




How to tackle standardization issues in the microbiome field

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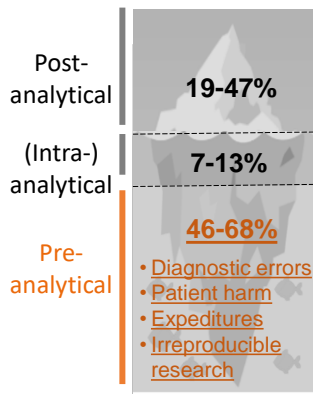


 This project has received funding from the European Union's Horizon Europe research and innovation programme under grant agreement No. 101094353

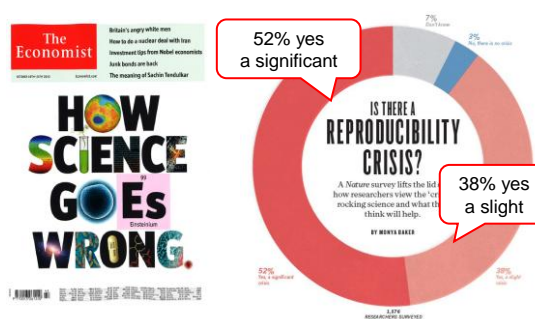


Why standardization & focus on pre-analytical phase?

Diagnostic testing process (%):



Research & development:



very critical but often neglected or underestimated

also in the microbiome field

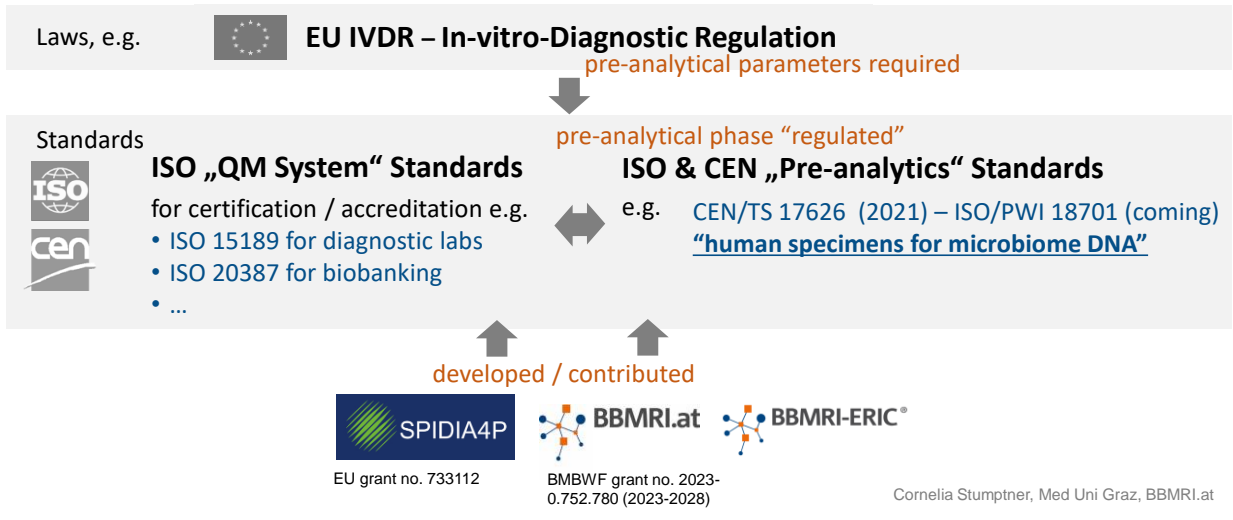
FREEDMAN LP et al, PLoS Biol 13(6): (2015) doi.org/10.1371/journal.pbio.1002165
 Economist, *How science goes wrong* (2013)
 BAKER M., Nature (2016) doi.org/10.1038/533452a

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 Green SF. Clin Biochem, (2013) <https://doi.org/10.1016/j.clinbiochem.2013.06.001>

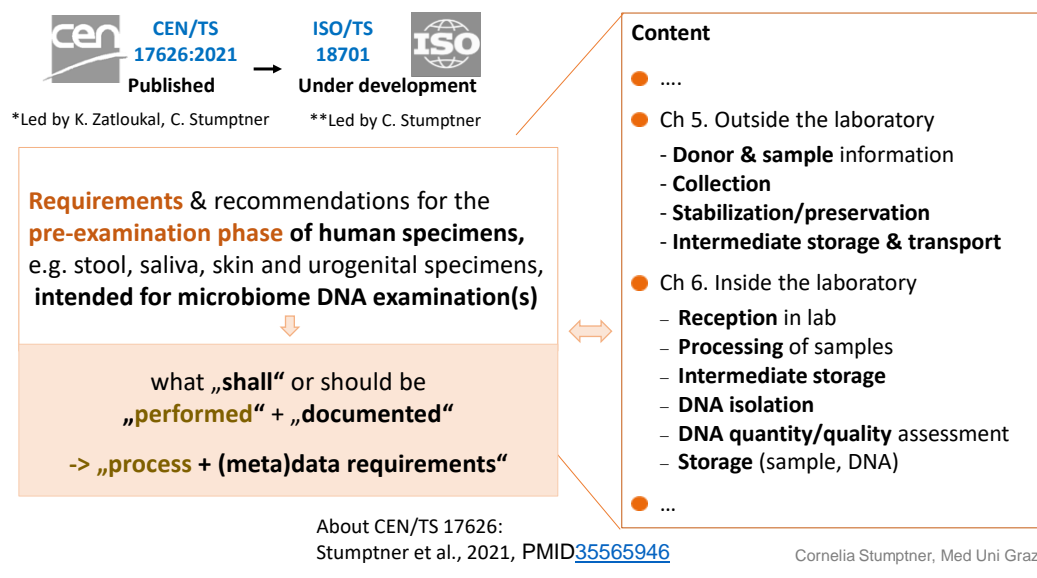
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Reducing pre-analytical errors



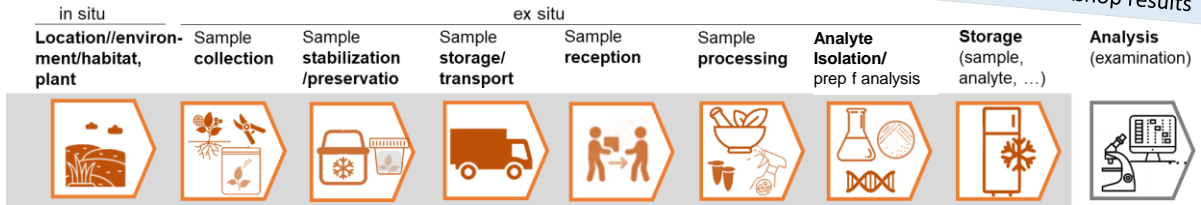
Molecular in vitro diagnostic examinations — Specifications for pre-examination processes for human specimen — Isolated microbiome DNA



Pre-analytical phase & variables – plant microbiome field



Incl. MICROBE/EPSo workshop results



Major pre-analytical factors that can have a dramatic effect on the analysis result

Location/environment/habitat/plant	Collection	Preservation	Storage/Transport	Processing	DNA extraction
<ul style="list-style-type: none"> • Geography (GPS) • Climate/weather • Agric./plant managem. • Former weather • Plant genetics, • Plant devel. stage • Plant health/disease • Soil • (Legal/regulatory) • ... 	<ul style="list-style-type: none"> • C. site heterogeneity • C. procedure • C. date/time • C. container • Plant heterogeneity • Weather during C. • Labelling • ... 	<ul style="list-style-type: none"> • Time-point (in field or lab) • Method • Reagents • Temp. • ... 	<ul style="list-style-type: none"> • Duration • Condition (Temp., ...) 	<ul style="list-style-type: none"> • Homogenization • Sterilization, ... • Aliquotting • ... 	<ul style="list-style-type: none"> • Method/Kit/reagents • Plant cell lysis • Microbiome lysis • Inhibitors • Host DNA • ..
<ul style="list-style-type: none"> • (Cross-)contamination 					

Posters
546 (8C)
549 (3A)

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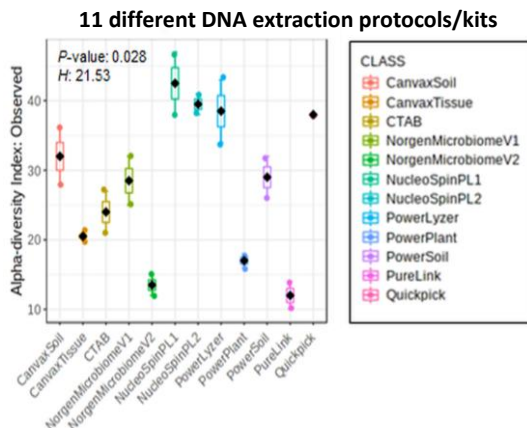
The importance of quality-controlled workflows & comprehensive metadata – plant microbiome field



Selected examples from literature

The effect of microbiome DNA extraction protocols/kits

⇒ DNA extraction protocol significantly affected bacterial community assessment



Richness alpha diversity index showed significant differences ($P < 0.044$) among the different DNA extraction methods applied to Olive xylem sap (i.e. internal fluid in olive tree tissue)

Part of fig. 1 from Haro et al., 2021 doi.org/10.3389/fpls.2021.640829

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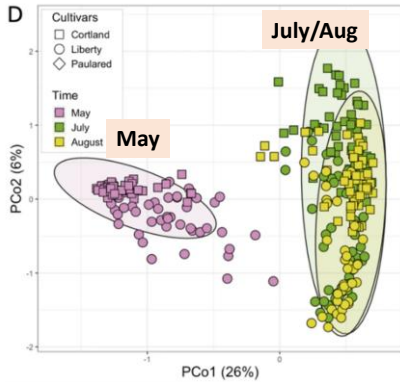
The importance of quality-controlled workflows & comprehensive metadata – plant microbiome field



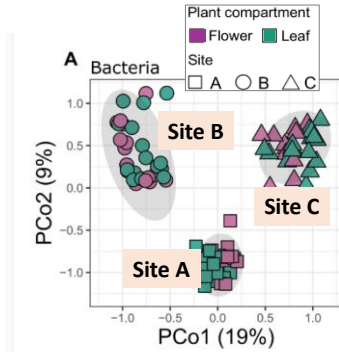
Effect of sampling time

Effect of site

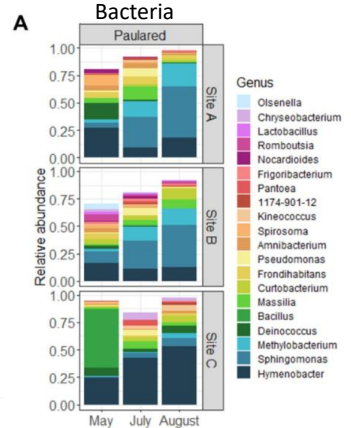
⇒ Time and site are strong drivers of bacterial community structure



(D) PCoA on leaf samples from apple trees ($n=292$) showing variation through time



(PCoA) on apple tree flower and leaf samples collected in May (45 trees) at different sites A,B,C



Leaf microbial community composition (A Bacteria); (May) same cultivar - different sites A,B,C

Parts of Fig. 1 from Boutin, 2025 (doi.org/10.1038/s41598-025-10729-0); & Fig. 1+3 from Boutin, 2022 (doi.org/10.1139/cjm-2023-0215)

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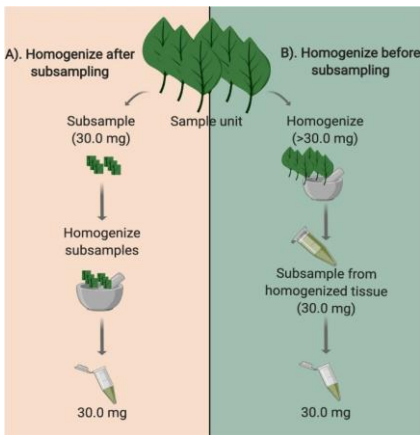
The importance of quality-controlled workflows & comprehensive metadata – plant microbiome field



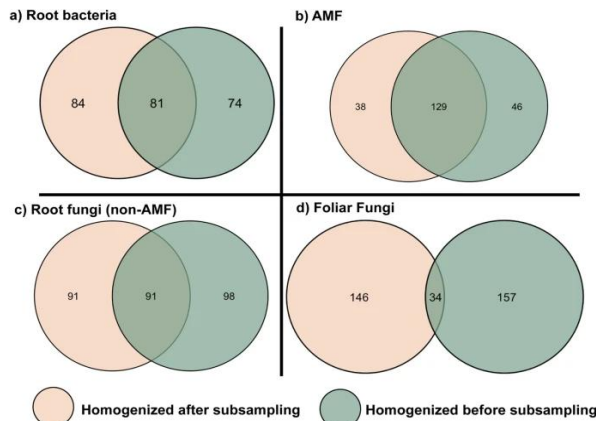
Selected examples from literature

The effect of plant sample processing

⇒ Different sample processing strategies yield different microbial communities



Bullington/Lekberg/Larkin, 2021 (doi.org/10.1038/s41598-021-83153-9)



Overlap of sequence variants (SVs) recovered from milkweed plants at a single site, when subsampling microbial communities using two different strategies.

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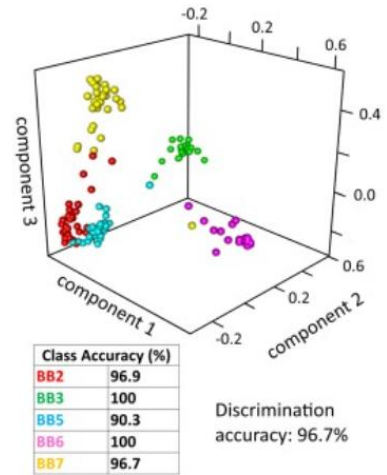
Pre-analytical effects – multi-centre study (human)



- Same sample types (blood -> **serum**)
- Collected & processed at different sites
- Metabolome analyzed at 1 site

Inter-centre variation significantly greater than inter-individual variations

Serum metabolomics RF spectra



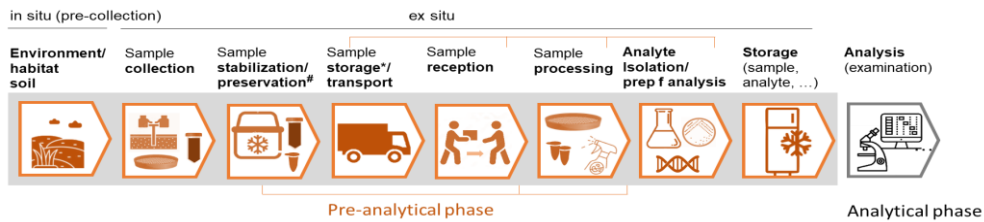
V Ghini (2022) DOI [10.1016/j.nbt.2022.01.006](https://doi.org/10.1016/j.nbt.2022.01.006) BBMRI-LPC project

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Pre-analytical workflow – ideal & reality

Ideal - simplified



Reality –

- Many pre-analytical steps
- In different order
- Several intermediate storage steps (different conditions/durations)
- Different methods
- + procedures
- + reagents (types, age)
- + handling
- ..
- Analysis

Workflows need to be harmonized/standardized, documented, and transparent

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(Pre-)analytical effects – multi-centre study (soil microbiome)

Unpublished data

MICROBE
the Microbiome
Biobanking (R1)
Enabler

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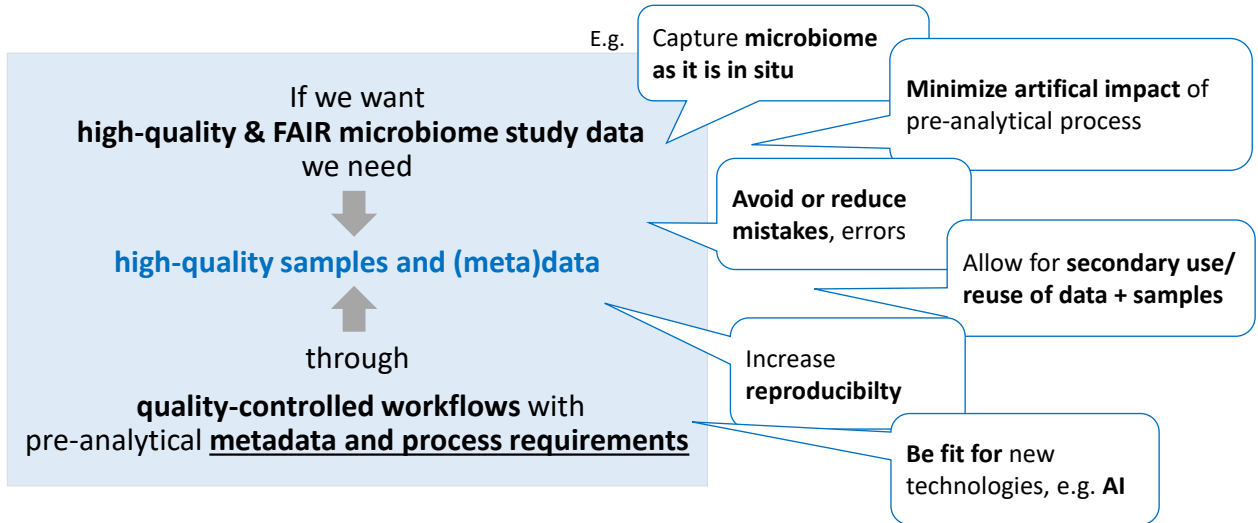
Not every pre-analytical variable can be controlled ...

Unpublished data

MICROBE
the Microbiome
Biobanking (R1)
Enabler



The need for pre-analytical metadata & process requirements



Thank you to all workshop participants!



Guidance document for sample pre-analytics for plant microbiome studies



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Image: <https://www.gutmicrobiotaforhealth.com/meet-your-microbiome-when-science-comes-to-youtube/rencontrez-votre-microbiome-quand-la-science-debarque-sur-youtube/>

Acknowledgements:

MICROBE project	Others

What to do?



Detailed study design

- aim
- entire workflow
- (pre-)analytical variables
- standards/guidelines



Risk assessment

- likelihood of impact
- + mitigation



- Use validated methods/
reagents/kits
(where available)



SOPs

- for all workflow steps;
- serve as **instructions** for use



Test

- where effects are unclear
- make upfront smaller tests



Meta(data)

- documentation



- Education, staff training and
supervision of junior staff by
seniors



- Consider the **entire workflow and variables**;
- Follow **standards/**
guidelines