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l'université de Bordeaux

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ENVIRONNEMENTS**

Spécialité : écologie évolutive, fonctionnelle et des communautés

Par David NEMER

**La relation entre la tolérance au stress et les
capacités de compétition et de facilitation
détermine les interactions entre les plantes le
long de différents gradients de stress**

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Titre : La relation entre la tolérance au stress et les capacités de compétition et de facilitation détermine les interactions entre les plantes le long de différents gradients de stress

Résumé

Le résultat des interactions plantes-plantes le long de gradients environnementaux est le résultat des effets des plantes sur les plantes voisines, de l'aptitude des plantes voisines à répondre à ces effets, mais aussi de la tolérance aux stress environnementaux des plantes qui produisent un effet ou répondent à cet effet. Les études concernées impliquent soit différentes espèces de plantes, soit différents écotypes qui sont des populations au sein d'une espèce donnée se développant dans des conditions environnementales différentes et ayant des adaptations spécifiques. Dans cette thèse, notre objectif principal était d'évaluer comment les aptitudes de tolérance au stress des plantes et les aptitudes compétitives et facilitatrices des plantes agissent ensemble pour déterminer le résultat des interactions plantes-plantes le long de différents gradients de stress. Nous avons d'abord étudié la relation entre les aptitudes de tolérance au stress et les effets et réponses compétitives des plantes dans un contexte où la compétition est l'interaction prédominante entre les plantes: le long d'un gradient hydrique induit par les différences de disponibilité en eau entre les substrats calcaires et siliceux dans le département de la Gironde (sud-ouest de la France). La relation entre la tolérance au stress et les effets et réponses facilitatrices des plantes a été étudiée dans les Pyrénées (sud de la France) dans un contexte où la facilitation est connue pour être l'interaction prédominante entre les plantes : dans des milieux dont les sols sont pollués par les métaux lourds. Nous avons utilisé des approches expérimentales (transplantation réciproque in situ et culture dans des zones contrôlées) aux niveaux interspécifiques et intraspécifiques. Nous avons également calculé des indices appropriés pour quantifier la contribution relative du stress et des interactions biotiques dans le résultat des interactions entre les plantes dans les deux systèmes. Nos résultats ont montré un compromis fonctionnel entre la tolérance au stress et les effets et réponses compétitives le long du gradient hydrique. En effet, les espèces tolérantes à la sécheresse, plus aptes à

résister au stress hydrique, subissait fortement les effets compétitifs des plantes voisines. Aussi, les effets compétitifs étaient faibles dans les communautés dominées par ces mêmes plantes tolérantes au stress. Inversement, les espèces non tolérantes au stress hydrique subissait peu les effets compétitifs des plantes voisines, et les effets compétitifs étaient forts dans les communautés dominées par ces mêmes espèces. Dans les systèmes pollués au métaux lourds, un compromis fonctionnel entre la tolérance au stress et la réponse à la facilitation a également été trouvé. Les espèces et écotypes les moins tolérants aux métaux lourds étaient plus facilités que les espèces et écotypes les plus tolérants. En revanche, nous avons trouvé une corrélation positive plutôt qu'un compromis fonctionnel entre la tolérance au stress et les effets facilitateurs des plantes. Les écotypes les plus tolérants au stress avaient des effets facilitateurs plus importants sur les espèces voisines que les écotypes moins tolérants au stress. Enfin, en ce qui concerne les indices utilisés, nous avons constaté que les stress environnementaux avaient une plus grande influence dans la détermination du résultat des interactions entre les plantes que l'effet compétitif des voisins le long du gradient d'eau et une plus grande influence que l'effet facilitateur des voisins à haut niveau de pollution par les métaux lourds.

Mot clés : tolérance au stress, effets compétitifs, réponses à la compétition, effets facilitateurs, réponses à la facilitation, effets de la sévérité environnementale, effets des voisins, métaux lourds, espèces métallophiles

Title: Relationship between tolerance to stress and both competitive and facilitative abilities drives plant-plant interactions along different stress gradients

Abstract

The interactions between plants along environmental gradients are not only the results of how plants affect their neighbours and how their neighbours respond to these effects, but also of how plants respond to environmental stresses. Related studies either involve different species or different ecotypes, which are populations of a species growing in different environments and with specific adaptations. In this thesis, our main objective was to assess how both plant stress-tolerance and plant competitive and facilitative abilities drive plant-plant interactions along different stress gradients. First, we investigated the relationship between plant stress-tolerance and plant competitive and response abilities in a context where competitive interactions dominate: along a water gradient driven by differences in water availability between calcareous and siliceous substrates in the Gironde department (South-Western France). Then, the relationship between plant stress-tolerance and plant facilitative effect and response abilities was explored in a context where facilitative interactions dominate: in sites polluted by large amounts of heavy-metals in soils in the Pyrenees mountains (South France), along metal pollution gradients. We conducted experimental approaches (reciprocal transplant experiments and common garden experiments) at the interspecific and intraspecific levels. We also calculated appropriate indices to quantify the relative contributions of environmental stress and biotic interactions in determining the outcome of plant-plant interactions in both systems. Our results demonstrated a trade-off between stress tolerance and competitive effect and response abilities along the water gradient. Drought-tolerant species were better able to withstand stress, while stress-intolerant species were better able to withstand competition. Additionally, competitive effects were higher in plant communities dominated by stress-intolerant competitive species. A trade-off between stress tolerance and facilitative response abilities was also found in metal-polluted sites, as the less metal-tolerant species and

ecotypes were more facilitated than the more metal-tolerant species and ecotypes. In contrast, we found a positive correlation instead of a functional trade-off between stress tolerance and plant facilitative abilities, since the most stress-tolerant ecotypes had greater facilitative effects on neighbouring species than the less stress-tolerant ecotypes. Finally, with respect to the indices used, we found that environmental stresses had a greater influence in determining the outcome of plant-plant interactions than the competitive effect of neighbours along the water gradient and a greater influence than the facilitative effect of neighbours at the highest pollution level.

Keywords: stress tolerance, competitive effects, competitive responses, facilitative effects, facilitative responses, environmental-severity effects, neighbor-trait effects, heavymetals, metalicolous species.

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“Wrong does not cease to be wrong because the majority share in it.”

— Leo Tolstoy

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CHAPTER 1

General introduction

Competition, facilitation, environmental stress, and disturbances are considered as the main biotic and environmental drivers shaping plant communities at local and regional scales (Lortie et al. 2004; Brooker et al. 2009; Michalet et al. 2015). Until the mid-nineties, mostly negative interactions between plants (i.e. competitive interactions) were considered in ecological theories and research (Grime, 1973; Connell 1983; Tilman, 1982; Goldberg and Barton 1992). However, despite the historical primacy of competition, interest in positive interactions between plants (i.e. facilitation) has increased over the past three decades, and there is now a consensus among ecologists that facilitation also plays a key role in maintaining diversity in plant communities (Callaway, 2007; Michalet et al. 2006).

More precisely, stress is defined by chemical or physical constraints that negatively affect plant growth and community productivity, such as cold temperature, and low light, water and nutrients availability, whereas, disturbance is defined as any biotic or physical constraint (e.g. grazing, burning, flooding) that destroys plant biomass (Grime 1973, 1997). Michalet et al. (2022) noted that nutrient-poor ecosystems that do not show other kinds of stress (like heathlands on sandy soils in temperate regions) can accumulate a high amount of biomass with time in case of low disturbance. This implies that community biomass and productivity vary independently in nature.

Competition is the negative effect of one organism on another due to deprivation of primary resources and is widely recognized as a major factor determining species diversity and dominance within plant communities, whereas facilitation can be defined as the positive effect of one organism on another due to modification of environmental conditions in its immediate vicinity. Facilitation has been considered particularly important in harsh environments (Bertness & Callaway 1994; Brooker & Callaghan 1998), increasing local diversity by allowing species that are not tolerant to stress or disturbance to persist in extreme habitats (Bruno et al. 2003, Liancourt et al. 2005, Callaway 2007, Cavieres et al. 2014; Michalet et al. 2015).

However, different attempts have been made to predict under which conditions, along environmental gradients facilitation or competition will predominate. The stress gradient hypothesis (SGH; Bertness and Callaway, 1994) proposes that net interactions between plants shift from predominantly negative (i.e. competition) to predominantly positive (i.e. facilitation) with increasing stress or disturbance. The SGH is the most widely reported view in the facilitation literature (Callaway, 1995; Holmgren et al. 1997; Bruno et al. 2003; Brooker et al. 2008; He et al. 2013), and is actually a prolongation of Grime's model in more stressful environments (1974). Grime proposed that competition prevail in benign environments. Throughout the distribution range of species, along environmental gradients, it is indeed unlikely that competition and environmental stress are equally important. Competitive exclusion should prevail in benign habitats where competitive species thrive, whereas in harsh environments stress is the most important factor that affects plant growth, and facilitation becomes the primary form of interaction at stake between plants (Grime 1974; Brooker et al. 2005). Factors related to species functional strategies (stress tolerance and competitive effect and response abilities) appear therefore to play a key role in determining the outcome of plant-plant interactions (Brooker and Callaghan, 1998).

1. Plant effect and response abilities

In plant communities, the direction and intensity of biotic interactions not only depend on the functional strategies of the species involved, but also on how plants affect and respond to each other (Goldberg and Werner, 1983; Goldberg et al. 1999). Therefore, to better understand the outcomes of plant-plant interactions in any community, it is important to separate the competitive effect from the competitive response abilities (Goldberg 1991, 1996; Peltzer and Köchy, 2001; Hager, 2004; Guido et al. 2019). The competitive effect refers to the capacity of one plant to inhibit the growth of another, mainly through resource deprivation, while the competitive response is the capacity of a species to withstand the negative effect generated by another plant. In contrast, few authors have highlighted the importance of distinguishing the same two components of plant facilitative abilities (Zhang and Tielbörger, 2019).

Indeed, facilitative abilities should be decomposed similarly into two different components, the

facilitative effects and the facilitative responses, to have a complete understanding of facilitative outcomes. The facilitative effect could be defined as the capacity of one plant (the benefactor or the nurse plant) to enhance the performance of another, whereas, the facilitative response refers to the potential of a plant (the beneficiary species or the target) to benefit stress or disturbance alleviation by the benefactor (Zhang and Tielbörger 2019; Delerue and Michalet, 2022). Functional traits most probably regulate both facilitative effects and responses and therefore regulate plant interactions variations with environmental variation (Butterfield et al. 2013). It has also been argued that nurse plant traits are more crucial than target traits in influencing the outcome of plant-plant interactions (Callaway, 2007; Gomez-Aparicio, 2009). However, there is still little empirical evidence of correlations between functional traits and facilitation.

Several studies have shown that biotic interactions can occur not only at the species level but also between ecotypes of the same species growing in more or less harsh environments (Espeland and Rice 2007, Liancourt and Tielbörger 2011). Ecotypes are defined as a variety of populations within a given species, genotypically adapted to specific environmental conditions, exhibiting phenotypic differences, and capable of interbreeding with other geographically adjacent ecotypes without loss of fertility (Turesson, 1922; Gregor, 1944; Briers, 2006). In light of this, Al Hayek et al. (2015a) highlighted the important role that ecotypic differentiation could potentially play in driving both the effect of a neighbour on a target individual and the response of a target individual to the effect of a neighbour. They found that there were differences in effects and response abilities between the two phenotypes of *Festuca gautieri* that were used in their experiments, with a tight phenotype having a stronger competitive effect and a loose phenotype having a greater competitive response. Altogether, this suggests that identifying traits relevant to facilitative effects and responses is of great importance to improving our overall understanding of positive interactions. However, there is little practical support for functional differentiation in facilitative responses and effects abilities at the intraspecific level, as most studies that have distinguished these two components have primarily addressed this issue at the interspecific level. Note that some studies did examined the response of beneficiaries (Espeland and Rice, 2007; Liancourt and Tielbörger, 2011) and the effect of benefactors (Crutsinger et al. 2010; Schöb et al. 2013) at the intraspecific level, but no study has assessed both components of species

facilitative ability simultaneously.

2. The relationship between stress tolerance and plant effect and response abilities

2.1. Competitive and facilitative response:

Many studies have demonstrated the existence of a trade-off between stress tolerance and plant responses, both in their facilitative and competitive component (Choler et al. 2001; Liancourt et al. 2005; Forey et al. 2010; Gross et al. 2010). Those studies have found that stress-tolerant species have low competitive-response abilities and low facilitative responses, whereas stress-intolerant species have high competitive-response abilities and high facilitative responses. Indeed, species that possess traits that enable them to thrive when competitive exclusion prevails in benign habitats are most likely to develop at the expense of traits that allow their persistence in highly stressed environments (Grime, 1974, Liancourt et al. 2005). Consequently, facilitative responses are more likely to occur with species from benign environments that have a low tolerance to stress but a greater competitive ability than species from severe environments that exhibit a high tolerance to stress but have a lower competitive ability. In fact, due to the higher dependence of competitive species on stress mitigation by their host plant, they tend to be more facilitated than species that are more tolerant of a given stress (Liancourt et al. 2005; Gross et al. 2010; Graff and Aguiar, 2017). For instance, Pennings et al. (2003) found in salt marshes, that species sensitive to salinity were more facilitated than salt-tolerant species.

However, it should be emphasized that the majority of the studies on plant-plant interactions have only focused on the occurrence of a trade-off at the interspecific level. In nature, there is no such thing as a steady environment, and plants must adapt to local changes in environmental conditions. Genetic variation and phenotypic plasticity are two possible ways through which plants can respond to a changing environment (Sultan 1995; Kawecki and Ebert 2004). These two mechanisms can allow species to be present under different environmental conditions by selecting the genotypes expressing the most suited phenotypes, leading to an ecotypic differentiation within the species (Linhart and Grant, 1996; Joshi et al. 2001; Sultan, 2004). Therefore, the outcome of plant interactions may depend more on genotypic identity than on species identity.

Furthermore, the concept of trade-off between stress tolerance and plant response abilities that occurs at the species level can be applied in the same manner at the intraspecific level, between populations of the same species inhabiting different environments (Liancourt and Tielbörger, 2011). In fact, ecotypes from benign environments have been shown to require more stress mitigation by nurse plants than those from harsh environments (e.g., Espeland and Rice, 2007; Liancourt and Tielbörger, 2011).

2.2. *Competitive and facilitative effect:*

All of the studies previously mentioned have focused on plant competitive or facilitative response abilities at the inter- and intraspecific levels. Thus much less is known on the nature of the relationship between stress tolerance and the facilitative and competitive effect abilities of plants. However, concerning competitive abilities, Michalet et al. (2022) recently showed that there is a trade-off between plant competitive effect abilities and stress tolerance, similar to the trade-off between stress tolerance and competitive response ability. When stress decreases, plant biomass increases significantly for stress intolerant species but not for stress tolerant species. Furthermore, since plant effects are size-dependent, plant competitive effect abilities increase when biomass increases.

Regarding facilitative effects, only few studies have evaluated if there are differences between loose and tight phenotypes of various alpine cushions under field conditions (Michalet et al. 2011, Al Hayek et al. 2014, 2015b, Jiang et al. 2017). However, since distinct phenotypes were always observed in different microhabitats, the authors of these experiments could not distinguish the facilitative effects of different phenotypes from the environmental effects. But, Al Hayek et al. (2015a) by comparing the differences in facilitative- and competitive-effect abilities in two common gardens that simulated the contrasting environmental conditions of two ecotypes of the cushion grass *Festuca gautieri*, were able to distinguish the influence of environmental factors from that of the nurse phenotype. However, the corresponding subalpine systems in the Pyrenees were not sufficiently stressed to properly investigate the relationship between facilitative effects and cushion phenotypic stress-tolerance.

Finally, studies investigating both facilitative and competitive effects are still scarce, and further researches across different ecosystems, species, and ecotypes are needed to better understand and conclude

on the relationship between stress tolerance and plant effect abilities.

3. About the measurements of plant effect or response abilities

3.1. Standard approaches:

Measurements of plant competitive or facilitative abilities, either response or effect abilities, require the use of dedicated metrics, mostly based on the comparison of target performance with or without neighbours. To study the difference in species or ecotype responses, the emphasis is on the variation in performance between several species or ecotypes in similar conditions (without neighbours or with the same neighbours). To study the difference in species or ecotype effects, the focus is on the variation in performance between the same targets growing without neighbours or with different species or ecotypes as neighbours. To do this, dedicated studies mostly used indices such as the Relative Interaction Index “i.e. RII” (Armas et al. 2004) which calculate the intensity of the interaction in a given environmental condition by comparing the performance of a target species (or ecotype) with or without the presence of a neighbouring species (or ecotype).

3.2. Limits in the use of the RII:

If RIIs can be calculated at different positions along environmental gradients, they describe the absolute impact of interactions on target performance, but do not distinguish between the relative effect of neighbours and that of the varying environment (Corcket et al. 2003; Brooker et al. 2005). That’s why several authors have stressed the necessity of using an index that has the ability to disentangle between biotic (i.e. competition or facilitation) and abiotic (e.g. environmental stress) factors when assessing the contribution of plant-plant interactions in shaping plant communities along environmental gradients (Welden and Slauson, 1986; Brooker et al. 2005, 2008). The “importance of interaction index” unlike the RII, measures the difference in an organism's performance in the presence of neighbours relative to the environmental effects (i.e., stress and disturbance) on plant performance (Welden and Slauson, 1986; Brooker and Kikvidze, 2008; Seifan et al. 2010).

Additionally, Michalet et al. (2014b), suggested another complementary methodological approach in biotic interactions studies that allows to assess the mechanism behind variation in competition and

facilitation along stress gradients. They split the absolute effect of the “RII” into two sub-effects, the environmental-severity effect and the neighbour-trait effect. The environmental-severity effect assesses the effect of a stress gradient on isolated target performance without neighbours, whereas the neighbour-trait effect provides an insight on the effect of the neighbour on the target performance as the level of stress varies. Indeed, a shift from competition to facilitation along environmental gradients could occur when: (i) target species' performance with neighbours remains unchanged, while its performance without neighbours decreases due to increased environmental severity (i.e., the environmental-severity effect; Figure 20b); (ii) target species' performance with neighbours increases with stress, while its performance without neighbours remains unchanged (i.e., the neighbour-trait effect; Figure 20a); (iii) both the environmental-severity effect and the neighbour-trait effect operate simultaneously (Figure 20). The increase in competition with decreasing stress is primarily caused by a neighbour-trait effect (i.e. the target species' performance with neighbours decreases with decreasing stress due to competitive exclusion, while its performance without neighbours remains unchanged, Michalet et al. 2022), whereas the increase in facilitation with increasing stress has often been shown to be the result of an environmental-severity effect (Chaieb et al. 2021). Consistently, Michalet et al. (2014b) found in the Alps, that the increase in competition associated with decreasing cold from the subalpine belt to lower altitude was due to a neighbour-trait effect, whereas the increase in facilitation with increasing cold from the subalpine to the alpine belt was driven by an environmental-severity effect.

4. Thesis objectives

The main objectives of this thesis were to investigate in different ecosystems and at inter- and intraspecific levels: (i) the nature of the relationship between stress tolerance and effect abilities (facilitative and competitive), and (ii) whether the well-documented trade-off between stress tolerance and plant response abilities is still valid. We chose to address these objectives in different systems affected by different types of stress, as the nature of stress is known to play an important role in determining the outcome of plant-plant interactions (Michalet 2007; Michalet et al. 2014a). For instance, along non-resource gradients, such as salt,

cold, or wind gradients, refined theories of facilitation (Michalet 2007, Maestre et al. 2009, Michalet et al. 2014a) have proposed that a collapse of facilitation was more likely to be supported than an increase of positive interactions in the most extreme environments. Along resource-related stress gradients, such as light, nutrients, or water, models predicting a switch back to competition appear to be supported instead of the SGH in the most extreme conditions.

The first system corresponds to a water gradient (resource-related) driven by difference in bedrocks. Calcareous soils are generally much drier than siliceous soils because of the greater fracturing of calcareous bedrock, which induces rapid drainage of water into the underlying soils (Michalet et al. 2001, 2002; Rolland et al. 1998). The second is a metalliferous system (non-resource related) located in the Pyrenees, in southwestern France, where several areas exhibit gradients of heavy metal pollution as a result of former mining activities that ended in the late 1960s.

In the first system, which corresponds to a water gradient induced by the difference in bedrock and where competition is known to be the main interaction between plants (Tielbörger and Kadmon 2000, Maestre and Cortina 2004, O'Brien et al. 2017), our specific objective was the following:

O1: At the interspecific level, what is the relative contribution of species stress tolerance and competitive effects in driving differences in plant composition along a water-availability gradient (between calcareous and siliceous communities)? (chapter 3)

In the second metalliferous system, where interactions between plants are predominantly positive (Zvereva and Kozlov 2004; Frérot et al. 2006; Eränen and Kozlov, 2007; Cuevas et al. 2013; Wang et al. 2014; Yang et al. 2015), we had two additional objectives and aimed to evaluate at inter- and intraspecific levels:

O2: If a trade-off exists between stress tolerance and plant facilitative responses? (Chapter 4 and 5)

O3: If a trade-off exists between stress tolerance and plant facilitative effects? (Chapter 5)

In addition, we also emphasized the importance to use methods that allow us to quantify the relative contribution of stress and biotic forces (competition and/or facilitation) in structuring plant communities and determining the outcome of plant-plant interactions.

In the last objective (**O4**): **we aimed to evaluate the respective contributions of environmental-severity**

effect (metallic stress) and neighbour-trait effect in determining the outcome of plant responses along pollution gradients (Chapters 4 and 5).

CHAPTER 2

Study sites and experimental designs

1. The first study site in the Gironde department related to the first objective

(O1): At the interspecific level, what is the relative contribution of species stress tolerance and competitive effects in driving differences in plant composition along a water-availability gradient (between calcareous and siliceous communities)

1.1. Study site and experimental design

We fulfilled this first objective implementing a reciprocal transplant-experiment. We used a water gradient (resource-related) driven by difference in bedrocks found in the Gironde department, in south-western France. Two distinct types of soils are located on the two sides of the Garonne River. Calcareous soils derived from calcareous bedrocks are located on the right east bank of the Garonne, while the siliceous sandy soils are located on the left west bank of the Garonne. Since calcareous soils are drier than siliceous soils (Michalet et al. 2001, 2002; Rolland et al. 1998), plant communities on these two types of soils have a very different species composition.

The reciprocal transplant-experiment was repeated four years from early March to late August, in 2016, 2018, 2019, and 2020. Personally, I contributed to the fourth year of the experiment. Four blocks spaced 10 km apart from each other were established along the Garonne River, roughly 50 kilometers between the cities of Bordeaux and Langon (Figure 1a). The experimental design included soil type as the main plot treatment (calcareous or siliceous soil), vegetation removal (with or without neighbouring vegetation), and species origin (calcareous or siliceous species) as subplot treatments (Figure 5b). Within each of the four blocks, one main plot was located on calcareous soil at the east of the river, while the second main plot was positioned on siliceous soil at the west of the river (Figure 1). Each year, inside each main plot, we conducted several vegetation removal treatments (Figure 1b). Finally, in each subplot, individuals of each of four target species

originating from calcareous or siliceous soils were transplanted each year (with or without neighbours).

The ability of calcareous and siliceous species to tolerate abiotic stress was assessed by comparing their performance without neighbouring vegetation in order to separate the effects of the environment from the effect of neighbours. On the other hand, the competitive effects abilities of calcareous and siliceous species were determined by comparing target species performance with and without neighbours using the relative interaction index, and the importance of interaction index. The latter index compares plant performance in the presence of neighbours relative to the environmental effects, and thus has also the ability to evaluate the relative contribution of species stress tolerance and competitive effects in driving differences in plant composition between calcareous and siliceous communities.

1.2. Target species

In order to assess the effects of the two community types on both calcareous and siliceous species, we chose four species of perennial grasses as targets, which are common dominant species of calcareous or siliceous communities in Western Europe. From calcareous soils we chose '*Bromus erectus*' (Figure 2) and '*Brachypodium sylvaticum*' (Figure 3) as target species. From siliceous soils we chose '*Pseudarrhenatherum longifolium*' (Figure 4) and '*Deschampsia flexuosa*' (Figure 5) as target species. *Bromus erectus* and *Pseudarrhenatherum longifolium* are more light-demanding species whereas *Brachypodium sylvaticum* and *Deschampsia flexuosa* are more shade-tolerant species (Rameau et al. 1989).

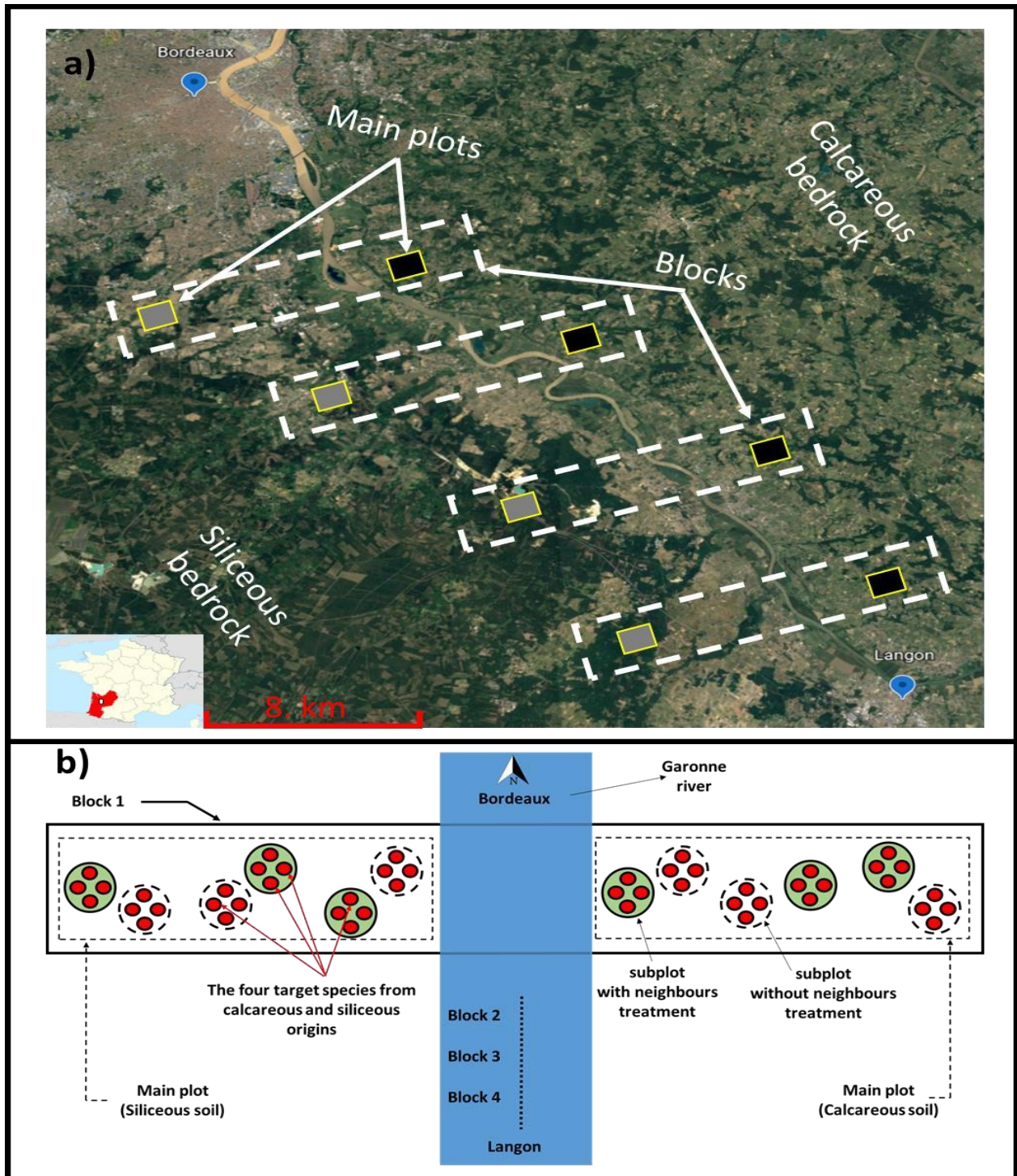


Figure 1. Experimental design. (a) Location of the four blocks along the Garonne River between the towns of Bordeaux (NW) and Langon (SE) in the south-west of France. Each block includes one main plot on calcareous soils (black rectangles) on the east side of the Garonne River and another one on siliceous soils (grey rectangles) on the west side of the river. (b) schema showing each block contain two main plot that includes subplots with neighbours (green circles) and without neighbours (white circles) treatments. Each subplot contains four target species from calcareous and siliceous origin.

Bromus erectus



Figure 2. *Bromus erectus* forms the background of many of dry meadows and is very common in calcareous grasslands. It is also found in abundance on the edges of roads and paths. It takes its name from its elongated and erect spikelets (height of the plant: 30-80 cm; flowering: from May to July; altitudes: 0 to 2100 meters). Photo sources: <https://www.floreAlpes.com>

Brachypodium sylvaticum



Figure 3. *Brachypodium sylvaticum* is frequently found on calcareous soils and can be recognized by its long upper ridges. The plant is heavily hairy on the leaves, sheaths, nodes and spikelets. It has very green leaves, soft and wide. It is found in deciduous woods at low and medium altitudes (height of the plant: 50-120 cm; flowering: from June to September; altitudes: 0 to 1700 meters). Photos source: <https://www.floreAlpes.com>

Pseudarrhenatherum longifolium



Figure 4. *Pseudarrhenatherum longifolium* is one of the most classic grasses in siliceous hayfields. It is a tall plant with flat, scabrous leaves. Loose panicles are composed of spikelets with two flowers, often marked with purple. It has soft hair on the leaves, sheaths, and nodes, with a grassy stump. (height of the plant: 60-150 cm; flowering: from May to July; altitudes: 0 to 2150 meters). Photos source: <https://jb.utad.pt>

Deschampsia flexuosa



Figure 5. *Deschampsia flexuosa* is very common on siliceous soils. It's is an herb with a very divided panicle and two-flowered spikelets shorter than the glumes. Spines long, clearly exceeding the glumes and inserted at the base of the lemmas. Ligule very short. Short and rolled leaves. (height of the plant: 20-60 cm; flowering: from June to August; altitudes: 300 to 2800 meters). Photo source: <https://www.florealpes.com>

2. The second study site the former mining area in the Pyrenees mountain related to the 2nd, 3rd and 4th objectives

2.1. The mining area and the pollution gradients

During my PhD, three experiments were carried out at various altitudes in the former mining area of Sentein (Ariège; 42°52'32"N, 0°57'19"E), in the French Pyrenees. These three experiments consist of two reciprocal transplant experiments conducted at the subalpine belt in 2020 and in 2021, and a common garden experiment carried out at the mountain belt in 2021. In these former mining areas, especially those exploiting metals, represent important sources of contamination dispersed in the environment, impacting the landscape and vegetation dynamics. Several locations in the region (Figure 6), exhibit a soil pollution gradient that is easily detectable with the naked eye (e.g., Figure 7). Along these gradients, the composition of the plant community gradually changes from the most polluted areas (hereafter referred to as Habitat 1 - H1) to the least polluted areas (hereafter referred to as Habitat 4- H4) (see Figure 7 and 8), forming an ideal system to fulfil our objectives. The major toxic metals in the area are Zn, Pb, and Cd, with concentration that varies along the gradients (see Table 1 for the concentration of major metals in location 2, 3 and 4 where our reciprocal experiments were carried out). Along the pollution gradient, the concentration of these major metal increases from H4 to H1 (Table 1).

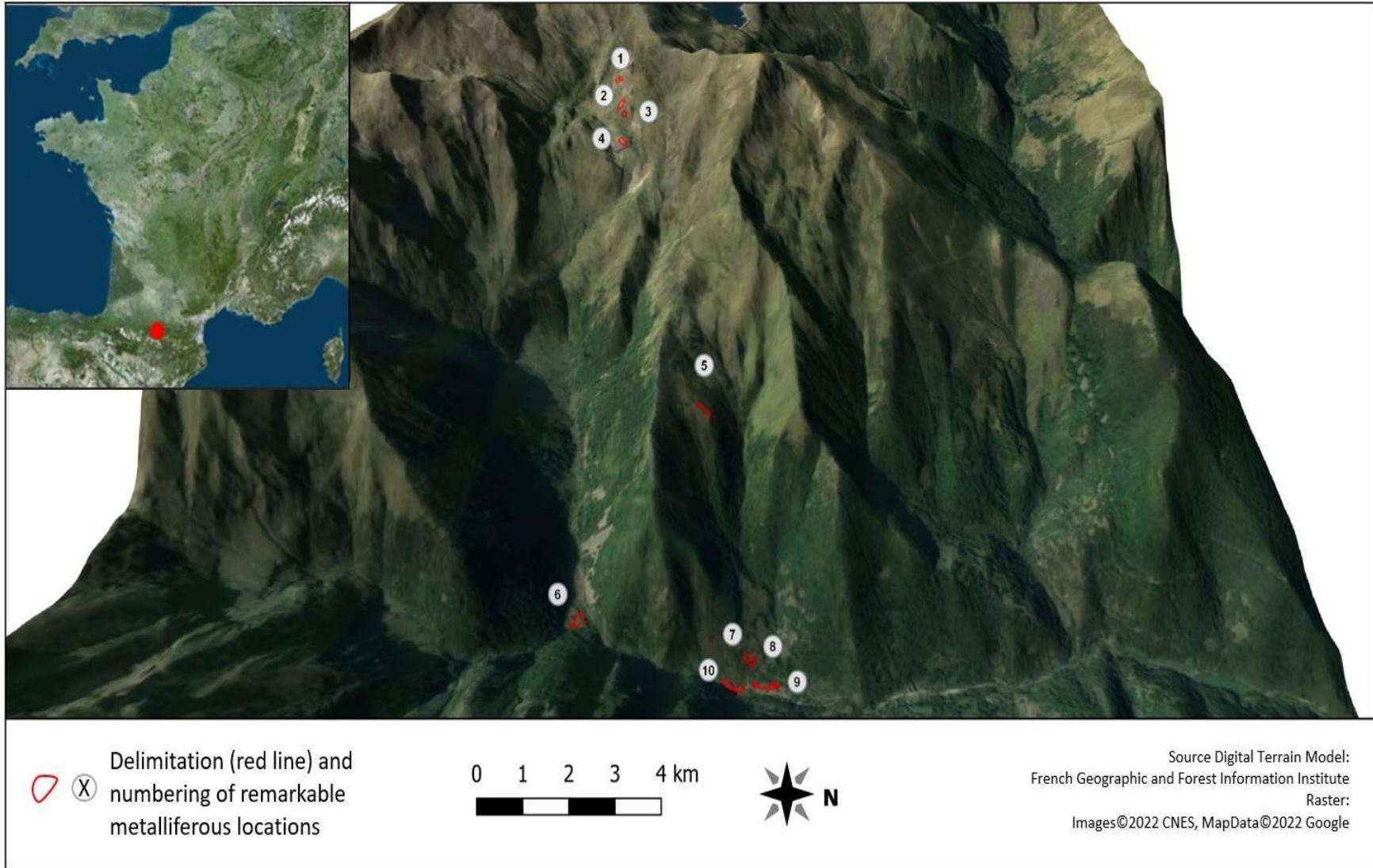


Figure 6. Location of the various remarkable metal-rich sites in the region. The subalpine area represented by location 2, 3 and 4 are where the reciprocal transplantation experiments took place along metal pollution gradients. The mountainous belt represented by location 10, a slag heap and where the common garden experiment was carried out.



Figure 7. Variation of plant communities along the metal pollution gradient at one of the experimental sites (see location 4 in Figure. 6). Four habitats can be distinguished. Habitat 1 (H1), without vegetation or only a few metallic species (*Armeria muelleri* and *Hutchinsia alpina* in this picture); Habitat 2 (H2): with dominant highly metal-tolerant species (*Arenaria multicaulis* and *Armeria muelleri*) and with few metal-intolerant or low metal-tolerant species (*Agrostis capillaris* and *Festuca rubra*); Habitat 3 (H3), with dominant low metal-tolerant species (*Agrostis capillaris* and *Festuca rubra*), with few highly metal-tolerant species, (*Armeria muelleri* and *Arenaria multicaulis*); and Habitat 4 (H4), with only low metal-tolerant species.

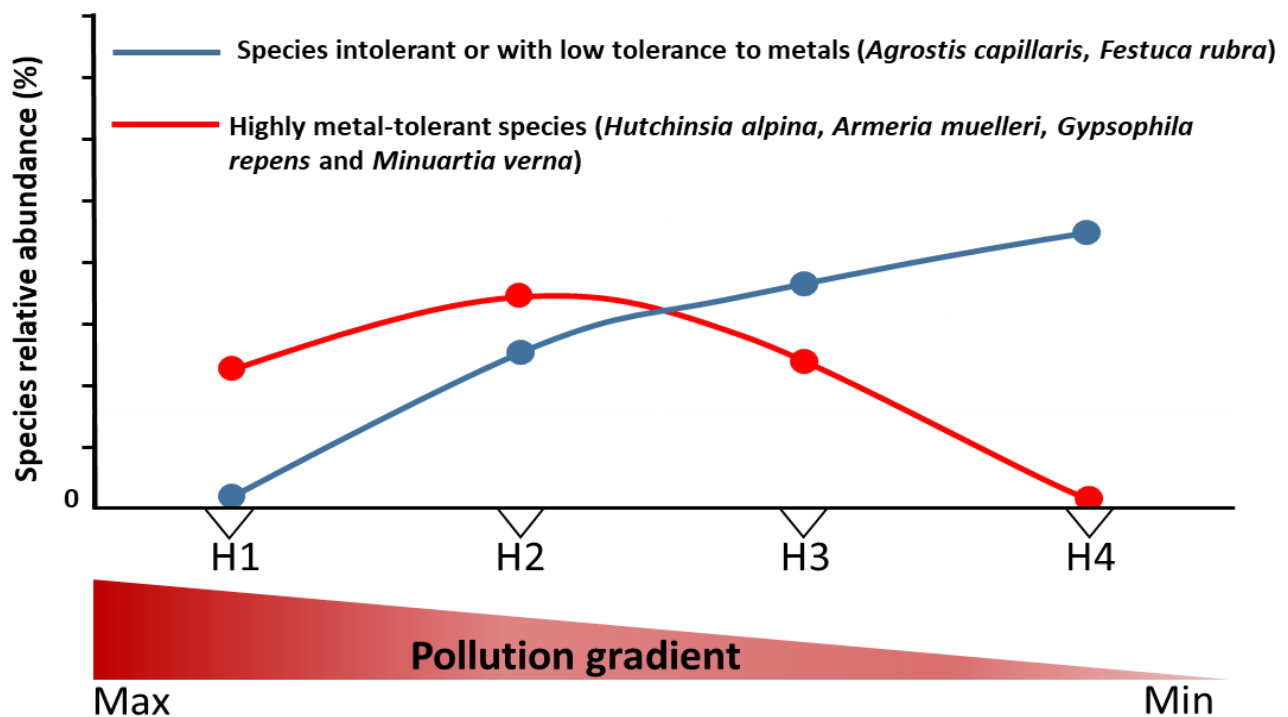


Figure 8. Schematic representation of the distribution range of highly tolerant and less tolerant species to metals along a pollution gradient. *Agrosits capillaris* and *Festuca rubra* are ubiquitous species often found in metal-polluted environments. But in our study area, these species are completely absent in the most extreme habitats (H1) and are only found in areas polluted by moderate levels of metal pollution (H2 to H4). Highly metal-tolerant or metallicolous species, dominate the most polluted habitats (H1) with a high content of heavy metals, especially lead, zinc, and cadmium, and they disappear from H4, the least polluted habitat.

Site	Metals type	unit	Habitats			
			1	2	3	4
Chichoué milieu haut (Location 2)	Pb _{TOT}	mg.kg ⁻¹	40065.2	31532.0	18417.1	3806.3
	Zn _{TOT}	mg.kg ⁻¹	53713.3	22972.5	9008.7	2995.1
	Cd _{AA}	mg.kg ⁻¹	45.9	20.6	12.0	2.4
	Pb _{AA}	mg.kg ⁻¹	660.2	444.4	179.9	4.9
	Zn _{AA}	mg.kg ⁻¹	3266.1	1232.7	432.0	55.9
Chichoué milieu bas (Location 3)	Pb _{TOT}	mg.kg ⁻¹	37837.1	46120.7	36239.9	18572.0
	Zn _{TOT}	mg.kg ⁻¹	20172.5	14578.7	10458.2	5173.4
	Cd _{AA}	mg.kg ⁻¹	19.1	12.0	8.3	6.7
	Pb _{AA}	mg.kg ⁻¹	931.42	793.41	676.18	452.51
	Zn _{AA}	mg.kg ⁻¹	1067.8	527.91	254.11	154.54
Chichoué bas (Location 4)	Pb _{TOT}	mg.kg ⁻¹	48930.3	6658.1	5233.2	3669.9
	Zn _{TOT}	mg.kg ⁻¹	170226.7	13031.2	7238.4	2930.8
	Cd _{AA}	mg.kg ⁻¹	32.0	13.6	8.8	2.6
	Pb _{AA}	mg.kg ⁻¹	589.5	52.0	30.5	2.1
	Zn _{AA}	mg.kg ⁻¹	2097.1	553.2	416.5	97.5

Table 1. Concentration of major metals in location 2, 3 and 4 where our reciprocal experiments were carried out. Pb_{TOT}: total Lead concentrations; Zn_{TOT}: total Zinc concentrations. Cd_{aa}: Cadmium extracted with acetic acid; Pb_{aa}: Lead extracted with acetic acid; Zn_{aa}: Zinc extracted with acetic acid.

2.2. Experimental designs for the second and fourth objective:

(O2): Evaluate at inter- and intraspecific if a trade-off exists between stress tolerance and plant facilitative and competitive response in metalliferous systems (Chapters 4 and 5).

(O4): Evaluate the respective contribution of environmental-severity effect (metallic stress) and neighbour-trait effect in determining the outcome of plant responses along pollution gradients (Chapters 4 and 5).

To fulfil these objectives, two reciprocal transplantation-experiments were conducted at the subalpine belt. The first reciprocal experiment was conducted in 2020, from early June to late September (see location 4 in Figure 6), followed by a second experiment in 2021, from early June to late September, covering a broader area than the first (see location 2, 3 and 4 in Figure 6).

In the first reciprocal transplantation experiment (figure 9), we evaluated the facilitative responses of two ecotypes of two perennial species (*Armeria muelleri*, Figure. 10 and *Agrostis capillaris*, Figure. 11) located at different positions along the pollution gradient, with different capacities of tolerance to metal stress. The two ecotypes of *Armeria muelleri* were chosen from Habitat 1 and Habitat 3, and the two ecotypes of *Agrostis capillaris* were chosen from Habitat 2 and Habitat 4 (see Figure. 8).

In the second reciprocal transplantation experiment, we assessed the facilitative responses of two ecotypes of two of *Festuca rubra* (Figure. 12) occurring at different positions along the pollution gradient, with different metal stress tolerance capacities. The two ecotypes of *Festuca rubra* were chosen in habitats corresponding to their highest (H2, See Figure. 8) and lowest (H4, See Figure. 8) levels of soil pollution.

To assess if a trade-off exists between stress tolerance and plant facilitative and competitive response along the pollution gradient, the performance of different species and ecotypes transplanted under different level of metal stress conditions and "with" and "without" neighbours was compared using the relative interaction index (RII). To evaluate the respective contribution of the environment and that of neighbours in determining the outcome of plant responses, the methodological approach of Michalet et al. (2014b) was used. This method consists in disentangling the effect of the "RII", into the environmental-severity effect and the neighbour-trait effect. The effect of environment and the effect of neighbours on the performance of

target species and ecotypes can be quantified separately, and thus the relative contribution of these two effects can be determined.

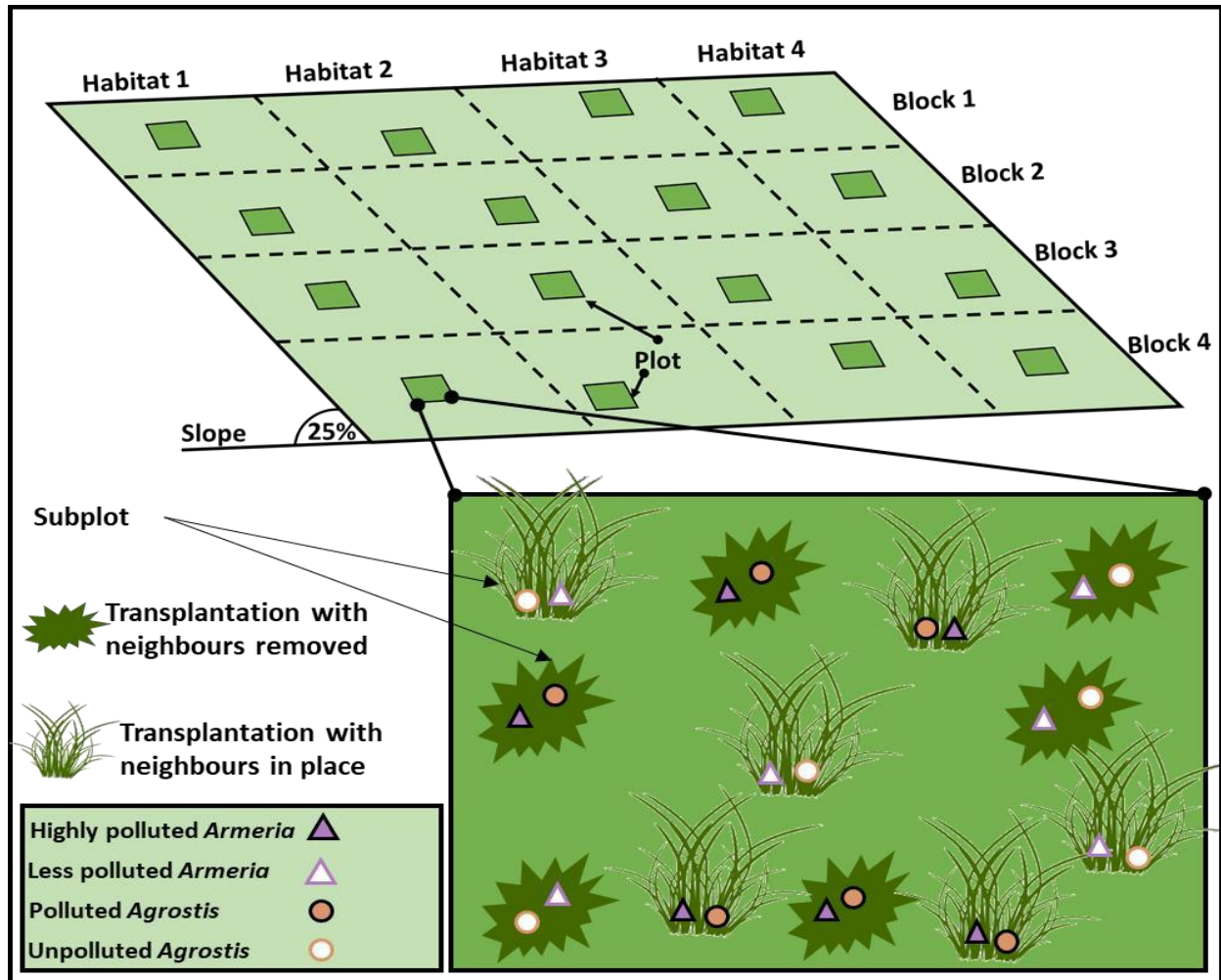


Figure 9. Schematic representation of the first reciprocal transplantation experiment in the subalpine belt in the Pyrenees.

Armeria muelleri



Figure 10. *Armeria muelleri* is morphologically very close to *Armeria alpina*, but seems to occur only in the eastern part of the Pyrenees. It was identified as a metallophyte growing on copper, zinc and lead mines. It has very narrow and normally single-veined leaves. (height of the plant: 10-30 cm; flowering: from June to August; altitudes: 2000 to 2700 meters). Photo taken by Florian Delerue.

Agrostis capillaris



Figure 11. *Agrostis capillaris* has a very dense panicle, with the branches remaining clearly spread out. Plant of the lawns and moors, generally on acid soils. It is capable to tolerate high levels of heavy metal contaminants such as As, Mn, Pb, Zn. (height of the plant: 15-80 cm; flowering: from June to September; altitudes: 0 to 2600 meters). Photo source: <https://www.florealpes.com>

Festuca rubra



Figure 12. *Festuca rubra* has non-scarious lemmas and very short ligules. Stems with almost completely fused sheaths Flat leaves with five prominent veins. It is tolerant of high levels of copper, lead, and zinc. (height of the plant: 20-90 cm; flowering: from May to October; altitudes: 0 to 2400 meters). Photo taken by Florian Delerue.

2.3. Experimental design for the third objective:

(O3) Evaluate at inter- and intraspecific levels if a trade-off exists between stress tolerance and plant facilitative effects in metalliferous systems (Chapter 5)

To fulfil this objective, a common garden experiment, was carried out at the mountain belt from May 25, 2020 to October 13, 2021 (see location 10 in Figure 6). An evaluation of the facilitative effects can only be achieved by an experimental garden, because different nurse plants with different metal tolerance must be grown under the same environmental conditions. The site of location 10 consists of a slag heap which is ideal for this type of experiment because of its homogeneous soil.

In this common garden experiment, we selected two ecotypes of four metal tolerant nurse species that have different metal-stress tolerance (*Armeria muelleri*, Figure. 10; *Hutchinsia alpina*, Figure. 13; *Gypsophila repens*, Figure. 14; and *Minuartia verna*, Figure. 15) to investigate the role of ecotypic variation on the facilitative effects of nurse species. The two ecotypes of these nurses were chosen from habitats with the highest (H1, See Figure. 8) and lowest (H3, see Figure S8) levels of soil pollution. Two ecotypes of

Agrostis capillaris, were selected as target for this common garden experiment. *Agrostis* ecotypes were selected in habitats corresponding to their highest (H2, See Figure. 8) and lowest (H4, See Figure. 8) levels of soil pollution to maximize the potential differences in their facilitative responses to nurse effects. In total, four blocks or nurse garden were placed 10 meters apart on a flat area of this slag heap (see Figure 16, Figure 17 and Figure 18a and b). Inside each block, nurse Individuals were transplanted in a systematic pattern and also several spots were preserved free of nurse individuals (open spots, see Figure 18b). Individuals of *Agrostis capillaris* from both highly polluted and unpolluted habitats were transplanted within the nurse plants and without the nurse plants into the open spots.

To assess the facilitative effects of nurse species and ecotypes that have different metal stress tolerance, the performance of target *Agrostis capillaris* individuals growing inside the nurse and in open spots was compared using the relative interaction index. This comparison allows us to determine the outcome of plant-plant interaction, and to identify whether a trade-off exists between stress tolerance and plant facilitative effects.

Hutchinsia alpina



Figure 13. *Hutchinsia alpina* grows at high altitudes and often forms metallicolous grasslands on soils with high levels of metallic elements such as copper, lead, arsenic and zinc. It has ferny evergreen leaves, smothered for many weeks by short sprays of tiny white flowers. (height of the plant: 2-10 cm; flowering: from May to August; altitudes: 0 to 3400 meters). Photo taken by Florian Delerue.

Gypsophila repens



Figure 14. *Gypsophila repens* has small white flowers and grows on limestone soils. It normally grows at higher elevations, and are abundant in metalicolous systems, but tends to follow the path of scree and sand, and is actually found along rivers at lower elevations. (height of the plant: 5-20 cm; flowering: from June to September; altitudes: 480 to 2900 meters). Photo taken by Hugo Randé.

Minuartia verna



Figure 15. *Minuartia verna* is a small plant that forms large clumps in lawns. It grows on heavy metal polluted soil and accumulate remarkable concentrations of copper and zinc in their leaves. It can be recognized by its narrow, flat leaves, its non-bicolored sepals and its non-cornered petals. (height of the plant: 2-10 cm; flowering: from June to August; altitudes: 1200 to 3000 meters). Photo taken by Hugo Randé.

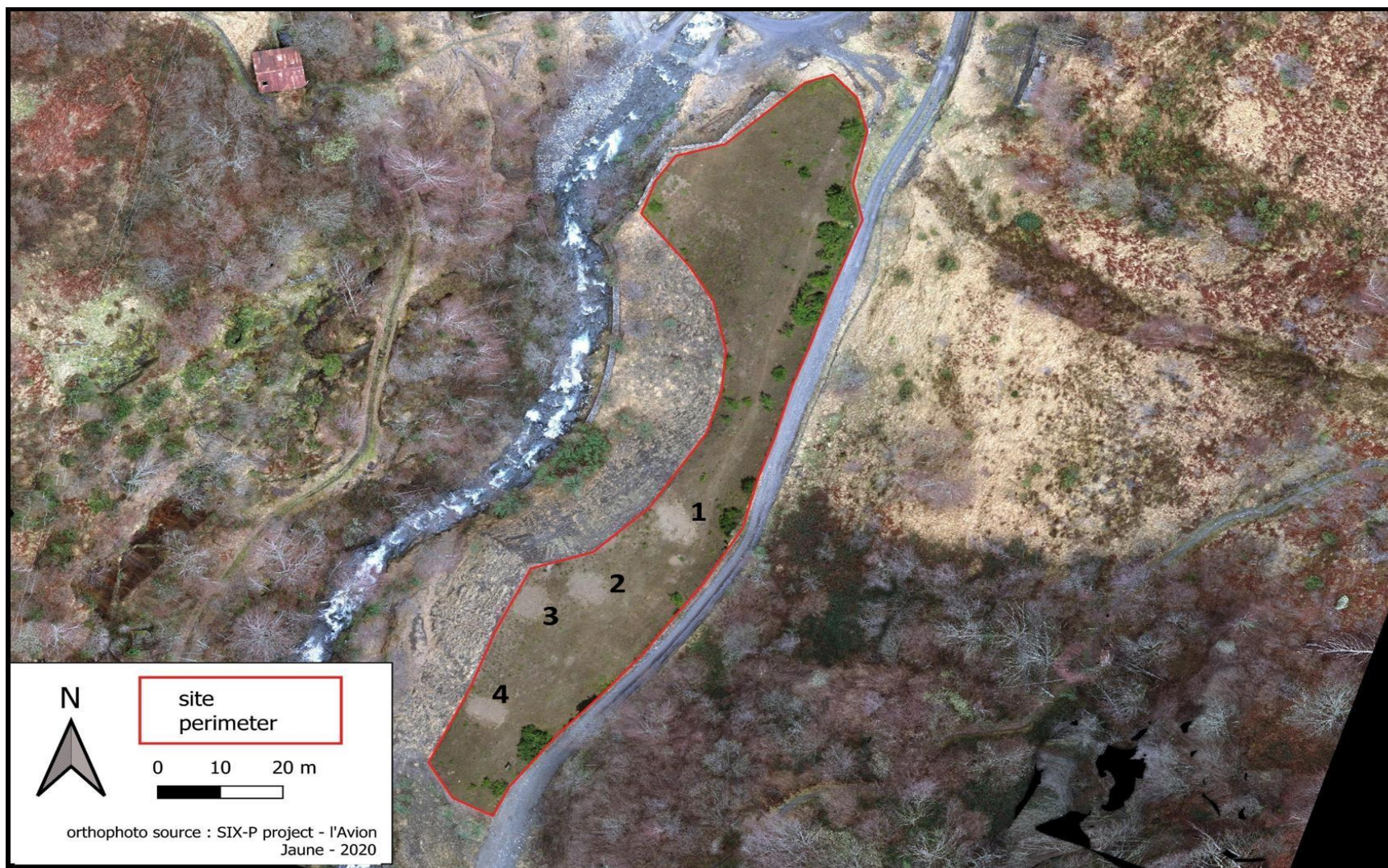


Figure 16. The common garden experiment at the mountain belt in the Pyrenees. Numbers 1,2 3 and 4 on the figure indicate the locations of the four blocks or nurse gardens being set up on the slag heap for the transplantation of nurse and target plants.



Figure 17. Photo of one common garden on the slag heap before the transplantation of nurse and target plants.

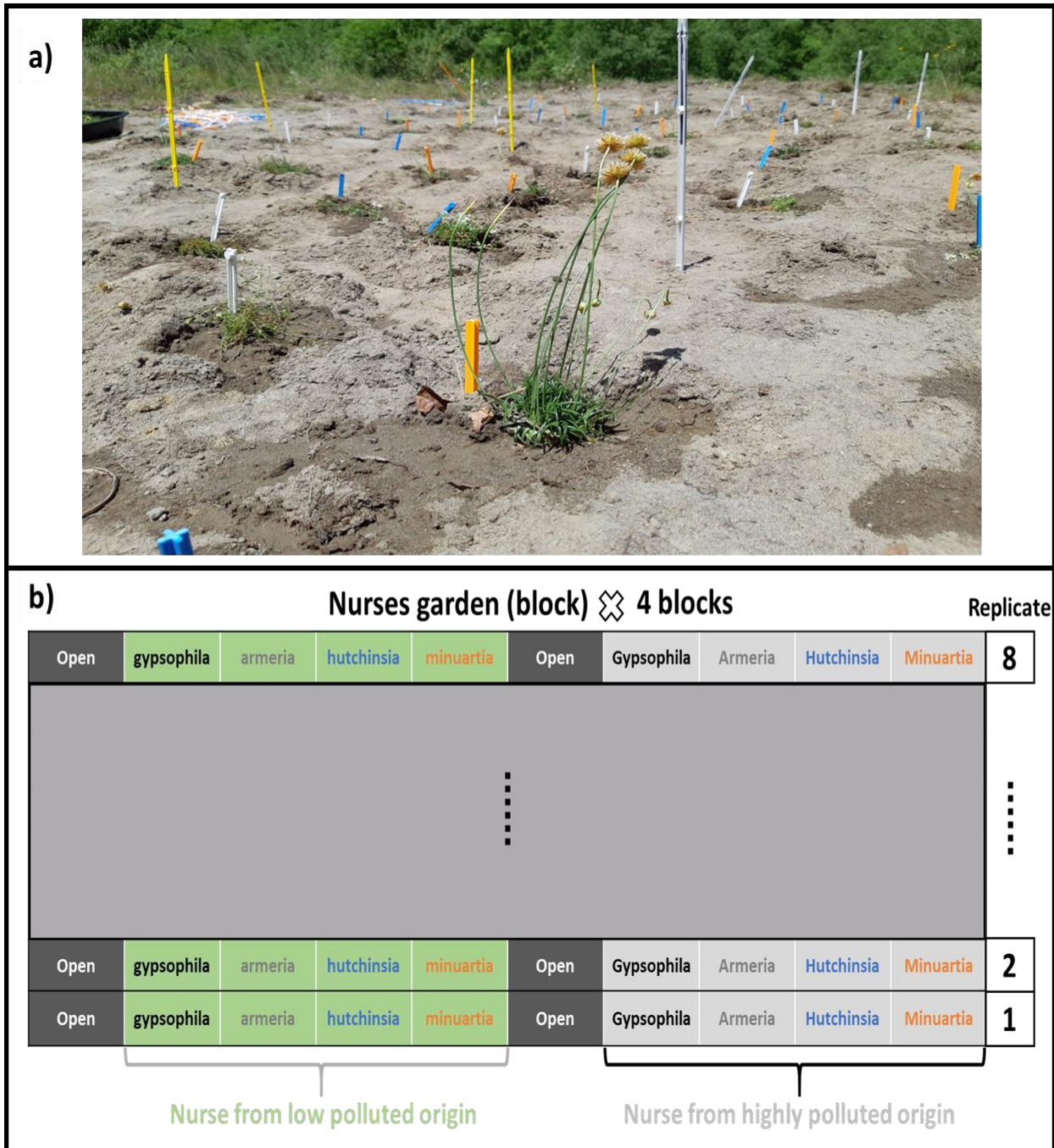


Figure 18. Presentation of the common garden experiment. (a) Transplantation of the nurses in the experimental garden on May 25, 2020. (b) Schema showing of a block containing individuals of each of two ecotypes of each nurse species, and open spots that are free from nurse individuals.

3. Indices used to quantify plant–plant interactions

One of the main objectives of this thesis was to assess changes in competition and facilitation along stress gradients. In all our experiments, to quantify the intensity of responses of the different species and ecotypes to the effects of neighbours, we used the relative interaction index (RII, Armas et al. 2004). This index is based on the relative difference in target performances with and without neighbours (Figure 19):

$$\text{RII}_{\text{neighbour}} = \frac{P_{+\text{neighbour}} - P_{-\text{neighbour}}}{P_{+\text{neighbour}} + P_{-\text{neighbour}}}$$

Where $P_{+\text{neighbour}}$ and $P_{-\text{neighbour}}$ represent target performance in the presence and absence of neighbouring vegetation, respectively (Figure 19). Values of RII vary between -1 and 1 , with negative values standing for competition and positive ones for facilitation.

Determining the relative effect of neighbours and environmental stress on target performance is of great importance in this thesis. However, relative interaction index (RII), does not have the ability to separate the two effects. Therefore, to achieve this goal, other metrics were used. In the first study site in the Gironde department, we used “Imp; importance of competition index” (Seifan et al. 2010), together with the RII “intensity of competition index”. The RII index only quantifies the effect of neighbours on the target species, independent of other environmental effects. However, the Imp index, quantifies the relative importance of competition for a target species as compared to other environmental constraints, in particular stress. The Imp index is computed as follow:

$$\text{Imp} = \frac{N_{\text{imp}}}{|N_{\text{imp}}| + |E_{\text{imp}}|}$$

Where N_{imp} and E_{imp} are the neighbours and environmental contributions to plant performance, respectively with: $N_{\text{imp}} = P_{+\text{neighbour}} - P_{-\text{neighbour}}$ and $E_{\text{imp}} = P_{-\text{neighbour}} + P_{\text{max} \pm N}$, where $P_{\text{max} \pm N}$ is the maximum value of plant performance.

In the second study site in the Pyrenees mountains, in addition to the aforementioned $RII_{\text{neighbours}}$, we compared plant performance under the highest and lowest stress conditions, in order to separate environmental-severity effects from neighbour-trait effects during changes in plant-plant interaction with increasing metal stress. We compared target performance under the highest stress conditions and no stress conditions (Figure. 19). Thus, we computed the following relative interaction index following Michalet et al. (2014b).

$$RII_{\text{stress}} = \frac{P_{+\text{stress}} - P_{-\text{stress}}}{P_{+\text{stress}} + P_{-\text{stress}}}$$

Where $P_{-\text{stress}}$ is the target performance without stress, and $P_{+\text{stress}}$ is the target response under high level of metallic stress (Figure 20). The increases in facilitation with increasing stress is either due to a neighbour-trait (Figure 20a) or an environmental-severity effect (Figure 20b) or both. Therefore, two RII_{stress} must be calculated, one for targets with neighbours comparing their performance with and without stress and the other for targets without neighbors comparing their performance with and without stress.

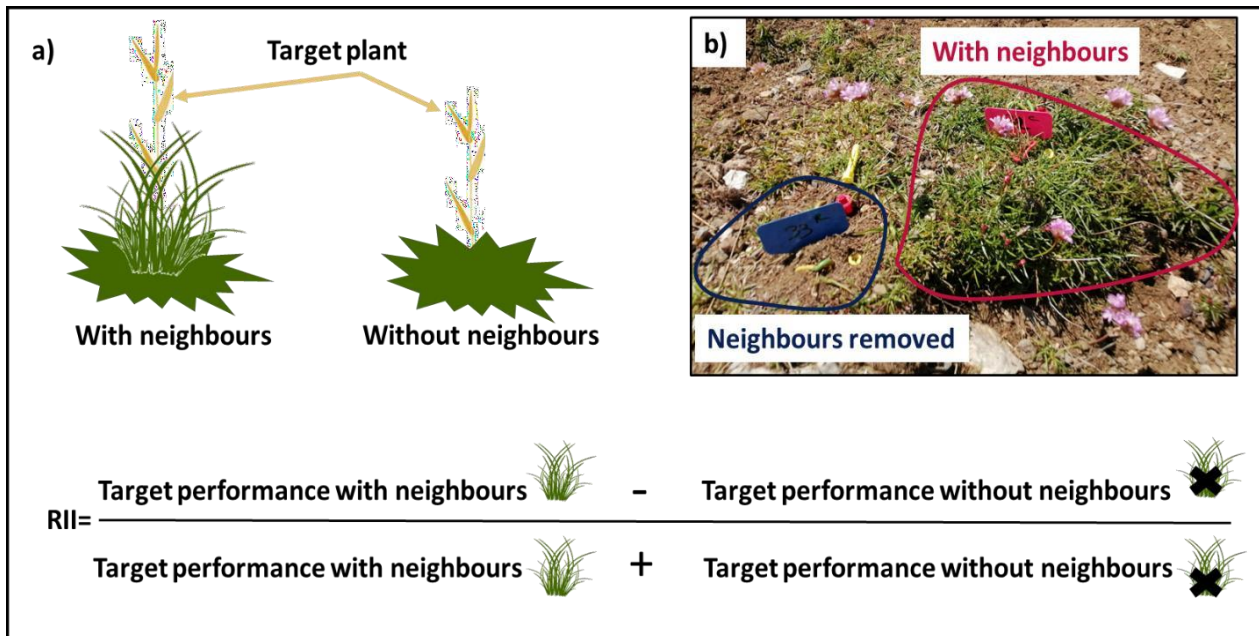


Figure 19. Plant-plant interaction measurement. (a) Schematic representation of target performance with and without neighbours. (b) Photos showing the two different neighbouring conditions.

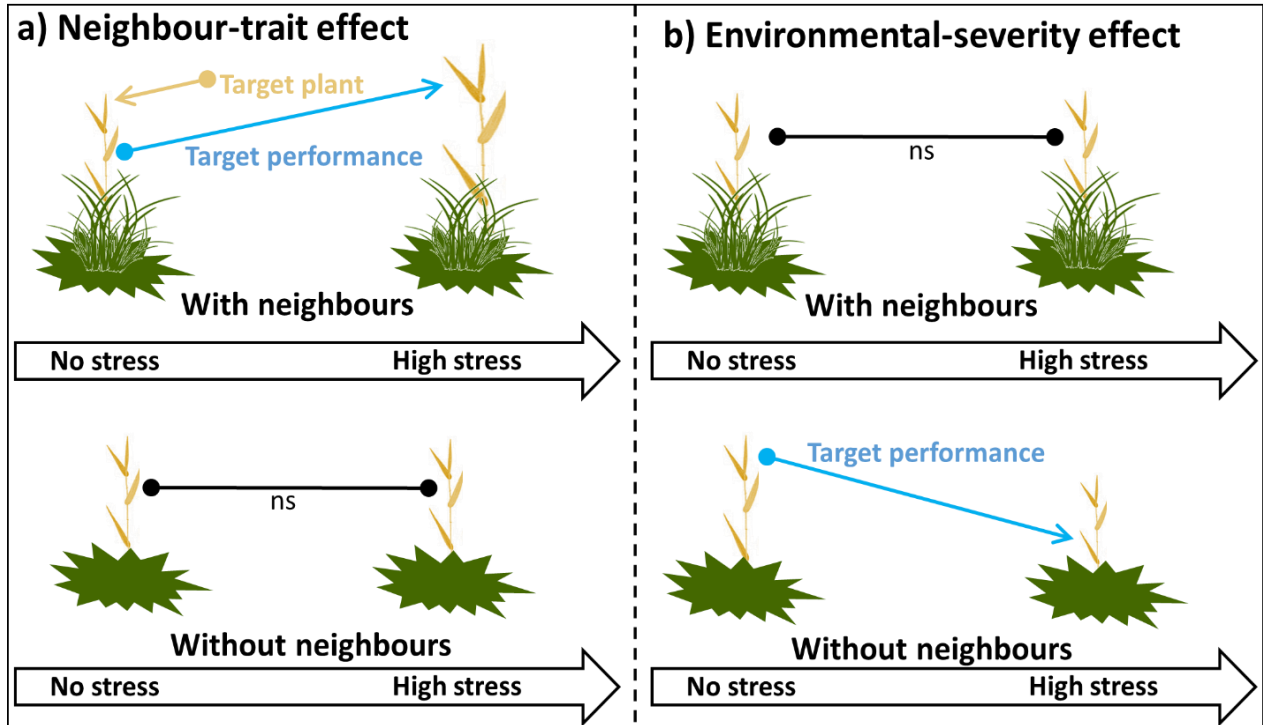


Figure 20. Changes in target performance along a stress gradient. (a) Neighbour-trait effect, (b) environmental-severity effect

CHAPTER 3

Species stress tolerance and community competitive effects drive differences in species composition between calcareous and siliceous plant communities

This chapter was the subject of an article published in *Journal of Ecology*, in which we present the results of a four-year reciprocal transplantation experiment conducted along a water gradient (resource-related) driven by difference in bedrocks, located in the Gironde department, in South-Western France. The aim of this multi-year experiments was to identify the relative contribution of species stress tolerance and competitive effects in driving differences in plant composition between calcareous and siliceous communities along a water-availability gradient.

Main results:

Meteorological variability over the four years of this study revealed that calcareous species were much more tolerant of dry episodes occurring some years than siliceous species but suffered the most from competition, highlighting a trade-off between stress tolerance and competitive response between calcareous and siliceous species. The higher stress tolerance abilities of calcareous species decreased their ability to cope with competition, while the higher competitive response of siliceous species decreased their ability to cope with stress.

Furthermore, in this study we showed that species from siliceous communities have strong competitive effects in their home low-stressed community. However, they are not tolerant to the higher stress occurring on calcareous bedrock. This highlighted the existence of a trade-off between plant competitive effect abilities and stress tolerance, similar to the trade-off between stress tolerance and competitive response ability.

Finally, we demonstrated that the relative contributions of the competitive effects of siliceous communities and high stress conditions on calcareous soils in determining differences in species distribution on the two soil types were not equal, with the effect of the former being less important than the latter. Competition never led to the exclusion of calcareous species from siliceous communities, whereas stress conditions on calcareous soils led to the almost complete exclusion of siliceous species.

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RESEARCH ARTICLE

Species stress tolerance and community competitive effects drive differences in species composition between calcareous and siliceous plant communities

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Abstract

1. Both chemical and physical properties of soils have been used for two centuries for explaining differences in species composition and diversity between plant communities from calcareous and siliceous soils. Here, we examine the prediction from the 'physical hypothesis', which proposes that species from siliceous soils are absent from calcareous soils because of high drought stress, while species from calcareous soils are absent from siliceous soils because of high competition due to higher water availability. This hypothesis therefore predicts a trade-off between stress tolerance and competitive response that allows calcareous and siliceous species to dominate their respective communities. There has been no attempt to experimentally assess these predictions.
2. We cross-transplanted with and without neighbours using a removal experiment, two dominant species from calcareous soils (*Bromus erectus* and *Brachypodium sylvaticum*) and two species from siliceous soils (*Pseudarrhenatherum longifolium* and *Deschampsia flexuosa*) in herbaceous communities from the two soils types. We repeated the experiment 4 years, taking advantage of inter-annual climatic variability to test the interplay between year quality (dry vs. wet years) and soil types on survival responses with and without neighbours. We quantified species stress tolerances, their competitive responses and the competitive effect of the two community types.
3. In the absence of neighbours, species from siliceous soils had much lower survival than species from calcareous soils during dry years, in particular on calcareous soils. It indicates a lower stress tolerance of siliceous species and a higher level of stress occurring on calcareous soils. Competition strongly reduced the survival of calcareous but not siliceous species, indicating a greater competitive response of the latter and competition was more important on siliceous soils than on calcareous soils during wet years. However, we found that species stress tolerances were more important than competitive effect of neighbours in driving differences in species composition.
4. *Synthesis.* The contrasting responses found for dominant species from calcareous and siliceous soils support the trade-off between stress tolerance and competitive

response proposed by the physical hypothesis. Our results bring additional evidence that differences in water availability and stress between the two soil types are driving species responses to bedrock types.

KEYWORDS

calcareous soils, climate variability, competitive effects, competitive responses, drought stress, siliceous soils, stress tolerance

1 | INTRODUCTION

Edaphic factors (e.g. water and nutrient availability, soil chemistry, soil texture, etc.) related to substrate types are known to be strongly correlated with community composition and species distribution, although causal relationships are not always identified (Austin & Smith, 1989; Whittaker, 1956). For example, Chauvier et al. (2021) showed that the contribution of soil and land cover on species distribution in the European Alps was as large as climate. However, since edaphic factors are known to interact with other complex environmental variables such as climate (e.g. Chaieb et al., 2020; Liancourt et al., 2013), they are likely to amplify or buffer the effect of climate. Thus, identifying the mechanisms by which edaphic factors interact with climate is therefore necessary for addressing the role of soils and substrate type for community composition.

Contrasting species composition of plant communities occurring on soils derived from calcareous and siliceous substrates has raised the attention of botanists, physiologists and ecologists, in particular in Europe, for almost 200 years (Bothe, 2015; Braun-Blanquet, 1964; Ellenberg, 1958; Gigon, 1987; Kinzel, 1983; Lee, 1999; Michalet et al., 2002; Thurmann, 1849; Unger, 1836). The work of Braun-Blanquet and Jenny (1926) has largely contributed to the wide acceptance of the idea among European scientists that this difference in species composition was mainly due to differences in soil chemistry. This is particularly the case for alpine communities due to the high correlation existing between pH, calcium and aluminium contents in soils and bedrock types (Duchaufour, 1997; Gensac, 1990; Reisigl & Keller, 1987).

This chemical hypothesis was further developed by Duchaufour (1989, 1997) in the context of the wet climate of the north-east of France. Briefly, he argued that soils derived from siliceous bedrocks have low pH (acidic) and clay content. These soil characteristics, leading to slow organic matter decomposition, low nutrient and high exchangeable aluminium contents, are considered as unfavourable for species from calcareous and neutral soils (Pinto & Gégout, 2005; Rameau et al., 1989).

However, the chemical hypothesis fails at fully explaining several empirical observations. For instance, the basis of the chemical hypothesis, the differences in organic matter decomposition between acidic and neutral soils, is not consistent with overwhelming evidence that have shown that litter quality and climate (and not soil chemistry) are the most important drivers of litter decay in terrestrial ecosystems (Cornwell et al., 2008; Coûteaux et al., 1995). It suggests that

pH is more likely the consequence of organic matter accumulation than its driver (Michalet et al., 2001). Moreover, species pH optima and amplitudes have been shown to vary geographically (Coudun & Gégout, 2005; Diekmann & Lawesson, 1999; Pakeman et al., 2008; Peřík & Bruelheide, 2006; Szymura et al., 2014), and the aforementioned tolerance to toxicity may only partly explain the pattern in the field. The interactions between soil moisture and pH have also been highlighted (Le Bagousse-Pinguet et al., 2017; Pakeman et al., 2008), suggesting that physical factors related to substrate types (such as water availability) may also drive differences in species and functional composition between communities occurring on siliceous and calcareous bedrocks.

The alternative 'physical hypothesis', first proposed by Thurmann (1849), posits that the physical structure of the substrate drives water percolation beneath the soil profile and soil moisture availability, which in turn determines community composition. Several authors have highlighted that climate and substrate type are complex factors both driving water availability (e.g. Gigon, 1987; Grime & Curtis, 1976), in particular for deep-rooted tree and shrub species that may benefit from the water flow existing at bedrock–soil interface. Although, there could be strong differences in physical structure within either calcareous or siliceous rocks, calcareous soils are overall drier than siliceous soils because of the higher porosity of calcareous bedrocks that induces rapid water percolation within the substrate (Michalet et al., 2001, 2002; Rolland et al., 1998).

Michalet et al. (2002) found at the subalpine belt of the French Alps that water stress on calcareous soils and competition on siliceous soils (due to lower drought stress) were the direct driving factors of richness, composition and structure of both community types. The more 'stress-tolerant' calcareous species may be favoured over 'intolerant to stress' siliceous species on calcareous soils, whereas calcareous species may be excluded in siliceous environments with dense vegetation due to the competitive effects of siliceous species (Gigon, 1971; Michalet et al., 2002; Thurmann, 1849). The existence of a trade-off between responses to stress and competition has important implication in ecological plant theory and has already been shown in contrasting conditions of water and light availability (Liancourt, Callaway, et al., 2005; Smith & Huston, 1989). Such trade-off has also been highlighted for other organisms than plants, such as animals and microbes (Connell, 1978; Dean & Connell, 1987; Garland, 2014; Peterson, 1991; Wissinger et al., 1999; Wood et al., 2018). However, to our knowledge, the implication of such trade-off in driving differences in species composition and diversity

between calcareous and siliceous communities has never been tested experimentally.

Our main objective in this study is to experimentally disentangle the relative contribution of stress tolerance and competition in determining the differences in plant composition between calcareous and siliceous communities by focusing on the responses of their dominant species. We set-up a field competition experiment in the south-west of France, cross-transplanting with and without neighbours two dominant species from calcareous origin and two dominant species from siliceous origin in two grasslands from the two soils types. For quantifying competition, we used the 'importance of competition index' together with the 'intensity of competition index' (Brooker et al., 2005; Seifan et al., 2010). The former index is particularly adapted to address our question, since it quantifies the relative importance of competition for a target species as compared to other environmental constraints, in particular stress. The latter only quantifies the effect of neighbours on the target species, independent of other environmental effects (Brooker et al., 2005; Corcket et al., 2003; Welden & Slauson, 1986). This experiment was repeated 4 years (2016, 2018, 2019 and 2020) to take advantage of the year-to-year variation in precipitation and evaluate the responses of our target species on the two soil types under 'wet' and 'dry' year conditions. We tested the two main predictions of the physical hypothesis:

1. Calcareous species are more tolerant to stress and less tolerant to competition than siliceous species, and, thus, both species types should respond differently to meteorological variations.
2. Competition is stronger on siliceous than on calcareous soils.

Finally, we expected that species stress tolerance and the difference in competitive effects between the two communities explain equal species distribution on their respective soil type.

2 | MATERIALS AND METHODS

2.1 | Study sites and target species

The study was conducted in the Gironde department, south-west of France. The climate is temperate oceanic with 931 mm of annual precipitation. As for other oceanic climates, winter is the rainiest season and temperatures are mild (the average seasonal winter temperature is 7.1°C; Michalet et al., 2021). Summer is less rainy but not dry (the average seasonal summer temperature is 20.7°C). The Gironde department is particularly adapted to answer our questions since it includes, in similar conditions of climate and topography, two lands having different soils types. The two lands occur at short distance on the two sides of the Garonne River, allowing the replication of soil type treatment in different blocks.

The ecosystems with calcareous soils are located on the Entre-Deux-Mers land, on the east bank of the Garonne River. The land is formed of small hills of less than a 100 m of elevation on a tertiary

calcareous bedrock. Soils are deep and fine-textured if topography is not too convex. Soil pH varied between 6 and 7.5, thus not acidic, due to the high content of exchangeable calcium and calcareous sands (between 50% and 60% of CaCO₃ in the upper soil horizon). The ecosystems with siliceous soils are located in the Graves land, on the west bank of the Garonne River. The land is at sea level and on flat quaternary terraces of siliceous deposits originating from the weathering of Pyrenean and Central Massif mountain ranges. Soils have a coarse sandy texture with lots of gravel. They are unfertile and acidic with pH lower than 4 (Wang et al., 2020). We selected two community types (one in each of the two soil types) occurring on poorly managed old fields with very low disturbance to avoid the confounding effects of grazing or mowing.

The community type on calcareous soil is a herbaceous shrubland dominated by *Erica scoparia* and *Brachypodium pinnatum*. Other common species are the shrubs *Prunus spinosa*, *Cornus sanguinea* and *Rosa rubiginosa*, and the grass *Bromus erectus*. Species richness is high (up to 25 species per m²) and community composition is the characteristic of a 'calcareous' vegetation (Rameau et al., 1989). The community type on siliceous soil is a shrubland dominated by *Ericaceae* species (*Erica scoparia*, *Erica arborea*, *Erica cinerea*, *Calluna vulgaris*), the fern *Pteridium aquilinum* and the grass *Pseudarrhenatherum elatius*. The average species richness is lower and community composition is the characteristic of a 'siliceous' vegetation (Rameau et al., 1989). We selected four perennial grass species as targets that are common dominant species of either calcareous or siliceous communities from western Europe '*Bromus erectus* (Huds.) Fourr.' and '*Brachypodium sylvaticum* (Huds.) Beauv.' for the former and '*Pseudarrhenatherum longifolium* (Thore.) Rouy.' and '*Deschampsia flexuosa* (L.) Trin.' for the latter. *Bromus erectus* and *Pseudarrhenatherum elatius* are dominant herbaceous species of calcareous and siliceous grasslands and heathlands, respectively, whereas *Brachypodium sylvaticum* and *Deschampsia flexuosa* are dominant herbaceous species of calcareous and siliceous forest understories and hedgerows respectively (Corcket et al., 2003; Michalet et al., 2015; Pagès & Michalet, 2006; Wang et al., 2020).

2.2 | Experimental design

Our field experiment was conducted from early March to late August and was repeated 4 years (2016, 2018, 2019 and 2020). The experimental design included soil type as main plot treatment (calcareous or siliceous soil), and vegetation removal (with or without neighbouring vegetation) and species origin (calcareous or siliceous species) as subplot treatments. Four blocks spaced 10 km apart were placed from upstream to downstream of the Garonne river between Langon (located 50 km south-east of Bordeaux, 44°33'23"N, 0°14'41"W) and Bordeaux (44°48'51"N, 0°32'37"W). Within each of the four blocks, one main plot was located to the east of the river on calcareous soil, and the second main plot was located to the west of the river on siliceous soil. Each year within each main plot, we randomly selected six circular subplots of 1 m² for the vegetation

removal treatment. Above-ground vegetation of half of the subplots was removed by hand. These subplots with no neighbours were kept free from vegetation during the experiment. Each year, we re-established new plots and applied the removal treatment at a slightly different location within each main plot to ensure that removal treatment did not affect our results on the long term (e.g. modification of nutrient dynamics, root decomposition). Tillers of the four target species were harvested in their own communities before transplantation. Finally, three replicates of individuals of each of the four target species originating from calcareous or siliceous soils were transplanted each year in all subplots (with or without neighbours). Each tiller of transplanted individual of the four species had from 2 to 10 leaves. All target individuals were tagged with metal wire and placed at a distance of at least 50 cm from each other within each subplot. In total, 768 transplants were performed in the experiment (4 blocks \times 2 soil types \times 2 neighbouring conditions \times 4 target species \times 3 replicates \times 4 years).

2.3 | Data collection and analysis

Each year, the survival of all target individuals was determined at the end of the experimental period (August). Survival rate was calculated at the block level in all combinations, per species and neighbour treatments. We did not analyse biomass because survival was too low in some treatments. We took advantage of the 4 years with different meteorological conditions to test the effect of different weather conditions (e.g. precipitation and temperature) on the performance and the outcome of plant–plant interactions in the two soil type conditions and for the species of the two origins. We used a drought index (the Standardized Precipitation Evaporation Index—hereafter ‘SPEI’) in the central location of our study (44.72N, –0.41W) to characterize meteorological conditions during the study. This drought index is based on the balance between precipitation and potential evapotranspiration and is more suitable than an index based on precipitation only to detect drought episodes compared to an historical background (Vicente-Serrano et al., 2010). The index allows estimates of how dry or wet a month is in comparison to the same month for other years. Corresponding climatic data were obtained on the ClimateDT web tool available at <https://ibbr.cnr.it/climate-dt/>. ClimateDT downscales historical monthly, seasonal and annual climate variables for any location in the Globe from a 1-km grid dynamically (see Marchi et al., 2020) for the European climate. Additionally, in order to characterize the 4 years as wet or dry climatic years, we calculated for each target species i growing without neighbours, per soil type j , per year k and per block l the survival anomaly as follows:

$$\text{Survival anomaly}_{i,j,k,l} = (\text{target survival}_{(\text{Soil type } j \times \text{block } l \times \text{Year } k)} - \text{average target survival}_{(\text{Soil type } j \times \text{block } l, \text{ all years})}) / \text{average target survival}_{(\text{Soil type } j \times \text{block } l, \text{ all years})} \quad (1)$$

For a given species in a given soil type treatment and in a given block, a positive anomaly indicates that the observed survival in the corresponding year is higher than the average of all years considered,

while a negative anomaly indicates that survival is lower than the average.

We computed two indices to characterize the intensity (RII) and the importance (Iimp) of plant–plant interactions. To analyse the variation in competition intensity between treatments, we used the relative interaction index ‘RII’ (Armas et al., 2004). This index is based on the comparison of target performance with and without neighbours:

$$\text{RII} = \frac{P_{+ \text{ neighbour}} - P_{- \text{ neighbour}}}{P_{+ \text{ neighbour}} + P_{- \text{ neighbour}}} \quad (2)$$

where $P_{+ \text{ neighbour}}$ and $P_{- \text{ neighbour}}$ represent target performances (survival) in the presence and absence of neighbouring vegetation respectively. Values of RII vary between –1 and 1, with negative values stand for competition and positive ones for facilitation.

Competition importance measures the difference in an organism's performance in the presence of neighbours relative to the impact of other factors such as abiotic environment factors (Brooker et al., 2005; Welden & Slauson, 1986). We calculated the importance of interactions using ‘Seifan et al., 2010’ index ‘Iimp’, in which the maximum value of plant performance is the highest average survival for each species in all the experiment treatments, in the four blocks and in all the year considered.

$$\text{Thus the importance of competition index is computed as: } \text{Iimp} = \frac{N_{\text{imp}}}{|N_{\text{imp}}| + |E_{\text{imp}}|} \quad (3)$$

where N_{imp} and E_{imp} are the neighbours and environmental contributions to plant performance, respectively, with: $N_{\text{imp}} = P_{+ \text{ neighbour}} - P_{- \text{ neighbour}}$ and $E_{\text{imp}} = P_{- \text{ neighbour}} + P_{\text{max} \pm N}$ where $P_{\text{max} \pm N}$ is the maximum value of plant performance (survival).

2.4 | Statistical analyses

All statistical analyses were done using R software (R Core Team, 2016). Mixed modelling with ‘Year nested within Blocks’ as a random factor was used in all cases. Dependent variables and model residuals were systematically checked for normality to respect the assumption of linear modelling.

Survival anomalies were compared using a linear mixed-effects model with ‘Year’, ‘Soil type’ and ‘Origin’ and their interactions as fixed factors. We also used one-sample t -tests to detect significant deviation of anomaly values from zero. Since results clearly showed that the 4 years of our experiment largely differed in their effects on plant survival (see the results section and Figure 1), ‘Year qual-

ity’ was therefore considered as a factor in the subsequent analyses where 2016 and 2018 were pooled and considered as favourable years (‘wet’), and 2019 and 2020 as unfavourable (‘dry’).

FIGURE 1 Survival anomalies (mean \pm SE, $n = 8$ – two species for each origin \times 4 blocks) for the species of the two origins for the 4 years on calcareous and siliceous soils. Black and grey bars represent species from calcareous and siliceous origin respectively. Results of one-sample t -tests are shown above or below bars when significant. (*), $p < 0.1$; *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$

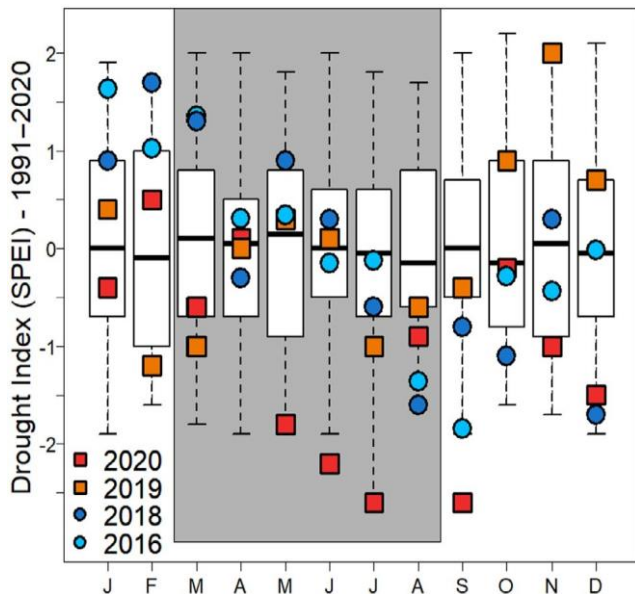
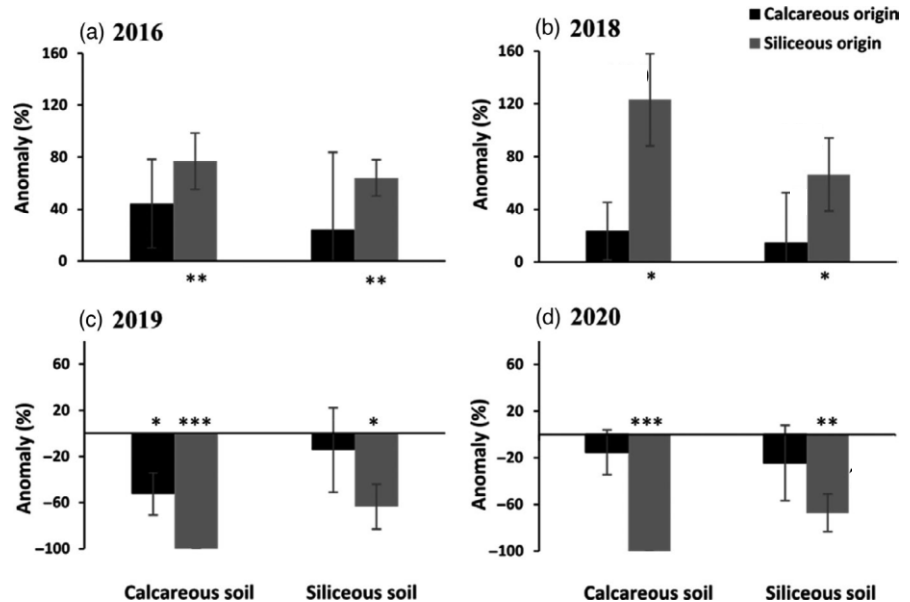


FIGURE 2 Boxplots for Monthly SPEI (Monthly drought index) for the period 1990–2020 in the central location of the study area (44.72N, -0.41 W). SPEI values indicate how much dry (negative value) or wet (positive value) a month is compared to the same month during a reference period (1961–1990 on the ClimateDT Tools) considering the balance P-PET. Data for the years of the study are shown (2016: light blue circles; 2018: dark blue circles; 2019: orange squares; and 2020: red squares). Grey area from March to August indicate monitoring period (from initial plant translocation to final harvest)

We then assessed the effect of the abiotic conditions ('Soil type' and 'Year quality'), alone, that is on the survival of target species growing without neighbours, originating from calcareous and siliceous soils ('Origin'), using a general linear mixed-effects model with a logit link function for binomial distribution. Additionally, planned

comparisons among 'Year quality', 'Soil type' and 'Origin' combinations were made using differences in least squares means.

We analysed RII and Iimp separately using linear mixed-effects models (followed by Tukey tests when necessary) with 'Year quality', 'Soil type', 'Origin' and their interactions as fixed factors. We used one-sample t -tests to detect significant deviation of RII and Iimp values from zero.

3 | RESULTS

3.1 | Wet and dry years

'Year' had a highly significant effect on survival anomaly, with positive anomalies in 2016 and 2018, and negative in 2019 and 2020 ($p < 0.001$, Tukey results: a, 2016, $52.4 \pm 17.8\%$, a, 2018, $56.9 \pm 17.1\%$, b, 2019, $-57.6 \pm 12.1\%$, b, 2020, $-51.7 \pm 11.5\%$, Figure 1). SPEI drought index for the 4 years of the study (Figure 2) showed: (a) an important water deficit in February and March in 2019, that is at the time of target translocation, and a similar water deficit in March 2020 (however less pronounced than in 2019). This was mainly due to a deficit in precipitation (Appendix S1); (b) the end of spring–early summer 2020 (May to July) was the driest of the last 30 years in the area (Figure 2); and (c) spring and early summer (from March to July) in 2016 and 2018 were wetter or close to the median of SPEI drought index of the last 30 years. Thus, the differences in survival anomalies over the 4 years delineating contrasting 'wet' and 'dry' years were likely to be explained by these differences in meteorological conditions. Additionally, there was a significant Year \times Origin interaction regarding anomaly responses ($p < 0.01$), because species from siliceous origin were more sensitive to the negative effect of dry years than species from calcareous origin (Tukey results: siliceous origin: a, for 2016, $70.6 \pm 12.5\%$ and 2018, $94.8 \pm 23.8\%$, and b, for 2019, $-81.8 \pm 10.5\%$ and 2020, $-83.6 \pm 8.84\%$, calcareous

origin, a, the 4 years, 2016, $34.2 \pm 33.2\%$, 2018, $19.1 \pm 21.3\%$, 2019, $-33.4 \pm 20.4\%$, 2020, $-19.85 \pm 18.2\%$, Figure 1). Finally, the one-sample *t*-tests showed that the negative anomalies of species from siliceous origin were higher and more significant on calcareous than on siliceous soils. This shows a tendency for species from siliceous origin to have more negative survival anomalies in the dry years on calcareous than on siliceous soils, although there was no significant Year \times Origin \times Soil type interaction.

3.2 | Survival without neighbours

Year quality and Soil type had a significant effect on survival without neighbours (Table 1). Survival was three times higher during wet than dry years ($p < 0.001$, Tukey results: a, $58 \pm 4.27\%$ for 2016 and 2018, and b, $19.1 \pm 3.52\%$ for 2019 and 2020, Figure 3) and on average higher on calcareous than on siliceous soil ($p = 0.012$; $42 \pm 4.82\%$ and $35.2 \pm 4.37\%$ respectively). There was also a significant Origin \times Soil type interaction, indicating that species from calcareous origin survived better on calcareous soil, whereas there was a tendency for species from siliceous origin to survive better on siliceous soil ($p = 0.032$; calcareous soils: a, $51.8 \pm 6.51\%$ for calcareous origin, and b, $32.1 \pm 6.76\%$ for siliceous origin, siliceous soils: b, $27.9 \pm 5.98\%$ for calcareous origin and ab, $42.4 \pm 6.20\%$ for siliceous origin). Additionally, the Origin \times Year quality interaction was also significant, because survival was much higher for calcareous species than siliceous ones during dry years, whereas there was no difference due to species origin during wet years ($p < 0.01$; wet years: ab, $49.8 \pm 6.86\%$ for calcareous origin and a, $66.3 \pm 4.76\%$ for siliceous origin, dry years: b, $30 \pm 5.83\%$ for calcareous origin and c, $8.25 \pm 2.96\%$ for siliceous origin). Survival for species from the two origins was reduced on both soils during dry years, resulting in no significant Origin \times Soil type \times Year quality interaction (Table 1). However, planned comparisons revealed that survival was equally high for the two origins during wet years on calcareous soil and equally low on siliceous soil during dry years ($p > 0.05$), whereas survival on siliceous soil was greater for

species from siliceous origin during wet years ($p < 0.001$), and only the species from calcareous origin survived on calcareous soil during dry years ($p < 0.001$).

3.3 | Intensity and importance of competition

RII survival results showed a significant Origin \times Year quality interaction, indicating that competition was always intense for species from calcareous origin, whereas competition was intense only during wet years for species from siliceous origin ($p = 0.010$, Table 2; wet years: ab, -0.19 ± 0.09 for calcareous origin and ab, -0.29 ± 0.08 for siliceous origin, dry years: a, -0.34 ± 0.1 for calcareous origin and b, -0.01 ± 0.07 for siliceous origin, Figure 4a). The effect of soil type was also marginally significant ($p = 0.058$) indicating a tendency for more intense competition on siliceous soil (RII: -0.29 ± 0.06 vs. -0.13 ± 0.05) in agreement with the results from one-sample *t*-tests (Figure 4a).

Results of Imp survival also showed a significant Origin \times Year quality interaction, indicating that competition was always important for the species from calcareous origin, but important only during wet years for species from siliceous origin ($p = 0.011$, Table 2, wet years: ab, -0.21 ± 0.09 for calcareous origin and ab, -0.26 ± 0.09 for siliceous origin, dry years: a, -0.31 ± 0.06 for calcareous origin and b, 0.01 ± 0.04 for siliceous origin, Figure 4b). In addition, there was a significant Soil type \times Year quality interaction, indicating that competition was more important on siliceous than on calcareous soils during wet years, whereas competition was as important on both soils during dry years ($p = 0.025$; Table 2; Figure 4b, wet years: a, -0.09 ± 0.105 for calcareous soil and b, -0.37 ± 0.07 for siliceous soil, dry years: ab, -0.17 ± 0.06 for calcareous soil and ab, -0.12 ± 0.06 for siliceous soil). The results of one-sample *t*-tests were consistent with these two two-way interactions, showing that the importance of competition was always significant for species for calcareous origin, except the wet years on calcareous soils, and significant for siliceous species only the wet years on siliceous soils.

TABLE 1 Results of generalized linear mixed-effects models (GLMM) for the effects of the Soil type, Origin, Year quality treatments and their interactions on survival without neighbours. Significant ($p < 0.05$) effects are indicated in bold

Effects	df	Survival	
		X^2	<i>p</i>
Origin	1	1.77	0.182
Soil type	1	6.27	0.012
Year quality	1	23.03	<0.001
Origin \times Soil type	1	4.56	0.032
Origin \times Year quality	1	7.27	0.007
Soil type \times Year quality	1	0.43	0.507
Origin \times Soil type \times Year quality	1	0.01	0.911

4 | DISCUSSION

The strong inter-annual variability of meteorological conditions during this study revealed important differences in stress tolerance and competitive response between both species types. Consistent with our first hypothesis, calcareous species were much more tolerant to dry climatic years than siliceous species, but suffered the most from competition. Consistent with our second hypothesis, competition on siliceous soils was stronger than that on calcareous soils, highlighting differences in competitive effects of the two communities. However, competitive effects of neighbours appeared to be less important than target species tolerance to stress for explaining differences in species distribution on both soil types. Overall, our results support the physical hypothesis, and highlight in particular

FIGURE 3 Survival (mean \pm SE, $n = 16$) of the species of the two origins during dry and wet years on calcareous and siliceous soils. Black and grey bars represent species from calcareous and siliceous origin respectively

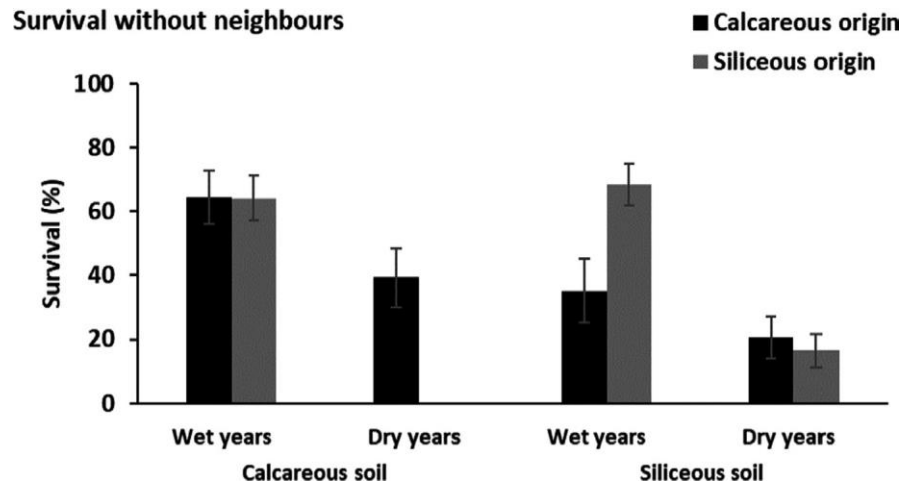


TABLE 2 Results of linear mixed-effects models for the effects of the Soil type, Origin, Year quality treatments and their interactions on RII and Iimp. Significant ($p < 0.05$) effects are indicated in bold and marginally significant are indicated in italic

Effects	df	RII		Iimp	
		F	p	F	p
Origin	1	1.77	0.185	3.01	<i>0.085</i>
Soil type	1	3.66	<i>0.058</i>	2.52	0.114
Year quality	1	0.29	0.593	0.71	0.413
Origin \times Soil type	1	0.46	0.498	0.44	0.505
Origin \times Year quality	1	6.81	0.010	6.57	0.011
Soil type \times Year quality	1	1.24	0.266	5.12	0.025
Origin \times Soil type \times Year quality	1	0.13	0.714	0.04	0.841
Residual error	120				

the importance of species drought tolerance for explaining the difference in species composition and diversity between calcareous and siliceous soils.

4.1 | Species with contrasting stress tolerance and response to competition respond to variations in environmental conditions differently

Several of our results support the trade-off for stress tolerance and response to competition between calcareous and siliceous species. First, unlike calcareous species, siliceous species were prone to year-to-year fluctuations, as their survival anomalies shifted from significantly positive during wet years (higher than the average survival) to significantly negative during dry years (2019 and 2020). Second, calcareous species had higher survival than siliceous species during dry years. These findings indicate that calcareous species were better able to cope with stress when environmental conditions became severe. The greater stress tolerance ability found in calcareous species is consistent with the physical hypothesis and the overall lower water availability of calcareous soils compared to siliceous soils (Michalet et al., 2002; Rolland et al., 1998; Thurmman, 1849). Consistently, Contran et al. (2013) and Hu et al. (2013) showed that oak species/populations from calcareous bedrocks were more adapted to drought stress than oak species/populations from

siliceous soils. Third, the higher stress tolerance abilities of the calcareous species that were used in our study appeared to decrease their capacity to cope with competition, confirming the occurrence of a trade-off. Calcareous species suffered from intense and important competition during wet and dry years, whereas for siliceous species, competition was intense and important during wet years only. This trade-off between stress tolerance and response to competition has been reported and recognized in a large number of studies (e.g. Forey et al., 2010; Liancourt, Callaway, et al., 2005; Lind et al., 2013; Qi et al., 2018; Suding et al., 2003).

4.2 | The relationship between community competitive effects and substrate type

In accordance with our second hypothesis, competition by neighbouring plants was both more intense and more important on siliceous soils than that on calcareous soils during the wet years. A possible explanation would be a greater availability of water on siliceous substrate than on calcareous substrate (Gigon, 1987; Thurmman, 1849), inducing a higher competition on siliceous soils, the least stressful habitat (Grime, 1973, 1974). This is also consistent with the study by Michalet et al. (2002) showing a higher biomass and lower species richness found on siliceous bedrocks. Similarly, Nicklas et al. (2021) have observed in the Alps, on siliceous bedrocks

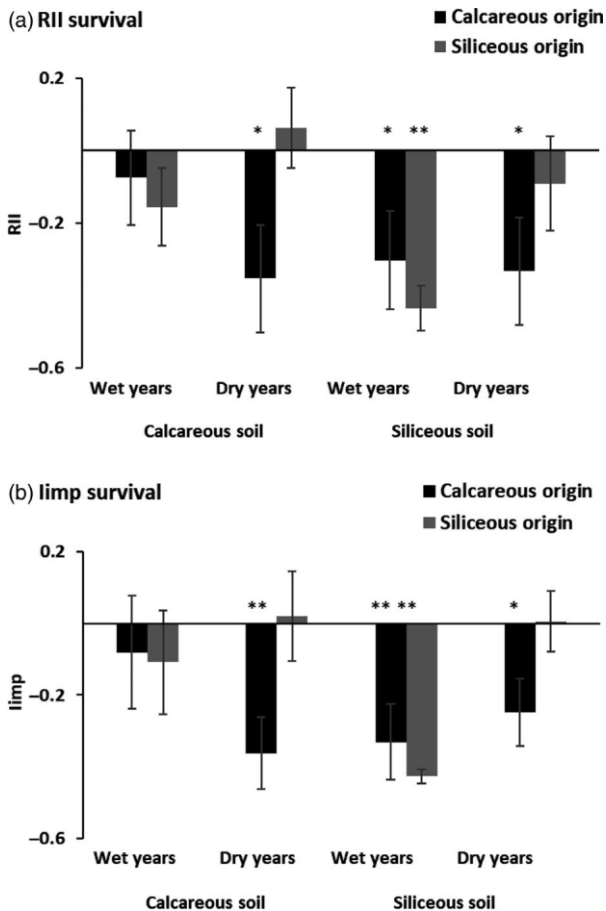


FIGURE 4 Means (\pm SE, $n = 16$) of Relative Interaction Index (RII survival) (a) and Competition importance index (Iimp survival) (b) for the species from the two origins on calcareous and siliceous soils during wet and dry years. Black and grey bars represent species from calcareous and siliceous origin respectively. Results of one-sample t -tests are shown below bars when significant. (*), $p < 0.1$; *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$

only, an increase in plant richness, related to a decrease in vegetation cover with increasing drought stress with climate change, suggesting a higher importance of competition on siliceous than on calcareous substrate. Strong competition on acidic siliceous soil has also been regularly reported in studies conducted in European heathlands (Aerts et al., 1991; Delerue et al., 2018).

Interestingly, Grime (1979) proposed two humped back model (HBM) of biomass/species richness relationships, one for calcareous regions with higher species richness and the other for siliceous regions with lower species richness, arguing that differences in species richness between both community types should be explained by differences in species pools related to different evolutionary history. Thus, he was considering himself that his CSR strategy model could not explain differences in species richness between the two community types. Nicklas et al. (2021) also proposed two separate HBMs for each substrate type. Instead, Michalet et al. (2002) proposed only one HBM merging the two community types, with calcareous communities on the stressful side and siliceous communities on the high-biomass side of the unimodal curve. Our results provide

additional support to this more synthetic view, with stress tolerance and competition being the two main drivers of differences in diversity between the two community types.

4.3 | The relative contribution of stress tolerance and community competitive effects in the physical hypothesis

Both higher stress tolerance of calcareous species and higher competitive effects in siliceous communities were observed in our experiment, but their relative importance was not equal. Stressful conditions on calcareous soils during the dry years lead to the almost complete exclusion of siliceous species only. Additionally, calcareous species during dry years suffered more from competition by neighbours than siliceous species as shown by RII and Imp results. However, competition never led to their complete exclusion from siliceous communities, unlike the effect of stress on siliceous species on calcareous soils. Thus, in our study, stress tolerance ability was more important than competitive effect for explaining contrasts in species composition between the two soil types. To our knowledge, no study before has attempted to disentangle the relative importance of the response to stress and the effect of competition in driving differences in species composition between calcareous and siliceous plant communities. Finally, our results support previous works that stressed the importance to distinguish competitive responses and effects, the latter being more important for understanding the patterns of composition and diversity in plant communities (Goldberg, 1990; Liancourt, Corcket, et al., 2005; Liancourt & Tielbörger, 2009).

Finally, concerning the two alternative hypotheses explaining differences in species composition and richness between communities from both bedrock types, the chemical hypothesis (greater availability of nutrients found on calcareous soils compared to siliceous soils) should have led to a more important competitive exclusion on calcareous soils (Grime, 1973, 1977) and a higher plant diversity on siliceous soils. This is in contradiction with the well-known higher diversity of calcareous grasslands (Michalet et al., 2002; Nicklas et al., 2021; Thurmann, 1849). Additionally, there should be reciprocal toxicity between the two soils and the two types of species. If the chemical hypothesis was true, more plants from siliceous communities would have died on calcareous soils (with and without neighbours), and more plants from calcareous communities would have died on siliceous soils (with and without neighbours). Thus, our results provide strong additional support to the physical hypothesis.

5 | CONCLUSIONS

By assessing the relative importance of stress tolerance and response to competition of calcareous and siliceous dominant species on the two types of soils during 4 years with contrasting climate conditions, our study clearly showed a trade-off between these two components.

However, stress tolerance had a greater effect than competitive effect of neighbours in the ability of the different species to develop in both communities. Additionally, our results add support to the physical hypothesis of the ecological determinism of difference in species composition and diversity between calcareous and siliceous soils. Our results have also important implications for studies forecasting how environmental factors influence plant species distributions in the context of climate change. They suggest that future modelling approaches focusing on plant responses to climate change should consider ecological interactions between biotic interactions and physical abiotic factors related to substrate types in order to improve their predictions.

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CONFLICT OF INTEREST

None of the authors have a conflict of interest.

AUTHORS' CONTRIBUTIONS

R.M. conceived the design and made field experiments during the 4 years; D.N. made field experiments during the fourth year and conducted statistical analyses with P.L. and H.R.; D.N. and P.L. wrote the first draft of the manuscript and F.D., P.L., H.R. and R.M. contributed critically to the writing.

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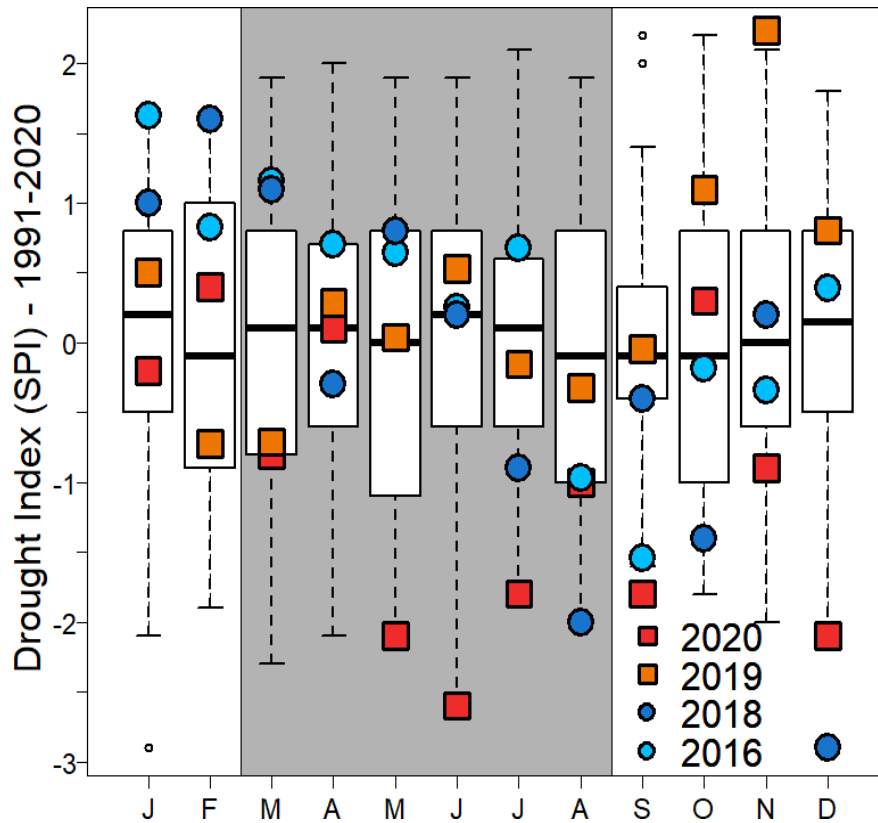
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SUPPORTING INFORMATION

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Supporting information



Appendix S1. Boxplot for monthly Drought index based on precipitation (SPI) for the period 1991 – 2020 in the central location of the study area (44.72N, -0.41W). As SPEI, SPI values indicate how much dry (negative value) or wet (positive value) a month is compared to the same month during a reference period (1961-1990 on the ClimateDT Tools) considering only precipitations. Data for the years of the study are shown (2016: light blue circles; 2018: dark blue circles; 2019: orange squares; 2020: red squares). Grey area from March to August indicate monitoring period (from initial plant transplantation to final harvest).

CHAPTER 4

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

This chapter was the subject of an article published in *Oikos*, in which we present the results of a field reciprocal transplant experiment along a pollution gradient in the French Pyrenees. Our goal in performing this experiment was to evaluate the following specific points: (i) the role of ecotypic variation on plant-plant interactions of two ecotypes of two species (*Armeria muelleri* the most metal-tolerant species and *Agrostis capillaris* the least metal-tolerant species in our experiment) occurring at different positions along a pollution gradient; (ii) the existence of a trade-off between facilitative responses and stress tolerance at the intra- and intra-specific level; (iii) the respective contribution of environmental-severity effects and neighbour-trait effects in determining the outcome of plant responses along pollution gradients.

Main results:

We showed that plant-plant interaction outcomes were not the same for *Armeria muelleri* the most stress-tolerant species and *Agrostis capillaris* the least stress-tolerant species. For *Armeria muelleri*, plant-plant interactions did not vary significantly along the metal pollution gradient. However, for *Agrostis capillaris*, plant-plant interactions shifted from negative to positive with increasing metallic stress consistent with the stress gradient hypothesis (SGH). In addition, at the intraspecific level, *Armeria muelleri* ecotype from highly polluted habitats showed a slight tendency to be more vulnerable to competition than the ecotype from less polluted habitats, whereas *Agrostis capillaris* ecotype from unpolluted habitats was more facilitated than the ecotype from polluted habitats at high pollution levels. These results confirmed the existence of a trade-off between stress tolerance and plant facilitative response abilities.

Furthermore, consistent with Michalet et al. (2014b) we showed that the increase in facilitation with increasing metal stress for *Agrostis capillaris* ecotype from unpolluted habitats was primarily due to an

environmental-severity effect, whereas the increase in competition with decreasing metal stress for polluted *Agrostis capillaris* from polluted habitats was primarily due to a neighbour-trait effect. Overall these results, showed that stress-intolerant species and ecotypes were more prone to environmental stress, the latter being the main factor explaining their higher facilitative responses in stressed habitats.

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Research

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



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 metallophilous 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 metallophilous 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 intra-specific 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

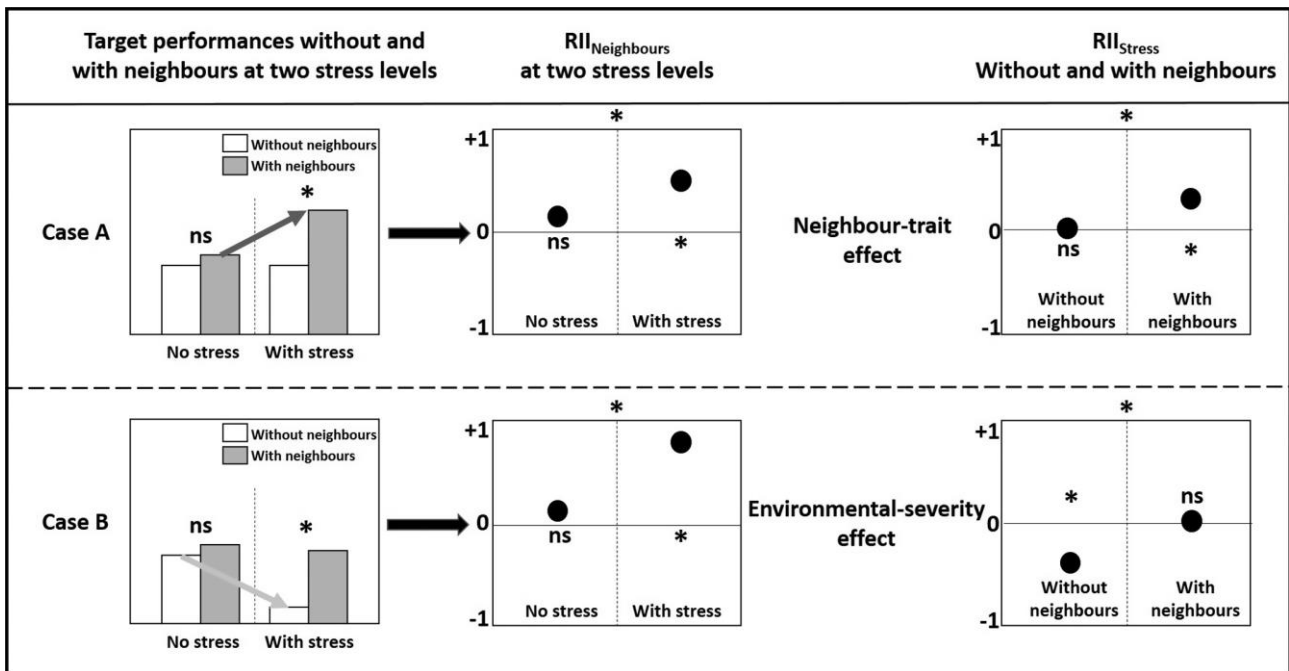


Figure 1. Changes in $RII_{Neighbours}$ along a stress gradient (increases in facilitation with increasing stress) either due to a neighbour-trait (case A) or an environmental-severity (case B) effect. In case A, the increase of $RII_{Neighbours}$ with stress is due to an improvement of the target performance 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 $RII_{Neighbours}$ 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). RII_{Stress} indicates the effect of stress on target plant performance (P) when grown with and without neighbours, with values below 0 indicating better target species performance with no stress ($P_{+stress} < P_{-stress}$), and values above 0 indicating better target species performance with stress ($P_{+stress} > P_{-stress}$).

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.

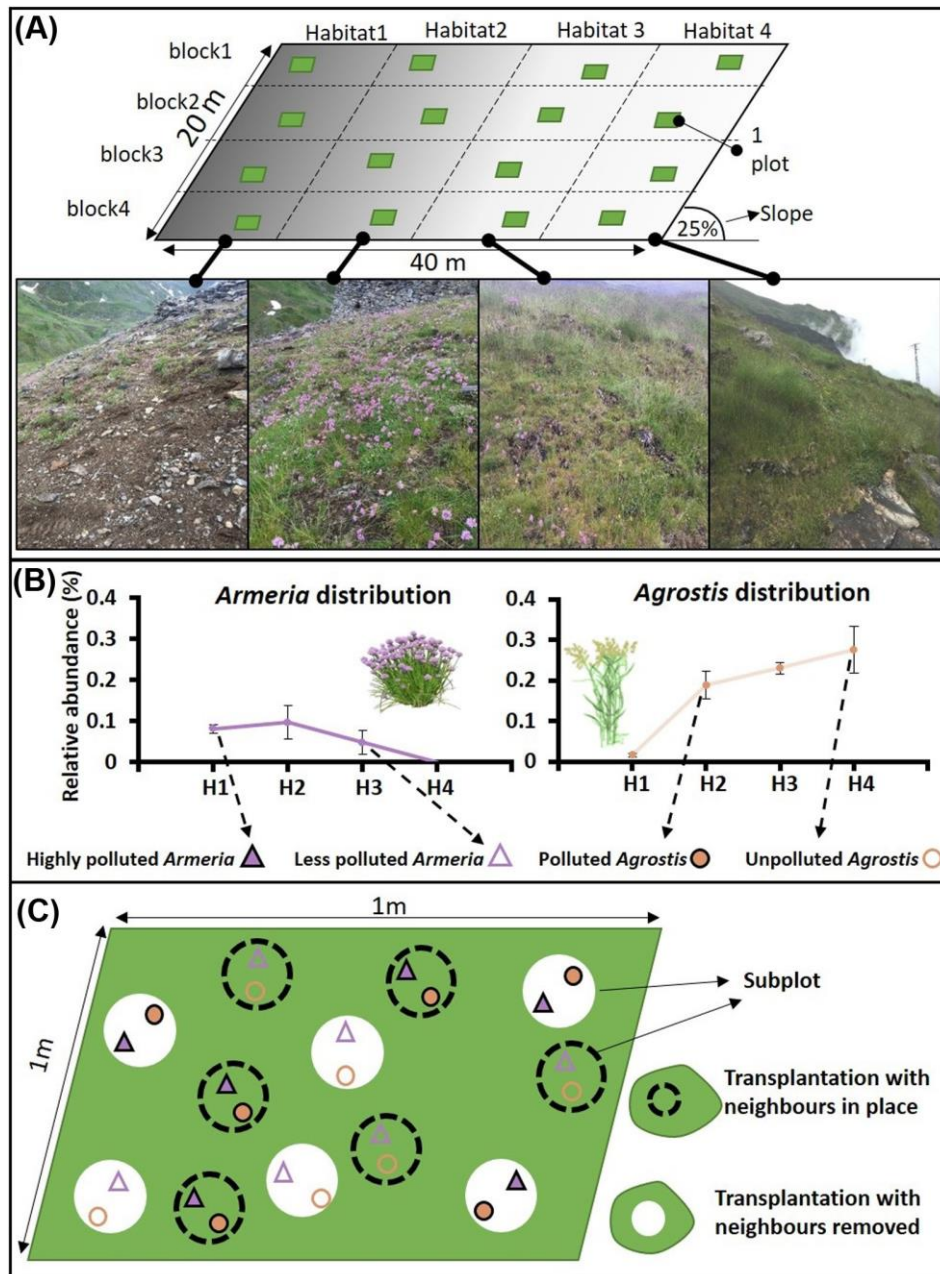


Figure 2. Presentation of the experimental design. (A) Schematic representation of the experimental sites and pictures of the four habitats showing the variation of plant communities along the metal pollution gradient studied. The pollution is indicated by the grey color gradient on the scheme. (B) Relative abundance of *Armeria muelleri* 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 where *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 m² 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 follows. 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:

$$RII_{\text{neighbour}} = \frac{P_{+\text{neighbour}} - nP_{-\text{neighbour}}}{P_{+\text{neighbour}} + P_{-\text{neighbour}}} \quad (1)$$

where $P_{+\text{neighbour}}$ and $P_{-\text{neighbour}}$ 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 $RII_{\text{neighbours}}$ (Eq. 1), we computed the following relative interaction index following Michalet et al. (2014b).

$$RII_{\text{stress}} = \frac{P_{+\text{stress}} - sP_{-\text{stress}}}{P_{+\text{stress}} + P_{-\text{stress}}} \quad (2)$$

where $P_{+\text{stress}}$ is the target response (proportion of transplants that survived in the plot) with metallic stress (in habitat 1) and $P_{-\text{stress}}$ is the target response without stress (in habitat 4). Two RII_{stress} were calculated independently, one for target species and ecotypes with neighbours, and one for target species and ecotypes without neighbours. RII_{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 ($P_{+\text{stress}} < P_{-\text{stress}}$), and values above 0 indicating better target species performance when metallic stress is present ($P_{+\text{stress}} > P_{-\text{stress}}$). The same $RII_{\text{neighbours}}$ 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, $RII_{\text{neighbours}}$ for target species and ecotypes under the highest stress level (in habitat 1), and $RII_{\text{neighbours}}$ for target species and ecotypes under no-stress level (in habitat 4). If $RII_{\text{neighbours}}$ differ significantly between no-stress and stress levels, then RII_{stress} can be used to attribute the differences to: 1) a neighbour-trait effect when RII_{stress} with neighbours is significantly different from 0 (Fig. 1, case A); 2) a significant environmental-severity effect when RII_{stress} without neighbours is significantly different from 0 (Fig. 1, case B) and 3) a combined environmental-severity and neighbour-trait effect if RII_{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 \times 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).

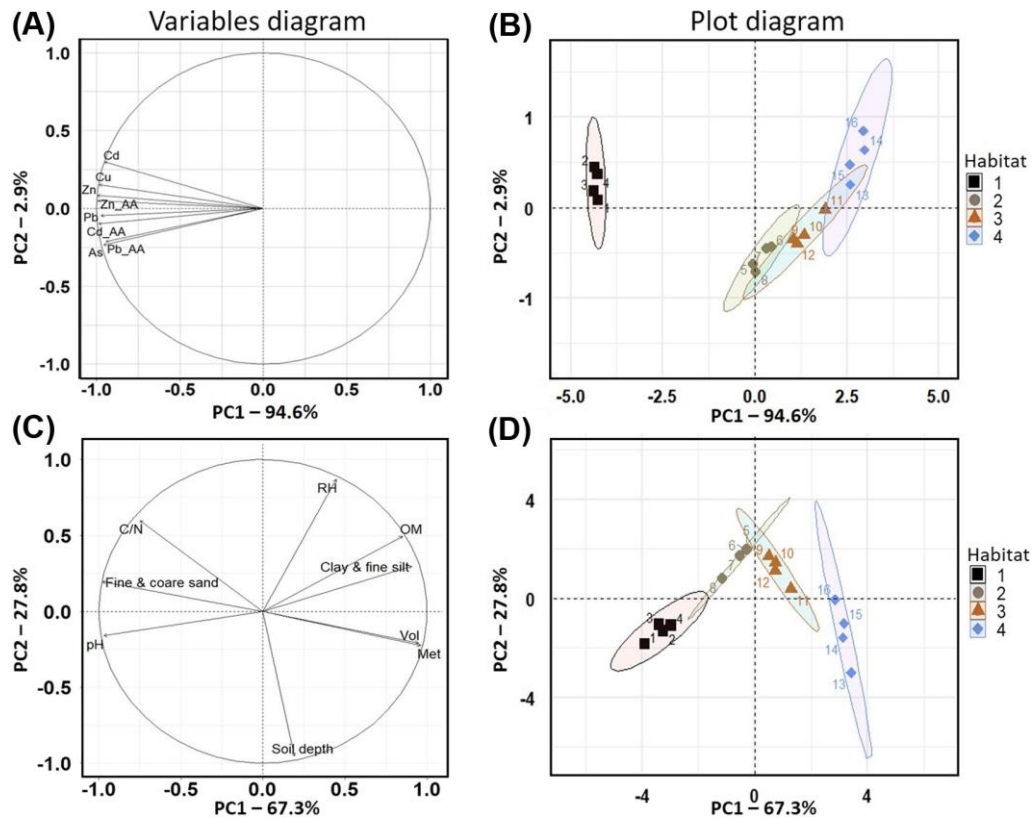


Figure 3. Principal component analyses (PCA) displaying the variability of soil metals variables and other soil variables between the four habitats along the studied gradient. (A) Variables factor map for soil metals concentration. Cd_AA: cadmium extracted with acetic acid; Pb_AA: lead extracted with 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

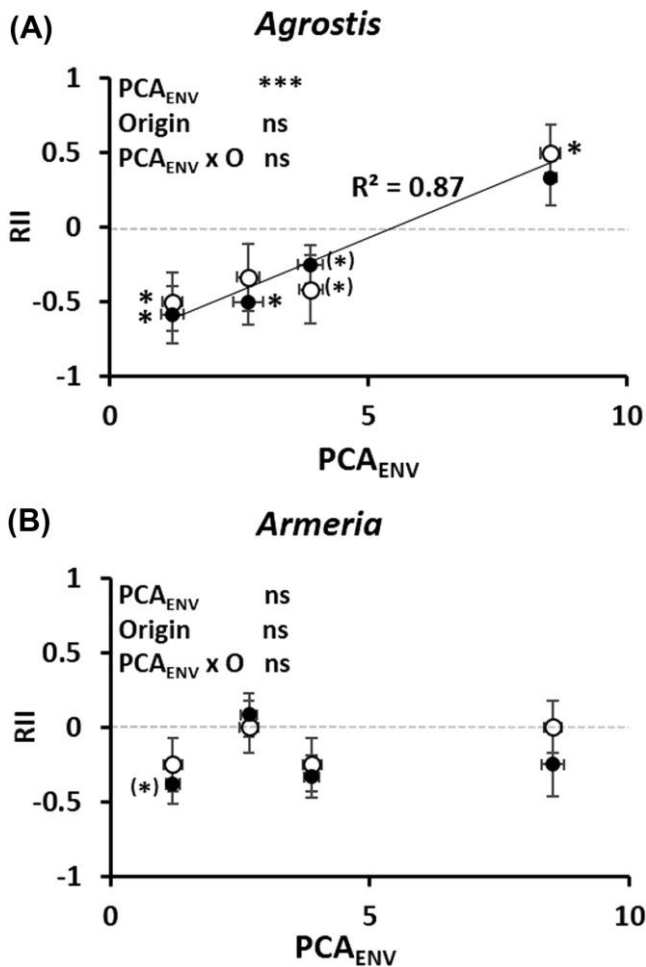


Figure 4. (A, B) Variation in $RII_{neighbours}$ (mean per habitat \pm SE) of *Agrostis* and *Armeria* along the metal pollution gradient. The metal pollution gradient is characterized by the PCA_{ENV} metric. In order to have increasing values of PCA_{ENV} indicating an increasing level of pollution, 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 sub-alpine 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.

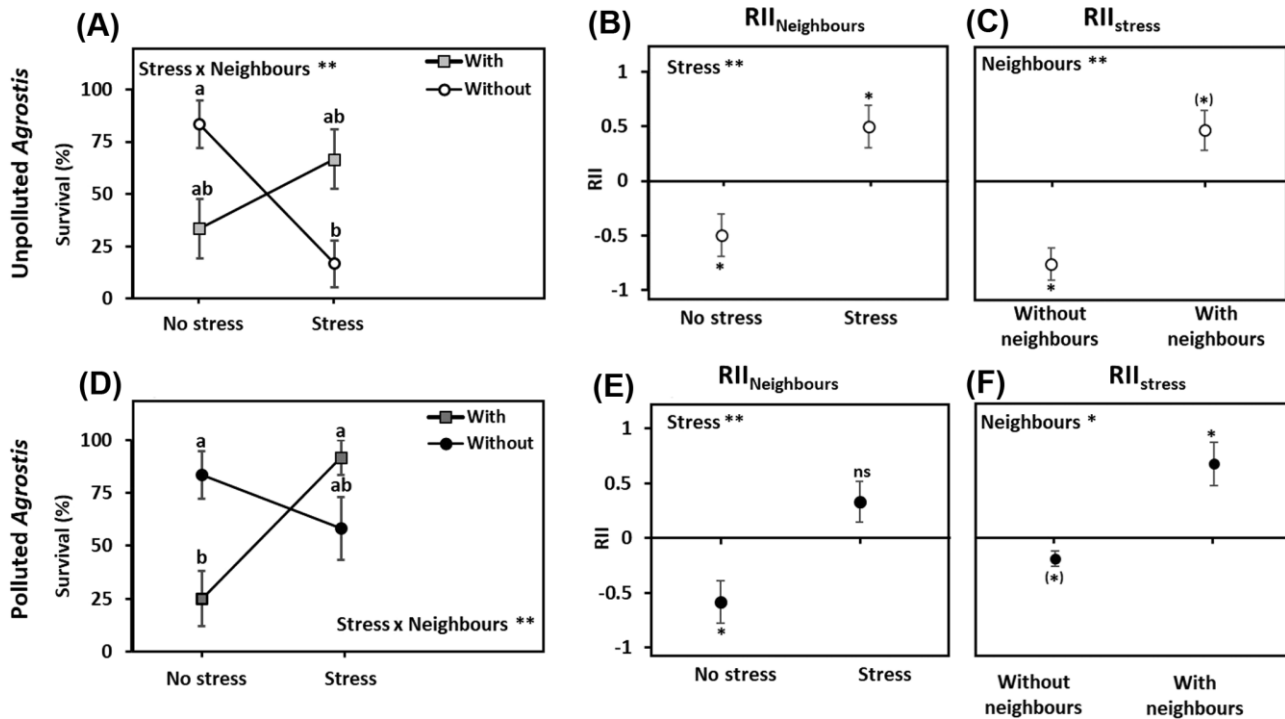


Figure 5. Survival of *Agrostis* ecotypes and corresponding RII at two contrasted stress levels and with and without neighbours. (A, D) Survival (mean \pm SE) of unpolluted ecotype (A) and polluted ecotype (D) at the two levels of stress. On the top left (A) and bottom right (D) corners, results of general linear mixed-effects model for the Stress \times Neighbours interaction on survival are shown. Different letters show significant difference between the corresponding situation (posthoc multiple Tukey comparisons). (B, C, E, F) RII_{neighbours} (mean \pm SE) for unpolluted (B) and polluted (E) ecotypes and RII_{stress} (mean \pm 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. Additionally, 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.8kpr4xrd>> (Nemer et al. 2022).

Supporting information

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

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Supporting information

Variables	unit	Habitats								P-value	
		1		2		3		4			
Metals	Cd _{TOT}	mg.kg ⁻¹	191.2 ± 16.9	a	13.6 ± 0.2	b	11.4 ± 0.4	b	11.1 ± 0.3	b	<0.001
	Pb _{TOT}	mg.kg ⁻¹	35000 ± 766.7	a	7300 ± 1394.7	b	3700 ± 431.1	bc	2000 ± 462.4	c	<0.001
	Zn _{TOT}	mg.kg ⁻¹	156000 ± 8125.8	a	6700 ± 808.2	b	3300 ± 254.1	b	1600 ± 156.2	b	<0.001
	Cu _{TOT}	mg.kg ⁻¹	240.9 ± 8.2	a	34.0 ± 1.9	b	19.7 ± 1.4	b	17.8 ± 1.04	b	<0.001
	As _{TOT}	mg.kg ⁻¹	1200 ± 39.5	a	406.0 ± 73.04	b	216.6 ± 35.4	bc	91.1 ± 13.8	bc	<0.001
	Cd _{AA}	mg.kg ⁻¹	34.5 ± 1.3	a	7.8 ± 0.8	b	5.5 ± 0.3	bc	2.4 ± 0.1	c	<0.001
	Pb _{AA}	mg.kg ⁻¹	701.5 ± 30	a	96.3 ± 30.8	b	39.4 ± 2.8	b	14.8 ± 8.5	b	<0.001
	Zn _{AA}	mg.kg ⁻¹	2900 ± 128.8	a	197.2 ± 39.7	b	119.1 ± 6.6	b	54.4 ± 2.4	b	<0.001
	PCA _{Met}		-4.46 ± 0.03	a	0.19 ± 0.12	b	1.40 ± 0.20	c	2.87 ± 0.11	d	<0.001
Environmental	Clay and fine silt	g.kg ⁻¹	150.7 ± 1.0	c	172.1 ± 3.1	b	171.04 ± 2.0	b	228.4 ± 2.5	a	<0.001
	Fine and coarse sand	g.kg ⁻¹	718.9 ± 7.3	a	621.8 ± 7.5	b	604.5 ± 1.8	b	559.3 ± 2.0	c	<0.001
	Organic matter	g.kg ⁻¹	65.9 ± 0.4	c	68.2 ± 3.6	bc	78.2 ± 2.2	b	109.5 ± 3.2	a	<0.001
	Soil depth	cm	20.9 ± 1.4	b	35 ± 0	a	31.9 ± 0.9	a	24.6 ± 2.9	b	<0.001
	Relative humidity	%	19.3 ± 0.1	ab	17.6 ± 0.2	b	18.5 ± 0.5	b	21.1 ± 0.8	a	<0.01
	Vegetation volume	dm ³	33.78 ± 3.9	c	70.34 ± 9.2	b	92.62 ± 3.3	ab	108.01 ± 3.1	a	<0.001
	pH _{H₂O}		7.3 ± 0.01	a	6.9 ± 0.02	b	6.3 ± 0.1	c	5.7 ± 0.1	d	<0.001
	C/N		13.3 ± 0.2	a	11.6 ± 0.3	b	11.1 ± 0.05	b	11.7 ± 0.1	b	<0.001
	PCA _{Env}		-3.51 ± 0.21	a	-0.58 ± 0.21	b	0.83 ± 0.17	c	3.25 ± 0.12	d	<0.001

Table S1. Soil variables in the four habitats (mean ± SE) and results of the one-way ANOVA on the effect of habitat. Different lowercase letters indicate significant difference for the considered variable between habitats (posthoc Tukey pairwise comparisons). PCA_{Met} and PCA_{Env} are synthetic variables that represent the soil pollution metric alone and all other environmental variables, respectively (see the methods section).

CHAPTER 5

The role of ecotypic variation for plant facilitation in a metal-polluted system: stress-tolerant nurse ecotypes are the best benefactors and stress-intolerant target ecotypes the best beneficiaries

This chapter is the subject of a research article submitted to the Journal “Science of The Total Environment”, in which we present the results of two experiments conducted in metalliferous habitats in the French Pyrenees. In a first field reciprocal transplant experiment, we investigated the response of two *Festuca rubra* ecotypes with different metal-stress tolerance along metal-stress gradients. In a second experiment, we assessed the facilitative effects of two ecotypes of four distinct metallicolous nurse species on the grass *Agrostis capillaris* in a highly polluted common garden. Our main objective was to assess whether there is a trade-off between stress tolerance and plant facilitative response (first experiment) and effect (second experiment) abilities.

Main results:

Our reciprocal transplantation experiment revealed that the outcome of plant-plant interactions for *Festuca rubra* from unpolluted habitats shifted from negative to positive when grown in polluted habitats, which is consistent with the Stress-Gradient Hypothesis (SGH). However, *Festuca rubra* ecotype from polluted habitat did not show any facilitative response when grown in polluted habitats, and did not experience more competitive exclusion by neighbours than the unpolluted ecotype when grown in unpolluted habitats. Our common-garden results showed that nurse ecotypes from highly polluted habitats had higher facilitative effects on target species than ecotypes from less polluted habitats. Overall, our results suggest that stress-intolerant ecotypes were the most sensitive to facilitation and thus are the best beneficiaries (results of first experiment) and stress-tolerant nurse ecotypes generated the highest facilitative effects and

were thus the best benefactors (results of the second experiment).

Finally, with respect to the objective of this study, we showed that a trade-off between stress tolerance and plant facilitative-response ability exist, as has been previously shown in the literature (Choler et al. 2001; Liancourt et al. 2005; Forey et al. 2010; Gross et al. 2010). However, we did not observe a trade-off between stress tolerance and plant, facilitative-effect ability, but instead, the facilitative-effect ability was positively correlated to the stress-tolerance of nurse plants.

Research article

The role of ecotypic variation for plant facilitation in a metal-polluted system: stress-intolerant target ecotypes are the best beneficiaries and stress-tolerant nurse ecotypes the best benefactors

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ABSTRACT

- Disentangling competitive-response and -effect abilities has strongly improved our understanding of the role of competition for the diversity and composition of plant communities. Much less is known about the relative importance of facilitative-effect and -response abilities in harsh ecosystems. Facilitation being the dominant plant-plant interactions in metal-stressed ecosystems, we aim to assess the facilitative-response and -effect abilities of different species and ecotypes in former mining sites in the French Pyrenees. This knowledge is crucial both for conceptual insights and for the use of facilitation for revegetation of metal-polluted ecosystems.
- In a first removal experiment, we studied the response of two ecotypes of *Festuca rubra* with contrasting metal-stress tolerance along metal-stress gradients. In a second experiment in a highly polluted common garden, we assessed the facilitative effects of two ecotypes with contrasting metal-stress tolerance of four different metallicolous nurse species on the grass *Agrostis capillaris*.
- In the first experiment, the response of the *Festuca* ecotype with lower metal-stress tolerance shifted from negative to positive as pollution increased, consistently with the stress-gradient-hypothesis. The ecotype with high metal-stress tolerance did not show any facilitative response. In the common garden, nurse ecotypes from highly polluted habitats had higher facilitative effects than ecotypes from less polluted habitats.
- Synthesis: Stress-intolerant ecotypes were the most sensitive to the positive effects of neighbouring nurses, while stress-tolerant nurse ecotypes were the best benefactors. Facilitative-response ability appeared to be driven by a trade-off between stress-tolerance and facilitative response of target ecotypes. In contrast, facilitative-effect ability was positively correlated to the stress-tolerance of nurse plants.

KEYWORDS: Facilitative effects, Facilitative responses, Ecotypes, Stress-tolerance, Heavy metals, Ecological restoration, Stress Gradient Hypothesis

1 | INTRODUCTION

Plant-plant interactions are important drivers of plant community composition and diversity (Grime, 1979; Bruno et al., 2003). In conditions of low stress and disturbance, communities have high biomass and species diversity is low due to the dominance of competitive species (Grime, 1979). In such condition, the distinction of competitive effects from competitive responses has strongly improved our understanding of plant-plant interactions in the corresponding communities (Goldberg & Landa, 1991). Competitive effects refer to the capacity of one plant to inhibit the performance of another, while competitive responses refer to the capacity of a species to tolerate the negative effects of a competitor. Michalet et al. (2022) have recently shown that the composition and diversity of herbaceous communities was more related to community biomass, the size of neighbours and their competitive effects, than to ecosystem productivity which was more related to competitive responses. When stress or disturbance increase competition decreases and is replaced by facilitation following the SGH. Consistently with the distinction between competitive effects and responses, facilitative effects and facilitative responses can be distinguished (Zhang & Tielbörger, 2019). Facilitative effects refer to the capacity of one plant (the benefactor or nurse plant) to improve the performance of a beneficiary plant (or the target plant) by mitigation of harsh conditions in its immediate vicinity, whereas facilitative responses refer to the potential of a plant to benefit from this stress mitigation (Zhang & Tielbörger, 2019; Delerue & Michalet, 2022).

Facilitative responses have been shown to be related to the trade-off between response to stress (i.e., stress tolerance) and competitive-response ability (Liancourt et al., 2005). Indeed, species from benign environments, with low stress tolerance but high competitive-response ability, benefit more from facilitation than species from harsh environments having high stress tolerance (Liancourt et al., 2005; Gross et al., 2010; Nemer et al., 2021). Indeed, the former stress-intolerant species depend more on stress amelioration by nurse plants, i.e., they show higher facilitative responses (Liancourt et al., 2005; Qi et al., 2018). In contrast, the latter stress-tolerant species are more negatively affected by competition in less severe environments, due to their low competitive-response ability (Nemer et al., 2021). This trade-off between stress tolerance and

competitive ability occurs not only at the species level but also at the intraspecific level (Michalet et al., 2011; Al Hayek et al., 2014; Nemer et al. 2022) because different populations may be more or less adapted to environmental severity. For example, Liancourt and Tielbörger (2011) found that, when transplanted in a dry environment, an ecotype of the annual grass *Brachypodium distachyon* from less severe environments, was very sensitive to facilitation since it could only survive under shrub canopies, whereas the ecotype from the dry environment was able to survive outside shrubs. In sum, ecotypes that thrive in harsh environments require less stress alleviation by nurse plants (sensu Liancourt et al., 2005) and are expected to have a lower facilitative response. Finally, most studies investigating the role of ecotypic differentiation in the context of facilitation between plants have focused either on the response of the beneficiaries (e.g. Liancourt & Tielbörger, 2011; Nemer et al., 2022), or on the effect of the benefactors alone (Crutsinger et al., 2010; Schöb et al., 2013). Note however, that such simultaneous investigation of effects and responses was performed by Al Hayek et al. (2015a) and shed light on the role of ecotypic differentiation in driving both effects and responses involved in plant-plant interactions.

Metalliferous ecosystems in general, including mine tailings, are often described as harsh environments for plants growth. This is due to the ecotoxic effect of metal/metalloids (hereafter referred to as “metals”) exposure on plant physiology and other diverse constraints for plant development. As a result, plant communities on mine tailings are characterized by a low diversity and a degraded vegetation with reduced plant cover. Restoration of these degraded habitats is frequently attempted in order to limit the dispersion of contaminated material in the environment including the food web and drinking water resources. However, effective restoration remains a challenge because of the presence of phytotoxic substrates. In metalliferous systems, several metallicolous species have the capacity to cope with high metal concentration in soils through various adaptations (Van der Ent et al., 2013). Such metallicolous species can promote the establishment and the growth of less metal-tolerant species by alleviating the toxic effects of metals (Frérot et al., 2006) and/or other constraints for plant growth in such degraded habitat (like low soil fertility or climatic stress; Navarro-Cano, 2019a). Consistently, most studies showed that facilitation is the dominant

plant-plant interaction in such systems (e.g. Frérot et al., 2006; Eränen & Kozlov, 2007; Yang et al., 2015). All these studies only investigated plant-plant interactions at the interspecific level. However, investigation of both effect and response abilities, at both the inter- and intraspecific levels is crucial to have a complete understanding of plant-plant interactions in metal-rich environments.

Here, we perform this complete evaluation by evaluating plant effects and responses for different species and ecotypes on several mine tailing showing important stress for plant development. Because, metal tolerance can vary between populations of the same species (Taylor & Macnair, 2006; Ehlers et al., 2016), this ecotypic difference in stress-tolerance may rationally influence both the facilitative-response and facilitative-effect abilities. In a recent study along a metal-pollution gradient, Nemer et al. (2022) found that the ecotype of the grass *Agrostis capillaris* with the lowest metal-stress tolerance was the most facilitated at high pollution levels. In the same study, the most metal-stress-tolerant ecotype of *Armeria muelleri* was the most sensitive to competition in unpolluted habitats. Therefore, depending on the level of metal-tolerance and soil pollution, the response to plant-plant interactions for metal-tolerant and less-metal-tolerant ecotypes may change. However, if the facilitative response was investigated in this field study, variations in the facilitative effects of neighbouring nurse species were not assessed, neither at the intra- nor interspecific level. Indeed, such investigation can only be done through an experimental garden since it requires to grow different nurse ecotypes (found spontaneously at different positions along environmental gradients) in a single environment (Al Hayek et al., 2015a).

We set up two experiments at the mountain and subalpine belts in a former mining valley in the French Pyrenees. The first experiment was a field reciprocal transplant experiment carried along metal-pollution gradients located at the subalpine belt. Its main objective was to assess the variation in competitive- and facilitative-response abilities of different ecotypes of the common grass *Festuca rubra* (hereafter referred to as *Festuca*) assumed to have different metal-stress tolerance. This experiment was conducted on contrasting soil types on two rock types, calcareous and siliceous, to generalize the findings of Nemer et al. (2022) who conducted their pioneer experiment on only a calcareous substrate. We assumed that: i) first hypothesis:

Festuca ecotype from polluted habitats should experience less facilitation than *Festuca* ecotypes from unpolluted habitats when grown in polluted habitats; ii) second hypothesis: *Festuca* ecotype from polluted habitats should experience more competitive exclusion by neighbours than *Festuca* ecotype from non-polluted habitats when grown in unpolluted habitats.

A common garden was settled in a second experiment on a mine slag heap located at the mountain belt. Its aim was to assess the role of ecotypic variation in the facilitative-effect abilities of different metallicolous nurses. Two ecotypes of four nurse species were harvested at low and high pollution levels and transplanted in the common-garden. To estimate nurse effects, two ecotypes of the common grass *Agrostis capillaris* (hereafter referred to as *Agrotis*), assumed to have different metal-stress tolerance, were transplanted with and without nurses. We assumed that: i) third hypothesis: the four metal-tolerant nurse species are not necessarily functionally equivalent and, thus, should have different facilitative effects; ii) fourth hypothesis: ecotypes of all nurse species from highly polluted habitats are less strained *sensu* Gross et al. (2010) at this highly polluted site, and, thus, should have a greater ability to generate facilitative effects than ecotypes from less polluted habitats.

2 | MATERIALS AND METHODS

2.1 | General description of metalliferous sites in the region.

This study was carried in a French Pyrenean valley with several remarkable locations with important metal pollution due to former mining activities (consisting mainly of the toxic elements Zn, Pb, Cd, Cu, and As) (Supplementary Figure S1). In these locations, metal-pollution gradients display a gradual change in plant community composition (Figure S2). These gradients extend from mining residues close to former mining galleries or mining plants dominated by metal-tolerant species (such as *Armeria muelleri*, *Minuartia verna*, *Arenaria multicaulis*, *Hutchinsia alpina* and *Gypsophila repens*) to traditional mountainous or subalpine plant community. Only the aforementioned metalliferous species are found on the most polluted soils whereas *Festuca gautieri*, *Festuca Eskia*, *Festuca rubra*, *Deschampsia flexuosa* and *Agrostis capillaris* are

the grass species predominant on the non-polluted soils. The plant communities in moderately polluted soils have an intermediate composition, with metalicolous species predominating closest to the source of the pollution and grass species predominating farthest from the source. This leads to the establishment of four distinct habitats (H1 to H4, from most to least polluted), each distinguished by a particular species composition (Figure S2).

2.2. Experimental design for the reciprocal transplant experiment.

2.2.1 Site description and pollution gradients involved.

The first experiment was conducted at approximately 2000m altitude at the subalpine belt in a large area of 60 ha including several mining galleries (42°49'30.3 "N, 0°53'53.3 "E, Supplementary Figure S1). The growing season starts in early June and lasts until late September at this altitude. A weather station (Delta-T Device® DLT/WGS-GPA) was installed at the study site in 2021 (in location 4, Figure S1). Average temperature during the growing season in 2021 was 11.9°C and precipitation 237.8 mm. Locations 2, 3 and 4 (Figure S1) form the three blocks of this experiment, each showing a marked gradient of pollution and vegetation. These three blocks were located between 1950 and 2050 m of altitude on two different soil types derived from calcareous (blocks in location 2 and 4, Figure S1) and siliceous bedrocks (block in location 3, Figure S1). Soil properties regarding metal-pollution and other environmental variables and their variation along the gradients involved are provided in Supplementary Table S1. In brief, the total concentration of Pb and Zn increased from 0.8% and 0.3% (H4) to 4% and 8% (H1), respectively. Acetic acid extractable concentrations for different metal showed similar patterns. Finally, pollution gradients were characterized by a synthetic metric “PCA_{ENV}” encompassing variables directly related to soil pollution and more complex ecological factors influenced by pollution (e.g. vegetation volume, soil C: N ratio, see Table S1 and Figure S3 for more information)

2.2.2 Transplantation and monitoring of target ecotypes

Our experiment was conducted from early June to late September 2021. We selected as targets two ecotypes

of the perennial grass *Festuca rubra*. Ecotypes were selected from habitats corresponding to their highest (H2, Figure S2b) and lowest (H4, Figure S2d) levels of soil pollution in the species distribution range. In each block and each of the four habitats, two plots of 1m² were randomly located avoiding obvious micro-topographic anomalies and the variables related to the pollution gradients in the plot were determined (Figure S4). Transplantation of the *Festuca* ecotypes with and without vegetation were performed in each plot as follow. On June 7, twelve individuals of each of the two ecotypes of *Festuca* were harvested. Their root systems were freed from soil and six individuals of each ecotype were transplanted “with neighbours” (where the vegetation remained intact) and “without neighbours” (where the vegetation was removed with scissors cutting aboveground vegetation over a surface of 0.15 m diameter). Transplanted individuals were tagged with plastic cable ties to ease their finding at the end of the experiment. In each block, a total of 192 individuals were transplanted (4 habitats x 2 plots x 2 ecotypes x 2 neighbouring conditions x 6 replicates) for a total of 576 transplants. Survival of all transplanted individuals was determined on September 20, 2021. Survival rates of the different species and ecotypes were calculated as the proportion of alive individuals relative to the six replicates transplanted initially. Complementary measurements of functional traits related to plant size and stress tolerance – Height (cm), Leaf Area (cm²), Specific leaf area (SLA, cm².mg⁻¹), Stem specific density (SSD, mg.cm⁻³), or Leaf dry matter content (LDMC, %) - were performed for both *Festuca* ecotypes to ease interpretation of their facilitative response (see Figure S5 for more information).

2.3. Experimental design for the common garden experiment:

2.3.1. Site description and level of pollution

The common garden of nurse plants was settled on a slag heap located at 950 m a.s.l. in the same valley (42°49'N, 0°56'E) and covering an area of 5000 m² (see Figure S6a and location 10 in Figure S1). At this altitude, the growing season starts in late April and lasts until mid-October. A weather station (Delta-T Device® DLT/WGS-GPA) was placed on the site in March 2021. Average temperature during the growing season in 2021 was 14.6 °C and precipitation 399 mm. *Gypsophila repens* and *Minuartia verna* are the most

dominant species encountered on the site. This study included four 15 m² blocks placed 10 meters apart on a flat area of the slag heap. In each block, 3 soils samples were harvested in September 2020 for measurements of soil characteristics such as soil texture, pH and metal content. These measurements confirmed the homogeneous soil properties among the different blocks and the high level of metal pollution (See details measurement in supplementary Table S2).

2.3.2. Transplantation and monitoring of the different species and ecotypes in the common garden.

We selected two ecotypes of four metal-tolerant species (*Hutchinsia alpina*, *Armeria muelleri*, *Gypsophila repens* and *Minuartia verna* - hereafter referred to as *Hutchinsia*, *Armeria*, *Gypsophila* and *Minuartia*, respectively) as nurse for this experiment. The two ecotypes of these nurses were chosen from habitats with the highest (H1) and lowest (H3) levels of soil pollution in their distribution range in close locations (location 8 and 9 in Figure S1). Two ecotypes of the grass *Agrostis capillaris*, abundant close to this site (in location 6, Figure S1), were selected as target and harvested in habitats corresponding to their highest (H2) and lowest (H4) levels of soil pollution.

In each block, the vegetation was removed on May 25, 2020. Then eight individuals of each of the two ecotypes of each nurse species were transplanted for a total of 64 nurse individuals into each block (8 replicates per block x 4 nurse species x 2 nurse ecotypes), and 256 nurse individuals in the experiment (see Figure S6b). Individuals were transplanted at a distance of 20 cm of each other with a systematic pattern ensuring presence of all nurses and ecotypes in all parts of the blocks. Twelve open spots were kept free of nurse individuals in each block to compare the performance of targets with or without nurse. These target individuals (two different *Agrostis* ecotypes) were transplanted one year later on April 15, 2021 to allow the nurses to develop and generate sufficient effects. Only one ecotype of *Agrostis* was transplanted within each nurse individual (Figure S6c,d). Transplant size (number of leaves and extended plant height) were measured initially and at the end of the experiment (October 13, 2021) enabling calculation of transplant Relative Growth Rates (RGR, see Table S3 for more details). Complementary measurements of functional traits related to stress tolerance were performed for *Agrostis* ecotypes as for *Festuca* ecotypes in the first

experiment. Nurse species and ecotype canopy size and relative growth rate (RGR) were also determined (see Table S5 for more information).

2.4 | Plant-plant interaction index calculation

For both experiments, we used the Relative Interaction Index (RII; Armas et al., 2004) to estimate plant-plant interaction, calculated as follow:

$$RII_{neighbour} = \frac{P_{+neighbour} - P_{-neighbour}}{P_{+neighbour} + P_{-neighbour}} \quad (1)$$

With P standing for target performance (survival rates or RGR in the presence “ $P_{+neighbour}$ ” and absence “ $P_{-neighbour}$ ” of neighbouring vegetation). RII values range from -1 to 1, with negative values denoting competition and positive values denoting facilitation. In the first experiment, $P_{-neighbour}$ conditions were represented by the removal treatment (see paragraph 2.2.2) and RII values were computed at the plot level according to *Festuca* ecotypes (two different RII values for each plot). In the second experiment, $P_{-neighbour}$ conditions were represented by the open spots (see paragraph 2.3.2) and RII values were computed at the block level according to the different treatments (four different nurse species with two ecotypes, two *Agrostis* ecotypes; 16 different RII values for each block).

The first experiment encompassed situations from high stress due to pollution (H1) to situations without pollution stress (H4). Along such large environmental gradients, Michalet et al. (2014) and Nemer et al. (2022) showed the importance to distinguish between modifications of plant-plant interactions due to changes in target performance without neighbouring nurses (i.e., ‘*environmental severity effects*’) from modifications due to changes in target performance with neighbouring nurses (i.e. ‘*neighbour trait effects*’). To take into consideration this important distinction, we computed the following index:

$$RII_{stress} = \frac{P_{+stress} - P_{-stress}}{P_{+stress} + P_{-stress}} \quad (2)$$

Where $P_{-stress}$ is the target performance (survival rates of *Festuca* in a given plot) without stress (H4), and $P_{+stress}$ is the response under pollution stress (H1). In each block, two RII_{stress} indices were calculated for each *Festuca* ecotypes: one in situations with neighbours, and another without neighbours (see Michalet et al.,

2014 and Nemer et al., 2022 for full details regarding these indices calculations and interpretations).

2.6 | Statistical analyses

All statistical analyses were done using R software (R Core Team, 2021). Linear mixed models were fitted using the “lme4” package. Normality and homoscedasticity of models residuals were systematically checked to respect assumption of linear modelling using the “performance” package. Tukey post-hoc comparisons were done using the “lsmeans” package. For all statistical analyses, we used mixed models to take into account the block effect as a random factor. Linear mixed models were used for analysis of different RII values variation, general linear models with a logit link function were used for survival rates analysis.

For the reciprocal transplant experiment, we first verified if the different *Festuca* ecotypes had different metal-stress tolerance analyzing their survival as pollution increased. Survival rates were analysed with and without neighbours separately, with “PCA_{ENV}” (i.e., the quantitative position of each plot along the pollution gradient), “*Festuca* origin” and their interactions as fixed factors. Then, the survival rates were analysed adding the neighbouring treatment in the model. Variations of RII_{neighbours} for *Festuca* ecotypes were analysed with “PCA_{ENV}”, “*Festuca* origin” and their interactions as fixed factors. Changes of RII_{neighbours} between the two different levels of stress, and changes of RII_{stress} between the two neighbouring conditions were also investigated. As for the effect of nurse species and their ecotypes on *Agrostis* responses in the common-garden experiment, we analysed RII based on RGR (RII_{growth}) and survival (RII_{survival}) with “Nurse_{species}”, “Nurse_{origin}”, “*Agrostis* origin” and their interactions as fixed factors, and followed by Tukey tests when necessary. For all RII analyses, complementary one-sample *t*-tests were used to detect significant deviations of RII values from zero.

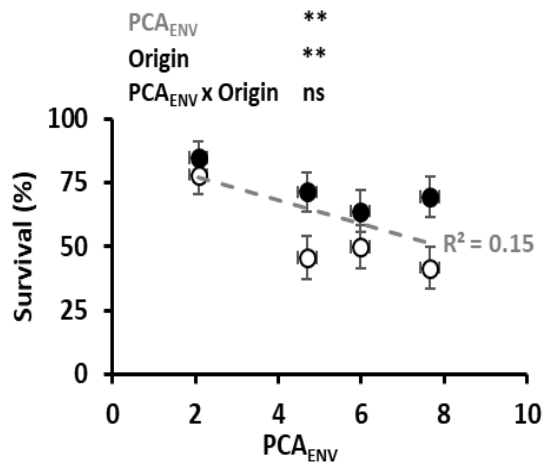
3 | Results

3.1 | Field reciprocal transplant experiment

The *Festuca* ecotype from highly polluted habitats had higher Specific Stem Density than the ecotype from unpolluted habitats (Figure S5d). There was a significant origin effect on *Festuca* survival without neighbours (Figure 1a, $P < 0.01$) because, in absence of neighbours, the ecotype from polluted habitats had a higher survival ($72.1 \pm 3.8\%$) than the other one ($53.2 \pm 4.25\%$). However, there was no effect of *Festuca* origin on survival with neighbours (Figure 1b). The metal pollution gradient (PCA_{ENV}) also had a significant effect on *Festuca* survival without neighbours which decreased significantly as pollution increased ($P < 0.01$, Figure 1a), whereas *Festuca* survival with neighbours did not vary when pollution increased ($P = 0.23$, Figure 1b). All together, these results suggest that the *Festuca* ecotype from polluted habitat is more tolerant to metal stress than the other one. Additionally, there was a significant interaction between the neighbour and metal- pollution gradient (PCA_{ENV}) treatments, because for both ecotypes *Festuca* survival was higher without neighbours, except in the most polluted habitats (see Table S4).

There was a significant effect of the metal-pollution gradient “ PCA_{ENV} ” treatment on $RII_{neighbours}$, because *Festuca* response shifted from competitive to more neutral or positive interaction with increasing pollution (Figure 2). Although the effect of *Festuca* origin treatment was not significant, results of one-sample t-tests showed that the “ $RII_{neighbours}$ ” of *Festuca* ecotype from unpolluted habitats shifted from marginally negative to significantly positive when pollution increased, whereas $RII_{neighbours}$ did not change significantly along the metal-pollution gradient for the ecotype from polluted habitats (Figure 2). Disentangling environmental-severity effects from neighbour-trait effects (Figure S7) revealed that the increase in facilitation with increasing metal stress for the ecotype from unpolluted habitats was primarily due to an environmental-severity effect, and to a lesser degree to a marginal neighbour-trait effect, as RII_{stress} was negative without neighbours and marginally positive with neighbours (Figure S7c).

a) *Festuca* survival without neighbours



b) *Festuca* survival with neighbours

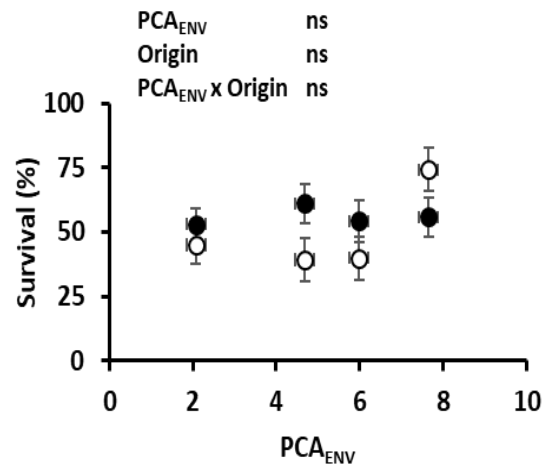


Figure 1. Variation in survival rates of *Festuca* ecotypes with and without neighbours along the metal pollution gradient. The gradient is characterized by the PCA_{ENV} metric, with higher values indicating a higher level of pollution. Survival rates and the PCA_{ENV} metric for the plots in the same habitat and in the three different blocks are averaged (mean per habitat \pm SE, N=6). a) *Festuca* survival without neighbours; b) *Festuca* survival with neighbours. Full circle: *Festuca* ecotype from polluted habitats; empty circles: *Festuca* ecotype from unpolluted habitats. Results of the generalized linear mixed-effects model for the effect of PCA_{ENV}, *Festuca* ecotype origin and their interaction are shown on the top left corners of the panels (**, $p < 0.01$; ns= not significant).

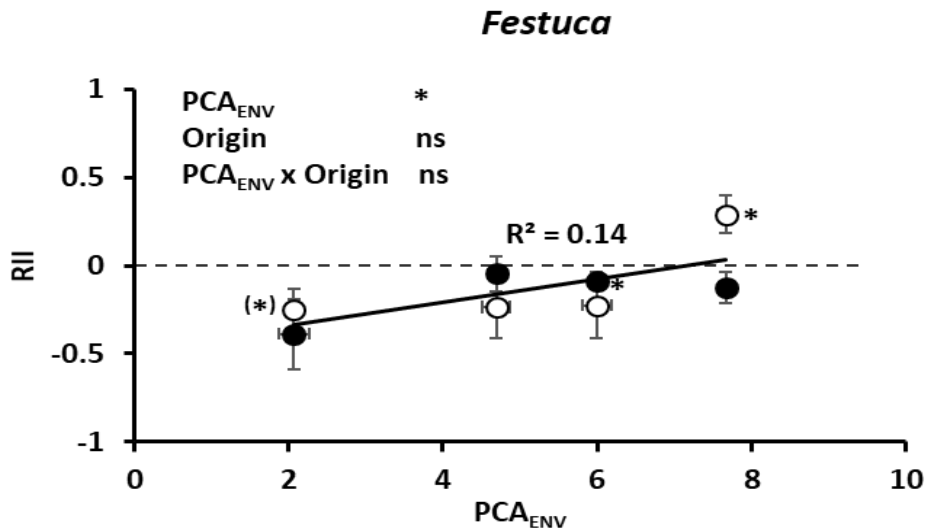


Figure 2. Variation in RII_{neighbours} of *Festuca* along the metal pollution gradient. RII and PCA_{ENV} metric for the plots in the same habitat and in the three different blocks are averaged (mean per habitat \pm SE, N=6). Full circle: *Festuca* ecotype from polluted habitats; empty circles: *Festuca* ecotype from unpolluted habitats. Results of linear mixed-effects model for PCA_{ENV}, *Festuca* ecotype origin and their interaction are shown on the top left corners of the panel. Significant differences of RIIs from 0 are shown next to each RII value (one sample t-test). (*, $P < 0.05$; (*), $P < 0.1$; ns= not significant).

3.2 | Common-garden experiment

Results on functional traits of the two *Agrostis* ecotypes showed that the ecotype from unpolluted habitats had higher height and leaf area and lower leaf dry matter content than those of the ecotype from polluted habitats (Figure S8a,b,e). Moreover, results on transplant survival showed that ecotype from polluted habitats survived better without neighbours (Figure S8g). As for *Festuca* ecotypes in the first experiment, these results suggest that the *Agrostis* ecotype from polluted habitats was the most stress-tolerant. Concerning the traits of the nurse plants, there was an effect of the nurse species on plant size traits (Table S5), but not of their ecotypic origin. *Gypsophila* surface area, cover and surface area x cover were significantly higher ($P < 0.05$) than those of all other nurse plants.

We did not observe differences in RII_{survival} (Table S6), or RII_{growth} (Table S7) between the two *Agrostis* ecotypes in response to nurse effects. However, nurses from highly polluted habitats had a significantly higher and positive effects on *Agrostis* revealed by positive RII_{growth} compared to nurses from less polluted habitats that produced negative RII_{growth} ($P < 0.05$ Table S7, and Figure 3). RII_{growth} were higher for nurse ecotype from highly polluted habitats for most nurse species (Figure S9).

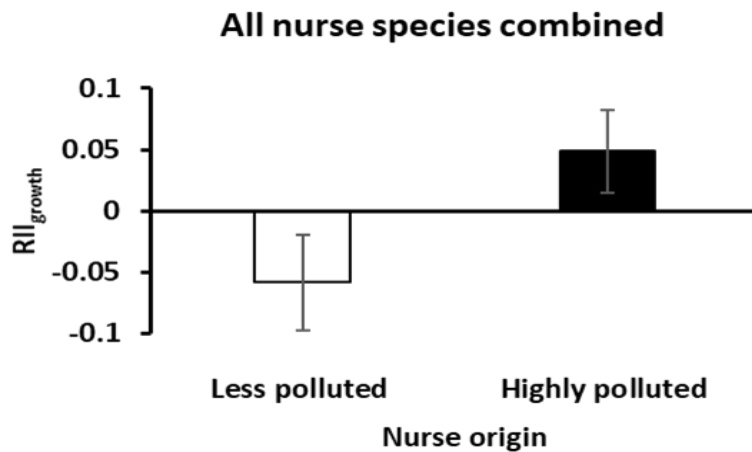


Figure 3. Relative Interaction Index of RGR_{growth} for *Agrostis* growing in nurses from highly polluted and less polluted habitats. *Agrostis* ecotypes and nurse species factors being not significant, RII values are averaged according to the two nurse ecotypes (mean per block \pm SE, $N=4$). RII for nurse from less polluted and highly polluted habitats are represented by black and white bars respectively and are significantly different ($P < 0.05$).

4 | DISCUSSION

Consistent to our first hypothesis, our reciprocal transplant experiment showed that, when grown in polluted habitats, the *Festuca* ecotype from unpolluted habitats was more facilitated than the ecotype from polluted habitats. However, in contradiction to our second hypothesis, when grown in unpolluted habitats, the ecotype from polluted habitat did not experience more competitive exclusion by neighbours than the unpolluted ecotype. In contrast to our third hypothesis, our common-garden results showed that the four metal-tolerant nurse species had similar effects on *Agrostis* ecotypes. However, in accordance with our fourth hypothesis, nurse ecotypes from highly polluted habitats had higher facilitative effects than nurse ecotypes from less polluted habitats. Overall, our results suggest that stress-intolerant target ecotypes are the best beneficiaries (first experiment) and stress-tolerant nurse ecotypes the best benefactors (second experiment).

Stress-intolerant target ecotypes are the best beneficiaries

For the *Festuca* ecotype from unpolluted habitats, the outcome of plant–plant interactions shifted from slightly negative to positive with increasing pollution stress. This result is consistent with the SGH (Bertness & Callaway, 1994) and in line with previous studies along pollution gradients (e.g., Yang et al., 2015). This result is also consistent with those of Nemer et al. (2022) who found similar results for an ecotype of *Agrostis capillaris* from unpolluted habitats. This suggest a trade-off between stress-tolerance and facilitative-response ability, consistently with previous studies (Liancourt, et al., 2005; Liancourt & Tielbörger, 2011; Qi et al., 2018). In contrast, the *Festuca* ecotype from polluted habitat did not show higher competitive exclusion in unpolluted habitats. An explanation could be than the overall level of stress at the high altitude of the experimental site limits vegetation development, and thus competition (Grime 1973, Michalet et al. 2022), even in unpolluted habitats. Additionally, a longer duration of our experiment might have revealed a higher sensitivity of this ecotype to competition.

Michalet et al. (2014), suggested that a shift from competition to facilitation along environmental gradients can occur because of: (i) a decrease of performance of targets without neighbours (i.e., an

environmental-severity effect); (ii) an increase of the target performance with neighbours (i.e., a neighbour-trait effect); and (iii) a combination of both. Here, we observed the occurrence of both effects (i.e., third case), although environmental severity effects were higher than neighbour-trait ones. Indeed, as stress increased, the performance of the *Festuca* ecotype from unpolluted habitats decreased significantly without neighbours, whereas the increase in its performance with neighbours was only marginally significant (Figure S7a,b,c). These findings are in agreement with former studies showing that increases in facilitation with increasing stress are generally due to environmental-severity effects (Michalet et al., 2014; Nemer et al., 2022). Rationally here, the less stress-tolerant ecotype appeared more sensitive to environmental-severity effects, which explains its higher facilitation.

In the second experiment, the *Agrostis* ecotype from polluted habitats was more tolerant to metal-stress since it survived better than the *Agrostis* ecotype from unpolluted habitats in the absence of neighbours. It also had a higher leaf dry matter content, showing its more conservative strategy, an important tenet of stress-tolerance (Liancourt et al., 2005; Fagundes et al., 2022). However, this did not translate into differences in responses to the effects of nurses. Transplanting *Agrostis* into the common garden was very easy due to the sandy loam soil and this resulted in a high survival rate in all treatments even for the ecotype from unpolluted habitats. These high survival rates might partly explain the similar survival responses of both ecotypes to neighbouring nurses. A longer duration of experiment might also be necessary to exacerbate differences in both growth and survival response between ecotypes.

Stress-tolerant nurse ecotypes are the best benefactors

In agreement with our fourth hypothesis, nurse ecotypes from highly polluted habitats had higher facilitative effects on targets than those from less polluted habitats. In contrast, Zhang and Tielbörger (2019) reported that a less salt-tolerant nurse ecotype of *Arabidopsis thaliana* had a stronger facilitative effect than a more salt-tolerant one. However, this latter study was a pot experiment conducted on the model species *Arabidopsis thaliana* exposed to salt-stress, and having specific mechanisms related to salt tolerance. This

likely explains the difference with our field study. Michalet et al. (2011) and Al Hayek et al. (2014, 2015b) have assessed in field conditions differences in facilitative and competitive effects of loose and tight phenotypes of different Alpine cushion species from Arizona (USA), Pyrenees (France) and Lebanon. In these studies, if the different phenotypes had contrasting facilitative or competitive effects, the authors could not separate the effect of environmental conditions from the effect of the phenotype, since the different phenotypes were always observed in different microhabitats. Al Hayek et al. (2015a) could separate the influence of environmental conditions from that of the nurse phenotype by assessing differences in facilitative- and competitive-effect abilities in two common-gardens simulating the contrasting environmental conditions of the natural habitats of two ecotypes of the cushion grass *Festuca gautieri*. However, the corresponding subalpine systems in the Pyrenees were not stressed enough to really assess the relation existing between facilitative effects and cushion phenotype stress-tolerance.

Thus, little is known from previous studies regarding the relationship between stress-tolerance and facilitative effects, in contrast to the known trade-off between stress-tolerance and competitive effects and between stress-tolerance and both competitive and facilitative responses. Our study is the first to show in a common-garden experiment that stress-tolerant nurse ecotypes are better facilitators than stress-intolerant ones. It is rational to propose that nurse-effect abilities should be higher in field experiments for nurse ecotypes the most adapted to the environmental conditions of the habitat under consideration. Indeed, nurse ecotypes from harshest habitats might have higher (positive) effects in harshest habitats than ecotypes from smoother habitats. Here, the slag heap used for this experiment corresponds to a strongly polluted environment (see Table S2). Therefore, higher positive effects for nurses from highly polluted habitats might be expected. However, the results found in our study might have been influenced by the particular nature of the metallic stress and more studies are needed in a variety of stressed systems to conclude on the relationship existing between stress-tolerance and facilitative-effect abilities along stress gradients.

We could not attribute these variations in facilitative effects to differences in functional traits between ecotypes, as found for example for alpine cushion studies (Al Hayek et al., 2015a,b). No differences in size

and growth traits were observed between ecotypes of any of the four nurse species. Since plant size did not affect the facilitation effect in this study, other mechanisms non evaluated here must have been at stake. For instance, an important functional trait related to nurse ability to cope with metal stress, and that can influence their effects on neighbouring plants in diverse ways is their leaf metal concentration (Van der Ent et al., 2013, Whiting et al. 2001, Randé et al. 2022). More generally, there is a large number of traits potentially involved in positive nurse effects in metal-rich environments. Navarro-Cano et al. (2018), found that C4 plant species with larger life-forms, with lesser root depth:laterality ratio and leaf C:N ratio appeared to have better facilitative effects than C3 or CAM species in metal-polluted sites. Thus, further investigation regarding nurse traits are required to have a more complete understanding of our results. This is true, at the intra-specific level to explain the higher positive effects of nurse ecotypes from highly polluted habitats, and at the inter-specific levels to explain the absence of significant difference between nurse species (third hypothesis).

CONCLUSION

We showed in a highly metal-polluted system that stress-tolerant nurse ecotypes are the best benefactors, and stress-intolerant target ecotypes the best beneficiaries. These results have important implications both for conceptual insight regarding the evolution of plant-plant interactions along ecological gradients and for restoration studies, as positive interactions between plants can be a cost-effective technique for restoring degraded habitats. We suggest that future programs that aim to use facilitation as a restoration tool should consider combining highly metal stress tolerant ecotypes as effect plants with less tolerant target ecotypes to achieve the best results. Consistently, Fagundes et al. (2022) showed that the highest facilitation is reached when conservative stress-tolerant nurses and acquisitive targets with higher competitive abilities interacted. Similarly, Navarro-Cano et al. (2019b) showed that facilitative effects increased with the difference in functional traits between the nurse and the beneficiary species. However, further studies associating plant-

plant interaction experiments conducted along different stress gradients and in common-gardens are needed to conclude on the relationship existing between stress-tolerance and nurse facilitative-effect abilities in stressed environments.

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AUTHORS' CONTRIBUTIONS

DN, FD, and RM conceived the study and designed it. DN, RM, FD, and HR implemented the field experiments. DN performed the analysis with the guidance of FD, HR, and RM. DN, RM, and FD wrote the manuscript.

CONFLICT OF INTEREST – None of the authors have a conflict of interest.

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Supporting information

		Habitats									
Variables	unit	1		2		3		4		P-value	
Metals	Pb _{TOT}	mg.kg ⁻¹	42278± 3461.4	b	28104± 1394.7	b	19963± 5722.8	ab	8683± 3267.4	a	<0.01
	Zn _{TOT}	mg.kg ⁻¹	81371± 29349.6	b	16861± 2450.1	b	8902± 895.0	b	3700± 472.1	a	<0.01
	Cd _{AA}	mg.kg ⁻¹	32 ± 5.2	b	15 ± 1.9	b	10 ± 1.2	b	4 ± 1.1	a	<0.001
	Pb _{AA}	mg.kg ⁻¹	727 ± 113.3	b	430 ± 142.5	ab	296 ± 127.5	ab	153 ± 100.9	a	<0.05
	Zn _{AA}	mg.kg ⁻¹	2144 ± 424.5	b	771 ± 168.9	b	368 ± 60.67	b	103 ± 22.9	a	<0.001
	PCA _{Met}		-2.4 ± 0.3	b	-0.6 ± 0.4	b	0.4 ± 0.4	b	2.6 ± 0.6	a	<0.001
Environmental	Clay & fine silt	g.kg ⁻¹	184 ± 12.2	a	180 ± 13.6	ab	230 ± 14.9	b	251 ± 20.2	b	<0.01
	Fine & coarse sand	g.kg ⁻¹	680 ± 11.6	c	631 ± 20.9	b	567 ± 13.6	a	477 ± 12.8	a	<0.001
	Organic matter	g.kg ⁻¹	58 ± 9.5	a	74 ± 6.7	b	91 ± 5.9	b	129 ± 11	b	<0.001
	Relative humidity	%	16 ± 1.5	a	19 ± 1	b	18 ± 0.6	ab	23 ± 1.1	b	<0.01
	Vegetation volume	dm ³	1418 ± 299	a	2279 ± 938.8	ab	3222 ± 763.7	ab	5979 ± 1823.9	b	<0.05
	pH _{H₂O}		7.6 ± 0.1	c	7.2 ± 0.1	bc	6.8 ± 0.2	ab	6.5 ± 0.2	a	<0.001
	C/N		13.9 ± 0.5	b	12.3 ± 0.7	ab	12.3 ± 0.7	ab	11.1 ± 0.7	a	<0.1
	PCA _{Env}		-2.6 ± 0.4	c	-0.9 ± 0.4	b	0.4 ± 0.2	b	3 ± 0.3	a	<0.001

Table S1. Soil variables in the four habitats mean ± SE (N=4) and results of the one-way ANOVA on the effect of habitat. Different lowercase letters indicate significant difference for the considered variable between habitats (posthoc Tukey pairwise comparisons). PCA_{Met} and PCA_{Env} are synthetic variables that represent the soil pollution variables alone (PCA_{MET}) and all environmental variables along the metal pollution gradient (PCA_{ENV}) (see

Figure S3). Total metals concentration in the soil (Pb_{TOT} , Zn_{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). To identify the available metals (Cd_{AA} , Pb_{AA} , Zn_{AA}), extraction with 0.11M acetic acid (Rauret et al., 1999), followed by analysis of the solutions by inductively coupled plasma atomic emission spectroscopy (ICP-AES) were used. 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 moisture (volume %) was measured in the field seven days after the last rain on a warm, sunny day on June 22 using a portable moisture probe (<https://delta-t.co.uk/product/ml3/>). The probe was inserted at four locations in each plots and the four values were averaged to estimate soil moisture. 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 $1m^2$ ($1\% = 1dm^2$) multiplied by mean vegetation height (dm)).

				Blocks				coefficient of variation between blocks (%)
Variables	unit	N sample per block	1	2	3	4		
Metals	Cd _{AA}	mg.kg ⁻¹	3	7.97	9.60	8.56	9.16	17.85
	Pb _{AA}	mg.kg ⁻¹	3	943.74	1183.69	894.11	959.37	25.04
	Zn _{AA}	mg.kg ⁻¹	3	1597.50	2347.65	1843.87	1914.71	27.76
Environmental	Clay & fine silt	g.kg ⁻¹	2	85.64	67.59	65.13	92.70	22.03
	Fine & coarse sand	g.kg ⁻¹	2	780.81	837.67	843.17	796.12	4.10
	Organic matter	g.kg ⁻¹	3	11.69	11.05	11.96	11.96	17.33
	pH _{H2O}		3	8.53	8.42	8.42	8.46	1.07
	C/N		3	24.37	26.07	21.27	25.1	17.38

Table S2. Soil variables in the four blocks of the common garden experiment on the slag heap. See Table S1 legend for variables names and methods for their measurement.

<i>Agrostis</i> origin	Neighbouring	R ²
Unpolluted	Open	0.74 ***
Polluted	Open	0.51 ***

Table S3. R² statistics of linear relationships fitted between measured *Agrostis* size traits (number of leaves and length) and dry biomass at the end of the common garden experiment. At the end of the experiment (October 13, 2021), surviving individuals were harvested and dried (48 hours at 65°C) before biomass measurement. Then we fitted the linear relationships between the final dry biomass (explained variable) and the final plant size (number of leaves and/or plant length, independent variable) and looked for the linear relationships that best fitted the final biomass. Number of leaves were chosen for *Agrostis* from unpolluted habitat and “length x number of leaves” for *Agrostis* from polluted habitat. These relationships were strongly significant (***, P < 0.001). Thus, they were used to estimate *Agrostis* biomass at the beginning of the experiment, and ultimately to calculate transplants Relative Growth Rate as follow:

$$RGR = \frac{B_f - B_i}{B_i} \quad (\text{Eq. S1})$$

where B_f and B_i are the final biomass measured and the initial biomass estimated, respectively. Note that to avoid the potential confounding effects of etiolation occurring during the experiment within nurse plants, these relationships were fitted for *Agrostis* ecotypes growing in the open spots without nurses.

factors	<i>Festuca</i> Survival		
	Df	X^2	P
PCA _{ENV}	1, 541	1.11	0.291
Neighbours	1, 541	4.88	< 0.05
Origin	1, 541	9.02	< 0.01
PCA _{ENV} x Neighbours	1, 541	11.78	< 0.001
PCA _{ENV} x Origin	1, 541	0.28	0.563
Neighbours x Origin	1, 541	2.85	<i>0.091</i>
PCA _{ENV} x Neighbours x Origin	1, 541	2.27	0.131

Table S4. Deviance table of the generalized linear mixed-effects model regarding *Festuca* survival rates in the first experiment. X^2 statistics and corresponding p-values are shown for the different factors (PCA_{ENV}, *Festuca* origin, neighbours treatment) and their interaction. Significant effects are indicated in bold and marginally significant are indicated in italic.

Factors	Df	Surface		Cover		Surface x Cover		RGR _{surface}	
		<i>F</i>	P	<i>F</i>	P	F	P	F	P
Nurse _{species}	3, 147	57.80	<0.001	10.39	<0.001	44.38	<0.001	1.2975	0.277
Nurse _{origin}	1, 147	0.08	0.775	0.87	0.352	0.02	0.878	0.1118	0.7385
Nurse _{species} x Nurse _{origin}	3, 147	0.23	0.871	0.27	0.843	0.25	0.864	1.6215	0.1868

Table S5. ANOVA table of the linear mixed-effects models regarding nurses size traits in the common garden experiment. F statistics and corresponding p-values are shown for the Nurse_{species}, Nurse_{origin}, and their interaction. Significant ($p < 0.05$) effects are indicated in bold. Surface canopy (cm²) of each nurse individual was obtained by the mean of two orthogonal measurements of canopy width (cm) on August 9, 2021. Nurse canopy cover, visually estimated in percentage, provided a complementary variable related to nurse canopy density. As another measurement of nurse canopy area was made on April 28, 2022, we used the two measurements to calculate nurse relative growth rate regarding their canopy surface (RGR_{surface}).

Factors	RII _{survival} (<i>Agrostis</i>)		
	Df	F	P
Nurse _{species}	3, 46	0.88	0.459
Nurse _{origin}	1, 46	0.89	0.350
Agrostis _{origin}	1, 46	1.03	0.316
Nurse _{species} X Nurse _{origin}	3, 46	0.47	0.704
Nurse _{species} X Agrostis _{origin}	3, 46	0.37	0.776
Nurse _{origin} X Agrostis _{origin}	1, 46	0.02	0.896
Nurse _{species} X Nurse _{origin} X Agrostis _{origin}	3, 46	0.49	0.688

Table S6. ANOVA table for the linear mixed-effects model regarding RII_{survival} of *Agrostis* in the common garden experiment. F statistics and corresponding p-values are shown for Nurse_{species}, Nurse_{origin}, Agrostis_{origin}, and their interaction.

Factors	RII _{growth} (<i>Agrostis</i>)		
	Df	F	P
Nurse _{species}	3, 46	0.96	0.422
Nurse _{origin}	1, 46	4.55	<0.05
Agrostis _{origin}	1, 46	1.22	0.275
Nurse _{species} x Nurse _{origin}	3, 46	0.41	0.747
Nurse _{species} x Agrostis _{origin}	3, 46	1.12	0.351
Nurse _{origin} x Agrostis _{origin}	1, 46	0.07	0.789
Nurse _{species} x Nurse _{origin} x Agrostis _{origin}	3, 46	1.20	0.319

Table S7. Results of linear mixed-effects models for the effects of Nurse_{species}, Nurse_{origin}, Agrostis_{origin}, and their interaction on RII_{growth} in the common garden experiment. Significant ($P < 0.05$) effects are indicated in bold.

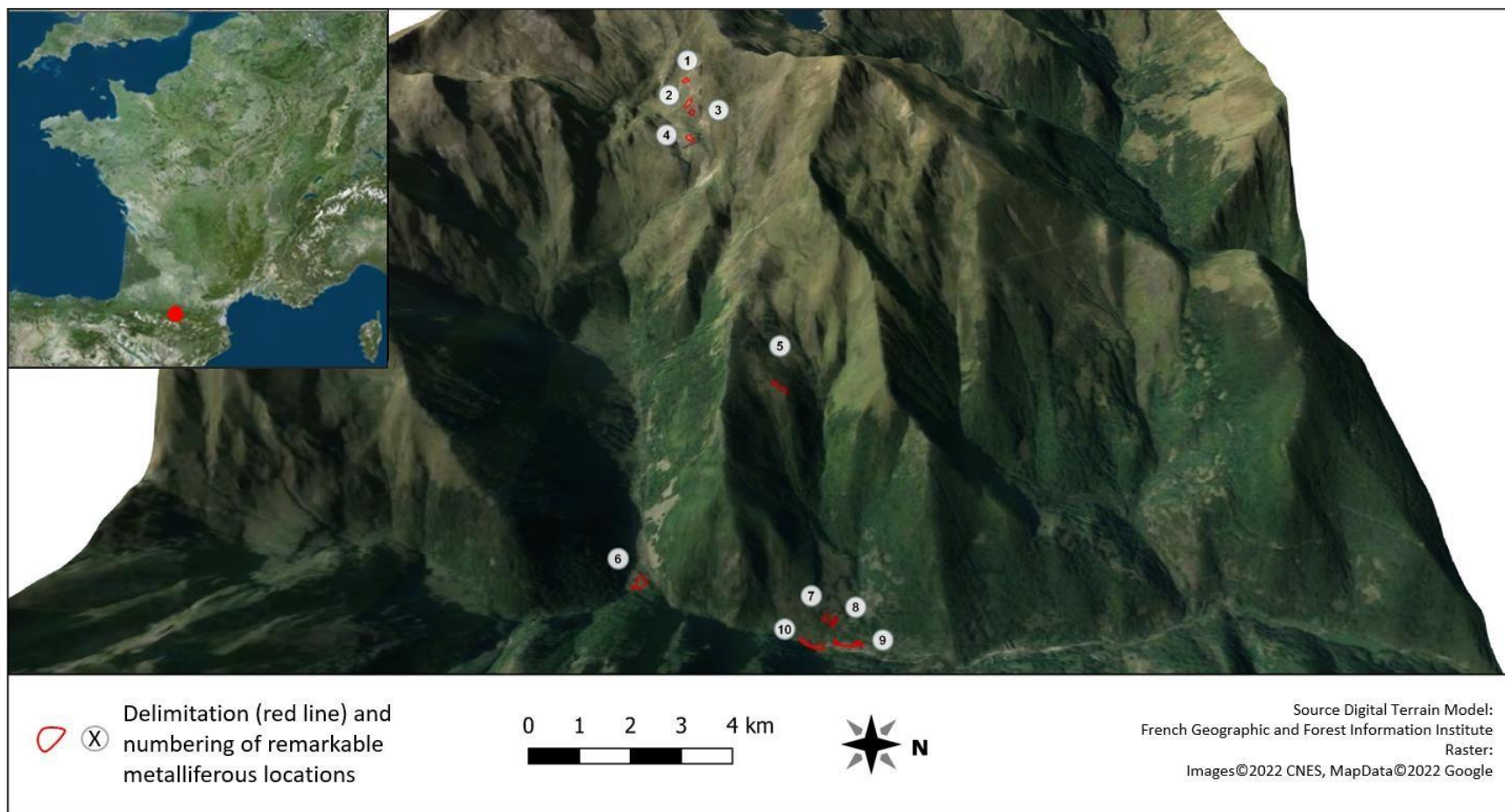


Figure S1. Position of different remarkable metalliferous locations in the region. The subalpine area where the first experiment was implemented shows several interesting locations due to several mining galleries. Locations 2, 3 and 4 form the three blocks of this experiment. The second experiment was implemented in the location 10 corresponding to the slag heap close to the former mining plant (location 9).



Figure S2. Illustration of changes in plant communities along the metal-pollution gradient in the study area. Pictures are from site 2 in Figure S1.

- a) Habitat 1 (H1) with only metal-tolerant species (*Armeria muelleri* and *Hutchinsia alpina* in this picture).
- b) Habitat 2 (H2) dominated by metal-tolerant species (*Arenaria multicaulis* and *Armeria muelleri* in the foreground) and with few metal-intolerant species (*Festuca rubra* inflorescences visible in the foreground).
- c) Habitat 3 (H3) dominated by metal-intolerant species including *Festuca rubra*, with few metal-tolerant species, such as *Armeria muelleri* and *Arenaria multicaulis*.
- d) Habitat 4 (H4) showing a standard subalpine grassland with only metal-intolerant species.

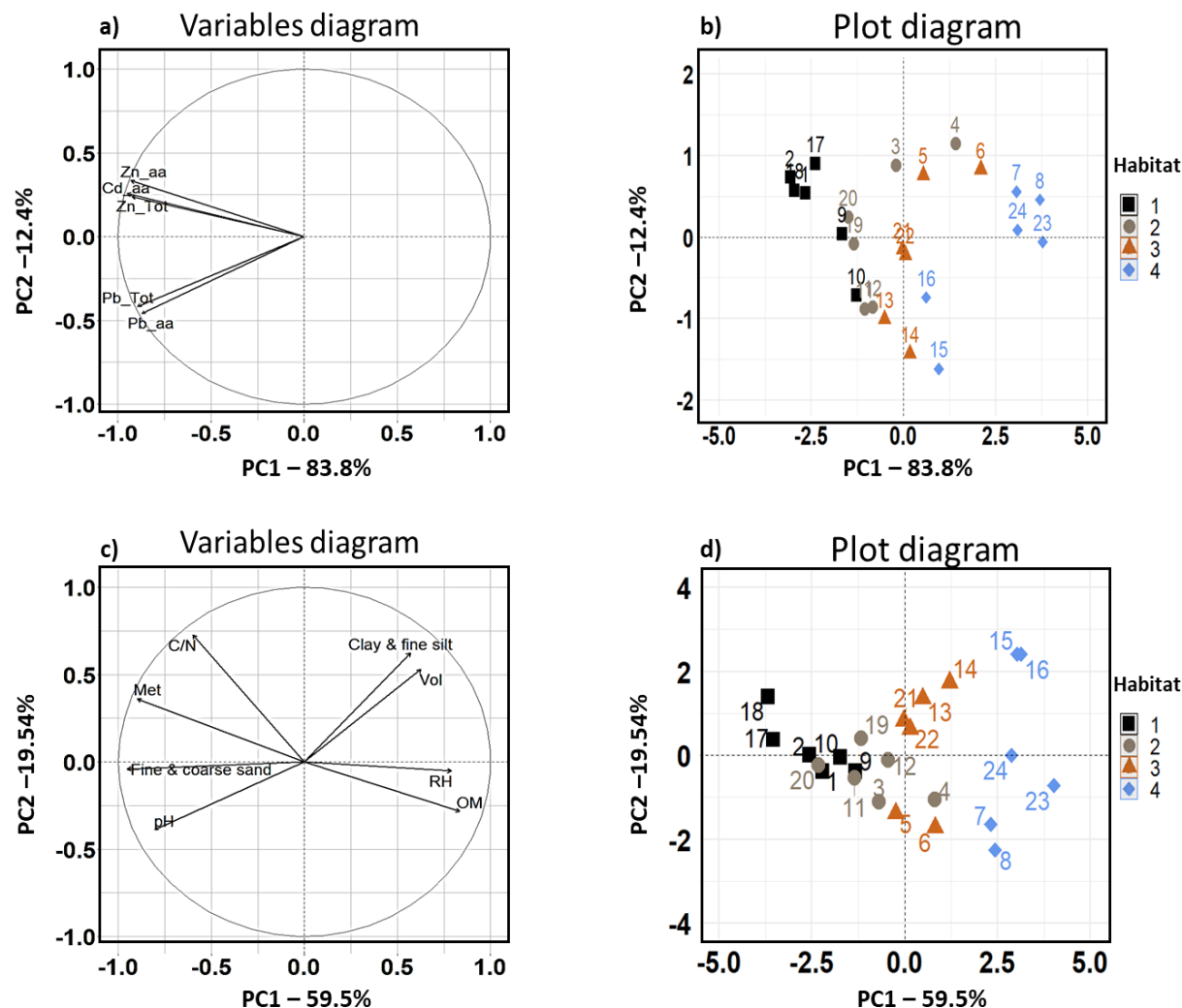


Figure S3. Principal component analyses (PCA) displaying the variability of soil metals variables and other variables between the four habitats along the studied gradient in the first experiment. (A) Variables factor map for soil metals concentration. Cd_aa: Cadmium extracted with acetic acid; Pb_aa: Lead extracted with acetic acid; Zn_aa: Zinc extracted with acetic acid; Pb_Tot: total Lead concentrations; Zn_Tot: total Zinc concentrations. (B) Plot position along PCA axes 1 and 2 for soil metal content. (C) Variables factor map for all variables including soil pollution. Met: synthetic estimation of metal concentration (PCA_{MET}) in soil from the PCA presented in panels (A) and (B). It corresponds to plot position on the first component of this PCA. RH: relative humidity; Vol: vegetation volume; OM: organic matter; Clay & fine silt; fine & coarse sand; C/N: carbon to nitrogen ratio. (D) Plot position along PCA axes 1 and 2 for all variables.

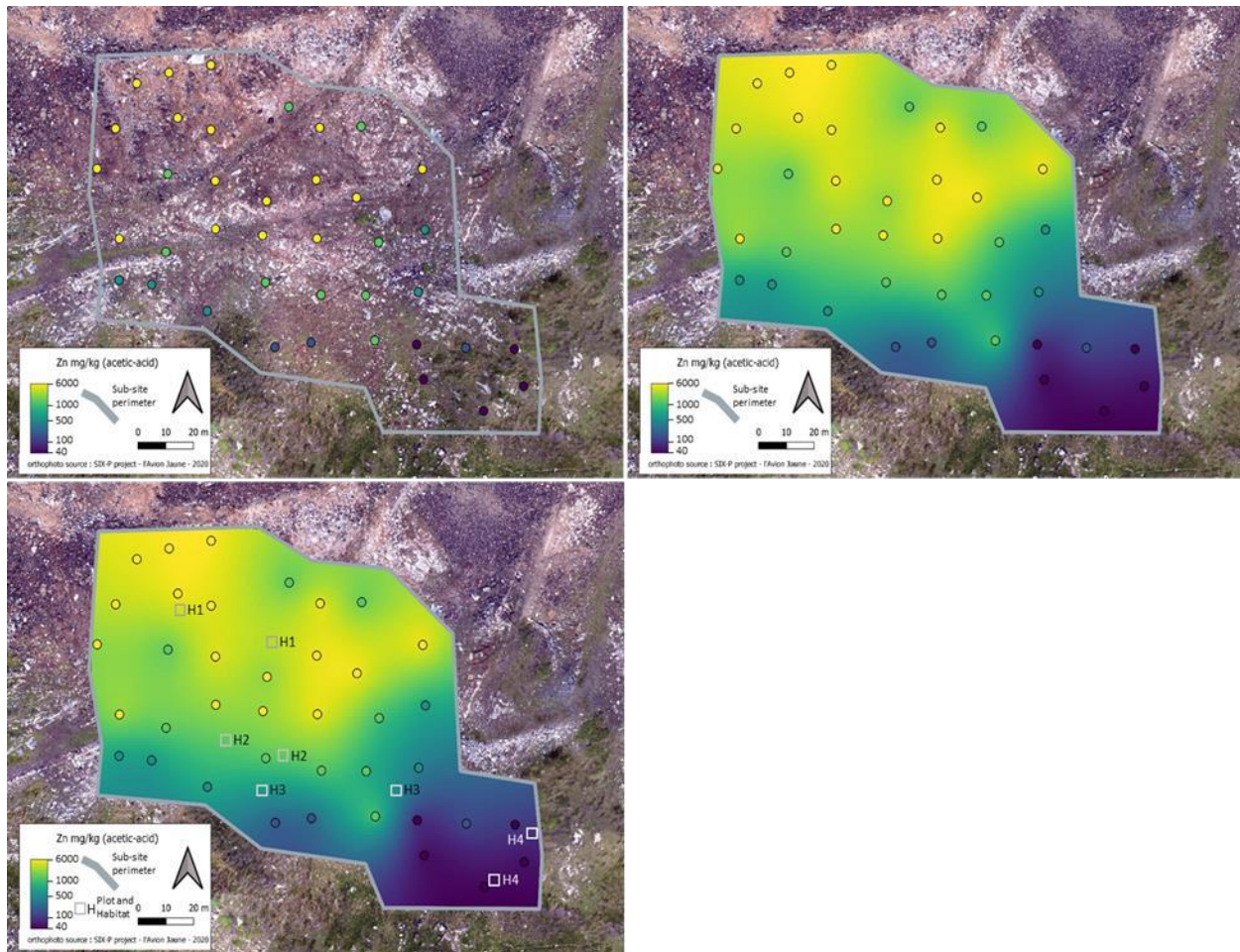


Figure S4. A three step procedure to estimate environmental variables in each plot. This procedure is based on a geographical information system built (QGIS 3.12) for the different remarkable locations studied in the valley. Raster orthophoto images were produced by UAV acquisition in June 2020. The example shown here concerns location 2 in the valley (see Figure. S1). a) Step 1: each sub-site perimeter was delineating by GPS (© garmin etrex 32x device) during preliminary field surveys, encompassing the whole vegetation gradient (see Figure S2) and avoiding important topographic or bedrock heterogeneities. Each sub-site was then divided with a systematic grid creating 40 different cells. In each cell, location of a specific point was randomly chosen. These points were visibly marked in the field and their precise position was recorded with a differential GPS (© Leica GS10, precision inferior to 3 cm, see the plain colored circles on the map). Different environmental variables were measured at each point, including those related to soil pollution (see Table S1). Here, Zn concentration determined by acid-acetic extraction (after soil collection and analysis) is shown at each point by the color scale. b) From the characterization of the desired variable on these 40 points and their geographical coordinates, we predicted its value in all positions within the site by ordinary kriging with the GSTools Python library (Müller et al., 2022). Preliminary distribution of the 40 values was checked to control for approximately normal distribution, and log-transformation was used when necessary to avoid strong departure from normality. c) Position of the experimental plots was precisely determined with the same differential GPS. Finally, plot values of each environmental variable correspond to the average value for the plot area on the kriged predictions.

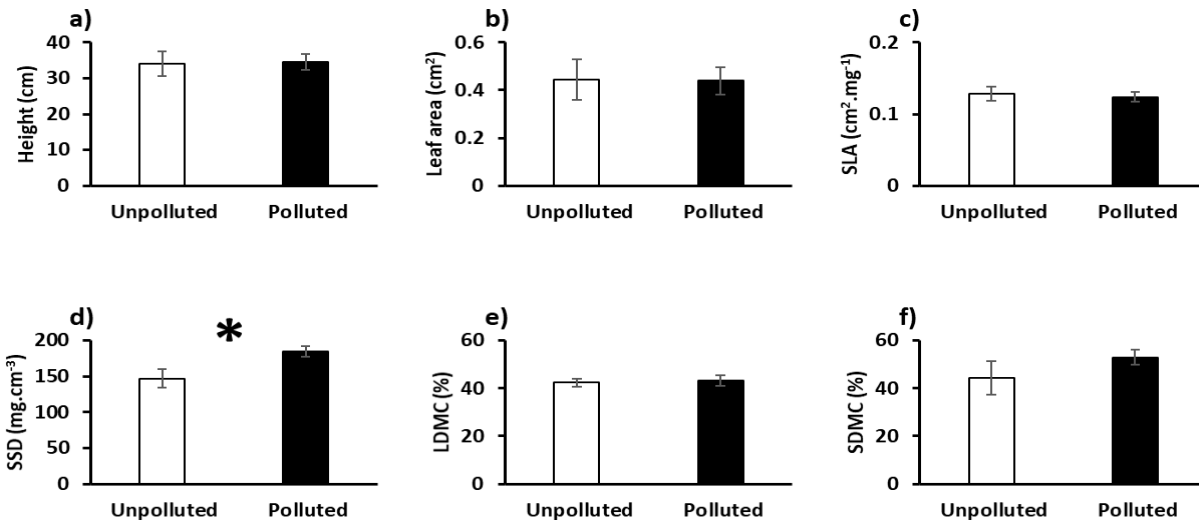


Figure S5. Morphological traits of *Festuca* ecotypes from unpolluted and polluted origin in the first experiment. Mean \pm SE are shown ($N_{\text{unpolluted ecotype}}=10$, $N_{\text{polluted ecotype}}=20$). a) Height, b) Leaf area, c) Specific leaf area (SLA), d) Stem specific density (SSD), e) Leaf dry matter content (LDMC), f) Stem dry matter content (SDMC). White and black bars represent *Festuca* ecotypes from unpolluted and polluted origin, respectively. Wilcoxon-Mann-Whitney tests were used to compare functional traits. Symbols (*, $P < 0.05$) indicate a significant difference between the two ecotypes.

The measurements of the different functional traits were done as follow. A minimum of five individuals of each ecotype were chosen to measure the different traits. The height of the top of the vegetative parts was used for plant height (cm). Three to five leaves were collected for each individual, as well as a 10 cm stem section. These samples were kept in a cool box in minigrip plastic bags for a few hours until fresh weight (g) measurement, leaf area (LA, cm²) and stem volume (cm³) determination with a portable scanner and Winfolia and Winrhizo softwares (Regents Instruments, Quebec, Canada). Leaf and stem dry weight (g) were measured after 48h of drying at 65°C. Then, we calculated leaf dry matter content (LDMC) and stem dry matter content (SDMC) [Dry weight x 100 / Fresh weight], specific leaf area (SLA, cm².mg⁻¹) and stem specific density (SSD, mg.cm⁻³).

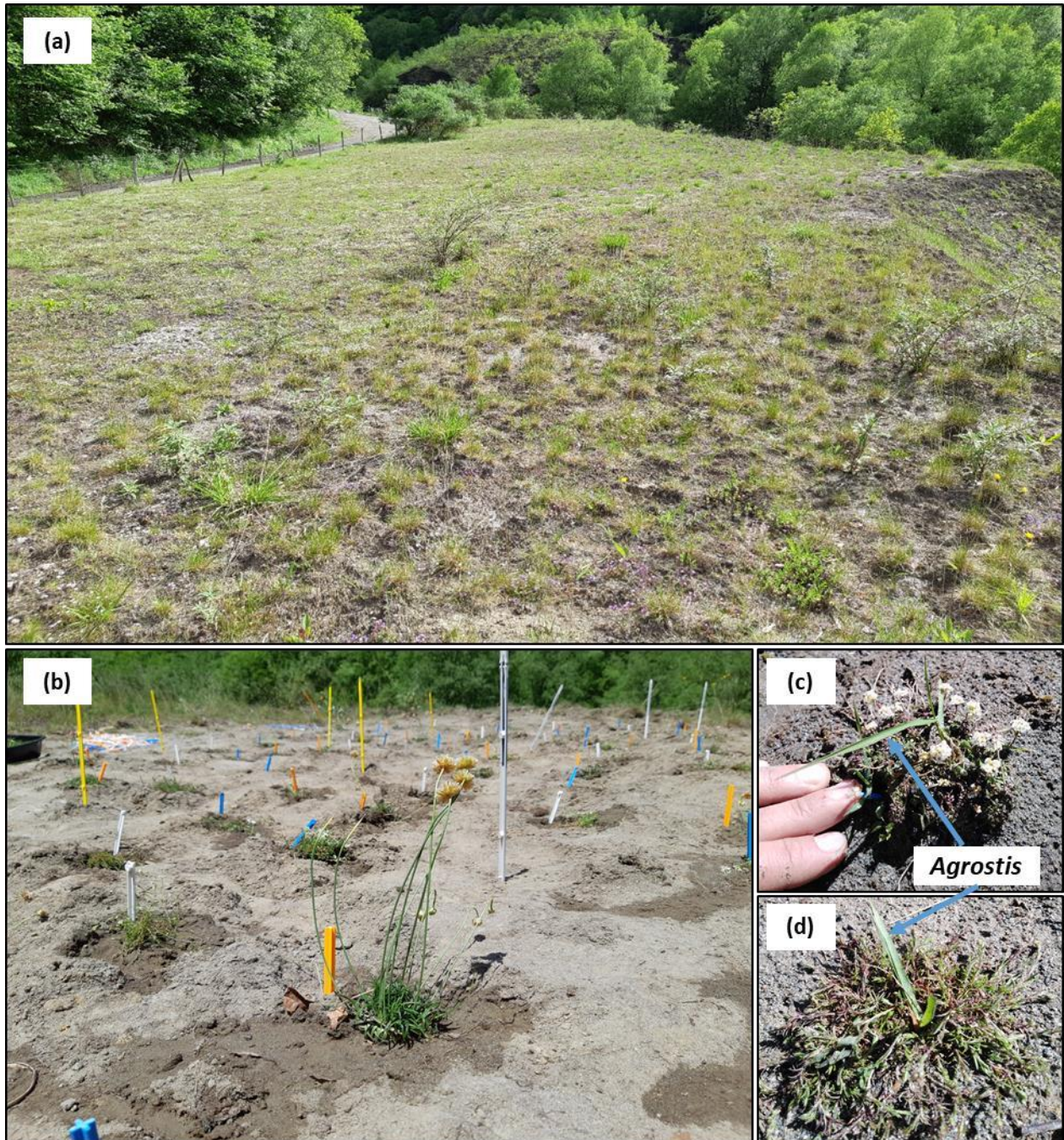


Figure S6. Presentation of the common garden experiment. a) The slag heap and its spontaneous vegetation prior to the nurse-garden experiment. b) Transplantation of the nurses in the experimental garden on May 25, 2020. Transplantation of an individual of *Agrostis* within the canopy of *Hutchinsia* (c) and *Minuartia* (d) on April 15, 2021. Only one ecotype of *Agrostis* was transplanted within each nurse individual, while both *Agrostis* ecotypes were transplanted in open spots, for a total of 88 individuals in each block (one in each of the 64 nurse individuals and two in the 12 open spots) and 352 individuals in the whole experiment.

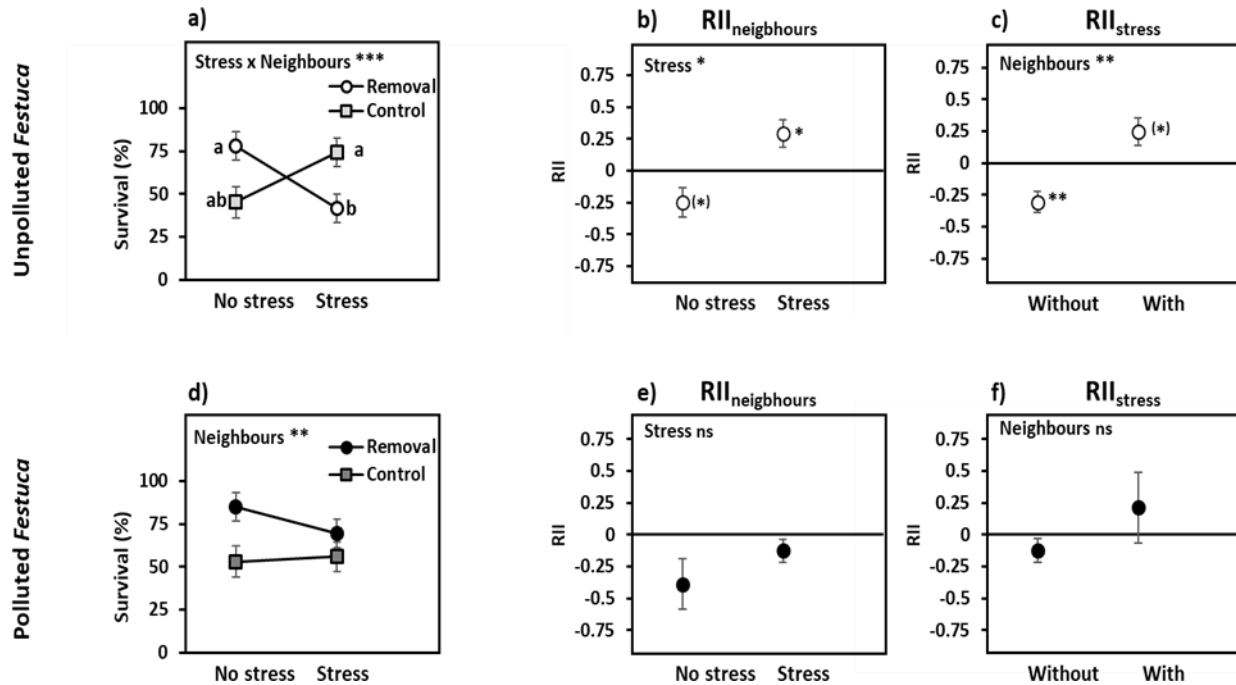


Figure S7. Survival of *Festuca* ecotypes and corresponding RII in contrasted stressed conditions and with and without neighbours in the first experiment. Survival rates (mean \pm SE, N=6) of *Festuca* ecotypes from unpolluted (a) and polluted (d) origin at the two extreme position along the metal stress gradient (less- H4 and most-H1 polluted situations). On the top left a, d) corners, results of general linear mixed-effects model for the Stress x Neighbours interaction on survival rates are shown. Different letters show significant difference between the corresponding situation (posthoc multiple Tukey comparisons). b, e) $RII_{neighbours}$ (mean \pm SE, N= 6) for *Festuca* ecotypes from unpolluted (b) and polluted (e) origin. c,f) RII_{stress} (mean \pm SE, N= 6) for *Festuca* ecotypes from unpolluted (c) and polluted (f) origin. 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. (*), $p < 0.1$; *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.

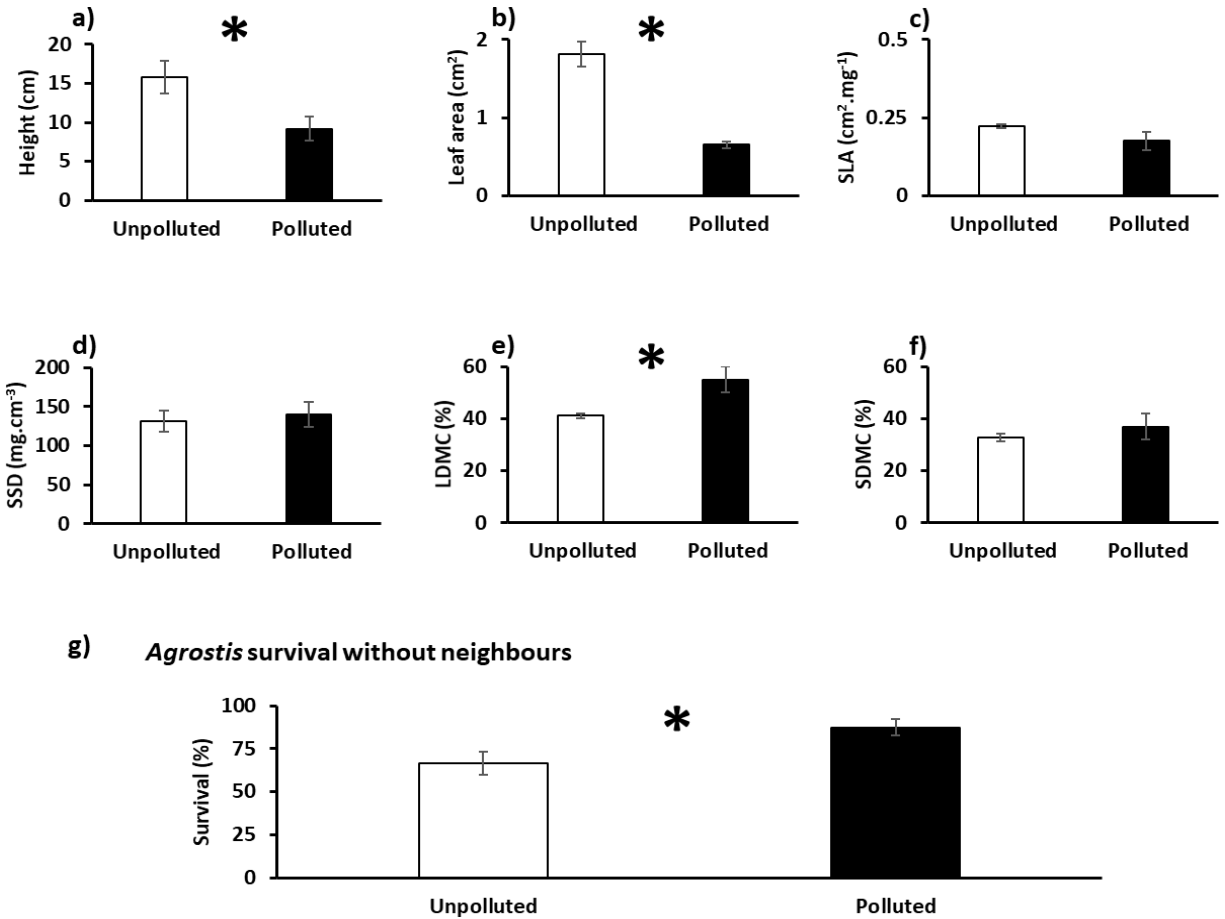
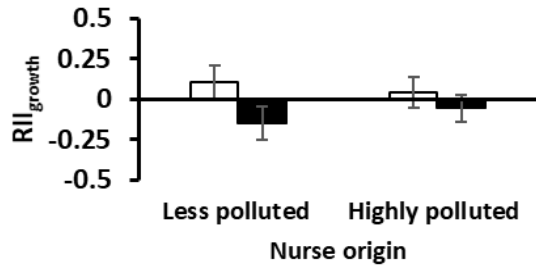
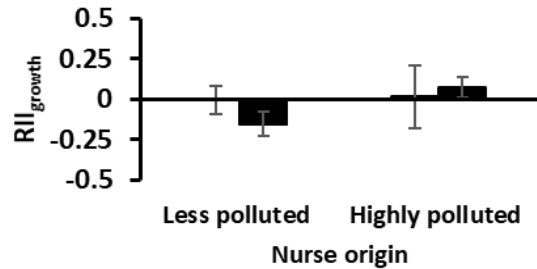


Figure S8. Functional traits and survival of *Agrostis* ecotypes from unpolluted and polluted origin in the common garden experiment. Mean \pm SE are shown (N =5) a) Height, b) Leaf Area, c) specific leaf area (SLA), d) Stem specific density (SSD), e) Leaf dry matter content (LDMC), f) Stem dry matter content (SDMC), g) Survival (mean \pm SE; N=48) of *Agrostis* ecotypes from unpolluted and polluted origin without neighbours in the open spots. White and black bars represent *Agrostis* ecotypes from unpolluted and polluted origin respectively. Results of non-parametric Mann–Whitney tests (for the effect of *Agrostis* origin on each functional trait) and of the general linear mixed-effects model (for the effect of *Agrostis* origin on survival) are shown on the panels when present (*, $P < 0.05$). Note that methods to measure these functional traits for *Agrostis* were strictly similar to the methods used for *Festuca* ecotypes (see Figure S5 legend).

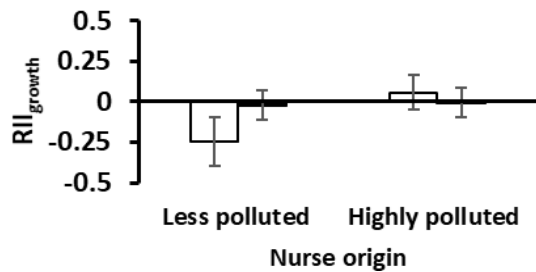
a) *Armeria*



b) *Gypsophila*



c) *Hutchinsia*



d) *Minuartia*

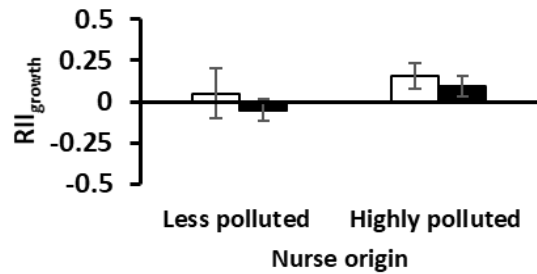


Figure S9. Relative Interaction Index of RGR_{growth} for *Agrostis* ecotypes from unpolluted and polluted origin according to the four nurse species and their ecotype in the common garden. Mean \pm SE are shown (N=4). White and black bars represent *Agrostis* ecotypes from unpolluted and polluted origin respectively.

CHAPTER 6

General discussion

1. The relationship between stress tolerance and plant effect and response abilities

1.1. Trade-off between stress tolerance and plant competitive effects and responses

The results of the four-year reciprocal transplanting experiment conducted along a water gradient in the Gironde department revealed that the contrasting water conditions between calcareous and siliceous soils led plant species related to each soil type to adopt different strategies. During dry years, especially on calcareous soils, species from calcareous origin performed better than species from siliceous origin that were more prone to environmental stress, indicating a greater ability of species from calcareous origin to tolerate drought-stress. Indeed, the low water availability on calcareous soils can further promote adaptive traits favouring calcareous species “stress-tolerant” over siliceous species “non-stress tolerant” (Thurmann, 1849; Michalet et al. 2001, 2002). However, the higher drought-stress tolerance abilities of species from calcareous origin decreased their competitive-response abilities as they suffered from competitive exclusion more than species from siliceous origin (**chapter 3, see also figure. 21a**).

On the other hand, previous studies have reported that the higher water availability on siliceous soils, can lead to the exclusion of stress tolerant calcareous species by more competitive siliceous species in siliceous environments with dense vegetation (Gigon, 1971, Michalet et al. 2002). This was the case during wet years in our study systems, as we found that competitive effects of neighbouring plants were higher in siliceous communities developed under lower drought-stress conditions on siliceous soils compared to calcareous communities developed under higher stress conditions on calcareous soils. This result is consistent with Grime (1974) that suggested a higher competition in benign environments, and also in agreement with other studies that found strong competition on siliceous soil (Aerts et al. 1991; Delerue et al. 2018). To summarize, we showed in this study that species from siliceous communities

growing in low-stress conditions have a strong competitive effect in their home community. However, they are not tolerant to the higher stress occurring on calcareous soils. Reversely, species from calcareous soils tolerant to the higher stressful conditions on calcareous soils have a low competitive response and are excluded by siliceous species in siliceous communities. These findings highlighted a trade-off between stress tolerance and competitive response and effect abilities between calcareous and siliceous species, consistent with previous studies (Liancourt et al. 2005; Forey et al. 2010).

Finally, as we predicted in our hypothesis, differences in species composition and richness between communities in calcareous and siliceous soils, are explained more by differences in soil physical factors related to substrate types (such as water availability) rather than differences related to soil chemistry. In our study, if the chemical hypothesis were the primary direct factor explaining differences in species composition, we would have observed more competitive exclusion on calcareous soils during wet years, since this hypothesis suggests greater nutrient availability on calcareous soils and thus species with higher competitive effects compared to siliceous soils.

1.2. Trade-off between stress tolerance and plant facilitative responses

The results of the two reciprocal transplantation experiments along the pollution gradient where facilitation is known to be the major interaction between plants followed the same patterns (**chapters 4 and 5**). In the first experiment, findings at the interspecific level, showed that with increasing pollution, *Agrostis capillaris*, the least tolerant species, exhibited a facilitative response, but not *Armeria muelleri*, the most stress-tolerant species (**see figure. 21b**). The plant-plant outcomes for *Agrostis capillaris* were consistent with the stress gradient hypothesis (Bertness and Callaway 1994) and several studies showing that facilitation is the dominant plant-plant interaction in metalliferous systems (Zvereva and Kozlov 2004; Frérot et al., 2006; Eränen & Kozlov, 2007; cuevas et al. 2013; Wang et al. 2014; Yang et al. 2015).

Of particular interest, we have shown in this thesis, regarding facilitative response ability, that what is relevant at the interspecific level is also valid at the intraspecific level. Both reciprocal transplantation experiments revealed that the least stress-tolerant ecotypes of *Agrostis capillaris* and *Festuca rubra* were

more facilitated than the more stress-tolerant ecotypes that did not show any facilitative responses (see **figure. 21b**). On the other hand, the higher stress tolerance abilities of *Armeria muelleri* ecotype from polluted origin decreased their ability to cope with competition, as they were more prone to competition than the *Armeria muelleri* ecotype from less polluted habitat in benign habitats. The higher facilitative responses of less stress-tolerant species and ecotypes is probably due to their higher dependence on stress mitigation (Espeland and Rice, 2007; Liancourt and Tielbörger, 2011).

In summary, we showed that species and ecotypes from more or less polluted habitats developed contrasting adaptive strategies to cope with the multiple selective constraints they faced. However, as already shown in **Chapter 3**, there was a cost to favoring one strategy over another, suggesting a trade-off between stress tolerance and plant facilitative-response abilities, consistent with previous studies (Choler et al. 2001; Liancourt et al. 2005; Forey et al. 2010; Gross et al. 2010).

1.3. Positive correlation between stress tolerance and plant facilitative effects

Contrary to the known trade-off between plant stress-tolerance and facilitative-response abilities, little is known about the relationship between stress-tolerance and facilitative-effect abilities. Our common garden experiment carried out on a highly polluted slag heap was especially designed to investigate the facilitative effects of ecotypes from highly and less polluted origin of four metallophilous species on target species. Fagundes et al. (2022) showed that facilitation increased when conservative stress-tolerant nurses and acquisitive targets interacted. In a similar manner, Navarro-Cano et al. (2019b) showed that the facilitative effect increased with the difference in functional traits between the nurse and the beneficiary species. So in our study it was reasonable to hypothesize that the more stress tolerant nurse ecotypes from highly polluted habitats should have a higher facilitative effects on target species than nurse ecotypes from less polluted habitats. Results of the common garden experiment confirmed our hypothesis, as we showed that stress-tolerant nurse ecotypes from highly polluted habitats were better facilitators than stress-intolerant nurse ecotypes from less polluted habitats (**chapter 5**). In contrast to what we found, Zhang and Tielbörger (2019) observed that nurse ecotypes of *Arabidopsis thaliana* with low salt tolerance

had a higher facilitative effect than nurse ecotypes with high salt tolerance. The latter study, however, was a pot experiment carried out on a model species, and it is reasonable to assume that facilitative effects are more likely to be directly quantified under stressful field conditions with actual nurse species that offer a more accurate depiction of natural plant communities. Previous studies have assessed differences in the facilitative and competitive effects of loose and tight phenotypes of different alpine cushion species under field conditions (Michalet et al. 2011, Al Hayek et al. 2014, 2015b and Jiang et al. 2017). In these studies, because the different phenotypes were always observed in different microhabitats, the authors could not separate the effect of environmental conditions from the trait effect related to the cushion phenotype, and it was therefore not possible to assess the facilitative or competitive effects of the different phenotypes.

However, Al Hayek et al. (2015a) were able to separate between the influence of environmental conditions and the nurse phenotype in the Pyrenean region by comparing facilitative and competitive effect abilities in two common gardens that replicated the contrasting environmental conditions of the natural habitats of the two ecotypes of the cushion grass *Festuca gautieri*. The tight and loose phenotypes of *Festuca gautieri*, occurs naturally in dry convex outcrops (with low species richness), and in wet concave slopes (with high species richness) respectively. Interestingly, they found that competition was increasing within both nurse ecotypes from the convex (i.e. “tight” habitat) to the concave (i.e., “loose” habitat) common-garden, but decreasing within common-gardens from the tight to the loose phenotypes. This showed that in natural habitats the diversity of the dependent community was low in the tight phenotype because of the high competitive effect of the tight phenotype and high in the loose phenotype not because of facilitation, but due to decreasing in the loose cushion competitive effects with increasing disturbance and improving environmental conditions (soil fertility).

Thus, previous studies provide weak support for the existence of a trade-off between stress tolerance and facilitative effects, as demonstrated for competitive effects and for both competitive and facilitative response. This is primarily because all of the aforementioned study systems were not sufficiently stressed to truly assess the relationship between facilitative effects and stress tolerance. Our study was the first to

show in a common-garden in metalliferous habitats that stress-tolerant nurse ecotypes are better facilitators than stress-intolerant ones, suggesting a positive correlation instead of a trade-off between stress tolerance and plant facilitative effect abilities (**chapter 5**). A reasonable explanation for these results is that, under stressful conditions, the stress-tolerant ecotype is the one most likely to maintain a higher development and biomass, allowing it to generate more facilitative effects. Conversely, under benign conditions, the more competitive ecotype will be the one most likely to reach higher development and biomass and thus have a greater competitive effect.

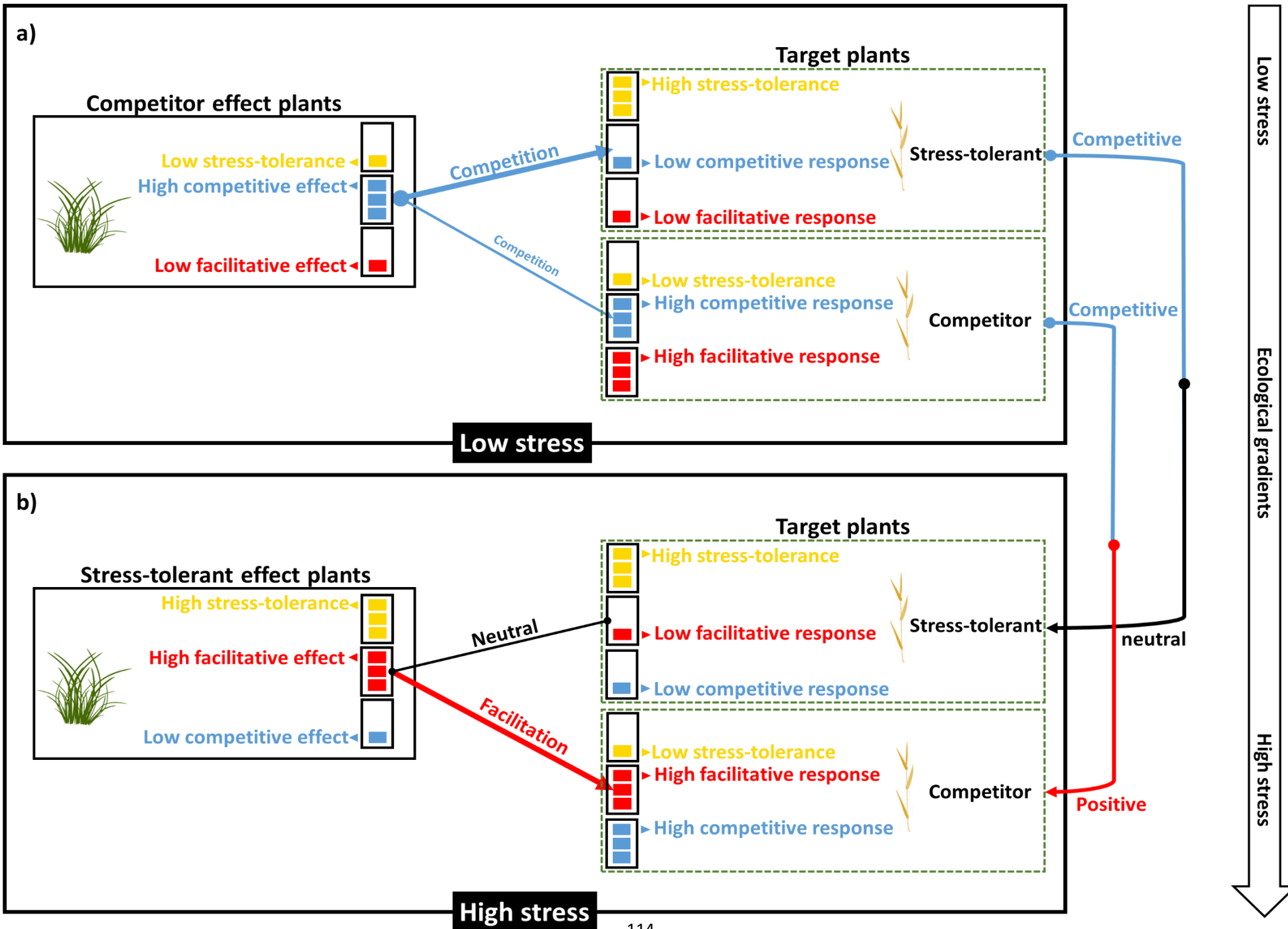


Figure 21. The relationship between tolerance to stress and plant facilitative and competitive abilities drives plant-plant interactions along ecological gradients. **(a)** At low stress levels, target plants with high stress tolerance and low competitive response abilities suffer more from competitive exclusion by neighbouring plants with high competitive effects than target plants with low stress tolerance but high competitive response abilities (**chapter 3**). **(b)** At high stress levels, target plants that have low stress tolerance but high facilitative response abilities are more facilitated by neighbouring plants with high facilitative effects than target plants that have high stress tolerance but low facilitative response abilities (**chapter 4 & 5**). In general, along ecological gradients, as stress increases, plant-plant interactions change from competitive to neutral for target plants with high stress tolerance, and from competitive to facilitative for target plants with low stress tolerance (**chapter 4 & 5**).

2. The relative contribution of environmental factors and biotic interactions

One of the main contributions of this PhD was to examine the relative contributions of environmental factors and biotic interactions (competition and/or facilitation) in structuring plant communities and determining the outcomes of plant-plant interactions.

In the first study system along the water gradient in the Gironde department, the two main selective pressures on plants were the high stressful conditions on calcareous soils and the high competitive exclusion on siliceous soils. Nevertheless, the relative effect of these selective pressures were not equally important. During the dry years, the stressful conditions on calcareous soils have led to the complete exclusion of species from siliceous origin but not the one from calcareous origin. On the other hand, species from calcareous origin suffered more from competitive exclusion by neighbours than species from siliceous origin during dry years (RII and Imp results), but competition never led to the complete exclusion of species from calcareous origin in siliceous communities. In summary, we showed in this study that along a water gradient the ability to withstand stress was likely a more important process for explaining differences in species composition between calcareous and siliceous soil than competition (**chapter 3**).

In the second metalliferous system, consistent with the observations of Michalet et al. (2014b) in alpine ecosystems, the increase in facilitation with increasing metal pollution for *Agrostis capillaris* and *Festuca gautieri* ecotype from unpolluted habitats was primarily due to an environmental-severity effect, and to a lesser degree to a marginal neighbour-trait effect (**chapters 4 and 5**). In other words, facilitation increased with increasing metal pollution because the performance of *Agrostis capillaris* and *Festuca gautieri* from unpolluted habitats with neighbours did not change significantly, but their performance without neighbours decreased significantly. Thus, the absolute difference between the performances with and without neighbours was positive, although the performance with neighbours did not change significantly. On the other hand, the increase in competition with the decrease in stress (sensu Grime, 1974), for the *Agrostis capillaris* ecotype from polluted habitats was primarily due to a neighbour-trait effect, and to a lesser degree to a marginal environmental-severity effect. Indeed, the performance of *Agrostis capillaris* from polluted habitats significantly decreased with neighbours with decreasing metal stress, while its performance without

neighbours did not change significantly. Thus, the absolute difference between the performances with and without neighbours was negative, although the performance without neighbours did not change significantly. These findings are consistent with those of Michalet et al. (2014b). In contrast, we did not detect any environmental-severity effects or a neighbour-trait effects on *Festuca rubra* from polluted habitats, as the performance with and without neighbours of this ecotype along the gradient did not vary significantly. A longer duration of our experiment might have revealed more about this ecotype's responsiveness to either effect.

In summary, our results suggest that when facilitation increases from an unstressed to a stressed environment (sensu Bertness and Callaway, 1994), this is primarily due to a worsening response to the effect of the environment and, to a lesser degree, to the improving facilitative effect of neighbours on target plants (neighbour-trait effect).

3. Implications of our results for climate change and restoration studies

In the context of climate change, global warming increases the risk of drought as water generally evaporates more quickly at higher temperatures and summer precipitation are predicted to decrease at mid-latitudes. Our findings along the water gradient driven by difference in bedrocks have important implications for studies forecasting how the climate influence plant species distributions. We highlighted in **chapter 3** that both environmental stress and competition had a negative influence on our targets, but the former effect had a more structuring influence on plant communities. Accordingly, stress-tolerant species from calcareous soils may be more favored than stress-intolerant species on siliceous soils with increasing drought with climate change. Therefore, future modeling approaches that aim for reliable predictions of plant responses to climate change should consider both plant-plant interactions and abiotic physical factors related to differences in substrate types as important predictors to incorporate into their models.

Furthermore, in this thesis, by simultaneously assessing variations in plant facilitative effects and responses in metalliferous systems, we were the first to show that stress-tolerant nurse ecotypes were the best benefactors (**chapter 5**), and less stress-tolerant target ecotypes were the best beneficiaries (**chapters 4 and**

5). These results have important implications for studies in restoration science, as positive interactions between plants have been proven to be a cost-effective technique for restoring degraded habitats. For a greater restoration success of metalliferous habitats, future restoration programs should take into account the importance of functional distance (stress tolerance and plant effect and response abilities) at the inter- and intraspecific level in an attempt to maximize facilitation and minimize competition between plants for greater restoration success. Our results imply that pairing highly metal-tolerant nurse ecotypes with less-tolerant target ecotypes should yield the best facilitative outcomes in terms of effect and response.

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Other contributions



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