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

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Abstract

Despite increasing metals and metalloids (MM) human-driven soil contamination, how it simultaneously alters biodiversity and ecosystem functioning remains unknown. We used a wide gradient of a 170-year-old MM soil multi-contamination in Mediterranean scrublands to assess the effects of soil multi-contamination on multiple plant biodiversity facets, microbial communities and ecosystem multifunctionality (EMF). We found an overall positive effect of plant biodiversity on EMF mediated by microbial communities, and allowing to offset the 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 by enhancing microbial communities, whereas the subordinate species richness altered EMF. By developing a holistic approach of these complex relationships between soil multi-contamination, plant biodiversity, microbial communities and ecosystem functioning, our results reveal the potential of plant biodiversity, and especially the diversity of evolutionary distant species, to offset the alteration of ecosystem functioning by MM soil multi-contamination. In this worldwide decade of ecosystems restoration, our study helps to identify relevant facets of plant biodiversity promoting contaminated ecosystem functioning, which is crucial to guide and optimize management efforts aiming to restore ecosystems and preserve human health.

Key words

Inorganic pollution, phylogenetic diversity, soil-plant-microorganism continuum, species dominance, soil functioning

1. Introduction

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 in the Mediterranean region (Panagos et al., 2013; van Liedekerke et al., 2014; Stolte et al., 2016; Merino et al., 2019). MM soil contamination could alter biodiversity and ecosystem functioning by impacting all the components of the soil-plant-microorganisms continuum, i.e., by affecting soil composition and functioning (Greger, 2004; Petruzzelli et al., 2020), microbial activities (Kızılkaya et al., 2004; Gülser & Erdoğan, 2008) and plant physiological processes (Cheng, 2003; Yadav, 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 multi-contamination gradient (see Steudel et al., 2012 for biodiversity-ecosystem functioning relationships along environmental stress gradients). Yet, how MM soil multi-contamination gradients simultaneously impact on taxonomic (species richness and their abundances within a community), phylogenetic (the diversity of evolutionary lineages within a community) and functional (the diversity of forms and functions within a community) facets of plant 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). Deciphering the relationships between biodiversity and EMF under soil multi-contamination could not only expand our fundamental understanding of the biodiversity-EMF relationships 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).

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

118 Different assembly processes (*sensu* Keddy, 1992) can shape the diversity patterns of
119 dominant plant species with high abundance within a plant community, and subordinate plant
120 species with low abundance within a plant community (Arnillas & Cadotte, 2019; Arnillas et
121 al., 2021). For instance, while environmental filtering can lead to functional similarity (i.e.,
122 reduced range of functional traits values) among dominant species, niche differentiation (i.e.,
123 differentiated use of resources in time and space) can lead to greater divergence (i.e., wide range
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
127 (Mariotte et al., 2013a). Finally, dominant and subordinate species can differently influence
128 ecosystem functioning (Gross et al., 2017; Le Bagousse-Pinguet et al., 2019; Le Bagousse-
129 Pinguet et al., 2021; Lyons et al., 2005). Even if dominant species highly influence individual
130 functions related to biomass production, carbon, and nutrient cycling (mass ratio hypothesis;
131 Grime, 1998), subordinate species can have significant effects on EMF (richness effect; Gross
132 et al., 2017; Le Bagousse-Pinguet et al., 2019; Le Bagousse-Pinguet et al., 2021). Thereby,
133 deciphering the diversity patterns of dominant *versus* subordinate species is critical to our
134 understanding of their respective implications on ecosystem functioning (Seabloom et al.,
135 2013).

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

151

152 ## 2. Material and methods

153 ### 2.1. Study area and sites

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

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

168 We chose to study scrubland communities, widely represented in the study area and
169 more broadly in Mediterranean areas, and especially woody species because of their perennial
170 and well-developed root systems subjected to MM soil multi-contamination. Thus, we first
171 restricted the study area to areas potentially occupied by scrubland by selecting “323
172 Sclerophyllous vegetation”, “333 Sparsely vegetated areas” and “332 Bare rock” land cover
173 types from CORINE Land Cover 2012 database (available 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

scrubland vegetation characterized by homogeneous abiotic conditions influencing plant and microbial communities structure and functioning: distance to the sea, elevation, aspect, and slope (Heckenroth et al., 2016). The first step was to remove areas inferior to 250m to the sea to avoid the influence of sea dispersal spray on plant and microbial communities, using a 250m radius buffer area around the coastline provided by the Histolitt® database (available at: https://www.data.gouv.fr/fr/datasets/trait-de-cote-histolitt-1/). The second step was to calculate aspect and slope raster layers using a 5m resolution elevation raster layer provided by the RGEalti® database (available at: https://geoservices.ign.fr/rgealti#telechargement5m). Then, 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 variables (Appendix A), we reduced our selection to the range between the 25^{th} and 75^{th} percentiles for each variable separately. As a result, our site selection encompasses sites from 123 m to 283 m of elevation, from 23 % to 38 % of slope and south aspect. Then, we based our study sites selection on MM soil multi-contamination, using Marséco project gridded spatial data at a 250 m resolution (Laffont-Schwob et al., 2016). For each grid cell, corresponding to a 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-contamination. Mapped MM contamination data relieved a spatially structured contamination gradient from West to East. These data are not used as value for the MM multi-contamination 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 multi-contamination levels: very high ($40<PLI<216$), high ($10<PLI<30$), intermediate ($5<PLI<10$) and low ($1<PLI<5$) multi-contamination. We positioned 7 or 8 plots of 32 m² each within each multi-contamination level, in different patches of scrubland vegetation with a minimum distance of 70m between two plots to avoid pseudo replication (Fig. 2). The 32 m²

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

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.

2.2.1. MM soil multi-contamination

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 HNO_3 + 2/3 HCl) and filtered through a 0.45 µm cellulose ester membrane filter (ISO 11466, 1995). Then, MM pseudo-total concentrations were determined by ICP-OES (Jobin Yvon Horiba, Spectra 2000). Quality controls and accuracy were checked using standard soil reference materials (CRM 049-050, from RTC-USA), with accuracies within 100 ± 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:

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

where $CF_{MM}=[MM]_{pseudo\text{-}total} / [MM]_{local\ background\ value}$ and $[MM]_{local\ background\ value}$ are 42.9, 4.9, 3.1, 66 and 7.5 $mg.kg^{-1}$ 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 ± 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 pseudo-

226 total concentrations and PLI (Appendix B). Therefore, we chose to keep MM pseudo-total
227 concentrations because they allow us to calculate the index of MM soil multi-contamination
228 (i.e., PLI) based on MM local background pseudo-total concentrations defined by Affholder et
229 al. (2014). Because of their skewed distribution, PLI values used in analysis were log-
230 transformed.
231
232 **2.2.2. Ecosystem multifunctionality**
233 We quantified EMF for each plot using five soil functions (Table 1): (i) total organic carbon
234 (TOC), (ii) total Kjeldahl nitrogen (TKN), (iii) available phosphorus (P) contents, (iv) cation
235 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
237 holding capacity (WHC). Total organic carbon (TOC) content was determined by the difference
238 between (i) the total carbon content, measured by non-dispersive infrared (NDIR) spectroscopy
239 after soil combustion at 900 °C in a furnace under oxygen flow, and (ii) the inorganic carbon
240 content, measured by NDIR spectroscopy after soil acidification with phosphoric acid (40 %
241 H_3PO_4) at 80 °C under oxygen flow (AnalytiK Jena N/C 2100 S, ISO 10694, 1995). Total
242 Kjeldahl nitrogen (TKN) content was determined by Kjeldahl method (ISO 11261, 1995): (i)
243 mineralization of the soil sample by concentrated sulfuric acid (H_2SO_4) during 4h at 300°C
244 (Büchi Speed Digester K-436), (ii) distillation of the obtained ammonium sulfate with a
245 concentrated soda solution during 7min and neutralization with an excess of sulfuric acid
246 solution (Büchi Distillation Unit 323), (iii) dosage of the excess of sulfuric acid by indirect
247 titration with soda solution (Titroline Easy, Schott Instruments). Available phosphorus (P,
248 Olsen method, ISO 11263, 1995) and cation exchange capacity (CEC, Metson method, NF X
249 31-130, 1999) measurements were performed by the Laboratoire Développement Méditerranée
250 (COFRAC accreditation n°1-5865, Alès, France). WHC was measured by soil immersion in

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):

$$Z \text{ score} = \frac{\text{Fij} - \text{Mean Fi}}{\text{SD Fi}} \quad \text{Eq. (2)}$$

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 Fi, calculated for the 30 scrubland plots. Finally, we calculated an index of EMF by averaging the Z-score of individual functions for each plot.

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_{cat}).

SBR and substrate-induced respiration (SIR) were measured to assess global microbial activity and biomass (Anderson, 2003; Anderson & Domsch, 1978). 10 g dry weight equivalent of fresh soil at 30 % of the WHC were placed in 117 mL slightly open ajar glass jars and incubated for 48 h at 23 °C. For SBR measurement, each sample was oxygenated for 4 min and incubated for 4 h at 23 °C in tightly closed glass jars. Then, 1 mL of their atmosphere was sampled in the headspace with a syringe and injected into a gas chromatograph (Chrompack CHROM 3 – CP 9001) to analyse CO_2 production by microbial oxidation of the soil organic matter. SIR was estimated using a procedure from Anderson (2003). Soil samples were 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

276 was oxygenated for 4 min and then incubated for 2 h at 23 °C in tightly closed glass jars. 1 mL
277 of their atmosphere was sampled in the head space with a syringe and injected into a gas
278 chromatograph to analyse CO_2 production by microbial oxidation of glucose. The gas
279 chromatograph was equipped with a thermal conductivity detector and a packed column
280 (Porapack). The carrier gas helium flow was regulated at 60 $mL.h^{-1}$. Ambient CO_2
281 concentrations were subtracted from sampled CO_2 concentrations and resulting values were
282 adjusted at 22 °C according to Ideal Gas Laws using a Q10 = 2. SIR was converted into
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
287 Garland & Mills (1991). For each soil sample, 100 mL of 0.1 % sodium pyrophosphate sterile
288 solution was added to 5 g dry weight equivalent of fresh soil in a 250 mL flask and the whole
289 was shaken for 2 h at 80 rpm. The obtained soil solution was diluted and standardized at optical
290 density (OD) 595 nm=0.03 with a sterile physiological solution (NaCl 0.85 %) and 125 µL were
291 used to inoculate each well of the plates. After an incubation period of 5 days at 25 °C, microbial
292 response was assessed by reading the absorbance at 590 nm using a TECAN®
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
296 the AWCD index:

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

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

299 H_{cat} was assessed using Shannon's index:

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

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.

2.3. Plant biodiversity facets

2.3.1. Phylogenetic and taxonomic diversity

In each plot, the presence of each species was recorded using a 2m² quadrat which was moved 16 times over the whole 32m² 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* 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 occurrences: (i) weighted and (ii) non-weighted mean phylogenetic distance (respectively 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 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; Oksanen et al., 2019) for each plot: (i) species richness and (ii) Pielou's evenness.

2.3.2. Functional diversity

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 and ecosystem functioning in Mediterranean scrublands (Gross et al., 2013; Valencia et al., 2015; Le Bagousse-Pinguet et al., 2015): Leaf Area (LA), Specific Leaf Area (SLA) and Leaf Dry Matter Content (LDMC). Traits such as LA are related to light interception and water stress tolerance (Westoby et al., 2002). SLA and LDMC are related to the leaf economic spectrum, reflects the relative growth rate of plants, and are associated with plant strategies to acquire, use and/or conserve resources such as light, nutrients and water (Wright et al., 2004).

In each plot, we sampled three mature individuals of each species at most to account for 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., 2010; Bolnick et al., 2011; Le Bagousse-Pinguet et al., 2014, 2015), and ecosystem processes (Fridley & Grime 2010). A total of 25089 leaves were sampled from 983 individuals. On each individual, a branch section with mature leaves was collected. We avoided sampling of leaves 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 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 the minimum LA per individual is 1 cm^2 (Appendix D). First, leaves were cut from the branch. We included or excluded the petiole depending on the species considered, based on the functional definition of a leaf, i.e., all leaf parts which are photosynthetic and then pigmented (Appendix D). Then, all the fresh leaves from a same individual were pooled, scanned, weighted (fresh mass), dried at 40°C until constant mass and reweighted (dry mass). The LA of each leaf 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 community-weighted 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) non-weighted functional dispersion (respectively wFDis and FDis). Because of their skewed distributions, functional trait values used in indices calculation were log-transformed.

2.4. Data analyses

2.4.1. Conceptual model

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

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

375 LA and SLA), or CWM LA, CWM SLA, CWM LDMC, FRic and FDis (including LA, SLA
376 and LDMC) for functional diversity.
377
378 **2.4.3. Best adequate initial model**
379 We tested all possible combinations of plant explanatory variables in 6 path analysis (2
380 combinations for phylogenetic diversity x 1 combination for taxonomic diversity x 3
381 combinations for functional diversity; *lavaan* R package; Rosseel, 2012), and kept the
382 combination of variables that provided the best adequate initial model according to Grace
383 (2006) and Rosseel (2012) criteria: Chi-squared test > 0.05, Comparative Fit Index (CFI) >
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
386 explanatory variables: (i) MNTD and wMPD for phylogenetic diversity, (ii) richness and
387 evenness for taxonomic diversity, and (iii) CWM LA, CWM SLA and FDis for functional
388 diversity (Fig. 3 and Table 1).
389
390 **2.4.4. Minimal parsimonious model**
391 From the best adequate initial model, we performed path analyses to assess: (i) the direct
392 and indirect effects of MM soil multi-contamination on the plant biodiversity facets, on
393 microbial biomass and activities and on EMF, (ii) the relationships between biodiversity facets,
394 each of the soil individual functions (i.e., TOC, TKN, P, CEC, WHC), and ultimately EMF,
395 and (iii) whether and how the relationships between plant biodiversity and EMF differ once
396 considering either dominant or subordinate species within each community. We defined
397 dominant and subordinate species within each plant community using a relative abundance
398 threshold of 0.06, based on the median of species relative abundances in all the 30 communities
399 (0.06306) and the median of median species abundances among the 30 plant communities

(0.06660). As a result, dominant species have a relative abundance between or equal to 0.06 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 (Akaike Information Criterion), until that a minimal parsimonious model was reached. Finally, 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 (> 0.90), a low RMSEA (< 0.10) and a low SRMSR (< 0.08) (Grace, 2006; Rosseel, 2012; Appendix F).

3. Results

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.

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

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 (r = 0.423, Fig. 5c) and WHC (r = 0.435, Fig. 5d). wMPD and CWM LA had indirect positive effects on EMF (r = 0.291 and r = 0.165 respectively; Fig. 4a), TOC (r = 0.280 and r = 0.159 respectively; Fig. 5a), P (r = 0.321 and r = 0.182 respectively; Fig. 5b), CEC (r = 0.267 and r = 0.151 respectively; Fig. 5c) and WHC (r = 0.275 and r = 0.156 respectively; Fig. 5d), and wMPD always showed the strongest effect. In contrast, species richness had an indirect negative effect on EMF (r = -0.204, Fig. 4a), TOC (r = -0.196, Fig. 5a), P (r = -0.224, Fig. 5b), CEC (r = -0.187, Fig. 5c) and WHC (r = -0.192, Fig. 5d). The negative effects of species richness on EMF, TOC, P, CEC and WHC were compensated by the positive effects of wMPD and CWM 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 negative effects of MM soil multi-contamination, respectively.

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

Considering dominant and subordinate species separately highlighted different patterns of biodiversity-EMF relationships. MM soil multi-contamination had a direct and negative effect on EMF when focusing on dominant species (r = -0.303, Fig. 4b), while no effect occurred when considering subordinate species. wMPD is the only biodiversity index which had a 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, the positive effect of wMPD on EMF partially offset the negative effect of MM soil multi-contamination on EMF (r = -0.303, Fig. 4b). In addition, species richness had a significant 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

450 species, the indirect negative effect of species richness on EMF was compensated by the
451 indirect positive effect of wMPD on EMF. Thus, plant biodiversity had an overall indirect
452 positive effect on EMF in subordinate species (r = 0.158, Fig. 4c).
453
454 **4. Discussion**
455 We investigated how MM soil multi-contamination influences the relationships between
456 multiple plant biodiversity facets and EMF in Mediterranean scrublands. Our results highlight
457 that plant biodiversity offset the negative impacts of MM soil multi-contamination on EMF
458 (Fig. 4a), underlining its potential for ecological restoration of contaminated ecosystems. We
459 also found antagonism between the positive effects of phylogenetic and functional facets and
460 the negative effects of taxonomic facet on ecosystem functioning, warning for the need to
461 consider multiple biodiversity facets for the restoration of contaminated ecosystems.
462 MM soil multi-contamination impacted differently the components of the soil-plant-
463 microorganisms continuum. On the one hand, MM soil multi-contamination altered EMF (Fig.
464 4a), and mostly soil water holding capacity (Fig. 5c) and total nitrogen content (Fig. 5d). The
465 observed soil alteration likely results from competition for adsorption onto soil particles
466 between toxic MM (e.g., lead and arsenic) and nutrients, resulting in changes in soil
467 composition and functioning (Greger, 2004; Petruzzelli et al., 2020). On the other hand, MM
468 soil multi-contamination did not impact plant biodiversity and microbial biomass and activities.
469 Since MM and water stresses lead to similar physiological responses of plants (e.g., synthesis
470 of osmoprotective compounds (Sharmila & Pardha Saradhi, 2002), reduction of leaves sizes,
471 thickness, and stomata density (Rucińska-Sobkowiak, 2016)), our results support the
472 environmental filtering hypothesis (Keddy, 1992; Weiher et al., 1998), i.e., the presence of
473 tolerant species to water stress preadapted to MM contaminated soils (Affholder et al., 2014;
474 Heckenroth et al., 2022; Salducci et al., 2019). Altogether our study highlights the complex

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

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

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.,

total organic carbon, available phosphorus, cation exchange capacity, and water holding capacity; respectively Fig. 5a, b, c and d) through microbial communities. Phylogenetic diversity can effectively encompass hidden biological traits (Flynn et al., 2011), such as those involved in root associations with rhizobia (Werner et al., 2015) and mycorrhizal fungi (Brundrett, 2002; Brundrett & Tedersoo, 2017) which highly influence soil functioning (Bardgett et al., 2014) and enhance MM plant tolerance (Gamalero et al., 2009) For example, *C. juncea* is a leguminous species (Fabaceae) in association with nitrogen-fixing bacteria (Robles et al., 2002; Heckenroth et al., 2022), and mycorrhizal fungi (Carrasco et al., 2011). These root associations can improve soil total nitrogen and bioavailable phosphorus contents, enhancing plants mineral nutrition and growth on MM contaminated soils. Since wMPD (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 that the effect of very distinct plant lineages diversity was particularly important, as reported for EMF in global drylands (Le Bagousse-Pinguet et al., 2019). This could be explained by the very ancient evolutionary history of plant-microorganisms associations (Brundrett, 2002; Brundrett & Tedersoo, 2017; Sprent, 2007). Thus, the coexistence of evolutionary distinct lineages within plant communities could ensure a high functional diversity, allowing for differentiated resources use in time and space (i.e., niche differentiation), and thus the maintenance of several ecosystem functions simultaneously (Gamfeldt et al., 2008; Isbell et al., 2011). Our results highlight the importance of considering plant phylogenetic diversity, in particular distant lineages, in restoration of contaminated ecosystems, not only to better understand MM tolerance mechanisms but also because the co-occurrence of distant lineages provides an important source of biodiversity to offset the negative effects of contamination on 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
526 found that EMF was impacted by MM soil multi-contamination but not by plant richness in
527 dominant species (Fig. 4b) and *vice versa* in subordinate species (Fig. 4c). Several studies have
528 reported that subordinate species can enhance microbial communities and soil functions
529 (Mariotte et al., 2013b; Peltzer et al., 2009). However, we found that subordinate species
530 richness altered EMF, potentially due to allelopathic effects (i.e., plant-plant or plant-
531 microorganisms biochemical interactions) of a pool of subordinate species: *Ruta angustifolia*
532 Pers., *Helichrysum stoechas* (L.) Moench, *Lavandula latifolia* Medik. and *Cephalaria*
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.*
535 *officinalis* (Akbarzadeh et al., 2013) and *C. syriaca* (Ali et al., 2012)). In addition, comparison
536 of plant diversity between communities in dominant *versus* subordinate species (i.e., beta
537 diversity; Appendix G) indicate that (i) communities shared a common dominant species pool
538 and (ii) differences in species composition among communities was mainly driven by
539 subordinate species. As a result, the effect of plant biodiversity on EMF was mainly driven by
540 the diversity of subordinate species. Our results highlight the importance of considering
541 subordinate species in restoration programs because they can strongly influence EMF (richness
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
544 particularly important in Mediterranean ecosystems where water stress is accentuated by MM
545 (Poschenrieder & Barcelo, 1999; Rucińska-Sobkowiak, 2016) and droughts will become more
546 and more intense and frequent in the context of global warming (Cramer et al., 2018).
547
548

5. Conclusion

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

Data availability

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

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921 **Figure legend**

922

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

928

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

934

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

940

941 **Figure 4.** Path analysis of the relationships between metals and metalloids soil multi-
942 contamination, biodiversity facets (phylogenetic, taxonomic, and functional diversity),
943 microbial biomass and activities and soil multifunctionality in (a) scrubland whole
944 communities, (b) dominant species and (c) subordinate species. Effect sizes and correlation
945 significance levels (*: $p \leq 0.05$; **: $p \leq 0.01$; ***: $p \leq 0.001$) are indicated on each arrow. Bar

charts show the effect sizes of the direct impact of metals and metalloids soil multi-contamination and of the indirect effects of biodiversity facets on ecosystem multifunctionality mediated through microbial biomass and activities. Indirect effects were calculated by multiplying the direct effect of biodiversity facets on biomass and microbial activities, and the direct effect of biomass and microbial activities on ecosystem multifunctionality.

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

971 **Table legend**

972

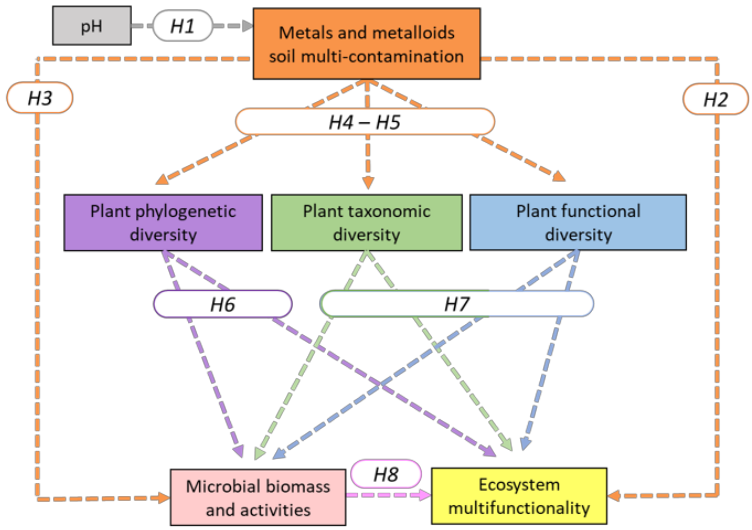
973 **Table 1.** Minimum, first quartile, median, third quartile and maximum of soil (pH, TOC: Total
974 Organic Carbon, TKN: Total Kjeldahl Nitrogen, WHC: Water Holding Capacity, P: available
975 phosphorus, CEC: Cation Exchange Capacity, PLI: Pollution Load Index), microbial
976 community (SBR: Soil 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	Minimum	First quartile	Median	Third quartile	Maximum
Soil	pH	7.88	8.08	8.19	8.28	8.38
	TOC (g.kg^{-1} of dry soil)	35.98	51.96	67.61	88.40	198.31
	TKN (g.kg-1 of dry soil)	1.89	4.13	4.80	6.03	11.58
	WHC (g.100 g^{-1} of dry soil)	59.00	78.75	91.50	105.75	152.00
	P (g.kg-1 of dry soil)	13.00	20.50	29.00	35.00	68.00
	CEC (méeq.100 g^{-1} of dry soil)	15.40	24.43	31.05	35.93	64.60
	PLI	1.91	4.85	7.81	13.58	171.41
Microbial community	SBR (µg CO_2-C.g^{-1}.h^{-1})	0.02	0.02	0.03	0.04	0.08
	MB (µg C_{mic}.g^{-1})	0.01	0.02	0.02	0.03	0.06
	AWCD	0.35	0.72	0.89	0.97	1.44
	H_{cat}	2.72	2.95	3.07	3.14	3.24
Plant community	Species richness	6.00	11.00	13.00	15.00	19.00
	Evenness	0.80	0.87	0.88	0.91	0.97
	CWM LA (mm²)	22.77	47.64	60.78	94.52	142.46
	CWM SLA (mm².mg^{-1})	15.50	42.51	49.05	57.59	90.83
	FDis	0.17	0.58	0.70	0.99	2.04
	wMPD	166.80	185.11	200.19	228.08	254.64

Figure1

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Hypotheses supporting the conceptual model

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

H2 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).

H4 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.

H5 Since dominant and subordinate species are not subjected to similar assembly processes (Arnillas & Cadotte, 2019; 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.

H6 *H8* 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.

H7 *H8* 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.

Figure2

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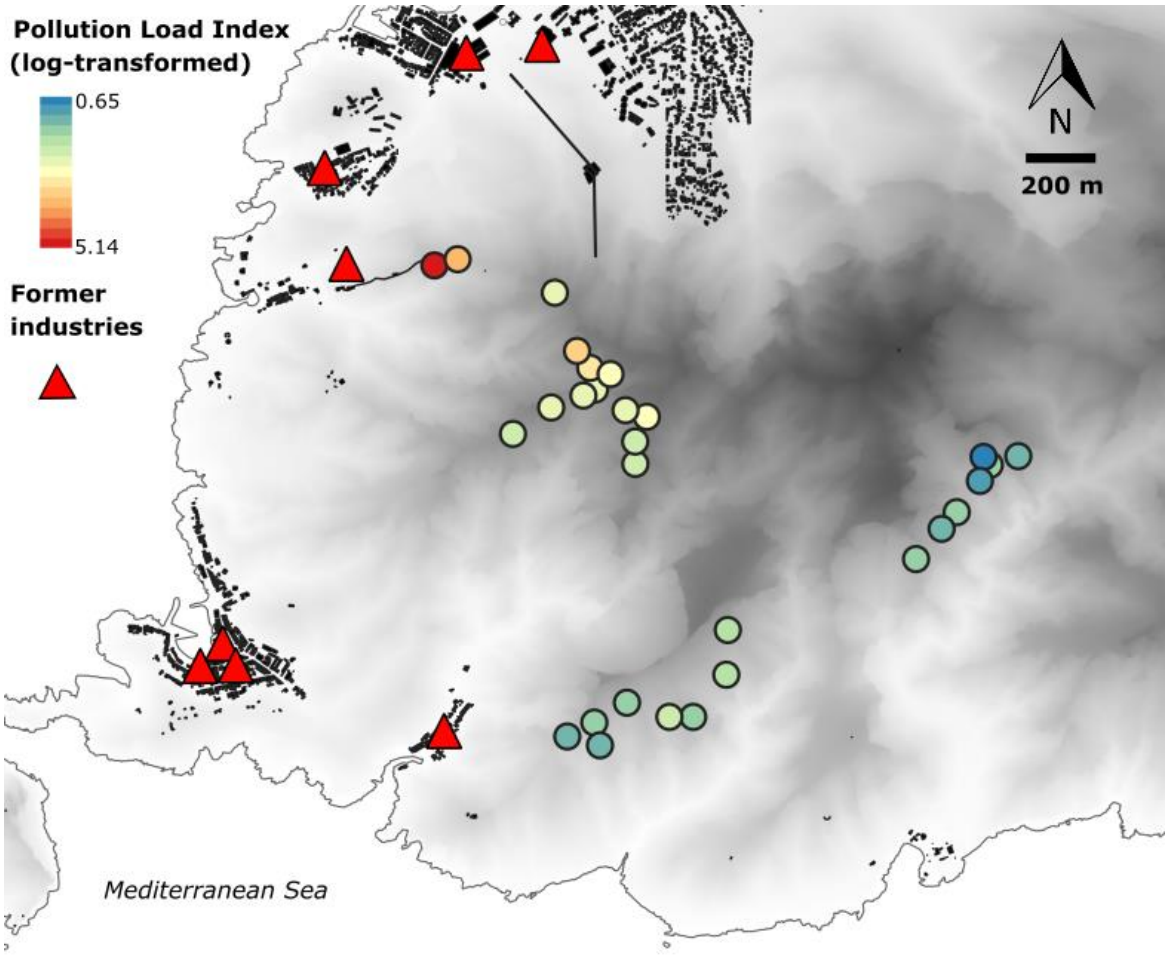


Figure3

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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

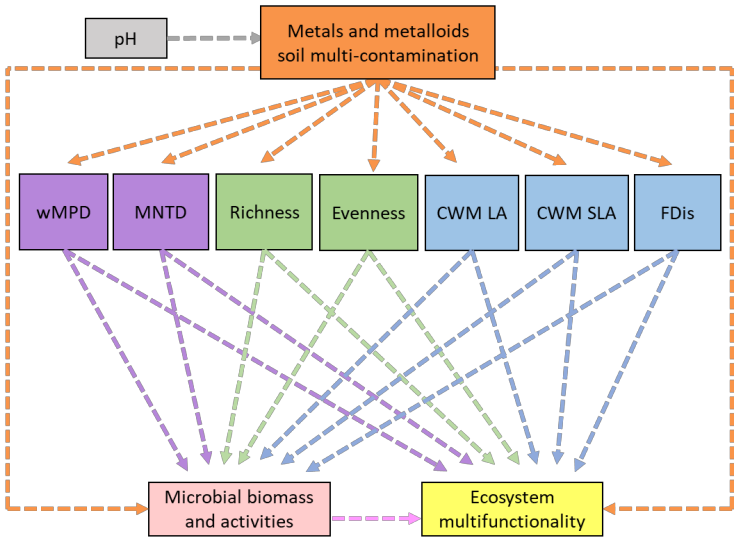


Figure4

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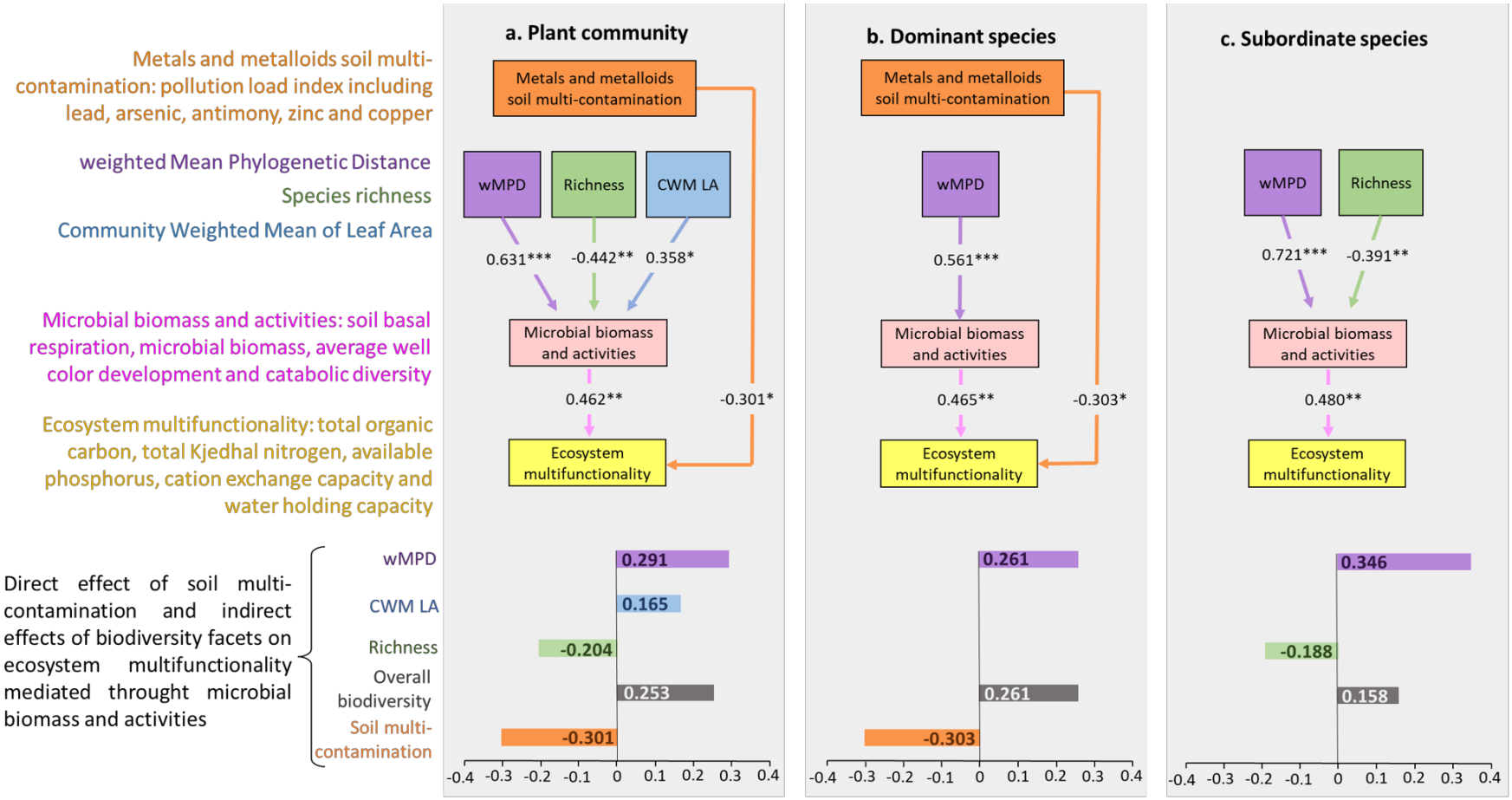
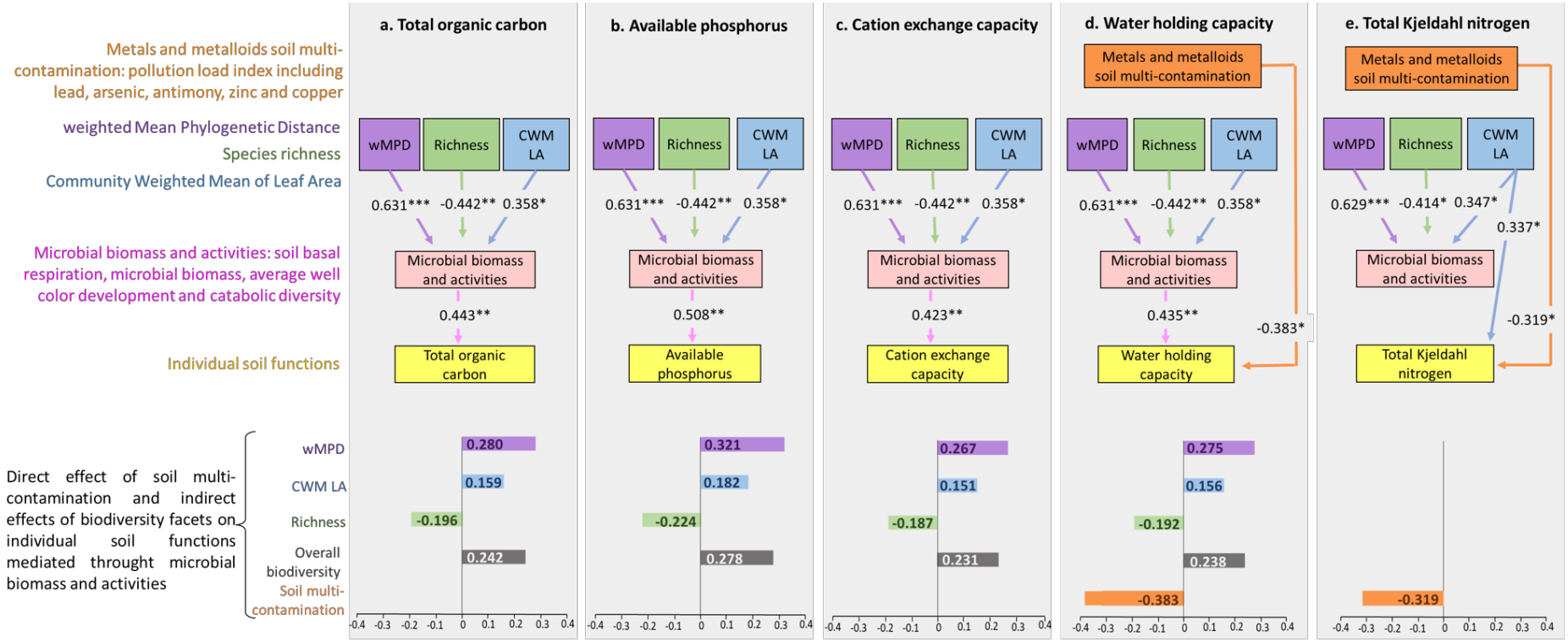


Figure5

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Appendices

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Supplementary Material
Appendices.docx

Declaration of Interest Statement

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.