

# Interactions between above- and belowground organisms modified in climate change experiments

## Supplementary Material

**Table S1 | Means and standard errors of the main response variables.** The table shows the overall mean, as well as the means of selected treatments. Standard errors are given in brackets. “Overall ambient” is the mean of all plots without herbivory and without global change treatments applied.

Variable	Overall mean	Overall ambient	No herbivory	Herbivory	Ambient CO <sub>2</sub>	Elevated CO <sub>2</sub>	No drought	Drought	No warming	Warming
Diff. in grass height (cm)	2.69 (0.467)	2.72 (0.471)	-0.11 (0.287)	5.16 (0.426)	3.22 (0.702)	2.09 (0.592)	2.38 (0.583)	3.05 (0.755)	2.56 (0.609)	2.83 (0.726)
Abovegrd. Biomass (g m <sup>-2</sup> )	172.20 (8.587)	188.25 (43.248)	194.33 (13.121)	150.07 (9.388)	158.63 (12.386)	186.90 (11.340)	186.31 (13.238)	156.91 (10.070)	181.93 (14.126)	161.65 (9.112)
Microbial biomass (mg C g soil <sup>-1</sup> )	1.78 (0.464)	1.78 (0.464)	1.78 (0.511)	1.78 (0.464)	1.78 (0.511)	1.78 (0.464)	1.78 (0.511)	1.78 (0.464)	1.78 (0.464)	1.78 (0.511)
Protozoan abund. (g soil <sup>-1</sup> )	24996.51 (2451.663)	24489.39 (2519.466)	18537.34 (2667.347)	30680.58 (3647.475)	26715.91 (3710.954)	23042.64 (3146.643)	20046.47 (2040.803)	30621.54 (4459.397)	24480.22 (3692.209)	25535.25 (3283.95)
Nematode abund. (g soil <sup>-1</sup> )	1242.41 (68.47)	1222.57 (70.273)	1249.43 (77.202)	1236.23 (110.905)	1201.09 (87.683)	1289.36 (108.426)	1199.09 (94.418)	1291.63 (100.718)	1318.89 (105.403)	1162.60 (85.698)
Micr. growth (ctrl)	0.78 (0.04)	0.76 (0.042)	0.81 (0.033)	0.75 (0.07)	0.70 (0.062)	0.87 (0.041)	0.79 (0.036)	0.76 (0.076)	0.78 (0.028)	0.77 (0.081)
Micr. growth on C	1.18 (0.032)	1.18 (0.034)	1.11 (0.038)	1.25 (0.048)	1.25 (0.046)	1.11 (0.041)	1.18 (0.04)	1.19 (0.053)	1.13 (0.043)	1.24 (0.047)
Micr. growth on CN	1.66 (0.034)	1.67 (0.036)	1.66 (0.062)	1.66 (0.034)	1.71 (0.054)	1.61 (0.038)	1.66 (0.045)	1.67 (0.053)	1.62 (0.045)	1.71 (0.05)
Micr. growth on CP	1.26 (0.027)	1.25 (0.029)	1.21 (0.038)	1.30 (0.038)	1.29 (0.044)	1.22 (0.03)	1.26 (0.041)	1.26 (0.036)	1.22 (0.034)	1.30 (0.043)
Micr. growth on CNP	1.72 (0.045)	1.73 (0.048)	1.71 (0.057)	1.73 (0.07)	1.78 (0.065)	1.64 (0.059)	1.75 (0.063)	1.68 (0.065)	1.71 (0.064)	1.72 (0.064)
Soil water ct. (%)	8.78 (0.281)	8.74 (0.295)	8.14 (0.431)	9.34 (0.337)	8.91 (0.431)	8.63 (0.357)	8.49 (0.399)	9.10 (0.392)	8.53 (0.401)	9.03 (0.395)
Soil org. matter (%)	4.02 (0.054)	4.01 (0.054)	4.01 (0.089)	4.03 (0.064)	4.07 (0.069)	3.97 (0.085)	4.04 (0.071)	4.00 (0.085)	3.95 (0.075)	4.10 (0.076)

**Table S2 | Parameter estimates from linear mixed-effects models fitted to data on grass height measured on 3<sup>rd</sup> and 12<sup>th</sup> September 2008.** Parameter estimates are expressed as differences between individual treatment means (as explained in column 2). Note that parameter estimates are tested marginally, i.e. in presence of all other terms in the model. The corresponding sequential F tests are given in the main text. The unit of all parameter estimates is cm grass height. Significant effects are given in bold.

Term	Explanation	Value [cm]	SE	DF	t	P
Overall mean	Mean grass height per cage (tested against 0)	8.94	0.77	68	11.61	0.0000
<b>Date (2008-09-12 minus 2008-09-03)</b>	<b>Difference in height over time (negative values indicate herbivory)</b>	<b>-3.35</b>	<b>0.42</b>	<b>68</b>	<b>7.97</b>	<b>0.0000</b>
Grasshopper	Marginal main effect of herbivory (Grasshopper minus control cage)	-1.37	0.87	68	1.57	0.1212
CO <sub>2</sub>	Marginal main effect of CO <sub>2</sub> (elevated minus ambient CO <sub>2</sub> )	0.87	0.96	4	0.91	0.4156
Drought	Marginal main effect of drought (drought minus no drought)	-1.06	0.93	5	1.13	0.3079
<b>Date:Grasshopper</b>	<b>Reduction in height due to herbivory over time</b>	<b>-5.76</b>	<b>0.58</b>	<b>68</b>	<b>9.87</b>	<b>0.0000</b>
Date:CO <sub>2</sub>	Interaction between date and CO <sub>2</sub>	0.98	0.61	68	1.60	0.1150
<b>Date:Drought</b>	<b>Interaction between date and drought (grasses are shorter in drought plots)</b>	<b>-0.82</b>	<b>0.36</b>	<b>68</b>	<b>2.28</b>	<b>0.0257</b>
Grasshopper:CO <sub>2</sub>	Interaction between herbivory and CO <sub>2</sub> (irrespective of date)	0.89	1.27	68	0.70	0.4875
<b>Date:Grasshopper:CO<sub>2</sub></b>	<b>Decrease in herbivory in elevated CO<sub>2</sub> over time (grasses are taller)</b>	<b>1.82</b>	<b>0.85</b>	<b>68</b>	<b>2.13</b>	<b>0.0365</b>

**Table S3 – Parameter estimates from linear mixed-effects models fitted to data on microbial biomass, microbial growth and protozoan abundance.** Note that parameter estimates are tested marginally, i.e. in presence of all other terms in the model. The first row in the table gives the overall mean; microbial growth is dimensionless. Rows give (i) main effect parameter estimates, expressed as differences: Herbivory minus control; elevated CO<sub>2</sub> minus ambient CO<sub>2</sub>; Drought minus no drought; (ii) interaction terms (differences between intercepts). For example, a negative CO<sub>2</sub> main effect for microbial biomass means that there were more microbes present under ambient than under elevated CO<sub>2</sub> (compare this with Figure 2).

Variable	Microbial biomass (mg C g <sup>-1</sup> soil)					Microbial growth* (Control)					Microbial growth* (N added)					Protozoan abundance (ln g <sup>-1</sup> soil)				
	Value	SE	DF	t	P	Value	SE	DF	t	P	Value	SE	DF	t	P	Value	SE	DF	t	P
Overall mean	1.92	0.16	36	12.36	<0.001	1.24	0.04	37	31.34	<0.001	1.69	0.07	36	22.64	<0.001	9.99	0.15	28	64.97	<0.001
CO <sub>2</sub> (elevated - ambient)	-0.28	0.16	4	1.74	0.156	-0.14	0.06	4	2.36	0.077	-0.19	0.10	4	1.87	0.134	-0.13	0.17	4	0.76	0.492
Drought (drought - ambient)	0.49	0.14	36	3.51	<b>0.001</b>	--	--	--	--	--	--	--	--	--	--	-0.2	0.24	28	0.83	0.411
Herbivory (herbivory - control)	-0.59	0.18	36	3.34	<b>0.002</b>	0.14	0.06	37	2.43	<b>0.02</b>	-0.09	0.08	36	1.14	0.262	0.5	0.22	28	2.26	<b>0.032</b>
Warming (warming - control)	--	--	--	--	--	--	--	--	--	--	0.09	0.04	36	2.13	<b>0.040</b>	0.14	0.23	28	0.62	0.542
Herbivory:CO <sub>2</sub>	0.85	0.25	36	3.33	<b>0.002</b>	--	--	--	--	--	0.25	0.11	36	2.16	<b>0.037</b>	0.34	0.32	28	1.08	0.289
Warming:Drought	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
CO <sub>2</sub> :Drought	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	-0.26	0.32	28	0.82	0.422
CO <sub>2</sub> :Warming	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	-0.15	0.33	28	0.46	0.651
CO <sub>2</sub> :Warming:Drought	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
Herbivory:CO <sub>2</sub> :Drought	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	1.94	0.63	28	3.06	<b>0.005</b>
Herbivory:CO <sub>2</sub> :Warming	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	1.41	0.64	28	2.21	<b>0.036</b>
Herbivory:Drought	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	-0.95	0.44	28	2.15	<b>0.040</b>
Herbivory:Warming	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	-0.77	0.45	28	1.72	0.097
Herbivory:Warming:Drought	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
Herbivory:CO <sub>2</sub> :Warming:Drought	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--

\*: (respiration rate 4-20h)/(respiration rate 0-4h)

**Table S4 | Effects of global change on chemical composition and morphology of *Deschampsia*.** Dry weight (g) of green leaves, senescent leaves, and roots; crude fibre (percent) and leaf diameter (mm) in green *Deschampsia* leaves from soil cores not exposed to herbivory. Treatment levels: A ambient, T warming, D drought, CO<sub>2</sub> elevated CO<sub>2</sub>, plus combinations.

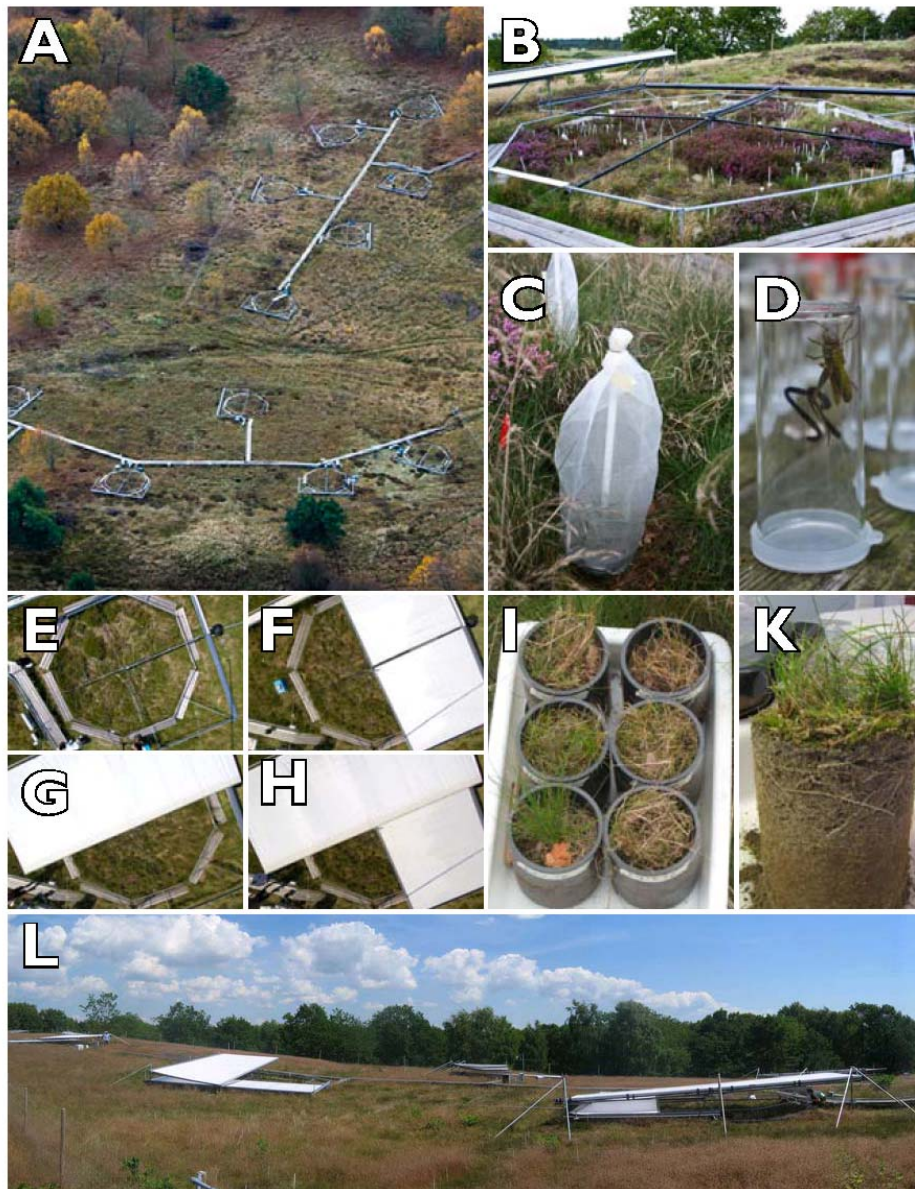
Treatment	Leaf weight				Root weight		Crude fiber		Leaf diameter	
	green (g)		senescent (g)		(g)		(percent)		(mm)	
	avg	s.e.	avg	s.e.	avg	s.e.	avg	s.e.	avg	s.e.
A	0.61	0.14	0.93	0.16	0.50	0.12	26.38	1.33	0.30	0.01
T	0.93	0.22	0.71	0.12	0.75	0.21	33.72	0.93	0.35	0.04
D	0.74	0.25	0.66	0.14	0.77	0.33	29.82	2.01	0.29	0.02
TD	0.43	0.13	0.41	0.04	0.34	0.14	28.71	1.03	0.28	0.03
CO <sub>2</sub>	0.62	0.31	0.88	0.42	0.71	0.14	31.68	0.24	0.26	0.01
TCO <sub>2</sub>	0.72	0.18	0.83	0.11	0.46	0.04	32.58	2.12	0.29	0.00
DCO <sub>2</sub>	1.03	0.49	1.19	0.49	0.38	0.13	32.39	4.14	0.29	0.01
TDCO <sub>2</sub>	0.38	0.19	0.81	0.42	0.51	0.13	33.41	4.69	0.28	0.01
Significance	n.s.		n.s.		n.s.		n.s.		P(CO <sub>2</sub> ) = 0.0750	

**Table S5 Silica concentration (percent of dry weight) in green leaves.** s.e., standard error of the mean

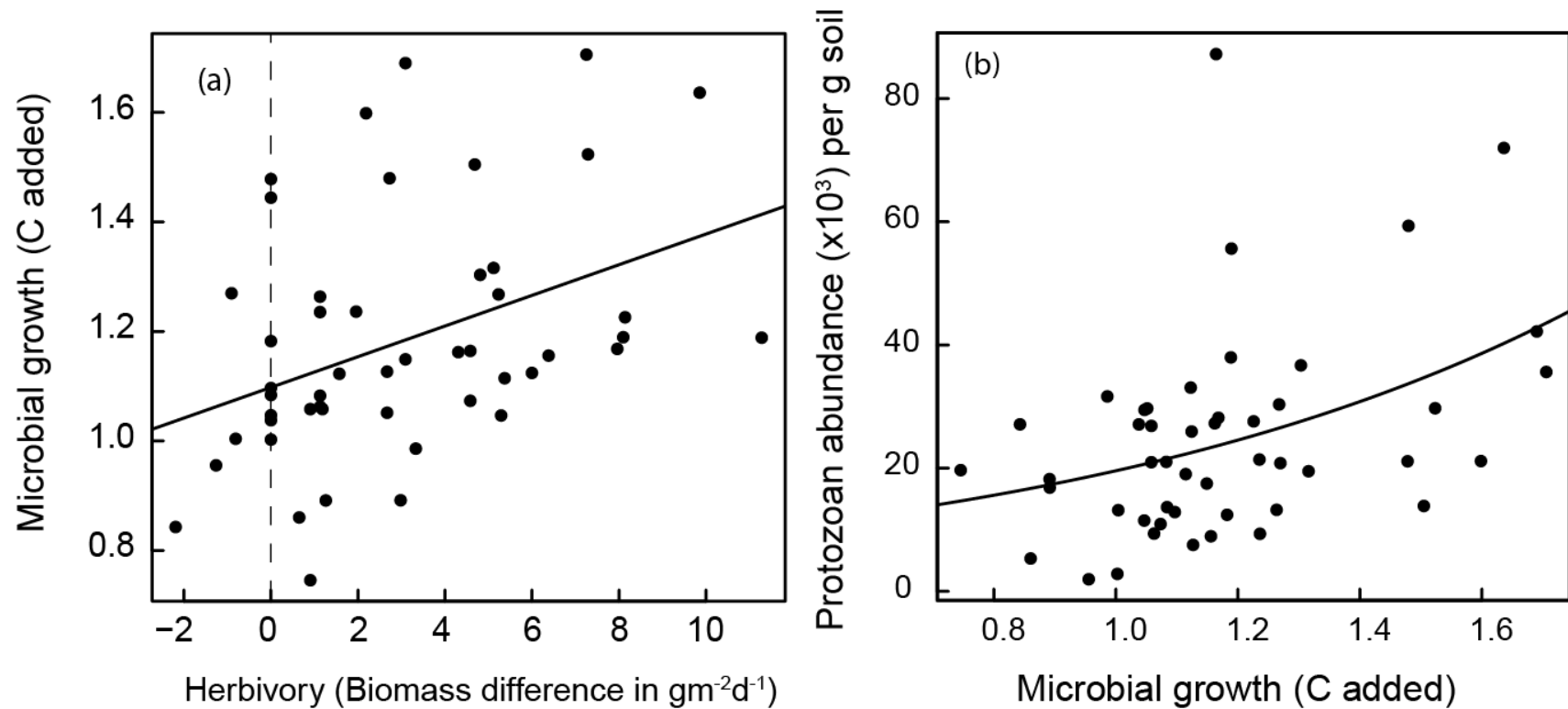
	Mean value	
	(%)	s.e.
Ambient CO <sub>2</sub>	0.89	0.04
Elevated CO <sub>2</sub>	0.89	0.01

**Table S6 | Number of replicates per treatment combination in the experiment.** Note that the design was a split-plot with CO<sub>2</sub> applied at the largest plot size, drought and warming at intermediate plot sizes, and herbivory at the smallest plot size.

<b>CO<sub>2</sub> treatment</b>	<b>Drought treatment</b>	<b>Warming treatment</b>	<b>Herbivory treatment</b>	<b>Number of replicates</b>
Ambient CO <sub>2</sub>	Drought	No warming	No herbivory	3
Ambient CO <sub>2</sub>	Drought	Warming	No herbivory	3
Ambient CO <sub>2</sub>	No drought	No warming	No herbivory	3
Ambient CO <sub>2</sub>	No drought	Warming	No herbivory	4
<b>Sum</b>				<b>13</b>
Elevated CO <sub>2</sub>	Drought	No warming	No herbivory	3
Elevated CO <sub>2</sub>	Drought	Warming	No herbivory	3
Elevated CO <sub>2</sub>	No drought	No warming	No herbivory	4
Elevated CO <sub>2</sub>	No drought	Warming	No herbivory	2
<b>Sum</b>				<b>12</b>
Ambient CO <sub>2</sub>	Drought	No warming	Herbivory	3
Ambient CO <sub>2</sub>	Drought	Warming	Herbivory	3
Ambient CO <sub>2</sub>	No drought	No warming	Herbivory	3
Ambient CO <sub>2</sub>	No drought	Warming	Herbivory	4
<b>Sum</b>				<b>13</b>
Elevated CO <sub>2</sub>	Drought	No warming	Herbivory	3
Elevated CO <sub>2</sub>	Drought	Warming	Herbivory	3
Elevated CO <sub>2</sub>	No drought	No warming	Herbivory	4
Elevated CO <sub>2</sub>	No drought	Warming	Herbivory	2
<b>Sum</b>				<b>12</b>
<b>Grand sum</b>				<b>50</b>

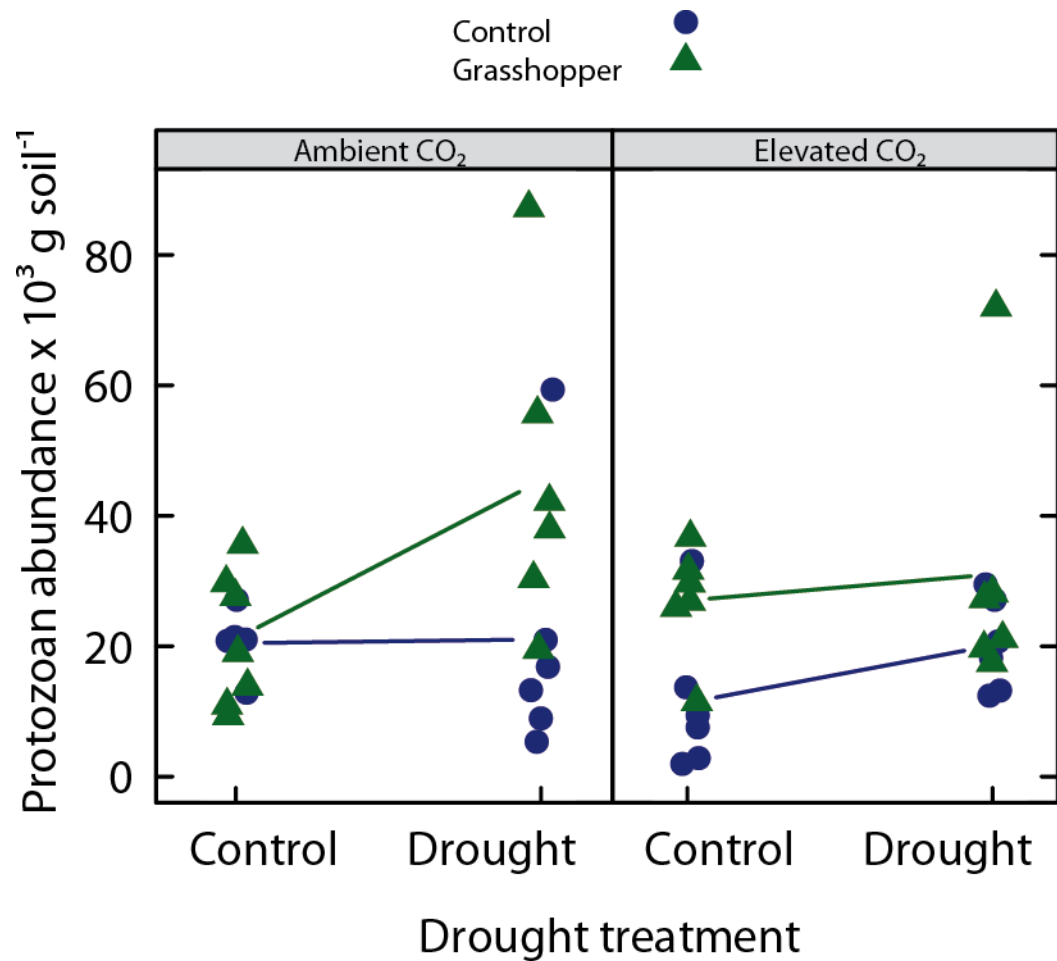


**Figure S1. Experimental setup.** (A) Aerial view of the CLIMAITE experiment, showing 10 out of 12 octagons (+/- CO<sub>2</sub>). (B) A single octagon surrounded by CO<sub>2</sub> pipes and split into four halves for warming and drought treatment combinations. (C) Close-up of a soil core covered with nylon netting; (D) Randomization of grasshopper (*Chorthippus brunneus* THUNB.) individuals; (E-H) A series showing the positioning of the curtains controlling warming (F), drought (G) and warming plus drought (H); (I) A set of six extracted soil cores (left: control, right: herbivory); (K) close-up of a soil core before post-processing; (L) Ground panorama view of the CLIMAITE experiment, showing curtains in action. Image copyright: David Gladbach (B,C,D), Søren Christensen (I,K), all others: CLIMAITE project.

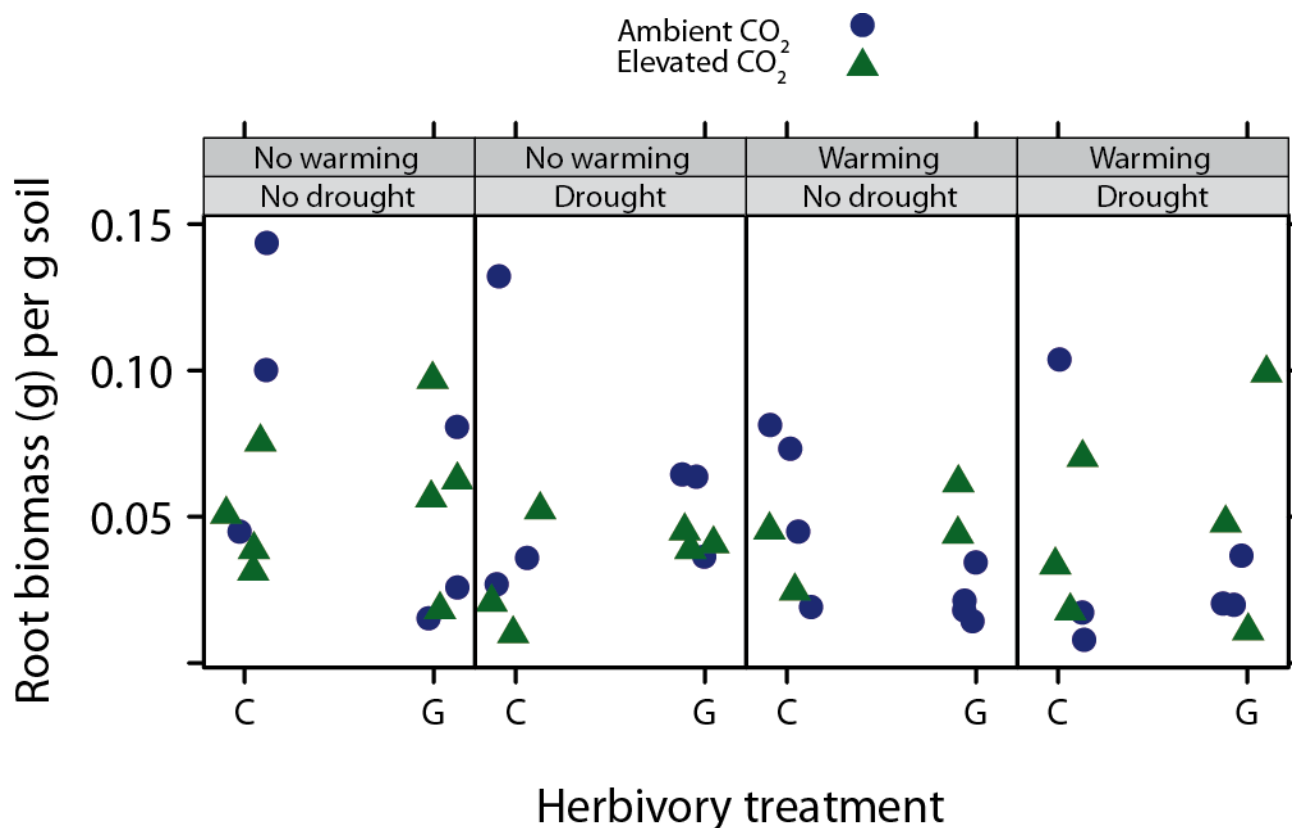


**Figure S2 | Relationships between herbivory, microbial growth and protozoan abundance.** (a) More herbivory translates into higher microbial growth [(respiration rate 4-20h)/(respiration rate 0-4h) during soil incubation] when Carbon is added as the only substrate; solid line is from a linear regression. overall  $P < 0.0005$ ; dashed line indicates a biomass difference of 0 between herbivory and control cages; (b) High microbial growth coincides with high protozoan abundance. Note that this relationship does not imply a causal relationship; both abundances could be driven by a third (unmeasured) factor. The non-linear curve was fitted using a generalized linear model with a log-link and microbial growth as the explanatory variable). The slope of the curve was  $1.13 \pm 0.004$ .  $|z| = 291.2$ .  $P < 2 \times 10^{-16}$ .

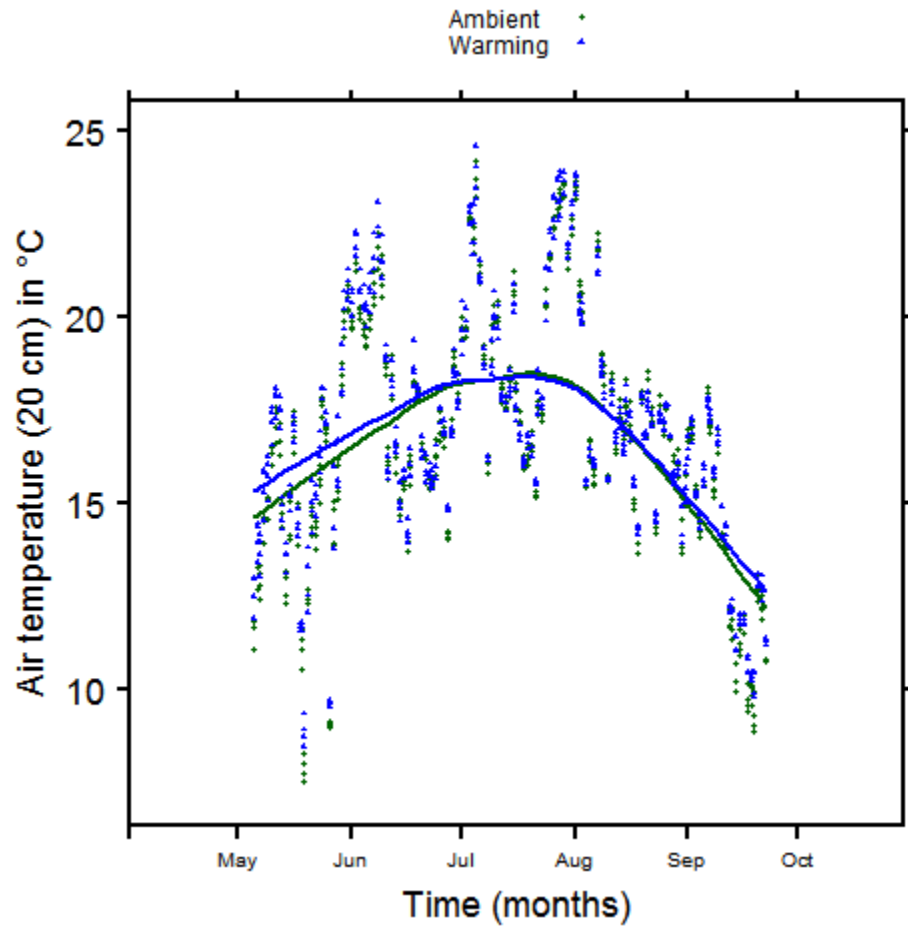




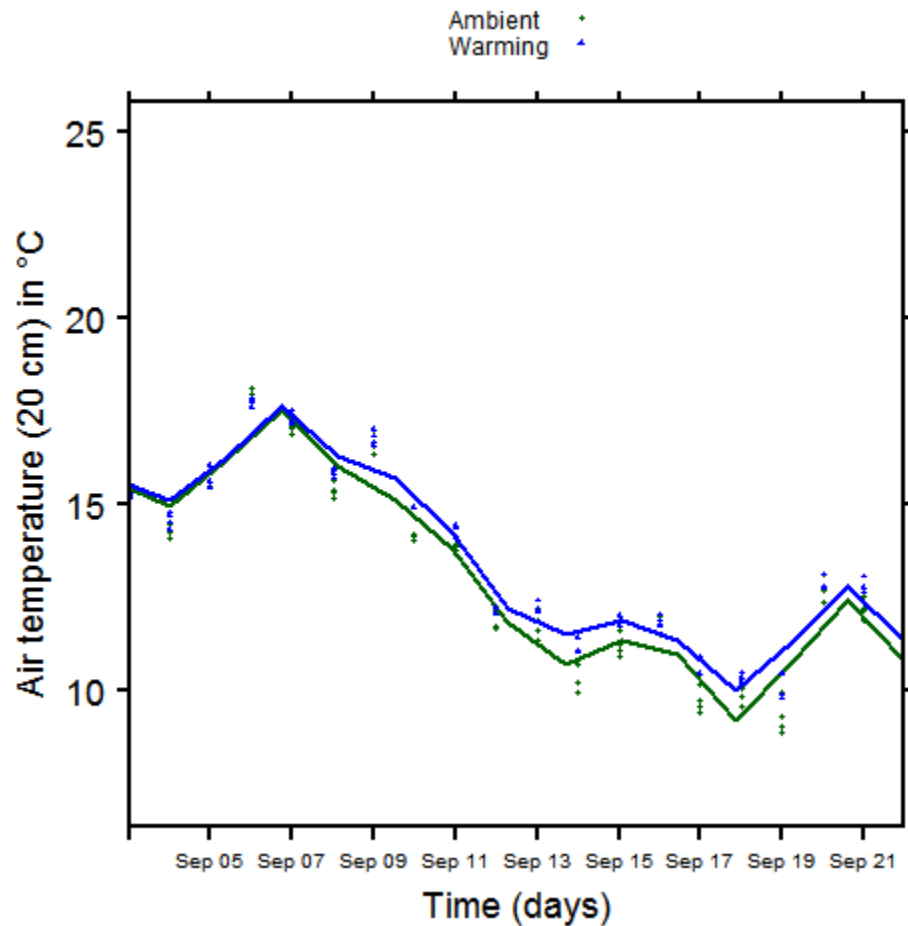
**Figure S3 | Global change and herbivory effects on protozoan abundance.** Abundance of protozoans as a function of herbivory (Herbivory: green triangles; No herbivory: Blue circles). drought treatment and CO<sub>2</sub> exposure (Ambient: left; elevated: right). Green and blue lines show averages for cages with herbivory and no herbivory respectively. Effects of grasshopper herbivory depended on CO<sub>2</sub> level and drought treatment (P=0.005, Table S2). Lines show least-squares fits (for illustrative purposes only).



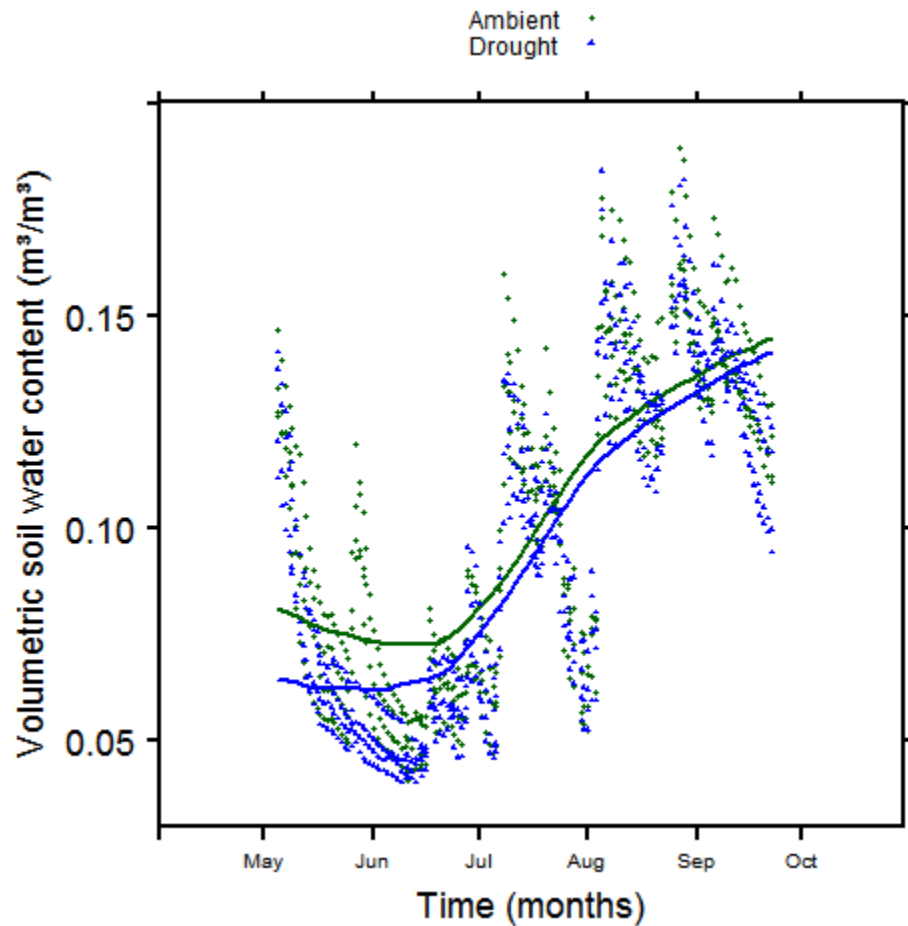
**Figure S4 | Treatment effects on root biomass.** The Figure shows that root biomass was slightly higher in ambient than in global change plots. However, none of these effects were statistically significant: P values for the effects of temperature, drought, and elevated CO<sub>2</sub> were 0.26, 0.66, and 0.59, respectively.



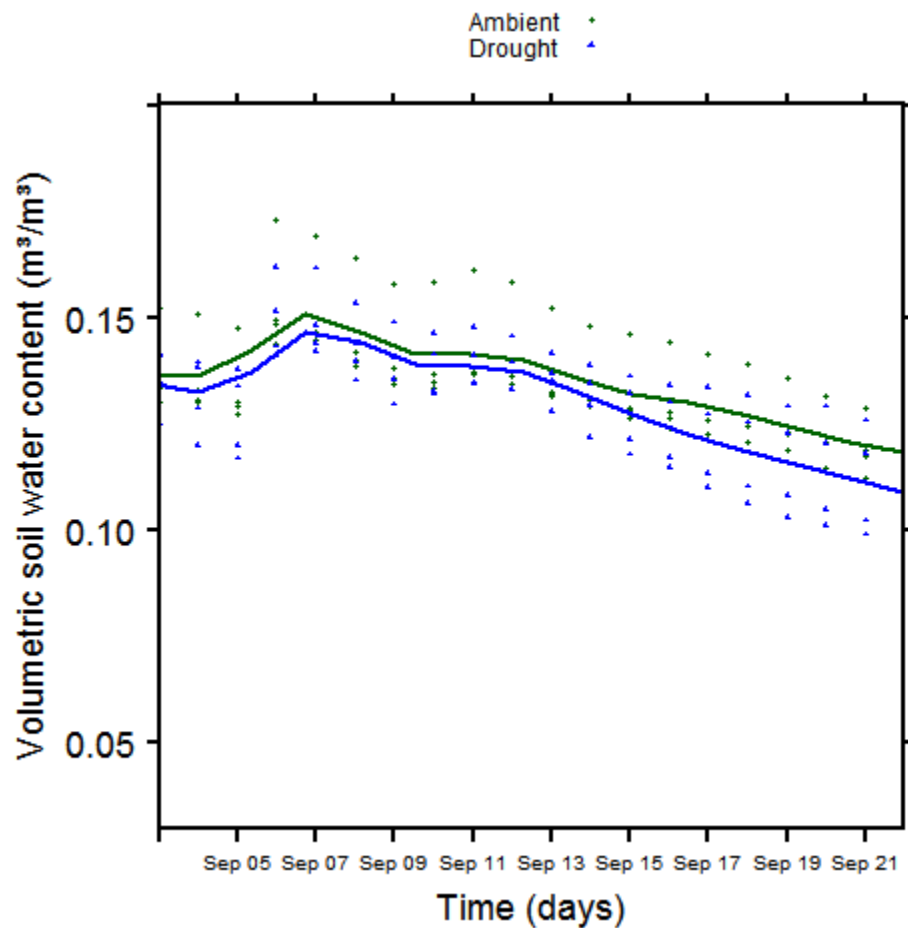
**Figure S5 | Daily air temperature in warmed vs. ambient plots (May-October 2008).** Air temperature was measured continuously at a height of 20 cm above the soil surface. Lines (for illustrative purposes) produced using a local non-parametric smoothing function.



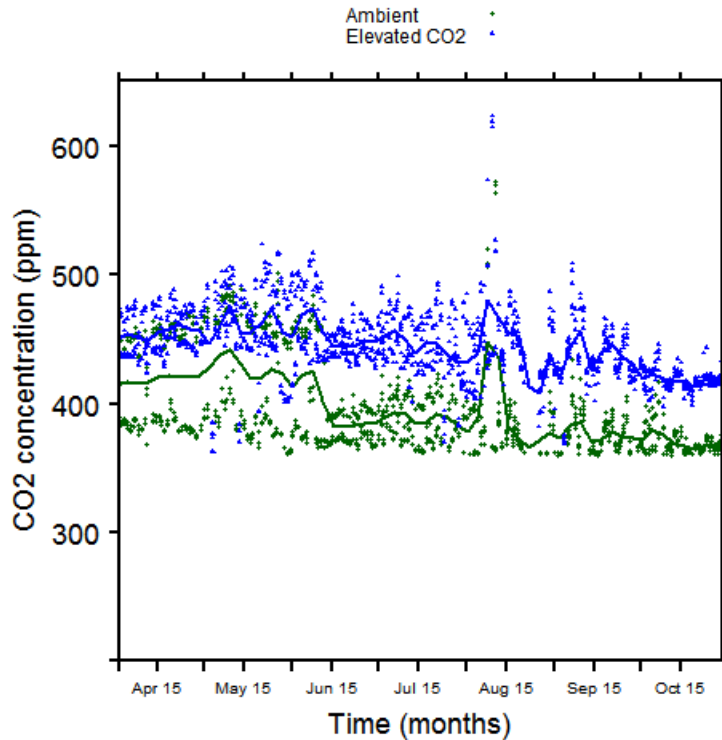
**Figure S6 | Daily air temperature during the period of grasshopper feeding, as recorded in warmed vs. ambient plots.** Air temperature was measured continuously at a height of 20 cm above the soil surface. Lines (for illustrative purposes) produced using a cubic spline function (3<sup>rd</sup> order polynomial).



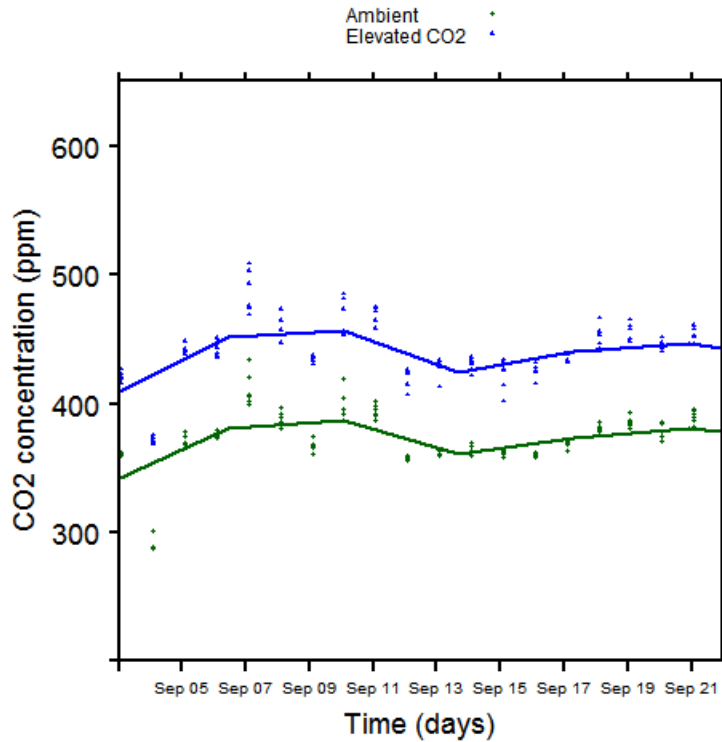
**Figure S7 | Daily soil water content in drought-treated vs. ambient plots (May-October 2008).** Volumetric soil water content was measured continuously using time domain reflectometry sensors. Lines (for illustrative purposes) produced using a local smoothing function.



**Figure S8 | Daily soil water content during the period of grasshopper feeding, as recorded in drought-treated vs. ambient plots.** Volumetric soil water content was measured continuously using time domain reflectometry sensors. Lines (for illustrative purposes) produced using a cubic spline function (3rd order polynomial).



**Figure S9 | Daily CO<sub>2</sub> concentrations in ambient vs. elevated-CO<sub>2</sub> plots (April-October 2008).** CO<sub>2</sub> concentration (ppm) was measured continuously using LI-820 CO<sub>2</sub> sensors LI-COR Inc., Lincoln, NE, USA). Lines (for illustrative purposes) produced using a local smoothing function.



**Figure S10 | Daily CO<sub>2</sub> concentrations in ambient vs. elevated-CO<sub>2</sub> plots during the period of grasshopper feeding.** CO<sub>2</sub> concentration (ppm) was measured continuously using LI-820 CO<sub>2</sub> sensors LI-COR Inc., Lincoln, NE, USA). Lines (for illustrative purposes) produced using a local smoothing function.