Quantifying bioturbation of a simulated ash fall event

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Abstract: Tephrochronology allows the establishment of “isochrons” between marine, lacustrine, terrestrial and ice cores, typically based on the geochemical fingerprint of the tephra. The development of cryptotephrochronology has revealed a vast inventory of isochrons which hold the potential to improve stratigraphic correlation and identify systemic leads and lags in periods of rapid climate change. Unfortunately, bioturbation acts to blur these isochrons, reducing the temporal resolution in marine and lacustrine records. In order to better resolve these event horizons, we require a better understanding of bioturbative processes, and the depth and time over which they operate. To this end, an ash fall event was simulated on the intertidal zone of the Eden Estuary, Fife, Scotland and sediment cores were collected over 10 days. A novel approach to tephra quantification was developed, using the imaging software ImageJ. Our results showed limited bioturbation (mixed depth = 18 mm), most likely owing to the fine grain size, low-energy environment and the resulting faunal composition of the sediments. These results imply a strong ecological control on bioturbation, and suggest that inferences may be made about palaeoenvironments from the observed bioturbation profiles.

Supplementary material: The ImageJ macro used in this study, as well as raw tephra concentration data and details of the method validation are available at http://www.geolsoc.org.uk/SUP18725.

In most marine or lacustrine benthic habitats, the surficial sediment layer is very likely to be mixed and reworked by its epifauna and infauna, creatures living on and in the sediment, respectively (e.g. Berger & Heath 1968), as well as by any fish or mammals which come into contact with it (Biles et al. 2002). The collective mixing effect of this biological activity is known as bioturbation. In terms of the processes involved, bioturbation occurs largely through burrowing, feeding, defecation and locomotion.

Bioturbation processes are of interest to researchers in many fields. For marine ecologists, bioturbation is an essential set of processes that enhance nutrient release from the sediment (Solan et al. 2004a) as well as increasing the surface area of the sediment–water interface (Biles et al. 2002). Within the context of evolutionary theory, bioturbation is a classic example of ecosystem engineering, the alteration of a habitat by its inhabitants (Meysman et al. 2006). However, to palaeoceanographers, bioturbation presents a problem because the processes of bioturbation act to mix younger material into the older sediments below, and vice versa. Bioturbation therefore acts to obfuscate the geological sedimentary record. This makes it difficult to identify boundaries between strata which may have been relatively clear at the time of deposition.

Previous bioturbation investigations (Solan et al. 2004b) have focussed on bioturbation at the scale of individual events using, for example, time lapse photography. However, carrying out such an investigation is expensive; furthermore, while individual bioturbation events are of interest to ecologists, geochronology tends to be concerned with the net effect of repeat events. Unlike ecologists, geochronologists are not primarily interested in the individual bioturbation events but, rather, the integrated result of this process through time.
Guinasso & Schink (1975) reviewed bioturbation from an oceanographic perspective, and described it in terms of three principle parameters: an eddy diffusion rate parameter, $D$; a maximum mixing depth, $L$; and sediment deposition rate, $v$. They also defined a dimensionless bioturbation index:

$$\text{bioturbation index} = \frac{D}{Lv}$$

whose value describes the homogeneity of the surface mixed layer, with low values describing limited mixing and vice versa. An important fact highlighted by this bioturbation index is that the extent of mixing is affected not only by the processes of bioturbation, but also by the rate of sediment burial.

**Tephrochronology**

Tephrochronology uses tephra layers from past volcanic eruptions as isochronous marker horizons, layers of the same age in different geological records. On geological time-scales, tephra deposition by a volcanic eruption is effectively synchronous and can occur over a wide geographical area, making it especially useful in studying the (a)synchrony of rapid climate transitions, between disparate sequences and where traditional dating methods lack sufficient resolution (Mangerud et al. 1984; Haflidason et al. 2000; Austin et al. 2004; Austin & Abbott 2010). For example, Austin et al. (2004) demonstrated how the correlation of the rhyolitic component (II-Rhy-I) of North Atlantic Ash Zone II between the GISP2 ice-core and a Northeast Atlantic marine core (MD95-2006) suggested the regional synchronicity of the abrupt cooling at the end of Greenland Interstadial-15.

One of the great values of tephrochronology lies in the recognition of globally synchronous ash fall events. Unfortunately, an inability to precisely define the position of a tephra isochron in a marine sequence owing to various source, transport, depositional and post-depositional processes, including bioturbation, may sometimes arise. This makes bioturbation particularly problematic for tephrochronologists, especially in the analysis of cryptotephras, whose tephra deposits are invisible to the naked eye. While a non-bioturbated cryptotephra may be detected in a sediment core as a distinct peak in shard concentration, it is often the case that shards from the same population are found through a considerable depth of the core. In these cases, there is some confusion in the literature as to where to define the actual depth of the marker horizon (i.e. isochrons; Lowe 2011; Davies et al. 2012).

The situation is further complicated by the fact that bioturbation is a highly variable process; the rate and maximum extent of bioturbation depends not only on the collection of species which make up the benthos and their abundance, but also on the thickness of the ash layer and the subsequent rate of deposition. The work of Carter et al. (1995) suggested that thicker (>1 cm) ash layers tend to smother the benthos, minimizing bioturbation. It also suggested that, if rapid deposition occurs after the ash fall event, the tephra layer will be isolated before the benthos can be re-established, also minimizing bioturbation.

By simulating an ash fall event on a marine estuary and then studying the bioturbation of tephra over a period of 10 days, this paper aims to produce a better understanding of the factors which affect the rate and extent of bioturbation, within the wider context of marine tephrochronology.

**Methods**

**Site description**

In order to explore the questions outlined above, an ash fall event was simulated in the intertidal zone of the Eden Estuary, Fife (56.355° N, 2.839° W, Fig. 1) in July 2010. The decision to study bioturbation in an estuarine environment was made for several reasons. From a practical perspective, intertidal zones are easily accessible and are often well documented; in this case, a commissioned report by Scottish Natural Heritage (Bates et al. 2004) outlines the spatial extent of different biotopes on the Eden Estuary, which are shown in Figure 1. This is particularly useful when studying ecosystem processes as it eliminates the need for a full site survey. Furthermore, unlike high-energy coastal systems where bioturbation is limited and disrupted by wave and tide activity (Cadée 2001), estuaries are relatively low-energy environments, analogous to the deep sea-bed.

Our study site is a sheltered estuarine mud-flat, with minimal wave activity, making it ideal for investigating bioturbation. The site is exposed for approximately 7 h of every tidal cycle (Austin 2003).

As illustrated in Figure 1, the study site is located near the shoreline, in a biotope classified as ‘LMU, HedMac’ (Connor et al. 1997a, b). This classification refers to a well-sheltered intertidal zone comprising sandy mud characterized by the presence of Nereis (Hediste diversicolor) and Baltic clam (Macoma baltica), as well as other oligochaetes, polychaetes and bivalves (Bates et al. 2004). A comprehensive database of all biotopes identified in Figure 1 can be found online at www.marlin.ac.uk. Previous efforts to quantify sediment bioturbation...
have suggested that the annelids (polychaetes and oligochaetes) play a significant role in estuarine bioturbation (Biles et al. 2002). Therefore, the dominance of Nereis and the presence of other annelids at this site suggested that bioturbation would indeed occur.

Characterization of tephra

Ash was collected from Seljavellir, South Iceland (63.6°N 19.7°W) in June 2010 following the main eruptions of Eyjafjallajökull earlier in the year. Laser grain size analysis was carried out on a tephra sample using a Beckman Coulter LS 230 Laser Diffraction Particle Size Analyzer at the Facility for Earth and Environmental Analysis at the University of St Andrews. Riffle box splitting was used to ensure a representative sub-sample was obtained. The results (Fig. 2) show that this proximal tephra sample is fairly coarse ($\bar{x} = 299.4\ \mu m$, median = $228.3\ \mu m$). This is coarser material than would be expected to occur in distal ash fall deposits (e.g. Abbott et al. 2011) as the size of airfall material decreases exponentially with distance from eruptive source (Sparks et al. 1981). The tephra was found to be a mix of pumaceous and glassy shards containing vesicles. There was no specific or diagnostic morphology; a mixture of spherical and elongate, platy and blocky shards was observed.

Simulating an ash fall event

Ash was spread in $20 \times 20$ cm square quadrats directly onto the exposed intertidal estuarine sediments. We visited the study site at low tide on 22 July and seeded 18 quadrats with 60 g ($37.8\ cm^3$) of ash each, equivalent to an ash layer of 0.95 mm thickness. So that results were comparable, quadrats were placed in a grid with 1 m spacing between each. This ensured that all quadrats were placed within an ecologically homogenous area and that all experienced the same tide and wave effects. Closer spacing was considered, but it was felt that this might lead to inter-quadrat ash contamination. Additionally, the possibility of a raised edge on the quadrat was considered, to prevent outwashing of tephra by waves and tide; however, it was felt that this would significantly increase the ecosystem disturbance. Furthermore, in such a low-energy environment, tephra loss is likely to be minimal. This point is addressed further in the Discussion.
Figure 3 shows a typical quadrat with signs of burrowing clearly visible in the bottom half of the image.

**Field sampling and laboratory sub-sampling**

Coring was carried out after 0, 2, 5 and 10 days, using a sample tube of 26 mm diameter with the base removed. This relatively simple setup allowed cores of 60 mm depth to be taken, from the middle of each quadrat. On day 0, six quadrats were cored and one control core was taken from an adjacent location on the mudflat, unseeded with tephra. The main purpose of the day 0 cores was to test the coring method; any ash found below the surface section of the day 0 cores will represent disturbance through corer insertion into the sediment. Three of these ‘day 0’ cores were frozen and three were maintained at room temperature, in order to determine the best method of storage before sub-sampling. However, it quickly became apparent that, if cores were not frozen, bioturbation would continue after collection, invalidating the resultant experimental data. Therefore, the three unfrozen cores were discarded. On days 2, 5 and 10, four replicate cores were taken and frozen immediately after collection. Cores were coded in a ‘day-sample number’ format (i.e. Core 0-2 refers to the second core taken on day 0). Frozen cores were sliced into 3 mm sections using a three-way clamp, and the sections were prepared for analysis as outlined in Figure 4. The samples were prepared for microscopy by wet sieving (63 μm) to remove the fine fractions; this facilitates the drying, disaggregation and counting of the samples. The aim of this process was to produce samples which could be accurately and rapidly counted by the process outlined below.

**Quantifying bioturbation**

As discussed above, we treat bioturbation as a diffusive process. Thus, we sought to analyse the presence of tephra such that it could be expressed as a change in concentration through depth and time. Therefore, we determined the number of tephra grains at specific depths in the cores relative to the overall number of mineral grains at these depths and expressed these as percentage counts. Typically, this counting is carried out manually using a picking tray and a tally counter. Repeated counts are carried out until a statistically reliable value is attained for tephra shard concentration. This method is extremely time-consuming; we estimate that to process all 300 sub-sampled sections, each requiring the counting of 15 squares, would take up to 400 h (i.e. 50 standard working days). In order to reduce the analysis time, the possibility of a faster, semi-automated method was investigated.

Costa & Yang (2009) presented a solution to a similar problem involving the counting of pollen grains. Rather than counting manually, they used...
Fig. 3. Microcosm on Eden Estuary (20 × 20 cm) at (a) day 0 and (b) day 2. By day 2, tephra is barely visible at the surface.
ImageJ, an open-source Java-based image analysis program to count thousands of grains at a time. Furthermore, they developed a macro for the program which would automatically open a specified series of microscope images, count all the pollen grains in each image and record the results in Excel format. They estimated a 5- to 60-fold saving in time using this method, depending on the number of grains in each image.

In order to determine the applicability of ImageJ to the problem of determining tephra shard concentration, a series of trials was carried out. The optimized process resulting from these trials is described below and illustrated in Figure 5, and the parameters and algorithms of choice are outlined in the macro file available in the Supplementary Material.

Using image analysis to quantify tephra concentrations

The first stage of automated counting of particles was image capture. Material from each processed section was placed on a piece of white paper to enhance contrast, then placed under a Zeiss Stemi 2000 optical stereomicroscope. Using an attached Deltapix Infinity X-21 CMOS camera, 10 unique images were taken of each sub-sampled section, each with a field of view of 10.67 × 8.53 mm (1280 × 1024 pixels). Although ImageJ can handle clustered particles, it does not deal well with large amalgamations of grains, and so care was taken to ensure that grain density was sufficiently low (<500 grains per image) that minimal clustering occurred. Care was also taken to ensure that the microscope’s light source produced a relatively uniform intensity across the field of view, as the performance of ImageJ is enhanced when analysing particles on a constant background. It should be noted, however, that the program is capable of ‘subtracting’ the image background.

As illustrated in Figure 5, particle analysis in ImageJ is a multistage process. First, the image is sharpened to better delineate the outline of individual grains. Then the image background is ‘subtracted’. ImageJ carries out background subtraction by identifying the predominant trend in colour, saturation and hue across the image, based on the assumption of a light background. Following background subtraction, the image is converted from RGB to 8-bit greyscale. The example in Figure 5a shows an image that has undergone the sharpening, background subtraction and 8-bit conversion, which are prerequisites for ‘autothresholding’ (Fig. 5b).

Autothresholding describes the use of an algorithm that automatically distinguishes particles from background without user input. It converts the greyscale image in Figure 5a into a binary image (Fig. 5b) where black represents ‘particle’ and white represents ‘background’. This feature is one of the two automatic functions that make ImageJ so useful for particle analysis, the second being the watershed function (Fig. 5c). The watershed function takes the dark patches on a light background produced by autothresholding and determines, based on their geometry, which patches are likely to be multiple particles touching each other. Finally, the program counts, measures and labels the particles (Fig. 5d) based on a preset size threshold (area >100 pixels/6940 μm²). This threshold corresponds, for a perfectly round particle, to a diameter of 93 μm; this is sufficiently above the 63 μm sieve size to ensure that small particles present owing to incomplete sieving are not counted.

Performing the individual steps shown in Figure 5 on each of the 3000 images in turn would still be very time-consuming; therefore, a macro was written, based on that of Costa & Yang (2009), which first prompts the user to select a directory containing images, and then proceeds to open each image in turn, perform each of the steps in Figure 5 and then save the results in an appropriately named Excel file. In this way, the images are processed at roughly one per second and all 3000 images can be processed in under an hour. Including the few hours required to capture all the images under the microscope, this represents an almost 100-fold saving in laboratory time and introduces an improved uniformity of analysis.

The method outlined above is suitable only for counting the total number of grains; identification of tephra shards in bulk sediment by eye is an acquired skill and could not be readily automated with this image analysis software. As such, the final stage of analysis in ImageJ is manual identification.
of tephra shards in the processed images. ImageJ is equipped with the software equivalent of a tally counter, and so this is simply a process of clicking on all the tephra shards in each image in turn and appending these counts to the Excel files produced by the macro described above.

In addition to automatic grain counting, ImageJ is also able to determine shard size automatically, based on edge detection. Thus, data were collected for shard size distribution for each of the sections, to determine if the rate of bioturbation affects the observed shard size or vice versa. For example, are smaller grains more readily bioturbated, leading to a varying size distribution of tephra through the depth of the core influenced by the mixing process?

**Testing the accuracy of the image analysis**

The automatic nature of particle analysis in ImageJ has the advantage of being a significantly less labour-intensive alternative to the traditional manual counting method. However, the automatic counting process is not completely accurate; in particular, the watershed function calculates ‘probable’ breaks, and sometimes cuts large particles into a few smaller particles, as can be seen in the bottom right side of the example in Figure 5d.

Furthermore, accuracy is also affected by the ability of the user to identify tephra shards from a 2D photograph. Sometimes, the distinction between tephra and bulk sediment is not clear and so the traditional counting method often involves inspection at higher magnification or turning particles in order to better distinguish the features thereof. Analysing still 2D images makes this process more difficult and, as such, it is to be expected that the rate of misidentification may be higher.

The above caveats to this new approach to tephra shard concentration analysis require that it be tested and compared with the traditional method to quantify accuracy and ensure validity. To accomplish this, repeat counts were carried out using both the manual counting method. However, the automatic counting process is not completely accurate; in particular, the watershed function calculates ‘probable’ breaks, and sometimes cuts large particles into a few smaller particles, as can be seen in the bottom right side of the example in Figure 5d.

**Fig. 5.** Particle analysis in ImageJ involves some pre-processing owing to the poor quality of the original photography. First the image is sharpened, converted to 8-bit color and the background subtracted (a). Particle detection is carried out using automatic thresholding (b). A watershed analysis is performed to split up grains which are touching (c), before the particles are counted and labelled (d).
Accuracy v. efficiency in image analysis of tephra

Our analysis of the ImageJ method of tephra identification, available in the Supplementary Material, suggests that it is less accurate than the traditional method. However, we must consider that the observed error of 23% is relative to the traditional method, which has itself been shown to have an inherent error of around 12% (see Supplementary Material). This figure, therefore, may be an exaggeration of the true error. Undoubtedly, with greater replication and an optimization of the image capture and processing protocols, this error could be considerably reduced. For example, images could be captured at a higher effective resolution, either by photographing at a higher magnification or by using a more specialized microscope camera. In particular, the possibility of taking ‘stacks’ of images with progressively greater depths of focus has been suggested; this could potentially allow 3D stack analysis to be carried out in ImageJ, which would better represent the morphology of grains and allow easier identification of tephra. This would make the structure of the shards (and their often conchoidal surfaces) more readily identifiable.

While the disadvantage of the ImageJ method is the potential loss of accuracy, the advantage is the speed at which analysis can be performed. The analysis takes roughly two orders of magnitude less time than the traditional method. This vast saving in time means that many more images could be analysed for each section, which would result in a reduction in the associated error. For example, one could carry out four times as many analyses on each section, which would result in a halving of the margin of error. This would result in a comparable error to the manual counting method, but a 25-fold saving in time. Our results show that errors tend to be large when tephra shard concentrations are low. In these cases, it would be advisable to analyse considerably more images of each section.

Results

Rate and maximum extent of bioturbation

Average tephra concentration profiles for each sampling day were produced by taking the mean tephra concentration from all the replicates at each depth. For each depth, one-way ANOVA was carried out to test for a significant change in tephra shard concentration over time. Where homogeneity of variance was not met, Welch’s ANOVA was also carried out. Post-hoc analysis was carried out using Tukey’s test to determine which sample days have significantly different tephra shard concentrations at each depth. Raw data are available in the Supplementary Material.

Visual inspection of the experimental quadrats on day 2 initially suggested that physical transport (i.e. advection) by wave and tide activity had largely removed the tephra from the quadrat surfaces. Figure 3 shows that, after only 2 days, tephra appears to be almost completely absent. However, the percentage tephra in the surface (i.e. 0–3 mm section) of quadrat 2-2, for example, is 13.41%, which is comparable with the percentage tephra found at the surface of all the day 0 cores (mean = 13.42%).

One would expect that physical mixing by wave and tide action would be associated with at least some degree of horizontal displacement of the tephra. However, the high tephra retention rate seen in the surface layer of successively sampled cores suggests that minimal horizontal displacement has occurred, and that displacement is primarily vertical. This suggests that, rather than having been removed by wave or tide activity, the tephra have largely been bioturbated downwards through the first 3 mm of the sediment. Similarly high tephra shard concentrations were found at the surface of all cores (Fig. 6), suggesting that, despite fears that open quadrats would lead to significant tephra loss, physical transport of tephra was actually a relatively minor process in this environment at the time of the experiment.

Figure 6a shows the tephra concentration profiles for all of the 15 cores investigated; Figure 6b shows tephra concentration profiles averaged for each sampling day. No tephra was found anywhere in the control core. Day 0 cores have a high percentage of tephra in the first 3 mm, which immediately drops to less than 1% at 3–6 mm depth; no tephra was found in day 0 cores below the 6–9 mm section. This suggests that the coring process causes only minimal downward disturbance.

Cores from days 2, 5 and 10 show progressively more tephra at increasing depths, down to a maximum depth of 15–18 mm. This pattern is interpreted as the result of progressive bioturbation and is illustrated in Figure 6. There appears to be little difference, at any depth, between cores from day 5 and day 10. Disregarding the day 0 control cores, the least bioturbation occurs in core 2-4, while the most bioturbation occurs in core 5-1; this implies a degree of spatial heterogeneity in the extent of the bioturbation processes in the intertidal environment.
Fig. 6. (a) Percentage tephra through the depth of all 15 cores. % Tephra = percentage of identified grains which are tephra. Quoted depths correspond to the top of each section. (b) Same as (a), but averaged for each sampling day. Error bars represent standard error of the mean (SEM).
Analysis of variance showed that, at the surface (0–3 mm), there was no significant difference between any of the sampling days. This supports the idea that tephra loss through physical transport away from each quadrat was minimal. At 3–6 mm depth, Welch’s one-way ANOVA found a significant difference in means ($F = 9.652$, $p = 0.012$). Tukey’s test showed a significant difference (90% confidence interval) between tephra concentration at days 0 and 5 ($p = 0.064$). At 6–9 mm depth, one-way ANOVA found a significant difference in means ($F = 3.587$, $p = 0.050$). Tukey’s test showed a significant difference (90% confidence interval) between tephra concentration at days 0 and 10 ($p = 0.056$). Below this depth, no statistically significant differences were found. The tephra concentration data and associated analysis of variance are therefore compatible with bioturbation of tephra from the surface to sub-surface layers at all sites.

Shard size analysis of the tephra grains revealed no statistical difference through the depth of any of the cores, suggesting that the rate of bioturbation is, in this case at least, independent of shard size. The data also suggest that the bioturbation process (downwards mixing) does not preferentially favour the size sorting of tephra.

### Discussion

Accurate quantification of bioturbation, in terms of both rate and maximum depth, is essential to the development of the discipline of tephrochronology, as it allows an opportunity to ‘work back’ from an observed tephra shard concentration profile to identify the point of initial deposition. While previous studies have quantified bioturbation using luminesphores (Biles et al. 2002; Solan et al. 2004b) or radioactive tracers (Gerino et al. 1998), this study represents the first direct investigation of bioturbation of a simulated ash fall event.

Perhaps the most striking feature of our results is the relatively shallow depth over which bioturbation has occurred. Our work suggests that bioturbation occurs over a maximum ‘mixed depth’ of 18 mm. The global dataset for bioturbation, however, seems to support the hypothesis of a global mean mixed depth of 9.8 cm (±4.5 cm, 1σ), largely independent of ecosystem type (Boudreau 1998). Of course, 18 mm lies within 2σ of this global mean and so is not statistically at odds with this observation. However, as described above (under ‘Site description’), our study site is dominated by Nereis and other active bioturbators. A previous investigation of bioturbation in an estuarine environment demonstrated the burrowing activity of polychaete worms down to 15 cm (Smith & Schafer 1999), presumably highlighting the highly variable nature of bioturbation and bioturbation depths within estuarine environments.

### Bioturbation in low-energy environments

Our site on the Eden Estuary was chosen as an ideal site for studying bioturbation based on the low energy level. Investigating in a low-energy environment minimizes the physical mixing signal which, in the context of a bioturbation study, could be regarded as a significant source of experimental ‘noise’. However, the relationship between energy, physical mixing and bioturbation is not straightforward, as illustrated by Figure 7 (Cadée 2001). While it is true that physical mixing does increase linearly with energy, bioturbation is actually greatest in mid-energy environments. If the energy of the environment is too high, vigorous wave and current activity make it difficult for infauna to survive, and so bioturbation is minimal. However, low energy levels also curtail biological activity. Our study site is mostly a very low-energy environment; not only does it lie in a sheltered estuary, but it also lies at the high-intertidal edge. Therefore, despite the presence of several species of active bioturbators, bioturbation depth is apparently severely restricted.

Our study site was chosen for ease of repeat access. However, future investigations into marine bioturbation should choose a sheltered site in the sub-tidal zone. Although this will make data collection significantly more challenging, it is also more likely to represent a clearer analogue to the deep sea, and any potential ‘washing out’ of tephra material by tidal activity may be reduced.

### Bioturbation and sediment grain size

Previous research into bioturbation and nutrient fluxes suggests that grain size exerts a control on the extent of bioturbation. Winston & Anderson (1971) conducted a semi-quantitative investigation of bioturbation in an estuarine environment and showed that more bioturbation occurred at the sandy study sites than at the fine-grained silt sites. Furthermore, in a quantitative study of gas transport in bioturbated sediments, Kristensen & Hansen (1999) showed that the transport of ammonia and carbon dioxide through sediment profiles is dependent on grain size. They found that, in coarser-grained sediments, gas transport fitted an eddy diffusive model. In other words, burrowing and locomotion led to physical movement of porewater, gases and sediment. However, in fine-grained sediments, this eddy diffusive model overestimated gas exchange and so was replaced with a molecular diffusive model. Implicit in the molecular diffusive
model is the lack of physical particle mixing by biological activity; the nonporous and cohesive nature of finer-grained sediments therefore tends to restrict vertical mixing. Qualitatively, the sediment material at the study site clearly contains a high silt content, and the Scottish Natural Heritage ecological survey reports that the entire lower Eden Estuary is mud-flats, while LMU.HedMac is defined as ‘sandy mud shores’ (Bates et al. 2004).

The importance of epifauna

Our results echo those of Solan et al. (2004b), who carried out bioturbation research in Gullmarsfjord, Sweden. They used time-lapse image analysis to investigate the rate and depth of bioturbation over a 16 h period. They found that, despite the abundance of several active infaunal bioturbators, it was actually the locomotion of a single crab of the species *Hyas araneus* that was responsible for almost all bioturbation. Epifaunal bioturbation of this type takes the form of a few discrete events, as opposed to an ongoing diffusive process, as is usually considered for infaunal bioturbation. No evidence of epifaunal activity was found at any of our experimental quadrats. Both this study and theirs, therefore, suggest that, over the relatively short time-scales considered, those infaunal species typically considered to be active bioturbators are incapable of mixing below a depth of c. 20 mm. This would imply that future studies investigating infaunal bioturbation would need to do so over longer time-scales (i.e. months rather than days). However, Figure 6 appears to imply that bioturbation halted after day 5 of the experiment. This would be at odds with the idea that infaunal bioturbation occurs over longer time periods. In fact, it is unlikely that the similarity of the profiles at days 5 and 10 represents a real cessation of bioturbation; we would expect that, once the maximum mixed depth has been reached, the tephra concentration would homogenize over the mixed-depth. We do not see this, which suggests that, for some reason, bioturbation has ‘paused’ or significantly slowed between days 5 and 10. This may, in fact, be a coincidental result, and might imply that future investigations would benefit from using a greater number of replicate quadrats or longer field sampling intervals.

The effects of community structure on bioturbation

In this investigation, we purposely chose to investigate a single biotope, LMU.HedMac, which describes sheltered, sandy mud shores dominated by *Hediste diversicolor* and *Macoma balthica*. Investigating a single biotope reduces the biological variable from the investigation, allowing a more detailed focus on how bioturbation progresses over time following a simulated ash fall event. However, the Eden Estuary and similar environments would make ideal study sites for future investigations into the role of ecosystem type on bioturbation. As shown in Figure 1, the estuary contains many different biotopes. Furthermore, the development and successful application of the new,
semi-automated method of tephra concentration analysis outlined here makes it feasible to carry out considerably more analyses than would previously have been possible, making a wider ranging study entirely feasible. It would be particularly interesting to consider how grain size varies along the length of the estuary, how this affects community structure and, in turn, the rate and extent of bioturbation. The same, of course, applies to the study of bioturbation in sub-tidal habitats.

**Investigating tephra burial**

The tephra concentration profiles resulting from the present study represent, in reality, only half of a bioturbation profile; it is equally important to consider how a tephra marker horizon is bioturbated upwards following burial. It would, for example, be incorrect to assume that the original marker horizon occurs at the highest point in the core where tephra is found. Is it therefore correct to assume that the peak tephra concentration represents the original marker horizon? Future research should seek to answer this question. For example, Winston & Anderson (1971) described an investigation where an area of estuarine sediment is excavated, a marker horizon is laid and then re-buried. Their research was not quantitative, but there is no reason why the approach followed in this paper could not, for example, be applied to a buried marker horizon.

**Conclusions**

Our results demonstrate that bioturbation of tephra begins immediately following deposition, even in a low-energy mid-estuarine environment. The rate of bioturbation does not appear to be dependent on the grain size of the tephra present. It is clear that the processes responsible for bioturbation are significantly depth-limited in the biotope (LMU HedMac) studied. Future investigations into bioturbation of tephra in low-energy environments should seek to study the process over longer time periods, perhaps over an entire seasonal cycle. Over this time period, we would expect to see tephra penetration to a maximum mixed depth, and this would be confirmed by the progressive homogenization of the tephra concentration profile over said mixed depth.

The extent to which these results are applicable to sub-tidal marine, as opposed to estuarine, bioturbation is uncertain. Certainly, the interrelated factors of sediment grain size distribution and the energy level of the system appear to play a critical role in determining the rate of bioturbation. The sediment at the study site had an oxic layer thickness of the order of 1 mm, underlain by dark anoxic sediments; this is typical of fine sediments with high organic content and a dense and active biofilm. It is likely that this very thin oxic layer impacts bioturbation rates and maximum extent. In this study, the depth of bioturbation may also have been limited by the fact that the study site is above the low-tide line, and is thus exposed for several hours a day; this effect is a subject worthy of further investigation.

Automatic image analysis using ImageJ was previously employed by Costa & Yang (2009) to carry out pollen counts, and it has been adapted here to count all types of particles, as well as assist in measuring and counting tephra particles. Our analysis of the accuracy of this new method suggests that it has a fairly high counting error associated with it. However, this reported error should be taken as a tentative figure, because the traditional method, against which our new method is tested, is far from error-free. Our results also suggest the need for more replication in order to produce statistically robust results. The new particle analysis method demonstrated in this paper has high significance and future applicability.

We measured significantly slower bioturbation rates than previous studies in other marine environments (Boudreau 1998), and we attribute this to the fine grain size and the low energy level. These two factors play a significant role in determining community structure and abundance. This strong ecological control on bioturbation implicitly hints at the possibility of developing a ‘bioturbation index’, and using it to infer information about palaeoenvironments, which is an exciting possibility for the future development of marine tephrochronology.

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