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Stable Isotope Ratio Analysis of timber to protect two forest concessions in Gabon

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Executive summary

Context
Traceability of timber has come under the spotlight during recent decades with consumers becoming increasingly aware of the damage caused by deforestation and illegal logging to forest ecosystems. Tropical rainforests possess some of the greatest biodiversity on earth, being home to many critically endangered species ranging from insects to mammals. They also act as significant CO₂ sinks and their loss is considered a significant contributor to climate change. Besides ecological impacts, the economic development of many poor nations is at stake. Illegally harvested timber leads to a loss of revenue for many developing economies and leads to hardship for many indigenous forest communities who depend on forest resources for their livelihoods and wellbeing. Misdeclarations, with respect to origin and species of timber also pose a risk to importing nations due to the variety of plant health pathogens and insects that are able to proliferate outside of their natural range. Ash Dieback (Hymenoscyphus fraxineus) and the Asian Long Horn beetle (Anoplophora glabripennis) are just a few examples where ecological damage has been accelerated due to imports of mislabelled timber. Timber export markets compound these problems by trading timber and wood products without ensuring responsible sourcing, or by failing to carry out enough due diligence in their supply chains. Research suggests that between 20% and 40% of all globally traded timber may be mislabelled with respect to origin and species declarations, equating to a volume of 650 million cubic meters of timber. The Worldwide fund for Nature (WWF) estimates that €3bn of illegally harvested timber is placed on European markets each year with most being sourced from the tropical forests in the Far East, and the Congo Basin of West Africa. During the last decade, scientific methods have increasingly played a part in role in verifying the origin and species of traded timber. Recent advancements in scientific techniques including wood anatomy, genetics (DNA), stable isotope ratio analysis (SIRA) and ambient mass spectrometry (DART) now enable regulatory bodies in both importing and exporting nations to provide scientific evidence with respect to the origin and species of traded timber. Moreover, through the dissemination of science-based techniques and implementation of capacity building in timber exporting nations, efforts to combat deforestation using scientific techniques can be implemented at both ends of the supply chain.

Relevance to policy
Since the beginning of the 21st Century, many governments have implemented statutory instruments as means to control the origin and species of imported timber. The US Lacey Act was amended in 2008 to include timber, making it the world’s first ban on trade in illegally sourced wood products. In the EU, regulations took the form of the European union Timber Regulation (EUTR) with the UK set to adopt a parallel version (UKTR) in the year 2021. Several organisations including the International Tropical Timber Organisation (ITTO), Environmental Investigation Agency (EIA), Global Witness, US Department of Justice (DOJ), Worldwide Fund for Nature (WWF), International Union for Conservation of Nature (IUCN) and World Resource Institute (WRI) work to expose illegal practices with respect to harvesting of timber and encourage governments and corporations to take actions against illegal logging. Several certification schemes including Forestry Stewardship Council (FSC) and Programme for the Endorsement of Forest Certification (PFEC) provide the timber industry with a means of carrying out due diligence checks within supply chains, providing paper-based evidence linked to forest and supplier audits. However, certification bodies are only able to audit a fraction of the world’s forests at a fraction of the time, and participation by the timber industry is only voluntary.

Method statement
Stable isotope analysis is a widely accepted analytical technique that has become well established around the world, and it is often used to verify the origin of biological samples. To verify if the origin claim for a piece of timber (test sample) is correct, the method of analysis typically involves comparison of the stable isotopes (C,H,N,S,O)
measured in the test sample against a timber reference dataset of stable isotopes for the same origin and species. Despite the growing number of research studies utilising authentication analyses to verify origin of timber, many have involved insufficient numbers of samples and have focused on single tree species. To address these shortfalls, World Forest ID (WFID) was conceived from the collaborative efforts of several key organisations including DEFRA, Royal Botanic Gardens at Kew, FSC, Agroisolab and the US Forestry Service, with the aim of co-ordinating the collection and analysis of timber reference samples from some of the most endangered forests around the world.

**Aims of the World Forest ID project**

World Forest ID aims to:

- Facilitate the co-ordination and development of timber tracking technologies.

- Provide governments with a scientific resource to carry out enforcement activities which are based on evidence established through laboratory analysis of seized timber.

- Carry out large-scale collections of timber reference samples from around the world, including ‘at risk’ categories of tree species.

- In collaboration with the Royal Botanic Gardens, Kew, provide the wider scientific community with a go-to resource of authentic georeferenced timber samples covering over 100 commonly traded taxa.

- Disseminate research findings, including all data outputs, to the wider scientific community.

**Project objectives in Gabon:**

- Collaborate with Royal Botanic Gardens, Kew (RBG, Kew) and FSC to obtain authentic georeferenced samples of endangered, commonly traded or species of interest of timber from two forest concessions in Gabon.


- Establish if differences occur in the stable isotope ratios of trees from different forest concessions within and outside Gabon.

- Assess what differences occur in the stable isotope ratios of single tree taxa from the same location within Gabon.

- Utilise isotopic methods to verify if the declared origin of the Gabonese timber is consistent with data from Gabon.

![](imageCredit: Charlie Watkinson, 2020.)
Results

• Significant differences in the sulfur isotope ratios of Okoume (Aucoumea klaineana), from PWG and CBG concessions were observed, suggesting that concession level classification in Gabon may be achieved with extensive sampling.
• Discrimination in the sulfur isotope ratios was evident between samples of Dacryodes taken from CBG and PWG concessions.
• Stable isotope ratios measured in samples of Aucoumea klaineana and Dacryodes buttneri are not directly comparable despite belonging to the Burseraceae family.
• Aucoumea spp. and Dacryodes spp. do not follow expected patterns with respect to correlation between δ18O and δD in cellulose and precipitation.

Conclusions

Despite the quantity of samples and species being relatively low, the data acquired establishes a basis of evaluation for assessing geographic origin claims of certain forest products including plywood and laminates from Gabon. The differences in the sulfur isotope ratios of Okoume and Dacryodes reference samples from Precious Woods Group (PWG) and Compagnie des Bois du Gabon (CBG) forest concessions suggest that regional scale origin classification may be realised with high enough frequency of reference sample collection. This study helped to establish the minimum number of reference samples (five per species per site) required in order to account for variance of isotopic distribution at a single sampling site. Low frequency of sampling per site and limited sample numbers mean that investigation of the Dunbar line hypothesis was not possible on this occasion. Further, higher resolution sampling of target species including Okoume and Dacryodes will address this much needed comparison as it may enable more efficient allocation of reference sampling resources. Further sampling of Burseraceae family timbers in the tropics may permit a global model for verifying their geographic origin.

Future work

Expanding the collection reference samples will be necessary to investigate regional classification further. It is hypothesised that having greater quantities of sample data will improve the discrimination between concessions or regions, as well as allowing for comparison of stable isotopes both within a single sampling site and single taxa. A higher frequency of reference sample collection will also enable assessment of the suitability of specific taxa to act as isotopic proxies for other species. Natural variability of isotopic distribution within a site is still not fully understood, and it is anticipated that large scale sample collections for specific taxa will also enable a better understanding of this. So far, the study has focused on whole wood and cellulose components of timber reference samples. Several alternative analytical methods detail procedures for analysing alternative metabolic fractions including lignin and proteins in the form of amino acids. Furthermore, isotope methods are already being used to measure the stable isotopes of position-specific atoms within a selection of molecules such as ethanol, providing higher resolution information on the source water incorporated during metabolism. Analysis of alternative fractions and position-specific isotope ratios within a molecule such as glucose may yield higher quality results and aid the discrimination of reference samples taken from concessions within the same country.
Abstract
The aim of this report was to address the differences in stable isotope ratio profile of bulk, homogenised wood samples collected from living or recently felled trees, between two FSC concessions in Gabon, which are approximately 240km apart, for the purposes of origin classification and protecting valuable forest commodities.

Forty-seven timber samples comprising ten genera of tropical trees were obtained using a Pickering Punch sampling device or chainsaw from two forest concessions in Gabon (PWG and CBG) during July 2019. Samples were subject to \(^{18}O/^{16}O\), D/H, \(^{13}C/^{12}C\), \(^{15}N/^{14}N\) and \(^{34}S/^{32}S\) stable isotope analysis using elemental analysis-isotope ratio mass spectrometry (EA-IRMS). Additional data from relevant taxa and geographic origins (altitude, soil type, geographic co-ordinates) were used to assess potential higher-order spatial patterns in the data.

Results show that significant differences are evident in the stable isotope ratios of Aucoumea klaineana between PWG (Precious Woods Group) and CBG forest concessions. Relationships are evident between climatic and geological variables and the stable isotope ratios of the samples suggesting that further degrees of origin classification may be achievable in Gabon. For other species that were sampled, insufficient numbers meant the possibility to determine discriminating factors between the two concessions was limited.

The data presented establishes a basis for evaluating origin claims of forest products and timber from the CBG and PWG concessions and lays a foundation for future development of timber tracking technologies in Gabon. The technique can be used for purposes of due diligence or forensic investigation by law enforcement as part of demand-side regulations such as EUTR, ILPA, or the Lacey Act.

Introduction
Gabon is a West African nation located on the Gulf of Guinea and Atlantic Ocean, bordering Cameroon, Equatorial Guinea, and the Republic of Congo. Its land mass is approximately 85% forested with deforestation rates of approximately 0.1% occurring annually [NEPcon, 2017]. The timber industry in Gabon accounts for nearly 20,000 jobs [EIA, 2019] and is the country’s most important export (5% of GDP) after oil [NEPcon, 2017]. Of the 22-23 million hectares of forested area, about 4 million ha is protected and 14 million ha are allocated for forestry [EIA, 2019]. All Gabon’s forests are managed by the Ministry of Forest, Environment and Natural Resource Protection who oversees the monitoring of forest resources, including the allocation of forest concessions. Two types of permits are issued by the ministry: Concession Forestière sous Aménagement Durable (CFAD), and Permis Forestier Associé (PFA). CFAD, allows for logging by corporations in land areas of between 50 000 and 200 000 hectares, whereas PFA - (which is reserved exclusively for Gabonese nationals) has a maximum size of 50 000 hectares [NEPcon, 2017]. As of 2017, China, followed by France, Belgium, and Italy were the largest export markets for timber sourced from Gabon [ITC, 2018]. The most important species for the Gabonese timber industry is Okoumé (Aucoumea klaineana) which accounts for most of the country’s timber exports. The timber is commonly manufactured into plywood and veneers at plants within Gabon and Asia [Timber Trade Portal, 2020]. A four-year investigation by the EIA established that illegally sourced timber from Gabon has routinely entered the United States (US) for over a decade and made its way to thousands of US consumers. An in-depth analysis of the okoume (Aucoumea klaineana) veneer imported directly into the US from Gabon indicates the complicity of the US importers [EIA, 2019]. Following a corruption scandal in 2010, the country moved to ban the export of logs and switched to the export of sawn logs and plywood as a means of stimulating the domestic economy [Kersenty, 2019]. Other economically important species for the Gabonese timber industry include Azobé, Bongossi (Lophira alata), Okan (Cyclodiscus gabunensis), Padouk d’Afrique (Pterocarpus soyauxii), Beli (Julbernardia pellegriniana), Tali (Erythrophleum ivorense), Missanda
(Erythrophleum suaveolens). These timbers, and forest products derivatives, are of great importance for protection.

Relevance of stable isotope analysis to other timber tracking technologies

Population genetics is an excellent method for timber tracking [Jolivet & Degen 2012], however, its application as a tool to verify the origin of timber-based products is dependent on the availability and accessibility of genetic material within a sample. For example, okoume is most commonly converted into plywood and is often used for boatbuilding due to its excellent properties [Negro et al., 2011]. Plywood is produced from the layering of multiple sheets (veneers) of rotary-peeled logs which are held together with thermosetting formaldehyde-urea-based resins [Desch & Dinwoodie 1996; Negro et al., 2011]. Typically, heartwood is used for veneer making. Heartwood can be defined as “the inner layers of the wood, which, in the growing tree, have ceased to contain living cells, and in which the reserve materials (e.g., starch) have been removed or converted into heartwood substance” [Hillis 1987]. The process of heartwood formation does not always lend itself to the preservation of genetic material either in plant cell nuclei, mitochondria or chloroplasts. The process of veneer-making involves boiling the wood, applying steam and hot-pressing at over 100°C to cure the thermosetting resin. This process also does not lend itself to the preservation of genetic material for later analysis by population geneticists. As such, there needs to be a method capable of evaluating the origin of the veneers in plywood so supply-chain stakeholders, enforcement and concerned parties can be assured of its legal origin as part of demand-side authentication. Nevertheless, population genetics is a vital technique and is suitable for addressing the origin of timber within Gabon where genetic material can be accessed for analysis.

Ecological importance of Gabonese trees

Aucoumea klaineana pierre (okoume) is a long-lived pioneer species capable of converting savannah into rainforest [White et al., 1996; Born et al., 2006] and is the only species of its genus. It grows relatively quickly and makes up a large proportion of the trees within Gabon. The natural range of okoume includes Gabon but also extends into Equatorial Guinea, southern Cameroon and parts of the Republic of Congo [Born et al., 2011]. Despite its relatively large coverage of Gabon, okoume can be difficult to grow in plantations; one study into the silviculture of young okoume trees found that saplings grew best in the soil of their original population. The growth of the saplings could be described in terms of a function of the distance from the sites where their seeds were harvested to the research site [Koumba Zaou et al., 1998]. This means that if okoume is harvested to the point of exhaustion in one region of Gabon, it will not be a straightforward task to simply replant the trees from another region. Genotypic mechanisms were proposed as a potential explanation for this phenomenon. However, phenotypes are ultimately the product of genetic expression, of which, the available genetic sequences are only part of the story; the mechanism phenotypic variation can also be considered in terms of epigenetics or other genetic regulatory mechanisms. Even so, there are clear examples of distinct genetic populations of okoume within Gabon; Muloko-Ntoutoume et al., 2000 revealed population differences in chloroplast DNA (cDNA), and differences in polymorphic microsatellites have also been identified [Born et al., 2006, Born et al., 2008].

Humans are not the only great apes that rely on okoume for survival; western lowland Gorillas have been observed eating the flowers of okoume. Though it is not a main food source for Gorillas, the availability of okoume during times of famine caused by shortages of other food sources may ensure that Gorillas survive [Williamson et al., 1990]. Nevertheless, in research where Gorillas have been observed, effect of logging did not appear to have affected the local population at the time meaning that logging the trees can still be acceptable if done so in a sustainable manner. Although Gabon constitutes a fraction of the Congo Basin, the country also shelters approximately 45,000 forest elephants, representing nearly 60 percent of Africa’s remaining population [EIA, 2019]. Natural forest is vital for the survival of forest elephants.
Project aims
The ambitions of this project were to define the ranges of stable isotope ratios from multiple species of trees from two forest concessions in Gabon by analysing timber samples extracted from living trees. Analysis of the analytical data should enable:

1. Assess what differences occur in the stable isotope ratios of trees from different forest concessions within Gabon.
2. Assess what differences occur in the stable isotope ratios of single tree taxa from the same location within Gabon.
3. Utilise isotopic methods to verify if the declared origin of the Gabonese timber is consistent with data from Gabon.
4. Collect and perform stable isotope analysis of authentic geo-referenced timber such as: Okoumé (Aucoumea klaineana), Azobé (Lophira alata) Okan (Cylicodiscus gabonensis) Padouk (Pterocarpus soyauxii) Sipo (Entandrophragma utile), Tiali (Entandrophragma angolense), Tali (Erythrophleum ivorene), Bilinga (Nauclea diderrichii), Kévazingo (Guibourtia tessmanii), Bubinga (Guibourtia pellegriniana), Owëngkol (Guibourtia ehie), Beli (Paraberlinia bifoliolate), Niangon (Heritiera densiflor), Douka (Tieghemella Africana), and Movingui (Distemonanthus benthamianus).

If significant differences in the stable isotope ratios of trees from within different concessions in Gabon are evident, this may be of great benefit to audit teams wanting to meet due diligence requirements and demonstrate sustainable practices. If the isotope ratios of trees from within different forest concessions within Gabon are relatively homogenous, this would mean that it may not be necessary to collect reference samples from all concessions to verify that the timber is from the Gabon.

Stable isotope analysis for provenance verification
Stable isotope analysis is widely accepted analytical discipline and has a long history, with many laboratories adopting the technology around the world. Since the beginning of the 21st century, the technique has become well established as a means of verifying the origin of food and drink [Kelly et al., 2002; Boner & Förstel, 2004; Heaton et al., 2008; Pilgrim et al., 2010; Li et al., 2014]. The same principles used to authenticate food were later applied to timber provenance research [Boner et al., 2007; Keppler et al., 2007; Kagawa et al., 2008; Horacek et al., 2009; Kagawa et al., 2010; Gori et al., 2013; Rees, 2015]. Verifying the origin of timber typically depends on comparing an unknown sample against an authentic reference database for a region or territory. The technique is used routinely to assess legality, compliance with labelling legislation, and its use to conduct due diligence is advocated by EUTR [Regulation (EU) No 995/2010].

Methodology
Sampling
Agroisolab liaised with several organisations including FSC, Royal Botanic Gardens at Kew, US Fish and Wildlife Service, World Resource Institute (WRI) and US Forestry Service to establish a taxa priority list and reach a consensus on which locations within Gabon should be sampled. It was agreed that two forest concessions, Compagnie des Bois du Gabon (CBG) and Precious Woods Group (PWG) would be selected for reference sample collection. The concessions are separated by approximately 240km and presented an opportunity to assess the variability in stable isotope ratios at higher resolution and the viability of stable isotope data to differentiate several taxa at concession level resolution.
15 taxa including a mix of high (green) and medium (yellow) priority species were selected for sampling:

**Table 1. List of priority timber species in Gabon.**

<table>
<thead>
<tr>
<th>Trade name</th>
<th>Scientific name</th>
</tr>
</thead>
<tbody>
<tr>
<td>Okoumé</td>
<td>Aucoumea klaineana</td>
</tr>
<tr>
<td>Azobé</td>
<td>Lophira alata</td>
</tr>
<tr>
<td>Okan</td>
<td>Cylcodiscus gabonensis</td>
</tr>
<tr>
<td>Padouk</td>
<td>Pterocarpus soyauxii</td>
</tr>
<tr>
<td>Sipo</td>
<td>Entandrophagma utile</td>
</tr>
<tr>
<td>Tiana</td>
<td>Entandrophagma angolense</td>
</tr>
<tr>
<td>Tali</td>
<td>Erythrophleum ivorense Erythrophleum suaveolens</td>
</tr>
<tr>
<td>Bilinga</td>
<td>Nauclea diderrichii</td>
</tr>
<tr>
<td>Kévazingo</td>
<td>Guibourtia tessmanii</td>
</tr>
<tr>
<td>Bubinga (Kévazingo)</td>
<td>Guibourtia pellegriniana, demeusii</td>
</tr>
<tr>
<td>Ovèngkol (Not available at Prescious Woods)</td>
<td>Guibourtia ehie</td>
</tr>
<tr>
<td>Beli</td>
<td>Paraberlinia bifoliolata</td>
</tr>
<tr>
<td>Niangon (Not available at Prescious Woods)</td>
<td>Guibourtia ehie</td>
</tr>
<tr>
<td>Douka</td>
<td>Heritiera densiflora, utilis</td>
</tr>
<tr>
<td>Movingui</td>
<td>Tieghemella africana</td>
</tr>
<tr>
<td></td>
<td>Distemonanthus benthamianus</td>
</tr>
</tbody>
</table>

Samples were taken from forty-seven trees covering two forest concessions (CBG, n= 33 and PWG, n=14) in Gabon during June 2019. Between 2 and 4 pieces of timber (heartwood and sapwood) were collected from each tree. In most cases, three samples were taken per tree as well as leaf and twig samples. Samples of heartwood/sapwood were submitted to Agrosolab for stable isotope ratio analysis whereas the remaining material was distributed between Conservation of Biodiversity at IRET/CENAREST in Gabon and the World Forest ID collection at the Royal Botanic Gardens at Kew. A Pickering Punch (Agrosolab UK, Welburn, UK), a type of hammer-driven bore, was used to collect 45 of the 47 cores of timber 9 to 11 cm in length and 1.5 cm wide. One sample (*Berlinia confusa*) was smaller than the recommended size due to the hardness of the tree. Samples were then transferred from the Pickering Punch into cardboard tubes which were sealed inside 500 mL evacuated bags and silica gel to aid drying and protect the sample from the humid air in the local environment. 2 of the 47 samples were collected with a chainsaw. Samples were dried in-transit to Conservation of Biodiversity at IRET/CENAREST using 30g silica gel per plug/core. No perforations were made in the acid-free cardboard tubes; field tests found this did not adequately facilitate drying and some cores presented with mould growth upon receipt at Plant Quarantine Unit, Jodrell Laboratory of the Royal Botanic Gardens, Kew. To eliminate the risk of pathogenic fungi entering the UK, samples were subject to 121°C heat at 15psi for 30 minutes before they were released into the WFID collection for analysis and storage.

GPS data, photographs of the trees and leaves, descriptions and comments about the sampled trees were recorded in a mobile phone application named TreeSnap [Staton, Condon & Almsaleed, University of Kentucky 2016]. The data from this collection has since been moved to the World Forest ID app [app.worldforestid.org Staton, Condon & Almsaleed, University of Kentucky 2019].

Data from Watkinson [et al. 2020] was also included in this study in order to evaluate the relationship between the quantity of wood samples collected from an area and the range of stable isotope ratios that can be observed from that collection. This was done with the aim to create a better understanding of limitations in stable isotope data and to define a minimum quantity that should be collected in future projects.
There are clear environmental differences between the two concessions (table 2) that may influence isotope ratios in timber. These are in addition to differences in elevation (figures 1 and 2) and distance to the sea. The CBG concession is situated next to a lagoon and approximately 20km from the Atlantic Ocean at its closest point. PWG concession is approximately 380km from the ocean at its nearest. The two concessions are approximately 240km apart.

Table 2. Environmental differences between the PWG and CBG concessions.

<table>
<thead>
<tr>
<th>Environmental variable</th>
<th>Spatial resolution</th>
<th>Compagnie des Bois du Gabon (CBG) concession</th>
<th>Precious Woods Group (PWG) concession</th>
</tr>
</thead>
<tbody>
<tr>
<td>$^{18}$O/$^{16}$O Annual precipitation [Bowen &amp; Revenaugh 2003]</td>
<td>0.25° x 0.25°</td>
<td>-3.6‰</td>
<td>-4.4‰</td>
</tr>
<tr>
<td>D/H Annual precipitation [Bowen &amp; Revenaugh 2003]</td>
<td>0.25° x 0.25°</td>
<td>-16‰</td>
<td>-22‰</td>
</tr>
<tr>
<td>$SO_4^2-$ tropospheric deposition, December (mean 1980-2018) [GMAO 2015]</td>
<td>0.5° x 0.625°</td>
<td>6.5mg/m²</td>
<td>7.8mg/m²</td>
</tr>
<tr>
<td>Precipitation, mean February 1983-2016 (multi-satellite method) [Huffman and Bolvin 2019]</td>
<td>0.5° x 0.5°</td>
<td>200-250mm/day</td>
<td>130-150mm/day</td>
</tr>
<tr>
<td>Soil types [Jones et al., 2013]</td>
<td></td>
<td>• Dystric fluvisol</td>
<td>• Ferralic cambisol</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Ferralic arenosol</td>
<td>• Xanthic ferralsol</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Ferralic cambisol</td>
<td>• Ferralic cambisol</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Xanthic ferralsol</td>
<td>• Xanthic ferralsol</td>
</tr>
</tbody>
</table>
**Figure 1.** Locations and taxa of samples collected in the CBG concession in Gabon. Data sources: Global Administrative Dataset (GADM), ASTER GDEM (ASTER GDEM is a product of METI and NASA), Interactive Forest Atlas Forest Management [WRI 2009], DIVA GIS.
**Figure 2.** Locations and taxa of samples collected in the Precious Woods Group (P.W.G) concession in Gabon. Data sources: Global Administrative Dataset (GADM), ASTER GDEM (ASTER GDEM is a product of METI and NASA), Interactive Forest Atlas Forest Management [WRI 2009], DIVA GIS.
Measurement

Samples were initially dried at 103°C before being coarsely ground by hand and placed into a Retsch MM400 ball-mill. The resulting fine powder was extracted in a soxhlet apparatus over 6 hours with non-polar (Dichloromethane) and polar (methanol) solvents which were then dried in a laboratory-type drying cabinet for at least 1 hour. Finally, the samples were stored in air-tight sample vials and weighed for analysis.

The method to isolate non-exchangeable hydrogen in cellulose is outlined by Cheung [2014]. Homogenised samples were nitrated using 4ml HNO₃ (90%) and 4ml of glacial H₂SO₄ (96%) in falcon tubes with 35ml distilled water. As the reaction is exothermic the acid/sample solution was refrigerated for 2 hours during the digest. The sample solution was agitated using an automatic shaker for two hours allowing the cellulose to precipitate. Precipitated cellulose was separated from the solution with an initial centrifugation for 1 minute at 3000rpm. The supernatant was then discarded, and the precipitate was resuspended using 40ml distilled water and subjected to another centrifugation for 1 minute at 3000rpm. This process was repeated until a pH of 6-7 was achieved. Finally, precipitated cellulose was resuspended in 2-3ml of distilled water, transferred to a glass vial and water decanted following centrifugation. Residual water was removed by gentle heating.

To avoid equilibration or humidity effects in the oxygen and hydrogen analysis, samples were equilibrated overnight in desiccators with a humidity of 10.6 %. Afterwards, the samples were vacuum dried for at least 2 hours. Three-point calibration was used to ensure the robustness of the measurements. Samples were compared at the beginning, end, and between each measurement run to a laboratory internal reference standard. Measurements are also reported relative to an internationally defined standard; for hydrogen and oxygen isotope ratio analysis, Vienna Standard Mean Ocean Water (VSMOW) is used. For carbon isotope ratio analysis, Pee Dee Belemnite (PDB) is used. For nitrogen isotope ratio analysis, air is used as the standard. For sulfur isotope ratio analysis, Canyon Diablo Troilite (CDT) was used. Measurements are reported in delta notation in accordance with the following equation:

$$
\delta = \frac{(\text{heavy isotope/light isotope})_{\text{sample}} - (\text{heavy isotope/light isotope})_{\text{standard}}}{(\text{heavy isotope/light isotope})_{\text{standard}}} \cdot 1000
$$

Measurements are reported in permille ($\delta$) and were made in accordance with processes outlined in Boner et al., 2007.

D/H and $^{18}$O/$^{16}$O measurement: The high-temperature application uses HT-PyrOH with a covalently bonded silicon carbide tube (patented by Agroisolab) filled with glassy carbon chips and coal powder. Working temperature for pyrolysis is typically >1530°C. To gain a higher precision the isotopes are measured in a master / slave configuration with two Isotope Ratio Mass Spectrometers/IRMS (Isoprime, Elementar - Cheadle, UK). Each IRMS only measures one isotope ratio: D/H or $^{18}$O/$^{16}$O. This configuration provides excellent stability because the magnetic field and accelerating voltage remain constant on each IRMS.


$^{15}$N/$^{14}$N measurement: Elemental Analysis (Carlo Erba NA 1500 series 2 elemental analyser (Thermo Fisher Scientific, Dreieich, Germany) in combination with a Nu Horizon IRMS (NU-Instruments – Wrexham, Wales).

$^{34}$S/$^{32}$S measurement: Elemental Analyser (EA3000, Eurovector – Milano, Italy) with IRMS (Isoprime - Cheadle, UK). A one tube combustion (oxidation and reduction in one tube) is used to solve issues caused by SO₂. Combustion water is directly trapped with magnesium perchlorate at the end of the tube. Working temperature: 1000°C.
Data analysis

As many of the species analysed in this project had not been previously analysed by Agroisolab or other laboratories, and the quantities of samples were relatively low, multi-variate analysis methods were not considered ideal to draw conclusions from the data but were still performed to at least make inferences. Univariate analyses were performed to show differences between distributions of means such as Student’s T-test and ANOVA, or to assess co-variance such as regression as well as boxplot visualisations (Orange 3.24, University of Ljubljana, Slovenia; Microsoft Excel 2019 ver.16.0.6742.2048, Redmond WA). *Aucoumea klaineana* and *Dacryodes buettneri* were assessed in SAGA GIS 2.3.2 (Departments for Physical Geography, Hamburg and Göttingen, Germany) for spatially related patterns using Inverse Distance Weighting to the 2nd order power and weighting was applied using all sampling points in the area.

Results

**18O/16O**

**Bilinga** (*Nauclea diderrichii*) is remarkably consistent in terms of its δ18O isotopic composition relative to other sampled taxa, and it also has the most depleted 18O/16O isotope ratio. Lophira has the most enriched and widest range of 18O/16O isotope ratios. This seems unusual because figure 3 shows the range of isotope ratios for *Aucoumea spp.* and *Dacryodes spp.* which cover two regions within Gabon as opposed to Lophira which was only collected from the CBG concession. Figures 4 and 5 (boxplots of oxygen and hydrogen stable isotopes of different species) show that the range in results is unexpectedly high for the quantity of samples collected. The reason for this range can be attributed to a single sample with a highly depleted 18O/16O isotope ratio. Of the four samples of *Lophira alata* that were collected, the sample in question was the only one collected with a chainsaw as opposed to a Pickering Punch. The samplers were contacted about the result and were able to provide further corroborating information about the authenticity of the sample. It is not clear at the time of writing what the explanation is to this outlier result, other than outliers exist in dataset and must be included.

There are significant differences between the 18O/16O isotope ratios of the different taxa (p=0.002). For the interpretation of this result, it should be considered that the Aucoumea and Dacryodes were collected in two sites, whereas the other taxa were collected in just one.

Based on the data from samples collected in this study, there is no strong relationship between the oxygen stable isotope ratios and the elevation of each sample. *Aucoumea klaineana* has the strongest relationship with elevation (R² = 0.2) (figure 9), although this value lacks explanatory power to be used as a sole predictor of δ18O isotopic composition in *Aucoumea klaineana*.

**D/H**

**Bilinga** (*Nauclea diderrichii*) has the narrowest range of D/H isotope ratios in comparison to other taxa that were analysed in this study. Contrary to the trend between taxa having the most depleted 18O/16O isotope ratios, Nauclea shows the most enriched D/H isotope ratios (figure 4). The differences in the mean D/H isotope ratios of the taxa are significant (P=0.009) however, there is a lot of overlap in the ranges.

The range of all taxa is approximately 20‰ (between -62 and -42‰). The typical range of D/H ratios is around 10‰, however, Pterocarpus (5 samples collected in in CBG concession, 1 sample collected in PWG concession) shows a range of 18‰ (table 3).

*Nauclea diderrichii* and *Pterocarpus soyauxii* appear to have strong, positive relationships with elevation (R² s of 0.79 and 0.55 respectively). This positive proportionality suggests that their D/H isotope ratios should
increase with increasing elevation if this trend persists. *Dacryodes buettneri, Lophira alata*, and *Aucoumea klaineana* do not show strong relationships between their D/H isotope ratios and elevation (figure 10).

**Non-exchangeable D/H from cellulose**

Relative to the D/H ratios of the extracted wood, analysis of the non-exchangeable D/H isotope ratios show some interesting patterns: *Aucoumea klaineana* has the most negative D/H isotope ratios in both cases, the remainder of the sampled taxa seemed to have switched places entirely. Table 3 shows that the typical ranges observed in the taxa are approximately 10‰ for D/H from extracted wood, whereas the ranges in the D/H isotope ratios for the non-exchangeable D/H are all greater than 10‰ for all well-sampled taxa.

*Nauclea diderrichii, Pterocarpus soyauxii* and *Lophira alata* appear to have positive relationships between their non-exchangeable D/H from cellulose and elevation with R²’s of 0.26, 0.73 and 0.31 respectively. This positive proportionality suggests that their D/H isotope ratios should increase with increasing elevation if this trend persists. *Aucoumea klaineana* has a negative relationship between its non-exchangeable D/H from cellulose and elevation with an R of -0.43. *Dacryodes buettneri* was the only taxa without a strong relationship between its D/H isotope ratios and elevation (figure 11).

**¹³C/¹²C**

Carbon isotope ratios of the sampled species have a range that varies between 1.5 and 4.0‰. The carbon isotope ratios of all species vary between -27 and -30.2‰ (table 3). The widest range evident in this study exists in *Pterocarpus* (4‰ range). It should be known that 5 samples of *Pterocarpus* were collected in the CBG concession and 1 in the PWG concession.

**¹⁵N/¹⁴N**

The range of nitrogen isotope ratios in all sampled taxa were approximately between -2‰ to 8.5‰. The most negative nitrogen isotope ratios belong to the Pterocarpus samples, whereas the most positive nitrogen isotope ratios belong to the Nauclea samples (figure 7). The nitrogen isotope ratios of each taxa had an individual range of around 1 to 4‰. There are significant differences in the mean nitrogen isotope ratios of the taxa sampled (P=0.000) implying a strong taxonomically related effect in the data.

**³⁴S/³²S**

All taxa show significantly enriched sulfur isotope ratios that are in excess of 6‰. The range of sulfur isotope ratios in all taxa range between 6 and 13.5‰ (figure 8) and have individual ranges of approximately 2-3‰ in each concession (table 3). The broadest range per taxa was evident in the Aucoumea samples which were collected in two concessions and also happen to be the most well-sampled timbers in this project. There are significant differences between the taxa (P=0.001) suggesting a strong taxonomically related effect in sulfur stable isotope ratio profile.

All analysed taxa show negative relationships between sulfur isotope ratio and elevation meaning that, with increasing elevation, a decreasing sulfur isotope ratio would be expected if this trend persists. The strongest relationship was evident in Aucoumea klaineana with an R of -0.79 (figure 14).
Table 3. Stable isotope data from taxa sampled from the two concessions in Gabon. Ranges are given when n>1, standard deviations (σ) are given when n>2.

<table>
<thead>
<tr>
<th>Origin</th>
<th>Taxa</th>
<th>N samples</th>
<th>$^{18}$O/$^{16}$O vs. VSMOW (‰)</th>
<th>D/H vs. VSMOW (‰)</th>
<th>D/H non-exchangeable vs. VSMOW (‰)</th>
<th>$^{13}$C/$^{12}$C vs. PDB (‰)</th>
<th>$^{15}$N/$^{14}$N vs. Air (‰)</th>
<th>$^{34}$S/$^{32}$S vs. CDT (‰)</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Mean</td>
<td>σ</td>
<td>Range</td>
<td>Mean</td>
<td>σ</td>
<td>Range</td>
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<td>CBG Concession, Gabon</td>
<td>Aucoumea klaineana</td>
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<td>1.1</td>
<td>-55.2</td>
<td>3.3</td>
<td>10.1</td>
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<td>-44.7</td>
<td>4.2</td>
<td></td>
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<tr>
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<td>Cylicodiscus gabonensis</td>
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<td></td>
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<td></td>
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<td>14.4</td>
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<td>-54.6</td>
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<td>8.8</td>
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<td>7.0</td>
<td>18.0</td>
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Figure 3. Boxplots of $^{18}$O/$^{16}$O ratios (‰) Aucoumea (n=16), Dacryodes (n=8), Lophira (n=4), Nauclea (n=5), and Pterocarpus (n=6).

Figure 4. Boxplots of D/H isotope ratios (‰) Aucoumea (n=16), Dacryodes (n=8), Lophira (n=4), Nauclea (n=5), and Pterocarpus (n=6).
Figure 5. Boxplots of D/H nitrated isotope ratios (‰) Aucoumea (n=16), Dacryodes (n=8), Lophira (n=4), Nauclea (n=5), and Pterocarpus (n=6).

Figure 6. Boxplots of $^{13}$C/$^{12}$C isotope ratios (‰) Aucoumea (n=16), Dacryodes (n=8), Lophira (n=4), Nauclea (n=5), and Pterocarpus (n=6).
Figure 7. Boxplots of $^{15}\text{N}/^{14}\text{N}$ isotope ratios (‰) Aucoumea (n=16), Dacryodes (n=8), Lophira (n=4), Nauclea (n=5), and Pterocarpus (n=6).

Figure 8. Boxplots of $^{34}\text{S}/^{32}\text{S}$ isotope ratios (‰) Aucoumea (n=16), Dacryodes (n=8), Lophira (n=4), Nauclea (n=5), and Pterocarpus (n=6).
Figure 9. Relationship between Elevation (metres) and the $^{18}$O/$^{16}$O (d$^{18}$O) isotope ratios (‰) of genera sampled in the PWG and CBG concessions from Gabon.
Figure 10. Relationship between Elevation (metres) and the D/H (dD) isotope ratios (‰) of genera sampled in the PWG and CBG concessions from Gabon.
Figure 11. Relationship between Elevation (metres) and the non-exchangeable D/H (dD) isotope ratios (‰) from cellulose of genera sampled in the PWG and CBG concessions from Gabon.
Figure 12. Relationship between Elevation (metres) and the $^{13}$C/$^{12}$C (d13C) isotope ratios (‰) of genera sampled in the PWG and CBG concessions from Gabon.
Figure 13. Relationship between Elevation (metres) and the $^{15}$N/$^{14}$N (d15N) isotope ratios (‰) of genera sampled in the PWG and CBG concessions from Gabon.
Figure 14. Relationship between Elevation (metres) and the $^{34}S/^{32}S$ (d34S) isotope ratios (‰) of genera sampled in the PWG and CBG concessions from Gabon.
Aucoumea klaineana (Okoume)

Significant differences are evident in the mean values of non-exchangeable D/H isotope ratios (P=0.036), and the $^{34}$S/$^{32}$S isotope ratios (P=0.000) of the Aucoumea klaineana samples between the CBG and PWG concessions. Figure 17 shows there is a lot of overlap in the non-exchangeable D/H isotope ratios between the two concessions limiting its applicability as a concession classifier. However, figure 20 shows there is very little overlap between the two concessions in the sulfur isotope ratios of the samples from the two concessions.

The FreeViz (figure 21) shows that the most useful measurements to classify Aucoumea to the Precious Woods concession are the oxygen and carbon stable isotope ratios. The most useful measurements to classify Aucoumea to the CBG concession are the non-exchangeable D/H isotope ratios from cellulose (dDnit). The Principle Component Analysis (PCA) highlights that, while there is good separation in the data between the two concessions, there are also similarities (figure 22). The k-means silhouette scores show that it is most likely that the data has two clusters (48% probability), however, there are also reasonable possibilities that there are more clusters to the data (3 clusters = 36.5%, 4 clusters 37.3%).

Figure 23 highlights the spatial patterns in the stable isotope ratios in Aucoumea between and within the CBG and Precious Woods Group concessions. The colour scales of the figures are set to maximise the visual differentiation in values rather than to reflect whether differences are significant or not. For example, there are no significant differences between the oxygen, hydrogen (extracted), carbon and nitrogen stable isotope ratios of Aucoumea between the two concessions. The scale for figure 23.1 shows insignificant differentiation in the oxygen isotope ratios, however, the scales of the isotope ratios of carbon (2), extracted hydrogen (3), non-exchangeable D/H from cellulose (4), nitrogen (5), and sulfur (6) all show good ranges in their respective values. Figure 23.2 shows that carbon isotope ratios may have been a more useful classifier to each concession were it not for a single sample in the north west of the main portion of the PWG concession having such an enriched carbon isotope ratio. Figure 23.3 and figure 23.4 do not show comparable spatial patterns in hydrogen isotope ratios, figure 23.3 shows that, generally, the hydrogen isotope ratios of Aucoumea are enriched in the CBG concession and depleted in the Precious Woods Group concession, whereas Figure 23.4 seems to show the opposite save for one sample in the CBG concession that has an enriched hydrogen isotope ratio in both situations. Figure 23.5 shows that the CBG concession has some populations of δ$^{15}$N enriched Aucoumea close to δ$^{15}$N depleted Aucoumea, whereas most of the samples collected in the Precious Woods Group concession are relatively depleted in δ$^{15}$N with the exception of a sample in the north west of the main body of the concession. This same sample had an enriched carbon isotope ratio. Figure 23.6 shows that the sulfur isotope ratios of Aucoumea within each concession are consistent. The CBG concession shows enriched sulfur isotope ratios whereas the PWG shows depleted. Only one sample in the CBG concession shows a relatively depleted sulfur isotope ratio.

Significant differences are evident between the mean values of non-exchangeable D/H (P=0.020), $^{15}$N/$^{14}$N (P=0.004), $^{34}$S/$^{32}$S (P=0.046) of Aucoumea by soil type. Significant differences are not evident in the oxygen, hydrogen and carbon isotopes by soil type (figures 24 – 29).
Figure 15. Boxplots of $^{18}$O/$^{16}$O stable isotope ratios (‰) of *Aucoumea klaineana* from the CBG (n=9) and PWG (n=7) concessions.

Figure 16. Boxplots of D/H stable isotope ratios (‰) of *Aucoumea klaineana* from the CBG (n=9) and PWG (n=7) concessions.
Figure 17. Boxplots of non-exchangeable D/H from cellulose stable isotope ratios (‰) of *Aucoumea klaineana* from the CBG (n=9) and PWG (n=7) concessions.

Figure 18. Boxplots of $^{13}$C/$^{12}$C stable isotope ratios (‰) of *Aucoumea klaineana* from the CBG (n=9) and PWG (n=7) concessions.
Figure 19. Boxplots of $^{15}$N/$^{14}$N stable isotope ratios (‰) of *Aucoumea klaineana* from the CBG (n=9) and PWG (n=7) concessions.

Figure 20. Boxplots of $^{34}$S/$^{32}$S stable isotope ratios (‰) of *Aucoumea klaineana* from the CBG (n=9) and PWG (n=7) concessions.
Figure 21. FreeViz of the analysed stable isotope ratios of *Aucoumea klaineana* samples from the CBG (n=9) and Precious Woods Group (n=7) showing which measurements are more relevant at identifying each concession. $d^{18}O = \frac{^{18}O}{^{16}O}$, $dD = D/H$, $dD_{\text{nit}} = \text{non-exchangeable } D/H \text{ from cellulose}$, $d^{13}C = \frac{^{13}C}{^{12}C}$, $d^{15}N = \frac{^{15}N}{^{14}N}$ and $d^{34}S = \frac{^{34}S}{^{32}S}$ isotope ratios.
Figure 22. Principle Component Analysis of Okoume (Aucoumea klaineana) from the Precious Woods and CBG concessions. Data used to generate the PCA were the $^{18}$O/$^{16}$O, D/H, non-exchangeable D/H from cellulose, $^{13}$C/$^{12}$C, $^{15}$N/$^{14}$N and $^{34}$S/$^{32}$S isotope ratios.
Figure 23. Inverse Distance Weighting (global) isoscapes of Aucoumea klaineana stable isotope data from samples collected in the CBG and PWG concessions. 1. $^{18}$O/$^{16}$O, 2. $^{13}$C/$^{12}$C, 3. D/H, 4. Non-exchangeable D/H from cellulose, 5. $^{15}$N/$^{14}$N, 6. $^{34}$S/$^{32}$S.
Figure 24. $^{18}\text{O}/^{16}\text{O}$ ($\%$) of *Aucoumea klaineana* compared to soil type of the sampling location [Jones et al, 2013].

Figure 25. D/H ($\%$) of *Aucoumea klaineana* compared to soil type of the sampling location [Jones et al, 2013].
Figure 26. non-exchangeable D/H of cellulose (‰) of *Aucoumea klaineana* compared to soil type of the sampling location [Jones et al, 2013].

Figure 27. $^{13}C/^{12}C$ (‰) of *Aucoumea klaineana* compared to soil type of the sampling location [Jones et al, 2013].
Figure 28. $^{15}\text{N}/^{14}\text{N}$ (‰) of *Aucoumea klaineana* compared to soil type of the sampling location [Jones et al, 2013].

Figure 29. $^{34}\text{S}/^{32}\text{S}$ (‰) of *Aucoumea klaineana* compared to soil type of the sampling location [Jones et al, 2013].
**Dacryodes buettneri results**

Significant differences in the oxygen, hydrogen, non-exchangeable hydrogen, and nitrogen stable isotope ratios were not evident between the CBG and Precious Woods concessions. Differences between the mean values of the carbon and sulfur stable isotope ratios between the two concessions were observed. These differences were close to being significant (P=0.143 for carbon isotope ratios, P=0.127 for sulfur isotope ratios), however, as the data overlapped clear segregation was not evident between the two concessions based on the eight samples of Dacryodes buettneri sampled (figures 30 – 35).

The first three principle components of the data show that there is good segregation in the data, though some datapoints from CBG and Precious Woods are more similar than they are different. This is also evident when observing the k-means silhouette scores of the PCA, there is a 24% probability that there are two clusters in the data, however, there is 24.7% probability that there are 3 clusters or 4 clusters within the data. The best multivariate model for the dataset tested so far, Naïve Bayes using the first three Principle components, is only able of achieving 6/8 correct assignment of the Dacryodes to the correct concession (figure 36).

The inverse distance weighted (IDW) isoscapes (figure 37) give insight into the spatial patterns of the Dacryodes data between and within the two concessions. Figure 37.1 shows that there is more variation of the oxygen stable isotopes of the Dacryodes within each concession than there is between the two. Figure 37.2 shows that there would be good segregation of the two concessions by the carbon isotope ratios of the Dacryodes were it not for the high variance in the CBG concession perhaps explaining why the Student’s t-test shows a nearly significant difference in carbon isotope ratios (P=0.127). Figure 37.3 and 4 show that there are similar spatial patterns in the hydrogen stable isotopes of the extracted wood and of the non-exchangeable hydrogen from cellulose of the Dacryodes. The relatively high variation of hydrogen isotope ratios in the CBG concession limit the statistical separation of Dacryodes using the hydrogen isotope ratios alone. Figure 37.5 shows that Dacryodes buettneri have lower nitrogen isotope ratios in the CBG concession relative to the Precious Woods Group concession save for a single sample in CBG that was particularly enriched in δ^{15}N. Figure 37.6 shows that Dacryodes follows a similar pattern to Aucoumea in its sulfur isotope ratios; the samples from the CBG concession are more enriched in δ^{34}S than in the Precious Woods concession save for one sample in CBG that had a depleted sulfur isotope ratio.

Significant differences in the mean values of the oxygen, hydrogen, and carbon stable isotope ratios were not observed between Dacryodes samples classified to different soil types. However, significant differences between the mean values of non-exchangeable D/H from cellulose(P=0.016), nitrogen (P=0.029) and sulfur (P=0.042) stable isotope ratios were observed classifying the Dacryodes samples by soil type. Dacryodes samples grown in Ferralic cambisol were notably different to those grown in Xanthic ferralsol and Ferralic arenosol in terms of the non-exchangeable D/H from cellulose and δ^{15}N/δ^{14}N isotope ratios (figures 40 and 42). Dacryodes in Ferralic arenosol had significantly different sulfur isotope ratios to those grown in Xanthic ferralsol and Ferralic cambisol (figure 43).

There is no relationship between the D/H and δ^{18}O/δ^{16}O isotope ratios of Aucoumea klaineana and Dacryodes buettneri (figure 44) which are both of the Burseraceae family.
Figure 30. Boxplots of $^{18}\text{O}/^{16}\text{O}$ stable isotope ratios (‰) of *Dacryodes buettneri* from the CBG (n=5) and Precious Woods Group (n=3) concessions.

Figure 31. Boxplots of D/H stable isotope ratios (‰) of *Dacryodes buettneri* from the CBG (n=5) and Precious Woods Group (n=3) concessions.
Figure 32. Boxplots of nitrated D/H stable isotope ratios (‰) of *Dacryodes buettneri* from the CBG (n=5) and Precious Woods Group (n=3) concessions.

Figure 33. Boxplots of $^{13}$C/$^{12}$C stable isotope ratios (‰) of *Dacryodes buettneri* from the CBG (n=5) and Precious Woods Group (n=3) concessions.
Figure 34. Boxplots of $^{15}\text{N}/^{14}\text{N}$ stable isotope ratios ($\delta$) of *Dacryodes buettneri* from the CBG (n=5) and Precious Woods Group (n=3) concessions.

Figure 35. Boxplots of $^{34}\text{S}/^{32}\text{S}$ stable isotope ratios ($\delta$) of *Dacryodes buettneri* from the CBG (n=5) and Precious Woods Group (n=3) concessions.
Figure 36. Principle components 1, 2 and 3 of the Dacryodes spp. data (variables used: $^{18}\text{O}/^{16}\text{O}$, D/H, non-exchangeable D/H from cellulose, $^{13}\text{C}/^{12}\text{C}$, $^{15}\text{N}/^{14}\text{N}$, $^{34}\text{S}/^{32}\text{S}$).
Figure 37. Inverse distance weighting (global) isoscapes of *Dacryodes buettneri* stable isotope data 1. $^{18}O/^{16}O$, 2. $^{13}C/^{12}C$, 3. D/H, 4. Non-exchangeable D/H from cellulose, 5. $^{15}N/^{14}N$, 6. $^{34}S/^{32}S$. 
Figure 38. $^{18}$O/$^{16}$O (‰) of *Dacryodes buettneri* compared to soil type of the sampling location [Jones et al., 2013].

Figure 39. D/H (‰) of *Dacryodes buettneri* compared to soil type of the sampling location [Jones et al, 2013]
Figure 40. Non-exchangeable D/H from cellulose (‰) of *Dacryodes buettneri* compared to soil type of the sampling location [Jones et al, 2013].

Figure 41. $^{13}$C/$^{12}$C (‰) of *Dacryodes buettneri* compared to soil type of the sampling location [Jones et al, 2013].
Figure 42. $^{15}\text{N}/^{14}\text{N}$ (%o) of *Dacryodes buettneri* compared to soil type of the sampling location [Jones et al, 2013].

Figure 43. $^{34}\text{S}/^{32}\text{S}$ (%o) of *Dacryodes buettneri* compared to soil type of the sampling location [Jones et al, 2013].
Figure 44. Relationship between the D/H and $^{18}O/^{16}O$ isotope ratios of Aucoumea klaineana and Dacryodes buettneri.
How many samples are required to describe natural variation on a site?

‘Sampling area’ is a term that is currently loosely defined. Some have evaluated highly defined areas [Mayer et al., 1995; Leavitt 2010; van der Sleen et al., 2017; and Gori et al., 2018] but none have used explicitly defined areas of land. Though the community would certainly benefit from defining a resolute area, there is no doubt this would be subject to controversy and does not lend itself to the practical limitations of sampling real forests. In this evaluation, sample areas of islands (Kolombangara, Guadalcanal), forestry management units (Taiwan) and concessions (CBG and Precious Woods) defined a ‘site’ category [Watkinson et al., 2020].

![Oxygen isotope range vs. samples](image)

**Figure 45.** Ranges of oxygen isotope ratios relative to how many samples were collected per site for multiple different taxa.

The majority of the natural range of oxygen isotope ratios on a site can be defined by approximately 5-10 samples as 90% of samples have a range of 1.2‰ irrespective of species (median range = 0.8‰). This is in contrast to some literature [Van der Sleen et al., 2017] which shows oxygen variation to be as great as 9‰ at 1500m altitude, though this seems to be an exceptional case that has not been encountered at all commonly. Leavitt [2010] mostly reviewed studies containing 4-6 samples per site, inter-tree variability ranged from 1-4‰ for oxygen stable isotope ratios.

![Hydrogen isotope range vs. samples](image)

**Figure 46.** Ranges of hydrogen isotope ratios relative to how many samples were collected per site for multiple different taxa.
Between 5 and 10 samples collected and analysed from a site is sufficient to define the range of D/H ratios on a site as judged by figure 46. 90% of site collections had ranges of up to 19.7‰ irrespective of species (median range = 10.1‰). Leavitt [2010] shows that hydrogen isotope ratios may vary from 5-30‰ between trees on the same site. The maximum range observed thus far is 25.3‰ with 9 samples collected from one site.

Figure 47. Ranges of carbon isotope ratios relative to how many samples were collected per site for multiple different taxa.

Relative to their natural range of approximately -24 to -34‰ in higher C3 plants [Smith and Epstein 1971], carbon stable isotopes vary significantly on a site. There is a strong logarithmic relationship between the number of samples collected and the range of carbon ratios defined by the collection. In some collections the range was as high as 4‰ with only 5 samples. 90% of sites had ranges of carbon isotope ratios up to 2.7‰ irrespective of species. The maximum site variation in Leavitt 2010 for carbon ratios was 3‰, Van der Sleen et al., 2017 also reviews that tropical tree rings have carbon stable isotope ratios that vary by up to 3‰ on a given site. According to figure 47, more than 10 samples per site would be needed to define the range of carbon ratios. It must be considered that site size (e.g. Kolombangara Island and Taiwan) and bias towards sampling in rainy tropical regions may influence this value for site carbon isotope variability. Regions with variable aridity may have greater carbon isotope variability as trees respond to changes in water stress and vapour pressure by adjusting stomatal conductance, thus, more enriched carbon isotope ratios may be more common [Farquhar et al., 1989].
Figure 48. Ranges of sulfur isotope ratios relative to how many samples were collected per site for multiple different taxa.

Sulfur isotope ratio variability ranged between 0.3‰ (CBG concession, Gabon) to 8.4‰ (Kolombangara Island, an extinct stratovolcano in the Solomon Islands archipelago) on a site. 90% of site collections had a sulfur isotope range of 3.8‰ irrespective of taxonomic classification. In order to capture natural variation in future sample datasets, up to 10 samples must be collected per ‘site’. It is challenging to put this result into context as it does not appear that there is a publication that has investigated sulfur isotope variance between trees on the same site despite the fact sulfur is one of the strongest geographic discriminators [Thompson et al., 2010; Rummel et al., 2010; Pianezze et al., 2019]. This evaluation represents a first attempt at defining the relationship between sulfur isotope ratios ranges in tropical trees and the quantity of samples collected on a site.

Discussion

Baillonella toxisperma, Cylcodiscus gabonensis, Guibourtia tessmannii, Lophira alata, Nauclea diderrichii, Pterocarpus soyauxii, Berlinia confusa, and Didelotia africana

Data presented in this report establishes a basis for evaluating geographic origin claims of the aforementioned species from the concessions where they were collected from within Gabon. However, little can be stated about what exactly the data means until a point where collections are large enough to permit thorough analysis and interpretation. There are clear, significant differences between these species even though they were collected mostly on the same sites. This means that data from one species cannot easily be applied to another at this stage. Watkinson et al., 2020] suggest that as collections of samples and data grow, it is likely that higher-order patterns will become evident and this may permit interpretation of the origin of various species using data from another species such as using Dunbar lines [Dunbar & Wilson, 1983] to convert between datasets, or using mechanistic models to forecast data [Roden et al., 2000; Cueni et al., 2019].

Of all sampled timbers, Pterocarpus soyauxii showed the most negative nitrogen stable isotope ratios. This was expected as Pterocarpus soyauxii is a nitrogen-fixing tree and perhaps acts as a primary nitrogen source in the areas where it was sampled. The results are supported by Heitz [2011] who demonstrated that leaves of leguminous trees had more negative \(^{15}\)N/\(^{14}\)N isotope ratios relative to non-leguminous trees. Variability in nitrogen isotope composition was also demonstrated by Heitz [2011] to be influenced by sun/shade. Results from nitrogen fixers are important from an ecological perspective as they are one of the nitrogen sources for other nearby trees due to the nitrogen they fix in the soil and the distribution of the nitrogen by mycorrhiza. Mycorrhizal type varies with climate, such as arbuscular mycorrhiza which are more abundant in tropical areas.
such as Gabon. Forecast arbuscular mycorrhiza populations in Gabon by Steidinger [2019] show that varying proportions of this mycorrhiza exist across Gabon and between the two concessions that were sampled in this project. This variable may give rise to geographic variation in nitrogen isotope ratios of Pterocarpus and other flora and fauna that source nitrogen through mycorrhiza networks [Williamson et al., 1990].

**Aucoumea klaineana**

*Aucoumea Klaineana* (Okoume) is a fast-growing, light-demanding pioneer tree [Zaou et al., 1998]. In growing conditions with favourable light, okoume can develop rapidly. In terms of photosynthesis, it can be assumed that the main source of water for incorporation into sugars is precipitation [Ohashi et al., 2016]. It is therefore difficult to understand why there is no strong relationship between the hydrogen and oxygen isotope ratios of okoume, and very little trend with respect to elevation and its water hydrogen and oxygen isotope ratios. The lack of a trend may suggest that the forces of nature may be conspiring against each other, such as the rainout effect and the continental effect [Ohashi et al., 2016], or that despite the fact there are differences in rainfall and elevation, perhaps there is little meaningful difference in relative humidity between the Precious Woods Group and CBG concessions [Roden et al., 2000; Fritts et al., 1971]. One of the most important activities of rainforests is regulating the temperature of the atmosphere by removing CO$_2$ and moderating rainfall. Provided that excessive evaporation does not occur, precipitation can be expected to follow the Global Meteoric Water Line [Craig and Boato, 1955; Craig 1961]. Evapotranspiration in rainforests produces rain for the forest and contributes to atmospheric cooling. However, this cyclical evapotranspiration may be considered as ‘excessive evaporation’ meaning that the precipitation tropical trees receive is effectively not meteoric precipitation [Marryanna et al., 2017]. One idea to investigate this further would be to sample *Aucoumea klaineana* under very different conditions (i.e. not just within Gabon, but elsewhere in the world), however, this is marred by the fact that Okoume chiefly grows in Gabon and surrounding countries. Okoume and Dacryodes are both of the Burseraceae family, this study offers the opportunity to see if the idea of nitrogen isotope ratios as an origin classifier is that Okoume exists as plywood in western markets. Veneers in plywood are typically glued using formaldehyde-urea-based resins [Desch &
Dinwoodie 1996; Negro et al., 2011], some also include melamine. Urea and melamine contain nitrogen, this exogenous nitrogen must be removed for the natural nitrogen to be accessed for comparison. The method of sample preparation posed in this study is also intended to mitigate the effect of resins and other secondary metabolic compounds and therefore is a practical solution to the problems that glues present in plywood.

Sulfur isotope ratios were the best discriminator of okoume between the two concessions. There are clear differences between CBG and PWG concessions in terms of distance from the sea, tropospheric sulfate deposition [Novak & Bottrell, 1996] and elevation that may explain the differences in observed values either individually or in combination. Soil type also appeared to be important in separating ranges of sulfur isotope ratios in okoume; different soils contain varying concentrations of sulfate. All these potential predictors suggest that there is a higher spatial structure of sulfur isotope ratios in okoume across Gabon that is likely to be useful at identifying the origin of okoume.

**Dacryodes buettneri**

Eight samples of *Dacryodes buettneri* were sampled in the Precious Woods (n=3) and CBG (n=5) concessions. As judged by the figures illustrating the range of isotope ratios relative to the quantity of samples collected from a ‘site’, there is insufficient sampling of *Dacryodes buettneri* to come to any resolute conclusions about the data. It is likely collecting more samples of Dacryodes from the CBG and Precious Woods concession would elucidate the true differentiation possibilities between the two origins. Though multiple multi-variate models were attempted with the Dacryodes data, it is statistically inappropriate to attempt to use three to six variables to classify eight samples to two origins. The Principle Component Analysis and the silhouette scores of the data show that there isn’t any real clustering of the data, this is because there aren’t enough samples to define clusters. Further interpretation of the differentiation possibilities of *Dacryodes buettneri* using stable isotope ratio measurements is beyond the scope of this paper.

Nonetheless, it is interesting that the sulfur isotope ratios of *Dacryodes buettneri* appear to follow a similar trend to that of the *Aucoumea klaineana* reference samples. It is not unreasonable to expect that further collection of this timber may also reveal a higher spatial structure within Gabon that can be used to classify the origin of Dacryodes timber.

**Conclusions**

Despite the quantity of samples and species being relatively low, the data acquired establishes a basis of evaluation for assessing geographic origin claims of certain forest products including plywood and laminates from Gabon. The differences in the sulfur isotope ratios of Okoume and Dacryodes reference samples from Precious Woods Group (PWG) and Compagnie des Bois du Gabon (CBG) forest concessions suggest that regional scale origin classification may be realised with high enough frequency of reference sample collection. This study helped to establish the minimum number of reference samples (five per species per site) required in order to account for variance of isotopic distribution at a single sampling site. Low frequency of sampling per site and limited sample numbers mean that investigation of the Dunbar line hypothesis was not possible on this occasion. Further, higher resolution sampling of target species including Okoume and Dacryodes will address this much needed comparison as it may enable more efficient allocation of reference sampling resources. Further sampling of Burseraceae family timbers in the tropics may permit a global model for verifying their geographic origin.

**Future work**

Expanding the collection reference samples will be necessary to investigate regional classification further. It is hypothesised that having greater quantities of sample data will improve the discrimination between concessions or regions, as well as allowing for comparison of stable isotopes both within a single sampling site and single taxa. A higher frequency of reference sample collection will also enable assessment of the suitability
of specific taxa to act as isotopic proxies for other species (Dunbar lines). Natural variability of isotopic distribution within a site is still not fully understood, and it is anticipated that large scale sample collections for specific taxa will also enable a better understanding of this. So far, the study has focused on whole wood and cellulose components of timber reference samples. Several alternative analytical methods detail procedures for analysing alternative metabolic fractions including lignin and proteins in the form of amino acids. Furthermore, isotope methods are already being used to measure the stable isotopes of position-specific atoms within a selection of molecules such as ethanol, providing higher resolution information on the source water incorporated during metabolism. Analysis of alternative fractions and position-specific isotope ratios within a molecule such as glucose may yield higher quality results and aid the discrimination of reference samples taken from concessions within the same country.

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Glossary

ANOVA: Analysis of variance is a collection of statistical models and their associated estimation procedures used to analyse the differences among group means in a sample.

Atom Percent (Atom %): the absolute number of atoms of a given isotope in 100 atoms of total element. For example, the nitrogen-15 content of air nitrogen is 0.3663 Atom %. For calculation, At% = $[Rs / (Rs + 1) * 100]$ where Rs is the ratio of the light isotope to the heavy isotope of the sample.

Combustion: a high-temperature exothermic redox chemical reaction between a fuel and an oxidant, usually atmospheric oxygen, that produces oxidized, often gaseous products.

Continuous-Flow (CF): refers to an automated preparation device and mass spectrometer in which sample analysis is conducted in a continuous stream of helium carrier gas.

Delta Units (d or δ): are expressed in molecules per thousand, or “per mil”. For example, δ$^{15}$NAir = 12 per mil means that the sample was analysed against a reference material and found to be 12 molecules per thousand more than Air - the accepted zero point for expression of nitrogen-15 in per mil notation. For calculation, d = $[(Rs / Rr) - 1] * 1000$ where Rs is the ratio of the heavy isotope to the light isotope of the sample and Rr is the ratio of the heavy isotope to the light isotope of the reference.

Elemental Analyser (EA): an automated sample preparation instrument in which samples are automatically converted into pure gases for isotope ratio analysis. An elemental analyser contains the following elements: (i) furnace for combustion, reduction or pyrolysis of sample material; (ii) chemical traps for analyte gas purification; (iii) gas chromatography for time separation of these analyte gases.

Equilibration: a sample preparation technique whereby an aqueous sample in a sealed container is allowed to exchange and equilibrate molecules and isotopes with a headspace gas.

Fractionation: the enrichment or depletion of a stable isotope caused by natural or artificial processes.

FSC: The Forest Stewardship Council is an international non-profit, multi stakeholder organization established in 1993 that promotes responsible management of the world's forests.

Genus: a taxonomic rank used in the biological classification of living and fossil organisms, as well as viruses, in biology. In the hierarchy of biological classification, genus comes above species and below family. In binomial nomenclature, the genus name forms the first part of the binomial species name for each species within the genus.
Isotopes: can be defined as atoms whose nuclei contain the same number of protons but a differing number of neutrons.

Isotope Ratio Mass Spectrometry (IRMS): a mass spectrometer is an instrument for separation of molecules based upon their mass-to-charge ratio. In IRMS the mass spectrometer used separates isotopes of different mass within a magnetic field and precisely measures the ratio of two, or more, isotopes.

Isotope Ratio: the ratio of the minor isotope over the major isotope. For example, nitrogen in air contains 0.3663 Atom % nitrogen-15 and 99.6337 Atom % nitrogen-14, giving an isotope ratio of 0.3663 / 99.6337 = 0.003676466).

METI: Ministry of Economy, Trade and Industry.

NASA: The National Aeronautics and Space Administration is an independent agency of the United States Federal Government responsible for the civilian space program, as well as aeronautics and aerospace research. NASA was established in 1958, succeeding the National Advisory Committee for Aeronautics.

Natural Abundance: the concentration of isotopes as found in nature.

NGO: Non-governmental organization.

Per Mil (‰): see Delta Units

Pyrolysis: a high temperature means of converting sample material to pure gas in the absence of oxygen.

Site: The area where samples have been collected. In this report ‘site’ functions as a categorical label that may refer to a concession, island, small sampling area or forest management unit.

Species: the basic unit of classification and a taxonomic rank of an organism, as well as a unit of biodiversity.

Stable Isotope: a non-radioactive isotope in which the number of protons and neutrons in the atomic nucleus is constant through time. Stable isotopes pose no known physiological risk.

Taxa: a group of one or more populations of an organism or organisms seen by taxonomists to form a unit.

United States Forestry Service: The United States Forest Service is an agency of the U.S. Department of Agriculture that administers the nation’s 154 national forests and 20 national grasslands.

USAID: The United States Agency for International Development is an independent agency of the United States federal government that is primarily responsible for administering civilian foreign aid and development assistance.

Vienna-Canyon Diablo Troilite (V-CDT): an FeS meteorite used as the accepted zero point for expression of sulfur-34 in delta units. V-CDT is thought to have a sulfur-34 value close to the universal mean.

Vienna-Pee Dee Belemnite (V-PDB): a belemnite from the Cretaceous Pee Dee formation of South Carolina, US which is used as the accepted zero-point standard for expression of carbon and oxygen isotope abundance in delta units.

Vienna-Standard Mean Ocean Water (V-SMOW): the accepted zero-point standard for expression of hydrogen and oxygen isotopes of water samples in delta units.

WRI: The World Resources Institute is a global research non-profit organization that was established in 1982 with funding from the MacArthur Foundation under the leadership of James Gustave Speth.