

# Plant biodiversity offsets negative effects of metals and metalloids soil multi-contamination on ecosystem multifunctionality

Lorène Tosini, Manuel Cartereau, Yoann Le Bagousse-Pinguet, Isabelle Laffont-Schwob, Pascale Prudent, Anne Marie Farnet da Silva, Nicolas Montès, Yoan Labrousse, Laurent Vassalo, Hélène Folzer

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#### 51 Abstract

52 Despite increasing metals and metalloids (MM) human-driven soil contamination, how it simultaneously alters biodiversity and ecosystem functioning remains unknown. We used a 53 wide gradient of a 170-year-old MM soil multi-contamination in Mediterranean scrublands to 54 assess the effects of soil multi-contamination on multiple plant biodiversity facets, microbial 55 communities and ecosystem multifunctionality (EMF). We found an overall positive effect of 56 plant biodiversity on EMF mediated by microbial communities, and allowing to offset the 57 58 negative impacts of MM soil multi-contamination, especially on soil water holding capacity and nitrogen content. The diversity of distant plant lineages was the key facet promoting EMF 59 by enhancing microbial communities, whereas the subordinate species richness altered EMF. 60 61 By developing a holistic approach of these complex relationships between soil multicontamination, plant biodiversity, microbial communities and ecosystem functioning, our 62 results reveal the potential of plant biodiversity, and especially the diversity of evolutionary 63 distant species, to offset the alteration of ecosystem functioning by MM soil multi-64 contamination. In this worldwide decade of ecosystems restoration, our study helps to identify 65 relevant facets of plant biodiversity promoting contaminated ecosystem functioning, which is 66 crucial to guide and optimize management efforts aiming to restore ecosystems and preserve 67 human health. 68

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#### 70 Key words

Inorganic pollution, phylogenetic diversity, soil-plant-microorganism continuum, speciesdominance, soil functioning

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#### 1. Introduction

77 Human-driven metals and metalloids (MM) soil contaminations are a major threat to soil functioning by representing 34.9% of soils contaminants in the European Union, especially 78 in the Mediterranean region (Panagos et al., 2013; van Liedekerke et al., 2014; Stolte et al., 79 2016; Merino et al., 2019). MM soil contamination could alter biodiversity and ecosystem 80 functioning by impacting all the components of the soil-plant-microorganisms continuum, i.e., 81 by affecting soil composition and functioning (Greger, 2004; Petruzzelli et al., 2020), microbial 82 83 activities (Kızılkaya et al., 2004; Gülser & Erdoğan, 2008) and plant physiological processes 84 (Cheng, 2003; Yaday, 2010). Since biodiversity and ecosystem functioning are tightly coupled (e.g., Gamfeldt et al., 2008; Isbell et al., 2011), their relationships may vary along a MM soil 85 86 multi-contamination gradient (see Steudel et al., 2012 for biodiversity-ecosystem functioning relationships along environmental stress gradients). Yet, how MM soil multi-contamination 87 gradients simultaneously impact on taxonomic (species richness and their abundances within a 88 community), phylogenetic (the diversity of evolutionary lineages within a community) and 89 functional (the diversity of forms and functions within a community) facets of plant 90 91 biodiversity, and, ultimately, on the capacity of ecosystems to perform multiple functions (i.e., ecosystem multifunctionality (EMF); Manning et al., 2018) remains unknown (Fig. 1). 92 Deciphering the relationships between biodiversity and EMF under soil multi-contamination 93 could not only expand our fundamental understanding of the biodiversity-EMF relationships 94 95 but could also help to identify the relevant biodiversity facets to improve management and restoration of contaminated ecosystems (Balvanera et al., 2014; Brum et al., 2017). 96

According to the pervasive environmental filtering hypothesis (Keddy, 1992; Weiher et al., 1998), MM soil multi-contamination can act as a strong environmental filter simultaneously decreasing phylogenetic, taxonomic, and functional diversity, and ultimately resulting in the reduction or loss of ecosystem functions (Fig. 1). MM soil multi-contamination can exacerbate 101 the effects of water stress in plants (Poschenrieder & Barcelo, 1999; Rucińska-Sobkowiak, 102 2016). This could result in the selection of few plant evolutionary lineages, forms and functions tolerant to MM-induced water stress, (e.g., synthesis of osmoprotective compounds (Sharmila 103 & Pardha Saradhi, 2002), reduction of leaf size, thickness, and stomata density (Rucińska-104 Sobkowiak, 2016). However, these predictions contrast with the often-high diversity that can 105 be observed in stressful and degraded environments, resulting from the co-occurrence of stress-106 avoidant and stress-tolerant plant species (Poorter et al., 2009; Gross et al., 2013), positive 107 interactions (e.g., Butterfield & Briggs, 2011; Butterfield & Munson, 2016), or spatial / 108 temporal storage effects (Chesson, 2000; Chesson et al., 2004). This discrepancy between 109 patterns and predictions reminds the "functional paradox" of water-limited environments 110 (Maestre et al., 2021) which could exhibit a low taxonomic, but remarkable plant phenotypic 111 diversity to cope with the environmental constraints of these areas (Chesson et al., 2004; Gross 112 et al., 2013; Gross et al., 2017; Le Bagousse- Pinguet et al., 2017). Thus, testing the 113 environmental filtering hypothesis and its discrepancies on the multiple facets of plant 114 biodiversity in contaminated environments is crucial to extend our understanding of the 115 116 relationships between biodiversity and EMF, one of the key challenges for the next generation of functional ecology research (Steudel et al., 2012). 117

Different assembly processes (sensu Keddy, 1992) can shape the diversity patterns of 118 dominant plant species with high abundance within a plant community, and subordinate plant 119 120 species with low abundance within a plant community (Arnillas & Cadotte, 2019; Arnillas et al., 2021). For instance, while environmental filtering can lead to functional similarity (i.e., 121 reduced range of functional traits values) among dominant species, niche differentiation (i.e., 122 differentiated use of resources in time and space) can lead to greater divergence (i.e., wide range 123 124 of functional traits values) among subordinate species, allowing them to coexist with dominant 125 ones (Maire et al., 2012). In addition, subordinate species can better tolerate water stress than

126 dominant ones (Mariotte et al., 2017) and enhance community resistance against drought (Mariotte et al., 2013a). Finally, dominant and subordinate species can differently influence 127 ecosystem functioning (Gross et al., 2017; Le Bagousse-Pinguet et al., 2019; Le Bagousse-128 Pinguet et al., 2021; Lyons et al., 2005). Even if dominant species highly influence individual 129 functions related to biomass production, carbon, and nutrient cycling (mass ratio hypothesis; 130 Grime, 1998), subordinate species can have significant effects on EMF (richness effect; Gross 131 et al., 2017; Le Bagousse-Pinguet et al., 2019; Le Bagousse-Pinguet et al., 2021). Thereby, 132 133 deciphering the diversity patterns of dominant versus subordinate species is critical to our understanding of their respective implications on ecosystem functioning (Seabloom et al., 134 2013). 135

136 Here we evaluated whether and how the relationships between biodiversity and EMF change along a wide gradient of a 170-year-old MM soil multi-contamination in Mediterranean 137 scrubland communities. We investigated (i) how MM soil multi-contamination impacts 138 taxonomic, phylogenetic and functional facets of plant biodiversity and EMF, (ii) how these 139 three plant biodiversity facets affect EMF, either directly or mediated by soil microbial 140 141 communities and (iii) whether dominant (mass-ratio effect) versus subordinate (richness effect) plant species reveal different patterns of biodiversity-EMF relationships. We assessed these 142 relationships using structural equation modeling approaches based on an index of MM soil 143 multi-contamination combining five different MM, seven complementary indices reflecting 144 145 taxonomic, phylogenetic and functional biodiversity facets, an index of microbial activities and biomass based on four microbial functions, and an index of EMF based on five soil functions. 146 We tested the core hypothesis that the environmental filtering exerted by MM soil multi-147 contamination selects for tolerant lineages and species with similar trait values within 148 communities (i.e., reducing plant biodiversity), resulting in decreased microbial activities and 149 biomass, and organic matter, nutrients, and water contents (i.e., reducing EMF, Fig. 1). 150

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#### 152 **2.** Material and methods

#### 153 **2.1. Study area and sites**

We studied 30 scrubland communities located in the protected area of the Marseilleveyre massif (Calanques national park, Marseille, SE France), characterized by a semi-arid Mediterranean climate, calcareous, skeletal and oligotrophic soils. The studied communities correspond to young rosemary scrublands with at least one occurrence of *Rosmarinus officinalis* L., and one occurrence among four other characteristic species, i.e., *Cistus albidus* L., *Coronilla juncea* L., *Erica multiflora* L., and *Globularia alvpum* L. (Pavon et al., 2018).

From the middle of the 19<sup>th</sup> century to the beginning of the 20<sup>th</sup> century, metallurgical 160 161 and chemical industries generated massive amounts of MM-contaminated ashes in the Marseilleveyre massif (Daumalin & Raveux, 2016). MM dispersal by eolian and hydric erosion, 162 influenced by topography, resulted in a diffuse, heterogenous and persistent MM soil multi-163 contamination (Laffont-Schwob et al., 2016). Soils are mainly contaminated by toxic elements 164 as lead (Pb, from 110 to 29,412 mg.kg<sup>-1</sup>), arsenic (As, from 16 to 3,926 mg.kg<sup>-1</sup>), antimony (Sb, 165 from 4 to 1,230 mg.kg<sup>-1</sup>), and essential elements for plant development as zinc (Zn, from 77 to 166  $6,420 \text{ mg.kg}^{-1}$ ) and copper (Cu, from 15 to 52 mg.kg<sup>-1</sup>). 167

We chose to study scrubland communities, widely represented in the study area and 168 more broadly in Mediterranean areas, and especially woody species because of their perennial 169 170 and well-developed root systems subjected to MM soil multi-contamination. Thus, we first restricted the study area to areas potentially occupied by scrubland by selecting "323 171 Sclerophyllous vegetation", "333 Sparsely vegetated areas" and "332 Bare rock" land cover 172 from CORINE Land Cover 2012 database (available 173 types at: 174 https://www.data.gouv.fr/fr/datasets/corine-land-cover-occupation-des-sols-en-france/).

175 Second, using a custom GIS procedure along with spatial databases, we selected areas with

176 scrubland vegetation characterized by homogeneous abiotic conditions influencing plant and microbial communities structure and functioning: distance to the sea, elevation, aspect, and 177 slope (Heckenroth et al., 2016). The first step was to remove areas inferior to 250m to the sea 178 to avoid the influence of sea dispersal spray on plant and microbial communities, using a 250m 179 radius buffer area around the coastline provided by the Histolitt® database (available at: 180 https://www.data.gouv.fr/fr/datasets/trait-de-cote-histolitt-1/). The second step was to calculate 181 aspect and slope raster layers using a 5m resolution elevation raster layer provided by the 182 183 RGEalti® database (available at: https://geoservices.ign.fr/rgealti#telechargement5m). Then, 184 we checked for statistical associations between elevation, aspect and slope using principal components analysis and a correlation matrix. Since there was no relationship among these 185 variables (Appendix A), we reduced our selection to the range between the 25<sup>th</sup> and 75<sup>th</sup> 186 percentiles for each variable separately. As a result, our site selection encompasses sites from 187 123 m to 283 m of elevation, from 23 % to 38 % of slope and south aspect. Then, we based our 188 study sites selection on MM soil multi-contamination, using Marséco project gridded spatial 189 data at a 250 m resolution (Laffont-Schwob et al., 2016). For each grid cell, corresponding to a 190 191 measurement of Pb, As, Sb, Zn and Cu soil concentrations, a pollution load index (PLI) was calculated (Rashed, 2010; see below for calculation details) to evaluate the level of MM multi-192 contamination. Mapped MM contamination data relieved a spatially structured contamination 193 gradient from West to East. These data are not used as value for the MM multi-contamination 194 195 explanatory variable but only to support the choice of study sites localization by ensuring a representative sampling of the whole multi-contamination range. Then, we distinguished 4 196 multi-contamination levels: very high (40<PLI<216), high (10<PLI<30), intermediate 197 (5<PLI<10) and low (1<PLI<5) multi-contamination. We positioned 7 or 8 plots of 32 m<sup>2</sup> each 198 within each multi-contamination level, in different patches of scrubland vegetation with a 199 minimum distance of 70m between two plots to avoid pseudo replication (Fig. 2). The 32 m<sup>2</sup> 200

201 plot surface was estimated as the optimal surface to detect all woody species within a202 community, using the nested plot method.

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204 **2.2. Soil analysis** 

For each plot 5 soil subsamples of 200mL each, collected in the top 20cm-layer after litter removal and sieved at 2 mm, were pooled in a composite soil sample.

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#### 208 2.2.1. MM soil multi-contamination

209 Soils samples were dried at 40 °C, grounded less than 0.2 mm (RETSCH 200, agate mortar),

digested in a microwave mineralizer (Milestone Start D) using aqua regia  $(1/3 \text{ HNO}_3 + 2/3 \text{ HCl})$ 

and filtered through a 0.45  $\mu m$  cellulose ester membrane filter (ISO 11466, 1995). Then, MM

pseudo-total concentrations were determined by ICP-OES (Jobin Yvon Horiba, Spectra 2000).

213 Quality controls and accuracy were checked using standard soil reference materials (CRM 049-

214 050, from RTC-USA), with accuracies within  $100 \pm 10$  %.

By using Pb, As, Sb, Zn and Cu pseudo-total concentrations, we calculated an index of MM
soil multi-contamination, the PLI (Rashed, 2010; Table 1) for each plot:

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$$PLI = \sqrt[5]{CF_{Pb} x CF_{As} x CF_{Sb} x CF_{Zn} x CF_{Cu}} Eq. (1)$$

where CF<sub>MM</sub>=[MM]<sub>pseudo-total</sub> / [MM]<sub>local background value</sub> and [MM]<sub>local background value</sub> are 42.9, 4.9,
3.1, 66 and 7.5 mg.kg<sup>-1</sup> for Pb, As, Sb, Zn and Cu, respectively (Affholder et al., 2014).

Since calcareous Mediterranean soils are characterized by relatively high pH which can reduce MM bioavailability (Petruzelli et al., 2020; Fig. 1, H1), we measured soil pH (soil/osmosed water, 1/5, V/V; ISO 10390, 2005; Orion 2 Star Thermo Scientific SM30B), and the concentrations of potentially bioavailable MM using 0.05 M EDTA solution (pH =  $7.0 \pm$ 0.1) as extractant with a 1/10 w/V ratio of soil/EDTA solution (Quevauviller, 1998; Appendix B). Concentrations of potentially bioavailable MM were strongly correlated with MM pseudototal concentrations and PLI (Appendix B). Therefore, we chose to keep MM pseudo-total
concentrations because they allow us to calculate the index of MM soil multi-contamination
(i.e., PLI) based on MM local background pseudo-total concentrations defined by Affholder et
al. (2014). Because of their skewed distribution, PLI values used in analysis were logtransformed.

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#### 2.2.2. Ecosystem multifunctionality

We quantified EMF for each plot using five soil functions (Table 1): (i) total organic carbon (TOC), (ii) total Kjeldahl nitrogen (TKN), (iii) available phosphorus (P) contents, (iv) cation exchange capacity (CEC) and (v) water holding capacity (WHC).

236 Soil physicochemical analyses were carried out on 40 °C dried soils, excepted for the water holding capacity (WHC). Total organic carbon (TOC) content was determined by the difference 237 between (i) the total carbon content, measured by non-dispersive infrared (NDIR) spectroscopy 238 after soil combustion at 900 °C in a furnace under oxygen flow, and (ii) the inorganic carbon 239 content, measured by NDIR spectroscopy after soil acidification with phosphoric acid (40 % 240 241 H<sub>3</sub>PO<sub>4</sub>) at 80 °C under oxygen flow (AnalytiK Jena N/C 2100 S, ISO 10694, 1995). Total Kjeldahl nitrogen (TKN) content was determined by Kjeldahl method (ISO 11261, 1995): (i) 242 mineralization of the soil sample by concentrated sulfuric acid (H<sub>2</sub>SO<sub>4</sub>) during 4h at 300°C 243 (Büchi Speed Digester K-436), (ii) distillation of the obtained ammonium sulfate with a 244 245 concentrated soda solution during 7min and neutralization with an excess of sulfuric acid solution (Büchi Distillation Unit 323), (iii) dosage of the excess of sulfuric acid by indirect 246 titration with soda solution (Titroline Easy, Schott Instruments). Available phosphorus (P, 247 Olsen method, ISO 11263, 1995) and cation exchange capacity (CEC, Metson method, NF X 248 31-130, 1999) measurements were performed by the Laboratoire Développement Méditerranée 249 (COFRAC accreditation n°1-5865, Alès, France). WHC was measured by soil immersion in 250

water during 48h, followed by soil re-wiping and drying during 48 h at 90 °C. Soils were
weighed after soil re-wiping and after drying. WHC correspond to the difference between soil
mass after re-wiping and dry soil mass, divided by the dry soil mass. WHC represents the
maximum mass of water absorbed per 100 g of dry soil.

Then, we Z-scored each individual function (i.e, TOC, NTK, P, CEC and WHC):  $Z = \frac{Fij-Mean Fi}{SD Fi}$ Eq. (2)

where  $F_{ij}$  is the value of the function *i* in the community *j*, and Mean  $F_i$  and *SD*  $F_i$  are respectively the mean and the standard deviation of the function  $F_i$ , calculated for the 30 scrubland plots. Finally, we calculated an index of EMF by averaging the Z-score of individual functions for each plot.

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#### 2 2.2.3. Microbial biomass and activities

We quantified microbial biomass and activities for each plot using four microbial functions (Table 1): (i) soil basal respiration (SBR), (ii) microbial biomass (MB), (iii) average well color development (AWCD) and (iv) catabolic diversity (H<sub>cat</sub>).

SBR and substrate-induced respiration (SIR) were measured to assess global microbial 266 activity and biomass (Anderson, 2003; Anderson & Domsch, 1978). 10 g dry weight equivalent 267 of fresh soil at 30 % of the WHC were placed in 117 mL slightly open ajar glass jars and 268 incubated for 48 h at 23 °C. For SBR measurement, each sample was oxygenated for 4 min and 269 incubated for 4 h at 23 °C in tightly closed glass jars. Then, 1 mL of their atmosphere was 270 271 sampled in the headspace with a syringe and injected into a gas chromatograph (Chrompack CHROM 3 – CP 9001) to analyse CO<sub>2</sub> production by microbial oxidation of the soil organic 272 matter. SIR was estimated using a procedure from Anderson (2003). Soil samples were 273 274 amended with 450 mg of talc and 50 mg of powdered anhydrous glucose to maximize the respiration rate and incubated for 2 h at 23 °C in slightly open ajar glass jars. Then, each sample 275

276 was oxygenated for 4 min and then incubated for 2 h at 23 °C in tightly closed glass jars. 1 mL of their atmosphere was sampled in the head space with a syringe and injected into a gas 277 chromatograph to analyse CO<sub>2</sub> production by microbial oxidation of glucose. The gas 278 chromatograph was equipped with a thermal conductivity detector and a packed column 279 (Porapack). The carrier gas helium flow was regulated at 60 mL.h<sup>-1</sup>. Ambient CO<sub>2</sub> 280 concentrations were subtracted from sampled CO<sub>2</sub> concentrations and resulting values were 281 adjusted at 22 °C according to Ideal Gas Laws using a Q10 = 2. SIR was converted into 282 283 microbial biomass (MB) using the relation established by Beare et al. (1990).

284 Community level physiological profiling (CLPP) of cultivable bacterial communities, i.e., 285 their potential to oxidize different C-substrates (Appendix C), was carried out using the 286 Ecoplate microplates (Biolog, Hayward, CA, USA) according to a procedure adapted from Garland & Mills (1991). For each soil sample, 100 mL of 0.1 % sodium pyrophosphate sterile 287 solution was added to 5 g dry weight equivalent of fresh soil in a 250 mL flask and the whole 288 was shaken for 2 h at 80 rpm. The obtained soil solution was diluted and standardized at optical 289 density (OD) 595 nm=0.03 with a sterile physiological solution (NaCl 0.85 %) and 125 µL were 290 291 used to inoculate each well of the plates. After an incubation period of 5 days at 25 °C, microbial response was assessed by reading the absorbance at 590 nm using a TECAN® 292 293 spectrophotometer (Tecan Trading AG, Switzerland). For each well, the absorbance value was 294 blanked against the control well and negative absorbance values were set to zero. The minimum 295 OD for a positive well was fixed at 0.1. The intensity of microbial C-use was assessed using the AWCD index: 296

297 AWCD = 
$$\frac{\sum OD_i}{N}$$
 Eq. (3)

where ODi is the OD of the  $i^{th}$  well and N the number of wells in the plate.

299 H<sub>cat</sub> was assessed using Shannon's index:

300 
$$H_{cat} = \sum_{i=1}^{N} (p_i x \ln (p_i))$$
 Eq. (4)

where pi is the ratio between the absorbance of the  $i^{th}$  well and the sum of the absorbances of all the wells of the plate.

Then, we Z-scored each individual function. Finally, we calculated an index of microbial
biomass and activities by averaging the Z-score of individual functions for each plot.

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- 307 **2.3. Plant biodiversity facets**
- 308 2.3.1. Phylogenetic and taxonomic diversity

In each plot, the presence of each species was recorded using a 2m<sup>2</sup> quadrat which was moved 16 times over the whole 32m<sup>2</sup> plot surface. 32 woody species, all native to the study area, have been recorded in total (Appendix D). According to the PNCal's regulations, two species were not included in our study because of their conservation status which prevent sampling: *Teucrium polium* L. and *Helianthemum syriacum* (Jacq.) Dum.Cours. The relative abundance of each species in each plot was calculated by dividing the number of quadrats in which it occurs by the sum of all species occurrences by quadrat.

We computed four phylogenetic diversity indices (picante R package, mpd and mntd 316 317 functions; Kembel et al., 2010) for each plot using a phylogenetic tree (V.Phylomaker R package, phylo.maker function, "S3" method; Jin & Qian, 2019) based on woody species 318 319 occurrences: (i) weighted and (ii) non-weighted mean phylogenetic distance (respectively 320 wMPD and MPD), which account for both closely related and distant evolutionary lineages, (iii) weighted and (iv) non-weighted mean nearest taxon distance (respectively wMNTD and 321 322 MNTD), which represent the diversity of evolutionary lineages at the tips, i.e., focusing on closely related lineages. We calculated two taxonomic diversity indices (vegan R package; 323 Oksanen et al., 2019) for each plot: (i) species richness and (ii) Pielou's evenness. 324

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#### 2.3.2. Functional diversity

327 We focused on three plant functional traits describing one of the major axes of plant form and function globally (Díaz et al., 2016), and that are key determinants of functional diversity 328 and ecosystem functioning in Mediterranean scrublands (Gross et al., 2013; Valencia et al., 329 2015; Le Bagousse-Pinguet et al., 2015): Leaf Area (LA), Specific Leaf Area (SLA) and Leaf 330 Dry Matter Content (LDMC). Traits such as LA are related to light interception and water stress 331 tolerance (Westoby et al., 2002). SLA and LDMC are related to the leaf economic spectrum, 332 333 reflects the relative growth rate of plants, and are associated with plant strategies to acquire, 334 use and/or conserve resources such as light, nutrients and water (Wright et al., 2004).

335 In each plot, we sampled three mature individuals of each species at most to account for 336 intraspecific functional trait variability (Albert et al., 2011), a key biotic component involved in species interactions (Kraft et al., 2014), community assembly and dynamics (Jung et al., 337 2010; Bolnick et al., 2011; Le Bagousse-Pinguet et al., 2014, 2015), and ecosystem processes 338 (Fridley & Grime 2010). A total of 25089 leaves were sampled from 983 individuals. On each 339 individual, a branch section with mature leaves was collected. We avoided sampling of leaves 340 341 with herbivory or pathogen attack symptoms. Then, the samples were wrap in moist paper, put in closed plastic bags and stored at 4 °C before processing in the next 24 h (Pérez-Harguindeguy 342 343 et al., 2016). The number of analyzed leaves per individual depended on the species considered. Preliminary tests allowed us to determine the minimum number of leaves to be analyzed so that 344 345 the minimum LA per individual is 1 cm<sup>2</sup> (Appendix D). First, leaves were cut from the branch. We included or excluded the petiole depending on the species considered, based on the 346 functional definition of a leaf, i.e., all leaf parts which are photosynthetic and then pigmented 347 (Appendix D). Then, all the fresh leaves from a same individual were pooled, scanned, weighted 348 (fresh mass), dried at 40°C until constant mass and reweighted (dry mass). The LA of each leaf 349 350 was measured with Fiji (Schindelin et al., 2012). SLA was calculated per individual as the ratio between the LA and the dry mass of all the leaves from a same individual. LDMC was calculated as the ratio between the fresh mass and the dry mass of all the leaves from a same individual.

We computed six functional indices (*FD* R package; Laliberté et al., 2014): the communityweighted mean of (i) LA (CWM LA), (ii) SLA (CWM SLA) and (iii) LDMC (CWM LDMC), and three multi-trait indices, i.e., (iv) functional richness (FRic), and (v) weighted and (iv) nonweighted functional dispersion (respectively wFDis and FDis). Because of their skewed distributions, functional trait values used in indices calculation were log-transformed.

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#### 360 **2.4. Data analyses**

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#### 2.4.1. Conceptual model

We developed an *a priori* conceptual model of the relationships among MM soil multicontamination, biodiversity facets (phylogenetic, taxonomic, and functional diversity), microbial biomass and activities and EMF ecologically meaningful and framed from current knowledge (Fig. 1).

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### 2.4.2. Plant explanatory variables selection

From this conceptual model, we avoided redundancy between plant explanatory variables by performing Pearson's correlation. If two variables were strongly correlated (Pearson coefficient > 0.70 or < -0.70), then only one of the 2 variables was retained (Appendix E). Based on this selection criterion, several associations of plant explanatory variables could have been retained for each biodiversity facet: (i) wMNTD and MPD or MNTD and wMPD for phylogenetic diversity, (ii) richness and evenness for taxonomic diversity, (iii) CWM LA, CWM SLA and FDis (including LA and SLA) or CWM LA, CWM SLA and FRic (including LA and SLA), or CWM LA, CWM SLA, CWM LDMC, FRic and FDis (including LA, SLA
and LDMC) for functional diversity.

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#### **2.4.3. Best adequate initial model**

We tested all possible combinations of plant explanatory variables in 6 path analysis (2 379 combinations for phylogenetic diversity x 1 combination for taxonomic diversity x 3 380 combinations for functional diversity; lavaan R package; Rosseel, 2012), and kept the 381 382 combination of variables that provided the best adequate initial model according to Grace (2006) and Rosseel (2012) criteria: Chi-squared test > 0.05, Comparative Fit Index (CFI) >383 384 0.90, Root Mean Squared Error (RMSEA) < 0.10 and Standardized Root Mean Square Residual 385 (SRMR) < 0.08. Our best adequate initial model retained included the following plant explanatory variables: (i) MNTD and wMPD for phylogenetic diversity, (ii) richness and 386 evenness for taxonomic diversity, and (iii) CWM LA, CWM SLA and FDis for functional 387 diversity (Fig. 3 and Table 1). 388

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#### 2.4.4. Minimal parsimonious model

From the best adequate initial model, we performed path analyses to assess: (i) the direct 391 and indirect effects of MM soil multi-contamination on the plant biodiversity facets, on 392 microbial biomass and activities and on EMF, (ii) the relationships between biodiversity facets, 393 each of the soil individual functions (i.e., TOC, TKN, P, CEC, WHC), and ultimately EMF, 394 and (iii) whether and how the relationships between plant biodiversity and EMF differ once 395 considering either dominant or subordinate species within each community. We defined 396 dominant and subordinate species within each plant community using a relative abundance 397 threshold of 0.06, based on the median of species relative abundances in all the 30 communities 398 (0.06306) and the median of median species abundances among the 30 plant communities 399

400 (0.06660). As a result, dominant species have a relative abundance between or equal to 0.06 401 and 0.28 and subordinate species have a relative abundance between 0.007 and 0.06. The initial model was simplified by stepwise exclusion of non-significant variables, as estimated by AIC 402 (Akaike Information Criterion), until that a minimal parsimonious model was reached. Finally, 403 404 the adequacy of the minimal parsimonious model was evaluated by non-significant differences between predicted and observed covariance matrices (p of Chi-squared test > 0.05), a high CFI 405 (> 0.90), a low RMSEA (< 0.10) and a low SRMSR (< 0.08) (Grace, 2006; Rosseel, 2012; 406 407 Appendix F).

408

409 **3. Results** 

#### 410 **3.1. Effects of MM soil multi-contamination on plant biodiversity facets and EMF**

The effects of MM soil multi-contamination on plant biodiversity facets and EMF were not influenced by the reduced range of soil pH values (7.88-8.38). MM soil multi-contamination did not affect TOC (Fig. 5a), P (Fig. 5b) and CEC (Fig. 5c), but it decreased WHC (r = -0.383, Fig. 5d) and TKN (r = -0.319, Fig. 5e), resulting in a direct negative effect on EMF (r = -0.301, Fig. 4a). In contrast, we did not observe any effect of MM soil multi-contamination on plant biodiversity (neither at the whole community level, nor when segregating between dominant and subordinate species) and on microbial biomass and activities.

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#### 419 **3.2. Effects of plant biodiversity facets on EMF**

Plant biodiversity had an overall indirect positive effect on EMF (r = 0.253, Fig. 4a), TOC (r = 0.242, Fig. 5a), P (r = 0.278, Fig. 5b), CEC (r = 0.231, Fig. 5c) and WHC (r = 0.238, Fig. 5d). Of the seven plant biodiversity indices considered in the initial model (Fig. 3), three significantly influenced EMF and soil individual functions, i.e., wMPD, CWM LA and species richness. Except for TKN, the significant effects of biodiversity facets on EMF and soil 425 individual functions were always mediated through the positive effect of microbial biomass and activities on EMF (r = 0.462, Fig. 4a), TOC (r = 0.443, Fig. 5a), P (r = 0.508, Fig. 5b), CEC (r426 = 0.423, Fig. 5c) and WHC (r = 0.435, Fig. 5d). wMPD and CWM LA had indirect positive 427 effects on EMF (r = 0.291 and r = 0.165 respectively; Fig. 4a), TOC (r = 0.280 and r = 0.159428 respectively; Fig. 5a), P (r = 0.321 and r = 0.182 respectively; Fig. 5b), CEC (r = 0.267 and r =429 0.151 respectively; Fig. 5c) and WHC (r = 0.275 and r = 0.156 respectively; Fig. 5d), and 430 wMPD always showed the strongest effect. In contrast, species richness had an indirect negative 431 effect on EMF (r = -0.204, Fig. 4a), TOC (r = -0.196, Fig. 5a), P (r = -0.224, Fig. 5b), CEC (r 432 = -0.187, Fig. 5c) and WHC (r = -0.192, Fig. 5d). The negative effects of species richness on 433 434 EMF, TOC, P, CEC and WHC were compensated by the positive effects of wMPD and CWM 435 LA. The overall indirect positive effect of plant biodiversity on EMF and WHC and the direct positive effect of CWM LA on TKN (r = 0.337, Fig. 5e) partially and fully offset the direct 436 negative effects of MM soil multi-contamination, respectively. 437

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#### 439 **3.3.** Effect of plant species relative abundance on biodiversity-EMF relationships

Considering dominant and subordinate species separately highlighted different patterns of 440 biodiversity-EMF relationships. MM soil multi-contamination had a direct and negative effect 441 on EMF when focusing on dominant species (r = -0.303, Fig. 4b), while no effect occurred 442 when considering subordinate species. wMPD is the only biodiversity index which had a 443 444 significant positive effect on EMF mediated through microbial biomass and activities in both dominant (r = 0.261, Fig. 4b) and subordinate (r = 0.346, Fig. 4c) species. In dominant species, 445 the positive effect of wMPD on EMF partially offset the negative effect of MM soil multi-446 contamination on EMF (r = -0.303, Fig. 4b). In addition, species richness had a significant 447 448 negative effect on EMF mediated through microbial biomass and activities in subordinate species (r = -0.188, Fig. 4c), but no significant effect in dominant species. In subordinate 449

species, the indirect negative effect of species richness on EMF was compensated by the indirect positive effect of wMPD on EMF. Thus, plant biodiversity had an overall indirect positive effect on EMF in subordinate species (r = 0.158, Fig. 4c).

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454 4. Discussion
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We investigated how MM soil multi-contamination influences the relationships between multiple plant biodiversity facets and EMF in Mediterranean scrublands. Our results highlight that plant biodiversity offset the negative impacts of MM soil multi-contamination on EMF (Fig. 4a), underlining its potential for ecological restoration of contaminated ecosystems. We also found antagonism between the positive effects of phylogenetic and functional facets and the negative effects of taxonomic facet on ecosystem functioning, warning for the need to consider multiple biodiversity facets for the restoration of contaminated ecosystems.

MM soil multi-contamination impacted differently the components of the soil-plant-462 microorganisms continuum. On the one hand, MM soil multi-contamination altered EMF (Fig. 463 4a), and mostly soil water holding capacity (Fig. 5c) and total nitrogen content (Fig. 5d). The 464 465 observed soil alteration likely results from competition for adsorption onto soil particles between toxic MM (e.g., lead and arsenic) and nutrients, resulting in changes in soil 466 composition and functioning (Greger, 2004; Petruzzelli et al., 2020). On the other hand, MM 467 soil multi-contamination did not impact plant biodiversity and microbial biomass and activities. 468 469 Since MM and water stresses lead to similar physiological responses of plants (e.g., synthesis of osmoprotective compounds (Sharmila & Pardha Saradhi, 2002), reduction of leaves sizes, 470 thickness, and stomata density (Rucińska-Sobkowiak, 2016)), our results support the 471 environmental filtering hypothesis (Keddy, 1992; Weiher et al., 1998), i.e., the presence of 472 tolerant species to water stress preadapted to MM contaminated soils (Affholder et al., 2014; 473 Heckenroth et al., 2022; Salducci et al., 2019). Altogether our study highlights the complex 474

responses of degraded ecosystems to soil contaminations, and thus calls for the need of holistic
approaches simultaneously considering the effects of contamination on above- and
belowground biodiversity and soil functions to target the relevant components of the soil-plantmicroorganisms continuum that restoration and management efforts should focus on.

Despite having both additive and antagonistic effects, depending on the facet 479 considered, the overall effect of plant biodiversity on ecosystem functioning mediated through 480 microbial communities was positive, allowing to partially offset the negative impacts of MM 481 482 soil multi-contamination. Of the seven plant biodiversity indices under consideration (Fig. 3), 483 three indices from phylogenetic, taxonomic and functional diversity influenced EMF (Fig. 4a) and soil individual functions (Fig. 5). wMPD and CWM LA had additive positive effects on 484 485 ecosystem functioning whereas plant richness altered it. Increasing CWM LA enhance litter inputs, i.e., substrates supporting microbial activities (Lange et al., 2015; Chen et al., 2019), 486 which can improve soil functioning (Le Bagousse-Pinguet et al., 2021). Numerous studies have 487 reported that the coexistence of many species within plant communities (i.e., high plant 488 richness) allows to support several functions simultaneously (e.g., Gamfeldt et al., 2008; Isbell 489 490 et al., 2011; Maestre et al., 2012). However, our results, as other studies (e.g., Creed et al., 2009), reconsider this prevailing assumption of a positive relationship between plant richness 491 and EMF because increasing number of plant species can also increase the probability of 492 493 recruiting species which have negative effects on ecosystem functioning (selection effect; 494 Tilman et al., 1997). Our results show that considering several facets of plant biodiversity is crucial to understand the complex overall effect of biodiversity on ecosystem functioning, and 495 ultimately guide restoration efforts toward the relevant plant biodiversity facets to enhance 496 ecosystem functioning. 497

498 Phylogenetic diversity was the key facet promoting EMF at all biological levels (i.e.,499 whole community, dominant and subordinate species; Fig. 4) and individual functions (i.e.,

500 total organic carbon, available phosphorus, cation exchange capacity, and water holding capacity; respectively Fig. 5a, b, c and d) through microbial communities. Phylogenetic 501 diversity can effectively encompass hidden biological traits (Flynn et al., 2011), such as those 502 involved in root associations with rhizobia (Werner et al., 2015) and mycorrhizal fungi 503 (Brundrett, 2002; Brundrett & Tedersoo, 2017) which highly influence soil functioning 504 (Bardgett et al., 2014) and enhance MM plant tolerance (Gamalero et al., 2009) For example, 505 C. juncea is a leguminous species (Fabaceae) in association with nitrogen-fixing bacteria 506 507 (Robles et al., 2002; Heckenroth et al., 2022), and mycorrhizal fungi (Carrasco et al., 2011). 508 These root associations can improve soil total nitrogen and bioavailable phosphorus contents, 509 enhancing plants mineral nutrition and growth on MM contaminated soils. Since wMPD 510 (accounting for both closely related and distant lineages) had a strong effect on ecosystem functioning, contrary to MNTD (biased towards closely related lineages), our result indicates 511 that the effect of very distinct plant lineages diversity was particularly important, as reported 512 for EMF in global drylands (Le Bagousse-Pinguet et al., 2019). This could be explained by the 513 very ancient evolutionary history of plant-microorganisms associations (Brundrett, 2002; 514 Brundrett & Tedersoo, 2017; Sprent, 2007). Thus, the coexistence of evolutionary distinct 515 lineages within plant communities could ensure a high functional diversity, allowing for 516 differentiated resources use in time and space (i.e., niche differentiation), and thus the 517 maintenance of several ecosystem functions simultaneously (Gamfeldt et al., 2008; Isbell et al., 518 519 2011). Our results highlight the importance of considering plant phylogenetic diversity, in particular distant lineages, in restoration of contaminated ecosystems, not only to better 520 understand MM tolerance mechanisms but also because the co-occurrence of distant lineages 521 provides an important source of biodiversity to offset the negative effects of contamination on 522 523 ecosystem functioning.

524 The relationships between MM soil multi-contamination, biodiversity and EMF 525 strongly differed between dominant and subordinate species within a given community. We found that EMF was impacted by MM soil multi-contamination but not by plant richness in 526 dominant species (Fig. 4b) and vice versa in subordinate species (Fig. 4c). Several studies have 527 reported that subordinate species can enhance microbial communities and soil functions 528 (Mariotte et al., 2013b; Peltzer et al., 2009). However, we found that subordinate species 529 richness altered EMF, potentially due to allelopathic effects (i.e., plant-plant or plant-530 531 microorganisms biochemical interactions) of a pool of subordinate species: Ruta angustifolia Pers., Helichrysum stoechas (L.) Moench, Lavandula latifolia Medik. and Cephalaria 532 533 leucantha (L.) Schrad. Ex Roem. & Schult. (known allelopathic effects of closely related 534 species, respectively: R. graveolens (Hale et al., 2004), H. italicum (Mastelic et al., 2005), L. officinalis (Akbarzadeh et al., 2013) and C. syriaca (Ali et al., 2012)). In addition, comparison 535 of plant diversity between communities in dominant versus subordinate species (i.e., beta 536 diversity; Appendix G) indicate that (i) communities shared a common dominant species pool 537 and (ii) differences in species composition among communities was mainly driven by 538 539 subordinate species. As a result, the effect of plant biodiversity on EMF was mainly driven by the diversity of subordinate species. Our results highlight the importance of considering 540 subordinate species in restoration programs because they can strongly influence EMF (richness 541 542 effect; Gross et al., 2017; Le Bagousse-Pinguet et al., 2019; Le Bagousse-Pinguet et al., 2021), 543 including by enhancing community resistance to drought (Mariotte et al., 2013a). This is particularly important in Mediterranean ecosystems where water stress is accentuated by MM 544 (Poschenrieder & Barcelo, 1999; Rucińska-Sobkowiak, 2016) and droughts will become more 545 and more intense and frequent in the context of global warming (Cramer et al., 2018). 546

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#### 5. Conclusion

Our results reveal the potential of plant biodiversity, and especially the diversity of 550 evolutionary distant species, to offset the alteration of ecosystem functioning by MM soil multi-551 contamination. These findings require the use of holistic approaches considering soil-plant-552 microorganisms continuum, multiple plant biodiversity facets, and plant species relative 553 abundance. In this worldwide decade of ecosystems restoration (FAO, IUCN CEM, SER, 554 2021), our study helps to identify relevant facets of plant biodiversity promoting contaminated 555 556 ecosystem functioning, which is crucial to guide and optimize management efforts aiming to 557 restore ecosystems without removing MM contaminated soils and preserve living soils and 558 human health.

559

#### 560 Data availability

561 Dataset and R codes are available from Figshare 562 (https://figshare.com/s/fd5a224d980758f2d049) and will be made public if the manuscript is 563 accepted.

564

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921 Figure legend

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**Figure 1.** Conceptual model and associated hypotheses based on scientific literature and assessing the relationships between metals and metalloids soil multi-contamination, biodiversity facets (phylogenetic, taxonomic, and functional diversity), microbial biomass and activities and ecosystem multifunctionality in the 30 Mediterranean scrubland communities. The dashed arrows represent the theorical relationships.

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Figure 2. Location of the 30 scrubland plots (represented by circles) along a wide gradient of
a 170-year-old MM soil multi-contamination (evaluated by the pollution load index including
lead, arsenic, antimony, zinc and copper) due to former industrial activities (represented by
triangles) in the currently protected area of the Marseilleveyre massif (Calanques national park,
Marseille, SE, France).

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Figure 3. The best adequate initial model resulting from variables selection and assessing the relationships between metals and metalloids soil multi-contamination, biodiversity facets (phylogenetic, taxonomic, and functional diversity), microbial biomass and activities and ecosystem multifunctionality in the 30 Mediterranean scrubland communities. The dashed arrows represent the potential relationships.

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Figure 4. Path analysis of the relationships between metals and metalloids soil multicontamination, biodiversity facets (phylogenetic, taxonomic, and functional diversity), microbial biomass and activities and soil multifunctionality in (a) scrubland whole communities, (b) dominant species and (c) subordinate species. Effect sizes and correlation significance levels (\*:  $p \le 0.05$ ; \*\*:  $p \le 0.01$ ; \*\*\*:  $p \le 0.001$ ) are indicated on each arrow. Bar 946 charts show the effect sizes of the direct impact of metals and metalloids soil multi-947 contamination and of the indirect effects of biodiversity facets on ecosystem multifunctionality 948 mediated through microbial biomass and activities. Indirect effects were calculated by 949 multiplying the direct effect of biodiversity facets on biomass and microbial activities, and the 950 direct effect of biomass and microbial activities on ecosystem multifunctionality.

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Figure 5. Path analysis of the relationships between metals and metalloids soil multi-952 contamination, biodiversity facets (phylogenetic, taxonomic, and functional diversity), 953 954 microbial biomass and activities and individual soil functions ((a) total organic carbon, (b) 955 available phosphorus, (c) cation exchange capacity, (d) water holding capacity and (e) total 956 Kjeldahl nitrogen) in the 30 Mediterranean scrubland communities. Effect sizes and correlation significance levels (\*:  $p \le 0.05$ ; \*\*:  $p \le 0.01$ ; \*\*\*:  $p \le 0.001$ ) are indicated on each arrow. Bar 957 charts show the effect sizes of the direct impact of metals and metalloids soil multi-958 contamination and of the indirect effects of biodiversity facets on individual soil functions 959 mediated through microbial biomass and activities. Indirect effects were calculated by 960 multiplying the direct effect of biodiversity facets on biomass and microbial activities, and the 961 direct effect of biomass and microbial activities on individual soil functions. 962

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## 971 Table legend

973	<b>Table 1.</b> Minimum, first quartile, median, third quartile and maximum of soil (pH, TOC: Total
974	Organic Carbon, TKN: Total Kjeldahl Nitrogen, WHC: Water Holding Capacity, P: available
975	phosphorus, CEC: Cation Exchange Capacity, PLI: Pollution Load Index), microbial
976	community (SBR: Soil Basal Respiration, MB: Microbial Biomass, AWCD: Average Well
977	Color Development, Hcat: catabolic diversity) and plant community (species richness,
978	evenness, CWM LA: Community Weighted Mean of Leaf Area, CWM SLA: Community
979	Weighted Mean of Specific Leaf Area, FDis: Functional Dispersion; wMPD: weighted Mean
980	Phylogenetic Distance) variables used in the path analysis.

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	Variables pH TOC (g.kg <sup>-1</sup> of dry soil) TKN (g kg-1 of dry soil)	Minir 7.8 35.	num 98	num First quartile 38 8.08 98 51.96 29 4.13	num First quartile Median 38 8.08 8.19 98 51.96 67.61 29 4.13 4.80
<u>.</u>	TKN (g.kg-1 of dry soil) WHC (g 100 g <sup>-1</sup> of dry soil)	1.89 59 NN		4.13 78 75	4.13 4.80 78 75 91 50
SOIL	יש אר (g. 100 g - סו מדץ soil) P (g.kg-1 of dry soil)	39.00 13.00		78.75 20.50	20.50 29.00
	CEC (méeq.100 g <sup>-1</sup> of dry soil)	15.40		24.43	24.43 31.05
	PLI	1.91		4.85	4.85 7.81
	SBR ( $\mu$ g CO <sub>2</sub> -C.g <sup>-1</sup> .h <sup>-1</sup> )	0.02		0.02	0.02 0.03
Microbial community	MB (μg C <sub>mic</sub> .g <sup>-1</sup> ) AWCD	0.01 0.35		0.02 0.72	0.02 0.02 0.72 0.89
	H <sub>cat</sub>	2.72		2.95	2.95 3.07
	Species richness	6.00		11.00	11.00 13.00
<u>)</u>	Evenness	0.80		0.87	0.87 0.88
Plant community	CWM LA (mm²)	22.77		47.64	47.64 60.78
	CWM SLA (mm <sup>2</sup> .mg <sup>-1</sup> )	15.50		42.51	42.51 49.05
	FDis	0.17		0.58	0.58 0.70
	WMPD	166.80		185.11	185 11 <u>200</u> 10



# Hypotheses supporting the conceptual model H1 Relatively high pH in calcareous soils can limit MM mobility and bioavailability (Greger, 2004; Petruzzelli et al., 2020).

MM soil multi-contamination generate physicochemical constraints on soil by adsorption of toxic MM (i.e., lead, arsenic, antimony) onto soil particles instead of nutrients, resulting in alteration of soil composition and functioning (Greger, 2004; Petruzzelli et al., 2020).

H3 MM soil multi-contamination generates stress on microbial communities resulting in decreased microbial biomass and activities (Kızılkaya et al., 2004 ; Gülser & Erdoğan, 2008).

MM soil multi-contamination can act as a strong environmental filter (Keddy, 1992; Weiher et al., 1998) inducing plant physiological dysfunctions, especially water stress (Poschenrieder & Barcelo, 1999; Rucińska-Sobkowiak, 2016). As a result, MM soil multi-contamination can select MM-tolerant plant evolutionary lineages, forms and functions, resulting in reduction of phylogenetic, taxonomic, and functional diversity.

Since dominant and subordinate species are not subjected to similar assembly processes (Arnillas & Cadotte, 2019; H5 Arnillas et al., 2021), MM soil multi-contamination can differently impact on biodiversity of dominant and subordinate species, resulting in different patterns of biodiversity-ecosystem functioning relationships.

Phylogenetic diversity can integrate plant-microorganisms coevolutionary processes and unmeasured biological traits
 (Flynn et al., 2011) as root associations with beneficial microorganisms (Brundrett, 2002; Werner et al., 2015) which highly influence soil functioning (Bardgett et al., 2014). As a result, decrease in phylogenetic diversity can lead to reduction of soil functioning.

Taxonomic and functional diversities influence the quantity and the diversity of litter inputs, i.e., microbial substrates (Lange et al., 2015; Chen et al., 2019). As a result, decrease in taxonomic and functional diversity can lead to reduction of soil functioning.







Metals and metalloids soil multi-contamination: pollution load index including lead, arsenic, antimony, zinc and copper

weighted Mean Phylogenetic Distance Mean Nearest Taxon Distance Species richness Evenness Community Weighted Mean of Leaf Area Community Weighted Mean of Specific Leaf Area Functional Dispersion

Microbial biomass and activities: soil basal respiration, microbial biomass, average well color development and catabolic diversity

Ecosystem multifunctionality: total organic carbon, total Kjedhal nitrogen, available phosphorus, cation exchange capacity and water holding capacity









Appendices

Click here to access/download Supplementary Material Appendices.docx

#### **Declaration of interests**

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.

□The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

#### **CRediT authorship contribution statement**

Lorène Tosini: Conceptualization, Methodology, Investigation, Software, Formal analysis, Writing – Original Draft, Writing – Review & Editing, Project administration, Funding acquisition. Manuel Cartereau: Conceptualization, Methodology, Investigation, Formal analysis, Writing – Review & Editing. Yoann Le Bagousse-Pinguet: Conceptualization, Methodology, Formal analysis, Writing – Review & Editing. Isabelle Laffont-Schwob: Conceptualization, Methodology, Investigation, Writing – Review & Editing, Project administration, Funding acquisition. Pascale Prudent: Conceptualization, Methodology, Investigation, Writing – Review & Editing. Anne-Marie Farnet Da Silva: Investigation, Writing – Review & Editing. Nicolas Montès: Methodology, Investigation, Software, Writing – Review & Editing. Yoan Labrousse: Investigation. Laurent Vassalo: Investigation. Hélène Folzer: Conceptualization, Methodology, Investigation, Writing – Review & Editing, Project administration, Funding acquisition.