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African C_3 grass n-alkane distributions & potential chemotaxonomy

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ABSTRACT

Reconstructing past vegetation is critical to understanding the timing and mechanisms of ecosystem change. Plant macro- and microfossils are used to study vegetation but suffer from production and taphonomic biases, such as underrepresentation of herbaceous vegetation components. Geochemical proxies such as leaf wax carbon isotopes ($\delta^{13}C$) in isolation are unable to distinguish between structurally different C_3 habitats, such as forests and grasslands. Thus, new geochemical methods to identify grassy C_3 ecosystems are necessary. We present n-alkane chain length distributions of 200 plant specimens from two Kenyan C_3-dominated ecosystems, representing a range of plant functional types (PFTs). We find that C_3 PACMAD grasses produce exceptionally high abundances of C_{33} and C_{35} n-alkanes (ACL = 32.7, mean $C_{33}+C_{35}$ relative abundance = 0.69), unlike other C_3 PFTs which produce low abundance of C_{33} and C_{35} (ACL = 28.9–30.3, mean $C_{33}+C_{35}$ relative abundance = 0.0–0.21). This finding highlights the importance of measuring and reporting the C_{35} n-alkane. We further demonstrate that n-alkane distributions can serve as proxies for African C_3 PACMAD grasses, offering a new paleoecological tool for distinguishing C_3 vegetation types.

1. Introduction

Records across Africa chart the evolution of [illegible] and provide insight into the region's ecological history. The proportion of woody vs. grassy/herbaceous biomes [illegible] climate and ecological factors that shape ecosystems—e.g., hydroclimate, temperature, fire regime, and herbivore community—that are still poorly understood (e.g., Tieszen et al., 1979; Sankaran et al., 2005; Staver et al., 2009, 2011; Good and Caylor, 2011; Lehmann et al., 2014). In addition, reconstructing the history of African ecosystems throughout the Neogene informs paleoanthropological research that connects hominin evolution to climate (e.g., Bonnefille, 2010; Kingston, 2007; Cerling et al., 2011; Levin, 2015).

Current understanding is that during the Oligocene and early Miocene, Africa had lower-[illegible] woody [illegible] supported [illegible] wetter, more homogeneous [illegible], although the structure of the C_3 woody ecosystems [illegible] (e.g., Andrews and Van Couvering, 1975; [illegible] 1993; Bonnefille, 2010; [illegible] 2017; Couvreur et al., 2021; Peppe et al., 2023). C_3 ecosystems [illegible] through the [illegible] Miocene (Jacobs et al., 2010; [illegible] 2014), with forests [illegible] habitat (Cerling et al., 1997; Wynn [illegible]; Retallack, 2001; Jacobs, 2004; Feakins et al., 2013; [illegible] 2017; Couvreur et al., 2021). Although there is some evidence of C_4 grasses being abundant in the early Miocene (Peppe et al., 2023), to both

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2. Materials and methods

2.1. Sample recovery and collection

Atotal of 208 angiosperm samples were collected from two Kenya sites: Nanyuki (n = 122) and Kakamega Forest (n = 87) in 2018 (see Appendix A). Kakamega Forest (KNF) is one of the easternmost Guineo-Congolian forests (Fig. 1) at ~1600 m above sea level (ASL), with mean annual precipitation (MAP) of 1984 mm and mean annual temperature (MAT) of 20 °C (Cerling et al., 2011). This site is 500 m below the expected treeline ... C$_3$ grass is less well documented in this high ... savanna (Tieszen et al., 1979). At Kakamega, samples were collected from a ... woodland and open grassland (n = 50), forest edge (n = 13) and ... closed canopy (n = 21). Two samples were opportunistically sampled from the road sides and, samples collected ... have a collection

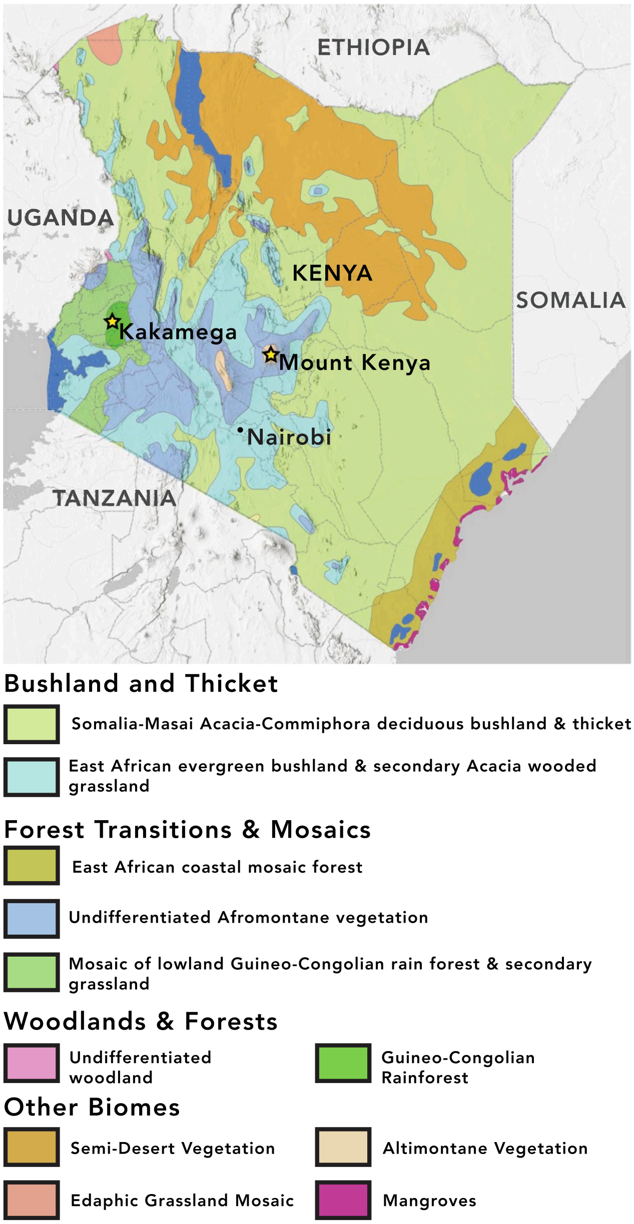

Fig. 1. Map of major vegetation zones in Kenya after White (1983). The Guineo-Congolian Rainforest (Kakamega NF) ecotone supports C_3 grasses (PACMAD, Pharoideae). The "Undifferentiated Afromontane" and "Altimontane vegetation" ecotones comprise Mount Kenya NP, and also support C_3 Poaceae (Bambusoideae, Pooideae, Panicoideae). Map made in ArcGIS using Basemaps from ESRI, used under license.

located on Mount Kenya National Park (NP), five different ecotones spanning White (1983) undifferentiated Afromontane and altimontane vegetation zones defined with high specificity (Niemelä and Pellikka, 2004; Rucina et al., 2009) were sampled along elevations from ~2400 m ASL to ~3600 m ASL. These were: lower montane forest (~2400 m ASL, n = 45), bamboo zone (~2600 m ASL, n = 10), upper montane forest (~3200 m ASL, n = 15), ericaceous shrubland (~3400 m ASL, n = 32), and Afroalpine moorland (~3600 m ASL, n = 18) — two sampling sites at each collection location were recorded. Along this transect from lowest to highest elevation, MAT ranges from 14.3 to 8.3 °C (Downing et al., 2023) — reflective of adiabatic lapse rates (Loomis et al., 2017) — and MAP ranges from 1240 to 1690 mm (Hijmans et al., 2005).

At both Kakamega National Forest and Mount Kenya National Park, the samples in these sites were selected to determine the effects of micro-habitat at each location, including regional community structure and composition. At each site, a 10 × 10 m sampling grid was drawn and all identifiable species within that area were collected. Different plant samples from each taxon represent combinations of species names and confident taxonomic identification.

Plant specimens were identified by botanists using appropriate identification manuals (e.g., Lind and Morrison, 1974; Blundell, 1987; Kabuye, 1988; Beentje, 1994; Agnew, 2013) and further authenticated at the East African Herbarium of the National Museums of Kenya in Nairobi through comparison with reference specimens. For each reconstructed specimen collected, two samples were collected (one for n-alkane analysis, one to be a voucher specimen for herbarium records). Grass and herb samples were gathered as entire specimens including roots, stems, and reproductive structures. Leaves were sampled from individual plants with woody hardened stems (trees, shrubs, lianas, bamboo). Sampling was conducted following plant collection protocols (Bridson and Forman, 1992), with the addition of care procedures to avoid hydrocarbon contamination. Each sample was stored in newspaper between two blotting papers and cardboard placed in plant presses that allowed samples to air dry. Upon return to the National Museums of Kenya's East African Herbarium, plant presses were placed in a 30 °C drying oven to fully dry. Voucher specimens are archived at the East African Herbarium, while n-alkane samples were exported to the United States for further work.

In total, this specimen set collected represents 12 PFTs: 62 C_3 shrubs, 45 C_3 trees, 27 C_3 vines, 27 C_3 herbs, 13 C_3 grasses (not including bamboo samples, which are classified as their own PFT), 11 C_4 grasses, 5 C_3 sedges, 9 bamboos, 3 C_4 sedges, and one each of Crassulacean acid metabolism (CAM) in C_3 species, epiphytic C_3s, palm. The C_3 grasses at Mount Kenya NP comprised of C_3 Pooideae grass species and other taxa. Five of these were collected from the high elevation Afroalpine moorland, upper montane and lower montane forest (see Table 1). Five Oldeania alpina bamboo samples were collected in the bamboo zone of Mount Kenya NP. The bamboo specimens included in analysis of the Poaceae family are grouped as C_3 PFTs due to their different canopy-forming habit. At Kakamega NF, nine C_3 grasses were collected (see Table 1). Of these nine samples, eight were early-diverging PACMAD grasses from the Panicoideae (n = 6), Micrairoideae (n = 1), and Arundinoideae (n = 1) subfamilies, all of which are species associated with forest ecotones. See Appendix S1 for a complete list of all taxa following World Flora Online (https://www.worldfloraonline.org).

2.2. Sample processing: n-alkanes

All samples were homogenized, using as much material (>1 g) when possible such that all parts of the plant collected were represented. Grass samples were homogenized by grinding in a Wiley Mill, while all other samples were homogenized using a mortar and pestle with liquid nitrogen. Lipids were extracted from 0.25 g of the ground samples using an ASE 350 and 9:1 dichloromethane:methanol. 200 μL of an internal standard (20 ng/μL of 5α-androstane) was added to the total lipid extract (TLE). n-Alkanes were isolated from the TLE using silica gel chromatography through 2.5 mL of hexanes.

n-Alkanes were identified and quantified using an Agilent 7890A gas chromatograph (GC) equipped with a 5975C mass selective detector (MSD) and flame ionization detector (FID). 1 μL of samples was injected into a multi-mode inlet (MMI) injector held at 60 °C for 6 s, then ramped at 600 °C per minute to 320 °C and held. The GC oven temperature was 60 °C for 1.5 min, then ramped at 15 °C per minute to 150 °C and finally to 320 °C at a rate of 4 °C per minute.

Table 1
Detailed breakdown of plant wax samples collected, including genus and species identification and local ecology. See Appendix A for further information.

Collection site	PFT	Clade	Subfamily	Genus and species	n	Local ecology	n
Kakamega National Forest	C_3 Grass	PACMAD	Panicoideae	*Oplismenus hirtellus*	3	Shaded forest floor	3
				Pseudechinolaena polystachya	3	Shaded forest floor	2
						Edge of open glade	1
			Micrairoideae	*Isachne mauritiana*	1	Shaded forest floor	1
			Arundinoideae	*Phragmites mauritianus*	1	Edge of open glade	1
		Early diverging	Pharoideae	*Leptaspis zeylanica*	1	Shaded forest floor	1
	C_4 Grass	PACMAD	Panicoideae	See Appendix	9	Open glade	6
						Shaded forest floor	3
			Chloridoideae	See Appendix	2	Open glade	1
						Shaded forest floor	1
Mount Kenya National Park	C_3 Grass	BOP	Pooideae	See Appendix	8	Lower montane forest ~2400 m ASL	1
						Upper montane forest ~3225 m ASL	2
						Afroalpine zone ~3600 m ASL	5
	C_3 Bamboo	BOP	Bambusoideae	*Oldeania alpina*	5	Bamboo zone ~2600 m ASL	5

helium-purged fluidic splitter divides the GC flow to the MSD and FID. Peaks were integrated using extracted ion chromatograms for n-alkanes (m/z 57) and 5α-androstane (m/z 245). Response factors for each n-alkane relative to 5α-androstane were determined by analysis of a bracketing homologous n-alkane series (n-C_{10}–C_{40}) and 5α-androstane. Response factors were applied to calculate n-alkane concentrations. Total n-alkane C_{25}–C_{35} concentrations were summed, and Average Chain Length (ACL) was calculated as shown below, where [C_i] represents the relative abundance of the odd-chain n-alkane homologue.

$$ACL_{25-35} = \sum_{25}^{35} i \times [C_i]$$

We report ACL alongside summed C_{33} + C_{35} n-alkane relative abundance (C_{33+35}), as ACL can be misleading when used in isolation (Polissar et al., 2025).

2.3. Principal component analysis and statistics

Principal component analysis (PCA) was performed on the n-alkane relative abundances for samples from Kakamega NF and, separately, Mount Kenya NP. Prior to PCA, the C_{25}–C_{35} relative abundances were normalised using centered log-ratio transformation (Aitchison, 1982), a normalization technique for compositional datasets.

Statistical significance between subsets (e.g. PFTs) were evaluated using the Hotelling's T-squared test, a multivariate extension of the Student's T-test. For example, this was used to compare the distribution of C_3 grasses from Mount Kenya NP to those of Kakamega NF to determine if they are significantly different.

For broad ecosystem-scale analyses, to address differential biomass in integrating different n-alkane distributions, we calculated a weighted mean for the ecosystem (e.g. a mean of all samples collected at Mount Kenya NP, removing uneven sampling of different PFTs). Hotelling's T-squared test was used to statistically compare the weighted mean distribution of each subset. For example, the weighted mean n-alkane distribution of Mount Kenya NP samples was compared to the n-alkane distribution of C_4 grasses from Mount Kenya NP and assessed for statistical differences.

For all comparisons of individual carbon numbers, variance was accounted for by variation within and across subsets, further testing the significance of the differences between the mean and standard deviation of groups. A post-hoc chi-squared probability distribution was used to evaluate the significance of the Hotelling's T-squared test. A Hotelling's T-squared statistic close to zero denotes no significant difference in the data, supporting the null hypothesis. A high Hotelling's T-squared statistic at the extreme of the chi-square function, indicating the alternative hypothesis. Comparisons were deemed significant at $p < 0.05$.

3. Results

n-Alkane distributions of C_3 woody (trees and shrubs) PFTs and C_4 grasses (e.g., Fig. 2) support previously reported patterns. The C_4 Panicoideae and Chloridoideae from the Guineo-Congolian forest at Kakamega NF closely follow the established pattern of high abundance of C_{33} and C_{35} in C_4 grasses (Rommerskirchen et al., 2006b; Bush and McInerney, 2013), with a mean ACL of 31.2 and mean C_{33} + C_{35} relative abundance of 0.47. The C_3 PFTs (excluding grasses) from both Kakamega NF and Mount Kenya NP follow the expected pattern (e.g. Vogts et al., 2009; Bush and McInerney, 2013), with C_{29} or C_{31} maxima and low/negligible C_{35} production. ACLs ranged from 28.9 (Mount Kenya NP C_3 epiphytes) to 30.3 (Mount Kenya NP C_3 shrubs), and mean C_{33} + C_{35} relative abundance ranged from 0.09 (Mount Kenya NP C_3 epiphytes) to 0.2 (Mount Kenya NP C_3 bamboo). Bamboo from Mount Kenya NP have unusual distributions, producing near-constant abundance of C_{25}–C_{33} homologues, rather than a clear maximum (ACL = 29.0, mean C_{33} + C_{35} = 0.2). Five C_3 shrub samples (four from Mount Kenya NP and one from Kakamega NF) had C_{35} relative abundance ≥ 0.1 (Fig. 2). However, this high C_{35} production is not consistent at the genus or family level, and the three shrub samples with the highest C_{35} abundances were statistical outliers, falling more than 2σ outside the mean C_3 shrub n-alkane distribution.

The n-alkane distributions of C_3 grasses do not follow the same general distribution as C_4 grasses and differ significantly ($p = 0.004$) between C_3 grasses. The PACMAD C_3 grasses from the Guineo-Congolian forest have a unique tendency to produce long-chain n-alkanes (ACL = 32.7, mean C_{33} + C_{35} = 0.69), with six of seven samples having C_{35} maxima (Fig. 2). The mean relative abundance of C_{35} in C_3 PACMAD grass n-alkane distributions (0.40) is over four times greater than the mean relative abundance of C_{35} in C_4 grasses (~0.11). The single early-diverging Pharoideae grass from the Guineo-Congolian forest has an intermediate ACL of 31.3, C_{31} maxima, and mean C_{33} + C_{35} relative abundance of 0.33 – similar to the typical C_4 grass n-alkane distribution. In contrast, C_3 grasses from the BOP clade, that is the subfamilies Bambusoideae, Oryzoideae and Pooideae from alti-/Afromontane contexts, produce n-alkane distributions that are distinct from one another but not distinguishable ($p = 0.444$) from those of C_3 woody PFTs (Figs. 2 and 3). The distributions of Guineo-Congolian C_3 PACMAD grasses significantly differ from the mean distribution of all PFTs by Hotelling's T-test ($p < 0.001$), while alti-/Afromontane Pooideae do not ($p = 0.372$).

4. Discussion

The different n-alkane distributions of C_3 grasses from the studied sites indicate that there is considerable variability within the PFT. The n-alkane distribution of C_3 PACMAD grasses from the Guineo–Congolian rainforest at Kakamega have a mean ACL of 32.7 and a $C_{33} + C_{35}$ relative abundance of 0.69, representing n-alkane distributions with C_{35} maxima that are a feature of these samples. These samples were defined as having "high" relative abundance of C_{35}, as C_4 grasses make only 25% as much C_{35}, and were previously considered as high C_{35} producers. The C_3 PACMAD grass n-alkane distributions of the Guineo–Congolian/Afromontane are distinguishable from the other C_3 PFT in this study when compared to the C_3 Pooideae, the whole C_3 PFT data ($p = 0.372$) and C_3 PFTs from the Afroalpine moorlands ($p = 0.617$); the ability of n-alkane distributions to differentiate PACMAD C_3 grasses from the Kakamega rainforest ecosystems ($p < 0.001$) is identified in Principal Component Analysis (PCA), which transforms the n-alkane homologue information into a lower-dimensional space. At Kakamega, the C_3 grasses clearly separate from the C_3 PFT because they have high PC1 dominating the $C_{33} + C_{35}$ vector (Fig. 3), indicating unique alkane signatures of the grasses in the transitional Guineo–Congolian/Afromontane setting, chemically distinguishing them from the Mount Kenya ecosystem (Fig. 3).

Without carefully checking the taxonomic information, practically after sampling, the n-alkane distributions within the C_3 grass PFT would have been lost if the Guineo–Congolian/Afromontane C_3 grasses were lumped as C_3 grasses and members of a distribution that closely follows the n-alkane distribution of C_4 grasses (see supplementary figure). Thus, we strongly recommend using sampling of PFTs that is representative of the members within a PFT, particularly sampling that reflects taxonomy, e.g. genus/subfamily.

There are several possible explanations for the n-alkane distributions observed in the C_3 grasses, such as phylogenetic relationships (e.g., C_3 vs. C_4), growth form, and

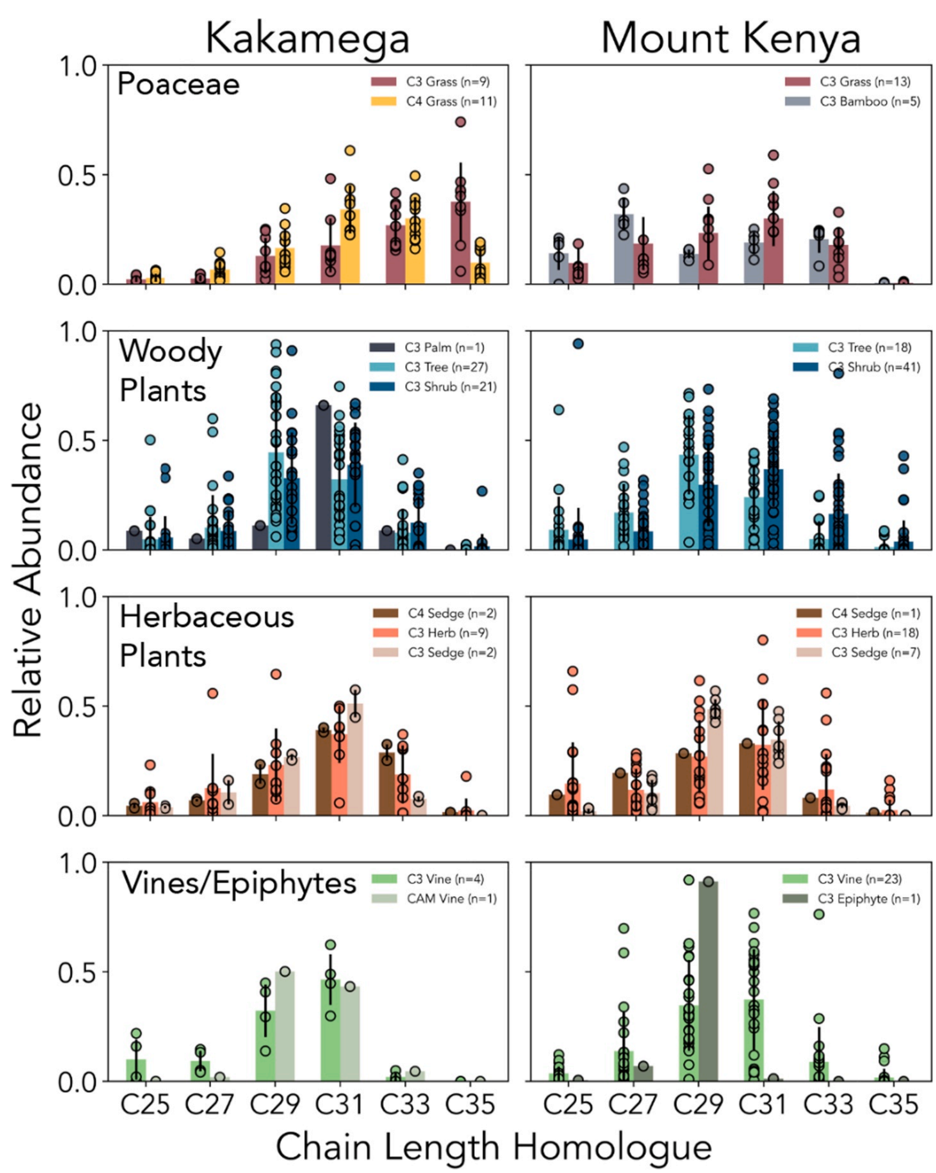

Fig. 2. n-Alkane distributions of Kakamega and Mount Kenya plant groups by PFT. Error bars show ... and individual relative abundances are shown as scatter points. Kakamega C_3 grasses are unique in their high C_{33} and C_{35} production, with C_{35} as the maximum abundance homologue.

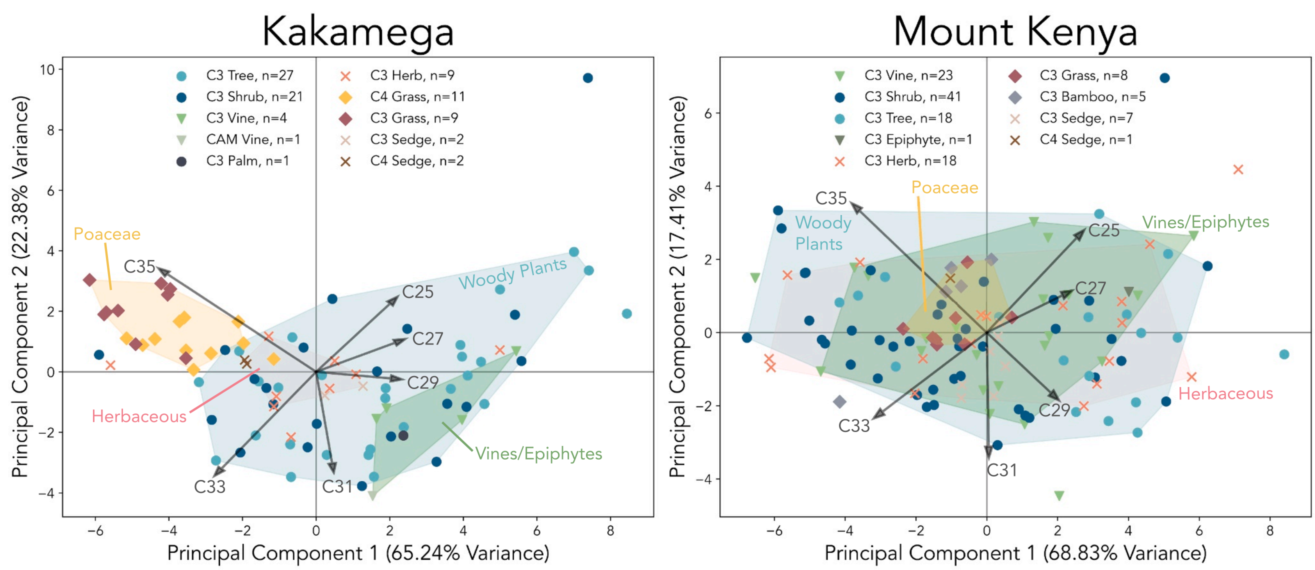

Fig. 3. Principal component analysis of n-alkane distributions for plants from Kakamega Forest and Mount Kenya. PCA convex hulls are defined around members of each PFT, with 2σ of the group mean, excluding … C3 grasses (Poaceae) and CAM … from Kakamega are the only PFT from either ecosystem that separate from the other C3 PFTs, and are defined by the combination of the C33+C35 vectors on PC1.

ecological function—reflecting changes in ambient temperatures or aridity—are needed to verify this. Taken together, … and water availability are able to alter n-alkane distributions … phylogenetic … n-alkane … chain length … making it difficult to … phylogenetic … ecological variation … interpretation of … proxy.

Although phylogenetic relationships may explain a lot of the observed variation in n-alkane distributions (Fig. 4), n-alkane distributions of the Poaceae … the following phylogenetic groups: PACMAD clade … high … C33+C35 values (0.52) … n-alkane maxima between … C31 … C29 … BOP clade has lower C33+C35 values … n-alkane maxima between C27–C31. The single … C33+C35 relative abundance … maximum … C31. This supports the … intermediate … PACMAD and BOP n-alkane distributions … consistent … pattern.

Despite the intermediate C33+C35 relative abundance and … different … n-alkane distributions of the Poaceae … phylogeny … observed … n-alkane distributions. For example, … PACMAD … n-alkane distributions … members of the early-diverging BOP clade. This … significant difference between the n-alkane distributions of C3 vs C4 PACMAD grass (p = 0.0017). C3 PACMAD grass distribution … Pharoideae … photosynthetic … C4 PACMAD … n-alkane … (Fig. 4) … the C4 pathway … PACMAD (Spriggs et al., 2014; … et al., 2019) … (Fig. 4) … n-alkane … C3 … C33+C35 … BOP clade Bambusoideae … phylogenetic …

… The pattern of converging C4 grass suggests that the possible drivers of n-alkane distributions … associated with the C3 or C4 photosynthetic pathway. The Panicoideae … support … C3 and C4 physiology … unique n-alkane distribution … (Figs. 4 and 5). A C3 Panicoideae … higher C33+C35 n-alkane (mean 0.67), while C4 Panicoideae produced lower amounts of C33+C35 (mean 0.39), in agreement with previously reported African C4 Panicoideae (Aleiti et al., 2005; Garcin et al., 2014; …, 2019). The distribution between C3 and C4 Panicoideae … particularly … C3 and C4 Panicoideae … independently … (Sinclair et al., 2018). Our data support … C4 photosynthesis … n-alkane distributions … pathway … associated … (… below).

Although … correlation between physiology and n-alkane distribution in the PACMAD clade and Panicoideae … difference … explain … C4 Panicoideae … (Table 1) … forest … C3 Panicoideae … shaded … (Table 1; Fig. 5). … separate … C3 and C4 Panicoideae … C33 … production … C4 … n-alkane distributions … photosynthetic … C35 … [illegible] …

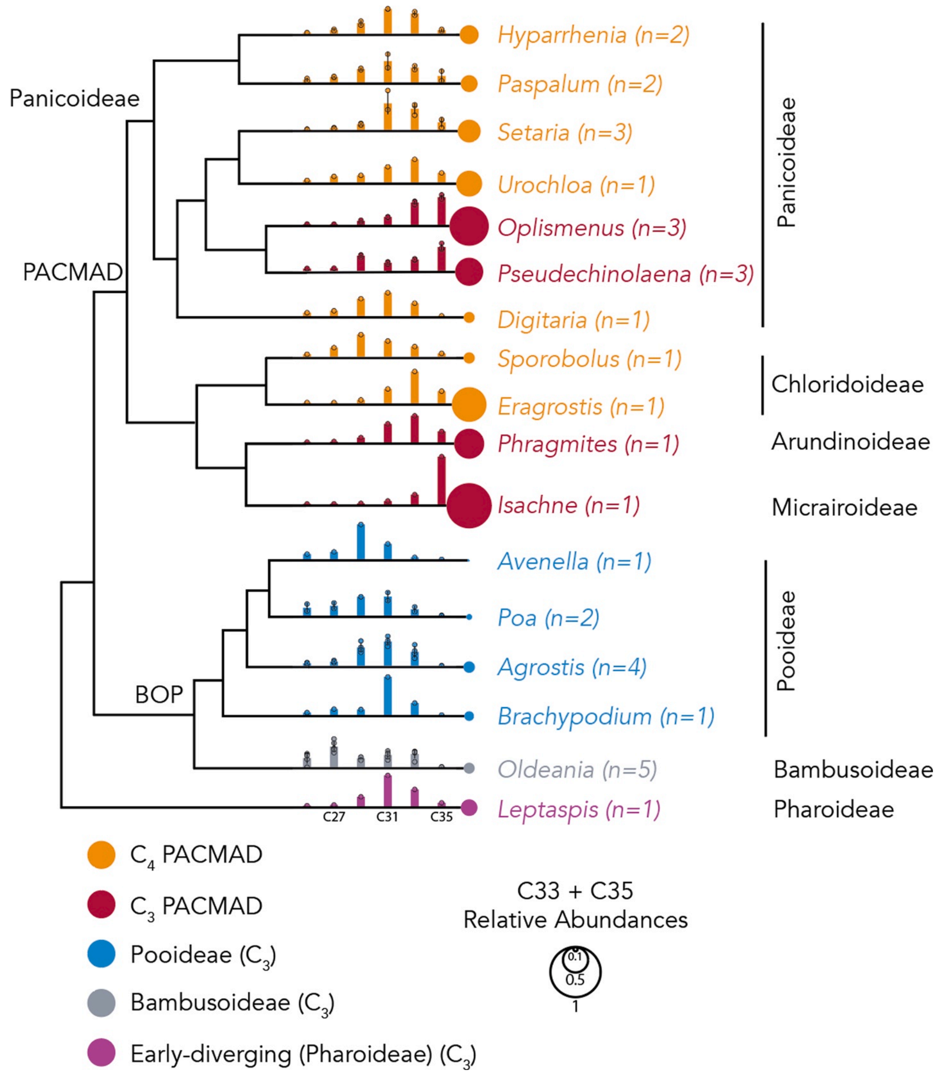

Fig. 4. Genus-level phylogenetic tree of Poaceae samples from Kakamega Forest and Mount Kenya. All PACMAD Poaceae are from Kakamega Forest and BOP samples are from Mount Kenya. Branches are annotated with the n-alkane distribution of each genus, and the diameter of the circle at the tip of each branch reflects the mean $C_{33}+C_{35}$ relative abundance for each genus. Original phylogeny from Gallaher et al., 2022, with Shorter et al., 2017 being used to place *Hyparrhenia*.

(Vogts et al., 2009), however, more data are needed to thoroughly test this relationship.

Finally, we evaluate the impact of growth temperature on n-alkane distributions. Previous studies have suggested that ACL or long chain length n-alkanes produced could be used as a proxy for temperature, finding that ACL in plants tracks MAT (Tipple and Pagani, 2013; Bush and McInerney, 2015; Wang et al., 2018). The altitudinal Afromontane Pooideae grasses from Mount Kenya were collected from three different elevations: the lower montane forest (~2400 m), two from the upper montane forest (~3200 m), and five from the Afroalpine moorland (~3600 m), and thus represent different MATs for the same grass subfamily (14 °C, 10.2 °C, and 8.3 °C, respectively; Downie et al., 2023). Were growth temperature the controlling factor, we would expect a consistent n-alkane distribution with increasing elevation. However, no consistent temperature trends are observed in distribution (Fig. 5C). The single sample from the lower montane forest has a C_{29} maximum and ACL of 28.7 and $C_{33}+C_{35}$ of 0.08 — the lowest Pooideae ACL and $C_{33}+C_{35}$ values of the cohort. The highest elevation sample has similar ACL and $C_{33}+C_{35}$ values, though the distribution has a C_{31} maximum, with greater C_{29} than C_{33} production. The mid-elevation n-alkane distribution from the upper montane forest has the highest Pooideae samples; however, ACL and $C_{33}+C_{35}$ production is higher at

lower MAT — the inverse of the "expected" pattern of previous works (Tipple and Pagani, 2013; Bush and McInerney, 2015; Wang et al., 2018). Our results do not support the hypothesis that chain length production is not positively correlated with MAT in Africa, though this does even validation studies mentioned above, which were conducted in differing geographical and ecological contexts, using ecosystem-scale global meta-analyses. Furthermore, given the small sample sizes (as low as n = 1) and the lack of consistency there between the mid-elevation (upper montane forest) and high-elevation (Afroalpine moorland) sites, more sampling is needed before robust assessments of n-alkane distribution/temperature relationships can be [illegible]. Natural habitats imply many confounding variables which make it challenging to disentangle – and is the reason for the differences in C_3 grass n-alkane distributions support the hypothesis that subfamily, or PACMADs differing physiology leads to distinct n-alkane distributions, despite phylogenetic relationships. However, physiology [illegible] similar n-alkane distributions in C_3 Poaceae [illegible] [illegible] growth habit (i.e., woody Bambusoideae are herbaceous [illegible] Pooideae adaptation for climate (e.g., warm-adapted PACMADs vs cold-adapted Pooideae)

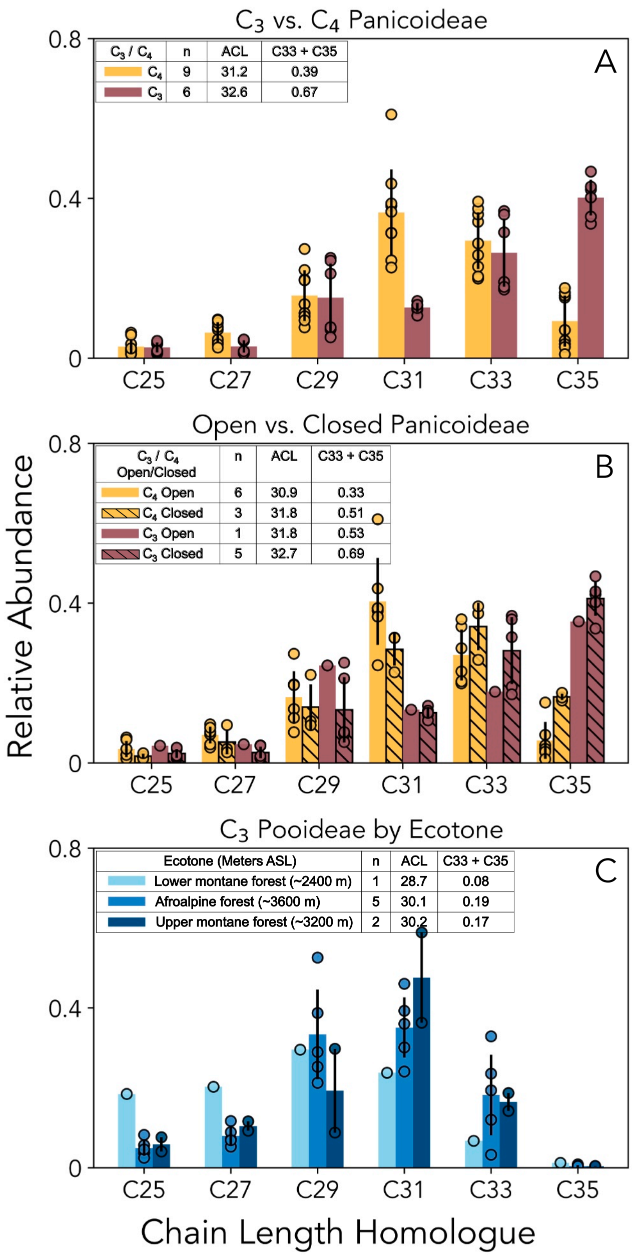

Fig. 5. n-Alkane chain length distributions across subsets of plots, portioning the fractional mean relative abundance (RA) comparing the distribution of C_3 and C_4 Panicoideae species (A), illustrating the distributions within the same subfamily (B) separating Panicoideae individuals (n) into open vs. closed habitat categories, C_3 vs. C_4 plant grasses (C) separated by elevation as a proxy for growth temperature to explore potential influence on n-alkane distributions.

habitat (open vs. closed). The influence of ecology (e.g., open vs. closed habitat, temperature) on n-alkane chain length distributions is not fully explored with our current dataset; however, limited findings support the hypothesis that long chain production is an adaptation to high MAT in open habitats suggested by previous studies (Vogts et al., 2009). Overall, the limited taxonomic and ecological data of this study means further research is needed to tease apart these complex relationships.

What is the significance of these results for the development of vegetation proxy of C_3 ecosystems? To be a suitable proxy, a PFT must produce an n-alkane distribution that is statistically different from that of the PFT distributions in soil and integrated sediment archives. For example, a peak in n-alkane C_{29} would represent a mixture of n-alkane distributions in a sediment archive integrating a number of PFTs (many of which produce C_{29}), complicating which un-mixing would be required to interpret the PFT (e.g., Yang and Bowen, 2022; Polissar et al., 2025), reducing the effectiveness of this proxy. It is therefore worth noting that the n-alkane distributions of C_3 PACMAD grasses are not possibly differentiable from other PFT distributions in soil and integrated sedimentary archive contexts, unless they are the only C_3 PFT which produce high amounts of C_{35}. Thus, the contribution of C_3 PACMAD grasses in sedimentary records would be possible to identify with their high C_{33} and C_{35} n-alkane production and their C_3 compound specific $\delta^{13}C$ values, making n-alkanes an effective proxy for C_3 PACMAD grasses.

Despite the promise of this new proxy, further refinement is prudent before application to non-Afromontane habitats, as C_3 grass habitats were presented as potential refugia of early C_3 grass in the Miocene. On a vegetation zone, the Guineo-Congolian region is likely a more suitable analog of Miocene ecosystems than Afromontane/Afroalpine vegetation at this time, when the Miocene was globally warm (e.g., Burls et al., 2021; Steinthorsdottir et al., 2021). However, Guineo-Congolian C_3 grasses are generally (but not exclusively) found in more closed ecosystems (as they are mainly shade-tolerant), the open C_3 grass that may have existed in the Miocene. Thus, long chain n-alkane proxy robustness in distinguishing ecosystems at the edge of forests remains unclear whether the diagnostic C_3 grass. Nonetheless, this proxy could be a tool to reconstruct the Miocene of African vegetation. Notably, it is extremely valuable when combined with pollen data that show the presence of grass but can not provide any additional ecological or morphological information that indicates the occurrence of Panicoideae grasses and its distinguishing photosynthetic pathway.

5. Conclusion

While the C_3 PFTs are indistinguishable in n-alkane distribution space, C_3 PACMAD grasses from Guineo-Congolian systems are unique in their elevated long chain production of C_{33} and C_{35}. Both C_3 and C_4 PACMAD grasses produce long chain n-alkanes, however, C_{35} production in C_3 PACMAD grasses is stronger than that in C_4 PACMAD grasses. Contributions from C_3 grasses to sedimentary archives would have a distinct and identifiable increase in long chain (C_{33} and C_{35}) abundance with a C_3 $\delta^{13}C$ value. Assuming such data supports Guineo-Congolian C_3 PACMAD grass as distinguishable in sedimentary records dominated by C_3 vegetation, it remains uncertain if this proxy can identify C_3 grass in the fossil record.

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work the author(s) used ChatGPT 4.0 in order to edit for grammar and clarity. After using this tool/service, the author(s) reviewed and edited the content as needed and take(s) full responsibility for the content of the publication.

CRediT authorship contribution statement

Ruth P. Tweedy: Data curation, Formal analysis, Investigation, Methodology, Visualization, Writing – review & editing, Writing – original draft. Caroline A.E. Strömberg: Writing – review & editing, Visualization, Investigation, Funding acquisition, Supervision, Methodology. [illegible]: Writing – review & editing, Investigation, Funding acquisition, Data curation. Rahab N. Kinyanjui: Writing – review & editing, Project administration, Data curation. [illegible]: Writing – review & editing, Investigation, Data curation. [illegible]: Writing – review & editing, Investigation. Kevin T. Uno: Writing – review & editing, Visualization, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

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.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.orggeochem.2025.104994.

Data availability

All data are available in Appendix A.

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