2nd SILVA doctoral student conference of the Faculty of Forest Sciences October 25th – October 26th, **2023** (Clarion Collection Uppsala hotel, Uppsala)

Time schedule for October 25

09:00 – 09:10: SILVA: Opening of the SILVA doctoral conference

09:10 – 09:30: ***Matilda Stein Åslund:*** Tree cellular and metabolic responses to infection by M. pinitorqua and D. sapinea in Scots pine

09:30 – 09:50: ***Jaime Luna:*** The biodiversity implications of fast-growing broadleaf trees

09:50 – 10:10: ***Emil Bengtsson:*** Emerging diseases in forest tree seedling production

FIKA: Coffee with organic yogurt & berries, sandwich, sweet and more

10:45 – 11:05: ***Kristina Berggren Nieto:*** Ecological effects and plant responses of novel plant protection against insect pests

11:05 – 11:25: ***Lukas Graf:*** "Micro to Macro - Ungulate forage at multiple scales and the effects of forage availability and ungulate densities on browsing damages

11:25 – 11:45: ***Isabell Rosenkranz:*** A GMO-free approach to boost plant production

11:45 – 12:05: ***Laura García Romañach***: Flowering time in trees: the role of the *miR156-SPL* pathway

12:05 – 12:25: ***Isak Ingerholt:*** Analyzing how allelic variation in Norway spruce resistance QTLs is linked to defense response against Heterobasidion spp.

LUNCH: Dish of the day with salad/raw food, butter & bread, coffee and homemade dessert

13:30 – 13:50: ***Carl Lehto:*** Seeing through their eyes: Revealing recreationists' landscape preferences through viewshed analysis and machine learning

13:50 – 14:10: ***Katharine King:*** Exploration of base cation transporter genes in Suillus genus in relation to mineral weathering capability

14:10 – 14:30: ***Sara López Fernández:*** Can we find new methods of forest health assessment applied to breeding?

14:30 – 14:50: ***Getachew Gemtesa Tiruneh:*** Effects of land use change from agriculture to market driven short-rotation forestry on carbon sequestration, soil acidity and available nutrient on a landscape scale

14:50 – 15:10 ***Lina Arnesson Ceder:*** Potential in creating an additional carbon sink by alternative forest management strategies combined with carbon credits.

15:10 – 15:30: ***Tinkara Bizjak:*** Presence and activity of nitrogen-fixing bacteria inside Scots pine needles in the Swedish boreal forest

FIKA: Energizing coffee, there is something for everyone here - fruity & fresh, sweet, sour & salty

16:00 - 16:20: ***Laura Tunnermann:*** Importance of organic nitrogen for shoot:root allocation

16:20 – 16:40: ***Alma Gustavsson Ruus:*** Pinus sylvestris and Picea abies grown in alternative substrates

Time schedule for October 26

This day is planned together with Mellanskog. We start at 08:00 sharp. More information to come.

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Tree cellular and metabolic responses to infection by M. pinitorqua and D. sapinea in Scots pine

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Background

Recent reports have highlighted the occurrence of young Scots pine stands infected by both Melampsora pinitorqua (pine twisting rust) and Diplodia sapinea (Diplodia tip blight) in various locations in Sweden. M. pinitorqua is a rust pathogen that alternates between Scots pine (Pinus sylvestris L.) and European aspen (Populus tremula), causing bending or breaking of the pine's annual shoots. The pathogen D. sapinea causes economically significant damage to conifers across the globe. Its impact in northern Europe, however, has only emerged as a concern in the last two decades. The dominating pine species in the Nordic countries, Scots pine, is among the species most susceptible to D. sapinea. It is known that D. sapinea can persist in a susceptible host without causing apparent disease, with an onset of disease symptoms when the host is subjected to abiotic stress. In this project, we study the ecological and physiological aspects of the interactions between Scots pine and these fungi. We hypothesise that 1) different metabolic pathways will be activated in M. pinitorqua-symptomatic compared to asymptomatic tissue, that 2) tissue with M. pinitorqua, but no D. sapinea, produce a different set of metabolites than those attacked by both pathogens, and that 3) D. sapinea is present in tissue from trees of all disease categories but with increased abundance after biotic stress (infection by M. pinitorqua).

Materials and Methods

Phenotyping was conducted on 567 trees to assess symptoms caused by M. pinitorqua and D. sapinea, and a subset of 15 trees was selected for sampling for metabolite profiling using Liquid Chromatography–Mass Spectrometry (LC-MS/MS) and quantification of the pathogens using quantitative Polymerase Chain Reaction (qPCR). Tissue samples were collected from trees categorised based on the previous year's symptoms – few and mild symptoms, M. pinitorqua symptoms but no D. sapinea symptoms, and trees showing symptoms of both pathogens. The sample collection occurred at three time points during shoot elongation, when M. pinitorqua is active on the host.

Results

The preliminary results show differences in the metabolic profiles for trees sampled at the end of M. pinitorqua's infection stage, depending on whether the fungus is still active or not. A few phenolic compounds and amino acids show distinct activation in trees infected by both pathogens compared to trees infected only by M. pinitorqua. D. sapinea was present in tissue from trees of all disease categories, but previous M. pinitorqua infection did not affect the biomass of D. sapinea.

Conclusions

This study contributes to a better understanding of the complex interactions between tree hosts and fungal pathogens, aiding in disease management and tree breeding programs. The results have implications for enhancing resistance to multiple pathogens and improving strategies for sustainable forest management.

The biodiversity implications of fast-growing broadleaf trees

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How can native, hybrid and exotic broadleaf tree species grown under short rotation contribute to forest biodiversity?

It is a goal from the Swedish government and certification agencies to increase the proportion and diversity of broadleaves and native tree species. The increase share of broadleaf trees will help us to achieve different environmental and societal goals. However, can we accelerate this by increasing the proportion of fast-growing broadleaves? Fast-growing broadleaf trees can provide us with a great amount of raw material in less time than conventional production stands while helping us to diversify the conifer-dominated Swedish landscape. It therefore has potential consequences for both biodiversity and recreational values. Yet, their potential negative or positive impacts are not known. Within this study, we are investigating the implications of native (birch), hybrid (hybrid aspen) and exotic (poplar) fast-growing broadleaves to forest biodiversity.

Emerging diseases in forest tree seedling production

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Approximately 400 million tree seedlings are produced in Sweden annually to replant harvested forest areas. These seedlings are cultivated in a highly industrialized manner, in which large monocultures of densely grown seedlings are subjected to intensive cultivation practices. Heavy fertilization and irrigation do create optimal conditions for their growth, but these practices may also stress seedlings and create conditions favourable for the establishment and rapid spread of fungal infections, resulting in tremendous economic losses. The use of chemical pesticides can also cause further plant stress making them even more susceptible to fungal infections.

The availability of effective fungicides in the European Union have been steadily shrinking during the last decades, thereby challenging the production of forest tree seedlings. Recently, there have been an emergence of previously neglectable diseases in Swedish forest nurseries, most notably species from the genera Phoma. These outbreaks have occurred in several forest nurseries form south to north in Sweden with infections taking place during the vegetation season as well as during winter storage. Besides, the nature of infections has changed. Today, infections of tree seedlings by Phoma are found on the shoots whereas previously infections were found on the roots.

Currently, there is a lack of knowledge regarding both the infection biology and the possible reasons of Phoma outbreaks. Changed cultivation practices, a warmer climate or new adaptations by these pathogens could all be possible explanations to the increased disease pressure. Other unknown factors should not be excluded as well. The objective of my research is to gain a better understanding of the Phoma population structure and the infection biology of the pathogen. Management methods to control this pathogen in Swedish forest nurseries will also be investigated.

Ecological effects and plant responses of novel plant protection against insect pests

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Background

Maximizing plant protection practices while ensuring viable and sustainable high-yield plant production and low negative ecological impact requires a deeper understanding of plant-herbivore interactions and plant defence mechanisms. Utilizing or manipulating plant's own defence system, e.g. enhancing plant resistance against pests, has the potential to become sustainable protection alternatives. For example, the plant defence signalling hormone methyl jasmonate (MeJA) has been proposed as a novel plant protection method since exogenous application of MeJA induces plant defences and has been shown to reduce pest damage in various crop species and in conifer trees. In addition, Norway spruce (Picea abies) plants propagated through the in vitro mass clonal technique called somatic embryogenesis (SE) have shown increased resistance to the major forest regeneration pests the pine weevil (Hylobius abietis). There is currently an increasing interest in using SE as a propagation method for conifer trees in the Nordic countries, thus, we aimed to explore the effects of SE and in combination with MeJA on plant resistance and tolerance, and the underlying mechanisms to their enhanced resistance.

Materials and methods

All experiments addressed differences between 2-3 years old Norway spruce plants produced via SE (emblings) or from seed in nurseries (seedlings). Prior to an experimental start, half of the plants of each plant type were treated once or twice with 10mM MeJA. We conducted five experiments:

1. Effect of MeJA on plant resistance to the pine weevil (attack and feeding damage) in a field (choice) and lab (no-choice) experiment (one season), as well as plant mortality (three years) in the field.
2. Incidence of constitutive and traumatic resin ducts (size, density and conductive area) in transversal stem sections.
3. Quantitative and qualitative terpene chemistry in stem bark (incl. mechanical damage). Extracts analyzed via Gas Chromatography–Mass Spectrometry (GCMS).
4. Pine weevil preference (no. of p.w. per tree and feeding damage) when exposed 48h to plants in two arenas in the lab; effects of emitted VOCs and visual cues on visiting and feeding choice.
5. Bark wound healing ability (onset of wound healing and wound healing rate) in the greenhouse.

Main results and conclusion

1. MeJA in combination with SE synergistically lowered pine weevil damage in the field the first year (86%), and mortality with 98%, compared to untreated seedlings, conferring a strong plant protection advantage. The positive effects on mortality remained for all three years.
2. Emblings displayed a slightly higher density of constitutive resin ducts, which may contribute to explain their higher resistance. On the contrary, MeJA-treated seedlings formed both larger and a higher number of traumatic resin ducts compared to emblings, implicating that traumatic resin ducts do not play a role in the greater resistance seen in MeJA-treated emblings.
3. Emblings and seedlings differed qualitatively and quantitatively in bark terpene chemistry, but specific compounds may be the drivers behind the higher resistance seen in emblings. In addition, MeJA treatment made emblings and seedlings more similar to each other. (*preliminary results*)
4. MeJA treatment but not plant type reduced the number of pine weevils seen on the plants, suggesting an

olfactory deterrent effect from MeJA-treated plants but not from emblings. Feeding damage correlated with the number of pine weevils per plant.

5. MeJA treatment accelerated the onset of wound healing but decreased overall wound healing rate, while non-treated emblings had the slowest onset but the highest overall healing rate. Hence, SE and MeJA displayed a trade-off between onset of healing and healing rate.

Our findings do not only increase the understanding of plant defence responses and pest resistance but also offer a sustainable approach to seedling protection, encouraging the use of our results in practical applications. They further underline the importance of continuing exploring plant-insect interactions and plant stress response mechanisms.

Micro to Macro - Ungulate forage at multiple scales and the effects of forage availability and ungulate densities on browsing damages

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Background

Forage availability and ungulate densities are main drivers of browsing damages in Swedish forests, yet precise data on forage availability is often lacking. The aim of my studies is to provide such data on scales relevant for management. In my first two studies I use data from small scale ecological experiments to investigate how we can improve forage availability using silvicultural techniques and large scale datasets from the National Forest Inventory (NFI) in combination with remote sensing data to predict forage availability for cervids on the national scale. The results from these studies will go into my third project, where I investigate the relationships between browsing damage, ungulate densities and forage availability, on the intermediate scale, i.e. Moose Management Areas (MMA).

The results of my studies will contribute insights into the temporal dynamics and spatial aspects of browsing damages, under consideration of silviculture and how to improve local forage availability to mitigate browsing damages. Further, I will develop a tool to efficiently estimate forage availability across large landscapes using remote sensing techniques and provide forage availability maps for decision makers in wildlife management.

Material and Methods

In my first study, I follow the development of approximately 1100 selected blueberry ramets, which are an important dwarf – shrub in ungulate diets in nine spruce dominated forest stands in Skåne and Småland after the stands have been thinned. I measure growth and yield related plant traits throughout over time, such as the height, growth rate and berry yield of individual ramets and relate them to change in canopy after thinning the spruce stand. Three different levels of thinning intensity are considered in this study: no thinning (the forest remains untouched), 30% thinning (30% of the basal area are removed) and 60% thinning (60% is removed) and four different browsing treatments to simulate browsing pressure by cervids.

In my second study, I combine Airborne Laser Scanning (ALS) data with NFI data to predict ungulate forage abundance. I use abundance data from approximately 40.000 NFI plots on AROW–species (Aspen, Rowan, Oak, Willow), birch and pine. I calculated several structural metrics describing the vertical structural composition of the NFI – plot using the ALS data. These metrics are canopy height (defined as the quantile were 95% of the echoes of the ALS data are accumulated), canopy cover (defined as the ratio between the number of echoes above 10m divided by all echoes) and shrub cover (the ratio of number of echoes between 0.5 and 2m). Further, I will incorporate climate data into the modelling process.

In my third study, I will use data from the NFI to investigate the relationships between browsing damages, forage availability and ungulate densities over time (approximately 20 years of available data). This study will be limited to few moose management areas and investigate spatial effects of ungulate densities and forage availability and their fluctuation over time on browsing damages in the respective MMAs, as few previous studies mainly have not incorporated variance of browsing damages over time into the analyses.

A GMO-free approach to boost plant production

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Background

The changing climate leads to challenges that plants need to overcome to maintain biomass production and yield. The temperature changes we are facing these days and which we will be facing over the next decades, are one of the most prevailing factors that cause stress for plants. To this day is the use of genetically modified organisms (GMO) under strict regulation in Europe. To overcome this hurdle, we are working on a mechanism of an endogenous protein to create a GMO-free technique to activate stress induced genes in plants. The goal is to activate user-defined genes such as the cold-responsive CBF genes by an addition of small RNAs (sRNA) that can bind and guide the protein Argonaute1 (AGO1) to specific genomic regions. This is a promising approach since AGO1 was found to be a fundamental player in activation of stress-related genes in the nucleus, besides its well-studied role in the silencing RNA pathway in the cytosol. This project aims to understand the role of AGO1 in the activation and expression of stress related genes in plants.

Material and Methods

The first big goal is to determine how AGO1 activates gene expression. We will use the model plant Arabidopsis thaliana to study the binding dynamics of AGO1 to its genomic targets. It is already known that AGO1 binds to the CBF region and to clarify the dynamics of the cold-induced interaction between AGO1 and the genomic sequence, we will expose the mutant line as well as the wild type to 4°C over a period of 12h. Samples will be processed for RNA-seq and CUT&Tag to find AGO1 binding sites in the DNA. Furthermore, we will try to identify the sRNA that is responsible for guiding AGO1 to the genomic region, the CBF genes in this case. This will be done by creating libraries of sRNAs after exposure to 4°C and deep sequencing. The promoter region of CBF1 might be a binding site for AGO1. To prove its importance, the sequence will be removed with CRISPR/Cas9 and the mutant line be studied. More mutant lines will be created to study and understand the involvement of AGO1 in activation of the CBFs and therefore cold acclimation.

AGO1 is conserved among eukaryotes which will allow us to prove the concept in other species such as aspen or spruce or crops. Therefore, we will insert stable constructs into the nuclear genome expressing sRNAs that guide AGO1 to its target. The final task is to achieve similar results as the stable insertion by just applying potential sRNAs exogenously to the plant. This project can have a major impact on plant biotechnology, the food industry, forestry, and society.

Flowering time in trees: the role of the *miR156-SPL* pathway

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The regulation of flowering in plants is a widely studied subject. The SQUAMOSA Promoter-binding protein-Like (SPL) family is known to promote flowering in annual and perennial plants such as Arabidopsis thaliana and Arabis alpina by regulating inflorescence meristem identity genes. At the same time, SPLs are negatively regulated by several factors involved in the vernalization pathway and the age pathway. In A. alpina, micro-RNA 156 (miR156) is a key member of the age pathway that determines flowering time in response to vernalization. Despite all the research, we know very little about this process in trees and how conserved it is between angiosperms and gymnosperms. Forest trees such as poplar and spruce are perennials that can take more than a decade to first set cones or flowers, which limits the speed of breeding programs in the forestry industry. To study the regulation of flowering time in conifers, a naturally occurring and early-cone setting variety of spruce (Picea abies var. acrocona) has been studied.

Homozygous acrocona plants can form seed cones after their second or third-growth period, while spruce usually initiates this process after 20-25 years. This phenotype in the acrocona variety has been linked to a mutation in the miR156 binding site of PaSPL1 (a member of the SPL family in spruce), which makes this gene resistant to miR156 degradation. To better understand this mechanism, several SPLs have been selected in Populus to perform functional studies: these candidate genes are more expressed during spring in old trees, which indicates that they might have a role in the vegetative-to-reproductive phase transition. miR156-resistant variants of the selected SPLs will be expressed in poplar to understand the regulation of flowering time in perennial species.

Analyzing how allelic variation in Norway spruce resistance QTLs is linked to defense response against Heterobasidion spp.

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Background

Trees must cope with an array of challenges during their long lifespans, a major stress being the attack of pathogens. In northern Europe, Heterobasidion parviporum and H. annosum root- and stem-rot are destructive diseases of Norway spruce (Picea abies) in production forests. These infections result in vast economic losses. The extent of research has disproportionately favored resistance towards H. parviporum, leaving resistance against H. annosum largely unexplored. This project aims to investigate how genetic components of resistance compares between the two pathogens.

A preceding study identified genes where an allelic variation associated with resistance to either or both pathogens. Specific alleles of these genes hence appear to increase resistance in a narrow (pathogen specific) or broad (both pathogens) sense. In the current project, we have selected three of these genes for a more comprehensive analysis. The chosen genes encode a LACCASE, a FATTY ACID EXPORTER and a MATAIRESINOL. Our underlying hypothesis is that these genes, and specific alleles of the genes, play pivotal roles in spruce defense response against the two pathogens. We will analyze the allelic variation of the selected trees. Then, specific alleles of the genes will be investigated for their contribution to resistance against H. parviporum and H. annosum using artificial inoculations.

Experimental Plan and Methods

Trees putatively homozygous for either allele of each gene will be re-sequenced. This will yield information about the allelic variation that serves at the starting point for the rest of the project. To determine if specific alleles of the genes confer resistance, trees homozygous for specific alleles will be inoculated with H. parviporum and H. annosum and spread of infection will be measured to quantify resistance.

To obtain detailed functional data of the genes and specific alleles, another inoculation experiment will be performed with more comprehensive analysis. Tempo-spatial differential gene expression data will be generated by performing RNA sequencing of samples at stages of infection. This will yield information about regulation of the genes of interest and how they are linked to regulation of other defense response genes. Further, the chemical profile of the samples will be analyzed using mass spectrometry. The composition of fatty acids and lignans is of special interest as we hypothesize this composition will differ dependent on the specific alleles of the genes.

Lastly, specific allele overexpression and knock out spruce transformants will be generated. The transformants will also undergo an inoculation experiment, followed by RNA-sequencing and chemical analysis similar to above but without collecting temporally and spatially separated samples..

Expected Outcomes and Implications

The data obtained in this project will specifically explain how the genes of interest are involved in resistance against H. parviporum and H. annosum. From a broader perspective, it may yield insight to how resistance towards the two pathogens is connected. This in turn may have critical implications for successfully breeding resilient and resistant trees in the future.

Seeing through their eyes: Revealing recreationists' landscape preferences through viewshed analysis and machine learning

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Background

The opportunity for outdoor recreation is an important cultural ecosystem service provided by the landscape. At the same time, land-use pressures are reducing people's opportunities for recreation, with the extent of green spaces available to people shrinking globally. For that reason, it has been suggested that green space indicators should be incorporated in physical planning, e.g. that all residents should have a certain area of green space accessible to them. However, focusing on the quantity of green space is not enough: simply that an area has vegetation might not be enough to supply high quality recreation. To achieve this we need knowledge on preferences and needs of recreationists: what landscape features are important?

To answer this we turn to landscape preference research. The field has been dominated mainly by ex situ studies, often by showing people pictures of landscapes and asking them to rate them. However, with recent technological advancements and the widespread adoption of smartphones, there are more studies on how recreationists actually utilize landscapes. Various methods have been used, such as GPS tracking, data scraping from social media, and online surveys with elements of Public Participatory GIS (PPGIS).

Material and Methods

In my research I have used survey data on peoples routes in the landscape when doing outdoor recreation of any kind. I compare characteristics of the landscapes people choose to be in with those of nearby landscapes, that they could have chosen instead. To get a closer estimation of what landscape each person has experienced, I use LiDAR data to calculate the visible landscape along the routes and sample various map data in this "experienced landscape". Analysis is performed with machine learning models (Boosted regression trees), which is a flexible method capable of handling a large amount of map data covariates.

Main results

I have shown that accessibility is a strong factor: we utilize the nature we have available to us. Further, landscapes containing water are highly selected for, recreational infrastructure (trails, shelters, toilets) are also positively correlated. Deciduous forests are preferred, while younger forests, clearcuts, urban and noisy environments are avoided.

Conclusion

Novel methodologies such as viewshed analysis with LiDAR data, along with machine learning methods can be useful tools to improve ecosystem service analysis. The results on which landscape features are important for recreationist also have policy implications: Water environments, recreational infrastructure, and deciduous forests are selected for, while urban environments, noise, forest clearcuts, and young forest are avoided. These outcomes suggest that increased recreational infrastructure could improve the conditions for recreation, especially in proximity to water. This also shows the importance of providing access to and hindering the exploitation of water environments and minimizing noise pollution. To manage forests for recreation, deciduous trees should be favored and clearcuts should be avoided, although felling trees to create viewsheds toward water could be positive for recreation.

Exploration of base cation transporter genes in Suillus genus in relation to mineral weathering capability

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Background

The role of ectomycorrhizal (ECM) fungi in biological weathering is increasingly recognised, although the quantitative significance of microbially mediated mineral dissolution for plant growth is debated. ECM fungi can weather minerals and mobilise essential nutrients such as base cations by physical force, extrusion of low molecular weight organic acids, free radicals and chelators. Suillus species in particular, are found to preferentially inhabit mineral soils and are frequently reported to possess weathering capabilities. Though studies growing ECM fungi with minerals have shown heightened nutrient content in mycelia compared to growth without minerals, the mechanistic understanding of nutrient mobilisation associated with weathering remains largely unknown.

Material and Methods

Here we focus on characterising the diversity and phylogenomic distribution of base cation transporters in ECM genomes. We hypothesise that species with extensive mineral weathering capabilities will have higher copy-numbers of transporter genes associated with the transport of base cations to enable rapid uptake and transfer of recently mobilised nutrients, and this has been selected for by transporter gene family expansions across a phylogenomic tree. We employed OrthoMCL, IQtree, r8s and CAFE5 software to identify orthogroups across 108 Agaricomycetes genomes, construct a dated phylogenomic tree and analyse evolutionary expansions and contractions of transporter gene families across the phylogeny.

Preliminary results

Preliminary results show large variations in copy-numbers in all transporter gene families across all species and there are rapid expansions of cation transporters at the Suillus node. Genome analyses will be complemented by pure culture studies comparing base cation mobilisation and uptake by Suillus spp. to that by other species with varying copy-numbers and ecology. Fungal cultures will be grown with and without mineral additions (granite and gabbro) and mycelial base cation uptake and biomass will be quantified. Additionally, a transcriptomic study of Suillus spp. when in synthesis with Pinus sylvestris seedlings will be conducted taking guidance from the genomic study results.

Can we find new methods of forest health assessment applied to breeding?

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Background

Forest health is critical for effective forest management. Climate change has increased the risk of forest diseases, particularly invasive pathogens. Identifying trees with exceptional health traits and integrating them into breeding programs is crucial to meet the forestry and societal needs. Conventional field assessments are labor-intensive and impractical on a large scale, failing to capture the spatiotemporal heterogeneity of pathogen attacks. To address this, our research aims to develop novel tools for assessing disease risk in Norway spruce forests and integrating this into breeding practices.

Material and Methods

We propose to use remote sensing (RS), Fourier transform infrared spectroscopy (FTIR) and sequencing technologies to link disease phenotypes to specific genotypes. This approach will speed up forest breeding efforts against economically significant pathogens and establish a flexible framework to accommodate new threats within breeding programmes.

To predict resistance against H. parviporum, we will monitor symptom development using Unmanned Aerial Vehicles (UAVs) and FTIR scanning until 50% of the trees exhibit visible symptoms. Subsequently, we will conduct destructive harvests to determine sapwood displacement, validating models built from UAV and FTIR data. These models will be tested on a separate validation set. We anticipate that sapwood displacement due to decay will correlate with progressive changes in Red-Blue-Green, Red edge, and Near-Infrared signals. Furthermore, symptom development will be associated with distinct chemical patterns detectable by FTIR, with variations across resistance levels.

Norway spruce needles host a diverse community of phyllosphere fungi, and some genotypes may be predisposed to pathogenic fungi. To investigate the relationship between tree phenological traits and colonization by phyllosphere pathogens, we are conducting a multi-season study, focusing on bud flush timing and the assembly of the phyllosphere mycobiome. Manual observation and UAV-based remote sensing determine bud flush timing. We collect samples from the local air spore community using spore traps and gather shoot samples from Norway spruce clones at defined stages of bud flush. The fungal community of these samples is characterized by high-throughput sequencing of the ITS2 region.

Expected outcomes

This project aims to improve forest health assessment for breeding practices, offering innovative solutions to address the challenges posed by climate change in sustainable forest management.

Effects of land use change from agriculture to market driven short-rotation forestry on carbon sequestration, soil acidity and available nutrient on a landscape scale

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Fagita Lekoma district in northwest Ethiopia has undergone rapid land-use change from cropland to short rotation forestry (SRF) using Acacia mearnsii, a species native to Australia, in the last two decades. The land-use change is primarily driven by the market demand for charcoal. The objective of this study was to assess the extent of land-use change, its impact on carbon stock change in soil and biomass, as well as effect on soil acidity and available nutrient status. Soil and biomass samples were collected between April and May 2022 from different land uses and plantation stands of different rotation cycles and stand ages. The results showed that a significant area of land had been converted from cropland to A. mearnsii cultivation over the last 15-20 years, with Acacia plantations being the dominant land use in 2017, covering 37% of the district.

The afforestation has led to an increase in the woody biomass carbon stock of 56.5 ± 5.3 ton/ha, corresponding to a sink 207.0 ± 19.6 ton CO2/ha in the study district. Soil carbon stock showed a decreasing trend with increased number of plantation rotation cycles and in young stand ages compared to cropland, but a gradual increase as the stands aged. However, these differences were not statistically significant. The study found that 0.98 MT of carbon was sequestered, equivalent to 3.6 MT of CO2 between 2002/5 and 2020/22. This translates to an annual sequestration rate of 0.18 MT of CO2 which makes it a sink corresponding to 2% of Ethiopia's total annual fossil fuel emissions in 2016. Soil acidity (both pH and exchangeable acidity) was higher with increasing number of rotation cycles; however, the results were not statistically significant. Active carbon got higher with stand age across all rotation cycles but was lower with an increased frequency of plantation. No notable difference in available soil N (1M KCl extractable NH4+ and NO3-) and phosphatase enzyme activity was observed among the different rotation cycles. Despite the fact that the tree benefits the soil through its nitrogen-fixing abilities, the export of other soil nutrients raises concern, as acidity seems to increase with cultivation frequency. Our results contradicts earlier observations that claim that the Acacia plantations are neutralizing soil acidity.

Potential in creating an additional carbon sink by alternative forest management strategies combined with carbon credits.

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The aim of my PhD project is to study forest management strategies and their effects on carbon pools in the forest ecosystem and in forest products. To transform silvicultural practices, such as using fertilizer or prolonging rotation periods to increase the carbon sink, can be costly for private forest owners. I study the effects of introducing carbon credits as a financial instrument to create an additional carbon sink. In addition to the private forest owner's perspective, I look into landscape and national level doing forecasting scenario analyses and also by applying a historical perspective using NFI data to study trends in carbon pools. I analyze land-use alternatives to better understand the potential of forestry to contribute to climate change mitigation and negative emissions of greenhouse gases. Moreover, there is a potential to increase the carbon sink with improved forest management combined with bioenergy and carbon capture and storage (BECCS) technology to create negative emissions. The Swedish forest sector could sequester millions of tons of CO2 by both acting as a carbon sink and by reducing CO2 with negative emission from wood-based products substituting fossil products.

Presence and activity of nitrogen-fixing bacteria inside Scots pine needles in the Swedish boreal forest

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Background

In recent years, endophytic nitrogen-fixing bacteria have been detected using sequencing and isolated using culturing methods from diverse native conifer trees, growing predominately in North American boreal forests. Furthermore, their nitrogen-fixing activity has been measured using acetylene-reduction assay. It has been suggested this could be especially beneficial for trees growing in nitrogen-limited environments. The aim of this study was to assess whether nitrogen-fixing bacteria are also present in Scots pine trees native to the nitrogen-limited Swedish boreal forest and if bacteria are actively fixing nitrogen inside the Scots pine needles.

Material and Methods

To assess whether nitrogen-fixing bacteria are also present in Scots pine trees native to the nitrogen-limited Swedish boreal forest, we isolated nitrogen-fixing bacteria on nitrogen-free media and confirmed their nitrogen-fixation ability by in vitro measurements of acetylene reduction. We used an immunodetection method to detect a subunit of the nitrogenase enzyme in needle protein extract to quantify the presence of nitrogen-fixing bacteria and we used acetylene-reduction assay to measure their activity inside Scots pine needles.

Results

The isolated bacteria belonged to Bacillus, Variovorax, Microbacterium, Sphingomonas, Novosphingobium and Priestia genera. 13 out of 10 isolated bacteria showed nitrogen-fixation activity measured in vitro. Additionally, we compared the presence and activity of nitrogen-fixing bacteria between control plots and nitrogen fertilized plots in a long-term inorganic nitrogen addition experimental forest. The hypothesis was that the activity of nitrogen-fixing bacteria would decrease in fertilized plots as was previously observed for nitrogen-fixing bacteria associated with mosses growing at the same field site. In contrast to our hypothesis of decreased nitrogen fixation in the fertilized forest plots, the presence and the rate of nitrogen fixation was similar between the two treatments.

Conclusion

The study proved that nitrogen-fixing bacteria are present in Scots pine needles in the Swedish boreal forest where they also fix nitrogen. The nitrogen fixation rates did not decline due to yearly forest inorganic nitrogen fertilization as the fixation rates and nitrogenase protein presence stayed similar.

Importance of organic nitrogen for shoot:root allocation

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Background

Soil nutrients are critical elements for plant growth and productivity. Nitrogen (N), in particular, is a limiting factor for primary production in most terrestrial ecosystems. Furthermore, nitrogen nutrition, its growth-promoting effects, and the importance for plant biomass allocation, when applied in its inorganic forms such as nitrate or ammonia, are well known. For a long time, the importance of inorganic N was highlighted in the scientific community and led to increased utilization of inorganic N fertilizers, which negatively impacted the environment. However, in the last decades, the focus shifted away from inorganic N as sole important N source towards organic N forms such as amino acids (AAs). In this study, I will focus on organic N in form of AAs and try to identify the effect of AA treatments on plant growth and biomass production.

Material and Methods

Different growth systems were developed to analyze the effect of organic and inorganic N sources on plant growth, shoot:root allocation, and nitrogen uptake of A. thaliana Col-0 plants. The usage of a split root system served to identify the influence of organic (glutamine) and inorganic N sources (nitrate) on the same plant. The plants were treated with the different N sources nitrate, glutamine (Gln) or both and were then analyzed regarding their 15N and 13C status, biomass, and root length.

Results

Here we demonstrated that Gln, affects plant growth positively, when used as sole N source, and leads to a distinctive plant phenotype, characterized by increased root biomass, compared to inorganic N treatment. Besides the effect on the root biomass, we observed positive effects of Gln on the presence of root hairs and on the N uptake of plants compared to inorganic N. Interestingly, similar responses could be observed when the plant was treated with a combination of organic and inorganic N. The observed phenotypic characteristics of Gln grown plants resemble those of N deficient plants which raises the question of the existence of a specific amino acid phenotype.

Outlook

Based on these results, we try to identify the role of carbon (C) provided by organic N and its effects on plant growth and development. We suggest that the observed phenotype was congruent with a significant contribution of C derived from organic N.

Pinus sylvestris and Picea abies grown in alternative substrates

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Background

The forestry industry is in constant need of seedlings for regeneration and Swedish nurseries produce 450 million seedlings annually. Nursery seedlings are predominantly containerized in Sweden and are mainly grown in peat. Peat derived from Sphagnum moss is the most common growing medium constituent in horticultural practices. Peat consists of decomposed plant material and accumulate in wetlands in the absence of oxygen. Peatlands are considered a carbon sink but give rise to greenhouse gas emission after drainage and extraction. There's rising incentive in EU to restore drained peatlands and limit extraction to reduce greenhouse gas emissions and reach sustainability goals. However, peat is difficult to substitute in horticultural practices because of its great properties as a growing medium. The nursery industry needs a sustainable growing medium that is economical and reliable in terms of quality, productivity, and availability. Furthermore, the growing medium needs to provide the plants with their requirements of water, air, mineral nutrients and physical support and not host pathogenic species.

Numerous materials have been tested to substitute peat. Forestry by-products are abundant which highlights their potential use as a peat-substitute in forest nurseries, while in addition providing circular bioeconomy. The current project aims to evaluate the usage of alternative substrates to peat in nursery production of pine (Pinus Sylvestris) and spruce (Picea abies). Plant growth and health including fungal diseases will be analysed. There is a lack of knowledge on alternative substrates' effect on fungi highlighting the importance of an in-depth analysis of the substrate's effect on the fungal community. Specifically, the substrate's interaction with mycorrhiza and potential hosting or promotion of pathogens. There's also a lack of studies with alternative substrates that have studied the plants whole life cycle- from nursery to forest. The project will therefore evaluate the storability and vitality after planting.

Material and Methods

The following materials will be used in the study: sawdust; hydrochar; biochar; milled bark; composted fiber sludge and wood-fiber. None of the mentioned materials have the same chemical and physical properties as peat. Therefore, I hypothesize that a growth medium that combines the alternative substrates can provide a sufficient replacement to peat. The substrates pH, EC, water capacity and porosity will be analysed and evaluated by recommendations for growing medium. The plants shoot-to-root ratio, Dickson's quality index (QID) and root formation will be analysed, providing a measurement of plant health. Mycorrhiza presence on roots and the fungal community in the growth substrate will be analysed during the study. Furthermore, in the study we will follow the plant vitality during storage and 1 year after planting.

The non-linear warming response of tundra heath nutrient dynamics and related plant and microbial characteristics

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My PhD project focuses on the response of cold-climate ecosystems against climate change, specifically the response of carbon C storage in tundra ecosystems against warming climate in the arctic regions of northern Sweden. The third chapter which is currently running specifically focuses on how intact tundra ecosystems (plants, soils, soil microbes) and the nutrients they contain will respond to warming. Under warming, plants and soil microbes should both increase their activity, but the former leads to higher carbon C fixation while the latter leads to increased C respiration and mineralization of soil nutrients such a nitrogen N and phosphorus P. The response of the tundra ecosystem is a sum of such individual responses, and we hypothesize that there should be a "threshold" level of warming at which the ecosystem response intensifies non-linearly, leading to drastic changes in carbon fixation and nutrient mineralization. To test this hypothesis, tundra heath have been cut out of the field as 20 cm deep 30 cm × 30 cm squares (mesocosms) with intact vegetation and soil structure. These will be incubated under five levels of warming scenarios (ambient – plus 9 degrees) for 20 weeks, which corresponds to the growing period in the field. Gas exchange will be measured periodically throughout the experiment, and soils, plant leaves, stems, and roots will be sampled at the end of the experiment. The utilization of microdialysis methods is also planned for in situ observation of concentrations of soil nutrients and related enzymes.

Towards integrated pest management in forest nurseries

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Background

In Sweden, about 400 million forest tree seedlings are produced annually and are primarily cultivated using a container system. In this system, intensive management practices may stress seedlings, thereby creating favourable conditions for the establishment and outbreak of fungal diseases. Fungal infections can have a high economic impact, and these are often controlled with different management methods including cultural and fungicidal applications. Better knowledge about local fungal communities, especially common pathogens, would improve preventative and directed control methods and ultimately reduce the use of chemical treatments. The overall goal with this project is to generate new knowledge and competence on integrated pest management (IPM) in Swedish forest nurseries. IPM includes several components and address aspects of pest management options, knowledge gain, monitoring, and timely action to prevent and control pests. The project includes four study areas: 1) introduction of fungal pathogens through seed material, 2) local spread and distribution of fungal pathogens, 3) fungal communities associated with nursery-grown Pinus sylvestris-seedlings, and the effect of microbial treatments on seedling growth and disease control.

Materials and Methods

Fungal communities were obtained from: 1) seed material collected from geographically separated seed orchards (both Swedish and non-Swedish origin) of Norway spruce (Picea abies), Scots pine (Pinus sylvestris), and Siberian larch (Larix sibirica); 2) filter papers collected weekly during 2020 and 2021 from spore trapes located at five geographically separated forest nurseries, and 3) needles and growth substrate from Pinus sylvestris-seedlings applied with four microbial treatments during 2019. Seedlings subjected to microbial treatments were planted in a southern and a northern area in Sweden during autumn 2019 and spring 2020 and sampled for needles to follow changes in fungal community after freeze storage and out-planting. DNA was extracted from collected samples, followed by amplification with fungal-specific primer-pairs and high-throughput sequencing of the internal transcribed spacer 2 (ITS2) region. Sequences were filtered for quality and clustered using the SCATA sequencing pipeline and fungal Operational Taxonomic Units (OTUs) were taxonomically identified through GenBank (NCBI) database.

Results

Results from study 1) showed host-specific fungal communities on forest tree seeds, with higher fungal diversity on P. abies seeds than seeds of P. sylvestris and L. sibirica. Additionally, a geographical variation of fungal community composition and species diversity were found within tree species. Results from study 3) showed changes in fungal community composition over time (MANOVA, $p<0.001$). Common fungal species occurred differently among the nurseries, suggesting a potential geographical variation. The fungal pathogens Phoma herbarum, Cladosporium sp., Sydowia polyspora, Botrytis cinerea, and Alternaria alternata were among the most abundant species. However, a low number of disease incidents were reported throughout the whole study period (overall infection rate $< 0.6\%$). Microbial treatments were not found to impact seedling growth or survival, nor the fungal community structure or species composition.

Conclusions

This project provides information about fungal communities associated with Pinus sylvestris and Picea abies seedlings, and occurrence of common pathogenic species within and around forest nurseries, which will be useful to improve disease control strategies.