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Effects of elevated CO_2 concentrations on ^{13}C fractionation during photosynthesis, post-photosynthesis and night respiration in mangrove saplings *Avicennia marina* and *Rhizophora stylosa*

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Abstract

Carbon fractionation ($\Delta^{13}C$) is well documented for various plants functional types. Yet, specific studies on $\Delta^{13}C$ on mangroves are particularly rare although they have a key role in coastal carbon (C) cycling. In this study, we investigated the ^{13}C exchanges between leaves and the atmosphere and between the main plant's organs in two common mangroves species, *Avicennia marina* and *Rhizophora stylosa* subjected to two different CO_2 concentrations. Two-years-old saplings were grown in mesocosms during one year under 400 ppm and 800 ppm of CO_2. At the end of the experiment, the isotopic value of the night-respired CO_2 was measured on six individuals for each species and CO_2 treatment. Then, 60 saplings were harvested to measure the organs $\delta^{13}C$ values, and, finally, carbon fractionation ($\Delta^{13}C$) during photosynthesis, post-photosynthesis and apparent $\Delta^{13}C$ during night respiration were calculated. Results indicated that elevated CO_2 reduced $\Delta^{13}C$ during photosynthesis by 13 % and during night respiration by 20 %. Alongside, within-plant $\Delta^{13}C$ was twice higher in the saplings grown under elevated CO_2 concentrations. These results showed that ongoing and future increases in atmospheric CO_2 concentrations have the potential to modify the $\delta^{13}C$ values of mangrove trees. These results could have important implications in Blue Carbon sciences, and particularly in the comprehension of future carbon cycling in coastal wetlands, mangroves being an essential link in terrestrial and marine food webs along tropical and subtropical coastlines.

Keywords: Mangrove, Carbon isotopes, Greenhouses experiment, Elevated CO_2 concentrations, New Caledonia

1. Introduction

Mangroves are forested ecosystems mainly composed of C_3 halophytic trees that develop in intertidal areas of tropical and subtropical coastlines. Mangroves are considered as major ecosystems in the coastal carbon cycle, and were therefore integrated into the “Blue Carbon” ecosystems list (Donato et al., 2011; Lovelock and Duarte, 2019; Mcleod et al., 2011). This specificity results from their particular ecological functioning, featuring (i) high primary productivity (Bouillon et al., 2008), (ii) permanent water logging favouring anaerobic conditions of their soils that strongly limits mineralisation processes (Kristensen et al., 2017), (iii) high quantity of recalcitrant lignin materials (Marchand et al., 2005), and (iv) large and long-term C sequestration capacities (Donato et al., 2011). Consequently, mangroves have been undergoing special attention for the last twenty years, being of great importance in climate change mitigation studies (Howard et al., 2017; Macreadie et al., 2019).

Plant carbon isotopes ratios ($\delta^{13}C$) is a powerful tool to study ecological processes at the ecosystem scale and thus to improve our knowledge of the past, current and future ecosystems functioning (*e.g.* Pypker *et al.*, 2008; Werner *et al.*, 2012), but also our comprehension of ecosystems-climate interactions (*e.g.* Arens *et al.*, 2000; Diefendorf *et al.*, 2010). At the scale of the plant individual, isotope studies allow the identification of the different metabolic mechanisms and carbon (C) pathways. As plants incorporate preferentially ^{12}C rather than the heavier ^{13}C, fractionation ($\Delta^{13}C$) occurs during photosynthesis, resulting in plants being generally ^{13}C-depleted compared to the atmosphere (Farquhar et al., 1982, 1989; O’Leary, 1981). In C_3 plants, C fractionation during photosynthesis has been modelled by Farquhar *et al.* (1989). In its simplest form, this model is based on the ^{13}C discrimination during: (i) CO_2 diffusion through the plant stomata (~4.4 ‰), and (ii) carboxylation by the enzyme Rubisco (~29 ‰). However, plants organic matter

(OM) $\delta^{13}C$ values not only reflect photosynthesis fractionation, but also integrate post-photosynthesis fractionation processes such as: (i) within-plant C fractionation that conducts to a $\delta^{13}C$ gradient throughout the leaves-roots continuum (Badeck et al., 2005; Cernusak et al., 2009), and (ii) apparent night-respiration fractionation, in which enriched ^{13}C-CO_2 is produced compared to leaves OM $\delta^{13}C$ (Bathellier et al., 2017; Ghashghaie et al., 2003; Ghashghaie and Badeck, 2014; Tcherkez et al., 2010; Werner and Gessler, 2011). C fractionation in plants showed to be highly variable, depending on many environmental factors (Cernusak et al., 2013). Particularly, the effects of atmospheric CO_2 concentrations on C fractionation have been investigated during the last decade. Unfortunately, no clear patterns were identified, as CO_2 concentrations showed to increase, decrease or not affect $\Delta^{13}C$ (see Schubert & Jahren, 2012 and Zhang *et al.*, 2019 and references therein).

If there is a growing interest in studying plants carbon fractionation, specific studies on mangroves are more rare and only a small handful of scientists focused on this topic so far (*e.g.* Saintilan et al 2013; Weiss et al 2016; Kelleway et al 2018). However, the comprehension of the effects of CO_2 concentrations on $\Delta^{13}C$ is of particular interest considering ongoing and future global changes. Indeed, if the current trend of extensive fossil fuel burning continues, CO_2 levels may reach between 794 and 1,150 ppm at the end of the century (Collins et al., 2013), which could have strong repercussions on Blue Carbon ecosystems functioning and their feedback on climate change.

Within this context, our objectives were to evaluate the effects of elevated CO_2 concentrations on the carbon isotope composition of the two most widespread mangrove species throughout the Indo-Pacific region, *Avicennia marina* and *Rhizophora stylosa* (Duke et al., 2008; Ellison et al., 2008). To address these objectives, we focused on three-year-old mangrove saplings, grown for two year in greenhouses prior being submitted to two different atmospheric CO_2 concentrations (ambient and double than ambient) for an additional year of growth. At the end of this growth period, the bulk $\delta^{13}C$ value of the leaves, stems and roots, and the $\delta^{13}C$ value of the CO_2 exchanged at the leaves level during the day and at night were measured.

2. Materials and Methods

2.1 Saplings growth and CO_2 enrichment

The present study was conducted in New Caledonia in a CO_2-enrichment complex constituted of three semi-open greenhouses of 72 m² each (22°13′49″S, 166°31′09″E). A circular closed chamber (36 m², 2.4 m height) was built inside each greenhouse, allowing atmospheric CO_2 concentrations to be controlled and monitored. This study was performed concomitantly to the studies of Jacotot *et al.* (2018, 2019b), in which readers can find a full description and pictures of the facility, as well as background information on the saplings and their response in term of growth, biomass and leaves-gas exchanges to elevated CO_2 concentrations. However and briefly, over 1,000 *Avicennia marina* and *Rhizophora stylosa* propagules, collected in 2014, were planted in a 2.5-liters mixture of mangrove peat and sand and placed on custom tidal tables that simulated the tidal variation naturally occurring in mangrove ecosystems. The water table level during high tide was fixed at 5cm above the soil surface, submerging completely the root system of the saplings. After two years of growing in a nursery, half of the saplings was assigned to ambient atmospheric CO_2 concentrations (ambient, 400 ppm) and the other half to elevated concentrations (800 ppm). The CO_2 enrichment started in June 2016 and lasted for one complete year, featuring periodical rotations of the saplings between tidal tables, greenhouses and closed chambers to avoid a positional effect.

2.2 $\delta^{13}C$ value of leaves respired CO_2 at night

Meantime, the $\delta^{13}C$ values of the night respired CO_2 ($\delta^{13}C\text{-}CO_{2\text{-}NR}$) were measured *in-situ* thanks to a G2131-*i* CRDS analyser (Picarro Inc., Santa Clara, CA, USA). Guaranteed precision by the manufacturer is for $\delta^{13}C\text{-}CO_2$ (5 min measurement, 1-σ) < 0.1 ‰ between 380 and 1000 ppm of CO_2. First, six individuals of each species and treatment were randomly chosen (two in each greenhouse and each closed chamber) and the youngest fully expended leaf of each chosen sapling was trapped in a transparent incubation chamber (9 cm^2; 9 cm^3) connected to the analyser. For each leaf, a 15-min incubation was performed with a permanent monitoring of the variation of the CO_2 concentration and the $\delta^{13}C$ value inside the chamber. Then, a Keeling plot mixing model was used to separate the $\delta^{13}C\text{-}CO_2$ value resulting from leaf respiration ($\delta^{13}C\text{-}CO_{2\text{-}NR}$) from the $\delta^{13}C\text{-}CO_2$ value of the background atmosphere (Keeling, 1961, 1958). Briefly, a linear regression is fitted to the relationship between the measured $\delta^{13}C$ value and the inverse of the corresponding CO_2 concentration ($1/CO_2$). Then, the intercept of the fitted line at the Y axis gives the value of $\delta^{13}C\text{-}CO_{2\text{-}NR}$ (Pataki et al., 2003). All measurements were realized at night, between 8:00 pm to 11:00 pm.

2.3 $\delta^{13}C$ value of saplings organs

After gas measurements, 60 saplings from both species and CO_2 treatments were randomly selected and harvested. Selected saplings were separated into leaves, stems and roots, dried at 60 °C until a constant weight was achieved and then were ground using a ball mill. Then, six batches of leaves, stems and roots were constituted for each species and CO_2 treatment, each batch containing 10 individuals. For each batch, one subsample (approximately 2 mg) was analysed for $\delta^{13}C$ using an isotope ratio mass spectrometer coupled with an elemental analyser (Integra2, Sercon, UK). $\delta^{13}C$ values of saplings organs were reported in per mil (‰) deviations from Pee Dee Belemnite (PDB). The analytical precision of the elemental analyser was checked using IAEA-600 caffeine standard (IAEA Nucleus) and was less than 0.3 % for $\delta^{13}C$.

2.4 Estimation of carbon discrimination during photosynthesis (Δ_P) and dark respiration ($\Delta_{NR}^{13}C$)

Carbon fractionation during photosynthesis has been calculated thanks to the simplified version of the linear model of Farquhar *et al.* (1989):

$\Delta_P{}^{13}C = (\delta^{13}C\text{-}CO_2 - \delta^{13}C_l) / (1 + \delta^{13}C_l / 1000)$

with $\Delta_P{}^{13}C$ the C fractionation during photosynthesis (‰), $\delta^{13}C\text{-}CO_2$, the $\delta^{13}C$ value (‰) of the atmospheric CO_2 surrounding the saplings and $\delta^{13}C_l$, the $\delta^{13}C$ value (‰) of the leaves OM. Apparent C fractionation during dark night respiration ($\Delta_{NR}{}^{13}C$) was calculated similarly as in Ghashghaie & Badeck (2014) and in Zhu and Cheng (2011) by the difference between the $\delta^{13}C$ values of the leaves and that of the background atmosphere. The $\delta^{13}C\text{-}CO_2$ values of the surrounding atmosphere in greenhouses (ambient treatment) and in closed chambers (high treatment) were monitored during the same days of gas exchange measurements using the CRDS analyser. Although it was not possible to monitor the values of $\delta^{13}C\text{-}CO_2$ throughout the enrichment year, the similar variation in CO_2 concentrations during the days of measurement (present study) compared to the full year (Jacotot et al., 2019a, 2018) suggests that the $\delta^{13}C\text{-}CO_2$ values followed a similar pattern and are therefore representative of the whole experiment. In this study, the $\delta^{13}C\text{-}CO_2$ values of the background atmosphere were respectively -8.2 ± 0.23 ‰ and -18.37 ± 0.69 ‰ for the ambient and elevated CO_2 concentrations.

2.5 Statistical analyses

Significant differences (P<0.05) in $\Delta_P{}^{13}C$ between the two CO_2 treatments for each species were tested thanks to Student t-tests after verification of normality and equality of variance using Shapiro and Fisher tests and thanks to a Mann-Whitney U test for As, gs and $\Delta_{NR}{}^{13}C$. A Kruskal-Wallis test was used to compare the $\delta^{13}C$ values between each saplings' organs. Both species were analyzed independently. All statistical analyses were performed using R software version 3.6.2 (R Development Core Team, Vienna, 2008). All values are reported with means ± SEM.

3. Results

3.1 $\delta^{13}C$ values of the saplings' organs

The $\delta^{13}C$ values of leaves, stems and roots for the two species and the two CO_2 treatments are presented in Fig. 1. Under ambient CO_2 concentrations, the $\delta^{13}C$ values ranged from -27.67 to -24.73 ‰, while they ranged from -35.28 to -30.77 ‰ under elevated CO_2 concentrations. For both species and both CO_2 treatments, a significant increase of the $\delta^{13}C$ values from the upper to the lower organs (leaves < stems < roots) was observed (Fig. 1 and Table 1), except for the $\delta^{13}C$ values of leaves and stems of *A. marina* for which the difference was not significant (Table 1). In addition, the difference in the $\delta^{13}C$ values between each organ within each species was twice higher in the plants that grown under elevated than under ambient CO_2 concentrations (Table 2), at the exception of the stems-roots difference for *A. marina* that decreased under elevated CO_2.

Table 1: *P*-values of the significant differences (<0.05) in the $\delta^{13}C$ *values* (‰) between the saplings' organs after a Kruskal-Wallis test (*NS*: Non-significant).

	Avicennia marina		*Rhizophora stylosa*	
	Stems	Roots	Stems	Roots
Ambient CO_2	$K_{(2)}=11.68$		$K_{(2)}=15.16$	
Leaves	0.133^{NS}	0.011	0.011	0.011
Stems		0.043		0.011
Elevated CO_2	$K_{(2)}=15.16$		$K_{(2)}=15.16$	
Leaves	0.011	0.011	0.011	0.011
Stems		0.011		0.011

Table 2: Within-plant fractionation (‰) in *Avicennia marina* and *Rhizophora stylosa*.

	Avicennia marina			*Rhizophora stylosa*		
CO_2 levels	Leaves-roots	Leaves-stems	Stems-roots	Leaves-roots	Leaves-stems	Stems-roots
Ambient (400 ppm)	1.55	0.61	0.94	1.66	1.40	0.26
Elevated (800 ppm)	3.09	2.55	0.54	3.82	2.90	0.92

3.2 δ^{13}C values of the CO_2 emitted during night respiration

The $\delta^{13}C\text{-}CO_{2\text{-}NR}$ values of the night-respired CO_2 measured in June 2017, the harvest month, were for *A. marina* and *R. stylosa* respectively, -21.64 ± 0.33 and -22.68 ± 0.38 ‰ under ambient CO_2 levels, and -30.37 ± 0.15 and -31.17 ± 0.21 ‰ under elevated CO_2 concentrations (Table 3).

Table 3: *$\delta^{13}C$ offset (‰) of the night-respired CO_2 ($\delta^{13}C$-CO_2 and* carbon fractionation *values (‰) during photosynthesis ($\Delta_P\delta^{13}C$) and night respiration* ($\Delta_{NR}\delta^{13}C$) in *Avicennia marina* and *Rhizophora stylosa* grown under ambient (400 ppm; *$\delta^{13}C$-CO_2 of the gas: -8.2 ± 0.23 ‰)* or elevated (800 ppm; *$\delta^{13}C$-CO_2 of the gas: -18.37 ± 0.69 ‰*) CO_2 concentrations.

	Avicennia marina		*Rhizophora stylosa*	
CO_2 levels	400 ppm	800 ppm	400 ppm	800 ppm
$\delta^{13}C$-$CO_{2\text{-}NR}$	*-21.64 ± 0.33*	*-30.37 ± 0.15*	*-22.68 ± 0.38*	*-31.17 ± 0.21*
$\Delta_P\delta^{13}C$	*19.91 ± 0.05*	*17.29 ± 0.07*	*19.05 ± 0.17*	*16.58 ± 0.11*
$\Delta_{NR}\delta^{13}C$	-4.88 ± 0.51	-3.89 ± 0.29	-5.10 ± *0.58*	-4.01 ± *0.26*

3.3 Calculation of $\Delta^{13}C$ during photosynthesis and night respiration

Calculated values of C fractionation during photosynthesis ($\Delta_P{}^{13}C$) and during night respiration ($\Delta_{NR}{}^{13}C$) at the time of measurement (in June 2017) are reported in Table 3. A significant decrease in $\Delta_P{}^{13}C$ was observed for *A. marina* ($t_{(10)}$=29.58, p<0.001) and *R. stylosa* ($t_{(10)}$=12.31, p<0.001) that grown under elevated CO_2 concentrations compared to the saplings grown under ambient ones. Similarly, elevated CO_2 levels significantly reduced $\Delta_{NR}{}^{13}C$ for both species (*A. marina*: U=10.5; p<0.05 and *R. stylosa*: U=5.5; p<0.05).

4. Discussion

4.1 Carbon post-photosynthetic fractionation throughout the plant-roots continuum

In our study, the organs' $\delta^{13}C$ values of the saplings grown in the greenhouses under ambient CO_2 concentrations (Fig. 1) were typical of the ones of mangrove species (Hayase et al., 1999; Jacotot et al., 2019b; Kelleway et al., 2018; Mckee et al., 2002; Reef et al., 2015;

Saintilan et al., 2013; Wei et al., 2008b, 2008a; Weiss et al., 2016). However, whatever the species or the CO_2 treatment, we observed a ^{13}C-enrichment from the leaves to the roots, the leaves being the more ^{13}C-depleted organs (Fig. 1). This gradient of $\delta^{13}C$ values along the plant-roots continuum indicates post-photosynthesis C fractionation within the plant tissues (Ghashghaie and Badeck, 2014). Post-photosynthesis C fractionation has been documented in terrestrial plants, however the mechanisms behind this process are not yet fully understood, and various hypotheses have been suggested (*e.g.* Badeck *et al.*, 2005; Brandes *et al.*, 2006; Cernusak *et al.*, 2013; Zhang *et al.*, 2017). Briefly, these hypotheses include for example: (i) a ^{13}C-enrichment of the leaves-respired CO_2 compared to a ^{13}C-depletion of the CO_2 respired by heterotrophic organs, (ii) C fractionation during phloem transportation, (iii) lower rates of C fixation by PEP (phosphoenolpyruvate) carboxylase enzyme in leaves than in heterotrophic organs, which discriminates over ^{13}C, (iv) different allocation between ^{13}C-enriched carbohydrates produced during the day and ^{13}C-depleted ones produced at night, or even (v) growth of heterotrophic tissues taking place during seasonal periods associated to lower ^{13}C discrimination in comparison to leaves growth. All details on the six main hypotheses can be found in the review of Cernusak *et al.* (2009), later summarized in Ghashghaie & Badeck (2014). In addition to these hypothesis, other specific mechanisms for salt-water species (such as mangroves) may be involved in post-photosynthesis C fractionation. For instance, the diffusion within the stems and roots tissues of allochthonous ^{13}C-enriched CO_2/HCO_3^- at the plant/water interface during high tide may be involved in the ^{13}C enrichment of these tissues in comparison to the leaves (Kelleway et al., 2018). Nevertheless, the ^{13}C enrichments of the stems and roots relatively to the leaves calculated in our study for *A. marina* and *R. stylosa* (Table 2 and Fig. 1) under ambient CO_2 concentrations were comparable to the values reported for C_3 plants (see the review of Badeck *et al.*, 2005). Concerning mangrove species, only three other studies have, so far, reported within-plant C fractionation values, or at least

$\delta^{13}C$ values for leaves and roots that allow its determination (Table S1 in Supplementary data). Of these studies, two were conducted in South Australia (29-38°S; Saintilan *et al.*, 2013; Kelleway *et al.*, 2018)) and one in Indonesia (02°N-07°S; (Weiss et al., 2016)). Since New Caledonia is located between these two areas (22°S), our study therefore provides complementary values of post-photosynthetic C fractionation in mangroves along their latitudinal and climatic distribution. Eventually, elevated CO_2 concentrations have modified the post-photosynthesis C fractionation by increasing its value for both species, at the exception of the stems to roots fractionation of *A. marina* (Table 2). One hypothesis that can explain this effect is that the saplings produced ^{13}C-enriched metabolites under elevated CO_2 because of the reduction of $\Delta_P{}^{13}C$. These enriched metabolites would then be transferred to the stems and roots, causing both an enrichment of the stems/roots $\delta^{13}C$ and a depletion of the leaves $\delta^{13}C$, increasing the difference of $\delta^{13}C$ between these organs. We suggest that this hypothesis should now be investigated in future studies.

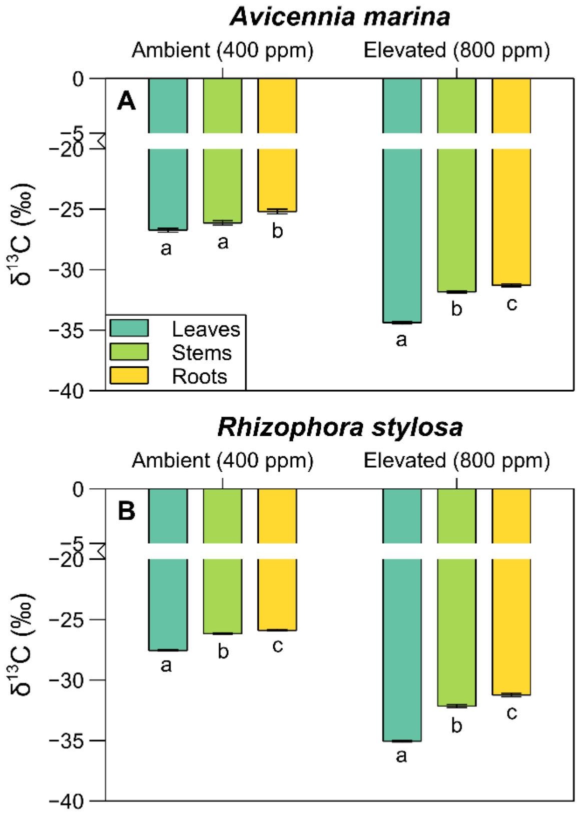

Figure 1: $\delta^{13}C$ values (‰) of leaves, stems and roots under ambient and elevated CO_2 concentrations. A) *Avicennia marina* and B) *Rhizophora stylosa*. Means ± SEM (*n*=6 for each species, organ and CO_2 level). Different letters indicate significant differences. CO_2 treatments must not be compared between each other.

4.2 Elevated CO_2 reduced carbon discrimination during photosynthesis

This study has provided the first values of C fractionation during photosynthesis ($\Delta_P{}^{13}C$) of the two common mangroves species, *Avicennia marina* and *Rhizophora stylosa.* When grown under ambient CO_2 concentrations, $\Delta_P{}^{13}C$ values in these two species were close to 20 ‰, which is typical of C_3 plants (Farquhar et al., 1982; Kohn, 2010; Vogel, 1980; Zhang et al., 2019). However, when grown under elevated CO_2 concentrations, $\Delta_P{}^{13}C$ values were significantly reduced in both species (Table 3). These results may suggest that $\Delta_P{}^{13}C$ and CO_2 concentrations were negatively correlated, which is in agreement with previously published studies (Zhang et al., 2019). The variation of stomatal conductance to CO_2 (gs) and of net assimilation (As), which are two parameters that vary with CO_2 concentrations, can be proposed to explain this reduction in $\Delta_P{}^{13}C$ with elevated atmospheric concentrations. First, we observed in our previous study a decrease by *ca.* 29 % of gs with 800 ppm of CO_2 in the atmosphere (Jacotot *et al.*, 2018). A negative correlation between gs and CO_2 concentration has already been reported in previous published studies (Del Amor, 2013; Franks and Beerling, 2009; Lammertsma et al., 2011), even for mangroves (Reef et al., 2015). The decrease in gs may have triggered the plant to use a higher fraction of the ^{13}C available in the leaves pore spaces, thereby reducing the $\Delta_P{}^{13}C$, as suggested by Lockheart *et al.* (1998). In their model, Farquhar *et al.* (1989) also suggested that the relationship between $\Delta_P{}^{13}C$ and CO_2 concentrations strongly depends on gs. Secondly, elevated CO_2 concentrations resulted in significant stimulation of As, by more than 76 and 93% for *A. marina* and *R. stylosa,* respectively , as observed in Jacotot *et al.*, 2018. These stimulations may have involved the use of a higher fraction of the available ^{13}C in the leaves, which in turn reduced $\Delta_P{}^{13}C$, as suggested in other studies (Assmann, 1999; Lockheart et al., 1998; Sekiya and Yano, 2008; Zhang et al., 2019). However, both gs and As can vary in response to the instantaneous micro-fluctuations of environmental factors such as for example temperature and light availability (Aasamaa and Sõber, 2011; Atkin and Tjoelker, 2003; Bunce, 1997; Lammertsma et al., 2011; Merilo et al., 2014; Sage and Kubien, 2007; Sharkey, 1985). Consequently, these two parameters highly fluctuate over the course of the day and may therefore drive the short-term variations of $\Delta_P{}^{13}C$ (*i.e.* the diurnal variation) but not the long-term ones. We, thus, suggest that a third physiological process may also be involved in the long-term variation of $\Delta_P{}^{13}C$ with CO_2 concentrations. In fact, plants reduce their stomatal density (SD) and/or adjust their aperture size in response to elevated CO_2 concentrations (Franks and Beerling, 2009; Wagner et al., 1996; Wagner-Cremer et al., 2004; Woodward, 1987), which in turn can reduce gs. Such a decrease in SD has effectively been observed for the saplings used in the present study, for which SD decreased by 19 % for *A. marina* and by 24 % for *R. stylosa* (Fig. 5 in Jacotot *et al.*, 2018). Eventually, the different atmospheric $\delta^{13}C$-CO_2 values between the two CO_2 treatments (-8.2 ± 0.69 ‰ *vs.* -18.37 ± 0.23 ‰ in the ambient and the elevated CO_2 treatment, respectively), may partly explain the decrease in $\Delta_P\delta^{13}C$ under elevated CO_2 concentrations. However, Zhang *et al.* (2019), who used similar $\delta^{13}C$-CO_2 values between their CO_2 treatments, have also observed this decrease, which comforts our results. Therefore, we hypothesize that a combination between the decrease of gs and SD, and the increase in As may be responsible of the decrease in $\Delta_P{}^{13}C$ under elevated CO_2 concentrations, and that this effect can have repercussions on saplings organs $\delta^{13}C$ values.

4.3 Elevated CO_2 reduced apparent C fractionation during night respiration

Whatever the treatment or the species, a ^{13}C–enrichment of the night-respired CO_2 has been observed in this study in comparison to the leaves OM δ^{13}C (Fig. 1 and Table 3). Although this is the first time that δ^{13}C-$CO_{2\text{-NR}}$ values are reported for mangroves plants, this result of a ^{13}C-enriched respired CO_2 is consistent with previous studies on C_3 species (*e.g.* Ghashghaie *et al.*, 2003; Xu *et al.*, 2004; Badeck *et al.*, 2005; Werner & Gessler, 2011; Ghashghaie & Badeck, 2014). This ^{13}C-enrichment of the respired CO_2 is believe to derive from the partial oxidation of hexose molecules that increases the ratio of ^{13}C-enriched compounds converted to CO_2 (Cui et al., 2015). The apparent C fractionation calculated for *A. marina* and *R. stylosa* in this study falls in the range of C_3 woody species reported in Ghashghaie and Badeck (2014). In our study, elevated CO_2 significantly reduced $\Delta_{NR}{}^{13}$C (Table 3), conformingly to our initial hypothesis. However, such a decrease in $\Delta_{NR}{}^{13}$C under elevated CO_2 was expected following the decrease in $\Delta_{P}{}^{13}$C under elevated CO_2 concentrations (Table 3). Indeed, as explained earlier, as $\Delta_{P}{}^{13}$C decreased under elevated CO_2 concentrations, the saplings used a higher fraction of ^{13}C to produce their metabolites that are then transferred to the other organs. At night, these ^{13}C-enriched metabolites are used as a substrate for leaf respiration, therefore producing ^{13}C-enriched CO_2 and reducing $\Delta_{NR}{}^{13}$C. Nevertheless, more data are needed to conclude precisely on the effect of elevated CO_2 concentrations on $\Delta_{NR}{}^{13}$C. In addition, it is quite possible that the reduced $\Delta_{NR}{}^{13}$C under elevated CO_2 concentrations had an implication on the increased post-photosynthesis fractionation between the leaves and the stems/roots (Table 2), as the release of ^{13}C-enriched CO_2 had potentially an implication in the depletion of leaves δ^{13}C compared to the other organs (Ghashghaie et al., 2003).

5. Conclusion

This study provides the first measurements of C fractionation in mangrove plants during photosynthesis and night respiration. Fractionation during photosynthesis resulted in leaves OM depleted in ^{13}C compared to atmospheric CO_2. At night, C fractionation caused the release of ^{13}C-enriched CO_2 by the leaves, further decreasing their $\delta^{13}C$ depletion in comparison to the other organs. In addition, an increasing gradient of the OM $\delta^{13}C$ values from the leaves to the roots have been observed for both species, which is indicative of post-photosynthesis C fractionation. Elevated CO_2 concentrations have significantly affected C fractionation during photosynthesis, night-respiration and ^{13}C discrimination between mangroves saplings' organs. First, fractionation during photosynthesis decreased under elevated CO_2, causing the plants to use a higher fraction of ^{13}C available in the leaves pore spaces. Then, within-plant C fractionation increased in the saplings grown under elevated CO_2 levels, resulting in a higher difference in $\delta^{13}C$ between the leaves, stems and roots. Finally, C fractionation during night respiration decreased with elevated CO_2 concentrations, releasing ^{13}C-enriched CO_2 to the atmosphere. The understanding of carbon fractionation in mangroves may have important repercussions for future blue carbon researches and in turn in future policies climate plans. We thus suggest that more prospects in C fractionation in mangroves should be conducted in future research efforts. In addition, the extraction of photosynthetic products and metabolites source materials to determine their specific $\delta^{13}C$ values will improve the comprehension of C fractionation in mangrove trees.

Declarations

Material collection permissions

Mangrove propagules, mangrove peat and sand were all collected and transported to the greenhouses with the permission of the Southern Province of New Caledonia.

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Conflicts of interest

The authors have no conflict of interest to declare.

Ethics approval

Not applicable

Consent to participate

Not applicable

Consent for publication

Not applicable

Availability of data and material

Data are available upon request.

Code availability

Not applicable

Authors' contribution

AJ, CM and MA designed the experiment. AJ conducted the fieldwork and data analyses with the help of IG. AJ, CM, IG and MA wrote the manuscript.

References

Aasamaa, K., Sõber, A., 2011. Responses of stomatal conductance to simultaneous changes in two environmental factors. Tree Physiol. 31, 855–864.

Arens, N.C., Jahren, A.H., Amundson, R., 2000. Can C3 plants faithfully record the carbon isotopic composition of atmospheric carbon dioxide? Paleobiology 26, 137–164. https://doi.org/10.1666/0094-8373(2000)026<0137:CCPFRT>2.0.CO;2

Assmann, S.M., 1999. The cellular basis of guard cell sensing of rising CO2. Plant Cell Environ. 22, 629–637.

Atkin, O.K., Tjoelker, M.G., 2003. Thermal acclimation and the dynamic response of plant respiration to temperature. Trends Plant Sci. 8, 343–351. https://doi.org/10.1016/S1360-1385(03)00136-5

Badeck, F.-W., Tcherkez, G., Nogués, S., Piel, C., Ghashghaie, J., 2005. Post-photosynthetic fractionation of stable carbon isotopes between plant organs—a widespread phenomenon. Rapid Commun. Mass Spectrom. 19, 1381–1391. https://doi.org/10.1002/rcm.1912

Bathellier, C., Badeck, F.-W., Ghashghaie, J., 2017. Carbon Isotope Fractionation in Plant Respiration, in: Tcherkez, G., Ghashghaie, J. (Eds.), Plant Respiration: Metabolic Fluxes and Carbon Balance, Advances in Photosynthesis and Respiration. Springer International Publishing, Cham, pp. 43–68. https://doi.org/10.1007/978-3-319-68703-2_3

Bouillon, S., Borges, A.V., Castañeda-Moya, E., Diele, K., Dittmar, T., Duke, N.C., Kristensen, E., Lee, S.Y., Marchand, C., Middelburg, J.J., Rivera-Monroy, V.H., Smith, T.J., Twilley, R.R., 2008. Mangrove production and carbon sinks: A revision of global budget estimates: Global Mangrove Carbon Budgets. Glob. Biogeochem. Cycles 22, n/a-n/a. https://doi.org/10.1029/2007GB003052

Brandes, E., Kodama, N., Whittaker, K., Weston, C., Rennenberg, H., Keitel, C., Adams, M.A., Gessler, A., 2006. Short-term variation in the isotopic composition of organic matter allocated from the leaves to the stem of Pinus sylvestris: effects of photosynthetic and postphotosynthetic carbon isotope fractionation. Glob. Change Biol. 12, 1922–1939. https://doi.org/10.1111/j.1365-2486.2006.01205.x

Bunce, J.A., 1997. Does transpiration control stomatal responses to water vapour pressure deficit? Plant Cell Environ. 20, 131–135. https://doi.org/10.1046/j.1365-3040.1997.d01-3.x

Cernusak, L.A., Tcherkez, G., Keitel, C., Cornwell, W.K., Santiago, L.S., Knohl, A., Barbour, M.M., Williams, D.G., Reich, P.B., Ellsworth, D.S., Dawson, T.E., Griffiths, H.G., Farquhar, G.D., Wright, I.J., 2009. Why are non-photosynthetic tissues generally 13C enriched compared with leaves in C3 plants? Review and synthesis of current hypotheses. Funct. Plant Biol. 36, 199. https://doi.org/10.1071/FP08216

Cernusak, L.A., Ubierna, N., Winter, K., Holtum, J.A.M., Marshall, J.D., Farquhar, G.D., 2013. Environmental and physiological determinants of carbon isotope discrimination in terrestrial plants. New Phytol. 200, 950–965. https://doi.org/10.1111/nph.12423

Collins, M., Knutti, R., Arblaster, J., Dufresne, J.-L., Fichefet, T., Friedlingstein, P., Gao, X., Gutowski, W.J., Johns, T., Krinner, G., Shongwe, M., Tebaldi, C., Weaver, A.J., Wehner, M., 2013. Long-term Climate Change: Projections, Commitments and Irreversibility, in: Stocker, T.F., Qin, D., Plattner, G.-K., Tignor, M., Allen, S.K., Boschung, J., Nauels, A., Xia, Y., Bex, V., Midgley, P.M. (Eds.), Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA, pp. 1029–1136. https://doi.org/10.1017/CBO9781107415324.024

Cui, H., Wang, Y., Jiang, Q., Chen, S., Ma, J.-Y., Sun, W., 2015. Carbon Isotope Composition of Nighttime Leaf-Respired CO2 in the Agricultural-Pastoral Zone of the Songnen Plain, Northeast China. PLOS ONE 10, e0137575. https://doi.org/10.1371/journal.pone.0137575

Del Amor, F.M., 2013. Variation in the leaf δ13C is correlated with salinity tolerance under elevated CO2 concentration. J. Plant Physiol. 170, 283–290. https://doi.org/10.1016/j.jplph.2012.10.019

Diefendorf, A.F., Mueller, K.E., Wing, S.L., Koch, P.L., Freeman, K.H., 2010. Global patterns in leaf 13C discrimination and implications for studies of past and future climate. Proc. Natl. Acad. Sci. 107, 5738–5743. https://doi.org/10.1073/pnas.0910513107

Donato, D.C., Kauffman, J.B., Murdiyarso, D., Kurnianto, S., Stidham, M., Kanninen, M., 2011. Mangroves among the most carbon-rich forests in the tropics. Nat. Geosci. 4, 293–297. https://doi.org/10.1038/ngeo1123

Duke, N., Kathiresan, K., Salmo III, S.G., Fernando, E.S., Peras, J.R., Sukardjo, S., Miyagi, T., Ellison, J., Koedam, N.E., Wang, Y., Primavera, J., Jin Eong, O., Wan-Hong Yong, J., Ngoc Nam, V., 2008. Avicennia marina: The IUCN Red List of Threatened Species 2010: e.T178828A7619457. https://doi.org/10.2305/IUCN.UK.2010-2.RLTS.T178828A7619457.en

Ellison, J., Duke, N., Kathiresan, K., Salmo III, S.G., Fernando, E.S., Peras, J.R., Sukardjo, S., Miyagi, T., 2008. Rhizophora stylosa: The IUCN Red List of Threatened Species 2010: e.T178850A7626520. https://doi.org/10.2305/IUCN.UK.2010-2.RLTS.T178850A7626520.en

Farquhar, G., O'Leary, M., Berry, J., 1982. On the Relationship Between Carbon Isotope Discrimination and the Intercellular Carbon Dioxide Concentration in Leaves. Funct. Plant Biol. 9, 121. https://doi.org/10.1071/PP9820121

Farquhar, G.D., Ehleringer, J.R., Hubick, K.T., 1989. Carbon isotope discrimination and photosynthesis. Annu. Rev. Plant Biol. 40, 503–537.

Franks, P.J., Beerling, D.J., 2009. Maximum leaf conductance driven by CO2 effects on stomatal size and density over geologic time. Proc. Natl. Acad. Sci. 106, 10343–10347. https://doi.org/10.1073/pnas.0904209106

Ghashghaie, J., Badeck, F.W., 2014. Opposite carbon isotope discrimination during dark respiration in leaves versus roots - a review. New Phytol. 201, 751–769. https://doi.org/10.1111/nph.12563

Ghashghaie, J., Badeck, F.-W., Lanigan, G., Nogués, S., Tcherkez, G., Deléens, E., Cornic, G., Griffiths, H., 2003. Carbon isotope fractionation during dark respiration and photorespiration in C3 plants. Phytochem. Rev. 2, 145–161. https://doi.org/10.1023/B:PHYT.0000004326.00711.ca

Hayase, S., Ichikawa, T., Tanaka, K., 1999. Preliminary Report on Stable Isotope Ratio Analysis for Samples from Matang Mangrove Brackish Water Ecosystems. Jpn. Agric. Res. Q. 33, 215–221.

Howard, J., Sutton-Grier, A., Herr, D., Kleypas, J., Landis, E., Mcleod, E., Pidgeon, E., Simpson, S., 2017. Clarifying the role of coastal and marine systems in climate mitigation. Front. Ecol. Environ. 15, 42–50. https://doi.org/10.1002/fee.1451

Jacotot, A., Marchand, C., Allenbach, M., 2019a. Increase in Growth and Alteration of C:N Ratios of Avicennia marina and Rhizophora stylosa Subject to Elevated CO2 Concentrations and Longer Tidal Flooding Duration. Front. Ecol. Evol. 7, 98. https://doi.org/10.3389/fevo.2019.00098

Jacotot, A., Marchand, C., Allenbach, M., 2019b. Biofilm and temperature controls on greenhouse gas (CO2 and CH4) emissions from a Rhizophora mangrove soil (New Caledonia). Sci. Total Environ. 650, 1019–1028. https://doi.org/10.1016/j.scitotenv.2018.09.093

Jacotot, A., Marchand, C., Gensous, S., Allenbach, M., 2018. Effects of elevated atmospheric CO2 and increased tidal flooding on leaf gas-exchange parameters of two common mangrove species: Avicennia marina and Rhizophora stylosa. Photosynth. Res. https://doi.org/10.1007/s11120-018-0570-4

Keeling, C.D., 1961. The concentration and isotopic abundances of carbon dioxide in rural and marine air. Geochim. Cosmochim. Acta 24, 277–298. https://doi.org/10.1016/0016-7037(61)90023-0

Keeling, C.D., 1958. The concentration and isotopic abundances of atmospheric carbon dioxide in rural areas. Geochim. Cosmochim. Acta 13, 322–334.

Kelleway, J.J., Mazumder, D., Baldock, J.A., Saintilan, N., 2018. Carbon isotope fractionation in the mangrove Avicennia marina has implications for food web and blue carbon research. Estuar. Coast. Shelf Sci. 205, 68–74. https://doi.org/10.1016/j.ecss.2018.03.011

Kohn, M.J., 2010. Carbon isotope compositions of terrestrial C3 plants as indicators of (paleo)ecology and (paleo)climate. Proc. Natl. Acad. Sci. 107, 19691–19695. https://doi.org/10.1073/pnas.1004933107

Kristensen, E., Connolly, R.M., Otero, X.L., Marchand, C., Ferreira, T.O., Rivera-Monroy, V.H., 2017. Biogeochemical Cycles: Global Approaches and Perspectives, in: Rivera-Monroy, V.H., Lee, S.Y., Kristensen, E., Twilley, R.R. (Eds.), Mangrove Ecosystems: A Global Biogeographic Perspective. Springer International Publishing, Cham, pp. 163–209. https://doi.org/10.1007/978-3-319-62206-4_6

Lammertsma, E.I., Boer, H.J. d., Dekker, S.C., Dilcher, D.L., Lotter, A.F., Wagner-Cremer, F., 2011. Global CO2 rise leads to reduced maximum stomatal conductance in Florida vegetation. Proc. Natl. Acad. Sci. 108, 4035–4040. https://doi.org/10.1073/pnas.1100371108

Lockheart, M.J., Poole, I., van Bergen, P.F., Evershed, R.P., 1998. Leaf carbon isotope compositions and stomatal characters: important considerations for palaeoclimate reconstructions. Org. Geochem. 29, 1003–1008. https://doi.org/10.1016/S0146-6380(98)00168-5

Lovelock, C.E., Duarte, C.M., 2019. Dimensions of Blue Carbon and emerging perspectives. Biol. Lett. 15, 20180781. https://doi.org/10.1098/rsbl.2018.0781

Macreadie, P.I., Anton, A., Raven, J.A., Beaumont, N., Connolly, R.M., Friess, D.A., Kelleway, J.J., Kennedy, H., Kuwae, T., Lavery, P.S., Lovelock, C.E., Smale, D.A., Apostolaki, E.T., Atwood, T.B., Baldock, J., Bianchi, T.S., Chmura, G.L., Eyre, B.D., Fourqurean, J.W., Hall-Spencer, J.M., Huxham, M., Hendriks, I.E., Krause-Jensen, D., Laffoley, D., Luisetti, T., Marbà, N., Masque, P., McGlathery, K.J., Megonigal, J.P., Murdiyarso, D., Russell, B.D., Santos, R., Serrano, O., Silliman, B.R., Watanabe, K., Duarte, C.M., 2019. The future of Blue Carbon science. Nat. Commun. 10, 3998. https://doi.org/10.1038/s41467-019-11693-w

Marchand, C., Disnar, J.R., Lallier-Vergès, E., Lottier, N., 2005. Early diagenesis of carbohydrates and lignin in mangrove sediments subject to variable redox conditions (French Guiana). Geochim. Cosmochim. Acta 69, 131–142. https://doi.org/10.1016/j.gca.2004.06.016

Mckee, K.L., Feller, I.C., Popp, M., Wanek, W., 2002. Mangrove isotopic (δ15N and δ13C) fractionation across a nitrogen vs. phosphorus limitation gradient 83, 11.

Mcleod, E., Chmura, G.L., Bouillon, S., Salm, R., Björk, M., Duarte, C.M., Lovelock, C.E., Schlesinger, W.H., Silliman, B.R., 2011. A blueprint for blue carbon: toward an improved understanding of the role of vegetated coastal habitats in sequestering CO $_2$. Front. Ecol. Environ. 9, 552–560. https://doi.org/10.1890/110004

Merilo, E., Jõesaar, I., Brosché, M., Kollist, H., 2014. To open or to close: species-specific stomatal responses to simultaneously applied opposing environmental factors. New Phytol. 202, 499–508. https://doi.org/10.1111/nph.12667

O'Leary, M.H., 1981. Carbon isotope fractionation in plants. Phytochemistry 20, 553–567. https://doi.org/10.1016/0031-9422(81)85134-5

Pataki, D.E., Ehleringer, J.R., Flanagan, L.B., Yakir, D., Bowling, D.R., Still, C.J., Buchmann, N., Kaplan, J.O., Berry, J.A., 2003. The application and interpretation of Keeling plots in terrestrial carbon cycle research. Glob. Biogeochem. Cycles 17.

Pypker, T.G., Hauck, M., Sulzman, E.W., Unsworth, M.H., Mix, A.C., Kayler, Z., Conklin, D., Kennedy, A.M., Barnard, H.R., Phillips, C., Bond, B.J., 2008. Toward using δ13C of ecosystem respiration to monitor canopy physiology in complex terrain. Oecologia 158, 399–410. https://doi.org/10.1007/s00442-008-1154-3

R Development Core Team, Vienna, 2008. R: A language and environment for statistical computing. R Foundation for Statistical Computing. Vienna, Austria.

Reef, R., Winter, K., Morales, J., Adame, M.F., Reef, D.L., Lovelock, C.E., 2015. The effect of atmospheric carbon dioxide concentrations on the performance of the mangrove *Avicennia germinans* over a range of salinities. Physiol. Plant. 154, 358–368. https://doi.org/10.1111/ppl.12289

Sage, R.F., Kubien, D.S., 2007. The temperature response of C3 and C4 photosynthesis. Plant Cell Environ. 30, 1086–1106. https://doi.org/10.1111/j.1365-3040.2007.01682.x

Saintilan, N., Rogers, K., Mazumder, D., Woodroffe, C., 2013. Allochthonous and autochthonous contributions to carbon accumulation and carbon store in southeastern Australian coastal wetlands. Estuar. Coast. Shelf Sci. 128, 84–92. https://doi.org/10.1016/j.ecss.2013.05.010

Schubert, B.A., Jahren, A.H., 2012. The effect of atmospheric CO2 concentration on carbon isotope fractionation in C3 land plants. Geochim. Cosmochim. Acta 96, 29–43. https://doi.org/10.1016/j.gca.2012.08.003

Sekiya, N., Yano, K., 2008. Stomatal density of cowpea correlates with carbon isotope discrimination in different phosphorus, water and CO2 environments. New Phytol. 179, 799–807. https://doi.org/10.1111/j.1469-8137.2008.02518.x

Sharkey, T.D., 1985. Photosynthesis in intact leaves of C3 plants: Physics, physiology and rate limitations. Bot. Rev. 51, 53–105. https://doi.org/10.1007/BF02861058

Tcherkez, G., Schäufele, R., Nogués, S., Piel, C., Boom, A., Lanigan, G., Barbaroux, C., Mata, C., Elhani, S., Hemming, D., Maguas, C., Yakir, D., Badeck, F.W., Griffiths, H., Schnyder, H., Ghashghaie, J., 2010. On the 13C/12C isotopic signal of day and night respiration at the mesocosm level. Plant Cell Environ. 33, 900–913. https://doi.org/10.1111/j.1365-3040.2010.02115.x

Vogel, J.C., 1980. Fractionation of the carbon isotopes during photosynthesis, in: Fractionation of the Carbon Isotopes During Photosynthesis. Springer, pp. 5–29.

Wagner, F., Below, R., Klerk, P.D., Dilcher, D.L., Joosten, H., Kürschner, W.M., Visscher, H., 1996. A natural experiment on plant acclimation: lifetime stomatal frequency response of an individual tree to annual atmospheric CO2 increase. Proc. Natl. Acad. Sci. U. S. A. 93, 11705–11708. https://doi.org/10.1073/pnas.93.21.11705

Wagner-Cremer, F., Kouwenberg, L., Hoof, T., Visscher, H., 2004. Reproducibility of Holocene atmospheric CO2 records based on stomatal frequency. Quat. Sci. Rev. 23. https://doi.org/10.1016/j.quascirev.2004.04.003

Wei, L., Yan, C., Guo, X., Ye, B., 2008a. Variation in the δ13C of Two Mangrove Plants is Correlated with Stomatal Response to Salinity. J. Plant Growth Regul. 27, 263–269. https://doi.org/10.1007/s00344-008-9054-7

Wei, L., Yan, C., Ye, B., Guo, X., 2008b. Effects of Salinity on Leaf δ13C in Three Dominant Mangrove Species along Salinity Gradients in an Estuarine Wetland, Southeast China. J. Coast. Res. 24, 267–272. https://doi.org/10.2112/06-0765.1

Weiss, C., Weiss, J., Boy, J., Iskandar, I., Mikutta, R., Guggenberger, G., 2016. Soil organic carbon stocks in estuarine and marine mangrove ecosystems are driven by nutrient colimitation of P and N. Ecol. Evol. 6, 5043–5056. https://doi.org/10.1002/ece3.2258

Werner, C., Gessler, A., 2011. Diel variations in the carbon isotope composition of respired CO $_2$ and associated carbon sources: a review of dynamics and mechanisms. Biogeosciences 8, 2437–2459. https://doi.org/10.5194/bg-8-2437-2011

Werner, C., Schnyder, H., Cuntz, M., Keitel, C., Zeeman, M.J., Dawson, T.E., Badeck, F.-W., Brugnoli, E., Ghashghaie, J., Grams, T.E.E., Kayler, Z.E., Lakatos, M., Lee, X., Máguas, C., Ogée, J., Rascher, K.G., Siegwolf, R.T.W., Unger, S., Welker, J., Wingate, L., Gessler, A., 2012. Progress and challenges in using stable isotopes to trace plant carbon and water relations across scales. Biogeosciences 9, 3083–3111. https://doi.org/10.5194/bg-9-3083-2012

Woodward, F.I., 1987. Stomatal numbers are sensitive to increases in CO 2 from pre-industrial levels. Nature 327, 617–618. https://doi.org/10.1038/327617a0

Xu, C., Lin, G., Griffin, K.L., Sambrotto, R.N., 2004. Leaf respiratory CO2 is 13C-enriched relative to leaf organic components in five species of C3 plants. New Phytol. 163, 499–505. https://doi.org/10.1111/j.1469-8137.2004.01153.x

Zhang, H.-Y., Hartmann, H., Gleixner, G., Thoma, M., Schwab, V.F., 2019. Carbon isotope fractionation including photosynthetic and post-photosynthetic processes in C3 plants: Low [CO2] matters. Geochim. Cosmochim. Acta 245, 1–15. https://doi.org/10.1016/j.gca.2018.09.035

Zhang, Y., Yu, X., Chen, L., Jia, G., 2017. Variations in δ13C of different plant organs: implications for post-photosynthetic fractionation. bioRxiv 238477. https://doi.org/10.1101/238477

Zhu, B., Cheng, W., 2011. 13C isotope fractionation during rhizosphere respiration of C3 and C4 plants. Plant Soil 342, 277–287. https://doi.org/10.1007/s11104-010-0691-9

Supplementary Information (SI)

Table S1 *: Comparison of leaves and roots $\delta^{13}C$ values* (‰) *and ^{13}C-enrichment* (‰) *between leaves-roots and leaves-stems in various mangrove species from this study and the values reported in the literature.*

$\delta^{13}C$ values (‰)			$\delta^{13}C$ offset (‰)			
Leaves	Stems	Roots	Leaves-roots	Leaves-stems	Species	Reference
-26.74	**-26.13**	**-25.19**	**1.55**	**0.61**	***Am***	**This study**
-27.56	**-26.16**	**-25.9**	**1.66**	**1.40**	***Rs***	
-		-	2.9*	2.4	*Am*	Kelleway *et al.* (2018)
-		-	1.3*	-0.4	*Am*	
-		-	1.97*	0.3	*Am*	
-		-	1.83*	0.6	*Am*	
-29.74		-29.1	0.64**	-	*Rs*	Weiss *et al.* (2016)
-30.35		-27.96	2.39**	-	*Ra*	
-28.83		-28.58	0.25**	-	*Bp*	
-32.7		-28.45	4.25**	-	*Bs*	
-31.15		-28.29	2.86**	-	*Xg*	
-30.65		-28.09	2.56**	-	*Sa*	
-27.59		-28.04	-0.45**	-	*Ac*	
-25.41		-25.86	-0.45**	-	*Nf*	
-27.9		-24.7	3.2**	-	*Am*	Saintilan *et al.* (2013)
-27.7		-24.4	3.3**	-	*Am*	
-27.03		-26.05	0.98**	-	*Jk*	
-27.3		-24.7	2.6**	-	*Am*	

-28.3	-24.3	4**	-	*Am*
-28.3	-23.3	5**	-	*Am*

*mean values of $\delta^{13}C$ *differences between leaves and* cable roots, fine roots and pneumatophores as reported in Kelleway *et al.* (2018)

**calculated values based on reported leaves and roots $\delta^{13}C$ *values*

Species: Am: *Avicennia marina*; Rs: *Rhizophora stylosa*; Ra: *Rhizophora apiculata*; Bp: *Bruguiera parviflora*; Bs: *Bruguiera sexangula*; Xg: *Xenocarpus granatum*; Sa: *Sonneratia alba*; Ac: *Aegiceras corniculatum*; Nf: *Nypa fruticans*; Jk: *Juncus kraussii*