

Limited effects of precipitation manipulation on soil respiration and inorganic N concentrations across soil drainage classes in northern Minnesota aspen forests

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SUPPLEMENTAL MATERIALS

This study investigated the effect of reduced precipitation on soil respiration and nitrogen dynamics via combined throughfall reduction and snow removal in aspen forests of northern Minnesota, USA. Throughfall reduction (50%) was applied during the growing seasons of 2020 and 2021, and snow removal was conducted during the winters of 2019/20 and 2020/21. Soil water content and temperature, *in situ* bulk soil respiration, and extractable inorganic nitrogen concentrations were measured during 2020 and 2021, and a laboratory incubation was conducted to measure soil respiration under controlled conditions.

Table S1: Mean percentages of pre-treatment carbon and nitrogen by site and drainage class.

Drainage class	Location					
	Aitkin		Itasca		St. Louis	
	%C	%N	%C	%N	%C	%N
WD	1.27	0.07	0.73	0.04	1.24	0.08
MWD	1.24	0.07	1.07	0.06	1.90	0.13
SPD	1.62	0.10	0.82	0.05	3.25	0.22
PD	1.94	0.14	1.12	0.08	5.25	0.39

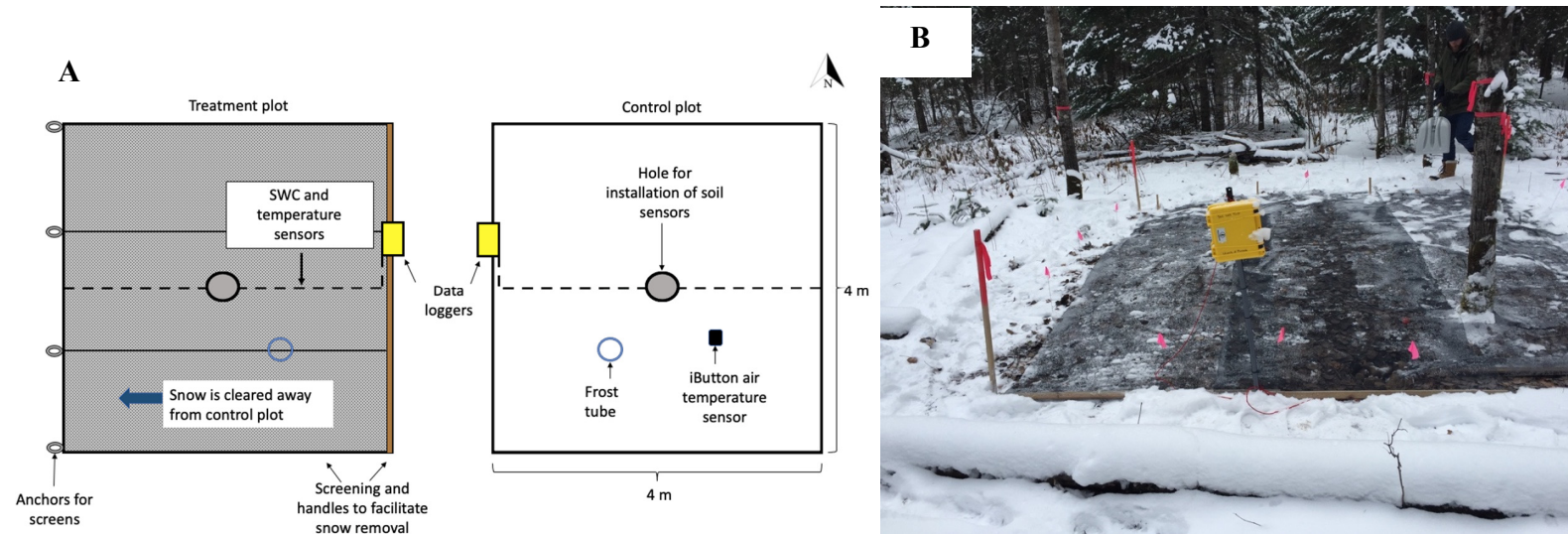


Figure S1: Paired-plot design schematic (panel A) and field photo (panel B) with snow removal treatment during the winter. (Photo credit: Alan Toczydlowski, University of Minnesota)

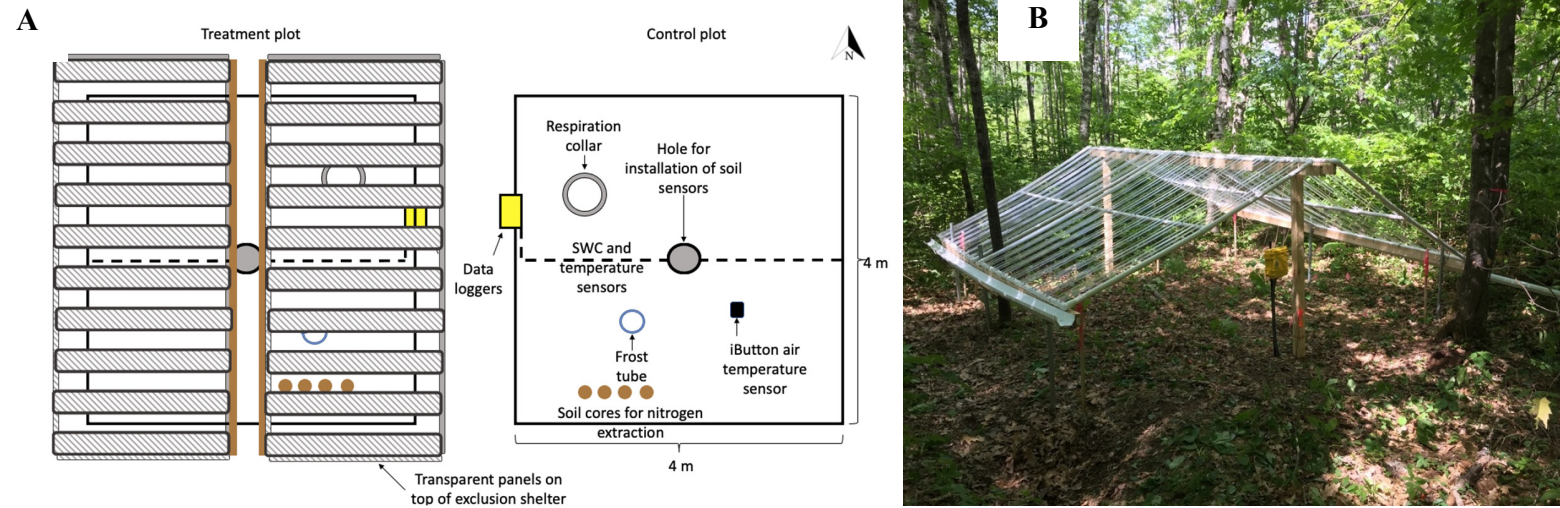


Figure S2: Paired-plot design with throughfall reduction treatment during the growing season is shown in panel A. All plots and transparent roof panels were oriented on an east-west transect, with the shelter ridgeline running north-south. Precipitation reduction shelters were designed to exclude 50% of throughfall. Plots that received treatment were randomized in each pair. Panel B shows the throughfall exclusion shelter on a treatment plot during the growing season. (Photo credit: Alan Toczydlowski)

Table S2: Four-way ANOVA summary for soil water content models for the growing seasons of 2020 and 2021. Model coefficient p-values are shown. Bolded values indicate a significant result (p-value < 0.05).

	2020	2021
Model term	p-value	p-value
Intercept	<0.001	<0.001
Drainage	<0.001	<0.001
Treatment	0.338	<0.001
Week	<0.001	<0.001
Depth	<0.001	0.220
Drainage:Treatment	<0.001	<0.001
Drainage:Week	0.941	<0.001
Treatment:Week	0.650	0.399
Drainage:Depth	<0.001	<0.001
Treatment:Depth	<0.001	<0.001
Week:Depth	1.000	0.974
Drainage:Treatment:Week	1.000	1.000
Drainage:Treatment:Depth	<0.001	<0.001
Drainage:Week:Depth	1.000	1.000
Treatment:Week:Depth	1.000	1.000
Drainage:Treatment:Week:Depth	1.000	1.000

Table S3: Four-way ANOVA summary for soil temperature models for the growing seasons of 2020 and 2021. Model coefficient p-values are shown. Bolded values indicate a significant result (p-value < 0.05).

	2020	2021
	05/03- 10/25	05/02-09/05
Model term	p-value	p-value
Intercept	<0.001	<0.001
Drainage	<0.001	<0.001
Treatment	<0.001	<0.001
Week	<0.001	<0.001
Depth	<0.001	<0.001
Drainage:Treatment	<0.001	<0.001
Drainage:Week	<0.001	<0.001
Treatment:Week	<0.001	<0.001
Drainage:Depth	<0.001	<0.001
Treatment:Depth	0.135	0.005
Week:Depth	<0.001	0
Drainage:Treatment:Week	<0.001	0.151
Drainage:Treatment:Depth	0.205	0.579
Drainage:Week:Depth	1	1
Treatment:Week:Depth	1.00	1
Drainage:Treatment:Week:Depth	1	1

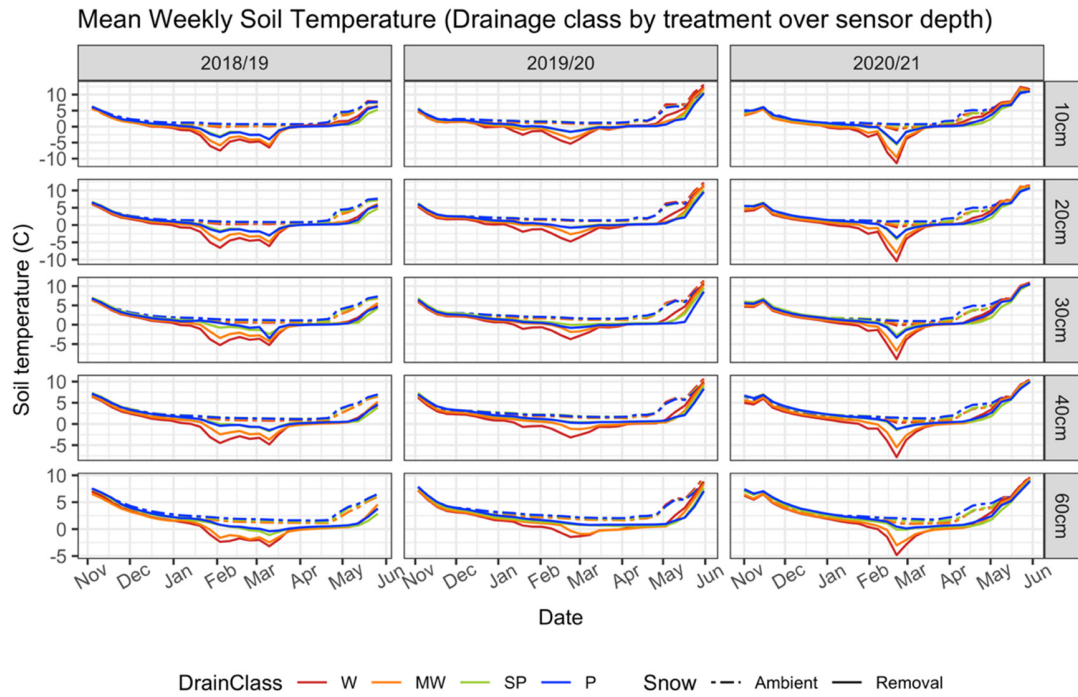


Figure S3: Mean weekly soil temperature during the winters of 2018/19, 2019/20, and 2020/21 across drainage class, treatment, depth, and week.

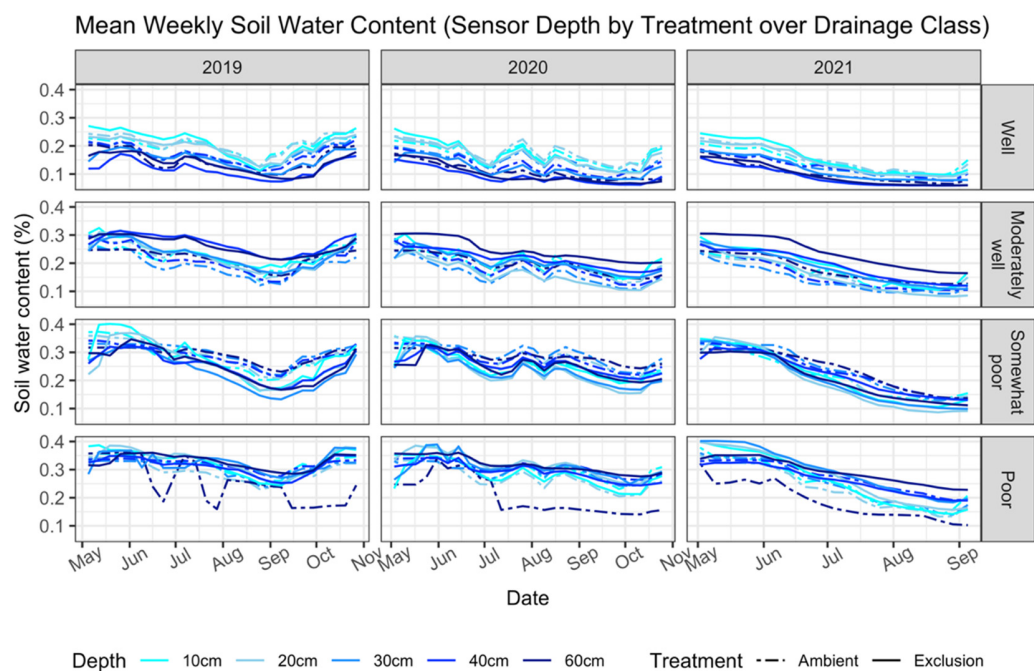


Figure S4: Mean weekly soil water content during the growing seasons of 2019, 2020, and 2021 by drainage class, treatment, and depth.

Table S4: Three-way ANOVA results summary for the field bulk soil respiration model. Numerator degrees of freedom and model coefficient p-values are shown. Bolded values indicate a significant result (p-value < 0.05).

	2020		2021	
	06/23 - 09/01		04/07 - 08/24	
Model term	Degrees of freedom	p-value	Degrees of freedom	p-value
Intercept	1	<0.001	1	<0.001
Drainage	3	0.091	3	0.006
Treatment	1	0.207	1	0.893
Date	5	<0.001	10	<0.001
Temperature	1	0.003	1	0.599
Percent clay	1	0.477	1	0.670
Pretreatment carbon	1	0.001	1	0.350
Drainage:Treatment	3	0.054	3	0.661
Drainage:Date	15	0.375	30	0.087
Treatment:Date	5	0.745	10	0.359
Drainage:Treatment:Date	15	0.991	30	0.940

Table S5: Mean soil respiration, total nitrogen, ammonium, and nitrate/nitrite for summers of 2020 and 2021. Superscript letters indicate significant differences between means within a given year. Level of significance (alpha) is 0.05. Confidence intervals are 95% confidence.

		2020		2021	
	Units	Mean	Confidence interval	Mean	Confidence interval
Respiration	$\mu\text{mol m}^{-2} \text{ s}^{-1}$	6.96 ^a	5.75 - 8.41	4.26 ^b	3.71 - 4.90
Total nitrogen	$\text{mg}^{-1} \text{ kg}^{-1}$	7.61 ^a	5.31 - 10.8	13.3 ^b	9.49 - 18.7
Ammonium	$\text{mg}^{-1} \text{ kg}^{-1}$	2.97 ^a	1.17 - 7.61	12.9 ^b	5.05 - 33.1
Nitrate/nitrite	$\text{mg}^{-1} \text{ kg}^{-1}$	3.03 ^a	1.19 - 7.69	5.10 ^b	2.05 - 12.8

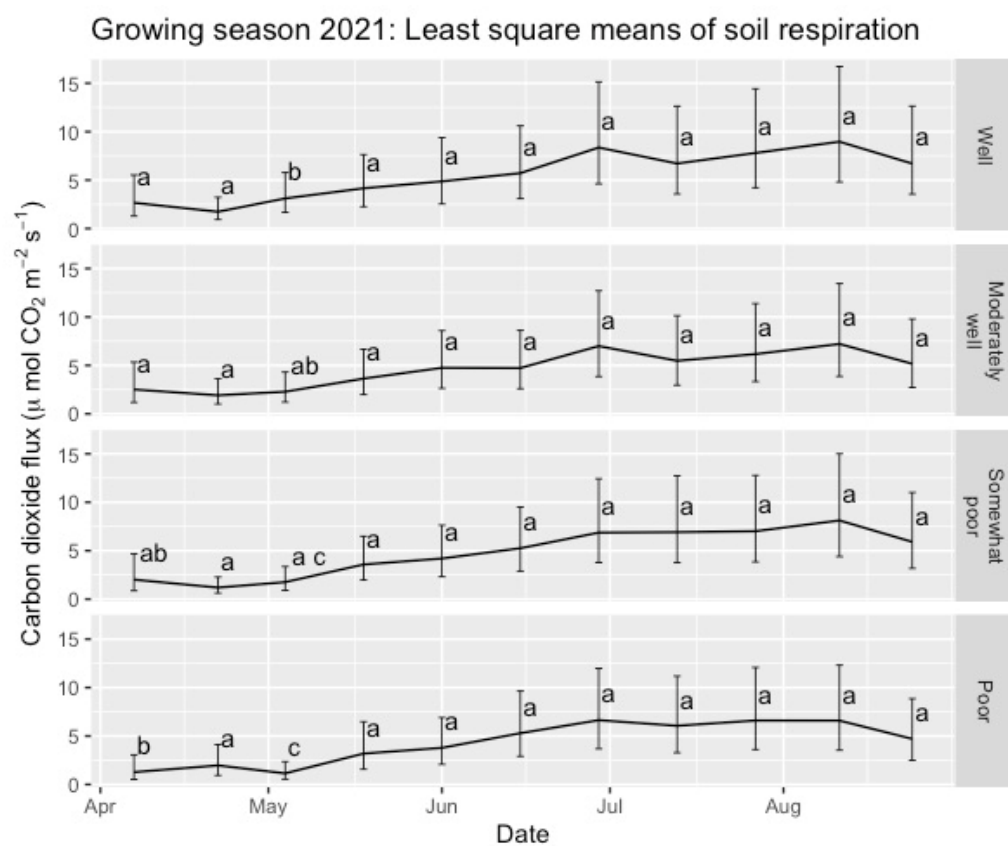


Figure S5: Soil respiration during 2021 across time and drainage classes. Letters indicate significant differences (p-value < 0.05).

Growing season 2021: Least square means of extractable ammonium

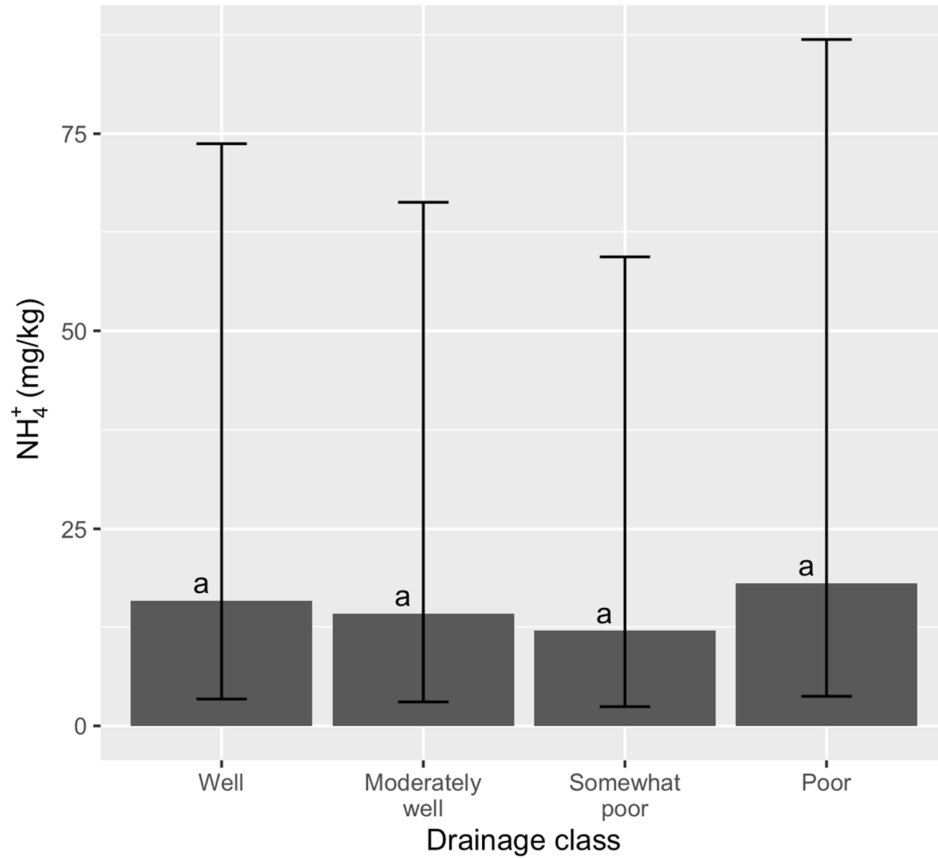


Figure S6: Least square mean values of extractable ammonium across drainage classes for 2021. Letters indicate significant differences between drainage classes (p-value < 0.05). Error bars represent 95% confidence intervals.

Table S6: Three-way ANOVA results for total nitrogen, ammonium, and nitrate/nitrite models. Pre-treatment nitrogen and percent clay were included as covariates in the models. Bolded values indicate a significant result (p-value < 0.05).

Model term	Total nitrogen		Ammonium		Nitrate/nitrite	
	2020	2021	2020	2021	2020	2021
	p-value	p-value	p-value	p-value	p-value	p-value
Intercept	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Drainage	<0.001	<0.001	0.014	0.372	<0.001	<0.001
Treatment	0.096	0.954	0.782	0.364	0.170	0.080
Date	0.139	<0.001	<0.001	<0.001	<0.001	<0.001
lnpretN	0.008	0.094	0.548	0.081	0.984	0.322
percentClay	0.797	0.545	0.159	0.831	0.458	0.016
Drainage:Treatment	0.222	0.053	0.632	0.569	0.068	0.495
Drainage>Date	0.662	0.067	0.423	0.025	0.296	0.413
Treatment>Date	0.331	0.347	0.918	0.460	0.651	0.888
Drainage:Treatment>Date	0.818	0.311	0.915	0.754	0.162	0.763

Vegetation communities

There were no significant differences in species richness or diversity between treatments or drainage classes (Supplemental materials Table S6). There were few clear patterns in species richness between treatments and among drainage classes. Control plots had a higher species richness than treatment plots for all but the St. Louis County sites (Supplemental Materials Table S8). The somewhat-poorly drained class had the highest species richness, followed by WD, MWD, and then PD (Supplemental Materials Table S8). There were also few consistent trends in Shannon's Diversity Index (SDI) across the plots (Supplemental Materials Table S9).

Table S7: Two-way ANOVA summaries of mixed models of species richness and Shannon's Diversity Index for 2021 vegetation community surveys. Site was included as a random variable in the models. Numerator degrees of freedom are shown. Bolded values denote significant result (p-value < 0.05).

Model Term	Degrees of freedom	Species richness	Shannon's Diversity Index
		p-value	p-value
Intercept	1	<0.001	<0.001
Drainage	3	0.4467	0.2953
Treatment	1	0.8587	0.6819
Drainage:Treatment	3	0.4716	0.1663

Table S8: Species richness (number of species) for all plots by location.

Drainage class	Aitkin		Itasca		St. Louis	
	Control	Treatment	Control	Treatment	Control	Treatment
WD	13	12	10	11	8	13
MWD	9	11	13	11	7	9
SPD	10	9	15	15	11	10
PD	12	6	10	9	11	11
Total	44	38	48	46	37	43

Table S9: Shannon's Diversity Index for all plots by location.

Drainage Class	Aitkin		Itasca		St. Louis	
	Control	Treatment	Control	Treatment	Control	Treatment
WD	2.87	2.83	2.93	2.69	2.27	2.92
MWD	2.04	2.95	2.96	2.52	2.07	2.65
SPD	2.88	2.62	3.16	3.25	2.85	2.29
PD	2.76	1.93	2.80	2.47	2.80	2.63

Table S10: Four-way ANOVA results for carbon dioxide model. Numerator degrees of freedom and model coefficient p-values are shown. Bolded values indicate a significant result (p-value < 0.05).

		Carbon dioxide
Model term	Degrees of freedom	p-value
Intercept	1	<0.001
Drainage	3	0.004
Treatment	1	0.438
H2O added	2	<0.001
Sample date	1	<0.001
Drainage:Treatment	3	0.740
Drainage:H2O added	6	0.949
Treatment:H2O added	2	0.240
Drainage:Sample Date	3	0.189
Treatment:Sample Date	1	0.956
H2O added:Sample Date	2	<0.001
Drainage:Treatment:H2O added	6	0.917
Drainage:Treatment:Sample Date	3	0.942
Drainage:H2O added:Sample Date	6	0.974
Treatment:H2O_added:Sample Date	2	0.561
Drainage:Treatment:H2O added:Sample Date	6	0.918