Ecological Rhizosphere Management for Enhanced Nutrient Efficiency, Stress Resilience and Biodiversity in Sustainable Agro(ecosystems)

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I agree that my report will be posted on the Co-operative Research Programme's website.

1. Major objectives and relevance of the research project

In this project, we explored how intercropping of grasses with different legume genotypes improves nutrient-acquisition efficiency and drought stress resistance through interspecific root interactions. The rationale behind this contention is that some legumes release carboxylates under nutrient (P, Fe, Mn) deficiency that mobilise phosphorus and trace nutrients from sparingly soluble element forms in soil. The carboxylates released mobilise not only essential elements but also an array of non-essential elements with potentially beneficial effects on plant growth under stress conditions such as silicon; however, the legumes cannot utilise the mobilised silicon due to a lack of transport mechanisms. Conversely, grasses cannot respond to nutrient deficiency with increased carboxylate release but can efficiently accumulate silicon in plant tissues that can alleviate drought stress. We hypothesised that intercropping cereals with legume genotypes characterised by high carboxylate release could improve overall nutrient efficiency and stress resistance in agroecosystems, especially on marginal soils with low element availability. If proven, this would have major implications for developing sustainable cropping systems in the face of climate change and finite fertiliser (phosphorus) resources. Thus, the primary objectives of this project were:

- Assessment of the availability of nutrients and beneficial elements in agriculturally used soils near Beauvais (France) and determination of the elemental composition of crop plants across these sites
- Evaluation of silicon dose-effect responses in crop species and legume genotypes under drought stress.
- Profiling of root exudates released by the plant species and genotypes under P deficiency and drought stress conditions.
- Evaluation of the ecological relevance of element fluxes between P-efficient legumes and grasses under conditions of nutrient deficiency and drought stress by comparing the performance of monocultures with mixed-cultures and crop rotation systems.

2. Results/Achievements

2.1 Assessment of element availability

In total, 45 soil samples and 90 plant samples were collected on agricultural field sites across the area of Beauvais. The samples were characterised regarding the total element concentrations. Given that total concentrations do not necessarily reflect plant availability, the samples are currently sequentially leached to characterise the distribution of nutrients and beneficial elements in potentially plant-available soil fractions. The sequential extraction is very time-consuming and is still ongoing. Preliminary results on the elemental composition of the collected plants show relatively low phosphorus and silicon contents in at least half of the sampling sites, suggesting a low element availability in the soil. The complete dataset on the distribution of elements in soil fractions will answer this research question conclusively.

2.2 Evaluation of silicon dose-effect responses

In a greenhouse, ten cultivars of *Lupinus albus*, *Sorghum bicolor*, *Zea mays*, *Triticum aestivum*, *Phalaris arundinacea* and *Festuca arundinacea* were cultivated on acid-washed quartz sand (Fig.

1) under conditions of different water supply (water content: 7% or 18%) and treated with nutrient solutions containing 0 mM Si (Reference), 0.04 mM Si; 0.2 mM and 1 mM Si (Fig. 1A). After eight weeks of plant growth, the shoots and roots were harvested. The shoot and root dry mass and water contents were determined. Fresh subsamples were stored at -80 °C for analysing enzyme activity. Dried samples were prepared for trace element analysis using ICP-MS. The ICP-MS device at UniLasalle had technical problems. Thus, the shoot element contents will be analysed at TU Freiberg until November 2023. The measurement of enzyme activity in plants is still ongoing at UniLasalle, and the data will be available in December 2023.
Considering data on biomass development, grasses and forbs responded very differently to the changes in Si availability, and this response clearly depended on the drought regime. Although lupin is considered a non-Si accumulator, adding low doses of Si (0.04 mM Si) increased shoot dry mass significantly compared to the reference plants, but only when the plants were sufficiently supplied with water (Fig. 1B). In drought-stressed legumes, there was no effect of Si on plant growth, nor did an increase in Si supply in adequately water supplied grasses have positive effects on biomass (Fig. 2). Surprisingly, *Z. mays* did not respond to changes in Si supply, irrespective of water supply. However, under drought stress, *S. bicolor* and the C3 grasses *T. aestivum*, *F. arundinacea*, and *P. arundinacea* showed significantly improved growth following increased Si supply with maximum plant biomass when 1 mM Si was added with the nutrient solution (Fig. 1C, D; Fig. 2).

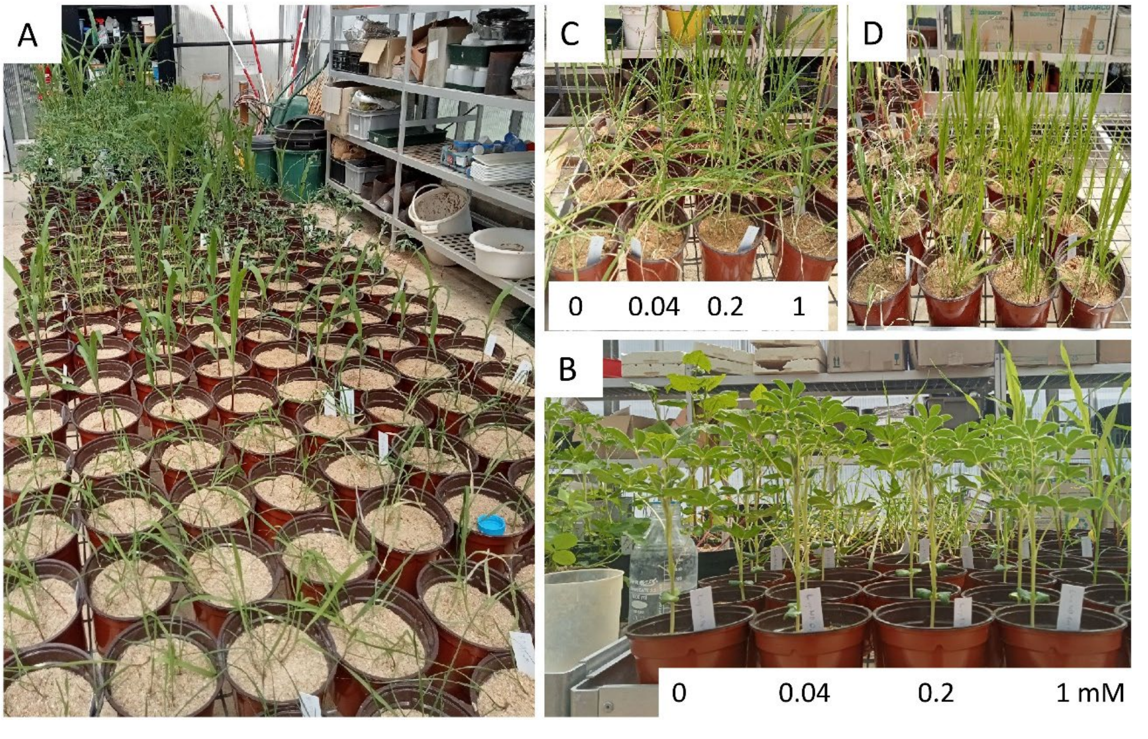

Fig. 1 A) Effect of Si availability (0 mM Si, 0.04 mM Si; 0.2 mM Si and 1 mM Si) on biomass and nutrition status of ten cultivars of *Lupinus albus*, *Sorghum bicolor*, *Zea mays*, *Triticum aestivum*, *Phalaris arundinacea* and *Festuca arundinacea* under conditions of drought (7% water content) or adequate water supply (18% water content). B) Effect of Si addition on adequately water-supplied *L. albus*; Effect of Si on drought-stressed *T. aestivum* (C) and *P. arundinacea* (D).

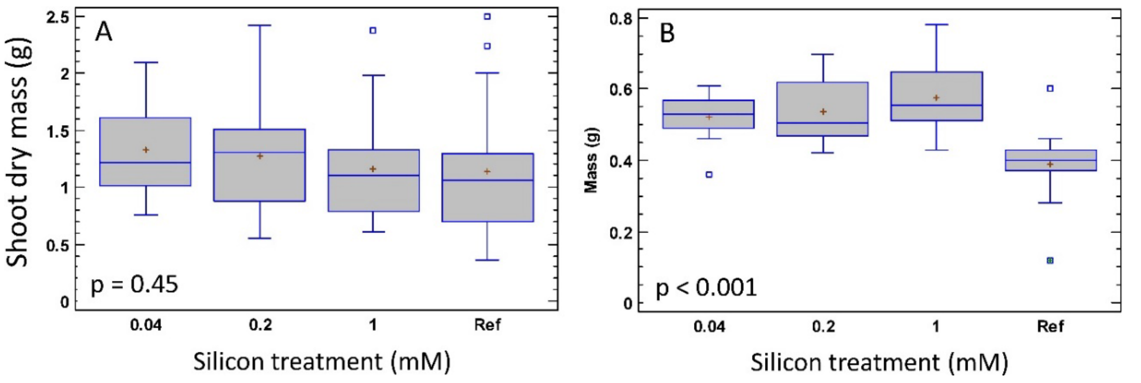

Fig. 2 Effect of Si addition on shoot biomass of *T. aestivum* (n = 5) without drought stress (A) or under drought conditions (B).

2.3 Profiling of root exudates

Fifteen cultivars/genotypes of six agricultural-relevant legumes and three selected grass species obtained from Deutsche Saatveredelung AG (DSV) were characterised regarding their root exudate composition under P deficiency and drought stress conditions. This screening included five genotypes of *Trifolium pratense* (e.g., Harmonie, Diplomat, Milvus, Saphir and Semperina), two genotypes of *Melilotus spec.*, *Lupinus albus*, *Medicago sativa*, *Festuca arundinacea, Zea mays, Phalaris arundinacea Sorghum bicolor*, Triticum aestivum and *Fagopyrum esculentum*. The plants were cultivated on acid-washed sand and treated with different levels of phosphate (P) (0.1 mM P, 0.01 mM P) in the nutrient solutions (5 replicates per treatment and species/genotype). After five weeks of growth, the plants were transferred to exudate collection vessels filled with 0.05 mM $CaCl_2$. After 1 h, the root exudat composition was determined by IC and HPLC.

The results on carboxylate release showed substantial differences between species and clover genotypes in their response to altered P-supply. *Medicago sativa*, *L. albus* and *Z. mays* showed the highest carboxylate release among all species (Fig. 3). The *L. albus* genotype responded to lower P supply with lower carboxylate release. In contrast, *M. sativa* released more carboxylates when the P-supply was low (Fig. 3). The red clover (*T. pratense*) genotypes Diplomat, Saphir and Semperina responded to a lower P-supply with declining carboxylate release. In contrast, when P-supply was low, the genotypes Harmonie and Milvus showed a higher carboxylate release. Notably, the genotype Harmonie showed the highest carboxylate release among all clover genotypes (Fig. 3). Moreover, the sweet clover genotypes (Melilotus 187014 and Melilotus 187030) showed similar carboxylate release compared to red clover; however, 187014 did not respond to changes in P supply whereas genotype 187030 released more carboxylates when P supply was low. All investigated legumes released predominantly di- and tricarboxylates (malate, oxalate and citrate), whereas cereals and grasses solely released dicarboxylates (malate, succinate, fumarate), indicating a higher nutrient acquisition efficiency of legumes.

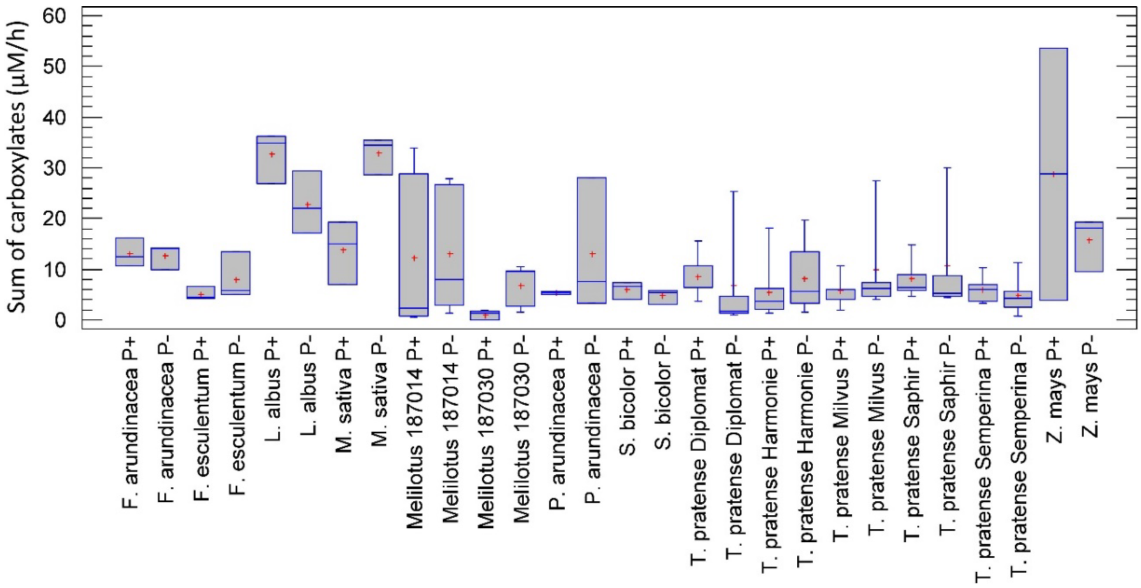

Fig. 3 Sums of carboxylates (µM citrate, malate, malonate, acetate, succinate, fumarate per unit time) released by plant roots of the investigated species and genotypes (n = 30) under conditions of P supply (P+) or P deficiency (P-).

2.4 Mixed cultures and crop rotation

Based on results from root exudate profiling, the legumes *T. pratense* cv. Harmonie, *Melilotus spec.* cv. 187014, *L. albus* and the grasses *S. bicolor*, *P. arundinacea* and *T. aestivum* were cultivated in monocultures and mixed cultures (30% legumes and 70% grass) on soil with low P-availability (pH 7.8). In parallel, crop rotation trials were conducted where the grasses were cultivated on unplanted soil or in sequence after the legumes. Micro-suction cups were installed in 20 cm soil depth to monitor changes in soil solution chemistry depending on culture form. Each treatment was set up under drought conditions (9 ± 4 % water content) or sufficient water supply (19 ± 6% water content). Each treatment was five-fold replicated. After eight weeks of growth, the shoots were harvested and dried for subsequent element analysis. Due to many samples, the analysis of plant samples from both experiments is still ongoing and will be accomplished by December 2023.

Compared to the monocultures, the presence of grasses (in this example, *T. aestivum*) decreased the biomass of both legumes *Melilotus spec.* and *T. pratense*, respectively, irrespective of water supply (Fig. 4A, 4B, 4D, 4E). This effect was most substantial when the plants suffered water deficiency (Fig. 4D, 4E). The legumes did not influence the growth of the grass when the plants were adequately supplied with water (Fig. 4C). However, under drought conditions, the grass developed significantly higher biomass when cultivated neighbouring to the legumes (Fig. 4F). The results on shoot elemental composition, enzyme activity and leaf water contents will clarify if these results derive from a better water supply and/or an improved nutritional status.

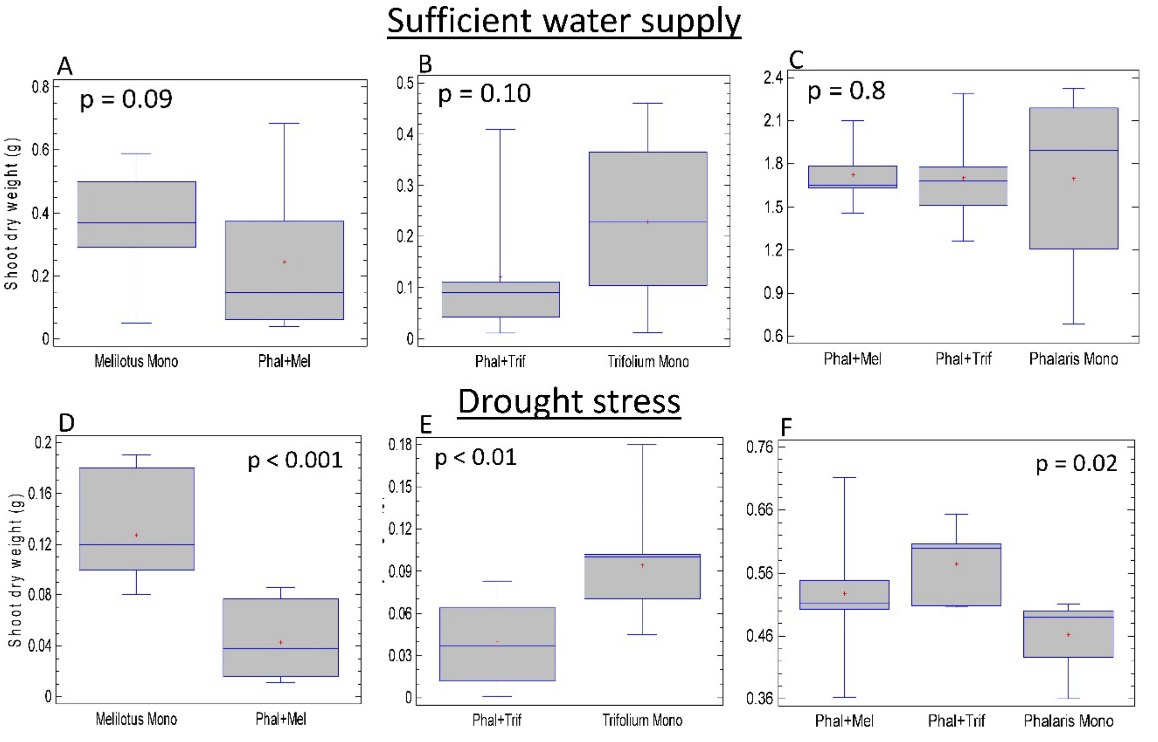

Fig. 4 Shoot biomass of Melilotus spec. cv. 187014, *Trifolium pratense cv. Harmonie and T. aestivum* in monocultures (Mono) and mixed cultures (Phal+Mel, Phal+Trif) under conditions of sufficient water supply (A, B, C) or drought stress (D, E, F). A, B, D, E shoot biomass of the legumes in monoculture and mixed culture; C, F shoot biomass of *T. aestivum* in monoculture and mixed culture.

3. Significant achievements of the fellowship

- Low silicon doses promote legume growth only when the plants are sufficiently supplied with water. In grasses, elevated silicon supply has no effect when the plants are adequately supplied with water. However, under drought stress, silicon alleviates drought stress, and the growth-promoting effect increases with increasing silicon availability.
- Legume species/genotypes strongly differ in their response to P deficiency. Under P deficiency, carboxylate release decreased in the order *M. sativa*, *L. albus* > *Melilotus spec.* > T. *pratense* and shows a high variability among *T. pratense* and *Melilotus spec.* genotypes.
- Intercropping of *T. aestivum*, *P. arundinacea* and *S. bicolor* with P-efficient *Melilotus spec.* cv. 187014 and *T. pratense* cv. Harmonie led to a higher drought tolerance compared to the monocultures.

4. Follow-up work

The analysis of samples and interpretation of the data obtained is not yet finished. The ongoing work is coordinated in regular online meetings. The promising results obtained under controlled conditions will be extrapolated to large-scale field conditions in the upcoming growth period, requiring a second research stay, which is envisaged next year. Thus, my research stay at UniLasalle formed a sound basis for a close collaboration between the research group AGHYL, UniLaSalle and the Institute of Biosciences, TU Freiberg. During my scholarship, I completed two publications, which will appear in the journals Plant and Soil and Environmental Science and

Pollution Research. I credited the funding program and my role as an OECD research fellow in these publications. In addition, at least two publications with results from the current research at UniLaSalle are prepared to be submitted to Plant and Soil, Journal of Environmental Management and/or New Phytologist until May 2023. Moreover, in April 2024, the results will be presented at the European Geoscience Union General Assembly in Vienna.

Publications submitted:

- Wiche O, Pourret O (2023) The role of root carboxylate release on rare earth element (hyper)accumulation in plants – a biogeochemical perspective on rhizosphere chemistry. Plant and Soil (in press). https://doi.org/10.1007/s11104-023-06177-2
- Zaffar N, Peiter E, Schirmer D, Samarska A, Lovynska V, Wiche O (under review) Effect of sewage sludge and digestate from anaerobic fermentation as soil additives on the nutritional status and accumulation of non-essential elements in plants with different nutrition strategies. Environmental Science and Pollution Research. 10.21203/rs.3.rs-3381617/v1

Forthcoming articles:

- Wiche O, Firmin S, Pourret O (2024) How root carboxylate release alleviates drought stress in legume–grass intercropping through interspecific root interactions (Journal of Environmental Management).
- Wiche O, Firmin S, Pourret O (2024) Comparing the effects of altered Si availability on growth and nutrient acquisition in legumes and grasses (Plant and Soil or New Phytologist).

5. Practical relevance of the results obtained

Higher average global temperatures, extreme weather events associated with climate change, and finite phosphorus resources for fertiliser production will seriously impact agriculture and food production. In this project, we could show that mixed culture cropping positively influences nutrient efficiency and crop plants' drought resistance, enabling crop production on marginal soils and in areas with a high probability of severe drought periods. Besides the development of drought-tolerant cereals, breeding legume genotypes most suitable for legume–cereal intercropping could reduce year-to-year yield fluctuations and improve food production reliability. The experimental seeds of clover genotypes were obtained from the Deutsche Saatveredelung AG. The published results will support trait-based plant breeding and the development of nutrient-efficient and drought-tolerant cropping systems.

6. Relevance to the objectives of the CRP

The cross-disciplinary research program covered all aspects of the three CRP pillars. The project was focused on sustainable agriculture in the face of climate change through sustainable management of natural resources, improved resilience in food and biomass production, and the development of novel rhizosphere-centred management techniques. Thus, the project addresses social, economic, and environmental topics required for a sustainable agriculture standard. The results will contribute to a paradigm shift in trait-based plant breeding and the implementation of novel, innovative agricultural practices targeting food and resource security in the context of globalisation and climate change.

7. Satisfaction

The fellowship exceeded my expectations. As a scientist, I always dreamt of going abroad and studying this research topic in a team of world-rank scientists. The fellowship allowed me to collaborate with reputed scientists and establish a close collaboration between my institute and the host institution. There is no dought that the OECD fellowship enormously improved my career opportunities, given that the OECD is an honourable institution, the results obtained will be published in at least two publications and orally presented at a world-rank conference. Shortly after my research stay in France, I was offered a position as an associate professor of applied geoecology at HS Zittau/Görlitz, Germany. I am convinced that the fellowship contributed to the positive evaluation during the assessment process.

8. Advertising the Co-operative Research Programme

I learned about the CRP via the program flyer sent by the head of our institute. I believe the most effective way to advertise the program is by sending a flyer to institutions and former fellows, which will spread the information in their professional environment.