

# Understanding the effect of disturbance on the function, structure and assembly of complex microbial communities, using activated sludge for wastewater treatment as a model system

*Ezequiel Santillan, Ph.D.*  @ezesantillan

Singapore Centre for Environmental Life Sciences Engineering

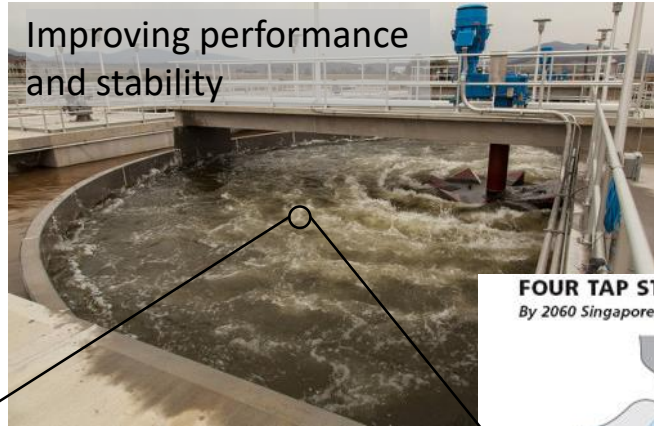


# Biological wastewater treatment, a model system for microbial ecology

Major role in the protection of water quality and public health

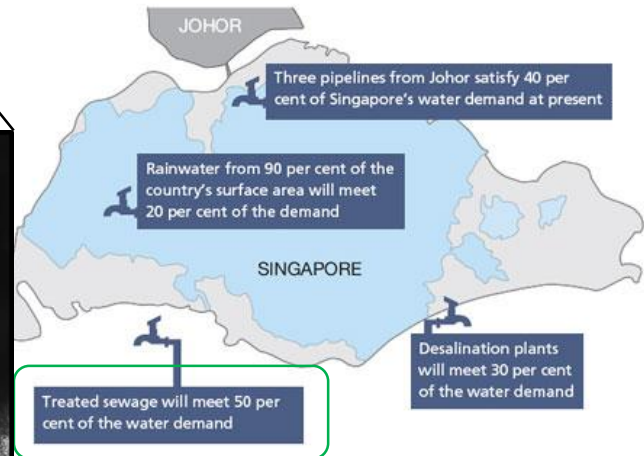
Renewable source of freshwater in urban centres with scarce water supplies

Improving performance and stability



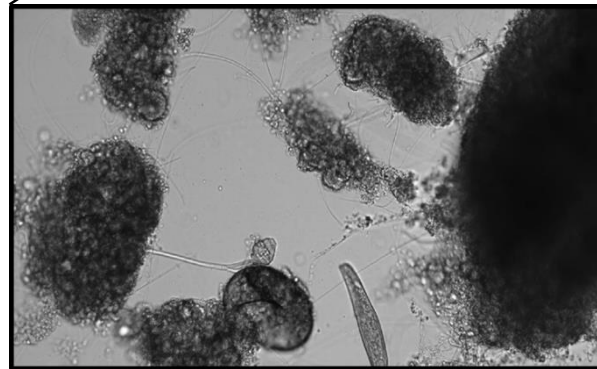
## FOUR TAP STRATEGY

By 2060 Singapore will be self-reliant in water



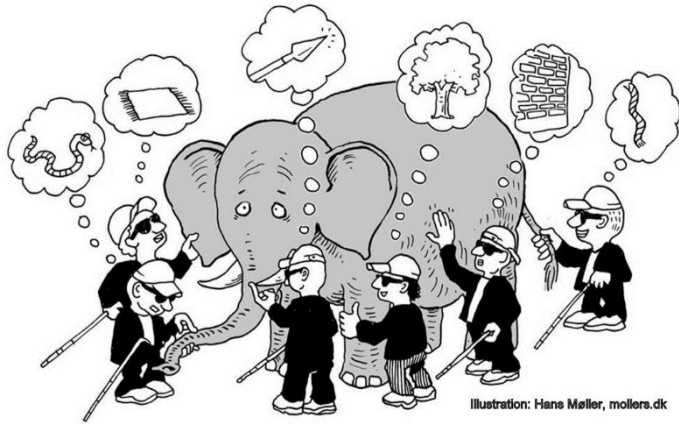
## Complex microbial system:

- Hundreds-thousands of taxa
- Thousands of genes
- Trillions of Individuals ( $10^{10}$  cells/mL)



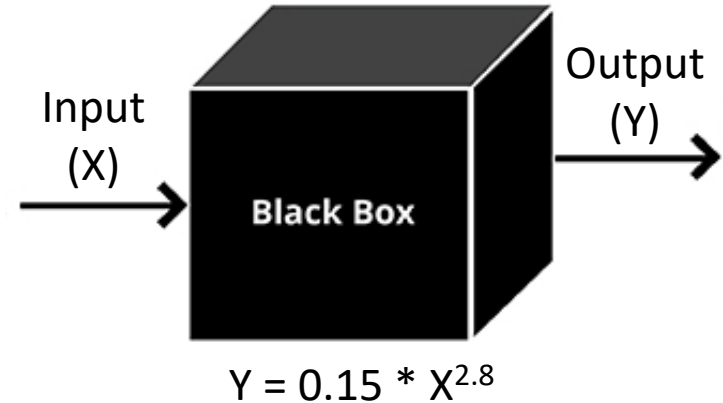
# How to understand complex microbial systems?

## 1. Reductionist approach



Limited  
cross-system  
relevance

## 2. Black box approach



3. Ecological theory: An innovative approach to predict outcomes in complex microbial systems!



# Ecological theory **can** lead to innovations in wastewater treatment!

“Ecological theory helps us draw **generalized conclusions** from specific observations of organisms in their environment, by allowing us to classify, interpret and **predict** the world around us”



# The Application of Ecological Theory Toward an Understanding of the Human Microbiome

Elizabeth K. Costello,<sup>1</sup> Keaton Stagaman,<sup>2</sup> Les Dethlefsen,<sup>1,3</sup>  
Brendan J. M. Bohannan,<sup>2</sup> David A. Relman<sup>1,3,4\*</sup>

*“Integration between theory and  
experiments is a crucial ‘missing link’  
in current microbial ecology”*

OPEN

The ISME Journal (2016) 10, 2557–2568  
© 2016 International Society for Microbial Ecology. All rights reserved.  
[www.nature.com/ismej](http://www.nature.com/ismej)

MINI REVIEW

## Challenges in microbial ecology: building predictive understanding of community function and dynamics

Stefanie Widder<sup>1</sup>, Rosalind J Allen<sup>2</sup>, Thomas Pfeiffer<sup>3</sup>, Thomas P Curtis<sup>4</sup>, Carsten Wiuf<sup>5</sup>,  
William T Sloan<sup>6</sup>, Otto X Cordero<sup>7</sup>, Sam P Brown<sup>8</sup>, Babak Momeni<sup>9,10</sup>, Wenying Shou<sup>10</sup>,  
Helen Kettle<sup>11</sup>, Harry J Flint<sup>12</sup>, Andreas F Haas<sup>13</sup>, Béatrice Laroche<sup>14</sup>, Jan-Ulrich Kreft<sup>15</sup>,  
Paul B Rainey<sup>3</sup>, Shiri Freilich<sup>16</sup>, Stefan Schuster<sup>17</sup>, Kim Milferstedt<sup>18</sup>, Jan R van der Meer<sup>19</sup>,  
Tobias Großkopf<sup>20</sup>, Jef Huisman<sup>21</sup>, Andrew Free<sup>22</sup>, Cristian Picioreanu<sup>23</sup>, Christopher Quince<sup>24</sup>,  
Isaac Klapper<sup>25</sup>, Simon Labarthe<sup>14</sup>, Barth F Smets<sup>26</sup>, Harris Wang<sup>27</sup>,  
Isaac Newton Institute Fellows<sup>28</sup> and Orkun S Soyer<sup>20</sup>

PERSPECTIVES

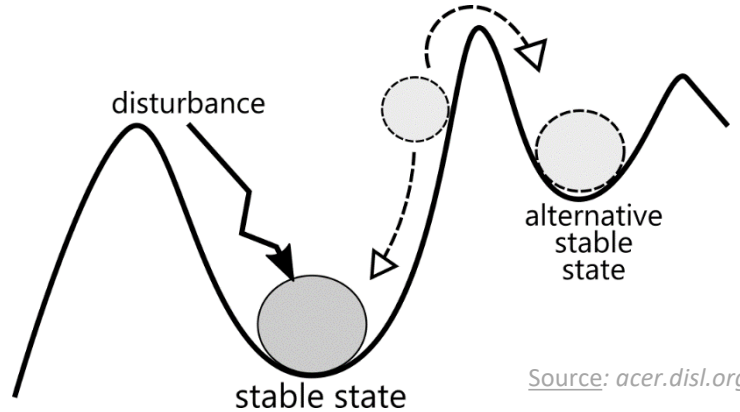
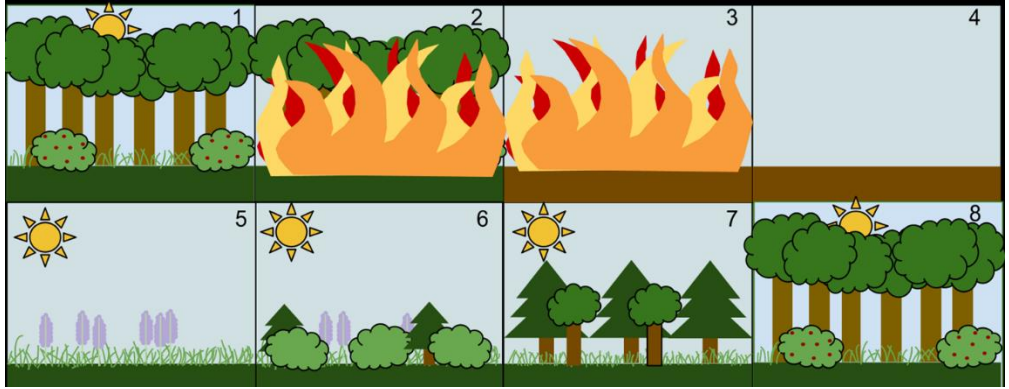
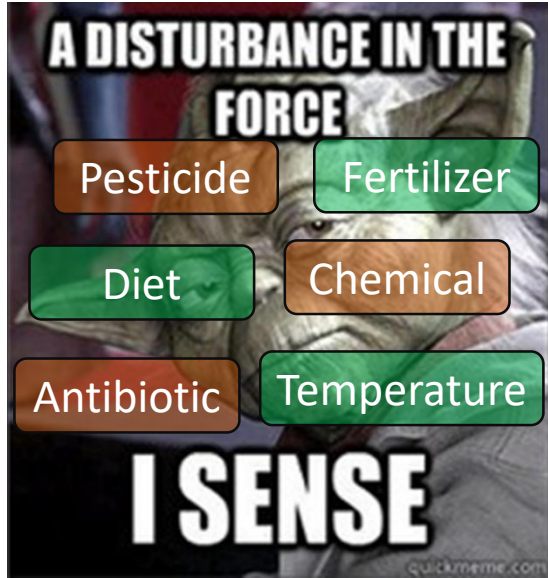
ESSAY

## The role of ecological theory in microbial ecology

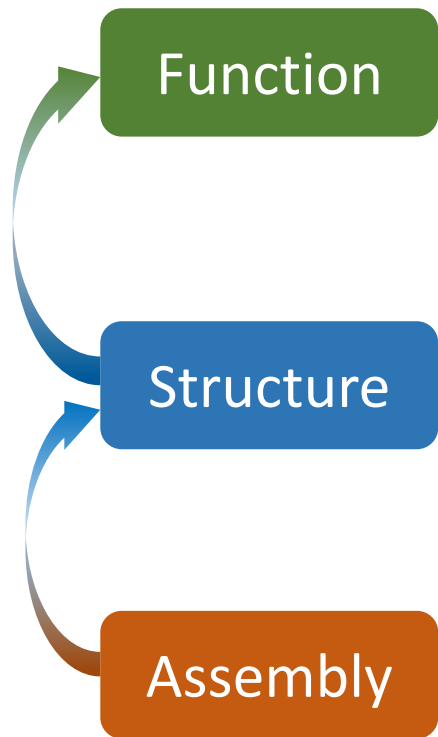
James I. Prosser, Brendan J. M. Bohannan, Tom P. Curtis, Richard J. Ellis,  
Mary K. Firestone, Rob P. Freckleton, Jessica L. Green, Laura E. Green,  
Ken Killham, Jack J. Lennon, A. Mark Osborn, Martin Solan,  
Christopher J. van der Gast and J. Peter W. Young

# Why disturbance effects on microbial communities? Wastewater treatment and beyond!

Disturbance *structures*  
ecosystems



# Ecological theory for microbial communities, from assembly to function



Ecosystem services

*Carbon removal, nitrification, etc.*

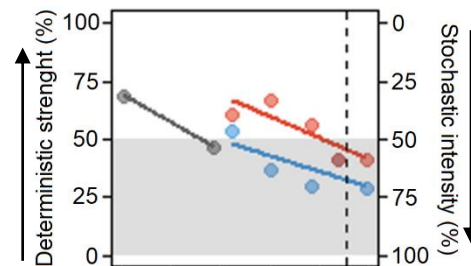
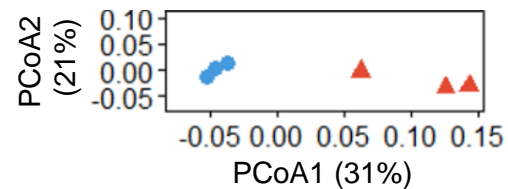
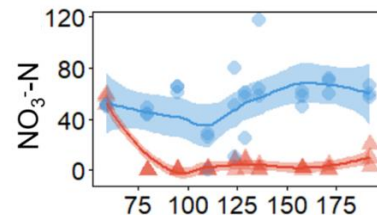
Composition and abundances

*Diversity*

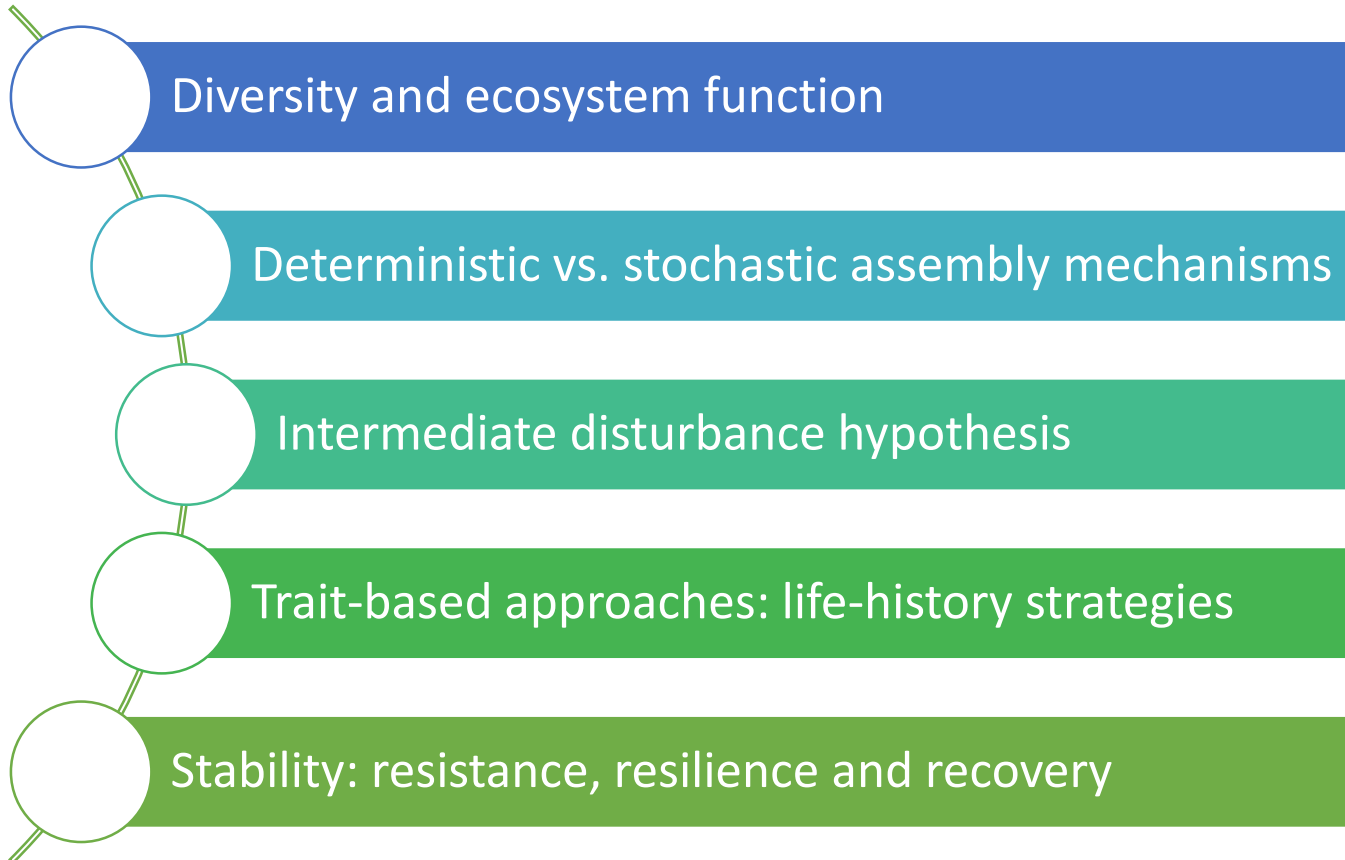
Underlying mechanisms

Deterministic / Stochastic

*Null models*

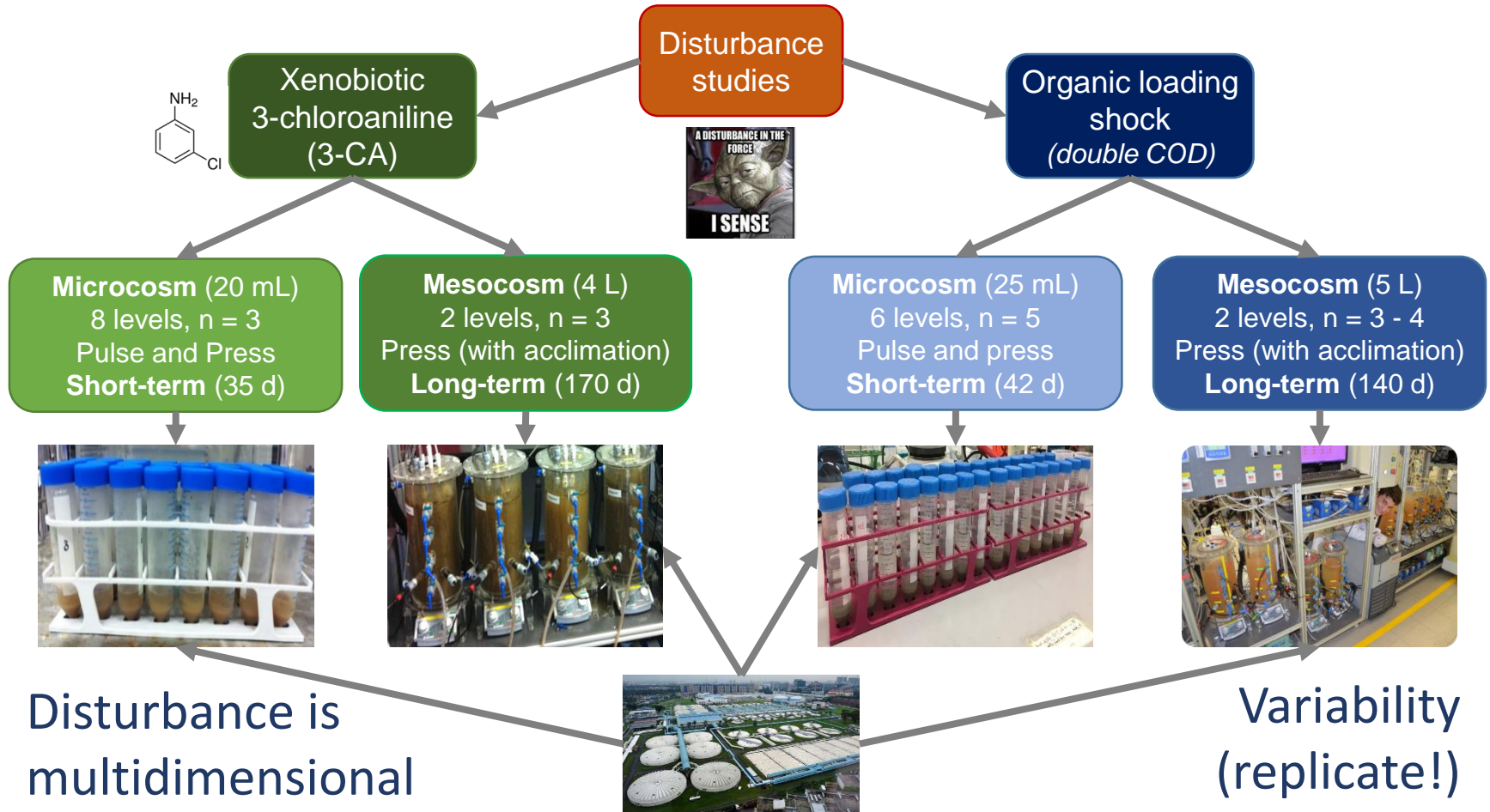


# Ecological theory of disturbance



Recommended read: Pulsford *et al.* 2016 *Biol. Rev.* 91: 148-167.

# Studies using wastewater treatment bioreactors





# Ecological theory of disturbance @SCElse

npj | Biofilms and Microbiomes

www.nature.com/npjbiofilms

ARTICLE OPEN

Frequency of disturbance alters diversity, function, and underlying assembly mechanisms of complex bacterial communities

Ezequiel Santillan <sup>1,2</sup>, Hari Seshan<sup>1,2,4</sup>, Florentin Constancias<sup>1</sup>, Daniela I. Drautz-Moses<sup>1</sup> and Stefan Wuertz <sup>1,2,3</sup>

[Link to publication](#)



RESEARCH ARTICLE  
Ecological and Evolutionary Science

Press Disturbance Alters Community Structure and Assembly Mechanisms of Bacterial Taxa and Functional Genes in Mesocosm-Scale Bioreactors

<sup>a,b</sup> Ezequiel Santillan, <sup>a</sup> Florentin Constancias, <sup>a,b,c</sup> Stefan Wuertz

[Link to publication](#)

pubs.acs.org/estwater

Article

Press Xenobiotic 3-Chloroaniline Disturbance Favors Deterministic Assembly with a Shift in Function and Structure of Bacterial Communities in Sludge Bioreactors

Ezequiel Santillan,<sup>§</sup> Hari Seshan,<sup>§</sup> and Stefan Wuertz<sup>\*</sup>

[Link to publication](#)





environmental  
microbiology



Environmental Microbiology (2019) 21(10), 3751–3764

doi:10.1111/1462-2920.14725

**Trait-based life-history strategies explain succession scenario for complex bacterial communities under varying disturbance**

Ezequiel Santillan <sup>1,2</sup> Hari Seshan,<sup>1,2</sup> Florentin Constancias <sup>1,3,4</sup> and Stefan Wuertz<sup>1,2,5\*</sup>

[Link to publication](#)

**Sustained organic loading disturbance favors nitrite accumulation in bioreactors with variable resistance, recovery and resilience of nitrification and nitrifiers**

Part of **SPRINGER NATURE**  
**SCIENTIFIC REPORTS**

E. Santillan, W. X. Phua, F. Constancias & S. Wuertz 

[Link to publication](#)

Microbiome assembly predictably shapes diversity across a range of disturbance frequencies

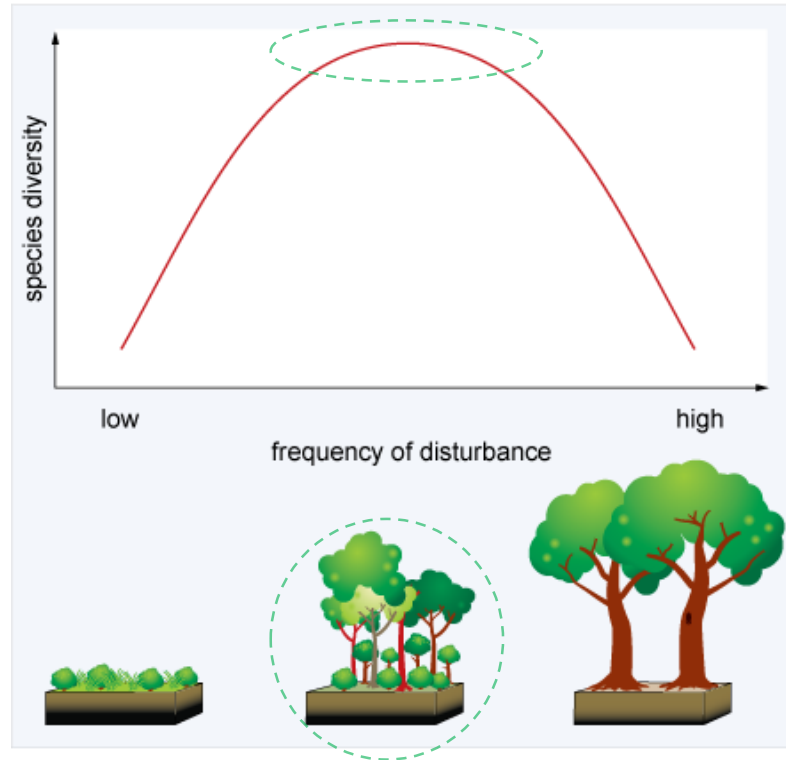
<sup>1</sup> Ezequiel Santillan, <sup>1</sup> Stefan Wuertz

doi: <https://doi.org/10.1101/2021.08.02.454702> (under revision)

[Link to preprint](#)

**bioRxiv**  
THE PREPRINT SERVER FOR BIOLOGY

# Ecological theory: the intermediate disturbance hypothesis (IDH)



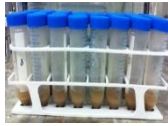
Competition-colonization  
trade-offs

Not yet tested for  
wastewater treatment  
systems until...

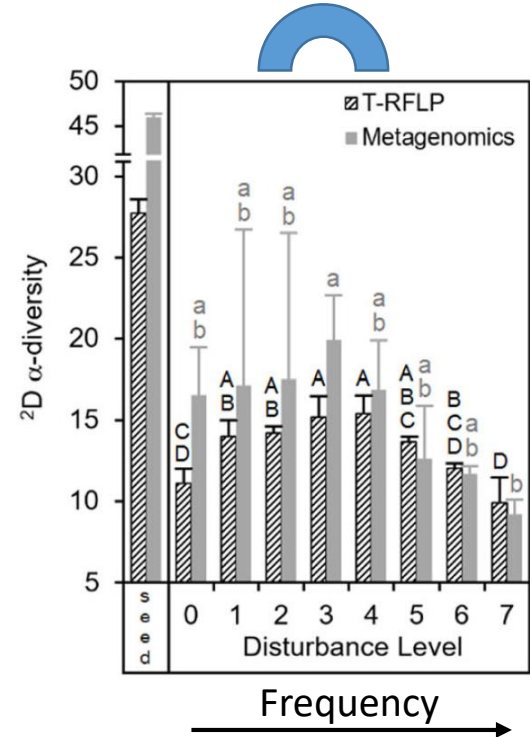
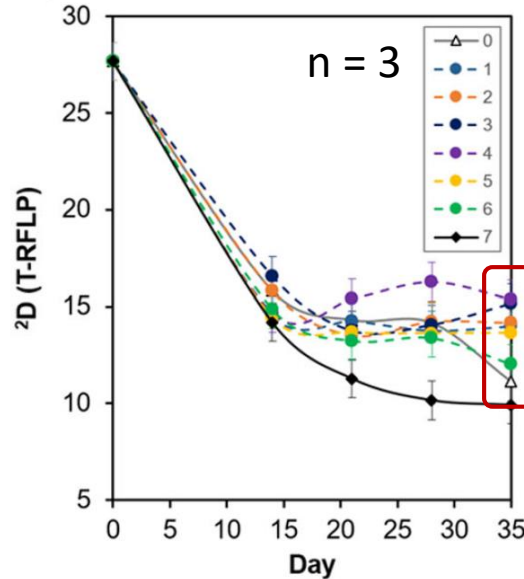
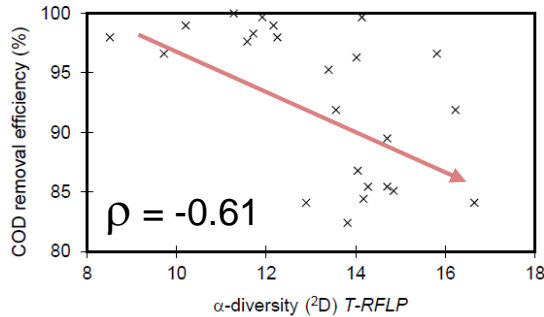
Connell (1978) *Science* 199(4335):1302-1310.

Based on tropical rain forests and corals on tropical reefs.

# Diversity varied with disturbance frequency reaching a parabolic pattern at d35



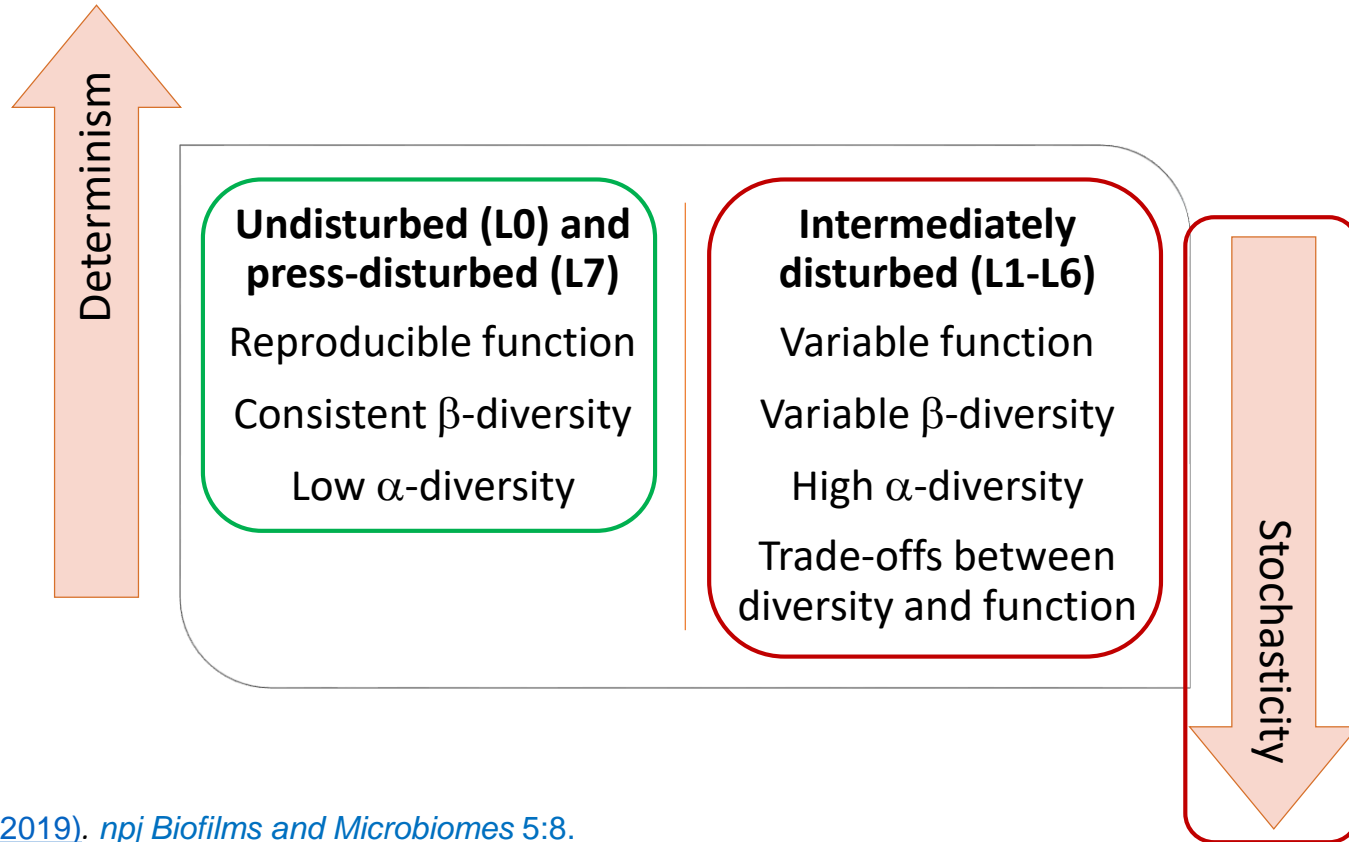
**Microcosm (20 mL)**  
8 levels (n = 3)  
Pulse and Press  
Short-term (35 d)



Disturbance: 3-CA addition at different frequencies.

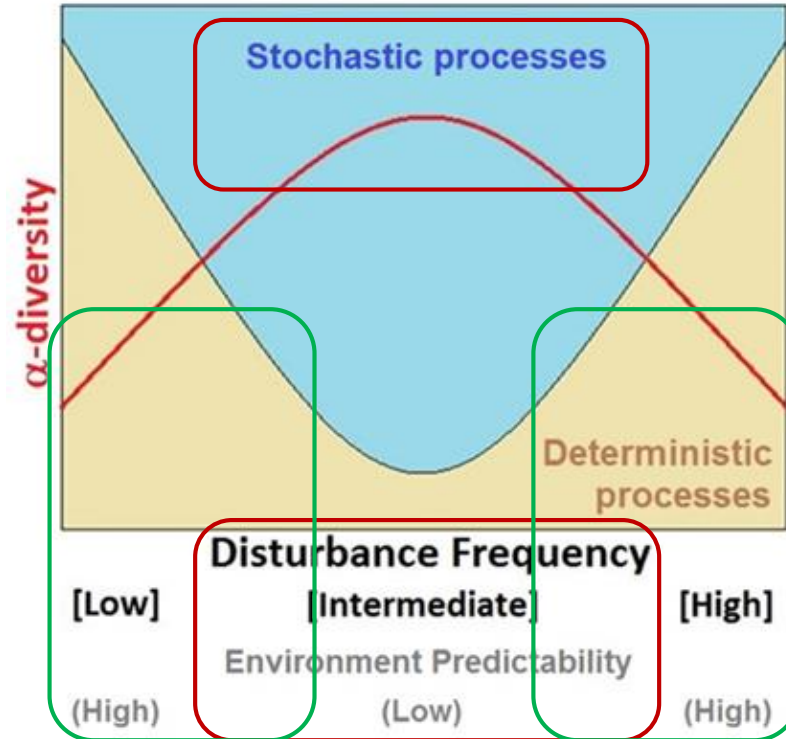
Levels: 0: undisturbed, 1-6: intermediately disturbed, 7: press-disturbed.

# Relationship between diversity, disturbance and function in complex biofilms



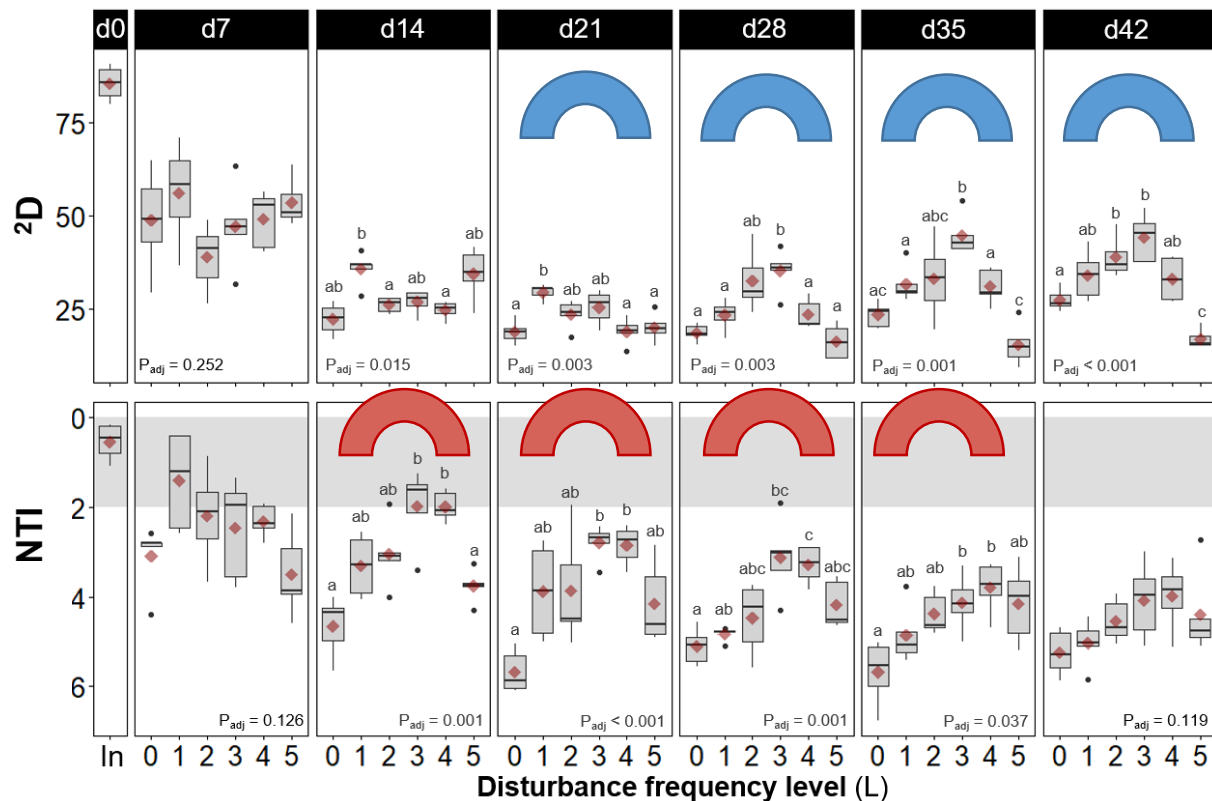
# Ecological theory:

## The Intermediate Stochasticity hypothesis



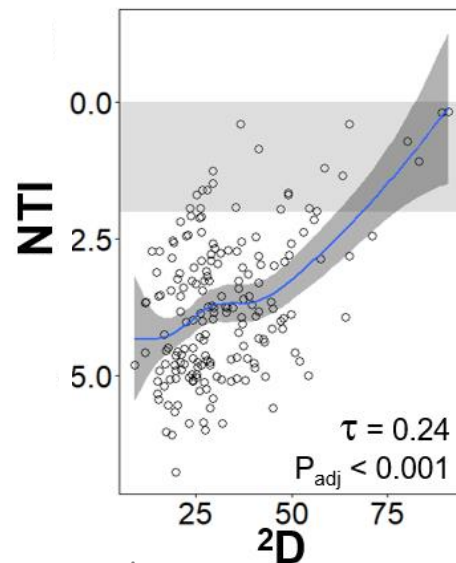
[Santillan E. et al. \(2019\)](#)  
*npj Biofilms and Microbiomes* 5:8.

# Testing the intermediate stochasticity hypothesis: assembly precedes structure



[Santillan & Wuertz 2021 \(under revision\)](#)

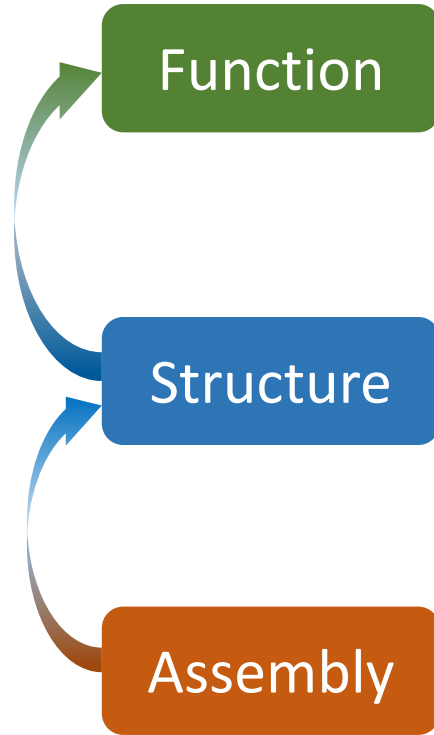
Microcosm (25 mL)  
6 levels (n = 5)  
Pulse and press  
Short-term (42 d)



Disturbance: Double organic-loading fed at different frequencies.

Levels: 0: undisturbed, 1-4: intermediately disturbed, 5: press-disturbed. 16S rRNA gene (v3-v4) amplicon sequencing

# What did we find?



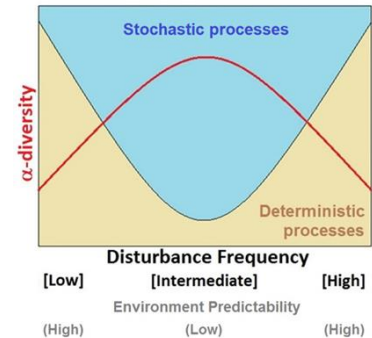
Ecosystem services

*More diverse*  $\neq$  *Better function*

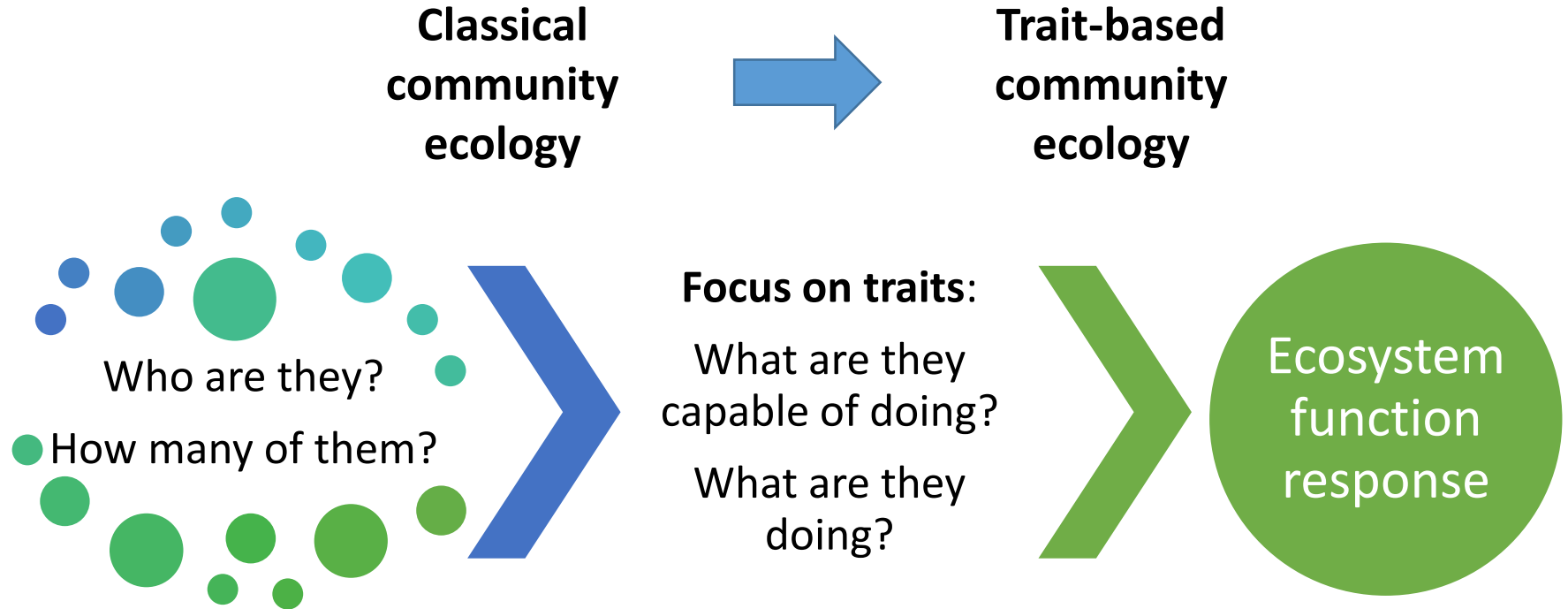
Composition and abundances  
Diversity

*Assembly precedes structure!*

Underlying mechanisms  
Deterministic / Stochastic



# Ecological theory: From community structure to traits



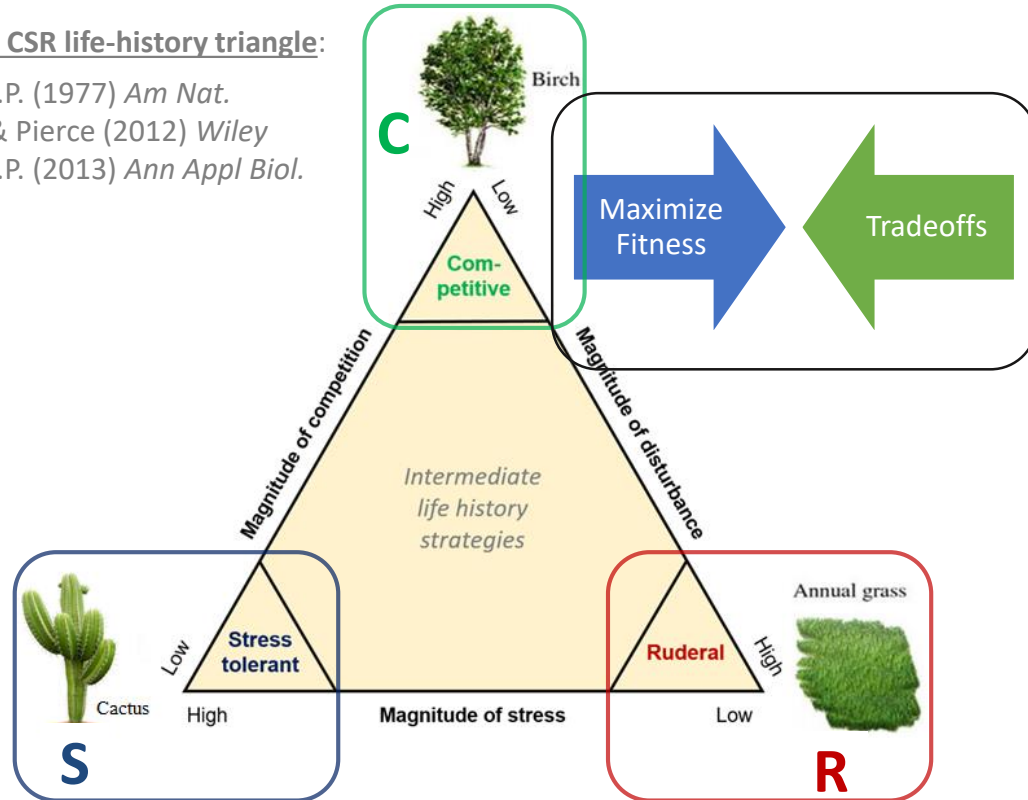
# Ecological theory: Three-way life-history strategies

## Grime's CSR life-history triangle:

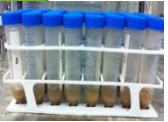
Grime J.P. (1977) *Am Nat.*

Grime & Pierce (2012) *Wiley*

Grime J.P. (2013) *Ann Appl Biol.*



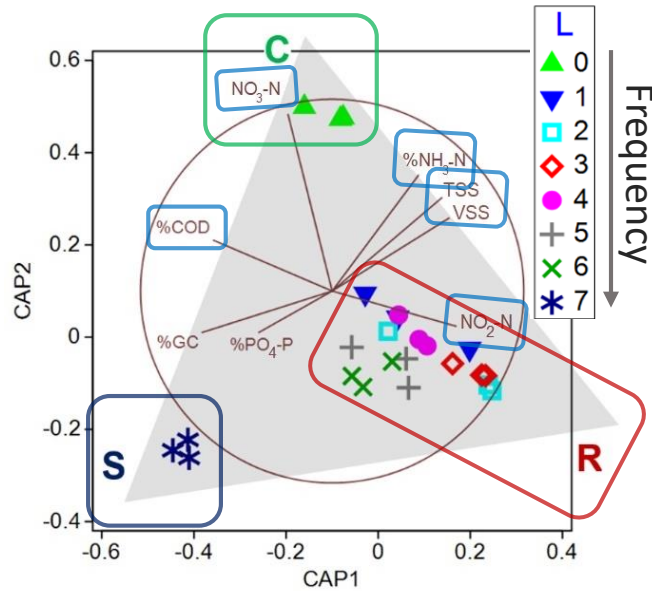
**Microcosm** (20 mL)  
8 levels (n = 3)  
Pulse and Press  
**Short-term** (35 d)



## Community aggregated traits:

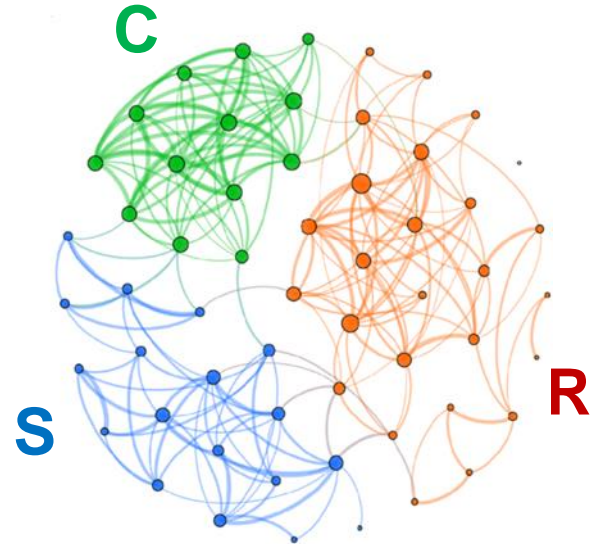
- Link microbial communities to ecosystem function.
- The whole is rarely just the sum of its individual parts.

# Trade-offs in function and genes: community-level life-history strategies



$p_{\text{PERMANOVA}} < 0.001$      $p_{\text{GLMMs}} = 0.015$

Metagenomics sequencing: genus level  
Bray-Curtis dissimilarities



Community aggregated traits  
(functional gene potential)

[Santillan E. et al. \(2019\).](#)  
*Environmental Microbiology*,  
21: 3751–3764.

# Press disturbance favours deterministic assembly with a shift in function and structure of communities

**Mesocosm (5 L)**

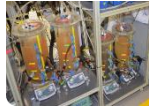
2 levels, n = 3 - 4

Press (with acclimation)

**Long-term (140 d)**

[Santillan et al. \(2020\)](#)

*mSystems* 5(4): e00471-20.



**Mesocosm (4 L)**

2 levels, n = 3

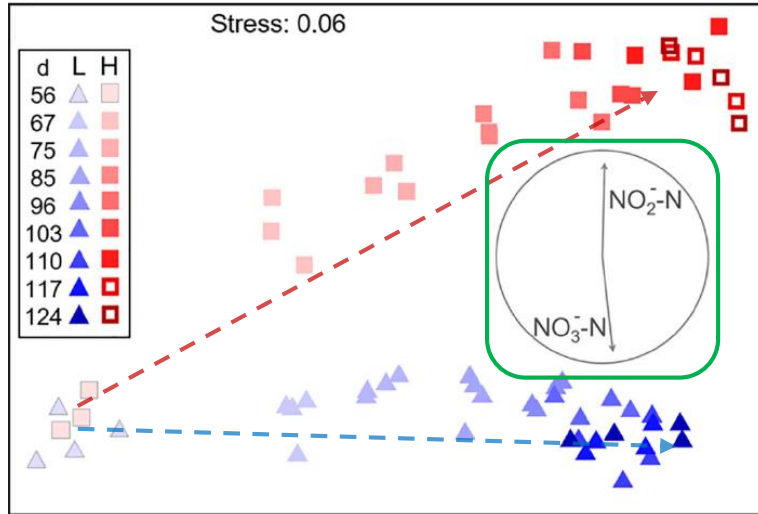
Press (with acclimation)

**Long-term (170 d)**

[Santillan et al. \(2021\)](#)

*ACS EST Water*

1(6):1429–1437.

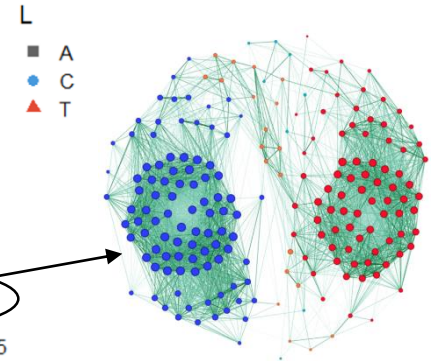
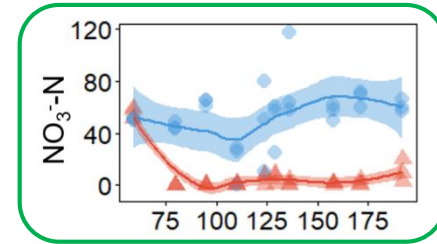
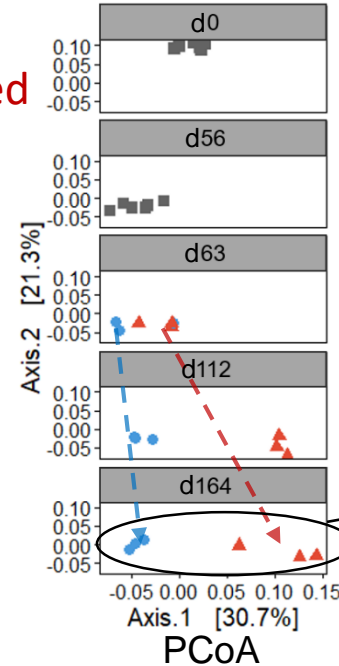


16S rRNA gene (v3-v4) amplicon sequencing data

$p_{\text{PERMANOVA}} < 0.001$

$p_{\text{PERMDISP}} > 0.05$

Undisturbed  
Press-disturbed



# Ecological theory: Stability

## Resistance

Ability to withstand a disturbance.

$$B > A$$

## Resilience

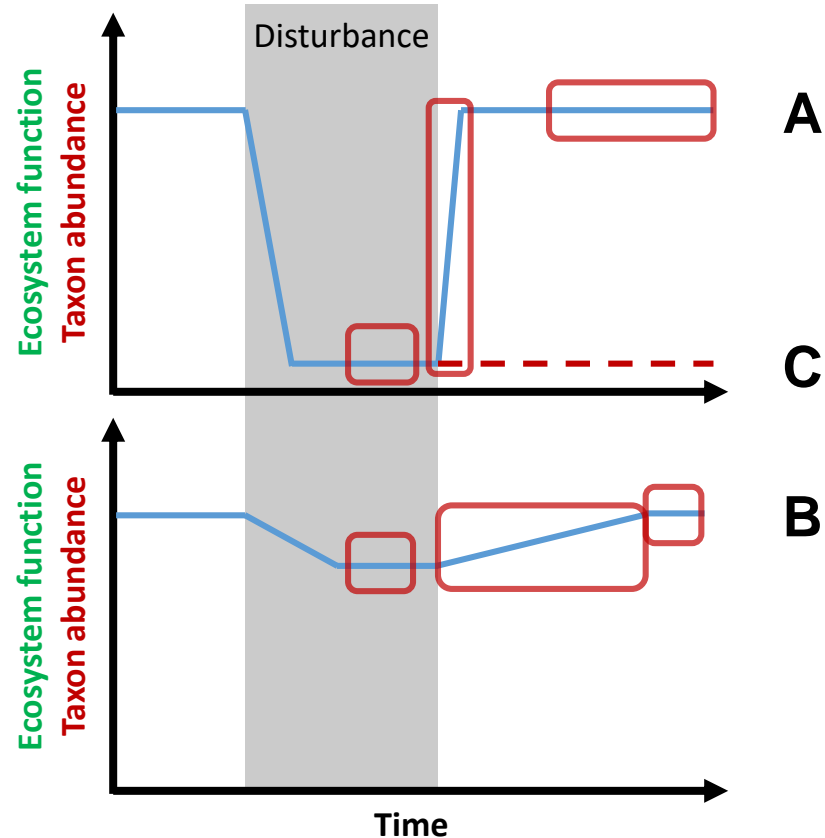
Speed of recovery from disturbance.

$$A > B$$

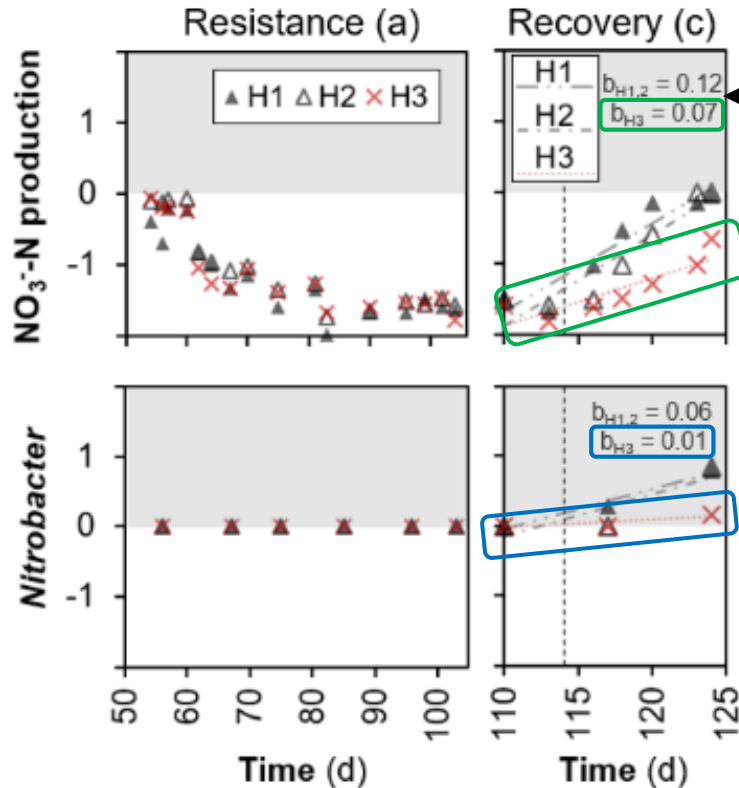
## Recovery

Ability to return to prior conditions after disturbance ceases.

$$A = B$$



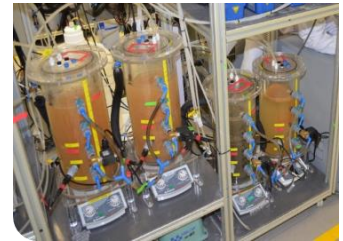
# Stability can vary across replicated systems: Resilience and recovery



Resilience (b)

Mesocosm (5 L)  
2 levels,  $n = 3 - 4$   
Press (with acclimation)  
Long-term (140 d)

[Santillan et al. \(2020\)](#)  
*Scientific Reports*



# Ecological theory relevance for practitioners: Feedback from SIWW 2021

Quote from Professor Glen Daigger,  
former chairman of the International Water Association

315 Sustained Organic Loading Disturbance Favors Nitrite Accumul  
(Ezequiel Santillan, Singapore Centre for Environmental Life Scienc  
WCP.1 Q&A Session  
6:00pm - 6:30pm

Leave Presentation

Glen Daigger

Current Questions

✓ Answered Question

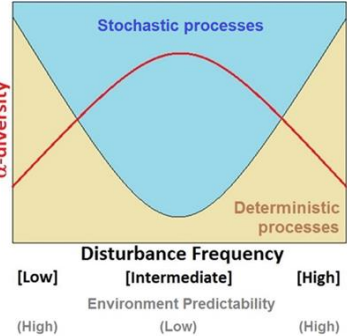
Delete All Question

Singapore International Water Week

“Your work is interesting in terms of opportunities to out-select NOB’s, but I really like the fact that you are reflecting the **variability of the response of biological systems** [...] The random and stochastic nature of these biological processes we work with”

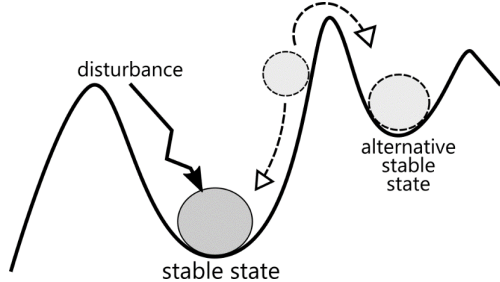
# So, what does this all mean?

## "MANAGING" MICROBIAL COMMUNITIES



Drawings adapted from @TwistedDoodles

Similar disturbance...  
**Different process outcome!?**




# Takeaways

Ecological theory helps us classify, interpret and predict the world around us.



The generality of established ecological theory can be tested using microbial systems for wastewater treatment.



Better controlled microbial systems can help generate **new theory** that is also relevant to plants and animals.

# Acknowledgements

- Prof. Stefan Wuertz [PI]
- Dr. Florentin Constancias [metagenomics bioinformatics]
- Dr. Hari Seshan [3-CA bioreactor work]
- Dr. Daniela Moses [sequencing-related advice]
- Sara S. Thi and Abeed F.B.M. Batcha [analytical chemistry]
- Larry Liew [sludge inoculum collection]
- Interns and FYP: Jessica Phua, Tan Jolene, Teo J. Qiang, Aquidah Latiff, Aw C. Keong, Adilah B.A. Aziz and Lim Joseph [lab work]
- Wuertz Lab group and SCELSE sequencing facility
- Funding entities



Ministry of Education  
SINGAPORE



NANYANG  
TECHNOLOGICAL  
UNIVERSITY  
SINGAPORE

NATIONAL  
RESEARCH  
FOUNDATION  
SINGAPORE

UCDAVIS  
UNIVERSITY OF CALIFORNIA

