Supplementary Information for

Detection of Per- and Polyfluoroalkyl Substances (PFAS) in Water using a Fluorescent Imprint-and-Report Sensor Array

Emily E. Harrison and Marcey L. Waters*

Department of Chemistry, CB 3290, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina 27599, United States

Synthesis and Characterization of Materials

Building Blocks. Exchangeable building blocks (BBs) E,[1] G,[2] N[3], and X[4] were synthesized according to previously described literature procedures. The prepared materials were stored in nitrogen-filled vials sealed with Parafilm to prevent oxidation. Extinction coefficients were measured for BB X in 50 mM borate buffer (pH 8.5) at 267 nm and 280 nm. Three measurements of each sample concentration were taken at each wavelength and averaged. Path length = 0.1 cm. Extinction coefficients for BBs E, G, and N have been previously reported.[5]

BB X: 2.62 mg was dissolved in 10.0 mL buffer. Serial dilutions were made to achieve the concentration range 500 µM - 31.3 µM.

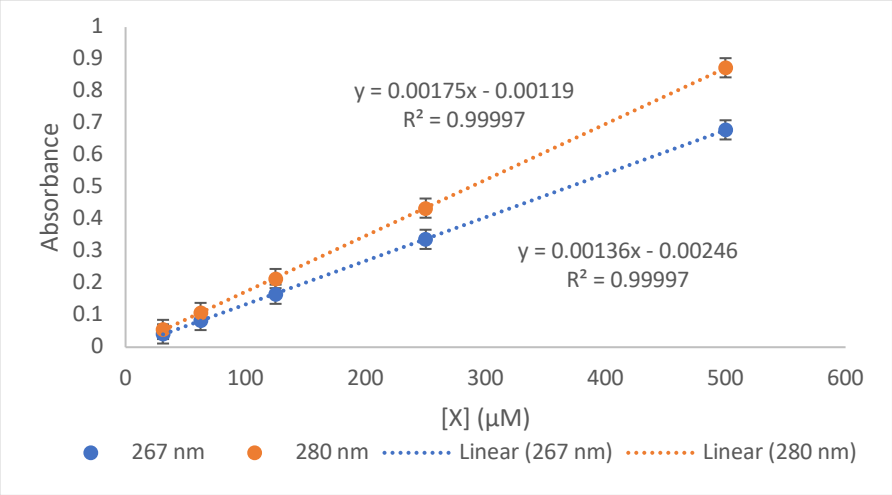


Figure S1. Plot of Abs versus concentration of BB X stocks to determine extinction coefficients. Abs was measured at 267 nm (blue) and 280 nm (orange). A standard error of +/- 0.03 for each Abs point was estimated.

Table S1. Extinction coefficients of BBs E, G, N, and X at 267 nm and 280 nm used for concentration determination.

BB	Extinction Coeff. @ 267 nm ($M^{-1}cm^{-1}$)	Extinction Coeff. @ 280 nm ($M^{-1}cm^{-1}$)
E[5]	8,750	11,100
G[5]	31,800	23,200
N[5]	11,000	9,900
X	13,600	17,500

Fluorophore and PFAS. Lucigenin (LCG) is a commercially available fluorophore that was purchased and used as received. The extinction coefficient used was 33,000 $M^{-1}cm^{-1}$ at 367 nm.[6]

Perfluorooctanesulfonic acid (CAS #1763-23-1), perfluorooctanoic acid (CAS #335-67-1), perfluoro-2-propoxypropanoic acid (CAS #13252-13-6), perfluoroheptanoic acid (CAS #375-85-9), perfluorohexanoic acid (CAS #307-24-4), perfluoropentanoic acid (CAS #2706-90-3), perfluorohexanesulfonic acid (CAS

#3871-99-6), and perfluorobutanesulfonic acid (CAS #375-73-5) were all purchased from commercial sources and used as received.

Dynamic Combinatorial Library Preparation and Characterization

The dynamic combinatorial libraries (DCLs) were prepared fresh for each experiment to allow disulfide exchange to occur in the presence of the template and to maintain consistency in equilibration time. BB stocks were measured by mass (2-3 mg) and dissolved in 1-2 mL 50 mM borate buffer (pH 8.5). Samples were sonicated to assist with dissolution. BB concentrations were validated using UV/Vis and their respective extinction coefficients (Table S1).

Dye stock solutions were made by dissolving lucigenin in 50 mM borate buffer (pH 8.5) and concentration was determined using UV/Vis and the reported literature extinction coefficient of the dye. The final concentration of dye in all templated libraries was 1.00 mM. Additionally, the total concentration of BB was 1.00 mM for all libraries. The concentrations of individual BBs in each library are as follows:

- E+G libraries: 0.50 mM E and 0.50 mM G
- G+N libraries: 0.50 mM G and 0.50 mM N
- E+N libraries: 0.50 mM E and 0.50 mM N
- E+G+N libraries: 0.25 mM E, 0.50 mM G, and 0.25 mM N
- X+G libraries: 0.50 mM X and 0.50 mM G
- X+N libraries: 0.50 mM X and 0.50 mM N
- X+N+G libraries: 0.25 mM X, 0.50 mM G, and 0.25 mM N

Libraries were equilibrated for 5 days with gentle shaking to allow thermodynamic equilibrium to be reached.

To characterize DCL speciation, the LCG-templated libraries were characterized on a Rapid Resolution LC-MSD system equipped with online degasser, binary pump, autosampler, heated column compartment, and diode array detector. We found that the large signal from free LCG interfered with our ability to characterize other species in the DCLs, so to prepare the DCLs for LC-MS analysis, they were first purified on a Waters semi-preparative RP-HPLC on a C18 column with a gradient of 0-100% B in 45 min (eluent A: 5 mM NH_4OAc in 100% water; eluent B: 5 mM NH_4OAc in 95% acetonitrile and 5% water). A large LCG peak eluted around 2 minutes, after which all species from the DCL were collected. Acetonitrile was evaporated from the samples, which were then frozen, lyophilized, and redissolved in borate buffer for LC-MS analysis. The X+N DCL purification of free LCG proved less effective, as the resulting LC-MS trace was messy and difficult to interpret. Thus, this DCL was purified via precipitation and redissolution using the following procedure (adapted from a literature procedure[7]) to eliminate as much free dye as possible. The DCL was first acidified with TFA to pH ~1-2 to form precipitate and then centrifuged at 6,000 rpm for 10 minutes. The liquid was decanted to yield a brown solid pellet, which was then resuspended in 5 mL milliQ water. After brief vortexing, the sample was centrifuged again, decanted, frozen, lyophilized, and redissolved in borate buffer (3 mL) for analysis.

Libraries were analyzed on a C18 column using a gradient of 0-100% B in 40 min with a flow rate of 0.3 mL/min (eluent A: 5 mM NH_4OAc in water; eluent B: 5 mM NH_4OAc in 10% water, 90% ACN). Mass spectra (ESI-) were acquired on a single quad mass spectrometer using a drying temperature of 350 °C, a nebulizer pressure of 45 psi, a drying gas flow of 10 L/min, and a capillary voltage of 3000 V. The untemplated and LCG-templated E+G, G+N, E+N, and E+G+N DCLs have been previously characterized and reported.[5]

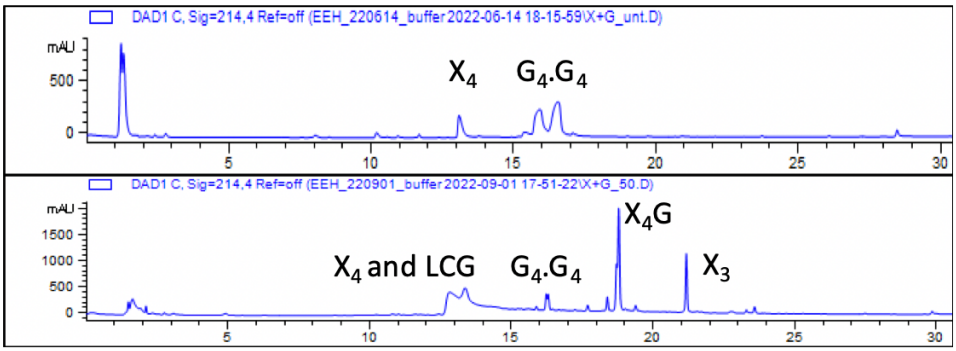


Figure S2. Absorbance (214 nm) traces of untemplated X+G DCL (top) and LCG-templated X+G DCL (bottom). Identified species are labeled on the spectra.

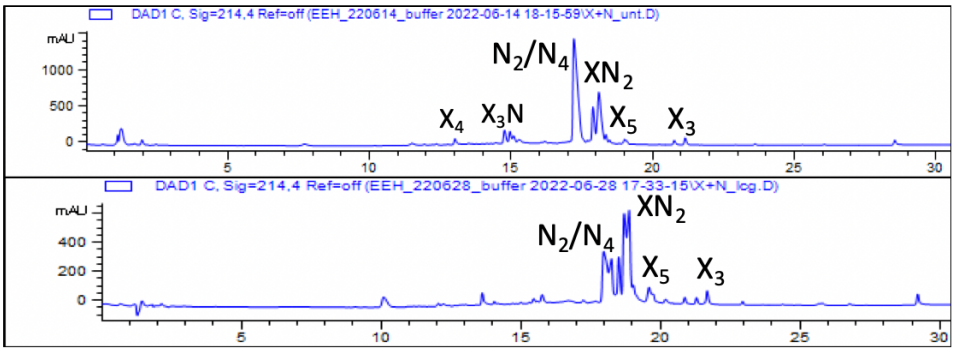


Figure S3. Absorbance (214 nm) traces of untemplated X+N DCL (top) and LCG-templated X+N DCL (bottom). Identified species are labeled on the spectra.

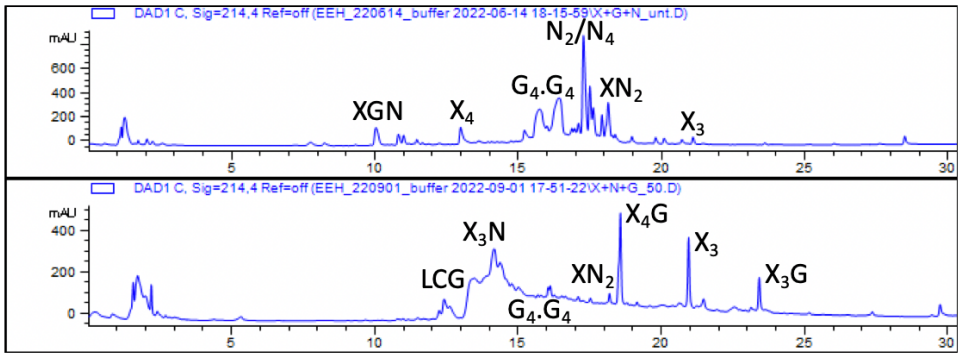


Figure S4. Absorbance (214 nm) traces of untemplated X+G+N DCL (top) and LCG-templated X+G+N DCL (bottom). Identified species are labeled on the spectra.

Fluorescent-DCC Sensor Array Assays

Individual PFAS assays. Stock solutions were made up of each of the six PFAS in 50 mM borate buffer (pH 8.5) and diluted to 500 nM with buffer. After DCLs equilibrated for 5 days, each one was diluted 100-fold (10 µL DCL + 990 µL buffer) and distributed among seven samples, each ultimately containing 10 µL diluted DCL, 10 µL 500 nM PFAS stock, and 980 µL buffer for the 5 nM PFAS assay. This resulted in final concentrations of 100 nM total BBs, 100 nM dye, and 5 nM PFAS. One sample contained 10 µL diluted DCL and 990 µL buffer to represent the 'No Guest' sample. For assays containing different PFAS concentrations, these volumes were adjusted and, in some cases, more dilute PFAS stocks (100 nM) were

used. These solutions were vortexed and left to incubate for 1 hour with gentle shaking. Six replicate samples (65 µL each) for each mixture were pipetted into Corning 384-well non-binding, black-bottom plates, and these plates were subsequently spun down at 4,000 rpm for 5 minutes.

Top-down fluorescence measurements were made using a BMG Labtech POLARStar Omega plate reader. The gain was set automatically by the Omega software using a 100 nM solution of dye in 50 mM borate buffer (pH 8.5) with no BBs or PFAS. Libraries were excited at 370 nm and emission was measured at 510 nm.

PFAS mixture assays. The same protocol as for *Individual PFAS assays* was adopted, except the PFAS mixture solutions were prepared at total PFAS concentrations of 500 nM in 50 mM borate buffer (pH 8.5), vortexed, and then added to the assay samples at the appropriate concentration. Further dilutions of the stocks were made as necessary. For the tap water assay, tap water from the lab sink was used in place of buffer.

Principal Component Analysis Workup

All assay data was visualized via principal component analysis (PCA). PCA was plotted with confidence ellipses using RStudio version 1.2.1335. Prior to being exported to Rstudio, the replicates of each data set were sorted from lowest to highest (Table S#), and each fluorescence value was normalized by dividing the raw fluorescence of each sample by the average fluorescence of the dye standard (Table S#). Since PCA requires the same number of data points for each analyte, removal of an outlier for a single analyte would require removing a data point for every analyte. To eliminate bias, a protocol was adopted where the highest and lowest data points were removed from each data set prior to PCA, resulting in 4 replicates per sample. Normalized fluorescence results were also visualized in bar graph form for facile visual comparison (Fig. S#).

Example of the data work-up for one DCL (E+G+LCG; 20 nM PFAS assay) is below.

Table S2. E+G+LCG raw fluorescence measurements with each PFAS guest. Each column is sorted from lowest to highest fluorescence.

Replicate	LCG Standard	No guest	PFOA	PFOS	GenX	PFHxA	PFPeA	PFHpA
1	230357	181793	185094	190283	180698	193580	189826	193603
2	232264	197251	191813	195923	185143	198514	193322	202123
3	232956	198336	197788	199656	189112	201741	193601	202564
4	234003	198756	200594	201085	191724	204090	194491	204005
5	234619	200392	201188	202920	192137	204461	195572	204247
6	235049	203926	201558	204064	193128	205495	201062	205469

Table S3. Data from Table S# normalized by dividing each fluorescence value by the average LCG standard fluorescence. The highest and lowest replicates have been removed, and the average and standard deviation have been included for each guest.

Replicate	No guest	PFOA	PFOS	GenX	PFHxA	PFPeA	PFHpA
2	0.846	0.822	0.840	0.794	0.851	0.829	0.867
3	0.850	0.848	0.856	0.811	0.865	0.830	0.869

4	0.852	0.860	0.862	0.822	0.875	0.834	0.875
5	0.859	0.863	0.870	0.824	0.877	0.870	0.876
Average	0.852	0.848	0.857	0.813	0.867	0.833	0.871
St. dev.	0.005	0.016	0.011	0.012	0.010	0.004	0.004

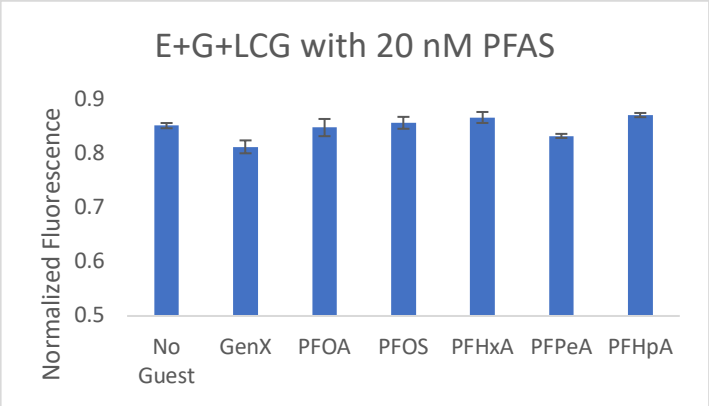


Figure S5. Bar graph of normalized fluorescence for each PFAS guest within the X+G+LCG DCL. Error bars represent standard deviation.

This process was repeated for each DCL that was analyzed. Data from Table S# was then formatted as a comma-separated list as follows:

0.846, 0.822, 0.840, 0.794, 0.851, 0.829, 0.867

0.850, 0.848, 0.856, 0.811, 0.865, 0.830, 0.869

0.852, 0.860, 0.862, 0.822, 0.875, 0.834, 0.875

0.859, 0.863, 0.870, 0.824, 0.877, 0.870, 0.876

Data lists in this format were input into Rstudio for each DCL. The code below was used to generate PCA plots with at least 90% confidence ellipses (***x*** = 0.90 or above). The 'PFAS' line was redefined with new sample names depending on the assay (individual, mixtures, etc.).

E/X_G <-c([insert comma-separated data list])

G_N <-c([insert comma-separated data list])

E/X_N <-c([insert comma-separated data list])

E/X_G_N <-c([insert comma-separated data list])

PFAS <-c("No Guest","PFOS","PFOA","GenX","PFHpA","PFHxA","PFPeA")

DCCdf <- data.frame(E/X_G, G_N, E/X_N, E/X_G_N, PFAS)

DCCdf.pca <- prcomp (DCCdf [, c(1:4)], center=TRUE, scale. = TRUE)

DCCdf.pca

str(DCCdf.pca)

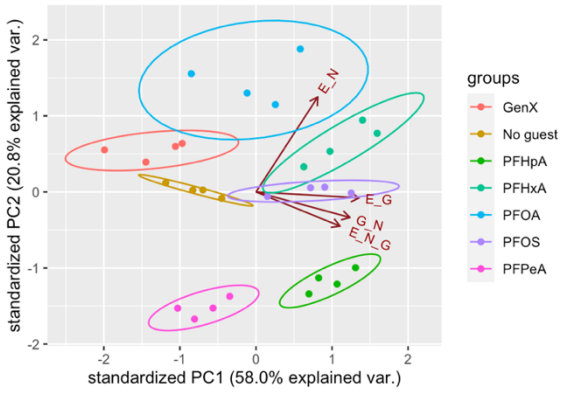
ggbiplot(DCCdf.pca, ellipse = TRUE, ellipse.prob = ***x***, groups = PFAS)

Sensor Array Results

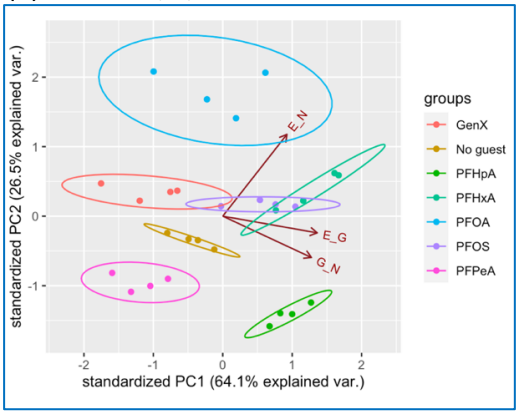
DCL data sets used to generate each PCA plot are denoted by the sensor labels defined in Figure 3 of the main text as follows: 1 = G+N, 2 = E+G, 3 = E+N, 4 = E+G+N, 2-X = X+G, 3-X = X+N, and 4-X = X+G+N. Plots outlined in blue are also shown in the main text.

Figure S6*. 100 nM E+G+N DCL set; 20 nM individual PFAS*

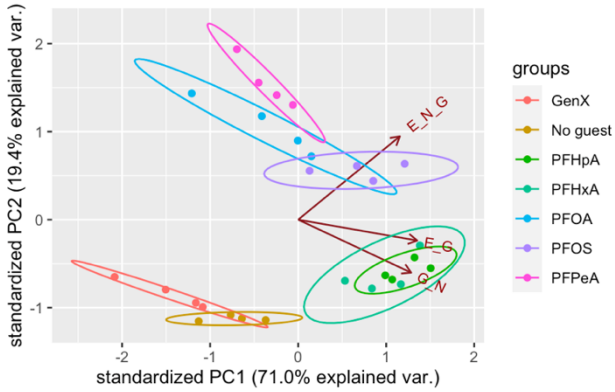
(a) Sensors 1, 2, 3, & 4:



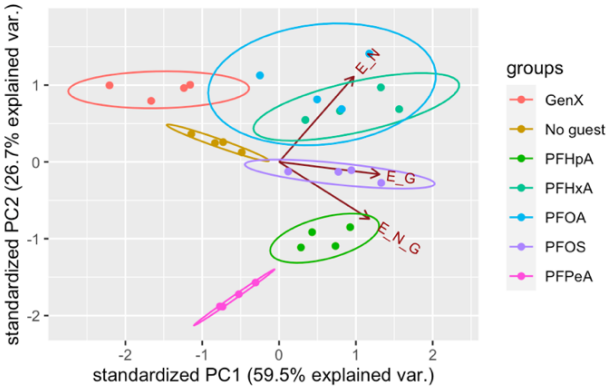
(b) Sensors 1, 2, & 3:



(c) Sensors 1, 2, & 4:



(d) Sensors 2, 3, & 4:



(e) Sensors 1, 3, & 4:

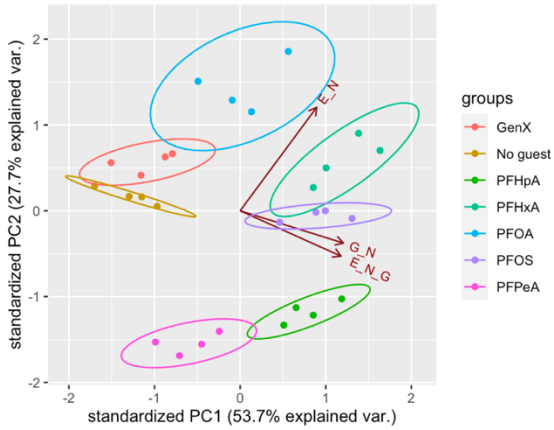
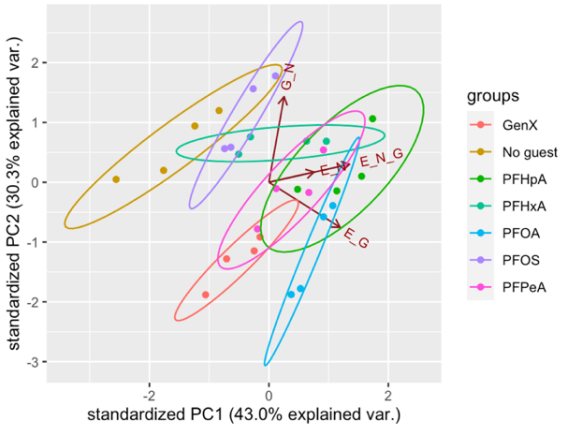
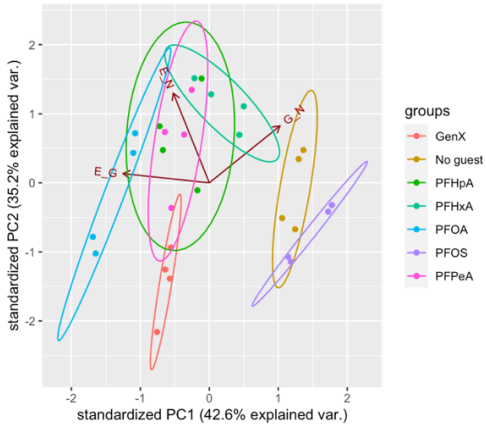


***Figure S7**. 100 nM E+G+N DCL set; 10 nM individual PFAS*

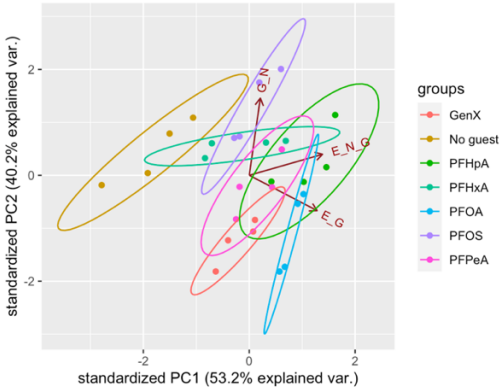
(a) Sensors 1, 2, 3, & 4:



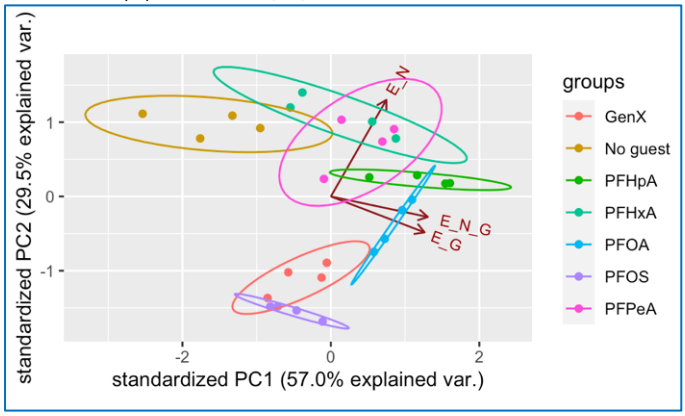
(b) Sensors 1, 2, & 3:



(c) Sensors 1, 2, & 4:



(d) Sensors 2, 3, & 4:



(e) Sensors 1, 3, & 4:

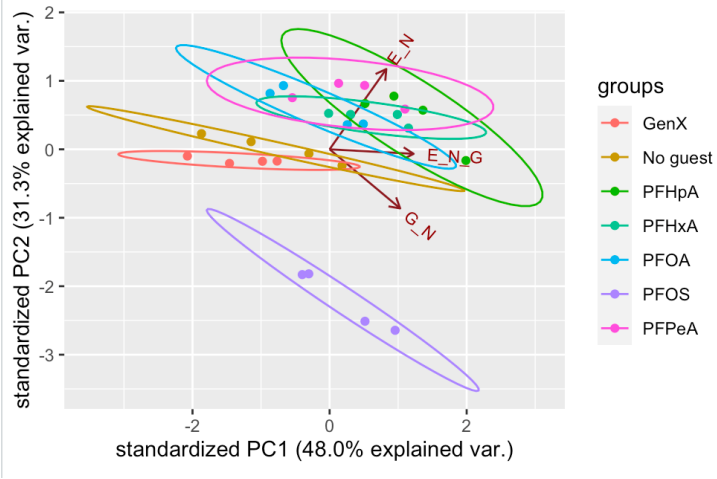
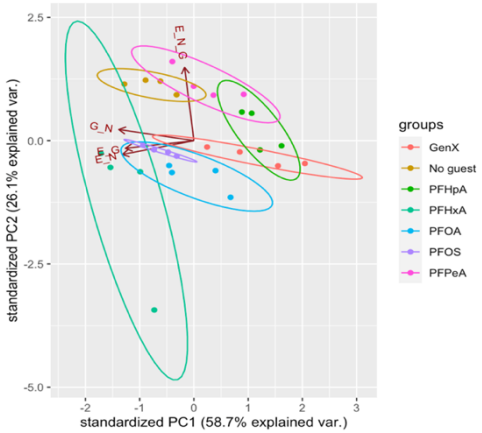
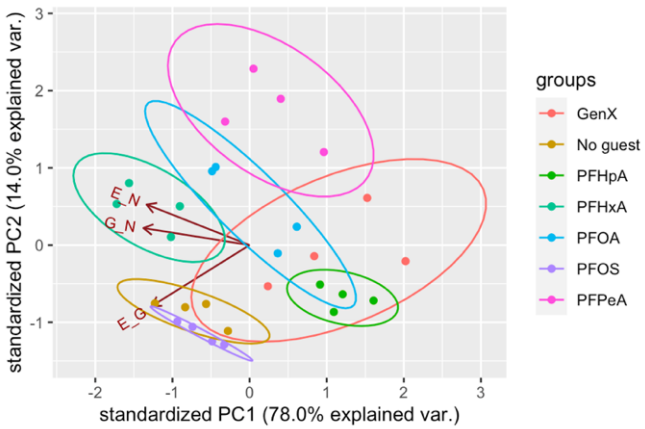


***Figure S8**. 100 nM E+G+N DCL set; 5 nM individual PFAS*

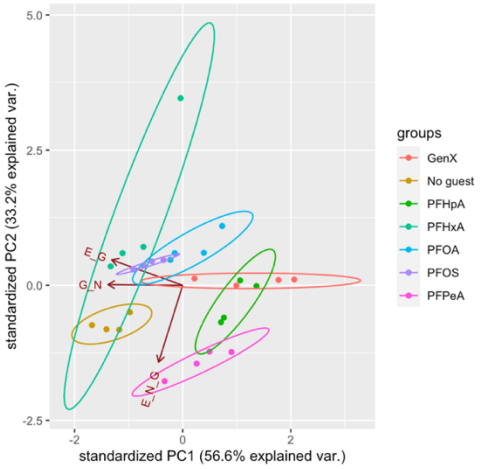
(a) Sensors 1, 2, 3, & 4:



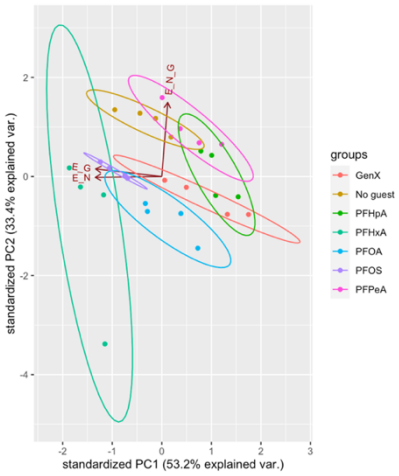
(b) Sensors 1, 2, & 3:



(c) Sensors 1, 2, & 4:



(d) Sensors 2, 3, & 4:



(e) Sensors 1, 3, & 4:

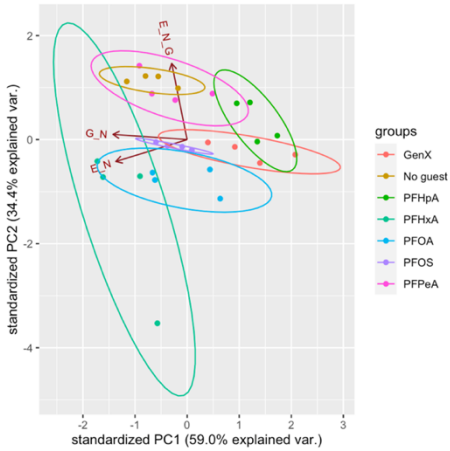
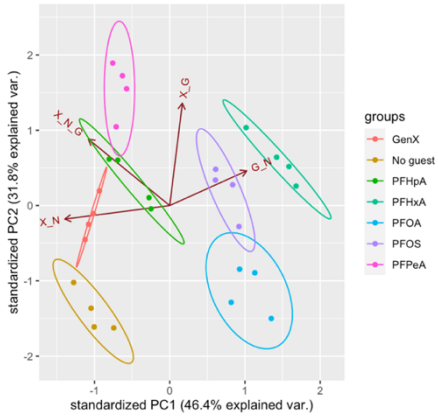
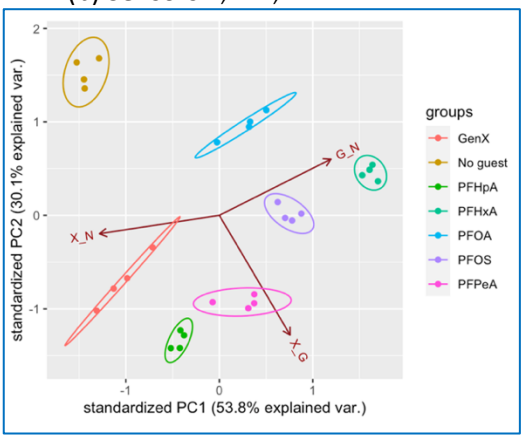


***Figure S9**. 100 nM X+G+N DCL set; 5 nM individual PFAS*

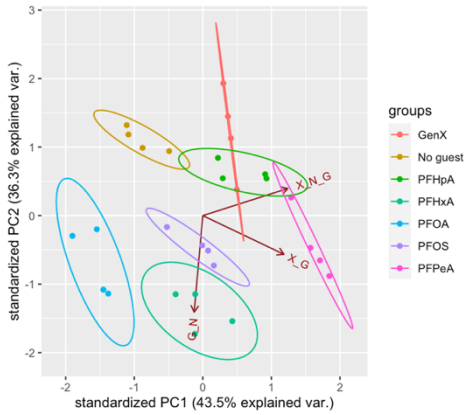
(a) Sensors 1, 2-X, 3-X, & 4-X:



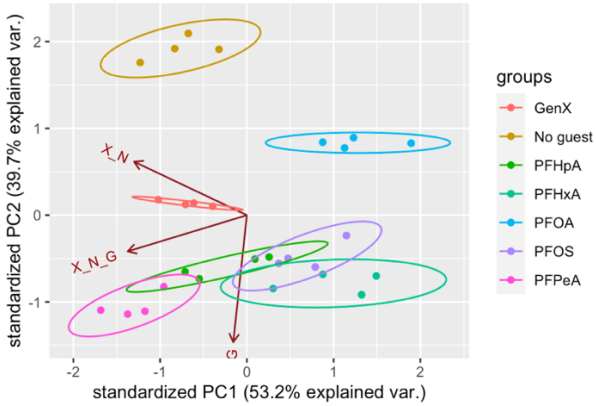
(b) Sensors 1, 2-X, & 3-X:



(c) Sensors 1, 2-X, & 4-X:



(d) Sensors 2-X, 3-X, & 4-X:



(e) Sensors 1, 3-X, & 4-X:

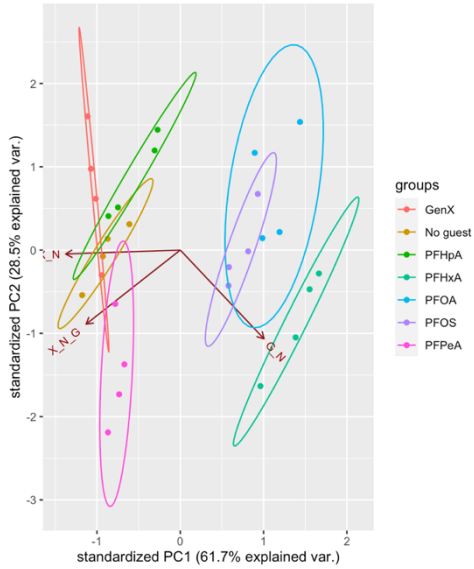
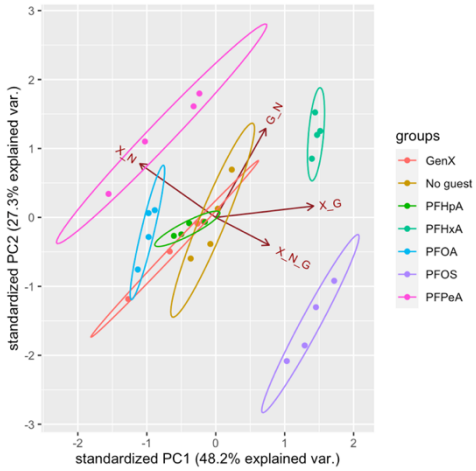
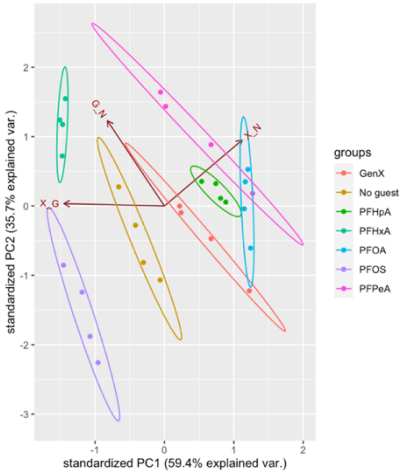


Figure S10*. 100 nM X+G+N DCL set; 1 nM individual PFAS*

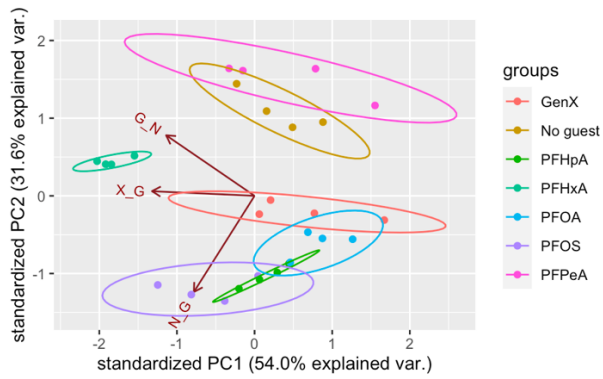
(a) Sensors 1, 2-X, 3-X, & 4-X:



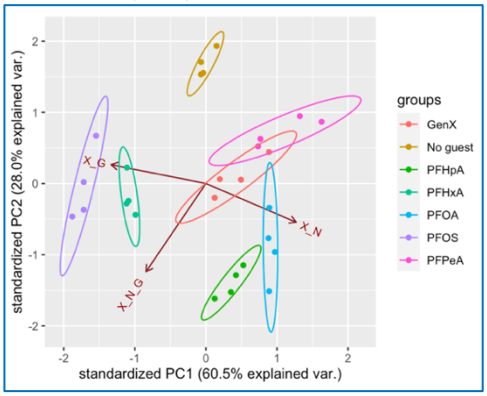
(b) Sensors 1, 2-X, & 3-X:



(c) Sensors 1, 2-X, & 4-X:



(d) Sensors 2-X, 3-X, & 4-X:



(e) Sensors 1, 3-X, & 4-X:

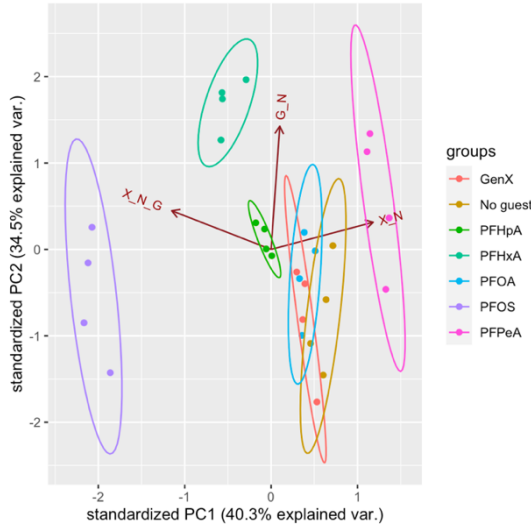
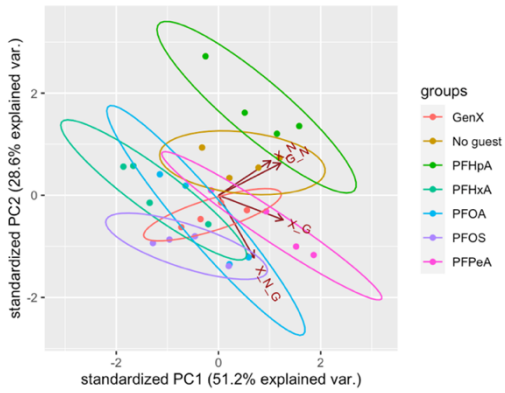
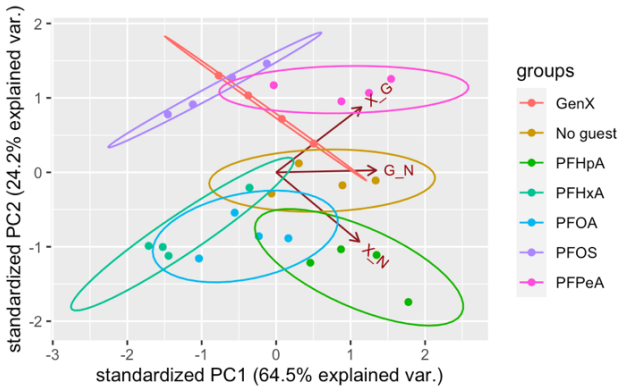


***Figure S11**. 100 nM X+G+N DCL set; 0.5 nM individual PFAS*

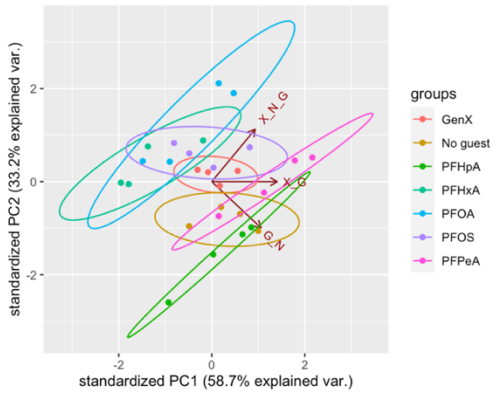
(a) Sensors 1, 2-X, 3-X, & 4-X:



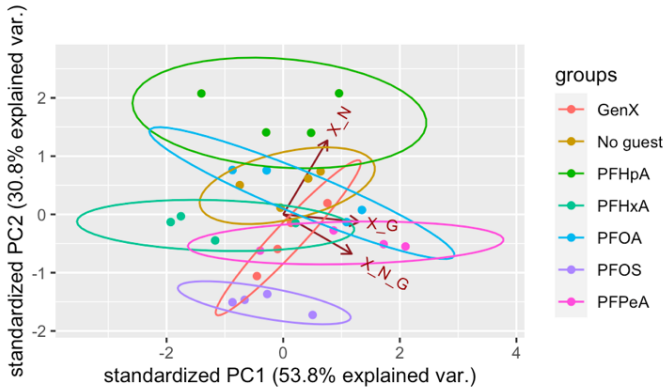
(b) Sensors 1, 2-X, & 3-X:



(c) Sensors 1, 2-X, & 4-X:



(d) Sensors 2-X, 3-X, & 4-X:



(e) Sensors 1, 3-X, & 4-X:

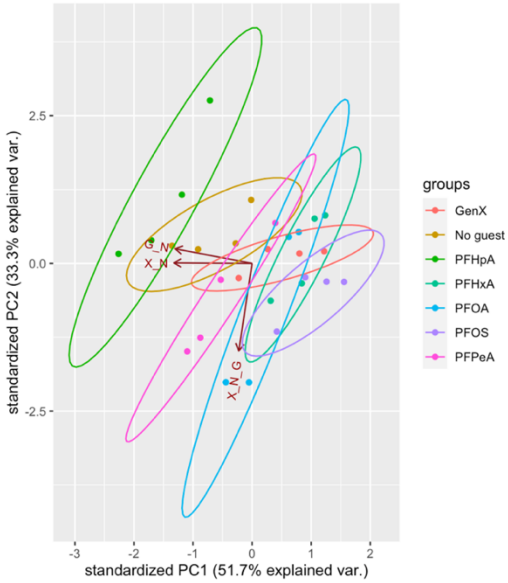
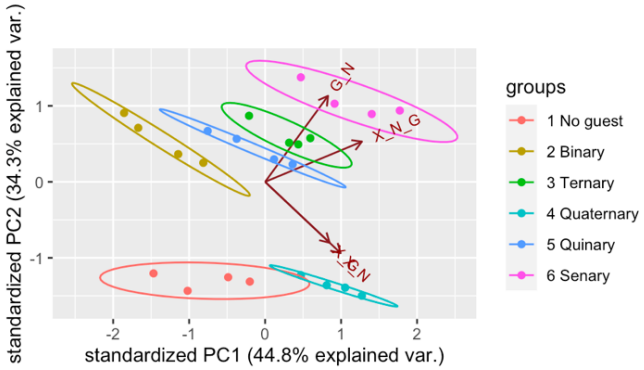
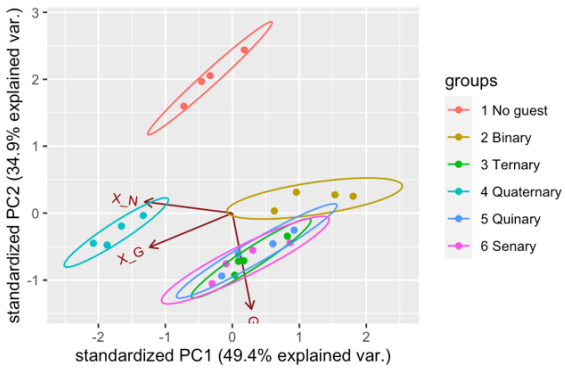


***Figure S12**. 100 nM X+G+N DCL set; 20 nM total PFAS mixtures (binary, ternary, quaternary, quinary, and senary)*

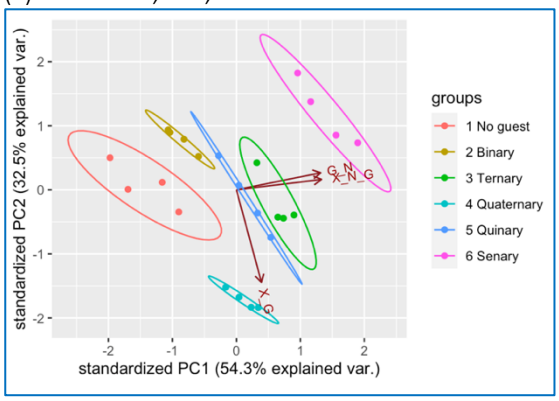
(a) Sensors 1, 2-X, 3-X, & 4-X:



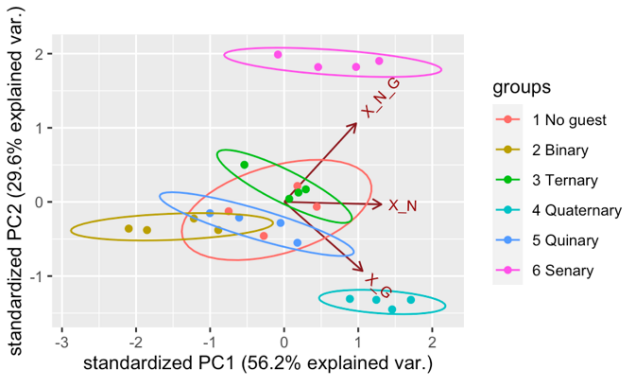
(b) Sensors 1, 2-X, & 3-X:



(c) Sensors 1, 2-X, & 4-X:



(d) Sensors 2-X, 3-X, & 4-X:



(e) Sensors 1, 3-X, & 4-X:

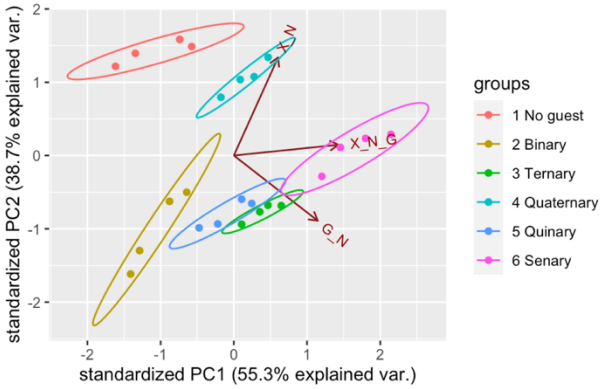
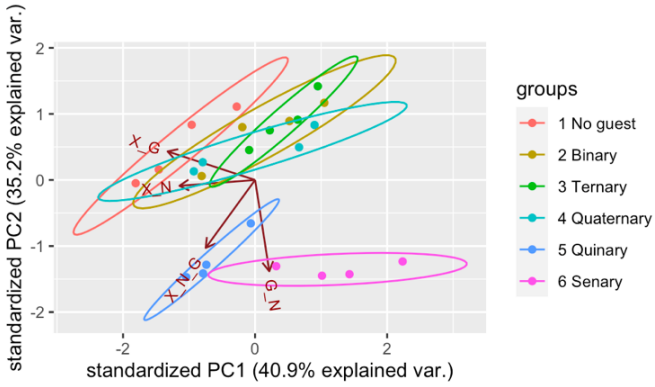
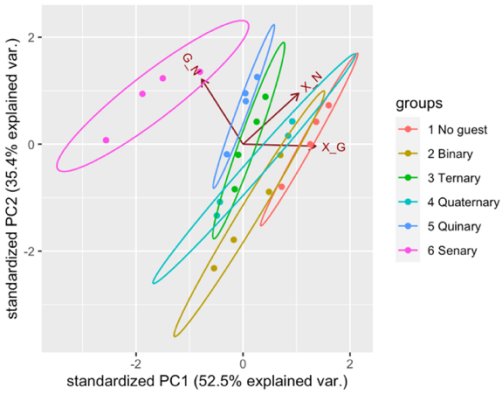


Figure S13*. 100 nM X+G+N DCL set; 10 nM total PFAS mixtures (binary, ternary, quaternary, quinary, and senary)*

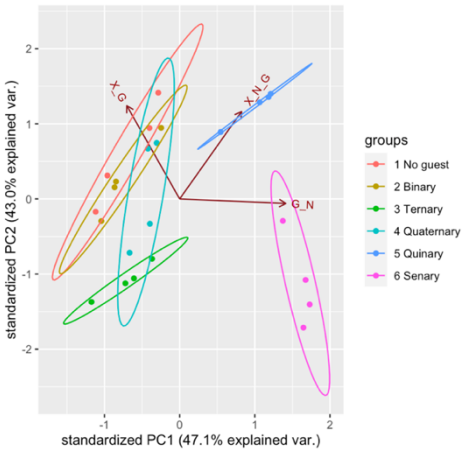
(a) Sensors 1, 2-X, 3-X, & 4-X:



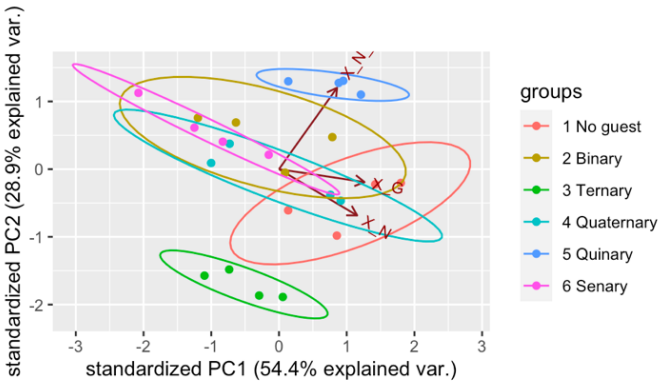
(b) Sensors 1, 2-X, & 3-X:



(c) Sensors 1, 2-X, & 4-X:



(d) Sensors 2-X, 3-X, & 4-X:



(e) Sensors 1, 3-X, & 4-X:

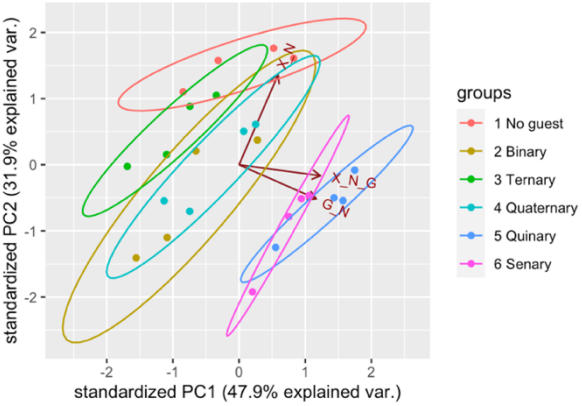
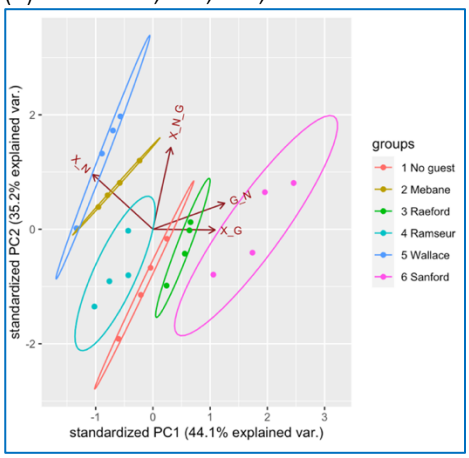
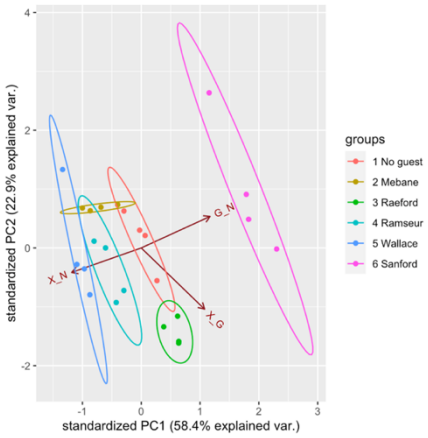


***Figure S14**. 100 nM X+G+N DCL set; 20-90 nM total PFAS mixtures (NC mixture set)*

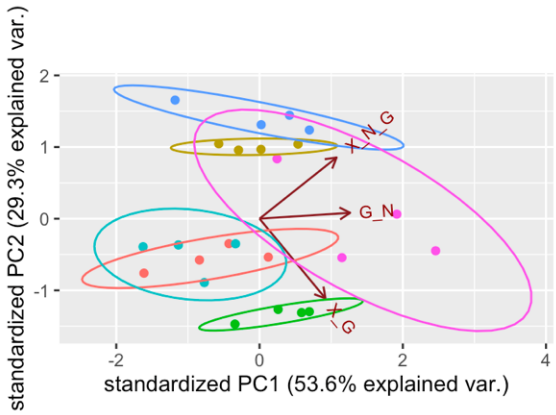
(a) Sensors 1, 2-X, 3-X, & 4-X:



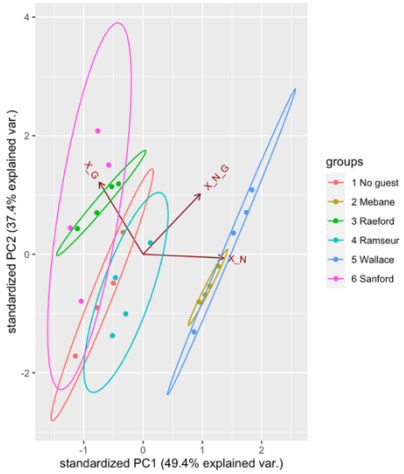
(b) Sensors 1, 2-X, & 3-X:



(c) Sensors 1, 2-X, & 4-X:



(d) Sensors 2-X, 3-X, & 4-X:



(e) Sensors 1, 3-X, & 4-X:

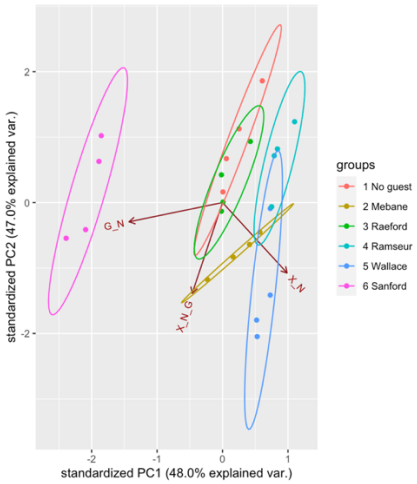
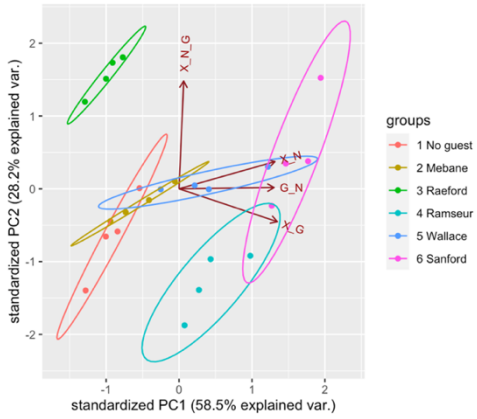
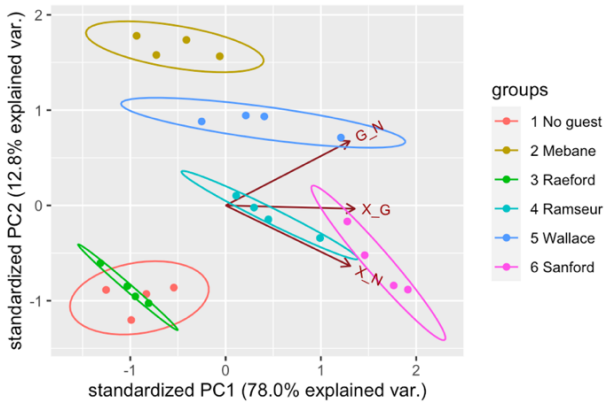


***Figure S15**. 100 nM X+G+N DCL set; 10-45 nM total PFAS mixtures (NC mixture set)*

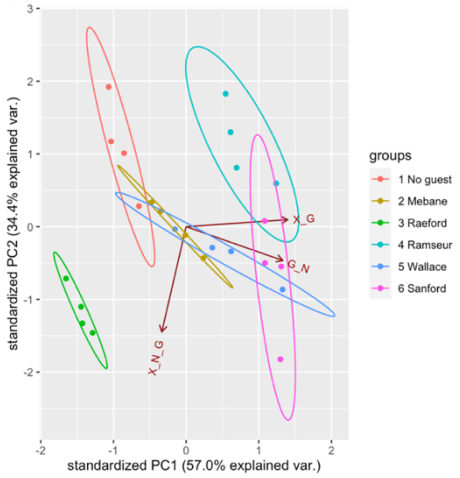
(a) Sensors 1, 2-X, 3-X, & 4-X:



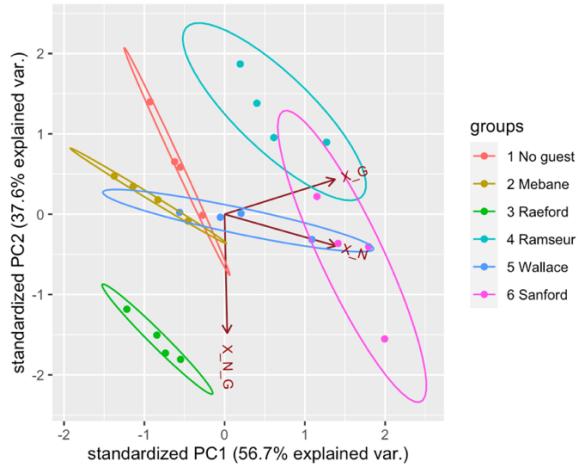
(b) Sensors 1, 2-X, & 3-X:



(c) Sensors 1, 2-X, & 4-X:



(d) Sensors 2-X, 3-X, & 4-X:



(e) Sensors 1, 3-X, & 4-X:

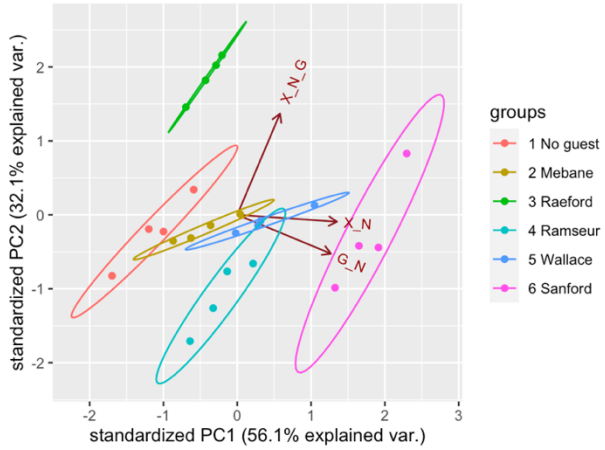
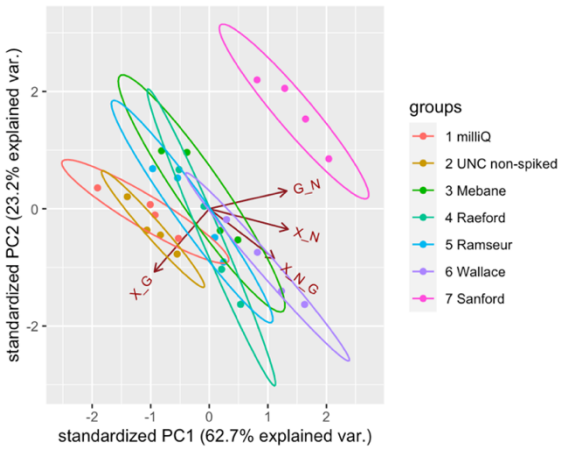
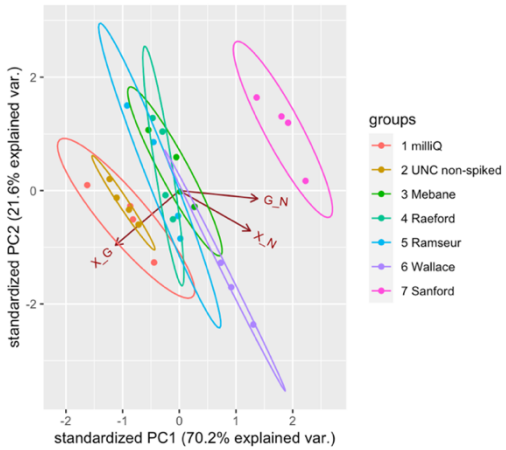


Figure S16*. 100 nM X+G+N DCL set; 40-180 nM total PFAS mixtures in tap water (NC mixture set)*

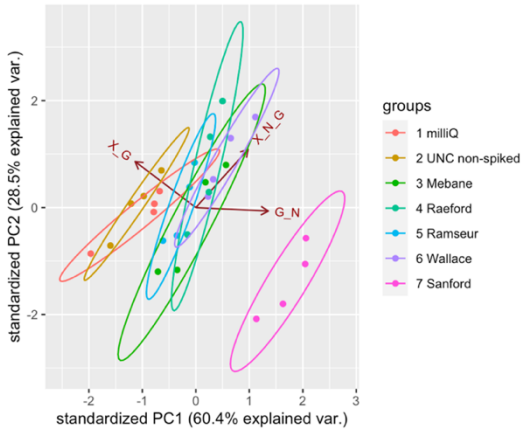
(a) Sensors 1, 2-X, 3-X, & 4-X:



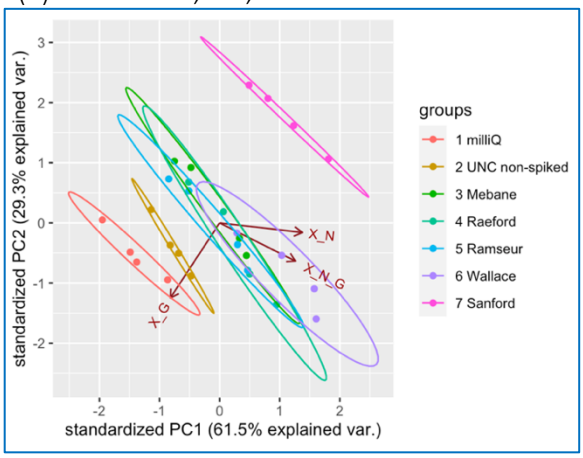
(b) Sensors 1, 2-X, & 3-X:



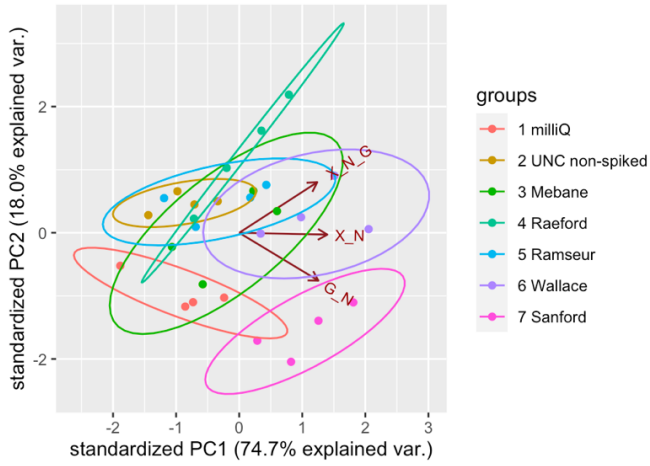
(c) Sensors 1, 2-X, & 4-X:



(d) Sensors 2-X, 3-X, & 4-X:



(e) Sensors 1, 3-X, & 4-X:

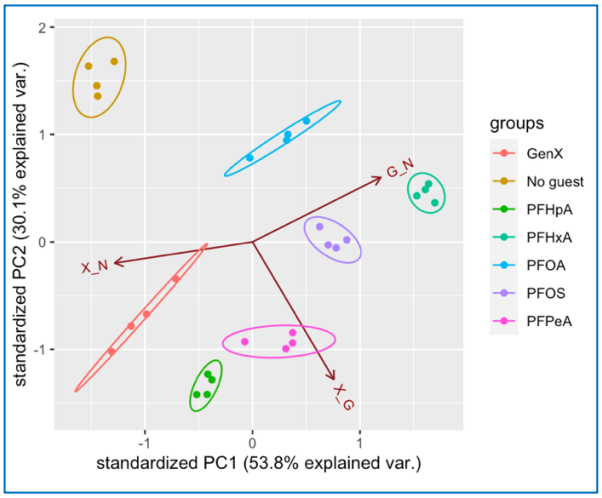


Sensor array results for reproducibility studies:

To test the reproducibility of the functional sensor arrays above, each assay was repeated with a fresh set of DCLs. The PCA plots from each trial are shown side-by-side for comparison. Plots outlined in blue are also shown in the main text.

***Figure S17**. 100 nM X+G+N DCL set; 5 nM individual PFAS*

Trial 1:



Trial 2:

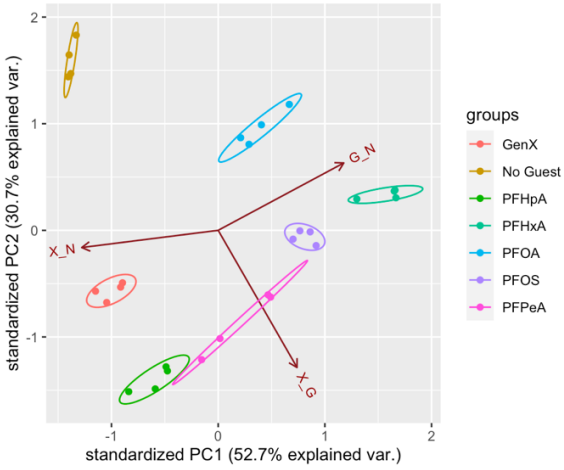
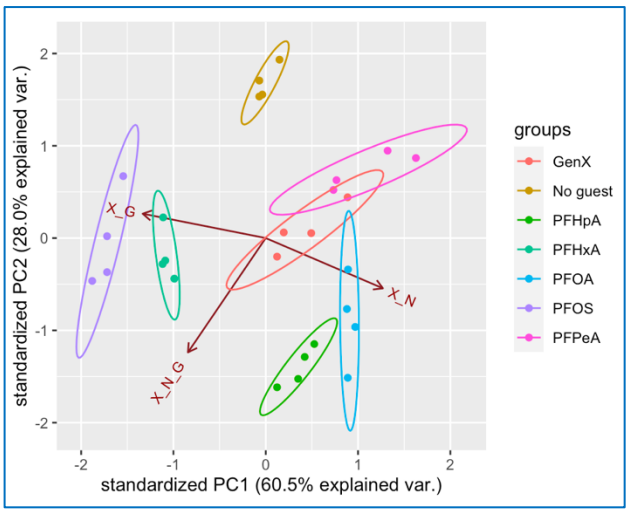


***Figure S18**. 100 nM X+G+N DCL set; 1 nM individual PFAS*

Trial 1:



Trial 2:

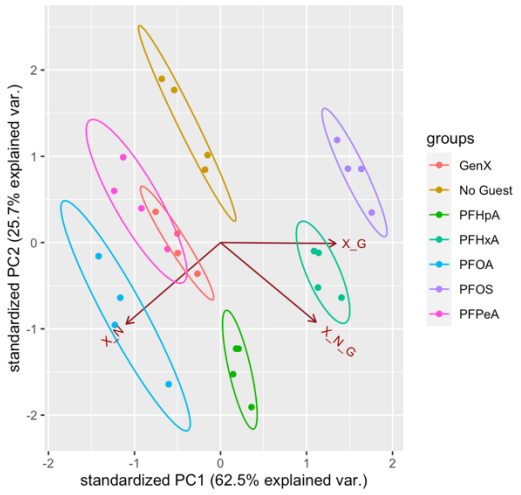
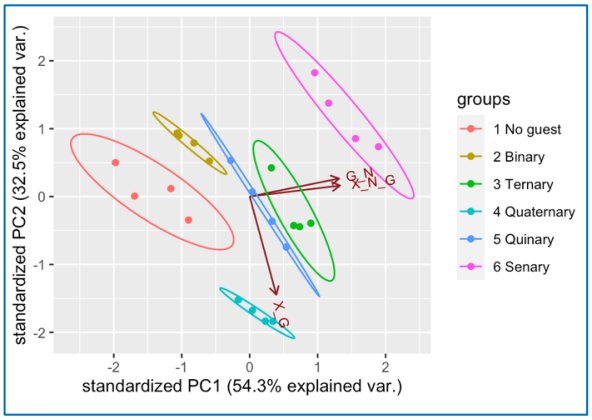


***Figure S19**. 100 nM X+G+N DCL set; 20 nM total PFAS mixtures (binary, ternary, quaternary, quinary, and senary)*

Trial 1:



Trial 2:

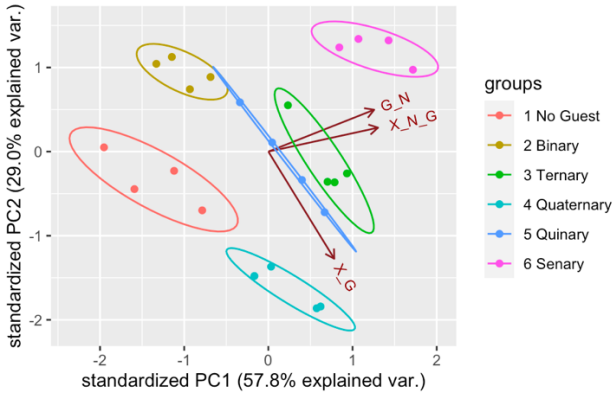
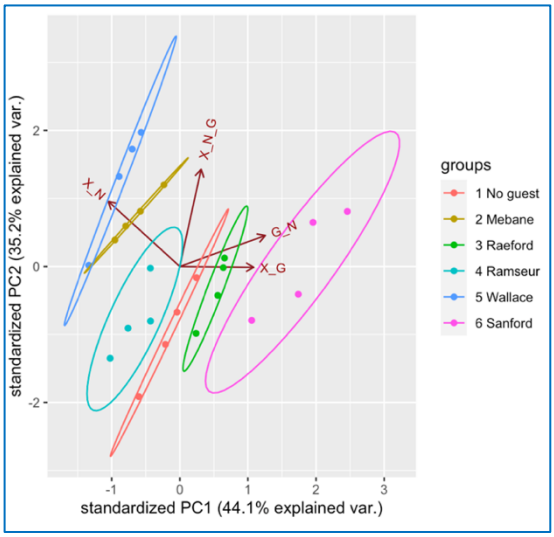


***Figure S20**. 100 nM X+G+N DCL set; 20-90 nM total PFAS mixtures (NC mixture set)*

Trial 1:



Trial 2:

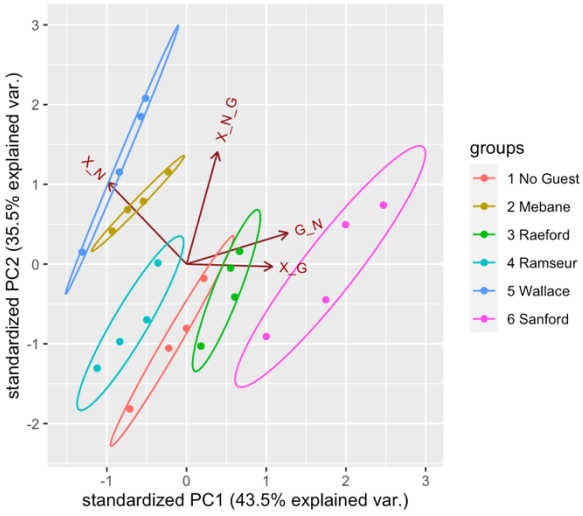
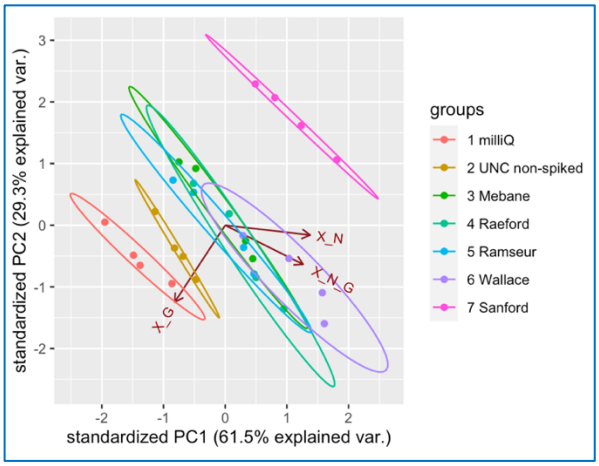


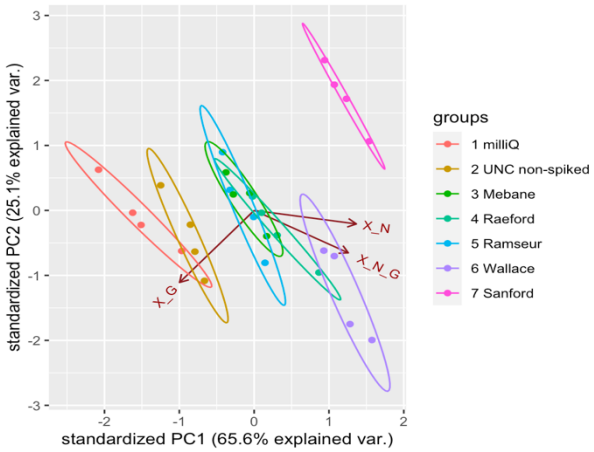
***Figure S21**. 100 nM X+G+N DCL set; 40-180 nM total PFAS mixtures in tap water (NC mixture set)*

Trials 1 & 2 were conducted with the same spiked tap water samples but different sets of DCLs. Trial 3 was conducted with both a fresh set of DCLs and freshly made spiked tap water samples, for which the tap water was collected during a different week.

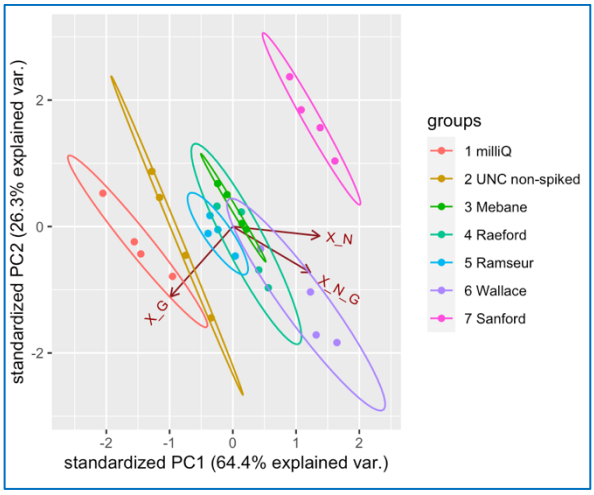
Trial 1:



Trial 2:



Trial 3



Supplementary Information References:

S1. Hamieh, S.; Saggiomo, V.; Nowak, P.; Mattia, E.; Ludlow, R. F.; Otto, S. *Angew. Chemie Int. Ed.* 2013, *52* (47), 12368–12372.

S2. West, K. R.; Ludlow, R. F.; Corbett, P. T.; Besenius, P.; Mansfeld, F. M.; Cormack, P. A. G.; Sherrington, D. C.; Goodman, J. M.; Stuart, M. C. A.; Otto, S. *J. Am. Chem. Soc.* 2008, *130* (33), 10834–10835.

S3. Pinkin, N. K.; Waters, M. L. *Org. Biomol. Chem.* 2014, *12* (36), 7059–7067.

S4. Umemoto, T.; Garrick, L. M.; Saito, N. *Beilstein J. of Org. Chemistry* 2012, *8*, 461-471.

S5. Harrison, E. E.; Carpenter, B. A.; St. Louis, L. E.; Mullins, A. G.; Waters, M. L. *J. Am. Chem. Soc.* 2021, *143* (36), 14845-14854.

S6. Peacor, B. C.; Ramsay, C. M.; Waters, M. L. *Chem. Sci.*, 2017, *8*, 1422–1428.

S7. Skowron, P. T.; Dumartin, M.; Jeamet, E.; Perret, F.; Gourlaouen, C.; Baudouin, A.; Fenet, B.; Naubron, J. V.; Fotiadu, F.; Vial, L.; Leclaire, J. *J. Org. Chem.* 2016, *81* (2), 654-661.