δ^{13}C SIGNATURES OF FLORA, MACROFAUNA AND SEDIMENT OF A MANGROVE FOREST PARTLY AFFECTED BY SEWAGE WASTES

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ABSTRACT

Stable carbon isotope ratios (δ^{13}C) of sediments, suspended particulate matter, and crab gut content from Maruhubi mangrove forest, Zanzibar, were determined. Stable carbon isotope ratios were used to determine sources of organic matter in the forest. Mangrove organic carbon (δ^{13}C±SD = -27.97±0.25) was found to be an important component of the sediment organic matter except at the marine fringe zone (δ^{13}C±SD = - 17.26±0.83). The areal distribution of δ^{13}C values show that the marine fringe zone was rich in organic carbon of marine origin. The terrestrial fringe zone was dominated by a mixed source of sediment organic carbon (δ^{13}C±SD =-24.89± 0.72). Vertical variations in δ^{13}C were not so obvious, showing a diminishing importance of mangrove organic matter below 60 cm in the sediments of the terrestrial fringe zone. Stable carbon isotope ratios of the major primary producers in the mangrove forest were reflected to a reasonable extent in the terrestrial fringe surficial sediment organic carbon. Stable carbon isotope ratios for plant and sediment organic carbon gave values in the range of – 27.67 to – 28.31 $^o/_{oo}$ for mangroves and – 17.57 to – 24.89 $^o/_{oo}$ for surface sediment (0 - 5 cm). Marine fringe sediments receive organic matter of marine origin to a large extent, in comparison to terrestrial fringe sediments, which contain mainly organic matter of mangrove origin.

INTRODUCTION

The advantage of using stable isotope ratios as a tracer of ecosystem energy flows is that isotopic composition of organic matter during natural transformation of organic matter through metabolic or ecological processes is

largely preserved (Parker 1964). Limitations of the technique apply where there are more than two sources of organic matter. In such a case, a simple mixing model of two end members can not be unambiguously applied to reveal the source of carbon in a sample. Reported $\delta^{13}C$ values for similar coastal and marine organic material are not constant but the differences, however, lie within 10 $^O/_{OO}$. For instance, a difference of 4.7 $^O/_{OO}$ has been observed for individual seagrass blades (McMillan *et al.* 1980). Stephenson *et al.* (1984) have reported a $\delta^{13}C$ range of 8 $^O/_{OO}$ for an individual algal frond. These wide within species variation of $^{13}C/^{12}C$ ratios can partly be influenced by environmental factors e.g. seasons, nutrients supply and geographical location (Fry & Sherr 1984). However, stable carbon isotope discrimination is a complex physiological process.

So far, there are adequate literature values to constrain the various sources of intertidal organic matter. Carbon isotope compositions of nearshore particulate organic carbon (POC) have been reported (Rezende *et al.* 1990, Rodelli *et al.* 1993, Middelburg & Nieuwenhuize 1998). This POC is mainly composed of benthic macrophyte organic carbon or phytoplankton carbon in productive marine areas. Generally, freshwater and brackish water POC has relatively $\delta^{13}C$ depleted values of between -24 $^O/_{OO}$ and -30 $^O/_{OO}$ (Sherr 1982, Tan & Strain 1983). Terrigenous C_3 organic matter with $\delta^{13}C$ of approximately -26 $^O/_{OO}$ may be an important source for highly negative $\delta^{13}C$ values. C_4 grasses that are common in basins of tropical rivers can be a source of high $\delta^{13}C$ values (~ -12 $^O/_{OO}$) in terrigenous organic matter (Marriotti *et al.* 1991, Bird *et al.* 1992, 1994). Published values for autotrophic and chemoautotrophic bacteria show highly depleted $\delta^{13}C$ values. For example, Rau and Hedges (1979) obtained a $\delta^{13}C$ of about -33 $^O/_{OO}$ for a hydrothermal vent mussel. It was suggested that the mussel assimilated the organic carbon from a chemoautotrophic source.

In the terrestrial environment, the isotopic signatures of soils and peat deposits depend mainly on the source of organic matter, which may be C_3 and/or C_4 plant material. The $\delta^{13}C$ values for this source may range from -11.6 $^O/_{OO}$ to -28.8 $^O/_{OO}$ (Emery *et al.* 1967, Schell 1983). In the marine areas, it is possible to characterise marine sediments with respect to the sources of organic matter using $^{13}C/^{12}C$ ratios (Torgersen & Chivas 1985). Possible sources of organic matter in tropical and subtropical intertidal marine areas include: adjacent terrestrial and marine macrophytes; benthic microflora and phytoplankton. In some cases, allochthonous input of organic matter of terrestrial origin may dominate in areas influenced by river mouth or

anthropogenic waste discharges (Calder & Parker 1968, Burnett & Schaeffer 1980, Sweeney *et al.* 1980). There is a close similarity between the C_3 dominated terrestrial organic matter and the predominantly phytoplankton derived marine organic matter. The former has a $\delta^{13}C$ value of about -26 $^O/_{OO}$ and the latter has a value in the range of -21 $^O/_{OO}$. Despite this small difference, it has been possible to detect a gradient of $\delta^{13}C$ values along riverine-marine transects (Hunt 1970, Hedges & Parker 1976, Schultz & Calder 1976, Tan & Strain 1983, Gearing *et al.* 1984). In such cases, terrigenous organic carbon is almost undetectable seawards of the fluvial-marine water mixing zones. Even in areas without prominent terrestrial runoff, similar gradients are manifested. Nissebaum and Kaplan (1972) found that tidal and subtidal sediments have varying strength of marine influence. The marine character was more conspicuous than the terrestrial finger print.

As a result of ^{13}C discrimination by cereal plants, sewage wastes have relatively ^{13}C depleted values, with $\delta^{13}C$ values close to -25 $^O/_{OO}$ (Faganeli *et al.* 1988). $^{13}C/^{12}C$ ratios as a tracer of material in an ecosystem takes advantage from the understanding that terrestrial organic matter is enriched in ^{12}C relative to marine sources. In the coastal zone, habitats that are characterised by a simple two end member mixing model are rare due to the present level of anthropogenic impacts. Multiple uses of catchment areas and coastal zones tend to complicate the sediment regime of the nearshore marine areas. Because of this and due to the inherent inadequacy of the $^{13}C/^{12}C$ technique in precisely determining the sources of organic matter, complimentary measurements or multiple isotope determinations may be necessary (Fry & Sherr 1984, Druffel & Williams 1992). For instance, Lin and Sternberg (1992) reported that physico-chemical factors, such as hypersalinity, can cause stress to *Rhizophora mangle* (L). Such a stress can affect growth and therefore influencing carbon isotope fractionation. The isotope measurements are useful in tracing sources, sinks and pathways of environmental organic matter. It has the advantage of accuracy in studies concerning food chain trophodynamics, and in metabolic and biogeochemical transformation processes of organic matter.

Fate of mangrove litter in the marine environment has received considerable attention in recent years (Machiwa 1999). Stable carbon isotopes signatures have been used to trace particle dynamics and the influence of refractory mangrove carbon in the sediment. Some results show that mangrove contribution to the particulate organic carbon (POC) pool of adjacent water can be 100%. This amount is variable depending on the hydrodynamics of the area. Studies have revealed that in mangrove dominated settings the ebb peak POC is essentially mangrove derived; in such areas the flood flow has a mixed input, but show a high marine influence (Haines 1976, Rezende *et al.* 1990).

Bird *et al.* (1995) have reported $\delta^{13}C$ values of riverine and delta carbon as low as -21 $^o/_{oo}$ in the gulf of Papua. Some delta sediment samples had values lower than -27 $^o/_{oo}$, depending on the extent of the influence of mangrove derived carbon.

In this study stable carbon isotopes of common flora, macrofauna and sediment are reported. Stable carbon isotopes are used to confirm the food preference of *Neosarmatium meinerti* (De Man) and *Cardisoma carnifex* (Herbst). The study evaluates the influence of crabs on the fate of mangrove litter in the terrestrial fringe mangrove area. Relative contribution of mangrove and non-mangrove sources of organic matter to the sediments of the mangrove forest was estimated using relevant mixing models (see section of data analysis below). The extent of detrital outwelling of the mangrove stand was also investigated.

MATERIALS AND METHODS

Study site
The ecological, environmental and geomorphological setting of the Maruhubi mangrove area is reported elsewhere (Machiwa & Hallberg 1995, Machiwa 1998, 1999). Sediment sampling stations are shown in Fig. 1. Maruhubi forest was subdivided into operationally defined zones comprising the marine fringe (MF), shoreline (SL), landward extending (LE) and terrestrial fringe (TF) zones. The zones correspond to characteristic mangrove species in each subarea (Fig.1).

Isotope composition of plant material, crab organic matter and sewage
Mature leaves were harvested from five mangrove species (*Sonneratia alba* (J.E. Smith), *Avicennia marina* (Forsk.) Vierh., *Ceriops tagal* (Perr.) C.B.Rob., *Bruguiera gymnorrhiza* (L) Lamk. and *Lumnitzera racemosa* (L) Gaertn.). Seagrass, *Thalassodendron ciliatum* (Forsk.) den Hartog and seaweed, *Ulva* spp specimens were collected from the forest floor. The plant material was washed, treated with 1 M HCl, re-washed and freeze dried, then the dried samples were crushed and homogenised. Triplicate samples, each with a pooled total of more than 10 mangrove leaves, seagrass blades and alga fronds were used for analysis.

Live crabs were harvested from the forest areas with and without sewage. Six crabs were collected from each of two areas. The specimens were immediately placed on ice in a cool box. In the laboratory the crabs were dissected in order to expose their alimentary canals. The contents of the proventriculus and rectum were suspended in 1 M HCl, washed and freeze-dried. Fresh sewage which had been disposed by trucks was treated with 1 M HCl, washed and

freeze-dried. After drying the samples were finely ground (about 80 mesh) and presented for analysis.
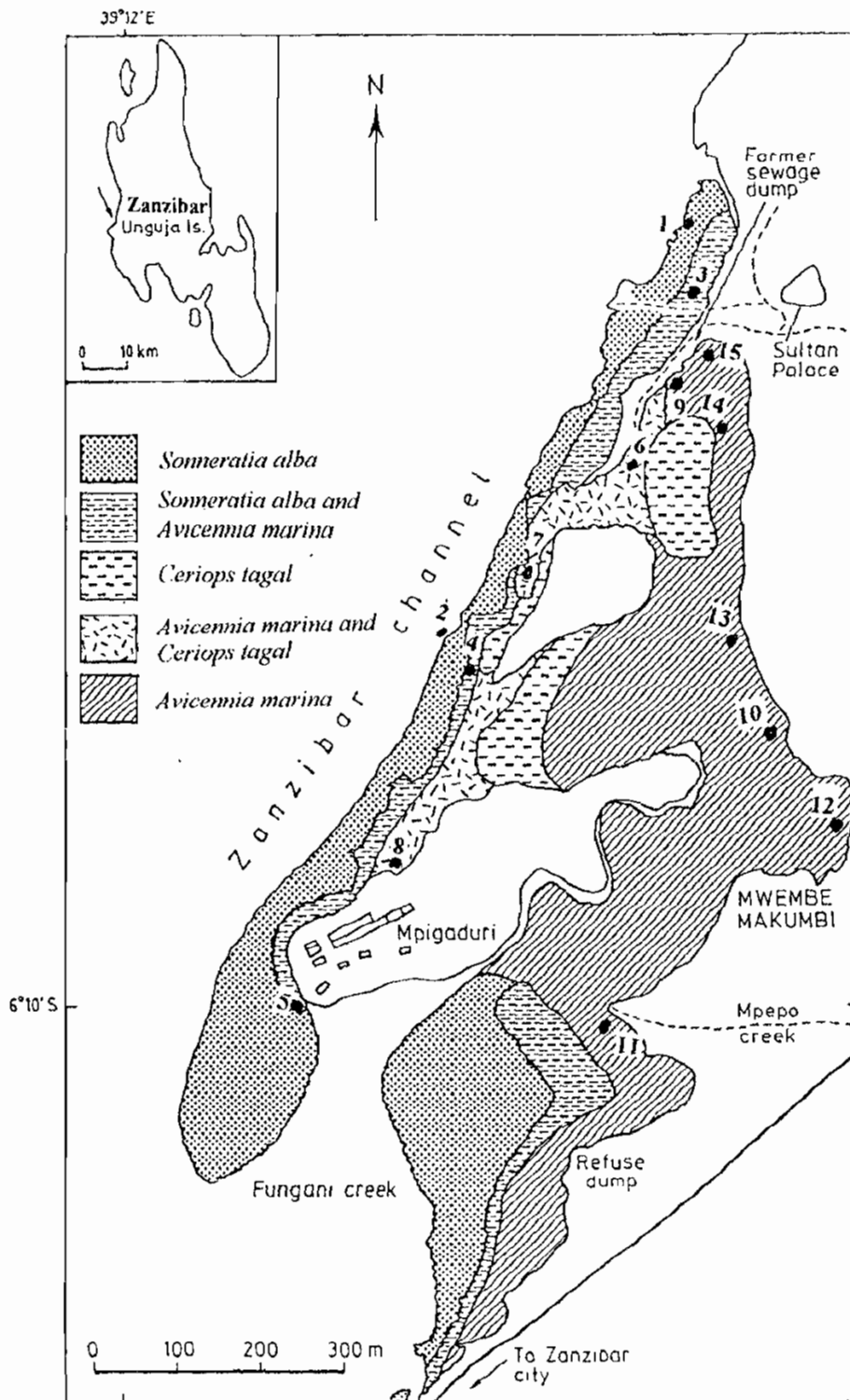

Fig. 1: Map of the study area showing sampling stations in the forest zones (*S. alba* zone = marine fringe; *S. alba/ A. marina* zone = shoreline; *A.marina/ C. tagal* zone = landward extending; *A. marina* zone = terrestrial fringe). Stations for spatial variation of $\delta^{13}C$ in the surface sediment are 1 – 11, and for vertical distribution of $\delta^{13}C$ in the sediments are 12 – 15.

Stable carbon isotopes of sediment and suspended particulate matter

Surface sediment (top 5 cm) samples that were used for isotope comparison between mangrove zones were wet sieved over a 125 μm sieve and freeze-dried. Triplicate sediment samples that were used for the examination of variation of isotope ratios with depth in the sediment were only passed through a 2 mm sieve to remove coarse plant debris. Suspended particulate matter (SPM) was obtained by centrifugation of seawater from the mangrove-inshore water boundary. SPM was sampled bimonthly at five stations for one year. The samples were pooled in order to form two categories of flood and ebb samples. Triplicate sub-samples for stable isotope analysis were drawn from each category. SMP and sediment samples were treated with 2 M HCl washed and then freeze-dried. The dry samples were finely powdered (80 mesh), homogenised and presented for stable carbon isotope analysis.

Stable Carbon Isotope analysis

Stable carbon isotope ratios were determined in fresh sewage, crab's gut material, mangrove leaves, seagrass, macrophytic algae and suspended solids in flood and ebb water. The inorganic carbon content of the samples was checked with a Shimadzu TOC-5000 analyser prior to analysis of stable carbon isotopes. A continuous flow IRMS (20-20 Stable Isotope Analyser, Europa Scientific Ltd), interfaced with an elemental analyser unit (ANCA-NT system, solids/liquids preparation module, Europa Scientific) was used for stable isotope analysis. The instrument was installed in a thermostated room (20°C) in order to achieve maximum temporal stability of analytical results. Change in the temperature is the principal cause of drift in the isotopic ratios. Pre-weighed samples were introduced in the elemental analyser by an autosampler. Each sample was completely combusted in an oxidising column containing chromium oxide, copper oxide and silver wool. A pulse of oxygen was added concurrently with sample introduction. The carbon and nitrogen content of the sample is quantitatively converted into CO_2 and N_2 respectively. The CO_2 and N_2 formed are separated using a Gas Chromatographic column, and transferred with a He carrier gas through a thermal conductivity detector to the IRMS inlet where a small amount of the sample gas enters the Mass Spectrometer.

The $^{13}C/^{12}C$ ratio is expressed in $\delta^{13}C$ notation as:

$$\delta^{13}C^o/_{oo} = \frac{R - R_s}{R_s} x1000$$

Where R and R_s are $^{13}C/^{12}C$ ratios in sample and standard respectively.

Results are here reported as per mille deviations from the carbon isotope composition of the international primary standard Pee Dee Belemnite (PDB), the Chicago cretaceous belemnite. For this study, secondary standards (wheat and corn flour) that had been calibrated with reference to the primary standard were used. Analytical uncertainty for the wheat and corn flour internal standard was ± 0.18 $^O/_{OO}$ and ± 0.12 $^O/_{OO}$ respectively.

Data analysis

Quantification of the relative contribution of marine and mangrove POC in SPM and sediment was calculated according to a two-end-member model (Rezende *et al.* 1990). Contribution of organic carbon to the mangrove sediments from several sources can be computed from the assumption that the end-members should have distinct isotopic signatures. The relative contribution of each end-member in a mangrove setting can be expressed quantitatively using a material balance equation as:

$$f_f = \frac{Rs - Rm}{Rf - Rm}.100$$

Where f_f = percentage contribution from mangrove organic carbon

$Rs = \delta^{13}C$ value of organic carbon in a sample

Rf = the assumed $\delta^{13}C$ value of mangrove organic carbon

Rm = the assumed $\delta^{13}C$ value of marine organic carbon

RESULTS AND DISCUSSION

Isotopic composition of crabs and plant material

Stable carbon isotope analysis data for the crab gut content show that the data are close to that of the mangrove leaves (Table 1). Hence, fractionation by the crabs was not evident, and the mangrove tissues appeared to be the major food item for *N. meinerti* and *Cardisoma carnifex*. Crabs collected from sewage and non sewage areas showed almost equal $^{13}C/^{12}C$ ($\pm$SD) values (-27.67$\pm$0.21 $^O/_{OO}$), which is identical to that of crabs fed on pure *A. marina* leaves in the laboratory (-27.28$\pm$ 0.14 $^O/_{OO}$). Stable isotope data support previous observations that the large crabs consume almost pure leaf material before the leaves are coated by the mud (Macnae 1968, Malley 1978, Leh & Sasekumar 1985, Camilleri 1989, 1992). However, this case may not be the same for the younger crabs, which are unable to hold the leaves properly. Young crabs can hardly lift the leaves from the forest floor, and thus eat them

on the sediment surface. $\delta^{13}C$ values in the surface sediment of the sewage area was $- 24.89\pm0.72$ $^{O}/_{OO}$ (Table 1). Other previous studies e.g. Parker (1964) in Texas, Black and Bender (1976) in Australian coral reef, also demonstrated that the carbon isotope composition of marine herbivorous and detritivorous fauna manifested that of their presumed food sources. For instance, one study showed that *Sesarma* sp. had a $\delta^{13}C$ signature nearly 3 $^{O}/_{OO}$ lower than *Uca pupilator* collected in the same area. The difference was attributed to the dissimilarity in the material they had been assimilating.

Table 1: Stable carbon isotope ratios of mangrove, seagrass and seaweed species, suspended particulate matter, sewage and crab gut content, Marubi mangrove stand, Zanzibar. 1995-1996

Mangrove species	$\delta^{13}C\pm SD$	n
Sonneratia alba	-27.67±1.67	3
Avicennia marina	-28.13±0.73	3
Ceriops tagal	-28.31±0.53	3
Bruguiera gymnorrhiza	-27.92±0.81	3
Lumnitzera racemosa	-27.82±0.87	3
Seagrass		
Thalassodendron ciliatum	-12.04±0.15	3
Macroalgae		
Ulva fasciata	-26.63±0.12	3
Suspended particulate matter		
Flood	-19.68±0.19	3
Ebb	-21.02±0.23	3
Sewage	-24.91±0.12	3
Crab gut content	-27.54±0.27	6

Isotopic composition of particulate matter

Marine SPM yielded ^{13}C-enriched values in contrast to ^{13}C depleted values of autochthonous mangrove organic matter. Stable carbon isotope analysis of SPM averaged -19.68± 0.19$^{O}/_{OO}$ for flood and $- 21.02\pm0.23^{O}/_{OO}$ for ebb tides. The $\delta^{13}C$ results show that a mixture of organic carbon from mangrove and marine origins was always present in the MF mangroves. For all tidal cycles, the results reveal the same trend, i.e. the flood peak $^{13}C/^{12}C$ ratio

(during 1 m depth at the MI boundary) showed a strong shift towards the marine value and the ebb - peak ratios reflected presence of mangrove POC.

Stable carbon isotope in the surface sediments

Spatial distribution of $\delta^{13}C$ in the sediment indicated that the mangrove zones had an average value of $- 22.50 \pm 3.54$ $^O/_{OO}$. This value probably represent a mixed origin of organic matter, which is composed of mangrove, marine and terrestrial (e.g. sewage) end-members. The $\delta^{13}C$ of organic matter in the sediments of the TF zone had almost similar values, $- 25.41 ^O/_{OO}$ in the northern part, $- 24.07$ $^O/_{OO}$ in the central and $- 25.19$ $^O/_{OO}$ in the southern parts. The TF zone is dominated by *A. marina* but the $\delta^{13}C$ signatures suggest that mangrove litter (Table 2) is not the only source of organic matter in the sediment. Terrestrial and marine inputs of organic matter significantly contribute to the sediment organic carbon pool. $^{13}C/^{12}C$ ratios of the MF and SL sediments showed enrichment of ^{13}C, the values were consistent with input marine and mangrove litter end-members. Published results estimate $\delta^{13}C$ values at $- 8$ to -27 $^O/_{OO}$ for benthic algae (Thayer *et al.* 1983); -3 to -23.8 $^O/_{OO}$ for seagrasses (McMillan *et al.* 1980). Most seagrasses lie in the range of -3 to -15 $^O/_{OO}$ with an average composition of -10 $^O/_{OO}$ for species like *Thalassia* and *Halodule* (Fry & Sherr 1984). Marine macroalgae and seagrass were isotopically distinct from mangrove leaves (Table 1). Average $\delta^{13}C$ for *S. alba, A. marina, C. tagal, L. racemosa* and *B. gymnorrhiza* was -27.97 ± 0.25 $^O/_{OO}$. Similar values ($-26.66 \pm 1.42^O/_{OO}$) have previously been reported for the mangroves in the region (Hemminga *et al.* 1994). Their work also showed that $\delta^{13}C$ for *Thalassodendron ciliatum* was -15.03 ± 4.18 $^O/_{OO}$.

Table 2: Spatial distribution of isotope ratios in the surface sediment (125 μm grain size fraction) of the forest zones (n = 3), Maruhubi mangrove stand, Zanzibar. 1996

Forest sub-area	$\delta^{13}C \pm SD$
Marine fringe	-17.57±0.83
Shoreline	-23.5±1.18
Landward extending	-24.34±1.14
Terrestrial fringe	-24.89±0.72

Comparison of the $\delta^{13}C$ values of *S. alba* growing in different forest zones suggested that there may be some intra-specific variation in the carbon isotope composition of the mangroves. Haines (1976) suspected similar variations in a salt marsh plant *Spartina alterniflora*. There was less discrimination against ^{13}C by the shoreline *S. alba* ($\delta^{13}C$ = -26.50 $^o/_{oo}$) indicating a certain degree of stress compared to the marine fringe *S. alba* ($\delta^{13}C$ = -28.85 $^o/_{oo}$). The relatively low $^{13}C/^{12}C$ ratio show a tendency for selective uptake of the heavier ^{13}C isotope during photosynthesis. Marine fringe zone was regularly flooded, while the shoreline zone was not completely inundated by some of the neap tides.

In the marine fringe zone, the surface sediment had a relatively low $\delta^{13}C$ value (-17.57±0.83 $^o/_{oo}$), suggesting that marine organic matter had a stronger influence in the surface sediment, seawards starting from the marine fringe. In general, the $\delta^{13}C$ values of the surface sediments increased seaward from the terrestrial fringe (-24.89±0.72 $^o/_{oo}$) to the marine fringe zone. This trend suggests a proportional increase of the heavier $\delta^{13}C$ of seagrass organic matter in the mangrove sediment. Sewage organic matter ($\delta^{13}C$ = -24.91±0.12 $^o/_{oo}$) was enriched in ^{13}C relative to mangrove organic matter in Maruhubi forest. Similar values for sewage have been reported, for instance, -25.0±0.05 $^o/_{oo}$ (Faganeli *et al.* 1988) and -26.7 $^o/_{oo}$ (Thornton & McManus 1994). The contribution of sewage organic matter to sedimentary organic content cannot be clearly shown using the present data.

Stable carbon isotope ratios for the long cores

The interest in temporal variations centres on the isotopic record of organic matter which is preserved in mangrove sediments as a potential record of fate of sewage in the mangrove forest. It was observed that $\delta^{13}C$ values for bulk sediment samples were constant (- 27 $^o/_{oo}$) from the surface to about 50 cm in the sediment. Further down the core the sediments were enriched with ^{13}C relative to mangrove leaf values ($\delta^{13}C$ = -26 $^o/_{oo}$). Thus enrichment possibly reflects the isotopic composition of the more refractory material (Deines 1980). Mangrove organic matter is the largest contributor to the sediment organic matter pool, partly due to preferential decomposition of the labile sewage and marine organic matter. Refractory mangrove organic matter is retained in the subsurface sediments. The vertical variation of $^{13}C/^{12}C$ ratios of the mangrove sediments reflects values in the same category as the mangroves, except at the sewage dumpsite ($\delta^{13}C$ = -26 $^o/_{oo}$). *In situ*

contribution of the mangroves to the sediment organic matter was prominent in the terrestrial fringe zone which had no sewage influence (Table 3). In this zone, the low $\delta^{13}C$ of *A. marina* (-28.13±0.73 $^{o}/_{oo}$) was reflected by a sediment ($\delta^{13}C$ = - 27.2±0.58 $^{o}/_{oo}$), which is lower than values found in other zones of the forest. In the area that was a former sewage dumpsite, the $\delta^{13}C$ values of the entire 1 m of the sediment was higher (- 26 $^{o}/_{oo}$) than that of the mangroves in the TF zone (- 29.07 $^{o}/_{oo}$). This suggests that the sediment at the dumpsite was enriched with sewage relative to mangrove organic carbon, reflecting the inadequacy of tidal flushing of sewage wastes.

Table 3: Vertical variation of stable carbon isotope ratios in the bulk sediment of the terrestrial fringe zone with sewage and without sewage influence (n = 3). Maruhubi mangrove stand, Zanzibar. 1996

Sediment depth	$\delta^{13}C$±SD	
(cm) area	Sewage area	Non sewage area
0 –5	-26.67±0.29	-27.20±0.58
15 - 20	-26.61±0.31	-27.54±0.49
20 – 25	-26.53±0.27	-27.25±0.81
35 – 40	-26.52±0.53	-27.62±1.10
60 – 70	-26.58±0.22	-26.63±0.74
90 – 100	-26.74±0.4	-26.75±0.05

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