

# Early warning of faecal recontamination in dune aquifers by online flow cytometry

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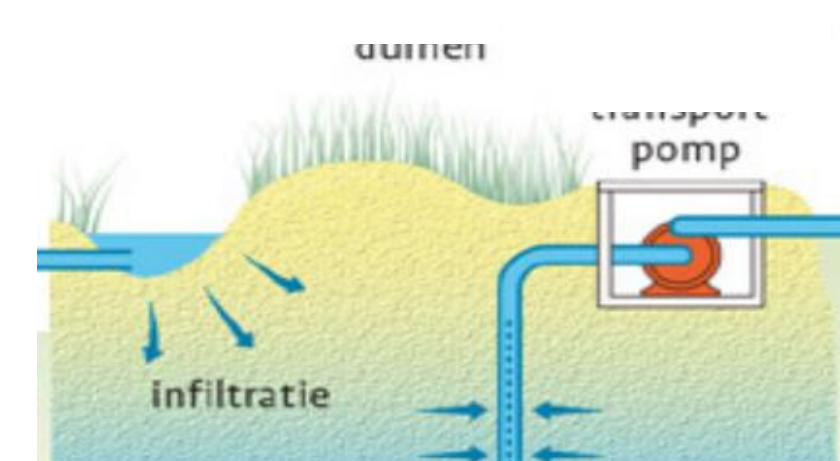
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## Introduction

Artificial recharge of dune aquifers in Dutch coastal area is applied for the production of safe drinking water. Incidental occurrence of faecal indicators indicates recontamination during the extraction. Value of online flow cytometry as an early warning tool to detect faecal recontamination in extracted water is evaluated.

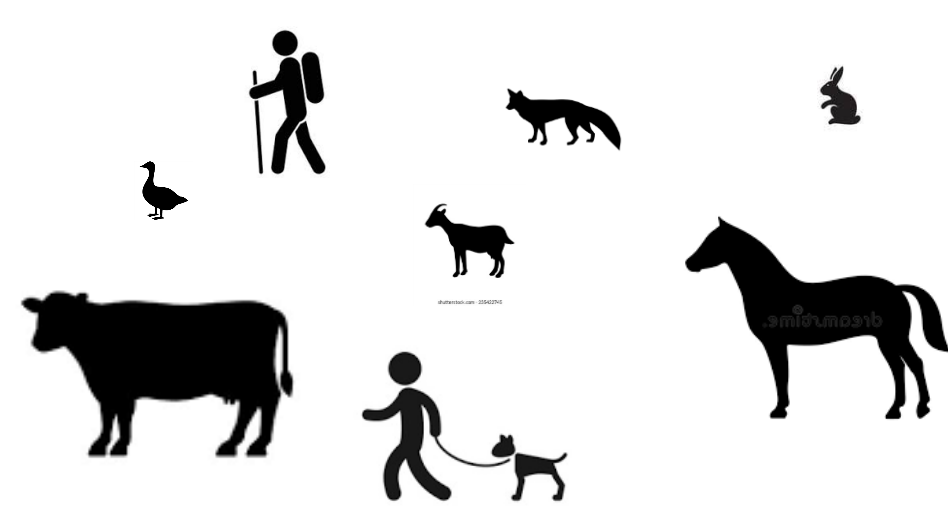
Dune filtration enables 8 log removal for pathogenic microorganisms

Treatment



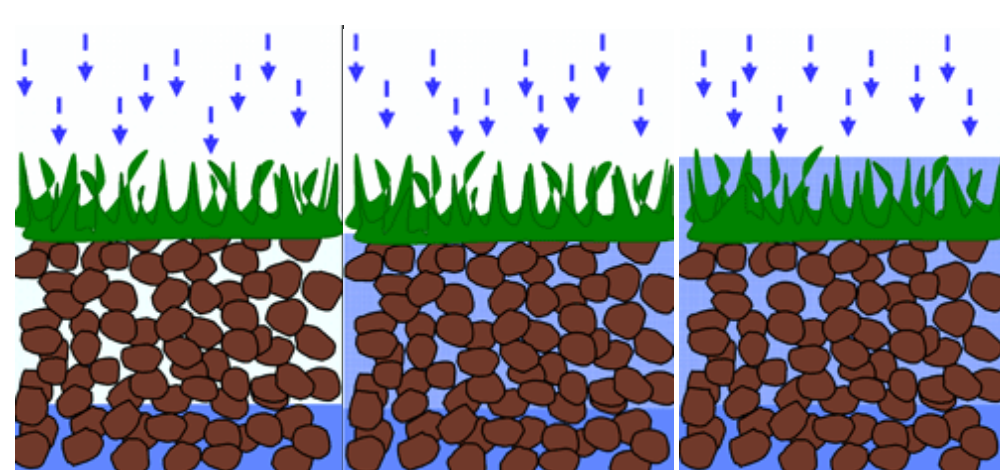
Recontamination of abstracted water by faecal depositions by natural fauna, grazers, human

Risks



Rainfall  
Thickness of unsaturated zone  
Groundwater level  
Abstraction rate

Factors



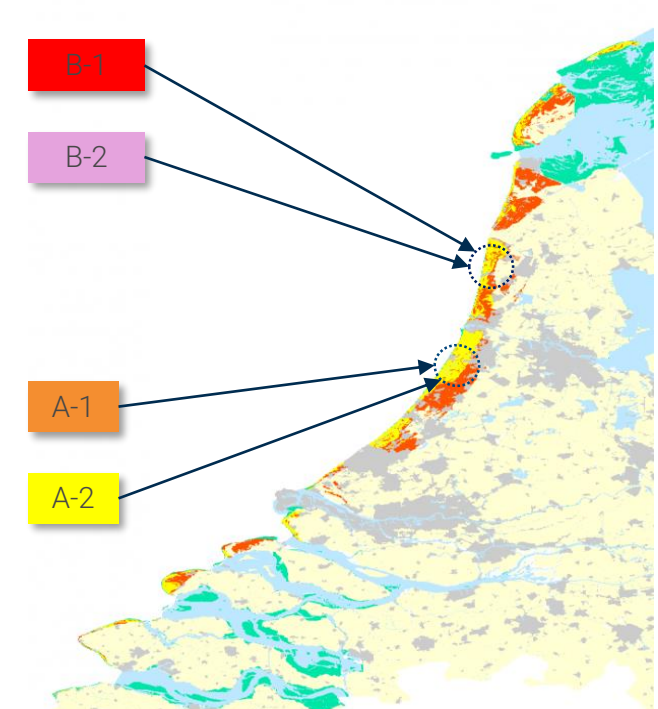
Water quality and safety

Monitoring



## Methods and materials

Sampling locations



Location	FIB*	Thickness of unsaturated zone [m]	Flow rate (min-max) [m <sup>3</sup> /h]
A-1	+++	Variable 0 - 0,6	60-120
A-2	-	Stable > 3	310-680
B-1	++	Variable 1-3	115-350
B-2	+	Mixture of well-series	330-1900

\*FIB=faecal indicator bacteria

Analysis

Measuring period: August – October 2021

Rainfall: The Royal Netherlands Meteorological Institute



Online:

Flow cytometry (FCM) BactoSense™ Flow Cytometer / 24 per day Applied Process Technology

- total number of intact cells (ICC)
- cell numbers with high (HNA) and low (LNA) content of nucleic acid

Off-line:

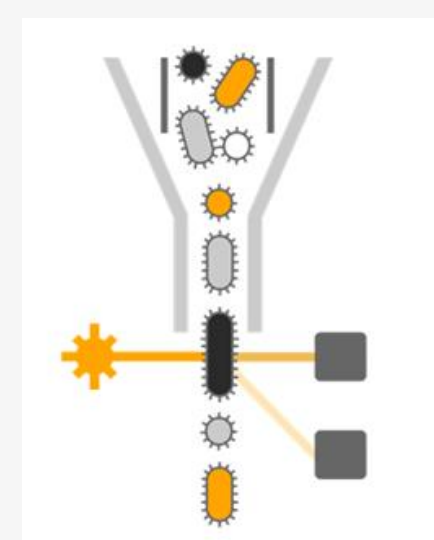
Faecal indicators bacteria (FIB)

Large volume (50 L) Coliforms (NEN-EN-ISO 9308-1:2014) twice per week

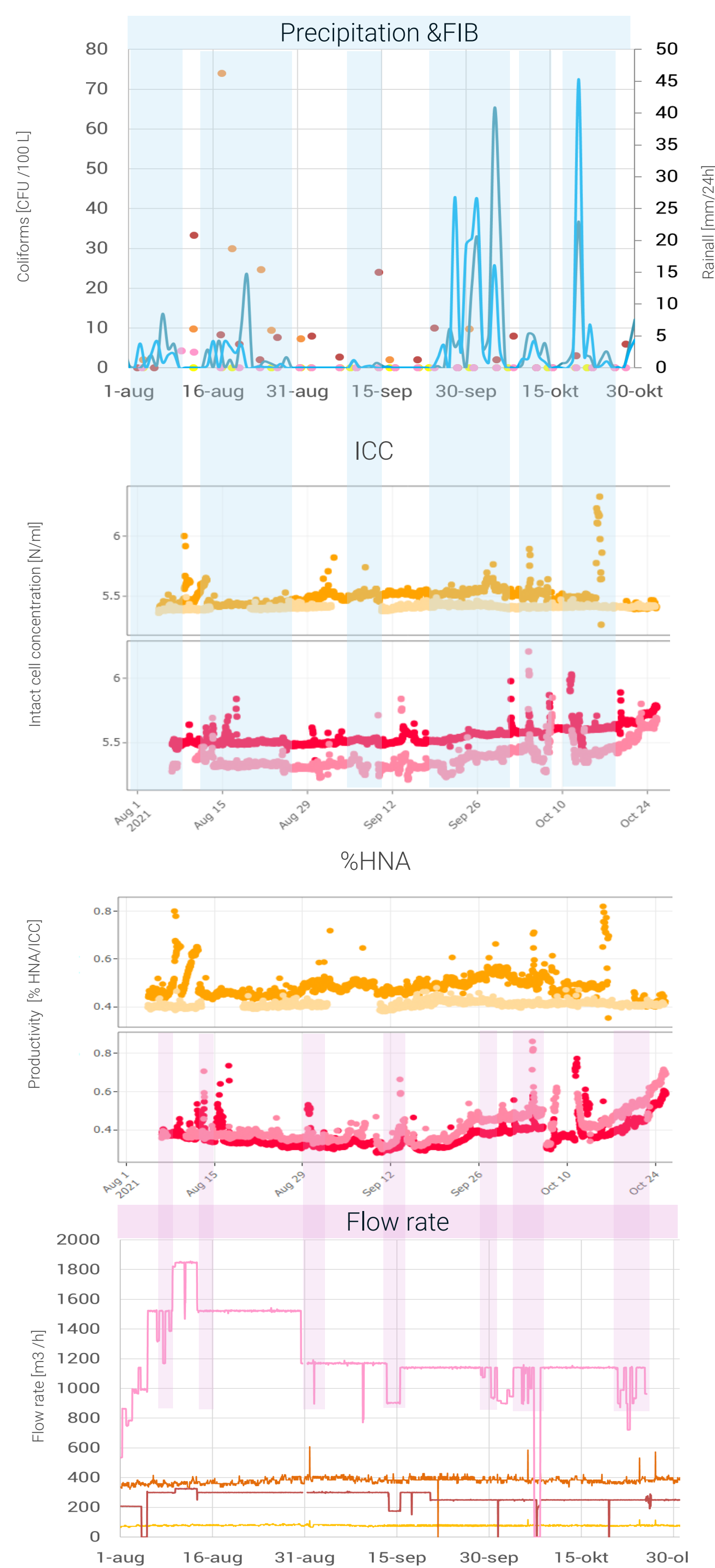
Data analysis

FCM-fingerprinting – KYTOS™

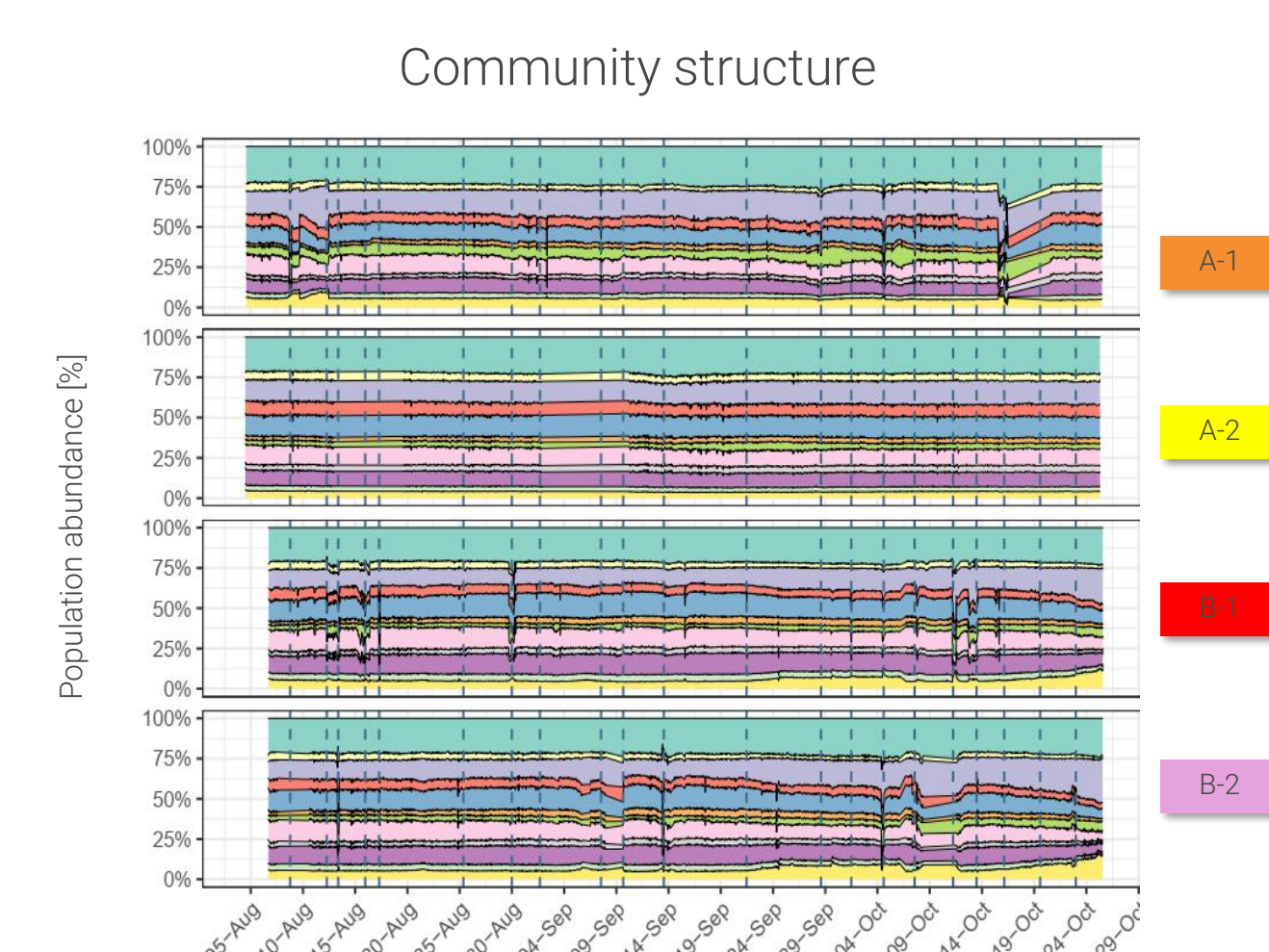
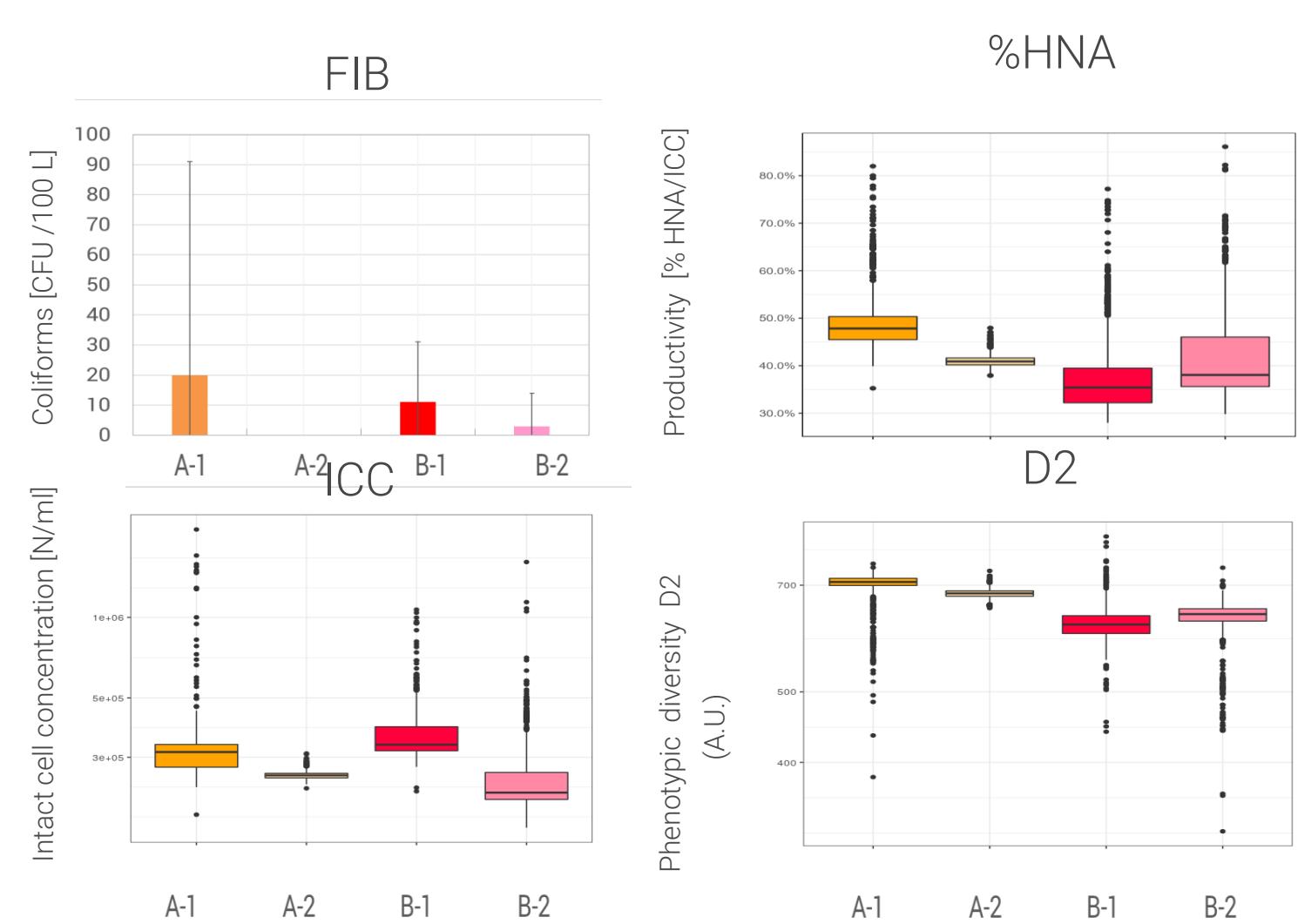
- Cell concentration – total, intact (ICC), HNA, LNA
- Diversity index – D2, unique cell types incl. abundance
- Microbiome type - KytoTypes
- Productivity index: % HNA
- Community structure – rel. abundance of KytoTypes



## Results



- Well series without FIB show stable community
- Response to rain, T°C and flow rate:
  - ICC, %HNA ↑
  - FCM phenotypic diversity ↑↓
- Flow rate and ground water level are related and affect thickness of unsaturated zone
- Community structure quickly restored
- Certain KytoTypes related to FIB



## Conclusions

**Online FCM combined with FCM-fingerprinting is suitable for rapid detection of quantitative and qualitative changes in general bacterial water quality. It can be used as efficient early warning tool for detection of faecal recontamination of shallow phreatic ground water during abstraction.**

- Stable abstraction process result in a stable, location specific baseline for FCM parameters
- Absence of FIB in abstracted water is reflected in low variation of FCM parameters
- Two types of deviations of FCM parameters in well-series with FIB co-occur with events of rainfall and flow rate change:
  - Recontamination – increase in ICC, %HNA and D2; intrusion of new types of micro-organisms and nutrients from surface and/or remobilisation of FIB from unsaturated zone triggered by increase in groundwater level
  - Regrowth – Increase ICC, %HNA accompanied by decrease in D2; enhanced microbial activity and shift in abundance of autochthonous populations
- FCM-fingerprinting (KytoTypes) useful to detect FIB contamination
- Molecular based research is required to confirm correlation of FIB recontamination with KytoTypes