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Using isotope labeling to partition sources of CO_2 efflux in newly established mangrove seedlings

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Abstract

Carbon dioxide (CO_2) flux is a critical component of the global C budget. While CO_2 flux has been increasingly studied in mangroves, better partitioning of components contributing to the overall flux will be useful in constraining C budgets. Little information is available on how CO_2 flux may vary with forest age and conditions. We used a combination of ^{13}C stable isotope labeling and closed chambers to partition CO_2 efflux from the seedlings of the widespread mangrove *Avicennia marina* in laboratory microcosms, with a focus on sediment CO_2 efflux in establishing forests. We showed that (1) above-ground part of plants were the chief component of overall CO_2 efflux; and (2) the degradation of sediment organic matter was the major component of sediment CO_2 efflux, followed by root respiration and litter decomposition, as determined using isotope mixing models. There was a significant relationship between C isotope values of CO_2 released at the sediment–air interface and both root respiration and sediment organic matter decomposition. These relative contributions of different components to overall and sediment CO_2 efflux can be used in partitioning of the sources of overall respiration and sediment C mineralization in establishing mangroves.

Mangroves contain variably thick organic sediments and are the most carbon (C) rich forests (Donato et al. 2011; Sanders et al. 2016). The high C accumulation capacity of mangroves has been recognized, and termed "blue C," along with saltmarsh and seagrasses (Mcleod et al. 2011; Duarte et al. 2013; Ouyang and Lee 2014). However, studies of mangrove carbon dioxide (CO_2) flux vary in the precision of their partitioning. CO_2 flux in mangroves may originate from the canopy, woody debris, root, litter and sediment organic matter (SOM), and is collectively called ecosystem respiration (E_e), which has been usually studied separately as canopy (above-ground parts, E_c) and sediment respiration (the other components, E_s).

Mangrove organic material such as leaf litter, if not exported, becomes incorporated in the sediment through decay and chemically modified by microbes inhabiting the mangrove forest floor (Kristensen et al. 2008). In contrast to the intensively studied and relatively established pattern of C exchange between mangroves and nearshore ecosystems (Lee 1995), the pattern of C gas flux released from mangrove sediment is less clear, although there is an increasing interest in this topic and C gas flux at the ecosystem scale (Lovelock 2008; Barr et al. 2010; Chen et al. 2010, 2012; Livesley and Andrusiak 2012; Barr 2013; Leopold et al. 2013, 2015, 2016; Bulmer et al. 2015). A key but poorly known aspect is the partitioning of E_s attributable to various components, i.e., root, litter, and SOM (including the microphytobenthos).

Laboratory microcosms have been used effectively in studies of mangrove energy pathways. For example, Bui and Lee (2014) evaluated relative contributions of organic matter from mangrove leaf litter and sediment to crab's diet via laboratory microcosms. Zhu et al. (2014) conducted a microcosm study to investigate the fate of two abundant congeners in polluted mangrove sediment. We use laboratory microcosms to partition different sources of E_e, and in particular E_s. The microcosms emulate field conditions with seedlings and sediments collected from mangrove forests, and then growing seedlings in the sediments. The study expands the horizon of current studies (e.g., Lovelock et al. 2015), which measure the portions of E_e in mature mangroves and do not completely partition E_s.

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Isotopic ($\delta^{13}C$) values can be used to distinguish photosynthetic pathways, shifts of vegetation and C sources supporting food chains (O'Leary 1981; Ouyang et al. 2015). Further, there is evidence that $\delta^{13}C$ values can differ among mangrove tissues, although no consistent patterns of variation has yet been demonstrated (Bouillon et al. 2008*a*). There is also evidence that the SOM pool in mangroves was consistently enriched in ^{13}C in relation to the mangrove litter in sites where litter was expected to be the sole input (Lallier-Verges et al. 1998). This difference is likely due to a rise in microbial and fungal residues (Ehleringer et al. 2000). However, the δ ^{13}C values of mangrove live tissues and litter are usually not distinguished. Boon et al. (1997) documented that the $\delta^{13}C$ values of the pneumatophores of *Avicennia*, a widely distributed species, were on some occasions depleted in $\delta^{13}C$ in relation to leaves by up to 3.1‰, while Vane et al. (2013) stated that the difference between leaves and pneumatophores was < 2‰. Rao et al. (1994) noted little difference in $\delta^{13}C$ values (< 1‰) between fresh and senescent leaves for five tree species of Kenyan mangroves, but for four other species, senescent leaves were significantly depleted in relation to fresh ones. However, Lee (2000) suggested that the direction and magnitude of this difference was opposite. Natural C isotope signals, therefore, may not be able to differentiate sources from roots and litter, suggesting that isotopic labeling might be preferable.

The enriched ^{13}C isotope technique has been used to identify food sources with similar ^{13}C signatures in food web research to overcome the drawback of natural ^{13}C (Lee et al. 2011), and been used in other ecosystems (Galván et al. 2008; Luo and Zhou 2010; Lee et al. 2012; Oakes et al. 2012). Similarly, it may be applicable in partitioning the sources of CO_2 flux if combined with the closed chamber technique (Luo and Zhou 2010; Ouyang et al. 2017), which has been used to measure CO_2 flux. The microcosms outweigh field experiments, for which it is difficult to perform isotopic enrichment in leaf litter and sediments under field conditions.

It is suggested that a relatively low proportion of the organic matter in leaves of *Avicennia* is lost by leaching, while most of the labile portion is present as non-leachable but easily decayed organic material. *Avicennia* leaves tended to be decayed through microbial action relative to crab consumption (Robertson 1988). Although decomposition rates of mangrove litter vary (Lee 1999), much of the important biochemical action occurs relatively quickly, with half-life period of just 10.5 d for *Avicennia* (Sessegolo and Lana 1991). The relatively short half-life period for *Avicennia* has been attributed to lower tannin content and higher initial N concentrations (Alongi 2009). Hence, it takes a short time to investigate the composition of E_s, attributable to leaf litter and their incorporated fraction into sediment for *Avicennia*.

This study aims to distinguish E_c and E_s, and focuses on partitioning E_s attributable to different components using laboratory microcosms. As E_s occurs at the sediment–air interface, tides were not set as a controlling factor in our laboratory microcosms. ^{13}C enrichment combined with the closed chamber technique was used to partition different sources of CO_2 efflux in microcosms with *Avicennia marina* seedlings simulating newly established stands. Our proposed method has the advantage of partitioning E_s without disturbing the sediment compared with directly measuring different components of E_s, e.g., the measurement of root respiration from detached roots (Lovelock et al. 2015).

Experimental materials and methods

Laboratory microcosms

Seeds of *A. marina* (a cryptoviviparous species) and sediments were collected in June 2015 from the mangrove forest on Tallebudgera Creek (28°6′22″S, 153°26′49″E) in southeast Queensland, Australia. The developing seedlings comprise cotyledons with fine roots at one side but no branching stems. Ninety healthy seeds were picked and planted in six glass chambers (40 × 30 × 50 cm) containing local sediment of 10 cm depth (*see* Fig. 1) and maintained at 24°C (∼ mean local ambient temperature) under fluorescent lighting in a constant temperature room. Another chamber just contained sediment without seedlings, established for the measurement of E_{SOM}. From the mangrove forest where the seedlings grew, sediments were collected, mixed and then put in the chambers. The initial volumetric water content of sediment is 32.6% ± 4.1% (mean ± SD), and sediment chlorophyll *a* concentration is 845.7 ± 212.4 μg L^{-1} (mean ± SD). Seawater was collected near the mangrove forest and injected in each chamber in equal quantities every 2 d to keep the sediment moist but not flooded. After injection, water either evaporated, or percolated through the sediment and could be absorbed by the seedlings for growth. After 1 month when leaves grew out of the cotyledons, polypropylene nets (1 cm mesh size) were hung in three of the chambers (over the sediment but under the cotyledons) to collect leaf litter. The netting was not set in the other three chambers. This net design prevented incorporation of leaf litter into the sediment, thus allowing separation of the contribution of leaf litter from E_s. When seedlings had 4–6 leaves by August 2015, they were enriched with ^{13}C using methods modified from Bui and Lee (2014) and Bromand et al. (2001). A bottle containing 25 mL of 1 M $NaH^{13}CO_3$ (99 atom% ^{13}C, Cambridge Isotope Laboratories) was put in each chamber before the chamber lid was tightly sealed. One milliliters of 1 M HCl acid was added to the bottle every 2 d for 45 d through a glass pipette passing through the lid of the chamber to generate $^{13}CO_2$ in situ. A small fan ($D = 8$ cm) was turned on for 30 min after the addition of acid to promote even dispersion of $^{13}CO_2$ within the growth chamber. At the end of the experiment, the seedlings grew to near the top of the chambers and were ∼ 40 cm height and the diameter of stems was 0.5 cm. After sampling at the end of the experiment, the plots were dug up and the roots were found to grow to the bottom of the chambers and some roots continued to extend horizontally in the sediments.

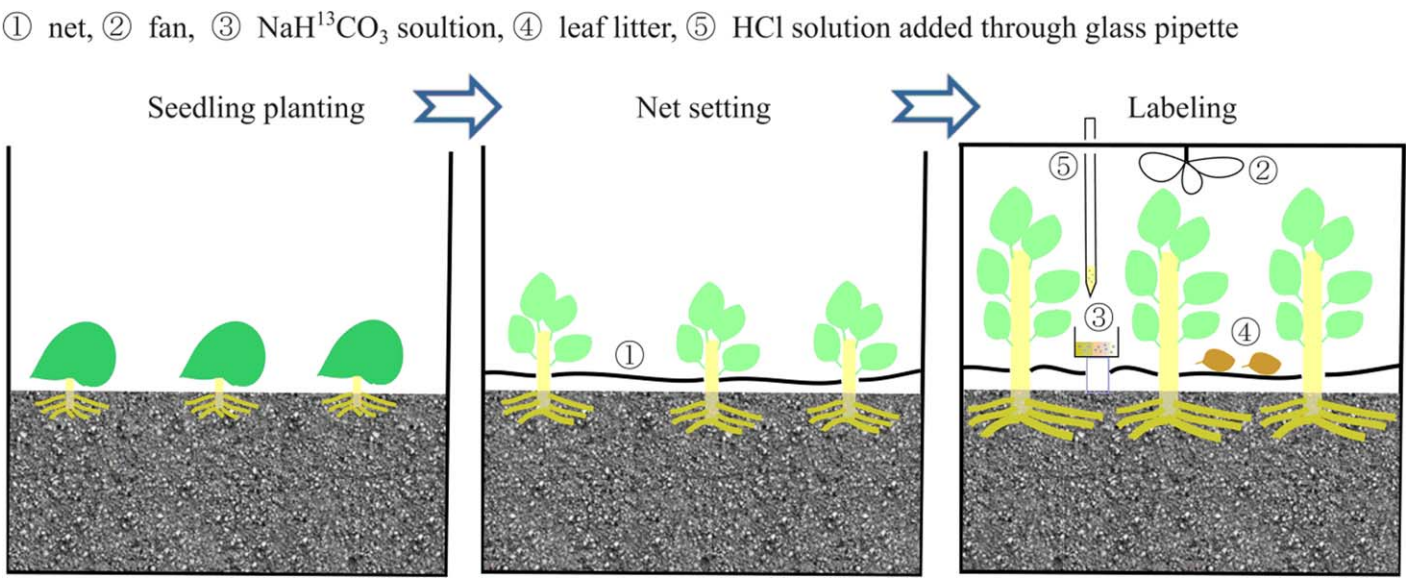

Fig. 1. Experimental setup in the three stages of the experiment.

Sample collection and analysis

In October 2015, samples were collected from the chambers to partition E_c and E_s, and to partition different sources of E_s. A SBA5 gas analyzer (PP system, U.S.A.) was employed to measure CO_2 efflux with a rotary pump, allowing air circulation within the closed loop. To begin with, CO_2 from the closed chambers was collected by 12 mL borosilicate vacutainers (Labco Limited, UK), followed by CO_2 efflux measurement. Likewise, CO_2 from two plots (replicates) of sediment in each chamber was collected with 200 mL containers. The containers were inserted into sediment and remained for 10 min before gas collection. E_s from each replicate was measured before a closed container was inserted in the sediment where it remained for 20 min. Then a pooled sample of new live roots from the seedlings was collected and frozen immediately. A pooled sample of leaf litter from the chambers without nets was collected and sealed in 5 mL polystyrene screw-cap vials. Similarly, a pooled sample of the top sediment from 0 cm to 5 cm below the bottom of the litter layer was collected from the chambers. The root samples were dried at 70°C for 48 h and then ground to pass through a 0.86 mm sieve. $\delta^{13}C$ value of CO_2 were measured by Cavity Ring-Down Spectrometry (DS–CRDS) at James Cook University, Queensland, Australia. The dried litter and sediment samples were individually ground to pass through a 20-mesh sieve for ^{13}C isotopic analysis. Sediment samples were acidified by 1M HCl until there was no effervescence to remove carbonate matter. The $\delta^{13}C$ values of mangrove roots, litter and sediment were analyzed by the Stable Isotope Laboratory, Griffith University.

Methods and principles of CO_2 efflux partitioning

E_e is composed of E_c and E_s. E_s consists of CO_2 efflux from root respiration (E_r) and decomposition of litter (E_l) and SOM (E_{SOM}) (Fig. 2). The closed chamber technique was used to partition E_c and E_s, with $E_e = E_c + E_s$. Nets were set in three of the six chambers to collect leaf litter, allowing $\delta^{13}C$ to partition sediment CO_2 into E_r and E_{SOM}. The difference of CO_2 efflux from sediments in chambers with and without nets, measured by the closed chamber technique, estimated E_l.

The labeling experiment exposed the above-ground portion of seedlings to the ^{13}C-labeled tracer inside the glass chambers. Photosynthesis incorporates ^{13}C-labeled CO_2 into carbohydrate immediately following exposure. The labeled carbohydrate within labile C pools is utilized for respiration over time, assimilated by structural substances of plant tissues via growth, then allocated to the rhizosphere, and transferred to SOM. Samples of mangrove tissues, sediment, and respired CO_2 were collected for the analysis of $\delta^{13}C$ to trace the fate of labeled C. Relative quantities of ^{13}C were employed to show partitioning of photosynthetically fixed C into various functional processes on the grounds of the mass conservation principle.

E_e and E_s of each chamber were combined to partition E_c. Meanwhile, an isotope mixing model was used to estimate average $\delta^{13}C$ of E_c.

$$\delta^{13}C_{en} = f_{sn}\delta^{13}C_{sn} + f_{cn}\delta^{13}C_{cn} \quad (1)$$

$$f_{sn} = \frac{E_{sn}}{E_{en}} \quad (2)$$

$$f_{sn} + f_{cn} = 100 \quad (3)$$

Where E_{sn} and E_{en} are CO_2 efflux from sediment and chambers with nets, $\delta^{13}C_{en}$, $\delta^{13}C_{sn}$, and $\delta^{13}C_{cn}$ are the $\delta^{13}C$ values of E_{en}, E_{sn}, and E_{cn} in chambers with nets, f_{sn} and f_{cn} are the fraction of E_{sn} and E_{cn} contributing to E_{en}.

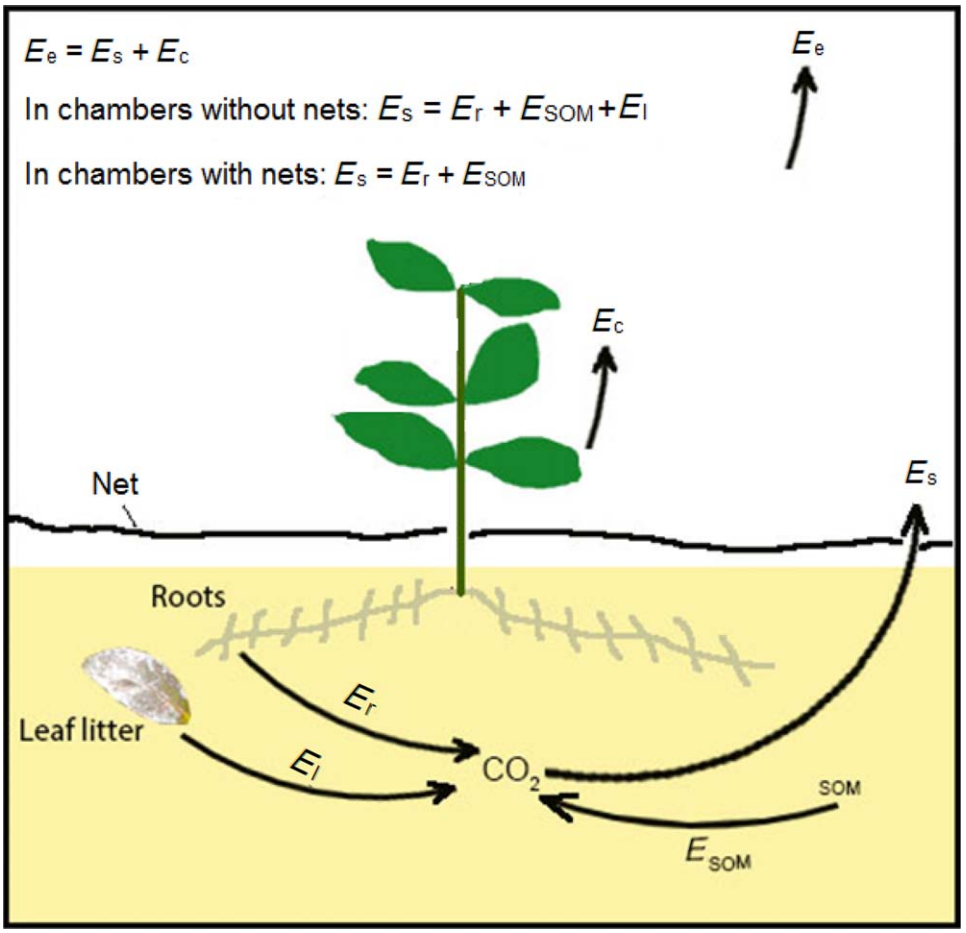

Fig. 2. A conceptual diagram describing the components of E_e and E_s. The net prevents leaf litter from accumulating on the sediment surface and contributes to efflux in the with-net treatment. E_e – ecosystem respiration, E_c – canopy respiration, E_s – sediment respiration, E_r – CO_2 efflux from root respiration, E_l – CO_2 efflux from decomposition of litter, E_{SOM} – CO_2 efflux from decomposition of SOM.

$$\delta^{13}C_e = f_s\delta^{13}C_s + f_c\delta^{13}C_c \quad (4)$$

$$f_s = \frac{E_s}{E_e} \quad (5)$$

$$f_s + f_c = 1 \quad (6)$$

Where $\delta^{13}C_e$, $\delta^{13}C_s$, and $\delta^{13}C_c$ are the $\delta^{13}C$ values of E_e, E_s, and E_c in chambers without nets, f_s and f_c are the fraction of E_s and E_c contributing to E_e, E_s and E_e are CO_2 efflux from sediment and chambers without nets.

The $\delta^{13}C$ of E_r, E_l, E_{SOM} and E_s were quantified to partition sediment E_s into autotrophic (plant respiration) and heterotrophic (decomposition) sources. The mixing model below was applied to estimate the proportion of E_r vs. E_s.

$$\delta^{13}C_{sn} = f_{rn}\delta^{13}C_{rn} + f_{SOMn}\delta^{13}C_{SOMn} \quad (7)$$

$$f_{rn} + f_{SOMn} = 1 \quad (8)$$

where $\delta^{13}C_{sn}$, $\delta^{13}C_{rn}$, and $\delta^{13}C_{SOMn}$ are the $\delta^{13}C$ values of E_s, E_r, and E_{SOM} in chambers with nets, f_{rn} and f_{SOMn} are the fraction of E_r and E_{SOM} contributing to E_s.

$$\delta^{13}C_s = f_r\delta^{13}C_r + f_{SOM}\delta^{13}C_{SOM} + f_l\delta^{13}C_l \quad (9)$$

$$f_l = \frac{E_s - E_{sn}}{E_s} \quad (10)$$

$$f_r + f_{SOM} + f_l = 1 \quad (11)$$

where $\delta^{13}C_s$, $\delta^{13}C_r$, $\delta^{13}C_{SOM}$ and $\delta^{13}C_l$ are the $\delta^{13}C$ values of E_s, E_r, E_{SOM}, and E_l in chambers without nets; f_r, f_{SOM}, and f_l are the fraction of E_r, E_{SOM}, and E_l contributing to E_s. The sampling strategy is described in Fig. 3.

The aforementioned mixing models are based on assumptions that $\delta^{13}C$ values of plant canopy, SOM, root, and litter may approximate those of each component of E_c and E_s. The assumptions lie in the fact that: (1) there is no C isotopic fractionation during heterotrophic microbial respiration (Lin and Ehleringer 1997); (2) there is little C isotopic fractionation during the early decomposing stage of fallen plant substances (Balesdent et al. 1993; Dehairs et al. 2000). Based on published litter turnover times, we limited the study period to 2–3 months such that only the first litterfall contributes to E_l, and afterwards there was little new litter formation

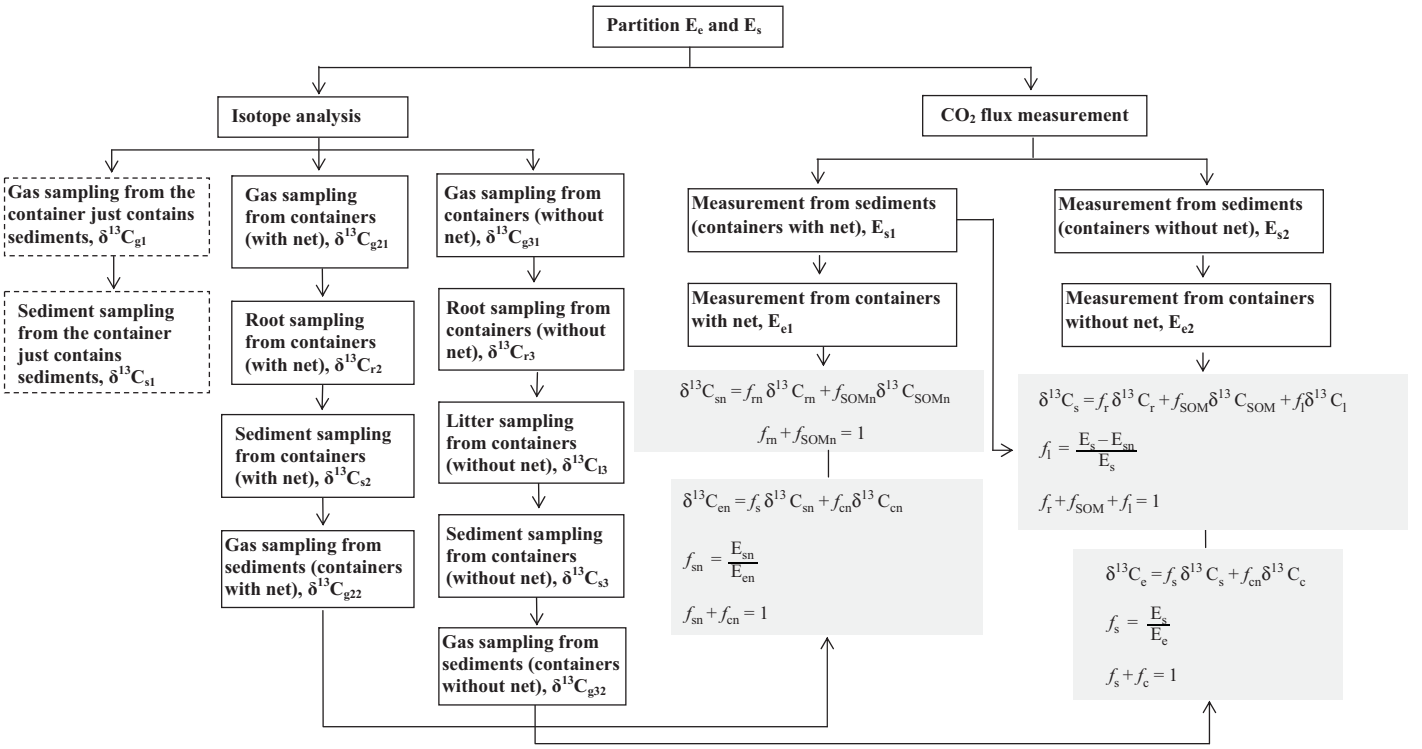

Fig. 3. A conceptual diagram describing the sampling strategy of the labeling experiment.

and decay in the chamber; and (3) there was a negligible difference between $\delta^{13}C$ values of sediment organic C in the surface layer and that of sediment released CO_2, and little difference of $\delta^{13}C$ values among different soil size fractions as suggested by Bird et al. (1996).

Data analysis

One-way analysis of variance (ANOVA) was used to examine (1) the difference in E_c, E_s, and E_{SOM} with or without nets hanging over sediment; and (2) the difference in source contribution to E_s from chambers with nets. Before ANOVA, the assumptions of normality and variance homogeneity were verified by the Shapiro–Wilk normality test and Bartlett test, respectively. Tukey's HSD test was applied when a significant treatment effect was found. Linear regression analysis was conducted to examine the relationship between $\delta^{13}C$ values of E_s and both E_r and E_{SOM}. Paired-sample t test was used to compare litter $\delta^{13}C$ values from chambers with and without nets. Student's t-test was performed to compare the contribution of E_{SOM} and E_r to E_S from all the samples.

Some previous studies investigated E_c and E_s via CO_2 efflux measurement or synthesis of different portions of E_e (Alongi 2009; Lovelock et al. 2015; Troxler et al. 2015). This prior information on the proportion of E_c to E_s was incorporated into a Bayesian framework to estimate the likely range of canopy or sediment contribution to $\delta^{13}C$ of E_e. Model fitting was undertaken by the Markov chain Monte Carlo (MCMC) method, which generated simulations of plausible values of isotopic source contribution to E_e in consistence with the data. Before running MCMC, $\delta^{13}C$ of E_e was assumed to be normally distributed (Moore and Semmens 2008) and verified for the normality assumption. The number of iterations of the Bayesian model was set at 5000.

R programming language was used to perform data analysis (R Core Team 2014). R package "SIAR" was applied to conduct Bayesian modeling of uncertainties in isotopic source contribution to total chamber respiration (Parnell and Jackson 2013). Data were expressed as mean ± standard error (SE).

Results

Carbon dioxide efflux from chambers and sediment

There was a highly significant difference among E_e, E_s, and E_{SOM} (ANOVA, $p < 0.01$, Fig. 4). Furthermore, E_e (785.0 ± 185.2 (SD) mmol m^{-2} d^{-1} with nets, 1160.8 ± 323.9 mmol m^{-2} d^{-1} without nets) was significantly higher than both E_s (170.1 ± 19.8 mmol m^{-2} d^{-1} with nets, 174.7 ± 22.8 mmol m^{-2} d^{-1} without nets) and E_{SOM} (79.8 ± 17.8 mmol m^{-2} d^{-1}) (Tukey's HSD test, $p < 0.05$). However, there was no significant difference between E_s and E_{SOM} (Tukey's HSD test, $p > 0.05$).

Figure 5 shows the result of Bayesian inference in terms of the posterior distribution of source contribution to E_e.

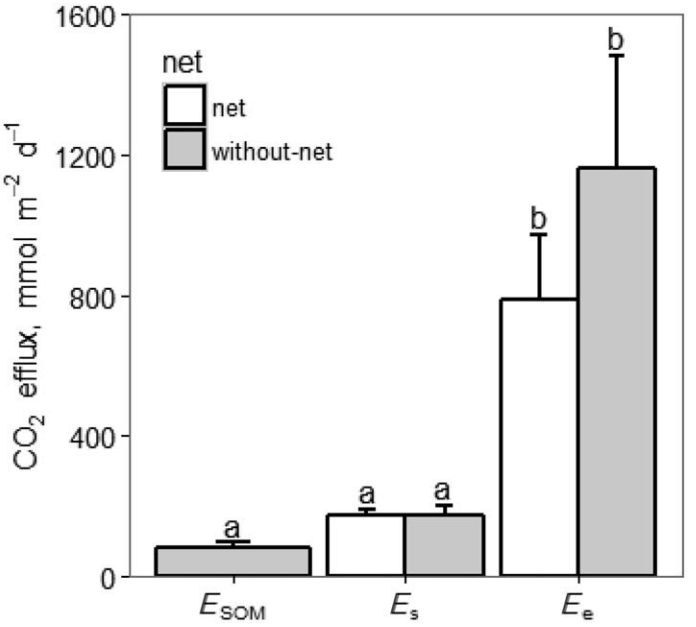

Fig. 4. E_e, E_s, and E_{SOM} of *A. marina*. Bars labeled with different letters have significantly different CO_2 effluxes.

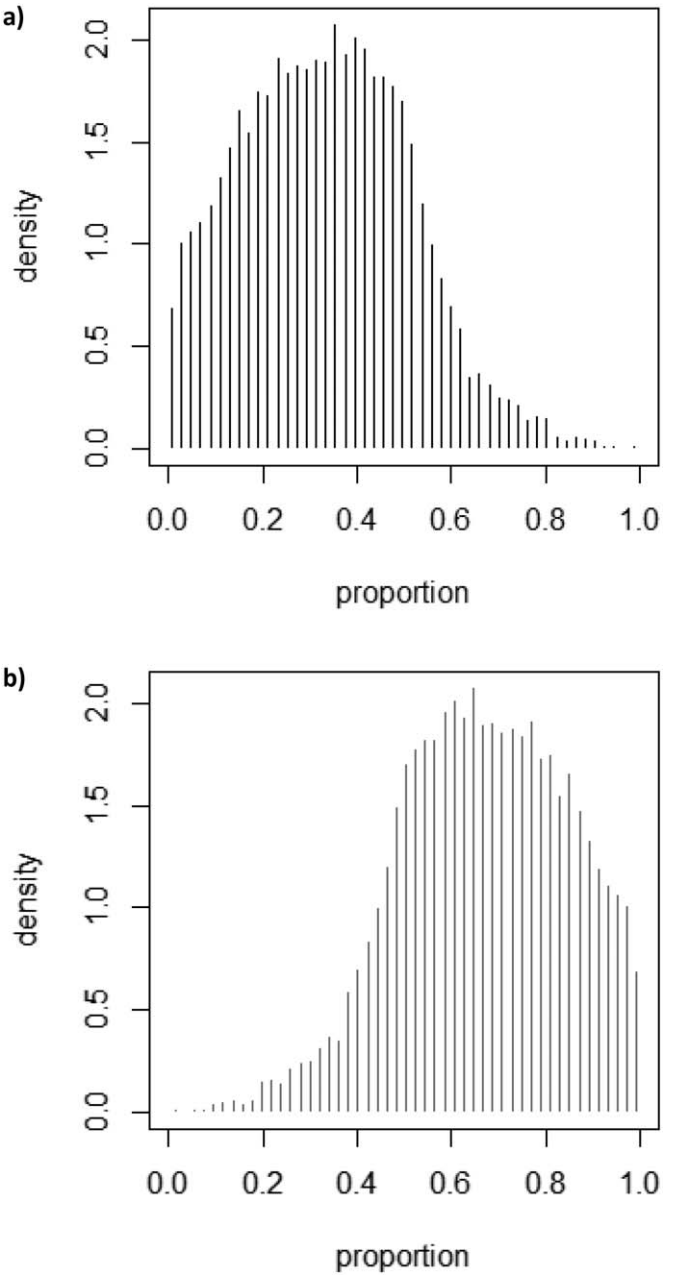

Fig. 5. A matrix of histogram from the Bayesian model describing uncertainties in the $\delta^{13}C$ source contributions of E_s (**a**) and E_c (**b**) to E_e.

Contribution of the two sources had different probability densities; the higher probability density occurs at > 50% contribution from E_c, but at < 50% contribution from E_s.

The sources of CO_2 efflux from the sediment surface

For both chambers with and without nets, E_{SOM} contributed 61.8% ± 9.2% and was the main component of E_s, followed by E_r (31.8% ± 9.7%). The difference between E_{SOM} and E_r was significant (Student's *t*-test, $p < 0.05$). E_l contributed the least to E_s. For chambers without nets, there was significant difference in the contribution of different components to E_s (ANOVA, $p < 0.05$). In particular, the contribution of E_{SOM} was significantly higher than that of both E_r and E_l (Tukey's HSD test, $p < 0.05$) but no significant difference was found between the contribution of E_r and E_l (Tukey's HSD test, $p > 0.05$) (Fig. 6). Additionally, there was a highly significant relationship between the $\delta^{13}C$ values of E_s and root ($R^2 = 0.59$, $p < 0.01$), as well as SOM ($R^2 = 0.62$, $p < 0.01$) (Fig. 7).

Isotopic ^{13}C values of litter

Table 1 shows the $\delta^{13}C$ values of E_e, E_s and different components. There was no significant difference in litter $\delta^{13}C$ values between chambers with and without nets (paired-sample *t* test, $p > 0.05$).

Discussion

Partitioning ecosystem CO_2 efflux

E_s contributes a minor proportion (20.9% ± 4.1%) to E_e, as is confirmed by the Bayesian inference. This suggests that E_c is the main component of E_e, generally in agreement with the global synthesis of mangrove C flow ($E_c : E_s = \sim 10 : 1$). Seedlings of *A. marina* were found to have a root/shoot ratio of ~ 0.5 under freshwater treatment (Burchett et al. 1984), which may support a relatively higher contribution from E_c since higher shoot biomass respires more CO_2 (i.e., E_c) than lower root biomass (i.e., E_r). Moreover, part of the decomposed C in sediment may be mineralized as inorganic C (e.g., DIC) in porewater (Maher et al. 2013), and thus gives rise to the difference between E_c and E_s; a global synthesis of

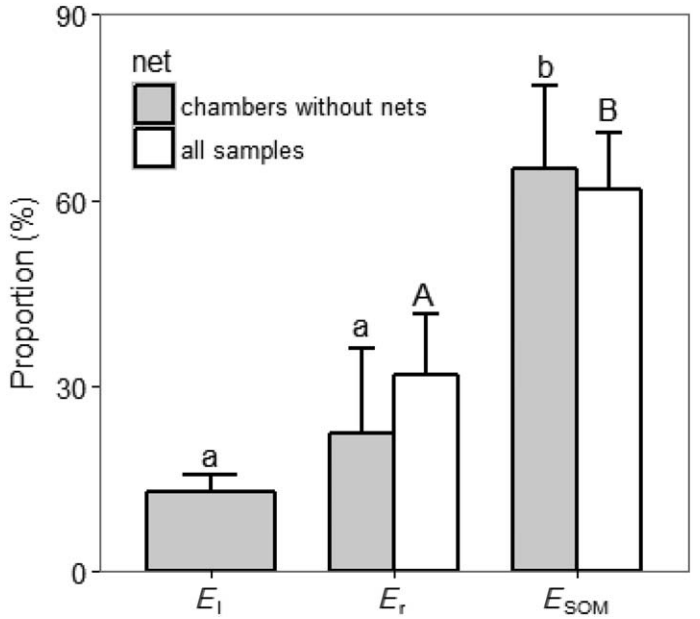

Fig. 6. Relative contributions of different components to E_s. Bars with different letters are significantly different. Relative contributions of different components were compared in chambers without nets (lower case letters) and all samples (upper case letters), including chambers with and without nets.

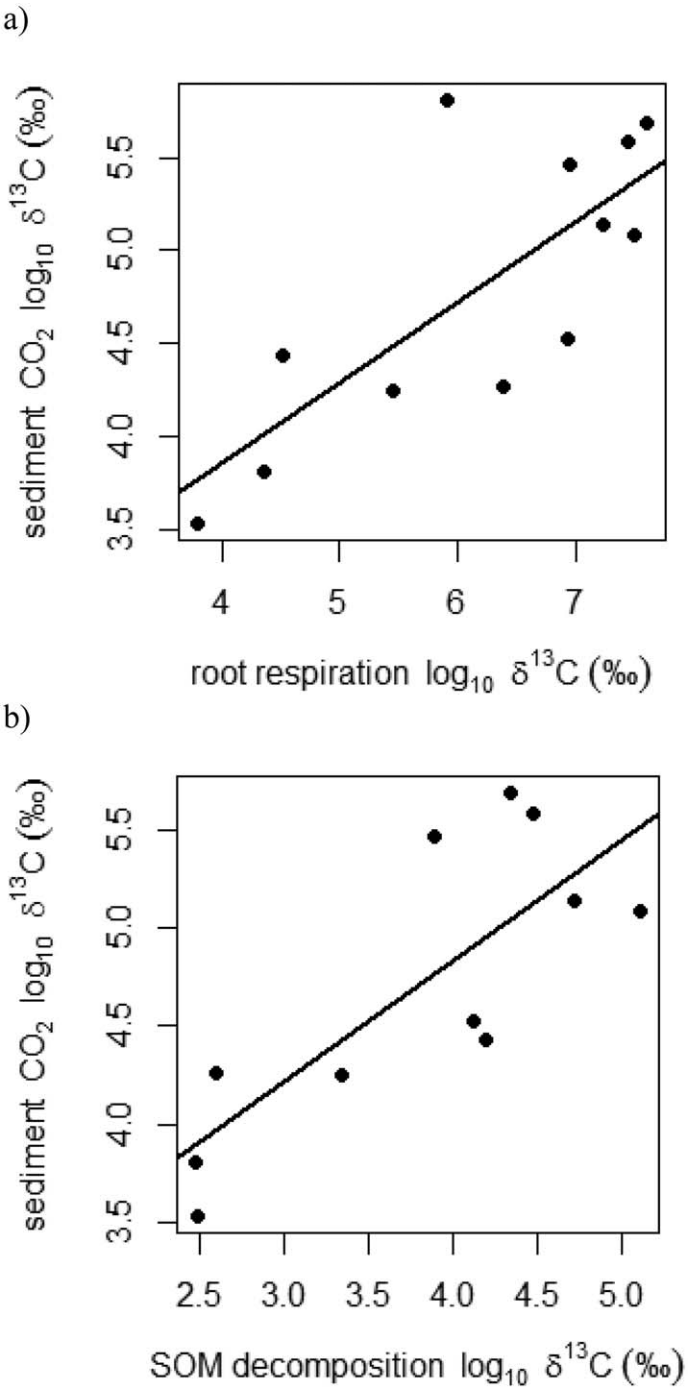

Fig. 7. Relationship between $\delta^{13}C$ values of E_s and *A. marina* roots (**a**), as well as SOM (**b**). The regression equation in (**a**): sediment CO_2 $\log_{10}\delta^{13}C = 0.43$*root respiration $\log_{10}\delta^{13}C + 2.12$ ($R^2 = 0.59$, $p < 0.01$). The regression equation in (**b**): sediment CO_2 $\log_{10}\delta^{13}C = 0.62$*SOM decomposition $\log_{10}\delta^{13}C + 2.36$ ($R^2 = 0.62$, $p < 0.01$).

mangrove C budget suggested that much of the C sinks in mangroves are still unaccounted for, and dissolved inorganic C in porewater may be a significant contributor to the unaccounted C (Bouillon et al. 2008*b*).

Further, the contribution of E_s reported herein (mean: 20.9%) is about double that in mature forests (~ 10%) (Alongi 2009). Aboveground biomass of the newly established mangrove seedlings is very low compared to mature forests. The aboveground biomass of *A. marina* seedlings growing for less than 1 yr was found to be rather low (14.4–58.2 g) (Downton 1982). However, for example, the aboveground biomass of mature *A. marina* trees may reach 39.7–557.9 kg (tree diameter at breast height 10–35 cm), estimated from the allometric equation of biomass proposed by Komiyama et al. (2008). The significantly lower aboveground biomass of mangrove seedlings may account for the lower contribution of E_c to E_e and thus higher E_s to E_e, in contrast to mature mangroves.

Partitioning sediment CO_2 efflux

This study suggests that E_r of young *A. marina* is low compared with E_{SOM}. This result is in contrast with the finding that generally E_r was higher than E_{SOM} in mature mangrove forests (Troxler et al. 2015). E_r comprises CO_2 respired by roots as well as that released in the process of microbial degradation of roots. The root biomass of *A. marina* seedlings growing for less than 1 yr was found to be rather low (14.8–51.2 g) (Downton 1982). However, for example, the root biomass of mature *A. marina* trees may reach 18.9–82.0 kg (tree diameter at breast height 10–35 cm), estimated from the allometric equation of biomass proposed by

Table 1. $\delta^{13}C$ values of chamber CO_2, sediment CO_2, SOM decomposition, and root respiration.

Sources	Components	Number of samples	$\delta^{13}C$ values (‰)
Chamber CO_2	E_e	6	245.3 ± 77.7
Sediment CO_2	E_s	12	154.5 ± 30.0
SOM decomposition	E_{SOM}	12	59.7 ± 13.4
Root respiration	E_r	12	815.7 ± 211.8
Litter decomposition	E_l	12	13.9 ± 9.9

Komiyama et al. (2008). The high biomass of root systems of mature trees definitely respire more CO_2 than the less-developed fine roots of establishing seedlings. In addition, mature *A. marina* has pneumatophores and contributes to CO_2 flux from the sediment–air interface. Furthermore, the high substrate supply of mature trees provides more energy for microbial communities to decompose roots, in comparison with the low substrate supply of seedlings.

This study also highlights the lower contribution of litter (12.8%) relative to roots to E_s in systems dominated by young trees. During the control experiment, litter production was very low, since isotopic fractionation must be minimized during the measurement period and thus the short incubation period did not allow significant accumulated litterfall. The small isotopic fractionation of litter is confirmed by the fact that there is little C isotopic difference of *A. marina* litter that fell on the sediment surface in chambers without nets, compared with litter segregated from sediments in chambers with nets. Therefore, the low litter production leads to low E_l, thereby contributing a lower portion than E_r to E_s. This is in agreement with published data showing that E_r contributed to approximately half of E_s (Luo and Zhou 2010).

In addition, our result implies that the $\delta^{13}C$ of E_s (154.5 ± 30.0‰) is closely related to $\delta^{13}C$ of E_r (815.7 ± 211.8‰) and E_{SOM} (59.7 ± 13.4‰). In our laboratory microcosms, mangrove seedlings took up enriched ^{13}C from CO_2 generated by the reaction between HCl and $NaH^{13}CO_3$. Subsequently, the assimilated ^{13}C was allocated to roots, the portion exuded by which was subsequently incorporated into SOM. Thus part of ^{13}C in SOM is derived from the ^{13}C of roots, explaining the close association between $\delta^{13}C$ of E_s and both E_r and E_{SOM}. The incorporation of ^{13}C from roots into the sediment is also mirrored by the highly enriched sediment $\delta^{13}C$ in chambers with seedlings, while sediment $\delta^{13}C$ values are significantly lower in chambers just containing sediment. This is consistent with earlier findings that mangrove roots can stimulate sediment sulfate reduction via root exudates (Alongi et al. 1998; Kristensen and Alongi 2006).

This study has implications for understanding the sources of ecosystem CO_2 efflux and CO_2 efflux from the sediment–air interface in global mangroves, especially those subjected to restoration after dieback or deforestation. When mature mangroves are replaced by monospecific mangrove plantations, the contributions of E_s to E_e and E_{SOM} to E_s increase while the contributions of E_c to E_e and E_r to E_s decrease in the short term. This study highlights the necessity to construct a temporal trajectory of ecosystem CO_2 efflux and CO_2 efflux from the sediment–air interface in mangrove ecosystems. Future studies may separate the contribution of microphytobenthos from E_{SOM} to further partition the role of microphytobenthic respiration from sediments (Leopold et al. 2013; Bulmer et al. 2015; Grellier et al. 2017; Ouyang et al. 2017).

The developed technique offers a safe and simple alternative to the ^{14}C isotope and dual stable isotope techniques proposed by Luo and Zhou (2010). It may be applied to the investigation of sources of CO_2 efflux from other vegetation and mature forests.

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Conflict of Interest

None declared.

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