Dear Reviewer 2,

We thank the reviewer for their time and suggestions which strengthen this manuscript. Below you will find our response to review #2 and the relevant changes made in the text. We hope we sufficiently responded to your thoughtful advice and suggestions.

The manuscript presents an interesting strategy on using machine learning probability estimates and pollen data to understand how source changes affect the distribution of brGDGTs and particularly the MBT'5ME index in sedimentary records. The study applies probability estimates from machine learning models to an extended modern global brGDGT database, to detect brGDGT contributions from different sources (soil, peat, lake) over time in two sediment archives and validate these findings through comparisons with independent pollen ad NPP data.

Although the study builds on previous research (Martinez-Sosa et al, 2023), it introduces several novel additions, including: addition of new modern br-GDGT samples to the training dataset, exploration of different probability calibration techniques and proposing a new "brGDGT wetland index". This index, if validated further, may help distinguish wetland-influenced brGDGTs from other depositional sources, with potential applications beyond brGDGT provenance analysis.

However, I find some aspects unclear or insufficiently explained:

1) The study uses five different machine learning models, but the criteria for selecting these specific models are not well justified. Why were these models chosen over others, such as deep learning approaches or ensemble methods beyond Random Forest? Also, the manuscript could benefit from a discussion of why certain models performed better and why others underperformed.

Response: In order to simplify the manuscript an ensure that the brGDGTs were the main focus we did not present an in-depth discussion of the selection of the ML models. However, early screening included utilizing other ensemble methods like XGBoost which performed similarly to Random forests but was computationally heavy and neural networks, which did not perform well. In the end we also stayed away from deep-learning models because they generally do not perform well on tabular datasets. We agree that some additional information is needed on model performance as well as model choice so we added the following text.

Introduction: "We test five popular parametric and non-parametric machine learning models based on their ability to handle small tabular datasets and produce reliable probability estimates when calibrated (Malley et al., 2012; Wang et al., 2019). Models utilizing different structures were chosen, including simple tree-based algorithms (CART), ensemble trees (RF), linear models (LR), margin-based classifiers (SVM), and instance-based lazy learners (K-NN) to evaluate performance. The best-performing model was then chosen to apply to two down-core sedimentary sequences."

Section added Methods:

"Five diverse algorithms were tested based on various methodological and practical reasons. Firstly, we choose algorithms that could produce reliable probability estimates and have been widely utilized and validated (Malley et al., 2012; Wang et al., 2019). Algorithms were also chosen by performance on smaller tabular datasets, low computing resource requirements, and their availability in the Scikit Learn Python library which is available publicly for download. These methods were chosen over more complex deep-learning methods which often underperform on small tabular datasets (Grinsztajn et al., 2022) and require significant time and expertise for hyper-tuning (Mohammed and Kora 2023), and other complex ensemble methods which can require more computing resources without increased accuracy."

In response to adding a discussion about why certain models performed we added the following into the model discussion

4.1 Probability estimates for chosen models and application to downcore records

4.1.1 Model accuracy

"The F1 score evaluates the accuracy of a model's predictions of both precision (how many predicted positives were positive) and recall (from all the positives, how many positives did the model predict) and can balance between understanding false positives and false negatives (Boozary et al., 2025). This score allows for a more robust accuracy when measuring each model. Many things may explain differences in F1 scores across our models. For example, K-NN, SVM, and CART models are prone to overfitting (Huang et al., 2005; Berk, 2008 Jadjav and Channe, 2013), which may have accounted for their lower F1 scores (Table 1). RF generally does not overfit due to its ability to handle noise in the datasets (Parmar et al., 2019), which may result in a higher F1 score. While LR does not typically overfit, the lower F1 score may be due to its assumptions of linearity (Nick and Campbell 2007), which may be problematic if there is no clear division in the dataset. The balanced versus unbalanced datasets may have also impacted performance. RF generally handles unbalanced datasets well (Anaissi et al., 2013), and the SMOTE dataset only offered marginal improvements to the F1 score, while CART's F1 score was significantly improved with the balanced SMOTE datasets."

2) The study primarily focuses on semi-arid and arid regions, but are the results generalizable to other climate settings? Note that one of the tested sediment records extends back to 35k years BP. Also, there is little discussion of how different environmental conditions might affect the model performance.

Response: Thank you so much for your insight and suggestions. It is true that we focus on arid and semi-arid regions due to the availability of the core records for our analysis. However, this model can indeed be utilized globally, but has not been tested in sediment archives from these contexts. The training dataset, however, is based on a global database, making it applicable globally. To address this, we have added a new section to address the issues with application of the models to sedimentary sequences and removed the words semi-arid and arid environments in the introduction, methods, and conclusion to reflect this insight. We also added a figure in the supplement that shows the distribution of the modern database across the *Köppen*-Geiger climate gradient.

4.2.4 Considerations for application to paleo-sedimentary sequences

“The samples from the modern training dataset come from a wide range of modern environmental contexts, making the model applicable to most brGDGT based paleoenvironmental reconstructions. The brGDGT based ML probability outputs on the Padul core, which is 36,000 years old, closely align with the independent pollen and XRF water depth reconstructions during the last glacial period (Figure 8), confirming the model's relevance beyond the Holocene. However, the distribution of modern samples across the Köppen-Geiger climate gradient is not well balanced (Figure S9), with temperate environments well represented, while tropical, arid, and arctic conditions are under-represented. Considering this, caution is advised when applying this model to sedimentary records from these climates. Additional caution is warranted for deep-time records where no modern analogs exist.”

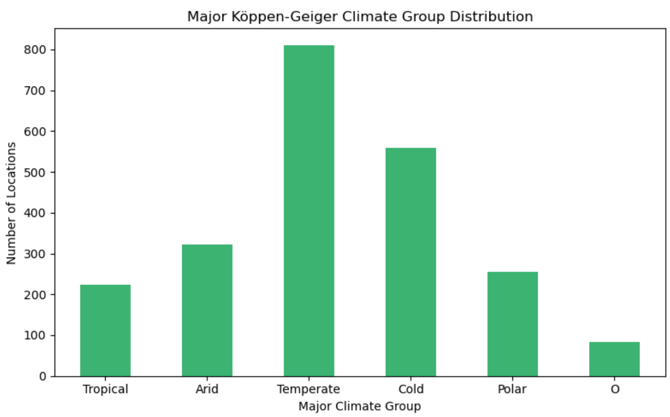


Figure S9. *Köppen*-Geiger climate distribution of samples in the brGDGT database created with the kgcpy (Yu et al. 2024) library in Python. O are samples without a label or coordinates assigned.

Citation: Yu, Xuanji, Julian Ascencio, and Roger French. "Open-Source Climate Classification Package: kgcPy." *2024 IEEE 52nd Photovoltaic Specialist Conference (PVSC)*. IEEE, 2024.

3) The results rely heavily on probability estimates to detect mixed-source environments, but their uncertainty and confidence intervals are not entirely clear. How robust are these estimates? How reliable would they remain in cases of overlapping depositional influences (ex. lake vs peat transitions)?

Response: Thank you for this feedback. Yes, we agree that more information is needed on the confidence intervals and the robustness of these estimates. Therefore, we have created 95% confidence intervals by bootstrap 500 times the probabilities on the downcore predictions. We have updated the text to include this information in the methods and discussion section, and what this may mean for applying our model to other sedimentary sequences and in mixed contexts. We have also updated Figure 4 to include these confidence intervals:

Text added:

Methods: "To estimate the 95% confidence intervals for each downcore record, we performed 500 bootstrap resampling on the probability predictions. These were computed separately for each record to reflect their individual variance.:"

Updated draft figure:

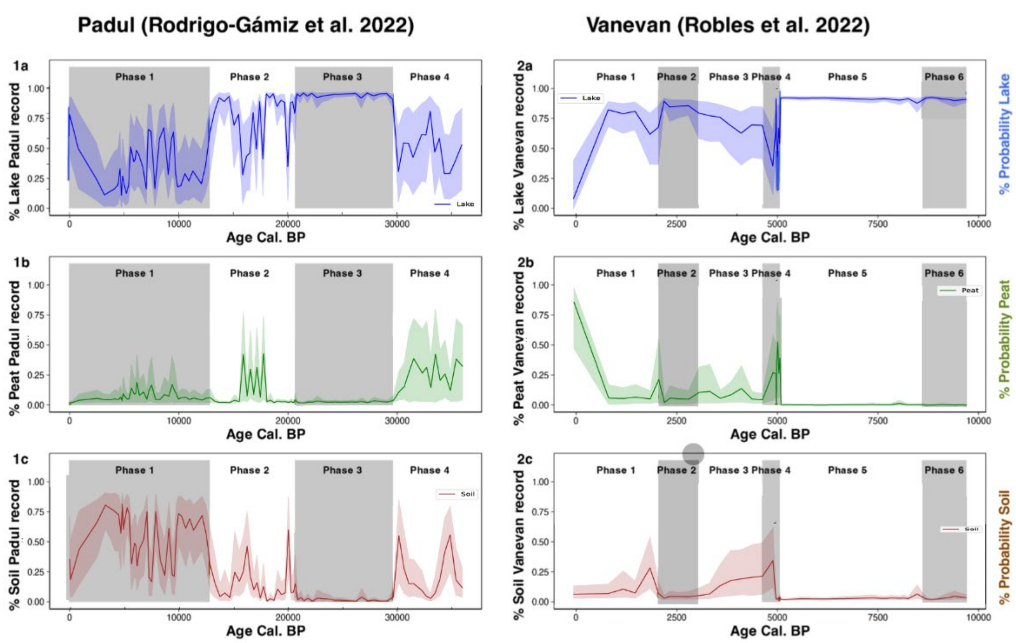


Figure 4: Downcore probability estimates with 95% confidence intervals and changepoint breaks from Random Forests (RF) on the SMOTE dataset with a sigmoid calibration. Results from the Padul (1) and Vanevan (2) records are broken down by lake probabilities (blue curves - a), peat probabilities (green curves -b), and soil probabilities (brown curves - c). Highlighted grey and white boxes indicate changepoint mean breaks identifying phases. Probability estimates from other models can be found in Supplement 1. (Fig. S5 – S8)

Text added:

4.2.4 Considerations for application to paleo-sedimentary sequences

"The log-loss score of 0.31 for the Random Forest model with a sigmoid calibration indicates robust performance in detecting provenance shifts, including sequences characterized by mixed sources. Nonetheless, the associated 95% confidence intervals exhibit variability along the sedimentary profiles, requiring caution when interpreting the model's output. Furthermore, given that the modern reference dataset is contingent on the accurate classification of the sedimentary context, as reported by original authors, and does not account for mixed provenance in the modern database (e.g., lake sample with high amounts of soil inputs) uncertainties in provenance classification remain possible despite the model's accuracy."

4) The study validates machine learning results using pollen and NPP data, but this comparison has uncertainties among which are variations in pollen/spore productivity and dispersal over time. These uncertainties could also affect the reliability of pollen-based reconstructions. A more extended comparison with established proxies of local relevance (e.g., geochemical elemental data, stable isotopes) would strengthen the argument that machine learning provides superior brGDGT provenance detection.

Response: While we understand the concerns with pollen productive and dispersal overtime, we disagree with the reviewer that pollen and NPPs are not relevant local proxy to measure changes in brGDGT provenance. Pollen dispersal and productivity are indeed an issue for pollen records and like all paleo-proxies are imperfect, but the water-depth estimates from these lakes are based on aquatic pollen and NPPs that are considered a local proxy. Both of these water-depth reconstructions were previously peer-reviewed in these separate studies done by separate non-coordinated researcher teams showing the accepted reliability of these reconstructions in the pollen communities. Most of the pollen and NPPs utilized in for these studies comes from aquatic taxa or taxa that is local to the lake, including Cypereaceae and Typha. The NPPs include aquatic algae, such as *Pediastrum* and *Botryococcus* that live in the lake. In addition, the spores utilized in these records are also of local origin. In addition, many gdgt articles have utilized pollen and NPPs to verify their GDGT results (see text below). Both cores, however, also had XRF analysis done which corresponds to the pollen water depth estimates and our datasets. We have provided an extended discussion in the manuscript including the XRF data from environments and updated figures (6 and 7).

We have added additional text to explain the validation.

Introduction:

This section now reads: "Aquatic pollen and NPPs has previously been used to verify changes in provenance in brGDGT communities from fossil records (i.e., Robles et al., 2022; d'Oliveira et al. 2023; Ramos Román et al., 2022; Barhoumi et al., 2023). In addition, we also compare our results with XRF core scanning data from the same sedimentary sequence to strengthen the analysis."

Methods section now reads:

“Although pollen and fungal spore dispersal can be an issue, the fossils of semi-aquatic plants, fungal and fern spores, along with algae should come primarily from around and inside the basin (Gelorini et al., 2013; Gill et al., 2013) which reflects their usage as local indicators of change. Percentages of the aquatic and NPP taxa were calculated by summing all relevant pollen types for each record and dividing each taxon by the total sum. We calculated and re-calculated key brGDGT-based indices (Table 1) to compare our machine learning results with the brGDGT record as well as the aquatic pollen and NPPs. In addition, we also compared our results with the principal components output on the XRF datasets, also taken from the same cores, published in Robles et al. (2022) and Camuera et al. (2018). The descriptions of this analysis can be found in the original publications.”

Citation added:

Gill, Jacquelyn L., et al. "Linking abundances of the dung fungus Sporormiella to the density of bison: implications for assessing grazing by megaherbivores in palaeorecords." *Journal of Ecology* 101.5 (2013): 1125-1136

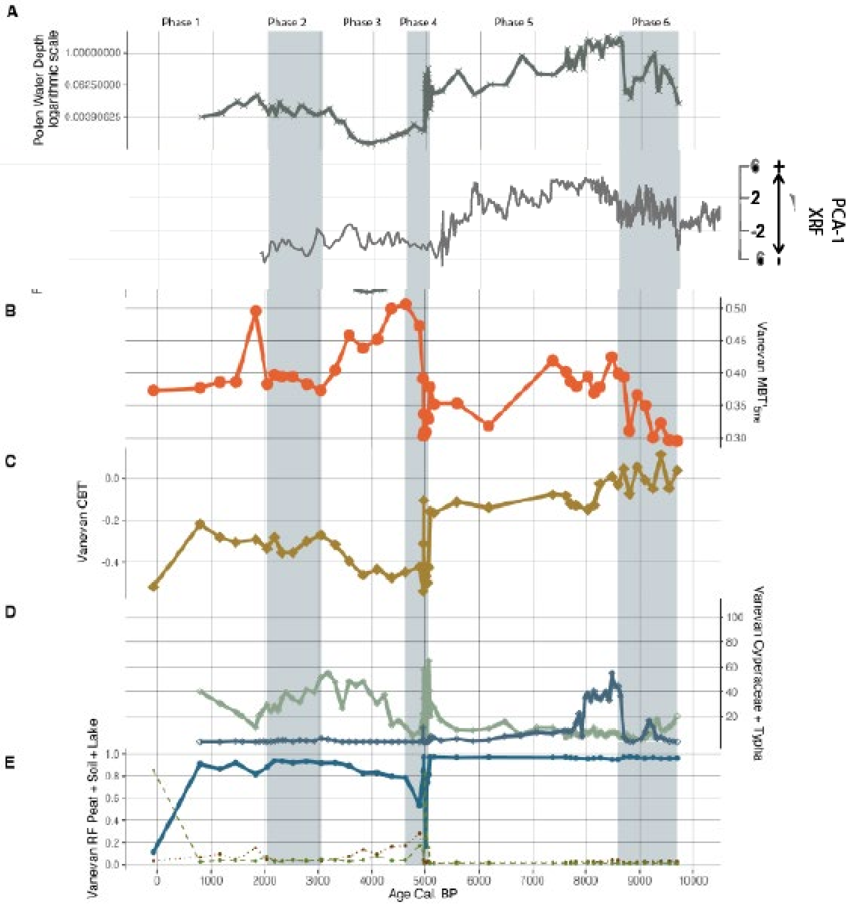
Gelorini, Vanessa, Immaculate Ssemmanda, and Dirk Verschuren. "Validation of non-pollen palynomorphs as paleoenvironmental indicators in tropical Africa: Contrasting~ 200-year paleolimnological records of climate change and human impact." *Review of Palaeobotany and Palynology* 186 (2012): 90-101.

This section now reads:

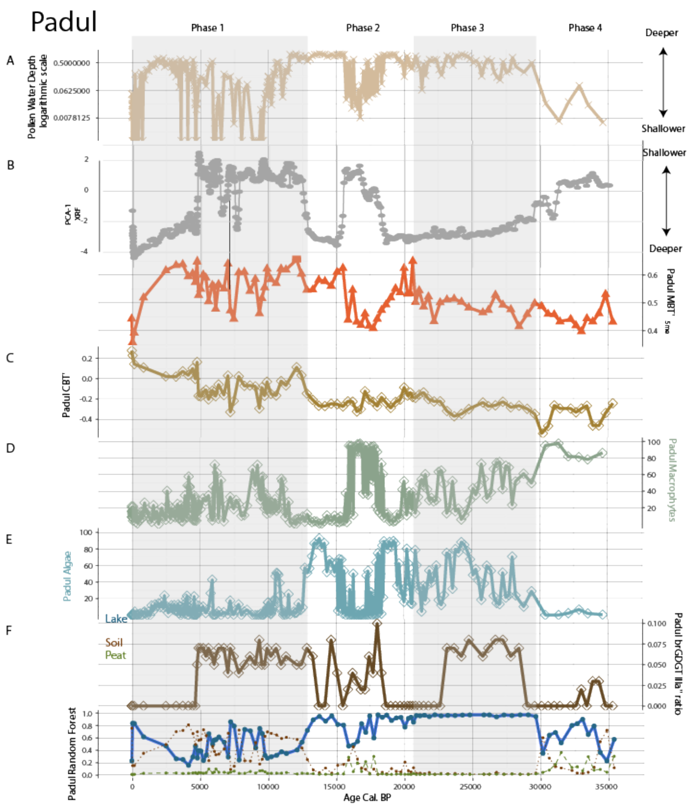
“PCA analysis was also conducted on the XRF dataset on both the Padul and Vanevan cores, and the authors used it as a proxy for lake-level change. For Padul Camerua et al., (2018) attributed negative loadings of PCA-1 with higher lake levels (Ca, Sr, Si, A, MS) and positive loadings with lower (Fe, S, Br, TOC, C/N). For Vanevan, Robles et al., (2022) associate higher lake levels with the PCA-1 and higher positive loadings (P, K, Al, Mg, Si, Ti, Fe) and negative (S) with lower lake levels. The probability estimates in the Padul record exhibit trends analogous to the Vanevan results, with an alignment with water depth as indicated by pollen, NPPs, and XRF (Fig. 7B). The estimates derive from the pollen data and XRF data from Camiera et al., (2018) indicating a low water stand in phase 4, a high water stand in phase 3, a fluctuating high to low to high stand in phase 2, and a high, fluctuating to low to high stand in phase 1. The observed trends are reflected in our brGDGT-based ML lake probability estimates. Similar to the Vanevan record, the Padul record predominantly features samples with brGDGT-based ML lake probabilities assigned to lakes. However, there is greater variation among categorical types. This is evident in phases 4 and 3, where peat and soil probabilities are combined with lake probabilities, and in phase 1, where notable fluctuations occur in soil and lake probabilities.”

Draft figures (these figures will be cleaned up prior to the manuscript being resubmitted, if accepted, but due to time we wanted to show that the PCA from the XRF data B corresponds to the waterdepth and brGDGTs).

Vanevan



A
Phase 1
Phase 2
Phase 3
Phase 4
Phase 5
Phase 6
Pollen Water Depth
logarithmic scale
1.00000000
0.06250000
0.00390625
6
2
-2
-6
+
-
PCA-1
XRF
B
Vanevan MBT'5me
0.50
0.45
0.40
0.35
0.30
C
Vanevan CBT
0.0
-0.2
-0.4
D
Vanevan Cyperaceae + Typha
100
80
60
40
20
E
Vanevan RF Peat + Soil + Lake
1.0
0.8
0.6
0.4
0.2
0.0
0
1000
2000
3000
4000
5000
6000
7000
8000
9000
10000
Age Cal. BP



Minor points

Introduction:

Could the authors elaborate more on the specific limitations of past methods in br-GDGT provenance detection?

Response: Thank you for this suggestion. We have gone ahead and added the following text in the introduction to give a brief history of past brGDGT provenance. This section now reads:

> "As climatic or successional changes occur concurrently with temperature variations, isolating the effects of source changes on the MBT'$_{5ME}$ is challenging. Several indexes and ratios have been developed to detect brGDGT provenance change. The BIT index (Hopmans et al., 2004), and later the IIIa/IIa ratio (Xiao et al., 2016), for example, were designed to identify terrestrial organic input in marine sediments (Hopmans et al., 2004). Although useful in marine contexts, these indexes have had limited success in lacustrine terrestrial environments (e.g., Martin et al., 2020). Ternary diagrams are commonly used to visualize brGDGT (e.g., Russell., et al. 2018; Naafs et al.), enabling the comparison between fossil and modern datasets. These diagrams, however, reduce the data size to three variables, limiting their usefulness in isolating the influence of provenance change on the individual brGDGT isomers. Recently Martínez-Sosa et al., (2023), employed supervised machine learning (ML) to identify changes in

provenance using classification models based on modern samples. Their success highlights the power ML applications can have in solving difficult issues. ML applications differ from traditional statistics applications by focusing on prediction rather than inference (Bzdok et al., 2018**)**. ML's power over these conventional methods lies in their ability to handle data with multiple variables for a few subjects while examining non-linear relationships within the datasets (Bzdok et al., 2018). Martínez-Sosa et al. (2023) models proved effective at identifying shifts in provenance; a limitation of their study, however, is the inability to detect periods of mixed provenance. This paper presents a strategy for identifying provenance changes across lacustrine, peat, and soil depositional environments, including mixed contexts, utilizing a new global brGDGT database, machine learning techniques, as well as environmental reconstructions based on pollen, non-pollen palynomorphs, and XRF datasets."

The discussion on machine learning techniques is somewhat broad; more details on how these approaches differ from traditional statistical methods would be very helpful.

Response: thank you for this response we have added the following sentence into the introduction, as seen integrated into the paragraph above.

"ML applications differ from traditional statistics applications by focusing on prediction rather than inference (Bzdok et al., 2018**)**. ML's power over these conventional methods lies in their ability to handle data with multiple variables for a few subjects while examining non-linear relationships within the datasets (Bzdok et al., 2018)."

Material and Methods:

- The manuscript states that models were trained and tested using a new modern database, but it does not provide sufficient details on data preprocessing and splitting strategies. E.g. What were the rationale behind dataset division (train-validation-test)?

Response: In order to test the machine learning we decided on a 60:20:20 split for training, validation, and testing based on the best practices. We initially tested these models with an 80:10:10 data split but did not find any improvement and found that our initial split was enough to provide data for training, validation, and testing. We have added the following to the text:

"The data was split into a 60:20:20 training, testing, and validation set. This provided enough data to train the model with high accuracy and ensure that testing and calibration could occur on datasets that were previously unseen during training."

The use of SMOTE seems suitable for handling class imbalance, but was there any testing to ensure it did not introduce artificial biases?

Response: SMOTE provided marginal gains in both the accuracy of the classification of the model and and probability estimates over the raw unbalanced datasets. However, testing occurred with both datasets to make sure that bias did not occur. The full probability models from both are in the supplement figure (S7 and S8) for comparison along with table 1.

In addition, we analyzed the distribution and used a Kolmogorov-Smirnov test comparing the original and smote datasets. These results show that bias was not introduced with smote. We have added the following text into the document:

Text added: lines 56-59 The distribution of the SMOTE samples and original database were plotted and a principal component analysis and Kolmogorov–Smirnov test were run to verify that no bias was introduced (results in supplement Figure S8 & S9 and Table S1).

Figures added:

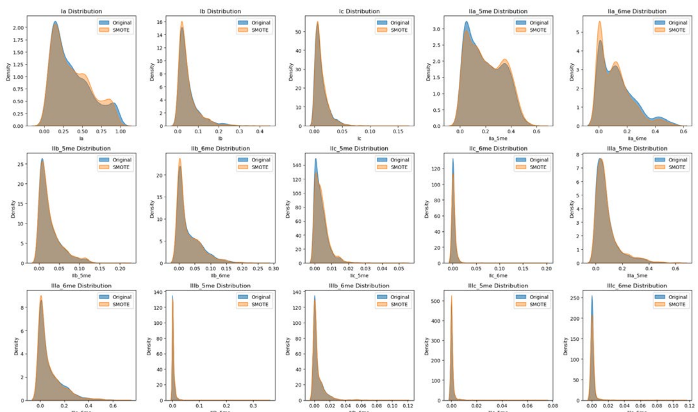


Figure S9. Distribution of brGDGTs between the original and smote datasets. Overlapping distributions suggests that bias was not introduced with the SMOTE samples.

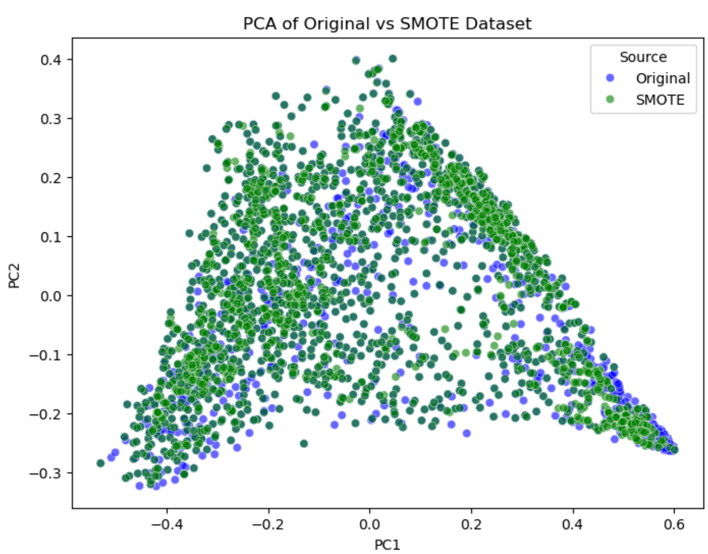


Figure S10. PCA distribution of the original and SMOTE datasets

A.	Original class distribution:	
	SampleTypeNum	
	1 0.507672	
	0 0.259097	
	2 0.233231	
	Name: proportion	dtype: float64
	SMOTE class distribution:	
	SampleTypeNum	
	0 0.333333	
	1 0.333333	
	2 0.333333	
	Name: proportion	dtype: float64
B.	Kolmogorov-Smirnov Test between original and SMOTE datasets (for each feature):	
	Ia: KS statistic = 0.0323	p = 1.8211e-01
	Ib: KS statistic = 0.0277	p = 3.4250e-01
	Ic: KS statistic = 0.0401	p = 5.0102e-02
	IIa_5me: KS statistic = 0.0300	p = 2.5261e-01
	IIa_6me: KS statistic = 0.0742	p = 6.9874e-06
	IIb_5me: KS statistic = 0.0161	p = 9.2414e-01
	IIb_6me: KS statistic = 0.0525	p = 3.6958e-03
	IIc_5me: KS statistic = 0.0658	p = 1.0150e-04
	IIc_6me: KS statistic = 0.0094	p = 9.9994e-01
	IIIa_5me: KS statistic = 0.0297	p = 2.6297e-01
	IIIa_6me: KS statistic = 0.0555	p = 1.7547e-03
	IIIb_5me: KS statistic = 0.0315	p = 2.0594e-01
	IIIb_6me: KS statistic = 0.0129	p = 9.9015e-01
	IIIc_5me: KS statistic = 0.0616	p = 3.4764e-04
	IIIc_6me: KS statistic = 0.0190	p = 7.9730e-01

Table S1. A counts of each class type in the dataset B. Kolmogorov-Smirnov Test between original and SMOTE datasets (for each brGDGT feature in the dataset)

Results:

The results section provides a detailed evaluation of model performance, but it lacks clarity on what is a meaningful improvement in classification accuracy. For example, what is the practical significance of a 0.72 vs. 0.90 F1 score in this context?

Response we have added an extended discussion on F1 accuracy and model comparison in the discussion (see above). This section now reads:

“The F1 score evaluates the accuracy of a model’s predictions of both precision (how many predicted positives were positive) and recall (from all the positives, how many positives did the model predict) and can balance between understanding false positives and false negatives (Boozary et al., 2025). This score allows for a more robust accuracy when measuring each model. Many things may explain differences in F1 scores across our models. For example, K-NN, SVM, and CART models are prone to overfitting (Huang et al., 2005; Berk, 2008 Jadjav and Channe, 2013), which may have accounted for their lower F1 scores (Table 1). RF generally does not overfit due to its ability to handle noise in the datasets (Parmar et al., 2019), which may result in a higher F1 score. While LR does not typically overfit, the lower F1 score may be due to its assumptions of linearity (Nick and Campbell 2007), which may be problematic if there is no clear division in the dataset. The balanced versus unbalanced datasets may have also impacted performance. RF generally handles unbalanced datasets well (Anaissi et al., 2013), and the SMOTE dataset only offered marginal improvements to the F1 score, while CART’s F1 score was significantly improved with the balanced SMOTE datasets.”

The comparison of sigmoid and isotonic calibration functions is interesting, but it is unclear why certain calibrations improved some models but worsened others. More discussion on the underlying reasons for these differences is needed (at least these could be included in the supplementary material).

Response: Thank you for this recommendation, the following has been added into the text under the discussion results

“For the logloss scores, logistic regression is already calibrated (Kull et al., 2017a) so calibrating may result in a lower log loss score, with both a sigmoid and isotonic calibration. SVM does not produce true probabilities by default and needs to be calibrated for these results (Kull et al., 2017b). By calibrating them with a sigmoid or isotonic regression, the output turns to true probabilities which may result in a lower log loss score. For K-NN, CART, and RF calibration improved the models on both datasets.”

Discussion:

The proposed "brGDGT wetland index" is an interesting addition, but more validation is required. The assumptions behind the brGDGT wetland index appear to be valid in modern datasets, but their applicability to fossil records is problematic mainly due to the fact that pollen productivity and dispersal (incl. source area) vary over time due to climatic, ecological, and taphonomic factors (incl. differential preservation). Because of these reasons, a water level reconstruction based solely on pollen/spores may also be problematic.

Response: We agree with both reviewer 1 and 2 that the WI needs more data to validate. We have decided to remove this index from this paper at this time and hope to publish it elsewhere at a later date. This section has now been replaced with a new section 4.2.4 Considerations for application to paleo-sedimentary sequences

Added citations:

Anaissi, Ali, et al. "A balanced iterative random forest for gene selection from microarray data." *BMC bioinformatics* 14 (2013): 1-10.

Berk, Richard A. "Support vector machines." *Statistical Learning from a Regression Perspective* (2008): 1-28.

Bzdok, D., Altman, N. & Krzywinski, M. Statistics versus machine learning. *Nat Methods* **15**, 233–234 (2018). https://doi.org/10.1038/nmeth.4642

Grinsztajn, Léo, Edouard Oyallon, and Gaël Varoquaux. "Why do tree-based models still outperform deep learning on typical tabular data?." *Advances in neural information processing systems* 35 (2022): 507-520.

Huang, Kaizhu, et al. "Local learning vs. global learning: An introduction to maxi-min margin machine." *Support vector machines: theory and applications* (2005): 113-131.

Jadhav, Sayali D., and H. P. Channe. "Comparative study of K-NN, naive Bayes and decision tree classification techniques." *International Journal of Science and Research (IJSR)* 5.1 (2016): 1842-1845.

Kull, Meelis, Telmo Silva Filho, and Peter Flach. "Beta calibration: a well-founded and easily implemented improvement on logistic calibration for binary classifiers." *Artificial intelligence and statistics*. PMLR, 2017a.

Kull, Meelis, Telmo M. Silva Filho, and Peter Flach. "Beyond sigmoids: How to obtain well-calibrated probabilities from binary classifiers with beta calibration." (2017)b: 5052-5080.

Nick, T.G., Campbell, K.M. (2007). Logistic Regression. In: Ambrosius, W.T. (eds) Topics in Biostatistics. Methods in Molecular Biology™, vol 404. Humana Press. https://doi.org/10.1007/978-1-59745-530-5_14

Parmar, Aakash, Rakesh Katariya, and Vatsal Patel. "A review on random forest: An ensemble classifier." *International conference on intelligent data communication technologies and internet of things*. Cham: Springer International Publishing, 2018.-65.

Wang, Xin, Hao Helen Zhang, and Yichao Wu. "Multiclass probability estimation with support vector machines." *Journal of Computational and Graphical Statistics* 28.3 (2019): 586-595