

# Meiofaunal Responses to Leaf Litter Added to Azoic Sediments in a Kenyan Mangrove Forest

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**Abstract** — Replicate azoic and organic-free sediments from a natural *Rhizophora mucronata* mangrove were enriched with similar amounts of mangrove and seagrass leaf litter to the natural organic concentration to establish which sources of litter most influence meiofaunal re-colonisation of reforested *R. mucronata* mangrove sediments in Gazi Bay. Sediments were incubated in 70 cc syringes with screened openings on the sides, allowing meiofaunal colonisation from the natural mangrove sediments in which they were buried. Controls were syringes filled with azoic sediment. The syringes were retrieved on days 1, 14, 30 and 60 days post-placement. Replicate cores were taken on the first day of the experiment to provide baseline data on the meiofaunal densities and community composition. Recolonisation occurred one day post-placement and meiofauna responded more to the addition of mangrove leaf litter, attaining the highest meiofaunal densities by the end of the experiment. ANOVA revealed a significant ( $p < 0.05$ ) litter source effect between mangrove and seagrass leaf litter, especially 30 days post-placement.

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## INTRODUCTION

Meiofauna, particularly nematodes, occur on all substrata in the marine environment. Detritus is a major energy source in many marine benthic systems and supports a high abundance and diversity of these meiofauna (Findlay & Tenore, 1982). In this regard, the phytal meiofaunal assemblages on mangrove leaf litter have been shown to be dynamic (Gee & Sommerfield, 1997; Zhou, 2001; Gwyther,

2003). Although the nitrogen content of detrital material may be the best measure of its nutritional quality, factors such as polyphenols (tannins) in mangrove leaf litter may lead to complex interactions between the tannins, the nitrogen content and age of the detritus (Tietjen & Alongi, 1990). This influences the utilisation of mangrove detritus by meiofauna, in particular nematodes. Mangrove leaves

initially undergo rapid leaching of dissolved organic matter (DOM) on the forest floor (Fell *et al.*, 1975). This is followed by slow decomposition of the remaining particulate organic matter (POM), facilitated by bacterial and fungal communities which condition the leaf litter for various invertebrate groups that utilise it as food. Gwyther (2003) indicated that the food value for meiofauna in leaf litter-derived particulate comprises the surface biofilm of bacteria, microalgae, protozoa and fungi. These are fed on by nematodes, in particular (Krishnamurthy *et al.*, 1984). Gee and Sommerfield (1997) showed that the initial chemical composition of leaves from different mangrove species is responsible for observed differences in meiofaunal communities during the decomposition process under similar conditions (sediment composition, salinity and tidal inundation). They also showed that a succession of meiofaunal communities occurs during the decomposition process.

Several meiofaunal colonisation studies have been published on mangrove leaf litter, including work by Zhou (2001) who investigated the responses of meiofauna in general and nematodes in particular to decaying mangrove leaf litter; Sommerfield *et al.* (1998) who investigated the relationship between meiofaunal communities and leaf litter from different mangrove species; and Gee and Sommerfield (1997) who investigated the effects of mangrove diversity and leaf litter decay on meiofaunal diversity. However, no study has investigated the effect of leaf litter of other origin on the meiofaunal re-colonisation of mangrove sediments. While mangrove leaves are naturally shed in this environment, seagrass leaves are also introduced to mangroves by tidal flooding in Kenya. This study was thus undertaken to elucidate the effect that different types of leaf litter have on meiofaunal re-colonisation of reforested *Rhizophora mucronata* mangrove sediments in Gazi Bay. Such information is needed to design restoration programmes for mangrove ecosystems after deforestation. The study thus focused on whether the availability of leaf litter or the origin of the leaf litter (mangrove or seagrass) affects meiofaunal re-colonisation of mangrove sediments.

## METHODS

The meiofaunal colonisation experiments were undertaken in a natural *Rhizophora mucronata* forest in Gazi Bay (4° 25'S; 39° 30'E; Fig. 1), located on the southern part of the Kenyan coast about 50 km from Mombasa. An area of approximately 50 m<sup>2</sup> was demarcated for the experiment.

Mangrove and seagrass leaf litter was used in the experiments for four incubation periods with two sets of controls. The latter comprised three cores from the experimental site as well as three azoic sediment samples, prepared as described below, which received no further treatment. Mangrove or seagrass leaf litter was added to the treated samples. Colonisation rates by meiofauna at different stages of the leaf litter decomposition were determined by sampling the experimental treatments at time intervals of 1, 14, 30 and 60 days post-placement. Each treatment was replicated four times.

Surface sediments were collected to a depth of 5 cm from the natural *R. mucronata* site for the preparation of the azoic, organic-free sediment. After collection the sediments were air-dried for two days and combusted in an oven at 600°C for six hours to obtain azoic and organic-free sediment. The total organic matter (TOM) of the natural mangrove sediment at the experimental site was determined in three replicate, dried (80°C for 24 h), similarly combusted, sediment samples (20 g). The TOM was calculated as the difference in dry weight of the sediment before and after combustion (Buchanan & Kain, 1971). This value was used to calculate the amount of leaf litter to be added to the experimental sediment.

Yellowish, senescent and ready to fall *R. mucronata* leaves were picked in the natural forest while seagrass leaves were collected along the beach in Gazi Bay. Senescent mangrove leaves were used instead of fresh green leaves because these are naturally shed on the forest floor. Seagrass leaves were collected from the beach since these are washed into the mangrove during tidal flooding. The leaves were air-dried for one week and granulated using an electric grinder. The C:N ratio of the mangrove and seagrass

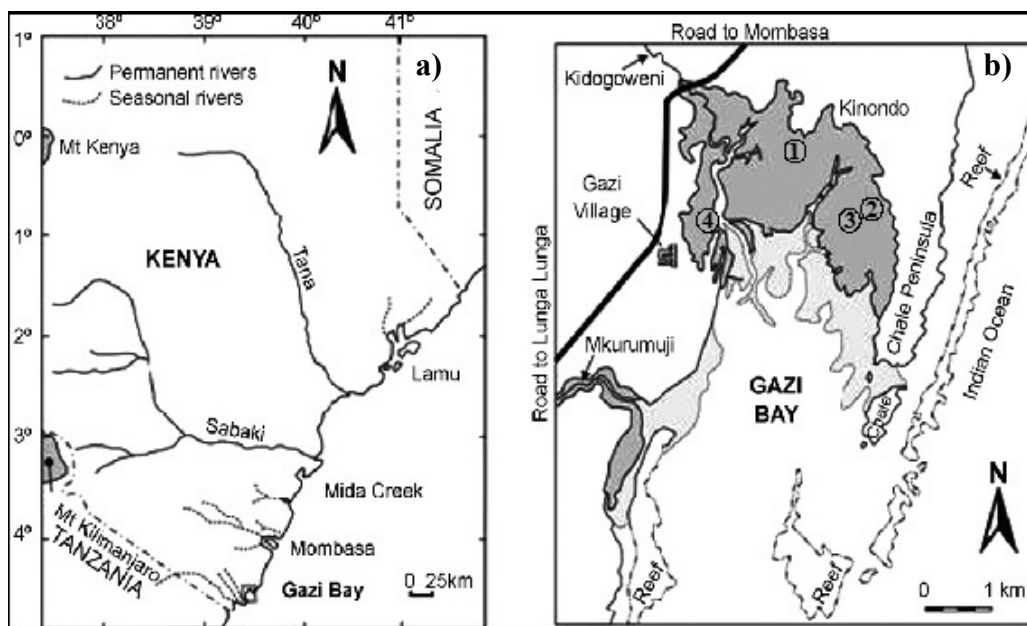


Figure 1. a) Map of the Kenyan coast and b) Gazi Bay showing the location of the experimental site (adapted from Bosire et al., 2004).

leaf litter was determined by first eliminating inorganic carbon from subsamples with dilute hydrochloric acid. The amounts of carbon and nitrogen were then analysed using a Carlo Erba element analyser, type NA-1500 (Nieuwenhuize & Mass, 1993-2002).

Plastic 70 ml (3 cm in diameter, 13.5 cm long) syringes were used as experimental vessels. Circular windows 2.5 cm in diameter were cut on opposite sides of each syringe and screened with plastic 2 mm mesh netting (Fig. 2) to allow water exchange with the surrounding natural sediment. The windows also enabled meiofauna to colonise the experimental sediments by horizontal migration, in addition to vertical movement from the overlying water column through each syringe top. The azoic, organic-free experimental sediments were put into the syringes and leaf litter was added on top. The syringes were labelled and randomly embedded in the sediment flush with its surface, covering a surface area of 7 cm<sup>2</sup>. They were fastened onto nearby roots or seedlings using nylon thread to avoid being washed away by tidal currents. Upon retrieval on days 1, 14, 30 and 60, they were immediately fixed in the field with 5% formalin.

In the laboratory, the meiofauna were rinsed from the samples with tap water through a 1 mm sieve to exclude macrofauna and debris, and retained on a 38 µm sieve. They were then centrifuged three times at 6000 rpm with MgSO<sub>4</sub> (specific density 1.28) for 10 minutes. After centrifuging, the supernatant was poured onto a 38 µm sieve, rinsed in tap water and stained with Rose Bengal. Meiofauna were identified under a dissecting microscope using Higgins and Thiel (1992) and enumerated.

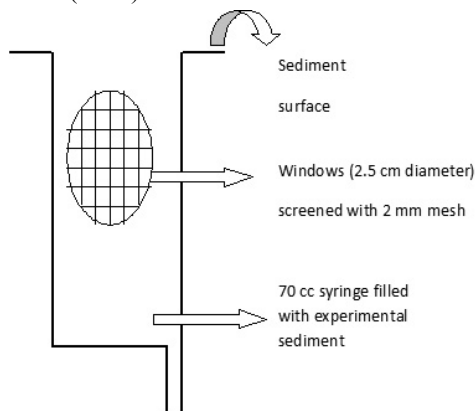


Figure 2. Diagram of the experimental sediment holders made from syringes (adapted from Zhou, 2001).

## RESULTS

Thirteen meiofauna taxa were recorded; seven taxa in the seagrass leaf treatments, and four and five taxa were recorded in the mangrove leaf and control treatments respectively. Nine taxa were recorded in the field control treatments. Nematoda were the most abundant fauna and accounted for 99% of the meiofaunal densities in the field control, 95% in the experimental control and 93% in both the seagrass and mangrove leaf litter treatments. Oligochaeta comprised the second most abundant taxon, accounting for 5% of the total densities in the seagrass leaf litter treatments, 3% in the experimental control and mangrove leaf litter treatments and 1% in the field control. Halacaroida occurred in relatively high numbers in the mangrove leaf litter treatment, accounting for 2% of the total meiofauna. The abundance of copepods was very low in the field control (<1%), and only comprised 1% of the meiofauna in the experimental control, seagrass and mangrove leaf litter treatments. Figure 3 depicts the re-colonisation trends of the meiofauna, nematodes in particular, in the leaf litter treatments and revealed that meiofaunal re-colonisation commenced in all the experimental treatments one day post-placement.

control ( $1209 \pm 198$  individuals.7 cm<sup>-2</sup>) on day one. The meiofaunal densities continued to increase during the course of the experiment, especially in the mangrove leaf litter. In fact, they surpassed those in the field control on day 30 ( $2071 \pm 958$  individuals.7 cm<sup>-2</sup>), and remained higher than the field control to the end of the experiment, although they declined between days 30 and 60. As stated, nematodes accounted for most of this increase in the mangrove leaf litter treatment, attaining  $2017 \pm 966$  individuals.7 cm<sup>-2</sup> on day 30, surpassing that of the field control and remaining higher to the end of the experiment, although they reflected the aforementioned decline between days 30 and 60. The above trends in meiofaunal and nematode densities in the mangrove leaf litter treatment coincided with a low C:N ratio recorded on days 30 and 60 (Fig. 4).

A meiofaunal increase occurred in the experimental control up to day 14, after which they remained more or less constant. Meiofaunal densities in the seagrass treatment remained below those of the field control throughout the experiment.

The differences in the meiofaunal densities, particularly of nematodes, were significant (ANOVA  $df=2$ ,  $F=19.511$ ,  $p<0.05$  and  $df=2$ ,  $F=14.712$ ,  $p<0.05$  respectively).

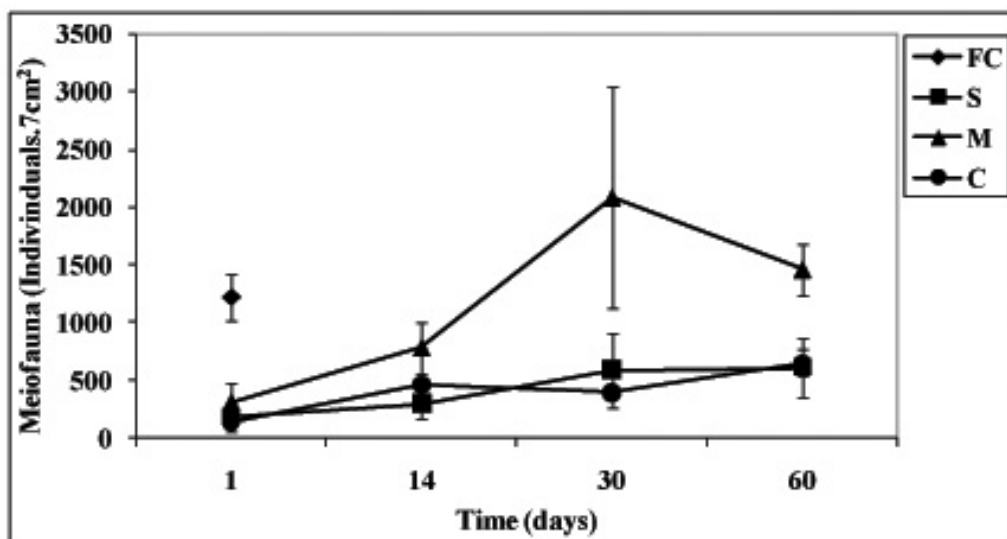


Figure 3. Colonisation rates expressed as densities (mean  $\pm$  SD) of a) meiofauna and b) nematodes during the experimental period (days). FC = field control, C = experimental control, S = seagrass leaf litter and M = mangrove leaf litter.

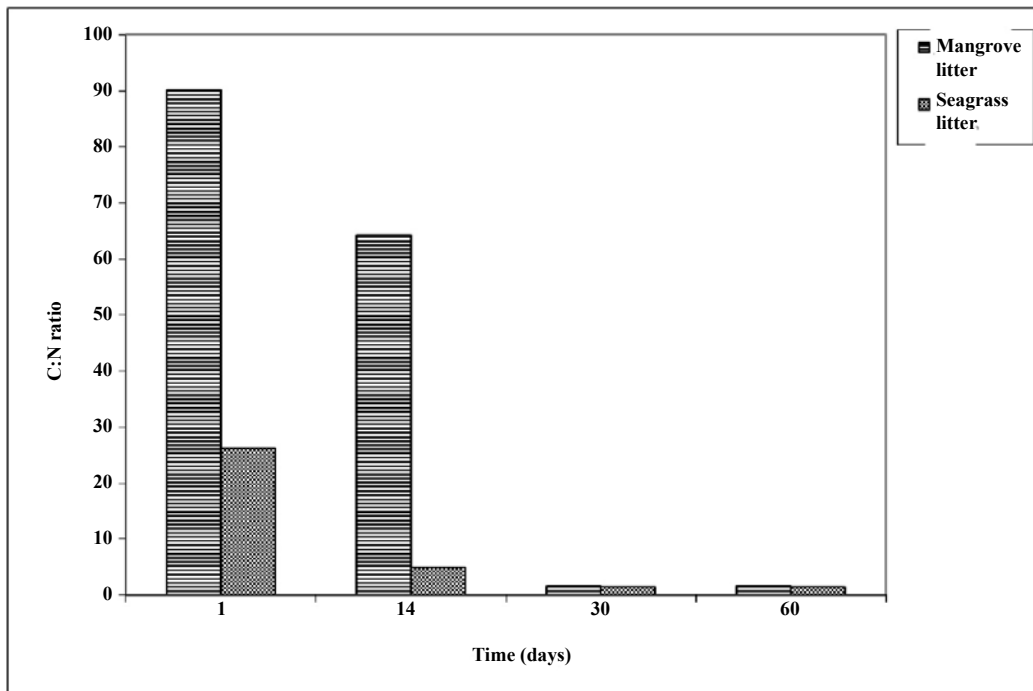


Figure 4. Variation in the C:N ratio over time (days) in the mangrove and seagrass leaf litter.

The re-colonisation rate on day one was higher in the organically enriched treatments (maximum  $307 \pm 160$  individuals  $7 \text{ cm}^{-2}$ ) compared to the organic-free control ( $128 \pm 75$

individuals  $7 \text{ cm}^{-2}$ ), the highest recolonisation being in mangrove leaf litter ( $307 \pm 160$  individuals  $7 \text{ cm}^{-2}$ ), which was nevertheless much less than the densities in the field

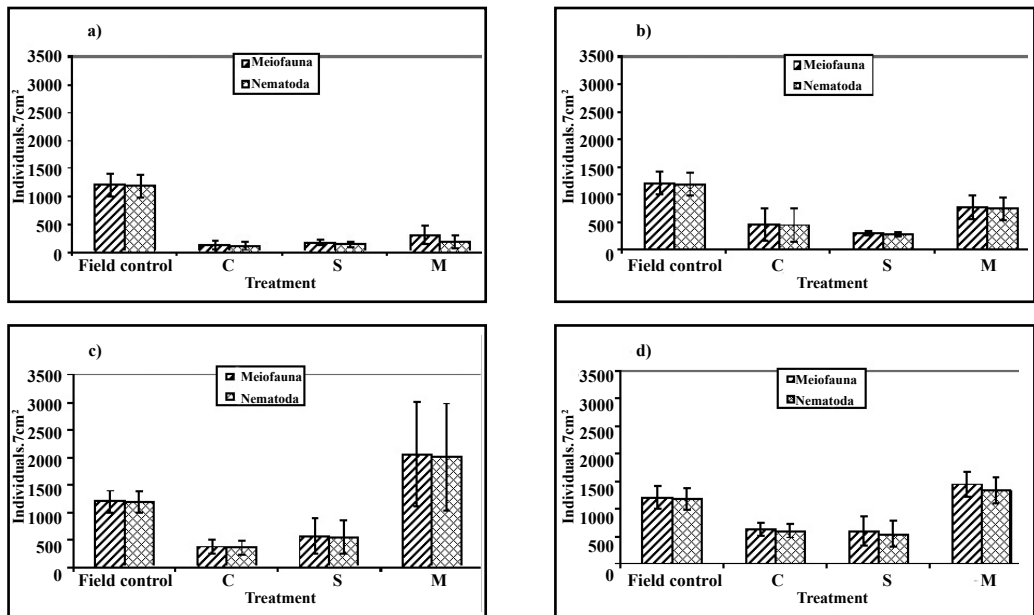


Figure 5. Densities of meiofauna and nematodes (mean  $\pm$  SD,  $n = 3$ ) on a) day 1, b) day 14, c) day 30 and d) day 60 in the different treatments. FC = field controls, C = experimental controls, S = seagrass leaf litter and M = mangrove leaf litter.

between the leaf litter sources. A Tukey HSD test revealed no significant differences between the litter sources on day one (Fig. 5a) but, on days 14, 30 and 60 (Figs. 5b, 5c & 5d), the mangrove leaf litter treatment yielded significantly higher meiofaunal and nematode densities than the seagrass leaf litter and the controls ( $p < 0.05$ ).

## DISCUSSION

As outlined in the introduction, there is a paucity of information on the influence of different detrital sources on meiofaunal abundance and community composition in mangrove benthic ecosystems and no literature was found on field experiments examining the meiofaunal re-colonisation of such detritus. This field experiment was thus the first to investigate the influence of different sources of detritus on meiofauna within mangrove ecosystems. The results show that meiofaunal re-colonisation of mangrove sediments is affected by the availability of leaf litter since much higher densities were attained in its presence compared to the experimental controls. Additionally, meiofaunal re-colonisation within the Gazi mangrove was affected by the source of the leaf litter since mangrove leaves yielded higher re-colonisation than seagrass leaves. Indeed, the meiofaunal densities became almost constant after day 14 within the control and seagrass leaf litter treatments.

The fact that meiofauna re-colonised the experimental controls devoid of organic matter one day post-placement indicates that meiofauna will occupy any available space, even in the absence of a food source. However, meiofaunal densities in the experimental controls remained low throughout the experiment.

The meiofaunal densities in the experimental treatments increased with time, which is possibly related to the decomposition process and the associated microflora. This implies that decomposition enhanced the nutritional value of the mangrove detritus, thereby attracting more meiofauna, especially nematodes. Macrophyte decomposition and detritus recycling have been shown to be important in mangrove ecosystems

(Lugo & Snedaker, 1974; Lee, 1995) and contribute much of the nutrition needed by grazers and filter feeders, while providing habitat for benthic fauna.

Although meiofaunal colonisation was observed one day after commencement of the experiment, the rate of colonisation of mangrove leaf litter was initially low, showing that there was a time lag before meiofauna could colonise the mangrove leaf litter and attain densities similar to the field controls. Alongi (1987) showed that the concentration of polyphenolic acids in mangrove leaf litter, mainly tannins, is negatively correlated with the associated meiofauna densities as these substances reduce the palatability of mangrove detritus. According to Zucker (1983) and Robbins *et al.* (1987), hydrolysable tannins impart a noxious taste to detritus, increasing the acidity of the plant material, and precipitating the plant proteins and the gastrointestinal enzymes of the meiofauna. Thus, these substances interfere with the feeding of benthic herbivores and detritivores. However, the tannins appear to be rapidly lost during the initial days of mangrove litter decay (Robertson, 1988; Tietjen & Alongi, 1990). Similarly, Gee and Sommerfield (1997) showed that meiofaunal proliferation may be affected and controlled by changes in leaf litter chemistry during its decomposition, influencing the subsequent successional development of the microbial community. Thus, while tannins may have been responsible for the slow initial colonisation of the leaf litter in the present study, their disappearance may have increased the palatability of the detritus, promoting meiofaunal colonisation, particularly by nematodes. This increased attractiveness is supported by the decrease in the C:N ratio in the mangrove leaf litter, implying that the nutritional value of the detritus improved as the nitrogen content increased (Skov & Hartnoll, 2002).

Nematodes were preponderant in the meiofaunal community that colonised the experimental sediment and leaf litter. According to Riemann and Helmke (2002), nematodes are believed to release hydrolytic enzymes in mucus which, together with bacterial enzymes,

assist in the breakdown of the detritus. The hydrolysed products can be directly consumed by the nematodes, garnering nutrition from the detritus. This, together with a decrease in tannin concentration and the stimulation of microbial growth probably accounts for the observed increase in meiofaunal and nematode densities within the experiment, most notably in the mangrove leaf litter.

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