

# SCGSR final report

2023-06-21

## Sample Summary

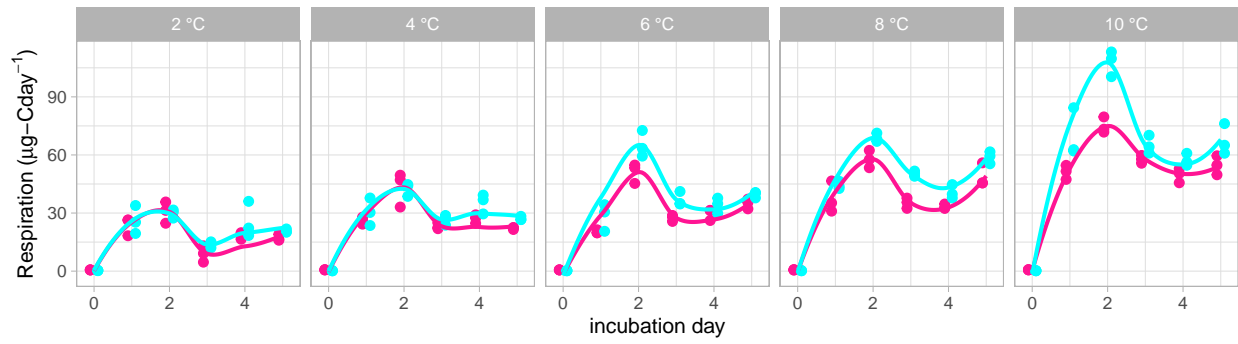
Soils from northwest Alaska were homogenized and pre-incubated at -2 and -6 degrees Celsius for three months after which they were incubated at 2,4,6,8,10 degrees Celsius for one week. After the week long incubation soils were extracted using 0.5M K<sub>2</sub>SO<sub>4</sub>, and chloroform extracted to measure microbial biomass and nutrient concentrations. Sub-samples were also sent to PNNL for MPLE<sub>x</sub> (Methanol chloroform extraction) to provide more comprehensive analysis of the molecular composition of organic matter using FT-ICR, NMR, GC-MS and LC-MS techniques. Lipidomics were also performed to ascertain if there were any significant shifts in microbial biomass.

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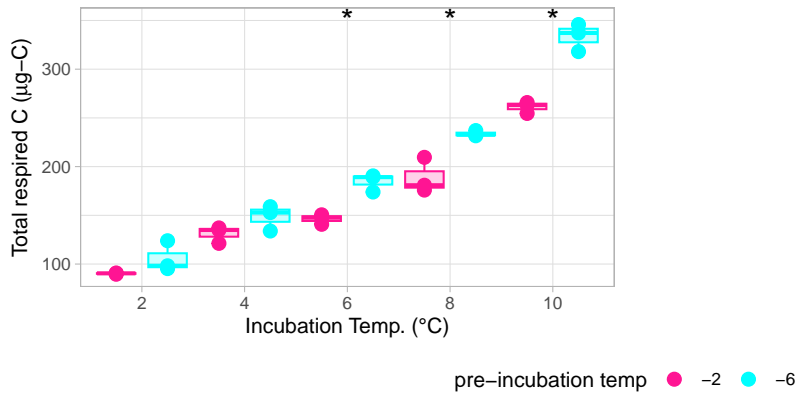
## Respiration Results

Respiration measurements were taken daily during the incubation using a Li-850 bench top respiration unit. Below are the respiration rates for each sample, as well as the calculates total C respired. Linear mixed effects model showed significant respiration variation by incubation and pre incubation temperatures. An asterisks indicates a significant ( $p \leq 0.05$ , ANOVA) difference in pre-incubation temperature.

## A Soil Respiration

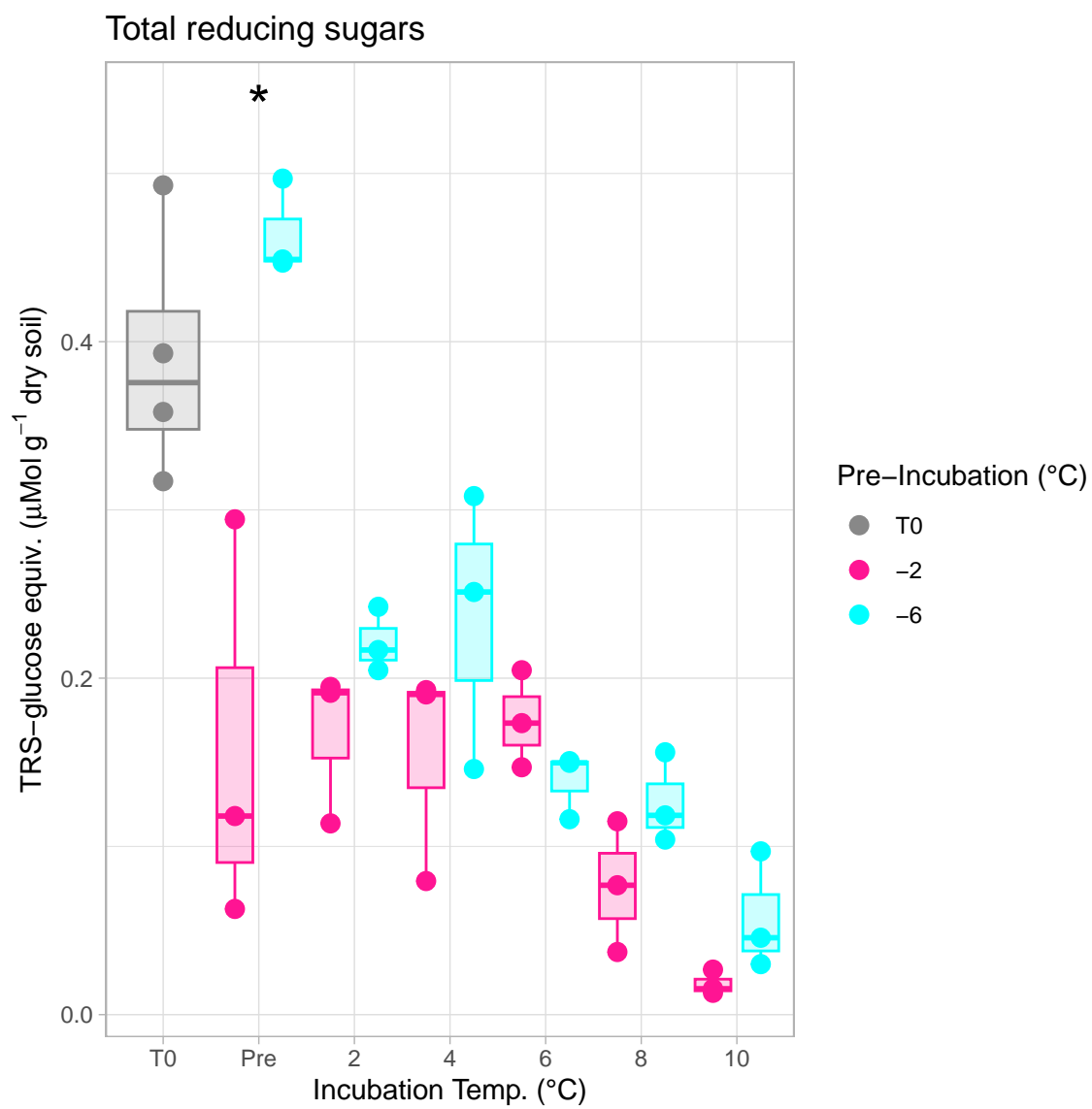


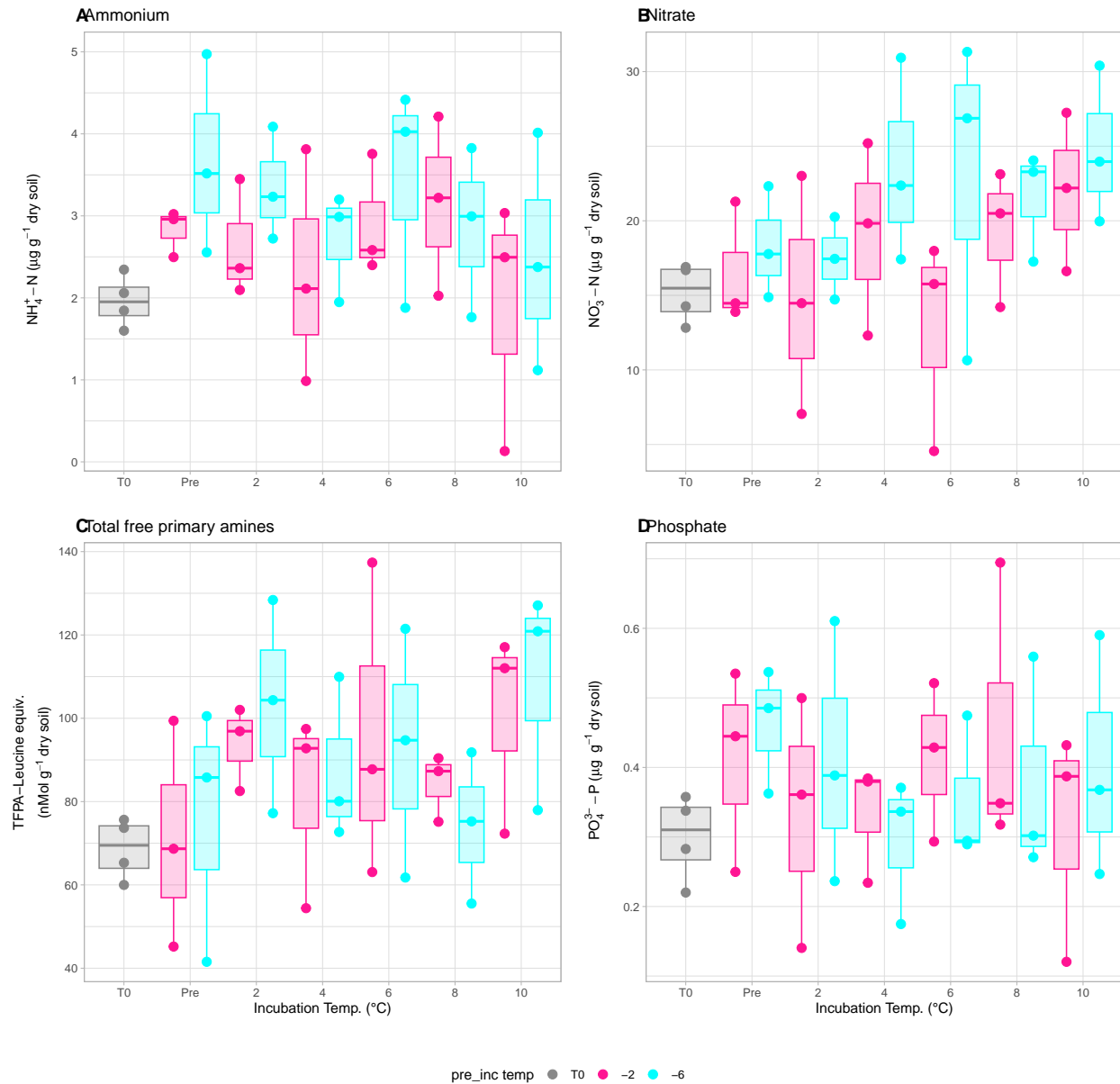
## B Cumulative respiration



## Soil Nutrients

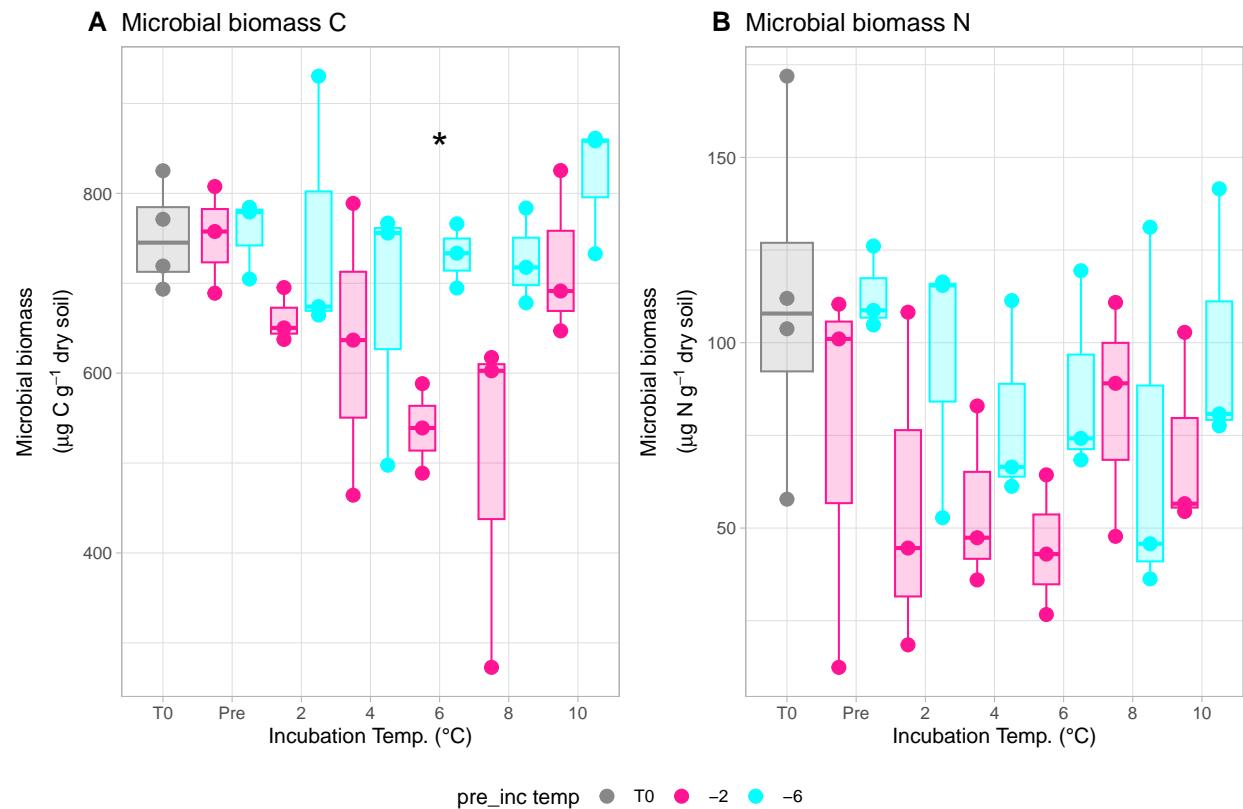
Soil K<sub>2</sub>SO<sub>4</sub> extracts were utilized to measure ammonium, Nitrate, Total free primary amines, phosphate, Total reducing sugars. Below are the concentration data. An asterisks indicates a significant ( $p \leq 0.05$ , ANOVA) difference in pre-incubation temperature.





## Microbial Biomass

Soil K<sub>2</sub>SO<sub>4</sub> extracts were utilized to measure ammonium, Nitrate, Total free primary amines, phosphate, Total reducing sugars. Below is the concentration data. An asterisks indicates a significant ( $p \leq 0.05$ , ANOVA) difference in pre-incubation temperature.



## GC-MS

Below is the relative quantification of compounds identified by gas chromatography within the MPLEx extracts. Little to no variation was identified that corresponds to the more broad metrics above in the soil nutrient section. The majority of compounds measured were unidentified. Volcano plot can be used to identify the compounds that are significantly greater between pre incubation temperature ( $p < 0.05$ , ANOVA). After which we used a PCA to visualize separation between the pre incubation temperatures across significantly different compounds. PERMANOVA results are displayed in the table below the PCAs to show variation between treatments.

## GC volcano

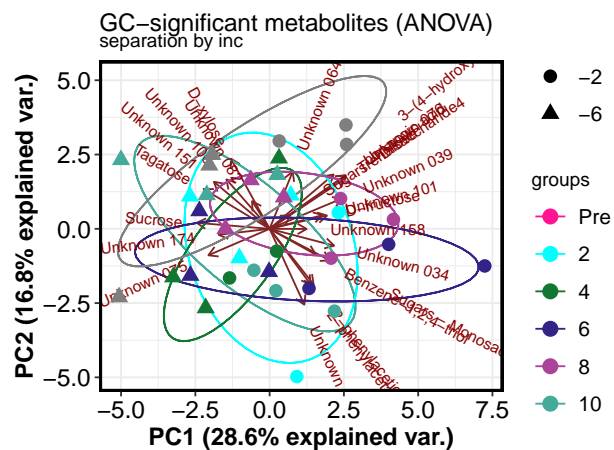
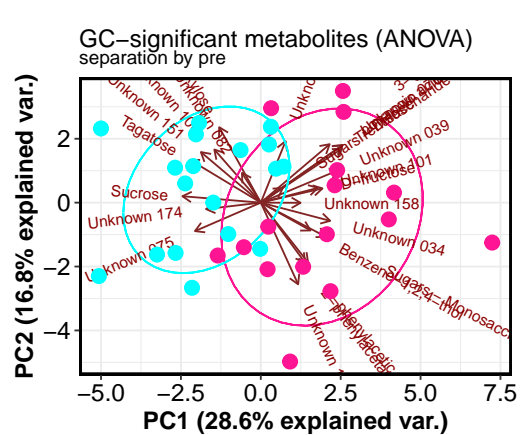
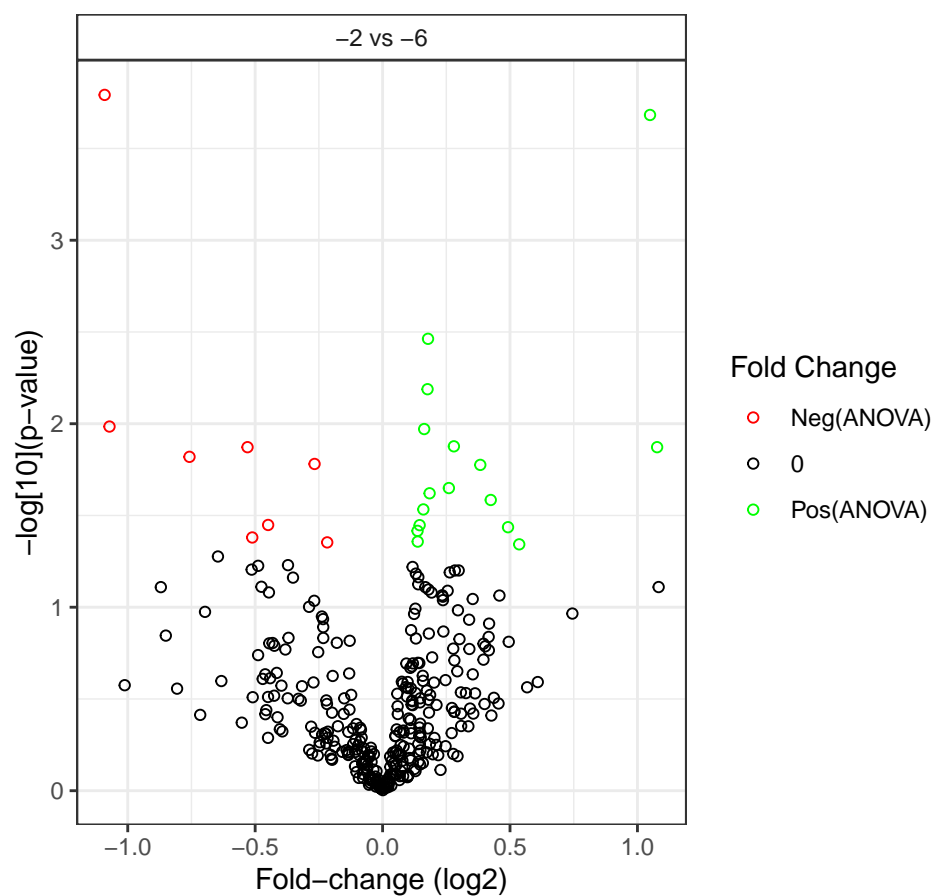
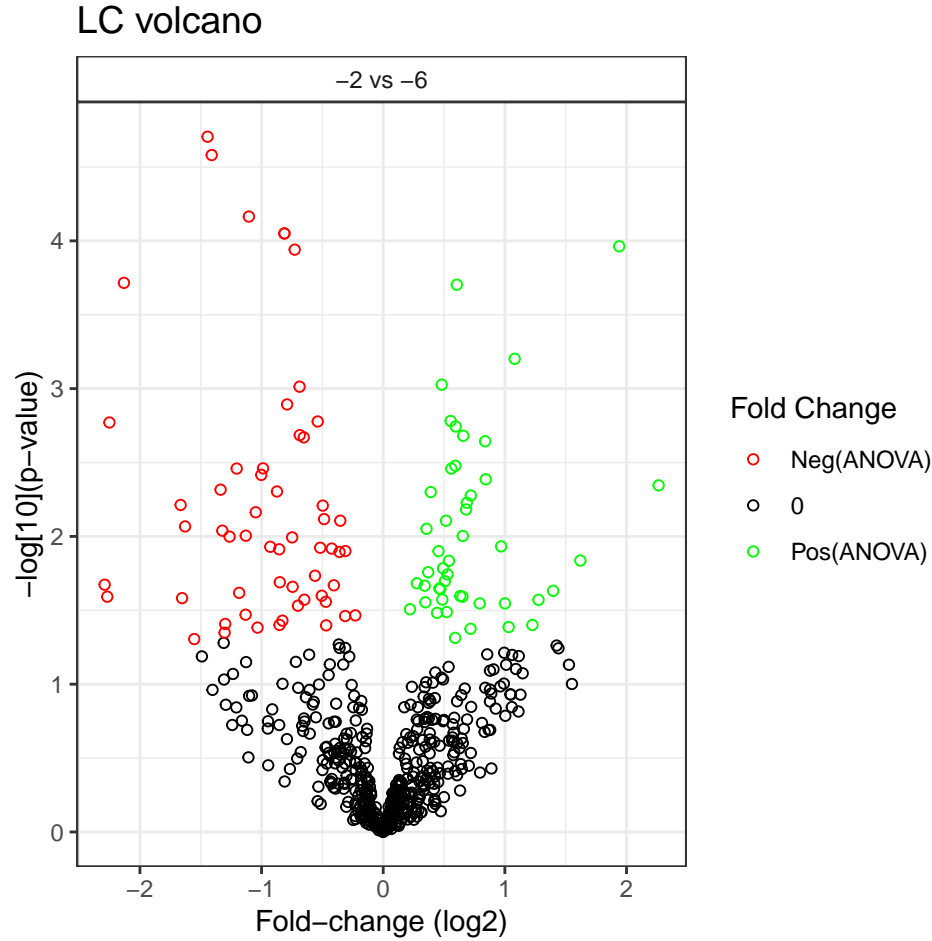


Table 1: Permanova results significant compounds only

	Df	SumOfSqs	R2	F	Pr(>F)
pre	1	0.0014341	0.1722985	7.519439	0.001
inc	5	0.0014313	0.1719666	1.500991	0.088
pre:inc	5	0.0012621	0.1516327	1.323509	0.152
Residual	22	0.0041957	0.5041023	NA	NA
Total	33	0.0083231	1.0000000	NA	NA

## LC-MS

Below is the relative quantification of compounds identified by liquid chromatography within the MPLEx extracts. Little to no variation was identified that corresponds to the more broad metrics above in the soil nutrient section. The majority of compounds measured were unidentified. Volcano plot can be used to identify the compounds that are significantly greater between pre incubation temperature ( $p < 0.05$ , ANOVA). After which we used a PCA to visualize separation between the pre incubation temperatures across significantly different compounds. PERMANOVA results are displayed in the table below the PCAs to show variation between treatments.



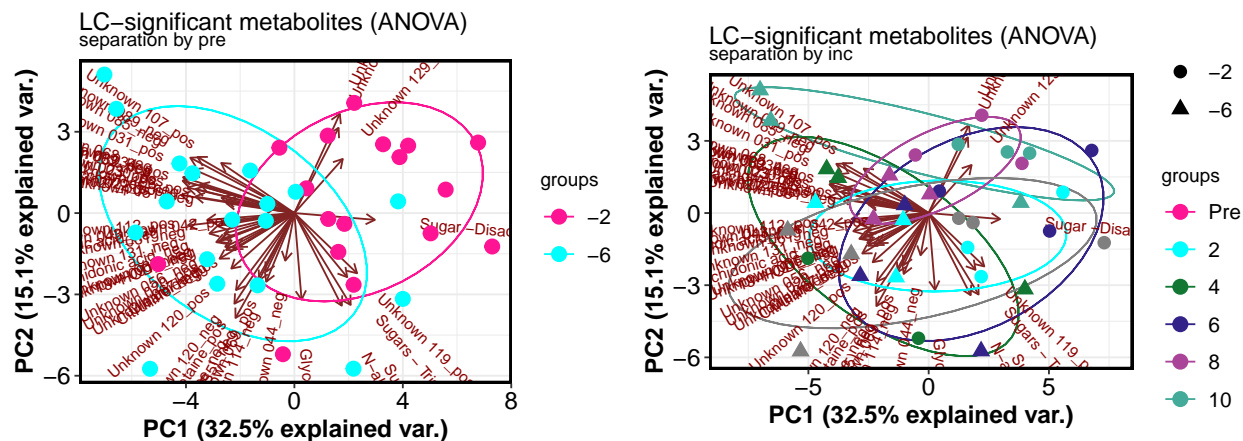


Table 2: Permanova results significant compounds only

	Df	SumOfSqs	R2	F	Pr(>F)
pre	1	0.0023887	0.1496193	7.120665	0.001
inc	5	0.0033118	0.2074340	1.974436	0.012
pre:inc	5	0.0025492	0.1596711	1.519810	0.079
Residual	23	0.0077157	0.4832756	NA	NA
Total	34	0.0159654	1.0000000	NA	NA

## Lipids

Lipid analysis was done via liquid chromatography on MEPLEx extracts. Some variation was identified between pre-incubation temperatures, though little was biologically significant. Conclusion that small changes in biomass were present but not significant. A big missing piece to this analysis would be community composition. Little no no variation was observed within this data set. PCAs below show little to no separation between incubation and pre incubation temperatures.



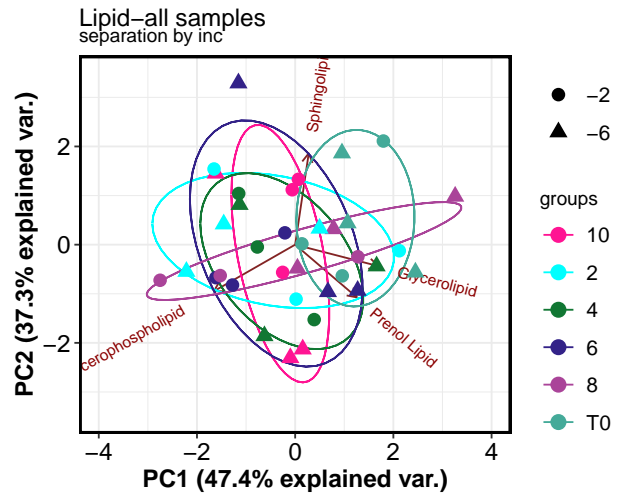
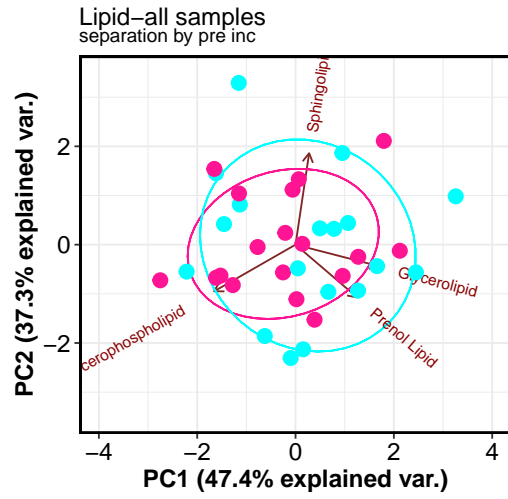


Table 3: Permanova results all

	Df	SumOfSqs	R2	F	Pr(>F)
Pre	1	0.0000127	0.0312988	1.259669	0.302
Inc	5	0.0000742	0.1823599	1.467871	0.175
Pre:Inc	5	0.0000773	0.1900165	1.529501	0.168
Residual	24	0.0002427	0.5963247	NA	NA
Total	35	0.0004071	1.0000000	NA	NA

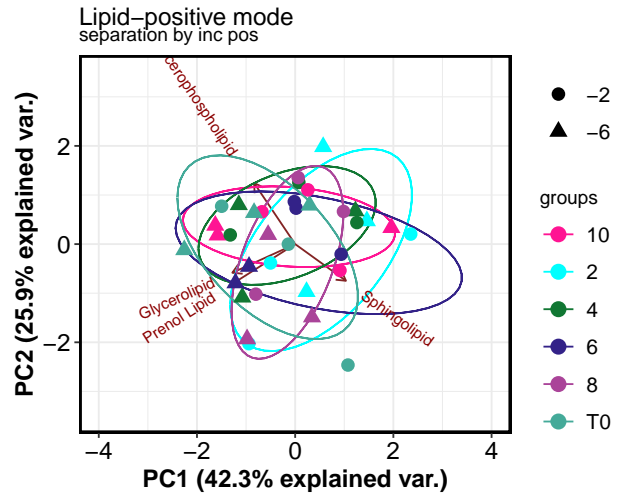
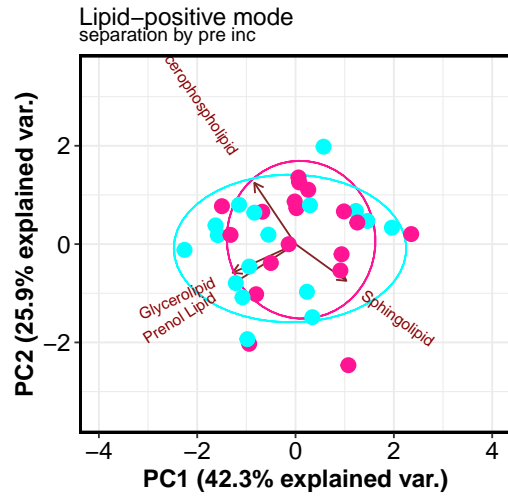


Table 4: Permanova results pos

	Df	SumOfSqs	R2	F	Pr(>F)
Pre	1	0.0000064	0.0153686	0.5551514	0.594
Inc	5	0.0000636	0.1537306	1.1106287	0.366
Pre:Inc	5	0.0000689	0.1664960	1.2028526	0.328
Residual	24	0.0002749	0.6644048	NA	NA
Total	35	0.0004138	1.0000000	NA	NA

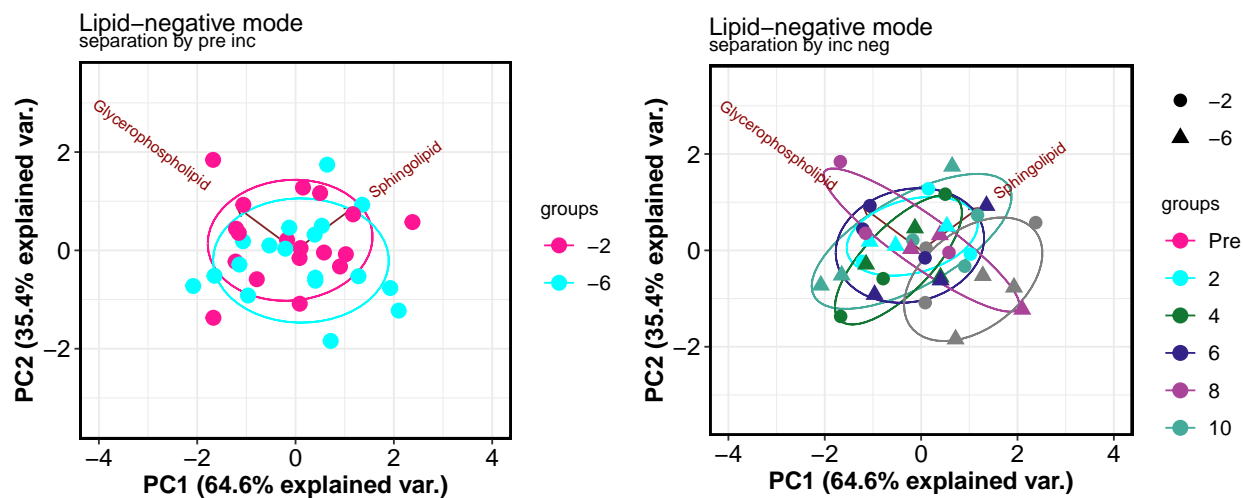


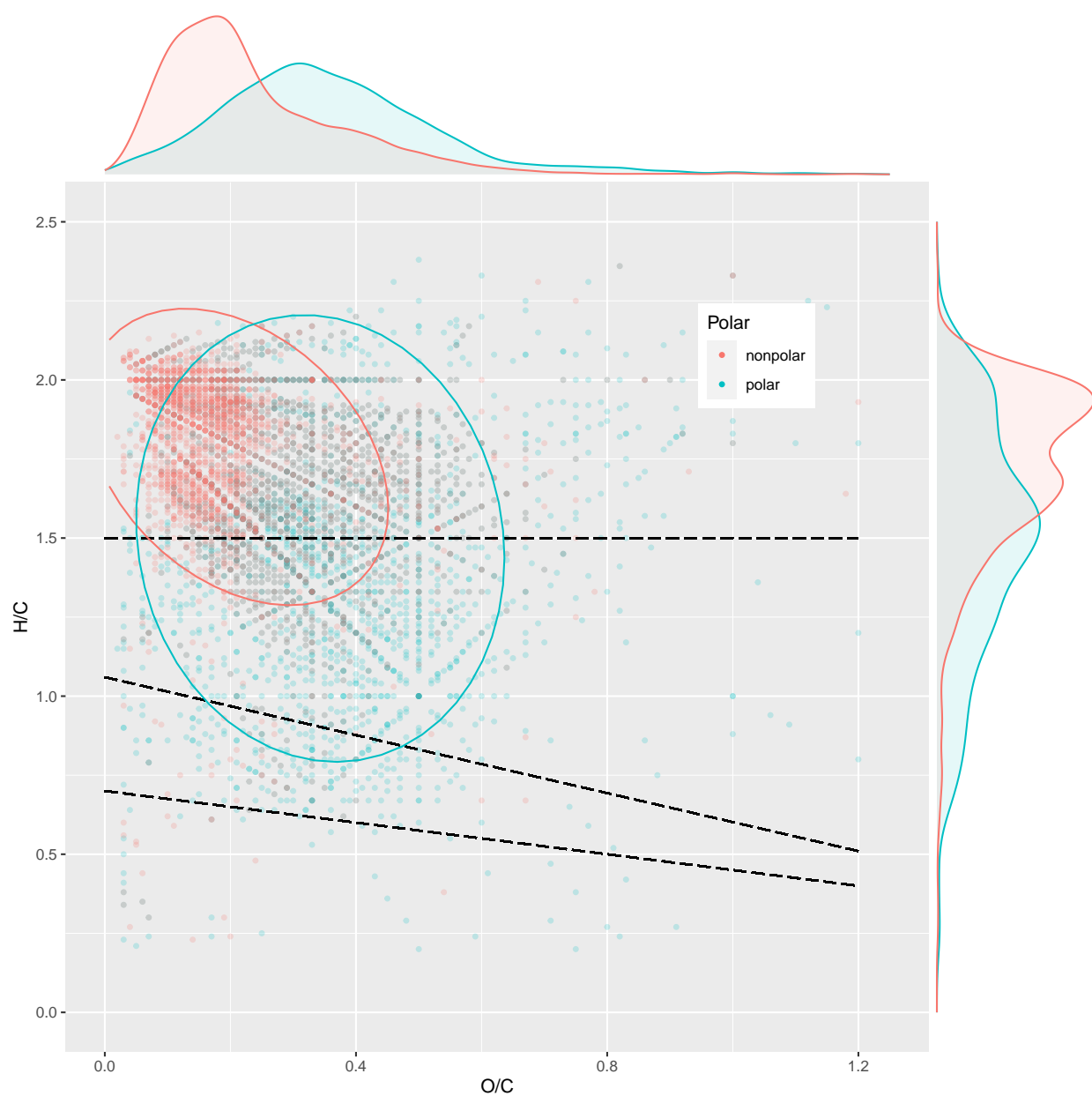
Table 5: Permanova results neg

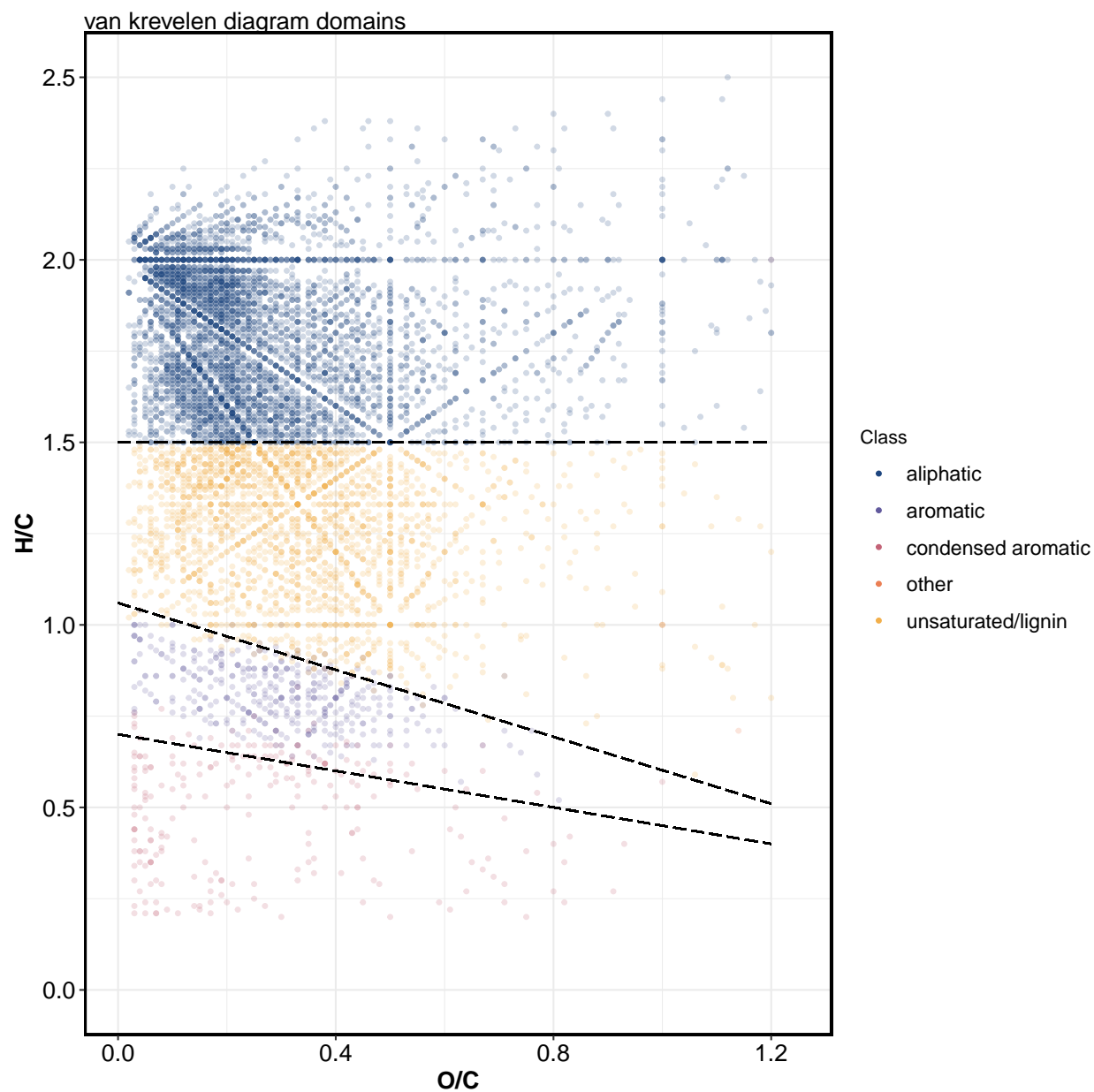
	Df	SumOfSqs	R2	F	Pr(>F)
Pre	1	0.0000268	0.0346902	1.933659	0.170
Inc	5	0.0002308	0.2982330	3.324750	0.009
Pre:Inc	5	0.0001830	0.2365126	2.636681	0.035
Residual	24	0.0003332	0.4305642	NA	NA
Total	35	0.0007739	1.0000000	NA	NA

## FT-MS (FT-ICR)

FTICR was performed on MEPLEx extracts to gain a qualitative understanding of the changes in organic matter composition after the incubation. There appear to be differences between pre-incubation temperatures, particularly in terms of the number of unique compounds, which could be indicative of microbial processing of organic matter and production of new organic compounds.

# FTICR Van krevelen diagrams:

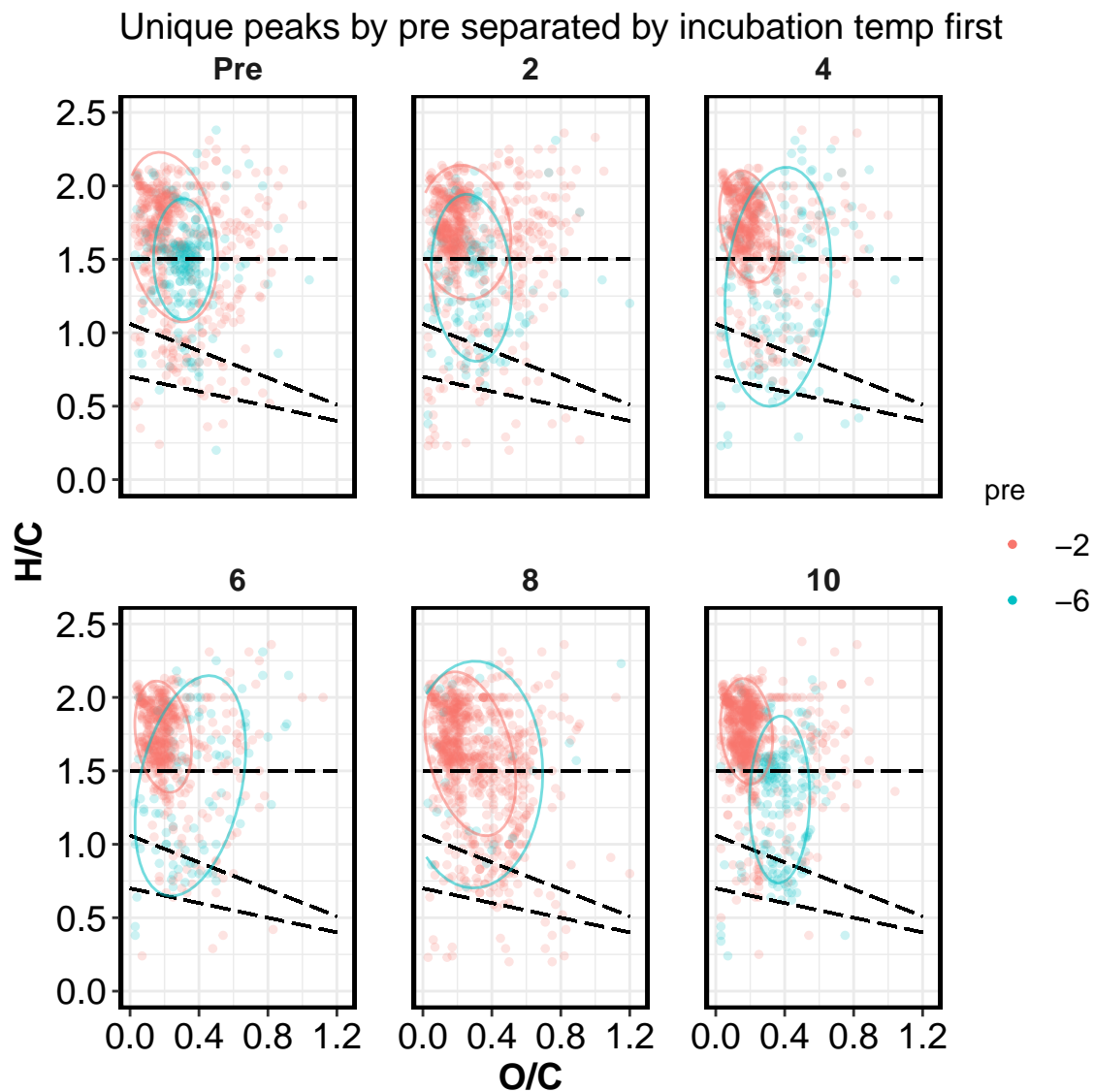




FTICR Common vs unique peaks by treatment:

All

## NULL



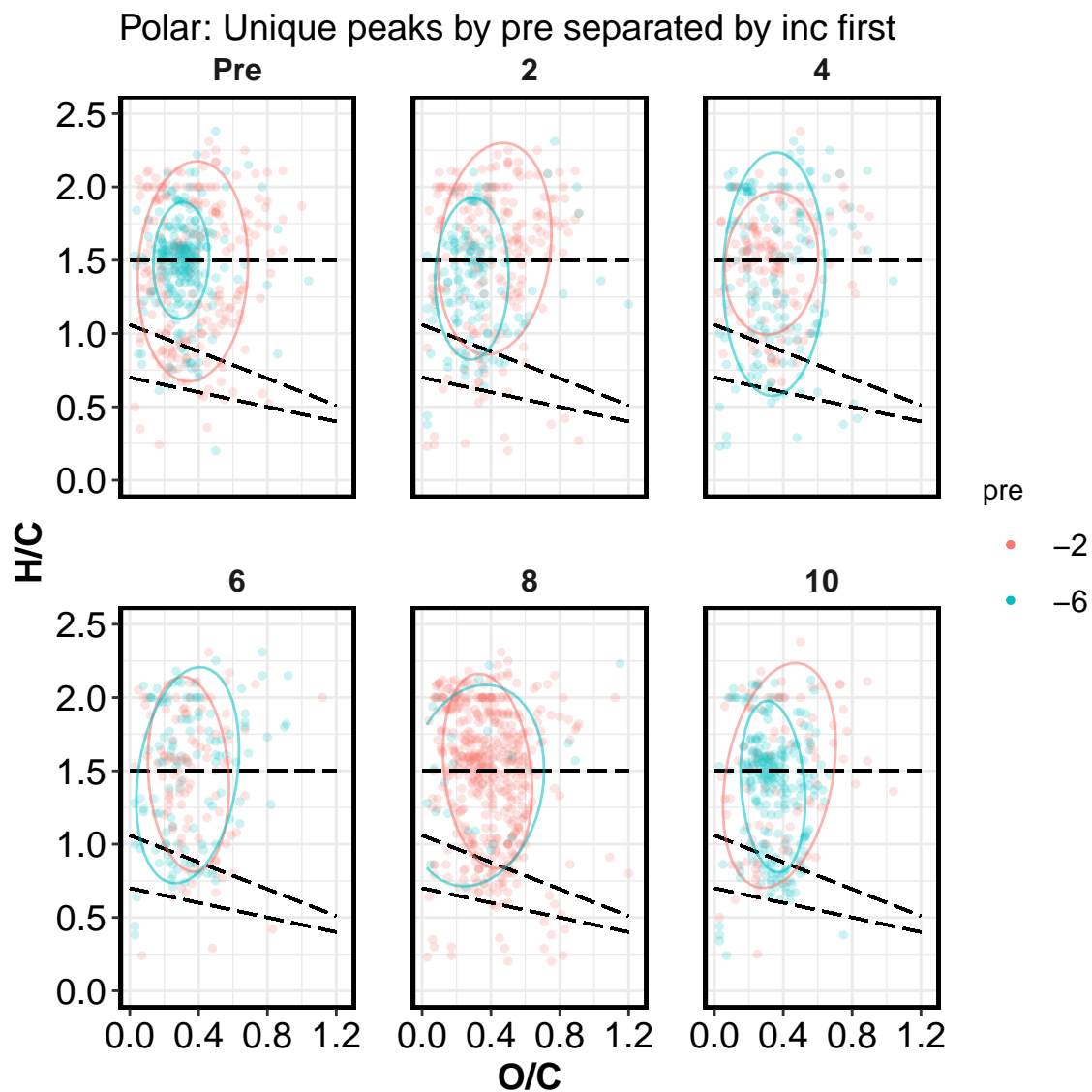
## NULL

Table 6: Unique between preincubation temperatures at each incubation temperature

Class	2_Pre	6_Pre	2_2	6_2	2_4	6_4	2_6	6_6	2_8	6_8	2_10	6_10
aliphatic	313	114	465	49	402	56	408	46	520	14	566	60
aromatic	34	13	18	16	21	14	13	18	48	3	21	35
condensed aromatic	15	2	27	3	NA	18	9	3	25	NA	7	9
unsaturated/lignin	85	79	86	54	69	42	57	27	166	9	69	75

Polar

## NULL



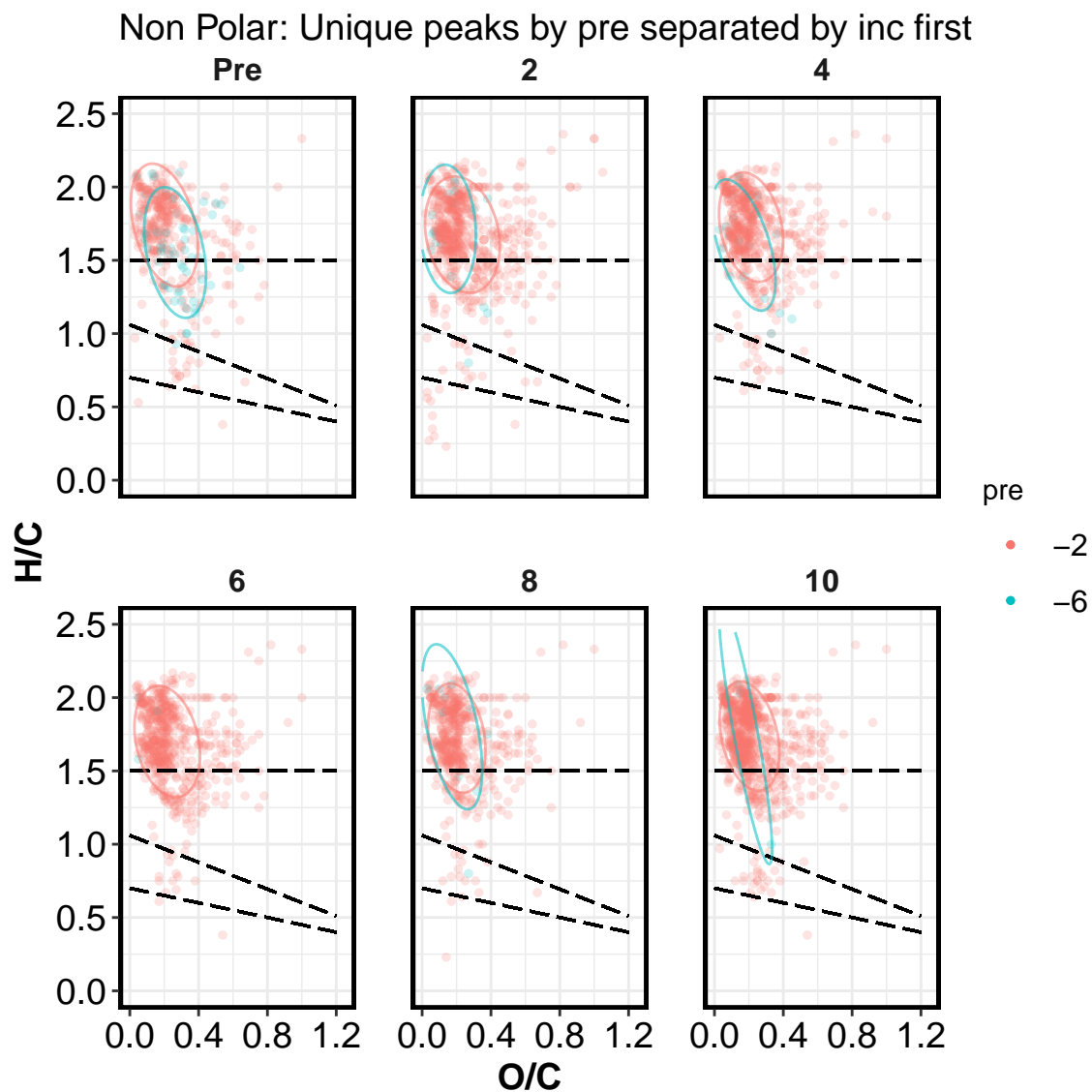
## NULL

Table 7: Unique between preincubation temperatures at each incubation temperature polar

Class	2_Pre	6_Pre	2_2	6_2	2_4	6_4	2_6	6_6	2_8	6_8	2_10	6_10
aliphatic	100	126	122	50	67	74	57	63	265	13	46	105
aromatic	28	14	10	17	12	14	8	20	42	3	10	38
condensed aromatic	13	3	18	3	NA	18	7	3	24	NA	4	10
unsaturated/lignin	67	84	42	60	45	43	31	28	142	9	28	84

Non-Polar

## NULL



## NULL

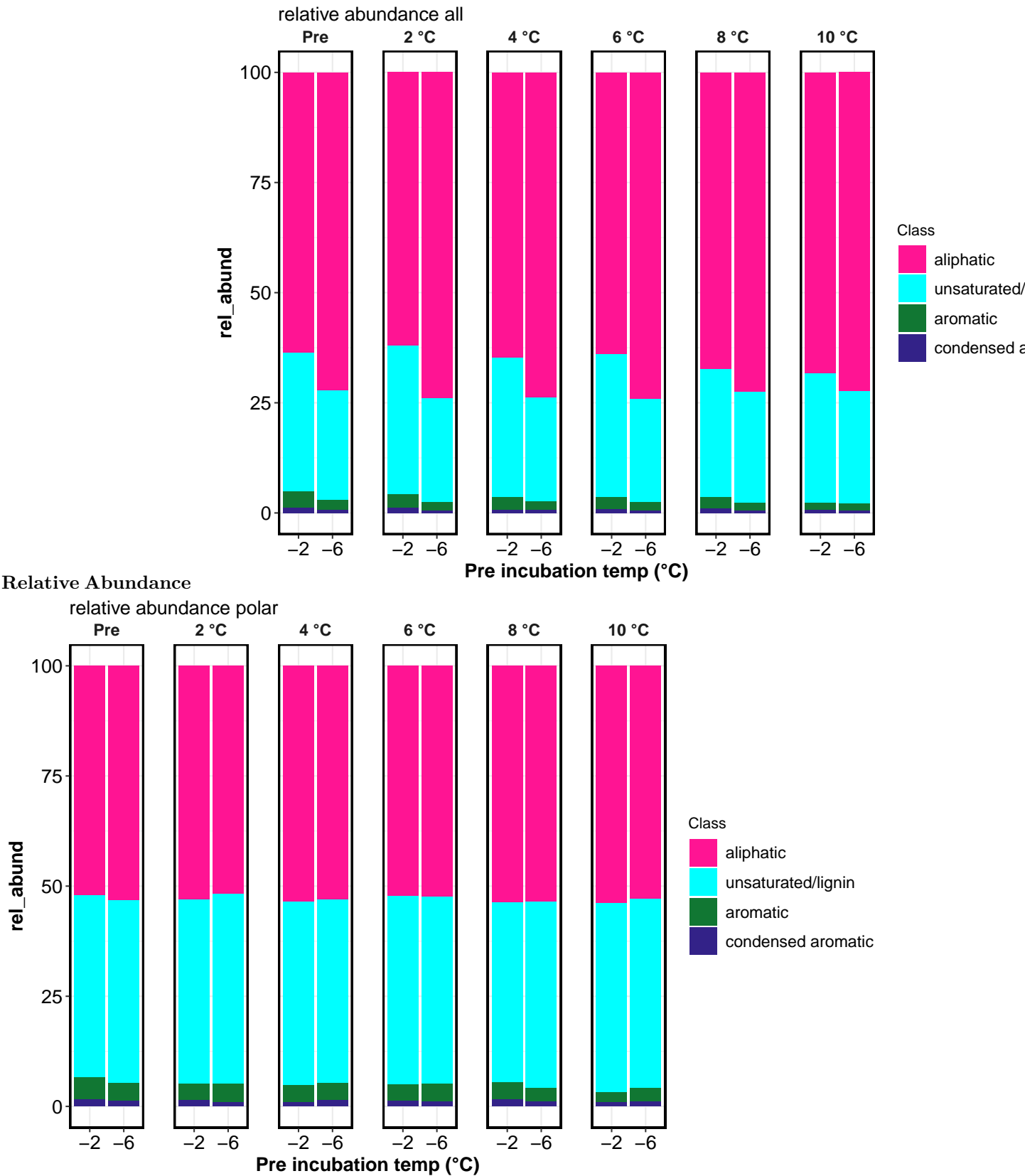
Table 8: Unique between preincubation temperatures at each incubation temperature nonpolar

Class	2_Pre	6_Pre	2_2	6_2	2_4	6_4	2_6	6_6	2_8	6_8	2_10	6_10
aliphatic	272	34	456	15	445	13	449	3	411	8	633	3
aromatic	13	1	13	1	14	NA	10	NA	11	1	20	NA
condensed aromatic	5	NA	11	NA	2	NA	3	NA	4	NA	4	NA

Class	2_Pre	6_Pre	2_2	6_2	2_4	6_4	2_6	6_6	2_8	6_8	2_10	6_10
unsaturated/lignin	49	20	111	3	77	6	102	NA	68	2	116	1



FTICR relative abundance and PCAs:



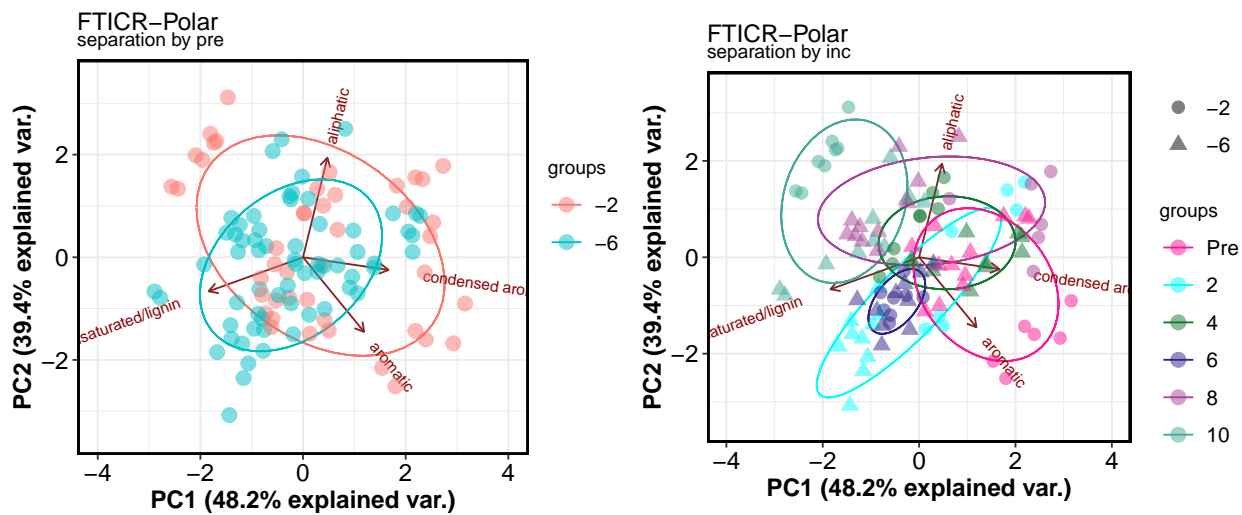
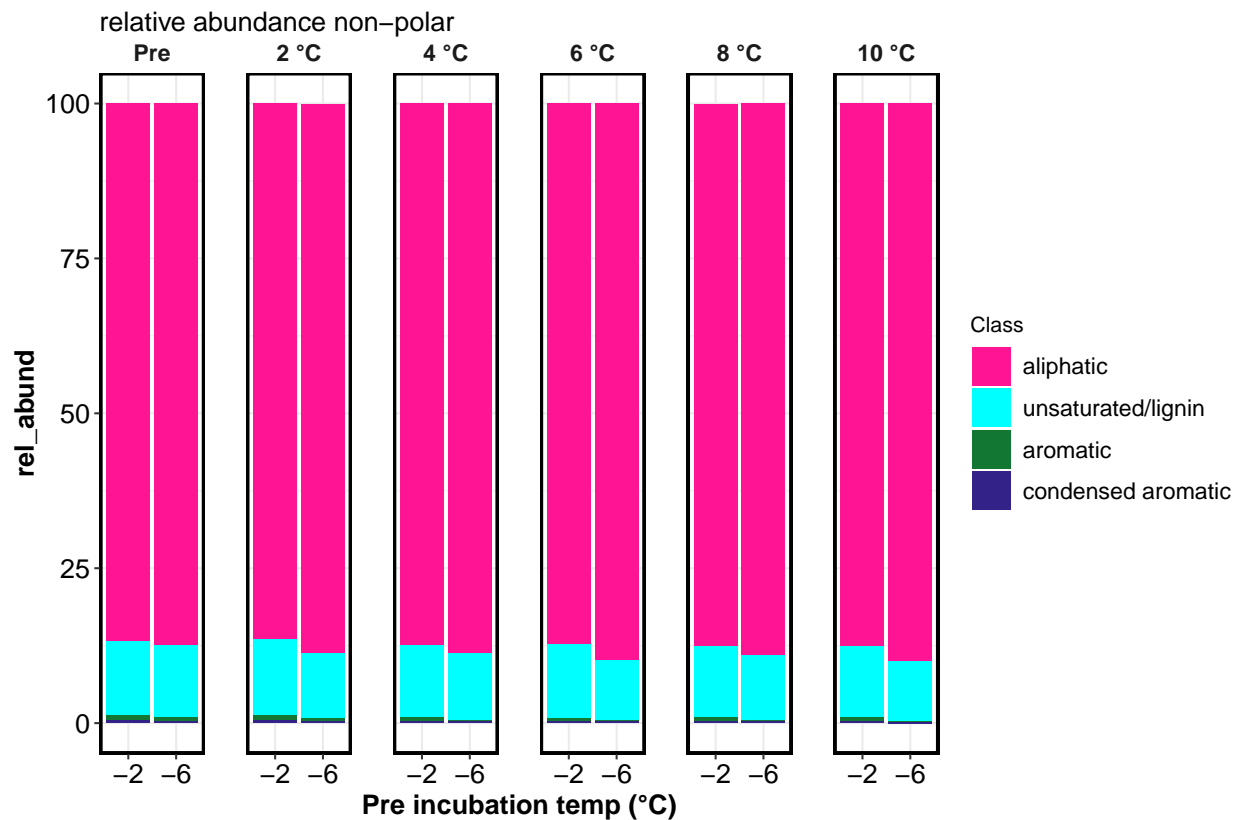


Table 9: Permanova results: Axis class Polar only

	Df	SumOfSqs	R2	F	Pr(>F)
pre	1	0.0004596	0.0321579	10.58298	0.001
inc	5	0.0066832	0.4676090	30.77754	0.001
pre:inc	5	0.0029803	0.2085238	13.72482	0.001

	Df	SumOfSqs	R2	F	Pr(>F)
Residual	96	0.0041692	0.2917093	NA	NA
Total	107	0.0142922	1.0000000	NA	NA

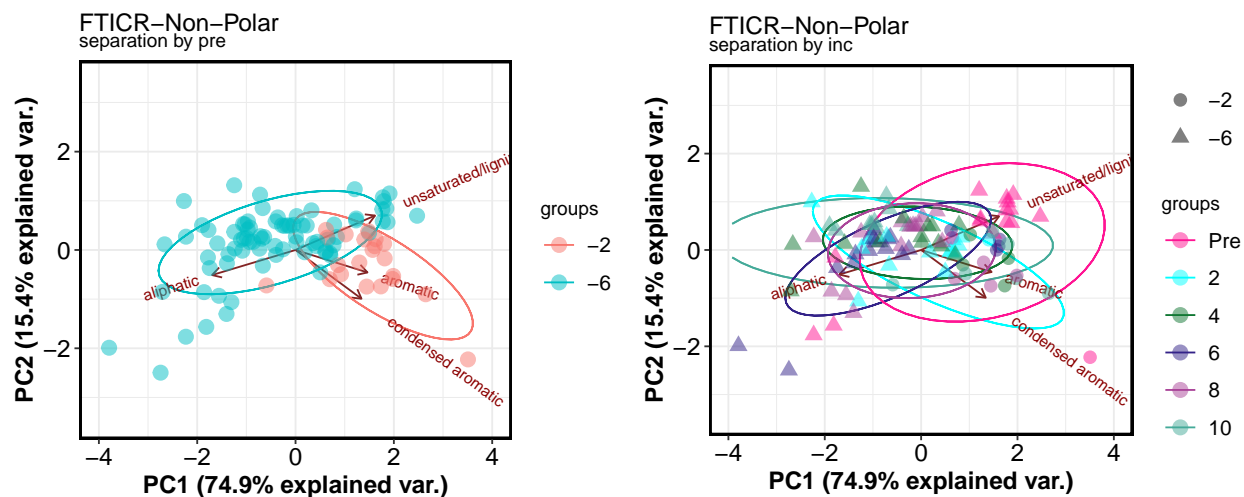


Table 10: Permanova results: Axis class Non-Polar only

	Df	SumOfSqs	R2	F	Pr(>F)
pre	1	0.0050061	0.1717359	26.4653827	0.001
inc	5	0.0052433	0.1798715	5.5438239	0.001
pre:inc	5	0.0009308	0.0319305	0.9841303	0.429
Residual	95	0.0179699	0.6164622	NA	NA
Total	106	0.0291500	1.0000000	NA	NA

## Session Info

Date run: 2023-06-21

```
## R version 4.2.3 (2023-03-15 ucrt)
## Platform: x86_64-w64-mingw32/x64 (64-bit)
## Running under: Windows 10 x64 (build 19045)
##
## Matrix products: default
##
## locale:
## [1] LC_COLLATE=English_United States.utf8
## [2] LC_CTYPE=English_United States.utf8
## [3] LC_MONETARY=English_United States.utf8
## [4] LC_NUMERIC=C
```

```

## [5] LC_TIME=English_United States.utf8
##
## attached base packages:
## [1] grid      stats      graphics  grDevices utils      datasets  methods
## [8] base
##
## other attached packages:
## [1] trelliscopejs_0.2.6 pmarR_2.3.0      agricolae_1.3-5
## [4] knitr_1.42          nlme_3.1-162     cowplot_1.1.1
## [7] ggpubr_0.6.0        janitor_2.2.0    pracma_2.4.2
## [10] reshape2_1.4.4      ggbiplot_0.55    scales_1.2.1.9000
## [13] plyr_1.8.8          vegan_2.6-4      lattice_0.20-45
## [16] permute_0.9-7       lubridate_1.9.2  forcats_1.0.0
## [19] stringr_1.5.0       dplyr_1.1.1      purrr_1.0.1
## [22] readr_2.1.4         tidyr_1.3.0      tibble_3.2.1
## [25] ggplot2_3.4.1       tidyverse_2.0.0  tarchetypes_0.7.6
## [28] targets_0.14.3
##
## loaded via a namespace (and not attached):
## [1] colorspace_2.1-0      ggsignif_0.6.4      ellipsis_0.3.2
## [4] mclust_6.0.0          snakecase_0.11.0    base64enc_0.1-3
## [7] fs_1.6.2              rstudioapi_0.14     farver_2.1.1
## [10] listenv_0.9.0         furr_0.3.1          fansi_1.0.4
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## [64] labeling_0.4.2        htmlwidgets_1.6.2   processx_3.8.0
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## [82] utf8_1.2.3            plotly_4.10.2.9000  rmarkdown_2.21
## [85] tzdb_0.3.0            future.callr_0.8.1  progress_1.2.2
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## [94] munsell_0.5.0         viridisLite_0.4.2

```