

Biological interpretations

Biological interpretations

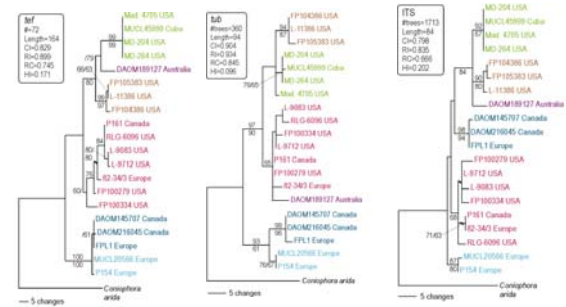
- Be aware of the limitations with DNA metabarcoding!

Biological interpretations

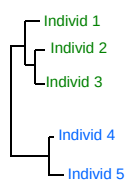
- Be aware of the limitations with DNA metabarcoding!
 - You are just working with one single locus!
 - In many cases, multi-locus data needed to make clear distinction between species

Coniophora olivacea

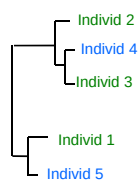
Good concordance across multiple gene phylogenies:



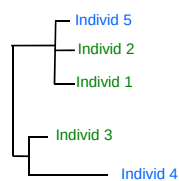
Gene phylogeny 1



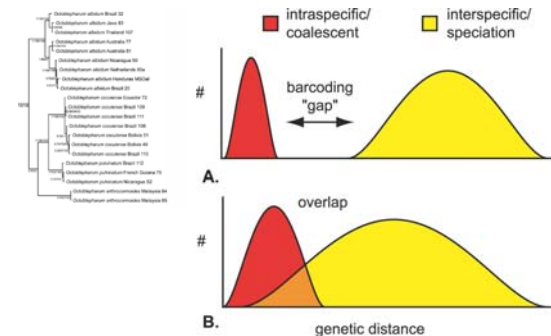
Gene phylogeny 2



Gene phylogeny 3



The barcoding gap



Biological interpretations

- Be aware of the biases:
 - Sampling
 - DNA extraction
 - Primers / marker
 - Chimeras and PCR mutations
 - OTU construction
 - Taxonomic annotation

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 - Sampling
 - DNA extraction
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Biological interpretations

- Marker
 - Conservative marker (e.g. 18S): > Species level → Error correction (DADA2, Swarm) might be enough
 - Variable marker (e.g. ITS): =species level → Have to take intraspecific variation into account → additional clustering step

Biological interpretations

- Be aware of the biases:
 - Sampling
 - DNA extraction
 - Primers / marker
 - Chimeras and PCR mutations
 - OTU construction
 - Taxonomic annotation
- The importance of biases depends on your research questions!

Biological interpretations

- Beta diversity

RESOURCE ARTICLE

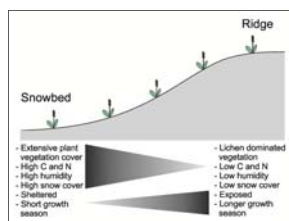
WILEY MOLECULAR ECOLOGY

Sequence clustering threshold has little effect on the recovery of microbial community structure

Synneve Smebye Botnen^{1,2} | Marie Louise Davey¹ | Rune Halvorsen³ | Håvard Kausrud¹

Name	Original name	Site	Recent of measured variable	Study system	Scale	Targeted region	Sequencing platform	Data availability
Dataset 1	Færø et al. (2013)	Finse, Norway	Yes, Snowbed/Ridge	Plant root associated fungi	Local	ITS1	Rosetta GS FLX Titanium Series 454	DRYAD accession: https://doi.org/10.5061/dryad.2189
Dataset 2	Aur AB, Dønnes ML, Mønstad A, Solheim T, Kjønnstad H (unpublished)	Finse, Norway	Yes, plant root	Fungal in plant roots and surrounding soil	Small	ITS2	Rosetta GS FLX Titanium Series 454	NCBI short read archive: Accession No. SRA000007
Dataset 3	Halvorsen RD, Dønnes ML (unpublished)	Swabard	Yes, treatment/control	Soil fungi	Local	ITS2	Rosetta v454 PE	DRYAD accession: https://doi.org/10.5061/dryad.79430
Dataset 4	Aur AB, Vrå U, Blaabjerg R, Halvorsen R, Carlsen T, Blomberg AB, Kjønnstad H, Dønnes ML (unpublished)	Finse, Norway	Yes	Fungal endophytes	Local	ITS2	Rosetta GS FLX Titanium Series 454	DRYAD accession: https://doi.org/10.5061/dryad.79430
Dataset 5	Dønnes ML and others (2018)	Trondheim, Norway	Yes, forest type	Fungal endophytes associated with bryophytes	Landscape	ITS2	Rosetta GS FLX Titanium Series 454	MG-ASST ID: 4517463-4517753 DRYAD accession: https://doi.org/10.5061/dryad.68005
Dataset 6	Dønnes et al. (2017)	Vindfjell, Norway	Yes, host species, forest variations, and nitrogen fertilization	Fungal endophytes associated with bryophytes	Local	ITS2	Rosetta GS FLX Titanium Series 454	MG-ASST ID: 4517463-4517753
Dataset 7	Botnen SS, Vrå U, Carlsen T, Halvorsen RD, Dønnes ML, Kjønnstad H (unpublished)	Swabard	No	Root associated bacteria	Local	V4 of 16S	Rosetta GS FLX Titanium Series 454	DRYAD accession: https://doi.org/10.5061/dryad.79430
Dataset 8	Vrå et al. (2018)	Finse, Norway	Yes, plant root/soil	Bacteria in plant roots and surrounding soil	Small	V4 of 16S	Rosetta GS FLX Titanium Series 454	NCBI short read archive: accession No. SRR020429
Dataset 9	Halvorsen RD, Dønnes ML (unpublished)	Swabard	Yes, treatment/control	Soil bacteria	Local	V4 of 16S	Rosetta v454 PE	DRYAD accession: https://doi.org/10.5061/dryad.79430

Finse



Biological interpretations

• Beta diversity

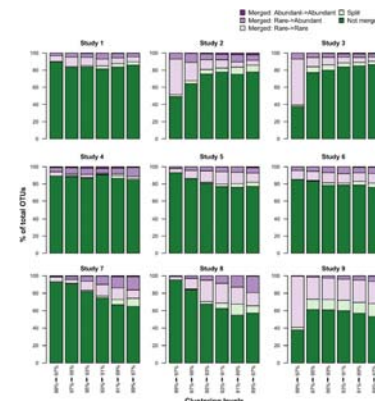
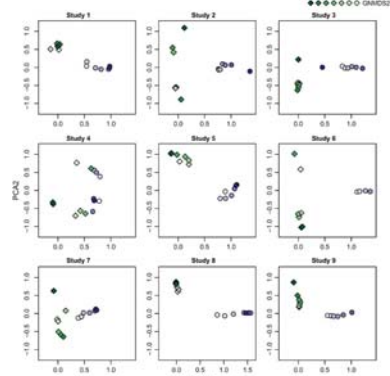
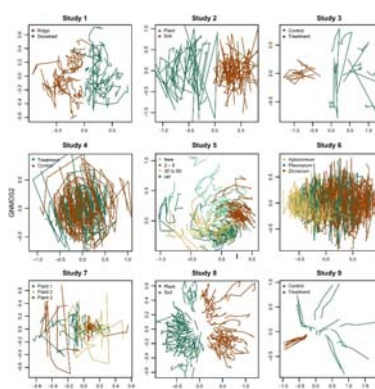
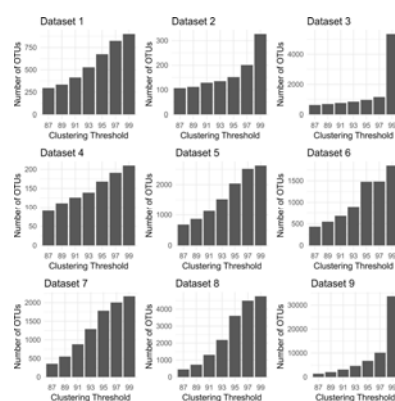
RESOURCE ARTICLE

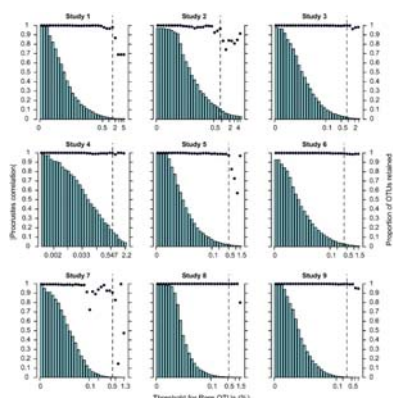
WILEY **MULTIPLIPLY ECOLOGY**

Sequence clustering threshold has little effect on the recovery of microbial community structure

Synneve Smebye Botnen^{1,2} | Marie Louise Davey¹ | Rune Halvorsen³ | Håvard Kausrud¹

- Clustered all datasets from 99 to 87% sequence similarity
- Ran multivariate analyses





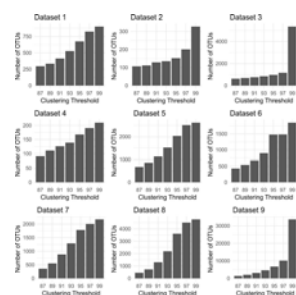
Biological interpretations

- Beta diversity

- Very robust!

Biological interpretations

- Alfa/gamma diversity



Biological interpretations

- Alfa/gamma diversity

- Relative (within-study) comparisons are fine
- Should be very (!) careful with using absolute values and comparisons across studies!

Biological interpretations

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 - Inclusion of internal mock communities (positives) in the setup are helpful then. To what degree is the known diversity re-shaped?

Biological interpretations

- Alfa/gamma diversity

- Relative (within-study) comparisons are fine
- Should be very (!) careful with using absolute values and comparisons across studies!
 - Inclusion of internal mock communities (positives) in the setup are helpful then. To what degree is the known diversity re-shaped?
 - LULU might be a good step to obtain more reliable absolute numbers, especially for variable markers.

ARTICLE

DOI: 10.1093/aib/abz012 OPEN

Algorithm for post-clustering curation of DNA amplicon data yields reliable biodiversity estimates

Tobias Goldberg-Frøsløv^{1,2}, Rasmus Kjeller¹, Hans Henrik Bruun¹, Rasmus Ejrnæs³, Ane Kirstine Brunbjerg³, Carlotta Pietroni² & Anders Johannes Hansen²

Biological interpretations

- Species/OTU abundances?
 - Relative comparisons ok (within your study)
 - Absolute comparisons not ok (across studies)
- May couple in other abundance data from e.g. qPCR or other biomarkers to say something about quantitative variation.

DNA metabarcoding: From 'wild west' towards an established approach?



Primary phase

- Poor replication
- Lack of controls
- Lack of insight into important biases
- Poor bioinformatics approaches
- Etc.

Editorial note for Molecular Ecology

DNA metabarcoding - need for robust experimental designs to draw sound ecological conclusions

Authors

Lucie Zupan¹, Jennifer Bousquet¹, Søren G. Årnes¹, Mikko Ballin¹, Holly Ball¹, Frédéric Buisson¹, Mathieu A. Chazotte¹, Simon Chou¹, Eric Coudrac¹, Steve D. Dwyer¹, Marie De Bie¹, Tim A. Ockler¹, Adam J. Dumbrell¹, Gemma Franceschi Fumagalli¹, Noah Fierer¹, Lars Forstner¹, M. Thomas P. Gilbert¹, Simon Harrison¹, Ari Happonen¹, Howard Hines¹, Ludovic J. Ojeda¹, John P. Parnell¹, Jan Pankovics¹, Lotta Tedenius¹, Philip Francis Thompson¹, Eike Wittenberg^{1,2,3}, Peter Zeman¹

Established scientific approach with a set of widely accepted guidelines

The importance of controls

- Technical replicates
- Extraction negatives
- PCR negatives
- Positive control(s)



Pierre Taberlet

