

## Reporting Summary

Nature Portfolio wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Portfolio policies, see our [Editorial Policies](#) and the [Editorial Policy Checklist](#).

### Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

| n/a                                 | Confirmed                                                                                                                                                                                                                                                                                      |
|-------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <input type="checkbox"/>            | <input checked="" type="checkbox"/> The exact sample size ( $n$ ) for each experimental group/condition, given as a discrete number and unit of measurement                                                                                                                                    |
| <input type="checkbox"/>            | <input checked="" type="checkbox"/> A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly                                                                                                                                    |
| <input type="checkbox"/>            | <input checked="" type="checkbox"/> The statistical test(s) used AND whether they are one- or two-sided<br><i>Only common tests should be described solely by name; describe more complex techniques in the Methods section.</i>                                                               |
| <input type="checkbox"/>            | <input checked="" type="checkbox"/> A description of all covariates tested                                                                                                                                                                                                                     |
| <input type="checkbox"/>            | <input checked="" type="checkbox"/> A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons                                                                                                                                        |
| <input type="checkbox"/>            | <input checked="" type="checkbox"/> A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals) |
| <input type="checkbox"/>            | <input checked="" type="checkbox"/> For null hypothesis testing, the test statistic (e.g. $F$ , $t$ , $r$ ) with confidence intervals, effect sizes, degrees of freedom and $P$ value noted<br><i>Give <math>P</math> values as exact values whenever suitable.</i>                            |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings                                                                                                                                                                      |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes                                                                                                                                                |
| <input type="checkbox"/>            | <input checked="" type="checkbox"/> Estimates of effect sizes (e.g. Cohen's $d$ , Pearson's $r$ ), indicating how they were calculated                                                                                                                                                         |

Our web collection on [statistics for biologists](#) contains articles on many of the points above.

### Software and code

Policy information about [availability of computer code](#)

|                 |                                                                                                                                                                                                                                             |
|-----------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Data collection | All data collection and processing was completing using open-source software (R and RStudio). Code is publicly available at <a href="https://github.com/arp264/HBEF_soil_respiration">https://github.com/arp264/HBEF_soil_respiration</a> . |
| Data analysis   | All data collection and processing was completing using open-source software (R and RStudio). Code is publicly available at <a href="https://github.com/arp264/HBEF_soil_respiration">https://github.com/arp264/HBEF_soil_respiration</a> . |

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Portfolio [guidelines for submitting code & software](#) for further information.

### Data

Policy information about [availability of data](#)

All manuscripts must include a [data availability statement](#). This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our [policy](#)

The soil and site characterization datasets used for all analyses in this study are available through the open access Hubbard Brook Data Catalog, hosted by the Environmental Data Initiative (<https://hubbardbrook.org/data-catalog/>).

## Research involving human participants, their data, or biological material

Policy information about studies with [human participants or human data](#). See also policy information about [sex, gender \(identity/presentation\), and sexual orientation](#) and [race, ethnicity and racism](#).

Reporting on sex and gender

NA

Reporting on race, ethnicity, or other socially relevant groupings

NA

Population characteristics

NA

Recruitment

NA

Ethics oversight

NA

Note that full information on the approval of the study protocol must also be provided in the manuscript.

## Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☐ Life sciences

☐ Behavioural & social sciences

☒ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see [nature.com/documents/nr-reporting-summary-flat.pdf](https://nature.com/documents/nr-reporting-summary-flat.pdf)

## Ecological, evolutionary & environmental sciences study design

All studies must disclose on these points even when the disclosure is negative.

Study description

Soil respiration was measured monthly during the growing season (May – October or November) using an in-situ chamber flux method at sites distributed along elevation gradients in reference and Ca-treated watersheds at Hubbard Brook Experimental Forest (4 landscape positions per watershed, 3 chambers per site) from 2002-2020. We explored relationships between soil respiration and microbial biomass and microbial CO<sub>2</sub> mineralization using measurements of these variables that were co-located with the in-situ soil respiration measurements.

Research sample

The primary dataset used in this study is in-situ gas measurements collected using permanent PVC collars at the soil surface, as well as soil cores collected in the vicinity of gas measurement locations. As such, the "research sample" includes soils across elevations and in two experimental watersheds.

Sampling strategy

Sampling was conducted monthly during May-October or November from 2002-2020. Soil locations spanned an elevation gradient and two experimental watersheds. This sampling frequency and spatial extent represented a balance of frequency to capture seasonal variation with logistical management of decadal long-term record.

Data collection

*Describe the data collection procedure, including who recorded the data and how.*

Timing and spatial scale

Sampling was conducted monthly during May-October or November from 2002-2020. Soil locations spanned an elevation gradient and two experimental watersheds. This sampling frequency and spatial extent represented a balance of frequency to capture seasonal variation with logistical management of decadal long-term record.

Data exclusions

Data were accessed from the Hubbard Brook repository within the Environmental Data Initiative archive. While some data were missing in these datasets, no data were excluded, with the exception of derived values of specific respiration, which were excluded if microbial mineralization rates were reported as 0 (resulting in undefined specific respiration).

Reproducibility

In this study, the primary focus is long-term changes in soil respiration fluxes (observational rather than experimental data); however, this study does include a contrast between manipulated watersheds (Ca-treated vs. reference). This study leverages application of an amendment to an entire watershed in 1999; while the scale of the manipulation is not easily replicated, the small-watershed treatment experimental approach has successfully been implemented in other areas of Hubbard Brook and is a feasible way to track whole-watershed behavior.

Randomization

NA (long-term observational data)

Blinding

NA

Did the study involve field work?

☒ Yes

☐ No

## Field work, collection and transport

|                        |                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |
|------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Field conditions       | This study reports a long-term (2002-2020) dataset of in-situ soil respiration measurements. The samples were collected across four landscape positions in two experimental watersheds, and paired with annual soil core collection. While field conditions at the time of sampling were variable across the long-term record, the study includes environmental data (air and soil temperature, soil pH) to provide context for changes in field conditions over time. |
| Location               | Hubbard Brook Experimental Forest, North Woodstock, NH                                                                                                                                                                                                                                                                                                                                                                                                                 |
| Access & import/export | In-situ soil respiration measurements and soil core collection was conducted following Research Approval Committee approval at the Hubbard Brook Experimental Forest, which is operated by the US Forest Service Northern Research Station.                                                                                                                                                                                                                            |
| Disturbance            | Permanent PVC rings were installed across landscape positions; maintaining permanent collars limits disturbance for long-term data collection. Soil cores were collected from replicate plots in the vicinity of the permanent collars; the footprint of soil core collection was determined to be the minimum possible for continuous soil core collection long-term.                                                                                                 |

## Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

### Materials & experimental systems

| n/a                                 | Involved in the study                                  |
|-------------------------------------|--------------------------------------------------------|
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Antibodies                    |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Eukaryotic cell lines         |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Palaeontology and archaeology |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Animals and other organisms   |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Clinical data                 |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Dual use research of concern  |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Plants                        |

### Methods

| n/a                                 | Involved in the study                           |
|-------------------------------------|-------------------------------------------------|
| <input checked="" type="checkbox"/> | <input type="checkbox"/> ChIP-seq               |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Flow cytometry         |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> MRI-based neuroimaging |

## Plants

|                       |    |
|-----------------------|----|
| Seed stocks           | NA |
| Novel plant genotypes | NA |
| Authentication        | NA |