Schlussbericht

Zuwendungsempfänger:	Förderkennzeichen:
Technische Universität München - TUM School of Life Sciences - Holzforschung München - Professur für Pilz-Biotechnologie in der Holzwissenschaft Hans-Carl-von-Carlowitz-Platz 2, 85354 Freising	**03LC2026A**

Vorhabenbezeichnung:

Verbundprojekt BiodivERsA: Innovative biotechnologische Strategien zur Verbesserung der Trockentoleranz und bodenmikrobiologischer Diversität bei Bäumen in der Wiederaufforstung

(**RESTORE**)

Laufzeit des Vorhabens:

01.04.2021 – 31.03.2025

Berichtszeitraum:

01.04.2021 – 31.03.2025

List of partners involved at the end of the project (company/organisation and principal investigator).	P1 - State University of Londrina, Brazil (UEL), PI: Halley Caixeta Oliveira P2 - Helmholtz Zentrum Muenchen, Germany (HMGU), PI: Michael Rothballer; Karin Pritsch P3 - Technical University of Munich (TUM), PI: J. Philipp Benz P4 - CNRS Research Federation ECCOREV FR3098, France (ECCOREV), PI: Ilja Reiter P5 - UMR7265 CEA CNRS AMU Institute of Biosciences and Biotechnology Aix-Marseille University, France (BIAM), PI: Catherine Santaella P6 - State University of Ponta Grossa (UEPG), PI: Jesiane Stefânia da Silva Batista P7 - State University of Maringá (UEM), PI: Lindamir Pastorini P8 - State University of Northern Paraná (UENP), PI: Alexandre Oliveira Fernandes da Silva PA - São Paulo State University, Brazil (UNESP), PI: Leonardo Fernandes Fraceto PB - University of Sorocaba, Brazil (UNISO), PI: Renata de Lima PC - Federal University of ABC, Brazil (UFABC), PI: Amedea Barozzi Seabra

GEFÖRDERT VOM

Bundesministerium für Bildung und Forschung

Erklärung: sämtliche Urheber/innen des Sachberichts gestatten dem Zuwendungsgeber die Nutzung.

Freising, den 02.06.2025

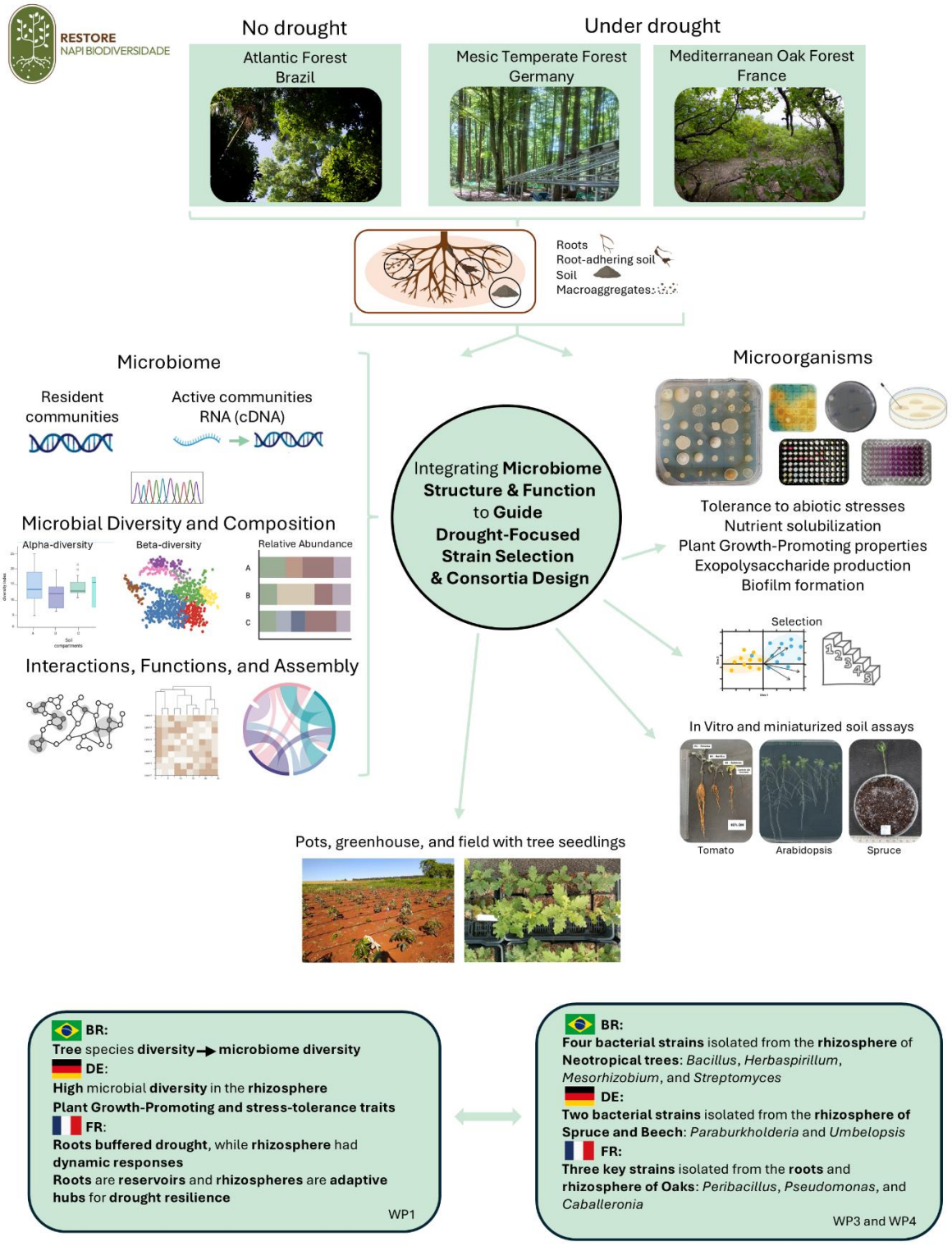

Fig. 1: Project overview

Inhaltsverzeichnis

Bemerkung:

Teil I (Kurzbericht) und II (Eingehende Darstellung) basieren auf dem Schlussbericht des Gesamt-Konsortiums, welcher (in englischer Sprache) am 5.5.2025 an BiodivERsA geschickt wurde. Teile, die spezifisch das deutsche Projekt betreffen, wurden in blau hinterlegt.

I. Kurzbericht

1.1. Public summary

The RESTORE project adopted a multidisciplinary and transnational approach to improve forest restoration under climate change, focusing on the responses of soil microbiota and tree seedlings to drought, microbial applications, the development of biomaterials, and stakeholder engagement. The analysis of soil and root microbiomes across three forest types—Brazil's Seasonal Semideciduous Atlantic Forest, France's Deciduous Mediterranean Oak Forest, and Germany's Mesic Temperate Forest—uncovered rich diversity and nuanced environmental responses. These microbiome insights drove targeted bioprospecting, resulting in over 2,800 bacterial and fungal isolates that were phylogenetically and functionally characterized for subsequent strain selection. The microbiome and screening data allowed the selection of strains with traits related to plant growth promotion and stress tolerance, which were tested on tree seedlings using different inoculation and drought-imposing protocols. For this, the responses of seedlings of almost 40 tree species to drought were screened by evaluating morphophysiological traits. In parallel, innovative nature-based materials were developed to deliver these microorganisms and plant growth regulators, such as biodegradable materials (composite foams, hydrogels and germination trays) made from agro-industrial residues. Nitric oxide-releasing chitosan nanoparticles, gibberellic acid-loaded alginate/chitosan nanoparticles and abscisic acid-containing lignin-zein nanoparticles have also been tested as effective tools to improve plant growth, development and/or stress responses. Field trials and a cost-benefit analysis confirmed the viability of nitric oxide-releasing nanoparticles in enhancing carbon assimilation and reducing seedling mortality in forest restoration efforts. The project benefited from the involvement of various stakeholders, mainly restoration practitioners who plan, implement and manage restoration projects. In Brazil, after mapping these priority stakeholders, a survey was conducted to assess the acceptance of nature-based solutions related to the project, as well as the needs and challenges faced by nurseries producing native seedlings. In addition, the general public and the scientific community were reached through various dissemination activities of the project thematic and results. Thus, in addition to contributing to the basic knowledge of the mechanisms of drought responses of trees and soil microbiota, the work carried out in RESTORE contributed to the improvement of reforestation programs through the use of nature-based solutions, with significant environmental, economic and social impacts.

1.2. Objectives of the research

The main objective was to explore plant-microbiota interactions in forest ecosystems facing increased drought stress due to climate change, allowing the development of biotechnologically-aided nature-based solutions to improve forest restoration initiatives. The project comprised the following specific objectives:

- evaluate the effect of drought stress on native trees and associated microbiota of European and Brazilian forest ecosystems;
- isolate microbial strains, capable of mitigating drought-related stress and/or promoting growth, from drought-stressed tree roots for their functional characterization;
- test the application of different plant associative microorganisms (both known and newly isolated) for increasing the tolerance of tree seedlings to drought stress aiming at the improvement of forest restoration;
- test the application of nature-based materials as carrier systems for plant associative microorganisms and plant growth regulators for increasing the tolerance of tree seedlings to drought stress and restructuring of tree-associated microbial communities aiming at the improvement of forest restoration;
- evaluate the social and economic cost-effectiveness of elaborated solutions.

1.3. Project activities and achievements

WP 1. Drought effects on soil microbiota

In Brazil, several attempts to determine the implications of drought on soil microbial diversity were carried out by P1. However, atypical rain regimen in the sampling years impaired the in-situ evaluation of the effects of drought on soil microbial diversity of Atlantic Forest fragments in Northern Paraná. In parallel, P6 evaluated the influence of biotic and abiotic soil factors on microbial communities of the Iguazu National Park, which is the second largest non-Amazonian tropical forest park in Brazil. The results pointed out that tree diversity drives soil bacterial community structures.

In Germany, for the analysis of the resident microbial soil diversity, root and root-adhering soil samples were collected by P2 and P3 at two mixed temperate forest sites (Norway spruce and European beech), in southern Germany (Kranzberg and Kelheim, which have different humidity conditions in the soil), after a drought period. Next Generation Sequencing (NGS) analyses revealed a broad on-site diversity. Combined with a functional prediction analysis, the metabarcoding approach indicated multiple plant growth-promoting and stress resistance traits to be present.

In France, long-term amplified versus natural drought effects on resident and active soil microbiomes were assessed by P4 and P5 in a Mediterranean oak forest by seasonally sampling roots and rhizospheres over two years. Root microbiomes remained stable with delayed richness gains under amplified drought, indicating buffering, while rhizospheres responded dynamically to drought and rewetting. Machine learning identified drought-adapted taxa. Integrated structural, functional, and predictive analyses showed roots as diverse microbial reservoirs and rhizospheres as flexible, compartment-specific contributors to drought resilience.

WP 2. Drought effects on trees

The partners have tested different methods to impose the drought conditions to the tree seedlings. In Brazil, by using *Cecropia pachystachya* and *Cariniana estrellensis* as plant models, P1 tested the gravimetric control of soil water content for moderate drought and the complete withholding of irrigation for severe drought. P1 also conducted a screening of the drought responses of seedlings of 34 tree species native to Atlantic Forest, by using the capillarity method adapted from Marchin et al. (Front Plant Sci 10:1715, 2020). In France, P4 and P5 observed that the control of soil moisture by weighing makes it possible to expose holm oak, downy oak and Aleppo pine seedlings to controlled, moderate or severe drought over periods of several days up to months. In collaboration with the ONF-PNRGF stakeholder, P4 and P5 were also able to develop a 4-line experimental setup, where all trees experience the same edge conditions, allowing to mimic better a reforestation setup. In Germany, different systems have been developed for axenic *Picea abies* cultivation, such as an agar plate system and a new glass tube system. In all countries, various traits have been measured to evaluate the seedling responses to drought, such as the evolution of wilting and necrotic symptoms, relative water content, shoot water potential, stomatal conductance, photosystem II activity, oxidative stress markers and morphometric traits (mass, length and diameter of plant organs).

The discussion among the partners has indicated that each method has its advantages and disadvantages and that the most suitable method varies according to objective of the experiment. For example, for formulations that induce rapid responses in plants (as the NO-releasing nanoparticles), the rapid and severe drought is a good model to provide the results of stomatal conductance, photosystem II activity and symptom evolution of a high number of tree species quickly. However, for the inoculation of microbial strains, long-term drought assays are necessary. The axenic cultivation is suitable for the rapid screening of the effects of microbial strains on plants, although pot experiments in greenhouses would be necessary with the selected strains to confirm the *in vitro* data.

WP 3. Isolation and characterization of plant associative microorganisms (PAMs) + WP 4. Application of PAMs to induce drought tolerance in tree seedlings

(The results of WP 3 and WP 4 are presented in a single topic, as it facilitates the understanding.)

In Brazil, a total of 516 bacterial strains were isolated by P1 from the rhizosphere of native Atlantic Forest trees. The bacterial isolates from this collection were fully characterized for the phylogenetic position and potential traits related to osmotic tolerance and plant growth-promotion (PGP). The screening led to the selection of 20 candidates, from which 12 were evaluated in planta through inoculation trials using different plant species (Atlantic Forest trees as well as crop species). The results indicated that at least 4 new bacterial strains presented high potential to be used as bio-input to improve tree seedlings production and survival under drought conditions. In parallel, experiments with 2 bacterial strains obtained from agricultural soil - *Azospirillum brasilense* (Ab-V5) and *Bacillus velezensis* (ZK) - demonstrated the potential of co-inoculation in inducing more positive effects on seedling growth. Additionally, inoculation protocols with the same strains have been developed for *C. pachystachya* and *C. estrellensis* seedlings.

In Germany, spruce/beech roots and adhering soil were sampled by P2 and P3 for the isolation of single strains (bacteria/fungi) on different media. Sanger sequencing was performed for the identification of part of the isolates on a genus level. Based on literature research, 40 bacteria and 20 fungi were chosen for further characterization in different *in vitro* assays assessing their stress resistance and PGP potential. For this, we developed a scoring system with high scores standing for a better performance in the *in vitro* assays and application of isolates with high scores to spruce seedlings. Promising microbes were applied to tree seedlings in axenic and non-axenic plant experiments under well-watered and drought conditions. The rhizosphere competence of bacteria and fungi were evaluated by CFU count, laser-scanning microscopy and epifluorescence microscopy.

In France, synthetic microbial communities (SynComs) were developed by P4 and P5 from strains isolated at O3HP Mediterranean forest in a plot under ten years of rain exclusion. Sampling roots, root-adhering and non-adhering soil, and root-driven macroaggregates captured niche diversity, focusing on exopolysaccharide (EPS) production for water retention. From 1,000 clones, 58 mucoid isolates were screened for EPS, phytohormone, siderophore, and osmotic-tolerance traits. Multivariate analysis selected the top 12 strains, assembled into four-strain consortia, tested for biofilm formation and growth promotion on Arabidopsis, then narrowed to four SynComs and four strains for greenhouse drought assays on *Quercus pubescens* and *Sorbus domestica*. One EPS-rich SynCom increased oak tolerance by 47%, and a hormone-producing mix reduced Sorbus symptoms by 71%. Predictive models identified strain identity and symptom-onset timing as key drought-mitigation predictors, with stem diameter increment as a physiological indicator. This niche- and function-informed approach offers a blueprint for climate-adaptive forest restoration.

WP 5. Nature-based materials as carrier systems for PAMs and plant growth regulators (PGRs)

Different types of innovative nature-based materials have been tested by the Brazilian team. P1 tested the effectiveness nitric oxide (NO)-releasing chitosan nanoparticles synthesized by PC in inducing the tolerance of Atlantic Forest tree seedlings to drought stress. P1 and P8 evaluated the effects of seed priming with gibberellic acid-loaded alginate/chitosan nanoparticles developed by PA and PB in inducing the early development of *C. estrellensis* and *C. pachystachya*. Different biodegradable composite materials (hydrogels, composite foams, and germination trays) were developed by P1 based on agroindustry wastes and designed to deliver PGP bacteria and bioactive compounds aiming to mitigate drought stress and improve the plant growth. These nature-based solutions were subjected to patent deposition and were also evaluated as bio-input for different plant species and growth conditions to validate the biotechnology. From these nature-based materials, NO-releasing nanoparticles were chosen for validation under field conditions using *Hymenaea courbaril* and *Amburana cearensis* seedlings.

In the studies carried out by P7, *Trichoderma harzianum* and *Trichoderma asperellum* were applied to induce drought tolerance in Atlantic Forest tree seedlings. Instead of carrying out studies with encapsulated *Trichoderma*, it was decided to use a novel *Trichoderma* isolated from Brazilian soil (*T. asperellum*), provided by researchers from UNICENTRO. Initially, the experiments were carried out with *C. pachystachya* and *C. estrellensis*, but they were then extended to other Atlantic Forest species (*Dahlstedtia muehlbergiana*, *Inga laurina*, *Inga vera*, *Plinia cauliflora*, *Eugenia gracillima* and *Schinus terebinthifolius*).

Abscisic acid (ABA) increases drought tolerance but suffers from poor water solubility and rapid environmental degradation. In France, P4 and P5 (with the collaboration of PA and P1) tested ABA loaded lignin/zein nanoparticles (NPABA) on *Quercus pubescens* and *Pinus halepensis* seedlings. NPABA increased symptom-free oaks by 17-23% compared to water or free ABA in Tween80, although empty nanoparticles exacerbated drought symptoms. A 1.13% ethanol treatment alone matched the benefit of ABA in 13% ethanol, increasing symptom-free oaks from 50% to 70%, while higher ethanol concentrations and NPABA provided no additional benefit. In drought-stressed pines, lignin nanoparticles induced complete stomatal closure, zein nanoparticles induced opening, and ABA-loaded matrices varied by carrier. These results show that low-dose ethanol priming is a cost-effective drought mitigation tool for nurseries, and that nanocarrier materials significantly influence outcomes - highlighting the need for integrated formulation, plant physiology and environmental testing to produce reliable, field-ready bioformulations.

WP6. Economic and social cost-effectiveness of elaborated solutions

Different from the European countries, there was not an organized list of nurseries provided by Brazilian governmental agencies. Thus, P1 and P6 mapped these priority stakeholders (nurseries cultivating Atlantic Forest tree seedlings) in three states where the biome occurs: São Paulo, Paraná, and Santa Catarina. For a better understanding of the needs of the native-seedlings producing nurseries and their challenges in the planting of those seedlings in restoration sites, P1 and P6 elaborated a survey in the form of a questionnaire, which evaluated also the acceptance of nature-based solutions related to the project. In parallel, P1 chose the NO-releasing nanoparticles as the nature-based solution to be evaluated regarding its cost-effectiveness. In collaboration with the start-up NanoFeed (which is specialized in the synthesis of nanoparticles for agriculture and livestock production), P1 elaborated a detailed table of costs regarding the implementation of NO-releasing nanoparticles for forest restoration purposes. Then, a field experiment was carried out with *C. pachystachya* seedlings treated with NO-releasing nanoparticles, which allowed to confirm the cost-effectiveness of this nanoformulation in enhancing carbon assimilation by the seedlings. The data from this experiment, as well as all other nature-based solutions developed during the project, will be exposed to the priority stakeholders in a workshop that will occur in Londrina in June 2025.

II. Eingehende Darstellung

2.1. Scientific Outcomes

WP 1. Drought effects on soil microbiota

Recognizing that tropical forests harbour a wide array of trees creating unique ecological niches for soil microorganisms, P6 carried out a study in the Iguazu National Park to analyze how plant and soil parameters shape soil bacterial communities. Results indicated that bacterial diversity varied across the vegetation gradient. Despite the park's diverse soil types, tree species were the primary drivers of bacterial diversity. Moreover, we analyzed the bacteria that were correlated with the trees from different ecological groups. Pioneers, Light-Demanding Climax (LDC), and Shade-Tolerant Climax species showed a high amount of Amplicon Sequence Variants (ASVs) shared among these tree types, suggesting a possible inheritance in forest succession. We also evaluated the relationship of tree species in soil microbial biogeochemical cycles. LDC species stood out because they were strongly correlated with the enzymes involved in the microbial carbon, nitrogen, phosphorus, and sulphur cycles. Overall, these results indicate that high plant diversity of tropical forests exerts a fundamental influence on shaping soil bacterial diversity, and certain tree taxa function as keystone species, exerting substantial effects on the structure and function of soil bacterial communities.

To evaluate the impact of drought stress on soil microbial diversity, soil samples from two fragments of the Atlantic Forest in Northern Paraná were collected by P1 in the rainy season (March 2022). Resampling was performed at the planned dry season (September 2022), but the sites were not under drought due atypical rainfall. Soil samples were collected in the same months of 2023, and again atypical rainfall impaired to obtain samples of soil under drought. Thus, the study of the drought effect on soil microbiomes was restricted to Germany and France. Even though, the microbiome associated to the rhizosphere of two neotropical tree seedlings grown in soils collected from two different Atlantic Forest physiognomies were studied, based on NGS and culturing. A total of 4,422 ASVs for bacteria and 1,765 ASVs for fungi were observed, with higher alfa and beta diversity for the rhizosphere microbiome of plants grown in soil collected from the Atlantic Forest fragment as compared to plants grown in a soil from Campos Gerais (a type of grassland), in agreement with the culturing data. In addition, the structure of the rhizosphere bacterial communities was similar for tree species grown in soil from a same site while the fungi community showed high similarity regardless the plant species or soil origin. The most abundant bacterial and fungal phyla was Firmicutes (60-90% relative frequency across samples) and Ascomycota (63-67% relative frequency), respectively.

In Germany, the spruce and beech rhizosphere microbiomes ware analyzed by P2 and P3 after drought periods based on DNA sequences to explore the potential for isolating PGP and stress resistant microbes. A broad on-site diversity was detected at both sites: 3,364 ASVs were found for bacteria and 4,357 ASVs for fungi. The resident root-associated microbial community was highly similar across both sites and tree species. A total of 30 bacterial phyla were identified in the soil microbiome. The top 10 most abundant phyla comprised Pseudomonadota (syn. Proteobacteria), Acidobacteriota, Actinomycetota, Verrucomicrobiota, Bacteroidota, Bacillota (syn. Firmicutes), Cyanobacteriota, Chloroflexota, Thermoproteota and Desulfobacterota (in descending order). Overall, 15 different phyla were found in the fungal microbiome. Ascomycota was the dominant phylum, followed by Basidiomycota and Mortierellomycota. The trait with the highest predicted abundance was auxin production followed by N fixation. The potential to harbor bacteria with the desired traits (PGP and stress resistance) was similar across both sites and tree species. Overall, amplicon sequencing combined with functional prediction analysis revealed that the rhizosphere community contained numerous taxa known for their potential to promote plant growth, with only minor variations between sites and tree species

In France, P4 and P5 investigated how long-term drought alters root- and rhizosphere-associated microbiomes in a Mediterranean oak forest (O3HP site) and in drought-stressed Aleppo pine seedlings under controlled conditions. In the field, soils were sampled across seasons and 2 years, from amplified and natural drought plots, targeting both resident (gDNA) and active (cDNA) microbial communities. While root microbiomes maintained structural stability and showed a delayed rise in richness under amplified drought, the active rhizosphere communities responded more dynamically, with marked

increases in both diversity and evenness following drought and rewetting. Volatility and beta-diversity analyses confirmed compartment-specific drought legacies and showed rhizosphere instability under amplified drought, while roots remained compositionally resilient. Machine learning revealed distinct drought-adapted taxa, and soil moisture strongly influenced their presence. In parallel, a greenhouse drought experiment with *Pinus halepensis* seedlings showed that drought-induced plant phenotypes (from asymptomatic to severely affected) correlated with significant microbial shifts. Active bacterial communities responded most to drought, with increased diversity and compositional changes tracking plant stress severity. Functional predictions revealed enhanced nitrogen cycling and saprotrophy, along with reduced fungal connectivity and symbiotrophic functions. Drought intensified deterministic microbial assembly, particularly in symptom-free stressed plants, suggesting hidden legacy effects. Together, these findings underscore the importance of integrating structural and functional microbiome data to capture plant–microbe responses to drought and guide SynCom design for resilience-focused applications.

WP 2. Drought effects on trees

Setting up a drought experiment can be challenging, particularly due to spatial heterogeneity in soil drying - seedlings at the edges often experience more rapid desiccation than those in the center. In collaboration with the ONF-PNRGF stakeholder, P4 and P5 developed a four-line experimental layout inspired from Armanhi et al. (Front Microbiol. (2021) 12: 747541), which ensures that all trees are equally exposed to edge effects. This design more accurately mimics real-world reforestation conditions and improves the consistency of drought treatments across seedlings. We tested this experimental system on four tree species: downy oak (*Quercus pubescens*), holm oak (*Quercus ilex*), and Sorb tree (*Sorbus domestica)*—three Mediterranean species known for their drought tolerance and contrasting iso- and anisohydric strategies for water use. Soil water content was progressively reduced from 80% (well-watered control) to 60% and 40% of field capacity (drought treatment). We monitored plant physiological responses by measuring stomatal conductance and photosynthetic activity. While stomatal conductance could be readily assessed in both downy oak and holm oak and pine, measurements in *S. domestica* were complicated by high variability as depending on leaf age and position, as well as for the position of the leaflets on the pinnate. In addition to gas exchange, we recorded drought symptoms and specific leaf area to evaluate stress responses. Visual symptoms proved useful for assessing drought effects in pine, downy oak, and *S. domestica*; however, holm oak showed no visible drought symptoms even at 40% field capacity, highlighting its remarkable tolerance under water-limited conditions.

In Brazil, *C. pachystachya* and *C. estrellensis* were used as plant models by P1 to establish the drought conditions and the morphophysiological traits for the following experiments. Both severe, short-term drought and moderate, long-term drought conditions were tested, with the measurement of different traits (evolution of wilting and necrotic symptoms, relative water content, shoot water potential, time-course of stomatal conductance, gas exchange parameters, photosystem II activity, oxidative stress markers and morphometric traits). Both experimental conditions were applied in tests of WP4 and WP5. In addition, P1 used an innovative capillarity method to make a screening of drought responses of seedlings of 34 tree species from the Atlantic Forest. Stomatal conductance, stem water potential and wilting/necrosis symptoms were monitored in this study. For these three parameters, a gradient of responses was observed, with multiple groups forming based on pairwise comparisons. The species that experienced shoot mortality most rapidly were *Trema micrantha, C. pachystachya, Aloysia virgata, Inga vera* and *Randia armata*, indicating that these species are more sensitive to drought. In contrast, *Ceiba speciosa*, *Ficus guaranítica*, *Enterolobium contortisiliquum* and *Parapiptadenia rigida* took the longest to reach shoot mortality, showing greater drought resistance. Different from the stomatal conductance, the stem water potential had a positive influence on the time to shoot mortality. Furthermore, after being rehydrated in the nursery, resprouting was observed in some species. These findings enhanced our understanding of how Atlantic Forest species respond to drought stress and contributed to identifying species with higher drought tolerance. This knowledge is expected to guide the strategic selection of species for forest restoration projects and improve predictions of ecosystem responses to climate change.

In Germany, pot experiments have been performed with spruce seedlings under drought stress: reduced fresh/dry weights as well as length of the seedlings were observed compared to well-watered control plants. Thus, these morphometrical traits were selected for the next experiments. Moreover, a 24-well-based in planta screening system was developed for spruce and axenic application of bacteria under well-watered and drought stress conditions. Other plant cultivation systems were tested: petri dish system for bacteria and agar plate system for fungi assays. For long-term, on-site data, the KROOF experiment conducted in Kranzberg/Freising (Germany) has performed a detailed analysis (see Grams et al. (2021) ***Ecosphere*** 12: e03399).

WP 3. Isolation and characterization of PAMs + WP 4. Application of PAMs to induce drought tolerance in tree seedlings

Initially, P1 carried out experiments evaluating the effect of coinoculation with 2 bacterial strains obtained from agricultural soil - *Azospirillum brasilense* (Ab-V5) and *Bacillus velezensis* (ZK) - on biometric, physiological and biochemical traits of *C. pachystachya* and *C. estrellensis* seedlings submitted to severe drought under greenhouse conditions. The coinoculation and inoculation with Ab-V5 or ZK led to an increment of nearly 40% in total biomass of *C. pachystachya* seedlings compared to non-inoculated seedlings, and coinoculation resulted in higher root dry mass compared to non-inoculated seedlings, regardless of water condition. No biomass increment was observed in *C. estrellensis* seedlings (co)inoculated with these bacteria, but the inoculation with ZK improved root biochemical parameters under drought stress. Part of these seedlings were transferred to a field experiment. Although this activity has been planned for the dry season, a lot of atypical rainfall interfered with the results. As an alternative, we are constructing a rain exclusion roof to simulate water deficit in the field experiments, which will be done in 2025.

In addition, P1 evaluated the efficacy of various inoculation protocols employing Ab-V5 and ZK in *C. pachystachya* and *C. estrellensis* seedlings under field capacity and moderate drought. Six treatments were tested: non-inoculated control (NIn), single inoculation at sowing (S), at transplanting (T), and after acclimation (P), double inoculation at sowing and transplanting (ST), and triple inoculation at all three stages (STP). Triple inoculation (STP) increased some physiological parameters of *C. pachystachya* seedlings, but it did not always result in greater biomass accumulation. Single inoculation protocols (S, T and P) often resulted in an increase in biometric traits, suggesting that one-off applications may be more effective than continuous inoculations. In *C. estrellensis* seedlings, S was the most effective treatment for biomass accumulation, especially under drought conditions, highlighting the importance of early colonization of the rhizosphere in optimizing plant-bacteria interaction.

Meanwhile, novel bacterial strains from forest soils were isolated by P1 resulting in a collection of 516 bacterial strains from two neotropical tree species and two soil types from Atlantic Forest. From these, a total of 347 strains were fully characterized and are deposited at the Growth-promoting Bacteria Culture Collection of UEL. This collection comprises 4 bacterium phyla (Pseudomonadota, Firmicutes/Bacillota, Actinomycetota and Bacteroidota) from 22 families and 37 genera. In 2024, P1 made another effort of isolation of associative bacteria that colonize endemic plants found in basalt outcrops in Atlantic Forest biome and fungi that live in the mulch of Atlantic Forest. From this effort, a total of 245 bacterial strains were obtained using selective culture medium for osmotic-resistant strains and 110 fungi were isolated from the forest mulch using diluted potato-dextrose-agar medium. The identification and characterization of these isolates are under way.

The strains obtained by P1 and other partners have been screened for traits associated with plant growth promotion or tolerance to drought, such as ability to form biofilms and produce exopolysaccharides, siderophore production, ACC deaminase activity, tolerance to abiotic stress (salt, water, pH, heat), ability to produce auxin and ABA, nitrogen fixation, siderophore synthesis, phosphate solubilization, and confrontation against plant pathogenic fungi. Thus, the strains with the most promising traits were used for assays with the plants. Inoculation trials performed by the P1 showed that *Bacillus* sp. strain 04CT. *Herbaspirillum* sp. strain 114JLB, *Mesorhizobium* sp. strain 112R1CT and *Streptomyces* sp. strain 09CL increased the growth of tree seedlings and crop species under limited water availability. The main mechanisms involved on the increased drought tolerance are related to the protection of the plant photosynthetic apparatus and increased root volume. Modification of endogenous phytohormonal balance by the inoculated strains is probably involved in these responses.

In Germany, 1,292 bacteria and 59 fungi were isolated from spruce/beech roots and adhering soil. A total of 390 bacteria and 39 fungi could be identified at genus level. The most abundant bacteria were *Paraburkholderia* (121) and *Bacillus* (43), while the most abundant fungi were *Penicillium* (8) and *Umbelopsis* (7). *In vitro* screening revealed multiple PGP traits for 33 bacteria and a high stress tolerance for 8 fungi. The highest scores were obtained for *Paraburkholderia* Ke296 and *Umbelopsis* F11. Overall, a number of bacterial and fungal isolates with strong stress resistance and PGP traits could be identified by *in vitro* screenings. Then, the most promising microbes were applied to tree seedlings in axenic and non-axenic plant experiments under well-watered and drought stress conditions. The newly developed *in planta* screening revealed six bacteria promoting spruce growth. *Caballeronia* Ke431 and *Paraburkholderia* Ke296 even significantly promoted seedling growth under drought stress. In the Petri dish system, 8 bacteria significantly promoted spruce growth under drought stress, while 3 fungi promoted seedling growth in the agar plate system fungi (although the effects were not significant). A pot experiment was carried out with the most promising isolates. *Caballeronia* Ke431 and *Paraburkholderia* Ke296 significantly promoted seedling growth under well-watered conditions, and *Paraburkholderia* Ke296 even under drought stress. Thus, at least two bacterial isolates were found to promote seedling survival and growth under drought stress in *in planta* systems. The strains *Caballeronia* Ke431, *Paraburkholderia* Ke296 and *Streptomyces* Ke434 were chosen for Whole Genome Sequencing (WGS) to identify mechanisms underlying the positive plant-microbe interaction (data analysis in progress).

In France, P4 and P5 developed a nature-based strategy by designing SynComs to boost drought tolerance in tree seedlings. Strains were isolated from O3HP Mediterranean forest, in a plot under ten years of rain exclusion, sampling four soil niches—roots, root-adhering soil, non-adhering soil, and root-driven macroaggregates—to maximize ecological and functional diversity. Selection prioritized exopolysaccharide (EPS) producers due to their role in improving soil water retention. From ~1,000 clones isolated on a nutrient gradient, 58 mucoid isolates were screened for EPS production, phytohormone synthesis (auxin, ABA), nutrient solubilization, siderophore release, ACC-deaminase activity and osmotic stress tolerance. Multivariate analysis then selected 12 top strains, which were combined into 8 four-strain consortia reflecting both complementary origin and functions. Biofilm formation and growth promotion were first screened on *Arabidopsis thaliana*, after which the most effective SynComs selected from multivariate analyses were tested on *Q. pubescens* and *S. domestica*, chosen for contrasting root architectures and ecological strategies. Outcomes depended on functional alignment: an EPS-rich consortium increased oak drought tolerance by 47%, while a hormone-producing consortia cut Sorbus symptoms by 71%. These SynComs consistently outperformed single-strain inoculations. Predictive models identified strain identity and symptom onset timing as key mitigation predictors, with stem diameter growth as a robust physiological indicator. This soil niche-informed, trait-driven approach offers a scalable blueprint for climate-adaptive forest restoration.

WP 5. Nature-based materials as carrier systems for PAMs and PGRs

Different types of innovative biomaterials have been tested by the Brazilian team:

- Chitosan nanoparticles containing the NO donor S-nitrosoglutathione were applied to *C. pachystachya* and *C. estrellensis* seedlings subjected to severe/short-term and moderate/long-term drought conditions. The pioneer species exhibited a positive response to the application of the technology, as evidenced by increased stomatal conductance, photosynthetic rate, and stem water potential, reaching values comparable to those of plants maintained at field capacity. Moreover, the treatment with NO-releasing nanoparticles delayed the development of wilting symptoms. In contrast, *C. estrellensis* did not show significant responses to the treatments at any of the tested concentrations. Additionally, a screening was conducted with 35 native species (and *Q. pubescens* in France by P5), among which 11 showed an increase in stomatal conductance following the application of NO donor nanoparticles. These results highlight the potential of the technology for use in ecological restoration programs, although its effects are species-dependent.
- In the field study, the treatment with NO-releasing nanoparticles improved the physiological responses of *H. courbaril* and *A. cearensis* seedlings to transplanting, as indicated by the highest values of CO_2 assimilation, stomatal conductance, and photosystem II activity. This formulation also induced a sharp decrease in the mortality of *H. courbaril* (from 50 to 0%) and *A. cearensis* seedlings (from 62.5 to 12.5%). These results suggest that the application of NO-releasing nanoparticles is a

promising strategy to increase the tolerance of tree seedlings to post-transplanting stress with a consequent mortality reduction, thereby improving the success of reforestation programs.

- Alginate/chitosan nanoparticles containing gibberellic acid were applied to *C. pachystachya* and *C. estrellensis* seeds through the priming technique. *Cecropia pachystachya* responded positively to nanopriming, with increased germination and seedling growth, outperforming the results obtained with hydropriming. However, *C. estrellensis* seeds showed no response to any priming treatment.
- Among the biodegradable materials, we obtained composite foams from corn starch and oat husk, with potential to enhance soil water retention and stimulate microbial activity. Furthermore, the foams supported live PGP bacteria and could be used to deliver them to seedlings. Experiments showed that the addition of foam to the substrate at 1% (w/w) improved the growth and physiology of *C. estrellensis* and *S. terebinthifolia* seedlings under different watering conditions. For example, under field capacity, the foam treatments increased the photosynthetic rate and the total dry mass of *C. estrellensis* seedlings by 95 and 34%, respectively, compared to control. In the drought condition, a positive effect in leaf relative water content was observed.

Regarding the application of *T. harzianum*, the fungus improved the performance of seedlings of different tree species under drought stress and enabled an improvement in the recovery process, as indicated by the analysis of chlorophyll and sugar contents, survival rate, and biometric traits. Plants under field capacity inoculated with *T. harzianum* also showed improved growth. The newly isolated *T. asperellum* strain showed effective results in protecting *C. pachystachya* seedlings from drought stress, with an increase in total dry mass and CO_2 assimilation. Interestingly, this plant species did not respond to *T. harzianum* inoculation, highlighting the importance of testing different microorganisms.

In a collaboration between France and Brazil, biopolymer-based ABA carriers were used to support drought acclimation in Mediterranean tree seedlings. ABA is known to enhance drought tolerance by closing stomata and reducing water loss, but its field use is hindered by poor solubility and rapid degradation. To overcome these issues, P4, P5, PA and P1 compared free ABA, ABA encapsulated in lignin–zein nanoparticles (NPABA), and low-dose ethanol treatments in downy oak (*Q. pubescens*) and Aleppo pine (*P. halepensis*). In 2022, *Q. pubescens* seedlings received free ABA (10 µM), NPABA (10 µM), solvent controls, or water before drought (40% FC). NPABA increased symptom-free plants by 18% compared to water controls (from 45% to 53%) and by 23% compared to free ABA (from 43% to 53%). However, empty nanoparticles hastened stress symptoms. A 2023 trial replaced Tween 80 by ethanol at 13.3% (ABA solvent) and 1.13% (reflecting potential NP residual). Early drought saw transient declines in PSII efficiency and stomatal conductance under free ABA and 13.3% ethanol, but not under NPABA or low-ethanol. At 50% FC, all plants maximally closed stomata. After two months, free ABA and 1.13% ethanol treatments cut symptom severity by 40%, increasing symptom-free individuals from 50% to 70%, while high-ethanol, NPABA, and empty NP treatments failed to improve outcomes. Low-dose ethanol appeared to prime stress tolerance via ABA-independent pathways. In *P. halepensis*, lignin NPs alone triggered full stomatal closure under drought, whereas zein NPs induced stomatal opening. ABA-loaded lignin–zein and zein NPs maintained tight stomatal regulation, but ABA-lignin NPs unexpectedly increased conductance—highlighting that carrier chemistry directly affects plant physiology. Logistic modeling revealed that treatment efficacy depended on interactions among soil moisture, temperature, and formulation. No treatment altered growth metrics (height, diameter) over the study period. These results show that simple ethanol priming (1.13%) offers a low-cost, practical drought-mitigation tool for nurseries, whereas ABA formulations require precise optimization. Importantly, delivery matrices are not inert: lignin and zein carriers exert distinct physiological effects. An integrative approach—testing solvent, carrier, and active compound under realistic environmental conditions—is essential to develop robust bioformulations for climate-adaptive forest restoration.

WP6. Economic and social cost-effectiveness of elaborated solutions

Aiming at understanding the need and acceptance of the proposed nature-based solutions by the forest restoration sector, P1 mapped nurseries cultivating trees native to the Atlantic Forest in São Paulo, Paraná, and Santa Catarina states, by using the economic activity marker of the registered companies (CNAE code), Google searches and information obtained from partners. During the mapping, it became clear that the main focus of most of the tree nurseries is to produce economically viable plant species, such as those used in the silvicultural and paper industry, namely *Pinus* sp. and *Eucalyptus* sp., other timber species, ornamental plants, and vegetables for human consumption. Thus, the focus on the production of reforestation-target species does not seem to be an economically attractive activity, and the sector may need the aid of restoration-focused policies besides scientifically driven solutions

for successful restoration practices. In the Paraná state, we reached also representatives of the public sector (nurseries of the environmental institute of the state, the IAT).

To achieve the goal of better understanding the needs of the native-seedlings producing nurseries and their challenges in planting those seedlings in restoration sites, we elaborated a survey in the form of a questionnaire entitled “Cultivation of seedlings of species native to the Atlantic Forest: characteristics, problems and needs”, with the sections: “Nursery characterization”, “Characteristics of the production of Atlantic Forest native seedlings in the nursery: difficulties and needs”, and “Planting seedlings in the field and executing reforestation projects: needs and difficulties”. We obtained the answers of representatives from 11 nurseries, which provided us a better scenario of native tree seedlings production and forest restoration mainly in Paraná State. We could perceive that the lack of water and excess radiation are among the main causes of mortality in the field. Moreover, the majority of the nurseries would accept nature-based solutions proposed by our project, in addition to having a preference for natural products and high concerns about their environmental impacts. Overall, the results pointed to a sector that needs improvement in the economic returns of the production of native Atlantic Forest species, is open to nature-based innovative technologies, and will benefit from the development of tools that will aid forest restoration success. Besides, the sector will benefit from partnerships with the academic sector and vice versa as means of developing nature-friendly technologies that can be successfully applied in restoration initiatives.

In parallel, we chose the NO-releasing nanoparticles as the nature-based solution to be evaluated regarding its cost-effectiveness. Considering all the processes related to the synthesis (raw material, power, workforce, and equipment), the cost was estimated at around 50 dollars per liter. The major part of this value (98%) is related to the raw material, which highlights the need for searching for cheaper alternatives in the market. Nevertheless, the cost-effectiveness of the nanoformulation was proven in the field experiment with *C. pachystachya* seedlings, given the very low volume required (0.35 mL per seedling) and significant gains of nearly 80% in leaf carbon assimilation. Moreover, the NO-releasing nanoparticles outperformed a commercial biostimulant available in the Brazilian market. These results reinforce the applicability of the technology in enhancing the growth of some tree species, particularly during the early stages of forest restoration projects.

For the final evaluation of the social acceptance of the implemented solutions, P1 is organizing a workshop with stakeholders, prioritizing those who responded to the questionnaire. This event will occur in Londrina on 26 and 27 June 2025, aiming at presenting all the nature-based solutions developed during the project in Brazil. In addition to short talks, the stakeholders will have access to prototypes of the solutions and examples of seedlings treated with them. The major goal is to engage stakeholders in joint experiments under realistic conditions that will be performed in the sequence of the project in Paraná State.

2.2. Outcomes for the consortium / added value

Outcome	Rating (3=major outcome, 2=moderate outcome, 1=minor outcome, n/a= not applicable)
1. Increased research capacity	3 New lab protocols for drought imposition and key markers to follow in plant seedlings. Diffusion of staff expertise in micro- or nanoformulation.
2. Improved scientific evidence base	3 Comprehensive drought-microbiome datasets from three forest types (7 sites) and greenhouse trials. Evidence of new active natural biopolymers that regulate stomatal aperture.
3. New method, data or technology	3 Metabarcoding and ecosystem-level functional profiling-based isolation of drought-tolerant, plant growth-promoting microbial strains.
4. New / improved product or service	3 Validated plant growth–promoting strains, SynCom consortia, and nanoformulations for mitigating drought stress in nurseries and after transplanting.
5. New technical process	3 Application of predictive modelling and AI algorithms to identify key predictors of drought tolerance in microbial community and plant seedlings.
6. New organisational process	3 Cross-partner data harmonization in protocols and FAIR database architecture under the PHENET project.
7. Better access to international networks / markets	3 Partnerships across three countries with industrial and policy stakeholders in forest restoration.
8. Better understanding of other European cultures / issues	n/a
9. Enhanced research network to compete for future European project funding	3 Established multi-infrastructure collaborations and nature-based solutions for drought mitigation testbed for Horizon Europe calls
10. Better understanding of stakeholder needs	3 Co-development with nurseries and private/state bodies involved in forest management defined real-world application requirements.
11. Other(s) (specify):	Awareness of climate change: three years of weather anomalies in Brazil and Western Europe highlighted the unpredictability of field drought trials and underscored the value of dedicated climate sites like O3HP (France) and Kroof (Germany).

2.3. Follow up activities and plans for further exploitation of the results

a) Did your project achievements lead to additional funding during or after the completion of the BiodivERsA project?	X 1. Yes (please specify) - I.A. (P4 and P5) received additional funding from the HORIZON EUROPE INFRA-2022-TECH project 'PHENET', grant number 101094587 (13/12/2024 to 12/12/2026) - M.F.H. postdoctoral fellowship (P1, P5, PA) was funded by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES), grant number 88887.712065/2022-00 in the framework of CAPES-COFECUB 8/2018 (01/01/2023 - 31/12/2023). - E.C.R. postdoctoral fellowship (PA and P5) was funded by CAPES grant number 88887.620205/2021 in the framework of CAPES-COFECUB 8/2018 (01/11/2020 – 30/10/2021) - P1, P6, P7 and P8 received additional funding from Araucaria Foundation through the NAPI Biodiversity program. - P1, PA, PB and PC received additional funding from CNPq and CAPES for the experiments with nanoparticles through the National Institute of Science and Technology in Nanotechnology for Sustainable Agriculture (INCT NanoAgro). - DAAD-FAPPR project between J.P.B. (P3) and A.L.M.O. (P1) - CNPq project led by H.C.O. (P1), with the collaboration of P3, P5, P6, PA, PB, and PC 2. No
b) If yes, does the follow-up project involve…	X 1. Further research See below X 2. Implementation of results obtained PHENET EU program grant number 101094587 will reuse the data obtained in the RESTORE project to build a database of forest soil microbiomes, explore the phenotype-microbiome relationships using artificial intelligence modeling. 3. Commercialisation of outcomes 4. Other (please specify)

c) Follow up activities and plans for further exploitation of the results:

- Microbiome data generated through the RESTORE project will be processed in the PHENET EU program, using generative and predictive AI to build a comprehensive database of forest microbiomes under drought conditions, enabling the identification of patterns and the extraction of high-level insights.
- Some project members will meet in France on June (after the BiodivClim final events) to discuss the follow up activities of the RESTORE project, including joint data analysis and publications, future projects and potential application to the 2025-2026 BiodivConnect call.
- In Brazil, a workshop with stakeholders will occur on 26 and 27 June 2025, aiming at presenting the nature-based solutions developed during the project. In addition to short talks, the stakeholders will have access to prototypes of the solutions and examples of seedlings treated with them. The major goal is to engage stakeholders in joint experiments under realistic conditions, thus leading to the implementation of the results obtained. A sequence of the RESTORE project in Paraná state will be applied to the Araucaria Foundation in the frame of the NAPI Biodiversity program.
- DAAD-FAPPR project between J. Philipp Benz and André Luiz Martinez de Oliveira to further develop the composite materials as nature based-solutions to mitigate the drought impact on plants. This new joint effort is part of the Brazilian funding agency Fundação Araucária and the German funding agency DAAD, involving the Universidade Estadual de Londrina (Brazil) and the Technical University of Munich (Germany). The project is titled "Development of mycelium-based composites from agroforestry residues as plant growth-promoting materials" (protocol DAD2024211000011) and foresees the mobility of researchers and students between the participant institutions.

- CNPq project led by Halley C. Oliveira approved in the call CNPq/MCTI/FNDCT nº 52/2022 – Knowledge production for solutions and technologies associated with mitigation and adaptation to climate change (protocol 405908/2022-9). The project is entitled “Nanotechnology and biotechnology as sources of innovative solutions for silviculture and forest restoration in a scenario of climate change”, involving most of the RESTORE’s network. It is allowing the further development of the nature-based solutions initiated during the RESTORE project, including the transference of technology to the productive sector.

2.4. Stakeholder engagement before, during and after project’s life

1. Stakeholders’ participation to project framing (including before the application) and implementation

Stakeholders actively involved in private and public nursery activities have provided important advices since the project elaboration in the 3 countries. Their participation included provision of seeds/seedlings for the experiments, advices concerning seed/seedling handling and plant cultivation, guided visit to nurseries to learn about their structure and practices, structure, data and personnel provision, suggestions regarding the survey questions, and help in the mapping of nurseries in Brazil.

Some specific examples:

- In France, the ONF-PNRGF Cadarache (Office National des Forêts – Pôle National des Ressources Génétiques Forestières), a stakeholder of P4 and P5, contributed to the project's design by sharing their expertise in setting up drought experiments and advising on the most suitable tree seedling species for reforestation under Mediterranean climate conditions.
- In Germany, P2 and P3 visited the tree nursery Samenklenge in Laufen. The stakeholder showed strong interest in the topic and provided seeds for the conducted experiments.
- In Brazil, the private nursery Flora Londrina (www.floralondrina.com.br) and the non-governmental organization Sociedade Chauá (https://www.sociedadechaua.org/) were priority stakeholders, helping in the definition of the Atlantic Foresty species and providing seeds/seedlings for the experiments. Flora Londrina also helped to improve the questionnaire applicability with an online meeting in which we simulated the questionnaire-based interview.

2. Provision of data by stakeholders; use of field / experiments allowed by stakeholders

- ONF–PNRGF of Cadarache (Office National des Forêts – Pôle National des Ressources Génétiques Forestières), a French stakeholder of P4 and P5, has played an active role in the RESTORE project (2021–2025) as part of its Missions d’Intérêt Général on forest adaptation to climate change. ONF has generously supported P4 and P5 partners by sharing expertise and data on the impact of drought on downy oak, providing free access to a 300 m² greenhouse for experimental purposes (Sept 2021 to Dec 2024), supplying tree seeds, and offering technical know-how in cultivating oaks, pines, and Sorbus species. The team also assisted with the setup of drought experiments, as well as the monitoring and watering of plants, contributing a total 11 person.month in 2021, 15.6 person.month in 2022, 12.4 person.month in 2023 and 7.5 person.month in 2024.
- INRAE (National Research Institute for Agriculture, Food and the Environment), through its RECOVER research unit, contributed its scientific expertise by granting access to P1, P4 and P5 to the experimental pine forest site of Saint-Mitre for seedling establishment studies. The team also shared on-site data and provided expert knowledge on regional mixed reforestation strategies.

- CNPF (National Centre for Forest Property) offered strategic guidance to P4 and P5 by identifying field challenges and bottlenecks in plantation implementation, particularly in relation to economic and ecological constraints specific to the Mediterranean region.
- DRAAF (Regional Directorate for Food, Agriculture, and Forestry of Provence-Alpes-Côte d'Azur) provided essential regulatory insight to P4 and P5, notably regarding the legal framework for applying bacterial inputs in forest environments.
- BioIntrant, a French biotechnology start-up specializing in biostimulants, supplied P4 and P5 with a patented plant growth-promoting bacterial strain for testing on forest tree seedlings.
- In Germany, the Bayerische Staatsforsten (www.baysf.de) has provided a flyer with information about beech cultivation.
- In Brazil, P1 carried out joint experiments with the Flora Londrina nursery, regarding the screening of the responses of seedlings of more than 30 Atlantic Forest tree species to drought stress and NO-releasing nanoparticles.
- P6 also had a very important role in the engagement of stakeholders in Paraná state (NGOs, private companies and the public sector), strengthening collaboration networks, ecological restoration and environmental education in the region. The actions concentrated in the validation of nature-based solutions in scenarios relevant to tree seedling production and forest restoration with a special focus in the plant-substrate interface. Some examples are: Mec Prec Indústria e Comércio Ltda (development of technologically-optimized substrates used in our experiments), Embrapa Florestas (exchange of information and technical support in silvicultural practices), Golden Tree Reflorestadora Ltda and NGO Sociedade Chauá (application of NbS for seedling production at the operational scale).

3. Involvement of stakeholders considered as research objects (e.g., Participatory workshop to assess stakeholder values of biodiversity and ecosystem services)

- The Brazilian team applied the questionnaire to detect the main difficulties faced in Atlantic Forest tree seedling production/planting, as well as the perception of the stakeholders about the nature-based solutions developed in the project (more details in the section 3 above). Moreover, stakeholders (including private nurseries and NGOs) participated in a specific session of the presential workshop of the RESTORE project that occurred in Brazil in 2023. During the event in Brazil, the project members also visited the Flora Londrina nursery, when many aspects of tree seedling production was discussed.

4. Other meetings and activities (to be specified)

- The fact that ONF-PNRGF hosted the experiments of P4 and P5 in France was an excellent opportunity to disseminate the objectives and results of the RESTORE project to all the ONF visitors. For example, at the end of September we presented the project to an Algerian delegation visiting the ONF.
- P5 participated in the 2022 Assises de la Forêt (Forest Conference), jointly organized by the French Ministry of Agriculture, the Secretary of State for Biodiversity, and the Ministry of Industry. This major forum provided a space for dialogue among stakeholders from across the forestry and wood sectors. It offered an opportunity to engage with key actors in the field, including a new stakeholder, Fransylva—the only organization representing all private forest owners in their diversity. The conference working groups addressed critical topics such as carbon neutrality, forest resilience and biodiversity, strategies to enhance the value of wood resources, and participatory approaches to local forest management.
- In Brazil, during the stakeholder mapping, we have organized virtual meetings with nursery managers that work with Atlantic Forest trees. In these meetings, we have briefly presented the project and proposed a stronger engagement in its activities, other than the participation in the survey.

- P1 presented the project during the IV Brazilian Conference of Ecological Restoration (November 2022), when we could reach important Brazilian stakeholders. The participation in agricultural fairs (e.g., ExpoLondrina and Show Rural Copavel) and innovation events (e.g., Summit Iguassu Valley) also allowed us to reach different stakeholders, including those from productive sector.
- P6 established strategic connexions the state environmental institute IAT and the NGO SPVS, through technical meetings and experimental demonstrations.

5. Follow-up activities with stakeholders

- In Brazil, a workshop with stakeholders will occur on 26 and 27 June 2025, aiming at presenting the nature-based solutions developed during the project. In addition to short talks, the stakeholders will have access to prototypes of the solutions and examples of seedlings treated with them. The major goal is to engage stakeholders in joint experiments under realistic conditions, thus leading to the implementation of the results obtained.
- During the meeting in France in June, the project members will discuss the strategies for implementing follow-up activities with stakeholders in Europe.

2.5. Dissemination of results

2.5.1 List of scientific publications (accepted incl. published pre-prints)

1. Campos EV, do ES Pereira A, Aleksieienko I, Do Carmo GC, Gohari G, Santaella C, Fraceto LF, Oliveira HC. Encapsulated plant growth regulators and associative microorganisms: Nature-based solutions to mitigate the effects of climate change on plants. ***Plant Science***. 2023 Jun 1;331:111688. https://doi.org/10.1016/j.plantsci.2023.111688
2. Moreira AA, de Carvalho FA, Bilck AP, de Paula MT, Mali S, Yamashita F, de Oliveira AL. Tannin improves the processability and delays the biodegradability of poly (lactic acid)-starch-based thermoset materials produced by injection molding made with renewable compounds. ***Journal of Applied Polymer Science***. 2023 May 10;140(18):e53815. https://doi.org/10.1002/app.53815
3. Oliveira HC, Melo AA, Fraceto LF. Opinion: Nanotechnology and agriculture in the tropical region: The perspective of the National Institute of Science and Technology in Brazil. ***Plant Nano Biology***. 2024 Nov 1;10:100103. https://doi.org/10.1016/j.plana.2024.100103
4. Vitali KF, Ximenez GR, Guerreiro RG, Silva TM, Romagnolo MB, Pastorini LH. Use of *Trichoderma harzianum* on the performance of young Inga laurina (Sw.) Willd.(Fabaceae) plants in response to drought. ***Brazilian Journal of Biology***. 2024;84:e289149. https://doi.org/10.1590/1519-6984.289149
5. Magosch S, Barrera C, Boelz A, Pritsch K, Rothballer M, Benz JP. Improving Seedling Survival for Forest Restorations: A Novel Screening Method to Identify Microbial Allies Against Drought Stress. ***bioRxiv***. 2025:2025-04. https://doi.org/10.1101/2025.04.24.650507
6. Aleksieienko I, Hertel MF, Reilhan J, De Castro M, Légeret B, Oliveira HC, Reiter IM, Santaella C. Soil-Gradient–Derived Bacterial Synthetic Communities Enhance Drought Tolerance in *Quercus pubescens* and *Sorbus domestica* Seedlings. ***Plants***. 2025 May;14(11); https://doi.org/10.3390/plants14111659

2.5.2. Dissemination of results to scientists and scientific organisations

The results of the RESTORE project have been presented as oral presentations and posters in many scientific events at national and international levels:

2021

- 8th Plant Nitric Oxide International Meeting (Hungary) Talk: “How nanotechnology can improve nitric oxide delivery to plants: current applications and perspectives” (P1)
- International Conference on Recent Advances in Agricultural Sciences (India) Talk: “The use of polymeric nanoparticles for the delivery of herbicides and plant growth regulators” (P1)

2022

- XVIII Brazilian Congress of Plant Physiology and I Ibero-latinoamerican Congress of Plant Biology (Brazil) Poster: “Applications of NO-releasing nanoparticles and associative bacteria for increasing seedling drought tolerance” (P1)
- IV Brazilian Conference of Restoration Ecology (Brazil) Poster: “Applications of NO-releasing nanoparticles and associative bacteria for increasing seedling drought tolerance” (P1)
- 14th Symposium of the Fungal Biology and Biotechnology (Germany) Poster: “Isolation, characterization and application of microorganisms to improve drought stress tolerance of trees for restoration projects” (P2 + P3)

2023

- 32nd Brazilian Congress of Microbiology Poster: “Applications of associative bacteria in forest restoration” (P6)
- 2nd NAPI Week (Brazil) Posters (P1 + P6)
- 6èmes Rencontres Plantes Bactéries (workshop international, France) Poster (P5)

2024

- 9th Plant Nitric oxide International Meeting (India) Talks: “S-nitrosoglutathione-loaded chitosan nanoparticles improve the physiological performance and reduce the mortality of neotropical tree seedlings after transplanting to the field” , “Effect of nitric oxide-releasing nanoparticles on the growth and physiology of *Cecropia pachystachya* Trécul seedlings subjected to moderate water deficit”, and “Nitric oxide-releasing polymeric nanoparticles in plants recent progress, perspective and challenges” (P1 + PC)
- 26th World Congress IUFRO (Stockholm, Sweden) Talks: “The application of nitric oxide-releasing chitosan nanoparticles as a nature-based solution to improve drought tolerance of neotropical tree seedlings” (P1) and “Isolation, characterization and application of microorganisms to improve drought stress tolerance of trees for restoration projects” (P2 + P3)

Fig. 2: Project group members at the IUFRO 2024 in Stockholm. From left to right: J. Philipp Benz, Sonja Magosch, Halley O. Caixeta, Karin Pritsch

- II Latin-American Workshop in Nano and Biotechnology (Chile) Talks: “Nanotechnology and Associative Microorganisms as Nature-Based Solutions to Improve Plant Responses to Climate Change”, “Cecropia pachystachya seedlings submitted to moderate drought stress: application of GSNO-loaded chitosan nanoparticles and screening the stomatal response of different tree species to the application of GSNO-loaded chitosan nanoparticles” and “Influence of growth-promoting bacteria on the germination of Embaúba-do-brejo seeds” (P1)
- II Workshop Microorganisms for a Sustainable Agriculture (Brazil) Talks: “Strategies based in green nanotechnology for the induction of tolerance to drought and other abiotic stresses” and “Microbial Biotechnology”
- 33rd Annual Scientific Initiation Meeting at the State University of Maringá (Brazil) Talk: “Application of *Trichoderma* and drought tolerance in young plants of tree species” (P7)

2.5.3. (Co-)Organized Scientific Events

P1 and P7 participated in the organization of the event Paraná Faz Ciência in 2023 and 2024, respectively, which is focused in the scientific community and the general public (more details in item 5.3). In 2023, P1 organized the 1st Workshop RESTORE, when the results of the project were disseminated to the scientific community and stakeholders.

2.5.4. Dissemination of results to the public - Scientific Communication and Outreach

1) Project website and social media

https://pos.uel.br/biologicas/restore-2/

https://napibiodiversidade.eco.br/

https://www.instagram.com/napibiodiversidade/

https://www.facebook.com/napibiodiversidade/

https://www.linkedin.com/company/napibiodiversidade

2) Short communication texts and flyers to announce the objectives and ambitions of the project to be disseminated on the websites of Institutions and stakeholders, and newsletters of Institutions

https://www.lse.ls.tum.de/en/fungbio/research-area/projects/restore/

https://www.cite-des-energies.fr/biam/projets/restore/

https://operobal.uel.br/ciencia/2020/10/05/projetodoccbuelintrnacional/

https://operobal.uel.br/pesquisas/2021/09/15/estado-pesquisas-conservacao-biodiversidade/

https://operobal.uel.br/inovacao/2023/03/28/seti-fundacao-araucaria-e-uel-apresentam-arranjo-de-pesquisa-e-inovacao-com-foco-na-biodiversidade/

3) Vulgarized articles on the project objectives

https://www.iaraucaria.pr.gov.br/napis/napi-biodiversidade-restore/

https://sites.uel.br/sustentabilidade/restore-biodiversidade/

https://napibiodiversidade.eco.br/sobre-napi-restore/

4) Video/photo reports and interviews

Lauch event of the RESTORE project and NAPI Biodiversity by Araucaria Foundation
https://www.youtube.com/watch?v=c3WC0iH2dwI

Report of TV UEL about the RESTORE workshop in Brazil
https://youtu.be/KkMSaTGsD8s

Report of TV UEL about the application of associative bacteria to improve Atlantic Forest tree seedling production
http://www.uel.br/tv/site/?videos=pesquisa-da-uel-estuda-aplicacao-de-bacterias-em-mudas-de-arvores-nativas

Report of TV UEL about the development of innovative biodegradable materials
http://www.uel.br/tv/site/?videos=projeto-da-uel-desenvolve-materiais-biodegradaveis-e-inteligentes

Report of TV UNESP about the applications of nanotechnology in agriculture and forest restoration
https://youtu.be/b3Bc2O1PQ6Y

TV show Campo Vivo with a report about the application of NO-releasing nanoparticles in agriculture and forest restoration
https://youtu.be/Z5PaoLvwL5A?si=H_Ot1Qap_2TMN8du

Report of TV UEL about the application of NO-releasing nanoparticles for improving plant drought tolerance
https://youtu.be/QOYbASqDXWU

Movie on the RESTORE project broadcast by CNRS images: “Trees for the future” https://lejournal.cnrs.fr/videos/les-arbres-du-futur

Movie on the RESTORE broadcast by a major French national daily newspaper “Le Monde”: Teaching trees to withstand drought https://www.lemonde.fr/sciences/video/2024/12/13/apprendre-aux-arbres-a-resister-aux-secheresses_6446692_1650684.html

Photo report on the RESTORE project “Trees of the future”
https://images.cnrs.fr/reportage-photo/rep001263

Interview about the application of NO-releasing nanoparticles for improving plant drought tolerance
https://open.spotify.com/episode/6dsiFNZqiwm4eFfyCxRAhg?si=BtNcY-WMRs6alut8gBbfoQ

Radio and video interview for France Info
https://www.francetvinfo.fr/meteo/secheresse/reportage-c-est-ce-qu-on-fait-avec-les-hommes-quand-on-les-vaccine-dans-les-bouches-du-rhone-l-office-national-des-forets-entraine-les-plantes-a-s-adapter-a-la-canicule_6725175.html

Radio and video interview for BFM TV
https://s3-eu-west-1.amazonaws.com/kmplus-account-files/85310885/2024/8/22/eIDcYw-yld0St74za5V3YHA.mp4

Radio interview for RCF
https://www.rcf.fr/articles/ecologie-et-solidarite/larbre-du-futur-un-arbre-plus-resilient-a-la-secheresse

5) Press releases about the project results and activities

https://globorural.globo.com/tecnologia-e-inovacao/noticia/2025/04/nanotecnologia-sustentavel-promete-proteger-lavouras-da-seca.ghtml

https://operobal.uel.br/jornal-noticia/2024/11/11/estudo-investiga-acao-de-nanoparticulas-para-proteger-plantas-da-seca/

https://operobal.uel.br/ciencia/2022/03/15/projeto-bacterias-arvores-neotropicais-combater-estresse-hidrico/

https://operobal.uel.br/sociedade/2025/02/04/professores-participam-de-portal-cientifico-internacional-sobre-estudos-de-ecologia-e-meio-ambiente/

https://www.fappr.pr.gov.br/Noticia/NAPIs-apresentam-suas-acoes-durante-o-Show-Rural

https://operobal.uel.br/internacional/2023/03/10/evento-tecnico-promovido-pelo-projeto-restore-esta-com-inscricoes-abertas/

https://napibiodiversidade.eco.br/2024/04/20/evento-arboriza-distribui-mais-de-12-mil-mudas/

https://napibiodiversidade.eco.br/2024/07/01/napi-biodiversidade-participa-do-summit-iguassu-valley/

https://napibiodiversidade.eco.br/2024/04/19/missao-de-pesquisadores-estrangeiros-ocorre-no-parana/

https://napibiodiversidade.eco.br/2024/09/25/cientistas-participam-do-ii-workshop-microrganismos-para-uma-agricultura-sustentavel/

https://napibiodiversidade.eco.br/2024/07/02/restore-visita-floresta-na-franca/

https://napibiodiversidade.eco.br/2024/07/03/nanotecnologia-e-tema-de-visitas-a-italia-e-suecia/

https://napibiodiversidade.eco.br/2024/07/08/parceiros-na-franca-e-alemanha-recebem-o-napi-rgb/

https://napibiodiversidade.eco.br/2024/06/10/pesquisadores-do-napi-participam-de-workshop-no-chile/

6) Vulgarized articles/videos on the project outcomes

https://theconversation.com/nanotecnologia-e-solucoes-baseadas-na-natureza-ajudam-a-aumentar-seguranca-alimentar-e-mitigar-mudancas-climaticas-254682?utm_medium=article_native_share&utm_source=theconversation.com

https://conexaociencia.com.br/o-combate-a-seca-tem-novos-e-incriveis-recursos/

https://conexaociencia.com.br/nanotecnologia-e-uma-das-tabuas-de-salvacao-da-mata/

https://conexaociencia.com.br/como-a-ciencia-potencializa-a-agricultura-brasileira/

https://conexaociencia.com.br/potenciais-biotecnologicos-sao-a-chave-da-conservacao/

Video for the international scientific dissemination channel EcolClips:

https://www.youtube.com/watch?v=PPTHIMYMW0M

7) Presentation of the project in agricultural fairs, showing to the public the importance of biodiversity conservation and forest restoration for crop production, as well as the nature-based solutions developed in the RESTORE

- Show Rural Coopavel 2025 (Cascavel): one of the greatest events of the agricultural sector in Latin America (nearly 350,000 attending people). https://www.fappr.pr.gov.br/Noticia/NAPIs-apresentam-suas-acoes-durante-o-Show-Rural
- Via Rural ExpoLondrina 2023, 2025 (Londrina): ExpoLondrina is event of the agricultural sector in Londrina joining nearly 240,000 people. The Via Rural is an educative exposition within the events, focused in students from primary schools (nearly 25,000 visitors). https://sites.uel.br/proex/participacao-na-via-rural-smart-farm-expolondrina/
- Municipal Fair of Native Seeds and Agrobiodiversity (Teixeira Soares) : fair attended by quilombolas, indigenous communities, and the community in general. The event brought together hundreds of people committed to preserving traditions and producing food free of pesticides while preserving agrobiodiversity.

8) Innovation events

- Summit Iguassu Valley – Latin America event held in Foz do Iguaçu, Paraná, Brazil, in June 2024: presentation of the results regarding the efficiency of the use of *Trichoderma* and nanoparticles in seedlings of tree plants kept under drought conditions. https://napibiodiversidade.eco.br/2024/07/01/napi-biodiversidade-participa-do-summit-iguassu-valley/

- Hackaton Smart Agro 2025: event that promotes the creation of science-based solutions for the biotechnological and agricultural sectors. Two teams with members of the RESTORE project were awarded in the competition with proposals about the biodegradable composite materials. https://napibiodiversidade.eco.br/2025/04/23/hackathon-smart-agro-2025-premia-pesquisadores-do-napi/
- Program of Market-Focused Intellectual Property (PRIME 2023): program from Paraná state government that promotes the approximation of the results from scientifc research to the market. One team with members of the RESTORE project participated with the NO-releasing nanoparticles.

9) Program Social Label 2024

- Recognition of the project in the State University of Maringá by its actions of social impact aligned to the SDGs of UN's 2030 Agenda. https://noticias.uem.br/index.php?option=com_content&view=article&id=29904:uem-conquista-selo-social-em-28-projetos-nas-areas-sociais-e-ambientais&catid=986&Itemid=211

10) Presentation for policy makers

- Workshop of contributions from academics to the update of Strategy and National Plans of Action for the Biodiversity (EPANB, Brasília, 2024). https://napibiodiversidade.eco.br/2024/07/15/napi-biodiversidade-participa-da-oficina-de-atualizacao-da-epanb/

2.5.5. Presentation of the RESTORE in education projects

- 18ª National Week of Science and Technology – Paraná Faz Ciência 2021 (online event): presentation of the project in the round table "Climate change: what do the science says?".

  https://www.youtube.com/watch?v=YLuItKmf_3o

- Feira das Profissões 2022, 2023, 2024 (UEL Profession Fair, Londrina): an event that receives about 16,000 students from primary school to learn about the courses and projects of UEL.

- I FisioPop 2022 (Porto Alegre): interactive scientific show that occurred during the XVIII Brazilian Congress of Plant Physiology to disseminate projects about Plant Physiology to a young public from primary schools.

- Arboriza Ponta Grossa 2023, 2024, 2025 (Ponta Grossa): this annual event aims at fomenting the socio-environmental awareness of the population through the distribution of native tree seedlings associated with the educational activities. The directly impacted public is higher than 100,000 people, including students, educators, farmers, technicians and the general population

  https://napibiodiversidade.eco.br/2024/04/20/evento-arboriza-distribui-mais-de-12-mil-mudas/

- Partnership with the Education Traffic School of DER-PR (Ponta Grossa): environmental education activities impacting nearly 5,000 people, mainly children from primary school.

- Paraná Faz Ciência 2023 (Londrina) and 2024 (Maringá): event receiving nearly 38,000 people, including 13,000 students from public and private primary schools and universities from Paraná state.

  https://napibiodiversidade.eco.br/2023/11/27/napi-biodiversidade-participa-do-parana-faz-ciencia-2023/

  https://napibiodiversidade.eco.br/2024/10/16/napi-biodiversidade-marca-presenca-no-parana-faz-ciencia-2024/

- Lecture entitled “The Role of Microorganisms in Promoting Plant Growth” presented by Gabriel Rezende Ximenez for the Maringá University Center (Unicesumar).

- Pritsch, K.: Technical University Munich, TUM School of Life Sciences, Module Soil Microbiology 1, Lectures on Soil Microbiology, part 2: Rhizosphere, Winter Semester 23/24, Winter Semester 24/25: Biodiversa rationale, methods and results have been presented.

- RESTORE climate change workshop for schools (primary and secondary) and the public, Fête de la Science in 2021 (6-8/10/2021 Manosque, France), 2023 (02-04/10 2023) St Michel l’Observatoire, 2024 (10-12/10/2024) Manosque.

2.5.6. Interactions with other BiodivERsA projects:

P1 and PA interacted with members of other BiodivERsa projects during the activities of the BiodivClim Knowledge Hub “Potential of Nature-based solutions for mitigating and adapting to climate change”, which included the participation in the launch event in 8-9 February 2023, monthly online meetings, and writing the Knowledge Gaps Paper. Moreover, P1 had interactions with members of the MixForChange project, during online meetings and participation in the event “Mixed Planting: Opportunities for the silviculture in a scenario of climate change”.