Modeling the diversity of interactions of *Prochlorococcus* (cyanobacteria) and Heterotrophs from the library

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# Introduction

*Prochlorococcus* in the lab exhibit a range of interaction phenotypes when co-cultured with diverse heterotrophic bacteria. Interactions range from synergistic to antagonistic interaction. When co-cultured with some strains, *Prochlorococcus* can survive under Nitrogen starvation stress, exhibiting positive interaction. Other strains inhibit *Prochlorococcus* growth entirely, resulting in no growth or in lower fluorescence.

Specifically, in the case of *Alteromonas*, both partners exhibit higher cell density (orders of magnitude higher), and *Prochlorococcus* can revive when transferred to fresh media (which it cannot do when grown axenically).

# Growth Phenotypes of *Prochlorococcus*

I have co-cultured *Prochlorococcus* med4 with 9 different bacteria strains for 129 days under nitrogen stress conditions (pro99 low N). Viability of the co-cultures was checked by transferring to a fresh media on days 42, 60, 81, 129. The resulting growth curves are clustered into 4 different interaction phenotypes.

*Prochlorococcus* growth was measured by auto fluorescence, a proxy for *Prochlorococcus* cell numbers during exponential growth and decline (but not around peak growth). Flow cytometry was run on the inoculated culture on days 20, 42, 60, 81, 129. The viability of the heterotrophs cultures was measured by MPN on day 129 of the inoculated culture.

Heterotroph Strains used are listed in the table below:

| Group | Name | Class | Reason Selected | Initial conc. cells/ml | MPN on day 120 cells/ml |
| --- | --- | --- | --- | --- | --- |
| Sustained | *Alteromonas macleodii HOT1A3* | γ proteo bacteria | Synergistic interaction. Inhibits some *Prochlorococcus* strains in a dose dependent manner | 1e6 | 5e7-5e10 |
| Sustained | *Pseudoalteromonas haloplanktis CIP 108707* | γ proteo bacteria | Cold-adapted, arctic, FBA model, chemotaxis to *Proch*. extracellular products. | 1e7 | 5e6-5e7 |
| Strong | *Ruegeria pomeroyi DSS-3* | α proteo bacteria | important model organism (e.g. DMSP degradation, mutation rate, quorum sensing), genetically tractable. Survives long term starvation in co-culture with Synechococcus. detoxifying DOM | 1e7 | 0 |
| Strong | *Sulfitobacter pseudonitzschiae SMR1* | α proteo bacteria | Associated with S.marinoi, pacbio genome, exclusive. Diatom attached, produces IAA and exchanges organosulfur compounds and ammonia with Diatom. Quorum sensing | 1e7 | 5e5-5e6 |
| Inhibited Viable | *Escherichia coli k-12* | γ proteo bacteria | Model organism | 1e6 | 0 |
| Inhibited Viable | *Marinovum HOT5\_F3* | TBD | Positive interaction in Sher, ISME 2011 | 1e7 | 5e4 |
| Inhibited | *Roseovarius HOT5\_C3* | TBD | Positive interaction in Sher, ISME 2011 | 1e7 | 5e2 |
| Inhibited | *Marinobacter adhaerens HP15* | γ proteo bacteria | Affects diatom aggregation and growth in co-culture | 1e7 | 5e6-5e7 |
| Inhibited | *Phaeobacter gallaeciensis* | α proteo bacteria | Remarkable surface (abiotic/biotic) colonizer, kill diatoms and other bacteria. Promotes growth than kills *Emiliania huxleyi* | 2.5e6 | 5e7-5e10 |

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| Figure 1: Growth phenotypes of 4 groups of heterotrophs. during initial inoculation and 4 subsequent transfers. Each line represents a replicate. Bold lines indicate group mean growth. |

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| Cells numbers of Prochlorococcus MED4 and heterotroph bacteria in the co-cultures. X – med4 cells, O – Heterotrophs. Small symbols indicate non-viable cultures, i.e., med4 did not grow when transferred to fresh media. Weak shaded line – culture fluorescence. |

# Mechanistic Modeling of the Interaction

Next, I set out to model the interactions between the phototrophs (*Prochlorococcus*) and the heterotrophs.

My goal is to model the mechanisms that may be involved in the interaction. If a given phenotype matches the simulations, we can hypothesize that similar mechanisms may occur in real life. If the model cannot be fitted to a given phenotype, it is likely that additional mechanisms are involved.

I elected to model the following mechanisms:

* **Nutrient exchange** – additional nutrients are available from exudates and recycled dead cells from the other species. *Prochlorococcus* has been shown to release organic matter (DOC, DON), fixed by photosynthesis. Heterotrophs may release inorganic DIN (e.g., NH4) and DIC. I modeled several mechanisms of nutrient exchange:
  + **Recycling**: dead cells are recycled as organic C and N
  + **Exudation**: a.k.a. “property tax”. A fixed portion of the biomass is released to the media. *Prochlorococcus* releases organic nutrients (DOC, DON). The heterotroph releases inorganic nutrients (DIC, DIN).
  + **Overflow**: Both species uptake the highest nutrient amount possible. However, to maintain stoichiometric ratios, the overflow uptake of the nutrient is released directly into the media. Again, *Prochlorococcus* releases organic nutrients (DOC, DON), while the heterotroph releases inorganic nutrients (DIC, DIN).
* **Competition**: Both strains compete for NH4 .
* **Mixotrophy**: By default, *Prochlorococcus* rely on inorganic nutrients. The model allows for additional consumption of organic nutrients to supplement.
* **Detoxification**: *Prochlorococcus* may release products that are toxic (e.g., ROS and excess DOC). The heterotroph may be able to uptake and break down these toxins.
* **Infochemicals/Signals**: Both strains may release infochemicals, secondary metabolites with no known major function in primary metabolism. These metabolites may have positive, neutral, or negative impact on the growth rate of the other organism in the co-culture.
* **Refractory**: only a subset of released biomass may be available for consumption.
* **Cell maintenance:** (e.g., breathing) requires energy and biomass. currently neglected.

Additional processes which are not implemented in the model:

* **Adaptation**: the heterotroph partner may enable *Prochlorococcus* to survive long enough to shift its metabolism to starvation mode
* **Density dependent release of infochemicals:** e.g., quorum sensing. turning on/off allelopathy based on cell density.
* **Stores of nutrients within the organism (quota)**

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| inhibition  uptake  overflow  death  DIN  DOC  DON  ROS  uptake  uptake  Exudation/Respiration  death  breakdown  release  exudation  DIC  uptake  CO2  overflow  overflow  SignalH  release  release  Promote/inhibit death  Promote/inhibit death  Exudation/respiration  SignalP  Respiration  Respiration |
| Figure 2: The Model |

# The Model

The model is based on Monod kinetics. It includes the following variables:

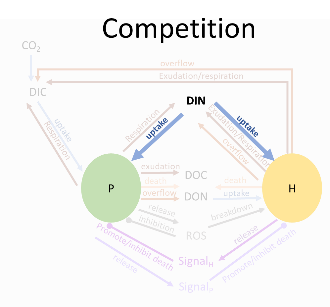
* There are 2 bacterial populations, **BP** (*Prochlorococcus*) and **BH** (heterotroph)
* nutrient pools: **DOC**, **DIC**, **DON**, **DIN**, **RDON, RDOC**
* **ROS**, produced by *Prochlorococcus*, which inhibits *Prochlorococcus* growth and which the heterotroph may break down, **ROS** inhibits *Prochlorococcus* growth.
* Two metabolites, **Sp** and **Sh**, produced by the *Prochlorococcus* and the heterotrophs respectively. These can either enhance or reduce the death rate of the other population.

The model is compared qualitatively to the growth phenotypes. We do not have sufficient information to attempt a quantitative fit. For example, we did not measure nutrients levels during the experiment. We do have measurements from other experiments that allow for ballpark estimates of concentrations and parameters.

Each population has a fixed stoichiometry (assigned a specific C/N ratio) and a fixed cell size (quota in umol/cell). Both are model parameters. Biomass is modeled in μmol N/L, using the C/N ratio parameter to convert to (implicit) C biomass of μmol C/L. Uptake and release are always synchronized to maintain a constant C/N ratio in the biomass. Cell numbers are implicit and computed post-simulation by dividing the biomass by the cell quota.

Each of the mechanism studied can be linked to a model configuration. I plan to run the model in a set of configurations, corresponding to different subsets of mechanisms. If a given phenotype can only be simulated when a specific mechanism is active, but not when that mechanism is inactive, this is a good indication that this mechanism may be at the heart of the interaction between this heterotroph and *Prochlorococcus*.

## Competition

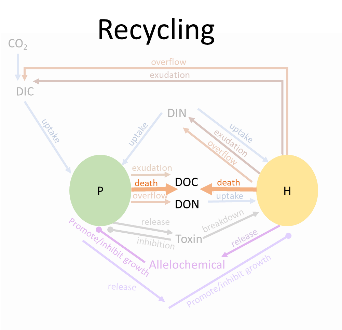
Modeled as competition over DIN (which stands for NH4). This is the nutrient resource which both *Prochlorococcus* and the heterotroph consume. The strength of competition for a given simulation run can be measured by

*competition = mean**(uptake(H, DIN) / uptake(P,DIN)) for all times where DIN > 0*

When the competition is close to 1, both populations have similar DIN uptake. When the competition is higher or lower than 1, the heterotroph or *Prochlorococcus* is outcompeting the other respectively.

Competition is disabled by setting *VmaxDINH* to a low value

## Nutrient Exchange – Recycling of Dead Cells

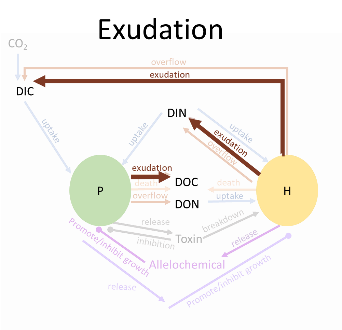
When cells die, a portion of the biomass is released as organic C and N from both populations. Death is modeled as first order; a fixed amount of the cells die each day.

In our experiments, decline rate of cocultures has a better match to quadratic rather than linear death rate. We are considering changing the death rate to be quadratic in the future.

The parameter *γi* controls the amount of recycled nutrients per population, biomass that cannot be recycled is transferred into a refractory DOM pool (RDON, RDOC). Recycling can be controlled by tuning the γ parameter, *γ = 0* will turn off recycling, *γ = 1* will recycle all dead biomass.

All dead matter is recycled as organic matter and not inorganic for the sake of simplicity of the model.

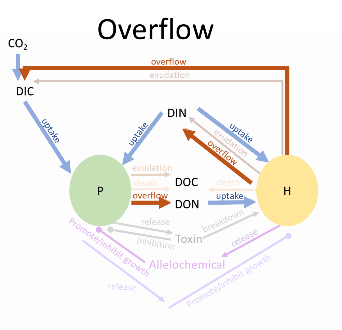
## Nutrient Exchange – Exudation

A fixed portion of the biomass is released to the media. *Prochlorococcus* releases organic nutrients (DOC, DON). The heterotroph releases inorganic nutrients (DIC, DIN).

Controlled by parameters EOP, EIP, EOH, EIH.

When these parameters = 0, exudation is disabled.

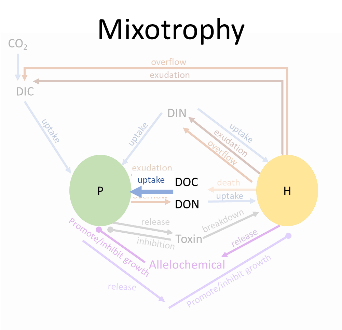
## Nutrient Exchange – Overflow

Both species uptake the highest nutrient amount possible. However, to maintain stoichiometric ratios, the overflow uptake of the nutrient is released directly into the media. *Prochlorococcus* releases organic nutrients (DOC, DON), while the heterotroph releases inorganic nutrients (DIC, DIN). Effectively, overflow converts nutrients from organic to inorganic form (heterotroph) or vice versa (*Prochlorococcus*). The nutrient pool affected depends on the current limitation. When the population is carbon limited, nitrogen overflow is released and vice versa.

An alternative mechanism could be to limit the uptake and growth depending on the current limitations. In both cases the net growth is the same, the main difference is that nutrients will not shift from one pool to another.

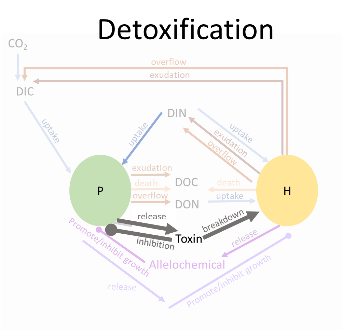
The parameter Oi controls the overflow and determines whether nutrients limitation will reduce the uptake of the non-limited nutrient (Oi = 0) or will result in conversion of nutrients to organic/inorganic through uptake and immediate overflow release (Oi = 1)

## Prochlorococcus mixotrophy

Mixotrophy in *Prochlorococcus* means that the organism can feed on organic nutrients as well as inorganic nutrients. Implemented by increasing *Prochlorococcus* Vmax for DOC and DON.

Mixotrophy probably does not play a major role in these interactions and therefore I plan to experiment with it only if all else fails.

## Detoxification of ROS

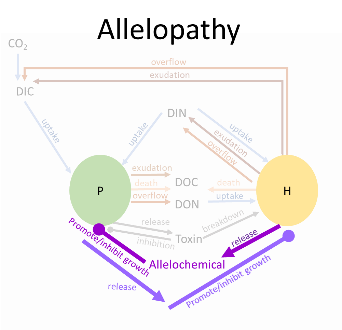
There are multiple studies on *Prochlorococcus* co-cultures in which *Prochlorococcus* produces reactive oxygen species (ROS) and is then unable to break them. These ROS inhibit *Prochlorococcus* growth while reducing ROS levels rescues the culture (ref). ROS levels are lower in co-culture with *Alteromonas* (ref). To implement this interaction, *Prochlorococcus* releases a fixed portion of its biomass as a toxic compound, which the heterotroph can break down (using Monod kinetics). When the toxin accumulates, it reduces the *Prochlorococcus* growth rate. The model currently does not impose cost for the release and subsequent breakdown of the toxin. The heterotroph does not benefit directly from the uptake of the toxin. I am assuming that the direct cost/benefit is negligible.

There are 3 sets of parameters related to detoxification:

* Rate of production of the toxin
* Vmax and K of the uptake
* Growth penalty.

There are several relevant model configurations: toxin could be produced and detoxicated, or toxin may be produced and accumulates in the media without detoxification, or toxin production is disabled completely.

## Infochemicals (Signals)

Both strains may release infochemicals, secondary metabolites with no known major function in primary metabolism. These metabolites may have positive, neutral, or negative impact on the death rate of the other organism in the co-culture. Another option is to have the infochemicals affecting the growth rate of the organism – I chose to go with changes to the death rate. Unlike toxins, infochemicals production reduces the overall biomass of the organism.

Both Prochlorococcus and heterotrophs release signal molecules (Sp, Sh). These Infochemicals increase the death rate of the other organism using Monod kinetics. There is no uptake or breakdown of the signals and they accumulate similarly to recalcitrant DOM.

Relevant parameters:

* MSp, MSh (1/day) – impact on the death rate, Positive values decrease the death rate while (beneficial Impact) while negative values increase the death rate (negative impact).
* KSp, KSh (umol N/L) – affinity of the impact on death rate of the other organism)
* ESp, ESh (% of biomass) – rates of release to the media

## DIC and respiration

CO2 is exchanged between the media in the air. Making the system open and not closed.

For simplicity, I assume a simplified DIC exchange mechanism. Where DIC is released/absorbed in sufficient amount to reach saturation level and the rate of exchange is controlled by tau.

dic\_uptake = - (DIC - c\_sat) / tau

Respiration is modeled relative to the growth (actual uptake of nutrients) and the biomass.

respirationp = b \* actual\_uptake + B \* r0

Respiration releases CO2, causing DIC to increase; however, to maintain fixed stoichiometry, an equivalent amount of nitrogen needs to be released as well. Therefore, respiration increases both DIC and DIN. I chose DIN and not DON somewhat arbitrarily.

# Model Definition

## Model Formulas

**Growth, uptake and overflow**

# nutrients limitation (no units)

# *n* stands for DIN, DIC, DON, DOC

Limni = ([n] / ([n] + Kni))

# gross uptake (max uptake)

# N uptake: umol N /L, C uptake: umol C /L

gross\_uptakeni = Vmaxni \* limni \* e-ω\*ROS \* Bi  # for PRO­­

gross\_uptakeni = Vmaxni \* limni \* Bi # for HET

# actual uptake (umol N / L)

actual\_uptakeNi = Min(gross\_uptakeINi + gross\_uptakeONi ,

(gross\_uptakeICi + gross\_uptakeOCi) / Ri)

# % of inorganic uptake

IOuptakeRateni = gross\_uptakeIni / (gross\_uptakeIni + gross\_uptakeOni)

# overflow (amount of nutrient that cannot be uptaken due to nutrient limitation on the other nutrient (umol N / L)

OverflowNi = gross\_uptakeINi + gross\_uptakeONi - actual\_uptakeNi

OverflowCi = gross\_uptakeICi + gross\_uptakeOCi - actual\_uptakeNi \* Ri

# overflow – the amount of nutrient converted and released

# to inorganic (het) or organic (pro) nutrients because it was uptaken

# but cannot be used without breaking the stoichiometry ratio

# of Oi is 0, the nutrient remains in the original (organic/inorganic) pull

# and is not converted

overflowINp =  (1 - Op) \* overflowNp \* IOuptakeRateNp

overflowONp = Op \* overflowNp + (1 - Op) \* overflowNp \* (1 - IOuptakeRateNp)

overflowICp =  (1 - Op) \* overflowCp \* IOuptakeRateCp

overflowOCp = Op \* overflowCp + (1 - Op) \* overflowCp \* (1 - IOuptakeRateCp)

overflowINh = Oh \* overflowNh + (1 - Oh) \* overflowNh \* IOuptakeRateNh

overflowONh = (1 - Oh) \* overflowNh \* (1 - IOuptakeRateNh)

overflowICh = Oh \* overflowCh + (1 - Oh) \* overflowCh \* IOuptakeRateCh

overflowOCh =  (1 - Oh) \* overflowCh \* (1 - IOuptakeRateCh)

**Respiration and DIC uptake**

# respiration umol N / L

# bi: respiration param, Bi biomass of organism *i*

respirationi = bi\* actual\_uptakeNi + Bi \* r0i

# DIC exchange with the air

dic\_uptake = - (DIC - c\_sat) / τ

**Death, signaling and exudation**

# signal release (umol N/L)

Sreleasei = Esi \* Bi

# signal limitation (impact on death)

limSi = (Si / (Si + KSi))

# death rate is changed based on signal S, with min of 0 and max of 1 /day

# positive­­ Msi decreases the death rate – positive interaction

# negative Msi increases the death rate – negative interaction

death\_ratei = Min(Max(Mi - MSi\*limSi, 0), 1 / seconds\_in\_day)

# death

deathi = death\_ratei \* Bi

# exudation (umol N /L)

exudationni = Eni \* Bi

# ROS release and breakdown (umol/L)

Treleasep = ε \* Bp

Tbreakdownh = VTmax \* ROS / (ROS + KTh) \* Bh

# conversion to cells/L

Xi = Bi / Qi

**Biomass and cell density**

# final equations

dBi/dt = actual\_uptakeNi – deathi - exudationOi - exudationIi

– respirationi – Sreleasei

**Nutrients in the media**

dDON/dt = Σ (deathi \* γi + exudationOi - gross\_uptakeONi + overflowONi)

dDOC/dt = Σ (deathi \* γi \* Ri + exudationOi \* Ri

- gross\_uptakeOCi + overflowOCi)

dRDON/dt = Σ (death­i \* (1 – γi))

dRDOC/dt = Σ (deathi \* (1 – γi) \* Ri)

dDIN/dt = Σ (exudationIi - gross\_uptakeINi + overflowINi + respirationi)

dDIC/dt = Σ (exudationIi \* Ri - gross\_uptakeICi + overflowICi

+ respirationi\* Ri)

+ dic\_uptake

**ROS and signals**

dROS/dt = Treleasep - Tbreakdownh

dSi/dt = Sreleasei

## Model Variables

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| --- | --- | --- | --- |
| Variables | Description | # vars | Units |
| Bi | Total Cell biomass | 2 | μmol N / L |
| DON, DIN | Nitrogen pools | 2 | μmol N / L |
| RDON | Refractory DON concentration | 1 | μmol N / L |
| DOC, DIC | Carbon pools | 2 | μmol C / L |
| RDOC | Refractory DOC concentration | 1 | μmol C / L |
| ROS | ROS/Toxin concentration | 1 | μmol / L |
| Si | Infochemicals/Signals | 2 | μmol N / L |
| Total | Number of variables | 11 |  |

## Model parameters

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| --- | --- | --- | --- |
| Parameter | Description | # params | Units |
| Kni | Nutrient uptake half-velocity constant | 8 | μmol N/L  μmol C/L |
| Vmaxni | Max uptake rate | 8 | μmol N/L/day / μmol N/L  μmol C/L/day / μmol N/L |
| Oi | % of nutrients above limit released as overflow | 2 | Ratio |
| Ri | C / N stoichiometric ratio | 2 | Ratio |
| Qi | Cell quota | 2 | μmol N/ cell |
| Mi | Mortality rate | 2 | 1/d |
| γi | % DON & DOC released when cells die | 2 | Ratio |
| Eni | % DIM/DOM released via exudation | 4 | 1/d |
| ε | Toxin release rate | 1 | μmol /L/day |
| VTmax | Max toxin breakup rate | 1 | μmol /L/day |
| KTh | Toxin half-velocity constant | 1 | μmol /L |
| ω | Inhibition of the toxin on Proch. Growth | 1 | 1 / μmol /L |
| KSi | Half saturation of signals impact on death | 2 | umolN/L |
| ESi | Signal release rate | 2 | 1/d |
| MSi | Signals impact on death | 2 | 1/d (between -1 to 1) |
| τ | DIC exchange (CO2), h / Kg, | 1 | No units |
| r0i | dark respiration | 2 | 1/d |
| Bi | respiration coefficient | 2 | no units |
| Total | Number of parameters | 34 |  |

# Model tuning

The model was implemented and simulated in python. I am using experimental results from a co-culture of *Prochlorococcus* mit9312 and *Alteromonas* HOT1A3 to do the initial test of the model and to assess its ability to represent real life processes. We are not aiming for quantitative fit and therefore there is no need to reach a 100% match to the measured data. However, testing against real experimental results gives a higher confidence that the model is in the correct direction and that the parameter settings are in the correct ballpark.

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| Figure 3: reference experimental results from co-culturing *Prochlorococcus* mit9312 and *Alteromonas* HOT1A3 in pro99 lowN. Also available, measurements from growing each organism axenically in monoculture. |

In axenic cultures, *Prochlorococcus* mit9312 reaches max growth around day 13, followed by a rapid decline and reaches a low level, below limit of detection on day 25. Axenic *Alteromonas* 1A3 reaches top growth on day 2, followed by a slow decline and stabilizing at 1e8 cells/L. In co-culture, mit9312 growth is similar to the axenic growth. The main difference is in the decline, which is slower and prolonged. At the end of the experiment, the mit9312 is viable at 1e10 cell/L. 1A3 seems to be initially inhibited in coculture, possibly due to competition with *Prochlorococcus* of NH4, but recovers around day 10 and is then able to sustain higher cell numbers, 1e10 cells/L, until the end of the experiment.

In comparison to the model, we observe similar dynamics. Although some observations are not recapitulated, e.g., long term *Prochlorococcus* survival.

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| Figure 4: dynamics of the model for axenic *Prochlorococcus* and *Alteromonas* and for the co-culture. dots: experimental measurements, lines: model simulation |

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| Figure 5: Dynamics of N pools in the three model simulations. From left to right: axenic *Alteromonas*, Axenic *Prochlorococcus*, co-culture of *Prochlorococcus* and *Alteromonas* |

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| Figure 5: Dynamics of C pools in the three model simulations. From left to right: axenic *Alteromonas*, Axenic *Prochlorococcus*, co-culture of *Prochlorococcus* and *Alteromonas* |

# Effect of Disabling Mechanisms on Model Simulations

The effect of disabling each of the mechanisms on the model is shown below.

These are the model settings used:

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| --- | --- | --- | --- |
| Mechanism | Change | Setting | Description |
| Competition | Disabled | VmaxIN,h = VmaxIN,h\* 1e-3 | Reduce heterotroph uptake of inorganic N |
| P Recycling | Disabled | γp = 0 | *Prochlorococcus* dead cells are not recycled. All dead biomass is converted to recalcitrant organic matter |
| H Recycling | Disabled | γh = 0 | Heterotrophs dead cells are not recycled. All dead biomass is converted to recalcitrant organic matter |
| P Exudation | Disabled | EO,p = 0  EI,p = 0 | Disable exudation of *Prochlorococcus* |
| H Exudation | Disabled | EO,h = 0  EI,h = 0 | Disable exudation of Heterotrophs |
| P Overflow | Disabled | Op = 0 | *Prochlorococcus* cells uptake upto the amount permitted by the limiting factor. |
| H Overflow | Disabled | Oh = 0 | Heterotrophs cells uptake upto the amount permitted by the limiting factor. |
| Mixotrophy | Enabled | VmaxON,p = VmaxON,p \* 50  VmaxOC,p = VmaxOC,p \* 50 | Increase *Prochlorococcus* uptake rate for DOC and DON |
| Detoxification | Disabled | ω = 0 | Disable ROS inhibiting effect on *Prochlorococcus* growth |

Note that mixotrophy is disabled by default and was added to the model (by increasing Vmax of *Prochlorococcus* DOC and DON uptake), this is the only mechanism that was disabled by default and enabled in the simulation below.

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| Figure 6: effects of disabling the different mechanisms on the model simulation . *Prochlorococcus* (left), Heterotroph (right) |

The closest *Prochlorococcus* match to measurements is the simulation during which overflow was disabled. None of the simulation matched the heterotroph dynamics.

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| Figure 7: dynamics of nitrogen nutrient exchange when disabling the different mechanisms |

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| Figure 7: dynamics of carbon nutrient exchange when disabling the different mechanisms |

## Next Steps

* 1. Add infochemicals/signals to the model
  2. investigate the model parameters and assign possible ranges per parameter
  3. run sensitivity analysis to assess the model sensitivity to changes in individual parameters
  4. define model configurations to simulate (for example: with/without detoxification, with/without exudation)
  5. Run Monte Carlo simulation on the different configurations, cluster the resulting simulations by phenotype, match the clusters to the experimental results

## Open Questions

* 1. How to add to the model beneficial effects of infochemicals? I know how to add inhibiting effects, not clear how to add beneficial effect
  2. It seems that some settings should influence multiple parameter values. For example, Ri (C/N ratio), Qi (cell quota), cell sizes (currently implicit). Should we define ‘super parameters’ that influence the allowed distributions of other parameters. What would these parameters and values be?