Supplementary Material for

Weston et al. (2024)

Physicochemical Perturbation Increases Nitrous Oxide Production in Soils and Sediments

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The following tables provide N_2O production, denitrification, and N_2O:DNF ratio data derived from the pulse physicochemical perturbation experiments in two agricultural soils from Lancaster, PA (Table S1) and in estuarine sediments sampled along a salinity gradient in the Scheldt River estuary (Table S2). Data from the press disturbance experiment in which tidal freshwater marsh soils were subjected to control (S = 0) or press salinity (S = 20) treatments with rates of N_2O production, denitrification, and N_2O:DNF ratios measured over time at various pulsed salinities is also provided (Table S3), along with the gene and transcript abundance and gene expression on non-acetylene amended samples from the press experiment (Table S4).

Table S1. Rates (average ± SD; n = 3) of nitrous oxide (N_2O) production and denitrification (N_2 + N_2O production), and the ratio of N_2O to total denitrification in soils collected from a conventional and organic farm in Lancaster, PA subjected to changes in salinity, pH, zinc, temperature, and moisture away from *in situ* conditions.

		N_2O ($\mu mol\ g^{-1}\ d^{-1}$)	Denitrification ($\mu mol\ g^{-1}\ d^{-1}$)	N_2O:DNF
Agricultral Soi - Conventional Farm	**Δ Salinity**			
	0	0.791 ± 0.072	1.737 ± 0.118	0.456 ± 0.072
	1	0.884 ± 0.092	1.782 ± 0.265	0.496 ± 0.125
	3	1.096 ± 0.096	1.885 ± 0.149	0.581 ± 0.097
	5	1.235 ± 0.102	1.830 ± 0.114	0.675 ± 0.098
	10	1.101 ± 0.160	1.585 ± 0.054	0.695 ± 0.124
	35	0.632 ± 0.081	0.689 ± 0.035	0.918 ± 0.165
	Δ pH			
	-3	1.033 ± 0.116	2.662 ± 0.115	0.388 ± 0.060
	-2	1.023 ± 0.125	2.847 ± 0.199	0.359 ± 0.069
	-1	0.661 ± 0.000	2.924 ± 0.092	0.226 ±
	0	0.806 ± 0.121	2.958 ± 0.232	0.273 ± 0.062
	1	1.092 ± 0.077	2.485 ± 0.392	0.439 ± 0.100
	2	1.105 ± 0.083	2.653 ± 0.276	0.417 ± 0.075
	3	1.168 ± 0.151	2.511 ± 0.143	0.465 ± 0.087
	Δ Zinc Concentration ($g\ L^{-1}$)			
	0	0.192 ± 0.058	1.396 ± 0.174	0.137 ± 0.058
	0.05	0.124 ± 0.015	1.252 ± 0.159	0.099 ± 0.024
	0.1	0.122 ± 0.037	1.363 ± 0.085	0.089 ± 0.033
	0.25	0.443 ± 0.050	0.960 ± 0.132	0.462 ± 0.116
	0.5	0.415 ± 0.079	0.709 ± 0.085	0.585 ± 0.182
	1	0.333 ± 0.052	0.552 ± 0.066	0.603 ± 0.167
	Δ Temperature (°C)			
	-17	0.136 ± 0.023	0.899 ± 0.058	0.151 ± 0.036
	-7	0.030 ± 0.009	1.659 ± 0.103	0.018 ± 0.007
	0	0.009 ± 0.001	1.333 ± 0.159	0.007 ± 0.001
	6	0.011 ± 0.006	1.052 ± 0.020	0.010 ± 0.006
	15	0.060 ± 0.060	0.985 ± 0.076	0.061 ± 0.066
	Δ Moisture (%)			
	-50.0	0.002 ± 0.000	0.002 ± 0.000	1.000 ± 0.287
	-45.2	0.011 ± 0.001	0.013 ± 0.002	0.870 ± 0.165
	-40.9	0.036 ± 0.006	0.040 ± 0.008	0.901 ± 0.335
	-33.3	0.069 ± 0.026	0.102 ± 0.024	0.672 ± 0.412
	-16.7	0.089 ± 0.013	0.209 ± 0.016	0.427 ± 0.093
	0.0	0.038 ± 0.006	0.207 ± 0.029	0.183 ± 0.055

		N_2O ($\mu mol\ g^{-1}\ d^{-1}$)	Denitrification ($\mu mol\ g^{-1}\ d^{-1}$)	N_2O:DNF
Agricultral Soil - Organic Farm	**Δ Salinity**			
	0	0.271 ± 0.039	1.075 ± 0.056	0.252 ± 0.049
	1	0.411 ± 0.036	1.091 ± 0.058	0.377 ± 0.053
	3	0.530 ± 0.063	0.958 ± 0.072	0.554 ± 0.107
	5	0.591 ± 0.053	0.870 ± 0.033	0.680 ± 0.086
	10	0.666 ± 0.007	0.985 ± 0.076	0.677 ± 0.060
	35	0.323 ± 0.027	0.340 ± 0.020	0.949 ± 0.135
	Δ pH			
	0	0.342 ± 0.050	1.410 ± 0.272	0.243 ± 0.082
	1	0.423 ± 0.015	1.717 ± 0.052	0.246 ± 0.016
	2	0.355 ± 0.088	1.780 ± 0.103	0.199 ± 0.061
	3	0.374 ± 0.073	1.586 ± 0.117	0.236 ± 0.064
	-1	0.407 ± 0.045	1.459 ± 0.283	0.279 ± 0.085
	-2	0.518 ± 0.100	1.529 ± 0.229	0.339 ± 0.116
	-3	0.604 ± 0.072	1.459 ± 0.201	0.414 ± 0.107
	Δ Zinc Concentration ($g\ L^{-1}$)			
	0	1.419 ± 0.121	2.507 ± 0.116	0.566 ± 0.075
	0.05	1.298 ± 0.126	2.958 ± 0.232	0.439 ± 0.077
	0.1	1.162 ± 0.063	2.111 ± 0.189	0.550 ± 0.079
	0.25	1.097 ± 0.088	1.617 ± 0.112	0.678 ± 0.101
	0.5	1.031 ± 0.039	1.121 ± 0.101	0.920 ± 0.118
	1	0.806 ± 0.121	0.995 ± 0.067	0.811 ± 0.176
	Δ Temperature (°C)			
	-17	0.011 ±	0.307 ± 0.017	0.036 ± 0.002
	-7	0.029 ± 0.020	0.876 ± 0.074	0.033 ± 0.026
	0	0.015 ± 0.000	0.761 ± 0.009	0.019 ± 0.001
	6	0.023 ± 0.011	0.654 ± 0.162	0.036 ± 0.025
	15	0.034 ± 0.017	0.501 ±	0.069 ± 0.034
	Δ Moisture (%)			
	-50.0	0.001 ± 0.000	0.002 ± 0.000	0.840 ± 0.265
	-45.2	0.015 ± 0.004	0.016 ± 0.006	0.919 ± 0.590
	-40.9	0.045 ± 0.012	0.057 ± 0.005	0.781 ± 0.280
	-33.3	0.089 ± 0.002	0.091 ± 0.011	0.973 ± 0.138
	-16.7	0.042 ± 0.010	0.263 ± 0.029	0.161 ± 0.057
	0.0	0.015 ± 0.003	0.174 ± 0.018	0.087 ± 0.025

Table S2. Rates (average ± SD; n = 3) of nitrous oxide (N_2O) production and denitrification (N_2 + N_2O production), and the ratio of N_2O to total denitrification in sediments collected along the salinity gradient in the Schelde Estuary subjected to changes in salinity.

	Δ Salinity	N_2O (μmol g^{-1} d^{-1})	Denitrification (μmol g^{-1} d^{-1})	N_2O:DNF
Tidal freshwater	0	0.003 ± 0.000	0.393 ± 0.073	0.008 ± 0.003
	1	0.004 ± 0.000	0.313 ± 0.064	0.013 ± 0.004
	3	0.004 ± 0.000	0.291 ± 0.025	0.013 ± 0.001
	5	0.004 ± 0.000	0.295 ± 0.007	0.014 ± 0.002
	10	0.011 ± 0.003	0.206 ± 0.025	0.056 ± 0.019
	15	0.029 ± 0.001	0.151 ± 0.057	0.193 ± 0.079
	30	0.050 ± 0.006	0.094 ± 0.023	0.536 ± 0.190
Oligohaline	-5	0.003 ± 0.001	0.052 ± 0.002	0.059 ± 0.027
	-4	0.003 ± 0.000	0.063 ± 0.007	0.053 ± 0.014
	-2	0.004 ± 0.001	0.087 ± 0.013	0.042 ± 0.016
	0	0.002 ± 0.000	0.110 ± 0.003	0.015 ± 0.005
	5	0.008 ± 0.004	0.105 ± 0.013	0.075 ± 0.050
	10	0.006 ± 0.001	0.106 ± 0.007	0.060 ± 0.014
	25	0.014 ± 0.011	0.094 ± 0.003	0.147 ± 0.119
Mesohaline	-24	0.038 ± 0.006	0.188 ± 0.029	0.203 ± 0.063
	-21	0.035 ± 0.001	0.252 ± 0.048	0.138 ± 0.032
	-19	0.028 ± 0.004	0.288 ± 0.033	0.096 ± 0.023
	-14	0.014 ± 0.007	0.433 ± 0.033	0.033 ± 0.019
	0	0.003 ± 0.000	0.641 ± 0.038	0.005 ± 0.000
	6	0.035 ± 0.003	0.571 ± 0.042	0.062 ± 0.010

Table S3. Rates of nitrous oxide (N_2O) production and denitrification (N_2 + N_2O production), and the ratio of N_2O to total denitrification in tidal freshwater soils in a 6-month experiment under control (S = 0) or press treatment (S = 20) conditions subjected to short-term pulsed changes in salinity.

Time (day)	Press Salinity Treatment	Pulse Salinity Treatment	N_2O ($\mu mol\ g^{-1}\ d^{-1}$)	Denitrification ($\mu mol\ g^{-1}\ d^{-1}$)	N_2O:DNF
0	Control (S = 0)	0.0	0.054 ± 0.027	1.363 ± 0.339	0.038 ± 0.010
0	Control (S = 0)	4.3	0.383 ± 0.307	1.668 ± 0.329	0.215 ± 0.142
0	Control (S = 0)	7.6	0.838 ± 0.549	1.537 ± 0.312	0.520 ± 0.252
0	Control (S = 0)	16.9	0.664 ± 0.213	0.877 ± 0.076	0.749 ± 0.177
0	Control (S = 0)	25.6	0.332 ± 0.126	0.374 ± 0.061	0.872 ± 0.193
0	Press (S = 20)	3.5	0.286 ± 0.048	1.724 ± 0.021	0.166 ± 0.030
0	Press (S = 20)	7.4	0.527 ± 0.022	1.129 ± 0.301	0.481 ± 0.109
0	Press (S = 20)	11.6	0.511 ± 0.182	0.895 ± 0.214	0.562 ± 0.069
0	Press (S = 20)	20.0	0.313 ± 0.097	0.478 ± 0.072	0.648 ± 0.105
0	Press (S = 20)	28.3	0.173 ± 0.029	0.230 ± 0.049	0.756 ± 0.037
7	Control (S = 0)	0.0	0.110 ± 0.010	1.607 ± 0.615	0.072 ± 0.021
7	Control (S = 0)	4.3	0.243 ± 0.067	2.079 ± 0.629	0.118 ± 0.003
7	Control (S = 0)	7.6	0.525 ± 0.172	1.552 ± 0.449	0.337 ± 0.014
7	Control (S = 0)	16.9	0.422 ± 0.172	0.848 ± 0.595	0.565 ± 0.193
7	Control (S = 0)	25.6	0.244 ± 0.030	0.436 ± 0.231	0.628 ± 0.262
7	Press (S = 20)	3.5	0.240 ± 0.051	1.158 ± 0.424	0.230 ± 0.128
7	Press (S = 20)	7.4	0.339 ± 0.015	1.363 ± 0.306	0.257 ± 0.069
7	Press (S = 20)	11.6	0.276 ± 0.027	1.079 ± 0.071	0.257 ± 0.042
7	Press (S = 20)	20.0	0.180 ± 0.125	0.778 ± 0.274	0.216 ± 0.085
7	Press (S = 20)	28.3	0.133 ± 0.098	0.617 ± 0.057	0.209 ± 0.139
14	Control (S = 0)	0.0	0.160 ± 0.003	1.261 ± 0.069	0.127 ± 0.009
14	Control (S = 0)	4.3	0.145 ± 0.047	1.807 ± 0.415	0.085 ± 0.046
14	Control (S = 0)	7.6	0.353 ± 0.062	1.498 ± 0.039	0.235 ± 0.035
14	Control (S = 0)	16.9	0.618 ± 0.064	1.084 ± 0.031	0.571 ± 0.075
14	Control (S = 0)	25.6	0.343 ± 0.049	0.439 ± 0.139	0.839 ± 0.376
14	Press (S = 20)	3.5	0.130 ± 0.084	1.811 ± 0.381	0.079 ± 0.063
14	Press (S = 20)	7.4	0.050 ± 0.001	1.555 ± 0.406	0.033 ± 0.009
14	Press (S = 20)	11.6	0.083 ± 0.034	1.543 ± 0.359	0.053 ± 0.010
14	Press (S = 20)	20.0	0.043 ± 0.018	1.565 ± 1.104	0.031 ± 0.010
14	Press (S = 20)	28.3	0.030 ± 0.001	2.391 ± 1.086	0.014 ± 0.007
21	Control (S = 0)	0.0	0.099 ± 0.013	1.777 ± 0.508	0.059 ± 0.024
21	Control (S = 0)	4.3	0.289 ± 0.163	1.599 ± 0.182	0.188 ± 0.123
21	Control (S = 0)	7.6	0.298 ± 0.111	1.266 ± 0.335	0.232 ± 0.026
21	Control (S = 0)	16.9	0.443 ± 0.034	0.961 ± 0.465	0.532 ± 0.293
21	Control (S = 0)	25.6	0.368 ± 0.107	0.411 ± 0.005	0.897 ± 0.274
21	Press (S = 20)	3.5	0.129 ± 0.081	1.153 ± 1.020	0.133 ± 0.047
21	Press (S = 20)	7.4	0.021 ± 0.011	1.398 ± 1.318	0.020 ± 0.011
21	Press (S = 20)	11.6	0.055 ± 0.005	0.751 ± 0.332	0.080 ± 0.028
21	Press (S = 20)	20.0	0.032 ± 0.002	1.161 ± 1.199	0.057 ± 0.057
21	Press (S = 20)	28.3	0.091 ± 0.098	0.711 ± 0.403	0.199 ± 0.250
35	Control (S = 0)	0.0	0.012 ± 0.012	0.418 ± 0.043	0.030 ± 0.032
35	Control (S = 0)	4.3	0.013 ± 0.013	0.641 ± 0.049	0.022 ± 0.022
35	Control (S = 0)	7.6	0.047 ± 0.056	0.646 ± 0.079	0.079 ± 0.097
35	Control (S = 0)	16.9	0.207 ± 0.046	0.350 ± 0.105	0.640 ± 0.324
35	Control (S = 0)	25.6	0.146 ± 0.027	0.162 ± 0.053	0.921 ± 0.129

Table S3. Continued.

Time (day)	Press Salinity Treatment	Pulse Salinity Treatment	N_2O (μmol g^{-1} d^{-1})	Denitrification (μmol g^{-1} d^{-1})	N_2O:DNF
35	Press (S = 20)	3.5	0.140 ± 0.062	0.469 ± 0.249	0.305 ± 0.029
35	Press (S = 20)	7.4	0.109 ± 0.102	0.579 ± 0.108	0.175 ± 0.143
35	Press (S = 20)	11.6	0.103 ± 0.120	0.486 ± 0.409	0.168 ± 0.105
35	Press (S = 20)	20.0	0.092 ± 0.111	0.759 ± 0.717	0.095 ± 0.056
35	Press (S = 20)	28.3	0.061 ± 0.069	0.721 ± 0.312	0.070 ± 0.066
49	Control (S = 0)	0.0	0.008 ± 0.006	0.258 ± 0.190	0.057 ± 0.067
49	Control (S = 0)	4.3	0.006 ± 0.000	0.129 ± 0.012	0.044 ± 0.002
49	Control (S = 0)	7.6	0.018 ± 0.020	0.130 ±	0.241 ±
49	Control (S = 0)	16.9	0.038 ± 0.034	0.167 ± 0.040	0.207 ± 0.152
49	Control (S = 0)	25.6	0.045 ± 0.046	0.125 ± 0.028	0.323 ± 0.293
49	Press (S = 20)	3.5	0.016 ± 0.011	0.089 ± 0.047	0.242 ± 0.247
49	Press (S = 20)	7.4	0.013 ± 0.000	0.083 ± 0.059	0.219 ± 0.158
49	Press (S = 20)	11.6	0.009 ± 0.000	0.123 ± 0.075	0.092 ± 0.053
49	Press (S = 20)	20.0	0.010 ± 0.003	0.130 ± 0.056	0.087 ± 0.057
49	Press (S = 20)	28.3	0.012 ± 0.008	0.085 ± 0.046	0.193 ± 0.195
70	Control (S = 0)	0.0	0.020 ± 0.002	0.947 ± 0.003	0.021 ± 0.002
70	Control (S = 0)	4.3	0.029 ± 0.005	0.710 ± 0.352	0.049 ± 0.031
70	Control (S = 0)	7.6	0.148 ± 0.022	0.704 ± 0.034	0.211 ± 0.042
70	Control (S = 0)	16.9	0.181 ± 0.016	0.442 ± 0.074	0.419 ± 0.107
70	Control (S = 0)	25.6	0.175 ± 0.012	0.196 ± 0.019	0.898 ± 0.023
70	Press (S = 20)	3.5	0.052 ± 0.033	0.178 ± 0.011	0.299 ± 0.204
70	Press (S = 20)	7.4	0.045 ± 0.034	0.232 ± 0.004	0.193 ± 0.149
70	Press (S = 20)	11.6	0.040 ± 0.030	0.314 ± 0.100	0.117 ± 0.057
70	Press (S = 20)	20.0	0.031 ± 0.016	0.349 ± 0.009	0.088 ± 0.043
70	Press (S = 20)	28.3	0.027 ± 0.013	0.241 ± 0.009	0.112 ± 0.049
110	Control (S = 0)	0.0	0.029 ± 0.038	0.614 ± 0.084	0.052 ± 0.069
110	Control (S = 0)	4.3	0.070 ± 0.071	0.518 ± 0.156	0.162 ± 0.185
110	Control (S = 0)	7.6	0.169 ± 0.028	0.523 ± 0.103	0.335 ± 0.119
110	Control (S = 0)	16.9	0.189 ± 0.017	0.346 ± 0.033	0.551 ± 0.102
110	Control (S = 0)	25.6	0.152 ± 0.058	0.168 ± 0.016	0.894 ± 0.260
110	Press (S = 20)	3.5	0.075 ± 0.046	0.221 ± 0.033	0.329 ± 0.158
110	Press (S = 20)	7.4	0.055 ± 0.015	0.238 ± 0.061	0.230 ± 0.002
110	Press (S = 20)	11.6	0.034 ± 0.032	0.261 ± 0.091	0.116 ± 0.082
110	Press (S = 20)	20.0	0.012 ± 0.001	0.337 ± 0.091	0.036 ± 0.007
110	Press (S = 20)	28.3	0.035 ± 0.019	0.128 ± 0.023	0.266 ± 0.098
181	Control (S = 0)	0.0	0.114 ± 0.065	2.293 ± 0.062	0.050 ± 0.030
181	Control (S = 0)	4.3	0.097 ± 0.034	1.862 ± 0.155	0.053 ± 0.023
181	Control (S = 0)	7.6	0.281 ± 0.118	1.434 ± 0.284	0.208 ± 0.123
181	Control (S = 0)	16.9	0.420 ± 0.051	0.732 ± 0.124	0.575 ± 0.027
181	Control (S = 0)	25.6	0.404 ± 0.112	0.438 ± 0.110	0.986 ± 0.503
181	Press (S = 20)	3.5	0.082 ± 0.069	0.405 ± 0.278	0.190 ± 0.039
181	Press (S = 20)	7.4	0.067 ± 0.076	0.505 ± 0.298	0.106 ± 0.088
181	Press (S = 20)	11.6	0.066 ± 0.081	0.491 ± 0.414	0.102 ± 0.079
181	Press (S = 20)	20.0	0.057 ± 0.069	0.872 ± 0.868	0.052 ± 0.028
181	Press (S = 20)	28.3	0.021 ± 0.014	0.301 ± 0.189	0.069 ± 0.004

Table S4. Average (± SD; n = 4) nitrate reductase (nirS) and nitrous oxide reductase (nosZ) gene abundance (DNA), gene transcripts (cDNA), and gene expression (cDNA/DNA) in soils from select samples taken from the press/pulse experiment.

Time (day)	Press Salinity Treatment	Pulse Salinity Treatment	DNA (10^9 copies g^{-1})		cDNA (10^9 copies g^{-1})		Expression	
			nirS	nosZ	nirS	nosZ	nirS	nosZ
7	Control (S = 0)	0.0	7.63 ± 2.55	0.08 ± 0.04	0.70 ± 0.78	0.08 ± 0.02	0.092	0.937
7	Control (S = 0)	25.6	4.74 ± 1.18	0.05 ± 0.04	0.45 ± 0.54	0.00 ± 0.00	0.094	0.000
7	Press (S = 20)	2.6	4.21 ± 3.08	0.07 ± 0.03	0.00 ± 0.00	0.00 ± 0.00	0.000	0.000
7	Press (S = 20)	28.0	1.39 ± 0.04	0.01 ± 0.00	9.98 ± 12.96	0.12 ± 0.00	7.181	10.092
35	Control (S = 0)	0.0	3.05 ± 1.33	0.04 ± 0.00	0.10 ± 0.11	1.55 ± 0.35	0.032	43.147
35	Control (S = 0)	24.8	2.09 ± 0.41	0.02 ± 0.00	1.03 ± 0.21	0.00 ± 0.00	0.490	0.000
35	Press (S = 20)	1.1	1.53 ± 0.81	0.03 ± 0.03	0.00 ± 0.00	0.09 ± 0.01	0.000	3.072
35	Press (S = 20)	19.5	7.32 ± 1.37	0.05 ± 0.00	5.49 ± 5.83	1.04 ± 0.51	0.750	21.612
110	Control (S = 0)	0.0	1.29 ± 0.08	0.01 ± 0.00	1.59 ± 1.32	0.16 ± 0.08	1.236	15.416
110	Control (S = 0)	24.8	3.45 ± 2.81	0.04 ± 0.03	0.02 ± 0.02	0.00 ± 0.00	0.006	0.000
110	Press (S = 20)	1.1	5.23 ± 2.44	0.05 ± 0.01	0.06 ± 0.08	0.01 ± 0.00	0.012	0.169
110	Press (S = 20)	19.5	3.41 ± 3.12	0.03 ± 0.03	0.85 ± 0.54	0.50 ± 0.44	0.250	18.643