scientific reports

Check for updates

OPEN

Evaluating the role of the understory mangrove fern *Acrostichum aureum* in mangrove sediment organic matter and carbon dynamics

Michael Kyei Agyekum[5,1,2]✉ & José M. Riascos[3,4,6]

Understory plants have long been considered rare in mangrove forests, leaving their ecological roles poorly understood. This limits our understanding of how below-canopy vegetation contributes to sediment carbon cycling and benthic habitat dynamics in mangrove ecosystems. We examined the widespread mangrove fern *Acrostichum aureum* in a neotropical mangrove system to test whether fern-derived organic matter influences sediment properties and benthic macrofaunal assemblages. We established a litter-manipulation experiment (fern litter exclusion, control, procedural control, and mangrove-litter plots) in Bahía Málaga, Colombia. Sediments were sampled in October 2016 and March 2017 for pH, total dissolved solids, carbon:nitrogen ratio, organic matter content, and organic carbon, while macrofauna (crabs, molluscs, and polychaetes) were sampled after five months of manipulation. Macrofaunal species composition did not differ significantly among stations or treatments, whereas multivariate sediment characteristics differed significantly among treatments. Organic matter showed a treatment-dependent temporal response, increasing in plots receiving litter inputs and remaining lower in fern-litter exclusion plots by March. In contrast, organic carbon and other sediment variables showed no significant treatment effects over the experimental period. Overall, fern-litter exclusion was associated with reduced maintenance of sediment organic matter but did not restructure macrofaunal assemblages over five months, indicating that longer timescales or broader environmental gradients may be required for detectable faunal responses to changes in understory-derived litter. Together, these findings highlight the contribution of mangrove understory vegetation to sediment organic matter dynamics and the importance of longer-term perspectives when evaluating faunal responses to understory change.

Keywords Mangrove fern, Organic matter, Organic carbon, Macrofauna, Sediment biogeochemistry, Detritivores

In forest ecology, the understory comprises shade-tolerant plants, epiphytes, vines, herbs, and shrubs that complete their life cycle beneath the tree canopy[1]. Since Janzen's classical observation of the virtual lack of an understory in mangroves[2], ecologists have often treated this as a defining feature of these systems, with several hypotheses proposed to explain the scarcity of shrubs, vines, and herbs[3]. Yet understory plants do occur in high-precipitation mangrove regions, where vines, herbs, ferns, and palms can be abundant along the landward fringe[3–5]. This long-standing view of mangroves as understory-deficient has likely contributed to the limited investigation of understory vegetation and its role in ecosystem functioning.

The mangrove fern *Acrostichum aureum* L. is a widespread understory species found across tropical and subtropical regions in both the Indo–West Pacific and Atlantic–East Pacific provinces[6,7]. It typically occupies high-intertidal habitats on the landward side of mangroves, where shading and salinity are relatively low[8]. In

[1]Faculty 2 Biology/Chemistry, University of Bremen, Bremen, Germany. [2]Leibniz Center for Tropical Marine Research, Bremen, Germany. [3]Departamento de Biología, Universidad del Valle, Cali, Colombia. [4]Corporation Center of Excellence in Marine Sciences – CEMarin, Bogotá, Colombia. [5]Present address: Helmholtz Centre for Environmental Research, Magdeburg, Germany. [6]Present address: Instituto de Ciencias del Mar, Universidad de Antioquia, Turbo, Colombia. ✉email: michaelk@uni-bremen.de

Colombia, along both Pacific and Caribbean coasts, *Acrostichum aureum* forms a conspicuous component of mangrove vegetation, often colonising canopy gaps and back-mangrove zones. Its distribution is closely linked to sediment characteristics such as grain size and salinity gradients[9,10]. Despite its abundance, the extent to which this fern influences sediment properties through litterfall, decomposition, and organic matter inputs remains poorly understood. Studies from Southeast Asian mangroves indicate that *Acrostichum aureum* litter can differ from mangrove tree litter in decomposition dynamics and litter quality, reflecting species-specific controls on organic matter turnover[11,12]. This suggests that understory-derived litter may follow distinct decomposition pathways and contribute differently to sediment carbon and nutrient dynamics. However, comparable field-based manipulations in neotropical mangrove systems, particularly those isolating understory litter inputs, remain scarce.

Environmental gradients in mangroves, driven by hydroperiod, tidal inundation, and freshwater inputs, shape forest composition and sediment conditions by regulating salinity, pH, redox potential, oxygen availability, and sediment texture[4,8]. Within these gradients, *Acrostichum aureum* occurs in zones where it reflects prevailing environmental conditions but may also contribute to local sediment processes through litter inputs and biomass accumulation[10]. However, empirical evidence for its role in sediment organic matter dynamics and associated biogeochemical processes in neotropical mangroves is still scarce.

Mangrove vegetation and sediment characteristics also regulate the distribution and composition of benthic macrofauna, which are typically dominated by crustaceans, molluscs, and polychaetes[13–15]. These organisms play key roles in nutrient cycling through bioturbation, burrowing, and detrital processing[16,17]. While the influence of mangrove tree species, hydrology, and canopy structure on macrofaunal assemblages has been widely studied[18,19], the contribution of understory vegetation has received comparatively little attention. Understory plants such as *Acrostichum aureum* may influence macrofauna indirectly by modifying sediment organic matter availability and directly by providing structural habitat and detrital resources.

Plant litter inputs represent a key pathway through which vegetation influences sediment biogeochemistry and benthic habitat conditions[20,21]. Through decomposition, leaching, and microbial processing, litter regulates the quantity and quality of organic matter in mangrove sediments[20], thereby influencing nutrient availability and the resources accessible to benthic organisms through detrital pathways and faunal processing[22].

In this study, we investigated the role of *Acrostichum aureum* in shaping sediment properties and benthic macrofaunal assemblages in a neotropical mangrove system in Bahía Málaga, Colombia (Fig. 1). Despite its widespread occurrence, the contribution of this mangrove fern to sediment carbon dynamics and associated ecological processes remains poorly quantified, limiting our understanding of how mangrove carbon cycling and benthic habitat conditions are influenced beyond canopy-derived inputs. To examine how these litter-driven processes are reflected in sediments, we focused on properties that capture organic matter dynamics and broader habitat conditions, including organic matter content and organic carbon as measures of bulk carbon pools, the carbon-to-nitrogen (C:N) ratio as an indicator of organic matter quality and decomposition potential, sediment pH as a control on nutrient speciation and microbial activity, and total dissolved solids (TDS) as a proxy for salinity-related ionic conditions that constrain both geochemical processes and macrofaunal tolerance[23–28]. We therefore tested whether the presence or absence of fern-derived litter influences (1) sediment organic matter and organic carbon dynamics, and (2) the composition and abundance of benthic macrofaunal communities. We hypothesised that plots receiving fern litter would show greater organic matter accumulation than litter-exclusion plots, and that any resulting changes in sediment conditions could be reflected in macrofaunal assemblages.

Results

Fern distribution across the salinity gradient

Acrostichum aureum showed a distinct spatial pattern along the estuarine gradient. Percent cover differed among the seven stations, with high cover in stations 2–4 and complete absence of the fern in stations 5–7 (Fig. 2). Stations with high fern cover corresponded to sites differing in inundation regime, creek proximity, and freshwater influence, whereas the most seaward stations lacked *Acrostichum aureum*.

Salinity varied along the estuary and covaried with fern distribution. Mean salinity at both low and high tide increased from the inner stations towards the outer bay, with stations 5–7 exhibiting higher salinities than stations 1–4 (Fig. 3). The stations selected for the experiment (2–4) therefore combined relatively low salinity with high fern cover.

Sediment characteristics (pH, OM, C:N, TDS, and OrgC)

Multivariate patterns

Multivariate differences in sediment properties were assessed using permutational multivariate analysis of variance (PERMANOVA) based on Bray–Curtis dissimilarities. No significant differences were detected among stations (Pseudo-F = 0.68, P = 0.74) or for the station × treatment interaction (Pseudo-F = 0.69, P = 0.88), whereas the main effect of treatment was significant (Pseudo-F = 3.17, P < 0.001; Table 1). Pairwise comparisons were conducted using permutation-based tests within the PERMANOVA framework and indicated that fern litter exclusion differed from the procedural control, control, and mangrove litter treatments. No difference was detected between procedural control and control, whereas a difference was detected between mangrove litter and control (P = 0.04; Table 1).

Principal component analysis (PCA) of temporal changes in sediment properties explained 56% of the total variance in the first two components. PC1 accounted for 32% of the variance and showed strong loadings for organic matter and organic carbon, whereas PC2 explained 24% and was associated mainly with C:N ratio and organic carbon. Fern litter exclusion plots were separated from the other treatments along PC1 (Fig. 4).

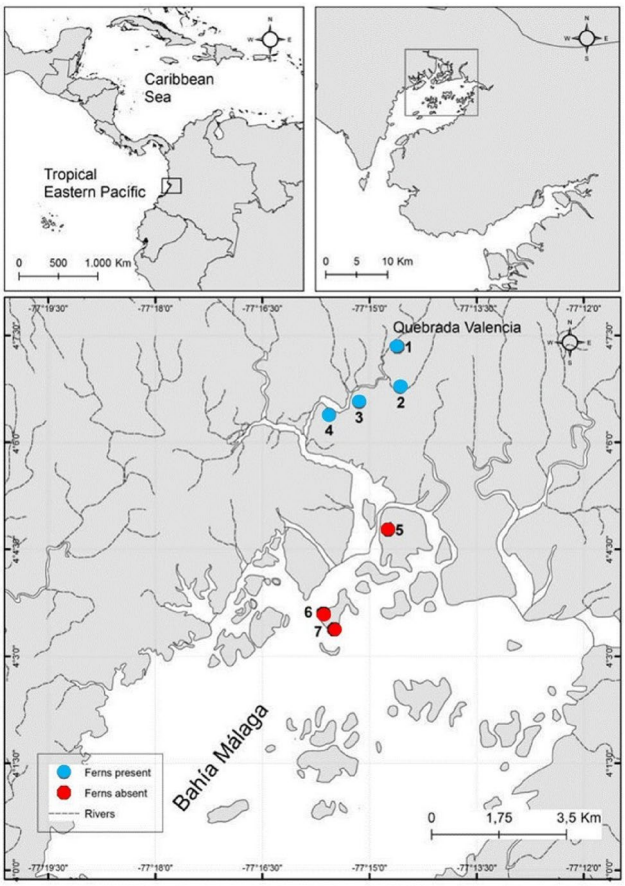

Fig. 1. Map of the study area and sampling stations in Bahía Málaga, Colombia. The map shows the location of Bahía Málaga on the tropical eastern Pacific coast of Colombia and the distribution of the mangrove forest where the experiment was conducted, with the positions of sampling stations along the estuarine gradient. Blue dots indicate sampling stations where *Acrostichum aureum* was present, and red dots indicate stations where the fern was absent. The map was produced in QGIS version 3.38.3 (QGIS Development Team; see https://www.qgis.org) using Colombia administrative boundary data from the Common Operational Datasets (COD-AB) published on the Humanitarian Data Exchange (https://data.humdata.org/dataset/cod-ab-col).

Organic matter and organic carbon

The dynamics of organic matter (OM) showed a significant Treatment × Time interaction ($\chi^2 = 30.24$, df = 3, $p = 1.23 \times 10^{-6}$) and a strong effect of Time ($\chi^2 = 40.99$, df = 1, $p = 1.53 \times 10^{-10}$), while the main effect of Treatment was not significant ($\chi^2 = 7.04$, df = 3, $p = 0.071$). From October to March, OM increased in the plots receiving litter inputs, with mean changes of + 4.15 percentage points in the Control, + 4.87 in the Procedural control, and + 3.02 in the Mangrove litter-plots (all Tukey-adjusted $p \leq 0.0005$; Table S1; Fig. 5a). Fern litter exclusion showed no significant temporal change (− 1.17 percentage points; $p = 0.183$; Table S1). By March, OM was lower in the fern litter exclusion treatment than in the Control, Procedural control, and Mangrove litter treatments (Table S2), whereas no treatment differences were detected in October (all $p \geq 0.599$; Table S2).

Organic carbon (OrgC) showed directional temporal changes consistent with OM, but these changes were small (Fig. 5b). Log-transformed (OrgC) showed no significant effects of Treatment, Time, or their interaction (Treatment: $\chi^2 = 0.96$, df = 3, $p = 0.811$; Time: $\chi^2 = 0.01$, df = 1, $p = 0.924$; Treatment × Time: $\chi^2 = 4.20$, df = 3, $p = 0.241$). Changes between October and March were small and not statistically significant across all treatments (all within-treatment contrasts $p \geq 0.081$; Table S1). Pairwise treatment comparisons within each sampling time were also not significant (all $p \geq 0.422$; Table S2).

C:N ratio

The C:N ratio showed a significant Treatment × Time interaction ($\chi^2 = 8.41$, df = 3, $p = 0.038$), while the main effects of Treatment ($\chi^2 = 6.31$, df = 3, $p = 0.097$) and Time ($\chi^2 = 2.05$, df = 1, $p = 0.152$) were not significant. Temporal changes varied among treatments, with a decline in the Mangrove litter treatment contributing to the interaction ($p = 0.0093$; Table S1**;** Fig. 5c). No treatment differences were detected in March (all $p \geq 0.997$; Table S2).

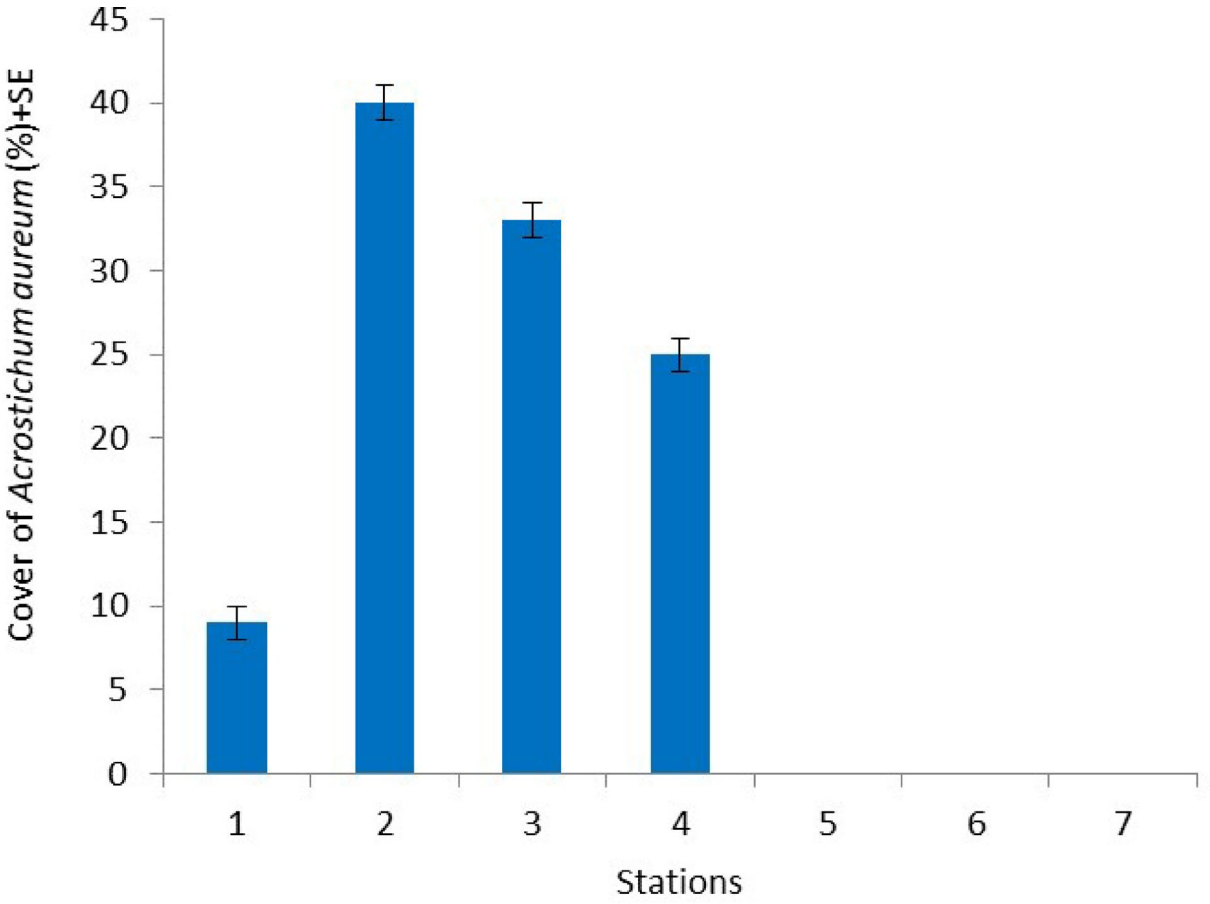

Fig. 2. Percentage cover of the mangrove fern *Acrostichum aureum* at the seven sampling stations in Bahía Málaga. The error bars represent standard errors. The bars represent the mean percentage cover from stations 1 to 4.

Total dissolved solids (TDS)

Log-transformed total dissolved solids (TDS) showed a strong effect of Time ($\chi^2 = 212.74$, df = 1, $p < 2 \times 10^{-16}$), with no significant effects of Treatment or interaction (both $p \geq 0.99$). TDS increased consistently across all treatments from October to March (Table S1; Fig. 5d).

Sediment pH

Sediment pH showed a small but significant effect of Time ($\chi^2 = 4.81$, df = 1, $p = 0.028$), with no significant Treatment or interaction effects. Changes in pH were minor across treatments (Fig. 5e), and no significant pairwise differences were detected (Table S1, S2).

Macrofauna species composition

Across all treatments and stations, 15 benthic macrofaunal taxa were recorded, including 8 crustacean species, 4 gastropods, 2 bivalves, and 1 polychaete family (Spionidae). The most abundant taxa were *Eurypanopeus transversus*, *Sesarma sulcatum*, and *Pitar* sp., which dominated total abundances across treatments (Table 2).

Multivariate differences in species composition were assessed using ANOSIM. No significant differences were detected among stations (Global R = −0.028, P = 0.773) or among treatments (Global R = −0.081, P = 0.994).

The non-metric multidimensional scaling (MDS) ordination showed strong overlap among samples from different stations and treatments, with very low stress (stress = 0.01), indicating a reliable representation of the multivariate structure (Fig. 6).

Discussion

Fern distribution and environmental gradients

In contrast to the long-standing view that understory components in mangrove forests are negligible, our results indicate that *Acrostichum aureum* can contribute to sediment carbon dynamics over seasonal timescales.

Acrostichum aureum exhibited a clear spatial structure, occurring in less saline areas, which is consistent with previous studies showing that osmotic tolerance is a key determinant of its distribution in mangrove forests[8,29]. In these areas, fern cover ranged between 10 and 40% of total vegetation, and previous work has shown that

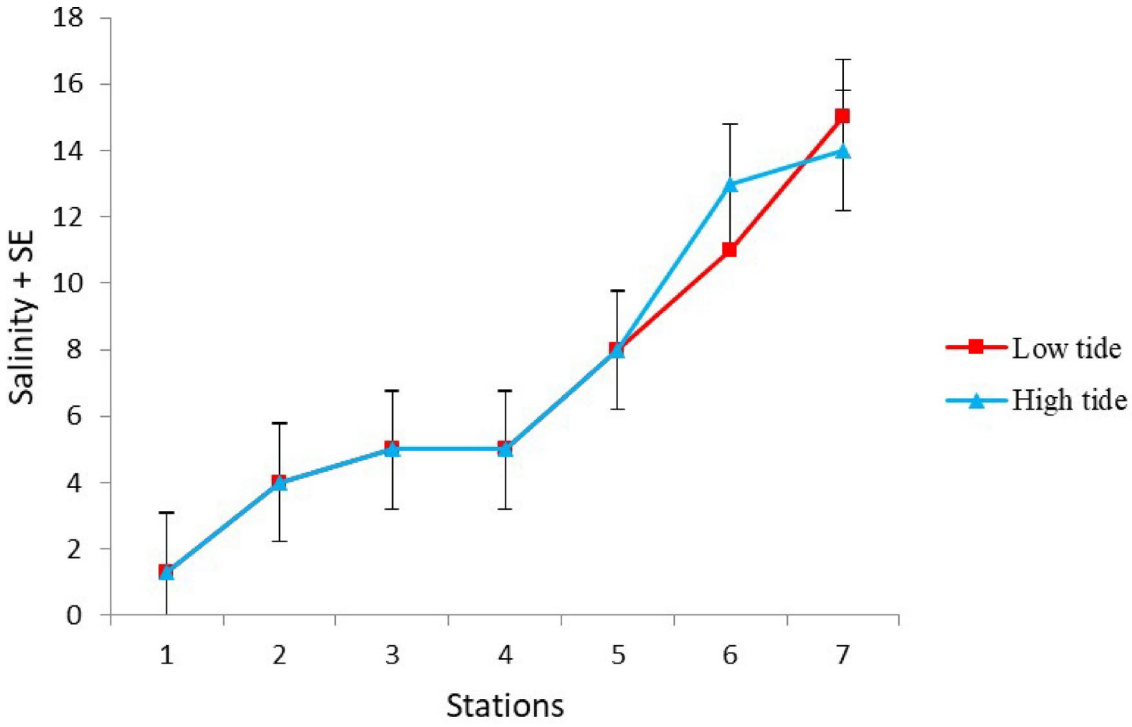

Fig. 3. Salinity at low and high tide across the seven stations in October 2016. Symbols (squares for low tide, triangles for high tide) show mean salinity values; error bars indicate standard errors.

Factors	Df	MS	Pseudo F	P(perm)
Stations	2	3.19	0.68	0.74
Treatments	3	14.81	3.17	**<0.001**
Stations x Treatments	6	0.69	0.69	0.88
Residual	45	4.67		
Treatment groups	**T**			**P(perm)**
Fern litter exclusion vs. Procedural control	2.36			**<0.001**
Fern litter exclusion vs. Mangrove litter	1.93			**<0.001**
Fern litter exclusion vs. Control	2.09			**0.002**
Procedural control vs. Mangrove litter	0.94			0.37
Procedural control vs. Control	1.60			0.51
Mangrove litter vs. Control	1.15			**0.04**

Table 1. PERMANOVA results for differences in multivariate sediment characteristics among stations and treatments, and pairwise tests among treatments.

Acrostichum aureum can represent a substantial understory component in mangrove systems[30,31]. This supports the view that the species can contribute meaningfully to vegetation structure in certain mangrove settings.

Sediment organic matter and carbon dynamics

The temporal trajectories of sediment organic matter (OM) indicate an influence of fern litter inputs on the sediment carbon pool. A significant Treatment × Time interaction was detected for OM, and by March the fern-litter exclusion plots had lower OM than the control, procedural control, and mangrove-litter treatments. Plots receiving litter inputs showed increases in OM over time, whereas fern-litter exclusion plots did not exhibit a significant temporal change. This pattern indicates that plots receiving fern litter inputs maintained or accumulated organic matter relative to exclusion plots. This is consistent with the interpretation that continued litter deposition contributes to the short-term maintenance of surface sediment organic matter. Regardless of the exact decomposition rate of fern litter, its continuous input may offset part of the seasonal loss of sediment carbon in plots where it is present[11,32,33].

Organic carbon exhibited directional changes consistent with organic matter across treatments, with decreases under fern litter exclusion and slight increases where litter inputs were maintained. However, these changes were small and not statistically significant, indicating a comparatively muted response of sediment carbon relative

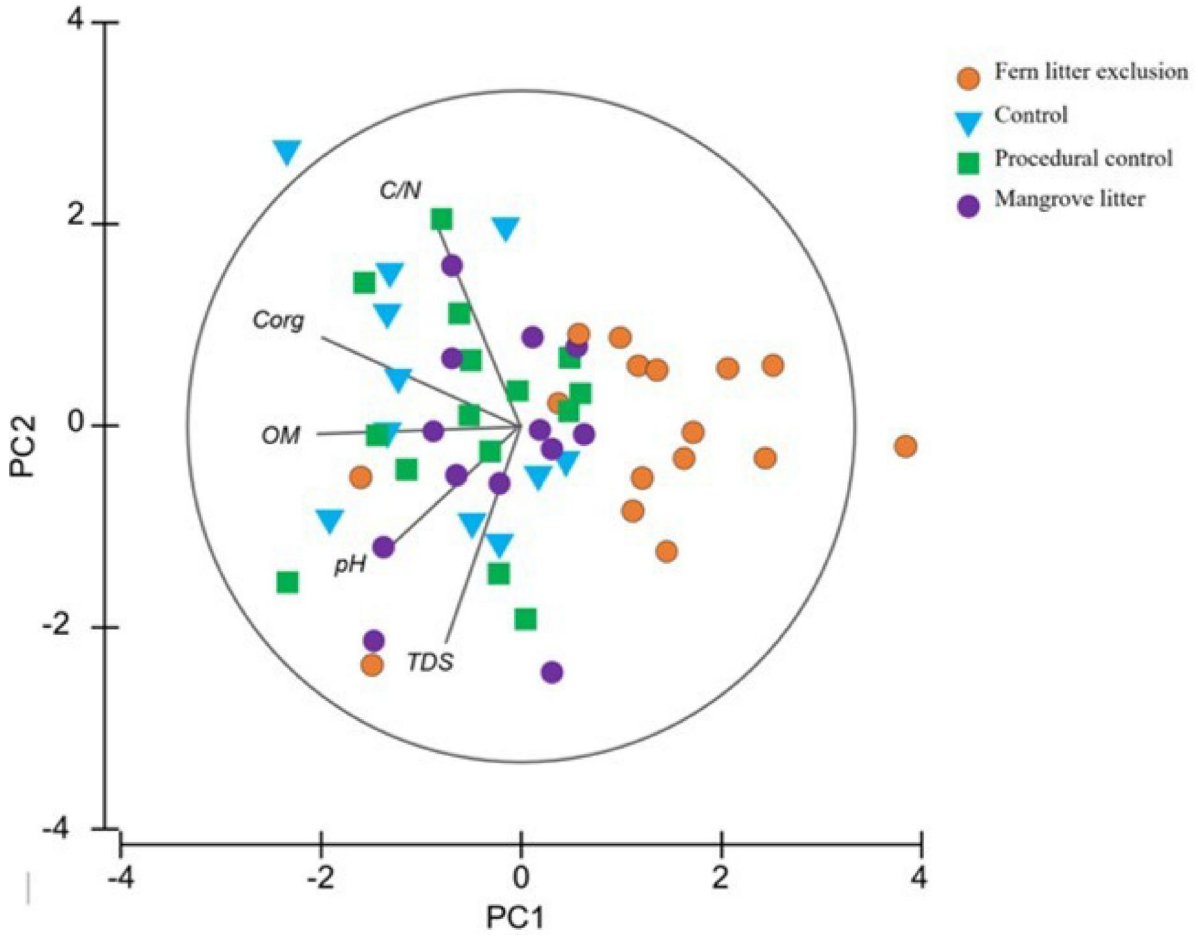

Fig. 4. Principal component analysis (PCA) of temporal changes in sediment pH, OrgC, OM, TDS, and C:N over the five-month experimental period. The first two components together explained 56% of the total variance (PC1: 32%, PC2: 24%). Orange circles represent fern litter exclusion, blue triangles represent control, green squares represent procedural control, and violet circles represent mangrove litter.

to bulk organic matter. This pattern suggests a coupled but dampened response, whereby increases in organic inputs are reflected in total organic matter accumulation but translate only weakly into measurable gains in sediment carbon. Such decoupling is consistent with the idea that loss-on-ignition integrates a broader suite of organic constituents, including labile compounds and non-carbon elements, whereas organic carbon reflects the carbon fraction that is retained after rapid microbial processing[21].

In tidally influenced mangrove sediments, high rates of microbial decomposition and frequent inundation can promote efficient turnover of newly deposited litter, leading to simultaneous inputs and losses of carbon through respiration and export of dissolved or particulate forms[20,21]. Under these conditions, increases in organic matter may partly reflect the accumulation of transient or non-carbon-rich material, or shifts in organic matter composition, rather than net carbon storage. The relatively small magnitude of change in organic carbon further suggests that the sediment carbon pool may be near steady state over the timescale of the experiment, with inputs largely balanced by mineralisation and hydrodynamic removal processes. Similar patterns have been reported in other mangrove systems, where substantial litter inputs do not necessarily translate into short-term increases in sediment carbon stocks due to rapid cycling and export pathways[34,35]. Together, these results indicate that while fern litter influences the accumulation of bulk organic material, its effect on sediment carbon storage is limited over seasonal timescales, highlighting the importance of considering both total organic matter and carbon-specific measures when evaluating biogeochemical responses in dynamic coastal systems.

The comparable OM and OrgC values observed in natural control and mangrove-litter plots should be interpreted in light of differences in vegetation structure. Natural control plots received litter from both *Acrostichum aureum* and mangrove trees, but fern dominance reduced available space and canopy cover for mangrove trees. Therefore, the similarity between these treatments suggests that fern-derived litter may compensate for reduced mangrove litter inputs, maintaining sediment organic matter and carbon pools at levels comparable to mangrove-dominated areas. This supports the view that Acrostichum litter is a non-negligible contributor to sediment organic inputs, even though its effect on measurable organic carbon accumulation was modest and not statistically significant over the five-month experiment.

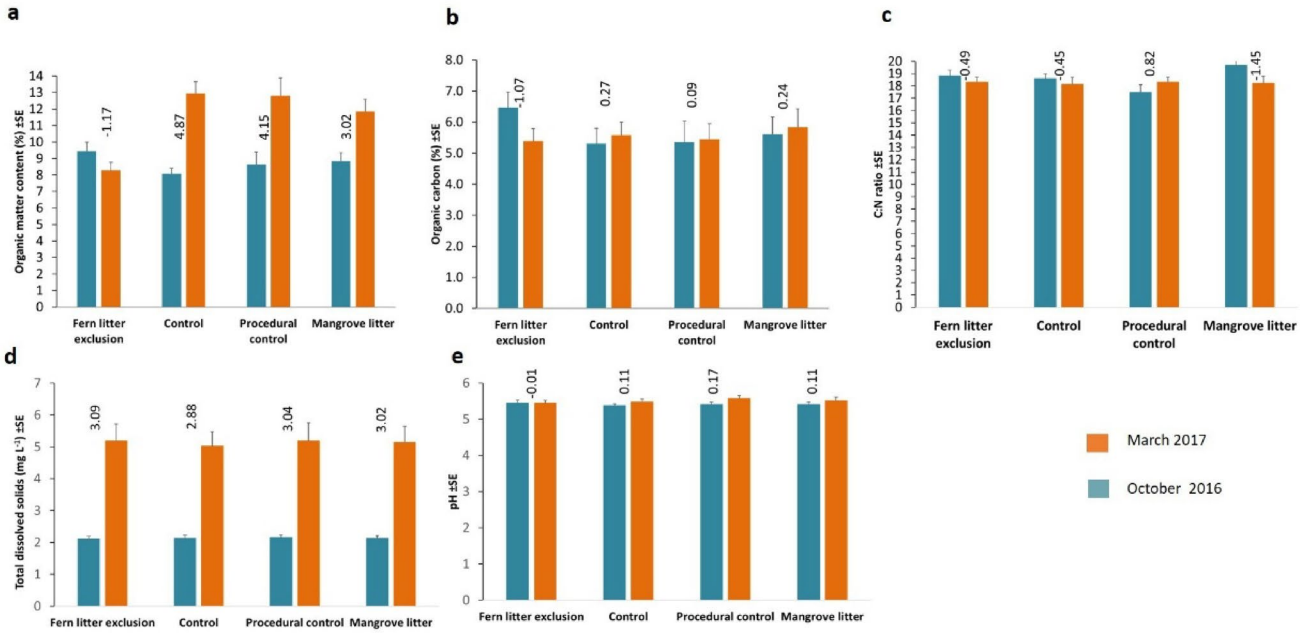

Fig. 5. Changes in (**a**) organic matter content **(b)** organic carbon **(c)** C:N ratio **(d)** total dissolved solids **(e)** pH between March 2017 and October 2016 for each treatment (fern litter exclusion, control, procedural control, and mangrove litter). The values above the graph correspond to mean change (Δ). Bars show mean values and error bars indicate standard errors.

Taxon/species	Treatments			
Gastropods	**Fern-litter exclusion**	**Control**	**Procedural control**	**Mangrove litter**
Nerita funculata (Menke, 1851)	0.0	0.2	0.0	0.2
Cerithidea mazatlanica (Carpenter, 1857)	0.0	0.2	0.0	0.0
Cerithidea pulchra (Adams, 1852)	0.2	0.0	0.0	0.2
Melampus castaneus (Megerle von Mühfeld, 1818)	0.2	0.2	0.0	0.0
Bivalves				
Pitar consanguineus (Adams, 1852)	0.2	0.0	0.0	0.0
Pitar sp.	0.4	0.6	0.4	0.8
Crustaceans				
Sesarma sulcatum (Smith, 1870)	1.6	0.6	0.4	0.0
Eurypanopeus transversus (Stimpson, 1860)	0.8	1.0	0.6	0.2
Uca sp.	0.4	0.4	0.2	0.2
Petruca panamensis (Stimpson, 1859)	0.0	0.6	0.4	0.2
Aratus pacificus (Thiercelin & Schubart, 2014)	0.0	0.2	0.0	0.2
Cardisoma crassum (Smith, 1870)	0.0	0.2	0.0	0.0
Panopeus sp.	0.0	0.2	0.4	0.0
Pinnotheridae	0.4	0.0	0.0	0.0
Polychaete				
Spionidae	0.2	0.4	0.0	0.0

Table 2. Mean abundance (ind. m^{-2}) of benthic macrofaunal taxa in each experimental treatment (fern litter exclusion, control, procedural control, and mangrove litter).

Other sediment properties (C:N, TDS, pH)

The other sediment variables showed weaker or largely treatment-independent responses. The C:N ratio displayed only a modest Treatment × Time interaction, mainly driven by a decline in C:N in the mangrove-litter treatment, while changes in the fern-litter exclusion plots were small. This suggests that litter inputs influenced the quantity of organic matter more than its bulk stoichiometry over the duration of the study. Accordingly, fern litter decomposition primarily increased labile organic material available in surface sediments, without producing large shifts in nutrient stoichiometry or pore-water chemistry over the timescale of the experiment.

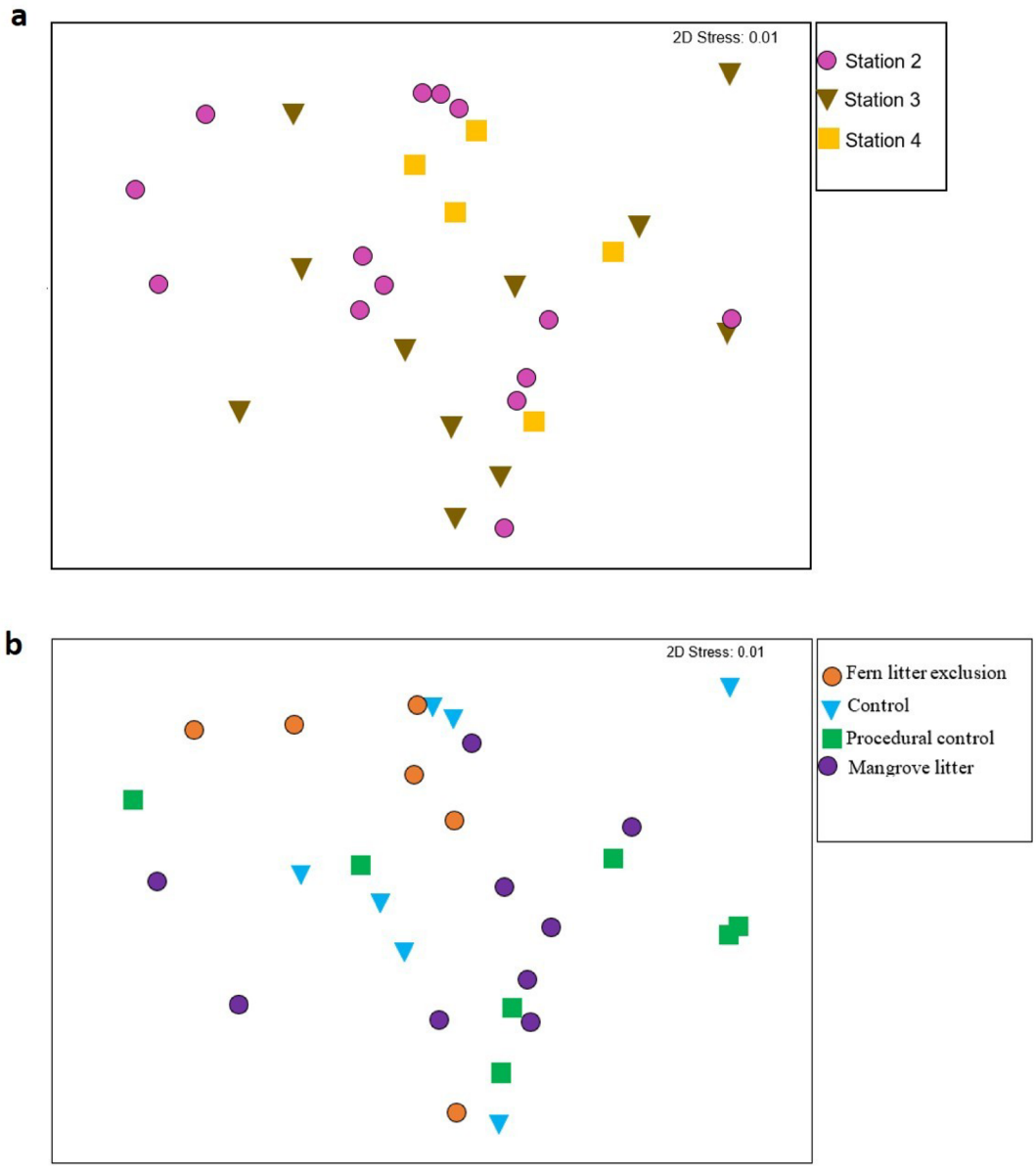

Fig. 6. Non-metric multidimensional scaling (MDS) ordination of macrofaunal assemblage composition among (**a**) stations and (b) treatments. In panel (**a**), Station 2 is represented by pink circle, Station 3 by inverted brown triangles, and Station 4 by yellow squares. In panel (**b**), orange circles represent fern litter exclusion, inverted blue triangles represent control, green squares represent procedural control, and violet circles represent mangrove litter.

Total dissolved solids (TDS) increased uniformly from October to March across all treatments, indicating a strong background temporal shift in pore-water or surface-water conditions[36]. Sediment pH varied only slightly and did not differ among treatments, suggesting limited influence of fern-derived litter on sediment acidity within the experimental timeframe.

Overall, the sediment results indicate that fern-litter exclusion is associated with reduced maintenance of surface sediment OM, while effects on C:N ratio, TDS, pH, and measured OrgC are comparatively small or not statistically detectable. These findings are consistent with a role for *Acrostichum aureum* as a steady contributor to surface organic matter in fern-rich mangrove stands, while broader geochemical changes were not evident over the seasonal timescale examined[2,3,5,36–40].

Hydrological conditions in Bahía Málaga—semi-diurnal tides, high precipitation, and frequent inundation[36]—likely influenced these patterns by enhancing leaching and fragmentation of litter[41]. Such processes can facilitate the rapid transfer of dissolved and fine particulate organic material into surface sediments, stimulating microbial processing and contributing to increases in OM without producing immediate changes in measured OrgC. This interpretation aligns with broader work showing that hydrological setting and litter inputs jointly influence sediment carbon dynamics in mangroves[38,39]. Although tidal inundation likely allowed some lateral transport

of organic material beneath the mesh structures, the lower organic matter observed in fern-litter exclusion plots relative to litter-receiving treatments indicates that net litter inputs differed among treatments at the plot scale. This suggests that, despite partial exchange, the manipulation was sufficient to generate detectable differences in sediment organic matter dynamics.

Macrofaunal assemblages

Several studies have shown that detritus derived from litter decomposition can influence macrobenthic community structure in mangrove systems[42–44]. In the present study, however, no detectable changes in macrofaunal assemblage composition were observed following fern-litter manipulation. This result is best interpreted in the context of spatial and temporal scale rather than as evidence of no effect.

Dominant taxa (*Sesarma sulcatum*, *Eurypanopeus transversus*, *Pitar* spp.) are mobile and generalist detritivores capable of exploiting diverse resources, including microbial biofilms, mangrove litter, and below-ground production[16,17]. Such flexibility may buffer these organisms against moderate changes in sediment organic matter. Moreover, macrofaunal distributions in mangroves are strongly influenced by salinity gradients, inundation regimes, and sediment redox conditions[45–47], which were not directly altered by the litter manipulation.

The absence of a detectable macrofaunal response should therefore be interpreted as a limitation of the spatial and temporal scale of the experiment. This limitation reflects an inherent trade-off in our design between logistical feasibility and the spatial extent required to elicit community-level responses, and should be considered when interpreting the macrofaunal results. The 1 m^2 plots and five-month duration may not have been sufficient to generate environmental changes large enough to restructure mobile benthic assemblages. Responses may require larger spatial scales, longer timeframes, or stronger shifts in sediment conditions to become detectable.

Temporal scale further constrains our inference. Five months is sufficient to capture initial leaching and early incorporation of litter-derived material, but not longer-term processes such as burial, stabilisation, or multi-generational faunal responses. Benthic assemblages often vary interannually, and community-level responses can lag behind changes in sediment conditions. Our experiment therefore likely represents an early phase in which sediment processes respond before macrofaunal patterns become detectable.

Limitations and implications

Several limitations of this study should be acknowledged. It was conducted in a single estuary and at three stations, so extrapolation to other geomorphological settings, climates, or fern densities should be made carefully.

In addition, the experiment focused exclusively on *Acrostichum aureum* as the dominant understory component. Mangrove forests in other regions often support more diverse understory vegetation or mixed litter inputs with differing chemical composition and decomposition dynamics. The extent to which the patterns observed here apply to more complex understory assemblages therefore remains uncertain.

Sampling targeted only the upper 5 cm of sediment and did not include below-ground inputs (roots, rhizomes, exudates)[37,40,48,49]. The spatial scale and sampling approach may also have limited the detection of responses by rare, mobile, or deep-burrowing taxa. Finally, the experiment did not explicitly quantify processes such as microbial activity or bioturbation, which may mediate responses to litter inputs[5,6,17].

Despite these constraints, the experiment provides direct evidence that *Acrostichum aureum* contributes to short-term changes in surface sediment organic matter in a neotropical mangrove system, and that its influence can be distinguished from that of the tree canopy[48,50,51]. These findings highlight understory vegetation as a relevant, though often overlooked, component of mangrove carbon dynamics. The apparent decoupling between sediment responses and macrofaunal patterns further suggests that changes in carbon pools may precede detectable shifts in benthic assemblages.

Future studies should extend similar manipulations over longer time periods and across multiple estuaries to test whether the modest sediment effects observed here accumulate into larger differences in carbon stocks. Experiments along gradients of fern density, combined with tracers or biomarkers, could help track the incorporation of fern-derived material into sediments and food webs. Integrating microbial, biogeochemical, and functional trait approaches would further clarify the mechanisms linking understory inputs to ecosystem functioning[11,17,52].

Conclusion

In conclusion, this study shows that *Acrostichum aureum* contributes to short-term dynamics of surface sediment organic matter in a neotropical mangrove system, while effects on measured organic carbon and macrofaunal assemblages were not statistically detectable over the five-month period. The results suggest that understory-derived litter can support the maintenance of sediment organic matter, but that detectable changes in carbon stocks and benthic community structure may require longer timescales, larger spatial scales, or stronger environmental gradients. These findings highlight the need to consider understory vegetation as a component of mangrove carbon cycling, while also emphasising that its influence is likely context-dependent and may not be captured by short-term experiments alone.

Methods

Study area

The study was conducted in mangrove forests of the Uramba-Bahía Málaga National Park, on the tropical eastern Pacific coast of Colombia (Fig. 1). Uramba–Bahía Málaga is a marine protected area encompassing a tectonic estuary with extensive mangrove stands, soft-sediment mud and sand flats, cliffs and rocky shores[53]. Mean water depth in the bay is approximately 13 m, with maxima of 40 m. Tides are semidiurnal with a range of 1–4 m[36].

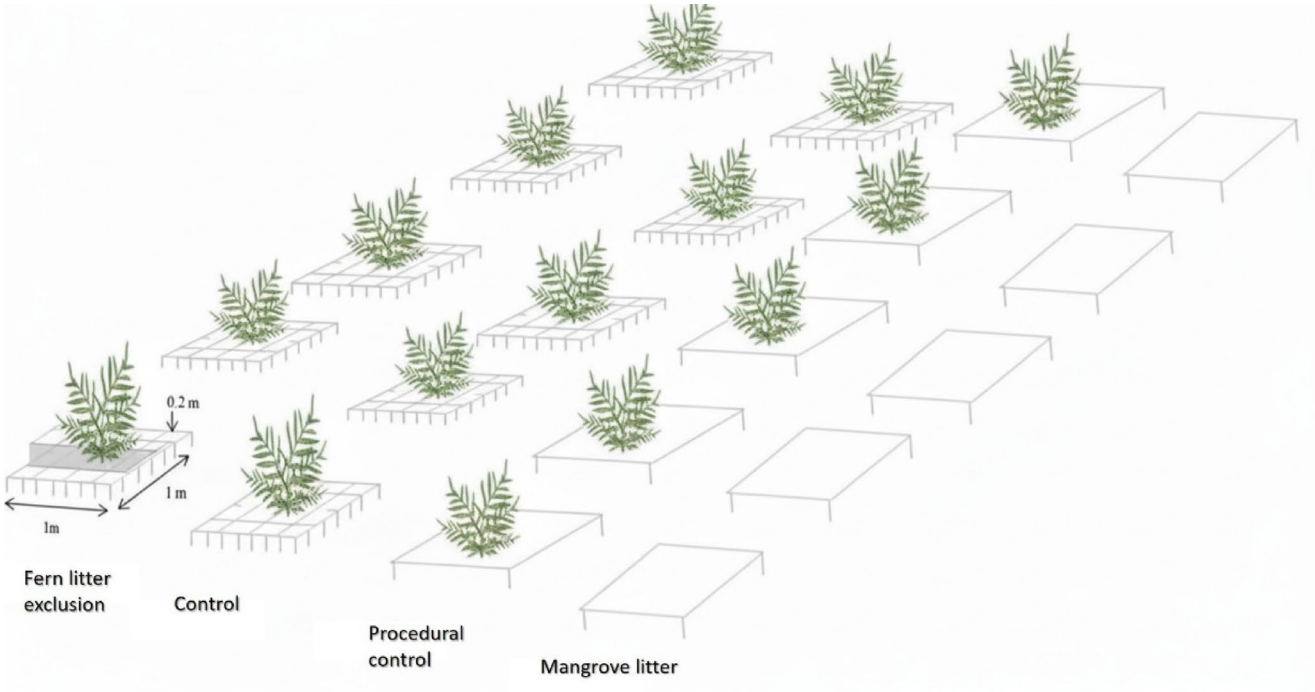

Fig. 7. Experimental treatments established at each sampling station. Plots (1 m^2) were spaced at least 5 m apart. The four columns represent, from left to right, the fern litter exclusion, control, procedural control, and mangrove litter treatments.

Treatment	Description	Litter input	Mesh structure	Purpose
Fern litter exclusion	Fern litter removed; mesh net installed	Mangrove litter only	Present	Isolate the effect of excluding fern litter
Control	Intact plots beneath *Acrostichum aureum* stands	Fern + mangrove	Absent	Baseline condition
Procedural control	Mesh net installed; all litter returned	Fern + mangrove	Present	Control for potential mesh (structure) effects
Mangrove litter	Plots without *Acrostichum aureum*	Mangrove litter only	Absent	Habitat reference (no fern; canopy litter only)

Table 3. Summary of experimental treatments used in the litter-manipulation experiment. Descriptions of treatment types, litter inputs, mesh structures, and their intended role in isolating the effects of fern-derived litter on sediment properties and benthic macrofaunal assemblages.

Water temperature typically ranges from 25 to 30 °C, and salinity varies from 19 to 28 at the bay mouth to 1–10 near freshwater inflows. The region receives among the highest annual rainfall in the western hemisphere (7000–11,000 mm yr^{-1})[36] and is strongly influenced by the Intertropical Convergence Zone, producing two main rainy seasons (April–June and September–November).

Field sampling and experimental design

In October 2016, a pilot survey assessed the distribution of the mangrove fern *Acrostichum aureum* at seven stations located along a salinity gradient within one estuarine branch (Fig. 1). At each station, a transect was established perpendicular to the creek from the seaward margin towards the landward boundary. Five 1 m^2 quadrats were positioned at variable locations along each transect to capture local variation in fern cover, and percent fern cover was estimated using Braun–Blanquet cover classes[54].

Stations dominated by *Rhizophora* spp. typically contained high fern abundance, whereas stations lacking ferns were characterised by mixed stands of *Rhizophora* spp., *Avicennia germinans*, *Mora oleifera*, and *Pelliciera rhizophorae*. Three stations (2–4), representing sites with high fern cover that differed in inundation regime, creek proximity, and freshwater influence, were selected for the litter-manipulation experiment. Fronds of *Acrostichum aureum* arise from rhizomes at or just below the sediment surface, and senescing material may accumulate around the plant base and decompose gradually, which could influence how litter interacts with the mesh structures.

Within each station, four treatments (Fig. 7; Table 3) were established in 1 m^2 plots: (1) "fern litter exclusion", in which all surface fern litter was removed and a 1 cm × 1 cm mesh net was suspended approximately 0.2 m above the sediment to prevent further fern litter input while allowing tidal flow, sediment exchange, and light penetration; mangrove leaf litter was manually returned to the plot surface beneath the mesh while fern litter was consistently removed during monthly plot inspections; (2) "control", consisting of intact plots located directly

beneath naturally occurring *Acrostichum aureum* stands without net installation or litter manipulation; (3) "procedural control", with an identical mesh structure as that in the first treatment installed but all litter (fern and mangrove) returned to control for potential installation effects associated with the mesh structure; and (4) "mangrove litter", consisting of plots established in mangrove areas where *Acrostichum aureum* was naturally absent, representing a habitat reference rather than an active litter-removal treatment. Plot placement within stations was constrained by vegetation structure and accessibility and was therefore not fully randomised (see Fig. 7 layout). Plots were inspected monthly throughout the experiment, and the netting was cleared of trapped debris during inspections to minimise shading and interference with local hydrodynamic conditions. Debris was removed from the mesh and discarded outside the plots and was not quantified by source. Plots were inundated on a semidiurnal tidal cycle, typically twice daily, resulting in frequent submergence of the mesh structures and potential lateral transport of litter beneath them. This could not be fully prevented and may have influenced litter inputs and redistribution within plots.

Each treatment was replicated five times per station (total n = 60 plots), and plots were spaced at least 5 m apart to minimise spatial interference. Because plots were nested within stations along the salinity gradient, the station was included as a spatial blocking factor in all subsequent analyses. Sediment cores were collected at low tide using a 2.5 cm diameter syringe to a depth of 5 cm at the start of the experiment (October 2016) and after five months (March 2017). October corresponds to the end of the relatively drier period in Bahía Málaga, whereas the following months are characterised by increased rainfall and freshwater input, which in many tropical mangrove systems coincide with higher litterfall and enhanced delivery of litter to the forest floor. Installing plots in October and resampling in March therefore allowed us to span a marked seasonal transition in hydroclimatic conditions and cumulative litter inputs, maximizing the likelihood of detecting treatment-related changes in sediment properties over a manageable experimental duration. Five cores were collected per plot and pooled to form one composite sample for laboratory analyses at each sampling time. Salinity of porewater (interstitial water) in the upper sediment layer was measured in situ using a refractometer.

Macrofauna sampling

Macrofauna (crabs, molluscs, and polychaetes) were quantified during the final month of the experiment using excavation and hand collection[50,52,55]. Dense debris and frequent inundation precluded visual or trap-based approaches. Each 1 m^2 plot was excavated within its boundaries for 5 min to an approximate depth of 10–15 cm, and excavated sediments were washed through a 1 mm mesh sieve[50]. All organisms were collected, identified, and counted. Species-level abundances were recorded. Individuals were identified to the lowest practical taxonomic level (typically species for crabs and molluscs and family level for polychaetes). This time-standardised approach provided comparable sampling effort across plots, although it may under-represent highly mobile or deep-burrowing taxa[17].

Organic matter (OM)

Organic matter content was estimated using the loss-on-ignition (LOI) method following Nelson and Sommers[48]. Approximately 2 g of dried sediment was placed in a crucible and combusted at 550 °C for 6 h in a muffle furnace[49]. Samples were then cooled in a desiccator and reweighed to obtain ash weight[56]. Organic matter (OM) was calculated as:

$$OM = \frac{Dryweight\,(g) - Ashweight\,(g)}{Dryweight\,(g)} \times 100\%$$

where "dry weight" is the mass of the dried sediment and "ash weight" is the mass of the combusted sediment.

Total dissolved solids (TDS) and pH

Dried sediment samples were mixed with distilled water at a 1:5 sediment-to-water ratio in a beaker. The suspension was shaken for 30 min using a mechanical shaker and then allowed to settle for 1 hour[56,57]. Electrical conductivity and pH were measured in the clear supernatant using a WTW conductivity probe (TetraCon 925) and a WTW pH sensor (Tix980)[57,58]. Electrical conductivity (μS cm^{-1}) was converted to total dissolved solids (mg L^{-1}) following established conversion procedures[59].

Carbon: Nitrogen ratio and organic carbon

Sediment samples were oven-dried at 65 °C for 72 h, gently disaggregated, ground to a fine powder, and homogenised. Subsamples were weighed into pre-cleaned silver capsules, acidified with 200 μL of 1 M HCl to remove inorganic carbon, and dried at 60 °C to constant mass.

Total organic carbon (OrgC) and total nitrogen (N) were then determined simultaneously by dry combustion using an elemental analyser (Euro EA 3000). OrgC and N were expressed as percentages of dry sediment mass, and C:N ratios were calculated from these measurements.

Statistical analysis

We first tested treatment effects on multivariate temporal changes in sediment properties using PERMANOVA on Δ-values and PCA for visualisation, and then used linear mixed-effects models to quantify univariate temporal trajectories for individual variables.

Sediment properties were analysed with linear mixed-effects models including Treatment, Time (October 2016 vs March 2017), and their interaction, with Station and SampleID (nested within Station) included as random intercepts to account for spatial structure and repeated measures. Total dissolved solids (TDS) and organic carbon (OrgC) were log-transformed prior to analysis to meet model assumptions; organic matter

(OM), C:N ratio, and pH were analysed on the original scale. Model assumptions were checked using residual diagnostics and tests of homogeneity of variance, and Treatment and Time effects were evaluated with Type III Wald χ^2 tests and Tukey-adjusted pairwise comparisons.

Macrofaunal counts were square-root transformed prior to analysis to reduce the influence of highly abundant taxa and improve homogeneity of variance[60]. Bray–Curtis resemblance matrices were constructed and community patterns visualised using two-dimensional non-metric multidimensional scaling (MDS). Differences in macrofaunal assemblage composition among stations and among treatments were tested using analysis of similarities (ANOSIM) with 9,999 permutations in PRIMER v7.

For temporal patterns, we calculated changes in each sediment variable as $\Delta T = T_1 - T_2$ (T_1 = Mar2017; T_2 = Oct2016) for each plot and used these Δ values (ΔOM, ΔTDS, ΔC:N, ΔOrgC) for PCA and PERMANOVA analyses. All absolute values reported in the text and figures refer to the original measurements from each sampling date.

Draftsman plots were used to assess collinearity, and variables were standardised where necessary. Principal component analysis (PCA) was applied for visualisation of multivariate patterns. Treatment effects on multivariate sediment resemblance matrices were tested using PERMANOVA, with treatment as a fixed factor and station as a random blocking factor, using 9,999 permutations under a reduced model. Pairwise comparisons were conducted using permutation-based tests within the PERMANOVA framework following Anderson[61]. Homogeneity of multivariate dispersion was assessed using PERMDISP[62]. Differences among treatments in organic matter and organic carbon were further tested using PERMANOVA on Euclidean distance matrices of temporal change values, again with treatment as a fixed factor and station as a random blocking factor. All analyses were conducted in PRIMER v7 with PERMANOVA + [63], and the datasets are publicly available at Zenodo[64].

Data and code availability

The minimum dataset required to reproduce all main results and figures of this study is publicly available at Zenodo (https://doi.org/10.5281/zenodo.18807835). The code used to fit the mixed-effects models is deposited in the same Zenodo repository (https://doi.org/10.5281/zenodo.18807835).

Received: 3 March 2026; Accepted: 29 June 2026
Published online: 07 July 2026

References

1. Gentry, A. & Emmons, L. Geographical variation in fertility, phenology, and composition of the understory of neotropical forests. *Biotropica* **1**, 216–227 (1987).
2. Janzen, D. H. Mangroves: Where's the understory?. *J. Trop. Ecol.* **1**, 89–92 (1985).
3. Lugo, A. E. Mangrove understory: An expensive luxury?. *J. Tropic. Ecol.* **2**(3), 287–288 (1986).
4. Krauss, K. W. et al. Environmental drivers in mangrove establishment and early development: A review. *Aquat. Bot.* **89**, 105–127 (2008).
5. Corlett, R. T. The mangrove understory: Some additional observations. *J. Trop. Ecol.* **2**, 93–94 (1986).
6. Zhong, Y., Yu, R., Chen, Y., Zhou, R. & Liu, Y. The complete chloroplast genomes of the mangrove fern *Acrostichum aureum*. *Mitochondrial. DNA Part B Resour.* **5**, 2258–2259 (2020).
7. Yamamoto, T. et al. Development and characterization of 27 microsatellite markers for the mangrove fern, *Acrostichum aureum* (Pteridaceae). *Appl. Plant Sci.* **4**, 1–5 (2016).
8. Medina, E., Cuevas, E., Popp, M. & Lugo, A. E. Soil salinity, sun exposure, and growth of *Acrostichum aureum*, the mangrove fern. *Bot. Gaz.* **151**, 41–49 (1990).
9. Blanco, J. F., Estrada, E. A., Ortiz, L. F. & Urrego, L. E. Ecosystem-wide impacts of deforestation in mangroves: The Urabá Gulf (Colombian Caribbean) case study. *ISRN Ecol.* **2012**, 1–14 (2012).
10. Castellanos-Galindo, G. A., Casella, E., Tavera, H., Zapata Padilla, L. A. & Simard, M. Structural characteristics of the tallest mangrove forests of the American continent: A comparison of ground-based, drone and radar measurements. *Front. For. Glob. Change.* **8**(4), 732468 (2021).
11. Pradisty, N. A., Amir, A. A. & Zimmer, M. Plant species- and stage-specific differences in microbial decay of mangrove leaf litter: The older the better?. *Oecologia* **195**, 843–858 (2021).
12. Liu, J. et al. Potential detritivorous diet of the invasive apple snail (Pomacea canaliculata Lamarck, 1822) in mangroves: The relationship between feeding indicators and chemical characteristics of decaying leaf litter. *J. Mar. Sci. Eng.* **11**(3), 672 (2023).
13. Li, P. et al. Community structure of benthic Macrofauna and the ecological quality of mangrove wetlands in Hainan China. *Front. Mar. Sci.* **9**, 1–9 (2022).
14. Kabir, M. et al. Effect of mangroves on distribution, diversity and abundance of molluscs in mangrove ecosystem: A review. *AACL Bioflux* **7**, 286–300 (2014).
15. Macintosh, D. J., Ashton, E. C. & Havanon, S. Mangrove rehabilitation and intertidal biodiversity: A study in the Ranong mangrove ecosystem. *Thailand. Estuar. Coast. Shelf Sci.* **55**, 331–345 (2002).
16. Nagelkerken, I. et al. The habitat function of mangroves for terrestrial and marine fauna: A review. *Aquat. Bot.* **89**, 155–185 (2008).
17. Kristensen, E. et al. What is bioturbation? The need for a precise definition for fauna in aquatic sciences. *Mar. Ecol. Prog. Ser.* **446**, 285–302 (2012).
18. Kon, K., Kurokura, H. & Tongnunui, P. Effects of the physical structure of mangrove vegetation on a benthic faunal community. *J. Exp. Mar. Biol. Ecol.* **383**, 171–180 (2010).
19. Bernardino, A. F., Gomes, LEdeO., Hadlich, H. L., Andrades, R. & Correa, L. B. Mangrove clearing impacts on macrofaunal assemblages and benthic food webs in a tropical estuary. *Mar. Pollut. Bull.* **126**, 228–235 (2018).
20. Alongi, D. M. Carbon cycling and storage in mangrove forests. *Ann. Rev. Mar. Sci.* **6**(1), 195–219. https://doi.org/10.1146/annurev-marine-010213-135020 (2014).
21. Kristensen, E., Bouillon, S., Dittmar, T. & Marchand, C. Organic carbon dynamics in mangrove ecosystems: A review. *Aquat. Botany.* **89**(2), 201–219 (2008).
22. Cannicci, S. et al. Faunal impact on vegetation structure and ecosystem function in mangrove forests: A review. *Aquat. Botany.* **89**(2), 186–200 (2008).

23. Smith, B. C., Rogan, T. A., Redding, M. R. & Rabbi, S. M. Carbon-to-nitrogen stoichiometry of organic amendments regulates microbial biomass growth and nitrogen mineralization in soil. *Soil Use Manag.* **40**(4), e13116. https://doi.org/10.1111/sum.13116 (2024).
24. Yamakura, T. & Sahunalu, P. Soil carbon/nitrogen ratio as a site quality index for some South-east Asian forests. *J. Tropic. Ecol.* **6**(3), 371–377 (1990).
25. Aguinaga, O. E., McMahon, A., White, K. N., Dean, A. P. & Pittman, J. K. Microbial community shifts in response to acid mine drainage pollution within a natural wetland ecosystem. *Front. Microbiol.* **27**(9), 1445 (2018).
26. Zhang, Y. *et al. pH Effect on Heavy Metal Release from a Polluted Sediment*. **2018**, (2018).
27. Timpano, A. J., Soucek, D. J. & Benfield, E. F. *Levels of Dissolved Solids Associated with Aquatic Life Effects in Headwater Streams of Virginia's Central Appalachian Coalfield Region*. (2011).
28. Weber-Scannell, P. K., & Duffy, L. K. *Effects of Total Dissolved Solids on Aquatic Organisms: A Review of Literature and Recommendation for Salmonid Species*. (2007).
29. Sharpe, J. M. Responses of the mangrove fern *Acrostichum danaeifolium Langsd. & Fisch*. (Pteridaceae, Pteridophyta) to disturbances resulting from increased soil salinity and hurricane Georges at the Jobos Bay National Estuarine Research Reserve. *Puerto Rico. Wetlands Ecol. Manag.* **18**(1), 57–68. https://doi.org/10.1007/s11273-009-9148-4 (2010).
30. A L Rumondang, C. K. and S. W. B. Species composition and structure of mangrove forest exposed to plastic waste in Angke Kapuk mangrove protected forest, Jakarta Species composition and structure of mangrove forest exposed to plastic waste in Angke Kapuk mangrove protected forest, Jakar. *Earth Environ. Sci.* (2022) https://doi.org/10.1088/1755-1315/950/1/012016.
31. Kimura, N. *et al.* Botany, uses, chemistry and bioactivities of mangrove plants V : Acrostichum aureum and A. Speciosum. **15**, 1–6 (2017).
32. Cuchietti, A., Marcotti, E., Gurvich, D. E., Cingolani, A. M. & Pérez Harguindeguy, N. Leaf litter mixtures and neighbour effects: Low-nitrogen and high-lignin species increase decomposition rate of high-nitrogen and low-lignin neighbours. *Appl. Soil Ecol.* **82**, 44–51 (2014).
33. Yam, R. S. W., Fan, Y. T. & Wang, T. T. Importance of macrophyte quality in determining life-history traits of the apple snails *Pomacea canaliculata*: Implications for bottom-up management of an invasive herbivorous pest in constructed wetlands. *Int. J. Environ. Res. Public Health* **13**, 1–17 (2016).
34. Adame, M., Neil, D., Wright, S. & Lovelock, C. Sedimentation within and among mangrove forests along a gradient of geomorphological settings. *Estuar. Coast. Shelf Sci.* **86**, 21–30 (2010).
35. Donato, D. C. et al. Mangroves among the most carbon-rich forests in the tropics. *Nat. Geosci.* https://doi.org/10.1038/ngeo1123 (2011).
36. Riascos, J. M., Cantera, J. R. & Blanco-Libreros, J. F. Growth and mortality of mangrove seedlings in the wettest neotropical mangrove forests during ENSO: Implications for vulnerability to climate change. *Aquat. Bot.* **147**, 34–42 (2018).
37. Sasmito, S. D. et al. Mangrove blue carbon stocks and dynamics are controlled by hydrogeomorphic settings and land-use change. *Glob. Chang. Biol.* https://doi.org/10.1111/gcb.15056 (2020).
38. Xiong, Y., Liao, B. & Wang, F. Mangrove vegetation enhances soil carbon storage primarily through in situ inputs rather than increasing allochthonous sediments. *Mar. Pollut. Bull.* **131**, 378–385 (2018).
39. Sasmito, S. D. et al. Organic carbon burial and sources in soils of coastal mudflat and mangrove ecosystems. *CATENA* **187**, 104414 (2020).
40. Suello, R. H. et al. Mangrove sediment organic carbon storage and sources in relation to forest age and position along a deltaic salinity gradient. *Biogeosciences* **19**, 1571–1585 (2022).
41. Wieder, W. R., Cleveland, C. C. & Townsend, A. R. Controls over leaf litter decomposition in wet tropical forests. *Ecology* **90**, 3333–3341 (2009).
42. Muskananfola, M. R., Purnomo, P. W. & Sulardiono, B. Impact of environmental factors on macrobenthos distribution and abundance in mangrove ecosystems on the Northern Coast of Java. *AACL Bioflux* **13**, 2745–2756 (2020).
43. Salimi, E., Sakhaei, N., Nurinezhad, M. & Savari, A. Composition, biomass and secondary production of the macrobenthic invertebrate assemblage in a mangrove forest in Nayband Bay. *Persian Gulf. Reg. Stud. Mar. Sci.* **42**, 101636 (2021).
44. Lee, S. Y. Mangrove macrobenthos : Assemblages, services, and linkages. **59**, 16–29 (2008).
45. Alongi, D. M. & Christoffersen, P. Benthic infauna and organism-sediment relations in a shallow, tropical coastal area: Influence of outwelled mangrove detritus and physical disturbance. *Mar. Ecol. Prog. Ser.* **81**, 229–245 (1992).
46. Dissanayake, N. & Chandrasekara, U. Effects of mangrove zonation and the physicochemical parameters of soil on the distribution of Macrobenthic Fauna in Kadolkele mangrove forest, a tropical mangrove forest in Sri Lanka. *Adv. Ecol.* **2014**, 1–13 (2014).
47. Frusher, S. D., Giddins, R. L. & Smith, T. J. Distribution and abundance of grapsid crabs (Grapsidae) in a mangrove estuary: Effects of sediment characteristics, salinity tolerances, and osmoregulatory ability. *Estuaries* **17**, 647–654 (1994).
48. Nelson, D. W. & Sommers, L. E. Total carbon, organic carbon, and organic matter. *Methods Soil Anal. Part 3 Chem. Methods* (1996) https://doi.org/10.2136/sssabookser5.3.c34.
49. Sutherland, R. A. Loss-on-ignition estimates of organic matter and relationships to organic carbon in fluvial bed sediments. *Analyzer* https://doi.org/10.1023/A:1003570219018 (1998).
50. Ashton, E. C., Macintosh, D. J. & Hogarth, P. J. A baseline study of the diversity and community ecology of crab and molluscan macrofauna in the Sematan mangrove forest, Sarawak. *Malaysia. J. Trop. Ecol.* **19**, 127–142 (2003).
51. Rayment, G. E. & Higginson, F. *Australian Laboratory Handbook of Soil and Water Chemical Method. Australian Laboratory Handbook of Soil and Water Chemical Methods* (1992).
52. Cannicci, S. et al. A functional analysis reveals extremely low redundancy in global mangrove invertebrate fauna. *Proc. Natl. Acad. Sci. USA.* **118**, 1–8 (2021).
53. Kintz, J. R. C. et al. Environmental issues of a marine protected area in a tectonic estuary in the tropical eastern Pacific: Uramba (*Malaga Bay Colombia*): Context, biodiversity, threats and challenges. *J. Water Resour. Prot.* https://doi.org/10.4236/jwarp.2013.51109 (2013).
54. Proctor, M. C. F. & Braun-Blanquet, J. *Pflanzensoziologie: Grundzuge Der Vegetationskunde. J. Ecol.* vol. 55 (Springer, 1967).
55. Guevara-Fletcher, C. E., Cantera Kintz, J. R., Mejía-Ladino, L. M. & Cortés, F. A. Benthic macrofauna associated with submerged bottoms of a tectonic estuary in tropical Eastern Pacific. *J. Mar. Sci.* **2011**(1), 193759 (2011).
56. Konare, H. *et al.* Loss on ignition: Measuring soil organic carbon in soils of the Sahel, West Africa. *AFRICAN J. Agric. Res.* (2010).
57. Miller, J. J. & Curtin, D. *Soil Sampling and Methods of Analysis* (Taylor & Francis Group, 2008).
58. Jönsson, U., Rosengren, U., Nihlgård, B. & Thelin, G. A comparative study of two methods for determination of pH, exchangeable base cations, and aluminum. *Commun. Soil Sci. Plant Anal.* **33**, 3809–3824 (2002).
59. Wolkersdorfer, C. & Hubert, E. Establishing a total dissolved solids : Electrical conductivity ratio for mine waters. *10th Int. Conf. Acid Rock Drain. IMWA Annu. Conf.* 1–8 (2015).
60. Clarke, K. R., Somerfield, P. J. & Chapman, M. G. On resemblance measures for ecological studies, including taxonomic dissimilarities and a zero-adjusted Bray-Curtis coefficient for denuded assemblages. *J. Exp. Mar. Biol. Ecol.* **330**, 55–80 (2006).
61. Anderson, M. J. A new method for non-parametric multivariate analysis of variance. 32–46 (2006) https://doi.org/10.1111/j.1442-9993.2001.01070.pp.x.
62. Anderson, M. J. Distance-based tests for homogeneity of multivariate dispersions. *Biometrics* **62**, 245–253 (2006).
63. Clarke, K. R. & Gorley, R. N. Getting started with PRIMER v7. *Primer-E* 20 at www.primer-e.com (2015).

64. Agyekum, M. & Riascos, J. Raw data and analysis outputs for a fern-litter manipulation experiment in a neotropical mangrove forest (Bahía Málaga, Colombia). at https://doi.org/10.5281/zenodo.18702427 (2026).

Author contributions

M.K.A. and J.M.R. conceived and designed the study. M.K.A. and J.M.R. conducted the fieldwork, performed the statistical analyses, and generated all figures and tables. M.K.A led the interpretation of the results and drafted the manuscript. All authors contributed to the interpretation of the results, critically revised the manuscript for intellectual content, and approved the final version for publication.

Funding

M.K.A. is grateful for having received funding from the German Academic Exchange Service (DAAD), Universidad del Valle, and the Leibniz Centre for Tropical Marine Research (ZMT) during his master thesis research project. J.M.R. was funded by the Ministerio de Ciencia, Tecnología e Innovación-Colombia, program "Tiempo de Volver" and Universidad del Valle.

Declarations

Competing interests

The authors declare no competing interests.

Additional information

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1038/s41598-026-60454-5.

Correspondence and requests for materials should be addressed to M.K.A.

Reprints and permissions information is available at www.nature.com/reprints.

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.