**January 22nd 2016**

# Zürich Mycology Symposium 2016

**Swiss Federal Institute for Forest, Snow and Landscape Research WSL,**
**Zürcherstrasse 111, Engler Saal, 8903 Birmensdorf**

**08:30 - 09:00** ***Arrival and Registration*** (fee CHF 10.-)

## *Morning session I (chaired by Daniel Rigling)*

**09:00 - 09:05** ***Opening remarks (by Markus Aebi)***

**09:05 - 09:25** **Salome Leibundgut-Landmann, VetSuisse, Uni ZH**:
Dynamic interactions between *Candida albicans* and its mammalian host in barrier tissues

**09:25 - 09:45** **Joana Beatrice Meyer, Phytopathology, WSL:**
Interaction between two invasive organisms on the European chestnut: does the chestnut blight fungus benefit from the presence of the gall wasp?

**09:45 - 10:05** **Saskia Bindschedler, Institute of Biology, Uni NE:**
Bacterial-fungal interactions and soil functioning: what do we know about the fungal partners?

**10:05 - 10:25** **Fanny Hartmann, Plant Pathology Group, ETH ZH:**
Genomic architecture of virulence: a segregating gene deletion polymorphism is linked to pathogenicity of a fungal wheat pathogen

**10:25 - 10:55** ***Coffee break***

## *Morning session II (chaired by Simone Prospero)*

**10:55 - 11:15** **Stefan Linder, Institute of Plant Biology, Uni ZH:**
Molecular Characterization of R-Gene/Effector Interaction in the Wheat/ Powdery Mildew System

**11:15 - 11:35** **Renate Heinzelmann, Phytopathology, WSL:**
Genome-wide SNP analysis to generate a genetic map for *Armillaria ostoyae* to be used for locating a natural mutation that severely affects mycelial growth

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**11:35 - 12:25** ***Microsynth-Lecture:***
**Harald Kellner, TU Dresden - Zittau DE**:
Dead wood decomposition in temperate forests – enzymatic and molecular aspects

**12:30 - 14:00** ***Lunch break (at the cafeteria WSL)***

## *Afternoon session I (chaired by Martina Peter)*

**14:00 - 14:20** **Simon Egli, Mycorrhiza Group, WSL:**
Truffle monitoring in Switzerland and Hungary

**14:20 - 14:40** **Anja Kombrink, Institute of Microbiology, ETH ZH**:
Bacteria-induced response in the fungus *Coprinopsis cinerea*

**14:40 - 15:00** **Florian Geschwend, Agroscope Reckenholz:**
Multi-year assessment of soil fungal diversity patterns at sites of the Swiss soil monitoring network

**15:00 - 15:20** **Mout de Vrieze, Agroscope Wädenswil:**
The potato microbiome and its potential impact on late blight resistance

**15:20 - 15:50** ***Coffee break***

## *Afternoon session II (chaired by Ivano Brunner)*

**15:50 - 16:10** **Klaus Schleppi, Agroscope Reckenholz:**
High resolution profiling of arbuscular mycorrhizal communities reveals that inoculation perturbs the native community structure

**16:10 - 16:30** **Coraline Praz, Institute of Plant Biology, Uni ZH:** Transcriptome analysis of the wheat/wheat powdery mildew interaction

**16:30 - 16:50** **Lucrezia Comensoli, Institute of Biology, Uni NE:**
The art of survival to the rescue of artworks

**16:50 - 16:55** ***Closing remarks (Markus Künzler)***

**Organisation:** Ivano Brunner, Daniel Rigling, Simon Egli (WSL), Markus Künzler (ETH)

## Abstracts - Zürich Mycology Symposium; January 22nd 2016, WSL Birmensdorf

**Salome Leibundgut-Landmann,** Section of Immunology, Vetsuisse VetSuisse, Uni Zürich

**Dynamic interactions between *Candida albicans* and its mammalian host in barrier tissues**

Fungal pathogens bear a serious health hazard for individuals with a weakened immune system. Although some fungi, such a *Candida albicans*, are present in the normal human microbiota, they can cause severe diseases if host defenses are breached. The continuous rise in fungal infections and the increase in resistance against available antifungal drugs urge the development of novel preventive and therapeutic strategies. For this, a detailed understanding of fungal pathogenicity and natural host defense mechanisms is of great importance.

It is generally believed that the host immune status determines the outcome of the interaction between the fungus and the host, resulting in health or disease. Specific host mechanisms including those mediated by the cytokine interleukin-17 are now known to regulate the antimicrobial response and thereby limit fungal overgrowth at the epithelial barriers. It remains less clear however how the genetic diversity of *C. albicans* that is detected in strains isolated from colonized individuals impacts on the outcome of the interaction of the fungus with its host. Here I will discuss the current view of dynamic host-pathogen interactions that adjust the fine balance between fungal colonization and pathogenic infection.

**Joana Beatrice Meyer**, Phytopathology, WSL Birmensdorf

**Interaction between two invasive organisms on the European chestnut: does the chestnut blight fungus benefit from the presence of the gall wasp?**

The impact of invasive fungal pathogens and pests on trees is often studied individually, thereby omitting possible interactions. In this study the ecological interaction between the chestnut blight fungus *Cryphonectria parasitica* and the chestnut gall wasp *Dryocosmus kuriphilus* was investigated. We determined if abandoned galls could be colonized by *C. parasitica* and thereby act as an entry point and a source of pathogen inoculum. Moreover we assessed the identity and diversity of other gall-colonizing fungal species. A total of 1973 galls were randomly sampled from 200 chestnut trees in eight Swiss stands. In a stand *C. parasitica* was isolated from 0.4–19.2% of the galls. The incidence of *C. parasitica* on the galls and the fungal diversity significantly increased with the residence time of *D. kuriphilus* in a stand. All but one *C. parasitica* cultures were virulent. The predominant fungus isolated from galls was *Gnomoniopsis castanea* whose abundance influenced negatively that of *C. parasitica*. This study shows that *D. kuriphilus* galls can be colonized by virulent strains of the chestnut blight fungus *C. parasitica*. This can have effects on the chestnut blight incidence even in chestnut stands where the disease is successfully controlled by hypovirulence. The gall wasp presence influences also the fungal species composition on chestnut trees.

**Saskia Bindschedler**, Institute of Biology, Uni Neuchâtel

**Bacterial-fungal interactions and soil functioning: what do we know about the fungal partners?**

Fungi and bacteria are both essential actors of organic matter turnover and nutrient cycling in soils, a crucial aspect of ecosystem functioning. While their individual roles are well described, the influence of bacterial-fungal interactions (BFI) in soil functioning is still poorly understood. Both organisms have shared the same environment for millions of years resulting in a myriad of interactions from mutualism to antagonism. Describing such interactions in complex systems such as soils is challenging, as their regulation depends on both biotic and abiotic factors. Consequently, both reductionist and systemic approaches are needed in order to get a comprehensive overview of BFI related to ecosystem functioning. First, by using model organisms in Petri dish experiments, we are starting to understand some basics regulatory elements of BFI in nature. In the systemic approach, our model is the oxalate-carbonate pathway (OCP), a natural process in which both fungi and bacteria play a crucial role. The OCP has a major impact on soil functioning by triggering alkalinisation in acidic soils, which positively impacts soil nutrient content. As a result, the OCP is a pertinent model to study the impact of microorganisms on soil functioning. Using soil microcosms studies we were able to highlight that BFI have a positive impact on OCP functioning. Traditional microbial ecology has usually focused more on bacteria than fungi. Therefore, in this presentation we will highlight the role of fungi in BFI. In the future we aim at understanding the complexity of BFI in soils and to decipher the instrumental role of BFI in soil nutrient bioavailability and plant health. Although there are still many aspects to disentangle, our studies highlight the overlooked role of BFI in biogeochemical processes. As a consequence, future studies aiming at unravelling the factors that drive soil functioning should definitely benefit from integrating BFI.

**Fanny Hartmann**, Plant Pathology Group, ETH Zürich

**Genomic architecture of virulence: a segregating gene deletion polymorphism is linked to pathogenicity of a fungal wheat pathogen**

The fungus *Zymoseptoria tritici* is the causal agent of *Septoria tritici* Blotch (STB) of wheat causing major economic losses. However, the genetic architecture for virulence in natural populations of the fungus is poorly understood. We performed a genome-wide association study (GWAS) to identify genetic variation linked to virulence in natural populations. We analysed 106 isolates originating from four geographical locations: Australia, Israel, Switzerland and the United States. Pycnidia production, that is a key indicator of virulence in *Z. tritici*, was assayed on two Swiss spring wheat cultivars differing in resistance to STB. Differences in virulence were observed within and among pathogen populations, but also between the two wheat cultivars as expected. Using Illumina whole-genome sequencing, we genotyped 779'178 high-quality single nucleotide polymorphisms (SNPs) segregating in multiple populations. GWAS analyses identified multiple chromosomal regions associated with virulence on both cultivars,

showing that virulence is based on a complex genetic architecture. We found little overlap in associated regions identified for each cultivar separately, suggesting that many virulence loci may be cultivar-specific. Significant associations localized to genes encoding for proteins of diverse functions, including transporters, nutrient degradation and general metabolism. The most significantly associated SNP was in near complete linkage disequilibrium with a deletion polymorphism of a gene encoding a small secreted protein. The gene is highly transcribed during leaf infection and is located at the boundary between a transposable element-rich region showing large-scale deletion polymorphisms within the species and a conserved, gene-rich region. Isolates lacking the gene showed higher virulence, suggesting that the gene plays a role in plant defense activation or recognition. The finding that a gene deletion polymorphism was strongly associated with virulence shows that chromosomal rearrangements in *Z. tritici* populations can be a major driver of virulence evolution.

**Stefan Linder**, Institute of Plant Biology, Uni Zürich
**Molecular Characterization of R-Gene/Effector Interaction in the Wheat/Powdery Mildew System**
The biotrophic fungal pathogen *Blumeria graminis* f. sp. *tritici (*Bgt), which causes the agronomically important plant disease wheat powdery mildew, is able to infect susceptible wheat lines by expressing effector proteins that suppress the basic defense mechanisms of the host. Resistant wheat plants can express resistance genes that recognize effectors and initiate the hypersensitive response (HR), a locally limited cell death that prevents the fungus from growing. One of the most studied resistance genes against powdery mildew is the allelic series of Pm3 in wheat. For one of these alleles, Pm3a, the complementary effector gene (AvrPm3a2/f2) has recently been cloned.
Pm3a encodes an NB-ARC-LRR protein that induces HR in the presence of the effector protein AVRPM3A2/F2 from Bgt, thus conferring resistance against powdery mildew. However, it is yet unknown how this recognition is working on the molecular level. Considering the mode of action of NB-ARC-LRR proteins from other plant species, it is very likely that the LRR domain of PM3A directly interacts with the effector. Therefore, we exchanged SNP-containing regions as well as single amino acids from the LRR domain of PM3A with the corresponding sequences from a susceptible allele, Pm3CS. These constructs were transiently expressed in tobacco leaves by *Agrobacterium tumefaciens* infiltrations, and the resulting HR was assessed. With that, we want to find single and/or combined amino acid positions responsible for the specificity of Avr recognition, downstream resistance signaling, as well as the intensity of the cell death response.

**Renate Heinzelmann**, Phytopathology, WSL Birmensdorf
**Genome-wide SNP analysis to generate a genetic map for *Armillaria ostoyae* to be used for locating a natural mutation that severely affects mycelial growth**
As saprotrophic white rot fungus *Armillaria ostoyae* decomposes dead wood, and as an aggressive pathogen it causes root and butt rot on healthy trees. Based on inoculation tests, variation in virulence among *A. ostoyae* strains is large. When basidiospores of a non-virulent *A. ostoyae* strain were plated, some progeny with an abnormal phenotype was observed. The mycelium of these cultures grew extremely dense and hardly expanded. This phenotype segregated in a 1:1 ratio (256 abnormal vs. 262 normal progenies) and the normal phenotype could be restored upon mating of an abnormal strain with a normal haploid strain. These observations suggest that the abnormal strains are likely carrying a mutation in a single gene, which severely affects mycelial growth. Any such gene might be interesting, not only in *Armillaria*, but also in other fungi, because its dysfunction leads to drastically reduced growth and likely also to reduced virulence. Double digest restriction-site associated DNA sequencing (ddRADseq) was used to sequence about 200 haploid progenies of the non-virulent *A. ostoyae* strain mentioned. The obtained sequence data was used to establish a set of several thousand single nucleotide polymorphisms (SNPs) distributed over the entire genome. With those SNPs a genetic map for *A. ostoyae* was constructed and 11 linkage groups were identified. The mutation causing abnormal growth was mapped to a single genome region of approximately 87’000 bp which harbors 31 genes.

## Microsynth-Lecture:

**Harald Kellner**, Technische Uni Dresden - Zittau, Deutschland
**Dead wood decomposition in temperate forests – enzymatic and molecular aspects**
A substantial part of terrestrial carbon is bound in wood. After die-back, this source is continuously degraded by microorganisms, hereby structuring forest ecosystems and influencing multiple ecosystem functions, like carbon sequestration, nutrient cycling, and habitation of wood-dwelling organisms. However, detailed analyses of the fungal molecular community composition and their extracellular “digestion” enzymes during the course of wood decay in situ are still largely lacking.
Currently we (TU Dresden-IHI Zittau and collaborators at the UFZ Halle and NP Bayrischer Wald) investigate the decomposition of 13 tree species in three larger long-term study sites in Germany, called “Biodiversity-Exploratories”, with the aim to I) understand the mechanisms of wood degradation, to II) identify the microbial keyplayers in this process and to III) evaluate potential effects of altered forest management.
We use “classical” approaches like photometric and HPLC-based assays to assess enzymatic activities of lignocellulolytic oxidoreductases and hydrolases, which we relate to chemical changes of the wood (e.g. lignin content, amount of organic extractives, element content, pH, etc.) during the course of decomposition. Using high-throughput sequencing approaches (e.g. Illumina MiSeq sequencing), we investigate in parallel the changes in the microbial communities (fungi, eubacteria).
A main finding of our research is the relation between fungal biomass in sap- and heart-wood and the activities of degradative enzymes. Furthermore, we were able to identify dominant fungal species during the decomposition process, which we are currently using for additional analyses in the laboratory and genome sequencing approaches. Since deadwood is a nitrogen-limited habitat, its acquisition is of general significance for the understanding of wood decomposition. Therefore, we follow, in a first approach, the presence of diverse N2-fixing bacteria, and could already detect their nifH-genes (encoding nitrogenase) in various wood samples from the Biodiversity Exploratories.

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**Simon Egli,** Mycorrhiza Group, WSL Birmensdorf
**Truffle monitoring in Switzerland and Hungary**
No abstract

**Anja Kombrink,** Martina Stöckli, Reiko Ueoka, Markus Aebi, Markus Künzler; Institute of Microbiology, ETH Zürich
**Bacteria-induced response in the fungus *Coprinopsis cinerea***
Fungi occur in diverse ecological habitats, where they encounter microbes that compete for nutrients and/or have antagonistic activity. While defense of plants and animals is well studied, little is known about defense mechanisms of fungi to cope with competitors and parasites. We investigate defense of the basidiomycete *Coprinopsis cinerea* towards various antagonistic organisms, including bacteria. It was previously demonstrated that genes encoding nematotoxic proteins are upregulated in *C. cinerea* mycelium challenged with fungivorous nematodes. To investigate the response of *C. cinerea* to bacteria, we compared the transcriptome of *E. coli*- and *B. subtilis*-challenged *C. cinerea* with unchallenged mycelium. The mycelium was grown on glass beads submerged in liquid medium to allow close and dynamic contact of the bacteria with the fungal hyphae. Eight hours after addition of the bacteria genes were found to be highly induced (>eight fold compared to the unchallenged control) in *C. cinerea* mycelium, with a larger set in response to *E. coli* than in response to *B. subtilis*. However, the response to the different bacteria seems not specific as the differentially expressed gene sets largely overlap. Among the highly induced genes in response to both *E. coli* and *B. subtilis* are eight members of a gene family that encodes eleven small cysteine-rich hypothetical proteins (SCRP) and five lysozymes whose N-termini share high homology with the SCRPs. One of the lysozymes was heterologously produced and showed anti-bacterial activity. Interestingly, not only live bacteria, but also filter-sterilized growth medium of *E. coli* and *B. subtilis* cultures induced expression of the putative defense genes in *C. cinerea*. By fractionation of the medium and chemical analysis of the active fractions we aim at the identification of the bacterial cue that is perceived by the fungus and triggers this response.

**Florian Gschwend[1]**, Martin Hartmann[1,2], Anna-Sofia Hug[1], Beat Frey[2], Andreas Gubler[1], Reto Giulio Meuli[1], Franco Widmer[1]; 1 Agroscope Reckenholz Zürich, 2 WSL Birmensdorf
**Multi-year assessment of soil fungal diversity patterns at sites of the Swiss soil monitoring network**
Since 2012 soil samples for microbial diversity analysis have been collected every spring at 30 sites of the Swiss Soil Monitoring Network (NABO). The goals are to investigate spatio-temporal patterns of soil microbial communities in different well characterized habitats and to assess the potential of soil microbial communities for use in soil quality monitoring. The sites were selected to represent various soil types and include arable land, grassland, and forest. Besides high-throughput amplicon sequencing targeting fungi and bacteria, several soil properties were determined, including pH, C/N ratio, bulk density, microbial biomass, and DNA quantity. In addition, information is available on the management history and climate parameters of each study site. Both, bacterial and fungal communities revealed strong site specificity and high temporal stability. For example, canonical analysis of principal coordinates (CAP) of fungal communities from samples of the first three years correctly reclassified 265 out of the 270 samples. Furthermore, PERMANOVA based on a distance matrix of fungal communities showed that 71% of the total variation can be explained by the factor site. Data revealed that the sites analyzed harbored characteristic and temporally robust fungal community structures. Deviations from these characteristic patterns may be informative for soil quality monitoring purposes.

**Mout De Vrieze[1]**, Ramona Gloor[1], Josep Massana Codina[1], Adithi Ravikumar Varadarajan[1], Tomke Musa[2], Christian H. Ahrens[1], Aurélien Bailly[4], Laure Weisskopf[1,3];
1 Agroscope, Institute for Plant Production Sciences; 2 Agroscope, Institute for Sustainability Sciences; 3 University of Applied Sciences and Arts, Western Switzerland, Changins, Viticulture and Oenology; 4 University of Zürich, Institute of Plant Biology, Microbiology
**The potato microbiome and its potential impact on late blight resistance**
Late blight caused by the oomycete *Phytophthora infestans* is a major threat for potato production worldwide. In organic farming, control is based on the use of copper-based fungicides, but in view of copper's environmental toxicity, the need to develop alternative organic control methods is evident. In natural and agro-ecosystems, plant roots and shoots are colonized by a diverse community of microorganisms. In the model plant *Arabidopsis thaliana*, a protective role of this plant microbiome against a number of phytopathogens has been demonstrated. However, the putative role of the potato microbiome in protecting the plant against pathogens such as the fast evolving *P. infestans* is still under investigation. In this project, in vitro screening of recently isolated strains from field-grown potatoes revealed differential antagonistic activity of these strains against *P. infestans*. 16 *Pseudomonas* strains were selected and further characterized for their in vitro effects on mycelial growth, sporangia germination, and zoospore production and behavior, and for their effects on symptom development by means of a leaf disc assay. Furthermore, 10 strains of varying anti-*Phytophthora* activity are being sequenced in order to identify the genomic determinants of this antagonistic activity. In addition, in order to assess the spectrum of activity of the bacteria, 3 strains were tested against a collection of *P. infestans* strains. For this, a monitoring of the Swiss *P.infestans* population was conducted during the summer of 2015. 22 *P. infestans* isolates were successfully retrieved from infected fields and are being characterized for morphological and virulence traits, as well as fungicide resistance. Altogether, these findings should provide better understanding of the mechanisms involved in the anti-*Phytophthora* activity of the *Pseudomonas*. The ultimate goal of this study is to improve our use of bacteria as biocontrol agents through a deeper understanding of their metabolic possibilities and of their needs.

**Klaus Schleppi,** Agroscope Reckenholz Zürich

**High resolution profiling of arbuscular mycorrhizal communities reveals that inoculation perturbs the native community structure**

There is increased interest to inoculate arbuscular mycorrhizal fungi (AMF) into agricultural ecosystem for enhancing plant productivity and improving sustainability. In order to successfully inoculate AMF into the field, we need to be able to trace the survival and persistence of inoculants and to quantify their effects on the native microbiome in a given soil environment. High-throughput sequencing using ribosomal markers enables the characterization of whole AMF communities. We have developed a method to capture AMF diversity at high resolution based on an AMF specific ~1.5 kb long ribosomal fragment spanning partial SSU, the ITS and partial LSU. The method allowed precise monitoring of AMF communities in different soil and plant root environments, enables tracing the inoculation of AMF strains into soils and also quantification of the effect of inoculation on native AMF communities. We were able to follow inoculations of *Rhizoglomus irregularis* to a field soils and we found that while inoculation did not affect the size it can affect the composition of the AMF community. Compositional changes could range up to a degree where the native *Rhizoglomus* strains where largely replaced the inoculated strain. The novel tool allows investigating AMF biogeography with unprecedented resolution, which in turn permit taxonomy-based and targeted AMF inoculations for optimizing inoculation success.

**Coraline Praz,** Institute of Plant Biology, Uni Zürich

**Transcriptome analysis of the wheat/wheat powdery mildew interaction**

*Blumeria graminis* f. sp. *tritici* is a pathogen causing powdery mildew of wheat. It is an obligate biotrophic fungus with high host specificity. In this interaction, effectors are believed to play an important role in suppressing wheat immunity and facilitating host reprogramming for nutrient uptake.

It is proposed that the genetic background, effector content, and transcriptional programs together play a role in tuning the virulence of the pathogen.

In this work, the nature and level of changes in gene expression upon wheat infection with three powdery mildew isolates are studied. An RNAseq approach was used to study the compatible interaction during formation of “haustorium”: a feeding structure mediating successful pathogen infection and disease progression. Sequencing data were generated from (i) uninfected wheat leaves, and (ii) wheat leaves infected with the mildew isolates 96224, JIW2 and 94202. On the pathogen side, we found significant differences between isolates in the nature and level of expression of effector genes suggesting isolate specific regulatory programs. Effectors are statistically overrepresented in highly expressed genes suggesting that the fungus is investing massively in their expression at this stage of the infection. They are also statistically overrepresented among the most differentially expressed genes between isolates. We propose that these differences between isolates could be associated to the fitness of the pathogen or to race specificity. Finally, we found that most of the differences are due to a few effector gene families suggesting that these families are more important for virulence at this stage than others. Based on this observation, we hypothesize that different effectors families distinctly contribute to different phases of disease development.

**Lucrezia Comensoli**, Institute of Biology, Uni Neuchâtel

**The art of survival to the rescue of artworks**

Without any conservation-restoration intervention copper-based and iron-based artworks are affected by corrosion that leads to structural modification and finally to irreversible damage. The conservation-restoration methods available for these two metals perform poorly concerning efficiency and time and in addition rely on toxic substances. The two projects presented here propose sustainable alternatives exploiting fungi to stabilize copper or iron corrosion layers. We take advantage of natural resistance mechanism to withstand harsh environmental conditions, exploiting the art of survival to preserve artworks.

For copper-based surfaces, the biological stabilization method is based on the use of a specific fungal strain isolated from vineyard soils highly contaminated with copper. Performance of the fungal treatment in terms of homogeneity, color variation, corrosion passivation and long-term behavior, were monitored. Very promising results were obtained and are now validated on real case studies such as archaeological objects and outdoor sculptures. An overview of the main results obtained during this 10-year research will be presented.

Treatment of iron-based objects relies on the study of alkaliphilic and/or alkalitolerant fungi that are able to tolerate chlorine and would actively remove this element from archaeological iron objects. In fact chlorine causes active corrosion that leads to a complete destruction of the object. Preliminary results demonstrating the ability of some fungi to uptake chlorine and to produce stable iron biominerals like iron oxalates will be presented.

The results obtained show that the remarkable survival capabilities of fungi have the potential to stabilize the corrosion layers of copper and iron objects in order to preserve artworks to be transmitted through generations.

## List of participants:

| Family name | First name | Institution - Town | E-mail |
|---|---|---|---|
| Aebi | Markus | ETH Zürich - Hönggerberg | aebi@micro.biol.ethz.ch, |
| Beenken | Ludwig | WSL Birmensdorf | ludwig.beenken@wsl.ch, |
| Berndt | Reinhard | ETH Zürich | reinhard.berndt@env.ethz.ch, |
| Bindschedler | Saskia | Uni Neuchâtel | saskia.bindschedler@unine.ch, |
| Blauenstein | Helene | WSL Birmensdorf | helene.blauenstein@wsl.ch, |
| Bourras | Salim | Uni Zürich | s.bourras@botinst.uzh.ch, |
| Brännhage | Jonas | WSL Birmensdorf | jonas.braennhage@wsl.ch, |
| Brunner | Ivano | WSL Birmensdorf | ivano.brunner@wsl.ch, |
| Brunner | Patrick | ETH Zürich | patrick.brunner@usys.ethz.ch, |
| Comensoli | Lucrezia | Uni Neuchâtel | lucrezia.comensoli@unine.ch, |
| Cornejo | Carolina | WSL Birmensdorf | carolina.cornejo@wsl.ch, |
| Croll | Daniel | ETH Zürich | daniel.croll@usys.ethz.ch, |
| de Freitas Pereira | Maíra | WSL Birmensdorf | mairadefreitaspereira@gmail.com, |
| De Vrieze | Mout | Agroscope Wädenswil | mout.devrieze@agroscope.admin.ch, |
| Degrune | Florine | WSL Birmensdorf | florine.degrune@wsl.ch, |
| Dennert | Francesca | ETH Zürich | francesca.dennert@usys.ethz.ch, |
| Dubach | Vivanne | ETH Zürich | vdubach@usys.ethz.ch, |
| Duffy | Brion | ZHAW Wädenswil | dufy@zhaw.ch, |
| Egli | Simon | WSL Birmensdorf | simon.egli@wsl.ch, |
| Enkerli | Jürg | Agroscope Reckenholz | juerg.enkerli@agroscope.admin.ch, |
| Fässler | Fabio | WSL Birmensdorf | fabiof@student.ethz.ch, |
| Fiori | Gioele | ETH Zürich | gfiori@student.ethz.ch, |
| Fouche | Simone | ETH Zürich | simone.fouche@usys.ethz.ch, |
| Freimoser | Florian | Agroscope Wädenswil | florian.freimoser@agroscope.admin.ch, |
| Frey | Beat | WSL Birmensdorf | beat.frey@wsl.ch, |
| Frossard | Aline | WSL Birmensdorf | aline.frossard@wsl.ch, |
| Gall | Anja | ETH Zürich | anja.gall@usys.ethz.ch, |
| Gamper | Hannes | ETH Zürich - Lindau | hannes.gamper@usys.ethz.ch, |
| Gloor | Ramona | Agroscope Wädenswil | ramona.gloor@agroscope.admin.ch, |
| Gross | Andrin | ETH Zürich | andrin.gross@env.ethz.ch, |
| Gschwend | Florian | Agroscope Reckenholz | florian.gschwend@agroscope.admin.ch, |
| Hartmann | Martin | WSL Birmensdorf | martin.hartmann@wsl.ch, |
| Hartmann | Fanny | ETH Zürich | fanny.hartmann@usys.ethz.ch, |
| Heinzelmann | Renate | WSL Birmensdorf | renate.heinzelmann@wsl.ch, |
| Herve | Vincent | Uni Neuchâtel | vincent.herve@unine.ch, |
| Herzog | Claude | WSL Birmensdorf | claude.herzog@wsl.ch, |
| Holdenrieder | Ottmar | ETH Zürich | ottmar.holdenrieder@env.ethz.ch, |
| Honegger | Rosmarie | Uni Zürich | rohonegg@botinst.uzh.ch, |
| Jamil | Isha | Uni Neuchâtel | isha.jamil@unine.ch, |
| Junier | Pilar | Uni Neuchâtel | pilar.junier@unine.ch, |
| Kaiser | Deborah | Agroscope Reckenholz | deborah.kaiser@agroscope.admin.ch, |
| Kälin | Noemi | ETH Zürich - Hönggerberg | nokaelin@ethz.ch, |
| Kaufmann | Lydia | WSL Birmensdorf | lydia.kaufmann@wsl.ch, |
| Keller | Beat | Uni Zürich | bkeller@botinst.uzh.ch, |
| Kellner | Harald | TU Dresden - Zittau DE | hkellner@ihi-zittau.de, |
| Kombrink | Anja | ETH Zürich - Hönggerberg | koanja@ethz.ch, |
| Künzler | Markus | ETH Zürich - Hönggerberg | mkuenzle@ethz.ch, |
| Leibundgut-Landmann | Salome | Uni Zürich - Irchel | salome.leibundgut-landmann@uzh.ch, |
| Leuchtmann | Adrian | ETH Zürich | adrian.leuchtmann@env.ethz.ch, |
| Lindner | Stefan | Uni Zürich | stefan.lindner@botinst.uzh.ch, |
| Lohberger | Andrea | Uni Neuchâtel | Andrea.Lohberger@unine.ch, |
| Massana Codina | Josep | Agroscope Nyon | josep.massana.codina@gmail.com, |
| Mayerhofer | Johanna | Agroscope Reckenholz | johanna.mayerhofer@agroscope.admin.ch, |
| McNally | Kaitlin | Uni Zürich | kait.mcnally@botinst.uzh.ch, |
| Meile | Lukas | ETH Zürich | lukas.meile@usys.ethz.ch, |
| Meyer | Joana Beatrice | WSL Birmensdorf | joana.meyer@wsl.ch, |
| Michalecka | Monika | ZHAW Wädenswil | monika.michalecka@inhort.pl, |

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| | | | |
|---|---|---|---|
| Molinier | Virginie | WSL Birmensdorf | virginie.molinier@wsl.ch, |
| Müller | Marion | Uni Zürich | marion.mueller@botinst.uzh.ch, |
| Peter | Martina | WSL Birmensdorf | martina.peter@wsl.ch, |
| Plissonneau | Clémence | ETH Zürich | plissonc@ethz.ch, |
| Pothier | Joël | ZHAW Wädenswil | joel.pothier@zhaw.ch, |
| Praz | Coraline | Uni Zürich | coraline.praz@botinst.uzh.ch, |
| Prospero | Simone | WSL Birmensdorf | simone.prospero@wsl.ch, |
| Queloz | Valentin | WSL Birmensdorf | valentin.queloz@wsl.ch, |
| Rigling | Daniel | WSL Birmensdorf | daniel.rigling@wsl.ch, |
| Sanchez Vallet | Andrea | ETH Zürich | andrea.sanchez@usys.ethz.ch, |
| Sandoz | Frédéric Alexandre | Uni Neuchâtel | frederic.sandoz@unine.ch, |
| Sanglard | Dominique | Uni Lausanne | Dominique.Sanglard@chuv.ch, |
| Schlaeppi | Klaus | Agroscope Reckenholz | klaus.schlaeppi@agroscope.admin.ch, |
| Schlegel | Markus | ETH Zürich | markus.schlegel@env.ethz.ch, |
| Schneebeli | Sandra | WSL Birmensdorf | beel.sa511@gmail.com, |
| Schöbel | Corine | WSL Birmensdorf | corine.schoebel@wsl.ch, |
| Schütz | Lukas | Uni Basel | lukas.schuetz@unibas.ch, |
| Schwarz | Janine | WSL Birmensdorf | schwarja@ethz.ch, |
| Senn | Beatrice | WSL Birmensdorf | beatrice.senn@wsl.ch, |
| Sieber | Thomas | ETH Zürich | thomas.sieber@env.ethz.ch, |
| Simon | Anaële | Uni Neuchâtel | anaele.simon@unine.ch, |
| Solly | Emily | WSL Birmensdorf | emily.solly@wsl.ch, |
| Stanley | Claire | ETH Zürich - Hönggerberg | claire.stanley@chem.ethz.ch, |
| Stewart | Ethan | ETH Zürich | ethan.stewart@usys.ethz.ch, |
| Stöckli | Martina | ETH Zürich - Hönggerberg | smartina@ethz.ch, |
| Stroheker | Sophie | ETH Zürich | sophie.stroheker@usys.ethz.ch, |
| Stuerchler | Alessandra | ETH Zürich | astuerch@student.ethz.ch, |
| Svercel | Miro | Protabaco, Burg AG | msvercel@dannemann.com, |
| Tayyrov | Annageldi | ETH Zürich - Hönggerberg | tayyrova@ethz.ch, |
| Tsykun | Tetyana | WSL Birmensdorf | tetyana.tsykun@wsl.ch, |
| van der Heijden | Marcel | Agroscope Reckenholz | marcel.vanderheijden@agroscope.admin.ch, |
| Ventura | Yolanda | Uni Neuchâtel | yolanda.ventura@unine.ch, |
| Wenger | Sandra | ETH Zürich | wengers@student.ethz.ch, |
| Widmer | Franco | Agroscope Reckenholz | franco.widmer@agroscope.admin.ch, |
| Wyler | Michele | ETH Zürich - Lindau | mwyler@student.ethz.ch, |

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