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Higher facilitation for stress-intolerant ecotypes along a metal pollution gradient are due to a decrease in performance in absence of neighbours

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The study of variation in plant–plant interactions along metal-pollution gradient is in its infancy, although this is worth to be assessed for both restoration and theoretical perspectives. Additionally, the mechanisms of facilitation at stake in these particular stressed conditions are poorly known. We aim at understanding the importance of species and ecotypes stress-tolerance in competitive and facilitative responses to neighbours along metal-pollution gradients. We addressed this goal in a field experiment conducted in a former mining area located in a subalpine grassland of the French pyrenees. Two ecotypes of *Armeria muelleri* (a highly tolerant species to metal stress) and *Agrostis capillaris* (a less stress-tolerant species) were harvested at the highest and lowest levels of pollution within their distribution range and transplanted with and without neighbours along a strong metal-pollution gradient. The relative interaction index (RII) was used to assess both the effect of neighbours at different stress levels and the effect of metallic stress with and without neighbours. With increasing pollution, plant–plant interactions shifted from negative to positive for *Agrostis*, but did not vary significantly for *Armeria*. At high pollution level, the unpolluted *Agrostis* ecotype was more facilitated than the polluted one, and in benign habitats the polluted *Armeria* ecotype was more sensitive to competition than the less polluted ecotype. Interestingly, the increase in facilitation with increasing metal stress for the stress-intolerant *Agrostis* ecotype was primarily due to a decrease in performance without neighbours, whereas the increase in competition with decreasing metal stress for the stress-tolerant *Agrostis* ecotype was primarily due to an increase in performance with neighbours. More generally, these results suggest that the high size-plasticity of competitive (and stress-intolerant) species or ecotypes may explain both their competitive effects in benign habitats through neighbour-trait effects and facilitative responses in stressed habitats in the context of environmental-severity effects.

Keywords: competition, ecotypes, environmental-severity effects, facilitation, heavy metals, metallophytes, neighbour-trait effects, stress gradient hypothesis, stress tolerance

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Introduction

Metalliferous habitats are common worldwide, resulting either from anthropogenic activities or the occurrence of spontaneous metal-rich soils derived from ultramafic rocks like serpentine (Harrison and Rajakaruna 2011). These habitats represent harsh environments for plant growth that typically exhibit high metal or metalloid (hereafter referred to as 'metals') concentrations with potential toxicity, but also low nutrient-availability and water holding capacity (Wong et al. 1998, Ye et al. 2002, Meeinkuirt et al. 2012). Mine tailings are one of the anthropogenic metalliferous habitats exhibiting strong constraints for plants (Wong et al. 1998, Ye et al. 2002, Meeinkuirt et al. 2012) and are an important part of the many polluted sites reported worldwide (Panagos et al. 2013, Hou et al. 2020). Plant communities on mine tailings are characterized by a low diversity and a degraded vegetation with reduced vegetation cover due to the ecotoxic impact of metal excess on plant physiology (Chatterjee and Chatterjee 2000, Ye et al. 2002, Oancea et al. 2005). Despite the fact that metals have a deleterious impact on non-tolerant species growth, various metallicolous species have the ability to survive with high amounts of metals through diverse mechanisms, such as enhanced complexation and stabilization of metals in soils, their storage and accumulation in roots or even in leaves in dedicated organs or cell compartments (McGrath and Zhao 2003, Ernst 2005, Marques et al. 2009).

Most studies and experiments conducted over the past two decades have focused on the use of these metallicolous species as a potential phytoremediation tool to clean up or stabilize polluted soils (Ghosh 2005, Jadia and Fulekar 2008, Marques et al. 2009, Meeinkuirt et al. 2013, Losfeld et al. 2015, Rodríguez-Seijo et al. 2016, Stanovych et al. 2019). Fewer have investigated the nature and outcome of biotic interactions in plant communities growing on metal-polluted soils, although environmental stresses and disturbances are known to drive the direction of plant–plant interactions and ultimately plant diversity (Grime 1973, 1974, Nemer et al. 2021). According to the stress-gradient-hypothesis (SGH) (Bertness and Callaway 1994), competition dominates plant–plant interactions in benign environments, whereas facilitation predominates in stressed or disturbed environmental conditions (Bertness and Callaway 1994, Liancourt et al. 2005, Sthultz et al. 2007, He et al. 2013, Pugnaire et al. 2015, Cavieres et al. 2018). Additionally, facilitation between plants is now widely recognized as an important practical tool for ecological restoration of degraded habitats with important environmental stress across a wide range of ecosystems and climates (Maestre et al. 2001, Egerova et al. 2003, Castro et al. 2004, Sánchez-Velásquez et al. 2004) including mine tailings (Navarro-Cano et al. 2019). In agreement with the SGH, Zvereva and Kozlov (2004) showed in a case study that the effect of tree canopy on dwarf shrubs shifted from negative in an unpolluted forest to generally positive in a polluted industrial barren (Eränen and Kozlov 2007). Similarly, Frérot et al. (2006) found evidence for facilitation between diverse interacting species on metal-polluted soils in a field experiment. Other investigations have reached a similar conclusion, namely that facilitation is the dominant plant–plant interaction found in metal-polluted soils (Cuevas et al. 2013, Wang et al. 2014, Yang et al. 2015).

Facilitation is more likely to benefit species from benign environments with low stress tolerance but high competitive response ability than species from harsh environments with high stress tolerance but low competitive response ability (Liancourt et al. 2005, Gross et al. 2010). Such distinction can occur not only at the species level but also at the intraspecific level, between populations of the same species growing in more or less harsh environments, which can lead to ecotype divergence within species (Espeland and Rice 2007, Liancourt and Tielbörger 2011). According to Liancourt and Tielbörger (2011), ecotypes from more benign environments have a higher facilitative response when grown in stressful conditions than ecotypes adapted to these stressed conditions. This is because ecotypes growing in stressful conditions are more stress tolerant and require less stress mitigation by neighbours, while having a higher cost of living close to neighbours due to competition for light (stress-tolerance/competitive ability tradeoff sensu Liancourt et al. 2005). Ecotypic differentiation with distinct stress tolerance to metalliferous habitats have been shown for different species (Nandillon et al. 2021). In these former studies, ecotypes that grow on metal-polluted soils are better able to cope with metal stress than those that grow on unpolluted soils. Thus, such ecotypic differentiation could be an important driver of plant sensitivity to facilitation by neighbouring plants in metalliferous habitats.

Plant–plant interactions are typically quantified comparing the performances of a dependent target species with and without neighbours with indices such as the relative interaction index (hereafter RII, Armas et al. 2004). However, Michalet et al. (2014b) have emphasized that different ecological processes may be involved when changes in facilitation and competition are detected along environmental gradients with such indices. Indeed, a switch from competition to facilitation along a stress gradient can result from: 1) a decrease in the performance of the target species without neighbours due to increased environmental-severity without changes in its performance with neighbours (Fig. 1, case B for detailed illustration). This effect is referred to as an 'environmental-severity effect'; 2) an increase in target species performance with neighbours as stress increases (Fig. 1, case A). This latter effect is referred to as a 'neighbour-trait effect' because it is due to the improvement of micro-environmental conditions in neighbours vicinity due to changes in neighbour-traits; 3) a combination of both environmental- and neighbour-trait effects. Michalet et al. (2014b) have shown that increases in competition with decreasing stress are often due to a neighbour-trait effect (i.e. to an increase in neighbour competitive effect, Goldberg 1990, Liancourt and Tielbörger 2009), whereas increases in facilitation with increasing stress are generally due to environmental-severity effects (Chaieb et al. 2021). Indeed, it is rational to propose that an increase in facilitation with increasing stress is unlikely to be due to a

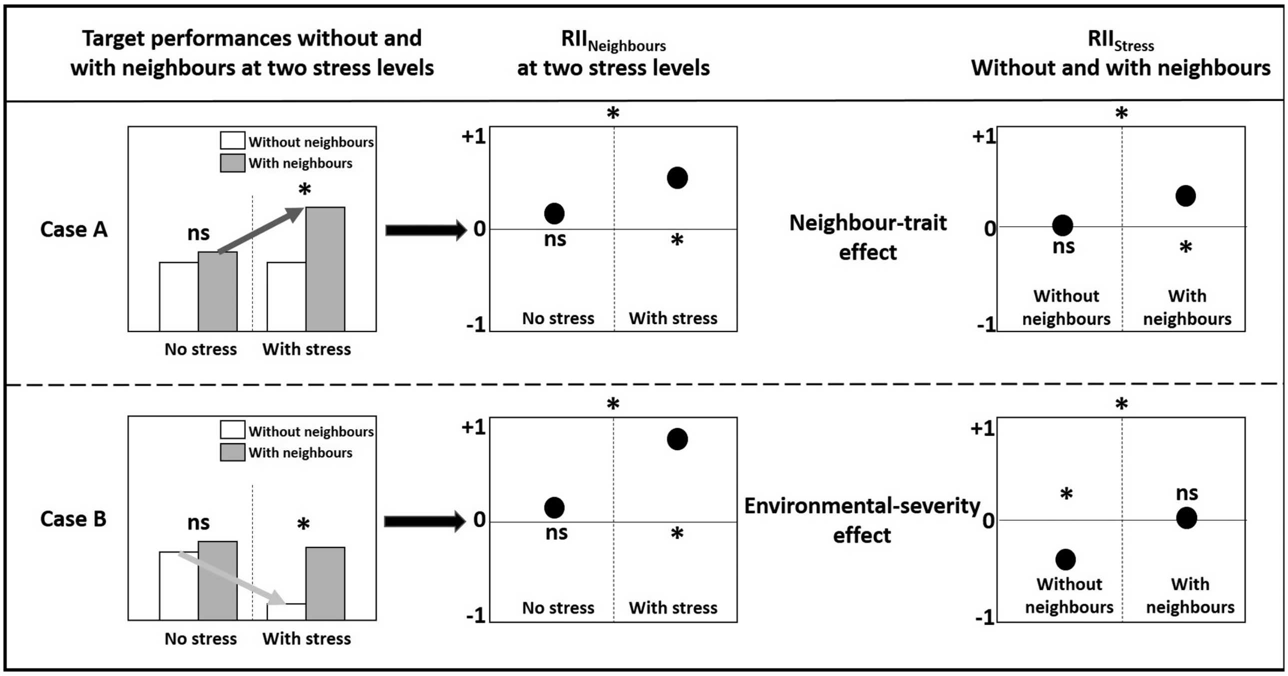

Figure 1. Changes in $\text{RII}_{\text{Neighbours}}$ along a stress gradient (increases in facilitation with increasing stress) either due to a neighbour-trait (case A) or an environmental-severity (case B) effect. In case A, the increase of $\text{RII}_{\text{Neighbours}}$ with stress is due to an improvement of the target performance with neighbours when the stress is present compared to situation without stress (see the dark grey arrow in the left panel). In case B, the increase of $\text{RII}_{\text{Neighbours}}$ with stress is due to a decrease of the target performance without neighbours when the stress is present compared to situation without stress (see light grey arrow in the left panel). Ns and stars in left panels indicate non-significant and significant neighbour effects at each stress level, respectively. Ns and stars below or above black dots in middle and right panels indicate non-significant and significant sample t-tests on RII values, respectively. Stars above panels in middle and right panels indicate significant stress and neighbour effects, respectively. Inspired from Michalet et al. (2014b). $\text{RII}_{\text{stress}}$ indicates the effect of stress on target plant performance (P) when grown with and without neighbors, with values below 0 indicating better target species performance with no stress ($P_{+\text{stress}} < P_{-\text{stress}}$), and values above 0 indicating better target species performance with stress ($P_{+\text{stress}} > P_{-\text{stress}}$).

neighbour-trait effect because nurse plants are also weakened by the increased stress level (but see Schöb et al. 2013).

Our overarching hypothesis in this study is that species and ecotypes from metalliferous habitats are less facilitated than species and ecotypes from non-metalliferous habitats when grown in harsh metalliferous habitats. The corollary of this hypothesis is that species and ecotypes from metalliferous habitats are more sensitive to competition than species and ecotypes from non-metalliferous habitats when grown in more benign habitats. Although this question is crucial both for our knowledge of plant populations dynamics and assemblage in metalliferous habitats and for potential phytoremediation technologies, it has not been investigated so far in the ecological literature. We make two predictions in line with our first hypothesis: 1) an increase in facilitation from a low- to highly metal-polluted habitat for a species or ecotype from low-polluted habitats should be due to an environmental-severity effect. Indeed, we expect these less stress-tolerant species or ecotype to strongly decrease their performance when growing without neighbours in condition of increasing stress; 2) an increase in competition from highly to low metal-polluted habitats for a species or ecotype from highly-polluted habitats should be due to a neighbour-trait effect. Indeed, we expect more stress-tolerant species or ecotypes from highly polluted habitats to strongly decrease their performance when growing with neighbours in low metal-polluted habitats because of their higher sensitivity to increasing neighbours competitive effects.

We set up a field reciprocal transplant experiment in a former mining sector located in the French pyrenees, cross-transplanting with and without neighbours two ecotypes of two species present at different positions along a pollution gradient (with assumed different tolerance abilities to metallic stress): 1) *Armeria muelleri* present from the most polluted area until intermediate levels of pollution. 2) *Agrostis capillaris* present from unpolluted areas until intermediate levels of pollution (Fig. 2A, B). To assess the role of ecotypic variation for these two species responses to plant–plant interactions, individuals of the two ecotypes were harvested for the two species at the highest and lowest levels of pollution within their distribution range along the pollution gradient and then transplanted all along the gradient within neighbours or in small areas where neighbours were removed. We predict that:

1) Plant–plant interactions should shift from negative (competition) to positive (facilitation) with increasing environmental stress imposed by pollution in agreement with the SGH.

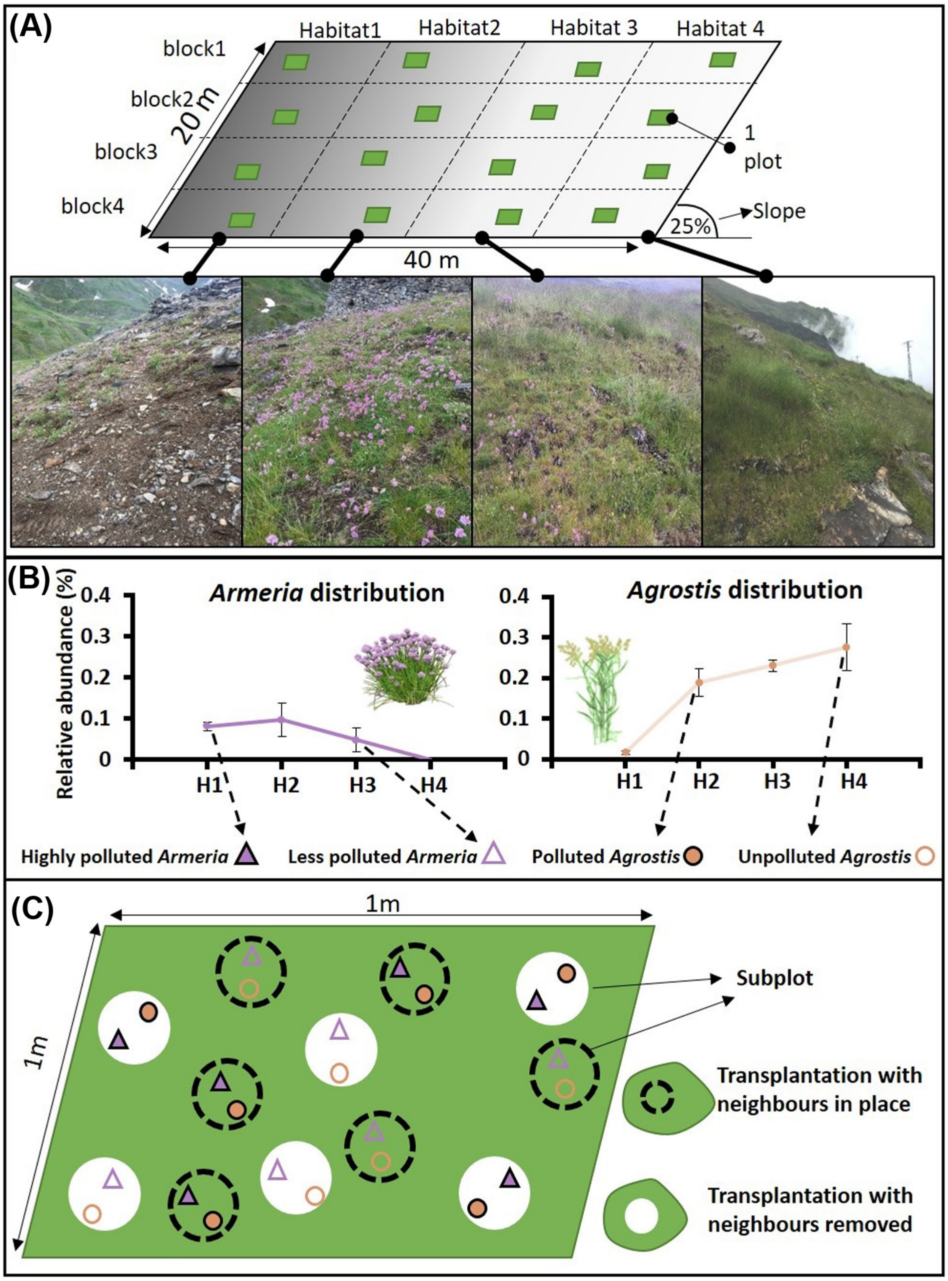

Figure 2. Presentation of the experimental design. (A) Schematic representation of the experimental sites and pictures of the four habitats showing the variation of plant communities along the metal pollution gradient studied. The pollution is indicated by the grey color gradient on the scheme. (B) Relative abundance of *Armeria mulleri* and *Agrostis capillaris* in the 4 habitats from the most polluted area (habitat 1) to the classic subalpine community (habitat 4). Symbols at the panel bottom (triangles and circles) indicate the habitats were *Armeria* (purple triangles) and *Agrostis* (brown circles) ecotypes were harvested. Full symbols are for ecotypes from higher level of pollution; empty symbols for ecotypes from lower level or absence of pollution. (C) Schematic representation of a plot where three replicates of the two ecotypes of both species were transplanted within neighbours or with neighbours removed.

2) Facilitation should likely occur at high level of pollution for *Agrostis capillaris* (the least stress-tolerant species according to its distribution range, Fig. 2B), while *Armeria muelleri* (the most stress-tolerant species, Fig. 2B) should be excluded from benign habitats by competition.
3) Facilitation should be higher for *Agrostis capillaris* ecotype from unpolluted habitats, while competition should be higher for *Armeria muelleri* ecotype from the highly-polluted habitats.
4) Increasing facilitation with increasing environmental stress should be due to environmental-severity effects, whereas

increasing competition with decreasing stress should be due to neighbour-trait effects.

Material and methods

Study site and target species selection

This experiment was carried out in the former mining area of Sentein (department of Ariège, southwest France, 42°49′30.3″N, 0°53′53.3″E), located at 2000 m altitude at the subalpine belt of the Pyrenees Mountains on a calcareous rock. At this altitude, the growing season begins early June and ends late September. The average temperature and precipitation during this period recorded in 2021 by a weather station located in the studied site (Delta-T Device® DLT/WGS-GPA) were 11.9°C and 237.8 mm, respectively. In the region, mining activities stopped in the late 1960s. However, zones of important metal pollution remain and foster vegetation dynamics (Supporting information). The main toxic elements related to mining activities are Zn, Pb, Cd, Cu and As (Stephant-Champigny et al. 2015). Metallicolous communities spontaneously colonized these polluted areas. The study site extends from a former zone of ore transportation (dominated by *Armeria muelleri* and other metal-tolerant species such as *Minuartia verna*, *Arenaria multicaulis*, *Hutchinsia alpina*) to a classic subalpine plant community growing on a calcareous rock. Thus it encompasses a gradient of vegetation cover with progressive change of plant community composition (Fig. 2A, B). On the most polluted soils, only the above mentioned metallicolous species are present, whereas in the non-polluted soils the subalpine community is dominated by *Festuca gautieri*, *Festuca rubra* and *Agrostis capillaris*. In the intermediately polluted soils, plant communities have an intermediate composition, with metallicolous species dominating the area close to the source of pollution, and subalpine grasses dominating the area more distant from the pollution. Thus, it forms four distinguishable habitats (H1–H4, from most to least polluted) characterized by distinct species composition and abundance (Fig. 2A, B).

In this study, we selected as targets for the transplantation experiment two ecotypes of two perennial species occurring at different positions along the pollution gradient, with different presumed metal stress tolerance capacities, (Fig. 2B). *Armeria muelleri* (hereafter referred to as *Armeria*) was the first target species. It is present from the most to the moderately polluted habitats and is thus presumed to have high metal-stress tolerance. It is an evergreen perennial plant forming clumps or mats of strap-like or linear leaves, with dense clusters of small cup-shaped flowers. *Agrostis capillaris* (hereafter referred to as *Agrostis*) was present from moderately polluted to unpolluted habitats that are farthest from the source of pollution (Fig. 2B) and is thus presumed to have a lower metal-stress tolerance than *Armeria muelleri*. Note that *Agrostis capillaris* is a grass species regularly found in polluted sites with moderate levels of metal pollution (Humphrey and Nicholls 1984). In this study, we focused on the response to plant–plant interactions of two ecotypes of both species. These ecotypes were selected in the habitats corresponding to their highest and lowest levels of soil pollution within their distribution range (Fig. 2B). Because the geographical distance between these habitats is limited, corresponding ecotypes are likely not genetically isolated. Still, the individuals harvested are those that were able to cope with the edaphic conditions locally, conditions that vary considerably along the studied gradient. Thus, ecotypes here are two different subpopulations of local species that were selected by contrasting environmental conditions.

Experimental design

Our field experiment was conducted from early June to late September 2020 to cover the entire growing season at this altitude. The site had a south east exposure with a slope of 25%. The pollution gradient (from the zone of high pollution to the unpolluted zone nearby) is orthogonal to the slope. The site was split in four blocks orthogonal to the slope in order that each block encompasses the whole pollution gradient (Fig. 2A). In each block and each of the four habitats a plot of 1 m^2 was randomly identified avoiding obvious microtopographic anomalies (e.g. particularly concave or convex zones) or zones dominated by barren rocks (Fig. 2A).

Transplantation of the studied species and ecotypes with and without vegetation were performed in each plot as follow. On 2 June, three individuals of each of the two ecotypes for each species were harvested at the highest and lowest pollution levels of their distribution range along the pollution gradient. Their root systems were freed from soil and all individuals were immediately transplanted in two different neighbouring conditions: 1) 'with neighbours': in three subplots where the vegetation remained intact (control conditions), and 2) 'without neighbours': in three other subplots (diameter 0.15 m and with similar vegetation composition than the controls) randomly selected for the vegetation removal treatment (Fig. 1C). This 'neighbour removed' treatment was done with scissors cutting aboveground vegetation as close as possible to the soil. In each subplot, two individuals were transplanted: one of each species, ecotypes from the highest pollution level or lowest pollution level being transplanted together (Fig. 1C). All transplanted individuals were tagged with metal wire to ease their finding at the end of the experiment. In each plot, a total of 24 individuals were thus transplanted (2 species × 2 ecotypes × 2 neighbouring conditions × 3 replicates, Fig. 2C) for a total of 384 transplants (24 individuals per plots × 4 habitats × 4 blocks).

Soil and environmental measurements for characterizing the pollution gradient

Soils were sampled (500 mg) next to each plot from the top soil layer [0–15 cm]. Then, total metals concentration in the soil (Zn_{TOT}, Pb_{TOT}, Cd_{TOT}, Cu_{TOT}, As_{TOT}), were determined after acid digestion of the soils with a mixture of hydrofluoric and perchloric acids (NF X 31–147, 1996) and subsequent analysis of the solutions by inductively coupled plasma mass spectrometry (ICP-MS). Extraction with 0.11 M acetic acid (Rauret et al. 1999), followed by analysis of the solutions by inductively coupled plasma atomic emission spectroscopy (ICP-AES) were used to identify the available metals (Cd_{AA}, Pb_{AA}, Zn_{AA}). To complete the characterization of the gradient, other soil properties from the same soil samples were measured. Soil texture (clay, silt and sand content) was estimated using the Robinson pipette method (NF X 31–107, 1983). Dry combustion was used to determine the carbon content (CTOT) and total nitrogen (NTOT) of the soil following carbonate correction (CTOT: NF ISO 10694:1995, NTOT: NF ISO 13878). The organic matter (OM) content was calculated by multiplying the organic carbon content of the soil (NF ISO 14235: 1998) by 1.72 (Pribyl 2010). Soil pH was measured in water with a 1:5 soil:water ratio (NF ISO 10390:2005). Soil depth was measured as the depth to which a 4 cm diameter metal probe could penetrate the soil. Soil moisture (volume %) was measured in the field seven days after the last rain on a warm, sunny day on 22 June using a portable moisture probe (<https://delta-t.co.uk/product/ml3/>). The probe was inserted at four locations in each plot and the four values were averaged to estimate soil moisture. Additionally, a 0.5 × 0.5 m quadrat was randomly placed next to each plot and relative abundance for both species in each habitat were quantified using the contact points method. The quadrat was composed by a 10 × 10 cm wire grid given 36 intersect points considering the quadrat edges. The presence of both species was recorded (0–1) at each corresponding points encompassing a zone of 3 cm in diameter. The abundance of each species (in %) was obtained by dividing the number of points with the species of interest over the total number of contact points (36). The vegetation cover (%) and mean vegetation height was also recorded in each plot and enable calculation of vegetation volume (dm^3) (volume dm^3 = vegetation cover % on 1 m^2 (1% = 1 dm^2) multiplied by mean vegetation height (dm). Full details regarding metals concentration in soils and other environmental variables along the experimental gradients are given in supporting information (Supporting information).

Plant–plant interactions measurement and analysis

Survival of all transplanted individuals was determined at the end of the growing season on 24 September. In each plot, survival rates of the different species and ecotypes were calculated as the proportion of alive individuals relative to the three individuals transplanted initially (i.e survival rates equal to 0, 33, 66 or 100%). Because of low survival rates in many plots, we did not analyse target biomass and growth.

In order to quantify the responses (survival rates) of the different species and ecotypes to the effects of neighbours along the metal pollution gradient, we calculated the relative interaction index (RII, Armas et al. 2004) in each plot. This index is based on the comparison of target performance with and without neighbours:

$$\mathrm{RII}_{\mathrm{neighbour}} = \frac{P_{+\mathrm{neighbour}} - nP_{-\mathrm{neighbour}}}{P_{+\mathrm{neighbour}} + P_{-\mathrm{neighbour}}} \quad (1)$$

where $P_{+\mathrm{neighbour}}$ and $P_{-\mathrm{neighbour}}$ represent target performance (the proportion of corresponding species and ecotype that survived in the plot) in the presence and absence of neighbouring vegetation respectively. Values of RII vary between −1 and 1, with negative values standing for competition and positive ones for facilitation.

To disentangle environmental-severity effects from neighbour-trait effects during changes in plant–plant interaction with increasing metal stress, we compared plant performance under the highest stress level (habitat 1) and no-stress level (habitat 4). In addition to the $\mathrm{RII}_{\mathrm{neighbours}}$ (Eq. 1), we computed the following relative interaction index following Michalet et al. (2014b).

$$\mathrm{RII}_{\mathrm{stress}} = \frac{P_{+\mathrm{stress}} - sP_{-\mathrm{stress}}}{P_{+\mathrm{stress}} + P_{-\mathrm{stress}}} \quad (2)$$

where $P_{+\mathrm{stress}}$ is the target response (proportion of transplants that survived in the plot) with metallic stress (in habitat 1) and $P_{-\mathrm{stress}}$ is the target response without stress (in habitat 4). Two $\mathrm{RII}_{\mathrm{stress}}$ were calculated independently, one for target species and ecotypes with neighbours, and one for target species and ecotypes without neighbours. $\mathrm{RII}_{\mathrm{stress}}$ indicates the effect of metallic stress on target plant performance when growing in similar neighbouring conditions, with values below 0 indicating better target species performance when no stress is present ($P_{+\mathrm{stress}} < P_{-\mathrm{stress}}$), and values above 0 indicating better target species performance when metallic stress is present ($P_{+\mathrm{stress}} > P_{-\mathrm{stress}}$). The same $\mathrm{RII}_{\mathrm{neighbours}}$ calculated to quantify plant responses to the effects of neighbours along the metal pollution gradient was also used here, but only at the two extremes of the gradient, $\mathrm{RII}_{\mathrm{neighbours}}$ for target species and ecotypes under the highest stress level (in habitat1), and $\mathrm{RII}_{\mathrm{neighbours}}$ for target species and ecotypes under no-stress level (in habitat 4). If $\mathrm{RII}_{\mathrm{neighbours}}$ differ significantly between no-stress and stress levels, then $\mathrm{RII}_{\mathrm{stress}}$ can be used to attribute the differences to: 1) a neighbour-trait effect when $\mathrm{RII}_{\mathrm{stress}}$ with neighbours is significantly different from 0 (Fig. 1, case A); 2) a significant environmental-severity effect when $\mathrm{RII}_{\mathrm{stress}}$ without neighbours is significantly different from 0 (Fig. 1, case B) and 3) a combined environmental-severity and neighbour-trait effect if $\mathrm{RII}_{\mathrm{stress}}$ with neighbours and without neighbours are both significantly different from 0 (see Michalet et al. 2014b for full details regarding these indices calculation and interpretation).

Statistical analyses

All statistical analyses were done using R software (<www.r-project.org>). To examine how soil metals and soil environmental properties differed among the experimental gradients, we conducted two principal component analyses on the soil variables measured close to each plot. The first PCA was for soil metal content (Supporting information, above part) and enabled the quantification of a synthetic variable representing metal pollution (PCA_{Met}, the position of the plots on the 1st PC). Besides toxicity, metal pollution can have cascading effect on soil structure and fertility, all potentially influencing plant–plant interaction. Thus, we performed a second PCA to combined this soil pollution metric (PCA_{Met}) with all other environmental variables characterized (Supporting information, bottom part). This enabled the quantification of a synthetic variable (PCA_{Env}, the position of the plots on the 1st PC of this second PCA) to have a complete characterisation of the environmental and pollution gradient at stake in this study. This synthetic PCA_{Env} metric has the advantage to capture all the complexity of the environmental gradient at stake, soil pollution being the most important driver along this gradient.

As to species and ecotypes response to plant–plant interactions, a first analysis focused on the variation of plant–plant interactions along the pollution gradient. We analysed the variations of $RII_{neighbours}$ for *Agrostis* and *Armeria* separately using linear mixed-effects models with PCA_{ENV} (i.e. the quantitative position of each plot along the pollution gradient), Ecotypes and their interactions as fixed factors, and block as random factor. Additionally, we used one-sample t-tests to detect significant deviation of RII values from zero for the four plots present in each habitats. Because this first analysis indicated that these variations were only detectable for *Agrostis*, delineation of environmental and neighbour-trait effects between the most (habitat 1) and less (habitat 4) polluted situations was done only for this species and its two ecotypes. First, for each *Agrostis* ecotype, survival of transplants was analyzed using a general linear mixed-effects with a logit link function for binomial distribution, with stress level (high-habitat 1 or low-habitat 4), neighbouring conditions (with or without) and their interactions as fixed factors (followed Tukey post hoc multiple comparisons) and block as random factor. Then, changes of $RII_{neighbours}$ between the two different levels of stress, and modifications of RII_{stress} between the two neighbouring conditions were investigated using linear mixed-effects with block as random factor. We used one-sample t-tests to detect significant deviation of $RII_{neighbours}$ or RII_{stress} values from zero. During all analysis regarding RII values, normality and homoscedacity of models residuals were systematically checked to respect the condition of application of linear modeling.

Results

Environmental and pollution gradient

The first PCA conducted on metal pollution variables clearly highlighted the pollution gradient with strong correlations between concentrations of all measured elements (Fig. 3A). The plots situated in the four habitats showed significant differences ($p < 0.001$) regarding soil metal concentration (Supporting information), with the first habitat having extremely high level of pollution with up to 15% and 3% of Zn and Pb in total concentration and high concentration in highly toxic element like Cd and As (Supporting information, Fig. 3B). In sum, the variation between plots regarding metal concentration in soils is well represented by the 1st PCA axis (PCA_{MET}) accounting for 94.6% of the whole variance between plots (Fig. 3A, B). Combination with the other environmental variables (2nd PCA) showed that the metal pollution gradient was also clearly correlated with a significant decrease of vegetation volume when pollution increased as well as an increase in soil pH and a sandier texture (Supporting information, Fig. 3C). The major axis of variation between plots for all environmental variables was related to this metal pollution gradient, and well represented by the 1st PCA axis (PCA_{ENV}) accounting for 67.3% of the whole variance between plots and showing a clear distinction between plots from the four different habitats (Fig. 3C, D).

Plant interactions along the metal pollution gradient

There was a highly significant effect of the metal pollution gradient (PCA_{ENV}) on $RII_{neighbours}$ for *Agrostis* (Fig. 4A), indicating an overall shift from competition to facilitation with pollution. In contrast, $RII_{neighbours}$ for *Armeria* was not affected by the metal pollution gradient. The ecotype effect was not significant in corresponding linear models for the two species (Fig. 4A, B). However, the results of one-sample t-tests showed that $RII_{neighbours}$ for unpolluted *Agrostis* ecotype shifted from significantly negative to significantly positive when pollution increased, and shifted from significantly negative to neutral for polluted *Agrostis* ecotype. Moreover, the results of one-sample t-test revealed that at the lowest pollution level, $RII_{neighbours}$ for highly polluted and less polluted *Armeria* ecotype, were marginally negative ($p = 0.054$) and neutral, respectively (Fig. 4B). According to this finding, there was a slight trend for the highly polluted *Armeria* ecotype to be more excluded from benign habitats by competition than the less polluted *Armeria* ecotype.

Neighbour-trait and environmental-severity effects

Because the influence of the pollution gradient was only significant for *Agrostis* in the previous results (Fig. 4A), we focus in this section on this species response to the different stress levels with and without neighbours. We found a significant stress level × neighbours interaction on survival for both *Agrostis* ecotypes (Fig. 5A–D). Survival of unpolluted *Agrostis* ecotype without neighbours was significantly higher under low stress (habitat 4) than under high stress (habitat 1) levels (Fig. 5A), whereas the survival rate of polluted *Agrostis* ecotype without neighbours did not differ significantly between no-stress and stress levels (Fig. 5D). In contrast, under high stress and low stress levels, the survival rate of unpolluted *Agrostis* ecotype with neighbours did not differ significantly (Fig. 5A), whereas the survival of polluted *Agrostis* ecotype with neighbours was significantly higher under high stress than low stress levels (Fig. 5D).

For unpolluted *Agrostis* ecotype, $RII_{neighbours}$ was negative under no stress levels and shifted to positive value under stress (Fig. 4A, 5B). RII_{stress} was negative without neighbours and shifted to marginally positive with neighbours (Fig. 5C).

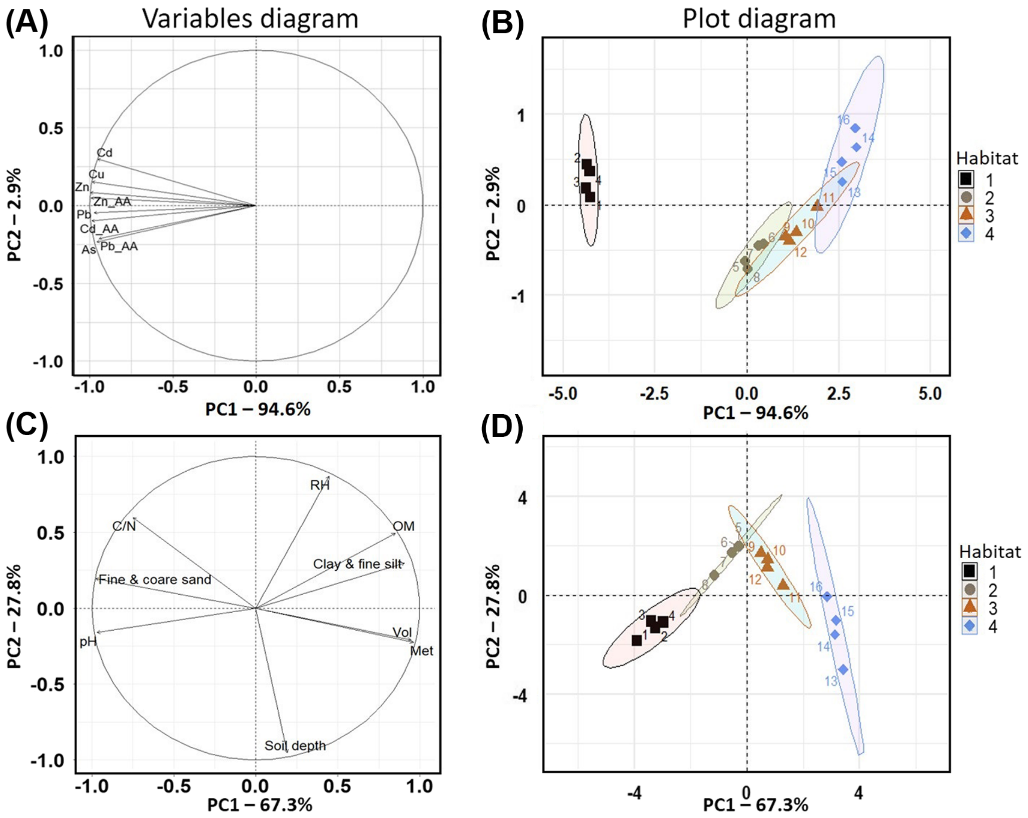

Figure 3. Principal component analyses (PCA) displaying the variability of soil metals variables and other soil variables between the four habitats along the studied gradient. (A) Variables factor map for soil metals concentration. Cd_AA: cadmium extracted with acetic acid; Pb_AA: lead extracted with acetic acid; Zn_AA: zinc extracted with acetic acid; Cd: total cadmium concentrations; Pb: total lead concentrations; Zn: total zinc concentrations; Cu: total copper concentrations; As: total arsenic concentrations. (B) Plot scores along PCA axes 1 and 2 for soil metal content. (C) Variables factor map for all soil variables including soil pollution. Met: synthetic estimation of metal concentration (PCA_{MET}) in soil from the PCA presented in (A) and (B). It corresponds to individuals' position on the first component of this PCA. RH: relative humidity; Vol: vegetation volume; OM: organic matter; Clay_Silt: clay and fine silt; Silt_Sand: fine and coarse sand; C/N: carbon to nitrogen ratio. (D) Plot scores along PCA axes 1 and 2 for all soil variables.

These findings show that when stress increases along the pollution gradient, the shift from competition to facilitation for the unpolluted ecotype was predominantly due to an environmental-severity effect, and to a lesser degree to a marginal neighbour-trait effect.

For polluted *Agrostis* ecotype, $RII_{neighbours}$ was negative under no stress level and shifted to neutral under stress level (Fig. 5E). RII_{stress} was marginally negative without neighbours and shifted to positive with neighbours (Fig. 5F). These results provide evidence that the increase in competition with decreasing stress for the polluted ecotype was primarily due to a neighbour-trait effect, and to a lesser degree to a marginal environmental-severity effect.

Discussion

The outcome of plant–plant interactions for *Armeria* did not vary significantly along the metal pollution gradient, contradicting our first hypothesis. However, in accordance with our first hypothesis, plant–plant interaction for *Agrostis* shifted from negative to positive, with increasing pollution stress. Consistent with our second hypothesis, *Agrostis*, the least stress-tolerant species according to its distribution range, was more facilitated than *Armeria* at high pollution level. Consistent with our third hypothesis, at the intraspecific level, the unpolluted *Agrostis* ecotype was more facilitated than the polluted one at high pollution level; and in benign habitats the highly polluted *Armeria* ecotype showed a slight tendency to be more prone to competition than the less polluted ecotype. Consistent with our fourth hypothesis, the increase in facilitation with increasing metal stress was primarily due to an environmental-severity effect, whereas the increase in competition with decreasing metal stress was primarily due to a neighbour-trait effect. Overall, our results improve our understanding of the mechanisms driving changes in competition and facilitation along metal-stress gradients.

Response of the two species to the effects of neighbours along the gradient

Variation of $RII_{neighbours}$ along the gradient revealed that as metal stress increased, plant interactions shifted from negative to positive for *Agrostis* (Fig. 4A). This result confirms previous

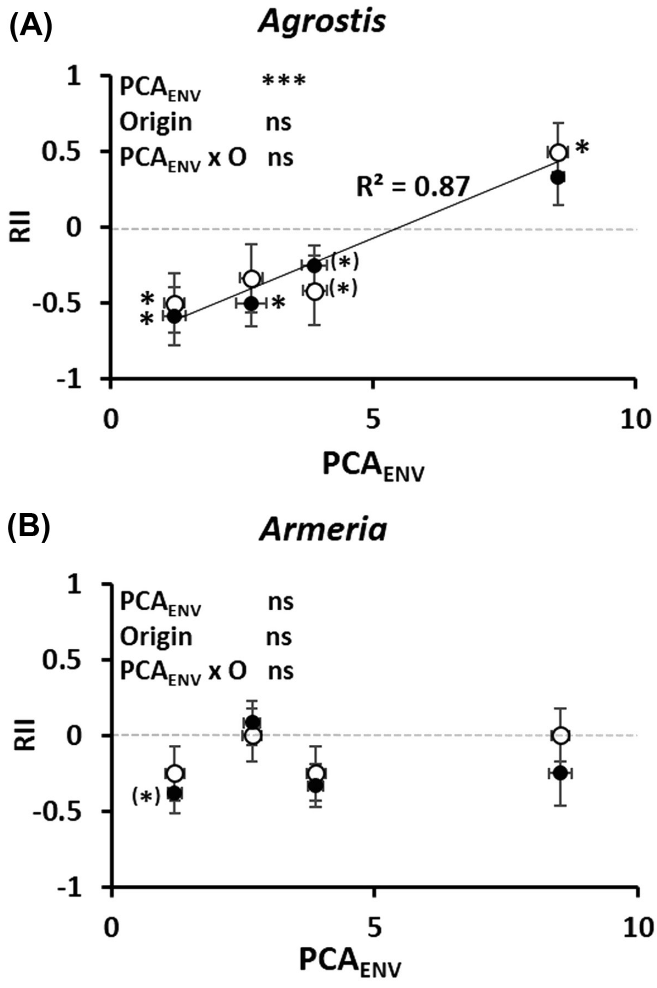

Figure 4. (A, B) Variation in $RII_{neighbours}$ (mean per habitat ± SE) of *Agrostis* and *Armeria* along the metal pollution gradient. The metal pollution gradient is characterized by the PCA_{ENV} metric. In order to have increasing values of PCA_{ENV} indicating an increasing level of pollution, we first multiplied all plot scores on the first PCA_{ENV} axis in Fig. 3D by −1 to reverse the values of PCA_{ENV} along the gradient. We then added the lowest plot score value (H4 value) +1 to the scores of all plots for visual comfort to finally obtain the PCA_{ENV} axis in this figure. Full circles: ecotypes harvested in habitats with highest level of pollution; empty symbols: ecotypes from lowest level of pollution. Significant differences of RIIs from 0 are shown next to each RII value (one sample t-test). On the top left corners of (A) and (B), results of linear mixed-effects model for the effect metal pollution gradient (PCA_{ENV}), ecotype origin and their interaction are shown. (*), $p < 0.1$; *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.

studies showing that plant–plant interactions change from competition to facilitation along pollution gradients consistent with the SGH (Domínguez 2015, Yang et al. 2015). Additionally, this is in agreement with refined facilitation theories (Michalet 2007, Maestre et al. 2009, Michalet et al. 2014a) that have proposed that the SGH is more likely to be supported along non-resource gradients, i.e. such as salt, cold or wind gradients (Bertness and Hacker 1994, Bertness and Ewanchuk 2002, Callaway et al. 2002, Pennings et al. 2003, Badano et al. 2007, Qi et al. 2018). However, unlike *Agrostis*, $RII_{neighbours}$ of *Armeria*, did not change along the pollution gradient (Fig. 4B). Several explanations can be proposed to explain these results. First, *Armeria* may have not been facilitated because it is a species strongly tolerant to the metal stress at stake in this system, consistent to results found by Callaway and Pennings (2000) in salt marshes or Noumi et al. (2015) and Liancourt et al. (2017) in very arid climates. Additionally, *Armeria* has an important primary tap-root with low development of secondary root branches. We cannot exclude that its low survival rate in all treatments was partly due to its root architecture less favourable for transplantation than a grass species with more fasciculate or branched root architecture. The potential impact of transplantation of *Armeria* in this study likely impeded to accurately assess the response of this species to plant–plant interactions. However, our result support previous studies that found that plants from benign habitats with low stress tolerance are more facilitated than plants from harsh environments with high stress tolerance (Liancourt et al. 2005, Wang et al. 2008, Gross et al. 2010). Similarly, Nemer et al. (2021) also found that the higher drought-tolerance of species from calcareous habitats compared to species from siliceous habitats reduced their competitive ability, and was therefore responsible for their exclusion from siliceous sites by competitive, high-biomass siliceous species. However, the very low species richness occurring in the most polluted habitat 1 impeded us to replicate this treatment to straightforwardly conclude on this species effect.

Difference of response between ecotypes

Liancourt and Tielbörger (2011) argued that a tradeoff between stress tolerance and competitive ability occurs not only at the species level but also at the intraspecific level, as also found by several authors (Michalet et al. 2011, Al Hayek et al. 2014). The difference in responses between *Agrostis* ecotypes are in line with this statement, since we found that at the highest level of soil pollution the unpolluted *Agrostis*, the least stress-tolerant ecotype, was more facilitated than the polluted one. Additionally, we found that the more stress-tolerant ecotype of *Armeria* was marginally more excluded from benign habitats by competition than the less stress-tolerant one. In line with our results, Al Hayek et al. (2014), found in a subalpine community from the French pyrenees that tight and loose *Festuca gautieri* phenotypes which naturally occur at two positions along a topographic gradient had contrasting competitive abilities. Additionally, they could separate the two components of species competitive ability, the competitive effect, which is the capacity of one plant to inhibit the growth of another, and the competitive response, which is the capacity of a species to withstand inhibition by its neighbours, Goldberg 1990, 1996). The tight *Festuca* phenotype was the strongest effect competitor and the loose *Festuca* phenotype the weakest response competitor.

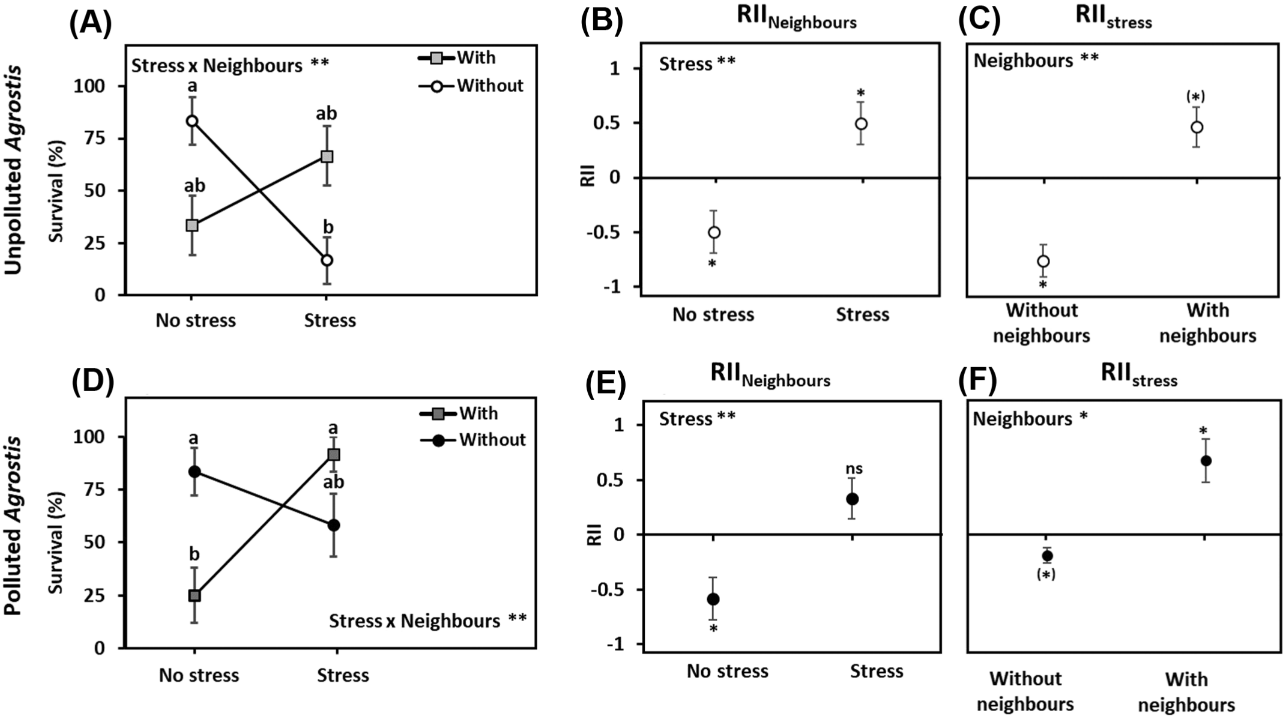

Figure 5. Survival of *Agrostis* ecotypes and corresponding RII at two contrasted stress levels and with and without neighbours. (A, D) Survival (mean ± SE) of unpolluted ecotype (A) and polluted ecotype (D) at the two levels of stress. On the top left (A) and bottom right (D) corners, results of general linear mixed-effects model for the Stress × Neighbours interaction on survival are shown. Different letters show significant difference between the corresponding situation (posthoc multiple Tukey comparisons). (B, C, E, F) $\text{RII}_{\text{neighbours}}$ (mean ± SE) for unpolluted (B) and polluted (E) ecotypes and $\text{RII}_{\text{stress}}$ (mean ± SE) for unpolluted (C) and polluted (F) ecotypes. Significant differences of RII from 0 are shown next to each RII value (one sample t-test). Results of linear mixed-effects model for the effect of stress (B and E) and neighbours (C and F) are shown on the top left corners. (*), $p < 0.1$; *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.

Environmental and neighbour-trait effects

In agreement with our fourth hypothesis the increase in facilitation for unpolluted *Agrostis* ecotype with increasing metal stress was mostly due to an environmental-severity effect, whereas the increase in competition for polluted *Agrostis* ecotype with decreasing metal stress was primarily due to a neighbour-trait effect (Fig. 5D–F). These findings are consistent with Michalet et al. (2014b) who found for alpine communities that increases in facilitation with increasing stress are generally due to a decrease in target performance without neighbours (i.e. an environmental-severity effect), whereas increases in competition with decreasing stress are generally due to a decrease in target performance with neighbours (i.e. a neighbour-trait effect). Indeed, as stress increases there are few reasons that neighbours increase their size and, thus, their positive effects on target species when facilitation increases. For instance, Chaieb et al. (2021a, b) have shown that a collapse in facilitation in extreme conditions of salinity was due to a strong decrease of halophytic nurse size at the dead end of the stress gradient. Additionnaly, it is reasonable to consider consistent with several competition theories (Grime 1973, Tilman 1982, Goldberg 1990) that competition increases with decreasing stress because neighbours increase their negative effects on other species with increasing their size traits. In addition to these results, we found in our study that: 1) the best candidates for environmental-severity effects during increases in facilitation in stressed habitats were stress-intolerant ecotypes; 2) the best candidates for neighbour-trait effects during increases in competition in benign habitats were stress-tolerant ecotypes. This highlights that the high size-plasticity of competitive (and stress-intolerant) species or ecotypes (Grime 1973, Liancourt et al. 2005, Liancourt and Tielbörger 2009) may explain both their competitive effects in benign habitats through neighbour-trait effects and facilitative responses in stressed habitats because of their higher sensitivity to environmental-severity effects.

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Author contributions

David Nemer: Conceptualization (supporting); Data curation (equal); Formal analysis (equal); Investigation (equal); Methodology (equal); Writing – original draft (equal);

Writing – review and editing (equal). **Richard Michalet**: Conceptualization (equal); Formal analysis (equal); Investigation (equal); Methodology (equal); Supervision (equal); Writing – original draft (equal); Writing – review and editing (equal). **Hugo Rande**: Formal analysis (equal); Investigation (equal); Validation (equal); Writing – review and editing (equal). **Valerie Sappin-Didier**: Formal analysis (supporting); Methodology (supporting); Writing – review and editing (equal). **Florian Delerue**: Conceptualization (equal); Data curation (equal); Funding acquisition (lead); Investigation (equal); Methodology (equal); Supervision (equal); Writing – original draft (equal); Writing – review and editing (equal).

Data availability statement

Data are available from the Dryad Digital Repository: <https://doi.org/10.5061/dryad.8kprr4xrd> (Nemer et al. 2022).

Supporting information

The Supporting information associated with this article is available with the online version.

References

Al Hayek, P. et al. 2014. Phenotypic differentiation within a foundation grass species correlates with species richness in a subalpine community. – Oecologia 176: 533–544.

Armas, C. et al. 2004. Measuring plant interactions: a new comparative index. – Ecology 85: 2682–2686.

Badano, E. I. et al. 2007. Ecosystem engineering facilitates invasions by exotic plants in high-Andean ecosystems. – J. Ecol. 95: 682–688.

Bertness, M. D. and Callaway, R. 1994. Positive interactions in communities. – Trends Ecol. Evol. 9: 191–193.

Bertness, M. D. and Hacker, S. D. 1994. Physical stress and positive associations among marsh plants. – Am. Nat. 144: 363–372.

Bertness, M. D. and Ewanchuk, P. J. 2002. Latitudinal and climate-driven variation in the strength and nature of biological interactions in New England salt marshes. – Oecologia 132: 392–401.

Callaway, R. M. and Pennings, S. C. 2000. Facilitation may buffer competitive effects: indirect and diffuse interactions among salt marsh plants. – Am. Nat. 156: 416–424.

Callaway, R. M. et al. 2002. Positive interactions among alpine plants increase with stress. – Nature 417: 844–848.

Castro, J. et al. 2004. Benefits of using shrubs as nurse plants for reforestation in mediterranean mountains: a 4-year study. – Restor. Ecol. 12: 352–358.

Cavieres, L. A. et al. 2018. The importance of facilitative interactions on the performance of *Colobanthus quitensis* in an Antarctic tundra. – J. Veg. Sci. 29: 236–244.

Chaieb, G. et al. 2021a. Shift from short-term competition to facilitation with drought stress is due to a decrease in long-term facilitation. – Oikos 130: 29–40.

Chaieb, G. et al. 2021b. A regional assessment of changes in plant–plant interactions along topography gradients in Tunisian Sebkhas. – Ecosystems 24: 1024–1037.

Chatterjee, J. and Chatterjee, C. 2000. Phytotoxicity of cobalt, chromium and copper in cauliflower. – Environ. Pollut. 6: 69–74.

Cuevas, J. G. et al. 2013. Nurse effect and herbivory exclusion facilitate plant colonization in abandoned mine tailings storage facilities in north-central Chile. – Rev. Chil. Hist. Nat. 86: 63–74.

Domínguez, M. T. et al. 2015. Facilitating the afforestation of Mediterranean polluted soils by nurse shrubs. – J. Environ. Manage. 161: 276–286.

Egerova, J. et al. 2003. Facilitation of survival and growth of *Baccharis halimifolia* L. by *Spartina alterniflora* Loisel. In a created Louisiana salt marsh. – Wetlands 23: 250–256.

Eränen, J. K. and Kozlov, M. V. 2007. Competition and facilitation in industrial barrens: variation in performance of mountain birch seedlings with distance from nurse plants. – Chemosphere 67: 1088–1095.

Ernst, W. H. O. 2005. Phytoextraction of mine wastes – options and impossibilities. – Geochemistry 65: 29–42.

Espeland, E. K. and Rice, K. J. 2007. Facilitation across stress gradients: the importance of local adaptation. – Ecology 88: 2404–2409.

Frérot, H. et al. 2006. Specific Interactions between local metallicolous plants improve the phytostabilization of pine soils. – Plant Soil 282: 53–65.

Ghosh, M. 2005. A review on phytoremediation of heavy metals and utilization of its byproducts. – Appl. Ecol. Environ. Res. 3: 1–18.

Goldberg, D. E. 1990. Components of resource competition in plant communities. – In: Grace, J. B. and Tilman, D. (eds), Perspectives on plant competition. Academic Press, pp. 357–364.

Goldberg, D. E. 1996. Competitive ability: definitions, contingency and correlated traits. – Phil. Trans. R. Soc. B 351: 1377–1385.

Grime, J. P. 1973. Competitive exclusion in herbaceous vegetation. – Nature 242: 344–347.

Grime, J. P. 1974. Vegetation classification by reference to strategies. – Nature 250: 26–31.

Gross, N. et al. 2010. Strain and vegetation effects on local limiting resources explain the outcomes of biotic interactions. – Perspect. Plant Ecol. Evol. Syst. 12: 9–19.

Harrison, S. P. and Rajakaruna, N. 2011. Serpentine: the evolution and ecology of a model system. – Univ. of California Press.

He, Q. et al. 2013. Global shifts towards positive species interactions with increasing environmental stress. – Ecol. Lett. 16: 695–706.

Hou, D. et al. 2020. Metal contamination and bioremediation of agricultural soils for food safety and sustainability. – Nat. Rev. Earth Environ. 1: 366–381.

Humphrey, M. O. and Nicholls, M. K. 1984. Relationships between tolerance to heavy metals in *Agrostis capillaris* L. (*A. tenuis* Sibth.). – New Phytol. 98: 177–190.

Jadia, C. D. and Fulekar, M. H. 2008. Phytoremediation of heavy metals: Recent techniques. – Afr. J. Biotechnol. 8: 921–928.

Liancourt, P. and Tielbörger, K. 2009. Competition and a short growing season lead to ecotypic differentiation at the two extremes of the ecological range. – Funct. Ecol. 23: 397–404.

Liancourt, P. and Tielbörger, K. 2011. Ecotypic differentiation determines the outcome of positive interactions in a dryland annual plant species. – Perspect. Plant Ecol. Evol. Syst. 13: 259–264.

Liancourt, P. et al. 2005. Stress tolerance abilities and competitive responses in a watering and fertilization field experiment. – J. Veg. Sci. 16: 713–722.

Liancourt, P. et al. 2017. SGH: stress or strain gradient hypothesis? Insights from an elevation gradient on the roof of the world. – Ann. Bot. 120: 29–38.

Losfeld, G. et al. 2015. Phytoextraction from mine spoils: insights from New Caledonia. – Environ. Sci. Pollut. Res. 22: 5608–5619.

Maestre, F. T. et al. 2001. Potential for using facilitation by grasses to establish shrubs on a semiarid degraded steppe. – Ecol. Appl. 11: 1641–1655.

Maestre, F. T. et al. 2009. Refining the stress-gradient hypothesis for competition and facilitation in plant communities. – J. Ecol. 97: 199–205.

Marques, A. P. G. C. et al. 2009. Remediation of heavy metal contaminated soils: phytoremediation as a potentially promising clean-up technology. – Crit. Rev. Environ. Sci. Technol. 39: 622–654.

McGrath, S. P. and Zhao, F.-J. 2003. Phytoextraction of metals and metalloids from contaminated soils. – Curr. Opin. Biotechnol. 14: 277–282.

Meeinkuirt, W. et al. 2012. Phytostabilization of a Pb-contaminated mine tailing by various tree species in pot and field trial experiments. – Int. J. Phytoremediat. 14: 925–938.

Meeinkuirt, W. et al. 2013. Phytostabilization potential of Pb mine tailings by two grass species, *Thysanolaena maxima* and *Vetiveria zizanioides*. – Water Air Soil Pollut. 224: 1750.

Michalet, R. 2007. Highlighting the multiple drivers of change in interactions along stress gradients. – New Phytol. 173: 3–6.

Michalet, R. et al. 2011. Phenotypic variation in nurse traits and community feedbacks define an alpine community: alpine community genetics. – Ecol. Lett. 14: 433–443.

Michalet, R. et al. 2014a. Two alternatives to the stress-gradient hypothesis at the edge of life: the collapse of facilitation and the switch from facilitation to competition. – J. Veg. Sci. 25: 609–613.

Michalet, R. et al. 2014b. Partitioning net interactions among plants along altitudinal gradients to study community responses to climate change. – Funct. Ecol. 28: 75–86.

Nandillon, R. et al. 2021. Contrasted tolerance of *Agrostis capillaris* metallicolous and non-metallicolous ecotypes in the context of a mining technosol amended by biochar, compost and iron sulfate. – Environ. Geochem. Health 43: 1457–1475.

Navarro-Cano, J. A. et al. 2019. Using plant functional distances to select species for restoration of mining sites. – J. Appl. Ecol. 56: 2353–2362.

Nemer, D. et al. 2021. Species stress tolerance and community competitive effects drive differences in species composition between calcareous and siliceous plant communities. – J. Ecol. 109: 4132–4142.

Nemer, D. et al. 2022. Data from: Reciprocal transplantation of two ecotypes of two species along a metal pollution gradient. – Dryad Digital Repository, <https://doi.org/10.5061/dryad.8kprr4xrd>.

Noumi, Z. et al. 2015. Limitations to the use of facilitation as a restoration tool in arid grazed savanna: a case study. – Appl. Veg. Sci. 18: 391–401.

Oancea, S. et al. 2005. Effects of heavy metals on plant growth and photosynthetic activity. – Anal. Stiin. Univ. AL. I. Cuza 1: 107–110.

Panagos, P. et al. 2013. Contaminated sites in Europe: review of the current situation based on data collected through a European network. – J. Environ. Public Health 2013: 158764.

Pennings, S. C. et al. 2003. Geographic variation in positive and negative interactions among salt marsh plants. – Ecology 84: 1527–1538.

Pribyl, D. W. 2010. A critical review of the conventional SOC to SOM conversion factor. – Geoderma 156: 75–83.

Pugnaire, F. I. et al. 2015. No evidence of facilitation collapse in the Tibetan plateau. – J. Veg. Sci. 26: 233–242.

Qi, M. et al. 2018. Competitive ability, stress tolerance and plant interactions along stress gradients. – Ecology 99: 848–857.

Rauret, G. et al. 1999. Improvement of the BCR three step sequential extraction procedure prior to the certification of new sediment and soil reference materials. – J. Environ. Monit. 1: 57–61.

Rodríguez-Seijo, A. et al. 2016. Pb pollution in soils from a trap shooting range and the phytoremediation ability of *Agrostis capillaris* L. – Environ. Sci. Pollut. Res. 23: 1312–1323.

Sánchez-Velásquez, L. R. et al. 2004. Nurses for *Brosimum alicastrum* reintroduction in secondary tropical dry forest. – For. Ecol. Manage. 198: 401–404.

Schöb, C. et al. 2013. Variability in functional traits mediates plant interactions along stress gradients. – J. Ecol. 101: 753–762.

Stanovych, A. et al. 2019. Depollution of mining effluents: innovative mobilization of plant resources. – Environ. Sci. Pollut. Res. 26: 19327–19334.

Stephant-Champigny, A. et al. 2015. Etude sanitaire et environnementale sur le secteur minier de Sentein, bassin versant du Lez (09). – Geoderis, p. 208.

Sthultz, C. M. et al. 2007. Shifts from competition to facilitation between a foundation tree and a pioneer shrub across spatial and temporal scales in a semiarid woodland. – New Phytol. 173: 135–145.

Tilman, D. 1982. Resource competition and community structure. – Princeton Univ. Press.

Wang, J. et al. 2014. Facilitation drives the positive effects of plant richness on trace metal removal in a biodiversity experiment. – PLoS One 9: e93733.

Wang, Y. et al. 2008. On the relevance of facilitation in alpine meadow communities: an experimental assessment with multiple species differing in their ecological optimum. – Acta Oecol. 33: 108–113.

Wong, J. W. C. et al. 1998. Acid-forming capacity of lead–zinc mine tailings and its implications for mine rehabilitation. – Environ. Geochem. Health 20: 149–155.

Yang, R. et al. 2015. Copper tolerant *Elsholtzia splendens* facilitates *Commelina communis* on a copper mine spoil. – Plant Soil 397: 201–211.

Ye, Z. H. et al. 2002. Evaluation of major constraints to revegetation of lead/zinc mine tailings using bioassay techniques. – Chemosphere 47: 1103–1111.

Zvereva, E. L. and Kozlov, M. V. 2004. Facilitative effects of top-canopy plants on four dwarf shrub species in habitats severely disturbed by pollution. – J. Ecol 92: 288–296.