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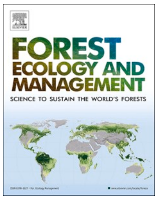

Stable isotope ratios in wood show little potential for sub-country origin verification in Central Africa

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ABSTRACT

Origin verification of timber is essential to expose origin fraud and reduce illegal timber trade. A promising forensic method for origin verification is based on stable isotope ratios in wood, but large-scale studies that test local and regional variation to apply the method at a sub-country scale are lacking.

We investigated the isotopic variation in wood in Central Africa for two economically important timbers (Azobé and Tali). We measured wood δ^{18}O, δ^{2}H, δ^{13}C and δ^{34}S at 17 locations in the main timber exporting countries (Cameroon, Republic of the Congo, Gabon). This is the first study to quantify both local and regional variation as well as species differences. We applied a novel statistical technique, Quantile Regression Forests, to develop spatial predictions of isotopic composition based on gridded covariables (isoscapes). This is a powerful method to develop isoscapes based on non-normally distributed and correlating covariables.

We found limited potential for origin assignment with stable isotope ratios in Central Africa, because the local variability of values for all four studied isotopes was generally higher than regional variation. This led to low site classification success (Azobé: 32.1%, Tali: 20.5%) and large uncertainty in the isoscapes. The limited origin signal can be attributed to low variation in the driving environmental factors in the region. The isotope ratios did differ between the three countries, country-level assignment was good (Azobé: 79.4%, Tali: 61.7%) and considerably higher than site-level values. Lastly, we found a significant species and site effect, stressing species-specific reference datasets might be needed for future isotope tracing studies.

These results show that the four isotopes applied here have limited potential for sub-country origin assignment of timber in this region (Cameroon, Republic of the Congo, Gabon). Nevertheless, the variation at large spatial scales resulted in promising accuracy of country-level assignment. These findings contribute to our understanding of forensic timber tracing methods and can help to identify the most appropriate method for a tracing question at hand.

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1. Introduction

Illegal timber trade harms people, ecosystems, and local economies and is especially prevalent in the tropics (Hoare and Uehara, 2022). In the Congo Basin, estimations of illegally exported timber go up to 90% for some countries (Hoare and Uehara, 2022), while law enforcement efforts to reduce this trade are often limited by a lack of available tools to verify the true origin of the wood (Lowe et al., 2016). Origin fraud occurs both within and between countries (Gan et al., 2016), so a verification of both country and region of origin could help to combat the illegal trade. A method that has been applied for origin verification of a variety of products is based on the use of stable isotope ratios. It has been successful for origin verification of for example cocaine (Ehleringer et al., 2000), elephant tusks (Ziegler et al., 2016), coffee (Driscoll et al., 2020) and other foodstuffs (Oulhote et al., 2011). Its application in tropical timber so far is relatively limited however (Dormontt et al., 2015; Low et al., 2022) and has yielded mixed results: some studies could distinguish between sites (Watkinson et al., 2022a,b) but others not (Paredes-Villanueva et al., 2022; Vlam et al., 2018a).

The stable isotope ratios mostly used for timber tracing are the bioelements δ^{18}O, δ^{2}H, δ^{13}C, δ^{15}N and δ^{34}S, from which an origin-specific isotopic fingerprint can be defined. The stable isotope ratios of a new sample are then compared to a reference database, allowing for the assignment of a sample to its most likely origin (Förstel et al., 2011). Additionally, isotopic composition can be mapped, producing so-called isoscapes (Bowen, 2010). These isoscapes predict the isotopic composition in between measurement points based on relevant drivers of isotopic variation as covariables, allowing for the assessment of isotopic variation at a landscape scale (Gori, Stradiotti, and Camin, 2018). Both of these methods have the potential to determine timber origin.

For successful origin assignment, geographical variation in stable isotope ratios is a prerequisite (Bowen, Wassenaar, and Hobson, 2005). In timber, this variation depends on the availability and uptake of the isotopes by the tree as well as further on fractionation processes within the tree. Both result in geographical isotopic variation as isotope ratios in wood are influenced by climate, geology and/or deposition, although the specific processes differ per isotope. These processes also define the relevant covariables to be used in isoscapes. Important factors that have been linked to geographic variation in isotopes include distance to the ocean (δ^{18}O, δ^{2}H, δ^{34}S), elevation (δ^{18}O, δ^{2}H, δ^{34}S), temperature (δ^{18}O, δ^{2}H, δ^{13}C), rainfall amount as well as rainfall isotopic composition (δ^{18}O, δ^{2}H, δ^{13}C), nitrogen source (δ^{15}N), bedrock (δ^{34}S) and deposition (δ^{15}N, δ^{34}S) (Allen et al., 2022; Brlík et al., 2022; Cernusak et al., 2022; Lehmann et al., 2022; Sleen, Zuidema, and Pons, 2017; West et al., 2010; Wynn, Loader, and Fairchild, 2014). Therefore, the method has shown high potential for timber tracing in areas where the natural variation of stable isotopes in wood was large between origins (Degen, Bouda, and Blanc-Jolivet, 2015; Förstel et al., 2011; Horacek, Jakusch, and Krehan, 2009; Lee et al., 2015).

For timber origin assignment in West and Central Africa, the use of stable isotopes has shown some potential: one report indicated country-level verification with 55%-75% accuracy for blind samples, including 10 blind samples per test (Degen, Bouda, and Blanc-Jolivet, 2015). At a sub-country scale, timber from two locations in Gabon could be distinguished, mostly based on δ^{34}S (Watkinson et al., 2022b). In contrast, this was not possible in Cameroon based on δ^{15}N, δ^{13}C and δ^{18}O (Vlam et al., 2018a). Studies on animal products in the area did show tracing potential, for example bird feathers have been traced using δ^{34}S (Brlík et al., 2022) and ivory using δ^{13}C, δ^{18}O, δ^{2}H, δ^{15}N and δ^{34}S (Ziegler et al., 2016) although both encompassed a larger study area. Studies on multiple timber species and multiple isotopes with high sampling coverage are lacking however, which is essential to further understand variation in isotopic composition across the area.

The aim of this study was to investigate the isotopic variation in timber from Central Africa in order to test the potential for timber tracing. Additionally, we aimed to improve isotopic origin assignment tests by applying machine learning techniques and by explicitly including prediction uncertainty. To achieve this, we developed an extensive dataset of stable isotope ratios from trees growing at 17 locations across the main timber exporting countries in the region: Cameroon, Gabon and Republic of the Congo. These three countries are the most important sources of timber exports in the Congo Basin, while at the same time illegal practices remain widespread throughout their timber supply chains (Hoare and Uehara, 2022). Increased transparency through scientific origin verification is thus an important step to reduce illegality in the region. We included two important timber species in this study: Azobé (*Lophira alata*) and Tali (*Erythrophleum ivorense* and *E. suaveolens*) to allow for species comparisons. We focused on δ^{18}O, δ^{2}H, δ^{13}C and δ^{34}S as they have been most extensively used for tracing in the area (Brlík et al., 2022; Degen, Bouda, and BlancJolivet, 2015; Watkinson et al., 2022b; Ziegler et al., 2016). δ^{15}N is mostly affected by the source of N_2 and local nitrogen cycling (Sleen et al., 2015), which is not expected to show strong geographical variation, and was thus not included here. This is the first study to test the feasibility of isotopic timber tracing using a sampling coverage that allows a quantification of species differences as well as local and regional variation for multiple isotopes.

As tracing can only be successful if regional variation is larger than local variation, we first quantified the local and the regional isotopic variation within the two species. We then developed isoscapes based on relevant covariables for all four stable isotope ratios to assess the variation in wood isotopic composition across our study area. To develop the isoscapes we made use of Quantile Regression Forests, a novel statistical technique for spatial analysis based on machine learning that has not yet been applied to map isotope variability. The strength of Quantile Regression Forests comes from the fact that it is a powerful method to develop prediction maps based on non-normally distributed, high-dimensional and possibly correlating covariables (the environmental variables driving variation in the stable isotopes). Furthermore, it estimates the conditional quantiles based on all random forest outcomes, which presents an accurate estimation of the uncertainty with a certain prediction (Meinshausen, 2006). Lastly, we tested assignment success by applying classification models based on the stable isotope ratios. Through this research, we aimed to contribute to the development of effective tools for assigning the origin of tropical timber and that could ultimately aid in the reduction of illegal timber trade.

2. Methods

2.1. Study design

The study was conducted on two valuable tropical timber species from Central Africa: Azobé (*Lophira alata* Banks ex C.F. Gaertn, Ochnaceae) and Tali (*Erythrophleum ivorense* A. Chev. and *E. suaveolens* (Guill. & Perr.) Brenan, Fabaceae). These are among the most important internationally traded species and represent a single-species timber, Azobé, as well as a multi-species timber, Tali. Tali was only identified to species level if leaf, flower and/or fruit material was available as it is hard to distinguish in the field. Both timbers grow in evergreen and moist deciduous forests. Samples were taken from trees at 17 study sites spread across the main timber exporting countries of timber from natural forests in Central Africa: Cameroon (7 sites), Gabon (4 sites) and the Republic of the Congo (6 sites), between September 2019 and April 2022 (Fig. 1). Samples were taken from both species at all sites except for two locations: at one site only Azobé was sampled (GAB1) and at one site only Tali was sampled (GAB4) due to low occurrence of the other species. All sites were natural forest concessions, accessed in collaboration with the operating forestry companies.

2.2. Sample collection

At each site, we sampled heartwood from 20 to 30 trees per timber

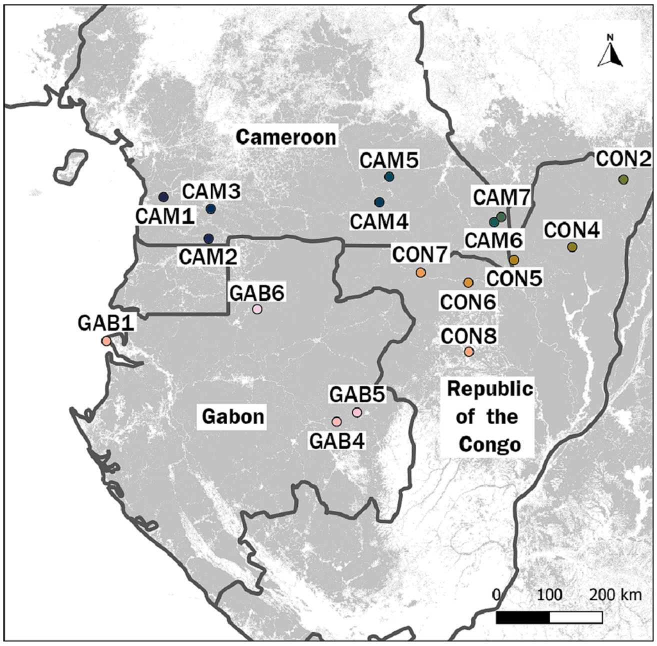

Fig. 1. Map of the study area and study sites. Primary tropical forest extend from Global Forest Watch in light grey (Turubanova et al., 2018).

species. Target trees within one site were located between 100 m and 5 km apart. Trees were either standing or recently felled and were of at least 30 cm diameter at breast height (DBH). We sampled trees of different sizes at all sites to minimize the effect of ontogenetic stage on any isotopic signal, as can be the case for δ^{13}C (Brienen et al., 2017). The heartwood sample was collected from each tree as an increment core (Haglöf Increment borer 350 mm × 5,15 mm; n = 27), with a FAMAG plug cutter of 15 mm diameter (n = 165), as a wood chunk (n = 11) or as a wood powder sample obtained with an electrical drill (n = 85). All samples were taken at least 14 cm into the tree. While in the field, the samples were stored in plastic straws or paper envelopes and properly ventilated to prevent mould. No additional drying steps were performed. Additionally, GPS-coordinates and DBH were recorded.

2.3. Stable isotope analysis

Between four and 10 trees per site were selected for stable isotope analysis, depending on the isotope: we measured δ^{34}S in four trees per species per site (total of 126), δ^{2}H in six or 10 (total of 254), δ^{18}O in six or 10 (total of 246) and δ^{13}C in six or 10 (total of 240) trees per species per site. To define the number of species per site per isotope we balanced between measurement costs and the expected local variation per isotope, local variation of δ^{34}S was expected to be lower than the other three isotopes for example as we assumed less fractionation takes place within the tree. This resulted in a geolocated database of 143 Azobé and 145 Tali trees in total. Measurements of the different isotopes were additive, meaning that all four isotopes were measured in the same tree in as many trees as possible (106 in total). Only if the amount of material was too limited, another tree of that site was analysed.

For the analyses, a subsample of heartwood from every tree was cut in radial direction including at least 3–5 cm to include wood formed during multiple years. δ^{13}C, δ^{18}O and δ^{2}H were measured in cellulose, following Vlam et al. (2018a) for cellulose extractions. δ^{13}C and δ^{18}O were measured at the Leicester Environmental Stable Isotope Laboratory, using an IRMS (Sercon Hydra 20–20). δ^{2}H was measured in duplo at the laboratory of WSL, Birmensdorf, applying an on-line equilibration technique with a DeltaPlus XP IRMS (Finnigan MAT, Schuler et al. (2022)). δ^{34}S was measured in whole wood at Agroisolab GmbH, where the wood was powderized and extracted as described by Watkinson et al. (2022b), after which measurements were performed with an IRMS (Isoprime). The stable isotope compositions were expressed in ‰, relative to an international reference standard (V-SMOW for δ^{18}O and δ^{2}H, V-PDB for δ^{13}C and CDT for δ^{34}S).

2.4. Statistical analysis

2.4.1. Linear models for origin and species differences

Statistical analyses were performed in R version 4.1.0 (R Core Team, 2021). Differences between countries and species as well as site and species were tested by two-way ANOVAs including Tukey post-hoc tests. Additionally, the association between the water isotopes (δ^{2}H and δ^{18}O) in wood and rainfall was tested by multiple regression, including species and rainfall isotopic composition as explanatory variables. The association between tree DBH and δ^{13}C was tested by linear regression including species and DBH as explanatory variables, to test the effect of ontogenic stage of the tree on the δ^{13}C ratio. Before all tests, box-cox or log transformations were applied to remove skewedness of the data after adding a constant value to move values above zero (MASS package, Venables and Ripley (2002)).

2.4.2. Individual isoscapes based on Quantile Regression Forests

We identified relevant environmental co-variables from stable isotope theory and prior studies and obtained those variables at the growth locations of all trees (Table S5.1). We then predicted the relationship between wood isotopic composition and the co-variables by applying Quantile Regression Forests, one model per isotope per species. Random Forest Regression, the underlying method for this analysis, is not influenced by co-linearity and non-normal data distributions and has been successfully applied for other isoscapes (Brlík et al., 2022). Another important advantage over other mapping techniques, such as co-kriging, is that no ‘one best model’ needs to be chosen based on the available data. Therefore, it remains more flexible, as additional data can be easily incorporated once it becomes available and the models can simply be updated by re-training the models. Furthermore, through applying a Quantile Regression Forest, the confidence interval around the predicted median is quantified as the values of all individual prediction trees are stored to calculate the interquantile range. This provides an estimation of prediction uncertainty, an essential step in origin assignment methods. Based on these Quantile Regression Forests, we predicted the spatial distribution of the isotopes in the study area by applying them to gridded layers of all covarables. This resulted in eight isoscapes of the predicted median (2 species, 4 isotopes) and their respective interquantile ranges.

2.4.3. Site and country classification models

Lastly, we applied Random Forest classification models to assign trees to their most likely site (models 1–3, Table 1) and country of origin (models 4–6) based on the four isotopes. As we did not measure all isotopes in all trees, the missing isotope ratios were imputed with the

Table 1
Random forest assignment success at the site and the country level. Trees were assigned to their respective origin based on reference data of Azobé only (1, 4), Tali only (2, 5) or including both species (3, 6). Classification variables are shown in decreasing order of importance.

		Site	Classification variables in order of importance		Country	Classification variables in order of importance
Azobé	1)	32.1%	δ34S, δ2H, δ18O, δ13C	4)	79.4%	δ18O, δ34S, δ2H, δ13C
Tali	2)	20.5%	δ34S, δ18O, δ2H, δ13C	5)	61.7%	δ18O, δ34S, δ2H, δ13C
Combined	3)	18.1%	δ^{34}S, δ^{18}O, δ^{2}H, Species, δ^{13}C	6)	63.7%	δ^{34}S, δ^{18}O, δ^{2}H, δ^{13}C, Species

mean of that site per species. To ensure a fully independent test set without imputed data, 25% of the trees in which all four isotopes were measured was set apart randomly first and these samples were not included in the Random Forest model development. Assignment success was then evaluated using 50-fold cross-validation (i.e., the procedure to form a random test set was repeated 50 times), calculated as: 100% minus the percentage of incorrect assigned trees. Furthermore, we calculated the geographical distance between the real and the assigned origin of all test samples in the 50 cross validations (distHaversine from the geosphere package, Hijmans (2022)).

3. Results

3.1. Species and sites differences

We measured the wood isotopic composition of 288 trees from two tree species at 17 sites, from an area that spanned about 900 km west to east and 450 km north to south across Cameroon, Republic of the Congo and Gabon. δ^{18}O varied between 24.7 and 30.9‰($n = 246$; Fig. 2), δ^{2}H between −25.8 and 36.6‰($n = 254$), δ^{13}C between −31.1 and −21.9‰ ($n = 240$) and δ^{34}S between 4.4 and 10.9‰($n = 126$). We found significant differences between species in all four isotopes, except for δ^{18}O which did not differ between Azobé and Tali when comparing the countries (two-way ANOVA with Tukey post-hoc, $p > 0.05$, Fig. 2a): Azobé had overall higher δ^{2}H ratios and lower δ^{13}C and δ^{34}S ratios than Tali, corresponding to more heavy δ^{2}H stable isotopes and less δ^{13}C and δ^{34}S isotopes relative to their lighter counterparts. Isotopic ratios also differed between countries (Fig. 2a) and individual sites (Fig. 2b). The δ^{18}O ratio in Azobé for example was significantly lower in Cameroon compared to Republic of the Congo and Gabon and was lower in site CAM1 specifically than in nine of the other sites.

In addition to the species and site effect, the interaction between the two was significant for three isotopes (δ^{18}O, δ^{13}C, δ^{34}S), indicating the two species did not share a geographical pattern across sites. Again, this was clear at site CAM1 for example, where δ^{18}O was lowest of all sites in Azobé but not in Tali. The interaction was not significant in δ^{2}H, indicating shared variation between sites in the two timbers. However, even if some sites were relatively unique in terms of stable isotopes, many

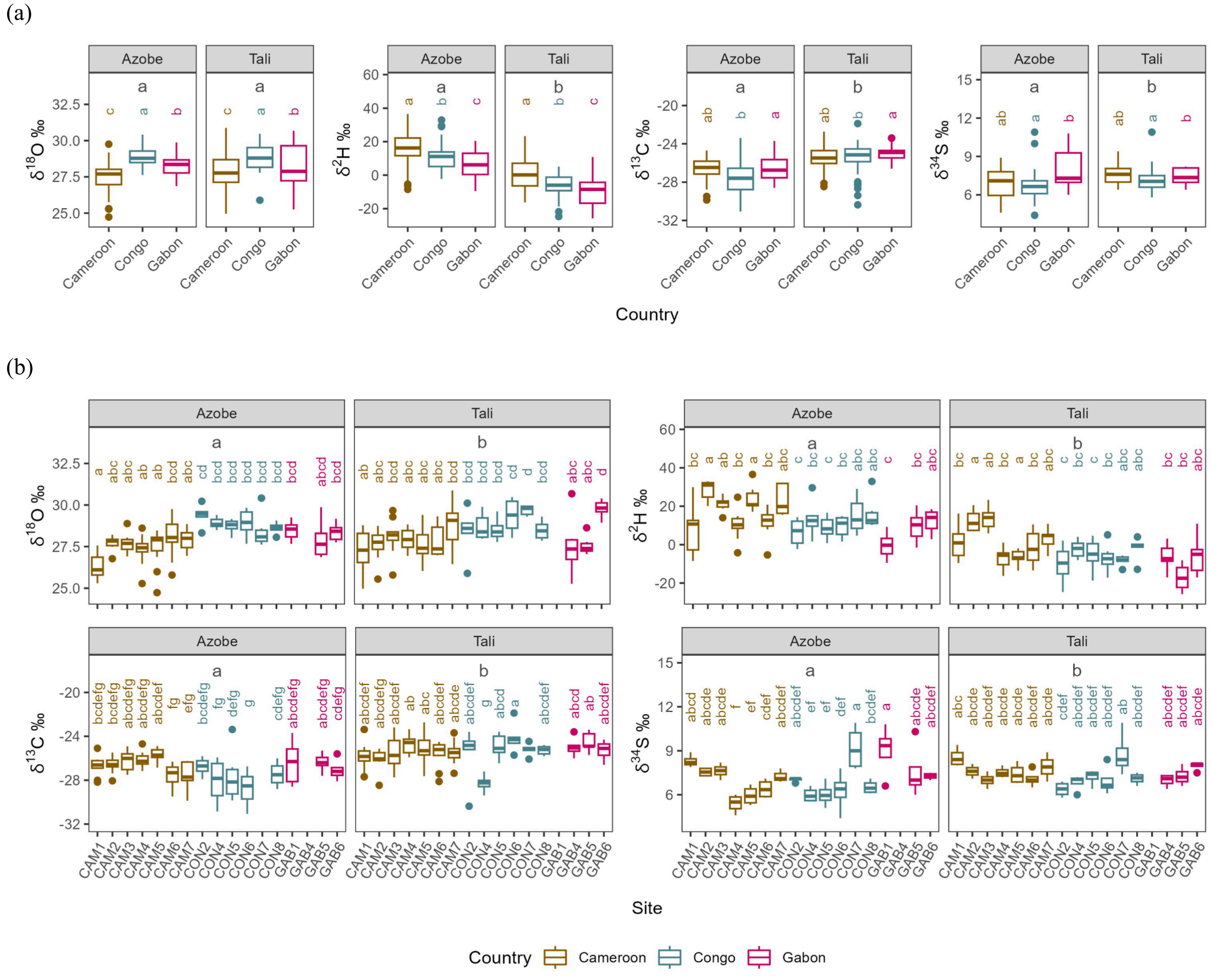

Fig. 2. Stable isotope ratios in the wood of Azobé and Tali across the countries (a) and study sites (b), expressed relative to the respective international standards in ‰. Letters indicate significant differences between species (grey, horizontal) and between sites or countries (colored, vertical, two-way ANOVA with Tukey post-hoc; $n = 246$ for δ^{18}O, $n = 254$ for δ^{2}H, $n = 240$ for δ^{13}C, $n = 126$ for δ^{34}S). None of the species * country interactions were significant so in (a) letters indicate shared country differences between Azobé and Tali. The species * site interaction was significant in δ^{18}O, δ^{13}C and δ^{34}S, therefore in (b) the letters indicate a unique species * site effect that is not shared between Azobé and Tali. In δ^{2}H, the interaction was not significant so the letters indicate shared site differences between Azobé and Tali.

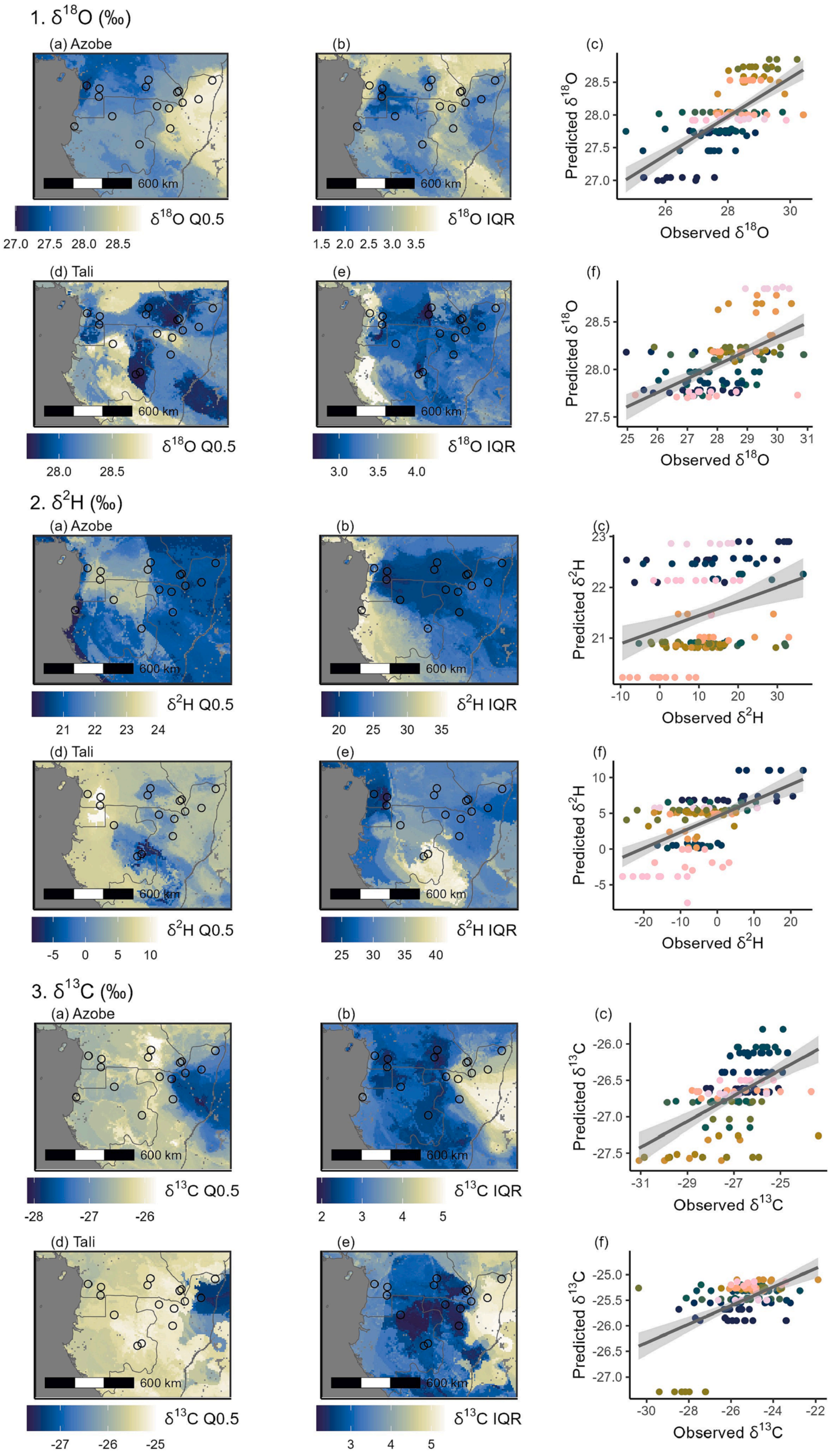

Fig. 3. Isoscapes based on Quantile Regression Forests for $\delta^{18}O$, $\delta^{2}H$, $\delta^{13}C$ and $\delta^{34}S$ (1–4) in Azobé (top) and Tali (bottom), in the study region across Cameroon, Republic of the Congo and Gabon. Isotope ratios are expressed relative to their international standards in ‰. Panels indicate the predicted median isotope ratio (a, d), the interquantile range of the predictions (IQR 0.1–0.9, b, e) and the observed versus predicted isotope ratios of the reference trees (c, f). IQR was calculated as the 0.9 quantile minus the 0.1 quantile. Circles indicate the study sites. For variable importance per Quantile Regression Forest please refer to Fig S5.1.

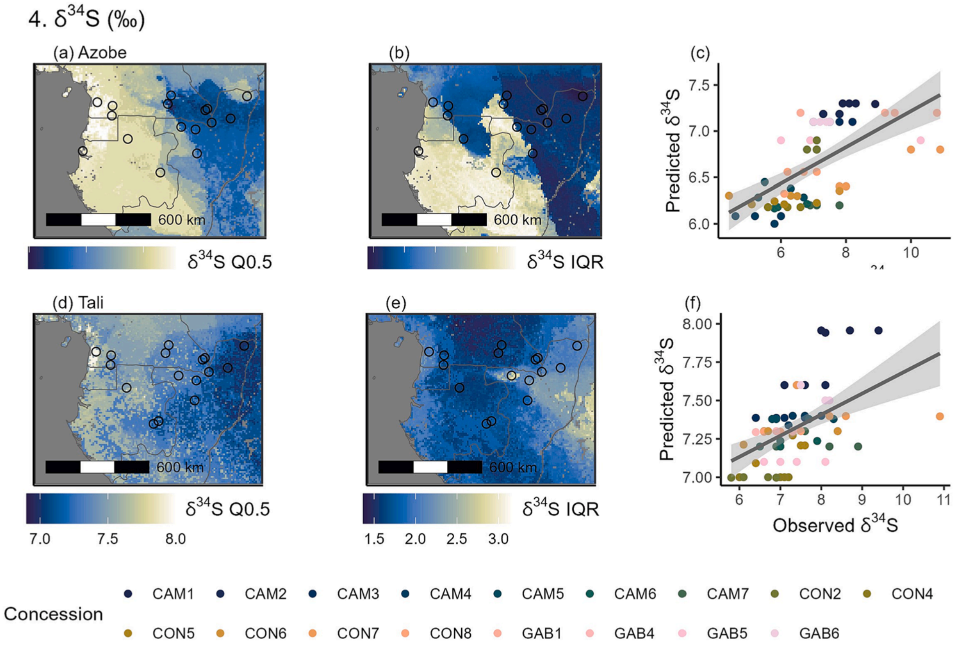

Fig. 3. (*continued*).

sites also showed a large within-site variation that overlapped strongly with the isotopic signal of other sites. As a result, many sites were not significantly different neither from other sites within the same country, nor from sites of the other countries.

3.2. Isotopic variation across the study area: individual isoscape models

We developed eight isoscapes predicting stable isotope ratios across the study area (panels a and d in Fig. 3), one per species for each isotope. All isoscapes showed a gradient from the ocean inwards, reflecting distance to the coast as one of the top predictors in all models (Fig S5.1). Sand content in the soil was another important variable, as well as elevation and several precipitation-related bioclimatic variables (bio 12–19) and temperature-related bioclimatic variables (bio 1–11, see Fick and Hijmans (2017) for the individual labels). Specifically for δ^{34}S, the SO_2 deposition occurred among the top variables as well.

Contrary to what we expected, the modelled stable isotopes in rainwater were not among the most important variables for δ^{18}O and δ^{2}H. δ^{2}H did largely follow the expected pattern of an increased light isotope concentration from the coast inland, and thus lower δ^{2}H for Azobé and Tali from the coast inland (Fig. 3.2a and d). However, this was much less clear in δ^{18}O (Fig. 3.1a and d), where the highest δ^{18}O values were found more inland, especially for Azobé. These patterns were also reflected in the associations between wood and modelled rainwater isotopic compositions for δ^{18}O and δ^{2}H individually: the water stable isotope ratios were significantly associated between rainwater and wood (Fig S5.2), but model fit was low for both isotopes (adj. R^2 of 0.04 for δ^{18}O and 0.19 for δ^{2}H). This indicates a strong local deviation in the isotopic signal in wood from the modelled isotopic signal in rainwater.

The predicted values at the locations of our measured trees all correlated well with the observed values (Fig. 3c and f). However, the range of predicted values was much smaller than the observed range. Even though the median prediction showed a geographical pattern based on the combination of covariables, the interquantile range around the predicted median of every model was large: sometimes the minimal uncertainty around the prediction was as large as the predicted variation across the study area. In Azobé for example, the δ^{2}H ratio was predicted between 20 and 23 ‰ across the whole study area (Fig. 3.2c), but the minimum IQR range was as high as 20 ‰ (Fig. 3.2b). Assigning origin based on these isoscapes will thus result in a large potential area of origin when the model uncertainty is taken into account.

3.3. Origin assignment with multiple stable isotope ratios

Site-level classification success based on the four stable isotopes was low: correct assignment was highest for the Random Forest model predicting Azobé with 32.1% (Table 1). Correct site assignment for Tali was 20.5%. Furthermore, there was no shared isotopic signal among the two species that could lead to increased correct assignment when combining both species, also reflected in the significant interaction term in the two-way ANOVAs (Fig. 2b). Still these results indicate that not all assignments were random, as that would have led to a correct assignment of (1/17 sites)*100 = 6%. In all models, some individual sites were predicted well, such as GAB1, CAM1 and CAM5 for Azobé. The same holds true for Tali, where the sites CAM1 and GAB5 were predicted well, and the combined model where CAM1 and GAB1 were predicted well. This was also reflected in their specific isotopic ratios (Fig. 2b).

Country-level classification performed better, the Random forest model yielded a correct assignment of 79.4% for Azobé and 61.7% or Tali (based on random assignment this would be (1/3 countries)*100%=33.3%). None of the three countries was predicted much better or worse than the others. These country-level differences were also found in the stable isotope ratios: both country and species were significantly different (Fig. 2a).

Lastly, we compared the distance between the predicted site and the real origin of a sample based on the individual random forest models (Fig. 4). A decrease in assignments further away from the real origin would indicate that the samples that were assigned incorrectly were still assigned relatively close-by. Such a finding would be important as it shows at what scale the assignments based on isotopic composition has the most distinguishing potential. However, we did not find that trees were assigned more to close-by sites than to sites that were further away. About 31% and 23% of the samples (Azobé and Tali) were assigned within 25 km of their respective origin, representing the correctly assigned samples. When not assigned correctly, samples were assigned close-by as well as far away (up to 800 km). For comparison, the 17 study sites themselves were on average 395 km apart (SD of 205 km), with a minimum of 16 km and a maximum of 1013 km.

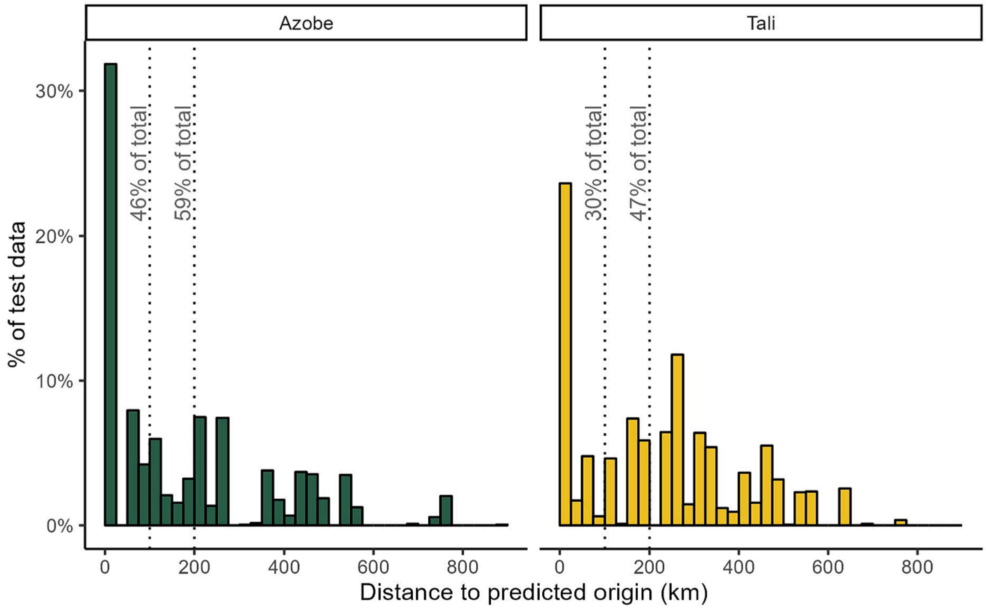

Fig. 4. Histogram of the distance between the predicted site and the real origin (km), based on Random Forest models 1 (Azobé) and 2 (Tali, Table 1). Distance to predicted origin was calculated for every sample in the test set per cross validation, performed for 50 random test sets. The percentage of Azobé and Tali trees that was assigned within 100 km and within 200 km of the real origin is indicated.

4. Discussion

This is the first study to test the feasibility of isotopic timber tracing with a sampling coverage that allowed quantifying species differences as well as local and regional variation. However, we found large local variation in all studied stable isotope ratios which led to low assignment accuracy at a sub-country scale in the main tropical timber production area in Africa (Cameroon, Republic of the Congo and Gabon): For the two studied timbers, Azobé and Tali, a few individual sites could be separated based on δ^{18}O, δ^{2}H, δ^{13}C and δ^{34}S, but for most sites the local isotopic variation was too large to distinguish sites statistically. As a result, isoscapes of the area did not provide sufficient isotope variability, and consequently classification models were unable to provide a satisfactory assignment accuracy for a forensic context. Nevertheless, the accuracy of country-level classification was higher.

4.1. Stable isotope tracing within countries in Central Africa

Our results showed that for all isotopes, site variation was almost as large as the variation in median isotope ratio across the entire study area (Fig. 2). The regional variation in wood stable isotopes was thus too limited to show sufficient differences between sites, which can be explained by a lack of spatial variation in driving environmental conditions. In Central Africa, differences in temperature, rainfall and seasonality are relatively small (Fick and Hijmans, 2017), variation in the stable isotopes in rainfall is limited within the study region (Bowen, 2023), elevation differences are mostly very gradual and not more than a few 100 m, and the bedrock mostly homogeneous (Persits et al., 1997). As a result, strong drivers for geographical variation in the isotopic composition of wood are limited. This shows that tracing with stable isotope analysis on a sub-country scale is more challenging in Central Africa compared to other areas where the gradients in isotope ratios are more defined, such as in Central Europe (Boner et al., 2007; Gori, Stradiotti, and Camin, 2018; Hobson et al., 2004) or across South America (Bowen, 2023; Ehleringer et al., 2000). The latter regions have strong variation in temperature, rainfall, the isotopic composition in rainfall, elevation and/or bedrock material, which can drive the geographical isotopic variation in wood.

We did find a more unique isotopic fingerprint at some individual sites, such as CAM1 in the coastal area of Cameroon (Fig. 2). The method could thus be used to distinguish specific combinations of sites. This is in line with previous work on timber in Central Africa, showing isotopic differences between two sites in Gabon of which one was close to the coast and the other was about 400 km inland (Watkinson et al., 2022b). δ^{34}S was found to be most important for site distinction there, which is especially driven by to distance to coast (Fig S5.1 and Nehlich (2015)). However, a pairwise site comparison is not always the most relevant question for tracing purposes. More often, a claimed origin needs to be verified, for which the claimed origin has to exhibit unique biochemical characteristics that are not found elsewhere. Based on our isoscape results we foresee that origin verification tests at the sub-national level will not improve by expanding the dataset with additional sites. On the contrary, as data collection increases to fill the sampling gaps in the area, the isotopic fingerprint of individual sites might become less unique. As a result, assignment accuracy between sites may go down further.

One of the factors most often described as a source of isotopic variation in δ^{18}O and δ^{2}H in wood is the isotopic composition in rainwater, so we tested the association between the two. However, we did not find strong positive correlations between isotope ratios in cellulose and those modelled in rainwater within our study area (Fig S5.2), contrasting what has been found on a global scale (Barbour, Andrews, and Farquhar, 2001; Lehmann et al., 2022). This lack of association could again arise from the low spatial variability in climatic conditions and origin of rainfall within the study region. The modelled δ^{18}O and δ^{2}H in rainwater are a function of rainfall amount and temperature (Bowen, 2023), both of which show little variation across the area. As a result, the range in modelled δ^{18}O in rainfall is 4 ‰and the range in δ^{2}H is 30‰, which is very small compared to the global variation of 61.7 ‰for δ^{18}O and 486.1 ‰for δ^{2}H (Bowen, 2023). This variation in modelled rainwater isotopes is even small compared to the modelled variation within the African continent, which is 15.5 ‰ for δ^{18}O and 108.2 ‰ for δ^{2}H. This lack of a strong gradient in the modelled isotopes in rainfall also explains their limited importance in the random forest models, both for the isoscapes (Fig. 3) and the classification models (Table 1).

4.2. Causes of local and temporal variation in isotopic ratios

In addition to naturally occurring variation, the observed local variation could also be partially attributed to methodological uncertainty and choices made in sampling design (Horacek et al., 2018). One source of uncertainty is measurement precision, as the variation may be due to measurement errors instead of actual patterns. The measurement precision of all four isotopes was high however and did not substantially contribute to the observed variation within species and sites: measurement errors were on average 0.2 ‰ for δ^{18}O, 3 ‰ for δ^{2}H, 0.1 ‰ for δ^{13}C and 0.3 ‰ for δ^{34}S. Therefore, although it may add some variation, it does not explain the large variation within our research sites.

Another source of variation are the year-to-year fluctuations in stable isotopes within a wood sample, which can cause variation between sampled trees if not enough years are pooled (Van der Sleen, Groenendijk, and Zuidema, 2015). To minimize this effect, we sampled 3–5 cm of the heartwood, thereby including multiple growth years. Tali and especially Azobé do not produce distinct annual rings but it is assumed that this sample size corresponds to at least 5 years of growth as these trees grow between 0.2–1 cm in diameter a year (Engone Obiang et al., 2012; Groenendijk et al., 2014). In all trees the sample was taken from the outer 10 cm of diameter growth of the heartwood, assuming limited additional variation in δ^{13}C due to changing atmospheric CO_2 composition as a result of the Suez effect (Cernusak and Ubierna, 2022). Therefore we are confident this methodological source of variation has a limited effect on our results.

Variation in δ^{13}C could also result from differences in tree age through variation in height and the amount of light it receives (Fonti et al., 2018). We only sampled trees of $>$30 cm DBH to exclude juvenile trees, but we still found a slight positive trend in δ^{13}C with increasing DBH (Fig S5.3), especially for Azobé. Trees of varying sizes were sampled at all sampling sites, which could thus partially explain the local variation in δ^{13}C. The Suez and juvenile effect highlight the complexity of applying δ^{13}C measurements for timber tracing, as the year of wood formation cannot be easily verified after processing at a sawmill. No trends were found between DBH and any of the other stable isotope ratios.

Lastly, we deliberately chose to measure the δ^{18}O, δ^{2}H and δ^{13}C isotope ratios in cellulose instead of whole wood, even though isotopic composition between the two is assumed to be correlated (Helle et al., 2022). Whole-wood measurements include more wood constituents such as lignin, extractives and resin, which have different isotopic signatures. As a result, the isotopic signal in whole wood is also affected by differences in wood composition such as higher lignin content (Helle et al., 2022). Cellulose, on the other hand, has a more stable isotopic signal as variation due to different biosynthetic pathways of wood constituents has been removed. This is especially relevant when aiming to identify origin in areas with low isotope variability. If measurements would be done on whole wood, a low signal of natural variability could then be masked by differences in wood composition and complicate tracing (Ferrio and Voltas, 2005). Furthermore, measuring cellulose rather than whole wood reduces differences between species as a result of wood composition, therefore facilitating a better species comparison.

4.3. Species differences

All four stable isotope ratios were different between Azobé and Tali (Fig. 2). This species difference is in line with isotopic studies on other tropical tree species in Bolivia Paredes-Villanueva et al. (2022) and Ghana (Förstel et al., 2011) and also suggested by data from the United States and Gabon (Watkinson et al., 2022a,b). Species differences can for example result from differences in fractionation processes due to differences in water use or differences in wood constituents. More importantly however, we also found a significant interaction between site and species for δ^{18}O, δ^{13}C and δ^{34}S. This indicates the two species responded differently to the environmental variables at these sites. Species specific differences such as rooting depth and water-use efficiency could have had a strong influence on the local isotopic signal, therewith suppressing a shared geographical pattern (Sleen, Zuidema, and Pons, 2017; Wynn, Loader, and Fairchild, 2014). For tracing purposes this complicates matters, since it means that multiple species cannot easily be combined into one reference dataset for origin classification: accuracy went down in the combined models (Table 1). This is especially important when defining sampling designs, which are often based on the assumption that multiple species share their isotopic patterns and are therefore aimed at a low amount of samples per species per site. This also warrants a check of the two species that are traded as Tali and regarded as one in this study. The differences between Azobé and Tali samples were clearly larger than any variation of the Tali samples across sites. Therefore we think that combining samples from the two botanical species traded as Tali did not have a strong effect on the results of our study. This lack of isotopic geographical correspondence among species should be tested further in areas with a stronger isotopic gradient (f.e. in terms of rainfall, elevation, etc), as any environmental signal may be reflected more clearly in the wood isotopic composition there.

4.4. Potential for stable isotope tracing in Central Africa

The potential for tracing at a sub-country level with stable isotopes in Central Africa has been a subject of debate in scientific literature in recent years. Site-level differentiation based on δ^{18}O, δ^{15}N and δ^{13}C was not possible for three sites in south Cameroon (Vlam et al. (2018a) and see Horacek et al. (2018) and Vlam et al. (2018b)), but two sites in Gabon could be distinguished mostly based on δ^{34}S and δ^{2}H (Watkinson et al., 2022b). Compared to previous studies, which were based on samples obtained from 2 to 3 sites within Cameroon or Gabon, our study is much more elaborate (17 sites) and covers a considerably larger area (across Cameroon, Gabon and Republic of the Congo). While the sets of isotopes differ among these studies (Vlam: δ^{18}O, δ^{15}N and δ^{13}C; Watkinson: δ^{18}O, δ^{15}N, δ^{13}C, δ^{2}H and δ^{34}S; this study: δ^{18}O, δ^{13}C, δ^{2}H and δ^{34}S), two to four isotopes overlap among them. With the four isotopes that have showed the highest potential for origin verification, we found limited potential for sub-country timber tracing with stable isotopes in Central Africa. This does not imply that timber tracing with stable isotopes has no potential at the sub-country scale in other areas, for example in the US (Kagawa and Leavitt, 2010; Watkinson et al., 2020), Central Europe (Gori, Stradiotti, and Camin, 2018; Horacek, Jakusch, and Krehan, 2009) or the East Mediterranean (Rich et al., 2016).

Despite the high local variability in the isotopic signal in wood hindering provenancing at the site level in the study area, we did find country-specific differences. Stable isotope ratios can thus still offer larger-scale origin identification, also in Central Africa, which can be relevant depending on the provenancing question and required scale of tracing. For example, this is relevant when international trade routes are coming together, as it is often a moment where fraud occurs (Lowe et al., 2016). These findings are in line with results on three timber species in West and Central Africa for example, where country verification success based on stable isotope ratios was between 55% and 75% (Degen, Bouda, and Blanc-Jolivet, 2015).

Still, logically, country verification can only be achieved when natural variation in stable isotope ratios is present between origins. As the drivers of isotopic variation do not always correspond to country borders, a clear isotope signal per country might not always be found. To improve our scientific understanding of the relevant drivers of variation, the established databases here could be extended to cover more countries in West Africa as well as the Democratic Republic of the Congo. This will allow for a comparison of local and continent-wide variation, further testing the scale at which isotopic composition can aid timber tracing in Africa. However, this is less relevant for origin verification in practice as by far the largest share of timber from natural forests in Africa originates from the three countries in this study (International Tropical Timber Organization, 2021).

5. Conclusion

The urgency for accurate timber tracing methods is high, especially in tropical regions such as Central Africa. However, our study shows limited potential to trace origin based on stable isotope ratios within the three most important timber exporting countries in the region (Cameroon, Republic of the Congo, Gabon). Isotopic composition may have more potential on larger scales (such as between countries) or in other regions, but here the regional variation was not strong enough to identify sub-country origin as individual sites already showed high local variability in isotopic composition. Future studies on timber tracing in Central Africa should consider at what scale tracing is most relevant, depending on the types of fraud that are occurring, and investigate methods that can identify such types of fraud. This also includes small-scale fraud such as timber movement close to the border (Nellemann, 2012), for which other methods such as genetic analysis (Jolivet and Degen, 2012; Vlam et al., 2018a) or multi-element analysis (Boeschoten et al., 2022) might have more potential. On the other hand, stable isotope ratios could still be a cost-effective method to identify timber fraud across larger scales and in parts of the world where environmental gradients show strong spatial variability.

CRediT authorship contribution statement

Laura E. Boeschoten: Conceptualization, Methodology, Investigation, Data curation, Formal analysis, Writing – original draft, Writing – review & editing, Visualization. **Mart Vlam:** Funding acquisition, Conceptualization, Methodology, Writing – review & editing. **Ute Sass-Klaassen:** Conceptualization, Methodology, Writing – review & editing. **Barbara Rocha Venâncio Meyer-Sand:** Investigation, Writing – review & editing. **Arnoud Boom:** Investigation. **Gaël U.D. Bouka:** Investigation. **Jannici C.U. Ciliane-Madikou:** Investigation. **Nestor Laurier Engone Obiang:** Investigation. **Mesly Guieshon-Engongoro:** Investigation. **Joël J. Loumeto:** Investigation. **Dieu-merci M.F. Mbika:** Investigation. **Cynel G. Moundounga:** Investigation. **Rita M.D. Ndangani:** Investigation. **Dyana Ndiade Bourobou:** Investigation. **Peter van der Sleen:** Methodology, Writing – review & editing. **Steve N. Tassiamba:** Investigation. **Martin T. Tchamba:** Investigation. **Bijoux B.L. Toumba-Paka:** Investigation. **Herman T. Zanguim:** Investigation. **Pascaline T. Zemtsa:** Investigation. **Pieter A. Zuidema:** Funding acquisition, Conceptualization, Investigation, Methodology, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

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