# Belowground carbon and hydrological dynamics of mangrove forests

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The candidate confirms that the work submitted is her own, except where work which has formed part of jointly-authored publications has been included. The contribution of the candidate and the other authors to this work has been explicitly indicated below. The candidate confirms that appropriate credit has been given within the thesis where reference has been made to the work of others.

The work in **chapter two** of the thesis has appeared in publication as follows:

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MA led the development of the EnRoot minirhizotron and conducted the laboratory tests, and wrote the manuscript. AJB and PJM contributed to the design of EnRoot. JH and AH developed the image correction technique. All authors contributed critically to manuscript development.

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MA was responsible for the study design, data analysis, preparation of figures, and writing the manuscript. All other authors contributed to the study design, data collection and contributed critically to manuscript development.

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MA was responsible for the study design, data analysis, preparation of figures, and writing the manuscript. AT, AJB and PJM lead the modelling part of the study. All other authors contributed to the study design, data collection and writing of the manuscript.

## Thesis by alternative format – rationale

This thesis is submitted in accordance with the Faculty of Environment's alternative style of doctoral thesis including published materials. This format is apposite for this thesis because chapters 2 and 3 have already been published in peer-reviewed journals, while chapters 4 and 5 are in manuscript format. Each manuscript is self-contained and includes an introduction, method, result and discussion, which may overlap with that in other sections of the thesis. Nevertheless, this PhD thesis constitute a continuous body of work including: a short introduction (chapter 1); four research articles (chapter 2, 3, 4 and 5); and a discussion and outlook chapter (chapter 6). The format described above is in line with the University of Leeds guidelines for the alternative style of doctoral thesis including published materials.

# Acknowledgments

A PhD thesis is signed by one author, but in reality, it is a collaborative work, including many people. Along the way of my PhD, I have received incredible help and support from a lot of people and I am really grateful for that.

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# List of abbreviations commonly used

| | |
|---|---|
| € | Euro |
| °C | Degree Celsius |
| a | Year |
| *AICc* | Akaike’s corrected information criterion |
| C | Carbon |
| CG1978 | Mangrove site reforested in 1978 |
| CG1986 | Mangrove site reforested in 1986 |
| CG1991 | Mangrove site reforested in 1991 |
| CGBR | Can Gio Biosphere Reserve |
| $CO_2$ | Carbon dioxide |
| d | Day |
| DIC | Dissolved inorganic carbon |
| DOC | Dissolved organic carbon |
| g | Gram |
| h | Hour |
| ha | Hectare |
| log | Logarithm |
| m | Meter |
| Pg | Petagram ($1 \times 10^{15}$ g) |
| s | Second |
| SOM | Soil organic matter decay |
| t | Metric tonne |
| VWC | Volumetric water content |

# Abstract

Mangroves are among the most carbon-dense ecosystems in the world. However, it is unclear whether natural and restored mangroves will continue to sequester carbon in the face of global environmental change. I used both laboratory mesocosms and field experiments to investigate the effect of global changes (reforestation, warming and sea level change induced by climate change) on fine root production (chapters 2 and 3) and on soil organic matter (SOM) decomposition (chapter 4). In addition, I identify the controls on soil water regimes in mangrove soils (chapter 5). My research showed that warming from 27 to 31°C increased SOM decomposition by 21 %, though this impact was reduced in mangrove soils affected by rising sea levels. Drought conditions (simulated as a suppression of soil inundation) sharply increased SOM decomposition (+66 %) and acted with warming to exaggerate this effect. Rising sea levels (simulated as an increase of inundation duration from two to six hours per day), alone or combined with warming, did not affect SOM decomposition. Therefore, the persistence of mangrove to sea level rise by accumulating SOM is likely driven by mangrove root production, and not by reduced SOM decay as previously assumed. Root production might be altered by additional global change factors (chapter 3). I showed that following mangrove restoration the fine root production declines monotonically with mangrove stand ages. This age-related pattern has been observed in aboveground production, but has not previously been reported in belowground carbon. Forecasts of changes to mangrove carbon storage and the persistence of these ecosystems in the face of rising sea levels need to account for stand ages in reforested mangroves (chapter 3). Finally, I showed that clayey mangrove sediment does not drain during ebb tides, even in locations with high densities of animal burrows. Only mangroves with coarser sediments seem to be sensitive to animal burrows. A possible shift toward coarser sediment with rising sea levels may lead to changes in lateral water flows within mangrove soils and their associated carbon loss (chapter 5). Overall, my thesis findings have important implications for understanding both mangrove carbon dynamics and the persistence of mangroves and other coastal wetlands under current and future environmental conditions.

# Chapter 1 Introduction

## 1.1 Project Rationale

### 1.1.1 Mangroves: a valuable ecosystem under threat

Mangroves are forested wetlands found along tropical and subtropical coastlines. They provide ecosystem services that have been estimated to be worth $194 000 per hectare per year (2011 figure: Costanza et al., 2014), such as provision of basic food protein for coastal communities, support of offshore fishery yield by being a spawning and nursery area and protection against storms (Costanza et al., 2014). In recent decades, mangroves have been reported to store a considerable amount of carbon (900 t C $ha^{-1}$: Alongi, 2012) and to be a very efficient carbon sink (226 ± 39 g C $m^{-2}$ $a^{-1}$: McLeod et al., 2011). The capacity of mangroves to store large amounts of carbon in their soil (5-10.4 Pg globally) makes these ecosystems as a low-cost option for mitigating climate change at the regional and national scale (Murdiyarso et al., 2015; Taillardat et al., 2018a). Since most of the human population is concentrated in coastal areas, mangrove ecosystem services have been suggested to directly benefit up to 200 million people globally (Hutchison et al., 2014). However, these valuable ecosystem services are under threat due to extensive mangrove losses.

While mangroves once occupied nearly 75 % of the world’s tropical and subtropical coasts, around 35 % of this has now been lost (Alongi, 2002). Duke (2007) predicted that by the end of the 21st century, the world will be without mangroves if no actions are taken.

In response, worldwide restoration and conservation projects have rapidly emerged (Lee et al., 2019). Here, I define restoration as actions aiming to re-establish mangrove ecosystem structures (biotic and abiotic factors) and functions (flow of energy and materials) (Temperton et al., 2004), and reforestation as planting mangrove trees. Restoration and reforestation have intensified since mangroves were recognised to be among the world's most carbon-dense ecosystems (Donato et al., 2011). Mangroves have been mostly reforested, rather than restored (Lee et al., 2019). However, there is considerable uncertainty about the sustainability of such mangrove reforestation schemes (Lee et al., 2019), and so far there is very little study on how mangrove functions and processes respond to reforestation (Bosire et al., 2008; Osland et al., 2012).

In addition, against a backdrop of changing future climates, it is uncertain whether natural and reforested mangroves will be resilient to sea level rise and remain a sink for atmospheric carbon (Gilman et al., 2008; Lovelock et al., 2015). Despite the large potential effects of global changes and reforestation on mangroves, relatively few studies have addressed this question, especially for belowground carbon cycling (Krauss et al., 2017; Osland et al., 2012)

### 1.1.2 Mangrove adjustment in response to sea level rise

Mangroves can withstand sea level rise by migrating landward, or by accreting soil vertically. These processes are referred to as the occupation of landward and vertical 'accommodation spaces' (Rogers et al., 2019). Landward migration is constrained by topographic features, such as hills (Ezcurra et al., 2016); and human developments, including urban areas and flood-protection infrastructure such as embankments (Schuerch et al., 2018). The vertical accretion of soil depends on the accumulation of soil organic matter (SOM) and sediments (Krauss et al., 2014). There are evidences of a sharp decrease in sediment supply to deltas worldwide, this is in part due to intensive sand mining in rivers, and dams trapping sediments (Anthony et al., 2015; Darby et al., 2016). However, the factors driving the accumulation of SOM are not fully understood. Specifically, it is unclear whether the enhanced soil accretion that enables mangroves to occupy vertical accommodation space results from increased root production or a reduction in SOM decay. Nor do we know how global changes will affect these processes. Therefore, suggestions that mangroves will persist as sea levels rise are yet to be thoroughly tested (Krauss et al., 2017).

### 1.1.3 Mangroves as carbon rich ecosystems: carbon stock and dynamics

Critical to our ability to evaluate the effects of global change on the persistence of mangroves is an accurate estimation of their carbon dynamics. Mangrove carbon can be separated into two major stocks: i) the aboveground carbon stock, encompassing the leaves, the trunks and the branches, and ii) the belowground carbon stock – including soil carbon and root biomass.

#### 1.1.3.1 Aboveground carbon stock and productivity

Aboveground carbon stocks and production have been used to predict and better understand the belowground carbon dynamics (Adame et al., 2017; Kauffman et al., 2012; Komiyama et al., 2000). The aboveground carbon stocks in mangroves have been relatively well studied and are estimated to be between 129 to 184 t C ha$^{-1}$, and 1.75 Pg globally (Hutchison et al., 2014; Simard et al., 2019). The spatial distribution of this aboveground carbon stock might be explained by precipitation, temperature and cyclone frequency (Simard et al., 2019). Aboveground productivity has been related to other factors, such as latitude, hydromorphological setting, interaction of stressors (e.g. salinity, inundation regime) and mangrove stand age (Alongi, 2009; Fromard et al., 1998; Twilley & Rivera-Monroy, 2009; Walcker et al., 2018). These factors can be classified into global, regional, ecosystem, and habitat scales (Figure 1-1, Twilley & Rivera-Monroy, 2009). At the global scale, mangrove aboveground production is greatest at the equator and declines with increasing latitude (Twilley & Rivera-Monroy, 2009). At the regional scale, mangrove geomorphological setting (e.g. riverine, fringe, basin, and scrub: Lugo & Snedaker, 1974) drives aboveground productivity with the greatest productivity in riverine forests and the lowest in scrub forests (Twilley & Rivera-Monroy, 2009). At the ecosystem scale, hydrology and topography interact to control aboveground production. For instance, tidal amplitude explains a large proportion of the variance in mangrove net primary production (Rovai et al., 2016). Finally, at the habitat scale, mangrove aboveground productivity responds to regulators (salinity, sulphide, pH and redox condition), resources (nutrients, light, space), and hydroperiods, with the greatest production occurring when the levels of stress-related to those factors are low (Figure 1-1) (see also Twilley & Rivera-Monroy, 2005). For instance, a mangrove stand will have low aboveground productivity if the soil salinity is high and the inundation duration long.

The interaction of stressors and regulators have been suggested as a theoretical framework to guide mangrove restoration to reach optimum aboveground growth (Twilley et al., 1999). Indicators of mangrove restoration success are commonly based on aboveground dynamics, but are mostly restricted to structural changes, such as the number of planted trees per area (Lee et al., 2019). Aboveground carbon biomass per unit area has also been proposed to identify priority areas for restoration efforts that maximise carbon storage (Hutchison et al., 2014). However, this focus on aboveground biomass may be misleading, because: i) most of the carbon (up to 90 %) is found in the soil (Donato et al., 2011), ii) low canopy mangroves have been shown to accumulate a large amount of carbon belowground (900-3000 t C ha$^{-1}$: Ezcurra et al., 2016), while little aboveground, and iii) areas with high densities of soil carbon do not always fully overlap with areas that have high densities of aboveground biomass (Atwood et al., 2017). Research efforts should, therefore, aim for a better understanding of the largest and most persistent carbon stock in mangroves: the soil and the roots (Alongi, 2014).

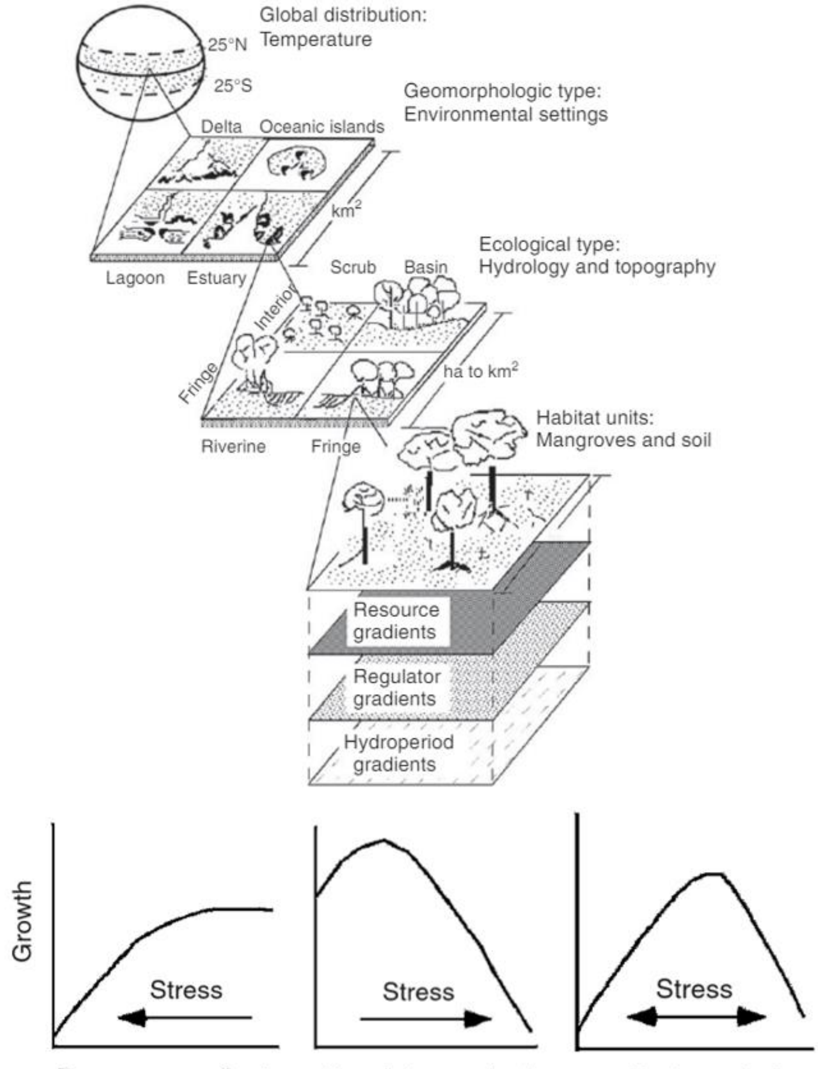

**Figure 1-1** Hierarchical classification of mangrove aboveground production based on factors at the global, regional, ecosystem and habitat scales (Twilley & Rivera-Monroy, 2009). At the habitat scale, the highest aboveground production occurs when all the level of stress related to those factors are low (modified from Twilley & Rivera-Monroy, 2005).

#### 1.1.3.2 Soil carbon stock

The average global mangrove soil carbon stock has been estimated to be between to 283 ± 193 t C ha$^{-1}$ (Atwood et al., 2017) to 361±136 t C ha$^{-1}$ (Sanderman et al., 2018). Atwood et al. (2017) used 1,230 sampling points from 48 countries covering 88 % of the global mangrove area. However, the general quality of the mangrove soil carbon data was variable. Twenty-two countries were identified as having poor-quality data; these were mostly in Asia and the Pacific islands, where the majority of mangroves are found. There was also an underrepresentation of soil carbon data from Africa, with few sites investigated. In addition, both Atwood et al. (2017) and Sanderman et al. (2018) restricted their soil carbon estimates to the top metre, whereas it is known that mangrove soil carbon stocks extend to depths well beyond this (Donato et al., 2011; Ezcurra et al., 2016; Murdiyarso et al., 2015; Nam et al., 2016; Sanders et al., 2016). Both of these estimations are therefore likely to be conservative.

An alternative approach to estimate soil carbon stock is to determine its controls. The soil carbon stock is believed to be linked with precipitation (quantity and regime), tides, river discharges, temperatures, plant diversity, latitude (as a combination of factors), and potential evapotranspiration (Alongi, 2009; Atwood et al., 2017; Rovai et al., 2018). Using soil carbon stock from neotropical mangroves, Rovai et al. (2018) showed that tidal amplitude is the main driver of mangrove soil carbon stock, followed by temperature. Precipitation, potential evapotranspiration and river discharge were not included in the predictive model of soil carbon stock by Rovai et al. (2018). The exclusion of these factors does not ultimately mean that they are not controlling the soil carbon stock, but rather that they are less important than tidal amplitude and temperature, or that the index used were not appropriate or of good enough quality (Rovai et al., 2018). Alternatively, the data chosen to define each index may not be appropriate. For example, Rovai et al. (2018) defined the temperature index by the minimum temperature of the coldest month, and the precipitation index as the minimum precipitation of the driest month. Different indexes, such as average monthly precipitation or average monthly temperature might have been better to predict the soil carbon stock. Those studies identified some factors correlated with soil carbon stock at large scales, but they do not explain the mechanisms leading to the formation of this stock. Accumulation of soil carbon is likely to result from the balance between organic matter decay and root production in carbon-dense mangroves (Alongi, 2009; Bouillon et al., 2003; Middleton & McKee, 2001). The aboveground litter production contributes little to the soil carbon, because the leaves are fast washed away by tides, rapidly eaten by crabs and less recalcitrant than roots (see 1.1.3.4). The role of algae has been little studied, but estimates of algae net primary production is two and half time less important than roots (Alongi, 2009). The source of soil organic matter is increasingly studied, but the mechanisms leading to the accumulation of carbon are not well understood, especially the factors controlling the SOM decay and the root production.

#### 1.1.3.3 Root production

Mangrove root production is a major input of carbon to the soil, but also one of the least studied components of the mangrove carbon cycle (Figure 1-2, Bouillon et al., 2008; Muhammad-Nor et al., 2019). The root production is defined as an increase of biomass or an increase of root area (length, diameter or total area) depending on the method of measurement (ingrowth core, sequential coring or (mini)rhizotron) (Johnson at al. 2001).Several tools have been developed for measuring root production, such as the minirhizotron (Johnson et al., 2001), but there is a need to adapt them for mangrove ecosystems. Minirhizotrons involve the installation of a transparent tube into the soil, into which a camera is inserted periodically to record root development. They have shown to have major advantages over other methods, such as reduced disturbances of soil after installation, non-usage of artificial soil matrix, and the ability to track individual root growth (Johnson et al. 2001). However, commercial minirhizotrons are not adapted to mangrove conditions, due to their size (large) and weight (heavy), and because they are not waterproof and need external power supply. The inherent difficulty of measuring root production in-situ has resulted in limited root production measurements in mangroves (Adame et al., 2014; Bouillon et al., 2008; Muhammad-Nor et al., 2019).

The few studies that have investigated mangrove root production have examined the response of root growth to nutrients, flooding and their interactions (Adame et al., 2014; Castañeda-Moya et al., 2011; Cormier et al., 2015; Naidoo, 2009; Poungparn et al., 2016; Torres et al., 2019). Removing nutrient limitation seems to increase root growth (Naidoo, 2009), but this varies with inundation frequency (Adame et al., 2014; Castañeda-Moya et al., 2011). For instance, McKee et al. (2007) and Adame et al. (2014) reported an increase of root production with high soil phosphorus levels (as superphosphate and orthophosphates, respectively); but Adame et al. (2014) observed this phosphorus-driven increase only in mangroves with infrequent tidal inundation and high soil salinity. There was no change in root production under higher phosphorus in frequently inundated mangroves (Adame et al., 2014). The effect of nutrients has also been tested on root biomass increase in combination with other factors, such as elevated atmospheric $CO_2$ (Reef et al., 2016). Under elevated atmospheric $CO_2$, the increase of root biomass was greater for the high-nutrient treatment relative to the low-nutrient treatment (Reef et al., 2016). However, Reef et al. (2016) did not distinguish between increase of root biomass and root production. Both are different, since root biomass results of root production and root decay together. However, root production and decay are likely to respond differently to nutrients and elevated atmospheric $CO_2$ (Adame et al., 2014; Pendall et al., 2013). Therefore, further research investigations of the effect of $CO_2$ and nutrients on root production alone are needed. Increasing coastal population and land fertilisation from agriculture (Reis et al., 2017) have resulted in unprecedented nutrient enrichment; therefore, we need to better understand fine root production response to nutrients alone and in combination with other factors in order to forecast mangrove fine root response to present and future environmental conditions.

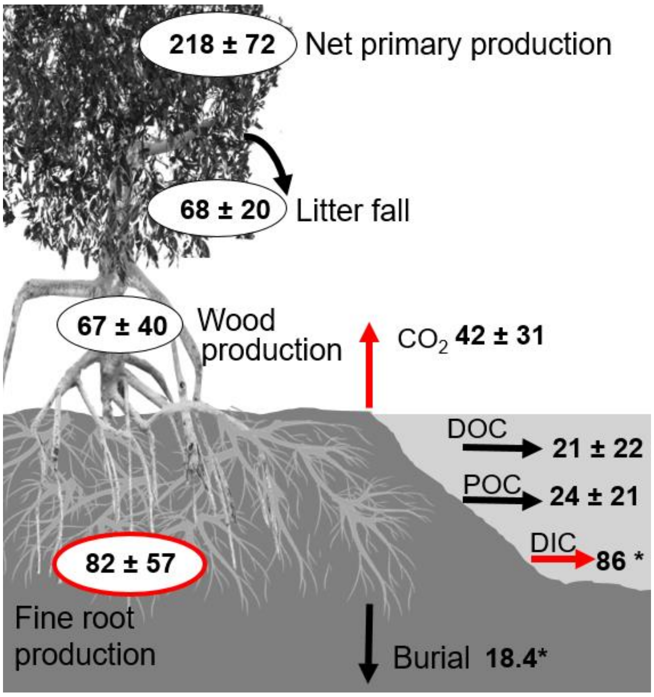

**Figure 1-2** Mangrove carbon budget from Bouillon et al. (2008) and Alongi et al. (2014). All values are reported in millions tonnes of carbon per year. Red indicates components of the budget that I am investigating in this thesis. Asterisks indicate no error estimate reported. The net primary production is allocated to leaves, woods and roots growth. This carbon is then buried, mineralised or transferred to adjacent ecosystem (see arrows).

Another major change that mangroves are now facing is reforestation (Lee et al., 2019). Only two studies have investigated the impact of mangrove reforestation on root production (McKee & Faulkner, 2000; Perez-Ceballos et al., 2018), and they show contrasting results. Perez-Ceballos et al. (2018) show that reforested mangroves have lower belowground production than natural mangroves; in contrast, McKee and Faulkner (2000) found that two reforested mangroves of 6- and 14-year-old (from two different locations) had similar root production to natural mangroves. However, the natural mangroves investigated were matures, while the reforested one unmature, and therefore both should have different root production as observed in other forests (Idol et al., 2000; Law et al., 2003). While we know the aboveground trajectory of mangroves (Fromard et al., 1998; Walcker et al., 2018), the trajectory of the belowground productivity after mangrove reforestation or restoration is, as yet, unknown. Understanding root production trajectory, in particular for fine roots, is important, because SOM accumulation results from fine roots accumulation, and mangrove soil carbon is mostly derived from fine roots (McKee, 2011; Middleton & McKee, 2001; Xiong et al., 2017). Considering the large investment in mangrove reforestation projects (Lee et al., 2019), there is a clear need for improved understanding of the fine root production trajectory to be able to forecast the carbon sink capacity of reforested mangroves over time, and to determine if they will withstand sea level rise in the future through SOM accumulation.

#### 1.1.3.4 SOM decay

SOM is formed by decaying materials from the above- and belowground component of ecosystems, such as decaying leaves, trunks and roots. In mangroves, leaves have been shown to be of minor importance for SOM formation, since they are rapidly washed away by the tides, eaten by animals such as crabs, and are less recalcitrant to microbial decomposition than are roots (Alongi, 2009; Black & Shimmield, 2005; Twilley et al., 1997). The decomposition of trunk and root material has been poorly studied. However, belowground roots are believed to be the main component of SOM (Ezcurra et al., 2016; McKee et al., 2007; Middleton & McKee, 2001; Van Der Valk & Attiwill, 1984), because unlike aboveground detritus they are: i) not consumed by crabs (Van Der Valk & Attiwill, 1984); ii) not washed away by tides; and iii) biochemically resistant to decay (Albright, 1976; Huxham et al., 2010; Middleton & McKee, 2001; Ono et al., 2015; Poret et al., 2007; Van Der Valk & Attiwill, 1984).

Recently, root decay has been estimated to occur at a rate of 0.14 % mass loss per day, but this estimate based on the literature review of Ouyang et al. (2017) needs to be taken with caution. The data used in this review were from 14 studies that employed a range of methods to estimate decay rates, and which were sometimes incorrectly interpreted. For instance, root decay was extracted from the state of peat decomposition (visual inspection with Von Post scale) (McKee & Faulkner, 2000); from primary production (Ewe et al., 2006) and from in-situ soil $CO_2$ (Middelburg et al., 1996) and oxygen efflux (Sweetman et al., 2010), which includes autotrophic biofilm, fauna and fine root respiration in addition of SOM decomposition. Factors controlling mangrove root decay are, therefore, not fully resolved.

The SOM is controlled by a multitude of factors, which might be altered by global changes. For instance, mangroves are experiencing eutrophication (nutrient enrichment), rising temperature, and sea level changes which are likely to modify SOM decay. Nutrients availability can limit microbial activity, and have shown to be important controls on SOM decay in mangroves (Feller et al., 2003). Most studies show that phosphorus (under enriched and natural gradient conditions) increases or has no effect on the decay of SOM (including root decay), while nitrogen seems not to increase the SOM decomposition in most cases (Feller et al., 2003; Huxham et al., 2010; Keuskamp et al., 2013; Poret et al., 2007). However, which nutrient is limiting the SOM decay is not straightforward, since the nutrient limiting the microbial activity can be different from the nutrient limiting the plant growth (Feller et al., 2003).

Temperature has been shown to control SOM decay in coastal wetlands, but few mangrove sites have been investigated (Kirwan et al., 2014; Lovelock, 2008; Morris & Whiting, 1986). Studies have reported a range from no effect from 100 % increase in SOM decay rate with a temperature rise of ~5°C (from 25 to 30°C; Jin et al., 2013; Lewis et al., 2014; Lovelock, 2008; Poungparn et al., 2009; Simpson et al., 2019). Most of those studies have used latitudinal gradients to examine the thermal sensitivity of mangrove SOM decay, meaning that this large range of thermal sensitivity is likely to reflect in part site-specific confounding factors (such as tidal inundation regime, soil nutrient status, water chemistry, substrate geology). In addition, most of the studies report soil respiration, and therefore include autotrophic (plant) respiration in addition to heterotrophic respiration (Ouyang et al., 2018). Controlled experiments are therefore required to isolate the effect of temperature on SOM decay (Twilley et al., 2017).

Flooding duration and frequency are assumed to be inversely related to decomposition rate in mangroves and other wetlands (Davidson & Janssens, 2006; Nyman & DeLaune, 1991; Rovai et al., 2016), but so far there is little evidence to support this hypothesis in mangroves. Some studies show a decrease of SOM decay under increasing flooding (Chambers, 2012; Lewis et al., 2014), while others show no effect or even a slight increase of SOM decay (Blum, 1993; Blum & Christian, 2004; Hackney, 1987; Kirwan et al., 2013). Mangroves will also experience temporary sea-level drops, also known as Taimasa events (Widlansky et al., 2015). Taimasa events occur in the tropical Pacific and is linked to El Niño–Southern Oscillation. During El Niño, weak equatorial trade winds cause the thermocline to shoal in the tropical western Pacific. The presence of cool water then causes the sea level to drop (e.g thermal contraction) as much as 0.30 m (Widlansky et al., 2015) for up to a year. With climate change, these events are forecasted to be more intense and frequent, even against a long-term backdrop of rising relative sea level (Widlansky et al., 2015). As a result, mangroves might experience drought conditions (Lovelock et al., 2017). However, so far the impact of drought events on SOM decay in mangroves is unknown (Chapman et al., 2019).

Warming and sea level changes are highly likely during the remainder of the 21st century (IPCC, 2013; Widlansky et al., 2015), but we do not know how these factors will affect mangrove SOM decay. A recent study shows that multiple factors in combination affect SOM decay in an unexpected way (Rillig et al., 2019). Therefore, understanding the impact of warming and different inundation regimes, individually and in combination, should be a priority for further mangrove research.

### 1.1.4 Mangrove soil hydrology and belowground carbon dynamics

Soil water content is a major control on the belowground carbon dynamics of mangroves (Spivak et al., 2019; Twilley & Chen, 1998). Compared to other carbon-dense ecosystems, such as northern peatlands, our understanding of mangrove soil hydrology is limited. It has been suggested that mangrove soils have a limited drainage capacity, given that mangrove sediments are mostly clays (Schwendenmann et al., 2006; Stieglitz et al., 2000). However, some mangrove soils have been shown to be dominated by coarser sediments, such as sand or silt (Banerjee et al., 2018; Sanders et al., 2012), which may make them more prone to drainage. However, few studies have reported the hydraulic conductivity of different mangrove soil types (Schwendenmann et al., 2006; Susilo et al., 2005; Susilo & Ridd, 2005; Xia & Li, 2012). The role of SOM on mangrove soil hydrology have also been overlooked (Mazda & Ikeda, 2006). Mangrove SOM is mostly composed of roots and that could increase the subsurface flow (Mazda & Ikeda, 2006), but few studies have directly quantified it in mangroves (Whelan et al., 2005).

A better-studied phenomenon is the influence of animal burrows on water flow in mangrove soils. Susilo & Ridd (2005) reported that crab burrows can increase the flux of water through mangrove soil by up to a factor of ten. Flushing of burrows occurs during tidal cycles, and results from a pressure difference between the openings of burrows due their different positions on sloping soil surfaces (Ridd, 1996; Stieglitz et al., 2000). Large-scale (forest scale) studies using radioisotope tracer techniques have estimated that a large amount of water ($16.3 \pm 5.1$ cm $d^{-1}$ in average; Tait et al., 2016) is exported from mangrove soils to adjacent creeks (Stieglitz et al., 2013; Tait et al., 2016; Taillardat et al., 2018b).This water seems to be highly enriched with dissolved inorganic carbon (DIC) (Taillardat et al., 2018b), and is suspected to represent one of the largest losses of mangrove carbon (Figure 1-2). DIC has been estimated to be a century-old at one site (Maher et al., 2017) and is believed to be the result of aerobic mineralisation of the sediments composing the walls of crab and other animal burrows, but this hypothesis has not been tested in the field yet.

While the role of animal burrows in increasing the area of soil exposed to air has been quantified, few studies have investigated if burrows cause the mangrove sediment matrix (the sediment between the burrows) to drain more readily. Since the sediment matrix is saturated with water by tidal inundation, and the burrows are flushed quickly (Stieglitz et al., 2000), it is likely that the water from the sediment matrix seeps into the empty burrows. This flow, if it occurs, will be generated by the pressure difference between the sediment matrix and the empty burrows at ebb tide (Darcy’s law). This process would result in the drainage of the sediment matrix in addition to the burrows, which would aerate the sediment matrix and might therefore result in higher rates of carbon mineralisation, which will increase the inorganic carbon flux. Alongi (2014) suggested that this process happens and that the mangrove sediment matrix is recharged and drained between flood and ebb. In contrast, other authors (e.g., Tait et al., 2016) have suggested that the water flow is restricted to animal burrows in mangrove soils, and that the sediment matrix does not drain. Better understanding the water flow paths is important, because if the sediment matrix is hydrologically isolated from the ocean then so are the solutes (e.g. DOC, DIC) that the water contains. These competing ideas have not been tested in the field so far, but have direct relevance to predicting the impact of global change on belowground carbon dynamics in mangroves.

## 1.2 Mangroves of South-East Asia

My PhD research aimed to investigate some of the key knowledge gaps identified above regarding the mangrove belowground carbon cycling and the soil hydrology of mangroves. My investigation took place in the Mekong Delta in south Vietnam, and below I explain why I chose this area to conduct my research.

South-East Asia holds the most carbon-dense mangroves in the world (Donato et al., 2011); but is also the region most heavily affected by mangrove deforestation (Richards & Friess, 2016), and likely one of the most vulnerable to climate change (Ward et al., 2016). Therefore, South-East Asia has been the focus of mangrove reforestation, with large projects implemented in Bangladesh or Philippines (Lee et al., 2019). Investigating the impact of reforestation projects on mangrove functions and structures requires waiting for planted mangrove trees to grow (López-Portillo et al., 2017), while there is an urgent need for an improved evidence-base to guide mangrove reforestation.

Mangroves in south Vietnam offer an opportunity to investigate the impact of reforestation projects on mangrove functions and structures, as well as the impact of global environmental changes in a short timescale. During the US-Vietnamese war, most of the mangroves there were destroyed by the spraying of herbicides and napalm (Hong & San, 1993). Since the war, extensive mangrove restoration efforts have been conducted in the Mekong Delta. This resulted in one of the largest mangrove reforestation projects in the world, covering more than 100,000 hectares (Hong & San, 1993). Since this project started in 1978, the replanted mangroves have had time to develop, providing a unique scientific opportunity to study the long term impact of reforestation on mature mangroves. The mangroves there have also been reforested at different times, providing an opportunity to investigate the trajectory of mangroves using a chronosequence approach (space-for-time substitution).

This reforestation project is similar to those that have been conducted in South-East Asia and worldwide in the last decade (Lee et al., 2019), because they used propagules of the *Rhizophora* genus, which is the most commonly used genus in reforestation projects worldwide (Ellison, 2000). In addition, the study of *Rhizophora* genus is globally relevant, since *Rhizophora* mangroves hold the second-largest stock of mangrove carbon worldwide (Atwood et al., 2017). Finally, the Mekong Delta has a rapidly-growing coastal population, intensive agriculture and a high rate of urbanisation, which is typical for coastal regions in South-East Asia.

## 1.3 Objectives and outline

The aim of my PhD was to investigate, both, the belowground carbon and the hydrological dynamics of mangroves. I defined three research objectives that I present below, along with their rationale and my strategy for fulfilling them. For each of my objectives, I summarise the originality of my findings. Finally, I provide an outline of my thesis.

### 1.3.1 Objective 1: Quantifying fine root production along a 40-year chronosequence of reforested mangroves using minirhizotrons (chapters 2 and 3)

#### 1.3.1.1 Rationale

Together with riverine sediments, mangrove roots are the main input of carbon to the soil, but also one of the least studied components of the mangrove carbon cycle (Bouillon et al., 2008; Muhammad-Nor et al., 2019). The inherent difficulty of measuring fine root production in situ is probably one of the main factors explaining the paucity of data regarding root production (Adame et al., 2014; Bouillon et al., 2008; Muhammad-Nor et al., 2019). This lack of knowledge prevents us from fully understanding carbon cycling in mangroves, and predicting the trajectories of soil carbon inputs from root production after mangrove reforestation. My aim was to quantify root production across a chronosequence of reforested mangroves with a new type of minirhizotron. My objectives were to: i) develop an inexpensive and easy-to-build minirhizotron suitable for use in mangroves (chapter 2), and ii) quantify the root production in a chronosequence of reforested mangroves (chapter 3).

#### 1.3.1.2 Strategy

I first developed EnRoot, a minirhizotron suitable for use in mangroves and tested it in the laboratory for accuracy and precision. Then, I used Enroot to quantify the root production of reforested mangroves in the Can Gio Biosphere Reserve in the Mekong in Vietnam. I installed seven minirhizotron tubes in each of three reforested mangroves of different ages, and measured monthly root production at several depths over a period of four months after a stabilisation period of more than five months post-installation.

#### 1.3.1.3 Originality of the findings

In chapters 2 and 3, I show for the first time that mangrove fine root production change with mangrove development, using EnRoot minirhizotron. Firstly, I show that minirhizotrons can be upgraded to be used in mangroves, and I provide a full methodology to build and use EnRoot. Secondly, I show that fine root production decline with stand age, as a result of mangrove self-thinning and that a large portion of fine root production might have been overlooked in mangroves carbon budget, because appearing below 30 cm. Together those findings help to address the knowledge gap in one of the least well understood parts of the mangrove carbon cycle; and improves our ability to forecast mangrove SOM accumulation after mangrove reforestation.

### 1.3.2 Objective 2: Quantification of the sensitivity of the SOM decay of mangroves to warming and sea-level change (chapter 4)

#### 1.3.2.1 Rationale

Mangroves will experience warming, and alteration to their inundation regime due to changes in sea level (IPCC, 2013; Widlansky et al., 2015). A recent study highlighted that combining multiple factors might affect SOM decay in an unexpected way (Rillig et al., 2019). Therefore, my aim was to quantify the sensitivity of organic matter decay to those future environmental conditions, individually and in combination. My objectives were to i) quantify the increase, if any, of SOM decay with global warming, ii) quantify the change of SOM decay in response to the alteration in the inundation regime associated with drought events and sea level rise, and iii) quantify the interactive impact of temperature and inundation regime on the SOM decay rates in mangroves.

#### 1.3.2.2 Strategy

I conducted an incubation experiment to simulate how warming and relative sea level change, individually and in combination, are likely to affect SOM decay in mangroves. Soil samples were incubated at two temperatures (27°C and 31°C) and under three inundation regimes (zero, two and six hours of inundation per day). Those factors were combined factorially and resulted in six treatments in total. I measured the soil $CO_2$ efflux as a proxy for SOM decay across the length of the experiment (22 days).

#### 1.3.2.3 Originality of the findings

In chapter 4, I provided a new understanding of how SOM will respond to climate changes in mangroves. I notably reveal the individual and combined effect of temperature and sea level change (simulated with different inundation regimes) on mangrove SOM decay. I show that simulated sea level rise does not result in a decrease of SOM decay, but that sea level fall events cause SOM decay rates to rise sharply (+ 66 %). A warming of 4°C caused SOM decay to increase by 21 %, but this temperature-driven increase was negated under simulated sea level rise. Finally, warming and simulated sea level fall acted together to increase the SOM decay. My results indicate that accumulation of SOM does not result from a decrease of SOM decay under sea level rise, and that SOM accumulation might be affected negatively by warming and sea level drop events, individually and in combination. My results have important implications for forecasting mangrove carbon dynamics and the persistence of mangroves and other coastal wetlands under future scenarios of climate change.

### 1.3.3 Objective 3: Developing a new conceptual model of mangrove hydrology (chapter 5)

#### 1.3.3.1 Rationale

One of the largest losses of mangrove carbon is thought to be via outwelling of carbon-rich groundwater into the ocean. While there is evidences that mangrove outwelling is large, there is still a lack of understanding of the factors controlling this process. Tait et al. (2016) suggested that most of this outwelling water comes from animal (e.g. crab burrows) and that the drainage of the sediment matrix is unimportant. In contrast, Alongi (2014) suggested that both are equally important. My aim was therefore to test these competing conceptual models of soil hydrology.

#### 1.3.3.2 Strategy

I took over 1,400 measurements of near-surface volumetric water content in the sediment matrix between burrows. Those measurements were taken over time during the tidal cycle and across 26 plots with a range of crab burrow densities. These data allowed me to investigate a potential relationship between soil drainage and the density of animal burrows. I used a directed partial sensitivity analysis with a groundwater model to 'sense check' my results. The model showed that clayey soils do not drain readily into crab burrows during the diurnal tidal cycle, even in the immediate proximity of the burrow.

#### 1.3.3.3 Originality of the findings

In chapter 5, I show that the drainage of the sediment matrix is negligible in clayey mangroves, independently of burrow density. I show that substantial drainage occurs between the sediment matrix and the burrows only in mangroves with coarser sediment fractions. With those findings, I reveal the heterogeneity of water flow paths in mangrove soil, as well as its controlling factors. My findings have important implications for the understanding and modelling of mangrove carbon cycling, because mangrove soil hydrology is closely linked to the lateral loss of carbon and carbon burial and decay in soils.

### 1.3.4 Strategy to control for confounding factors

I have carried laboratory (chapter four) and field experiments (chapter five and six). In each chapter, my experimental design allowed me to control for factors known to have an influence on the studied process. In my third chapter, I have controlled for cofounding factors by selecting sites with similar elevation, tree genus, temperature and precipitation regimes. Only the age of the sites (e.g. my treatment) was known to be different. Similarly, in chapter 5, I chose sites with similar features to excluded confounding factors (e.g. similar slope, sediment grain size distribution, and limited amount of fine roots). Only the studied factors (e.g. the number and density of burrows) were known to be different across sites. In contrast, I have deliberately reduced the complexity of the mangrove soil to control for confounding factors in chapter four. I have created a homogeneous soil matrix (e.g. the same fine root biomass, salinity, grain size distribution, and nutrient and carbon content) and applied inundation and temperature treatments. In addition, I have excluded macrofauna (e.g. crabs) and live roots, which produce CO2 that is not created by soil organic matter decomposition.

### 1.3.5 Outline of my research

In this chapter (chapter 1), I identified key knowledge gaps regarding the belowground carbon cycling and the soil hydrology of mangroves, and I defined my research objectives (Figure 1-3). The next chapters (chapters 2, 3, 4 and 5) are four research articles that answer the objectives defined in chapter 1 (Figure 1-3). In chapter 6, I summarise and highlight the connections and implications of the findings from the previous chapters. I also show the relevance of my work to filling the current knowledge gaps associated with the belowground carbon dynamics and the soil hydrology of mangroves, discuss the limitations of those results and suggest directions for future studies (Figure 1-3).

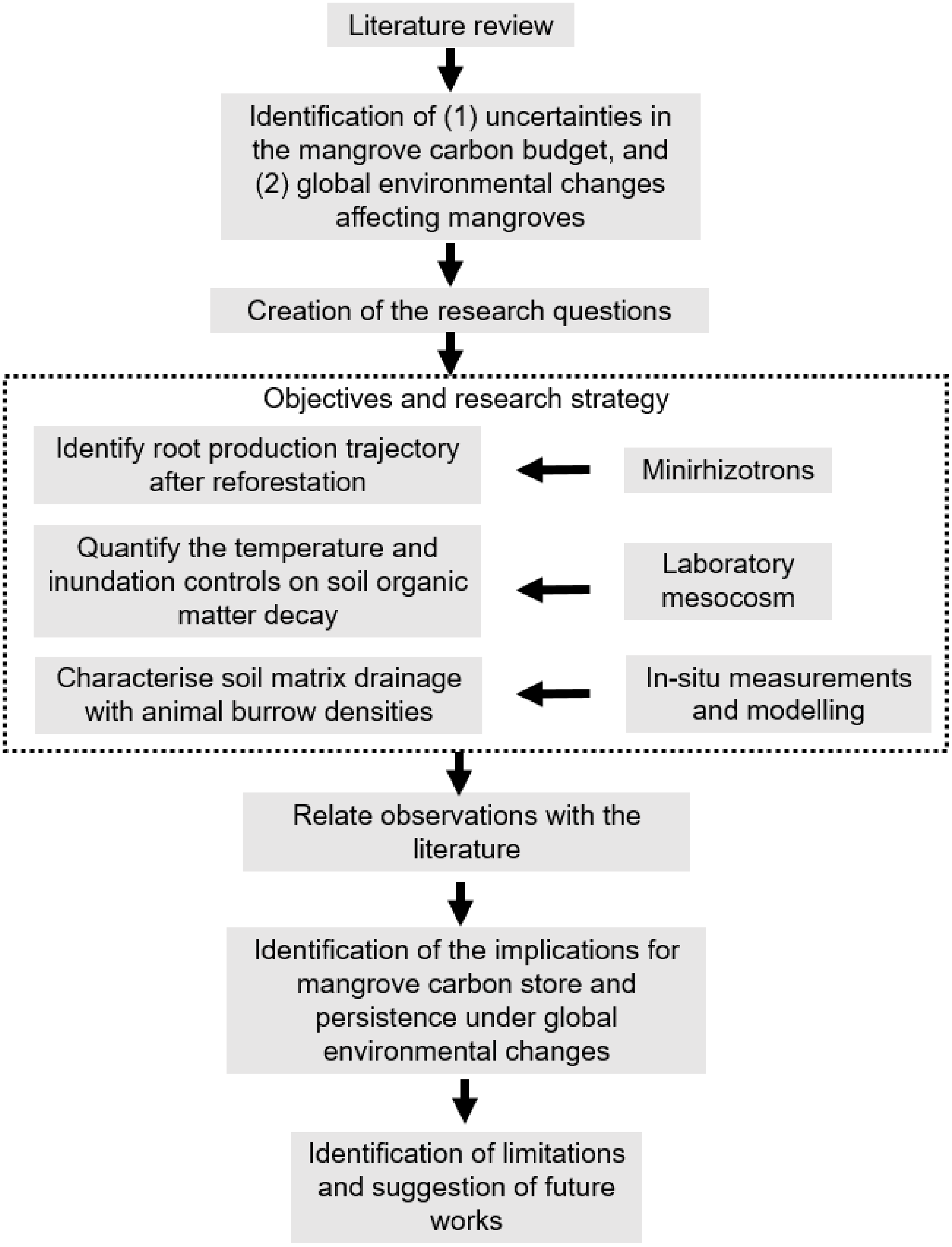

**Figure 1-3** Summary of the conceptual approach to understanding the belowground carbon and hydrological dynamics of mangroves. The first three boxes represents the steps of the introduction (chapter 1). The dashed box includes the analytical chapters (chapters 2, 3, 4 and 5). The last three boxes are the steps of the chapter 6.

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# Chapter 2 EnRoot: a narrow, inexpensive and partially 3D-printable minirhizotron for imaging fine root production

Arnaud, M., Baird A. J, Morris P. J., Harris A., Huck J.J. (2019) EnRoot: a narrow-diameter, inexpensive and partially 3D-printable minirhizotron for imaging fine root production. *Plant Methods*; 15, 101. https://doi.org/10.1186/s13007-019-0489-6

## Abstract

Fine root production is one of the least well understood components of the carbon cycle in terrestrial ecosystems. Minirhizotrons allow accurate and non-destructive sampling of fine root production. Small and large scale studies across a range of ecosystems are needed to have baseline data on fine root production and further assess the impact of global change upon it; however, the expense and the low adaptability of minirhizotrons prevent such data collection, in worldwide distributed sampling schemes, in low-income countries and in some ecosystems (e.g. tropical forested wetlands).

We present EnRoot, a narrow minirhizotron of 25 mm diameter, that is partially 3D printable. EnRoot is inexpensive (€150), easy to construct (no prior knowledge required) and adapted to a range of ecosystems including tropical forested wetlands (e.g. mangroves, peatlands). We tested EnRoot's accuracy and precision for measuring fine root length and diameter, and it yielded Lin’s concordance correlation coefficient values of 0.95 for root diameter and 0.92 for length. As a proof of concept, we tested EnRoot in a mesocosm study, and in the field in a tropical mangrove. EnRoot proved its capacity to capture the development of roots of a legume (*Medicago sativa*) and a mangrove species (seedlings of *Rhizophora mangle*) in laboratory mesocosms. EnRoot's field installation was possible in the root-dense tropical mangrove because its narrow diameter allowed it to be installed between larger roots and because it is fully waterproof. EnRoot compares favourably with commercial minirhizotrons, and can image roots as small as 56 µm.

EnRoot removes barriers to the extensive use of minirhizotrons by being low-cost, easy to construct and adapted to a wide range of ecosystem. It opens the doors to worldwide distributed minirhizotron studies across an extended range of ecosystems with the potential to fill knowledge gaps surrounding fine root production.

**Key words:** Minirhizotron, Root production, Belowground carbon, Fine roots, 3D minirhizotron, Root monitoring, Belowground biomass, Carbon sequestration, Root dynamics

## 2.1 Introduction

Root production is one of the least studied components of terrestrial ecosystems, despite being likely to represent a third of net primary production (Jackson et al., 1997). Several techniques exist to measure *in situ* fine root production, but quantifying such subterranean processes remains difficult and often expensive. Minirhizotrons involve the installation of a transparent tube into the soil, into which a camera is inserted periodically to record root development (Iversen et al., 2011). Net root production is estimated by calculating changes in root diameters and lengths between successive images (Johnson et al., 2001).

Minirhizotrons have proven to be accurate for root production measurements (Johnson et al., 2001) and overcome limitations associated with other methods because: i) the same roots and soil profile are sampled repeatedly, reducing the spatial component of experimental error (Hendricks & Pregitzer, 1996); ii) root production and mortality are measured simultaneously, minimising the likelihood of missing any roots with a fast turnover (appearance, growth and death, Hendricks et al., 2006); iii) they do not use artificial soil substrate, which might modify the root production unlike ingrowth cores (Vogt et al., 1998); and iv) they are non-destructive; once the minirhizotron tubes have been installed, no subsequent disturbance is required to take repeated measurements (Majdi, 1996). The minimal disturbance is advantageous for both mesocosm studies and long-term field experiments.

Minirhizotrons do, however, have limitations. They are expensive (Mohamed et al., 2017) and lack a standardised design; consequently, they are not used in worldwide distributed sampling schemes, such as RAINFOR and GEM networks plots (Marthews et al., 2014) and are rarely used in low-income countries. Additionally, they are not well adapted to wetland conditions (Iversen et al., 2011), including tropical forested wetlands. While working in waterlogged wetland soils or during heavy rain (e.g. monsoon), commercial minirhizotron cameras, being non-waterproof, can be easily damaged. Large above- and belowground roots, also make the installation of commercially-available minirhizotron tubes (with diameters of over 50 mm) in tropical forested wetlands difficult or impossible. As a result, minirhizotrons are either not used (e.g., in mangroves) or are located away from tree trunks, resulting in potentially unrepresentative fine root production estimates. Narrow minirhizotrons would be easier to install. Hand-made minirhizotrons might partially overcome the price limitation, but are usually produced in small numbers for a specific application, are hard to construct (prior knowledge is required), and often are not well documented. There is a need for a minirhizotron that overcomes the limitations identified above and that can be made cheaply and easily to a reproducible specification that allows comparison among and between sites and ecosystems.

Here, we report the development and testing of a narrow, waterproof and inexpensive minirhizotron that can be repeatedly and easily made to a standardised specification: EnRoot. With its narrow diameter and waterproof camera, EnRoot is easy to install and suitable for a large range of ecosystems, including tropical forested wetlands. The material costs – including the camera – are less than €150 per unit. EnRoot is easy to assemble and to reproduce to a standard with its 3D printable components costing less than €35, and has similar imaging capabilities to commercial minirhizotrons.

## 2.2 Results and discussion

### 2.2.1 The new minirhizotron system: EnRoot

#### 2.2.1.1 Description and set up of EnRoot

EnRoot has two main components – an imaging module and a soil tube (Figure 2-1). Both are very narrow; the module has an outside diameter of 25mm, and the soil tube an outside diameter of 32 mm. EnRoot's soil tube is left permanently buried in the soil to allow the development of roots around it (Figure 2-2, a). The tube is made from clear acrylic (2 mm thick; purchased from theplasticshop.co.uk) and has a rubber bung fitted in its base (Figure 2-1). The imaging module is composed of an indexing handle and a camera apparatus (Figure 2-1); both have and inside diameter of 21 mm, and an outside diameter of 25 mm. The camera apparatus is 15 cm long and the indexing handle 45 cm long. The handle extension length is adjustable so that the camera apparatus can reach the bottom of soil tubes. The holes of the indexing handle and its extension are drilled every centimetre with a pillar drill (AJVBM 4, Ajax) using a drill bit of 4 mm. The distance between the drilled holes can be adjusted to take pictures with no or a range of overlaps. The camera apparatus has a window through which the roots are directly observed with a camera (Potensic® 2-in-1 USB Endoscope with LEDs) and a mirror orientated at 40° relative to the soil tube's long axis (Figure 2-3).

We developed EnRoot with the aim that anyone can reproduce it easily. The assembly of EnRoot does not require prior training and takes less than an hour.

Its components – the connectors, adaptor and two camera-apparatus supports – are 3D printable (Figure 2-1). The 3D files required to fabricate these are freely available for use and modification (Appendix A2.2). We printed these components in polylactic acid thermoplastic using the 3D Hubs printing platform (https://www.3dhubs.com). We chose this platform because of its low price and because it is available in 140 countries, making EnRoot reproducible almost everywhere.

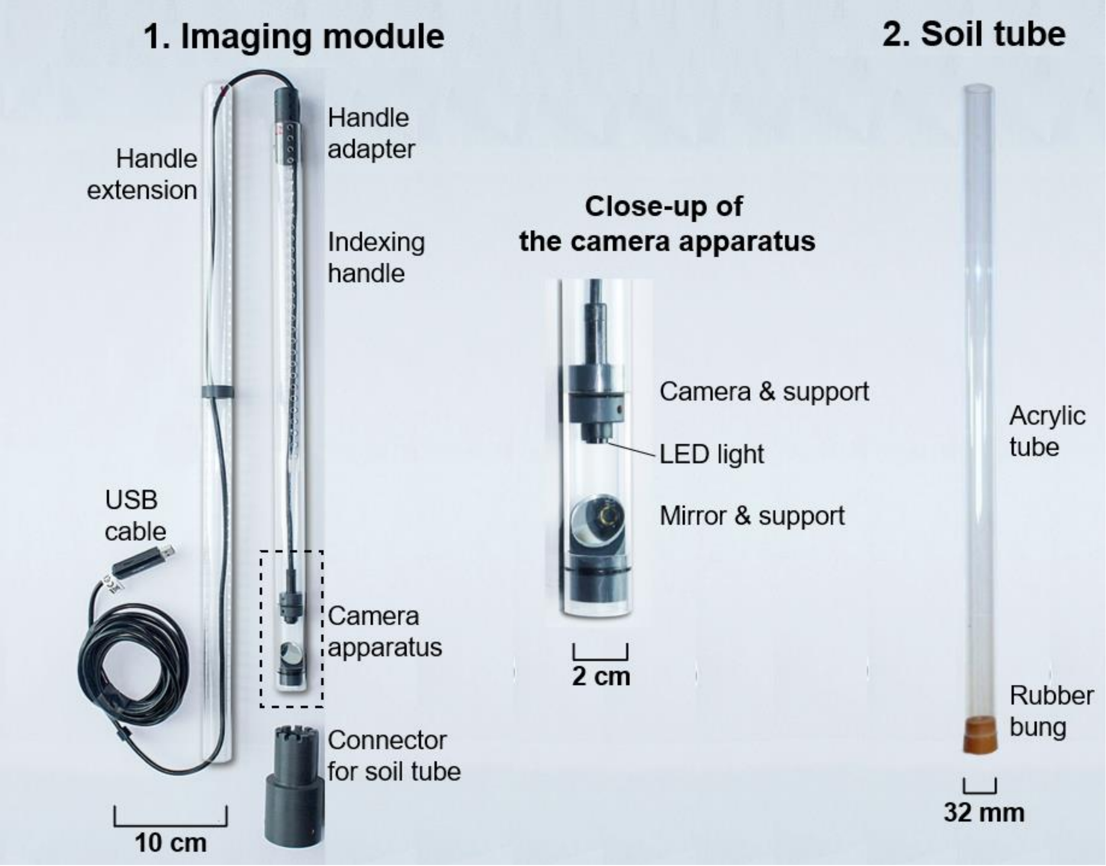

**Figure 2-1** EnRoot's components. All the grey plastic components are 3D printable.

We use EnRoot by inserting the imaging module into the soil tube. The full circumference and length of the tube (around where the roots are developing) is imaged by incrementally rotating and moving up and down the imaging module within the soil tube (Figure 2-2c, Figure 2-5). The position of the imaging system can be recorded by inserting a metal rod at a known position into i) the connector castellated every 5 mm for 360° coverage, and ii) the indexing handle perforated every centimetre to reach any depth in the soil tube (Figure 2-5). The record of the position allows repeated measurements of the same roots and soil area over time.

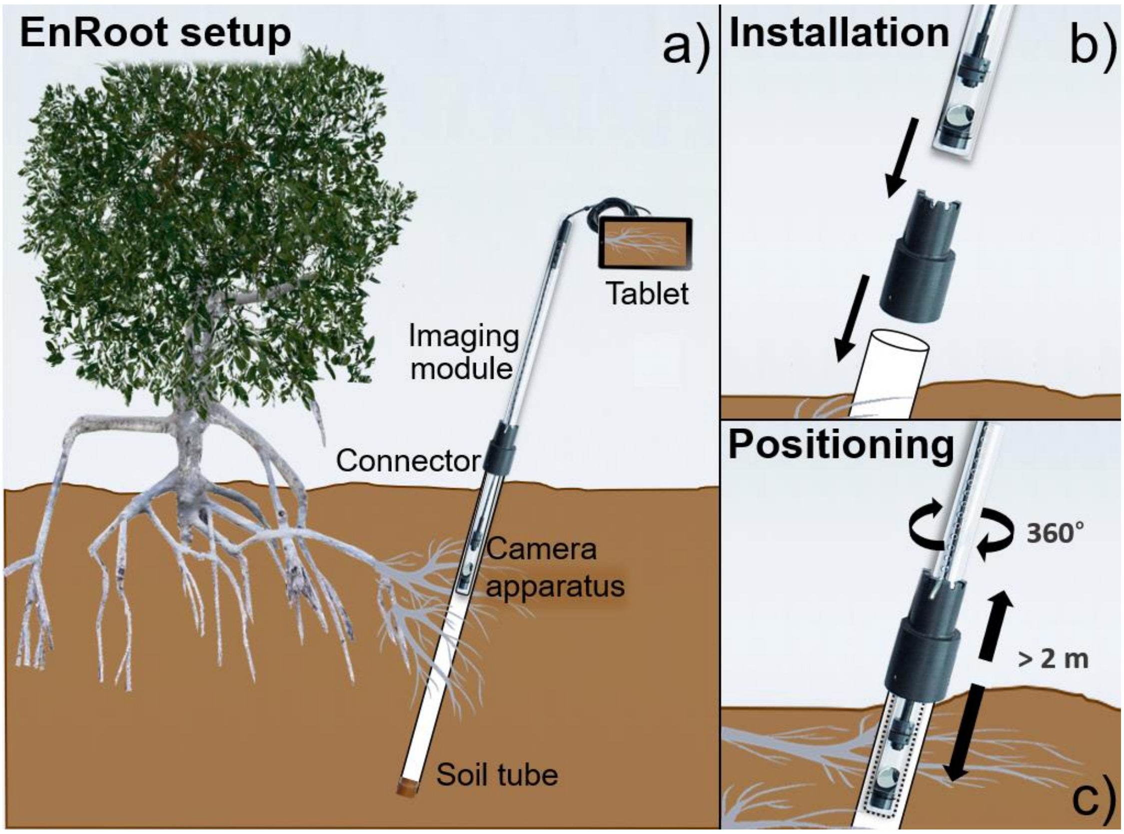

**Figure 2-2** EnRoot's setup and usage.

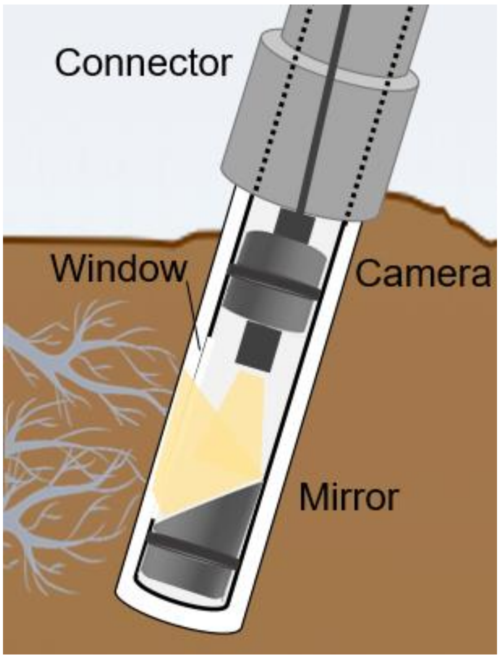

**Figure 2-3** The design of the camera apparatus used in EnRoot.

Once the minirhizotron is set up, EnRoot is connected to a computer, a tablet or a smartphone via its in-built USB cable (Figure 2-2). No extra source of power is required. Roots are observed in real-time on the monitor's screen and saved with an image-acquisition program. We used the Smart Camera software, which is the software provided with the endoscope camera, but any image-acquisition software can operate the camera (e.g. digiCamControl, simpleCV or VideoCapture).

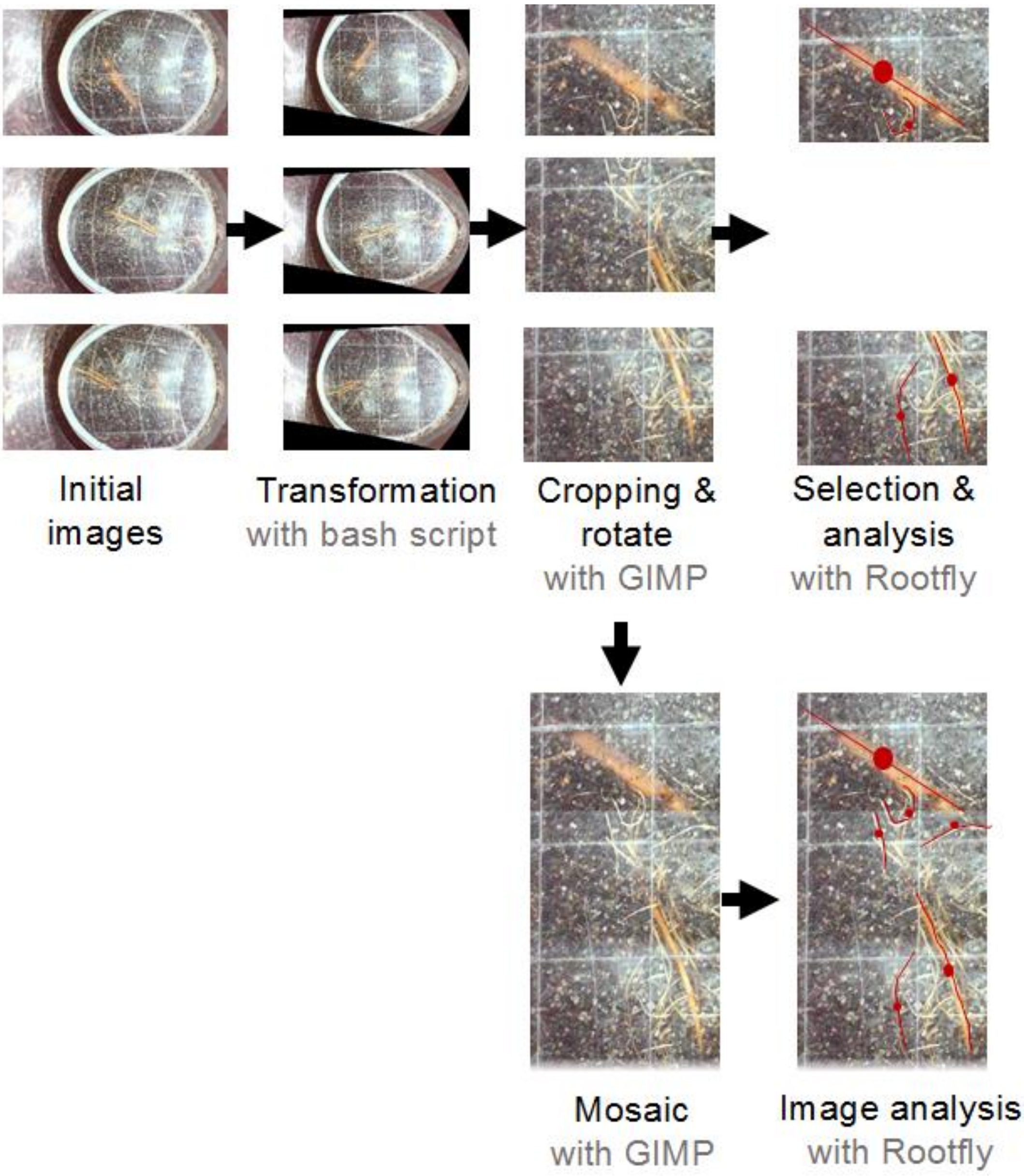

**Figure 2-4** EnRoot's image processing and analysis. The initial images are transformed with EnRoot's bash script, then cropped and rotated in batch with GIMP. A selection of those images can be analysed with Rootfly or if the images of the full tube are required, the images can be assembled as a mosaic (with GIMP or other software) and analysed with Rootfly.

The collected images are corrected using a geometric transformation to compensate for distortion from the cylindrical soil tube and the camera lens; correction is automated using the EnRoot bash script that we developed and have made freely available (Appendix A2.1, Figure 2-4). EnRoot's bash script uses GDAL (GDAL contributors, 2018) and a Python script (included in the repository). A step by step guide to install GDAL and a guide to use the bash script is provided in the Appendix A2.1. The images are then cropped. We recommend using the bash mode of GIMP (www.gimp.org) to crop the images (<1 second per image). Generally, only every other image is analysed to reduce analysis time (Figure 2-4, Johnson et al., 2001). Subsampling images from different depths of a minirhizotron tube showed to have little effect on the experimental results provided the numbers of minirhizotron tubes used are sufficient (Johnson et al., 2001; Iversen et al., 2011). If the subsampling method is used, the selected images can be readily analysed using any root-analysis software (e.g. Rootfly, WinRHIZO, rhizoTrak or SmartRoot). However, if the user requires a mosaic of images covering the full soil tube, we recommend using software to create panoramas, such as GIMP, Image Composite Editor (Microsoft) or PowerPoint (Microsoft).”

### 2.2.1.2 Testing EnRoot's performance

The resolution of EnRoot exceeds requirements for imaging tree roots and is adequate for small roots, such as grass roots. Its maximum resolution is 1,600 × 1,200 pixels, equivalent to 28 µm per pixel in our setup. Since two pixels are required to identify a root, EnRoot can theoretically detect roots with a minimum size of 56 µm. The camera can also be set to a lower resolution to save disk space, for example at 1,280 × 720 pixels, allowing for roots of a minimum size of 74 µm to be imaged.

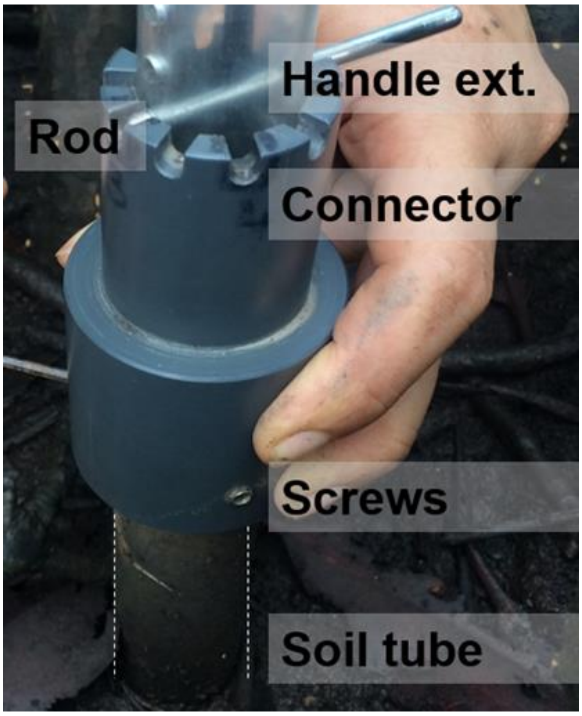

**Figure 2-5** EnRoot’s indexing handle and soil tube connector allow images to be taken at precise depths and radial directions within the soil tube. The screws hold the connector in place.

### 2.2.2 EnRoot’s accuracy and precision

The measurements of fine root production using minirhizotrons are made by extracting the diameter and length of roots from a series of root images. In order to test EnRoot's accuracy and precision, we compared root lengths and diameters obtained with EnRoot with measurements from a high-resolution flat scanner (see 2.4.1). The root diameters and lengths obtained using EnRoot and the high-resolution scanner were very similar, producing concordance correlation coefficients of 0.95 for root diameter and 0.92 for root length (Lin’s Concordance Correlation Coefficient, Figure 2-6). Depending on the descriptive scale used, these values of concordance can be described as moderate to excellent (McBride, 2005; Altman, 1991). Despite these encouraging results, there are differences in measurements between the methods (Figure 2-6), which we suspect are mainly due to i) the semi-manual method used by Rootfly to trace and extract root lengths and diameters, and ii) some alteration of the roots during their attachment to the tube and their installation in the test pot (see Methods).

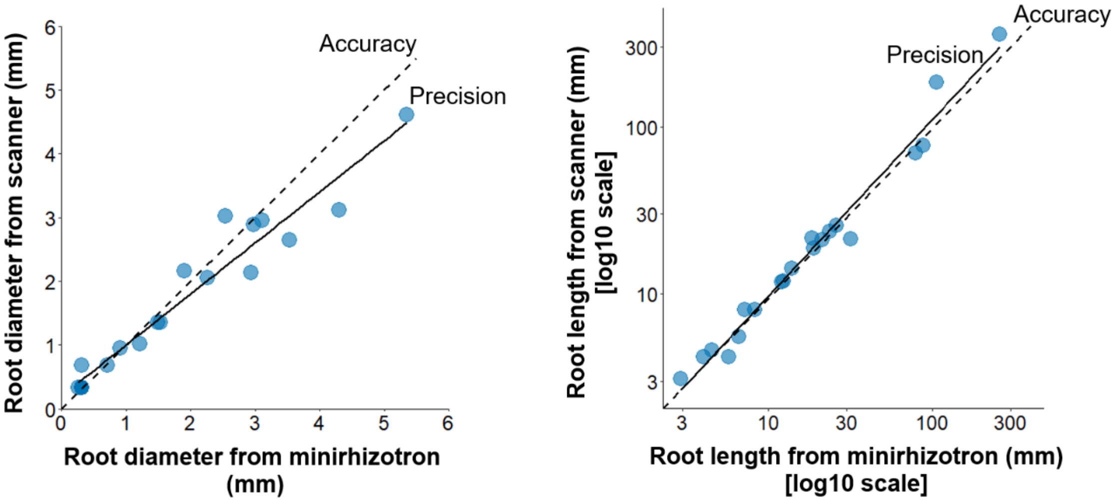

**Figure 2-6** Estimation of root diameter and length with images from EnRoot and a high-resolution scanner. The solid line represents the precision, and the dashed line the accuracy.

### 2.2.3 Using EnRoot in mesocosms and in-situ

We used a mesocosm experiment, and installed EnRoot soil tubes in mangroves (see 2.4.2), to test the system’s practicality and capacity to image complex rooting systems under different environmental conditions. From the images captured with EnRoot we determined the lengths, diameters, total area and total biomass of the roots in each mesocosm (Table 2-1). The high resolution, low glare and full colour of the images made it easy to distinguish roots from the substrate (Figure 2-7) and delineate root length and diameter using Rootfly (Figure 2-7). Specular reflection of the light from the LEDs against the soil tube caused some glare but did not impede the detection and measurement of roots. The initial distortion of the pictures was properly corrected with EnRoot's bash script.

EnRoot was practical and easy to use. The movement of the imaging module through the soil tube was easily controllable with the indexing handle. It was possible to stop the minirhizotron movement with the help of the connector at any time to capture high resolution images (Figure 2-7). The soil tubes remained sealed with no water ingress, and the images were acquired almost instantaneously.

**Table 2-1** Maximum root length and diameter recorded within each mesocosm with the accumulated area of roots imaged with EnRoot and the associated estimated biomass.

| | **Mesocosm** | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
| ***Rhizophora mangle*** | | | | | | | | | | |
| Maximum Root length (mm) | 20.6 | 35.5 | 20.3 | 39 | 23.8 | 32.3 | 36.4 | 23.5 | 24.6 | 42.8 |
| Maximum Root diameter (mm) | 1.3 | 1.9 | 1 | 2.4 | 1.3 | 3 | 2.0 | 2.3 | 2.0 | 2.2 |
| Total area (mm²) | 70 | 77.19 | 75.6 | 93.11 | 102.5 | 122.62 | 154.52 | 190.07 | 194 | 244.5 |
| Biomass (g wet weight) | 0.10 | 0.11 | 0.11 | 0.13 | 0.14 | 0.17 | 0.22 | 0.27 | 0.27 | 0.34 |
| ***Medicago sativa*** | | | | | | | | | | |
| Maximum Root length (mm) | 40.22 | 42.65 | 41.34 | 36.01 | 86.33 | 95.39 | | | | |
| Maximum Root diameter (mm) | 1.20 | 0.76 | 1.17 | 1.23 | 1.40 | 0.99 | | | | |
| Total area (mm²) | 48.46 | 50.50 | 59.83 | 100.59 | 219.17 | 227.38 | | | | |

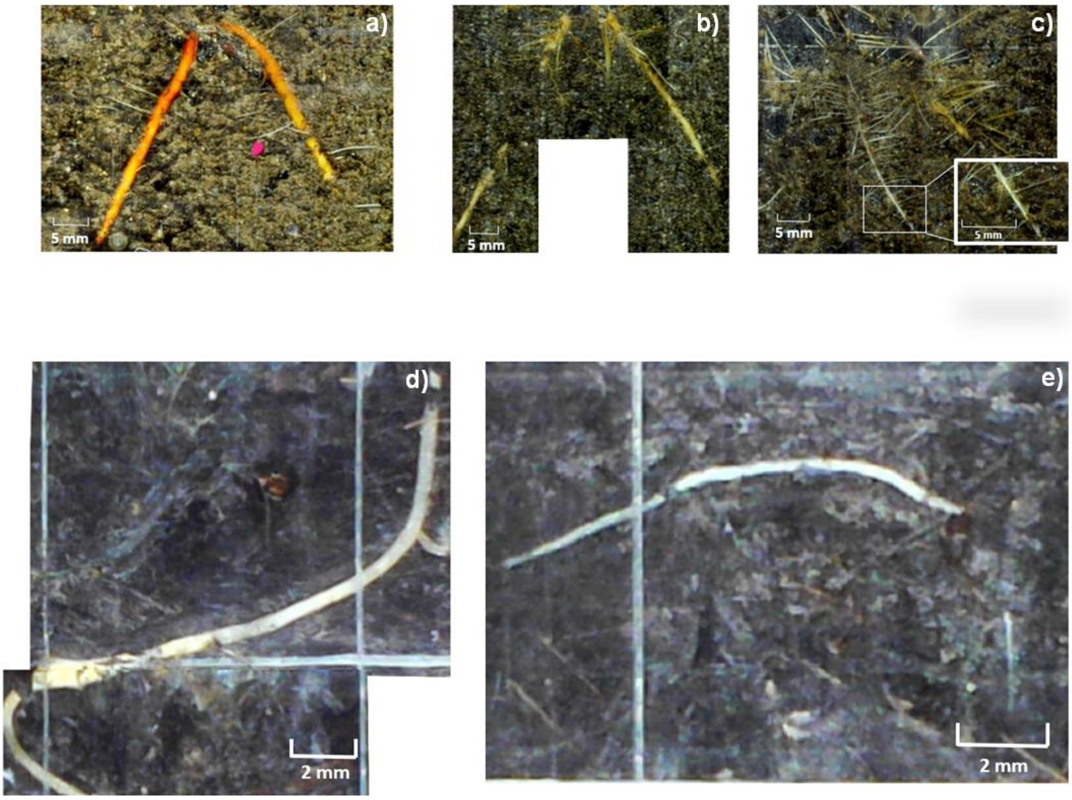

**Figure 2-7** Images of Rhizophora mangle roots with a magnified root in the white box (a, b and c) and Medicago sativa roots (d and e) using EnRoot.

In the field, EnRoot soil tubes could easily be installed in-between the aerial and belowground roots of mangroves (a tropical forested wetland, Figure 2-8). After four months of installation, the roots had developed around the minirhizotron soil tube and were clearly visible in the video we recorded (Appendix A2.3). There was no water ingress in 59 EnRoot soil tubes after 10 months of installation. Only one tube, that was unknowingly damaged prior to installation, had water ingress. The EnRoot imaging module was deliberately inserted while water was within this soil tube to test for module's robustness, but it did not cause any damage because the camera is waterproof. The tops of the soil tubes were closed in the field sites with a rubber bung, sealed with aquarium sealant, to prevent ingress of tidal water.

**Figure 2-8** An EnRoot soil tube installed between stilt roots of mangrove trees in Vietnam.

### 2.2.4 Comparison of EnRoot specifications with other systems

EnRoot has similar or better specifications than commercial minirhizotrons (Table 2-2). The resolution of the images is comparable to other minirhizotrons, but EnRoot is much cheaper than commercial minirhizotrons, at approximately one hundredth to one sixtieth of their price. EnRoot is not, however, suitable for studying hyphae and mycorrhizae. Use of a higher-resolution camera has the potential to extend the system's capacity to studying such smaller features, albeit at an increased cost. The capture times and the size of the images captured with EnRoot was similar to or better than commercial minirhizotrons. The advantages of EnRoot over commercial minirhizotrons are its: low weight, waterproof camera, small diameter and that it does not require an additional energy source because the system is powered by the computer, tablet or smartphone that it is connected to. EnRoot is also more flexible than commercial minirhizotrons, because it can be easily and freely adapted to different soil tube sizes and image-acquisition software (e.g digiCamControl, simpleCV or VideoCapture).

**Table 2-2** Comparison of EnRoot with the commercial minirhizotrons most cited in the literature

| | **Minirhizotron system** | | | |
|---|---|---|---|---|
| **Characteristics** | **EnRoot (this article)** | **CID bioscience CI-600** | **Bartz technology BTC-100X** | **RhizoSystems™, LLC Manual minirhizotron** |
| Price (~€) | 150 | 14,500 | 17,500 | > 13,500 |
| Waterproof camera | Yes | No | No | No |
| Theoretical resolution[1] (µm/pixels) | 28 | 42 | 25 | 13 |
| Image size (mm) | 17 × 12 | 216 × 196 | 13.5 x 18 | 8.4 x 6.3 |
| Capture time (s) | 0 to 3 | 30 to 480[2] | Not indicated | Not indicated |
| Weight (imaging system only) (g) | 250 | 750 | 450 | 6800 |
| Size (mm) | Diameter: > 25<br>Length: all possible | Diameter: 63.5<br>Length: 1830 | Diameter: 51<br>Length: 1820 | Diameter: 50<br>Length: 2000 |
| Battery life (hours) | No battery needed | > 4 | 8 | 11 |
| Magnifier | No | No | Yes | Yes |

[1] Theoretical resolution was calculated by dividing the size of the picture by the maximum resolution. [2] for scanning an image of 21.6 × 19.6 cm.

## 2.3 Conclusion

EnRoot opens the minirhizotron method to i) new usage, particularly in large scale, distributed sampling schemes; ii) new users, such as researchers in low income countries or those with limited equipment budgets; and iii) new, carbon-rich ecosystems, such as tropical forested wetlands. The small diameter and waterproofness of EnRoot increases the range of application of minirhizotrons without compromising the quality of the image; EnRoot's resolution allows theoretical identification of roots with diameters of 56 µm and greater. EnRoot’s lightweight, small diameter tube and no need for external battery offer extra advantages in remote sites. The components of EnRoot are also highly customisable and replacements can be easily built or bought, or 3D printed at low cost.

EnRoot avoid some methodological artefacts compared to other root production methods, because EnRoot i) does not use artificial soil substrate, ii) allows for a soil settling period and iii) permits frequent samplings with little soil disturbances. When the soil is cored, the roots are severed, and nutrients are released (Johnson et al., 2001). Those disturbances induce abnormal root production (Johnson et al., 2001). No settling period can be used to recover initial soil condition with ingrowth bags. It is therefore likely that root production measurements with ingrowth bags reflect abnormal root growth, especially at the beginning of the measurements. In contrast, EnRoot allows to begin the measurements months or years after the tubes are installed. During this lag time, the soil is likely to recover from disturbances. In addition, substrates in which roots are growing have shown to influence root production (Johnson et al., 2001). For instance, the presence of old roots showed to be a major source of nutrients for new root production in mangroves. The artificial substrate used with ingrowth bags are therefore likely to induce some artefacts. Finally, it is important to measure the root production frequently, because roots might growth and die before they are measured. While it is possible to measure frequently with little disturbance the root production with minirhizotrons, it is not possible with ingrowth bags. EnRoot has some limitations, notably because it provides root production measurements in terms of length or area increment. It is possible to convert those measurements into biomass, but the conversion is prone to errors and inaccuracies. Some studies have compared minirhizotron and ingrowth bags, but it is almost impossible to define which method is most accurate because all root production methods have their own biases.

EnRoot can be used for any ecosystems, because it is adapted to local and remote sites with its light weight and integrated power supply. EnRoot can also be used in wet soil, because its camera is waterproof. EnRoot soil tubes are also durable, because it is made of acrylic, which is resistant to corrosion and hardly degradable. For instance, previous minirhizotron studies have left those acrylic tubes in soils for several years. The durability of Enroot's imaging module is sufficient, but can be further improved. The acrylic material of the module might be replacing by PVC to increase its durability. The durability of the camera will depend of the brand used, but if deteriorated the camera can be replace for little cost and efforts. EnRoot is easily reproducible, because all its components are open-source (including software to process the images before analysis) and is easy to build.

EnRoot could potentially be enhanced if operated with an external computer program, such as OpenCV-Python, or with rhizoTrak (e.g., for image cropping, creating a mosaic of images and image analysis). Recent progress in the automatic detection and measurement of objects with computer programs means that it is likely that, in the future, the root images could be analysed automatically in order to extract root length, diameter and area directly in the field. Some programs have already been developed in this direction and could be used with EnRoot (e.g., SegRoot or the multiple instance learning algorithms). Such an improvement would save processing image time in the laboratory and remove the lag between image collection and the obtaining of root production data.

Because EnRoot is cheap to build, freely reproducible and easy to use, it has the potential to close our knowledge gap regarding fine root production. Finally, we have focused primarily on root production measurements, but EnRoot could also be used for other applications, such as root phenology studies.

## 2.4 Methods

### 2.4.1 Evaluation of EnRoot for accuracy and precision

To test EnRoot's accuracy and precision, we used a high-resolution flat scanner (2,400 × 4,800 dpi, Expression 11000XL, Epson) to scan 20 roots of Red mangrove (*Rhizophora mangle*) with a range of diameters and lengths. The same roots were then wrapped with a transparent plastic film around an EnRoot soil tube (32 mm diameter, 50cm long) subsequently placed in a test pot (60 cm long and 110 mm diameter, for a total volume of 5702 $cm^3$) filled with a peaty soil and then saturated with water. EnRoot was then used to image the same 20 roots. The length and the diameter of the roots were extracted using the freely available software Rootfly (Zeng et al., 2008). We used Lin's Concordance Correlation Coefficient to compare the output from both instruments (Lin, 1989). This metric incorporates both accuracy and precision to quantify the level of agreement between paired measurements and is commonly used to assess bias between instruments or human operators. Accuracy is incorporated through a bias correction factor that represents the gradient of the best-fit line compared to the 1:1 line; while precision is incorporated through the use of Pearson's Correlation Coefficient (Figure 2-6). The value of Lin's Concordance Coefficient increases towards one as the compared data approach perfect agreement.

### 2.4.2 EnRoot trial

The mesocosm experiment was undertaken for six months to generate a range of root lengths and diameters representing different stages of fine root production. In the first batch of mesocosms, we mimicked field conditions of mangrove forests. In a greenhouse, 10 *Rhizophora mangle* propagules were planted in ten mesocosm pots (60 cm long and 110 mm diameter, for a total volume of 5702 $cm^3$) filled with a mix of sandy and peaty substrate, which was periodically saturated with water. The temperature was maintained at 26°C with a relative air humidity of 70 %. In each mesocosm, an EnRoot soil tube (32 mm diameter and 50 cm long) was installed. After the saplings exhibited first leaf out, we used EnRoot to image the roots of one mesocosm per day at 10 random dates over six months. We imaged only the area with roots. We repeated the same experiment with an Alfalfa crop (*Medicago sativa*) in six mesocosms of a peat-only substrate, with temperature maintained between 24 and 26°C and an average relative air humidity of 30 %.

Each set of root images was corrected for distortion using EnRoot's bash script. The images were then cropped using GIMP (www.gimp.org) and assembled as a mosaic with GIMP and PowerPoint (Microsoft). The length and diameter of roots within each mesocosm were extracted from mosaics of images using Rootfly. In each mesocosm we identified the longest root and the thickest root, as well as the cumulative area of all the roots imaged. The longest root was defined by the longest continuous segment of root visible. We estimated the fine root biomass of each mangrove mesocosm. Root wet biomass was calculated with a simplified area:biomass coefficient that we calculated for the *Rhizophora mangle* roots. The installation of EnRoot soil tubes was tested in three mangrove sites in the Can Gio Biosphere Reserve in the Mekong Delta in Vietnam where we installed 60 EnRoot soil tubes at 1m depth. We generated 60 random locations and installed at each an EnRoot soil tube (1.2 m long, so 0.2 m left above the ground surface) using a screw auger of 31 mm diameter. We changed the initial location of three tubes, because we could not core due a very hard substrate – probably large belowground roots. All of the tubes were installed vertically in the soil (90°).

### 2.4.3 Comparing EnRoot with commercial minirhizotrons

EnRoot was compared with 3 other commercial minirhizotrons in terms of nine characteristics that we deemed to be important, such as camera resolution, weight and price (see Table 2-2 for full list). Details of the commercially-available minirhizotrons were provided by suppliers, manufacturers' online documentation, and peer-reviewed publications.

**Acknowledgements:** Development of the prototype system received support from Antony Windross, from the School of Earth and Environment's workshop at the University of Leeds. The 3D pictures and files were made using the 3D hub supplier Gateros Plating and were further modified by Johan Fruh. David Ashley helped assemble the mesocosms. Rakesh Tiwari took the picture of the device (Figure 2-1).

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# Chapter 3 Fine root production along a 40-year chronosequence of reforested mangroves

## Abstract

Globally, one-third of mangroves have been lost since 1950. In response, mangrove reforestation projects have rapidly emerged with the aim of reinstating mangrove provision of ecosystem services, notably for carbon storage and sequestration. Monitoring and assessment of mangrove reforestation have mostly focused on aboveground carbon stocks, yet the majority of mangrove carbon is found belowground, and little is known about its dynamics. In particular, changes in fine root production following mangrove reforestation is unknown. Fine root production is a major sink for carbon and likely a major control of soil surface accretion. Therefore, our current lack of knowledge on fine root production inhibits meaningful forecasts of mangrove carbon sequestration and resilience to sea level rise. Using minirhizotrons, we investigate how fine root production varied along a chronosequence of mangroves planted in 1978, 1986 and 1991, and with depth. We found that fine root production declines with: i) stand age, as a result of mangrove self-thinning; and ii) soil depth, likely due to a vertical gradient in soil nutrient availability. In addition, a major fraction of fine root production (up to 40 %) was deeper than 30 cm, layers that are commonly omitted from most calculations of mangrove carbon budgets. Our findings have important implications for better characterising belowground carbon dynamics in mangroves, and highlight the need to account for forest stand age when forecasting carbon dynamics and resistance to sea level rise of reforested mangroves.

**Key words:** belowground carbon, blue carbon, restoration, coastal wetland, soil organic matter

## 3.1 Introduction

### 3.1.1 Background and rationale

Mangroves provide ecosystem services, such as flood protection and carbon sequestration, that have been estimated to be worth US$194,000 per hectare per year (Costanza et al., 2014 using data from 2011). However, these valuable coastal wetlands are being lost rapidly (Duke et al., 2007; Richards & Friess, 2016). In response, reforestation projects have emerged worldwide (Lee et al., 2019). Mangrove reforestation has particularly intensified since it was recognised that mangroves are among the world's most carbon-dense ecosystems (Bosire et al., 2008; Donato et al., 2011). Compared to the above-ground carbon store, belowground carbon dynamics remain poorly understood and monitored after mangrove reforestation. However, up to 90 % of the carbon in mangroves is found in the soil, and fine roots are believed to be the main source of soil carbon with riverine sediments (Bouillon et al., 2003; Ezcurra et al., 2016; McKee, 2011; Middleton & McKee, 2001). As such, the resistance of reforested mangroves to sea-level rise, and the long-term trajectory of the carbon store in reforested mangroves, is unclear.

Mangroves have been shown to resist sea-level rise by accreting soil through increased SOM accumulation and sedimentation (Krauss et al., 2014; McKee, 2011; Middleton & McKee, 2001). In doing so, they occupy vertical "accommodation space" (Rogers et al., 2019). The sedimentation and the soil organic matter accumulation are separate mechanisms for soil accretion. The relative importance of both mechanisms is not fully understood, because the contribution of soil organic matter to soil accretion has been little studied (Krauss et al., 2014). Nevertheless, a couple of studies have shown that accumulation of soil organic matter can be the main mechanism of soil surface elevation in mangroves (Krauss et al., 2014). SOM accumulation results from the balance between SOM decay and root production (Ezcurra et al., 2016; McKee, 2011; Middleton & McKee, 2001). Several studies have indicated that SOM decay does not decrease with sea-level rise in mangroves and saltmarshes (Arnaud et al., 2019; Blum, 1993; Kirwan et al., 2013), meaning that mangroves require enhanced root production or sedimentation to access this vertical accommodation space and to persist during sea-level rise. However, root production is one of the least studied components of the carbon cycle in mangroves, and little is known about its controlling factors, especially following reforestation (Alongi, 2009; Bouillon et al., 2008; McKee & Faulkner, 2000; Muhammad-Nor et al., 2019; Perez-Ceballos et al., 2018).

Most studies have investigated the response of root production to nutrients, flooding and their interactions (Adame et al., 2014; Castañeda-Moya et al., 2011; Cormier et al., 2015; Naidoo, 2009; Poungparn et al., 2016; Torres et al., 2019). The root production is believed to be high in soils deprived of nutrient and freshwater, and low if those resources are available (Reef & Lovelock, 2015). In-situ measurements showed similar results, with a high root production in soils having a high salinity, a low nutrient concentration and a high frequency of inundation (Castañeda-Moya et al., 2011). The root production also showed to decrease when resources become available or when stress was alleviated (Adame et al., 2014). For instance, nutrient enrichment of mangrove soils (being not nutrient deprived) showed to reduce the root production of mangroves (Naidoo, 2009). In contrast, nutrient enrichments showed to increase the root production in mangroves experiencing stress, such as a high salinity and a high frequency of inundation (Adame et al., 2014). In comparison, the effects of mangrove reforestation on root production are understudied, while recent years have seen a proliferation of mangrove reforestation projects (e.g. in Bangladesh, Philipines and Senegal) (Lee et al., 2019). In addition, the only two studies that have investigated the impacts of reforestation on root production show contrasting results. McKee and Faulkner (2000) found that reforested mangroves had similar belowground productivity to natural mangroves; in contrast, Perez-Ceballos et al. (2018) showed that reforested mangroves were much less productive than natural mangroves. The effect of reforestation on belowground carbon production is also likely to change as the replanted mangrove stand ages (Fromard et al., 1998; Walcker et al., 2018), but no study have considered the time after reforestation. Aside from decades-long monitoring programmes, a chronosequence approach allows the convenient study of the effects of stand age upon belowground carbon cycling. However, finding a chronosequence of mature reforested mangroves with comparable environmental conditions is challenging. Indeed, to date no study has investigated the temporal development of belowground production following mangrove reforestation.

The depth-distribution of mangrove root production is also poorly understood but is important. It regulates major soil processes, such as carbon input into the soil from fine roots, mangrove tree resource acquisition and vertical input of rhizodeposition (release of organic compounds by the roots in the rhizosphere). For instance, a major source of slow-cycling organic carbon in soil is thought to come from rhizodepositions (Sokol et al., 2019), and this process seems prevalent in ecosystems with dense rhizospheres (Sokol & Bradford, 2019), such as mangroves. Therefore, root production distribution seems likely to be involved in the primary mechanism through which recalcitrant carbon accumulates in mangrove soils. Rhizodepositions have also been shown to prime or enhance soil carbon decomposition (Kuzyakov, 2010). It is currently unclear whether rhizodepositions increase or decrease the soil carbon pool under different environmental conditions (Dijkstra et al., 2013), but ecosystems, such as mangroves, exposed to $CO_2$ enrichment and nutrient limitation have been shown to exhibit increased rhizodepositions stimulating carbon mineralisation (Phillips et al., 2011; Sadowsky & Schortemeyer, 1997). Understanding the depth-distribution of root production would therefore improve future projections of the impacts of global environmental change on mangroves.

In common with other subterranean measurements, in situ measurement of fine root growth is operationally challenging. Unlike other root-production measurement techniques, minirhizotrons allow repeated observations of the same roots or soil space to be made over extended periods, with limited soil disturbance after an initial settling-in period. Minirhizotrons also have the advantage of distinguishing root production from the simultaneous decay of root detritus and to avoid artefacts from artificial or disturbed soil substrates that can affect root ingrowth tubes. We are not aware of any previous studies that have reported the use of minirhizotrons in mangroves, due to their lack of flexibility and high costs. However, a recently developed type of minirhizotron, EnRoot, tailored to mangrove conditions, enables the growth of individual roots to be tracked in situ at multiple depths, with little disturbance (Arnaud et al., 2019), and was used in the study reported below.

### 3.1.2 Aim and hypotheses

In this study, we used the EnRoot minirhizotron technique described by Arnaud et al. (2019) to investigate fine root production in one of the largest restored mangroves in the world in the Mekong Delta in Vietnam. We used a 40-year chronosequence to test whether:

i) fine root production differed with stand age in reforested mangrove;

ii) fine root production had vertical patterns that accorded with reforested mangrove stand age.

## 3.2 Methods

### 3.2.1 Study area: restored mangroves in South Vietnam

The Mekong Delta, in south Vietnam offers unique opportunities for investigating the trajectory of mangrove root production after reforestation. The mangroves there were reforested more than 40 years before our measurements, allowing us to study the decadal impact of reforestation on mature mangroves. The reforestation efforts were spread over several decades resulting in mangrove stands of different ages within the same area. The close proximity of reforested stands allowed us to investigate the impact of mangrove stand age on fine root production, while controlling for important confounding factors such as climate and inundation regime. The mangroves there were also likely representative of other reforested mangroves, since i) they have been planted with trees from the *Rhizophora* genus, which is the most common genus used in mangrove reforestation worldwide (Ellison, 2000; López-Portillo et al., 2017); ii) they were dominated by *Rhizophora* mangroves, which are the second most important mangroves in terms of soil carbon stock globally (Atwood et al., 2017).

Before reforestation, the mangroves in the Mekong Delta had been extensively damaged, and in many cases completely destroyed, during the US-Vietnam war (1955–1975) through the spraying of napalm and herbicides, mostly the so-called Agent Orange (2,4,5-Trichlorophenoxyacetic acid and 2,4-Dichlorophenoxyacetic acid, both with traces of dioxins) and Agent White (2,4-dichlorophenoxyacetic acid and picloram) (Hong & San, 1993). Our study site, Can Gio, was one of the most heavily affected areas (Hong & San, 1993), in which > 80 % of the original mangrove forest was destroyed (Oxmann et al., 2010). The current soil concentration of agent orange is very limited (Kishida et al., 2010). There is no study having investigated the impact of the agent orange on soil structure. However, if there was any impact, it is likely to be similar across our sites, since our sites have been sprayed evenly (Oxmann et al., 2010). Can Gio has been extensively reforested, starting in 1978, with the genus *Rhizophora* (Hong & San, 1993), and is now protected under the statute of the Can Gio World Biosphere Reserve (UNESCO-MAB). The mangroves of Can Gio are nationally important because they are the only buffer between the sea and Ho Chi Minh City, the most populous city in Vietnam (Figure 3-1).

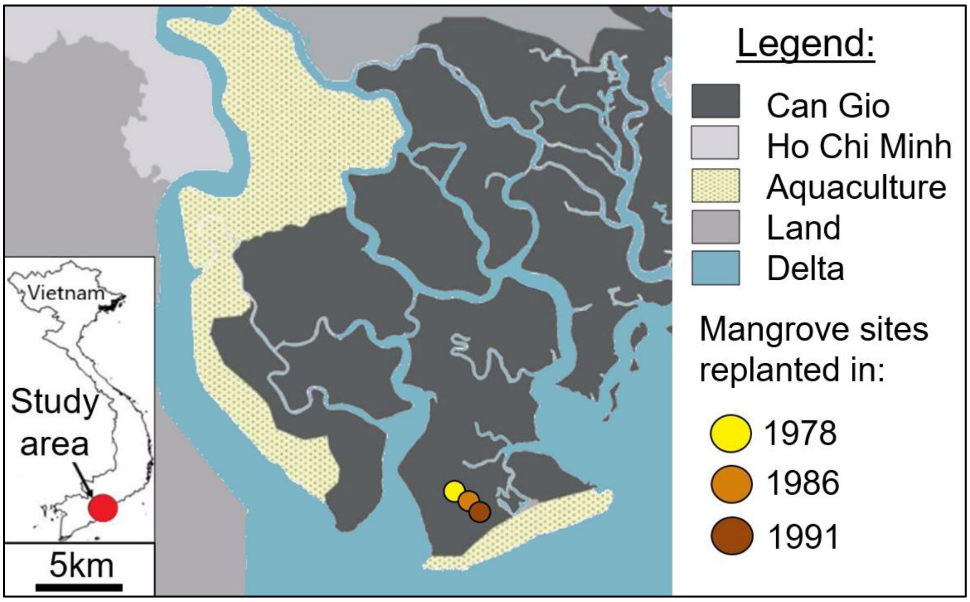

**Figure 3-1** Location of our study area and sites. Our study was located in Can Gio, which is the only area separating Ho Chi Minh city from the sea.

In Can Gio, we chose three sampling locations that fulfilled the following criteria: i) the management board of Can Gio were able to identify confidently the date of reforestation, corroborated by the date of reforestation observable in historical images from Google Earth; ii) the hydrological conditions of the three sites were similar; and iii) the three sites were reforested with *Rhizophora* propagules. The sites were restored in 1978 (CG1978), 1986 (CG1986) and 1991 (CG1991). The sites were located within 2 km of one another. At each site, we established a monitoring plot of around 250 m².

### 3.2.2 Fine root production measurements

We measured fine root production using the EnRoot system, a minirhizotron designed for use in mangroves (see full description in Arnaud et al., 2019). Commercially-available minirhizotrons were unsuitable because their soil tubes were too large to fit between the stilt roots of *Rhizophora* and were impractical for remote swamps (too heavy, too large, camera not waterproof, need for power supply). Between February and March 2018, we installed 21 EnRoot minirhizotron soil tubes at randomly-selected locations across our three monitoring plots (seven minirhizotrons tubes per plot) following the procedure described by Iversen et al. (2012) and Johnson et al. (2001). The tubes were 120 cm long and had an outside diameter of 3.2 cm. We painted the uppermost 20 cm to avoid light penetration. We also sealed the tops of the tubes with rubber bungs and non-toxic aquarium-grade silicone sealant to prevent water ingress between sampling (Iversen et al., 2012).

We installed all the tubes vertically into pre-augered holes with a slightly smaller diameter than the minirhizotron tubes. The bottoms of the tubes reached a depth of 100 cm. Installation of the tubes at 45° has been shown to maximise root capture for some grass and tree species (Johnson et al., 2001); however, due to the aerial roots of *Rhizophora*, this was not possible at our sites. Because of the vertical installation of our minirhizotron tubes, our measurements of fine root production might be conservative; however, our installation procedure was consistent across our three plots. In all three sites, we allowed the soil to settle around the tubes for more than five months (March to September 2018) between initial installation and first data collection. This helped limit fine root production artefacts, such as abnormal fine root production occurring in response to the severing of roots or in response to nutrients released during soil tube installation (Iversen et al., 2012; Johnson et al., 2001).

We took monthly measurements from September to December 2018. All measurements were carried out at low tide and in the morning. In total, we measured root growth during three intervals: September–October, October–November and November–December (Figure 3-2). We considered root growth to constitute either the longitudinal extension of existing roots, or the appearance of new roots, between the measurement periods (Figure 3-2) (Iversen et al., 2012; Johnson et al., 2001). Due to the large number of roots, we limited our measurements of root growth to four depth intervals: 8–10, 30–32, 50–52 and 70–72 cm. In each depth interval, two pictures were taken. Each depth interval was sampled for all minirhizotrons on all sampling dates. Lengths and diameters of individual roots were traced using a labour-intensive, manual procedure (see Arnaud et al., 2019), which placed a constraint on the number of samples that could reasonably be processed.

For consistency, all minirhizotron images for all plots and on all dates were collected and processed by the same operator (M. Arnaud), using a standardised protocol (see Appendix A3.1) and ROOTFLY version 2.0.2 (Zeng et al., 2007). The root growth was converted into a length increment of root production in mm per day per square centimetre of observed soil. We used this measure of production, hereafter referred to as 'root production', in our statistical analysis (see below). In total, 504 root production measurements per square centimetre of soil were recorded and used in our analysis.

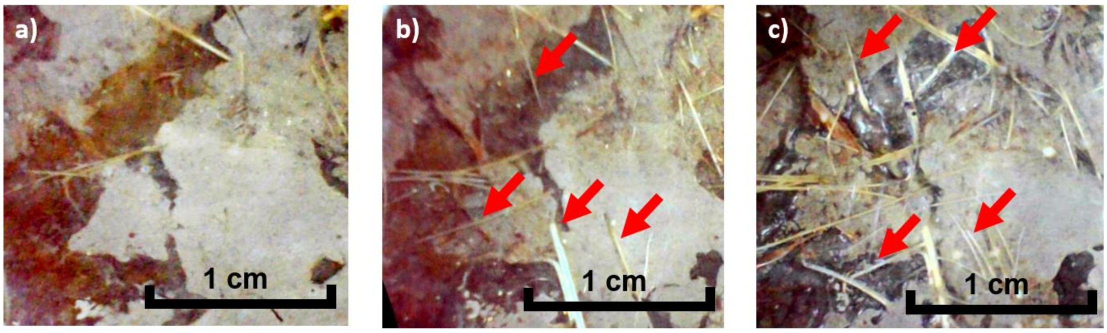

**Figure 3-2** Root production images from the EnRoot minirhizotron. Each letter represents a measurement period: a) initial image; b) one month post-installation; and c) two months post-installation. Red arrows indicate selected new roots that appeared between images.

### 3.2.3 Environmental conditions

We measured a number of environmental variables that might exert some control over fine root production over time and with soil depth.

At each of the three reforested mangrove plots, we measured: i) water-table depth and shallow groundwater temperature continuously at 1.5 metres below the surface, using a dipwell fitted with a water-level datalogger (Solinst Levelogger Edge 3001), corrected for barometric pressure (using a Solinst Barologger Edge); ii) mean bulk soil salinity at 20 random points, using a Decagon GS3 probe (Dettmann & Bechtold, 2018); and iii) average tree stem density for the whole study site (250 $m^2$). In addition, we measured soil nutrient (ammonium, nitrogen and phosphorus) contents in each plot at two depths (8 and 70 cm) in seven soil cores, collected from randomly-chosen locations using a gouge auger (126 samples in total). After extraction, all samples were transported to the laboratory in a cool box, and were then frozen prior to analysis. We determined total soil nitrogen and phosphorus, and soil ammonium after its extraction using KCl, using a SKALAR SAN++ auto-analyser. We measured air temperature and humidity continuously, using an Extech RHT10 Data Logger at a central point between the three monitoring plots. Finally, we obtained total daily precipitation from the Can Thanh weather station (situated < 10 km from our three plots) from the Vietnamese Center for Hydro-meteorological Data.

### 3.2.4 Statistical analyses

#### 3.2.4.1 Fine root production and distribution across mangrove stand ages

We used a linear model to investigate how fine root production is affected by the year of reforestation, the depth of the soil, and any interaction between the two.

First, we fitted a linear mixed effects model to fine root production to test for any specific artefacts induced by the period of measurement. We chose this approach because measuring fine root production repeatedly raised the possibility of a hierarchical structure in our data (e.g. a greater similarity of measurement within measurement periods than between measurements). Such a situation would violate the assumption of independent measurements required by linear regression. To begin with, we fitted both main effects (year of reforestation and depth) simultaneously. We then added an interaction term between year of reforestation and depth, followed by a random intercept that was allowed to vary between measurement periods. At each stage, we tested whether any of those combinations led to a significant improvement in model performance using the corrected Akaike information criterion (*AICc*, $p < 0.05$ threshold) (Zuur et al., 2007). The combination with a random intercept did not lead to any significant improvement in our models' fit (*AICc* = +74, $p < 0.01$), so the assumption of independence appeared reasonable and we proceeded with a standard multiple linear regression model. The linear model with an interaction between year of reforestation and depth had the best fit (lowest *AICc*), but it displayed strong heteroscedacity, which would have prevented it from being generalised beyond our sampling locations (Field et al., 2012). Transforming the data using logarithmic and exponential functions did not lead to any improvement; therefore, we complemented our linear model results with a robust linear model. Robust linear models correct for unequal variances of regression residuals using covariance matrix estimators (Zeileis, 2004). Both the robust and the linear model provided similar results, so we cautiously used the linear model to test our hypotheses. We used the least square means *post hoc* test to examine differences according to the categorical factor of year of reforestation (Lenth, 2016).

#### 3.2.4.2 Environmental characteristics of the mangrove site and stands

We analysed the environmental conditions using ANOVA, and when the ANOVA assumptions were not met, we used a Kruskall-Wallis *H*-test (one-way

ANOVA on ranks). We investigated: i) whether differences exist in soil nutrients between the sites, ii) whether differences exist in soil nutrients between depth intervals; and ii) if temperature, relative humidity and precipitation were different between the measurement periods. We used the least square means post hoc test (Lenth, 2016). We then used the linear model that we built for fine root production (see above) and added the precipitation as a main effect to see if it improved the fit of the model in predicted fine root production using the *AICc*.

All statistical analyses were performed using r (r core Team, 2013). We used the lmer function from the r package lme4 (Bates et al., 2015), the *AICc* function from the r package MuMin (Barton, 2019), the lsmeans function from the r package lsmeans (Lenth, 2016), the kruskal.test and lm function from the stat package (r core Team, 2013), and the coeftest function from the r package sandwich (Zeileis, 2004).

## 3.3 Results

Average fine root production varied by a factor of seven according to year of reforestation, and decreased with increasing depth. More than 90 % of all measured roots had a diameter < 1 mm.

### 3.3.1 Time since reforestation

Fine root production decreased monotonically with increasing age since reforestation ($p < 0.001$, $F_{(2)} = 27.06$, Figure 3-3). The greatest fine root production was at CG1991 (0.124 mm $cm^{-2}$ $d^{-1}$), which had almost twice the fine root production than CG1986 (0.063 mm $cm^{-2}$ $d^{-1}$) ($p < 0.001$, least square means = 0.06, $t_{(492)} = 4.21$), and seven times that in CG1978 (0.017 mm $cm^{-2}$ $d^{-1}$) ($p < 0.001$, least square means = 0.11, $t_{(492)} = 7.33$). The fine root production of CG1978 and CG1986 were significantly different ($p < 0.01$, least square means = 0.04, $t_{(492)} = 3.12$).

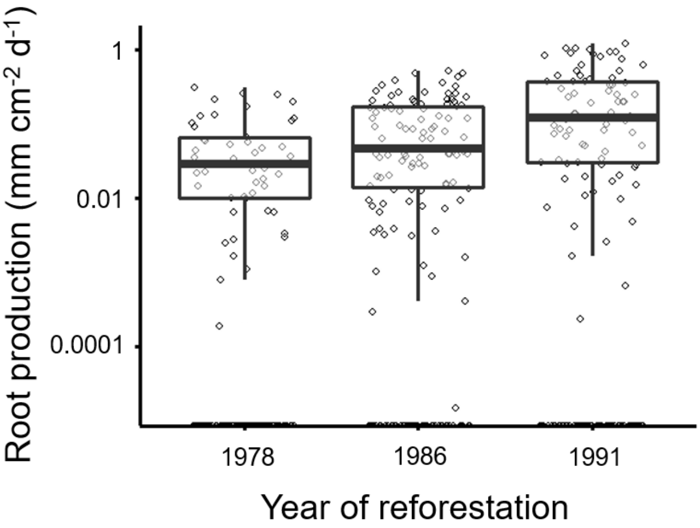

**Figure 3-3** Fine root production across the age-gradient of mangrove sites (mean daily production per frame area of the three periods of measurements for each year of reforestation). Bold, horizontal lines show sample medians. The lower and upper hinges correspond to the first and third quartiles of the sample. The upper whisker extends from the hinge to the largest value of the sample, but no further than 1.5 times the sample inter-quartile, while the lower whisker extends from the hinge to the smallest value at most 1.5 times the sample inter-quartile range. Note logarithmic vertical axis scales.

### 3.3.2 Depth variation

Fine root production decreased strongly and significantly with increasing depth ($p < 0.001$, $F_{(3)} = 39.44$, Figure 3-4). The interaction between depth and year of reforestation was significant ($p < 0.001$, $F_{(6)} = 16.64$). In CG1986 and CG1991, the greatest fine root production was at the shallowest depth (0.35 mm $cm^{-2}$ $d^{-1}$ for CG1991; 0.15 mm $cm^{-2}$ $d^{-1}$ for CG1986) and the lowest at 50-52 cm for CG1986 (0.013 mm $cm^{-2}$ $d^{-1}$) and at 70-72 cm for CG1991 (0 mm $cm^{-2}$ $d^{-1}$). The difference between the shallowest and deepest depths was significant for CG1986 ($p < 0.001$, least square means = 0.13, $t_{(492)} = 4.63$) and CG1991 ($p < 0.001$, least square means = 0.35, $t_{(492)} = 12.07$). In contrast, there was no clear vertical pattern in fine root production at CG1978, which ranged from 0.02 to 0.01 mm $cm^{-2}$ $d^{-1}$ across all soil depths (none of the production at each soil depths were different from each other $p > 0.99$).

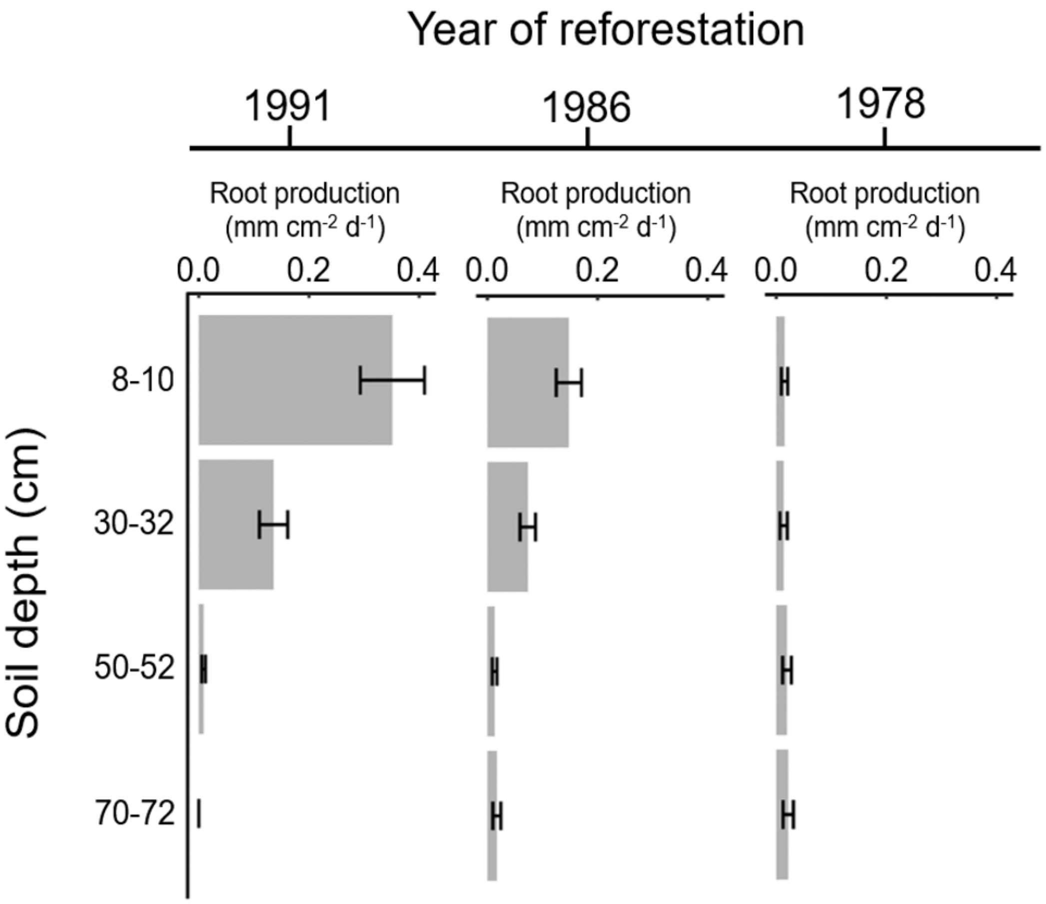

**Figure 3-4** Mean fine root production over soil depths across reforested mangroves of different ages. Error bars show standard deviation.

### 3.3.3 Environmental conditions

Soil ammonium was not significantly different across the year of reforestation ($p = 0.57$, $\chi^2_{(2)} = 1.11$) and ranged between 0.26 and 0.35 mg $l^{-1}$ . Total soil nitrogen ranged from 2.27 to 3.50 mg $g^{-1}$ ($p < 0.01$, $F_{(2, 36)} = 14.6$). Only CG1986 had a total soil nitrogen concentration different from the two others sites (CG1978: $p < 0.01$, least square means = 1.05, $t_{(36)} = 4.43$; CG1991: $p < 0.01$, least square means = 1.24, $t_{(36)} = 4.97$). Soil phosphorus ranged from 0.17 to 0.42 mg $g^{-1}$ and was different across year of reforestation ($p < 0.01$, $F_{(2, 36)} = 14.71$). The highest phosphorus concentration was in CG1978, followed by CG1986 and then CG1991.

Between the shallowest (0–15cm deep) and deepest (50–74 cm deep) sampling depths in each site, soil nutrients decreased by almost 20 %. The difference was most pronounced for soil phosphorus (-47 %, $\chi^2_{(1)} = 13.97$). Total soil nitrogen decreased by 22 % between the shallowest and deepest layers ($p < 0.001$, $p < 0.01$, $F_{(1,37)} = 8.63$). Only for soil ammonium was there no difference between the shallowest and deepest layers. However, if CG1978 was excluded from the analysis (the fine root production did not have a vertical spatial gradient in CG1978), the difference in concentration between the shallowest and deepest layers was significant (-47 %, $p < 0.001$, $\chi^2_{(1)} = 15.85$).

Soil water temperature, tidal inundation frequency and soil salinity were similar between the years of reforestation. Average soil water temperature ranged from 27.1 to 27.8°C between the years of reforestation. All the sites were inundated on average twice a day and their soils were highly saline (>12 bulk mS $cm^{-1}$).

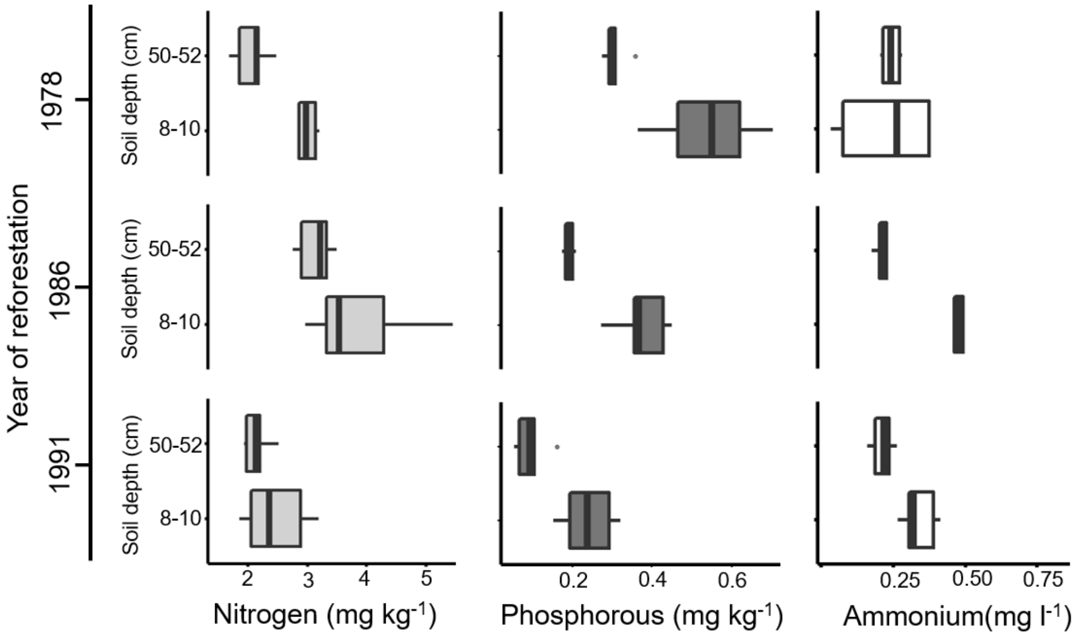

**Figure 3-5** Mean soil nitrogen, phosphorous and ammonium over soil depths across reforested mangroves of different ages. Bold, horizontal lines show sample medians. The lower and upper hinges correspond to the first and third quartiles of the sample. The upper whisker extends from the hinge to the largest value of the sample, but no further than 1.5 times the sample inter-quartile, while the lower whisker extends from the hinge to the smallest value at most 1.5 times the sample inter-quartile range.

Daily precipitation was highly variable between the three measurement periods, while mean air temperature (27.0°C) and humidity (91.6 %) remained almost constant. The average daily precipitation ranged from 3.2 to 12.3 mm $d^{-1}$ between measurement periods. The first measurement period (Sept-Oct) had the highest average amount of daily rainfall. The last period (Nov-Dec) had the second-highest average amount of daily rainfall, but almost 90 % of the rain occurred in one day during a tropical storm (Usagi, 24/11/2018). While the daily precipitation was significantly different between the period of measurement ($p < 0.001$, $\chi^2_{(2)} = 22.69$), the increase in precipitation did not improve the linear model for root production ($AICc$ =+ 0.03, $p = 0.14$, $F_{(1,491)} = 2.10$). The tree density varied across year of reforestation, with the largest tree density in CG1991 (0.37 trees·m$^{-2}$), followed by CG1986 (0.25 trees·m$^{-2}$) and CG1978 (0.09 trees·m$^{-2}$).

**Table 3-1** Environmental conditions of each mangrove reforested stands

| | Year of reforestation | | |
|---|---|---|---|
| | 1978 | 1986 | 1991 |
| Tree density (trees m$^{-2}$) | 0.09 | 0.25 | 0.37 |
| Precipitation (mm d$^{-1}$)$^{1}$ | 7.7 | 7.7 | 7.7 |
| Air Temperature (°C) $^{1-2}$ | 27 | 27 | 27 |
| Air humidity (%)$^{1-2}$ | 91.6 | 91.6 | 91.6 |
| Soil salinity (bulk mS cm$^{-1}$) $^{3}$ | 13.2 | 12.6 | 12.7 |
| Soil ammonium (mg l$^{-1}$) $^{3}$ | 0.33 | 0.36 | 0.28 |
| Soil phosphorous (mg g$^{-1}$) $^{3}$ | 0.42 | 0.28 | 0.17 |
| Soil nitrogen (mg g$^{-1}$) $^{3}$ | 2.46 | 3.50 | 2.27 |
| Soil water temperature (°C)$^{3}$ | 27.1 | 27.8 | 27.5 |
| Inundation frequency per day (nb d$^{-1}$) | 2 | 2 | 2 |

$^{1}$ Reported as mean for the whole measurement period. $^{2}$ Measured at a central point between the three sites. $^{3}$Mean of all the measurements per stand.

## 3.4 Discussion

Fine root production decreased monotonically with increasing age of reforestation, and did so independently of confounding factors (e.g. inundation regime or temperature). Our results are relevant to: i) the prediction of fine root production and SOM dynamics in reforested mangroves; and ii) understanding the vulnerability of reforested mangroves to future sea level change, through their ability to occupy vertical accommodation space by accumulating fine roots.

### 3.4.1 Time since reforestation

Fine root production decreased with increasing age of reforested mangroves (Figure 3-3). No other study has investigated belowground production during mangrove development, either in primary or secondary mangroves. However, this age-related decrease of fine root production is in accordance with the development trajectory of natural mangroves, in which the aboveground production declines gradually after stand maturity until senescence at about 70 years (Alongi, 2009; Fromard et al., 1998; Jimenez et al., 1985; Walcker et al., 2018). Earlier work in natural mangrove forests showed that, as mangrove stands mature, they begin to self-thin and tree density declines (Fromard et al., 1998); similar observations were seen at our sites (section 3.3.3). The association between tree density and fine root production has previously been reported (Adame et al., 2014). Our results substantiate this relationship showing a concomitant decline of tree density and fine root production Table 2-1). Although, we did not measure basal area density per site, we observed large trees in the old stand, and thin trees in the young stand. Therefore, this decrease in root production might be a consequence of tree number decline rather than a decline of root production per tree, because we expect larger trees to produce more roots. Further investigation defining the root production at the tree scale are necessary to confirm this hypothesis.

A second potential explanation of this age-related decrease of fine root production is a reduction of soil nutrients with increasing mangrove stand age (Figure 3-4). In others forests, nitrogen, phosphorous and ammonium have shown to be reduced with forest ages (Yuan & Chen, 2010). In our study area, the total soil nitrogen and ammonium did not change significantly with the age of the reforested mangroves, and thus likely did not control the decrease of fine root production. This result agrees with previous findings for 15- and 60-year-old reforested *Rhizophora* mangroves, where soil ammonium content did not differ with stand age (Alongi et al., 1998). However, total phosphorus decreased with the ages of the reforested stands, and was inversely related to fine root production, as has been observed in other mangroves (Castañeda-Moya et al., 2011; Cormier et al., 2015). This is in accordance with previous studies showing that fine root production respond to nutrient limitation (Feller et al., 2003; Naidoo, 2009).

### 3.4.2 Depth variation

The fine root production declined with increasing depth in GG1986 and GG1991 (no significant change in  CG1978), which is consistent with what has previously been observed in natural mangroves (Castañeda-Moya et al., 2011; Xiong et al., 2017). In our site, this vertical pattern of fine root production likely results from the higher concentration of soil nutrients in the surface than deep in the soil (Castañeda-Moya et al., 2011). Phosphorus and nitrogen decreased with depth at all sites, while ammonium decreased in two sites (CG1986 and CG1991). Only in CG1978, the ammonium was not significantly different between depths. CG1978 was also the only site with no significant difference in the distribution of root production. Therefore, it might be that ammonium concentration influences the depth distribution pattern of root production. However, further studies need to verify this hypothesis. Another reason could be an opportunistic distribution of the roots at shallow depths to increase uptake of precipitation-derived fresh water  (Reef & Lovelock, 2015). However, fine root production in the shallowest soil layers did not respond to the temporal variation in precipitation in any of our stands (see 3.3.3).

The depth-distribution of fine root production varied between the reforested mangroves of different ages. The young site had most of its production in the topsoil, while the most mature site exhibited an even depth- distribution of fine root production. No other study has investigated the influence of mangrove age on the depth-distribution of fine root production. However, this has important implications for  our understanding of the soil carbon distribution at depth, because fine root production i) controls the soil carbon accumulation in mangroves (Ezcurra et al., 2016; McKee, 2011; Middleton & McKee, 2001; Arnaud et al., 2019); and ii) is associated with rhizodepositions (e.g. exudates from live roots), which are thought either to exert a disproportionate influence on the formation of stable soil organic carbon (Sokol et al., 2019), or to prime old carbon (Kuzyakov et al., 2000).

### 3.4.3 Implications for carbon budget

Across our sites, up to 40 % of the fine root production was deeper than 30 cm depth. One could argue that is due to our sampling method limited to a small portion of soil. However, previous research suggest that it is a recurrent pattern in mangroves. The only two studies reporting root production at depth below 30 cm found a significant amount of fine root production in those soil layers (up to 45% found in Castañeda-Moya et al., 2011; and up to 43% found in

Muhammad-Nor et al., 2019, both studies used the ingrowth core method). Additionally, live roots have been commonly reported below 30 cm in mangrove soils (Komiyama et al., 1987, 2000; Tamooh et al., 2008). This depth-distribution of fine roots if associated with Rhizophora genus is also likely to be applicable to other sites, since mangroves with Rhizophora genus are distributed globally, and hold the second largest stock of mangrove carbon globally (Atwood et al., 2017). Mangrove carbon budgets are commonly calculated from root production in the uppermost 30 cm of the soil profile (Bouillon et al., 2008). Fine root production has therefore be grossly underestimated in mangrove carbon budget (Bouillon et al., 2008; Alongi, 2009), and the inclusion of fine root production at depth below 30 cm might increase the contribution of root production to carbon budget by a factor of almost two. In addition, the input of carbon by roots was believed to occur only in topsoil layer. Our study in conjunction with previous one reveal that the depth-distribution of roots can be much deeper (> 70 cm deep), and that ages might be a factor controlling this depth-distribution.

Fine root production might disproportionally contribute to soil carbon burial. If the decay rate of root is 0.14% per day (e.g. 51.1 % per year: Ouyang et al. 2017) and the annual root production 164 Tg of carbon per year (e.g. doubling of the root production estimate of Bouillon et al. 2008, since ~half is unaccounted), the annual root contribution to carbon burial would be 83 Tg of carbon annually. Estimations of mangrove burial rate are ranging from 18.6 (Bouillon et al., 2008) to 38 Tg of carbon per year (Chmura et al., 2003), which is two time less than our estimate of carbon burial by roots (e.g. 83 Tg). This discrepancy between our estimate of root contribution to carbon burial and the reported carbon burial rates might results from unaccounted loss of decomposed root, high spatial variability of mangrove root production and lateral loss of carbon in form of dissolved organic and inorganic carbon (Maher et al., 2013; Taillardat et al., 2018), may be originating from root exudation.

## 3.5 Conclusions

1. Production of fine roots decreased with the age of reforested mangrove stands. While this pattern has previously been observed in aboveground production, our findings are the first to demonstrate it for belowground production. This pattern was shown for Rhizophora. Further studies should investigate root production trajectory for other genera. However, it is likely that a similar pattern will occur, since the

aboveground carbon trajectory of non- Rhizophora species showed to be similar than Rhizophora species (Alongi, 2009).

2. Fine root production decreased with depth in the two youngest sites, likely due to nutrient limitations; but this depth variation was not apparent in the oldest site, where fine root production was slow for all depths. The vertical pattern of fine root production may have important implications for understanding mangrove soil accumulation and rhizosphere processes.

3. Large amounts of fine root production (up to 40 %) were found deeper than 30 cm in the soil, yet these layers are commonly omitted from mangrove carbon budget calculations. Our results highlight the need to give due consideration to these deeper layers.

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# Chapter 4 Sensitivity of mangrove soil organic matter decay to warming and sea level change

Arnaud, M, Baird, AJ, Morris, PJ, Dang, TH, Nguyen, TT. Sensitivity of mangrove soil organic matter decay to warming and sea level change. *Global Change Biol*ogy; 00: 1– 9. https://doi.org/10.1111/gcb.14931

## Abstract

Mangroves are among the world's most carbon-dense ecosystems, but they are threatened by rapid climate change and rising sea levels. The accumulation and decomposition of soil organic matter (SOM) are closely tied to mangroves' carbon sink functions and resistance to rising sea levels. However, few studies have investigated the response of mangrove SOM dynamics to likely future environmental conditions. We quantified how mangrove SOM decay is affected by predicted global warming (+4°C), sea level changes (simulated by modification of the inundation duration by zero, two and six hours per day), and their interaction. Whilst changes in inundation duration between two and six hours per day did not affect SOM decay, the treatment without inundation led to a 60 % increase. A warming of 4°C caused SOM decay to increase by 21 %, but longer inundation moderated this temperature-driven increase. Our results suggest that i) sea level rise is unlikely to decrease the SOM decay rate, suggesting that previous mangrove elevation gain, which has allowed mangroves to persist in areas of sea level rise, might result from changes in root production and/or mineral sedimentation; ii) sea level fall events, predicted to double in frequency and area, will cause periods of intensified SOM decay; iii) changing tidal regimes in mangroves due to sea level rise might attenuate increases in SOM decay caused by global warming. Our results have important implications for forecasting mangrove carbon dynamics and the persistence of mangroves and other coastal wetlands under future scenarios of climate change.

**Keywords:** belowground carbon, blue carbon, climate change, coastal wetland, greenhouse gas production, soil elevation

## 4.1 Introduction

Mangroves are among the most carbon dense ecosystems in the world (Donato et al., 2011), and provide ecosystem services worth US$194,000 per hectare per year (Costanza et al., 2014). The conservation and restoration of mangroves are increasingly being promoted (Lee et al., 2019; McLeod et al., 2011). Several countries have already committed to using coastal wetlands as part of their mitigation and adaptation strategies for climate change (Herr et al., 2015) and re-forestation and conservation projects are taking place widely (Lee et al., 2019). In Bangladesh and Vietnam, for example, more than 200,000 ha of damaged or destroyed mangroves have been reforested in the last 50 years. However, against a backdrop of changing future climates it is unclear whether natural and reforested mangroves will be resilient to sea level rise, and in particular whether they will continue to sequester soil carbon (Gilman et al., 2008; Lovelock et al., 2015).

The accumulation of soil organic matter (SOM) controls both the carbon sink capacity of mangroves and their resistance to sea level rise (Alongi, 2009; Rogers et al., 2019). In carbon-dense mangroves such as our study site (see next section), SOM accumulation results primarily from the balance between organic matter decay and root production (Alongi, 2009; Middleton and McKee 2001; Bouillon et al., 2003). Since SOM represents up to 80 % of the carbon stored in mangroves (Alongi, 2009), the alteration of SOM decay by climate change is likely to affect their carbon sink capacity in the future. Accumulation of SOM in mangroves causes the soil surface to rise within the tidal prism. If the rate of soil accretion in mangroves equals or is faster than the rate of sea level rise, mangroves can persist and avoid being submerged (Rogers et al., 2019). In contrast, an increase of root decay might reduce or even reverse SOM accumulation, leading to subsidence, thus causing relative sea levels to rise. Temperature and inundation regimes are major factors controlling SOM decay in mangroves, and will be modified by climate and sea level change (Alongi, 2009; Kristensen et al., 2008; Lovelock, 2008); Yet, compared to many others ecosystems, little is known about how mangrove SOM dynamics will respond to future environmental conditions (Jennerjahn et al., 2017; Song et al., 2019; Twilley et al., 2017).

Global mean temperature is expected to rise between 1.0 and 4.8°C by 2100 under RCP2.6 and RCP8.5, respectively, relative to the 1986–2005 baseline (IPCC, 2013). Rising temperatures have been shown to increase rates of SOM decay in coastal wetlands (Kirwan et al., 2014; Morris & Whiting, 1986), but only a few mangrove sites have been investigated. Field studies have used latitudinal gradients to infer the thermal sensitivity of mangrove SOM decay, and therefore incorporate site-specific confounding factors such as tidal inundation regime, root respiration, biofilm development, and soil nutrient status (Lovelock, 2008; Jin et al., 2013; Lewis et al., 2014; Poungparn et al., 2009). Since it is challenging to isolate the effect of temperature in the field, a more controlled laboratory approach is necessary (Lovelock, 2008; Twilley et al., 2017).

Global sea level is projected to rise between 0.28 and 0.98 m by 2100 (RCP2.6 and RCP8.5, respectively, relative to the 1986–2005 baseline, IPCC, 2013) and incidences of extreme sea levels are expected to become more frequent (IPCC, 2013), yet the impacts of these changes upon SOM decay in mangrove soils are understudied. As sea level rises, mangroves can migrate landward or can accumulate organic matter and sediments. Through these mechanisms mangroves can occupy landward and vertical 'accommodation space' (Rogers et al., 2019). In the past, some mangroves have adjusted to sea level rise and avoided submergence by accreting soil, notably SOM (McKee et al., 2007; Krauss et al., 2014). However, it is unclear whether the vertical accretion of mangrove soil (where it occurs) is driven primarily by an increase of root production and sedimentation, reduced SOM decay rates, or both. Landward accommodation space for mangroves is limited in many countries due to coastal urbanisation; while vertical soil elevation may be limited by a lack of riverine sediment inputs to the coast (Lovelock et al., 2015; Schuerch et al., 2018). Some mangroves therefore seem likely to experience an increasing duration of tidal inundation, or will become fully submerged with continued sea level rise (Lovelock et al., 2015; Ward et al., 2016).

There is a dominant view that increased flooding is inversely related to decomposition rate in coastal wetlands (Davidson & Janssens, 2006; Nyman & DeLaune, 1991; Reed, 1995; Miller et al., 2001), but there is a surprising lack of empirical data that may be used to test such a hypothesis (Mueller et al., 2016), particularly for mangroves. In some settings, mangroves will experience prolonged and repeated sea-level drops, for up to a year (Widlansky et al., 2015). These incidences of extreme sea levels, known as Taimasa, lower the sea level by as much as 0.30 m and reduce the inundation of inter-tidal mangrove habitats (Widlansky et al., 2015). Taimasa events have already been shown to contribute to drought conditions in mangroves, resulting in large areas of mangrove dieback (Lovelock et al., 2017). Taimasa events are predicted to become more intense and frequent with future climate change, even against a long-term backdrop of rising relative sea level (Widlansky et al., 2015). However, the impacts of Taimasa events or other types of drought (e.g., those caused by reductions in riverine flows into coastal water) on SOM decay in mangroves remain unexplored (Chapman et al., 2019).

Here, we conducted a fully factorial mesocosm experiment to reveal the direction and magnitude of change in mangrove SOM decay rate in response to important global environmental changes. We posed the following research questions:

1) How much will rates of SOM decay increase with global warming?

2) How will changes in inundation associated with Taimasa events and sea level rise affect rates of SOM decay?

3) How will SOM decay rates respond to the combined impact of increases in temperature and changes in inundation?

## 4.2 Methodology

### 4.2.1 Experimental design and setup

We conducted an incubation experiment to simulate how warming and relative sea level change might affect SOM decay rates in mangrove soil. We used $CO_2$ efflux from the mesocosms as a proxy for rates of SOM decay. The mesocosm approach allowed us to limit confounding factors, while revealing the independent effect sizes of temperature and inundation, as well as their interaction. We incubated samples for 22 days at two temperatures (27°C and 31°C) and under three inundation regimes (zero, two and six hours of inundation per day). Those factors were combined factorially and resulted in six treatments in total. The 4°C difference between our two temperature treatments is within the range of surface warming predicted for 2100 under RCP8.5, both globally (RCP8.5- IPCC, 2013; Ward et al., 2016), and locally at our study site in the Mekong Delta, Vietnam (MONROE, 2009; Katzfey et al., 2014) (see below). The six-hour inundation treatment represents an increase of inundation duration due to sea level rise; the two-hour treatment is the baseline condition; while the zero hour inundation treatment represents a drought event, such as that caused by falling sea level during a Taimasa event. Likely impacts of sea level change on the duration of inundation in mangroves have not yet been established due to a lack of understanding of soil surface rise in response to SOM inputs in these ecosystems, so we took a simplified approach. We chose two hours of inundation as our baseline condition because the mangrove trees (from the genus *Rhizophora*) found at our site are inundated for a minimum of two hours per day (van Loon et al., 2007). We chose the six hour treatment to represent the longest duration of inundation that can be found for the genus *Rhizophora* at our study site (van Loon et al., 2007), and because the difference in altitude between these durations of approximately 60 cm (van Loon et al., 2007) corresponds to the projected sea level rise in the Mekong Delta region (MONROE, 2009).

For each of our six treatments, we had ten replicate mesocosms, each of which we sampled five times during the course of the experiment, for a total of 300 $CO_2$ efflux measurements. The mesocosms were constructed from PVC tubes 11 cm long and 6 cm in diameter, drilled at their top to allow lateral exchange of water into and out of the mesocosm. Each mesocosm was filled with 330 grams of wet soil to a height of 7 cm, and packed to match field bulk density as closely as possible. We homogenised the soil and removed any large pieces of organic matter (> 5 mm). At the bottom of each mesocosm, we installed a mesh screen to allow the vertical exchange of water. We controlled temperature with two environmental chambers set to a constant temperature of 27 and 31°C. The soil samples were placed in tanks in which the inundation regimes were controlled in two ways. Flood tides were created by pumping water gradually into the tanks using a peristaltic pump, and the ebb tide simulated by gradually reducing the water level with a flush mechanism activated by an automatic arm. Both flood and ebb were programmed using a Python script and a Raspberry Pi microcomputer. The soil samples were placed at different levels in the tanks to give the different inundation treatments. The two- and six-hour treatments were kept inundated for the whole two or six hours. The inundation depth was two cm in the mesocosm inundated two hours, and ranged from two to four centimetres for the mesocosm inundated for six hours (four hours at two centimetres and two hours at four centimetres). We used artificial sea water, prepared by mixing Instant Ocean® with distilled water to prevent any additional input of organic matter that could have potentially been added by using in-situ sea water (Lewis et al., 2014). The salinity of the artificial seawater was 30 ppt, which is within the range of the salinity in our field site (Dung & Duc, 2016) and in mangrove tidal water more generally (Chen et al., 2014; Frusher et al., 1994; Lara & Cohen, 2006; Marchand et al., 2004). The bases of the mesocosms in the zero-hour treatment (< 5 mm) were wetted daily for two hours to prevent any gas loss during flux measurement (see below). We conducted the experiment in an environmental room with no natural or artificial light to inhibit any photosynthetic activity.

### 4.2.2 Field study site description and sample collection

The soil for the experiment was collected in the mangroves of Can Gio, located in the north of the Mekong Delta, Vietnam (10°30′ N, 106°52′ E). The tidal regime of Can Gio is semi-diurnal. The frequency with which mangroves are inundated ranges from twice per month to twice per day (van Loon et al., 2007). The mangroves of Can Gio occupy an area that extends from below the mean sea level to more than two meters above it. Organic carbon content, sediment particle size distribution and nutrient concentrations of the soil used in the experiments were comparable to other carbon rich mangroves (Table 4-1). The organic matter content of the soil, estimated by loss on ignition, was 10.5 % of the bulk soil weight, which is similar to that found in the most carbon-rich deltaic mangroves in the Indo-Pacific region (Donato et al., 2011). Grain size distribution of our samples was dominated by clay and silt, which is also typical for deltaic mangroves (Table 4-1). Soil nutrient content was 0.39 % N and 0.03 % P of bulk dry weight. After forest destruction caused by the war between the USA and Vietnam, the area was reforested in 1986 with *Rhizophora apiculate* Blume*,* a common species used in mangrove reforestation projects in the Mekong Delta and elsewhere. Soil sampling from three sites took place in February 2018. After removing the surface litter, we collected 14 kg of wet soil from the top soil layer (0-15 cm) at each site. The wet samples were transported to the laboratory and stored at 4°C to limit losses of SOM.

**Table 4-1** Soil characteristics

| **Grain size distribution (%)** [1] | | **Nutrient and Carbon (%)**[2] | |
|---|---|---|---|
| Clay | 67.01 | Total nitrogen | 0.39 |
| Silt | 32.97 | Total phosphorus | 0.04 |
| Sand | <0.02 | Total organic carbon | 10.19 |

[1] Grain size distribution was measured with a laser diffraction particle size analyser on wet samples treated with hydrogen peroxide.[2] TN and TOC were analysed with an elemental combustion analyser (Vario Micro Cube) after removal of the inorganic carbon with hydrochloric acid, and TN and TP were analysed with a continuous flow autoanalyser (Skalar SAN + + auto analyser). % of the bulk dry weight.

### 4.2.3 Gas fluxes measurement and calculation

SOM decay was estimated by measuring carbon gas ($CO_2$ and $CH_4$) fluxes from the mesocosms using mini flux chambers. After an initial seven-day stabilisation period, we sampled on five occasions: days 1, 2, 5, 9 and 16. The $CH_4$ fluxes during the experiment were entirely un-measurable. Only four positives $CH_4$ fluxes on 300 gas fluxes measurements were recorded (Appendix A4.2). This lack of $CH_4$ is explained by a suppression of methanogenesis by sulphate reduction under anoxic condition and by aerobic respiration under oxic condition (Kristensen et al., 2008). Therefore we do not consider $CH_4$ further. The gas flux measurements were taken under dark conditions when the soil was not inundated, one to three hours after ebb. Gas concentrations in the mini chambers during flux tests were measured using off-axis integrated cavity output spectroscopy (Los Gatos Research Ultra-portable GHG Analyzer, model 915-0011, California). During the tests, ppmv gas concentrations in the mini flux chambers were measured at 1 Hz. The chambers were sealed to the top of the mesocosm for a total of 150 seconds. During the tests, the temperature and pressure of the environmental chamber in which the mesocosms were housed were recorded (pressure accuracy of ~ ± 0.5 hPa and precision of ~ 0.2 hPa; temperature accuracy of ~ ± 0.5°C and precision of 0.2°C; Commeter C4141 probe: Comet Systems, Rožnov pod Radhoštěm, Czech Republic) to allow calculation of gas fluxes. Before each test, the water-exchange ports (drilled holes) in the sides of the mesocosms were blocked with gas-tight rubber bungs. We also wore a respirator (3 M 7501 Silicone half mask respirator) connected to the outside of the environmental chamber to remove any exhaled $CO_2$. To calculate the gas fluxes we converted ppmv gas concentrations in the mini flux chambers into masses using the ideal gas equation. Ordinary least squares regression was then used to estimate the rate of change in gas mass over time which could then be converted into a mass flux when the volume of the mini chamber and area of the soil samples were taken into account. Fluxes were calculated only if the slope of the linear regression was i) significantly different from zero ($p < 0.05$), ii) the coefficient of determination ($r^2$) was > 0.70, and iii) the difference between the minimum and maximum gas concentration were above the error range (7.15 ppmv) of the Los Gatos instrument. In instances where measured concentrations did not satisfy criterion iii), we recorded a zero flux. In total, 296 fluxes from 300 were valid and used in our statistical analysis.

### 4.2.4 $CO_2$ flux as an estimate of SOM decay

$CO_2$ fluxes from soils are controlled by microbial decomposition of organic matter, autotrophic root respiration, algae, chemolithotrophs and macrofauna (Kuzyakov, 2006; Lovelock, 2008). The largest $CO_2$ flux is likely to be from microbial decomposition of organic matter, followed by root respiration (Ouyang et al., 2018). Our measurements would have excluded autotrophic root respiration because no living roots were present in them (and larger roots were, anyway, removed, Bloom & Caldwell, 1988). In some mangroves, autotrophic biofilms have been shown to contribute to soil $CO_2$ efflux (Jacotot et al., 2019; Lovelock, 2008), although they do not exert any direct control over the decay of SOM itself. Visual checks were carried out before each gas measurement, but no biofilm development was apparent. Macrofauna were excluded when we extracted the soil in situ, and we did not notice any macrofauna when the soil was mixed prior to being put in the mesocosms. Between measurements, the soil was kept in dark conditions to limit any phototrophic respiration. Thus we are confident that our measured $CO_2$ emissions represent SOM decay alone.

### 4.2.5 Experimental limitations

Our use of homogenised mangrove soil has reduced the complexity of the soil to avoid confounding factors (e.g., different initial SOM contents, root densities or faunal respiration). For instance, faunal burrowing activities in natural soils might be expected to increase soil aeration and so aerobic respiration; while live fine roots in natural soils might release root exudates. Such factors are likely to exert some influence on SOM decay in coastal wetlands (Kristensen et al. 2008, Mueller et al., 2016) and are also likely to respond to inundation duration and temperature changes. These differences between our soils and natural mangrove soils should be borne in mind when interpreting our results.

### 4.2.6 Statistical analysis

A linear mixed model was used to investigate how SOM is affected by temperature, inundation and the interaction of temperature and inundation. The reason for using a mixed model is because we took repeated measurements in each soil mesocosm, thereby raising the possibility of a hierarchical structure in our data set. Initial visual inspection of the data clearly indicated that measured rates of $CO_2$ efflux declined throughout the experiment, meaning that our data exhibited obvious clustering by date of measurement (Appendix A4.1). Such a situation violates the assumption of independence of measurements required by regression analysis and ANOVA, but using a mixed model overcame this problem because it explicitly models the non-independence of our data (Appendix A4.1).

To construct our linear mixed model, we began with a baseline model, and used a forward addition method adding one predictor (fixed or random effects) at a time. Doing so allowed us to assess the effect of each predictor on the overall performance of the model using the Akaike information criterion (*AICc*) and the likelihood ratio (Zuur et al., 2007; $p < 0.05$ threshold). We set the day of measurement to be the subject variable, and experimented with a random intercept and random slopes for the effects of temperature and inundation period, that were allowed to vary between measurement days. Doing so accounted for the possibility that measurements were more similar within days than they were between days (i.e., representing any long-term drift in $CO_2$ efflux during the course of our experiment). All the $CO_2$ flux data were transformed using a $\log_{10}$ function in order to ensure linear, heteroscedastic fits.

The baseline model consisted solely of a fixed-effect intercept, which is equivalent to the grand mean of the entire dataset. Next we added a random intercept that varies by date of measurement, in order to assess the effect of long-term drift; but no other predictors. The random intercept model is equivalent to a one-way ANOVA that predicts the mean $CO_2$ efflux across all treatments according to date of measurement. This random intercept led to a significant improvement in model performance (-67 of the *AICc*, $p < 0.001$), reflecting the important role of date of measurement. We then proceeded to experiment with adding fixed slopes for temperature and inundation regimes, and their interaction; and then random slopes for temperature, inundation and their interaction. Each time we re-specified the model, we re-evaluated its performance compared to the previous iteration to ascertain whether the alteration had significantly improved its fit according to *AICc*. All models that included random slopes were numerically intractable, and either failed to converge or had singular fits (Appendix A4.1). $CO_2$ efflux was best described by a model that has fixed effect slopes for temperature, inundation, and their interaction; and the random intercept for date of measurement. Visual inspection of residuals did not reveal any obvious deviations from normality, homoscedasticity or linearity (Appendix A4.1). We used ANOVA to investigate the effect of the main treatments and their interaction on SOM decay (Appendix A4.1), and the least square means with a Tukey's adjustment of *p*-values as a post hoc test to investigate differences between the levels of each factor

(Lenth, 2016), including both main effects and interactions. All statistical analyses were performed using r (r core Team, 2013). We built the linear mixed model using the lmer function from the r package lme4 (Bates et al., 2015). *AICc* was computed using the function *AICc* from the r package MuMin (Barton, 2019), and least square means were computed with the function lsmeans from the r package lsmeans (Lenth, 2016). Full details of the analysis, the r scripts and our data are provided as Appendix A4.1 to ensure the full reproducibility of our analysis.

## 4.3 Results

The $CO_2$ efflux data are summarized in Figure 4-1. Average $CO_2$ efflux varied by a factor of two across our treatments, with the highest averages from samples exposed to high temperature and zero hours inundation treatment; and the lowest $CO_2$ efflux from samples inundated for two hours per day at 27°C (Figure 4-1c).

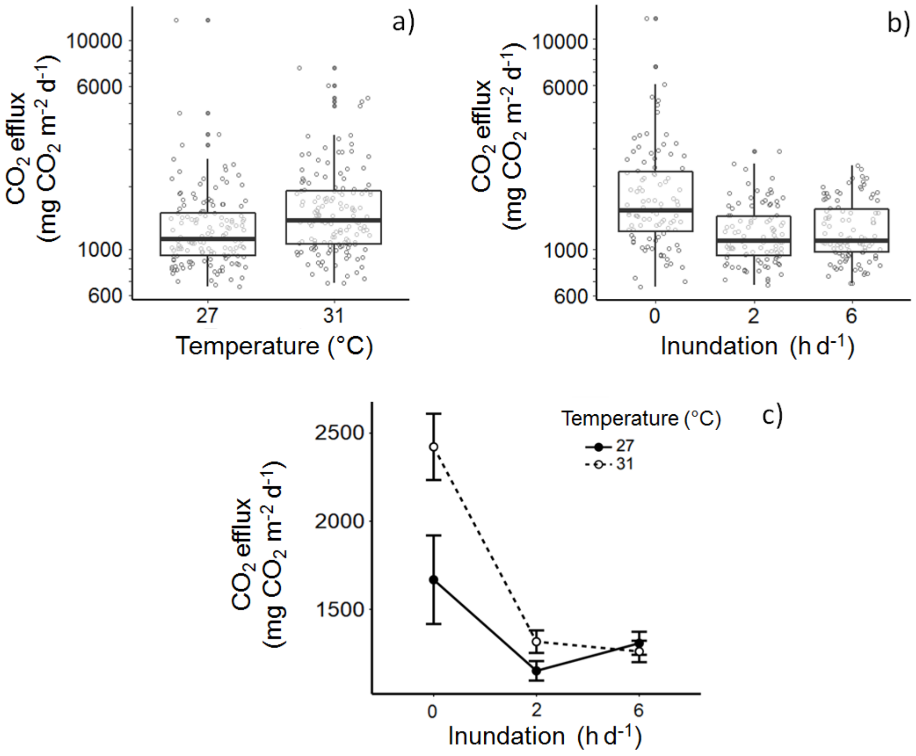

**Figure 4-1** The response of SOM decay of mangrove ($CO_2$ efflux) to (a) temperature, (b) inundation regime, and (c) the interaction of temperature and inundation regime. Bold, horizontal lines in (a) and (b) show sample medians. Note logarithmic vertical axis scales in (a) and (b). Symbols in (c) show sample means; error bars show standard errors. For (a), (b) and (c) n = 296.

A rise of temperature by 4°C increased mean soil $CO_2$ efflux by 21 % ($p < 0.001$, $F_{(1,291)} = 27.83$). The inundation regime also affected the mean soil respiration ($p < 0.001$, $F_{(2,291)} = 47.72$). The zero hours inundation treatment emitted 66 % more $CO_2$ than the two-hour inundation treatment ($p < 0.001$, least square means = 0.168, $t_{(296)} = 8.774$), and 60 % more than the six-hour treatment ($p < 0.001$, least square means = 0.152, $t_{(296)} = 7.931$). In contrast, the $CO_2$ fluxes from the two-hour and six-hour treatments were not significantly different from one another ($p = 0.691$, least square means = -0.016, $t_{(296)} = -0.820$). Temperature and inundation interacted significantly to affect soil respiration ($p < 0.001$, $F_{(2,291)} = 16.41$). In the samples that were never inundated, the higher-temperature treatment increased $CO_2$ emissions by 45 % ($p < 0.001$, least square means = 0.201, $t_{(296)} = 7.440$). For the two-hour inundation treatment, the higher-temperature treatment increased soil respiration by 14 %, ($p = 0.039$, least square means = 0.056, $t_{(296)} = 2.070$). For the six-hour inundation treatment there was no effect of warming upon soil respiration ($p = 0.666$, least square means = -0.012, $t_{(296)} = -0.433$). See Figure 4-1, Table 4-2 and Table 4-3 for details.

**Table 4-2** Effects of the inundation, temperature and their interaction on the SOM decay rate ($\log_{10}$ $CO_2$ efflux) using ANOVA. Significant effects ($p < 0.05$ threshold) shown in bold. Dfn and Dfd are degrees of freedom in numerator and denominator, respectively.

| **Treatments** | **Dfn** | **Dfd** | ***F*-value** | ***p*-value** |
|---|---|---|---|---|
| **Temperature** | 1 | 291 | 27.83 | **< 0.001** |
| **Inundation** | 2 | 291 | 47.72 | **< 0.001** |
| **Temp:Inundation** | 2 | 291 | 16.41 | **< 0.001** |

**Table 4-3** Effects of each inundation levels and the temperature treatments with each inundation level on the SOM decay rate (log10 $CO_2$ efflux) with the least square means as post hoc test for the linear mixed model. Significant effects ($p < 0.05$ threshold) shown in bold.

| **Treatments** | | **Estimated marginal means ($\log_{10}$ mg $CO_2$ $m^{-2}$ $d^{-1}$)** | **Df** | ***t*-value** | ***p*-value** |
|---|---|---|---|---|---|
| **0 h - 2 h $d^{-1}$ inundation** | | 0.168 | 296 | 8.774 | **< 0.001** |
| **0 h - 6 h $d^{-1}$ inundation** | | 0.152 | 296 | 7.931 | **< 0.001** |
| 2 h - 6 h $d^{-1}$ inundation | | -0.016 | 296 | -0.820 | 0.691 |
| **31 - 27° C** | **0 h $d^{-1}$ inundation** | 0.201 | 296 | 7.440 | **< 0.001** |
| | **2 h $d^{-1}$ inundation** | 0.056 | 296 | 2.070 | **0.039** |
| | 6 h $d^{-1}$ inundation | -0.012 | 296 | -0.433 | 0.666 |

## 4.4 Discussion

We reveal the independent effect sizes of temperature and inundation, and their interaction, upon SOM decay rates in mangroves, independently of confounding factors. Our results have a direct relevance to: i) the prediction of soil carbon fate under future environmental conditions; and ii) understanding the likely persistence of mangroves under sea level change and global warming.

### 4.4.1 Thermal sensitivity of mangrove SOM decay

SOM decay was affected by warming, as has been observed in other coastal wetlands and in some mangroves; however, the thermal sensitivity of the SOM decay determined here was in the lower range of what has been described for mangroves. Other studies have reported an increase in SOM decay ranging between 0 and 100 % with a temperature rise of ~5°C (from 25 to 30°C, Jin et al., 2013; Lewis et al., 2014; Lovelock et al., 2008; Poungparn et al., 2009; Simpson et al., 2019). This large range of SOM thermal sensitivity reported for mangroves is likely attributable to some combination of: i) the inclusion of autotrophic respiration in addition to heterotrophic respiration in field studies (Ouyang et al., 2018); and ii) the assumption that temperature is the dominant control on SOM decay, which our results do not entirely support. Field studies have used latitudinal gradients but did not control for other co-varying factors in the field such as tree species, inundation regime, soil nutrients and initial root biomass. Those factors are likely to contribute to soil organic matter decomposition. For instance, the interaction between temperature and inundation had a significant effect on SOM decay in our study, whereby increasing inundation duration reduced the thermal sensitivity of SOM decay (Figure 4-1). A potential explanation for the reduced thermal sensitivity of SOM decay with longer inundation could be that the input of water during inundation reduced the soil temperature in the mesocosms (Dalva & Moore, 1993), but the artificial seawater used to inundate the soils in our experiment was kept at air temperature in each of the environmental chambers; therefore, this is not a plausible explanation here. Rather, we might conjecture that the large effect of temperature in the mesocosms without inundation is likely due to increased soil aeration and drying at 31°C compared to 27°C (based on visual observations). In contrast, the inundated mesocosms (two and six hours per day) might have remained waterlogged between the inundation events due to slow drainage from our fine-grained clay soils (Table 2-1, Schwendenmann et al., 2006). The low to absent thermal sensitivity of SOM decay in frequently-inundated soil suggests that: i) SOM of low inter-tidal mangroves might be less sensitive to warming induced by climate change; and ii) more general sea level rise is likely to counterbalance the impact of warmer temperatures on SOM decay, at least in part, and provide some protection to mangrove soil carbon stocks under climate change.

### 4.4.2 Response of SOM decay to an inundation gradient

SOM decay did not decrease monotonically with an increased duration of inundation, as is often assumed in coastal wetlands (Davidson & Janssens, 2006; Nyman & DeLaune, 1991; Reed, 1995; Miller et al., 2001). The SOM decay showed rather a dichotomous response between inundated (the two-hour and six-hour per day treatments) and not-inundated soil (zero-hour per day inundation). The suppression of tidal inundation – a potential consequence of Taimasa or other drought events – caused increases of 66 % in $CO_2$ emissions from the mangrove soil. To our knowledge, no previous studies have looked at the effect of drought on mangrove SOM decay, but studies on tropical forested peatlands have shown similar rises of $CO_2$ fluxes when artificially drained (e.g., Hooijer et al., 2012). The lowering of the water table in carbon-rich soils induces a shift from anaerobic to aerobic conditions resulting in faster SOM decay, because aerobic respiration is more energetically favourable than anaerobic respiration (Chapman et al., 2019; Hooijer et al., 2012; Wolf et al., 2007). Taimasa events might therefore represent a major threat to mangrove carbon stocks, since they are projected to double in frequency, and nearly double in the area that they will affect, over the next 100 years (Widlansky et al., 2015).

Our representation of sea level rise, as an increase in inundation duration from two to six hours per day, had no significant effect upon rates of SOM decay, a finding that contrasts with the only two previously reported studies on mangroves, in both of which SOM decay was suppressed by increasing inundation (Lewis et al., 2014; Chambers et al., 2014: see treatment under ambient salinity). The mesocosms that were inundated for two and six hours per day might have remained close to waterlogged between inundation events due to the slow drainage of the fine-grained sediments, which might explain the similar response of soil $CO_2$ efflux for those two treatments (Schwendenmann et al., 2006). Our results are  similar to those reported in many studies on saltmarshes where increases in inundation duration had a limited effect on SOM decay (Kirwan et al., 2013; Hackney, 1987; Blum, 1993; Blum &Christian, 2004). We might conjecture that, like in saltmarshes, the input of saline water during our flooding treatments promoted the decay of SOM through sulphate reduction, and this compensated for the slower decomposition of SOM found normally under anaerobic conditions (Kirwan et al., 2013; Weston et al., 2011). Similar results have been observed by Chambers et al. (2014) in an outdoor mangrove mesocosm experiment that used similar levels of salinity – 15 ppt and 35 ppt – to us. Chambers et al. (2014) found that under low salinity the increased inundation duration resulted in lowering of SOM decay, while under high salinity, the SOM decay was not affected by the inundation duration increase. This suggests that increase of sulphate concentration of the water inundating the soil explains the variability of the SOM decay. Other factors are also likely to have contributed, because the increase of inundation with water of low salinity (< 12 ppt) has previously been shown to have no impact on SOM decay in a saltmarsh (Kirwan et al., 2013). Our results demonstrate that, like in saltmarshes, increased inundation in mangroves does not necessarily lead to a decrease in SOM decay rate. This implies that the persistence of mangroves to sea level rise, by vertical soil building and elevation in the tidal frame, does not exclusively rely on a reduction of SOM decay. Root production or mineral sedimentation are also likely to play a major role and require further investigation. Human impacts on those processes might represent additional threats to mangroves resilience to sea level rise; for instance, multiple river dams trapping sediments and large-scale commercial sand mining decreasing considerably the suspended sediments input to the coasts (Dai et al., 2009; Anthony et al., 2015), are highly likely to compromise the capacity of some mangroves to keep pace with relative sea level rise in the future. Our study was limited to the change of inundation duration; however the increase of sea level rise is likely to increase the extend and the frequency of tidal inundations. The impact of more frequent tidal inundations on SOM decay has not been studied to our knowledge. In contrast, the extend of the tidal inundation have already caused the mangroves to migrate landward in some mangrove settings (e.g. mangrove encroachment, see Gilman et al., 2008).

### 4.4.3 Sea level change and temperature effect on methane emissions

Independently of our treatments, the methane fluxes were un-measurable. This is in accordance with Cabezas et al. 2008 showing that water level fluctuations have no effect on methane emissions, but contrast with other studies reporting that methane emissions are high in low tide and low in high tide (Al-Haj & Fulweiler, 2020). Since sea level rise will increase the duration of inundation, it is likely that methane emissions might be reduced. Methane emissions have also shown to be reduced with root respiration and macro-fauna increasing soil oxygenation (Al-Haj & Fulweiler, 2020). Sea level rise can result in a reduction or suppression of root respiration and macro-fauna activities (Gilman et al., 2008) and by this process might increase methane emissions – however so far, no study has demonstrated it. In addition, sea level rise might result in mangrove encroachments into salt marshes, which might increase methane emissions, since mangroves have shown to produce more methane than saltmarshes (Al-Haj & Fulweiler, 2020).

## 4.5 Conclusions

By isolating the effects of temperature and inundation, and also considering them in combination, we reveal that:

1) Rates of SOM decay do not decrease with simulated sea level rise in our experiments. Previous reports of mangroves adapting to sea level rise through increased net SOM accumulation (McKee et al., 2007; Krauss et al., 2014) therefore seem likely to have been the result of an increase in root production rather than a reduction in SOM decay.

2) Drought conditions are likely to sharply raise SOM mineralisation of mangroves, and global warming might exaggerate this effect.

3) Climatic warming is likely to increase SOM decay rates, but the impact of rising temperatures may be lower than previously suspected, especially in mangrove soils that become inundated by rising relative sea levels.

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# Chapter 5 A new conceptual hydrological model of mangrove soils

## Abstract

Many mangrove ecosystem services, such as carbon sequestration, are closely linked to mangrove soil water content, which has been increasingly suggested to vary with animal burrow density. We tested this hypothesis by measuring the water content in the sediment matrix between crab burrows across 26 plots in a typical mangrove soil dominated by clay. We found that the water content of the sediment matrix remained more or less constant throughout the tidal cycle, and was independent of burrow density. Our results suggest that burrows act as a network of connected pipes transporting water through the mangrove soil, but that there is little exchange of water between the pipes and the sediment matrix. Using a numerical groundwater model, we confirm that fine-grained mangrove soils are resistent to drainage via animal burrows. We propose a new conceptual model of subsurface water flow in fine-grained mangrove soils with important implications for biogeochemical processes.

**Keywords:** subsurface flow, pore water discharge, blue carbon, sediment permeability, bioturbation, coastal wetland

## 5.1 Introduction

Mangroves provide ecosystem services worth US$ 194,000 per hectare, such as carbon sequestration and export of nutrients supporting offshore biological production (Costanza et al., 2014; Alongi, 2009). The drainage of mangrove soil is thought to be an important control on their carbon and nutrient cycling (Wolanski et al., 1992; Xiong et al., 2018). Near-surface water tables in mangroves are believed to prevent high rates of organic matter mineralisation, resulting in mangroves being one of the most carbon dense ecosystems in the world (Donato et al., 2011). Discharge of water from mangrove soils into the ocean through outwelling means that mangroves may also export a large quantity of organic matter, inorganic carbon  and nutrients offshore, notably supporting coastal fisheries (Lee, 1995; Dittmar et al., 2006; Maher et al., 2013).

Soil water content in mangroves controls biogeochemical cycling by determining i) soil redox conditions, and ii) the potential exchange and storage of solutes with adjacent ecosystems (e.g. coral reefs, open ocean, Alongi, 2009; Lee, 1995). Soil water content, in turn, is controlled by the drainage capacity of the soil, and the main fluxes of water (tides, evapotranspiration, precipitation, river floods and groundwater upwelling: Wolanski et al., 1992). The drainage of a mangrove soil will be influenced by its slope and the bulk hydraulic conductivity of the sediment matrix and the animal burrows (Mazda et al., 2007; Mazda & Ikeda, 2006). The soil hydraulic conductivity controls the rate of water flowing through, into and out of the soil. Several factors influence the hydraulic conductivity of soils, such as the grain-size distribution of the sediments, and macropores created by burrowing activities, such as by crabs (Susilo & Ridd, 2005). Hereafter, we use soil to refer to the entirety of the mangrove soil, i.e., animal burrows and the material between them. We term the latter the 'sediment matrix'.

Mangrove soils have been described as having low rates of drainage due to their fine-grained sediment matrix (Schwendenmann et al., 2006), but small-scale studies have shown that animal (e.g., crab) burrows are often interconnected and allow the rapid transport of water through the soil (Hollins et al., 2000; Stieglitz et al., 2000). Although few studies have investigated mangrove soil hydrology, mangrove soils have recently been conceptualised as a sponge that is drained and replenished with the tides (Figure 5-1, Alongi, 2014).

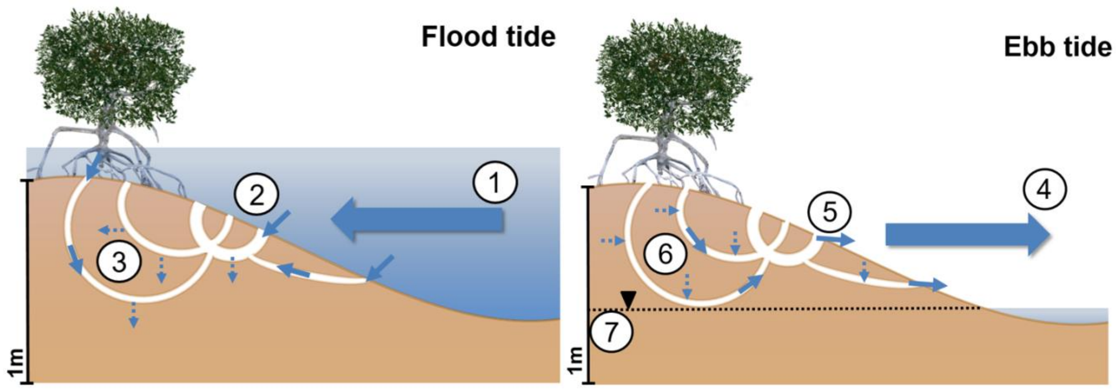

**Figure 5-1** The sponge model reproduced from Alongi (2014). Water from the incoming tide (1) enters the burrows (2), flows through the burrow network and infiltrates into the sediment matrix. At ebb tide, there is an outwelling of solute-enriched groundwater (4) resulting from the drainage of the burrows (5) and the sediment matrix (6). This drainage causes the water table in the soil, including the sediment matrix, to fall (7).

In this sponge model, water flows in through the burrows during the rising tide, and percolates through the sediment matrix surrounding the burrows. During low tide water drains through both the burrows and the sediment matrix (Figure 5-1, Alongi, 2014). In the model, these fluxes cause large variations in water table position between the burrows across tidal cycles (Figure 5-1, Alongi, 2014). Large-scale studies (several km$^2$) have also revealed that the discharge of water flowing out of mangrove soils is much larger than expected (Tait et al., 2016). Alongi's (2014) model attaches equal importance to drainage through crab burrows and the sediment matrix. Tait et al. (2016), however, suggested that drainage of the sediment matrix is unimportant, with most water flow into and out of mangrove soils occurring in animal burrows. However, these competing ideas are yet to be tested in the field.

Here, we tested these conceptual models in a Vietnamese mangrove by measuring the drainage of the mangrove sediment matrix along a gradient of animal burrow densities and relative surface areas.

## 5.2 Methods

We took more than 1,400 measurements of water content in the sediment matrix between burrows, over time and across 26 plots in the mangroves of the Can Gio Biosphere Reserve (CGBR), in south Vietnam, to investigate a potential relationship between soil drainage and animal burrow density and surface area (coverage). To help interpret our results, we built a groundwater model of flow between the sediment matrix and a crab burrow.

### 5.2.1 Study site

Our study site in CGBR is typical of Southeast Asian mangroves, possessing: i) a high carbon store (910.7 ± 32.2 t C ha$^{-1}$: Dung et al., 2016) (see also Donato et al., 2011); ii) a sediment matrix mostly composed of clay-sized grains (90 % of sediment by weight), which is typical of deltaic or riverine mangroves (Woodroffe, 1992); and iii) an average groundwater outwelling discharge between 3.1 and 7.1 cm day$^{-1}$, which is within the range of what has been previously reported in mangroves more broadly (Taillardat et al., 2018; Tait et al., 2016). The macrofauna associated with burrowing activities found in this site appear to be dominated by sesarmid (family Grapsidae) and fiddler crabs (genus *Uca*; family Ocypodidae) (Diele et al., 2013). Burrows of fiddler crab are J- or L-shaped and between 20 to 40 cm deep (Kristensen, 2008). In contrast, burrows of sesarmid crabs range from simple, straight burrows with few branches to complex, labyrinthine structures with multiple openings and a depth of 55 to 110 cm (Kristensen, 2008). CGBR has a low lying topography with altitudes ranging from 0.5 to 1.5 m above mean sea level. All our plots were located at similar altitudes in the mudflat (mid-intertidal zone) and all had a shallow slope.

### 5.2.2 Water content measurements

We measured over 1,400 soil water contents across 26 1 m × 1 m plots located in the inter-tidal area of CGBR. The plots had a range of densities and surface areas of animal burrows. In each plot we established between 15 and 20 fixed points from which we took repeated measurements of soil volumetric water content (VWC) during that part of the tidal cycle when the plot was not covered in water or too dangerous to access (hours of darkness). We chose plots with similar altitudes above the mean sea level, and that were mostly free of belowground roots to limit potential confounding effects. We took all the *in-situ* measurements from floating platforms. When grounded, these platforms exerted low pressures on the soil surface and had no obvious effects on the water content of the sediment matrix between the burrows. We measured VWC of the sediment matrix using a ThetaProbe (±1% accuracy, DeltaT Devices; Cambridge, UK). We took measurements at approximately one-hour intervals for up to 6 hours. The ThetaProbe measures VWC in a cylinder of sediment 3 cm in diameter and 6 cm in depth (Gaskin & Miller, 1996). We calibrated the ThetaProbe with sediments collected from our field site. We dried the calibration sediments in an oven set at 30°C. During the drying, we took ThetaProbe readings of VWC from the sediments, and reweighed them each time to calculate change in water contents.

### 5.2.3 Determination of burrow aeral coverage and density

We measured the number of burrows per unit area, and the aeral coverage of their openings, from digital photographs of the plots. In total, we measured more than 2000 burrows. We corrected each picture for image distortion with the GNU Image Manipulation Program (GIMP) (https://www.gimp.org/) and then extracted the area and the number of burrows in each image using the ImageJ software (Rueden et al., 2017). We measured only burrows with a minimum area of 0.50 cm$^2$ (~8 mm diameter), because smaller burrows could not be differentiated in GIMP from crab defecation pellet shadows.

### 5.2.4 Modelling of water flow into an idealised burrow

The model simulated flow into a single vertical burrow that was 50 cm long (Figure 5-2), had a diameter of 6 cm, and in which the water level was fixed at a depth of 5 cm from the burrow base (45 cm depth) as shown in Figure 5-2.

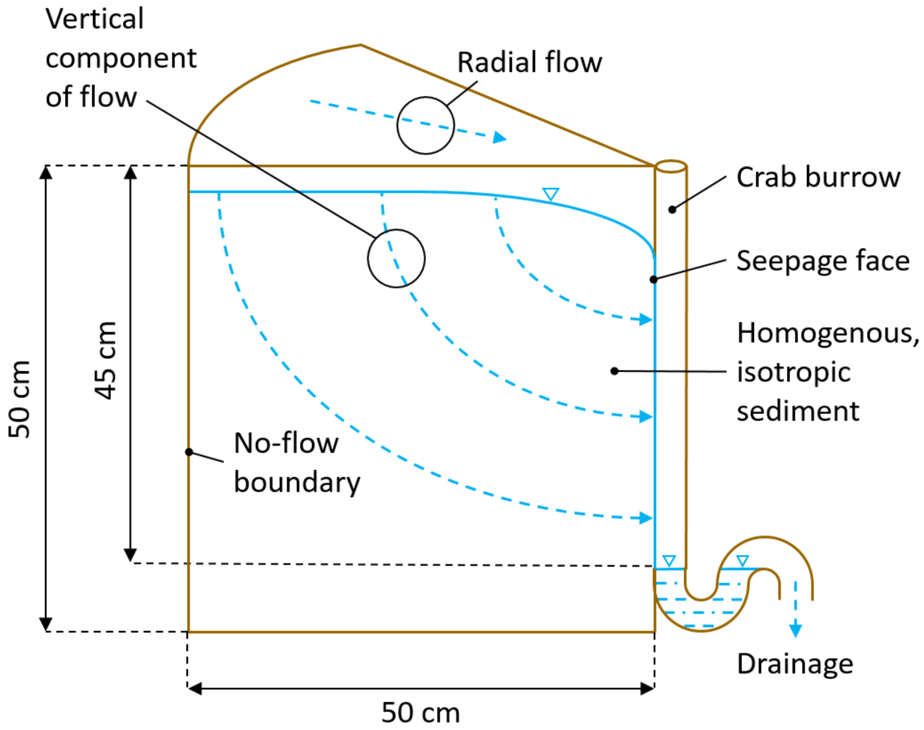

**Figure 5-2** Geometry of the idealised crab burrow represented in the numerical model**.**

The model accounted for radial flow, both in plan and with depth, and for the formation of a seepage face on the burrow wall. It was set up using Modflow 6 (Appendix A5.1). In each model run, we assumed the sediment matrix had a single value of hydraulic conductivity and specific yield (Table 5-1). The model was run for six hours assuming an initial condition in which the sediment matrix beyond the burrow was saturated (the water table was at the mangrove surface). Further details of the model are provided in the Appendix A5.1.

**Table 5-1** Hydraulic conductivity and specific yield values used in the numerical model simulations. [a] denotes values taken from Table 3.2 and [b] from Table 4.2, both from Domenico and Schwartz (1990). The hydraulic conductivity for the medium silt was set to be 100× greater than the value for the fine silt.

| | **Fine clay**[a,b] | **Coarse clay**[a,b] | **Fine silt**[a,b] | **Medium silt** | **Coarse silt**[a,b] |
|---|---|---|---|---|---|
| **Hydraulic conductivity ($m\ s^{-1}$)** | $1 \times 10^{-11}$ | $4.7 \times 10^{-9}$ | $1 \times 10^{-9}$ | $1 \times 10^{-7}$ | $2 \times 10^{-5}$ |
| **Specific yield (-)** | 0.03 | 0.03 | 0.08 | 0.08 | 0.08 |

## 5.3 Results and discussion

Across our plots, our *in-situ* measurements showed that the water content of the sediment matrix remained more or less constant through time, and was unaffected by animal burrow density or area (Figure 5-3). Contrary to the sponge model, this suggests that the burrows act as a network of pipes that transports water through the mangrove soil and that the burrows are hydrologically isolated from the surrounding sediment matrix.

### 5.3.1 Animal burrows in the soil and moisture content of the sediment matrix

The minimum VWC of the sediment matrix was 51.5 % and the maximum was 52.2 % across our plots. In all plots, the water content (Figure 5-3) hardly varied through time and there was no obvious difference between the plots, despite i) the number of burrows per plot differing by more than an order of magnitude (min= 11, max=198), and ii) the area of the burrows being more than two orders of magnitude higher in the plot that was most heavily burrowed compared to plots that were almost free of burrows (Figure 5-3).

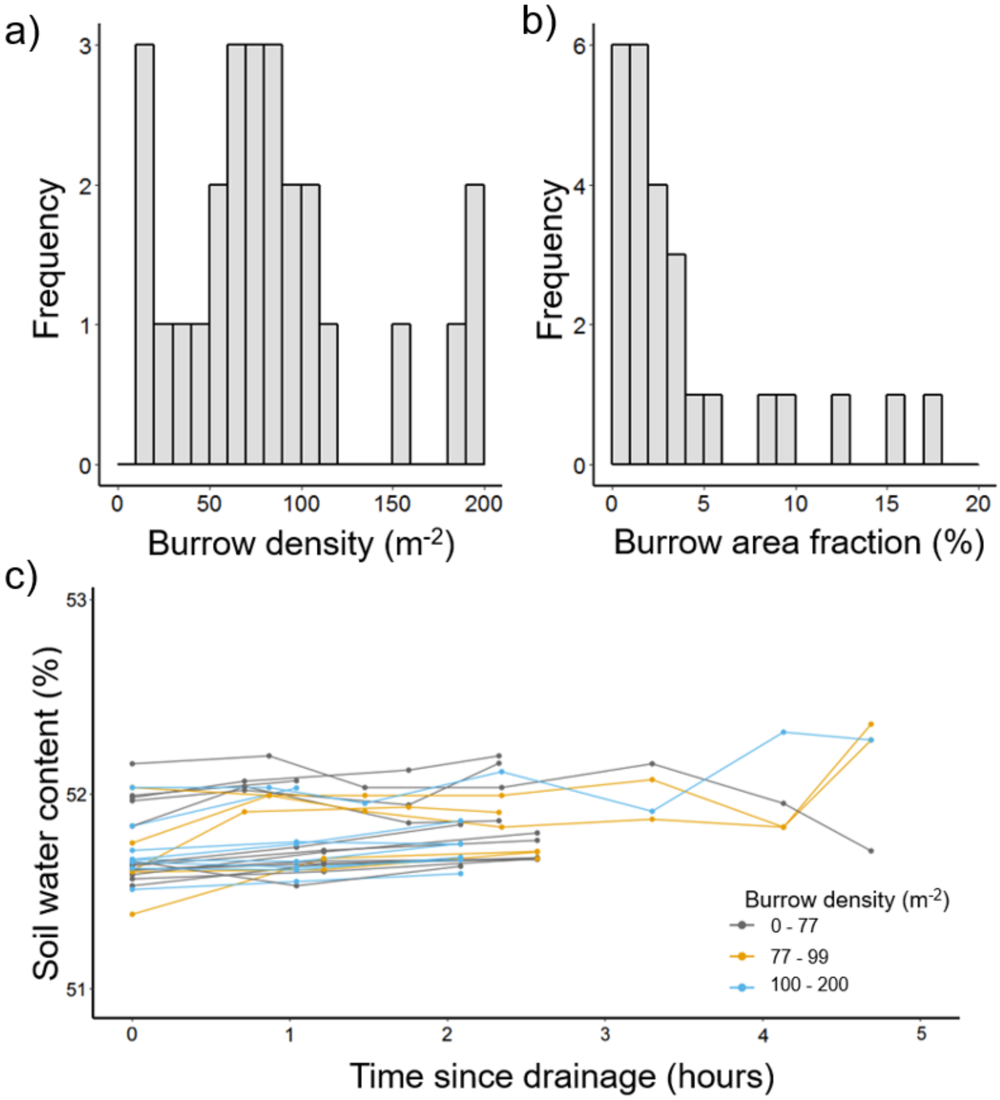

**Figure 5-3** The burrow density and area fraction do not affect the drainage of the sediment matrix. (a) Burrow density, (b) burrow area fraction, (c) mean VWC of the sediment matrix in each soil plot grouped by burrow density.

### 5.3.2 Revisiting the hydrological framework of mangrove soils

The all-but constant VWC of our sites indicates that the sediment matrix remained saturated or close to saturated at all times, and possesses a low permeability (Schwendenmann et al., 2006). We propose that the sponge model does not apply to our site or other similar sites, and requires substantial modifications, since: i) little or no water is transported through the sediment matrix; and ii) the water table level of the sediment matrix is unlikely to vary substantially between the ebbing and flooding tides.

Several studies (Taillardat et al., 2018; Tait et al., 2016) have shown a large exchange of water between mangrove forest and adjacent water bodies. Because the water content of the sediment matrix at our site did not change over time, most of the water exchange there must, therefore, take place in a secondary permeability system, namely the one formed by the animal burrows. In our site, the largest burrows exceeded 12 cm in diameter, which is consistent with the findings of Stieglitz et al. (2000) that burrows can form large soil macro-pores and accommodate large quantities of water.

A video recorded at our sites (Appendix A5.2) shows crab burrows filling with tidal water from their base. Some burrows were almost entirely filled before the tidal water reached their surface openings. This shows that some animal burrows have at least two openings at different levels within the tidal prism, and suggests that some burrows might form a network of interconnected soil macropores as observed by Stieglitz et al. (2000). Such a burrow geometry might act as a pipe network transporting water and solutes through mangrove soils; while the low permeability of the surrounding sediment matrix, as indicated by our volumetric wetness measurements, prevents or greatly reduces water exchanges between the burrows and the sediment matrix. Therefore, we propose a new model, based on Stieglitz et al. (2000), with a dual permeability system (Figure 5-4), in which the primary permeability (i.e., that of the sediment matrix) is negligible, and in which the secondary permeability afforded by the burrow macropores dominates groundwater exchanges.

Our model is similar to previous dual porosity models developed for soils with macropores (Armstrong & Arrowsmith, 1986; Beven & Germann, 1982). The sediment matrix seems to be best described by hydraulic principles based on Darcy’s law and the macropores as a subcompartment in which turbulent or non-Darcian flow may occur (Beven & Germann, 1982). The limited interaction between these two domains is likely to result from the fine-grained sediment resulting in an almost impermeable sediment matrix as described by the model of Armstrong & Arrowsmith (1986) for a soil with a matrix dominated by clay and silt fractions. Given the large and frequent input of tidal water and the low permeability of the sediment matrix, the primary effect of macropores is to increase the volume of the water storage, rather than to increase the water infiltration in the sediment matrix as previously reported by Beven & German (1982).

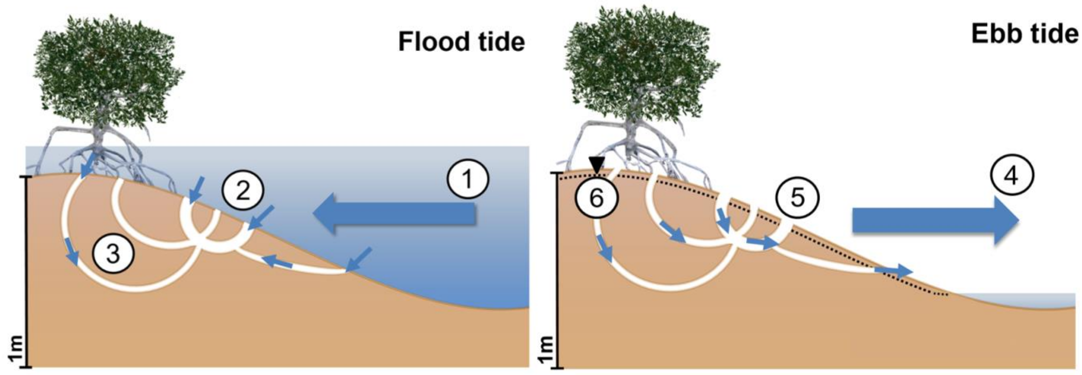

**Figure 5-4** Our proposed piping model. During flood tide, incoming water (1) enters the burrows at their surface openings (2). Because the low hydraulic conductivity of the matrix prevents downward and lateral flow of water from the burrows (3), the water is stored in the burrows. At ebb tide, the water stored in the burrows, enriched in solutes derived from the burrow, drains with the falling tide (4). While the burrows drain (5), the water table in the sediment matrix remains stable (6).

### 5.3.3 Modelling water exchanges between the sediment matrix and crab burrows

Our numerical model results (Figure 5-5) agree with our conceptual piping model. The simulations show that, in a crab burrow surrounded by a clay, there is little or no drainage between the sediment matrix and the burrow. The ranges of hydraulic conductivity values reported for clays and silts (Figure 5-5) overlap. For the bottom end of the clay range (fine clay in Figure 5-5 and Table 5-1) and the bottom end of the silt range (fine silt), virtually no water drains between the sediment and the burrow. For the high end of the clay range, which is higher than the lower end of the silt range, a small amount of sediment drainage occurred within 15 cm of the burrow. What these results confirm is that for the low hydraulic conductivities of most clays and fine silts, our conceptual piping model applies. However, our simulation for the higher end of hydraulic conductivities for coarse silt did show substantial drainage to the crab burrow, and is consistent with the sponge model. As shown in Figure 5-5, even after only 1.5 hours, more than half of the sediment within the vicinity of the burrow had drained, releasing water representing 8 % of the total volume. Overall, therefore, our numerical model simulations illustrate conditions in which the sponge model does and does not apply. Clearly, our simulations are idealised and do not include multiple burrows, which would prove numerically challenging or intractable, but suggests the sponge model does not apply to clays, where our piping model is more appropriate. Since clay and fine silt are the dominant grain size fraction of mangrove sediments (Woodroffe, 1992), our piping model will describe most of mangrove soils, while the sponge model will be restricted to sandy mangrove soils.

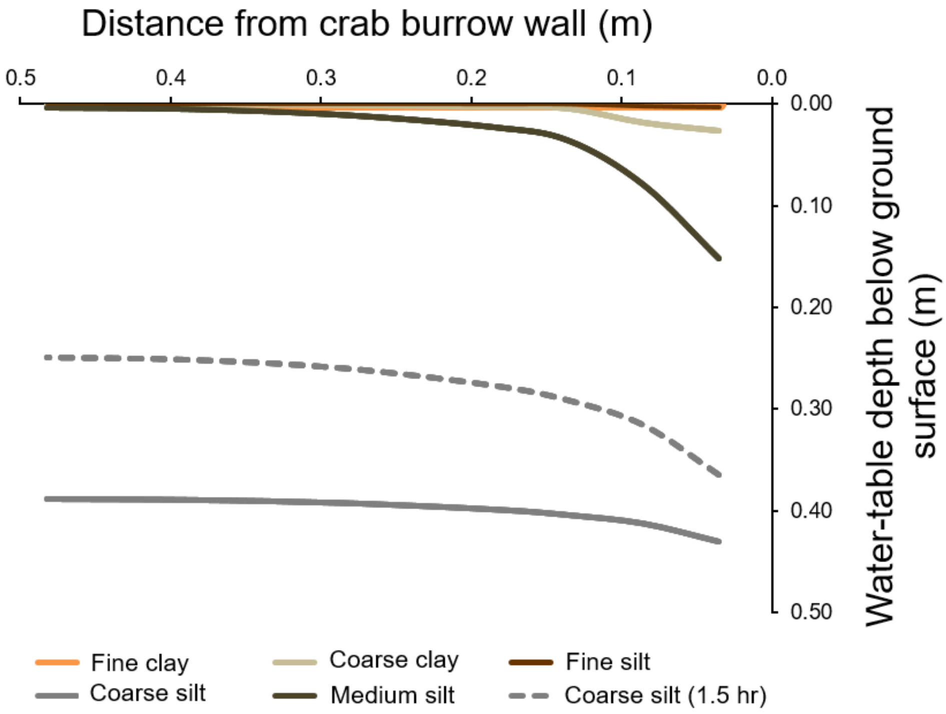

**Figure 5-5** Results from the numerical model runs for the situation depicted in Figure 5-2. All water tables are for six hours after the commencement of drainage, except the dashed line, which shows the position of the water table after 1.5 hours.

### 5.3.4 Similarity of hydrological processes in mangrove and marine sediments

There was no water transport in clayey mangrove soils, which is similar to that observed in marine clay sediments (Huettel et al., 2003). It is likely that like in fine grained marine sediments, the transport of solutes is mostly through diffusion in clayey mangroves (Huettel et al., 2003; Aller and Aller 1998). Mangrove soils having coarser sediments seem to drain similarly as permeable marine sediments (Huettel et al., 2003). It is therefore likely that like in marine sediments, the transport of solutes is dominated by advection in mangroves having coarser sediments, and by diffusion in mangroves having finer sediments. The diffusion rate of solutes showed to increase in relation with burrow density but not with burrow radius in marine sediments (Aller and Aller 1998), and this might be the same in mangroves. The large loss of dissolved carbon (DIC and DOC) reported in mangroves, are therefore likely to result from diffuse transport, which might increase as the distance between burrows decrease (e.g. when the burrow density increase). Further researches will be necessary to constrain the controlling factors of solutes diffusion in mangroves, but the literature on marine sediments are likely to provide some interesting insights.

### 5.3.5 Implication for biogeochemical processes

Our new piping model has several implications for biogeochemical cycling in mangroves. In contrast to the sponge model (Alongi, 2014), we show that the water table does not fluctuate substantially in the sediment matrix. This implies that the mangrove sediment matrix is mostly anaerobic, which likely aids mangrove carbon sequestration by supressing mineralisation rates. Our new model also suggests that most of the carbon and nutrients outwelling from the mangrove likely originate from the interior surface walls of the animal burrows and from the mangrove soil surface (e.g., leaves, propagules, dead stems, animal faeces). Burrow walls may act as hot-spots of carbon mineralisation and nutrient cycling, by increasing the total surface area of the sediment matrix exposed to the air (Kristensen et al., 2012). Crabs ingest organic matter and redeposit it in the form of defecation pellets at the surface of the sediment matrix or inside burrows (Kristensen et al., 2012). This mineralised carbon is readily available for daily tidal export in fringe mangroves. The twice-daily tidal inundation that brings fast-flowing water through the burrow pipe network transports this matter to adjacent ecosystems, and is also likely to cause erosion of the burrow wall surface or its collapse, increasing the quantity of matter exported.

## 5.4 Conclusions

We found that the upper layer of a mangrove sediment matrix does not drain during ebb tides and that near-surface water contents are unaffected by animal burrows. We argue that the animal burrows do not allow extensive water flow through the mangrove sediment matrix, and rather act as a separate or independent pipe network through which water enters and leaves according to the tidal cycle**.**

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# Chapter 6 Discussion and outlook

## 6.1 Summary of key findings and their implications

### 6.1.1 Summary of key findings

In this thesis, I investigated the impact of global change on the belowground carbon dynamics of mangroves, and the controls on water flow in mangrove soils. The accumulation of carbon in mangroves results from the balance between: i) carbon input to the soil through root production; and ii) carbon loss through SOM decay and lateral transport to the ocean. I quantified the impact of current conditions and future global change on both of those dynamics — in particular, the impact of large-scale mangrove reforestation on fine root production (chapter 3) and the impact of global warming and sea-level change on SOM decay (chapter 4). In addition, I identified the factors controlling water flow in fine-grained mangrove soils (chapter 5). I found that belowground carbon dynamics are shaped by individual global change factors and their interactions, and that the sediment matrix of mangroves has limited drainage, even with a high density of animal burrows. In the following paragraphs, I summarise the main findings of each analytical chapter, explaining how they contribute to achieving the objectives of this thesis.

#### 6.1.1.1 Fine root production response to mangrove age

Fine root production was controlled by the age of the reforested mangrove. The production of fine roots decreased through time after reforestation, likely responding to the self-thinning of the mangroves as they mature. A large amount (up to 40 %) of this production was deeper than 30 cm, an important finding considering that most researchers limit their investigations to depths of 15 to 30 cm. The distribution of root production at different soil depths varied with the age of the reforested mangroves. There was no discernible pattern of root production in the oldest mangrove stand, while the largest root production was at the shallowest portion of the soil for the younger mangrove stands. Mangrove age has not been considered as a driver of belowground carbon dynamics before, but my results demonstrate that the stand age of mangrove affects the belowground production and hence potentially the carbon accumulation and the persistence of mangroves to sea level rise.

#### 6.1.1.2 SOM decay sensitivity to warming and sea level change

SOM decay was affected by the interaction of global change factors. However, SOM decay did not decrease with simulated sea-level rise as it is often assumed. In contrast, drought events strongly increased the decay of SOM in the mangroves. This increase was greater at higher temperatures, showing that global warming and drought act together to affect mangrove SOM decay. Warming affected the increase in SOM decay rate, but less than has been previously reported for other mangroves and coastal ecosystems such as salt marshes. The thermal sensitivity of the mangrove soil decreased with longer inundation showing potential mitigation of a warming effect on SOM decay by sea level rise. Those results demonstrate that SOM decay is probably not the primary control of carbon accumulation in mangroves affected by sea-level rise, and that root production is likely to be more important in affecting the resistance of mangroves to increases in sea levels.

#### 6.1.1.3 Lateral transport of water in mangrove soil

The matrix in the upper layer of a clayey mangrove soil did not drain during the tidal cycle, even in the presence of a dense network of animal burrows. The hydraulic gradient was high between the empty burrows and the saturated clayey soil during low-tide, but it did not compensate for the low hydraulic conductivity of the fine-grained sediment; therefore, no water flow was generated between the two domains. The animal burrows seem to act as an independent network of pipes, transporting water in and out of the mangrove soil, but exerting little influence on the drainage capacity of the sediment matrix. The groundwater modelling work showed that animal burrows might only affect the matrix domain when it is composed of larger-grained sediments (with relatively high hydraulic conductivities). The lateral loss of carbon is therefore likely to be limited to the animal burrow walls.

### 6.1.2 Implications

My findings have implications for understanding i) the response of mangrove carbon stores to global change, such as sea-level rise, global warming and mangrove reforestation; ii) controls on the accretion of mangrove soils, and the persistence of mangroves to sea-level rise; and iii) mangrove soil hydrology, which influences the mangrove carbon cycle.

#### 6.1.2.1 An update of the mangrove carbon budget

I found that up to 40% of the fine root growth occurs below 30 cm deep in soil. This agrees with the few studies having investigated root production at depth over 30 cm, but contrasts with the most commonly cited mangrove carbon budget having restraining mangrove root production at the depth of 30 cm (Bouillon et al., 2008). This commonly used mangrove carbon budget (e.g. Bouillon et al., 2008) might have underestimated by roughly 80 Tg carbon per year the root carbon input in mangrove soils. This deep root production might disproportionally contribute to soil carbon burial in mangroves, because the decay rate is likely to slow down at depth (e.g. due to reduced microbial biomass, stabilisation of soil organic matter by minerals and reduced supply of fresh carbon priming soil carbon). My estimate of root production is roughly five times higher than the carbon burial rate of mangroves. Only few studies have investigated the annual root decay. For instance, Ouyang et al. 2017 estimated that the global mangrove root decay is ~ 51.1% of the annual fine root production per year. However, it is worth to reemphasized that this root decay estimation might not be completely accurate (see section 1.1.3.4). Nevertheless, other carbon losses might have been underestimated or not measured at all, such as the root exudations.

### 6.1.2.2 Mangrove carbon sinks and store under future environmental conditions

Mangroves may accumulate carbon over thousands of years (Spivak et al., 2019). This carbon accumulation results primarily from the balance of fine root inputs and SOM decay in carbon rich mangroves (Bouillon et al., 2003; McKee, 2011; Middleton & McKee, 2001). Therefore, my findings about fine root production and SOM decay have important implications for understanding the mangrove carbon sink and store under current and future environmental conditions (Spivak et al., 2019).

#### 6.1.2.1.1 Mangrove carbon sinks and stock under global warming and sea level change

I showed that warming (+4°C) may increase SOM decay by 20%, which is in the lower range of previous estimates (Jin et al., 2013; Lewis et al., 2014; Lovelock et al., 2006; Poungparn et al., 2009; Simpson et al., 2019). This carbon loss may be compensated by an increase in net primary production with higher temperatures. Warming has been shown to increase net primary production by increasing rates of photosynthesis in mangrove leaves up to 30°C (Ball et al., 1988), but no study has investigated the effect of warming on mangrove carbon allocation between above- and belowground biomass. Above 33°C, photosynthetic rate declines, sometimes gradually and sometimes sharply (Ball et al., 1988). Therefore, under these conditions, the future carbon sink function of mangroves is uncertain.

I found that a decrease in soil inundation (simulated by a change in inundation duration between two and zero hours per day) strongly increased SOM decay (+ 60 %), and this effect was exacerbated with warming. To my knowledge, the impact of drought on mangrove SOM dynamics has not been studied to date; however, a similar rise in SOM decay (as $CO_2$ efflux) has been documented for drained tropical peatlands (Cooper et al., 2020; Hooijer et al., 2012). It is unlikely that drought conditions would result in higher net primary production, since the last drought event in Australia resulted in large mangrove dieback (Lovelock et al., 2017). Combined with warming, droughts are likely to be more intense, and Mafi-Gholami et al. (2017) showed that drought intensity was correlated with a reduction in aboveground biomass (e.g. loss of mangrove area). Therefore, it is probable that drought combined with global warming would reduce the net primary productivity of mangrove stands, and, thus, the carbon sink and store function of mangroves in the long term.

On the other hand, it is possible that drought could result in a pulse of SOM inputs from dead wood in the short term, but its effect on the soil carbon stock of mangroves remains unknown. The death of mangrove trees caused by drought may also lead to rejuvenation of mangrove stands. Young mangrove trees are likely to develop after a drought, because of a reduction in competition for space enabling greater access to nutrients and light. However, young (immature) mangrove stands would probably need several years to reach the same belowground root production as mature stands. In chapter 3, I showed that fine root production of mangrove stands is higher in younger mangroves, but the investigated stand was already mature (28 years old). Therefore, under drought, the mangrove carbon store and sink are likely to be reduced, and recovery to initial carbon sink and store levels is likely to take several decades, if it happens.

The most prominent scenario of future environmental conditions is a rise in both temperature and sea level (Ward et al., 2016). To date, no study has investigated the response of net primary production (above and belowground biomass) to these factors in combination. Nevertheless, an increase in belowground carbon accumulation has been observed in natural and restored mangroves with sea level rise (Ezcurra et al., 2016; Krauss et al., 2017; McKee et al., 2007), but the response of root production to sea level rise is unclear, and may depend on several factors (e.g., chapter 3). In contrast, SOM decay response is clearer, since I showed that under sea level rise (simulated by a change in inundation duration between two and six hours per day), SOM decay is not reduced at ambient and warmer temperatures (chapter 4). While these findings contrast with those of the only two previous studies on mangroves, they agree with results reported for many studies on salt marshes (Blum, 1993; Blum & Christian, 2004; Hackney, 1987; Kirwan et al., 2013). If the SOM is not reduced, and the belowground productivity is stable or higher under warming and sea level rise, the carbon sink and store functions of mangroves may be protected (provided the mangrove soil is not eroded). However, in other cases, mangrove trees may die due to submergence of their habitat, which will supress the mangrove carbon sink, and potentially slowly decrease the soil carbon stock. Indeed, I showed that soil carbon decay under rising inundation is not significantly different from under ambient conditions, but nor is it non-existent (chapter 4). It is also likely that under full submergence, the soil carbon stock is preserved, at least partly, as observed in submarine peatlands (Kreuzburg et al., 2018), but this needs to be further investigated.

In addition, it is likely that following the grain size distribution of the mangrove sediments, the effect of sea level rise will be different. In chapter 5, I show that clayey mangrove does not drain, and this independently of crab burrows. However, I show that mangroves with coarser sediments are more sensible to drainage and that crab burrows increase their drainage. This means that an increase of the inundation duration by sea level rise will increase the water content of mangrove soil dominated by silt or sand. In contrast, an increase of the inundation duration in clayey mangroves might have a limited effect on the soil water content, because the sediment matrix is already saturated with water. The water content of mangrove soils is a large control over the decomposition of the soil organic matter (Spivak et al., 2019). Therefore, the sediment grain size distributions will affect the sensitivity mangroves to sea level rise through control of soil organic matter accretion and soil surface elevation.

#### 6.1.2.1.2 Belowground production trajectory after reforestation and implication for belowground carbon dynamics and budgets

I showed that fine root production decreases with stand age, which is in accordance with the aboveground biomass trajectory of reforested mangroves

(Saenger et al., 2002). No other study has investigated belowground production during mangrove development, either in primary or secondary mangroves. However, my results showed a belowground carbon trajectory similar to that of other secondary forests, such as those dominated by ponderosa pine or oak and hickory stands (Idol et al., 2000; Law et al., 2003). However, white-birch-dominated forest showed a slightly different trajectory; in this case, root production increased in forests from age 20 to 36 years, and then decreased (Sun et al., 2015). The decrease in belowground carbon production is often linked to canopy closure, which may differ between tree species. The canopy is said to be closed when tree density is so high that only little direct sunlight can reach the forest floor. The competition for light between trees then results in high tree mortality, with the forest self-thinning and the tree density gradually declining. These dynamics have been previously described in mangroves (e.g. mangrove self-thinning process; Fromard et al., 1998), and showed to result in a reduction in aboveground biomass (Walcker et al., 2018). My site exhibited similar declines in tree density with mangrove-stand age. Canopy closure is, therefore, likely to be the starting point of the decline in belowground production in mangroves. While there is no other study to confirm this age-related hypothesis, a higher tree density in mature forest resulted in higher fine root production (Adame et al., 2014). Therefore, it is likely that this pattern recurs in mangroves; however, the age at which it occurs may vary across species and location, since other factors might interact with age.

In contrast to fine-root production, SOM decay has been reported to remain unchanged across mangrove reforestation ages, but only one site has been investigated (Alongi et al., 1998). If SOM decay does not change with mangrove age, then mangrove fine root production is likely to control the accumulation of SOM. In fact, autochthonous organic carbon accumulation has been shown to decrease after mangrove stands reach maturity (Marchand, 2017), exactly like fine root production, adding to the evidence that fine root production controls SOM accumulation. Fine root production varies with mangrove stand age and seems likely to control SOM accumulation; therefore, prediction of the carbon store and sink would be considerably improved by accounting for mangrove age in any carbon budget calculations.

In addition to helping explain key mechanisms of carbon accumulation in mangrove soils, my findings also invite reassessment of current mangrove carbon budgets. I showed that root production is likely to vary strongly between mangrove stands. While I focused on mangrove age, results of other studies complement these findings by showing that fine root production is ecologically plastic and responds to local environmental conditions such as nutrient availability or flooding frequency (Adame et al., 2014; Castañeda-Moya et al., 2011; Naidoo, 2009; Reef et al., 2016). Therefore, global estimates of fine root production based on only a few available studies (Bouillon et al., 2008) are likely to be misleading. In addition, my findings revealed that a large part of fine root production is below 30 cm, which is consistent with Castañeda-Moya et al.'s (2011) investigation of fine root production and other studies reporting live root biomass below 30 cm (Komiyama et al., 1987, 2000; Tamooh et al., 2008). These findings are likely to be applicable to other sites, since the tree genus, *Rhizophora*, in the site I studied is distributed worldwide, and *Rhizophora* mangrove stands hold the second largest stock of mangrove carbon globally (Atwood et al., 2017). Therefore, previous mangrove carbon budgets (Bouillon et al., 2008) using fine root production estimates for only the first 30 cm of soil are likely to have grossly underestimated the production of fine roots.

In addition, the restoration of mangrove soil hydrology is important, because it controls the lateral loss of soil carbon and its mineralisation. Yet, little studies have investigated the role of mangrove ages or the role of sediment grain size restoration on soil hydrology (see Chapter 5). I show in Chapter 5 that clayey mangrove sediment matrix has very limited drainage implying that most of the lateral carbon loss and the aerobic decomposition are restricted to crab burrows. A shift toward a matrix with larger sediment grain size is likely to induce greater soil carbon mineralisation and lateral loss. In addition, mangrove ages might affect the structure of the soil due to differences in root density. Yet, the relation between soil water flow and the soil root density remains to be investigated.

#### 6.1.2.3 The resilience of mangroves to sea level rise by SOM accumulation

There is evidence that mangrove roots are an important or even dominant part of SOM accumulation, allowing mangroves to occupy vertical accommodation space to withstand sea level rise. For instance, Middleton & McKee (2001) suggested that SOM accumulation resulted from the accumulation of abundant and refractory mangrove roots. In addition, McKee et al. (2007) showed that past peat-building was tightly coupled with sea level rise over the Holocene and allowed Caribbean mangroves to persist. Ezcurra et al. (2016) confirmed these findings in their study of mangrove peat remains. The peat was mostly composed of root fragments and was shown to have accumulated in response to sea level rise. However, none of these studies explained how the peat formed. It is often assumed that the SOM decay rate is reduced with sea level rise, resulting in peat accretion. In contrast to this assumption, I found that SOM decay does not decrease with sea level rise. If SOM decay does not reduce with sea level rise, root production needs to increase so that SOM can accumulate at higher rates and build up soil.

Root production is likely to have a role in the persistence of mangroves against sea level rise, but as stated earlier, only a few studies have quantified mangrove root production. In addition, concurring strands of evidence support the idea that mangrove root production will be affected by the interactions of multiple global changes (e.g. eutrophication, reforestation, $CO_2$ fertilisation) in addition to sea level rise (Adame et al., 2014; Castañeda-Moya et al., 2011; Cormier et al., 2015; Naidoo, 2009; Poungparn et al., 2016; Reef et al., 2016; Torres et al., 2019). For instance, I showed that fine root production strongly varies following mangrove reforestation (chapter 3), but the response of fine root production in stands of different ages to other global changes (e.g. sea level rise and warming) remains unexplored. Therefore, forecasts of mangrove persistence in the face of sea level rise by SOM accumulation will benefit by accounting for mangrove stand age; however, the response of root production to future environmental conditions (e.g. warming, $CO_2$ enrichment, sea level rise) also needs to be considered.

#### 6.1.2.4 Water transport in mangrove soil and implication for soil carbon dynamics

Soil water content and movement influence the belowground carbon cycle by changing the geochemical environment (such as redox conditions) and allowing the transport of dissolved carbon offshore (Spivak et al., 2019). For example, mangrove soil water drainage might lead to large losses of mangrove soil carbon through lateral flow (Taillardat et al., 2018) or through an increase of SOM decay (see chapter 4). Animal burrows (e.g., those of crabs) have been suggested to increase the drainage of the mangrove sediment matrix (Alongi, 2014). However, in chapter 5, I challenge this conceptual understanding, since I show that no, or very little, water seepage occurred from the sediment matrix to the burrows. Those results support evidence from previous studies showing that the groundwater flow is likely restricted to the macropores created by animal burrows (Schwendenmann et al., 2006; Stieglitz et al., 2013; Tait et al., 2016). The limited interaction between the sediment matrix and the burrows may be explained by the fine-grained sediment making up the matrix having a low permeability as described by the model of Armstrong & Arrowsmith (1986). However, I show that if burrows are in more permeable sediments (with larger grain size), then flow might occur between both domains as suggested by Alongi (2014). My work reveals the importance of mangrove sediment size distribution for the transport of water in mangrove soils. Those results are important because future sea level rise might lead to an increase in the particle size of mangrove sediment (Sanders et al., 2012), and thus an increase in the lateral transport of water, which is a major control of mangrove belowground carbon dynamics (Spivak et al., 2019).

My findings have implications for understanding biogeochemical cycles, in particular the carbon cycle, in mangroves. I suggest that burrows act as a network of pipes transporting water in and out of mangrove soil, and that most of the sediment matrix remains constantly anoxic. If the model of Alongi (2014) had applied, the SOM decay rate would be high like that reported in chapter 4 under drought condition (e.g. soil water content was reduced under this condition). In contrast, my piping model implies continuous saturation of the soil matrix with water, and hence a low SOM decay rate, which is consistent with the reported high burial rate of carbon in mangroves (McLeod et al., 2011). Even though the quantity of dissolved inorganic carbon loss reported is large (Maher et al., 2013; Taillardat et al., 2018), its origin is likely to be restricted to burrow walls. Burrows are oxygenated at low tide and may act as hot spots of carbon mineralisation (Kristensen et al., 2012). Another source of dissolved inorganic carbon could be from the digested carbon contained in defecation pellets of crabs, or from collapsed burrows increasing the quantity of matter exported. This carbon lost from mangroves is not a net loss to the atmosphere, since it will be partly emitted back as $CO_2$ to the atmosphere, but also partly remain in the open ocean as dissolved inorganic carbon with a potentially long residence time (e.g. Emerson & Hedges, 2008; Maher et al., 2018).

## 6.2 Limitations

### 6.2.1 Limitations of the mesocosm approach to studying belowground carbon decay

In my mesocosm study, I revealed the effects of temperature and sea level change, and their interactions, on mangrove SOM decay. However, by using a simplified soil matrix (homogenised soil with no live fine roots), I excluded some important soil processes. For instance, animal burrows may result in aerated soil space. This aerated soil would have a different microbial community from that of anaerobic soil (Booth et al., 2019), and may, therefore, respond differently to temperature changes and sea level rise. However, in chapter 5, I showed that the aerated portion of the soil matrix is likely to be limited in most mangroves. I also excluded the influence of plant-mediated processes on SOM decay. Temperature change and sea level rise may increase fine root production, which in turn may affect SOM decay, as previously observed in a salt marsh (Mueller et al., 2016). For instance, an increase in fine root production may affect SOM decay by two processes. First, roots release oxygen, creating aerated pockets of soil which support aerobic respiration (Wolf et al., 2007). Under aerobic conditions, the decay of SOM is greater. Second, roots release exudates, which may prime SOM decay (Kuzyakov et al., 2000). A recent study in tropical peatlands revealed that root exudates increase soil carbon decay (Girkin et al., 2018). Finally, I investigated only two global change factors (across six treatments) while in reality many more are likely to interact to affect SOM decay and soil processes in the future (Rillig et al., 2019). To cite only a few, mangroves are experiencing pollution (e.g. by microplastics, nutrients, or antibiotics: Ivar do Sul et al., 2014; Reis et al., 2017; Zhao et al., 2019), atmospheric $CO_2$ enrichment and storm events (Ward et al., 2016). Multiple factors in combination can result in synergistic, antagonistic or additive effects on SOM decay, but these responses are hardly predictable with modelling, and have been shown to vary across different ecosystems (Jackson et al., 2016; Komatsu et al., 2019; Rillig et al., 2019). In addition, climate change and its impact are likely to modify the plant-soil interactions (Kuzyakov et al., 2000). Free-Air Carbon dioxide Enrichment of forests have shown that an increase of atmospheric $CO_2$ might increase rhizodepositions, which might accelerate soil carbon mineralisation (Pendall et al, 2004). Even old carbon that was thought to be protected showed to be sensitive to the decomposition induced by rhizodeposition (e.g. priming effect) (Kuzyakov et al., 2000; Keiluweit et al., 2015). Few ecosystems have been investigated, but it seems that ecosystems respond differently (Kuzyakov et al., 2000). In some sites, the rhizodepositions accumulate in the soil at greater rate than they prime carbon, and in other sites most rhizodepositions are mineralised and there is a net loss of soil carbon through priming (Kuzyakov et al., 2000). Further investigations on rhizodepositions are needed to assess under which conditions rhizodepositions result in net soil carbon priming or in net soil carbon accumulation.

### 6.2.2 Limitations of the field-based approach to studying belowground productivity

I showed that fine root production changes with mangrove age. This pattern was assessed by measuring root production over three months between the wet and dry season. Despite the relatively short measurement period, the large difference in fine root production between the sites, and the stable rate of root production throughout the measurement period, confirm the robustness of the results. However, it would have been interesting to measure root production for a full year, and to have included several sites across Southeast Asia, in order to evaluate whether there is a seasonal influence on root production and if this pattern varies across sites. This was not possible due to the time and resource constraints associated with my PhD. In addition, the sites studied were chosen to have the same tree genus, as well as similar flooding frequencies and durations. It might be the case that sites less frequently inundated or hosting other species would have different root production patterns (Ezcurra et al., 2016). The general trend of a belowground root production reduction with mangrove age might be similar, but the ages at which the mangrove stands reach maturity and senesce might differ, depending on the hydromorphological setting or tree genus. Finally, minirhizotron studies are highly time consuming. The images are analysed semi-automatically and this requires extensive processing time. This prevented me from analysing a large number of samples. Development of automatic image analysis could prevent this sample-size limitation.

### 6.2.3 Limitations of the field-based approach to studying the soil-water dynamics

I measured the soil-water content of the mangrove soils across plots with different animal-burrow densities. This approach provided a relatively high temporal and spatial resolution, with restricted soil disturbance. However, this investigation was restricted to the top 6 cm of the soil profiles. Despite those relatively shallow measurements, our findings might be generalizable to deeper layers, since the soil layer was homogeneous and dominated by clay (>1 m deep). In addition, I evaluated the influence of animal burrows on root-free soil; however, as explained in chapter 3, fine roots may occupy a large portion of the subsurface soil, and so further research will be required to evaluate the dual influence of burrows and roots on the drainage of the mangrove sediment matrix. Finally, I used a simple model, with no field calibration for the soil matrix. Confirmation of the modelling results using broader field observations would enhance this approach. For instance, it would have been interesting to compare measured drainage capacity in sandy sediments with modelled one. In addition, informing the model with real hydraulic conductivity measurements for each type of mangrove sediment matrix type could considerably increase the accuracy of our model, but this was prevented by the lack of measurements in the literature. However, the results from my field investigation in a clayey mangrove were in accordance with the modelling outputs, and clayey soil is the most common type of mangrove sediment matrix.

## 6.3 Further work required

I have provided key evidence of the belowground carbon dynamics of mangroves, and some of its drivers. There is now a need to consider the full complexity of mangrove soils and to scale up these findings by including other hydromorphological types of mangroves and other global-change factors (Ward et al., 2016). Making further progress would involve performing similar studies at different mangrove locations and moving this study from the laboratory to a controlled, field-based experiment. In the section below, I detail how I envisage the future of research into the belowground carbon and water dynamics in mangroves.

### 6.3.1 A mangrove free-air carbon dioxide enrichment (FACE) experiment using active warming and simulated sea-level rise

More in-situ research needs to be carried on the belowground carbon dynamics of mangroves under global change scenarios. Ecosystem-plot experiments using FACE and warming have indicated unexpected belowground carbon dynamics, notably in saltmarshes (Noyce et al., 2019). Those studies have considerably increased the understanding of soil carbon processes (Norby et al., 2004; Pendall et al., 2004), but they are not scalable to mangroves, and have potentially overlooked the greatest threat for mangroves — sea-level rise (Lovelock et al., 2015). One of the next steps is therefore to develop a multifactorial experiment involving manipulated sea levels, warming and FACE. Sea level could be manipulated using in-situ mesocosms, where the water residence time would be artificially increased with the help of a lock (Figure 6-1). This setup could be coupled with measurements of root production, SOM decay and soil-water dynamics.

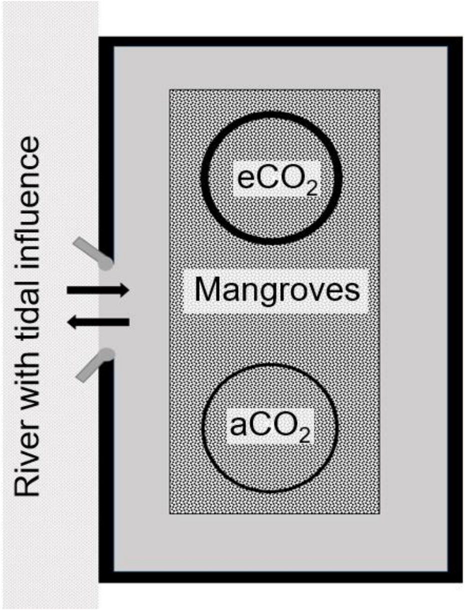

**Figure 6-1** Outdoor mesocosm experiment to simulate sea level rise and FACE for mangroves. Black arrows shows the water circulating between the experimental unit and the adjacent river. At the top and the bottom of the arrows are the lock doors. The doors of the lock can be closed before ebb tide to artificially increase the inundation time by retaining the water within the experimental unit. The circles represent the mesocosms, including one enriched with $CO_2$ ($eCO_2$) and one under ambient $CO_2$ ($aCO_2$). This outdoor mesocosm experiment can be upgraded by including active warming and ambient conditions within each mesocosm.

### 6.3.2 Studying SOM and moving to in-situ approach

Future studies should investigate whether mangrove plants mediate the response of SOM decay to temperature and sea-level rises, as has been reported for saltmarshes (Mueller et al., 2016; Wolf et al., 2007). For that, the impact of both fine root exudates and fine root oxygen release on SOM decay would need to be evaluated. Since root exudates and root oxygen release might interact with other factors, this approach would be more robust if applied under controlled field-experiment conditions, such as those described in section 6.3.1. The impact of live roots on SOM matter decay can be quantified by using carbon stable isotopes allowing us to trace the source of $CO_2$ emitted from the soil and to differentiate between the plant- and SOM-derived $CO_2$ production (e.g. $\delta^{13}CO_2$). Those measurements could be related with measurements of the release from fine root of oxygen, $CO_2$ and exudates to examine their effect on SOM decay. $CO_2$ and oxygen can be quantified at the level of the roots using optode cameras (Figure 6-2) (Koop-Jakobsen et al., 2018; Santner et al., 2015), and root exudates at several depths using micro-suction syringes (Figure 6-2) (Dessureault-Rompré et al., 2006). Those four measurements could be done within tailored rhizoboxes (similar to a minirhizotron but larger and rectangular, Figure 6-2), installed in controlled field-experiment conditions (section 6.3.1). The equipped rhizoboxes are likely to reveal how fine roots modify the soil conditions and how they affect the SOM decay under current and future environmental conditions.

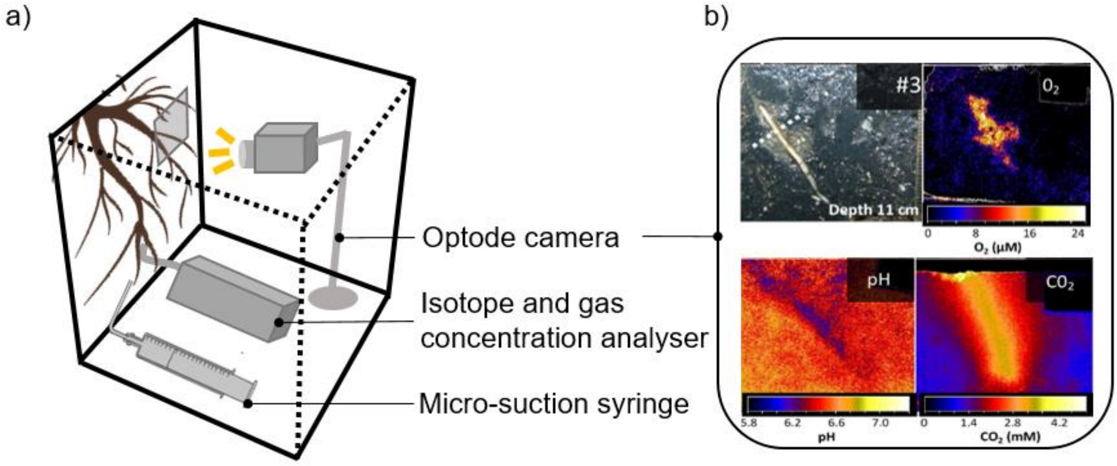

**Figure 6-2** a) Rhizobox equipped with a micro-suction syringe to extract and quantify root exudates, an isotope and gas concentration analyser to measure the $\delta 13CO_2$ and $CO_2$, and an optode camera, which is imaging the optode foil for $O_2$, $CO_2$ and pH. b) optode foils imaged with an optode camera in a saltmarsh from Koop-Jakobsen et al., 2018.

### 6.3.3 Studying fine root production at several sites using fine-scale spatial resolution of biotic and abiotic factors

Only a few studies have investigated the fine root production in mangroves, but much more data are needed to draw conclusions on the factors controlling the variation of fine root production spatially and temporally. Future root production studies should focus on the influence of other important global-change factors, such as rises in sea-levels, temperatures and atmospheric $CO_2$, all in relation to surface elevation changes of mangrove soils. Such an investigation would preferably be carried out on an outdoor mesocosms experiment, such as the one described in section 6.3.1. Minirhizotrons could be used to track the root dynamics, and the processing of the images could be automated. To reveal control factors of fine root production at the local scale, the measurements of fine root production in the soil profile could be coupled with non-invasive investigations of biotic and abiotic factors, such as nutrient concentrations (e.g. with an optode camera). Ideally, this approach would be complemented with globally-distributed fine root growth sampling plots, equipped with surface elevation tables (method to measure sediment accretion and belowground subsurface change: Callaway et al., 2013) allowing for a robust forecast of the resilience of diverse mangrove types. Surface elevation tables have been already installed in several mangroves worldwide (Lovelock et al., 2015), and such a network could be used for measuring fine-root production with minirhizotrons.

## 6.4 Outlook for mangrove restoration

In response to the possibility of a world without mangroves, several mangrove conservation organisations have set the goal to increase mangrove cover by over 20 % by 2030 (Lee et al., 2019; Lovelock & Brown, 2019). As a result, mangroves have been reforested at a fast pace as outlined section 1.1.1. However, these large investments in reforesting have not resulted in a significant increase in mangrove areas in the long term (e.g. a lot of trees planted are now dead), nor in restoration of mangrove functioning (Lee et al., 2019). Here, I will address some of the reasons for these failures and assess whether the scientific knowledge of mangrove restoration is sufficient to support restoration efforts, and how my results can contribute to the improvement of mangrove restoration guidelines.

Most of the reforestation projects have consisted of planting propagules of the *Rhizophora* genus. However, plantings have often failed because the environmental conditions are unsuitable or have not been restored to enable seedlings to develop. For instance, a large number of projects have planted *Rhizophora* propagules in lower intertidal areas, despite their hydrological conditions being unsuitable. Several projects did not remove environmental and socio-economic stressors (e.g. altered hydrology and land-use and land-tenure issues) preventing mangrove establishment in the long term (Lee et al., 2019; Lovelock & Brown, 2019). Reasons are multi-faceted, but cannot be fully attributed to a lack of scientific knowledge.

Science-based protocols, in particular the Community-Based Ecological Mangrove Restoration (CBEMR) programme, have been developed with detailed steps and a monitoring strategy. The CBEMR provides scientific guidance on how to conduct projects to restore mangrove functioning (Figure 6-3), and has led to several successful mangrove restoration projects (Bosire et al., 2008). However, this protocol has not been used widely because the funding to restore mangroves is short term, and the funding agencies have set project evaluation metrics favouring the number of trees planted rather than recovery of mangrove ecosystem functioning or socio-economic benefits (Lovelock & Brown, 2019). The gap between funders' objectives and scientific evidence, and the lack of long-term funding are, therefore, likely to have induced large mangrove plantings without the aim of restoring the ecosystem functions and services.

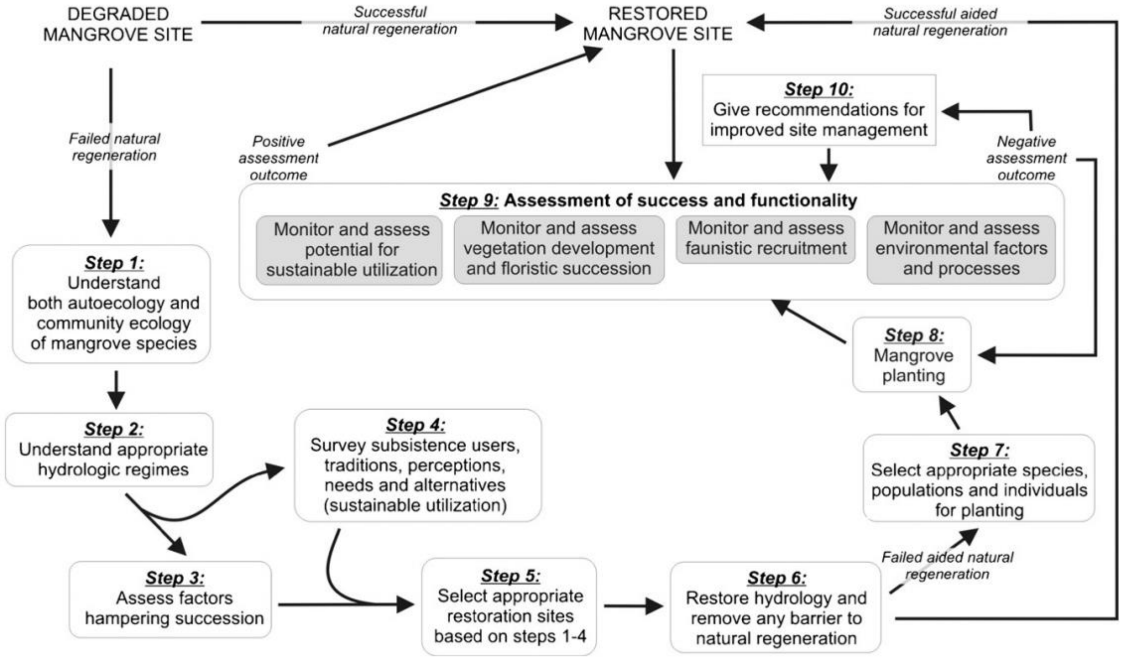

**Figure 6-3** Decision tree and recommended steps and tasks to restore mangrove (reproduced from Bosire et al., 2008).

CBEMR provides a strong theoretical basis for mangrove restoration, but it also has some limitations. CBEMR lacks empirical evidences and fails to address the impact of climate change. In my thesis, chapter 3 and 4 could contribute to the development of an improved guideline. The monitoring of mangrove developmental trajectories has been largely guided by theory (Duarte et al., 2015), because there are few observations on the recovery of mangrove ecosystems after restoration projects. In chapter 3, I show with empirical data the development of mangrove root production following mangrove restoration. I provide some evidences that after reaching maturity, the root production of mangrove might decrease. This belowground trajectory needs to be explicitly integrated to forecast the persistence of mangroves, as well as predict the carbon dynamics of restored mangroves over time. In addition, monitoring the development trajectory of newly restored mangroves could be used to generate assessment metrics of restoration. In chapter 2, I provide an open-source and affordable minirhizotron, which could be used for this purpose.

In addition, the CBERM has not addressed climate change and its impact while I show in chapter 4 that climate change will impact mangroves. Not all mangroves will resist sea level rise and cope with warming in the future. Specific guidelines should be given to prioritise mangroves likely to sustain these changes. In chapter 3 and 4, I provide some evidences to support this new guideline. In chapter 3, I show that with ages mangroves produce less roots, and might therefore accrete less soil and be less persistent to sea level rise. In chapter 4, I reveal no reduction of SOM decay with increased duration of inundation. Therefore, the prioritisation of mangroves for restoration should not be based on a potential reduction of SOM decay allowing mangroves to accrete soils and persist sea level rise. Low-lying areas prone to high sea level rise and increased inundation or submergence should not be a priority for mangrove restoration projects, if there is no evidence that mangroves can withstand these conditions. In addition, climate change will increase mangrove stress, such as temperature stress, which needs to be considered when choosing the location of mangrove restoration projects. I show that mangroves being less inundated are likely to be more sensitive to global warming, and likely to experience a greater increase of SOM decay (Chapter 4). This knowledge needs to be taken into account to forecast the persistence and the green-house gas emission of mangroves. In addition, previous studies have also shown that at 38- 40°C mangrove photosynthesis ceases (Ward et al., 2016; Ball et al., 1988), meaning that mangrove restoration in areas projected to reach those temperatures for a long period of the year might not be sustainable. The method of restoration should also be adapted to enable a degree of persistence to mangroves so that they can sustain future environmental conditions. At the same time, the scientific community needs to continue to investigate the impact of climate change on restored mangroves, and to study mangrove ecosystem development, especially belowground as outlined section 6.3. These pressing issues should be resolved collectively, with coordinated agendas and efforts, notably by i) using monitoring plots spread across biomes; ii) carrying out targeted experiments which can provide quick results on the impact of global change (e.g. mesocosm experiments, such as the one in chapter 4, and the one suggested in section 6.3.1); and iii) modelling mangroves as socio-ecosystems to predict future usages of mangroves, as well as delivery of ecosystem services.

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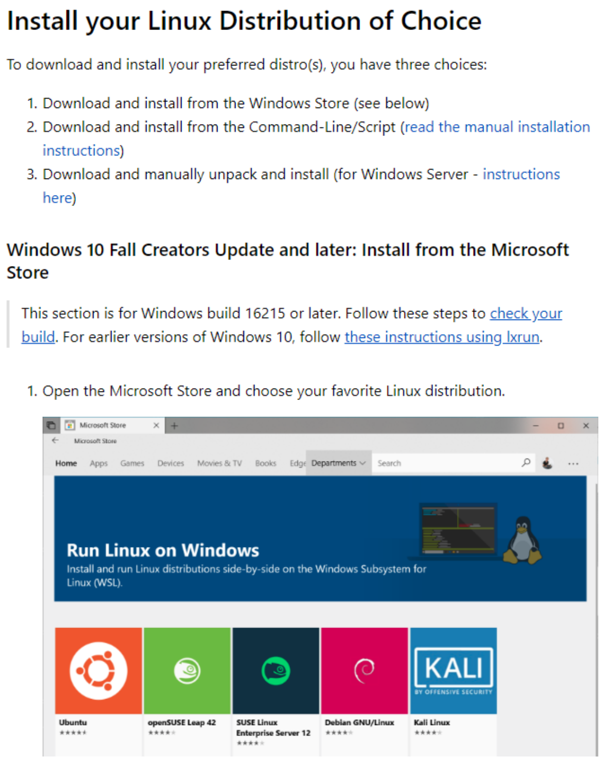
# Appendices

## A2.1 Running EnRoot's bash script on Windows 10

The bash script can be found in github: https://github.com/jonnyhuck/EnRoot

1. Install Ubuntu on Windows 10 as a windows subsystem for Linux. The instructions are here https://docs.microsoft.com/en-us/windows/wsl/install-win10
2. See also https://docs.microsoft.com/en-us/windows/wsl/user-support for information on setting up a Ubuntu user account and permissions.



### Install your Linux Distribution of Choice

To download and install your preferred distro(s), you have three choices:

1. Download and install from the Windows Store (see below)
2. Download and install from the Command-Line/Script (read the manual installation instructions)
3. Download and manually unpack and install (for Windows Server - instructions here)

**Windows 10 Fall Creators Update and later: Install from the Microsoft Store**

> This section is for Windows build 16215 or later. Follow these steps to check your build. For earlier versions of Windows 10, follow these instructions using lxrun.

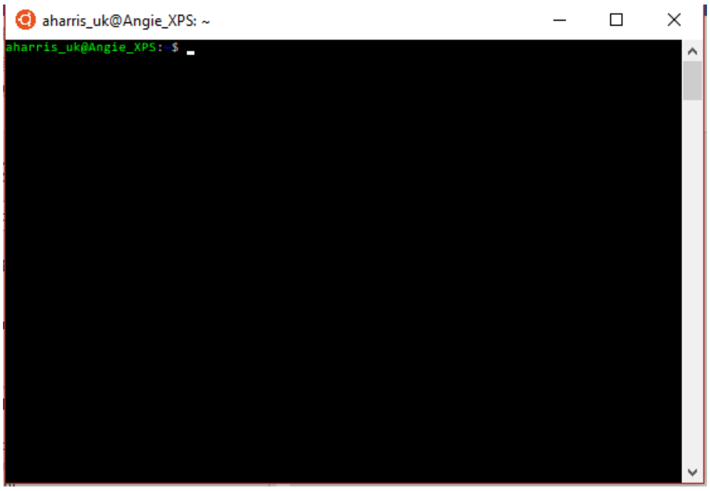
1. Open the Microsoft Store and choose your favorite Linux distribution.

**Run Linux on Windows**

Install and run Linux distributions side-by-side on the Windows Subsystem for Linux (WSL).

3. Install GDAL via Ubuntu. The instructions can be found here http://www.sarasafavi.com/installing-gdalogr-on-ubuntu.html

1. It should be possible to open Ubuntu from the Start menu in Windows 10 or by typing wsl in the Windows command prompt (or Windows PowerShell). The Windows shell can be used to control Ubuntu.



```
aharris_uk@Angie_XPS:~$ _
```

2. The following pages may be helpful for those not familiar with Linux commands
    - How do I access my C drive? https://docs.microsoft.com/en-us/windows/wsl/faq
    - Moving between directories etc https://help.ubuntu.com/community/UsingTheTerminal

3. Create a folder and place the EnRoot bash script and the images to be corrected in the same folder as each other. To make things as easy as possible, avoid long file pathnames (i.e., avoid putting the folder deep within the Windows system) and avoid using spaces when naming a folder. For example, a folder named ‘my fieldwork photos’ should be named my_fieldwork_photos with no spaces.

4. In the bash shell, navigate to the directory containing the new folder. Consider the example where the data have been placed in a Windows directory C:\Users\Angela\Documents. To get there in Ubuntu, type the address as

    ```
    cd /mnt/c/Users/Angela/Documents
    ```

5. Type pwd to see the file path or ls to see the list of files to check the directory is the correct one.

6. Now make the warp.sh script “executable” (i.e. enable it to run) by typing

    ```
    chmod +x ./warp.sh
    ```

7. Now the script can be run (again assuming that the user has correctly navigated to the directory where the files are located, as outlined in the steps above) using

```
./warp.sh
```

The script can be run each time new images are added to the directory (it is not necessary to install Ubuntu and GDAL again, or to make the script executable again).

The script can also be run directly from Windows without opening the Ubuntu shell. To do that, follow the instructions on this website

https://www.howtogeek.com/265900/everything-you-can-do-with-windows-10s-new-bash-shell/.

## 10. Results of the geometric transformation of EnRoot pictures

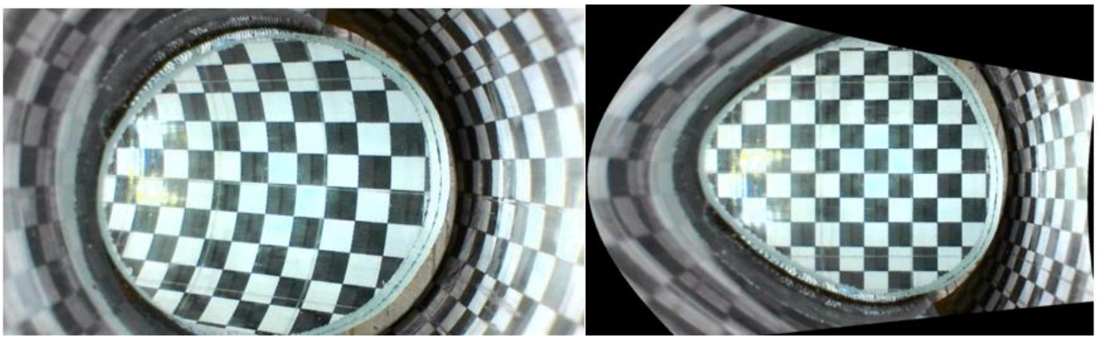
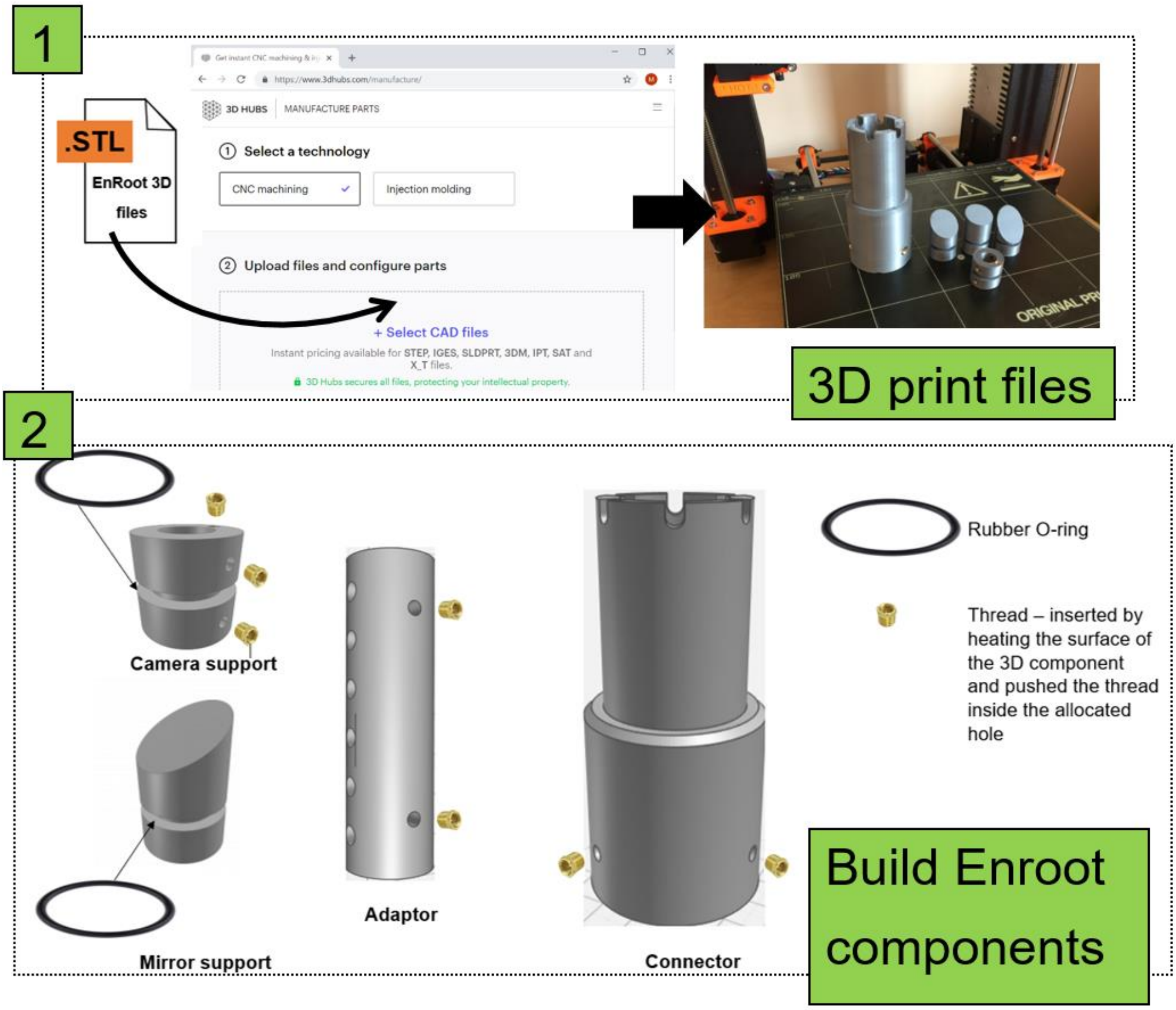


**Figure A2.1.1** An illustration of a captured image from a calibration grid before (left) and after (right) transformation using the EnRoot’s bash script.

### A2.2 Quick 3D printing guide

The 3D files can be found in:

https://springernature.figshare.com/articles/MOESM2_of_EnRoot_a_narrow-diameter_inexpensive_and_partially_3D-printable_minirhizotron_for_imaging_fine_root_production/9745988/1



Instant pricing available for STEP, IGES, SLDPRT, 3DM, IPT, SAT and X_T files.

3D Hubs secures all files, protecting your intellectual property.

Thread – inserted by heating the surface of the 3D component and pushed the thread inside the allocated hole

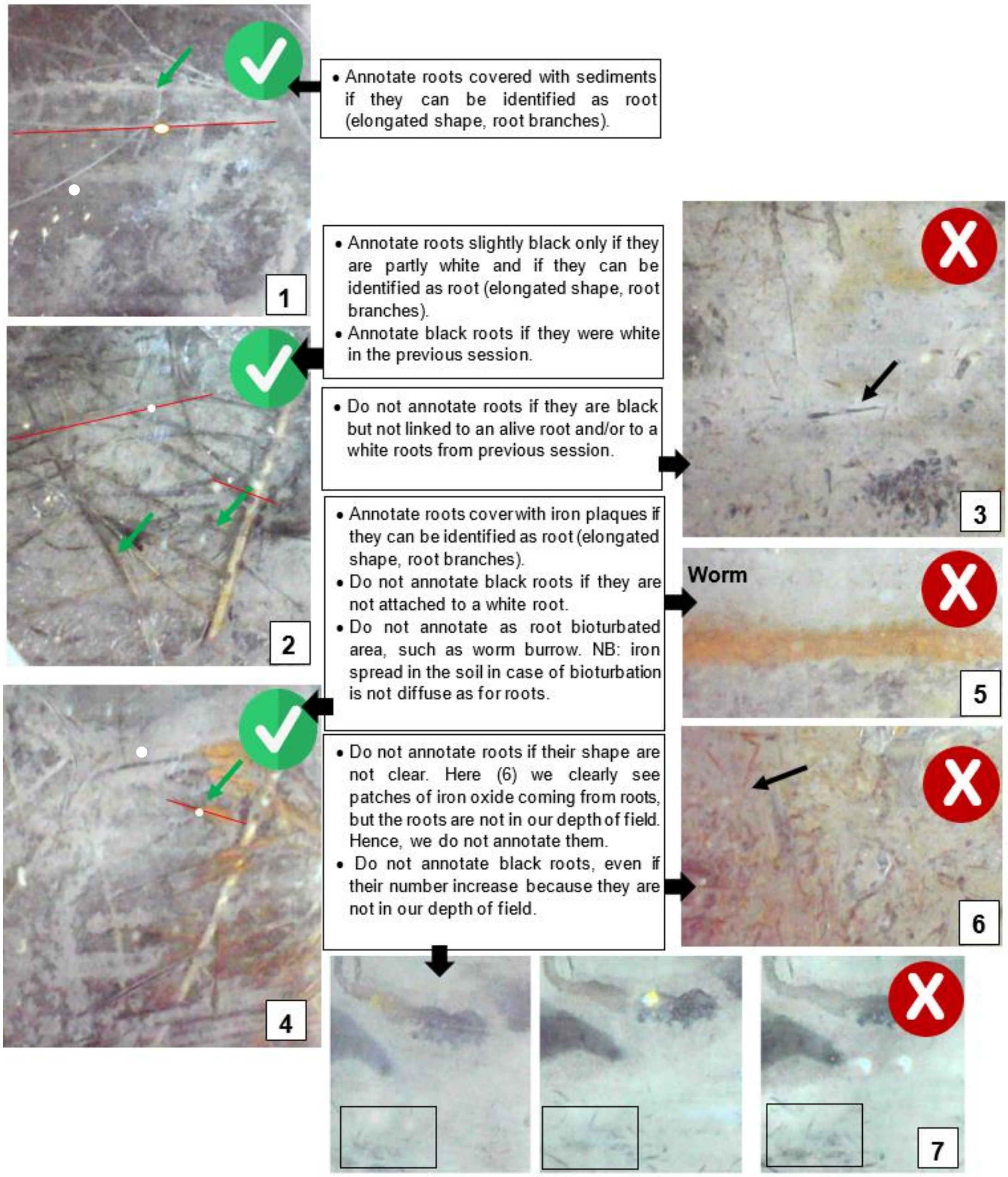
**Figure A2.2.1** Quick 3D printing guide**.**

## A2.3 Video of mangrove roots development recorded with EnRoot imaging device

The video can be found in:

https://springernature.figshare.com/articles/MOESM3_of_EnRoot_a_narrow-diameter_inexpensive_and_partially_3D-printable_minirhizotron_for_imaging_fine_root_production/9745994/1

## A3.1 Root identification protocol



- Annotate roots covered with sediments if they can be identified as root (elongated shape, root branches).

- Annotate roots slightly black only if they are partly white and if they can be identified as root (elongated shape, root branches).
- Annotate black roots if they were white in the previous session.

- Do not annotate roots if they are black but not linked to an alive root and/or to a white roots from previous session.

- Annotate roots cover with iron plaques if they can be identified as root (elongated shape, root branches).
- Do not annotate black roots if they are not attached to a white root.
- Do not annotate as root bioturbated area, such as worm burrow. NB: iron spread in the soil in case of bioturbation is not diffuse as for roots.

- Do not annotate roots if their shape are not clear. Here (6) we clearly see patches of iron oxide coming from roots, but the roots are not in our depth of field. Hence, we do not annotate them.
- Do not annotate black roots, even if their number increase because they are not in our depth of field.

**Figure A3.1.1** Root identification protocol

## A4.1 Full details of the analysis and the R scripts

The full script can be found here:

https://onlinelibrary.wiley.com/action/downloadSupplement?doi=10.1111%2Fgcb.14931&file=gcb14931-sup-0001-MaterialS1.html

## A4.2 Experimental set up, $CH_4$ fluxes and time series of mangrove SOM decay

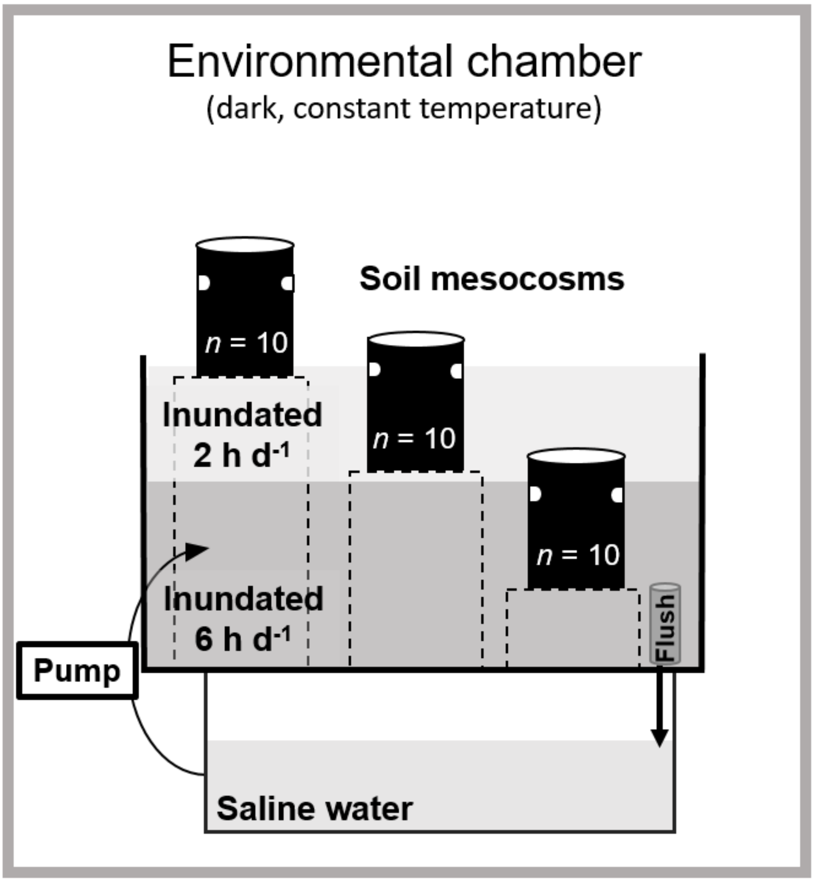


**Figure A4.2.1** Experimental set up in each of the environmental chambers.

**Table A4.2.1** Number of positive $CH_4$ fluxes per treatment (from $n = 296$). We defined positive fluxes as a $CH_4$ flux greater than zero and where variations in $CH_4$ concentrations in the mini flux chambers were above the error range of 0.03 ppm.

| Treatments | Positive fluxes |
|---|---|
| 27°C, 2 h $d^{-1}$ inundation | 0 |
| 27°C, 4 h $d^{-1}$ inundation | 0 |
| 27°C, 6 h $d^{-1}$ inundation | 0 |
| 31°C, 2 h $d^{-1}$ inundation | 0 |
| 31°C, 4 h $d^{-1}$ inundation | 0 |
| 31°C, 6 h $d^{-1}$ inundation | 4 |

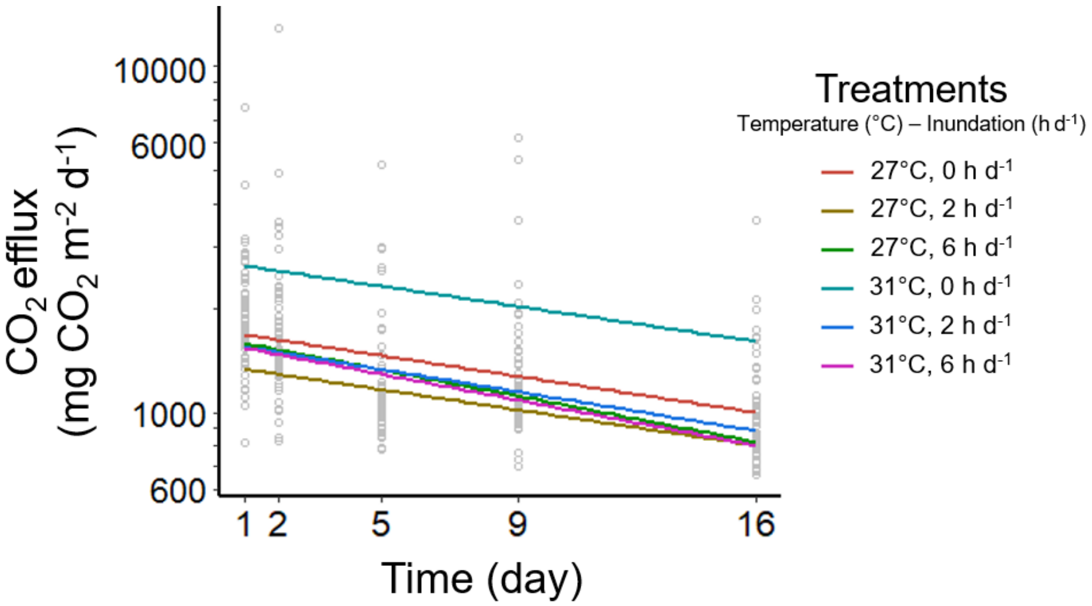


**Figure A4.2.2** Time series of mangrove SOM decay ($CO_2$ efflux) (day indicates sampling occasion after initial settling period) for all six experimental treatments. Hollow grey circles show individual measurements of $CO_2$ efflux. Coloured trendlines are regression lines between $CO_2$ efflux and time. Note logarithmic scale on vertical axis.

## A5.1 Modelling of water flow into an idealised burrow

A groundwater model was used to estimate the potential impact of crab burrows on the drainage of mangrove sediments. The model was constructed using the United States Geological Survey (USGS) groundwater modelling software, Modflow (or MODFLOW) 6 and is based on a generic model developed for pumping test analysis.

The model setup and results are accessed through an Excel spreadsheet to facilitate ease of model use. The original version of the spreadsheet model was developed for pumping test analysis and has been benchmarked against Theis (1935), which is an analytical solution for pumping test analysis of a confined aquifer under idealised conditions. Modifications were made to the model setup to allow it to represent unconfined conditions, and to allow a pressure head to be specified for the well instead of a pumping rate.

The model setup makes use of the Newton-Raphson formulation and upstream weighting capabilities of Modflow 6 which allow much better simulation of the partially-saturated conditions which can occur as water tables are lowered. The model also makes use of the unstructured mesh capabilities of Modflow 6 which allows a polar coordinate system to be defined, based on appropriate definition of the node dimensions and connections. Note that the best agreement to the Theis analytical solution was obtained when the node areas were calculated using a simplified approximate formula (centroid circumference multiplied by annular width) rather than more rigorous area calculations. The approximate formula was therefore used for the crab burrow simulations.

The model calculates groundwater heads for multiple distances from the well (burrow) and for multiple depths belowground surface for each time step. For the purposes of this study, a total of 167 model columns were used to represent different distances from the burrow (with higher resolution close to the burrow), 20 layers were used to represent different depths below the ground surface, and 22 time steps were used, with higher resolution during the early parts of the simulation. The overall dimensions and parameterisation of the model are as described in the main paper.

The groundwater flow equations have been solved using a modified version of the ‘complex’ solver parameters .The modifications were made to ensure that the solver produced well-converged results for the small-scales and specific ranges of parameter values of interest in this study. In all cases the cumulative water balance error for the model simulations was less than 1 %.

## A5.2 Crab burrows filling with tidal water from their base

The video can be found here: https://youtu.be/HzMimf8oY9I