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RESEARCH ARTICLE

Contrasting soil- and canopy-nurse effects in metalliferous systems may be explained by dominant plant functional strategies

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Abstract

1. Plant-plant interaction studies in metalliferous systems have focused either on the role of facilitation or on negative effects of elemental allelopathy. However, no studies have investigated both of these effects in the same system, and their relationships with the functional strategy of the nurse species, although this is crucial for the ecological restoration of polluted sites.
2. We assessed the effects of two dominant nurse species, with apparent contrasting strategies, on two target species on a slag heap in the Pyrenees (France). We quantified both long-term soil-engineering and short-term canopy effects on the growth of two target species. We also measured morphological traits and leaf metal concentration of the two nurse species and their effects on air moisture, temperature and vapour pressure deficit.
3. Nurse functional strategies, as inferred from morphological traits and leaf metal concentration seem to drive their long-term soil engineering effects on target species: *Gypsophila repens*, the relative larger and more exploitative species with high leaf metal concentration, had negative long-term effects likely due to elemental allelopathy, whereas *Minuartia verna*, the relative smaller and more conservative species with lower amount of metals in leaves, had neutral long-term effects. Although *Gypsophila repens* had a slightly stronger positive effect on microclimate than *Minuartia verna*, this did not turn into higher short-term effects for the target species.
4. *Synthesis and applications*: Our study highlights the diverse long and short-term nurse effects on companion species in metalliferous systems. Different nurse species can improve the performance of companion species by mitigation of climatic stress, and ultimately help speed-up the restoration of polluted environments. However, species status regarding leaf metal accumulation has to be verified. For species having a higher metal concentration in their leaves, positive microclimatic effects may be offset by local increase of soil contamination on the long-term.

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KEYWORDS
elemental alellopathy, functional strategies, leaf economic spectrum, metal hyperaccumulation, micro-climate amelioration, soil engineering, stress gradient hypothesis, ecological restoration

1 | INTRODUCTION

Metalliferous systems, either spontaneous (on ultramafic rocks) or related to anthropogenic activities, are characterized by an important presence of metal(loid)s in soils. In Europe alone, metal-contaminated sites are an important part of the 350,000 polluted sites requiring remediation because of the important risk for human and ecosystem health (Panagos et al., 2013). Phyto-management provides affordable and sustainable alternatives for ecological rehabilitation of these sites. However, in heavily polluted sites, phyto-management is impeded by the slow plant development (Mench et al., 2010). Meanwhile, an increasing number of studies have shown that positive interactions between plants can facilitate vegetation dynamics, thus, providing a nature-based solution for ecosystem rehabilitation of these heavily-polluted sites.

To date, no general pattern regarding plant-plant interactions in metalliferous systems has emerged, due to two contrasting working directions. First, in relation with the restoration of metal-contaminated sites, several authors have investigated the potential role of facilitation. Corresponding studies have confirmed that positive interactions are frequent, consistent with the Stress Gradient Hypothesis (SGH, Bertness & Callaway, 1994). The underlying mechanisms are diverse with possible: (i) microclimate ameliorations (wind speed decrease or drought stress alleviation) by nurse plants (Domínguez et al., 2015; Zvereva & Kozlov, 2007); (ii) improvement of soil nutrient availability (Frérot et al., 2006; Yang et al., 2015) and (iii) decrease in metal stress by lowering metal bio-availability and toxicity (Frérot et al., 2006; Whiting et al., 2001). Second, the remarkable ability of some metallicolous species to (hyper) accumulate metals in their leaves (Krämer, 2010) has led to the proposition that it provides a competitive advantage for (hyper)accumulating plants by elemental allelopathy (accumulation of metals in the leaves and release of important amount of bio-available toxic compounds through litter decomposition that decrease the development of neighbouring plants; Boyd & Jaffré, 2001; Morris et al., 2009). This theory received support in both controlled and field studies (El Mehdawi et al., 2011; Mohiley et al., 2020), although it appears contradictory with former references showing facilitation by nurses on target species performances in metalliferous systems. However, both climatic and edaphic stress can co-occur in metalliferous systems (Navarro-Canoet al., 2019). It stresses the need for studies simultaneously investigating both potential positive and allelopathic effects in order to quantify their relative importance, thus, addressing a crucial topic in the restoration of metal-contaminated sites: what makes a good nurse plant?

A better understanding of plant-plant interaction outcome needs the consideration of the functional strategies of the species producing the effect. A general assertion is that positive effects increase with increasing plant size and vigour (Michalet et al., 2006). For instance, Navarro-Cano et al. (2018) showed in several mine tailings that the most efficient nurse species are tall plants. They also have a lower leaf C:N ratio, thus, being tall exploitative species. These exploitative species (also referred as "acquisitive" in the trait literature) have the potential to improve soil fertility, thanks to their short-lived leaves and high litter quality. However, exploitative species are also good candidates for elemental allelopathy effects if they accumulate metals in their short-lived leaves and have high litter decomposition rates. By contrast, conservative species with slow turn-over rates may have no such allelopathic effect, and could even decrease soil toxicity by storing metals in their organs (Navarro-Cano et al., 2018). Thus, we should take into account both metal accumulation (i.e. metal concentration in leaves) and leaf life-span in relation with litter decomposition rate (Wright et al., 2004) if we want to progress in our knowledge of the effect of dominant plant species of metalliferous sites on target species.

Considering the array of processes potentially at stake in plant-plant interactions in metalliferous systems, a full investigation is a complex task. Recent studies have developed an innovative approach to delineate a first group of effects, mostly occurring at a short-term time scale and related to the effects of living dominant plants (mainly micro-climate modifications, light interception, water and nutrient uptake, called "Short-Term Effect", STE) from soil modifications due to the presence of dominant plants on the long term and related to the soil-engineering effects operating during ecological succession (like soil organic matter enrichment, called "Long-Term Effect", LTE; Michalet et al., 2015). This requires the measurement of a target species performance in three neighbouring conditions: (i) with neighbours (inside nurse canopy); (ii) in a similar microhabitat where the neighbours canopy is removed; (iii) in a close naturally open area with similar environmental conditions but without neighbours. This distinction is particularly adapted to metalliferous systems because elemental allelopathy is a specific case of negative soil-engineering effect for the target species but positive for the plant producing the effect through a long-term enrichment of soil by toxic compounds.

Here, we present a study conducted in a mine tailing in the Pyrenees (France) in relation with former mining activity in the region. This tailing was dominated by two species with apparent important functional differences, *Gypsophila repens* with relatively larger size traits (plant height, width and leaf area) and likely more exploitative strategy, and *Minuartia verna*, with lower size traits and a more conservative strategy. The other smaller and less dominant species present both within the dominant species canopy and in open areas were *Achillea millefolium* and *Campanula rotundifolia*. We investigated the functional strategy of the two dominant species

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in regards to metal accumulation and the leaf economic spectrum, together with microclimatic measurements of the effects of their canopy. Because canopy (short-term) and soil (long-term) effects can co-occur, our main objective was to delineate these two effects for the two dominant species, and to relate them to their functional strategies. Short and long-term effects were estimated assessing the growth of *Achillea millefolium* and *Campanula rotundifolia* in the three different neighbouring conditions stated above. More precisely, we assumed that:

1. Long-term effects of the two dominant species should depend on both their metal-accumulation ability and leaf-turnover rate. Following Navarro-Cano et al. (2018), the combination of important accumulation together with a short leaf life span should create favourable conditions for negative long-term effects (likely through elemental allelopathy).
2. Short-term effects of the two dominant species should also depend on their functional strategies. Taller and more exploitative species (with short leaf life span) should provide more pronounced microclimatic amelioration due to a higher transpiration rate than shorter conservative species.

The net effect of the dominant plants should be better understood when delineating short from long-term effects. Both effects are not necessarily consistent. They may reinforce or offset each other.

2 | MATERIALS AND METHODS

2.1 | Study site and soil characterisation

This field study did not require permission for fieldwork nor ethical approval. The study site (Figure 1a) is located in Sentein, Ariège, France (42°49′N, 0°56′E, 950 m a.s.l.). Climate is warm oceanic climate, with an average mean temperature of 9.5°C and average annual precipitation of 1280 mm (ClimateDT tool; https://ibbr.cnr.it//climate-dt/; Marchi et al., 2020). The site is a slag heap composed of fine processing residues (mainly sands and silts, see Table 1) from the gravimetric operations of the nearby ore washing plant operated until the 1960s. 28 soil samples were collected from the top 0–20 cm of soil in September 2020 to characterize main soil properties (pH, texture, cationic exchange capacity, carbon content, total nitrogen and available phosphorus) and metal contamination (total metal content and available metals determined using acetic acid extraction). Full details regarding soil analyses are given in Supplementary Material S1.

Briefly, the main toxic elements in this systems are Zn, Pb, Cd and As: with high total concentration (10,942.5 ± 1908.2 mg/kg for Zn, 4706.5 ± 681.9 mg/kg for Pb, 29.9 ± 5 mg/kg for Cd, 47.2 ± 8.1 mg/kg for As, see Table 1). Ore bodies in the valley being located within a calcareous sheath, the slag heap pH is slightly basic (8.49 ± 0.08, Table 1). The site covers an area of 5000 m^2, and present herbaceous vegetation naturally established after the last reprofiling to level the slag heap and minimize erosion in 2004.

2.2 | Nurse and target species

To test our assumption regarding soil and canopy effect of plant in metalliferous systems, we selected the two most dominant species of this spontaneous vegetation *Minuartia verna* (hereafter *M. verna*, Figure 1b) and *Gypsophila repens* (hereafter *G. repens*, Figure 1c). We chose these two dominant species as the effect neighbours but we will refer to them as nurse species in the rest of the paper for simplicity. *Minuartia verna* and *Gypsophila repens* are frequent in metallicolous systems in the Pyrenean and Alpine ranges, and are abundant in several mine tailings in the region. Both are perennial mat-forming species that are widely distributed in Europe in open grasslands, generally from stressful sites such as alpine grasslands from dry calcareous rocks. To study the effect of these two nurse species, we used as targets *Achillea millefolium* (hereafter *A. millefolium*) and *Campanula rotundifolia* (hereafter *C. rotundifolia*), two species commonly found in more fertile and favourable grasslands, raising the question of their potential facilitation by nurse plants. We choose these species as targets because they can be found both within patches of the two nurse species and in open areas.

2.3 | Experimental design

At the end of May 2020, for each nurse species, we randomly selected 18 zones (0.5 to 1 m^2) with at least two individuals of the nurse and three individuals of the two target species (present within nurse plants canopy and in open areas) for a total of 36 zones over the whole slag heap for both nurses. In each zone, and for both target species, different neighbouring conditions were created as followed: (i) one individual of each target species within nurse plants canopy was tagged ('With neighbour' condition); (ii) aerial parts of one nurse plant individual in the zone was cut at the ground level, to create removal areas including one individual of each target species ('Neighbour removal' condition); (iii) an individual of each target species was tagged in an open area with a minimum distance of 20 cm from nurse plant ('Open condition'), a reasonable distance to get rid of the influence of neighbours in stressful systems with very small plants (Pistón et al., 2018). For each target species, a total of 108 observed individuals was considered in this study (two nurse species x three neighbouring conditions × eighteen replicates).

2.4 | Nurse traits

At the end of May 2020 (at the beginning of the growing season), five individuals of each of the nurse species were randomly chosen on the slag heap to measure morphological traits related to the leaf economic spectrum and plant stature (Díaz et al., 2016). The height

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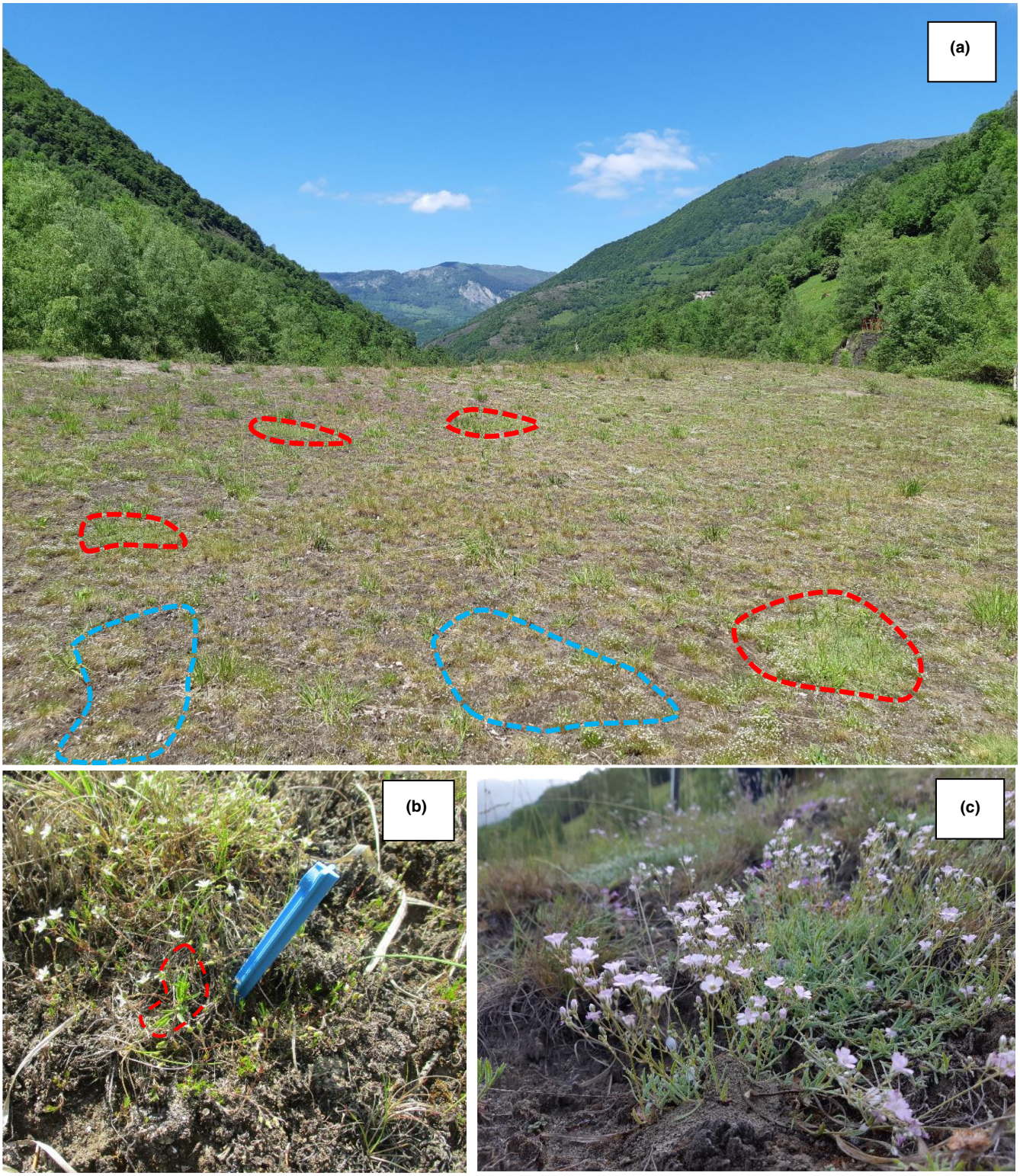

FIGURE 1 Presentation of the study system. (a) The slag heap and its open grassland with low vegetation development and the two nurse species: *Gypsophila repens* (red circles) and *Minuartia verna* (blue circles); (b) *Minuartia verna* with an individual of *Campanula rotundifolia* (red circle) growing within its canopy; and (c) *Gypsophila repens*.

of the top of the extended vegetative parts was used for plant height (cm). The mean of two orthogonal measurements of plant horizontal width was used for lateral width (cm). A minimum of 10 leaves were harvested for each *G. repens* individual and a minimum of 30 leaves for *M. verna* due to smaller leaves. These fresh leaves were stored in minigrip plastic bag and in a cool box. Then, within a few hours after sample collection, leaf fresh weight was measured with a portable scale and leaf area (LA, cm^2) was determined with a portable scanner

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TABLE 1 Soil properties of the slap-heap ($n = 28$)

Factors	Units	Mean	SD[a]	Median	RSD(%)[b]
Clays (0 to 0.002 mm)	%	3.65	0.70	3.50	19
Silt (0.002 to 0.05 mm)	%	19.68	6.72	20.10	34
Sands (0.05 to 2.0 mm)	%	76.67	7.18	76.40	9
N_{TOT}	$g.kg^{-1}$	0.24	0.06	0.23	27
C_{TOT}	$g.kg^{-1}$	44.87	2.21	45.19	5
C/N	–	25.83	4.74	25	18
P_{olsen}	$mg.kg^{-1}$	3.94	1.59	3.58	40
Cation exchange capacity (CEC)	$cmol+.kg^{-1}$	1.19	0.12	1.16	11
Zn_{TOT}	$mg.kg^{-1}$	10942.51	1908.28	10272.36	17
Cd_{TOT}	$mg.kg^{-1}$	29.99	5.04	28.86	17
Pb_{TOT}	$mg.kg^{-1}$	4706.54	681.98	4719.80	14
As_{TOT}	$mg.kg^{-1}$	47.29	8.21	49.37	17
Zn_{aa}	$mg.kg^{-1}$	2914.70	2082.87	2355.36	71
Cd_{aa}	$mg.kg^{-1}$	12.79	7.79	10.24	60
Pb_{aa}	$mg.kg^{-1}$	1368.81	1004.51	1028.94	73
pH	–	8.49	0.08	8.49	1

Note: See Supplementary Material S1 for soil analysis methods.

Abbreviations: Cdaa, Znaa and Pbaa, available metals determined using acetic acid extraction; Cd_{TOT}, Pb_{TOT}, Zn_{TOT} and As_{TOT}, Total metal content in soil; CEC, Cationic exchange capacity; C_{TOT}, Total carbon content; N_{TOT}, Total nitrogen content; P_{Olsen}, Available phosphorus.

[a]SD is for standard deviation.

[b]RSD is for relative standard deviation.

and the Winfolia software (Regents Instruments, Quebec, Canada). Leaves were dried at 65°C during 48h before weighting. Then, we calculated Leaf Dry Matter Content (LDMC, %) ([Fresh weight/Dry weight] × 100) and Specific Leaf Area (SLA, $cm^2.g^{-1}$; leaf area/leaf dry weight).

In order to determine metal (Cd, Zn, Pb, As) concentration in nurse species leaves, the same spring, leaves of seven randomly chosen *G. repens* individuals on the slag heap were harvested, dried (48 h at 65°C) and grinded. Full details of leaf metal concentration determination are given in Supplementary Material S2. For *M. verna*, the same protocol was followed in a preliminary survey in 2019.

2.5 | Effects of the two nurse species on environmental conditions

We used hygrochron DS1923-F5 IButton dataloggers (Maxim Integrated) to measure temperature (T, °C) and relative humidity (RH, %). IButton loggers were placed at the soil level under the nurse species canopy ('With neighbour' condition) and in the 'Open' condition nearby, with eight replicates for each nurse species for a total of 32 loggers. We recorded RH and T during a warm and sunny day two consecutive summers (from 10 am to 6 pm on July 29, 2021, and from 11.30 am to 5 pm on August 2, 2022) to evaluate nurse plants effect on micro-climate. Ibuttons records occurred each 2 min, and values of each IButton were then averaged per hour. Temperature measurements with IButton can be biased in sunny areas due to logger heating with solar radiations. Thus, following Maclean et al. (2021), we considered temperature records during the same time intervals by WS-GP1 weather station (Delta-T Devices) placed at 2 m from the soil as references. Then, correction of temperature measurements obtained with hygrochron loggers were corrected as follow. For each time interval (each hour, e.g. 13:00-13:59), we calculated the anomaly of all IButtons (i.e. their difference in °C from the average of the 32 sensors). Then, for each IButton we added their anomaly to the reference mean temperature over the same time interval given by the weather station. Then, for each of the time interval and each IButton, we calculated a vapour pressure deficit (VPD, Pascal) value according to the following equation:

$$\mathrm{VPD} = \frac{100 - RH}{100} \times 610.7 \times 10^{\frac{7.5T}{237.3+T}}. \quad (1)$$

2.6 | Target plant growth

On 28 May 2020, plant size of each target species individual was measured (number of leaves and extended height of the plant). The same traits were measured at the end of the growing season on the same individuals (23 September 2020). Note that survival was very high since only seven individuals out of 216 died during the experiment. Thus, results presented here only concern plant growth. All living individuals by the end of the experiment were cut at the ground level and oven dried at 65°C during 48 h before dry aboveground biomass measurement.

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In order to calculate the initial biomass of each individual, we fitted linear relationships between the final dry biomass (explained variable) and final plant size (length × number of leaves; independent variable; Table S1). Initial biomass was then estimated using these relationships and initial plant length and leaves. We calculated these relationships for each target species. Finally, we calculated the relative growth rate (RGR) of each individual as follows:

$$\mathrm{RGR} = \frac{B_f - B_i}{B_i}, \tag{2}$$

where B_f and B_i are the final and initial biomass, respectively.

2.7 | Net, short-term and long-term effects

We used the Relative Interaction Index (RII, Armas et al., 2004) to quantify the effects of the nurse species on target species. The NET effect was calculated as follows:

$$\mathrm{RII}_{\mathrm{Growth-Net}} = \frac{\left(\mathrm{RGR}_{\mathrm{With\ neighbour}} - \mathrm{RGR}_{\mathrm{Open}}\right)}{\left(\mathrm{RGR}_{\mathrm{With\ neigbour}} + \mathrm{RGR}_{\mathrm{Open}}\right)}, \tag{3}$$

where $\mathrm{RGR}_{\mathrm{With\ neigbour}}$ is the value of the RGR for the individual in "With neighbour" neighbouring condition and $\mathrm{RGR}_{\mathrm{Open}}$ is the value of the RGR for the individual in "Open" neighbouring condition.

We calculated short and long-term effects, following the study of Michalet et al. (2015):

$$\mathrm{RII}_{\mathrm{growth-Short-term}} = \frac{\left(\mathrm{RGR}_{\mathrm{With\ neighbour}} - \mathrm{RGR}_{\mathrm{Neighbour\ removal}}\right)}{\left(\mathrm{RGR}_{\mathrm{With\ neighbour}} + \mathrm{RGR}_{\mathrm{Neighbour\ removal}}\right)}, \tag{4}$$

$$\mathrm{RII}_{\mathrm{growth-Long-term}} = \frac{\left(\mathrm{RGR}_{\mathrm{Neigbour\ removal}} - \mathrm{RGR}_{\mathrm{Open}}\right)}{\left(\mathrm{RGR}_{\mathrm{Neighbour\ removal}} + \mathrm{RGR}_{\mathrm{Open}}\right)}, \tag{5}$$

where $\mathrm{RGR}_{\mathrm{Neighbour\ removal}}$ is the RGR for the individual in "Neighbour removal" neighbouring condition. The three RII indices are varying between - 1 and 1, with positive values showing a positive effect of the neighbour on the RGR, negative values showing a negative effect and values close to zero indicating no effect.

2.8 | Statistical analysis

All analyses were done using R software version 4.0.3 (https://www.r-project.org). All nurse plant traits values (height, width, leaf area, specific leaf area, leaf dry matter content, metal concentration in leaves) were compared by Wilcoxon–Mann–Whitney *U* tests to test for differences between nurse species for these traits (nonparametric tests were preferred because of small sample sizes). As to microclimatic variables, we performed one-way ANOVA (with posthoc Tukey pairwise comparison) to test differences of RH, T and VPD between open, under *G. repens* and under *M. verna* canopy situations at each time interval. Regarding plant-plant interactions, we tested the effects of the nurse species, the target species, the effect type (Net, short-term and long-term effects) and their interactions on the different RIIs with linear mixed model (LME4++ package). We used mixed modelling with the nurse zone as random effect to take into account the dependence between RIIs calculated for target species individuals present in the same zone (see experimental design paragraph). Tukey post hoc pairwise comparisons were used in case of significant interactions to highlight differences between each combination of treatments. We checked the homogeneity and normality of model residuals to respect the conditions of application of linear modelling. Sample *t*-tests were used to test difference of RIIs from zero.

3 | RESULTS

3.1 | Nurse traits

The two nurse species had contrasting size and growth traits. *G. repens* was 300 % wider ($p < 0.01$, 40.60 ± 5.57 cm versus 12.00 ± 1.64 cm), 100% taller ($p = 0.01$, 13.20 ± 0.66 cm versus 6.00 ± 0.94 cm), and had 900% higher leaf area ($p < 0.01$, 40.86 ± 7.13 cm^2 versus 3.71 ± 0.32 cm^2) than *M. verna* (Figure 2A), whereas *M. verna* had a 250% higher LDMC ($p < 0.01$, 34.24 ± 1.40 % versus 13.16 ± 0.84%) and 25% lower SLA ($p = 0.02$, 0.19 ± 0.01 $cm^2.g^{-1}$ versus 0.26 ± 0.01 $cm^2.g^{-1}$) than *G. repens* (Figure 2B). We also found important differences in leaf metal concentration, with an overall higher concentration for *G. repens* than *M. verna* (Figure 2C). Noticeably, concentration for Zn, Pb and Cd were 150% ($p = 0.01$, 3281 ± 400 and 1406 ± 376 mg $Zn.kg^{-1}$), 1400 % ($p < 0.001$, 1504 ± 214 and 96.82 ± 39.34 mg $Pb.kg^{-1}$) and 200% ($p < 0.001$, 14.17 ± 1.67 and 4.57 ± 0.84 mg $Cd.kg^{-1}$) higher for *G. repens* than *M. verna*, respectively. There were no significant differences in As ($p = 0.14$). All *p*-values are from the Wilcoxon–Mann–Whitney *U* tests.

3.2 | Microclimate modification

On 29 July 2021, at all hours except 13:00, RH was significantly higher below the canopy of *G. repens* than in the Open (Figure 3A). *Minuartia verna* had overall intermediate relative air humidity values (Figure 3A). No significant effects were detected for T between the three conditions (Figure 3B). Finally, *G. repens* had significant lower VPD values than the Open from 10:00 to 12:00 and from 17:00 to 18:00 (Figure 3C). *Minuartia verna* had also overall intermediate VPD values. Thus, the effects of the two nurse species on VPD were mainly due to RH modifications. These effects were even more pronounced on 2 August 2022. Relative air humidity was significantly higher than the Open under the canopy of both nurses during all day (Figure 3D). T was lower under the canopy of both nurses than in the Open during all day. In addition, temperature was lower below *G. repens* than below *M. verna* at the end of the afternoon (Figure 3E). In sum during this second period, the higher RH and lower T under

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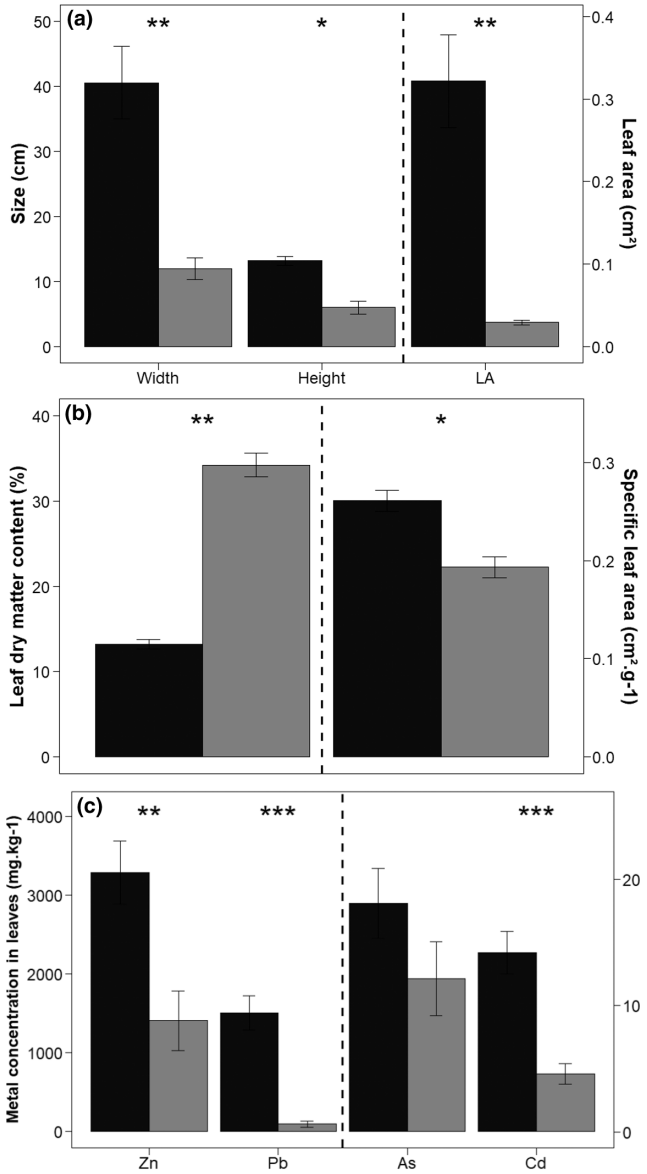

FIGURE 2 Leaf traits of the two nurse species (Means ± SE). (A) Growth traits: height, width and leaf area; (B) growth traits related to the leaf economic spectrum: specific leaf area and leaf dry matter content; (C) metal concentration: zinc (Zn), lead (Pb), arsenic (As) and cadmium (Cd) concentration in leaves. *Gypsophila repens* values are shown by black bars, *Minuartia verna* by dark grey bars. The dotted vertical lines separate response variables that do not share the same *y* axis or scale. Results of nonparametric Mann–Whitney tests are shown (**p* < 0.05; ***p* < 0.01; ****p* < 0.001)

the canopy of both nurses contributed to the lower VPD values compared to the Open (Figure 3F).

3.3 | Target responses to the short- and long-term effects of the two nurse species

There was a significant nurse species effect in the three-way ANOVA due to overall positive RII values for *M. verna* (i.e. facilitation) versus negative RII values for *G. repens* (i.e. competition, Table 2). Effect type (net, short-term or long-term effects) had a significant effect (Table 2) with positive short-term versus negative long-term effects. However, there was a significant nurse species per effect type interaction (Table 2) because short-term were more positive (Figure 4) than long-term effects (which were negative, Figure 4) for *G. repens*, whereas all effects were slightly positive and not different among them for *M. verna* (Figure 4). These responses were mainly consistent for both target species since the target species effect was not significant (Table 2).

4 | DISCUSSION

Here, we provide evidence that considering nurse species with different functional strategy and short-canopy versus long-term soil effects in a single approach may improve our understanding of interactions at stake in a metalliferous system. Consistent to our first hypothesis, the direction of long-term effects depended on nurse species with different strategies regarding nutrient use and metal accumulation, with negative long-term effects for the exploitative and metal-accumulating nurse species only (*G. repens*). In contrast, our second hypothesis was only partially validated. Although we found positive microclimatic ameliorations of both nurse species, they were only slightly stronger for the taller and more exploitative species than for the shorter conservative species. In addition, for the target species there were no differences between the short-term effects of the two nurse species. Ultimately, the net outcome of plant-plant interactions was the balance between these different effects and, thus, positive in the patches of *M. verna* only, due to the absence of negative long-term effects.

4.1 | Nurse strategies

Two major axes of variations are recurrently identified in plant functional space based on plant trait characterization, one related to growth and nutrient availability, the second to plant size and regeneration (Díaz et al., 2016). According to growth traits, species with high SLA and low LDMC are exploitative species producing physiologically efficient but short-lived leaves (Wright et al., 2004). Conservative plant species have opposite characteristics. In our study, the two species used as nurses differed regarding both growth and size traits: *G. repens* had exploitative and large size traits, whereas *M. verna* had more conservative and smaller size traits. These two species also differed in their strategy regarding adaptation to metal stress. Specifically, the Zinc content in *G. repens* leaves (3281 mg.kg^{-1}) was higher than the concentration threshold used to classify metallicolous species as hyper-accumulators (i.e. 3000 mg.kg^{-1}; Krämer, 2010). The concentration found for the other elements did not suggest hyperaccumulation but they clearly showed contrasting behaviours regarding metal accumulation between the two nurses, with *G. repens* accumulating much more metals than *M.*

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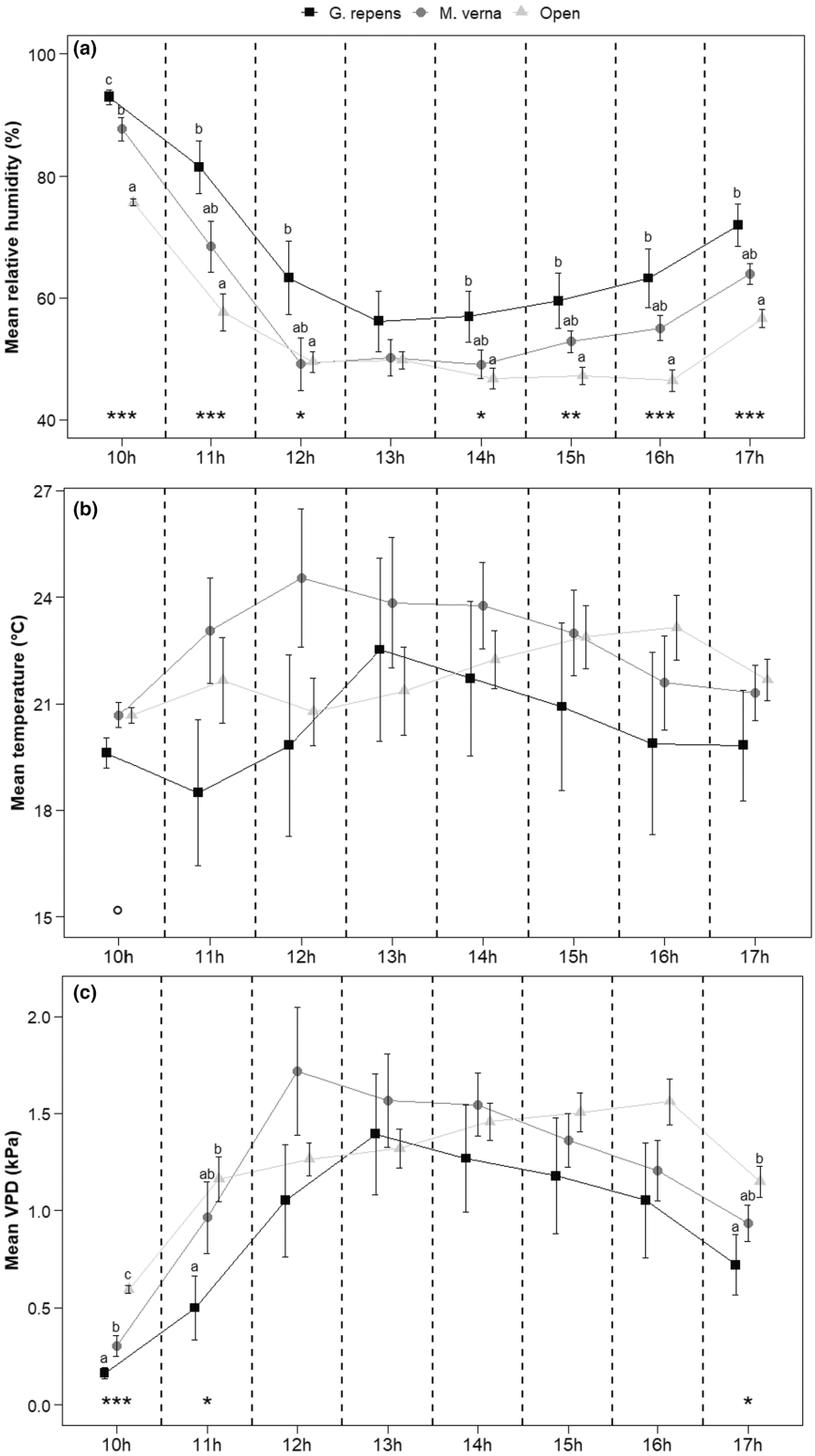

FIGURE 3 Microclimatic changes under the nurse species canopy during two days in dry summer conditions. (A) Relative humidity, (B) temperature, (C) VPD on 29/07/21, (D) relative humidity, (E) temperature and (F) VPD on 02/08/22. Hourly means ± SE are represented. Results of the one-way ANOVAS are shown (°$p < 0.1$; *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$). Different letters indicate significant difference between groups (post hoc Tukey HSD multiple comparisons)

verna. To summarize, *G. repens* appeared as a species producing an important amount (taller species with larger leaves) of short-lived leaves (exploitative species) with important concentration of metals, relatively to *M. verna* having a lower amount of longer-lived leaves with lower level of metal concentration.

4.2 | Long-term soil effects are related to the leaf economic spectrum and metal accumulation

Modification of soil quality by nurses and cascading effects on their neighbours in metalliferous systems are diverse. On the one hand,

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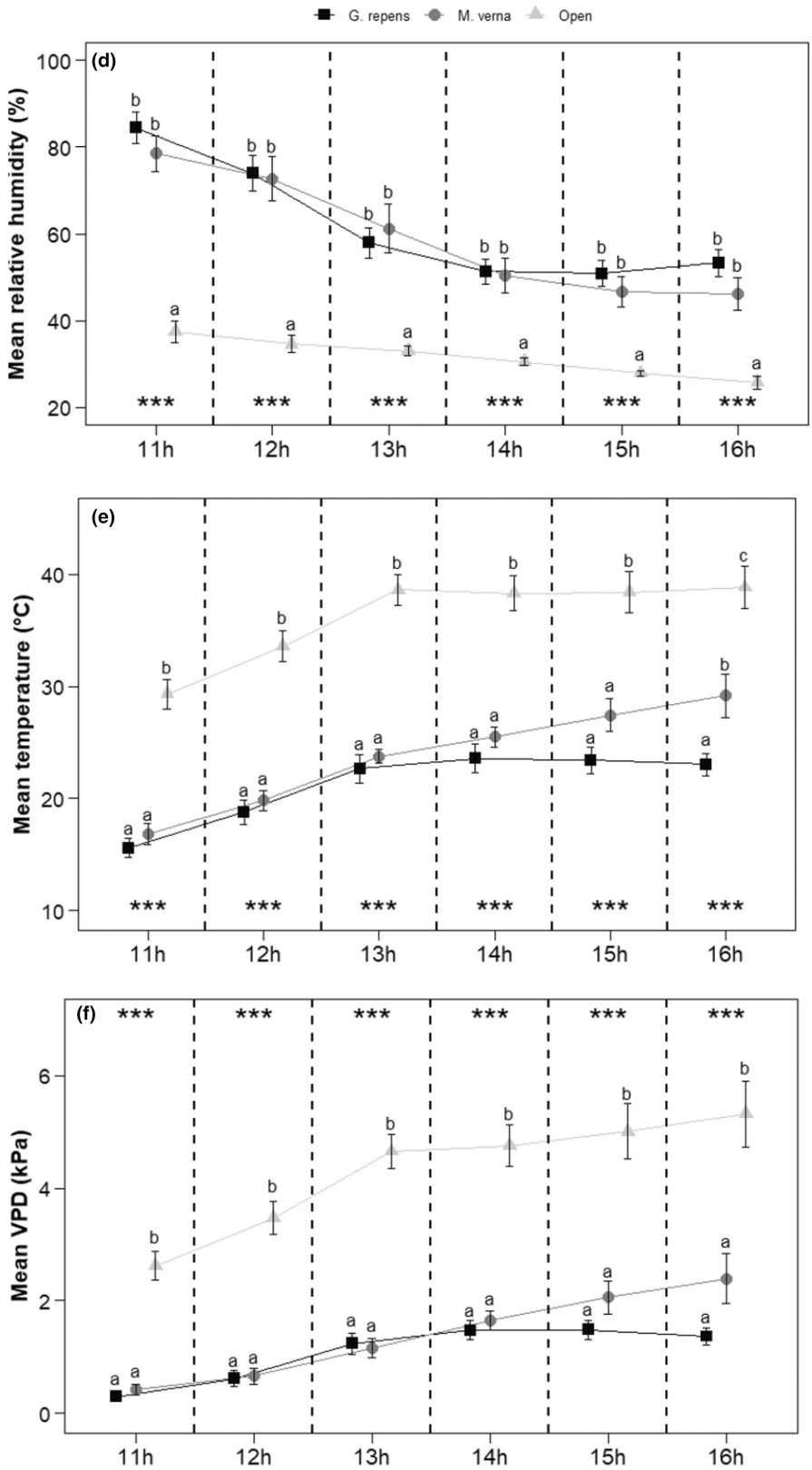

FIGURE 3 (Continued)

some species are known to create positive plant soil feedbacks by supplying litter and root exudates to decomposers that enhance soil microbial activity and soil fertility (Navarro-Cano et al., 2014, 2018; Yang et al., 2015), or even decreasing metal bioavailability and toxicity (Antoniadis et al., 2017). On the other hand, the elemental allelopathy hypothesis and related studies relied on an increase of soil metal bioavailability and toxicity for neighbours by metal accumulating species. Our results are consistent with these latter soil effects with negative

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long-term effects for *G. repens*. Additionally, our results shed new light on the relationship between soil effect and nurse strategies, not only regarding metal stress adaptation, but also for more global functional strategies regarding plant stature and the leaf economic spectrum. The negative soil effect of *G. repens* is likely due to its strong capacity to accumulate metals related to its fast growing strategy and relatively higher size traits combined with short-lived leaves, thus, creating conditions favourable for increasing metal bio-availability and elemental allelopathy, consistent with our first hypothesis. Also consistent with this hypothesis, the null soil effect of *M. verna* is certainly related to its low metal accumulation behaviour: it does not create conditions for higher metal concentration and availability. Although in our study differences in long-term effects between the two nurses with contrasting strategies were clear, a direct assessment of nurse functional trait effects, with more nurse species, on the long-term responses of targets should confirm this.

Note that the role of accumulating species on the modification of metal-bioavailability in soils is not clear in the literature. The active absorption of metals in the rhizosphere solution can deplete metals availability locally (Álvarez-López et al., 2021) and even improve the performance of metal sensitive neighbouring plants (Whiting et al., 2001). Additionally, production of acidifying roots exudates to enhance metal uptake can increase metal availability in soil water (Wenzel et al., 2003), which could have adverse effect on sensitive neighbours. Whatever the conclusion of these studies, they focused on short-term rhizospheric processes occurring during plant soil resources uptake. Elemental allelopathy is a different process that encompass a more integrative view of metal cycle (metal uptake and then metal release in soils through litter decomposition) in relation with accumulating species. This implies a long-term effect. Noteworthy, in this study we did not investigate the fine modifications of soil properties by the nurses. One possibility would have been to estimate metal bio-availability concentration in the surrounding soil of both nurses. While this is a limitation of this study, the abundant literature regarding the methods to estimate metal bio-availability shows that there is no unique and standardized approach to measure this bio-availability (Degryse et al., 2009; Viala et al., 2017). It depends not only on dynamic and complex geochemical processes, but also on the species considered and its rhizospheric processes potentially mitigating exposure to metal contaminant (Antoniadis et al., 2017). Thus, in this study, we focused on the biological response of targets as phytometers that integrate all soil effects on the long-term. Negative effect being detected only for the nurse accumulating species, elemental allelopathy remains the most rational explanation for our long-term effects results.

TABLE 2 Results of the three-way ANOVA (bold values: $p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$) on the effects of the nurse species, effect type, target species treatments and their interactions on Relative Interaction Index (RII Growth)

	RII (Growth)		
Factors	***df***	***F***	***p***
Nurse species	1	19.694	**<0.001*** **
Effect type	2	10.348	**<0.001*** **
Target species	1	0.026	0.870
Nurse species × effect type	2	6.516	**0.0018** **
Nurse species × target species	1	1.271	0.261
Effect type × target species	2	0.404	0.667
Nurse species × effect type × target species	2	0.182	0.833

4.3 | Short-term micro-climate amelioration

Short-term micro-climate amelioration is a process involved in many studies showing facilitation between plants in various climates (Gómez-Aparicio et al., 2005; Saccone et al., 2009). Micro-climate ameliorations are also suggested by studies in metal-polluted context (Domínguez et al., 2015; Zvereva & Kozlov, 2007). One of the

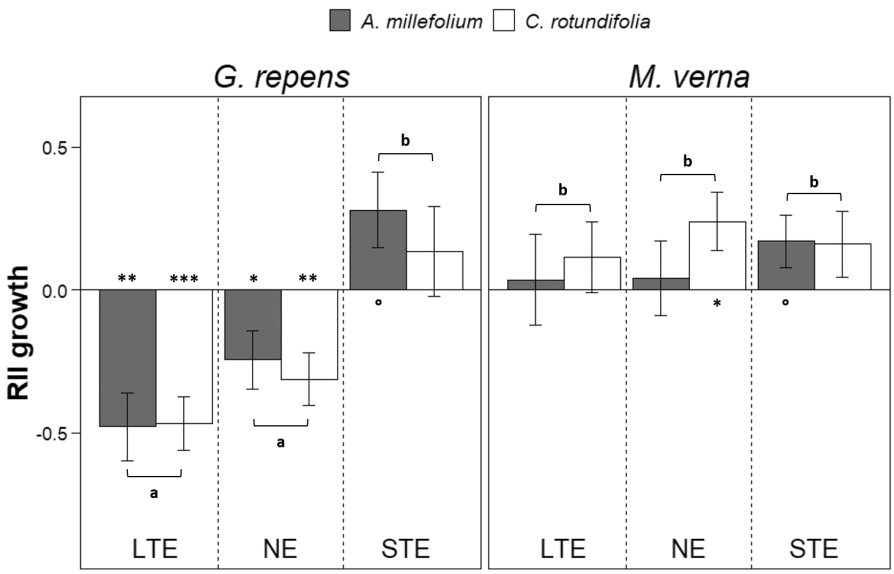

FIGURE 4 Means ± SE of Relative Interaction Index (RII Growth ± SE) of the two target species for long-term (LTE), net (NE) and short-term (STE) effects of the two nurse species. *Achillea millefolium* values are shown by light grey bars, *Campanula rotundifolia* by white bars. See Table 2 for detailed results of the corresponding three-way ANOVA. Different letters show the results of the post hoc Tukey HSD tests for the effect type × nurse species interaction. Difference of RII from 0 value (one-sample *t*-test) are also shown (°$p < 0.1$; $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$).

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corner-stone processes in dry conditions is the mitigation of atmospheric drought (i.e. the decrease of VPD, Aguirre et al., 2021; Guignabert et al., 2020; Muhamed et al., 2013). Thus, in line with previous facilitation studies we found in our experiment a lower VPD below the canopy of both nurse species than in the open. This effect was due to both an increase in RH and a decrease in T below nurse canopies in particular during the hottest conditions. Consistent with our second hypothesis, *G. repens* being the tallest nurse and having largest leaves, there was a tendency of higher microclimate amelioration below its canopy than *M. verna*'s one. However, this difference in microclimatic amelioration between both species was slight and not always observed during our two sampling periods. Thus, these differences in canopy effects should be assessed over longer periods of time to get a more robust conclusion.

In addition, these positive effects on microclimate of the two species did not turn into clear positive short-term effects on targets since short-term RII were only marginally significantly positive. Although atmospheric drought mitigation by nurse canopies very likely benefited target species, this beneficial effect could have been offset by negative light interception effects. Liancourt et al. (2005) and Forey et al. (2010) have shown that the balance of drought mitigation and negative light interception effects strongly drive plant-plant interaction net outcome.

4.4 | Short-term, long-term and net effects

The strength of our approach distinguishing short- and long-term effects highlights the diversity of simultaneous and potentially opposite effects involved in species interactions. This is consistent with former studies in Mediterranean climates (Michalet et al., 2017; Pistón et al., 2018) where this approach allowed to distinguish positive short-term and negative long-term effects (litter interference). As to metalliferous systems, Domínguez et al. (2015) found that the increase of target species survival in the shade of a dominant shrub occurred along with an increase of metal concentration in target species leaves. It suggests that opposite soil and micro-climate effects were also at stake. Future studies interested in elemental allelopathy should estimate other potential processes simultaneously involved in plant-plant interactions. It will enable to better estimate the importance of such kind of effects in the net outcome of plant-plant interactions.

5 | CONCLUSIONS

Studies of plant-plant interactions in metalliferous systems are still in their infancy compared to other harsh systems largely explored by plant ecologists like alpine or dry ecosystems. By dissociating the short- and long-term effects on target species of two nurse species with different strategies, our experiment improved our knowledge on mechanisms at stake in plant-plant interactions in metal-rich environments. Evidence of facilitation between plants in metalliferous systems are increasing in the literature, thus providing additional support to the Stress Gradient Hypothesis (SGH). However, the specific adaptation to metal stress by some species accumulating metals in their leaves, and the corresponding elemental allelopathy, creates conditions where the SGH is less likely to be verified. The focus made here on the nurse strategy enables to capture this complexity. The corresponding results should help determine what makes a good nurse in harsh metalliferous systems and ultimately help ecological rehabilitation of metal-contaminated sites. It has been suggested that tall exploitative species are the most likely to act as pioneer species improving soil fertility conditions (Navarro-Cano et al., 2018). Tall species are also more likely to improve micro-climatic harsh conditions which are frequent in many metal-polluted environments (Domínguez et al., 2015; Mendez & Maier, 2008). As VPD and temperature stresses will increase with climate change, micro-climatic amelioration by nurses will be even more crucial for ecological restoration of many metalliferous environments. However, the status of such species regarding metal accumulation have to be verified with care. If it comes along with a higher metal concentration in their leaves, their positive effects may be offset by local increase of soil contamination on the long-term. However, other studies with replications of the two nurse types are needed to go beyond the proof of concept provided by our experiment including only one species of each nurse type.

AUTHOR CONTRIBUTIONS

Florian Delerue and Richard Michalet conceived the study and design. Hugo Randé, Richard Michalet, Florian Delerue and David Nemer collected field data. Valérie Sappin-Didier carried out the soil analysis. Hugo Randé performed the analysis with inputs from Florian Delerue, David Nemer and Richard Michalet. Hugo Randé, Richard Michalet, Valérie Sappin-Didier and Florian Delerue wrote the manuscript.

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CONFLICT OF INTEREST

The authors have no conflicts of interest to declare.

DATA AVAILABILITY STATEMENT

Data available via the Dryad Digital Repository: https://doi.org/10.5061/dryad.gf1vhhmt0 (Randé et al., 2022).

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