots (NA). Black symbols represent plants in amended plots (A). Circles are *D. pentaphyllum*; up triangles are *M. senegalensis*; down

triangles are *R. lycioides*; squares are *P. halepensis*; diamonds are *T. articulata*. Dashed line shows significant correlation across species ($p<0.01$)

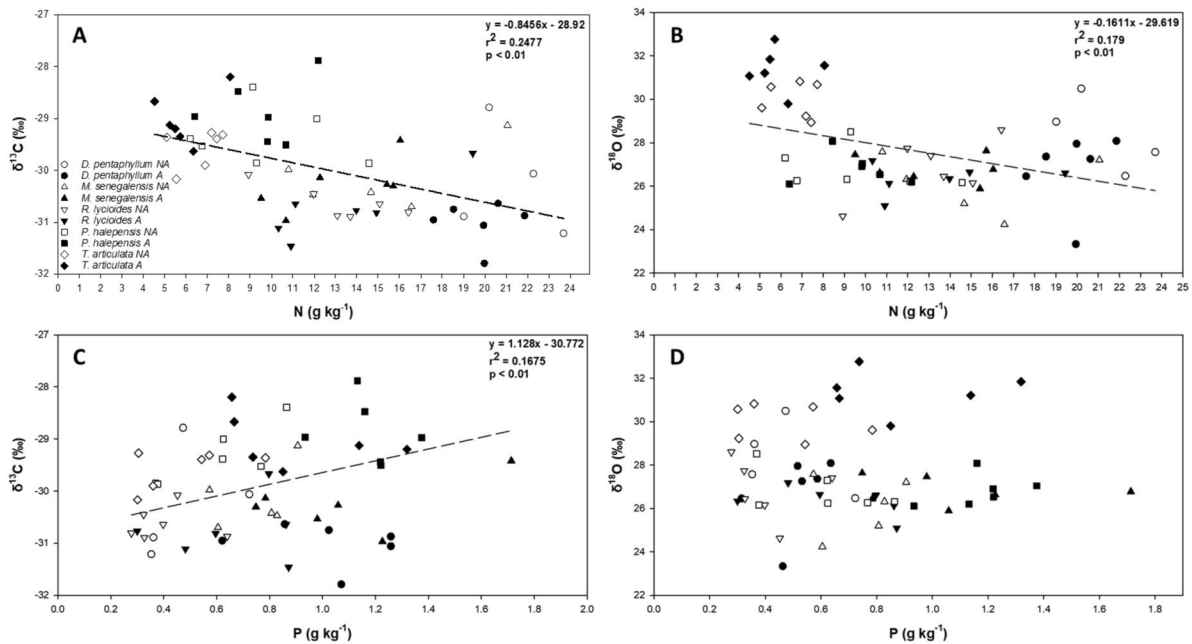


Fig. 2 Foliar $\delta^{13}\text{C}$ vs. foliar N (A), foliar $\delta^{13}\text{C}$ vs. foliar P (C), foliar $\delta^{18}\text{O}$ vs. foliar N (B) and foliar $\delta^{18}\text{O}$ vs. foliar P (D) for each plant species. White symbols represent plants in unamended plots (NA). Black symbols represent plants in

amended plots (A). Circles are *D. pentaphyllum*; up triangles are *M. senegalensis*; down triangles are *R. lycioides*; squares are *P. halepensis*; diamonds are *T. articulata*. Dashed lines show significant correlation across species ($p < 0.01$)

low (ppb–ppm range), consistent with previous findings in neutral to slightly alkaline mine tailings (e.g., Párraga-Aguado et al. 2013; Conesa and Párraga-Aguado 2022; Peñalver-Alcalá et al. 2021). The low soil:water ratio (1:2.5) used, intentionally selected to mimic near-field leaching, may also have contributed to these low values.

For Cu, its affinity for organic matter (Strobel et al. 2001) may have contributed to this trend, as indicated by the strong positive correlation between WSOC and soluble Cu ($p < 0.01$). Conversely, Zn and Cd decreased in the amended plots, consistent with their retention by biochar, as shown in previous studies (Conesa and Párraga-Aguado 2022). These contrasting responses underline the element-specific interactions between MOSW composition, biochar adsorption properties and site soil chemistry.

How did the amendment affect foliar metal(loid)s uptake?

Foliar concentrations of Cu and Mn remained within normal ranges, whereas As and Cd exceeded

background values but generally remained below phytotoxic thresholds, with the exception of Cd in *M. senegalensis* (Kabata-Pendias 2001) (Table 4). Lead levels approached or surpassed thresholds for phytotoxic risk, while Zn concentrations exceeded toxic levels in all C3 species and were particularly high in the C4 species *A. halimus*. Despite this, no visible toxicity symptoms were recorded (Figure SM2).

Foliar metal and As concentrations, regardless of amendment addition (Table 4), fell within the ranges previously reported for *D. pentaphyllum* (Párraga-Aguado et al. 2014b), *M. senegalensis* and *R. lycioides* (Oná et al. 2021), *P. halepensis* (Párraga-Aguado et al. 2014a, 2014c; Navarro-Cano et al. 2018), *T. articulata* (Párraga-Aguado et al. 2014a; Oná et al. 2021), and *A. halimus* (Clemente et al. 2012; Párraga-Aguado et al. 2014b; Navarro-Cano et al. 2018) growing in metalliferous tailings in the Cartagena-La Unión mining area.

Most species accumulated Cd and Pb above the legal thresholds (European Commission 2002) for animal feed, raising concerns for phytomanagement. Although grazing is uncommon in such sites, wild

fauna may be at risk. (Ali et al. 2013). For this reason, our strategy included fencing and seedling protectors (Figure SM1), and most species selected are unpalatable to wildlife, with the exception of *A. halimus*. The elevated metal concentrations in this halophytic species appear linked to its high accumulation of cations such as K, Ca, Mg and Na (Table SM6), which may be explained by the coupled uptake of salts and metal(loid)s (Otte 2001; Manousaki and Kalogerakis 2011; Shahrokh et al. 2022). This characteristic, together with its potential risk for wildlife, questions the suitability of *A. halimus* in phytomanagement schemes.

While metal(loid) toxicity can impair ecophysiology, the concentrations observed here are below those typically causing such effects (Guo et al. 2023). Thus, other factors, such as nutrient availability and water stress, are more likely to determine plant performance under these conditions (Conesa et al. 2024).

How did the amendment affect plant nutritional and ecophysiological status?

Given the distinct photosynthetic pathways of C3 and C4 plants, our ecophysiological discussion focuses on comparisons among the selected C3 plants: both trees *P. halepensis* and *T. articulata* and the shrubs *D. pentaphyllum*, *M. senegalensis* and *R. lycioides*.

Foliar $\delta^{13}\text{C}$ is indicative of intrinsic Water-Use Efficiency (WUE), as it reflects the balance between stomatal conductance and photosynthetic CO_2 assimilation. Higher $\delta^{13}\text{C}$ values result from reduced discrimination against ^{13}C when stomata are more closed (Paillassa et al. 2020; Querejeta et al. 2022). A significant positive correlation between foliar $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ across species (Fig. 1) further supports this interpretation. The $\delta^{18}\text{O}$ concentrations in plant organic matter primarily reflects the isotopic enrichment of leaf water during transpiration. It is particularly sensitive to stomatal regulation: tighter stomatal control reduces evaporative flux and enhances $\delta^{18}\text{O}$ enrichment (Barbour 2007). Therefore, the concurrent increase in $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ observed in some species suggests coordinated adjustments towards more conservative water-use strategies.

The interpretation of the $\delta^{18}\text{O}$ values for each plant species between treatments was based on differences in the stomatal conductance control rather than variations in the $\delta^{18}\text{O}$ of shallow water sources, as root

depths were similar (0–20 cm) among the plants studied. Differences in $\delta^{18}\text{O}$ signature between co-existing plant species due to varying water source depth are more evident when comparing contrasting adult plant forms in semiarid areas, e.g. shrubs vs trees (Querejeta et al. 2006; Párraga-Aguado et al. 2014a; Prieto et al. 2018). However, field observations during harvesting (authors' personal observations, Figure SM3) confirmed that the roots of all experimental plants were confined to the upper 20 cm of soil, growing parallel to the surface along the tillage furrows. This supports the assertion that differences in $\delta^{18}\text{O}$ among plants were not attributable to variations in water uptake depth.

This previous framework helps explain the contrasting behaviour of trees and shrubs. The two tree species, *P. halepensis* and *T. articulata*, showed higher $\delta^{13}\text{C}$ values than the shrubs under amended conditions, consistent with more conservative water-use strategies (Prieto et al. 2018). In particular, *T. articulata* exhibited the highest $\delta^{18}\text{O}$ values across treatments, suggesting very tight stomatal control. These results are in line with mesocosm studies showing higher WUE and more restricted stomatal conductance in *T. articulata* compared to *P. halepensis* under similar water availability (Conesa et al. 2024). Moreover, under amended conditions, *T. articulata* presented higher $\delta^{18}\text{O}$ together with lower foliar Ca concentrations (Table SM6). Because Ca uptake largely depends on transpiration-driven mass flow (Gilliam et al. 2011; Yin et al. 2022), this pattern indicates reduced transpiration rates in the amended treatment. Such plasticity in stomatal regulation and nutrient acquisition highlights the ability of *T. articulata* to adjust to semiarid tailings, making it a strong candidate for phytomanagement interventions in these environments.

Contrastingly, the three shrub species (*D. pentaphyllum*, *M. senegalensis* and *R. lycioides*) exhibited lower $\delta^{13}\text{C}$ values than the trees, reflecting more spendthrift water-use strategies (Prieto et al. 2018). Although Amendment alone did not significantly alter $\delta^{13}\text{C}$ or $\delta^{18}\text{O}$ (Table SM7), the interaction between Species and Amendment was significant for $\delta^{18}\text{O}$ and marginally significant for $\delta^{13}\text{C}$, indicating that physiological responses depended on species identity. This may be linked to specific plant strategies to adapt water use and nutrient acquisition patterns to the presence of available nutrients in the soil (Prieto et al.

2023) and emphasises the need to evaluate ecophysiological traits when designing amendment-based phytomanagement strategies, as responses cannot be inferred solely from biomass or foliar chemistry. The lack of a consistent treatment effect across all species may be attributed to the moderate rate of amendment application and the complex interplay between species-specific traits and soil conditions under field settings.

According to Prieto et al. (2023) plant water use-strategies are tightly linked to nutrient acquisition, as stomatal conductance and transpiration fluxes influence nutrient uptake via mass flow. This is especially relevant in moist soils and for those chemicals primarily acquired through mass flow (e.g. Ca^{2+} , NO_3^-) but it may not result so determinant for dry soils or low mobility nutrients (Conesa et al. 2024). In our study, negative correlations between $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ and foliar N (Fig. 2A, B) indicate that species with conservative water-use strategies (trees) had lower foliar N, while species with rapid water-use strategies (shrubs) exhibited higher foliar N, suggesting that water availability may critically affect N uptake during early phytomanagement stages (Cramer et al. 2009).

Nitrogen dynamics provided further insights. While foliar N concentrations did not change significantly with amendment (Table 3), foliar $\delta^{15}\text{N}$ values increased in *M. senegalensis*, *P. halepensis* and *T. articulata*. $\delta^{15}\text{N}$ signatures trace the sources and transformations of N in soils: enriched values in plants from amended plots reflect the input of ^{15}N -rich organic matter from the MOSW (Table 1). Changes in foliar $\delta^{15}\text{N}$ values despite similar foliar N concentrations suggest a shift in the signature of the N source under similar N demand and supply conditions (Ruiz-Navarro et al. 2016; Martínez-Oró et al. 2019). In contrast, the leguminous *D. pentaphyllum*, showed minimal changes in $\delta^{15}\text{N}$, consistent with its ability to access atmospheric N through symbiotic fixation, which has a different $\delta^{15}\text{N}$ signature compared to soil mineral N (Wanek and Arndt 2002; Gubsch et al. 2011). *Pinus halepensis* exhibited the lowest overall $\delta^{15}\text{N}$ values, likely due to ectomycorrhizal associations that preferentially deliver ^{15}N -depleted N forms to the host (Craine et al. 2009). All these results demonstrate that $\delta^{15}\text{N}$ can disentangle plant-specific N acquisition strategies, complementing the concentration-based

analyses. Although *Species* strongly influenced foliar N concentrations, *Amendment* itself did not significantly alter them (Table SM7), despite increasing soil N availability (Table 2). In contrast, both *Species* and *Amendment* and their interaction significantly affected foliar $\delta^{15}\text{N}$, with the *Amendment* exerting the strongest effect (Table SM7). This indicates that the *Amendment* shifted the isotopic signature of N uptake, reflecting a change in N sources, but did not increase foliar N concentrations. These results suggest that N was not a limiting factor for plant growth, underscoring the importance of considering species-specific N acquisition strategies when designing phytomanagement interventions.

Phosphorus played a pivotal role in shaping isotopic and nutritional responses. Under unamended conditions, foliar P concentrations in most species fell below values considered adequate, leading to high N/P ratios (> 14–16; Table SM9) indicative of strong P limitation (Olde Venterink et al. 2003). The amendment, enriched in P (Table 1), alleviated this constraint, as reflected by significant increases in foliar P and reductions in C/P and N/P ratios. Importantly, foliar $\delta^{13}\text{C}$ correlated positively with foliar P (Fig. 2C), suggesting that improved P nutrition enhanced photosynthetic performance and that improved water-use efficiency (Zeng 2023). The trade-off was particularly evident in *P. halepensis*, where higher $\delta^{13}\text{C}$ values under amended conditions coincided with lower C/P ratios, indicating a shift from P-use efficiency to water-use efficiency as P limitation was relieved (Wright et al. 2001, 2003; Dijkstra et al. 2016). This trade-off has been shown in manipulative studies under controlled conditions, where water and nutrient inputs can be precisely modified (Conesa et al. 2024). However, demonstrating this effect under field conditions is more challenging due to the inherent variability of soil and environmental factors.

In contrast, plants in unamended plots showed lower water use efficiency (lower $\delta^{13}\text{C}$ values), likely as a strategy to promote nutrient acquisition through mass flow by enhancing transpiration. The significant effect of *Amendment* in foliar P concentrations together with the marginally significant effect of *Species* $\times$ *Amendment* in foliar $\delta^{13}\text{C}$ may reflect a plant specific nutrient-mediated physiological adjustment influencing carbon isotope

discrimination. Thus, these results may imply that differences in WUE are largely governed by species-specific traits, yet may be subtly modulated by soil conditions modified through the addition of amendments.

Conclusions

The addition of an amendment pairing biochar and municipal organic waste to a metal-enriched mine tailings pile proved effective in improving soil conditions and enhancing biomass production (both aboveground and belowground) in *Atriplex halimus*, *Dorycnium pentaphyllum*, *Rhamnus lycioides*, *Pinus halepensis*, and *Tetraclinis articulata* (after an 18-month growth period). Foliar metal concentrations were barely affected in the amended plots. The amendment mitigated foliar P deficiency status compared to the unamended plants. However, although N availability was higher in the amended treatment, this was not reflected in increased foliar N concentrations. The enhanced P uptake determined in the amended plants may have contributed to improved water-use efficiency as it was proved by the existing trade-off between water use efficiency and phosphorus use efficiency in *P. halepensis*.

The contrasting water-use and nutrient acquisition strategies between C3 trees and C3 shrubs may provide important consequences in the employment of plant species for the phytomanagement of semiarid mine tailings. Plant species exhibiting conservative water-use strategies, such as the trees *P. halepensis* and especially, *T. articulata*, may show greater resilience under prolonged drought conditions common in semiarid environments. In contrast, species with a more spendthrift water-use strategy, such as the shrubs *D. pentaphyllum*, *M. senegalensis*, and *R. lycioides*, may enhance nutrient acquisition via mass flow, thereby favouring faster growth and potential early-stage soil amelioration. This divergence in water and nutrient acquisition strategies suggests that not all species respond equally to amendments, emphasizing the need for species-specific approaches in successful phytostabilization.

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Authors contributions *Héctor M. Conesa*: Conceptualisation, Investigation, Methodology, Writing – original draft, Writing – review & editing.

José-Antonio Franco: Conceptualisation, Investigation, Writing – review & editing.

Matías Ceacero-Moreno: Investigation, Writing – review & editing.

Juan-José Martínez-Sánchez: Conceptualisation, Investigation, Writing – review & editing.

M. Nazaret González-Alcaraz: Conceptualisation, Investigation, Methodology, Writing – review & editing.

José Álvarez-Rogel: Conceptualization, Funding acquisition, Investigation, Methodology, Project administration, Supervision, Writing – original draft, Writing – review & editing.

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Data availability The datasets generated during the study are available at <https://zenodo.org/records/15075003>

Code availability No application.

Declarations

Ethics approval No application.

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