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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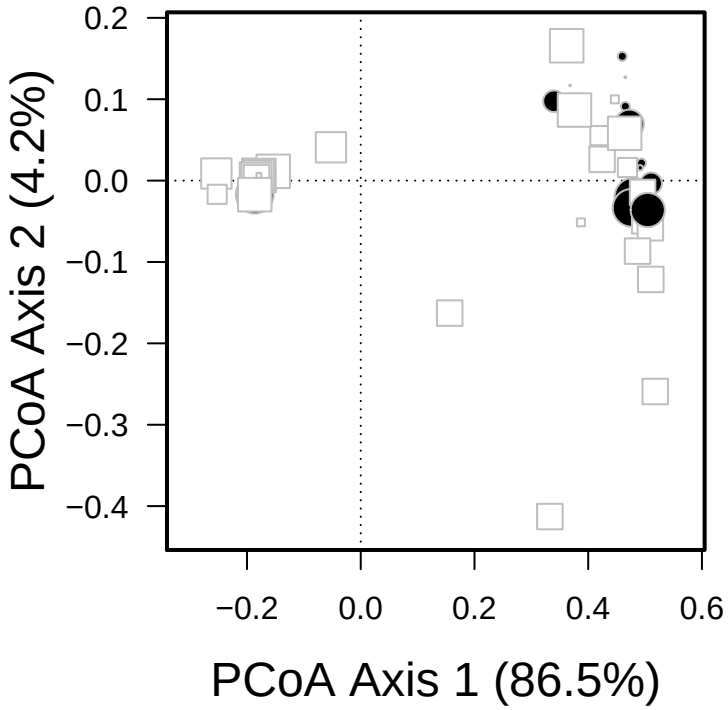

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2 Physiological growth: Does nutrient stoichiometry affect the growth rate of *Synechococcus*?

Overview: In this experiment, we tested for growth enhancement with the addtion of N or P to our stoichiometrically amended AN media (Lennon *et al.* 2007; see Larsen *et al.* 2016 Table S1).

2.1 Summary of Major Results

1. Addition of N or P to our N-limited or P-limited media increased *Synechococcus* in comparison to the control treatment (Figure 1, Table 1).

Analysis Notes:

1. Population growth curve data was collected on a Biotek Synergy Mx instrument loaded with softward version 2.01.12.
2. Growth rate calculations were completed using lab generated code for bacterial growth rate analysis for the above instrument and can be found at `http://github.com/LennonLab/Growth_Curves`.

2.2 *Synechococcus* growth rates with response to nutrient addition

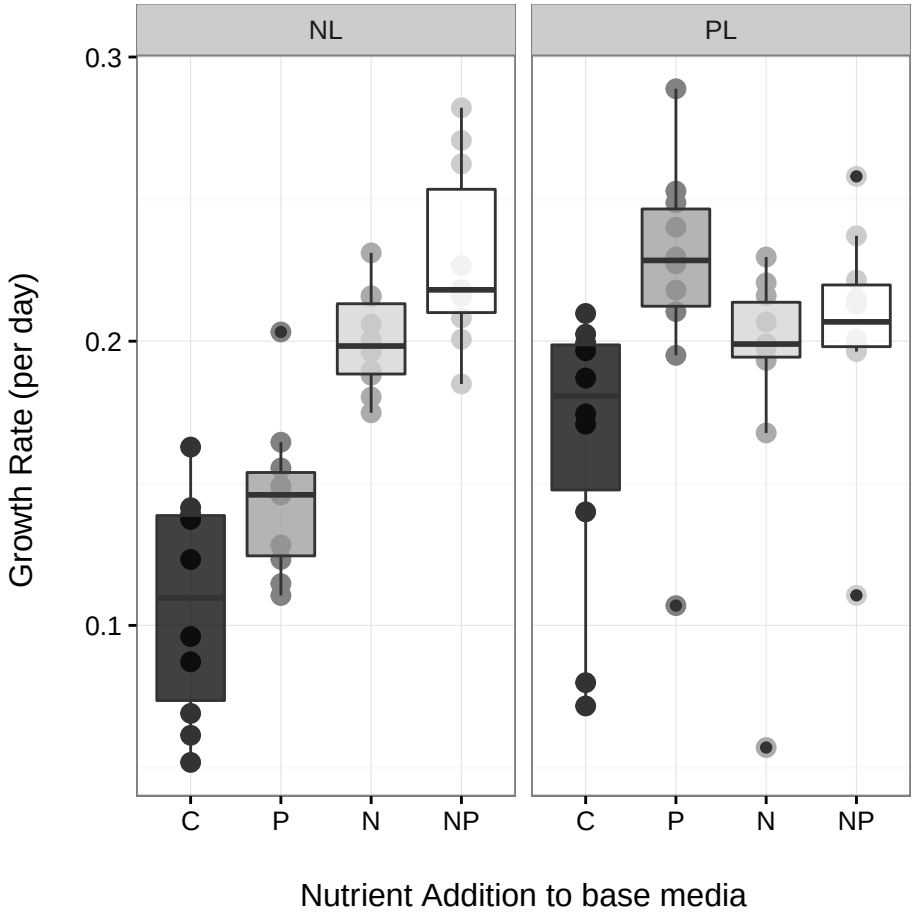


Figure 1: Nitrogen (N), phosphorus (P), or NP addition to the base N-limited and P-limited media used in the chemostat experiment. Culture controls (C) did not contain additional N or P.

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

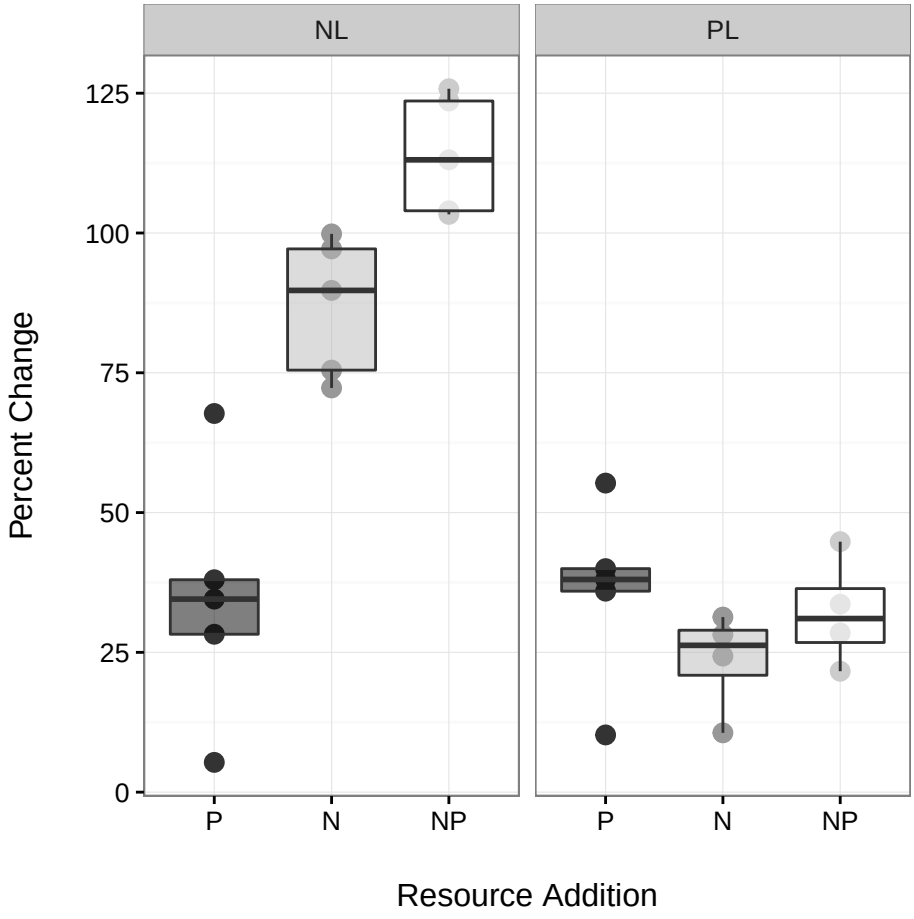


Figure 2: Percent change in growth rate between control and nutrient additions(N, P, or NP) cultures. NL = N-limited; PL = P-limited

2.3.1 Growth rate ANOVA tables

N-limited

Table 5: ANOVA table for NL nutrient addition

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
med.add	2	16206	8103	31.59	1.653e-05
Residuals	12	3078	256.5	NA	NA

Table 6: Posthoc comparisons using Tukey HSD

	diff	lwr	upr	p adj
NP-N	**27.06**	**0.03951**	**54.08**	**0.04966**
P-N	**-52.14**	**-79.16**	**-25.12**	**0.0006541**
P-NP	**-79.2**	**-106.2**	**-52.18**	**1.316e-05**

P-limited

Table 7: ANOVA table for PL nutrient addition

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
med.add	2	343.4	171.7	1.079	0.3765
Residuals	10	1592	159.2	NA	NA

Table 8: Posthoc comparisons using Tukey HSD

	diff	lwr	upr	p adj
NP-N	8.522	-15.93	32.98	0.6197
P-N	12.29	-10.91	35.49	0.3532
P-NP	3.764	-19.44	26.96	0.8978

3 Population Dynamics: Does nutrient stoichiometry affect temporal 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/CV), and cross-correlation analyses on whitened data.

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

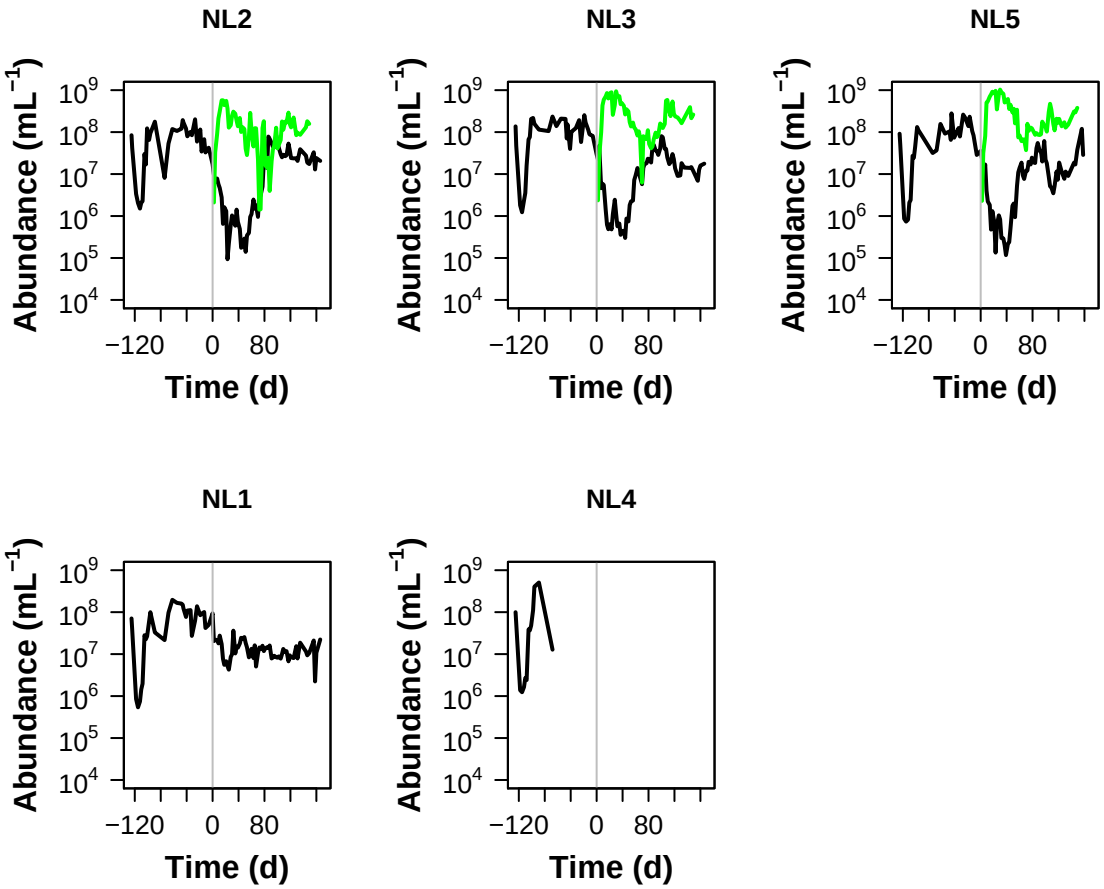


Figure 3: N-Limited Chemostats

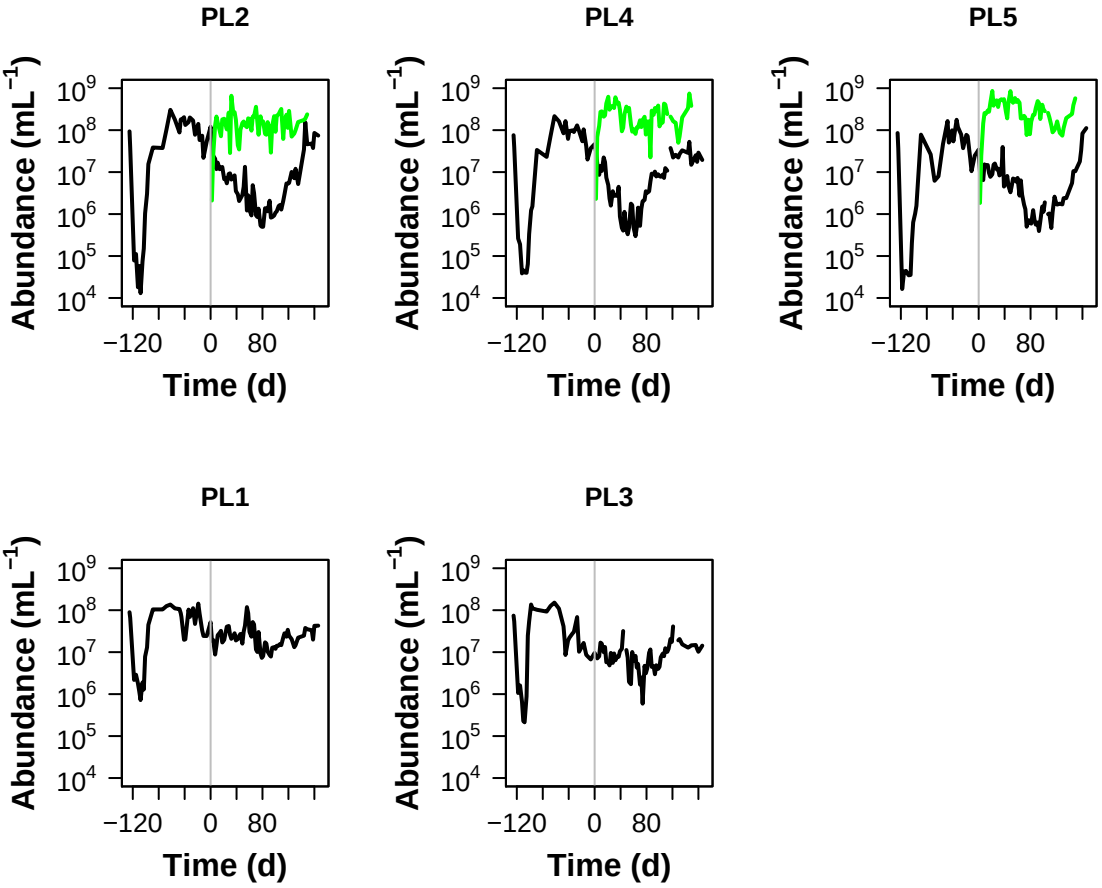
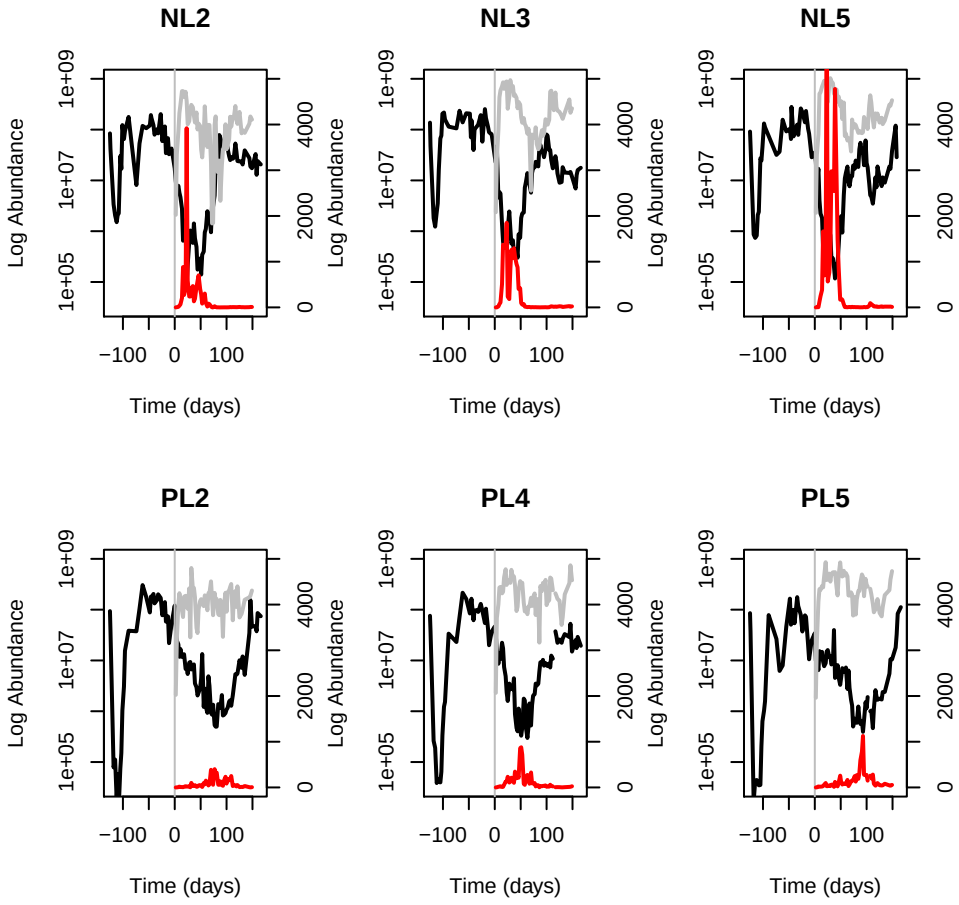


Figure 4: P-Limited Chemostats

3.2.2 Phage to bacteria ratio



3.3 Treatment-level comparisons

3.3.1 Controls

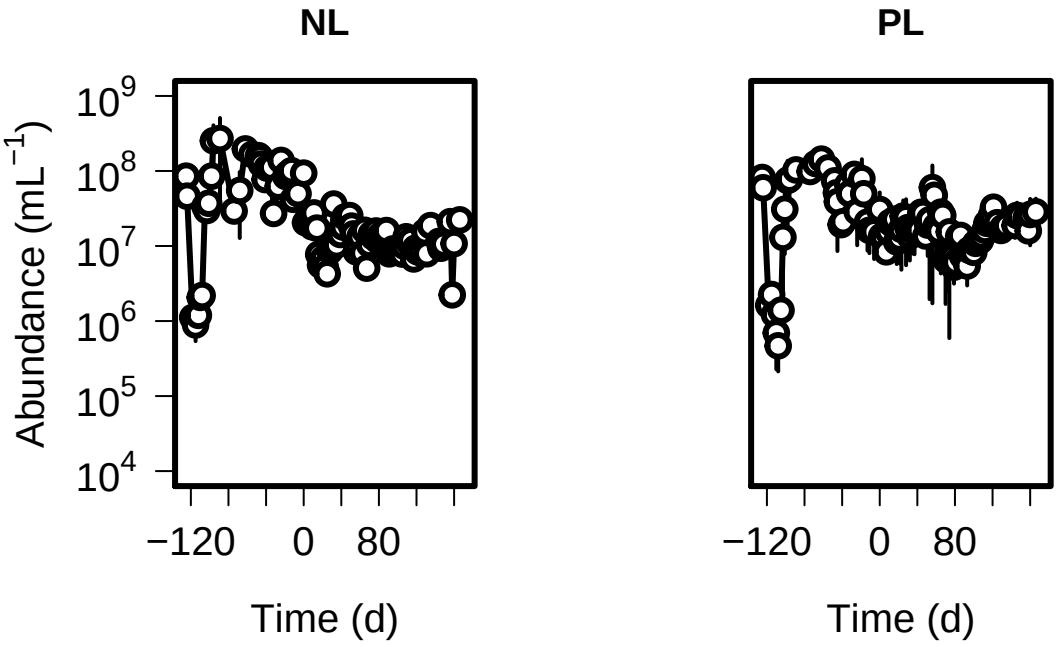


Figure 5: Control Chemostats

3.3.2 Treatment (i.e. Exposed) population abundances

RMANOVA for control cyanobacteria

RMANOVA for control vs exposed cyanobacteria densities

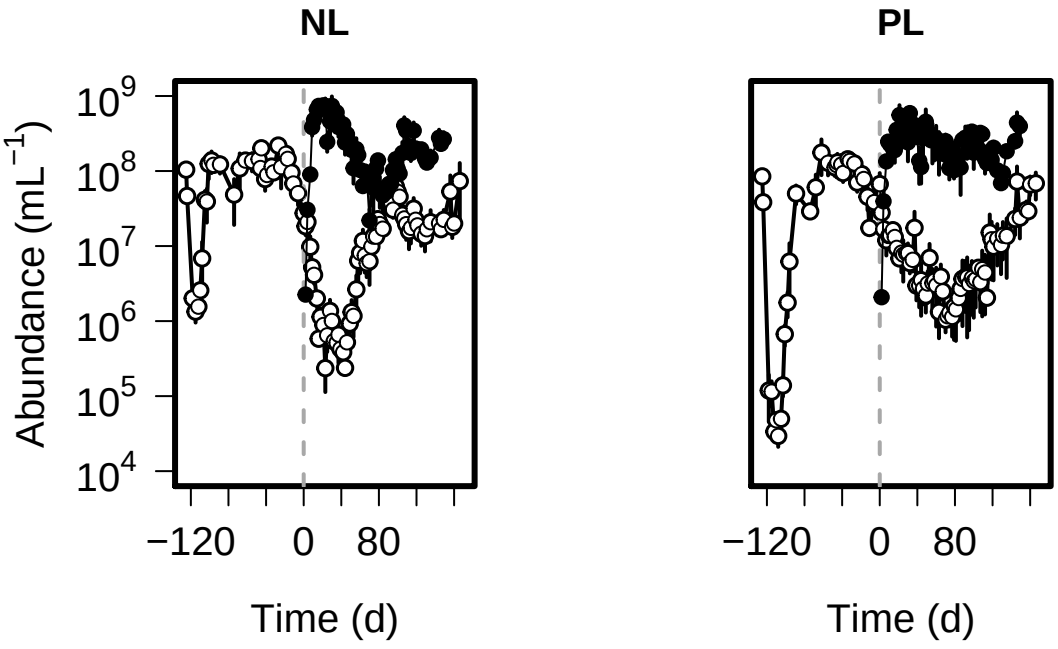
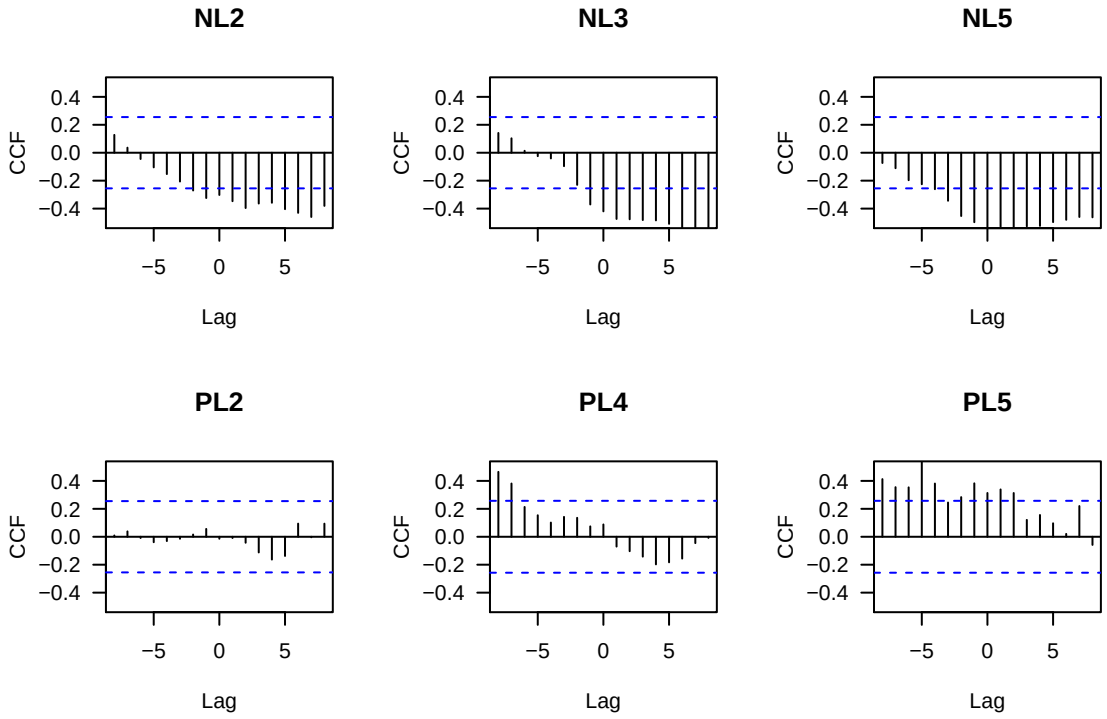
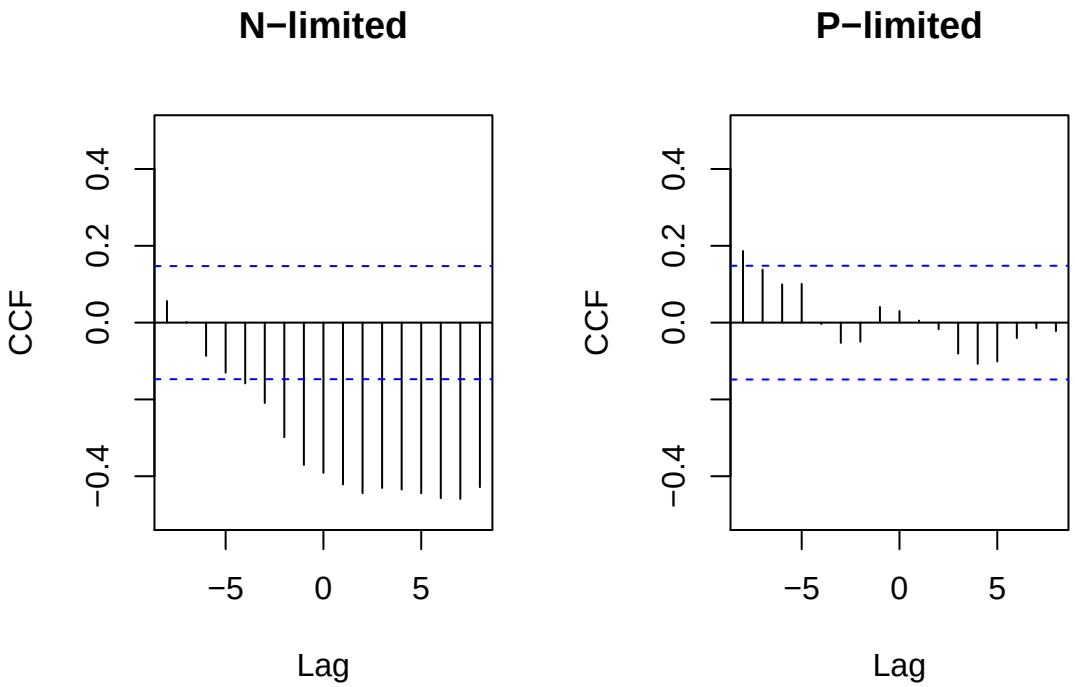


Figure 6: Treatment Chemostats

3.3.3 Temporal autocorrelation

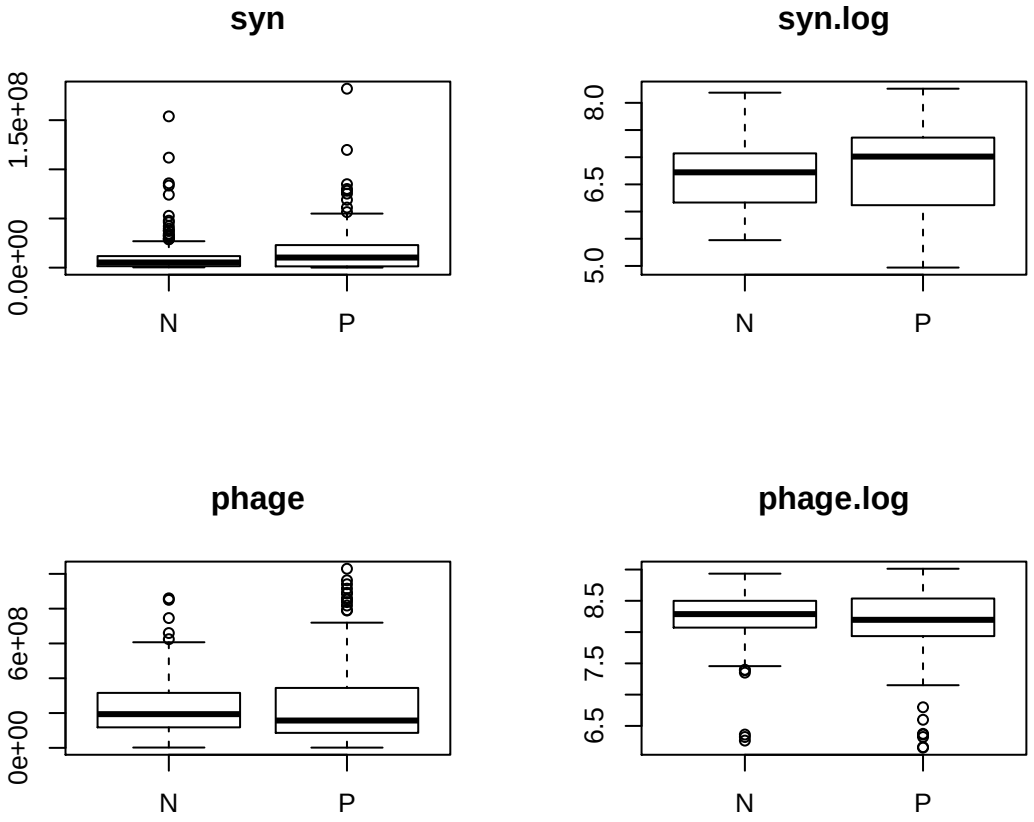



N−limited
P−limited
CCF
0.4
0.2
0.0
−0.4
−5
0
5
Lag

3.4 Statistical analyses

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

3.4.1 Heteroskedaskicity (i.e skewness) with treatment data only



3.4.2 Reapeated Measures ANOVA (RMANOVA)

	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

```
##             numDF denDF  F-value p-value
## (Intercept)     1   245 987.2110  <.0001
## lim             1     4   0.1869  0.6878
## day.fac        62   245   3.3834  <.0001
## lim:day.fac    62   245   2.4368  <.0001
```

	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

3.4.3 Despcriptive statistics

lim	cID	microbe	mean	var	sem
N	NL2	Phage	151575375	1.881e+16	79190498
N	NL3	Phage	292205382	6.38e+16	145831435
N	NL5	Phage	312565642	7.765e+16	160881119
P	PL2	Phage	161267374	1.047e+16	59073946
P	PL4	Phage	243403403	2.342e+16	88361764
P	PL5	Phage	312523940	3.66e+16	110450827
N	NL2	Syn	17594096	4.371e+14	12070003
N	NL3	Syn	16110168	3.023e+14	10039048
N	NL5	Syn	16985817	8.152e+14	16484799
P	PL2	Syn	12944893	6.116e+14	14277915
P	PL4	Syn	10812795	1.329e+14	6655700
P	PL5	Syn	9399556	3.232e+14	10380093

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

3.4.4 Topographic statistics

Table 14: Table continues below

lim	cID	microbe	mean	var	sem	stab	start.abd
N	NL2	Phage	151575375	1.881e+16	79190498	1.105	2102121
N	NL3	Phage	292205382	6.38e+16	145831435	1.157	2362910
N	NL5	Phage	312565642	7.765e+16	160881119	1.122	2299688
P	PL2	Phage	161267374	1.047e+16	59073946	1.576	2102121
P	PL4	Phage	243403403	2.342e+16	88361764	1.59	2299688
P	PL5	Phage	312523940	3.66e+16	110450827	1.634	1841331
N	NL2	Syn	17594096	4.371e+14	12070003	0.8416	9599786
N	NL3	Syn	16110168	3.023e+14	10039048	0.9265	22353787
N	NL5	Syn	16985817	8.152e+14	16484799	0.5949	23186422
P	PL2	Syn	12944893	6.116e+14	14277915	0.5234	42190080
P	PL4	Syn	10812795	1.329e+14	6655700	0.938	31571541
P	PL5	Syn	9399556	3.232e+14	10380093	0.5228	9874066

final.abd	min.day	min.abd	max.day	max.abd
157137459	72	1422488	16	573074216
261895765	2	2362910	30	940323547
378381691	2	2299688	30	1.029e+09
237940680	2	2102121	32	659870095
378302664	2	2299688	146	7.46e+08
5.74e+08	2	1841331	21	859404750
20629744	23	93339	98	84902597
17710626	44	300299	100	80035767
1.82e+08	39	117089	166	1.82e+08
74192632	79	496642	146	153895345
19439567	63	297912	146	52700866
111891383	93	393297	166	111891383

microbe	analysis	df	pvalue
syn	mean	2.68	0.01793
syn	stability	3.63	0.5036
phage	mean	3.92	0.855
phage	stability	3.94	4e-05

3.4.5 Temporal Synchrony

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

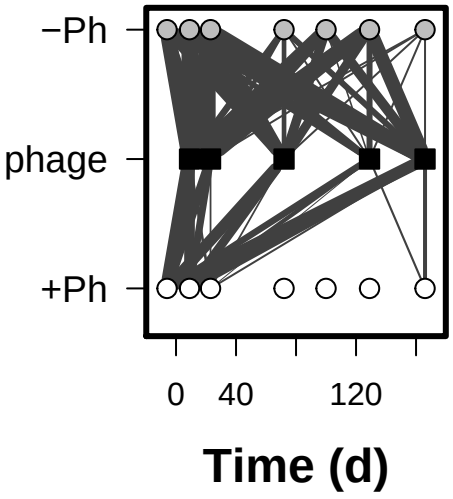
	Std.Error	t-value	p-value
(Intercept)	**0.08346**	**9.123**	**4.674e-18**
trtT	**0.1136**	**-2.871**	**0.004325**
limP	**0.1155**	**-3.857**	**0.01819**
phage.time	0.0006714	-1.743	0.08219
bac.time	0.001086	0.0845	0.9327
trtT:limP	0.1588	1.295	0.196
trtT:phage.time	0.0009219	0.7362	0.4621
limP:phage.time	**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

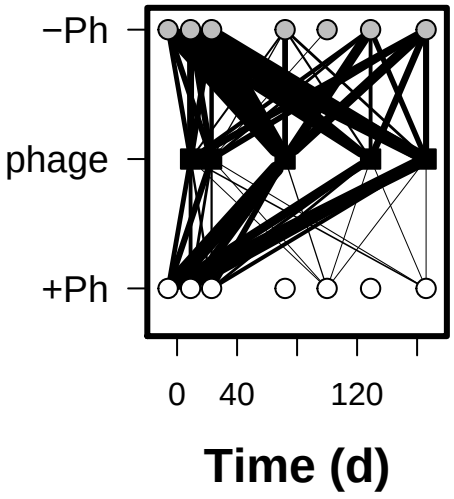
4.4 Infection dynamics by chemostat

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
```

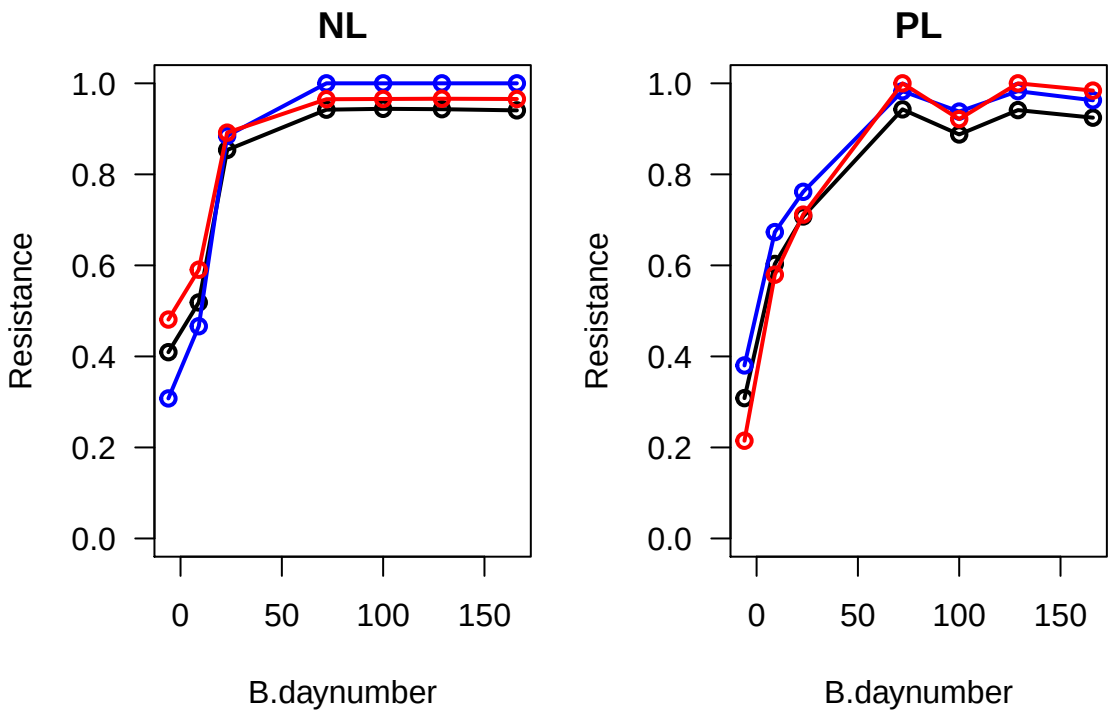
4.6 Community Networks

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
```

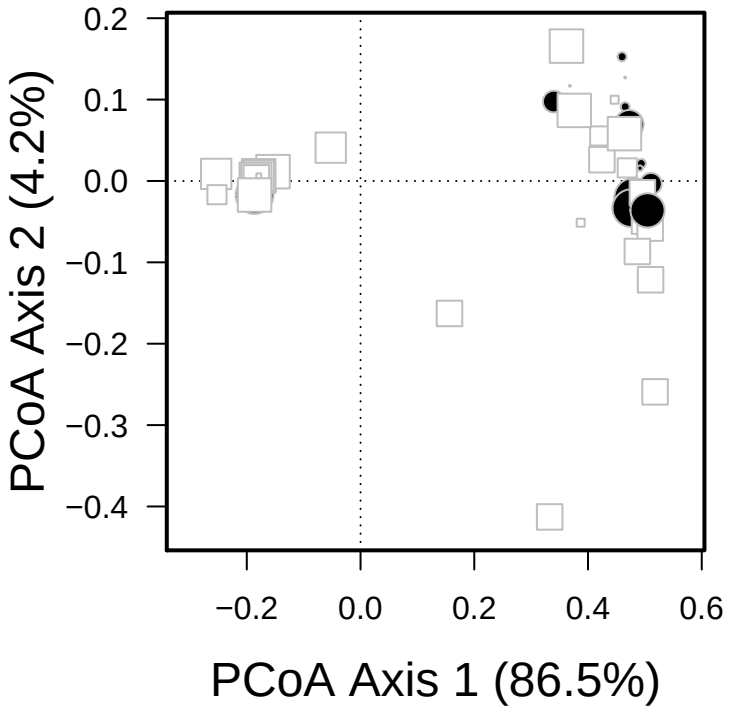
	numDF	denDF	F-value	p-value
(Intercept)	1	97	2184	0
B.trt	1	4	0.05589	0.8247
B.daynumber	6	97	31.82	0
B.trt:B.daynumber	6	97	0.5104	0.7992

	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 0.017

Time*Limitation 1 0.07149 0.07149 1.205 0.006572 0.263

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	