

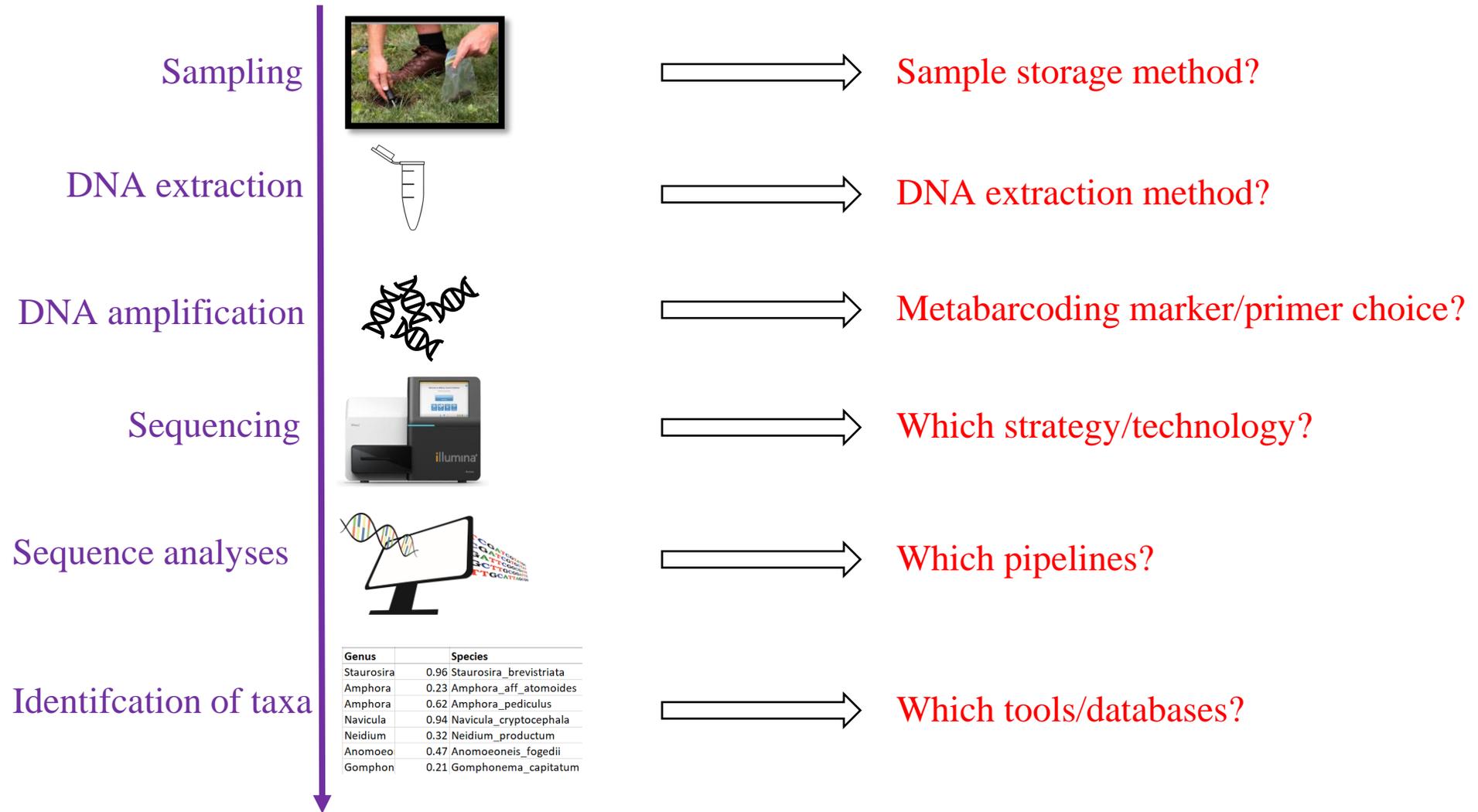


Is there such thing as
the „best“ metabarcoding workflow?

Sten Anslan <sten.anslan@ut.ee>



Experimental design



Genus	Species
Staurosira	0.96 Staurosira_brevistriata
Amphora	0.23 Amphora_aff_atomoides
Amphora	0.62 Amphora_pediculus
Navicula	0.94 Navicula_cryptocephala
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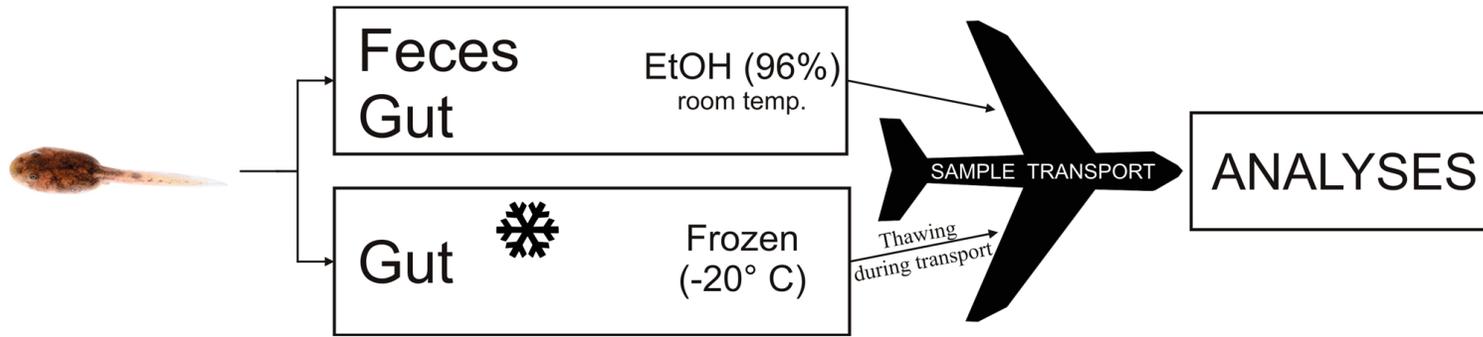
Biological interpretation



Sample storage

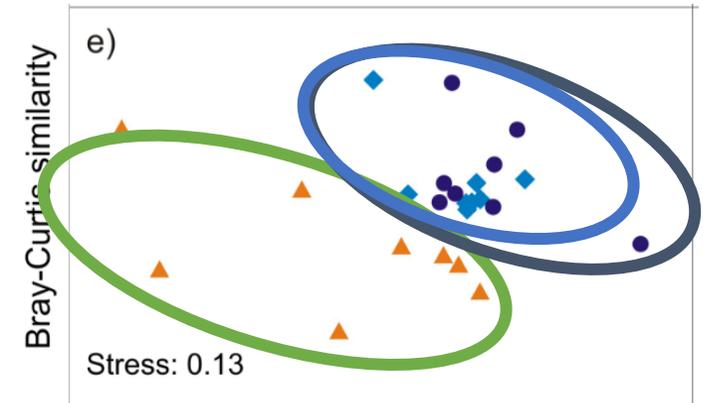
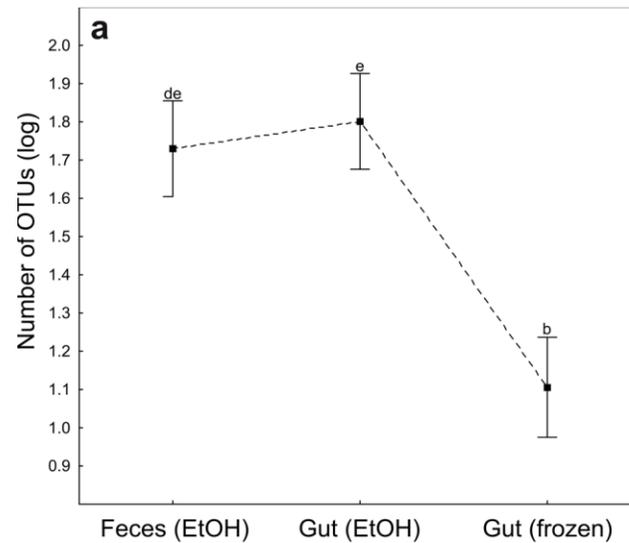
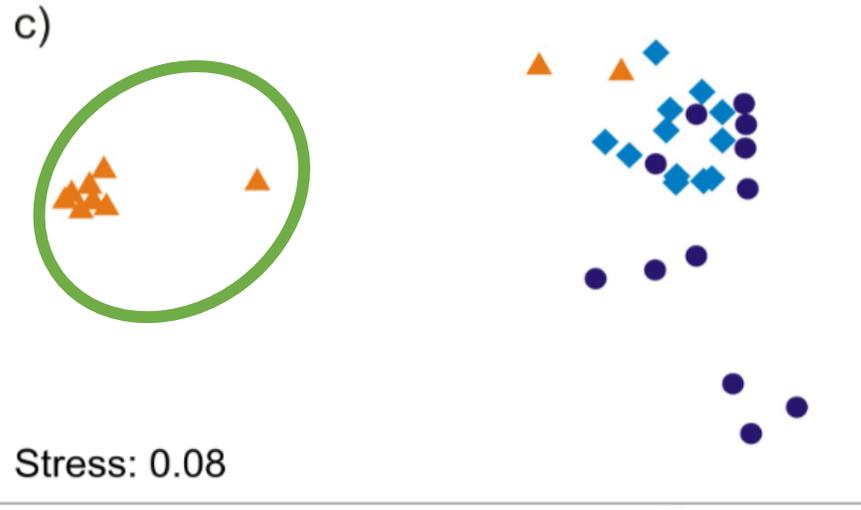
Storage at **-80 °C**; „The Golden Standard“

Sample storage



Microbiomes from feces vs. gut in tadpoles: distinct community compositions between substrates and preservation methods

STEN ANSLAN¹, HUAN LI², SVEN KÜNZEL³ & MIGUEL VENCES¹



▲ Gut (frozen) ● Gut (EtOH) ◆ Feces (EtOH)



Storage for soil samples

Soil stabilisation for DNA metabarcoding of plants and fungi.
Implications for sampling at remote locations or via third-parties

Lina A. Clasen¹, Andrew P. Detheridge¹, John Scullion¹, Gareth W. Griffith¹

Freeze-thaw

Storage at **-80 °C** = CONTROL

1. Storing and shipping **at 4°C** (14 days)
2. **Air dry at room temperature (23°C)** prior to shipping



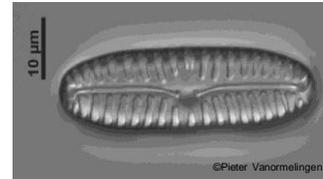
X grams ???

the more the merrier?



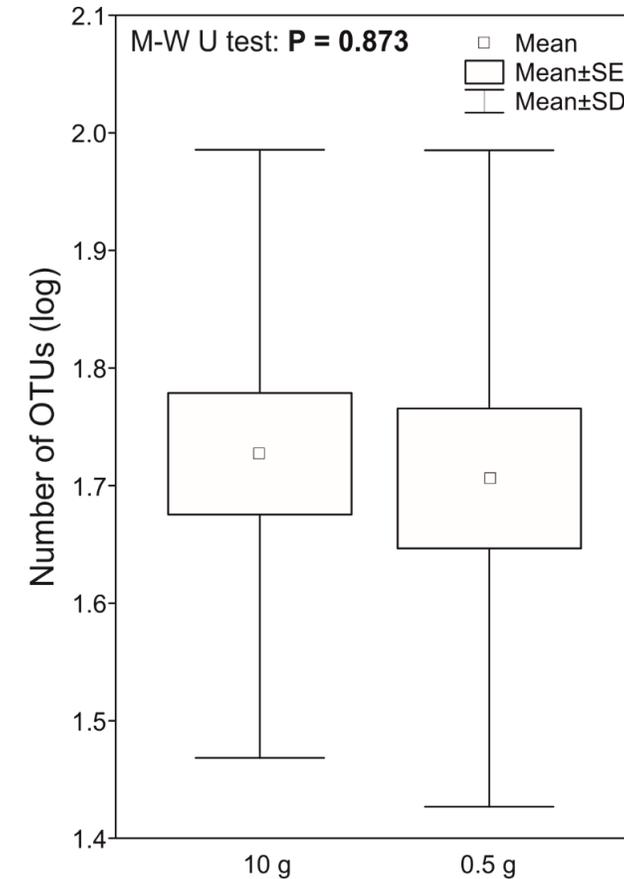
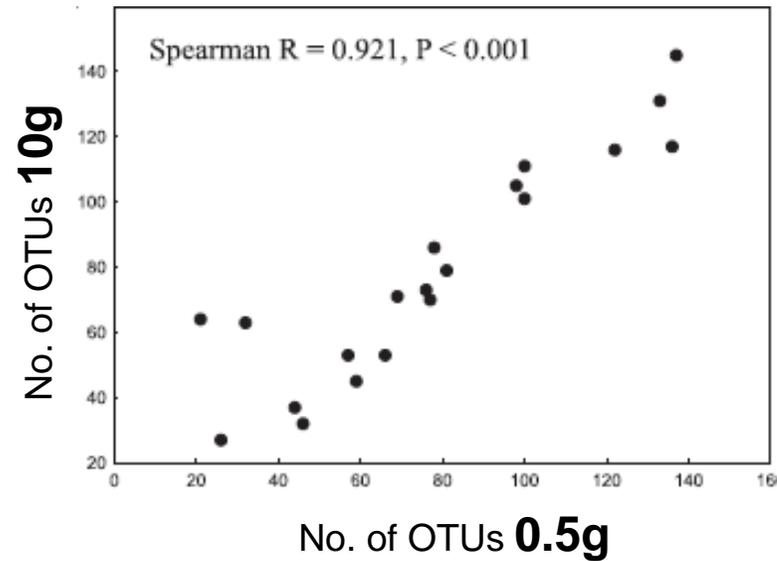
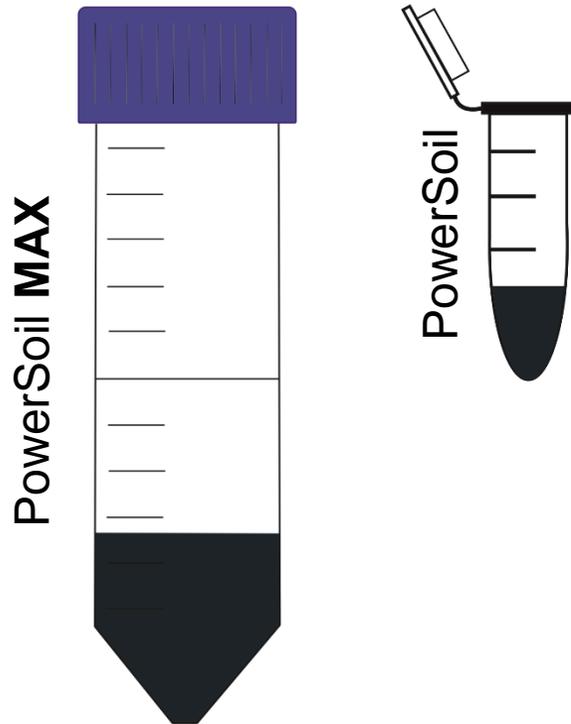
10 grams vs 0.5 grams of substrate

- differences in detecting diatom **richness**?



Diatom metabarcoding and microscopic analyses from sediment samples at Lake Nam Co, Tibet: The effect of sample-size and bioinformatics on the identified communities

Wengang Kang^a, Sten Anslan^{b,*}, Nicole Börner^a, Anja Schwarz^a, Robin Schmidt^b, Sven Künzel^c, Patrick Rioual^{d,f}, Paula Echeverría-Galindo^a, Miguel Vences^b, Junbo Wang^c, Antje Schwalb^a

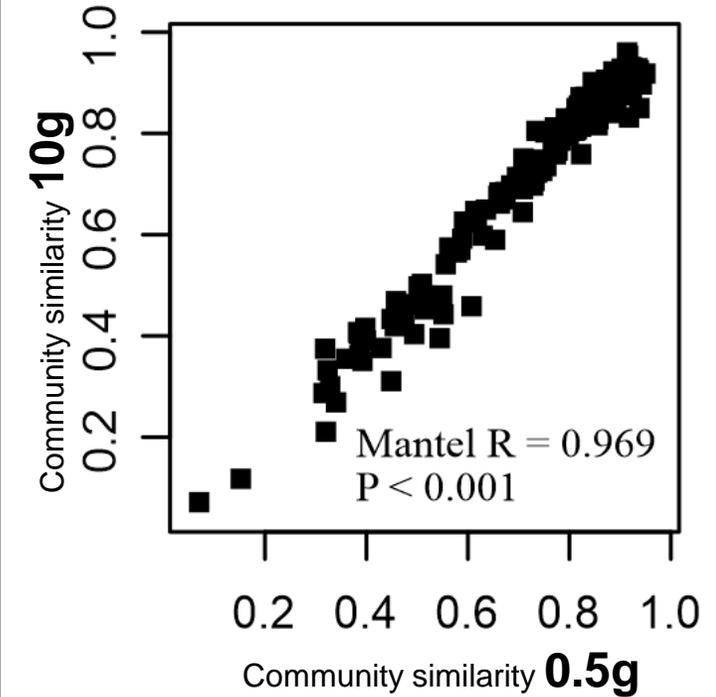
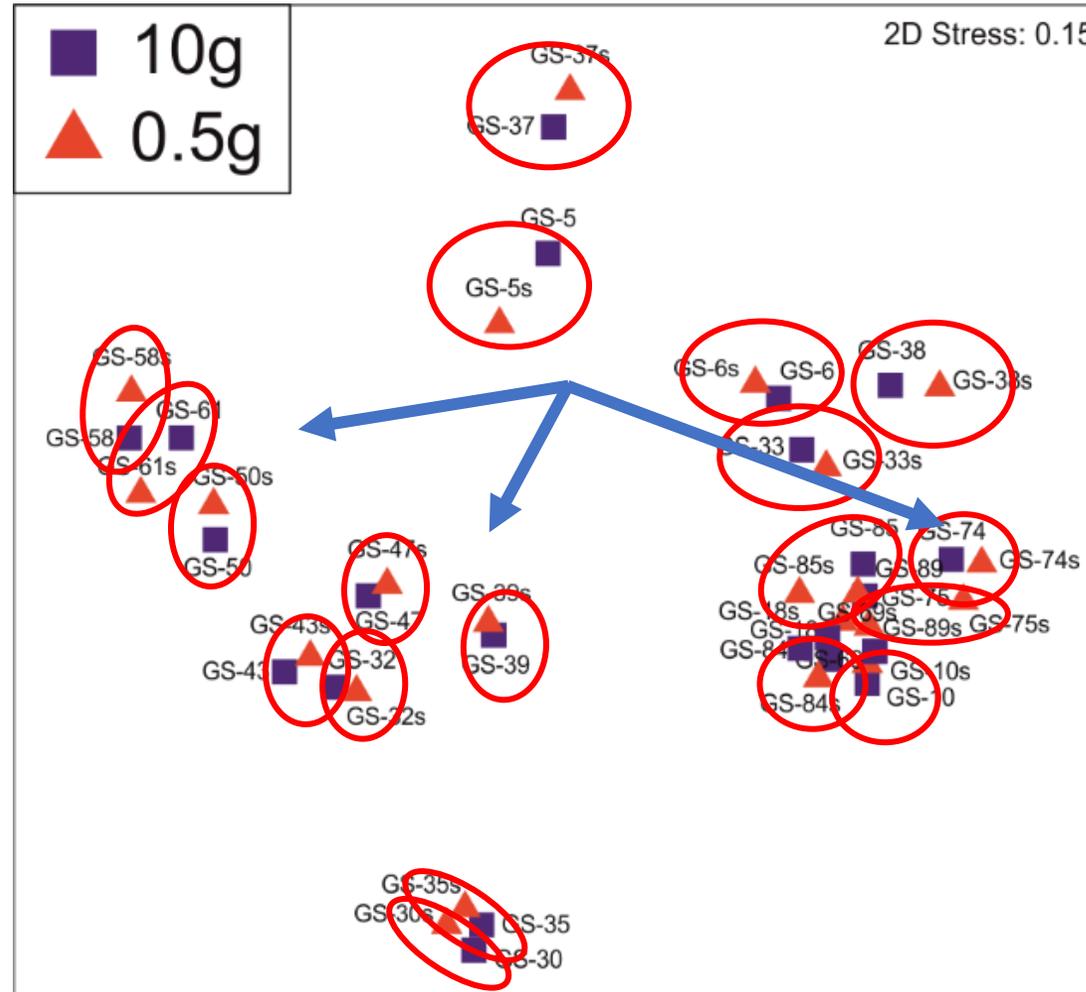
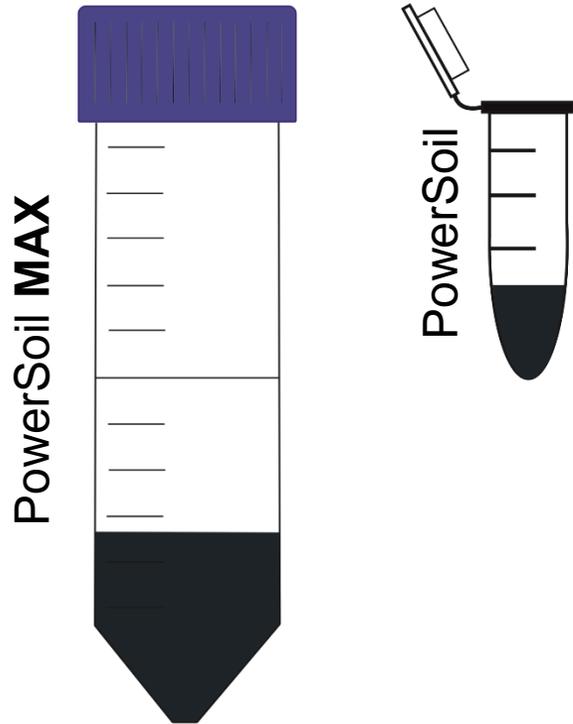


10 grams vs 0.5 grams of substrate

- differences in diatom **community structure**?

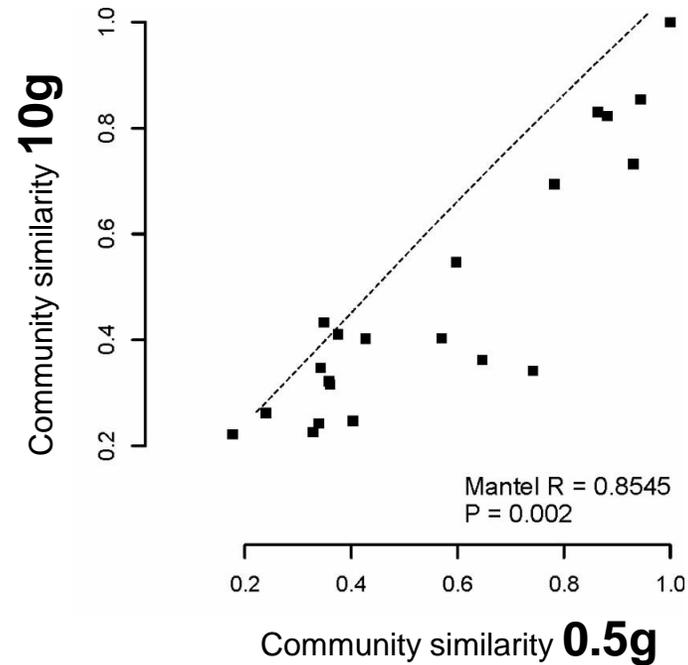
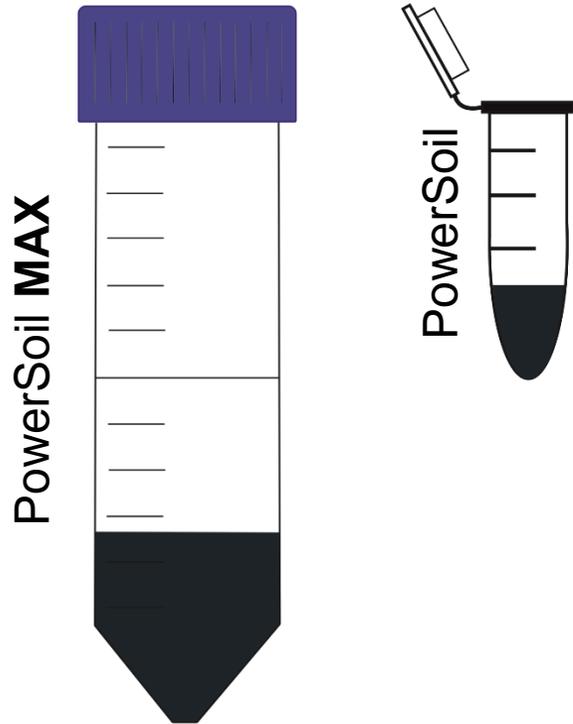
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10 grams vs 0.5 grams of substrate

- no differences in ostracode community structure



Ostracodes





Size Matters: Assessing Optimum Soil Sample Size for Fungal and Bacterial Community Structure Analyses Using High Throughput Sequencing of rRNA Gene Amplicons

C. Ryan Penton^{1,2*}, Vadakattu V. S. R. Gupta³, Julian Yu¹ and James M. Tiedje⁴

10 g

SCIENTIFIC REPORTS

OPEN

Sample size effects on the assessment of eukaryotic diversity and community structure in aquatic sediments using high-throughput sequencing

Francisco J. A. Nascimento^{1,4}, Delphine Lallias², Holly M. Bik³ & Simon Creer⁴

1: 26 January 2018
2: 23 July 2018
3: online: 06 August 2018

>1 g



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Journal of Microbiological Methods 66 (2006) 242–250

Journal
of Microbiological
Methods

www.elsevier.com/locate/jmicmeth

The effect of sample size in studies of soil microbial community structure

Sanghoon Kang¹, Aaron L. Mills^{*}

Laboratory of Microbial Ecology, Department of Environmental Sciences, 291 McCormick Road, P.O. Box 400123, University of Virginia, Charlottesville, VA 22904-4123, USA

Received 20 October 2005; received in revised form 30 November 2005; accepted 30 November 2005
Available online 19 January 2006

0.25 g

RESEARCH ARTICLE

Methods in Ecology and Evolution

Impacts of DNA extraction and PCR on DNA metabarcoding estimates of soil biodiversity

Andrew Dopheide^{1,2,3} | Dong Xie⁴ | Thomas R. Buckley^{1,3} |
Alexei J. Drummond⁴ | Richard D. Newcomb^{1,2}

Larger soil volumes = higher biodiv estimates for Arthropods but not for prokaryotes or microeukaryotes.

But which kit?



Metabarcoding and Metagenomics 2: 1–12
DOI 10.3897/mbmg.2.26664

Research Article



Choice of DNA extraction method affects DNA metabarcoding of unsorted invertebrate bulk samples

Markus Majaneva^{1,2}, Ola H. Diserud³, Shannon H.C. Eagle², Mehrdad Hajibabaei², Torbjørn Ekrem¹

Application of high-throughput sequencing (HTS) metabarcoding to diatom biomonitoring: Do DNA extraction methods matter?

Valentin Vasselon^{1,3}, Isabelle Domaizon^{1,4}, Frédéric Rimet^{1,5}, Maria Kahlert^{2,6}, and Agnès Bouchez^{1,7}

¹CARTELE, INRA, Université de Savoie Mont Blanc, 74200, Thonon-les-Bains, France

²Department of Aquatic Sciences and Assessment, Swedish University of Agricultural Sciences, P.O. Box 7050, 75007, Uppsala, Sweden

DNeasy PowerSoil as a universal DNA extraction method

nature
biotechnology

Assessment of variation in microbial community amplicon sequencing by the Microbiome Quality Control (MBQC) project consortium

Rashmi Sinha¹, Galeb Abu-Ali^{2,3}, Emily Vogtmann¹, Anthony A Fodor⁴, Boyu Ren², Amnon Amir⁵, Emma Schwager^{2,3}, Jonathan Crabtree⁶, Siyuan Ma^{2,3}, The Microbiome Quality Control Project Consortium⁷, Christian C Abnet¹, Rob Knight^{5,8}, Owen White⁶ & Curtis Huttenhower^{2,3}

RESOURCE ARTICLE

WILEY MOLECULAR ECOLOGY
RESOURCES

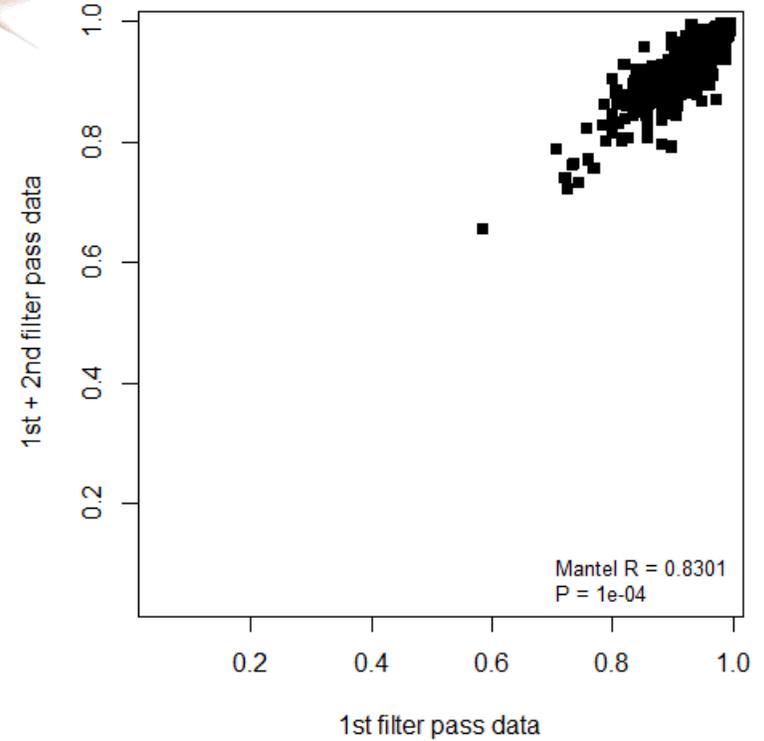
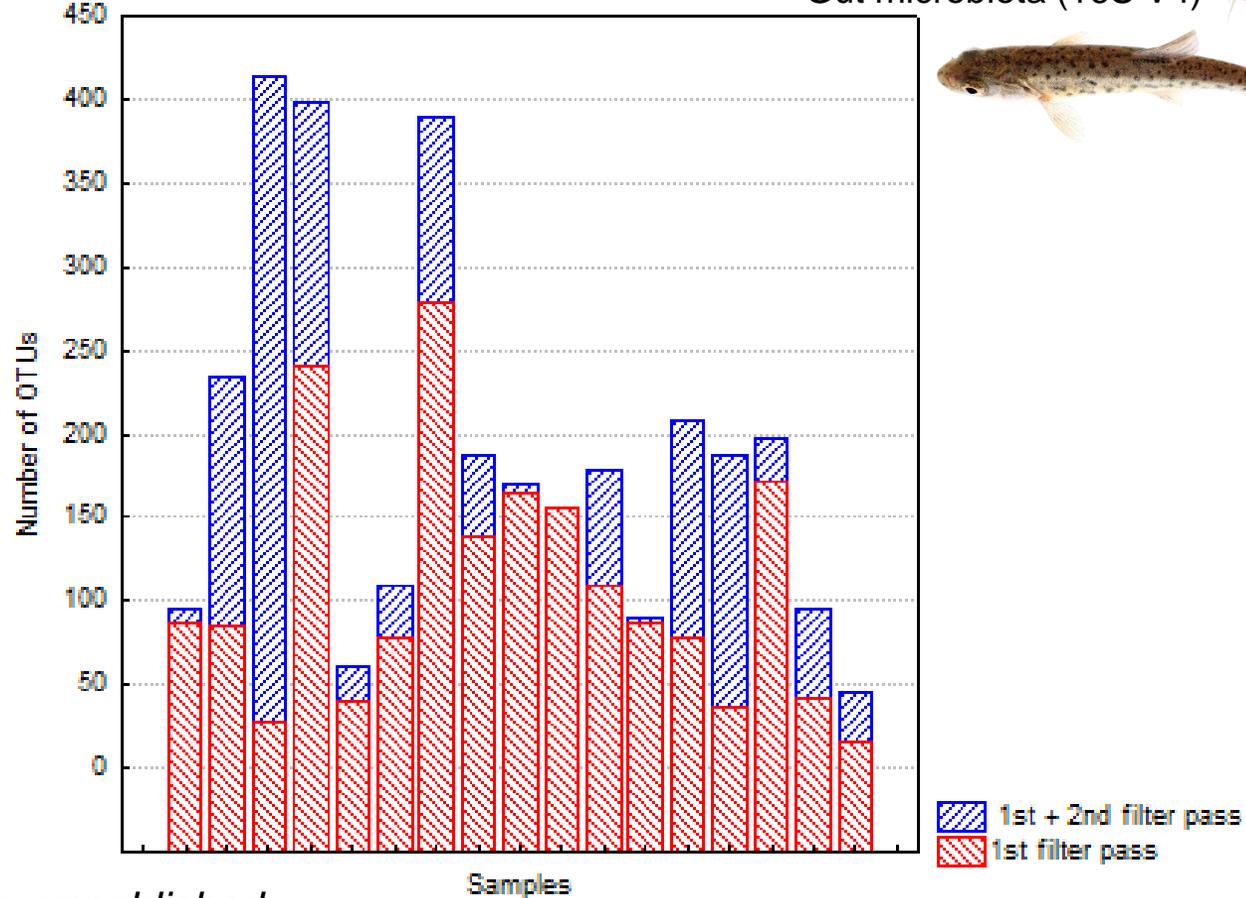
Optimal extraction methods for the simultaneous analysis of DNA from diverse organisms and sample types

Syrie M. Hermans¹ | Hannah L. Buckley² | Gavin Lear¹





Gut microbiota (16S V4)



Anslan, unpublished.

longer incubation time = higher DNA yields
(more gene copies)

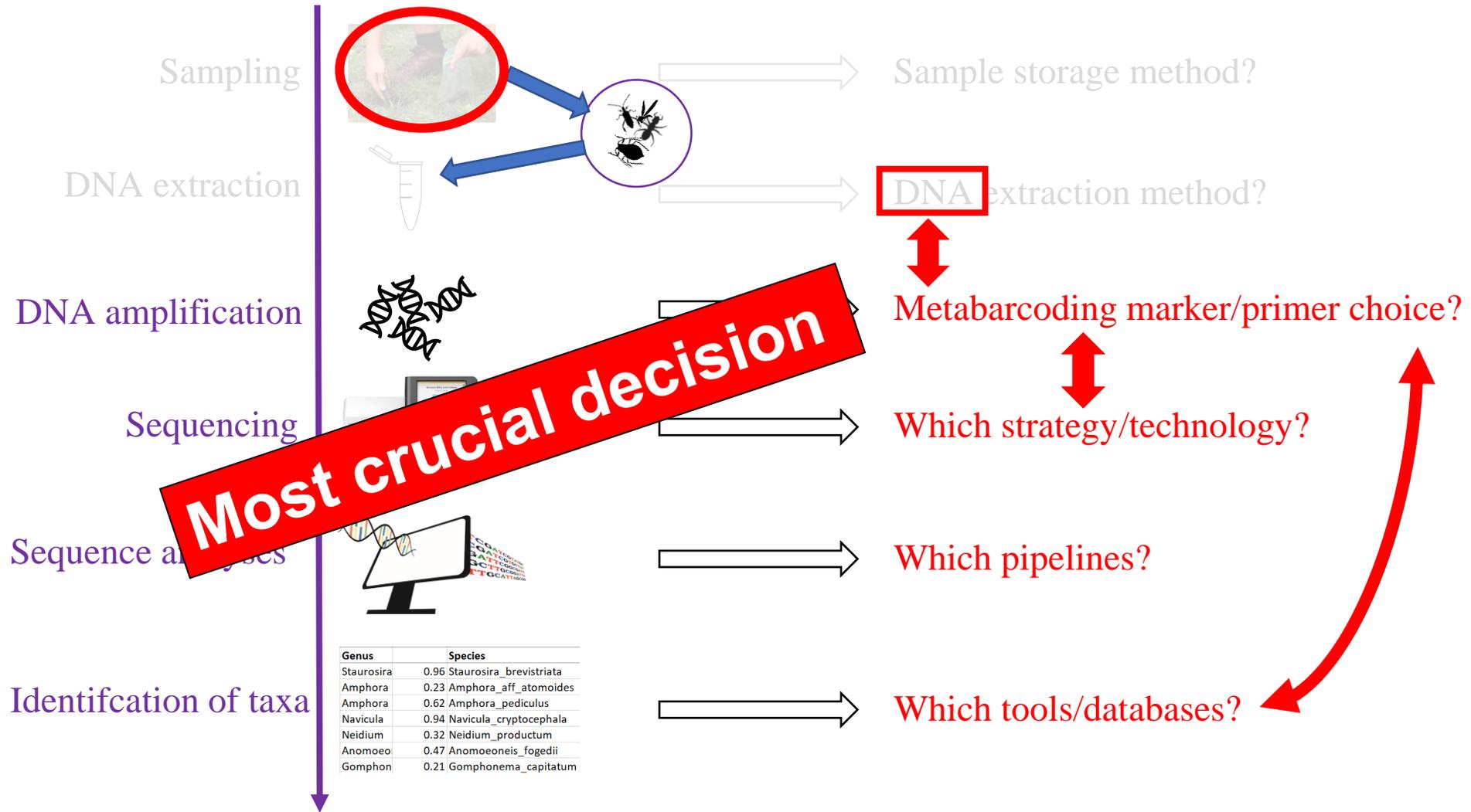
ORIGINAL RESEARCH

Ecology and Evolution WILEY

Keeping it cool: Soil sample cold pack storage and DNA shipment up to 1 month does not impact metabarcoding results

Camille S. Delavaux^{1,2} | James D. Bever^{1,2} | Erin M. Karppinen³ | Luke D. Bainard³

Experimental design



Genus	Species
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Amphora	0.23 Amphora_aff_atomoides
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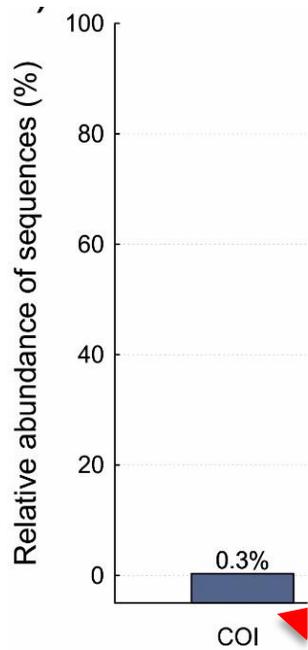
Biological interpretation



Ostracodes metabarcoding from sedimentary DNA

Highest *in-silico* amplification rate
for **COI** gene primers

(matched with **99.3%** of ostracodes ref seqs in the database)

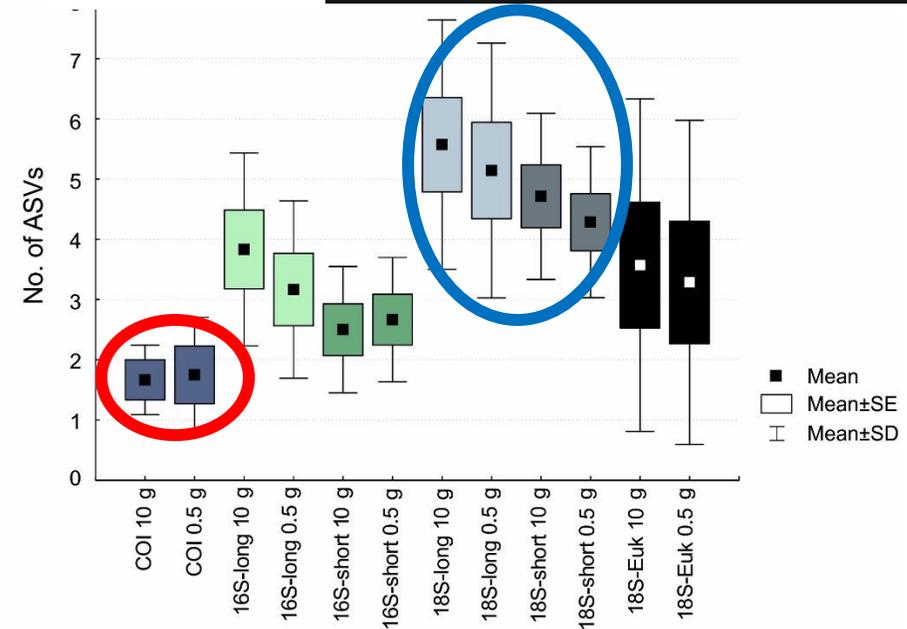
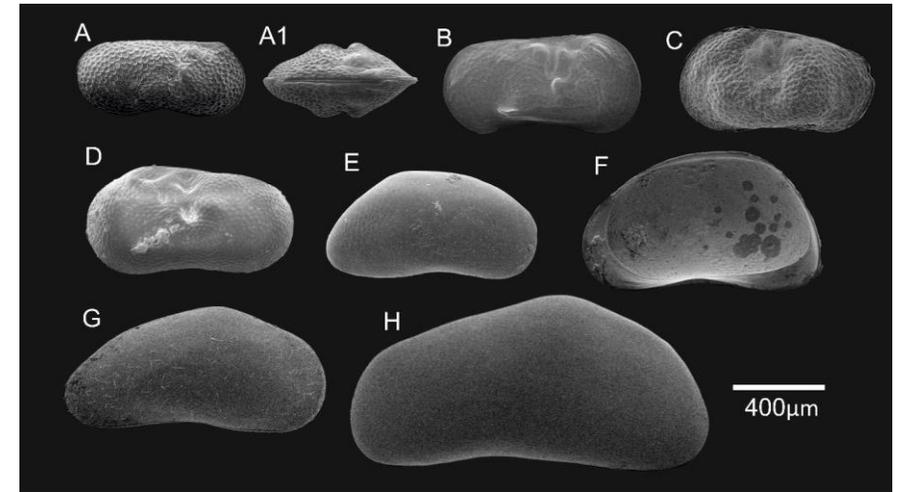


Higher sequencing depth would resolve this issue???

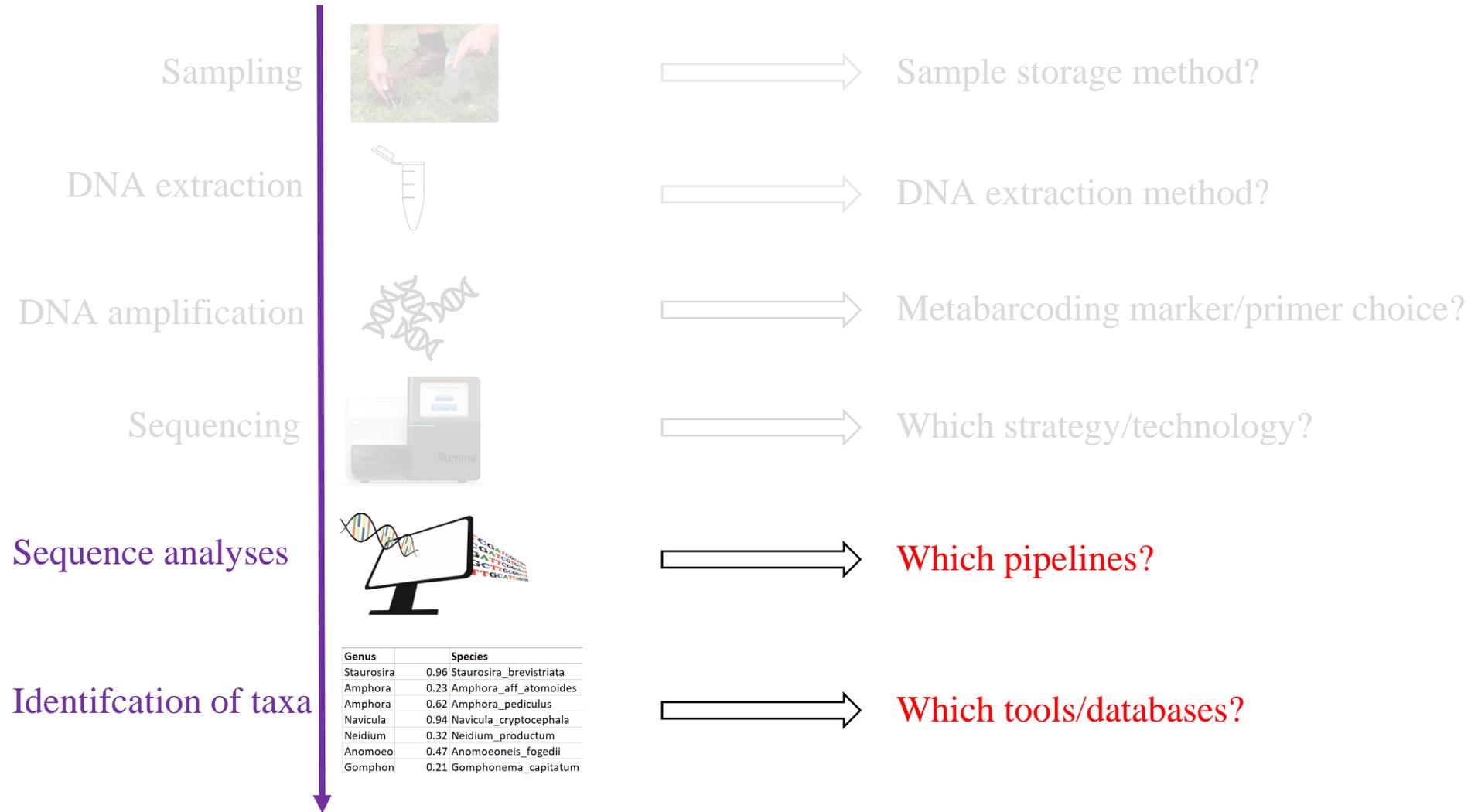
High-throughput identification of non-marine Ostracoda from the Tibetan Plateau: Evaluating the success of various primers on sedimentary DNA samples

Paula Echeverría-Galindo^{1#}, Sten Anslan^{2##}, Peter Frenzel³, Wengang Kang¹, Nicole Börner¹, Anja Schwarz¹, Ping Peng⁵, Sven Künzel⁴, Miguel Vences², Liseth Pérez¹, Liping Zhu⁵, Antje Schwalb¹

under review



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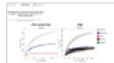


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MycoKeys 39: 29–40 (2018)
doi: 10.3897/mycokeys.39.28109
<http://mycokeys.pensoft.net>

RESEARCH ARTICLE



Great differences in performance and outcome of high-throughput sequencing data analysis platforms for fungal metabarcoding

Sten Anslan¹, R. Henrik Nilsson², Christian Wurzbacher³, Petr Baldrian⁴, Leho Tedersoo⁵, Mohammad Bahram^{6,7,8}



Contents lists available at ScienceDirect

Fungal Ecology

journal homepage: www.elsevier.com/locate/funeco



Bioinformatics matters: The accuracy of plant and soil fungal community data is highly dependent on the metabarcoding pipeline



Charlie Pauvert^a, Marc Buée^b, Valérie Laval^c, Véronique Edel-Hermann^d, Laure Fauchery^b, Angélique Gautier^c, Isabelle Lesur^{a,e}, Jessica Vallance^f, Corinne Vacher^{a,*}



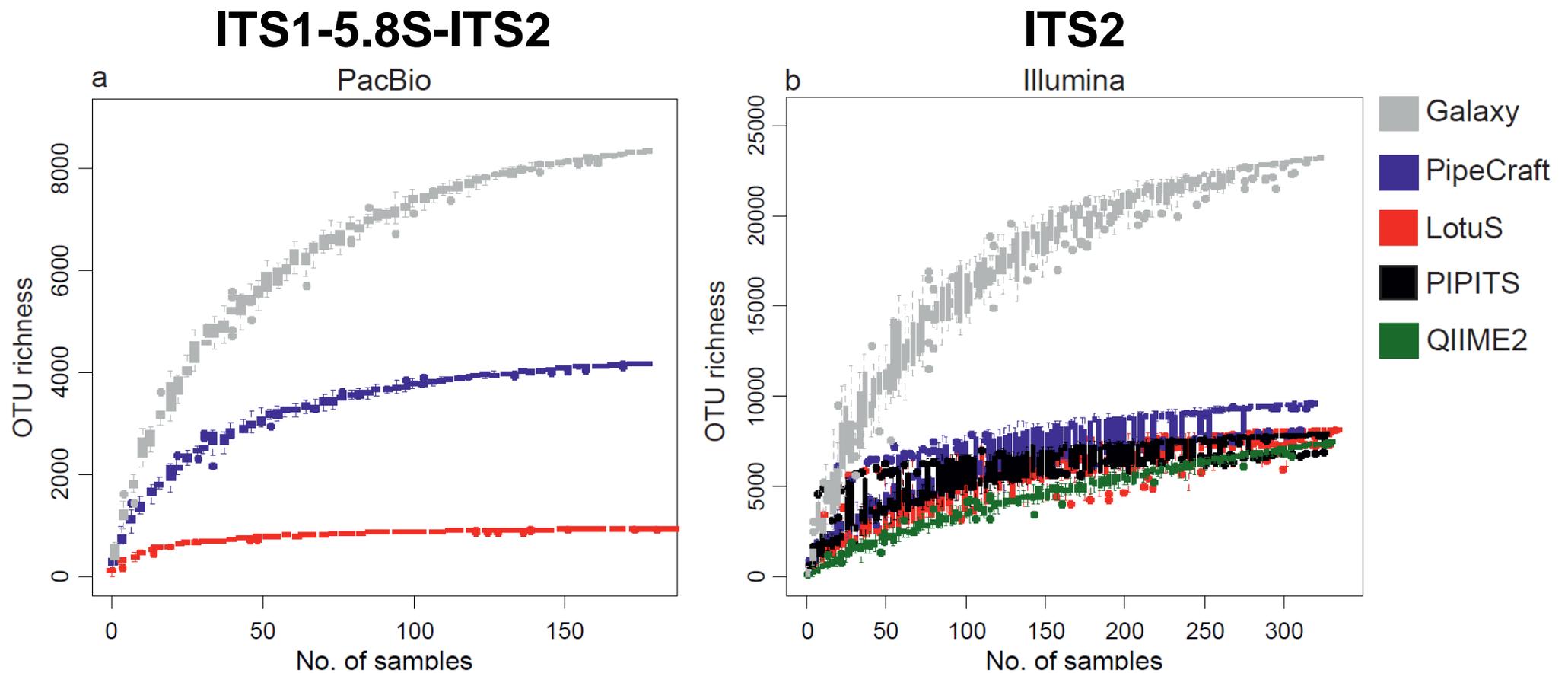
RESEARCH ARTICLE

Bioinformatic Amplicon Read Processing Strategies Strongly Affect Eukaryotic Diversity and the Taxonomic Composition of Communities

Markus Majaneva^{1,2,*}, Kirsi Hyytiäinen^{1,2}, Sirkka Liisa Varvio³, Satoshi Nagai⁴, Jaanika Blomster¹

Great differences in performance and outcome of high-throughput sequencing data analysis platforms for fungal metabarcoding

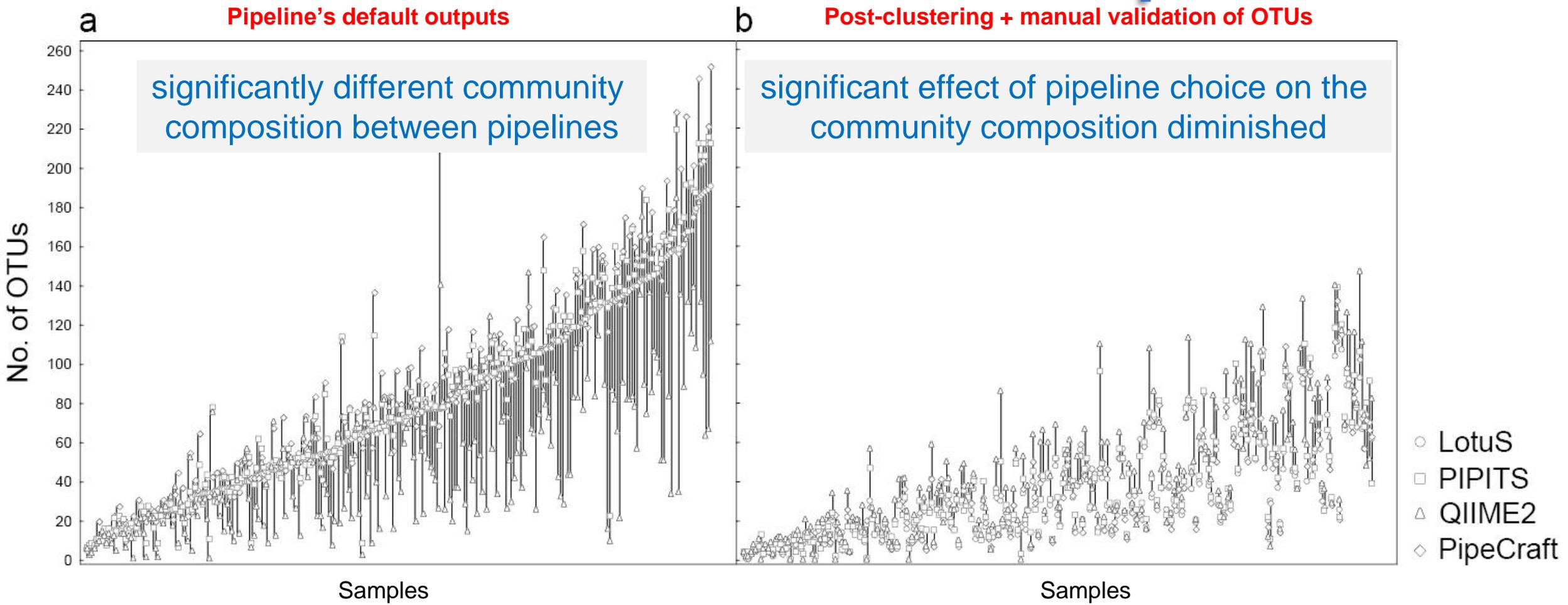
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Based on taxonomy assignment values



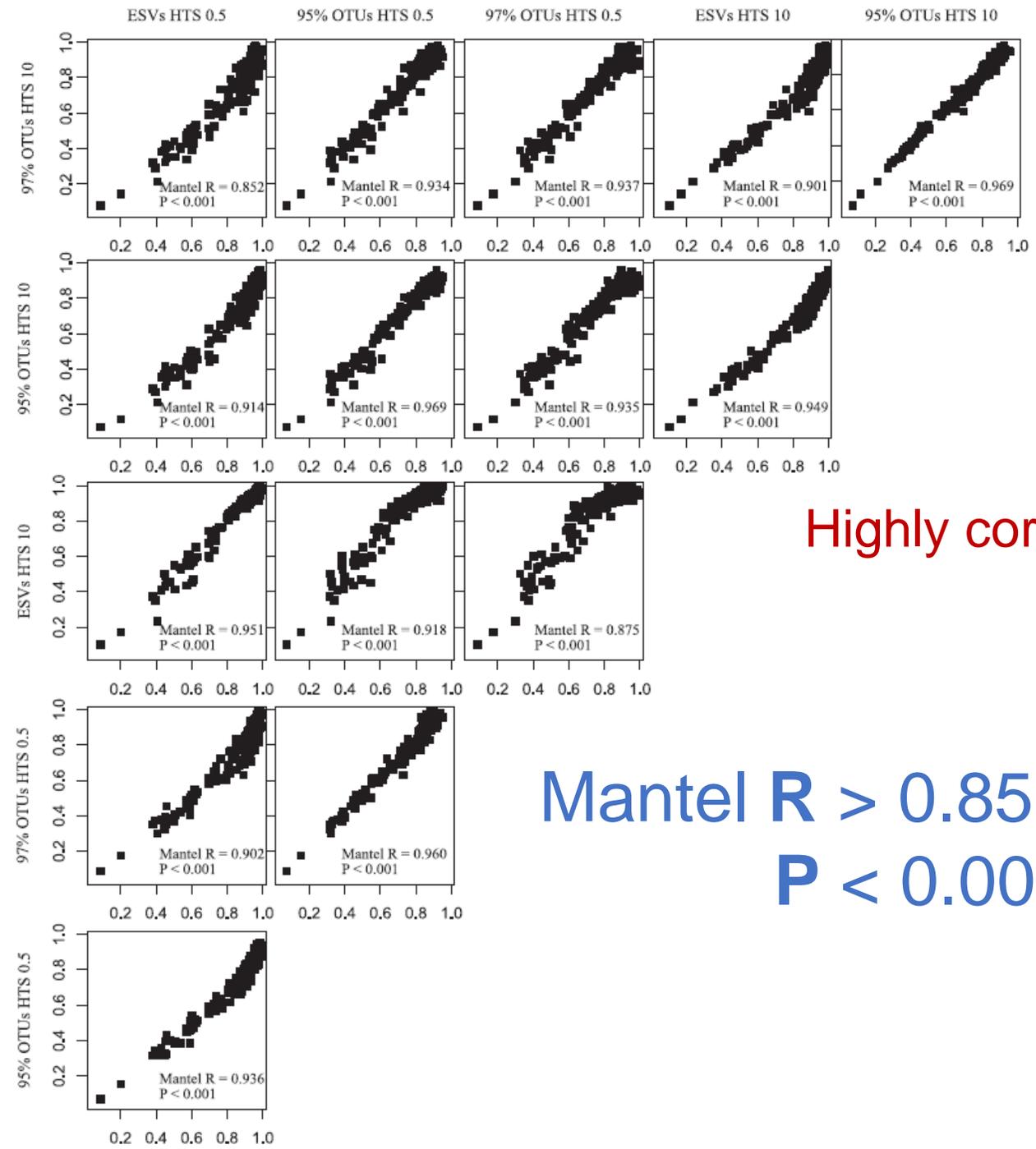
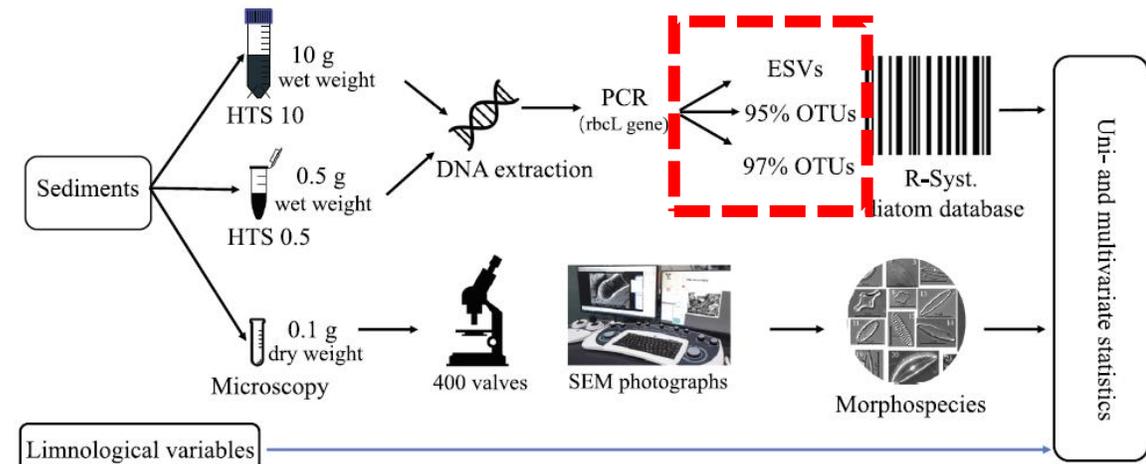
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Highly correlating results from different pipelines

DADA2 pipeline → ESVs
 mothur pipeline → 95% OTUs
 PipeCraft pipeline → 97% OTUs

Mantel $R > 0.85$
 $P < 0.001$



Experimental design

Sampling



DNA extraction



DNA amplification



Sequencing



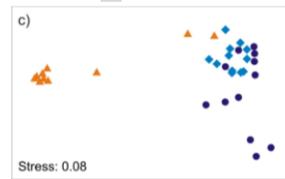
Sequence analyses



Identification of taxa

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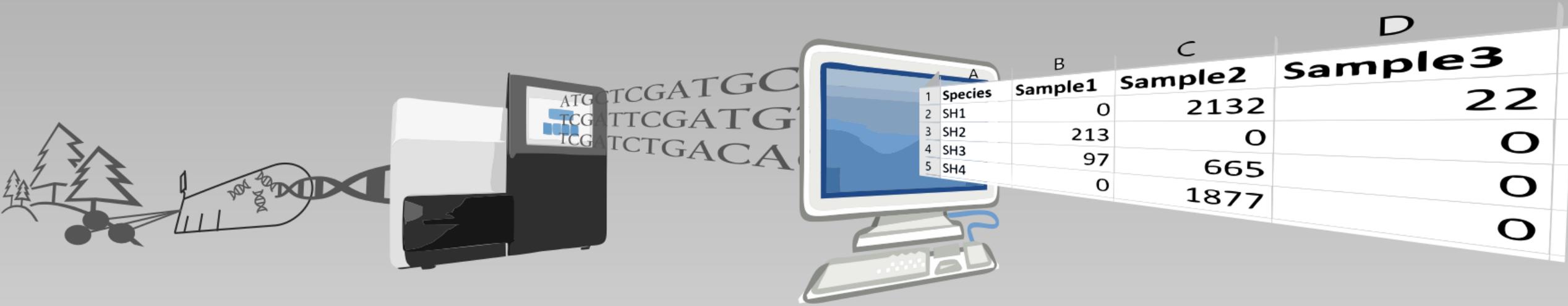
Biological interpretation



unite

community

Is there such thing as the „best“ metabarcoding workflow?



There are appropriate and inappropriate ones!

Is there such thing as the „best“ metabarcoding workflow?



There are appropriate and inappropriate ones!

THANK YOU!