Heliyon 5 (2019) e02816

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.heliyon.com

Heliyon

Research article

Atmospheric respiratory CO_2 efflux by aquatic suspended particle-bound microbial communities: A laboratory experimental study

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ARTICLE INFO

Keywords:
Environmental science
Ecology
Microbiology
Microbial ecology
Aquatic biology
Aquatic respiratory CO2
Estuaries
Microbial communities
Water column particulates

ABSTRACT

Natural sources of atmospheric CO_2 are of increasing interest as possible contributors to global climate warming. This study documents the amount of respiratory CO_2 contributed by microbial communities associated with suspended particulates in aquatic water columns. Microcosms containing three different sources of water (pond freshwater, NY East River estuary and Hudson River estuary) were used to experimentally determine the atmospheric respiratory CO_2 released from particle-associated microbes. Two different approaches were used. In the first, finely powdered dried cereal leaves (alfalfa) were added to each of the three microcosms as a consistent source of particulate organic matter (POM). In the second, only Hudson River estuary water samples were used with natural densities of POM. Respiration rates associated with two sizes of particles were assessed: 1) $\geq$ 200 μm and 2) $\geq$ 50 μm but less than 200 μm. The total respiration rate for the three microcosms with cereal leaf POM ranged from 5.09 to 14.87 μmol CO_2 min^{-1} L^{-1}. Of this, the amount contributed by larger particulates was in the range of 55–63%; and for smaller particulates ranged from 18 to 32 %. Data for microcosms containing water from the Hudson River estuary, with natural particulates, was as follows: total respiration ranged from ~3 μmol CO_2 min^{-1} L^{-1} to ~3.73 μmol CO_2 min^{-1} L^{-1}. Larger particulates contributed approximately 40% of total respiration, and that of smaller particulates was substantially less (4–5% of total). Overall, these results indicate that microbial communities associated with particulates in the water column (especially larger particulates) may contribute substantial amounts of CO_2 to the atmosphere.

1. Introduction

Estuaries and other coastal aquatic environments are often major sources of atmospheric respiratory CO_2 (Anderson, 2016a; Cai, 2011) and in locales with high loads of dissolved organic matter (DOM) or particulates (POM) causing diminished light penetration, the metabolic regime is largely heterotrophic (Caffrey, 2004; Howarth et al., 1992; Massicotte et al., 2017; Smith and Mackenzie, 1987). Current evidence points to water column particulates as a likely major source of microbial activity and a substantial proportion of total respiratory CO_2 efflux to the atmosphere from organically enriched aquatic environments (Meinhard et al., 2002; Ploug et al., 2002; Valliéres et al., 2008; Zimmermann--Timm, 2002). Laboratory experimental microcosm research has contributed insights into the role of organic matter (DOM and POM) in aquatic microbial ecosystems. This includes water quality, role of particulates derived from decaying plant matter (Bayarsaikhan et al., 2016), and resulting microbial community structure and respiratory CO_2 loss from the organic, particle-laden aquatic systems (Anderson, 2016b; Wörner et al., 2000). However, less is known about how much of the total respiratory CO_2 efflux from the water column is contributed by microbial communities associated with particulates of different size fractions in the water column. The objective of this research was to assess how much of total respiratory CO_2 released into the atmosphere from organically enriched water was contributed by particle-associated microbial communities in freshwater and estuarine water samples using laboratory microcosms as have been productively used in prior research (Anderson, 2016b; Bayarsaikhan et al., 2016; Ploug et al., 2002; Wörner et al., 2000).

Two experimental approaches were used to assess respiratory CO_2 emissions: 1) microcosms, containing either filtered pond water or estuarine water, were augmented with finely-fragmented, dried cereal leaves (alfalfa) simulating natural sources of plant, particulate organic matter (POM) as a consistent carbon source (Bayarsaikhan et al., 2016) for metabolism within the microcosms, and 2) microcosms containing estuarine water with naturally occurring suspended particulates, freshly

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https://doi.org/10.1016/j.heliyon.2019.e02816
Received 17 August 2019; Received in revised form 14 September 2019; Accepted 6 November 2019

collected from the natural environment. A pond water microcosm was used, because the upper tributaries of some river estuaries, such as the Hudson river, contribute largely freshwater influx to the estuary and represent a low salinity end point along the estuary salinity gradient, terminating at the ocean margin. This study examined the proportion of total respiratory CO_2 efflux contributed by microbial communities in the microcosm cultures associated with larger (≥200 μm) and smaller (≥50 μm, but less than 200 μm) particulates under controlled climate conditions. Although the main objective was to assess the amount of atmospheric CO_2 contributed by respiration of microbes associated with water column particulates, estimates of the densities of bacteria associated with the particulates are also reported.

2. Materials and methods

2.1. Water samples

Natural pond water, collected from a freshwater pond at Burlington, NC (salinity = zero, pH = 7.28), was obtained from Carolina Biological (Cat. No. 163380). Samples of naturally occurring water with suspended particulates were collected from the East River estuary (New York City) and the Hudson River estuary (Fig. 1) during the summer months when the warmer water yields the most release of Respiratory CO_2 to the atmosphere. East River estuary water (salinity = 25 $^{o}/_{oo}$, pH = 8.01) was collected in June, 2018 in bottles from surface water using a boat at mid channel off the east edge of Manhattan Island (40.741951 N, -73.964937 W). Hudson River estuary water (salinity = 10 $^{o}/_{oo}$, pH = 7.36) was collected at two sampling times between July to August (2018) near the Piermont, NY pier (41.041040 N, -73.920620 W) using a bucket specifically dedicated to Hudson River sampling and deposited in 1 gal. plastic bottles. The bucket is thoroughly cleaned with distilled water and dried to ensure there are no particulates remaining before new water samples are taken. All plastic bottles were identical to those supplied by Carolina Biological Co. and used solely for Hudson River sampling.

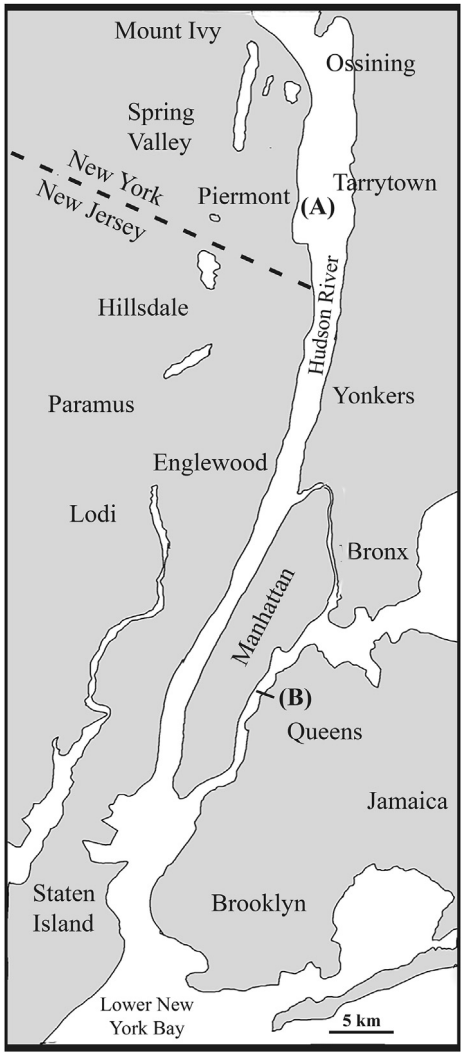

Fig. 1. Sampling sites in the Hudson River at Piermont Pier, New York (A), and in the East River between Manhattan and Queens boroughs of New York City (B).

2.2. Microcosm experiments

2.2.1. Unamended natural water

Microcosms containing water collected directly from the natural environment (Hudson River estuary water) were set up using 200 mL of water in 250-mL Nalgene bottles. The containers of sampled water from the Hudson River estuary were gently and thoroughly mixed to suspend all particulates before distributing 200-mL aliquots into each of the Nalgene culture bottles. To ensure adequate sources of respiratory substrate, 0.1 g of glucose was added to each microcosm culture bottle. All water samples were brought to 20 °C before use in the experiments. Bottles were loosely capped and incubated in a dark, walk-in constant temperature room at 20 °C. All of the cultures were maintained at 20 °C for three days, except for the second Hudson River sample that was collected following a major rain event (as explained in the table of Results) when the water was particularly heavily concentrated with particulates. To ensure that the available glucose added was not depleted before the respiration analyses, the second Hudson River sample was cultured for two days at 20 °C. Three replicate experiments were used for the East River estuary water where we were able to obtain one sample, and six replicates for the Hudson River water; i.e., three replicates per sampling date and two different sampling days. Six replicates were obtained using the Carolina pond water (two preparations, with triplicate prepared culture bottles for each of the two preparations).

2.2.2. Natural water amended with powdered cereal grass leaves

To experimentally determine the effects of adding a known quantity of particulate organic matter in the microcosm cultures, a defined weight of finely powdered dried cereal leaves prepared from alfalfa as explained below was added to three sources of water as described in the preceding subsection on water samples: 1) natural pond water, 2) Hudson River estuary water, or 3) East River estuary water. The water sample from each site was passed through a 50-μm pore size Nitex mesh filter to remove as much naturally occurring particulates as possible before addition of the powdered cereal grass leaves in the experimental microcosms. Each microcosm (250 mL-Nalgene bottle) contained 50 mL of the water sample, plus 0.03 g glucose as a supplemental C source and 1 mL of suspended pond water sediment (collected from a pond on the Lamont-Doherty Earth Observatory campus) as a source of natural microbial community. Particulate dried alfalfa leaves (Carolina Biological Catalog no. 132376) were prepared before use by passage through a 200-μm, pore-size mesh, to produce fine-pulverized, organic particulates that were ≤200 μm in size. An aliquot of 0.15 g of the fine powdered leaves was added to each Nalgene culture bottle containing the 50-mL water sample as described above. After gently mixing the contents of each culture bottle, they were loosely capped and incubated at 20 °C in a dark, walk-in culture room for two to three days before respiration measurements were made. Six replicate experiments were used for the pond water samples.

2.2.3. Respiration measurements

All respiration measurements were made using an infrared gas analyzer (Vernier Software Technology, Beaverton, OR) inserted, and

sealed, in the neck of the 250-mL microcosm Nalgene bottle, maintained at 20 °C in a constant temperature bath (Isotemp model 3006D; Fisher Scientific, Pittsburgh, PA). Respiration rates were recorded as μmol CO_2 min^{-1} L^{-1}. Total respiration measurements were made by direct measurement of the water in the microcosm Nalgene bottle. Additionally, respiration was measured in each microcosm after filtering out suspended particles that were ≥200 μm using a Nitex mesh filter (200-μm mesh size). The difference in rate of respiration between the unfiltered and filtered suspension provided an estimate of the respiration contributed by the ≥200-μm-particulate microbial community and available sources of soluble organic substrate associated with the particulates. Likewise, a second filtration step was used to remove remaining particles that were ≥50 μm from the remaining microcosm suspension obtained in the prior step to obtain an estimate of the respiration associated with these smaller particles and their available soluble organic nutrients. A Nitex mesh filter with 50-μm mesh pore size was used. The difference in respiration rate between the 200-μm filtrate and the 50-μm filtrate provided an estimate of the contribution of the particulates in the 50–200 μm range.

The filtration apparatus (Fig. 2) used to gently filter out the particulates was the same as previously published to obtain aquatic particulates to estimate the amount, and identification, of particle-bound naked amoebae (Anderson, 2011). This apparatus consisted of a sheet of Nitex filter of specified pore size, attached to a cylindrical piece of polystyrene tubing. The filter apparatus was suspended in a shallow, concave watch glass at a position slightly above the surface of the watch glass. This assembly was placed above a funnel fitted into a Nalgene bottle to capture the filtrate as it flowed out of the watch glass under the filtration apparatus. The water sample to be filtered was gently poured into the cylindrical filter apparatus, while the overflow of the filtrate passing out of the watch glass was collected in the funnel. The position of the filter assembly above the watch glass ensured that the particulates were maintained in a small volume of fluid during the filtering process, thus preventing damage due to impact on the filter until the final few milliliters of filtrate were gently released into the watch glass and subsequently deposited into the Nalgene bottle – thus, the total filtrate was recovered. Respiration measurements were made immediately on each filtrate in the Nalgene bottle as described above.

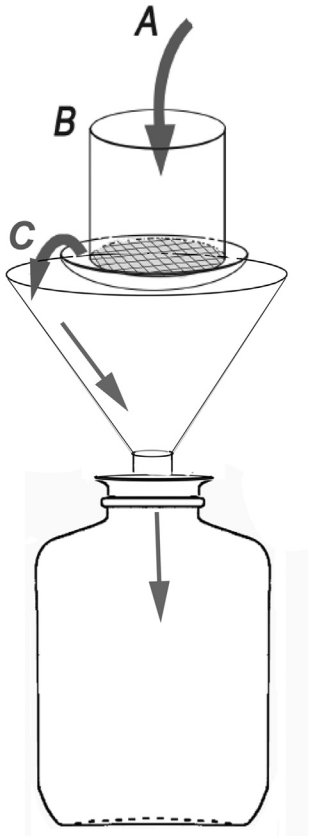

Fig. 2. Diagram of filter apparatus used to collect particulates from the microcosm suspension. The suspension (A) is gently poured into the filter unit (B), containing a Nitex mesh of the specified pore size (either 200 μm or 50 μm), while suspended above a concave watch glass that maintains a small volume of suspension above the filter surface, thus preventing compaction of the particulates on the filter. Overflow of the filtrate passing out of the watch glass (C) is collected in the receiving respiration flask by way of a large funnel. The final small volume of filtrate remaining in the cover glass is decanted into the funnel, thus quantitatively recovering all of the filtrate while the particulates are retained on the filter mesh and back washed into a 15-mL centrifuge tube using micro-pore filtered deionized water to be used in subsequent analyses. Adapted from Anderson (2011).

Immediately after filtration, the particulates on the Nitex mesh were gently back washed into a 15 mL-conical graduated plastic centrifuge tube using micropore-filtered water from the sample collection site and brought to a final volume of 5 mL. The suspension of recovered particulates was fixed with glutaraldehyde as described above for counting of bacteria and protists. The fixed suspension was allowed to settle in the graduated conical centrifuge tubes by gravity for at least 2 h, and the volume of the sedimented pellet was measured and expressed as cm^3. The volume of sedimented particulates for each microcosm preparation is presented in the footnotes to Tables 1 and 2 in the Results.

2.2.4. Estimation of bacterial densities

Bacterial densities were estimated based on fluorescent microscopic counts of SYBR Green-stained aliquots of microcosm water samples that had been preserved with unbuffered, pure glutaraldehyde (3% w/v) and stored in the refrigerator for counting of bacteria. To ensure that the particle-bound bacteria were released into suspension, the fixed water samples in 15-mL conical centrifuge tubes were treated with Tween-80 and EDTA and vortexed for 6 min at the highest speed of a Vortex-Genie 2 (Scientific Industries, Bohemia, NY) according to the method of Suter et al. (2011). The SYBR Green-stained aliquots of the treated, preserved water were filtered on black 25-mm diameter, 0.20-μm pore size polycarbonate filters (GVS Life Sciences, Sanford, ME), observed with a Labrophot-2 fluorescent microscope (Nikon Instruments, Melville, NY) using oil immersion lenses, and the number of bacteria per microscopic field was counted and converted to the number of particle-bound bacteria per mL in the original water sample using the methods of Hobbie et al. (1977).

2.3. Statistical analysis

Descriptive statistics were obtained using Excel spread sheets. Pearson product-moment linear correlations were obtained using StatPlus:mac (AnalystSoft Inc., Walnut, CA).

3. Results

3.1. Respiration

The results of the respiration studies are presented in Tables 1 and 2. For the three microcosm experiments augmented with cereal leaf powder (pond freshwater, Hudson River, and East River), the same quantity (0.15 g) of leaf powder was added to each of the microcosms to ensure that the same POM experimental treatment was used in all of the microcosm experiments. The total respiration rate (Table 1) ranged from 5.09 to 14.87 μmol CO_2 min^{-1} L^{-1}. Of this, the amount contributed by the larger particulates (≥200 μm) was in the range of 55–63%. While the

Table 1
Mean respiration rates (μmol CO_2 min^{-1} L^{-1}) in microcosm studies of pond water, Hudson River estuary water, and NY East River estuary water augmented with fine granular dried leaf particulates.

	Fresh water pond[a]			Hudson River estuary[b]			East River estuary[c]		
	Total	>200 μm	> 50 μm	Total	> 200 μm	> 50 μm	Total	>200 μm	> 50 μm
Mean	5.65	3.14	0.96	14.87	8.18	4.28	5.09	3.13	1.61
S.E	0.4	0.4	0.4	2.0	0.8	0.4	0.6	0.4	0.4
Percent of Total		56	18		55	29		63	32

Total = total respiration before filtering out particulates; >200 μm = contribution to respiration of particulates ≥200 μm; > 50 μm = contribution to respiration of particulates in the range of ≥50 μm, and smaller than 200 μm in size.
[a] Mean values for six replicate experiments, sedimented volume of ≥200 μm particles = 0.3 cm^3.
[b] Mean values for three replicate experiments, sedimented volume of ≥200 μm particles = 0.8 cm^3.
[c] Mean values for three replicate experiments, sedimented volume of ≥200 μm particles = 0.8 cc. In all experiments, particulates ≥50 = ~ 0.1 cm^3.

Table 2
Mean respiration rates (μmol CO_2 min^{-1} L^{-1}) in two microcosm studies of Hudson River estuary surface water containing naturally occurring particulates.

	Hudson River estuary			Hudson River estuary[a]		
	Total	> 200 μm	> 50 μm	Total	> 200 μm	> 50 μm
Mean	3.01	1.33	0.12	3.73	1.37	0.17
S.E.	0.4	0.2	0.2	0.9	0.6	0.2
Percent of Total		44	4		37	5

Total = total respiration before filtering out particulates, >200 μm = contribution to respiration of particulates ≥200 μm; > 50 μm = contribution to respiration of particulates in the range of ≥50 μm, and smaller than 200 μm in size. All values are means for three replicates in each experiment. Mean value of sedimented total particulates = 0.6 cm^3.
[a] Sample taken after recent heavy precipitation with turbulent suspension in the water column.

respiration contributed by smaller particulates (≥50 μm) ranged from 18 to 32 %. Overall, the proportion of respiration contributed by the combined small and large particulates ranged from 74 to 95% of the total respiratory CO_2 released in each microcosm.

The data for microcosms containing water from the Hudson River estuary, without further organic particulate augmentation (Table 2), showed that total respiration ranged from ~3 μmol min^{-1} L^{-1} to ~3.73 μmol CO_2 min^{-1} L^{-1}. The contribution of the larger particulates in the two microcosms was 44 and 37%, of the total respiration respectively. The contribution of the smaller particulates was substantially less (4–5% of total). Thus, overall, the combined contribution of larger and smaller particulate-based respiration was 42–48% for the Hudson River estuary.

3.2. Particle-bound bacterial densities

Densities of bacteria associated with the particulates expressed as 10^{10} L^{-1} were as follows: 1) pond water plus cereal leaf powder ≥200 μm particles (0.73) and ≥50 μm particles (0.70); 2) East River with natural particulates ≥200 μm particles (1.28) and ≥50 μm particles (0.82); and 3) Hudson River estuary with natural particulates >200 μm particles (1.60) and ≥50 μm particles (0.86). Bacterial densities are consistent with prior published reports for particulate-rich Hudson River water in the range of 10^9 to 10^{10} L^{-1} (Findlay, 2006; Lesen et al., 2010; Taylor et al., 2003). The linear correlation of particle-bound, bacterial densities with CO_2 respiration rate, combining data for the two size ranges of particulates in the three sources of microcosm water, was r = 0.79.

4. Discussion

There is a substantial published evidence that water column particulates support robust bacterial and protist communities (Ploug et al., 2002; Simon et al., 2002), and that particle-bound bacteria tend to be highly metabolically active, including high respiration rates (Harvey and Young, 1980; Kirchman and Mitchell, 1982). Moreover, the quantity and quality of particulate organic matter can be directly related to bacterial production in estuaries (Crump et al., 2017). However, particle-bound bacteria may be more susceptible to predation by some heterotrophic protists (Caron, 1987; Pernthaler, 2005) and thus particle surfaces are highly dynamic microecological systems involving complex interactions of bacteria and protists (Simon et al., 2002), in some cases varying seasonally in community dynamics (Zimmermann-Timm et al., 1998). In some marine systems, the complexity and diversity of prokaryotes attached to particulates in the water column increase with increasing particle size (Mestre et al., 2017); thus, particle size may be a significant factor in microbial activity and possibly respiratory CO_2 release to the atmosphere.

A goal of this study was to experimentally examine how much of total respiratory CO_2 released from freshwater and estuarine aquatic mesocosms is attributable to microbial communities associated with two size groups of particulates: those ≥200 μm and those that were ≥50 μm in size, but less than 200 μm. Samples were collected during the summer months when respiratory activity is relatively high (Raymond et al., 1997), and the experiments were incubated at 20 °C consistent with this seasonal sampling. Overall, the main purpose of the research was to provide evidence of differences in respiration associated with larger and smaller particles, and was not intended to assess seasonal changes in respiratory efflux to the atmosphere. The annual seasonal cycles of respiratory CO_2 evasion to the atmosphere from the tidal Hudson where some of the sampling was done for this study has been published previously (Howarth et al., 1992; Raymond et al., 1997). The CO_2 emission released from the Hudson to the atmosphere ranged from 665 g C m^{-2} yr^{-1} to 984 g C m^{-2} yr^{-1} and was positively correlated with seasonal temperature of the water. However, further studies of particulate samples taken from the Hudson and East River at other seasons, and incubated in laboratory experiments at temperatures consistent with the water temperature for that season, may provide additional evidence of how the particle-bound respiratory activity varies with season.

The data from both fresh water and estuarine microcosms showed that the respiratory CO_2 released from the larger particulate fraction was substantially greater than from the smaller particulate fraction in all of the experiments. As might be expected, the volume of the sedimented larger particles was greater than that of the smaller particles. However, overall, the combined contribution of particulate fractions (large and small) accounted for a substantial proportion of the total respiration relative to their combined settled volume (~0.5–1 cm^3), while the total volume of the water varied between 50 and 200 cm^3 across the different experiments – thus, the volume of the particulates accounted for only approximately 0.5–1.0 % of the total volume of the water suspension in the microcosms, while the contribution of their microbial communities to respiratory CO_2 release was substantially greater. In the experiments using freshwater microcosms enriched with fine powdered, dried cereal leaves, the proportion of total respiratory CO_2 released by the particulates was in a range of 75–95%, even though the proportional volume of the particulates was only ~1% of the total volume of the microcosm

suspension. However, for the two microcosm studies using Hudson River estuary water, the proportion was closer to 50% of total released respiratory CO_2. These results are consistent with prior research that indicated a substantial amount of respiratory activity is associated with particulates in the water column relative to the bulk volume of the total water.

For example, Ploug et al. (2002) studying the particulates in the Elbe Estuary, reported that the respiration rate on aggregates larger than 400 μm accounted for 84–94% of the estimated total respiration in the upper water column. This is close to the percentages reported here in Table 1. Crump and Barros (1996) reported sharp peaks in bacterial production in the estuarine turbidity maxima in the Columbia River estuary, largely due to particle-attached bacteria, suggesting that increased particle-bound bacterial production may account for the enhanced respiratory activity of particle-dwelling microbes compared to those in the bulk phase of the water column. Moreover, in this study, particle-bound bacterial densities were positively correlated with the respiratory CO_2 efflux from the microcosms ($r = 0.79$) as might be expected if bacteria are the main source of respiratory CO_2 (Ploug et al., 2002).

The current study used water samples from the East River estuary and Hudson River estuary during the summer months when the warmer water typically releases a higher amount of respiratory CO_2 to the atmosphere; however, further research at other seasons of the year would be useful to determine possible seasonal variations. Additional research is needed to determine how the chemical composition, structure, and surface area of organic particulates in aquatic environments may influence associated microbial communities and the amount of respiratory CO_2 released to the atmosphere. Presumably, the greater the amount of DOM released from the particulates may influence bacterial productivity and respiratory metabolism, as well as the density and composition of the protists inhabiting the particulates. In this respect, the relative contribution of allochthonous versus autochthonous (by primary producers) sources of organic matter within the particles of varying composition needs to be elucidated in relation to microbial community composition, growth and resulting respiratory CO_2 release from the aquatic suspended particulates to the atmosphere.

Declarations

Author contribution statement

O. Roger Anderson: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Funding statement

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

Acknowledgements

This is Lamont-Doherty Earth Observatory Contribution Number 8362.

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