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## RESEARCH ARTICLE

# Combined effects of soil multi-contamination by metals and metalloids and drought slow down the ecological restoration of a Mediterranean scrubland

Lorène Tosini<sup>1,2</sup>, H el ene Folzer<sup>1,2</sup>, Manuel Cartereau<sup>2</sup>, Pascale Prudent<sup>3</sup>, Laura March<sup>1</sup>, Alma Heckenroth<sup>1</sup>, Laurent Vassalo<sup>3</sup>, Marie-Dominique Salducci<sup>2</sup>, Yoan Labrousse<sup>1</sup>, Isabelle Laffont-Schwob<sup>1,4</sup>

In Mediterranean ecosystems, soil contamination by metals and metalloids and drought represent major abiotic stresses whose combined effects on plant communities remain poorly understood, hindering effective ecological restoration practices. Here we evaluated the responses of plant community and species to combined stresses during a 4-year ecological restoration operation in Mediterranean scrubland. We conducted monthly monitoring of plant communities across 20 restoration plots, analyzing taxonomic and phylogenetic diversity attributes alongside soil contamination levels and drought indices using mixed-effects and hurdle models. Our results highlight that both stresses combined reduced taxonomic diversity and the diversity of distant lineages within the plant community by exerting a strong environmental filtering on species. Synergistic effects revealed that species richness decreased more strongly under high drought at low contamination levels than at high contamination levels, suggesting prior selection for tolerant species. Annual species were primarily impacted by drought through stress-avoidance strategies, while perennial species were more affected by metal and metalloid contamination despite their stress-tolerance mechanisms. Our findings highlight the importance of considering combined stress effects in restoration planning and identify phylogenetic diversity as a powerful criterion for species selection. Several perennial species, particularly *Coronilla juncea*, showed high tolerance and restoration potential for metal and metalloid-contaminated Mediterranean ecosystems facing increasing drought under climate change.

**Key words:** combined abiotic stresses, environmental filtering hypothesis, Mediterranean plant biodiversity, phylogenetic diversity, phytostabilization, soil contamination, stress-tolerance

## Implications for Practice

- Using a combination of plant community indices encompassing multiple attributes of biodiversity, especially phylogenetic ones, helps implementing efficient plant assemblages for ecological restoration and the evaluation of ecological restoration success.
- In Mediterranean scrublands heavily contaminated by metals and metalloids, ecological restoration practices based only on planting native species (without soil or water inputs) may not be sufficient to cope with increased droughts associated with global warming.
- More human intervention may be needed by adapting ecological restoration and phytoremediation practices to enhance plant community resilience face to global warming.

## Introduction

In Mediterranean ecosystems, soil contamination by metals and metalloids (MM) and drought represent major abiotic stresses whose combined effects drastically impact biodiversity and ecosystem functioning. While individual effects of these stresses on plants have been extensively studied, their combined impacts

on plant communities remain poorly understood (Mittler 2006; Hirve et al. 2020). This knowledge gap hinders effective ecological restoration practices needed to establish plant communities that sustainably immobilize soil contaminants and preserve ecosystem and human health.

Combined soil contamination by MM and drought creates complex plant responses that can be additive (independent effects, e.g. de Silva et al. 2012) or synergistic (mutually reinforcing effects, e.g. Disante et al. 2011), potentially constituting a

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novel stress factor (*sensu* Mittler 2006). Since MM induce water stress-like responses in plants (Poschenrieder & Barcelo 1999; Rucińska-Sobkowiak 2016), their combination with drought often produces similar plant responses: synthesis of osmoprotective compounds (Sharmila & Pardha Saradhi 2002), reduced leaf size, thickness, and stomata density (Rucińska-Sobkowiak 2016). However, stress-specific responses also occur, particularly metal-dependent synthesis of phytochelatins and metallothioneins (Hall 2002). Plant responses depend largely on ecological strategy (*sensu* Grime 1974; Grime 2001) related to life cycle: annuals avoid stress through seed dormancy during unfavorable seasons (Jenks & Hasegawa 2014), while perennials develop physiological and metabolic tolerance mechanisms (Jenks & Hasegawa 2014).

Understanding these combined stresses requires examining multiple biodiversity attributes, that is, taxonomic (species richness and abundance), phylogenetic (evolutionary lineage diversity), and functional diversity (form and function), which may respond differently to identical stressors (Brum et al. 2017; Tucker et al. 2018; Tosini et al. 2023). Environmental filtering theory (Keddy 1992; Weiher et al. 1998) suggests that contamination by MM and drought should alter diversity measures by selecting few tolerant plant evolutionary lineages and species with limited functional range. However, high biodiversity persists in metal-rich (Baker et al. 2010) and water-limited environments (Le Bagousse-Pinguet et al. 2017; Maestre et al. 2021), through coexistence of stress-avoidant and stress-tolerant species (Poorter et al. 2009; Gross et al. 2013), positive interactions (e.g. Butterfield & Briggs 2011; Butterfield & Munson 2016; Muszyńska & Labudda 2019) and functional convergence among distantly related species (Maestre et al. 2021).

The French Mediterranean Marseilleveyre massif (Marseille, SE France) exemplifies these challenges. Industrial activities from the nineteenth to the twentieth centuries, particularly the Escalette former lead-smelting factory, created diffuse metal and metalloid soil multi-contamination through ash dispersal (Laffont-Schwob et al. 2016). Despite contamination, this area became part of the Calanques National Park in 2012, offering opportunities to test restoration solutions. Sparsely vegetated, heavily contaminated scree at the Escalette site underwent joint ecological restoration and phytostabilization operation in 2015 (Heckenroth et al. 2017) to establish native Mediterranean scrubland capable of immobilizing contaminants. After 3 years, while community resilience was observed, target goals of dense perennial cover and soil health recovery were not attained (Tosini et al. 2020).

This study evaluates vegetation response to combined soil multi-contamination by MM and drought during restoration operations using 4 years of monthly monitoring data. We investigated how individual, additive, and/or synergistic effects of soil multi-contamination by MM and drought impact taxonomic and phylogenetic community attributes, as well as species occurrence and abundance patterns based on life cycle strategies. Results inform optimization of ecological restoration and phytoremediation practices in metal and metalloid-contaminated Mediterranean ecosystems.

## Methods

### Study Site

The study site is included in the protected area of the Marseilleveyre massif (Calanques National Park, Marseille, SE France, Fig. 1A), characterized by a semiarid Mediterranean climate (Quézel & Médail 2003), calcareous, skeletal, oligotrophic, and steep soils subject to significant karstic erosion (Knoerr 1960), and a cold, dry, and often violent wind, the Mistral (Aillaud & Cruzet 1988). The Marseilleveyre massif is mainly covered by Mediterranean scrublands and *Pinus halepensis* Mill. pinewoods. The study site is located at the outlet of the horizontal chimney of the Escalette former lead-smelting factory (WGS84 coordinates:  $x = 5.3533$ ,  $y = 43.2268$ ; Fig. 1B). The site corresponds to a steep scree with an average slope of 23%, located below a ridge at an altitude of 140 m. This site is defined as a hotspot of soil multi-contamination by MM (Testiati et al. 2013; Heckenroth et al. 2016), mainly by lead (Pb) and arsenic (As) but also by zinc (Zn) and copper (Cu) (for metal and metalloid concentrations in soil see Table 1). The soil, whose depth varies between 10 and 30 cm, consists of a mixture of limestone, clayey limestone, marl (Knoerr 1960), and contaminated ashes and can therefore be defined as an anthroposol.

In 2015, an active ecological restoration was carried out at the Escalette site (Heckenroth et al. 2017), in accordance with the Calanques National Park regulations prohibiting both the introduction of non-local plant material and the use of amendments to preserve local biodiversity. First, 20 plots of 1 m<sup>2</sup> cultivation terraces were created by using limestones from the unvegetated scree to facilitate plant rooting and limit water run-off. Then, we transplanted an assemblage of seedlings from eight plant species from the local floristic background defined as metal-tolerant species in each plot: *Brachypodium retusum* (Pers.) P. Beauv. (*Poaceae*), *Cistus albidus* L. (*Cistaceae*), *Coronilla juncea* L. (*Fabaceae*), *Dianthus caryophyllus* L. (*Caryophyllaceae*), *Globularia alypum* L. (*Plantaginaceae*), *Lobularia maritima* (L.) Desv. (*Brassicaceae*), *Salvia rosmarinus* Spenn. (syn. *Rosmarinus officinalis* L. [*Lamiaceae*]), and *Teucrium polium* L. (*Lamiaceae*) (Fig. 1C). The soil surface was covered with small limestones to reduce soil water loss by evapotranspiration. No addition of amendments or water was done after transplanting.

### Soil Multi-Contamination by Metals and Metalloids

For each plot, four soil subsamples of 100 mL each were collected 3 years after implementation, in the top 20 cm layer after litter removal, sieved at 2 mm, and pooled in a composite soil sample. Soil samples were dried at 40°C, ground to less than 0.2 mm (RETSCH RM 200 ©, agate mortar), digested in a microwave mineralizer (Milestone Start D ©) using aqua regia (1/3 HNO<sub>3</sub> + 2/3 HCl), and filtered through a 0.45 µm cellulose ester membrane filter (procedure adapted from standard ISO 54321, 2020). Then, metal and metalloid pseudo-total concentrations were determined by ICP-OES (Inductively Coupled Plasma Optical Emission Spectrometry, Horiba Jobin Yvon, JY2000 Ultratrace ©). Quality controls of

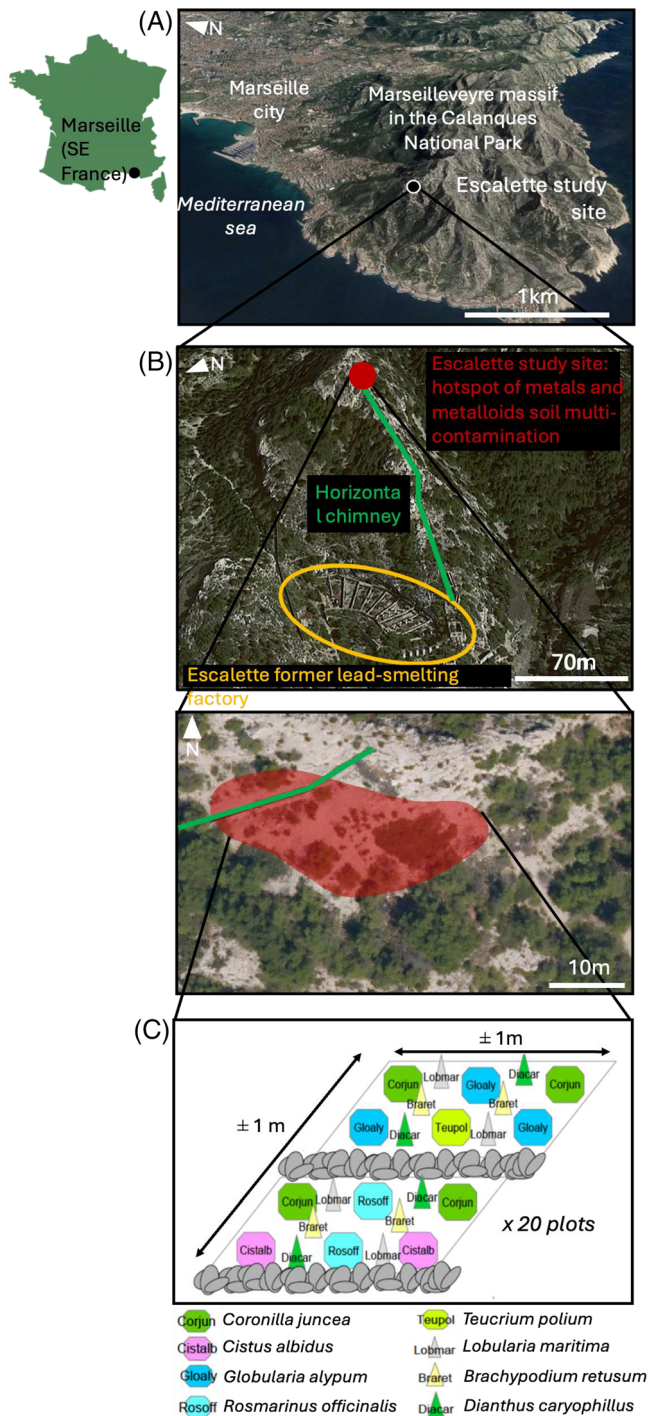


Figure 1. Location of (A) the Escalette site in the Marseilleveyre massif and the Calanques National Park, (B) the Escalette former lead-smelting factory, the horizontal chimney, and the study site (Source: Google Earth). (C) Representation of the ecological restoration plots at the Escalette site organized in cultivation terraces and planted with seedlings from eight native metal-tolerant species (Source: Heckenroth et al. 2017).

elemental quantification were done using certified reference material (soil CRM 049-050, from RTC-USA), with accuracies within  $100 \pm 10\%$ .

Pb, As, Zn, and Cu soil concentrations were highly correlated (all Spearman's  $r^2$  values were above 0.6) and highly correlated to the Pollution Load Index (PLI) (all Spearman's  $r^2$  values were above 0.7). Therefore, soil multi-contamination by MM can be estimated for each plot by using PLI (Table 1) according to the formula (Rashed 2010):

$$PLI = \sqrt[4]{CF_{Pb} \times CF_{As} \times CF_{Zn} \times CF_{Cu}} \quad (1)$$

Contamination factor (CF) was calculated as follows:

$$CF_{MM} = \frac{[MM]_{\text{pseudo-total}}}{[MM]_{\text{local background value}}} \quad (2)$$

$[MM]_{\text{local background value}}$  are 42.9, 4.9, 66, and 7.5 mg/kg for Pb, As, Zn, and Cu, respectively (Affholder et al. 2014).

### Drought

For each month from February 2018 to December 2021, we calculated a xerothermic index ( $XTI_j$ ) based on the Gaussen index approximating drought and water availability for living organisms (Gaussen & Bagnouls 1952; Bagnouls & Gaussen 1957; Table 1):

$$XTI_j = -(P_j - 2T_j) \quad (3)$$

where  $j$  corresponds to the month of record,  $P_j$  to the total amount of monthly rainfall and  $T_j$  to the average monthly temperature (Table 1) recorded from the Marignane (13) weather station (WGS84 coordinates:  $x = 5.3533$ ,  $y = 43.2268$ ; available at: [https://www.meteociel.fr/climatologie/obs\\_villes.php?code=7650&mois=5&annee=2025](https://www.meteociel.fr/climatologie/obs_villes.php?code=7650&mois=5&annee=2025)). Gaussen index values above zero indicate drought periods with very few available water resource for living organisms.

Since there can be a time lag between weather conditions and their effects on plant communities, we calculated nine other xerothermic indices for each month, each corresponding to the mean XTI over the month of record ( $j$ ) plus the  $n - 1$  preceding months:

$$XTI_{j,n} = \frac{1}{n} \sum_{i=1}^n XTI_i \quad (4)$$

$j$  corresponds to the month of record and  $n$  to the number of months included in the calculation.

For instance,  $XTI_{\text{february2018},n=3} = (1/3) (XTI_{\text{february2018}} + XTI_{\text{june2018}} + XTI_{\text{december2017}})$ .

Based on absolute values of Spearman's correlation coefficients (Table S1), we determined that the index including the month of record and the 4 months preceding the month of record (i.e.  $XTI_{j,n=5}$ ) influenced the most plant community attributes. Therefore, we selected this index for the statistical analyses.

### Plant Species and Community Attributes

At the Escalette site, we considered plant communities both species planted during the ecological restoration operation and

**Table 1.** Minimum, first quartile, median, third quartile, and maximum of metal and metalloid pseudo-total concentrations in soil, pollution load index, total amount of monthly rainfall, average monthly temperature, xerothermic index, species richness, evenness, weighted mean nearest taxon distance, and weighted mean phylogenetic distance.

Variables		Minimum	First quartile	Median	Third quartile	Maximum
MM soil multi-contamination	Pseudo-total lead concentration in soil (mg/kg)	23,200	64,463	72,220	78,436	102,959
	Pseudo-total arsenic concentration in soil (mg/kg)	2905	8463	12,317	17,589	34,858
	Pseudo-total zinc concentration in soil (mg/kg)	8667	17,159	26,583	30,731	61,549
	Pseudo-total copper concentration in soil (mg/kg)	24	51	57	77	107
	Pollution Load Index	111	216	324	410	603
Drought	Total amount of monthly rainfall (mm)	0.0	9.4	38.5	74.4	263.0
	Average monthly temperature (°C)	6.0	11.0	16.6	22.9	27.0
	Xerothermic index	-87.4	-57.0	-17.9	-0.2	25.1
Plant community attributes	Species richness	2	3	4	6	15
	Pielou's evenness	0.10	0.66	0.77	0.87	1.00
	Weighted Mean Nearest Taxon Distance (million years)	15.4	187.7	223.0	241.2	650.1
	Weighted Mean Phylogenetic Distance (million years)	5.9	113.4	152.6	176.1	229.1

spontaneously established species in the plots. In each plot, the occurrence and the relative abundance of each species were recorded once a month from February 2018 to December 2021, using a 0.8 m<sup>2</sup> quadrat (excluding 0.2 m<sup>2</sup> of plots' limestone borders) divided in 80 sub-quadrates of 10 × 10 cm (Table S2). Plant species responses to soil multi-contamination by MM and drought depend on their ecological strategies, which are closely linked to their life cycle (annual or perennial). Therefore, life cycle information for each species was collected from regional floras (Tison & De Foucault 2014; Tison et al. 2014; Pavon & Pires 2020; Table S2). Two taxonomic diversity indices were calculated for each plant community (i.e. for each plot at each monthly survey) using *vegan* R package (Oksanen et al. 2020): species richness and Pielou's evenness (Table 1). Two phylogenetic diversity indices were computed using *picante* R package (Kembel et al. 2010) based on a chronogram built with *V.Phylomaker* R package (Jin & Qian 2019) and species abundances (Table 1): weighted Mean Phylogenetic Distance (wMPD) accounting for both closely related and distant evolutionary lineages, and weighted Mean Nearest Taxon Distance (wMNTD) representing the diversity of evolutionary lineages at the tips, that is, focusing on closely related lineages. In total, on the 20 plots studied monthly for 4 years, 880 observations were collected for each plant community attribute (i.e. species richness, evenness, wMPD, and wMNTD).

### Data Analysis

The aim of this study was to evaluate the effects of soil multi-contamination by MM (estimated by the PLI) and drought (estimated by the  $XTI_{j,n=5}$ ) on taxonomic and phylogenetic attributes of plant communities (estimated by species richness and evenness, and by wMNTD and wMPD, respectively), and on the absence probability and the abundance of each plant species. The validity of model assumptions (i.e. normality of residuals, normality of random effects, linear relationship, homogeneity of variance, and multicollinearity) was checked using the *performance* R package (Lüdecke et al. 2021).

### Effects of Soil Multi-Contamination by Metals and Metalloids and Drought on Taxonomic and Phylogenetic Attributes of Plant Communities.

First, the plots from the community dataset which did not contain any living plant or only one species at a given month of record (85 observations out of a total of 880) were removed. Second, the dataset was centered and scaled because variables were in different units. Third, a linear mixed-effects model was performed for each of the four plant community attributes using the *lme4* R package (Bates et al. 2015) as follows:

$$\text{Plant community attribute} \sim \text{PLI} + XTI_{j,n=5} + \text{PLI} \times XTI_{j,n=5} + (1|\text{Year}) \quad (5)$$

Plant community attribute corresponds to either species richness, evenness, wMNTD, or wMPD; PLI corresponds to the fixed effect of MM soil multi-contamination index;  $XTI_{j,n=5}$  corresponds to the fixed effect of xerothermic index including the month of record and the 4 months preceding the month of record;  $\text{PLI} \times XTI_{j,n=5}$  corresponds to the synergistic effect of PLI and  $XTI_{j,n=5}$ , and  $(1|\text{Year})$  corresponds to the random effect of the year of record (accounting for temporal autocorrelation).

### Effects of Soil Multi-Contamination by Metals and Metalloids and Drought on the Absence Probability and the Abundance of Annual and Perennial Plant Species.

First, species with less than 60 non-zero occurrences, that is, 11 species out of 29 were removed from the dataset (Table S2) because their low number of non-zero occurrences did not allow for building robust models. Second, the explanatory variables (i.e. PLI and  $XTI_{j,n=5}$ ) were centered and scaled because they were in different units. Third, hurdle mixed-effects models were used to model count data for each of the 18 remaining species. Hurdle models allow for modeling count data with an excess of zeros and manage overdispersion in both zeros and non-zero counts (Bolker et al. 2009; Brooks et al. 2017). Indeed, hurdle models are two-part models: (1) a binomial model to model the probability of measuring a zero, with no distinction between true

(i.e. structural errors) and false zeros (i.e. observation errors) (sensu Martin et al. 2005), (2) a truncated negative binomial model to model non-zero observations. The zeros in our dataset were assumed to be true zeros (sensu Martin et al. 2005), that is, a species from the local pool is absent because the environmental conditions (i.e. MM soil contamination and drought) are not suitable for its development at a given plot at a given record time. Thus, a hurdle mixed-effects model was performed for the probability of absence and the abundance of each plant species using the *glmmTMB* R package (Brooks et al. 2017) as follows:

$$\text{Count data for a species} \sim \text{PLI} + \text{XTI}_{j,n=5} + \text{PLI} \times \text{XTI}_{j,n=5} + (1|\text{Year}) \quad (6)$$

PLI corresponds to the fixed effect of MM soil multi-contamination index,  $\text{XTI}_{j,n=5}$  corresponds to the fixed effect of xerothermic index including the month of record and the 4 months preceding the month of record,  $\text{PLI} \times \text{XTI}_{j,n=5}$  corresponds to the interacting effect of PLI and  $\text{XTI}_{j,n=5}$ , and  $(1|\text{Year})$  corresponds to the random effect of the year of record (accounting for temporal autocorrelation) (Table S3, Fig. S1).

Finally, either the positive or negative impact of MM soil multi-contamination and drought, respectively, was assigned

to each species based on its effect on absence probability and abundance estimated from the hurdle model. A positive effect on species absence probability corresponds to a negative impact on species presence and vice versa. A positive effect on species abundance corresponds to a positive impact on species abundance and vice versa. For each stress (i.e. MM soil multi-contamination and drought), the number of effects showing non-significant, positive, and negative impacts on annual versus perennial species, irrespective of species absence probability or abundance, was counted.

## Results

### Effects of Soil Multi-Contamination by Metals and Metalloids and Drought on Plant Community Attributes

Soil multi-contamination by MM and drought had significant additive effects on the four plant community indices considered (Figs. 2 & 3). Soil multi-contamination by MM and drought had negative effects on species richness ( $\beta = -0.61^{***}$  and  $-0.37^{***}$ , Fig. 2A1 & 2B1, respectively), on evenness ( $\beta = -0.13^{***}$  and  $-0.21^{***}$ , Fig. 2A2 & 2B2, respectively), and on wMPD ( $\beta = -0.37^{***}$  and  $-0.30^{***}$ , Fig. 3A1 & 3B1, respectively). In contrast, soil multi-contamination by MM and drought had positive effects on wMNTD ( $\beta = 0.33^{***}$  and  $0.27^{***}$ , Fig. 3A2 & 3B2, respectively).

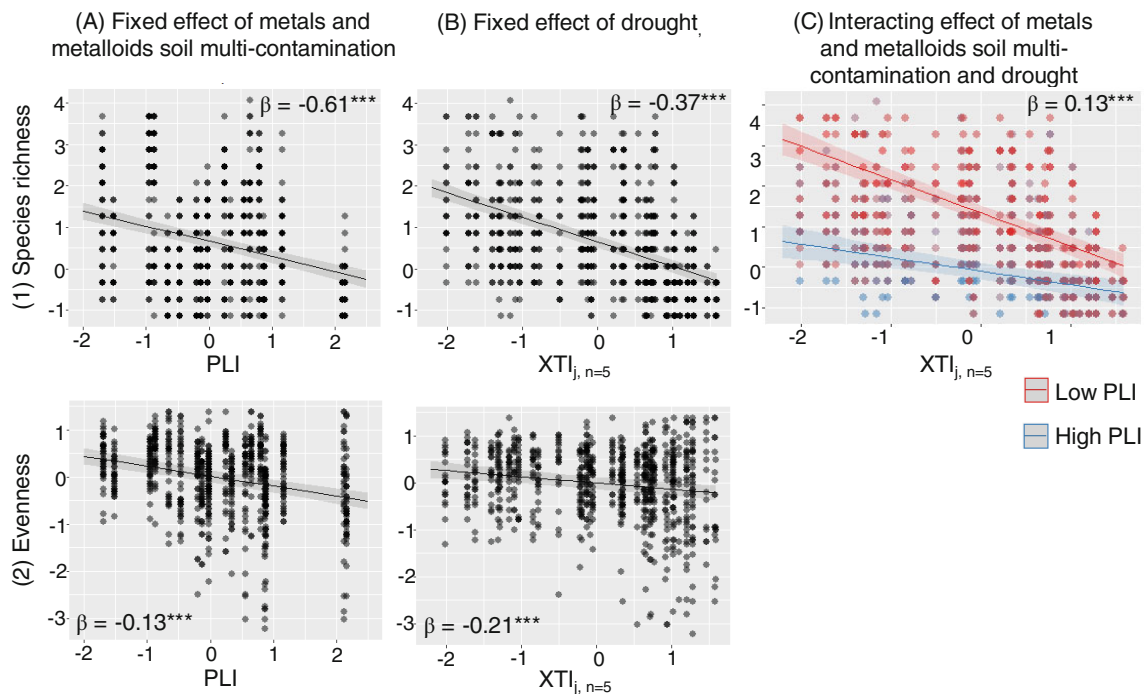


Figure 2. Linear mixed-effects models of the relationships between taxonomic attributes of plant community ([1] species richness and [2] evenness), soil multi-contamination by metals and metalloids, and drought. All the data were centered and scaled. (A) Fixed effects of soil multi-contamination by metals and metalloids (estimated by the PLI: Pollution Load Index) on taxonomic attributes of plant community. (B) Fixed effects of drought (estimated by the xerothermic index  $\text{XTI}_{j,n=5}$  including the month of record and the 4 months preceding the month of record) on taxonomic attributes of plant community. (C) Interacting effect of soil multi-contamination by metals and metalloids and drought on species richness. Dots and solid lines represent the observed and predicted values, respectively. The shaded areas around the solid lines represent the 95% CI. The model's estimate and the level of significance ( $***p \leq 0.001$ ) are indicated on each graph.

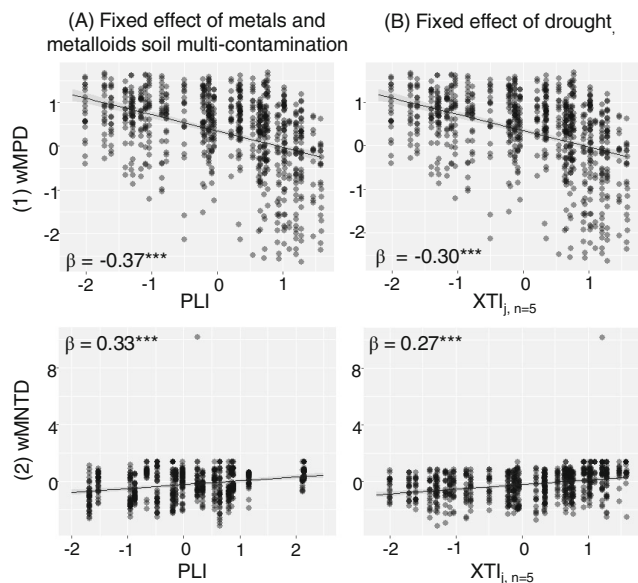


Figure 3. Linear mixed-effects models of the relationships between phylogenetic attributes of plant community ([1] wMPD: weighted Mean Phylogenetic Distance accounting for both closely related and distant evolutionary lineages, and [2] wMNTD: weighted Mean Nearest Taxon Distance focusing on closely related lineages), soil multi-contamination by metals and metalloids, and drought. All the data were centered and scaled. (A) Fixed effects of soil multi-contamination by metals and metalloids (estimated by the PLI: Pollution Load Index) on phylogenetic attributes of plant community. (B) Fixed effects of drought (estimated by the xerothermic index  $XTI_{j,n=5}$  including the month of record and the 4 months preceding the month of record) on phylogenetic attributes of plant community. Dots and solid lines represent the observed and predicted values, respectively. The shaded areas around the solid lines represent the 95% CI. The model's estimate and the level of significance ( $***p \leq 0.001$ ) are indicated on each graph.

Soil multi-contamination by MM and drought had a significant synergistic effect on species richness ( $\beta = 0.13***$ , Fig. 2C1): as drought increased, species richness decreased more strongly at low levels of soil multi-contamination by MM than at high levels. However, whatever the level of soil multi-contamination by MM, communities converged to a very low species richness at the highest levels of drought.

#### Effects of Soil Multi-Contamination by Metals and Metalloids and Drought on Plant Species

Irrespective of species absence probability or abundance, the number of modeled effects supporting a negative versus positive impact of soil multi-contamination by MM and drought on annual versus perennial species was counted.

Out of the 14 modeled effects (seven species  $\times$  two response variables) of MM soil multi-contamination on annual species (Fig. 4A), 10 were significant, of which 4 showed a positive impact and 6 a negative impact on species presence and abundance. In contrast, out of the 22 modeled effects (11 species  $\times$  two response variables) of MM soil multi-contamination on perennial species (Fig. 4A), 16 were

significant, of which most showed a negative impact (14) and only 2 a positive impact on species presence and abundance.

Out of the 14 modeled effects of drought on annual species (Fig. 4B), 13 were significant and always showed a negative impact on species presence and abundance. In contrast, out of the 22 modeled effects of drought on perennial species (Fig. 4B), only 8 were significant and showed a negative impact on species presence and abundance.

Soil multi-contamination by MM and drought had significant interacting effects on the probability of absence of the annual *Bromus madritensis* L. (*Poaceae*) ( $\beta = -0.266**$ , Fig. 5), and the perennials *Lobularia maritima* (L.) Desv. (*Brassicaceae*) and *Sonchus bulbosus* (L.) N.Kilian & Greuter (*Asteraceae*) ( $\beta = -0.200*$  and  $-0.347*$ , respectively, Fig. 5). As drought increased, species absence probability increased more strongly at low levels of MM soil multi-contamination than at high levels. However, whatever the level of MM soil multi-contamination, species converged to a high probability of absence at the highest levels of drought.

## Discussion

This study investigated how combined soil multi-contamination by MM and drought impacts plant community attributes and species dynamics during ecological restoration in Mediterranean scrublands. Our results demonstrate that these co-occurring stresses act as strong environmental filters, reducing species richness, evenness, and phylogenetic diversity of distant lineages while favoring closely related, stress-tolerant species. This environmental filtering process may reflect the compounding effects of metal and metalloid-induced water stress combined with natural drought conditions, both of which impose similar physiological and metabolic constraints on plants.

#### Community-Level Responses: Additive and Synergistic Effects

Soil multi-contamination by MM and drought had negative additive effects on plant species richness, evenness, and wMPD (accounting for both closely and distant lineages), and a positive additive effect on wMNTD (biased toward closely related lineages). Combined MM soil multi-contamination and drought acted as a strong environmental filter by selecting a pool of evolutionarily closely related species tolerant to both contamination by MM and drought and eliminating very distinct lineages and less tolerant species. This environmental filtering process can result from the increase of the negative effects of drought by MM soil multi-contamination due to the additional MM-induced water stress in plants (Poschenrieder & Barcelo 1999; Rucińska-Sobkowiak 2016). Among plant community attributes, diversity of very distinct lineages (i.e. wMPD) decreasing in the face of combined MM soil multi-contamination and drought is particularly significant because wMPD is a key facet promoting ecosystem functioning in water-limited (Le Bagousse-Pinguet et al. 2019) and MM-contaminated environments (Tosini et al. 2023).

The synergistic effect of MM soil multi-contamination and drought on plant species richness showed that as drought

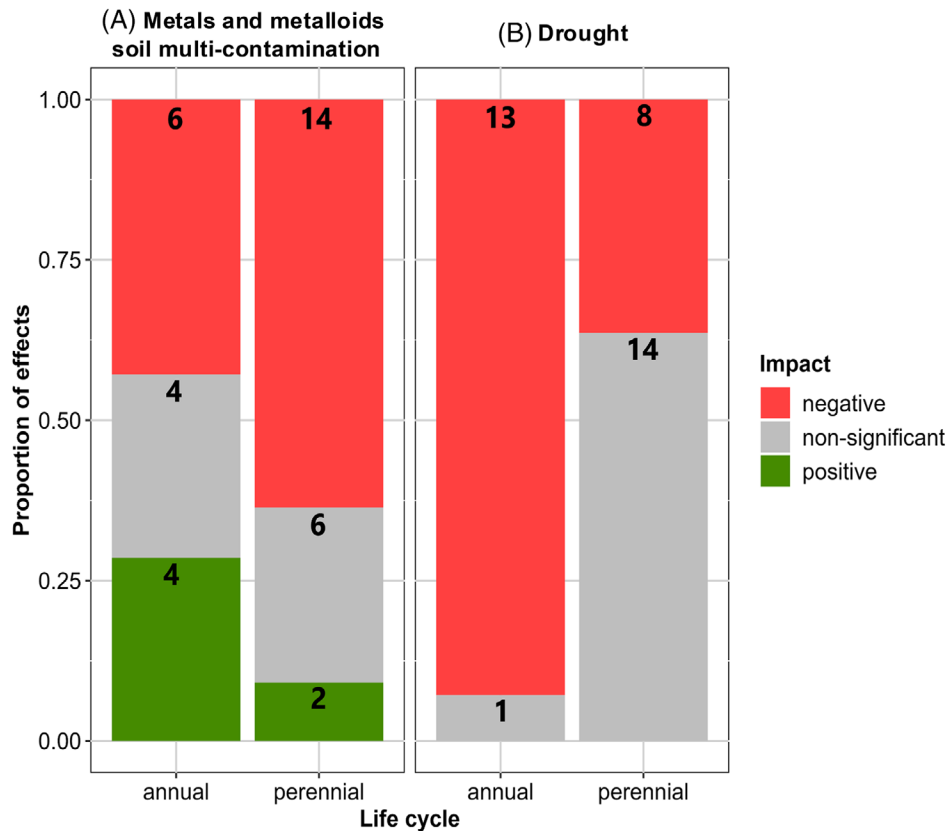


Figure 4. Proportion (on y-axis) and number (on bar plots) of effects showing non-significant versus positive and negative impacts of (A) soil multi-contamination by metals and metalloids (estimated by the PLI: Pollution Load Index) and (B) drought (estimated by the xerothermic index  $XTI_{j,n=5}$  including the month of record and the 4 months preceding the month of record) on annual versus perennial species. Displayed additive effects are from hurdle mixed-effects models, either on absence probability or abundance.

increased, species richness decreased more strongly at the lowest levels of multi-contamination than at the highest. At the lowest level of MM soil multi-contamination, the increase of drought resulted in a significant species loss because of the disappearance of less tolerant species. At the highest levels of MM soil multi-contamination, the increase of drought resulted in a low species loss, suggesting that contamination had already selected for the most tolerant species to both MM multi-contamination and drought.

#### Species-Specific Responses

The combined stresses differentially affected annual versus perennial species, reflecting their distinct ecological strategies. Annual species were mainly impacted by drought, whereas perennial species were affected by MM soil multi-contamination. Indeed, drought had 2.6 times more negative impacts on annual than on perennial species. Annual species complete their development cycle during favorable periods and spend dry periods as seeds as a temporal stress-avoidance strategy, whereas perennial species have to cope with drought by using a water stress-tolerance strategy (Jenks &

Hasegawa 2014). Even if the tolerance of perennial species to drought can enhance their tolerance to MM by limiting water stress in plants (Poschenrieder & Barcelo 1999; Rucińska-Sobkowiak 2016), MM soil multi-contamination had 1.8 times more negative impacts on perennial species than drought. This result suggests that soil MM multi-contamination can generate more complex effects on plant community than simply selecting tolerant species through biotic and abiotic filters.

The differential responses of plant species to combined MM contamination and drought are also closely linked to their water resource acquisition. Metal contamination can significantly impair root hydraulic conductivity and water uptake efficiency, forcing plants to invest more energy in water acquisition mechanisms (Rucińska-Sobkowiak 2016). Mediterranean perennial species often rely on deep root systems and efficient water storage tissues to cope with seasonal drought (Padilla & Pugnaire 2006), but these adaptations may be compromised by MM-induced damage to root cell membranes and xylem functionality (Perfus-Barbeoch et al. 2002). Furthermore, the ability of plants to form beneficial associations with mycorrhizal fungi, which significantly enhance water and nutrient uptake in water-limited environments, can be severely reduced under high metal

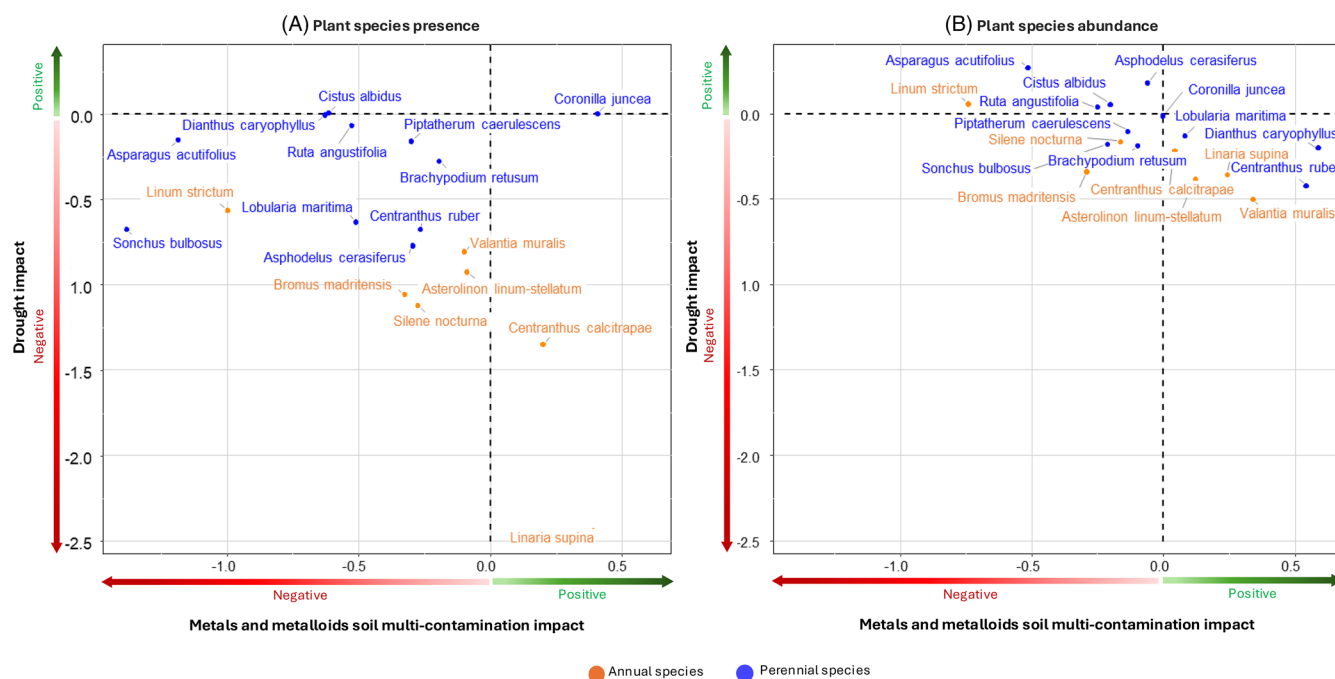


Figure 5. Estimates from the hurdle mixed-effects models of soil multi-contamination by metals and metalloids (estimated by the pollution load index) and drought (estimated by the xerothermic index  $XTI_{j,n=5}$  including the month of record and the 4 months preceding the month of record) effects on (A) presence and (B) abundance of plant species.

contamination (Mitra et al. 2021). This disruption of plant-mycorrhizal partnerships may explain why perennial species, despite their inherent drought tolerance mechanisms, showed greater sensitivity to metal and metalloid contamination than to drought alone in our study.

However, soil multi-contamination by MM had a positive impact on two perennial species (*Coronilla juncea* and *Dianthus caryophyllus*) and three small annual species (*Centranthus calcitrapae* [L.] DuRoi. [Caprifoliaceae], *Linaria supina* [L.] Chaz. [Plantaginaceae], and *Valantia muralis* L. [Rubiaceae]), potentially due to competition relaxation processes. The perennial species *C. juncea* and *D. caryophyllus* could take advantage of highly contaminated and unoccupied ecological niches to grow, underlining their tolerance to MM soil multi-contamination, as shown by the relatively low impact of MM soil on *C. juncea* root development (Heckenroth et al. 2022). Because of their very small size, the annual species *C. calcitrapae*, *L. supina*, and *V. muralis* could take advantage of the micro-heterogeneity of soil contamination to grow in less contaminated and unoccupied micro-niches (i.e. spatial stress-avoidance strategy).

### Restoration Implications

Our results highlight the significance of investigating the effects of combined MM soil multi-contamination and drought at both community scale and species scale to better understand the environmental filtering process through plant species' ecological strategies.

As drought and MM soil contamination can both generate water stress in plants (Poschenrieder & Barcelo 1999; Rucińska-Sobkowiak 2016), our results support the hypothesis that plant species can share similar responses to these two abiotic stresses (de Silva et al. 2012), e.g. synthesis of osmoprotective compounds to maintain cell homeostasis (Sharmila & Pardha Saradhi 2002), reduction of leaf sizes, thickness, and stomata density to limit water loss (Rucińska-Sobkowiak 2016), associations with mycorrhizal fungi to increase water supply (Gamalero et al. 2009). This result highlights the potential of scrubland plant communities for ecological restoration of MM-contaminated Mediterranean ecosystems. However, although species tolerance to drought can enhance their tolerance to MM soil multi-contamination, extreme levels of drought strongly reduce species richness within plant communities, regardless of the level of multi-contamination. Since droughts will become increasingly intense and frequent in Mediterranean ecosystems (Cramer et al. 2018) and the cooccurrence of MM soil contamination and drought can drastically impact plant biodiversity, it is necessary to adapt ecological restoration and phytoremediation practices to enhance plant community resilience in the face of global warming.

Our results help to identify relevant plant community attributes and species for ecological restoration of MM-contaminated Mediterranean ecosystems. Among plant community attributes, phylogenetic diversity appeared to be a powerful criterion. Phylogenetic indices including both closely related and distant lineages (e.g. wMPD and wMNTD) can be antagonistically impacted by combined soil multi-contamination by

MM and drought. As a result, considering different types of phylogenetic diversity indices can be relevant for ecological restoration. In addition to being a non-destructive and relatively easy to measure metric, phylogenetic diversity can integrate biological traits (Flynn et al. 2011), especially root associations with beneficial microorganisms (Brundrett 2002; Werner et al. 2015) improving MM plant tolerance (Gamalero et al. 2009) and ecosystem functioning (Bardgett et al. 2014). The cooccurrence of distant lineages has been identified as a key attribute of plant biodiversity to improve ecosystem functioning in a Mediterranean scrubland contaminated with MM (Tosini et al. 2023).

Although no plant species was favored by both MM soil multi-contamination and drought, the diversity of species response to these combined abiotic stresses revealed a tolerance gradient. Overall, perennial species, in addition to being potential candidates for phytostabilization (Bolan et al. 2011), appeared to be more tolerant in the face of combined MM soil multi-contamination and drought than annual species. *C. juncea* was the most tolerant to combined drought and high MM soil multi-contamination. *C. juncea* has already been identified as a good candidate for the ecological restoration of MM-contaminated Mediterranean ecosystems (Heckenroth et al. 2022) because of its engineer and nurse effect (sensu Padilla & Pugnaire 2006) facilitating other plant species establishment by improving soil nitrogen content through its root association with nitrogen-fixing bacteria and endomycorrhizal fungi (Carrasco et al. 2011). Among perennial species, other species present: (1) a good tolerance to drought combined with a moderate tolerance to MM soil multi-contamination (e.g. *Cistus albidus* and *Ruta angustifolia* [Rutaceae]), or (2) a moderate tolerance to both MM soil multi-contamination and drought (e.g. *Brachypodium retusum*, *D. caryophyllus*, *Lobularia maritima*, and *Piptatherum caeruleum* [Poaceae]). Out of the 8 planted native species (*C. juncea*, *C. albidus*, *Globularia alypum*, *Salvia rosmarinus*, *Teucrium polium*, *D. caryophyllus*, *B. retusum*, and *L. maritima*), five (*C. juncea*, *C. albidus*, *D. caryophyllus*, *B. retusum*, and *L. maritima*) can be relevant for the ecological restoration of metal-contaminated Mediterranean ecosystems, as well as two other species (*R. angustifolia* and *P. caeruleum*) which grow spontaneously on the Escalette ecological restoration plots. When combined in a plant community, these species exhibit a diversity of foliar and root functional traits (Gachet et al. 2005; de la Riva et al. 2017; Shihan et al. 2022), which may allow for differentiated resource use in time and space (i.e. niche differentiation), improving ecosystem functioning (Gross et al. 2017).

Further investigations on the role of phylogenetic diversity and plant functional traits in ecological restoration and phytostabilization processes could optimize criteria for selecting plant assemblages and evaluating the success of ecological restoration and phytostabilization in metal and metalloids-contaminated Mediterranean ecosystems.

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## Supporting Information

The following information may be found in the online version of this article:

**Table S1.** Coefficients of Spearman correlation and level of significance.

**Table S2.** Number of zero and non-zero observations for each of the 29 annual and perennial species present on the ecological restoration plots of the Escalette site from February 2018 through December 2021.

**Table S3.** Estimates and standard errors from the hurdle models performed with species count data.

**Figure S1.** Estimates of hurdle mixed-effects models between plant species counts, soil multi-contamination by metals and metalloids and drought.

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