南方海洋科学与工程广东省实验室（珠海）
Southern Marine Science and Engineering Guangdong Laboratory(Zhuhai)

croucher summer courses

Synthetic ecology of nitrogen cycling microorganisms

Zhili He

Email : hezhili@sml-zhuhai.cn

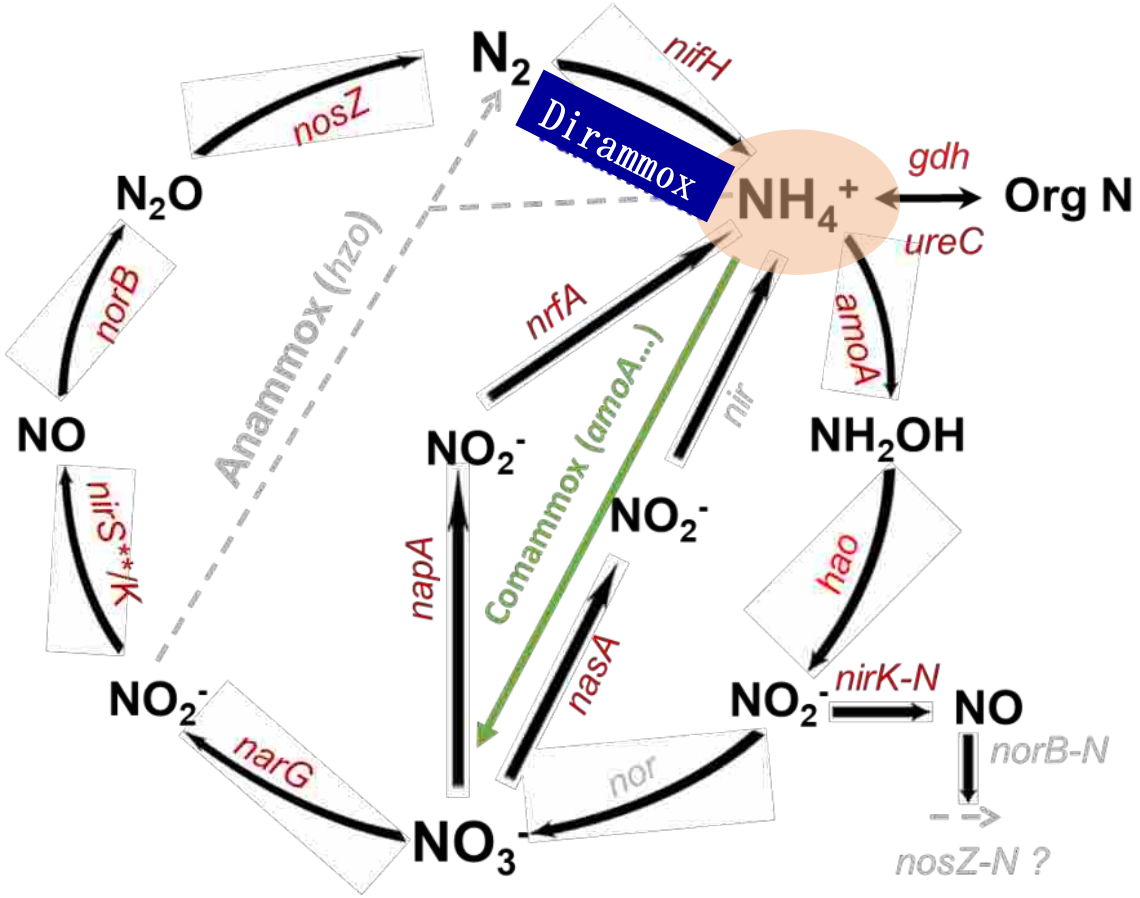
WeChat: zhilihe65

Hong Kong University of Science and Technology

Hong Kong

July 1, 2025

Importance of nitrogen cycling in the biosphere



- The nitrogen (N) cycle is one of the most biogeochemical cycles and plays critical in **ecosystem functioning**
- Including **nitrification**, **denitrification**, N fixation, and anaerobic ammoinum oxidization
- Coupling with carbon (C), sulfur (S), phosphorus (P), and metal cycling processes (e.g., **N_2O** and **CH_4** production and removal)
- **NO_2^-** accumulation in aquaculture ecosystems

How do N cycling microorganisms interact?

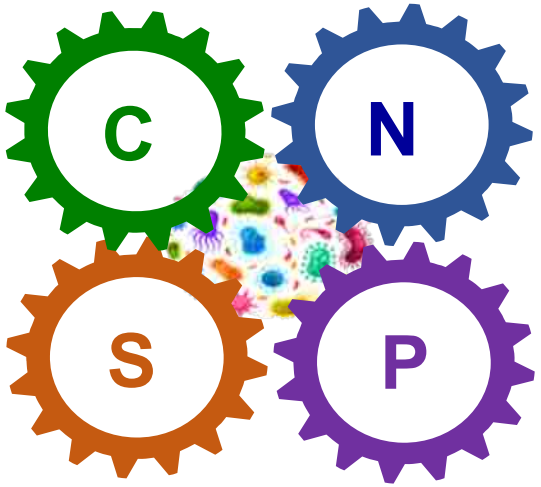
(He et al., 2010, *Ecol Lett*; Kartal et al., 2011, *Nature*; van Kessel et al. 2015, Nature; Wu et al., *Environ Microbiol*)

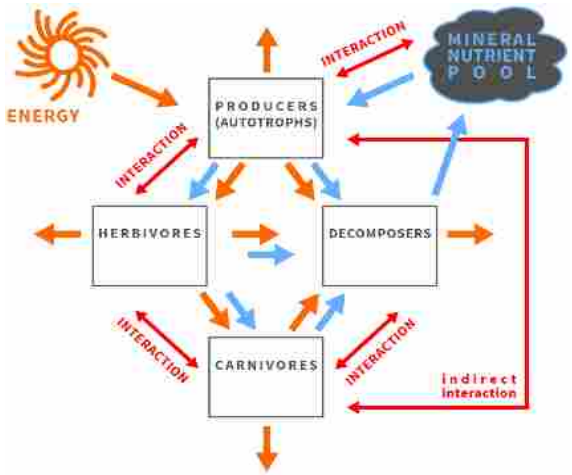
Outline

- Synthetic microbial ecology
- Synthetic nitrifying community niche differentiation and mechanisms
- Biodiversity and community function relationships of synthetic denitrifying communities
- Challenges and perspectives

Key ecology questions : biotic interactions and mechanisms

Who is where with whom doing what, when and why?





- Element cycling & coupling
- Food web interactions
- Biological stoichiometry

Effect	Ecological	Metabolic	References
0/+	Commensalism	Food chain	Freilich *et al.* [22], Xu *et al.* [34]
-/-	Competition	Substrate competition	Foster & Bell [19]
-/+	Predation	Food chain with waste product inhibition	Balagadde *et al.* [23] Kerr *et al.* [24]
0/0	No interaction	No common metabolites	
+/+	Cooperation	Syntrophy	Shou *et al.* [25], Kerner *et al.* [26], Hillesland *et al.* [28], Summers *et al.* [29],
0/-	Amensalism	Waste product inhibition	

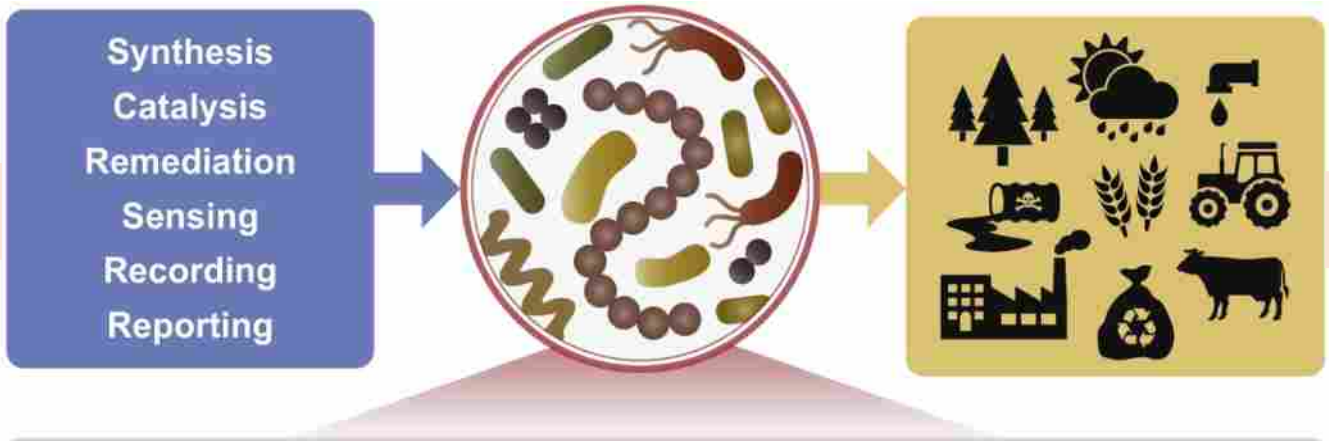
(Elser et al., 2000, *Ecol Lett*; Elser et al., 2006, *Am Nat*; Großkopf and Soyer, 2014, *Curr Opin Microbiol*)

Synthetic microbial ecology is a new interdiscipline

Pure culture studies

Field studies

No interactions!



High complexity!

Synthetic microbial ecology

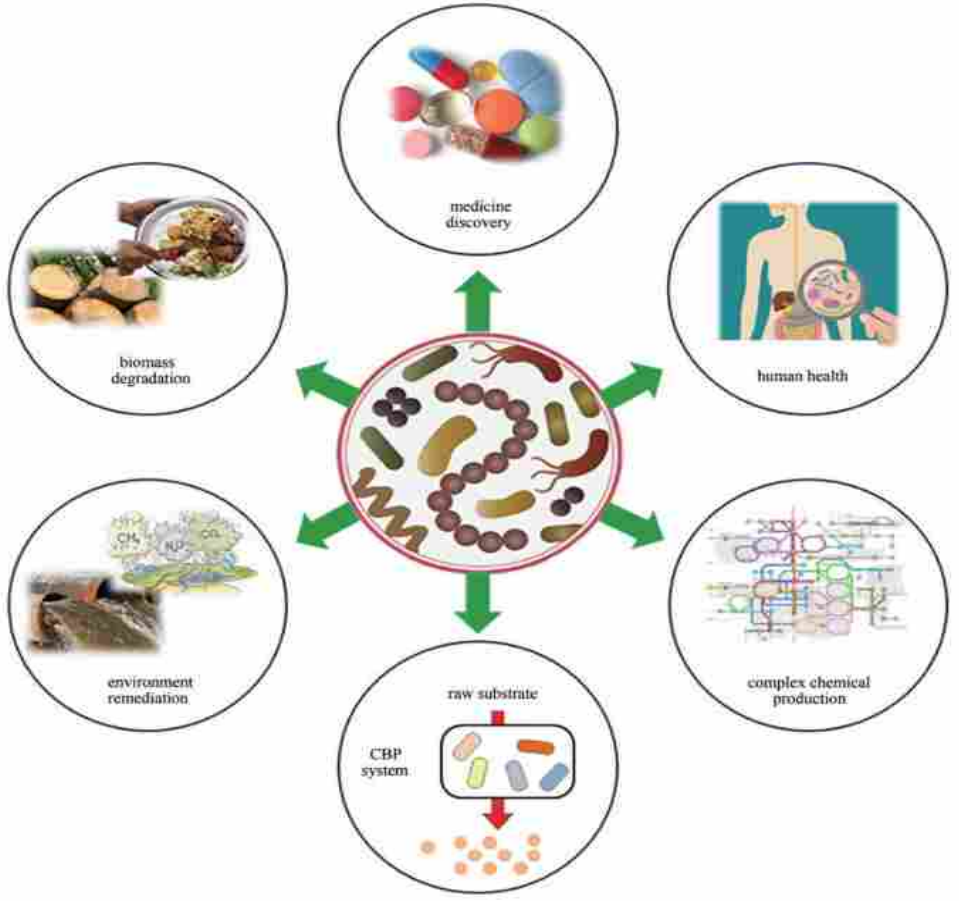
Bridge pure culture and field studies

- Changing community composition
- Understanding biotic interactions
- Enhancing community functionality

Two objectives of synthetic microbial ecology

1. Testing ecological theories and hypotheses: understanding the general principles and rules of the dynamic interplay among different biological genotypes

2. Applications of synthetic communities: rationally engineering synthetic microbial communities to enable, control or optimize a desired biotransformation

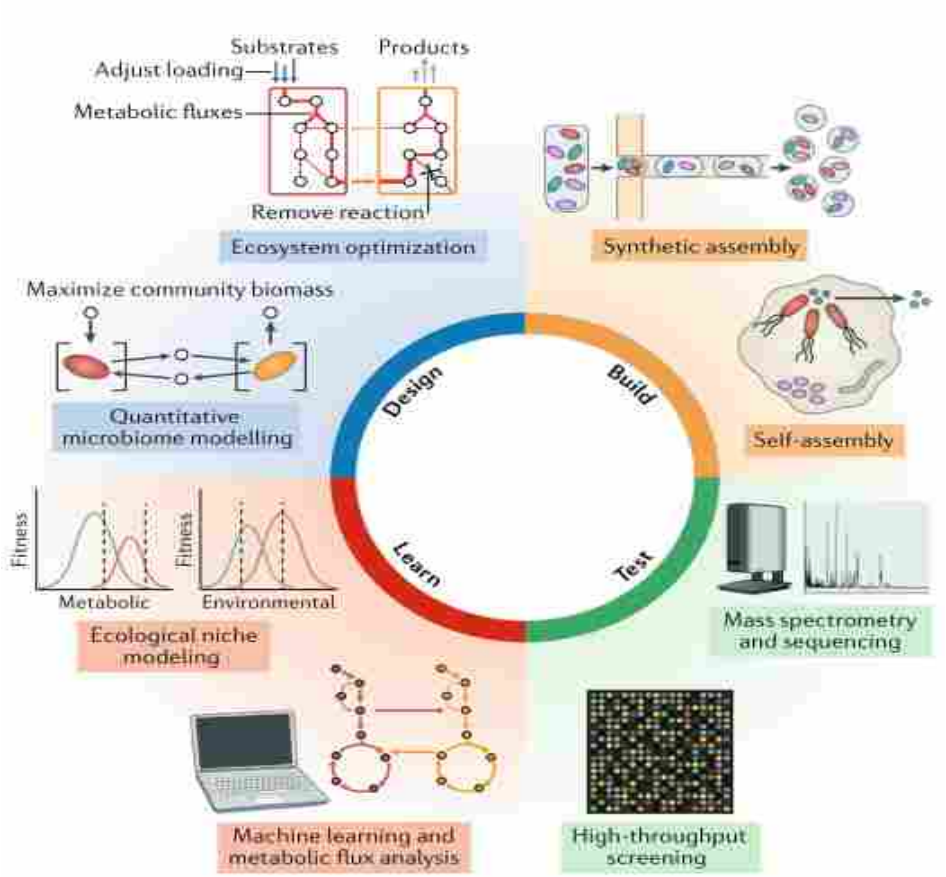


→ Microbiome engineering

Synthetic microbial community construction

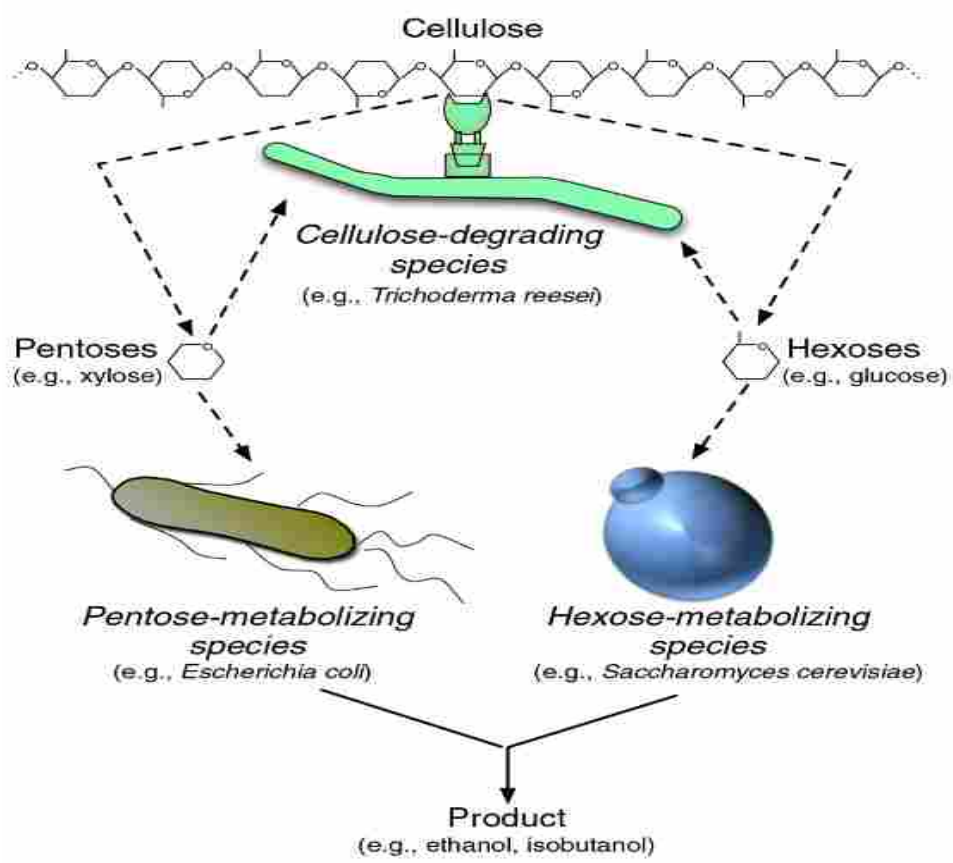
Design-Build-Test-Learn model

- **Focusing on interactions and dynamics**
- **Enhancement of community-level functional traits**

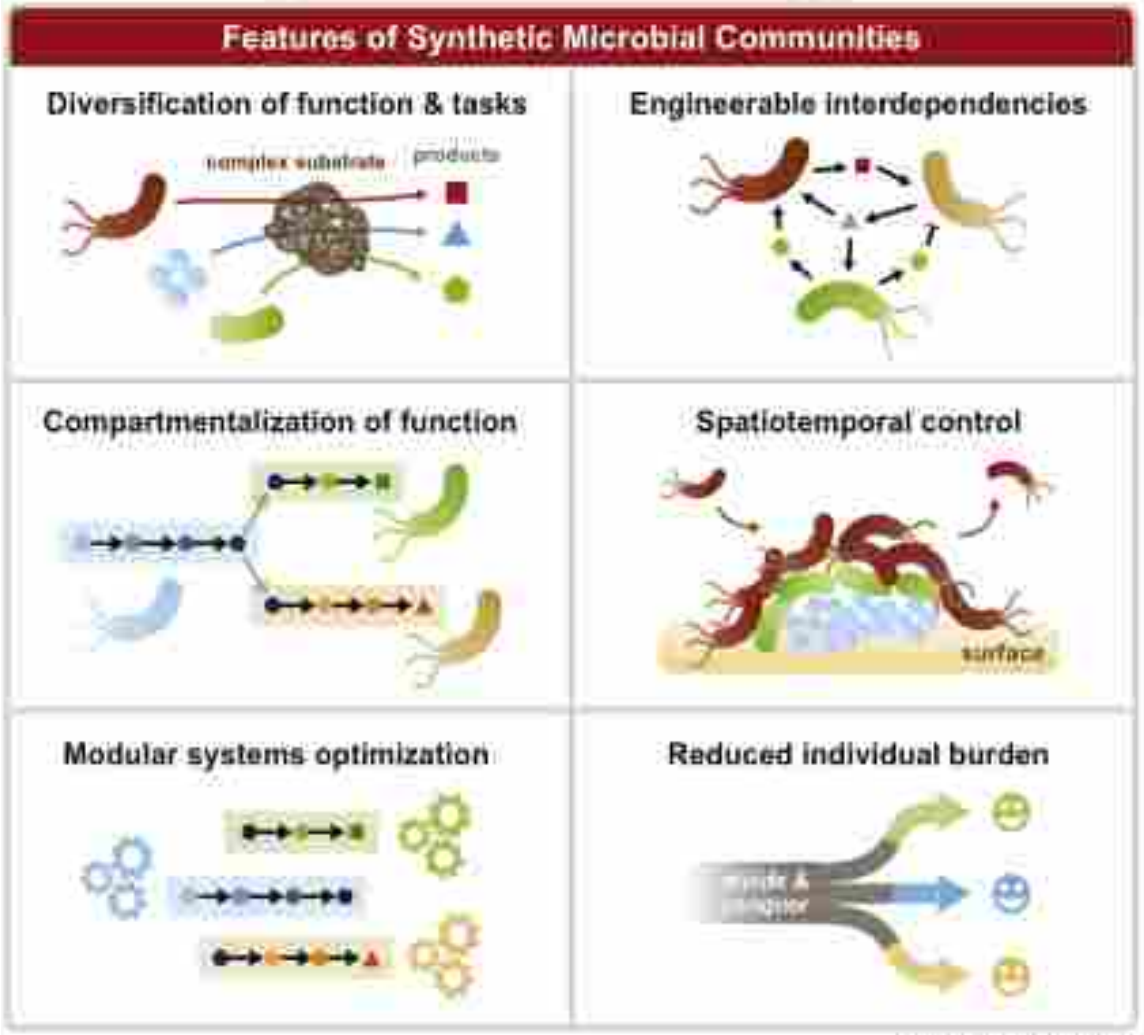


(Johns et al., 2016, *Curr Opin Microbiol*; Lawson et al., 2019, *Nat Rev Microbiol*)

Characteristics of synthetic microbial communities



- Reducing community complexity
- Increasing system controllability



(Zomorrodi and Segrè, 2016, *J Mol Biol;* Grosskopf and Soyer, 2014, *Curr Opin Microbiol)*

Research and application of synthetic communities

nature microbiology

Perspective

https://doi.org/10.1038/s41564-024-01827-2

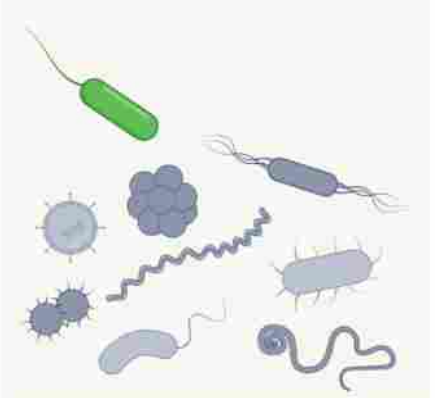
A cross-systems primer for synthetic microbial communities

Received: 2 February 2024

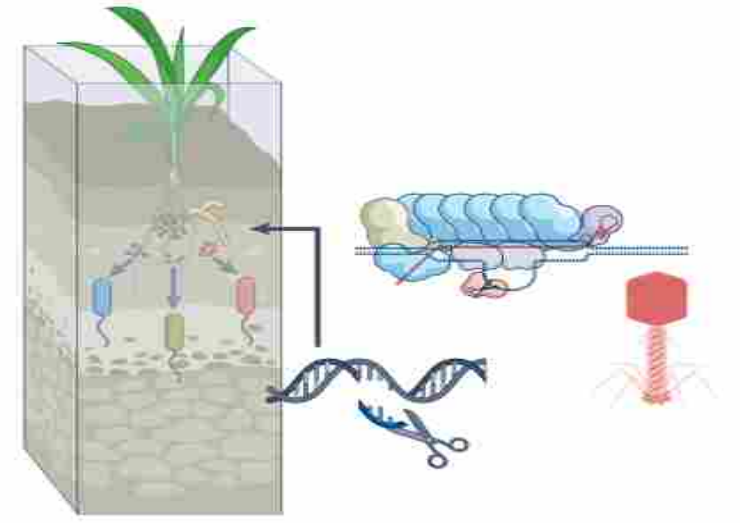
Accepted: 11 September 2024

Published online: 30 October 2024

Elijah C. Mehlferber[1,2], Gontran Arnault[3,9], Bishnu Joshi[4,9], Laila P. Partida-Martinez[5,9], Kathryn A. Patras[4,6,9], Marie Simonin[3] & Britt Koskella[7,8]



Microbial interactions and emergent properties in complex communities



nature microbiology

Perspective

https://doi.org/10.1038/s41564-024-01833-4

Community standards and future opportunities for synthetic communities in plant–microbiota research

Received: 30 October 2023

Accepted: 16 September 2024

Published online: 30 October 2024

Check for updates

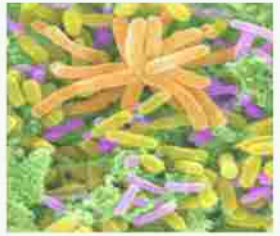
Trent R. Northen[1,2], Manuel Kleiner[3], Marta Torres[1], Ákos T. Kovács[4,5], Mette Haubjerg Nicolaisen[6], Dorota M. Krzyżanowska[7], Shilpi Sharma[8], George Lund[9], Lars Jelsbak[5], Oliver Baars[10], Nikolaj Lunding Kindtler[11], Kathrin Wippel[12], Caja Dinesen[4,5], Jessica A. Ferrarezi[13], Malek Marian[14], Adele Pioppi[4,5], Xinming Xu[4], Tonni Andersen[15,16], Niko Geldner[17], Paul Schulze-Lefert[15,16], Julia A. Vorholt[18] & Ruben Garrido-Oter[15,16,19]

A framework for synthetic microbial ecology

Selection of microbial species



Sequenced species



Enriched or reduced communities

Rational design of microbial communities



Microbial interactions

$$\frac{d}{dt}x_i^{(k)}(t)=\sum_{j=1}^{m_k}W_{ij}^{(k)}\cdot x_j^{(k)}(t)$$

Mathematic modeling and simulation

Divide and conquer

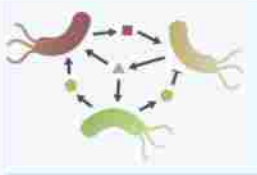
Compartmentation of functions

Temporal and spatial dynamics

Optimization of growth and functionality

Optimizing local conditions

- Defined common media
- Control populations
- Exchange metabolites



Optimizing functional processes

- Functions/subfunctions
- Regulatory circuits
- Key species/genes

Long-term evolution and analysis

Community analysis

- Species abundance, composition and dynamics
- Community resilience, resistance and stability
- Ecosystem productivity and functioning

Functional analysis

- Gene expression
- Protein/enzyme activity
- Metabolites
- End products

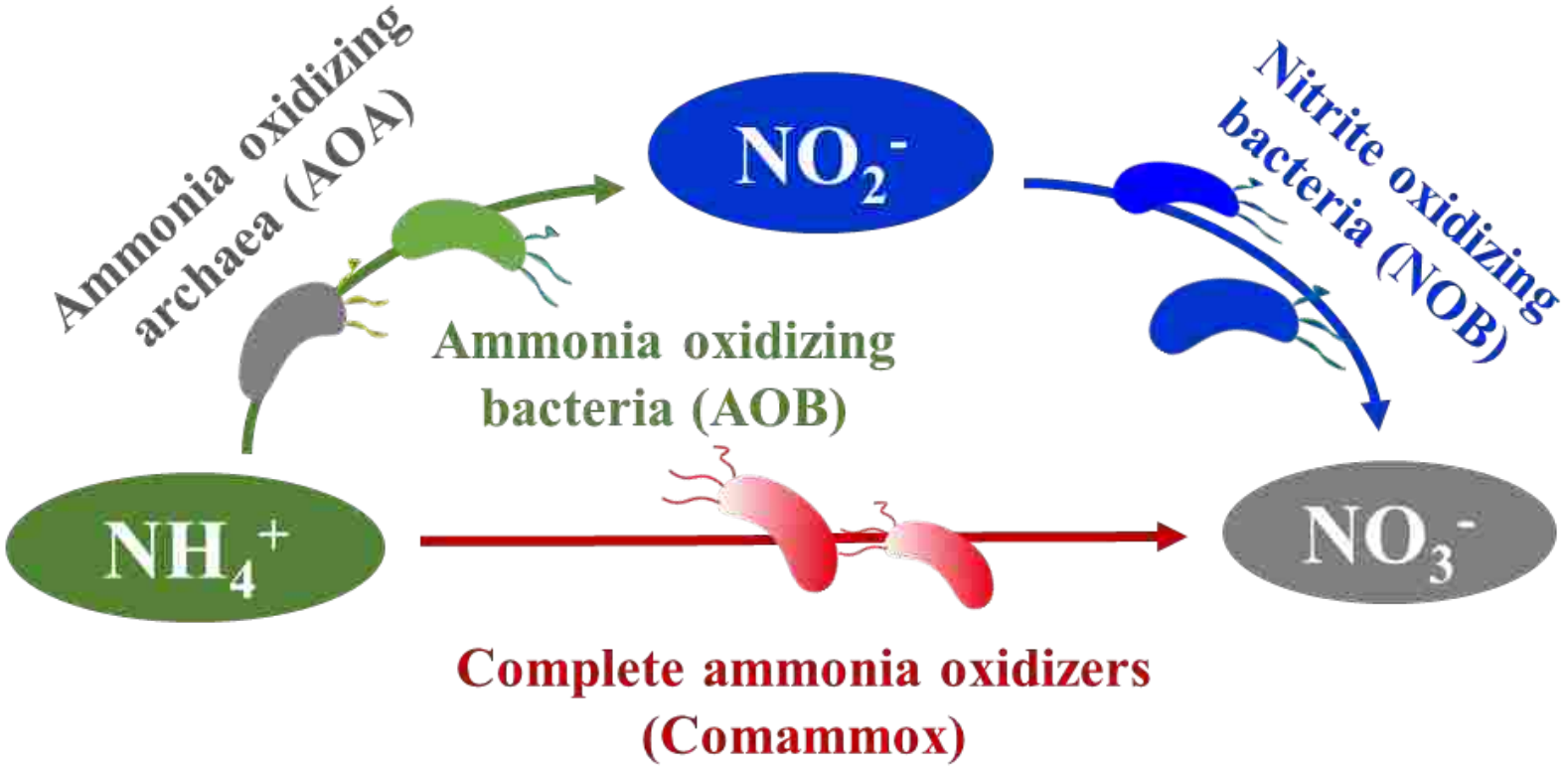
Synthetic ecology vs. synthetic biology

	Synthetic ecology	Synthetic biology
Scientific question	Composition-interaction-function	Structure-function-regulation
Basic element	Individual organisms	DNA sequences (genes/pathways)
Key technique	Optimization of community composition, biotic interactions, community metabolic modelling, and process couplings	Individual metabolic modelling, genetic engineering, gene/protein expression
Regulation	Community composition, dynamics, interactions and evolution	Gene functions and metabolic regulation
Controllability	Community and ecosystem levels	Gene expression, metabolic products

Outline

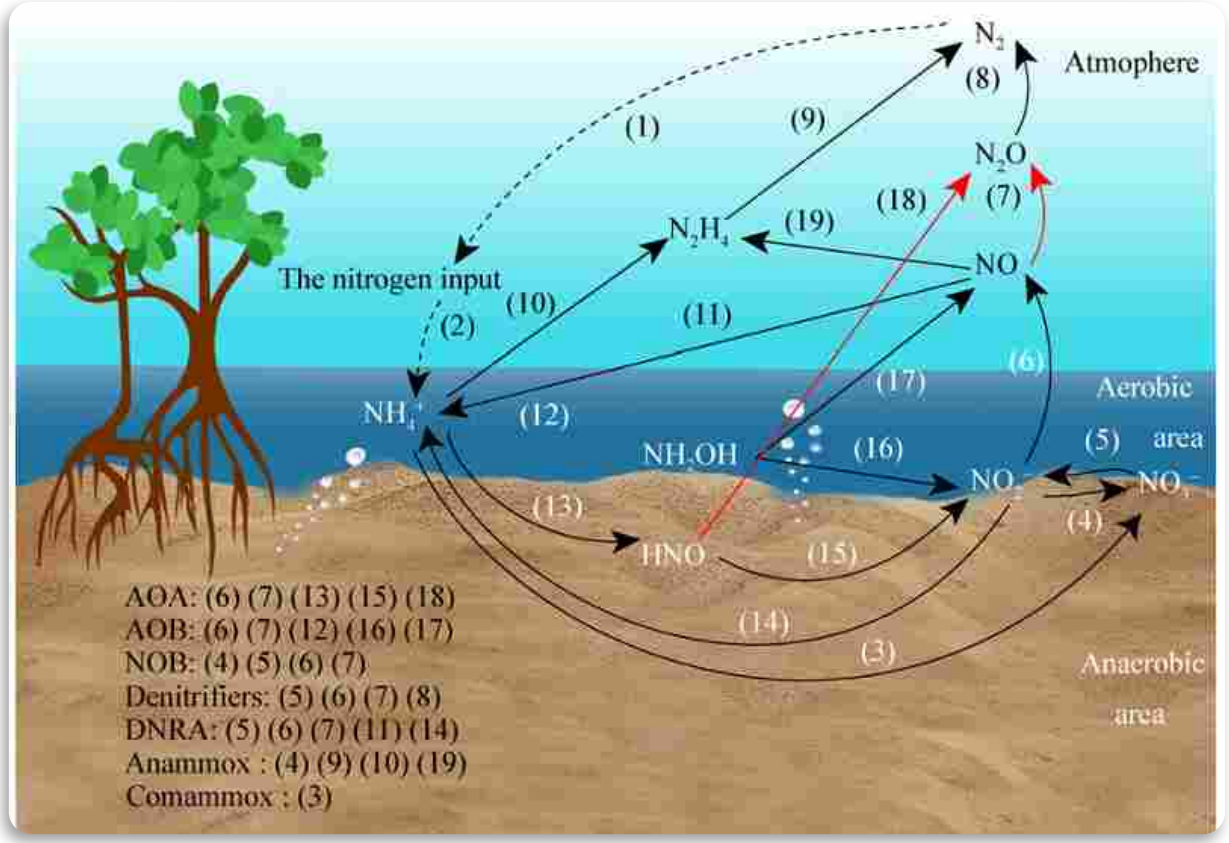
- Synthetic microbial ecology
- Synthetic nitrifying community niche differentiation and mechanisms
- Biodiversity and community function relationships of synthetic denitrifying communities
- Challenges and perspectives

Nitrification process and nitrifiers

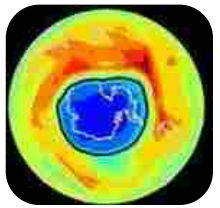


How do those four types of nitrifiers differentiate in niche and interact in the environment?

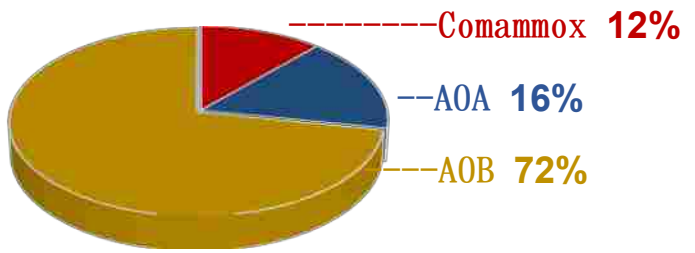
Microbially-driven N_2O production in coastal wetlands







The greenhouse effect of N_2O is 300 times of CO_2



N_2O production

- **Nitrifiers and dentrifiers** mainly regulate the production of N_2O
- **Comammox bacteria** produce less N_2O than AOA and AOB

(Zhuang *et al*., 2019, *Microbiol China*; Jiang *et al*., 2023, *Glob Change Biol*)

Scientific questions and hypotheses

How do NH_4^+concnetrations affect nitrifiers' niche differentiation ?

Nitrifiers differentiate in their niches under an NH_4^+ gradient largely due to their affinity to NH_4^+ or NO_2^-, growth rate, and substrate inhibition:

- AOA are dominant at low NH_4^+ , AOB at high NH_4^+, while Comammox are between
- Comammox and NOB compete for NO_2^-, and NOB prefer higher ammonium than Comammox

Tested by synthetic nitrifying communities

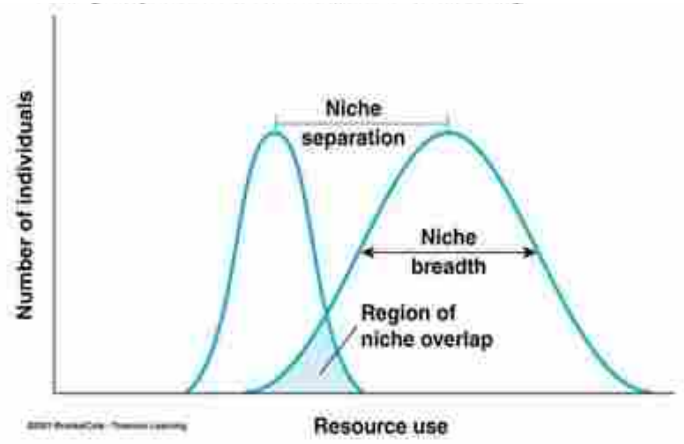
Niche differentiation

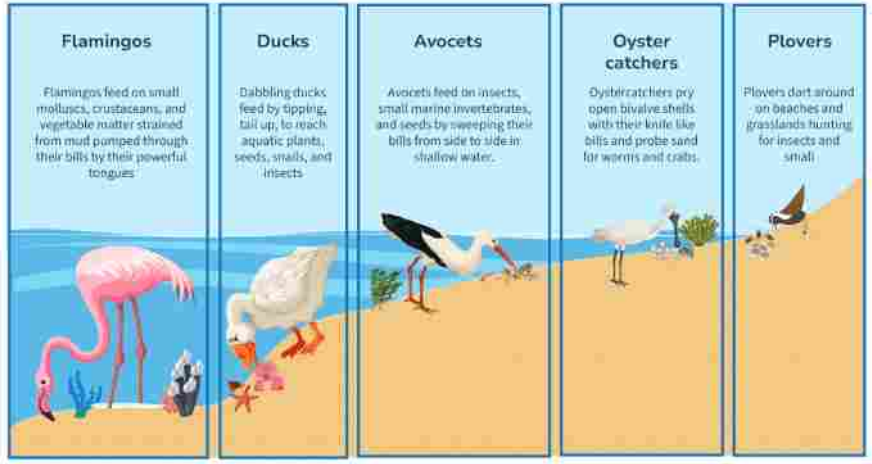
Niche: The niche of an organism is the functional role that it plays within an ecosystem.

Ecological niche is determined by the biotic factors (e.g., animals, plants, fungi, microorganisms), and abiotic factors (e.g., sunlight, water, nutrient resources).

Niche differentiation:

- Habitat differentiation
- Territorial differentiation
- Feeding differentiation
- Physiological differentiation
- Body-size differentiation



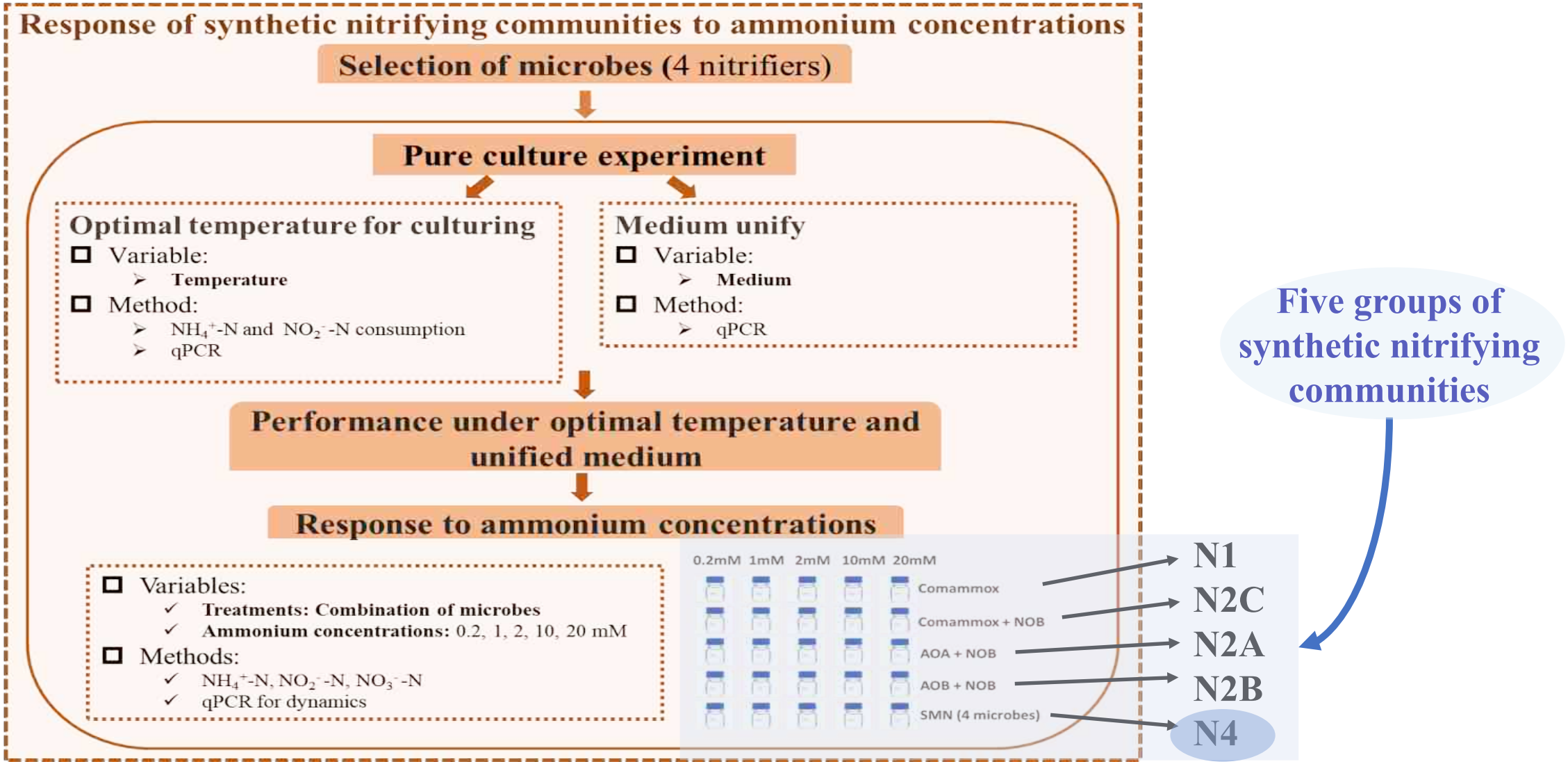


Synthetic nitrifying community construction

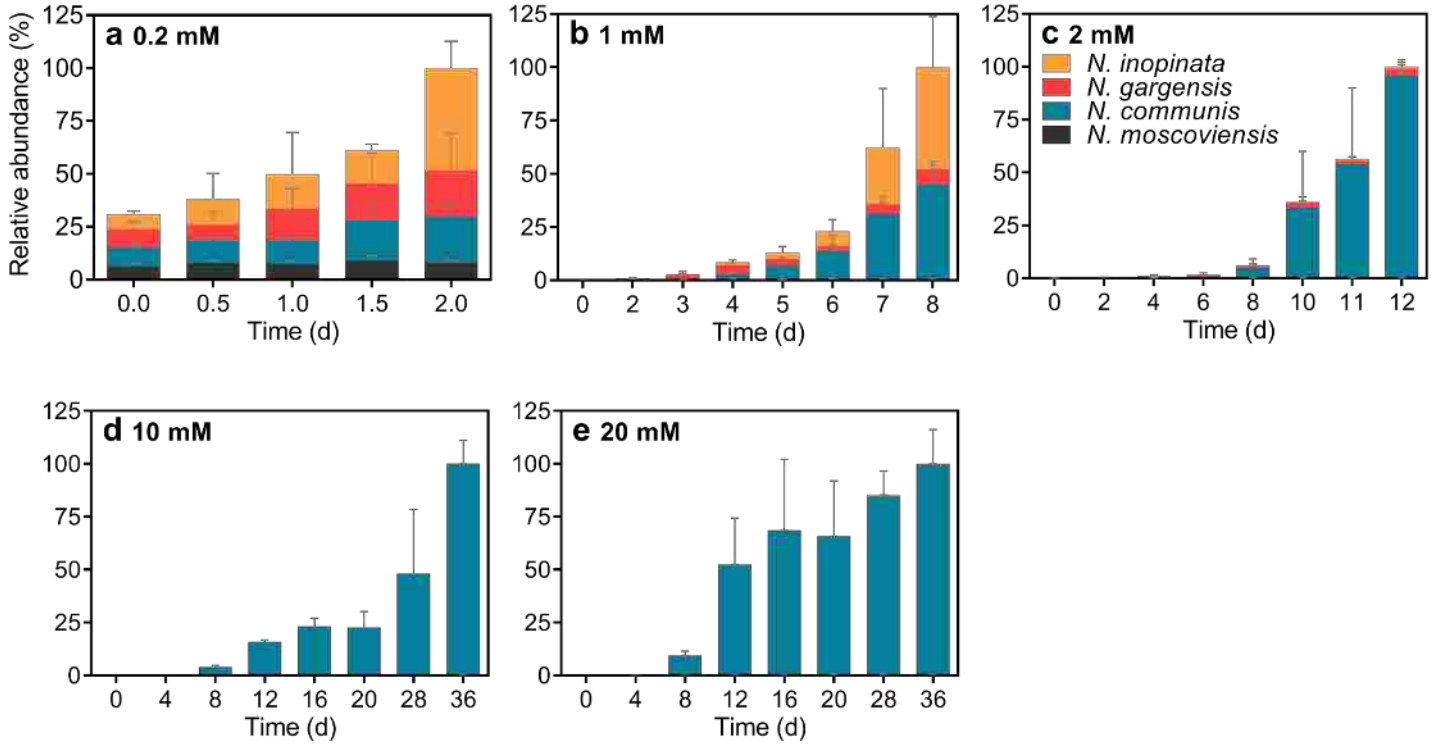
Characteristics	*N. inopinata*[1, 2]	*N. gargensis*[1, 3, 4]	*N. communis*[5, 6]	*N. moscoviensis*[7, 8, 9]
Sources of strain	A 1200-m deep oil exploration well	Hot spring	Soil	Heating water system
Cell morphology	Spiral-shaped cell with a flagellum	Round cell with a flagellum	Short rods or ellipsoidal with round ends	Irregularly shaped cells or spiral-shaped rods
Cell dimension (width × Length, μm)	0.18-0.3 × 0.7-1.6	Around 0.3 × 0.3	1.0-1.4 × 1.7-2.2	0.2-0.4 × 0.9-2.2
Optimal Temperature (□)	37	46	28	37
Saturation constant for activity (K_m)	0.65 [a]; 372 [b]	5.6 [a]	Around 1000 [a]	9 [b]
Maximum specific activity (V_{max})	12.8 [c];16.9 [d]	28.7 [c]	ND	18 [d]
Saturation constant for growth (K_s)	ND	ND	ND	ND
Maximum specific growth rate (μ_{max})	0.032 h^{-1}	0.028 h^{-1}	0.084 (0.055) [e]	0.031 h^{-1}
	Comammox	**AOA**	**AOB**	**NOB**

(Kits et al., 2017, *Nature*; Daims et al., 2016, *Trends Microbiol*; Spieck et al., 2006, *Environ Microbiol*)

Experimental design



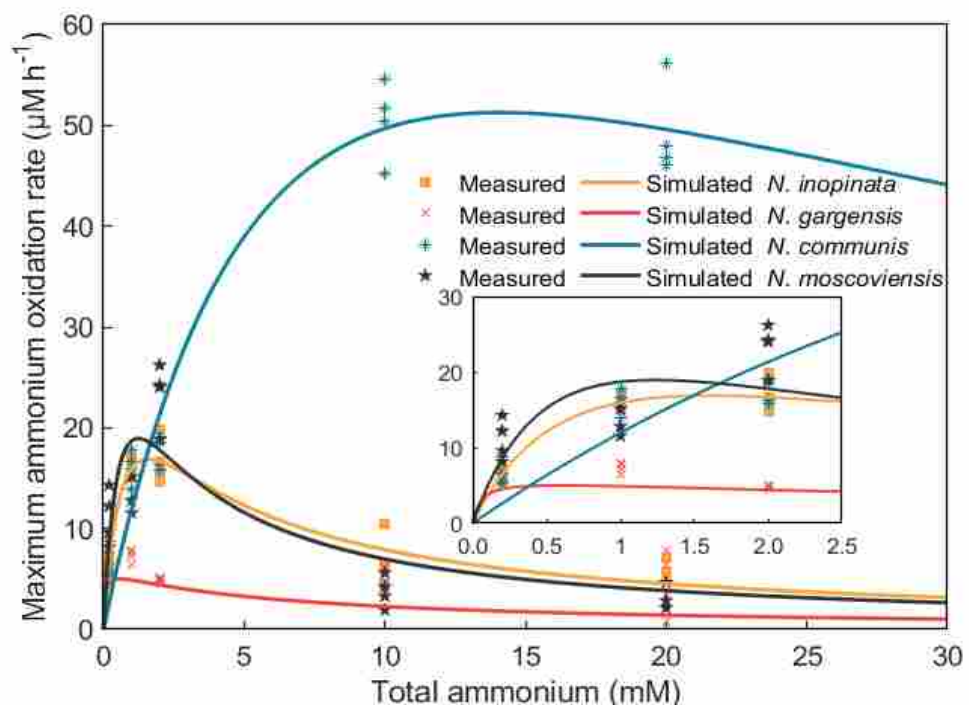
The dynamics of synthetic nitrifying community N4



- At 0.2 mM NH_4^+ , Comammox > AOB/AOA > NOB
- At 1 mM NH_4^+ , Comammox > AOB > AOA > NOB
- At ≥ 2 mM NH_4^+ , AOB were dominant

(Yang et al, 2022, *Front Microbiol*)

Nitrifiers' substrate affinity and niche differentiation



(Fitted by the Haldane Model)

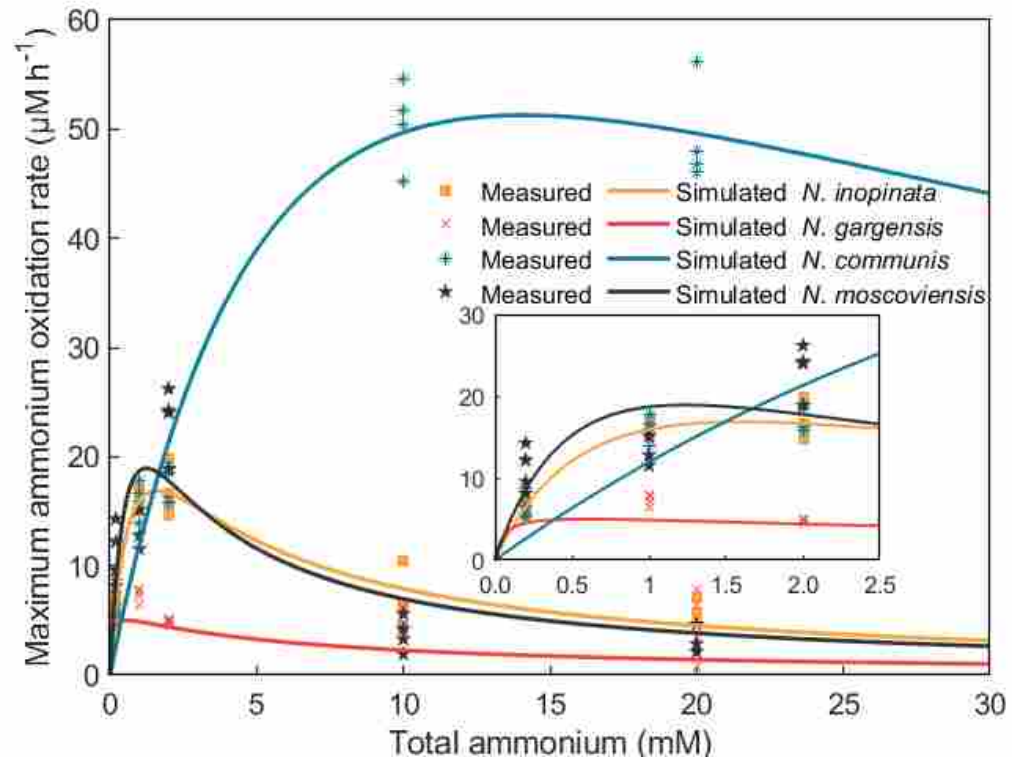
	r_m	K_{S2}	K_I	V_m	$K_{m(app)}$	R^2
N. inopinata	34.93 [a]	857 [b]	3002 [b]	16.88 [a]	282 [b]	0.976
N. gargensis	5.97 [a]	58 [b]	6191 [b]	5.00 [a]	42 [b]	0.778
N. communis	111.84 [a]	8310 [b]	23826 [b]	51.28 [a]	2553 [b]	0.971
N. moscoviensis	42.32 [a]	763 [c]	2004 [c]	18.94 [a]	228 [c]	0.732

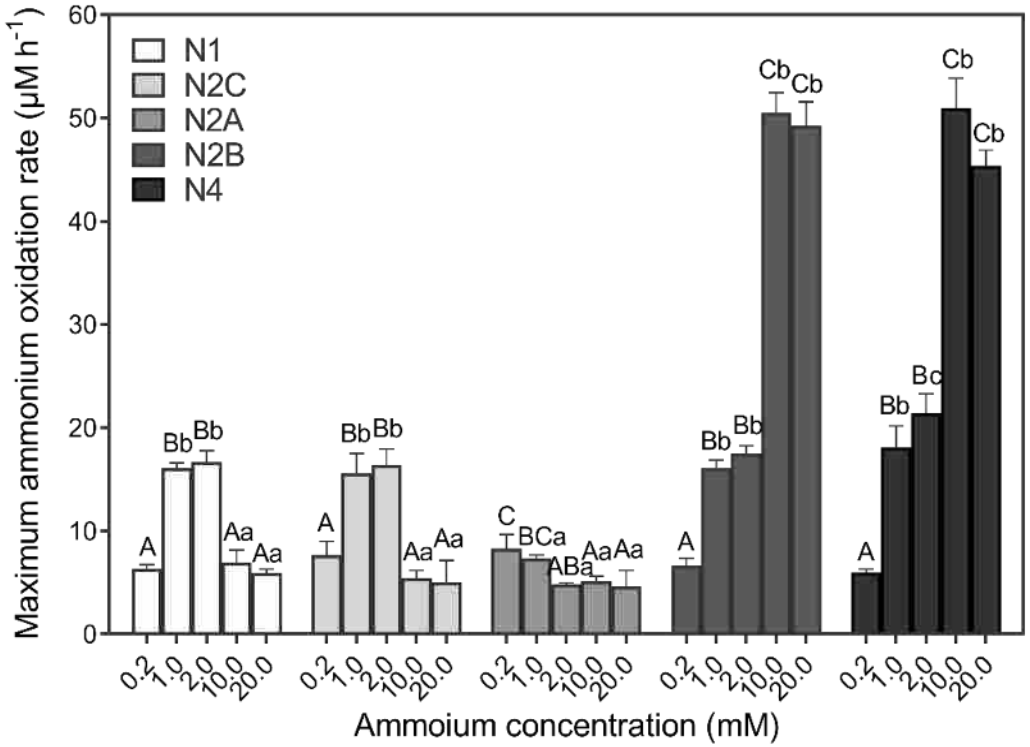
[a] μM ammonium or nitrite h^{-1}; [b] μM total ammonium; [c]μM nitrite.

AOA had the highest affinity to NH_4^+ , then Comammox , and then NOB and

(Sheintuch et al., 1995; Yang et al, 2022, *Front Microbiol*)

Nitrifiers' substrate inhibition and niche differentiation

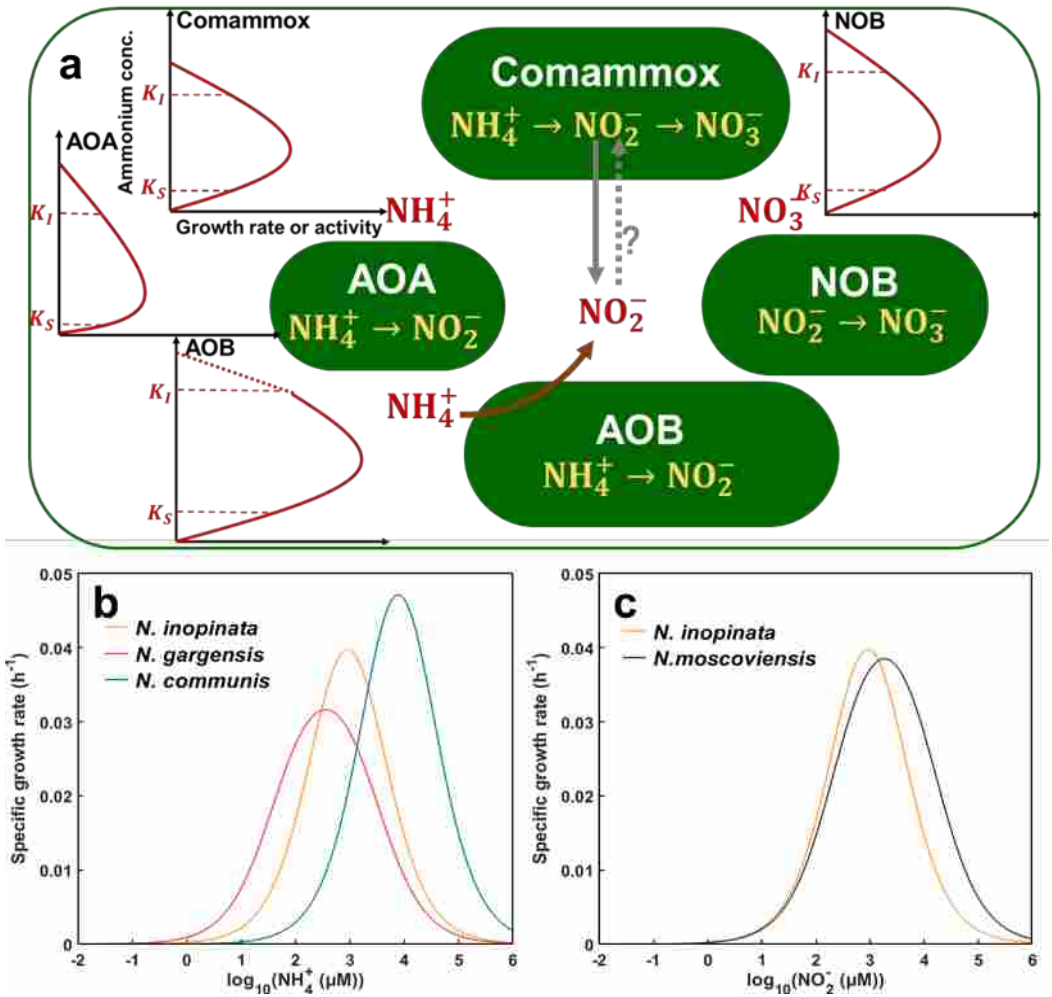




- At high NH_4^+, the growth of AOA, Comammox, and NOB was inhibited
- Even at 20 mM NH_4^+, AOB were not significantly inhibited.

(Yang et al, 2022, *Front Microbiol*)

A conceptual model for nitrifiers' niche differentiation



- ✓ **Substrate affinity**
- ✓ **Growth rate**
- ✓ **Substrate inhibition**

- AOA prefer low NH_4^+ , AOB prefer high NH_4^+ , and Comammox prefer middle NH_4^+
- NOB and Comammox compete for NO_2^- with lower NO_2^- for Comammox
- They had well overlapped niches

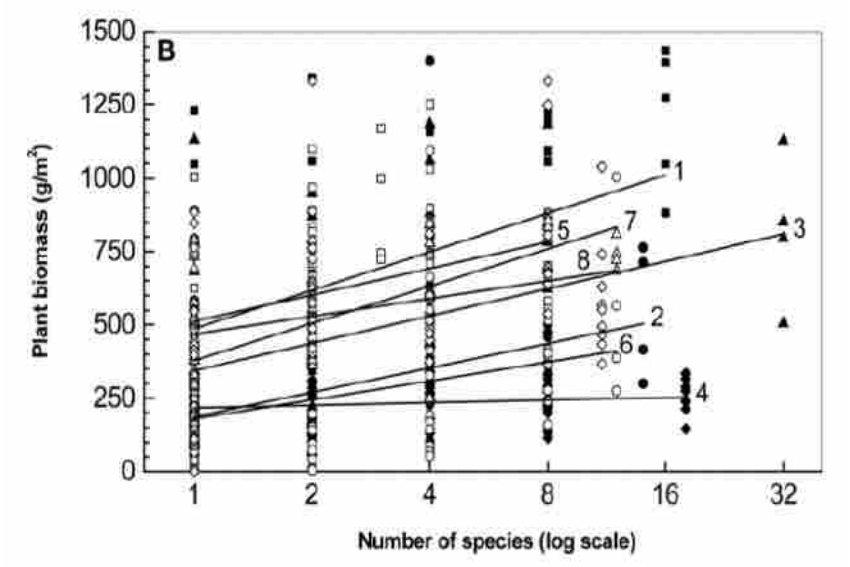
(Yang et al, 2022, *Front Microbiol*)

Outline

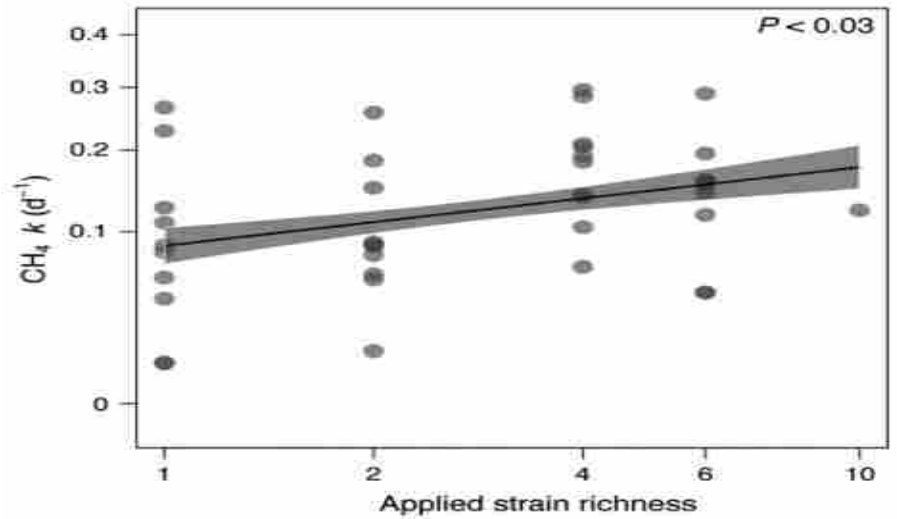
- Synthetic microbial ecology
- Synthetic nitrifying community niche differentiation and mechanisms
- Biodiversity and community function relationships of synthetic denitrifying communities
- Challenges and perspectives

Biodiversity-ecosystem function (BEF) relationship

- In plant and aminial studies, BEF was positive, negative, or not related
- Methanogen richness was positively correlated with methane production



(Loreau et al., 2001, *Science*)



(Fiegna et al., 2015, *ISME J*)

BEF relationships and underlying mechanisms remain elusive for microorganisms

Long-term experimental evolution

E. coli experimental evolution

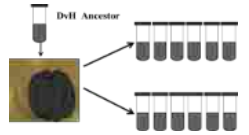


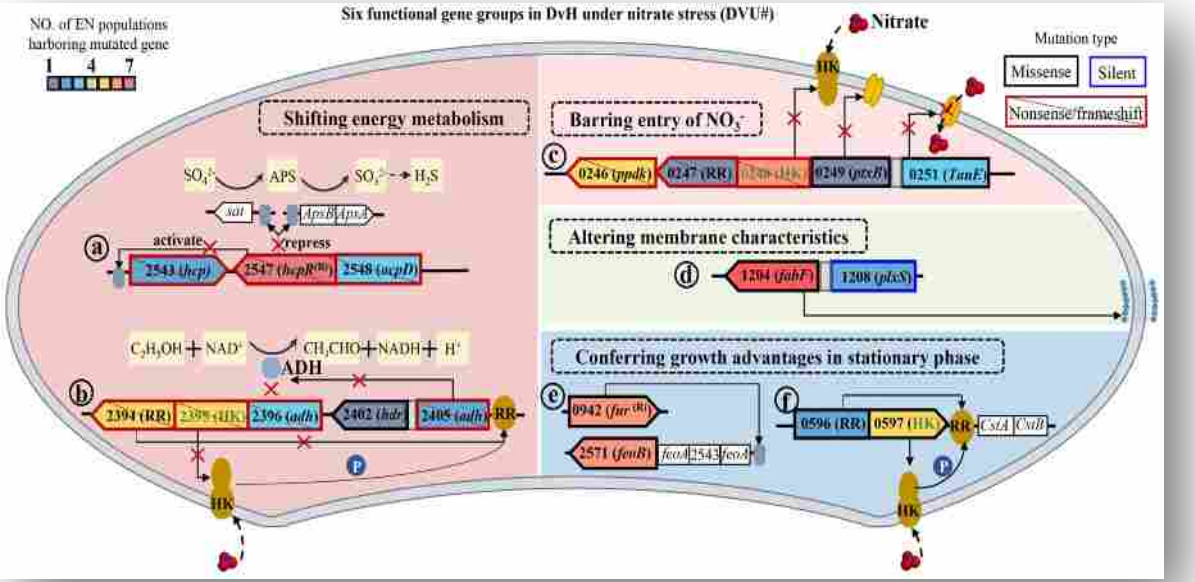
Richard E. Lenski

- **Evolved >90,000 generations**
- **~ 36 years**

(Lenski et al., 1991, *Am Nat*)

Desulfovibrio vulgaris experimental evolution



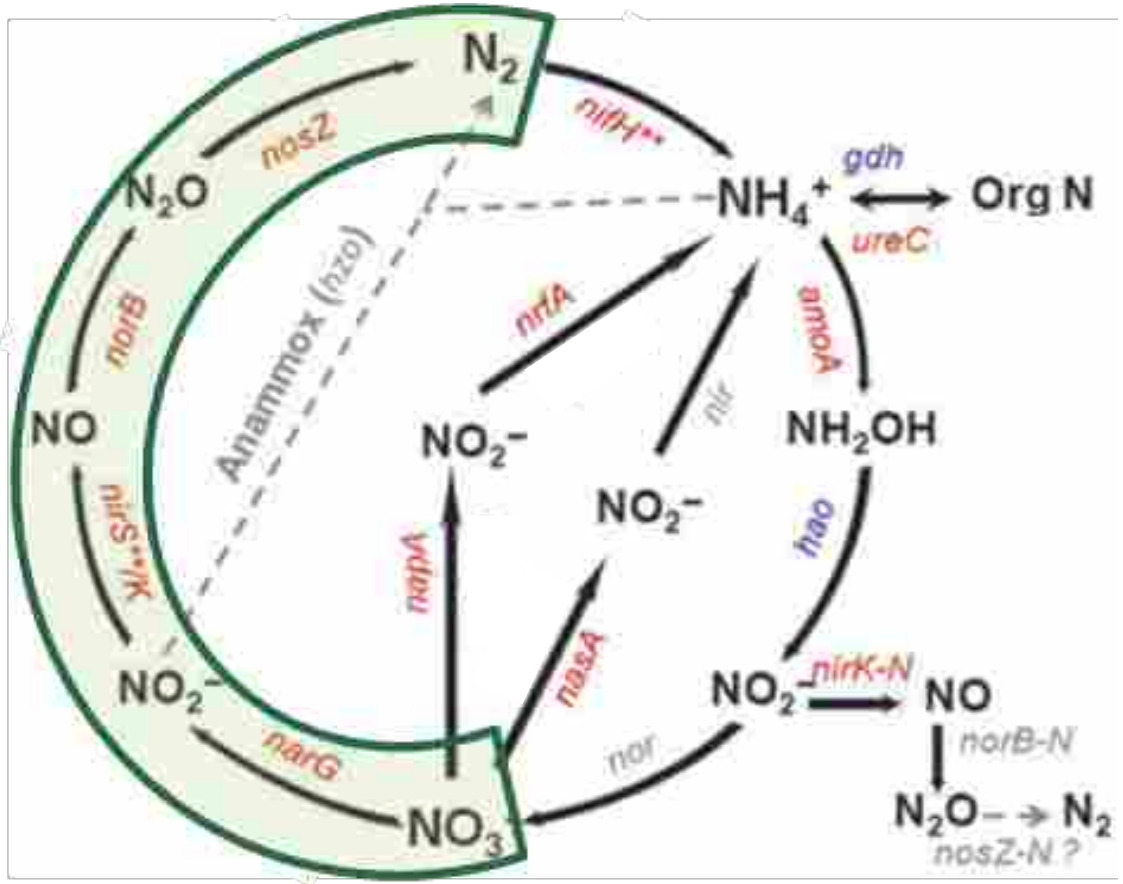


Salt tolerant mechanisms for *D. vulgaris*

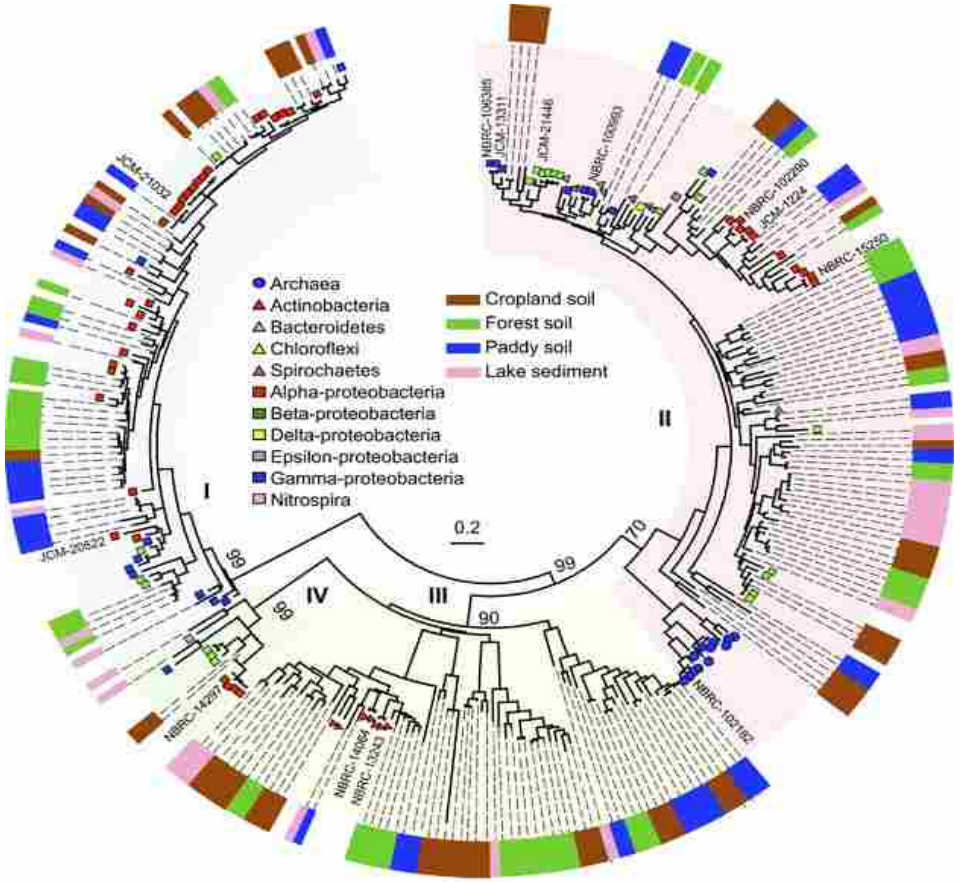
(Zhou et al., 2013, *ISME J*; Wu et al., 2020, *ISME J*)

Few studies with microbial community evolution

Dentrification and denitrifers in the environment



Denirtrifcation process and key functional genes



Denitrifiers are widely distributed in the environment

(He et al., 2010, *Ecol Lett*; Wei et al., 2015, *ISME J*)

Key scientific questions and hypotheses

Q1: What are the BEF relationship and underlying mechanisms of synthetic denitrifying communities?

H1: The productivity and denitrification of synthetic denitrifying communities would be positively correlated with their richness due to more corssing-feeding and interactions at higher richness communities

Q2: How does long-term evolution affect the BEF relationship?

H2: Long-term experimental revolution would decrease biotic competence and enhance community functions but differs with different richness levels due to complex biotic interactions

Experimental design and analysis

Three types of synthetic denitrifying communities:

BEF relationship

Richness and community function

- Richness of 1 、 2 、 4 、 8 and 12 *Shewanella* SDCs
- Microbial interactions (Rivett et al., 2016, *ISME J*)

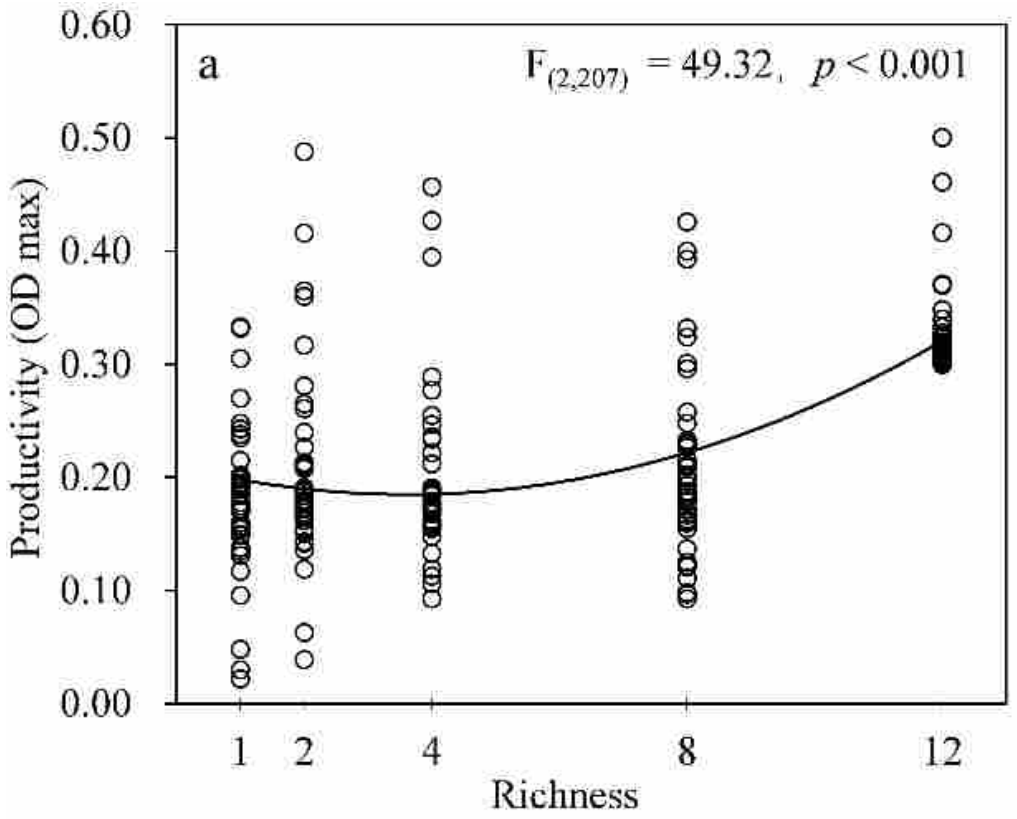
Phylogenetic diversity and community function

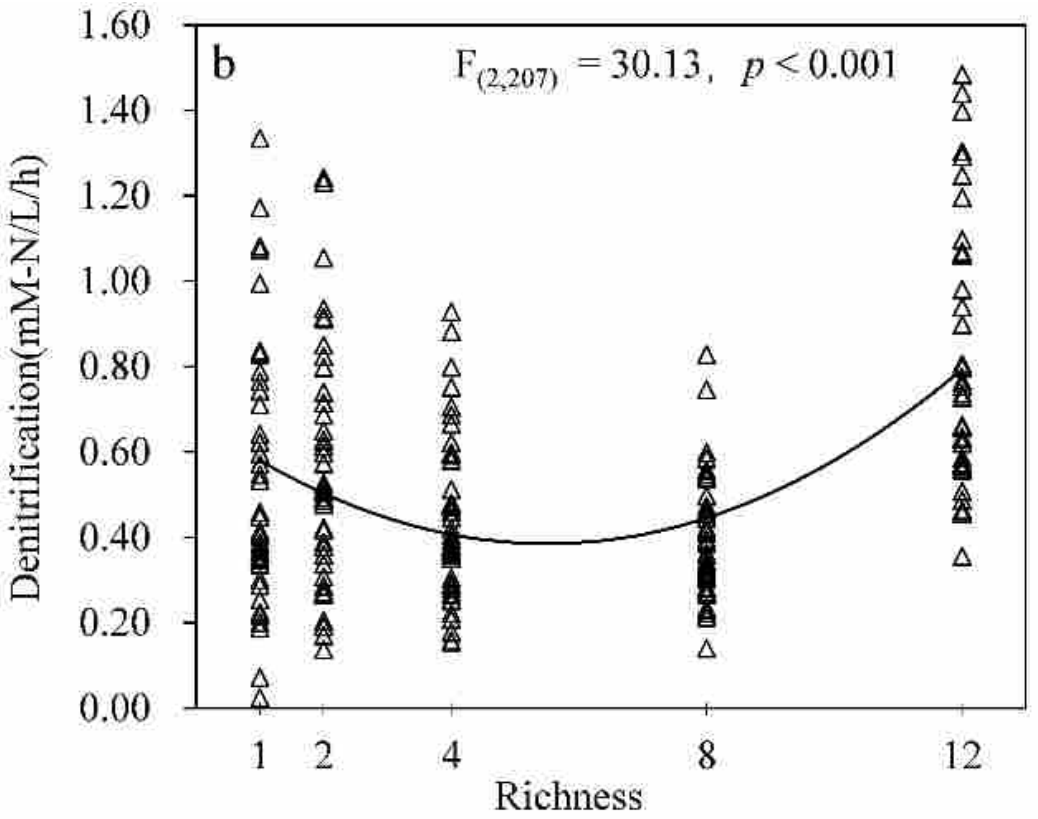
- Distantly/closely related SDCs (DR vs. CR)
- Community function and stability

Interaction and evolution mechanism

- SDC1, SDC2 and SDC6
- Genome/metagenome re-sequencing analysis
- Mutation analysis

Richness and denitrification function relationship

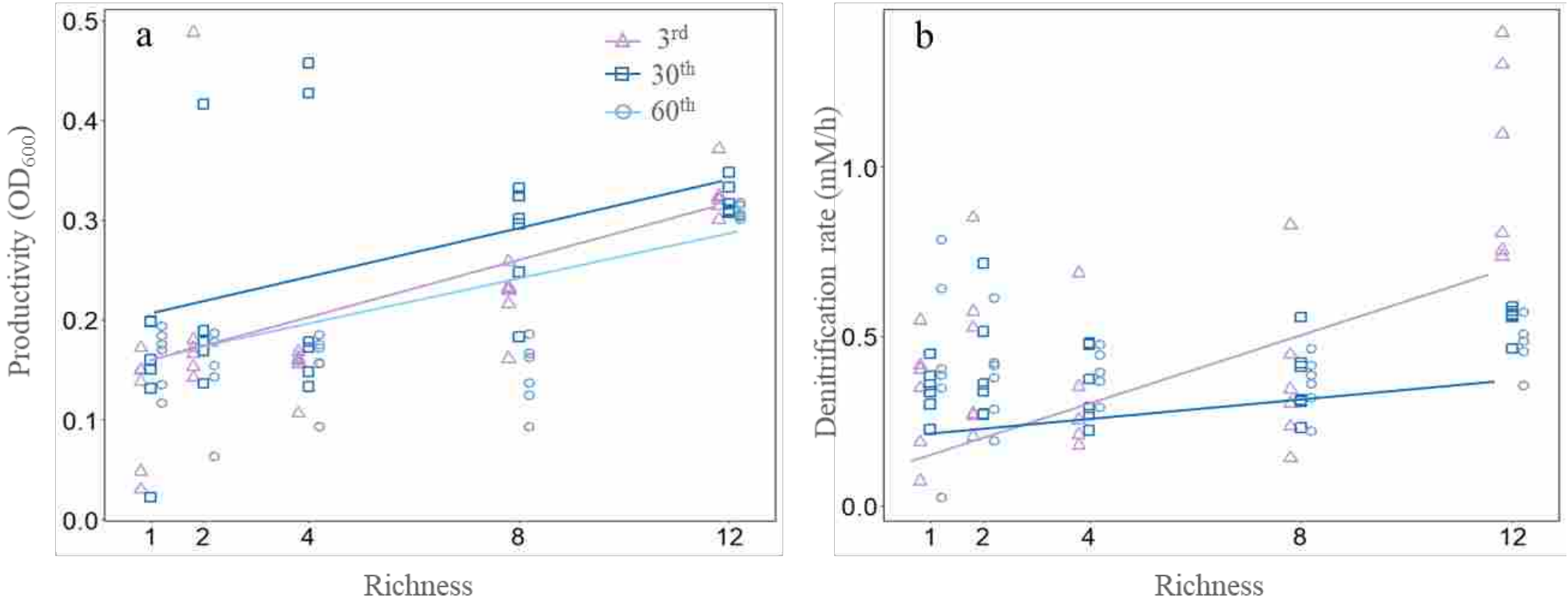




Richness of SDCs was positively correlated with biomass or denitrification rate (Spearman's correlation)

(Wu et al, 2023, *Microbiol Spectr*)

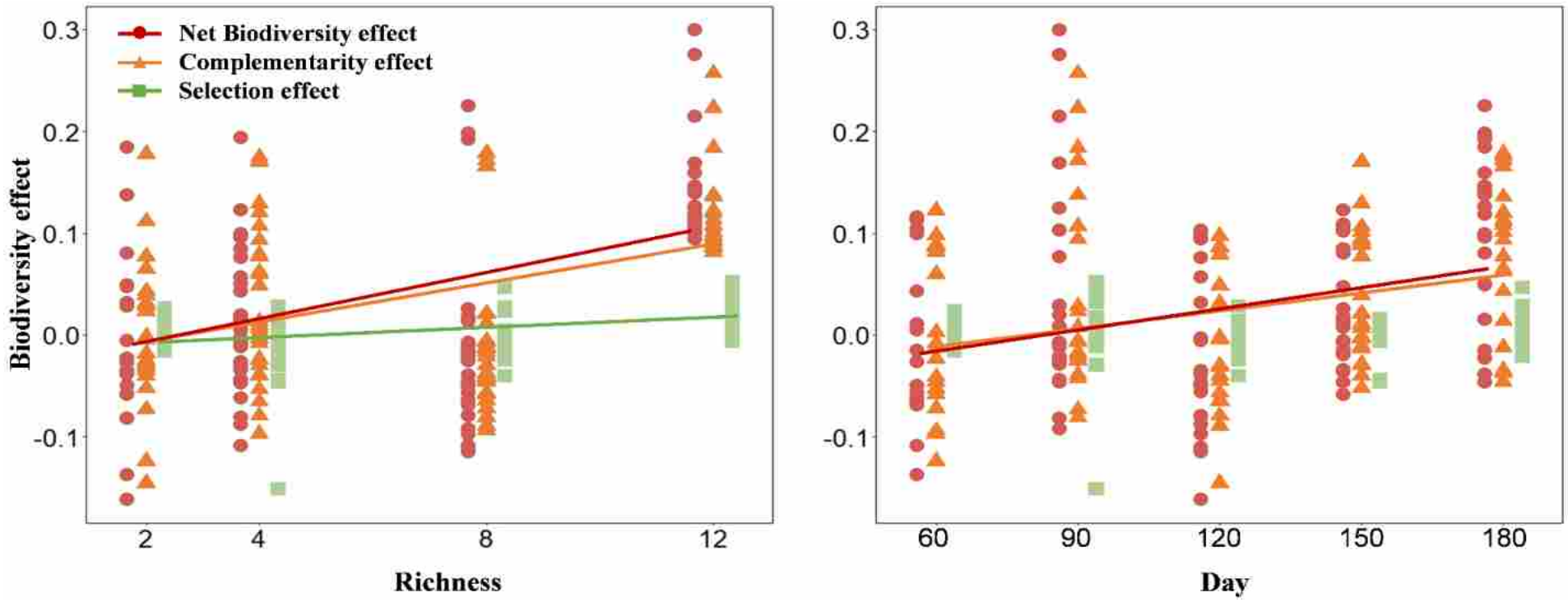
Denitrification function increased during evolution



Positive correlations between richness and function were more evident in the early stages (3-60 days)

(Wu et al, 2023, *Microbiol Spectr*)

Effects of richness and evolution on community functioning



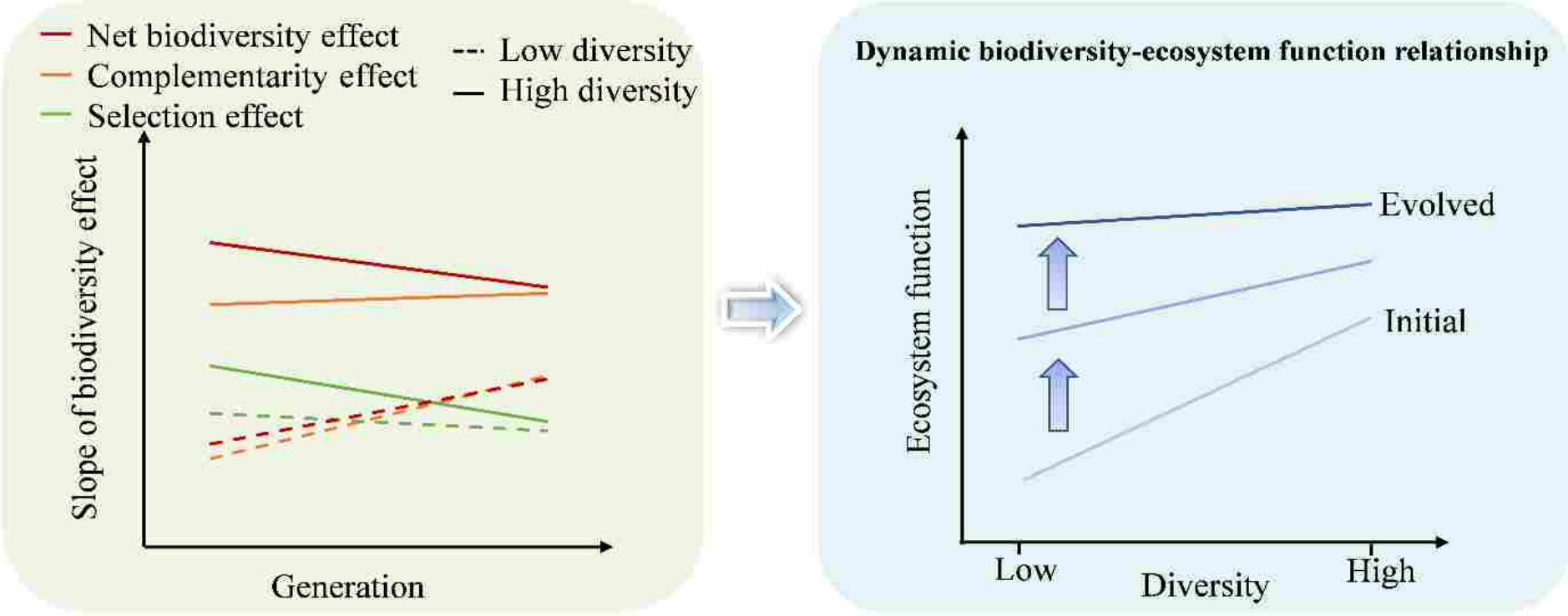
Net biodiversity = complementarity + selectivity

$$\Delta Y = N\overline{\Delta RY}\,\overline{N} + N\,\mathrm{cov}(\Delta RY, M)$$

(Loreau and Hector 2001)

Net biodiversity and complementarity effects generally increased with richness and evolution

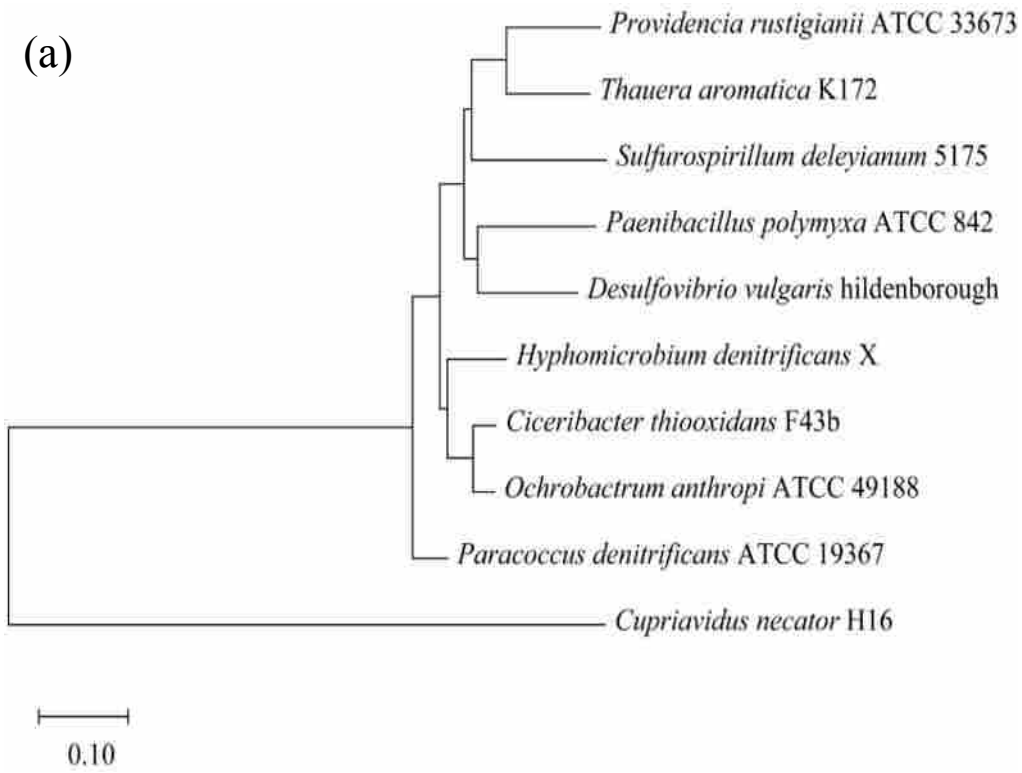
Dynamic BEF relationships during experimental evolution

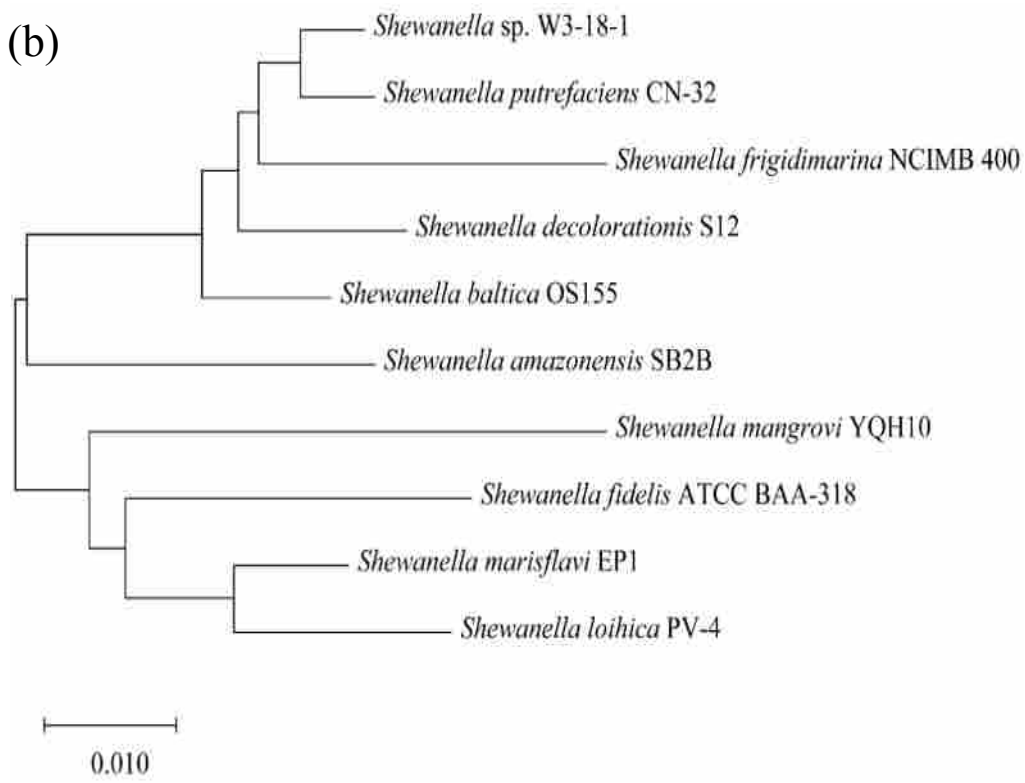


- **Complementary effect of low-richness communities increased rapidly, while the selection effect decreased slowly**
- **Ecosystem functions were high in high-richness communities, but increased slowly**
- **Complementary effect increased with experimental evolution, one of mechanisms for increased in SDC functions**

(Wu et al, 2023, *Microbiol Spectr*)

SDCs with low and high phylogenetic diversity





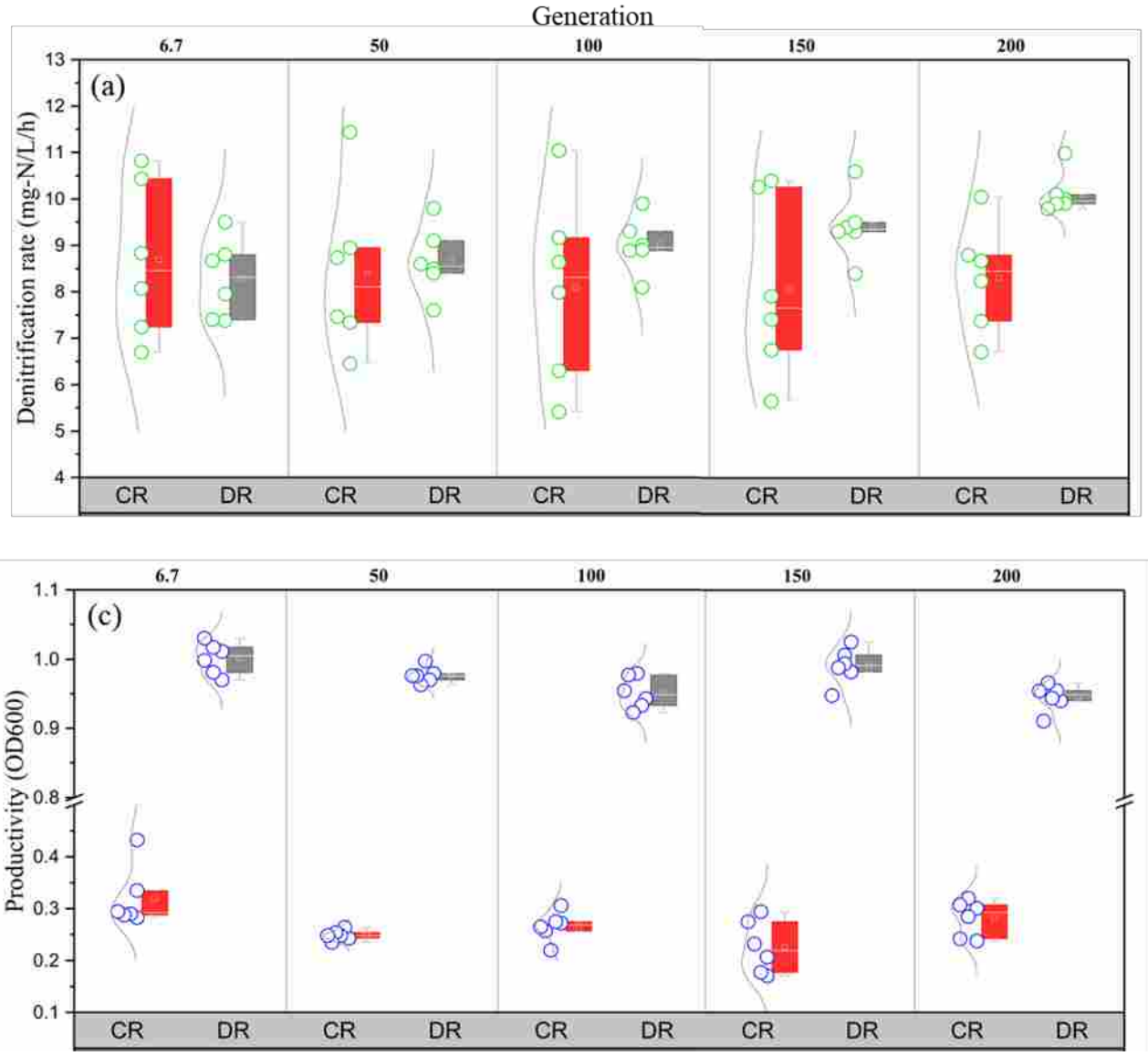
10 strains of different genera

Distantly related (**DR**) SDC

10 strains in the same genus (*Shewanella*)

Closely related (**CR**) SDC

SDC functions with low/high phylogenetic diversity

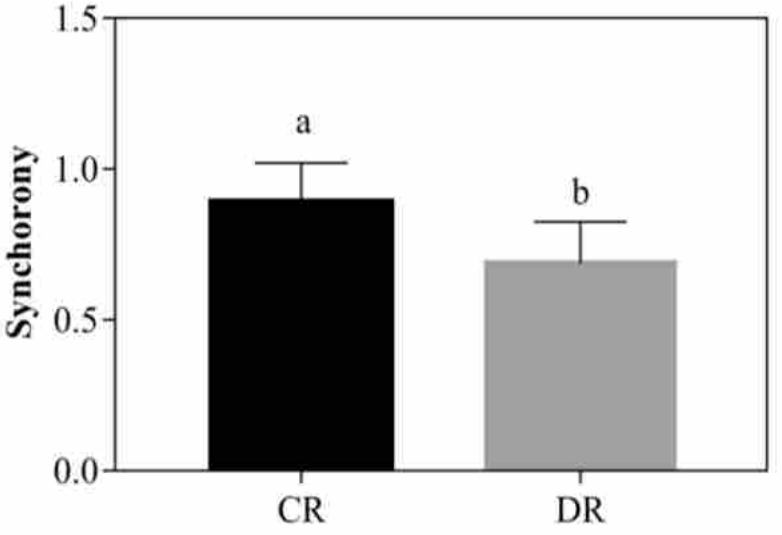


- The DR community had significantly ($P < 0.05$) **higher denitrification rates** than the CR community
- The DR Community had significantly ($P < 0.05$) **higher productivity** than the CR community (~3.6 times)

(Zhou et al, 2023, *Environ Res*)

Species survival rate and synchronicity

Factors	SS	DF	MS	F (DFn, DFd)	*P* value
Phylogenetic diversity	0.551	1	0.551	F (1, 60) = 106.100	$p < 0.001$
Generation	0.955	5	0.191	F (5, 60) = 36.750	$p < 0.001$
Phylogenetic diversity × Generation	0.141	5	0.0283	F (5, 60) = 5.439	$p < 0.001$

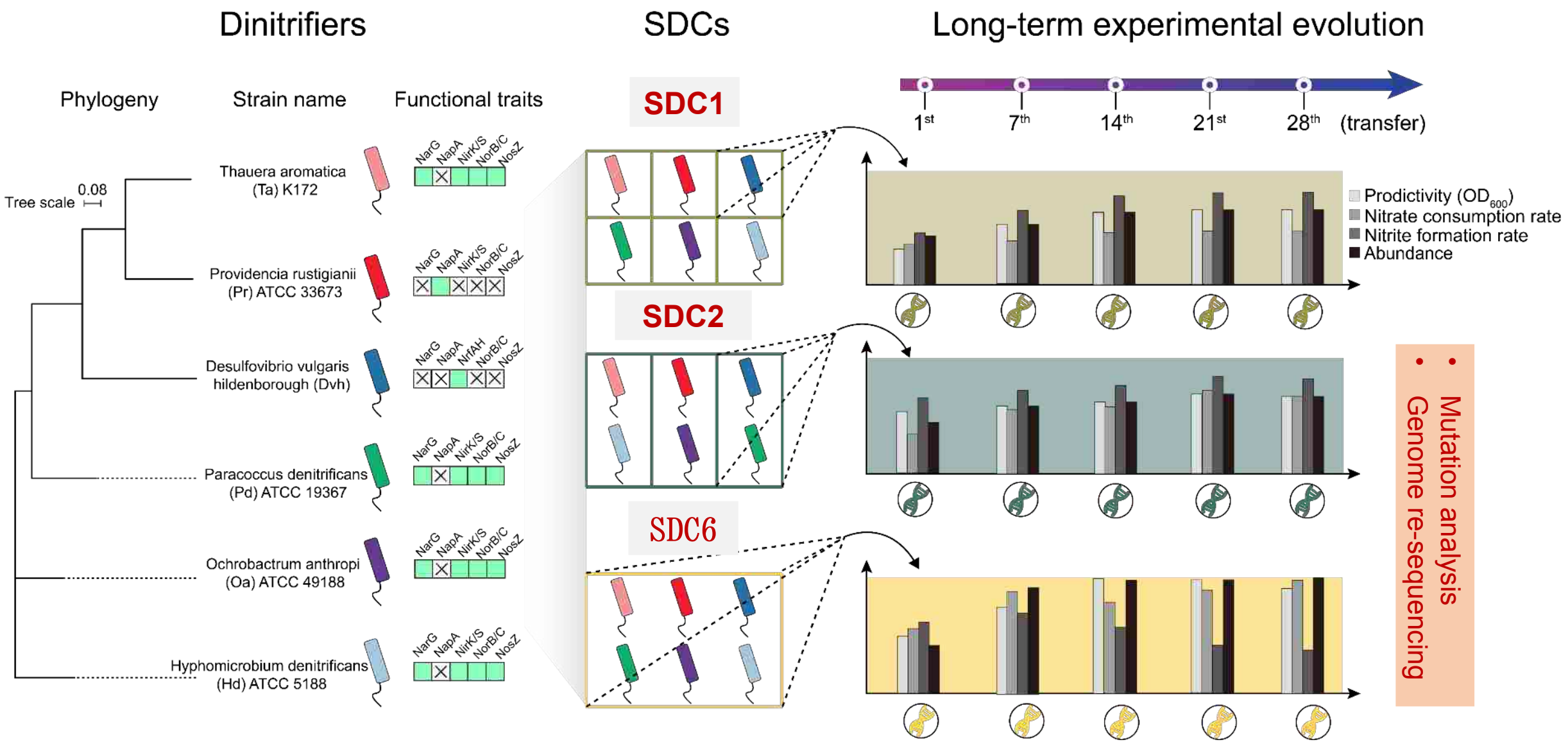


- **Phylogenetic diversity, evolution and their interactions** had significant (P < 0.01) effects on the survival rate of community species
- The CR community was **more synchronized** than the DR community (P < 0.01)

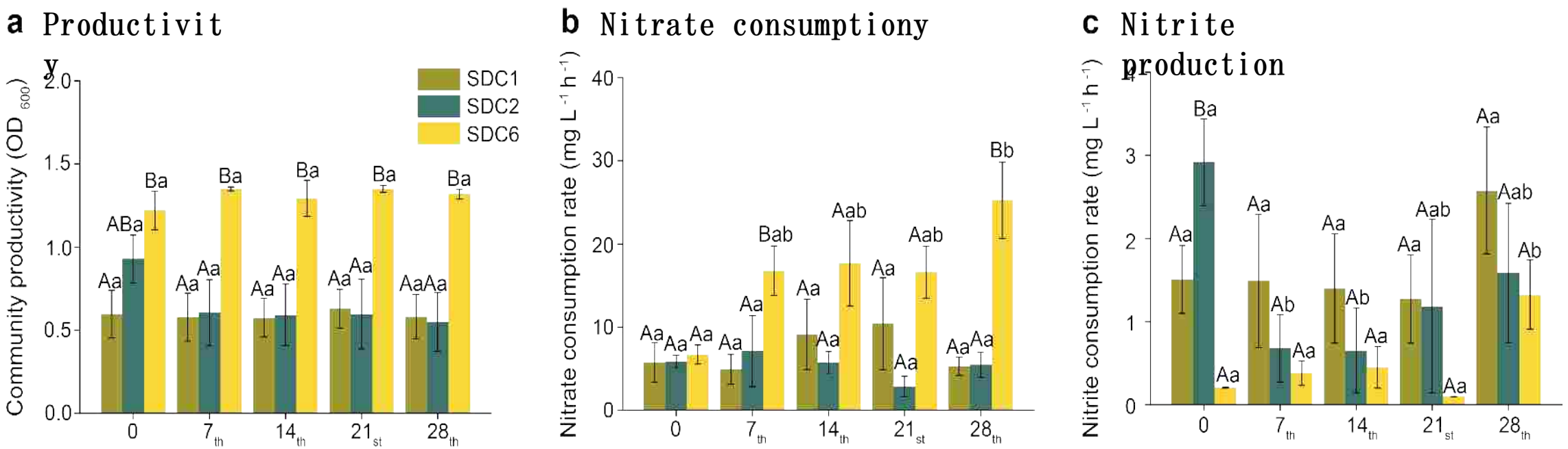
- **Implications:** more phylogenetically diverse microorganisms should be used for synthetic community construction
- **In-depth studies:** interaction-driven mechanisms for enhanced community functions

(Zhou et al, 2023, *Environ Res*)

Experimental design for interaction mechanisms



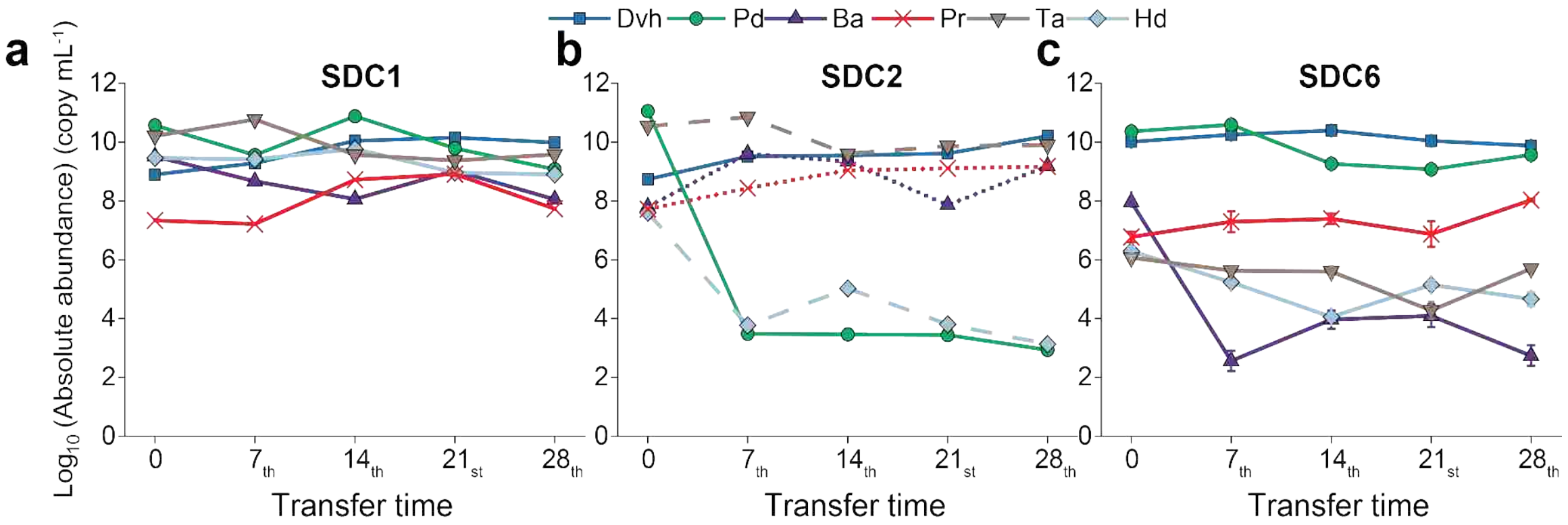
Productivity and denitrification functions



- SDC6 had higher productivity, nitrate consumption rate and lower nitrite accumulation rate than SDC1 and SDC2
- Nitrate consumption and nitrite production of SDC6 gradually increased during experimental evolution

(Zhao et al., unpublished)

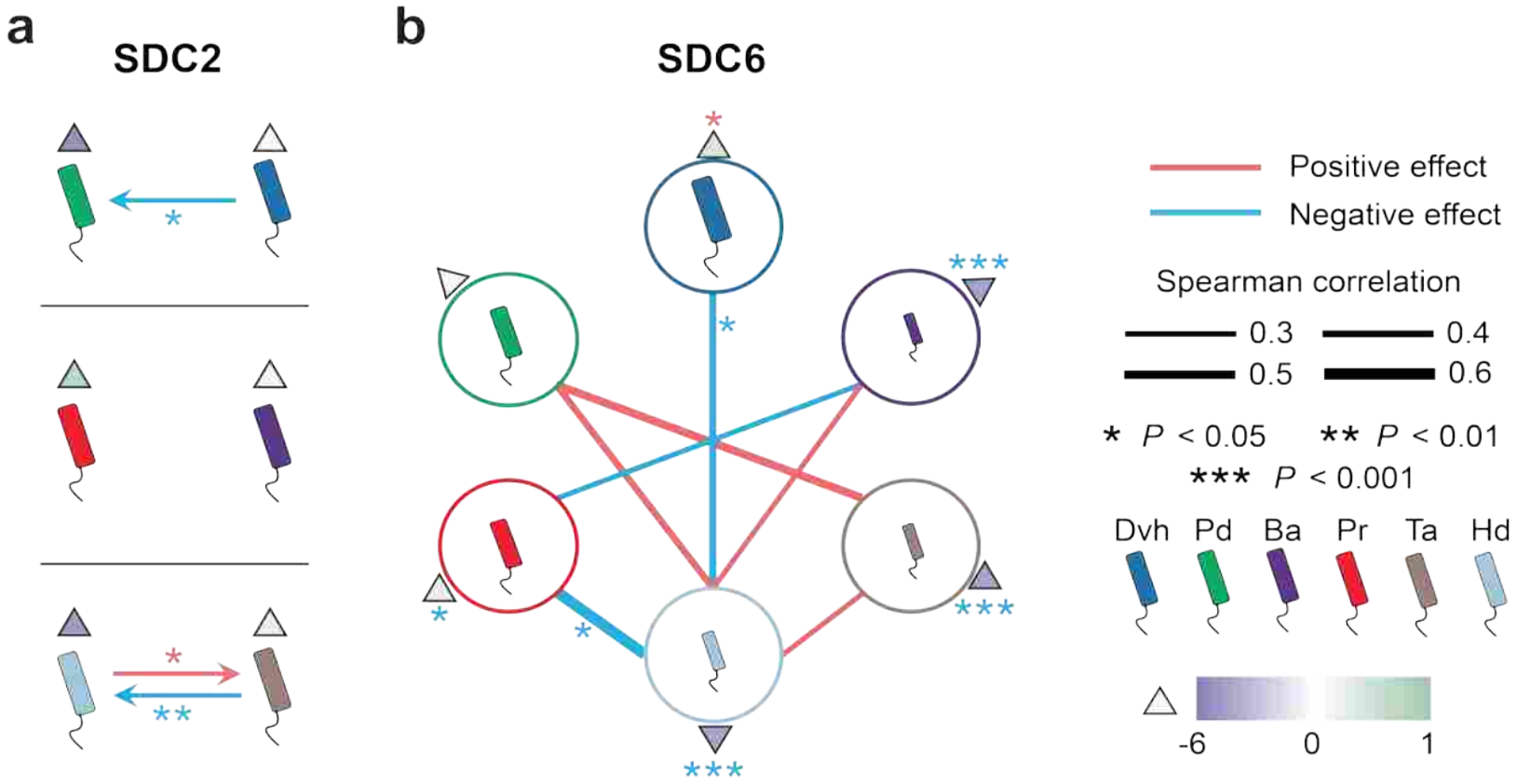
Abundance changes in individual denitrifiers



Compared with SDC1, the abundance of most strains decreased in SDC2 and SDC6

(Zhao et al., unpublished)

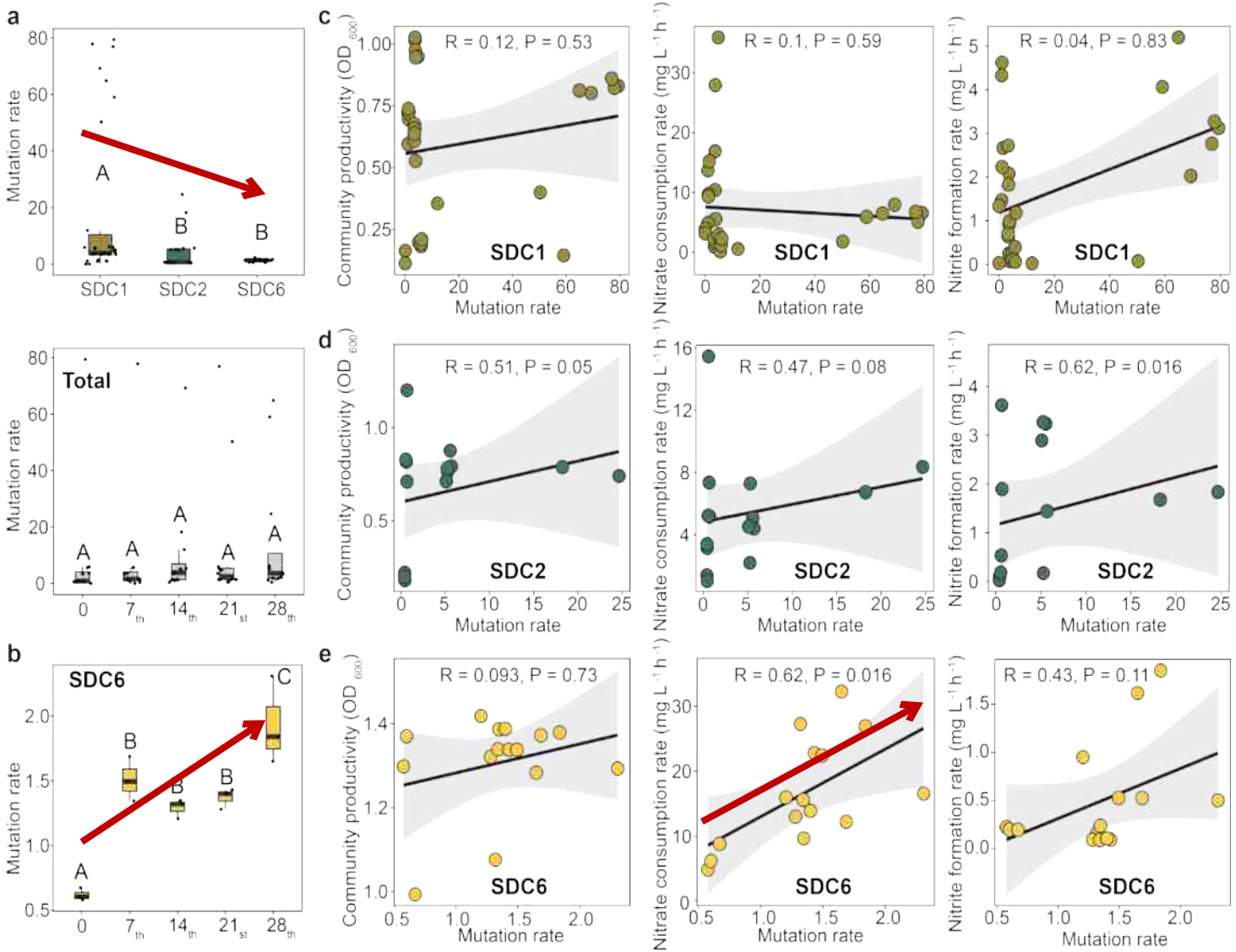
Interactions among denitrifiers in SDCs



Interactions between denitrifiers were both competition and facilitation, but competition appeared to be dominant.

(Zhao et al., unpublished)

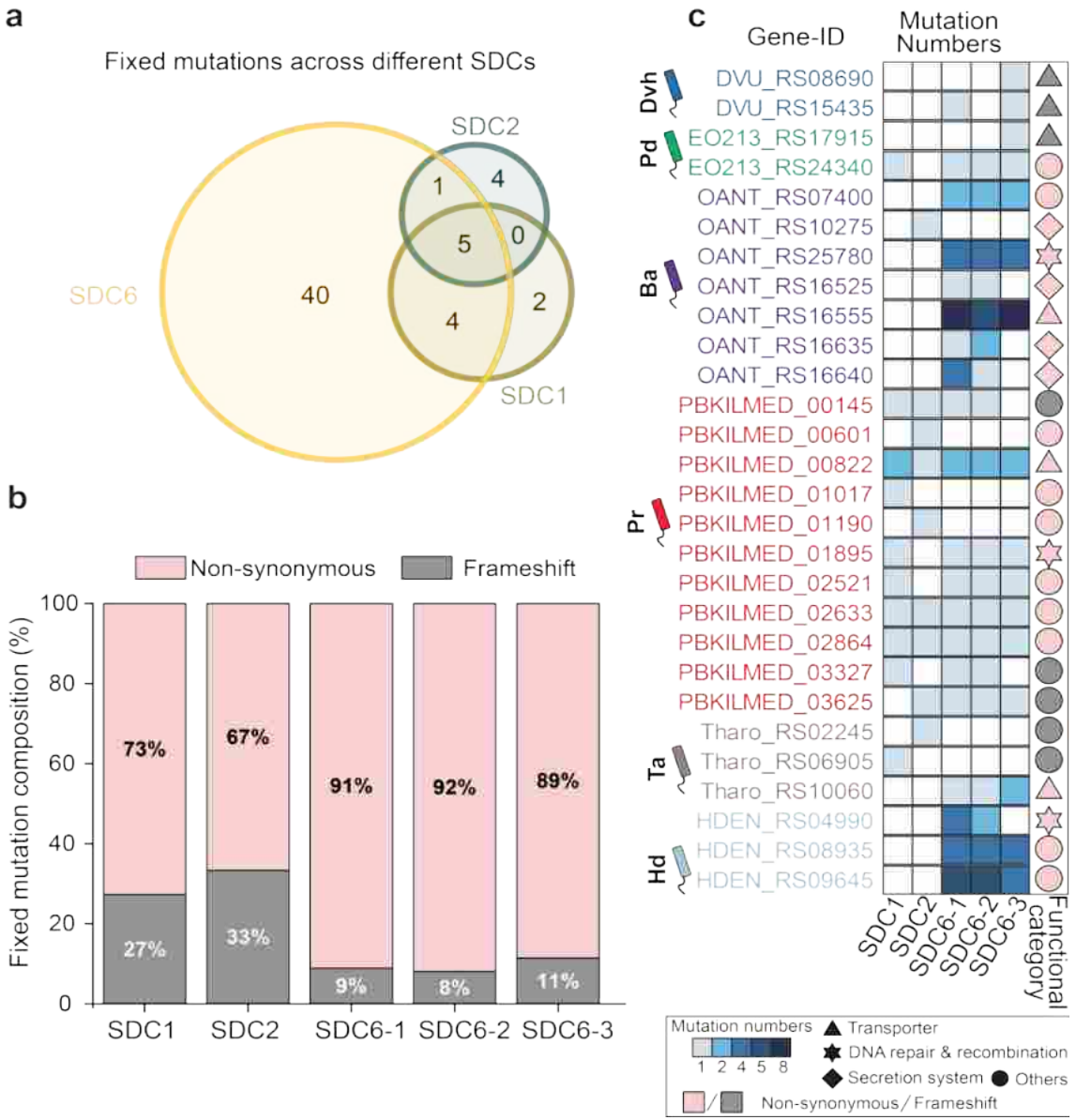
Rates of total mutations in SDCs



- SDC1 had the **highest** rate of total mutations among SDCs
- The mutation rate **increased** with experimental evolution in SDC6
- A **positive correlation** was observed between the mutation rate and nitrate consumption

(Zhao et al., unpublished)

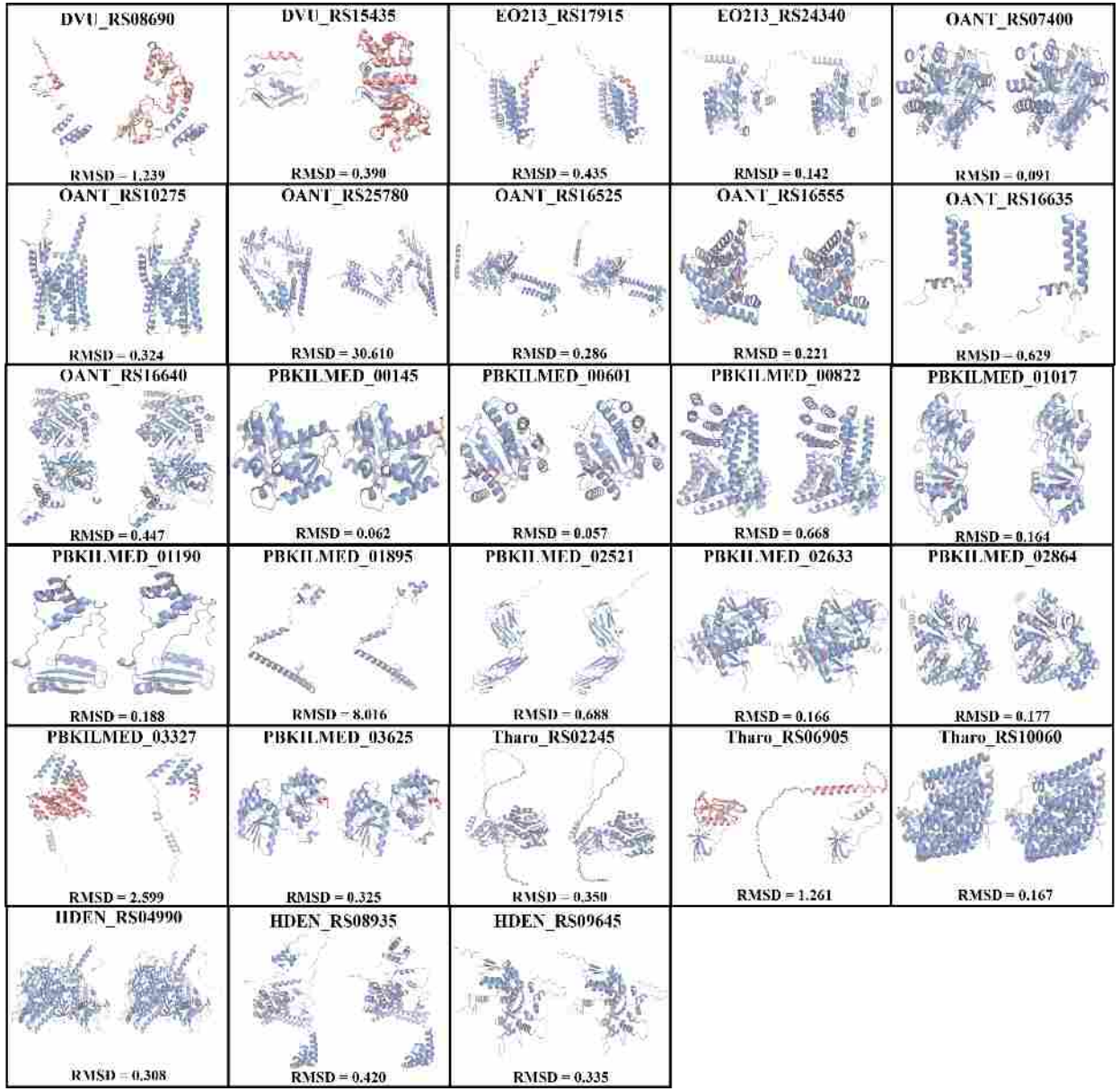
Rates of fixed mutations in SDCs

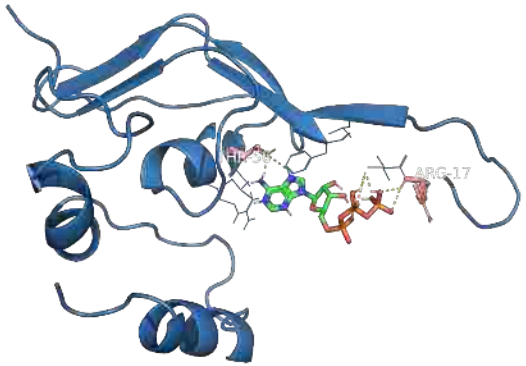


- **Fixed mutation:** a mutation with frequency >90%
- Some fixed mutations **occurred in all SDCs**
- SDC6 had the **highest** fixed mutations
- Genes with most fixed mutations involve **transportation**, **DNA repair and recombination**, and **secretion systems**

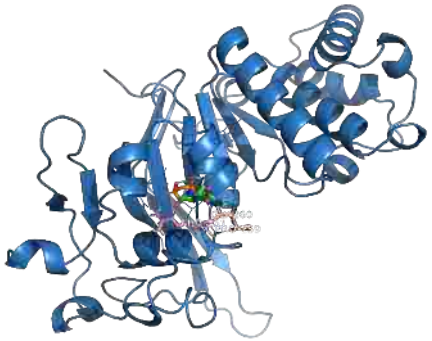
(Zhao et al., unpublished)

Predicted protein structure before and after mutation

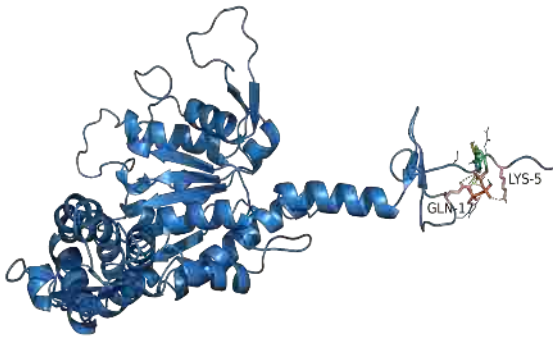




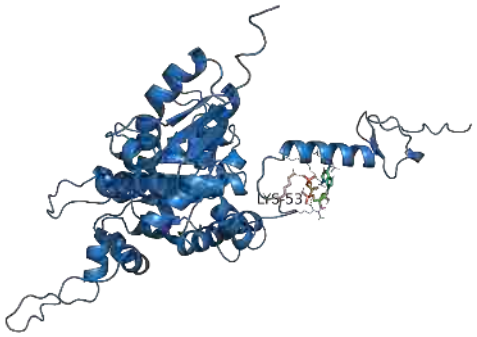
Binding energy = -1.32



Binding energy = -1.46

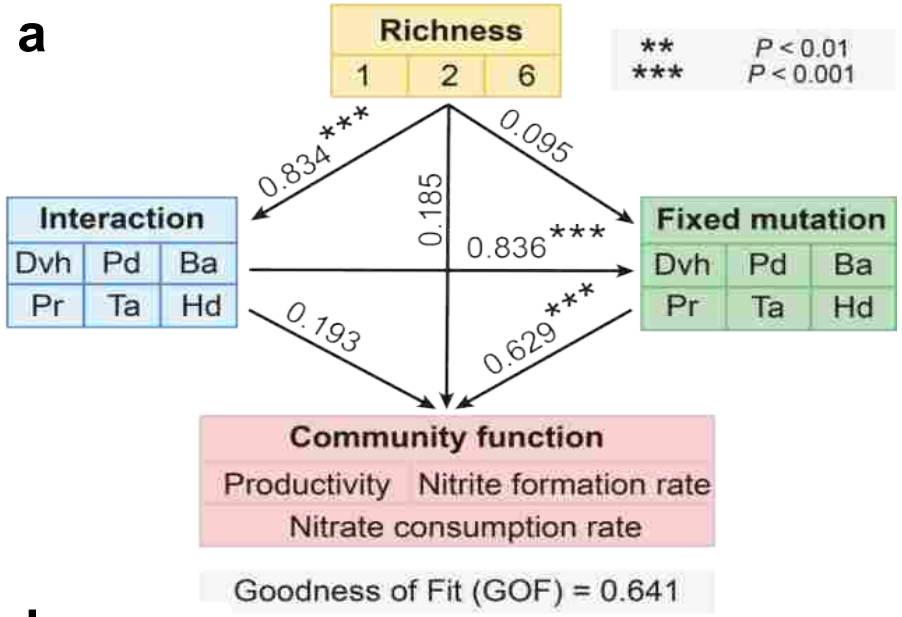


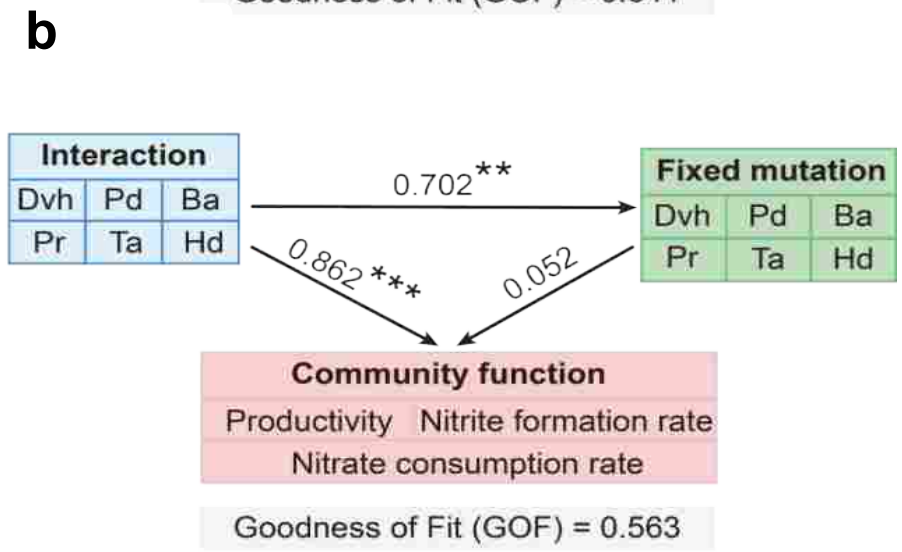
Binding energy = 1.29



Binding energy = 1.2

What factors drive community function enhancement?



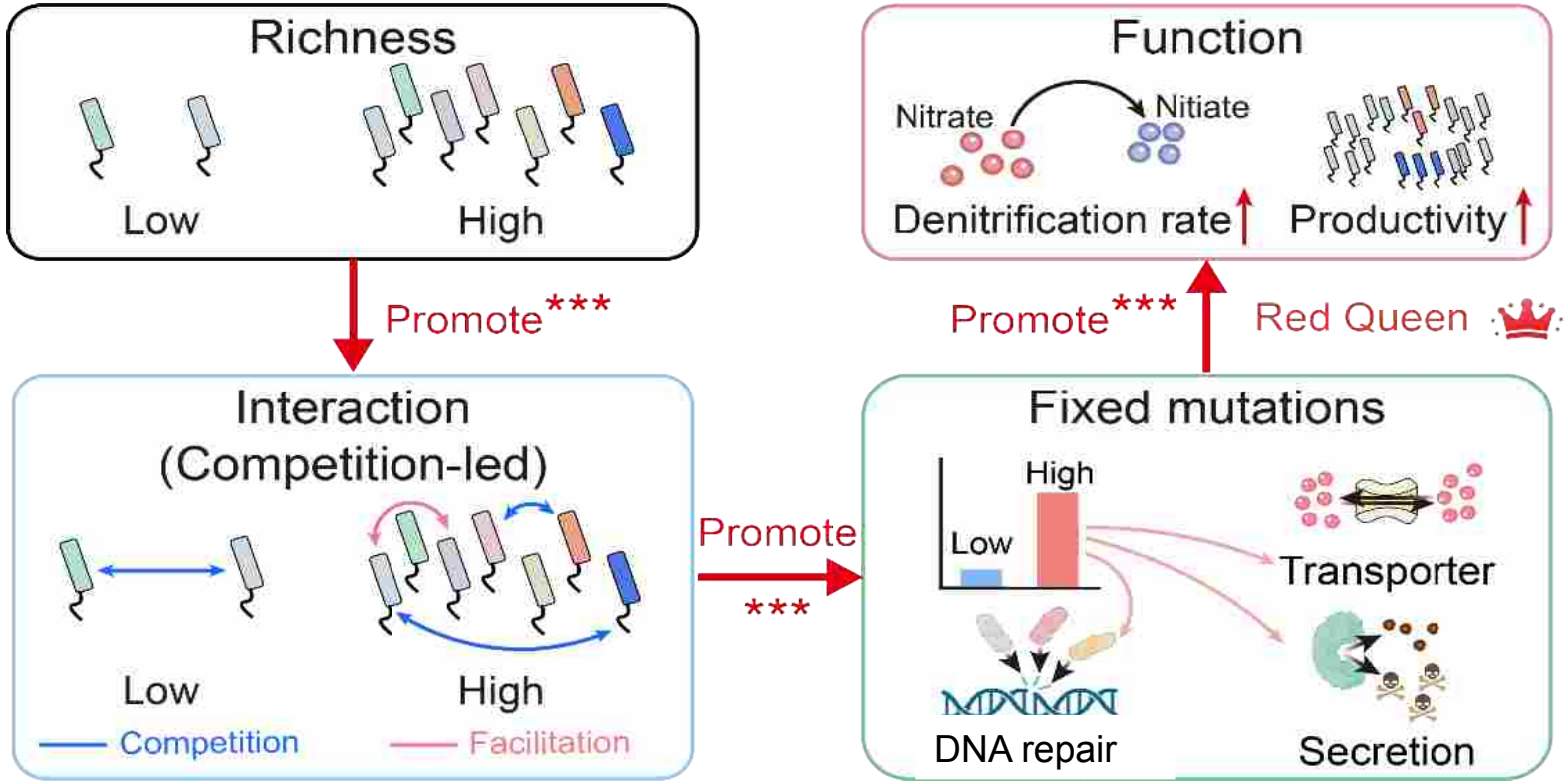


Partial least squares path modeling:

- Fixed mutations directly affected community functions in all SDCs
- Interaction directly affected fixed mutations and community functions in SDC6

(Zhao et al., unpublished)

Interactions and mutations enhance community functions



- **Denitrifiers interacted (generally by competition, especailly in SDC6), and increased beneficial fixed mutations (e.g., transporters, DNA repair proteins, and secretion proteins) , thus enhancing community functions.**

(Zhao et al., unpublished)

Outline

- Synthetic microbial ecology
- Synthetic nitrifying community niche differentiation and mechanisms
- Biodiversity and community function relationships of synthetic denitrifying communities
- Challenges and perspectives

Challenges

- **Microbial resources**： specific functional strains for synthetic community construction
- **Genome- and community-level modelling**： selection and optimization of microbial species/strains
- **Experimental validation of metabolic modelling**
- **Microbial interactions and underlying mechansims**
- **Microbiome engineering and applications in the environment**

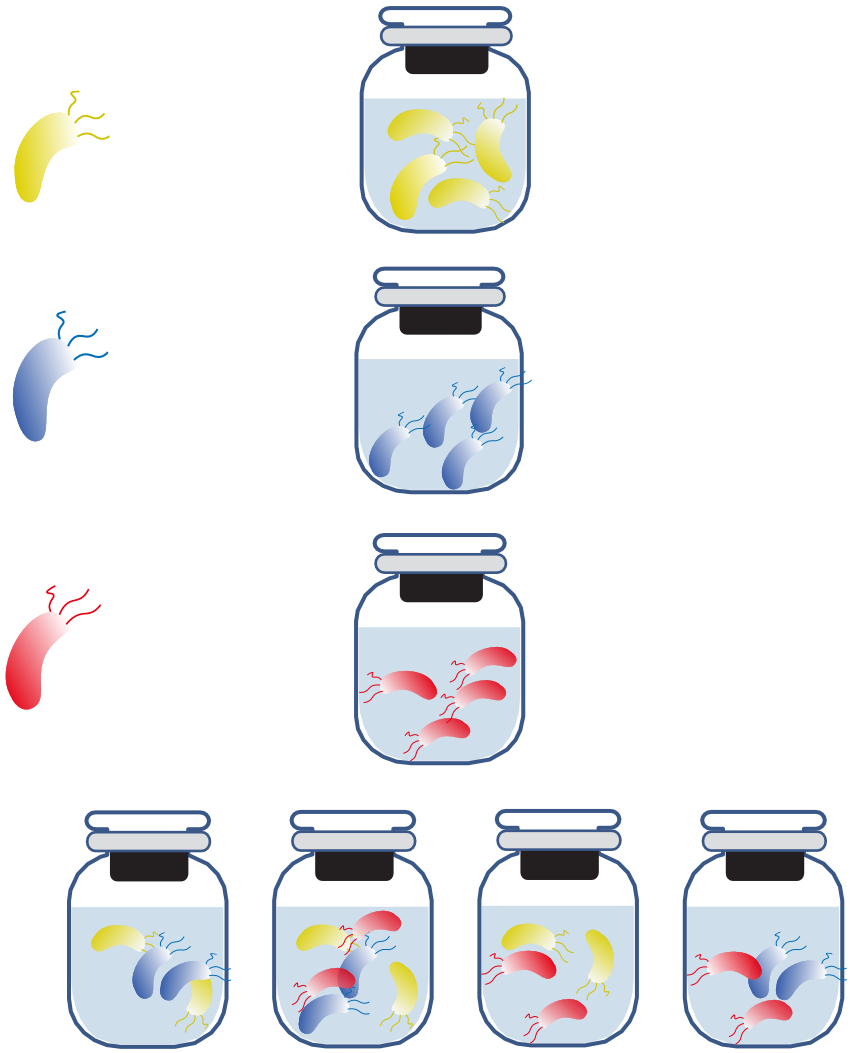
N_2O emission-reducing communities

nosZ I denitrifiers

nosZ II denitrifiers

Comammox bacteria

Synthetic communities

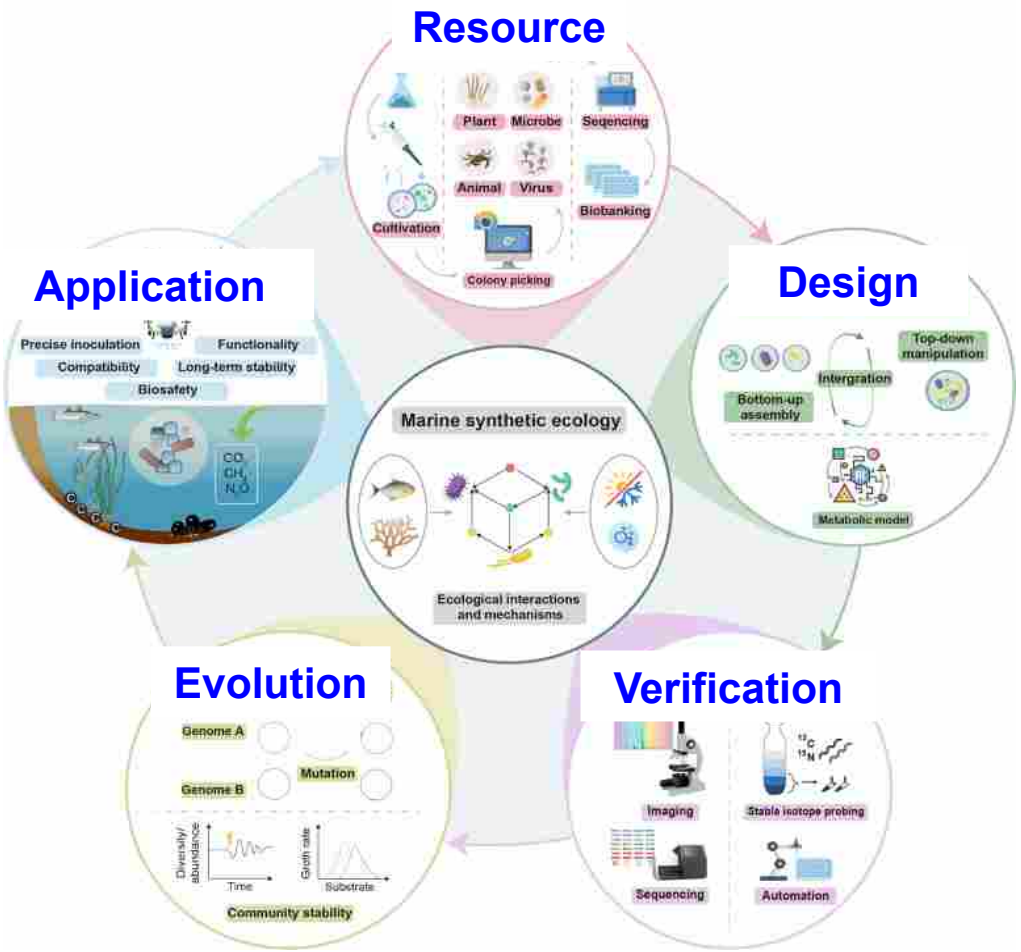


Marine synthetic ecology

Synthetic ecology is a new interdiscipline emerging ecology, biology, engineering, and technology to make sustainable ecosystems using **natural strategies and mechanisms,** enabling artificial ecosystems to be **strong, resilient** and **adaptable.**

- **Ecology theories and methods**
- **Applications of synthetic communities or ecosystems**

The RDVEA framework



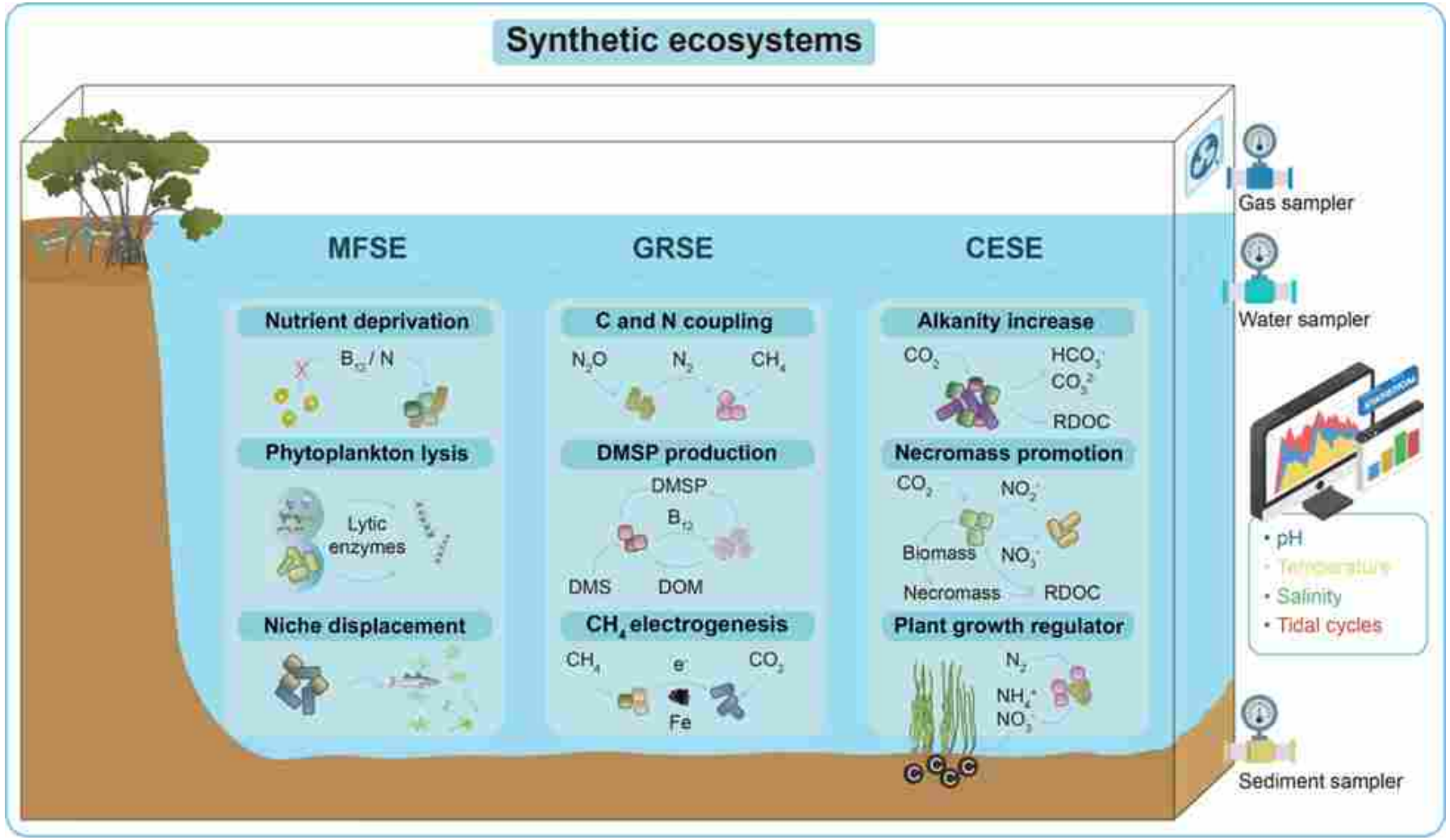
(Su et al., unpublished)

From synthetic communities to synthetic ecosystems

MFSE: marine food web synthetic ecosystem

GRSE: greenhouse gas reduction synthetic ecosystems

CESE: carbon sequestration enhancement synthetic ecosystems



(Su et al., unpublished)

Summary

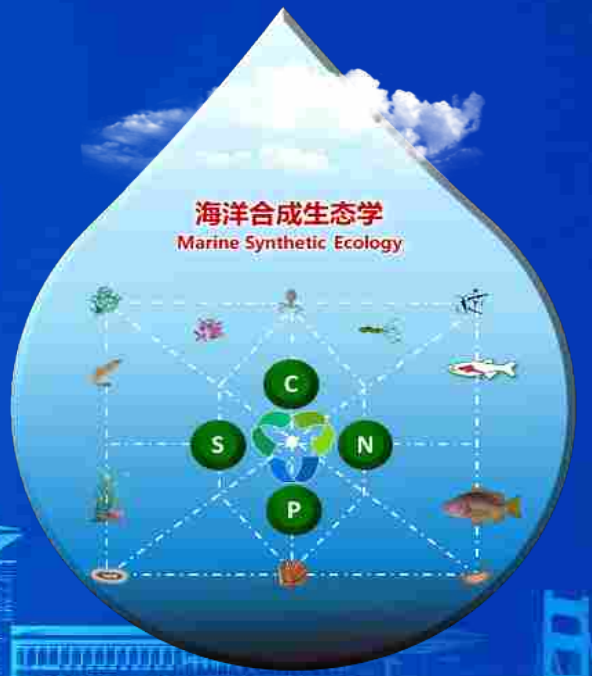
- Nitrifers' niche differentiation was determined by their differences in the substrate affinity, substrate inhibition and growth rate: AOA < Comammox < AOB for ammonium, and Comammox < NOB for nitrite
- Analysis of SDCs (with a richness of gradient) indicated positive, dynamic BEF relationships, and high phylogenetic diversity communities had strong functionality and stability, respectively
- A combination of metabolic modelling and experimental validation will provide novel insights into biotic interactions and underlying mechanisms, realizing accurate prediction of ecosystem functions

Thanks!



海 洋 合 成 生 态 学 研 究 中 心

Marine Synthetic Ecology Research Center (MSEC)



南方海洋科学与工程广东省实验室（珠海）

Southern Marine Science and Engineering Guangdong Laboratory(Zhuhai)

国家自然科学基金委员会

National Natural Science Foundation of China