

Influence of Microbial Weathering on the Partitioning of Per- and Polyfluoroalkyl Substances (PFAS) in Biosolids

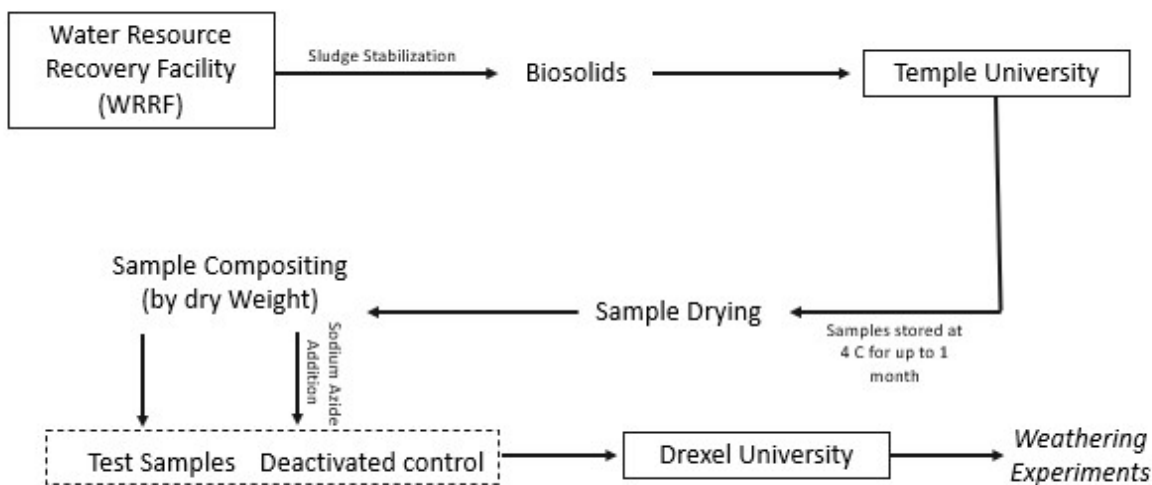
Asa J. Lewis^a, Farshad Ebrahimi^b, Erica R. McKenzie^b, Rominder Suri^b, Christopher M. Sales^a

(Corresponding author – Asa J Lewis - +1 207-323-8600 – ajl394@drexel.edu)

a. Department of Civil, Architectural, and Environmental Engineering, Drexel University, 3100 Market St.,
Philadelphia, PA, 19104, USA

b. Department of Civil and Environmental Engineering, Temple University, 1947 N 12th St., Philadelphia,
PA, 19122, USA

S1. Sample flow schematic of recruited biosolid samples from recruitment to weathering experiments.



S2. Calculation of various PFAS terminology concentrations.

$PFCA = \sum PFCA_i$ Data from unoxidized samples; i = species (or chain length)

$PFCA (from TOP) = \sum (PFCA_{i,ox} - PFCA_{i,unox})$ Data from oxidized and unoxidized samples; i = species (or chain length)

$PFSA (from TOP) = \sum (PFSA_{i,ox} - PFSA_{i,unox})$ Data from oxidized and unoxidized samples; i = species (or chain length); expected to be ~0

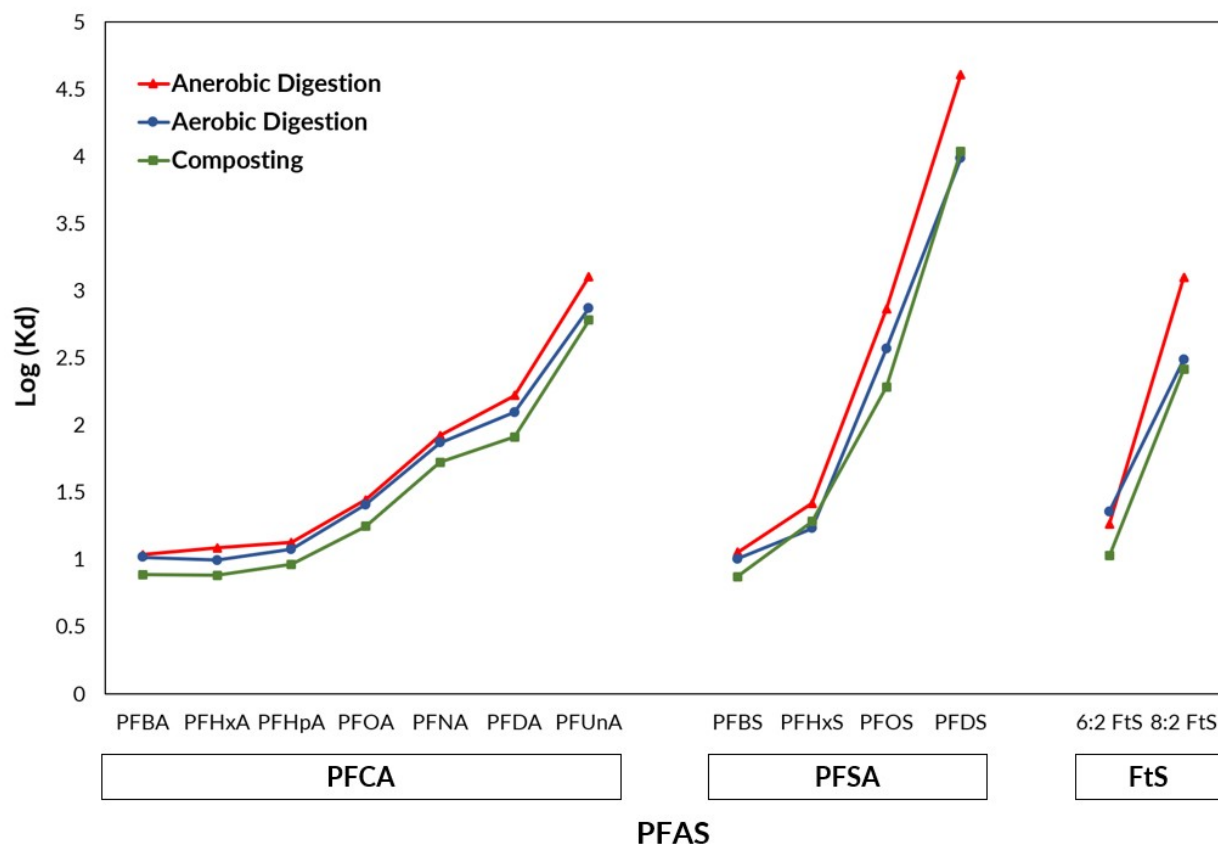
$Total\ PFAS = PFCA + PFSA$ Data from unoxidized samples (above)

$Precursor\ PFAS = \sum Precursor\ PFAS_{quant}$ Data from unoxidized samples, quantifiable compounds are 4:2 FtS, 6:2 FtS, 8:2 FtS, N-MeFOSAA, N-EtFOSAA, and PFOSA

S3. Precursors quantified for unoxidized samples concentrations over the weathering experiment.

		4:2 FtS (µg/kg)	6:2 FtS (µg/kg)	8:2 FtS (µg/kg)	N-MeFOSAA (µg/kg)	N-EtFOSAA (µg/kg)	PFOSA (µg/kg)
Aerobic	1	ND	72.51	5.26	61.39	21.40	38.99
	4	ND	61.14	4.56	60.27	14.70	38.09
	10	ND	37.20	3.16	50.40	16.22	29.05
	32	ND	ND	ND	28.48	2.46	15.47
	91	ND	ND	ND	37.20	3.31	18.59
	% Decrease	N/A	100	100	39.4	84.5	52.3
Anaerobic	1	ND	7.38	1.71	62.28	15.54	20.95
	4	ND	5.20	2.11	58.83	13.81	22.54
	10	ND	5.38	2.33	0.00	12.83	23.43
	32	ND	2.345	ND	74.82	5.02	16.52
	91	ND	2.10234	ND	50.60	3.99	16.93
	% Decrease	N/A	71.5	100.00	18.8	74.3	19.2
Composting	1	ND	35.15	10.17	93.71	28.88	47.80
	4	ND	30.61	7.48	106.13	23.30	45.43
	10	ND	30.14	4.61	87.70	23.80	43.19
	32	ND	18.32	ND	68.14	9.17	36.00
	91	ND	20.15	0.82	54.13	9.49	26.22
	% Decrease	N/A	42.7	91.9	42.2	67.1	45.1

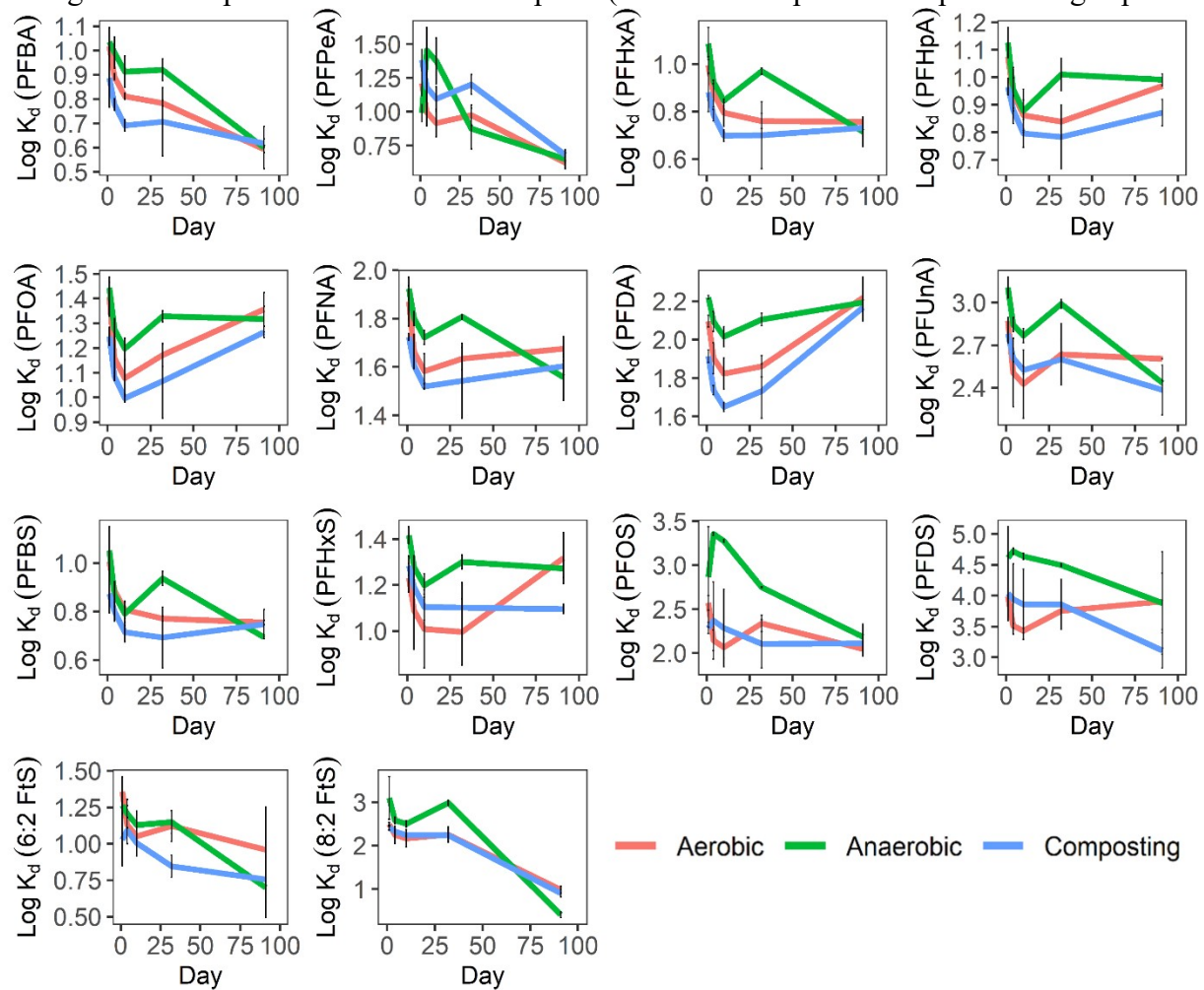
S4. Sludge Stabilization method and its impact on biosolids-water K_d values. Reported values are from Day 1 of the experiment (Samples directly from the WWTP and stored at 4°C).



S5. General characterization of aerobic, anaerobic, and composting digestion class B biosolid composite samples.

Stabilization Method	Day	Organic Matter (%)	Lipids (%)	Proteins (%)	Extractable Dissolved Organic Carbon (mg/L)	Extractable Total Nitrogen (mg/L)	pH
Aerobic	1	57.37 ± 1.03	9.3 ± 0.06	10.77 ± 0.24	291.37 ± 28.15	103.14 ± 6.40	6.2 ± 0.03
Aerobic	4	55.12 ± 0.71	9.21 ± 0.08	9.67 ± 0.71	215.23 ± 14.98	75.28 ± 5.35	6.26 ± 0.01
Aerobic	10	52.21 ± 0.54	8.42 ± 0.08	8.20 ± 0.37	207.7 ± 5.81	85.35 ± 3.05	6.26 ± 0.03
Aerobic	17	45.39 ± 1.53	8.01 ± 0.02	8.46 ± 0.32	169.54 ± 57.02	62.57 ± 16.86	6.32 ± 0.01
Aerobic	32	44.10 ± 3.00	7.8 ± 0.05	8.15 ± 0.20	165.97 ± 15.91	71.57 ± 4.73	6.32 ± 0.03
Aerobic	91	42.18 ± 1.45	7.55 ± 0.10	8.09 ± 0.12	152.37 ± 2.37	68.56 ± 1.37	6.32 ± 0.02
Anaerobic	1	65.33 ± 1.27	7.91 ± 0.09	11.62 ± 0.83	274.5 ± 86.72	92.56 ± 22.08	6.30 ± 0.02
Anaerobic	4	63.13 ± 1.17	6.84 ± 0.12	10.57 ± 0.42	226.2 ± 48.75	90.92 ± 19.07	6.93 ± 0.02
Anaerobic	10	57.48 ± 1.15	6.28 ± 0.13	9.44 ± 0.68	91.37 ± 61.48	38.11 ± 19.03	6.98 ± 0.02
Anaerobic	17	52.57 ± 2.63	6.1 ± 0.08	9.10 ± 0.85	66.18 ± 30.89	39.74 ± 23.26	6.96 ± 0.06
Anaerobic	32	50.08 ± 1.29	5.84 ± 0.04	8.88 ± 0.62	79.22 ± 7.88	34.69 ± 4.81	7.18 ± 0.02
Anaerobic	91	47.84 ± 2.06	5.64 ± 0.08	8.85 ± 0.07	79.33 ± 2.96	27.71 ± 1.71	7.31 ± 0.02
Composting	1	62.12 ± 1.18	5.66 ± 0.18	9.49 ± 0.83	21.43 ± 4.70	12.25 ± 2.71	6.74 ± 0.04
Composting	4	55.14 ± 1.22	5.4 ± 0.14	9.03 ± 0.85	51.67 ± 4.23	27.03 ± 2.20	6.87 ± 0.06
Composting	10	43.53 ± 0.92	4.75 ± 0.09	7.80 ± 0.32	115.77 ± 53.62	47.84 ± 18.92	6.95 ± 0.03
Composting	17	42.65 ± 1.62	4.66 ± 0.13	7.74 ± 0.68	191.59 ± 96.47	73.19 ± 32.97	6.97 ± 0.03
Composting	32	40.46 ± 0.97	4.55 ± 0.18	7.65 ± 0.39	154.94 ± 43.30	85.93 ± 5.40	6.77 ± 0.04
Composting	91	34.82 ± 2.23	4.48 ± 0.18	7.68 ± 0.13	201.33 ± 15.77	97.47 ± 2.37	6.90 ± 0.02

S6. Time series for change in PFAS biosolid-Water K_d for all PFAS compounds analyzed throughout the experiment. $n = 9$ for each point (microcosm triplicate and partitioning triplicate).

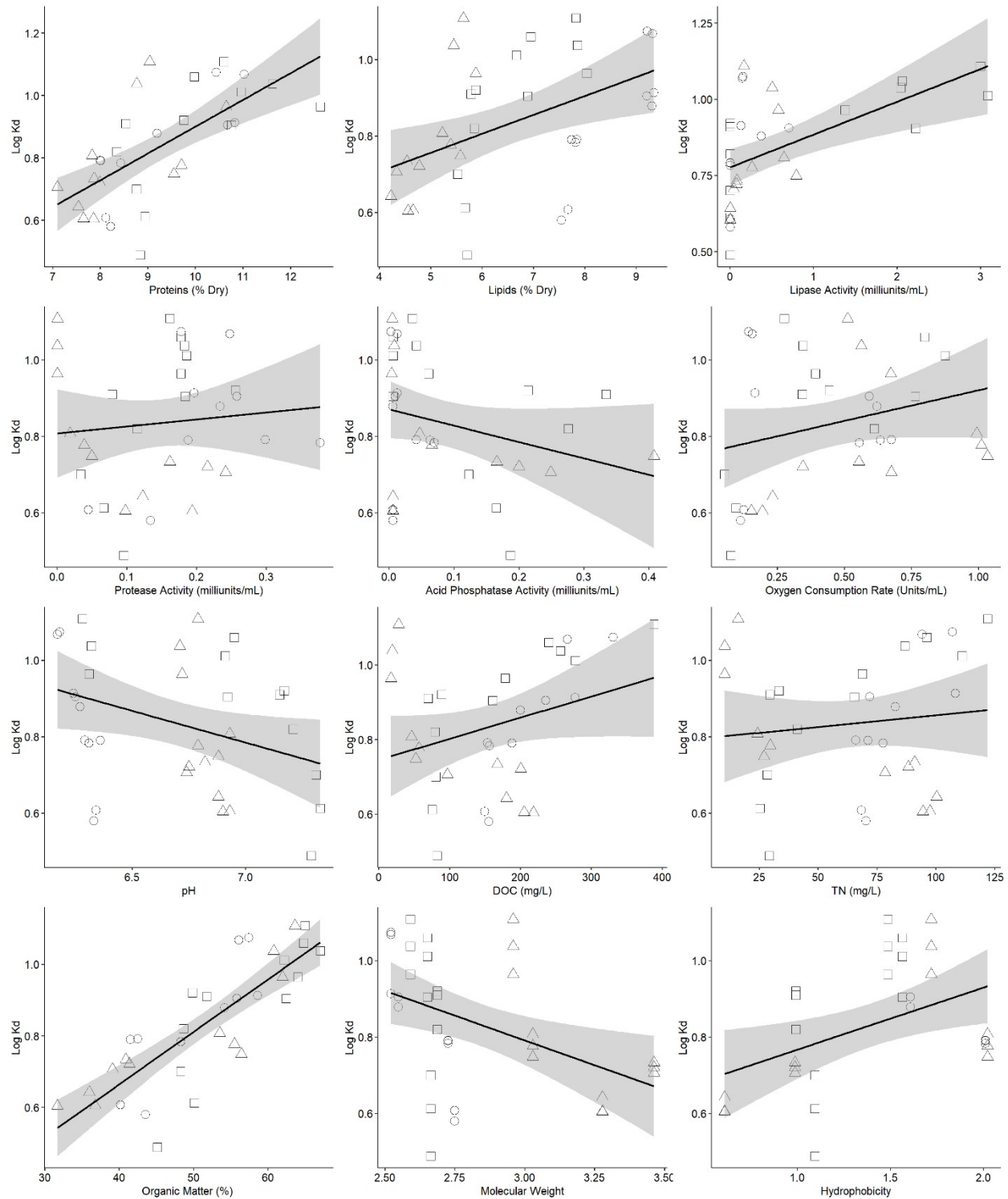


S7. Calculation of rate change of total PF_{AA}.

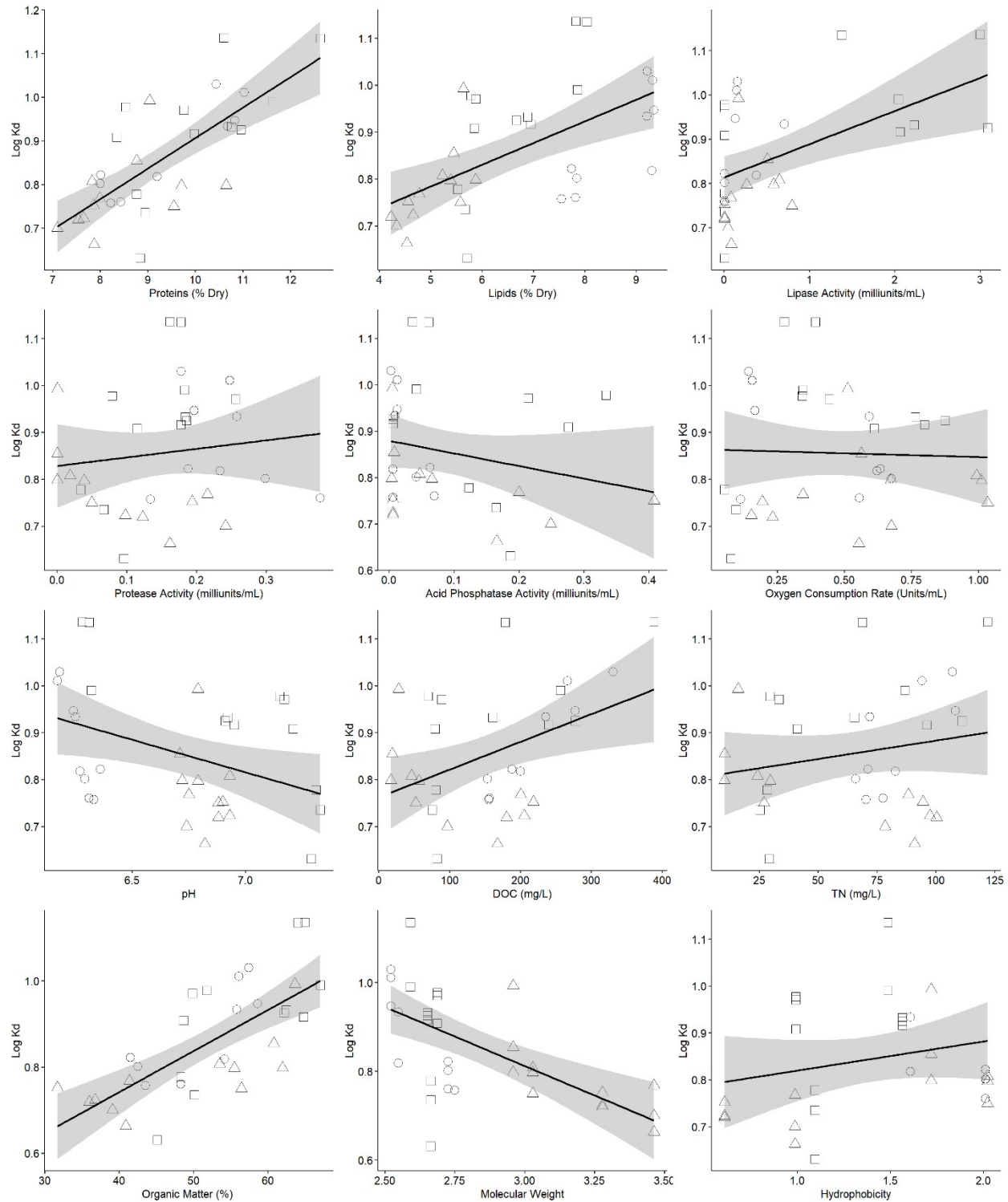
$$\text{Rate Change in Total PF}_{AA} = \frac{\text{Total PF}_{AA} \text{ Concentration } (\frac{ug}{kg})}{\text{Days } (d)}$$

The above formula was used to analyze changes in total PF_{AA} over the course of the experiment, represented in Figure 4 of the main manuscript. Since change in total PF_{AA} increased after Day 10 of analysis, two different rate changes were calculated for the first 10 days (Total PF_{AA} concentration Day 1 – Day 10) and for the last 81 days (Total PF_{AA} concentration Day 10 – Day 91) of the experiment to realize the change.

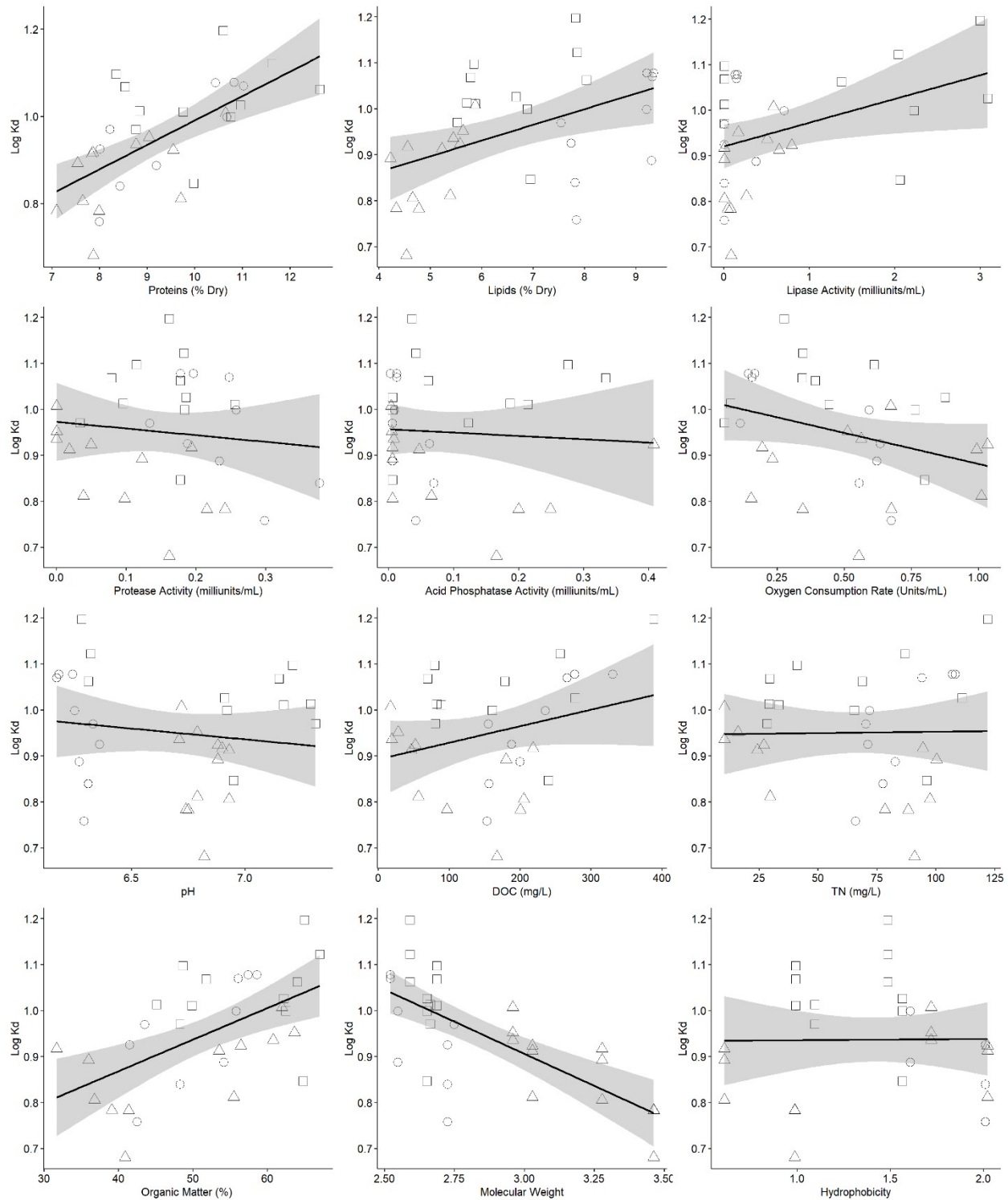
S8. PFBA single variable linear regressions. Gray band is a confidence band (95% confidence). Vertical stacking for some figures is a result of non-detect values for the assay. Triangles represent composting samples, squares represent anaerobic samples, and circles represent aerobic samples.



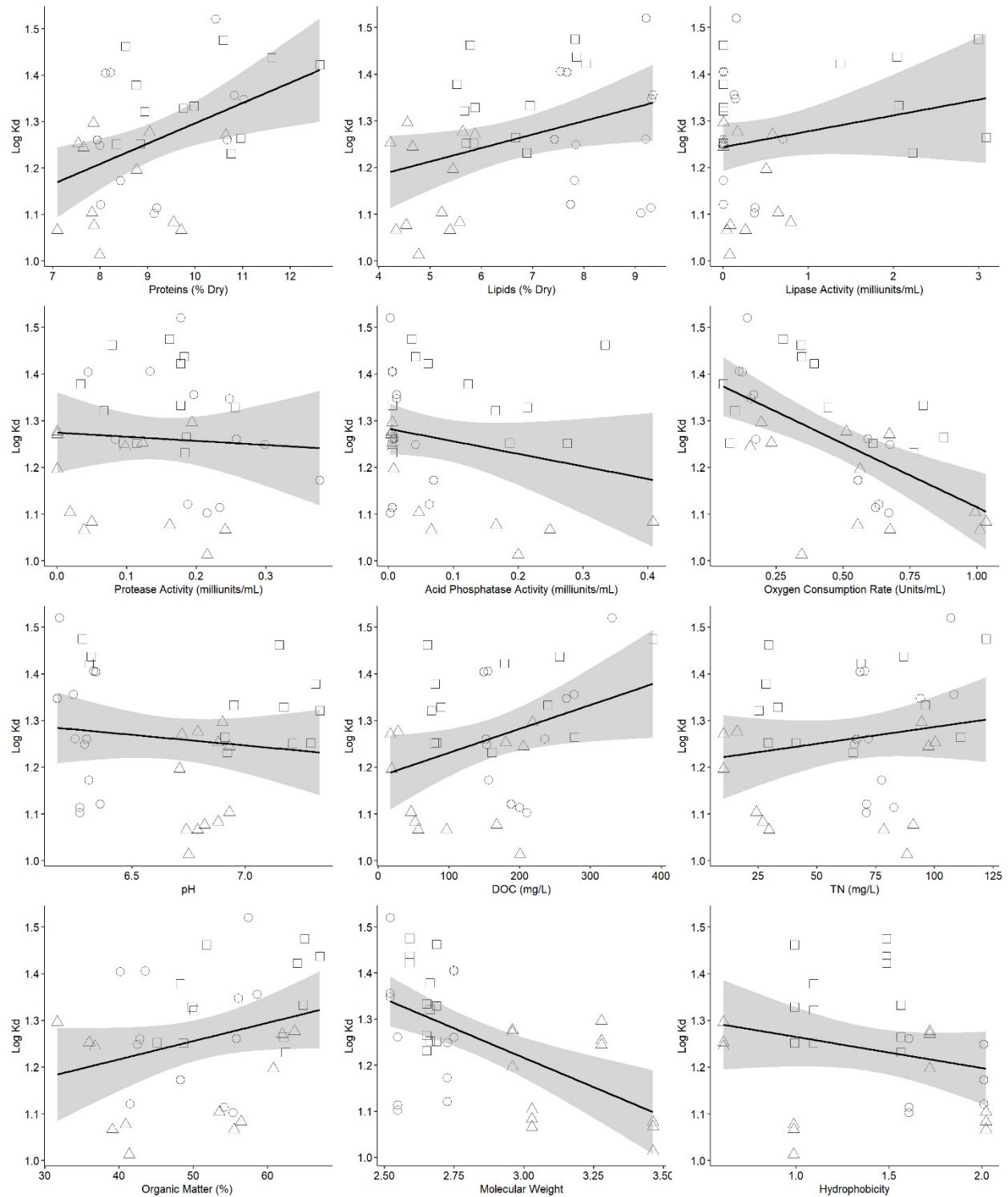
S9. PFHxA single variable linear regressions. Gray band is a confidence band (95% confidence). Vertical stacking for some figures is a result of non-detect values for the assay. Triangles represent composting samples, squares represent anaerobic samples, and circles represent aerobic samples.



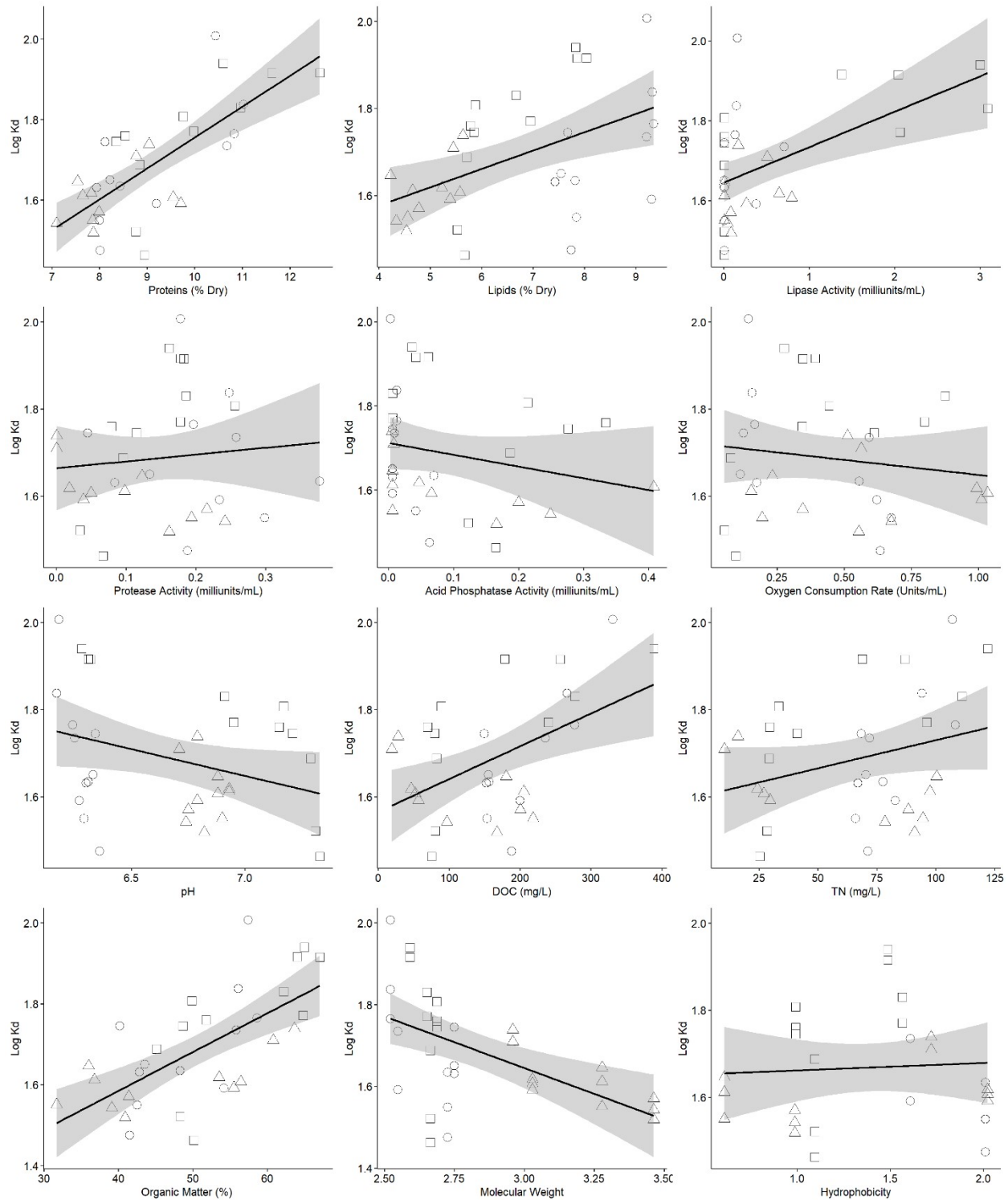
S10. PFHpA single variable linear regressions. Gray band is a confidence band (95% confidence). Vertical stacking for some figures is a result of non-detect values for the assay. Triangles represent composting samples, squares represent anaerobic samples, and circles represent aerobic samples.



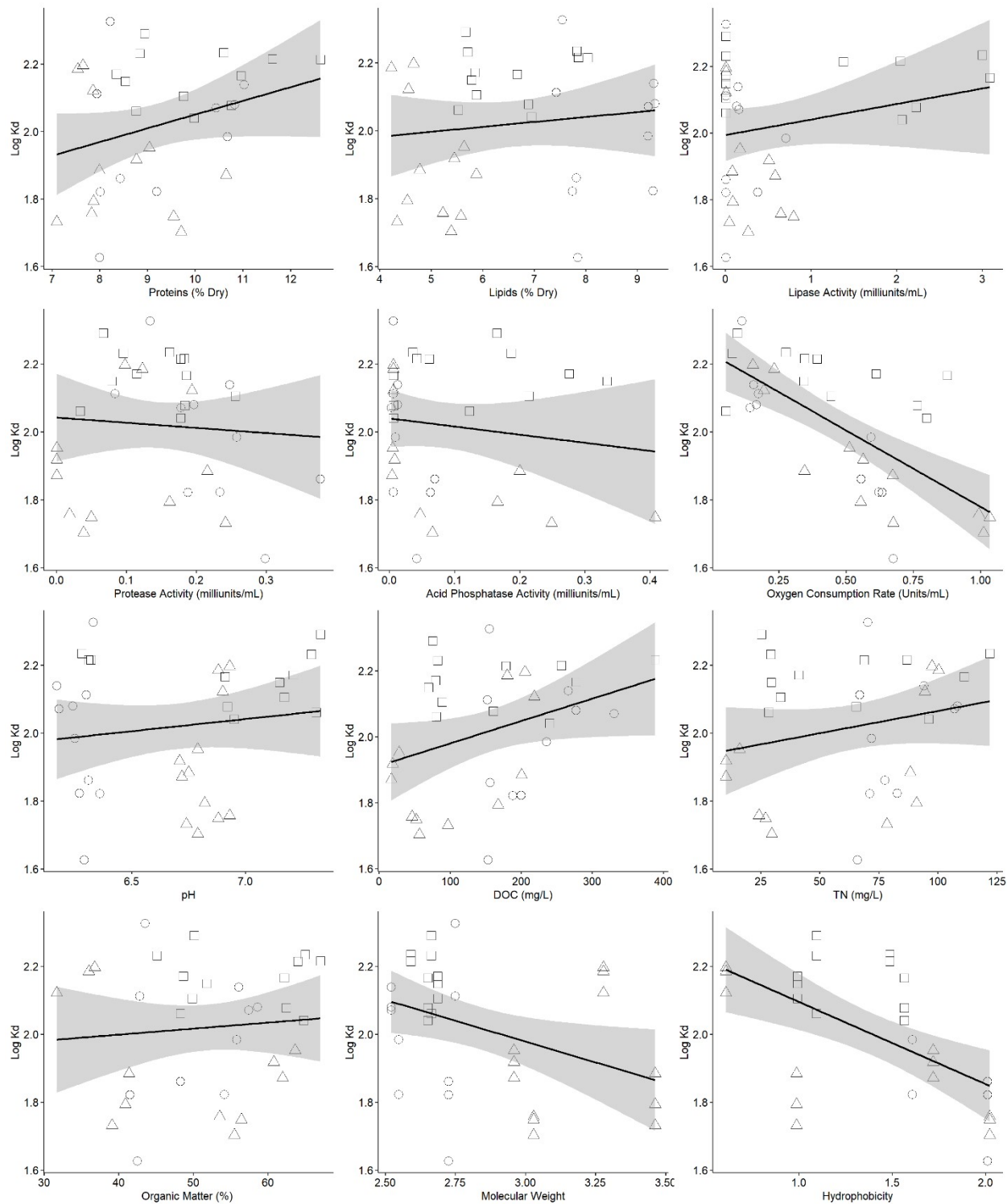
S11. PFOA single variable linear regressions. Gray band is a confidence band (95% confidence). Vertical stacking for some figures is a result of non-detect values for the assay. Triangles represent composting samples, squares represent anaerobic samples, and circles represent aerobic samples.



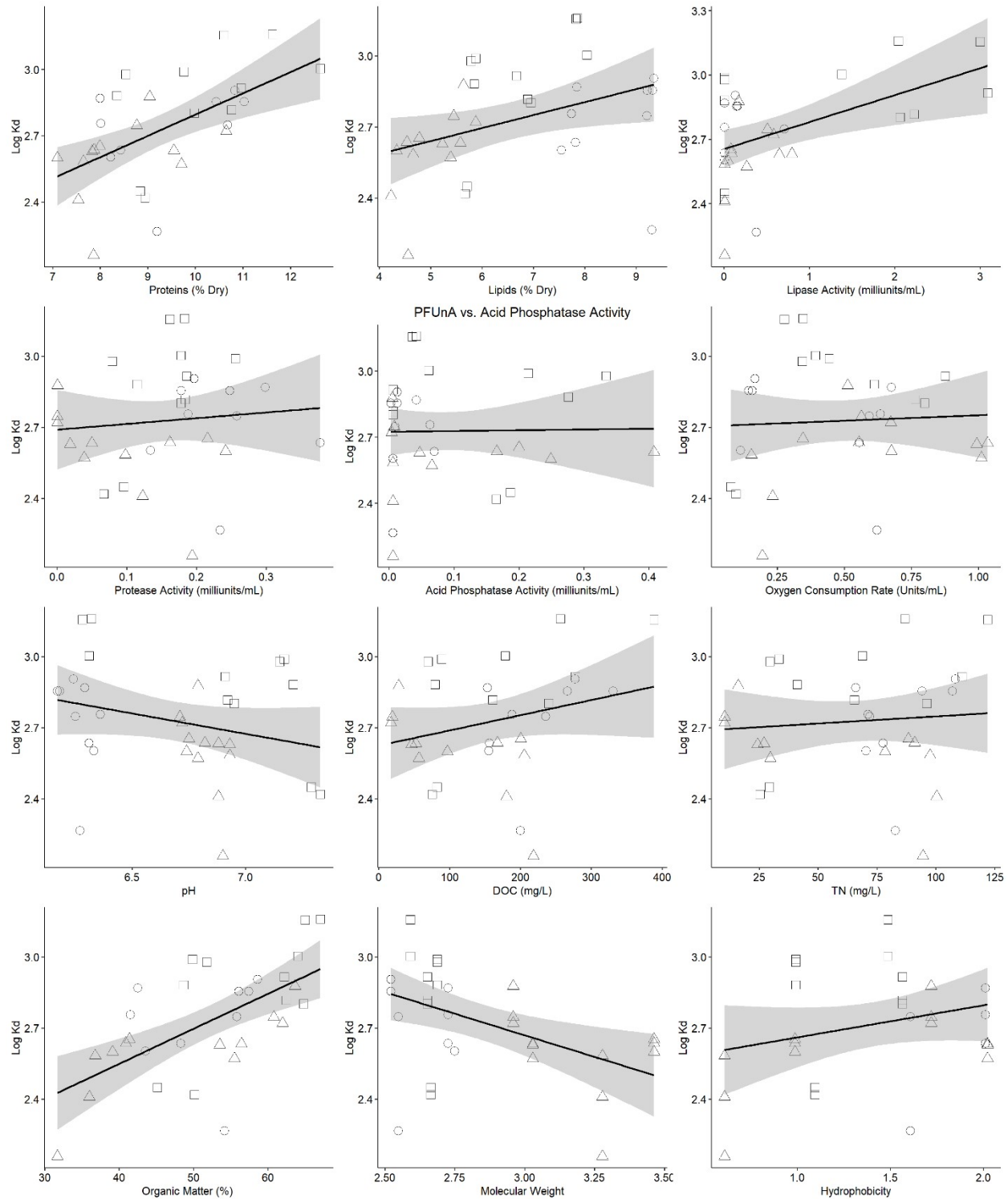
S12. PFNA single variable linear regressions. Gray band is a confidence band (95% confidence). Vertical stacking for some figures is a result of non-detect values for the assay. Triangles represent composting samples, squares represent anaerobic samples, and circles represent aerobic samples.



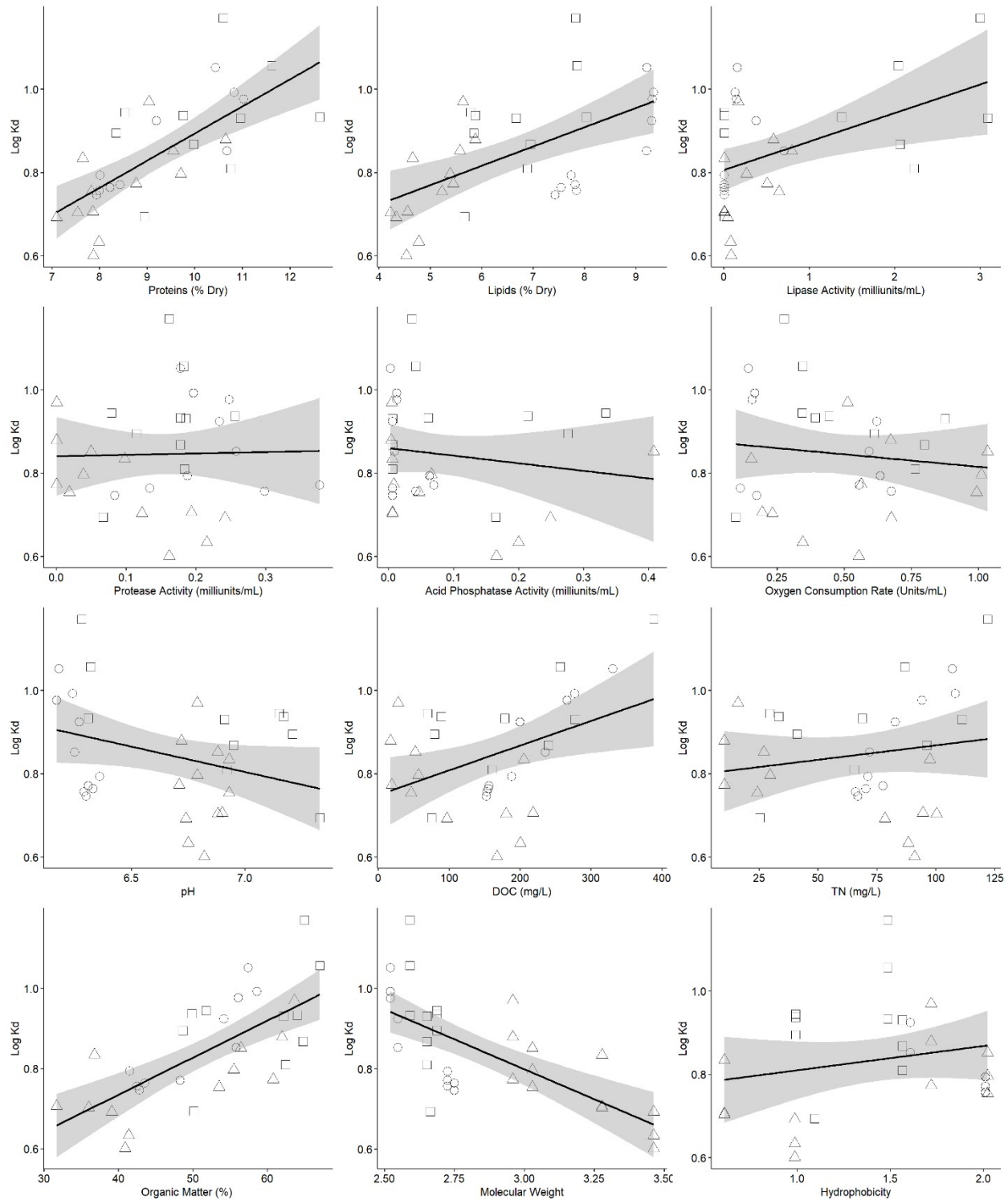
S13. PFDA single variable linear regressions. Gray band is a confidence band (95% confidence). Vertical stacking for some figures is a result of non-detect values for the assay. Triangles represent composting samples, squares represent anaerobic samples, and circles represent aerobic samples.



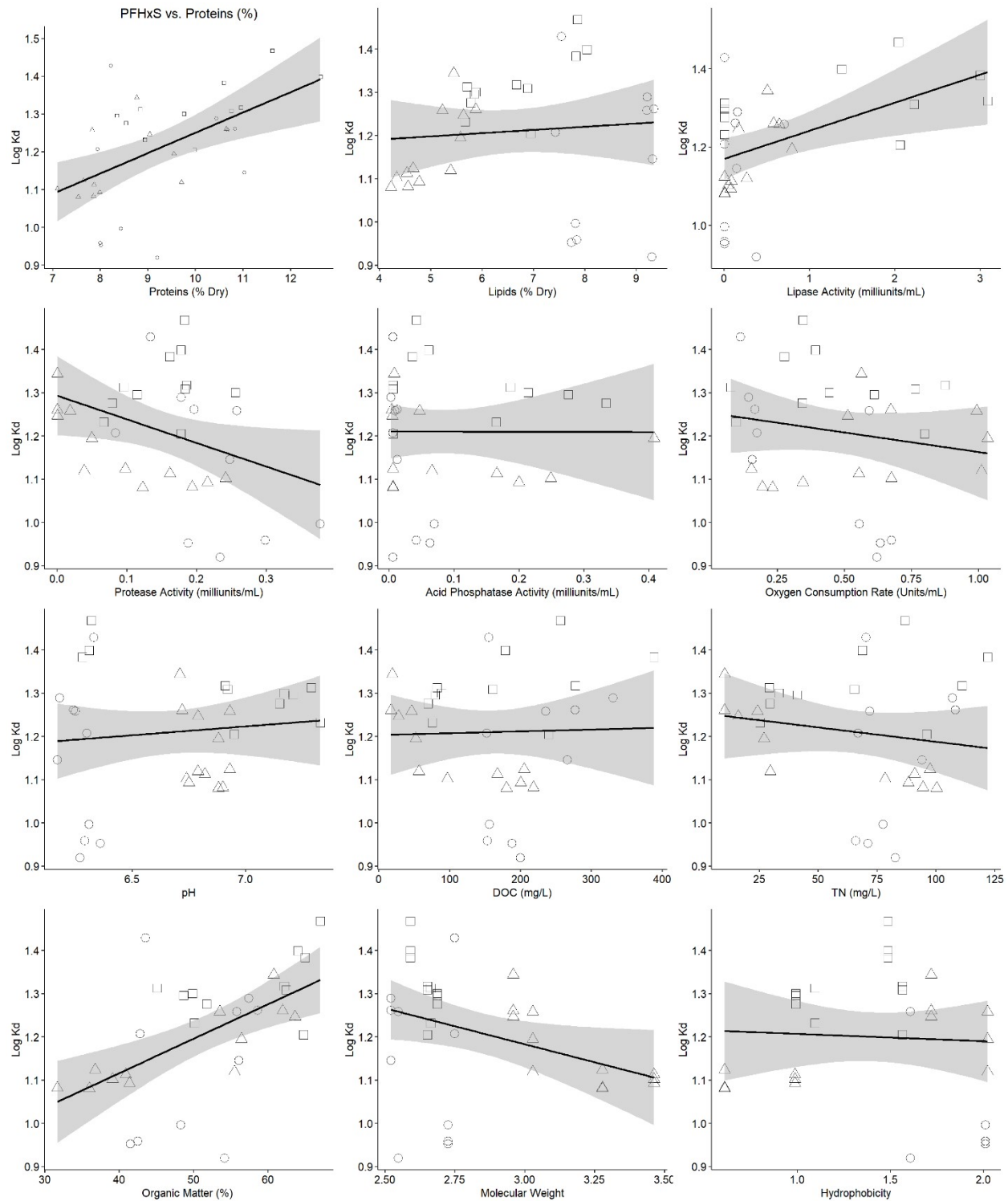
S14. PFUnA single variable linear regressions. Gray band is a confidence band (95% confidence). Vertical stacking for some figures is a result of non-detect values for the assay. Triangles represent composting samples, squares represent anaerobic samples, and circles represent aerobic samples.



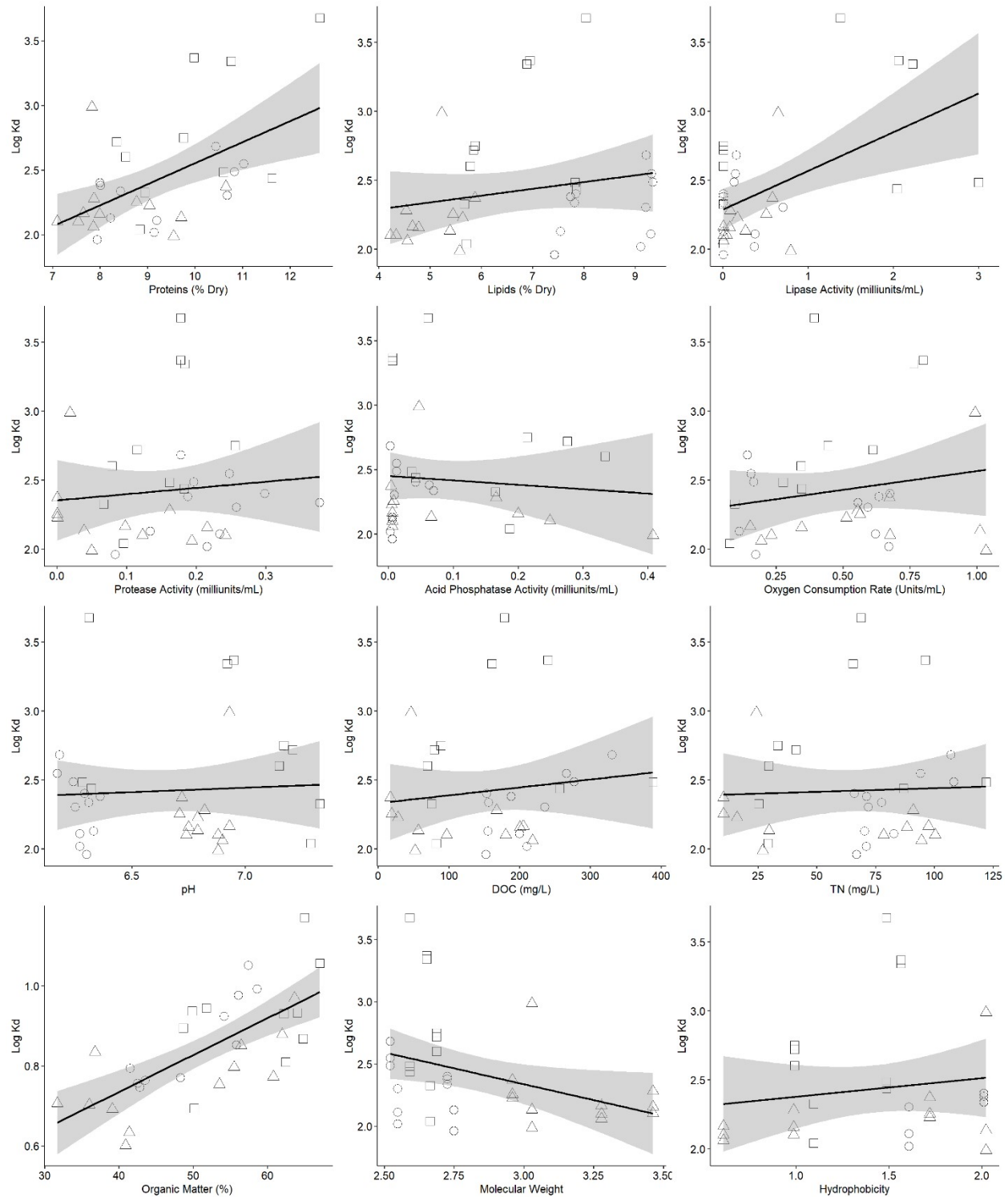
S15. PFBS single variable linear regressions. Gray band is a confidence band (95% confidence). Vertical stacking for some figures is a result of non-detect values for the assay. Triangles represent composting samples, squares represent anaerobic samples, and circles represent aerobic samples.



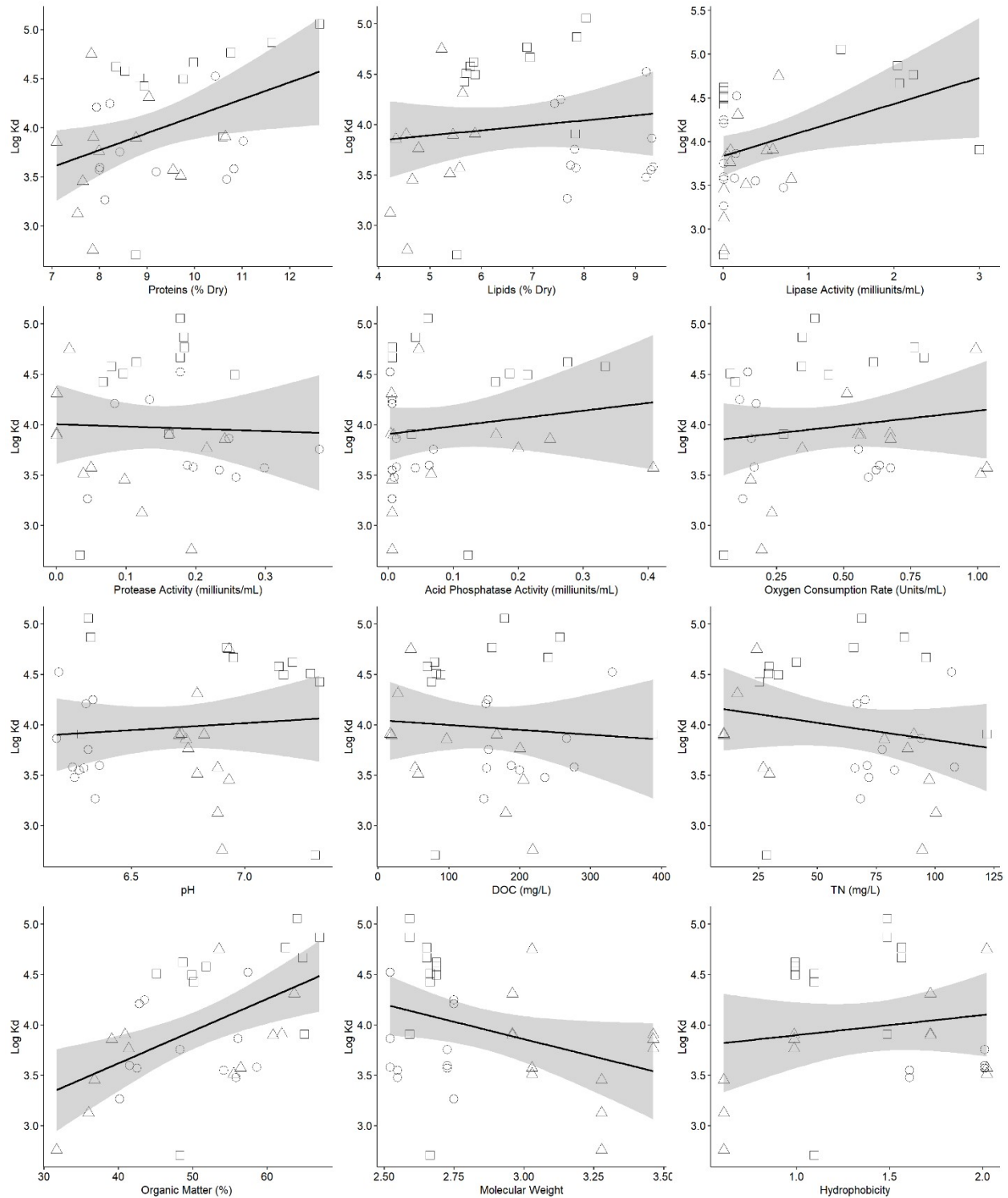
S16. PFHxS single variable linear regressions. Gray band is a confidence band (95% confidence). Vertical stacking for some figures is a result of non-detect values for the assay. Triangles represent composting samples, squares represent anaerobic samples, and circles represent aerobic samples.



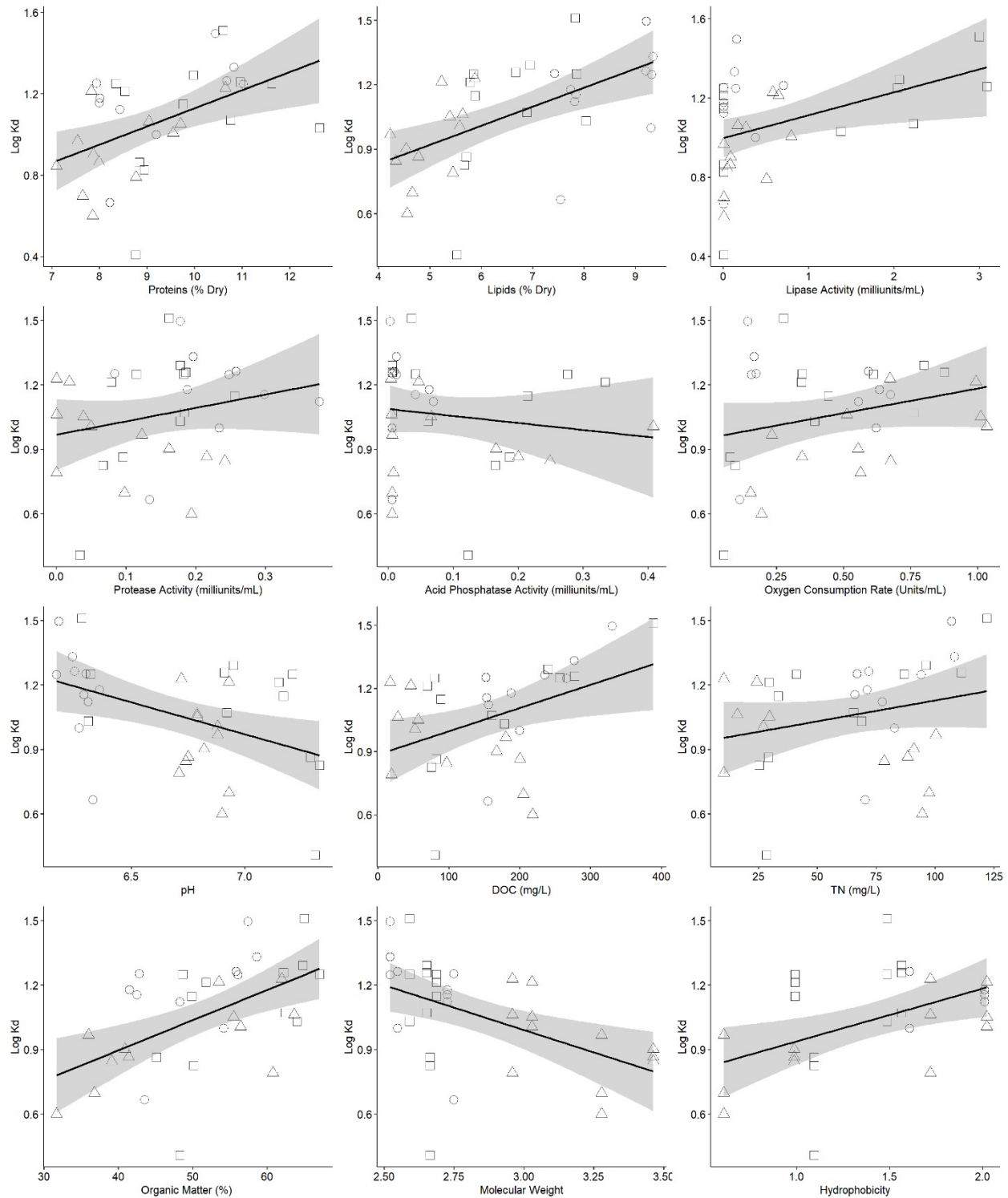
S17. PFOS single variable linear regressions. Gray band is a confidence band (95% confidence). Vertical stacking for some figures is a result of non-detect values for the assay. Triangles represent composting samples, squares represent anaerobic samples, and circles represent aerobic samples.



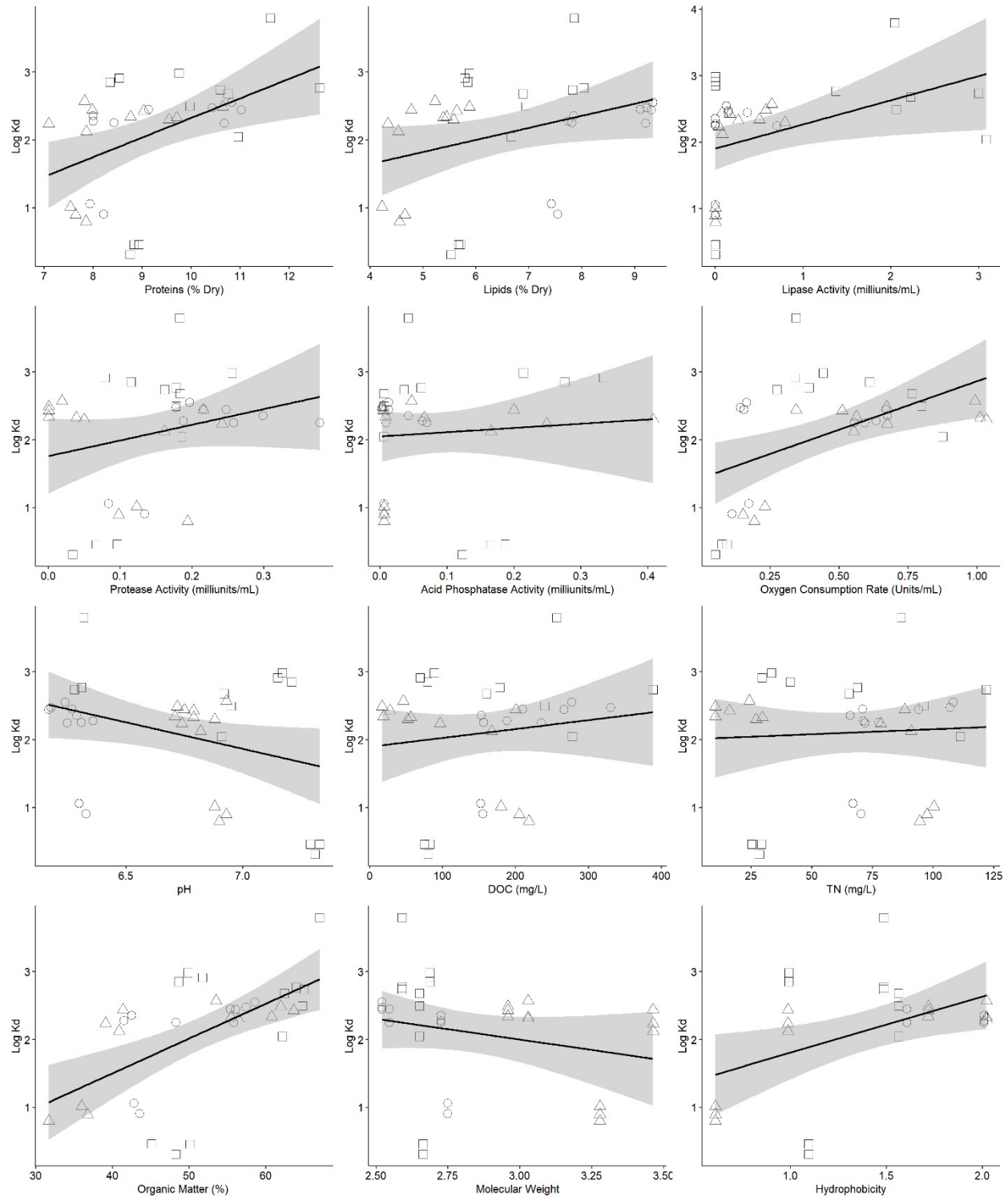
S18. PFDS single variable linear regressions. Gray band is a confidence band (95% confidence). Vertical stacking for some figures is a result of non-detect values for the assay. Triangles represent composting samples, squares represent anaerobic samples, and circles represent aerobic samples.



S19. 6:2 FtS single variable linear regressions. Gray band is a confidence band (95% confidence). Vertical stacking for some figures is a result of non-detect values for the assay. Triangles represent composting samples, squares represent anaerobic samples, and circles represent aerobic samples.



S20. 8:2 FtS single variable linear regressions. Gray band is a confidence band (95% confidence). Vertical stacking for some figures is a result of non-detect values for the assay. Triangles represent composting samples, squares represent anaerobic samples, and circles represent aerobic samples.



S21. Single Variable linear regression results.

	PFBA	PFHxA	PFHpA	PFOA	PFNA	PFDA	PFUnA	PFBS	PFHxS	PFOS	PFDS	6:2 FtS	8:2 FtS
Lipids													
p-value	0.0063	0.0004	0.007	0.0322	0.0036	0.489	0.0294	5.0e-4	0.632	0.268	0.449	0.0005	0.0501
Estimate	0.0495	0.0463	0.0341	0.0289	0.0421	0.0145	0.0548	0.0463	0.0074	0.0492	0.0496	0.0886	0.1778
Standard error	0.0169	0.0117	0.0118	0.0130	0.0133	0.0208	0.0240	0.0119	0.0153	0.0436	0.0647	0.0227	0.0873
Proteins													
p-value	1.1e-5	7.7e-7	5.4e-5	0.0061	6.9e-7	0.0914	5.5e-4	6.9e-6	0.0011	0.0014	0.0220	0.0033	0.0046
Estimate	0.0858	0.0670	0.0560	0.0437	0.0767	0.0407	0.0963	0.0651	0.0539	0.1628	0.1727	0.0892	0.2887
Standard error	0.0165	0.0113	0.0119	0.0150	0.0124	0.0234	0.0249	0.0120	0.0150	0.0464	0.0717	0.0280	0.0948
Lipase Activity													
p-value	7.1e-4	0.0020	0.0256	0.188	0.0011	0.216	0.0045	0.0060	0.0064	0.0018	0.0246	0.0156	0.0239
Estimate	0.1077	0.075	0.0521	0.0340	0.0888	0.0466	0.1258	0.0685	0.0712	0.2805	0.2976	0.1159	0.3641
Standard error	0.0288	0.022	0.0222	0.0253	0.0246	0.0369	0.0409	0.0232	0.0245	0.0820	0.1261	0.0453	0.1535
Protease Activity													
p-value	0.58	0.468	0.538	0.72	0.568	0.679	0.602	0.897	0.0388	0.58	0.84	0.189	0.147
Estimate	0.1828	0.1820	-0.145	-0.088	0.1569	-0.142	0.2419	0.0338	-0.546	0.4507	-0.229	0.6244	2.3111
Standard error	0.3265	0.2480	0.2329	0.2440	0.2720	0.3630	0.4591	0.2579	0.2533	0.8060	1.1229	0.4656	1.5566
Acid Phosphatase Activity													
p-value	0.125	0.198	0.717	0.198	0.22	0.44	0.927	0.4	0.99	0.617	0.427	0.424	0.647
Estimate	-0.428	-0.272	-0.072	-0.269	-0.280	-0.239	0.0343	-0.182	-0.003	-0.340	0.7714	-0.326	0.6252
Standard error	0.2715	0.2067	0.1977	0.2048	0.2234	0.3061	0.3824	0.2134	0.2255	0.6728	0.9593	0.4022	1.3545
Oxygen Consumption Rate													
p-value	0.128	0.843	0.0717	1.0e-4	0.423	7.0e-6	0.76	0.484	0.3	0.309	0.417	0.128	0.0027
Estimate	0.1605	-0.016	-0.134	-0.273	-0.069	-0.451	0.0459	-0.060	-0.090	0.2721	0.2991	0.2300	1.4309
Standard error	0.1012	0.0802	0.0723	0.0620	0.0848	0.0841	0.1492	0.0843	0.0856	0.2632	0.3639	0.1468	0.4408
pH													
p-value	0.0394	0.0221	0.4431	0.451	0.0571	0.4310	0.142	0.0696	0.5542	0.755	0.625	0.0090	0.0447
Estimate	-0.166	-0.139	-0.047	-0.045	-0.123	0.0715	-0.172	-0.122	0.041	0.06417	0.1385	-0.298	-0.781
Standard error	0.0775	0.0577	0.0604	0.0600	0.0623	0.0897	0.1139	0.0647	0.0686	0.2040	0.2807	0.1069	0.3739
Extractable Dissolved Organic Carbon													
p-value	0.0799	0.713	0.114	0.0338	0.0036	0.0543	0.147	0.0145	0.875	0.482	0.678	0.0155	0.408
Estimate	0.0006	0.0002	0.0004	0.0005	0.0008	0.0007	0.006	0.0006	4.2e-5	0.0006	-0.001	0.0011	0.0013
Standard error	0.0003	0.0005	0.0002	0.0002	0.0002	0.0003	0.004	0.0002	2.7e-4	0.0008	0.0012	0.0004	0.0016
Extractable Total Nitrogen													
p-value	0.513	0.256	0.931	0.304	0.0938	0.195	0.638	0.34	0.379	0.823	0.298	0.142	0.745
Estimate	0.0006	0.0008	5.8e-5	0.0007	0.0013	0.0013	0.0006	0.0007	-0.001	0.0005	-0.003	0.0019	0.0015
Standard error	0.0009	0.0007	6.6e-4	0.0007	0.0007	0.0010	0.0013	0.0007	0.007	0.0023	0.0032	0.0013	0.0045
Organic Matter													
p-value	1.3e-9	2.7e-6	7.3e-4	0.0891	2.4e-5	0.611	1.3e-4	7.7e-6	4.8e-4	0.0028	0.0015	8.4e-4	0.0002
Estimate	0.0145	0.0095	0.0068	0.00391	0.0096	0.0018	0.0148	0.0092	0.0080	0.02157	0.0320	0.0141	0.0513
Standard error	0.0017	0.0017	0.0018	0.0022	0.0019	0.0034	0.0034	0.0017	0.0021	0.0066	0.0092	0.0038	0.0122
Molecular Weight													
p-value	0.0090	1.5e-4	7.3e-6	3.4e-4	0.0013	0.0266	0.0063	1.4e-5	0.408	0.0345	0.0499	0.0032	0.2209
Estimate	-0.260	-0.266	-0.279	-0.255	-0.253	-0.245	-0.364	-0.298	-0.167	-0.510	-0.689	-0.417	-0.612
Standard error	0.0933	0.0615	0.0512	0.0640	0.0712	0.1055	0.1240	0.0576	0.0780	0.2306	0.3380	0.1306	0.4901
K_{ow}													
p-value	0.0131	0.246	0.957	0.203	0.767	0.0012	0.183	0.286	0.781	0.469	0.449	0.0084	0.0144
Estimate	0.1630	0.0622	0.0028	-0.067	0.0172	-0.242	0.1358	0.0584	-0.017	0.1337	0.2005	0.2464	0.8290
Standard error	0.0614	0.0524	0.0510	0.0511	0.0573	0.0667	0.0993	0.0535	0.0602	0.1820	0.2607	0.0867	0.3171

S22. Spearman's Rank Correlation Coefficient results (ρ -values).

Compound	Lipids	Proteins	pH	Dissolved Organic Carbon	Total Nitrogen	Organic Matter	Molecular Weight	Hydrophobicity
PFBA	0.518	0.692	-0.381	0.233	0.107	0.835	-0.555	0.398
PFHxA	0.668	0.709	-0.405	0.328	0.135	0.745	-0.698	0.271
PFHpA	0.540	0.680	-0.121	0.237	0.051	0.580	-0.716	-0.026
PFOA	0.433	0.439	-0.110	0.285	0.171	0.348	-0.529	-0.251
PFNA	0.535	0.678	-0.287	0.383	0.296	0.662	-0.605	0.048
PFDA	0.168	0.230	0.173	0.312	0.244	0.121	-0.370	-0.535
PFUnA	0.526	0.596	-0.221	0.248	0.114	0.615	-0.523	0.102
PFBS	0.633	0.765	-0.280	0.337	0.161	0.729	-0.741	0.147
PFHxS	0.249	0.550	0.085	0.003	-0.141	0.609	-0.429	-0.035
PFOS	0.352	0.469	0.044	0.151	0.035	0.485	-0.442	0.107
PFDS	0.190	0.690	0.147	-0.106	-0.217	0.543	-0.313	0.081
6:2 FtS	0.633	0.525	-0.405	0.399	0.302	0.562	-0.573	0.398
8:2 FtS	0.416	0.485	-0.196	0.074	-0.028	0.605	-0.380	0.203

S23. Multiple Linear Regression fits. The adjusted R^2 values represent the multiple linear regression fit for PFAS compound-specific K_d values and organic matter, molecular weight, proteins, and lipids.

Compound	Adjusted R^2
PFBA	0.700
PFHxA	0.560
PFHpA	0.561
PFOA	0.353
PFNA	0.506
PFDA	0.328
PFUnA	0.324
PFBS	0.556
PFHxS	0.416
PFOS	0.259
PFDS	0.234
6:2 FtS	0.368
8:2 FtS	0.395