



ACTIONr: Research Action Network for Reducing Reactive Nitrogen Losses from Agricultural Ecosystems

Grant Agreement No. 101079299

Document Type:	Agenda
Workshop ID:	Training Workshop: <i>Microbial Diversity Analysis with High-throughput (HT) Sequencing of PCR Amplicons</i>
Date of Workshop:	3-9 January 2024
Workshop Venue:	Teleconference Room, 1st floor, Department of Biochemistry and Biotechnology, University of Thessaly, Viopolis, 41500 Larissa, Greece
Workshop URL:	https://teams.microsoft.com/l/team/19%3awGF P1bE1LlIXsSsnUs5sl9aElUxNlItIp6EwRntEqr01%40thread.tacv2/conversations?groupId=7e3bd339-cad0-4248-b4ac-ccddd7330b89&tenantId=3180bf70-17cc-44f6-90a4-5c9476625295
Workshop access code:	r18h122
Workshop organized by:	University of Thessaly (UTH)
Workshop Facilitator:	Dr Sotirios VASILEIADIS



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Agenda

03-09 January 2024

Day	Description
03 January 09:00 -17:00	Background on microbiome analysis with metabarcoding & R/RStudio basics <ul style="list-style-type: none">• Microbiome analysis<ul style="list-style-type: none">○ The past and the era of big data○ Generations of sequencing: Chemistry, errors and uses○ Next generation sequencing (NGS) of marker gene amplicons (MGA): from sampling to raw data, to data quality control, to annotation and microbial data matrices.○ Units of phylogenetic markers: OTU, phylotype, genotype/ASV○ Quantitative/qualitative ecological aspects: alpha/beta diversity, core microbiome, differential microbial data features, correlations (between phylogenetic markers and environmental variables/features)○ Random forests for assessing and predicting classification of samples and assessing importance of microorganisms/features• Next generation sequencing (NGS) of marker gene amplicons (MGA) with R<ul style="list-style-type: none">○ Why R? Intro to R, installation of tools for working with R, R objects, packages
04 January 09:00 -17:00	R/RStudio basics hands on <ul style="list-style-type: none">• R coding bootcamp<ul style="list-style-type: none">○ R coding bootcamp (1): R as a calculator, installing/loading packages, objects, variables, operators and operations, location, command structure, getting help○ R coding bootcamp (2): Data types, conditional statements○ R coding bootcamp (3): loops, building a function, illustrations

<p>05 January</p> <p>09:00 -17:00</p>	<p>R/RStudio basics hands on, and hands on at microbiome analysis with R</p> <ul style="list-style-type: none"> • MGA analysis hands on <ul style="list-style-type: none"> ○ MGA analysis hands on (1): Generating an ASV table with dada2 and preparing a phyloseq object ○ MGA analysis hands on (2): Reducing the dataset and assessing treatment effect with random forests ○ MGA analysis hands on (3): Alpha diversity analysis with ANOVA and non-parametric equivalents; multivariate approaches (hierarchical clustering, nMDS, assessing treatment effects with CCA/RDA and PERMANOVA) for beta diversity ○ MGA analysis hands on (4): Differential abundance of taxa/features between treatments, core microbiomes, correlations among features and between features and parameters
<p>06 & 07 January</p>	<p>Break...for the tutor only!</p>
<p>08 & 09 January</p> <p>09:00 -17:00</p>	<p>Microbiome analysis with R (hands on and wrap up)</p> <ul style="list-style-type: none"> • Review... questions & answers