The effect of ozonation on RAS biofilters

Håkon Dahle - University of Bergen
(BRAS project)

Ozone: A strong oxidant and good disinfectant

Ozone

$O_3 + OH^- \rightarrow HO_2^- + O_2$

$O_3 + HO_2^- \rightarrow O_3^{-\bullet} + HO_2^-$

$O_3 + O_2^{-\bullet} \rightarrow O_3^{-\bullet} + O_2$

$O_3^{-\bullet} + H^+ \rightarrow HO_3^\bullet$

$HO_3^\bullet \rightarrow {}^\bullet OH + O_2$

Targets:

> Cell wall

> Cytoplasmic material
(e.g. proteins and DNA)

Lysis
and death

Ozone attacks reactive chemical groups, such as carbon–carbon double bonds, and breaks larger organic molecules into smaller compounds. Products can include aldehydes, ketones, and carboxylic acids.

https://www.sciencedirect.com/science/article/pii/S0023643818309484?via%3Dihub

Units of ozone treatments

How much ozone is added by mass: **mg** / **L**

What oxidation reduction potential (ORP) is reached: **mV**

A high dosage of ozone may be easily consumed if the organic load is high, leading to a modest response in ORP.

Ozonation can be very effective for inactivation of planktonic cells

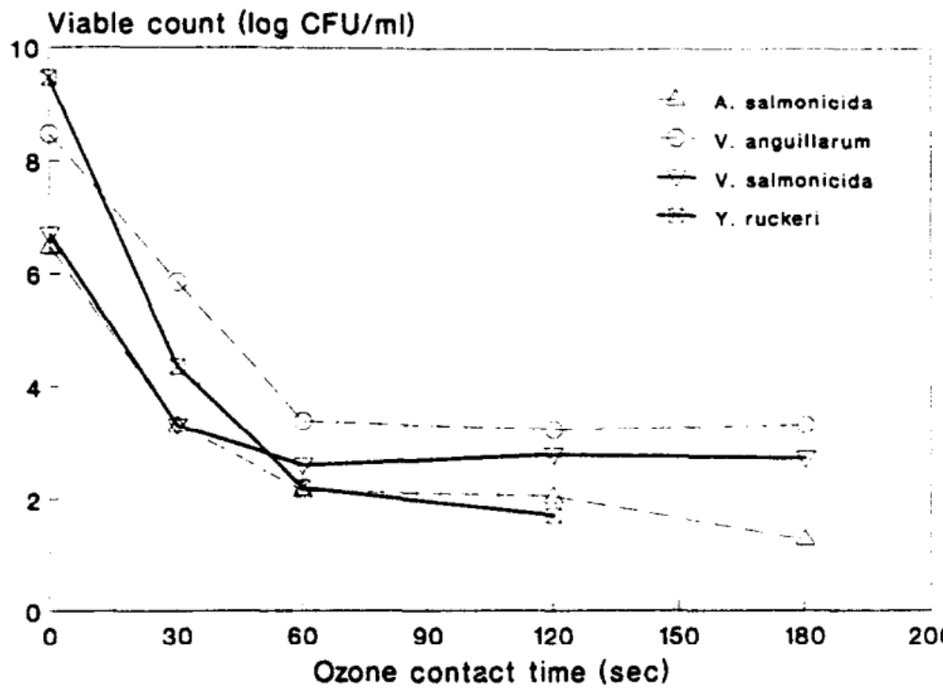


Fig. 1. Inactivation of *A. salmonicida* subsp. *salmonicida*, *V. salmonicida*, *V. anguillarum*, and *Y. ruckeri* in ozonated lake water. The measured residual ozone concentration was 0·20 mg/liter. Each datum point represents one exposure and the mean value of duplicate platings.

99.99% inactivation

Liltved et al., 1993

https://www.sciencedirect.com/science/article/pii/0144860994P4430J

Ozonation is less effective in

- organic rich RAS water

"RAS process water requires high amounts of ozone to inactivate bacteria, as the oxidative power of ozone and residual oxidants is consumed in reactions with organic matter and other components of the rearing water."

Attramadal et al., 2012

https://www.sciencedirect.com/science/article/pii/S0044848611009318

- and in biofilms

"Immobilization on biocarriers increased the tolerance of AOB and NOB dramatically, suggesting the biofilm matrix to be highly protective against… [ozonation]"

Schroeder et al., 2015

https://www.sciencedirect.com/science/article/pii/S0144860914001009?via%3Dihub

Our question

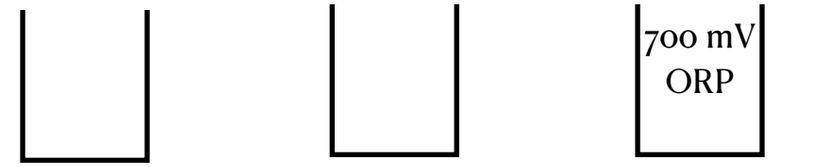
Will acute high-dose ozonation (**700 mV ORP**) reduce unwanted microorganisms without largely disrupting the beneficial microbial community that keeps the biofilter functioning?

Sampling for 16S rRNA gene amplicon analyses (V3-V4 region)

Experiment 1
(ISAV HPRdel experiment)

RAS biochips

Do



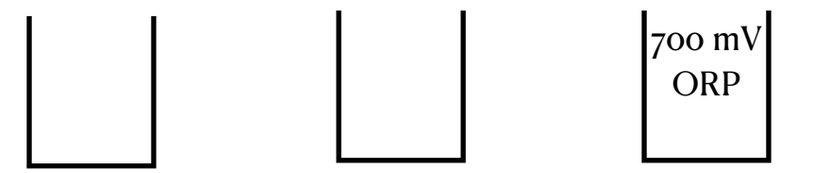
T1	T2	T3
No Treatment	Water Exchange	Water Exchange & Ozonation
D1	D1	D1
D17/21	D17/21	D10

x3

Experiment 2
(Branchiomonas experiment)

RAS biochips

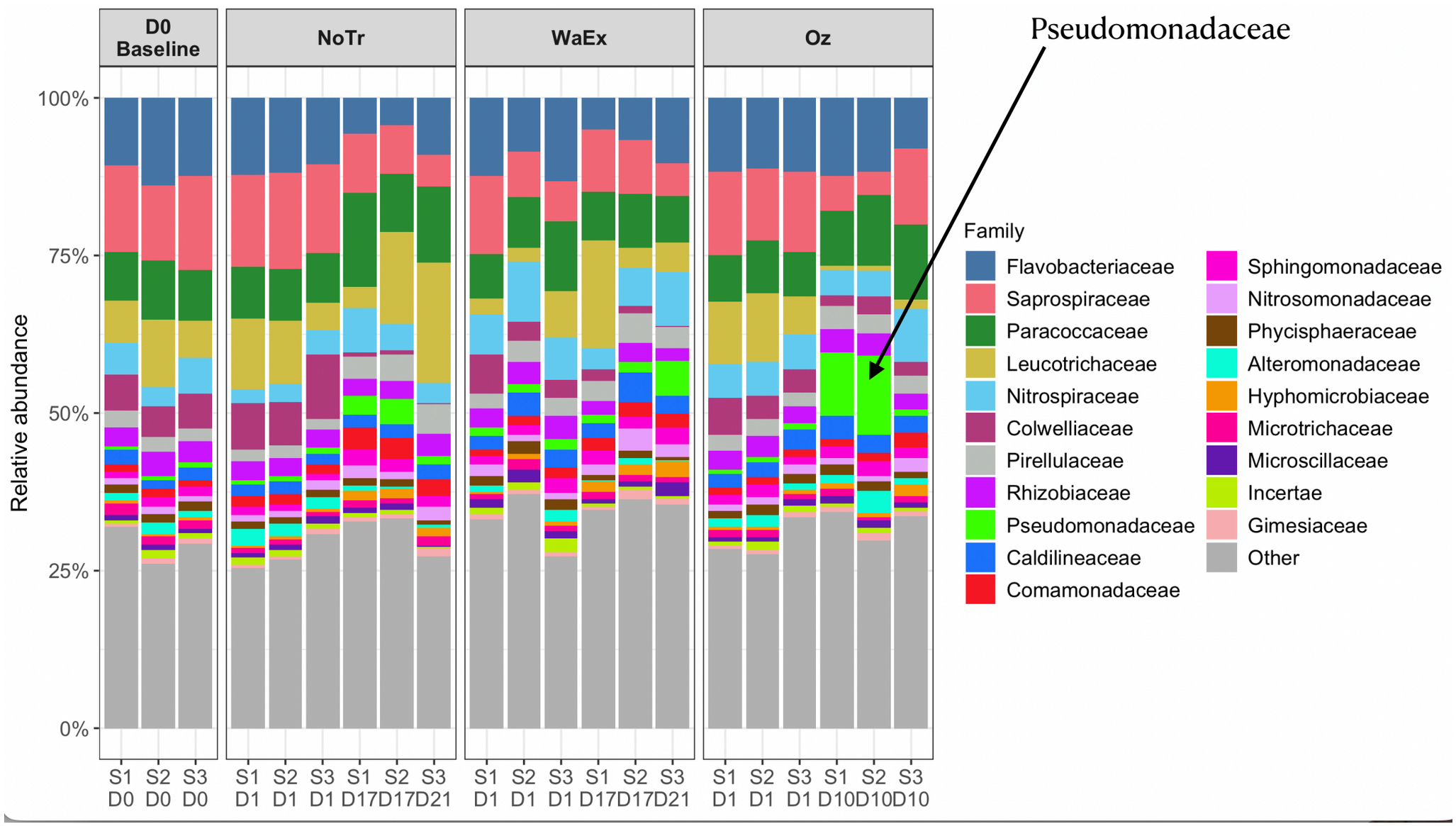
Do



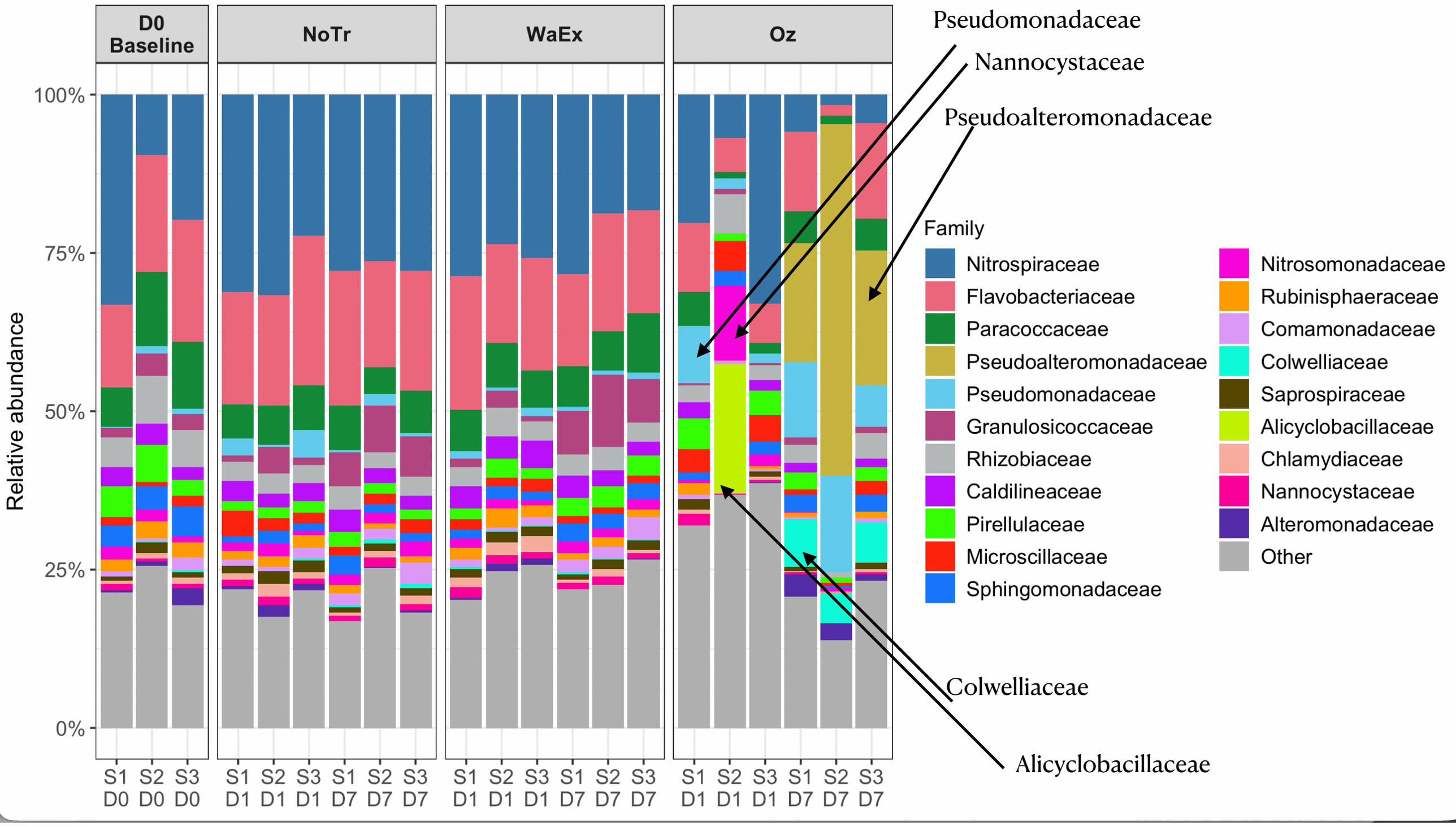
T1	T2	T3
No Treatment	Water Exchange	Water Exchange & Ozonation
D1	D1	D1
D7	D7	D7

x3

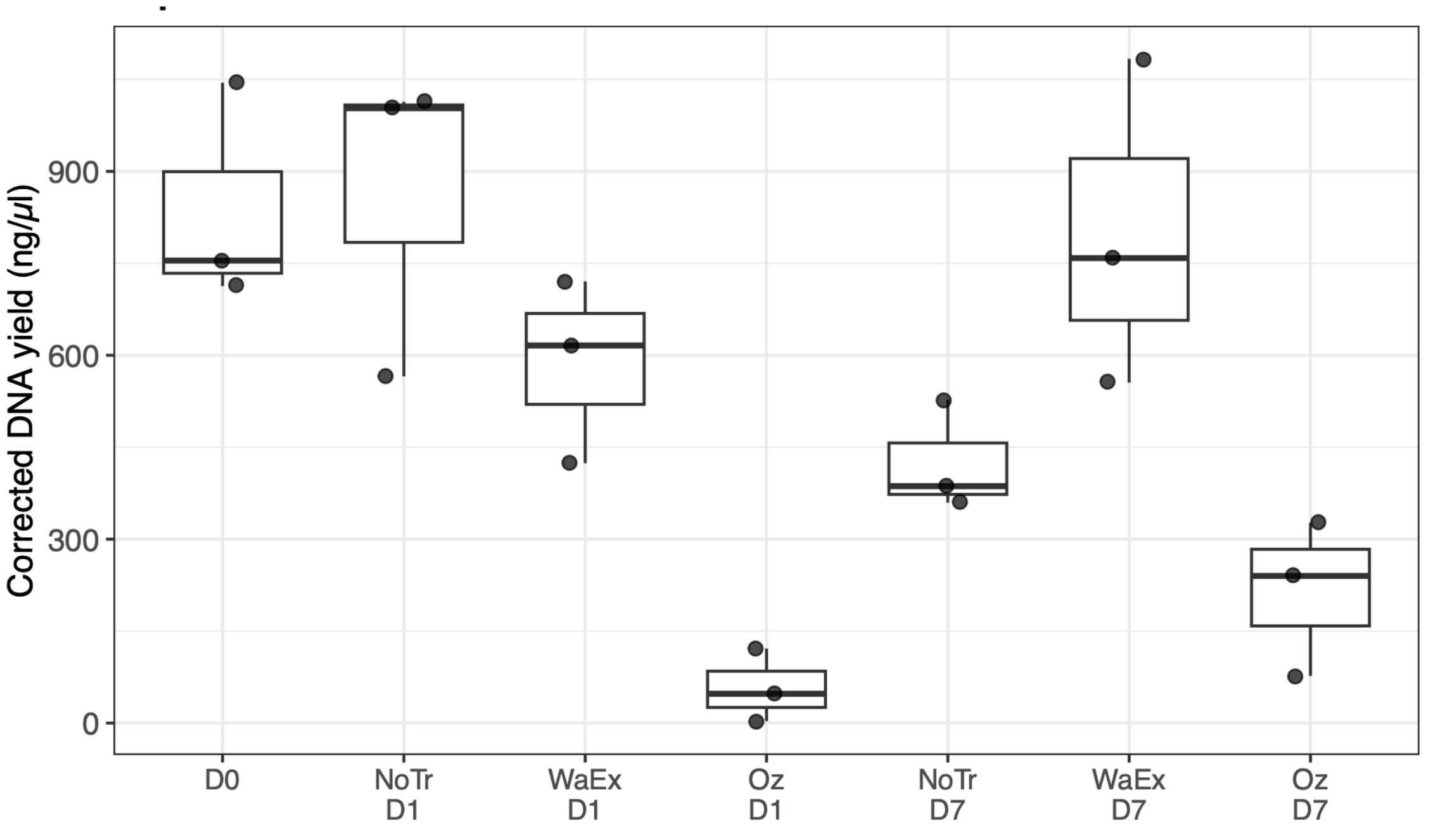
Taxonomic profiling (Experiment 1)



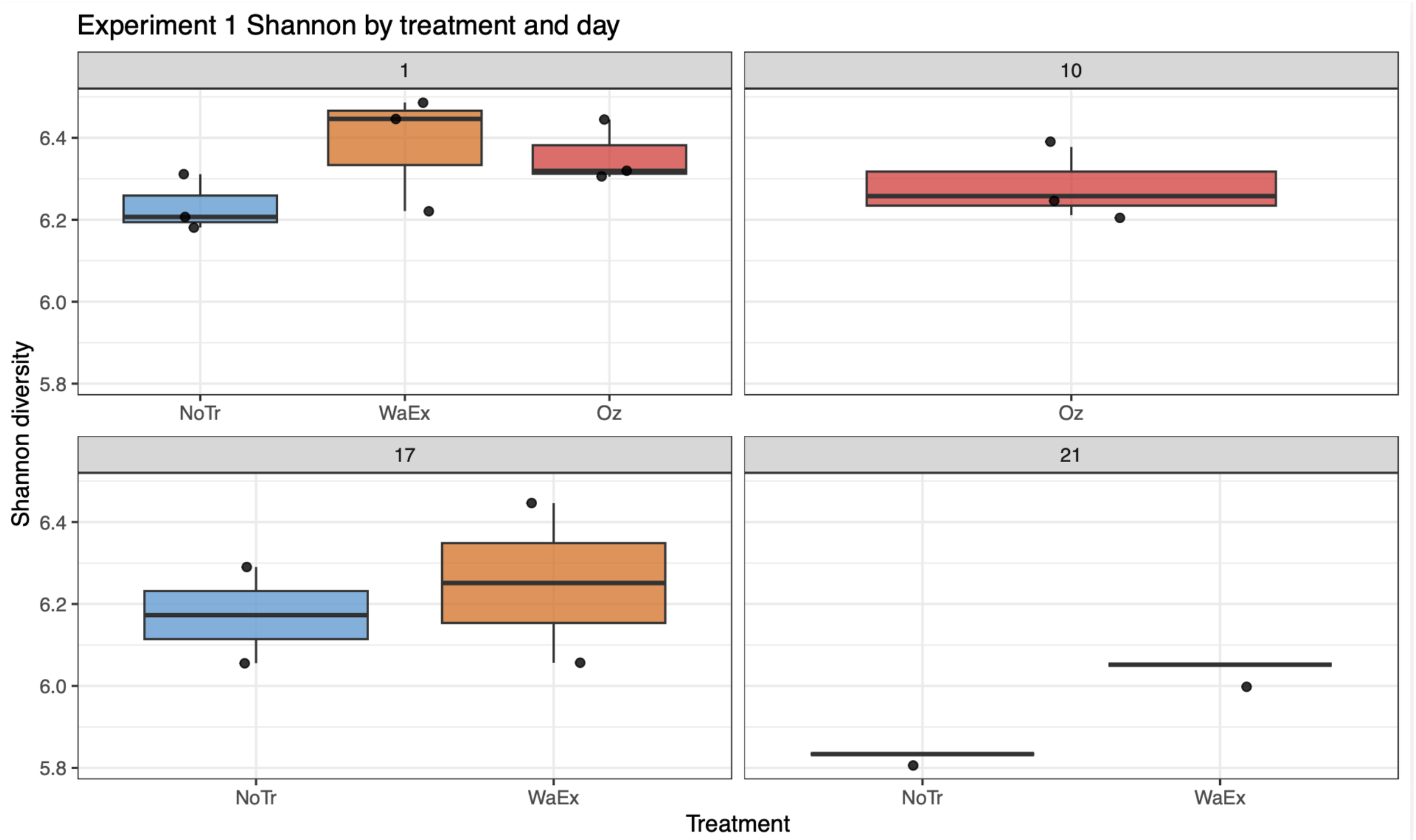
Taxonomic profiling (Experiment 2)



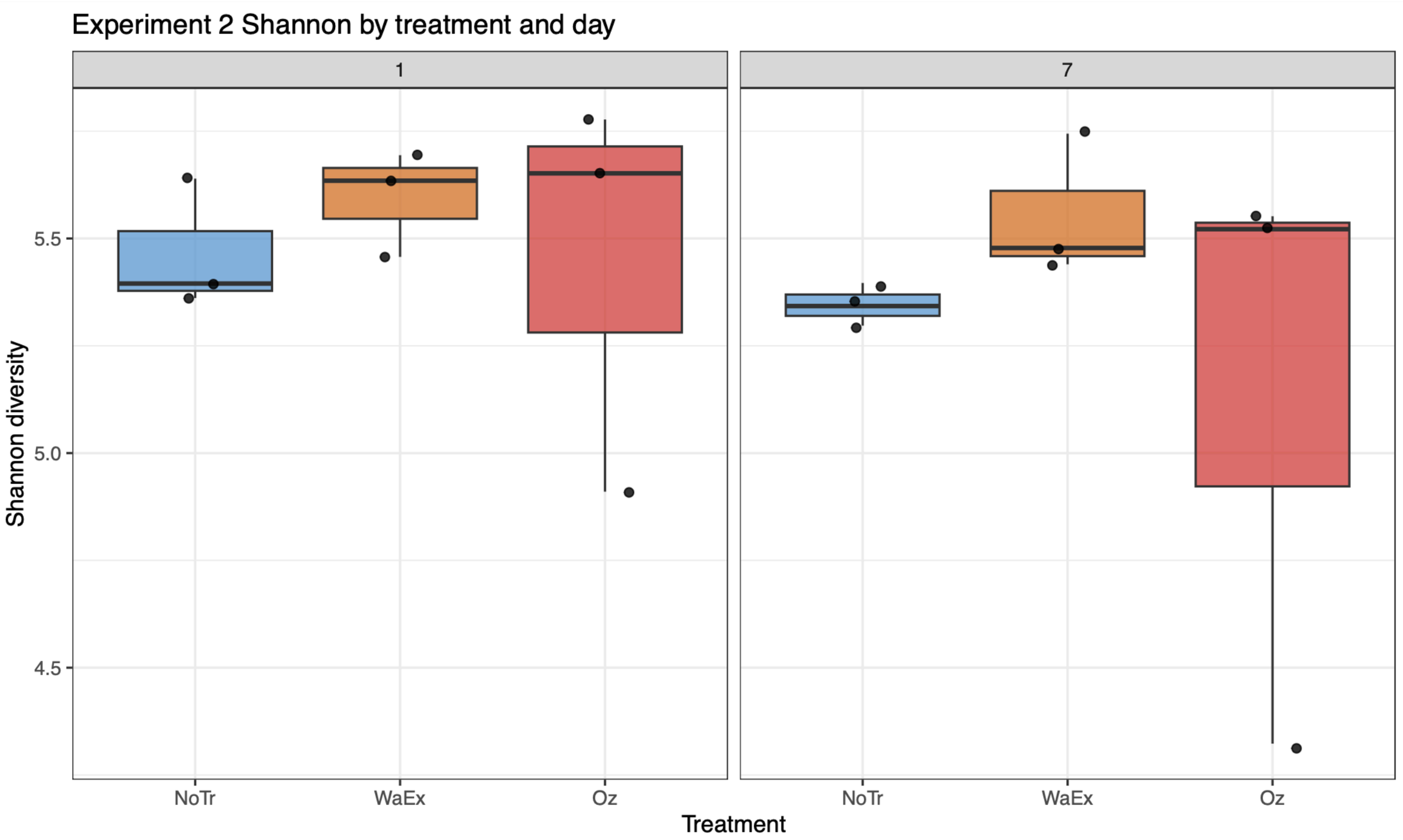
DNA yield Experiment 2



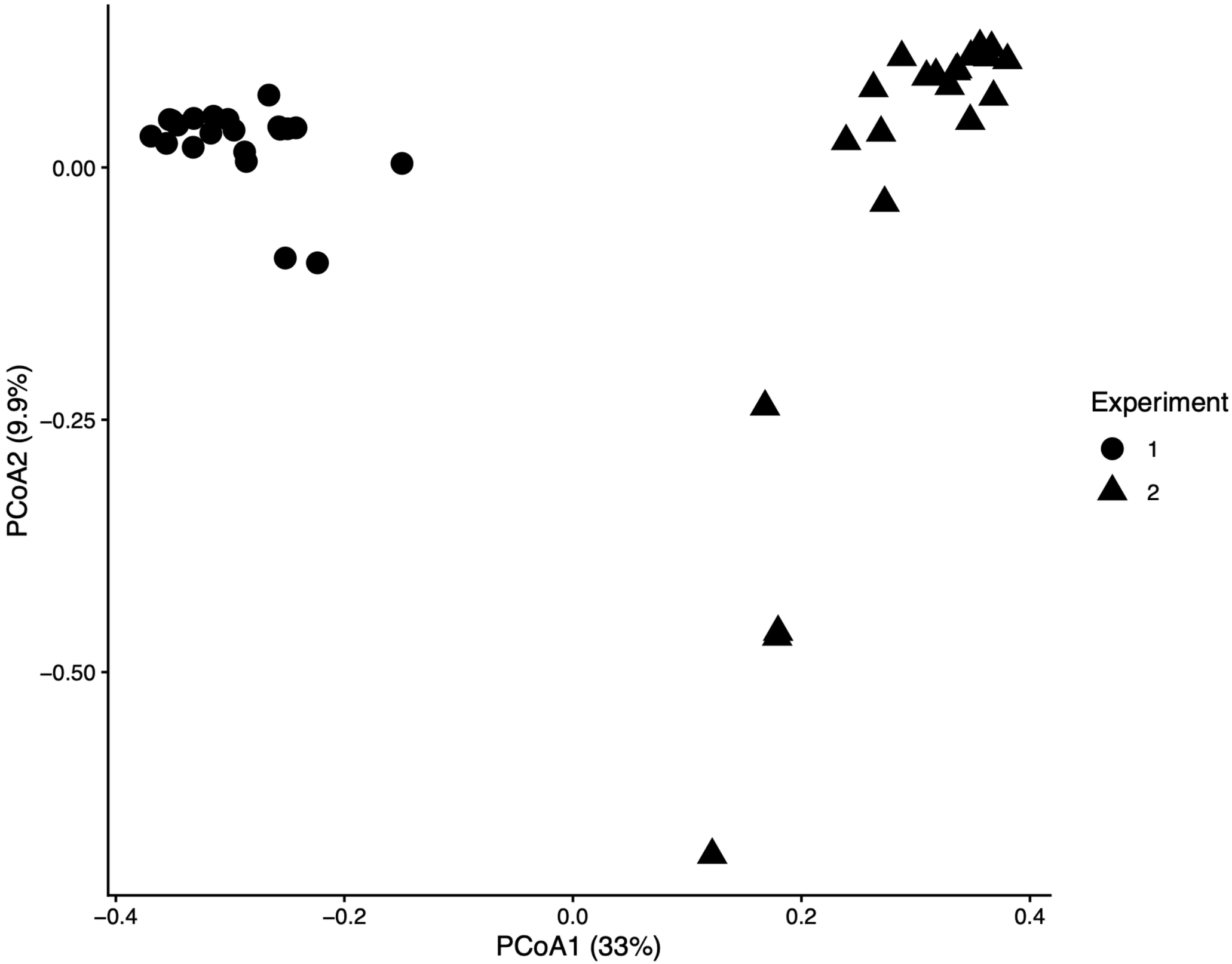
Alpha-Diversity (Experiment 1)



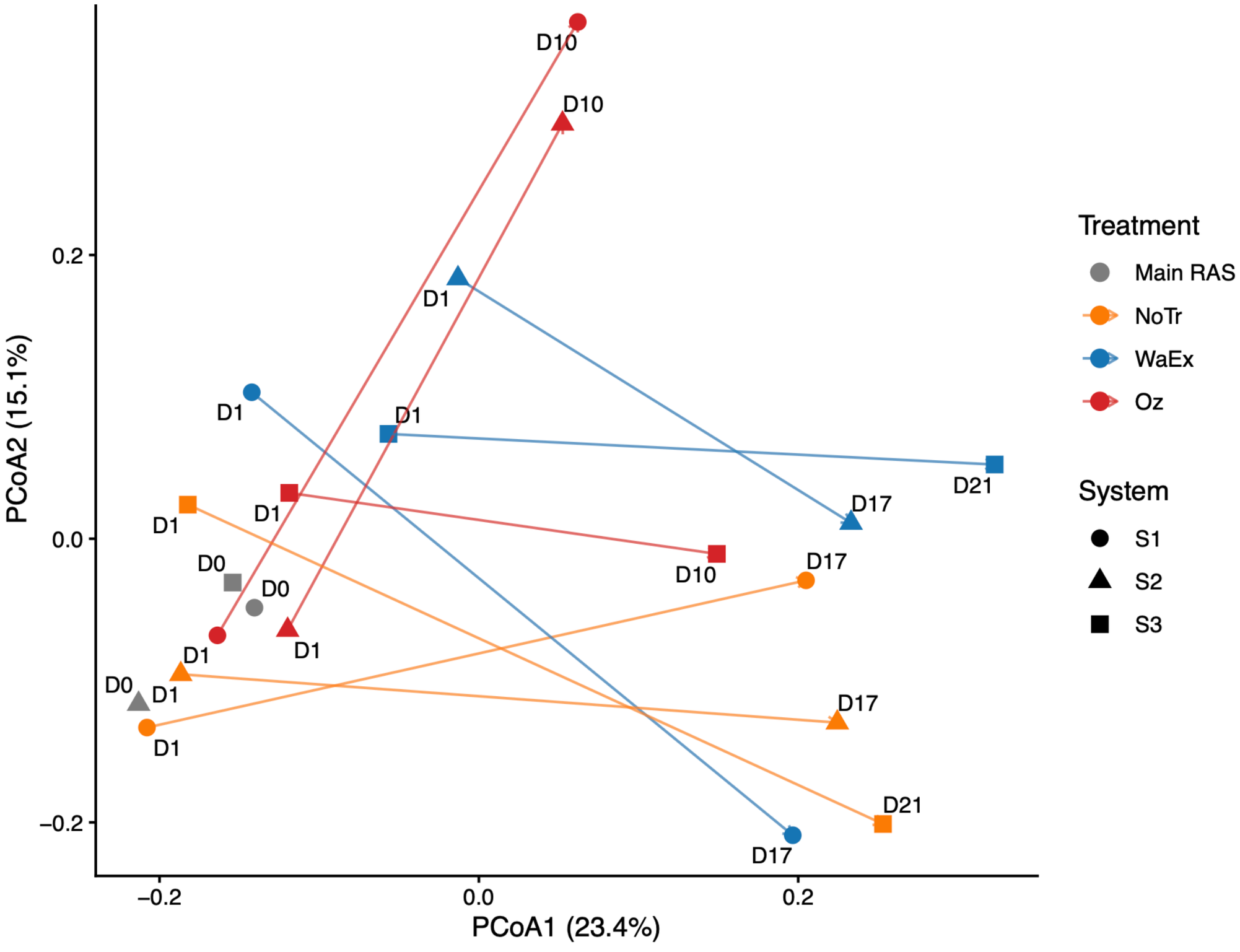
Alpha-Diversity (Experiment 2)



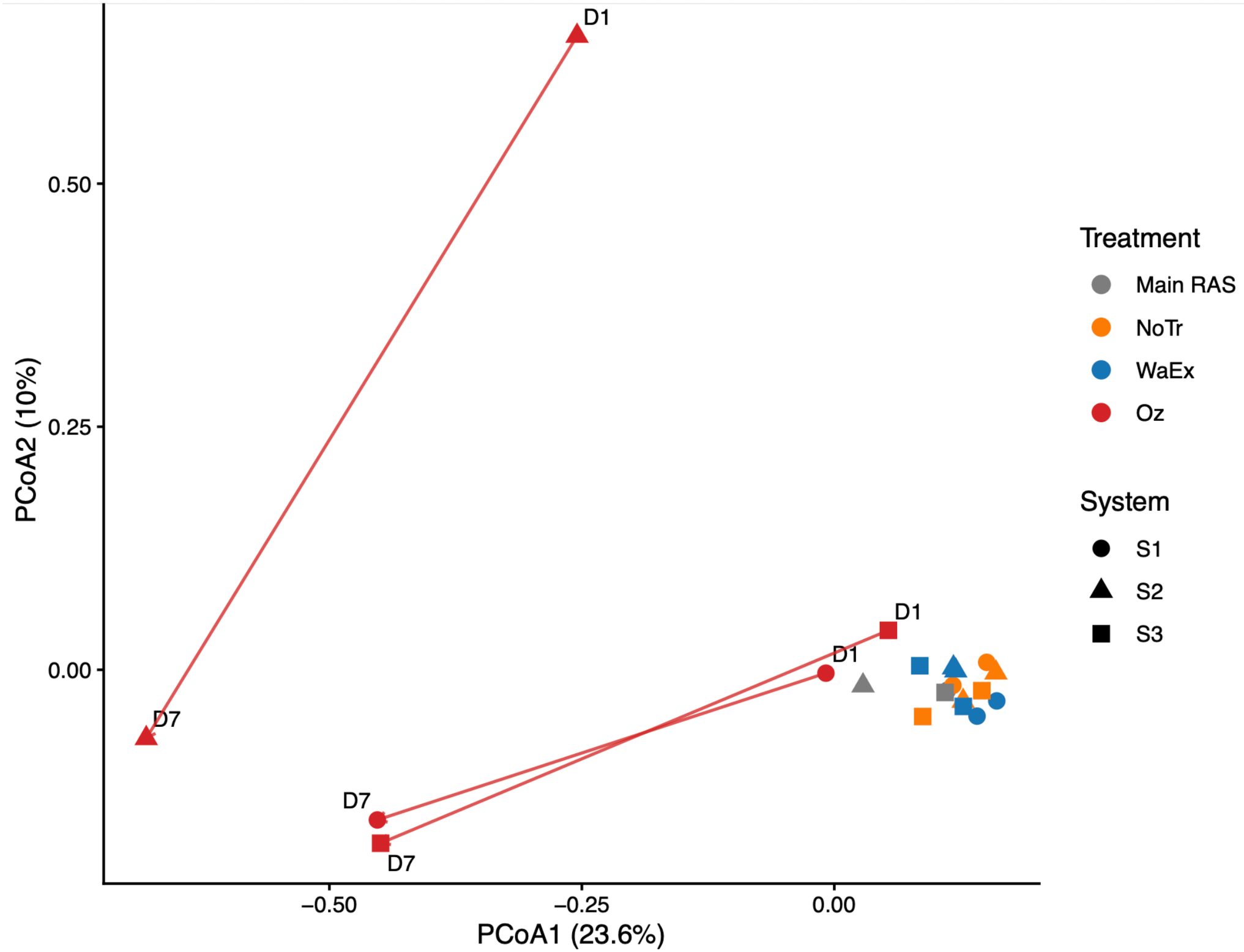
PCoA of all samples (both experiments)

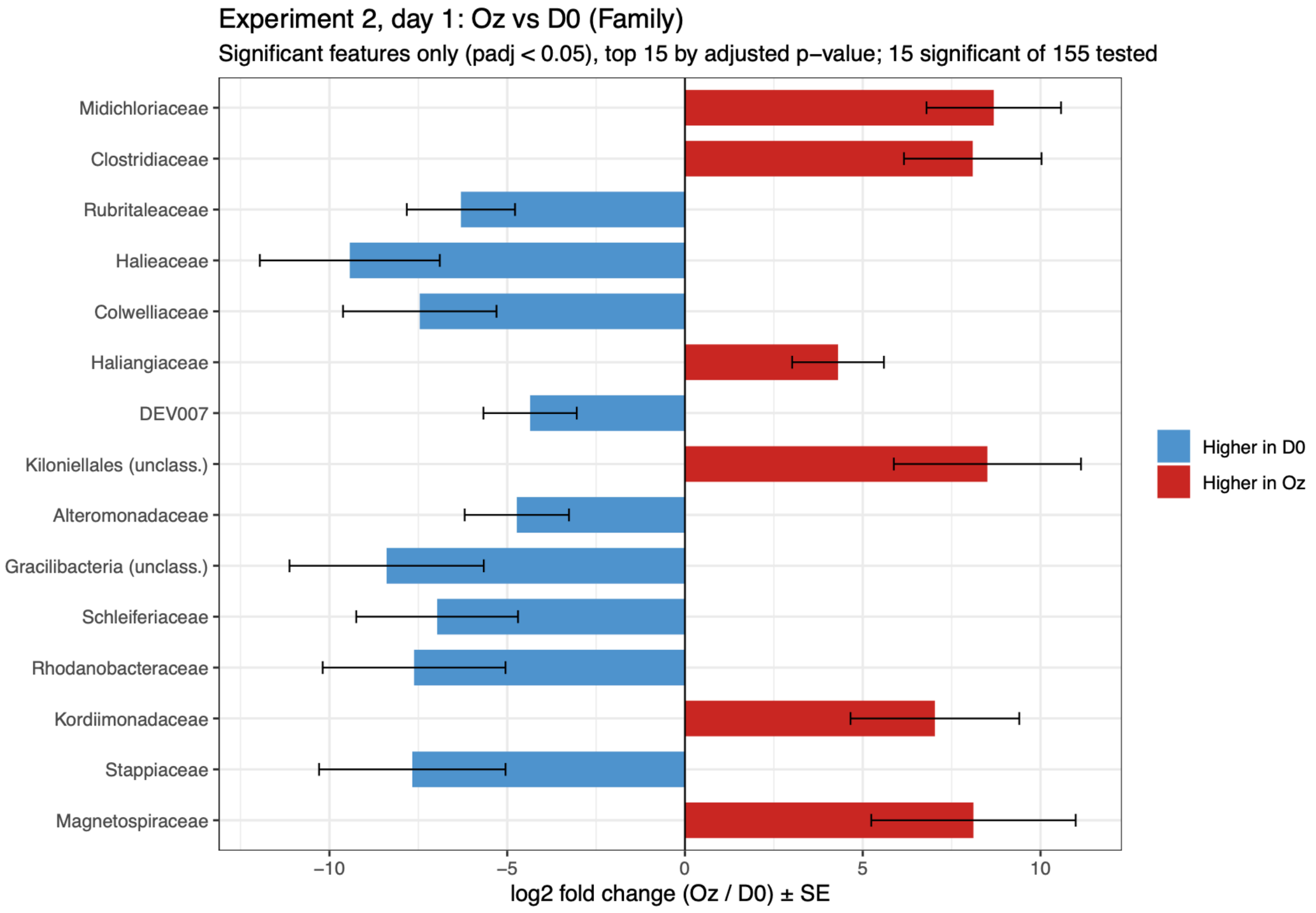


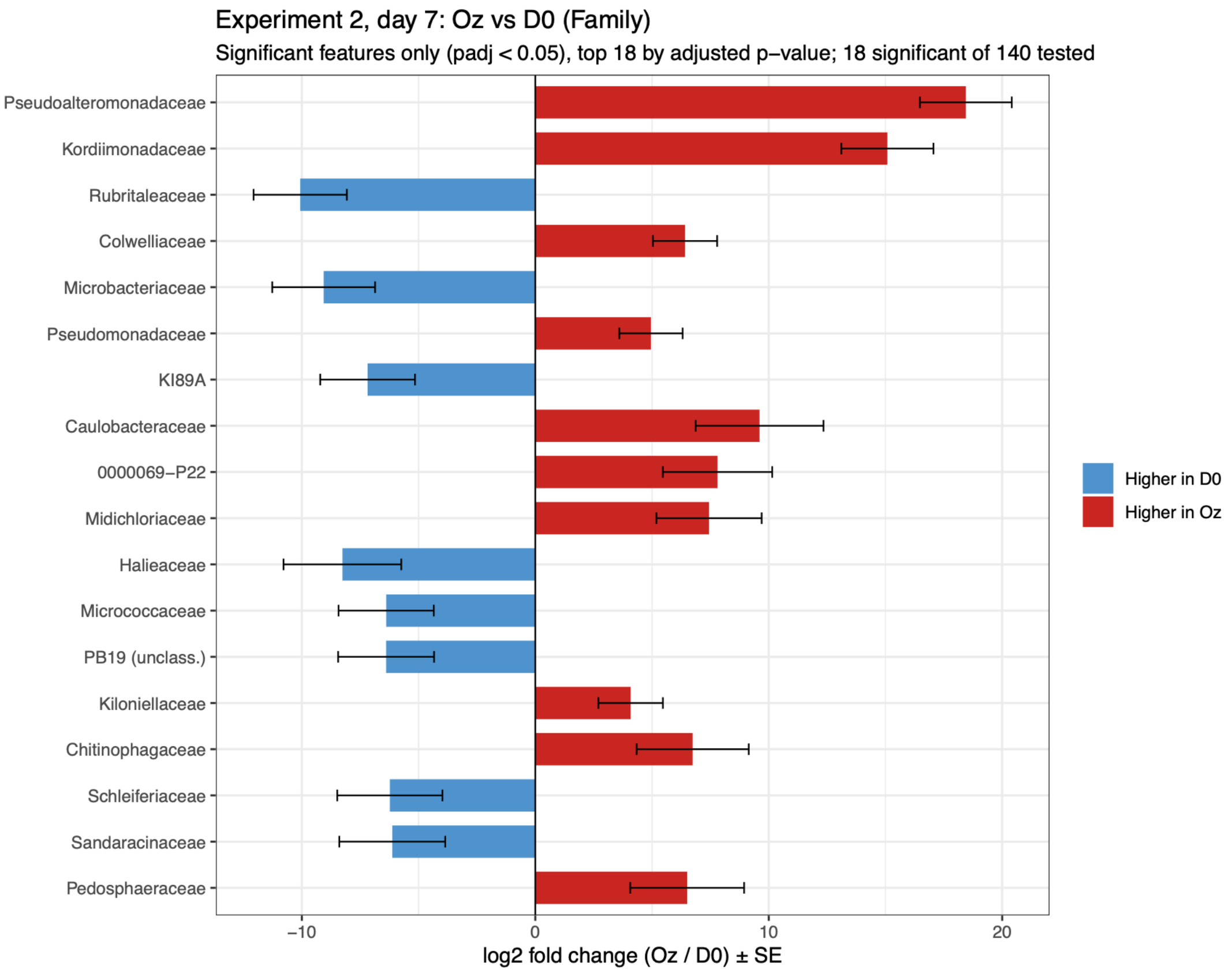
Experiment 1



PCoA Experiment 2

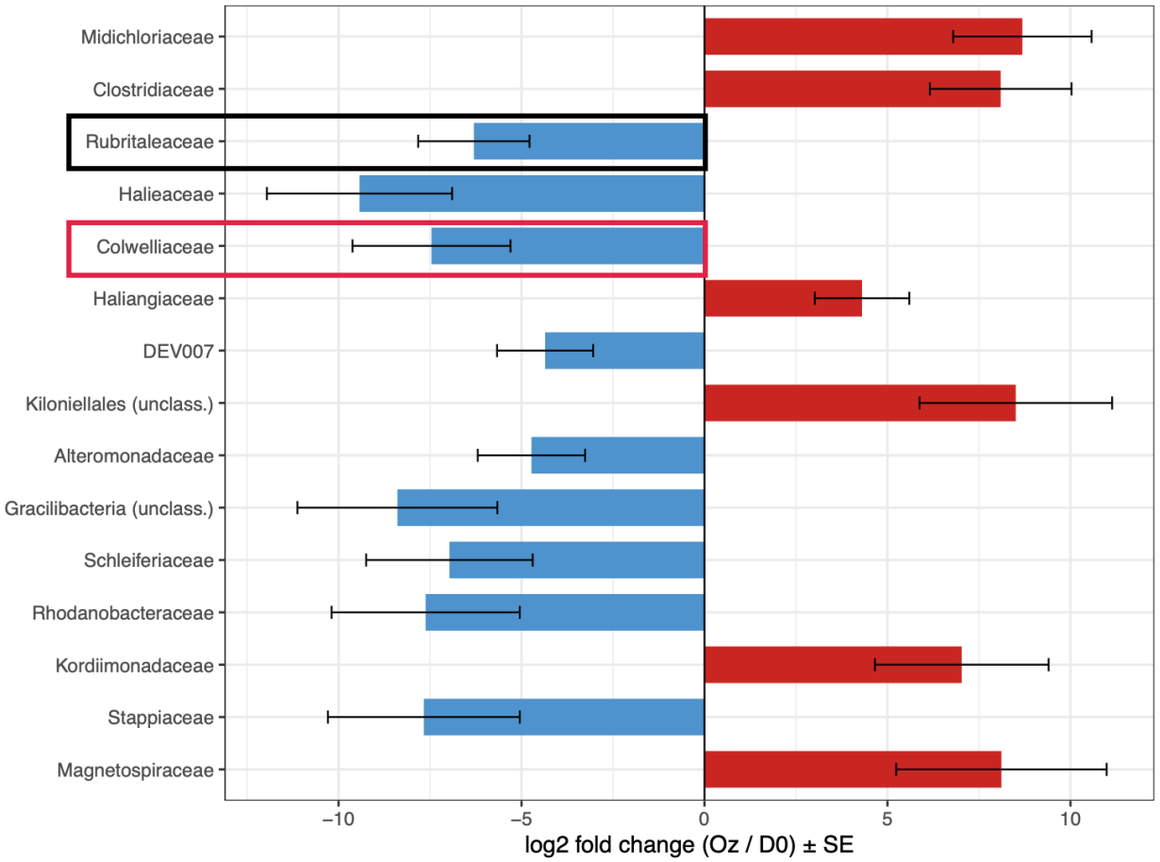



Experiment 2, day 1: Oz vs D0 (Family)
Significant features only (padj < 0.05), top 15 by adjusted p−value; 15 significant of 155 tested
Midichloriaceae
Clostridiaceae
Rubritaleaceae
Halieaceae
Colwelliaceae
Haliangiaceae
DEV007
Kiloniellales (unclass.)
Alteromonadaceae
Gracilibacteria (unclass.)
Schleiferiaceae
Rhodanobacteraceae
Kordiimonadaceae
Stappiaceae
Magnetospiraceae
−10
−5
0
5
10
log2 fold change (Oz / D0) ± SE
Higher in D0
Higher in Oz


Experiment 2, day 7: Oz vs D0 (Family)
Significant features only (padj < 0.05), top 18 by adjusted p–value; 18 significant of 140 tested
Pseudoalteromonadaceae
Kordiimonadaceae
Rubritaleaceae
Colwelliaceae
Microbacteriaceae
Pseudomonadaceae
KI89A
Caulobacteraceae
0000069–P22
Midichloriaceae
Halieaceae
Micrococcaceae
PB19 (unclass.)
Kiloniellaceae
Chitinophagaceae
Schleiferiaceae
Sandaracinaceae
Pedosphaeraceae
Higher in D0
Higher in Oz
–10
0
10
20
log2 fold change (Oz / D0) ± SE

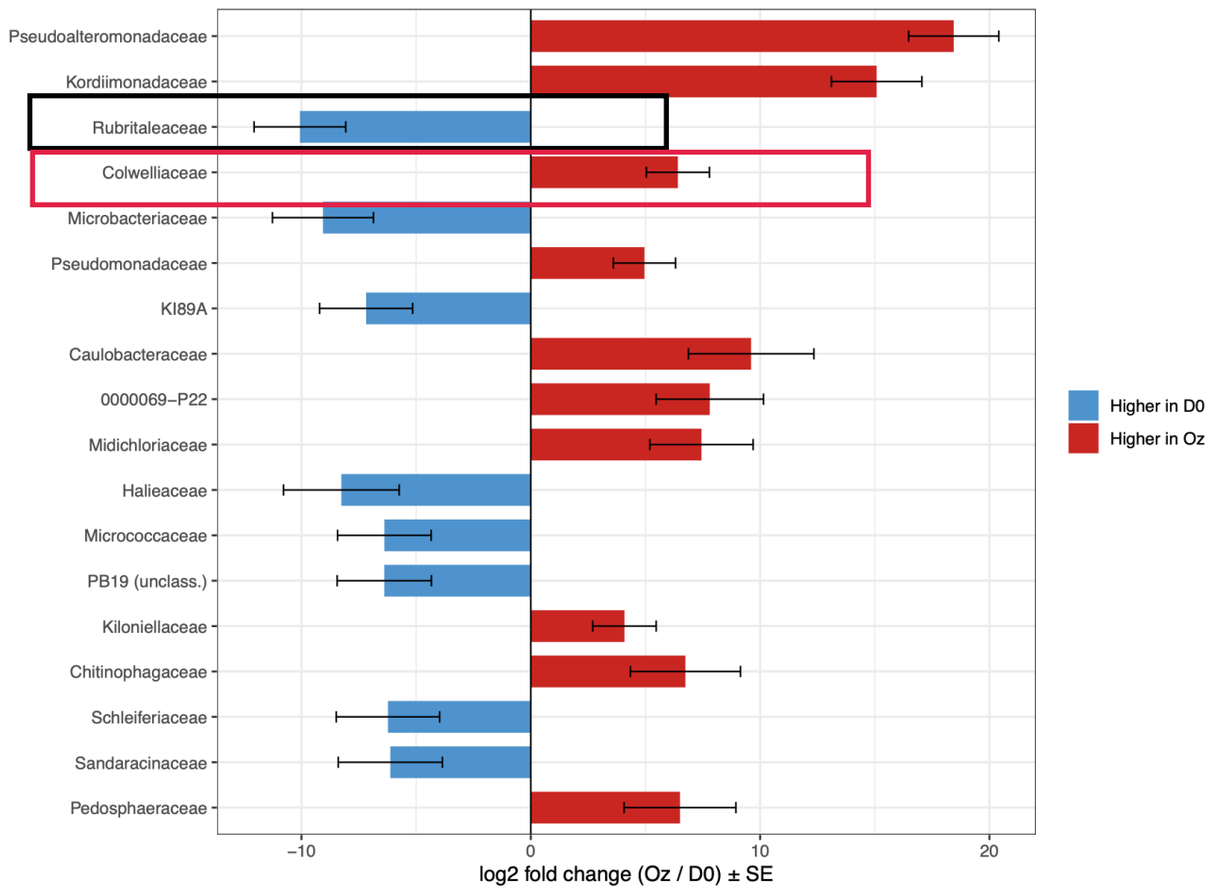
Experiment 2, day 1: Oz vs D0 (Family)

Significant features only (padj < 0.05), top 15 by adjusted p−value; 15 significant of 155 teste



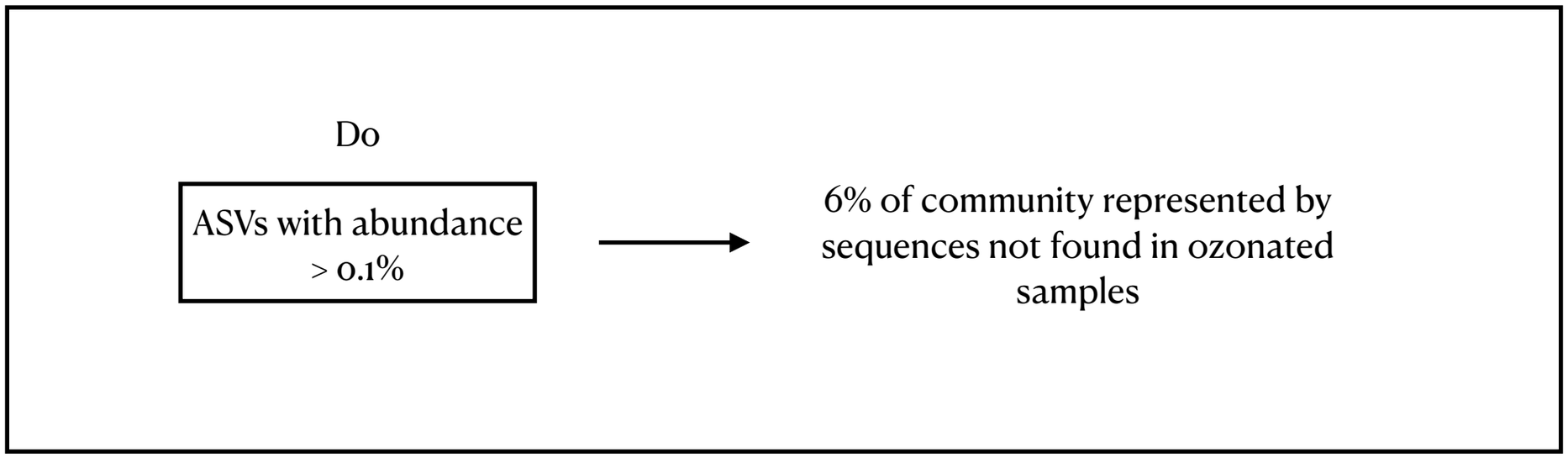
Experiment 2, day 7: Oz vs D0 (Family)

Significant features only (padj < 0.05), top 18 by adjusted p−value; 18 significant of 140 tested

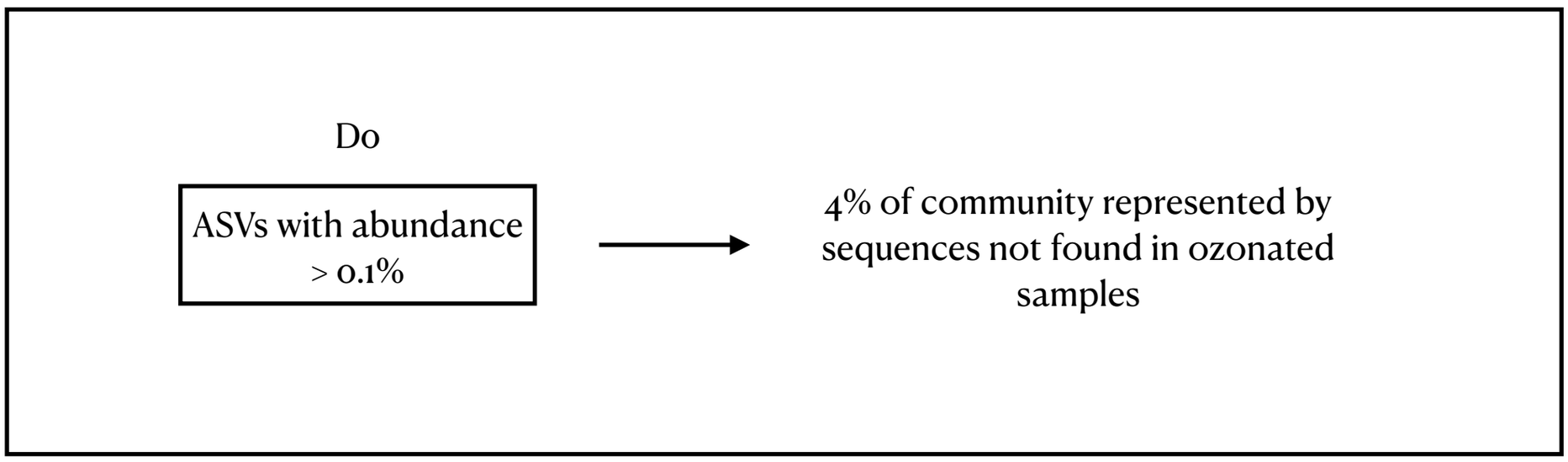


ASV loss (Experiment 2)

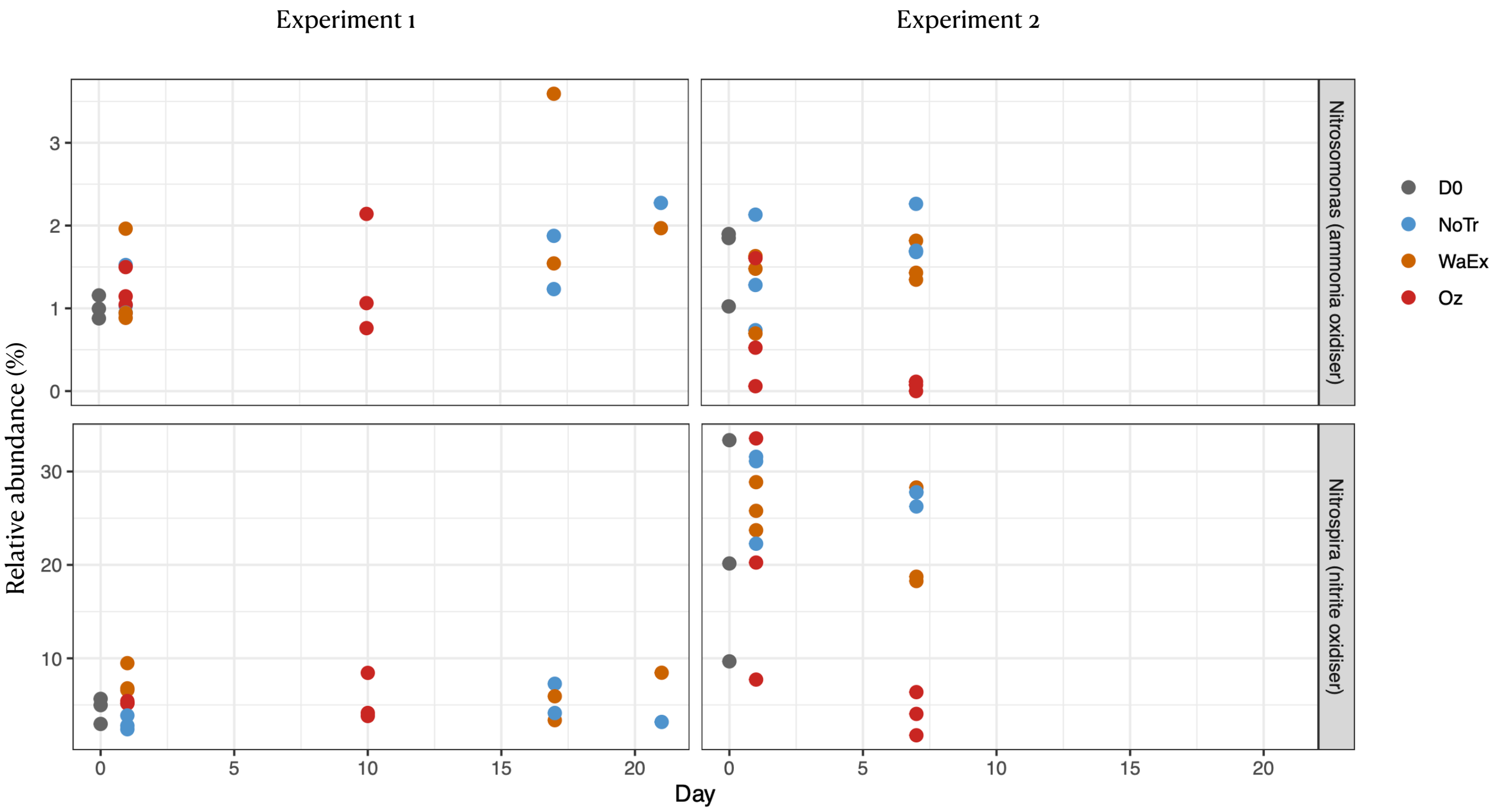
Experiment 2



Experiment 1



Relative abundances of Nitrosomonas and Nitrospira



Conclusion

> Ozonation attacks the biofilter, releases carbon and stimulate the growth of opportunistic organotrophs ('r-strategists')

> Strong ozonation seems to have limited effect of alpha diversity and richness.

> Still, strong ozonation can damage the biofilm severely and one should be careful with using this strategy if it is important to preserve biofilm functions.

> The effect of strong ozonation on RAS biofilms is highly variable
    > Depending the physical properties of the biofilm and
      the effectiveness of the ozonation attack?

Thanks

Norwegian Veterinary Institute — Sonal Patel, Duncan Colquhoun, Simon Weli, Brit Tørud, Merete Sture Gåsvær, Miriam Furne, Saima Mohammad, Henriette Kvalvik, Marta Moutinho

NIVA — Ole Kristian Hess-Erga, Paulo Fernandes, Stephanie Delacroix, Kamilla Furseth

Akvaplan niva — Sondre Veberg Larsen

PATOGEN — Elisabeth Midtbø Viik, Martin Huun-Røed

IAB — Reidar Handegård, Melanie Andrews, Mai-Linn J. Aga

UNIVERSITETET I BERGEN — Håkon Dahle, Loie Bonnet, Selma Grotaas, Birte Topper

PURE salmon Technology — Morten Lund, Erik Sterud, Ragnhild Hasche

VESO — Sigmund Sævetdal

Nofitech — Kari Attramadal

Funds

FHF - 091792

FHF

SOA15 A2-1

Co-funded by the European Union

EUP AH&W European Partnership on Animal Health and Welfare

«Co-funded by the European Union under project 101136346 EUPAHW. Views and opinions expressed are however those of the author(s) only and do not necessarily reflect those of the European Union or the European Research Executive Agency. Neither the European Union nor the granting authority can be held responsible for them.»