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Supporting Information for:

**Impacts of Drying and Rewetting on the Radiocarbon Signature of Respired
CO₂ and Implications for Incubating Archived Soils**

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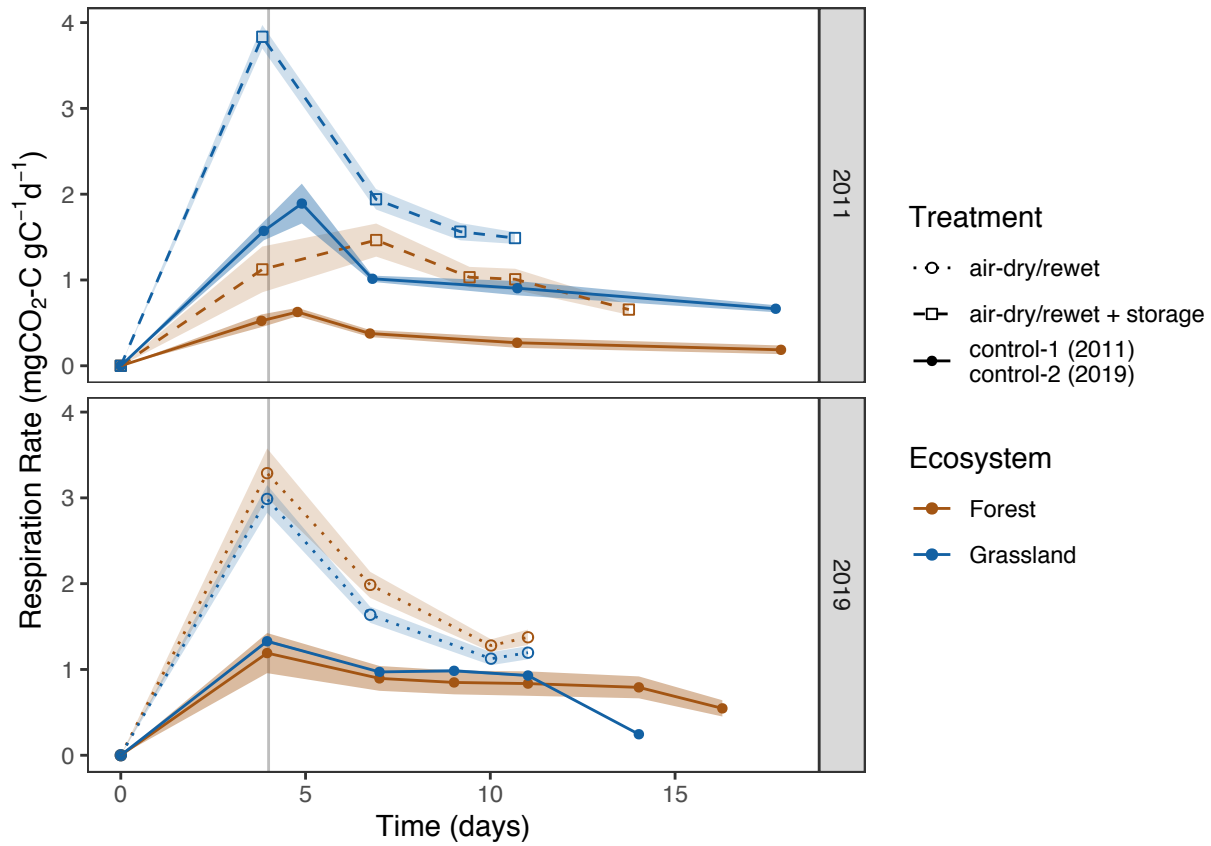
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Respiration rates

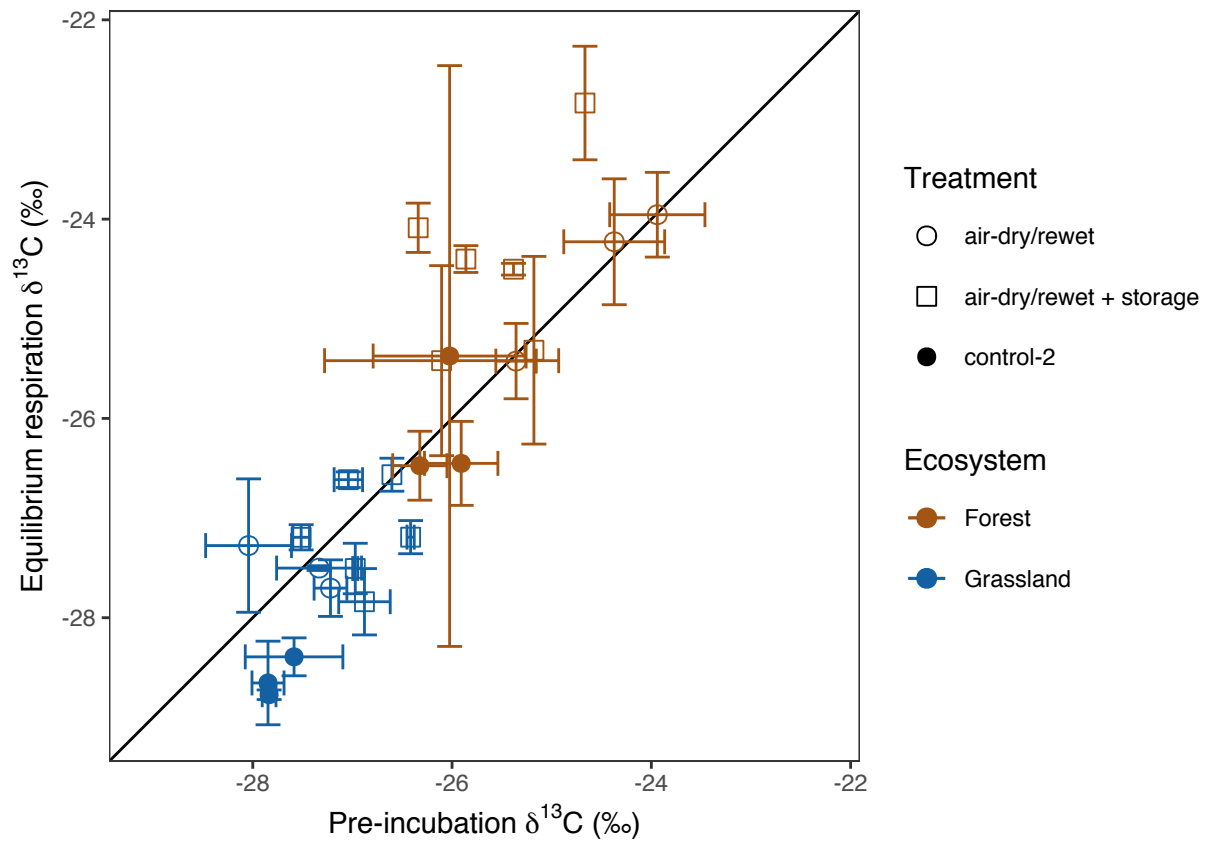
Figure S1



Supplemental Fig 1. Respiration rates for Experiment 1 and Experiment 2 (pre-incubation respiration rates shown as a cumulative average for all samples)

Caption: CO_2 concentrations for Experiment 1 control samples were only measured once during the pre-incubation period, in contrast to daily measurements for all other samples. Pre-incubation respiration rates are shown here calculated as cumulative averages for the whole pre-incubation period for ease of comparison across all treatments in both Experiment 1 and Experiment 2.

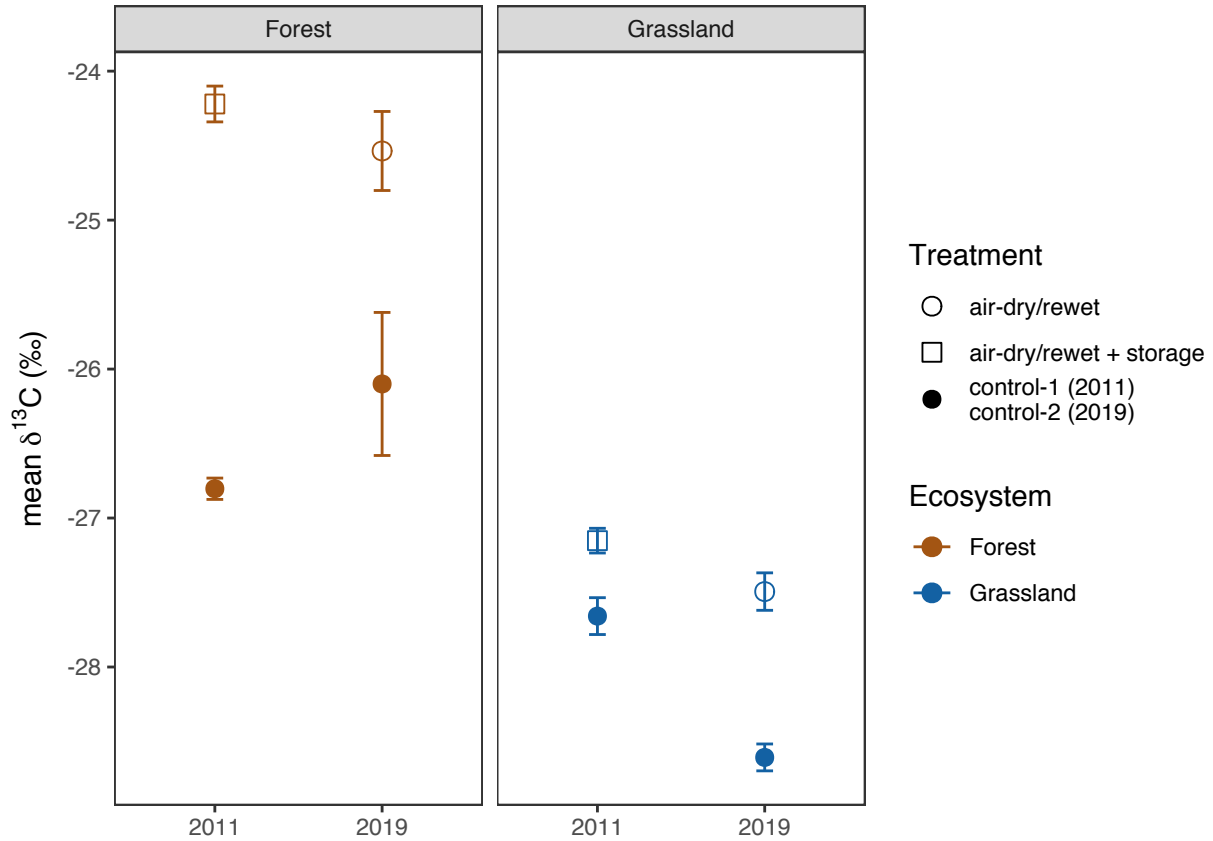
Treatment effects on $\delta^{13}\text{C}\text{-CO}_2$ (Figures S2, S3)



Supplemental Fig 2. Rewetting pulse (pre-incubation period) $\delta^{13}\text{C}\text{-CO}_2$ versus equilibrium respiration $\delta^{13}\text{C}\text{-CO}_2$

Caption: Points are means and error bars show the minimum and maximum of laboratory duplicates.

Figure S3



Supplemental Fig 3. Time series of control and treatment $\delta^{13}\text{C-CO}_2$ (Experiments 1 and 2)

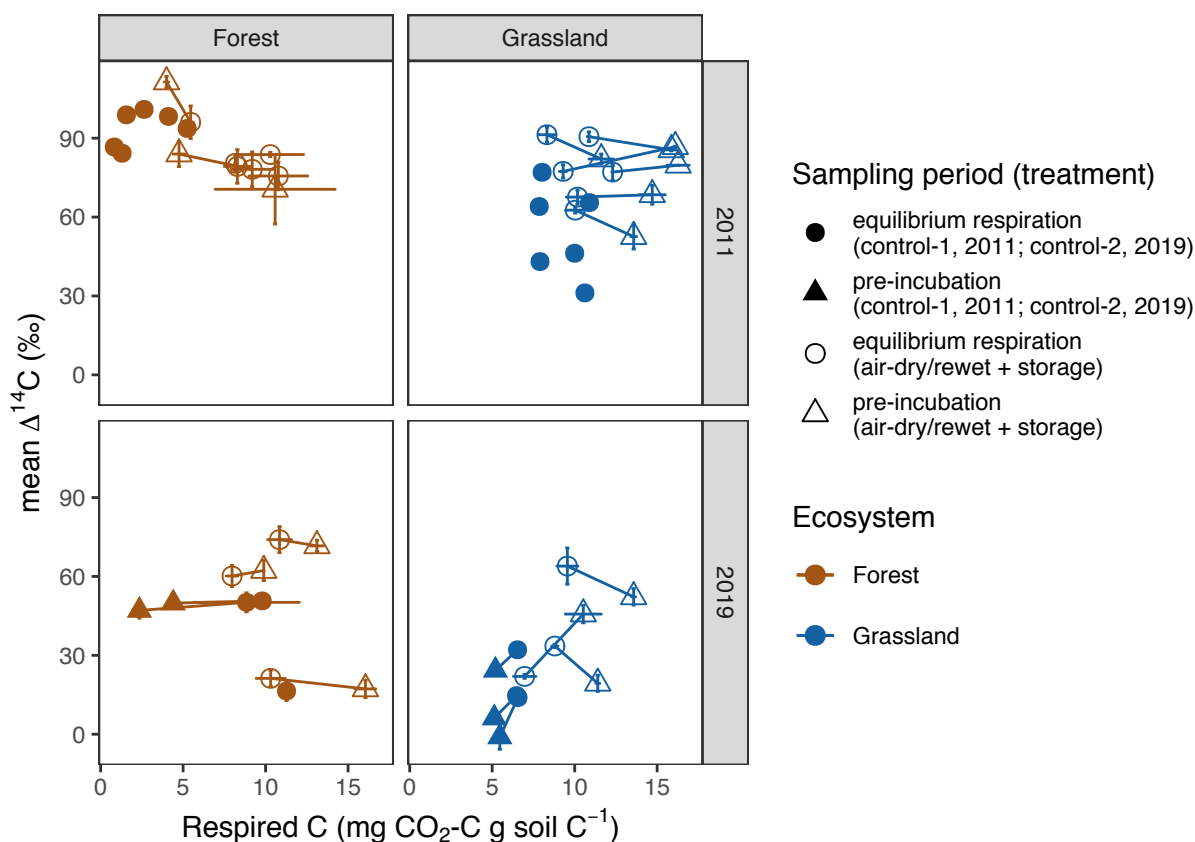
Caption: Points are means and error bars show 2x standard error.

Additional factors influencing treatment effects on $\Delta^{14}\text{C-CO}_2$

Text S1: Change in $\Delta^{14}\text{C-CO}_2$ relative to the difference in carbon respired

We looked at the possible effect of the difference in the amount of carbon respired ($\text{mg CO}_2\text{-C g soil C}^{-1}$) on the differences between control and treatment $\Delta^{14}\text{C-CO}_2$ using a linear regression model, but it was not significant overall. When data from Experiment 1 and Experiment 2 were considered separately, we observed a slight positive trend between the difference in respired carbon and the difference in $\Delta^{14}\text{C-CO}_2$ within Experiment 2, but it was only marginally significant ($p = 0.063$).

Figure S4



Supplementary Fig. 4. Change in $\Delta^{14}\text{C-CO}_2$ in relation to cumulative soil carbon respired

Caption: Error bars show minimum and maximum values measured for laboratory duplicates, while points show the mean. Lines connect mean pre-incubation and equilibrium respiration observations for a single sample. Lines parallel to the x-axis indicate a lack of trend in $\Delta^{14}\text{C-CO}_2$ with the amount of carbon respired, while differences between open and filled symbols show the impact of treatments on both the amount of carbon respired and $\Delta^{14}\text{C-CO}_2$. Note that pre-incubation $\Delta^{14}\text{C-CO}_2$ was not measured for the control-1 samples in 2011. Plot limits exclude outlier point (HEW22 control-2, pre-incubation) for improved legibility.

Text S2: Change in equilibrium respiration $\Delta^{14}\text{C-CO}_2$ as a function of field-moisture content

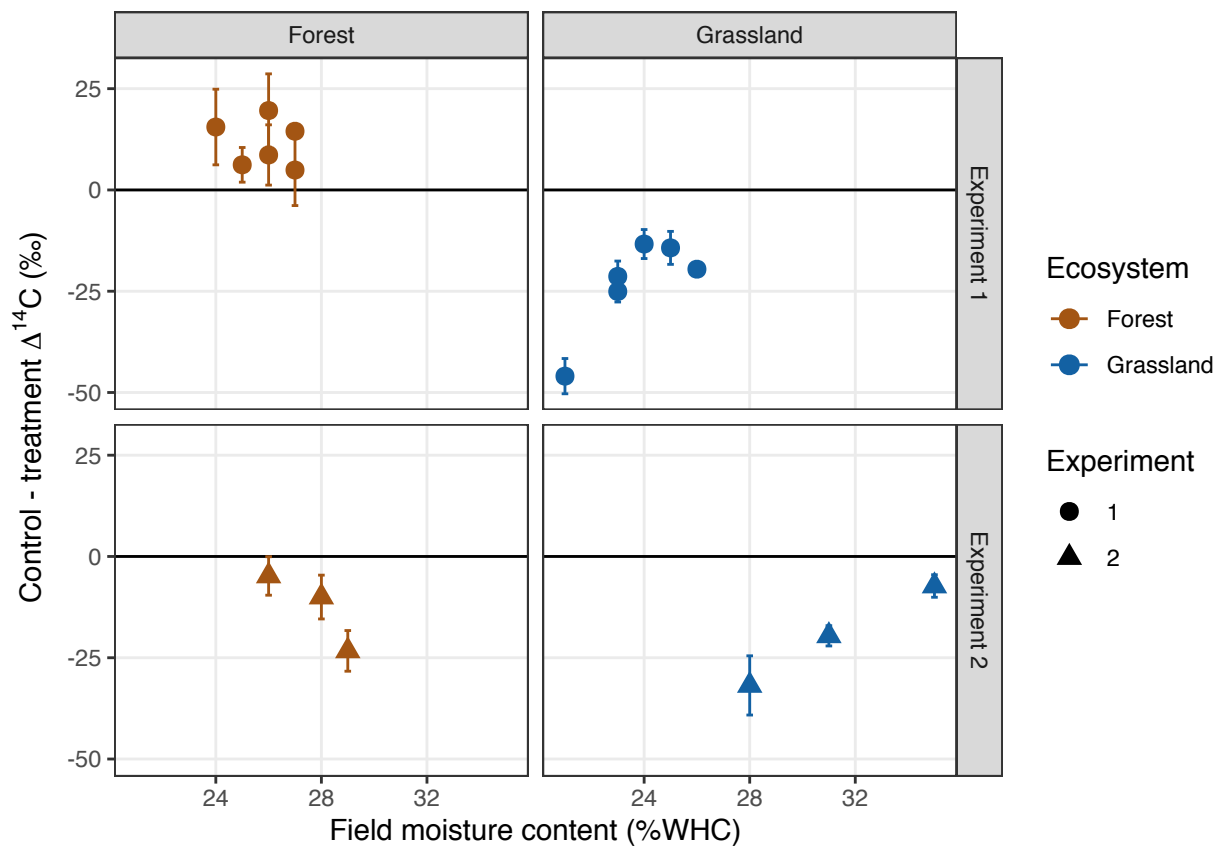
Differences in field moisture content of samples could be related to the magnitude of the shift in $\Delta^{14}\text{C-CO}_2$ observed between control and treatment sample, as control sample field moisture content varied. All treatment samples were air-dried in the laboratory prior to rewetting; a change in moisture content of zero percent water holding capacity (%WHC) to 60 %WHC. In contrast, moisture adjustment of control samples was made from field moisture, thus, for example, control samples with lower field moisture contents received a correspondingly greater water addition than wetter control samples.

In order to control for the variance in field moisture content of control samples, we looked at the relationship of the difference in equilibrium respiration period $\Delta^{14}\text{C-CO}_2$ observed between control and treatment samples and the change in moisture content of the control samples. If the shift in $\Delta^{14}\text{C-CO}_2$ observed in response to the air-drying and rewetting treatment were a linear function of the change in moisture content, the differences between control and treatment $\Delta^{14}\text{C-CO}_2$ should be smaller for samples with lower field moisture.

However, we did not observe any consistent relationship between the difference in $\Delta^{14}\text{C-CO}_2$ and field moisture (Supplemental Fig. 5).

We observed the strongest trend in the Experiment 2 grassland samples, but the trend was opposite to what we expected: differences in $\Delta^{14}\text{C-CO}_2$ between treatment samples and control samples were greater for drier samples than wetter samples (Supplemental Fig. 5). Experiment 2 forest samples showed the expected trend, but it did not appear to be linear (Supplemental Fig. 5). Given the relatively low sample number when considered within treatment and ecosystem groups (Experiment 1 $n = 6$, Experiment 2 $n = 3$), we do not consider these trends to be significant, but the data from Experiment 2 suggest that the relationship between the change in $\Delta^{14}\text{C-CO}_2$ and the magnitude of rewetting warrents further study.

Figure S5



Supplemental Fig. 5. Change in $\Delta^{14}\text{C-CO}_2$ (control - treatment) relative to the change in moisture content

Caption: Differences in $\Delta^{14}\text{C-CO}_2$ are shown as means; error bars show one standard deviation of the difference in means. All samples were moisture-adjusted to 60% of water holding capacity (WHC) prior to incubation, but control samples were adjusted from field moisture whereas treatment samples were moisture adjusted after air-drying. Data from Experiment 3 are not shown as field moisture content was unknown for the majority of samples (Supplemental Table 1).

Site data, soil properties, and supporting references for all samples (Experiments 1, 2, and 3)

Table S1

Supplementary Table 1. Site data and soil properties for all soils (Experiments 1, 2, and 3)

Experiment	Collection date	Control Incubation Laboratory*	Latitude	Longitude	Site Name	Land cover	ID	Incubation replicates	Soil order	Sieved	Depth		Field		Organic C	Total N	Particle size distribution			Reference**	
											Top	Bottom	Moisture	Incubation Moisture			Sand	Silt	Clay		
														percent water							holding capacity
year									WRB name	< 2mm	cm	gravimetric	gravimetric		g kg-1	g kg-1	g kg-1	g kg-1	g kg-1		
1	2011	MPI-BGC	53.09	13.63	Schorfheide-Chorin (Germany)	forest	SEW11	2	Cambisol	Yes	0	10	0.26	0.26	60	31.3	1.3	884	85	31	Solly et al. 2014
1	2011	MPI-BGC	52.9	13.85	Schorfheide-Chorin (Germany)	forest	SEW34	2	Albeluvisol	Yes	0	10	0.24	0.24	60	16.4	0.7	889	69	42	Solly et al. 2014
1	2011	MPI-BGC	52.9	13.93	Schorfheide-Chorin (Germany)	forest	SEW43	2	Cambisol	Yes	0	10	0.3	0.3	60	18.4	1.1	810	121	69	Solly et al. 2014
1	2011	MPI-BGC	53.12	13.68	Schorfheide-Chorin (Germany)	grassland	SEG38	2	Cambisol	Yes	0	10	0.25	0.27	60	22.8	2.2	838	72	89	Solly et al. 2014
1	2011	MPI-BGC	53.12	13.84	Schorfheide-Chorin (Germany)	grassland	SEG40	2	Luvisol	Yes	0	10	0.26	0.27	60	21.3	2	710	192	98	Solly et al. 2014
1	2011	MPI-BGC	52.98	13.83	Schorfheide-Chorin (Germany)	grassland	SEG46	2	Cambisol	Yes	0	10	0.31	0.34	60	24.3	2.3	644	210	146	Solly et al. 2014
1, 2	2011	MPI-BGC	51.34	10.36	Hainich-Dün (Germany)	forest	HEW22	2	Luvisol	Yes	0	10	0.38	0.37	60	23.3	1.7	68	747	184	Solly et al. 2014
1, 2	2011	MPI-BGC	51.11	10.45	Hainich-Dün (Germany)	forest	HEW41	2	Luvisol	Yes	0	10	0.4	0.42	60	23.4	1.9	34	754	210	Solly et al. 2014
1, 2	2011	MPI-BGC	51.1	10.46	Hainich-Dün (Germany)	forest	HEW42	2	Stagnosol	Yes	0	10	0.34	0.36	60	24.3	1.7	60	760	184	Solly et al. 2014
1, 2	2011	MPI-BGC	51.28	10.45	Hainich-Dün (Germany)	grassland	HEG10	2	Vertisol	Yes	0	10	0.47	0.61	60	43.7	4	30	532	436	Solly et al. 2014
1, 2	2011	MPI-BGC	51.08	10.57	Hainich-Dün (Germany)	grassland	HEG32	2	Cambisol	Yes	0	10	0.52	0.54	60	40	3.8	17	640	340	Solly et al. 2014
1, 2	2011	MPI-BGC	51.29	10.38	Hainich-Dün (Germany)	grassland	HEG48	2	Cambisol	Yes	0	10	0.55	0.56	60	41.6	4	50	488	465	Solly et al. 2014
1, 2	2019	MPI-BGC	51.34	10.36	Hainich-Dün (Germany)	forest	HEW22	2	Luvisol	Yes	0	10	0.38	0.37	60	23.3	1.7	68	747	184	Solly et al. 2014
1, 2	2019	MPI-BGC	51.11	10.45	Hainich-Dün (Germany)	forest	HEW41	2	Luvisol	Yes	0	10	0.4	0.42	60	23.4	1.9	34	754	210	Solly et al. 2014
1, 2	2019	MPI-BGC	51.1	10.46	Hainich-Dün (Germany)	forest	HEW42	2	Stagnosol	Yes	0	10	0.34	0.36	60	24.3	1.7	60	760	184	Solly et al. 2014
3	2009	UCI	37.03	-119.27	Musick (Sierra Nevada, CA)	forest	MA	3	Ultic Haploxeralf	Yes	5	20	0.07	0.33	50	27.4	1	600	270	150	Koarashi et al. 2012
3	2009	UCI	37.03	-119.27	Musick (Sierra Nevada, CA)	forest	MB	3	Ultic Haploxeralf	Yes	55	70	0.08	0.21	50	1.1	0	670	180	170	Koarashi et al. 2012
3	2009	UCI	37.03	-119.19	Shaver (Sierra Nevada, CA)	forest	SA	3	Pachic Xerumbrept	Yes	5	20	0.07	0.31	50	29.4	1.2	800	150	50	Koarashi et al. 2012
3	2009	UCI	37.03	-119.19	Shaver (Sierra Nevada, CA)	forest	SB	3	Pachic Xerumbrept	Yes	40	60	0.06	0.22	50	0.4	0	790	170	40	Koarashi et al. 2012
3	2008	UCI	35.98	-79.09	Duke, NC	forest	120	1	Ultic Alfisol	Yes	5	15	0.95	0.04		16.6	0.8				Hopkins et al. 2012
3	2004	UCI	35.94	-84.33	Tennessee Valley Authority (Oak Ridge, TN)	forest	TVA 6E C	1	Inceptisol	No	0	5	0.28	0.2		24.9	1.2				Cisneros-Dozal et al. 2005
3	2004	UCI	35.94	-84.33	Tennessee Valley Authority (Oak Ridge, TN)	forest	TVA 2B C	1	Inceptisol	No	0	5	0.3	0.2		24.9	1.2				Cisneros-Dozal et al. 2005
3	2004	UCI	35.94	-84.33	Tennessee Valley Authority (Oak Ridge, TN)	forest	TVA 3B C	1	Inceptisol	No	0	5	0.49	0.2		24.9	1.2				Cisneros-Dozal et al. 2005
3	2004	UCI	35.94	-84.33	Tennessee Valley Authority (Oak Ridge, TN)	forest	TVA 5B C	1	Inceptisol	No	0	5	0.26	0.2		24.9	1.2				Cisneros-Dozal et al. 2005
3	2004	UCI	35.97	-84.27	Walker Branch (Oak Ridge, TN)	forest	WB 4B C	1	Ultisol	No	0	5	0.34	0.2		24.9	1				Cisneros-Dozal et al. 2005
3	2004	UCI	35.97	-84.27	Walker Branch (Oak Ridge, TN)	forest	WB 5B C	1	Ultisol	No	0	5	0.25	0.2		24.9	1				Cisneros-Dozal et al. 2005
3	2004	UCI	35.97	-84.27	Walker Branch (Oak Ridge, TN)	forest	WB 8B C	1	Ultisol	No	0	5	0.34	0.2		24.9	1				Cisneros-Dozal et al. 2005
3	2004	UCI	35.97	-84.27	Walker Branch (Oak Ridge, TN)	forest	WB 3E C	1	Ultisol	No	0	5	0.36	0.2		24.9	1				Cisneros-Dozal et al. 2005
3	2004	UCI	35.97	-84.27	Walker Branch (Oak Ridge, TN)	forest	WB 7E C	1	Ultisol	No	0	5	0.18	0.2		24.9	1				Cisneros-Dozal et al. 2005
3	2011	MPI-BGC	51.34	10.51	Hainich-Dün (Germany)	forest	HEW26	2	Luvisol	Yes	0	10	0.34	0.36	60	24.4	1.6	54	796	150	Solly et al. 2014
3	2011	MPI-BGC	51.18	10.38	Hainich-Dün (Germany)	forest	HEW47	2	Stagnosol	Yes	0	10	0.43	0.45	60	32.5	2.4	46	632	323	Solly et al. 2014
3	2011	MPI-BGC	51.22	10.37	Hainich-Dün (Germany)	grassland	HEG20	3	Stagnosol	Yes	0	10	0.47	0.45	60	27.2	2.3	102	661	239	Solly et al. 2014
3	2011	MPI-BGC	51.11	10.43	Hainich-Dün (Germany)	grassland	HEG33	3	Cambisol	Yes	0	10	0.47	0.47	60	40.1	3.8	29	618	353	Solly et al. 2014
3	2011	MPI-BGC	51.21	10.39	Hainich-Dün (Germany)	grassland	HEG6	3	Stagnosol	Yes	0	10	0.41	0.45	60	20.8	2	45	698	257	Solly et al. 2014
3	1999	UCI	42.54	-72.18	Harvard, MA	forest	WN-1 Ap (ba)	1	Inceptisol	Yes	0	16			60						Gaudinski et al. 2001
3	1999	UCI	42.54	-72.18	Harvard, MA	forest	WN-1 Ap #2	1	Inceptisol	Yes	0	16			60						Gaudinski et al. 2001
3	1999	UCI	42.54	-72.18	Harvard, MA	forest	WN-2 Ap #3	1	Inceptisol	Yes	0	16			60						Gaudinski et al. 2001
3	1999	UCI	42.54	-72.18	Harvard, MA	forest	WN-1 Ap #4	1	Inceptisol	Yes	0	16			60						Gaudinski et al. 2001
3	2004	UCI	35.94	-84.33	Tennessee Valley Authority (Oak Ridge, TN)	forest	TVA 4E	1	Inceptisol	No	0	5			24.9	1.2					Cisneros-Dozal et al. 2005
3	2004	UCI	35.94	-84.33	Tennessee Valley Authority (Oak Ridge, TN)	forest	TVA 6E	1	Inceptisol	No	0	5			24.9	1.2					Cisneros-Dozal et al. 2005
3	2004	UCI	35.94	-84.33	Tennessee Valley Authority (Oak Ridge, TN)	forest	TVA 8E	1	Inceptisol	No	0	5			24.9	1.2					Cisneros-Dozal et al. 2005
3	2004	USGS Menlo Park	35.94	-84.33	Tennessee Valley Authority (Oak Ridge, TN)	forest	TVA2B-C_IT2	1	Inceptisol	No	0	5			24.9	1.2					Cisneros-Dozal et al. 2005
3	2004	USGS Menlo Park	35.94	-84.33	Tennessee Valley Authority (Oak Ridge, TN)	forest	TVA3-C_IT1	1	Inceptisol	No	0	5			24.9	1.2					Cisneros-Dozal et al. 2005
3	2004	USGS Menlo Park	35.97	-84.27	Walker Branch (Oak Ridge, TN)	forest	WB4B-C_IT2	1	Ultisol	No	0	5			24.9	1					Cisneros-Dozal et al. 2005
3	2004	USGS Menlo Park	35.97	-84.27	Walker Branch (Oak Ridge, TN)	forest	WB5-C_IT2	1	Ultisol	No	0	5			24.9	1					Cisneros-Dozal et al. 2005
3	2004	USGS Menlo Park	35.97	-84.27	Walker Branch (Oak Ridge, TN)	forest	WB8-C_IT2	1	Ultisol	No	0	5			24.9	1					Cisneros-Dozal et al. 2005

* Not all data were available (e.g. moisture content, texture) for control samples incubated at UCI and USGS Menlo Park

** See references section in main text for full citations