Gain in biodiversity but not in phytostabilization after 3 years of ecological restoration of contaminated Mediterranean soils

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Highlights

- Metal and metalloid soil contamination slows down the ecological restoration dynamics
- Native plants transplanted in Mediterranean contaminated soils persist after 3 years
- Active ecological restoration allows the creation of favorable micro-niches for plants
- Metal and metalloid phytostabilization efficiency is not evidenced after 3 years

Abstract

Recent studies consider the native flora as a potential source of plant candidates for phytostabilization of metal and metalloid (MM) contaminated soils, but ecological restoration is not the main objective of these researches. However, in contaminated areas, phytostabilization should be considered as a useful tool for ecological restoration. The present study takes stock of 3 years of a Mediterranean pilot site implementation using native plant species to recover plant and microbial communities (diversity and functions) together with soil remediation in the Calanques National Park. To determine the success of this operation, three *in situ* treatments were compared: ecological restoration plots characterized by the handling of physical environment (creation of cultivation terraces) and plants, negative control plots without vegetation but with the same physical environment handling as ecological restoration plots, and positive control plots with natural vegetation and no

handling. The results suggest that an ecological restoration trajectory is initiated in the ecological restoration plots, characterized by a partial permanent plant cover. However, there is no evidence of a significant improvement of soil quality (evaluated by soil texture, pH, nutrients and organic carbon contents, cation exchange capacity, microbial biomass and activities) and phytostabilization efficiency after 3 years. Native plant communities and their associated microorganisms may need more time before improving soil quality and MM stabilization under the drastic Mediterranean conditions. Any amendment addition to accelerate restoration and MM immobilization was forbidden in this protected area. Under such conditions, an active restoration need to be carried out in this contaminated area even if resilient dynamics of the native plant communities may sporadically occur over a long period of time.

Keywords: active restoration, field experiment, Mediterranean protected area, metals and metalloids, native plant communities, resilient dynamics

1. Introduction

Human activities generate a significant degradation of ecosystems and specifically of soils. Indeed, in 2015, 33 % of soils were considered as moderately or severely degraded by erosion, nutrient depletion, acidification, salinization, compaction and chemical pollution (FAO, 2015). The European Strategy for Biodiversity expects at least 15% of degraded ecosystems to be restored by 2020 (EU, 2011). In this context, some scientists are currently working on ecological restoration of polluted environments (Wong, 2003; Li, 2006) particularly in the Mediterranean region (Heckenroth et al., 2016b). Ecological restoration is the intentional activity that assists ecosystem restoration in order to regain its structural and functional integrity, allowing it to be resistant and/or resilient to normal stress and environmental disturbance levels (SER, 2004). Ecological restoration can be active or passive depending on the level of ecosystem degradation (SER, 2016). Facing weak degradation, stopping the disturbance is often enough for spontaneous plant recolonization of the site (i.e. spontaneous regeneration through passive ecological restoration). When degradation is significant, active restoration operations are required by restoring abiotic components (i.e. assisted regeneration) or both abiotic and biotic components (i.e. reconstruction). In the case of contaminated soils, ecological restoration can be combined with phytoremediation techniques to decontaminate the environment or to avoid pollutants dispersion (Wong, 2003; Li, 2006). Phytostabilization consists of using plants and their associated microorganisms to

stabilize contaminants in the roots and/or rhizosphere to limit their transfer *via* erosion, leaching or runoff processes and their bioavailability (Pilon-Smits, 2005) and to promote restoration of contaminated ecosystem especially by metals and metalloids (MM) (Ma et al., 2011; Marchiol et al., 2013). The success of an ecological restoration combined with phytostabilization is based on several criteria. Plant species selection from the local floristic background is essential. The selection must consider plant adaptations to local conditions and their MM tolerance which promote plant survival and development (Baker et al., 2010; Bolan et al., 2011; Cortina et al., 2011). Moreover, permanent and large root systems and plant cover, as well as plant MM immobilization capacities, permit to reduce soil erosion and MM bioavailability, thus limiting contaminant dispersion and transfer into the environment (Pilon-Smits, 2005; Bochet et al., 2006; Bolan et al., 2011). In addition, consideration of biotic interactions is essential, in particular in harsh environments (Padilla and Pugnaire, 2006; Cortina, 2011). Facilitation by nurse plants, favoring micro-niche formation by improving microclimatic and edaphic conditions (Callaway, 2007), permits establishment of other plant species on unfavorable environments (Frérot et al., 2006). Plant associated microorganisms, besides potentially participate in MM immobilization, improve plant MM tolerance by enhancing nutrient and water absorption (Khan, 2005; Ma et al., 2011). Better consideration of soil quality, its contamination level and the contamination spatial heterogeneity are necessary to think how to improve soil properties involved in contaminant retention in order to reduce their bioavailability (Adriano et al., 2004; Bolan et al., 2011; Kumar Yadav et al., 2018). However, the use of ecological restoration tools combined with those of phytostabilization is still very recent and these success criteria are still to be assessed. Moreover, ecological restoration in Mediterranean dry habitats is a challenging topic and implies inventiveness in the developed processes to enhance vegetation establishment (Jaunatre et al., 2012; Rey and Burylo, 2014; Ballesteros et al., 2017). However, in addition to biogeoclimatic constraints framing the tools used, accessibility, pollution, topographical and regulatory constraints may restrict possible actions.

Mining areas are relevant study cases of such approaches and literature on MM phytoremediation by using autochthonous plant species is becoming more important this last decade (Ilunga wa Ilunga et al., 2015; Marrugo-Negrete et al., 2015; Ghazaryan et al., 2019). However, fewer restoration trials are conducted in brownfields generating diffuse pollution of soils. One of those brownfields, the Escalette site, is located on the Mediterranean coast of Marseille (south-eastern France) at the outskirt of the city center. This site sheltered a lead-

smelting factory that operated from 1851 to 1925 using galena imported from Mediterranean countries (Daumalin and Raveux, 2016). This activity contributed to a diffuse MM contamination of surface soils of the surrounding area by slag deposits and by dispersion of contaminated ashes from the factory horizontal chimney *via* erosion and bioalteration processes (Testiati et al., 2013; Heckenroth et al., 2016a; Laffont-Schwob et al., 2016). The brownfield is now included in the protected area of the Calanques National Park (PNCal), the first French peri-urban National Park founded in 2012. This territory gathers several socio-environmental issues (environmental health, biodiversity conservation and management, regulatory issues) linked with anthropogenic disturbances (industrial and urban pollution, soil erosion, over-frequentation), affecting this urban and natural protected and biodiversity-rich area (Affre et al., 2015). At the Escalette site, the natural resilience capacity of the original ecosystem has been strongly affected by MM soil contamination, which is currently reflected by the presence of unvegetated scree which can constitute a source of contamination transfer by water erosion and/or wind (Heckenroth et al., 2016b). In November 2015, a joint active ecological restoration and phytostabilization trial has been implemented in this site (Heckenroth et al., 2016a). This pilot-scale experiment aims at stabilizing MM soil contamination sustainably by favoring a permanent native vegetation cover to limit its transfer, while considering restoration of plant and soil biodiversity and functionalities. Three years after the implementation of this experiment, two main questions remain: (i) At which step along an ecological restoration trajectory correspond the implemented plant communities? (ii) Do they ensure effective stabilization of MM soil contamination? To answer these questions, a comparative *in situ* monitoring of soil properties, MM concentrations in soils and in runoff water and characteristics of plant communities was performed. These parameters were monitored over three *in situ* treatments: ecological restoration plots characterized by the handling of physical environment variables (creation of cultivation terraces) and plants, negative control plots without vegetation but with a physical environment variable handling (terraces) such as ecological restoration plots, and positive control plots with natural vegetation and nor abiotic neither biotic variables handling.

2. Material and methods

2.1. Study site

The study site is located in the Marseilleveyre calcareous massif, characterized by significant karstic erosion and skeletal oligotrophic soils (Knoerr, 1959). The study site has a semi-arid

Mediterranean climate, characterized by hot and dry summers, mild and wet winters (Quézel and Médail, 2003) and by a prevailing cold, dry and often violent wind, called Mistral (Aillaud and Crouzet, 1988), accentuating the environment's aridity (average annual rainfall of 500 mm, average annual temperature of 16 °C). The study site is located at the Escalette industrial brownfield, at the outfall of the horizontal chimney of a former lead smelting factory, in the protected area of the PNCal (WGS84 co-ordinates: 43.22578°N, 5.35059°E, Fig. A). The site corresponds to a steep scree with an average slope of 23 %, located down below a ridge at 140 m of altitude. This site is defined as a hotspot of contamination mainly by lead (Pb) and arsenic (As) but also by copper (Cu) and zinc (Zn) (Testiati et al., 2013). The soil, whose depth varies between 10 and 30 cm, corresponds to a mixture of limestone, clayey limestone, marl (Knoerr, 1959) and contaminated ashes. A layer of ashes from a few to several centimeters covers the soil at the outfall of the horizontal chimney, which can then be defined as anthroposol.

In November 2015, an active ecological restoration has been carried out at the Escalette site by environmental handling and planting. This operation is in accordance with the PNCal's regulations, which prohibit the introduction of non-local plant material and the use of amendments to conserve local biodiversity. 20 plots of 1 m² were created on the unvegetated steep scree. These plots correspond to cultivation terraces made up of two strips, one upstream and one downstream. Limestones present on the site were removed from the soil surface and used to shape the terraces by installing a row of stones between upstream strip and downstream strip of cultivation terraces. Limited soil handling was targeted. Eight plant species from the local floristic background (constituted of calcareous xero-thermophilous shrubland: Mediterranean scrubland, grasslands and stands of Aleppo pine) and defined as pseudo-metallophytes according to the definitions given by Pauwels et al. (2006) and Faucon et al. (2009) were transplanted in each plot: *Brachypodium retusum* (Pers.) P.Beauv., 1812, *Cistus albidus* L., 1753, *Coronilla juncea* L., 1753, *Dianthus caryophyllus* L., 1753, *Globularia alypum* L., 1753, *Lobularia maritima* (L.) Desv., 1815, *Rosmarinus officinalis* L., 1753 and *Teucrium polium* L., 1753 (Heckenroth et al., 2017, Fig. B). Soil surface was covered with limestones to limit water stress. A unique watering was done to the plant's earth clod at the transplantation. Nor amendment adding neither watering was carried out after transplantation.

2.2. Experimental design

Three treatments (E: ecological restoration plots, Cn: negative control plots, Cp: positive control plots), each consisting of 5 replicates, were tested *in situ* (Fig. 1). E and Cn plots have been located on the unvegetated scree at the beginning of the study and correspond to active restoration with different levels of intervention. E plots are characterized by environmental and plant handling (i.e. creation of cultivation terraces and planting, respectively). E plots correspond to 5 plots selected from those implemented in November 2015 and contain planted and spontaneous plant species. These E plots were selected for their well-established perennial vegetation and the persistence of planted *C. juncea* individuals, assuming that their highly developed root systems act on soil contamination fixation. Cn plots are characterized by the same environmental handling as E plots, i.e. 1 m²-cultivation terraces located on the unvegetated scree, but without planting. E and Cn plots were equipped with runoff water collectors in November 2018. Runoff water collectors consist of a plastic chute placed at the level of the downstream band of the plots, closed at the end and connected to a can of water recovery by a funnel. A plastic tarpaulin was installed at ground level, under the stones of the downstream band of the terraces and, attached to the chute. Another tarpaulin was placed over the chute to recover only runoff water and not rainwater. This installed system can be easily dismantled as required by the PNCal regulation. Cp plots correspond to the reference plant community of this ecological restoration operation, i.e. a natural vegetation of young Mediterranean scrubland that has sporadically persisted during past industrial activities until today. No ecological restoration work was carried out on these plots, they have only been spatially delimited by limestones. Thus, Cp plots have not been structured in cultivation terraces or equipped with runoff water collectors to avoid disturbance and degradation due to abiotic handling. Cp are located close to E and Cn plots to maintain comparable soil contamination levels between the active restoration plots (E and Cn) and reference plots (Cp). This allows a realistic ecological restoration objective to be defined on MM contaminated soil.

2.3. Soil analysis

Soil samples were collected in 4 points at each plot corner in the top 15 cm-layer and pooled (mix of an equal weight of the 4 subsamples on each plot). The obtained composite soil samples for each E, Cp, and Cn plots were sieved at 2 mm and analyses of agronomic characteristics, MM contamination and microbial properties were performed.

2.3.1. Soil agronomic characteristics

Soil agronomic analyses were carried out on 40 °C dried soil. pH was determined with the Orion 2 Star Thermo Scientific SM30B device, in a solution of soil and distilled water (1/5, V/V, NF ISO10390, 2005). Total nitrogen content (TKN) was estimated by the Kjeldahl method (NF ISO 11261, 1995). Total organic carbon (TOC) was determined by the difference between total carbon content and inorganic carbon content (NF ISO 10694, 1995). Granulometry and texture (NF X 31-107, 2003), available phosphorus (P, NF ISO 11263, 1995) and cation exchange capacity (CEC, NF X 31-130, 1999) measurements were performed in the Laboratoire Développement Méditerranée (Alès, France).

2.3.2. Soil contamination

Soil samples were dried at 40 °C and ground at 0.2 mm (RETSCH zm 1000 with tungsten blades and titanium sieve) in order to evaluate soil contamination level and MM mobilization in soil. In order to determine pseudo-total MM concentrations (MM_{tot}), samples were digested in a microwave mineralizer (Milestone Start D) using aqua regia (1/3 HNO_3 + 2/3 HCl), then filtered through a 0.45 µm cellulose ester membrane filter (NF ISO 11466, 1995). For mobile MM concentrations (MM_{mob}), 0.05 M EDTA (pH 7.0 ± 0.1) solution was used as extractant following the method approved by the Community Bureau of Reference (CBR) (Quevauviller, 1998). A ratio of soil/EDTA solution corresponding to 1/10 w/V was used. The solution was stirred at room temperature for 1 h, then centrifuged during 10 min at 8,000 rpm and filtered at 0.45 µm. MM_{tot} and MM_{mob} concentrations were determined by ICP-AES (Jobin Yvon Horiba, Spectra 2000) for As, Cu, Pb and Zn. Quality controls and accuracy were checked using standard soil reference materials (CRM 049-050, from RTC-USA), with accuracies within 100 ± 10 %. Contamination factors (CF) were calculated according to the formula:

Eq. 1. $CF_{MM}=[MM]_{tot}/[MM]_{local\ background\ value}$, where $[MM]_{local\ background\ value}$ are 4.9, 7.5, 42.9, 3.1 and 66 $mg.kg^{-1}$ for As, Cu, Pb, Sb and Zn, respectively, as previously reported with the same method used in the present study (Affholder et al., 2014).

Then, the multi-contamination level was estimated by calculating the pollution load index (PLI) according to the formula (Rashed, 2010; Affholder et al., 2014):

Eq. 2. $PLI=\sqrt[4]{CF_{As} \times CF_{Cu} \times CF_{Pb} \times CF_{Zn}}$

Percentage of mobile MM in soil were calculated according to the formula (Affholder, 2013):

Eq. 3. $\%MM_{mob} = ([MM]_{mob}/[MM]_{tot})\ x100$

2.3.3. Soil microbial analysis

Soil microbial analyses were performed in order to assess the eco-physiological state of soil microbial communities.

Soil basal respiration (SBR) and substrate-induced respiration (SIR) were measured to assess global microbial activity and biomass (Anderson and Domsch, 1978; Anderson, 2003). Ten grams dry weight equivalent of fresh soil at 30 % of water holding capacity 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 and Domsch (1978). 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 was oxygenated for 4 min and then incubated for 2 h at 23 °C in tightly closed glass jars. Then, 1 mL of their atmosphere was sampled in the head space with a syringe and injected into a gas chromatograph (Chrompack CHROM 3 – CP 9001) to analyse CO_2 production by microbial oxidation of glucose. The gas chromatograph was equipped with a thermal conductivity detector and a packed column (Porapack). The carrier gas helium flow was regulated at 60 $mL.h^{-1}$. Ambient CO_2 concentrations were subtracted from sampled CO_2 concentrations and resulting values were adjusted at 22 °C according to Ideal Gas Laws using a Q10 = 2. SIR was converted into microbial biomass (MB) using the relation established by Beare et al. (1990). Then, the metabolic quotient (qCO_2), which is a sensitive eco-physiological indicator of soil stress induced by environmental conditions (Anderson, 2003), was obtained by calculating the SBR/MB ratio.

Glomalin-related soil protein (GRSP), a fungal glycoprotein synthetized by certain arbuscular mycorrhizal fungi involved in soil quality (Zhu and Miller, 2003; Rillig et al., 2004) and MM chelation (Cornejo et al., 2008; Vodnik et al., 2008), was quantified. Three analytical replicates were made for each sample. GRSP was extracted by repeated autoclaving extraction (Advantage-Lab ALO2-05) from 0.5 g of 40 °C dried soil in 4 mL of 50 mM

citrate pH 8.0 at 121 °C for 60 min. After each autoclaving cycle, the supernatant was removed by centrifugation (Hettich Zentrifugen Universal 320R) at 7000 g for 20 min. The extraction cycles were repeated until the supernatant had lost its dark brown-red color (8 cycles). Supernatants of the different extraction cycles of the same analytical replica were pooled and then centrifuged at 7000 g for 10 min. GRSP content was measured in 1 mL supernatant by spectrophotometric dosage (595 nm) according to Bradford method, by using a Bovin Serum Albumin solution as standard. Finally, GRSP concentration was converted to $g.kg^{-1}$ of dry soil.

2.4. Runoff water analysis

Runoff water were collected on each E and Cn plots over the period from 24 January 2019 to 15 April 2019 during which there was 97.75 mm of rainfall. MM concentrations in runoff water (MM_{water}) were determined by ICP-AES (Jobin Yvon Horiba, Spectra 2000) for As, Cu, Pb and Zn, for each E and Cn plot in order to evaluate MM transfer by hydric erosion. Before that, 20 mL of runoff water were filtered to 0.45 µm, and 0.2 mL of nitric acid (Trace metal grade HNO_3) were added to them. Quantity of each MM exported in runoff water (MM_{water}) was calculated according to the formula:

Eq. 4. $MM_{water} = [MM]_{water}$ / water volume of the collector

2.5. Vegetation analysis

Vegetation analysis was performed in order to characterize the structure (plant cover), the composition (richness, diversity, equitability) and the sustainability (Raunkier plant life-form, Grime adaptative strategy) of each plant community. Cn plots, which were unvegetated at the beginning of this study, were considered in plant community analysis because plants established during the experiment.

Plant cover was estimated by different percentage class: < 10 %, 10-25 %, 25-50 %, 50-75 %, > 75 % (Braun-Blanquet et al., 1952). The following indices were estimated along with presence/absence and abundance field data: specific richness, Shannon's diversity index, and Pielou's equitability index (Wheater et al., 2011).

Plant functional traits (Garnier and Navas, 2013; Pérez-Harguindeguy et al., 2016) were studied from BASECO database (Gachet et al., 2005). Percentages of plant species in a plot per Raunkier plant life-form (geophyte, chamaephyte, hemicryptophyte, therophyte, phanerophyte) correspond to the ratio of the number of species in a plot with a given Raunkier

life-form on the total number of species in a plot. Percentages of plant species in a plot per Grime adaptative strategy (stress tolerant, competitive stress tolerant, ruderal stress tolerant, competitive ruderal stress tolerant) correspond to the ratio of the number of species in a plot with a given Grime adaptative strategy on the total number of species in a plot.

2.6. Statistical analysis

Statistical analyses were conducted with R (3.4.2 version). *P*-values were considered as significant when they were less than or equal to 0.05.

The two plant categories in E plots (i.e. planted and spontaneous) have been considered without distinction in statistical analysis because it was not possible to discriminate on field planted individuals from spontaneous individuals which came from vegetative propagation or seedlings from seeds of planted individuals or from the soil seed bank.

Kruskal-Wallis tests, followed by Dunn's post-hoc tests, were carried out to study the impact of experimental treatment (Cn, E, Cp) on soil (physicochemical and microbial) and plant community characteristics. Mann-Whitney tests were performed to study the impact of experimental treatment (Cn, E) on MM transfer in runoff water.

A path analysis was performed to evaluate the impacts of MM soil contamination on ecological restoration dynamic and the impacts of vegetal succession on agronomic and microbial soil quality. Path analysis is a particular case of structural equation modelling (SEM). Its permits to represent causal networks between several measured variables and to test model data consistency (Grace, 2006). Path analysis was performed by using the *lavaan* package (Rosseel, 2012). A conceptual model was developed from *a priori* knowledge consistent with our data and having biological sense (Fig. C). Five groups of measured variables were created by using axis 1 coordinates of principal component analysis (PCA): MM soil contamination ($[As]_{tot}$, $[Cu]_{tot}$, $[Pb]_{tot}$, $[Zn]_{tot}$; PCA axis 1 explaining 84.5 % of the variation), MM mobilization in soil and transfer by water erosion ($[As]_{mob}$, $[Cu]_{mob}$, $[Pb]_{mob}$, $[Zn]_{mob}$, $[As]_{H2O}$, $[Cu]_{H2O}$, $[Pb]_{H2O}$, $[Zn]_{H2O}$; PCA axis 1 explaining 49.6 % of the variation), agronomic soil quality (pH, TOC, TKN, C/N, P, CEC, clay percentage; PCA axis 1 explaining 55.9 % of the variation), microbial activities and biomass (SBR, MB, GRSP; PCA axis 1 explaining 88.2 % of the variation), plant community parameters (plant cover, specific richness, Shannon's diversity index, Pielou's equitability index, chamaephytes, hemicryptophytes and competitive stress tolerant species; PCA axis 1 explaining 74.2 % of the variation). The full model was simplified by stepwise exclusion of non-significant variables until a minimal adequate model was reached. The adequacy of the model was

determined by non-significant differences between predicted and observed covariance matrices (chisquared test, $P > 0.05$), a low Root Mean Squared Error (RMSEA < 0.10), a high Comparative Fit Index (CFI > 0.90) and a low Standardized Root Mean Square Residual (SRMR < 0.08) (Grace, 2006; Rosseel, 2012). The selected model does not consider MM mobilization in soil and transfer by hydric erosion because these variables do not permit to obtain an adequate model. Thus, Spearman correlation tests (correlation between two variables) were performed in order to test the relationship between MM mobilization in soil and transfer by hydric erosion and MM soil contamination, agronomic soil quality, microbial activities and biomass, plant cover, diversity and sustainability.

3. Results

3.1. Soil physicochemical characteristics

Six parameters were significantly affected by the experimental treatment (Tables 1 and A). pH was 11 % higher for Cn than for Cp plots (Table 1). TOC content was 3.9 and 1.9 times higher in Cp than in Cn and E plots, respectively (Fig. 2a, Table 1). CEC was 2.1 and 1.4 times higher in Cp than in Cn and E plots, respectively (Fig. 2b, Table 1). TKN was 2 times higher in Cp and E than in Cn plots (Fig. 2c, Table 1). As_{mob} percentage was 2.1 times higher in Cp than in Cn plots (Table 1). Zn_{mob} percentage was respectively 1.7 and 1.4 times higher in Cp and E than in Cn plots (Table 1).

3.2. Soil microbial properties

Two parameters were significantly affected by the experimental treatments (Tables 1 and A). SBR was 4.2 and 5.2 times higher in Cp than in Cn and E plots, respectively (Table 1). GRSP content was 2.8 and 2.1 times higher in Cp than in Cn and E plots, respectively (Fig. 2d, Table 1).

3.3. Vegetation characteristics

Ten parameters were significantly affected by the experimental treatments (Tables 1 and A). Plant cover was respectively 40.0 and 21.7 times higher in Cp and E plots than in Cn plots (Fig. 3a); specific richness was respectively 6.7 and 9.0 times higher in Cp and E plots than in Cn plots; Shannon's diversity index was respectively 8.3 and 9.6 times higher in Cp and E plots than in Cn plots (Fig. 3b); Pielou's equitability index was respectively 26.3 and 25.0 times higher in Cp and E plots than for Cn plots; percentage of hemicryptophyte species respectively 18.1 and 9.0 times higher in Cp and E plots than for Cn plots (Fig. 3c);

percentage of competitive stress tolerant species respectively 83.3 and 29.8 times higher in Cp and E plots than for Cn plots (Fig. 3d); percentage of chamaephyte species respectively 28.7 and 25.0 times higher in Cp and E plots than for Cn plots (Table 1). Cp plots presented a percentage of competitive stress tolerant species 2.8 times higher than in E plots (Fig. 3d, Table 1). E and Cn plots presented a percentage of therophyte species respectively 16.7 and 14.7 times higher than in Cp plots (Fig. 3e, Table 1). E plots had a percentage of ruderal stress tolerant species 3.3 and 7.0 times higher than in Cp and Cn plots, respectively (Fig. 3f, Table 1). In E plots, most of the transplanted native plant species remained and other spontaneous plant species appeared such as *Sonchus bulbosus* (L.) N. Kilian & Greuter, 2003, *Linaria supina* (L.) Chaz., 1790 *or Centranthus calcitrapae* (L.) Dufr., 1811. Spontaneous plant species also appeared in Cn plots (Table B).

3.4. Correlations between soil, runoff water and plant community characteristics

Path analysis (Fig. 4) showed that MM soil contamination negatively affected vegetation characteristics (i.e. plant cover, diversity and sustainability) and agronomic soil quality. Vegetation characteristics positively affected agronomic soil quality. Agronomic soil quality positively affected soil microbial properties (i.e. microbial biomass and activities). MM soil contamination and vegetation characteristics showed indirect effects on soil microbial properties mediated by degradation and improvement of agronomic soil quality, respectively.

Spearman correlation tests showed that MM soil contamination was positively correlated to MM mobilization in soil and transfer by hydric erosion ($r = 0.89$, $P < 0.001$).

4. Discussion

The aim of this study was to evaluate the evolution of the ecological restoration trajectory and the effectiveness of phytostabilization at the peri-urban and protected site of the Escalette by a comparative *in situ* monitoring of soil properties, MM concentrations in soils and in runoff water and, characteristics of plant communities.

4.1. Evaluation of ecological restoration success after three years

Three attributes are mainly used to evaluate the success of an ecological restoration operation (Ruiz-Jaen and Mitchell Aide, 2005) out of the nine defined by the Society of Ecological Restoration (SER, 2016): diversity of the organism communities (richness, abundance), vegetation structure (cover, height, biomass) and ecological processes (biotic interactions,

nutrient and organic matter cycles). Compared to the reference plant community (i.e. a young Mediterranean scrubland for the present study), these features are milestones enabling to assess the ecosystem position on the restoration trajectory. When ecosystem disturbances are due to industrial pollution such as in this study site, the level of soil contamination is also an important parameter to consider in assessing the environmental physical conditions because it affects the composition, structure and functioning of the ecosystem (Wong, 2003; Li, 2006).

At the Escalette site, plant communities in the reference plots (Cp plots, young Mediterranean scrubland) and in the ecological restoration plots (E plots) had similar composition, relatively low diversity and high equitability during the study. However, they differed by their plant community structure and functions. In the reference plots, plant communities formed a completely permanent vegetation cover mainly shaped by hemicryptophytes and chamaephytes with competitive stress tolerant strategy. However, only a partial permanent plant cover occurred in ecological restoration plots (planted and spontaneous hemicryptophytes and chamaephytes with competitive stress tolerant strategy) with also annual species forming a more or less temporary vegetation cover (therophytes with ruderal and tolerant stress strategies) (Garnier and Navas, 2013; Pérez-Harguindeguy et al., 2016). Establishment of a dense and permanent vegetation cover is a central element for the success of an ecological restoration operation (Cortina et al., 2011). Indeed, this allows the creation of favorable micro-niches to the establishment of new local plant species by improving environmental conditions, notably edaphic ones, thus favoring plant succession and soil microbial functions (Padilla and Pugnaire, 2006; Padilla et al., 2009). At the Escalette site, the establishment of a large, diversified and sustainable plant cover is negatively affected by soil MM contamination. Indeed, soil contamination can constrain plant establishment and persistence because MM may cause toxic, oxidative and/or hydric stresses on plants leading to disruption of physiological processes (i.e. respiration, photosynthesis, water and nutrients absorption) essential for plant growth and development (Baryla et al., 2001; Yadav, 2010; Michalak, 2016). Moreover, MM contamination adds to intrinsic abiotic stress (i.e. scarce and scattered rain events, high summer temperatures, wind) which can strongly constraint plant establishment, growth and development (Bolan et al., 2011; Kumar Yadav et al., 2018).

Soil quality is also an important parameter to consider in an ecological restoration operation because soil functioning recovery is essential to pass the resilience threshold (Heneghan et al., 2008). Overall, the Escalette soils were characterized by poor agronomic quality (low organic matter, nutrient contents, high C/N ratio) (Thomas et al., 2006; Gobat et al., 2013). The high

MM soil contamination in this site negatively impacted the agronomic soil quality. A competition for adsorption onto soil particles can occur between toxic MM and essential cations for plant development (Adriano et al., 2004; Bolan et al., 2011; Kumar Yadav et al., 2018). This competition is governed by physicochemical soil parameters (MM concentrations, redox potential, cation exchange capacity, organic matter content and in particular pH) which influence MM distribution between solid soil phase and soil solution thus affecting MM mobility and bioavailability (Adriano et al., 2004; Bolan et al., 2011). Moreover, microbial communities were submitted to significant stress (high metabolic quotient whatever the treatment) due to the indirect negative effect of high MM contamination on microbial properties mediated by degradation of soil quality. This resulted in low microbial biomass as previously reported by Kızılkaya et al. (2004) and Gülser and Erdoğan (2008). This low biomass can be explained by a metabolic quotient increase. This indicates that sensitive microorganisms decrease whereas resistant microorganisms increase their respiration activity to maintain their basal metabolism at the expense of biosynthesis, resulting in their biomass decrease (Fließbach et al., 1994; Friedlová, 2010).

However, plant community differences between the reference plots, the ecological restoration plots and the unvegetated plots (Cn plots) induced soil properties variations. The completely permanent vegetation cover in the reference plots improved agronomic soil quality and therefore soil microbial activities and biomass compared to the ecological restoration and unvegetated plots. This may be due to regular litter input resulting in soil enrichment in organic matter and nutrients which increase microbial activities and biomass as reported by Kızılkaya et al. (2004), Gülser and Erdoğan (2008) and Friedlová (2010). Among these microbial activities, glomalin secretion by arbuscular mycorrhizal fungi was higher in the reference plots compared to the ecological restoration and unvegetated plots. This glycoprotein is known to enhance soil organic matter content (Rillig et al., 2001; Zhu and Miller, 2003), soil aggregation and structural stability (Rillig, 2004). In the ecological restoration plots, soil nitrogen content was higher than in the unvegetated ones. The occurrence of *C. juncea* individuals in the ecological restoration plots, a leguminous engineer plant in association with nitrogen-fixing bacteria and endomycorrhizal fungi (Carrasco et al., 2011), may facilitate other plant establishment by enhancing soil quality in particular nitrogen content (Padilla and Pugnaire, 2006).

Results of this study suggest that an ecological restoration trajectory was initiated at the ecological restoration plots (Fig. 5) according to the floristic composition and vegetation analysis processed in the Calanques National Park by Heckenroth et al. (2016b). Several hypotheses on ecological restoration trajectory evolution can be emitted. On the one hand, similarities of plant communities between the reference and the ecological restoration plots and presence of a partial annual plant cover at the ecological restoration plots may indicate that these plant communities are at an earlier stage of vegetation succession relative to the reference plots (*sensu* Aronson et al., 1993). Thus, ecological restoration trajectory may tend towards reference state, i.e. a young Mediterranean scrubland. On the other hand, differences in ecological niches between the reference plots and the ecological restoration plots (MM contamination heterogeneity, the occurrence of Aleppo pines close to Cp plots creating shading and litter inputs) can lead to the establishment of alternative stable states at the ecological restoration plots. Indeed, during an ecological restoration operation in Mediterranean area with limited human intervention, return to the reference state is rarely reached because aridity may keep the ecosystem below the irreversibility threshold (Aronson, 1993; Young et al., 2001, 2005).

4.2. Evaluation of phytostabilization success after three years

MM mobilization in soil and MM transfer by water erosion were not significantly different between the unvegetated and the ecological restoration plots. It was therefore not possible to demonstrate the stabilizing effect of soil contamination by plant communities and their associated microorganisms. This may be due to the temporary and removable runoff water collectors which were not really adapted to the scarce and scattered rain events which occurred during the study period. However, this system implementation was restricted by PNCal regulations in this protected area. Most of the studies used microcosms equipped with a downstream water collector to recover only runoff water from the plot (Bochet et al., 1998; Zhang et al., 2004; Martínez Raya et al., 2006). But, in this study, plots were not isolated from the local ecosystem. Thus, the runoff water recovered in collectors came from the plots themselves but also may come from upstream areas, generating a response more at the level of the ecosystem watershed than the plots.

After three years, no significant effect of soil agronomic quality, microbial and plant communities on MM immobilization and transfer were detected, although the literature indicates that improvement of soil quality, microbial functionalities, plant cover and

sustainability allow enhancing MM immobilization (Mendez et al., 2007; Mendez and Maier, 2008; Valentín- Vargas et al., 2014). This result may be due to several parameters. Firstly, in this study, improvement of soil physicochemical properties was only related to the action of engineering native plant species and associated microorganisms, contrary to most phytostabilization studies using amendments (Rizzi et al., 2004; Radziemska et al., 2018). Thus, at the ecological restoration plots, improving the soil quality exclusively by plants and their impact on MM retention may take more time than when using amendments which hasten MM toxicity reduction and limit their bioavailability by adsorption and complexation processes (Rizzi et al., 2004; Mendez and Maier, 2008; Epelde et al., 2009; Radziemska et al., 2018). Then, MM immobilization by microorganisms by biosorption, bioaccumulation and transformation (Adriano et al., 2004; Khan, 2005; Ma et al., 2011) is limited by low biomass and microbial activities at the ecological restoration plots. Finally, the temporary vegetation cover of annual species with shallow roots may have not allowed a sustainable stabilization of MM contamination at the ecological restoration plots. Phytostabilization is enhanced by persistent and dense root systems and plant cover that reduce soil erosion by decreasing runoff water, protecting soil surface, increasing water infiltration and soil quality (Bochet et al., 1998; 2006; Durán Zuazo et al., 2006; 2008; Bolan et al., 2011) and immobilizing MM in roots and/or rhizosphere (Adriano et al., 2004; Pilon-Smits, 2005; Bolan et al., 2011). Therefore, without amendment inputs and under drastic Mediterranean environmental conditions, native plant communities and their associated microorganisms may need more time before improving MM stabilization by enhancing physicochemical soil parameters involved in MM retention and by biological mechanisms. Thus, a longer observation period may be necessary to conclude on a potential phytostabilization effect of these plant communities. It would also be interesting to take into account other aspects of soil microbial communities (litter decomposition, C and N mineralization, functional diversity) which are driving parameters for good management of soil MM contamination (Brookes, 1995; Friedlová, 2010, Colin et al., 2019).

5. Conclusions

The objectives of this study were to evaluate the evolution of restoration trajectory at the ecological restoration plots and the effectiveness of soil MM stabilization *via* soil, runoff water and plant community analyses. Three years after the implementation of the ecological restoration plots at the Escalette site, it appears that the mobilized ecological engineering tools have enabled the establishment of perennial and annual plant communities at these plots.

However, there is no evidence of a significant improvement of soil quality and phytostabilization efficiency. This may be due to the non-use of amendments and a too short observation period.

The active restoration actions carried out in this Mediterranean peri-urban and MM contaminated area, based on abiotic (creation of cultivation terraces) and biotic (native plant species planting) filter handling by less intrusive techniques on the environment adequate with regulations of a protected area, seem to allow the creation of micro-niches favorable to the establishment of resilient dynamics of the native plant communities. The obtained results support the fact that it is preferable to intervene in these environments rather than to leave in place unvegetated screes with risks of erosion of contaminated soil particles and transfer to the food web.

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Figure captions

Figure 1. Location of (a) the Escalette site in the Calanques National Park (modified after Heckenroth et al., 2016b), (b) the Escalette lead smelting factory, horizontal chimney and study area (source: Google Earth), (c) Cn plots (red, negative control), E plots (yellow, ecological restoration plot) and Cp plots (green, positive control) plots in the study area (source: Géoportail) and (d) characteristics of Cn, E and Cp plots.

Figure 2. Total organic carbon (TOC) concentration, total Kjeldahl nitrogen (TKN) concentration, cation exchange capacity (CEC) and glomalin-related soil protein (GRSP) concentration in Cn plots (negative control, n = 5), E plots (ecological restoration plots, n = 5) and Cp plots (positive control, n = 5). Different letters above boxplots indicate a significant difference between plots for a given parameter ($P \leq 0.05$).

Figure 3. Plant cover, Shannon's diversity index, percentage of hemicryptophyte species, percentage of therophyte species, percentage of competitive stress tolerant species and percentage of ruderal stress tolerant species in Cn plots (negative control, n = 5), E plots (ecological restoration plots, n = 5) and Cp plots (positive control, n = 5). Different letters above box plots indicate a significant difference between plots for a given parameter ($P \leq 0.05$).

Figure 4. Path analysis performed between agronomic soil quality, MM soil contamination, microbial activities and biomass, plant cover, diversity and sustainability. The arrows represent the relationships between the different parameters. Red arrow indicates a negative relationship and green arrow a positive relationship. The thicker is the arrow, the greater is the correlation. Correlation coefficients and significance levels of the correlations (*: $P \leq 0.05$; **: $P \leq 0.01$; ***: $P \leq 0.001$) are indicated at each arrow.

Figure 5. Ecological restoration process and perspectives after three years at the Escalette site.

Table captions

Table 1. Averages ± standard errors (n = 5) of soil (TOC: total organic carbon, TKN: Kjeldahl nitrogen, CEC: cation exchange capacity, $[As]_{tot}$, $[Cu]_{tot}$, $[Pb]_{tot}$, $[Zn]_{tot}$: arsenic, copper, lead and zinc pseudo-total concentrations in soil, PLI: pollution load index, $[As]_{mob}$, $[Cu]_{mob}$, $[Pb]_{mob}$, $[Zn]_{mob}$: arsenic, copper, lead and zinc mobile fraction concentrations in soil, and % (in brackets) of mobile versus pseudo-total MM in soil, SBR: soil basal respiration, MB: microbial biomass, qCO_2: metabolic quotient, GRSP: glomalin-related soil protein), runoff water (As_{water}, Cu_{water}, Pb_{water}, Zn_{water}: quantities of arsenic, copper, lead and zinc exported in runoff water) and plant communities parameters of Cn plots (negative control, n = 5), E plots (ecological restoration plots, n = 5) and Cp plots (positive control, n = 5). Different letters indicate a significant difference between plots for a given parameter ($P \leq 0.05$).

Appendix captions

Figure A. Localization of the Escalette study site in Marseilleveyre massif (Calanques National Park).

Figure B. Schematic representation of Escalette's E plots organized in two terraces and planted with eight pseudo-metallophytes species from the local floristic background in November 2015 (modified from Heckenroth, 2017).

Figure C. Conceptual model of path analysis taking into account agronomic soil quality, MM soil contamination, microbial activities and biomass, plant cover, diversity and sustainability. The arrows represent the relationships between the different parameters. Red arrow indicates a negative relationship and green arrow a positive relationship.

1 – MM soil contamination impacts negatively plant cover, diversity and sustainability. MM soil contamination may cause oxidative and hydric stresses disturbing essential physiological process of plant growth and development (respiration, photosynthesis, water and nutrients absorption) (Baryla et al., 2001; Yadav, 2010; Michalak, 2016).

2 – MM soil contamination impacts negatively agronomic soil quality. MM soil contamination potentially created a competition between essential cations for plant development and toxic MM for adsorption to soil particles (Bolan et al., 2011).

3 – MM soil contamination impacts negatively microbial activities and biomass. MM soil contamination may cause a reduction of microbial number, diversity and activities (Fließbach et al., 1994; Brookes, 1995; Kızılkaya et al., 2004; Gülser et Erdoğan, 2008).

4 – Increase in plant cover, diversity and sustainability impacts positively agronomic soil quality. A permanent, dense and diversified plant cover improves soil organic matter, nitrogen and nutrient contents by regular litter inputs (Gobat et al., 2013).

5 – Improvement of agronomic soil quality, in particular organic matter quantity and quality, impacts positively microbial activities and biomass (Kızılkaya et al., 2004; Gülser et Erdoğan, 2008; Friedlová, 2010; Gobat et al., 2013).

6 – The higher the MM soil contamination is, the more MM are mobilizable and transferable (Bolan et al., 2011).

7, 8, 9 - Improvement of soil quality, microbial functionalities and plant cover, diversity and sustainability allows to enhance MM immobilization by physicochemical and biological

mechanisms. This reduces MM mobilization in soil and transfer by hydric erosion (Adriano et al., 2004; Pilon-Smits, 2005; Bolan et al., 2011).

The adequacy of the full model was not good (*P* of $\chi^2 = 0.020$). Thus, we removed non-significant relationships (*P*-value of relationship 3 = 0.656; *P*-value of relationship 7 = 0.864; *P*-value of relationship 8 = 0.306; *P*-value of relationship 9 = 0.696) from this model. Exclusion of non-significant relationships did not permit to obtain a good adequacy of the new model (*P* of $\chi^2 = 0.010$, CFI = 0.850, SRMR = 0.111). Finally, by removing the relationship 6, we obtained an adequate model (*p* of $\chi^2 = 0.061$, RMSEA = 0.067, CFI = 0.904 and SRMR = 0.063) and this final model was selected.

Table A. (a) *P*-values of Kruskal-Wallis and Dunn tests performed for soil characteristics (TOC: total organic carbon, TKN: Kjeldahl nitrogen, CEC: cation exchange capacity, $[As]_{tot}$, $[Cu]_{tot}$, $[Pb]_{tot}$, $[Zn]_{tot}$: arsenic, copper, lead and zinc pseudo-total concentrations in soil, PLI: pollution load index, As_{mob}(%), Cu_{mob}(%), Pb_{mob}(%), Zn_{mob}(%): percentages of mobile arsenic, copper, lead and zinc in soil, SBR: soil basal respiration, MB: microbial biomass, qCO_2: metabolic quotient, GRSP: glomalin-related soil protein) and plant communities parameters of Cn plots (negative control, n = 5), E plots (ecological restoration plots, n = 5) and Cp plots (positive control, n = 5), (b) *P*-values of Mann-Whitney tests performed for runoff water parameters (As_{water}, Cu_{water}, Pb_{water}, Zn_{water}: quantities of arsenic, copper, lead and zinc exported in runoff water) of Cn and E plots. Significant difference between treatments are indicated by stars (*: $P \leq 0.05$; **: $P \leq 0.01$; ***: $P \leq 0.001$).

Table B. Floristic inventory on E plots (ecological restoration plots), Cp plots (positive control) and Cn plots (negative control).

Graphical Abstract (for review)

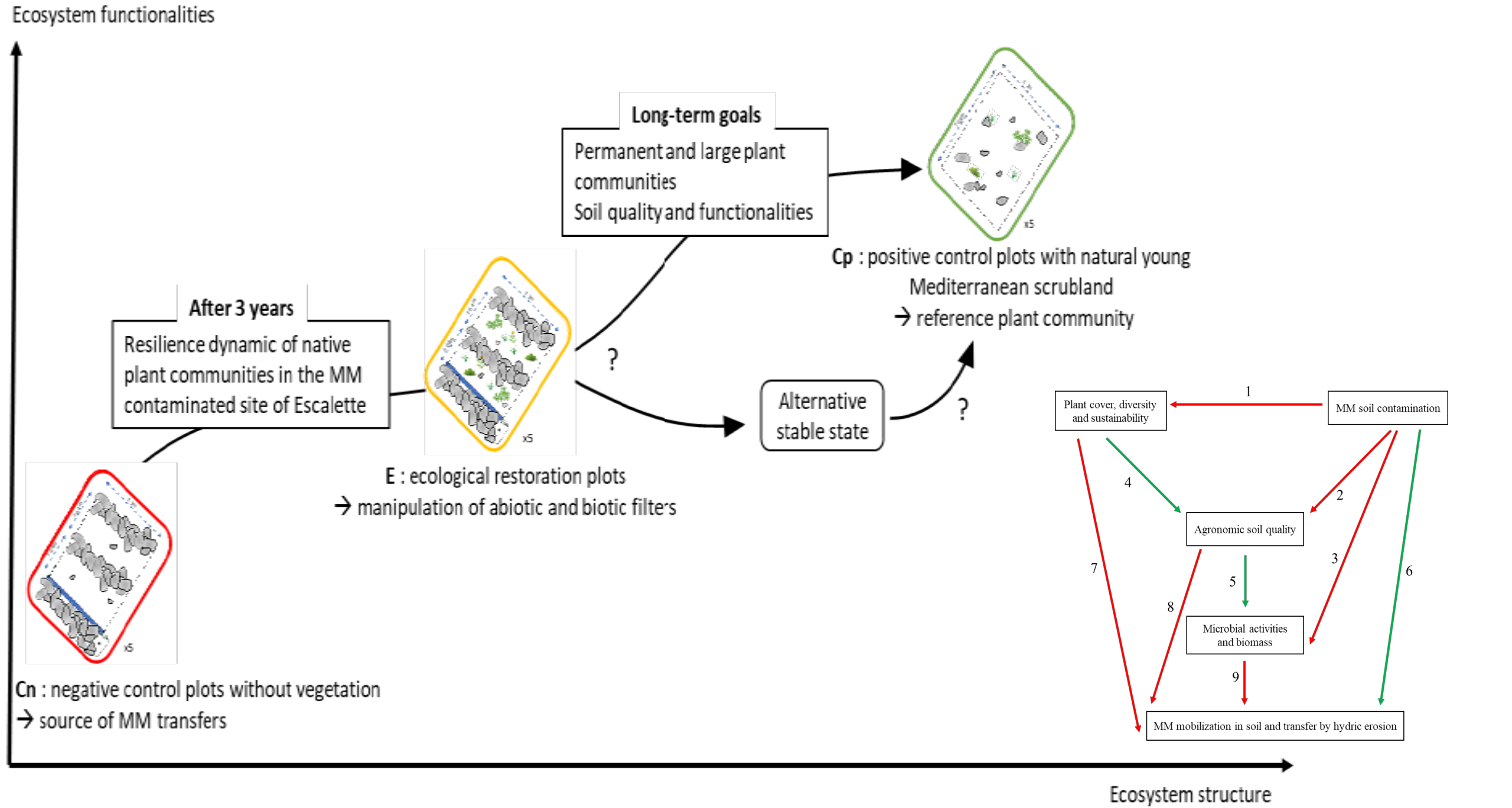

Figure1

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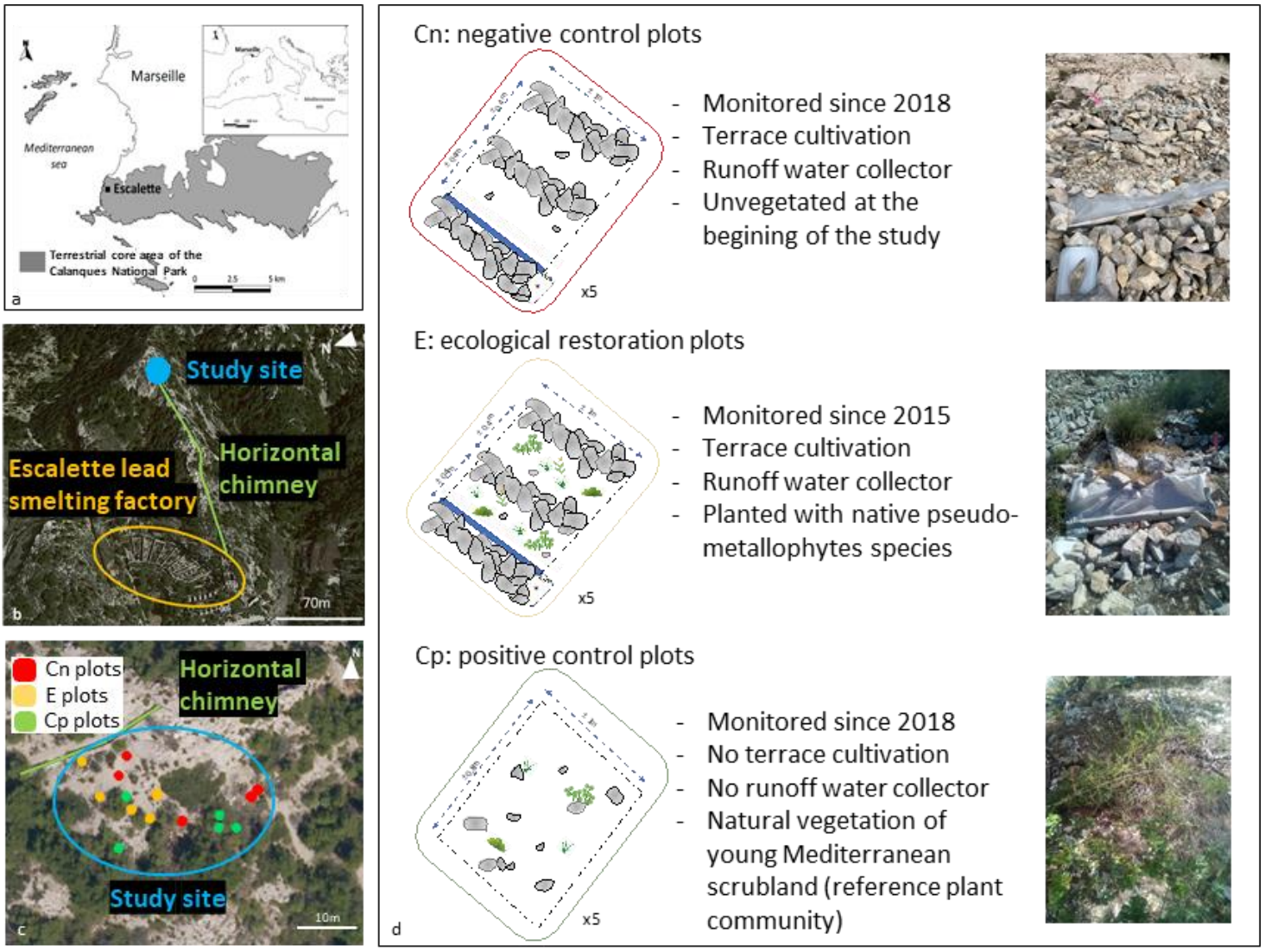

Figure2

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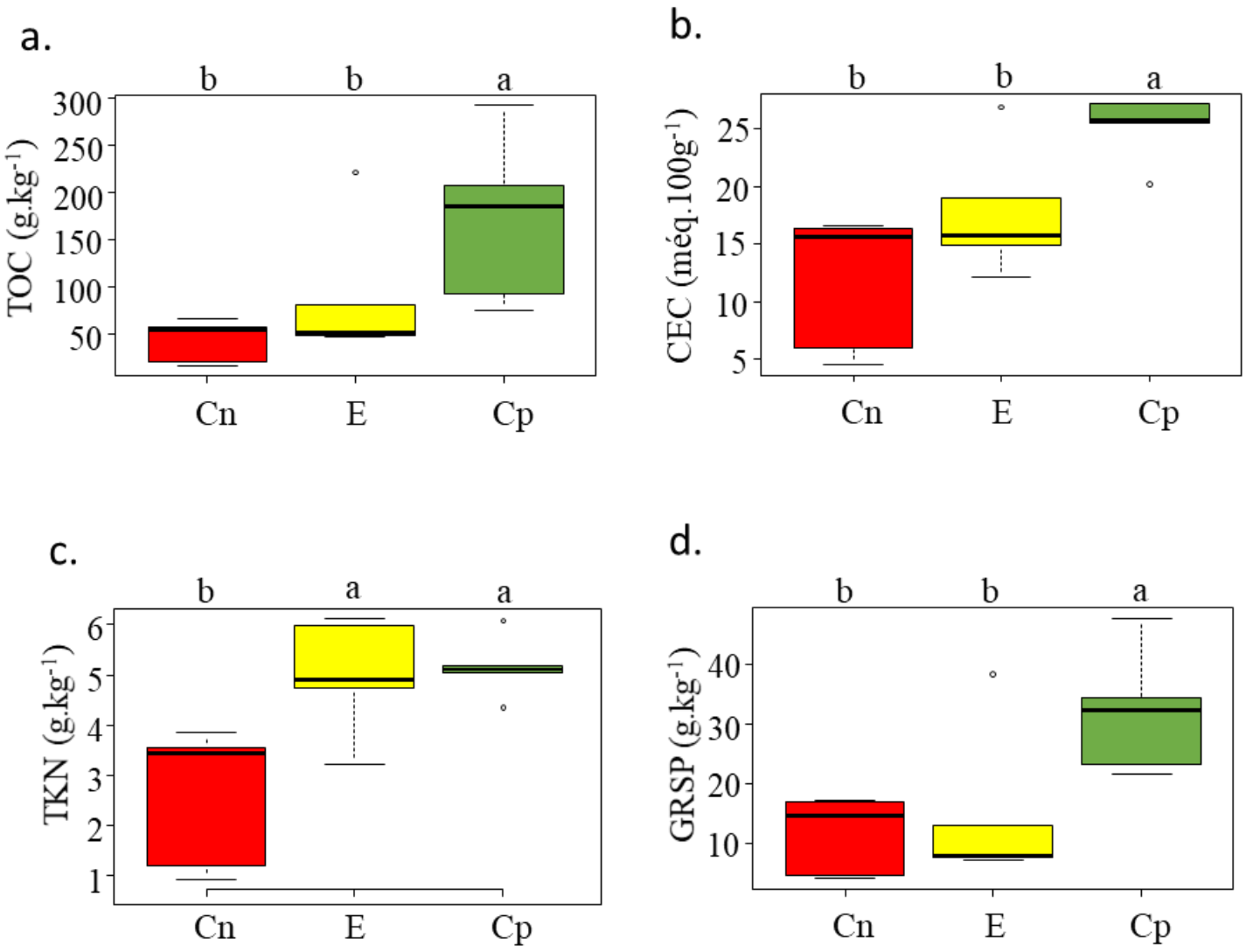

Figure3
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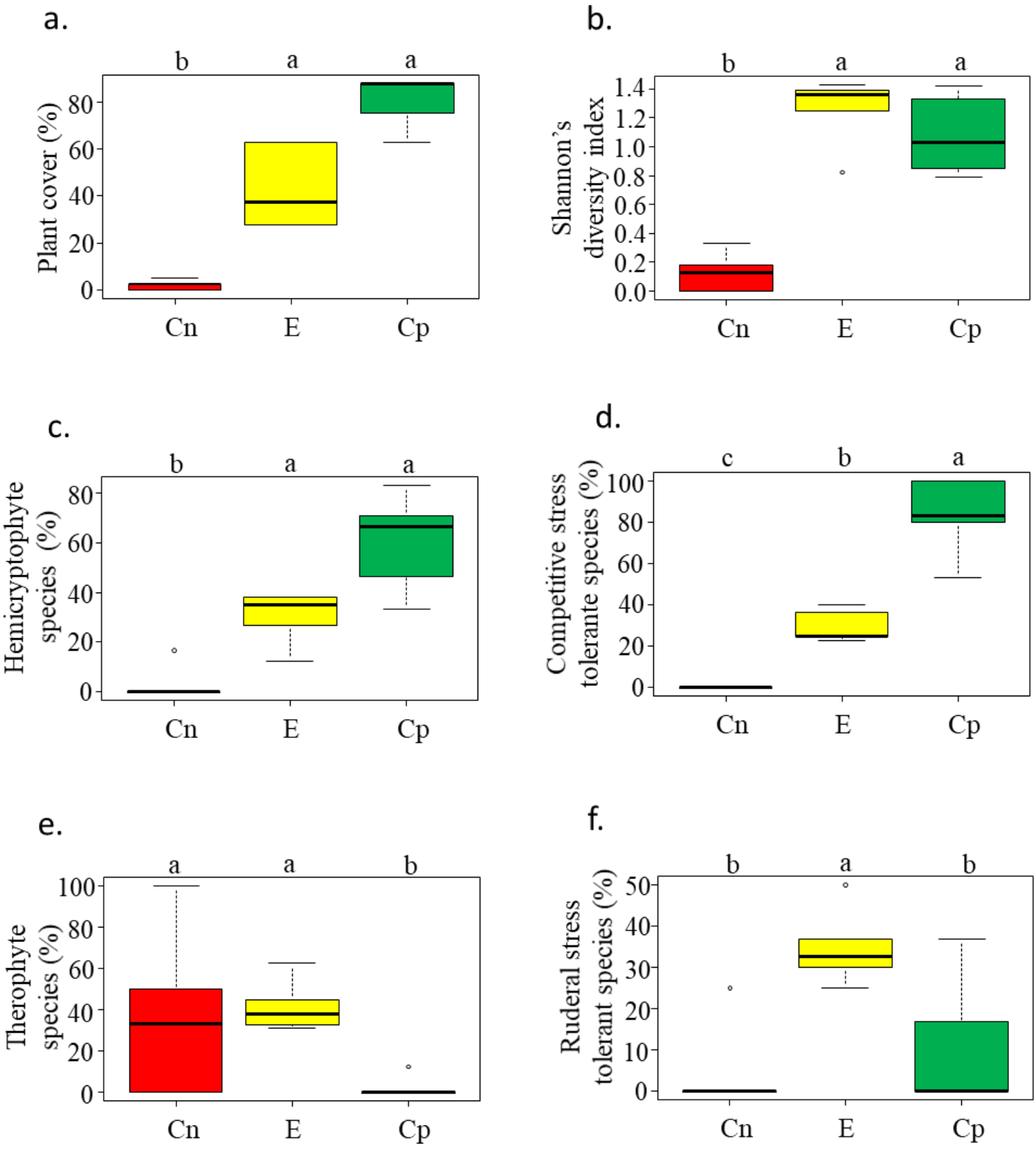

Figure4

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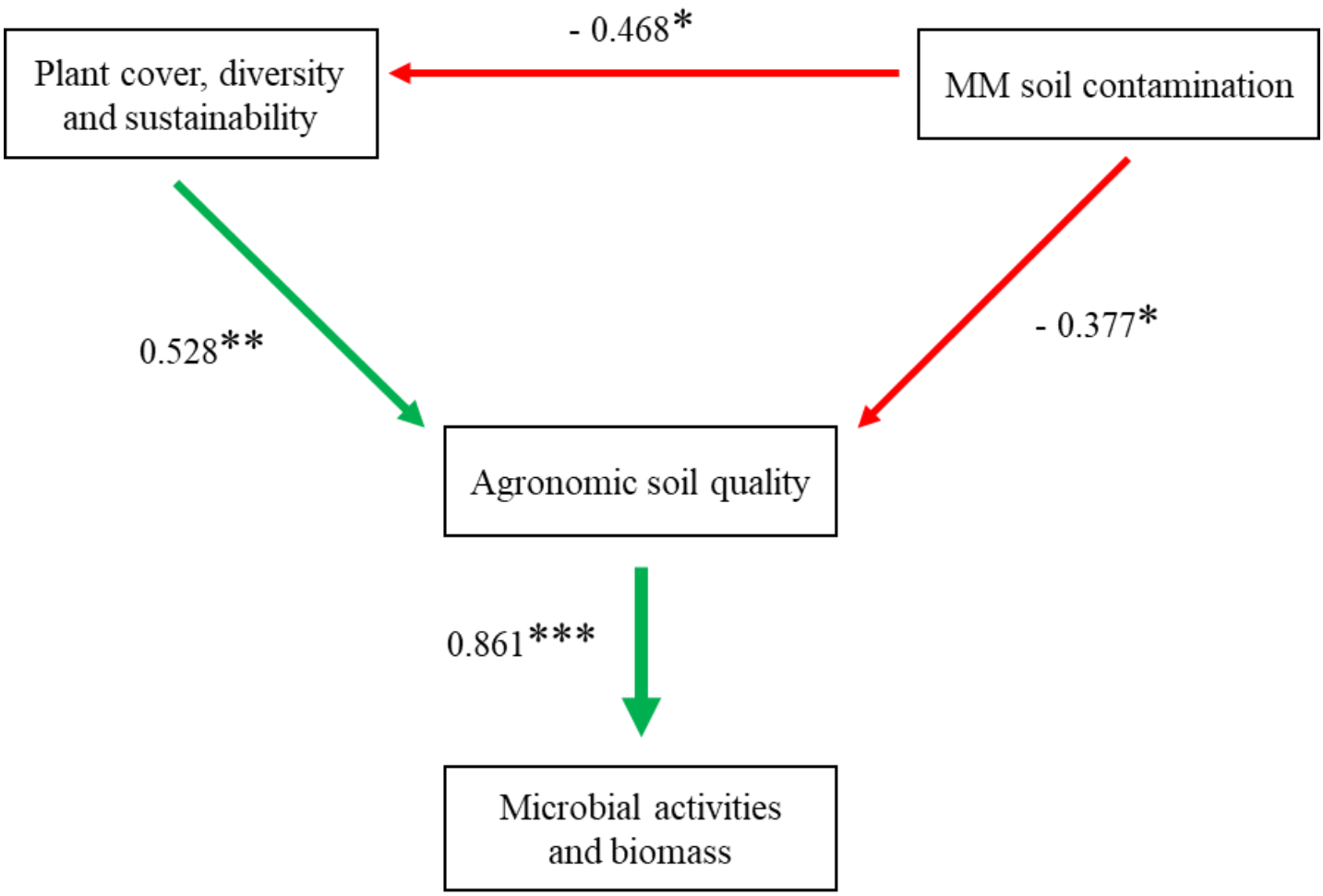

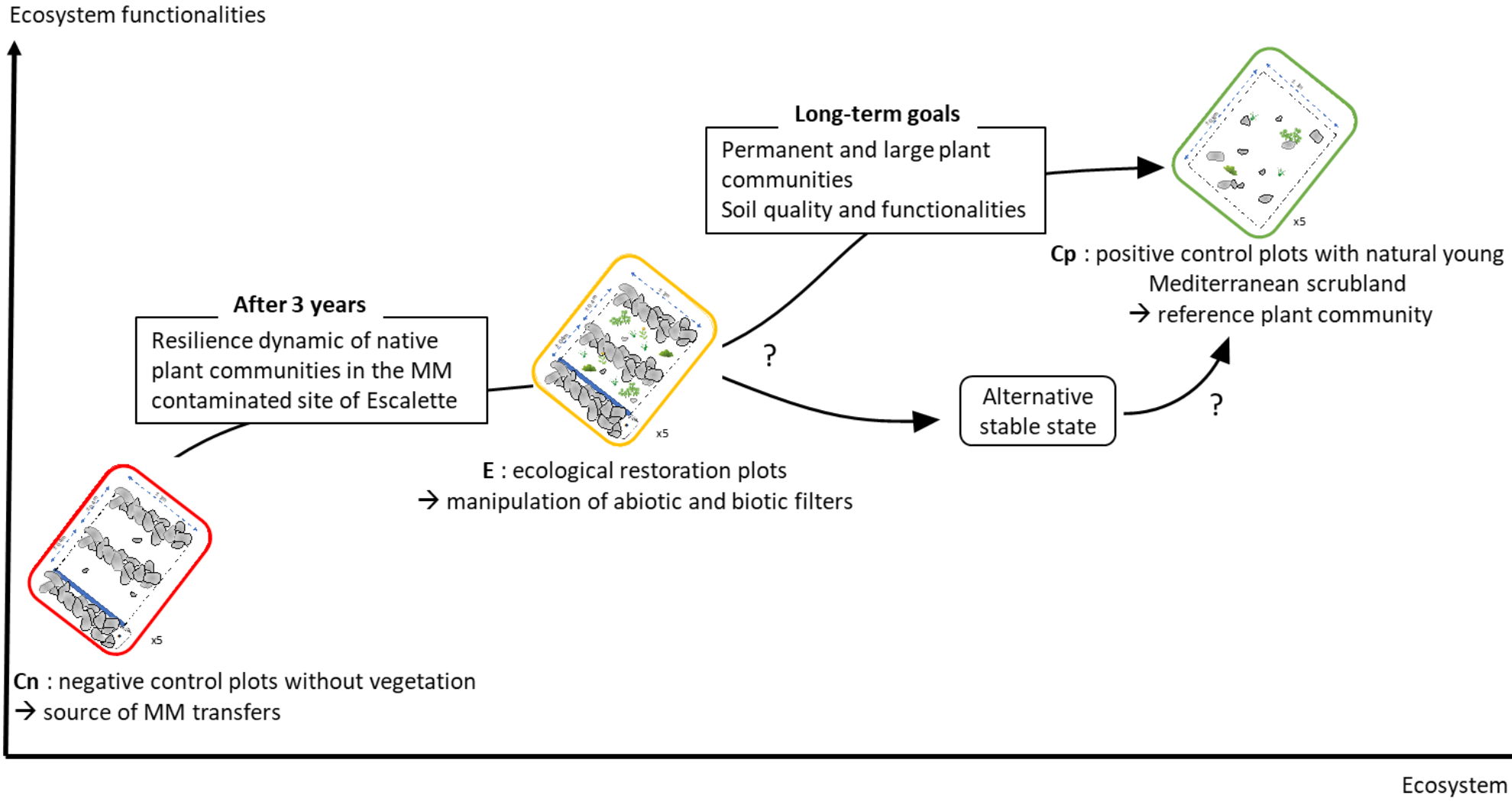

Table1

	Parameters	Cn plots	E plots	Cp plots
Soil characteristics	pH	7.68 ± 0.06^{a}	7.41 ± 0.11^{ab}	6.93 ± 0.15^{b}
	TOC (g.kg^{-1})	43.58 ± 10.25^{b}	90.28 ± 33.25^{b}	171.36 ± 39.58^{a}
	TKN (g.kg^{-1})	2.59 ± 0.63^{b}	4.99 ± 0.52^{a}	5.15 ± 0.27^{a}
	C/N	35.86 ± 4.53^{a}	24.58 ± 6.78^{a}	36.65 ± 6.35^{a}
	P (g.kg^{-1})	0.04 ± 0.00^{a}	0.04 ± 0.00^{a}	0.05 ± 0.01^{a}
	CEC (méq.100g^{-1})	11.84 ± 2.66^{b}	17.70 ± 2.53^{b}	25.12 ± 1.28^{a}
	Clay (%)	14.96 ± 2.75^{a}	21.24 ± 2.48^{a}	21.18 ± 3.75^{a}
	Lime (%)	19.65 ± 2.57^{a}	21.37 ± 1.83^{a}	17.65 ± 1.04^{a}
	Sand (%)	22.86 ± 3.29^{a}	18.02 ± 2.62^{a}	21.76 ± 1.65^{a}
	$[As]_{tot}$ (mg.kg^{-1})	19770.04 ± 7082.15^{a}	13640.33 ± 1174.80^{a}	8579.00 ± 2250.43^{a}
	$[Cu]_{tot}$ (mg.kg^{-1})	75.54 ± 15.98^{a}	60.51 ± 3.28^{a}	51.40 ± 5.73^{a}
	$[Pb]_{tot}$ (mg.kg^{-1})	63470.68 ± 10902.03^{a}	75600.15 ± 3780.47^{a}	55685.76 ± 10653.43^{a}
	$[Zn]_{tot}$ (mg.kg^{-1})	33754.54 ± 11515.45^{a}	25944.16 ± 2664.65^{a}	14457.50 ± 4061.24^{a}
	PLI	406.54 ± 107.11^{a}	351. 45 ± 23.02^{a}	239.33 ± 50.23^{a}
	$[As]_{mob}$ (mg.kg^{-1}) (%)	3306.47 ± 987.35 (18.65 ± 1.49)^{b}	3568. 09 ± 423.65 (27.29 ± 5.12)^{a}	2821.20 ± 312.74 (39.35 ± 7.27)^{a}
	$[Cu]_{mob}$ (mg.kg^{-1}) (%)	26.62 ± 5.34 (35.28 ± 2.30)^{a}	22.17 ± 0.78 (36.98 ± 1.73)^{a}	21.04 ± 1.64 (41.62 ± 1.87)^{a}
	$[Pb]_{mob}$ (mg.kg^{-1})(%)	40357.01 ± 8421.02 (62.96 ± 6.78)^{a}	45668.26 ± 2261.84 (60.67 ± 2.90)^{a}	37579.61 ± 5272.05 (70.29 ± 3.97)^{a}
	$[Zn]_{mob}$ (mg.kg^{-1}) (%)	11553.41 ± 3546.00 (36.17 ± 2.82)^{b}	12398. 93 ± 1075.45 (49.37 ± 5.10)^{a}	8014.85 ± 1582.89 (63.34 ± 8.53)^{a}
	SBR (µg CO_2-C.g^{-1}.h^{-1})	0.048 ± 0.024^{b}	0.042 ± 0.010^{b}	0.209 ± 0.024^{a}
	MB (µg C_{mic}.g^{-1})	0.033 ± 0.015^{a}	0.033 ± 0.011^{a}	0.095 ± 0.009^{a}
	qCO_2 (µg CO_2-C.µg C_{mic}^{-1}.h^{-1})	2.237 ± 0.333^{a}	1.434 ± 0.147^{a}	2.224 ± 0.353^{a}
	GRSP (g.kg^{-1})	11.42 ± 2.95^{b}	14.73 ± 5.98^{b}	31.75 ± 4.69^{a}
Water runoff characteristics	As_{water} (µg)	16.09 ± 5.44^{a}	44.65 ± 20.80^{a}	NA
	Cu_{water} (µg)	3.06 ± 0.91^{a}	3.52 ± 2.47^{a}	NA
	Pb_{water} (µg)	47.79 ± 30.05^{a}	103.89 ± 65.44^{a}	NA
	Zn_{water} (µg)	489.72 ± 188.40^{a}	252.77 ± 40.16^{a}	NA
Plant communities characteristics	Plant cover (%)	2.00 ± 0.94^{b}	43.50 ± 7.87^{a}	80.00 ± 5.00^{a}
	Specific richness	0.60 ± 0.29^{b}	5.40 ± 0.51^{a}	4.00 ± 0.45^{a}
	Shannon's diversity index	0.13 ± 0.06^{b}	1.25 ± 0.11^{a}	1.08 ± 0.13^{a}
	Pielou equitability index	0.03 ± 0.03^{b}	0.75 ± 0.04^{a}	0.79 ± 0.03^{a}
	Geophyte (%)	0.00 ± 0.00^{a}	3.10 ± 3.10^{a}	7.00 ± 5.17^{a}
	Chamaephyte (%)	0.00 ± 0.00^{b}	25.02 ± 4.36^{a}	28.67 ± 5.13^{a}
	Hemicryptophyte (%)	3.33 ± 3.33^{b}	30.07 ± 4.87^{a}	60.17 ± 8.93^{a}
	Therophyte (%)	36.67 ± 18.56^{a}	41.81 ± 5.73^{a}	2.50 ± 2.50^{b}
	Phanerophyte (%)	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	1.67 ± 1.67^{a}
	Stress tolerant (%)	35.00 ± 18.71^{a}	28.00 ± 2.76^{a}	4.00 ± 2.45^{a}
	Competitive Stress tolerant (%)	0.00 ± 0.00^{c}	29.83 ± 3.53^{b}	83.33 ± 8.56^{a}
	Ruderal Stress tolerant (%)	5.00 ± 5.00^{b}	34.83 ± 4.24^{a}	10.67 ± 7.26^{b}
	Competitive Ruderal Stress tolerant (%)	0.00 ± 0.00^{a}	5.33 ± 3.43^{a}	2.00 ± 2.00^{a}