Supplementary Information

Increasing soil respiration in a northern hardwood forest indicates symptoms of a changing carbon cycle

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Supplementary Methods

Assessment of possible methodological bias in long-term measurements

To address concerns that the increase in soil respiration that we measured is not related to changes in experimental conditions, we present information on changes in measurement timing, changes in *in situ* chambers, and changes in instrumentation over the study period.

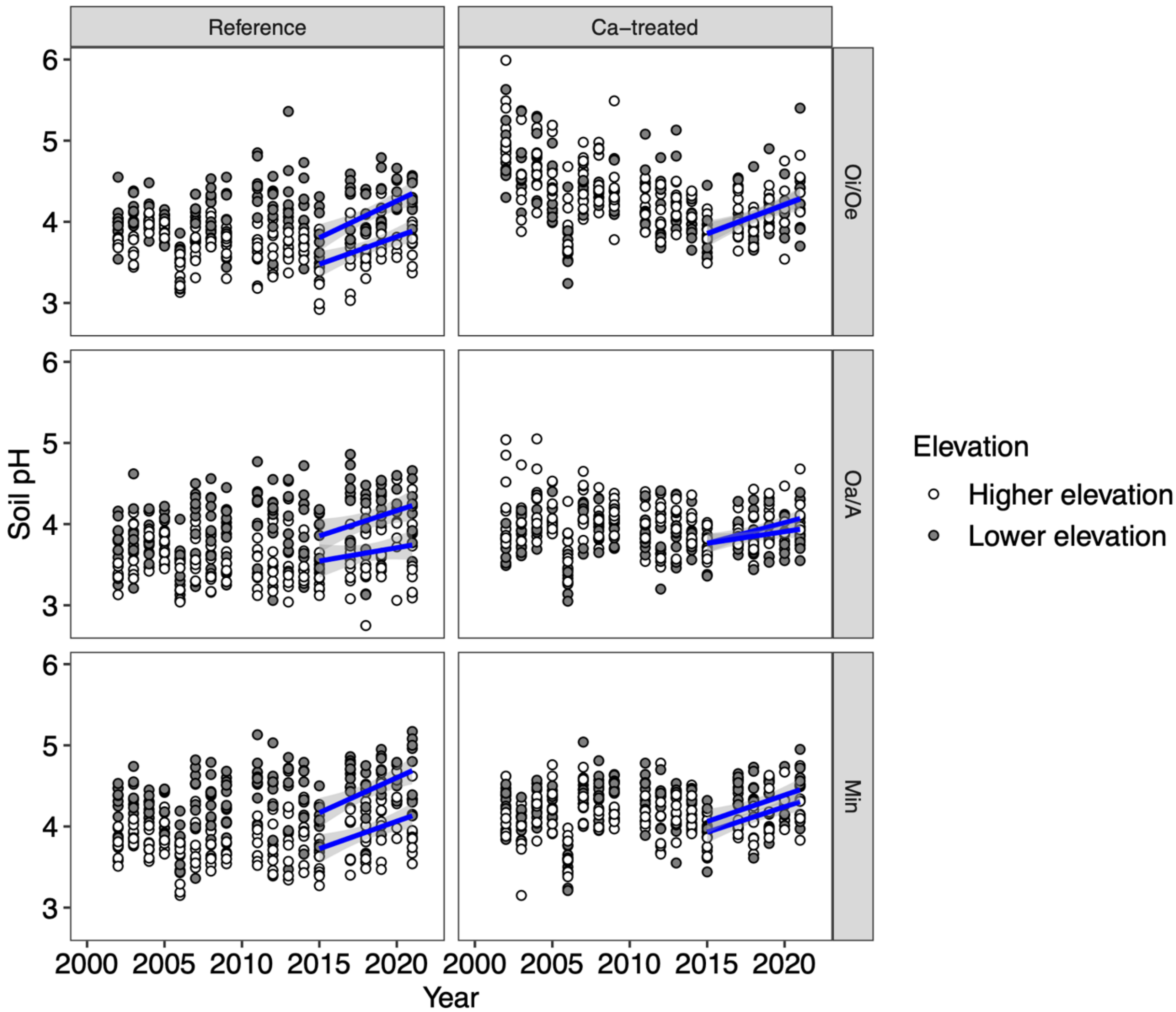
Changes in sampling months. *In-situ* trace gas measurements were collected from May-October throughout the time series. November measurements were added in 2014 (11-06-2014), but were only collected again once, in 2017. November measurements were not incorporated into summer cumulative measurements. In addition, we determined that November rates were not particularly influential on overall respiration trends because (1) there are only 2 timepoints (2014 and 2017) and (2) the soil respiration increase is mainly driven by July/August rates (manuscript **Fig. 3; Supplementary Fig. 10**).

Changes in sampling time of month or day. We checked if there was a systematic change in the timing of sampling within a month over the long-term record. There is no directional pattern in sampling that would affect this relationship (e.g., consistently earlier sampling in fall, consistently later sampling in spring) (**Supplementary Fig. 11**), and we are not aware of any systematic changes in the timing of measurements within a given day. In addition, potential biases driven by sampling time of day are minimized by our use of soil temperature measurements within a daily sampling time window (10 am – 4 pm), and assessment of trends in not only mean daily soil temperature but also minimum and maximum within this window (**Supplementary Fig. 6).**

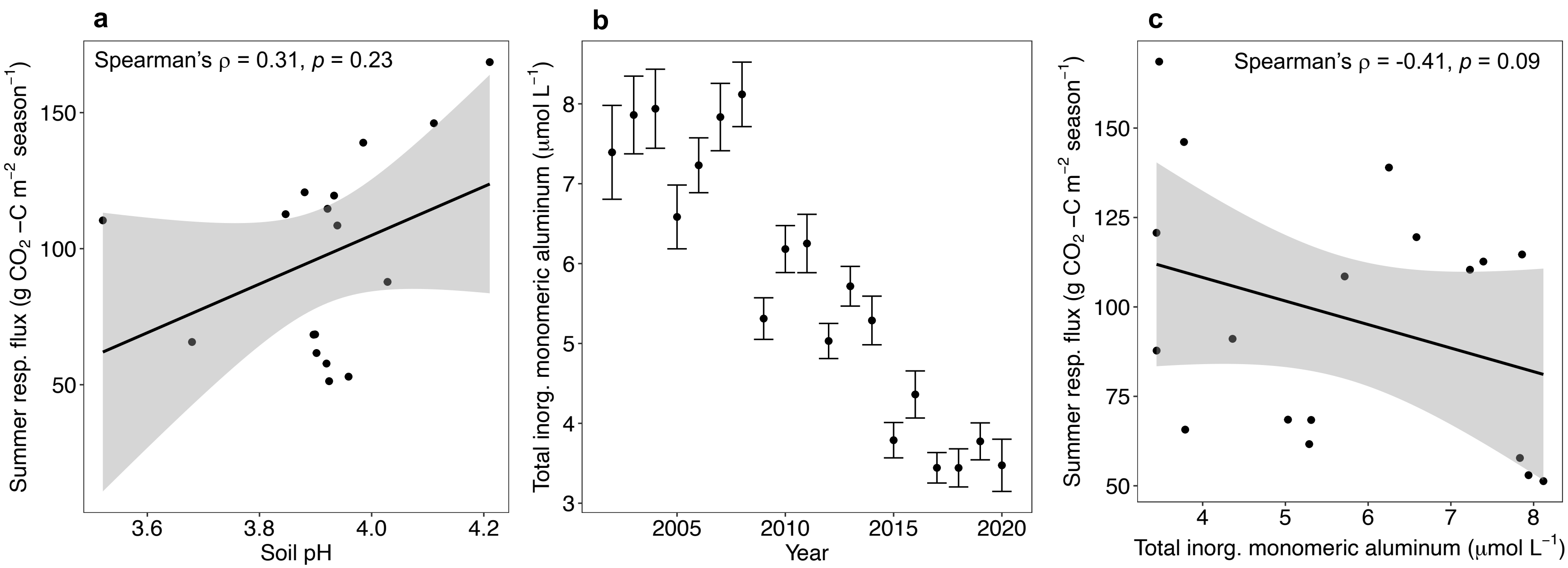
Changes in trace gas sampling and instrumentation. Potential effects of wear and tear on PVC collars were assessed by installing new collars and comparing with the collars that had been previously installed for a longer period. Ni and Groffman[1] published long-term data on methane fluxes for the same *in-situ* chambers used in this study, with comparison to identical chambers in forest sites in the Baltimore metropolitan area. This analysis included installation of two new chambers at two of the long-term sites in Baltimore and comparison with four old chambers at each of these sites over 7 sampling dates between June 2015 and February 2016. There was no difference in methane flux between new and old chambers at either site (see **Supplementary Material** in Ni and Groffman[1]). Carbon dioxide and nitrous oxide fluxes also did not differ between new and old chambers (unpublished data from Ni and Groffman[1]). A similar test was also conducted at the Multiple Element Limitation in Northern Hardwood Ecosystems (MELHNE) experiment by Mann et al.[2] Neither collar test showed an installation effect; i.e. the older collars matched the new ones.

All gas samples were analyzed by gas chromatography (GC) with thermal conductivity detection using a Shimadzu GC-14 (2002-2015) or Shimadzu GC-2014 (2015-2020) instrument (Shimadzu Corp., Kyoto, Japan). While the instrument shift does occur at a similar time to the average detected increase in soil respiration, we considered several factors related to instrument changes and conclude that the increase is not due to this change in GC instrument model: (1) sampling trip standards and certified external calibration standards were used to verify that instrument bias did not affect gas measurements over time; (2) both GC instruments use the same mechanism of detection and detector type; (3) a change in GC instrumentation would likely result in a step-change (e.g., a baseline shift) rather than a consistent increase over the 2015-2020 period; (4) our observations are consistent with Mann et al.[2], who reported an overall increase in ambient soil respiration at HBEF using an *in-situ* Li-COR 8100 Soil Respiration System (Licor Biosciences, Lincoln, NE) rather than gas sampling and GC analysis.

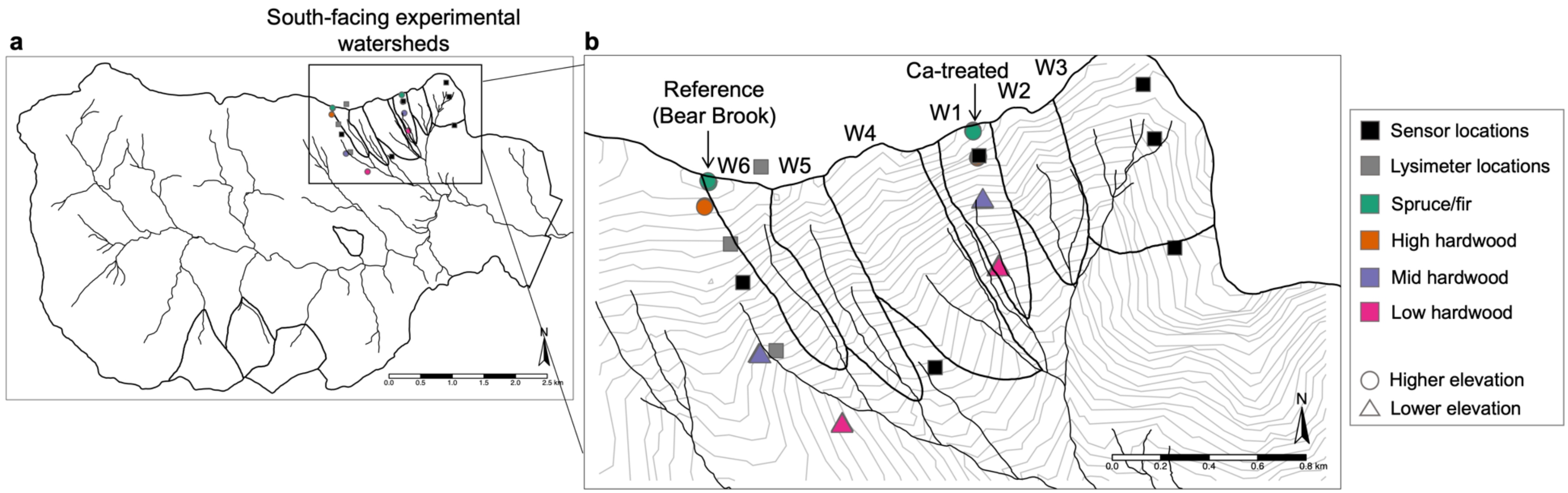
Supplementary Figures



Supplementary Fig. 1. **Soil pH in Oi/Oe, Oa/A and mineral soils at Hubbard Brook Experimental Forest[3].** Point fill indicates elevation group, where higher elevation includes spruce-fir and high hardwoods and lower elevation includes mid- and low hardwoods (**Supplementary Fig. 3**). Best-fit lines are shown only from 2015 on only. Shaded region indicates standard error.

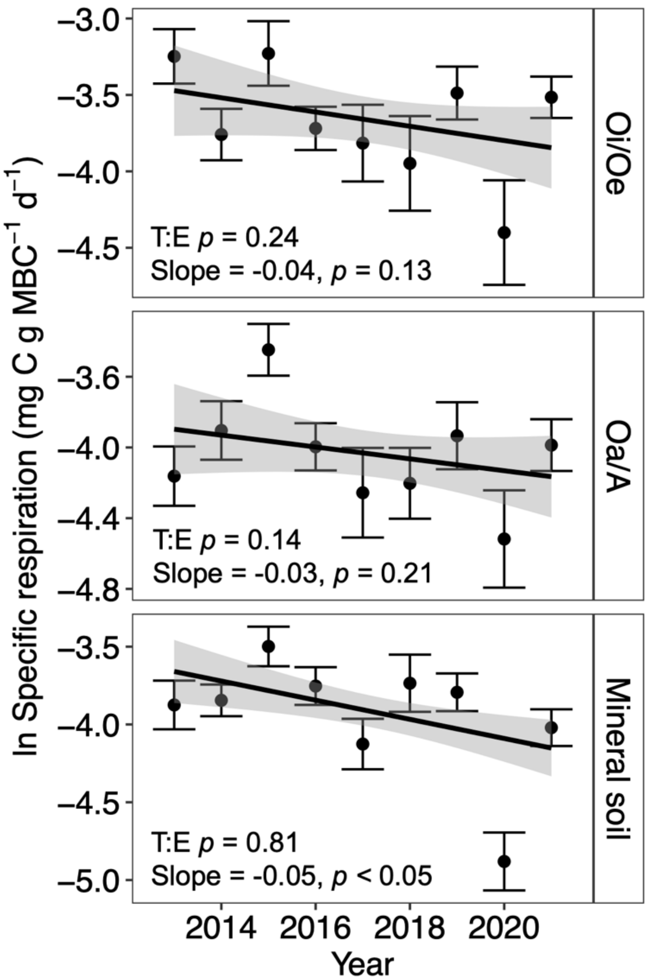


Supplementary Fig. 2. **Soil pH, soil solution monomeric aluminum, and soil respiration relationships**. **a** Relationship between mean soil pH[3,4] and cumulative summer soil respiration flux (2002-2020). Best-fit line is a simple linear regression for visualization only; shaded areas indicate standard error. **b** Annual (2002-2020) mean (± standard error) inorganic monomeric aluminum (Al^{3+}) concentration in freely-draining pore water in organic (Oa horizon) and mineral (Bh and Bs horizons) soils, collected at low hardwood, high hardwood and spruce-fir landscape positions in the reference watershed (locations are shown in **Supplementary Fig. 3**)[4]. Each point represents the mean of three horizons, three landscape positions, and 12 sampling months. **c** Relationship between annual mean inorganic monomeric Al^{3+} and cumulative summer soil respiration flux (2002-2020). Best-fit line is a simple linear regression for visualization only; shaded areas indicate standard error.

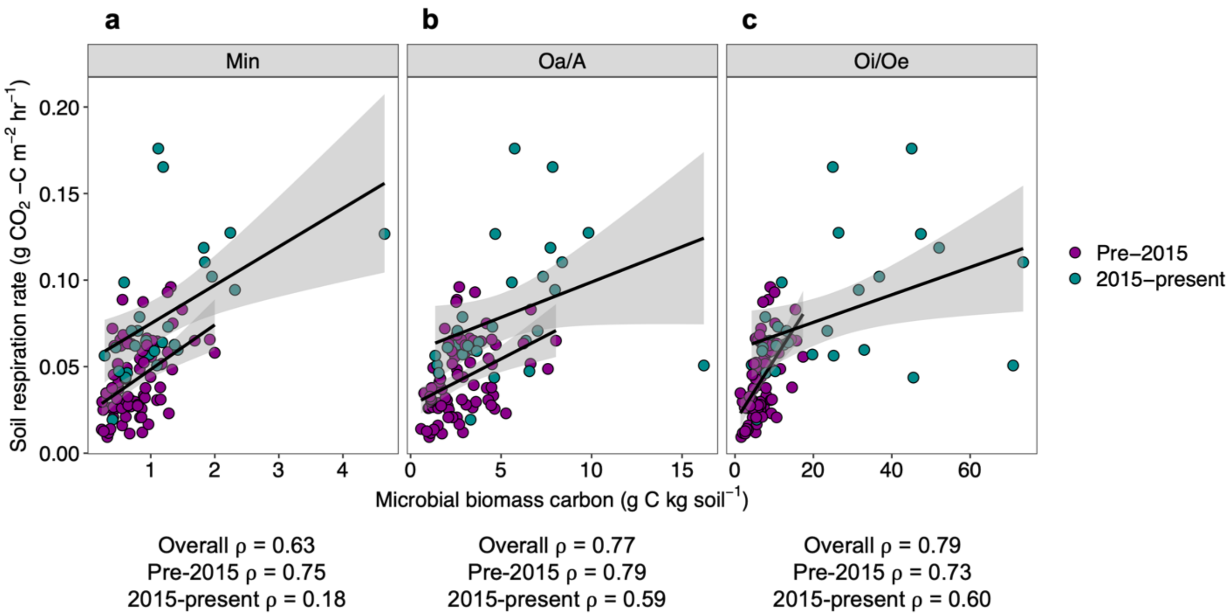


Supplementary Fig. 3. Hubbard Brook Experimental Forest study sites. a Overview of Hubbard Brook Experimental Forest. Watershed boundaries and hydrography derived from the USDA Forest Service[5,6]. **b** Detail of *in-situ* soil respiration measurement and soil core collection elevation transects in the calcium (Ca)-treated and reference watersheds. W1-W6 refers to experimental watersheds 1-6. Topography is shown by 12.2 m contours[7].

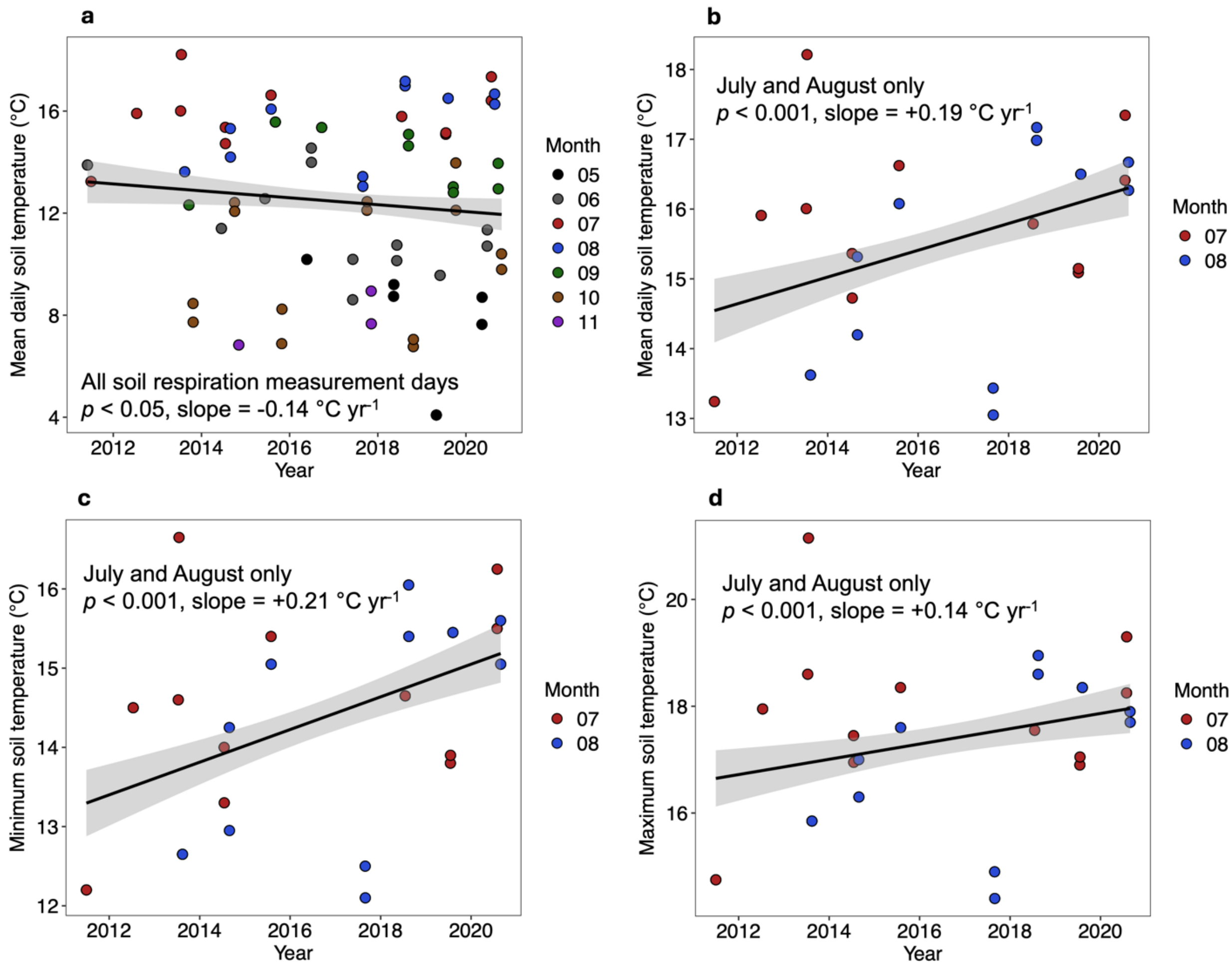
Supplementary Fig. 4. **Dissolved inorganic carbon in soil solution at Hubbard Brook.** Monthly (2002-2020) dissolved inorganic carbon (DIC) concentration of freely-draining pore water in organic (Oa horizon) and mineral (Bh and Bs horizons) soils, collected at low hardwood, high hardwood and spruce-fir landscape positions in the reference watershed (n = 9 for each monthly measurement; locations are shown in **Supplementary Fig. 3**)[4]. Trendlines show change in mean yearly DIC over time; shaded regions indicate standard error. The rate of increase in mean yearly DIC over time was significantly higher in the 2015-2020 time period (multiple linear regression interaction $p < 0.001$)



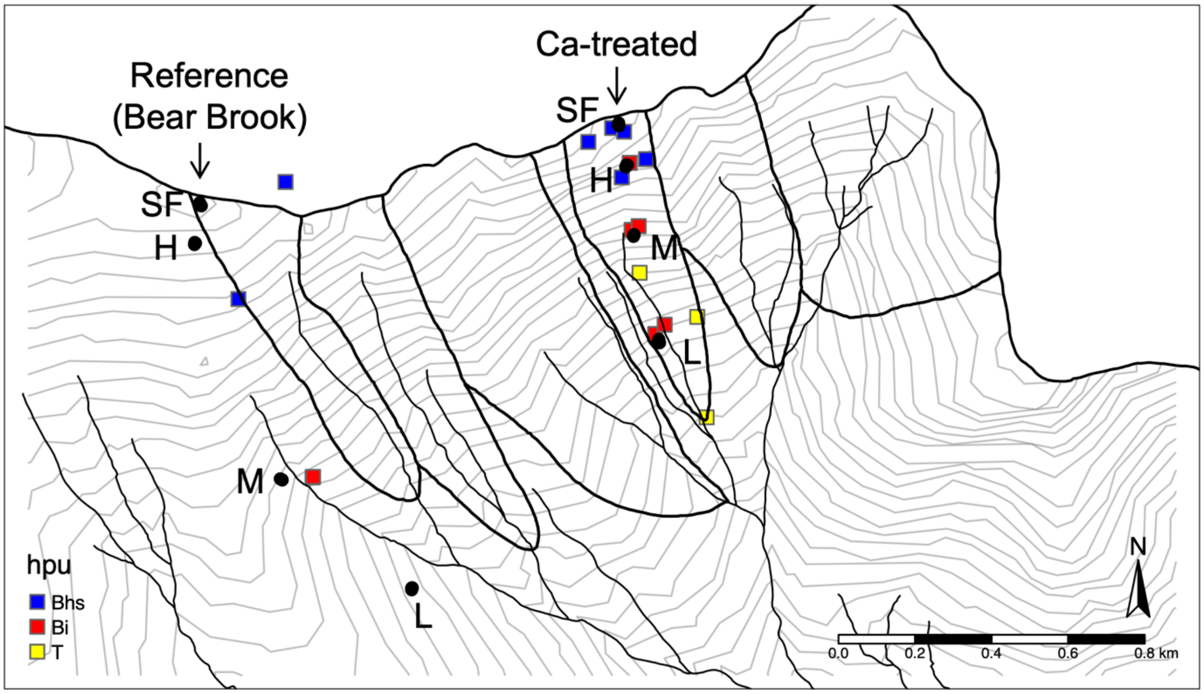
Supplementary Fig. 5. Decrease in specific respiration (microbial respiration normalized to microbial biomass carbon, MBC) post-breakpoint (*ln*-transformed). Time by elevation interaction effects (T:E) are derived from linear mixed models (**Supplementary Table 3**). Because no significant elevation interactions were detected, each point is the mean of up to 5 replicate plots, 2 watersheds, and 4 landscape positions (± standard error). Best-fit lines and slope estimates for visualization of effect direction and magnitude are derived from simple linear regression, with shaded area indicating standard error.



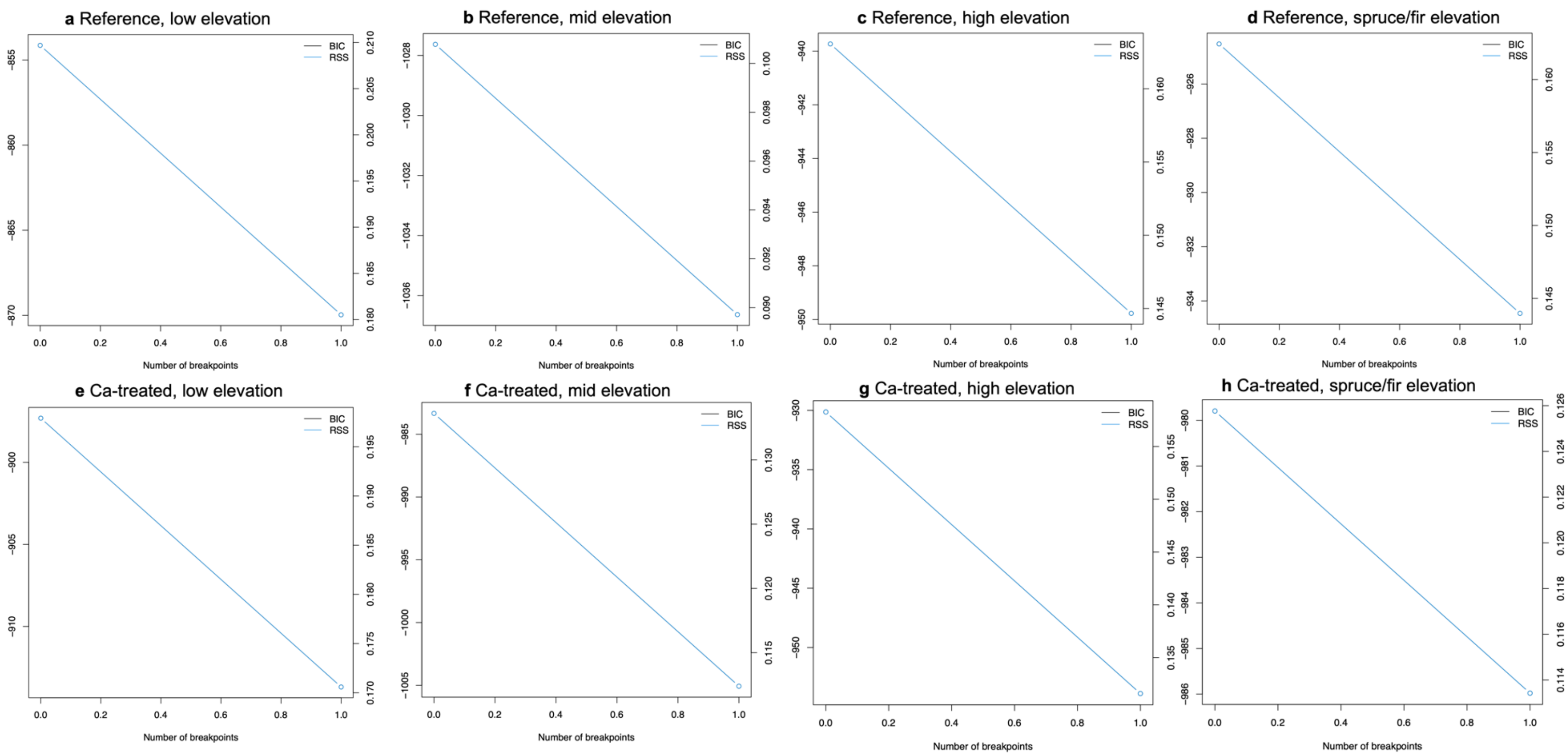
Supplementary Fig. 6. Correlation between microbial biomass carbon (MBC) and *in-situ* soil respiration rates. All points are from July soil sampling and soil respiration measurement dates, and each point is the mean across all landscape positions, watersheds, and replicate cores (for soil samples) or collars (for soil respiration). Spearman rank coefficients (ρ) are associated with overall, pre-2015, or 2015-present MBC and soil respiration relationships for a given soil horizon; *p*-values for all Spearman rank estimates are significant at $\alpha = 0.005$. Best-fit lines (for visualization only) are simple linear regressions within the pre-2015 and 2015-present periods. Shaded areas indicate standard error.



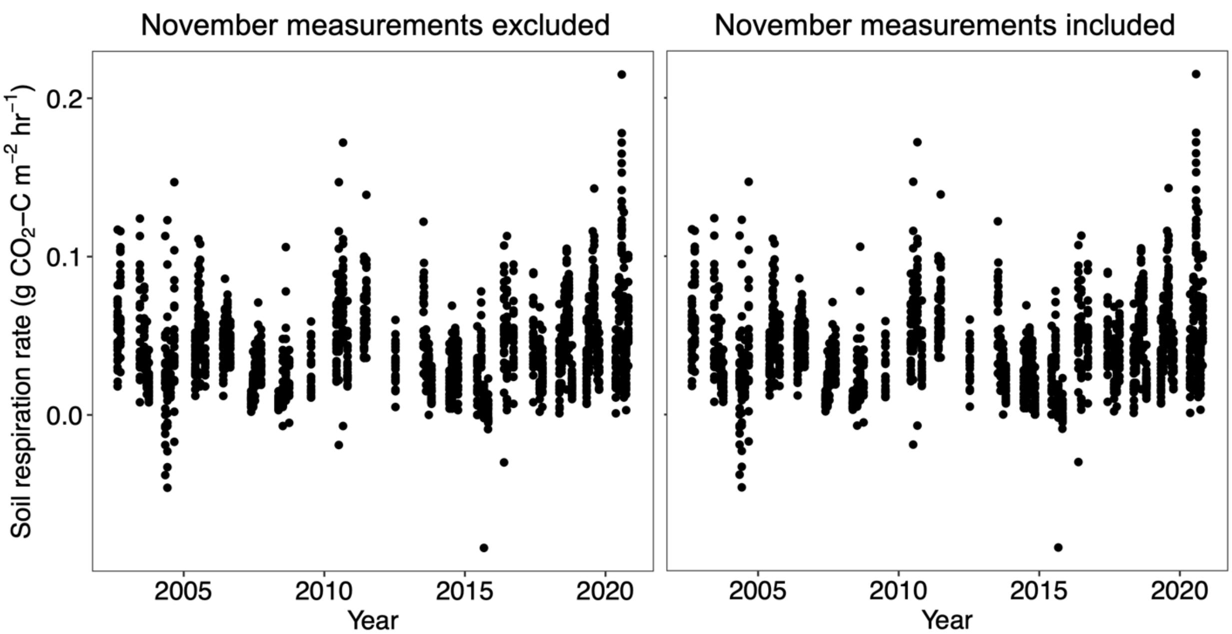
Supplementary Fig. 7. Soil temperature and soil respiration relationships. For all plots, the best-fit line is a simple linear regression; shaded areas indicate standard error. **a** Daily mean soil temperature (2011-2022) on all soil respiration measurement dates, filtered by the soil respiration field measurement window (10am-4pm) (mean across 2 soil sensors per location and 6 locations, **Supplementary Fig. 3**). **b** Daily mean soil temperature for July and August soil respiration measurement dates (mean across 2 soil sensors per location and 6 locations). **c** Daily minimum soil temperature for July and August soil respiration measurement dates (mean across 2 soil sensors per location and 6 locations). **d** Daily maximum soil temperature for July and August soil respiration measurement dates (mean across 2 soil sensors per location and 6 locations).



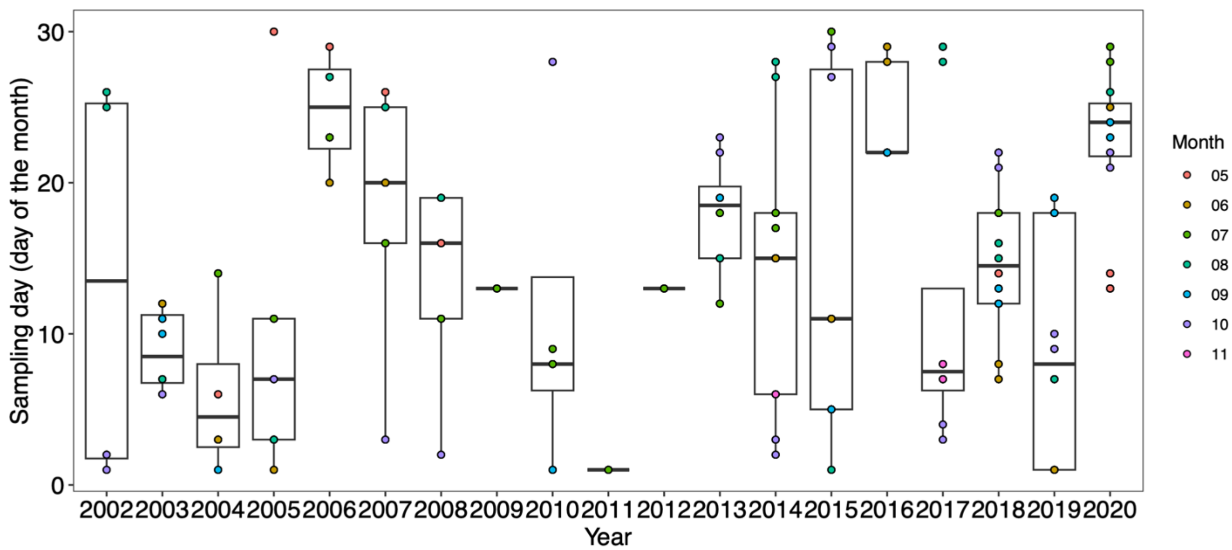
Supplementary Fig. 8. Hydropedological unit classification of soil profiles co-located with *in-situ* respiration measurement locations across the reference (Bear Brook) and Ca-treated (Watershed 1) elevation transects. HPU = hydropedological unit[8]; Bhs = Bhs podzol HPU; Bi = bimodal podzol HPU; T = typical podzol HPU; SF = spruce-fir site; H = high hardwoods; M = mid hardwoods; L = low hardwoods. Based on landscape position and distance from predicted shallow soils and bedrock outcrops[9], we conclude that the reference watershed low hardwood location does not experience frequent saturation cycles (i.e., is not a Bhs podzol).



Supplementary Fig. 9. Model residual sum of squares for breakpoint models. Decrease in model residual sum of squares (blue line) for total soil respiration flux models with one or without a breakpoint. **a-h** Each plot shows model fit for three replicate collars at each elevation-by-watershed combination.



Supplementary Fig. 10. All soil respiration rates (all collars, elevations, and watersheds combined), showing pattern with and without November measurements (in 2014 and 2017 only).



Supplementary Fig. 11. Day of month soil respiration measurements were collected over the time series used in this study.

Supplementary Tables

Supplementary Table 1. Change in soil respiration over time in the post-breakpoint (~2015) time period. Linear mixed model F-tests are included for main effects of time and the interactions between time and elevation group (higher or lower) or watershed (Ca-treated or reference). Denon. DF = denominator degrees of freedom. *** = p-values < 0.001, ** = p < 0.05, * = p < 0.10.

Variable	Model term	Denom. DF	F-value	p-value
Cumulative summer CO_2 flux	Time	155	34.0	***0.000
	Time by elevation	155	0.15	0.704
	Time by watershed	155	0.13	0.717
July and August respiration rate	Time	400	30.76	***0.000
	Time by elevation	400	0.16	0.692
	Time by watershed	400	0.21	0.646
May-November respiration rate	Time	774	55.25	***0.000
	Time by elevation	774	0.12	0.734
	Time by watershed	774	0.04	0.850

Supplementary Table 2. Change in microbial biomass carbon, C:N, and C mineralization over time in the post-breakpoint (~2015) period. Linear mixed model F-tests are included for main effects of time and the interactions between time and elevation group (higher or lower) or watershed (Ca-treated or reference). Denon. DF = denominator degrees of freedom. *** = *p*-values < 0.001, ** = p < 0.05, * = p < 0.10.

Variable	Horizon	Model term	Denom. DF	F-value	*p*-value
Microbial biomass carbon	Oi/Oe	Time	287	12.94	***0.000
		Time by elevation	287	3.90	**0.049
		Time by watershed	287	0.15	0.700
	Oa/A	Time	287	3.35	*0.068
		Time by elevation	287	0.18	0.668
		Time by watershed	287	0.21	0.646
	Mineral soil	Time	286	2.53	0.113
		Time by elevation	286	5.35	**0.021
		Time by watershed	286	0.25	0.616
Microbial biomass C:N ratio	Oi/Oe	Time	287	13.43	***0.000
		Time by elevation	287	3.15	*0.077
		Time by watershed	287	0.10	0.756
	Oa/A	Time	286	3.65	*0.057
		Time by elevation	286	0.50	0.479
		Time by watershed	286	0.91	0.340
	Mineral soil	Time	286	2.43	0.120
		Time by elevation	286	0.69	0.408
		Time by watershed	286	0.35	0.552
Microbial mineralization rate	Oi/Oe	Time	287	7.40	**0.007
		Time by elevation	287	1.20	0.275
		Time by watershed	287	0.20	0.658
	Oa/A	Time	288	0.00	0.963
		Time by elevation	288	1.46	0.228
		Time by watershed	288	0.53	0.468
	Mineral soil	Time	287	1.94	0.165
		Time by elevation	287	2.21	0.138
		Time by watershed	287	0.01	0.921

Supplementary Table 3. Decrease in specific respiration over time in the post-breakpoint (~2015) period. Linear mixed model F-tests are included for main effects of time and the interactions between time and elevation group (higher or lower) or watershed (Ca-treated or reference). Denon. DF = denominator degrees of freedom. *** = *p*-values < 0.001, ** = *p* < 0.05, * = *p* < 0.10.

Variable	Horizon	Model term	Denom. DF	F-value	*p*-value
Specific respiration	Oi/Oe	Time	287	2.35	0.126
		Time by elevation	287	1.39	0.240
		Time by watershed	287	0.02	0.897
	Oa/A	Time	287	3.84	*0.051
		Time by elevation	287	2.22	0.137
		Time by watershed	287	0.01	0.922
	Mineral soil	Time	286	5.65	**0.018
		Time by elevation	286	0.06	0.810
		Time by watershed	286	0.28	0.597

Supplementary Table 4. Forest floor carbon pool, Ca-treated watershed. The carbon pool in the forest floor (sum of Oie and Oa horizons) was calculated using measured soil mass and carbon content at each of the sampling sites. Annual means and standard deviation (in parentheses) are shown. The total forest floor carbon pool is the sum of the Oie and Oa pools, and the median forest floor pool is the median over both sampling years and across the range of estimates (i.e., plus or minus standard deviation within each year).

Year	Oie carbon pool	Oa carbon pool	Forest floor carbon pool	Number of observations
	kg C m^{-2}	kg C m^{-2}	kg C m^{-2}	
2014	1.25 (0.7)	1.19 (1.3)	2.44 (1.5)	100
2018	1.37 (0.8)	1.41 (1.3)	2.78 (1.5)	100
2014-2018 median			2.58	

Supplementary References

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