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ORIGINAL ARTICLE

Mangrove trees survive partial sediment burial by developing new roots and adapting their root, branch and stem anatomy

Judith Auma Okello[1,2,4] · **James Gitundu Kairo**[1] · **Farid Dahdouh-Guebas**[2,4] · **Hans Beeckman**[3] · **Nico Koedam**[2]

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Abstract

Key message **Large sedimentation events resulting in partial burial may negatively affect mangrove trees' growth and survival. However, mangroves can adapt to respond dynamically within months to sediment burial, attributes which confer resilience.**

Abstract Mangrove forests are generally sites of sediment deposition at the coast facilitated by the unique root structure of the trees, thus serving to help the system keep pace with rising sea level. However, at high levels it can cause anoxia in sediments and consequently tree mortality. This study evaluates the morphological and anatomical response of bark and roots of three mangrove tree species (*Avicennia marina*, *Ceriops tagal* and *Rhizophora mucronata*), commonly found along the Kenya coast and around the Indian Ocean, to partial burial by sediment. This was done through simulation of natural and rapid sedimentation in an experiment involving 15, 30 and 45 cm burial levels. Partial sediment burial resulted in an increase in root density which also aided survival particularly in *C. tagal*, where 5 of the 17 buried trees that did not form new roots in the 45 cm treatment died. Air lacunae in the root cortex became larger in pneumatophores following burial, while the ray parenchyma and cylinder of secondary xylem showed increased widths in cable roots of *C. tagal*. There was also an induction of the phellogen which produced more outer tissue in the buried section of stems in all three studied species (two fold increase in *A. marina* and *C. tagal* and fourfold in *R. mucronata*). The results suggest that the observed morpho-anatomical adaptations could lead to enhanced performance or recovery of biological processes in the burial-affected trees.

Keywords Mangrove roots · Adaptation · Anatomy · Sedimentation · Bark

Introduction

The mangrove environment is highly variable owing to a combination of periodic fluctuations and extremes in physicochemical variables caused by the tides and coastal currents (Tomlinson 1994). As a result, mangrove tree species have developed various adaptive mechanisms to deal with the demands of the mangrove habitat (Krauss et al. 2008). Moreover, the possession of adaptive features has been attributed to the fidelity of these plants to their environment (Saenger 2002). One of the important forms of morphological adaptation is the complex root networks for anchorage and facilitation of gaseous exchange in the hypoxic mangrove environment (Kathiresan and Bingham 2001). The process of air storage and transport is facilitated by the presence of aerial roots, a feature highly characteristic of more specialised mangroves in which some part of the root system remains exposed to the atmosphere at least at low tide (Tomlinson 1994). Mangrove roots are characterised

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✉ Judith Auma Okello
Judith_okello2003@yahoo.com

[1] Kenya Marine and Fisheries Research Institute (KMFRI), Mombasa, Kenya

[2] Laboratory of Plant Biology and Nature Management (APNA), Vrije Universiteit Brussel, 1050 Brussels, Belgium

[3] Laboratory of Wood Biology and Xylarium, Royal Museum for Central Africa (RMCA), 3080 Tervuren, Belgium

[4] Laboratory of Systems Ecology and Resource Management, Université libre de Bruxelles, 1050 Brussels, Belgium

by extensive coverage of lenticels (Blasco et al. 1996) and a spongy cortex made up of large aerenchyma lacunae (Metcalfe and Chalk 1957; Pi et al. 2009) which enhances efficient internal oxygen transfer (Colmer 2003; Jackson and Armstrong 1999). Since low oxygen concentrations can impede root growth (Armstrong and Drew 2002; Crawford 1992; Mckee 1996; Drew 1997), such adaptations that aid in root aeration can be critical for plant survival (Colmer and Voesenek 2009).

Aerial roots may take five major forms which are characteristic of specific mangrove tree species or genera (Tomlinson 1994): (1) stilt roots are the most conspicuous forms of the aerial roots, characterised by branched loops originating from the tree trunk, typical of the genus *Rhizophora*; (2) knee roots, which form pronounced loops appearing as a blunt, knoblike structure raised from the soil surface are found in *Ceriops* spp. and *Bruguiera* spp.; (3) pneumatophores of *Avicennia* spp. show negative geotropism with pencil-like structures protruding from the ground rarely exceeding 20 cm in height; (4) peg roots in *Sonneratia* spp. are similar to the pneumatophores in *Avicennia* spp. but much stouter and may become knob or mushroom like in some cases; and (5) plank roots in *Xylocarpus* spp. which are vertically wavy and plank-like. The aerial roots (apart from those of *Rhizophora* spp.) are attached to cable roots which run laterally below the soil surface.

Apart from their role in aeration and providing anchorage to the trees, mangrove roots also provide a mechanism for trapping sediment which may be introduced to coastal areas through river discharge, dumping of dredged material and floods (Kathiresan 2003; Wolanski 1995). Mangrove trees create a turbulent zone, maintaining the sediments in suspension and letting them be deposited just before slack tide. The aerial roots also ensure that the deposited sediments are not re-suspended during ebb by creating an opposite force to the ebbing tides (Alongi 2009). As a result, fine particles are not just passively imported into the mangroves, but the trees structurally capture silt, clay, and organic matter (Alongi 2009; Furukawa and Wolanski 1996; Kitheka et al. 2003) contributing to vertical sediment accretion (Alongi 2009; Kimeli 2013; Ouyang et al. 2017). In addition, mangrove root detritus makes a significant contribution to sediment carbon within the profile (Lovelock et al. 2014).

Through the process of accretion, mangrove trees stabilise the mud flats and create new mud banks, facilitating further colonisation by this plant community (Alongi et al. 2004; Furukawa and Wolanski 1996). Long-term accumulation of sediment through accretion and subsurface accumulation of refractory mangrove roots results in raising the elevation of the soil surface, and hence may help mangroves adjust to sea level rise (McKee et al. 2007; Smoak et al. 2013). Coupled to sediment accretion, mangroves also help in filtration and retention of sediments and adsorbed nutrients from terrestrial areas (Tam and Wong 1994), thereby protecting the offshore ecosystems (i.e. seagrass beds and corals) against siltation and eutrophication (Ewel et al. 1998; Fabricius 2005). However, with increasing demand for wood and wood products and cropland uplands, there has been rampant clearance of vegetation coverage including forests, leaving the land bare and prone to soil erosion. This has increased riverine delivery of sediment from bare land and agricultural fields to the coastal areas with a potential of causing enhanced rates of sedimentation in mangrove habitats (Alongi et al. 2004; Mohamed 2008). Coupled with the likelihood of increased mean annual rainfall, punctuated with flash floods in East Africa (Christensen et al. 2007; IPCC 2014), the situation may worsen. Increased sediment inputs into the coastal areas consequently pose a major threat to mangrove forests and adjacent ecosystems (Gray 1997).

Although mangrove root structures are an important agent in the accretion process, they may also be vulnerable to extreme sedimentation events which may result in burial. A number of studies have attempted to document belowground development of mangroves under normal accretion conditions (Pi et al. 2009; Purnobasuki and Suzuki 2005; Young and Harvey 1996), following an oil spill (Snedaker et al. 1981) and under arid conditions (Saifullah et al. 2004), but the effects of increased sedimentation on mangrove root development are not fully understood. Ellison (1998) reviewed cases of sediment burial of roots of different mangrove tree species, and noted that in cases where accretion rates do not go beyond 10 mm year^{-1}, these trees show some degree of tolerance. However, burial events within ranges of 10–70 cm show large variation in responses by tree species and by location (Ellison 1998). This study therefore evaluated the response of mangrove trees to experimental sediment burial mimicking large sedimentation events and their probable contribution to survival and/or mortality in the affected trees. The study assessed two hypotheses: (1) increased sedimentation leads to mangrove mortality through interference with the functioning and development of mangrove roots and the buried stem section; and (2) after sediment burial, the mangrove tree root system has to regain a similar amount of aerial roots to guarantee sufficient oxygen uptake. Specifically, the study focused on describing: (1) morphologic change in aerial roots, (2) root anatomy and (3) bark development, in experimentally partially buried *Avicennia marina*, *Ceriops tagal* and *Rhizophora mucronata* mangrove trees in their natural setting.

Materials and methods

Study site and experimental design

The study was based on a field experiment set up in plantations of *C. tagal*, *R. mucronata* and *A. marina* planted in 1994, 1998 and 2001, respectively, at Gazi Bay, Kenya (4º22′S, 39º30′E), shown in Fig. 1. The three species selected for this study make up the dominant formation of mangroves in Gazi Bay (Matthijs et al. 1999) and had been replanted in sites where they occurred before clearing following the appropriate respective inundation classes. The experimental setups for *C. tagal* was established in December 2010, *A. marina* in April 2011, while *R. mucronata* was put up in October 2011.

Squared plots of 2 m by 2 m were selected within the plantations, surrounded with a netting material of 0.5 mm mesh size and filled with terrestrial sediment to 15, 30 and 45 cm levels as described in Okello et al. (2014). In the controls, no sediment was added but the plots were surrounded with the same netting material and restrained to the ground using pegs as in the treatments. A total of four plots were established for each of the tree species apart from *C. tagal* for which eight plots were made (four closer to the forest edge and the others more to the interior). This is because as compared to the other two species, *C. tagal* had been planted over a large area with varying inundation frequency. For the purpose of this study, the *C. tagal* plots closer to the plantation edge are termed landward (Fig. 1). The total number of trees in each of the plots and their structural attributes are given in Supplementary Table SI.

Field sampling

Field surveys

Survival All branches that were already dead (completely dry) at the beginning of the experiment were removed from all the trees within each of the plots and the total number of living branches noted. The trees were then assessed for signs of wilting (by observing drooping leaves) on a monthly basis and any new dry branch noted (denoted ‘branch mortality’).

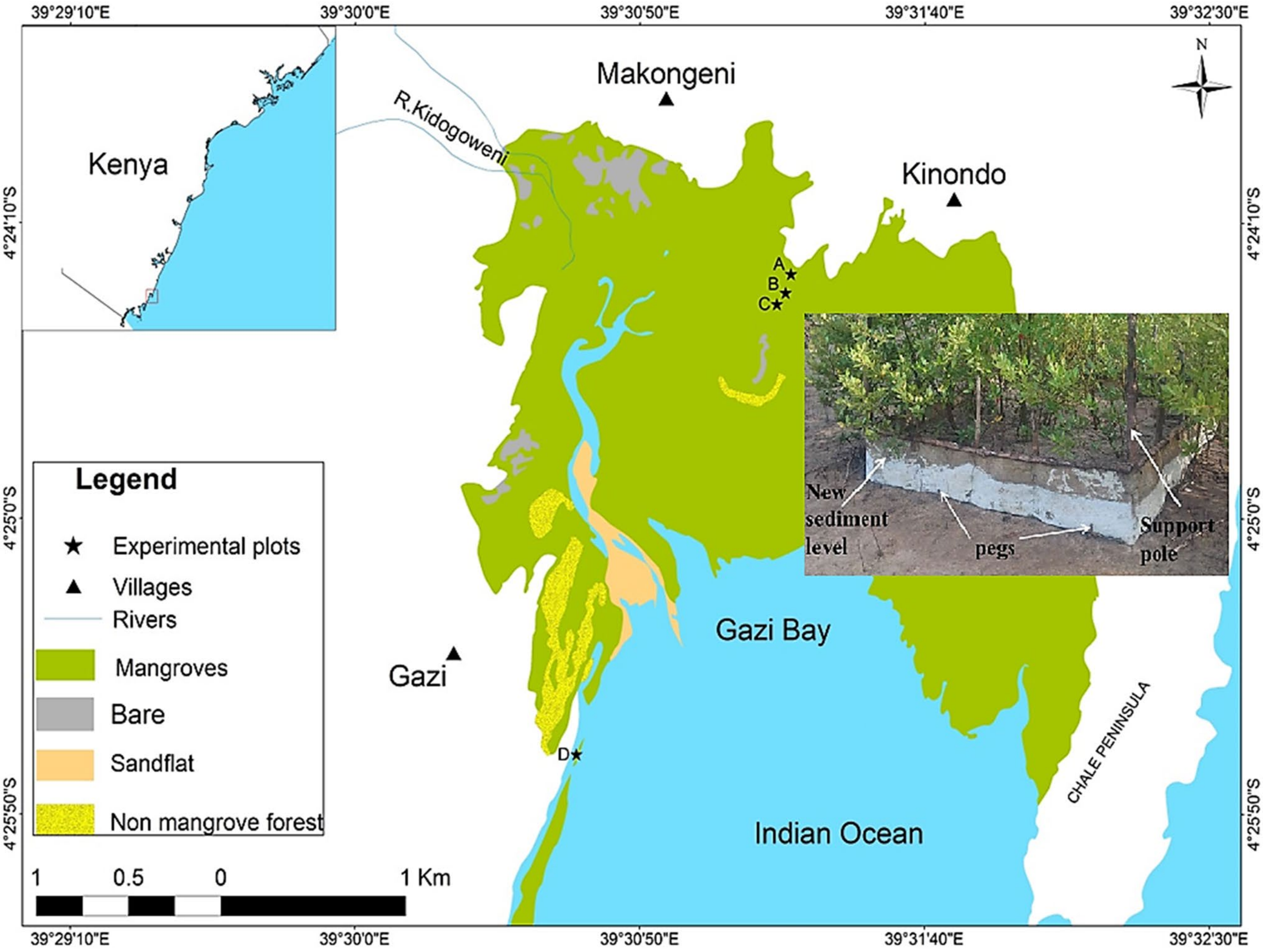


Fig. 1 Map of Gazi Bay locating the different study sites: A: *A. marina*, B: *C. tagal* landward, C: *C. tagal* seaward and D: *R. mucronata*. The inset shows where the study area is located within Kenya (Kenya Marine and Fisheries Research Institute database), including a picture of one of the experimental sedimentation *A. marina* plots in the study site

Trees that remained standing but died were also noted and are described in the results as 'whole tree mortality'.

Root development Enclosures in *A. marina* were subdivided into four, 1 m by 1 m quadrats, using short (20 cm) pegs and assigned roman numbers i, ii, iii and iv. Monitoring of pneumatophore was only done in two diagonal quadrats (ii and iii) while standing on an adjacent one, thus (and generally) avoiding trampling the pneumatophores within the sampling quadrats. All pneumatophores (where present after burial) were counted at the beginning of the experiment and the process repeated monthly over a period of 1 year. In each consecutive sampling campaign, all the roots were counted and cumulative increase in the number of pneumatophores recorded (Table S1).

All aerial roots of *C. tagal* were buried by sediment at the beginning of the experiment (Table S1). Subsequent sampling was done when the first aerial roots were observed above the sediment (after 20 months). Assessment of development was based on ease of visibility of the knee roots appearing above the new sediment level and reported as (1) not visible: no roots seen, (2) slightly visible: just appearing from the tree trunk and only seen through slight removal of some sediment, and (3) visible: seen clearly protruding above the sediment.

Stilt roots in *R. mucronata* that remained exposed after burial at the onset of the experiment were counted per tree and grouped as hanging and fixed at that time (hanging roots usually becoming fixed subsequently). New roots and dead ones were assessed and grouped accordingly during the subsequent monthly sampling. These were done in all the trees within the plots.

After 2 years of partial sediment burial, two living trees of each of the three species, one from the 45 cm and the other from controls of each of the three studied species, were excavated. A slit was made on the side of the net close to the tree to be excavated in the burial treatment plots, using a pair of scissors. The sediment was then carefully removed around the tree using sharp pegs while ensuring that the root structures were maintained. The structural development of the aerial roots was observed in the sections that were once exposed to the atmosphere and buried after the treatment, and any anomaly with respect to controls recorded. The sediment was also dug deeper using a hoe to access the original cable roots that were present before sedimentation.

Roots and stem sampling for anatomical analysis

Small pieces (5–8 cm) of the different sections of the aerial and belowground roots, both the original (present before sedimentation) and new (developed after burial), were then sampled from the partially buried trees. Cable roots (in *A. marina* and *C. tagal*) and stilt roots (in *R. mucronata*) were cut from two points, 5 cm away from the trunk and mid distance from the root tip to the trunk (Fig. 2a). Two to three samples of the different root types were collected from the aforementioned sections giving a total of 21. Pneumatophores in *A. marina* were sampled from both new cable roots and original cables, as well as above and below the sediment level. Two to three samples in each case were collected giving a total of 32. These were cut 4 cm from the tip of the exposed section and 4 cm below the sediment level. Similar samples were also collected from the controls for comparison. The samples were cleaned with water and stored in a mixture of 70% ethanol (CH_3CH_2OH) with a few drops of glycerol ($C_3H_8O_3$) for anatomical analysis.

The trunk below the new sediment level was also assessed for any observable changes between the periods before and after burial. All the trees from which root samples were collected (including the controls) were then cut down, and an 8 cm piece of the main stem was cut 5 cm below and above the sedimentation level (Fig. 2a). Three 0.5 cm^3 pieces were then cut as shown in Fig. 2b and immediately stored in an alcohol–glycerol mixture. The remaining piece of about 3–5 cm long was air dried.

Root and bark anatomy

Sample preparation

The alcohol–glycerol preserved root samples were cut into 8–10 mm long segments. In the case of large stilt/cable roots, the samples were further cut radially from the centre for ease of handling. These were then soaked in polyethylene glycol (PEG) with molecular weight 1500 (Pure, VWR International, Prolabo) in the oven at 60 °C for 24 h. The soaked samples were then embedded in fresh PEG 1500 for another 24 h at room temperature to enhance the cells' integrity and avoid tearing of sample during sectioning (Wolosewick 1980). Transverse sections of 25–30 µm thickness were made from the PEG-embedded samples using a microtome (Lab-Microtome, Holger Gätner) and stained using safranin–alcian blue mixture. The staining solution was prepared by dissolving 0.35 g safranin in 35 ml 50% alcohol and mixed with 0.65 g alcian blue dissolved in 65 ml distilled water. Sections were then mounted on glass slides using Canada balsam (Merck). The 0.5 cm^3 alcohol–glycerol mixture-preserved stem cubes were also impregnated with PEG to prevent the bark from peeling away during sectioning. Sectioning, staining and mounting were done following similar steps described above for the roots.

The air-dried pieces of stem were sanded using a series of sandpaper from 50 to 1200 grit to smoothen the transverse sections to improve the visibility of the tissues (Fig. 2b). The wood samples were then further cut using a hand saw into discs of about 1.5 cm thickness.

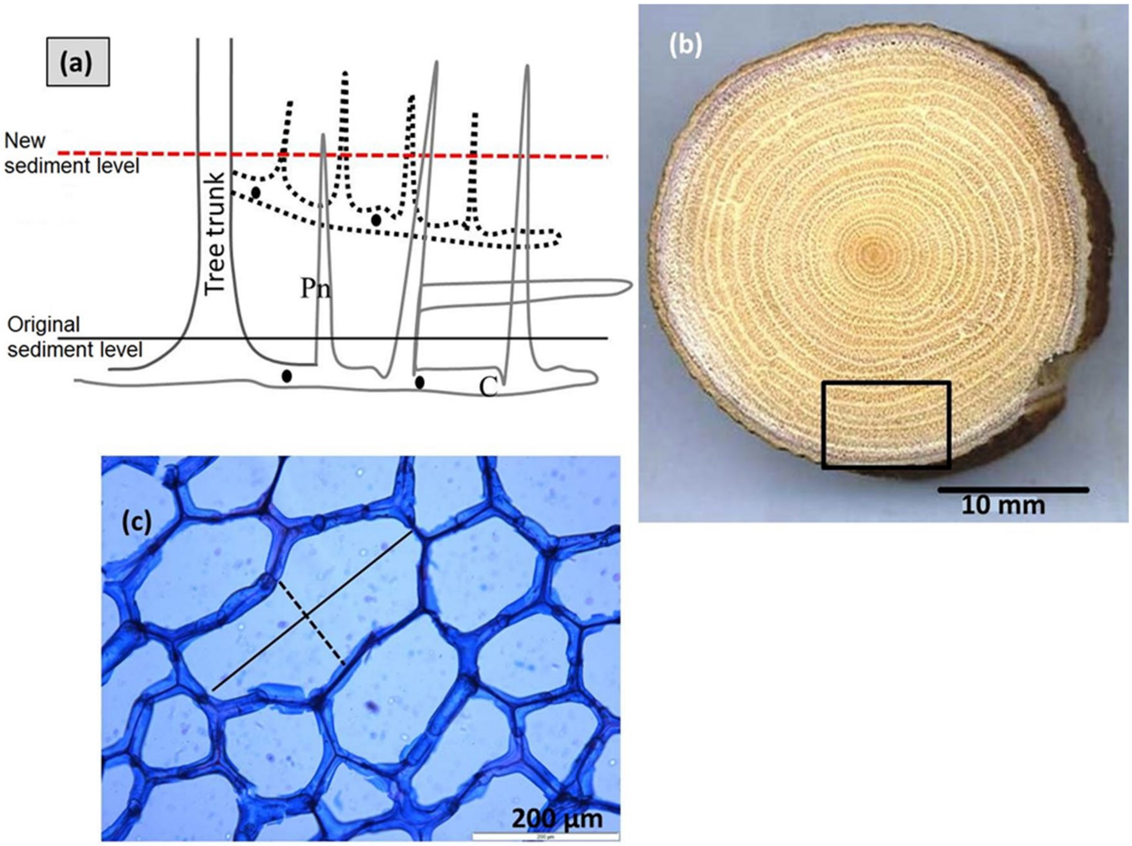


Fig. 2 **a** A diagrammatic representation of the pneumatophores in *A. marina* after 2 years of partial sediment burial (C = cable root, Pn = pneumatophores from the original roots, while the dotted lines are the new roots). Sampling of different root parts was done at the points indicated by black dots. **b** Piece of stem disc of *A. marina* showing the position of sawing for sectioning. **c** Cortex in one of the *A. marina* pneumatophore sampled below the sediment. Continuous line and dotted lines are the first and second measurements of an air lacuna, respectively

Anatomical observations and measurements

Observations of the stem discs were made under a stereo microscope (Olympus SZ) and measurements of the cork tissue done using ImageJ 1.45s (Wayne Rasband, National Institute of Health, USA). The micro-sections of the bark were viewed under a microscope (Olympus BX60) fitted with a camera (Olympus UC30) and any anatomical variations between the discs cut below and above sediment noted. The root sections were also viewed under the microscope and measurements done using basic image-acquisition and archiving software CellB (Olympus Cell^B Soft Imaging System GmbH, Münster, Germany). Air lacunae 'diameter' was obtained by averaging the longest distance across the aperture of the intercellular spaces and the mid perpendicular length across the first measurement (Fig. 2c). Additionally, the width of the cortex and the cylinder of the secondary xylem were also measured from which a ratio was obtained to determine their relative sizes.

In *R. mucronata* roots, the barks of the aerial roots were assessed for any observable differences in thickness and the widths of ray parenchyma were measured at four random points along each set of rays under four different microscopic fields of view. All the microscopic measurements were done in four fields of views of about 0.385 mm^2 (objective × 10) or 2.387 mm^2 (objective × 4).

Analysis

Data analysis was done in Excel (Microsoft office 2010) and STATISTICA 7.0 (StatSoft Inc., Tulsa, USA). Data sets were tested for normality and homogeneity of variances and, where conditions were met, a parametric test (one-way ANOVA) was used to test for differences across treatments; otherwise, a non-parametric test (Kruskal–Wallis ANOVA by ranks) was applied. This was with exception to *C. tagal* where factorial ANOVA was used to test differences between landward and seaward plots as well as across treatments. Mann–Whitney *U* test was used to test for differences between the bark thicknesses of the stem below and above the sediment. The test was also used for determining the difference in ray parenchyma thickness between new roots in silted and controls of *R. mucronata*. Spearman rank correlation was then used to determine the relationship between root visibility and branch mortality in *C. tagal*. Since the difference between cortex cylinder to secondary xylem ratio in cable roots from sections close to the trunk (5 cm) and away from the trunk was not significant, the two sets of data were pooled in a subsequent analysis.

Results

Mortality

Branch mortality with respect to partial burial varied among species. In *A. marina*, the highest mortality of branches was in the lowest sedimentation treatment and the controls, while the lowest was in trees under sediment burial 30 and 45 cm (Fig. 3a). The highest percentage reduction (8.5%) in branch mortality relative to the controls was observed in the 30 cm buried trees. In *C. tagal*, branch mortality increased with increase in sedimentation level in both the seaward plots and the landward plots. There was, however, no significant difference between the two sets of plots ($F = 1.025$. $P > 0.05$) despite the visual differences, indicating relatively higher branch mortality in the seaward than the landward treatments (Fig. 3b). Highly significant differences were, however, noted across treatments ($F = 11.57$. $P < 0.001$) with post hoc Tukey HSD test revealing significantly higher branch mortality in the 45 cm partially buried trees than the controls and all the other sedimentation levels (Fig. 3b).

Whole tree mortality was only observed in *C. tagal* trees under the 45 cm sedimentation level. In the seaward plot, two out of the eight buried trees died, while three out of the ten died in the landward plot. The first death was observed in the landward plot 3 months into the experiment, while the first seaward tree died after 5 months. It was also noted that all the *C. tagal* trees that died had not developed new roots, but instead the stem section below the new sediment level rotted away. Moreover, a highly significant negative correlation was established between root visibility in this species and percentage branch mortality ($rs = -0.58$, $P < 0.001$). In *R. mucronata*, exceptionally high branch mortality was only observed in the 45 cm sedimentation plot and in only one of the partially buried trees (36.8% of the total branch count). The rest of the *R. mucronata* trees in all the treatments had very low branch mortality ranging between 0 and 3.0% of the total branch count.

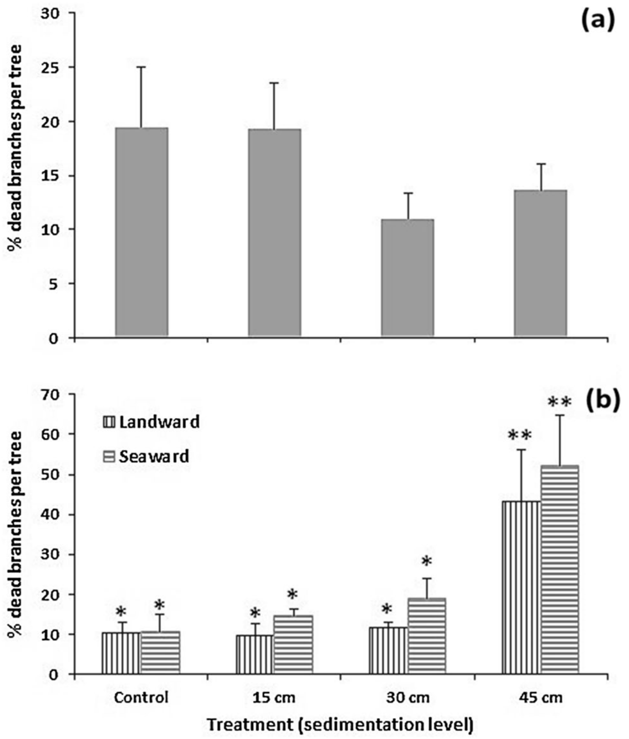


Fig. 3 Branch mortality in mangrove trees: **a** *Avicennia marina* and **b** *Ceriops tagal*, under partial sediment burial of different levels. The bars marked with two asterisks are significantly different from the others (Tukey's HSD, $P = 0.05$)

Root morphology

Whereas the trees in the controls did not show large variation in the number of aerial roots over the experimental period (Fig. 4a, b), the partially buried trees produced new roots which could be observed above the new sediment level after different periods of time depending on the sedimentation level and the species (Fig. 4).

A. marina formed new cable roots that emerged from the stem about 2–5 cm below the new sediment level. From this cable root, pneumatophores developed which could be seen from above the sediment, while the pneumatophores from the original cable root also grew longer to reach the new sediment surface thus contributing to the counts recorded (Fig. 4a). Branching of pneumatophore was more frequent in the partially buried trees as compared to the control. This branching was observed in the pneumatophores from the original cable root, which also became fluffy in nature and developed numerous feeding roots. Partially buried *C. tagal* trees formed new knee roots which could be observed close to the trunk above the sediment (Fig S2). New cable roots also developed about 2 cm below the new sediment level and these too had several feeding roots. The cable roots, however, appeared to be positively geotropic (growing

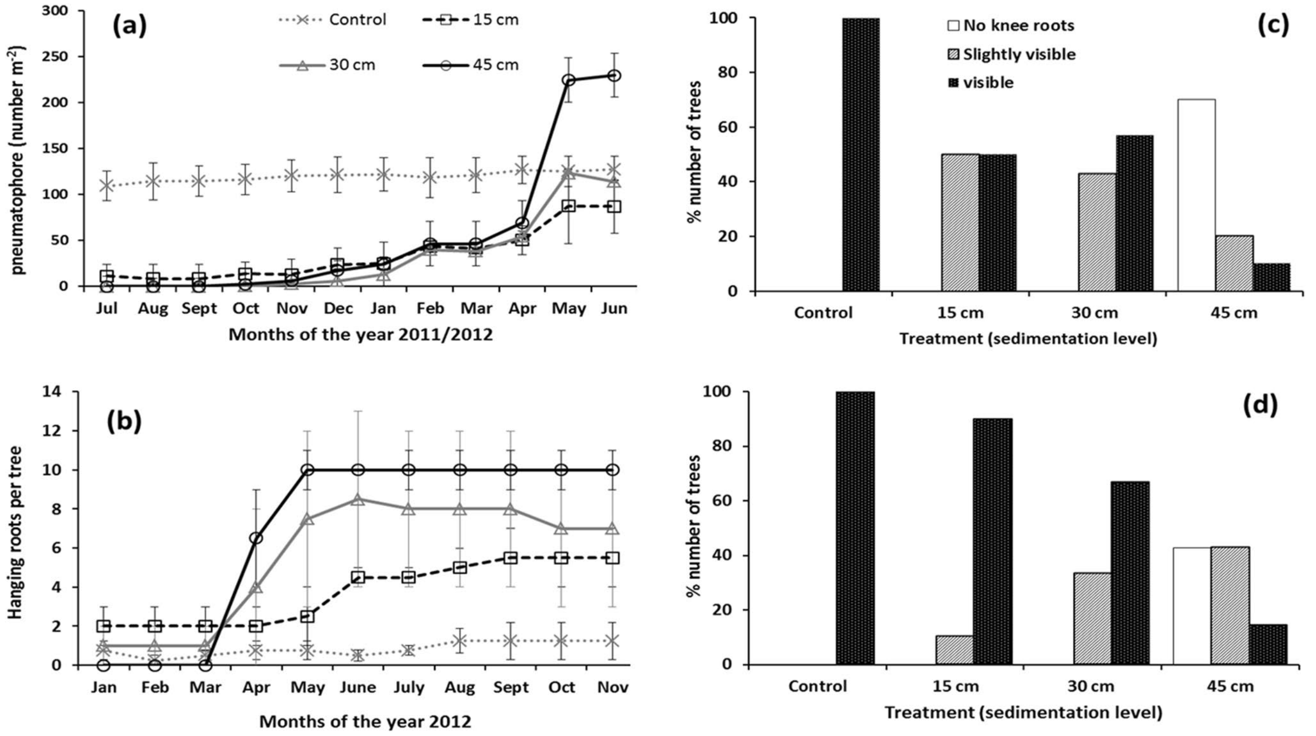


Fig. 4 Mean ± SE; **a** pneumatophore density per m^2 in partially buried *A. marina* trees, **b** hanging stilt roots per tree in partially buried *R. mucronata* during 1 year of burial; root visibility in *C. tagal* after 20 months of exposure to sedimentation in **c** landward and **d** seaward plots (the numbers of trees per plot are as in Table SI)

downward), unlike the original cable roots and those in the control which lay parallel to the ground surface. In *R. mucronata*, new stilt roots were formed above the new sediment level at higher rates than in control trees (Fig. 4b). Below the new sediment level were also new roots with numerous feeding roots, while the original stilt roots produced hypertrophied lenticels on the portion that was covered below the sediment. The originally exposed stilt roots also developed root branches which were equally covered with feeding roots below the sediment.

The first new aerial roots appearing above the sediment in the highest sedimentation level in *A. marina* were observed after 4 months of burial (Fig. 4a), while in *R. mucronata* trees, the first observations of new prop roots were made after 5 months (Fig. 4b). This was then followed by a further increase in densities with time, the peaks levelling out after 11 months in *A. marina* and 6–7 months in *R. mucronata* (Fig. 4a, b). The rate of development of the new aerial roots was also higher with increase in the level of partial burial in both *A. marina* and *R. mucronata*. New roots in *C. tagal* were first seen in the 45 cm seaward plot after 9 months of burial, while the others appeared much later in the experiment (Table 1). After 20 months into the experimental period, the visibility above the sediment of the new roots in the trees was as shown in Fig. 4c, d.

Root anatomical structure

In all the three studied species, the original roots in the partially buried trees remained alive despite the formation of new ones. Table 2 give a pictorial summary of the anatomical changes observed in the roots of the different studied species. The basic anatomical features of the new roots formed as a result of the sedimentation were similar to their respective originals and controls. However, variations were observed in air lacunae sizes, ray parenchyma widths as well as cortex cylinder of secondary xylem ratio following partial burial treatment.

Table 3 shows the variations observed in pneumatophores upon subjecting *A. marina* trees to partial burial.

Table 1 The time of first observations of knee roots above the sediment in partially buried *Ceriops tagal* trees

Treatment	Period after burial	
(sedimentation level)	Landward	Seaward
Control	Roots not buried	Roots not buried
15 cm	11 months	10 months
30 cm	10 months	11 months
45 cm	12 months	9 months

Significantly larger air spaces were noted in the cortex of pneumatophores from the original cable roots and the new ones as compared to those of the controls ($F = 78.86$, $P < 0.001$). Tukey HSD post hoc test further revealed significantly larger air lacunae in cortex of the buried section of the original pneumatophores, as compared to the exposed original root section ($P < 0.001$) as well as the newly formed pneumatophores ($P < 0.05$). Sedimentation also resulted in significantly larger intercellular air spaces in the pneumatophores piths (though not always present) as shown in Table 3 ($F = 96.73$, $P < 0.001$). The cylinder of secondary xylem region was significantly larger in both the new and original pneumatophores above the sediment in the partially buried trees as compared to the controls ($H = 29.03$, $P < 0.001$; Table 2). Newly formed cable roots in *A. marina* trees, as a result of partial burial, were similar to those from the controls. The roots had internal phloem within the cylinder of secondary xylem [typical of this species (Robert et al. 2011)].

In partially buried *C. tagal* trees, the original cable root formed a larger cylinder of secondary xylem as compared to the cortex (Table 3). The new cable roots had larger cortex/cylinder of secondary xylem ratio similar to observations made in the controls. Further, the cortex/cylinder of secondary xylem ratio for the controls was significantly higher than in the original roots ($H = 28.95$, $P < 0.001$), but were not significantly different from the newly formed roots ($P > 0.05$).

In *R. mucronata*, a difference between the newly developed prop roots and the controls was observed in the xylem region and the pith. New roots formed below the sediment had smaller-sized air lacunae in the pith and cortex than in the controls. The new roots also had narrower bands of ray parenchyma as compared to those of the controls ($U = 106$, $P < 0.001$). The mean diameter of the ray parenchyma in the control roots was 176 ± 1.52 µm, while that in the newly formed prop roots of buried trees was 81.55 ± 5.95 µm.

Table 3 Mean (± SE) diameter of the air lacunae and cortex cylinder of secondary xylem ratio in the pneumatophores of silted *A. marina* trees after 2 years of exposure to partial burial

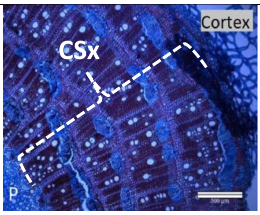
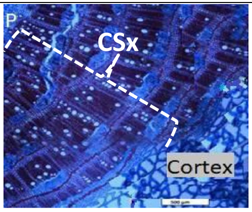
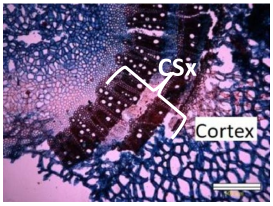
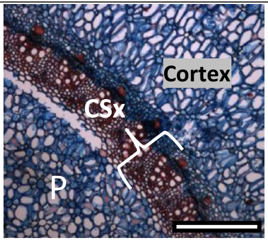
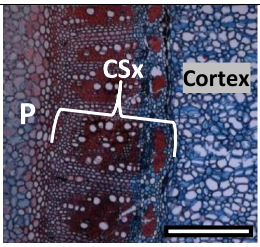
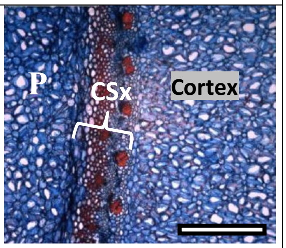
Pneumatophores	Air lacunae diameter[c] (µm)		Mean ± SE cortex-cylinder of secondary xylem ratio
	Cortex	Pith	
New[a]			
Below sediment	70.0 ± 2.4	35.9 ± 1.3	4.6 ± 0.1
Above sediment	57.9 ± 3.6	Absent	1.1 ± 0.1
Original[b]			
Below sediment	133.3 ± 8.4	61.5 ± 2.4	3.1 ± 0.2
Above sediment	55.1 ± 1.7	Absent	1.0 ± 0.1
Control			
Below sediment	62.8 ± 1.6	27.8 ± 4.8	3.8 ± 0.2
Above sediment	48.9 ± 2.2	Absent	3.4 ± 0.1

[a] Pneumatophores emerging from cable roots formed after partial burial

[b] Pneumatophores from the original cable root in partially buried trees

[c] Obtained from mean of shortest and longest aperture length

Table 2 Images of the cable roots of the *A. marina* and *C. tagal* (scale bar = 500 µm)

Species	Control	Original	New root
A. marina	 (1.6 ± 0.1) µm	 (1.5 ± 0.3) µm	 (4.5 ± 0.2) µm
C. tagal	 (15.0 ± 0.3) µm	 (8.2 ± 0.2) µm	 (28.1 ± 0.6) µm

Values in parenthesis are mean (± SE) cortex cylinder of secondary xylem ratio. P = pith, CSx = cylinder of secondary xylem

The anatomical structure of the bark in stem and root

The partially buried trees in all the three species developed a substantial amount of bark tissue on the stems below the new sediment level (Table 4).

The difference between the bark thickness of stem below and above the new sediment level was highly significant in all the three studied species ($P < 0.001$), while this was not the case for the stems from control trees ($P > 0.05$). The periderm layers on stem portions below the new sediment level were on average twofold thicker in *A. marina* and *C. tagal* and fourfold in *R. mucronata* (Table 4).

The buried stems developed both an extended phellem (cork) and phelloderm with more spongy areas in the buried stems of all the species, resulting in increased bark thickness. The buried section of the original stilt roots of *R. mucronata* developed more layers of periderm, resulting in the formation of a rhytidome (Fig S4). In addition, *A. marina* and *R. mucronata* formed hypertrophied lenticels on the bark below the sediment, while *C. tagal* developed a thick powdery substance that covered the bark. The powdery substance which was not identified was not present on stems of trees in the control even below the sediment.

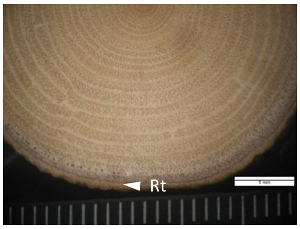
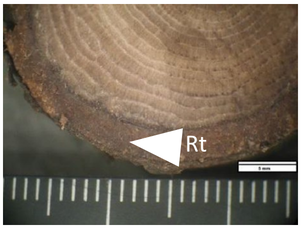
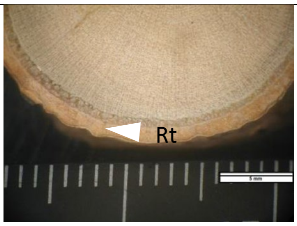
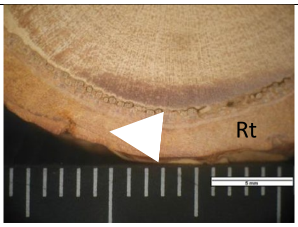
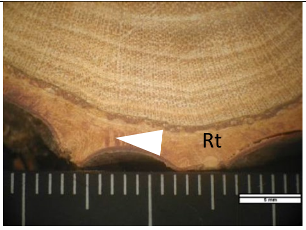
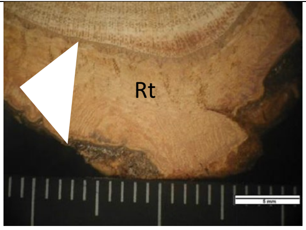
Discussion

Mangrove species can withstand considerable levels of sediment burial, much higher than indicated by Ellison (1998). Experimental sedimentation triggered a series of morphological and anatomical reactions in roots and bark (including stilt roots) of buried mangrove trees, features that are likely to enhance survival, hence facilitating resilience. Similar to other environmental stressors such as oil pollution which results in hypoxic conditions by slowing down rates of oxygen diffusion (Sang-Hwan et al. 2007) and flooding, which also displaces air from the sediment (Armstrong and Drew 2002), sediment accumulation above the normal profile is associated with oxygen deficiency.

How useful are the morpho-anatomical modifications in partially buried mangrove trees

Partial sediment burial in the studied mangrove tree species resulted in morpho-anatomical adjustments aimed at enabling the affected trees cope with the negative changes associated with the treatment. This was particularly evident in *C. tagal* where trees that did not develop new roots

Table 4 Transverse section of stem showing the development of bark in stems of partially buried trees of the three studied species indicating the rhytidome (Rt) (scale bar = 5 mm)

Species	Transverse section of stem	
	Above sediment	**Below sediment**
A. marina	 574.1 ± 29.8 µm	 2045.4 ± 21.7 µm
C. tagal	 1023.8 ± 57.8 µm	 2979.6 ± 103.3 µm
R. mucronata	 2630.9 ± 1347.1 µm	 5665.1 ± 449.9 µm

Values below are mean ± SE bark tissue thickness in the sediment burial treatments died. *Ceriops tagal* is generally a slow-growing tree species (Okello 2008), hence aerial roots appeared above the new sediment level much later in the experimental period. The consequence is seen in the higher branch mortalities in treated plots as compared to the controls of both landward and seaward plots. In *R. mucronata*, the trees showed low branch mortalities which could be attributed to natural death of parts of a tree with development. The high branch mortality observed in one of the highest partially buried *R. mucronata* trees (45 cm) signifies limited tolerance as a result of less root development by the particular tree.

Although partial burial may result in stress in some species and settings, the associated modifications as highlighted in the subtitles below may also enhance functioning of biological processes in the affected trees which may facilitate growth. For instance, adaptations have been observed to improve water absorption by roots in trees exposed to prolonged flooding, leading to increased photosynthetic rates in a seasonal wetland in Venezuela (Herrera 2013). This could explain the reduced branch mortality observed in *A. marina* with increased sedimentation levels.

Aerial roots

At organ level, there was increased production of aerial roots in *A. marina* and *R. mucronata* with increase in sedimentation levels. Although sedimentation could be beneficial to mangroves, there is need to maintain the aerial roots above the sediment for continued gaseous exchange. The increased aerial root density in partially buried trees thus ensures the maintenance of balance between the benefits of aeration and the drawbacks of sediment accretion (Young and Harvey 1996). Further, the results reveal pneumatophore density increase in *A. marina* not only from the original cable root, but also through development of new cable roots, a feature which has never been reported before. The observation of positively geotropic roots observed in this study is also unique, as cable roots are generally expected to spread horizontally in the soil (Yáñez-Espinosa and Flores 2011). Increased cumulative rate of growth and branching nature of pneumatophore observed in the partially buried trees have earlier been reported in two separate studies. The pneumatophores of mangroves of the Arabian Gulf began to branch after an oil spill (Böer 1993), while increased pneumatophore elongation was reported by Saifullah and Elahi (1992) in oil-polluted mangroves of Karachi. These adjustments are likely to maintain oxygen diffusion from aerated root parts to the buried sections and facilitate elimination of by-products of anaerobic respiration which could have been initiated in the buried root section (Srikanth et al. 2015).

Feeding roots

The production of numerous feeding roots from new prop roots below the sediment in *R. mucronata*, buried portions of pneumatophores in *A. marina* and cable roots in *C. tagal* have also been observed in flooded trees. According to Srikanth et al. (2015), feeding roots, anchor roots, cable roots and pneumatophores (including other types of aerial roots) facilitate gaseous exchange during inundation. Similarly, feeding roots in pneumatophores were observed in mangroves of the Arabian Gulf after the 1991 Gulf war (Böer 1993).

Cork tissue and lenticels

At tissue level, increase in cork tissue improves the trees' capacity to tolerate partial burial, as more cork aerenchyma are formed creating more intercellular spaces for oxygen transport and storage (Lambers et al. 1998; Wong 2004). The resultant production of additional cork could provide an increased protective layer, restricting the entrance of pathogens which may come from the sediment now covering the stem (Beck 2010). Other than the cork tissue, *A. marina* stem buried below the sediment developed hypertrophied lenticels which have also been observed in the stem portions of *A. germinans* and *Rhizophora mangle* stilt roots immersed under water over prolonged periods of time (Yáñez-Espinosa and Flores 2011). These features were also found to be present in a number of mangrove associates' stems when exposed to flooding (Mielke et al. 2005). Since lenticels are important for the supply of oxygen for the underground roots, the hypertrophied lenticels are likely created to enhance gaseous exchange in the otherwise increased hypoxic conditions due to the additional sediment load.

Air lacunae

Formation of larger air lacunae in the cortex and pith of the buried pneumatophores could also be attributed to need for efficient air supply in response to increased hypoxic conditions initiated by partial burial. Similar observations have been made in trees without cork aerenchyma in their bark, which developed larger air spaces in the phloem parenchyma and primary cortex with flooding (Roth 1981 cited in Yáñez-Espinosa and Flores 2011). In addition, for purposes of stability, the increased intercellular air spaces in the lower pneumatophore sections which became fluffy were compensated for by an increased xylem ratio in the exposed section.

What were the probable triggers of the observed changes?

Partial sediment burial has been noted to result in stimulation of growth of bark tissue, leading to increased thickness in buried stem sections and in stilt roots. The observed modifications of bark tissue are similar to those that occur under prolonged flooding condition (Yáñez-Espinosa and Flores 2011), including in mangrove seedlings (Xiao et al. 2010) and in submerged portions of *Fraxinus* sp. stems (Voesenek et al. 2004). Such increase in tissue has been associated with increased cambial activity resulting in increased parenchyma tissue production. This reaction is influenced by the phytohormone, ethylene (Junghans et al. 2004), which is associated with cambium activity (Savidge 1988) and whose production has been found to increase under flooding conditions (Jackson et al. 2003; Vidoz et al. 2010). Ethylene production could have also resulted in the formation of feeding roots and the development of aerenchyma in partially buried trees, as has been observed during prolonged flooding (Drew et al. 1979; Jackson et al. 1985; Visser et al. 1996). This is in addition to hypertrophied lenticels produced on the portion of *A. marina* stem and the original stilt roots that were covered below the sediment (Yáñez-Espinosa and Flores 2011; Mielke et al. 2005; Bruna et al. 2012), suggesting a role of this hormone in partially buried mangrove trees.

In addition to ethylene, auxins also play an important role in signalling ethylene-mediated production of feeding roots (Srikanth et al. 2015; Bruna et al. 2012). The entire process may be facilitated by the ability of the root cap to sense different forms of stimuli from its immediate surrounding, which triggers a series of reactions that direct growth towards or away from un/favourable environmental cues (Kumpf and Nowack 2015). The result is the continual propagation of new meristems in the root cap, an attribute also known to be responsible for the plastic growth of roots (Williamson et al. 2001) and hence modifications in root system architecture and anatomy as observed with sediment burial.

Conclusion

In contrast to the findings of the effects of sedimentation by Ellison (1998), mangrove trees can survive sediment burial at least under the conditions in the current experiment, aided by the associated morpho-anatomical changes. What is remarkable is the fact that the trees had 8–33% of their initial height buried, yet only a few trees from one of the species died. The plasticity of roots and bark observed on exposure to induced sedimentation could therefore be considered as an important trait in ensuring the survival of the trees following a spontaneous large increase in sediment levels. However, the findings suggest that increased levels of sedimentation would affect different species' distribution according to their threshold tolerance levels, with mortality being recorded at relatively lower burial levels in the more sensitive species. Though tree mortality occurred (only in *C. tagal*), we have not reached the threshold of full mangrove loss with the sedimentation conditions we imposed.

Author contribution statement This work was done by a team of researchers from four different institutions. Dr. Judith Okello as the first author was at the centre of carrying out the field data collection, laboratory analysis and write-up of the manuscript. Prof. Nico Koedam was the head of the supervisory team and together with Dr. James Kairo designed the experiment; Dr. Hans Beeckman was instrumental in guiding all the anatomical analysis both in the laboratory and during the write-up. Prof. Farid Dahdouh Guebas was mainly engaged during the write-up and back and forth reviews.

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Compliance with ethical standards

Conflict of interest We do hereby declare that this work has not been published previously, except as an oral presentation during the 10th WIOMSA symposium held in Dar-es Salaam Tanzania in 2017. The publication has been approved by all authors and the institutions where the work was carried out. If accepted for publication in this journal, this work will not be published elsewhere including electronically in the same form, in English or in any other language, without the written consent of the copyright holder. We further state that there is no conflict of interest.

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