SUPPLEMENTARY INFORMATION APPENDIX

Hawkes et al. Historical climate controls soil respiration responses to current soil moisture

Supplementary Methods

Soil Biogeochemistry

Total organic soil C was determined on air-dried soils acidified with H_2SO_3 to remove inorganic carbonates and on litter samples run on an Apollo 9000 TOC analyzer with boat sampler (Teledyne Tekmar, Mason, OH). Inorganic N was extracted from soils using 2M KCl (1) and quantified colorimetrically (2, 3) on a DTX 880 microplate spectrophotometer (Beckman Coulter, Brea, CA, USA). Soil microbial biomass C was measured via chloroform fumigation and direct extraction with 0.5 M K_2SO_4 (4, 5) and quantification by combustion on an Apollo 9000 TOC analyzer with liquid autosampler (Teledyne Tekmar, Mason, OH). Moisture content was determined gravimetrically by drying soil subsamples to constant weight at 105 °C. Soil pH was determined in a 1:1 slurry of soil and distilled water. Soil percent clay was quantified with the hydrometer method (6).

Soil Microbial Community Characterization

For analysis of microbial community composition, DNA was extracted from frozen soils in duplicate 0.25 g subsamples using MoBio PowerSoil extraction kits (MoBio Laboratories, Carlsbad, CA). DNA extracts were amplified with the universal SSU ribosomal primers 515F (7) and 907R (8) targeting the V4 hypervariable region, using Invitrogen Platinum PCR Supermix chemistry (Invitrogen, Carlsbad, CA, USA). The reverse primer was barcoded with a 12-base site-specific barcode sequence. Amplicons were cleaned using the MoBio UltraClean PCR Clean-Up kit (MoBio Laboratories, Carlsbad, CA) and quantified on a Bioanalyzer (Agilent Technologies, Loveland, CO, USA) before pooling equimolarly. Pooled libraries were purified of fragments < 150 bp in size using Agencourt AmPure XP magnetic beads (Beckman Coulter, Brea, CA). Samples were sequenced on a 454 GS FLX sequencer with titanium chemistry (Roche, Branford, CT) at the University of Texas Genome Sequencing and Analysis Facility.

We obtained 227,243 raw sequences, which were analyzed using QIIME v. 1.5.0 (9) and Mothur v.1.26.0 (10). Quality filtering was used to remove sequences with quality scores below 25, fewer than 150 bases, homopolymer runs of more than 10 bases, any ambiguous bases, or an anomalous barcode (any barcode that differed from the specified length by 1 bp or contained a mismatch). All singletons (unique to the entire dataset) were removed, and putative chimeras were removed with USEARCH 6.1 (11). Operational taxonomic units (OTUs) were defined at 97% sequence similarity using the UCLUST algorithm (11). Representative sequences from each OTU were searched against the SILVA v1.1.1 database (12) to identify and eliminate any non-bacterial taxa. To avoid bias in sampling effort, we rarefied to the sample with lowest sequencing depth after filtering (2490). All raw sequence data were deposited into the National Center for Biotechnology Information (NCBI) Sequence Read Archive and are available under the project accession number PRJNA379880.

Lab Microcosm Moisture Response Tests for Field Experiments

After the field common garden harvest, 15 g of air-dried soil (dry weight) from each of the 156 cores was added to individual glass microcosms (67 ml vials) equipped with PTFE-silicone septa caps. Three cores in each factorial treatment combination were randomly assigned to each soil moisture treatment. After initial adjustment of soil water content, vials were sealed with Parafilm and allowed to equilibrate for 1 week at 24 °C. Thereafter, respiration rates were measured once every 2 weeks for 8 weeks. Soil moisture was adjusted weekly by weight to maintain treatments within 0.01%. After 8 weeks, microcosms were destructively harvested for the determination of microbial biomass C as described above.

Soils (15 g) from the field rainfall manipulation experiment were added to microcosms (67 ml vials as above) and maintained at 5 or 25% moisture for 8 weeks, with moisture adjusted by weight and soil respiration measured weekly. Vials were destructively harvested and sampled for microbial biomass C as described earlier. Note that we also sampled soils in the field plots from two plant communities in the rain treatments (tallgrass and shortgrass), but because there were no differences in respiration rates between them we collapse those data here for simplicity.

Microcosm Headspace Sampling

In all three lab incubations, microcosms were sealed for 2 to 24 hours (depending on volume) before 15 ml air samples were collected from the headspace of each microcosm through septum inserted in the lid. Headspace samples and standards for each time point were stored in 12-ml borosilicate vials with butyl rubber septa until CO_2 was quantified on a SRI 8610C gas chromatograph (SRI Instruments, Santa Monica, CA).

Supplementary Tables

Table S1. Gradient site information and experiments that used soils from these sites. Experiments are numbered as follows: (1) long-term reciprocal transplant lab microcosms, (2) field common gardens, and (3) field rainfall manipulation. Abbreviations are as follows: Lat = latitude, Lon = longitude, MAP = mean annual precipitation, T max = maximum annual temperature, T min = minimum annual temperature, Elev = site elevation, SOC = soil organic C, MBC = microbial biomass C when soils were collected in experiments 1, 2; Expt = experiment. Climate data are from the PRISM Climate Group using the 30-year record from 1981-2010 (Oregon State University, http://prism.oregonstate.edu). Sites are listed from high to low MAP.

Site	Site code	Lat.	Lon.	MAP (mm)	T max (°C)	T min (°C)	Elev (m)	pH	SOC (%)	Clay (%)	MBC (mg g^{-1})	Expt.
Ladybird Johnson Wildflower Center	WFC	30.18	-97.88	886.8	26.3	13.6	244	7.9	5.5	26.7	1.255, 0.883	1, 2, 3
SCO Ecolab	SCO	30.17	-98.36	854.6	25.5	12.3	421	7.7	10.9	18.5	0.294	2
ING Ecolab	ING	30.33	-98.45	852.3	25.6	12.4	385	7.4	2.4	15.3	0.476	2
Hill Country State Natural Area	HCS	29.63	-99.19	832.8	26.0	11.9	463	8.5	2.4	30.8	0.487	1
COL Ecolab	COL	30.33	-98.44	814.2	25.8	12.5	382	8.1	3.2	20.6	0.902	2
Old Tunnel State Park	OTS	30.10	-98.82	812.3	24.8	11.1	521	8.7	5.1	40.0	1.236	1
Lost Maples State Natural Area	LMS	29.83	-99.59	796.5	25.0	11.4	641	7.9	4.8	40.4	0.957	1
Heart of the Hills Research Station	HOH	30.18	-99.35	762.2	24.8	10.6	604	7.6	4.7	41.1	0.774	1
SPE Ecolab	SPE	30.53	-98.72	750.7	25.8	12.1	367	7.8	3.6	39.4	2.048	1
Kerr Wildlife Management Area	KER	30.09	-99.49	726.1	24.6	10.6	639	8.2	5.6	24.5	1.074	1
MOR Ecolab	MOR	29.74	-100.10	631.6	26.7	13.3	433	8.2	4.1	34.9	0.573	2
Walter Buck Wildlife Management Area	WBW	30.43	-99.80	606.0	25.5	11.1	620	8.0	4.7	38.0	0.737	1
SIE Ecolab	SIE	29.49	-100.27	605.7	26.9	13.6	442	8.0	4.8	39.7	0.945	2
Kickapoo Caverns State Park	KCS	29.62	-100.44	604.7	26.3	12.9	555	8.2	4.2	47.6	2.086	1
Devils River State Natural Area	DRS	29.94	-100.92	536.0	26.7	12.4	557	8.2	2.4	42.2	1.274, 1.166	1, 2
Seminole Canyon State Park	SCS	29.69	-101.32	442.3	27.5	13.9	427	8.1	5.7	31.9	0.154, 0.468	1, 2
Fort Lancaster State Historic Site	FLS	30.67	-101.70	407.4	26.7	11.5	636	8.3	2.2	27.9	0.776	1

Table S2. Repeated measures ANOVA for soil respiration in the long-term lab reciprocal moisture experiment as a function of home precipitation region (Region), site nested within region (Site(Region)), soil moisture treatment (Moisture), litter addition (Litter), and sample date (Date).

	df	MS	*F*	*P*
Between subjects				
Region	2	0.1554	3.641	0.069
Moisture	3	0.3602	51.861	**<0.001**
Litter	1	0.5524	86.090	**<0.001**
Site(Region)	9	0.0427	26.748	**<0.001**
Region*Moisture	6	0.0272	3.911	**0.006**
Region*Litter	2	0.0193	3.012	0.100
Moisture*Litter	3	0.0753	19.236	**<0.001**
Moisture*Site(Region)	27	0.0069	4.353	**<0.001**
Litter*Site(Region)	9	0.0064	4.021	**0.001**
Region*Moisture*Litter	6	0.0058	1.493	0.229
Moisture*Litter*Site(Region)	21	0.0039	2.452	**0.003**
Error	69	0.0016		
Within subjects				
Date	11	0.1788	111.122	**<0.001**
Date*Region	22	0.0045	2.848	**0.026**
Date*Moisture	33	0.0304	29.030	**<0.001**
Date*Litter	11	0.0317	56.028	**<0.001**
Date*Site(Region)	99	0.0016	6.652	**<0.001**
Date*Region*Moisture	66	0.0019	1.825	**0.032**
Date*Region*Litter	22	0.0009	1.668	0.165
Date*Moisture*Litter	33	0.0052	8.578	**<0.001**
Date*Moisture*Site(Region)	297	0.0011	4.340	**<0.001**
Date*Litter*Site(Region)	99	0.0005	2.337	**<0.001**
Date*Region*Moisture*Litter	66	0.0005	2.376	**0.001**
Date*Moisture*Litter*Site(Region)	231	0.0006	2.505	**<0.001**
Error(Date)	759	0.0002		

Table S3. Results of repeated-measures ANOVA for soil respiration from the common garden lab incubation experiment as a function of region of soil origin (Region), common garden location (Garden), soil moisture treatment (Moisture), site nested in region (Site(Region)), date of sampling (Date), and their interactions. Microbial biomass, initial and final, were included as covariates. Factors significant at $P < 0.05$ are in bold.

	df	MS	*F*	*P*
Between subjects				
Microbial Biomass Initial	1	0.0008	0.286	0.594
Microbial Biomass Final	1	0.0010	0.341	0.560
Region	1	0.0639	35.415	**0.004**
Garden	1	0.0147	4.463	0.102
Moisture	1	0.0401	6.155	0.068
Region*Garden	1	0.0005	0.166	0.704
Region*Moisture	1	0.0003	0.042	0.849
Garden*Moisture	1	0.0025	3.425	0.138
Region*Garden*Moisture	1	0.0048	6.658	0.061
Site(Region)	4	0.0018	0.607	0.659
Garden*Site(Region)	4	0.0033	1.109	0.357
Moisture*Site(Region)	4	0.0065	2.190	0.076
Garden*Moisture*Site(Region)	4	0.0007	0.243	0.913
Error	95	0.0030		
Within subjects				
Date	3	0.0034	2.118	0.151
Date*Microbial Biomass Initial	3	0.0043	1.534	0.206
Date*Microbial Biomass Final	3	0.0024	0.872	0.456
Date*Region	3	0.0007	0.409	0.749
Date*Garden	3	0.0041	2.033	0.163
Date*Moisture	3	0.0031	1.181	0.358
Date*Region*Garden	3	0.0033	1.629	0.235
Date*Region*Moisture	3	0.0010	0.368	0.777
Date*Garden*Moisture	3	0.0064	1.101	0.387
Date*Region*Garden*Moisture	3	0.0001	0.010	0.999
Date*Site(Region)	12	0.0016	0.577	0.860
Date*Garden*Site(Region)	12	0.0020	0.732	0.720
Date*Moisture*Site(Region)	12	0.0026	0.955	0.493
Date*Garden*Moisture*Site(Region)	12	0.0058	2.085	**0.018**
Error(Date)	285	0.0028		

Table S4. Results of repeated measures ANOVA for soil respiration (µmol CO_2 g^{-1} h^{-1}) over 8 weeks for soils from the field rainfall experiment. Independent variables were field rain treatment (FieldRain; 1331 or 326 mm yr^{-1}), field plant community (FieldPlant; tallgrasses or shortgrasses), lab soil moisture treatment (LabMoist; 5 or 25%), and their interactions. Microbial biomass at the conclusion of the incubation experiment was included as a covariate. All *P* values reflect Greenhouse-Geisser correction (epsilon = 0.31); factors significant at $P < 0.05$ are in bold.

	df	MS	*F*	*P*
Between subjects				
Microbial biomass	1	0.00144	1.136	0.298
FieldRain	1	0.00030	0.240	0.629
FieldPlant	1	0.00173	1.368	0.254
LabMoist	1	0.04051	32.042	**<0.001**
FieldRain*LabMoist	1	0.00061	0.482	0.494
FieldRain*FieldPlant	1	0.00005	0.037	0.849
FieldPlant*Moisture	1	0.00013	0.099	0.756
FieldRain*FieldPlant*LabMoist	1	0.00027	0.217	0.646
Error	23	0.00126		
Within subjects				
Date	7	0.00131	2.848	0.064
Date*Microbial biomass	7	0.00016	0.356	0.718
Date*FieldPlant	7	0.00036	0.79	0.468
Date*FieldRain	7	0.00070	1.534	0.225
Date*LabMoist	7	0.00364	7.943	**0.001**
Date*FieldPlant*FieldRain	7	0.00014	0.295	0.762
Date*FieldPlant*LabMoist	7	0.00011	0.239	0.805
Date*FieldRain*LabMoist	7	0.00088	1.915	0.155
Date*FieldPlant*FieldRain*LabMoist	7	0.00003	0.056	0.955
Error(Date)	161	0.00046		

Table S5. Results of PERMANOVA examining bacteria community composition as a function of region of soil origin (Region), common garden location (Garden), soil moisture treatment (Moisture), site nested in region (Site(Region)), and their interactions. Factors significant at $P < 0.05$ are in bold. Full data are reported in Waring (33).

	df	MS	*F*	R^2	*P*
Region	1	1.526	1.447	0.031	0.225
Garden	1	0.454	1.427	0.009	0.922
Region*Garden	1	0.323	1.016	0.007	0.455
Site(Region)	6	1.055	3.476	0.130	**0.001**
Garden*Site(Region)	4	0.318	10.495	0.026	0.054
Error	128	0.303			

Table S6. ANOVA results for initial microbial biomass from soils collected for the lab incubation experiment analyzed as a function of region of origin and site nested in region. Factors significant at $P < 0.05$ are highlighted in bold.

	df	MS	*F*	*P*
Region	2	0.061	0.078	0.925
Site(Region)	9	0.780	16.596	**<0.001**
Error	12	0.047		

Table S7. ANOVA results for initial microbial biomass from the common garden harvest, measured prior to the lab incubation, analyzed as a function of region of origin, garden location, site nested in region, and their interactions. Factors significant at $P < 0.05$ are highlighted in bold.

	df	MS	*F*	*P*
Region	1	2.088	1.093	0.355
Garden	1	1.848	9.681	**0.036**
Region*Garden	1	0.004	0.020	0.893
Site(Region)	4	1.911	12.665	**<0.001**
Garden*Site(Region)	4	0.191	1.266	0.287
Error	117	0.151		

Table S8. ANOVA results for final microbial biomass in the common garden lab incubation experiment. Independent variables were region of soil origin (Region), common garden location (Garden), soil moisture treatment (Moisture), site nested in region (Site(Region)), date of sampling (Date), and their interactions. Error mean squares and degrees of freedom are reported under each heading. Factors significant at $P < 0.05$ are highlighted in bold.

	df	MS	*F*	*P*
Region	1	0.34	0.077	0.796
Garden	1	34.988	63.847	**0.001**
Moisture	1	11.651	5.599	0.077
Region*Garden	1	0.135	0.246	0.646
Region*Moisture	1	0.296	0.142	0.725
Garden*Moisture	1	0.393	0.227	0.659
Region*Garden*Moisture	1	1.066	0.615	0.477
Site(Region)	4	4.444	8.663	**<0.001**
Garden*Site(Region)	4	0.548	1.068	0.376
Moisture*Site(Region)	4	2.081	4.057	**0.004**
Garden*Moisture*Site(Region)	4	1.734	3.380	**0.012**
Error	114	0.513		

Table S9. Results of three-way ANOVA for soil microbial biomass from the field rainfall experiment. Independent variables were: field rain treatment (FieldRain), field plant community (FieldPlant), lab moisture treatment (LabMoist), and their interactions. Error mean squares and degrees of freedom are reported under each heading. F-ratios significant at $P < 0.05$ are in bold.

	df	MS	*F*	*P*
FieldRain	1	973.990	0.175	0.680
FieldPlant	1	60421.547	10.845	**0.003**
LabMoist	1	44416.958	7.972	**0.009**
FieldRain*FieldPlant	1	281.025	0.050	0.824
FieldRain*LabMoist	1	4102.544	0.736	0.399
FieldPlant*LabMoist	1	17280.791	3.102	0.091
FieldRain*FieldPlant*LabMoist	1	2663.877	0.478	0.496
Error	24	5571.516		

Supplementary Figures

Figure S1. Map of sites across the Edwards Plateau (white outline) in Texas. Site codes refer to Table S1. Images are examples of sampling areas at the sites, with MAP indicated on each image (for full list of MAP, see Table S1). Photo credits: BGW except HCO, LMS = JDR; COL, MOR = J Fontenot; WFC = EW Connor. Map created using ggmap in R (34).

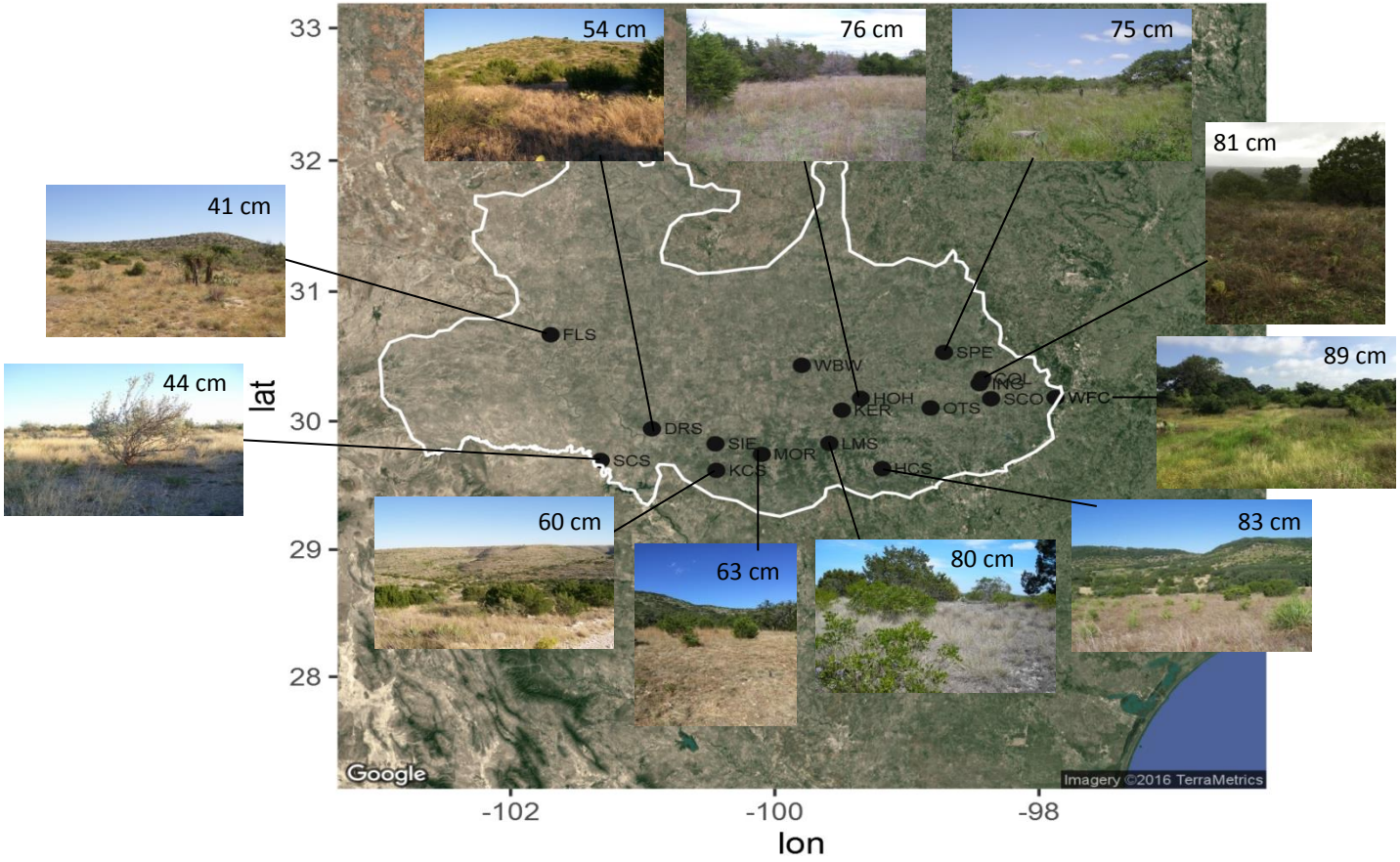

Figure S2. Soil respiration moisture response by site nested within region across the four moisture treatments. Litter elevated all respiration responses (note the difference in the y-axis scale for panels a-c and d-f), but the variation among sites was consistent. Site codes refer to Table S1.

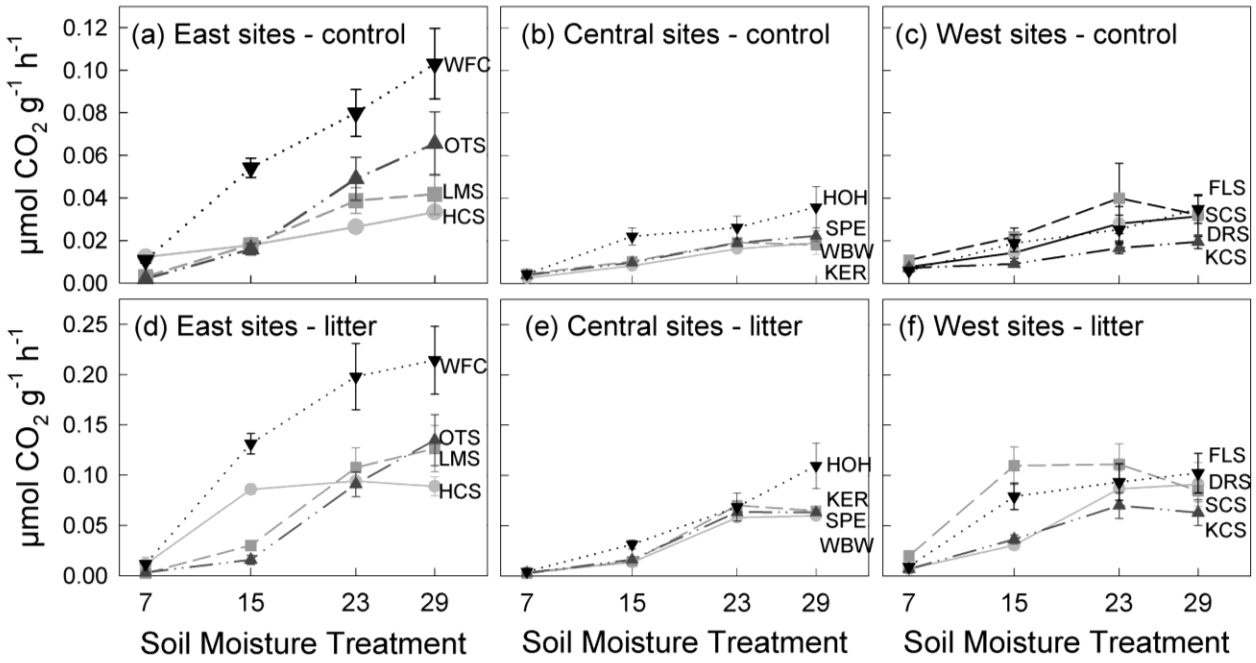

Figure S3. Soil respiration moisture response by region of origin for control microcosms (no litter added) across all 12 sample dates. Differences among regions were largely maintained over time, with eastern regions respiring more than others at 15, 23, and 29% soil moisture and western regions respiring more (or the same as) eastern regions at 7% soil moisture. However, the magnitude of differences among regions declined over time.

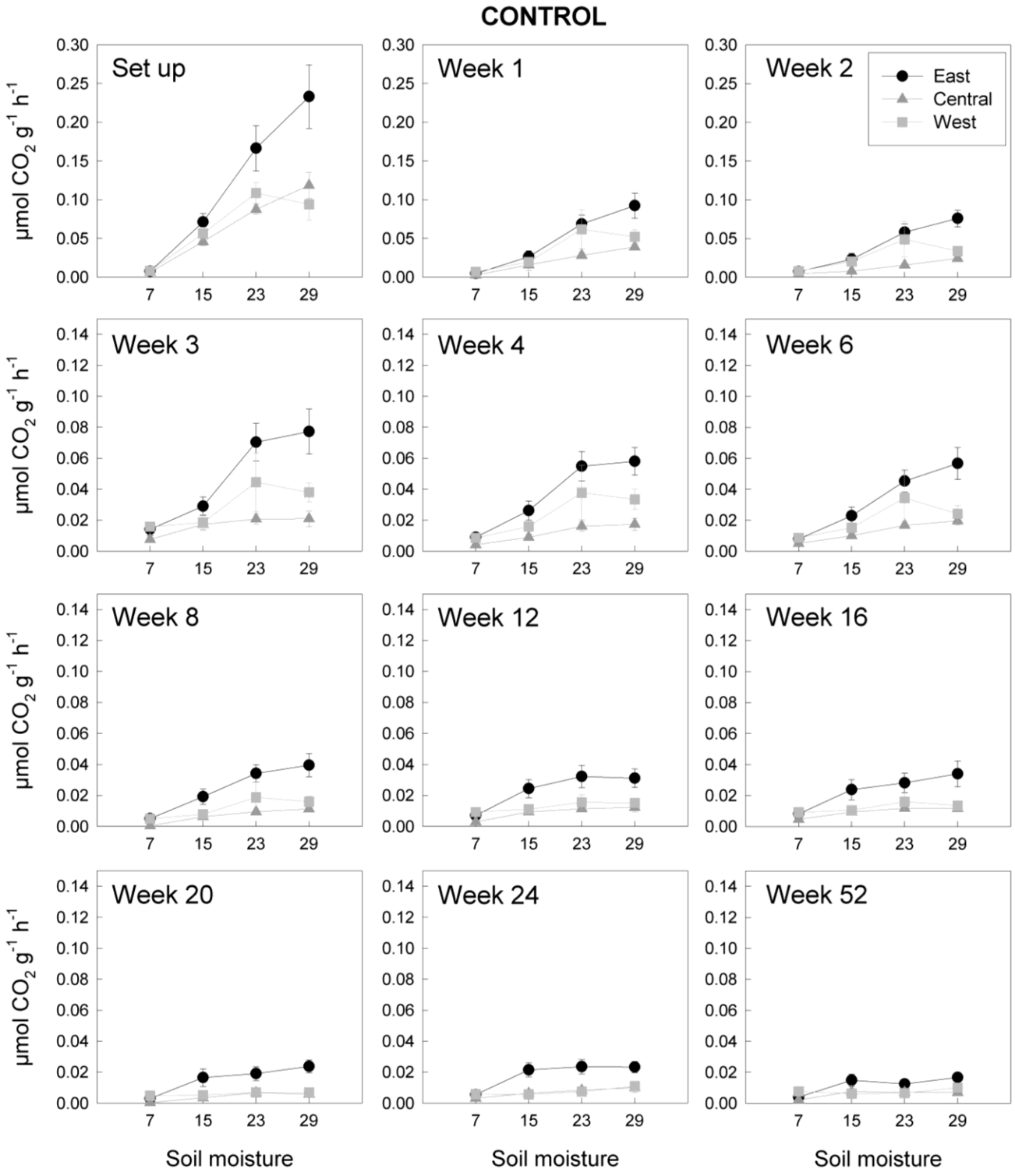

Figure S4. Soil respiration moisture response by region for litter addition microcosms across all 12 sample dates. Litter addition increased the overall respiration response when moisture was maintained at 15% or more, but did not affect respiration at 7% moisture and did not change the relative ranking of regions. The effect of litter declined over time.

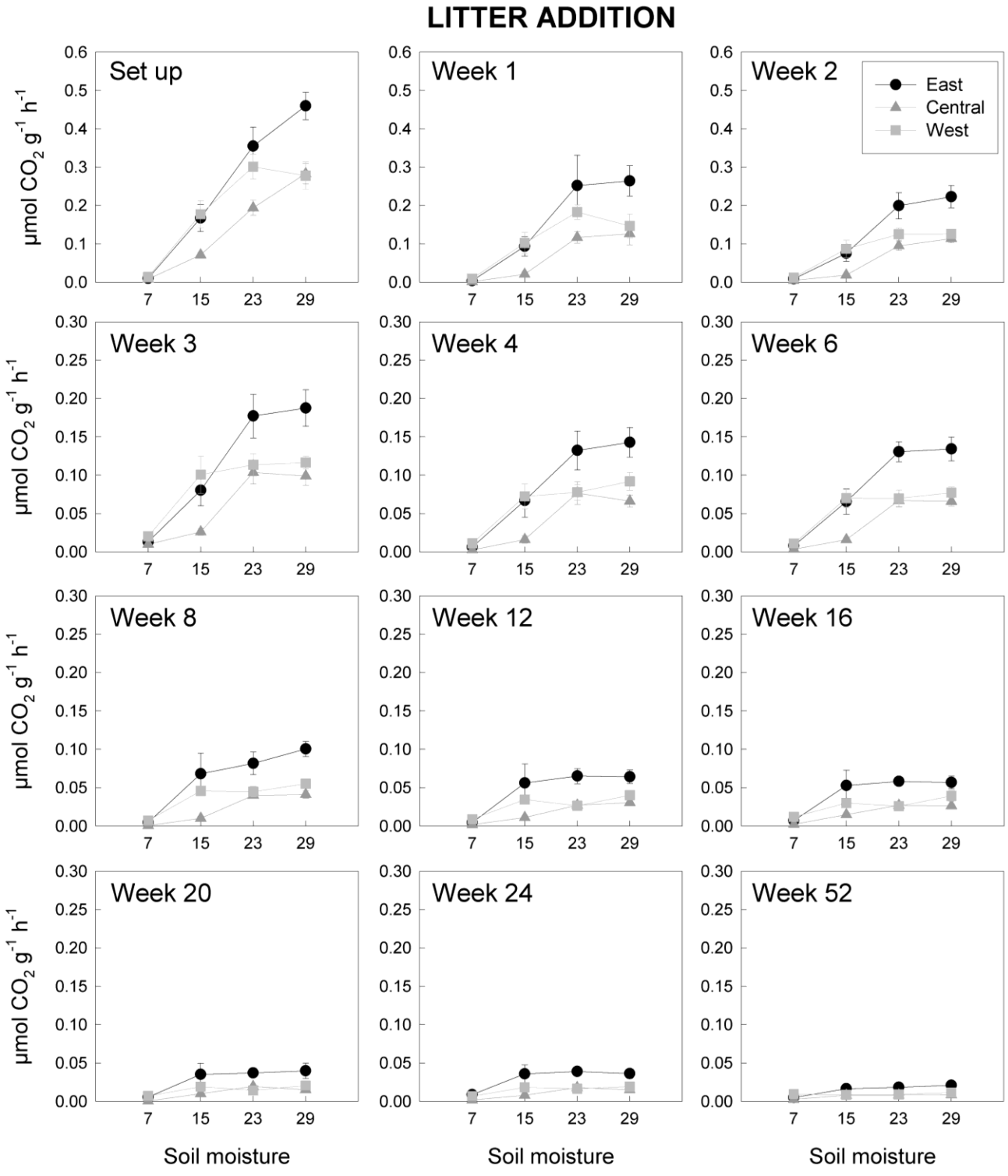

Figure S5. Field rainfall manipulation experiment at the WFC site. Photo credit: CVH.

Supplementary References

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