Baikal Fatty Acids

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Contents

1 Background

This document is meant to serve as a compilation of fatty acid analyses that MFM performed as it pertains to the study: *Effects of spatially heterogeneous lakeside development on nearshore biotic communities in a large, deep, oligotrophic lake (Lake Baikal, Siberia)*

The three main goals of the study are:

1. to identify areas of wastewater pollution using robust sewage indicators,

2. to assess the relationship between sewage indicators and littoral periphyton and macroinvertebrate community composition, and

3. to evaluate how food webs may restructure with increasing sewage pollution.

The fatty acid data largely pertain to objective #3. As the manuscript stands, we hypothesized that increased sewage loading into Baikal's nearshore would cause an increase in relative abundance of filamentous green algae (i.e. *Ulothrix* and *Spirogyra*) relative to diatoms, which would cause macroinvertebrate feeding guilds to change or cause macroinvertebrate to consume different resources (e.g., detritus, other amphipods, MAYBE the filamentous taxa).

For clarity, fatty acids are grouped according to this taxonomy:

fatty_acid_group	fatty_acid_included
SAFA	c12_0, c14_0, a_15_0, c15_0, i_15_0, c16_0, a_17_0, c17_0, i_17_0, c18_0, c20_0, c22_0, c24_0
MUFA	c14_1n5, c15_1w7, c17_1n7, c16_1w5, c16_1w6, c16_1w7, c16_1w8, c16_1w9, c18_1w7, c18_1w9, c20_1w7, c20_1w9, c22_1w7, c22_1w9
SCUFA	c16_2w4, c16_2w6, c16_2w7, c16_3w3, c16_3w4, c16_3w6, c16_4w1, c16_4w3, c18_2w6, c18_2w6t, c18_3w3, c18_3w6, c18_4w3, c18_4w4, c18_5w3
LCUFA	c20_2_5_11, c20_2_5_13, c20_2w6, c20_3w3, c20_3w6, c20_4w3, c20_4w6, c20_5w3, c22_2w6, c22_3w3, c22_4w3, c22_4w6, c22_5w3, c22_5w6, c22_6w3

Some detail on the algal taxa and invertebrates

- *Drapa spp* is an endemic filamentous green alga. As far as we know, nothing really eats it. There is anecdotal (experiential) evidence that small amphipods (e.g., *Gmeloides*) live on the *Drapa spp*, but I do not know of studies detailing what eats it.

- *Eulimnogammarus cyaneus* (Dybowsky, 1874) is a small (11-15 mm), endemic amphipod and is representative of the upper littoral zone. Reproduction takes place three times during the summer, starting May, when females first appear with eggs (Gavrilov, 1949). This species has an enhanced thermal response compared to *E. verrucosus* (Bedulina et al., 2013; 2016) and prefers temperatures approximately 11-12°C (Timofeev & Kirichenko, 2004).

- *Eulimnogammarus verrucosus* (Gerstfeldt, 1858) is a common, larger (up to 36 mm), endemic species in Lake Baikal, largely inhabiting the littoral and sublittoral zones (e.g., 0-15 m) (Kravtsova et al., 2003). *E. verrucosus* is a stenobiotic species, reproducing during the winter when temperatures are lowest (0-6°C). Females with eggs appear at the end of October, and juveniles appear at the end of May (Gavrilov, 1949). At that time, the juveniles are found at the upper littoral zone, but adults migrate to the deeper parts of the littoral zone (Weinberg & Kamaltynov, 1998). This species is thermosensitive and stenothermal (Bedulina et al., 2013; 2016), and in laboratory experiments, the adults preferred a narrow temperature zone of 5-6°C (Timofeyev & Shatilina, 2007).

- *Eulimnogammarus vittatus* (Dybowsky, 1874) is one of most common species in the littoral zone. It is thought to have similar thermosenstivity as *E. verrucosus*, although the level of study on *E. vittatus* is not as extensive as *E. verrucosus*.

- *Pallasea cancellus* (Pallas, 1776) is less common in the littoral zone than *E. cyaneus*, *E. verrucosus*, and *E. vittatus*, but they are by no means uncommon. Far less is known about *Pallasea spp*, but trophic data suggest that they are more predatory than *Eulimnogammarus spp*.

- *Snail* samples were not sorted to species, so this one is quite tricky. They were likely *Valvatidae spp* or *Planorbidae spp* based off community abundance data.

2 Summary of fatty acids

2.1 Summary statistics for all fatty acids across all taxa

Below are mean, variance, and coefficient of variation values for all fatty acid proportins across all sites and taxa.

	Mean	Variance	Var_Mean_Ratio
c18_3w3	0.1602368	0.0224841	0.1403179
c18_1w9	0.1407172	0.0121024	0.0860054
c16_4w3	0.0647749	0.0037803	0.0583604
c20_5w3	0.0955193	0.0052015	0.0544545
c20_4w6	0.0138800	0.0006002	0.0432400
c18_4w3	0.0705442	0.0026924	0.0381656
c16_1w5	0.0029166	0.0000940	0.0322270
c20_2w6	0.0056434	0.0001112	0.0196961
c16_1w7	0.0610762	0.0012012	0.0196668
c22_6w3	0.0205393	0.0003545	0.0172582
c16_0	0.1615245	0.0026012	0.0161042
c22_5w3	0.0116894	0.0001815	0.0155287
c18_0	0.0166529	0.0001983	0.0119068
c18_2w6	0.0463715	0.0004775	0.0102967
c14_0	0.0361117	0.0002687	0.0074408
c16_4w1	0.0060933	0.0000415	0.0068086
c16_3w3	0.0096756	0.0000656	0.0067835
c22_5w6	0.0024051	0.0000156	0.0064974
c18_1w7	0.0223507	0.0001301	0.0058198
c16_2w6	0.0074717	0.0000358	0.0047926
c20_0	0.0074988	0.0000249	0.0033217
c20_3w3	0.0054603	0.0000161	0.0029463
c22_0	0.0017523	0.0000046	0.0026005
a_17_0	0.0008646	0.0000022	0.0025322
c12_0	0.0029665	0.0000069	0.0023131
c16_3w4	0.0040396	0.0000089	0.0022032
c22_4w6	0.0015241	0.0000024	0.0015996
i_17_0	0.0011237	0.0000018	0.0015833
c20_3w6	0.0008363	0.0000012	0.0014270
c18_3w6	0.0034940	0.0000048	0.0013815
c17_0	0.0014715	0.0000019	0.0012587
c20_4w3	0.0048747	0.0000054	0.0011129
c16_1w9	0.0027836	0.0000019	0.0006930
c16_2w7	0.0005724	0.0000003	0.0005825
c15_0	0.0012930	0.0000006	0.0004506
c14_1n5	0.0009590	0.0000004	0.0004459
i_15_0	0.0012401	0.0000005	0.0004246
i_16_0	0.0003008	0.0000001	0.0003009
a_15_0	0.0004238	0.0000001	0.0002416
i_14_0	0.0003269	0.0000001	0.0001997

2.2 Summary statistics for all fatty acids for periphtyon

Below are mean, variance, and coefficient of variation values for all fatty acid proportins across all sites for periphyton.

	Mean	Variance	Var_Mean_Ratio
c18_3w3	0.1414195	0.0259395	0.1834228
c16_4w3	0.0493075	0.0038629	0.0783440
c18_1w9	0.1644968	0.0097339	0.0591738
c20_5w3	0.1207568	0.0064459	0.0533788
c20_4w6	0.0199291	0.0008352	0.0419088
c16_1w5	0.0032677	0.0001026	0.0313865
c18_1w7	0.0268061	0.0008102	0.0302263
c22_6w3	0.0286780	0.0005085	0.0177315
c22_5w3	0.0108428	0.0001907	0.0175867
c22_4w6	0.0035025	0.0000605	0.0172701
c20_2w6	0.0070071	0.0001168	0.0166674
c16_1w7	0.0709082	0.0010019	0.0141297
c16_0	0.1467322	0.0019309	0.0131596
c18_0	0.0193262	0.0002285	0.0118232
c14_0	0.0336907	0.0002880	0.0085492
c16_4w1	0.0072157	0.0000403	0.0055878
c22_5w6	0.0035092	0.0000192	0.0054660
a_17_0	0.0014606	0.0000075	0.0051423
c18_2w6	0.0382883	0.0001952	0.0050978
c18_4w3	0.0443305	0.0001727	0.0038949
c22_0	0.0014442	0.0000049	0.0033872
c20_0	0.0062735	0.0000211	0.0033589
c16_2w6	0.0092997	0.0000306	0.0032857
c12_0	0.0031444	0.0000071	0.0022716
c20_3w3	0.0063334	0.0000136	0.0021497
c18_3w6	0.0033713	0.0000052	0.0015398
c16_3w4	0.0047392	0.0000072	0.0015269
i_17_0	0.0015671	0.0000023	0.0014688
c17_0	0.0017828	0.0000020	0.0011276
c16_3w3	0.0054306	0.0000058	0.0010755
c20_3w6	0.0011533	0.0000012	0.0010590
c20_4w3	0.0055552	0.0000038	0.0006776
c16_1w9	0.0024596	0.0000016	0.0006666
c14_1n5	0.0011064	0.0000005	0.0004359
c16_2w7	0.0008028	0.0000003	0.0004216
i_15_0	0.0013142	0.0000005	0.0004146
c15_0	0.0014525	0.0000006	0.0004114
i_16_0	0.0004043	0.0000001	0.0003321
a_15_0	0.0005030	0.0000001	0.0002571
i_14_0	0.0003871	0.0000001	0.0002093

2.3 Summary statistics for all fatty acids for Drapa

Below are mean, variance, and coefficient of variation values for all fatty acid proportins across all sites for Drapa.

	Mean	Variance	Var_Mean_Ratio
c16_1w7	0.0335356	0.0004103	0.0122358
c16_3w4	0.0056091	0.0000166	0.0029673
c18_2w6	0.0404100	0.0000935	0.0023135
c18_3w3	0.4327014	0.0009560	0.0022095
c16_0	0.1759665	0.0003788	0.0021526
c16_4w3	0.1605045	0.0003292	0.0020507
c20_5w3	0.0044554	0.0000063	0.0014044
c18_4w3	0.0510055	0.0000568	0.0011134
c18_1w9	0.0414599	0.0000426	0.0010282
c14_0	0.0089052	0.0000086	0.0009682
c20_4w3	0.0046424	0.0000033	0.0007150
c16_3w3	0.0080142	0.0000048	0.0006022
c18_1w7	0.0044257	0.0000020	0.0004541
c16_4w1	0.0007239	0.0000003	0.0004236
c16_2w6	0.0011326	0.0000005	0.0004062
c22_0	0.0053349	0.0000014	0.0002564
c18_0	0.0069311	0.0000016	0.0002274
c22_6w3	0.0006111	0.0000001	0.0002058
c20_4w6	0.0004226	0.0000001	0.0001886
c16_1w5	0.0014810	0.0000002	0.0001392
c20_3w3	0.0013723	0.0000002	0.0001303
a_17_0	0.0000733	0.0000000	0.0000979
c20_2w6	0.0002323	0.0000000	0.0000842
i_15_0	0.0007686	0.0000001	0.0000692
c18_3w6	0.0011739	0.0000001	0.0000588
c22_5w6	0.0000394	0.0000000	0.0000568
c12_0	0.0002813	0.0000000	0.0000453
c16_1w9	0.0045466	0.0000002	0.0000393
c20_0	0.0014115	0.0000001	0.0000391
c22_5w3	0.0000090	0.0000000	0.0000280
c14_1n5	0.0002769	0.0000000	0.0000184
i_17_0	0.0001663	0.0000000	0.0000130
i_14_0	0.0000752	0.0000000	0.0000114
c15_0	0.0006408	0.0000000	0.0000078
i_16_0	0.0000570	0.0000000	0.0000049
c17_0	0.0005132	0.0000000	0.0000045
a_15_0	0.0000896	0.0000000	0.0000031

2.4 Summary statistics for all fatty acids for macroinvertebrates

Below are mean, variance, and coefficient of variation values for all fatty acid proportins across all sites for all macroinvertebrates.

	Mean	Variance	Var_Mean_Ratio
c20__4w6	0.0256185	0.0009398	0.0366842
c16__1w5	0.0037888	0.0001325	0.0349627
c18__1w9	0.2003826	0.0067343	0.0336070
c18__1w7	0.0333337	0.0008594	0.0257822
c20__5w3	0.1546781	0.0030887	0.0199687
c22__4w6	0.0045241	0.0000741	0.0163737
c16__0	0.1382055	0.0020838	0.0150777
c20__2w6	0.0089832	0.0001343	0.0149488
c22__5w3	0.0140026	0.0002026	0.0144676
c18__0	0.0229415	0.0002372	0.0103408
c22__6w3	0.0368641	0.0003536	0.0095908
c16__1w7	0.0818085	0.0006507	0.0079542
c18__2w6	0.0376694	0.0002284	0.0060641
c16__4w3	0.0168751	0.0000920	0.0054505
a__17__0	0.0018652	0.0000090	0.0048457
c18__4w3	0.0423836	0.0001929	0.0045509
c22__5w6	0.0045212	0.0000203	0.0044867
c18__3w3	0.0564622	0.0002308	0.0040878
c16__4w1	0.0091091	0.0000359	0.0039459
c14__0	0.0409198	0.0001319	0.0032244
c20__0	0.0076915	0.0000182	0.0023634
c12__0	0.0039795	0.0000061	0.0015306
c18__3w6	0.0040122	0.0000049	0.0012100
c16__2w6	0.0116817	0.0000135	0.0011569
i__17__0	0.0019757	0.0000022	0.0011288
c16__3w4	0.0044854	0.0000048	0.0010699
c20__3w3	0.0077804	0.0000080	0.0010329
c17__0	0.0021531	0.0000020	0.0009232
c16__3w3	0.0046770	0.0000037	0.0007987
c20__3w6	0.0014897	0.0000011	0.0007184
c20__4w3	0.0058215	0.0000037	0.0006384
i__15__0	0.0014733	0.0000006	0.0003936
c15__0	0.0016893	0.0000005	0.0003073
c14__1n5	0.0013483	0.0000004	0.0002650
i__16__0	0.0005055	0.0000001	0.0002525
c22__0	0.0003095	0.0000001	0.0002375
c16__1w9	0.0018509	0.0000004	0.0002052
c16__2w7	0.0010370	0.0000002	0.0001814
a__15__0	0.0006236	0.0000001	0.0001626
i__14__0	0.0004780	0.0000001	0.0001406

2.5 Summary statistics for essential fatty acids across all taxa

Below are mean, variance, and coefficient of variation values for essential fatty acid proportins across all sites and taxa.

	Mean	Variance	Var_Mean_Ratio
c18_3w3	0.3457248	0.0673328	0.1947584
c20_5w3	0.2603856	0.0367428	0.1411090
c20_4w6	0.0317146	0.0018416	0.0580665
c18_4w3	0.1674879	0.0091712	0.0547572
c22_6w3	0.0577539	0.0026625	0.0461009
c22_5w3	0.0265994	0.0005438	0.0204430
c18_2w6	0.1103338	0.0014907	0.0135112

2.6 Odd-numbered carbons

Below are intersite mean and standard deviation of odd-numbered carbon chains.

Aaron suggested odd-numbered carbons as an indicator of bacterial fatty acids.

There may be some evidence that *Eulimnogammarus cyaneus* has higher odd-numbered fatty acid chains, but that's also only with two data points across the transect. Otherwise, proportions of odd-numbered fatty acid chains seems pretty constant across all sites.

Genus	Species	mean_odd_chains	sd_odd_chains	n
Drapa	NA	0.0021409	0.0002235	4
Eulimnogammarus	cyaneus	0.0132318	0.0031237	2
Eulimnogammarus	verrucosus	0.0049136	0.0018961	6
Eulimnogammarus	vittatus	0.0068628	0.0015207	6
Pallasea	cancellus	0.0052439	0.0008387	3
Periphyton	NA	0.0041032	0.0021653	7
Snail	NA	0.0202758	0.0018310	3

2.7 Mean Fatty Acid proportions across all sites and taxa

AG suggested having some summary statistics, and I think this could be really interesting for showing breakdowns by taxanomic group. Personally, I found the following information really cool:

- Snails (although only three samples) have very high PUFA levels relative to amphipods. This could support the reasoning that snails cannot get proper nutrition near sites with high filamentous algae, so that could explain some (although not all) of decreased mollusc abundance.

- The amphipods seem to be more or less consistent in proportions, except *E. cyaneus* and *P. cancellus* appear to be higher in LCUFAs. Not sure why, but interesting. Additionally, *Eulimnogammarus spp* tend to be similar in SAFAs in comparison to *Pallasea spp*. Again, not sure why, but I know very few people looking at fatty acids of amphipods in Lake Baikal, so the potential discprepacnies are pretty neat.

- Periphyton has really high SCUFA realtive to the LCUFA, especially considering the macroinvertebrates' high abundance of LCUFA realtive to SCUFA. Could this support our conclusions in the manuscript with respect to selective grazing or trophic upgrading?

Genus	Species	n	Branched	LCUFA	MUFA	SAFA	SCUFA
Drapa	NA	4	0.0001148	0.0124741	0.0884231	0.1891418	0.7098462
Eulimnogammarus	cyaneus	2	0.0016153	0.2591262	0.3093047	0.2479450	0.1820088
Eulimnogammarus	verrucosus	6	0.0004095	0.1880347	0.3846434	0.2397720	0.1871404
Eulimnogammarus	vittatus	6	0.0007293	0.1705910	0.3711423	0.2413189	0.2162185
Pallasea	cancellus	3	0.0006872	0.2821854	0.3591169	0.1868748	0.1711357
Periphyton	NA	7	0.0004632	0.0731230	0.0922999	0.2839179	0.5501959
Snail	NA	3	0.0016824	0.4699080	0.1234863	0.1937771	0.2111462

2.8 Coefficient of variation in Fatty Acid proportions across all sites

I included the coefficient of variation for each taxon just to show that overall variation in fatty acid proportion is quite low.

Genus	Species	n	Branched	LCUFA	MUFA	SAFA	SCUFA
Drapa	NA	4	0.0000011	0.0013065	0.0099875	0.0010546	0.0027990
Eulimnogammarus	cyaneus	2	0.0001365	0.0054490	0.0002902	0.0008776	0.0058999
Eulimnogammarus	verrucosus	6	0.0000184	0.0136463	0.0007862	0.0156517	0.0068321
Eulimnogammarus	vittatus	6	0.0000939	0.0061678	0.0040677	0.0025979	0.0020210
Pallasea	cancellus	3	0.0000034	0.0015247	0.0020506	0.0207463	0.0153438
Periphyton	NA	7	0.0002408	0.0004876	0.0311680	0.0082393	0.0188555
Snail	NA	3	0.0000352	0.0024525	0.0001746	0.0016232	0.0020064

3 Univariate analyses

3.1 Comparing fatty acids by taxon over sewage gradient

I first started looking at how proportions of each fatty acid group change over the sewage gradient.

LCUFA: It's interesting that snails are just so much higher in LCUFA in comparison to amphipods, but this figure also shows how consistent amphipods remain across the gradient.

MUFA: Again, consistency in amphipods (and all taxonomic groupings really) across the gradient.

SAFA: Even though periphtyon is showing a decreasing line, the confidence envelope was quite wide, so I hesitate to speak about real trends here. Otherwise, things are pretty consistent.

SCUFA: Here, we see the peripyhyton really have an uptick in proportion. Although the fit is not the tightest, I think that it's a much stronger relationship than the SAFA relationship. I made plots of SCUFA/SAFA, but the resulting figure is similar to the current figure.

Personally, I really like a plot like this (although I will clean it up), and I think that this could be useful to inlcude in the supplement. It can be referenced in the main analysis for snails have a high nutritional demands, amphipods being consistent across the gradient, *Drappa spp.* being high in SCUFA, and periphyton increasing across the gradient.
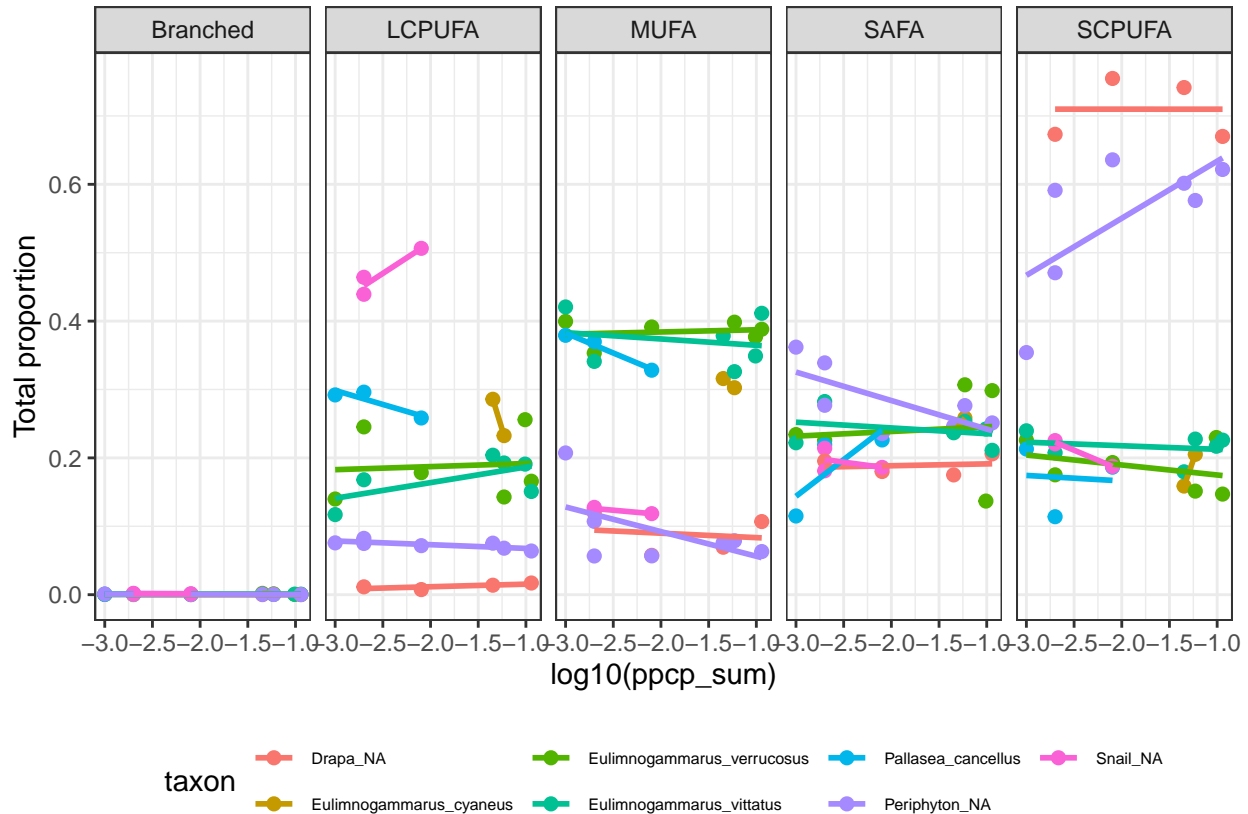
3.2 EFA Omega-3:Omega-6

AG recommended comparing ω-3:ω-6 for taxa. I did not remember there being a pattern way back when we first started, and personally I am not seeing a pattern.
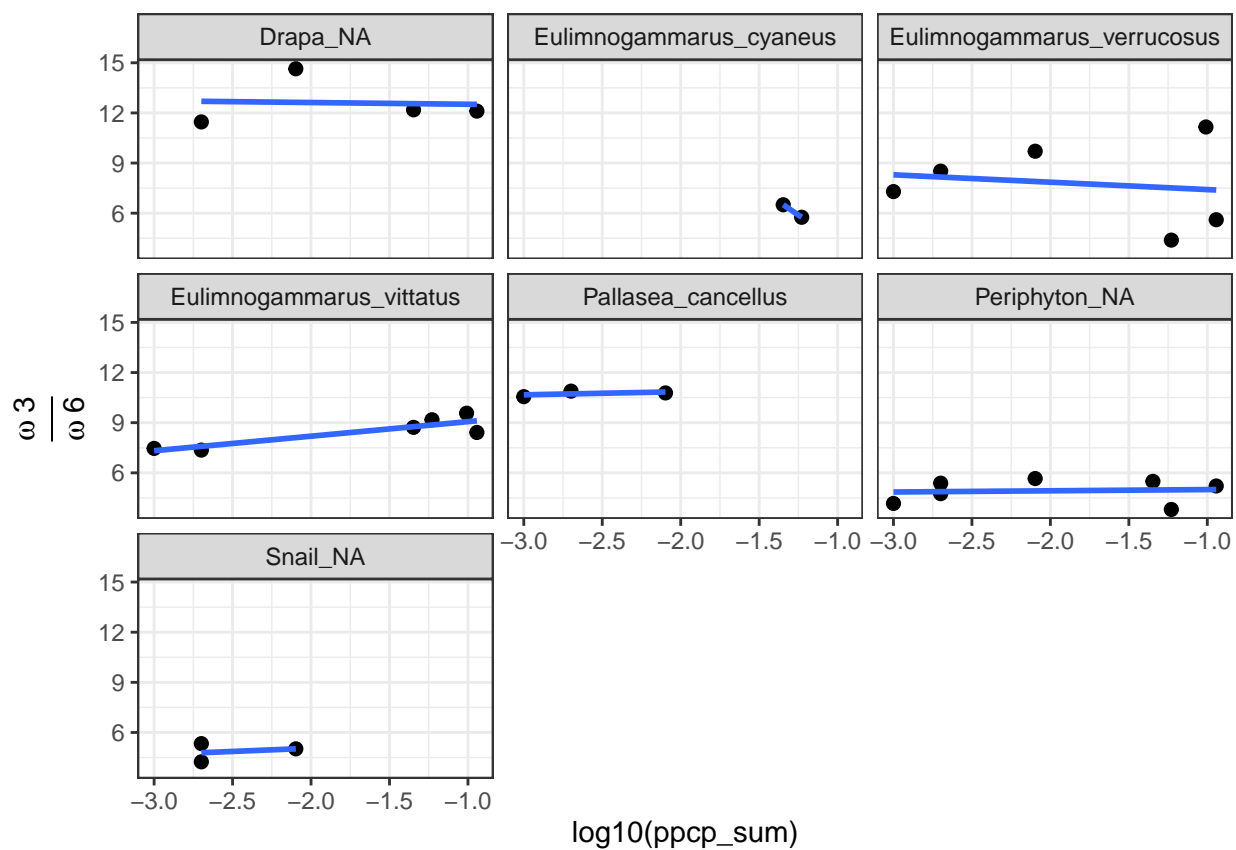
3.3 LCUFA Omega-3:Omega-6
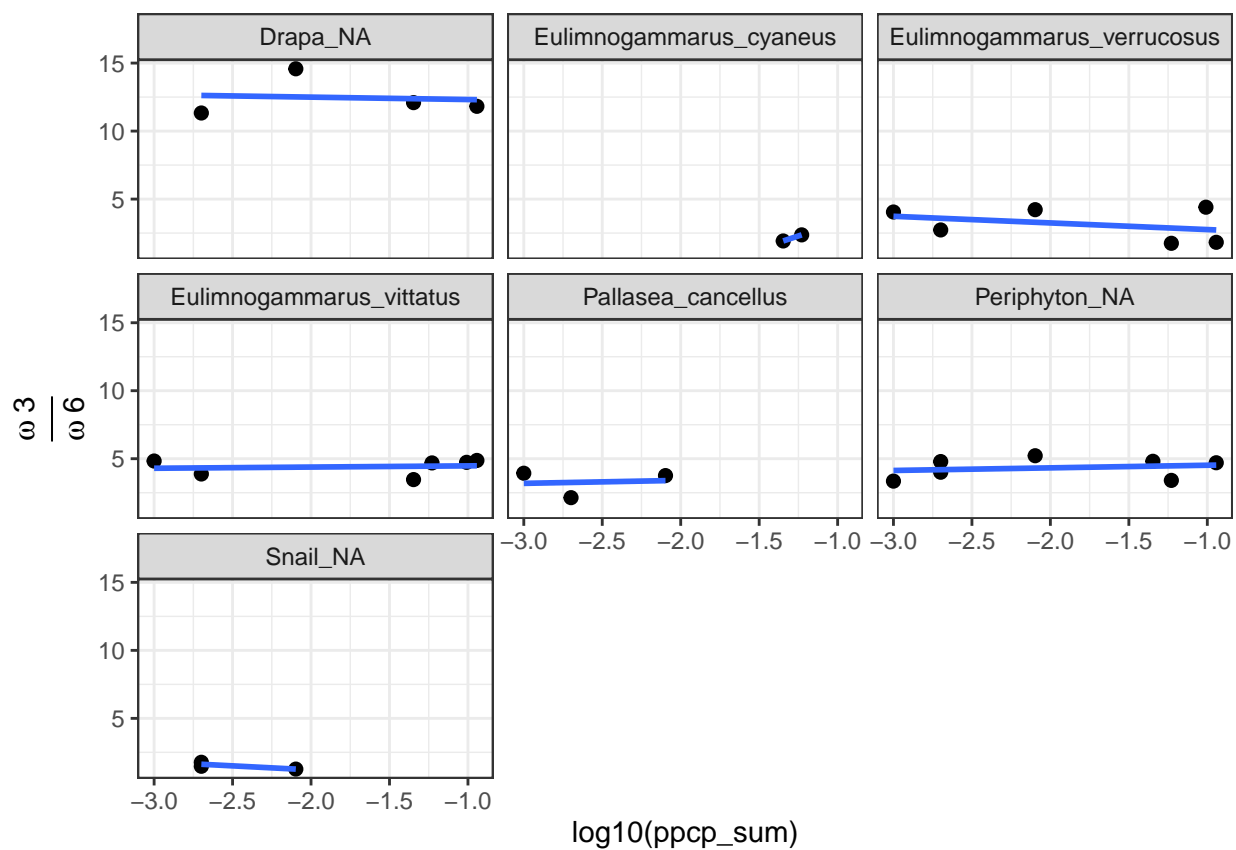
AG recommended comparing ω-3:ω-6 for taxa. I did not remember there being a pattern way back when we first started, and personally I am not seeing a pattern.

log10(ppcp_sum)
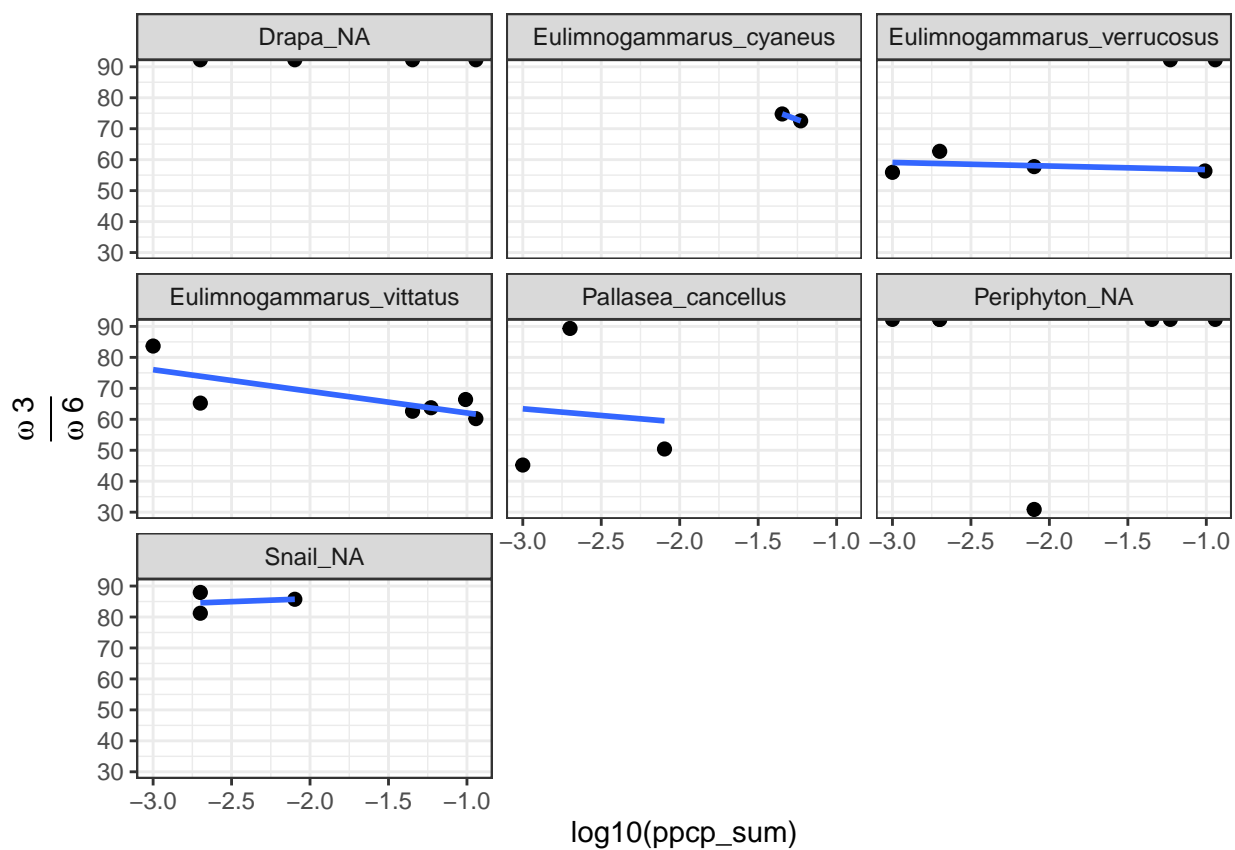
3.4 SCUFA Omega-3:Omega-6

AG recommended comparing ω-3:ω-6 for taxa. I did not remember there being a pattern way back when we first started, and personally I am not seeing a pattern.

3.5 SCUFA:SAFA

Given the variation in SCUFAs from Figure 3.1, I decided to try a SCUFA:SAFA ratio. We see that it tells a similar story as in the ms, but the relationship is not necessarily the cleanest. I also refrained from including this analysis in the ms because I was unsure as to what a SCUFA:SAFA ratio could tell us.

I am definitely open to interpretation though.
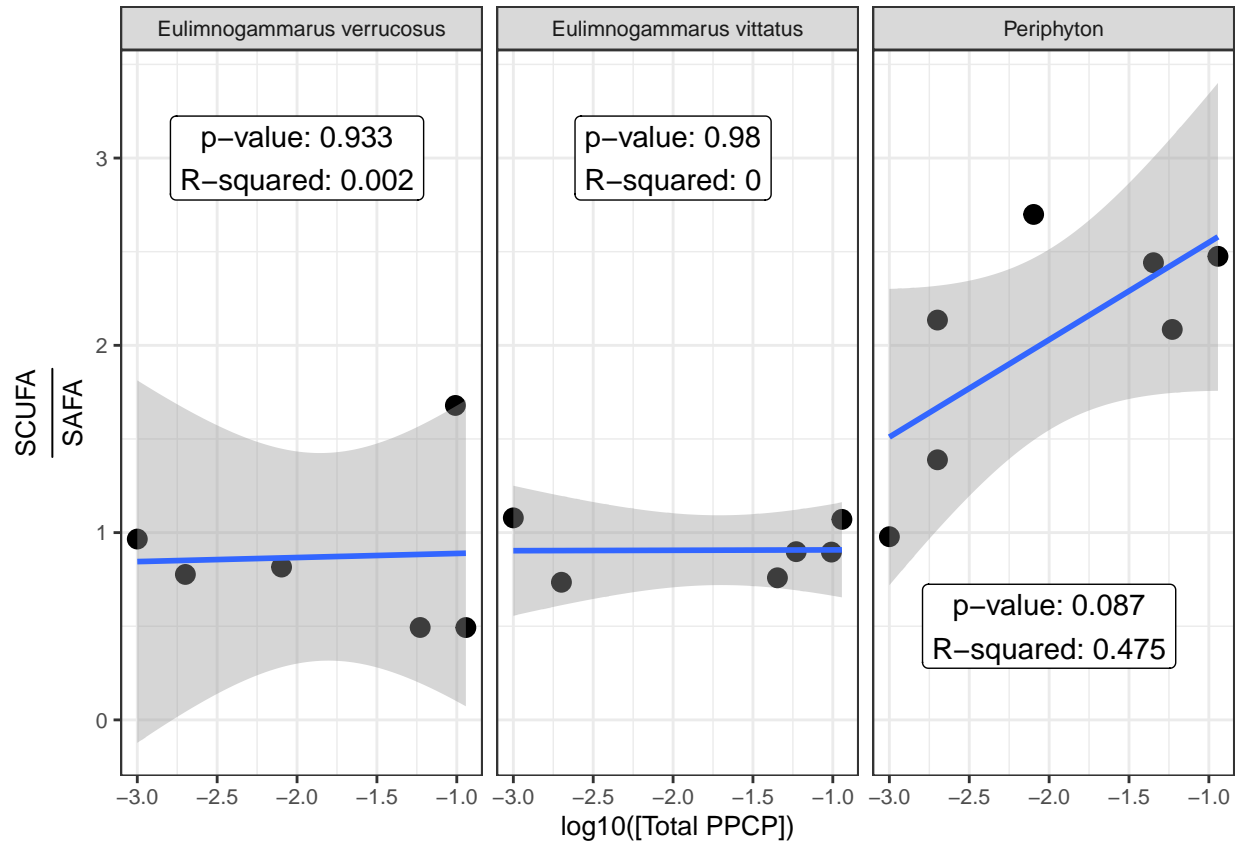
3.6 ALA:EPA

This is an updated figure to replace Figure 6 in the manuscript. It follows a similar logic as in the manuscript, where $18:3\omega3$ is meant to represent green, filamentous algal abundance and $20:5\omega3$ is meant to represent diatom abundance.

Personally, not only does is this fit much better, it also reflects the community composition patterns we saw across the sewage gradient, so we have some logic as to why we should have observed these patterns.

I removed $18:4\omega3$ from this analysis because that particular fatty acid had a lower coefficient of variation in comparison to $18:3\omega3$ and $20:5\omega3$.
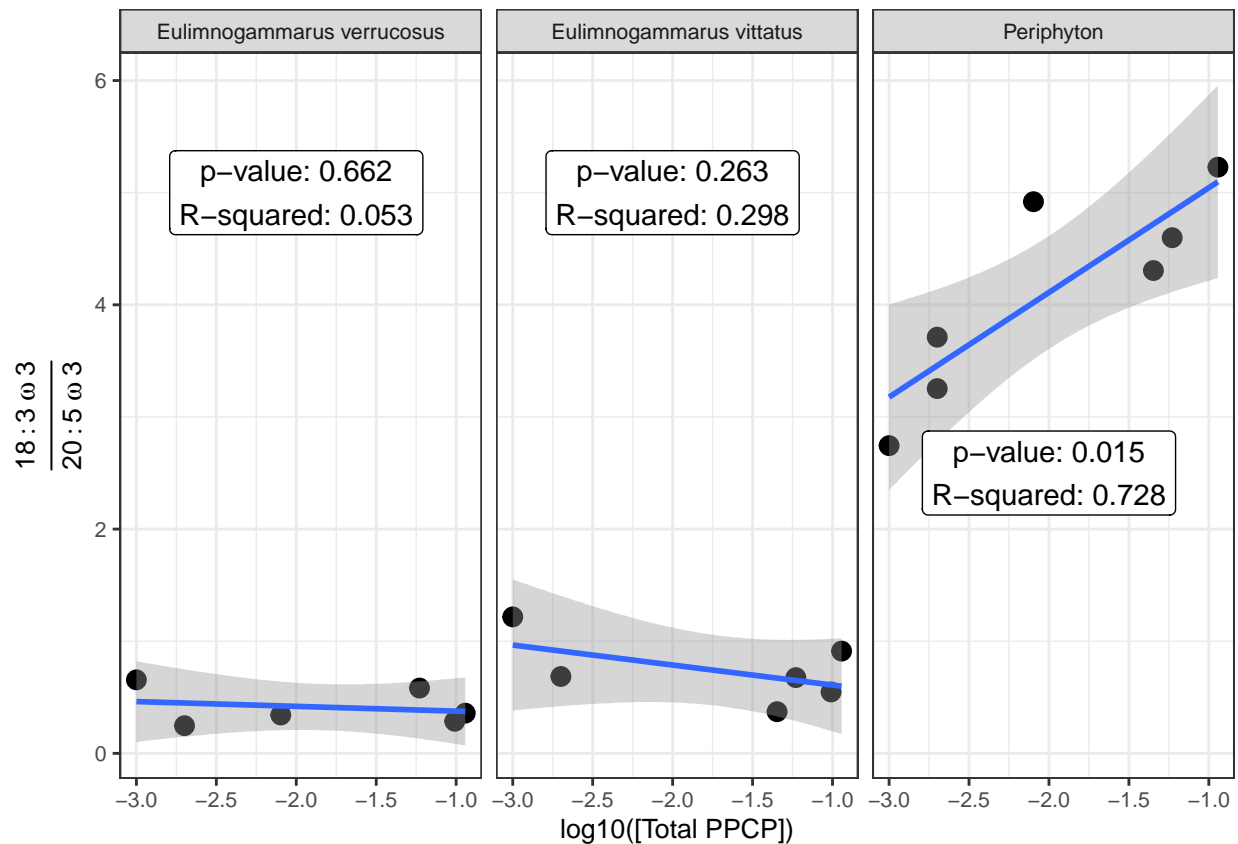
4 Multivariate FA approach

These plots are updated from the supplemental information. AG suggested that we use symbols and colors. Personally, I am not a fan of two forms of data representation meaning the same thing, so I got rid of colors in place of symbols.

Aesthetically, I like the color-version better, but I do think that the symbols help show how individual taxa are similar and where some taxa overlap slightly.

In both of these plots, 16 and 18 carbon fatty acids tend to be on the right side, whereas 20 and 22 carbon fatty acids tend to be on the left side.

4.1 Entire Fatty Acid Profile

NMDS with Entire FA Spectrum

4.2   Essential Fatty Acid Profiles

NMDS with Essential FA Spectrum
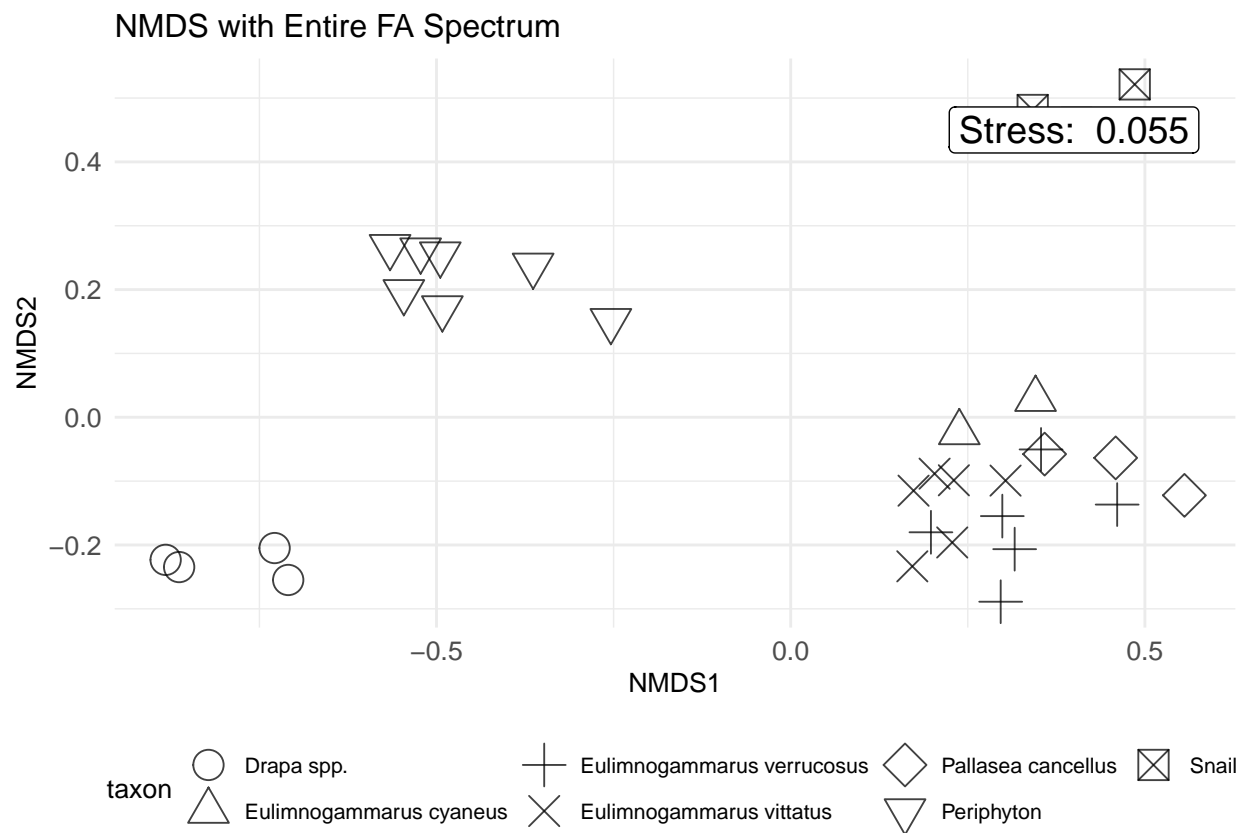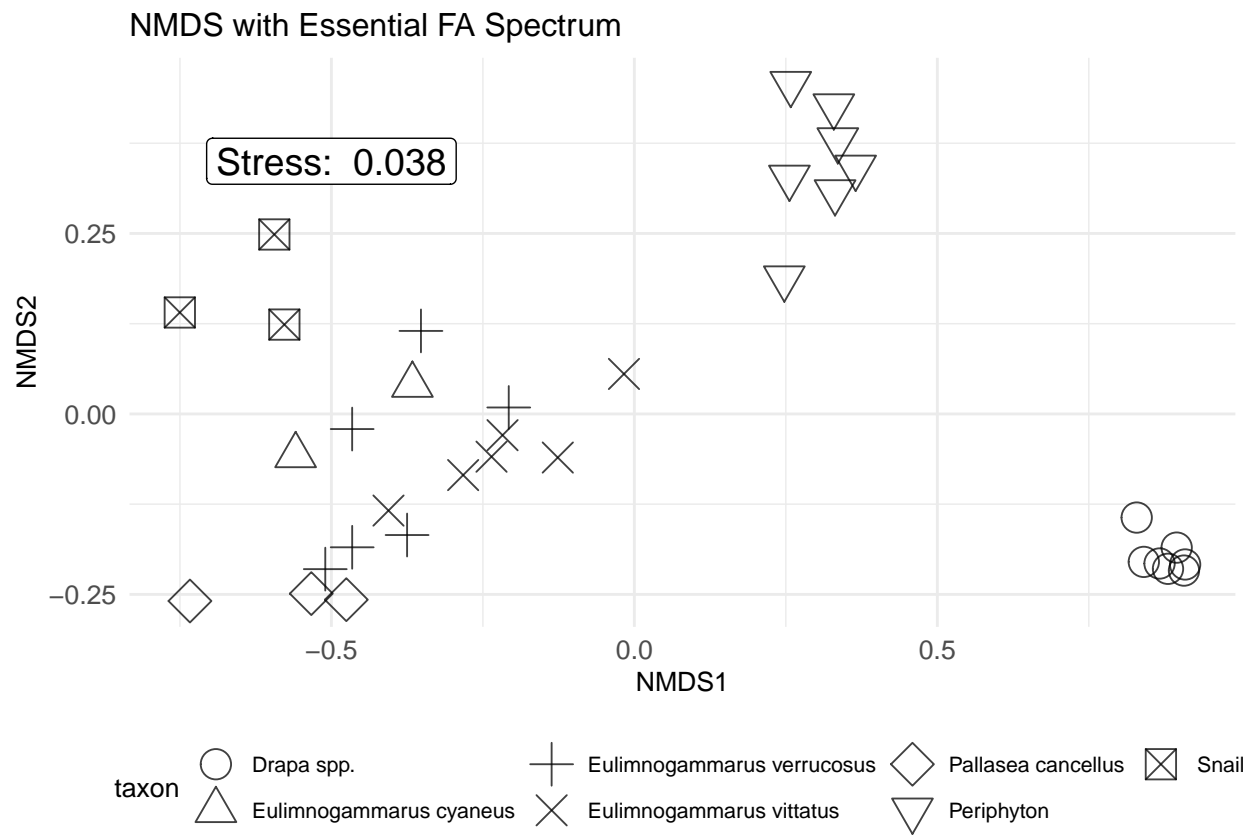
4.3 PERMANOVA

AG suggested a type of PERMANOVA to separate which fatty acids were varying most across sites.

Admittedly, I am not sure how I would structure that formula, but here's what I tried.

First, I did a normal PERMANOVA with total PPCP concentration and taxon as predictors. This test suggested that both PPCP and taxon were each significant but not the interaction.

```
## Permutation test for adonis under reduced model
## Terms added sequentially (first to last)
## Permutation: free
## Number of permutations: 999
##
## adonis2(formula = permanova_data[, 19:77] ~ ppcp_sum * taxon, data = permanova_data, method = "bray")
##                Df SumOfSqs      R2       F Pr(>F)
## ppcp_sum        1  0.03629 0.01448  3.4811  0.035 *
## taxon           6  2.25149 0.89845 35.9939  0.001 ***
## ppcp_sum:taxon  6  0.04095 0.01634  0.6546  0.809
## Residual       17  0.17723 0.07072
## Total          30  2.50596 1.00000
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

4.4 SIMPER

I then went through the SIMPER results both of total PPCP concentrations and taxons.

For PPCP concentrations alone, here were the more influential fatty acids:

pairwise_comparison	significant_fatty_acids
0.0_0.0	c16_0, c12_0
0.0_0.045	c14_1n5, i_14_0
0.0_0.098	c16_4w1
0.008_0.0	c16_1w7, c12_0, c22_5w3
0.008_0.045	i_15_0, i_14_0
0.008_0.098	c16_4w1
0.045_0.098	c16_4w1
0.059_0.098	c16_4w1
014_0.0	c18_3w6
014_0.045	i_14_0

For taxonomic concentrations alone, here are the significant ($p <= 0.05$) influential fatty acids as pairwise comparisons:

Comparison	significant_fatty_acids
Drapa E. cyaneus	c18_3w3, c16_4w3, c20_5w3, c22_6w3, c22_5w6, c22_0, c22_4w6, c15_0, i_15_0, c16_2w7, a_15_0, i_14_0, i_16_0
Drapa E. verrucosus	c18_3w3, c18_1w9, c16_4w3, c20_5w3, c14_0, c18_1w7, c16_4w1, c16_2w6, c22_0, c18_3w6, c16_1w9, c14_1n5
Drapa E. vittatus	c18_3w3, c18_1w9, c16_4w3, c14_0, c18_1w7, c20_3w3, c22_0, c12_0, c16_1w9, c14_1n5, c16_2w7, a_15_0
Drapa P. cancellus	c18_3w3, c20_5w3, c18_1w9, c16_4w3, c22_6w3, c18_1w7, c14_0, c16_2w6, c16_4w1, c12_0, c22_0, c22_4w6, c16_1w9, c20_3w6, c14_1n5, c16_2w7
Drapa Periphyton	c18_3w3, c18_4w3, c18_2w6, c14_0, c18_1w7, c16_3w3, c20_0, c16_3w4, c22_0
Drapa Snail	c18_3w3, c20_5w3, c16_4w3, c20_4w6, c22_5w3, c22_6w3, c18_0, c20_2w6, c18_1w7, c20_0, c20_3w3, c22_5w6, c16_2w6, a_17_0, c20_4w3, c22_0, c17_0, i_17_0, c20_3w6, c22_4w6, c16_1w9, c15_0, i_16_0, c16_2w7, a_15_0, i_14_0
E. verrucosus E. cyaneus	c22_5w6, i_15_0, c15_0, i_14_0, a_15_0
E. verrucosus P. cancellus	c12_0
E. verrucosus Periphyton	c18_1w9, c18_4w3, c16_4w3, c16_1w7, c18_2w6, c16_3w3, c16_4w1, c16_2w6, c16_3w4, c16_1w9, c14_1n5
E. verrucosus Snail	c18_1w9, c20_4w6, c22_5w3, c18_2w6, c20_2w6, c18_0, c20_0, c16_4w1, c22_5w6, c20_3w3, a_17_0, c20_4w3, i_17_0, c17_0, c20_3w6, c15_0, i_16_0
E. vittatus E. cyaneus	c22_5w6, i_15_0, i_14_0
E. vittatus Snail	c18_1w9, c20_4w6, c18_2w6, c22_5w3, c20_2w6, c18_0, c20_0, c22_5w6, a_17_0, c20_4w3, c17_0, i_17_0, c20_3w6, i_16_0
P. cancellus E. cyaneus	c18_1w7, c22_5w6, c12_0, i_15_0, c15_0, a_15_0, i_14_0
P. cancellus Periphyton	c18_1w9, c20_5w3, c18_4w3, c16_0, c16_1w7, c18_2w6, c22_6w3, c16_3w3, c16_2w6, c16_4w1, c12_0, c16_3w4, c20_4w3, c22_4w6, c20_3w6, c14_1n5, c16_2w7
P. cancellus Snail	c18_1w9, c20_4w6, c18_2w6, c22_5w3, c20_2w6, c18_0, c16_4w1, c22_5w6, c20_0, c12_0, c20_3w3, a_17_0, c17_0, i_17_0, c15_0, i_16_0
Periphyton E. cyaneus	c18_1w9, c18_4w3, c22_6w3, c16_3w3, c22_5w6, c15_0, i_15_0, c16_2w7, a_15_0, i_14_0
Periphyton E. vittatus	c18_1w9, c18_4w3, c16_1w7, c18_2w6, c16_3w3, c20_3w3, c14_1n5, c16_2w7
Periphyton Snail	c20_5w3, c16_0, c18_4w3, c16_4w3, c20_4w6, c22_6w3, c20_2w6, c18_0, c22_5w3, c16_3w3, c20_3w3, c22_5w6, c16_2w6, c20_4w3, a_17_0, i_17_0, c17_0, c20_3w6, c22_4w6, c15_0, c16_2w7, i_16_0
Snail E. cyaneus	c20_4w6, c22_5w3, c20_2w6, c20_0, c20_3w3, c20_4w3, a_17_0, i_15_0

4.5 dbRDA

I then started thinking that we may be interested in constraining fatty acid variation to PPCP and taxonomic variance. So, I figured that a dbRDA could be useful for that.

First when we look just at the variance explained by taxon and PPCPs, we see that there are some really good fits. 93% of the variance can be explained by these two predictors, both of which are significant.

```
## Call: dbrda(formula = permanova_data[, 19:77] ~ 0 + ppcp_sum * taxon,
## data = permanova_data, method = "bray")
##
##               Inertia Proportion Rank
## Total         1.41360    1.00000
## Constrained   1.31335    0.92909   13
## Unconstrained 0.10024    0.07091   17
## Inertia is squared Euclidean distance
## Some constraints were aliased because they were collinear (redundant)
##
## Eigenvalues for constrained axes:
##  dbRDA1  dbRDA2  dbRDA3  dbRDA4  dbRDA5  dbRDA6  dbRDA7  dbRDA8  dbRDA9 dbRDA10
##  0.9466  0.2047  0.1335  0.0119  0.0087  0.0030  0.0022  0.0012  0.0007  0.0004
## dbRDA11 dbRDA12 dbRDA13
##  0.0002  0.0001  0.0000
##
## Eigenvalues for unconstrained axes:
##     MDS1    MDS2    MDS3    MDS4    MDS5    MDS6    MDS7    MDS8
## 0.04966 0.02231 0.01533 0.00423 0.00278 0.00207 0.00130 0.00110
## (Showing 8 of 17 unconstrained eigenvalues)

## Permutation test for dbrda under reduced model
## Terms added sequentially (first to last)
## Permutation: free
## Number of permutations: 999
##
## Model: dbrda(formula = permanova_data[, 19:77] ~ 0 + ppcp_sum * taxon, data = permanova_data, method
##               Df SumOfSqs       F Pr(>F)
## ppcp_sum       1  0.01818  3.0823  0.053 .
## taxon          6  1.27118 35.9289  0.001 ***
## ppcp_sum:taxon 6  0.02399  0.6781  0.777
## Residual      17  0.10024
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

If we look at the ordination plot, it appears that the first axis separates by trophic levels, where consumers are on the left and primary producers are on the right.

The second axis seems to correspond with responses to sewage, where snails and periphyton are on top (the groups that changed the most across the sewage gradient), whereas amphipods and Drapa are lower on the y axis (taxa that remained present/consistent acros the sewage gradient).

Personally, I think this is a really cool story – one that I only realized going back to the data. I do worry however that a potential reviewer would say that we have too few data points for such an analysis, and it is more complicated of an analysis than the univariate approach. I am open to expanding this analysis, but I do think that it would add another complex layer to an already complex story. At the same time, I find it super cool that the snails separate in the same direction as periphtyon (which could have been inferred from the NMDS maybe but is made more evident in the dbRDA).
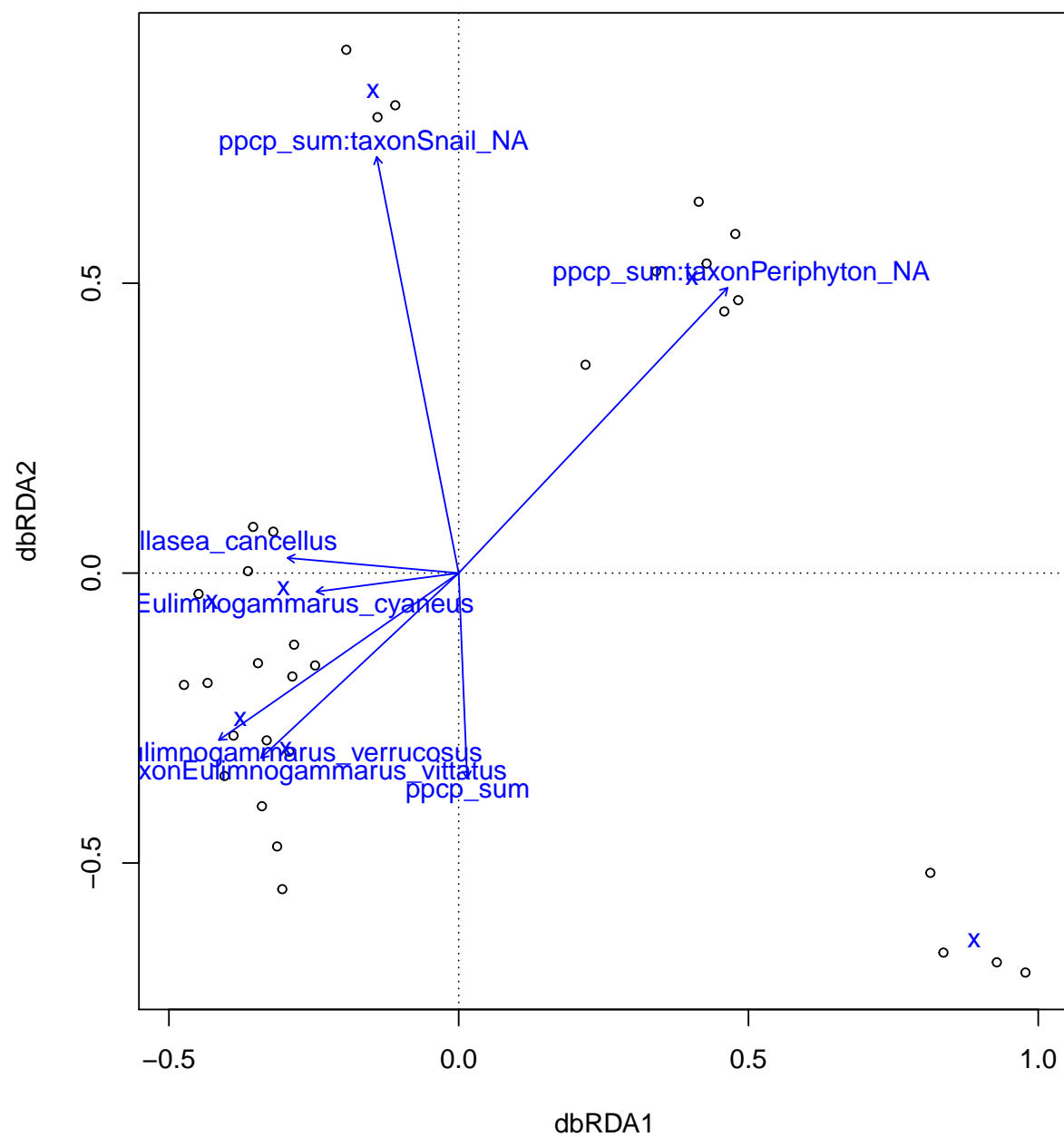
5 Follow-up analyses

During the meeting on 28 September 2020, Aaron and Julie suggested a few different approaches where we could draw a more explicit line between fatty acid profiles and the periphyton community composition. In general, the big takeaway for MFM was to use more "taxon-specific" indicators, whereas fatty acids $18:3\omega3$ and $20:5\omega3$ are not necessarily indicative of green algae and diatoms in and of themselves. Rather, the text should be worded that given our observed periphtyon communities, we can expect a certain fatty acid profile.

5.1 Green-filamentous-specific:Diatom-specific

Below is another variant of Figure 6 in the manuscript and Figure 3.6 in this document.

I went back to Taipale et al (2013), and looked at the four major fatty acids in both green algae and diatoms. I then made a ratio of $\frac{18:3\omega3+18:1\omega9+18:2\omega6+16:0}{20:5\omega3+16:1\omega7+16:0+14:0}$. I then regressed this ratio against log-transformed PPCP concentrations.

At first, I was unsatisfied with this fit not being as tight as simply $\frac{18:3\omega3}{20:5\omega3}$. However, I do think that it does a more explicit job of looking at the green:diatom ratio that we are trying to get at. Additionally, I poked around the Baikal diatom fatty acid literature, and apparently diatoms in Baikal can have quite a range of PUFA signatures (see Shishlyannikov et al. 2018). In particular $16:1\omega7$ is prominent in endemic Baikal diatoms, but it's abundance can be variable within a species. Additionally, *Drapa spp.* has low $16:1\omega7$ proportions, but a similar coefficient of variation as periphyton (see tables 2.2 and 2.3). So, this is a long way of saying that $16:1\omega7$ messess with the cleanliness of this trend BUT variation in $16:1\omega7$ is expected and demonstrated before in Baikal diatoms.
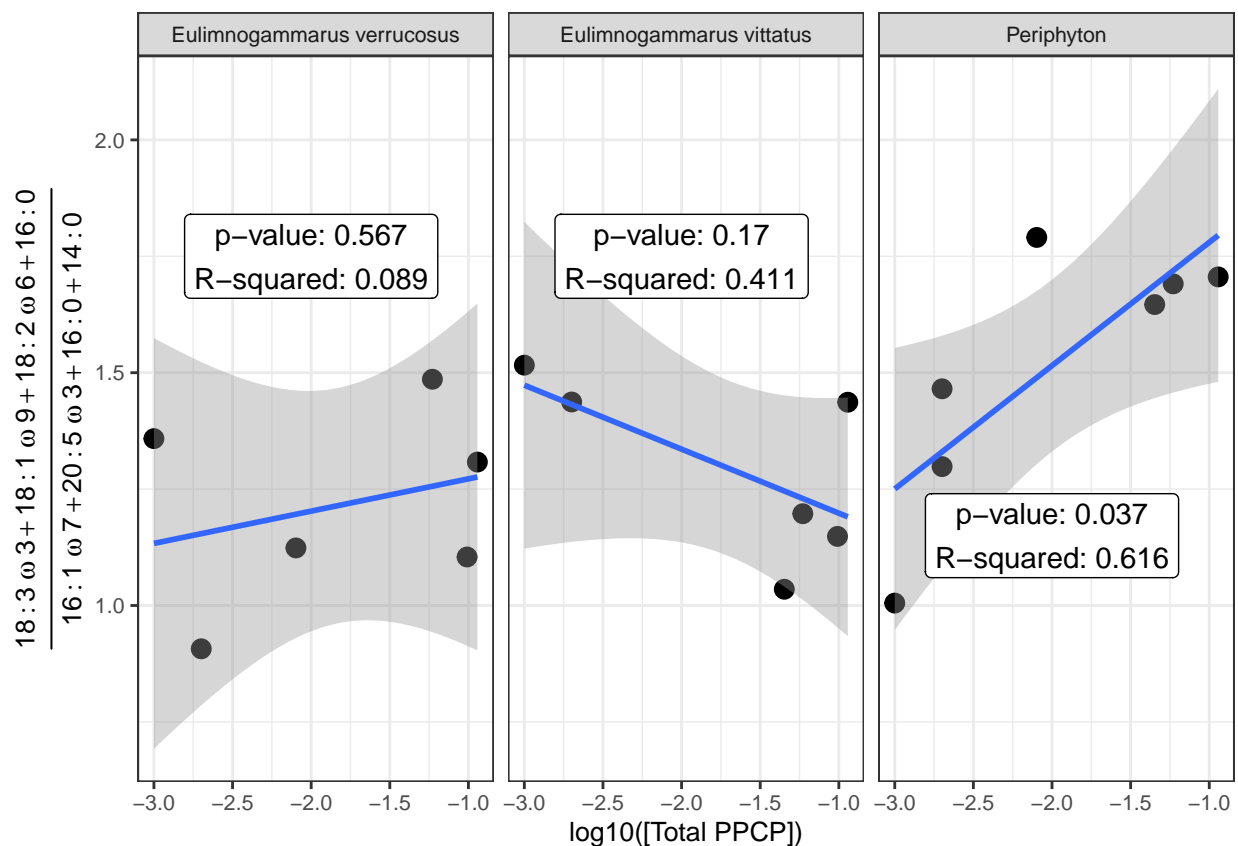
5.2 16-Carbon:18-Carbon ratios

Aaron recommended separating the C16-PUFAs and C18-PUFAs. Like section 3.1 in this document, the combined short-chain PUFAs (as defined here) vary most intensely across our gradient in the periphyton.
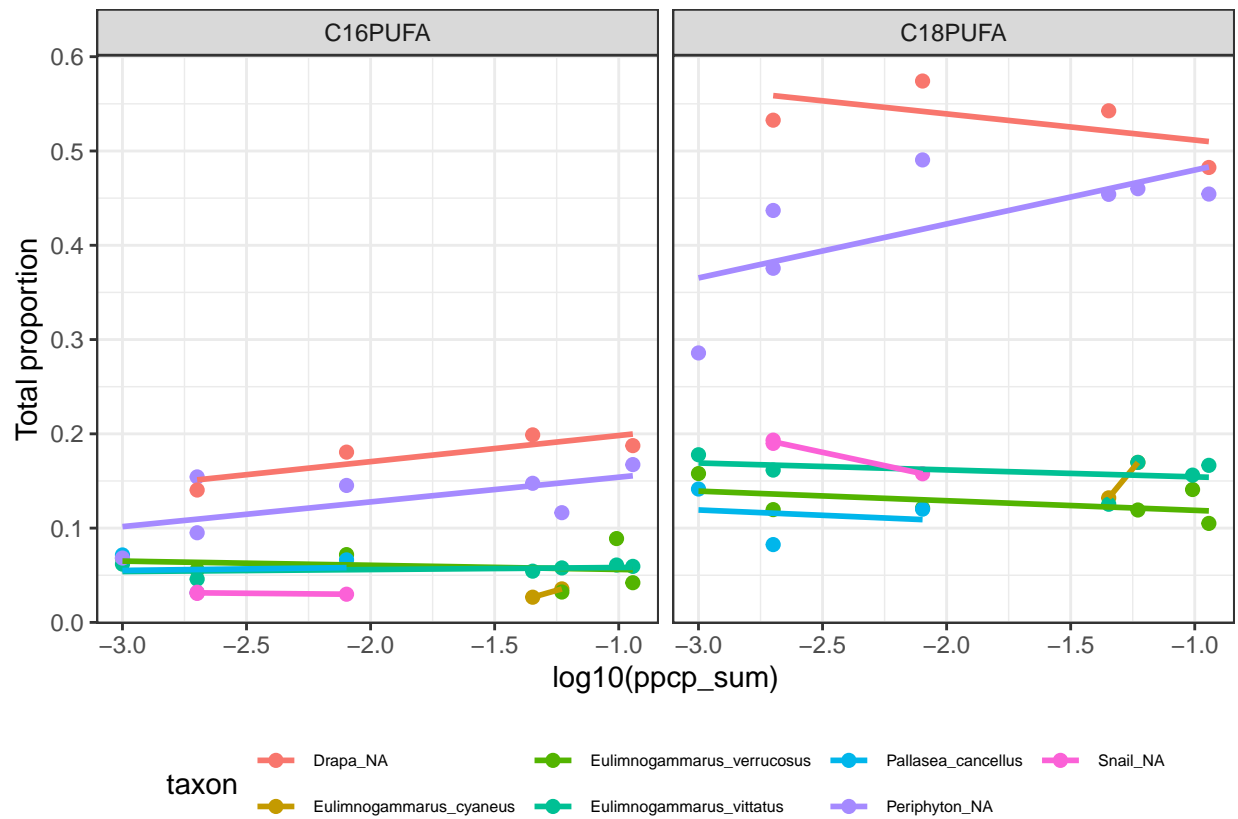
5.3 Branched and Odd-Carbon Fatty Acids

Julie recommended looking just as branched and odd-carbon fatty acids. I ran this analysis just on the periphyton (as the other taxa were consistent across the gradient). Periphtyon Branched and Odd-Carbon Fatty Acids tend to decrease across the gradient slightly yet significantly.

Personally, I am not sure what this means (if it means anything). We should expect Branched and Odd-Carbon Fatty Acids to increase with sewage, but this could be evidence of the PPCPs actually having an effect by decreasing bacterial community abundance – something that Emma noted in her 2013 study.
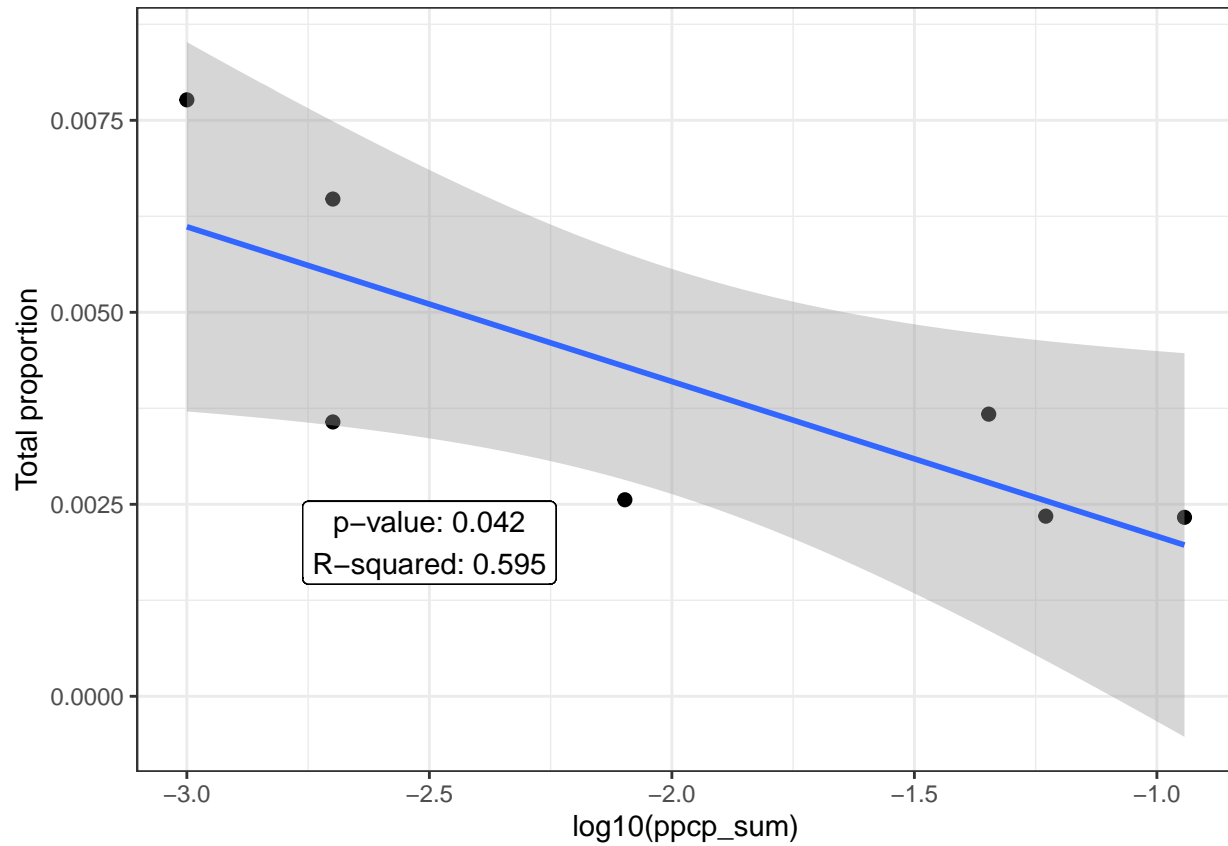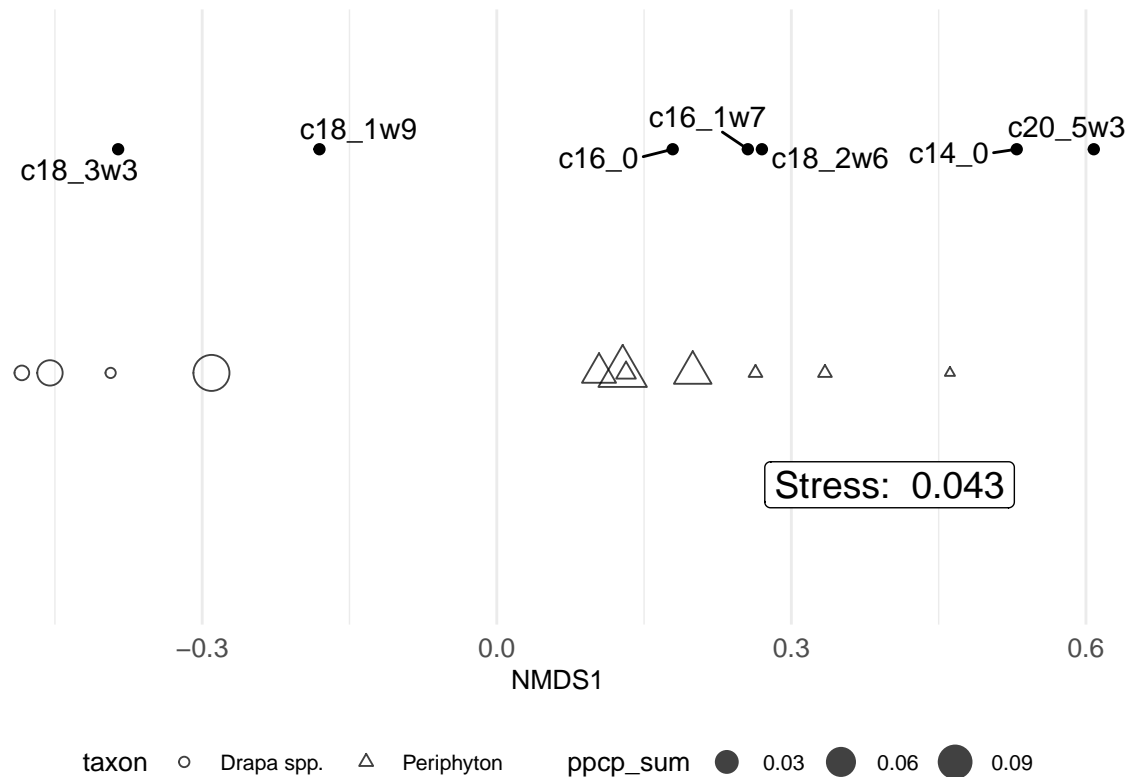
5.4   Multivaraite primary producer analysis

Here I tried an NMDS just with $18:3\omega3$, $18:1\omega9$, $18:2\omega6$, $16:0$, $20:5\omega3$, $16:1\omega7$, $16:0$, and $14:0$ from *Drapa spp.* and periphyton. The analysis only converged when I used one dimension.

The y-axis mean nothing – just something I fixed in place. The plot shows that as we move from left to right, periphyton tend to have a higher green-filamentous signature. Dots above the shapes demonstrate directionality of the fatty acids (think of this as the first axis of PCA with the corresponding eigenvalues compressed to one dimension).

NMDS with Filamentous:Diatom Fatty Acids

5.5 Multivaraite amphipod analysis

Here I tried an NMDS just with $18:3\omega3$, $18:1\omega9$, $18:2\omega6$, $16:0$, $20:5\omega3$, $16:1\omega7$, $16:0$, and $14:0$ from macroinvertebrates.

Personally, I am not seeing any patterns with species or PPCP concentration, which would be expected since the amphipods see to be compensating somehow.

NMDS with Filamentous:Diatom Fatty Acids

marus cyaneus △ Eulimnogammarus verrucosus + Eulimnogammarus vittatus × Pallasea cancellus ppcp_sun