Measuring the microbial biodiversity by single-cell analysis

Pieter Monsieurs, Ruben Props, Mohamed Mysara, Lieven Clement, Nico Boon, Natalie Leys

Belgian Nuclear Research Center

pmonsieu@sckcen.be
Hot topic...

Bacterial diversity amplifies nutrient-based plant–soil feedbacks

Shifts in microbial diversity through land use intensity as drivers of carbon mineralization in soil

Testing biodiversity-ecosystem functioning relationship in the world’s largest grassland: overview of the IMGRE project

Marine biodiversity and ecosystem function relationships: The potential for practical monitoring applications
Assessing biodiversity

The microbial community

Ecological metrics
- Applications
- Ecological theory testing
- System monitoring

Taxonomic fingerprint

Sequencing workflow
- Filtration
- DNA extraction
- Amplicon sequencing
- Bioinformatics to OTU-table
Flow Cytometry

10,000 cells s\(^{-1}\)
Phenotypic markers

Multivariate data

Morphology ~ Scattering
DNA content ~ Fluorescence
...

Green Fluorescence

Red Fluorescence

# cells

2 parameters

© SCK•CEN, 2018
“The community landscape represents a phenotypic state of the whole microbial community.”
Rationale

Hill Order \( (q) \)

<table>
<thead>
<tr>
<th>Diversity metric ( (D_q) )</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
</tr>
<tr>
<td>1</td>
</tr>
<tr>
<td>2</td>
</tr>
</tbody>
</table>
Oligotrophic ecosystem model

- Control
- Startup
- Operation
- Shutdown

- Ion exchange
- Cooling tower
- Central storage
- BR2 nuclear test reactor
- Ground water
Both a dynamic and stable system

- $60 \text{ m}^3 \text{ h}^{-1} \rightarrow 4,000 \text{ m}^3 \text{ h}^{-1}$
- $15 \, ^\circ\text{C} \rightarrow 30 \, ^\circ\text{C}$
- $1 \, \mu\text{S cm}^{-1} \rightarrow 7 \, \mu\text{S cm}^{-1}$
Both a dynamic and stable system
Monitoring the biodiversity

Props, R. et al., submitted.
Monitoring the biodiversity

Phenotypic diversity ($D_2$)

<table>
<thead>
<tr>
<th>Reactor cycle (days)</th>
<th>0</th>
<th>10</th>
<th>20</th>
<th>30</th>
</tr>
</thead>
<tbody>
<tr>
<td>800</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1,200</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1,600</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2,000</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Monitoring the biodiversity

✓ Phenotypic diversity indicates dynamic changes

✓ So does the taxonomic diversity...
Monitoring the biodiversity

Diversity indices derived from:
- ≠ Analysis methods
- ≠ Data types
- ≠ Processing pipelines

*indicate similar community dynamics*
Other Ecosystems
Absolute quantification of OTUs

Relative abundance (%)

Absolute abundance (cells µl⁻¹)

Survey 1

Survey 2
✓ In-situ real-time monitoring of biodiversity

✓ Intelligent experimental design

✓ Complementary tool to sequencing platform

https://github.com/rprops/Phenoflow_package
Acknowledgements

Ir. Ruben Props

Dr. Mohamed Mysara
Dr. Natalie Leys

Dr. Emma Hernandez Sanabria
Prof. Dr. Nico Boon

Prof. Dr. Vincent Denef

University of Michigan