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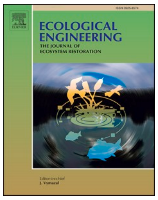

The relationships between functional and physicochemical soil parameters in metal(loid) mine tailings from Mediterranean semiarid areas support the value of spontaneous vegetation colonization for phytomanagement

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ABSTRACT

Spontaneous growth of native vegetation in abandoned metal(loid) mine tailings can be valuable for phytomanagement restoration projects. This study aimed to assess the degree to which spontaneous plant colonization of abandoned metal(loid) mine tailings from Mediterranean semiarid areas led to functional soil improvement, and to identify, if possible, a critical level indicating that this functionality was moving towards that of the vegetated soils from the surroundings. Vegetation ecological indexes, plant life forms and species functional roles, together with physicochemical and functional soils parameters, were studied in metal(loid) mine tailings abandoned ~40 years ago and surrounding forests in SE Spain. Vegetation patches showed only small differences in physicochemical parameters related to soil abiotic stress conditions (pH, salinity and metals), regardless of the vegetation. However, vegetation patches with greater species diversity and richness and presence of plants with contrasted life forms and functional traits that facilitate the growth of less stress-tolerant species showed an increase of the soil microbial functionality (higher microbial biomass C, β-glucosidase activity, bacterial metabolic activity and functional diversity). Moreover, these vegetation patches showed a functional soil status comparable to that of the forests outside the mine tailings. In this sense, the present study showed the value of preserving these vegetation patches since they may act as nucleation spots favoring positive plant-soil feedbacks that may help to accelerate the functional recovery of these degraded areas. Furthermore, strategies to promote the creation of new vegetation patches including a variety of species with contrasted life forms and functional traits should be considered in phytomanagement restoration projects for abandoned metal(loid) mine tailings.

1. Introduction

Metal mining is one of the most environmentally detrimental activities worldwide, among others, due to the high load of (hazardous) wastes disposed (Lottermoser, 2010). An important part of these wastes is often stored as muddy residues in open-air piles (mine tailings). In particular, sulfidic tailing wastes are characterized by high metal(loid) levels, extreme pH values (from acid to basic), high salinity, organic matter and nutrients deficiency, and poor physical structure, leading to low water retention capacity and aeration (Wong, 2003; Mendez and Maier, 2008). The hostile conditions offered by these wastes often hinder plant colonization and lead to low-biologically active tailing soils (i.e., soils with low capacity to shelter living organisms and, consequently, to support biological processes), which restricts their functionality and the provision of ecosystem services (Niemeyer et al., 2012; Brown et al., 2014).

Bare mine tailings are very prone to be eroded by wind and water, spreading polluted particles to surrounding areas, which pose major

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risks for environmental and human health (Gutiérrez et al., 2016; Wang et al., 2019). Conventional techniques such mine wastes removal and washing, or the use of capping materials and afforestation for *in situ* wastes isolation, are expensive and often technically difficult to implement (Mendez and Maier, 2008). Alternative options, useful for ecosystem functions restoration, include a set of cheaper and environmentally friendly alternatives such as phytomanagement by phytostabilization (Mendez and Maier, 2008; Robinson et al., 2009; Antoniadis et al., 2017; Burges et al., 2018). Phytostabilization aims to reduce pollutant fluxes to the environment (Robinson et al., 2009) to enhance ecosystem functions, and to integrate mine tailings within the surrounding landscape (Tordof et al., 2000; Mendez and Maier, 2008; Navarro-Cano et al., 2018). The implementation of this option can take advantage from the study of the native vegetation that spontaneously colonizes mine tailings and the soils beneath (i.e., passive restoration) (Prach and Hobbs, 2008; Gutiérrez et al., 2016; Prach and Tolvanen, 2016).

Under field conditions, sulfidic tailing wastes undergo hydrogeochemical stabilization due to the action of atmospheric factors such as rain, which implies a decrease in the capacity to provide extreme acidity, salinity, and soluble metals (Huang et al., 2012; You et al., 2018). Early microbial colonizers, adapted to extreme conditions, may grow in the raw materials, so contributing to the initial alteration phases (Colin et al., 2019 and references cited therein). The amelioration of the initial extreme conditions favors the colonization by adapted native plants. This is followed by a concomitant evolution of soil microorganisms and vegetation, leading to the development of ecological linkages between root zone microorganisms and aboveground plant communities (Huang et al., 2012). These vegetation patches (i.e., fertility islands) can have different shape, cover and species composition (Parraga-Aguado et al., 2013; Navarro-Cano et al., 2018), and their soils show higher contents of soil organic C and N and higher microbial enzyme activity than the adjacent surrounding barren areas (Parraga-Aguado et al., 2013, 2014; Navarro-Cano et al., 2018; Risueño et al., 2020). So, these fertility islands act as soil microbial hotspots (i.e., small soil volumes with higher microbial abundance, activity and/or diversity compared to the average soil conditions leading to faster and intensive functioning process rates) (Kuzyakov and Blagodatskaya, 2015). In fertility islands the so-called nurse species play a major role. Nurse species are stress-resistant pioneer plants with functional traits that allow them not only to colonize bare soils but also to facilitate the growth of less stress-tolerant species, hence promoting the development of fertility islands with facilitation-driven plant communities (Navarro-Cano et al., 2015). These authors identified relationships between soil bacterial community shifts and predominance of nurse plants with different functional traits (trees, shrubs, dwarf shrubs and perennial grasses).

The aim of the present study was to assess to what degree spontaneous plant colonization of abandoned metal(loid) mine tailings from semiarid Mediterranean areas led to functional soil improvement. Furthermore, the aim was also to identify, if possible, a critical level indicating that this functionality was moving towards that of the natural vegetated soils from the surrounding areas. We did not intend to establish cause-effect relationships relative to successional trajectories of vegetation and soil microorganisms, but to assess the benefits of preserving these spontaneous vegetated patches as potential nucleation spots favoring positive feedbacks between vegetation and soils for ecosystem restoration (Corbin and Holl, 2012). Moreover, the gained knowledge will help to highlight the value of restoration strategies based on promoting the creation of new vegetation patches in mine tailings phytomanagement programs. For this purpose, we carried out an evaluation of the vegetation and the analysis of a set of microbiological soil parameters in tailings vegetated patches with different physiognomy and plant composition and surrounding forests, within an abandoned mining area in southeast Spain, and related to the physicochemical soil conditions.

2. Materials and methods

2.1. Description of the study area and selection of study environments

The former mining district of La Unión-Sierra de Cartagena is located in southeast Spain (Fig. S1, Suppl. Mat.), which comprises one of the main drylands in continental Europe. The area has a Mediterranean semiarid climate (mean annual precipitation ~200–300 mm, mean annual temperature ~17 °C, and mean annual evapotranspiration rate ~850 mm). The natural vegetation is mainly constituted by xerophytic shrubs and small formations of pine trees (*Pinus halepensis*). The mining district was one of the most important metal mining centers in the Iberian Peninsula, especially during the 19th and 20th centuries, until its closure in 1991. The principal metals extracted were Fe, Pb and Zn, obtained from minerals such as carbonates, sulfides, and sulfates. At present, 89 mine tailings storages remain in the area. Most of these tailings were abandoned without any type of intervention since the mining exploitations occurred before the implementation of the current legislation on restoration and, at that time, economic activity had priority over environmental issues (Conesa et al., 2008; Conesa and Schulin, 2010).

The study was conducted in the spring of 2018 on two mine tailings ~2000 m apart built by mid-60's to store wastes from mines exploiting galena ore (IGME, 2002), and in the surrounding forest areas (Fig. S1, Suppl. Mat.). The tailings were abandoned ~40 years ago and have been partially colonized by native vegetation in a patchy structure with covers between ~20% and ~50%. In tailing 1 (total surface area ~6.4 ha) the plots were located in an area of ~0.9 ha, and in tailing 2 (total surface area ~1.5 ha) in two areas of ~0.1 and ~0.3 ha respectively (Figs. S2 and S3, Suppl. Mat.). Micro-topographical depressed areas with visual evidence of water accumulation were avoided. One environment devoid of vegetation and five different types of vegetated environments (according to their physiognomy and plant composition) were selected. Because we aimed to evaluate whether soil functionality in mine tailing's vegetation patches was comparable to that of surrounding pine forests, *P. halepensis* was present in all the vegetated study environments, but with different species under canopy (Figs. S1-S3, Suppl. Mat.). The six environments studied were: A) Four inside the mine tailings: 1. Bare soils (B); 2. Patches with small groups of *P. halepensis* trees ~2.5–5 m high growing scattered (P); 3. Patches formed by isolated *P. halepensis* trees > ~4–5 m high, with scattered shrubs and herbs under the canopy (P+S); 4. Dense patches including several *P. halepensis* trees > ~4–5 m high, with shrubs and herbs under the canopy (DP+S). B) Two outside the mine tailings: 5. Forest located next to the mine tailings with *P. halepensis* trees > ~5 m high, with shrubs and herbs under the canopy (FN); 6. Forest located away from the mine tailings (~1600–1800 m) with *P. halepensis* trees > ~5 m high, with shrubs and herbs under the canopy (FA). Based on previous experience in the study area (e.g., Parraga-Aguado et al., 2013) four plots (2 m × 2 m) were established within each study environment. We acknowledge that the proximity of the four sampling plots located in each environment could not represent the variability of the vegetation in the whole area. But the sampling design was not stablished to study and discuss the factors that affect plant colonization of mine tailings, but to study the soils beneath vegetation patches with different species composition.

2.2. Vegetation evaluation

For each study environment and plot established, the plant cover and the number of species and individuals per species were recorded ($n = 4$). Moreover, the family, life form (Table S1, Suppl. Mat.), functional group (Paula et al., 2009; Colin et al., 2019), and functional role in relation with mine tailing colonization (pioneer or nurse) (Navarro-Cano et al., 2018) were identified for each plant species. The Margalef richness index – R (Margalef, 1958), Shannon-Weaver heterogeneity index – H' (Shannon and Weaver, 1963), and Pielou evenness index – J' were calculated.

2.3. Soil sampling

Within each study environment, five soil subsamples (upper ~15 cm) were randomly collected per plot with a shovel and mixed to get a representative, composite sample per plot ($n = 4$). Aliquots of these samples were air-dried, sieved (2 mm mesh) and stored at room temperature. In parallel, aliquots of these samples were collected with sterilized spoons, placed in Falcon tubes, and taken to the laboratory on ice inside a portable cooler for water-soluble determinations and microbiological analyses. Some Falcon tubes were stored at −20 °C and the rest were frozen with liquid N and stored at −80 °C.

2.4. Physicochemical soil analyses

Soil texture was determined in the dried, sieved samples with the Bouyoucos's densimeter method (Gee and Bauder, 1986). Soil aliquots were ground in an agate mortar for measuring total $CaCO_3$, total organic C (TOC) and total N (TN) contents with an elemental analyzer (LECO CHN628) (ISO, 1995), and total metal(loid)s by X-ray fluorescence (Bruker S4 Pioneer).

Soil:water suspensions were generated from material stored at −20 °C by thawing, mixing (1:2.5 w:v) and 2 h shaking. The required water volume was adjusted based on the field soil moisture content. Suspensions were filtered through nylon membrane syringe filters (0.45 μm, WICOM) and pH and electrical conductivity (EC) measured (Crison Basic 20 pH meter and Crison Basic 30 conductivity, respectively). Water-soluble As, Cd, Cu, Mn, Pb and Zn concentrations ($Element_{H2O}$) were analyzed by ICP-Ms (Agilent 7500A, detection limit 0.002 mg L^{-1}). Dissolved organic C (DOC) and total dissolved N (TDN) concentrations were analyzed with a TOC analyzer (TOC-VCSH Shimadzu) and soluble NO_3^- and NH_4^+ concentrations by ion chromatography (Metrohm 861). Then, NO_3^- and NH_4^+ concentrations were pooled together and subtracted from the TDN to obtain the dissolved organic N (DON) fraction. Only DOC and DON data are shown. Unless otherwise noted, the concentrations are shown on a soil dry weight basis.

2.5. Microbiological soil analyses

2.5.1. Microbial biomass C and β-glucosidase activity

Both parameters were analyzed in the soil aliquots stored at −20 °C and reported on a soil dry weight basis. Microbial biomass C – MBC (indicator of soil microbial abundance) was determined following the fumigation-extraction method (Vance et al., 1987; Wu et al., 1990). Once thawed, soil samples were fumigated with $CHCl_3$, incubated for 24 h and organic C extracted with 0.5 M K_2SO_4 and measured with a TOC analyzer (TOC-VCSH Shimadzu). The activity of the β-glucosidase enzyme (an important enzyme in soil C cycle as it catalyzes the final step in cellulose degradation) was determined following the modification of Ravit et al. (2003) proposed by Reboreda and Caçador (2008). Thawed samples were incubated with *p*-nitrophenyl-*β*-D-glucopyranoside (≥99% purity, Sigma-Aldrich) at 37 °C for 60 min and the released *p*-nitrophenol (pNF) was measured by spectrophotometry at 410 nm (Thermo Fisher Scientifc Multiskan GO).

2.5.2. Community-level physiological profile

The metabolic activity and functional diversity of soil microorganisms (bacteria) was evaluated by means of the community-level physiological profile (CLPP) technique. The Biolog EcoPlate™ system was used (Biolog, Hayward, CA, USA). Each Biolog EcoPlate contains 31 different substrate wells of common C sources present in soil and a blank well, each replicated three times. The different substrates were classified within six C source groups according to Sala et al. (2006): amines and amides, amino acids, carbohydrates, carboxylic acids, phenolic acids, and polymers (Table S2, Suppl. Mat.).

Soil aliquots stored at −80 °C were used. Three grams of thawed soil were shaken (orbital shaker) with 27 mL of sterile deionized water and 20 sterile glass beads for 10 min at 200 rpm and room temperature (Samarajeewa et al., 2017). Soil:water suspensions were diluted 100-fold to attain a cellular density of approximately 10^6 cell mL^{-1} (Preston-Mafham et al., 2002). Afterwards, 100 μL of the diluted suspensions were inoculated into the Biolog EcoPlates (one plate per plot) and incubated for 192 h in the dark at 20 °C. Substrate utilization rate was determined by measuring the color development due to the reduction of a tetrazolium violet redox dye. Color intensity was recorded every 24 h by spectrophotometry at 590 nm (Biolog MicroStation System, Hayward, CA, USA). Absorbance values were corrected using the blank well at the corresponding measurement time. Substrate utilization was considered positive when the corrected absorbance value was >0.06 (Preston-Mafham et al., 2002; Classen et al., 2003; Dumontet et al., 2017; Deary et al., 2018).

The data collected for the whole incubation period (0–192 h) was used to calculate the average well-color development – AWCD (average of absorbance values for all the substrates consumed that provides a measurement of the total metabolic activity) and the substrate average well-color development – SAWCD (average of substrate consumption by C source groups) (Garland, 1997; Sofo et al., 2010). In addition, the corrected absorbance values >0.25 were used to derive the substrate richness index – S (number of different substrates consumed), Shannon-Weaver heterogeneity index – H′ (diversity of substrates consumed), and Pielou evenness index – J′ (equitability/dominance of activities across all substrates consumed) (Garland, 1997; Sofo et al., 2010; Gryta et al., 2014).

2.6. Statistical analyses

Detrended Correspondence Analysis (DCA) was applied to plant species cover in the study environments with 'CANOCO for Windows' v4.02 (ter Braak and Smilauer, 1999). The rest of the statistical analyses were performed with IBM SPSS Statistics 24 and PRIMER v6 software packages. Data were transformed when they did not fulfil the assumptions of normal distribution (Shapiro-Wilk's test) and/or homogeneity of variances (Levene's test). One-way ANOVA followed by Tukey post-hoc test was used to check for differences among the study environments. Repeated measures ANOVA (RM-ANOVA) was used to check for the evolution of AWCD over time. The factors included in the analysis were: 1) an inter-subject factor, the environment, with six levels (B, P, P+S, DP+S, FN, and FA); 2) an intra-subject factor, the time - repeated factor, with many levels as sampling times (9). Univariate F statistics using a corrector index of epsilon were applied when data did not fulfil the Mauchly's sphericity requirement (SPSS Inc, 2006). A significant effect of time indicates that AWCD evolves significantly over the incubation period. A significant effect of time x environment interaction indicates that the evolution of AWCD over the incubation period differed among the study environments. A significant effect of environment indicates that the average AWCD value differed among the study environments. When the environment was a significant factor, Bonferroni post-hoc test was used to identify differences. Pearson's correlations were performed to evaluate the relationships among all the assessed parameters.

A two-dimensional Principal Coordinate Analysis (PCoA) was used to examine the whole dataset. The PCoA was performed based on the Bray-Curtis similarity after the Biolog EcoPlate matrix was square root transformed (without restrictions – 0.06 and 0.25). A matrix gathering the carbon utilization by the microbial community (31 carbon substrates) per study environment (B, P, P+S, DP+S, FS, and FA) was used to calculate a Euclidean distance similarity matrix. This similarity matrix was simplified through the calculation of the distance among centroids matrix based on the carbon utilization per each environment. Briefly, the centroid is the arithmetic mean for a group of data points in an n-dimensional space. Thus, in this study, 4 replicates per each environment were used to construct these centroids. The soil parameters presenting good correlation with the Biolog EcoPlate matrix (Pearson's correlation coefficient ≥ 0.5) are indicated as vectors in the PCoA plot.

3. Results

3.1. Vegetation characteristics

In mine tailings bare soils (B) the only plant species found was the hemicryptophyte *Zygophyllum fabago* (Table S3, Suppl. Mat.). The individuals recorded (19 in total) were very small (<~2.5 cm), with a low plant cover (<~1%), and they died after few weeks. Therefore, these data were not considered for the evaluation of the vegetation. In the case of the vegetated environments (P, P+S, DP+S, FN, and FA) a total of 36 plant species belonging to 20 families were recorded (Table S3, Suppl. Mat.). Fewer species appeared in P (2; significantly lower) and FA (5) compared to the other vegetated environments (8); and no significant differences were observed for the number of individuals (~12–61) (Table S4, Suppl. Mat.). In the P environment only two plant families were growing (Compositae and Pinaceae), while the rest of the vegetated environments counted with 7–11 families (Table S3, Suppl. Mat.). Therophytes (annual plants) were only present in P (17%) and P+S (3%), but the other life forms were found in the rest of the vegetated environments (Tables S3 and S4, Suppl. Mat.). The number of pioneer and nurse plants were higher in P+S and DP+S (Tables S3 and S4, Suppl. Mat.). Among the pioneer species appeared the therophyte *Sonchus tenerrimus* (annual plant), hemicryptophytes such as *Leontodon taraxacoides* (dwarf shrub), and chamaephytes such as *Brachypodium retusum* (perennial grass) and *Helianthemum syriacum* (dwarf shrub) (Table S3, Suppl. Mat.). Apart from the tree species *P. halepensis*, other nurse plants present in tailing environments included the hemicryptophytes *Lygeum spartum*, *Stipa tenacissima*, *Hyparrhenia sinaica* and *Piptatherum miliaceum* (perennial grasses), the nanophanerophyte *Chamaerops humilis* (a shrub-like clumping palm), and the chamaephytes *Teucrium carthaginense*, *Helichrysum decumbens* and *Thymus hyemalis* (dwarf shrubs) (Table S3, Suppl. Mat.). The Shannon-Weaver (H′, diversity) and Margalef (R, richness) indexes were different in P+S and DP+S from the forests outside the tailings (FN and FA) (H′ ~1.8–2.6; R ~1.6–2.4), but Pielou evenness index was quite similar in all of the vegetated study environments (J′ ~0.7–0.9) (Table S4, Suppl. Mat.).

The different types of vegetated study environments (P, P+S, DP+S, FN, and FA) were segregated by the DCA ordination analysis based on species cover (27.2% of the total variance explained by the first two axes; Fig. 1A), and the results revealed the predominance of species belonging to different functional groups in each environment. FA plots were depicted in the positive side of Axis 1, characterized by shrubs and the tree species *Tetraclinis articulata*, while plots from the mine tailings (P+S and DP+S) and forest nearby (FN) were depicted in the negative side of Axis 1. Dwarf shrubs species tended to increase towards P+S plots, while perennial grasses and shrubs were more related with DP+S and FN plots. A second DCA was applied after excluding FA environment. The results (33.4% of the total variance explained by the first two axes; Fig. 1B) clearer reflected changes in predominant biological forms from P+S to FN environments.

3.2. Soil texture, pH, salinity, total $CaCO_3$ and metal(loid) concentrations

All the study environments had a sandy loam texture (~59–72% sand, ~19–23% silt, and ~6–19% clay), except the P environment which showed a loamy fine sand texture (~86% sand, ~10% silt, and ~5% clay) (data not shown). Soil pH was slightly acid in mine tailings bare soils (B) (~6.4; significantly lower than in the other environments), while the rest of the study environments showed neutral or slightly basic pH values (~7.5–7.9) (Table 1). Bare soils (B) had significantly higher salinity (EC ~6.3 dS m^{-1}) than the vegetated environments inside the mine tailings (EC ~1.3–2.3 m^{-1}) (Table 1). The P+S and DP+S environments were the ones showing the closest EC values to the forest soils outside the mine tailings (FN and FA, EC ~0.5–1.0 dS m^{-1}; no

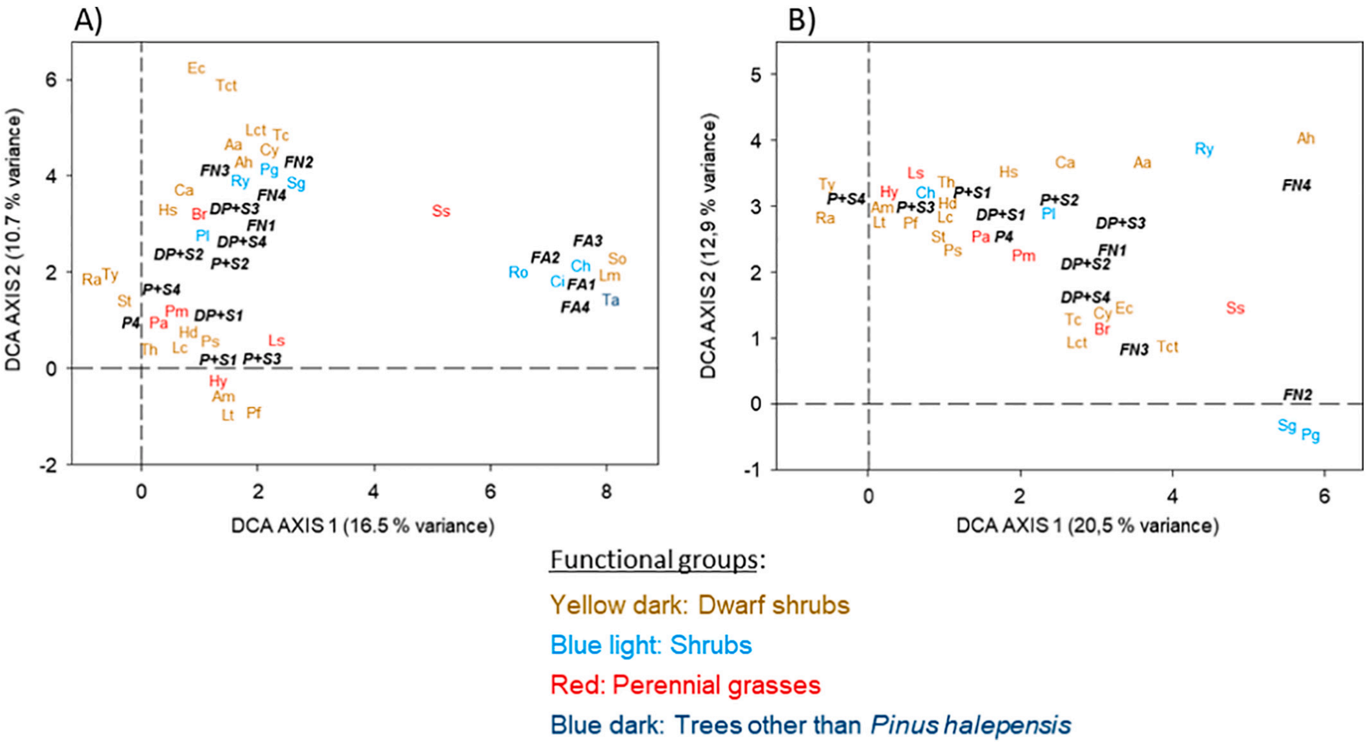

Fig. 1. Results of Detrended Correspondence Analyses (DCA) carried out with data of plant cover: A) P, P+S, DP+S, FN and FA environments; B) P, P+S, DP+S and FN environments. See Table S3 (Suppl. Mat.) for species abbreviations. See Materials and Methods section for meaning of the environments (P, P+S, DP+S, FN and FA). Three P plots with the only presence of *Pinus halepensis* do not appear since this species (common to all environments) was excluded from the analysis.

Table 1
Soil characterization of the study environments (average ± SE, n = 4).

Parameter	Environment					
	B	P	P+S	DP+S	FN	FA
pH	6.37 ± 0.35 a	7.55 ± 0.07 b	7.46 ± 0.26 b	7.93 ± 0.12 b	7.77 ± 0.15 b	7.47 ± 0.08 b
EC (dS m^{-1})	6.28 ± 1.29 e	2.31 ± 0.08 d	2.01 ± 0.28 cd	1.33 ± 0.35 bc	0.53 ± 0.03 a	1.0 ± 0.09 b
$CaCO_3$ (g kg^{-1})	0.64 ± 0.07 a	28.2 ± 1.4 b	35.9 ± 11.0 b	56.0 ± 3.8 bc	89.8 ± 10.0 cd	149 ± 35 d
As_{Total} (mg kg^{-1})	223 ± 28 b	253 ± 21 b	1251 ± 165 d	794 ± 24 c	623 ± 27 c	70 ± 11 a
Cd_{Total} (mg kg^{-1})	32.5 ± 8.3 a	56.0 ± 4.1 b	40.5 ± 6.6 ab	38.8 ± 10.2 a	27.8 ± 1.4 a	<10
Cu_{Total} (mg kg^{-1})	233 ± 4 d	277 ± 9 e	198 ± 12 c	179 ± 8 c	142 ± 3 b	49 ± 3 a
Mn_{Total} (mg kg^{-1})	1828 ± 159 a	3253 ± 59 b	9634 ± 1190 c	10,830 ± 268 c	7435 ± 234 c	1497 ± 254 a
Pb_{Total} (mg kg^{-1})	5345 ± 373 b	7961 ± 228 c	14,570 ± 804 e	10,196 ± 243 d	8649 ± 149 cd	1343 ± 243 a
Zn_{Total} (mg kg^{-1})	8596 ± 2213 b	17,858 ± 685 c	12,210 ± 963 b	13,235 ± 704 bc	9227 ± 362 b	758 ± 72 a
As_{H2O} (μg kg^{-1})	1.09 ± 0.14 a	4.22 ± 0.44 b	6.36 ± 0.82 bc	11.2 ± 2.1 c	27.6 ± 4.8 d	162 ± 17 e
Cd_{H2O} (μg kg^{-1})	1878 ± 685 c	126 ± 29 b	50.7 ± 30.1 ab	2.51 ± 0.69 a	2.51 ± 0.52 a	2.44 ± 0.21 a
Cu_{H2O} (μg kg^{-1})	13.2 ± 4.4 a	23.3 ± 4.3 ab	23.1 ± 4.6 ab	48.8 ± 3.8 bc	93.5 ± 23.9 cd	158 ± 9 d
Mn_{H2O} (μg kg^{-1})	11,502 ± 4379 b	991 ± 211 ab	6442 ± 3461 ab	638 ± 280 a	245 ± 92 a	922 ± 96 ab
Pb_{H2O} (μg kg^{-1})	1891 ± 1090 d	19.6 ± 3.2 ab	23.7 ± 10.3 ab	14.7 ± 3.3 a	99.9 ± 21.6 bc	181 ± 11 c
Zn_{H2O} (μg kg^{-1})	119,161 ± 41,229 c	3312 ± 963 b	2529 ± 1456 ab	371 ± 121 a	331 ± 72 a	617 ± 56 ab

Values are expressed on a soil dry weight basis. See Materials and Methods section for meaning of the environments (B, P, P+S, DP+S, FN and FA). EC (electrical conductivity). Metal(loid)$_{TOTAL}$ (total metal(loid) concentration). Metal(loid)$_{H2O}$ (water-soluble metal(loid) concentration). Different letters indicate significant differences among environments (one-way ANOVA followed by Tukey post-hoc test, $p < 0.05$; Table S5, Supp. Mat.).

significant differences between DP+S and FA). Total $CaCO_3$ content was significantly lower in B (~0.6 g kg^{-1}) than in the vegetated environments within the mine tailings (P, P+S and DP+S, ~28–56 g kg^{-1}) (Table 1). Of the latter, the DP+S environment was the one that showed the closest values to the forest soils outside the mine tailings (FN and FA, ~90–149 g kg^{-1}; significantly higher in FA).

In general, total metal(loid) concentrations were higher in mine tailing soils than in forest soils (Table 1). The highest concentration of As_{Total} (~1251 mg kg^{-1}) and Pb_{Total} (~14,570 mg kg^{-1}) were found in the P+S environment, of CdT_{otal} (~56 mg kg-1), Cu_{Total} (~277 mg kg^{-1}) and Zn_{Total} (~17,858 mg kg^{-1}) in the P environment, and of Mn_{Total} (~10,830 mg kg^{-1}) in the DP+S environment. Forest away (FA) soils had significantly lower As_{Total} (~70 mg kg^{-1}), Cd_{Total} (<10 mg kg^{-1}), Cu_{Total} (~49 mg kg^{-1}), Pb_{Total} (~1343 mg kg^{-1}) and Zn_{Total} (~758 mg kg^{-1}) than the rest of the study environments. Similar to total metal(loid)s, mine tailings soils generally showed higher concentrations of water-soluble metal(loid)s than forest soils (Table 1). Inside the mine tailings, bare soils (B) showed significantly higher concentrations of Cd_{H2O} (~1878 μg kg^{-1}), Mn_{H2O} (~11,502 μg kg^{-1}), Pb_{H2O} (~1891 μg kg^{-1}) and Zn_{H2O} (~119,161 μg kg^{-1}). The water-soluble concentrations of these elements tended to be lower in the DP+S environment that showed the closest values to the forest soils, especially for Cd_{H2O} (~2.5 μg kg^{-1}), Mn_{H2O} (~638 μg kg^{-1}) and Zn_{H2O} (~371 μg kg^{-1}). However, the highest water-soluble concentrations of As and Cu were found in the forest soils outside the mine tailings (FN and FA, As_{H2O} ~28–162 μg kg^{-1} and Cu_{H2O} ~94–158 μg kg^{-1}).

3.3. *Soil organic C and N contents*

Mine tailings soils had lower TOC (~3–36 fold) and TN (~3–14 fold) contents than forest soils (Fig. 2A and B). Inside the mine tailings, the B and P environments showed significantly lower TOC and TN contents (~3–4 g kg^{-1} and ~0.31–0.38 g kg^{-1}, respectively) than P+S and DP+S (TOC ~11–13 g kg^{-1} and TN ~0.66–0.71 g kg^{-1}). Outside the mine tailings, forest away (FA) soils had the highest TOC (~99 g kg^{-1}) and TN (~5 g kg^{-1}) contents. TOC:TN ratios (Fig. 2C) significantly increased within the mine tailings from bare soils (B) (~7) to the P+S and DP+S environments (~12–17). The latter ones had similar ratios to that of the forest soils outside the mine tailings (FN and FA, ~20–21).

Mine tailing soils showed lower DOC (~2–153 fold) and DON (~4–15 fold) concentrations than forest soils (Fig. 2D and E). Within the mine tailings, there was a progressive and significant increase of DOC concentrations from B (~8 mg kg^{-1}) to P (~50 mg kg^{-1}), P+S (~73 mg kg^{-1}) and DP+S (~165 mg kg^{-1}) (Fig. 2D). The concentrations of DON were very low in the B and P environments (<1.5 mg kg^{-1}), while increased in P+S and DP+S (~1.2–5.2 mg kg^{-1}) (Fig. 2E). For both DOC and DON, the DP+S environment showed concentrations closest to the forest soils outside the mine tailings, mainly to FN (DOC ~371 mg kg^{-1} and DON ~18 mg kg^{-1}; no significant differences). Outside the tailings, forest away (FA) soils had the highest concentrations (DOC ~1223 g kg^{-1} and DON ~61 g kg^{-1}; significant). In relation to the DOC:DON ratios (Fig. 2F), the mine tailings soils showed higher ratios (~89 in P+S and ~38 in DP+S) than the forest soils (~20–22).

3.4. *Soil microbial biomass C and β-glucosidase activity*

Mine tailings soils generally showed lower MBC and β-glucosidase activity than forest soils (Fig. 3). Inside the mine tailings, a progressive and significant increase of MBC occurred from B (~12 mg C kg^{-1}) to P (~132 mg C kg^{-1}), P+S (~263 mg C kg^{-1}) and DP+S (~292 mg C kg^{-1}) (Fig. 3A). Outside the mine tailings, the FA environment showed the highest MBC content (~1489 mg C kg^{-1}; significant). Similarly, bare soils (B) had much lower β-glucosidase activity than the vegetated environments inside the mine tailings (Fig. 3B). Among the latter ones, the β-glucosidase activity increased from P (~0.38 μmol pNF g^{-1} dry soil h^{-1}) to P+S and DP+S (~1.02–1.50 μmol pNF g^{-1} dry soil h^{-1}). In this case, the P+S environment showed the closest β-glucosidase activity values to the forest soils outside the mine tailings (FN and FA, ~2.10–2.29 μmol pNF g^{-1} dry soil h^{-1}).

3.5. *Soil community-level physiological profile*

Average well-color development (AWCD) showed a 24 h delay before metabolic reactions began to take place (Fig. 4). After that, AWCD tended to increase with time in most of the study environments (significant effect of time, RM-ANOVA) and reached the highest values upon 168–192 h of incubation. This was not the case of mine tailing bare soils (B) that showed a very low consumption of carbon substrates throughout the incubation period (absorbance values at 590 nm <0.06; significant effect of environment and time x environment interaction, RM-ANOVA). Within the vegetated mine tailing soils, AWCD progressively increased from P (from ~0.06 at 48 h to ~0.48 at 192 h) to P+S

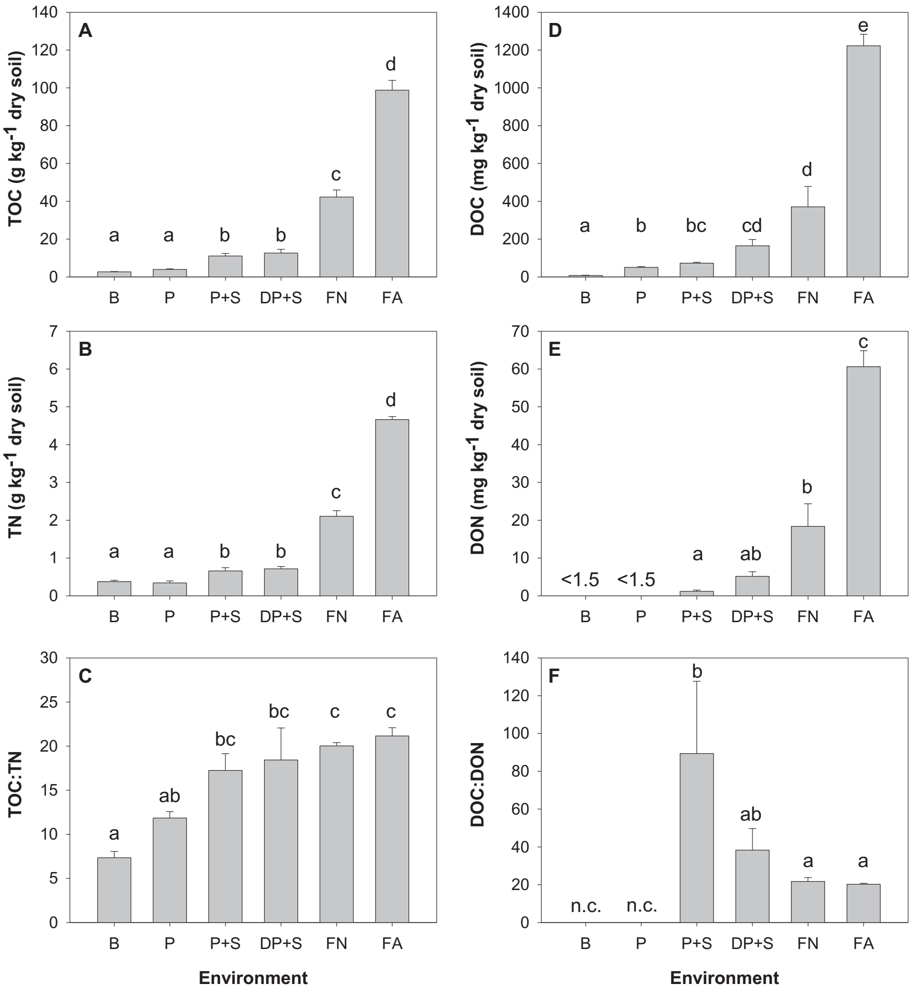

Fig. 2. Carbon and nitrogen contents in the study environments. Columns represent average values and bars on columns standard error ($n = 4$). Values are expressed on a soil dry weight basis. TOC (total organic carbon). TN (total nitrogen). DOC (dissolved organic carbon). DON (dissolved organic nitrogen). Different letters indicate significant differences among environments (one-way ANOVA followed by Tukey post-hoc test, $p < 0.05$). n.c. (not calculated). See Materials and Methods section for meaning of the environments (B, P, P+S, DP+S, FN and FA).

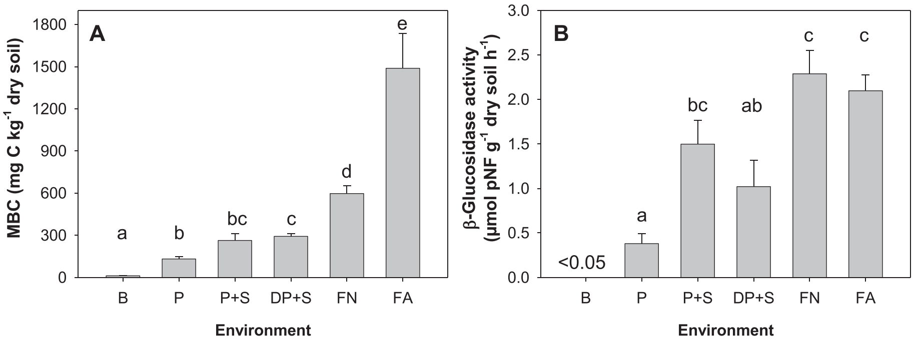

Fig. 3. Microbial biomass carbon (MBC) and β-glucosidase activity in the study environments. Columns represent average values and bars on columns standard error (n = 4). Values are expressed on a soil dry weight basis. See Materials and Methods section for meaning of the environments (B, P, P+S, DP+S, FN and FA). Different letters indicate significant differences among environments (one-way ANOVA followed by Tukey post-hoc test, $p < 0.05$).

(from ~0.09 at 48 h to ~0.70 at 192 h) and DP+S (from ~0.12 at 48 h to ~0.93 at 192 h). The DP+S environment showed the closest AWCD values to the forest soils outside the mine tailings (FN and FA, ~0.19–0.33 at 48 h to ~0.88–1.14 at 192 h). From all the incubation times registered, the greatest differences observed among the study environments were found at 144 h (Fig. 4). Therefore, this incubation time was selected to show the remaining parameters derived from the CLPP analysis (Li et al., 2016).

No significant differences were found for SAWCD among the vegetated study environments (Fig. 5), which indicates that they showed similar patterns in terms of consumption of C source groups. In the mine tailings soils the most consumed substrates were polymers (~26–30% in P and DP+S) and amino acids (~23% in P+S), while in the forest soils polymers (~23% in FN) and carbohydrates (~23% in FA). In all the cases, amines/amides and phenolic acids were the least consumed substrates (~6–13%). The SAWCD was not calculated for mine tailings bare soils (B) due to the low absorbance values registered. Despite the similar SAWCD values for the vegetated study environments, significant differences were observed for the consumption of some specific substrates (L-asparagine, *L*-phenylalanine, α-D-lactose, β-methyl-D-

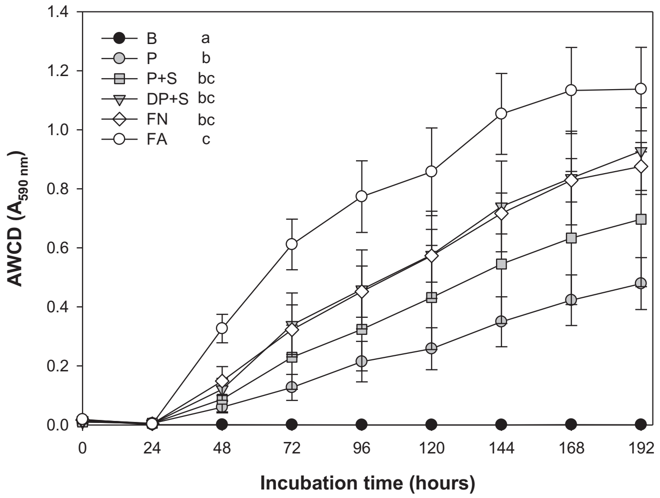

Fig. 4. Average well-color development (AWCD) at 590 nm in the study environments at the different incubation times (average ± SE, n = 4). See Materials and Methods section for meaning of the environments (B, P, P+S, DP+S, FN and FA). Different letters indicate significant differences among environments (repeated measures ANOVA followed by Bonferroni post-hoc test, $p < 0.05$; Table S6, Supp. Mat.).

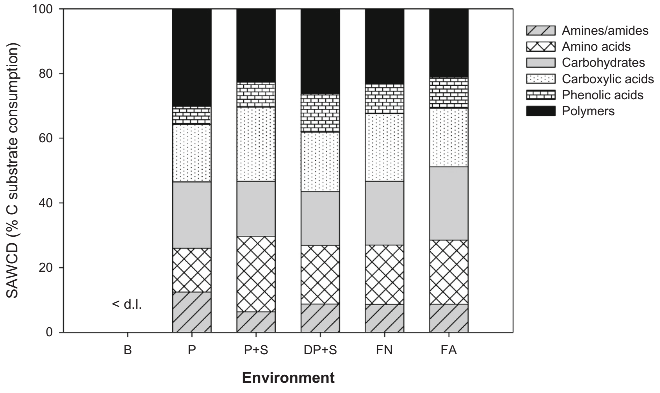

Fig. 5. Substrate average well-color development (SAWCD) in the study environments at 144 h of incubation expressed as percentage of substrate consumption (n = 4). See Materials and Methods section for meaning of the environments (B, P, P+S, DP+S, FN and FA). d.l. (detection limit: absorbance values at 590 nm <0.06).

glucoside, D-mannitol, D,L-α-glycerol phosphate, *N*-acetyl-D-glucosamine, and glycogen) (Table S7, Suppl. Mat.). For these specific substrates, lower consumption was found in the mine tailings soils compared to the forest soils. Inside the mine tailings, the P+S and DP+S environments were those that showed the closest substrate consumption values to the forest soils.

The ecological diversity indices calculated were generally lower in the mine tailings soils than in the forest soils (Table 2), although the differences were not so marked as for the other parameters derived from the CLPP analysis. Inside the mine tailings, all the indices increased from P to P+S and DP+S (S: from ~19 to ~21–24; H′: from ~2.6 to ~2.8–3.0; J’: from ~0.91 to ~0.93–0.94). No significant differences were observed among the P+S, DP+S, FN, and FA environments for S and J’. As for H′, no significant differences were observed among all the study environments.

3.6. Principal coordinate analysis

The results of the PCoA analysis (79.7% of the total variance explained by the first two axes) showed the spatial distribution of each study environment in relation with the metabolic profiling of the soil bacterial communities (based on the combined C substrate utilization values) (Fig. 6A). The primary axis (PCoA 1, total variance explained 68.8%) depicted bare soils (B) at the positive side, as the most acidic and saline sites and with the highest concentrations of Cd_{H2O}, Mn_{H2O}, Pb_{H2O} and Zn_{H2O}. The secondary axis (PCoA 2, total variance explained 10.9%) affected vegetated environments. The P environment was depicted on the positive side while the forest away (FA) was highlighted on the negative side as the sites with the greatest values of total $CaCO_3$, TOC, TN, DOC, DON, MBC and β-glucosidase activity, but also with the highest As_{H2O} and Cu_{H2O} concentrations. To gain more insight about the differences among the vegetated study environments, a second PCoA was performed excluding mine tailings bare soils (B) (Fig. 6B). The results (72.2% of the total variance explained by the first two axes) separated FA environment on the negative side of the primary axis, again as the sites with better soil physicochemical and microbiological conditions, opposite to P and P+S environments, which were depicted on the positive side with higher total metal(loid)s and Cd_{H2O} concentrations (Fig. 6B). The DP+S and FN environments were clustered on the negative side of the primary axis, closer to FA than to P and P+S.

Table 2
Substrate richness (S), Shannon-weaver index (H′) and Pielou index (J′) in the study environments at 144 h of incubation (average ± SE, n = 4).

Index	Environment					
	B	P	P+S	DP+S	FN	FA
Substrate richness (S)	<d.l.	19.00 ± 3.44 a	20.50 ± 3.80 ab	23.75 ± 2.17 ab	26.00 ± 0.82 ab	28.75 ± 0.63 b
Shannon-Weaver (H′)	<d.l.	2.62 ± 0.22 a	2.76 ± 0.20 a	2.95 ± 0.09 a	3.05 ± 0.05 a	3.20 ± 0.04 a
Pielou (J′)	<d.l.	0.91 ± 0.01 a	0.93 ± 0.01 ab	0.94 ± 0.00 ab	0.94 ± 0.01 ab	0.95 ± 0.01 b

See Materials and Methods section for meaning of the environments (B, P, P+S, DP+S, FN and FA). Different letters indicate significant differences among environments (one-way ANOVA followed by Tukey post-hoc test, $p < 0.05$; Table S5, Supp. Mat.). d.l. (detection limit: absorbance values at 590 nm <0.06).

4. Discussion

We evaluated the relationships between physicochemical and functional soil parameters and plants in vegetation patches of metal(loid) mine tailings abandoned ~40 years ago, relative to forest areas outside the tailings. It is assumed that microclimatic/microtopographic conditions in all the tailing study sites, and vegetation cover close to each tailing, could be non-identical, and this could influence biota colonization (plants and soil microorganisms). However, it is reasonable to think that the current situation, after ~40 years, represents a realistic picture of the effects of spontaneous plant colonization and the concomitant changes in soil functionality. The results indicated that metal(loid) mine tailings soils may reach a functional state comparable to that of the surrounding forests along with spontaneous plant colonization. This shows the value of preserving spontaneously formed vegetation patches when implementing restoration programs in areas degraded by mining activities, as valuable nucleation spots not only for vegetation but also for soil microbiota. Even more, the creation of new vegetation patches by favoring the establishment of nurse species could be a suitable strategy to trigger soil functional restoration. This could be implemented through the placement of local woody and organic debris, to ameliorate the extreme conditions of these environments, as suggested by other authors (Oreja et al., 2020).

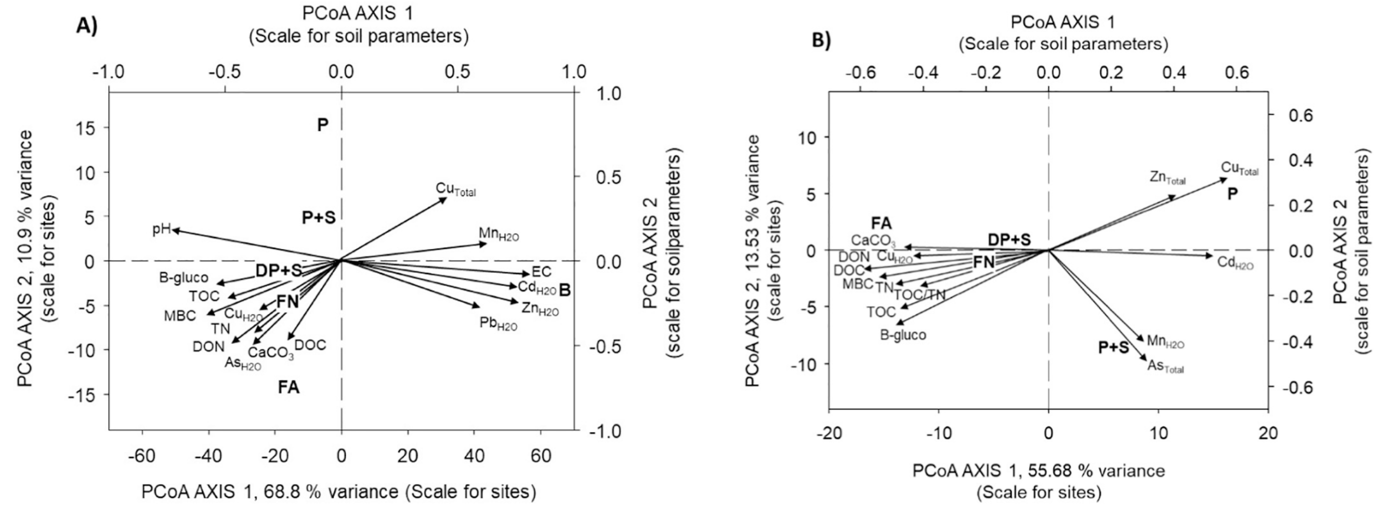

Fig. 6. Results of the two-dimensional Principal Component Analyses (PCoA). Centroids for all the study environments (A) and vegetated environments (B) are represented. Vectors imposed for those soil parameters with Pearson's correlation coefficient ≥ 0.5. See Materials and Methods section for meaning of the environments (B, P, P+S, DP+S, FN and FA). EC (electrical conductivity). Metal(loid)$_{Total}$ (total metal(loid) concentration). Metal(loid)H_2O (water soluble metal(loid) concentration). TOC (total organic carbon). TN (total nitrogen). DOC (dissolved organic carbon). DON (dissolved organic nitrogen). MBC (microbial biomass carbon).

4.1. Soil physicochemical conditions and vegetation in the metal(loid) mine tailings

High levels of total metal(loid)s were found throughout mine tailings in the study area, without a consistent pattern of decreasing concentrations from barren to vegetation patches or among these patches (Table 1). Compared to bare soils (B), vegetation patches (P, P+S, and DP+S) showed better conditions in terms of higher pH and $CaCO_3$ and lower salinity (EC) and water-soluble metals (Table 1). Higher soil pH in vegetation patches led to decreasing Cd_{H2O}, Mn_{H2O}, Pb_{H2O} and Zn_{H2O} concentrations (negative correlation; $r \leq -0.608$, $p \leq 0.002$) and, consequently, to lower potential toxic effects on soil microorganisms (Giller et al., 2009; Abdu et al., 2017). Plants could also contribute to reductions in the concentrations of water-soluble metals in the underlying soils by taking up and/or immobilizing them in their rhizospheres (Antoniadis et al., 2017). Particularly, in the case of the P environment, the coarser texture (attributable to the accumulation of sand particles blown up by wind around pine trunks) (Navarro-Cano et al., 2018), could contribute to lower salinity and water-soluble metal concentrations, but also lower water availability for plants. Reduced water-soluble metal concentrations were, in general, more evident in the DP+S environment, which showed similar concentrations of Cd_{H2O}, Mn_{H2O} and Zn_{H2O} to the forest soils outside the mine tailings (Table 1). Nevertheless, despite the low total metal(loid) levels, the forest soils outside the mine tailings showed the highest concentrations of As_{H2O} and Cu_{H2O} from all the study environments (Table 1). The latter could have been related to the greater solubility of these elements with increasing labile organic C, as shown by the positive correlation with DOC ($r \leq 0.929$, $p < 0.001$), and, in the case of As_{H2O}, to the limited soil fixation with high $CaCO_3$ content (positive correlation between As_{H2O} and $CaCO_3$; $r = 0.780$, $p < 0.001$) (Simón et al., 2010, 2015; Pardo et al., 2017; Parraga-Aguado et al., 2017).

Vegetation presence on mine tailings was mainly reflected in higher levels of soil TOC, TN, DOC and DON (Fig. 2a, b, d and e). Moreover, among the vegetation patches under study, these parameters tended to increase in P+S and DP+S, which were the environments with the greatest plant diversity and richness values (Shannon-Weaver index – H′ and Margalef index – R) and the highest number of total plant families and different life forms (Table S3 and S4, Suppl. Mat.). The tendency to higher values for vegetation indexes in P+S, DP+S and even in FN than in FA can be explained because ecosystem perturbations facilitate the development of opportunistic species, so increasing diversity, while invasive plants tend to disappear in mature stages. These findings are of interest for restoration approaches that consider assemblages of species with distinct characteristics particularly suitable for the establishment of native perennial vegetation (Brown et al., 2014). In fact, significant positive correlations between H′ and R with TOC, TN and DON ($r \geq 0.596$, $p \leq 0.05$), and marginally significant between H′ and DOC ($r = 0.574$, $p = 0.083$), were found when only considering the data from the vegetation patches. In particular, the DP+S environment showed the closest conditions to the forest soils outside the mine tailings, especially for DOC and DON concentrations (Fig. 2d and e).

Similar to TOC, TN, DOC and DON, the C to N ratios (TOC:TN and DOC:DON) also showed differences between vegetation patches and barren areas (Fig. 2c and f). The tendency of higher TOC:TN ratios in P+S and DP+S than in B and P suggests a greater incorporation and stabilization of soil organic matter in vegetation patches with higher plant diversity and richness (i.e., better balance between organic matter mineralization and immobilization) (Brust, 2019), reaching similar values to those of the forest soils. This could be favored by the abundance of nurse plants in these environments (Tables S3 and S4, Suppl. Mat.). Plant species with this functional role often generate leaf litter with a low C:N ratio and, therefore, greatly contribute to increase soil fertility and bacterial abundance (Colin et al., 2019).

DOC and DON composition in forest soils is a complex issue that is controlled by several factors (Kooch et al., 2018). The DOC:DON ratio is often related to the decomposition of soil organic matter and soluble organic matter leached from senescent leaves, fine roots, and living fine root exudates (Uselman et al., 2012; Vestgarden et al., 2010; Kooch and Bayranvand, 2017). Uselman et al. (2012) reported lower DOC:DON ratios from root-derived soluble organic matter than from leaf litter. In our study, the occurrence of dense pine stands with continuous understory shrubs in the DP+S environment could have led to higher amounts of root-derived soil organic matter and lower DOC:DON rations than in the P+S environment (vegetation patches formed by isolated pines trees). Lower DOC:DON ratios could imply more easily accessible soil organic matter for microorganisms and, hence, favor C and nutrients cycling (Uselman et al., 2012). Moreover, an increase in the amount and diversity of root exudates has been related with the presence of nurse plants (Eisenhauer et al., 2017; Colin et al., 2019).

4.2. Soil functional conditions and vegetation in metal(loid) mine tailings

Vegetation not only provides organic matter and nutrients to the soil through plant debris and root exudates, but also offers active root surfaces for microorganisms to adhere to and function (Caffery and Kemp, 1990; Hinsinger et al., 2009). The latter can trigger and/or accelerate organic matter turnover and biogeochemical cycling in these degraded environments, which, in turn, helps providing a more suitable habitat to other soil-dwelling (micro)organisms (Wall et al., 2012; Morgado et al., 2018). Relief from soil abiotic stress conditions of barren metal(loid) mine tailings with spontaneous plant colonization, along with increased C and N levels, could be simultaneously accompanied by an improvement in the functional status of the soil microbial communities. Most of the soil microbiological parameters determined were positively correlated with pH, $CaCO_3$, TOC, TN, TOC:TN, DOC and DON ($r \geq 0.424$, $p \leq 0.039$), while negatively with EC, Cd_{H2O}, Mn_{H2O} and Zn_{H2O} ($r \leq -0.465$, $p \leq 0.022$). Compared to bare soils (B), higher MBC, β-glucosidase activity, overall bacterial metabolic activity (AWCD) and functional diversity (ecological diversity indices) were found in vegetation patches (Figs. 3 and 4; Table 2). Our results agree with other studies showing increasing functionality of soil microorganisms in the rhizosphere of plants spontaneously growing on metal mine tailings (Li et al., 2011) and in areas affected by metal mine wastes after the introduction of vegetation as a phytomanagement strategy (Zhang et al., 2007; Zhou et al., 2020). Higher resource availability coming from plants could have provided energy and nutrients for microbial colonizers, stimulating the transition from the autotrophic structure typically found in barren tailings to more heterotrophic communities beneath vegetated spots (Huang et al., 2012; Colin et al., 2019; Risueño et al., 2020). This could have led to more abundant microbial communities, with greater capability to consume more diverse organic substrates and, hence, functionally more diverse.

The degree of functional improvement in tailing soils seemed to be related not only to the mere presence of plants but also to the characteristics of the species growing in vegetation patches. The different environments considered showed similar plant cover, but the vegetation of P+S and DP+S had greater diversity and richness and more pioneer and nurse species than that of P (Tables S3 and S4, Suppl. Mat.). The establishment of a variety of plants with different life forms triggers a cascade of benefits on ecosystem functioning, increases resilience and stimulates positive plant-soil feedbacks and synergistic interactions between soil microbial communities and spontaneous vegetation colonizing metal(loid) mine tailings, hence promoting microbial functionality and providing longer stability to these vegetated spots (Parraga-Aguado et al., 2014; Navarro-Cano et al., 2018). Moreover, previous studies have shown concomitant successional trajectories of vegetation and soil bacterial communities in spontaneously colonized mine tailings, with trees, shrubs, and perennial grasses significantly increasing bacterial diversity, but not dwarf shrubs (Colin et al., 2019). Our work was not specifically designed to study the relationships between the functional role of plants and soil function, but the results showed that shrubs and perennial grasses were more relevant in DP+S than in P+S (Fig. 1), coinciding with a tendency of higher bacterial metabolic activity (AWCD) in the first environment (Fig. 4). In fact, DP+S, and in less extent P+S, showed a microbial functional status closer to that of the forest soils outside the mine tailings (Fig. 6). In this sense, the P+S and DP+S environments, or something in between (i.e., patches with intermediate vegetation composition), could represent a soil microbial functional threshold or tipping point within mine tailings. In a previous work focused on successional trajectories of soil bacterial communities in mine tailings, Colin et al. (2019) identified root architecture as a plant functional key trait for soil bacterial community structure during primary succession. Although it is well known that rhizosphere environment provides a better structure and resources for microorganisms (Hinsinger et al., 2009), competition with plants for resources in dense rhizospheres can negatively affect bacterial communities (Moreau et al., 2015; Colin et al., 2019). Hence, not very dense root systems could favor species coexistence in vegetated patches, which would lead to higher diversity of roots exudates and, consequently, higher microbial diversity. The greater vegetation diversity and richness in P+S and DP+S environments could facilitate microbial functionality to move towards that of the forest soils outside the mine tailings. Furthermore, the presence of more species with more favorable functional traits, such as shrubs and perennial grasses, could provide more suitable organic compounds to the soil medium, which, in turn, could favor more diverse heterotrophic microbial communities (Hartmann et al., 2009; El Moujahid et al., 2017; Sun et al., 2018; Risueño et al., 2020).

Regardless of the increasing soil microbial functionality with vegetation composition, no differences were found for the consumption pattern of the different C source groups (SAWCD) among the vegetation patches (Fig. 5). Moreover, the forest soils outside the mine tailings had similar metabolic fingerprints to the tailing vegetation patches (Fig. 5). In all the cases (P, P+S, DP+S, FN, and FA), polymers, carbohydrates and amino acids were the most widely used C groups while amines/amides and phenolic acids the least used. This overlap in the C use potential could indicate a certain degree of functional redundancy of the soil bacterial communities present in the vegetated study environments (i.e., communities capable of performing similar metabolic functions even if they differ in composition) (Louca et al., 2018; Waymouth et al., 2020), regardless of their location and/or their soil contamination status. Nevertheless, given that the methodology used in this study is culture-based and so only a small fraction of the soil microbial community is evaluated, specific studies must be done to prove this functional redundancy.

5. Conclusions

Different types of vegetation patches formed by spontaneous plant colonization in metal(loid) mine tailings under Mediterranean semiarid climate showed only small differences in physicochemical parameters related to soil abiotic stress conditions (pH, salinity, and total and water-soluble metals). However, vegetation patches with greater vegetation diversity and richness, and presence of plants with contrasted life forms and, particularly, abundance of shrubs and perennial grasses, showed an improvement in terms of soil microbial functional-related parameters (higher MBC, β-glucosidase activity, bacterial metabolic activity, and functional diversity). Furthermore, these vegetation patches showed scores of functional-related parameters comparable to that of the forest soils outside the mine tailings.

Our findings contribute to support the value of spontaneously colonized patches as valuable components of phytomanagement strategies for abandoned metal(loid) mine tailings. Vegetation patches should be preserved during restoration programs since they can act as nucleation spots not only for plant recruitment but also for biological propagules that may help to accelerate the functional recovery of these degraded environments. Management practices should consider the establishment of species with diverse life forms and functional roles, to create vegetation patches with the capacity to trigger plant and soil microbial succession, which would contribute to the functional restoration of these environments.

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.ecoleng.2021.106293.

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