Statistical analysis

1 Project Summary

Collaborators

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Project questions

1. Does resource stoichiometry affect the growth rate of *Synechococcus*?
2. How does resource stoichiometry alter ecological dynamics?
3. Does stoichiometry alter phenotypic (co)evolution in cyanobacteria and phage?

Data collection

Briefly, all data for this project was collected during a long term continuous culture experimental evolution study with *Synechococcus* and SRIM-8 cyanomyophage.

For a complete description of the materials and methods for this repository, see Larsen *et al.* 2016.

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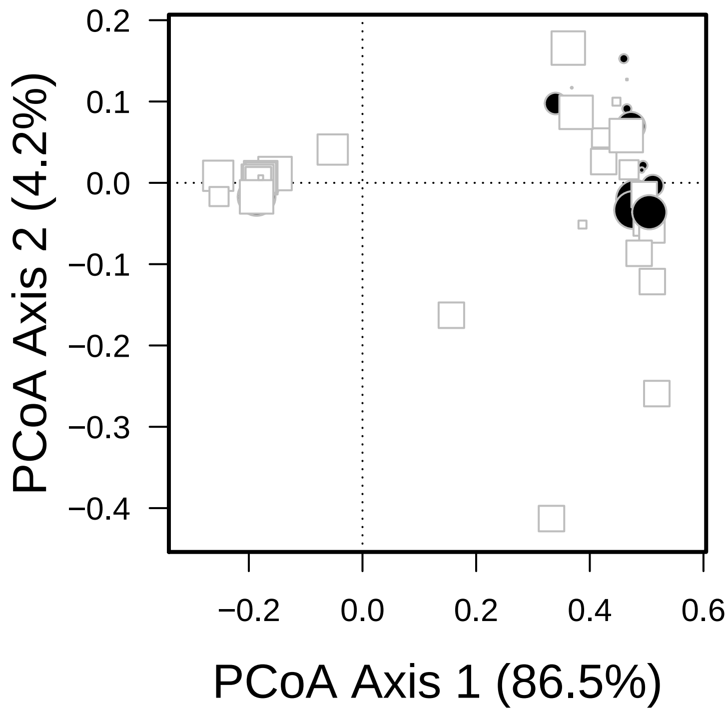
Contents


0.2
0.1
0.0
−0.1
−0.2
−0.3
−0.4
PCoA Axis 2 (4.2%)
−0.2
0.0
0.2
0.4
0.6
PCoA Axis 1 (86.5%)

2 Physiological growth: Does nutrient stoichiometry affect the growth rate of *Synechococcus*?

Overview: In this experiment, we tested for growth enhancement with the addtion of nitrogen (N), phosphosphorus (P), or the addition of both nutrients to our stoichiometrically modified AN media (Lennon *et al.* 2007; see Larsen *et al.* 2016 Table S1). Population growth curve data was collected on a Biotek Synergy Mx instrument loaded with software version 2.01.12.

2.1 Summary of Major Results

1. Addition of N or P to the N-limited or P-limited base medium, respectively, increased *Synechococcus* maximum growth rate (Figure 1) and percent change in growth (Figure 2) in batch culture as compared to control cultures without the addition of N or P.

2.2 *Synechococcus* growth rates with response to nutrient addition

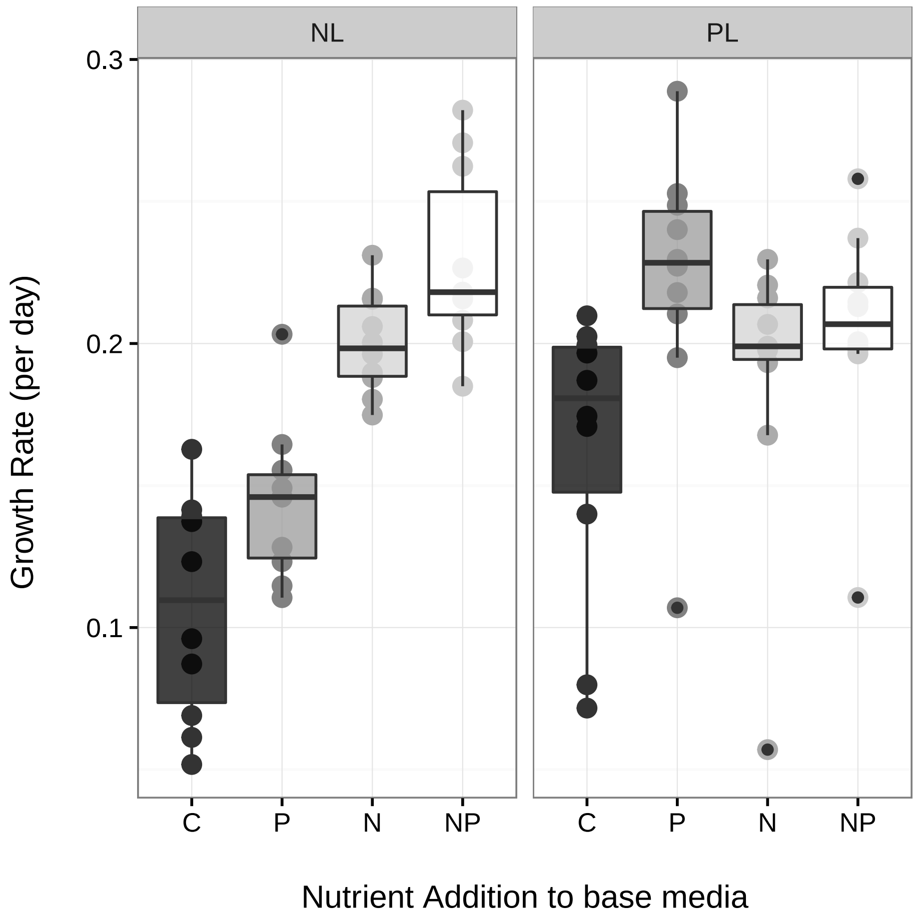


Figure 1: Nitrogen (N), phosphorus (P), or NP addition to the base N-limited and P-limited media used in the chemostat experiment. Culture controls (C) did not contain additional N or P.

2.2.1 Growth rate ANOVA tables

N-limited

Table 1: ANOVA table for NL nutrient addition

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
med.add	3	0.08993	0.02998	33.3	1.743e-10
Residuals	36	0.0324	0.0009001	NA	NA

Table 2: Posthoc comparisons using Tukey HSD

	diff	lwr	upr	p adj
N-C	**0.0929**	**0.05677**	**0.129**	**2.426e-07**
NP-C	**0.1218**	**0.0857**	**0.158**	**4.542e-10**
P-C	**0.03716**	**0.001027**	**0.0733**	**0.04188**
NP-N	0.02893	-0.007204	0.06507	0.1551
P-N	**-0.05574**	**-0.09188**	**-0.01961**	**0.001056**
P-NP	**-0.08467**	**-0.1208**	**-0.04854**	**1.564e-06**

P-limited

Table 3: ANOVA table for PL nutrient addition

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
med.add	3	0.01865	0.006215	2.845	0.05117
Residuals	36	0.07864	0.002184	NA	NA

Table 4: Posthoc comparisons using Tukey HSD

	diff	lwr	upr	p adj
N-C	0.02548	-0.03081	0.08178	0.619
NP-C	0.04166	-0.01463	0.09796	0.2094
P-C	**0.05857**	**0.002277**	**0.1149**	**0.03881**
NP-N	0.01618	-0.04011	0.07247	0.8656
P-N	0.03309	-0.02321	0.08938	0.4008
P-NP	0.01691	-0.03939	0.0732	0.8498

2.3 Percent Change in Growth

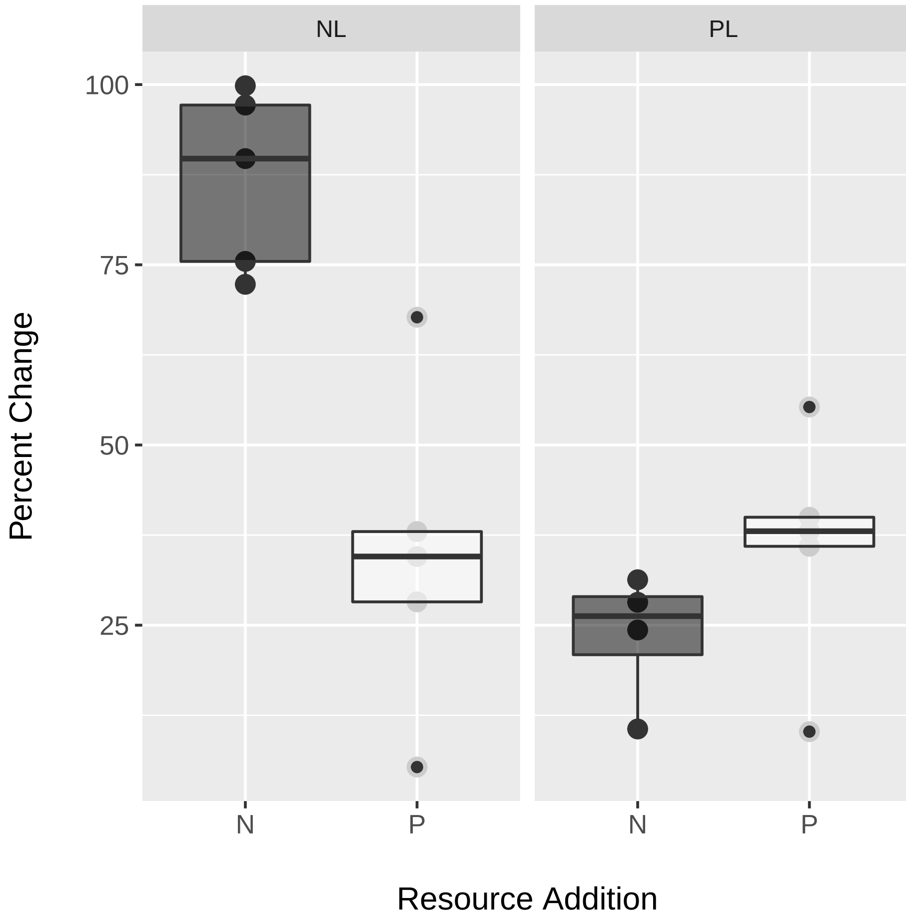


Figure 2: Percent change in growth rate between control and nutrient additions(N, P, or NP) cultures. NL = N-limited; PL = P-limited

2.3.1 Growth rate t-test tables

N-limited

Table 5: t-test table for NL nutrient addition

Test statistic	df	P value	Alternative hypothesis
4.546	6.271	0.001749 * *	greater

P-limited

Table 6: t-test table for PL nutrient addition

Test statistic	df	P value	Alternative hypothesis
1.432	6.442	0.09939	greater

3 Population Dynamics: Does nutrient stoichiometry affect temporal population dynamics?

Overview: In this experiment, whole samples were collected from each chemostat system three times per week for ~5 months. Each sample was processed, stained, and counted using epi-fluorescence on a Zeiss microscope and quantified using Axiovision software. Statistics for these data include repeated measures anova (RMANOVA), stability (1/Coefficient of Variation), and cross-correlation analyses on whitened data using SAS.

3.1 Summary of Major Results

1. Stoichiometry significantly affected *Synechococcus* and phage densities. RMANOVA
2. Altered mean and stability of the populations
3. Modified the temporal coherence, or synchrony, of the *Synechococcus*-phage dynamics, suggesting ecological ramifications of stoichiometry.

3.2 Chemostat-level comparisons

3.2.1 Population dynamics

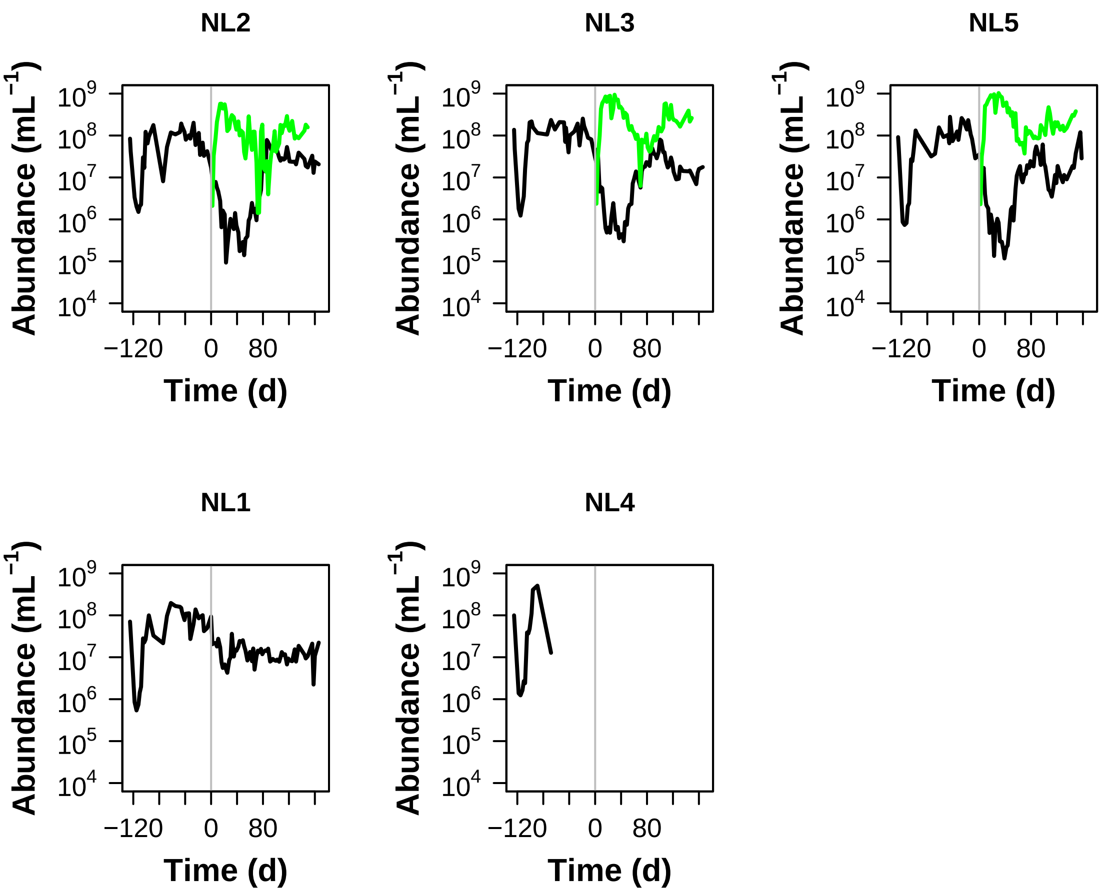


Figure 3: Individul N-Limited chemostat replicates. Chemostats NL2, NL3, and NL5 contained both *Synechococcus* (black) and SRIM8 phage (green) while chemostats NL1 and NL4 contained only *Synechococcus*. Chemostat NL4 was lost due to fungal contamination prior to phage addition.

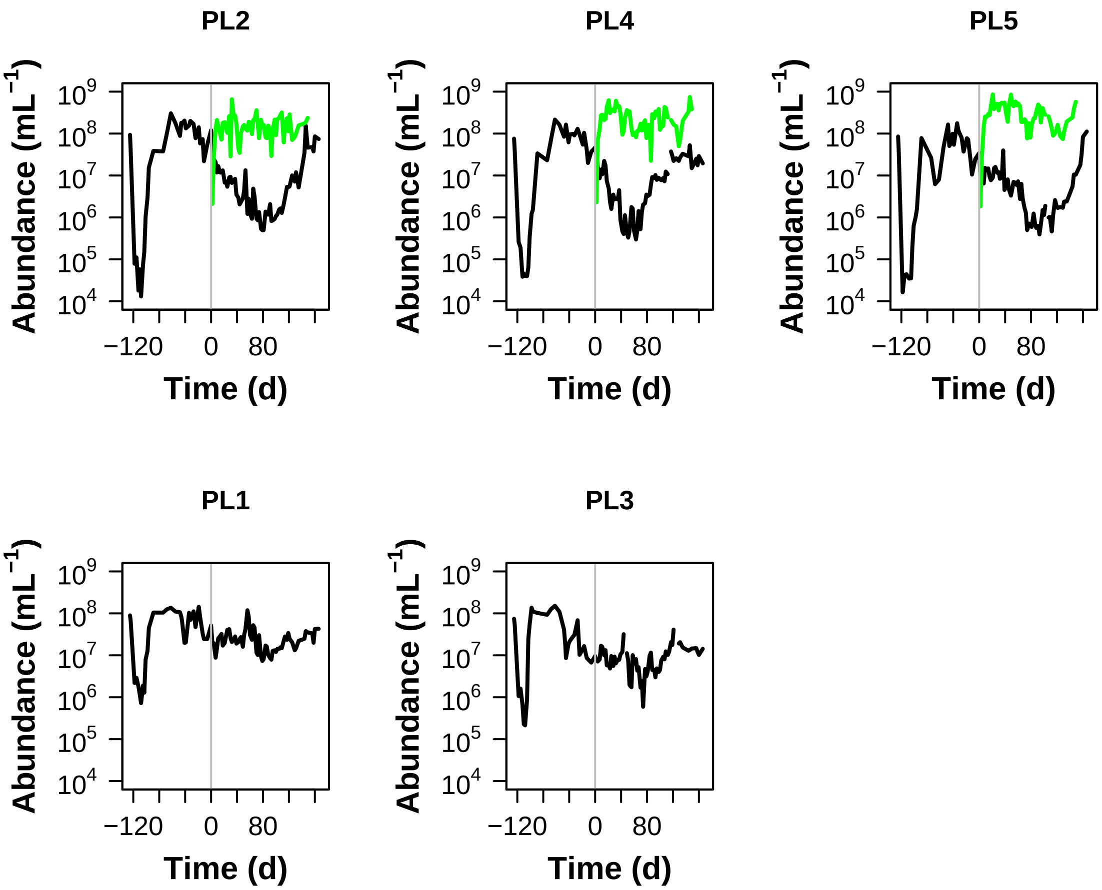
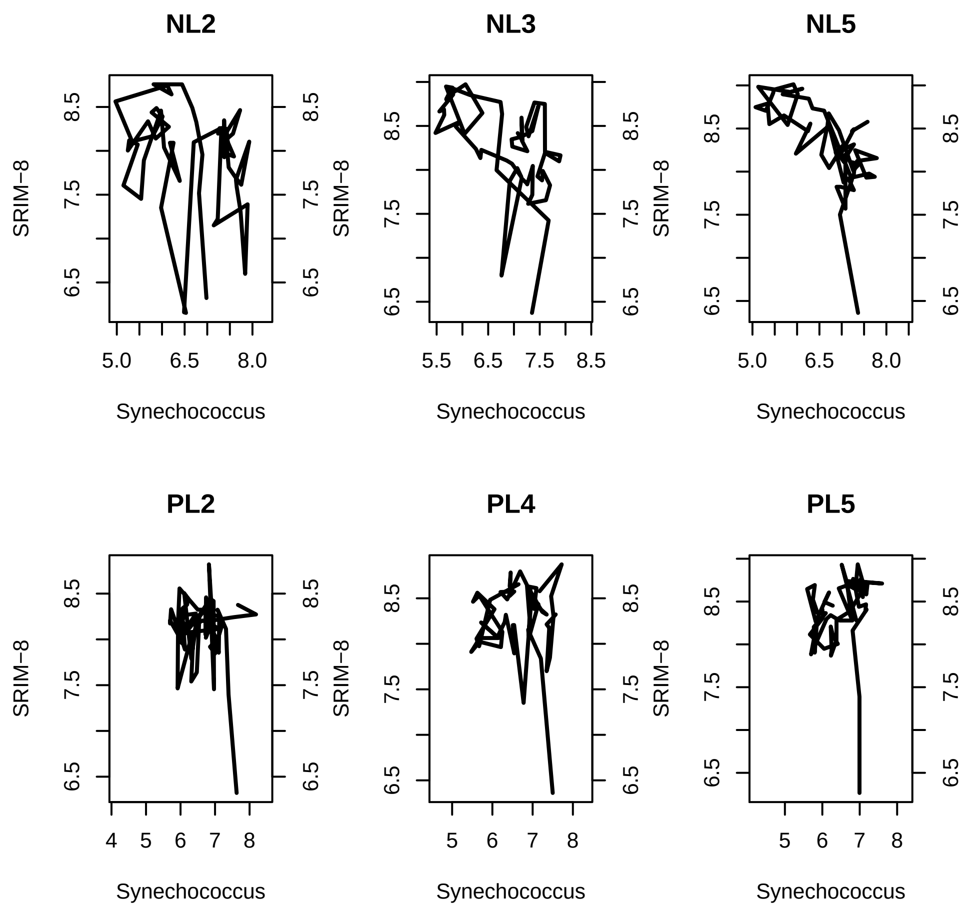


Figure 4: Individul P-Limited chemostat replicates. Chemostats PL2, PL4, and PL5 contained both *Synechococcus* (black) and SRIM8 phage (green) while chemostats PL1 and PL3 contained only *Synechococcus*.

3.2.2 Chemostat phase plane diagrams



3.3 Treatment-level comparisons

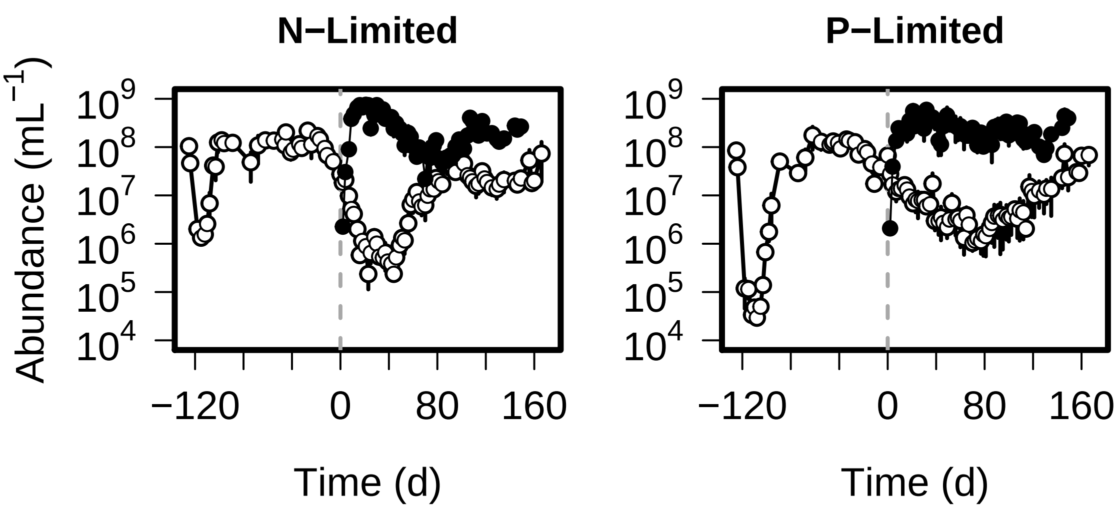
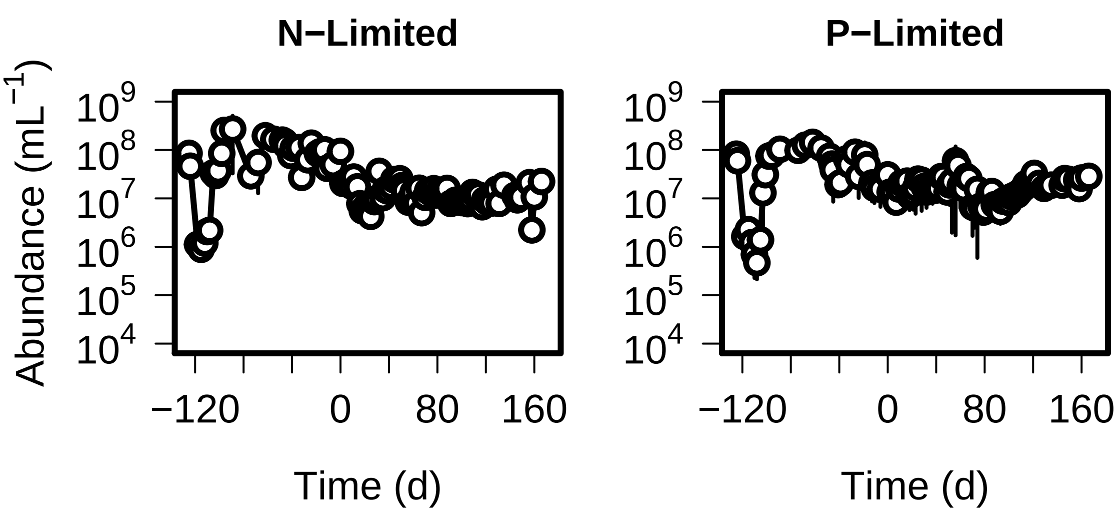
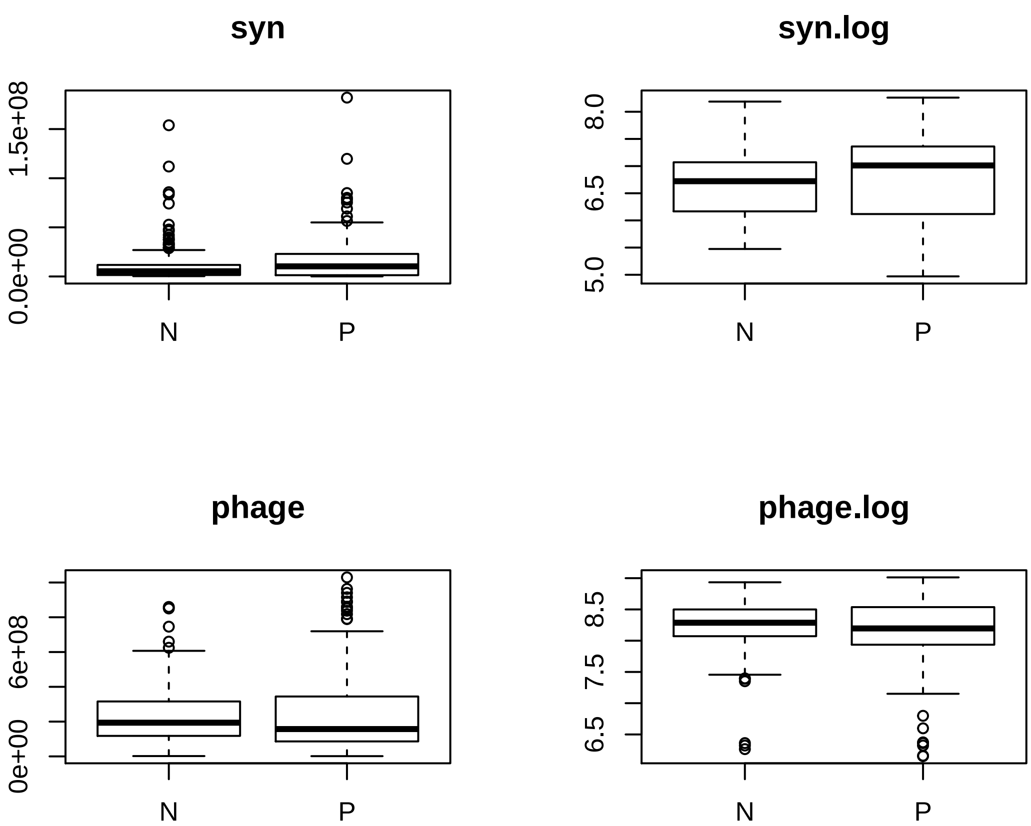


Figure 5: Average population dynamics of Synechococcus (white) and SRIM8 phage (black) in N-limited and P-limited nutrient treatments. The dashed line at day 0 indicates the time in which phage were added to the chemostat system.



3.3.1 Heteroskedaskicity (skewness)



3.3.2 Reapeated Measures ANOVA (RMANOVA)

+Ph *Synechococcus* and phage

Table 7: RMANOVA table for SRIM8 phage

	numDF	denDF	F-value	p-value
(Intercept)	1	230	14010	0
lim	1	4	0.3592	0.5812
day.fac	58	230	10.22	0
lim:day.fac	58	230	2.588	2.771e-07

Table 8: RMANOVA table for +Ph Synechococcus

	numDF	denDF	F-value	p-value
(Intercept)	1	245	12432	0
lim	1	4	0.5225	0.5098
day.fac	62	245	3.354	1.14e-11
lim:day.fac	62	245	2.437	6.993e-07

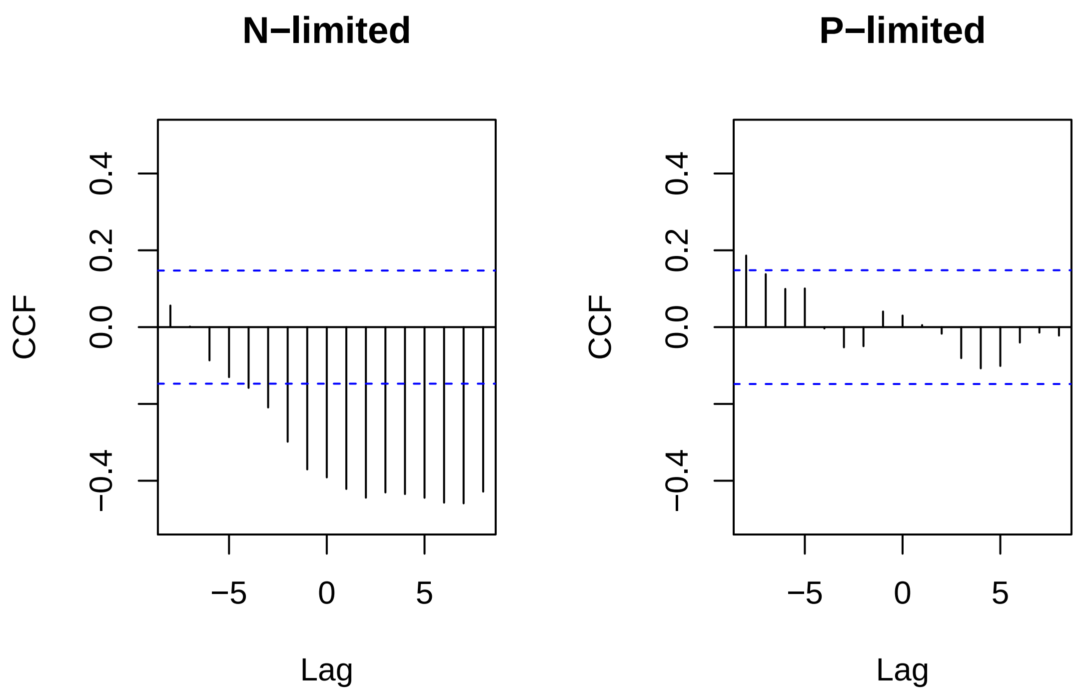
Table 9: RMANOVA table comparison between -Ph and +Ph Synechococcus

	numDF	denDF	F-value	p-value
(Intercept)	1	305	19415	0
lim	1	5	0.002236	0.9641
day.fac	62	305	2.87	1.107e-09
type	1	5	8.787	0.03136
lim:day.fac	62	305	2.354	8.796e-07
lim:type	1	5	0.3204	0.5958
day.fac:type	62	305	1.244	0.1198
lim:day.fac:type	62	305	1.121	0.2639

Table 10: RMANOVA for -Ph Synechococcus

	numDF	denDF	F-value	p-value
(Intercept)	1	60	1629	0
lim	1	1	0.01077	0.9342
day.fac	62	60	0.9176	0.6315
lim:day.fac	62	60	0.9089	0.6454

3.3.3 Temporal autocorrelation



NOTE:
Cross-correlation analyses and RMANOVA were also completed in SAS

3.4 Despcriptive statistics

lim	cID	microbe	mean	var	sem
N	NL1	Syn	13134483	4.093e+13	3693536
P	PL1	Syn	25428955	3.159e+14	10261999
P	PL3	Syn	9864044	4.838e+13	4015664
NA	NA	NA	NA	NA	NA
NA	NA	NA	NA	NA	NA
NA	NA	NA	NA	NA	NA
NA	NA	NA	NA	NA	NA
NA	NA	NA	NA	NA	NA
NA	NA	NA	NA	NA	NA
NA	NA	NA	NA	NA	NA
NA	NA	NA	NA	NA	NA
NA	NA	NA	NA	NA	NA

Limitation	Treatment	Synechococcus mean densitiy (+/- SEM)	Synechococcus mean stability	Phage mean density (+/- SEM)	Phage mean stability
N	Control	1.3e+07(4e+06)	2.1	NaN(NA)	NA
N	Infect	1.7e+07(1e+07)	0.75	2.5e+08(1.4e+08)	1
P	Control	1.8e+07(9e+06)	1.1	NaN(NA)	NA
P	Infect	1.1e+07(1e+07)	0.59	2.4e+08(9.5e+07)	1.5

Chemostat	Treatment	Synechococcus mean densitiy (+/- SEM)	Synechococcus mean stability	Phage mean density (+/- SEM)	Phage mean stability
NL1	Control	1.3e+07(4e+06)	2.1	NaN(NA)	NA
NL2	Infect	1.8e+07(1e+07)	0.84	1.5e+08(7.9e+07)	1.1
NL3	Infect	1.6e+07(1e+07)	0.93	2.9e+08(1.5e+08)	1.2
NL5	Infect	1.7e+07(2e+07)	0.59	3.1e+08(1.6e+08)	1.1
PL1	Control	2.5e+07(1e+07)	1.4	NaN(NA)	NA
PL2	Infect	1.3e+07(1e+07)	0.52	1.6e+08(5.9e+07)	1.6
PL3	Control	9864044(4e+06)	1.4	NaN(NA)	NA
PL4	Infect	1.1e+07(7e+06)	0.94	2.4e+08(8.8e+07)	1.6
PL5	Infect	9399556(1e+07)	0.52	3.1e+08(1.1e+08)	1.6

3.5 Topographic statistics

Table 14: Descriptive statistical summary of population data. (continued below)

lim	cID	microbe	mean	var	sem	stab	start.abd

final.abd	min.day	min.abd	max.day	max.abd

4 Infection Dynamics: Does stoichiometry alter phenotypic (co)evolution in cyanobacteria and phage?

Overview: To examine how nutrient stoichiometry impact evolutionary interactions, I collected cross-infectivity data from 96 phage and ~200 *Synechococcus* strains. Each challenge was recorded based on cellular growth where lysis = 1 (i.e. infectious interaction occured) or no lysis (i.e. no evidence of infection; cell line is resistant). This data was incorporated into network-based metrics.

4.1 Summary of Major Results

1. **Are temporal infection dynamics affected by stoichiometry?**
2. **Do community infection networks change as a result of the environment?**
3. **How are the dynamics affected? Through changes in overall resistance/infectivity? Changes in compositional resistance?**

4.2 Degree of interaction

4.3 RMANOVA

	AIC	BIC	logLik	L.Ratio	p-value
model.ar	254.3	329	-108.2	NA	NA
model.arma1	233.7	312.3	-96.86	22.59	2.003e-06
model.arma2	225.7	308.2	-91.84	10.05	0.001523

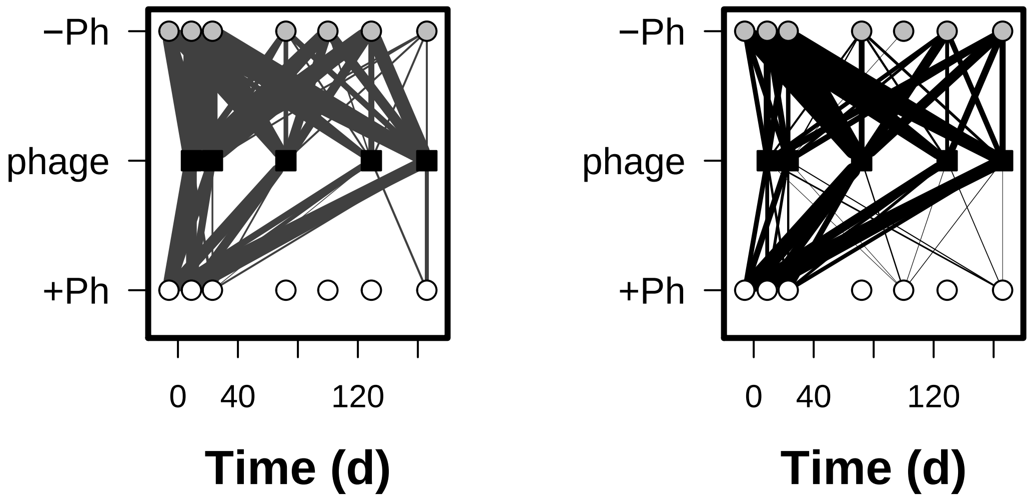
	Std.Error	t-value	p-value
(Intercept)	**0.08346**	**9.123**	**4.674e-18**
trtT	**0.1136**	**-2.871**	**0.004325**
limP	**0.1155**	**-3.857**	**0.01819**
phage.time	0.0006714	-1.743	0.08219
bac.time	0.001086	0.0845	0.9327
trtT:limP	0.1588	1.295	0.196
trtT:phage.time	0.0009219	0.7362	0.4621
limP:phage.time	**0.0009172**	**2.812**	**0.005188**
trtT:bac.time	**0.001379**	**-2.373**	**0.01815**
limP:bac.time	0.001367	-0.06095	0.9514
phage.time:bac.time	9.204e-06	-1.641	0.1016
trtT:limP:phage.time	0.001273	-0.6152	0.5388
trtT:limP:bac.time	0.001816	1.275	0.2029
trtT:phage.time:bac.time	1.15e-05	1.662	0.09738
limP:phage.time:bac.time	1.145e-05	0.3174	0.7511
trtT:limP:phage.time:bac.time	1.498e-05	-1.448	0.1484

4.4 Infection dynamics by chemostat

4.5 Infection dynamics by treatment

4.5.1 Network plots

```
##       [,1] [,2] [,3] [,4] [,5] [,6] [,7] [,8] [,9] [,10] [,11] [,12] [,13]
##  [1,]    0    0    0    0    0    0    0    0    0     0     0     0     0
##  [2,]    0    0    0    0    0    0    0    0    0     0     0     0     0
##  [3,]    0    0    0    0    0    0    0    0    0     0     0     0     0
##  [4,]    0    0    0    0    0    0    0    0    0     0     0     0     0
##  [5,]    0    0    0    0    0    0    0    0    0     0     0     0     0
##  [6,]    0    0    0    0    0    0    0    0    0     0     0     0     0
##  [7,]    0    0    0    0    0    0    0    0    0     0     0     0     0
##  [8,]    0    0    0    0    0    0    0    0    0     0     0     0     0
##  [9,]    0    0    0    0    0    0    0    0    0     0     0     0     0
## [10,]    0    0    0    0    0    0    0    0    0     0     0     0     0
## [11,]    0    0    0    0    0    0    0    0    0     0     0     0     0
## [12,]    0    0    0    0    0    0    0    0    0     0     0     0     0
## [13,]    0    0    0    0    0    0    0    0    0     0     0     0     0
## [14,]    0    0    0    0    0    0    0    0    0     0     0     0     0
## [15,]    0    0    0    0    0    0    0    0    0     0     0     0     0
## [16,]    0    0    0    0    0    0    0    0    0     0     0     0     0
## [17,]    0    0    0    0    0    0    0    0    0     0     0     0     0
## [18,]    0    0    0    0    0    0    0    0    0     0     0     0     0
## [19,]    0    0    0    0    0    0    0    0    0     0     0     0     0
##       [,14] [,15] [,16] [,17] [,18] [,19]
##  [1,]     0     0     0     0     0     0
##  [2,]     0     0     0     0     0     0
##  [3,]     0     0     0     0     0     0
##  [4,]     0     0     0     0     0     0
##  [5,]     0     0     0     0     0     0
##  [6,]     0     0     0     0     0     0
##  [7,]     0     0     0     0     0     0
##  [8,]     0     0     0     0     0     0
##  [9,]     0     0     0     0     0     0
## [10,]     0     0     0     0     0     0
## [11,]     0     0     0     0     0     0
## [12,]     0     0     0     0     0     0
## [13,]     0     0     0     0     0     0
## [14,]     0     0     0     0     0     0
## [15,]     0     0     0     0     0     0
## [16,]     0     0     0     0     0     0
## [17,]     0     0     0     0     0     0
## [18,]     0     0     0     0     0     0
## [19,]     0     0     0     0     0     0
```




```
## null device
##           1
```

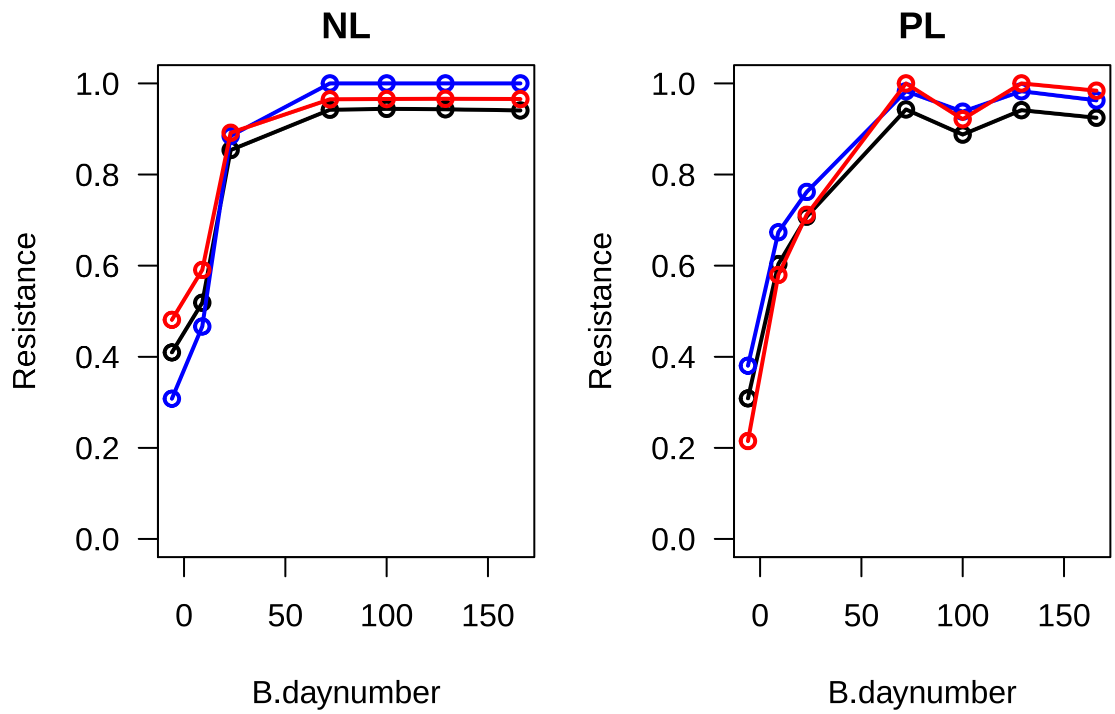
4.6 Community Networks

4.7 BiWeb estimates for nestedness and modularity

	statistic.t	parameter.df	p.value
connectance	1.456400410208	3.89610299683149	0.220826873899216
modularity.qb	-3.5488938188692	3.00184431086153	0.0380832752236465
modularity.qr	-0.337865126206578	3.62274149633006	0.754122338605035
nodf	0.371973397721244	3.80924523421393	0.729674513225951
ntc	-0.848020202172062	3.96441380258424	0.444591439999469

4.8 Synechococcus resistance

4.8.1 global; sympatric vs. allopatric resistance




```
##                      numDF denDF  F-value p-value
## (Intercept)              1    97 43.84393  <.0001
## B.trt                    1     4  1.45906  0.2936
## B.daynumber              6    97 14.27125  <.0001
## B.trt:B.daynumber        6    97  0.51041  0.7992
```

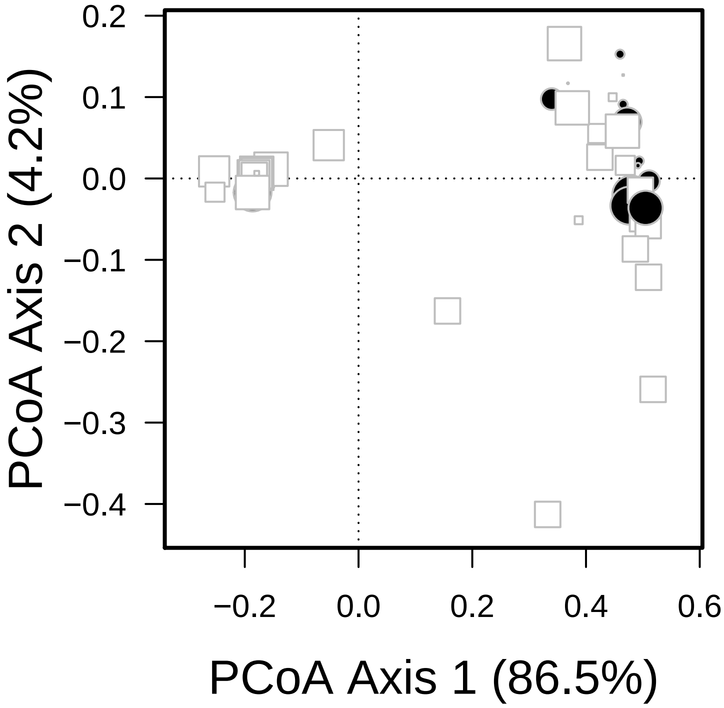
	numDF	denDF	F-value	p-value
(Intercept)	1	97	2184	0
B.trt	1	4	0.05589	0.8247
B.daynumber	6	97	31.82	0
B.trt:B.daynumber	6	97	0.5104	0.7992

	numDF	denDF	F-value	p-value
(Intercept)	1	97	1645	0
B.trt	1	4	1.962	0.2339
B.daynumber	6	97	27.78	0
B.trt:B.daynumber	6	97	0.7992	0.5729

	numDF	denDF	F-value	p-value

	numDF	denDF	F-value	p-value
(Intercept)	1	97	2394	0
B.trt	1	4	0.4009	0.561
B.daynumber	6	97	36.72	0
B.trt:B.daynumber	6	97	1.557	0.168

4.8.2 Compositional resistance



4.9

	Df	SumsOfSqs	MeanSqs	F.Model	R2	Pr(>F)
Time	1	4.242	4.242	71.5	0.39	0.001

Limitation 1 0.03804 0.03804 0.6411 0.003496 0.018

Time*Limitation 1 0.07149 0.07149 1.205 0.006572 0.267

```
**Residuals**     110     6.527     0.05933     NA       0.6      NA

  **Total**       113     10.88       NA        NA        1       NA
```

Table: Blocks: strata

4.10 Phage Host Range

4.10.1 global; sympatric vs. allopatric host range

4.10.2 Compositional infectivity

4.11 Treatement level degree of infection

5 Appendix

5.1 R and packages

All analyses were completed using R version 3.2.5 (2016-04-14)

5.2 References

5.3 Appendix

5.3.1 Key term definitions

Word	Abbreviation	Definition
Nitrogen	N	
Phosphorus	P	
Nitrogen Limited	NL	
Phosphorus Limited	PL	
chemostat	cID	