

FED-AMR

The role of free extracellular DNA in dissemination of antimicrobial resistance over ecosystem boundaries along the food/feed chain



Werner Ruppitsch

werner.ruppitsch@ages.at

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Background

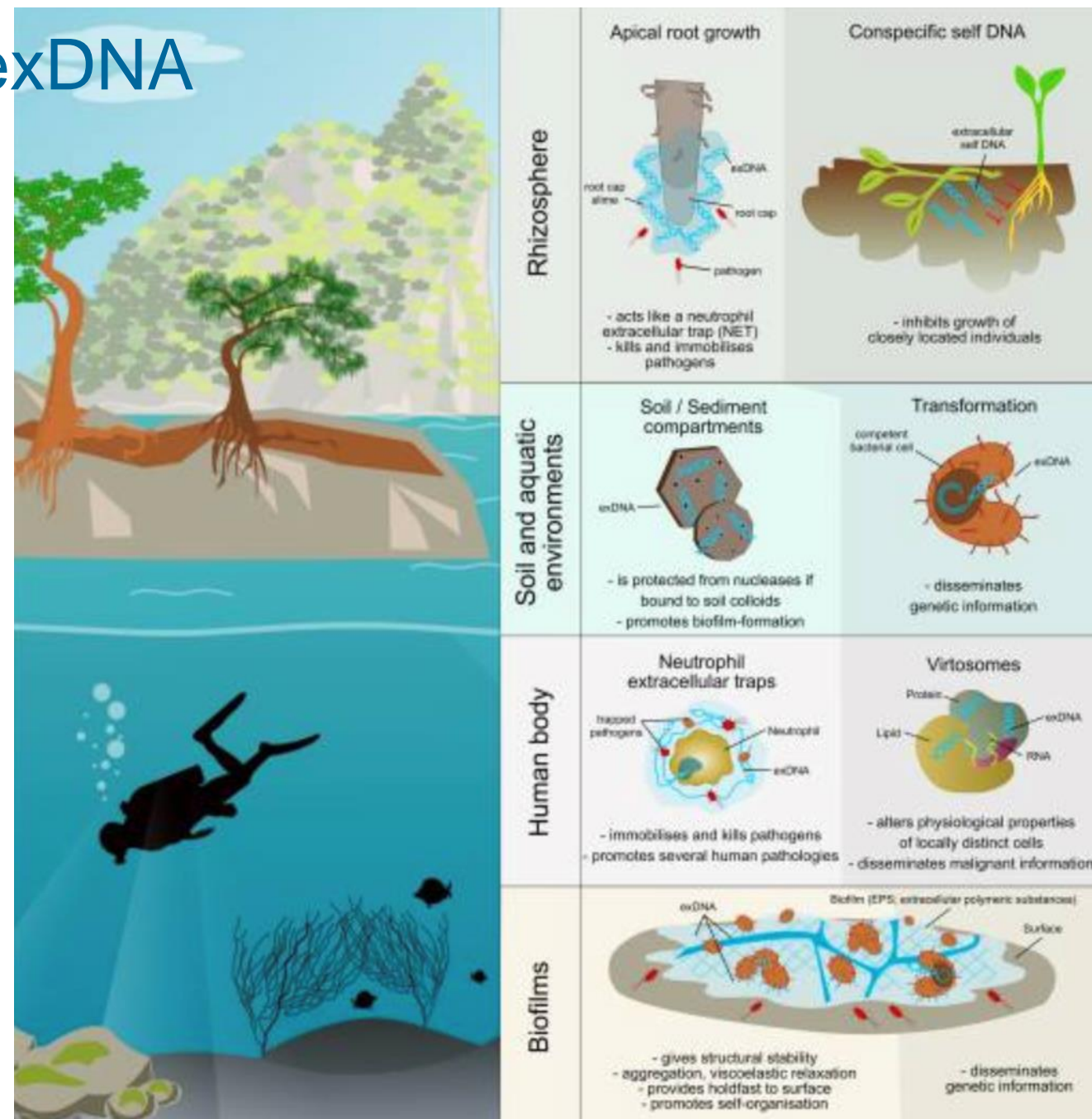
- Antimicrobial resistance gene (ARG) pollution is a global threat to environment and public health
- Risk evaluation of pathogens, antibiotic resistance genes (ARGs), mobile genetic elements (MGEs) in diverse environments are **mainly based on total DNA** (tDNA) data
- The role of **exDNA still neglected** in most environmental studies
- **Limited knowledge – technological challenges** (exDNA isolation, ARG detection and data analysis)



What we know about exDNA

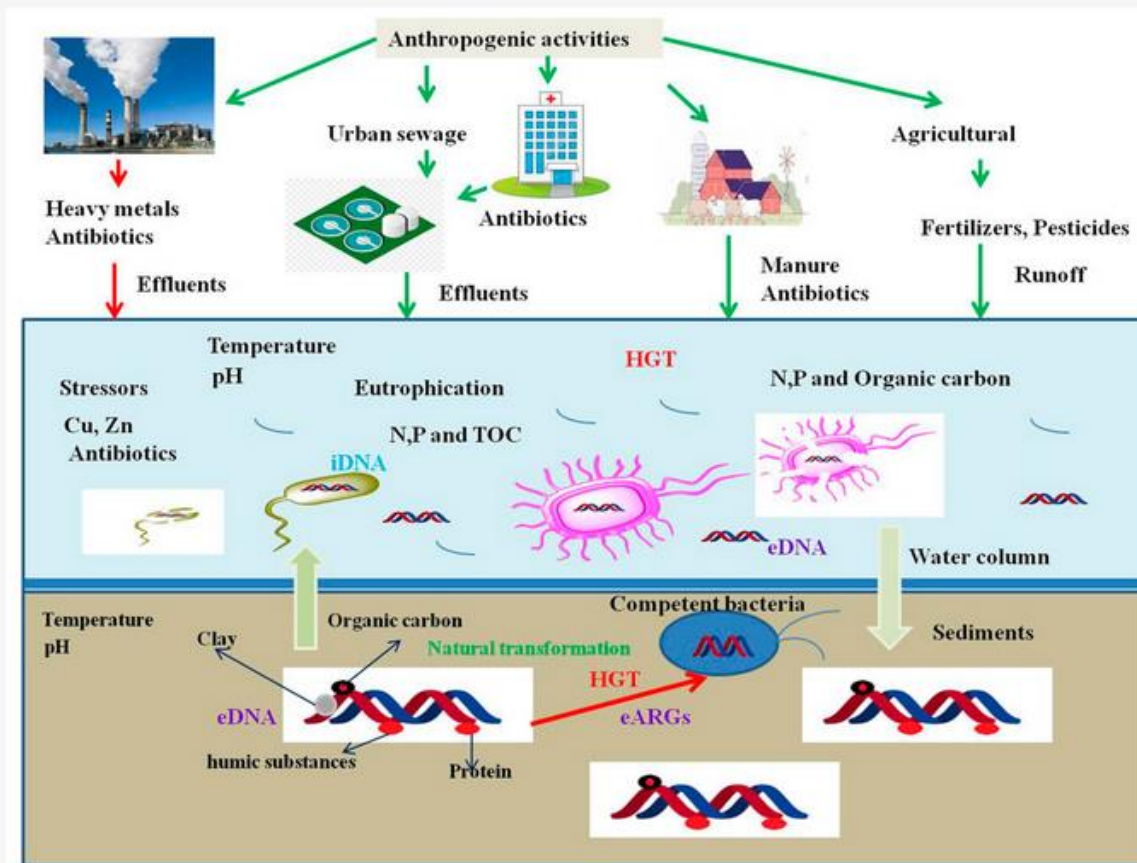
- exDNA is defined as DNA not enclosed in living cells
- Can persist 21.000 – 900.000 years
- Size 80 – 20.000bp
- Low abundance in comparison to intracellular DNA (100 – 1000 ARG/ MGE copies/ mL water)
 - Natural transformation is not likely
 - But long persistence and „hot spots“ increases probability of HGT
- Important structural component of (bacterial) biofilms
 - dissemination of genetic info
 - actively contributes to AMR in biofilms

Nagler M (2018) Appl. Microbiol Biotech





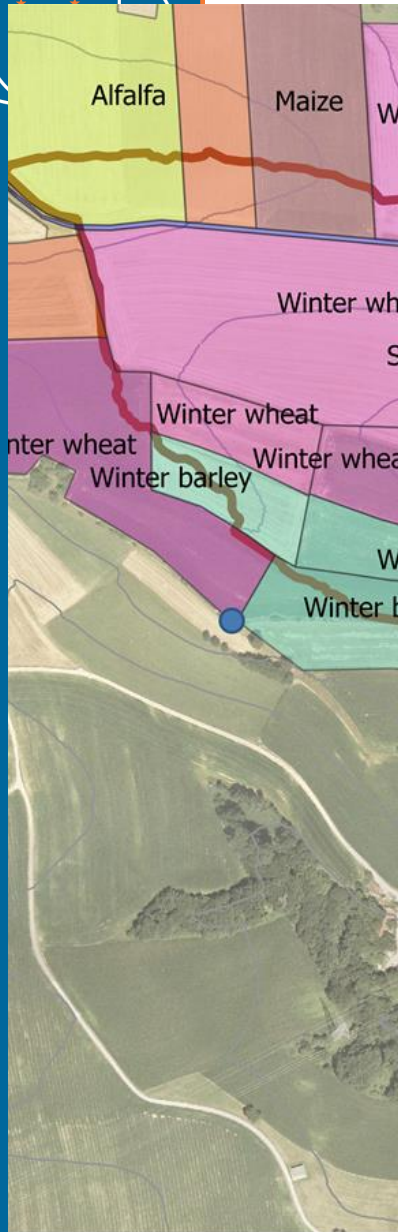
exDNA release, persistence, natural transformation in the environment - influence of various biotic and abiotic stresses.



FROM: Sivalingham et al.
Pathogens 2020, 9(11), 874;
<https://doi.org/10.3390/pathogens9110874>

- Utmost importance to study the role of exDNA in the spread of ARGs in the environmental matrices
- **FED-AMR** will contribute to improve the methods and knowledge in environmental exDNA research and it's role in AMR transmission

Open Air Lab (OAL) study



G. Blöschl et al.: The Hydrological Open Air Laboratory



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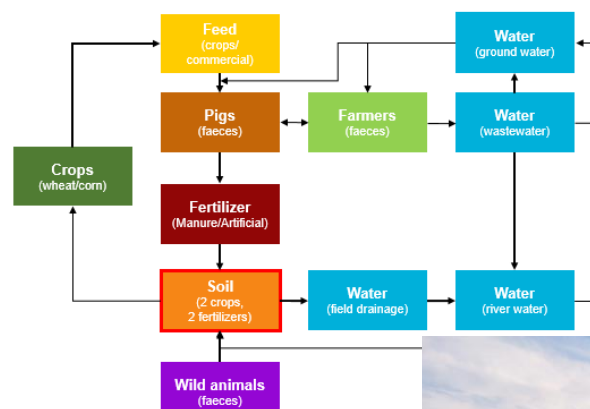


Outlet - MW

barriers



FED-AMR overview



> 500 samples

Austria, Czech Rep., Estonia,
Great Britain, Ireland,
Portugal, Norway

- Antibiotics, pesticides, heavy metals
- exDNA; tDNA
 - Microbial diversity
 - Resistome (GeneEnrichment)
- Clinically relevant (and other) bacterial species
 - WGS, ARG, susceptibility testing, phylogeny
- *C. difficile* as a model (pig-human)
- Mathematic modelling





Clinically relevant bacteria (WHO-listed, Austria)

- **89 clinically-relevant** isolates were obtained
- Forty-nine (55.1%) isolates were *E. coli*, 27 (30.33%) were *E. faecalis* and 6 (6.74%) *E. faecium*.
- **No MRSA and no *Salmonella* sp** isolates were found.
- **Wildlife** (n= 29) and **wastewater** (n= 28) accounted for the majority
- High diversity based on classical MLST(*E. coli* 36 STs, *E. faecalis* 10 STs)



> Lett Appl Microbiol. 2022 Jun;74(6):1008-1015. doi: 10.1111/lam.13685. Epub 2022 Mar 15.

Outbreak of *Cronobacter turicensis* in European brown hares (*Lepus europaeus*)

Annika Posautz¹, Michael P Szostak¹, Adriana Cabal Rosel², Franz Allerberger², Anna Stöger², Gerhard Rab^{3,4}, Andrea T Feßler^{5,6}, Joachim Spargser¹, Anna Kübber-Heiss¹, Stefan Schwarz^{5,6}, Stephen J Forsythe⁷, Werner Ruppitsch², Igor Loncaric¹

Affiliations

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- ¹ University of Veterinary Medicine, Vienna, Austria.
- ² Austrian Agency for Health and Food Safety, Vienna, Austria.
- ³ Institute of Hydraulic Engineering and Water Resources Management, Vienna, Austria.
- ⁴ Institute for Land and Water Management Research, Federal Agricultural University of Applied Sciences, Petzenkirchen, Austria.
- ⁵ Freie Universität Berlin, Berlin, Germany.
- ⁶ Department of Veterinary Medicine, Veterinary Centre for Resistance Research, University of Veterinary Medicine, Berlin, Germany.
- ⁷ Foodmicrobe.com, Keyworth, Nottingham, UK.

the 1st time in Austria
ice gene

, *K. pneumoniae* 20.6

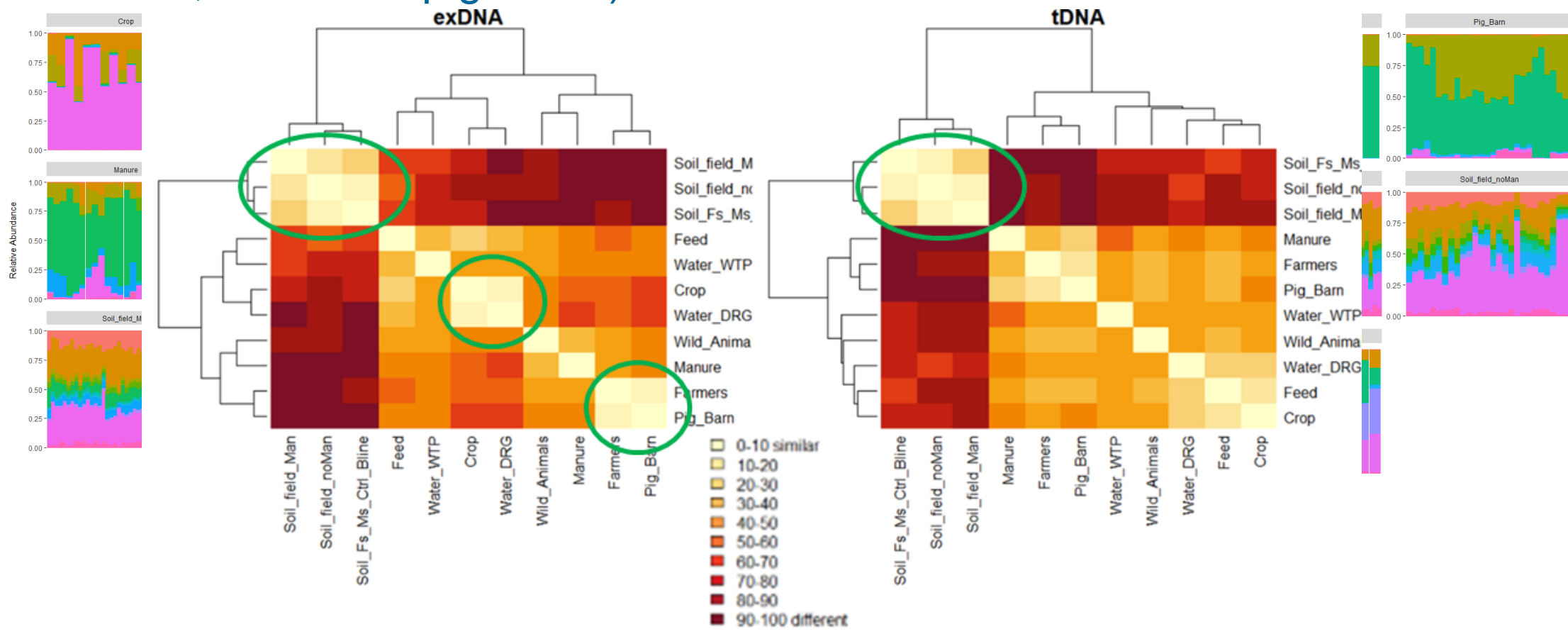
average 48.6 genes, for
undwater (14 genes) a

- ❖ 7 hare isolates genetically unrelated
- ❖ 5 from dead hares, 2 from hare feces
- ❖ But relatedness to human isolates
- ❖ All carry new *mcr* gene



Results 16S AmpSeq (tDNA – exDNA)

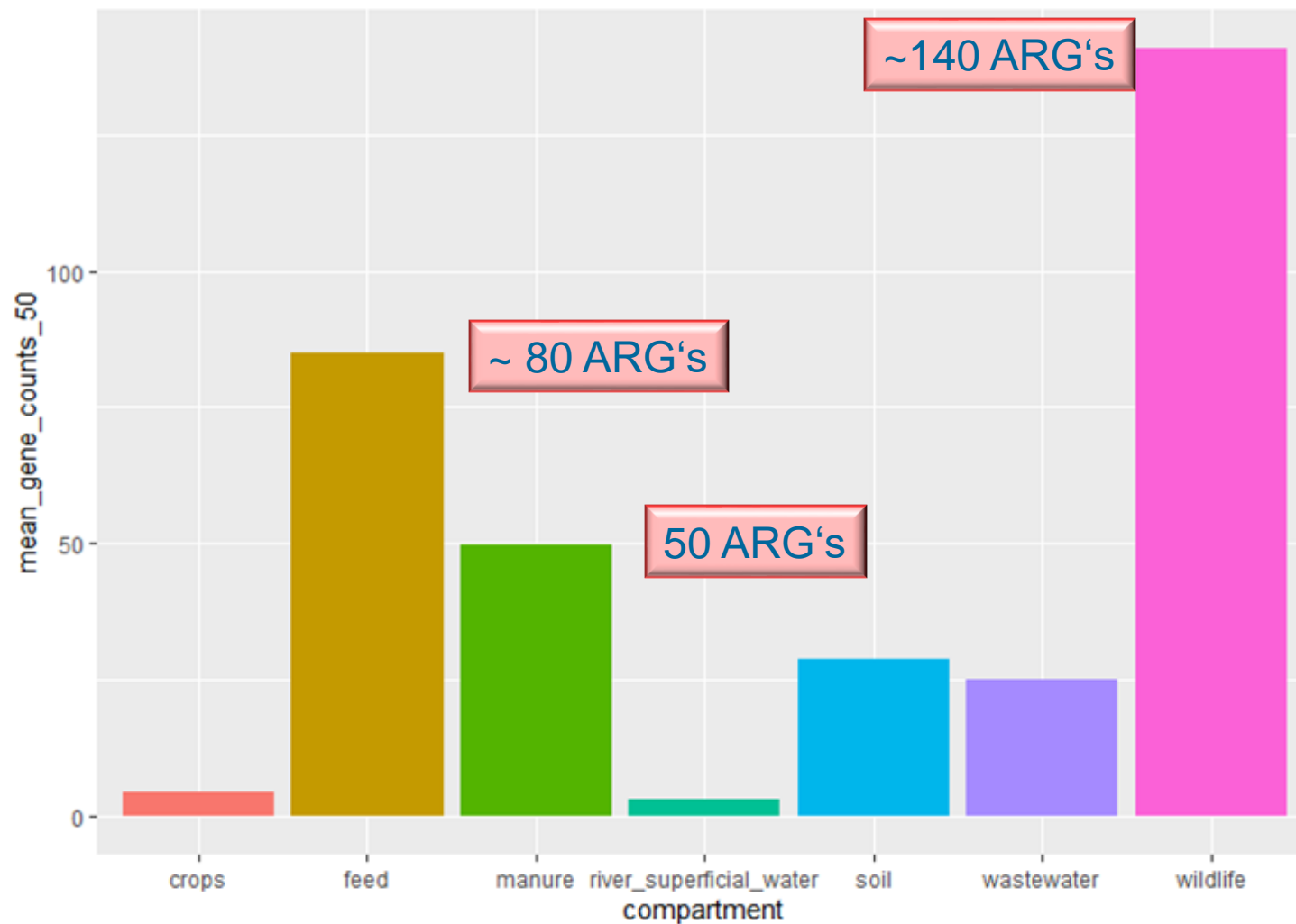
- **High α -diversity** (similar for ex and tDNA; except drainage water and crops) 16 – ~1Mio. species specific reads/ sample; average 90.000/ sample
- **Low β -diversity** (except for diverse soils, (exDNA: crops – drainage water, farmers – pig barns))





ARG detection - GeneEnrichment

First results – ARG's/ compartement - Austrian samples





Conclusions

- **Large amount of data and metadata** from an agricultural environment
- **The combination of all data** can help to elucidate the **transfer and development of resistances** in microbial communities in the environment
- Identification of **new potential sources** of emerging resistant microorganisms along the feed/food chain with a focus on the environment and **wildlife as a typically neglected** component of One Health
- Results influence the **development of strategies** to use and to ensure availability of new and old antimicrobial agents, as per requirement of the ECDC technical report on One Health and AMR, 2021





Conclusions – shortcomings/ optimization

- **Large amount of data**
 - **still not large enough** for development of very good mathematical models?
- **Sampling**
 - One year growing season is **too short!**
 - More time points
 - Logistics!
- **Development & application of new technologies**
 - Technical improvement!
 - extraction methods exDNA
 - gene enrichment
 - ultra deep sequencing!
 - Analysis exDNA (free and bound i.e. in biofilms) & iDNA & tDNA)



FED-AMR Consortium



Veterinærinstituttet
Norwegian Veterinary Institute

Solveig Mo
Thomas Haverkamp



**INSTITUT
PASTEUR**
Cristelle Mazuet
Laure Diancourt



Christian Seyboldt
Ines Dost
Mostafa Abdel-Gliel



Mark Chambers
Giovanni Lo Iacono
Roberto La Ragione
Brian Gardner
Marwa Mohsen Hussain Ali Hassan
Monica Felipe-Sotelo



Tanel Tenson
Julia Jeremejeva
Kaidi Telling
Veljo Kisand
Viia Kõiv
Krõõt Arbo



**STATENS
SERUM
INSTITUT**

Søren Persson
Semeh Bejaoui



Sven Maurischat
Anissa Scholtzek
Pascal Witt
Gaby Raatz



NUI Galway
OÉ Gaillimh

Alexandre De Menezes
Gavin Collins
Collin Lawton
Florence Abram



Marta Kořínová
Martina Štěpánková
Magdaléna Zimová
Ladislava Matějů
Karolína Hochmalová
Barbora Macková



Anna Gajda
Tomasz Bladek
Ewelina Nowacka-Kozak
Monika Banaszek-Urban
Małgorzata Gbylik – Sikorska
Magdalena Bilecka
Iwona Szymanek – Bany
Aleksandra Kuśmierz



Werner Ruppitsch
Adriana Cabal Rosel
Karin Rainer
Martin Brandtner
Markus Wögerbauer
Krista Rathammer
Nadine Peischl
Harald Bock

Instituto Nacional de Saúde
Doutor Ricardo Jorge



Manuela Caniça
Mónica Oleastro
Tania Rosado
Vera Manageiro
Daniel Olivença



Delft University of Technology



Thank you for your attention!

werner.ruppitsch@ages.at

