Positive Plant-Plant interactions and spatial Patterns in Pyrenean Post-mine tailings

I. Pre-proposal's context, positioning and objective(s)

In France, mine tailings are witnesses of exploitation of ore bodies that took place several decades ago. For instance, in the Sentein area (100 km²) in the Pyrenees, 20 sites contain high level of heavy metals (up to several percent of Zn and Pb among others). This raises two issues. **First, tailings represent a sizeable source of contaminated material dispersible in the environment**. To limit this dispersion, costly restoration of a vegetation cover is often undertaken. But achieving recolonization success remains a challenge due to the phyto-toxic substrate, and to the harsh climatic constraints in mountains. **Second, despite toxic substrates, rare heritage plant communities spontaneously colonized tailings after the end of mining activities**. The Pyrenean endemic metallicolous lawn of *Armeria muelleri* and *Noccea caerulescens* is present on several mine tailings. This project intends to study the role of plant-plant interactions in tailings, both as a key stress system to understand variation in plant-plant interactions along stress gradients and as a likely restoration tool.

Mine tailings represent harsh environments for plant growth. When phyto-availability increases, growth limitation is due to direct phyto-toxicity and indirect impact on soil organisms involved in nutrient recycling. In such conditions, the most important expected mechanism of plant coexistence is positive interactions (i.e. facilitation) according to the "Stress Gradient Hypothesis (SGH)" [1]. The SGH is a leading theory in plant ecology which has been supported by many experiments conducted in different stressed systems worldwide [2]. It proposes that plant-plant interactions shift from negative in benign environments to positive in stressful environments where plant productivity is limited. Several case studies confirmed the occurrence of facilitation in polluted system indeed [e.g. 3]. Nonetheless, **the facilitating mechanisms have not been elucidated.**

Despite the general acceptance of the SGH, there is an **active field of research aiming at better defining its conditions of applications.** Four research directions arise from the literature: i) specifying plant-plant interactions at **extreme stress levels**; ii) disentangling the respective role of the **multiple stresses** along natural gradients; iii) characterizing the **interacting species strategies** and adaptation to stressful conditions; iv) **scaling up to the community level** the observed effects on pairs of interacting species**.** First, positive interactions between plants in most extreme environments are not systematic. It has been proposed that extreme stress can affect the growth of the benefactor species and lead to a collapse of facilitation [4]. Alternatively, when the stress factor is resources-based (e.g. water), extreme stress can lead to competition for the limiting resource [5]. Second, multiple-stressed systems are common in nature because complex geographical gradients influence different direct ecological factors affecting plant growth and physiology. Experimental designs that aim to disentangle the respective role of the different stresses have a great potential to infer the mechanisms of facilitation [6]. Third, the outcome of plant-plant interactions not only depends on the stress level, but also on the strategies of the interacting species regarding stress tolerance and resources acquisition [4]. When the stress level is important, interacting species are expected to have similar stress-tolerance abilities (S). At intermediate stress level, strategies of present species are more diverse, which can lead to different responses to the effects of neighbors [7]. These first three points can be straightforwardly addressed with experiments focusing on pairs of interacting species. However, plant-plant interactions can vary with time, in relation to meteorological variations [8], to the date of plant emergence [8] and to plant ontogeny. The ultimate proposal of the SGH is that positive interactions are a fundamental mechanism structuring plant communities in stressed environments. This justifies a complementary approach based on plant spatial distribution to propose a more integrative assessment of the long-term effects of plant-plant interactions [9]. Shift from competition to facilitation along stress gradients can be revealed by patterns of segregation or aggregation, respectively [10].

The SixP project aims to address the need to better define the conditions of application of the SGH, thanks to characterization of plant-plant interactions along phyto-toxicity gradients, an overlooked system. A second altitudinal gradient**,** where a solid knowledge regarding the SGH have been accumulated [11,12], will be considered. SixP focuses on post-mine tailings, an ideal system to address timely questions relative to plant-plant interactions. Tailings show very high metal concentrations, creating **extreme stress levels** conditions. They form **complex gradients with multiple stresses** (phyto-toxicity, indirect decrease of nutrient availability, and presence at low or high altitude). **Plant strategies occurring along these two gradients are well identified**. When productivity decreases due to cold or drought, species develop stress tolerant strategies based on nutrient conservation and slow resource acquisition. When phyto-toxicity increases, metal-tolerance strategies have also been described (e.g. metal hyperaccumulation of *Noccaea caerulescens*). The project will be implemented in four mine tailings of the Sentein Valley from low (700 m) to high altitude (2400 m). At each site, zones with null to very high phyto-toxicity will be prospected. Standard plant-plant interactions experiments will be designed and full species distribution will be determined thanks to multi-millimeter-resolution close-range airborne data analysis. This will enable full evaluation of plant-plant interactions. This combined approach remains rare because of the complexity to measure multiple species interactions. At two sites, additional experimentation will be conducted to complete the stress gradient studied: i) rainfall exclusion (at the lowest site) to exacerbate summer drought, thus simulating climate change effects; ii) uncoupling of nutrient and phyto-toxic constraints by adding fertilizers (at the second lowest site).

SixP is based on three complementary objectives and four hypotheses.
- The **first objective (O1)** is to characterize variations in plant-plant interactions along phyto-toxic gradients. Accordingly, the first hypothesis (H1) is that plant-plant interactions change according to the SGH and its recent revision focusing on extreme stress levels. We assume a collapse of facilitation in case of severe stress considering that phyto-toxicity is a non-resource stress factor [5].
- The **second objective (O2)** is to define the outcome of species interactions in case of co-occurring multiple stresses, and considering diverse plant strategies. We assume that along a phyto-toxicity gradient (H2):

- when another stress factor occurs, collapse of facilitation will occur at lower toxicity level because of the cumulative or synergic impact of the combined stresses.
- when another stress factor is alleviated (e.g. by improving nutrient availability) plant-plant interactions are not strongly modified because high toxicity remains an important limiting factor; but we expect more intense competition in case of low toxicity.

Additionally, we assume (H3) that positive plant-plant interactions are more likely to imply stress tolerant species - metal-tolerant species in conditions of phyto-toxicity - as benefactor because competitive species should not dominate in those conditions. As to beneficiary species, other stress tolerant species are probable in case of high stress, and less tolerant and more competitive species are probable at moderate stress level.
- The **third objective (O3)** is to combine airborne data and *in-situ* species characterization in a deep learning framework to ensure species mapping. We assume (H4) that using such multiple sources in a multi-task, deep neural network will allow to derive high-resolution information beyond the standard land cover mapping achieved with semantic segmentation networks (that usually fail to extract precise object contours). It will make possible the analysis of the emerging pattern of multiple plant interactions at the community scale, and illustrate the potential of Artificial Intelligence (AI) in ecology.

Studying **species interactions** rules and their role in structuring **plant communities**, SixP falls directly into the scope of the axis **"1.2 – Terre Vivante"**. SixP studies specific **ecosystems that were not disturbed for several decades**, and where **rare species** associations spontaneously occured. It will focus on **species adaptation** and modification of their **interactions** along several stress gradients, coupling **observation** of species pattern distribution with experimentations. Through its methodological contributions in computer vision, SixP has connections with the axis "5.2 – Intelligence Artificielle" and the related national priority.

II. Partnership

The project leader (PL, Florian Delerue) belongs to the multidisciplinary GE lab. He has a strong experience in project management acquired abroad in management of natural resources (Mongolia and Haïti from 2005 to 2010) before his PhD thesis. His research activities focus on the ecology of stressed environments, and more specifically on biotic interactions along productivity gradients [8,13]. He wrote and co-managed the project ECODUNE (ECOlogy of maritime pine natural regeneration in DUNE) funded by the former Aquitaine Region (2015-2018, see for instance [14]). The present project corresponds to new scientific perspectives in one of the main research topics of the GE lab: polluted sites management.
SixP is based on a consortium with expertise in ecology, metals biogeochemistry, airborne data acquisition, computer vision, and management of post-mining sites. At EPOC ecology lab, the main person involved is Richard Michalet who has been studying plant-plant facilitation for two decades in many systems, including mountain areas, producing strongly impacting research in the field of plant community ecology. At ISPA biogeochemistry lab, the main persons involved are Valérie Sappin-Didier and Christophe Nguyen. They study

the transfer of metal contaminants from the soil to the plants, and develop modelling approaches to estimate phyto-availability from soil parameters [15]. BRGM is the national geological survey of France. BRGM is responsible for monitoring and action to prevent risks and pollution arising from mine closures. The main persons involved are Gaël Bellenfant and Thomas Dewez having expertise in tailing managements, and interpretations of geomorphological data from LIDAR [16]. At L'Avion Jaune (AJ), a remote sensing company with experience in research projects for environment and geomorphology, the main person involved is the CEO Michel Assenbaum. At IRISA computer science lab, the main person involved is Sébastien Lefèvre, leading the OBELIX group in machine learning and computer vision for Earth and environment observation, especially with deep learning [17,18]. Other persons from GE will be involved to contribute to phyto-availability interpretation (Olivier Atteia) and machine learning (Samia Boukir) strengthening the collaborations between partners.

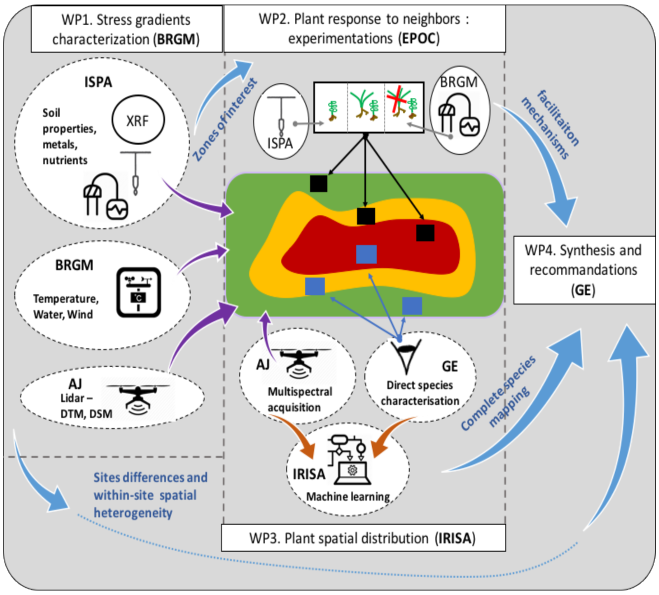

Figure 1 : Organization of the project and main responsibilities of the different partners. The colored zone represents a site with high (red) to low (green) contamination

We consider **three main tasks** to answer the project's objectives: i) to characterize the stress gradients; ii) to perform experiments on pairs of interacting species; iii) to identify plant spatial distribution. We propose a simple project structure with 3 Work Packages (WP) dedicated to these tasks. An additional coordination WP (WP0) ensure common understanding of the project objectives and methodologies. A last synthesis WP (WP4) will bring together all results regarding plant-plant interactions to conclude about the project hypotheses (Fig1).

WP0. Coordination (F. Delerue – GE lab, project leader). Coordination of the project encompasses the intern organization as well as communication with stakeholders. As to the intern organization, scientific committees will be more frequent the first year to confirm the sites of the project, to ensure a shared view of the methodology, and to ensure good scientific practices for data acquisition and publication. Noteworthy, the PL will finalize the identification of the most suitable sites before it starts (i.e. in 2019) thanks to a dedicated internship. During the rest of the project, yearly scientific committees will ensure that the different tasks are progressing and that any adaptation is compatible with the other WPs. As to communication with stakeholders, it has already started with information of the Ministry of the Environment (and the mining police) and the Regional Direction of the Environment (DREAL) about the SixP project. Regular meetings will be planned with local officials, managers of the Regional Natural Parc of the Pyrénées Ariégoises, and herders to inform them about the project and potential recommendations. In addition, the PL will stimulate exchanges with other teams interested in metallicolous lawns (e.g. Maxime Pauwels, Evo-Eco-Paleo lab of Lille University).

WP1. Stress gradients characterization (G. Bellenfant - BRGM). This WP encompasses: i) information about soil physico-chemical properties including metals phyto-availability (ISPA); ii) meteorological instrumentation of each investigated site (BRGM), iii) drone-borne LIDAR to build multi-centimeter Digital Terrain Models and Digital Surface Models (DSM) (AJ). These models will enable mapping of geomorphic context and assessment of plant productivity (spring and summer DSM will show volumetric plant growth). The first year of the project, characterization of metals content (X-Ray Fluorescence method) will enable identification of zones suitable for WP2 experiments. Phyto-availability will be characterized in a second step.

WP2. Plant response to neighbors: experiments (R. Michalet, EPOC lab). A PhD under the supervision of R. Michalet and the PL is planned and will start the second year of the project (once the zones of interest will be identified in WP1). All pairs formed by four species with contrasted strategies found in each site will be scrutinized. Two species from contaminated areas (metallophytes) and two species from peripheral areas (stress tolerant or more competitive according to the pedoclimatic context) will be chosen. Effects of one potential benefactor species on the three-other species will be investigated with conventional methods in facilitation studies (neighbors removal experiments, transplantation in similar zones with and without neighbors). The alleviation of the stress factors by benefactor species will be characterized. Soil and air humidity and temperature probes will be used and soil collection in the rhizosphere of hyperaccumative species will help to evaluate the potential phytoprotection (i.e. the decrease of metal phyto-availability for the beneficiary species as shown for *Noccea caerulescens* in controlled condition [19]). In sum, the intensity and mechanisms of facilitation will be identified in this WP.

WP3. Plant spatial distribution (S. Lefèvre, IRISA lab). This WP aims to give a complete view of species distribution on the sites, from which will be derived plant spatial patterns. Knowledge extraction will rely on high resolution multispectral imagery (RGB+NIR) acquisition (AJ) to build sub-centimeter-scale ortho-images and *in-situ* recognition of species (PL). These data sources will feed deep learning models designed by IRISA for mapping task. Given the complexity of objects under study (individual plants of different species and mix of different species), this WP requires a postdoctoral position in computer science (under the supervision of S. Lefèvre). The postdoctoral fellow will work in association with the AJ company to develop its skills in AI for vegetation monitoring. He/she will have the opportunity to integrate the company by the end of the project.

WP4. Synthesis and recommendations (F. Delerue, GE lab). Interpretation of plant spatial patterns will be put in regards of site heterogeneity (soil parameters and vegetation productivity) measured in WP1. The main objective of this WP is to evaluate if facilitative mechanisms identified in WP2 are integrated and visible at the community level, when considering larger time scales and multiple species interactions. Finally, by the end of the project, we aim to propose several species associations and species strategies associations to be tested to restore or improve vegetation cover in mine tailings or metal polluted sites.

III. References (**Scientists** that are member of the consortium)

1. Bertness MD, Callaway R. Positive interactions in communities. Trends Ecol Evol. 1994;9: 191–193. online access
2. He Q, Bertness MD, Altieri AH. Global shifts towards positive species interactions with increasing environmental stress. Ecol Lett. 2013;16: 695–706. online access
3. Domínguez MT, Madejón E, López-Garrido R, et al. Shrubs for the remediation of contaminated Mediterranean areas: Is the nurse effect mediated by increases in soil enzyme activities? Ecol Eng. 2016;97: 577–581. online access
4. **Michalet R**, Brooker RW, Cavieres LA, Kikvidze Z, Lortie CJ, Pugnaire FI, et al. Do biotic interactions shape both sides of the humped-back model of species richness in plant communities? Ecol Lett. 2006;9: 767–773. online access
5. **Michalet R**, Le Bagousse-Pinguet Y, Maalouf J-P, Lortie CJ. Two alternatives to the stress-gradient hypothesis at the edge of life: the collapse of facilitation and the switch from facilitation to competition. J Veg Sci. 2014;25: 609–613. online access
6. He Q, Bertness MD. Extreme stresses, niches, and positive species interactions along stress gradients. Ecology. 2014;95: 1437–1443. online access
7. **Michalet R**, Chen S, An L, Wang X, Wang Y, Guo P, et al. Communities: are they groups of hidden interactions? J Veg Sci. 2015;26: 207–218. online access
8. **Delerue F**, Gonzalez M, **Michalet R**, Pellerin S, Augusto L. Weak Evidence of Regeneration Habitat but Strong Evidence of Regeneration Niche for a Leguminous Shrub. PLoS ONE. 2015;10: e0130886. online access
9. Schöb C, Kammer PM, Kikvidze Z. Combining observational and experimental methods in plant–plant interaction research. Plant Ecol Divers. 2012;5: 27–36. online access
10. Haase P. Can isotropy vs. anisotropy in the spatial association of plant species reveal physical vs. biotic facilitation? J Veg Sci. 2001;12: 127–136. online access
11. Callaway RM, Brooker RW, Choler P, Kikvidze Z, Lortie CJ, **Michalet R**, et al. Positive interactions among alpine plants increase with stress. Nature. 2002;417: 844–848. online access
12. **Michalet R**, Schöb C, Lortie CJ, Brooker RW, Callaway RM. Partitioning net interactions among plants along altitudinal gradients to study community responses to climate change. Funct Ecol. 2014;28: 75–86. online access
13. **Delerue F**, Gonzalez M, Achat DL, Puzos L, Augusto L. Competition along productivity gradients: news from heathlands. Oecologia. 2018;187: 219–231. online access
14. Guignabert A, **Delerue F**, Gonzalez M, Augusto L, Bakker MR. Effects of Management Practices and Topography on Ectomycorrhizal Fungi of Maritime Pine during Seedling Recruitment. Forests. 2018;9: 245. online access
15. Cornu J-Y, Denaix L, Lacoste J, **Sappin-Didier V**, **Nguyen C**, Schneider A. Impact of temperature on the dynamics of organic matter and on the soil-to-plant transfer of Cd, Zn and Pb in a contaminated agricultural soil. Environ Sci Pollut Res. 2016;23: 2997–3007. online access
16. **T. J. B. Dewez**, J. Leroux, S. Morelli. Cliff collapse hazard from repeated multicopter UAV acquisitions: return on experience. Int Arch Photogramm Remote Sens Spat Inf Sci. 2016;XLI-B5. online access
17. **Lefèvre S**, Tuia D, Wegner JD, Produit T, Nassar AS. Toward Seamless Multiview Scene Analysis From Satellite to Street Level. Proc IEEE. 2017;105: 1884–1899. online access
18. Audebert N, Le Saux B, **Lefèvre S**. Beyond RGB: Very high resolution urban remote sensing with multimodal deep networks. ISPRS J Photogramm Remote Sens. 2018;140: 20–32. online access
19. Whiting SN, Leake JR, McGrath SP, Baker AJM. Hyperaccumulation of Zn by Thlaspi caerulescens Can Ameliorate Zn Toxicity in the Rhizosphere of Cocropped Thlaspi arvense. Environ Sci Technol. 2001;35: 3237–3241. online access