Annex to the report

European Plant Science Organisation
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EPSO Workshop
Implementing a Plants and Microbiomes Strategy in Europe
Online, January 2021

Brussels, 1.9.2021

The 'Report with recommendations' as well as this 'Annex to the report' are available at https://epsoweb.org/working-groups/plants-and-microbiomes/ .

Annex I – Session reports

Theme 2: Addressing R&D bottlenecks - status of today | update recommendations |

Parallel Group 1 Report

Participants: Angela Sessitsch (chair); Sofie Thijs (rapporteur)

Harald Mikkelsen, Koppert, NL (d2)
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Joëlle Ronfort, INRAE, FR
Soledad Sacristan, UPM-INIA, Madrid, ES
Cristiana Sbrana, CNR, IT
Angela Sessitsch, AIT, AT
Ágnes Szebesi, HU
Sofie Thijs, Univ. Hasselt, BE
Corinne Vacher, INRAE, FR (apologies)
Marc Viñas, IRTA, ES
Mette Haubjerg Nicolaisen, Uni Copenhagen, DK
Živko Jovanović, Uni Belgrade, RS
Judith Lundberg-Felten, SLU, SE
Borjana Arsova, FZ Juelich, DE
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Dragana Nikolic, Univ. Belgrade, RS
Raffaella Balestrini, CNR, IT
Davide Pacifico, CNR, IT

R3 - Moving <u>from correlation to causation</u> under lab, greenhouse and field conditions

Timeframes: how can we translate what we observe in the lab, to what happens in the field. We currently look at short time frames (4-5 weeks of growth), how representative is this for the full growth cycle? We need to study the whole growth cycle, over different growth cycles, multi-annual studies in the field.

Recommendations: The ability to apply for re-current funding (not limited to 4 years), or a funding plan that secures long-term continuation (e.g. crop rotation takes 4 years, before a similar crop returns to the same field, if we want to study this aspect, we need multi-year plans).

How much can we translate from model plants to crops: you can use the model plant to study how long does the microbiome brings beneficial functions to the plant and use findings from the model plant to crops in the field. The microbes need to be persistent for some time to see the effect (related to the above).

Lab + field: For application we go to the field, discovery science we need to be in the lab. Joining up lab + field

Model plants + crops needed: for mechanism understanding, wheat is too complex, we need the model plants for mechanism understanding. Complex field = complex subject. So merge the two.

Study the effect of plant diversity on the plant microbiome interaction

Use the plant genetics

Understand rhizosphere competence: reductionist approaches and field scale experiments.

Insight in the microbes and the capacity to make use of it

Environmental gradients in the field (Marc Viñas): Environmental gradients in field experiments are challenging. Extra inputs of alochthonous microbial populations cause complexity for correlations to plant functions.

<u>Summary:</u>

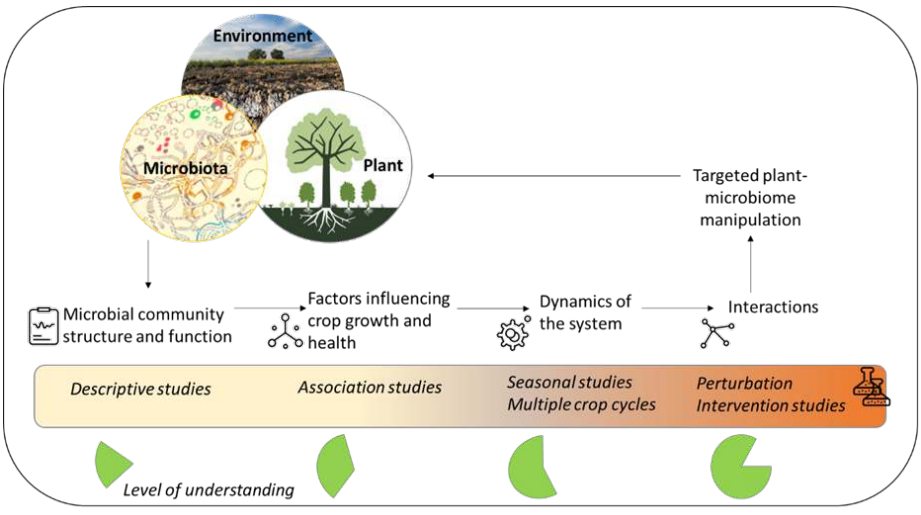
We need the molecular toolbox: plant genetics, mutants, access to syncoms, culture collections

Different microbiota communities: study the dynamics of the system, longer term experiments

Look at the plant diversity on the plant and microbiome interaction

Link molecular to ecological findings: robustness, rhizosphere competence, how long does a microbial function persists.

I tried to schematize it: from correlation to causation, we need to move away from doing (only) descriptive studies and do also more association studies (~MWAS, GWAS, factor analyses), and study the dynamics of the system (so longitudinal study designs, over crop cycles), and for mechanistic understanding, more perturbation, intervention, causal study designs are needed, to better understand the 'interactions' (plant diversity on the plant and microbiome interaction). Then we move from rather correlation to more causation and more predictable or targeted microbiome manipulation of our crops. It will give us competitive insights to efficiently identify the best practices to improve crop yield, health, taste, quality.



R4 - More understanding on the complexity of the ecosystem-plant-microbiome system is needed

Effect of inoculant on the residing microbes: look at the microbiome and combine with nutritional composition, what is the effect of the inoculant on the residing microbes. There is a relation between disease suppression and amount of nitrogen you apply. The test of inoculants on microbial communities is usually not published.

Maintain soil health in general: what is soil health from biotic and abiotic perspective, what do management processes have as effect on this. What does it mean stable microbial communities, on the functional levels, what are we measuring, what is soil health.

Do we need a (new) definition for soil health and what should it include?

Look at the full cycle: turnover of roots, litter, perennial plants. We are too much focussed on the first part of the growth. How can we ensure that crops give a reproducible effect from season to season. Instability of the phenotype, lack of mechanism. What part is stochastic and what part we do can take into control, manage, predict.

Characteristics of a resilient microbiome: what are the traits that a microbiome needs to have to be resilient to perturbations, how can we control that adding different inputs to the crop is going to affect the microbe or not.

Whole system model: plant, microbes, genes, nutrients in one model to better make a marker toolkit for making crops, soil, microbes, more resilient.

Time scale + spatial scale is important

Microbes + insects: Include also the insects in studies

Mono to polyculture: the transition from mono- to poly-culture (agroforestry) is a new challenge for holobiont studies (soil plant rhizosphere).

Linking plant diversity to microbial diversity

R5 - Plant mechanisms to attract / interact with microbiota require understanding

Link between basic and applied research: if we see a trait in the field, we can use model systems to spit it out, trace back. Which traits to focus on: those which are linked close to evolution (flowering time, fruit, seed size?). We need to pull in all the different aspects: microscopy also next to metabolomics, molecular mechanisms.

Root traits and phenotyping: we need to apply these lab-techniques also to soil and to microbes

Do not disregard the microbiota: Microbes in agriculture are extremely important, question is how to use it smartly. Current diagnostics in agriculture focus on plants and nutrients, but abuse of nitrogen leads to change in microbiota. How to get to a breeding system in which you implement the microbes back in. You need to have a microbial system to get the proper exudates as well. Resilience. A system or model where you combine/consider both biostimulants and nutritional composition.

Sustainability: in rice fields, some microbiota are active at different times related to exudates. Take into account the sustainability, greenhouse gas emissions from microbes, archaea (culture more archaea?)

We need plant molecular markers for breeding: root exudate engineering, regulation for the microbial community for soil is difficult. Use root exudates as a breeding target. Understanding microbial interactions that change the root exudates.

Plant genotypes matter: to maximise the microbe functions in the field.

Breeding ignores the microbial component

Developing methods to analyse exudates in soil-grown plants, see also https://www.sciencedirect.com/science/article/abs/pii/S0038071720300407"; Our expert in the field in the project is https://www.slu.se/en/ew-cv/sandra-jamtgard/

Going back to evolutionary pre-decessors (pre-selection): using CRISPR based approaches like done for tomato to create edible cultivar by editing wild species?

R7 - <u>Precompetitive research</u> should address the identification of microbiome-based plant health and resilience indicators and microbiome understanding needed by the industry

Soil disease suppressiveness: Best practices to take, what is needed to make a soil suppressive, that is a question from industry (see also Jos Raaijmakers, Wim van de Putten).

Inoculant predictability + best practice: prediction, based on certain indicators why you can apply it in soil 1, but it's not successful in soil 2. Best practice for application in the field (effectiveness varies with season, multiple applications, timing of applications): to better explain or reduce observed-effect variabilities.

•Precision farming: fertilise plants + modelling. Too few mathematicians, statisticians, access to raw datasets and metadata

Suggestion: We have currently within our university a specific type of grant which stimulates to work with (as many) groups or institutions together to solve one 'sustainability issue' at the cross-border of our institutes. The project which shows to be the most interdisciplinary, and proof complementarity between partners (not data go from partner A, to B to C), gets the grant. This can be a good format to perhaps roll out larger? Horizon calls are already interdisciplinary 'in nature', but perhaps something can be included in the call text to stimulate cross-institutional collaboration (as we need more statisticians, informaticians to make our complex modelling systems for crops feasible?)

On another note: We have a new educational master called 'materiomics' (to make a new generation of students, cross-breed between material sciences, engineers, and physics). What about asking for a 'phytobiomics master' to deliver students of the future, getting a tailored curriculum, who can introduce or deliver the concept that we need microbes in agricultural systems (as now the microbes are largely neglected because the agronomists have 'never' heard of it). Agency of Nature and Forest, comes with a similar program 'Forests for Health Coaches', an Erasmus program with the same goal, to educate people in what sustainable forestry is (cfr. what is needed to maintain soil health in agriculture?)

Rhizosphere competence: is important for industry. What to select for, at which time to add.

Soil health indicators: We need soil health indicators (capacity for carbon sequestration, use profile of volatile compounds as indicators for soil health, core microbiome as an indicator)

Parallel Group 2 Report

Participants: Paul Schulze-Lefert (chair), Robert Koller (rapporteur)

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Rita Grosch, IGZ, DE
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Gabriel Castrillo, Univ. Nottingham, UK
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Islam Abd Eldaim, AIT, AT
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Marcel van der Heijden, Agroscope, CH
Martin Hartmann, ETH, CH
Merike Somera, Tallinn Univ. Technol., EE
Michael Schloter, Helmholtz Center Munich, DE
Paul Schulze-Lefert, Max-Planck-Inst., DE
Robert Koller, Forschungszentrum Jülich, DE
Alessandra Turrini, Univ. Pisa, IT (d2)
Anna Maria Pirttilä, Univ. Oulu, FI
Giovanni Bubici, CNR, IT

Erica Lumini, CNR, IT

R3 - Moving <u>from correlation to causation</u> under lab, greenhouse and field conditions

Idea (e.g. recently gained knowledge / publications/ other research areas) to fill the gap/ tackle the challenge:

- Culture collection exists and can be used to establish causal links to a given microbial or plant trait. These links may either indirect or direct, like for pathogen protection and mineral uptake.
- Apply more computational approaches (modelling) that can help to move from correlation to causation
- Development of genetic/molecular tool box to validate interactions:
  - Mutant development: for proving genetic implication into interactions.
  - Tools to track beneficial traits in a community (not only in mono-association) to check if it is maintained and if the phenotype is stable
- Local and Temporal aspects of rhizosphere (plants and microbial perspective) interactions should be further acknowledged
- Elucidate the role of invasion and persistence for stabilizing the function of a beneficial association / or to better understand beneficial association
- Develop (plant) stress conditions to validate microbial traits and plant phenotypes before going to the field
- Link 'genetic/molecular' with 'ecological' scientific communities
- hierarchical approach to link field and lab, e.g. how is the microbiome communities is assembled in different plant species
- In the lab we may identify to causation. But what determines the robustness of a specific link or trait? Robustness of such a link/ trait may be key for understanding when transferred to the field.

⇨ <u>Recommendations / conclusions:</u>

- We need more "genetics/molecular" tools in the field to overcome the correlation-mechanism gap
- No single 'best' microbial community exists: different microbial communities for different plant hosts and abiotic conditions should be considered
- dynamics and different activities within a plant microbiome need to be considered
- We need to further link 'genetic/molecular' with 'ecological' scientific communities to make progress in conceptual approaches and competences

R4 - More understanding on the complexity of the <u>ecosystem</u>-plant-microbiome system is needed

Idea (e.g. recently gained knowledge / publications/ other research areas) to fill the gap/ tackle the challenge:

- Integration of abiotic conditions e.g. climate in order to understand where to apply the product.
- Intermediate steps between lab and field are needed. Potential approaches are
  - 'Garden' / field experiments across Europe
  - Glasshouse environments
- Ecological principles 'may guide approaches'
- Strains specific resolution in field experiments are needed to link lab and field
- Microorganisms are under evolutional pressure on the species level and this is not much acknowledged, especially in the field
- Spatio-temporal / Functional aspects should be considered

⇨ Recommendations / conclusions:
- Further develop the link between 'reductionistic' and 'ecological' scientific communities
- 'hierarchical approach' to link field and lab, e.g. how are the microbiome communities (specially) assembled in different plant species (approaches from both perspective, i.e. plant and microbiome as driver)
- Food web implications to understand microbiome structure ('interkingdom') and function in the long run.
- On the other hand, we need to increase resolution of microbial communities as far as possible
- Garden experiments in between field and lab: define 'bridging' plant species to compare approaches across Europe
- Develop and promote management advices to improve microbiota-plant interactions for stakeholders/ farmers

R5 - Plant mechanisms to attract / interact with microbiota require understanding

Idea (e.g. recently gained knowledge / publications/ other research areas) to fill the gap/ tackle the challenge:
- Metabolite networks needs to be more investigated (for example in the framework of abiotic stress, pathogen defence and mineral nutrition)
- Ionome and metabolome analysis are complex, but technologies are available for analysis. Interactions of Ionome and metabolome are key in the R5 and need to be further developed
- our scientific community may further learn from human microbiome approaches in this context
- Further genetic approaches are necessary to improve understanding of mechanisms

⇨ Recommendations/conclusions:
- Develop a combined plant-microbiota metabolism approach is key for understanding interactions and needs to be further developed
- technologies linking Ionome and metabolome needs further be developed (including computational approaches for predictions)

R7 - Precompetitive research should address the identification of microbiome-based plant health and resilience indicators and microbiome understanding needed by the industry

Idea (e.g. recently gained knowledge / publications/ other research areas) to fill the gap/ tackle the challenge:
- Economic perspective: customisation of biological fertilizers needs to be economically relevant
- Industry may help to improve the robustness of microbial communities (e.g. by developing additives / formulation)
- Products should not be disappointing
  - Microbiome management: industry needs to consider the quality of products transferred to the marked
  - Products could first be tested in and applied under (less complex) greenhouse conditions
- Knowledge precedes application

⇨ Recommendations/conclusions:
- Develop tool box (strains, plants, soil, abiotic conditions) for specific farming areas / regional conditions
- Standards for microbial products needs to be set (➔ this topic needs to be further discussed in one of the next meeting of the working group)

Theme 3: Addressing infrastructure bottlenecks & next steps

Participants: all; Moderated by Corné Pieterse, Rapporteur: Islam Abd El-Daim

R17-European infrastructure recommended for plant microbiome research

Developing EU infrastructure for the following **aspects (a-c):**

a) ***Common EU repositories for cultures collections***

Suggestions:

- Establish protocols to link the already available national culture collections together
- Establish a common EU metadata for each strain ''strain passport'' deposited on national culture collection to facilitate access to strains
- Consider the developing of a genome-based culture collection where the strain genome data will be digitally stored and only physically store the reference strain
- Improve protocols for strains storage

Recommendation and task: make strong efforts to develop the EU culture collections. ♦ Paul and Angela will draft a concept to discuss with concerned parties (via Robert K) and RI funders (national and Biodiversity partnership)

b) ***EU phenotyping platforms for microbiome related experiments***

Suggestions:

- Establish new systems for high throughput phenotyping of plant microbiome related research in the EU
- Use the currently available Plant Phenotyping networks (EPPN, national PNs) to use and adapt the existing facilities more for microbiomes related experiments
- Invite industry specially plant breeding firms to discuss potential cooperation in field testing
- Discuss how a compromise could be found to make results from microbiome work publicly accessible

Recommendation and task: ♦ Robert and Paul facilitate better collaboration with two existing networks / facilities, draft 1-page concept and invite a representative to the next WG meeting

c) ***Protocols for metabolite profiles***

Suggestions:

- Develop common protocols to improve metabolites profiling specially for root exudates

Recommendation and task: ♦ All interested WG members discuss with ♦ Harro B who will lead the exchange and development of a concept (including protocols) and report back at the next WG meeting.

R15 - Open access databases integrating (plant) microbiome and meta-data are required

The following aspects were discussed

- Establishing a common EU database to deposit tools and bioinformatics pipelines used for microbiome analysis (e.g. Elixir database)
- Establish a common EU database for plant markers

- Consider bilateral collaboration between countries (not only on the EU level) to run open access databases

Recommendation and task:

♦ Corné facilitates combining own datasets at European level, drafting of one-page concept

- Step 1: ♦ Harald M, Mette HN and Paul SL link datasets from their respective national projects / programmes and discuss with their national funders a joint effort
- Step 2: ♦ More interested WG members from other countries join in and discuss with their national funders to join the effort

Annex II Workshop participants and programme

Workshop participants (d1 = day 1; d2 = day 2)

Islam Abd El-Daim, AIT, AT	Karin Metzlaff, EPSO
Hanna-Leena Alakomi, VTT, FI	Harald Mikkelsen, Koppert, NL (d2)
Magdalena Arasimowicz-Jelonek, AM Univ, PL (d1)	Christophe Mougel, INRAE, FR
	Lionel Moulin, IRD, FR (d1)
Borjana Arsova, FZ Juelich, DE	Giuseppina Mulè, CNR, IT (d1)
Pruthvi Balachandra, SLU, SE (d1)	Federico Navarro Garcia, UCM, ES
Raffaella Balestrini, CNR, IT	Dragana Nicolic, Uni Belgrade, SB
Loredana Barbarossa, CNR, IT (d1)	Davide Pacifico, CNR, IT (d1)
Carmen Bianco, CNR, IT (d1)	Kalliope Papadopoulou, Uni Thessaly, GR
Harro Bouwmeester, Uni Amsterdam, NL	Anna Maria Pirttilä, Uni Oulu, FI
Giovanni Bubici, CNR, IT	Corné Pieterse, Utrecht Univ, NL
Gabriel Castrillo, Univ. Nottingham, UK	Maruša Pompe-Novak, Natl. Inst. Biology, SI (d1)
Lemong Dong, Uni Amsterdam, NL	
Hilde Eggermont, Bioversity, BE (d1)	Mandeep Poudel, NMBU, NO (d1)
Kerrie Farrar, Aberystwyth Univ, UK	Simona Radutoiu, Uni Aarhus, DK
Morina Filis, CZ Acad Sci, CZ	Joëlle Ronfort, INRAE, FR
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Alfred Grand, Grand Farm, AT (d1)	Paul Schulze-Lefert, MPIZ, DE
Kristina Gruden, Uni Ljubljana, SI	Angela Sessitsch, AIT, AT
Rita Grosch, IGZ, DE	Merike Somera, Tallin UT, EE
Martin Hartmann, ETH Zurich, CH	Ágnes Szebesi, HU
Mette Haubjerg Nicolaisen, Uni Copenhagen, DK	Sofie Thijs, Uni. Hasselt, BE
	Friederike Trognitz, AIT, AT
Maria Adelaide Iannelli, CNR, IT	Alessandra Turrini, Univ. Pisa, IT (d2)
Valentina Iori, CNR, IT (d1)	Corinne Vacher, INRAE, FR (apologies)
Živko Jovanović, Uni Belgrade, RS	Marcel van der Heijden, Agroscope, CH
Agnieszka Klonowska, IRD, FR (d1)	Jaco Vangronsfeld, Uni Hasselt, BE (d1)
Robert Koller, FZ Juelich, DE	Ramesh Vetukuri, SLU, SE (d1)
Hendrik Kuepper, CZ Acad Sci, CZ (d1)	Marc Viñas, IRTA, ES
Erica Lumini, CNR, IT	Anouk Zancarini, Uni Amsterdam, NL
Judith Lundberg-Felten, SLU, SE	

Workshop programme

DAY 1 (09:00 – 12:00)

- **Welcome** by Angela Sessitsch, Paul Schulze-Lefert and Karin Metzlaff

- **Theme 1: First highlights from (multi)national plants and microbiomes initiatives** (09:15 – 10:45) moderated by Paul Schulze-Lefert
    - Welcome
    - Main R&I initiatives in DE, DK, NL – highlights and update (20 min each: 15' + 5')
    - Paul Schulze-Lefert, Mette Haubjerg Nicolaisen, Harro Bouwmeester
    - Member States and EU initiatives: Soil health and food mission (10' scope + 10' discussion)
    - Alfred Grand / AT (Grand Farm; Member of the EU Mission *Soil and Food Health*)

- **T1:** First highlights from (multi)national plants and microbiomes initiatives cont. (11:15 – 11:50) moderated by Karin Metzlaff
    - Member States & EU initiatives cont.: Biodiversity partnership, Agroecology partnership (20 min each: 10' scope + 10' discussion)
    - Hilde Eggermont (Belgian Biodiversity Platform), Chantal Gascuel (INRAe*)*

- **Instructions for day 2** by Angela Sessitsch **(**11:55 – 12:00)

DAY 2 (09:00 – 13:15)

- **Introduction to Themes 2 and 3** (09:00 - 09:15) by Paul Schulze-Lefert

- **Theme 2: Addressing R&D bottlenecks** (09:15 - 10:00)
  Further discuss recommendations from 2nd Workshop and how best advance these by developing specific and representative cases, which can be used for recommendations:
  Group 1 (Chair 1: Angela Sessitsch; Rapporteur: Sofie Thijs):
  (A. Sessitsch, S. Thijs, H.-L. Alakomi, B. Arsova, R. Balestrini, L. Dong, K. Farrar, K. Gruden, M. Haubjerg Nicolaisen, M. A. Iannelli, CNR, IT Ž. Jovanović, J. Lundberg-Felten, C. Pieterse, H. Mikkelsen, D. Nikolic, D. Pacifico, K. Papadopoulou, A.-M. Pirttilä, S. Radutoiu, J. Ronfort, S. Sacristan, C. Sbrana, Á. Szebesi, M. Viñas)
    - **R3-Moving from correlation to causation under lab, greenhouse and field conditions**
    - **R4-More understanding on the complexity of the ecosystem-plant-microbiome system is needed**

  Group 2 (Chair 2: Paul Schulze-Lefert; Rapporteur: Robert Koller):
  (P. Schulze-Lefert, R. Koller, I. Abd El-Daim, H. Bouwmeester, G. Bubici, G. Castrillo, M. Hartmann, K. Metzlaff, C. Mougel, F. Navarro Garcia, M. Schloter, M. Somera, F. Trognitz, M. van der Heijden, A. Turrini, A. Zancarini)
    - **R5-Plant mechanisms to attract / interact with microbiota require understanding**
    - **R7-Precompetitive research should address the identification of microbiome-based plant health and resilience indicators and microbiome understanding needed by the industry**

- **Theme 2:** Addressing R&D bottlenecks cont. (10:00 - 10:45)
  Further discuss recommendations from 2nd Workshop and how best advance these by developing specific and representative cases, which can be used for recommendations:
  Group 2 (Chair 2: Paul Schulze-Lefert; Rapporteur: Robert Koller):
    - R3-Moving from correlation to causation under lab, greenhouse and field conditions
    - R4-More understanding on the complexity of the ecosystem-plant-microbiome system is needed

  Group 1 (Chair 1: Angela Sessitsch; Rapporteur: Sofie Thijs):
    - R5-Plant mechanisms to attract / interact with microbiota require understanding
    - R7-Precompetitive research should address the identification of microbiome-based plant health and resilience indicators and microbiome understanding needed by the industry

- **Theme 2:** Addressing R&D bottlenecks & next steps **cont**. (11:15 – 11:45)
  Reports (by the rapporteurs) to the plenary and agree next steps (Angela Sessitsch, Paul Schulze-Lefert and Karin Metzlaff)

- **Theme 3: Addressing infrastructure bottlenecks & next steps** (11:45 – 13:00)
  Moderated by Corné Pieterse, Rapporteur: Islam Abd El-Daim
  Further discuss recommendations from 2nd Workshop and how to best advance these:
    - **R17-European infrastructure recommended for plant microbiome research**
    - **R15-Open access databases integrating (plant) microbiome and meta-data are required**
    - **All plenary, no breakout sessions**

- **AOB** (13:00 – 13:15) (discuss progress / ideas regarding the other recommendations as appropriate) and **closure** by Angela Sessitsch, Paul Schulze-Lefert and Karin Metzlaff