Electronic supplementary materials

Title: The effect of a freeze–thaw cycle on dissolved nitrogen dynamics and its relation to dissolved organic matter and soil microbial biomass in the soil of a northern hardwood forest

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Figures S-1 – S-6
Tables S-1 & S-2

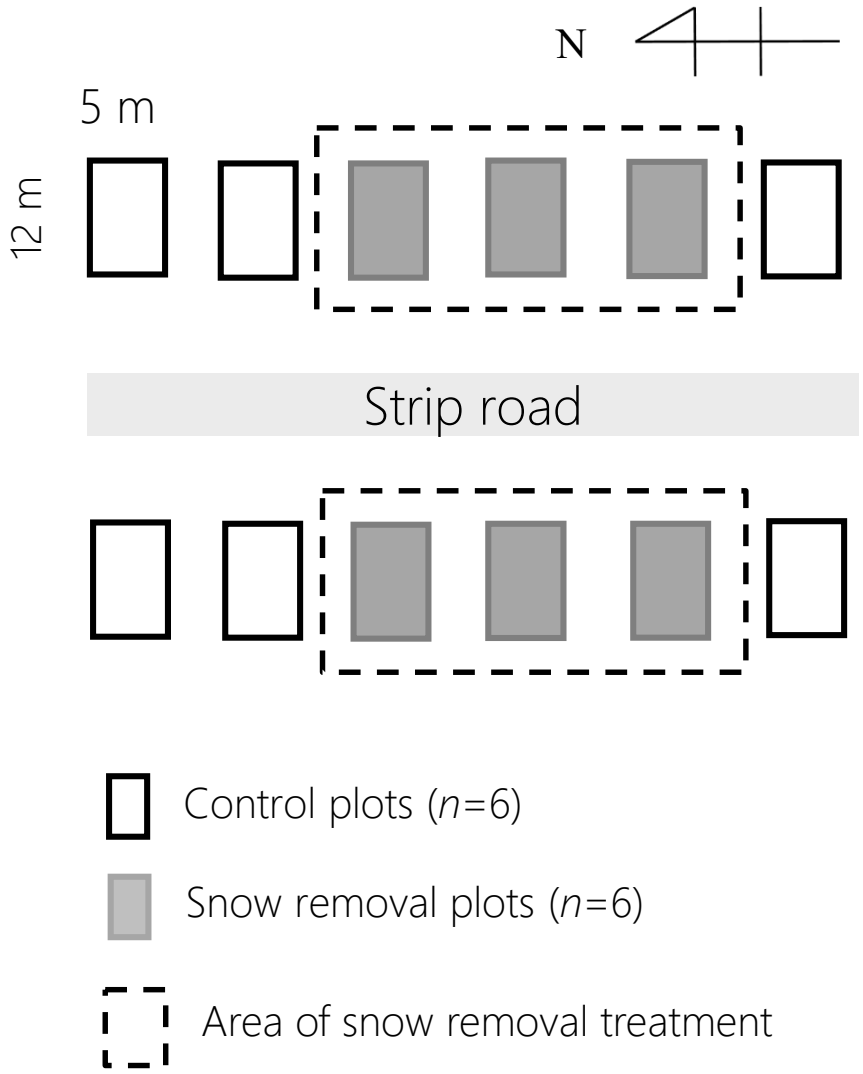

Figure **S-1**. Design and spatial location of the experimental plots.

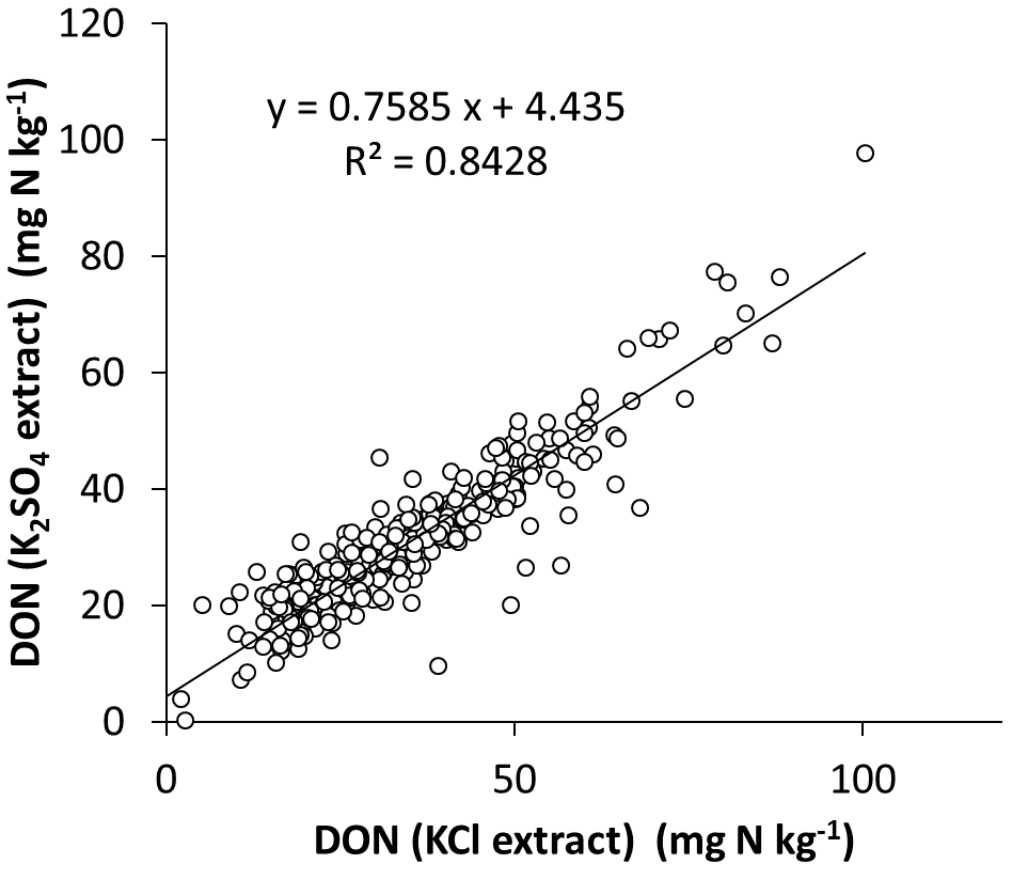

Figure **S-2**. Empirical relationship of dissolved organic nitrogen (DON) contents in soil between two different methods for dissolved inorganic nitrogen (DIN) (=NH_4^+ + NO_3^-) analysis. DON was calculated as DON = TDN (total dissolved N) – DIN. The DON (KCl extract) was calculated using DIN data extracted by KCl, while the DON (K_2SO_4 extract) was calculated using DIN data extracted by K_2SO_4. The TDN content was obtained using the same data extracted by K_2SO_4 for all samples. Refer to the methods section in the manuscript for detailed extraction methods for each. The empirical equation in this figure was applied for DON data on 7 and 16 April and 14 May 2014 because of the technical errors in the chemical analysis for the DIN contents.

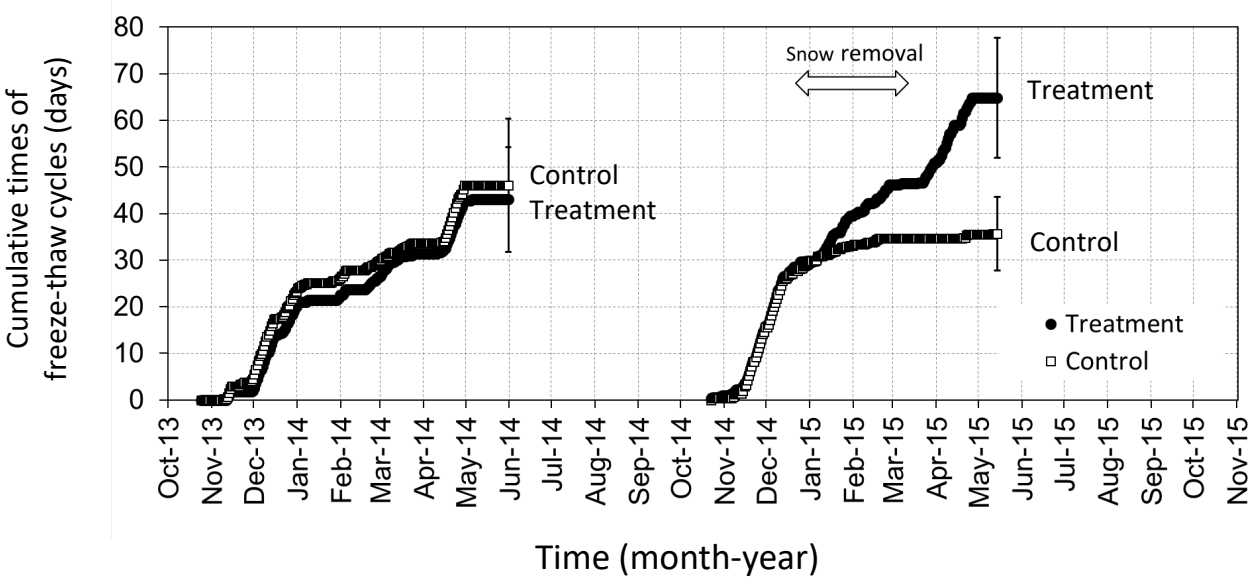

Figure **S-3**. Cumulative numbers (days) of freeze–thaw cycles at the control and treatment plots for pretreatment and treatment periods. The bar of the last data point in each period represents the standard deviation for the total number in each period (n = 6).

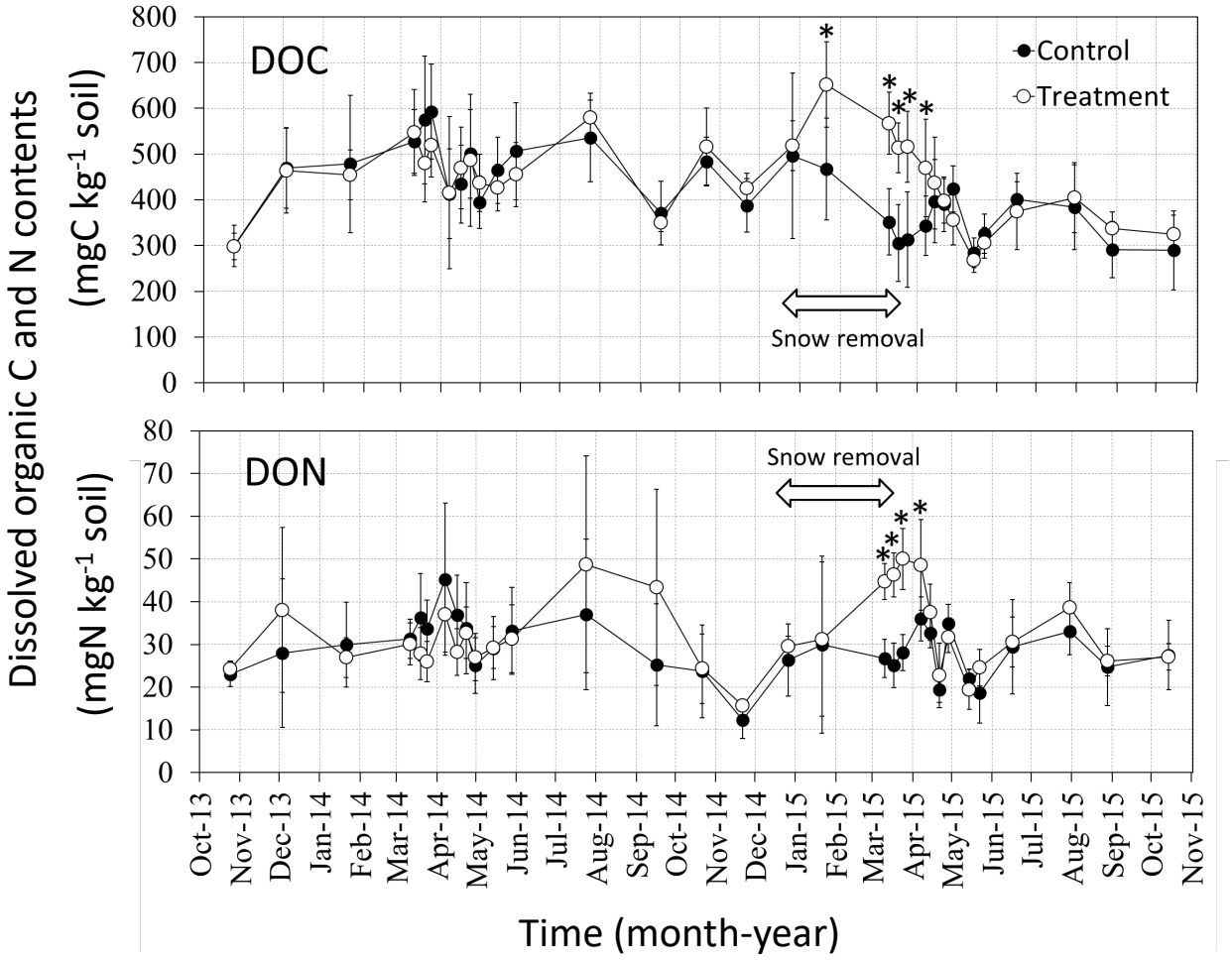

Figure **S-4**. Temporal changes in dissolved organic carbon (DOC) (upper) and dissolved organic nitrogen (DON) (lower) content in 0–10 cm depth soil at the control and treatment plots. Each bar of the plot indicates the standard deviation of six plots. The asterisks near the plot indicate significant differences (t-test, $P<0.05$) between the control and treatment plots for each observation day.

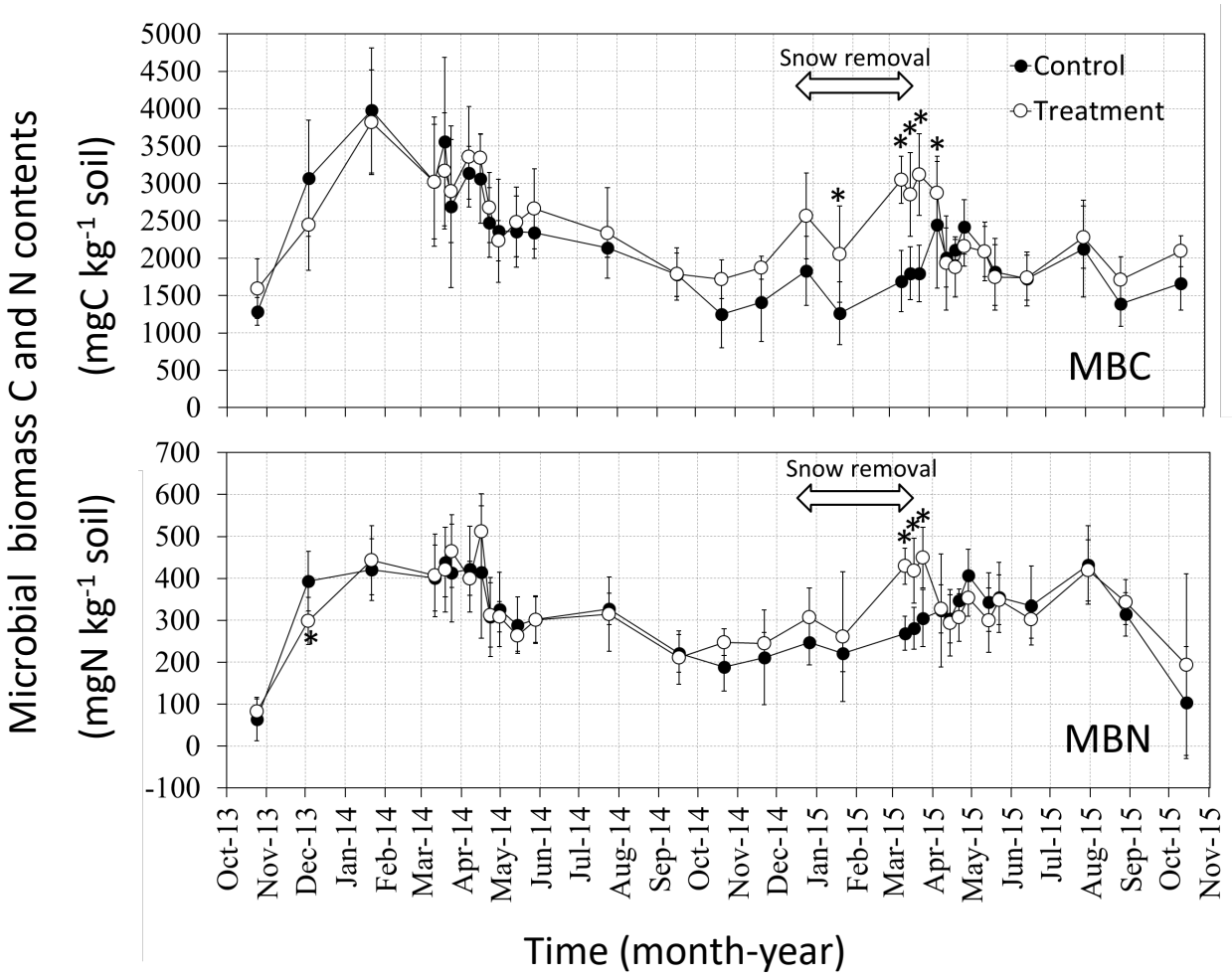

Figure **S-5**. Temporal changes in soil microbial carbon (MBC) (upper) and soil microbial nitrogen (MBN) (lower) content in 0–10 cm depth soil at the control and treatment plots. Each bar of the plot indicates the standard deviation of six plots. The asterisks near the plot indicate significant differences (t-test, $P<0.05$) between the control and treatment plots for each observation day.

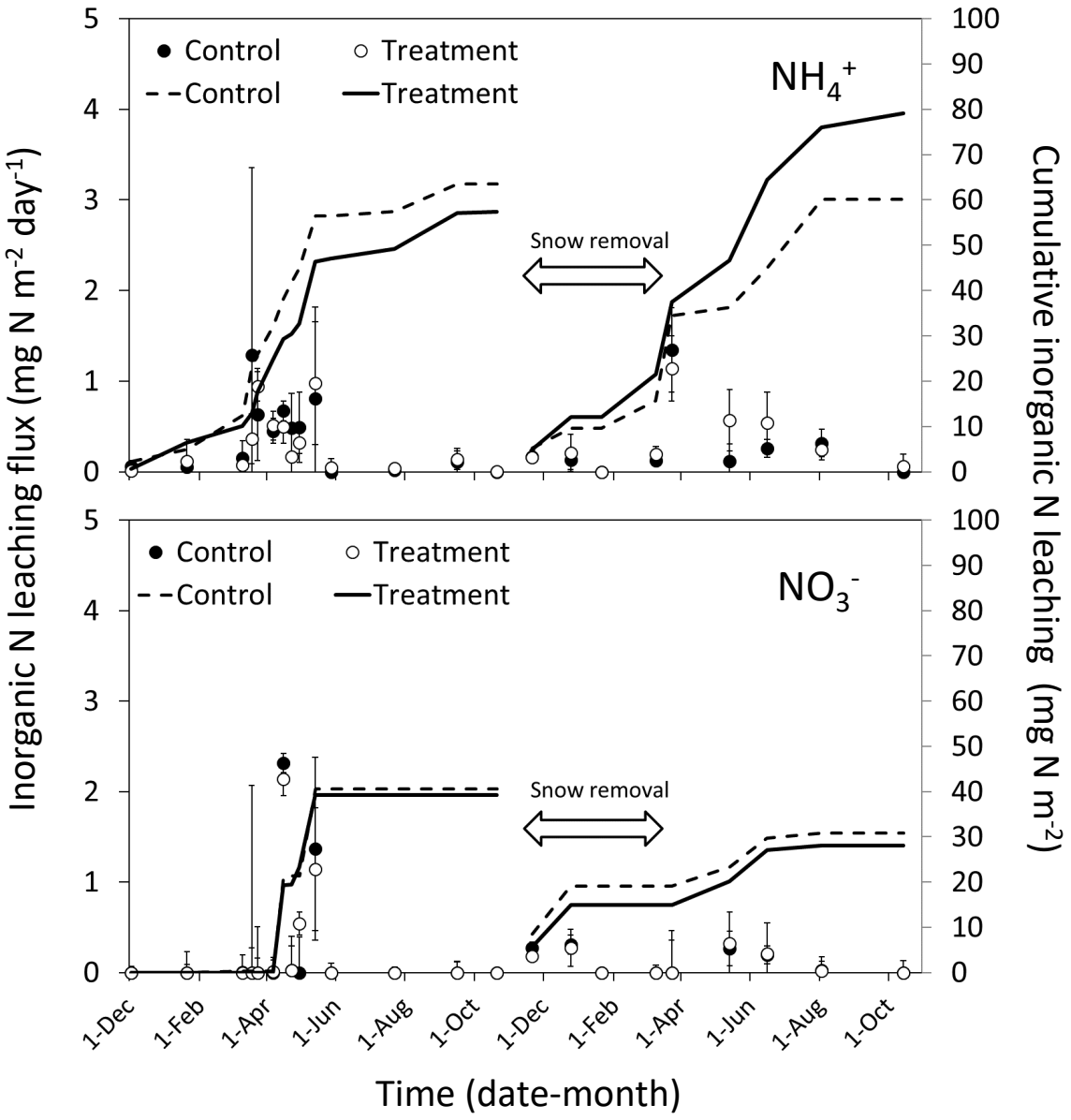

Figure **S-6**. Ammonium (upper) and nitrate (lower) leaching from soil at 20 cm depth at the control and treatment plots. The plots represent the leaching rate (mg m^{-2} day^{-1}) in each incubation period. The bar on the plot indicates the standard deviation of six plots. The lines represent the cumulative leaching amount (mg m^{-2}) in the pretreatment and treatment periods.

Table S-1. Net production rates (mg N kg^{-1} $period^{-1}$) of ammonium (NH_4^+) and nitrate (NO_3^-) in soil at 0-10 cm depth in each plot during the dormant and growing seasons (average ± standard deviation) and the results of three-way ANOVA.

Period	Net NH_4^+–N production				Net NO_3^-–N production			
	Dormant		Growing		Dormant		Growing	
	Control	Treatment	Control	Treatment	Control	Treatment	Control	Treatment
Pre-treatnent	27.3±32.1a	13.7±11.9ab	-21.9±20.1b	12.1±25.7ab	36.8±13.4b	21.5±12.3b	142.6±63.7a	160.4±55.9a
Post-treatment	1.08±25.2a	11.0±13.9a	4.86±7.54a	10.9±31.2a	17.9±7.63b	26.4±8.26b	134.8±25.2a	156.5±48.8a

Three–way ANOVA

Net NH_4^+–N production		Net NO_3^-–N production	
Treatment	n.s.	Treatment	n.s.
Year	n.s.	Year	n.s.
Season	n.s.	Season	P<0.001
Treatment × Year	n.s.	Treatment × Year	n.s
Treatment × Season	n.s.	Treatment × Season	n.s.
Year × Season	P<0.05	Year × Season	n.s.
Treatment × Year × Season	n.s.	Treatment × Year × Season	n.s.

Different lower letter indicatea significant difference amomng plots in each year and each inorganic N production, respectively (Tukey's HSD test, n=6). NS means insignificant difference (P>0.05).

Table S-2. Gross rates of nitrate (NO_3^-) and ammonium (NH_4^+) production, consumption, and their net production in each plot during the dormant and growing seasons.

Year	Day	NH_4^+–N (mg kg^{-1} day^{-1})						NO_3^-–N (mg kg^{-1} day^{-1})					
		Production		Consumption		Net rates		Production		Consumption		Net rates	
		Control	Treatment	Control	Treatment	Control	Treatment	Control	Treatment	Control	Treatment	Control	Treatment
	Dormant season (incubated at 4 °C)												
2014	21-Jan.	2.93±2.68	1.23±1.10	6.56±1.38	1.27±0.42***	-3.63±1.30	-0.03±1.30*	1.89±1.33	0.91±0.54	0.70±0.15	0.76±0.52	1.19±1.33	0.15±0.57
	17-Mar.	2.95±0.88	1.90±1.06	1.63±0.84	1.61±0.76	1.32±0.72	0.29±0.41*	0.25±0.09	0.26±0.23	0.47±0.26	0.29±0.13	-0.22±0.24	-0.03±0.17
	7-Apr.	2.47±0.59	1.44±0.53*	2.50±0.97	1.55±0.64	-0.03±0.97	-0.12±0.91	0.15±0.09	0.25±0.17	0.23±0.27	0.25±0.15	-0.08±0.31	-0.003±0.11
	28-Apr.	1.68±0.00	2.18±0.96	0.01±0.07	1.55±0.64	0.04±0.00	-0.58±0.75	0.15±0.04	0.14±0.12	0.01±0.07	0.05±0.03	0.14±0.03	0.09±0.10
2015	21-Jan.	1.47±0.65	0.73±0.42	2.33±1.11	2.02±1.66	-0.85±0.72	-1.29±1.83	0.65±0.53	0.26±0.26	1.06±1.05	0.72±0.55	0.38±1.56	-0.75±0.92
	17-Mar.	1.54±0.63	3.05±1.27†	1.05±0.37	2.22±1.99	0.48±0.77	0.82±1.29	0.46±0.23	0.52±0.31	0.10±0.07	0.59±0.27**	0.41±0.25	-0.07±0.45†
	7-Apr.	1.87±0.71	1.96±1.23	0.97±0.92	1.68±1.17	0.90±1.14	0.28±0.99	0.26±0.19	0.40±0.30	0.16±0.10	0.32±0.26	0.19±0.33	0.42±0.34
	28-Apr.	2.65±0.50	2.00±0.96	2.06±1.15	1.59±0.81	0.59±1.36	0.42±0.60	0.26±0.13	0.31±0.29	0.07±0.05	0.10±0.09	0.22±0.14	0.32±0.38
	Growing season (incubated at 9–17 °C)												
2015	31-Jul.	3.84±1.10	4.10±1.50	4.17±1.31	3.19±1.28	-0.32±0.46	0.90±2.39	1.49±0.57	1.34±0.52	0.52±0.29	0.42±0.16	1.16±0.63	1.19±0.33
	28-Aug.	3.74±1.03	3.26±0.61	4.24±1.14	3.29±0.94	-0.49±0.96	-0.03±0.60	1.32±1.04	0.85±0.70	0.47±0.30	0.61±0.34	1.43±0.93	1.45±1.02
	14-Oct.	1.62±0.43	1.42±0.73	1.41±0.55	1.91±0.79	0.21±0.84	-0.49±1.43	0.54±0.33	0.30±0.20	0.42±0.36	0.13±0.07	0.76±0.40	0.28±0.25

Asterisks indicate significant difference at the control and snow removal plots (*t*-test, n=6, †P＜0.1; *P＜0.05; **P＜0.01; ***P＜0.001).