Estimating bioturbation from replicated small-sample radiocarbon ages

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Key Points:

- Age-heterogeneity within sediment layers adds hidden uncertainty to radiocarbon-based age estimates.
- The amount of age-heterogeneity depends on the sedimentation rate and bioturbation mixing depth.
- We present a method to estimate ^{14}C age-heterogeneity and lookup figure to estimate age uncertainty.

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Abstract

Marine sedimentary records are a key archive when reconstructing past climate; however, mixing at the seabed (bioturbation) can strongly influence climate records, especially when sedimentation rates are low. By commingling the climate signal from different time periods, bioturbation both smooths climate records, by damping fast climate variations, and creates noise when measurements are made on samples containing small numbers of individual proxy carriers, such as foraminifera. Bioturbation also influences radiocarbon-based age-depth models, as sample ages may not represent the true ages of the sediment layers from which they were picked. While these effects were first described several decades ago, the advent of ultra-small-sample ^{14}C dating now allows samples containing very small numbers of foraminifera to be measured, thus enabling us to directly measure the age-heterogeneity of sediment for the first time. Here, we use radiocarbon dates measured on replicated samples of 3-30 foraminifera to estimate age-heterogeneity for five marine sediment cores with sedimentation rates ranging from 2-30 cm kyr^{-1}. From their age-heterogeneities and sedimentation rates we infer mixing depths of 10-20 cm for our core sites. Our results show that when accounting for age-heterogeneity, the true error of radiocarbon dating can be several times larger than the reported measurement. We present estimates of this uncertainty as a function of sedimentation rate and the number of individuals per radiocarbon date. A better understanding of this uncertainty will help us to optimise radiocarbon measurements, construct age models with appropriate uncertainties and better interpret marine paleo records.

1 Introduction

Proxy records recovered from sediments are an important source of information about the history of the Earth's climate prior to the instrumental era. For example, the ratio of magnesium to calcium (Mg/Ca) in the shells of marine organisms such as foraminifera contains information about the temperature of the environment in which calcification took place (Nürnberg et al., 1996; Lea, 2014; Rosenthal et al., 2000). These shells settle to the sediment surface and are buried as further sediment accumulates. Over time this produces an archive of recorded (proxy) temperatures that can be read in sequence by taking a sediment core and measuring the Mg/Ca ratio of shells found at progressively deeper, and therefore older, positions in the core.

To obtain a down-core proxy record, samples of foraminiferal shells (hereafter foraminifera) are picked from a series of sediment slices or down-core samples. Assuming, for example, that these slices are 1 cm thick and come from a core location with a constant sedimentation rate of 5 cm kyr^{-1}, foraminifera from a single slice would have a uniform distribution of ages with a width of 200 years, with a corresponding standard deviation (SD) of 58 years. However, wherever oxygenated, the surface layer of marine and freshwater sediments is mixed or bioturbated by the burrowing and feeding actions of benthic organisms, thus increasing the age-heterogeneity of material at a given depth (Guinasso & Schink, 1975; Boudreau, 1998). For simple models of sediment mixing, the standard deviation of ages at a given depth is simply the ratio of the mixed depth L and the sediment accumulation rate s (Guinasso & Schink, 1975). For a core with a 5 cm kyr^{-1} sedimentation rate and 10 cm bioturbation depth, $L/s = 2000$ years, and therefore bioturbation greatly increases the expected age-heterogeneity of a sediment slice from 58 to approximately 2000 years.

The additional age-heterogeneity created by bioturbation has important implications for sedimentary proxy records. Proxies measured on samples containing multiple individual signal carriers (e.g. foraminifera) will represent means over the time periods that have been mixed together. This has a smoothing or filtering effect on any signal, so that the observed amplitude of climate variations is reduced (Anderson, 2001). In addition to this smoothing effect, if proxies are measured on samples containing only a small number of individual signal carriers, the resulting values will be noisy means of the climate state over the time interval that has been mixed together (Schiffelbein & Hills, 1984; Kunz et al., 2020; Dolman et al., 2020). It would therefore be very useful to have an estimate of the degree of age-heterogeneity when interpreting proxy climate records.

Radiocarbon dating is the principle method used to estimate the age of sediment material younger than about 50 ka BP. The age inferred from the measured radiocarbon content is an estimate of the mean age of the particles in a given sample, and similarly, the reported machine error represents uncertainty in the mean age of the specific sample. However, the particles in a given sample are themselves only a sub-sample of the material from a given depth, and there is therefore additional, hidden, uncertainty about how representative the sample is of the age of the rest of the material from the same depth. Traditionally, radiocarbon dating required large samples of material that would necessarily include 100s of individual foraminifera (typically the equivalent of 1-5 mg C). Therefore, although it would give no indication of the heterogeneity in the age of the material, a single radiocarbon date would be a good estimate of the mean age of material at a given depth. However, the advent of ultra-small sample radiocarbon dating (Wacker et al., 2010) means that samples consisting of very small numbers of foraminifera can now be dated. With fewer individuals per sample, radiocarbon measurements become noisier estimates of the mean age of material at a given depth. However, by radiocarbon dating replicated samples of just a few individual foraminifera we can use this "noise" to estimate the age-heterogeneity of the sediment and to aid our interpretation of proxy climate records.

As described above, assuming a simple sediment mixing model, age-heterogeneity can be estimated from the ratio of the mixing depth and sedimentation rate, L/s. However, while the sedimentation rate for a given core can be readily determined using a series of down-core radiocarbon dates, the mixing depth is harder to estimate. Direct measurements using particle tracers show that L is highly variable in space (8.37 +- 6.19 cm, Teal et al., 2010) and mixing intensity may be particle size dependent (Wheatcroft, 1992; Thomson et al., 1995). Short life-span tracers, such as ^{210}Pb (half-life 26 years) may simply miss sporadic mixing events that compound over time to produce the long-term mixing behaviour. Additionally, these direct estimates of mixing depth are rarely available at proxy record core sites and in any-case give an estimate of the current mixing depth and cannot inform us about mixing depths in the past when the sediment archive was formed. Mixing depth can also be inferred from the "kink" in a series of down-core ^{14}C measurements (e.g., Trauth et al., 1997), but this requires a large number of measurements in the first 0-20 cm of the sediment core, and for gravity and piston cores the upper few centimetres are often lost during recovery. Although they integrate mixing over a longer time period than tracer experiments, kink based estimates also cannot tell us about mixing depths in the past.

Here we propose and test a method to directly estimate the age-heterogeneity of sediment by radiocarbon dating replicated samples of small numbers (3-30) of foraminifera and using the age-variation between these samples to estimate inter-individual age-heterogeneity. From this we can further infer bioturbation depths in these cores at the time the dated material was deposited. The wider use of this method would allow for a more rigorous interpretation of proxy climate records by providing direct estimates of age-heterogeneity and its smoothing effect on a per-core basis. The hidden uncertainty in radiocarbon based age-control points can also be estimated, resulting in better age-depth models. With this knowledge we can also further optimise future drilling campaigns sampling strategies. We examine the necessary conditions to use this method and estimate correction factors for the bias due to the exponential relationship between radiocarbon activity and age.

2 Materials and Methods

2.1 Physical Sampling and Radiocarbon Dating

We used foraminifera picked from five sediment cores recovered that span a range of sediment accumulation rates (approximately 2-30 cm kyr^{-}1). The sites were sampled as part of the SO184, SO213/2 and OR1-1218 cruises (Table 1, Figure 1) (Hebbeln & cruise participants, 2006; Tiedemann et al., 2014).

Radiocarbon dating was performed on samples of single species of foraminifera picked from discrete 1 cm thick sediment slices. With the exception of one sample from GeoB 10066-7, a single species was used from each core, either *Globigerina bulloides* (SO213-84-2, 250-400 μm size fraction) or *Trilobatus sacculifer* without sac-like final chamber (GeoB 10054-4, GeoB 10058-1, GeoB 10066-7, 250-400 μm size fraction; and OR1-1218-C2-BC, 300-355 μm or 315-355 μm) (Table 2).

To estimate sediment age-heterogeneity, replicated "small-n" radiocarbon dates were measured on samples consisting of between three and thirty individual foraminifera, n_f, with multiple replicate samples taken from each sediment slice, n_{rep}. We use the term "small-n" to refer specifically to samples consisting of a small number of discrete particles, or individuals, rather than samples with a small mass of carbon, but which may contain parts from a great many individuals. Additional radiocarbon dating was performed on non-replicated "bulk" samples consisting of larger numbers of foraminifera, to provide down-core age control points for estimating sediment accumulation rates. With the exception of the bulk samples from core SO213-84-2, all Accelerated Mass Spectrometry (AMS) ^{14}C dates were generated using a Mini Carbon Dating System (MICADAS) at the Alfred Wegener Institute, Bremerhaven, Germany (Wacker et al., 2010). MICADAS' capability of analysing a gas target was used for small-n samples (Ruff et al., 2010), larger samples were measured using a graphite target. Radiocarbon dating of the bulk samples from core SO213-84-2 was carried out at NOSAMS, Woods Hole Oceanographic Institution and Keck Carbon Cycle AMS Laboratory, University of California, Irvine.

Radiocarbon dates were converted to calendar ages using the Marine13 calibration (Reimer et al., 2013) and the R package Bchron (Haslett & Parnell, 2008). The Marine13 calibration includes a time-varying global marine reservoir effect. We did not adjust for local marine reservoir effects as this should not influence the variance in ages found in a given sediment slice. For each sample, the probability density function (PDF) for calendar age was summarised by its mean and standard deviation, as none of the PDFs were bi- or multi-modal.

Sediment accumulation rates were estimated by linear regression of calibrated calendar age on depth. Bulk and small-n dates from the depth range 15-100 cm (10-37 cm for OR1-1218-C2-BC) were used so as to exclude the mixed layer and to estimate the sediment accumulation rate over the range of depths for which replicated ^{14}C measurements were made. For replicated small-n dates, a mean date was first calculated for each depth. The multicore GeoB 10058-1 and gravity core GeoB 10054-4 were intended to be taken at the same site, but due to technical difficulties were in fact taken on subsequent days at locations 3 km apart (Hebbeln & cruise participants, 2006). However, their down-core radiocarbon data indicate very similar sedimentation rates (approximately 16 cm kyr^{-1}) and we combined these to create a single more robust sedimentation rate estimate.

2.2 Estimation of Age-Heterogeneity

For each sediment slice, we calculated the variance between replicated calendar age estimates, σ^2_{rep}. From this we subtracted the mean measurement error reported by the MICADAS lab for samples from that slice, σ^2_{meas}. As the ages of the individuals are in-

Table 1. Sediment cores used in this study with their locations and the research cruise during which the core was taken.

Core	Cruise	Latitude	Longitude	Water depth [m]
GeoB 10054-4	SO184	8°40'54"S	112°40'6"E	1076
GeoB 10066-7	SO184	9°23'33.6"S	118°34'31.8"E	1635
OR1-1218-C2-BC	OR1-1218	10°54'1.8"N	115°18'27.6"E	2208
GeoB 10058-1	SO184	8°40'S	112°38'E	1103
SO213-84-2	SO213/2	45°7'28.2"S	174°35'11.4"E	992

Table 2. Summary of radiocarbon dating per core and depth. Sub-core or tube is indicated in parentheses when appropriate. n_f is the number of individual foraminifera per radiocarbon dated sample, n_{rep} is the number of replicated radiocarbon dated samples.

Core	Core depth [cm]	Species	Size fraction [μm]	n_f	n_{rep}
GeoB 10054-4	28-29	*T. sacculifer*	250-400	50	1
GeoB 10054-4	48-49	*T. sacculifer*	250-400	50	1
GeoB 10054-4	68-69	*T. sacculifer*	250-400	10	10
GeoB 10054-4	88-89	*T. sacculifer*	250-400	50	1
GeoB 10058-1	11-12	*T. sacculifer*	250-400	5-6	20
GeoB 10058-1	17-18	*T. sacculifer*	250-400	110	1
GeoB 10058-1	20-21	*T. sacculifer*	250-400	110	1
GeoB 10058-1	23-24	*T. sacculifer*	250-400	110	1
GeoB 10058-1	26-27	*T. sacculifer*	250-400	110	1
GeoB 10058-1	29-30	*T. sacculifer*	250-400	5-6	20
GeoB 10066-7	23-24	*T. sacculifer*	250-400	50	1
GeoB 10066-7	48-49	*T. sacculifer*	250-400	49	1
GeoB 10066-7[a]	53-54	*G. bulloides*	250-400	10	10
GeoB 10066-7	98-99	*T. sacculifer*	250-400	53	1
OR1-1218-C2-BC (1)	36-37	*T. sacculifer*	315-355	5	10
OR1-1218-C2-BC (1)	36-37	*T. sacculifer*	300-355	30	1
OR1-1218-C2-BC (1)	36-37	*T. sacculifer*	315-355	200	3
OR1-1218-C2-BC (7,8,9)	10-12	*T. sacculifer*	315-355	200	6
SO213-84-2 (1)	1-2	*G. bulloides*	250-400	5-6	10
SO213-84-2 (1)	18-19	*G. bulloides*	250-400	>350	1
SO213-84-2 (1)	23-24	*G. bulloides*	250-400	5-6	10
SO213-84-2 (1)	23-24	*G. bulloides*	250-400	>350	1
SO213-84-2 (2)	17-18	*G. bulloides*	250-400	>350	1
SO213-84-2 (2)	20-21	*G. bulloides*	250-400	>350	1
SO213-84-2 (3)	17-18	*G. bulloides*	250-400	>350	1
SO213-84-2 (3)	21-22	*G. bulloides*	250-400	3	12
SO213-84-2 (3)	21-22	*G. bulloides*	250-400	5-6	10
SO213-84-2 (3)	21-22	*G. bulloides*	250-400	30	8
SO213-84-2 (3)	22-23	*G. bulloides*	250-400	>350	1

[a] *G. bulloides* were picked from a single slice from GeoB 10066-7

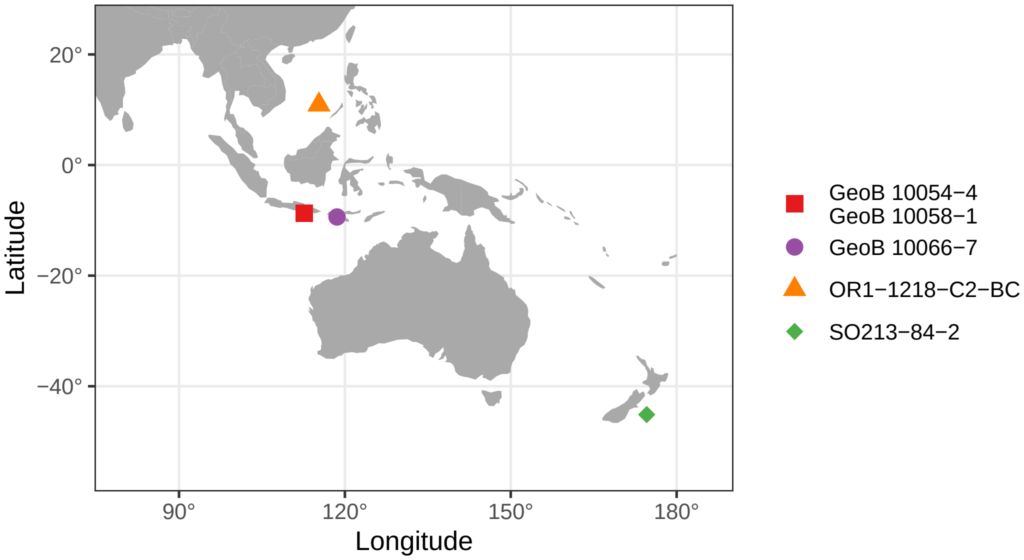

Figure 1. Locations of cores used in this study. Additional data published in Lougheed et al. (2018) from a core in the mid-Atlantic (29°59'150 W, 37°8'130 N) are included in the discussion but the core location is outside the range of this map and not shown. GeoB 10054-4 and GeoB 10058-1 are a gravity core and multicore respectively, taken at sites approximately 3 km apart.

dependent, the variance between individuals, σ^2_{ind}, can be inferred as the variance between replicates of size n_f multiplied by n_f.

$$\sigma^2_{ind} = n_f(\sigma^2_{rep} - \sigma^2_{meas}) \tag{1}$$

The inter-individual variance contains a component from the finite sediment width τ_{slice} (here 1 cm) and additional variation due to sediment mixing. We can estimate the variance due to the slice thickness using equation (2), where the 1/12 comes from the formula for the variance of a uniform distribution. After subtracting the variance due to the slice thickness we attribute the remaining excess variance to bioturbation, assuming that the radiocarbon age during deposition was the same for all particles.

$$\sigma^2_{slice} = \frac{1}{12}\left(1000 \cdot \frac{\tau_{slice}}{s} \frac{[\text{cm}]}{[\text{cm kyr}^{-1}]}\right)^2 \tag{2}$$

$$\sigma^2_{bioturbation} = \sigma^2_{ind} - \sigma^2_{slice} \tag{3}$$

To interpret this value, we use the simple bioturbation model proposed by Berger and Heath (1968) to infer a mixing depth from $\sigma^2_{bioturbation}$. Assuming that the upper L centimetres of sediment are fully and instantaneously mixed but below this level there is no further mixing, and in which the sedimentation rate and flux of foraminifera is assumed to be constant (Berger & Heath, 1968; Matisoff, 1982; Officer & Lynch, 1983), the bioturbation depth required to produce this excess age-variance is given by:

$$L = \frac{s}{1000}\sqrt{\sigma^2_{bioturbation}} \tag{4}$$

2.3 Bias Correction

Due to the exponential relationship between age and radiocarbon activity, estimates of both mean age, and age-variance between multiple samples, are biased because younger individual particles contribute exponentially more to the mean $^{14}C/^{12}C$ ratio. When the underlying age distribution is exponential, and there are infinitely many particles in the sample, there is an analytical formula for the bias in the mean radiocarbon age (Andree, 1987), however, we are not aware of a general solution for finite sample sizes. To address this we carried out a Monte-Carlo simulation study to investigate the properties of this bias and to obtain correction factors to adjust our measured age-heterogeneity estimates.

We simulated the process of sampling foraminifera from discrete depths by sampling replicated sets of n_f foraminifera from an exponential age distribution with a standard deviation corresponding to a given combination of L and s. For the purpose of the simulation we ignored the difference between calendar and radiocarbon age and convert the age of each foraminifera to an $F^{14}C$ value with the expression $F^{14}C = e^{\frac{age}{-8033}}$. For each replicate of n_f foraminifera we then calculated its mean age and mean $F^{14}C$ value. Mean $F^{14}C$ values were then back-transformed to (radiocarbon) ages, $age_{F^{14}C}$. The standard deviation between mean age and mean $age_{F^{14}C}$ values were then calculated for the replicated groups. We repeated this process for a range of underlying age variances and for groups with differing number of foraminifera per $F^{14}C$ "measurement". The difference between the standard deviation in age and standard deviation in $age_{F^{14}C}$ represents the expected bias in estimates of age-heterogeneity.

To adjust for this underestimation of age-heterogeneity we calculated correction factors by which to multiply biased estimates of age-heterogeneity (Figure 2). These correction factors likely represent an upper limit on the potential bias, as the bias depends on the shape of the underlying age distribution. If the true age-distribution differs from the assumed exponential, it is probably less skewed than an exponential and hence would produce a smaller bias. In the results we present both adjusted and un-adjusted age-heterogeneities and implied bioturbation depths. The simulation was written in R code and carried out with R version 3.6.2 (R Core Team, 2019). For more detail see Supporting Text S1 and Figure S1.

3 Results

3.1 Age-Heterogeneity in Core SO213-84-2

We first examine radiocarbon dates from the multicore SO213-84-2, for which we made measurements on groups of 3, 6 and 30 individual foraminifera, all picked from a single depth of multicore tube 3 (21-22 cm). For samples of 30 individuals, calendar ages range from 7.50 to 9.93 ka BP, with a standard deviation (σ_{rep}) of 726 years, a value far greater than the reported measurement error of about 150 years. Variation in age between samples is even greater for replicates of 6 foraminifera (range = 6.57 - 12.23 ka BP, σ_{rep} = 1514 years) and 3 foraminifera (range = 4.32 to 13.99 years BP, σ_{rep} = 2895 years). Clearly, the calibrated calendar ages of these replicated samples do not agree with each other within their reported uncertainties and this excess variation decreases strongly with the number of foraminifera per measurement (Figure 3). Additional measurements on replicated samples of 5-6 individuals taken from multicore tube 1 at depths of 1-2 and 23-24 cm have similarly large σ_{rep} values of 1187 and 1575 years.

The relationship between σ_{rep} and the number of individuals per measurement very closely follows an inverse relationship (Figure 4). This is a strong indication that inter-individual age variation (σ_{ind}) is the major component of the between sample variation and allows us to infer σ_{ind} by scaling for the number of foraminifera per sample, after first subtracting the much smaller reported measurement error (Equation 1). Inferred age-heterogeneity between individuals, σ_{ind}, from core SO213-84-2 ranges from 2854 to

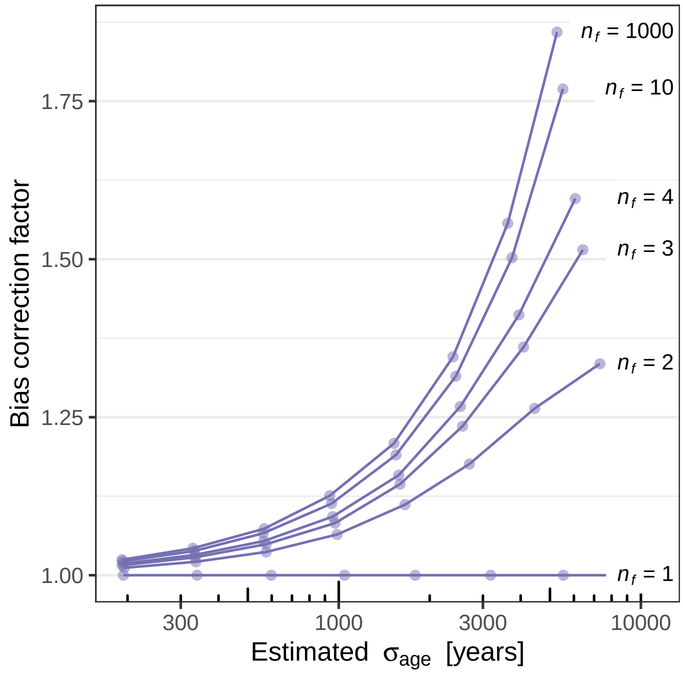

Figure 2. Bias correction factors to correct for the underestimation of age-heterogeneity due to the exponential relationship between radiocarbon activity and age.

4990 years (Table 3). Bias correction factors for SO213-84-2 estimated by simulation vary between 1.36 and 1.51, depending on the number of foraminifera per sample. Adjusting for the bias, the range of $\sigma^2_{ind_{adj}}$ increases to 3881 - 6847 years. Also shown in Table 3 is the much smaller age-heterogeneity of approximately 100 years expected due to the 1 cm thickness of the slice and the 2.9 cm kyr^{-1} sedimentation rate. After subtracting this, and assuming a simple sediment mixing model (Berger & Heath, 1968), the excess age-heterogeneity implies a mixing depth of 11.2 - 19.8 cm (8.3 - 14.4 before bias adjustment) (Equations 1-4, Table 3). Age-heterogeneity is somewhat lower for the samples from 1-2 cm deep, which would be in the active mixing layer, than for the other deeper samples.

3.2 Age-Heterogeneity Across Multiple Cores

To test the generality of this result we performed similar replicated small-n radiocarbon measurements at 4 additional sites with sediment accumulation rates of approximately 2, 16 (2 sites), and 29 cm kyr^{-1}. We again adjust the measured age-heterogeneity for bias assuming an exponential age distribution and present both adjusted and un-adjusted age-heterogeneities and bioturbation depths for comparison. To examine the relationship between age-heterogeneity and sedimentation rate across cores, we additionally present the inter-individual age-heterogeneity and implied bioturbation depth for core T86-10P from the North Atlantic using data published in Lougheed et al. (2018).

Estimated age-heterogeneity is again much higher than the measurement error in most cases, with between replicate standard deviations of 287, 603 and 3208 years, compared to measurement errors of 153, 110, and 304 years (Table 3). The one exception is core GeoB 10066-7 for which σ_{rep} is only 172 years (+- 40 SE) compared to a measurement error of 185 years. While this could imply no mixing at all ($L = 0$ cm), because this core has a relatively high sedimentation rate of 29 cm kyr^{-1}, and because the value

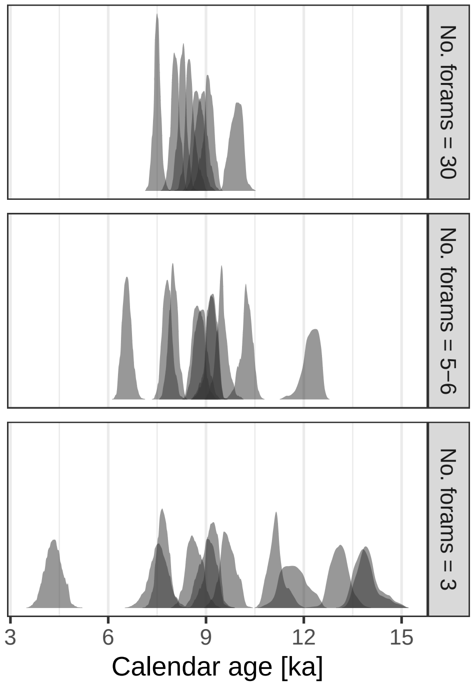

Figure 3. Replicated radiocarbon dates converted to calendar ages from a single 1 cm thick sediment slice, taken at a depth of 21-22 cm, from core SO213-84-2. Each individual density plot shows the probability density function of calendar age obtained by calibrating a radiocarbon age measured on a sample consisting of 3, 5-6 or 30 individual foraminifera (^{14}C age +- 1 SD) with the Marine13 calibration curve (Reimer et al., 2013). No local adjustment was made to the global marine reservoir effect contained in Marine13.

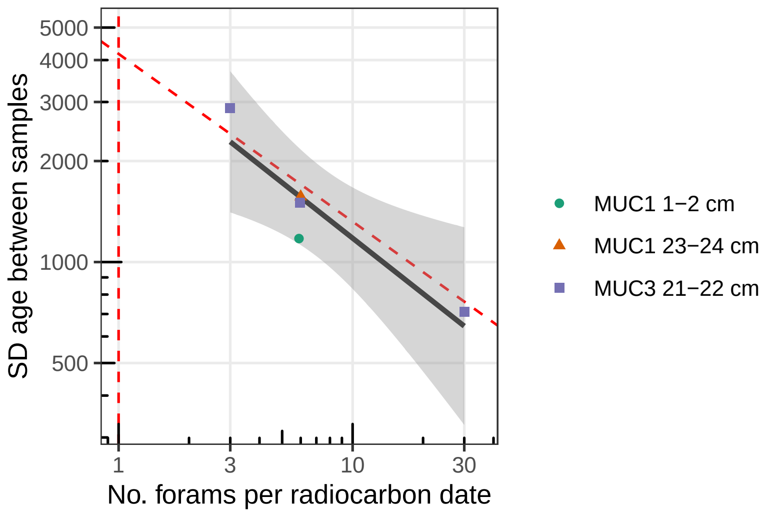

Figure 4. Standard deviation in age between radiocarbon dated samples from core SO213-84-2 as a function of the number of foraminifera they contain. The dashed red lines show extrapolation back to samples of single individual foraminifera assuming the theoretical proportional relationship between standard deviation and the square root of sample size. The samples came from two different multicore tubes of the same deployment.

Table 3. Measured standard deviation between replicated ^{14}C measurements on small-n samples of foraminifera, inferred age-heterogeneity between individual foraminifera and the implied bioturbation depth. n_f is the number of individual formaminifera per radiocarbon measurement, n_{rep} is the number of replicate radiocarbon measurements made on samples of n_f individuals, σ_{rep} is the standard deviation between replicated radiocarbon measurements made from samples from the same sediment slice, $SE_{\sigma_{rep}}$ is the standard error of the estimate of σ_{rep}, σ_{meas} is the reported measurement error, σ_{ind} is the inferred standard deviation in age between individuals. *Bias* is the estimated proportional bias in σ_{age} due to the exponential relationship between radiocarbon activity and age. Values with the subscript *adj* have been corrected for this bias. s and L are the sediment accumulation rate [cm kyr^{-1}] and inferred bioturbation depth [cm], respectively. Several samples at 11-12 cm from core GeoB 10058-1 had negative radiocarbon dates indicating the presence of modern material and could not be calibrated.

Core	Depth [cm]	n_f	n_{rep}	σ_{rep}	$SE_{\sigma_{rep}}$	σ_{meas}	σ_{ind}	Bias	$\sigma_{ind_{adj}}$	σ_{slice}	s	L	L_{adj}
GeoB 10054-4	68.0	10	10	287	58	153	766	1.07	820	18	16.3	12.5	13.4
GeoB 10058-1	11.5	5-6	20	..	..	98	..	1.10	..	18	16.3	..	..
GeoB 10058-1	29.5	5-6	20	603	97	110	1327	1.10	1458	18	16.3	21.7	23.8
GeoB 10066-7	53.0	10	10	172	40	185	526	1.05	553	10	28.9	15.2	16.0
OR1-1218-C2-BC	36.5	5	10	3208	763	304	7142	1.66	11855	169	1.7	12.2	20.2
SO213-84-2	1.5	5-6	10	1187	281	169	2854	1.36	3881	100	2.9	8.3	11.2
SO213-84-2	23.5	5-6	10	1575	374	168	3836	1.36	5218	100	2.9	11.1	15.1
SO213-84-2	21.5	3	12	2895	621	283	4990	1.37	6847	100	2.9	14.4	19.8
SO213-84-2	21.5	5-6	10	1514	359	176	3668	1.36	4989	100	2.9	10.6	14.4
SO213-84-2	21.5	30	8	726	193	152	3888	1.51	5858	100	2.9	11.3	17.0

Table 4. Sediment accumulation rate s (cm kyr^{-1}) and estimated bioturbation depth L (cm) at 4 sites measured in this study, plus one (T86-10P) previously published by Lougheed et al. (2018). SE_s is the standard error of the estimate of s, L_{adj} is the inferred bioturbation depth adjusted for the bias due to the exponential relationship between age and radiocarbon content.

Core/Site	s	SE_s	L	L_{adj}
GeoB 10054-4/58-1	16.3	1.8	16.3	17.7
GeoB 10066-7	28.9	2.4	15.2	16.0
OR1-1218-C2-BC	1.7	0.1	12.2	20.2
SO213-84-2	2.9	0.7	11.1	15.5
T86-10P	2.2	..	10.8	10.8

of σ_{meas} is itself an estimate with its own uncertainty, it is also consistent with mixing of several centimetres. For example, assuming a 15 cm bioturbation depth and given the 10 foraminifera per sample, the expected σ_{rep} would be just 164 years. To provide an upper estimate on the inter-individual age-variance and bioturbation depth for this core, we subtract only the error due to the binomial counting statistics for $^{14}C/^{12}C$ (45 years), essentially assigning all additional error to age-heterogeneity. Additionally, several samples taken from GeoB 10058-1 at 11.5 cm deep could not be calibrated with Marine13 as they were younger than the minimum 448 radiocarbon years that can be calibrated with Marine13, including some with negative radiocarbon dates indicating the presence of modern material down to at least 11-12 cm.

Across all analysed cores we found a strong negative relationship between sedimentation rate s and inter-individual age-heterogeneity, a clear indication that sediment mixing influences age-heterogeneity. Due to this negative relationship, the implied bioturbation depths for all sets of replicated samples fall within a relatively narrow range of 11.2 - 23.8 cm (Figure 5, Table 3). At the site level, after combining estimates for the same core taken from different depth layers, and combining GeoB 10054-4 and GeoB 10058-1 which come from two sites less than 3 km apart, implied bioturbation depths for the individual sites range from 15.5 - 20.2 cm (Table 4). For core T86-10P, Lougheed et al. (2018) report a mixing depth of 10.8 cm.

The relationship between s and σ_{ind} is only slightly altered by the bias adjustment, which is small compared to other sources of variation in age-heterogeneity. Adjustment is largest for core OR1-1218-C2-BC, for which the simulation study indicated a factor of 1.66, and which has the lowest sedimentation rate and highest estimates of individual age-heterogeneity. The adjustment shifts the implied bioturbation depth from 12.4 to 20.2 cm.

4 Discussion

We found variation in radiocarbon ages between replicated small-n samples of foraminifera that far exceeded the reported machine uncertainty at three of the four sites we examined. Between-replicate age-variation was only within the machine uncertainty for core GeoB 10066-7, which has a comparatively high sedimentation rate of 29 cm kyr^{-1}. Age-heterogeneity also far exceeds measurement error for a fifth core examined by Lougheed et al. (2018). This excess age-variation can be interpreted as within-sediment-layer heterogeneity caused by bioturbation. Assuming the classical Berger and Heath (1968) mixing model, the implied mixing at the five sites is 11-20 cm. This is somewhat higher than the 10 cm often assumed as typical value in literature (Boudreau, 1998) and consider-

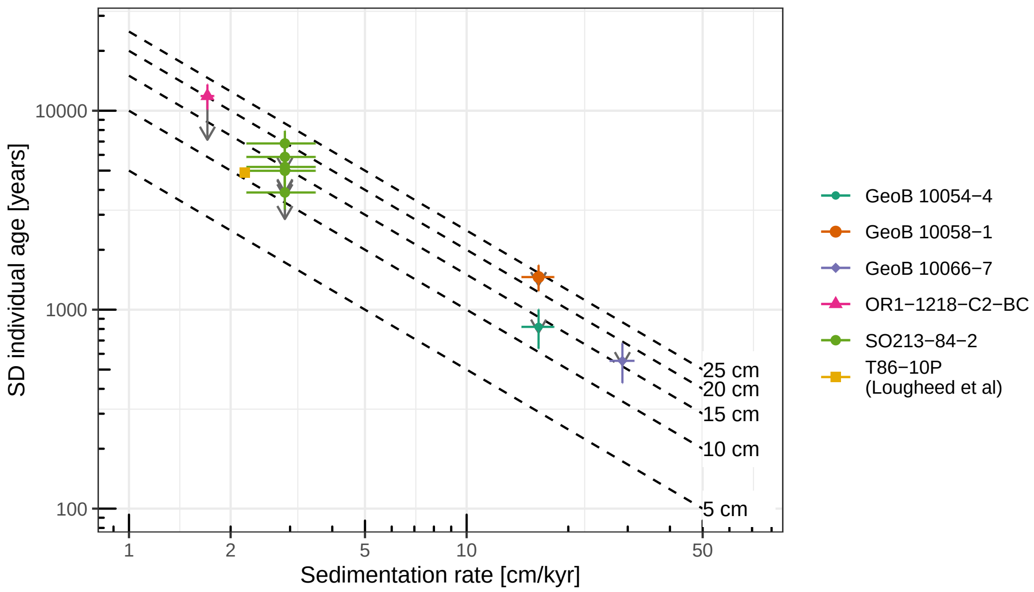

Figure 5. Inferred standard deviation in age between individuals $\sigma_{ind_{adj}}$ plotted against sediment accumulation rate s. Error bars indicate one standard error of the standard deviation and sedimentation rate estimates. The dashed isolines indicate bioturbation depths L consistent with a given sedimentation rate and σ_{ind}. The grey arrows indicate σ_{ind} prior to correcting for the bias due to the exponential relationship between age and radiocarbon content. The bias adjustment is much larger for cores with low sedimentation rates and high estimates of σ_{age}.

ably higher than the bioturbation assumed in the interpretation of most paleoclimate records.

Age-heterogeneity of this magnitude has important implications for proxy records recovered from these cores. The climate signal is strongly smoothed by the mixing together of time periods, reducing the inferred amplitude of climate variations (e.g., Anderson, 2001), but, if the proxy measurements are made on small numbers of foraminifera, records can also become noisier as the signal from different climate states is mixed together. In extreme cases measurements can include both glacial and interglacial material. This noise is especially problematic when the variance itself is of interest, for example in individual foraminiferal analyses (Groeneveld et al., 2019; Wit et al., 2013; Koutavas & Joanides, 2012; Thirumalai et al., 2019, 2013). Estimates of age-heterogeneity from replicated small-n radiocarbon dates can be used to parametrise proxy forward models to quantitatively assess this smoothing and noise generation (Lougheed, 2020; Dolman & Laepple, 2018).

A further implication is that radiocarbon dates used for age-depth modelling may require much larger uncertainties than the reported machine errors that are typically used. Although they may correctly quantify the uncertainty in the age of the sample, they ignore the uncertainty in how representative the sample may be of mean age of material at the depth from which it was recovered (Heegaard et al., 2005). The size of this effect will depend on the bioturbation depth, the sedimentation rate and the sample size. We can see this effect for the low sedimentation rate multicore SO213-84-2, for which a series of down-core radiocarbon dates were made in each of 3 sub-cores. These replicated age-depth series show very little overlap within their reported age-uncertainties (Figure 6a), despite having been measured on samples of approximately 350 foraminifera each.

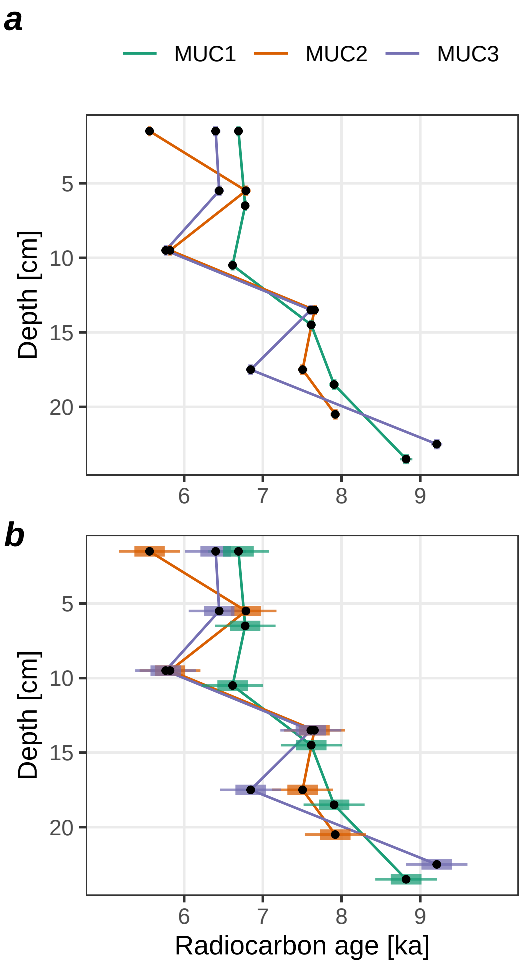

Figure 6. Replicated down-core radiocarbon age estimates for SO213-84-2. Each down-core record corresponds to a separate multicore tube or half tube from the same deployment. Age-uncertainties in subplot (a) are +- 2 times the reported machine error, whereas those in (b) include the inferred σ_{age} between individuals, scaled for samples of 350 individuals.

However, adding the expected uncertainty due to age-heterogeneity brings the three down-core age-depth series into much closer agreement (Figure 6b). Radiocarbon dating small-n samples, either because the sediment material contains only few foraminifera or to save picking and processing time, risks further inflating this additional error. To guide the choice of sample size, we have created lookup figures, based on equation 5, for mixing depths of 5, 10, 15 and 20 cm (Figure 7, S2). These can be used to get a rapid idea of the number of individual foraminifera per sample required to reduce the additional age-uncertainty below a desired level, or inversely, given a radiocarbon date we can estimate the additional hidden uncertainty from age-heterogeneity from the sedimentation rate and an estimate of the number of individuals in the sample.

$$n_f = \left(\frac{1000L}{s \cdot \sigma_{rep}} \right)^2 \quad (5)$$

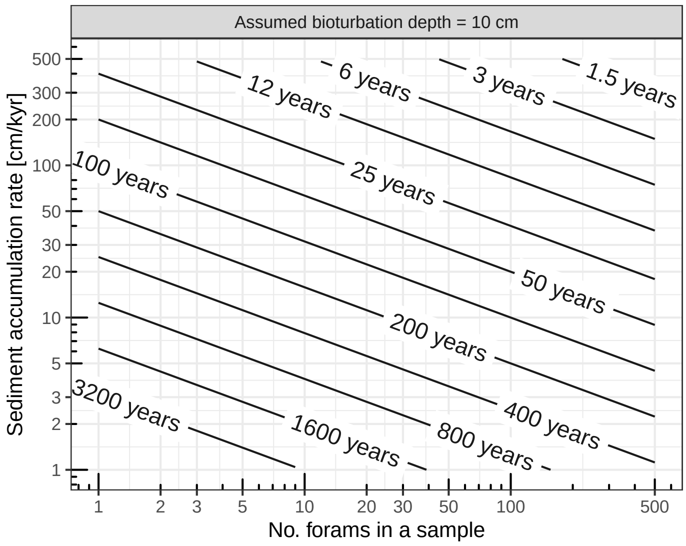

Figure 7. A reference chart to obtain estimates of the additional age-uncertainty σ_{age} for a sample measured on a given number of foraminifera, from the sedimentation rate of the core s, and assuming a bioturbation depth L of 10 cm. Or alternatively, an estimate of the number of foraminifera per sample needed to reduce σ_{age} below a given level. E.g. for a core with s = 5 cm kyr^{-1}, to get the additional age-uncertainty below 200 years you need at least 100 foraminifera; if s were 20 cm kyr^{-1} you would need only 6-7 foraminifera. The σ_{age} values of the isolines are proportional to L, so if a larger, 20 cm, bioturbation depth is suspected, double the isoline values. Note however, altering the mass of material processed and measured may also influence the reported instrument error - and the characteristic sizes of different foraminiferal taxa will impose their own constraints on the number of specimens required.

4.1 The Physical Mixing Process and Outliers

The concept of a bioturbation depth is an obvious simplification; however, as the age-heterogeneity is related to the sedimentation rate regardless of the precise mixing process (Matisoff, 1982), the specific mixing model assumed is not particularly important for the main conclusions here. We can still however question the extent to which our measured radiocarbon dates are consistent with the Berger and Heath (1968) mixing model. In contrast to Lougheed et al. (2018), who estimated that around 10% of their foraminifera had ages inconsistent with a simple mixing model, we found very few extreme outlying dates which might be evidence of unusually deep mixing events like *Zoophycos* burrows (Küssner et al., 2018). However, as we dated samples containing multiple foraminifera, individuals with aberrant ages may be hidden, as every distribution will converge towards a Gaussian distribution as the number of individuals increases (Figure S3). Therefore it is unclear the extent to which additional disturbance by *Zoophycos*, or other deep mixing mechanisms, contribute to the age-heterogeneity we measure. The single clear outlier we did obtain was measured on just three individuals, and was too young by about 5000 years in a core with sedimentation rate of 2.9 cm kyr^{-1} (core SO213-84-2). This implies a relative displacement of approximately 43.5 cm for one of the three foraminifera, which would be consistent with the known size of *Zoophycos* burrows (Wetzel & Werner, 1980). Additional displaced individuals hidden inside multi-individual measurements would mean that we have overestimated the depth of the well mixed layer.

The specific form of mixing and its resulting probability distribution of ages does have implications for the bias generated by the exponential relationship between age and the $^{14}C/^{12}C$ ratio. We calculated biases for the highly skewed exponential distribution resulting from the Berger and Heath (1968) mixing model; less skewed distributions, resulting for example from incomplete mixing or a smooth transition between the mixed layer and the unmixed sediment, will generate a smaller bias. Therefore our bias correction which assumes an exponential distribution may be too strong and probably represents an upper limit. This bias could potentially be eliminated by dating individual larger foraminifera (e.g., Lougheed et al., 2018), which would also remove the issue of hidden outliers.

In principle, $\Delta^{14}C$ variations across the water column also cause some apparent age-heterogeneity due to differences in the calcification depth of the individual foraminifera. However, even assuming a strong $\Delta^{14}C$ gradient (0.2 permille change per meter) and a highly variable calcification depth (uniform probability of calcifying between 0 and 100 m), the resulting heterogeneity ($\sigma = 50$ years) is small compared to the age-heterogeneity found in this study. Over most of the ocean the $\Delta^{14}C$ gradient is weaker than this (Key, 2001), and individual foraminifera may incorporate carbon over a range of depths during their calcification.

4.2 Practical Considerations When Applying This Method

We have demonstrated the use of small-n radiocarbon measurements to estimate site and core-depth specific bioturbational mixing. This knowledge is especially important when a high-resolution analysis or the analysis of individual foraminifera (IFA) is planned, and it is our hope that bioturbation estimates will become routine in these applications. However, there are some practical considerations when applying this method.

Firstly, the estimation only works if the age-heterogeneity is larger than the measurement error. For the data presented here, measurement error ranged from about 80 to 400 years. At sedimentation rates below about 2 cm kyr^{-1}, age-heterogeneity from bioturbation will far exceed this measurement error, even for relatively small bioturbation depths. However, as s rises, the expected age-heterogeneity between individuals (Figure 5, dashed lines), or samples (Figure 7, contour lines), falls rapidly. Furthermore, for many foraminifera taxa, single specimens cannot be dated, even with MICADAS, and

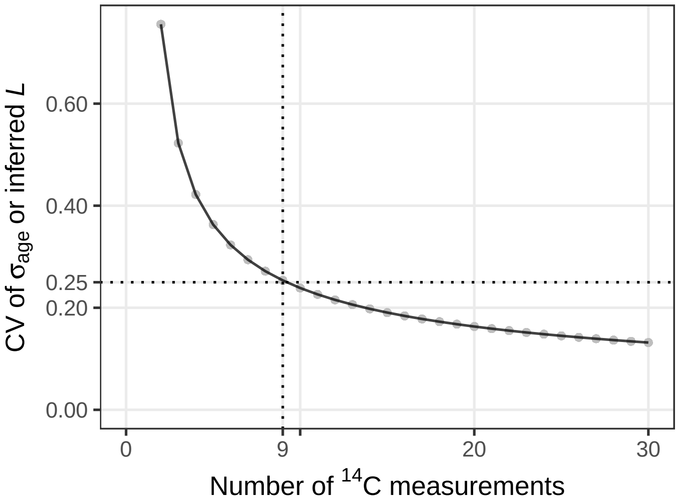

Figure 8. Coefficient of variation for estimates of σ_{age} or the implied bioturbation depth L as a function of the number of dated samples n_{rep}. Dashed lines indicate that for 9 replicated ^{14}C measurements there would be a 25% uncertainty in the estimated values of σ_{age} and L.

so the approach of dating small-n samples has to be used - reducing the signal of age-heterogeneity by a factor of n_f.

Secondly, the uncertainty, or standard error (SE), of a standard deviation depends on the number of samples measured (Equation 6), hence a sufficient number of small-n samples needs to be measured in order to get a reliable estimate of σ_{ind}, and in turn to estimate L with a given precision. For example, with approximately 9 samples, the proportional uncertainty (or coefficient of variation) of the standard deviation is approximately 1/4 (Figure 8), therefore with true bioturbation depths of 10 or 2 cm we would expect estimates of 10 +- 2.5 cm or 2 +- 0.5 cm respectively.

$$SE_{\sigma_{ind}} = \frac{\sigma_{ind}}{\sqrt{2(n_s - 1)}} \tag{6}$$

5 Conclusions

An awareness of bioturbation and its potential influence on sedimentary proxy records due to the age-heterogeneity it causes is not new (e.g., Schiffelbein, 1985; Andree, 1987; Keigwin & Guilderson, 2009; Steiner et al., 2016; Goreau, 1980); however, it has only recently become possible to directly measure the age-heterogeneity in sediment slices of the medium that is radiocarbon dated, e.g. foraminifera. We measured age-heterogeneities that imply much deeper mixing than is typically assumed in the paleo-climate literature. At the same time, we found that between core variation in age-heterogeneity could largely be explained by sedimentation rates, which implies a relatively consistent mixed layer depth. It is conceivable that the "paleo" bioturbation depth is larger and less variable than measurements of contemporary bioturbation depths would imply (e.g., Solan et al., 2019), as integrated over time, a long period of shallow mixing would be obliterated by a subsequent period of deep mixing; where "long" is relative to the sedimentation rate. The availability of small-n radiocarbon dating will allow us to assess how consistent bioturbation depths really are, in addition to obtaining independent estimates of age-heterogeneity to aid our interpretation of proxy climate records.

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Radiocarbon dates have been submitted to Pangaea (www.pangaea.de - DOI pending). Additionally, the data and R code to reproduce the analyses are supplied as Supporting Material to this manuscript (Data Set S1).

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