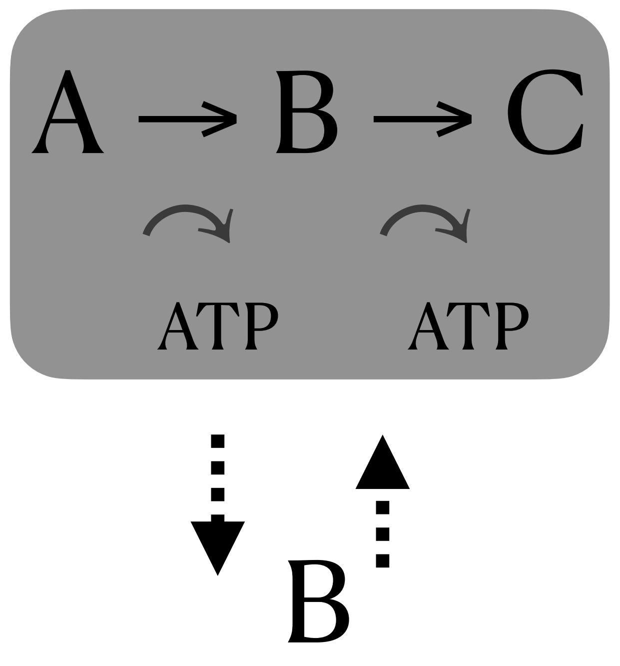
# Pathway splitting in denitrifying bacterial communities

Karna Gowda / Kuehn Lab / KITP EcoEvo21 / 20210728

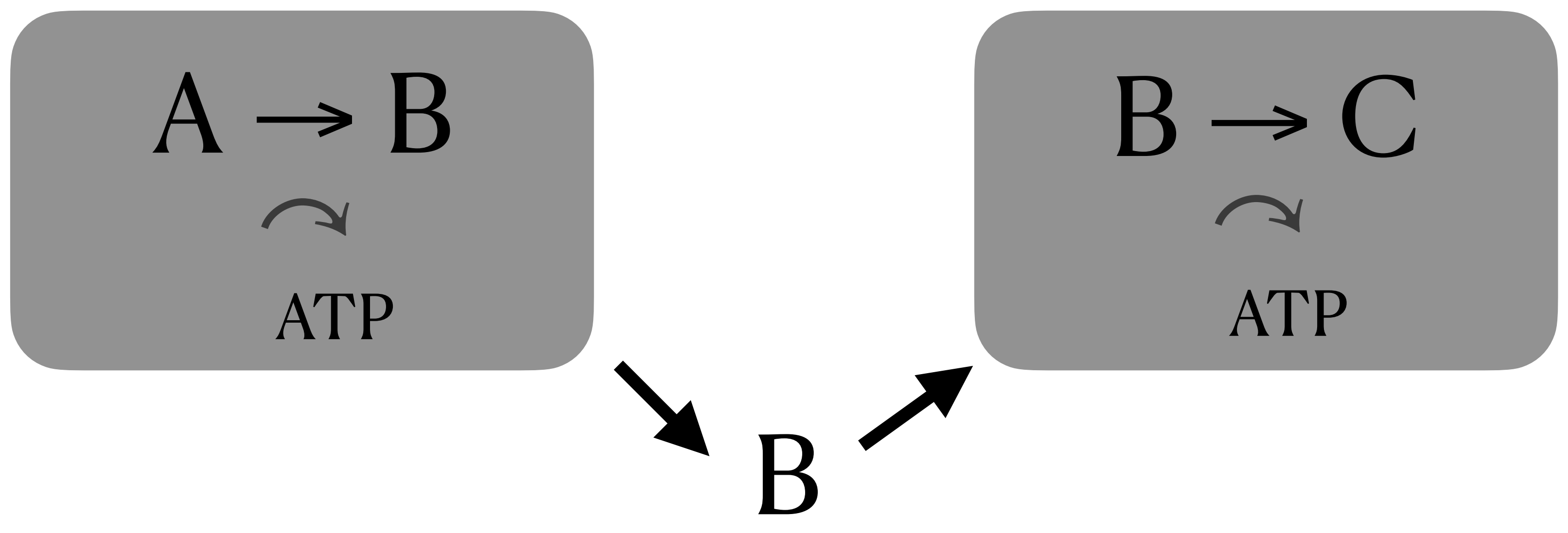
# Pathway splitting: obligate cross-feeding

full pathway:




cross-feeding possible through transient excretion of intermediates

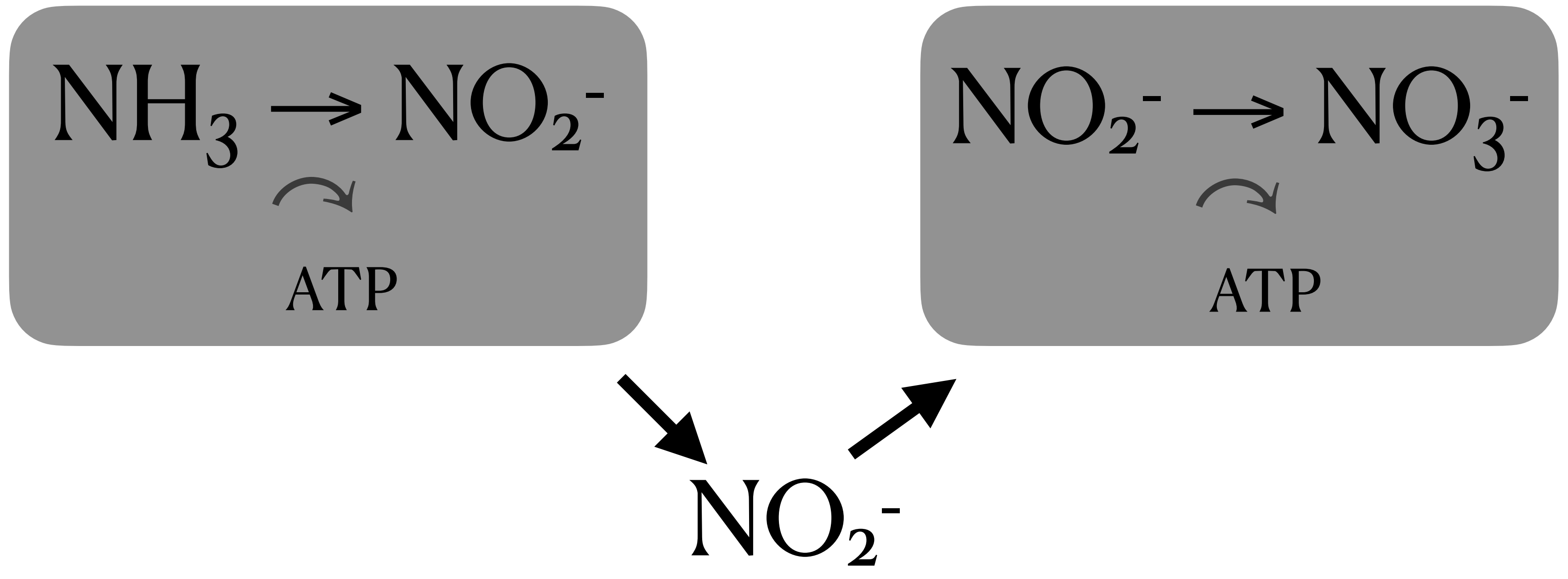
split pathway:




cross-feeding obligatory

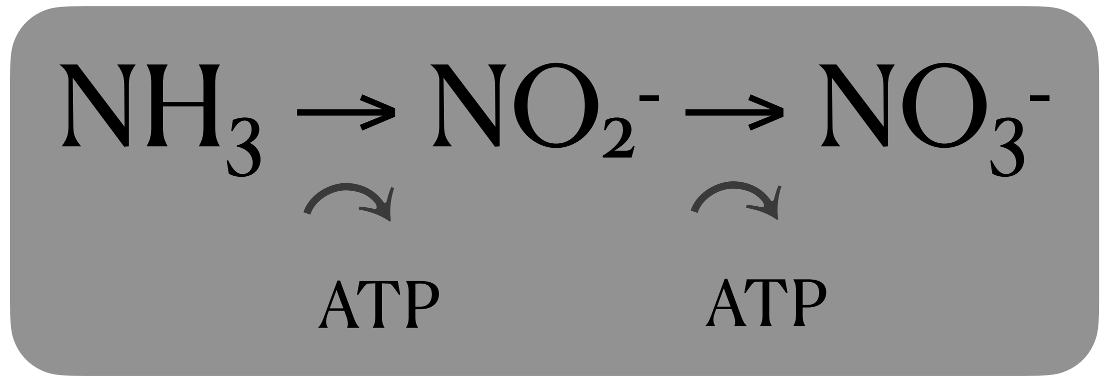
# Nitrifiers as an example

Traditional view of **nitrification**:




putatively under selection
for rate of ATP generation

Complete nitrifiers (comammox) found in 2015:

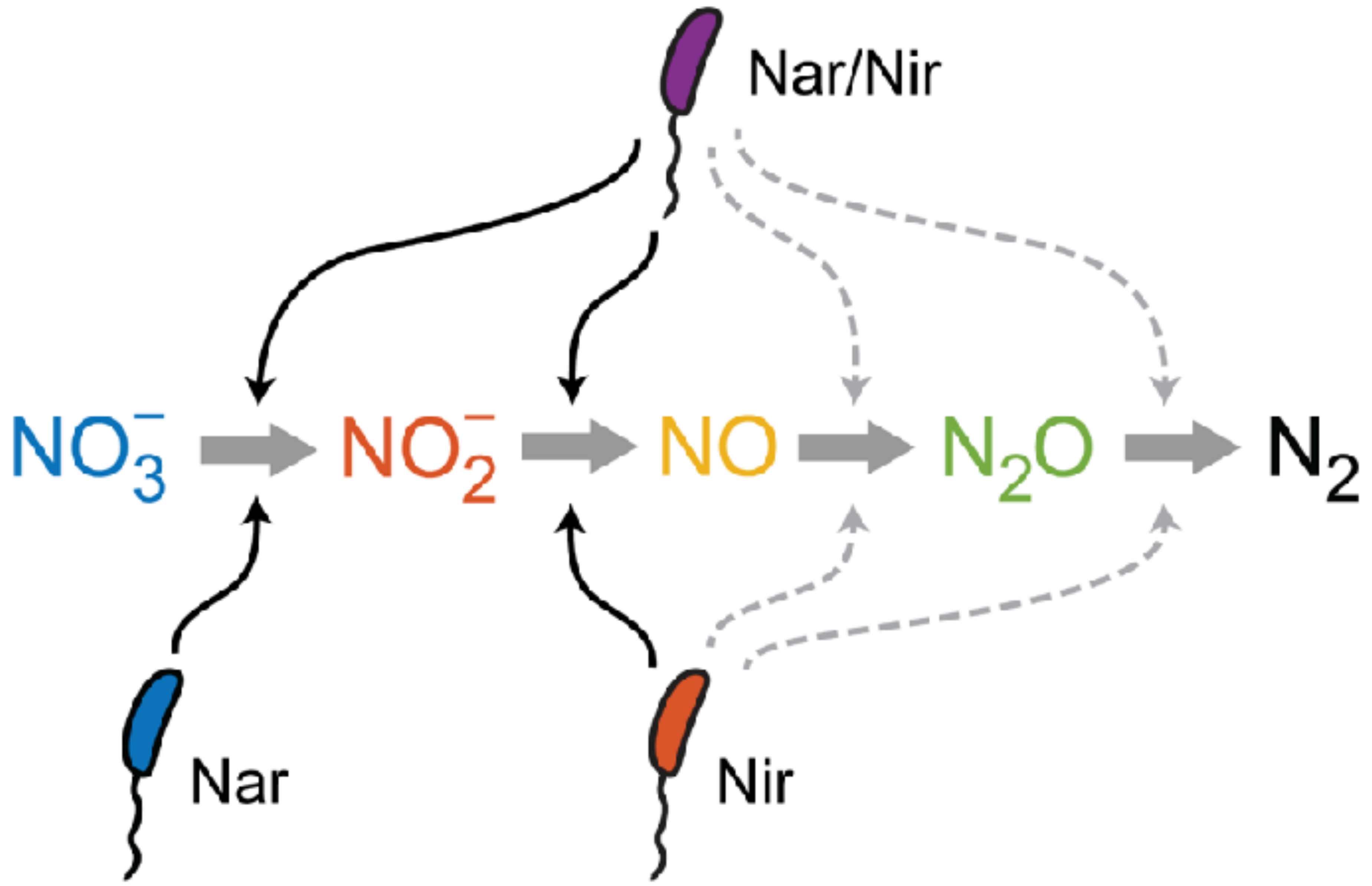



isolated from biofilms, putatively
under selection for yield

Costa et al., 2006; Daims et al., 2015; van Kessel et al., 2015

See also Pfieffer & Bonhoeffer, 2004; Tsoi et al., 2017

# Why do denitrifiers split pathways?




| | NAR | NIR | NOR | $N_2OR$ | pH 7.4 soil series A | pH 3.7 soil series B | pH 3.7 soil series C |
|---|---|---|---|---|---|---|---|
| Full-fledged | $N_2$ | | | | 8 | 0 | 1 |
| NIR, NOR, $N_2OR$ | | $N_2$ | | | 0 | 0 | 3 |
| Only $N_2OR$ | | | | $N_2$ | 2 | 1 | 0 |
| NAR*/NIR*, $N_2OR$ | $NO_3^-$ and $NO_2^-$ reduced | | | $N_2$ | 0 | 0 | 2 |
| NAR, NIR, NOR | $N_2O$ | | | | 1 | 2 | 0 |
| NIR, NOR | | $N_2O$ | | | 1 | 4 | 4 |
| NAR, NIR | NO | | | | 4 | 0 | 0 |
| Only NIR | | NO | | | 0 | 2 | 0 |
| Only NAR | $NO_2^-$ | | | | 19 | 13 | 1 |
| DNRA | $NO_3^-$ and $NO_2^-$ reduced to $NH_4^+$ | | | | 1 | 1 | 0 |

Gowda et al., 2021

Lycus et al., 2018

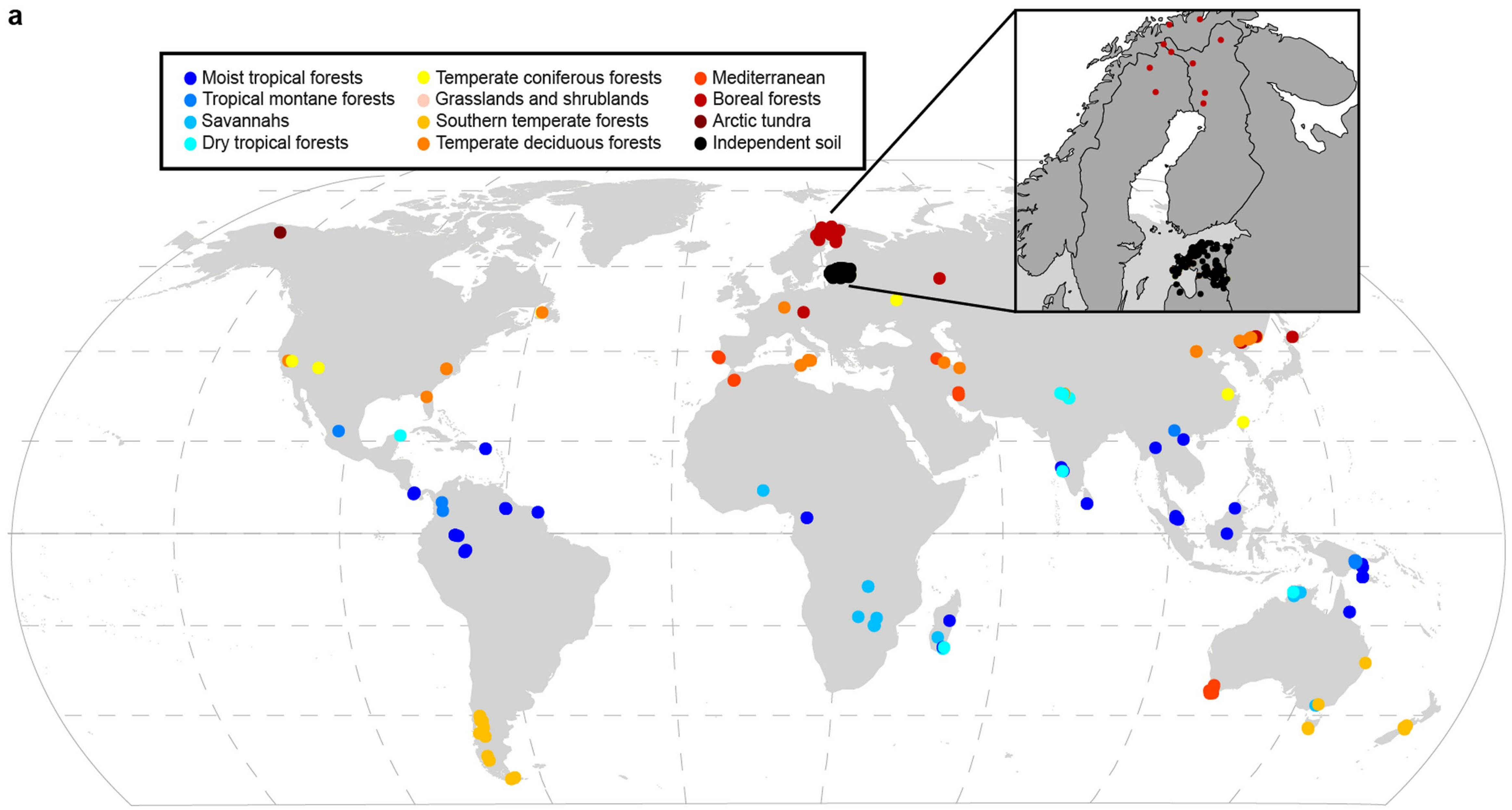
# Overview

1. What environmental factors drive denitrification pathway splitting?

2. An attempt to select for pathway splitting in the lab

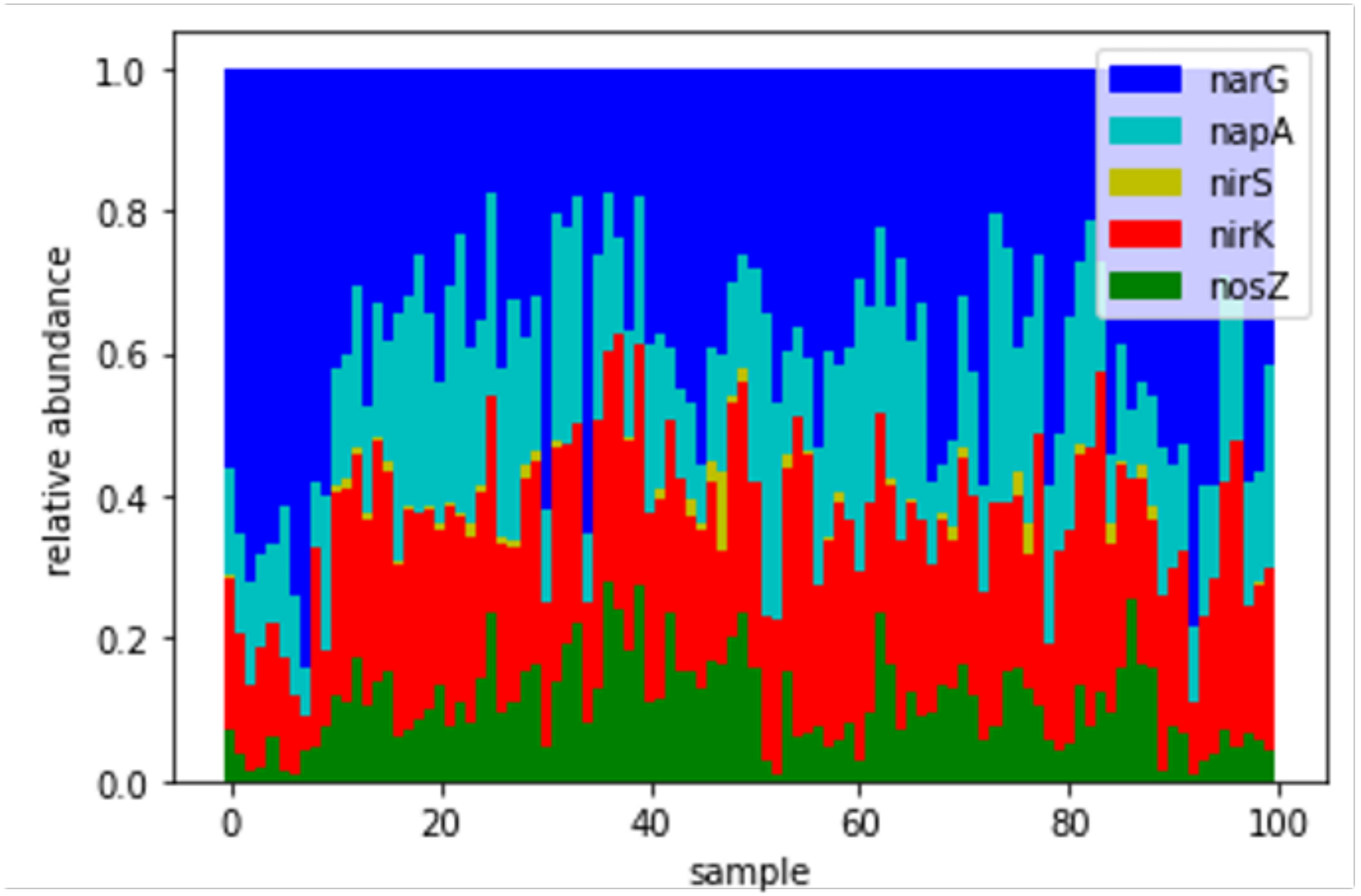
3. A proposed theory connecting environment to physiology and selection

# What environmental factors might drive pathway splitting?




Bahram et al., 2018

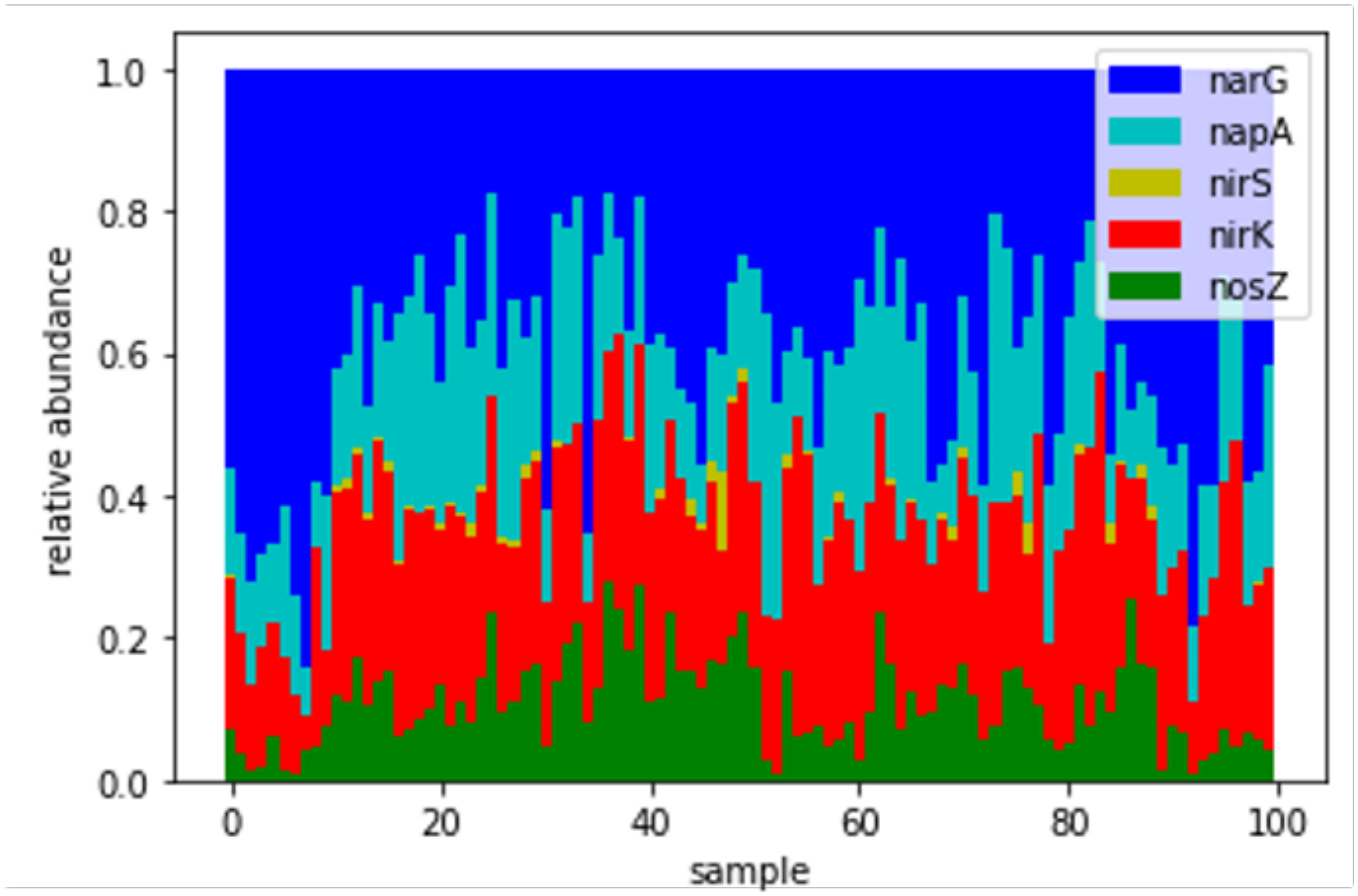
metagenomic "g-vectors"




data courtesy of Mo Bahram

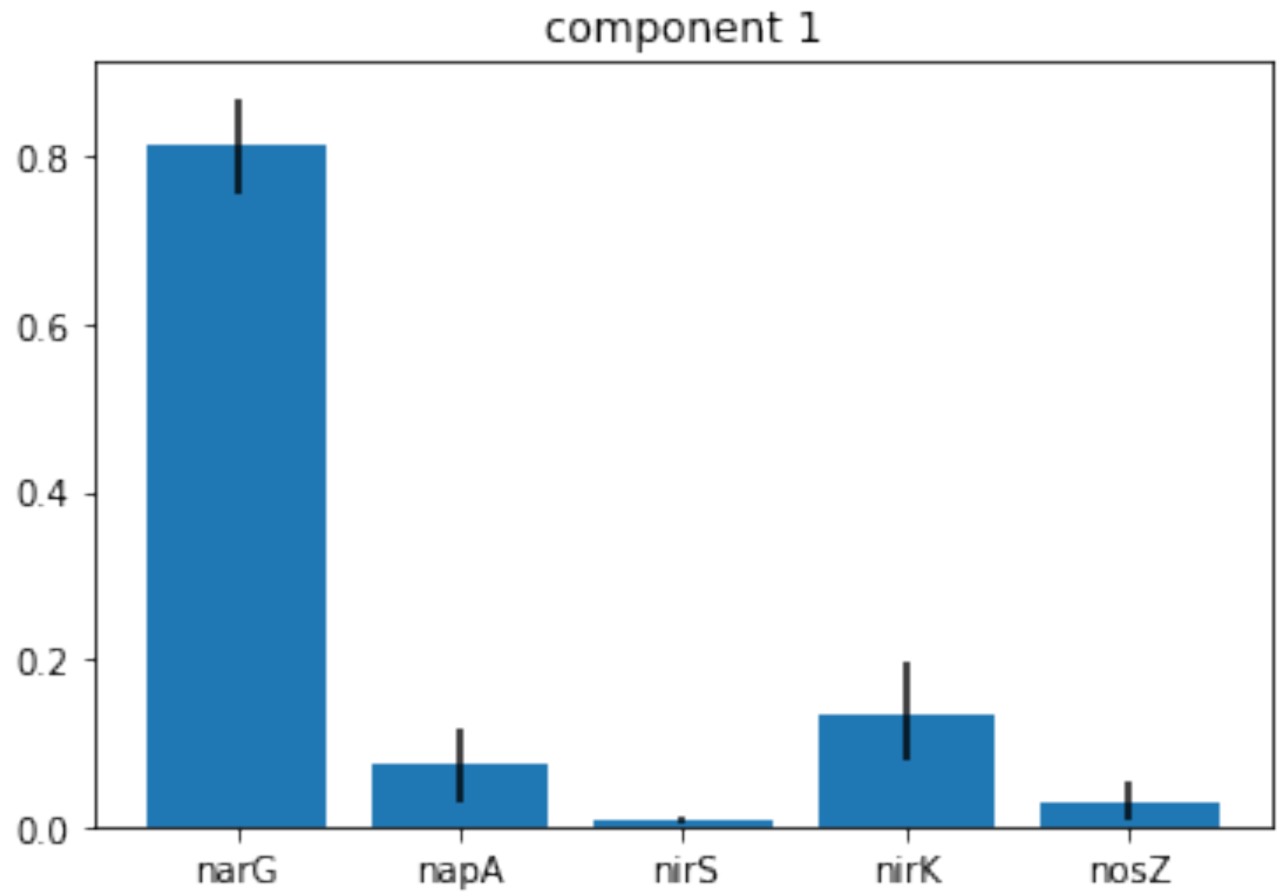
- 189 topsoil samples
- **Soil characterization (C, N, P, K, pH, ...)**
- Shotgun metagenomes (KO abundances, ...)
- Limitation: too shallow for MAGs (Zeqian Li)

# Two modes of variation in pathway composition

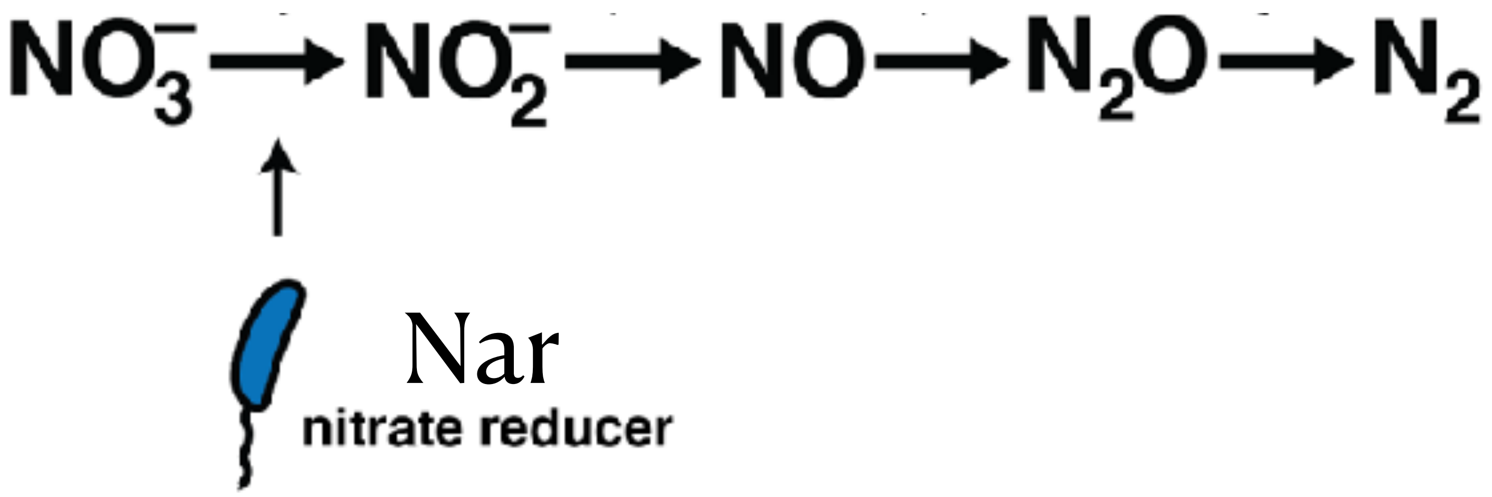


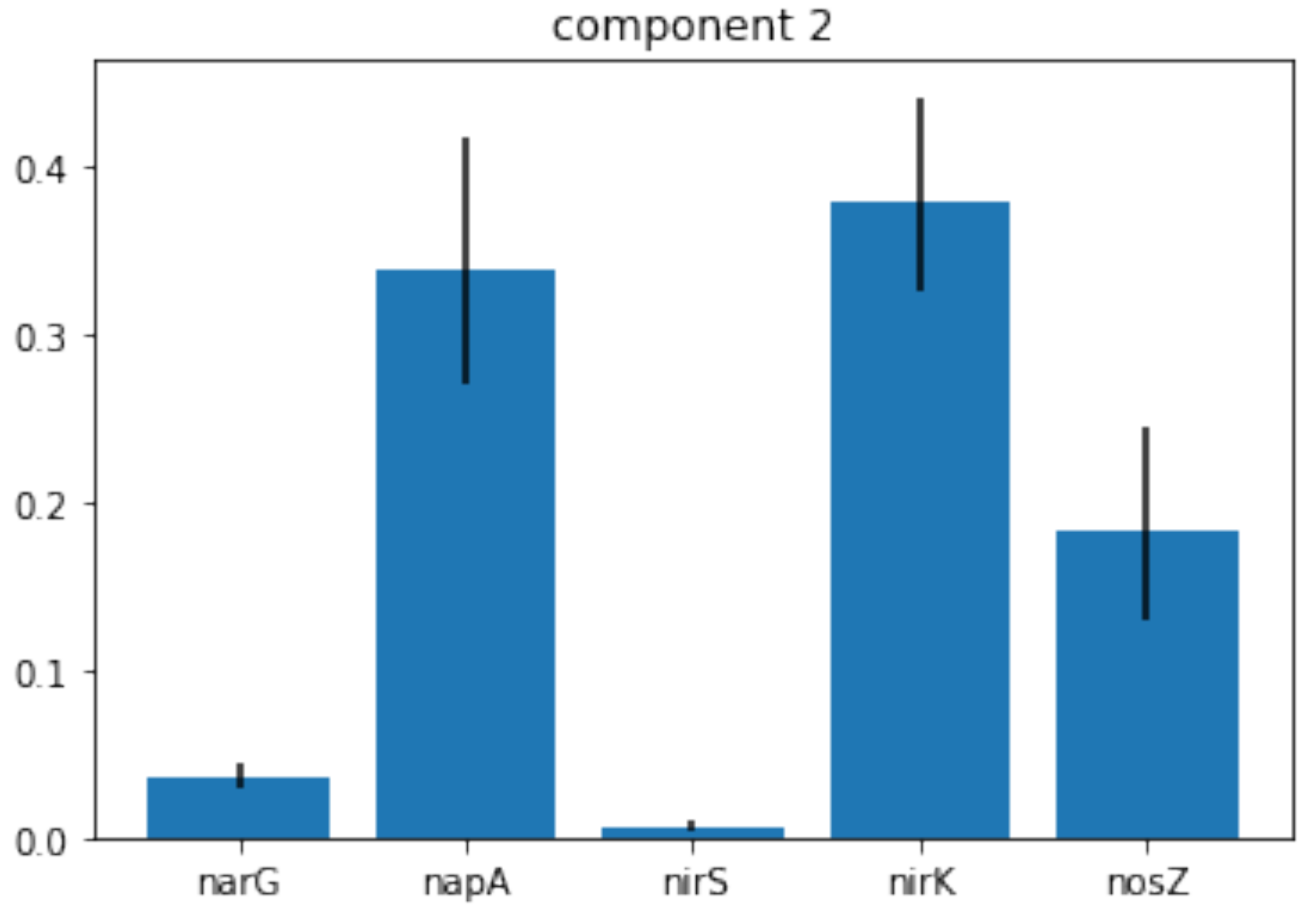

$$\vec{x} = w_1\vec{h}_1 + w_2\vec{h}_2$$
$$h_i, w_i \geq 0$$

Non-negative matrix factorization (Lee & Seung, 1999)

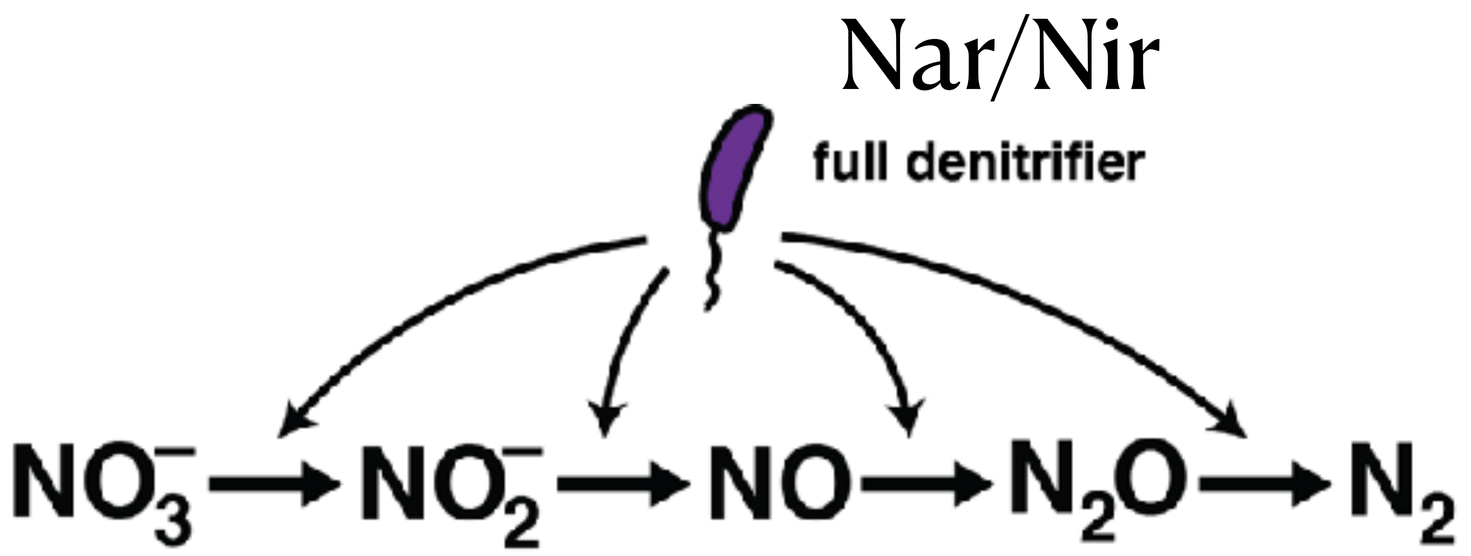



Fast/high yield nitrite reductase



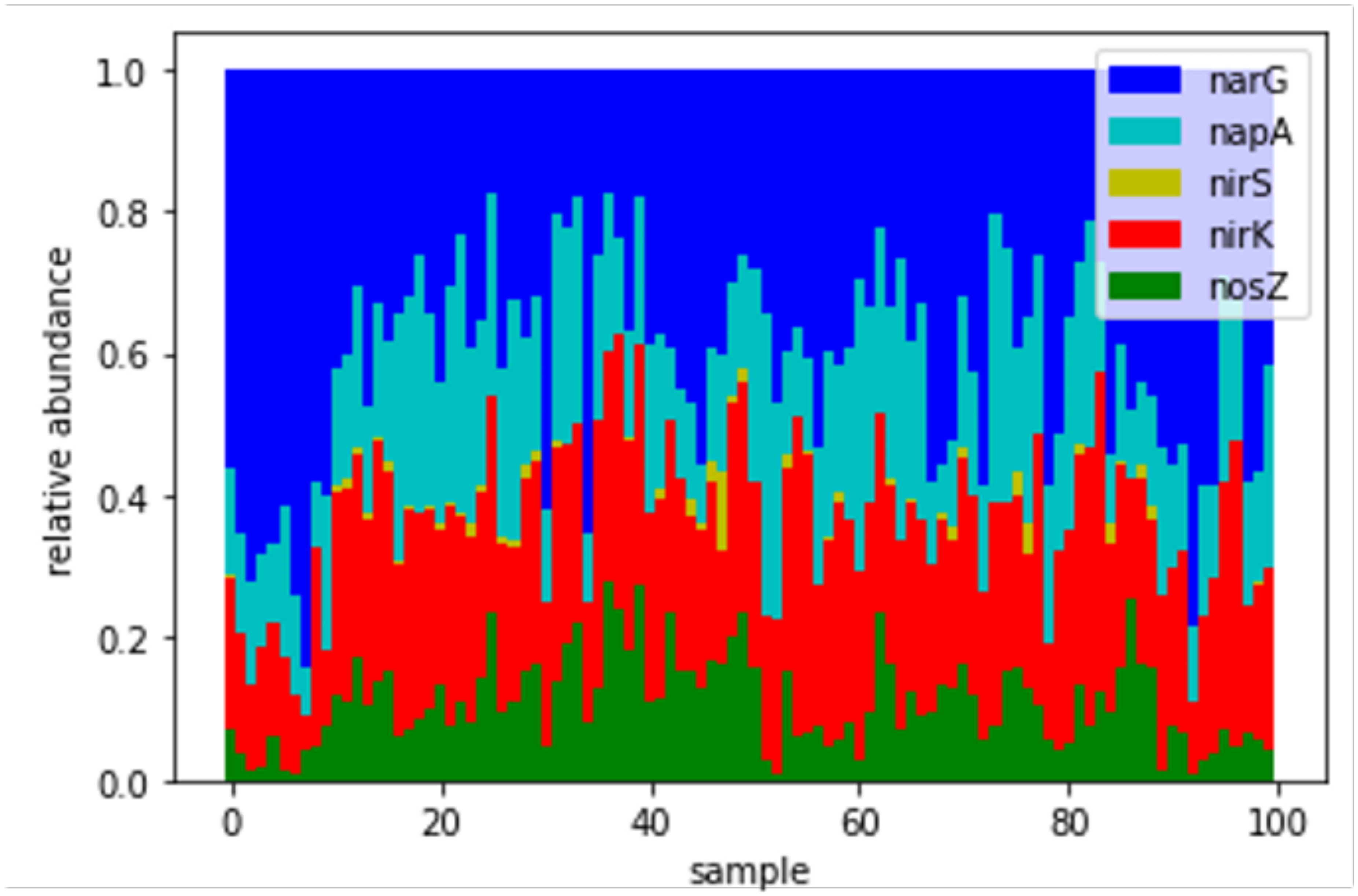




Slow/low yield nitrite reductase

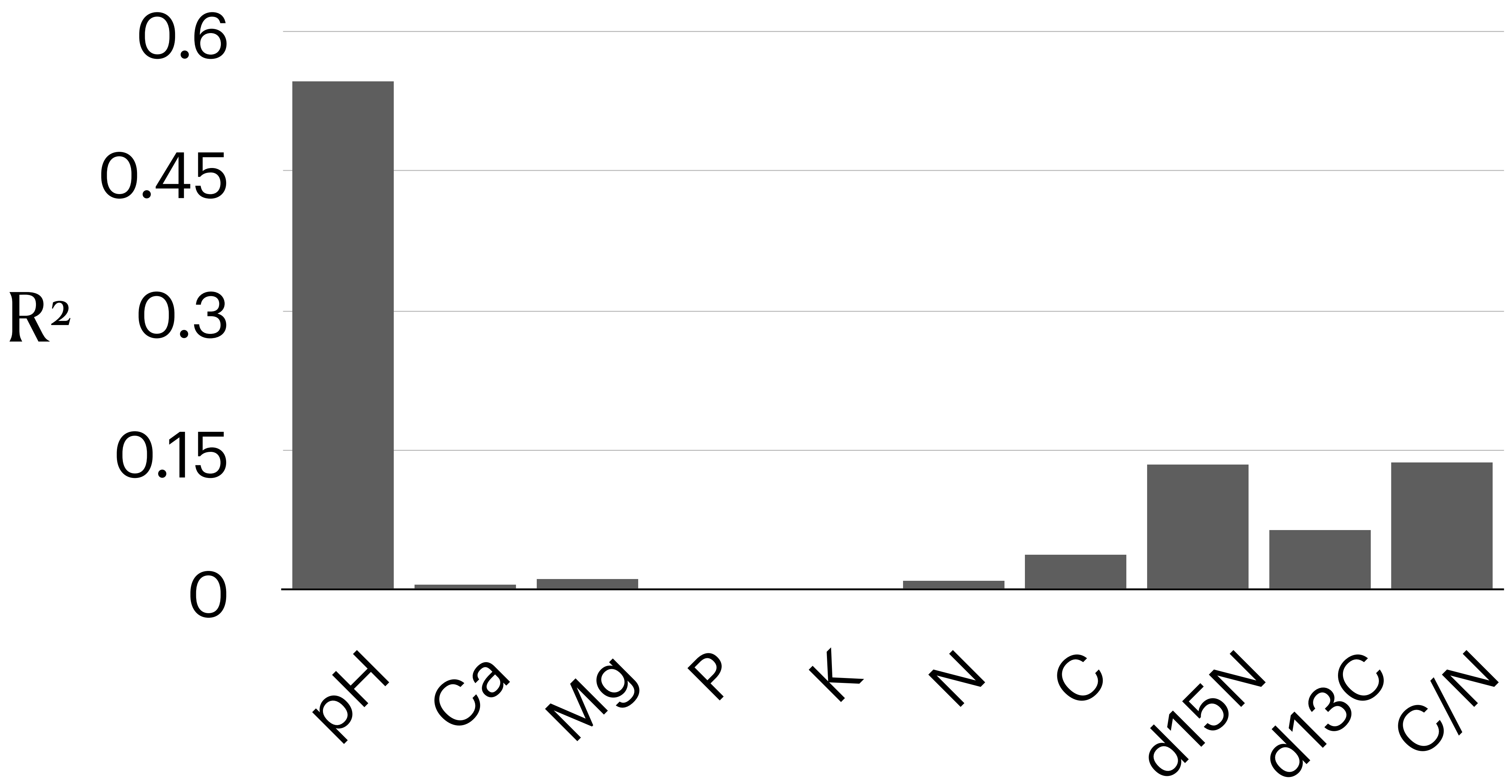



(nitrite specialist)

# pH best predictor of variation in pathway structure

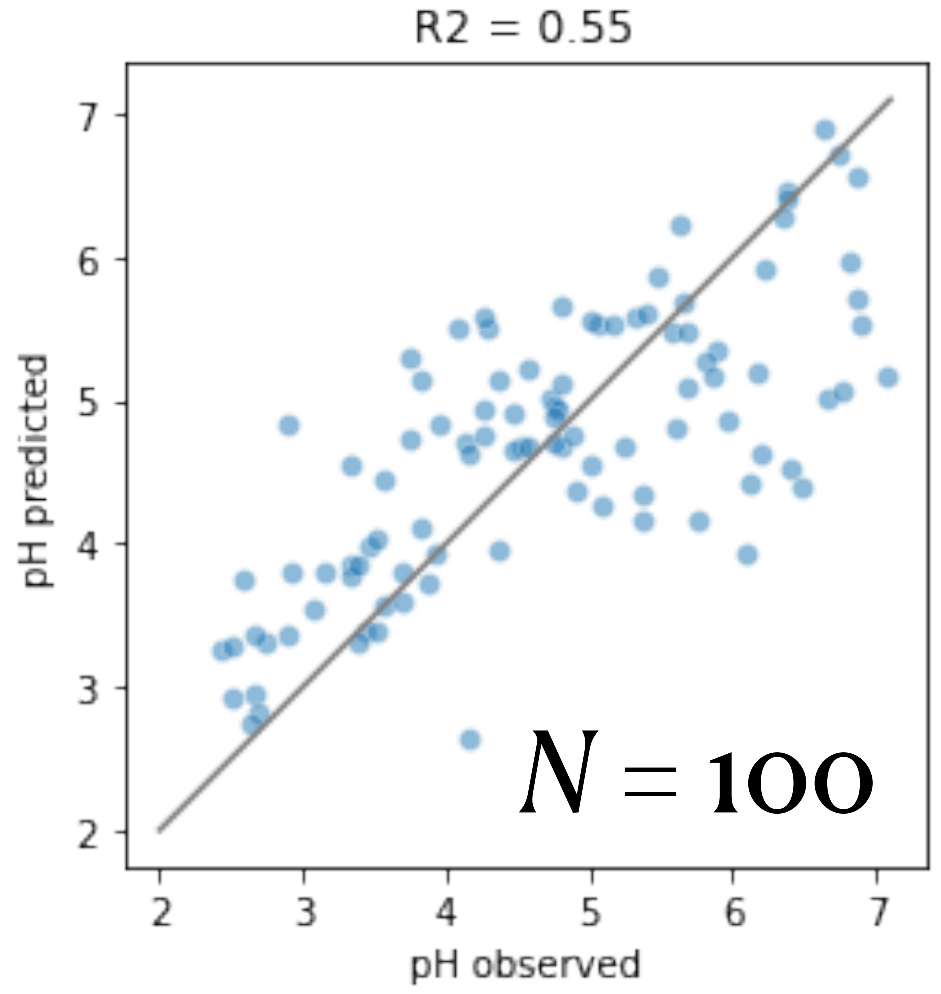



$$\vec{x} = w_1 \vec{h}_1 + w_2 \vec{h}_2$$

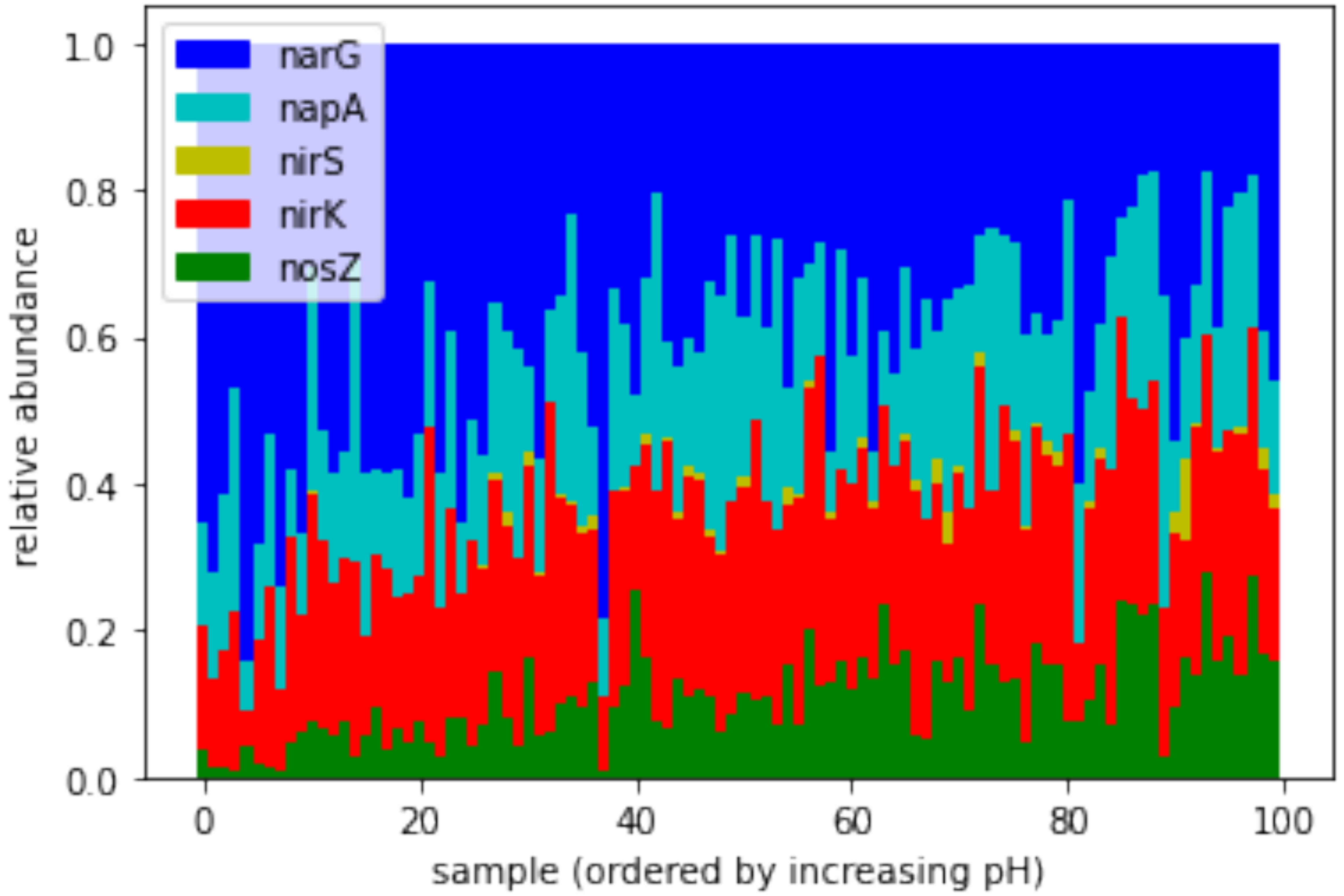



$$\mathrm{pH}^i = \beta_0 + \beta_1 w_1^i + \beta_2 w_2^i$$

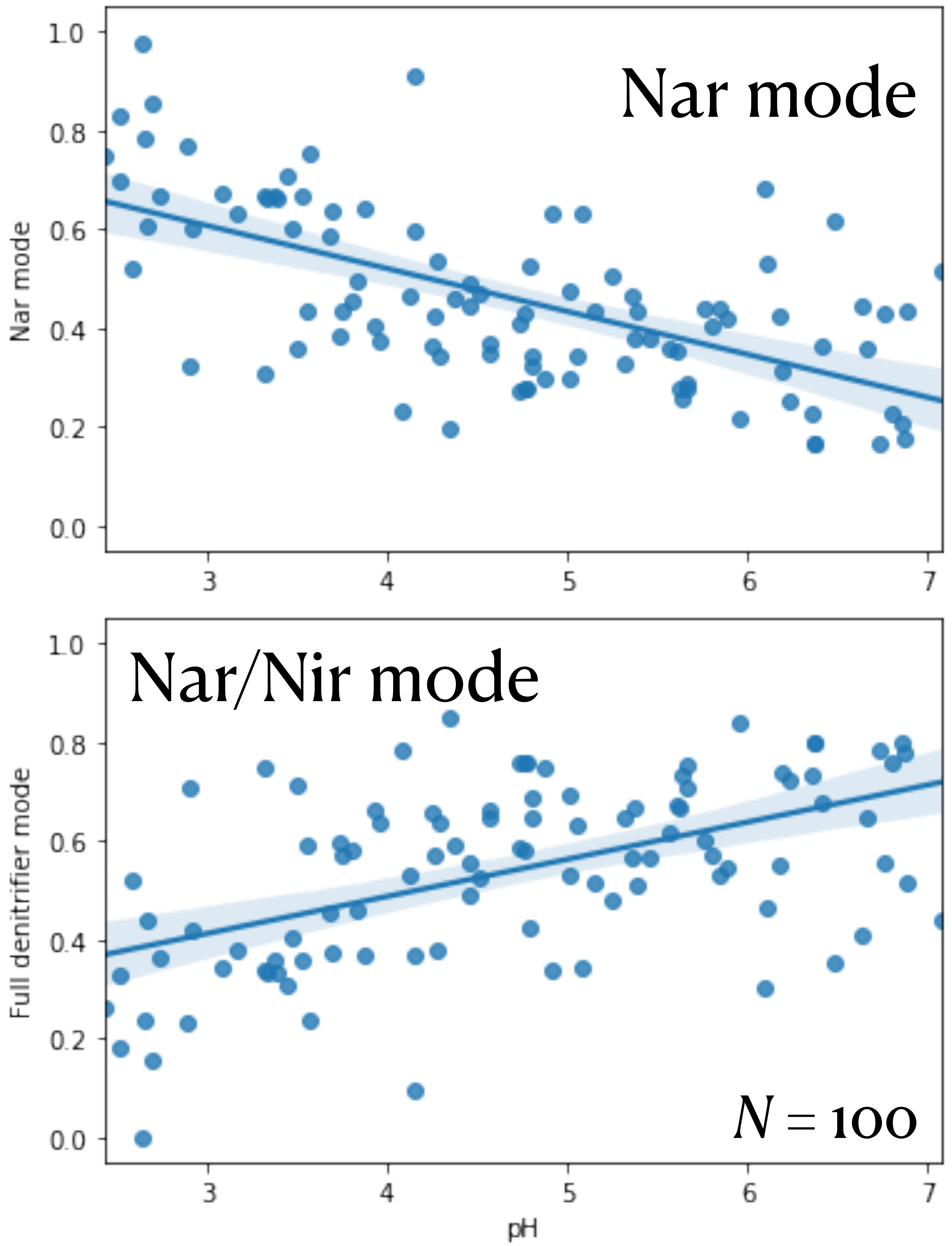
Predict each environmental variable, *E*, from weights *W* (OLS)



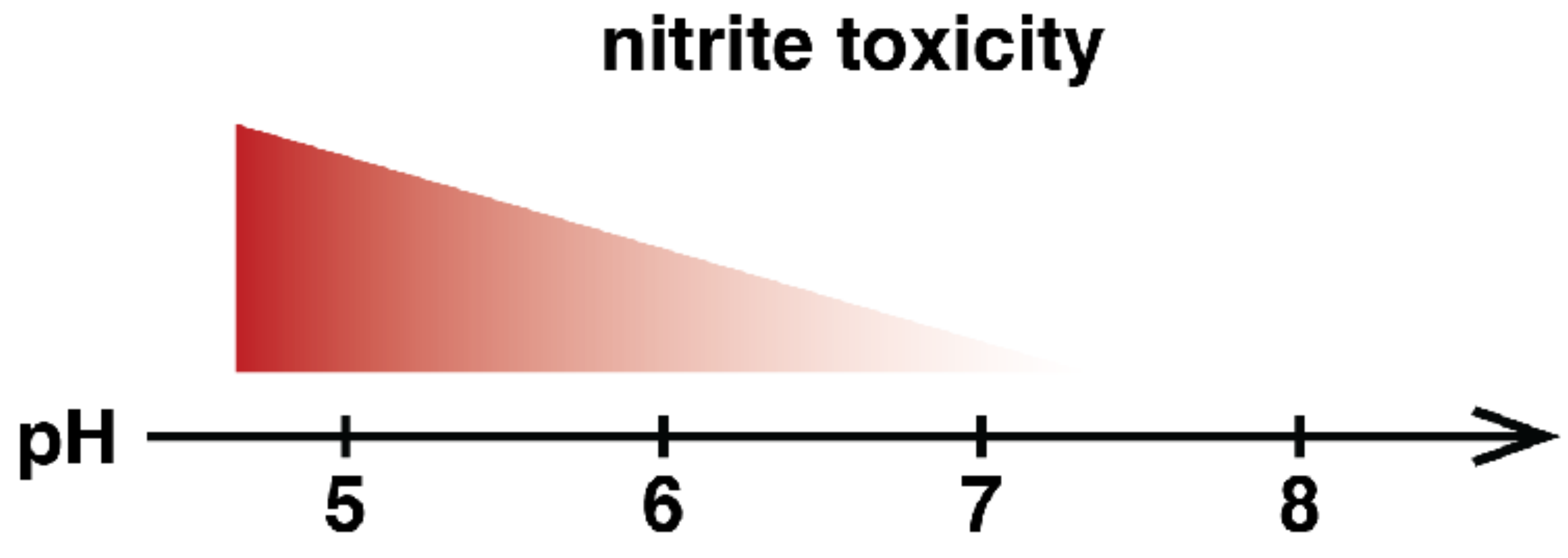
# pH controls which mode dominates



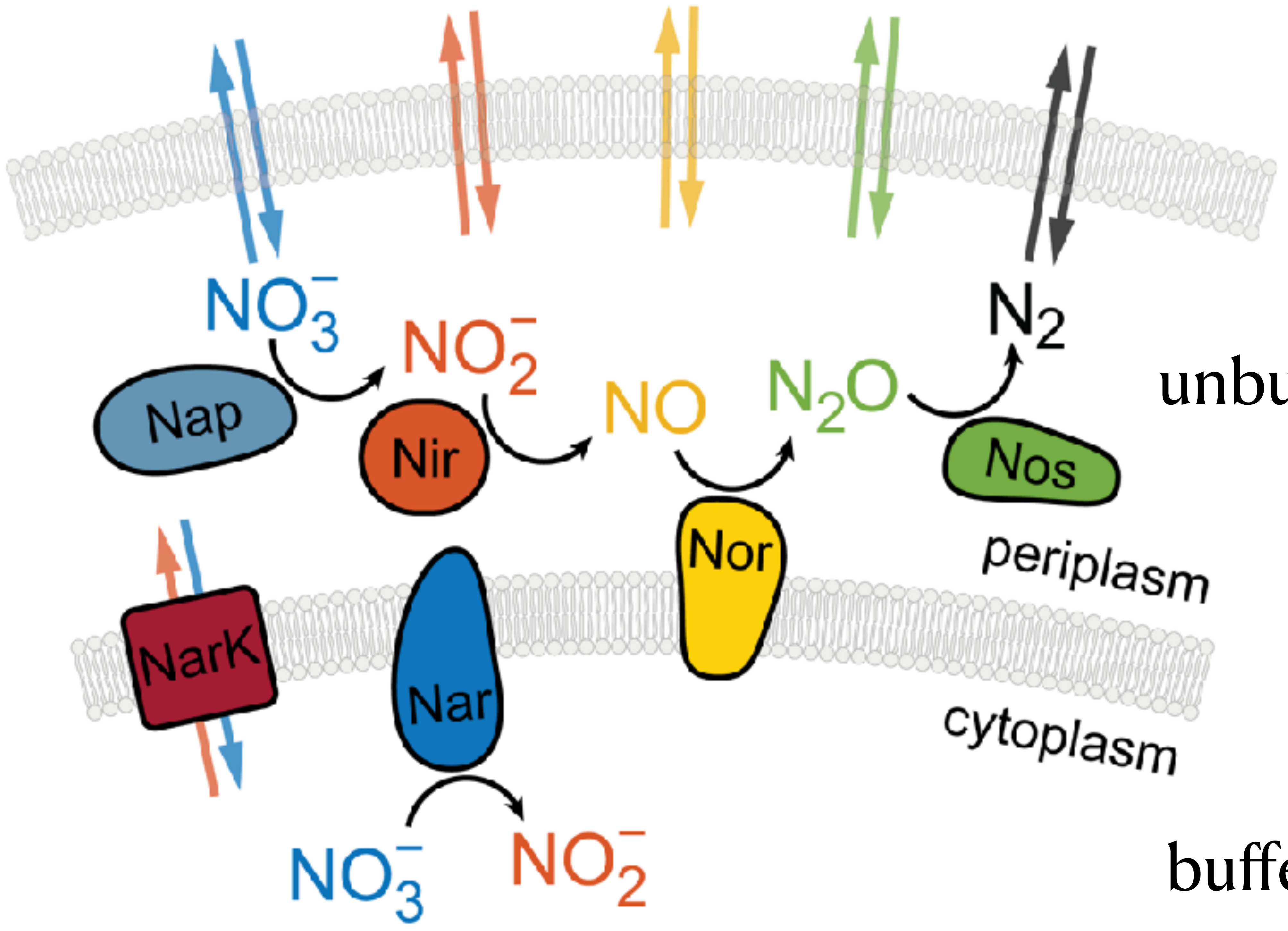

pH →



# Potential mechanism: pH affects nitrite toxicity




spontaneous protonation to
nitrous acid, nitric oxide
affects enzyme metal centers

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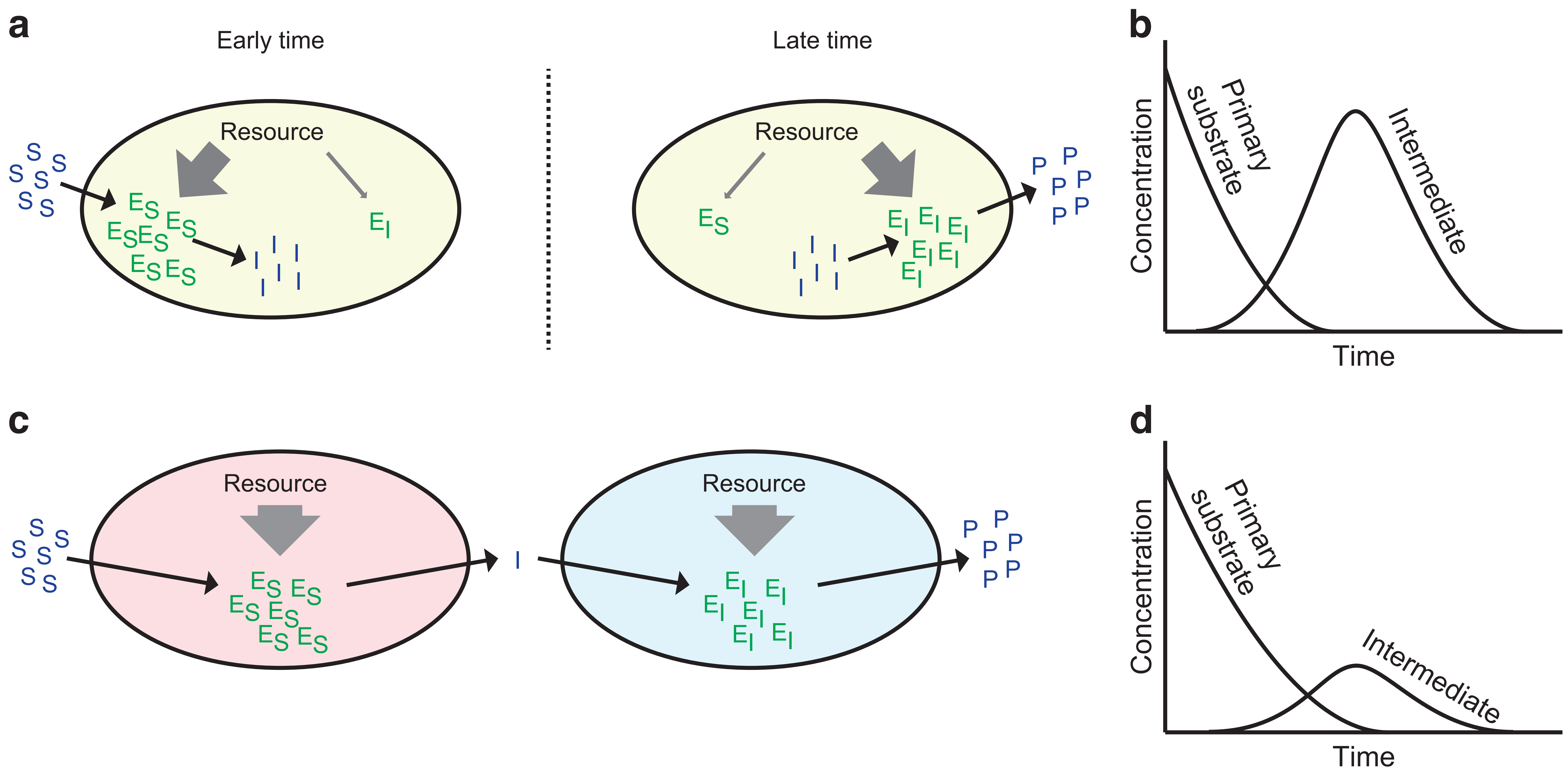
*see for ex. Wilks & Slonczewski, 2020

# Pathway splitting a possible solution to nitrite toxicity



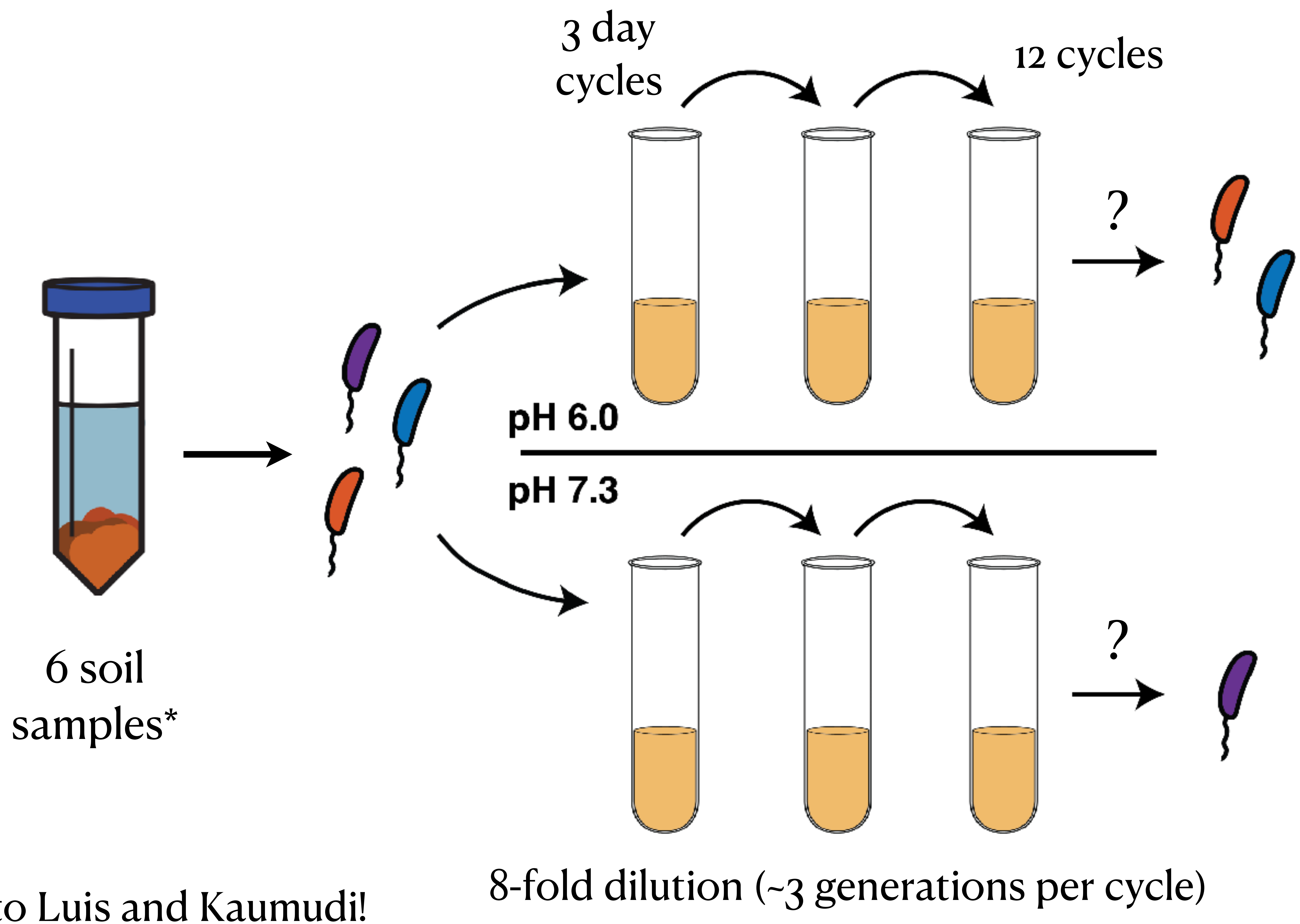

Splitting the pathway reduces intracellular resource competition

→

reduces accumulation of nitrite

Segregating metabolic processes into different microbial cells accelerates the consumption of inhibitory substrates, Lilja & Johnson, 2016

# Can we select for pathway splitting in an enrichment experiment?



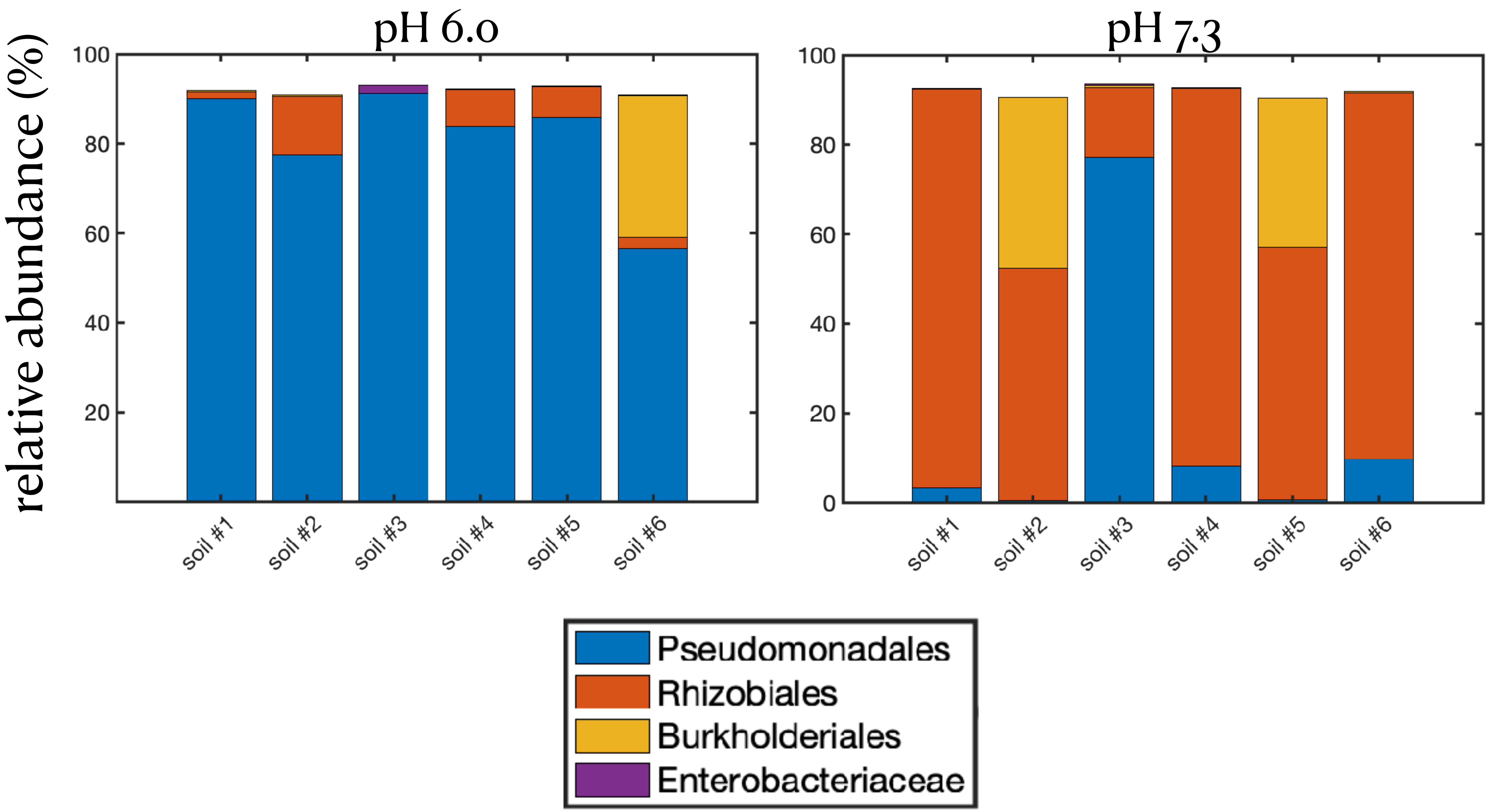

infer genotypes and relative abundances via metagenomics and binning

Do split pathways outcompete the full pathway at lower pH?

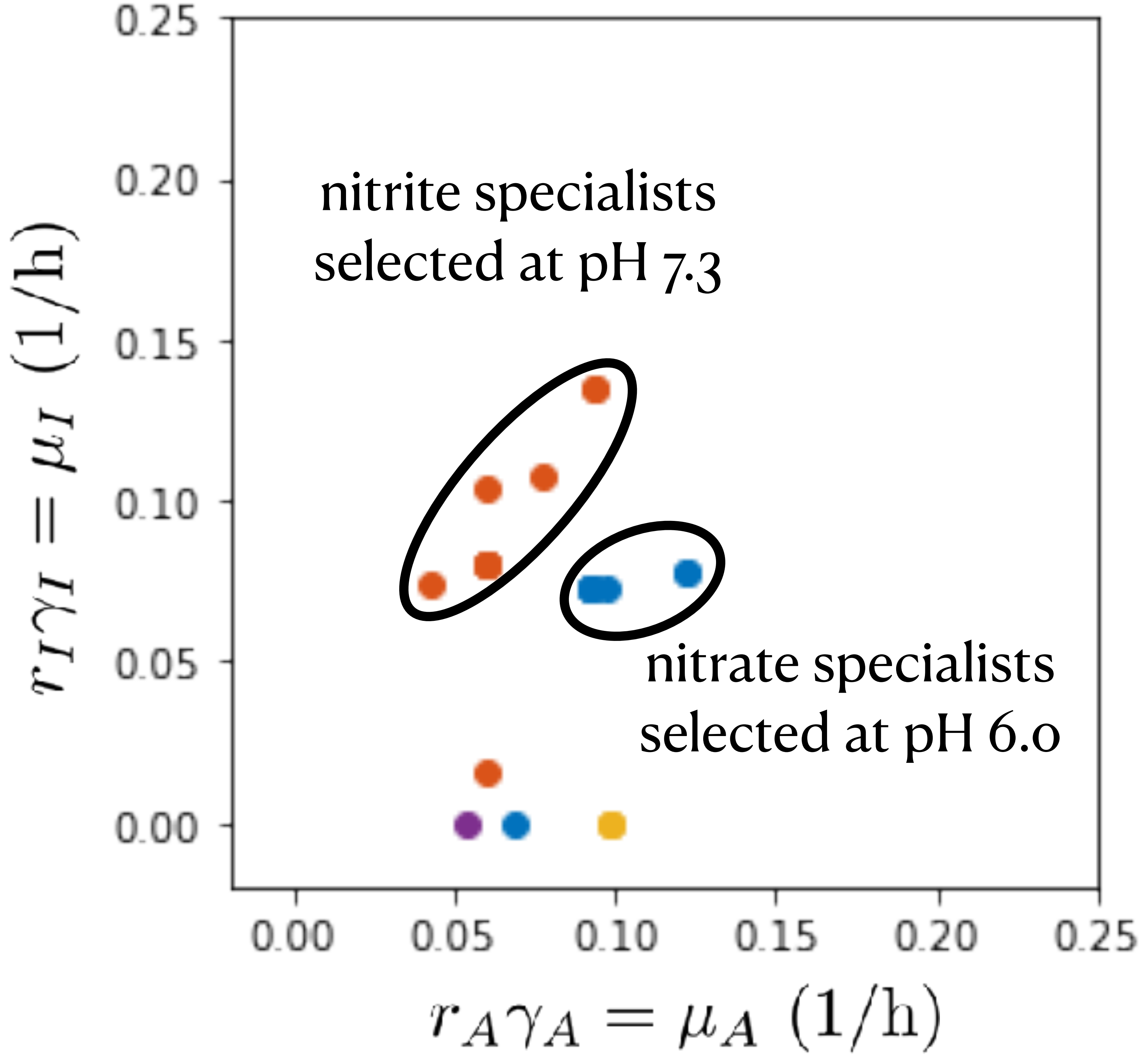
*Thanks to Luis and Kaumudi!

# Enrichments select for full pathways, but pH selects different specializations

Communities at cycle 12




Phenotypes at pH 7.3
**predicted* from MAGs**




*Gowda et al. 2021

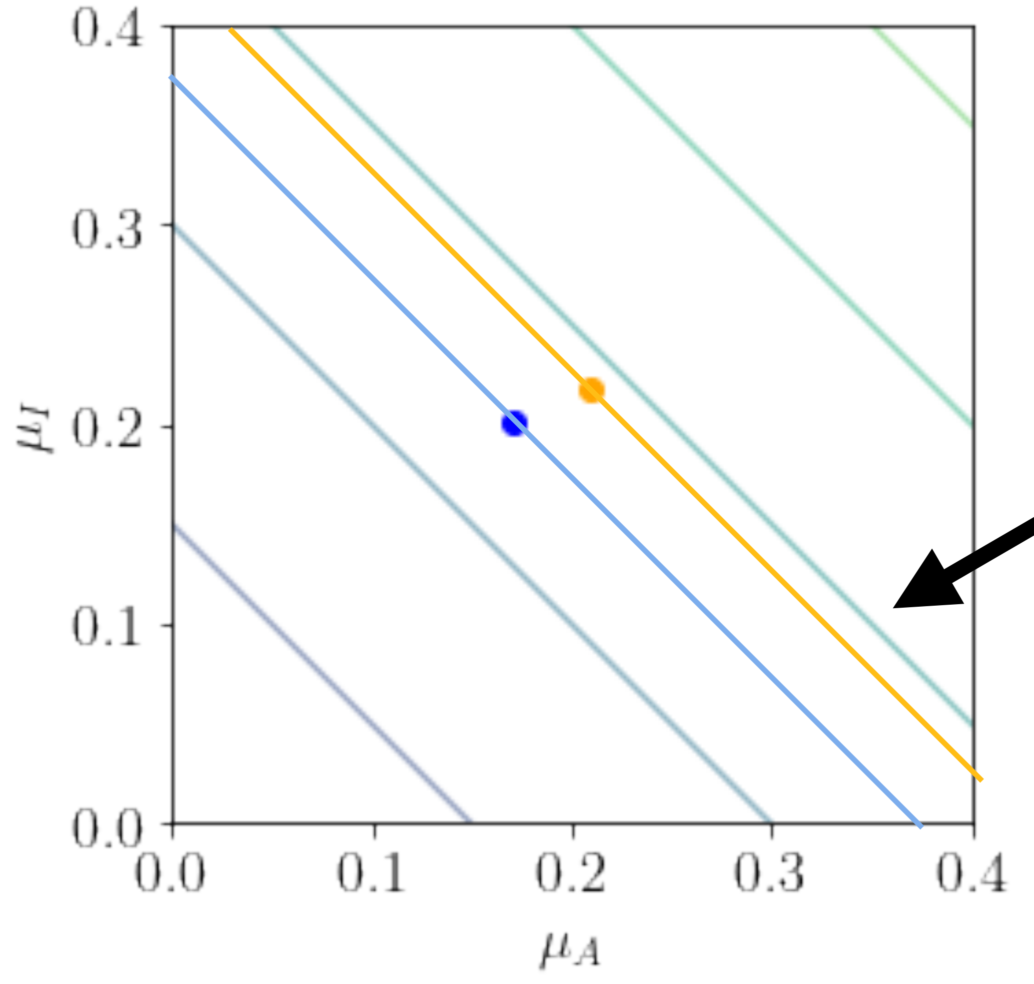
# Approximating fitness in the enrichment experiment

$$\frac{dx}{dt} = \left(\gamma_A r_A \frac{A}{K_A + A} + \gamma_I r_I \frac{I}{K_I + I}\right) x$$

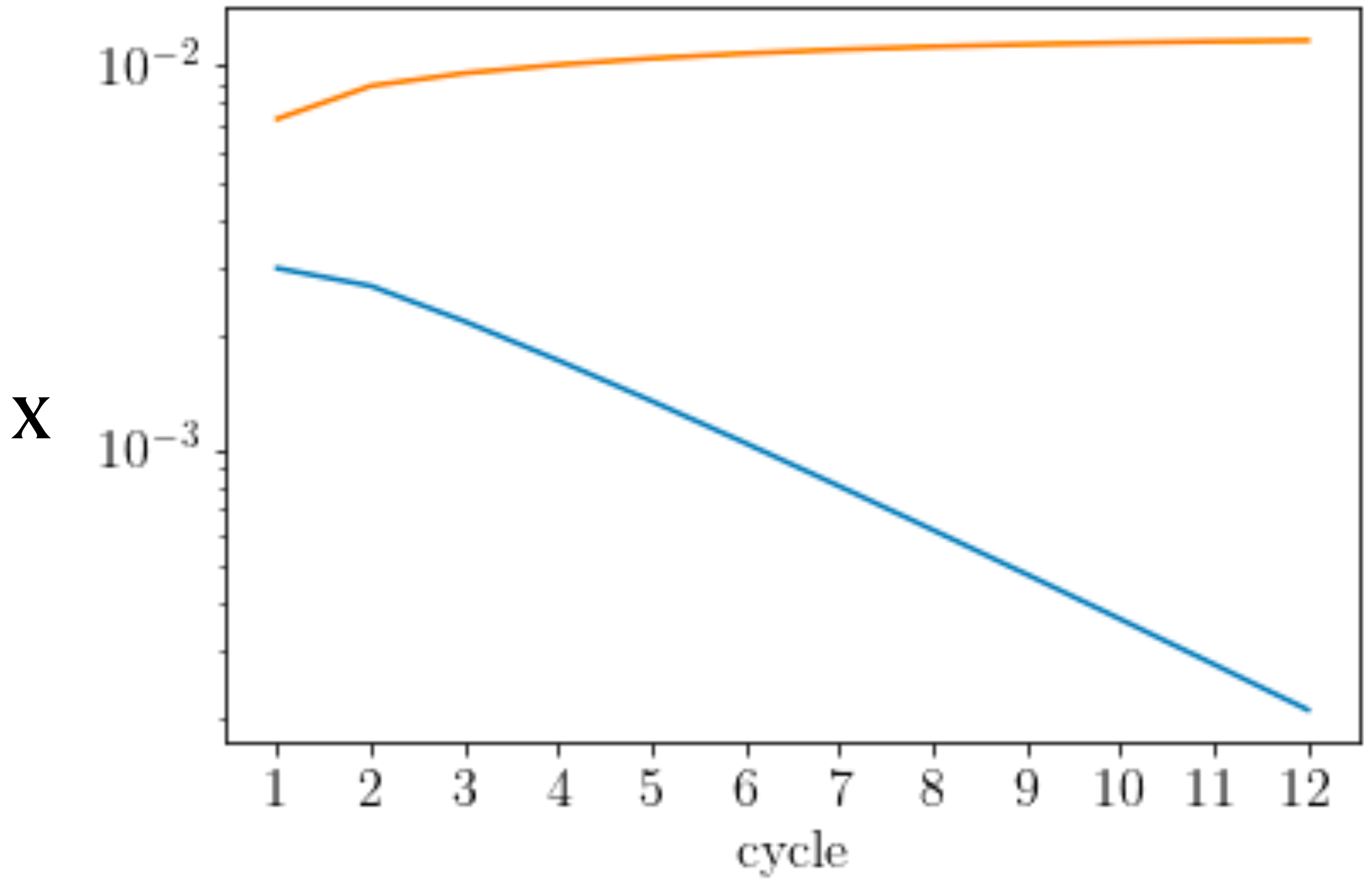
$$A \gg K_A, I \gg K_I$$

$$= (\gamma_A r_A + \gamma_I r_I)x = (\mu_A + \mu_I)x$$

x - biomass density
A - nitrate concentration
I - nitrite concentration

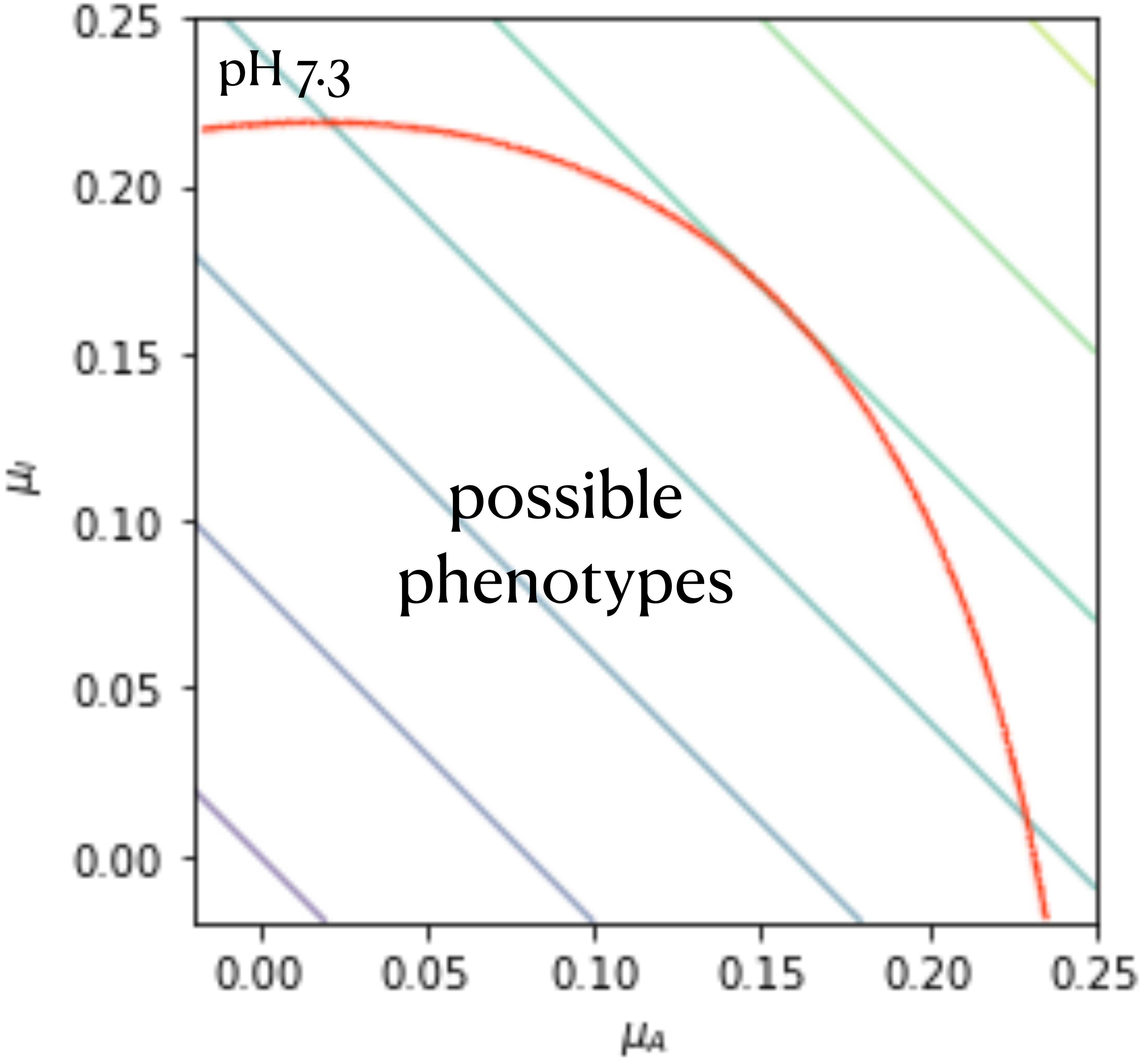



$$\mu_A + \mu_I = c$$

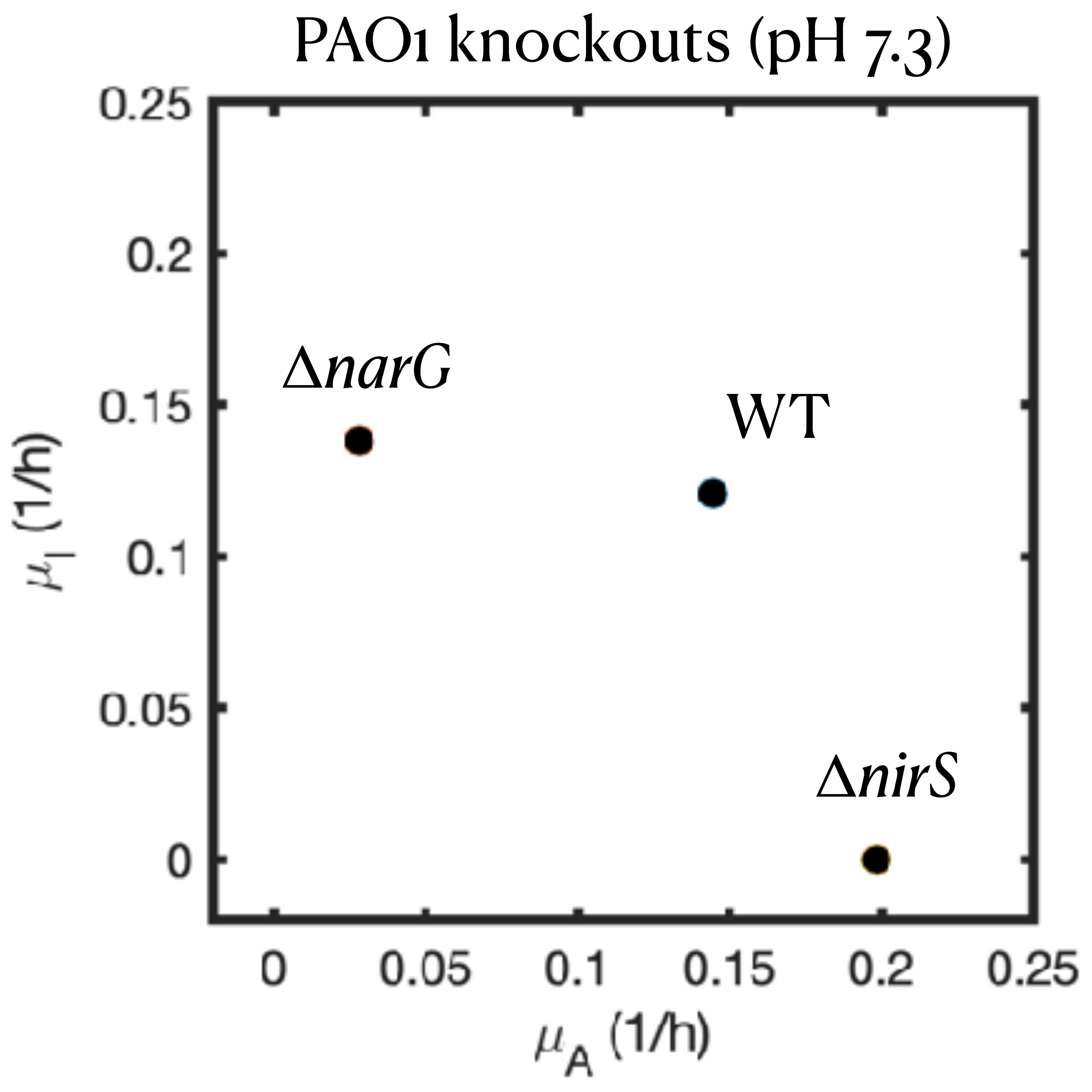



simulating full equations for 12 cycles

# Proposal for a theory

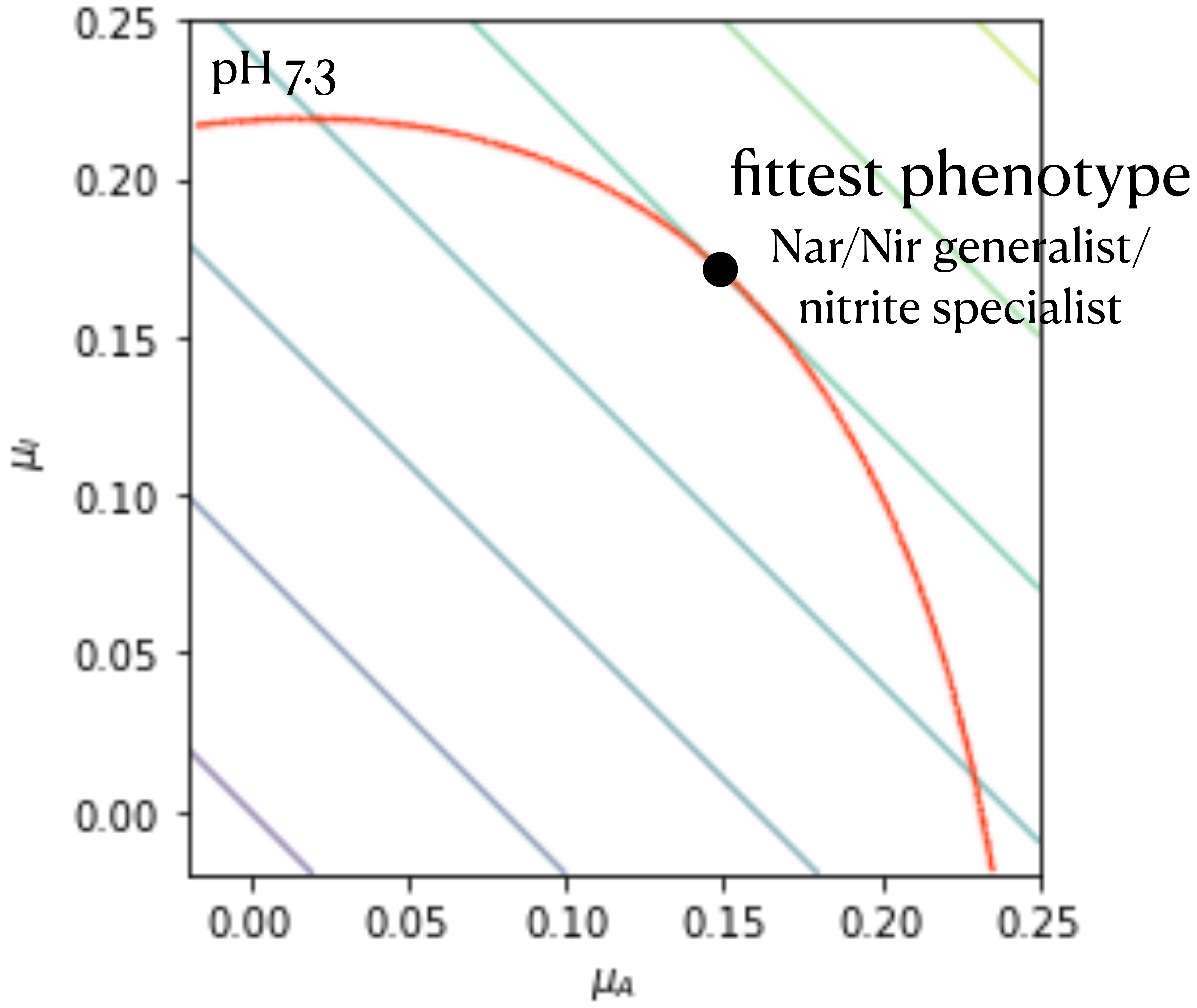



1. Phenotypes are constrained by pH-specific tradeoff curve



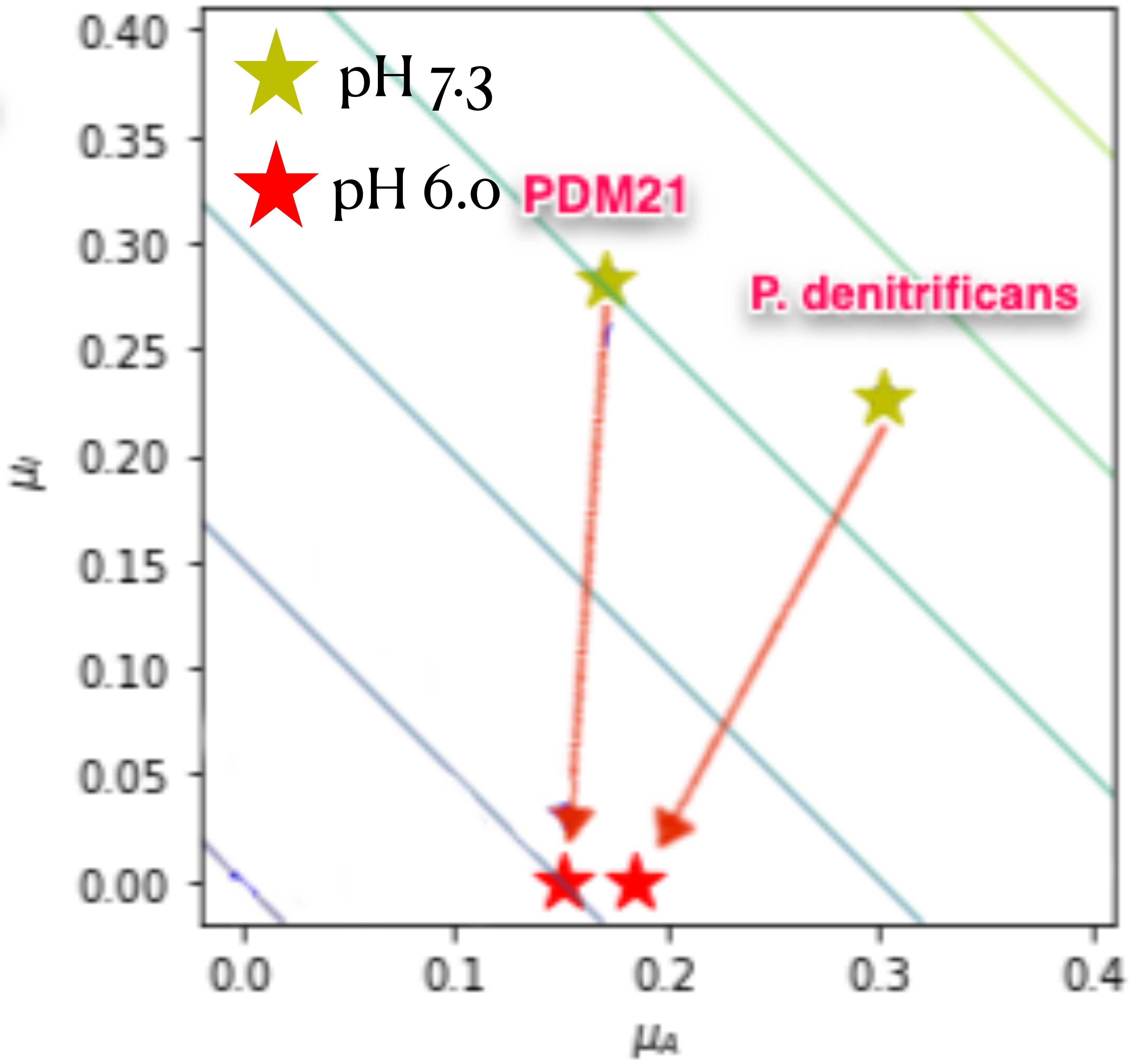

Competition for intracellular resources. See also Johnson et al., 2012; Lilja & Johnson, 2016.

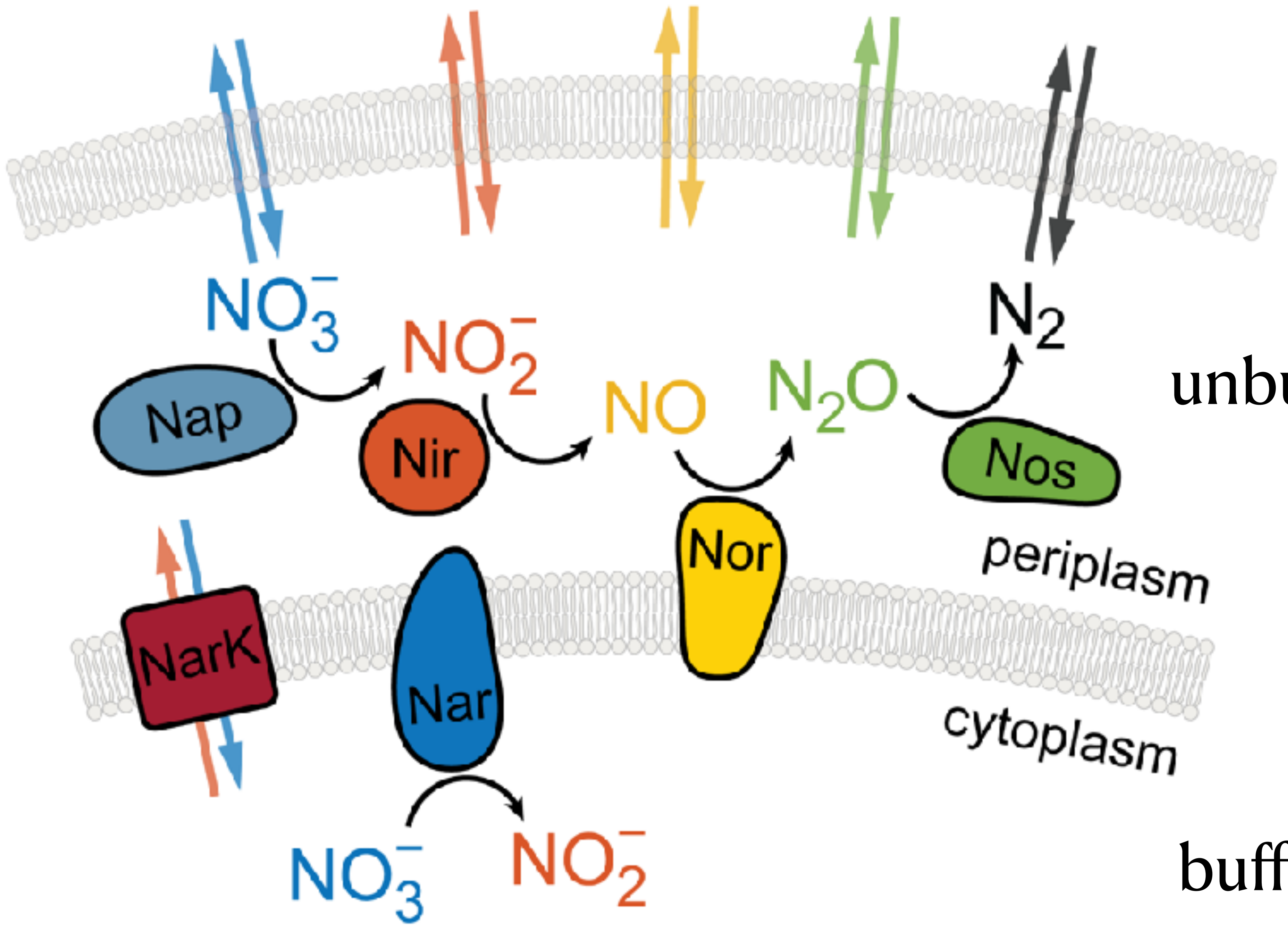
# Proposal for a theory




1. Phenotypes are constrained by pH-specific tradeoff curve
2. The curve is concave down at neutral pH

# How might pH impact the tradeoff curves?

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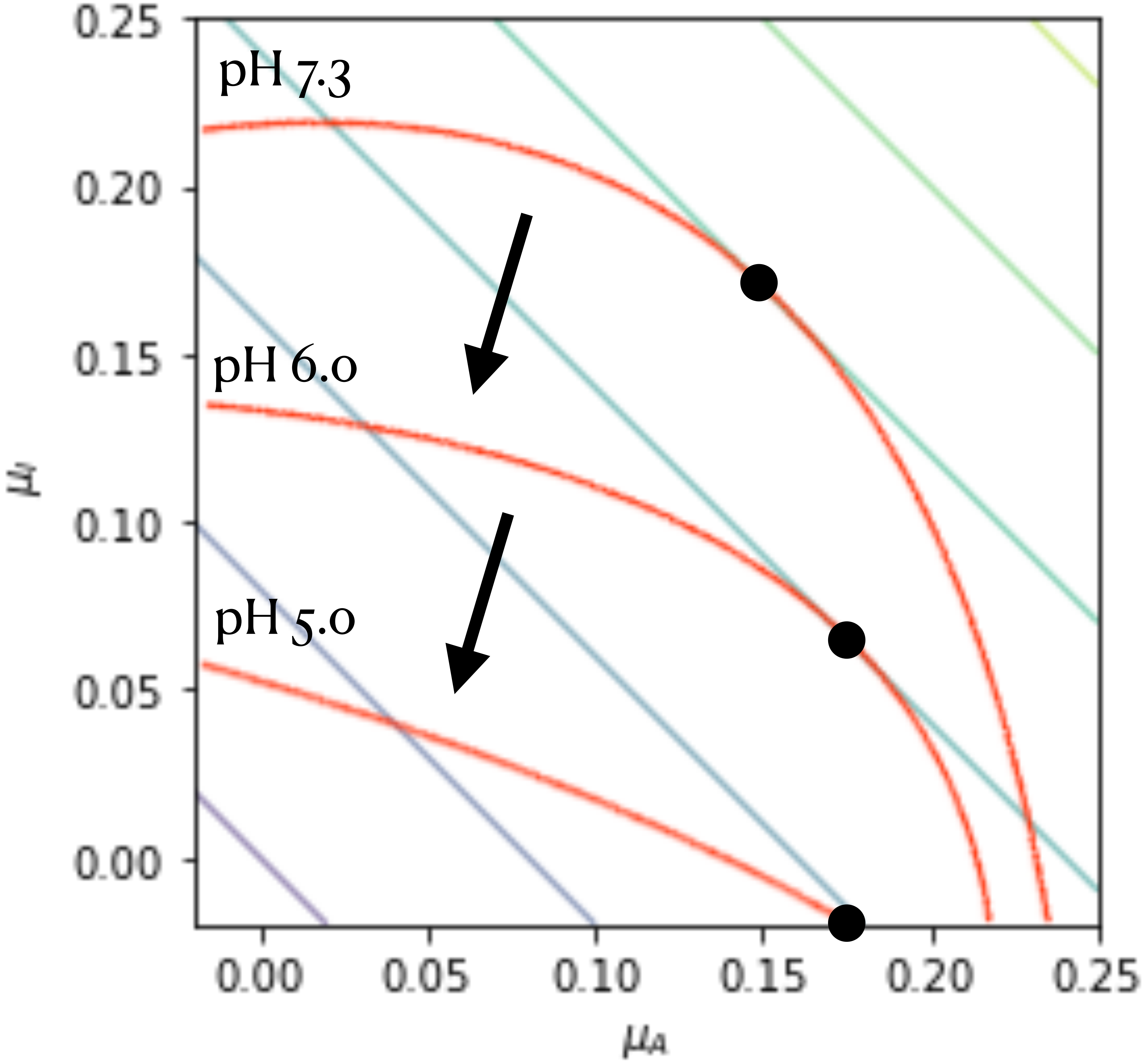
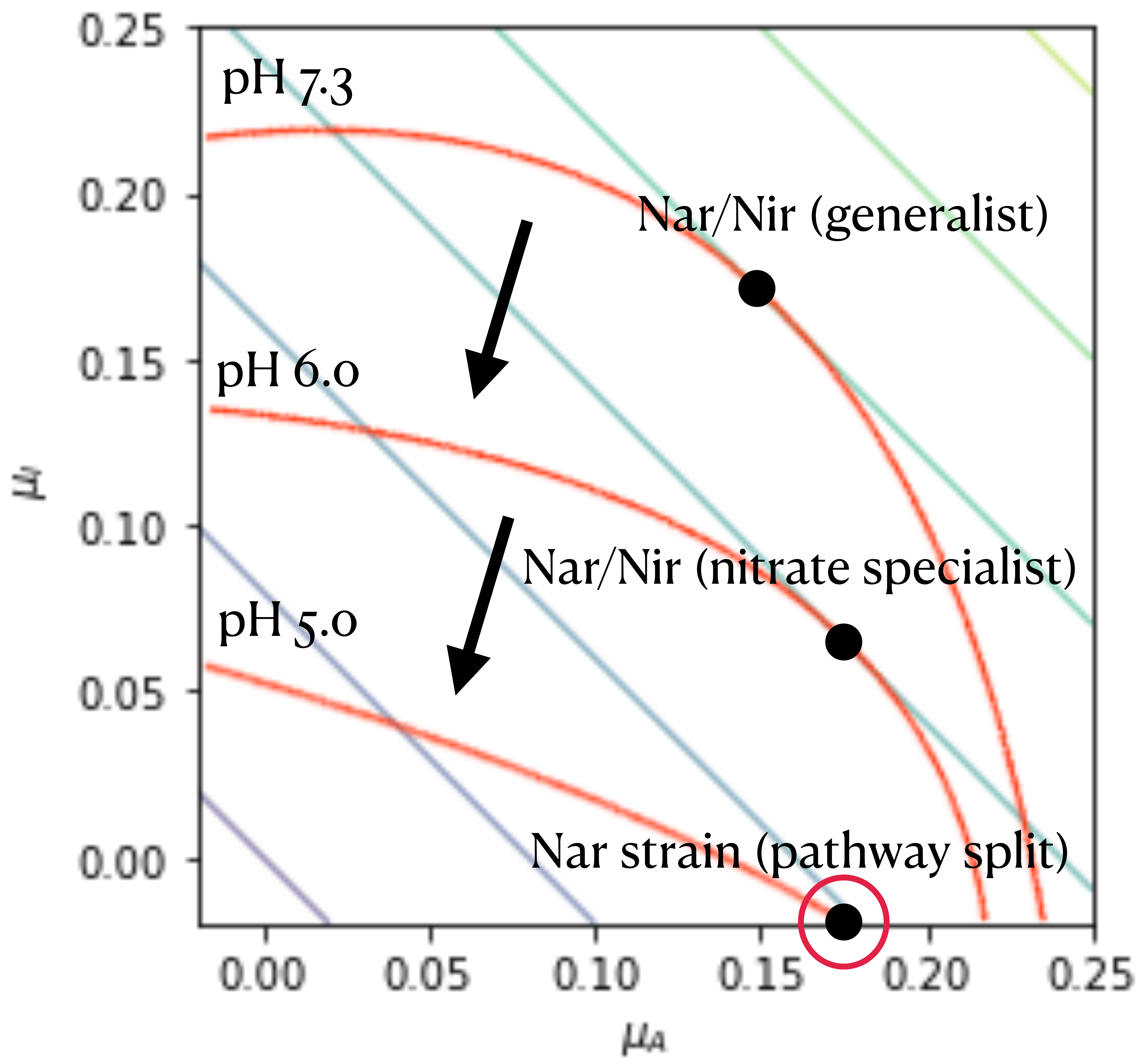
# Proposal for a theory




1. Phenotypes are constrained by pH-specific tradeoff curve
2. The curve is concave down at neutral pH
3. The curve is deformed asymmetrically as pH decreases

Assumption: nitrite toxicity harms $\mu_I$ more than $\mu_A$

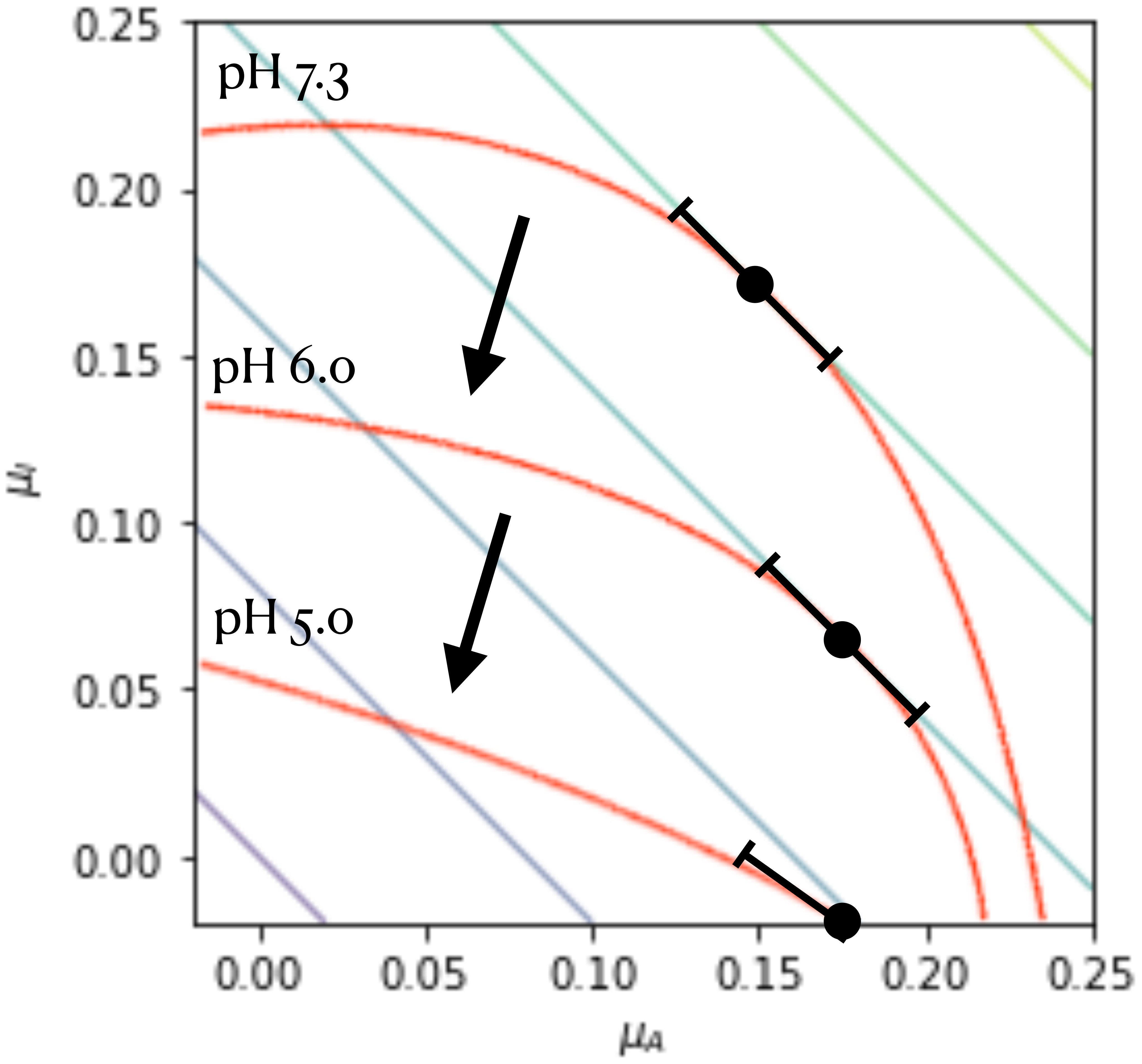
# Proposal for a theory




1. Phenotypes are constrained by pH-specific tradeoff curve
2. The curve is concave down at neutral pH
3. The curve is deformed asymmetrically as pH decreases

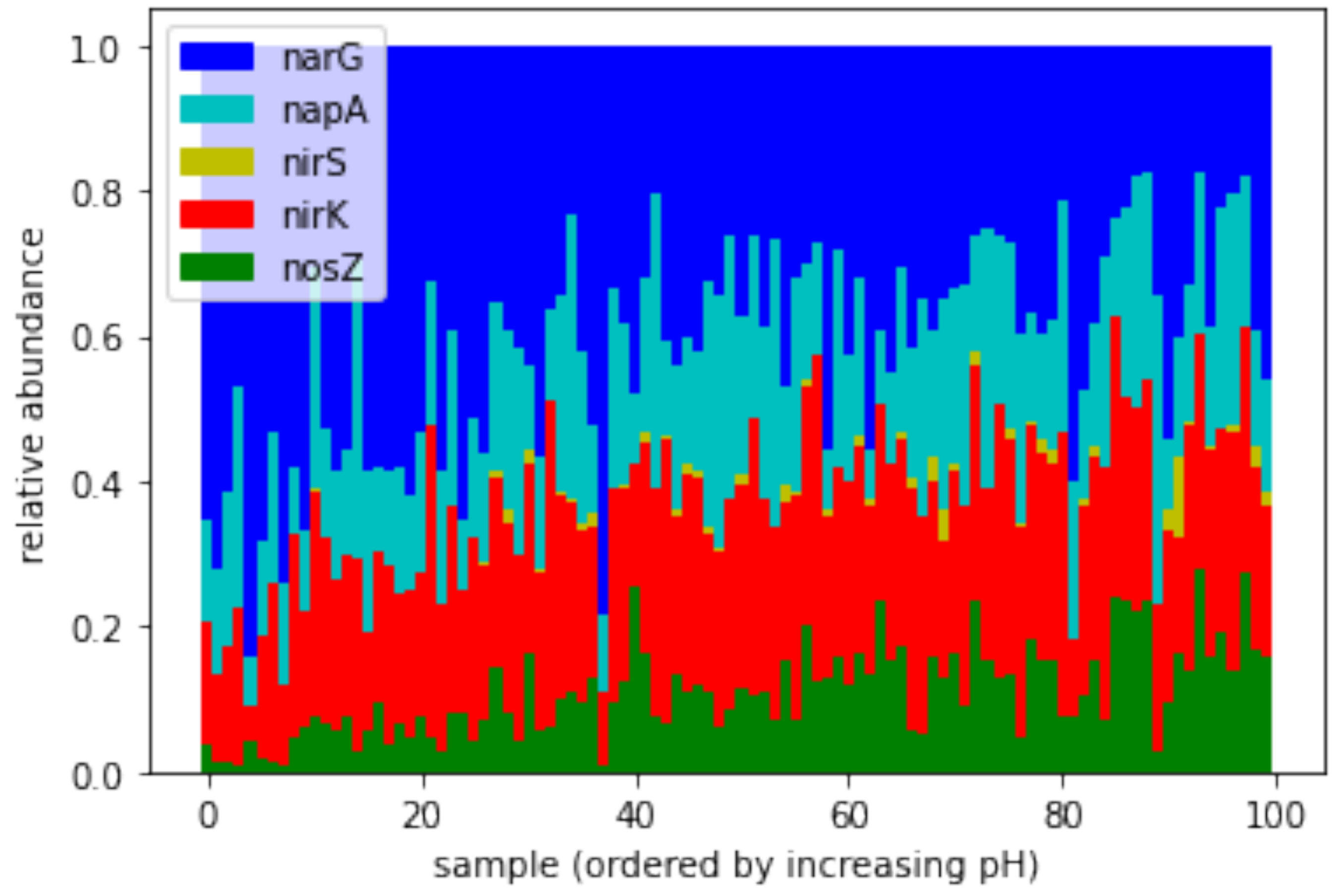
Pathway splitting via asymmetric tradeoff

# Next steps

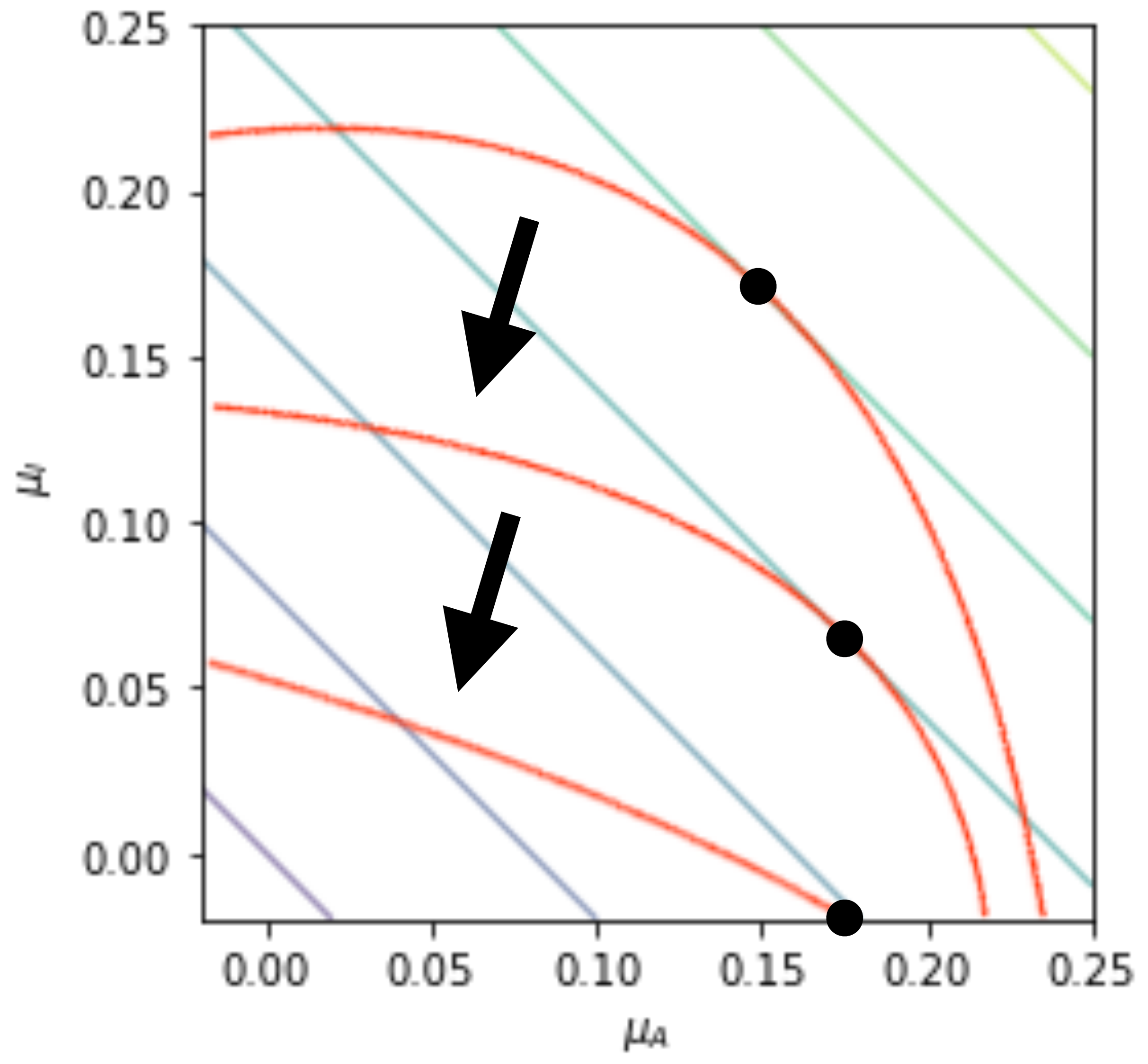



- Enrichments reveal “fittest points” on tradeoff curve.
- Perform enrichment experiments at lower pH levels
- Isolate/phenotype strains
- Locally characterize tradeoff curves

Kyle Crocker




pH →




# Summary

- pH drives variation in denitrification metagenome structure consistent with pathway splitting
- Possible mechanism: nitrite toxicity increases as pH decreases from neutral
- Proposed theory: pathway splitting occurs via asymmetric tradeoff induced by toxicity

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Kyle Crocker
Postdoc
U. Chicago

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