Silva Nova - Restoring soil biology and soil functions to gain multiple benefits in new forests

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Abstract

Afforestation is proposed as one of the most effective climate solutions for carbon sequestration. As a majority of threatened species are linked to forests, afforestation can also contribute to mitigate the biodiversity crisis. There is however a caveat: the agricultural legacy (high nutrient availability, altered soil biota structure and function) of new forests constrains the development of forest-adapted species, affects tree growth and stability, and delays environmental benefits from afforestation.

We hypothesize that inoculation of former arable land with soil (including microbiome, fauna and seeds/rhizomes of understory vegetation) from old forests along with targeted tree species mixtures will improve productivity and more rapidly restore forest-adapted communities. This will ultimately result in diverse, stable and resilient multifunctional forests.

We will test this hypothesis and develop applied inoculation methods by: i) exploring soil biota and benchmarking biodiversity in existing afforestation research platforms (chronosequences and sites with increasing distance to other forests); ii) conducting inoculation experiments in mesocosms to measure seedling performance and, above- and belowground linkages; iii) establishing field-scale inoculation experiments in new and existing afforestations to test short- and long-term inoculation success on forest productivity, biodiversity and soil functioning at the ecosystem scale; iv) incorporating the landscape context into guidelines and tools for spatially explicit prioritization of areas for assisted dispersal.

The aims are to resolve barriers for successful restoration and develop landscape-scale afforestation strategies that optimize productivity and biodiversity for the planning and implementation of green infrastructure; and produce basic knowledge on the tree, understory vegetation, soil fauna and microbiome nexus and its effect on forest productivity, biodiversity and soil functions (N-retention, C-sequestration, methane uptake).

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Keywords

Afforestation, agricultural legacy, ecosystem services, forest ecosystem, understory vegetation, soil fauna, soil GHG exchange, soil inoculation, soil microbiome

Challenge

Climate and land-use changes exert increasing pressure on ecosystems, degrading their biodiversity and ecosystem services. There are major national (Miljø- og Fødevareministeriet 2018) as well as global (United Nations 2017) plans to increase the forest area to sequester carbon (C) as a means to mitigate climate change (Doelman et al. 2020). At the same time, there is a demand for protecting and restoring forests to halt the decline in biodiversity (Convention on Biological Diversity 2011). Given that forests host a major part of the species pool and in particular many threatened species, planting new forests is thought to meet both the climate and the biodiversity challenges. While new forests sequester C and provide wood products (Vesterdal et al. 2002, Barcena et al. 2014) forest-adapted plant communities develop poorly and tree productivity may also be constrained by an agricultural legacy characterized by high soil nutrient content (Isbell et al. 2019, Ritter et al. 2003) and host microbial communities that differ distinctly from those of forest soils (Zornoza et al. 2009). Furthermore, the new forests are often single-species (sometimes exotic) stands, which lack variation in vertical structure that supports forest species, and experience dispersal limitation due to fragmentation (Brunet 2007, Verheyen et al. 2003). Most temperate trees are ectomycorrhizal plants that have evolved intimate rhizosphere interactions with soil fungi and bacteria (microbiome) for a range of beneficial functions including nutrient acquisition, pathogen resistance and stress tolerance (Brundrett and Tedersoo 2018). Planted trees may thrive in nutrient-rich cropland soils, but the transition to a diverse and robust forest ecosystem, if at all occurring, is very slow without their natural soil microbiome. Without a forest-adapted microbiome, there may be biotic and abiotic constraints on plant and soil community succession, biodiversity (Fig. 1A) and productivity (Lance et al. 2020). A visible sign is the understory vegetation composition in Danish afforestation chronosequences dominated by species characteristic of the arable landscape (Fig. 1B). Similarly, soil fauna as well as the soil microbial community may still resemble that of cropland soils. Furthermore, high nitrogen (N) leaching losses (Gundersen et al. 2009, Hansen et al. 2007) and low methane oxidation (Barcena et al. 2014a) several decades after afforestation reflect surplus N and agricultural legacy of the microbiome. We propose that this situation can be efficiently improved by adding forest soil inoculum.

Steering of soil communities to improve restoration is a novel tool gaining increasing attention from scientists and land managers. We have recently demonstrated that the soil community is an important driver of plant community development and that inoculation of cropland with soil from indigenous grassland or heathland successfully steers soil communities and restores the target plant communities within a few years (Wubs et al. 2016). We will, for the first time, develop and test forest soil inoculation as a restoration tool in new forests in former cropland and address the combined challenges of forest biodiversity loss and climate change through integrated research across disciplines using state-of-the-art molecular identification methods (Bahram et al. 2018, Tedersoo and Anslan 2019).

Aim and objective

Our overall **aim** is to develop ecosystem- and landscape-scale afforestation strategies (involving soil inoculation) that support forest productivity and resilience, improve forest-related species diversity and increase benefits from ecosystem services (pure water, C-sequestration, GHG emission reduction). We will provide fundamental understanding of the tree, understory vegetation, soil fauna and microbiome nexus and its effect on forest productivity, biodiversity, and soil functions.

The project will bring **significant novel insights** to the temporal and spatial development of forest- adapted flora, fauna and microbial communities after afforestation with and without soil inoculation to accommodate biomass production, biodiversity and soil-related functions. Outcomes will be relevant to implement in afforestation of millions of hectares to sequester CO_2 in a Green Transition. In Denmark, a doubling of the forest area is anticipated within this century, which implies afforestation of >5000 ha/yr. As forests harbor a major part of the threatened species, steering restoration of forest-adapted communities through assisted dispersal will be a pivotal contribution to halt biodiversity loss. The project will provide key answers to local and global challenges for land managers, policy makers and other users outside academia, e.g. best practice guidelines for spatially explicit selection of land and model tools to map areas for which production, biodiversity and soil functions will be improved faster and with greater success by use of assisted dispersal.

The specific **objectives** of the project are to

- evaluate temporal and spatial responses in biomass production, groundflora and fauna, soil microbiota and soil functions to afforestation of former cropland in the baseline situation without assisted dispersal (WP1)
- quantify effects of inoculation methods regarding donor, amount and application mode of inoculum to different soil types and tree species using a mesocosm approach (WP2)
- expand knowledge of inoculation methods from mesocosm to field-scale implementation and documentation (WP3), and
- synthesize previous WPs to develop concepts for assessment and planning of afforestation at the landscape scale and provide a decision tool for afforestation strategies including use of assisted dispersal (WP4).

Research Plan

The project has 3 experimental work packages (WP1-3) that feed into a landscape oriented analysis and synthesis work package (WP4; Fig. 2). Microbiologists, taxonomists and functional ecologists will work together in common sampling campaigns across experiments to investigate specific hypotheses for each organism group and function. For each WP, harmonised sampling and analytical protocols are developed.

In **WP1**, we will use **explorative platforms** to examine the baseline situation for afforestation of former croplands without mitigation of abiotic and biotic constraints. Insights to the temporal and spatial development of forest-adapted understory vegetation, soil fauna and microbial communities after afforestation will serve as the foundation for experimental work in WPs 2-3.

We **hypothesize** that

1. understory vegetation and soil biota diversity and composition will slowly approach old forest conditions;
2. distance to old forests is a limiting factor due to dispersal constraints;
3. higher tree species diversity will be conducive to higher below-ground soil biota diversity; and
4. C sequestration, N- retention and methane oxidation will increase with age due to establishment of forest-associated taxa.

We will explore above- and belowground biota succession in well-documented internationally unique afforestation and natural succession platforms belonging to the involved research institutions: *Chronosequence platforms* in DK and NL including cropland and old-forest references in relevant tree species such as beech, oak, maple and Norway spruce and selected mixed stands at different soil types (Vesterdal et al. 2002, Barcena et al. 2014b). In EE, 60 natural succession and planted afforestation sites aged 2-65 years, including monospecific alder, willow, oak, birch, hybrid poplar, aspen, spruce, pine and mixed stands (n>4), nearly half of which have been sampled for soil microbiomes in 2016.

In addition to tree species and temporal aspects of afforestation, the spatial aspects in terms of distance to old-growth forest will be specifically addressed in *three Danish site networks;* a National Forest Inventory (NFI) subset, a network with 33 afforestation areas across the country, as well as a network of beech forests 25-100 years old (previously evaluated for flora and birds) to evaluate spatial constraints related to dispersal of forest-adapted plant species, soil fauna and mycorrhizal fungi.

Distance to old-growth forests is key for dispersal of forest-adapted plant species (Brunet 2007, Verheyen and Hermy 2001), but there is sparse knowledge of important landscape-scale dispersal constraints for soil fauna and microbiomes (Boeraeve et al. 2018). Long-distance dispersal will be studied in *Greenland* using two explorative sites in the southwestern part of the country with no forest continuity supporting tree growth under current climatic conditions (Normand et al. 2013). Mixed conifer plantations and adjacent shrubland as control will serve as a case study for testing development of forest specific soil microbiomes in high-latitude areas with no forest legacy.

We will evaluate baseline temporal development of biodiversity indices, e.g., tree species composition, composition of understory vegetation, soil fauna and microbial communities in the abovementioned sites and relate these to biomass production. This will be supplemented by analyses of soil conditions (C, N, P, pH and base saturation) to assess abiotic filtering as potential constraint. Soil biota will be assessed as a potential biotic constraint for the first time. The chronosequences in all three countries will be evaluated using most up-to-date molecular identification methods (second- and third-generation sequencing platforms). Further analyses will be conducted in the most intensively studied sites focusing on microbial functioning, especially enzymatic activities and functional genes using metagenomics and metatranscriptomics approaches, and soil functions, e.g. C and N stock dynamics (assisted by ^{13}C and ^{15}N isotopic signatures), N_2O, CO_2 and methane oxidation fluxes, to evaluate the link between microbial and soil functioning.

The outcomes of WP1 are temporal and spatial responses in biomass production, understory vegetation, soil biota and key soil functions to afforestation with different tree species composition in variable distance to old forests. These outcomes serve as reference for the inoculation experiments in WP2 and WP3.

In **WP2**, we will conduct **inoculation experiments in mesocosms** to measure the function of tree-specific microbiomes, flora and fauna, focusing on mutualists as well as parasites/ pathogens as major drivers of development of biodiversity and biomass production. Specifically, we will study dispersal vs environmental filtering, tree seedling performance and resistance to biotic and abiotic drivers, above-belowground linkages, and effects on soil processes (N transformations, methane oxidation).

We hypothesize that 1) trees in inoculated soil will establish with greater success, have higher growth, and be more resistant to biotic and abiotic stress; 2) these effects will be strongest with inoculum from old forests; and 3) in presence of a tree host, inoculated organisms will multiply and a small amount of inoculum will be sufficient.

In climate chambers/greenhouses, we will examine tree germination and seedling growth and abiotic stress tolerance using different amounts of inoculum (1, 5, 10, 20%) from different origins (2 tree identities x 2 soil types x 3 forest ages) into background cropland soil with 4 tree species in monoculture + mixtures x ±understory plants. Soil inoculum will be collected from the afforestation chronosequence sites and their old forest and cropland reference. Seedling size and germination success will be recorded. In a common garden experiment, we will examine the effect of 10% inoculation into cropland soil of the above mentioned inocula on tree growth with and without ground vegetation, soil nutrient dynamics and presence/diversity of aboveground and belowground higher trophic levels of fauna in 20L containers. Measurements include insect/visible pathogen presence recorded weekly and above-ground insect communities sampled repeatedly and identified to groups/ trophic level/feeding guilds or species. Soil-dwelling insects will be collected at the end of the season. Leaf nutrient content, soil nutrients and microbiome will be determined as in WP1. Root endophytes (bacteria/fungi composition) will be determined using amplicon sequencing and microscopy for mycorrhizal colonization.

In separate experiments, we will examine the *role of soil biota* and the efficiency of inoculation for soil functions, biodiversity and tree growth by

- comparing inoculation with live and sterilized soil and
- extracting and adding different soil community groups using established wet-sieving approaches to remove mesofauna, fungi etc.;
- host preference by insect release-recapture;
- damage and performance (caged seedlings);
- the effects of inoculation on drought resistance;
- nutrient constraints.

The outcomes of WP2 include evaluation of the amount and type of inoculum, the role of tree-specific microbiome for seed germination, establishment, growth and responses to abiotic constraints, susceptibility and resistance to biotic stress. Plant-soil-microbiome interactions will be revealed, e.g. the role of tree species diversity and ground vegetation for microbiome, insect diversity and soil functions.

In **WP3, field-scale inoculation experiments,** we use existing small-scale inoculation experiments in afforestations to explore the effect of soil inoculation in the early phase of the project. We will establish multiple inoculation treatments in a new Afforestation Experimentarium in Denmark for field-scale testing of methods identified in WP2 and their short- and long-term effects on productivity and biodiversity in forests.

We hypothesize that

1. inoculation in the field will lead to persistent changes in soil biota towards old forest communities;
2. trees planted in field conditions inoculated with soils from old forests will establish with greater success, have higher growth rate and be more resistant to biotic and abiotic stress;
3. inoculated organisms will multiply with time, and a small amount of inoculum will be sufficient;
4. inoculation will be most efficient at nursery stage for the microbiome and in the forest phase after canopy closure for higher organisms; and
5. inoculation of rewetted organic cropland soils will reduce the emission of methane (i.e., the initial burst).

We will use existing experiments in DK and NL: DK, *assisted dispersal trial*, established in 2015 in beech and oak forests aged 10 and 20 years on loamy and sandy soil (Fig. 3) by transplanting 10 cm top soil, addition of rhizomes or soil inoculum from an old mixed beech-oak forest. We will use this experiment to assess the potential for establishment and further dispersal of the soil microbiome and micro- and mesofauna via simple soil inoculation in the field. NL, *Terra-Dunes soil inoculation experiment* established in a coastal dune area in 2018 to study the role of soil biota by adding sterilized (gamma irradiated) or live grassland and forest donor soil to the field plots, and ectomycorrhizal fungi or not. We will plant tree seedlings and study tree growth, nutrient content, microbiome composition, mycorrhizal infection, insect herbivory and diversity, foliar pathogens, etc., linking biodiversity, biomass production and stress tolerance.

The *Afforestation Experimentarium* with field-scale inoculation experiments will be designed during year 2 based on results from WP1, WP2, the preliminary analysis of the NFI data in WP4 as well as the existing inoculation experiments. An established afforestation area will be selected that allows testing of soil inoculation at the ecosystem scale in both new plantings (inoculation at the nursery stage), closed canopy stands where ground vegetation associated with open land is constrained by shade and after the first thinning when forest adapted ground vegetation species can establish. We compare inoculation methods:

- none;
- patch;
- row;
- mixed-ploughed in monoculture vs mixtures of commonly used tree species;
- planted tree islands in natural successions.

To scale up the restoration potential, different soil texture and moisture conditions will be investigated. We test mechanized and feasible methods of donor soil application using local donor material in the smallest possible amounts. For next generation experiments, we disperse saproxylic organisms by transplanting dead wood to newly thinned stands, acknowledging that up to 25% of forest adapted species are linked to dead wood.

In DK, many drained organic cropland soils will be rewetted and left for succession or afforestation in the coming years to mitigate climate change. Transitional high emissions of methane are expected, and we will examine whether inoculation with natural wet forest soil microbiome will alleviate the effect. Inoculation treatments will be performed at rewetted sites currently studied in other projects.

The expected outcome is evidence-based documentation of soil inoculation effects on biodiversity and biomass production in new and younger afforestations. The Afforestation Experimentarium will have an important role in dissemination of results, e.g. as management guidelines for inoculation. The Experimentarium will be included in the portfolio of long-term forest trials at IGN.

In **WP4, landscape scale analysis and afforestation concepts**, we synthesize our results to provide experimentally justified recommendations on landscape-level afforestation strategies in Denmark and more broadly in the temperate and hemiboreal climatic zone. We integrate the information from the previous work packages with land cover datasets to design spatially explicit afforestation planning considering the landscape fragmentation in Denmark.

In WP1-3 the emphasis is on the agricultural legacy and its mitigation, but productivity and biodiversity at a site are also shaped by other factors (e.g. forest fragmentation, landscape position, stand structure, management). To understand this complexity, we use the relevant platforms (WP1) and the Danish NFI in landscape analyses. Since a significant fraction of the forest area (20-25%) is located on former cropland (<70 years since abandonment) and because of its spatial coverage, the NFI is ideal to investigate the importance of the agricultural legacy at a landscape scale. The NFI database holds data on tree species composition, tree growth, forest structure, soil types and landscape position for >9.000 15-m radius plots of which ⅓ are resampled every 5 years. Ground vegetation is recorded (c. 3000 plots), and soil is repeatedly sampled in a subset of c. 300 plots. The latter subset of NFI plots, the natural succession and beech forest networks (WP1) will be further studied to address soil functions and insect and bird diversity (bioacoustics), as these mobile taxa often respond to landscape level processes. We will extract a suite of spatially explicit variables (30x30m) describing relevant local and landscape elements characterizing the surroundings of each plot (e.g. 500x500 m). These country-wide layers include information on forest structure (Lidar), topography (soil moisture), land cover (matrix quantity), forest continuity, high nature value forest (matrix quality), tree species distribution and soil types.

The first outcome will be a spatial evaluation resulting in a map of existing afforestation areas showing their current provision of different functions, while considering the landscape context. The map will be based on an analysis of spatial patterns of biodiversity, productivity and soil functions, while exploring emerging trade-offs and synergies (e.g. productivity vs biodiversity). We will use Zonation, a decision tool to identify the most efficient configuration of sites to represent different features. This map will be used to select different landscape level strategies (sharing vs sparing) to achieve specific goals (maximize provision of functions, increase landscape connectivity).

The second outcome is a model identifying the most relevant landscape and local factors for land managers. These will be used to select the most suitable cropland areas depending on different scenarios (e.g. conversion of most suitable areas vs conversion of marginal fields only) by modeling (Random Forest machine learning) the spatial relationship between below- and above-ground biodiversity, soil functions and productivity and the predictor layers.

To examine afforestation practices, we contrast natural succession afforestation exploratories in DK and EE (WP1) to existing planted afforestation to identify important controls on forest productivity and understory species diversity and on possible trade-offs between them. Results are used in designing the Afforestation Experimentarium (WP3). Finally, we assess the potential of soil inoculation methods. Based on the quantitative effects of soil inoculation methods in different tree species, soil types, forest ages (WP3) and distance to sources (WP1), we will identify areas where these methods can have the largest impact in improving biodiversity, forest growth and soil functions. These factors will be integrated in a simple landscape and local level Decision-Support tool for land managers.

Analyses and maps will inform policy decisions on the design of future afforestation programs and guide spatially-explicit prioritization of areas for afforestation and selection of potential sites for microbiome inoculation in existing and new afforestations. This information will be crucial for designing cost-effective, spatially targeted future afforestation plans. The stakeholder forum (see below) will be involved in the translation of results into management guidelines.

Consortium, outreach and training

University of Copenhagen (UCPH), Department of Geosciences and Natural Resource Management coordinates the project and bring in i) a cross disciplinary research team on forest biodiversity, biogeochemistry, and remote sensing of forest productivity and landscape attributes for biodiversity; working at scales from molecules to landscapes; ii) a unique ensemble of long-term field experiments and experimental platforms and site networks, and iii) advanced biogeochemical, genetic and entomological laboratories as well as space for controlled facilities in growth chambers and greenhouses. The research team has worked on aspects of afforestation for more than two decades (e.g. Gundersen et al. (2009), Hansen et al. (2007), Barcena et al. (2014b), Barcena et al. (2014a), Ritter et al. (2003), Schmidt and Brandbyge (2018), Vesterdal et al. (2002))

The University of Tartu (UTARTU), Mycology and Microbiology Center contributes

- a survey of an Estonian forest chronosequence;
- molecular analysis of soil samples; and
- bioinformatics analysis of the metabarcoding, metagenomics and metatranscriptomics data.

UTARTU has 12-year experience in handling massive amounts of samples and performing state of the art molecular analyses (Bahram et al. 2018, Tedersoo and Anslan 2019, Tedersoo et al. 2014a, Tedersoo et al. 2014b, Tedersoo et al. 2020).

Leiden University, Institute of Biology has ample experience in mesocosm experiments (from 0.5 to 200L) to study plant/tree-soil-insect interactions and microbiomes. At Leiden, there are advanced facilities available for extraction and identification of soil fauna as well as climate chambers, greenhouses and state of the art microbial and molecular labs. Leiden University contributes to the project by initiating mesocosm experiments and by making use of existing field experiments and site networks. Current research focuses on steering soil communities via soil inoculation to improve nature restoration on former arable land (Bezemer and Putten 2007, Heinen et al. 2020, Wubs et al. 2016).

We use scientific and popular science dissemination activities including best practice guidelines and a strong stakeholder involvement to reach all groups. A stakeholder forum is formed to discuss all aspects of the project to qualify the practical planning and research. This to ensure to incorporate the needs and vision of the stakeholder groups with regard to the physical planning of the new forests, design of forest at stand level and landscape scale. Target stakeholders are land managers, forest organizations, agencies and politicians.

At the start of the project, we discussed and decided on a communication plan. A project web page (https://ign.ku.dk/english/silvanova/) was released where information from the platforms is provided for the scientific communities, stakeholders, students and users outside academia. The website also introduces the rationale for the project and visualizes the research platforms, announces research opportunities and ensures easy access to background data from the platforms. Selected field sites and experiments will serve as demonstration sites and we will organize workshops with field excursions for scientists and stakeholders/end users. We will organize special sessions during conferences on afforestation management for enhancement of above- and belowground species diversity and key ecosystem functions, and at the end of the project we will organize a workshop where the team will present the project results, and finally produce professional videos about the scientific achievements.

Sixteen Early Stage Researchers (ESRs) are trained during the project. We employ nine PhDs and six postdocs each responsible for a specialist activity (organism group or soil function) across the experimental WPs (Fig. 2). To ensure collaboration and multidisciplinarity, we will have common sampling and field campaigns for each experiment, where the ESRs work together with the involved experienced scientists using harmonised protocols to obtain samples and datasets that facilitate synthesis work. All data will be stored on an online platform using standardized protocols and accessible to all team members. For ESR training we develop and implement three common PhD courses (open to external ESRs) on soil functionality (DK), microbiome functioning, analysis and bioinformatics (EE) and above-belowground interactions (NL) with the aim to provide the ESRs a broad in-depth insight into the array of experimental approaches and methods used in the project. During team meetings we also have specific training sessions with the ESRs on experimental design, data analysis and writing skills. We offer student projects, excursions and on-site exercises at BSc and MSc level, throughout the project and beyond. It is a great opportunity for students to be part of an international and multidisciplinary group.

Lay project description

Climate and land-use changes exert increasing pressures on ecosystems, degrading their biodiversity and ecosystem services. Afforestation is proposed as one of the most effective natural climate solutions. Given that the majority of threatened species are linked to forest habitats, afforestation can also contribute to mitigate the biodiversity crisis, while maintaining provision of forest production for societal needs. There is however a caveat. Areas available for afforestation are of agricultural origin with high nutrient availability and altered soil biota structure and function. These conditions can constrain forest growth and delay the benefits from afforestation. It is our hypothesis that addition of a thin layer of donor soil (including its biota) from mature forests will improve tree growth, accelerate restoration of biodiversity, and enhance other environmental benefits. This will ultimately result in diverse, stable and resilient multifunctional forests.

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Conflicts of interest

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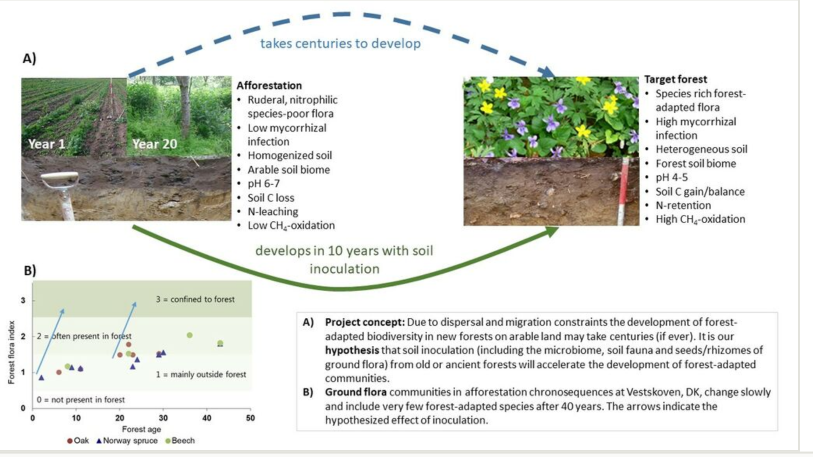

Figure 1.

Concept and hypothesis.

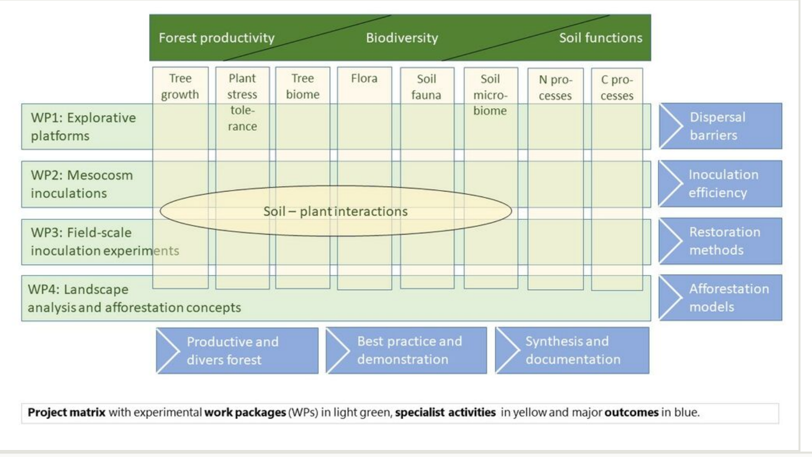

Figure 2.

Project matrix.

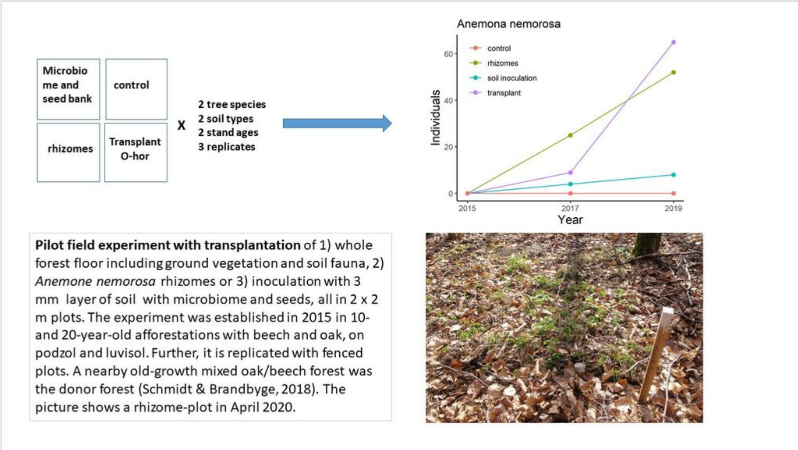

Figure 3.

Pilot experiment with soil transplantation and inoculation (Schmidt and Brandbyge 2018).