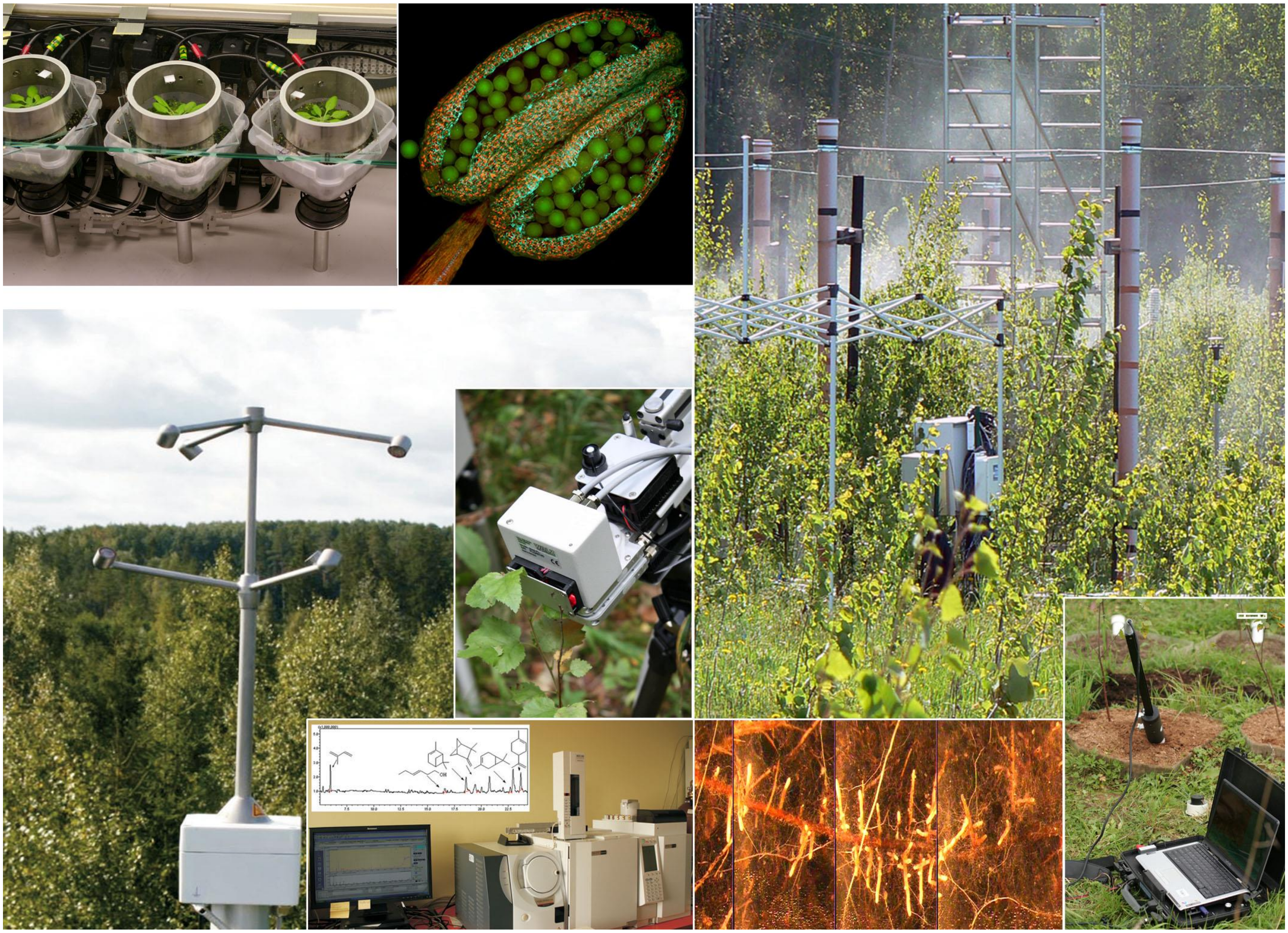

# Environmental Adaptation: from Molecules to the Planet

## The Estonian Centre of Excellence in Environmental Adaptation ENVIRON. Final Conference

## Abstract Book

October 1-3, 2015 ● Dorpat Conference Centre ● Tartu, Estonia

Environmental Adaptation: from Molecules to the Planet:
The Estonian Centre of Excellence of Environmental Adaptation ENVIRON. Final Conference.
Abstract Book

Tartu, 2015

Edited by Ivika Ostonen and Tiia Kurvits

Scientific Committee:
Ülo Niinemets, Estonian University of Life Sciences
Hannes Kollist, University of Tartu
Ülo Mander, University of Tartu
Anu Sõber, University of Tartu
Erkki Truve, Tallinn University of Technology

Organizing Committee:
Tiia Kurvits, Estonian University of Life Sciences
Ivika Ostonen, University of Tartu
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# Preface

Ecosystems have a large capacity to adapt to environmental perturbations, but so far, most of the future projections of global change ignore the adaptation responses. The Center of Excellence (CoE) of Environmental Adaptation (ENVIRON) was launched in August 2011 for a period of almost five years to study mechanisms of environmental adaptation from molecular to global scales, and feedbacks between plant and ecosystem adaptation and climate change.

The CoE encompasses five teams from three Estonian universities: Plant physiology and biosphere-atmosphere interactions (Prof. Ülo Niinemets, Estonian University of Life Sciences), Plant signals (Dr. Hannes Kollist, University of Tartu), Plant-pathogen interactions (Prof. Erkki Truve, Tallinn Technical University), Plant ecophysiology (Dr. Anu Sõber, University of Tartu) and Landscape ecology and ecotechnology (Prof. Ülo Mander). ENVIRON currently involves altogether 55 senior staff members and 50 PhD students, and has developed excellent field and laboratory setups to gain insight into the influences of stress on plants and ecosystems, including SMEAR Estonia and FAHM, the free air humidification facility. Interdisciplinary experimental and modeling approaches have been used to scale from molecular stress response mechanisms to ecosystem adaptation by analyzing abiotic and biotic stress effects at phenotypic, physiological and molecular levels, and determining the limits of adaptation to multiple sequential and interacting stresses.

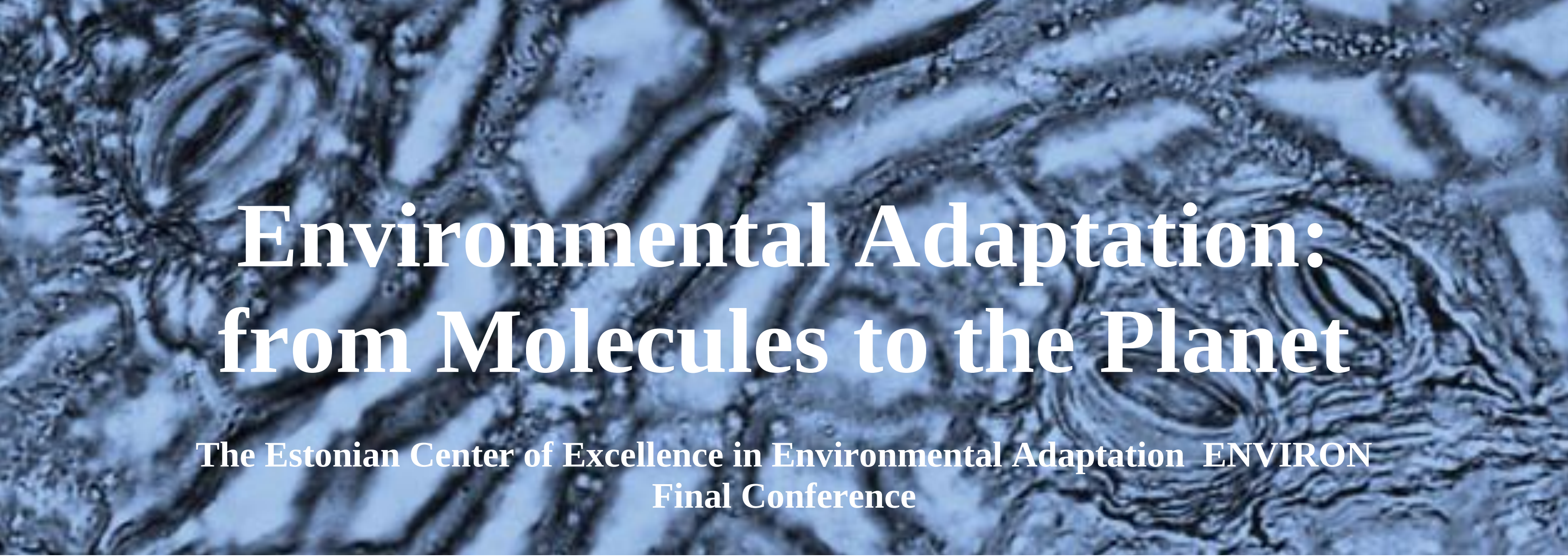
The key tasks of the ENVIRON were:

- studying the molecular mechanisms of key abiotic and biotic stress sensing and downstream signaling leading to adaptation in several classical model systems in controlled conditions, e.g., *Arabidopsis*, *Triticum* and *Populus*;
- characterizing the mechanisms of stress elicitation and synergistic and antagonistic effects of multiple stressors at ecosystem level and concomitant modifications in trace-gas emission patterns, carbon, nitrogen and water balance, carbon sequestration and vegetation capacity to alter atmospheric quality, transmissivity and cloud formation;
- studying changes in the gene expression patterns and of the population genetic structure to gain insight into local adaptations associated with modifications in genotype frequency under different long-term stresses;
- characterizing the role of the phylogenetic, structural and functional structure of soil microbial community (soil metagenome) in determining the adaptive capacity of soil microbial community and the effects of these key microbial community characteristics on biogeochemical cycling of carbon and nitrogen;
- simulating the effects of soil-vegetation-atmosphere feedbacks at ecosystem, regional and biome scales.

As the keynote talks and poster presentations at this conference demonstrate, ENVIRON has made major progress in all these directions and contributed to the training of a new generation of scientists who will keep working at the most exciting question: *How does the biosphere adapt to climate change?* Apart from the excellence in basic science, the consortium has importantly contributed to development of the Estonian National Adaptation Strategy for Climate Change that will serve as a foundation for sustainable management of natural resources of Estonia.

Hereby I welcome the participants of the final conference of ENVIRON and hope that the next three days will bring lots of stimulating presentations and insightful discussions. It has been a pleasure and privilege to lead ENVIRON and witness the hard work of its members that have guaranteed the success of the consortium in addressing its research agenda. Wishing you an excellent conference!

Ülo Niinemets
Head of the Centre ENVIRON



# Environmental Adaptation: from Molecules to the Planet

### The Estonian Center of Excellence in Environmental Adaptation ENVIRON
### Final Conference

**October 1-3, 2015 ● Dorpat Conference Centre ● Tartu, Estonia**

## PROGRAM

### Oct 1 (Thursday)

9:00 – 9:45 Registration

**9:45 – 12:35** **Global change from mechanisms to patterns,** moderator Ülo Mander

9:45 **Ülo Niinemets** (Estonian Univ. of Life Sciences, Head of the ENVIRON)
„How ENVIRON tries to understand adaptation to global change“.

10:45 –11:05 Coffee/tea break

11:05 **Markku Kulmala** (Univ. of Helsinki, Finland, ENVIRON board member):
„Biogenic volatile compounds enhancing aerosol load“.

**11:50** Rainer **Matyssek** (Technische Univ. München, Germany, ENVIRON board member):
„Drought – Challenge of the Future, for Trees and Science".

12:35 – 13:35 Lunch

**13:35 – 15:25 I session: Role of volatile messengers in plant adaptation,** moderator Ülo Niinemets

13:35 **Michael Staudt** (CNRS, Montpellier, France, invited keynote):
„BVOC emissions and Global Change“.

14:20 **Jörg-Peter Schnitzler** (Helmholz Gesellschaft, Germany, invited keynote):
„Plants under environmental stress - biosynthesis and function of plant volatiles“

15:05 **Astrid Kännaste** (Estonian Univ. of Life Sciences):
"Plants' chemotypes and their resistance against environmental stress factors"

15:25 – 15:45 Coffee/Tea break

**15:45 - 17:35** **II session: Plant-microbe interactions in the changing environment,** moderator Hannes Kollist

15:45 **Corné Pieterse** (Utrecht University, Netherlands, ENVIRON board member):
“The root microbiome and plant health”.

16:30 **Denis Fargette** (Université de Montpellier, France, invited keynote):

„Virus emergence in rice in the changing environment of Africa“.

17:15 **Miroslav Valarik** (Institute of Experimental Biology of the Czechoslovak Academy of Sciences):
„The Central Europe, a brewing pot of cereal powdery mildew disease.“

17:35 **Anders Kvarnheden** (Swedish Univ of Agricultural Sciences):
„Plant-infecting viruses in the Nordic countries and the possible effects of climate change“.

17:55 – 18:10 Break

18:10 – 19:00 Posters session, moderator Ivika Ostonen (Univ. of Tartu, Estonia)
1 minute poster presentations

19:00 – 21:00… and refreshments.

## Oct 2 (Friday)

### 8:45 – 11:55 III session: Adaptation at cell and plant level, moderator Erkki Truve

8:45 **Stefan Jansson** (Univ. of Umeå, Sweden, ENVIRON board member):
"Natural variation in aspen"

9:30 **Timothy Brodribb** (Univ. of Tasmania, invited keynote):
„Evolution of stomatal signaling“.

10;15 – 10:35 Coffee/Tea break

10:35 **Hannes Kollist** (Univ. of Tartu, Estonia):
„Stomatal regulation in response to environment“.

10:55 **Hanna Hõrak** (Univ. of Tartu, Estonia): „Stomatal $CO_2$ signaling“.

11:15 **Arne Sellin** (Univ. of Tartu, Estonia):
"Tree growth under increasing atmospheric humidity: possible mechanisms behind the growth retardation".

11:35 **Tiina Tosens** (Estonian Univ. of Life Sciences):
"The long and winding road of $CO_2$ from leaf mesophyll to chloroplasts - an evolutionary obstruction to plant functioning".

11:55 – 12:55 Lunch

### 12:55 – 15:00 IV session: Carbon and nitrogen cycles of forests and wetland ecosystems, moderator Jaak Truu

13:55 **Markus Reichstein** (Max Planck Inst. for Biogeochemistry, Germany, invited keynote): "Ecosystem functional responses to the environment - a FLUXNET perspective".

14:40 **Ivika Ostonen, Marika Truu** (Univ. of Tartu, Estonia):
“Ecosystem-level adaptation to environmental changes - ENVIRON results from local to regional scale”.

15:00 – 15:30 Coffee/Tea break

### 15:30 – 17:00 IV session continues, moderator Ebe Merilo

15:30 **Ülo Mander et al** (Univ. of Tartu, Estonia):
„Climate change and greenhouse gas emissions – ENVIRON results from local to global scale”.

15:50 **Jaak Truu** (Univ. of Tartu, Estonia):
„Climate change and Earth microbiome: understanding the processes and their controls“

16:10 **Steffen M. Noe** (Estonian Univ. of Life Sciences):
"Perspectives from SMEAR Estonia: First long term measurements of biosphere-atmosphere interactions in Estonia"..

16:30 **Krista Lõhmus et al** (Univ. of Tartu, Estonia):
"Elevated atmospheric humidity changes deciduous forest carbon and nitrogen cycling".

16:50 – 17:00 Closing, Ülo Niinemets

18:30 – 22:00 Conference dinner

## Oct 3 (Saturday)

### 8:45 – 17:15 Excursion

**Excursion route**

- Rõka: FAHM - Free Air Humidity Manipulation experiment site;
- Järvselja: SMEAR Estonia - a Station for Measuring Ecosystem-Atmosphere Relations, 130m tower;
- Värska (Culture of Setomaa and Setos – a small nation living on the border of Estonia and Russia http://www.visitsetomaa.ee/en,)
- Lunch in Värska (Seto cuisine)
- Pikalombi bog http://www.matkajuht.ee/et/pikalombi-matkarada/ (boardwalk)
- Podmotsa (the closest point to see the Russia over a small bay of Lake Peipsi; a chapel)
- Tartu

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# HOW **ENVIRON** HAS CONTRIBUTED TO UNDERSTANDING ADAPTATION TO GLOBAL CHANGE

**Ülo Niinemets**

Institute of Agricultural and Environmental Sciences, Estonian University of Life Sciences, Estonia, ylo.niinemets@emu.ee
Head of the Centre ENVIRON

Ecosystems have a large capacity to adapt to environmental perturbations, but so far, most of the future projections of global change effects do not consider the adaptation responses. This is a key shortcoming of current model predictions as adaptation responses can alter global carbon and nitrogen cycle as well as vegetation capacity to emit volatile organic compounds and contribute to secondary organic aerosol formation, with potentially important implications for the extent and rate of global change. Likewise, little is known of adaptation of vegetation-mediated emissions of greenhouse gases other than $CO_2$ and the relationships of these emissions to soil microbiological community composition and adaptability. Thus, understanding the capacity of ecosystems to adapt to environmental modifications is the key to predict ecosystem responses to global change. The Center of Excellence on Environmental Adaptation to Global Change (ENVIRON) is a truly interdisciplinary consortium that was formed in 2011 to address the adaptation of terrestrial ecosystems to environmental alterations. The specific foci of the work program were the effects of globally changing environmental drivers on biological activities ecosystems and the feedbacks between soil, vegetation, and biotic interactions and atmospheric processes. In the core of the proposed centre of excellence was the Estonian Science Roadmap Initiative project „Estonian Environmental Observatory“. In frames of this large infrastructure project of national significance, unique experimental facilities had become available, including establishment of a SMEAR (Station for Measuring Forest Ecosystem – Atmosphere Relations) station, and updating a FAHM (Free Air Humidity Manipulation) platform, allowing for direct monitoring of soil-vegetation-atmosphere feedbacks and assessment of the influences of a potential global change driver, air humidity, on forest ecosystems. In addition, the partners were using lab-based facilities for studying phylogenetic and functional diversity of soil micro-organisms as well as pathogens and pests. Genetic resources such as large collection of *Arabidopsis* ecotypes and recombinant inbred and near isogenic lines as well as collection of wheat cultivars and introgressed lines, and *Populus* and *Salix* genotypes were used to address molecular mechanisms behind natural variation to different abiotic and biotic stresses. Biotic stress studies considered both the effects of pathogens (plant viruses, different powdery mildew genotypes, rust fungi) and the effects of herbivores. The work conducted is currently being used to scale from molecular stress response mechanisms to ecosystem adaptation by modeling effects of abiotic and biotic stress at phenotypic, physiological and molecular levels to reveal the scope and mechanisms of stressor action and determining limits of adaptation to multiple sequential an interacting stresses. The major outcomes of ENVIRON include: (i) identification of key mechanisms controlling adaptation to abiotic and biotic stress factors and their interactions under global change, (ii) mapping molecular mechanisms of and adaptation to stress-induced events at major functional levels, (iii) prediction of the adaptation of carbon, nitrogen and water balances of terrestrial ecosystems in globally changing environmental conditions.

**Invited keynote/ Opening Session:** Global change: from mechanisms to patterns

# BIOGENIC VOLATILE COMPOUNDS ENHANCING AEROSOL LOADING

**Markku Kulmala**

University of Helsinki, Department of Physics,Finland, markku.kulmala@helsinki.fi
ENVIRON Advisory Board member

Secondary organic aerosols are crucial for global climate and air quality and their interactions. A key phenomenon associated with the atmospheric aerosol system is the formation and growth of new atmospheric aerosol particles via gas-to-particle conversion (GTPC). GTPC consists of a complicated set of processes including the production of condensable vapours (where organic vapours are important) via chemical reactions of volatile gases with oxidants, the formation of nanometer-size clusters from those precursor vapours, the growth of these clusters to detectable sizes via condensation of very low volatile vapours (like ELVOCs) and the removal of growing clusters by coagulation with the pre-existing aerosol particle population. Once formed, aerosol particles need to grow further to sizes >50-100 nm in diameter until they are able to influence climate, even though smaller particles affect human health and atmospheric chemistry. While atmospheric nucleation has been observed to take place almost everywhere with a significant contribution to aerosol particle number concentrations and indirect radiative effects, several gaps in our knowledge regarding this phenomenon still exist. These gaps range from the basic process-level understanding of GTPC to its various impacts on atmospheric chemistry, climate, human health and environment.

In my presentation I will focus on

- global environmental challenges
- 40 year time window
- biogenic feedbacks: from carbon sink to aerosol source
- GTPC
- Global aerosol load

**Invited keynote/ Opening Session:** Global change: from mechanisms to patterns

# DROUGHT – CHALLENGE OF THE FUTURE FOR TREES AND SCIENCE

**Rainer Matyssek**

Technische Universität München, Life Science Centre Weihenstephan-Freising/Germany,
matyssek@wzw.tum.de
ENVIRON Advisory Board member

Climate change may foster intensity and duration of drought in southern Germany/Bavaria. Mean annual air temperature would need, in the long term, to increase by about 2°C only, and annual precipitation decrease by 200 mm to prevent closed-canopy forests in that region to persist any longer. Such fluctuations have occurred in Bavaria sporadically, as e.g. in 2003, when both European beech and Norway spruce distinctly declined in productivity, staying inhibited even for another three years of "regular climate".

Obviously, drought challenges trees – albeit also science, as uncertainty about species-related die-off processes hinders management strategies to reliably provide for silviculture in a warming, water-limited future. Three experimental studies will be highlighted in overcoming knowledge deficits, on (i) the capacity of thinning for enabling wide-spread, but drought-susceptible spruce monocultures for water savings conducive both to survival and productivity; (ii) reforestation upon wind-throw with juvenile beech, being under light stress and competitive drought by residual spruce trees; and (iii) mechanisms of drought-associated die-off in neighbouring maturing beech and spruce trees through an automated rain exclusion experiment. Explored are extents of isohydric versus anisohydric strategies in trees' control of water shortage, and perhaps of soil water re-distribution and emerging facilitation rather than competition.

Will tree interaction under drought be driven in the future by facilitation? Does the latter underlie over-yielding of mixed beech-spruce forests relative to monocultures each, as widely observed in Bavaria today on resource-poor rather than rich sites? Understanding interaction principles will enable meeting silvicultural challenges of the future.

**Invited keynote/ Opening Session:** Global change: from mechanisms to patterns

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# ENVIRONMENTAL ADAPTATION TO GLOBAL CHANGE DRIVERS: UPSCALING FROM MOLECULES TO THE PLANET

**Josep Peñuelas**

CSIC- CREAF Barcelona, ENVIRON board member, josep.penuelas@uab.cat
ENVIRON Advisory Board member

Organisms and ecosystems are currently responding to global change through phenotypic plasticity, genotypic evolution, changes in growth, mortality, recruitment, and distribution and, in some cases, local extinction. Organisms alter their gene expression and metabolism to change their physiology, phenology, growth and reproduction in response to global environmental change. Rapid adaptation and microevolution occur at the population level. Together with these phenotypic and genotypic adaptations, the movement and the turnover of populations can lead to migration toward habitats with better conditions unless hindered by barriers. Both migration and local extinction of populations have occurred. However, many unknowns for all these processes remain. The application of omic techniques to ecological studies will greatly favor this research. It remains poorly understood how global change will result in asymmetrical responses of species and how the different drivers of global change, climate change, eutrophication, atmospheric change, and loss of biodiversity will interact with each other increasing global impacts in structure and functioning of ecosystems. The biogeochemical and biophysical feedbacks on climate of all these changes in vegetation are also poorly understood. We will present the current evidences of responses to global change, and will discuss the perspectives for increasing our knowledge of the interactions between global change and ecosystems. The emerging consistency of the relationship between biochemical, optical and odorous signals emitted by plants offers promising prospects for continuous local and global monitoring of their energetic status, and therefore of their processing of energy and matter.

**Invited keynote** /Session I: **Role of volatile messengers in plant adaptation**

# BVOC EMISSIONS AND GLOBAL CHANGE

**Michael Staudt**

Centre for Functional Ecology and Evolution CNRS, michael.staudt@cefe.cnrs.fr

VOC emissions from terrestrial ecosystems affect the earth's climate system by influencing the formation of aerosols and ozone, and the degradation of methane in the atmosphere. Therefore, studying the effects of man-made global environmental changes on VOC release is of major concern to anticipate potential feedbacks on climate forcing. While much research has focussed on the short-term effects of single global change factors on emission, fewer efforts were made to assess long-term effects and factor interactions, and the indirect effects associated with changes in the standing biomass and distribution of VOC-emitting species. In this context I will first provide a concise overview on the currently available information. Secondly I will present results from an exemplary field experiment, in which isoprene and monoterpene emitting Mediterranean oak saplings were grown under future warmer and dryer climates in competition with herbaceous species. We observed that warming treatments enhanced emissions by increasing the actual and the potential VOC emission rate of oak leaves and by extending their life span. On the other hand leaf level emissions were clearly attenuated by drought stress. In addition, biomasses and survival rates of oak saplings were reduced, basically due to the strong competitiveness of herbs including the potentially invasive C4-grass *Setaria parviflora*. Together these observations suggest that future climate conditions can positively and negatively affect VOC fluxes from Mediterranean oak forests depending on the temporal and spatial scale. The lack of forest regeneration and the associated reduced ability to adapt to climate change may trigger large shifts of biogenic VOC sources in this area.

**Invited keynote** /Session I: **Role of volatile messengers in plant adaptation**

# PLANTS UNDER ENVIRONMENTAL STRESS – BIOSYNTHESIS AND FUNCTION OF PLANT VOLATILES

Jörg-Peter Schnitzler

Research Unit Environmental Simulation, Institute of Biochemical Plant Pathology, Helmholtz
Zentrum München GmbH, Ingolstädter Landstr. 1, 85764 Neuherberg, Germany,
jp.schnitzler@helmholtz-muenchen.de

Plants synthesize and emit a large variety of volatile organic compounds (VOCs), with terpenes and fatty-acid derivatives being the dominant classes. Whereas some volatiles are probably common to almost all plants (e.g. C6 aldehydes, alcohols, and esters, as well as acetaldehyde or methanol), others are specific to only a few related taxa like isoprene that is predominately emitted by tree such as oaks and poplars, or emitted under specific stress situations. Our current progress in understanding plant volatile functions is due to general advances in biochemical and molecular techniques, i.e. availability of genetically modified plants and to the development of new instrumentation for the analysis of VOCs. The presentation will introduce some of these techniques and will demonstrate examples of stress-induced emissions of trees in urban areas. Moreover the talk will summarize our actual knowledge on the biological and ecological function(s) of isoprene and will discuss future directions of BVOC research.

# PLANTS´ CHEMOTYPES AND THEIR RESISTANCE AGAINST ENVIRONMENTAL STRESS FACTORS

**Astrid Kännaste**.
Estonian University of Life Sciences, department of plant physiology, astrid.kannaste@emu.ee

Plants´ vital functioning is constantly affected by simultaneously or consecutively acting stress factors stress. In general, the high concentration of greenhouse gases (GHGs) in the atmosphere is one the factors, which contributes in evolving of plant stress by environmental stress. For example the heat -, drought-, flooding-, ozone-, high $CO_2$-, wind - and smog stresses are either directly or indirectly related to GHGs. Additionally in agricultural regions abiotic plant stress can be caused by frost during night time (known as cold stress) or by various chemicals used either as pesticides or herbicides. Plant stressors affect the composition of volatile organic metabolites. Hence understanding within-species variability in VOCs content is essential in order to be able to predict plants´ ability to cope with the stressors and to acclimatize under changing environment.

**Invited keynote** /Session II: **Plant-microbe interactions in the changing environment**

## THE ROOT MICROBIOME AND PLANT HEALTH

**Corné M.J. Pieterse**

Utrecht University, C.M.J.Pieterse@uu.nl
ENVIRON Advisory Board member

Plants nurture a large community of plant growth-promoting rhizobacteria (PGPR) that provide them with essential services, such as enhanced mineral uptake, nitrogen fixation, growth promotion, protection against pathogens and insects, and adaptation to changes in the environment [1,4]. These plant microbiota are predominantly hosted by the root system. Selected PGPR promote plant health by stimulating the plant’s immune system, a phenomenon called induced systemic resistance (ISR) [2]. The molecular mechanisms underpinning ISR have been intensively studied in the interaction between the model plant *Arabidopsis thaliana* (Arabidopsis) and the PGPR strain *Pseudomonas fluorescens* WCS417 (WCS417) [2, 3]. WCS417-mediated ISR is effective against a broad variety of pathogens and even insect herbivores. Large scale gene expression analyses revealed that the establishment of WCS417-ISR in foliar tissues is not associated with major changes in gene expression. Instead, ISR-expressing leaves are primed for accelerated defense gene expression, which only becomes apparent after pathogen or insect attack. This phenomenon is known as priming and provides a cost-effective mechanism of protection against pathogens and pests. Recently, we demonstrated that volatile organic compounds produced by PGPR in the rhizosphere play an important role in both growth and protection of the plant, highlighting the potential role of soil microbes in the adaptation of plants to their often hostile environment.

1. Berendsen, RL, Pieterse, CMJ and Bakker, PAHM.(2012). The rhizosphere microbiome and plant health. ***Trends Plant Sci.*** 17:478-486.
2. Pieterse, CMJ, Zamioudis, C, Berendsen, RL, Weller, DM, Van Wees, SCM and Bakker, PAHM (2014). Induced systemic resistance by beneficial microbes. ***Annu. Rev. Phytopathol.*** 52: 347–375
3. Zamioudis, C and Pieterse, CMJ (2012). Modulation of host immunity by beneficial microbes. ***Mol. Plant-Microbe Interact.*** 25: 139-150
4. Zamioudis, C, Mastranesti, P, Dhonukshe, P, Blilou, I and Pieterse, CMJ (2013). Unraveling root developmental programs initiated by beneficial Pseudomonas bacteria. ***Plant Physiol.*** 162: 304-318.
5. Zamioudis, C, Korteland, J, Van Pelt, JA, Van Hamersveld, M, Dombrowski, N, Bai, Y, Hanson, J, Van Verk, MC, Ling, H-Q, Schulze-Lefert, P and Pieterse, CMJ (2015). Rhizobacterial volatiles and photosynthesis-related signals coordinate MYB72 in Arabidopsis roots during onset of induced systemic resistance and iron deficiency responses. ***Plant J.*** doi: 10.1111/tpj.12995.

**Invited keynote** /Session II: **Plant-microbe interactions in the changing environment**

## VIRUS EMERGENCE IN RICE IN THE CHANGING ENVIRONMENT OF AFRICA

**Denis Fargette**

Institut de Recherches pour le Développement (IRD), UMR IPME (IRD, CIRAD, Université de Montpellier), Montpellier, France, denis.fargette@ird.fr

*Rice yellow mottle virus* (RYMV) is the main pathogen of rice in Africa. RYMV has a narrow host range, restricted to the two cultivated rice species *Oryza sativa* and *O. glaberrima*, and a few related wild grasses. RYMV is propagated by beetles, but is not seed transmitted. Reported for the first time in 1966 in Kenya, RYMV has now been detected in all rice producing countries of sub-tropical Africa. A representative sample of isolates from over 20 African countries have been collected from 1966 to 2015, sequenced and subjected to phylogeographic analyses. It was estimated that RYMV emerged in Eastern Tanzania in the mid 19th century and spread throughout Africa within ca. 150 years. The patterns and rates of spread were inferred. They were related to the changing rice environment in Africa by applying two complementary approaches. An "historical" approach based on the available information on rice history in Africa encapsulated in historical maps. A "statistical" approach using rice production statistics and a present rice density landscape map. Both approaches concluded that rice intensification in Africa was the main driver of RYMV emergence and expansion. In complement to the role of these anthropogenic changes in virus emergence, the role of climatic changes will be discussed throughout spatio-temporal modelling of RYMV spread.

Pinel-Galzi A, Traoré O, Séré Y et al. 2015. The biogeography of viral emergence: rice yellow mottle virus as a case study. Current Opinion in Virology 10, 7-13.
Trovao N, Baele G, Vrancken B. et al. 2015. Host density determines the dispersal patterns of a plant virus. Virus Evolution, in press.

**Invited keynote** /Session II: **Plant-microbe interactions in the changing environment**

# THE CENTRAL EUROPE, A BREWING POT OF CEREAL POWDERY MILDEW DISEASE

Eva Komínková[1], Eva Malečková[1], Hana Vanžurová[1], Antonín Dreiseitl[2], Jaroslav Doležel[1]
& **Miroslav Valárik[1]**

[1]Institute of Experimental Botany, Centre of the Region Haná for Biotechnological and Agricultural Research, Šlechtitelů 31, CZ-783 71 Olomouc - Holice, Czech Republic, Valarik@ueb.cas.cz

[2]Agrotest Fyto Ltd., Havlíčkova 2787, CZ-767 01 Kroměříž, Czech Republic

The barley powdery mildew disease is caused by air-born fungus *Blumeria graminis* f. sp. *hordei* (*Bgh*). The pathogen can cause 40% yield and quality losses and is responsible for 50% of disease epidemics on barley. Until now, molecular diversity studies based on “house-keeping” genes have not provided sufficient resolution when applied to isolates from geographically limited regions. This is restricting studies of population structures and theirs changes in time due to changing agronomical approaches and environmental conditions. We developed efficient genotyping system capable to discriminate between isolates within the Czech Republic. Whole genome sequence data were employed to design a panel of molecular markers based on microsatellites and insertion sites of transposable elements which represent an abundant part of the genome. The marker panel comprises 16 SSR, 14 SNP and 2 ISBP/RJM markers. Its application on a set of 97 isolates originating from the Czech Republic, 50 Australian isolates and a collection of 11 isolates representing global *Bgh* diversity provided sufficient resolution power to discriminate the Czech originating isolates. The analysis of phylogenetic relationship performed by neighbor-joining algorithm for 97 Czech isolates resulted in 87 separate clades and revealed high genetic diversity of the pathogen population within a small geographical area. After supplementing with data on virulence of individual isolates and environmental conditions in different seasons, this study might open new opportunities of studying the host-pathogen relationship and patterns of the pathogen spatial distribution based on time and environmental conditions.

This work has been supported by the Czech Ministry of Education, Youth and Sports (grant awards LD14105, LO1204).

**Invited keynote** /Session II: **Plant-microbe interactions in the changing environment**

# PLANT-INFECTING VIRUSES IN THE NORDIC COUNTRIES AND THE POSSIBLE EFFECTS OF CLIMATE CHANGE

**Anders Kvarnheden**

Swedish University of Agricultural Sciences and Estonian University of Life Sciences,
anders.kvarnheden@slu.se

In northern Europe, virus infections of plants are often limited by the winter, which reduces the numbers of host plants and virus vectors. The viruses then have to persist in overwintering plants or vectors. With the predicted longer autumns, milder winters and earlier springs, there is an increased risk for virus infections of crop plants. The possible period for virus inoculation by vectors is then longer in the autumn and mild winters will increase the possibility for aphids to survive the winter as adults allowing early spread of virus in the spring. In the Nordic countries, among the most important viruses transmitted by insect vectors are wheat dwarf virus (WDV), barley yellow dwarf virus (BYDV) and potato virus Y (PVY). In 2015, there was a high incidence of barley yellow dwarf (BYD) disease in winter crops of cereals in southern Sweden as well as in Denmark and northern Germany. The high incidence was correlated with high levels of the oat bird-cherry aphid (Rhopalosiphum padi) in the previous autumn. Serological tests revealed infection with three types of BYD-associated viruses: BYDV-PAV, BYDV-MAV and Cereal yellow dwarf virus-RPV (CYDV-RPV). Our sequence studies on virus isolates from previous years have shown that there is a high diversity of BYD-associated viruses in grasses and cereals. Also for WDV, we have found infections in grasses and cereals, even during years with a low disease incidence. This suggests that these viruses persist in the region and may cause disease outbreaks when conditions are favourable for virus spread.

**Invited keynote** /III session: **Adaptation at cell and plant level**

# NATURAL VARIATION IN ASPEN

**Stefan Jansson**

Umeå Plant Science Centre, Department of Plant Physiology, Umeå University, 901 87 Umeå, Sweden, stefan.jansson@umu.se
ENVIRON Advisory Board member

As DNA sequencing technology becomes cheaper high-throughput studies becomes possible also on other plants than traditional model species like *Arabidopsis* and rice. This extends the range of scientific questions that can be raised and increases the possibilities to generalize findings but poses new challenges. Trees species differ from *Arabidopsis* in many ways and the genus *Populus* is the most developed tree model system. We have developed the genetics and genomics tools for one of the most widespread and ecologically important plant species on earth, aspen (*Populus tremula*). The hurdles for generating a high-quality aspen reference genome sequence have been massive – the genome is very heterozygous and variable – but we can now use resequencing data from a natural population of aspens with vast amounts of phenotypic data to perform Genome Wide Association Studies (GWAS). This talk will describe challenges and opportunities, local adaptation of aspen in particular focusing on phenology traits like bud flush, bud set and autumn senescence, but also how it is possible to use field experiments with transgenic trees to test predictions.

**Invited keynote** /III session: **Adaptation at cell and plant level**

# INSIGHTS FROM 400 MILLION YEARS OF WATER MANAGEMENT

**Tim Brodribb**

School of Biological Science, University of Tasmania, tim.brodribb@gmail.com

Since the earliest days of vascular plant evolution terrestrial plants have paid for photosynthetic carbon by spending extravagant amounts of water. The high costs associated with mining, transporting and storing water make plant "water management" one of the most pervasive adaptive dimensions of plant evolution. Here I will discuss long-term evolutionary changes in the function of plant tissues involved in the regulation of water loss (stomata) and water delivery (xylem) in land plants. Investigations into the co-evolution of these regulated systems provide a new context to understand diversity in the responses of plant species to changes in local and global climate.

# STOMATAL REGULATION IN RESPONSE TO CHANGES IN THE ENVIRONMENT

**Hannes Kollist**, Mikael Brosche, Hanna Hõrak, Liina Jakobson, Pirko Jalakas, Kristiina Laanemets, Ebe Merilo, Maris Nuhkat, Priit Pechter, Bahtijor Rasulov, Yana Sindarovska, Kadri Tõldsepp, Triin Vahisalu, Yuh-Shuh Wang, Dmitri Yarmolinsky, Olena Zamora

Plant Signal Research group, Institute of Technology, University of Tartu, Hannes.kollist@ut.ee

Guard cells that form stomatal pores are an attractive experimental system in plant adaptation to changes in its environment; besides balancing $CO_2$ uptake with loss of water they also control the entrance of harmful air pollutants and pathogens. Thus traits that affect stomatal regulation are essential for plant adaptation to ever-changing environment and are targets of the breeding programs aimed to develop crops with improved water-use efficiency. The emerging model of stomatal regulation that involves abscisic acid receptors, type 2 C protein phosphatases, calcium dependent and independent protein kinases and guard cell slow and rapid type of anion channels will be discussed. More specifically, recent progresses in understanding molecular events that control stomatal movements in response to environmental factors, the major knowledge gaps in the system and our ongoing approaches to fill those will be addressed.

# STOMATAL $CO_2$ SIGNALLING

Mikael Brosché[12], Adrien Gauthier[2], **Hanna Hõrak**[1], Liina Jakobson[1], Jaakko Kangasjärvi[2], Hannes Kollist[1], Kristiina Laanemets[1], Mart Loog[1], Ebe Merilo[1], Maris Nuhkat[1], Kirk Overmyer[2], Priit Pechter[1], Maido Remm[1], M. Rob G. Roelfsema[3], Jarkko Salojärvi[2], Julian I. Schroeder[4], Maija Sierla[2], Yana Sindarovska[1], Kadri Tõldsepp[1], Lauri Vaahtera[2], Triin Vahisalu[2], Ervin Valk[1], Cun Wang[4], Yuh-Shuh Wang[1], Cezary Waszczak[2], Dmitry Yarmolinsky[1](Authors in alphabetical order)

[1] - University of Tartu; hhorak@ut.ee
[2] - University of Helsinki;
[3] - University of Würzburg;
[4] - University of California, San Diego

Increasing atmospheric $CO_2$ concentrations affect climate and environment. $CO_2$ is the limiting substrate for photosynthesis and an important regulator of plant responses. $CO_2$ enters plants via stomata, pores formed by specialized guard cells, which function as regulators of plant water loss via transpiration as well as $CO_2$ uptake. Stomatal opening is regulated in response to environmental cues, such as changes in air and soil humidity, changes in $CO_2$ concentration, light-dark transitions, pathogens and air pollutants. Plant hormone abscisic acid (ABA) is a central regulator of stomatal responses. While there have been major developments in understanding guard cell ABA-signalling in recent years, the molecular mechanisms of stomatal $CO_2$-signalling have remained elusive.

We used different methods to address the regulation of $CO_2$-responses in *Arabidopsis thaliana*. A mutant screen for ozone-sensitivity, analysis of an ozone-sensitive *Arabidopsis thaliana* natural accession and mapping of a secondary mutation in a T-DNA line all led to identification of core stomatal $CO_2$-signalling components. We identified a mitogen activated protein kinase as a regulator of *Arabidopsis thaliana* $CO_2$-responses and propose a model for guard cell $CO_2$-signalling as well as suggest the points of convergence between stomatal ABA and $CO_2$-signalling pathways. These findings bring further insight into the molecular mechanisms of plant stomatal regulation and could be used for developing crops with increased water use efficiency and yield in changing and challenging environmental conditions.

# TREE GROWTH UNDER INCREASING ATMOSPHERIC HUMIDITY: POSSIBLE MECHANISMS BEHIND THE GROWTH RETARDATION

**Arne Sellin**, Priit Kupper, Krista Lõhmus, Anu Sõber, Arvo Tullus

Institute of Ecology and Earth Sciences, University of Tartu, arne.sellin@ut.ee

Increase in atmospheric relative humidity, the inevitable result of more frequent rainfall events predicted for northern Europe, will reduce water fluxes through the vegetation. This has impact on various physiological processes causing a decline in trees' increment observed both in silver birch and hybrid aspen growing at the Free Air Humidity Manipulation (FAHM) site. We propose alternative mechanisms to explain the growth deceleration observed in one or both experimental species.

- Reduced water flux through the trees diminishes nutrient uptake, leads to lower leaf nutrient status and unbalanced foliar P:N ratio causing a decline in leaf photosynthetic capacity.
- Increased soil water content causes hypoxia bringing about metabolic stress; disturbed nitrogen metabolism (significant changes in amino acid contents) may reduce both photosynthetic capacity and growth rate.
- Retardation of foliage development in response to elevated air humidity observed at individual leaf or whole-tree foliage levels; the biomass production of trees is directly proportional to their foliage area.
- Larger investments in vascular tissues in relation to foliage area cause an increase in the ratio of non-photosynthetic to photosynthetic tissues leading to larger maintenance respiration costs determined by the volume of living parenchyma cells.
- A probable increase in proportion of living parenchyma cells in relation to dead xylem elements in sapwood, resulting in higher stem respiration.
- Reduced hydraulic conductance of stems and leaves becoming a critical factor in case of weather extremes (heat wave, severe drought).
- Top shoot dieback and breakage induced by fungal pathogen *Cryptosporella betulae* contributes to decreased height growth.

# THE LONG AND WINDING ROAD OF $CO_2$ FROM LEAF MESOPHYLL TO CHLOROPLAST

**Tiina Tosens**, Ülo Niinemets.

EMÜ, tiina.tosens@emu.ee

Photosynthesis differ across ecosystems more than 30-folds. Traditionally, only biochemical and stomatal limitations have been considered as limiting factors of photosynthesis in terrestrial plants. Diffusional limitations are important limiting factors for plant functioning in developing or stress adapted leaves or in evolutionary old plants such as ferns and fern allies.

In Populus during leaf maturation mesophyll conductance increased 2.6-fold in control leaves and 3.5-fold in leaves stressed either by shading or by drought. Increased mesophyll conductance was associated with increases in mesophyll surface area per unit of leaf area (Sc/S) during leaf maturation. Detailed structural analyses with Populus demonstrated that in water stressed conditions under high light, mesophyll cell wall thickness increased 27% and this was the main reason for reduction in mesophyll conductance. Shading in Populus reduced Sc/S by 50% and consequently halved mesophyll conductance. Across 35 Australian sclerophyll species mesophyll conductance varied 4-fold, with this variation being most strongly driven by differences among species in Sc/S and the thickness of mesophyll cell walls.

Low mesophyll conductance to $CO_2$ is the main cause for low photosynthesis in ferns and fern allies species, which in turn was associated with very thick cell walls and reduced chloroplast distribution towards intercellular mesophyll air spaces.

**Invited keynote** /IV session: **Carbon and nitrogen cycles of forests and wetland ecosystems**

# ECOSYSTEM FUNCTIONAL RESPONSES TO THE ENVIRONMENT – A **FLUXNET** PERSPECTIVE

**Markus Reichstein**

Max-Planck-Institute for Biogeochemistry, Jena Department Biogeochemical Integration Hans-Knöll-Str. 10, D-07745 Jena, markus.reichstein@bgc-jena.mpg.de

Recent continuous observations of this exchange of CO2, H2O and sensible heat within the global observation network FLUXNET have enabled us to quantify ecosystem function in response to large eco-climatological spatial gradients and temporal variability of climate. We see that ecosystem function co-varies strongly with climate, but that climate alone does not suffice to explain the variation in total. Instead vegetation biophysical and structural parameters co-determine the exchange of carbon, water and energy between the ecosystem and the atmosphere. By combining ecosystem level observation and information of spatial meteorological and vegetation remote sensing covariates we can infer global patterns of ecosystem atmosphere fluxes and derive key ecosystem functional properties globally. While this approach is powerful and meteorological and vegetation structural predictors explain more than 70% of the spatial variation of monthly fluxes at FLUXNET sites, it ignores the effect of ecophysiological vegetation properties, which is expected from plant physiological leaf or whole-plant studies.

Hence, future research has to more strongly link the organismic trait information with ecosystem functional properties. For this we propose a framework that involves 1) the correlation of community aggregated traits with flux-derived ecosystem properties across a range of ecosystems, 2) the up-scaling of vegetation traits using spatially distributed geo-ecological co-variates and the comparison with global ecosystem functional properties and their co-variation with climate, 3) the use of vegetation traits instead of vegetation classes for empirical up-scaling of ecosystem-atmosphere fluxes from ecosystem to globe. We hypothesize that these studies will emphasize the superiority of a trait based approach over classical structural PFT concepts for modeling global ecosystem functioning, but will also highlight the scale-emergent properties at ecosystem level, which cannot be explained by plant traits alone.

# ECOSYSTEM-LEVEL ADAPTATION TO ENVIRONMENTAL CHANGES - ENVIRON RESULTS FROM LOCAL TO REGIONAL SCALE.

**Ivika Ostonen, Marika Truu**

Institute of Ecology and Earth Sciences, University of Tartu, ivika.ostonen@ut.ee, marika.truu@ut.ee

The root-mycorhizosphere interface of forest trees is the nexus of a variety of associations between bacteria, fungi and the host plant, and the health of these root dependent consortia plays a key role in adaptation of forest ecosystems along globally changing environments.

Our special focus was on ecosystem –level adaptation and on the role of rhizosphere bacteria, EcM fungi and absorptive fine roots in acclimation/adaptation of ecosystems. Here we aimed to analyze the complementary shifts in the community structure of ectomycorrhizal fungi and rhizosphere bacteria in relation to the changes in plant absorptive root tip morphological and functional traits in the light of changing environment. We analyzed the results from site-based free-air manipulation studies and climate gradient studies to ascertain the universal trends and general mechanisms in tree root-mycorhizosphere adaptation to climate change.

The results of our studies revealed strong relations between absorptive root morphology, the dominant EcM fungal colonizers and the bacterial community structure in the bulk soil and the rhizosphere.

Complementary mechanisms within roots, mycorrhizal networks and the rhizobiome lead to the adaptation of the root-mycorhizosphere under global change, although it may cause essential changes in C allocation and cycling in forest ecosystems.

# CLIMATE CHANGE AND GREENHOUSE GAS EMISSIONS – ENVIRON RESULTS FROM LOCAL TO GLOBAL SCALE

**Ülo Mander,** Jaak Truu, Kaido Soosaar, Martin Maddison, Jaan Pärn, Marika Truu, Ivika Ostonen, Teele Ligi, Järvi Järveoja, Raili Hansen, Kuno Kasak, Kristjan Oopkaup, Mikk Espenberg, Jens-Konrad Preem, Hiie Nõlvak, Jüri-Ott Salm, Alar Teemusk, Kalev Repp, Gert Veber etc

Department of Geography, Institute of Ecology and Earth Sciences, University of Tartu,
ulo.mander@ut.ee

We will present a short overview of results of 1-2 year-long chamber-based studies $CO_2$, $CH_4$ and $N_2O$ emission and related environmental factors from 10 natural, drained, and excavated bogs and fens, 9 drained peatland forests, four alder forests (two riparian and two grown on abandoned agricultural land), two restored peat extraction areas, two experimental constructed wetlands (CWs) for wastewater treatment (all located in Estonia), two created riverine wetlands (one in Columbus, Ohio, USA, another one in Rampillon, Brie, France), and one experimental young deciduous forest (FAHM). Also, data from short-term studies in 120 various peatland sites (25 study areas) from Eurasia, North America, South America, Africa, Tasmania and New Zealand, and from 1-3-years-long eddy-covariance based C balance studies in 3 hemi-boreal forest sites in Estonia are available.

For wastewater treatment CWs, drained peatland forests, one restored peat extraction area, created riverine wetlands in Ohio, and a tropical fen in French Guiana results of metagenomic studies (microbial activity and structure of microbial communities, abundance and diversity of denitrification-related genes *nirS*, *nirK* and *nosZ I* & *II*, and methanogenesis-related genes *mcrA*) in relation to critical physical-chemical characters of these systems will be presented.

We also discuss the possible impact of climate change on critical environmental conditions controlling GHG emissions in studied ecosystems.

# CLIMATE CHANGE AND EARTH MICROBIOME: UNDERSTANDING THE PROCESSES AND THEIR CONTROLS

**Jaak Truu**

Institute of Ecology and Earth Sciences, University of Tartu, jaak.truu@ut.ee

The relationships between GHG emission, global climate change and the environment require better understanding of the processes that govern GHG efflux. Behind the biogeochemical cycles are the largely hidden genetic and physiological characteristics of individual microorganisms that give rise to population dynamics and community-level interactions, all of which drive the biogeochemical processes including GHG emission. Microorganisms mediate key steps in biogeochemical cycles through the production of particular enzymes, which are encoded by functional genes. We have conducted research about microbial community structure and abundance, and diversity of functional genes in different natural and treatment wetlands, abandoned peatlands, arable and forest soils. Particularly, microbial community and functional genes related to carbon and nitrogen transformation processes in soil were targeted at different ecosystem levels. Cultivation-independent molecular techniques such as amplicons-based and shot-gun metagenomics sequencing, and quantitative PCR were applied for characterization of phylogenetic and functional structure of microbial communities. The main outcome of such studies is the knowledge about microbial capacity at the taxonomic, functional and genomic levels to impact nutrient cycling and GHG emission in different ecosystems.

# PERSPECTIVES FROM SMEAR ESTONIA: FIRST LONG TERM MEASUREMENTS OF BIOSPHERE-ATMOSPHERE INTERACTIONS IN ESTONIA

**Steffen M., Noe[1]**; Ahto, Kangur[1]; Ülo, Niinemets[1], Urmas, Hõrrak[2]; Alisa, Krasnova[1]; Dmitrii, Krasnov[1]; Beate, Noe[1]; H. Peter E., Cordey[1].

1) Estonian University of Life Sciences, steffen.noe@emu.ee
2) University of Tartu

The SMEAR Estonia (Station for Measuring Ecosystem-Atmosphere Relations, Hari and Kulmala, 2005) was build since 2011. Its purpose is to measure concentrations and fluxes of energy and matter that are exchanged between the biosphere and the atmosphere in a comprehensive way.

The station measures concentrations of greenhouse gases ($CO_2$, $CH_4$, $H_2O$), reactive trace gases ($O_3$, $NO_x$, $SO_2$) and volatile organic compounds (Bourtsoukidis *et al*., 2012) utilizing a 130m high mast and different, up to 30m high, scaffolding towers. Gases are measured on five heights (30,50,70,90 and 110m), Eddy covariance fluxes on 30 and 70m. Aerosols and air ions are measured within and above the forest canopy. Meteorological parameters like precipitation, temperature, atmospheric pressure and relative humidity are measured on several heights accompanying the turbulent wind flow and radiation measurements. Direct access to the trees and the canopy is available and gas exchange and light measurements within the canopy are planned to start later this year. Dendrometers are deployed and track changes in stem diameter growth and diurnal dynamic. Fluxes of aboveground biomass are measured utilizing a litter trap system and belowground biomass by ingrowth nets. Soil flux chambers, moisture and temperature sensor system are distributed in diverse permanent sample plots representing the major forest types around the station. The station is located in a hemiboreal mixed forest (Noe *et al*., 2011) and major tree species are Scots pine, Norway spruce and Birch.

Bourtsoukidis E., Bonn B. and Noe S.M. 2014. On-line field measurements of BVOC emissions from Norway spruce (Picea abies) at the hemiboreal SMEAR-Estonia site under autumn conditions. Boreal Env. Res. 19: 1-15.
Hari, P and Kulmala, M. 2005. Station for Measuring Ecosystem–Atmosphere Relations (SMEAR II). Boreal Env. Res. 10: 315-322.
Noe, S.M. *et al*. 2011. Ecosystem-scale biosphere–atmosphere interactions of a hemiboreal mixed forest stand at Järvselja, Estonia. Forest Ecology and Management 262: 71-81.

# ELEVATED ATMOSPHERIC HUMIDITY CHANGES DECIDUOUS FOREST CARBON AND NUTRIENT CYCLING

**Krista Lõhmus[1],** Ivika Ostonen[1], Katrin Rosenvald[1], Mai Kukumägi[1], Arvo Tullus[1], Raili Hansen[1], Priit Kupper[1], Jaak Sõber[1], Veiko Uri[2], Jürgen Aosaar[2], Mats Varik[2], Marika Truu[1], Kaido Soosaar[1], Ülo Mander[1], Arne Sellin[1], Anu Sõber[1]

[1]Institute of Ecology and Earth Sciences, University of Tartu, Krista.Lohmus@ut.ee
[2]Institute of Forestry and Rural Engineering, Estonian University of Life Sciences

FAHM – Free Air Humidity Manipulation of forest ecosystem was established to simulate more humid future climate predicted for higher latitudes of northern hemisphere (Kupper et al, 2011) and to analyze deciduous forest response at different organization levels. The main question at ecosystem level was: is forest ecosystem in more humid climate sequestering more or less carbon?

Annual input fluxes in 5-yr-old silver birch stands after two years of humidification: above- and belowground sequestration in plants (trees and understory), litter input into soil, $CH_4$ oxidation and output fluxes: soil respiration ($R_s$), DOC leaching were assessed.

Humidification has increased plant production (NPP) a third as belowground NPP of understory was redoubled (Kukumägi et al, 2014), but has decreased tree NPP except fine roots (< 2mm) in which NPP was three times higher. Simultaneously $R_s$ formed in humidified plots 80% from that in ambient conditions. DOC leaching and $CH_4$ oxidation were small. The smaller $R_s$ occurred despite the 1.6 times bigger litter flux and 1.3 times higher soil microbial activity; the possible explanation is decrease in autotrophic respiration.

After two years of misting humidified plots acted as C sink, and control ones as C source. Concerning the nitrogen budget (Annual plant N demand = Net N mineralization in soil + Retranslocation + Deposition – Leaching), Net N mineralization (NNM) and net nitrification were both smaller in misting plots. NNM in humidified plots formed 74% from the flux in control plots. Hence both C and N cycling were strongly affected by elevated humidity.

Kupper, P.; Sõber, J.; Sellin, A. et al. (2011). An experimental facility for Free Air Humidity Manipulation (FAHM) can alter water flux through deciduous tree canopy. Environmental and Experimental Botany, 72(3), 432 - 438.

Kukumägi, M.; Ostonen, I.; Kupper, P. et al. (2014). The effects of elevated atmospheric humidity on soil respiration components in a young silver birch forest. Agricultural and Forest Meteorology, 194, 167 - 174.

## POSTER SESSION (In alphabetical order)

### IMPACT OF ELEVATED ATMOSPHERIC HUMIDITY ON RAY PARENCHYMA IN HYBRID ASPEN

**Meeli Alber**, Arne Sellin

Institute of Ecology and Earth Sciences, University of Tartu, meeli.alber@ut.ee

Future climate models predict rise in precipitation amounts, frequency and air humidity at high latitudes. Trees need to cope with changing conditions by means of acclimation. One way of studying the influence of climate change on trees' functioning and forest ecosystems is manipulative experiments. Artificially increased relative air humidity at Free Air Humidity Manipulation (FAHM) site causes decline of transpirational flux through the trees. Reduced transpirational flux induces changes in xylem anatomy, including proportions of living and dead tissues in sapwood. We studied amount of ray parenchyma of 1-year-old hybrid aspen (*Populus* × *wettsteinii* Hämet-Ahti) coppice. Ray parenchyma cells are responsible for most of the storage function of xylem. The measured traits were: parenchyma ray height and number of cells, total parenchyma ray length, total area of rays – all measured on tangential sections, and total area of parenchyma rays, number of rays on cross sections. We also calculated volume index of ray parenchyma.

Our results indicate that increasing atmospheric humidity predicted for high latitudes will result in moderate changes in parenchyma properties. Area of parenchyma rays in tangential view was significantly ($P = 0.005$) larger in humidification treatment. Other characteristics showed statistically non-significant shifts, although all changes were in the same direction – towards the increase of parenchymatous tissue in response to increased air humidity. Larger proportion of living tissues brings along increase in maintenance respiration costs, which may be one of the causes of growth retardation.

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### IMPACT OF REED CANARY GRASS CULTIVATION AND MINERAL FERTILISER APPLICATION ON POTENTIAL OF SOIL MICROBIAL COMMUNITY METHANE PRODUCTION IN ABANDONED PEAT EXTRACTION AREA

**Mikk Espenberg**, Marika Truu, Jaak Truu, Järvi Järveoja, Martin Maddison, Hiie Nõlvak, Ülo Mander

Institute of Ecology and Earth Sciences, University of Tartu, mikk.espenberg@ut.ee

Peat is an important resource and is being extracted widely from regions rich in peatlands, which inevitably leads to the problem of abandoned peatlands. These areas emit greenhouse gases ($CH_4$, $N_2O$, $CO_2$) for decades if restoration action is not implemented. The potential of methane production of abandoned peatlands can be evaluated by the abundance of *mcrA* genes which encodes the alpha-subunit of the enzyme that catalyses the last step in $CH_4$ synthesis in all methanogens.

The purpose of this study is to assess the effect of energy crop (*Phalaris arundinacea* L.) cultivation and fertilisation on the abundance of soil bacterial and archaeal 16S rRNA genes, and to evaluate the methanogenic potential of the microbial communities in the soils of the experimental plots on the abandoned peat extraction area.

The experiment was carried out in 2012-2014 and samples were collected from 12 experimental plots at the vegetation period. Besides the widely used statistical methods, linear mixed effects modelling was used to test relationships between gene parameter values and chemical variables and in cases of different grouping factors.

Various physicochemical parameters changed the abundance of peat methanogenic community in vertical profiles. Methane emission rates correlated with the abundance of peat deeper layers methanogenic community in uncultivated plots while without *Phalaris* cultivation there are less constraining factors. These findings are important in the assessment of global warming potential while using abandoned peat extraction areas to produce biomass for energy.

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## LEAF ISOPRENE EMISSION FROM *DE NOVO* FIXED $CO_2$ OF DECARBOXYLATION PROCESS

**Sabrina Garcia**[1], Vinicius F. de Souza[1], José F.C. Gonçalves[1], Antônio O. Manzi[1], Niro Higuchi[1], Jeffrey Chambers[2], Kolby Jardine[2]

[1] National Institute for Research in the Amazonon (INPA), Ave. André Araújo, 2936, P.O. Box 478, CEP 69011-970, Manaus, AM, Brazil, sabrinagarcia.sg@gmail.com
[2] Climate Science Department, Earth Science Division, Lawrence Berkeley National Laboratory, One Cyclotron Rd, building 64-241, Berkeley, CA 94720, USA

Isoprene ($C_5H_8$) is a reactive hydrocarbon gas emitted at high rates by tropical vegetation, which affects atmospheric chemistry and climate. Under optimal conditions for photosynthesis, the majority of carbon used for isoprene biosynthesis is a direct product from recently assimilated atmospheric $CO_2$. However, the contribution of 'alternate' carbon sources, that increase with leaf temperature have been demonstrated and isoprene emissions from 'alternate' carbon sources under ambient $CO_2$ below the compensation point for photosynthesis have been observed. In this study, we investigated the released $CO_2$ from decarboxylation process as an 'alternative' carbon source for isoprene synthesis. At constant leaf temperature (30 °C) and $CO_2$ free atmospheres, leaves of the tropical species *Inga edulis* showed net emissions of $CO_2$ and light-dependent isoprene emissions which stagnated at low light levels (75 $\mu$mol $m^{-2}$ $s^{-1}$ PAR). Under constant light (1000 $\mu$mol $m^{-2}$ $s^{-1}$ PAR) and $CO_2$ free atmospheres, increase leaf temperatures from 25 - 40 °C resulted in net emissions of $CO_2$ and temperature-dependent isoprene emissions which reached values up to 26% of those under 450 ppm $CO_2$. Providing $^{13}CO_2$ by sodium bicarbonate ($NaH^{13}CO_3$), under $CO_2$ free atmospheres, isoprene labeled molecules could be observed. Our observations confirms a contribution of the *de novo* fixation of released $CO_2$ from decarboxylation process as an 'alternate' carbon source for isoprene under high light/temperature and drought stress (when the stomata close and the amount of internal $CO_2$ decreases); these processes can maintain photosynthesis rates resulting in the isoprene emission, independent of atmospheric $CO_2$.

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## MUTATIONAL ANALYSIS OF *Arabidopsis thaliana* RN-ase L INHIBITOR

**Jelena Gerassimenko**; Siim, Maiste; Erkki, Truve; Cecilia, Sarmiento

Tallinn University of Technology, Department of Gene Technology, jelena.gerassimenko@gmail.com

RNA silencing is a mechanism of sequence-specific RNA degradation conserved across eukaryotes. Through different pathways RNA silencing mediates developmental gene regulation and responses to biotic and abiotic stress (Melnyk *et al.*, 2011). Recently, it was demonstrated that RNase L inhibitor (RLI), a protein present in all eukaryotes, beside its role in translation, is a highly conserved RNA silencing suppressor (Kärblane *et al.*, 2015). RLI belongs to the class of ABC ATPases possessing two characteristic nucleotide binding domains (NBD) and a unique ferredoxin (FeS) domain (Braz *et al.*,

2004; Karcher *et al.*, 2008). The aim of the current study is to explore the role of *Arabidopsis thaliana* RLI2 (AtRLI2) domains in the suppression of RNA silencing. Using agroinfiltration assay, AtRLI2 mutants are transiently overexpressed together with the inducer of GFP silencing in GFP transgenic *Nicotiana benthamiana* leaves. The influence of mutations is analysed by *in vivo* imaging of GFP and Northern blot analysis of GFP siRNA accumulation. Our results show that FeS domain is important for AtRLI2 suppressor activity, since the mutant lacking this domain is a weaker suppressor, although its expression levels are higher. Interestingly, in a recent report, the substitution of a conserved proline amino acid in NBD1 of *Cardamine hirsuta* RLI orthologue was identified to be important for leaflets development (Kougioumoutzi *et al.*, 2013). Here we demonstrate that the same mutation in AtRLI2 affects its suppressor function, linking its role in RNA silencing pathway to plant development.

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## CO-EFFECT OF INCREASED HUMIDITY AND METEOROLOGICAL CONDITIONS ON GREENHOUSE GAS FLUXES IN A YOUNG HYBRID ASPEN FOREST

**Raili Hansen**[1], Ülo Mander[1], Priit Kupper[2], Kaido Soosaar[1], Martin Maddison[1], Jaak Sõber[2], Krista Lõhmus[2]

[1]Department of Geography, Institute of Ecology and Earth Sciences, University of Tartu, raili.hansen@ut.ee
[2]Department of Botany, Institute of Ecology and Earth Sciences, University of Tartu

Due to the global warming, climate events (heat waves and drought) will become more frequent, also higher precipitation and an increase in air humidity is expected in northern Europe in the coming decades. In 2006 a unique free-air humidity manipulation (FAHM) facility was established in Estonia to investigate the effect of artificially increased humidity on the performance of hybrid aspen (Populus tremula L. x P. tremuloides Michx.) trials. We measured soil $CO_2$, $CH_4$ and $N_2O$ emissions from the FAHM system using closed chamber and gas-chromatograph techniques from July 2009 to November 2012 during snow free periods. We monitored soil temperature, soil water potential (SWP) and precipitation. The vegetation period was rainy in 2009; droughty and exceptionally hot in 2010 and 2011 (according to SWP the drought was severe in 2011); moist and not very warm in 2012.
Soil respiration was the lowest in 2011 both in ambient and humidified plots; however $CO_2$ flux was significantly higher in humidified plots in 2011 and 2012. The soil acted as a sink for methane. Less $CH_4$ was oxidized in the soil of humidified plots. $N_2O$ emissions were higher in ambient plots in 2010, although one could observe a tendency of bigger $N_2O$ fluxes when soil was continuously water-saturated. Expectedly, $CO_2$ emission and $CH_4$ consumption demonstrated clear seasonal pattern, $CO_2$

fluxes had a strong positive correlations with soil temperature; and $CH_4$ fluxes with SWP. Hence, interaction of humidification and meteorological conditions affected greenhouse gas fluxes.

## TRITICUM MILITINAE INTROGRESSIONS INTO BREAD WHEAT AFFECT HOST RESPONSES TO POWDERY MILDEW CHALLENGE

**Bulat Islamov**, Hilma Peusha, Irena Jakobson & Kadri Järve

Department of Gene Technology, Tallinn University of Technology, Akadeemia tee 15, 12618 Tallinn, Estonia, bulat.islamov@ttu.ee

Obligate plant parasitic fungi like powdery mildew *Blumeria graminis* f. sp. *tritici* (Bgt) reduce crop yield quantity and quality. Plant disease can be managed by introduction of resistance into modern cultivars, which can be achieved either by introduction of race-specific resistance genes (R-genes) or non-race-specific quantitative trait loci (QTLs). In contrast to race-specific resistance that can be overcome by a mutation in avirulence gene of the pathogen, non-race-specific quantitative resistance is durable, although the mechanisms of quantitative resistance remain obscure. We used bread wheat lines carrying introgressions from *Triticum militinae* (2n=28) on 1A, 4A, 5A, 5B and 7A chromosomes and two powdery mildew isolates to identify microscopically the effects of resistance QTLs on Bgt development dynamics and host cell responses. Wheat line having a combination of all five QTLs exhibits the slowest powdery mildew progression, line with QTL on 4A (main QTL) chromosome outperforms the background cv.Tähti, a combination of four QTLs on 1A, 5A, 5B, 7A (minor QTLs) chromosomes is not different from background. Introgression of all five QTLs effectively decreases primary and secondary host cell penetration by the Bgt, main QTL negatively affects secondary penetration, minor QTLs decrease primary penetration and do not have significant effect on secondary penetration efficiency. No significant difference between penetration efficiency of two Bgt isolates was found. Inability of Bgt to establish compatible interaction with the host was accompanied by cell death and hydrogen peroxide production in wheat leaf epidermis. Data provided by this study contributes to understanding of quantitative disease resistance mechanism.

## IMPACT OF WATER TABLE LEVEL ON ANNUAL CARBON AND GREENHOUSE GAS BALANCES OF A RESTORED PEAT EXTRACTION AREA

**Järvi Järveoja**, Matthias Peichl, Martin Maddison, Kaido Soosaar, Kai Vellak, Edgar Karofeld, Alar Teemusk, Ülo Mander

University of Tartu, Department of Geography, jarvi.jarveoja@ut.ee

Peatland restoration may provide a potential after-use option to mitigate the negative climate impact of abandoned peat extraction areas; currently, however, knowledge about restoration effects on the annual balances of carbon (C) and greenhouse gas (GHG) exchanges is still limited. The aim of this study was to investigate the impact of contrasting water table levels (WTL) on the annual C and GHG balances of restoration treatments with high (Res-H) and low (Res-L) WTL relative to an unrestored bare peat (BP) site. Measurements of carbon dioxide ($CO_2$), methane ($CH_4$) and nitrous oxide ($N_2O$) fluxes were conducted over a full year using the closed chamber method and complemented by measurements of abiotic controls and vegetation cover. The main results suggest that three years following restoration

differences in the re-established WTL strongly affected vegetation development and plant-related $CO_2$ fluxes (i.e. production and respiration), however, effects on the net $CO_2$, $CH_4$ and $N_2O$ exchanges were small. In comparison to the two restored sites, greater net $CO_2$, similar $CH_4$ and greater $N_2O$ emissions occurred in BP. On the annual scale, Res-H, Res-L and BP were C sources of 111, 103 and 268 g C $m^{-2}$ $yr^{-1}$ and had positive GHG balances of 4.1, 3.8 and 10.2 t $CO_2$ eq $ha^{-1}$ $yr^{-1}$, respectively. Thus, the different WTLs had a limited impact on the C and GHG balances in the two restored treatments three years following restoration. However, the C and GHG balances in Res-H and Res-L were considerably lower than in BP owing to the large reduction in $CO_2$ emissions. This study therefore suggests that restoration may serve as an effective method to mitigate the negative climate impacts of abandoned peat extraction areas; however, longer time spans may be needed to return these sites into net C sinks.

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## COCKSFOOT MOTTLE VIRUS PX PROTEIN LOCALISES INTO THE PLASMA MEMBRANE

**Kristjan Kamsol**, Allan Olspert, Erkki Truve

Tallinn University of Technology, kristjan.kamsol@ttu.ee

Sobemoviruses are plant viruses with small icosahedral particles and a plus-oriented single-stranded single-component RNA genomes. The most 5’ proximal open reading frame (ORF) of the genome encodes protein P1. ORF 2a encodes the viral serine protease and VPg protein. Through a -1 programmed frameshift mechanism 2ab encodes the viral RNA dependent RNA polymerase. ORF3 encodes the viral coat protein (Sõmera et al. 2015).

A recent study revealed a hidden ORF in the genome of sobemoviruses named ORFx which encodes the Px protein (Ling et al. 2013). The necessity of Px was shown in the same study as mutant viruses that do not produce full-length Px were unable to establish viral infection (Ling et al. 2013).

In the current study we analysed the localisation of Cocksfoot mottle virus (CfMV) Px in onion epidermal cells. A plasmid was constructed where CfMV Px separated from EGFP by a 4 amino acid residue linker was expressed under the control of 35S promoter. Our preliminary results showed that Px-EGFP localises into the plasma membrane. These results were furthermore confirmed using plasmolysis of the onion cells which showed the movement of EGFP signal along with the contracting plasma membrane. In addition, Px-EGFP localisation was corroborated using a plasma membrane marker fused with mCherry. Px also localises to Hechtian strands during plasmolysis and seems to reduce the speed of this process in the cells where Px is expressed.

Sõmera, M.; Sarmiento, C.; & Truve, E. Overview on Sobemoviruses and a Proposal for the Creation of the Family Sobemoviridae. Viruses 2015, 7, 3076-3115
Ling, R.; Pate, A.E.; Carr, J.P.; Firth, A.E. An essential fifth coding ORF in the sobemoviruses. Virology 2013, 446, 397–408.

## ENVIRONMENTAL FACTORS PREVENTING PREDAWN WATER POTENTIAL EQUILIBRATION BETWEEN SOIL AND LEAVES IN HYBRID ASPEN

**Ott Kangur**, Arne Sellin

Institute of Ecology and Earth Sciences, University of Tartu, ott.kangur@ut.ee

The study was performed on coppice shoots and 9-year old saplings of hybrid aspen (*Populus tremula* × *P. tremuloides*) growing at the Free Air Humidity Manipulation (FAHM) site in 2013 and 2014. Main aims of our study were to determine the difference in water potentials between soil and leaves before dawn, and the major environmental factors responsible for this phenomenon. Additionally, the FAHM site enabled us to investigate the impact of increased relative air humidity (RH) – a climate trend predicted for Northern Europe. A relatively small predawn disequilibrium (PDD) was recorded both on coppice shoots and 9-year old trees: 0.042 and 0.066 MPa, respectively. In both years atmospheric conditions were the prevalent factors determining the degree of PDD. We found significant correlations between PDD and vapour pressure deficit (VPD) of ambient air ($R^2$=0.80 and 0.66 for respective years). The experiment revealed that 75% (2013) and 47% (2014) of the PDD are attributable to nocturnal transpiration. After cool and humid nights, when leaves were usually dew-wetted, the PDD was zero or close to it. We did not find significant difference in PDD values between humidified and control plots. Our findings suggest that stomata of hybrid aspen remain open at night, which enables nocturnal transpiration and water loss from foliage. The potential ecological significance of nocturnal water loss is still debatable, however we suggest that this phenomenon may provide hybrid aspen with enhanced nutrient uptake from the soil and hence support more rapid growth, which may give an advantage in competition with slow-growing species.

## RELATIONS BETWEEN CARBON FLUXES, VEGETATION AND SITE FACTORS IN DISTURBED BOGS

**Helen Karu**[1], Margus Pensa[2], Eva-Ingrid Rõõm[3], Angelika Portsmuth[2], Triin Triisberg[1]

[1]Institute of Ecology and Earth Sciences, University of Tartu, helen.karu@ut.ee
[2]Institute of Ecology, Tallinn University
[3]Centre for Limnology, Estonian University of Life Sciences

Bog ecosystems are sensitive to anthropogenic disturbance, including drainage and air pollution. Carbon (C) balance measurements to determine the effect of disturbance on bog functioning are laborious; therefore reliable proxies for C fluxes that could facilitate upscaling from single studies to a larger scale would be valuable.

The aim of our study was to investigate complex and still poorly understood relationships between peat C fluxes, environmental factors and vegetation dynamics in disturbed bog ecosystems. Study was conducted in forested bog margins that formed a human-impact gradient. Three of the study sites were situated in North-East Estonia (Kalina and Selisoo bogs) and affected by drainage, mining and alkaline air pollution; reference site was chosen from the Alam-Pedja Nature Reserve. Study sites and measurements of vegetation parameters and C gas fluxes are described in detail in Karu et al. (2014).

We expected that sites experiencing higher human impact (i.e., the vegetation was more distinct from that of a natural bog) would have higher peat $CO_2$ emissions ($R_s$) and lower $CH_4$ emissions, but differences in peat C emissions between the most disturbed (Kalina) and pristine site were not significant. $R_s$ and $CH_4$ emissions were primarily determined by water table depth that was strongly related also to tree stand productivity. Therefore, stand structural parameters, but not species composition, could serve as indicators of peat C emissions from poorly drained forested bogs, although underlying relationships require further investigation.

Karu, H., Pensa, M., Rõõm, E.-I et al. 2014. Carbon fluxes in forested bog margins along a human impact gradient. Wetl. Ecol. Manag. 22: 399–417

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## NITROGEN GAS EMISSIONS FROM TROPICAL PEATLANDS IN FRENCH GUIANA

**Kuno Kasak,** Teele Ligi, , Järvi Järveoja, Martin Maddison, Marika Truu Jaak Truu, Ülo Mander

Institute of Ecology and Earth Sciences, University of Tartu, kuno.kasak@ut.ee

This study is aimed to analyse nitrogen gas ($N_2$, $N_2O$) emissions from tropical peatlands in French Guiana (South America) and its response to the soil bacterial community and thereafter assess the use of denitrifying gene abundances for determining nitrous oxide emissions from soil. Measurements of greenhouse gas (GHG) emissions using static closed chambers (and He-O method for $N_2$), groundwater analysis, gas and peat sampling for laboratory analysis have been made. Two study sites were analysed in October 2013, one undisturbed peatland and one peatland with drainage influence, both in northern part of French Guiana. In both study sites 3 transects along the groundwater depth gradient and 3 rows of sampling sites were established. In each transect GHG emissions were measured during 3 days in 6 sessions. Emissions of $N_2$-N and $N_2O$-N were significantly ($p<0.05$) different between undisturbed and disturbed peatlands (mean values 1477.3 and 637.2 $\mu g\ m^{-2}\ h^{-1}$ for $N_2$-N; -0.3 and 9.9 $\mu g\ m^{-2}\ h^{-1}$ for $N_2O$, respectively). Molecular techniques were used to evaluate the abundances of nitrogen fixing and denitrifying bacteria. Microbiological analysis showed that $N_2O$ reduction potential was higher in natural area which was also estimated due to the low mineral N in peat and high groundwater level. In addition, microbiological analysis showed remarkably higher amount of denitrifies in drained area which was also estimated due to the higher $N_2O$ and lower $N_2$ emission. These results indicate that denitrification is a main process of N transformation in these tropical peatlands.

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## DISTURBANCES THAT AFFECT *Brassica nigra* L. VOLATILE EMISSIONS

**Kaia Kask**, Astrid Kännaste, Ülo Niinemets

Institute of Agricultural and Environmental Sciences, University of Life Sciences, kaiak@emu.ee

Plants in nature are affected several disturbances, what are affecting volatile organic compounds emission and are generally called as abiotic and biotic stress factors. Black mustard (*Brassica nigra* L.) belongs together with *Brassica napus*, *Brassica rapa* var. *oleifera* to the family of Brassicaceae and contain secondary metabolites that have a major role in nature and are supposed to be beneficial to humans.

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## SPATIAL VARIABILITY OF SOIL PROPERTIES IN HEMIBOREAL FOREST (SMEAR ESTONIA)

**Dmitrii Krasnov**, Steffen M. Noe, Alisa Krasnova, Ülo Niinemets

Estonian University of Life Sciences, dmitrii.krasnov@emu.ee

Soil is an essential component of terrestrial ecosystems, impacting on ecosystem processes in multiple ways. It is a non-homogeneous body with specific patterns in both spatial and temporal variability. While temporal variation mostly depends on climatic conditions, spatial variability is a result of complex inter-related factors and therefore a challenge for investigation.

Soil respiration is an indispensable part of the ecosystem carbon cycle and can be considered as an indicator of soil activity. Higher rates of soil CO2 flux reflect quite often more intensive processes taking place inside the soil. Soil respiration is a combination of related processes: root respiration, organic matter decomposition. The main factors affecting autotrophic respiration are the vegetation period, plant photosynthetic activity, nutrient availability and root mycorrhiza. Heterotrophic respiration is mainly influenced by soil moisture, temperature and available substrate. A non-homogeneous distribution of these factors leads to different spatial patterns of soil CO2 flux. To avoid over- or under-estimation of carbon balance when upscaling spot measured data to the whole ecosystem level such unequal spatial distributions of parameters and processes need to be taken into account.

In this research, we studied the forest microtopography and spatial distribution of several soil properties (soil respiration, moisture and temperature, thickness of organic horizons) in a mixed hemiboreal forest stand at SMEAR Estonia.

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## THE THREE TOWERS: A STUDY OF $CO_2$ FLUX IN HEMIBOREAL FORESTS

**Alisa Krasnova**[1], Kaido Soosaar[2], Steffen M. Noe[1], Kalev Repp[2], Dmitrii Krasnov[1], Ülo Mander[2], Ülo Niinemets[1]

[1]Estonian University of Life Sciences, alisa.krasnova@emu.ee
[2]University of Tartu

Forest ecosystems play essential role in global carbon turnover, being the main terrestrial $CO_2$ sinks. Although forest carbon cycle has been researched in details, transitional forest zones are still understudied. Hemiboreal forests have complex structure, often featuring a co-domination of tree species and several vegetation levels. These features reflect in the properties of $CO_2$ exchange under different conditions.

In this study, we analyzed eddy-covariance $CO_2$ flux data from 3 towers located in different types of hemiboreal forest in Estonia. Soontaga research station is located in pine-dominated forest with spruce trees in a second vegetation layer. Liispõllu site is a spruce/birch mixed forest with single trees of alder, common aspen, and lime. SMEAR Estonia tower has 2 EC systems: 30m height system footprint is a pine/spruce mixed forest with clearcut areas; 70m height system footprint includes additionally a birch-dominated forest site.

For all 4 systems, we compared mean diurnal cycles of $CO_2$ fluxes as well as their change throughout the middle of growing season. To compare the influence of main environmental factors (light and temperature) we used parameters obtained from Michaelis-Menten equation for day-time data and Lloyd and Taylor's and empirical logistic models for night-time ecosystem respiration.

This case study of one summer gave an overview of the possible differences between the sites carbon balance and possible directions of further research.

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## FOREST BELOWGROUND CARBON CYCLE – LINKAGES BETWEEN SOIL RESPIRATION, FINE ROOT AND LITTER PRODUCTION AND DECOMPOSITION RATES IN VARYING STAND FERTILITY AND MOISTURE CONDITIONS IN ESTONIA

**Kaie Kriiska**, Jane Frey, Ülle Napa, Naima Kabral, Ivika Ostonen

Institute of Ecology and Earth Sciences, University of Tartu, kaie.kriiska@ut.ee

Belowground processes play an important role in the global carbon cycle through the regulation of soil carbon. Present-day, boreal and temperate forests act as sinks for $CO_2$, however it is uncertain whether forests will persist as C sinks or become sources due to climate change. Climate change, accompanied by increasing temperature and precipitation in the Boreal region is likely to alter soil fertility, accelerate decomposition of soil organic matter and increase soil-borne $CO_2$ emissions.

The aim of current study was to analyze the main processes and estimate carbon fluxes related to soil carbon budget: above- and belowground litter input, litter decomposition and soil respiration in four spruce (*Polytrichum, Myrtillus, Oxalis* and *Calamagrostis* alvar) and four pine (*Cladonia, Vaccinium, Myrtillus, Fragaria*) stands with varying soil moisture and fertility conditions. Spatial gradient study provides information for predicting climate change impact on forest soil carbon cycle. Selected stands are *ICP Forests* and *ICP Integrated Monitoring* programme monitoring plots.

Soil respiration varied during the vegetation period from 0.237 to 1.185 t C $ha^{-1}mo^{-1}$, and was more intensive in stands with high soil fertility. Highest soil respiration variability occurred in the *Vaccinium* pine stand – from 0.269 to 1.185 t C $ha^{-1}mo^{-1}$. The aboveground/belowground litter ratio, which reflects soil carbon accumulation, was twice as high in spruce stands compared to pine stands. No significant difference was found between the decomposition rates of fine roots and litter in spruce and pine stands, however annual needle decomposition was twice as fast as fine root decomposition.

Present study indicates that the intesity of soil respiration increases with the increase of soil moisture and fertility, whereas the fine root litter production, thus C accumulation increases at less fertile sites.

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## THE EFFECTS OF INCREASED AIR HUMIDITY ON BUD DEVELOPMENT AND OUTGROWTH POTENTIAL

**Maarja Kukk**, Anu Sõber

Institute of Ecology and Earth Sciences, University of Tartu, maarja.kukk@ut.ee

The initiation, development and subsequent fate of buds shape the architecture of tree crowns. As architecture is a key component of plant growth modelling, insights into the control of bud development are valuable in predicting the growth of trees and forests in the future. Climate change

scenarios project not only a warmer but also a moister future climate at high latitudes. Recently, changes in air humidity were highlighted as a potential driver of bud break in spring, but the implications of increased air humidity on bud development and outgrowth potential remain little explored. The current data set was collected from an experimental forest ecosystem subjected to free-air humidity manipulation during summer months; measurements were carried out on young silver birch (*Betula pendula* Roth) and hybrid aspen trees (*Populus tremula* L. × *P. tremuloides* Michx.). Increased air humidity reduced bud size, by restricting the growth rather than the organogenesis of the incipient shoot within a bud, and increased the frequency of bud break. However, the responses were species-specific. As parent shoot growth as well as tree height increments offered little explanation for the reduction of bud length in silver birch, further study is needed to elucidate the underlying mechanisms. Although the effect of stimulated bud break was subtle in a given year, cumulative effects may modulate crown structure in the long term, facilitating the acclimation of tree growth to rising humidity in the future.

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## FACTORS AFFECTING SOIL RESPIRATION IN SILVER BIRCH AND NORWAY SPRUCE STANDS

**Mai Kukumägi**

Institute of Ecology and Earth Sciences, University of Tartu, mai.kukumagi@ut.ee

Soil respiration is affected by various abiotic and biotic factors. Components of soil respiration, autotrophic and heterotrophic respiration, can respond differently to climatic conditions. Thus, it is important to understand the mechanisms that determine changes in soil respiration. The objectives were: to examine the seasonal dynamics of soil respiration and its components in relation to soil temperature and soil moisture; to estimate temperature sensitivity and analyse age-related changes in soil respiration in three silver birch and three Norway spruce stands. Soil $CO_2$ effluxes were measured monthly during growing seasons using a closed dynamic chamber method. Trenching method was applied to distinguish heterotrophic respiration from total soil respiration. Autotrophic respiration was calculated as the difference between total soil respiration and heterotrophic respiration.

Significant seasonal variation of soil $CO_2$ effluxes was observed in all studied stands. Soil temperature was the main climatic factor explaining the temporal variation of total soil respiration as well as of heterotrophic respiration. In general, the descriptive force of soil temperature was weak or missing for autotrophic respiration. Soil moisture had a weak positive effect on soil respiration. The temperature sensitivity of soil respiration components was dissimilar showing opposite responses for birch and spruce. Non-linear relationship was found between respiration fluxes and stand age (Varik et al. 2015), which could be explained by changes in biotic factors rather than by changes in soil temperature and moisture. The results highlight the importance of partitioning soil respiration, estimating temperature sensitivity for both components and considering forest age in carbon budgeting studies.

Varik, M., Kukumägi, M., Aosaar, J. et al., 2015. Carbon budgets in fertile silver birch (*Betula pendula* Roth) chronosequence stands. Ecological Engineering 77, 284–296.

# DOES ELEVATED AIR HUMIDITY AFFECT TREE’S NITROGEN UPTAKE IN A FREE AIR HUMIDITY MANIPULATION (FAHM) EXPERIMENT?

**Priit Kupper**[1], Arvo Tullus[1], Ivika Ostonen[1], Katrin Rosenvald[1], Meeli Alber[1], Kaarin Parts[1], Kristina Lubenets[1], Gristin Rohula[1], Jaak Sõber[1], Mai Kukumägi[1], Reimo Lutter[2], Aigar Niglas[1], Raili Hansen[1], Maarja Kukk[1], Ingmar Tulva[1], Pille Mänd[1], Veiko Uri[2], Anu Sõber[1], Arne Sellin[1], Krista Lõhmus[1]

[1]University of Tartu, Priit.Kupper@ut.ee
[2]Estonian University of Life Sciences

Our earlier findings from the FAHM experiment suggest that rise in air relative humidity (RH) alters the ability to take up nitrogen (Tullus et al. 2012; Sellin et al. 2013). However, the mechanism of the observed changes in nitrogen (N) acquisition is unclear. We set up the hypothesis that reduced transpiration rate under increased RH may cause concomitant decrease in mass flow of N in soil. To investigate the possible impact of elevated RH on interactions between plant water supply and nutritional status the data of hybrid aspen and silver birch were analysed. Our results show that tree water uptake (as the estimate of mass flow) does not explain the high variability in N uptake between different years and tree species. However, the humidification treatment led to a shift in the ectomycorrhizal colonizers towards the dominance of hydrophilic taxa (Parts et al. 2013) and drove changes in short root morphological traits. Nevertheless, there was also shift in the uptake of different N forms: in control 65% of N was taken up in the form of nitrate, while in humidification plots only 50%. In addition, the trees from humidification treatment had greater stem-wood N concentration than the control trees (Tullus et al. 2014) and they allocated relatively more biomass into the fine roots (Rosenvald et al. 2014) and woody vascular tissues than into photosynthesising tissues. Our findings confirm that elevated RH and its concomitant factors affect tree’s nitrogen uptake whereas plant adaptations to environmental change will likely modify the plant responses.

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Tullus, A., Kupper, P., Sellin, A., *et al*. 2012. Climate Change at Northern Latitudes: Rising Atmospheric Humidity Decreases Transpiration, N-uptake and Growth Rate of Hybrid Aspen. PLoS ONE, 7(8), e42648.

Tullus, A., Sellin, A., Kupper, P., *et al*. 2014. Increasing air humidity – a climate trend predicted for northern latitudes – alters the chemical composition of stemwood in silver birch and hybrid aspen. Silva Fennica, 48 (4), article id 1107.

## ANATOMICAL CHANGES IN PLANT LEAVES IN DIFFERENT ENVIRONMENTAL CONDITIONS AND THROUGH ONTOGENY.

**Vivian Kuusk**

Estonian University of Life Sciences, vivian@emu.ee

Plant leaves adapt to the environment in many different ways to overcome unfavourable conditions and to be more fit for the growth conditions.
Model species were *Populus* sp. and *Pinus* sp. Growth conditions were modified for CO2 concentration and tempereature.  The influence of herbivore attack and age differences were also analyzed

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## SLOW STOMATA: FERN STOMATAL REACTIONS IN RESPONSE TO ENVIRONMENTAL CHANGE

**Liisa, Kübarsepp**. Tiina, Tosens. Ülo, Niinemets

Estonian Univesity of Life Sciences, liisa.kybarsepp@gmail.com

The pteridophyte stomata are considered less sensitive to changes in the environmental conditions due to less or no sensitivity to absicic acid. In previous studies passive regulation of stomata in response to low air humidity and no significant reaction to above-ambient CO2 concentrations has been suggested.
This study addresses two main questions considering stomatal reaction kinetics in ferns. Firstly which is the reaction speed of stomatal conductance of different species? Secondly is the reaction speed dependent on the stress applied? The response to light, CO2 concentration and decreased air humidity were tested upon different fern species from various habitat habitat types and geographical distribution. The reaction speed of stomatal conductance in response to changed environmental conditions was detected using gas exchange measurement equipment.
Besides the chemical signals regulating stomatal kinetics there is a vast amount of studies suggesting that stomatal morphology also has an important impact on the reaction speed of stomata. In many cases a negative correlation between stomatal size and density has been observed. Often smaller stomata appear to be more agile, enabling faster closing in response to stress conditions and rapid opening in favorable conditions, therefore minimizing the time plants have to endure disadvantageous conditions. In order to see if the fern species under focus also exhibit a correlation between stomatal kinetics and morphology, stomatal size and density was also measured. Enabling not only to say whether size and density in these species are correlated but also if morphological parameters are linked to reaction speed of stomata.

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## $Ca^{2+}$-ROS AMPLIFICATION LOOP

**Kristiina Laanemets**

Institute of Technology, University of Tartu, kristiina.laanemets@ut.ee

Plants have stomatal pores which regulate the balance between water loss and $CO_2$ uptake. Stomata are surrounded by two guard cells which can rapidly change size and shape to give plants the advantage of reacting to changes in the surrounding environmental conditions. Stomatal opening and closure have been extensively studied and several signaling molecules of those reactions have been identified. It has been found that cytosolic calcium oscillations and reactive oxygen species (ROS), especially $H_2O_2$, are involved in stomatal closure reaction but there are still many unanswered questions about the sequence of events concerning guard cell cytosolic calcium concentration oscillations, increase in guard cell ROS content and stomatal closure. Two separate pathways have been described for $H_2O_2$ dependent $Ca^{2+}$ amplification and two for $Ca^{2+}$ dependent $H_2O_2$ amplification. This makes up the $Ca^{2+}$-ROS amplification loop, which is necessary because both of these signals disappear easily. Our objective was to confirm $Ca^{2+}$-ROS amplification loop in vivo and test if it is necessary for stomatal closure reaction. We tested stomatal closure reactions of mutants where ROS production is affected. We also measured guard cell ROS production induced by extracellular $Ca^{2+}$ treatment.

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## BACTERIAL COMMUNITY STRUCTURE AND GENETIC POTENTIAL OF NITROGEN REMOVAL PROCESSES IN THE SOILS AND SEDIMENTS OF A CREATED RIVERINE WETLAND COMPLEX TREATING POLLUTED RIVER WATER

**Teele Ligi**, Marika Truu, Kristjan Oopkaup, Jaak Truu

Institute of Ecology and Earth Sciences, University of Tartu**,** Teele.Ligi@gmail.com

In order to protect aquatic ecosystems from excessive nitrogen, implementation of treatment wetlands (TWs) is proposed.

Microbial communities have a key role in the nitrogen cycle and hereby it is crucial to understand the relationships between microbial communities and environmental parameters in TWs. Favourable conditions for processes with maximum nitrogen removal and minimum $N_2O$ emission should be created in TWs.

In this study, the bacterial community structure and its nitrogen removal potential were characterised in the soils and sediments of a polluted river water treating wetland complex in relation to site-specific characteristics (soil chemical parameters, water regime, and soil type).

Water regime was an important factor determining the structure of the bacterial communities in the studied TW soils - the communities in the occasionally flooded areas were more diverse and complex compared to those of the permanently flooded areas.

Genetic potential for denitrification was detected in all the studied soils and it was dependent on the site's hydrological conditions. Nitrite reduction potential was higher in the permanently flooded zones, while potential for $N_2O$ emission was greater in the bacterial communities of occasionally flooded areas.

Genetic potential for ANAMMOX and n-damo processes was detected in the soils of the TWs and the most suitable conditions for those bacteria were in the permanently flooded areas.

The results of this study indicate that the bacterial communities of TW soils have the genetic potential for several nitrogen removal processes; still, denitrification is the main process performing this function and a creation of the occasionally flooded areas would decrease the $N_2O$ emission from the TWs soils.

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## METABOLITE RESPONSES OF SILVER BIRCH TO ELEVATED AIR HUMIDITY

**Jenna Lihavainen**[1], Viivi Ahonen[1], Markku Keinänen[1], Sarita Keski-Saari[1], Sari Kontunen-Soppela[1], Anu Sõber[2], Elina Oksanen[1]

[1]University of Eastern Finland, Department of Biology, PO Box 111, 80101 Joensuu, Finland, jenna.lihavainen@uef.fi
[2]University of Tartu, Institute of Ecology and Earth Sciences, Lai 40, 51005 Tartu, Estonia

Climate change scenarios predict that relative humidity (RH) may increase in northern Europe in association with increasing precipitation, cloud cover, atmospheric water vapor and more frequent wet days. RH affects plant growth and nutritional status; under high RH, plants can assimilate carbon with a relatively small cost of water, but at the same time mineral nutrient uptake is impaired due to decreased transpiration rate.

We studied the effects of elevated RH on the leaf metabolites of silver birch (Betula pendula Roth.) in a controlled growth chamber experiment and in a long-term Free Air Humidity Manipulation (FAHM) field experiment. In the chamber experiment, RH was elevated from 60% to 95%, and in the FAHM study RH was elevated by 7-8% over the ambient. Leaves were sampled after 26 days (chamber) and during the 4th growing season (field).

Leaf starch content increased under elevated RH in both experiments. Untargeted metabolic profiling with GC-MS revealed that glutamate and aspartate increased, whereas shikimic acid, ribonic acid, glucose, sedoheptulose, glutamine, alanine and valine decreased under elevated RH in both experiments. In addition, elevated RH induced the production of secondary metabolites such as coumaroyl quinic acids and flavonol glycosides in birch leaves in chamber and field experiments.

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## CIRCADIAN PATTERNS OF HYDRAULIC AND XYLEM SAP PROPERTIES: IN SITU STUDY ON HYBRID ASPEN

**Annika Meitern (Karusion),** Eele Õunapuu-Pikas and Arne Sellin

Institute of Ecology and Earth Sciences, University of Tartu, annika.meitern@ut.ee

Physico-chemical properties of xylem sap and tree hydraulic traits are being studied more extensively year by year. Nevertheless, to date complex circadian characterization of these characteristics measured in situ is still lacking. The aim of this study was to characterize circadian rhythms of tree hydraulic traits and physico-chemical properties of xylem sap in hybrid aspen (*Populus tremula* L. × *P. tremuloides* Michx.). In addition the study aimed to clarify which environmental drivers govern the daily dynamics of these parameters.

All studied hydraulic characteristics including whole-branch hydraulic conductance ($K_{wb}$), bare-branch hydraulic conductance ($K_B$), leaf blade hydraulic conductance ($K_{lb}$), petiole hydraulic conductance ($K_P$) and leaf blade relative resistance ($R_{lb}$) as well as all xylem sap properties like $K^+$ concentration ($[K^+]$), electrical conductivity ($\sigma_{sap}$), osmolality (Osm) and pH showed clear daily dynamics. Environmental factors like photosynthetic photon flux density (PPFD), atmospheric water vapor pressure deficit (VPD), air temperature ($T_A$) and relative humidity (RH) had significant impact on both hydraulic and xylem sap properties. Xylem sap pH was negatively correlated with soil water potential ($\Psi_S$), which also affected other measured characteristics. Most of the hydraulic traits and xylem sap physico-

chemical properties depended on tree size and branch height. We detected strong correlations between $[K^+]$ and $K_{wb}$, $[K^+]$ and $K_{lb}$, $\sigma_{sap}$ and $K_{wb}$, $\sigma_{sap}$ and $K_{lb}$. However, correlation between $[K^+]$ and $K_B$ was not seen, as $K_B$ was depressed at midday. Other hydraulic properties did not show midday depression.
The present study demonstrates an unequivocal coupling between circadian patterns of hydraulic conductance and xylem sap physico-chemical properties. The primary environmental factor responsible for those circadian patterns appears to be photosynthetic photon flux density (PPFD).

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## ABA SIGNALING AND TRANSPORT IN GUARD CELLS TO INDUCE STOMATAL CLOSURE: PERSPECTIVES IN RELATION TO PLANT GROWTH CONDITIONS AND SPECIES

**Ebe Merilo**[1], Pirko Jalakas[1], Priit Kupper[2], Mikael Brosche[1,3], Pedro Rodriguez[4] & Hannes Kollist[1]

[1]Institute of Technology, University of Tartu, ebe.merilo@ut.ee
[2]Institute of Ecology and Earth Sciences, University of Tartu
[3]Division of Plant Biology, Department of Biosciences, University of Helsinki
[4]Instituto de Biología Molecular y Celular de Plantas, Consejo Superior de Investigaciones Cientificas-Universidad Politecnica de Valencia

Abscisic acid (ABA) is the most important phytohormone to counteract drought-induced yield losses - it serves to prevent water loss from leaves through stomatal closure and promotes starch accumulation during grain filling. Most of ABA's cellular responses are elicited by a direct binding to cytosolic PYR/RCAR receptors that initiates inhibition of clade A type 2C protein phosphatases and stomatal closure. These receptors and their ABA signaling mechanism is highly conserved in land plants, crops included. Nine of the 14 total Arabidopsis PYR/RCARs are expressed in guard cells (PYR1 and PYLs1-9 except PYL3) and are thus potentially involved in stomatal regulation (Yang et al. 2008).
We have developed a new method for foliar application of ABA to intact plants simultaneously with measurements of stomatal conductance using custom-made flow-through gas exchange devices (Merilo et al., 2015). The assay works in low micromolar range, and the response is absent in the *ost1* mutant, showing ABA specificity.
Here, we present data on ABA-induced stomatal responses of: 1) different PYR/RCAR receptor mutants in order to clarify how individual receptors differ in specificity at guard cell level; 2) ABA transporter mutants in order to enlighten the role of active ABA transport in stomatal regulation; 3) other species besides Arabidopsis, crops and trees included, to identify the potential differences in ABA-responsiveness among species. We will also present results and discuss on the environmental fine-tuning of stomatal ABA-responsiveness.

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## SEED GERNIMATION CHARACTERISTICS TO COLD STRATIFICATION PERIOD IN *Fagus orientalis* Lipsky

**Azimeh Motallebi**[1], Masoud Tabari[2]

[1]Institute of Forestry and Rural Engineering, Estonian University of Life Sciences,
Azimeh.motallebi@emu.ee
[2]Institute of Forestry, Tarbiat Modares University, Iran.

Oriental beech (*Fagus orientalis* Lipsky.) forest is situated in a belt which lies between 700 to 2000 m a.s.l. in Caspian region of northern Iran. Improved understanding of the influence of cold stratification period on seed germination is necessary for sustainable forest management. The main aim of the present study was to determine the most suitable cold stratification period of oriental beech collected from different elevations (600, 1200 and 1800 m). Initial seed viability was checked using Tetrazolium test. Then, four replication of 50 seeds each, from three elevations were stored in dark and humid conditions at 4 °C in refrigerator with three different length of stratification period including one, two and three months. As a control, 200 seeds from each elevation were planted without stratification. In order to find out if seeds germinate or not, stratified seeds were checked once a week for whole the stratification period and in case of germination, seeds were planted in pots. Totally, 2400 seeds were planted and at the end of growing season i.e. August, germination characteristic, survival and height of seedlings were measured. The results of this study indicated the significant effects of cold stratification period and elevations on germination characteristics.

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## THE EFFECT OF ELEVATED AIR HUMIDITY AND SOIL MOISTURE ON NITROGEN ALLOCATION OF ASPENS

**Pille Mänd**[1], Anu Sõber[1], Marge Sepp[1], Priit Kupper[1], Arvo Tullus[1], Krista Lõhmus[1], Arne Sellin[1]

Institute of Ecology and Earth Sciences, University of Tartu, pille.mand@ut.ee

In northern regions an increase in atmospheric humidity is predicted (IPCC 2007). As relative air humidity (RH) most likely is accompanied with changes in soil moisture (SM), the combined effect of RH and SM on leaf nitrogen, carbon and chlorophyll content on hybrid aspens was studied in a Free Air Humidity Manipulation (FAHM) experiment (Kupper et al. 2011). During July and August 2014, the SM changed from near waterlogged at midsummer to 20% at August. The RH was approximately 7 % higher than ambient at manipulated plots.

We found significant changes in leaf nitrogen partitioning over time and over SM contents, showing significant co-effect between RH and SM.  Although, N content in aspen leaves was generally lower at humid air plots, no significant link between SM and leaf N content, neither whole tree foliage nitrogen, was found. Instead, Chl/N ratio appeared to be related with differences in SM over longer period of time, being lower when SM was high. Although, during first two weeks of high SM (and quite high RH at control plots) no effect of humidification was found, but later higher values of Chl/N were evident at plots of higher RH. However, the difference in Chl/N between treatments was much less than differences in Chl/N between time periods and SM. Differences in leaf carbon:nitrogen ratio (C/N) give insight into various sources of limitation in plants. Average C/N ratio decreased over time and over

decreasing SM in humidified plots, but increased over time at control plots indicating interacting effects between RH and SM on C/N.

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## CD, CU, PB, and ZN CONCENTRATION AND ACCUMULATION IN SOIL ORGANICS AND RETENTION IN FINE ROOTS OF CONIFEROUS STANDS.

**Ülle Napa**, Naima Kabral, Kaie Kriiska, Ivika Ostonen, Jane Frey.

Institution: University of Tartu, ulle.napa@ut.ee

ICP Integrated Monitoring and ICP Forests programmes enable the quantification of fluxes and pools of different element cycles, including heavy metals. We present the input and uptake balances of four heavy metals (HM) of 6 coniferous stands. Samples of different media and deposition were collected during 2009-2013. Highest concentrations for Cd and Pb were in soil organics, highest concentrations of bio elements (Cu and Zn) were measured in the second year´s fine roots. In fine roots the average concentrations were 5 times higher for Cd and Pb; 50 % for Cu and 30 % for Zn than in litterfall. The greatest HM concentration difference between living needles and litterfall occurred in regard to Pb indicating a strong tendency to accumulate with needle age. The largest stocks of HMs were embedded in organic layer of soil, where regional differences in accumulation occurred for Pb, Cu and Zn.

HMs like Cd and Pb are strongly bond to organic material – accumulating in organics of soil and retaining in fine roots and litterfall. Decomposition rate of root litter is lower in comparison to needle litter, therefore HMs in older coniferous forests are released more easily and taken back to cycling from litterfall than from fine roots – therefore the changes in the forest carbon cycle affect directly the heavy metal fluxes and pools.

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## STOMATAL ACCLIMATION TO HIGHER AIR HUMIDITY

**Aigar Niglas**[1], Meeli Alber[1], Eele Õunapuu-Pikas[1], Priit Kupper[1], Anna K. Jasińska[2], Arne Sellin[1].

[1]Institute of Ecology and Earth Sciences, University of Tartu, aigar.niglas@ut.ee
[2]Institute of Dendrology, Polish Academy of Sciences

Climate changes during the 21st century lead to higher air humidity at higher latitudes. Laboratory experiments have shown that stomata of trees growing under increased air relative humidity (RH) are acclimated to growing conditions – high RH-grown plants have lower stomatal density, bigger stomata. Besides effects on stomatal morphology, stomatal responses change as well – stomata of high RH-grown plants are less sensitive to leaf dehydration, increasing ABA concentration and decreasing light intensity. Our objective was to find out how acclimation to higher RH affects stomatal morphology and sensitivity in light-demanding deciduous tree species growing in field conditions.

Experiments were conducted in Free Air Humidity Manipulation (FAHM) experimental area, eastern Estonia, with silver birch and hybrid aspen saplings growing under ambient and elevated RH. Trees' sap flow measurements indicated that canopy conductance of plants grown under elevated RH responded more steeply to changes in soil water potential. Stomata of humidity-treated trees were also more sensitive to fast decreases in branch water potential. Results from the experiment by applying rapidly decreasing RH showed that stomata of trees grown in high RH conditions closed much faster when air humidity decreased. Our experiments suggest that stomata of plants growing in natural conditions (i.e. in diurnally and seasonally variable environment) under increased RH, are acclimated being more sensitive to fluctuations in water availability and air relative humidity, while the changed stomatal sensitivity is not associated with changes in stomatal morphology but in stomatal physiology.

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## CONSTITUTIVELY ACTIVE GUARD CELL PLASMA MEMBRANE $H^+$-ATPASE IMPAIRS OZONE-, ELEVATED $CO_2$- AND DARKNESS-INDUCED STOMATAL CLOSURE

**Maris Nuhkat[1],** M. Rob G. Roelfsema[2], Hannes Kollist[1]

[1]Institute of Technology, University of Tartu, marisnuhkat@gmail.com
[2]Molecular Plant Physiology and Biophysics, Julius-von-Sachs Institute for Biosciences, Biocenter, University of Würzburg

Ozone acts as an air pollutant in the lower levels of the atmosphere, and has negative effects on crop yield and overall carbon fixation by vegetation. Ozone degrades in the apoplastic space rapidly into reactive oxygen species (ROS), and has been therefore used as a tool to study ROS-induced processes. Application of ozone triggers a rapid transient decrease in stomatal conductance (RTD) which coincides with the burst of ROS in guard cells. RTD is abolished in plants that have impaired guard cell S-type anion channel activity.

In order to study what is the role of guard cell plasma membrane $H^+$-ATPase deactivation for ozone-induced RTD, we used dominant *ost2* mutants that have constitutive $H^+$-ATPase activity (Merlot et al. 2007). This revealed that both *ost2-1D* (L*er* bg) and *ost2-2D* (Col-0 bg) did not have RTD in response to ozone. The mutants also did not respond to elevation of $CO_2$ concentration and application of darkness. It was found that $Ca^{2+}$- and hyperpolarization-induced activation of S-type anion channel activity was intact in *ost2-2D* mutants. These results suggest that ozone/ROS-, $CO_2$- and darkness-induced stomatal closure requires deactivation of $H^+$-ATPase.

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## P1 INDEPENDENT REPLICATION OF RICE YELLOW MOTTLE VIRUS IN SEVERAL PLANT SPECIES

**Grete Nummert** and Erkki Truve

Department of Gene Technology, Tallinn University of Technology, grete.nummert@ttu.ee

The genus Sobemovirus belongs to the group of positive sense single stranded RNA genome plant viruses. The genome of sobemoviruses is common among all species and consists of five open reading frames (ORFs). ORF1 encodes P1 protein which has been characterized as a suppressor of posttranscriptional gene silencing (Sarmiento *et al.* 2007). Systemic virus spread and infection requires P1 protein, but it is not necessary for viral replication (Bonneau *et al.* 1998; Meier *et al.* 2006). In addition, P1 protein is the less conserved protein among sobemoviruses. The aim of this study was to experimentally test the hypothesis that P1 is indeed the host-range determinant for sobemoviruses. To test this hypothesis wild type and recombinant viruses of *Cocksfoot mottle virus* (CfMV) and *Rice yellow mottle virus* (RYMV) were used to infect oat, rice, wheat, barley, *Arabidopsis thaliana* and *Nicotiana benthamiana* plants. Viral RNA was synthesized *in vitro* and plants were biolistically inoculated. After total RNA extraction the viral RNA was detected using reverse transcription polymerase chain reaction. Wild type RYMV and two recombinants, RYMV without P1 and RYMV with CfMV P1, were detected in inoculated leaves of all tested plant species. These results show that P1 gene exchange between RYMV and CfMV does not change the host-range. The experiments unraveled new data on the requirement of P1 for RYMV replication in various plant species. It is important to note that all tested plant species, except rice, are not known for being natural nor experimental hosts of RYMV.

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## MYOSINS XI-K, XI-1, AND XI-2 ARE REQUIRED FOR DEVELOPMENT OF PAVEMENT CELLS, TRICHOMES, AND STIGMATIC PAPILLAE IN ARABIDOPSIS

**Eve-Ly Ojangu**, Krista Tanner, Pille Pata, Kristel Järve, Kristiina Talts, Erkki Truve, Heiti Paves

Department of Gene Technology, Tallinn University of Technology, eve-ly.ojangu@ttu.ee

The positioning and dynamics of vesicles and organelles is mediated by the acto-myosin system. In *Arabidopsis* there are 13 class XI myosins which mediate intracellular transport in different cell types. Simultaneous depletion of *Arabidopsis* class XI myosins XI-K, XI-1, and XI-2 in *triple* mutant plants affects the cell expansion during plant development: growth of root hairs, trichomes, leaf pavement cells and the elongation of stigmatic papillae (Peremyslov et al., 2010). Reduced cell size leads to size reduction of shoot organs, affecting fertility. The reduced fertility of *triple* mutant plants is caused by insufficient development of pistils (Ojangu et al., 2012).

The actin array is less dynamic in leaf midvein epidermal cells and in root hairs of *triple* mutant (Peremyslov *et al*. 20101, Ueda *et al*. 2010, Cai *et al*. 2014). Also leaf trichomes show randomly oriented and more bundled actin filament arrays in the *triple* mutant. Changes in actin organization and dynamics in class XI myosin *triple* mutant indicate that myosins motors influence the actin tracks they use for transport. Proper dynamics of actin cytoskeleton is necessary for efficient responses to phytohormone auxin, primarily for localization and intracellular dynamics of polar auxin transporters, AUX1 and PIN1 proteins (Kleine-Vehn *et al*. 2006, Nick *et al*. 2009). As morphological changes in *triple* mutant indicated possible disorders in auxin transport, the auxin responsiveness was measured quantitatively using auxin responsive reporter system IAA2::GUS in *triple* mutant and wild type. GUS activity assay showed significant decrease in auxin responsiveness during reproductive development of *triple* mutant. Polar auxin efflux vizualisation with PIN1::PIN1-GFP fusion showed heterogenous pattern in *triple* mutant in comparison with wild type.

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# ISOLATION OF ISOPRENE SYNTHASE (ISPS) GENE FROM DIFFERENT SPECIES OF FABACEACE FAMILY

**Leila Pazouki**[1*], Siamak Ghaffaripour[2] and Ülo Niinemets[1]

[1]Department of Plant Physiology, Estonian University of Life Sciences, leila.pazouki@emu.ee
[2]Department of Bioengineering, University of Antwerp, Belgium

Isoprene synthase is a chloroplastic enzyme that catalyzes formation of isoprene from dimethylallyl diphosphate. Isoprene is a biogenic hydrocarbon which has significant impacts on tropospheric chemistry. It is emitted by a number of plant species, including *Pueraria montana*, *Robinia pseudoacacia* and *Populus tremuloides*.

The goal of this study is isolation of isoprene synthase (ISPS) gene from different warm temperate to tropical species of Fabaceae family such as *Tamarindus indica*, *Gleditsia rolfei*, *G. caspica*, *G. triacanthos*, *Acacia longifolia*, *A. aneura*, *A. salicina* and *A. ramulosa*. Isoprene synthase sequences of related Fabaceace species were analyzed, the conserved motifs were indentified after sequence alignment of ISPS genes. The sequence alignment was carried out by software MEGA5 and degenerate primers were designed and synthesized on the basis of conserved regions of known cDNAs of Fabaceace. Healthy leaves of the plants were used for RNA extraction and cDNA synthesis. RT-PCR was done and the results showed a band in expected size and confirmed by sequencing. Based on our knowledge, this is the first report of isolation of partial ISPS sequences in these plants. Work towards obtaing the full sequence of all studies species is currently under process.

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## PLANTS AGAINST HERBIVORES: TRADE-OFFS BETWEEN DEFENCE STRATEGIES

**Triinu Remmel**

Estonian University of Life Sciences**,** triinu.remmel@emu.ee

Plants have two main strategies of defense against herbivores: resistance and tolerance. Neither of these lines of defense come cheap, they both have a cost. Therefore, it is assumed that there must be a trade-off between the two. However, such trade-offs have turned out to be hard to prove. Negative genotypic correlations between resistance and tolerance – considered to be the most reliable indicators of trade-offs – have been found in some studies, while others have yielded positive or no correlations. The problem may lie in the use of negative genotypic correlations: if some genotypes have inherently higher fitness in a given environment than others, they may be able to invest more than others in both defense strategies, thus producing a positive correlation, even if the trade-off exists. I performed an experiment with a wild plant species (*Fragaria vesca*) to find genotypic indices for resistance and tolerance and demonstrate if accounting for genotypes' average fitness in statistical models that test for genotypic correlations can shift the resultant correlations towards more negative values.

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## CARBON BALANCE OF AN OLD HEMI-BOREAL SOONTAGA PINE FOREST IN SOUTHERN ESTONIA BY USING DIFFERENT METHODS

**Kalev Repp[1]**, Kaido Soosaar[1], Krista Lõhmus[1], Veiko Uri[2], Kaire Rannik[3], Alisa Krasnova[3], Ivika Ostonen[1], Mai Kukumägi[1], Martin Maddison[1], Ülo Mander[1]

[1]Institute of Ecology & Earth Sciences, University of Tartu, kalev.repp@ut.ee
[2]Institute of Forestry & Rural Engineering, Estonian University of Life Sciences
[3]Institute of Agricultural & Environmental Sciences, Estonian University of Life Sciences

The Soontaga Forest Station (South Estonia; 58°01'N 26°04'E) is located in a hemi-boreal old Scots pine forest with a second layer of Norway spruce. The station has the instrumentation to assess the exchange of carbon dioxide (net ecosystem exchange, NEE), soil respiration, tree biomass (above and below ground biomass) and different environmental and meteorological parameters.

In this study we quantified carbon balance by analyzing eddy-covariance $CO_2$ flux data (carbon exchange) in combination with chamber-based measurements (ecosystem respiration) and $CO_2$ assimilation (soil and biomass).

Annual NEE, which quantifies the change in carbon storage of canopy, including growth of woody biomass and changes in soil carbon, is -2.3 t C ha yr$^{-1}$. It means that this mature pine forest is a carbon sink. The NEE values showed a clear diurnal and seasonal trend. During the daytime in summer the forest sequestered $CO_2$, while during the night and late night $CO_2$ emitted from the ecosystem to the atmosphere. Within the growing period, the sequestration of $CO_2$ by plants was greater than soil respiration. Hence, the ecosystem sequestered carbon. Most of the carbon is bound in tree biomass (above and below ground biomass) but as well in soil, while the sequestration in soil increases with stand age. In addition, the biomass of understory, especially belowground litter, is playing essential part in carbon input.

Carbon accumulation in ecosystem over long time periods provides a sink for atmospheric $CO_2$ and is very important to those studying global carbon budgets. Studies on C cycling in Soontaga are ongoing,

and future challenges are related to modelling and assessing long-term C budget in ecosystem by using more precise correlations between the carbon dioxide fluxes and environmental parameters.

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## IMPACT OF ELEVATED AIR HUMIDITY ON NIGHT-TIME AND PREDAWN WATER RELATIONS IN HYBRID ASPEN SAPLINGS

**Gristin Rohula**, Priit Kupper, Ingmar Tulva, Anu Sõber

Institute of Ecology and Earth Sciences, University of Tartu, gristin.rohula@ut.ee

The understanding how high air humidity impacts pre-dawn stomatal opening or dynamics of night-time water relations has remained unclear. The aim of the current study was to investigate how long-term exposure to increased air humidity is related to endogenous regulation of night-time water relations and gas exchange in shoots of hybrid aspen saplings in controlled conditions of a growth chamber. We observed that the predawn leaf conductance ($g_{n5}$) was 1.6 times higher ($P < 0.01$) in humidified (H) than in control (C) treatment. Furthermore, the $g_{n5}$ in the H treatment was higher than the early night-time leaf conductance ($g_{n1}$) in H. Thus, the shoots grown under higher air humidity conditions opened the stomata and lost more water in predawn hours. Although early night-time and predawn leaf conductance are low compared with daytime g, we suppose that pre-opened stomata may reach their maximum aperture faster in plants grown in elevated air humidity. However, in C treatment the predawn leaf conductance was positively related to average area of a single leaf ($P < 0.05$) and to leaf nitrogen (N) content ($P < 0.05$). Thus, trees with higher growth potential (bigger leaves, higher leaf N content) demonstrated more intensive predawn stomatal opening in control conditions. Nevertheless, the shoots taken from elevated air humidity treatment did not show similar relationship. Although elevated air humidity treatment did not increase either night-time or daytime stomatal conductance the predawn stomatal opening was more intensive under humidification probably because of altered endogenous regulation of leaf cell aquaporins.

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## YOUNG BIRCHES CHANGE ACCLIMATION STRATEGIES TO ELEVATED AIR HUMIDITY

**Katrin Rosenvald**, Ivika Ostonen, Arvo Tullus, Kaarin Parts, Priit Kupper, Krista Lõhmus

Institute of Ecology and Earth Sciences, University of Tartu, Katrin.Rosenvald@ut.ee

Consequences of increasing relative air humidity (RH) on forest ecosystems is a greatly understudied aspect of climate change. The study aims to find out acclimation strategies and effect on biomass accumulation of silver birch (*Betula pendula* Roth) in increased RH conditions.

Biomass, leaf and root specific areas and stem density measurements were done in FAHM study area, in 5- and 7-year-old birches, after two (in 2009) and four (in 2011) years of humidification, respectively.

Specific leaf area and stem taper were higher under humidification in both ages. Two years of elevated RH increased birch ectomycorrhizal root specific area (Parts et al. 2013) and decreased stem wood density and leaf N%, but the biomass partitioning among tree parts (leaves, branches, stems, and fine roots) remained unaffected. However, after four years of humidification, fine root proportion in root system of 7-year-old birches was 40% bigger in humidified plots (Rosenvald et al. 2014). The elevated RH decreased the tree biomass accumulation of 5-year-old trees, but after two years, differences in

biomasses and stem diameter increments levelled out between humidified and control plots (Rosenvald et al. 2014). Initially, birches had to use energetically cheaper acclimation strategy: to adjust leaf and root morphology, but after four years of humidification, 7-year-old birches were able to change also biomass allocation– to increase the proportion of absorbing EcM roots – and additionally to increase leaf N concentration, leading to efficient growth and biomass accumulation also under elevated humidity condition.

We conclude that young silver birches can efficiently acclimate to increased RH, and the birch forests will retain biomass productivity in future climate.

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## THE EFFECTS OF GROWTH CONDITIONS ON STOMATAL REGULATION OF *ARABIDOPSIS THALIANA*

**Liis Rüütel,** Hanna Hõrak, Pirko Jalakas, Ebe Merilo

Institute of Technology, University of Tartu, liisryytel@gmail.com

Stoma is a small pore, which is bordered by a pair of specialized epidermal cells known as guard cells, which regulate the size of stomatal aperture. Stomata mediate gas exchange – $CO_2$ uptake and water loss between the atmosphere and the plant. Plants open and close their stomata to different environmental stimuli. For example, stomata close in response to increased $CO_2$, darkness, abscisic acid (ABA), low humidity and ozone.

The aim of this research was to analyze, if growth conditions have an effect on stomatal regulation and how the stomatal conductance changes in response to different environmental stimuli (increased $CO_2$, darkness, low humidity and ABA) in differently grown plants. The plants were grown in a different air humidity environment, where relative air humidity was 20%, 70% and 90%, respectively. Half of the plants were watered and the other half were not watered, thus inducing drought stress in these plants. Stomatal conductance of plants was measured with a whole-rosette gas-exchange device[1].

All the plants closed their stomata in response to different stimuli, but the growth conditions affected the responses. Drought stressed plants had lower stomatal conductance compared with well-watered plants. Plants that were grown at high relative air humidity developed malfunctioning stomata, which were unable to close as efficiently in response to $CO_2$, darkness and ABA as did the stomata of plants grown in standard and low relative air humidity conditions. Well-watered plants which were grown in low relative air humidity showed increased ABA-sensitivity.

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# IDENTIFICATION OF PROTEINS INVOLVED IN $CO_2$-INDUCED STOMATAL REGULATION

**Yana Sindarovska**, Liina Jakobson, Yuh-Shuh Wang, Mikael Brosche, Hannes Kollist

Plant Signal Research Group, Institute of Technology, University of Tartu, sindarovskaya@ukr.net

Stomata are pores in epidermal plant tissues, which are formed by surrounding guard cells and control gas exchange in plants. Stomatal regulation is under intense research; however there are still gaps in understanding molecular details of guard cell signalling. Previous research showed that the *Arabidopsis* protein kinase HT1 regulates $CO_2$-induced stomatal movements (Hashimoto et al. 2006). HT1 is classified as a mitogen-activated protein kinase kinase kinase (MKKK). Our recent studies have identified MPK12 as an interactor of HT1 that lies upstream of HT1 in the signaling cascade. This is an unexpected result, since typically MPKKK should be at the top of the signal cascade.

The objective of this research is to study signalling cascade(s) involved MPK12 and HT1. Both MPK12 and MPK9 are preferentially expressed in guard cells and have functional redundancy. Thus *mpk9mpk12* double mutant was generated and will be extensively characterized for stomatal $CO_2$ response. Being protein kinase MPK12 has to interact with MKKs. Thus some MKKs may play an essential role in $CO_2$-induced stomatal movements. *Arabidopsis* MKK knockout mutants will be analyzed for $CO_2$ responses and other stimuli that trigger stomatal movements. MKKs having clear response will be used for pair-wise interaction tests with MPK12 and/or HT1. As protein kinases may interact with phosphatases, knock-out mutants of phosphatases related to MPK signaling also will be identified and analyzed for their stomatal $CO_2$ response. Additionally new MPK12 interactors will be isolated and characterized.

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# CARBON AND ENERGY: SOURCE AND SINK FOR ISOPRENE SYNTHESIS

**Vinícius F. de Souza**[1], Sabrina Garcia[1], José F.C. Gonçalves[1], Antônio O. Manzi[1], Niro Higuchi[1], Jeffrey Chambers[2], Ülo Niinemets[3],Kolby Jardine[2]

[1] National Institute of Amazonian Research (INPA), Ave. André Araújo, 2936, P.O. Box 478, CEP 69011-970, Manaus, AM, Brazil, viniciusfernandes11@yahoo.com.br
[2] Climate Science Department, Earth Science Division, Lawrence Berkeley National Laboratory, One Cyclotron Rd, building 64-241, Berkeley, CA 94720, USA
[3] University of Life Sciences, Institute of Agricultural and Environmental Sciences, Department of Plant Physiology, Riia 23, Tartu 51011, Estonia

Greater fraction of carbon (C) used in isoprene synthesis is recently assimilated. However, high temperature and [$CO_2$] may cause an uncoupling among synthesis isoprene and Calvin cycle. The uncoupling may be offset by extrachloroplastidic C sources. Furthermore, the increase in temperature and [$CO_2$] change photosynthetic processes, affecting the energy budget between the Calvin cycle and isoprene biosynthetic. To explain the mechanisms that control the effect of the both [$CO_2$] and temperature on isoprene synthesis, we done 1) Photosynthetic carbon-response curves (T=30ºC, PAR=1000 µmol $m^{-2}$ $s^{-1}$); 2) Photosynthetic temperature-response curves on four [$CO_2$] (150, 450, 750 and 1050 ppm and PAR=1000 µmol $m^{-2}$ $s^{-1}$). We used tropical evergreen tree (*Inga edulis*). At constant temperature, lower values of emission rates were find close the $CO_2$ compensation point. We fond maximum isoprene emission rates between 150 and 300 ppm of $CO_2$ after it decreased, but photosynthesis rate had a continuous increment until 650ppm of $CO_2$. During photosynthetic temperature-response curve under different [$CO_2$] conditions, the isoprene emission rates always increase together temperature. However, the isoprene emission rates were greater in lower [$CO_2$]. Our results suggest that 1) low [$CO_2$] conditions (below $CO_2$ compensation point), the isoprene emissions may be limited by carbon availability; 2) High [$CO_2$] further increased competition between Calvin cycle and isoprene synthesis for energy cofactors; 3) High temperatures may mitigate the negative effect of high [$CO_2$] on isoprene synthesis rates. However, some cases have demonstrated that high [$CO_2$] can change the chloroplastidic and extrachloroplastidic partitioning carbon sources for isoprene synthesis.

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## RISING SOIL WATER CONTENT CAN CHANGE THE DIRECTION OF AIR HUMIDITY EFFECT ON TREE GROWTH

**Anu Sõber**, Pille Mänd, Marili Sell, Marge Sepp, Priit Kupper, Jaak Sõber, Mai Kukumägi, Krista Lõhmus

Institute of Ecology and Earth Sciences, University of Tartu, anu.sober@ut.ee

As air humidity can increase in Northern latitudes and modify the effects of global climate change on forest trees, the free air humidity manipulation (FAHM) experiment was established, where silver birch and hybrid aspen trees were treated with ca 7% higher relative humidity (RH) in 2008-2012.

The effect of elevated RH on annual aboveground growth varied (Tullus et al, 2012, Rosenvald et al, 2014). Accompanying differences in N-uptake can be explained by changes in mass flow of water (Kupper et al, 2011, Tullus et al, 2012), biomass allocation (Rosenvald et al., 2014), or by adaptation of fine roots and mycorrhiza (Parts et al 2013).

The impact of soil water content (SWC) on RH effect was analyzed in current study. Treatment-difference in annual hight growth correlated negatively with average SWC during first half of growing season. As effect of RH on growth turned from positive to negative at certain SWC (60% and 70% of soil maximal water capacity for birch and aspen respectively), the influence of SWC was studied further in growth chambers. We have not analyzed aspen yet, but sharp optimum in growth rate at SWC=0,6 was found for birch. If the effect of RH at FAHM was mediated by the rise in SWC, this can really cause positive effect on growth in dry soil, but negative effect at over-optimal RWC values, explaining the change in direction of RH effect. The importance of inter-annual and misting-caused changes of SWC in determining tree's growth in different soils is discussed.

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Rosenvald, K., Tullus, A., Ostonen, I., *et al*. 2014. The effect of elevated air humidity on young silver birch and hybrid aspen biomass allocation and accumulation. Forest Ecology and Management, 330, 252–260.

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## VIRUSES OF POACEAE IN ESTONIA IDENTIFIED BY USING THE NEXT GENERATION SEQUENCING

**Merike Sõmera**, Mark Gantsovski, Anna Akkerman, Erki Aun and Erkki Truve

Department of Gene Technology, Tallinn University of Technology, merike.somera@ttu.ee

Traditional diagnostic tools like inoculation assays, electron microscopy, ELISA, PCR or microarrays using the virus-specific sequences, are powerful tools in diagnostics of known viruses or their close relatives. However, none of these traditional methods addresses a discovery of unknown viruses. Recently developed novel molecular method known as the next generation sequencing (NGS) provides universal way to identify viruses without prior knowledge about their particle shape and size, their type of genome or their taxonomy.

We were using the siRNA-based NGS approach that does not need virus particle purification but uses virus-derived small RNAs (siRNAs) generated by host RNA silencing mechanism. RNA silencing is an antiviral defense mechanism in many organisms, including plants, that detects and destroys virus-derived double-stranded RNA resulting in accumulation of virus-derived siRNAs of 21–24 nucleotides. Not only viruses with a dsRNA genome but also the double-stranded intermediates of ssRNA virus replication are targeted. Moreover, incomplete or non-polyadenylated copies of the viral genomes or gene transcripts may be detected by cellular RdRP that synthesizes a complementary strand and generates a dsRNA.

In 2013-2014, the first environmental screenings were performed to identify the viruses of *Poaceae* in Estonia. The grass samples showed 3 new virus species belonging into the genus *Tritimovirus* and the families *Tombusviridae* and *Amalgaviridae*. The analysis of cereal samples identified 4 species of Barley yellow dwarf viruses (BYDV-PAV-CN-like virus, BYDV-PAV, BYDV-GAV, CYDV-RPS) from family *Luteoviridae*, *Brome mosaic virus* from genus *Bromovirus*, and 3 species of new viruses belonging into the genera *Tenuivirus*, *Fijivirus* and *Closterovirus*.

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## LARGE-SCALE MUTANT SCREEN FOR STUDYING STOMATAL REGULATION IN RESPONSE TO ENVIRONMENTAL FACTORS

**Olena Zamora[1]**, Dmitry Yarmolinsky[1], Cezary Waszczak[2], Triin Vahisalu[2], Maija Sierla[2], Jaakko Kangasjärvi[2], Hannes Kollist[1]

[1]Plant Signal group, Institute of Technology, University of Tartu; olena.zamora@ut.ee
[2]Division of Plant Biology, Department of Biosciences, University of Helsinki, FI-00014 Helsinki, Finland

We are carrying out large-scale screening in collaboration with Prof. Jaakko Kangasjärvi lab (University of Helsinki), which is aimed at identification of new components in $O_3$/ROS-induced stomatal regulation. $O_3$ treatments of EMS-mutagenized GC1:YC3.6 mutant population was carried out in University of Helsinki and Helmholz Centrum in Munich, Germany in 2012-2013. $O_3$-sensitivity was used as a proxy to find plants with defects in stomatal regulation since mutants with more open stomata have higher ozone sensitivity. Altogether roughly 400 000 plants were screened and nearly one thousand putatively interesting plant lines were selected for further analysis. Currently, we perform further analysis of the selected mutants using our custom made gas-exchange systems in Tartu. We study stomatal responses of the mutant plants to elevated level of $CO_2$, abscisic acid, low relative air humidity and ozone pulse. Furthermore, water loss during 2 h in detached leaves was measured. Up to now, we have analyzed 296 lines; 204 of them showed mutant phenotypes. Impaired responses to elevated $CO_2$, abscisic acid spray and $O_3$ pulse were observed in 44%, 37% and 34% of the studied lines, respectively. We found 127 lines to have impaired responses to several stimuli, while 51 lines showed weakened or absent response to only one stimulus. Additionally, 24 lines had enhanced water loss and normal stomatal responses, while 2 mutants showed only moderately elevated stomatal conductance. The most interesting lines which demonstrated impaired responses to the applied stimuli will be afterwards used for identification of novel proteins which are involved in $O_3$/ROS-induced stomatal closure.

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## EFFECTS OF NITROGEN FERTILIZATION ON POLLEN BEETLE (*MELIGETHES AENEUS* FAB.) PARASITOIDS IN A CONTROLLED ENVIRONMENT.

**Valentina, Zolotarjova**[a]; Triinu, Remmel[a]; Eve, Veromann[a]; Ülo Niinemets[a].

[a]Estonian University of Life Sciences, valentina.zolotarjova@emu.ee

Pollen beetle (*Meligethes aeneus* Fab.) is one of the greatest *Brassica napus* L. enemies. It is known, that the amount of herbivours is dependent on ammonium (N) level on a field. On account of this, it is possible to reduce the damage from the pests by changing the amount of N fertilizer. Pollen beetle larvae and their parasitoids dependence on the intensity (0, 80, 100, 160 kg $ha^{-1}$) of fertilization in a controlled environment were tested in the current research. It was discovered that fertilization impact was significant for *B. napus* yield and for the amount of pollen beetle larvae but not for parasitoids. Generally the amount of larvae and parasitoids was positively correlated, however in the most N-rich samples, where the larvae amount was the highest, the number of parasitoids was small. This result could be related to N influence on emission intensity and bouquet of plant volatile organic compounds, which could be utilized by parasitoids in host selection. Further studies are required to test this hypothesis.

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## GRAVITROPIC RESPONSE OF ARABIDOPSIS INFLORESCENCE STEMS IN MYOSIN MUTANT PLANTS

**Kristiina Talts**, Eve-Ly Ojangu, Birger Ilau, Erkki Truve and Heiti Paves

Tallinn University of Technology, Department of Gene Technology, kristiina.talts@ttu.ee

Gravitropism is directional movement of the plant in response to gravity. Higher plants show positive (downward growth) and negative (upward growth) gravitropism. Actin cytoskeleton has been considered to be one of the significant components in gravitropic perception. Actin together with molecular motor proteins called myosins comprise actomyosin system which mediates the positioning and dynamics of organelles, vesicles and macromolecules. The positioning of statoliths and organelles in gravisensing is also considered to be a role of actomyosin system in plant cells. So far studies on the role of actin cytoskeleton and cytoskleletal proteins in regulating plant gravity sensing have not been conclusive.

To investigate possible involvement of myosins in negative gravitropism we used single and multiple T-DNA mutants of all 17 myosin members from classes VIII and XI of Arabidopsis. We characterized gravitropic response of inflorescence stems and our results show that all single mutants had normal responses to stimulation at room temperature. However, the gravitropic bending of well described class XI triple mutant *xi-1/xi-2/xi-k* was delayed, curvature was reduced and average speed of upward moving was also reduced. At the same time, there were no abnormalities in the response of class VIII quadruple mutant *viii-1/viii-2/viii-a/viii-b*. These results suggest that class XI myosins contribute to negative gravitropism in *Arabidopsis* inflorescence stems whereas myosins of class VIII do not have a role in this process. Mechanisms of retarded gravitropic response of myosin mutants is possibly related to discrepant building of cell walls in mutant plants.

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## MOSSES AND LEAF AREA INDEX

**Mari Tobias**, Ülo Niinemets

Institute of Agricultural and Environmental Sciences, Estonian University of Life Sciences,
mari.tobias@emu.ee

The interaction between the plant and atmosphere is to a large extent determined by the plant canopy. The key characteristic describing this canopy-atmosphere interface is a leaf area index (LAI), which is the amount of one-sided leaf area per unit ground area.

Due to the simple structure and lack of adaptations to store water mosses represent the colonial life forms where the shoots are packed together forming dense canopies. Their LAI values show a great variation reaching as high as 129 $m^2$ $m^{-2}$ (Simon 1987) to as low as 4,1 $m^2$ $m^{-2}$ (Waite and Sack 2010). The small size of foliage elements has made the determination of LAI difficult and so far determined only for 17 species (Niinemets and Tobias 2014).

Our main goal was to understand the causes underlying this large variation in LAI. We hypothesized that 1) LAI increases under high light due to the tighter backing of the shoots; and 2) there are differences in LAI values due to the different life form types.

We measured leaf area ($A_L$ – $mm^2$), leaf frequency ($F_{N,S}$ – leaves per $cm^{-1}$), shoot density ($N_N$ – shoots per $cm^{-2}$) and cumulative LAI ($L_c$ – $m^2 m^{-2}$) in 11 species from 5 life form.

Our results show that changes in habitat light environment can greatly influence intraspecies LAI values. Greater aggregation of the shoots with increasing habitat light availability creates more clumped canopies under high light supporting more than 3 times larger LAI compared to the canopies under low light. Among the life forms studied the small turfs have significantly higher LAI than tall turfs.

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## SEED-ORIGINATED SILVER BIRCHES ACCLIMATE DIFFERENTLY TO ELEVATED AIR HUMIDITY THAN MONOCLONAL HYBRID ASPEN IN THE FAHM EXPERIMENT

**Arvo Tullus**, Priit Kupper, Katrin Rosenvald, Ivika Ostonen, Anu Sõber, Krista Lõhmus, Arne Sellin

Institute of Ecology and Earth Sciences, University of Tartu, arvo.tullus@ut.ee

Global warming will bring more precipitation in Northern latitudes and warmer air can hold more water vapour, meaning that air humidity will increase. We clarified the effects of increasing air humidity on above-ground growth, chemical properties of stemwood and autumn leaf fall of silver birch (*Betula pendula* Roth) and hybrid aspen (*Populus tremula* L. × *P. tremuloides* Michx.). Data were collected during five years in the FAHM experiment, where air humidity is artificially elevated by *ca* 7%.

During the first experimental years, above-ground growth rate of both species was significantly lower under elevated humidity compared to the ambient (Tullus *et al*. 2012, Sellin *et al*. 2013). This can be partly explained by a decrease in transpiration-driven mass flow of $NO_3^-$ in soil evidenced by smaller N content and photosynthetic capacity of the foliage. During the following seasons, growth rate of birches recovered and eventually turned faster under elevated humidity, while growth of aspen remained suppressed (Rosenvald *et al*. 2014), which is partly attributed to higher genetic diversity of seed-originated birches compared to monoclonal aspens. Prolonged life span of the foliage was one of the acclimatory mechanisms in birches resulting in 1-3 weeks longer leaf retention (Godbold *et al*. 2014). Delayed leaf fall could have been caused by higher level of cytokinins. Significantly higher concentrations of N and P were observed in the stemwood of both species grown under elevated humidity (Tullus *et al*. 2014), which could be the result of a change in the content of living parenchyma cells and/or enhanced resorption of nutrients.

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# FUNCTIONAL ROLES OF HT1 AND MPK12 KINASES IN $CO_2$-INDUCED STOMATAL RESPONSES

**Kadri Tõldsepp**, Ervin Valk, Hanna Hõrak, Liina Jakobson, Mart Loog, Yuh-Shuh Wang, Mikael Brosche, Hannes Kollist

Plant Signal Research group, Institute of Technology, University of Tartu, kadri.toldsepp.001@ut.ee

Plants are immobile organisms and to survive they need to respond to the constantly changing environment. The communication between plants and surroundings is mediated by guard cells, highly specialized cells that form stomatal pores. Guard cells sense various signals from the environment and adjust the stomatal pore size to maximize $CO_2$ uptake for photosynthesis and to minimize the water loss by transpiration. The opening and closure of stomatal pores are regulated by dynamic changes of protein phosphorylation in guard cells. Plants possessing several kinase mutants have been shown to be defective in regulating guard cells movement but the direct substrates and roles of these kinases are often unknown.

We have been studying two protein kinases, HT1 and MPK12, as key regulators in $CO_2$-induced stomatal responses. Using *in vitro* kinase assays we show that protein kinase HT1 phosphorylates well-known proteins associated with stomatal pore movement. Additionally, we revealed that HT1 kinase activity is inhibited by MAP kinase MPK12. In the light of these findings we provide a new, more detailed model for $CO_2$ signalling and guard cells movement.

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# LATE BLIGHT RESISTANCE OF THE ESTONIAN POTATO CULTIVAR ANDO

**Karmen Tõnismann[1]**, Terje Tähtjärv[2, 3], Aide Tsahkna[2], Eve-Runno Paurson[3], Anu Aaspõllu[1], Kadri Järve[1]

[1]Tallinn University of Technology, Department of Gene Technology, karmen.tonismann@ttu.ee
[2]Estonian Crop Research Institute, Department of Plant Breeding
[3]Estonian University of Life Sciences, Institute of Agricultural and Life Sciences

The Estonian potato cultivar Ando has demonstrated late blight resistance to most of the local *Phytophthora infestans* isolates for over two decades. The putative candidate genes of Ando's resistance are NB-LRR class resistance genes (R genes). Based on the conserved NB-ARC domain motifs of the published potato genome (DM1-3 516R44) R gene sequences, the research aims to identify late blight R genes in cultivar Ando by NGS sequencing and develop functional marker(s) for the disease resistance.

The expressed R genes were identified from a late blight resistant cultivar Ando and susceptible cultivars Agra and Frila by paired-end sequencing (2x250 bp) of the 3' ends of the expressed resistance genes using the Illumina MiSeq sequencing platform. Local paired-end alignment of Ando's TruSeq quality controlled reads identified 6% of reads (163537 reads) mapping to 622 NB-LRR gene sequences out of 755 described by Jupe *et al*. (2013). The greatest number of reads mapped to chromosomes 4, 5, 6, 8, 10 and 11 and to unanchored superscaffolds which corresponds to the high density of R genes on those chromosomes. In addition, 452 reads unique to Ando were identified. Based on the sequencing results, particular sequence variations of resistance genes characteristic for cultivar Ando will be determined and the segregation of candidate R genes will be studied in two potato F1 populations (Ando x Agra and Ando x Frila).

Jupe, F. *et al*. (2013). The Plant Journal 76:530-544.

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## ANALYSIS OF POLYPROTEIN P2A C-TERMINAL PART OF COCKSFOOT MOTTLE SOBEMOVIRUS

**Gabriela Uffert**, Merike Sõmera and Erkki Truve

Tallinn University of Technology, gabriela.uffert@ttu.ee

*Cocksfoot mottle virus* (CfMV) belongs to the genus *Sobemovirus*. It has a positive-sense polycistronic RNA genome. An ORF2a of sobemoviruses encodes polyprotein P2a. It has been demonstrated that P2a of *Sesbania mosaic virus* (SeMV) is processed to five functional proteins: transmembrane anchor, serine protease, VPg and two C-terminal proteins – p10 with ATPase activity and p8 with nucleotide binding properties. Similar domain distribution has been predicted also for *Rice yellow mottle virus* (RYMV). However, there is no sequence conservation within the C-terminus of P2a.

In case of SeMV, the distribution of ordered and disordered regions is in accordance with the p10 and p8. The cleavage between p10 and p8 occurs at their border. Our predictions show the sequence profiles of CfMV C-terminal domains are different in the length of disordered segment and therefore, probably these proteins are cleaved differently.

Stop mutations in CfMV infectious clone were generated to test the importance of CfMV P2a C-terminal part for virus viability and those mutants were used to infect oat plants. Results of a study about C-terminal stop mutants suggest that the first 77 amino acids are needed and the last 20 amino acids are not needed for viral infection. The latter are a part of potential nucleotide binding region, also shown for SeMV, but this do not affected CfMV viability. Our predictions show the nucleotide binding region in CfMV C-terminus is broader than in case of SeMV.

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## LOOKING THROUGH THE PAST: MESOPHYLL AND STOMATAL CONDUCTANCES, AND THE RATE OF PHOTOSYNTHESIS IN EVOLUTIONARILY OLD PLANTS

**Linda-Liisa Veromann,** Tiina Tosens, Lauri Laanisto, Eero Talts, Ülo Niinemets

Estonian University of Life Sciences, linda-liisa.veromann@student.emu.ee

Although, there are less than 1,000 extant gymnosperm species, these plants have important roles in ecosystems ranging from boreal to rainforests. In addition, several confers are key forestry species making studying these species both ecologically and economically critical in the changing environment. Despite the significance of the former, proportionally much fewer species have been studied, especially from the aspect of diffusional limitations of photosynthesis ($A_n$). Furthermore, ferns and fern allies have been studied even less, with only recent research into their mesophyll conductance ($g_m$), while both groups have once dominated the planet and have persisted after the Jurassic period when angiosperms first appeared.

Our work adds to the data about diffusional limitations in evolutionarily old plants with an emphasis on $g_m$ and anatomical characteristics it depends on. We completed a broad assessment of foliar gas-exchange and the mesophyll anatomy of the photosynthetic organ involved in photosynthetic function in 13 species, with unprecedented results about cell wall thickness ($T_{cw}$), and novel research on the amount of structure present in mesophyll ($F_{str}$, percentage of cell walls in cross-section). In addition, we analyzed the leaf economics spectrum, e.g. relationship between assimilation and LMA.

The studied plants had, on average, a low $A_n$ and $g_m$, but high LMA and $T_{cw}$ compared to angiosperms, still, exhibited a large range of values for some characteristics illustrating the need for research a broader spectrum of taxa.

## NOVEL ASPECTS OF ISOPRENOIDS IN STOMATAL REGULATION

Priit Pechter and **Yuh-Shuh Wang**

Plant Signal Research Group, Institute of Technology, University of Tartu, yuh.shuh.wang@ut.ee

Stomata are natural openings on the plant surface, and their aperture is influenced by a number of environmental and biotic factors. Two of the well-known compounds that promote stomata closing and opening are the phytohormone abscisic acid (ABA) and the fungal toxin fusiccosin, respectively. Both of them are isoprenoids, which are the most diverse natural products. To explore the possibility that other isoprenoids are also capable of modulating stomatal movement, we will develop plant expression systems that enable overproduction of specific isoprenoids without creating metabolic burdens by a novel synthetic protein scaffold approach. Transgenic plants with specific isoprenoid profiles will be generated and their stomatal responses to various abiotic and biotic stresses will be inspected. Results from this project will expand our knowledge on stomatal regulation and provide valuable tools in isoprenoid bioengineering.

## DETECTION OF REACTIVE OXYGEN SPECIES IN ARABIDOPSIS PLANTS USING GENETICALLY ENCODED FLUORESCENT PROBES

**Dmitry Yarmolinsky**, Olena Zamora, Hannes Kollist

Plant Signal group, Institute of Technology, University of Tartu, dmitry.yarmolinsky@ut.ee

Reactive oxygen species (ROS) are shown to play a role as second messengers in the regulation of various processes in plants. We are establishing a system for ROS detection in Arabidopsis plants under exposure to various environmental factors, focusing on ROS signaling in guard cells. We use genetically encoded fluorescent probes which provide high sensitivity for ROS visualization and can be expressed in transgenic organisms with different subcellular localization. In addition to HyPer, which has been successfully used in plants, we are using new genetically encoded fluorescent probes for ROS detection that have been recently developed by V. Belousov group. HyperRed is the first genetically encoded red fluorescent sensor for hydrogen peroxide detection (Ermakova et al. Nat Commun. 2014 Oct 21;5:5222). NeonOx ROS sensors are bright green intensiometric probes which fluorescence is pH insensitive (V. Belousov, unpublished data). In our study, we constructed vectors for expression of HyPer3, HyperRed and NeonOx in Arabidopsis using ubiquitin 10 and guard-cell-specific promoters. Preliminary data indicate that HyperRed and NeonOx can be effectively expressed in plants and their fluorescence depends on the hydrogen peroxide concentration in plant cells. Generated plants will be used for detection of ROS in plants, especially in guard cells during stomatal movements under changing conditions. In addition, plants expressing HyperRed or NeonOx will be crossed with known stomatal mutant lines (ost1, ghr1, ht1, abi1, abi2 and others) to dissect roles of stomatal regulators in the amplification of ROS signal in guard cells.