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Influence of Climatic Factors on Vegetation Distribution along the Altitudinal Gradient in Western Kenya

Murono Dorca Auma[1] , Moses Gichua[1], Victoria Ngumi[1], Peter Njenga[1] and Stephen Sikolia[2]

[1]Department of Botany, Jomo Kenyatta University of Agriculture and Technology.
P.O BOX 62000 -00200 Nairobi –Kenya.

[2] Department of Plant Sciences, Maseno University.
P.O BOX 333 Maseno - Kenya

Corresponding author* muronodorca@gmail.com

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ABSTRACT

Distribution of C_3 and C_4 plant species in the western region of Kenya was studied along the altitude from Chwele in Bungoma through Mt. Elgon in Trans Nzoia to Baringo County. The study area experiences variable climatic conditions, and the distribution of various plant species is predicted to follow distinct patterns which reveal the adaptive advantages of the different photosynthetic pathways. Carbon isotope method was used to establish carbon isotopic values of the plant species. The values were used to distinguish between C_3, C_4 and CAM plant species. Vegetation was sampled along an elevation gradient and samples taken at 100 metre intervals using a nested design for trees, shrubs, and herbaceous species. Species were identified in the field and verified with taxonomic databases, while altitude, geographic coordinates, and climatic data were recorded. Species frequency was calculated per belt to evaluate distribution patterns along the gradient. The distribution of plants was strongly associated with temperature and rainfall. At 1100–1600 m a.s.l., C_3 and C_4 species occurred in similar proportions. However, C_3 species became more common as elevation increased. In contrast, C_4 species were mostly found at elevations lower than 3100 m.a.s.l. This pattern reflects cooler temperatures and higher rainfall at higher altitudes, which limit C_4 occurrence and promote C_3 dominance. Data was first recorded in a Microsoft Excel spreadsheet and later exported to the Statistical Package for Social Sciences (SPSS) software version 26.0. Pearson correlation was used to quantify the strength of linear relationship between photosynthetic pathways and climatic variable, while regression analysis was used to express the relationship of these variables inform of an equation. The findings were presented in tables and scatter plots. Of 313 plant species in the whole area 234 were C_3, 69 were C_4, and 10 were CAM species. Since temperature and rainfall strongly influence the distribution of C_3, C_4, and CAM species along altitudinal gradients, long-term ecological monitoring including incorporation of climate change models should be established. This would help detect shifts in plant distribution patterns under ongoing climate change, particularly warming trends that may allow C_4 species to expand into higher elevations.

Key words: Photosynthetic pathways, Carbon Isotopic values, Climatic factors, Altitude.

INTRODUCTION

Vegetation distribution in Kenya is strongly shaped by the country's diverse climate zones and its pronounced altitudinal gradients, which range from low-lying arid and semi-arid lands to cool, moist highland regions (Parracciani *et al.,* 2023). These environmental variations play a key role in determining the relative abundance of C_3 and C_4 plant species, whose contrasting photosynthetic pathways, influence their adaptation to heat, moisture availability, and atmospheric conditions (Ma *et al.,* 2023). However,

despite the crucial ecological roles of these plant groups, there remains limited understanding of how climatic factors and altitude influence their distribution, especially in areas experiencing rapid climatic shifts.

These C_3 and C_4 plant species are distinguished by their carbon isotopic discrimination values ($\delta^{13}C$ values) or signatures generated during the assimilation of carbon dioxide (Munroe *et al.*, 2021). According to Cernusak *et al.,* (2013), these $\delta^{13}C$ values of carbon are inherently integrated and transferred through the plants species and provide a potential quantitative scale to trace carbon sources, both in the soils and in the vegetative form of the plant tissues. Since plants form part of the ecosystem processes stable isotopes of the carbon can be traced to estimate or quantify the carbon changes in the atmosphere by climatic factors like temperature and rainfall sensitive to C_3 and C_4 species (Zhiguo *et al.,* 2017; Ehleringer *et al.*, 1997). Stable isotope technique provides a method to examine proportions of C_3 and C_4 at a given site, and the biodiversity change. This method has been applied in different ecological scenarios to collect biodiversity functional data. For example, it has been used to trace the origin and migration of organisms (Zimmo *et al.,* 2012), carry-out forensic studies as fingerprints of biological agents (Horita and Vass, 2003) and reconstruction of palaeodiets (Mora, 2022). Therefore, stable isotope method is one way to investigate the course of carbon under different ecosystems processes.

Studies have shown that temperature and rainfall (Ma *et al.,* 2023) influence the distribution and relative abundance of C_3 and C_4 plants along altitudinal gradients (Ma *et al.,* 2023; Sikolia, 2016; Tieszen, 1979). C_3 plants species generally thrive in cool, humid, and high-altitude habitats, whereas C_4 plants species are better adapted to warm, dry, and lowland conditions (Ma *et al.,* 2023). With rising global temperatures and increasingly erratic rainfall, the balance between C_3 and C_4 vegetation is expected to shift. These shifts may alter ecosystem productivity, carbon sequestration processes, forage quality, and biodiversity. However, significant gaps persist regarding how these species respond along climatic and elevational gradients, as well as how their distribution patterns differ across ecological regions. This limited knowledge limits our ability to predict future vegetation dynamics under continued climate change. This study therefore addresses the critical problem of understanding how Kenya's climatic factors and elevation shape the spatial distribution of C_3 and C_4 vegetation. By analyzing these interactions, the research aims to provide insights that can enhance ecological modeling, inform land-use planning, and strengthen climate adaptation strategies for Kenyan vulnerable ecosystems and community.

MATERIALS AND METHODS

Study area

The study was conducted along a broad altitudinal gradient extending from Pekerra, Baringo County (approximately 1000 m a.s.l.) to the peak of Mount Elgon (approximately 4200 m a.s.l.) in Kenya. The area lies between latitude 0^0 55'N -1^0 10' N and longitude 34^0 33'E-36^0 00'E (Figure 1). The change in elevation is linked to the corresponding changes in climate, soil and vegetation. The general trend of physiographic features influences climate, soil, ecoclimatic zones and land use patterns. The spatial rainfall distribution in the area and its temperature pattern is easily correlated with the topography. Rainfall and temperature distribution in the region closely correspond to the topography, with annual rainfall averaging around 1800 mm in the highlands and dropping to less than 600 mm a year down at the northern valley floor around Lake Baringo. The mean annual temperature in the highlands is 14^0C and in the lowlands 24^0C. Climatic parameters shift from tropical humid in the highlands to semi-arid in the lowlands. The vegetation of the area may be described fairly as tropical-savanna type punctuated by drought conditions.

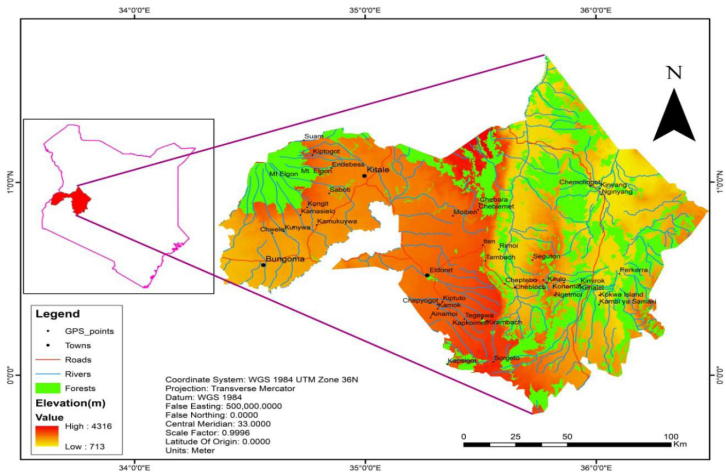

Figure 1: Map of the study area.

Collection of ecological data of the sampling sites

Sampling sites were randomly distributed along belt transects and stratified by vegetation life-form. The elevational gradient of the study area was partitioned into 100 m altitudinal intervals. Within each interval, sampling plots were systematically established along vertical line transects aligned with major road networks. Forty sampling points were randomly selected along the elevational gradient extending from Chwele to Perkerra. From each point, a 10 km horizontal transect oriented along feeder roads was delineated, along which five quadrats were randomly positioned, yielding a total of 200 sampling units. Vegetation sampling employed a nested plot design based on plant life-forms: trees were sampled in 20 × 20 m plots, shrubs in 5 × 5 m subplots nested within tree plots, and herbaceous species in 1 × 1 m quadrats nested within shrub subplots. Plot sizes were standardized to ensure comparability across altitudinal zones.

Plant species were identified in the field using regional floras, and scientific nomenclature was verified and standardized using recognized taxonomic databases, including the Plant List and Plants of the World Online (POWO). Altitude and geographic coordinates for each plot were recorded using a handheld GPS receiver equipped with altimetric capability. Climatic data, including mean annual temperature (°C) and total annual rainfall (mm), were obtained from in situ meteorological stations and supplemented with long-term records from the Kenya Meteorological Department. These climatic parameters include temperature (^{0}C) and rainfall (mm).

Species frequency was calculated independently for each altitudinal belt to assess distribution patterns along the elevation gradient. Frequency was expressed as the percentage of plots in which a species occurred using the formula:

$$\text{Frequency}(\%) = \frac{Number of\ qudrats\ in\ which\ a\ species\ occured.}{Total\ number\ of\ qudrats\ sampled\ within\ an\ altitudinal\ zone} X100$$

Identification, selection and collection of plant species

Plant species were identified in the field by experienced taxonomist using regional floras and standard field guides. Three leaves were collected from each plant. They were dried in natural conditions between 24^{0}C and 34^{0}C in the field and stored in paper bags for δ^{13}C analysis. Name of the site of collection and altitude were recorded on the paper bags containing the leaves. The collected samples were stored in wooden box for transport to the laboratory.

Preparation of leaves for carbon isotope analysis.

Dried leaves in labeled paper bags were used for carbon isotope values measurements. Air oven at 30^{0}C circulation was used to dry the leaves until there was no change in weight. Each leaf sample was milled into powder using isotopic ball mill operating at a 90 shaking speed per minute. Samples were weighed using an electronic weighing balance. Sample weights of 80µg were enclosed in aluminium capsules for δ^{13}C analysis.

Carbon Isotope value analysis

Leaf samples were sent to the stable carbon isotope laboratory, at the Australia National University in Canberra Australia for analysis of carbon stable isotope ratios. Samples were placed in an element analyzer (Carlo Erba – CE1110EA), to measure the total carbon concentration. The gases produced by the samples' combustion were purified and introduced directly into a mass spectrometer (Micromass Isoprime – CF-IRMS), for determination of isotope ratios. Isotope ratios were computed using the following equation:

$$\boldsymbol{\delta} = (R_{sample}/R_{standard}\text{-}1) \times 1000(‰)$$

Where, δ represents the isotope ratio of carbon in delta units relative to the international standards, while R_{sample} and $R_{standard}$ represent the $^{13}C/^{12}C$ ratios of the samples and standards, respectively.

The aim was to establish whether the plant species analyzed used the C_3 or C_4 photosynthetic pathway. Species were classified as C_3 when their δ^{13}C values ranged between -22‰ and -33‰ and as C_4 when δ^{13}C values ranged between -9‰ and -16‰ and CAM when δ^{13}C values ranged between -10‰ and -20‰. Each sample was measured in duplicate to ascertain the reliability of the results.

Statistical analysis

Data was first entered into Microsoft Excel spreadsheet and then exported to the Statistical Package for Social Sciences (SPSS) software version 26.0. Mean± and standard error of the mean was used to represent descriptive statistics. Pearson correlation was applied to quantify the strength of linear relationship between photosynthetic pathways and climatic variable, while regression analysis was used to express the relationship of these variables inform of an

equation. A p- value of less than 0.005 was set as the level of the significance. The findings were presented in tables and scatter plots.

RESULTS

Frequency distribution of C3 and C4 plant species along the altitude

A total of 313 (Table 1) plant species were collected between 1000m.a.s.l and 4100m.a.s.l. Plant distribution along the transect indicate that lowlands approximately 1000m.a.s.l to 1400m.a.s.l were dominated by semi-arid plants like *Balanites aegyptica, Vachellia seyal, Prosopis juliflora* and *Euphorbia candelabrum.* The middle altitude which is approximately 1500m.a.s.l to 2300m.a.s.l was dominated by agricultural crops like *Zea mays, Coffee robusta, and Mangifera indica.* The highlands which were about 2500m.a.s.l were predominantly occupied by *Podocarpus latifolius, Hagenia abyssinica* and *Aningeria adolfi-friederici.* Bamboo zone (2200m.a.s.l to 3000m.a.s.l) was mainly characterized by *Yushania alpina* which formed a dense canopy. Lower alpine zone approximately 3200m.a.s.l. was characterized by tussock grass (*Chionochloa rubra*) alongside the woody shrub *Erica arborea*. *Dendrosenecio elgonensis, Lobelia deckenii, Artemisia afra and Erica trimera* dominated the upper alpine (above 3600m.a.s.l.).

The frequency of plant species was analyzed at an interval of 100m (Figure 1). Plant species were further categorized into C_3, C_4 and Crassulacean acid metabolism (CAM) photosynthetic pathways according to the $\delta^{13}C$ value (Table 1). Percentage frequency was 74.7%, 21.8% and 3.4% for C_3, C_4 and CAM plant species respectively. The $\delta^{13}C$ values ranged from -30.89‰ for *Achyranthes aspera* (1500m.a.s.l – 2500m.a.s.l) to -10.10‰ for *Digitaria ciliaris* (1000m.a.s.l – 2301m.a.s.l) (Table 1). Sixty nine (69) plant species had their $\delta^{13}C$ values between -10.10‰ to -16.32‰ with a mean of -13.21‰ ± 0.56‰. Two hundred and thirty seven (234) plant species had more negative $\delta^{13}C$ values between -24.38‰ to -30.79‰ with a mean of -25.26±0.52‰. These plant species were categorized as C_4 and C_3 respectively. Ten (10) plant species had their isotopic values between -17.25‰ and -18.75‰ with a mean of -18.20±0.29‰. This group of plant species was categorized as CAM. These results were presented as Mean±Standard Error of Mean (SEM). Distribution of CAM plant species is limited and it was therefore not used in this study.

Table 1: List of the plant species, altitude, $\delta^{13}C$ value of the plant species and their CO_2 fixation pathway along the altitudinal gradient.

Plant Species	Altitude (in Metres a.s.l.)	$\delta^{13}C$ Values	CO_2 Fixation Pathway
Cyperus articulatus L.	1100-2100	-11.68	C_4
Euphorbia hirta L.	1000-2300	-13.74	C_4
Tribulus terrestris L.	1000- 2500	-12.98	C_4
Cyperus rotundus L.	1000-1900	-12.47	C_4
Euphorbia hirta L.	1300-2300	-13.28	C_4
Alternanthera pungens Kunth	1100-2100	-12.3	C_4
Euphorbia prostrata Aiton	1300-2300	-12.12	C_4
Amaranthus spinosus L.	1300-2500	-12.6	C_4
Cyperus difformis L.	1100-2500	-12.35	C_4
Setaria plicatilis (Hochst.) Hack.	1100-2700	-12.78	C_4
Cymbopogon citratus (DC) Stapf	1300-2300	-12	C_4
Digitaria ciliaris (Retz.) Koeler	1000-2500	-10.1	C_4
Panicum maximum Jacq.	1300-2300	-12.45	C_4
Panicum miliaceum L.	1300-2500	-16.0	C_4
Setaria viridis (L.) P.Beauv	1300-2300	-12.93	C_4
Euphorbia forskaolii J.Gay	1300-2100	-11.12	C_4
Euphorbia prostrata Burch. ex Hemsl	1000-2500	-12.1	C_4
Cleome gynandra L.	1000-2300	-13.5	C_4
Amaranthus albus L.	1000-2300	-14.74	C_4
Amaranthus cruentus L.	1000-1900	-12.4	C_4
Amaranthus dubius Mart. ex Thell.	1100-2300	-12.8	C_4
Amaranthus spinosus L.	1100-2100	-12.6	C_4
Gomphrena celosioides Mart.	1000-2300	-14.3	C_4
Gisekia pharnaceoides L.	1100-2300	-10.6	C_4
Boerhavia procumbens Banks ex Roxb.	1000-1900	-11.97	C_4
Trianthema portulacastrum L.	1000-2100	-12.3	C_4

Hypertelis cerviana (L.) Thulin	1000-1700	-13.3	C_4
Portulaca grandiflora Hook.	1000-2100	-11.7	C_4
Delosperma abyssinicum (Regel) Schwantes.	1000-1700	-13.75	C_4
Cyperus articulatus L.	1000-2300	-11.02	C_4
Cyperus papyrus L.	1000-2300	-13.3	C_4
Fimbristylis dichotoma (L.) Vahl.	1100-2300	-11.04	C_4
Cymbopogon caesius (Hook. and Arn.) Stapf	1000-2100	-12.9	C_4
Hyparrhenia hirta (L) Stapf	1000-2300	-13.9	C_4
Imperata cylindrica (L.) Raeuch	1000-2500	-13.51	C_4
Microstegium nudum (Trin.) A.Camus	1000-2100	-14.42	C_4
Aristida congesta Roem. and Schult.	1000-1900	-12.9	C_4
Eragrostis cilianensis (All.) Vignolo ex Janch.	1000-2300	-15.4	C_4
Sporobolus panicoides A. Rich	1100-2500	-13.05	C_4
Panicum maximum Jacq	1300-2300	-12.45	C_4
Cyperus kyllingia Endl.	1100-2500	-12.99	C_4
Themeda triandra Forssk.	1100-2700	-12.37	C_4
Agrostis stolonifera L.	1500-1700	-13.9	C_4
Commelina cyanea R.Br	1500-1700	-14.3	C_4
Zea mays L.	1100-2500	-13.02	C_4
Coffea robusta L. Linden	1500-1900	-11.99	C_4
Agrocharis pedunculata (Baker f.) Heywood and Jury	1500-1700	-13.98	C_4
Andropogon distachyos L.	1000-2700	-12.0	C_4
Sporobolus pyramidalis P. Beauv.	1000-2500	-11.78	C_4
Pennisetum clandestinum Hochst. ex Chiov.	1000-2500	-13.85	C_4
Cynodon plectostachyus (K.Schum.) Pilg	1500-2500	-12.99	C_4
Agrocharis stolonifera L.	1100-2300	-13.9	C_4
Eleusine coracana (L.) Gaertn.	1300-2500	-12.2	C_4
Hyparrhenia rufa (Nees) Stapf	1100-2500	-13.03	C_4
Cenchrus ciliaris L.	1100-2500	-13.7	C_4
Saccharum officinarum L.	1000-2100	-12.45	C_4
Cyperus rotundus L.	1000-1900	-13.85	C_4
Chloris gayana Kunth	1700-1900	-12.2	C_4
Cynodon dactylon (L.) Pers.	1000-2500	-13.68	C_4
Eragrostis curvula (Schrad.) Nees	1100-2700	-11.6	C_4
Panicum maximum Jacq.	1100-2700	-12.36	C_4
Pisum sativa L.	1500-2700	-13.45	C_4
Sorghum bicolor (L.) Moench	1300-2300	-13.31	C_4
Amaranthus spinosus L.	1000-2100	-13.98	C_4
Holcus lanatus L.	1100-2500	-12.93	C_4
Tillandsia bulbosa Hook.	1300-2700	-13.12	C_4
Amaranthus hybridus L.	1000-2300	-12.93	C_4
Digitaria scalarum (Schweinf.) Chiov.	1300-2700	27.92	C_4
Portulaca oleracea L.	1000-1900	-14.98	C_4
Aloe elgonica Bullock	1300-3100	-17.15	CAM
Kalanchoe longiflora Schltr.	1000-2100	-18.75	CAM
Aloe lateritia Engl.	1300-2300	-16.01	CAM
Opuntia ficus indica (L.) Mill.	1000-2100	-17.89	CAM
Kalanchoe densiflora Rolfe	1500-2500	-17.46	CAM
Dracaena trifasciata (Prain) Mabb	1500-2100	-17.48	CAM
Kalanchoe mitejea Leblanc and Raym. Hamet	1300-2500	-18.78	CAM
Euphorbia candelabrum Trémaux ex Kotschy	1000- 2900	-17.68	CAM
Euphorbia granulata Forssk	1300-3500	-18.12	CAM
Agave sisalana Perrine	1000-2500	-16.21	CAM
Lobelia deckenii (Asch) Hemsl	3100-4100	-29.38	C3
Euphorbia tirucalli L.	1300-2100	-23.28	C_3
Grewia trichocarpa Hochst. ex A.Rich.	1100 2900	-27.51	C_3
Periploca linearifolia Quart.-Dill. and A.Rich.	1300-2700	-16.61	C_3
Cissampelos mucronata A.Rich.	1100 2300	-28.28	C_3
Croton megalocarpus Hutch.	1500-2900	-24.89	C_3

Buddleja polystachya Fresen.	1000-1900	-13.85	C_3
Basella paniculata Lam.	1300-2500	-17.79	C_3
Basella alba L.	1300-2500	-17.68	C_3
Ipomoea hildebrandtii Vatke	1100- 2500	-24.38	C_3
Daucus incognitus (C.Norman) Spalik, Reduron and Banasiak	1300- 3900	-27.97	C_3
Vachellia xanthophloea (Benth.) P.J.H.Hurter and Mabb.	1300- 2500	-28.35	C_3
Senna didymobotyra (Fresen.)	1000-2700	-25.79	C_3
Balanites aegyptiaca (L.) Delile	1100-2700	-23.42	C_3
Lamium galeobdolon (L.) L.	1300-2300	-23.65	C_3
Annona senegalensis Pers.	1300-1900	-18.20	C_3
Phragmanthera rufescens (DC.) Balle	1500-2500	-17.25	C_3
Loranthus usuiensis Engl.	1300-1700	-17.45	C_3
Jatropha multiflora Müll.Arg.	1100-2300	-26.12	C_3
Cascabela thevetia ((L.) Lippold).	1100-2300	-28.51	C_3
Datura stramonium L.	1300-2500	-26.45	C_3
Carica papaya L.	1000-2700	-28.25	C_3
Mangifera indica L.	1100-2500	-28.78	C_3
Vachellia lahai (Steud. and Hochst. ex Benth.) Kyal. and Boatwr.	1000-2500	-28.36	C_3
Psidium guajava L.	1100-2300	-27.73	C_3
Neltuma juliflora (Sw.) Raf.	1000-2500	-28.95	C_3
Delonix regia (Hook.) Raf.	1000-2100	-28.88	C_3
Acanthus polystachyus Delile	1000-2500	-30.24	C_3
Vangueria madagascariensis J.F.Gmel.	1500-1900	-27.49	C_3
Catharanthus roseus (L.) G.Don	1500-2700	-25.14	C_3
Ricinus communis L.	1000-2900	-27.39	C_3
Gloriosa superba L.	1300-2700	-31.62	C_3
Ficus sycomorus L.	1000-2300	-27.63	C_3
Tradescantia zebrina Bosse	1100-2900	-27.00	C_3
Coleus comosus Hochst. ex Gürke.	1300-2700	-27.59	C_3
Lumnitzera racemosa Willd.	1500-2100	-26.15	C_3
Senna siamea (Lam.) H.S. Irwin & Barneby.	1100-2100	-27.84	C_3
Syzygium cumini (L.) Skeels	1300-2300	-28.16	C_3
Gymnanthemum auriculiferum (Hiern) Isawumi.	1300-2500	-27.83	C_3
Ficus sur Forssk	1000-2900	-29.91	C_3
Grevillea robusta A. Cunn. ex R.Br.	1300-2700	-28.04	C_3
Syzygium cordatum Hochst. ex Krauss	1300-2500	-28.49	C_3
Prunus africana (Hook.f.) Kalkman	1300-2500	-26.09	C_3
Podocarpus latifolius (Thunb.) R.Br. ex Mirb.	1100-2500	-24.65	C_3
Hesperocyparis lusitanica (Mill.) Bartel	1300-2100	-25.31	C_3
Pteris vittata L.	1300-2300	-26.71	C_3
Musa paradisiaca L.	1100-2700	-28.06	C_3
Eucalyptus saligna Sm.	1000-2500	-28.16	C_3
*Searsia natalensis (*Bernh. ex C. Krauss) F.A. Barkley.	1000-2300	-28.22	C_3
Cordia ovalis R.Br. ex A.DC.	1700-2500	-27.36	C_3
Searsia vulgaris (Meisn.) F.A.Barkley	1000-2100	-27.18	C_3
Pentas longiflora (Oliv.) Vatke	1300-2100	-26.62	C_3
Pinus patula Schiede ex Schltdl	1700-2500	-30.78	C_3
Caesalpinia decapetala (Roth) Alston	1300-2700	-25.56	C_3
Parthenocissus quinquefolia (L.) Planch.	1300-2500	-23.7	C_3
Momordica foetida Schumach.	1300-2500	-26.56	C_3
Phytolacca dodecandra L'Hér. ex Aiton	1100-2900	-28.02	C_3
Microglossa pyrifolia (Lam.) Kuntze	1500-3100	-29.91	C_3
Lantana trifoliata L.	1100-2700	-25.79	C_3
Albizia coriaria (Welw. ex Oliv.) Oliv.	1500-3100	-28.73	C_3
Tithonia diversifolia (Hemsl.) A.Gray	1500-2300	-28.04	C_3
Psidium guajava L.	1500-2500	-28.95	C_3
Senna spectabilis (DC.) H.S.Irwin & Barneb	1000-2300	-28.41	C_3
Chenopodium album L.	1000-2700	-29.09	C_3

Bougainvillea spectabilis Willd.	1000-2700	-28.52	C_3
Centella asiatica (L.) Urb.	1000-2300	-30.46	C_3
Gliricidia sepium (Jacq.) Steud.	1000-2300	-22.68	C_3
Plectranthus comosus Gürke	1000-2300	-28.96	C_3
Indigofera arrecta A.Rich.	1000-2300	-25.75	C_3
Lycium europaeum L.	1000-2500	-26.0	C_3
Tecoma stans (L.) Juss. ex Kunth	1000-2300	-30.41	C_3
Azadirachta indica A. Juss.	1300-2500	-24.45	C_3
Cucurbita pepo L.	1000-2300	-28.56	C_3
Jatropha curcas L.	1100-2300	-25.87	C_3
Bambusa vulgaris Schrad. ex J.C.Wendl.	1300-2300	-27.58	C_3
Ocimum kilimandascharicum Gürke	1300-2500	-25.44	C_3
Olea europaea L.	1300-2300	-28.59	C_3
Acanthus eminens Engl.	1300-2300	-25.0	C_3
Persicaria capitata (Buch.-Ham. ex D.Don) H.Gross	1300-2500	-28.94	C_3
Croton macrostachyus Hochst. ex Delile	1300-2700	-29.99	C_3
Erythrina abysinnica Lam.	1000-2300	-28.89	C_3
Erigeron bonariensis L.	1100-2100	-29.76	C_3
Leonotis nepetifolia (L.) R.Br. ex Benth.	1100-2100	-28.98	C_3
Vangueria madagascariensis J.F.Gmel	1300-2300	-27.5	C_3
Urtica dioica L.	1500-2700	-28.52	C_3
Cyathula uncinulata (Schrad.) Schinz	1300-2500	-31.58	C_3
Asystasia mysorensis (Roth) T.Anderson	1500-2700	-30.4	C_3
Phylica pubescens L.	1300-2500	-29.14	C_3
Solanum nigrum L.	1500-2500	-28.6	C_3
Ocimum basilicum L.	1300-2500	-30.32	C_3
Daucus carota L.	1300-2300	-29.63	C_3
Eriobotrya japonica (Thunb.) Lindl.	1300-2500	-26.79	C_3
Cupressus funebris Endl.	1000-2500	-28.4	C_3
Medicago sativa L.	1000-2500	-30.06	C_3
Mentha piperita L.	1300-2300	-26.67	C_3
Thunbergia alata Bojer ex Sims	1500-2500	-30.55	C_3
Aleurites moluccanus (L.) Willd.	1700-2900	-29.31	C_3
Vigna radiata (L.) R.Wilczek	1500-2700	-29.17	C_3
Solanum tuberosum L	1300-2500	-27.93	C_3
Crotalaria brevidens Benth.	1500-2300	-30.23	C_3
Lactuca perennis L.	1300-2700	-28.93	C_3
Derris trifolia Lour.	1500-2900	-30.79	C_3
Bersama abyssinica Fresen.	1700-2700	-29.17	C_3
Dodonaea angustifolia L.f.	1700-2500	-28.78	C_3
Dombeya torrida (J.F.Gmel.) Bamps	1700-2900	-26.01	C_3
Melaleuca citrina (Curtis) Dum. Cours.	1300-2700	-28.91	C_3
Myrica gale L.	1500-2500	-30.82	C_3
Vitex keniensis Turrill	1100-2900	-29.14	C_3
Jacaranda mimosifolia D.Don	1500-2700	-27.85	C_3
Clerodendrum myricoides (Hochst.) Vatke	1300-2300	-28.68	C_3
Olea capensis L.	1300-2500	-30.09	C_3
*Olea welwitschii (*Knobl.) Gilg and Schellenb.	1100-2500	-28.17	C_3
Impatiens tinctoria A.Rich.	1500-2700	-28.56	C_3
Yushania alpina (K. Schum.) W.C. Lin	2100-3500	-25.45	C_3
Moringa oleifera Lam.	1000-2900	-29.18	C_3
Achyranthes aspera L.	1500-2700	-27.61	C_3
Solanum lycopersicum L.	1700-1800	-29.35	C_3
Steganotaenia araliacea Hochst.	1500-2900	-28.31	C_3
Lantana trifolia L.	1500-2700	-25.79	C_3
Acalypha stuhlmannii Pax	1500-2900	-28.85	C_3
Myrsine melanophloeos (L.) R.Br. ex Sweet.	1700-2700	-26.44	C_3
Toona ciliata M.Roem.	1100-2700	-30.12	C_3
Spathodea campanulata P. Beauv.	1500-2700	-27.2	C_3

Macadamia integrifolia Maiden & Betche.	1300-2300	-26.86	C_3
Datura metel L.	1100-2500	-27.19	C_3
Galinsoga parviflora Cav.	1100-2500	-29.9	C_3
Croton macrocarpus Rchb. ex Mull.Arg.	1500-2900	-26.56	C_3
Hagenia abyssinica (Bruce) J.F.G.mel	1700-2500	-25.66	C_3
Rumex nepalensis Var.	1500-2500	-27.3	C_3
Leonotis ocymifolia (Burm.f.) Iwarsson	1500-2700	-26.8	C_3
Aningeria adolfi –fredrichi (Engl.) Robyns and Gilbert	1700-3100	-28.34	C_3
Persicaria senegalensis (Meisn.) Soják.	1300-2900	-31.0	C_3
Dendrosenecio elgonensis (T.C.E.Fr) E.B. Knox	1500-4100	-26.98	C_3
Clutia abyssinica Jaub. & Spach.	1500-2900	-27.68	C_3
Artemisia afra Jacq. ex Willd.	1500-3000	-28.9	C_3
Erica arborea L.	2500-3500	-27.08	C_3
Chionochloa rubra Zotov.	2700-3500	-29.11	C_3
Stephania abyssinica (Quart.-Dill. and A.Rich.) Walp..	2900-3900	-27.06	C_3
Rubus apetalus Poir.	1500-3900	-29.73	C_3
Erica trimera (Engl.) Beentje	2900-3900	-27.85	C_3
Oxalis corniculata L.	1700-2700	-30.67	C_3
Solanum nudum Humb. & Bonpl. ex Dunal	1500-2700	-28.87	C_3
Mesosphaerum pectinatum (L.) Kuntze.	1500-2500	-29.04	C_3
Clausena anisata (Willd.) Hook.f. ex Benth.	1700-2500	-30.76	C_3
Zanthoxylum asiaticum (L.) Appelhans, Groppo and J.Wen	1500-2500	-29.09	C_3
Abelmoschus esculentus (L.) Moench	1100-2500	-26.87	C_3
Citrus aurantium f. aurantium	1300-2500	-25.96	C_3
Melia azedarach L.	1300-2500	-26.21	C_3
Manihot esculentum Crantz	1500-2300	-30.56	C_3
Citrullus lanatus (Thunb.) Matsum. and Nakai	1500-2100	-26.77	C_3
Vigna unguiculata (L.) Walp.	1500-2300	-29.3	C_3
Albizia gummifera (J.F.Gmel.) C.A.Sm.	1000-2500	-29.44	C_3
Ochroma pyramidale (Cav. ex Lam.) Urb.	1000-1700	-30.04	C_3
Nuxia congesta R.Br. ex Fresen.	1300-2100	-26.16	C_3
Spathodea campanulata P.Beauv.	1300-2100	-30.23	C_3
Chaetacme aristata Planch.	1100-2100	-29.08	C_3
Kylinga bulbosa P.Beauv.	1100-2500	-28.92	C_3
Persea americana Mill.	1100-2500	-29.41	C_3
Erigeron floribundus (Kunth) Sch.Bip..	1100-2300	-30.69	C_3
Ipomoea batatas (L.) Lam.	1100-2300	-25.9	C_3
Aspilia pluriseta Schweinf. ex Engl.	1100-2300	-27.81	C_3
Rumex nepalensis Spreng.	1100-2300	-30.98	C_3
Solanum mauritianum Scop.	1700-2500	-27.01	C_3
Bidens pilosa L.	1500-2300	-30.98	C_3
Leucas calostachyus Oliv.	1700-2300	-29.30	C_3
Mimosa pudica L.	1500-1700	-30.46	C_3
Markhamia lutea (Benth.) K.Schum.	1500-2500	-26.96	C_3
Tylosema fassoglense (Kotschy ex Schweinf.) Torre and Hillc	1500-2100	-26.91	C_3
Allophylus abyssinicus (Hochst.) Radlk..	1500-1700	-26.07	C_3
Steganotaenia araliacea Hochst.	1300-2500	-26.5	C_3
Rotheca myricoides (Hochst.) Steane & Mabb.	1300-2300	-27.42	C_3
Piliostigma thonningii (Schumach.) Milne-Redh.	1300-2500	-26.98	C_3
Tagetes minuta L.	1500-2300	-30.89	C_3
Dryopteris filix-mas (L.) Schott	1300-2300	-27.04	C_3
Ficus lutea Vahl.	1500-2500	-29.34	C_3
Combretum molle R.Br. ex G.Don	1300-2300	-27.6	C_3
Rumex cordifolius Hornem. ex Rchb	1500-2500	-28.45	C_3
Sesbania sesban (L.) Merr.	1300-2300	-28.24	C_3
Casuarina equisetifolia L.	2300-2300	-30.91	C_3
Bridelia micrantha (Hochst.) Baill.	1500-2500	-29.45	C_3
Senecio vulgaris L.	1500-2900	-30.26	C_3
Ipomoea batatas (L.) Lam.	1500-2300	27.95	C_3

Passiflora edulis Sims	1300-2500	-29.56	C_3
Cassia didymobotyra (Fresen.) Irwin & Barneby	1100-2900	-29.91	C_3
Rumex abyssinicus Jacq.	1500-2700	-28.36	C_3
Rumex triangulivalvis (Danser) Rech. f.	1500-2500	-27.76	C_3
Paramollugo nudicaulis (Lam.) Thulin	1500-2300	-25.89	C_3
Achyranthes aspera L.	1300-2700	-30.93	C_3
Chenopodium album L.	1300-2500	-25.44	C_3
Hilleria latifolia (Lam.) H. Walt.	1500-2700	-29.01	C_3
Phytolacca dodecandra L'Hér.	1500-2700	-23.49	C_3
Persicaria pulchra Soják	1300-2700	-27.94	C_3
Rumex bequaertii De Wild	1300-2300	-27.3	C_3
Mirabilis jalaba L.	1700-2500	-30.34	C_3
Persicaria amphibian (L.) Delabre	1500-2700	-21.46	C_3
Commicarpus plumbagineus (Cav.) Standl.	1700-2900	-30.34	C_3
Persicaria decipiens (R.Br.) K.L.Wilson	1300-2500	-26.67	C_3
Polygonum aviculare L.	1500-2900	-25.37	C_3
Arenaria montana L.	2300-3500	-25.66	C_3
Pollichia campestris Aiton.	2300-3100	-25.75	C_3
Fallopia convolvulus (L.) Á. Löve	2100-2700	-29.76	C_3
Cerastium afromontanum T.C.E.Fr.	2300-3900	-25.68	C_3
Guizotia abyssinica (L.f.) Cass.	2500-3100	-30.5	C_3
Phytolacca octandra L.	1700-3100	-23.32	C_3
Rumex acetosa L.	1900-3100	-23.79	C_3
Centella asiatica (L.) Urb.	1500-2300	-27.9	C_3
Alchemilla vulgaris L.	2700-3900	-29.45	C_3
Carduus afromontanus (R.E.Fr.) N.Garcia, Moreyra and Susanna	2500-3700	-27.56	C_3
Podocarpus gracilior Pilg.	1900-3300	-25.81	C_3
Rununculus keniensis Milne-Redh. and Turrill.	1700-2900	-28.69	C_3
Persicaria senegalensis (Meisn.) Soják	1500-2700	-27.04	C_3
Sambucus adnata Wall. ex DC.	1900-2900	-28.23	C_3
Erigeron floribundus (Kunth) Sch.Bip.	1700-3100	-28.0	C_3
Warburgia ugandensis Sprague	1300-2900	-28.48	C_3
Baccharoides lasiopus (O.Hoffm.)	1500-2500	-29.27	C_3
Mimulopsis alpina Chiov.	1900-4100	-29.6	C_3
Cyathula polycephala Baker	1700-2900	-26.73	C_3
Micromeria biflora (Buch.-Ham. ex D.Don) Benth.	2300-3100	-27.25	C_3
Neoboutonia macrocalyx Pax.	1700-2700	-30.39	C_3
Hibiscus fuscus Garcke	1900-2500	-28.49	C_3
Chenopodium opolifolium Schrad. ex W.D.J.Koch and Ziz	1900-2500	-25.72	C_3
Bergia decumbens Planch. ex Harv.	1500-2700	-25.17	C_3
Agrostis gracilifolia C.E.Hubb.	3100-4100	-29.19	C_3
Festuca simensis Hochst. ex A.Rich..	2700-3700	-28.37	C_3
Alchemilla microbetula T.C.E.Fr.	3100-3700	-25.64	C_3
Trifolium multinerve (Hochst.) A.Rich.	2900-3700	-30.42	C_3
Phytolacca octandra L.	1300-2500	-23.32	C_3
Striga hermonthica (Delile) Benth.	1300-2500	-26.7	C_3
Romulea congoensis Bég.	1700-4100	-28.6	C_3
Rubus keniensis Standl.	1300-2500	-28.66	C_3
Vachellia seyal (Delile) P.J.H. Hurter	1600-2000	-30.4	C_3
Cordia africana Lam.	1501-2700	-27.57	C_3
Glinus oppositifolius (L.) Aug D.C	1500-2500	-25.04	C_3
Tipuana tipu (Benth)	1500-1700	-30.74	C_3

The pattern of distribution of the plant species along the altitude was variable, with C_4 species dominating at low altitude 1000m and 1200m decreasing between 1300m to 3000m and did not occur above 2700m (Figure 2). C_3 plant species were present along the whole length of the altitudinal transect but dominated in the high altitude (Figure 2). At medium altitude species composition was almost balanced but C_3 species were more abundant. Transition zone associated with dominance was between 1600m- 1700m. Transition zone is a point at where C_4 abundance falls below 50% in relation to climate variable. Most plants had C_3 pathway as compared to C_4 pathway.

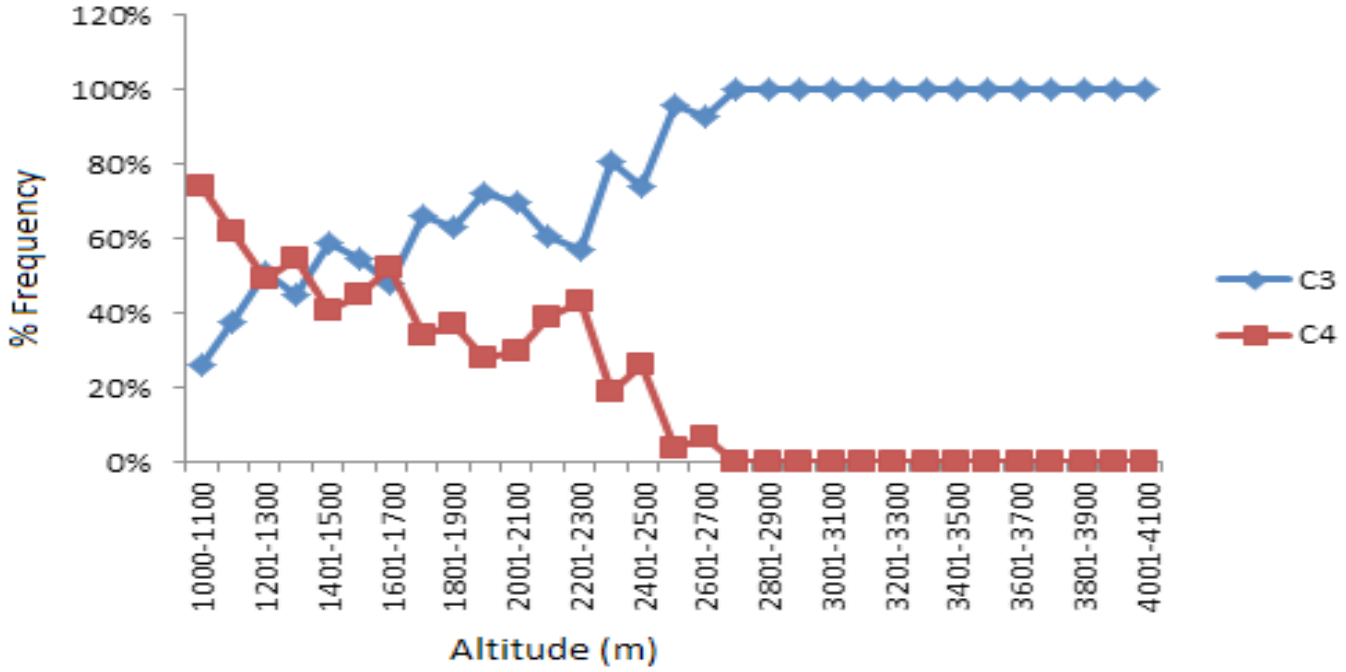

Figure 2: Frequency distribution of C_3 and C_4 plant species along the altitude.

This distribution of C_3 and C_4 plants is further illustrated by Figure 3a and b that displays the correlation of vegetation with altitude. There was a strong positive correlation between altitude and percentage of C_3 species, r([N(31)2]) = 0.919, p = < 0.001. Conversely, altitude exhibited a strong negative correlation with the percentage of C_4 species (r[31] = –0.900, p < 0.001).

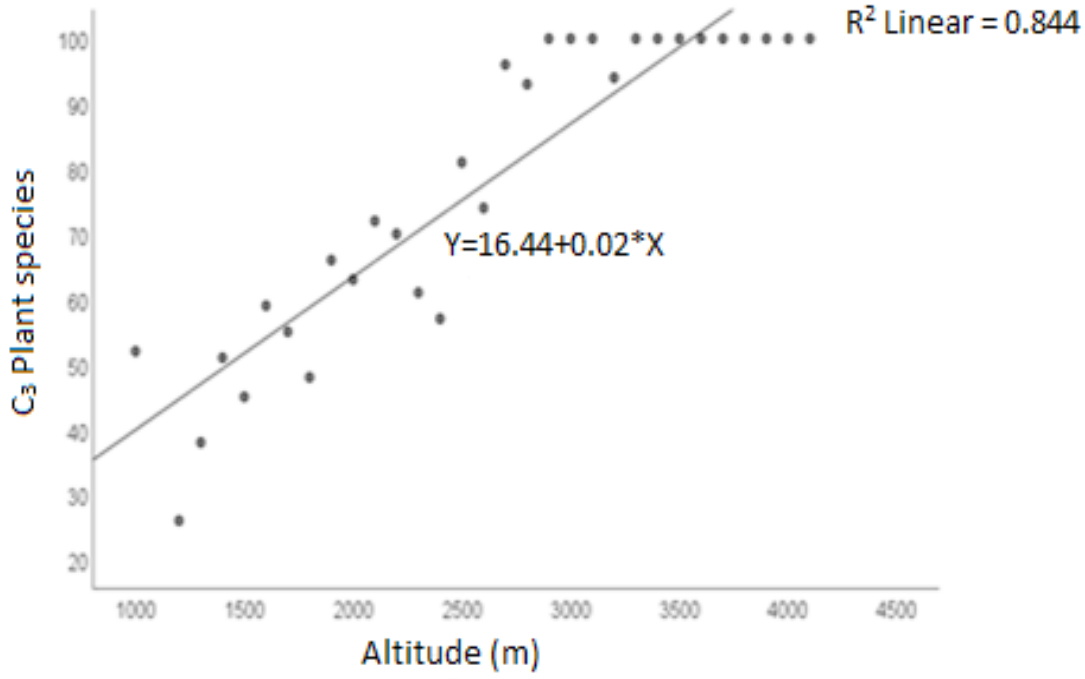

Figure 3a: Relationship between altitude and percentage of C_3 species

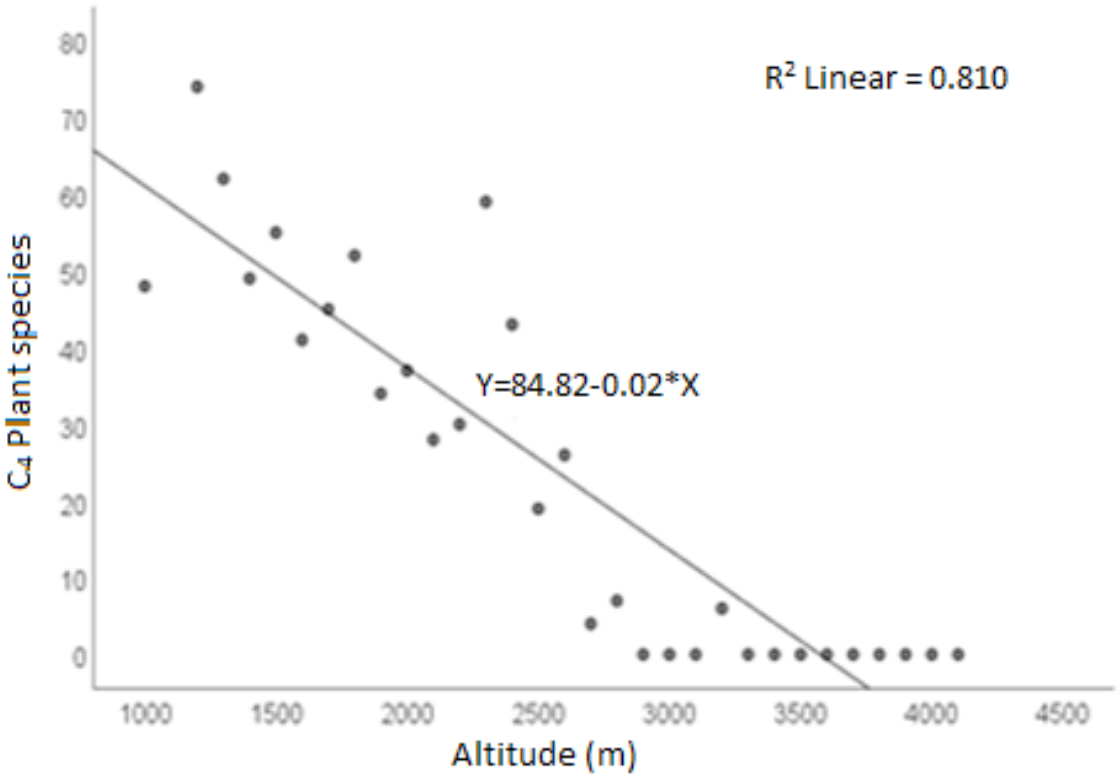

Figure 3b: Relationship between altitude and percentage of C_4 plant species.

Relationship between plant δ13C values and climatic variables

The mean annual rainfall and percentage of C_3 species had a significant moderate positive relationship, r([N(31)2]) = 0.658, p = < 0.001 (Figure 4a) while the mean annual rainfall and percentage of C_4 species had a significant moderate negative relationship, r([N(31)2]) = -0.634, p = < 0.001 (Figure 4b). Mean annual temperature exerted a significant influence on the distribution of both C_3 and C_4 plants with C_3 plants showing a strong significant negative relationship, r([N(31)2]) = -0.867, p = < 0.001 while C_4 plant species had a significant strong positive relationship r([N(31)2]) = 0.850, p = < 0.001 (Fig 5a,b).

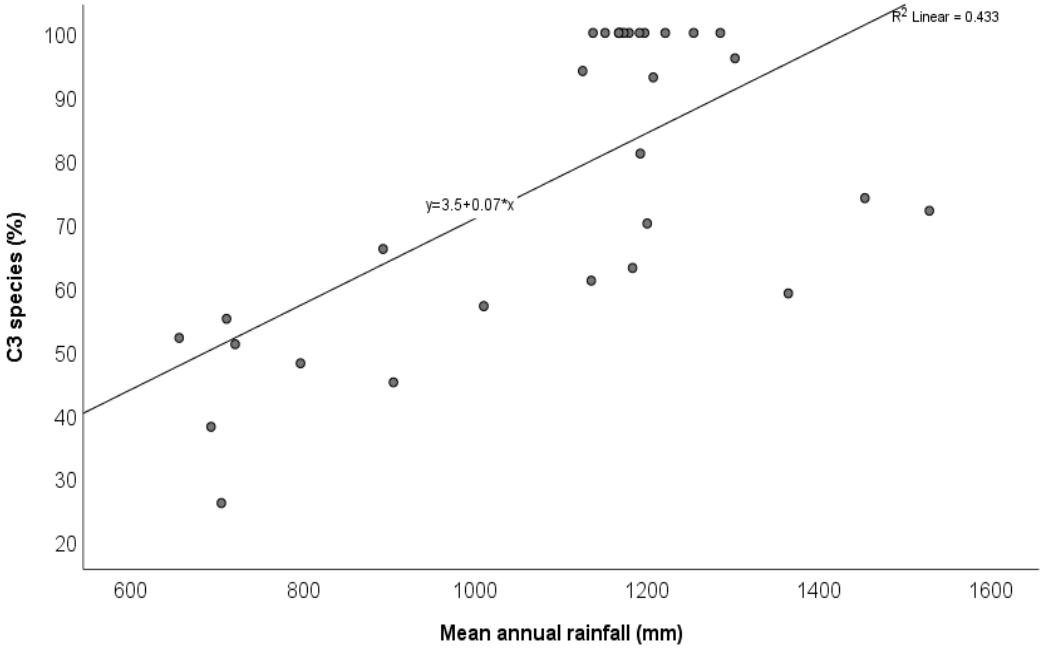

Figure 4a: Relationship between mean annual rainfall and percentage of C_3 species.

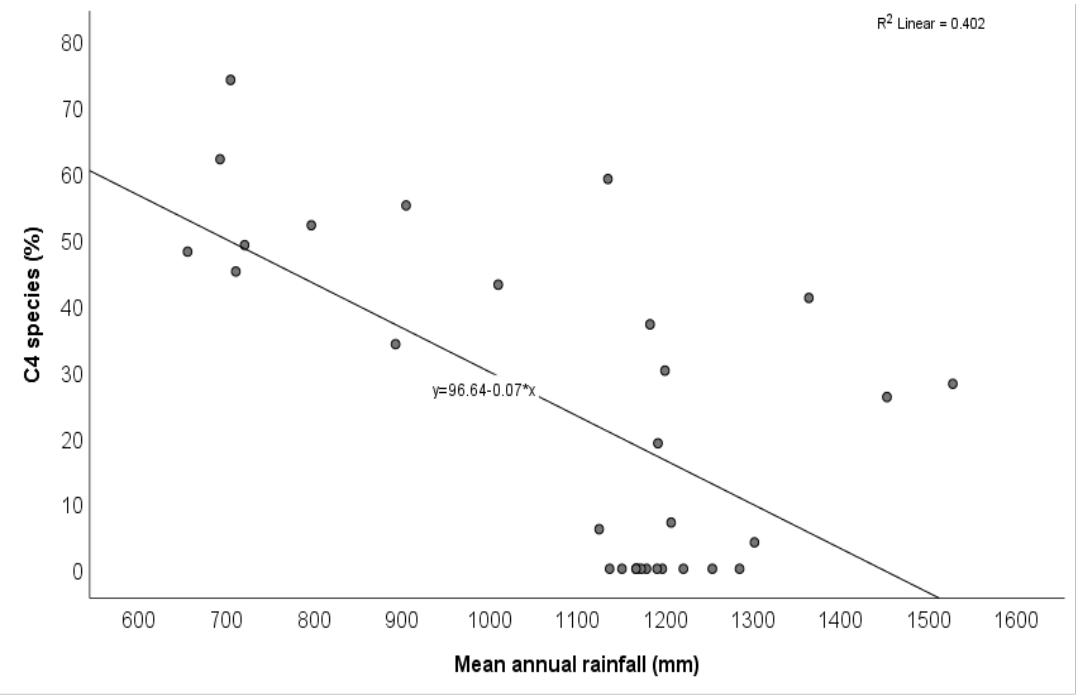

Figure 4b: Relationship between mean annual rainfall and percentage of C_4 species.

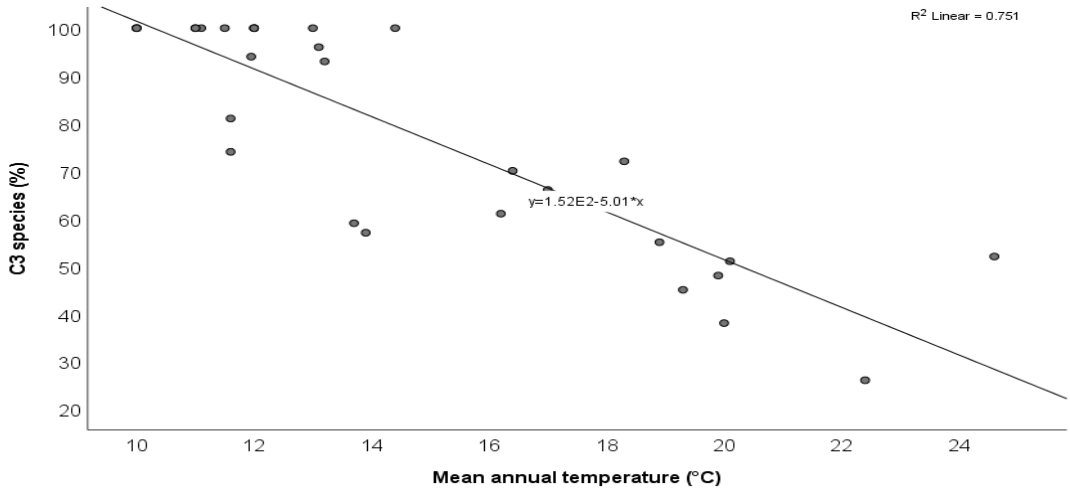

Figure 5a: Relationship between mean annual temperature and percentage of C_3 species

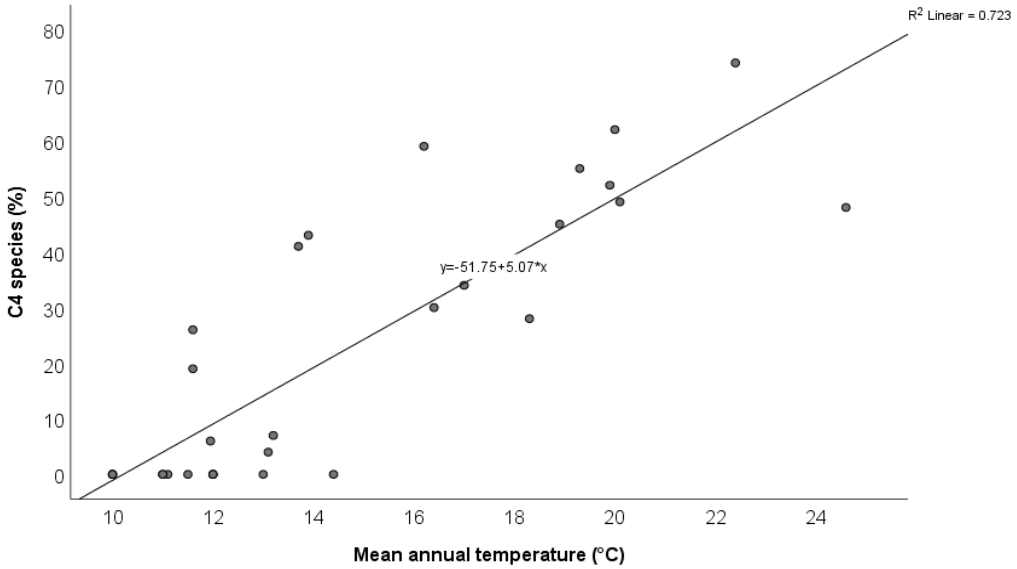

Figure 5b: Relationship between mean annual temperature and percentage of C_4 species.

DISCUSSION

The results indicated that C_4 plants declined in abundance while C_3 plants increased with altitude (Figure 2). The plant species composition across different altitudes agrees with earlier findings obtained by (Tiezsen *et al.,* (1979); Masrahi, *et al.*, (2011); Cabido *et al.*, (2003). At low elevations C_4 species are dominant, the relative abundances are comparable at medium altitudes, and at the highest elevation C_3 species predominate. The transition in dominance of C_3 and C_4 plant species occurred between 1700m and 1800 m, closely matching the elevations reported by Cabido *et al.*, (2003).

The current study has demonstrated a significant correlation between climatic parameters and the relative abundance of C_3 and C_4 plant species across elevations. Findings indicate that mean temperature and rainfall determines the distribution of C_4 plants along the altitudinal gradient. Low-altitude species are linked to high temperatures, increased humidity, intense radiation, high evaporation, and low rainfall (Zhang *et al.,* 2023). These environmental conditions favor a greater representation of C_4 plant species. These plants possess higher temperature optima for CO_2 assimilation and high water-use efficiency compared to C_3 plants (Bräutigam and Gowik, 2016; Lara and Andreo, 2011).

Studies on tropical plant distribution across the altitudinal gradients have shown that C_4 species were absent in areas where minimum air temperatures fall below 8 °C (Long, 2006; Osborne *et al.*, 2008). Similarly, in arid regions, C_4 plants do not occur where growing-season temperatures were below 8°C (Zhiguo *et al.,* 2012; Sayed and Mohamed, 2000). This suggests that the competitive relationships between C_3 and C_4 plants are strongly influenced by the altitudinal variation in air temperature. The present study aligns with these observations, reinforcing the idea that changes in air temperature with altitude influence how C_3 and C_4 plant species compete with each other. This gains support from similar results from earlier publications (Opoku *et al.,* 2024). Furthermore, higher abundance of C_4 plants at low altitudes is linked to the temperature sensitivity of phosphoenol pyruvate carboxylase to low temperature which makes it difficult for C_4 plants to compete at high altitudes (Chen *et al.*, 2009).

The present study shows that C_3 species perform better in highland areas characterized by cooler temperatures, while C_4 species are more successful in warmer lowland environments. This pattern is consistent with previous studies that have demonstrated fundamental physiological differences between the two photosynthetic pathways. Due to their comparatively high affinity for CO_2, plants utilizing the C_4 photosynthetic pathway can continue to produce large amounts of oxygen even in situations when there is little water available (Bräutigam and Gowik, 2016). Even with the stomata partially closed, they can still photosynthesize (Ghannoum, 2009). Under similar light and temperature conditions, C_4 species plants demonstrate greater water-use efficiency than C_3 plants due to their lower stomatal conductance. Ehleringer, (1978) used computer simulations to demonstrate that the quantum yield of C_4 species, declined as temperatures decreased below 32^0C at which point the quantum yield of C_3 plants surpass that of C_4 plants below this temperature. Using computer simulations, Ehleringer (1978) demonstrated that the quantum yield of C_4 plants declines when temperatures fall below 32°C, at which point C_3 species surpass them in efficiency. While C_4 grasses are better adapted to hot, dry environments, they lose out to C_3 plants in cool, moist conditions. These temperature-driven differences in photosynthetic performance provide a strong explanation for the observed altitudinal segregation of C_3 and C_4 species in this study.

The dominance of C_3 species in cooler highland environments and the prevalence of C_4 species at warmer low altitudes reflect fundamental differences in the thermal sensitivity of their photosynthetic pathways (Yamori *et al.*, 2014). In C_3 plants, high temperatures reduce photosynthetic rates and carbohydrate reserves, with carboxylation reactions being the initial site of damage (Hu *et al.*, 2020; Sage and Kubien, 2007). Heat sensitivity of C_3 plants is largely attributed to heat sensitivity of the enzymes ribulose bis-phosphate carboxylase/oxygenase (rubisco) and rubisco activase (Sharkey *et al.*, 2001; Demireuska-Kepova and Feller, 2004) with rubisco activation by rubisco activase identified as the most heat-sensitive step (Cen and Sage, 2005; Ristic *et al.*, 2009). This heat-induced impairment of rubisco activity explains the sensitivity of the C_3 pathway to high temperature (Ristic *et al.*, 2009). This mechanism provides a sufficient explanation for the observed low abundance of C_3 plant at low altitudes in the study area. In contrast, the cooler conditions at higher altitudes limit the occurrence of C_4 species and hence shift the competitive balance in favour of C_3 species.

The general pattern of $\delta^{13}C$ values distribution across altitude indicates that values ranging from -10.1‰ to -16.62‰, -17.15‰ to -18.87‰ and -22.68‰ to-32.42‰ occur at low altitudes (1000m-1500m a.s.l.), intermediate (1550m-1700m a.s.l.) and high altitude (1800m-4200m a.s.l.) respectively. The C_4 species had $\delta^{13}C$ values ranging from –10.1‰ to -16.55‰, while the C_3 had $\delta^{13}C$ values ranging from -22.68‰ to -32.42‰ (Table 1). These altitudinal distributions for plant species is well within the range reported by Basu *et al*, (2015) and

Kirkels *et al.,* (2022). The low altitudes (< 1550m a.s.l.) are associated with water drought, high temperatures and low relative humidity as aspects of aridity index where C_4 species are well adapted. In contrast, less water drought, low temperature, high relative humidity and high radiation are experienced at high altitudes (> 1700m a.s.l.) where a high percentage of C_3 species thrive. This altitudinal trend based on the $\delta^{13}C$ values is fully supported by the distributional pattern on floristic information of C_3 and C_4 species in this study.

Variation in $\delta^{13}C$ value may occur at several sites, including during diffusion of CO_2 from the leaf atmosphere into the chloroplast, during carboxylase-catalyzed fixation, and through subsequent metabolic processes (Snyder *et al.,* 2022). While environmental variables may affect each of these stages to some extent, their strongest effect is usually on the enzyme-catalyzed kinetic steps. Consequently, the carboxylase reaction and subsequent metabolic processes might be expected to be more strongly influenced by external selective variables that act cumulatively. A species-dependent variation in $\delta^{13}C$ values was noted in this study. This is because the availability of CO_2 to Ribulose bisphosphate carboxylase/oxygenase (RuBisCO) is determined by both external and internal structural traits of the plant. RuBisCO favors the fixation of $^{12}CO_2$ over $^{13}CO_2$ due to the kinetic isotope effect, whereas Phosphoenolpyruvate (PEP) carboxylase is less discriminating against $^{13}CO_2$ compared to RuBisCO (Mateus and Lavres, 2025).

Carbon ratios are increasingly being used as a helpful scientific method and instrument in the investigation of various biological issues in the environment and associated conditions. The $\delta^{13}C$ value have been used in partitioning the photosynthetic types (Kirkels, *et al*., 2022), forensic studies as fingerprints of biological agents (Horita and Vass, 2003), dietary biomarker studies in health research (O'brien, 2015), reconstruction of past environments and for the interpretation of plant physiological responses to climate (Zhang, 2019), tracing valuable information about the origin of greenhouse gases (Meija, 2023) and identifying the point of origin of illicit drugs (Ehleringer *et al.,*2002; Meikle *et al*., 2019). The above examples confirm the contributions of $\delta^{13}C$ value study in the science world. In the present study, it has been used to establish the different photosynthetic types, their abundance and distribution across the altitudinal gradient in Western Kenya. The distinct and clear separation between C_4 plants at lower elevations and C_3 plants at high elevations may be of special significance for understanding Kenya's ecology and paleoecology (Bremond *et al.,* 2008). This suggests that $\delta^{13}C$ values can be used to reconstruct Kenya's past vegetation patterns and potentially infer aspects of past climatic regimes.

Since temperature and rainfall strongly influence the distribution of C_3, C_4, and CAM species along altitudinal gradients, this research recommends long-term ecological monitoring. This would help detect shifts in plant distribution patterns under ongoing climate change, particularly warming trends that may allow C_4 species to expand into higher elevations. Conservation strategies should prioritize high-altitude zones dominated by C_3 species, which may be particularly vulnerable to climate warming. While temperature and rainfall were key drivers, future research should also consider other ecological factors such as soil type, soil moisture, nutrient availability, and land-use practices, which may further influence plant distribution along elevation gradients. Similar studies should be conducted across broader geographic areas and over wider altitudinal ranges to test the generality of the observed patterns and to improve regional and national vegetation classification models.

CONCLUSION

The distribution of plants was strongly associated with temperature and rainfall along the studied altitudinal gradient. While C_3 and C_4 species occurred in similar proportions at 1100–1600 m.a.s.l, C_3 species became more abundant with rising elevation. C_4 species were confined to elevations below 3100 m a.s.l., where cooler temperatures and higher rainfall at higher altitudes limited their occurrence and promoted C_3 dominance.

ACKNOWLEDGEMENTS

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CONFLICT OF INTEREST

Authors have declared that no competing interests exist**.**

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