Article title: Nutrient availability measurement techniques in arctic tundra soils: in situ ion exchange membranes compared to direct extraction

Submitted to Plant and Soil

Authors: Qian Gu and Paul Grogan

Department of Biology, Queen's University, 116 Barrie Street, Kingston, Ontario K7L 3N6, Canada

Corresponding author: Qian Gu (15qg1@queensu.ca)

Analyte	Incubation	Elution concentration	Elution concentration	Recovery variability (%)	
	concentration (mg/L)	(mg/L) mean	(mg/L) standard deviation		
NH4-N	0.02	-0.01	0.04	153	
	0.1	0.15	0.08	46.0	
	0.5	0.54	0.07	7.1	
	1.0	0.93	0.10	6.8	
	2.5	2.5	0.24	0.7	
	8.0	8.0	0.82	0.0	
NO₃-N	0.004	0.04	0.14	941	
	0.02	-0.06	0.03	388	
	0.1	0.09	0.03	13.4	
	0.5	0.53	0.10	6.5	
	1.0	1.06	0.08	5.5	
	2.0	1.97	0.10	1.7	
PO ₄ -P	0.004	0.03	0.01	696	
	0.02	0.01	0.05	35	
	0.1	0.12	0.01	20.3	
	0.5	0.45	0.08	9.4	
	2.5	2.50	0.09	0.1	
	5.0	5.00	0.01	0.1	

Table S1 Variability (%) between known incubated and mean recovered amounts (mg/L of incubation/extraction solution) for each set of replicate incubations for NH₄-N, NO₃-N, and PO₄-P (n = 6, 3, and 3 respectively) in the IEM methodological sensitivity tests. See Fig. S3 for graphical display of these data (in µg/cm² of membrane area) and their associated correlation equations.

Table S2 Statistical analyses of differences in IEM NH₄-N flux, PO₄-P flux, and NH₄-N:PO₄-P flux ratio (the latter is reported for control plots only), among the three serial incubations (R1, R2, and R3) in each of the fertilization treatments (n = 4-10). Each incubation period was 14 days, and soil element fluxes are expressed as μ g/cm²/day for each ion. See main text for detailed IEM incubation dates and statistics. The arrows illustrate the directions of the effects, symbols following arrows indicate the significance levels: P > 0.1: no symbol, P ≤ 0.1⁺, P ≤ 0.05⁺, P ≤ 0.01^{+*}.

	Treatment		R1	R2	R3	Average	Z	Z	Z
							(R2 vs.R1)	(R3 vs. R2)	(R3 vs. R1)
NH ₄ -N flux	Control	Mean	0.126	0.073	0.058	0.109	-4.44 ↓**	-0.06	-4.19 ↓* *
		(SD)	(0.177)	(0.104)	(0.064)	(0.122)			
	LN	Mean	0.328	0.318	0.374	0.340	-0.55	1.16	0.61
		(SD)	(0.418)	(0.450)	(0.527)	(0.458)			
		t	1.81 ††	2.67 ↑*	3.01 ↑**	4.35 ↑*			
	LP	Mean	0.066	0.062	0.033	0.068	-0.87	-0.84	-1.88
		(SD)	(0.066)	(0.042)	(0.037)	(0.049)			
		t	-0.91	-0.81	-0.58	-0.22			
	HN	Mean	17.88	16.36	15.42	16.55	-0.42	-0.37	-0.79
		(SD)	(10.27)	(7.13)	(7.25)	(7.20)			
		t	W = 0 ↑**	W = 0 ↑**	W = 0 ^**	19.08 ↑**			
	HP	Mean	0.223	0.196	0.213	0.211	-0.13	0.17	0.30
		(SD)	(0.133)	(0.080)	(0.101)	(0.091)			
		t	2.09 1+	W = 12 ↑**	W = 8 ↑**	4.87 *			
PO₄-P flux	Control	Mean	0.037	0.013	0.005	0.019	-2.81 ↓**	-2.57 ↓*	-5.38 ↓**
		(SD)	(0.041)	(0.011)	(0.003)	(0.016)			
	LN	Mean	0.019	0.008	0.003	0.010	-2.22↓†	-3.91 ↓**	-6.13 ↓**
		(SD)	(0.015)	(0.003)	(0.001)	(0.006)			
		t	-1.31	-1.01	-2.29 ↓*	-2.01			
	LP	Mean	0.091	0.102	0.059	0.078	1.34	-1.47	-0.18
		(SD)	(0.077)	(0.064)	(0.028)	(0.043)			
		t ,	1.71	6.50 1**	W = 1 ↑**	3.79 1+			
	HN	Mean	0.064	0.035	0.029	0.043	-1.66	-0.52	-2.18↓†
		(SD)	(0.045)	(0.020)	(0.013)	(0.017)			
		t	1.64	3.31 ***	W = 2 ↑**	4.18 *			
	НР	Mean	2.814	4.176	4.326	3.772	2.77 ↑*	0.97	3.73 ↑**
		(SD)	(2.340)	(3.097)	(1.899)	(2.236)			
		t	10.61 **	(3.057) W = 0 ↑**	W = 0 ^**	11.35 ***			
NH4-N:PO4-P	Control	Mean	18.82	17.63	16.94	18.33	-0.86	-2.43 ↓*	-1.71
flux ratio	20	(SD)	(51.49)	(42.63)	(19.38)	(36.35)	0.00	2.75 ¥	, _

Table S3 Statistical analyses of differences in soil water-extractable NH ₄ -N pool, PO ₄ -P pool, and NH ₄ -N:PO ₄ -P pool ratio (in control plots only),
among the three serial collections (R1, R2, and R3) in each of the fertilization treatments (n = 4-10). See main text for detailed sample collection
dates and statistics. Soil element pools are expressed as µg/g dw soil for each ion. The arrows illustrate the directions of the effects, symbols
following arrows indicate the significance levels: P > 0.1: no symbol, $P \le 0.1^+$, $P \le 0.05^*$, $P \le 0.01^{**}$.

	Treatment		R1	R2	R3	Average	Z	Z	Z
							(R2 vs. R1)	(R3 vs. R2)	(R3 vs. R1)
NH₄-N pool	Control	Mean	1.62	1.07	1.06	1.23	-3.03 ↓**	-0.19	-3.33 ↓**
		(SD)	(0.67)	(0.46)	(0.24)	(0.38)			
	LN	Mean	3.90	4.25	2.96	3.69	0.08	-1.55	-1.52
		(SD)	(4.80)	(4.93)	(4.36)	(3.37)			
		t	W = 32	W = 7 ↑**	W = 28	2.88 ↑+			
	LP	Mean	1.25	1.28	1.00	1.18	0.21	-1.95	-1.74
		(SD)	(0.42)	(0.30)	(0.35)	(0.24)			
		t	1.21	-1.54	-0.70	-0.16			
	HN	Mean	61.48	72.94	86.35	71.00	0.92	1.09	2.03 ↑†
		(SD)	(18.38)	(36.37)	(23.40)	(16.21)			
		t	W = 0 ↑**	W = 0 ↑**	W = 0 ^**	25.58 ↑**			
	HP	Mean	3.94	1.85	1.04	2.07	-0.58	-1.49	-2.05 ↓†
		(SD)	(6.84)	(1.14)	(0.25)	(2.37)			
		t	W = 49	2.32 ↑*	W = 42	1.33			
PO ₄ -P pool	Control	Mean	0.50	0.63	1.17	0.77	0.83	5.16	4.52 ↑**
		(SD)	(0.27)	(0.31)	(0.30)	(0.21)			
	LN	Mean	0.38	0.59	1.23	0.80	1.15	3.80 ^**	4.60
		(SD)	(0.57)	(0.27)	(0.29)	(0.16)			
		t	-1.29	-0.35	W = 46	0.87			
	LP	Mean	1.55	1.35	2.70	1.87	-0.23	1.53	1.30
		(SD)	(1.81)	(1.30)	(2.95)	(1.40)			
		t	1.10	0.99	W = 34	1.99			
	HN	Mean	3.17	4.05	2.69	3.30	0.92	-1.43	-0.51
		(SD)	(2.59)	(4.13)	(1.81)	(2.45)			
		t	3.61 ↑**	5.62 ↑**	W = 6 ↑**	4.79 ↑*			
	HP	Mean	151.02	228.47	172.67	191.03	2.67 ↑*	-1.80	0.94
		(SD)	(82.94)	(152.87)	(121.80)	(111.26)			
		t	W = 0 ^**	W = 0 ↑**	W = 0 ^**	19.31 ***			
NH4-N:PO4-P	Control	Mean	3.37	2.23	0.94	1.48	-1.93	-3.83 ↓**	-4.94 ↓**
pool ratio		(SD)	(1.40)	(1.51)	(0.21)	(0.19)			



Fig. S1 Procedures for IEM preparation, incubation, retrieval, and elution. (a): Measure out 5 cm x 5 cm sections and cut them out with a clean knife while the IEM sheet is still in its plastic covering. (b): Soak the IEM squares in the charging solution. (c): Tag one corner of each IEM with a plastic cloth barb and flagging tape. (d): Cut a vertical slit in the soil to the specific depth. (e): Insert the IEM down into the slit until it reaches the chosen depth. (f): Pack down the soil on either side to secure the IEM. (g): Rinse the IEM with distilled water. (h): Cut off the cloth barb and flagging tap from the IEM. (i): Store the IEM in a pre-labelled clean bag. (j) Place the multiple IEM squares from within the same replicate sampling area in a Petri dish and fill with elution solution to cover them. (k): Place the Petri dishes securely on the shaker table and shake for 2h.

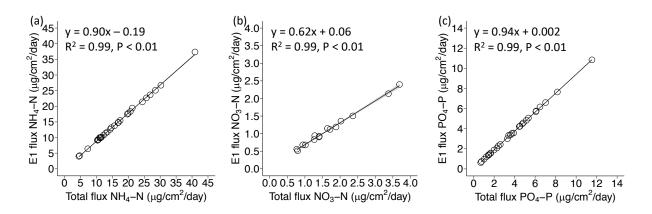


Fig. S2 IEM elution efficiency equations (the proportions of nutrient ions eluted from the first elution relative to the total cumulative amounts eluted when successive elutions with fresh elutant each time were included) for NH₄-N (a), NO₃-N (b), and PO₄-P (c), respectively. Data were based on IEM samples deployed in the high level nitrogen addition plots (for NH₄-N and NO₃-N) or in the high level phosphorus addition plots (for PO₄-P). These equations could be used in future IEM studies with soils that have large amounts of nutrient ions (e.g. heavily fertilized soils) to extrapolate the flux rates derived from a single initial elution to total flux rates (thereby avoiding the need for multiple successive elutions).

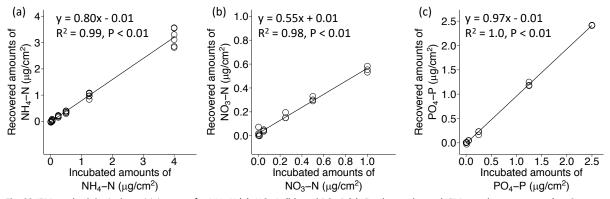


Fig. S3 IEM methodological sensitivity tests for NH₄-N (a), NO₃-N (b), and PO₄-P (c). Fresh pre-charged IEM membrane squares (n = 2 per individual solution, total one-sided membrane area of 50 cm²) were incubated in a range of NH₄-N, NO₃-N, or PO₄-P replicate solutions (25 mL in volume) of known concentrations (n = 6 for each NH₄-N concentration, and n = 3 for each NO₃-N and each PO₄-P concentration) for 1 h. Specifically, the concentrations were: 0, 0.02, 0.1, 0.5, 1, 2.5, and 8 mg/L for the NH₄-N solution; 0, 0.004, 0.02, 0.1, 0.5, 1, and 2 mg/L for the NO₃-N solution; and 0, 0.004, 0.02, 0.1, 0.5, 2.5, and 5 mg/L for the PO₄-P solution. The incubated membranes were then eluted with 2 *M* NaCl in 0.1 *M* HCl solution for 2 h. Ion recoveries from the membranes into the eluted solutions were compared with the known amounts of each ion added in the initial incubation solutions (all in units of μ g/cm² of membrane area). See Table S1 for quantitative data on accuracy (i.e. variability between known incubated and recovered amounts) for each set of replicate concentrations in the above graphs.

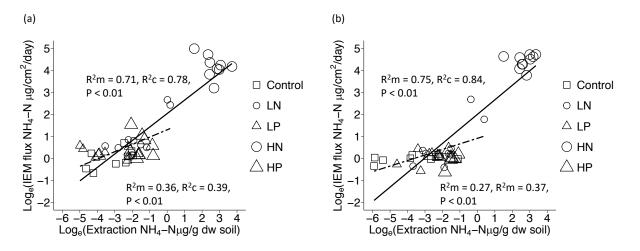


Fig. S4 Correlations between the IEM and direct water-extraction method determinations for soil NH₄-N availability during mid-growing season (a), and late-growing season (b), in the various experimental fertilization treatment plots (n = 6-10). Solid lines are linear regressions based on data from all the experimental plots (Control, LN addition, LP addition, HN addition, and HP addition), whereas dashed lines are linear regressions based on data from control, LN addition, and LP addition plots only. Linear mixed models were used, with both marginal R² (R²m), conditional R² (R²c), and P values of the fixed effects reported (Nakagawa and Schielzeth 2013). No figures for PO₄-P are shown since the regressions were not statistically significant. Note the log-scale of the X- and Y-axes.