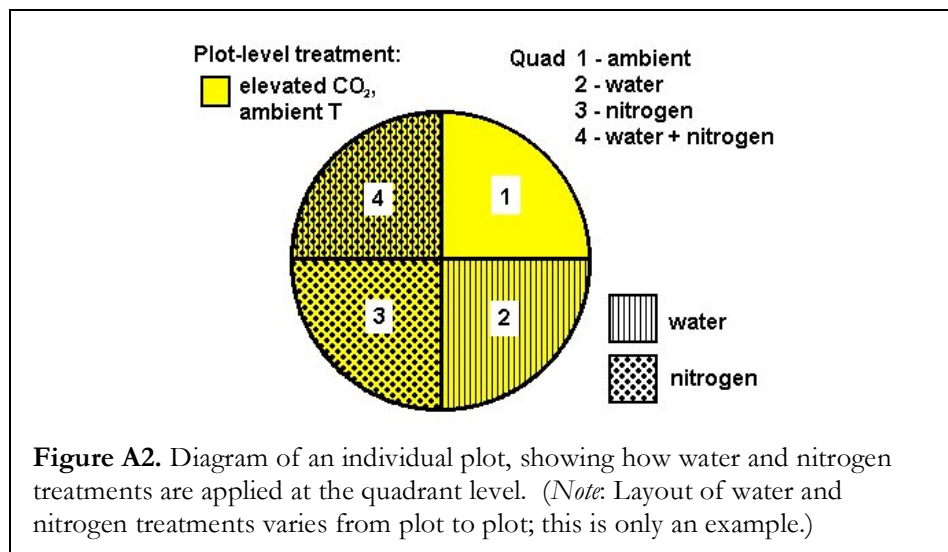
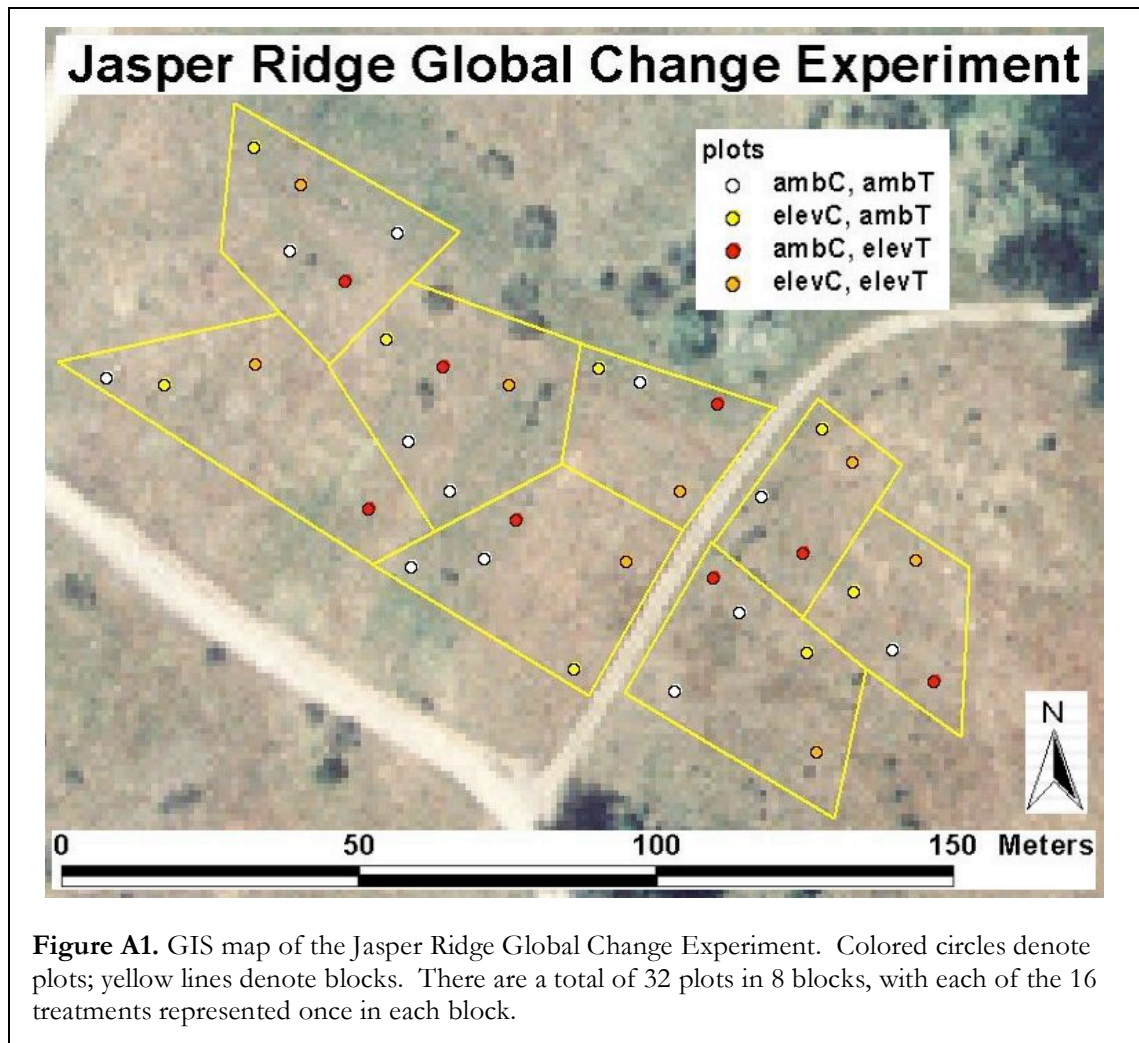


Appendix 2: Layout of the Jasper Ridge Global Change Experiment



Appendix 3: Ion Exchange Resin Specifications

AG-1-X8 anion exchange resin

Manufacturer: Bio-Rad Laboratories, 2000 Alfred Nobel Drive, Hercules, CA 94547.

Active group:	R-CH ₂ N ⁺ (CH ₃) ₃
Order of selectivity:	phenolate > ClO ₃ ⁻ > NO₃⁻ > Br > CN > HSO ₃ ⁻ > NO ₂ ⁻ > Cl > HCO ₃ ⁻ > IO ₃ ⁻ > H ₂ COO > Ac > OH > F (see table)
Thermal stability:	OH ⁻ form, fair to 50° C; Cl ⁻ and other forms, good to 150° C
Solvent stability:	Very good
Particle size:	20-50 dry mesh size (see Table A3 for further physical specifications)

Table A3. Physical characteristics of AG-1-X8 anion exchange resin.

Catalog Number	Ionic Form	Dry Mesh Size	Wet bead Diam. (µm)	Capacity (meq/ml)	Density (gm/ml)
140-1421	Chloride	20-50	300-1,180	1.2	0.75
140-1422	Hydroxide	20-50	300-1,180	1.2	0.75
140-1431	Chloride	50-100	180-425	1.2	0.75
140-1441	Chloride	100-200	106-180	1.2	0.75
140-1443	Acetate	100-200	106-180	1.2	0.75
140-1444	Formate	100-200	105-180	1.2	0.75
140-1451	Chloride	200-400	45-106	1.2	0.75
140-1453	Acetate	200-400	45-106	1.2	0.75
140-1454	Formate	200-400	45-106	1.2	0.75

Table A2. Relative selectivity of AG-1-X8 anion exchange resin for different anions.

Counter-ion	Relative selectivity
OH ⁻	1.0
Benzene sulfonate	500
Salicylate	450
Citrate	220
I ⁻	175
Phenate	110
HSO ₄ ⁻	85
ClO ₃ ⁻	74
NO₃⁻	65
Br ⁻	50
CN ⁻	28
HSO ₃ ⁻	27
BrO ₃ ⁻	27
NO ₂ ⁻	24
Cl⁻	22
HCO ₃ ⁻	6.0
IO ₃ ⁻	5.5
HPO₄⁻	5.0
Formate	4.6
Acetate	3.2
Propionate	2.6
F ⁻	1.6

Since this anion exchange resin has a higher affinity for NO₃⁻ than for Cl⁻, the counterion we used for recharging the resin (see Table A2), we expect that it was effective at collecting NO₃⁻ in the field. However, for the same reason, we may not have been entirely successful in displacing the collected NO₃⁻ when extracting with 2M KCl solution. This extremely high concentration of Cl⁻

would have helped to overcome its lower relative affinity, but some NO_3^- would still have remained on the resin. Previous work (S. Thayer, unpublished data) has estimated that this procedure results in an NO_3^- extraction efficiency of 60%, meaning that 60% of collected NO_3^- ions are successfully removed from the resin. In future studies, it would be advisable to improve this efficiency, because imperfect extraction efficiency can lead to accumulated errors when resin bags are reused (Skogley 1996)

The above table makes it clear why the resin was ineffective at collecting phosphate (HPO_4^-): the resin's relative affinity for HPO_4^- is very low, and therefore HPO_4^- ions would have great difficulty displacing Cl^- ions. A different technique should be used when attempting to measure phosphate.

HCR-W2 Na^+ form cation exchange resin

Manufacturer: Dow Chemical Company, Liquid Separations, P.O. Box 1206, Midland, MI 48641.
Tel. 1-800-447-4369.

General information: strong acid cation exchange resin, produced by sulfonation of styrene divinylbenzene copolymers, with high degree of cross-linking.

Exchange capacity: 2.0 eq/L

Water content: 45 – 49%

Bead size distribution: less than 2% bigger than 1.2 mm for 16 mesh, less than 1% smaller than 0.42 mm for 40 mesh.

Uncracked beads: at least 95%

Total swelling: 8%

Particle density: 1.28 g / mL

Max operating T: 120° C

pH range: 0 – 14

This cation exchange resin has a higher affinity for NH_4^+ than for Na^+ (see Table A4), and so can be expected to efficiently collect NH_4^+ when recharged with Na^+ and placed in the field.

Furthermore, the resin's affinity for K^+ is greater than for NH_4^+ , therefore extracting the collected

NH_4^+ with a KCl solution should have effectively removed all the NH_4^+ from the resin. Previous work (S. Thayer, unpublished data) estimates that this procedure results in an NH_4^+ extraction efficiency of 90%.

Table A4. Selectivity coefficients of various cations (compared with H^+) on sulfonated polystyrene Dowex cation exchange resins.

Counterion	Degree of crosslinking			
	4% DVB	8% DVB	10% DVB	16% DVB
Li^+	0.76	0.79	0.77	0.68
H^+	1.00	1.00	1.00	1.00
Na^+	1.20	1.56	1.61	1.62
NH_4^+	1.44	2.01	2.15	2.27
K^+	1.72	2.28	2.54	3.06
Rb^+	1.86	2.49	2.69	3.14
Cs^+	2.02	2.56	2.77	3.17
Ag^+	3.58	6.70	8.15	15.6
Tl^+	5.08	9.76	12.6	19.4
UO_2^{2+}	1.79	1.93	2.00	2.27
Mg^{2+}	2.23	2.59	2.62	2.39
Zn^{2+}	2.37	2.73	2.77	2.57
Co^{2+}	2.45	2.94	2.92	2.59
Cu^{2+}	2.49	2.03	3.15	3.03
Cd^{2+}	2.55	3.06	3.23	3.37
Ni^{2+}	2.61	3.09	3.08	2.76
Ca^{2+}	3.14	4.06	4.42	4.95
Sr^{2+}	3.56	5.13	5.85	6.87
Pb^{2+}	4.97	7.80	8.92	12.2
Ba^{2+}	5.66	9.06	9.42	14.2

Appendix 4: Example of Raw Data

Table A5. Raw data output from Alpkem RF/2 continuous flow analyzer.

CHANNEL NAME:					NO3-N		NH4-N		
RUN DATE:					6/26/02		6/26/02		
CALIBRATION TYPE:					LINEAR		LINEAR		
NUMBER OF STANDARDS:					8		8		
INTERCEPT:					-15		-28		
LINEAR COEF:					605.8528		1014.161		
CORRELATION COEF:					0.999812		0.999371		
					CONCENTRATION				
CUP#	SAMPLE ID	DIL	WGT	HEIGHT	mg/l	EF	HEIGHT	mg/l	EF
1	SYNC	1	1	490	0.833	s	1062	1.075	s
2	WASH	1	1	11	0.042	l	19	0.047	l
3	B	1	1	0	0.024	b	0	0.028	b
4	S1: .25	1	1	136	0.249		245	0.269	
5	S2: .5	1	1	283	0.491		478	0.499	
6	S3: 1	1	1	587	0.993		1055	1.068	
7	S4: 3	1	1	1779	2.960	N	2984	2.970	
8	WASH	1	1	38	0.087	l	21	0.049	l
9	B	1	1	0	0.024	b	0	0.028	b
10	04-24-02 1	1	1	271	0.471		230	0.255	
11	2	1	1	1457	2.429	N	447	0.469	
12	3	1	1	125	0.230	l	355	0.378	
13	4	1	1	91	0.174	R	109	0.135	R
14	5	1	1	94	0.179	R	93	0.119	R
15	6	1	1	68	0.136	R	828	0.844	
16	7	1	1	56	0.116	R	1283	1.293	
17	8	1	1	76	0.150	R	91	0.118	R
18	9	1	1	75	0.148	R	153	0.179	R
19	10	1	1	67	0.135	R	198	0.223	R
20	11	1	1	104	0.196	R	120	0.146	R
21	12	1	1	68	0.136	R	79	0.106	R
22	13	1	1	142	0.258		296	0.320	
23	14	1	1	292	0.506		255	0.279	
24	15	1	1	97	0.184	R	76	0.103	R
25	16	1	1	64	0.130	R	106	0.132	R
26	17	1	1	58	0.120	R	81	0.108	R
27	18	1	1	90	0.173	R	80	0.107	R
28	19	1	1	62	0.126	R	71	0.098	R
29	20	1	1	1725	2.871	N	737	0.755	
30	21	1	1	100	0.189	l	161	0.187	R
31	22	1	1	82	0.159	R	151	0.177	R
32	23	1	1	124	0.229	R	205	0.230	R
33	24	1	1	108	0.202	R	160	0.186	R
34	25	1	1	103	0.194	R	149	0.175	R
35	26	1	1	64	0.130	R	71	0.098	R
36	27	1	1	64	0.130	R	73	0.100	R
37	28	1	1	64	0.130	R	108	0.134	R

Red = standards

Blue, green = samples

Table A5 shows a typical output from the Alpkem RF/2 continuous flow analyzer, included here to show the considerable variability between samples. In several of the middle-season runs, many samples were indistinguishable from blanks (these samples were collected in late April). In general, over the year, concentrations were low but measurable.

Appendix 5: ANOVA Results

Table A6.

Nutrient availability ANOVA results, year-long and seasonal (using Erika Zavaleta's split-plot Systat model)

Ion:		ALL		EARLY		MIDDLE		LATE		
		trt effect	p-value	trt effect	p-value	trt effect	p-value	trt effect	p-value	
NO ₃ ⁻	CO ₂	–	0.911	–	0.658	⑨	0.801	⑦	0.822	
	HEAT	⑦	0.698	⑨	0.594	⑦	0.472	⑦	0.447	
	HEAT x CO ₂	⑦ –	0.433	⑦⑨	0.095	⑦⑦	0.699	⑦⑦	0.758	
	WATER	⑦	0.609	⑨	0.280	⑦	0.166	⑦	0.011	
	N	⑦	0.000	⑦	0.000	⑦	0.001	⑦	0.000	
	WATER x N	⑦ –	0.227	– ⑨	0.138	⑦⑦	0.582	⑦⑦	0.741	
	CO ₂ x WATER	– –	0.364	– ⑨	0.203	⑨⑦	0.081	⑨⑦	0.032	
	CO ₂ x N	⑦⑨	0.177	– –	0.655	⑨⑦	0.235	– ⑦	0.913	
	HEAT x WATER	– –	0.190	⑨ –	0.176	⑦⑦	0.654	⑦⑦	0.981	
	HEAT x N	⑦ –	0.237	– ⑨	0.425	⑦⑦	0.971	⑦⑦	0.838	
	CO ₂ x HEAT x WATER	⑦⑦⑨–	0.815	⑦ – ⑨⑨	0.359	⑨⑦⑨⑨	0.834	⑨⑦⑨⑦	0.332	
	CO ₂ x HEAT x N	⑦⑦⑨–	0.476	⑦⑦⑨⑨	0.435	⑨⑨⑨⑦	0.716	⑨⑦⑦–	0.422	
	CO ₂ x WATER x N	⑨⑦–⑦	0.237	⑨⑦–⑨	0.046	⑨––⑦	0.417	⑨⑨⑦⑦	0.696	
	HEAT x WATER x N	– – ⑦ –	0.166	⑨⑨⑦ –	0.767	⑦⑦⑦⑦	0.204	⑦⑦⑦–	0.540	
	CO ₂ x HEAT x WATER x N	n/a	0.285	n/a	0.692	n/a	0.635	n/a	0.088	
	NH ₄ ⁺	CO ₂	⑦	0.501	–	0.783	⑦	0.583	⑦	0.133
		HEAT	⑦	0.414	⑨	0.290	⑦	0.197	⑦	0.006
HEAT x CO ₂		⑨⑦	0.063	⑨ –	0.420	– ⑦	0.228	⑦⑦	0.050	
WATER		⑦	0.618	⑨	0.657	–	0.991	⑦	0.029	
N		⑦	0.000	⑦	0.000	⑦	0.270	⑦	0.027	
WATER x N		⑦⑨	0.236	⑦⑨	0.161	– –	0.723	⑦⑦	0.852	
CO ₂ x WATER		⑦ –	0.489	– –	0.962	⑦ –	0.735	⑦⑦	0.588	
CO ₂ x N		– ⑦	0.137	⑨⑦	0.120	⑦⑦	0.519	⑦⑦	0.881	
HEAT x WATER		⑦⑦	0.787	– ⑨	0.569	⑦ –	0.393	⑦⑦	0.958	
HEAT x N		⑦ –	0.193	⑨ –	0.584	⑦ –	0.107	⑦⑦	0.094	
CO ₂ x HEAT x WATER		⑨⑨⑦⑦	0.664	⑨⑨⑦ –	0.770	⑨⑨⑦⑦	0.761	⑨ – ⑦⑦	0.160	
CO ₂ x HEAT x N		⑨⑦⑦⑦	0.050	⑦⑦⑦–	0.055	⑨–⑦–	0.098	– ⑨⑦⑦	0.148	
CO ₂ x WATER x N		– ⑦⑨⑦	0.617	⑨ – ⑨⑦	0.631	⑦⑦⑦⑨	0.496	⑦⑦⑦⑦	0.652	
HEAT x WATER x N		⑦ – ⑦ –	0.828	– – ⑨ –	0.651	⑦⑦⑦⑨	0.059	⑦⑦⑦⑦	0.198	
CO ₂ x HEAT x WATER x N		n/a	0.643	n/a	0.150	n/a	0.635	n/a	0.966	
<p>Note : For a two-factor interaction effect, the first arrow represents treatment A's effect in the <u>absence</u> of factor B, and the second arrow represents treatment A's effect in the <u>presence</u> of factor B.</p> <p>For a three-factor interaction effect, the arrows represent factor A's effect with: 1) neither factor B or factor C; 2) C but not B; 3) B but not C; 4) both B and C.</p>										

Single-factor effects

As shown in Table A6, nitrogen deposition (N) dominates the ANOVA results for both nitrate and ammonium. Water has a significant effect for both ions, but only at the end of the growing season; CO₂ shows no significant effects for either ion. Heat becomes an important factor in

ammonium availability at the end of the growing season, but heat has no effect on nitrate availability at any time.

Interaction effects

Nitrate appears to show an interaction between CO₂ and water that becomes more important as the growing season progresses. The other significant interaction effects for nitrate (CO₂ × water × N, and CO₂ × heat × water × N) should probably be disregarded, as they occur only once during the year, and/or the direction of the effect is inconsistent.

For ammonium, there is an interaction between CO₂ and heat at the end of the growing season. There also appears to be a CO₂ × heat × N interaction during the early and middle growing season, but the direction of the effect changes. There is no reason to believe that the other two apparent interactions (heat × N, heat × water × N) indicate any meaningful effect.

Two-factor ANOVA

We ran a separate two-factor ANOVA only on treatments 1, 5, 9, and 13 (control, warming, elevated CO₂, and warming + CO₂). Results are given in Table A7.

Table A7.

ANOVA Results for CO₂ and warming treatments (using Systat 2-factor ANOVA)

NOTE: Data have been log-transformed from nmol / g resin / day.

Ion:		all			early			middle			late		
		ambient	elevated	p-value	ambient	elevated	p-value	ambient	elevated	p-value	ambient	elevated	p-value
NO ₃ -	CO ₂	2.881	2.706	0.065	3.895	3.814	0.298	1.770	1.179	0.130	0.894	0.728	0.208
	HEAT	2.804	2.784	0.826	3.892	3.817	0.343	1.408	1.541	0.728	0.768	0.854	0.511
	CO ₂ x HEAT	2.794	2.968	0.042	3.819	3.971	0.007	1.725	1.816	0.912	0.858	0.929	0.911
		2.814	2.599		3.964	3.663		1.091	1.267		0.678	0.778	
NH ₄ ⁺													
NH ₄ ⁺	CO ₂	2.559	2.627	0.790	3.388	3.197	0.561	0.965	1.173	0.546	1.781	2.086	0.322
	HEAT	2.461	2.724	0.305	3.337	3.248	0.785	0.835	1.303	0.180	1.659	2.208	0.080
	CO ₂ x HEAT	2.672	2.446	0.062	3.542	3.233	0.503	1.035	0.895	0.085	1.705	1.856	0.198
		2.251	3.002		3.132	3.262		0.635	1.712		1.612	2.559	

In this analysis, CO₂ slightly decreased nitrate availability, but had no effect on ammonium availability. Warming had an effect on ammonium at the end of the growing season, but had no effect on nitrate at any time. Both ions showed a significant CO₂ × warming interaction effect, but in opposite directions: the presence of both CO₂ and warming tended to decrease nitrate availability, but tended to increase ammonium availability.

Appendix 6: Phosphate Results

Phosphate (H_2PO_4^- and HPO_4^{2-}) is an essential nutrient for plants, and may be a limiting resource in the JRGCE ecosystem. For this reason, we hoped to measure availability of phosphate along with availability of ammonium and nitrate. However, phosphate is relatively immobile in the soil (Treseder and Vitousek, 2001), and our methods proved inadequate to capture meaningful amounts of phosphate. The phosphate concentrations of most of the resin bag extracts were below the autoanalyzer detection level.

Despite the low values, we did analyze 2 of the 11 batches for phosphate (dates: 12/24/01 and 02/04/02). It should be emphasized that the sample size is too small and the values are too uncertain to draw anything but very tentative conclusions. With that said, there are some indications of treatment effects which deserve further investigation. Results are given below (see Figure A3).

It appears as though each of the four global change factors (elevated CO_2 , warming, increased precipitation, and nitrogen deposition) may increase phosphate availability, though no results are significant (Table A8). Data was insufficient to test for interaction effects.

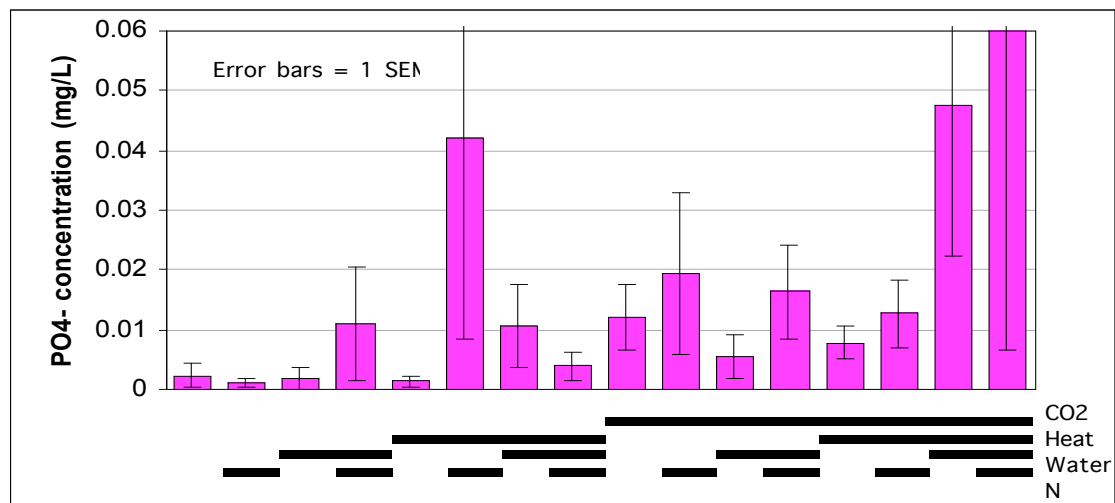


Figure A3. Phosphate availability by treatment (raw data, uncorrected for resin weight or surface area). Though the data are too incomplete to be conclusive, it appears that one or more of the global change treatments (especially CO_2 and heat) may increase phosphate availability.

For future studies on phosphate in this ecosystem, it will probably be necessary to leave resin bags in the ground for at least several weeks, or to use an anion exchange resin which collects phosphate more efficiently. Interpretation of results should take into account differences between how plants acquire P (for example, phosphatases and mycorrhizal symbiosis) and how resin bags acquire P (diffusion and mass transport). Nevertheless, more complete phosphate data from resin bags in this ecosystem could provide valuable insight into how global change affects nutrient dynamics.

Table A8. Effects of global change factors on phosphate availability. (Note: *p*-values uncorrected for multiple comparisons.)

T-test results for phosphate			
	mean	std dev	p-value
ambient	0.009	0.031	0.150
CO2	0.020	0.053	
ambient	0.008	0.019	0.126
heat	0.020	0.058	
ambient	0.011	0.030	0.346
water	0.018	0.054	
ambient	0.010	0.025	0.278
N	0.018	0.056	