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A standard sample method for controlling microfossil data precision: A proposal for higher data quality and greater opportunities for collaboration

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ABSTRACT

Using standard samples is a simple yet very effective method to control data quality. In the field of Quaternary science, it is a commonly applied approach for isotope and geochemical analyses, greatly increasing the general level of confidence in these analytical methods. Microfossil analysis, by contrast, does not normally have any such system to secure data precision and accuracy objectively and routinely. In this paper, we propose a relatively easy and inexpensive way to adopt such a standard sample method for microfossil analyses. Rejected material from core subsampling was collected and homogenised, and was used for standard samples. One standard sample was treated and analysed for every seven sediment samples. Departure of the standard data from the long-term average quantitatively indicates the error. If the error was correlated with time (*i.e.* the error has a long or short term trend within the time spent for analysis), the additional information gathered from the standard data can then be used to correct raw data to improve data precision. The standard sample method in microfossil analysis is not only very effective in monitoring and improving data quality, but also enhances opportunities for collaboration, as it significantly reduces systematic data offsets between all parties involved in the analysis.

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1. Introduction

High precision and accuracy of data is a prerequisite for reliable science. However, this is more difficult to achieve in practice because real world conditions, such as errors from the analyst, analytical instruments, and laboratory pre-treatments are not always constant. High data precision is particularly important for the high or very high resolution analyses for modern Quaternary scientific studies, both because of the required signal to noise ratio, and the analytical costs involved being high or very high accordingly.

Using standard samples is a typical approach to circumvent such problems. A very large mass of samples with known (or fixed by consensus) isotopic and/or chemical properties is shared by international laboratories and analytical results are expressed in terms of the departure from the standard value (cf. Vienna Standard Mean Ocean Water: VSMOW; Vienna Pee Dee Belemnite: VPDB) (Coplen, 1994, 1996). The data from standard and real samples both have associated errors caused by the same (or at least very similar) laboratory, machine and human factors. Therefore, normalising data against standards significantly helps to correct these error factors, and hence improves data accuracy. This approach can also help improving data precision if the measurement error is correlated with time (*i.e.* moment when the analysis was performed; this is typically the case with a set of data deriving from different analytical batches). The very active use of such a standard sample approach is one of the reasons why isotope and geochemical analyses have acquired a high level of confidence for data consistency, enabling active inter-laboratory comparison and collaboration, and has allowed the methods to become a main stream of Quaternary science.

Microfossil analysis is also an important area of modern Quaternary science, and the principles of the standard sample method described above are theoretically applicable to any analyses (*i.e.* not only isotopic or geochemical). However, data quality control using standard samples has not become common practice in microfossil analyses mainly for two reasons:

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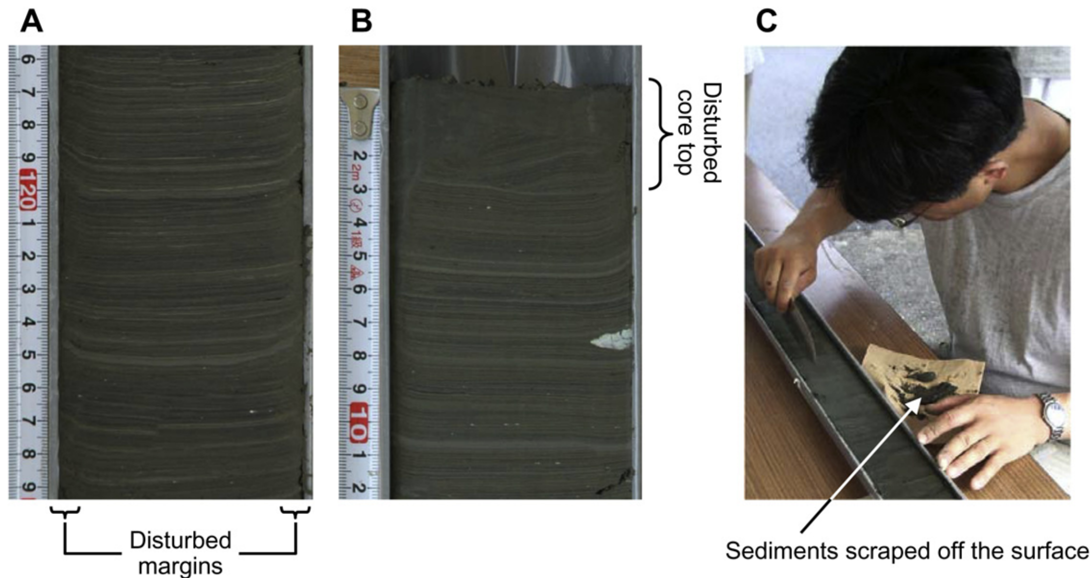

Fig. 1. Examples of materials that are normally rejected for analysis but are still useful for the generation of standard samples. **A**: The marginal few mm of the cores are often disturbed by the friction between the sediment and the sampling tube. **B**: The upper few cm of the cores are not suitable for analysis if the cores were extracted from sampling tubes by mechanical pressure. **C**: Core section surfaces scraped off for photography eventually accumulate to a considerable quantity.

1. *The regional nature of data*: Isotopic or geochemical measurements normally do not have any region-specific factors, enabling the use of one (or very few) universal standard(s) to be shared and analysed amongst international laboratories. This is not the case with microfossil data. For example, a pollen assemblage from one region (e.g. East Asia) contains a series of taxa that are significantly different from other continents (e.g. South America). This prevents any organisations from preparing a single "standard" pollen-bearing sediment that can be used universally.
2. *The multivariate nature of data*: Isotope ratio data are typically mono-dimensional, which are easy to normalise against standard data. Typical microfossil data, by contrast, contain tens of taxa percentage values; some are abundant and others are rare and, moreover, they are all correlated to each other. This multivariate nature of microfossil data makes it relatively complicated to normalise whole sets of data against standards.

Despite all of these difficulties, however, the need for a system to secure microfossil data quality and consistency is ever increasing as modern Quaternary sciences require higher and higher resolution analyses. That almost inevitably requires collaboration between multiple analysts (unless still experimental automatic microfossil identification systems might eventually be implemented in practice – Chen et al., 2006; Ranzato et al., 2007; Costa and Yang, 2009; Holt et al., 2011), making it a critical issue to normalise data across involved parties.

Analysing the same sample twice is a method of precision control sometimes used for relatively new analytical methods where an internationally quality-controlled standard sample is not available (e.g. Koutavas et al., 2002). However, this method requires as much as 100% extra analytical time, which researchers would otherwise be able to use for making the analytical resolution twice as great.

This paper proposes a relatively simple, yet effective method for improving (or monitoring) microfossil data quality using the concept of standard samples. The method is low-cost, relatively non-labour intensive, not specific to any type of fossils (pollen, diatoms, foraminifera, etc.) or sediments (marine, lacustrine, peat, etc.) to be analysed, and does not require establishing any international service organisations. According to our experience, the extra analytical time required to employ this method is in the range of 14% which, given the obvious benefit of data precision control, is acceptable for most professional analysts.[2]

2. Preparation of standard samples

The SG06 sediment core was recovered from four parallel bore holes, and was immediately divided into LL-channel subsamples at the field workshop near the coring site of Lake Suigetsu (Nakagawa et al., 2012). Through this subsampling procedure, the core produced not only includes a number of healthy 'samples' suitable for analyses, but also a rather large quantity of rejected material. Typical examples of such 'rejects' are:

1. Marginal parts of the core (Fig. 1A). The outer few mm of the core, which were in direct contact with the inner wall of the coring tubes, have a high risk of contamination and hence are inappropriate for analysis;
2. Core segment tops (Fig. 1B). As the cores were mechanically extracted from the bottom of coring tubes using a piston, the top *ca.* 5 cm of each core underwent mechanical compaction (which we could check by correlation to parallel core sections);
3. The scraped surface from split sections. Each recovered core segment was split longitudinally into a pair of half cylinders. The newly exposed section surface was scraped with a sharp blade to make a smooth surface suitable for high resolution photography (Fig. 1C). The volume of sediment matrix wasted by this scraping is normally more significant than is assumed

[2] ***Note***: For clarity, in this paper we explain the method that we used for the pollen analysis of the SG06 sediment core from Lake Suigetsu, Japan (Nakagawa et al., 2012). This is essentially arbitrary and the method can be applied to other microfossil analyses or other study sites with no or very little modification.

(typically *ca.* 100 cm^3/m) (scraping 2/3 mm from 100 × 7.5 cm^2 section of both half cylinders would yield 100 cm^3);

4. Residue on sampling tools. Spatulas and knives are used to recover clean and tidy subsamples. After recovering subsamples, these tools still have a small amount of sediment matrix attached to their surface. This residue on the tools is well representative of the analysed sediment because (although in small quantity) it derives from every sampling depth.

We recovered these rejects and grouped them by major palaeoclimatic and sedimentological units, *i.e.* (i) Holocene, (ii) Lateglacial, (iii) Last glacial maximum (LGM), (iv) Marine isotope stage (MIS) 3–4, and (v) MIS-5. This classification roughly corresponds to the sedimentary units recognised at Lake Suigetsu by Takemura et al. (1994). The rationale of not mixing everything was: (i) to prevent the much stronger pollen signal of warmer periods from 'masking' the weaker signal of cold periods (*i.e.* the pollen concentration in the Holocene sediment is about 10^2–10^3 times greater than that of the LGM), and (ii) to have different standard samples for different sediment types and/or different palaeoclimatic regimes.

If there are too many environmental 'zones', each of which needs to be represented by different standards, the simplest solution is to mix several standards and generate more 'universal' standard that can be more widely used. The mixing ratio of standards from different ages, however, needs to be controlled reflecting the difference in pollen concentration.

After grouping (and mixing where applicable), we thoroughly homogenised the rejects in each group by hand. Homogenisation was performed by very simple spreading, folding and squeezing in plastic bags for at least 2 h. Distilled water was sometimes sprayed to 'soften' the mixture but only in very limited volumes to avoid secondary grain sorting in the mixing bag. Light-blue or pink water colour paint was occasionally applied to the mixture to visually ensure that the subsequent mixing procedure was suitably homogenising the sediment.

Once thoroughly homogenised, the mixture is ready for use as a standard sample. The standard samples were then sealed in an air-tight bag or container and stored in the dark at 4 °C to prevent/minimise growth of algae and fungi (which produce spores). The standard sediment and package needs to be weighed together before storage so that any subsequent change of the water content (desiccation, condensation, etc.) during storage can be detected.

The justification for using rejects, which are highly likely to be contaminated, is that the standard samples only need to be homogeneous and vaguely similar to real samples. Contamination within reason is not harmful for standard samples.

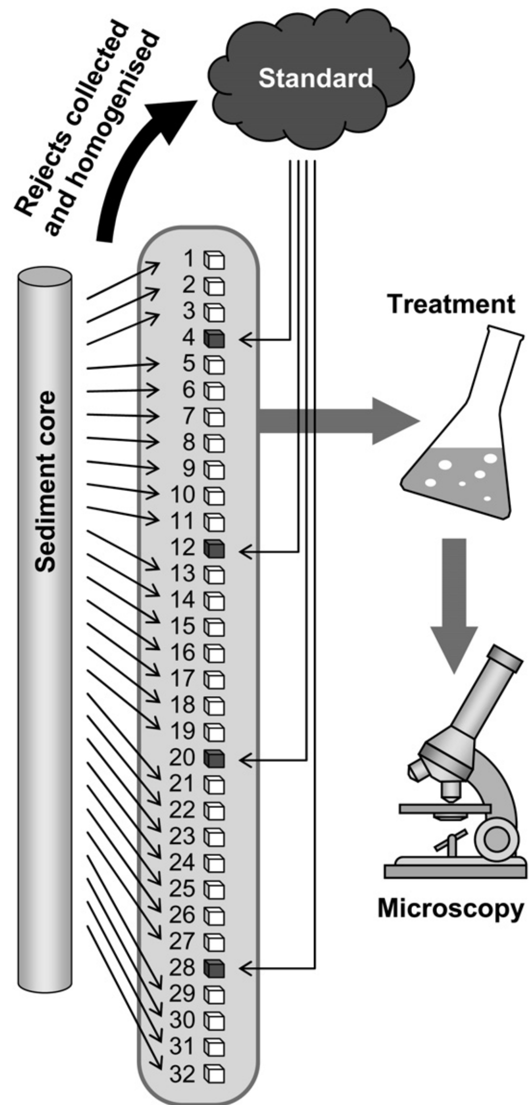

Fig. 2. Schematic explanation of the standard sample method adopted for pollen analysis for the Suigetsu 2006 Varved Sediment Core (SG06) project. One analytical batch consists of 32 samples among which four are standards and the rest are true samples. The four standard samples are evenly spaced. Both standards and true samples are treated and analysed in precisely identical ways.

3. Procedure

For the pollen analysis of the Suigetsu 2006 Varved Sediment Project (SG06 project), 32 sediment samples were treated in each laboratory batch, using a slightly modified method from Nakagawa et al. (1998) (a heavy liquid density of 1.85 g/cm^3 was used instead of 1.88 g/cm^3). We allocated slot numbers 4, 12, 20, and 28 for standard samples and the remaining 28 slots for actual sediment samples (Fig. 2). The four standard samples should be chosen from preferably only one, and maximum two, standard type(s) according to the sedimentological unit of the actual sediment samples in the batch. For example, if the batch predominantly consisted of Holocene samples, then the 'Holocene' standard should be used. If the batch was a mixture of 14 samples from the Lateglacial and another 14 from MIS-3, then two 'Lateglacial' and two 'MIS-3' standards should be used. In the latter case, the Lateglacial samples and standards should be grouped to slots No. 1–16 and the MIS-3 samples and standards should fill slots No. 17–32.

The volume of standard samples to be treated should also be analogous to the actual samples. For the first 800 pollen samples analysed for the SG06 project, the average sample size was 1.25 g (wet weight) and the average standard size was 1.07 g. It would have been ideal if these values had been identical (especially if there were any indication that error is correlated with sample size).

The 28 samples and 4 standards in the same batch were treated and analysed for pollen in precisely the same way. In other words, we added one dummy (*i.e.* standard) sample for every 7 real samples and performed our ordinary analytical routine without even thinking that some of the samples were not actual samples but were standards. Because no modification was made to the preparation and analytical procedures, the extra time and cost required to employ the standard method was about 14% (~ 1/7).

For data correction using such standards, it is important to record the time when the sample was analysed. This can be

achieved automatically if the counting was performed using the PolyCounter software (http://dendro.naruto-u.ac.jp/~nakagawa), a fast microfossil counting device that was also developed for the SG06 project (Nakagawa et al., 2007, 2012).

4. Data correction

4.1. Ideal approach

Ideally, pollen data produced from multiple standard samples must be always identical. In reality, this is almost never the case because of analytical and statistical errors (Fig. 3). The errors can be quantified as the departure from the 'expected' value. In more mathematical terms, an error matrix $\boldsymbol{E_t}$ at time t can be defined by the following equations:

$$S_t = \boldsymbol{E_t} S_0 \quad (1)$$

$$S_0 = (S_{t1} + S_{t2} + S_{t3} + \ldots + S_{tn})/n \quad (2)$$

$$S = (p_1, p_2, p_3, \ldots) \quad (3)$$

where S_t is the standard data vector (which constitutes taxa percentage values) obtained at time t, S_0 is the expected standard data vector (which is an average of multiple standard data vectors), n is the number of standard samples so far analysed (excluding obvious outliers), and p_1, p_2, p_3… are the pollen percentage values of taxa No. 1, 2, 3…

Once the error matrix at time t ($\boldsymbol{E_t}$) has been established, then fossil data obtained at time t can be corrected using the following equations:

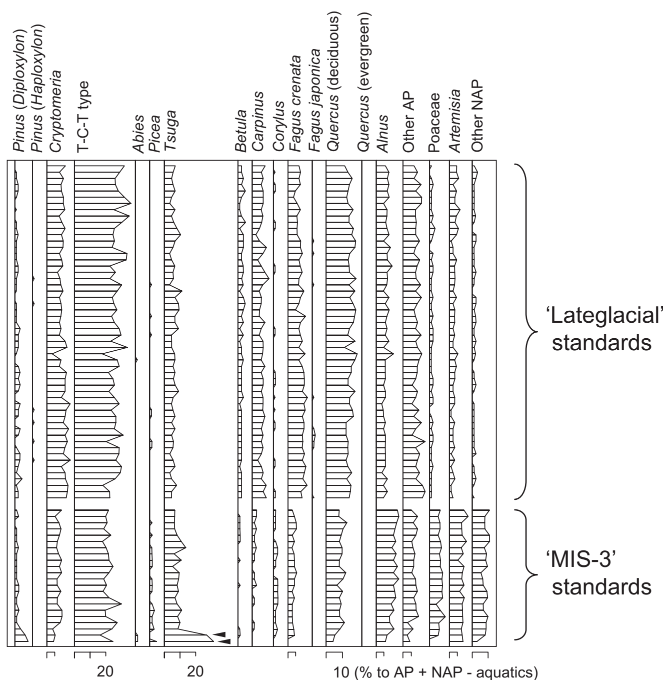

Fig. 3. Pollen diagram of 54 'Lateglacial' and 22 'MIS-3' standard samples. Data precision is generally good but Taxodiaceae–Cupressaceae–Taxaceae (T–C–T) type shows relatively high variability, implying greater human error with identification of this pollen type. Additionally, the final two 'MIS-3' standard data (both deriving from the same batch) clearly show abnormally high percentage values of *Tsuga* (black triangles), which indicate a laboratory error that took place only with this batch.

$$F_{ct} = k\boldsymbol{E_t}^{-1} F_{rt} \quad (4)$$

$$F = (p_1, p_2, p_3, \ldots) \quad (5)$$

where $\boldsymbol{E_t}^{-1}$ denotes the inverse of matrix $\boldsymbol{E_t}$, F_{ct} denotes corrected fossil data obtained at time t, F_{rt} denotes raw fossil data at time t, p_n is the pollen percentage value of taxa No. n, and k is an arbitrary coefficient to make the sum of all corrected percentages equal 100%.

In practice, both $\boldsymbol{E_t}$ and F_{rt} are never available from the same time t (it is not possible to analyse a fossil sample and a standard sample at the same time using the same person and same microscope). Therefore S_t in Equation (1), which is the basis for $\boldsymbol{E_t}$, needs to be produced by interpolation from successive standard data using the following equation:

$$S_t = \{(t - t_{-1})S_{t+1} + (t_{+1} - t)S_{t-1}\}/(t_{+1} - t_{-1}) \quad (6)$$

where t is the time for which interpolated S_t is required, t_{-1} is the time when the last standard data S_{t-1} were obtained before time t, and t_{+1} is the time when the first standard data S_{t+1} were obtained after time t. This is the simplest linear interpolation using only nearby time series data points, which is applicable in almost any circumstance, unless either S_{t-1} or S_{t+1} is an obvious outlier. More complicated methods, such as parabolic interpolation or spline fitting can also be considered depending on circumstances. In theory, this approach is most suitable when the whole standard dataset shows a long term trend (which typically tends to be the case with beginners).

Alternatively, if the raw standard dataset does not show any overall trend through time, but shows obvious outliers in one particular batch (which is likely to indicate irregularity in one batch), S_t for that particular batch can be obtained more simply as the average of the standard data treated and analysed in the same batch.

It is worth noting here that the ability of the standard method to check not only counting error but also irregularities in sample preparation is a considerable advantage over more conventional data quality checking methods such as re-counting and/or comparison between analysts.

One obvious limitation in this 'full' approach is the more than likely presence of zero values in the components of standard (S) and fossil (F) data vectors, in which case the $\boldsymbol{E_t}^{-1}$ in Equation (4) cannot be determined uniquely. A technique to circumvent this problem is to apply the method to only the major taxa, which are always present in both S and F vectors (this is also the reason why it is recommended to use standard samples as analogous to fossil samples as possible, for example Holocene standards for Holocene samples). Alternatively, it would be more sophisticated to apply the method to PCA scores (obtained using a variance–covariance matrix rather than a correlation coefficient matrix) of percentage values. Scores need to be weighted by PCA loading before standard correction, and inversely converted to percentage values after standard correction.

4.2. More practical approach

The 'full' approach described above is idealistic, but requires mathematical skill and pre-selection of major taxa (standard correction of minor taxa does not make much sense as the variance of percentage data between standard and fossil samples is more likely to be within statistical error). In reality, the imprecision of pollen data generated by experienced researchers is normally with only very selected taxa, such as subdivision between *Pinus* subgen. *Diploxylon* and *Pinus* subgen. *Haploxylon*, deciduous and evergreen

Quercus, Taxodiaceae–Cupressaceae–Taxaceae (T–C–T) type and *Cryptomeria*, etc. Correction of such subdivisions between two pollen types using standard data is easier than the 'full' method.

If absolute pollen concentration data are available from standard data, then the correction between pollen types X and Y can be more easily achieved than the 'full' method using the following simple equations:

$$x_{ct} = X_{ct}(x_{rt} + y_{rt})/(X_{ct} + Y_{ct}) \quad (7)$$

$$y_{ct} = Y_{ct}(x_{rt} + y_{rt})/(X_{ct} + Y_{ct}) \quad (8)$$

$$X_{ct} = (S_{x0}/S_{xt})X_{rt} \quad (9)$$

$$Y_{ct} = (S_{y0}/S_{yt})Y_{rt} \quad (10)$$

where $x_{ct}(y_{ct})$ is the corrected percentage value of pollen type $X(Y)$ in the fossil sample analysed at time t, $x_{pt}(y_{pt})$ is the raw percentage value of pollen type $X(Y)$ in the fossil sample analysed at time t, $X_{ct}(Y_{ct})$ is the corrected pollen count of pollen type $X(Y)$ at time t, $X_{rt}(Y_{rt})$ is the raw pollen count of pollen type $X(Y)$ at time t, $S_{xt}(S_{yt})$ is the absolute concentration of pollen type $X(Y)$ in standard sample analysed at time t, and $S_{x0}(S_{y0})$ is the average absolute concentration of pollen type $X(Y)$ in a number of standard samples analysed (excluding obvious outliers). Similarly to the 'full' method, standard data at the same time t as the fossil data are not available. This needs to be generated either by interpolation of time series standard data (when the standard data are showing a gradual change), or by averaging of all standard data analysed in the same batch (when one batch is obviously irregular).

If the absolute pollen concentration data are not available (or are not precise enough), then the percentage data would need to be corrected directly using the following equations:

$$x_{ct} = k(P_{x0}/P_{xt})x_{rt} \quad (11)$$

$$y_{ct} = k(P_{y0}/P_{yt})y_{rt} \quad (12)$$

$$x_{ct} + y_{ct} = x_{rt} + y_{rt} \quad (13)$$

where $x_{ct}(y_{ct})$ denotes the corrected percentage value of pollen type $X(Y)$ in the fossil sample analysed at time t, $x_{pt}(y_{pt})$ denotes the raw percentage value of pollen type $X(Y)$ in the fossil sample analysed at time t, $P_{xt}(P_{yt})$ denotes the percentage value of pollen type $X(Y)$ in the standard sample analysed at time t, $P_{x0}(P_{y0})$ denotes the average percentage value of pollen type $X(Y)$ in a number of standard samples analysed (excluding obvious outliers), and k is an arbitrary coefficient to satisfy Equation (13). Standard percentage data, P_t, need to be generated either by interpolation or averaging of all standard data from the same batch.

5. Application and discussion

Fig. 3 is a simplified pollen diagram of 54 'Lateglacial' and 22 'MIS-3' standard samples analysed in 38 different batches for the SG06 project (Nakagawa et al., 2012). Ideally, all curves should be perfectly straight and vertical. The departure from the vertical lines gives the quantified imprecision of the data caused by laboratory, identification, and statistical errors. Oscillations of real fossil pollen data beyond this variability range are considered to be significant, reflecting real changes in vegetation and/or the sedimentary environment. The variability of values in the diagram (Fig. 3) is generally very small (in the region of a few percent). Because the level of imprecision is fairly close to the statistical error (which is unavoidable, and is approximated by $1/n^{0.5}$ where n denotes the number of grains of the concerned pollen taxa), along with the observation that the error does not seem to be correlated with time but is more like random noise, it would not make much sense to correct fossil data using standard data (in other words, laboratory and identification errors are sufficiently small).

An exception to the above statement is T–C–T type, which clearly shows greater variability of its percentage value. The difference between maximum and minimum values exceeds 10%. The standard deviation excluding obvious outliers (see below) is 3.5%, whereas the average standard deviation of all other taxa is 0.6%. As this pollen type does not have much characteristic structure or ornamentation to aid identification (Demske et al., 2013), this greater variability is most likely to be due to identification (=human) error. Any oscillations of T–C–T type pollen percentages below ±5% should not be considered significant unless the oscillations are represented by a sufficiently large number of data points coherently showing a similar trend. Alternatively, it would be worth considering the standard correction technique described in Section 4 of this paper.

The last two 'MIS-3' standard samples are clearly problematic, showing much higher *Tsuga* percentages (black triangles), as well as generally lower percentage values of other taxa. Because these two standard samples both belong to the same analytical batch, this is almost certainly caused by a laboratory failure. Fig. 4A shows the fossil pollen diagram containing samples treated in the same batch as the two 'outlier' standards (black triangles). *Tsuga* percentage values of the samples derived from this batch systematically show significantly higher values than neighbouring samples from other batches. We therefore performed standard correction (we corrected the division between *Tsuga* and all other taxa using absolute concentration) and obtained the diagram in Fig. 4B. Most of the former outliers are now aligned to the overall trend of the change (grey triangles), indicating that the errors caused by the laboratory failure have been significantly reduced. Such correction cannot be achieved objectively unless standard data are available. Moreover, without using the standard method, it is likely that the laboratory failure would not even have been detected. This is especially the case if the samples in the same batch derive from a block of adjacent horizons, in which case the data offset due to the laboratory failure would look like a meaningful oscillation. Our recommendation is to use standard samples and to randomise the depths of the samples to include in the same batch.

Another advantage of the standard method is that it facilitates the merging of datasets generated by multiple persons/institutions. This is especially useful when a very high analytical resolution is intended and the number of samples to be analysed is accordingly so large that it is not realistic for a single person to complete it within a reasonable period of time. If the same standard sample is shared and analysed routinely by all parties involved in the analysis of the same sample set (for example a sediment core), then any data offset between the parties can be quantitatively detected by comparison of their standard data, and can be corrected using the same technique as described in Section 4. In this way, the standard method outlined above would significantly reduce data noise associated with involvement of multiple analysts and multiple laboratories to do sample treatment, and enhance opportunities for collaboration and facilitate participation of new members in collaborative projects of microfossil analyses.

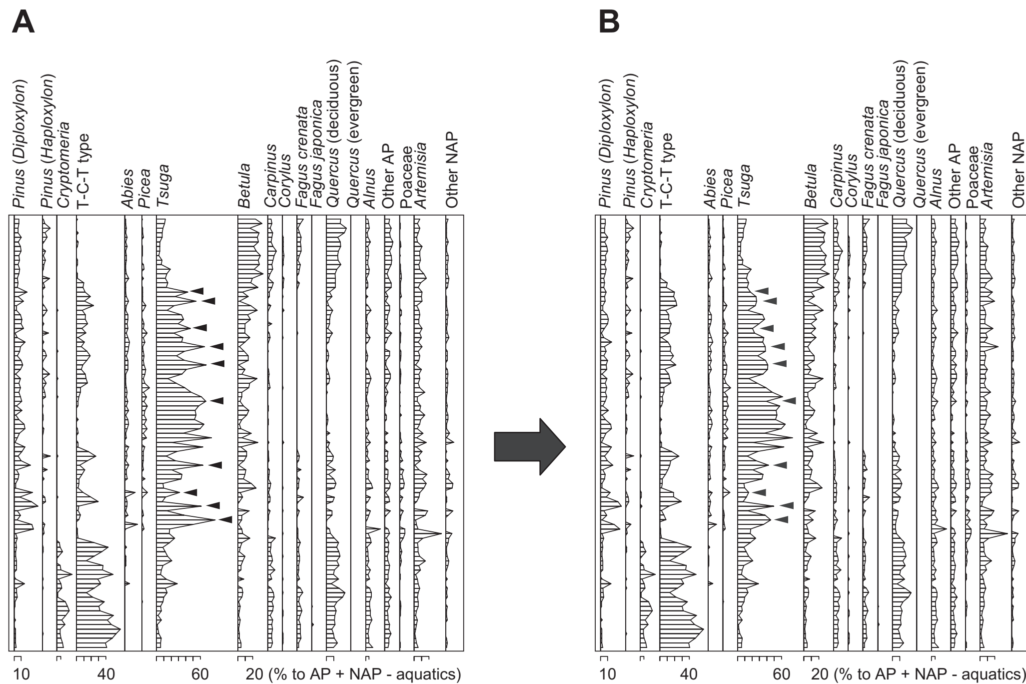

Fig. 4. Correction of pollen data using standard data (Fig. 3). **A**: Original pollen diagram before standard correction. The vertical axis gives the sample number in depth order. *Tsuga* percentage values of the samples treated and analysed in the same batch as the two final standard samples in Fig. 3 all show much higher values than neighbouring samples (black triangles). This is more likely to be a laboratory failure rather than true oscillations in the pollen data. **B**: Pollen diagram after standard correction. The correction was made for the ratios between *Tsuga* and all other pollen taxa by the absolute pollen concentration method described in the text. Most of the abnormal *Tsuga* peaks that existed before the correction are no longer 'outliers', indicating that the errors caused by the laboratory failure have been successfully reduced.

6. Concluding remarks

1. The standard method that we propose in this paper is relatively easy, inexpensive, and very effective to monitor (and improve where appropriate) the quality of microfossil data.
2. Rejected materials which derive from coring and subsampling can be homogenised and used as standard samples.
3. Data correction using standard data can be applied to either the whole dataset or the ratio between two particular taxa (or taxa groups).
4. The method was tested with the pollen data from the SG06 sediment core. The results showed that the precision of the raw dataset is significantly improved by the standard correction.
5. Standard samples shared by multiple parties would enhance opportunities for collaboration to achieve higher analytical resolution.

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