



# Changes in Leaf Litter Quality During Its Decomposition in a Tropical Planted Mangrove Forest (Can Gio, Vietnam)

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Mangroves are highly productive ecosystems, being key components of trophic food chains along tropical coastlines. In Southern Vietnam, planted *Rhizophora apiculata* develops in the upper intertidal zone, while *Avicennia alba* naturally colonizes areas at lower elevation along tidal creeks. The main objective of this study was to quantify the seasonal variability of leaf litter decomposition rates considering that in previous studies we observed a strong influence of the monsoon on C and nutrients cycling within the mangrove and in adjacent creeks. We were also interested in changes in the quality of the leaf litter during its decomposition, focusing on nutrients,  $\delta^{13}\text{C}$ , and trace metals. To reach our goals, we used the litterbag technique. During the monsoon, leaf litter decay rates were increased in the *Avicennia* stand, most probably because of enhanced leaching resulting from a higher level of the river due to heavy rainfall. In addition, the enhanced decay rates measured in the *Avicennia* stand during the rainy season resulted in a strong  $^{13}\text{C}$  depletion, while  $^{13}\text{C}$  enrichment was measured in the other conditions. Elemental concentrations changes during decay were not clearly influenced by season, but depended on the element considered. K, Mg, Na, and Ca had  $t_{50}$  values lower than the one of the bulk material. P and S contents also rapidly decreased during the first decomposition stages, but these elements had longer  $t_{50}$  values than the bulk leaves. Conversely, an enrichment in most trace metals was observed during leaf litter decomposition whatever the season. We suggest that these metals were either trapped from the water column and the forest floor by organic molecules composing the litter, or subjected to litter decomposers uptake. Consequently, this study shows that the release or the uptake of elements during mangrove leaf litter decomposition varied with different parameters, which may have ecological implications on the nature of the elements exported toward adjacent ecosystems, and on their primary productivity.

**Keywords:** mangrove, leaf litter, decomposition, monsoon, Vietnam

## INTRODUCTION

Mangrove forests area was estimated at 150,790 km<sup>2</sup>, representing <1% of tropical forests and 0.4% of global forest surfaces (Spalding, 2010). However, despite this limited extension, they are a valuable sink for atmospheric CO<sub>2</sub> and play a key role in energy flow, nutrient, and carbon cycling in the coastal ocean (Alongi, 2002). Unfortunately because of anthropogenic pressure, mangroves are being lost globally at a mean rate close to 1% per year (Alongi, 2002; Duke et al., 2007; Spalding, 2010); and in some countries, this rate can be as high as 8% (Polidoro et al., 2010). Mangroves destruction may strongly influence carbon and nutrient dynamics along tropical coastlines.

Mangrove plants can fix and store elevated amount of carbon in their biomass (Saenger, 2002). Part of their productivity can be exported to adjacent estuarine ecosystems and to the ocean by the tidal action (Bouillon et al., 2008; Maher et al., 2013; Stieglitz et al., 2013), but some mangrove-derived organic compounds can accumulate in their soils during several 100 or 1000 years (Lallier-Vergès et al., 2008; Adame et al., 2015; Jacotot et al., 2018). Processes of litterfall decomposition on mangrove forest floors are influenced by several parameters, including mangrove species, season, and position of the stand in the intertidal zone (Duke et al., 1981; Saenger and Snedaker, 1993; Feller et al., 1999; Mfilinge et al., 2002; Kristensen et al., 2017). The most important components of all litter materials are the leaves; they can represent 40–85% of the litterfall (Mackey and Smail, 1996; Ochieng and Erfteimeijer, 2002). They are, therefore, the main source of nutrients for the forest floor or the adjacent ecosystems. The initial chemical composition of the leaves, and specifically their C/N ratios, were describe to have a key influence on decay rates (Mfilinge et al., 2002). Sherman et al. (1998) showed that low N concentrations in the senescent leaves reduced the rate of microbial decomposition and macrofaunal consumption of the leaf litter. Additionally, nutrient release from litter during decay processes depends on climate, elevated temperatures increase decomposition rates (Sánchez-Andrés et al., 2010).

In mangrove ecosystems, sedimentary organic matter is a blend of allochthonous and autochthonous sources, deriving from algae to higher plants. Kristensen et al. (2008) compiled available data, and showed that, on the one hand, 58% of  $\delta^{13}\text{C}$  values measured in mangrove sediments were lower than  $-25\text{‰}$ , suggesting an important input of mangrove litter, and on the other hand that the significant number of relatively high  $\delta^{13}\text{C}$  values indicate large inputs of imported phytoplankton, seagrasses and microphytobenthos. In terrestrial ecosystems,  $^{13}\text{C}$  enrichment, depletion, or negligible shifts in the residual litter were reported during OM decomposition due to physical, chemical, and biological processes (Gioacchini et al., 2006; Gautam et al., 2016). These changes depend on several parameters, including litter quality, environmental conditions, and microbial biomass (Connin et al., 2001; Zhang et al., 2018). Feng (2002) suggested that for a system containing multiple organic components, like cellulose and lignin, which have different quality and different isotopic compositions, the temporal evolution of isotopic ratios reflected the competition

between isotopic fractionation and preferential preservation of organic components. Considering that  $\delta^{13}\text{C}$  is commonly used to trace OM sources in tropical coastal ecosystem, we consider relevant to understand changes in  $\delta^{13}\text{C}$  during mangrove litter decomposition.

Organic-rich mangrove soils can act as long-term sinks for most trace metals, because of their precipitation with sulfide minerals during sulfato-reduction processes (Harbison, 1986). However, trace metal speciation in mangrove soils is highly variable, depending on tide, season, and mangrove species (Marchand et al., 2006, 2012). When bioavailable, trace metals can be subject to an uptake by mangrove plants. The latter can store high amount of trace metals in their tissues (Marchand et al., 2012, 2016), which can return to the soil through litterfall during decay processes (Silva et al., 1998; Abohassan, 2013).

The Can Gio mangrove forest is the largest contiguous mangrove forest in Vietnam. It develops between a densely populated megacity (Ho Chi Minh City, the biggest industrial city in Vietnam) and the East Sea (the Vietnamese name is Bien Dong). Can Gio is known as the “green lungs and kidney of Ho Chi Minh City.” Can Gio mangrove forest covered an area of about 40,000 ha in 1964 (Hong and San, 1993), and had a high biodiversity with more than 200 species of fauna and 52 species of flora. Unfortunately, the forest was almost completely destroyed by the spraying of a mixture of herbicides and defoliant during the Vietnam War (1964–1971) (Ross, 1975), with only *Avicennia* and *Nypa* able to survive and naturally regenerate. Fortunately, since 1978, a vast reforestation program, mainly using *Rhizophora apiculata*, has been implemented by the Ho Chi Minh City Forest Department. When *Rhizophora* stands established, the mangrove area extended through natural regeneration and rapid colonization of mudflats along riverbanks notably by *A. alba*. In January 2000, Can Gio became the first mangrove forest in Vietnam to be included in the World Network of Biosphere Reserves by the United Nations Educational, Scientific and Cultural Organization (UNESCO). The importance of nutrients recycling via mangrove-derived organic matter mineralization in sustaining coastal food webs in the Can Gio estuary was demonstrated in previous studies using fatty acid biomarkers and dual stable isotopes ( $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ ) (David et al., 2018a, 2019). Additionally, higher NH<sub>4</sub> concentrations (Taillardat et al., 2019), pCO<sub>2</sub> and CO<sub>2</sub> emissions (David et al., 2018b; Taillardat et al., 2018; Vinh et al., 2019) were measured during the monsoon due to enhanced microbial activity. The monsoon also increased metal loads in the estuary (Thanh-Nho et al., 2018), and it was demonstrated that their transfer to the biota differed between mangrove stands (Thanh-Nho et al., 2019). Consequently in previous studies concerning Can Gio mangrove, we were able to show: (i) the importance of mangrove-derived organic matter in the functioning of this estuary, (ii) the seasonal variability of OM quality and mineralization, (iii) the influence of OM on trace metals cycling; however up to now, the first step of mangrove leaf litter decomposition was not studied. Consequently, the main objectives of the present study were: (i) to determine the influence of the monsoon on leaf litter decomposition rates, and (ii) to assess nutrients and trace metals dynamics, as well

the changes in  $\delta^{13}\text{C}$  for the mangrove leaves during litter decay processes. We hypothesize that the monsoon season would increase decay rates because of enhanced temperature and rainfall. We also hypothesized that changes in elemental and isotopic compositions of the leaves will depend on the element considered.

## MATERIALS AND METHODS

### Study Area

Can Gio Estuary is a deltaic confluence of the Sai Gon, Dong Nai, and Vam Co Rivers. It is located between  $10^\circ 22'$  and  $10^\circ 40'$  N and  $106^\circ 46'$  and  $107^\circ 00'$  E, and between the biggest industrial city in Vietnam (Ho Chi Minh City) and the East Sea (Figure 1). This estuary is subject to semi-diurnal tides, and the tidal amplitude ranges from 2 to 4 m. The climate is monsoonal, with a dry season lasting from December to April and a wet season from May to November (Figure 2). The total annual rainfall is  $1,816 \text{ mm yr}^{-1}$ , with most of the rain occurring during the rainy season ( $1,599 \text{ mm yr}^{-1}$ ) over a total of 154 days per year. The average annual temperature is  $27.4^\circ\text{C}$ , with the highest ( $37.6^\circ\text{C}$ ) occurring in October and the lowest ( $21.7^\circ\text{C}$ ) in February (Figure 2). The mean annual humidity is 86%, ranging from 80.8 to 91.4%. During the rainy season, representing  $\sim 80\%$  of the annual rainfall, the rivers bring much more silt, accreting the plain with an annual particles flux estimated at around 160 million tons (Milliman and Meade, 1983; Schwarzer et al., 2016). During the dry season, the fresh water flow is strongly reduced, and the coastal areas are severely affected by saline intrusion. The water salinity of the Long Tau River is higher (26.06‰) during the dry season than during the wet season (16.52‰), as observed by Thanh-Nho et al. (2018). Currently, the Can Gio mangrove forest area covers  $\sim 32,125 \text{ ha}$  of mangrove forests, including 23,508 ha of interior forest and 8,617 ha of mixed forest (Vinh, 2018). The *A. alba* stands develop between +20 and +30 above MSL, while the *Rhizophora* stand develops between +40 and +75 cm above MSL (Vinh, 2018). A transitional forest develops between these two stands, mainly composed of *R. apiculata*, *A. alba*, and *Althaea officinalis*, as well as, sparse *Excoecaria agallocha* and *Sonneratia alba*.

### Data Collection and Methods

#### Litterbags Experiment

The decomposition of leaf litter was determined using the litterbag technique. This method may underestimate actual decomposition, because of a slightly different microhabitat and microclimate. In addition, the mesh size of the bags excluded breakdown by large invertebrates, such as crabs, which play a role in leaf degradation. However, this method reflects trends and allows comparison among species and sites (Ashton et al., 1999). These experiments were conducted in the field during the two seasons: from December 2016 to February 2017 during the dry season, and from June 2017 to August 2017 during the rainy season. *R. apiculata* and *A. alba* yellow leaves were picked on several trees at each season. Leaves were stored in a frozen box in the field and then taken back to the laboratory, where they were rinsed with purified water to remove any excess salt, and then air

dried until reaching a constant mass. For each season, the leaves were split into 90 litterbags ( $15 \times 20 \text{ cm}$  and 1.0 mm mesh size). Forty five litterbags containing *Rhizophora* leaves were installed in the *R. apiculata* stand, and 45 litterbags containing *Avicennia* leaves were installed in the *A. alba* stand. Each bag contained 8 g dry weight (DW) of *R. apiculata* leaves or 6 gDW of *A. alba* leaves. In each mangrove stand, three random points were chosen, and at each point, three bags were installed. Bags were deposited on forest floor and tightly attached to *Avicennia* trunk or *Rhizophora* prop roots. Consequently, for each collection period, nine bags were removed from the field, representing three samples; one sample was composed of tree bags. Collection occurred 7, 14, 28, 56, and 84 days after the beginning of the experiment and during the two seasons. After collection, the leaf samples were rinsed carefully with purified water, placed in zipper bags, sealed in aluminum foil in order to minimize gas exchange, stored in a frozen box in the field, and then taken back to the laboratory. Samples were dried by Freeze Drying Vacuum at  $-52^\circ\text{C}$  until reaching a constant mass at the LAMA Lab IRD—Noumea, New Caledonia.

#### Analytical Methods and Calculations

For each site and each collection date, three leaf litter samples (composed of the detritus from three bags) were analyzed. Each sample was ground to  $250 \mu\text{m}$  with a cutting mill (Fritsch, Pulverisette 15, Germany) and divided into two sub-samples. One subsample was used to determine the total concentrations of Ca, Mg, K, S, Fe, Al, Ni, Cr, Co, Cu, and Zn by inductively coupled plasma optical emission spectrometry (ICP-OES) after extraction in a solution of 1% w/v solution of  $\text{FNH}_4$  and nitric acid (70%) (Bourgeois et al., 2019). The second sub-sample was used to determine the total C and N concentrations, as well as  $\delta^{13}\text{C}$  values, using a Flash EA1112 elemental analyzer coupled to a Thermo Finnigan DELTA plus XP isotope ratio mass spectrometer using a ConFlo III interface. The analytical precisions of the analyzer were checked using the IAEA-600 caffeine standard (IAEA Nucleus) and were  $<1\%$  for TOC, 0.15% for N, and 0.3% for  $\delta^{13}\text{C}$ . All the analyses were performed at the LAMA Lab IRD—Noumea, New Caledonia. Carbon isotope ratios are expressed as  $\delta^{13}\text{C}$  values in ‰ (per million) using the following equation:

$$\delta^{13}\text{C} (\text{‰}) = \left( \frac{R_{\text{sample}}}{R_{\text{standard}}} - 1 \right) \times 1000 \quad (1)$$

$R_{\text{sample}}$  and  $R_{\text{standard}}$  refer to the  $^{13}\text{C}/^{12}\text{C}$  ratio in the sample and the standard, respectively. The C/N ratios were determined for each sample.

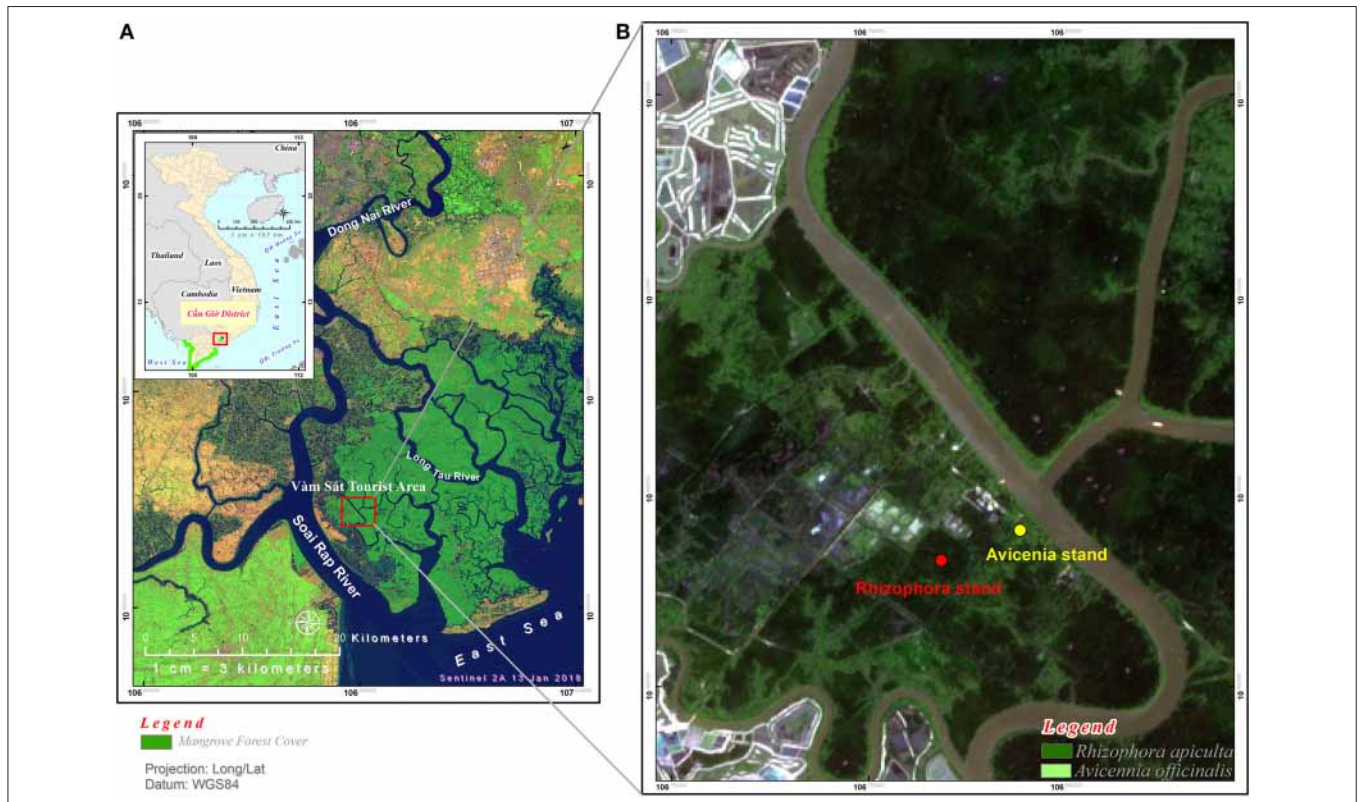
The percent of mass, nutrients, and trace metals remaining in the litter were calculated using a single negative exponential equation:

$$W_t = W_o \cdot e^{-kt} \quad (2)$$

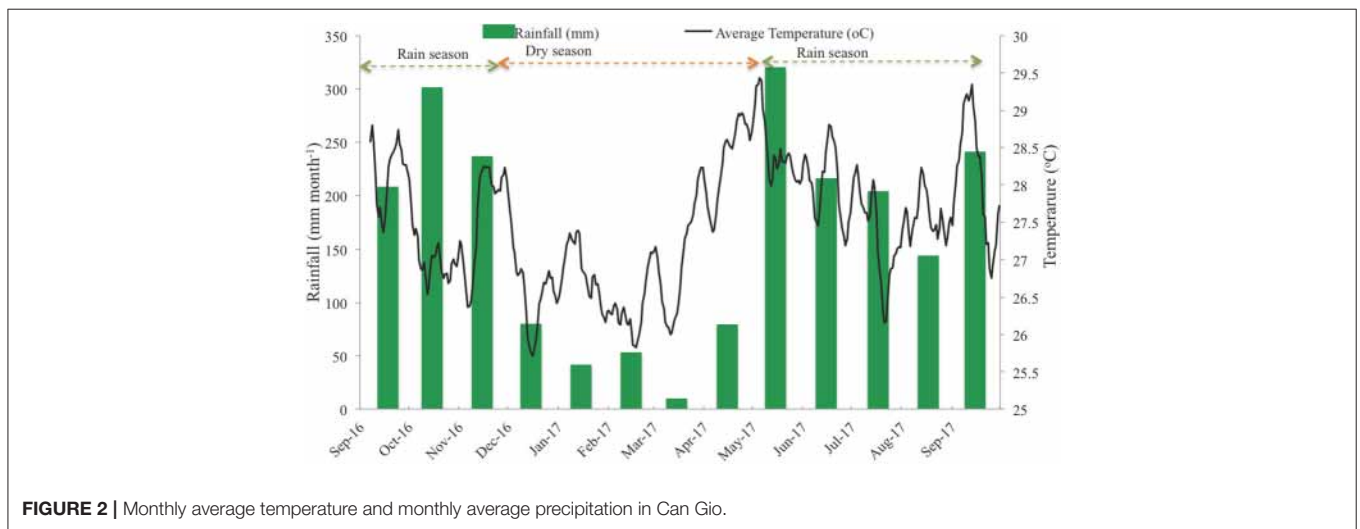
where  $W_t$  was the remaining mass at time (t),  $W_o$  was the initial leaf mass, and k was the decay constant. The half-time ( $t_{50}$ ) was calculated using the formula proposed by Olson (1963):

$$t_{50} = \ln(2)/k \quad (3)$$





**FIGURE 1 |** Study sites location: **(A)** Can Gio mangrove forest (in dark green color) in Southern Vietnam, **(B)** positions of the *Rhizophora* stand and the *Avicennia* stand studied.



**FIGURE 2 |** Monthly average temperature and monthly average precipitation in Can Gio.

Because of the rapid initial loss due to leaching, a double exponential model may be more suitable for some elements. However, the single exponential model, which was used in this study, is an easily applicable and robust method. Decay constants and half-lives could be compared within this study and with other experiments conducted elsewhere (Ashton et al., 1999). In addition, the rapid initial loss measured may have been enhanced

by the two different methods we used to dry the samples; freeze-drying tends to be more effective than air-drying (used for the T0 leave samples) in removing water content even if we wait until obtaining constant mass for both methods.

A parametric two-way analysis of variance (ANOVA) was used to test the effect of seasons and sites on decomposition rates. This method was also applied to assess the difference in  $\delta^{13}\text{C}$

**TABLE 1** | Two-way ANOVA tests showing the effect of species and seasons on decomposition rates.

Parameters	N	Source of variation		
		Species	Season	Interaction
$\delta^{13}\text{C}$ signature	30	0.08 <sup>ns</sup>	21.4 <sup>***</sup>	40.6 <sup>***</sup>
C/N ratios	30	67.0 <sup>***</sup>	0.5 <sup>ns</sup>	0.6 <sup>ns</sup>
$\delta^{13}\text{C}$ signature		Species	Season	Interaction
<i>Avicennia</i> sp	15	–	37.2 <sup>***</sup>	–
<i>Rhizophora</i> sp	15	–	2.2 <sup>ns</sup>	–
C/N ratios		Species	Season	Interaction
<i>Avicennia</i> sp	15	–	0.1 <sup>ns</sup>	–
<i>Rhizophora</i> sp	15	–	0.6 <sup>ns</sup>	–
Decomposition		Time	Season	Interaction
<i>Avicennia</i> sp	90	35.0 <sup>***</sup>	197.9 <sup>***</sup>	12.9 <sup>***</sup>
<i>Rhizophora</i> sp	90	168.7 <sup>***</sup>	4.4 <sup>*</sup>	1.2 <sup>ns</sup>

\* and \*\*\* indicate statistically significant effects for  $p < 0.05$ , and  $< 0.001$ , respectively. ns means no statistically significant difference ( $p > 0.05$ ).

values and C/N ratios among mangrove forests (Table 1). Data analyses were performed using XLSTAT software version 2017.4 for Mac OS.

## RESULTS

### Leaf Litter Decomposition in the *Rhizophora* Stand

In the *Rhizophora* stand, leaf litter was degraded rapidly during the first week, with decay rates of  $4.65 \pm 0.39\% \text{ d}^{-1}$  and  $3.62 \pm 0.35\% \text{ d}^{-1}$  during the dry and the rainy season, respectively; and then decomposition rates decreased with time. After 84 days, the leaf litter lost  $77.5 \pm 10.9$  and  $74.8 \pm 7.1\%$  of its original DW during the dry and the rainy season, respectively (Figure 3). Mean  $t_{50}$  values were similar between seasons (43.3 days). OC mean  $t_{50}$  values were 36.5 and 40.8 days during the dry and rainy seasons, respectively, with a steady decrease with time irrespective of the season (Figure 4A). The N content in the leaf litter increased during the first week irrespective of the season, reaching 107.5 and 112.9% of the original DW during the dry and the rainy season, respectively (Figure 4). After the first week, N contents decreased slowly, and  $t_{50}$  were higher than 69 days irrespective of the season. The initial C/N ratios of the *Rhizophora* leaves ranged from 61.2 to 68.2 (Figure 5). During decay, C/N ratios decreased to 28.5 and to 27.5, during the dry and the rainy season, respectively. The  $\delta^{13}\text{C}$  values of the original yellow leaves ranged from  $-29.43$  to  $-28.60\%$ . Whatever the season, an enrichment was observed during the first week, followed by a stabilization and a depletion after 2 months. The enrichments during the first week were  $\sim 1\%$  during the dry season and  $\sim 0.5\%$  during the rainy season, while the depletions at the end of the experiment ranged between 0.2 and 0.7%.

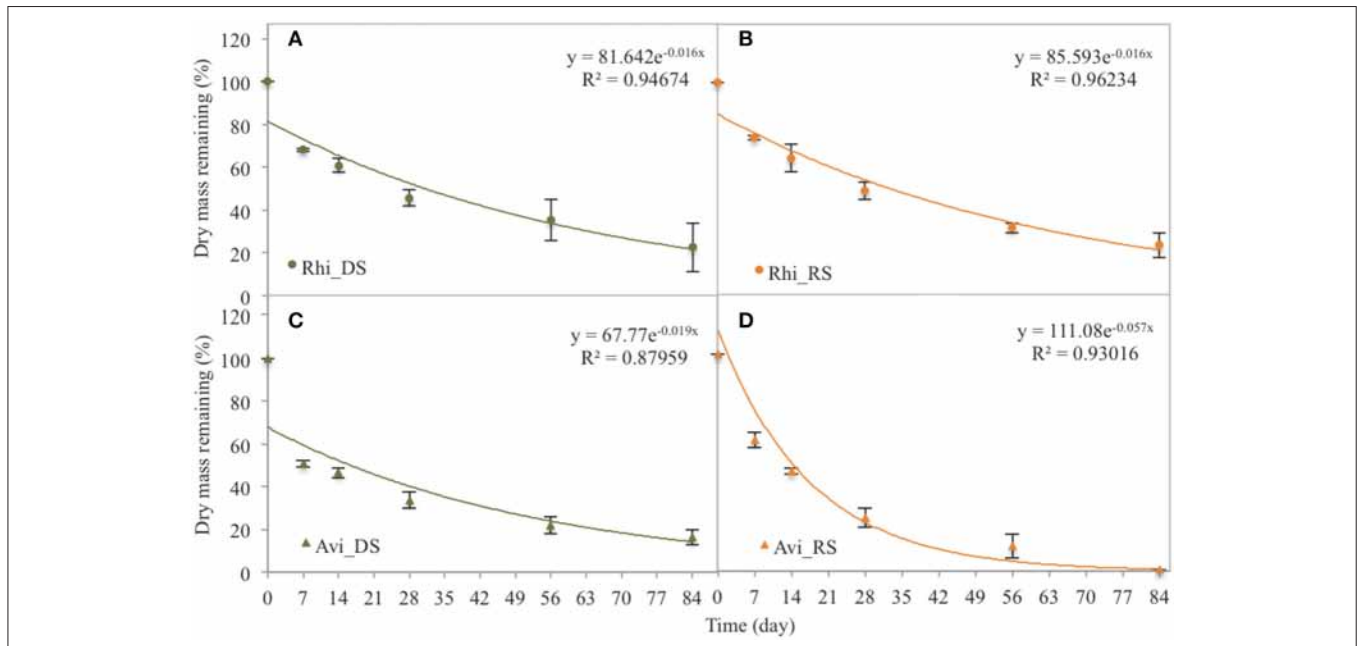
K decomposition rates were the highest of all the elements during the first week, with only 7.6 and 14.1% of original DW remaining during the dry and the rainy season, respectively

(Figure 4). However, Na presented the shortest mean  $t_{50}$  values, with 19.8 and 24.8 days during dry and rainy season, respectively; while K  $t_{50}$  values of 36.5 and 46.2 days during the rainy and dry seasons, respectively (Table 2). Mg releases were rapid during the first week as 70.4 and 57.4% of the original DW remained after the first week during the dry and the wet season, respectively. This rapid decrease was followed by a slow immobilization, with quite elevated  $t_{50}$  values,  $\sim 69$  days irrespective of the season. P releases during the first week were similar between seasons, with  $\sim 60\%$  of the original DW remaining. However, after this first week, P evolution differed between seasons: P contents slightly decreased during the rainy season, but during the dry season they increased to day 56 and decreased after (Figure 4C). P mean  $t_{50}$  values were 77.0 and 90.0 days during rainy and dry seasons, respectively. Like N, Ca content increased during the first week during the dry season, reaching 111.5% of the original DW (Figure 4G); and after the first week, Ca dynamics showed a slow decrease. During the rainy season, Ca content steadily decreased during the decomposition process. S showed a very specific trend; after a rapid release during the first week (50% of the original DW remained), S contents increased during both seasons.

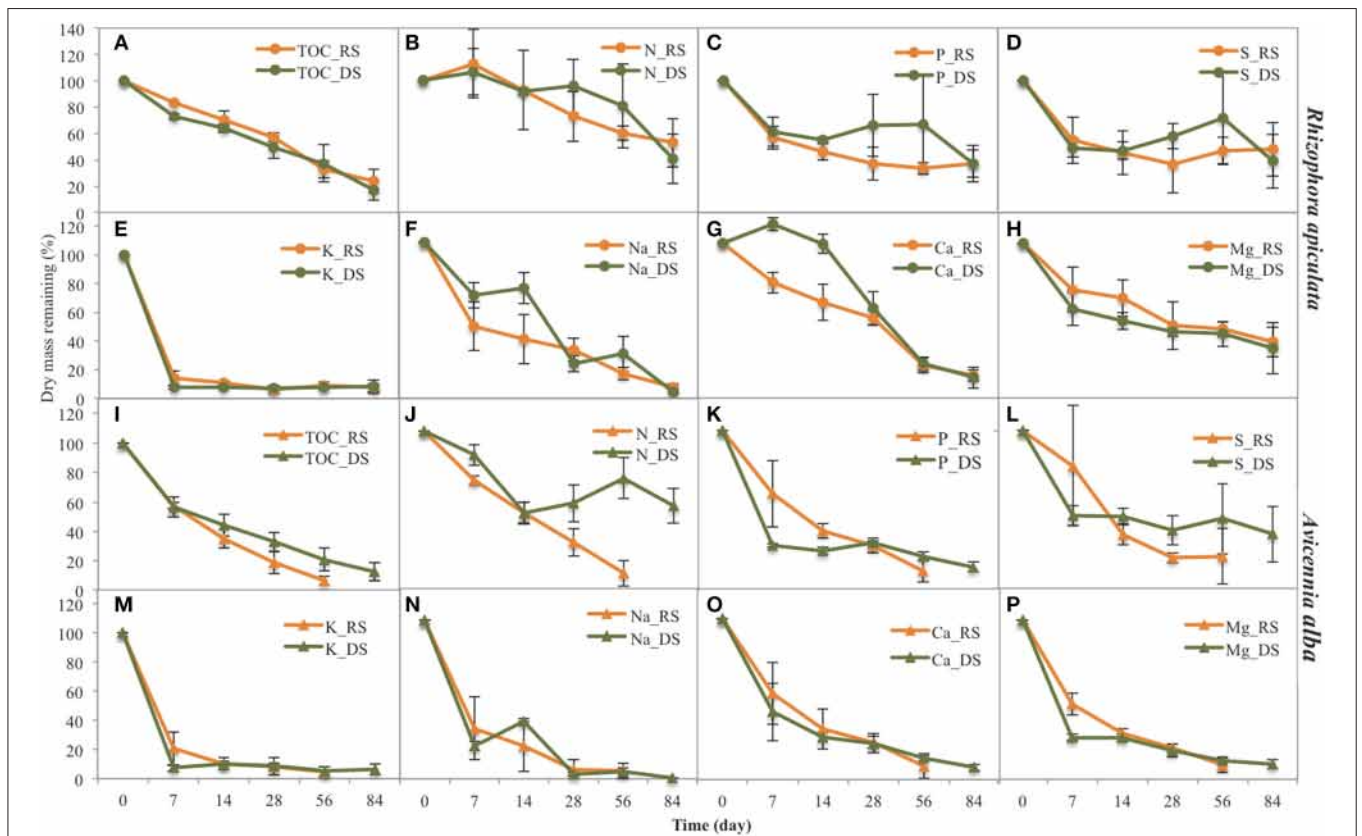
In the *Rhizophora* stand, most trace elements contents increased during the decay process for either season (Figure 6). During the rainy season, Co, Al, and Fe contents sharply increased during the first week, to  $\sim 80$ , 21, and 12 times that in the original DW, respectively. Cr, Ni, Cu, and Zn concentrations also increased during the first week, but not sharply. After the first week, the remaining Zn and Ni contents increased; whereas, Cr and Cu contents decreased before a rapid increase. During the dry season, most metal contents steadily increased during the decay process, with contents after 84 days being  $\sim 92$ , 29, 21, 8, 1, 3, and 3 times higher in the original DW for Co, Al, Fe, Cr, Ni, Cu, and Zn, respectively.

### Leaf Litter Decomposition in the *Avicennia* Stand

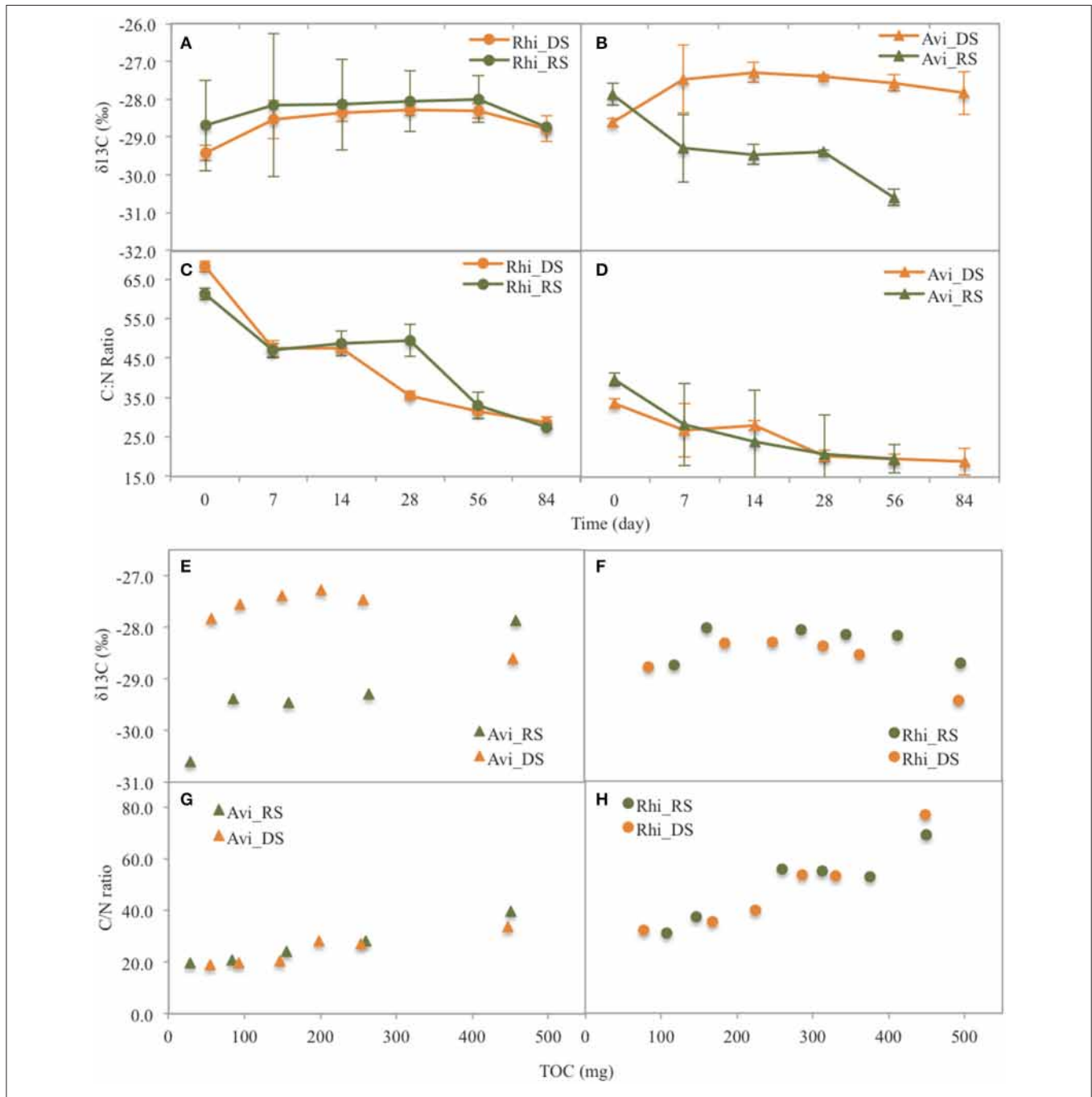
In the *Avicennia* stand, a higher decomposition rate was measured during the rainy season (with a  $t_{50}$  value of 12.2 days) compared to that measured during the dry season (with a  $t_{50}$  value of 36.5 days). At the end of the experiment, the leaf litter lost  $83.6 \pm 5.5\%$  during the dry season, and was completely decomposed during the rainy season (Figure 3). Decomposition rates decreased with time all along the experiment. OC mean  $t_{50}$  values were 31.5 and 14.7 days during the dry and rainy seasons, respectively. OC was released rapidly during the first week, decreasing of more than 40% DW irrespective of the season (Figure 4I). N was released throughout the experiment and at a faster rate during the rainy season, with mean  $t_{50}$  of 19.3 and 43.3 days during the rainy and the dry season, respectively. The initial C/N ratios ranged from 33.5 to 39.6 for the *Avicennia* leaves (Figure 5). C/N ratios decreased to 18.9 and to 19.6, during the dry and the rainy season, respectively. The  $\delta^{13}\text{C}$  values of the original yellow leaves ranged from  $-28.61$  to  $-27.86\%$ . Two different trends of  $\delta^{13}\text{C}$  shifts were observed during leaf litter decomposition (Figure 5). During the dry season, an enrichment was observed during the first week, followed by a stabilization



**FIGURE 3 |** Dry mass remaining of *Rhizophora* (Rhi) and *Avicennia* (Avi) leaves during the 84 days of the decomposition experiment. DS is the dry season, and RS is the rainy season. (A) *Rhizophora* dry season. (B) *Rhizophora* rainy season. (C) *Avicennia* dry season. (D) *Avicennia* rainy season.



**FIGURE 4 |** Remaining dry mass of several elements during leaf litter decomposition of the two species studied (*Rhizophora* and *Avicennia*) and at the two seasons (RS: rainy season, DS: dry season). (A) TOC for *Rhizophora*, (B) N for *Rhizophora*, (C) P for *Rhizophora*, (D) S for *Rhizophora*, (E) K for *Rhizophora*, (F) Na for *Rhizophora*, (G) Ca for *Rhizophora*, (H) Mg for *Rhizophora*, (I) TOC for *Avicennia*, (J) N for *Avicennia*, (K) P for *Avicennia*, (L) S for *Avicennia*, (M) K for *Avicennia*, (N) Na for *Avicennia*, (O) Ca for *Avicennia*, (P) Mg for *Avicennia*.



**FIGURE 5 |** Changes (with time and with TOC) of  $\delta^{13}\text{C}$  values and C/N ratios of the leaf litter of the two species *Rhizophora* (Rhi) and *Avicennia* (Avi) during leaf litter decomposition at the two seasons (DS: dry season, RS: rainy season). (A)  $\delta^{13}\text{C}$  with time for *Rhizophora*, (B)  $\delta^{13}\text{C}$  with time for *Avicennia*, (C) C/N with time for *Rhizophora*, (D) C/N with time for *Avicennia*, (E)  $\delta^{13}\text{C}$  with TOC for *Avicennia*, (F)  $\delta^{13}\text{C}$  with TOC for *Rhizophora*, (G) C/N with TOC for *Avicennia*, (H) C/N with TOC for *Rhizophora*.

and a depletion after 2 months. During the rainy season, we observed a  $\delta^{13}\text{C}$  depletion during the entire experiment, with the final value being 2.7‰ more negative than the original value (Figure 5).

K decomposition rates were the fastest of all the elements during the first week, with only 7.5 and 20.6% remained during the dry and the rainy season, respectively (Figures 4E,M). Na

showed a rapid release during the first week, with 20.8 and 31.8% of the original DW remained during the dry and rainy seasons, respectively. Na mean  $t_{50}$  values were 12.6 days and 14.1 during the dry and the rainy season, respectively (Table 2). K  $t_{50}$  values were 15.8 and 34.7 days during the rainy and dry seasons, respectively. Mg releases were rapid during the first week, and this decay was even more rapid during the rainy



**TABLE 2** | Simple negative exponential regression equations ( $X = ae^{-kt}$ ), % of litter mass remaining in the litterbags (X) with time (t) during decomposition of leaf litter in the studied *Rhizophora* and *Avicennia* stands.

Stands	Elements	Rainy season			Dry season		
		R	K	t <sub>50</sub>	R	K	t <sub>50</sub>
<i>Rhizophora</i>	Ca	0.979	0.023	30.1	0.970	0.027	25.7
	Mg	0.819	0.010	69.3	0.694	0.010	69.3
	Na	0.946	0.028	24.8	0.871	0.035	19.8
	K	0.356	0.019	36.5	0.207	0.015	46.2
	P	0.531	0.009	77.0	0.531	0.007	99.0
	S	0.210	0.005	138.6	0.210	0.005	138.6
	N	0.903	0.009	77.0	0.792	0.010	69.3
	OC	0.984	0.017	40.8	0.962	0.019	36.5
<i>Avicennia</i>	Ca	0.946	0.041	16.9	0.862	0.026	26.7
	Mg	0.906	0.039	17.8	0.703	0.022	31.5
	Na	0.768	0.049	14.1	0.842	0.055	12.6
	K	0.689	0.044	15.8	0.347	0.020	34.7
	P	0.951	0.036	19.3	0.570	0.016	43.3
	S	0.700	0.029	23.9	0.414	0.008	86.6
	N	0.997	0.036	19.3	0.930	0.016	43.3
	OC	0.976	0.047	14.7	0.930	0.022	31.5

R, coefficient of determination; k, decomposition constant; t<sub>50</sub>, half-life time.

season (Figures 4H,P), as 47.1 and 26.1% of the original DW remained after the first week during the dry and the rainy season, respectively. Mg mean t<sub>50</sub> were 31.5 and 14.7 days during dry and rainy seasons, respectively. P contents also showed a rapid decrease during the first week, but this decrease was higher during the dry season than during the rainy, with 27.7 and 60.0% of the original DW remaining (Figure 4K). P mean t<sub>50</sub> were 43.3 and 19.3 days during dry and rainy seasons, respectively. Ca was rapidly released during the first week irrespective of the season, with 42.3 and 53.4% of the original DW remaining during the dry and the rainy season, respectively. Considering the entire length of the experiment, Ca decomposition rates were higher during the rainy season than the dry season, with mean t<sub>50</sub> of 19.3 and 43.3 days (Figure 4O), respectively. S contents in the leaf litter also showed a rapid decrease during the first week and a slow immobilization afterward irrespective of the season (Figures 4D,L). S releases were higher during the rainy than during the dry season, with mean t<sub>50</sub> of 23.9 and 86.6, respectively.

In the *Avicennia* stand during the rainy season, Co, Cr, Ni, Fe, Cu, and Al contents sharply increased during the first week, reaching up to 20 times that in the original DW (Figure 7). Their contents rapidly decreased during the second week and then slightly decreased until the end of the experiment. During the dry season, most metal content in the leaf litter of *Avicennia* (Co, Cr, Ni, Fe, Cu, and Al) steadily increased during the decomposition process. The contents in the leaf litter after 84 days were ~2, 2, 2, 4, 1, and 5 times that in the original DW for Co, Cr, Ni, Fe, Cu, and Al, respectively. Only the Zn content slightly decreased during the first week and then showed a rapid increase at the end of decomposition process during the rainy season, reaching 224% of that in the original DW. However, during the dry season,

the Zn content slightly decreased during the first week before increasing to reach ~90% of that in the original DW at the end of the experiment. Conversely, the Mn content decreased during leaf litter decay. After the first week, 5.8 and 30.7% of that in the original DW remained in the *Rhizophora* stand, during the dry and the rainy season, respectively. In the *Avicennia* stand, 11.7 and 28.1% of that in the original DW remained after the first week during the dry and the rainy season, respectively. After the first week, the remaining Mn content ranged between ~20 and 40% irrespective of the season.

## Statistical Analysis

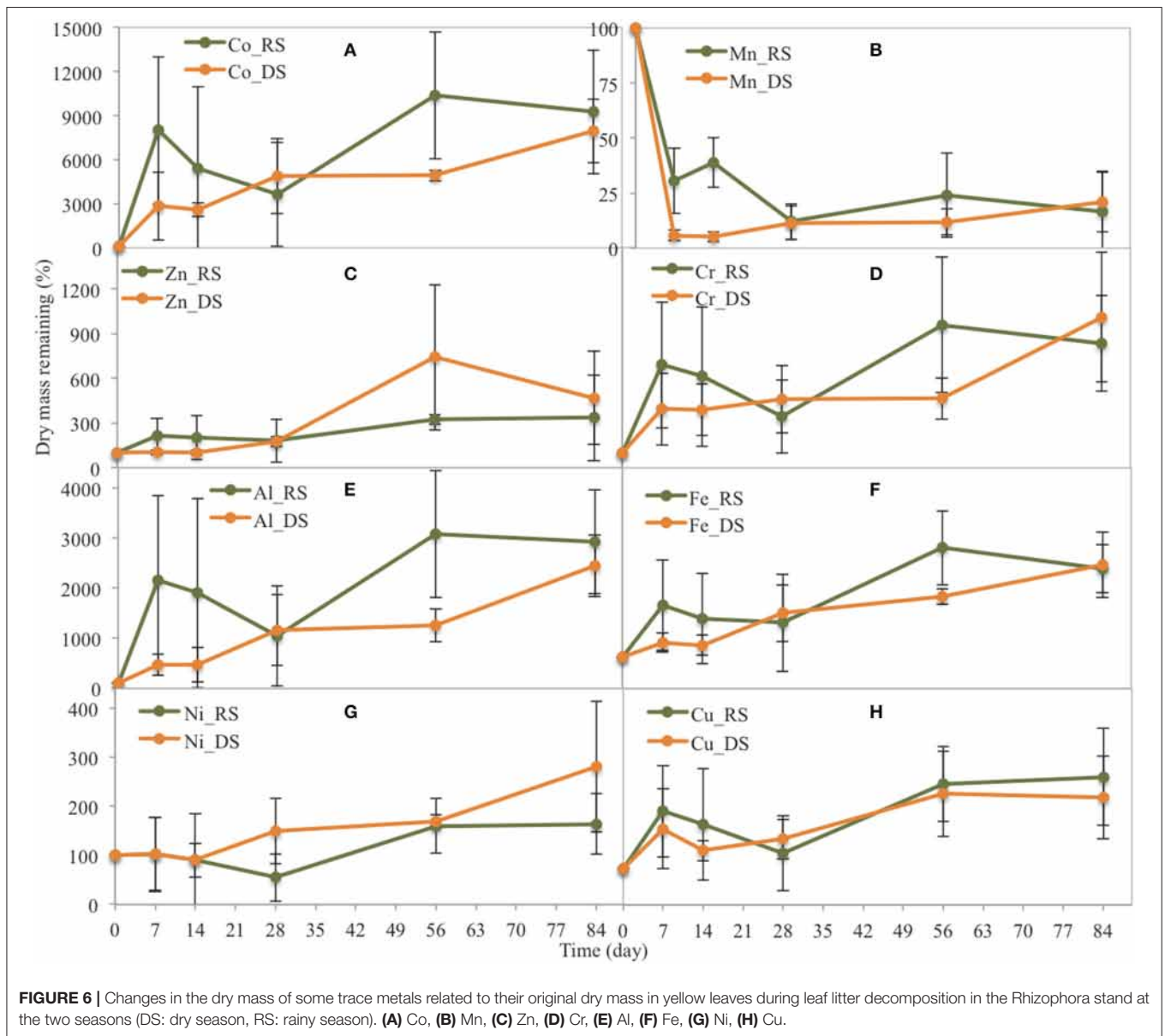
Decomposition rates were significantly different between mangrove species ( $p < 0.001$ ) and between seasons ( $p < 0.001$ ). The interaction between season and species also significantly influenced decomposition rates ( $p < 0.001$ ; Table 1). Decomposition rates were significantly higher in the *Avicennia* stand than in the *Rhizophora* stand in both seasons (Table 1 and Figure 3). Eventually, leaf litter decomposition rates of both species significantly varied during the 3 months of the experiments ( $p < 0.001$ ; Table 1), and were faster during the first week. The organic carbon (OC) decay rates of the leaf litter were significantly different between the *Rhizophora* and *Avicennia* stands ( $p < 0.001$ ). C/N ratios evolution during the decay processes were significantly different between species ( $p < 0.001$ ) but were not affected by season ( $p > 0.05$ ), and the interaction between species and season was also not significant ( $p > 0.05$ ). The  $\delta^{13}\text{C}$  values of the leaf litter during decay processes were significantly different between seasons ( $p < 0.001$ ) and were not affected by mangrove species ( $p > 0.05$ ). However, the interaction between season and species significantly influenced the  $\delta^{13}\text{C}$  values ( $p < 0.001$ ; Table 1).

## DISCUSSION

### Mangrove Leaf Litter Decay Rates

Decay rates vary with temperature, and thus decrease with latitude (Tam et al., 1998). In the present study, t<sub>50</sub> for *Rhizophora* leaves was ~43 days whatever the season, which was similar to previous studies for the same species at the same latitude in Malaysia or in Thailand (Boonruang, 1978; Ashton et al., 1999). For *Avicennia* leaves, t<sub>50</sub> reported in a Chinese mangrove was similar to the one we measured during the rainy season (<20 days) (Zhou et al., 2010), while in Pakistan, Shafique et al. (2015) reported a t<sub>50</sub> close to 50 days, little bit higher than what we measured during the dry season. During the rainy season, the river level was really higher, as observed in other studies (Schwarzer et al., 2016), and thus the *Avicennia* stand, which develops at a lower elevation than the *Rhizophora* one, was subject to an increased frequency of tidal inundation that is probably responsible for the fast decay rates measured. Ashton et al. (1999) reported that leaf litter immersed in seawater decomposed five times more quickly than one exposed to air. When leaves are wetted, there is initially a very rapid leaching of the most labile organic matter, increasing decomposition rates (Battle and Golladay, 2001). In addition, the tide's physical impact on the leaves may cause fragmentation and increased weight loss. Consequently, the position of the stand in the tidal zone and the

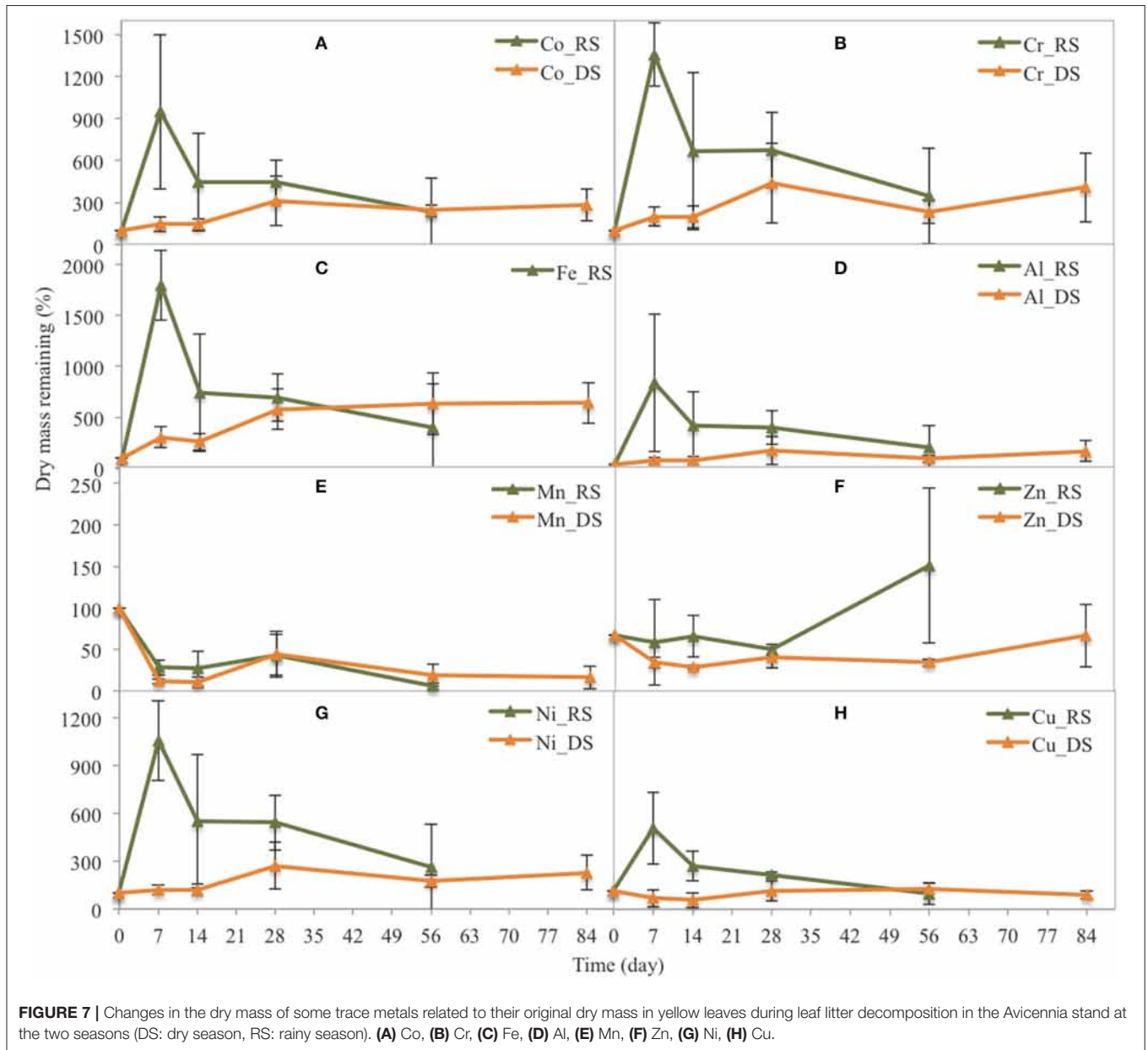




related length of tidal inundation may have an influence on the residence time of mangrove leaf litter, as observed by Ong et al. (1982) and then Twilley et al. (1986). Since the seasonal variation of leaf litter decay rates was almost negligible in the *Rhizophora* stand, while in the *Avicennia* stand the decay rate was almost twice as fast during the rainy season as compared to the dry season, we suggest that the intense rainfall occurring during the monsoon in Southern Vietnam strongly influenced decay rates by increasing the level of the river, while the role of temperature was limited.

In addition, the composition of the leaves of the two species differed, with the *Avicennia* leaves being less refractory to decomposition, with lower C/N values. This result is consistent with previous studies that demonstrated that litter

decomposition was faster with higher N concentrations in the leaves because of a higher nutritional value for decomposers (Pelegrai et al., 1997; Wafar et al., 1997). Conversely, *Rhizophora* leaves are rich in tannins (Kristensen et al., 2008), which are known to be aversive to detritivores and inhibit microbial activity. The rapid initial loss during the first week was already reported in previous studies that showed that mangrove leaf litter could lose half of its original DW during the first week (Twilley et al., 1986; Imgraben and Dittmann, 2008; Sánchez-Andrés et al., 2010). This initial loss represented the leaching of the most labile organic matter by the tidal action. The remaining leaf biomass, being more refractory, was decomposed more slowly and depended on the action of decomposers, bacterial, and fungal communities.



### Changes in TOC, C/N, and Major Elements During Mangrove Leaf Litter Decomposition

Through mangrove leaf litter degradation, nutrients are released to adjacent environments sustaining their primary productivity (Mfilinge et al., 2002). In the present study, during the decomposition process, we observed that the nutrient contents in the leaf litter of *Avicennia* and *Rhizophora* varied with time, mangrove species, and season. Nutrient releases were significantly faster in the *Avicennia* stand than in the *Rhizophora* stand irrespective of the season (Table 2). In addition, in the *Avicennia* stand, nutrient discharges were also significantly higher during the rainy season due to enhanced leaching as explained earlier, and all elements showed similar decay rates

than the bulk material. However, some differences between elements appeared during leaf litter decomposition in the *Rhizophora* stand during both seasons and in the *Avicennia* stand during the dry season. During mangrove leaf litter decomposition, C releases were very similar to the DW losses, while N releases were slower. Additionally, during the first week, *Rhizophora* leaves, with a lower initial N content than *Avicennia* leaves, gained N from external sources, reaching almost 120% of that in the original DW. This N gain most probably reflected the colonization of leaf litter by diverse microbial communities (Gautam et al., 2016). Nordhaus et al. (2017) also suggested an increasing portion of labile nitrogenous OM and total N in decomposing leaves over time were partly related to the activity of leaf-colonizing bacteria. Twilley et al. (1986) observed that during litter decomposition, the leaves of *Rhizophora* species sequestered

N from the environment, while *Avicennia* leaves released N to the environment. Consequently, C/N ratios decreased during leaf litter decomposition; and despite lower bulk decay rates in the *Rhizophora* stand, the C/N ratio decrease was more pronounced in this stand due to the original composition of the leaves. Bonanomi et al. (2010) suggested that litter C/N ratio progressively decreases until a threshold value is attained, which then allows a direct N release into the soil.

If OC decay rates were similar to the bulk material, some elements were characterized by faster rates of decomposition. K release was the fastest of all elements, losing more than 80 % of its original DW value during the first week regardless of the season in both the *Avicennia* and the *Rhizophora* stands (Figures 4E,M). Previous studies observed that K was the most mobile cation during leaf litter decay and that its leaching in the early stages of organic matter decomposition was very efficient (Alvarez et al., 2008; Bonanomi et al., 2010; Gautam et al., 2016). Bonanomi et al. (2010) suggested that the rapid K leaching reflected the presence of this element in plant tissues as a water-soluble salt not bound to complex organic molecules. This hypothesis may also be applied to Ca, Mg, and Na, which were characterized by higher decay rates than the bulk leaf litter. All these elements were characterized by a rapid leaching during the first week of the experiment, followed by a phase of immobilization or a slower release of nutrients possibly governed by the changes in refractory components. The P dynamic in the decomposing leaf litter was specific, characterized by both a rapid leaching during the first week, and decay rates during the length of the experiment lower than that of the bulk material. This result suggests that part of the initial P content was associated with non-structural components rapidly leached by the tidal action, but that another part was associated with refractory molecules. Another hypothesis was that P evolution during the later phase might be related to the P transfer from the forest floor to the decomposing leaf litter through fungal hyphae and/or bacterial immobilization (Lindahl et al., 2007). S showed the lowest decay rates. Similar to the other elements, S was characterized by a rapid leaching during the first week, and its mass stabilized very rapidly in the *Avicennia* stand but increased in the *Rhizophora* stand. Schroth et al. (2007) indicated that the highest fraction of S in the leaf litter was organic sulfide, which was the most reactive fraction, associated with a highly labile pool of organic matter. They also showed that the sulfonate fractional abundance increased during humification. We suggest that the soil surface in the *Rhizophora* stand may be more favorable to sulfato-reduction processes (Kristensen et al., 2017), with low Eh values (Vinh et al., 2019); and that consequently, sulfur compounds can be formed and be incorporated in the decomposing organic leaf material. It is generally accepted that inorganic S species react with functionalized molecules under very mild conditions (Damste and De Leeuw, 1990).

## Changes in Natural $^{13}\text{C}$ Abundance During Mangrove Leaf Litter Decomposition

OM sources in mangrove ecosystems have historically been identified using isotopic tools, notably  $\delta^{13}\text{C}$  (Bouillon et al.,

2008). However, to our knowledge, little attention has been paid to isotopic shift during mangrove leaf litter decomposition processes. In the *Avicennia* stand during the rainy season,  $^{13}\text{C}$  depletion was observed throughout the experiment. We suggest that fast organic matter decay rates, with  $t_{50}$  of the bulk material lower than 15 days, was responsible for this depletion. Gioacchini et al. (2006) observed that the decrease in  $\delta^{13}\text{C}$  during OM decomposition corresponded to the period of maximum mass loss, which would fit well with our observations. They explained this as the preferential microbial breakdown of the more easily degradable C sources, such as carbohydrates and cellulose, which are commonly  $^{13}\text{C}$  enriched compared to the more resistant C components, such as lignin (Benner et al., 1987). Consequently, enhanced preferential decomposition processes due to intense leaching are suggested to be responsible for the  $^{13}\text{C}$  negative shift. Conversely, during the first week of decomposition in the *Rhizophora* stand during both seasons and in the *Avicennia* stand during the dry season,  $^{13}\text{C}$  enrichment was measured. This may result from the incorporation of C originated from microbial biomass, characterized by higher  $\delta^{13}\text{C}$  values, into the decomposed litter. This hypothesis may be supported by the N gain of *Rhizophora* leaf litter during the first week of the experiment (Figures 4B). Recently, Yang et al. (2018) suggested that  $^{13}\text{C}$  enrichment during mangrove leaf litter decomposition was related to fungal and bacterial respiration. However, Preston et al. (2009) suggested that microbial biomass constituted only a small fraction of decomposing litter and may not have any major influence on  $\delta^{13}\text{C}$  dynamics, which may limit our hypothesis. In these stands at those seasons,  $^{13}\text{C}$  depletion was measured after 2 months of decomposition, which may reflect the preferential preservation of lignin compounds characterized by lower  $\delta^{13}\text{C}$  values, as observed for the *Avicennia* stand during the rainy season. This process may occur later as lower decomposition rates were measured in these conditions. Consequently, in our experiment, we measured both  $^{13}\text{C}$  depletion and enrichment during leaf litter decomposition that were possibly related either to microbial fractionation or to selective preservation of lignin compounds. The main driver of these  $\delta^{13}\text{C}$  shifts in our study seemed to be the decomposition rates.

## Trace Metals Enrichment During Leaf Litter Decomposition

An enrichment of trace metals (Fe, Al, Co, Cr, Ni, Zn, and Cu) in the decomposing leaf litter of *A. alba* and *R. apiculata* was observed irrespective of the season. Only Mn had the same evolution as the major elements described earlier with rapid leaching during the first week and <10% of the value in the original DW remaining, followed by an immobilization. Silva et al. (1998) observed that Mn was more susceptible to leaching than other metals, which were frequently associated with refractory structural compounds. Manganese is an essential micronutrient, participating in the structure of photosynthetic proteins and enzymes (Mukhopadhyay and Sharma, 1991). Foliar tissue contains predominantly  $\text{Mn}^{2+}$ , which can be solubilized during leaf litter decomposition, explaining its fast release. However, in a terrestrial forest, it was also observed that Mn

can be rapidly immobilized as  $Mn^{3+/4+}$  oxides, resulting in an enrichment of the remaining litter (Keiluweit et al., 2015). The specific conditions of the mangrove forest floor, the tidal immersion, and its richness in organic matter that lead to anoxic conditions (Vinh et al., 2019) may prevent  $Mn^{2+}$  oxidation and its subsequent immobilization.

Regarding the other trace metals, we suggest that the main cause for metal enrichment in the litter was the trapping of dissolved metals that were present in the water column at high tide and at the soil surface, which contains trace metals in different forms depending on the redox conditions. Previous studies observed enrichment of trace metals of the remaining litter during its decomposition, up to several hundred times (Zawislanski et al., 2001; Scheid et al., 2009). Mangrove soils are known to be a sink for trace metals due to their richness in organic matter and possible sulfide precipitation (Noel et al., 2014; Marchand et al., 2016). Dissolved metals can be complexed by organic molecules present in the litter or by the uptake by bacteria decomposing the litter. Thanh-Nho et al. (2018) described the distribution of trace metals in the Can Gio estuary, demonstrating that during the monsoon season enhanced amounts of trace metals were delivered to the estuary, both in particulate and dissolved phases. In addition, we observed that trace metal enrichment during mangrove leaf litter decomposition was higher in the *Rhizophora* stand than in the *Avicennia* stand. Taking into account that the *Rhizophora* stand was less frequently inundated than the *Avicennia* stand, we would suggest that the forest floor was the main source of trace metals, or that the organic molecules composing the *Rhizophora* leaves and their decomposers are more efficient in trapping trace metals. In the Can Gio mangrove forest beneath the *Rhizophora* stand, Thanh-Nho et al. (2018) measured lower trace metal concentrations in the solid phase compared to a higher level in the dissolved phase, which may explain the results obtained in the present study, dissolved metals being more bioavailable. In addition, as described in a previous section, the N content increase in the *Rhizophora* litter during the first week may translate the rapid development of bacteria, inducing enhanced metal uptake by decomposers. In the *Avicennia* stand during the rainy season, when decomposition processes were the most efficient, trace metal releases were measured after the first week of the experiment. We suggest that part of the organic molecules that had trapped trace metals during the first week may have been decomposed, leading to metals releases.

## CONCLUSIONS

The tropical planted mangrove studied (Can Gio, Vietnam) is ~40 years old, and is characterized by a specific zonation of the ecosystem, with planted *Rhizophora* trees developing at the highest elevation of the intertidal zone and *Avicennia* trees naturally colonizing the lower intertidal zone along rivers and creeks. The litterbags experiments conducted during this study provide evidence that changes in leaf litter quality during decomposition processes on forest floor depended on the mangrove species, its position in the intertidal zone, and the

season. The intense rainfall occurring during the monsoon strongly increased the frequency of tidal inundation of the *Avicennia* stand, which possibly resulted in an enhanced leaching and explaining the faster decay rates measured. We also suggest that *Avicennia* leaves characterized by higher N content and lower C/N ratios, were more labile than the *Rhizophora* leaves. Whatever the season or the species, some elements (K, Ca, Mg, and Na) were subject to a rapid leaching during the first week of the experiment, reflecting their probable presence in plant tissues as a water-soluble salt not bound to complex organic molecules. These elements can thus be rapidly available for different organisms or adjacent ecosystems. Conversely, S mass increased in the *Rhizophora* leaf litter, most probably because sulfur compounds were formed in anoxic conditions in the forest floor and then incorporated in the decomposing organic leaf material. All trace metals studied, except Mn, also showed enrichment in the leaf litter during their decomposition irrespective of the stand or the season, possibly trapped by organic molecules and/or the decomposers. Eventually, both  $^{13}C$  depletion and enrichment during mangrove leaf litter decay processes were observed. Depletion may be related to the enhanced decay rates in the *Avicennia* stand during the rainy season, and reflects the fast decomposition of the most labile compounds and the preferential preservation of lignin compounds. The enrichment observed in the other conditions may possibly result from the development of bacteria, but more studies must be performed to confirm this hypothesis. The seasonal and species differences in leaf litter decay processes may have impacts on the ecological functioning of the Can Gio estuary. Due to the position of the *Avicennia* stand, close to tidal creeks, and the lability of their leaves, their decomposition products may be useful in sustaining heterotrophic activity in adjacent waters and may be the basis of the trophic food chain. Conversely, *Rhizophora* leaf litter may accumulate in soils, and their decomposition products may be more available for mangrove plants and detritus consumers within the soil.

## DATA AVAILABILITY STATEMENT

All datasets generated for this study are included in the article/supplementary material.

## AUTHOR CONTRIBUTIONS

CM, TV, and MA performed the experimental design. TV and KL performed data collection and statistical analyses. CM and TV performed the data interpretation. TV, CM, KL, and MA wrote the paper.

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**Conflict of Interest:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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