ANNUAL REPORT 2021

TREES FOR THE FUTURE CROPS FOR THE FUTURE /TC4F

Trees and Crops for the Future, TC4F

Trees and Crops for the Future – TC4F – develops knowledge on sustainable plant production and plant based product development within agricultural and boreal forest systems with the main objective to support the development of a new circular bioeconomy in Sweden.

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Trees and crops for the future, TC4F Annual report 2021

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Chairpersons of the boards / Göran Ericsson & Christina Lunner Kolstrup

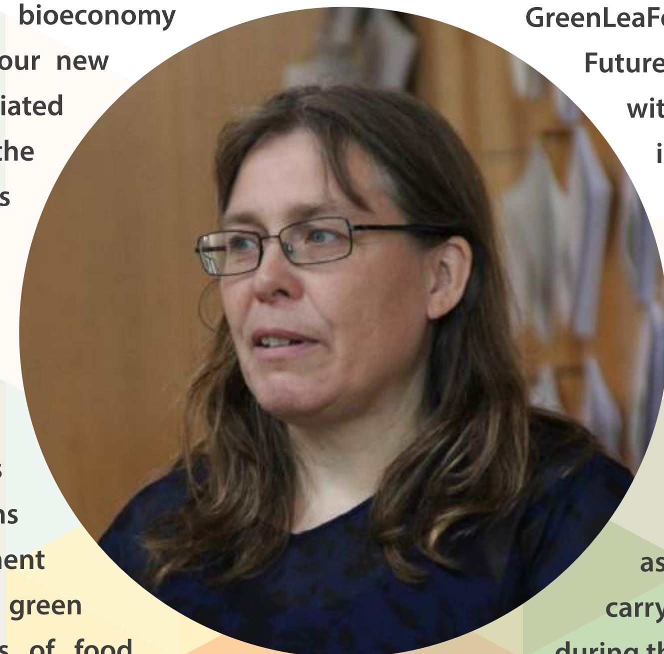
Trees and Crops for the Future (TC4F) is a research program coordinated by SLU that has been successfully addressing some of society's most important needs for more than 12 years. The TC4F program is funded by the Swedish Government as a Strategic Research Area with substantial contribution from Umeå University and Skogforsk. Most notably, TC4F has addressed the goal to maintain food and forest biomass production, while at the same time shift away from a fossil-based economy towards a more sustainable bio-based economy. As climate change accelerates, and geopolitical uncertainty intensifies, this sustainable transition is more important than ever. The program focuses heavily on research frontiers in plant molecular biology ,plant product quality, plant biotechnology, genetics, plant breeding, silviculture, agriculture, and cross-connections between these fields. TC4F is organized into two sub-programs, Crops For The Future (C4F) and Trees For The Future (T4F). For TC4F, 2021 was an exciting year, as we entered our third phase of operation (Phase III). Our Phase III research plan will run until 2025, and will focus on a new research agenda focused on a wide range of key questions related to sustainable bio-based management systems, covering scales from genes, individual plants, to whole agricultural and forested landscapes. During Phase III, the program will continue to deliver urgently needed knowledge for development of sustainable and resilient land management system for the future, which will contribute to a strong and vital Swedish Society.

Text: C. Lunner Kolstrup, G. Ericsson and M. Gundale

C4F Program director / Eva Johansson

C4F – Crops for the Future

The focus of C4F is to produce high level research in the area of sustainable new plant-based products development, using novel technologies, thereby contributing to a circular bioeconomy in Sweden. During 2021, four new projects have been initiated within C4F, also including the entrance of three new PIs into the program. Thus, we welcome Thomas Prade, Galia Zamaratskaia and Pär Ingvarsson as new PIs within C4F. The topics of the new projects cover research questions related to the development of superabsorbents from green biomass, protein structures of food produced by a mixture of legumes and cereals, and structure-function relationships between model proteins and breeding of high quality forage based on timothy. The new projects are all well connected to the strategy of C4F as well as to other large programs within the research area, such as SLU Grogrund, The Plant Protein Factory, GreenLeaFood, Green2Feed, Mistra Future Food, etc. Together with novel projects started in 2020, covering areas such as plant protein fractionation, Cd and bread-making quality, quality and quantity improvements of oils and proteins in oilseed crops and authophagy, C4F is creating a strong asset of strong research to carry the success of C4F further during the third phase of TC4F. C4F also sees several of the phase 2 projects successfully coming to an end with strong research publications, thesis defenses and sustainable new plant-based products as results from the program.



Text: Eva Johansson

T4F Program director / Michael Gundale

T4F – Trees for the Future

The Trees and Crops for the Future (TC4F) program is focused on developing new knowledge for the sustainable management of biomass production systems. The Trees for the Future (T4F) component of this program is focused on forests, which provide a wide range of ecosystem services that society is reliant on. Historically, sustainable forest management focused primarily on a single ecosystem service, wood production.

Over the past century, a gradual transition has occurred where society increasingly recognizes and values additional ecosystem services, including the role forests play as a net carbon sink in the global carbon cycle, and the role that biodiversity has in promoting ecosystem stability and resilience. Recognition of the global importance of forests in the global carbon cycle, as well as recognition that climate change is accelerating, has created a huge knowledge demand regarding understanding the trade-offs and potential synergies between managing forests for wood production, carbon sequestration, and maintenance of biodiversity. I am very excited about the progress the T4F program is making to fulfill these critical knowledge gaps.

In 2021, T4F has directly supported 15 junior and 24 senior researchers, resulting in the publication of 46 articles in international research journals, spanning a wide range of topics in forest science within the overarching theme of sustainable forest management.

For T4F, 2021 was also a year of many changes to the program. Firstly, at the beginning of the year, I started as the new program director. I am thrilled at the opportunity to work with such a diverse and talented group of scientists that participate in the T4F program. Secondly, 2021 marked the beginning of a new planning phase (Phase III) that sets the research agenda until 2025, during which a new program structure and scientific agenda will be implemented. The program will continue to serve as a research bridge between highly productive research environments at SLU, Umeå University, and Skogforsk; however, we have reorganized how these research environments will interact, aiming for an even higher level of synergy than we achieved previously. Regarding the scientific agenda, we have redefined the program focus into three general subjects, including Breeding and Genetics, Future Forest Composition, and Forest Carbon Sequestration and Soils. While breeding and genetics was a key theme in the previous T4F phases, the Future Forest Composition and Forest Carbon Sequestration and Soils are completely new research areas, and the Phase III plan will target new research questions and supports new analytical tools in each of these three areas.

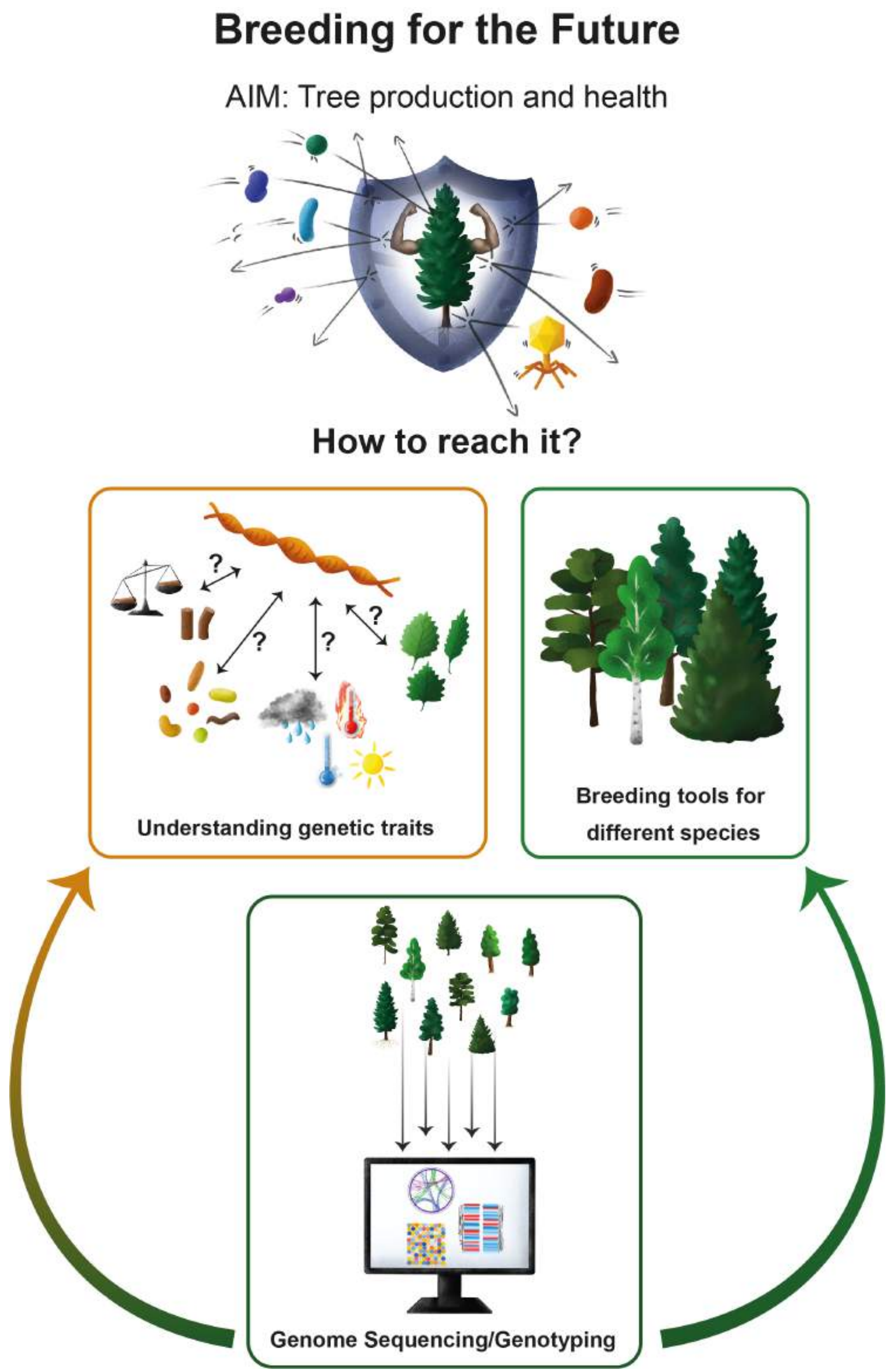
Each research theme will focus on cutting edge research and new analytical tools that will help push new boundaries in each thematic area. For Genetics and Breeding (see page 10), this will include researching efficient genetic evaluation methods and selection tools for wood production and climate adaptation. Further, we have identified a gap in experimental infrastructure that enable researchers to evaluate ecosystem level impacts of genetic and breeding enhancement. In this light, T4F will establish a new stand level experiment at three locations in Sweden (see page 26), where different levels of genetic improvement can be compared. Regarding the second research area, Future Forest Composition (see page 22), Phase III will aim to understand how interactions between tree species and environmental conditions influence biomass production; and further, will investigate how tree species mixtures impact forest productivity and resilience relative to monocultures. Research in this area will utilize existing research infrastructure, including field experiments and National Forest Inventory data. T4F will also initiate a completely new forest diversity experiment at multiple locations in Sweden (see page 28), so that the potential benefits of forest species mixtures can be evaluated under a range of environmental conditions. Regarding Carbon Sequestration and Soils, the theme will seek to understand how forest management activities affects the net carbon balance of forests, which includes not only forest biomass production, but also changes in soil C stocks. This theme will seek to understand how genetic and species compositional changes to forests, as well as other forest management activities, impact their net ecosystem carbon balances, including the often neglected soil carbon component of this. Work on soil C will focus on controlling mechanisms, including carbon inputs and outputs, and soil microbial community composition and activity.

In summary, it is a very exciting time for the T4F program as we transition to a completely new research agenda for the next five-year period. Many new personal will join the program during Phase III, and this team of researchers will continue to develop new tools and infrastructure to address the most pressing scientific questions regarding sustainable forest management for future society.

Text: Michael Gundale

THEME REPORTS AND RESEARCH PORTRAITS

Photo: Anke Carius



BREEDING FOR THE FUTURE

The boreal region is experiencing some of the most rapid effects of climate change. There are also increasing needs being placed on boreal forests to supply wood feedstock for industrial uses as well as for boreal forests to serve as carbon sinks and stores. To ensure maintained or improved productivity, improvements to feedstock quality and resilience to changing climate conditions requires rapid genetic improvement, which cannot be achieved using traditional breeding approaches with >30 years breeding cycles. Coupled to this, there is a pressing need to understand how ecosystems function in response to climate change. For example, we need to know how the economics of carbon-for-nitrogen exchange between ectomycorrhizal fungi (EMF) and trees will be impacted by the interaction of genetic improvement and climate change.

Developing such understanding requires forming links from molecules through to the ecosystem, employing genetic and genomics approaches and integrating these to landscape scale data and models. A fundamental requirement for this on both the breeding and microbial community sides is the need for genomic tools including genome assemblies, population genetics data, suitable analysis pipelines and user-friendly tools for utilising and exploring the data. Novel approaches are also needed to exploit these diverse data types to advance understanding of adaptive responses, to identify genetic markers for targeted breeding and to develop predictive models. The genetics and breeding theme of T4F includes work on these aspects, developing and supporting genomics resources as well as using those data and pipelines to perform original research.

THEME UPDATE AND OUTLOOK

This theme has two major components: developing and improving genomics resources and deploying those resources in genetics and breeding studies. Work in the theme to support development of the genomics resources is focused primarily on aspen, Norway spruce and Scots pine but also includes pipeline and analysis approaches for metatranscriptomics and metagenomics studies. These resources are also essential to support and enable the use of genomic approaches in other themes.

For aspen, the focus will be on establishment of a new field trial and subsequent development of genomics data. For Norway spruce and Scots pine T4F is supporting the ongoing KAW-funded genome project by generating transcriptomics data from abiotic stress studies. These data support gene annotation and comparative genomics studies and will identify candidate genes underlying abiotic stress regulation.

This information can then be used within genetics and breeding studies to identify adaptive alleles and to subsequently implement this information in e.g. genomics selection. There is also ongoing work to study gene and genome regulation (chromatin conformation) during Norway spruce somatic embryogenesis. An aim of this work is to better understand differences in zygotic and somatic embryo development and underlying genomic differences among somatic lines with contrasting initiation efficiencies.

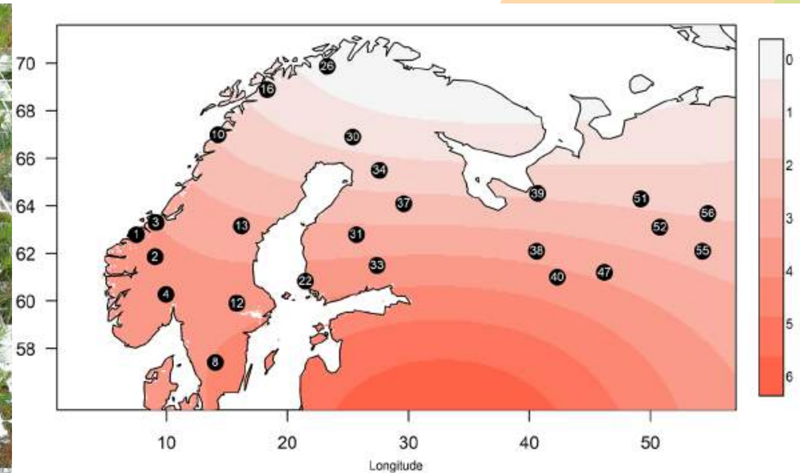
The theme will continue to support work on developing and expanding associated web resources for community exploration and utilisation of the genomics data generated by this theme, by all genomics projects at UPSC and by the wider community.
In this report we detail publications directly arising from the T4F project from this theme. However, as the theme supports the UPSC bioinformatics, genomics and transformation platforms more generally the scope and impact of the project is much broader than only these direct outputs.

MAIN FINDINGS/HIGHLIGHTs

LONG INTERGENIC NON-CODING RNA IN NORWAY SPRUCE

Conifer genomes are composed of >95% repetitive DNA elements, which have been shown to give rise to non-coding RNAs that can have regulatory roles. Given the extent of repeats in conifer genomes, we are interested to determine whether there is a higher abundance of long non-coding RNAs (lncRNAs) than in previously published studies on angiosperm species and to link these to potential biological function. For this we are using an RNA-Seq dataset generated from Norway spruce somatic embryogenesis during the previous T4F phase.

We have identified an extensive set (>6000) of high confidence lncRNAs and have explored their expression relationship to protein coding genes and miRNAs as a first step to assigning biological function. This work is being written up as a paper to be included in the thesis of PhD candidate Camilla Canovi. This initial analysis is being used to select a subset of candidate lncRNAs for functional validation studies using the genome editing facilities developed and funded by the previous phase of T4F. Complementary to this work, we have also generated data giving insight into the three-dimensional structure of chromosomes and how this changes during somatic embryogenesis. This will give greater insight into how the large genomes of conifers function and the relationship between genomics repeats, different classes of genes and chromosome conformation dynamics.



Scots pine freezing test and cold hardiness clines along latitudes and longitudes

During 2021, this has involved a lot of protocol optimisation work as available published methods do not work 'out of the box' in conifer genomes. This optimisation will also enable other groups to subsequently use the same approaches for their own study questions.
T4F is working closely with the KAW-funded genome project, which is generating new genome assemblies for Norway spruce and Scots pine. Within T4F during 2021 we have focused on generating additional transcriptomics data to complement the genome project and to enable comparative genomics using data generated for Norway spruce during the previous phase of T4F. We have therefore performed cold and drought stress studies in Scots pine and during 2022 will use this to perform comparative gene expression network studies to identify conserved and diverged abiotic stress regulators.

DIVERGENT PATTERNS BETWEEN PHENO-TYPIC AND GENETIC VARIATION IN SCOTS PINE (EMG)

In boreal forests, autumn frost tolerance in seedlings is a critical fitness component because it determines survival rates during regeneration. To understand the forces that drive local adaptation in this trait, we conducted freezing tests in a common garden setting for 54 Pinus sylvestris (Scots pine) populations (>5000 seedlings) collected across Scandinavia into western Russia, and genotyped 24 of these populations (>900 seedlings) at >10 000 SNPs. Variation in cold hardiness among populations, as measured by QST, was above 80% and followed a distinct cline along latitude and longitude, demonstrating significant adaptation to climate at origin.

In contrast, the genetic differentiation was very weak (mean FST 0.37%). Despite even allele frequency distribution in the vast majority of SNPs among all populations, a few rare alleles appeared at very high or at fixation in marginal populations restricted to north-western Fennoscandia.

Genotype–environment associations showed that climate variables explained 2.9% of the genetic differentiation, while genotype–phenotype associations revealed a high marker-estimated heritability of frost hardiness of 0.56, but identified no major loci. Very extensive gene flow, strong local adaptation, and signals of complex demographic history across markers are interesting topics of forthcoming studies on this species to better clarify signatures of selection and demography. (Hall et al 2021)

BREEDING AND GENOMIC SELECTION (SKOGFORSK)

Tree breeding programs generally conduct early selections at ages around 20% of a species rotation cycle to obtain higher genetic gains, assuming that correlations between early ages and final rotation age are high. We have now determined that these trends in genetic parameters and breeding value ranks over time in Scots pine are indeed high. Based on estimated genetic correlations, rankings of breeding value would be similar across ages up to mid rotation age indicating the early selections at 20% of the rotation length are relatively accurate for Scots pine. This work is currently being written up as a manuscript: Evaluation of genetic parameters and genotype-by-environment interactions up to mid-rotation age from improved Pinus sylvestris L. Ainhoa Calleja-Rodríguez, Mari Suontama, Torgny Persson (manuscript).
We have performed a genomic selection study in Scots pine, which indicates that nonadditive genetic variance may have a significant role in the variation of selection traits and additionally, confidence in the role of nonadditive genetic effects in this breeding program should be pursued in the future, using GS.

VARIATION IN NON-TARGET TRAITS IN GENETICALLY MODIFIED HYBRID ASPENS DOES NOT EXCEED NATURAL VARIATION

There is considerable interest in the application of genetically modified trees in forestry, especially in terms of potential ecological consequences. We have worked for many years with genetically modified hybrid aspen (Populus tremula L. x P. tremuloides Michx.) as a system in which to test basic science such as the function of genes, but also for improvement of stem yield traits. We selected genetically modified aspen genotypes for improved growth under controlled conditions and tested them in replicated field trials in southern Sweden to evaluate how the target trait (growth) translated to natural conditions. We compared the target trait variation among 16 transgenic genotypes in two contrasting field trials.

We also assessed ecologically important non-target traits: number of shoots, bud set, pathogen infection, amount of insect herbivory, composition of the insect herbivore community and flower bud induction and compared them to variation in the target trait. The variation among transgenics was then compared with the variation in a population of randomly selected natural *P. tremula*, to estimate how any “unintended variation” present in transgenic trees, which in the future may be commercialized, compares with natural variation in the native parent species.

The variation in the transgenic genotypes did not exceed the variation in the *P. tremula* genotypes. These data suggest that when authorities evaluate the potential risks associated with a field experiment or commercial introduction of transgenic trees, risk evaluation should focus on target traits. These results can guide policymakers on the use of genetically modified trees in future forestry practice.

This research line continues to yield numerous publications, for example:

Kathryn M. Robinson, Linus Möller, Rishikesh P. Bhalerao, Magnus Hertzberg, Ove Nilsson, Stefan Jansson (2021) Variation in non-target traits in genetically modified hybrid aspens does not exceed natural variation. *New Biotechnology 64:27-36. https://doi.org/10.1016/j.nbt.2021.05.005.*

SOCIAL BENEFIT

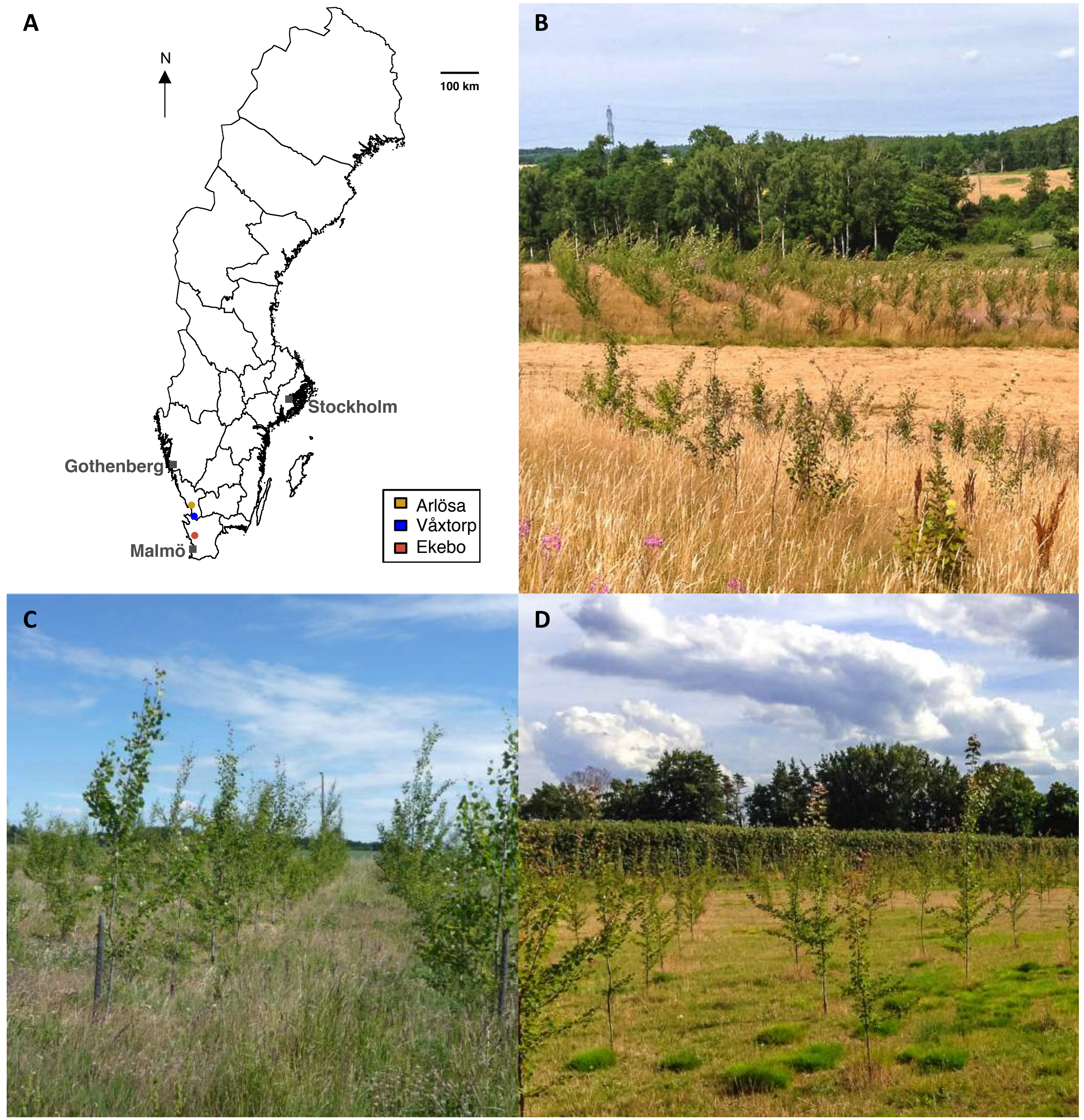
Forestry in Sweden faces increasing pressures from climate change, management policies and the need for biodiversity maintenance and ecosystem services such as carbon sequestration and storage. These often conflicting pressures must be balanced against the need to maintain economic viability of the industry. Forests also serve important societal and cultural roles and the influence of changes in forestry practise must be balanced against these needs.

One of the most pressing issues is the need to develop, either through breeding or genome engineering, climate resilient trees for replanting of forests after clearcutting. This theme is developing resources to enable both approaches and to further deploy the developed tools in breeding programmes. The genomic resources we are establishing are essential to enabling genomic based selection, a breeding approach that massively reduces the breeding cycle period by enabling selection of elite or desirable trees at the early seedling stage using only genetic marker information.

Text by T4F coordinator group: Vaughan Hurry, Nathaniel Street, Mari Suontama, Urban Nilsson and Michael Gundale

Illustration: Daria Chrobok

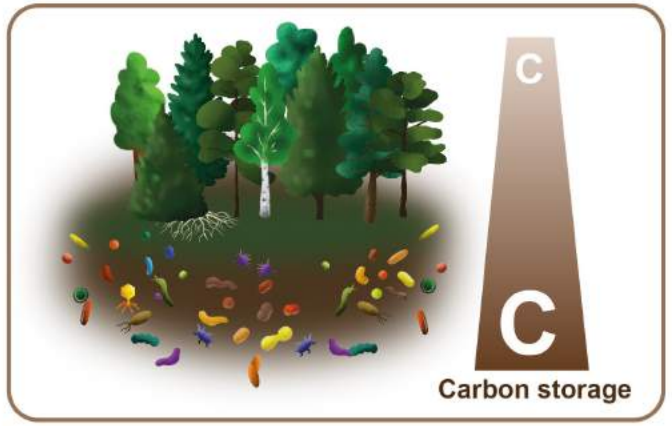
Photos: by authors

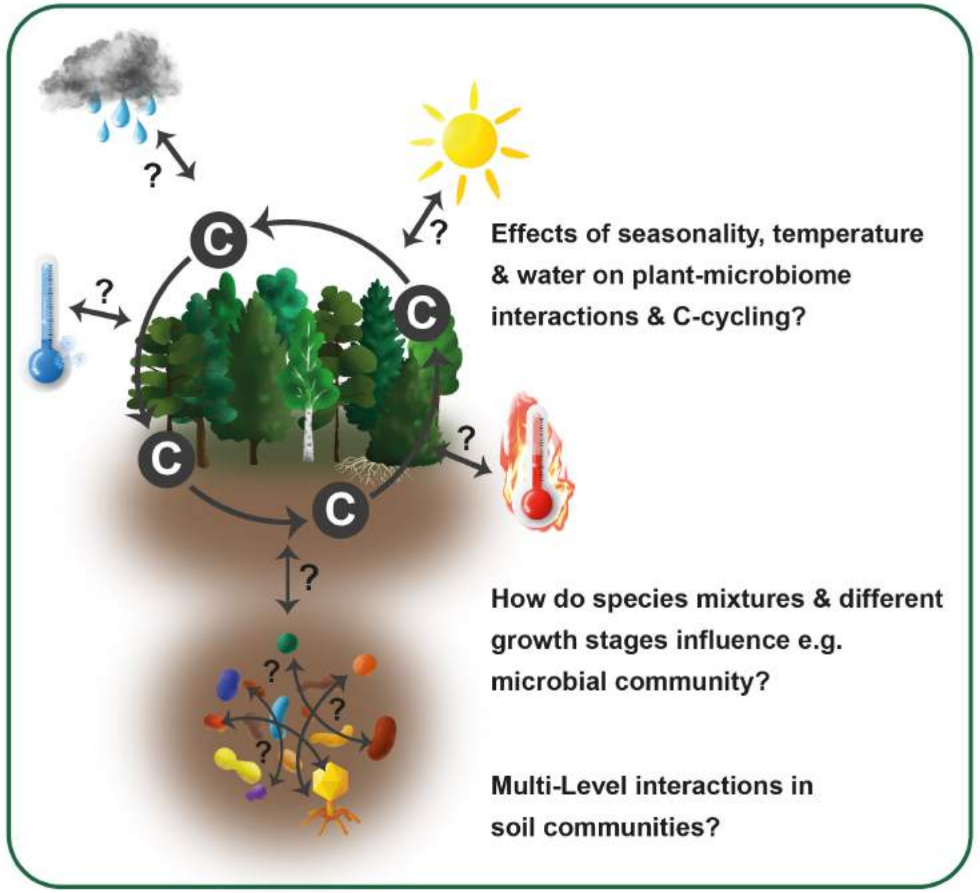


Map (left) indicating location of trials of transgenic and natural aspens in southern Sweden. Field trials of transgenic aspens at Arlösa (above) and Våxtorp (right).

Plant soil interactions & soil carbon stocks

AIM: Understanding forestry activities that influence soil carbon stocks





PLANT - SOIL INTERACTIONS AND SOIL CARBON STOCKS

Boreal forests have more than twice as much carbon stored in the soil than is found in the living tree biomass. The soil microbiome serves as a very important control on soil carbon by controlling the decomposition of soil organic matter. The regulation of soil organic matter dynamics not only influences soil C stocks, but also influences tree growth through their nutrient recycling activities that supply trees with nutrients.

Both climate change and forest management have a strong potential to influence soil carbon stocks by altering both the quantity and quality of carbon inputs to soil, as well as by impacting the composition and activity of the soil microbiome. The focus of theme C within T4F is to address the following key questions:

- How will global change drivers (e.g. climate warming, temperature, drought, and atmospheric nitrogen deposition) affect plant-microbial interactions and carbon cycling?
- How do changes in species composition and mixtures in the forest, the type of N used for fertilization at different growth stages, and shifts in the genetic makeup of planted material, influence the microbial community, carbon storage, and carbon and nutrient cycling?
- Genomic selection will potentially cause rapid shifts in the gene pool at the landscape scale as well as corresponding changes in tree-level traits – how will this influence the composition and functioning of the microbial community and the outcome of plant and forest exposure to abiotic stress?
- How do fungi, bacteria, and other soil organisms interact with each other to control soil C accumulation and nutrient cycling in response to forest management and climate change?

To develop the knowledge base to achieve this goal, it is essential that we understand how trees interact and feedback with soils. This research theme will invest in new research methods and infrastructure that are needed to addresses these questions.

MAIN FINDINGS

The use of DNA-based amplicon methods developed in T4F to profile microbial communities (metagenomics) provides insight into which microbial species are present in soils, and their relative abundance. Describing these types of microbial community changes is critical because it allows us to better understand how soils function, and how functioning is impacted by management or environmental change. However, information is still lacking for many soil microbial species regarding how they contribute to important soil functions.

This limitation can be alleviated through use of RNA based methods where relative expression (activity) of genes within each species is assayed and with functional annotations of those transcripts providing insight into active and responsive biological processes, such as shifts in metabolism. In Schneider et al. (2021) we developed a pipeline to use RNA-Sequencing (RNA-Seq) data from field samples of Norway spruce fine roots to measure fungal transcript expression and to compare community composition information to that provided by traditional DNA amplicon approaches.

Our analysis showed that at the community level expression of genes involved in starch and sucrose metabolism, biosynthesis of amino acids, and pentose and glucuronate interconversions was changed between control and nutrient enriched plots at the Flakaliden experiment. We further demonstrated that taxonomically refined insights can be obtained by showing that genes involved in processing of organic macromolecules, including aromatic and heterocyclic compounds, was enriched in transcripts assigned to the genus Cortinarius.

This work demonstrated that while RNA-Seq does provide comparable insight into community composition and shifts in composition, the taxonomic resolution of that insight is currently limited by the availability of reference genomes. To overcome this limitation, we have used our DNA data to identify nine highly abundant species at the Flakaliden and Rosindeal sites for which there is no reference genome available. We have sampled sporocarps and have sourced cultures from collaborators for DNA extraction, and in 2022 will sequence and assemble these genomes.

These types of molecular tools have been deployed in a variety of study systems, which have enabled us to show that atmospheric pollution (N-deposition) and forest fertilization change the composition and function of the fungal and bacterial microbial communities associated with Norway spruce and Scots pine stands. This has generated important insight into how these changes in microbial communities alter basic soil processes that impact the capacity of boreal soils to cycle and store carbon (e.g. Castro et al., 2021; Grau-Andres, et al. 2021; Forsmark et al. 2021). Initial studies identified how altered nutrient conditions not only change soil community composition but also identify the fungal species and the specific fungal genes that encode elicitor proteins that drive the changes in the Norway spruce root transcriptome (Law et al., 2022). These studies are now being followed up for Scots pine (Schneider et al, in progress; Law et al. in progress) and are also being used to study how, and how quickly, forest soil microbiomes recover from nitrogen addition once fertilization is halted. The methods developed in this program are also being applied to studies of how seedlings recruit their microbiome during establishment on clear-cut sites, and how this can be enhanced through local additions of organic and inorganic nitrogen (Castro et al, 2021; Schneider et al, in progress).

During 2021, we further developed the application of the microdialysis technique within the T4F program, which is a tool that can be used to understand the chemical interactions between fungi and plant roots during the initiation of ectomycorrhizal symbiosis. A study by Plett et al. (2021) first established the methodology for this type of sampling, which will allow this tool to be used further in 2022 and onward. In addition, the employment of a research engineer has enabled optimization of DNA extraction and sequencing of soil microbes from the microdialysis probes used for root simulation, and from soil. This will greatly improve and push this research forward in 2022.

A future goal is to add sequencing of bacteria to the toolbox in addition to the previously sequenced fungi to study the function of micro-scale diversity of microbes colonizing the microdialysis probes used as artificial roots. The low amount of DNA is a challenge but great progress is being made to increase the precision of this analysis. The long-term goal is to unravel the function of other soil organisms that influence plant nitrogen availability and uptake, and include them in the research toolbox. The novel approach for future studies on mechanisms involved in carbon-nitrogen exchange in plant-microbial interactions in investigating the importance of nitrogen for future forest growth under increased atmospheric CO_2 concentrations was accepted for publication during 2021 and will be published in Geoderma in 2022.

These interactions between plants and soil microbial communities have great potential to help us better understand forest regeneration and growth. In 2021 several studies were published within the T4F that shed greater light on the consequences of these plant soil interactions. For example, Nuske et al. (2021) showed that site-to-site variation in the composition of soil fungal communities can strongly influence growth of introduced *Pinus contorta* trees. Ibanez et al. (2021) showed wildfires of different levels of severity impact soil microbial communities differently, which subsequently impacts the growth rate of seedlings. New research in 2021 has also shown how a shift in plant genetic composition also can impact soil processes. Senior et al. (2021) showed that genetic improvement of Norway spruce that promoted aboveground productivity led to a corresponding increase in soil carbon accumulation, and a shift in the soil microbial community. Further, new work began in 2021 to quantify how different boreal tree species affect soil microbial communities differently, and the consequences of this for soil C accumulation.

SOCIETAL VALUE

While forest production has long been valued, society increasingly values forests for other ecosystem services, including carbon sequestration. For society to make informed decisions about synergies and trade-offs between production and whole ecosystem carbon sequestration (which includes soil C), we need a detailed mechanistic understanding of how plant nutrient acquisition processes that sustain aboveground growth affects the soil microbial community, and in turn, how this impacts soil carbon stability and accumulation. This detailed understanding will inform us about the fundamental relationships between aboveground production and soil carbon accumulation, which is needed to inform this discussion. The metagenomics and micro-dialysis tools we have established within the T4F program allow us to study the direct connection between aboveground production and soil process are being applied to a wide range of forest management factors that provide a landscape perspective on productivity and carbon sequestration. This type of information is in high demand by society, and in some cases has even resulted in collaboration with industry, for example Holmen Skog AB and SweTree Technologies, representing direct industrial application and benefit.

Text by T4F coordinator group: Vaughan Hurry, Nathaniel Street, Mari Suontama, Urban Nilsson and Michael Gundale

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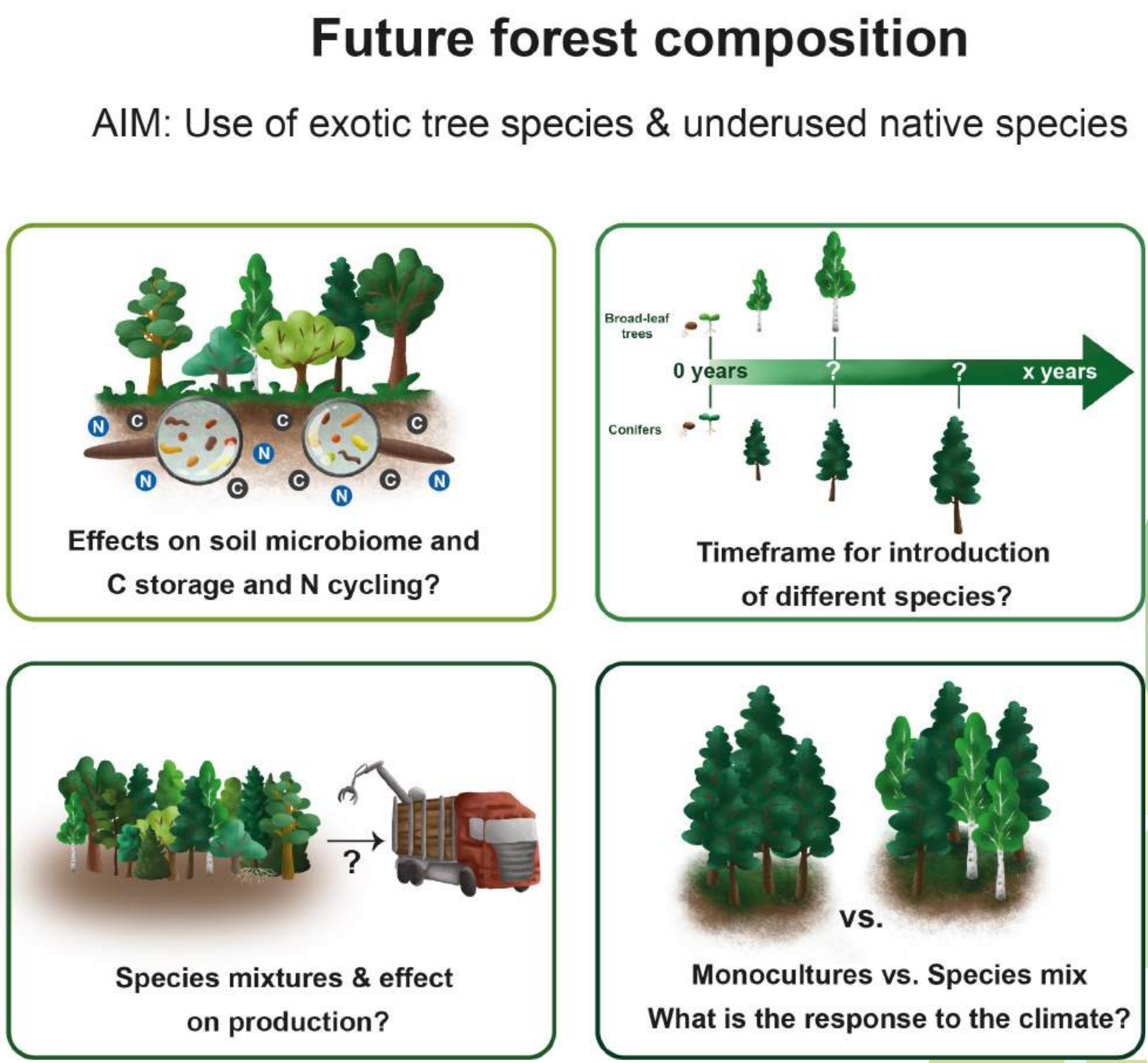
Illustration: Daria Chrobok
Photos: Anke Carius



FUTURE FOREST COMPOSITION

Modern forestry practices in Sweden primarily focus on managing Norway spruce and Scots pine. All other tree species have been considered of minor importance. However, there are indications that this focus has led to problems, for example, in southern Sweden Norway spruce is severely damaged by wind, bark-beetles and root-rot. In the north, young Scots pine trees are damaged by browsing and several different pathogens to a level that makes it difficult to establish new stands. We need to understand better where and when native species perform best, as well as the role that some non-native species may have to enhance forest production under increased environmental pressure.

Further, Swedish forests have traditionally been managed as mono-cultures, yet there is increasing evidence from non-forested ecosystems that species diversity can enhance productivity due to complimentary resource use between species and/or complimentary tolerance to disturbance or stress. While it is proposed that species mixtures may serve as an insurance policy for forest growth under changing climates, surprisingly little research has addressed the potential use of species mixtures to enhance forest growth and resilience.

Theme Update and Outlook

During 2021, we begun establishing several large experiments to evaluate how forest composition impacts forest growth. The first experiment (see page 28 for more detail) will evaluate how 2-way and 4-way species crosses of Birch, Pine, Spruce, and Larix grow compared to monocultures. Further, a large experiment focused on regeneration was started in northern Sweden, where regeneration success has been low for many decades. We published a paper showing that due to poor regeneration, less than 40% of planted Scots pine forest will develop into Scots pine monocultures. Most stands will develop into mixed species stands with a large component of birch and Norway spruce, or very open stands with low production.

New regeneration approaches are needed to increase survival of planted seedlings. In the new experiment, several tree species and combinations of tree species will be evaluated with different scarification methods. In addition, we are evaluating how fertilization at the time of planting may be used to enhance regeneration success of contrasting species. The experiment will be installed in ten sites from Jämtland in the south to Tornedalen in the north.

During the past year we also completed a nationwide study comparing growth of Norway spruce and Scots pine, which will be presented in a dissertation in April 2022. The study shows that Scots pine is a good choice for production even on relatively fertile sites in southern Sweden, which is opposite to current recommendations. This unexpected finding also means that new regeneration treatments need to be tested in these fertile environments. In this light, we used a new shelterwood experiment set up in Tagel, Sweden, to examine the effectiveness of various regeneration approaches, which was described in two manuscripts that will be submitted for publication during 2022.

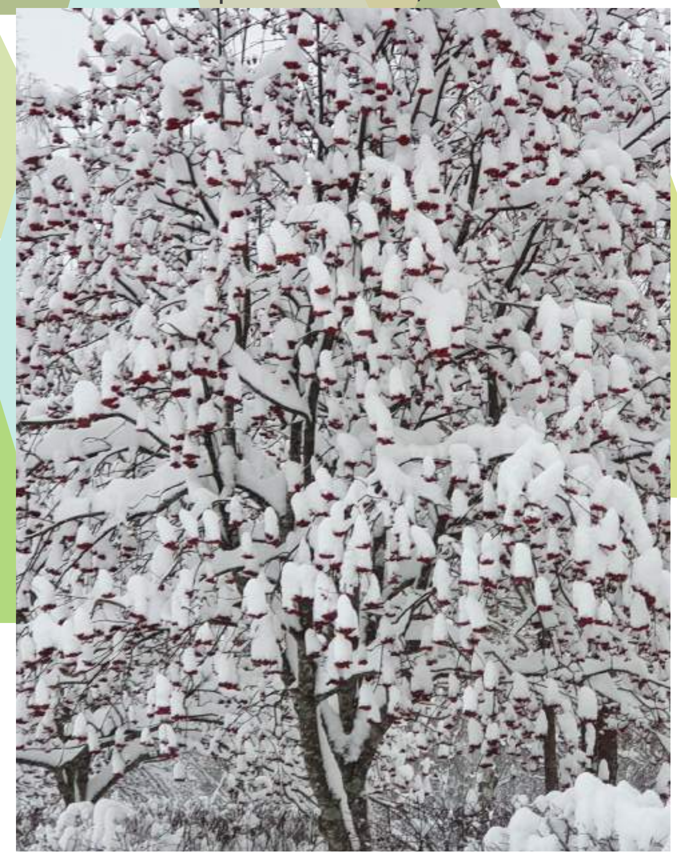
Further, a post doc supported by the T4F program published a manuscript describing the interaction between tree species and climate on forest growth. The study was carried out in northern Sweden using a gradient of landscape aspects and slopes to serve as a proxy for climate. Results show interactions between tree-species and landscape position, and that Siberian larch and lodgepole pine may be good alternatives to Scots pine on sites with harsh climates. This work will lead to more informed decision making regarding where different tree species will perform best in Northern Sweden.

In addition to work focused mainly on Spruce and pine, genetically improved birch is an interesting tree species that may serve as alternative in almost all of Sweden. Skogforsk in Sävar will soon be able to provide seeds from improved birch material for northern Sweden and Skogforsk in Ekebo have been producing seeds for southern Sweden for many years. However, planted birch is a new tree species for Swedish forestry, and adaptions may be needed compared to silvicultural treatments for Norway spruce and Scots pine. An ongoing PhD-project is examining the effects of browsing on growth and quality of newly planted birch, extent of browsing in practical birch-plantations and effect on growth and quality of spacing at planting.

In 2021 we also started a new Post doc position focused on disease resistance of Larch. Larch is of interest because one approach for adaptation of forests to climate change is to diversify them with other tree species, including natives and exotics. Among those include various Larix species, including hybrid larch, European larch and Siberian larch. European larch is no longer used in Swedish forestry due to its high susceptibility to larch canker disease. However, putatively resistant material from central Europe brings about a renewed possibility for the use of European larch. In addition, there is greater interest in using Siberian larch, considered native to Sweden, and the potential for increasing the use of all three Larix species needs to be assessed. The overall aim is to support the current and potential use of Larix species.

During the past year, we also recruited a new Post doc to use Swedish national forest inventory data to evaluate how different tree species grow in mixtures versus mono-cultures. The work is inspired by past research that has shown mixed species stands grow at a higher rate than mono-cultures, which has been a hotly debated conclusion. While this past debate has been largely focused at evaluating stand scale growth, the new project initiated in 2021 will look at the tree-level, with the goal to identify which tree species benefit the most and least from growing in mixtures with other species. The project will further explore how these effects of mixture vary depending on site fertility and climate.

Finally, in 2021 we also developed and used a hybrid model for estimating Scots pine and Norway spruce growth. Hybrid models combine traditional empirical models, which relies on



historical growth patterns, with mechanistic models, which can include additional factors such as effects of future climate change. In our new model, we have replaced time with useable absorbed radiation. In this way, we could explain historical growth with climate variables and make a model that is sensitive to climate change. The model gave satisfactory results when compared to traditional empirical models and will be developed further for broadleaves and mixed species forests. In another project, we have combined traditional empirical models in Heureka with a mechanistic model 3PG. The combined model, 3PG-Heureka, has been parametrized for the entire forest landscape of Sweden, and is now implemented in the decision support system Heureka. The model will be used in the upcoming scenario-analysis SKA22 made by the Swedish Forest Agency.

Societal Benefit

Swedish forests serve many purposes. Production of raw material for the forest industry is of course important, but it must be done without jeopardizing biodiversity or production of other ecosystem services. In the theme Forest Composition, all projects address this multifunctional aspect of forests. In addition, the aim for increased diversification will help to ensure sustainable production of many ecosystem services. As described above, current debate questions the sustainability of today's mono-culture approach focused on two tree species. Therefore, this research theme explores new frontiers for how a more variable forest composition can be utilized to achieve a wide range of forest management objectives, which are desired by forest owners and managers.

Text by T4F coordinator group: Vaughan Hurry, Nathaniel Street, Mari Suontama, Urban Nilsson and Michael Gundale, Illustration: Daria Chrobok, Photos: Anke Carius

T4F Field Experiments:

Ecosystem Genetics Experiment Implemented by the T4F program

Tree breeding programs are designed to enhance forest growth by selecting the best performing individuals, and then cross breeding them to produce more vigorous offspring. For Norway Spruce, testing and selection of tree material is based on a multiple breeding population strategy, defined by the latitude and temperature regime.

For Norway Spruce, there are 24 different breeding populations across the country. Deployment of improved genetic material from the breeding program originates from seed orchards for specific zones which are defined by the climatic and environmental variables. Genetic improvement of seed orchards is now on its third cycle, and moving towards a fourth cycle of breeding. Predicted genetic gains for the first, the second and the third cycle of improved seed orchards is 10, 15, and 25% respectively, in comparison to the base line unimproved forest stand material (0%). There is increasing interest in the ecosystem consequences of variation in tree growth; however, there are almost no existing stand scale experiments of improved tree material that allow ecosystem pools (e.g. above and belowground C and N pools) or fluxes (e.g. Gross Primary Production, Net Primary Production, Net Ecosystem Exchange) to be assessed. For this reason, T4F is implementing a new experiment to provide a comparison of how genetic improvement may influence realized forest ecosystem properties at different levels of genetic improvement.

The experiment will focus on Norway Spruce (*Picea abies*), and will focus on four different levels of genetic treatments (% growth increase), including: 1) unimproved forest stand material (0%), 2) the first breeding cycle seed orchard material (10%), 3) second breeding cycle seed-orchard material (15%), and, 4) the cross-control material from the latest breeding cycle (25-30%). The experiment will be set up in each of three locations at SLU research park sites, Svartberget (64 N), Siljanfors (61N) and Tönnersjöheden (57N). The seed material that will be planted at each location will come from the specific breeding zones for which the genetic improvements have been made. The experiment will consist of 16 plots in each trial, including 240 trees per plot, total 3840 trees per site and 11 520 trees in total across the three sites. Ecosystem response variables such as soil carbon and nitrogen stocks in the organic and mineral soils, soil pH and understory vegetation composition as well as tree growth and adaptation including survival and resistance to diseases and pests will be assessed at each experiment. This will provide unique knowledge of how genetically improved material will respond to surrounding ecosystems and their capability to mitigate factors affecting climate change.

This experiment will be the first of its kind in Sweden, allowing a comparison of how different levels of genetic improvement for specific environments affects a wide range of important ecosystem response variables, such as carbon uptake and storage in aboveground biomass and soils. Since it will be possible to have genetic background information for each tree, the trials will also provide the possibility to estimate the influence of genetic variation in these ecosystem measurements, which to our knowledge has never been done before in the breeding program material of Swedish Norway Spruce. Ultimately, the use of ecosystem level measurements in this experiment will serve to quantify genetic gain, and contribute to an overall knowledge of the status of Norway Spruce breeding in adaptation to climate change.

Text by T4F coordinator group: Vaughan Hurry, Nathaniel Street, Mari Suontama, Urban Nilsson and Michael Gundale
Photo: Anke Carius

T4F Field Experiments:
New mixed forest experiment

Recently, researchers and society have debated the potential benefits of mixed species forest management, as an alternative to traditional monoculture forestry. Compared to monocultures, several positive outcomes of mixed species stands have been identified, including increased biodiversity, water quality, esthetical and recreational values and reduced stand vulnerability to pest and pathogens (Felton et al. 2016). In the Swedish database for long-term forest field experiments (Silva boreal), there are 4151 experiments in total listed. An absolute majority of these experiments are focused on homogeneous monocultures of either Norway spruce or Scots pine, and less then 50 experiments in total represents mixed forests.

Therefore, the scientific base is much stronger for recommendations of stand treatment for pure Norway spruce and Scots pine than for mixed species forests. About one third of mature forests in Sweden are mixed species stands, but current recommendations for pre-commercial and commercial thinnings are designed mainly to promote homogeneous monocultures.

In the future, new remote sensing data will give a much more detailed description of species composition on a higher spatial resolution than previous stand-level data. With this new data, it will be possible to apply treatments to small areas for promoting stand development depending on desired future stand structure. However, doing so requires information about species and stand level growth in mixtures, which is currently done primarily via model simulation approaches. Empirical data are sorely needed describing species and forest level growth patterns in mixed forests in comparison to monocultures.

Because of the apparent advantages of mixed forests, and because of the lack of data for decision-making about stand management treatments in mixed forests, it is urgent to implement a new experiment to support future research and management in mixed species stands. To address this knowledge gap, T4F will begin installation of a new experiment in 2022 that will experimentally mix 4 different species, as well as mono-cultures of each species at 5-10 locations spanning a range of site conditions in all regions of Sweden. The aim of the experiments is foremost to secure long-term data of stand development and carbon sequestration in mixed species stands, including in aboveground biomass and soils. However, the experiments will also yield short-term data on establishment of the different tree species in mixtures and monocultures in varying site conditions in different regions of Sweden, as well as possibilities to measure a wide range of other ecosystem services.

Tree-species that will be included in the experiment include Norway spruce, Scots pine, birch, and Siberian larch. All four species are designated as native to Sweden, and their use in regenerations are therefore not restricted by certification rules. In the experiment, plots with monocultures of each species will be compared with six two-species mixtures and one four-species mixture. In addition, we will establish monocultures and mixtures with two initial spacings, 1.5 x 1.5 m and 2.2 x 2.2 m, to understand how positive effects of species mixing might change with increasing competition intensity. These treatments sum up to 22 plots in total on each of the three sites.

Because the main aim of the experiment is to provide long-term stand level data on mixed forests, all treatments will be set up in large 0.1 ha plots, which includes buffer zones for eventual pre-commercial and commercial thinning that will retain the mixtures throughout the rotation. We will measure seedling establishment after the first, third, and fifth growing season, and ecosystem carbon stock and other ecosystem properties and processes episodically. The experiments will also be managed and measured according to normal protocols at the Unit for Field-based Research at SLU.

Text by T4F coordinator group: Vaughan Hurry, Nathaniel Street, Mari Suontama, Urban Nilsson and Michael Gundale
Photo: Anke Carius

Ewa Mellerowicz

How modifications in the cell wall can influence tree productivity

Cell walls in plants have several important tasks to fulfill. Trees allocate their assimilated carbon in their woody stems. Therefore cell wall synthesis is an important factor in tree productivity. Trees have primary and secondary cell walls, the secondary walls being thicker and providing structural stability. Secondary cell walls are built by cells that have stopped dividing, but they are still metabolically active. Cell wall synthesis regulation is complex and many signaling pathways are not yet well understood. One of them is the cell wall integrity sensing, especially of the secondary cell wall.

It has previously been shown that modifications in xylan structure in Arabidopsis and Aspen trees can have both growth enhancing and growth suppressing effects. Whether a specific modification is positive or negative for biomass production over time seems to depend partially on how extensive the modification to the xylan structure is. For extensive defects, the effect is more likely compromising growth, for minor modifications it is more likely to be beneficial. However, even minor modifications can have further benefits for the tree and for biobased products to be produced from it. Some modifications have shown to improve the biotic resistance of the trees and also the saccharification, meaning the degradation to sugars for utilisation of biomass can be facilitated through certain modifications.

This postdoc project focuses on the hypothesis that the cell wall integrity signaling pathway is responsible for detecting the modifications in the xylan structures and increasing the biomass productivity and growth in these trees, as a response to minor defects in the xylan. From a previous trial, transgenic aspen trees with modified xylan structure and enhanced productivity were selected for a transcriptome study that will reveal important players in the regulatory pathways.

The wood of these trees will also be investigated for their extractability, and analytical saccharification to see which of the modifications would be most suitable for further processing toward bio-based products such as biofuel.

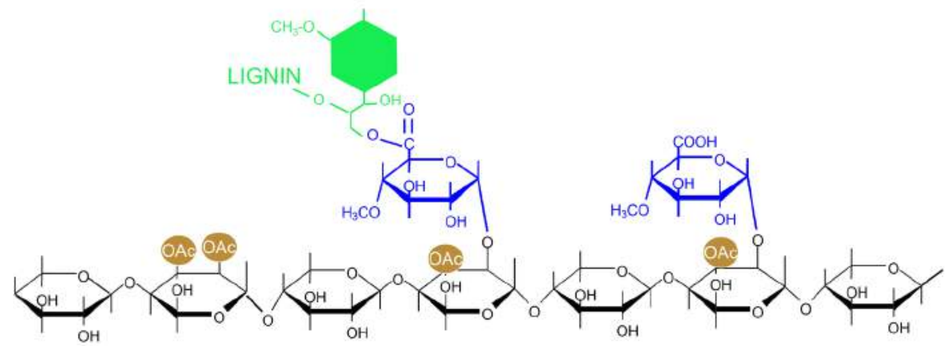
Genetically modified trees that were doing really well in the green house have previously shown to become difficult to maintain once planted out in the field. Modifications that seem to only influence a small part of the plant will inevitably influence the whole metabolism and it is impossible for scientists to foresee, how the plant will perform under natural conditions outside of the greenhouse.

Greenhouse experiment with transgenic aspen (Photo: Ewa Mellerowicz)

Here, the modified aspen trees are doing exceptionally well. They are still fast growing, with better biomass productivity than their control group. Project leader Ewa Mellerowicz is very confident that these trees will be quite applicable in plantations in the future.

This is the first time a project with directed breeding towards a specific, industrially desired trait is incorporated in the TC4F project. Previously, directed breeding was mostly directed towards improving general traits of the trees, selecting the most resistant, fastest growing or otherwise best individuals for further propagation or crossing. T4F projects encompass different levels ranging from the cell and molecule level, the tree level up to the stand and landscape level whereas this project addresses mostly the scale from the molecular to the individual tree level.

Text: Anke Carius, Photos and illustrations E. Mellerowicz



The structure of xylan. (Illustration Ewa Mellerowicz)

Petter Axelsson

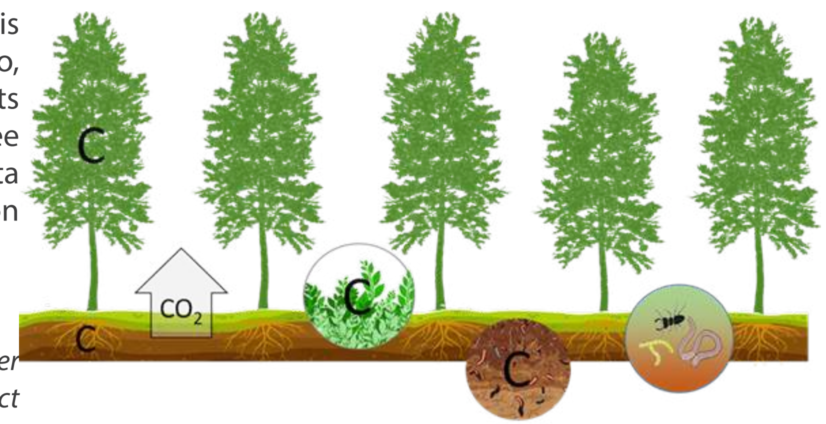
More biomass production means more carbon sequestration, right?

For many decades the main goal for forest tree breeding has been the increase of above ground biomass productivity. By now many methods are available for natural and directed breeding of boreal tree species and many lines are available that have significantly improved biomass production and hence carbon sequestration above ground. However, a large part of carbon sequestration in forests is due to accumulation of carbon in the ground, as molecules like lignin and humic acids that are hard to degrade for microbes, fungi, and even earthworms and other animals. Hence, the idea that above ground carbon sequestration is linear to below ground sequestration may not hold true for improved trees.

Changes in tree metabolism that are required to make trees grow better can also have flow on effects on the interactions between the tree with its environment. A genetically iproved tree might have different mycorrhiza, take up micronutrients in a different way, deprive the soil more, have deeper roots or many other possibilities. The genetic control of these below ground interactions are widely unknown, and this project will shed some light on how genetic gains in tree growth might alter their environments, and how well they do for net carbon sequestration. The project will utilize an already established long term experiment replicated across a climate gradient to assess the dual influence of genetics and climate on carbon sequestration. Within this experiment, carbon assimilation and release will be measured in several different places, assessing carbon storage in the tree, in the understory vegetation, soil carbon and litter input. As it has been shown in some studies, increased photosynthetic activity can lead to increased soil respiration, CO_2 efflux from soil, which will be determined in our study. In further work packages, the earthworm and the fungal community will be assessed. Genetic experiments replicated over climatic gradient, such as this one, comprise powerful tools to assess impacts of climate change on ecosystem function. The collection of weather data during the measurement of soil respiration will give valuable information about the impact of climate changes on the release of carbon from the soil. "Economics and environmental sustainability can often appear to be competing goals; however, the T4F strategy highlights that the goal to increase productivity in Sweden's forests can be hugely beneficial for both.

Picture 1: Measurement of carbon release from the soil, Picture 2: Soil sampling for microbes and animals.

This project is directly relevant to this statement as we will assess if, and if so, how genetic gains in tree growth in Scots pine, one of the prime targets for tree breeding in Sweden, may effect soil biota and C-sequestration in soils – a question that is currently unresolved."



Text: Anke Carius, Photos and illustration: Petter Axelsson, quote: Petter Axelsson, from project application.

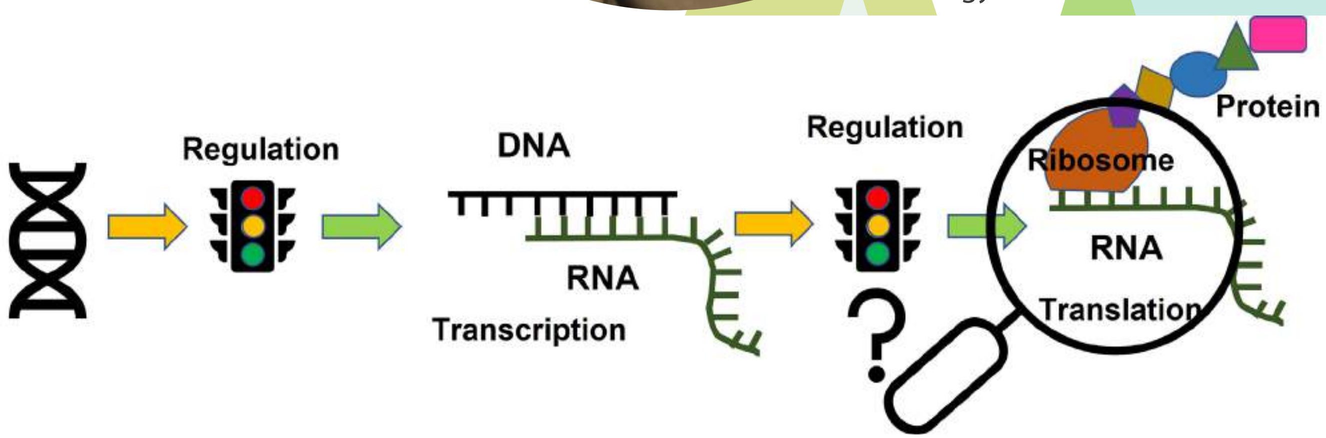
Ribo-Seq: Illuminating a new regulating point in the genetic expression of plants

Johannes Hanson

To run a complex organism as a tree, for example, genetic expression must be highly regulated. Researchers have made significant progress in understanding how plants work by measuring expression levels of mRNA and abundance of proteins. Whilst the existence of mRNA strands is the absolute prerequisite for making the corresponding protein, it does not necessarily mean that they actually get translated. The amount of abundant protein in the cell is regulated by even more factors than just the amount that gets translated, for example by how stable it is and how quickly it gets degraded. This reveals that even though we maybe know mRNA-levels and final protein levels, we have no clue what is going on in between.

Johannes Hanson started to work with this gap in information when Hannele Tuominen came to him with a mRNA measurement for lignin monomer synthesising enzymes in spruce, that was exorbitantly high during the winter, where trees have hardly any metabolism. There are two reasons why the tree does this: It needs these enzymes during winter to repair the lignin or it needs these enzymes quickly in the beginning of the spring to get a head start as soon as possible.

Hannele wanted to know if Johannes could find out when those mRNAs actually were translated. To prepare for things that are coming up in the future by pre-making mRNAs is not a unique phenomenon: Seeds are stuffed with mRNAs that allow them to germinate quickly. This option of storing mRNA and not translating it, reveals a regulatory step between transcription and translation that is still mostly a blind spot in plant biology.



To illuminate this, Johannes adjusted the Ribo-Seq technique to conifers and other trees (now the methodology is available for Aspen and Spruce). Ribo-Seq only analyses the mRNA that, in the moment of sampling, is attached to a ribosome and therefore definitely being translated into a protein. Comparing the new Ribo-Seq data sets with mRNA data and protein data will reveal new regulatory pathways that can turn into new breeding targets! In the future, the methodology will be especially interesting in regard to teaching trees to be prepared to rapidly changing conditions as hot and cold snaps that are expected to become more common as climate change progresses.

Ribo-Seq data will in the future become a fixed part of plant sequencing databases, however, as the technology is quite new and needs to be adjusted to every new species that it is used for, it is quite expensive still and fewer samples need to be sufficient to start building the databases and screening for information.

Text and illustration: Anke Carius,
Pictures: Bernard Wessels

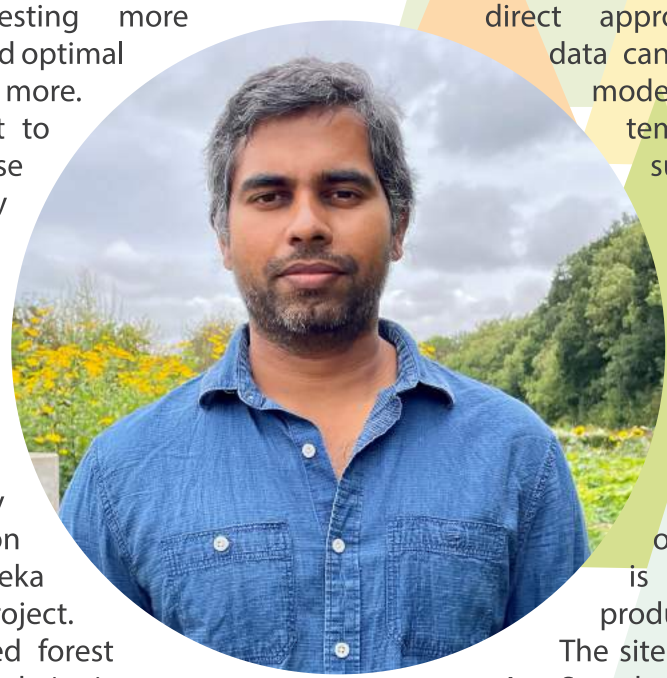
Pictures: Samples are taken quite high up in the trees: Postdoc Bernard Wessels is not afraid of heights.

Narayanan Subramanian

Improving climate change predictions in the HEUREKA forest management model

Intensive forest management will be needed as in a biobased economy the demand for wood will increase. As the area of forest will likely not increase, more productivity is necessary. Therefore, forests will need to be meticulously managed to meet the demand. This has of course some positive effects on the forest but will also have very negative effects, for example leaching of nutrients from fertilization into the ground and to streams and groundwater.

The main aim of this project is to determine, whether we can attain higher carbon sequestration by forest management and how the chosen forest management strategy contributes to that. There are a variety of forest management methods to choose from, for example short rotation, planting hybrid material, harvesting more frequently, final felling, and optimal fertilization and many more. It is therefore important to find out how each of these methods individually contribute to the forest carbon sequestration. Together with this, the negative effects of the forest management techniques will be addressed.



Narayanan has already been working on developing the Heureka model during his PhD project. Heureka is a widely used forest management model and it is an added value system. Right now climate change is integrated as an ad hoc method, which does not display extreme events very well, and it overestimates the future growth of forests with climate change. Now, the modelling approach has been changed from an indirect approach that implemented climate change as increased temperatures into the growth model to a more direct approach. Monthly climate data can be fed directly into the model, then including real temperature variations, such as cold snaps or hot snaps, drought and other extreme events instead of smooth temperature increase. This should address the current growth overestimation and reflect the actual situation more accurately. This project focusses on Norway spruce and is aiming toward biomass production optimization.

The site used for this project is in Asa, Sveaskogs 1100Ha area. This park is established aiming to improve forest productivity by 50% in 50 years. It was established in 2009.

When the focus is really on achieving as much production as possible, to avoid thinning in combination with final felling as early as possible is expected to be most efficient. When you want to change to an exotic tree species for example the whole forest system would need to be changed. But to avoid thinning and harvesting trees as early as possible in the spruce dominated system that exists, it will be the quickest way to adapt to increased demands.

Also making use of harvesting residues like stumps would be a quickly applied option to retrieve more from the existing forest. The economics of this is however unclear. Fertilization is another management option than can be applied to existing forests. Currently, the focus of the study solely focuses on the use of nitrogen, which is the most commonly used fertilizer in forestry with the biggest impact on biomass production.

Text: Anke Carius, Photos: N. Subramanian, Anke Carius

C4F LEADERS: EVA JOHANSSON AND LI-HUA ZHU

C4F- Crops for the Future

C4F program has been functioning as a research platform since its start, supporting a large number of projects, connected to other large projects. During 2021, about half of the projects were finished and new projects have been initiated, in which the involvement of young researchers (PhD students, postdoc and project leaders) has been further highlighted among the other important criteria. The overall progress of the research within C4F has been in principle as planned in 2021.

A number of peer-reviewed articles of high quality have been published, while a number of manuscripts have been submitted or are in the pipeline for publication. A couple of young researchers, PhD students, or postdocs have been recruited and new young project leaders have been appointed. The Grogrund research school associated with C4F has been established. A number of large grants have been received, partially due to the C4F program, such as Formas food, 8 milj, Energimyndigheten 5 milj, BSRC (bioresource center) – SLU together with Linköping university. Xue Zhao has defended her thesis in October 2021, in which she has developed a simplified method for determination of the branching density in amylopectin.

Research on innovatively designed wheat gluten fibers as potential absorbents of biofluids and impact of climate on Swedish wheat protein quality have resulted in accepted publications. The new results on the use of neutron techniques to monitor Cd stress and water uptake in wheat for food uses have been generated. The results on the relationship between amylopectin internal molecular structure and its thermoanalytical properties have been generated. A 3-D printing technique has been applied in legume research. Studies on purification methods for mung bean protein, spinning protein nanofibrils in flow celles into threads, utilization of SAXs at DESY and texture and microstructure of mixed faba bean protein/starch/fiber gels have generated data for publications.

Based on the study of carbon allocation in rice, the mechanism of methane emission in rice paddies was reviewed, 5 low methane varieties through hybrid breeding with 70% methane reduction from rice paddies were bred, which could generate high biofuel rice straw and build up a win-win cultivation model with low-temperature-adaptive rice in Uppsala.

The latest genome editing technique, CRISPR/Cas9, has been one of the major advanced breeding technologies used in studies on the oil crops and potato within C4F, as an efficient molecular tool for research and crop improvement. The well-established protoplast protocols established within the program have enabled generation of transgene-free mutants of the target crops by CRISPR/Cas9. CRISPS-edited mutation lines of rapeseed and field cress have been obtained for improving the seedcake and oil qualities. RNA-seq analysis in relation to changes in oil and starch on greenhouse grown CRISPR-edited potato with redirected carbon flow has progressed. Moreover, genome sequencing information and RNA-seq profiles have also been used in analyzing evolutionary perspectives of oil accumulation in *Cyperus* tubers.

Successful transient expression of gene combinations to optimize production of novel molecules in pheromone work was achieved and stable transformants of oilseeds were obtained. RNAseq analysis and gene mining of *Lindera* species have been progressed for potentially improving incorporation of medium chain pheromone precursors in camelina.

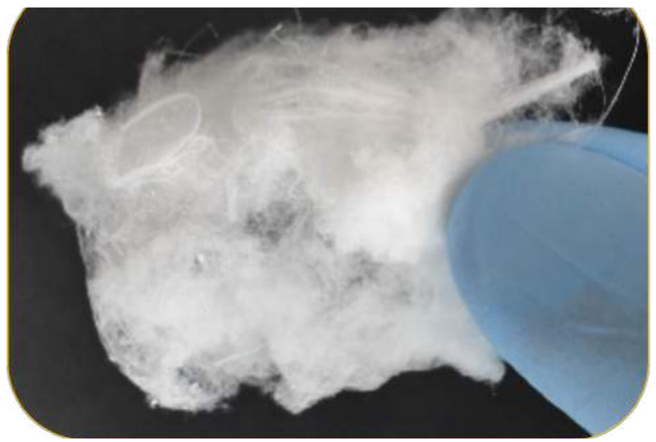
A significant progress in establishing the methods and tools required for elucidating plant autophagy dynamics and the molecular mechanism underpinning the effect of chemical compounds, which we identified previously as selective plant autophagy regulators, has been achieved.

Research outcomes and associated outreach activities deal with new knowledge and information on novel potential uses of plant oils, proteins, starches and other compounds from side streams, which can be used as food, feed and industrial applications. The program contributed to emerging of new research areas such as bio-based composites for food and non-food uses, possibility of crop improvement by regulating autophagy process, and renewable sources of plant produced insect pheromones for pest management. SLU Grogrund has continued to support more novel projects in 2021. Due to Covid-19, physical participation in national and international conferences has not been possible in 2021.

Detailed research findings and progress

Protein fractionation of green biomass, its problems and opportunities in terms of both technologies and economy were elucidated. The major finding is that the fractionation from the pulp has to be further resolved. The structure of protein isolate from lupin and from innovatively processed quinoa was further evaluated by the most novel methods. Functionalization of potato and wheat proteins to obtain superabsorbent materials was further evaluated and described.

A new method was developed to design sustainable microfibers from wheat gluten protein using a non-reducing solvent and electrospinning. The wheat fibers containing 20% of gluten were relatively strong and very promising as blood and biofluid absorbents (Fig. 1). Heat treatment of gluten fibers improved chemical bonding in the proteins and the functional properties of fibers. Varying climate such as, prolonged heat and drought, increased gluten polymers and large monomers, and the ratio of monomers vs polymers, and decreased protein concentration in the wheat material grown during the two years. The cool climate resulted into a longer grain development time, while the hot and dry climate resulted into much shorter grain development period. The yield was impacted negatively, while the grain microstructure was found to be similar due to the heat and drought stresses. The recent neutron results indicated that Cd stress impacted negatively the wheat root development and this differed in the two studied growing media.



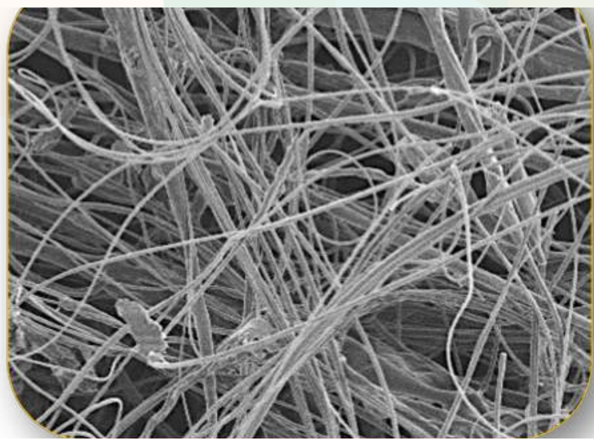


Fig 1: Wheat gluten fibers as potential absorbents of biofluids (Photos by Faraz Muneer)

The results on characterization of secondary structure of 7 different PNFs along with how protein size and purity affect the ability to form PNFs were published. Plant-based PNFs' ability to form stable foams at different pH–values was investigated and the ability of the PNFs to form stable threads was investigated by SAXS.

Faba bean starch, side-stream product, was structurally and chemically characterized and correlated with the thermal behavior. Faba bean starch is amylose rich, has high proportion of long-branch-chain-lengths and a homogenous granular size distribution causing higher gelatinization temperatures and relative viscosities. Isolated faba bean fractions; starch, protein and fiber, were combined to create an edible bio ink. Inks with higher proportion of carbohydrates were more stable. Faba bean protein films were reinforced with cellulose-nanocrystals from pine cones. Pea protein gels at different pH and concentration of unrefined lentil fraction were characterized in terms of texture and microstructure. Results showed that starch was responsible for changes in viscoelasticity and insoluble cell wall fragments acted as fillers in the gels. Similarly, mixed faba bean gels were characterized and the effect of starch and fiber on texture and microstructure of heat induced protein gels was investigated (Fig 2).

The simplified method for determination of the branching density in amylopectin developed by Xue is very significant for connecting starch structure and its physical properties. We strongly believe that the branching density is one of the most important features for the rate of retrogradation. The fact that we now can measure the branching density in a large number of samples makes it possible to provide good statistical evidence for correlations with physical properties. This method has been applied on barley lines with a systematic variation in a transcription factor controlling the starch synthesis.

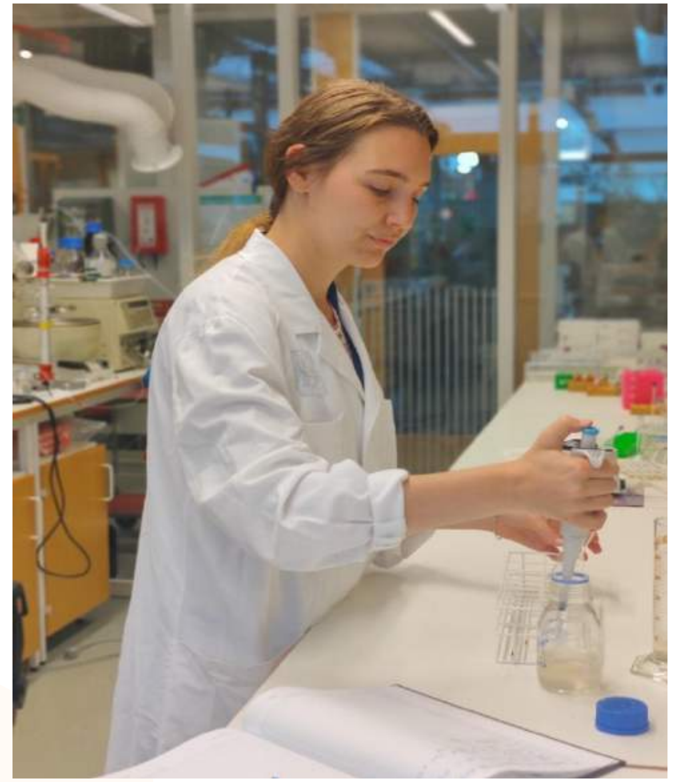
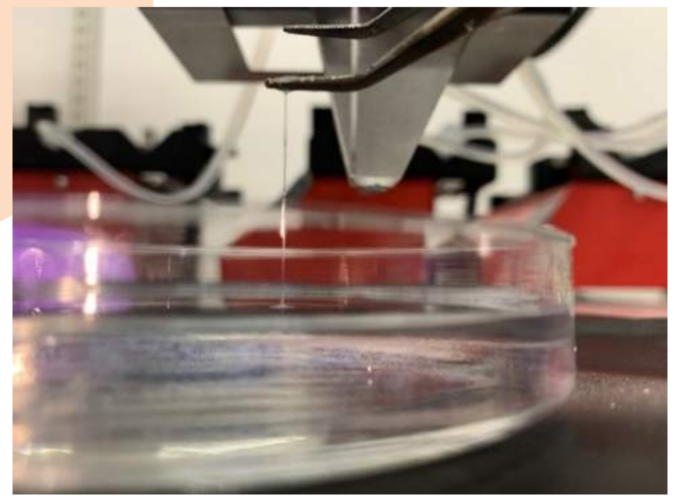


Fig 2. above: PhD-student Anja Herneke working with protein nanofibrils in the lab (Photo by Klara Nilsson).
below: Thread made from spinning mung bean protein nanofibrils. (Photo by Anja Herneke)



The results are very promising and will be published together with data describing gene activities and beta-glucan levels.

Biomethane production in *WRI1*-rice straw has been detected with a 20% increase compared with wild type without any grain yield defect. A win-win cultivation modus of Nordic climate adapting rice has been shown. We have also shown that endogenous fumarate and ethanol dictate methane emission in rice paddies, and obtained low-methane high-yield rice lines based on our discovery through hybrid breeding for sustainable agriculture with less environmental impact. Three manuscripts have been submitted in these research areas.

Manuscripts on the results of proteomic analysis in relation to oil in *Cyperus* and *WRINKLED1* overexpression in rice, respectively, have been re-submitted, while a manuscript on decipherment of transcription factor domains important for induction of fatty acid synthesis has been submitted. A couple of manuscripts are in preparation regarding interactions between transcription factors affecting seed structure, seed filling and seed maturation, oil induction in wheat endosperm with seed X-ray imaging and nutritional evolution, evolution of oil accumulation in *Cyperus* and genome editing as a methodology to redirect carbon flow by modifying promoters of transcription factors.

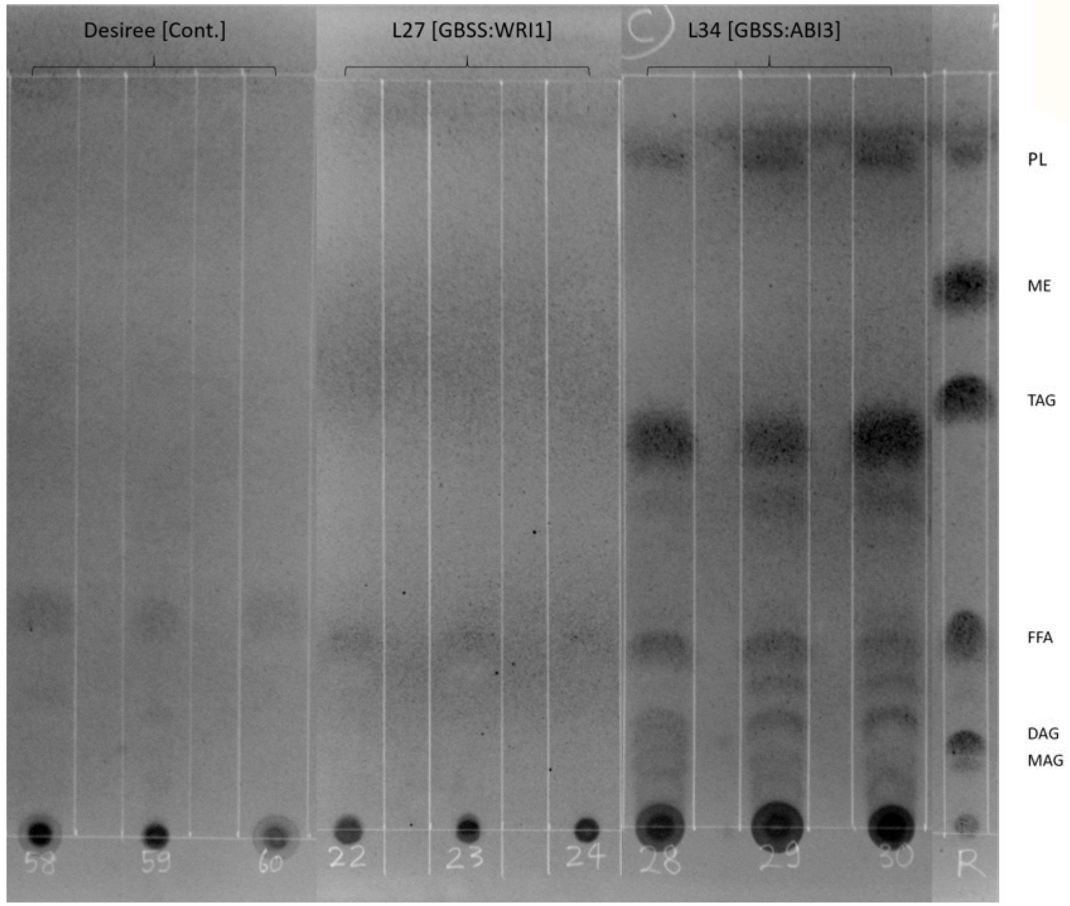


Fig. 3 Lipid accumulation induced by CRISPR-Cas9 modulation of promoter of StABI3 from seed to tuber expression (Photo by Shrikant Sharma)

Fig. 4 CRISPR-edited rapeseed (left) and filed cress (right) plants grown in biotron (Photos by Li-Hua Zhu)

Efficient protoplast regeneration protocols with successful transfection and generation of CRISPR-edited lines of field cress and rapeseed, respectively, have been published. Using these protocols, a number of mutation lines targeting on glucosinolate genes have been now obtained for both species. Further screening for homozygous lines in biotron (Fig 4) is ongoing, which would provide materials for phenotypic, chemical and molecular analyses of these lines on the target trait. Cloning of new target genes on new target traits are ongoing on both species.

One manuscript in pheromone research has been published, one is under revision and one is under submission regarding detecting release of pheromones from engineered plants and validating the whole production process of pheromone precursors from field trial as oil constituents including their processing and evidenced application in pest control. Characterization of novel genes and compounds related to insect pheromone production are progressing in collaboration with LU partners (Fig 5).

We have established a new method for non-invasive imaging of autophagic reporters in the model organism Arabidopsis thaliana (Fig 6). The method and our initial observations on the cell-type specific autophagy are summarized in the manuscript currently under review in Scientific Reports.

Using the established state of the art method we have obtained results on the differences in autophagic activity in the shoot and roots upon different conditions impacting the pathway activity, i.e. inhibition of the TORC kinase complex, nitrogen or carbon starvation.

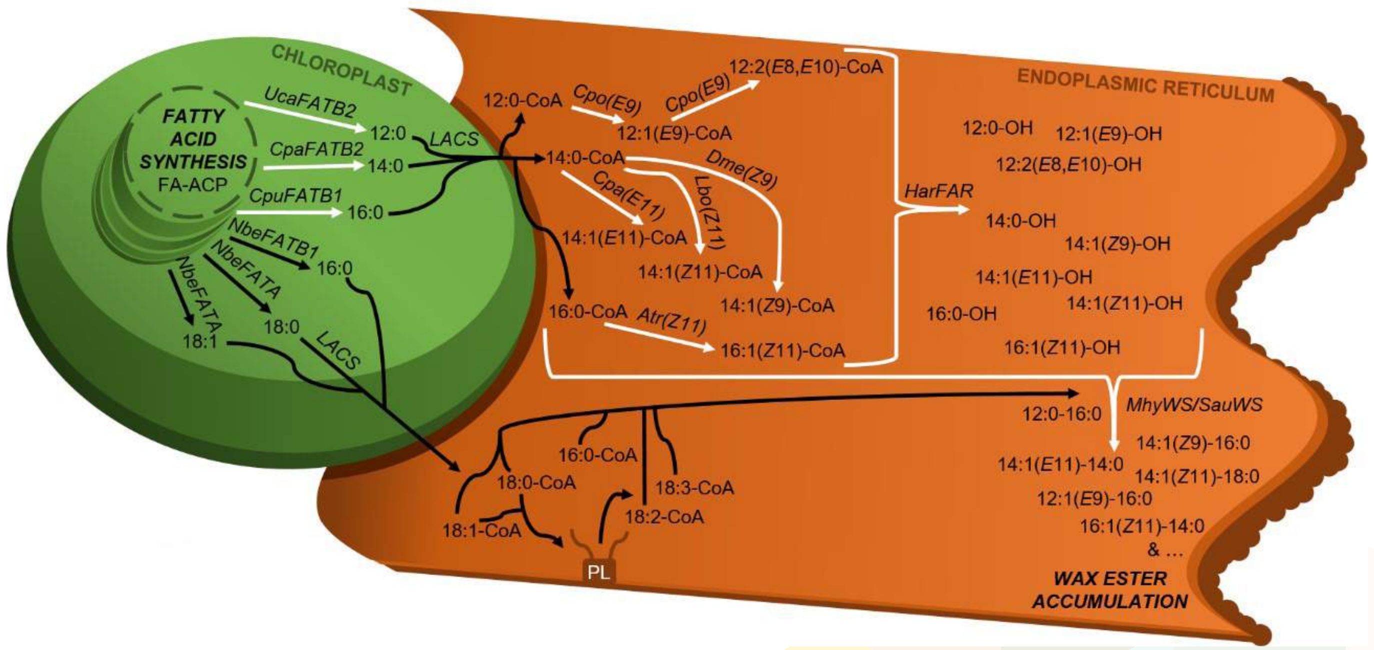


Fig 5. Strategy for producing novel medium chain wax esters with applications for pheromone production. White arrows indicate novel activities added to the plant. (Photo by Kamil Demski)

We have developed SPIRO, a state of the art automated plant phenotyping platform, which enables rapid assessment of the autophagy modulation on plant seedlings under standard growth conditions. The platform and its application is described in the manuscript currently under review in the Plant Cell.

We have established the first prototype of non-invasive bioluminescence reporters for plant autophagic activity in collaboration with Planta LLC in order to introduce a fungal bioluminescence pathway that recruits plant metabolite caffeic acid to produce substrate for bioluminescence reporter. The reporter has been modified to decrease its activity upon plant autophagy upregulation. We have successfully obtained bioluminescence activity in plant cells and are currently working on modifying intracellular localization of the pathway components to be optimal for autophagic activity detection.

We made a significant progress in elucidating the mechanism of action of the small organic compounds that we have previously identified as specific regulators of plant autophagy. We have short listed a number of proteins potentially interacting with these compounds in plant cells and are currently confirming the occurrence of the interaction and its impact on plant autophagy. These information will not only enable efficient tuning of plant autophagic activity using the compounds, but will also guide implementation of genetic tools for regulating the same targets and might contribute to establishing new molecular markers for breeding.

Currently most of the experiments are carried out using the model plant Arabidopsis. The C4F program will allow us to then expand our conclusion also on crop species closely related to Arabidopsis, i.e. *Brassica napus* and *Lepidium campestre*. For this we established a collaboration with Dr. Selvaraju Kanagarajan (Prof. Li-Hua Zhu's group, SLU, Alnarp), which will be carried out in a form of a jointly supervised PhD project.

In what way the research has contributed to social benefit

Within C4F program, some projects are closely connected to or have been transferred to UDIs or EIPs, one way to transfer TC4F knowledge into product-based projects, thus benefiting to the society. Whereas some other projects have potential significant social benefits, thus benefiting the society in long-run. For instance,
The plant protein factory was transmitted to a pilot facility at the faculty, to be available for uses of interested users. A patent of the superabsorbent material was filed.

Faba beans, oat, peas, rapeseed, potato are Swedish crops with a good nutritional profile, which can be a good plant based protein alternative to soy-products and gluten. Legumes can help with nitrogen fixation when incorporated into an intercropping system, which will thus enrich the soil and reduce over fertilization.
The starch research aims to improve product quality in food as well as non-food applications. Starch with increased amylose content have nutritional benefits since it has more slow carbohydrates. Slow carbohydrates can potentially decrease our insulin response and thereby reduce the risk to develop type II diabetes.

Our research on retrogradation can in the long run reduce food waste by prolonging product shelf life. Crystallizations and other inter-molecular interactions are also important for material applications, that will be studied further in this project.

Social benefits include two open-access scientific publications that communicating a new knowledge on the latest findings in the area of bio-based absorbents and wheat quality breeding for climate stability. The paper focusing on innovative green and sustainable materials from plant protein by-products is a high value information for the Swedish society and the world aiming to be independent from petroleum resources in nearest future. The paper focusing on wheat gluten protein quality aspects in a varying climate is a valuable information to obtain when breeding wheat crops for future climate stability and aiming to make Sweden self-securing of quality wheat.

The new research has discovered the mechanism of methane emission in rice paddies, and based on the research, we have bred 5 independent low methane rice lines, with 70% methane reduction, which could dramatically mitigate the effect of rice production on global warming. The research has achieved two international patents and at least two high profile publications.

Novel CRISPR-edited mutation lines of oilseed crops with improved oil qualities contribute to increased plant oil production for food, feed and industrial purposes, and consequently reducing the fossil use and benefiting the environment.

Insect pheromones for pest management is non-toxic and produced from a renewable resource contrasting to pesticides. Camelina derived pheromone blends have now been proven to work in the field.

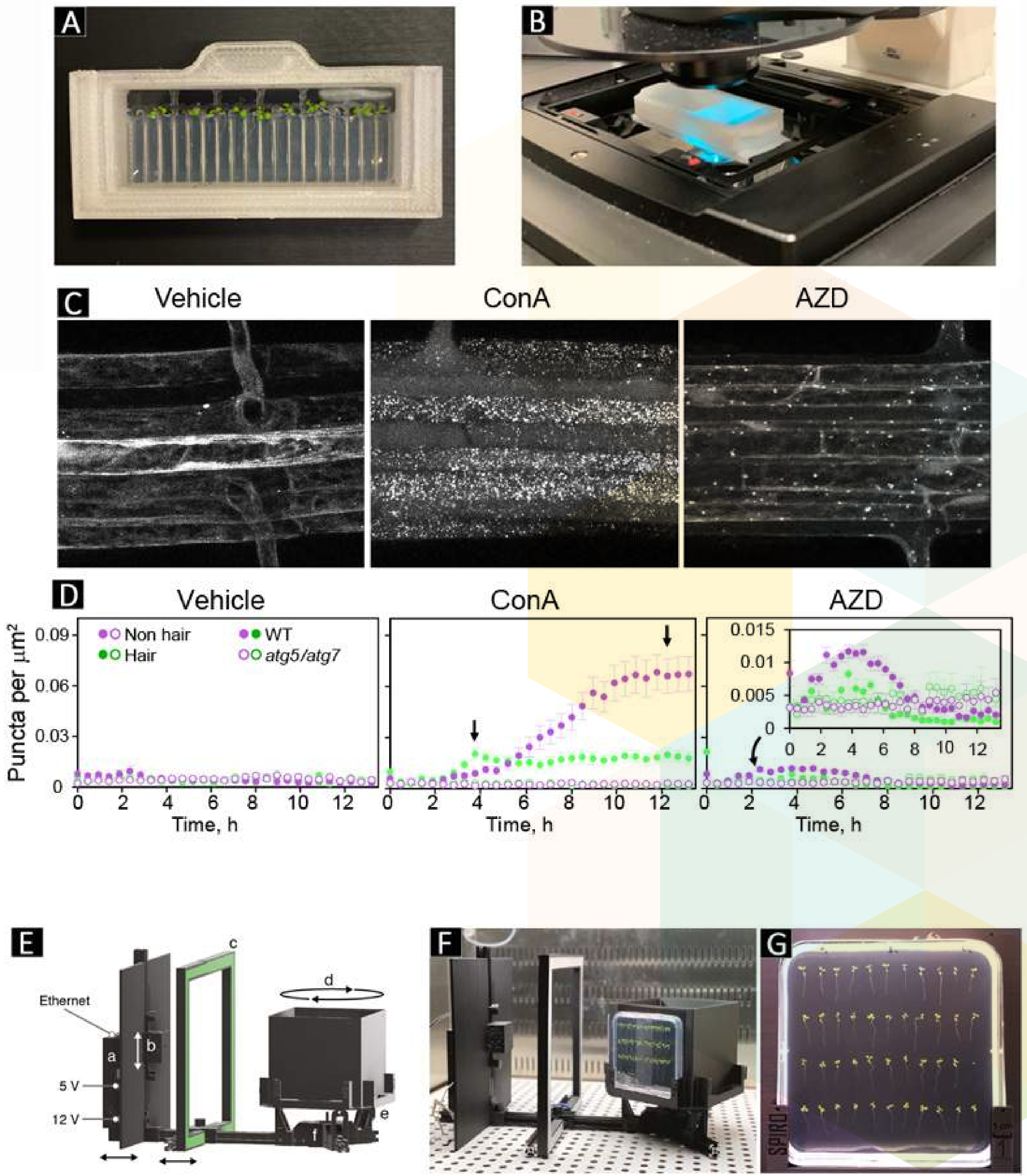


Fig 6. State-of-the-art tools developed for the project. (A-D) RoPod toolkit for non-invasive monitoring of autophagic activity in Arabidopsis thaliana roots. **A.** RoPod, a 3D printed chamber custom designed for growth and imaging of Arabidopsis seedlings. **B.** RoPod mounted on a stage of a confocal laser scanning microscope, not that seedlings are grown and imaged inside the same chamber, which alleviates mechanical stress usually imposed by mounting seedlings on the glass slides for microscopy assays. **C.** Detection of the fluorescent reporter for plant autophagy in the epidermal root cells using RoPod toolkit. Under non-inducing conditions (vehicle) the reporter is diffusely distributed in the cytoplasm. Treatment with AZD8055 (AZD) triggers formation of puncta-like autophagosomes in the cytoplasm. Implementation of Concanamycin A (ConA) leads to accumulation of autophagic bodies in the plant vacuoles. **D.** Time resolved dynamics of cell-specific autophagy obtained using RoPod toolkit and the fluorescent reporter illustrated in C. (E-G) SPIRO, Smart Plate Imaging Robot, an automated platform for phenotyping plant seedlings grown on Petri plates. **E.** a schematic representation of the imaging platform that comprises Raspberry Pi-driven components: camera, stepper motor and LED light source. Petri plates are mounted on the cube-shaped staged and imaged at user-defined time intervals. **F.** Photo of a SPIRO robot inside a standard growth incubator. The small footprint of the robot allows automated phenotyping under normally used conditions. **G.** An example of high-resolution image obtained by the SPIRO, the downstream pipeline includes automated image processing for seed germination and root growth detection.

At least one example on how C4F takes basic research to application to be used

The most of the projects in C4F have a character of more towards applied research, meaning that we have tried to transfer the known knowledge from basic research in oil, protein and starch as well as material science into potential applications in one way or another. Some examples are:

1. Protein factory or superabsorbent material
2. Bio-based sustainable absorbents
3. Wheat gluten quality for breeding climate stable wheat
4. Cd stress in wheat- management strategies
5. By understanding the structure and properties of the different fractions ; protein , starch and fibre new foods can be developed and optimised. Mixtures will also be valuable to show how pure fractions is needed. Previous knowledge on the structure and behaviour of the faba bean fractions was used in the bio-ink development in the 3D-printing project.
6. Starch is a useful part in a composite material where the starch can provide oxygen barrier functionality and something else gives strength and water resistance.
7. A single gene characterised in rice could be used to generate a high-bioenergy-rice for fuel production by increasing the oil content in rice straw.
8. Pheromones from a plant production source for pest management is a good example of going from basic science on specific pheromone compounds their genetic background in e.g. moths to applications in production and pest management.
9. Identified target genes in model species or the same species from basic research have been used in improving target traits in oil crops by CRISPR/Cas9.

Text, photos and illustrations: Li-Hua Zhu and C4F researchers

Vice program leader Li-Hua Zhu

Alyona Minina: Boundaries between life and death in plants cells

Alyona Minina studies the border of life and death in plants cells
On the third floor in the Uppsala BioCenter at SLU in Ultuna, Alyona Minina and her colleagues study the self-degradation process in plants, called autophagy. In the C4F project their goal is to make use of autophagy for growing better crops in the future.

Sometimes unexpected findings about fundamental things can lead to useful inventions. Associate professor Alyona Minina´s work on autophagy - a degradation and recycling mechanism in plants - started off as basic research about the programmed cell death that is needed for proper plant embryo development. Now she and her colleagues strive to find out how to take advantage of this specific molecular machinery, when regulating the growth of crops.

– Autophagy allows plant cells to degrade their superfluous or damaged contend and upcycle the products of degradation in the form of energy and building blocks that can be used for other things, for example for the plant to grow bigger, live longer, produce more seeds or become more resistant to pathogens, Alyona Minina explains.

By fine-tuning the autophagy pathway in the right way, crop yields can be increased.

– Since too much degradation and recycling is not good, and since autophagy is involved in a lot of different things happening in plants, we have to figure out what tissues and organs, and what stages of plant growth, we should target, she says.

One way to regulate autophagy in crops is by spraying the plants with specific chemicals. Another way would be to modify the crops' autophagy traits genetically.

– We have used a library of small organic molecules, provided by Umeå University, to discover chemical compounds that are regulators of plant autophagy. Now, we investigate how to apply them on crops, says Alyona Minina.

Growing genetically modify crops with enhanced autophagy, is not a realistic alternative in Europe today, given the current legislation surrounding gene technology.

– It would probably be better for nature if we could use genetically modified plants, but I hope it will be possible in the observable future to argue with politicians about that. Meanwhile we are trying out the chemical treatments and we will do experiments to make sure these chemicals do not damage ecosystems.

Plant cells are surrounded by cell walls, and when it's time to die, the cell has to kill itself from the inside, in order not to leave a mess for the neighboring cells to deal with.

– I have established methods for how to measure autophagy in this stage, and for studying how it is linked to programmed cell death.

In 2018-2020 Alyona Minina was at the Heidelberg University in Germany, running her Marie Skłodowska-Curie Actions project.

– I came up with questions about how autophagy works on the subcellular level. As researchers we often separate whatever happens inside the cell by specializing on our own pathways. I try to look at the bigger picture of autophagy.

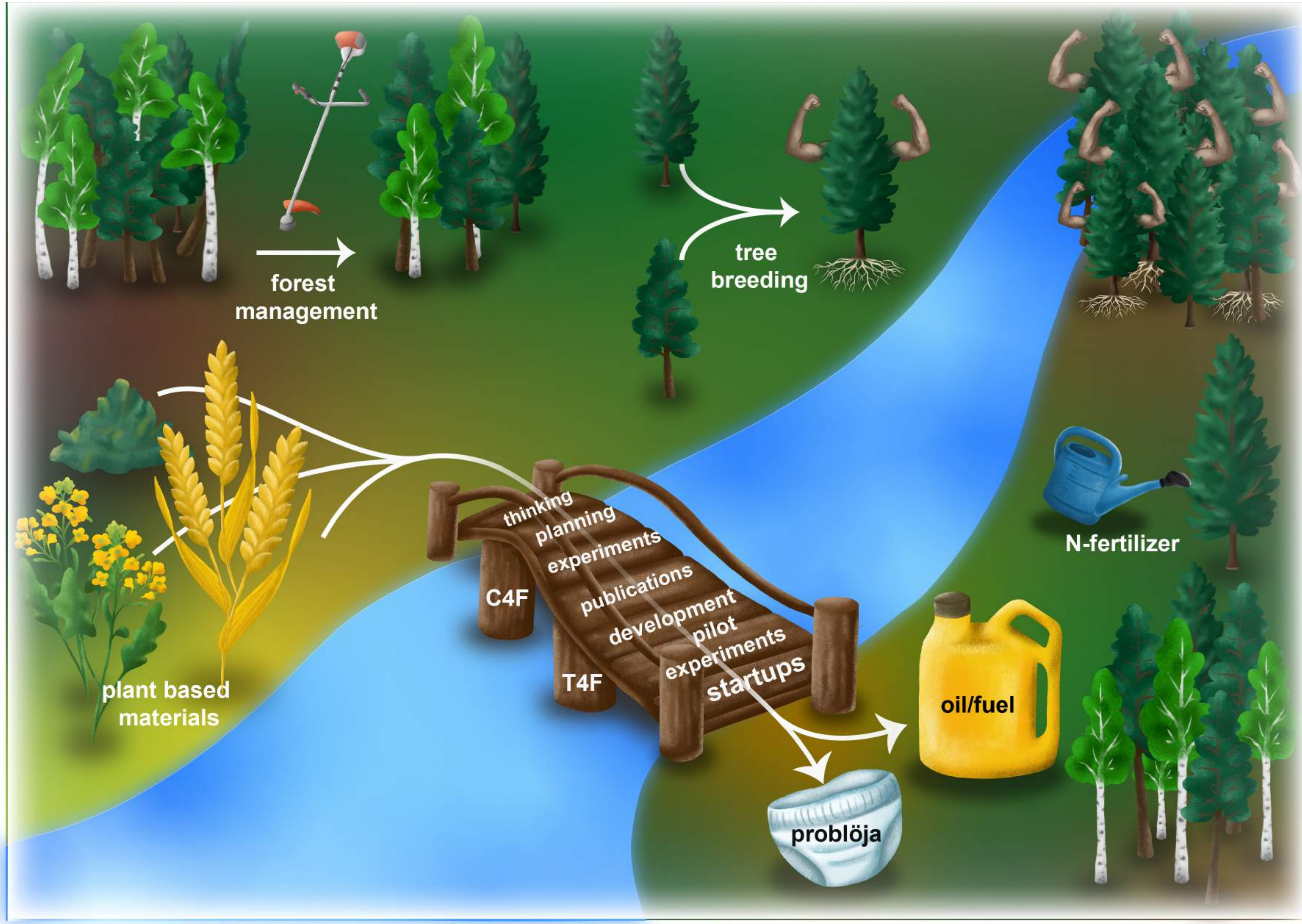
Alyona Minina was born and raised in Moscow, Russia. She came to SLU as a post-doc in 2008. Running ideas together with co-workers that are contributing with different skills, is Alyona Minina's favorite part of being a researcher.

– I especially like it when we have a very thorough plan, and then find something completely unexpected. When we think that we know how things work, and then something comes up that blows our minds, she says.

A couple of years ago Alyona Minina and her colleagues invented a robot that they named Spiro (Smart Plate Imaging RObot).

– It all started with us having a coffee in the morning, discussing how to improve the process of scanning seedlings without changing a lot of factors, like illumination, humidity and temperature that cause stress to the plants. We decided to become engineers and developed the small robot. It fits into our growth chambers, and allows us to get better images of the plantlets under controlled conditions. It was meant as a hobby project, but now we provide instructions for how to build it to biologists all over the world.

Text: Lisa Beste
Photo: Upendo Lupanga



Bridging Basic Research to Application in TC4F

The research program TC4F takes fundamental research to application in many different ways. Here, some examples are illustrated.

Theme 1: "Forest genetics and next generation of forest trees" uses genomic research for applied tree breeding, developing more efficient and directed breeding technologies.

Theme 2: "Growth and interaction with the environment - current and future" explores trees in their natural context, describing effects of biotic and abiotic factors and using modelling, machine learning and remote sensing technologies, the theme adresses how climate change will affect our future forests.

Theme 3: "Sustainable and adaptive forest management" investigates, how forest should be taken care of in regard to the purpose it should fulfill. Modelling is used to optimize management for aims like productivity and sustainability.

Program part **C4F** develops many plant related products, for example superabsorbent materials from wheat protein and plant based oils and fuels.

Text Anke Carius,

Illustration Daria Chrobok and Anke Carius

TC4F publications and activities 2021

The five institutions involved in TC4F have published 46 articles in T4F and 24 in C4F in peer-reviewed scientific journals. Read here how many have been involved with supervision of students, teaching, received other grants and contri buted to popular scientific activities. For T4F, the information is now presented via institution instead of per theme as the themes according to the program plan involve several institutions.

Authors marked in **bold** represent researchers that have been financed by, or are associated to, TC4F.

T4F - Department of Forest Ecology and Management

Scientific publications

During 2021 Department of Forest Ecology and Management has published 18 peer reviewed scientific articles in international journals. Authors marked in bold represents researchers that have been financed by, or are associated to, the research program.

1. Bandau F, Albrectsen BR, Robinson KM, **Gundale MJ**. European aspen with high compared to low constitutive tannin defenses grow taller in response to anthropogenic nitrogen enrichment. Forest Ecology and Management. 2020;487:118985.

2. **Forsmark B, Nordin A**, Rosenstock NP, Wallander H, **Gundale MJ**. Anthropogenic nitrogen enrichment increased the efficiency of belowground biomass production in a boreal forest. Soil Biology and Biochemistry. 2021;155:108154.

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15. Senior JK, **Gundale MJ**, Iason GR, Whitham TG, Axelsson EP. Progeny selection for enhanced forest growth alters soil communities and processes. Ecosphere. 2022;13(2):e3943.

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Theresa Ibanez	Postdoc
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Zsofia Stangl	Postdoc
Lina Nilsson	Researcher

Scientific presentations

Disentangling Effects of Soil Abiotic and Biotic Factors on Tree Seedling Regeneration Following Boreal Forest Wildfire

TS Ibáñez, DA Wardle, MJ Gundale, MC Nilsson

EGU General Assembly Conference Abstracts, EGU21-5604

Other funding that has been received partially or fully due to the TC4F research

Gundale 16 000 000kr Formas – Assessing relationships and trade-offs between productivity, climate impacts and biodiversity in northern Swedish forests.

Investments in research infrastructure

200 000 kr Three climate change chambers

Education

Maja Sundqvist, Michael Gundale, and Marie-Charlotte Nilsson, Zsofia Stangl -- Forest Ecology Level 3

Michael Gundale, Zsofia Stangl, Teacher Forest Ecosystem Ecology – MSc program.

T4F - Department of Plant Physiology, Umeå Universitet

Scientific publications

During 2021 Department of Plant Physiology has published 18 peer reviewed scientific articles in international journals. Authors marked in bold represents researchers that have been financed by, or are associated to, the research program.

1. **Bag P**, **Lihavainen J**, Delhomme N, Riquelme T, **Robinson KM**, **Jansson S**. An atlas of the Norway spruce needle seasonal transcriptome. Plant J. 2021;108(6):1815-29.

2. Bandau F, Albrectsen BR, **Robinson KM**, Gundale MJ. European aspen with high compared to low constitutive tannin defenses grow taller in response to anthropogenic nitrogen enrichment. Forest Ecology and Management. 2020;487:118985.

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Alisa Kravtsova	PhD-student

POPULAR SCIENTIFIC PUBLICATIONS (REPORTS ETC)

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Jansson, S. 2021. The role of scientists in society - GMOs in agriculture. Workshop during MolBio symposium "Science Outside the Box", Heidelberg, Germany 1/10 2021

Jansson, S. 2021. CRISPR – a genetic scissor that can be used to reprogam organisms. Seminar during Symposium "AI, biotech and genetics in the future". 14/10 2021

Jansson, S. 2021. CRISPR – a genetic scissor that can be used to reprogam organisms. Seminar during SPPS-PPL webinar. 4/11 2021

Jansson, S. 2021. Why has it been so hard for the EU to decide about regulation of genome editing? – Seminar during SPPS-PPL webinar. 4/11 2021

Calleja- Rodriguez A, 2021-03-22. Webinar Forest Genetics . 15 min.SLU and Brazilian Government, SLU, Skogstyrelsen and Brasilian Universities and Government.

Calleja-Rodriguez A, 2021-10-07. T4F workshop presentation. SLU/T4F.

Suontama M, 2021-10-07. T4F workshop presentation. SLU/T4F.

POPULAR SCIENTIFIC PRESENTATIONS

Jansson, S. 2021. Gensaxen och EU:s GMO-lagstiftning - Föredrag för Rotary Umeå Stockholm 23/1 2021
Jansson, S. 2021. GMO-debatten. - Föredrag för Kunskapsskolan 2/6 2021

Jansson, S. 2021. GMO träd i Sverige – på lab och i fält. Föredrag på Genteknikmyndigheternas möte 27/10 2021

Jansson, S. 2021. What is NGT and how is it regulated? – Seminar at the webinar "New Genomic Techniques and its role in the EU", European parliament, Brussels. 16/11 2021

Jansson, S. 2021. Miljöbalkens inverkan på en universitetsforskare – Seminar at Gentekniknämnden, Stockholm. 6/12 2021

OTHER FUNDING THAT HAS BEEN RECEIVED PARTIALLY OR FULLY DUE TO THE TC4F RESEARCH

- Jansson S: "Trees that grow better", SSF, 2022-2026.
- Jansson S: How do trees survive the winter? VR 2022-2025
- Jansson S: How do trees know it is autumn? Formas 2022-2024

EDUCATION

PHD THESES, MSC THESES, BACHELOR THESES SUPERVISION AND TEACHING DEPARTMENT OF PLANT PHYSIOLOGY

Jansson S. Main supervisor for PhD-candidate Pushan Bag Tentative title: How do Christmas trees remain evergreen? - Photosynthetic acclimation of Scots pine and Norway spruce needles during winter. Expected date for dissertation: May 2022.

Jansson S. Main supervisor for PhD-candidate Sanchali Nanda. Tentative title: Regulation of light harvesting in Arabidopsis and Populus. Expected date for dissertation: October 2024

Street N. Main supervisor for PhD-candidate Camila Canova. Tentative title: The role and origin on long non-coding RNAs in conifer genomes. Expected defence date for dissertation: June 2025.

Street N. Main supervisor for PhD-candidate Elena van Zalen. Tentative title: Comparative genomics of conifers. Expected defence date for dissertation: Nov 2025.

T4F - Department of Forest Genetics and Plant Physiology

During 2021 the Department of Forest Genetics and Plant Physiology has published 7 peer reviewed scientific articles in international journals. Authors marked in bold represents researchers that have been financed by, or are associated to, the research program.

Scientific publications

1. **Castro D, Schneider AN,** Holmlund M, **Näsholm T, Street NR, Hurry V.** Effects of Early, Small-Scale Nitrogen Addition on Germination and Early Growth of Scots Pine (Pinus sylvestris) Seedlings and on the Recruitment of the Root-Associated Fungal Community. Forests. 2021;12(11):1589.

2. Haas JC, **Vergara A**, **Serrano AR**, Mishra S, **Hurry V, Street NR**. Candidate regulators and target genes of drought stress in needles and roots of Norway spruce. Tree Physiol. 2021;41(7):1230-46.

3. Le K, **Egertsdotter U**. Scale-up of Somatic Embryogenesis Plant Production of Hybrid Larch (Larix x eurolepis) Using Temporary Immersion Bioreactors. . In Vitro Cellular & Developmental Biology-Animal, 57(SUPPL 1): S38–S39.

4. Ranade SS, **Egertsdotter U.** In silico characterization of putative gene homologues involved in somatic embryogenesis suggests that some conifer species may lack LEC2, one of the key regulators of initiation of the process. BMC Genomics, 22(1): 392

5. Schneider AN, Sundh J, Sundström G, Richau K, Delhomme N, Grabherr M, et al. Comparative Fungal Community Analyses Using Metatranscriptomics and Internal Transcribed Spacer Amplicon Sequencing from Norway Spruce. mSystems. 2021;6(1).

6. **Vergara A**, Haas JC, **Aro T,** Stachula P, **Street NR, Hurry V.** Norway spruce deploys tissue-specific responses during acclimation to cold. Plant Cell Environ. 2022;45(2):427-45.

7. Zhu L, Bloomfield KJ, Asao S, Tjoelker MG, Egerton JJG, Hayes L, et al. Acclimation of leaf respiration temperature responses across thermally contrasting biomes. New Phytol. 2021;229(3):1312-25.

Personnel

Vaughan Hurry	Professor
Simon Law	Post-doc
Alonso Serrano	Computer engineer
David Castro	PhD-student
Tuuli Aro	PhD-student
Ulrika Egertsdotter	Professor
Sofie Johansson	Researcher
Annika Nordin	Professor
Tinkara Bizjak	PhD-student

Joint Efforts and Collaborations

SE transformation platform:

The SE transformation platform utilizes early-stage somatic embryo cultures for genetic transformation with Agrobacterium. The platform performs transformations as a service primarily for the UPSC community but also has international collaborations. The main focus for the platform's internal activities is to develop the technology for efficient CRISPR/Cas transformation

T4F - Skogforsk

During 2021 Skogforsk has published 3 peer reviewed scientific articles in international journals. Authors marked in bold represents researchers that have been financed by, or are associated to, the research program.

Scientific publications

1. **Calleja-Rodriguez A,** Chen Z, **Suontama M,** Pan J, Wu HX. Genomic Predictions With Nonadditive Effects Improved Estimates of Additive Effects and Predictions of Total Genetic Values in. Front Plant Sci. 2021;12:666820.

2. **Hall D**, Olsson J, **Zhao W**, Kroon J, Wennström U, **Wang XR.** Divergent patterns between phenotypic and genetic variation in Scots pine. Plant Commun. 2021;2(1):100139.

3. Samils B, Kaitera J, **Persson T,** Stenlid J, Barklund P. Relationship and genetic structure among autoecious and heteroecious populations of Cronartium pini in northern Fennoscandia. Fungal Ecology. 2021;50:101032.

Personnel

Ainhoa Calleja Rodriguez	Researcher
Mari Suontama	Researcher
Henrik Hallingbäck	Researcher
Torgny Persson	Researcher
Ulfstand Wennström	Researcher
Sara Abrahamsson	Researcher

Education

Abrahamsson, S. 2021-08-11. Fältresa av kursen Sverigesa norr. SLU field trip for students. Forest Ecology and Management.

Suontama, M. 2021-02-08. Lecture Tree breeding methods and strategies to mitigate - climate change.2 tim. SLU, Umeå.

Suontama, M, 2021-05-21. Lecture Tree Breeding. UMU studenter föreläsning/virtual studiebesök. 3 tim. Umeå University students.

Persson, T. Suontama, M, Wennström, U. 2021-11-18. Student Visit to Tree Breeding demonstration trial at Vännfors and to Skogforsk research station. SLU, Umeå.

T4F - Southern Swedish Forest Research Centre

During 2021 the Southern Swedish Forest Research Centre has published 9 peer reviewed scientific articles in international journals. Authors marked in bold represents researchers that have been financed by, or are associated to, the research program.

Scientific publications

1. Appiah Mensah A, **Holmström E**, Petersson H, Nyström K, Mason EG, **Nilsson U**. The millennium shift: Investigating the relationship between environment and growth trends of Norway spruce and Scots pine in northern Europe. Forest Ecology and Management. 2021;481:118727.

2. Ara MT, Barbeito I, Elfving B, Johansson U, **Nilsson U**. Varying rectangular spacing yields no difference in forest growth and external wood quality in coniferous forest plantations. Forest Ecology and Management. 2021;489:119040.

3. Ara M, Barbeito I, Kalén C, **Nilsson U**. Regeneration failure of Scots pine changes the species composition of young forests. Scandinavian Journal of Forest Research. 2021:1-9.

4. Barbeito I, Eskelson BNI, Carsky G. Trade-offs across densities and mixture proportions in lodgepole pine-hybrid spruce plantations. Forest Ecology and Management. 2021;490:119095.

5. Dahlgren Lidman F, **Holmström E**, Lundmark T, fahlvik n. Management of spontaneously regenerated mixed stands of birch and Norway spruce in Sweden. Silva Fennica. 2021;55.

6. Hahn T, Eggers J, **Subramanian N,** Toraño Caicoya A, Uhl E, Snäll T. Specified resilience value of alternative forest management adaptations to storms. Scandinavian Journal of Forest Research. 2021;36(7-8):585-97.

7. **Holmström E**, Carlström T, **Goude M**, Lidman FD, Felton A. Keeping mixtures of Norway spruce and birch in production forests: insights from survey data. Scandinavian Journal of Forest Research. 2021;36(2-3):155-63.

8. Lula M, Trubins R, Ekö PM, Johansson U, **Nilsson U**. Modelling effects of regeneration method on the growth and profitability of Scots pine stands. Scandinavian Journal of Forest Research. 2021;36(4):263-74.

9. Siipilehto J, Allen M, **Nilsson U**, Brunner A, Huuskonen S, Haikarainen S, et al. Stand-level mortality models for Nordic boreal forests. Silva Fennica. 2020;54.

10. **Skovsgaard JP,** Johansson U, **Holmström E,** Tune RM, Ols C, Attocchi G. Effects of Thinning Practice, High Pruning and Slash Management on Crop Tree and Stand Growth in Young Even-Aged Stands of Planted Silver Birch (Betula pendula Roth). Forests. 2021;12(2):225.

Personnel

Jorge Aldea	Postdoc
Michelle Cleary	Senior Lecturer
Martin Goude	Researcher
Annika Felton	Senior Lecturer
Karin Hjelm	Senior Lecturer
Emma Holmström	Senior Lecturer
Narayanan Subramanian	Researcher
Diana Marciulyniene	Postdoc
Urban Nilsson	Professor
Friday Ogana	Postdoc
Andis Zvirgzdins	PhD-student

Collaboration with industry and other parts of society

Experiments in northern Sweden are established in collaboration with Forest Agency, Sveaskog, SCA and Holmen. Experience of Siberian larch in northern Sweden will be investigated in collaboration with Forest Agency, Sveaskog and SCA. Establishment of mixtures of Scots pine and Norway spruce is studied

in collaboration with Södra. Establishment of spacing experiments in birch and oak is established in collaboration with Sveaskog. An update of available experiments in clear-cut free forestry is done together with Sveaskog. Models for quantifying the effects of forest edges on the risk for wind damage is constructed in collaboration with the Forest Agency.

Other funding that has been received partially or fully due to the TC4F research

Many projects have received funding because of base research in T4F. But the most important is Trees For Me where the application builds on research in T4F. TFM will run for five years and has received a total of 113 million SEK from the Energy Agency, Universities and forest sector

Investments in research infrastructure

During 2021 we have established several long-term field experiments including:

- Regeneration and tree species experiments in northern Sweden
- Pre-commercial tinning experiments in northern Sweden
- Scarification and mixed species experiments in southern Sweden
- Continuous cover forestry experiments in southern Sweden
- Thinning experiments in Scots pine in central Sweden

Education

All researchers in T4F in the Southern Swedish Forest Research Centre are involved in teaching at the department. In SSFRC we teach at Euroforester (master), Forest & Landscape (bachelor), Skogsbruk med många mål (master), Sverigeresan (bachelor). Michelle Cleary, Karin Hjelm, Emma Holmström, Narayanan Subramaninan, Martin Goude and Urban Nilsson have course responsibilities for courses at these programs.

C4F - Crops for the Future

Scientific publications

1. Capezza AJ, **Muneer F**, Prade T, **Newson WR**, Das O, Lundman M, et al. Acylation of agricultural protein biomass yields biodegradable superabsorbent plastics. Communications Chemistry. 2021;4(1):52.

2. **Ceresino EB**, **Johansson E**, Sato HH, Plivelic TS, Hall SA, Bez J, et al. Lupin Protein Isolate Structure Diversity in Frozen-Cast Foams: Effects of Transglutaminases and Edible Fats. Molecules. 2021;26(6):1717.

3. Guichard M, Holla S, Wernerová D, Grossmann G, **Minina EA**. RoPod, a customizable toolkit for non-invasive root imaging, reveals cell type-specific dynamics of plant autophagy. bioRxiv. 2021:2021.12.07.471480.

4. **Herneke A**, Lendel C, Johansson D, Newson W, Hedenqvist M, Karkehabadi S, et al. Protein Nanofibrils for Sustainable Food–Characterization and Comparison of Fibrils from a Broad Range of Plant Protein Isolates. ACS Food Science & Technology. 2021;1(5):854-64.

5. Hussain A, Larsson H, **Johansson E**. Carotenoid Extraction from Locally and Organically Produced Cereals Using Saponification Method. Processes. 2021;9(5):783.

6. Jasieniecka-Gazarkiewicz K, Demski K, Gidda SK, Klińska S, Niedojadło J, **Lager I**, et al. Subcellular Localization of Acyl-CoA: Lysophosphatidylethanolamine Acyltransferases (LPEATs) and the Effects of Knocking-Out and Overexpression of Their Genes on Autophagy Markers Level and Life Span of A. thaliana. International Journal of Molecular Sciences. 2021;22(6):3006.

7. **Johansson E**, Prieto-Linde ML, Larsson H. Locally Adapted and Organically Grown Landrace and Ancient Spring Cereals—A Unique Source of Minerals in the Human Diet. Foods. 2021;10(2):393.

8. **Johansson M**, Xanthakis E, **Langton M**, Menzel C, Vilaplana F, Johansson DP, et al. Mixed legume systems of pea protein and unrefined lentil fraction: Textural properties and microstructure. LWT. 2021;144:111212.

9. **Kanagarajan S**, Carlsson MLR, Chakane S, Kettisen K, Smeds E, Kumar R, et al. Production of functional human fetal hemoglobin in Nicotiana benthamiana for development of hemoglobin-based oxygen carriers. International Journal of Biological Macromolecules. 2021;184:955-66.

10. Klionsky DJ, Abdel-Aziz AK, Abdelfatah S, Abdellatif M, Abdoli A, Abel S, et al. Guidelines for the use and interpretation of assays for monitoring autophagy (4th edition)1. Autophagy. 2021;17(1):1-382.

11. **Kuktaite R**, Repo-Carrasco-Valencia R, de Mendoza CC, Plivelic TS, Hall S, **Johansson E**. Innovatively processed quinoa (Chenopodium quinoa Willd.) food: chemistry, structure and end-use characteristics. J Sci Food Agric. 2021.

12. **Li X**, **Sandgrind S**, **Moss O**, Guan R, Ivarson E, Wang ES, et al. Efficient Protoplast Regeneration Protocol and CRISPR/Cas9-Mediated Editing of Glucosinolate Transporter (GTR) Genes in Rapeseed (Brassica napus L.). Frontiers in Plant Science. 2021;12.

13. **Minina EA**, Dauphinee AN, Ballhaus F, Gogvadze V, Smertenko AP, Bozhkov PV. Apoptosis is not conserved in plants as revealed by critical examination of a model for plant apoptosis-like cell death. BMC Biology. 2021;19(1):100.

14. **Minina EA**, Scheuring D, Askani J, Krueger F, Schumacher K. Light at the end of the tunnel: FRAP assay reveals that plant vacuoles start as a tubular network. bioRxiv. 2021:2021.05.13.444058.

15. **Muneer F**, Hovmalm HP, **Svensson S-E**, **Newson WR**, **Johansson E**, Prade T. Economic viability of protein concentrate production from green biomass of intermediate crops: A pre-feasibility study. Journal of Cleaner Production. 2021;294:126304.

16. **Nynäs A-L**, **Newson WR**, **Johansson E**. Protein Fractionation of Green Leaves as an Underutilized Food Source—Protein Yield and the Effect of Process Parameters. Foods. 2021;10(11):2533.

17. Ohlsson JA, Leong JX, Elander PH, Dauphinee AN, Ballhaus F, Johansson J, et al. SPIRO – the automated Petri plate imaging platform designed by biologists, for biologists. bioRxiv. 2021:2021.03.15.435343.

18. Prade T, **Muneer F**, Berndtsson E, **Nynäs A-L**, **Svensson S-E**, **Newson WR**, et al. Protein fractionation of broccoli (Brassica oleracea, var. Italica) and kale (Brassica oleracea, var. Sabellica) residual leaves — A pre-feasibility assessment and evaluation of fraction phenol and fibre content. Food and Bioproducts Processing. 2021;130:229-43.

19. Ramachandran P, Augstein F, Mazumdar S, Nguyen TV, **Minina EA**, Melnyk CW, et al. Abscisic acid signaling activates distinct VND transcription factors to promote xylem differentiation in Arabidopsis. Current Biology. 2021;31(14):3153-61.e5.

20. Rojas-Lema S, **Nilsson K**, Trifol J, **Langton M**, Gomez-Caturla J, Balart R, et al. "Faba bean protein films reinforced with cellulose nanocrystals as edible food packaging material". Food Hydrocolloids. 2021;121:107019.

21. **Sandgrind S**, **Li X**, Ivarson E, Ahlman A, **Zhu L-H**. Establishment of an Efficient Protoplast Regeneration and Transfection Protocol for Field Cress (Lepidium campestre). Frontiers in Genome Editing. 2021;3.

22. Xia Y-H, Wang H-L, Ding B-J, Svensson GP, Jarl-Sunesson C, Cahoon EB, et al. Green Chemistry Production of Codlemone, the Sex Pheromone of the Codling Moth (Cydia pomonella), by Metabolic Engineering of the Oilseed Crop Camelina (Camelina sativa). Journal of Chemical Ecology. 2021;47(12):950-67.

23. **Zhao X**, **Andersson M**, **Andersson R**. A simplified method of determining the internal structure of amylopectin from barley starch without amylopectin isolation. Carbohydrate Polymers. 2021;255:117503.

24. **Zhao X**, **Jayarathna S**, Turesson H, Fält A-S, Nestor G, González MN, et al. Amylose starch with no detectable branching developed through DNA-free CRISPR-Cas9 mediated mutagenesis of two starch branching enzymes in potato. Scientific Reports. 2021;11(1):4311.

Popular scientific publications (reports etc)

In Zamaratskaia, G. 2021. Forskning: växtbaserad köttanalog med struktur som liknar biff. Livsmedel i Fokus, 20 Aug 2021

Nilsson, K., Johansson, M. & Langton, M. 2021. Popular science article about the 3D-printing project published on the Molecular Science web-page: 3D-printning av livsmedel baserat på åkerböna | Externwebben (slu.se)

Interviews and presence in media

- Jakten på perfekta proteiner. Lantmannen nr 1, 2021
- Ökat intresse för växtförädling. Jordbruksaktuellt, May 2021
- Gluten istället för petroleumbaserade superabsorbenter i blöjor. Jordbruksaktuellt. 5 Aug 2021.
- Forskare hoppas på blöjor av gluten och potatis. DN. 4 Aug 2021.
- Engångsblöjor med gluten i stället för petroleumbaserade superabsorbenter. Örebronyheter. 4 Aug 2021.
- Blöjor av vete och potatis istället för petroleumprodukter kan snart bli verklighet. Epoch Times Sverige. 4 Aug 2021.
- Miljövänligare engångsblöjor med gluten istället för olja. Forskning. Aug 3 2021.
- Engångsblöjor med gluten i stället för petroleumbaserade superabsorbenter. IT-Hälsa. 5 Aug 2021.
- Potatisbaserade blöjor uppfunna i Sverige. Aktuell hållbarhet. 6 Aug 2021.
- Gluten och potatisprotein i blöjan – istället för olja. Tidningen Syre 13 Aug 2021.
- Om X antal år. Podcast. Medicon Village 21 Okt 2021.
- Hon gör framtidens mat av gräs. Sydsvenskan 21 Okt 2021.
- SLU Växtprotein – ny hub för vegetabiliskt (växt)protein. Biodiverse 22 Okt 2021.
- Nav ska sprida kunskap om växtproteiner. Lommabladet V44, sida 4, november 2021

- Sverigesradio.se: https://sverigesradio.se/avsnitt/safar-vi-tillrackligt-med-mat-i-en-varmare-varld-med-torka-och-oversvamningar; November 2021

- Forskning: växtbaserad köttanalog med struktur som liknar biff. https://www.livsmedelifokus.se/forskning-vaxtbaserad-kottanalog-med-struktur-som-liknar-biff/

Scientific presentations

EA Minina. 2021. A talk "Degrade to develop: the crosstalk between plant autophagy and developmental programs". November 24th, Linnean Centre for Plant Biology Symposium.

Li-Hua Zhu, 2021. CRISPR-Cas9 editing in rapeseed, Invited keynote speech, 2nd PlantEd conference in Lecce, Italy, on 20-22 Sept.

Oliver Moss, 2021. Genome editing of rapeseed for reducing phytic acid in seeds. Oral presentation. C4F-Workshop. Grand Hotel in Lund, 9 Dec.

Collaboration with industry or other parts of society

- Lantmännen
- Gasum
- Oriflame
- Grönsaksmästarna
- Region Skåne
- Lilla Harrie Valskvarn
- Orkla
- Havredals Biodevelop AB
- RISE
- KTH
- Chalmers
- KI
- Sveriges Stärkelseproducenter Förening
- MariboHilleshög Research
- Kalmar Ölands Trädgårdsprodukter
- Findus
- FoodHills and ISCA Technologies
- LU
- Gunnarshögs Gård AB
- Syngenta
- Planta LLC
- SLU Grogrund – a number of research projects connected to the C4F program

Other funding that has been received partially or fully due to the TC4F research

- Formas Food – GreenLeaFood
- Energimyndigheten – Green2Feed
- Energimyndigheten – BSRC
- Vinova - Olika växtproteinkällors effekt på strukturen hos nanofibriller studerad med röntgenljusspridning
- Novo Nordisk Foundation- Optimization of medium-chain fatty acid accumulation in Camelina (Camelina sativa) for biotechnological insect pheromone production.
- Formas - Sustainable production of insect pheromones in plant factories.
- Formas -Plant autophagy for sustainable agriculture

Investments in research infrastructure

- Proteinfabriken
- Rheometer
- 3-D Printer

Education

a) PhD theses, MSc theses, Bachelor theses

Belsing, Axel. (Male) 2021. Masters thesis: Swedish wheat in the changing climate: screening for stable spring wheat genotypes from 2017 and 2018 with focus on the protein quality for bread-making defended at the Department of Plant Breeding, https://stud.epsilon.slu.se/16558/

Xue Zhao. (Female) 2021. Doctoral thesis: Novel potato starch - New structure and beneficial qualities. SLU Uppsala, Acta Universitatis agriculturae Sueciae, 2021:65. ISBN 978-91-7760-807-3

b) Supervision and teaching (include supervision of finished and on-going students, include teaching and organization of courses)

Langton, Maud Main supervisor for PhD-student Nilsson, Klara. Title: Integral valorisation of Faba beans molecular compounds to nutritional texturized food products

Langton, Maud Main supervisor for PhD-student Herneke, Anja. Title: Functionalization of nanofibers from plant based proteins

Langton, Maud Main supervisor for PhD-student Johansson, Mathias. Title: Legume based Gels – Microstructure and Texture

Hofvander, Per. Supervisor for PostDoc Shrikant Sharma

Hofvander, Per. Supervisor for PostDoc Kamil Demski

Zhu, Li-Hua. Supervisor for PhD candidate Sjur Sandgren. Tentative title: Genome editing of oil crops. Expected date for dissertation: 2022.

Zhu, Li-Hua. Supervisor for PhD candidate Oliver Moss. Tentative title: Improvement of seedcake quality of rapeseed for high quality food and food uses. Expected date for dissertation: 2025.

Johansson, Mathias and Nilsson, Klara supervision for intermentship student Fanny Knab. Title "Faba Bean Fractions For 3d Food Printing", defense September 2021.

Roger Andersson. Main supervisor for PhD-candidate Xue Zhao. Title: Novel potato starch - New structure and beneficial qualities. Defended October, 2021.

Roger Andersson. Main supervisor for PhD-candidate Shishanthi Jayarathna. Tentative title: New starch for novel applications. Expected date for dissertation: January, 2024.

Kanagarajan, Selvaraju. Co-supervisor for PhD candidate Sjur Sandgrind. Tentative title: Genome editing of oil crops. Expected date for dissertation: 2022.

Kanagarajan, Selvaraju. Supervisor for PhD candidate Oliver Moss. Tentative title: Improvement of seedcake quality of rapeseed for high quality food and food uses. Expected date for dissertation: 2025.

Kuktaite, Ramune. Main supervisor for PhD-candidate Sbatie, Lama. Tentative title: Wheat quality in a varying climate. Expected date for dissertation: June, 2023.

Minina, EA. Course organizer and teacher at the course "Real Time Quantitative PCR – theory, experimental design and data analysis", (PNS0215), 3.5 ECTS, SLU.

Herneke Anja. Teaching at the course "Food Technology", (LV0101) 15 ECTS, Ultuna

Herneke Anja. Teaching at the course "Food chemistry and physics", (LV0110) 15 ECTS, Ultuna

Nilsson, Klara. Teaching at the course "Food Technology", (LV0101) 15 ECTS, Ultuna

Nilsson, Klara. Course leader at the course "Introduction course food agronomist", (LV0100) 15 ECTS, Ultuna

Nilsson, Klara. Course leader at the course "Basic course Food Science", (LV0117) 7.5 ECTS, Ultuna

Johansson, Mathias. Lab supervisor at the course "Food chemistry and physics", (LV0110) 15 ECTS, Ultuna

Roger Andersson. Course organizer and teaching at the course "Plant food science", (LV0113), 15 ECTS, SLU.

Hofvander, Per. Teaching at the course "Applied Plant Biotechnology" (BI1344), Alnarp.

Hofvander, Per. Teaching at the course "Advanced Plant Breeding and Genetic Resources" (BI1345), Alnarp

Hofvander, Per. Teaching at the course "Växters kemi och biokemi" (KE0070), Alnarp.

Grimberg, Åsa. Teaching at the course "Växtförädling och växtfysiologi" (BI1367), Alnarp.

Grimberg, Åsa. Teaching at the course "Odling och kvalitet" (TD0010), Alnarp.

Grimberg, Åsa. Teaching at the course "Advanced plant breeding and genetic resources" (BI1345), Alnarp.

Zhu, Li-Hua. Course organiser and teaching at the course "Advanced Plant Breeding and Genetic Resources" (BI1345), Alnarp

Zhu, Li-Hua. Course organiser and teaching at the course "Applied Plant Biotechnology" (BI1344), Alnarp.

Zhu, Li-Hua. Teaching at the course "Växtförädling och växtfysiologi" (BI1367), Alnarp.

C4F- Crops for the Future, Personnel

Name	Gender & Position	Part of full time financed by TC4F
Eva Johansson	F, Professor, C4F leader	15%
Li-Hua Zhu	F, Professor, C4F vice leader	10%
Maud Langton	F, Professor	0
Roger Andersson	M, Professor	0
Anna Schnurer	F, Professor	0
Chuanxin Sun	M, Docent	0
Folke Sitbon	M, Professor	0
Mariette Andersson	F, Researcher	0
Ramune Kuktaite	F, Researcher	5%
Åsa Grimberg	F, Researcher	0
Ida Lager	F, Researcher	0
Selvaraju Kanagarajan	M, Researcher	8%
Alyona Minina	F, ass. Professor	17,5%
Per Hofvander	M, Researcher	0
Jia Hu	F, PhD	30%
Sjur Sandgrind	M, PhD student	50%
Yunkai Jin	M, Postdoc	20%
Kamil Demski	M, Postdoc	40%
Shrikant Sharma	M, Postdoc	40%
William (Bill) Newson	M, Postdoc	0
Elaine Ceresino	F, PhD student	25%
Anna-Lovisa Nynäs	F, PhD student	50%
Oliver Moss	M, PhD-student	50%
Anja Herneke	F, PhD student	0
Klara Nilsson	F, PhD student	61%
Silvana Moreno	F, PhD student	30%
Xue Zhao	F, PhD student	30%
Shishanthi Jayarathna	F, PhD student	0
Mathias Johansson	M, PhD student	0
Sven-Erik Svensson	M, PhD student	17%
Joel Markgren	M, PhD student	25%
Faraz Muneer	M, PhD student	25%
Lekan Jolayemi	M, PhD-student	50%
Lan Yuzhou	M, PhD-student	25%
Sbatie Lama	F, PhD-student	0
Xueyuan Li	M, Research assistant	0

*Researchers listed with 0% have received financing from TC4F earlier which resulted in projects with independent financing.

TC4F Economy 2021

In 2021, TC4F received 28,5 mio SEK of funding which were distributed according to the budget and 75% were used. The surplus was caused by delays in recruitment due to the Covid-19 pandemic.

	SLU	UmU	Skogforsk	Total
Distributed Funds (tkr)				
Coordination	1 884			2 576
Plant Physiology (UMU)		5100		5 100
Forest Genetics and Plant Physiology	4 454*			4 454
Southern Swedish Forest	4 454*			4 454
Forest Ecology and Management	3 504			3 504
Wildlife, Fish and Environmental Studies	950*			950
Skogforsk			1 100	1 100
C4F (LTV)	7 149			6 457
TOTAL	22 395	5 100	1 100	28 595
Costs, spent funds (tkr)				
Coordination	764			764
Plant Physiology (UMU)		2 262		2 262
Forest Genetics and Plant Physiology	2 432			2 432
Southern Swedish Forest	3 242			3 242
Forest Ecology and Management	4 042			4 042
Wildlife, Fish and Environmental Studies	0			0
Skogforsk			1 100	1100
C4F (LTV)	7 658			7 658
Total	18 120	2 262	1 100	21 482
RESULT T4F	4 784	2 262		
RESULT C4F	- 509			
RESULT	4 275	2 838	0	7 113

*assigned postdoc grants