Decomposition and soil carbon sequestration in mangrove ecosystems

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Decomposition and soil carbon sequestration in mangrove ecosystems

De gevolgen van eutrofiëring op afbraak in mangroven

(met een samenvatting in het Nederlands)

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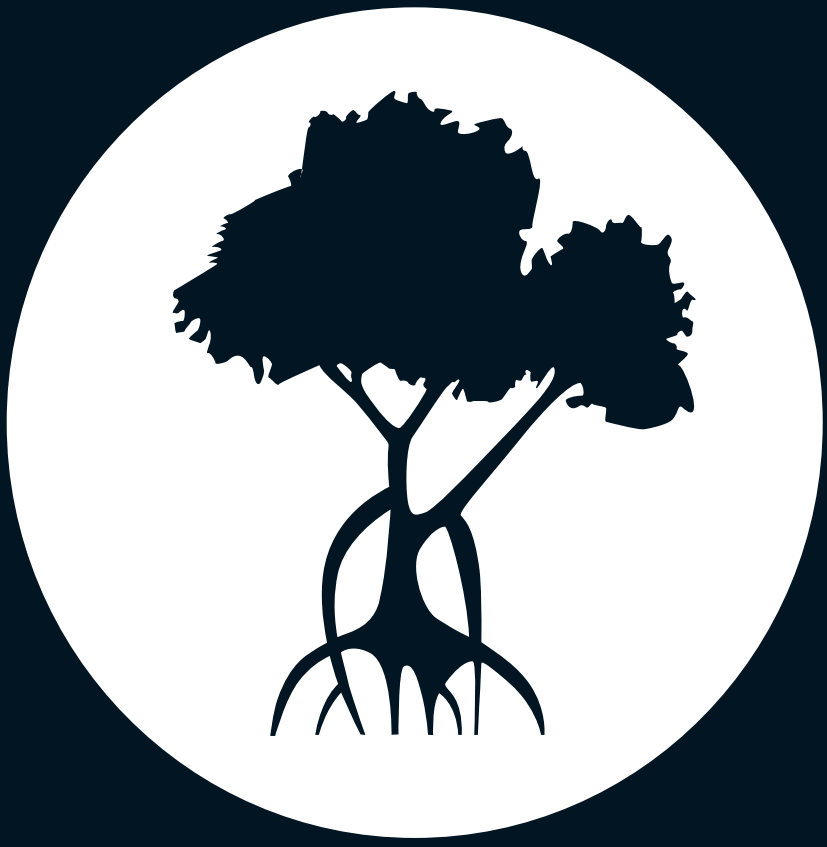

Chapter 1

General Introduction

1.1 Introduction

Mangroves are a heterogeneous group of woody plants that are able to grow in the saline, frequently flooded conditions of the intertidal zone. They are confined to the (sub)tropics, where they cover substantial parts of the coastlines, river mouths and oceanic islands (Fig.1.1). Mangrove forests are amongst the most productive terrestrial ecosystems (Kristensen et al., 2008) and are highly valued for their ecosystem services, such as coastal protection, wood production, sediment capture and increment of fish production. Due to their position at the interface of land and sea, mangroves play an important role in coastal nutrients and carbon exchange, acting as a buffer between terrestrial and marine ecosystems (Rivera-Monroy and Twilley, 1996; Wolanski et al., 2000; Valiela and Cole, 2002; Alongi and McKinnon, 2005).

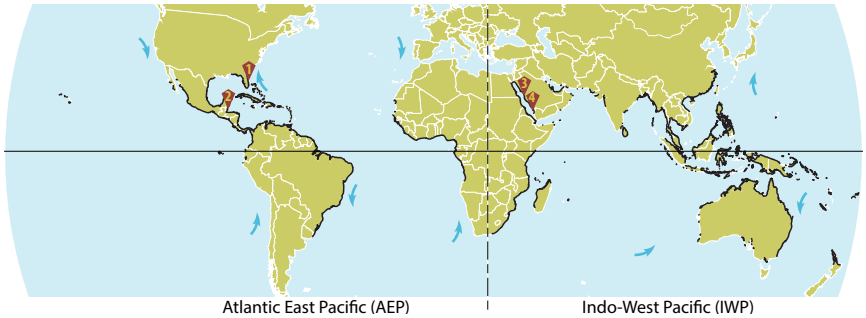

Figure 1.1: Mangrove distribution in the world as indicated by the black lines after Duke (1992), Spalding et al. (2010) and Saintilan et al. (2014). Mangrove distribution is confined to areas with water temperatures above 20 °C in winter (Duke et al., 1998). This roughly coincides with the tropics, but is modified by sea currents (arrows) (Duke et al., 1998). The distribution area is divided in two biogeographic regions separated by the pacific and the African continent as indicated by the dashed line. The Atlantic East Pacific region is markedly poorer in number of species than the Indo-West Pacific region (Duke, 1992) and only one mangrove species is found in both regions (Duke et al., 1998). The red diamonds indicate the field locations used in the present study: 1) North Hutchinson Island, Florida, USA; 2) Twin Cays, Belize; 3) Thuwal, Saudi-Arabia; 4) Farazan Islands, Saudi-Arabia

Mangroves sequester substantial amounts of carbon at a global scale, in spite of their relatively minor areal coverage. The high rates of primary production combined with low rates of decomposition in mangrove soils, result in the build-up of large quantities of soil organic matter (SOM) (Alongi et al., 2000; Kristensen et al., 2008), making mangroves amongst the carbon-richest forests in the world (Donato et al., 2011). Recent studies estimate the mean carbon burial rates of mangroves to be $163\,\mathrm{g\,C\,m^{-2}\,yr^{-1}}$ (Breithaupt et al., 2012), seven times higher than the estimated global average for peatlands (Yu et al., 2011). Although there are many case studies of mangrove SOM dynamics, a more fundamental understanding of the mechanisms involved in carbon sequestration and nutrient cycling is still lacking (Kristensen et al., 2008; Mcleod et al., 2011).

Due to coastal development, mangroves receive increasing amounts of pollutants and nutrients (Matson, 1997; Downing et al., 1999; Valiela et al., 2001; Erisman et al., 2013). Nutrient inputs are generally considered to be one of the lesser threats to mangroves, as they generally increase primary production (Lovelock et al., 2007; McKee et al., 2007; Naidoo, 2009) and mangroves even have been proposed to be suitable for wastewater polishing (Wong et al., 1997; Tam et al., 2009). However, nutrients potentially also increase decomposition rates of contemporarily and historically produced SOM, turning mangroves from sinks into sources of carbon. Suárez-Abelenda et al. (2014) found that nutrient-rich shrimp pond effluents decreased soil carbon content in Brazilian mangrove forests by more than 50%. Even if the average decline in soil carbon as a result of eutrophication would be much smaller, the resulting CO_2 efflux could be significant on a global scale. In addition, an increased turnover of organic carbon may disturb the resilience of peat-forming mangroves to sea level rise (Middleton and McKee, 2001), further accelerating mangrove habitat loss.

Despite the large potential effects, relatively few studies have addressed the effects of nutrient enrichment on decomposition in mangroves, and results are also context-dependent. Litter decomposition was found to increase after enrichment with either N (Huxham et al., 2010) or P (Feller et al., 1999, 2002). Wastewater effluent was found to increase soil microbial activity (Tam, 1998), and to decrease SOM content (Suárez-Abelenda et al., 2014). Holmboe et al. (2001) and McKee et al. (2007), in contrast, did not find an effect of either N or P amendment on SOM turnover. Apparently, predicting the consequences of nutrient enrichment on carbon sequestration in mangrove ecosystems requires a more mechanistic understanding of the way in which external disturbances affect internal nutrient and carbon processing by microbial decomposers.

1.2 From litter input to carbon sequestration — mechanisms and interactions

Organic matter inputs to mangroves comprise locally produced mangrove litter, algal or bacterial biomass, as well as organic material that was transported from nearby terrestrial and marine ecosystems (Kristensen et al., 2008; Bouillon et al., 2008b). Once incorporated, part of this organic matter is readily decomposed, while another fraction is sequestered in the soil as SOM. In mangroves the SOM concentration is highly variable: While on average mangrove soils are estimated to contain 2 % organic matter (Kristensen et al., 2008), oceanic mangrove islands build up layers of peat ranging up to 10 m in thickness (McKee et al., 2007). Biochemical recalcitrance of the organic matter, capability of decomposers to use organic compounds as resources, environmental factors modifying rates of decomposition (e.g. the availability of electron acceptors such as oxygen and nitrate) and physical protection of the organic matter (e.g. through burial by sediment) are important determinants of the fraction of organic matter input that is sequestered as SOM (Baldock et al., 2004). While some of these factors

such as temperature and pH are fairly stable throughout the biome, others vary widely within and between mangroves and may be modified by increased nutrient availability. Below I will discuss a number of factors which are important to consider when studying decomposition in mangrove ecosystems.

Carbon oxidation pathways

Heterotrophic decomposition involves oxidation of organic matter through a catalysis reaction with an electron acceptor. Although microorganisms are capable of using a great variety of electron donors, reactions involving molecular oxygen as electron acceptor (aerobic decomposition) are usually an order of magnitude faster than reactions involving alternative electron acceptors (anaerobic decomposition).

In mangrove soils oxygen is scarce, thus promoting anaerobic decomposition. The low diffusion rate of oxygen in water saturated soils and its high consumption in heterotrophic activity leads to suboxic bulk soil conditions, while only the upper few millimetres at the top of the soil are completely oxic (Kristensen, 2000). Aerating roots (Thibodeau and Nickerson, 1986) and crab burrows (Kristensen, 2008), facilitate oxygen transport down to deeper layers in the soil, exposing organic matter in deeper soil layers to oxic conditions. Additional oxygen transport to the bulk soil is mediated by tidal pumping: With rising tides, oxygen-rich water flows into the soil (Ovalle et al., 1990; Kostka and Luther III, 1995), while fresh air is sucked into the soil with receding tides (Colmer et al., 2013).

Despite the low influx of oxygen, about half of the decomposition in mangrove soils is aerobic (Alongi et al., 2000), while oxidation with iron and sulphate accounts for the other half of decomposition activity (Kristensen et al., 2008). As the bulk of SOM is exposed to anoxic conditions, a change in rates of anaerobic decomposition would have a dramatic effect on the turnover time of historically sequestered SOM. On the other hand, a large part of fresh organic matter input is initially exposed to oxic conditions, so that the rate at which aerobic decomposition takes place is an important determinant of SOM content as well. Aerobic decomposition is arguably more sensitive to exogenous pollution, as it occurs in soil layers which are more exposed to possibly polluted surface water and terrestrial runoff.

Exoenzymes

Microorganisms produce a wide array of enzymes, which are excreted into the environment to facilitate the depolymerisation of otherwise non-bioavailable macromolecular organic compounds (Vetter et al., 1998). These exoenzymes catalyse rate-limiting steps in decomposition (Sinsabaugh and Moorhead, 1994; Schimel and Bennett, 2004) and are a key factor in driving nitrogen and carbon cycling (Schimel and Weintraub, 2003). Most hydrolytic exoenzymes are substrate-specific, so that the activity of a specific enzyme can be linked to the rate at which product becomes available for microbial uptake.

Being exposed to the soil matrix, exoenzymes are relatively sensitive to the edaphic environment. Phenol oxidase, one of the few enzymes capable of breaking down important SOM constituents like lignin, requires elemental oxygen to func-

tion (Freeman et al., 2004). As a result, the decay of lignin and other phenolic compounds almost comes to a halt under anoxic conditions, thereby increasing SOM build-up.

Plants are capable of altering ecosystem scale nutrient fluxes by influencing exoenzyme activity (Kraus et al., 2003). Especially under oligotrophic circumstances, plants decrease nitrogen mineralisation rates through the production of exoenzyme-inhibiting polyphenolic substances such as tannins (Northup et al., 1995; Schimel et al., 1998; Bragazza et al., 2012). In mangroves, the suppression of exoenzyme activity by the production of (condensed) tannins may play a central role in decreasing leaching of otherwise mobile organic nitrogen by seawater (Maie et al., 2007).

Litter chemistry

Mangrove litter forms an important input to mangrove SOM, and its chemistry influences carbon sequestration in multiple ways. Firstly, there is a direct effect of litter chemistry on the decomposition rate and stabilisation of organic matter (Berg et al., 2003). Decomposition rate has a strong positive correlation with high initial nutrient and low initial lignin contents (Melillo et al., 1982; Prescott, 2010). Secondly, litter composition has an altering effect on turnover times of recalcitrant SOM (priming effects). These priming effects can be either positive or negative: An example of positive litter priming is the stimulation of energetically unfavourable, but N-releasing decomposition of recalcitrant SOM by the influx of energy-rich litter (Fontaine et al., 2003; Moorhead and Sinsabaugh, 2006). Negative litter-induced priming occurs when decomposition of SOM is hampered by litter-contained compounds such as tannins or other phenolic compounds. An extreme example of negative priming is found in northern peatlands, where the combination of anoxic conditions and high production of polyphenols brings the decomposition virtually to a halt, so that plant material accumulates as peat (Freeman et al., 2001). Negative priming by soluble tannins may also be responsible for peat formation in mangroves.

Microbial community: r- versus K-strategists

Broadly speaking, decomposing microorganisms can be divided into two functional groups: r-strategists consuming easily degradable material and K-strategists consuming recalcitrant SOM (Fontaine et al., 2003). The r-strategists depend on fresh organic material and have rapid growing rates and high turnover times. K-strategists, in contrast, are slow growing microorganisms with low turnover times, which invest a large fraction of their metabolic activity in the production of protective substances and exoenzymes. Unlike r-strategists, K-strategists are able to sustain themselves on SOM (Fontaine et al., 2003).

The more successful K-strategists are in competing with r-strategists for easily degradable compounds, the more they will dominate the microbial community and the faster decomposition of SOM will be. Alteration of the competition outcome between r- and K-strategists is the driving factor of priming effects (Fontaine et al., 2003; Chena et al., 2013) including those of nutrient enrichment and tannins.

Effects of nutrient enrichment

Mineral nutrient inputs affect litter and SOM decomposition by changing microbial biomass, enzyme activity and microbial community composition (Milcu et al., 2011). Hessen et al. (2004) note that external nutrient amendments can be expected to increase decomposition rates by compensating for the low nutrient content of litter as compared to microbial biomass. This is however not always the case. Especially the effects of mineral nitrogen amendment vary widely, and negative, neutral, as well as positive effects have been reported (Knorr et al., 2005). Generally, labile litter decomposition is stimulated by increasing nitrogen availability, while decomposition of more recalcitrant litter and SOM is inhibited (Knorr et al., 2005; Hobbie et al., 2012).

According to the nitrogen-mining theory presented by Moorhead and Sinsabaugh (2006), microorganisms have to invest in the energetically unfavorable decomposition of recalcitrant, nitrogen-containing organic matter to fulfill their need for N. Consequently, when nitrogen is externally added, these recalcitrant compounds are no longer decomposed. Similar negative effects of increased phosphorus amendment on decomposition are not expected, because recalcitrant organic matter does not contain phosphorus. On the contrary, phosphorus enrichment has been found to stimulate decomposition of recalcitrant litter (O'Connell, 1993). This effect may however rather depend on the induction of nitrogen stress than on the increased availability of phosphorus itself.

Mineral nutrient inputs also change the amount of litter that is produced, as well as its chemical composition. The carbon-nutrient balance hypothesis predicts a decrease of the concentration of carbon-rich secondary metabolites such as tannins when plants are no longer limited by nutrients (Bryant et al., 1983). This has indeed been confirmed by studies in various ecosystems (Entry et al., 1998; Bragazza and Freeman, 2007). In mangroves, tannin concentration (Lin et al., 2009) as well as C:N and C:P ratios of litter (Feller et al., 2007) has been found to decrease as a result of nutrient enrichment. Hence, using the same conceptual model, the litter-mediated effects of nutrient enrichment on microbial decomposition can also be predicted.

Both direct and litter-mediated effects on decomposition can be understood in terms of competitive interactions between r- and K-strategists (Chena et al., 2013), where the effect of amendments on the competitive outcome between those two functional groups determines the rate of SOM decomposition. These changes in decomposition rate are expected to be largest in the biologically active, oxic zones of mangrove soils. Especially during initial phases of decomposition, newly produced litter will first be exposed to aerobic decomposition, before being buried. Nutrient input from agricultural runoff or wastewater will preferentially become available to aerobic micro-organisms. Tidal pumping movement readily flushes the soil with surface water and increased nitrogen concentrations will therefore most likely be found in the top layers of the soil, which experience this pumping. Phosphate is mobile under oxic conditions, but precipitates under anoxic circumstances, preventing its movement to deeper layers.

Box 1.1: Mangrove genera - *Avicennia* and *Rhizophora*

A mangrove is a tree, shrub, palm or ground fern, generally exceeding one half metre in height, which normally grows above mean sea level in the intertidal zone of marine coastal environments, or estuarine margins (Duke, 1992). This taxonomically heterogenous group comprises 72 species from 20 families (Spalding et al., 2010). All mangroves possess adaptations to cope with saline and hypoxic conditions, although the ranges in which these stresses are tolerated vary widely (Ball, 1988).

Species of the genera *Rhizophora* and *Avicennia* are dominant and virtually ubiquitous within the complete area of mangrove distribution (FAO, 2007). The adaptations required to deal with high salt concentrations and waterlogged, reduced soil conditions have evolved independently in both genera, leading to functionally homologous but structurally different adaptations (Liang et al., 2008) (Fig. 1.2). The stilt roots of *Rhizophora* spp. and the pneumatophores of *Avicennia* spp. enabling the mangroves to transport oxygen to their subsurface root systems are a notable example of such adaptations. Due to the different root structure, soil redox potential is generally higher in *Avicennia*- than in *Rhizophora*-dominated forests (Thibodeau and Nickerson, 1986; Alongi et al., 2000).

Another major difference between the two genera is the decomposability of organic matter that they produce. Generally speaking, *Rhizophora* litter decomposes slower than *Avicennia* litter (Lacerda et al., 1995; Robertson, 1988; Middleton and McKee, 2001) as a result of a lower nitrogen content and a higher amount of tannins (Alongi, 1987; Robertson, 1988; McKee, 1995a; Alongi et al., 2005).

Due to these genus-specific traits, the amount of SOM sequestered in the stands below these genera as well as the effect of nutrient amendment on decomposition dynamics are expected to differ between *Rhizophora* spp. and *Avicennia* spp. stands.

Figure 1.2: *Avicennia germinans* (l) and *Rhizophora mangle* (r). Clearly visible are the salt glands of *Avicennia* and the stilt roots of *Rhizophora*, two typical adaptations to the frequently waterlogged, saline environment of the mangrove habitat.

1.3 Outline of this thesis

Mangrove forests are amongst the most carbon-rich forests in the world (Donato et al., 2011) and form a significant global carbon sink despite their low areal coverage (Breithaupt et al., 2012). As a result of ongoing coastal development, mangroves receive increasing amounts of nutrients contained in agricultural runoff and wastewater (Erisman et al., 2013). The nutrient loading of mangroves may change internal carbon and nutrient cycling and lead to the emission of substantial amounts of CO_2 on a global scale. Despite the large consequences, there is very little mechanistic knowledge of the interaction between wastewater influx and decomposition rate of litter and soil organic matter (SOM). Although wastewater fluxes have been shown to increase microbial biomass (MB), and to decrease SOM content (Tam, 1998; Suárez-Abelenda et al., 2014), it is not clear what drives these processes. This thesis deals with a number of experiments with mangrove soil and litter to shed more light on mechanisms behind differences in decomposition rate. A combination of field and laboratory studies was carried out to unravel the direct and indirect effects of nutrient loading on heterotrophic microbial activity in mangrove soils, and the resulting changes in decomposition rates. In addition to N and P amendments, the study focuses on labile organic carbon (LOC) as a modifier of decomposition processes as this common constituent of wastewater has large potential impacts on decomposition. The study involves comparative studies of *Rhizophora* spp.- and *Avicennia* spp.-dominated mangroves, two dominant genera with contrasting litter chemistry (Box 1.1). Several experiments were conducted with material collected at sites where long-term fertilisation experiments had revealed the role of nutrient limitation for the vegetation.

Within this framework, several research questions are addressed:

- Is decomposition in mangroves limited by energy or by nutrient availability?
- How does enrichment of labile organic carbon, nitrogen, and phosphorus modify decomposition rate of mangrove litter and soil organic matter?
- Is decomposition rate determined by the chemical composition of organic matter or rather by environmental effects such as hydroperiod and external nutrient availability?
- Is there a fundamental difference in decomposition dynamics below stands of *Avicennia* spp. an *Rhizophora* spp.?

After the more general introduction of the study in **Chapter 1**, **Chapter 2** explores the short-term effects of labile organic carbon and nutrient enrichment on microbial stoichiometry, biomass, and respiration rates in *Avicennia*- and *Rhizophora*-dominated mangrove forests of Saudi-Arabia. The use of short-term incubations allowed me to study the direct effects of nutrients and organic material on the microbial community, without the interference of indirect effects such as alterations in litter production by primary producers. The questions investigated were, whether microbial activity is either energy- or nutrient limited and whether short-term additions increase decomposition of historically sequestered SOM (so-called priming effects).

Chapter 3 focuses on long-term effects of nutrient amendments and the role of exoenzymes. I conducted this study in a highly organic soil from an oceanic mangrove island in Belize, where the quality rather than the quantity of soil organic carbon determines microbial activity. The site was subject to long-term experimental fertilisation with nitrogen or phosphorus. This facilitated the detection of long-term effects of nutrient input on decomposition dynamics driven by changes in input quality. In this study, microbial limitation was detected by assessing exoenzyme activity rather than growth in order to specifically study functional changes not associated with rapid growth and microbial community change.

Decomposition in a broader context is the focus of **Chapter 4**. This chapter functions as an intermezzo to the other chapters and describes a novel low-cost method to measure decomposition at a high resolution. The method was tested in a large number of ecosystems of different types, amongst which a mangrove forest in Florida. The outcome of this experiment gives an indication of decomposition rates in mangroves as compared to other terrestrial ecosystems.

Chapter 5 deals with the nutrient dynamics of litter decomposition in mangroves of Belize and Florida. It assesses whether chemistry of newly produced litter is altered through external nutrient enrichment, and whether the impact of such indirect, litter-mediated enrichment on decomposition differs from that of direct nutrient additions to the microbial community.

Another aspect of carbon sequestration in mangroves is explored in **Chapter 6**. In this chapter a mechanism for cyclic succession between *Avicennia germinans* and *Rhizophora mangle* is proposed on the grounds of a difference in decomposition dynamics of peat underlying these two species.

In **Chapter 7** the results found are discussed and placed in a broader context. This chapter finishes with an outlook and suggestions for further research.

Chapter 2

Nutrient amendment does not increase mineralisation of sequestered carbon during incubation of a nitrogen-limited mangrove soil

***Soil Biology and Biochemistry*, 57, pp. 822–829 (2013)**

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J.T.A. Verhoeven and M.M. Hefting

Abstract Mangrove forests are sites of intense carbon and nutrient cycling, which result in soil carbon sequestration on a global scale. Currently, mangrove forests receive increasing quantities of exogenous nutrients due to coastal development. The present chapter quantifies the effects of nutrient loading on microbial growth rates and the mineralisation of soil organic carbon (SOC) in two mangrove soils contrasting in carbon content. An increase in SOC mineralisation rates would lead to the loss of historically sequestered carbon and an enhanced CO_2 release from these mangrove soils.

In an incubation experiment we enriched soils from *Avicennia* and *Rhizophora* mangrove forests bordering the Red Sea with different combinations of nitrogen, phosphorus and glucose to mimic the effects of wastewater influx. We measured microbial growth rates as well as carbon mineralisation rates in the natural situation and after enrichment. The results show that microbial growth is energy limited in both soils, with nitrogen as a secondary limitation. Nitrogen amendment increased the rate at which labile organic carbon was decomposed, while it decreased SOC mineralisation rates. Such an inhibitory effect on SOC mineralisation was not found for phosphorus enrichment.

Our data confirm the negative effect of nitrogen enrichment on the mineralisation of recalcitrant carbon compounds found in other systems. Based on our results it is not to be expected that nutrient enrichment by itself will cause degradation of historically sequestered soil organic carbon in nitrogen-limited mangrove forests.

2.1 Introduction

Mangroves are highly productive ecosystems, growing at the interface of land and sea along much of the tropical and subtropical coastlines, estuaries and river mouths. Their position between land and sea makes them critical in land-sea nutrient exchange. Due to their high primary productivity and biological activity they can be considered a hotspot for nutrient and carbon cycling so that changes in their functioning will have a substantial influence on coastal nutrient and carbon dynamics.

Mangrove soils are a large sink for carbon with estimated average carbon burial rates three to ten times higher than those of northern peatlands (Duarte et al., 2005; Bouillon et al., 2008a; Limpens et al., 2008). Soil carbon content varies in mangroves, but many mangroves are peat-forming with peat layers up to several meters thick (Middleton and McKee, 2001; McKee et al., 2007) therefore containing significant amounts of carbon per unit of area.

During the past decades, nutrient influx to coastal systems has been increasing due to anthropogenic activity and could be considered a component of global change (Duarte, 2009; Nixon, 2009). These nutrient influxes affect several major processes in the mangrove carbon cycle, amongst which mangrove growth (Feller, 1995), peat build-up (McKee et al., 2007), and decomposition of leaf (Feller et al., 1999) and root litter (Huxham et al., 2010). Soil organic carbon (SOC) decomposition is potentially enhanced, changing mangroves from a carbon sink to a carbon source, especially if large amounts of historically sequestered carbon are mineralised. In peat-forming mangroves, this ultimately causes the system to collapse through elevational loss resulting in increasing inundation times and dieback of mangrove trees.

Nitrogen (N) and phosphorus (P) are the major limiting nutrients for mangrove tree growth (Reef et al., 2010) and their inflow rates to coastal waters have dramatically increased over the past decades (Seitzinger et al., 2010). The other macronutrients — potassium, calcium, magnesium and sulphur — are less likely to be limiting in a marine environment, as they are major constituents of seawater. We will therefore focus on nitrogen and phosphorus enrichment as a potentially moderating factor on SOC decomposition rates.

Additions of nitrogen and phosphorus stimulate plant growth if either of these are limiting. Likewise, nitrogen and phosphorus additions can be expected to stimulate decomposition when either of these elements is limiting microbial activity. Feller et al. (1999) indeed found enhanced litter decomposition after P addition in a P-limited mangrove. The effect of nitrogen on decomposition rate is however rather complex, since SOC mineralisation has been shown to be either increased, unaffected or decreased by addition of N. Many studies have revealed that decomposition of recalcitrant litter (Knorr et al., 2005; Berg and Laskowski, 2005) and SOC (Neff et al., 2002) is inhibited by external nitrogen addition, while the decay of easily degradable litter or labile organic carbon (LOC) is stimulated, as also predicted by the nitrogen-mining theory (Moorhead and Sinsabaugh, 2006; Craine et al., 2007). The net effect on total SOC is not always clear: Mack et al.

(2004) have demonstrated that long-term fertilisation in a tundra peatland leads to a dramatic loss of soil carbon through increased SOC decomposition rates while Shaver et al. (2006) find lower respiration rates in the same plots.

To our knowledge, the direct effects of nutrient and LOC addition on microbial growth and mineralisation rates in mangrove ecosystems have not been elucidated yet. In our research we assessed the effects on microbial growth and activity by measuring microbial respiration rates in soils from two common mangrove genera *Avicennia* and *Rhizophora* after amendments of nutrients and glucose. We expect that nitrogen addition increases microbial growth rate and LOC mineralisation in both genera, but that overall microbial activity is lower in the *Rhizophora* soil due high content of tannins, known to inhibit decomposition (Robertson, 1988).

The effect of nutrient additions on SOC mineralisation rates was studied more detailed in the *Avicennia* soil by measuring the change in microbial biomass and respiration upon nitrogen, phosphorus, and LOC amendment. Here we expect that the amended nutrients differentially modify LOC and SOC decomposition rates: nitrogen as well as phosphorus addition will stimulate decomposition of LOC, while nitrogen but not phosphorus will inhibit SOC mineralisation.

2.2 Material and Methods

Study site characteristics

The soils used for incubation were collected from mangrove stands dominated by either *Avicennia marina* or *Rhizophora mucronata* on Saudi Arabian islands in the Red Sea. The sampled *Avicennia* site was located on a small island just outside the campus of the King Abdullah University of Science and Technology (KAUST), near the village of Thuwal, Jeddah. The *Avicennia* covering the island varied in height, with tree sizes from ± 0.5 m in the dwarf zone up to 5 m in the fringe. The soil cores were taken in the western part of the island (22°19′52″N, 39°05′59″E) where tidal floods could freely enter. In this area, trees had an average height of 3.5 m.

The *Rhizophora* site was located at the Farazan Islands, an archipelago of coral islands in the southernmost part of Saudi Arabia. This group of islands probably supports the largest population of *Rhizophora* in the Red Sea (El-Demerdash, 1996). The soils were taken at the north-eastern part of Farazan Kebir, the largest of the Farazan Islands, at 16°47′24″N, 42°05′59″E. This site was protected from high-energy waves by a number of land-tongues. Average tree height in this stand was similar to that of the *Avicennia* site.

Soil collection and analysis

Within each study site, nine sampling locations were selected to account for small-scale variation. Soils were classified using the WRB soil classification system (IUSS Working Group WRB, 2007). At each location, eight 10 cm soil samples were taken using a stainless steel soil corer with a diameter of 9.6 cm. Directly after sampling, redox conditions were measured at 5 cm from the top of the core using a Sentix PtR electrode (WTW GmbH, Weilheim, Germany). Pore water

was sampled using 10 cm long Rhizon soil moisture samplers (Eijkelkamp BV, Giesbeek, the Netherlands). On the day of sampling, pore water pH was measured using a Sentix 41 pH electrode (WTW GmbH, Weilheim, Germany) and salinity was determined using an optical refractometer with automatic temperature correction. Pore water and soil samples were sent to the Utrecht University, The Netherlands, and stored at 4 °C until further analysis and incubation. The *Rhizophora* soils were sampled in November 2009; the *Avicennia* soils were sampled one year later in November 2010. Incubations started within five weeks after sampling. Bulk density of the soil was calculated from the core weight and volume of the corer. It does therefore include the effect of crab holes and other tertiary structures. Soil moisture content was determined by weight loss after a 48 h drying period at 70 °C. C/N ratios were determined using an EA/110 CHNS-O analyser (Interscience BV, Breda, The Netherlands). In preparation for the C/N determination soils were homogenised and ground using an MM200 mixer mill (Retsch GmbH, Haan, Germany) at 20 RPS during two minutes. After grinding, the soils were washed with a 32 % HCl solution to remove $CaCO_3$ and dried for 48 h at 70 °C to evaporate excess HCl. Measured C and N concentrations were corrected for weight changes due to the HCl washing. Pore water was analysed for PO_4^{3-}, NH_4^+, NO_3^-, dissolved organic nitrogen (DON) and dissolved organic carbon DOC using a continuous flow auto analyser (Skalar SA-40, Breda, The Netherlands).

Incubation experiment

As mangrove soils are oxygenated with fresh air when the tide lowers, while mangrove roots oxygenate their surroundings during high tides, incubations were conducted in atmospheric circumstances as we believe this most closely matches the prevalent conditions in the sampled top layer. Before incubation, soils were allowed to drain with gravitation to mimic field conditions just after a flooding event.

Collected soils were incubated to measure Substrate Induced Respiration (SIR) after Anderson and Domsch (1978) and subsequent growth respiration to calculate microbial growth rates. Respiration was measured after amendment with different combinations of glucose, ammonium, and phosphate to alleviate energy and/or nutrient limitations. Soil samples of both *Avicennia* and *Rhizophora* stands were subjected to five treatments: Control, glucose (C), glucose plus ammonium (CN), glucose plus phosphate (CP), and glucose plus ammonium plus phosphate (CNP). The *Avicennia* soil additionally received an ammonium plus phosphate (NP) treatment, without glucose. In preparation for the incubations, soil cores were manually cleared from roots and shells and homogenised. The homogenised soil was allowed to acclimatise for three days at 20 °C in a dark box covered with a moist cloth to minimise evaporation from the soil.

To start the incubations, 0.1 ml treatment solution per gram soil fresh weight (FW) was thoroughly mixed through the soil and 10 g of the amended soil was put in 600 ml flasks. D^+glucose was added as a source of labile organic carbon in the C treatment (0.6 mg C g soil FW^{-1}). In the CNP treatment, an equal amount of carbon was added together with 0.06 mg g soil FW^{-1} nitrogen (as NH_4Cl)

and 12 µg g soil FW^{-1} phosphorus (as Na_3HPO_4). Relative molar amounts of C, N and P were similar to that of aquatic microbial biomass (C:N:P= 50:10:1, Fagerbakke et al. (1996)) to ensure that microbial nutrient limitation was released. After amendment, soils were incubated for 6 days at 20 °C. During incubation, CO_2 production was measured at intervals of 130 minutes using a respirometer (Biometric Systems, Germany) equipped with optical CO_2 and O_2 sensors. The incubation flasks were flushed with fresh air whenever CO_2 levels exceeded 4.5 ml l^{-1} or O_2 levels decreased to less then 180 ml l^{-1}.

Additional to these treatments, we also incubated the above-mentioned treatments with 7.5 mmol g soil FW^{-1} commercially available tannic acid to mimic the soluble tannin concentration of mangrove soils as reported by Alongi (1987), in order to assess whether these inhibit microbial activity.

Fumigation-extraction

In order to determine microbial biomass, 10 g of soil was subjected to a fumigation-extraction procedure according to Vance et al. (1987). We followed this procedure at the start and after 56 h of the incubation. In short, the procedure consisted of a 24 h treatment with an ethanol-free chloroform atmosphere followed by extraction with 50 mL of a 0.5 M K_2SO_4 solution.

After the extraction, DOC and DON concentrations were measured in fumigated and non-fumigated control soils using a continuous flow auto analyser (Skalar SA-40, Breda, The Netherlands). Following Vance et al. (1987), microbial carbon was estimated by multiplying the amount of DOC liberated by fumigation by an empirically derived factor of 2.64 reflecting the relative amount of non-extractable to extractable carbon in microbial biomass. DON was also measured to quantify nitrogen fluxes and calculate C/N ratios of microorganisms. Following Brookes et al. (1985), we calculated microbial N as the difference in extractable DON before and after fumigation divided by 0.54. Only the *Avicennia* soils were subjected to the fumigation-extraction procedure as the necessary resampling for the *Rhizophora* soil was not logistically possible.

Data analysis & Statistics

CO_2 production rates increased exponentially after C amendment. The rate of exponential increase was quantified using a logarithmic growth function (Simkins and Alexander, 1984), by fitting the initial, rising part of the respiration peak to an exponential growth function $R_{CO_2}(t)$ analogous to Colores et al. (1996):

$$R_{CO_2}(t) = p \cdot e^{\mu_{max}(t-b)} \tag{2.1}$$

with p being the initial respiration rate, b the delay (i.e. lag time) before exponential growth starts and μ_{max} the relative growth rate. The fitting was done using a least squares fitting procedure in Mathematica 7.0.0 (Wolfram research, Champaign (IL), USA). For each plot, the initial respiration rate p was determined by first fitting the CO_2 respiration rate of the control treatment to a negative value for specific growth rate μ_{max}; the value found for p then was used as a fixed

parameter in the fitting procedure for the amended samples originating from the same plot.

Other values used for further analysis were obtained from directly measured respiration rates or cumulative respiration as calculated by integrating respiration rates to time. Initial microbial biomass carbon (C_{micr}) was determined following the relationship given by Anderson and Domsch (1978) recalculated to standard units:

$$C_{micr} = 81.84 \cdot R_C + 3.7 \tag{2.2}$$

with C_{micr} in µg microbial C per g soil DW, and R_C the average carbon respiration over the first 4 hours in µg g CO_2-C h^{-1} g soil DW. We measured basal respiration (BR) as an averaged respiration rate in soils without amendment over the first 24 h after the pre-incubation. The metabolic quotient (q_{CO_2}), i.e. the relative respiration rate for microorganisms, was calculated as BR/C_{micr} (Anderson and Domsch, 1985). Respiration quotient q_C, the amount of carbon respired per unit available SOC, was calculated as $BR/SOC\ content$ (Anderson and Domsch, 1986). Microbial respiratory quotient RQ was measured as CO_2/O_2 in mol mol^{-1} (Dilly, 2001) for both amended and unamended soils.

All data were analysed using R version 2.12.1 (R Core Team, 2010). Some outliers occurred in the fumigation procedure and short-term CO_2 measurements. These were identified manually and removed upon confirmation by Grubbs test. Homogeneity of variances was confirmed using Levene's test. Normality was tested with the Shapiro-Wilk test. Treatment effects were tested using an ANOVA on a linear model with replicate as a random factor to account for soil heterogeneity. Post-hoc testing for group differences was done with Tukey HSD for group differences where applicable. Differences between two groups were judged using Welch's t-test in case of normal distributions. The Mann-Whitney U test was used for group differences between non-parametric data such as redox conditions and temperature in Table 2.1. Experimental data were tested on measured values per unit fresh weight (FW) rather than units dry weight as we performed all experiments keeping units fresh weight equal between treatments. Values after ± indicate standard error.

2.3 Results

Edaphic properties and initial nutrient status

The *Avicennia marina*- and *Rhizophora mucronata*-derived soils were comparable with respect to pH, redox, temperature and salinity (Tab. 2.1). The *Rhizophora* soil was a black moderately organic silt loam, while the *Avicennia* soil consisted of coarse calcareous sand and contained little organic material. The difference between the soils was also reflected in the lower bulk density and nutrient content of the *Avicennia*-derived soil. The pore water phosphate and ammonium concentrations were three to eight times higher in the *Rhizophora* soil as compared to the *Avicennia* soil, as were the soil organic carbon and nitrogen contents. The N:P ratio in the pore water of *Avicennia* was 26, that of *Rhizophora* was 12. In both

the *Rhizophora* and *Avicennia* systems, surface water nutrient concentrations (*Rhizophora*: NO_3: $< 0.02\,mg\,l^{-1}$; NH_4^+: $0.1\,mg\,l^{-1}$; PO_4: $0.1\,mg\,l^{-1}$, *Avicennia*: NO_3^-, NH_4^+, and PO_4^{-3}: $< 0.02\,mg\,l^{-1}$) were lower than those of pore water (Tab. 2.1). Moisture content at the time of incubation was close to field capacity with $0.32 \pm 0.01\,g\,g^{-1}$ and $0.60 \pm 0.03\,g\,g^{-1}$ for the *Avicennia* and the *Rhizophora* soil, respectively.

Initial microbial biomass and activity

To compare microbial activity between the soils from the *Avicennia* and *Rhizophora* sites at the start of the incubations, we determined microbial biomass (C_{micr}), microbial respiration rate (BR), and metabolic quotient (q_{CO_2}) (Tab. 2.2). Microbial respiration and SIR estimated microbial biomass per gram soil were markedly higher for the *Rhizophora* than for the *Avicennia* soil (BR: $df = 6.7$, $p < 0.001$, C_{micr}: $df = 5.1$, $p < 0.05$); note that these differences are not significant when expressed per unit volume. The respiration per unit carbon in the soil (q_C) was significantly lower for the more organic *Rhizophora* soil ($df = 5.6$, $p < 0.005$), whereas microbial respiratory quotient (RQ) was for both soil types well below 1, which is the RQ expected for aerobic consumption of reduced carbon substrates without growth (Dilly, 2003).

Respiration response to glucose and nutrient amendments

Glucose additions induced a clear respiration peak between 10 and 40 h after amendment (Fig. 2.1: all treatments except Control and NP). The induced peak initially follows a curve similar to that for logistic growth, with an initial exponential growth phase followed by an exponential decrease of the specific growth rate to zero. This first exponential phase showed a good fit to the exponential growth rate function (eq. 2.1, $R^2 > 0.98$). μ_{max}, the microbial specific growth rate in this equation describes change in time and hence has unit h^{-1}. Unlike absolute measures such as respiration rate, μ_{max} is suitable to compare microbial growth rates between systems as it does not depend on weight or volume units and is therefore insensitive to differences in moisture content or bulk mass. Overall, μ_{max} is higher in *Avicennia* than in *Rhizophora* soil ($F = 67.0$, $p < 0.001$) (Fig. 2.2a; Tab. 2.3). Because there was a significant interaction effect, we analysed the treatment effect separately for the two soil types.

In the *Avicennia* soil, we tested if nitrogen plus phosphorus (NP) application, without glucose addition, would induce a respiration peak, but this was not the case; in fact, like in the control treatment, respiration just followed a negative exponential curve after amendment (Fig. 2.1: Control and NP treatments). Addition of both nitrogen and phosphorus together with glucose (CNP) increased μ_{max} significantly compared to glucose only (C) treatments in both *Avicennia* and *Rhizophora* soil.

Lower stimulatory effects on glucose-induced growth were observed with single nutrient additions (N or P) in both soils: phosphorus plus glucose additions (CP) did not increase μ_{max} as compared to the C treatment, while glucose plus nitrogen (CN) addition only increased μ_{max} in the *Avicennia* soil (Fig. 2.2a; Tab. 2.3).

Table 2.1: Edaphic and other site-specific properties as measured at two mangrove stands in Saudi Arabia (n = 9). Significant (quantitative) differences between sites are indicated with symbols (* $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$). Numbers after ± indicate standard errors.

Property	*Avicennia marina*	*Rhizophora mucronata*		Unit
location	Thuwal (SA)	Farazan (SA)		
soil type	Tidalic Gleyic Solonchaks	Tidalic Mollic Fluvisol		
texture	Sand	Silt		
tree height	3–4	3–4		m
pH	6.9 ± 0.1	7.0 ± 0.1		−
salinity	50 ± 0	46 ± 1	*	$mg\,g^{-1}$
redox	69 ± 53	-22 ± 20	*	mV
temperature	30.3 ± 0.2	32.9 ± 0.4	*	℃
bulk density	1.15 ± 0.11	0.50 ± 0.3	*	g soil DW cm^{-3}
SOC	18 ± 2	79 ± 6	***	mg g soil DW^{-1}
SON	1.4 ± 0.1	4.7 ± 0.2	***	mg g soil DW^{-1}
DOC	32 ± 3	27 ± 1		$mg\,l^{-1}$
DON	<0.2	<0.2		$mg\,l^{-1}$
NO_3^-	<1	<1		$\mu mol\,l^{-1}$
NH_4^+	13 ± 3	49 ± 9	**	$\mu mol\,l^{-1}$
PO_4^{3-}	0.5 ± 0.2	4 ± 1	**	$\mu mol\,l^{-1}$

SOC: Soil Organic Carbon, DOC: Dissolved Organic Carbon, SON: Soil Organic Nitrogen, DON: Dissolved Organic Nitrogen

Table 2.2: Microbial characteristics as measured in soils from two mangrove sites in the Red Sea dominated by either *Avicennia marina* or *Rhizophora mucronata*. Significant differences between sites are indicated in the first column ($^{*}p < 0.05$, $^{***}p < 0.001$). Numbers after ± indicate standard errors.

Property	*Avicennia*	*Rhizophora*		Unit
BR	1.7 ± 0.1	4.0 ± 0.3	***	µg CO_2-C g soil $DW^{-1}\,h^{-1}$
C_{micr}	228 ± 54	559 ± 95	*	µg C_{mic} g soil DW^{-1}
q_{CO_2}	8 ± 2	9 ± 1		mg CO_2-C h^{-1} g ${C_{mic}}^{-1}$
$C_{mic} : C_{org}$	12 ± 4	7 ± 1		mg C_{mic} g SOC^{-1}
q_C	104 ± 16	52 ± 4	*	mg CO_2-C h^{-1} g SOC^{-1}
RQ	0.49 ± 0.05	0.47 ± 0.04		mol CO_2 mol ${O_2}^{-1}$

BR = basal respiration, C_{micr} = SIR determined microbial biomass, q_{CO_2} = metabolic quotient, $C_{mic} : C_{org}$ = mass ratio of (SIR determined) microbial biomass C and SOC, q_C = relative carbon use, RQ = microbial respiratory quotient.

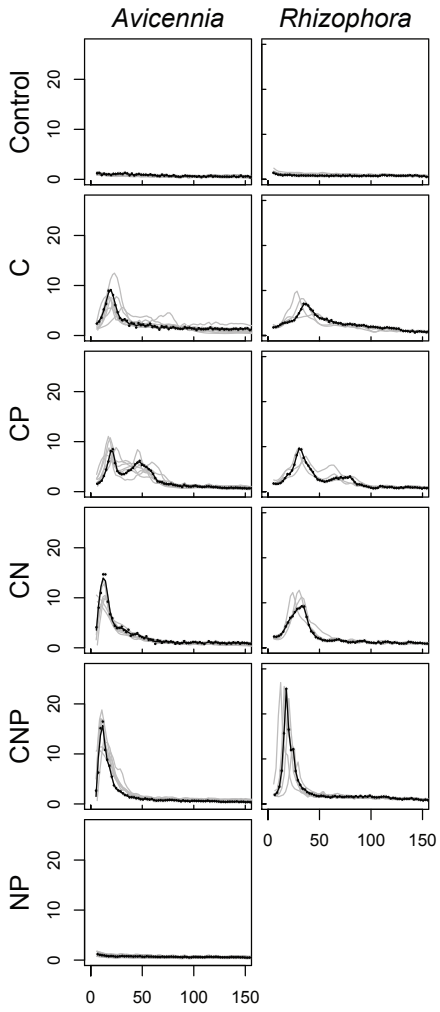

Figure 2.1: Respiration responses (µg CO_2-C h^{-1}) in time (h) of soils from *Avicennia marina*- and *Rhizophora mucronata*-dominated sites in the Red Sea after amendment of 0.6 mg glucose-C g soil FW^{-1} (C), ammonium (N) and/or phosphate (P) at $t = 0$. CO_2 concentrations are measured every 2 hours. In each panel, one of the five to eight replicates is plotted in black with its measured values shown as black dots.

Table 2.3: Experimental results of an incubation of soils from monospecific *Avicennia marina* and *Rhizophora mucronata* mangrove stands. Results are obtained during 150 h of incubation. The treatment consisted of a glucose addition (0.05 mmol C g soil FW^{-1}), with or without nitrogen (N) or phosphate (P). Relative amounts of C:N:P were 50:10:1. Both soil types were analysed separately by ANOVA with treatment as a fixed factor, significant differences are indicated by letters. For μ_{max} all differences indicated are significant at $p < 0.001$, for R_{cum} this is $p < 0.01$.

Species	Treatment	μ_{max}	R_{cum}
Avicennia marina	Control	-0.005 ± 0.000 [a]	0.18 ± 0.01 [a]
	NP	-0.005 ± 0.001 [a]	0.18 ± 0.01 [a]
	C	1.2 ± 0.01 [b]	0.61 ± 0.06 [bc]
	CP	1.3 ± 0.01 [b]	0.70 ± 0.03 [b]
	CN	2.6 ± 0.02 [c]	0.59 ± 0.03 [c]
	CNP	3.8 ± 0.01 [d]	0.63 ± 0.03 [bc]
Rhizophora mucronata	Control	-0.088 ± 0.004 [A]	0.45 ± 0.03 [A]
	C	0.6 ± 0.1 [B]	1.15 ± 0.07 [B]
	CP	0.6 ± 0.1 [B]	1.23 ± 0.06 [B]
	CN	1.0 ± 0.2 [B]	1.20 ± 0.08 [B]
	CPN	2.8 ± 0.3 [C]	1.25 ± 0.11 [B]

After the exponential growth phase, respiration rates come to a maximum rate R_{max} after which respiration declines again. R_{max} is equivalent to the inflection point of a logistic growth curve, where a limitation starts to reduce growth rates. The amount of CO_2 produced at R_{max} was about one-third higher ($df = 37.0$, $p < 0.001$) in *Rhizophora* (1.21 ± 0.06 mg CO_2-C) than in *Avicennia* with 0.83 ± 0.04 mg CO_2-C, but did not differ significantly between nutrient treatments.

In the CP treatment, a second respiration peak occurs consistently in both *Rhizophora*- and *Avicennia*-derived soil, this second peak is small as compared to the first one, but similar in shape (Fig. 2.1: CP). Such a second respiration peak is also observed in a number of *Avicennia* soil samples receiving the C treatment. The total respiration is elevated by glucose ($p < 0.001$). Within the glucose treatments, P has a stimulatory effect on total respiration ($F = 7.3$, $p < 0.05$).

The microbial respiratory quotient (RQ) over 150 hours was significantly lower for *Rhizophora* in comparison to *Avicennia* soil in all treatments (Fig. 2.2b; $F = 157.1$, $p < 0.001$). In the control treatment, RQ over the whole incubation was equal to initial values of RQ in *Rhizophora* soil, but it almost doubled to 0.83 ± 0.05 for *Avicennia* soil. Glucose addition elevated RQ significantly in both *Avicennia* ($F = 11.9$, $p < 0.01$) and *Rhizophora* ($F = 75.0$, $p < 0.001$) soil, but supplementary nitrogen or phosphorus amendment did not alter RQ significantly (Fig. 2.2b). Tannic acid addition slightly increased total respiration with 0.10 ± 0.02 mg CO_2-C g soil FW^{-1} in both *Avicennia* and *Rhizophora* soil ($F = 28.3$, $p < 0.001$), but did not modify μ_{max} in any of the treatments (data not shown).

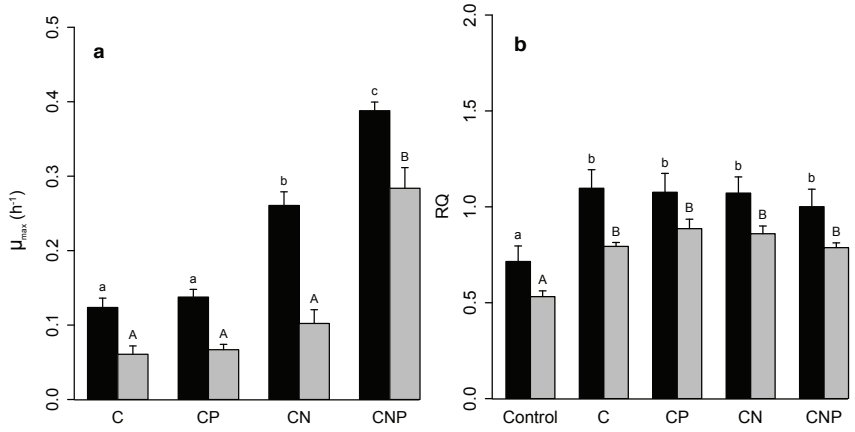

Figure 2.2: a) Microbial growth rate response and b) microbial respiratory quotient *RQ* (CO_2/O_2) of soils from *Avicennia marina*- (black bars) and *Rhizophora mucronata*- (grey bars) dominated sites in the Red Sea. Responses are measured after amendment with 0.6 mg glucose-C g soil FW^{-1} (C) in combination with ammonium (N) and/or phosphate (P). Bars represent standard errors. Letters indicate differences between bars. All differences indicated are significant at $p < 0.001$, differences between species are significant at $p < 0.001$ in panel a and $p < 0.05$ in panel b.

Carbon and nitrogen budget

We accounted for all net carbon fluxes during incubation by measuring changes in DOC, C_{micr}, and cumulative respiration losses over the incubation period (Fig. 2.3a). Similarly, mineralisation of soil organic nitrogen can be quantified by measuring changes in nitrogen pools (Fig. 2.3b) and correcting for nitrogen amendment where needed. We did not measure N_2O and N_2 efflux through denitrification, as earlier tests on these soils showed that gaseous nitrogen loss was negligible under the experimental conditions (data not shown). For both carbon and nitrogen the only unknown pool is soil organic matter, so that all changes in total pool can be attributed to either experimental addition or decomposition of soil organic matter.

In the treatments where no glucose was added, C_{micr} slightly decreased while DOC concentration remained the same. Respiration slightly exceeded the loss of microbial carbon so that a net SOC mineralisation was observed (Tab. 2.4). In the glucose-amended treatments (C, CP, CN, CNP), carbon fluxes were markedly higher as a result of the added glucose: C_{micr} tripled and respiration almost quintupled. The DOC pool did not change during incubation with the exception of the C treatment where an increase in DOC indicated an incomplete consumption of the added glucose (Fig. 2.3a). To calculate SOC decomposition in these treatments, the amended glucose-carbon was subtracted from the total flux. In Figure 2.3a this is graphically shown by the horizontal bar: the lower border

Table 2.4: Microbial response to carbon and nutrient amendments in soils from an *Avicennia marina*- dominated system. Results are obtained after 80 hours of incubation at 20 °C followed by fumigation-extraction. The treatments consisted of glucose (C), ammonium (N) and phosphorus (P) additions in various combinations. Glucose addition was 0.05 mmol C g soil FW^{-1}, relative amounts were 50:10:1 for C:N:P.

Treatment	G_{eff}	C_{micr}	N_{micr}	C/N	SOC_{min}	SON_{min}
initial	—	202 ± 28	17 ± 2	11 ± 1	—	—
control	—	174 ± 51	15 ± 5	14 ± 5	69 ± 51	-1 ± 4
NP	—	216 ± 6	68 ± 17	3 ± 1	89 ± 51	5 ± 16
C	0.67 ± 2	621 ± 27	24 ± 5	22 ± 4	133 ± 66	6 ± 3
CP	0.49 ± 5	683 ± 114	37 ± 9	30 ± 7	233 ± 143	7 ± 4
CN	0.49 ± 3	617 ± 69	109 ± 7	6 ± 1	36 ± 76	34 ± 8
CNP	0.39 ± 3	508 ± 52	86 ± 12	6 ± 1	-28 ± 69	-3 ± 11

G_{eff} = growth efficiency (g C_{mic} g C consumed^{-1}), C_{micr} = microbial biomass C (µg C_{mic} g soil DW^{-1}), N_{micr} = microbial biomass N (µg N_{mic} g soil DW^{-1}), C/N = Microbial C/N ratio, SOC_{min} = mineralised SOC (µg C g soil DW^{-1}), SON_{min} = mineralised SON (µg N g soil DW^{-1}).

of this bar represents the amendment of glucose-carbon, the height of the bar indicates the SOC mineralisation in the control treatment for comparison.

We tested the effects of carbon additions with a one-way ANOVA on the control and the glucose treatment. Within each carbon treatment we tested for the effect of nutrient addition with a two-way ANOVA with nitrogen and phosphorus addition as main effects. Since these two comparisons are orthogonal, ordinary F-tests without corrections were used. There were no significant effects of P or C, but N did show a significant inhibition on SOC mineralisation ($F = 7.9$, $p < 0.05$) in the glucose-amended treatments. In Figure 2.4 we summarised the effects of C and N on LOC and SOC decomposition.

Growth efficiency (G_{eff}) (Δ microbial C/consumed C) indicates the amount of carbon needed to produce a certain amount of microbial biomass. Phosphorus and nitrogen additions additively lowered growth efficiencies (nitrogen: $F = 5.6$, $p < 0.05$, phosphorus: $F = 5.3$, $p < 0.05$) so that the C treatment had the highest and the CNP treatment the lowest growth efficiency (Tab. 2.4).

The nitrogen budget in Figure 2.3b shows changes in dissolved and microbial nitrogen pools during the incubation. In the nitrogen amended treatments DON + DIN as well as microbial N are clearly elevated indicating partial consumption of the added N. The relative amount of immobilised nitrogen (microbial N/(DIN + DON)) was increased by glucose addition ($F = 5.2$, $p < 0.05$). Microbial C/N ratios in the control treatments did not change significantly during incubation. The microbial C/N ratios in the other treatments were strongly determined by the ammonium and glucose additions and differed almost tenfold from 22-30 for samples receiving carbon without nitrogen to 3 for samples receiving nitrogen without carbon.

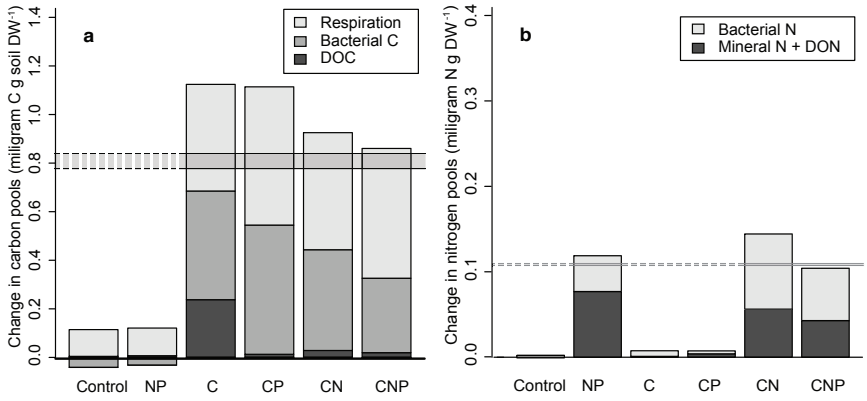

Figure 2.3: Total carbon (a) and nitrogen (b) budgets of soils from *Avicennia marina*-dominated sites after 80 h of incubation. Soils were enriched with glucose (C), phosphate (P) and ammonium (N) in various combinations. The horizontal bar shows the effect of amendment of C (a) or N (b) on the total budget. The lower border of this bar represents the direct effect of amendment; the height of the bar is the net budget change in the control treatment. N lowered total carbon mineralisation significantly compared with the C and CP treatments ($p < 0.05$, panel a).

2.4 Discussion

Basal nutrient status and microbial activity

The microorganisms in the studied mangrove soils are primarily energy-limited, even though a substantial amount of organic carbon is locked in the surrounding soil as SOC (*Avicennia*: 2 %, *Rhizophora*: 8 %). The high metabolic quotient (q_{CO_2}) reveals low energy use efficiency as compared to other terrestrial (Anderson and Domsch, 1993) or submerged soils (Torres et al., 2011). At the same time, heterotrophic activity in the *Avicennia*- and *Rhizophora*-dominated soils largely depends on the oxidation of refractory compounds as shown by low *RQ* values (Dilly, 2001). We therefore hypothesise that the microbial community of the studied soils are severely energy stressed and largely composed of K-strategists: slowly growing microorganisms oxidising mainly recalcitrant materials.

Differences in soil carbon dynamics were found between the two mangrove genera, despite comparable edaphic properties. In the *Rhizophora* site, mineralisation rates are twice as high as compared to the *Avicennia* soil, while carbon storage is four times higher. Assuming that the carbon sequestration rate is not negative at the moment of sampling, the input of carbon to the *Rhizophora* soil is therefore at least twice as high as encountered in the *Avicennia*-dominated system. Relative carbon use (q_C) and the microbial biomass per unit carbon, were lower in the *Rhizophora*-derived soil. This translates to a higher average residence time of carbon in the *Rhizophora* as compared to the *Avicennia* soil, and therefore a higher recalcitrance of organic matter in the *Rhizophora* system.

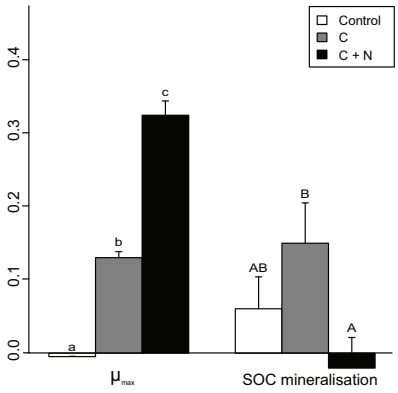

Figure 2.4: Relative microbial growth rates μ_{max} (h^{-1}) and net SOC mineralisation (mg g soil DW^{-1}) of soils from *Avicennia marina*-dominated sites receiving 0.06 mg g soil FW^{-1} of glucose in the C treatments and 0.06 µg g soil FW^{-1} of nitrogen in the N treatments. Significance was tested using ANOVA for C and N within C, letters indicate significant differences at $p < 0.05$.

This finding is in accordance to what is generally found for litter of these genera (Robertson, 1988; Sessegolo and Lana, 1991; Middleton and McKee, 2001) and is often ascribed to the higher tannin and lower nitrogen content of *Rhizophora* litter. Due to the larger carbon pool and higher recalcitrance, priming effects of labile organic carbon and inhibition of nitrogen on SOC decomposition are suspected to be stronger in the *Rhizophora* site.

Microbial growth rate, energy, and nutrient limitations

The hypothesised energy limitation for heterotrophic microbial activity was confirmed in the incubation experiment as adding labile organic carbon (LOC) induced exponentially growing respiration rates, indicating microbial growth at a constant rate μ_{max}. Nitrogen addition only increased respiration rates when added in combination with LOC (in *Avicennia*) or in combination with LOC and phosphate (in *Rhizophora*). This is in accordance to what could have been expected based on pore water N:P ratios, when assuming a microbial N:P of 10 as estimated for aquatic microorganisms by Fagerbakke et al. (1996) or 6-16:1 by Vrede et al. (2002) for marine bacteria. In future assays it would be worthwhile to assess if pore water nutrient concentrations are a good predictor of nutrient limitation for heterotrophic growth.

In all treatments, including those with tannic acid amendment, a lower μ_{max} and *RQ* was found in the *Rhizophora* soil as compared to the *Avicennia* soil. This is not thought to be caused by differences in LOC or O_2 availability between the two soils: μ_{max} does not decline with respiration rate, indicating that carbon uptake rate is limited by uptake capacity and growth and not by LOC availability

or diffusion rates. The *RQ* is never larger than one, showing that decomposition was aerobic. As the lower μ_{max} and *RQ* found for *Rhizophora* was independent of nutrient status or soluble tannin concentrations, the prevailing microbial communities must underlie this effect. The lower microbial growth rate found for *Rhizophora* indicates an even larger dominance of microbial K-strategists in these soils as compared to the *Avicennia*-derived soil.

A priming effect, a long-term change in decomposition rate due to a one-time addition of a resource, could not be confirmed in any of the treatments, as microbial activity did not reach equilibrium and still declined towards the end of the incubation period of 180 hours. Nevertheless, from our glucose-amended treatments it is clear that, when sufficient energy is supplied, nitrogen addition increases microbial growth rates and LOC mineralisation in microbial communities of *Avicennia* and *Rhizophora* mangrove soils. During continuous loading with LOC and nitrogen, which would be a realistic scenario for pollution by wastewater, we expect these dynamics to be the same. Whether this has consequences for the SOC pool was assessed for the *Avicennia*-dominated soil where we measured changes in microbial biomass, DOC and CO_2 efflux as well as soil nitrogen pool.

Carbon and nitrogen budgets

For the *Avicennia*-derived soil, a carbon and nitrogen budget was constructed by measuring microbial consumption, growth and respiration. In the glucose-amended treatment not all glucose carbon was consumed, confirming that heterotrophic activity was not energy-limited after amendment. Strikingly, both nitrogen and phosphorus increased LOC use, while at the same time decreasing growth efficiencies. The decreased energy efficiency suggests increased synthesis of energy-rich substances. Possible mechanisms include the synthesis of polyphosphate to store energy (Kortstee et al., 1994) in the P-enriched soils and an increased internal enzyme production in the nitrogen-enriched soils. A hint towards polyphosphate accumulation is the secondary peak in respiration consistently observed not only in *Avicennia*-but also in *Rhizophora*-derived soil after P addition (Fig. 2.1). This could be caused by delayed synthesis of polyphosphate as an energy store, but the results do not provide a definite answer as to which mechanisms are involved.

Added nitrogen was readily absorbed by microbial biomass, even in the absence of growth, and the soil microorganisms proved quite plastic with respect to their relative nitrogen content. Initially the microbial C/N ratio was 11; after N addition, when carbon was limiting the C/N ratio decreased to as low as 3. At that point the microbial C/N ratio is as high as that of proteins, so that a higher N content is unlikely to occur (Fagerbakke et al., 1996). In the C and CP treatments, where nitrogen was made limiting, C/N increased to 22-30, a value much higher than previously reported for marine bacteria (Vrede et al., 2002), indicating internal carbon storage as glycogen or some other polysaccharide.

While nitrogen in itself did not significantly stimulate or inhibit SOC decomposition in these systems, nitrogen amendment clearly inhibited SOC mineralisation when energy limitation was released. Our observations fit the nitrogen-mining theory (Craine et al., 2007), which states that under nitrogen limitation, recalcitrant material is broken down to obtain nitrogen, even though this is energetically not favourable. Whether the same suppression holds on larger timescales is subject to speculation, but given the larger fraction of r-strategists in the nitrogen amended soils and similar observations in long term laboratory incubations of Fontaine et al. (2004) and Yamasaki et al. (2011) on forest and savannah soils respectively, we do expect behavior on larger timescales to be the same.

Effects of nutrient enrichment on mangrove carbon sequestration
The microbial communities of mangrove soils form a large sink for carbon, while enhancing nitrogen availability through decomposition of soil organic matter. At the same time, the microbial community forms a large sink for exogenous nitrogen and most likely also phosphorus when these are available in higher concentrations. The size of this sink increases as long as there is a concurrent input of labile organic carbon. Although nutrients stimulate growth of microorganisms and increase the mineralisation rates of labile organic carbon, soil organic matter decay is not stimulated. It is therefore not expected that increased nutrient exposure of the microbial communities in mangrove soils will deteriorate existing soil organic carbon pools in mangroves.

Even more so, as primary production in mangroves is nutrient-limited while decomposition is energy-limited, there is a differential limitation between carbon production and carbon decomposition. This suggests an increased carbon sequestration rate of mangrove forests upon exposure to nutrients. From preliminary results (data not shown) it is clear that such an increase in primary production upon nutrient enrichment takes place in the *Avicennia* site from our research. Mangroves have been proposed to be usable as wastewater polishing facilities by a number of authors (Wong et al., 1997; Tam et al., 2009). One could speculate that this could work without losing carbon sequestration capacity: the nitrogen supplied increases primary production thus increasing carbon input to the soil, while excess LOC is readily decomposed by microorganisms. The extra microbial activity does not lead to CO_2 release from SOC decomposition as long as sufficient nitrogen is supplied.

Caution is required when extrapolating above conclusions up to the ecosystem scale, as soil-plant feedbacks were not included in this study. Reef et al. (2010) point out that nutrient enrichment potentially decreases primary production. In addition, increasing nutrient availability decreases tannin production (Lin et al., 2009) thereby potentially increasing decomposability. Moreover, relative root production also decreases with rising nutrient availability (Naidoo, 2009) while mangrove roots decompose slowly and are important in peat formation (Middleton and McKee, 2001). On the long term this may dramatically decrease the amount of sequestered carbon especially in mangroves depending on soil accretion through accumulation of dead root material.

2.5 Acknowledgements

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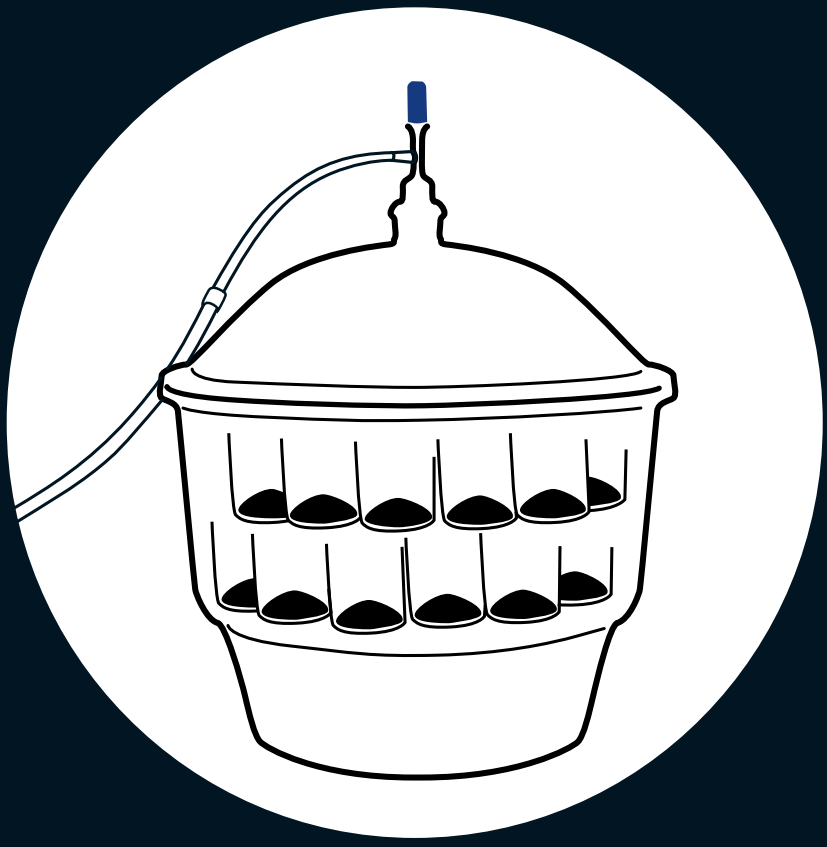

Chapter 3

Short- and long-term effects of nutrient enrichment on microbial exoenzyme activity in mangrove peat

Submitted

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Abstract Mangrove forests exhibit intense carbon and nutrient cycling with globally relevant rates of carbon sequestration. In oceanic mangrove systems, carbon deposition in peat layers is essential to avoid inundation due to sea level rise. In many parts of the world, mangroves are being exposed to increasing quantities of external nitrogen due to coastal development, and it is not known how nutrient cycling and carbon sequestration will be affected. We hypothesised that tannins induce a nitrogen limitation on microbial decomposition even when plant growth is limited by phosphorus, creating a situation of differential nutrient limitation between plants and microbial communities. To examine such differential nutrient limitation, we quantified the short- and long-term effects of nitrogen and phosphorus enrichment on microbial biomass and decomposition-related enzyme activities in a *Rhizophora mangle*-dominated mangrove, which had received fertilisation treatments for fifteen years at the time of this study. We compared microbial biomass, stoichiometry and potential enzyme activity in dwarf and fringe-type *R. mangle*-dominated sites, where primary production is limited by phosphorus or nitrogen depending on the proximity to open water.

Even in phosphorus-limited mangroves, microbial activity was nitrogen-limited. Such a differential nutrient-limitation between microbial decomposers and primary producers implies that the impact of eutrophication on carbon sequestration is nutrient-specific. In addition, this chapter shows that phenol oxidase activities in this system decreases through P, but not through N enrichment. Furthermore it is argued that the often used division between N-harvesting, P-harvesting, and C-harvesting exoenzymes needs to be reconsidered.

3.1 Introduction

Mangrove ecosystems are commonly found in tropical and subtropical coastal zones, where they are of major importance to local nutrient- and carbon-cycling (Alongi, 1996). Mangroves typically grow in the subtidal and intertidal zones with their lower range being imposed by the need to have their aerenchymatous roots exposed to the atmosphere during low tides (Ball, 1988). Therefore, in the absence of soil elevation, rising sea levels lead to dieback of mangroves. In systems where sediment input is not substantial, survival of mangroves depends mostly on the build-up of peat (McKee, 2011), resulting from the imbalance between primary production and decomposition. Nutrient enrichment in these systems can influence both these processes. Eutrophication, therefore, may have unforeseen effects on mangrove stability (McKee et al., 2007) leading to habitat loss if soil accumulation is negatively affected.

Decomposition is catalysed by several key exoenzymes that allow for extracellular conversion of complex organic matter into simpler products, such as glucose, amino acids, and phosphate. Mangrove litter, especially from *Rhizophora* spp., contains large amounts of dissolvable and non-dissolvable tannins (Alongi, 1987; Maie et al., 2006; Zhang et al., 2010). As protein-binding phenolic compounds, tannins inhibit microbial activity and lower nutrient mobilisation via substrate deprivation and enzyme inhibition (Schimel et al., 1996; Kraus et al., 2003; Joanisse et al., 2007). The immobilisation of exoenzymes in tannin-rich soils decreases their activity (Ximenes et al., 2011), resulting in reduced return of degradation products per unit of investment of nitrogen (N), carbon (C), and energy for producing exoenzymes. This induces energy and N limitation in microorganisms producing these enzymes, thereby suppressing microbial decomposing activity.

We hypothesise that decomposer microorganisms in mangroves are ultimately N-limited, due to tannin-protein complexation. If primary production is phosphorus (P)-limited, tannin-induced N limitation for microorganisms will lead to a differential nutrient limitation (DNL) for plants versus decomposing microorganisms. DNL would result in nutrient-specific biogenic controls of soil level in mangrove ecosystems. In such a system, enrichment in the plant-limiting nutrient (P) would result in net peat accumulation, whereas enrichment in the microbe-limiting nutrient (N) would result in net peat loss. The latter poses a large threat to mangrove systems on a global scale, because many coastal systems are experiencing increasing N inputs from anthropogenic activity (Howarth and Marino, 2006).

Earlier studies have attempted to quantify the effects of nutrient enrichment on peat decomposition in mangrove systems, using decomposition rates of roots (McKee et al., 2007) or tensile strength loss of cotton-strips (Feller et al., 2002) as indicators. These studies suggested that decomposition was either P-limited (Feller et al., 2002) or not sensitive to nutrient enrichment (McKee et al., 2007). However, (McKee et al., 2007) observe peat decline upon N fertilisation which is possibly attributable to increased decomposition rates (Lovelock et al., 2011).

A mechanistic approach that employs the measurement of both hydrolytic

and oxidative exoenzymes involved in the breakdown of organic matter provides a means to reconcile these seemingly contradictory results. Microorganisms catalyse the rate-limiting step in nutrient-cycling and decomposition of organic matter contained in peat (Sinsabaugh, 1994; Freeman et al., 2004) and their activities correlate with the rate of soil organic C decay and microbial nutrient demand (Sinsabaugh et al., 2009). Hydrolytic enzymes catalyse specific reactions and are frequently used to determine whether decomposition is limited by N or P (Sinsabaugh and Moorhead, 1994). Oxidative enzymes, on the other hand, are much less specific but show strong correlations with decomposition rate of recalcitrant organic compounds in peat (Freeman et al., 2004; Limpens et al., 2008).

To gain insight into the effects of nutrient-enrichment on enzyme mediated decomposition processes in mangrove systems, we used two complementary approaches: i) measuring the effects of long-term nutrient fertilisation in field experiments, and ii) tracking the short-term impact of nutrient enrichment in laboratory incubations.

The fertilisation field experiment allows one to examine the long-term, in-situ consequences of nutrient enrichment on enzyme activities in the context of complex interactions between microorganisms and plants competing for nutrients. The short-term amendments serve to assess the response to nutrient and C enrichment without the confounding effects of plant-soil interactions. These results can also be used as a benchmark to interpret the responses in enzyme activities to in the long-term fertilisation experiment. In addition to quantifying potential enzyme activities, we compared microbial biomass, elemental stoichiometry, and metabolic activity to test the hypothesis that tannin production induces a N limitation in decomposing microorganisms regardless of the limitations for primary production

The present study was conducted at Twin Cays, a mangrove-dominated oceanic island group in the Caribbean Sea, 16 km off the coast of Dangriga, Belize. The islands consist of mangrove-derived peat, which is up to 10 m thick (McKee et al., 2007). The dominant mangrove species on the island, *Rhizophora mangle* shows a clear zonation with respect to growth form and nutrient limitation: Trees on the inland parts of the island show stunted growth and are strongly P-limited, while trees are near the fringes of the island are much taller, and are generally N-limited, depending on proximity to open water (Feller et al., 2002).

Following from our hypothesis, we expected microbial decomposition to be ultimately N-limited in both the dwarf and the fringe zone, so that the dwarf zone has DNL, while the fringe zone has not. We expected the short-term response to nutrient amendments to reflect the direct effect of nutrients on enzyme production, which allows revealing any differences with the long-term effects to be attributed to plant-mediated influences such as changes in tannin production and litter quality change. The results from this study can be used to evaluate the use of enzyme activities to assess microbial nutrient limitations (Sinsabaugh et al., 2008), and to qualify the potential consequences of eutrophication with respect to peat decomposition in mangroves.

3.2 Materials and Methods

Study Site and field experiment

Soil and water samples were collected at Twin Cays, Belize (16°49′N, 88°06′W). An extensive description of the hydrology, climate, primary production, and soil properties can be found in (Feller, 1995; Feller et al., 1999, 2002; McKee et al., 2007; Lee et al., 2008; Feller et al., 2009).

At the moment of sampling, the *R. mangle*-dominated sites had been fertilised with either N or P for over 15 years using the method as described in Feller (1995). In short, the fertilisation consisted of the semiannual burial of two pieces of dialysis tubing filled with 150 g of either urea for N fertilisation or triple superphosphate for P fertilisation at opposing sides close to the base of each fertilised tree. This procedure led to a total annual amendment of 335 g NH_3 in the N treatment and 452 g PO_4 in the P treatment.

Feller et al. (2002) gives a detailed explanation of the lay-out of the long-term fertilisation experiment. Transects with a maximum length of 50 m perpendicular to the coastline were established on three randomly chosen positions (Feller, 1995). At each position, three parallel transects with a lateral distance of about 10m were established and a fertilisation treatment (control, P fertilisation and N fertilisation was randomly assigned to each of these transects. Each transect comprised of a fringe zone where *R. mangle* trees showed tall (5-6 m) growth, a narrow transition zone with intermediate tree heights, and a dwarf zone, where *R. mangle* trees showed stunted growth with distinctively lower tree height (<1.5 m) and density.

Some basic soil properties of dwarf and fringe zones are given in Table 3.1. The two zones did not differ significantly with respect to pH and bulk density, but salinity and temperature were lower in the fringe sites, which had markedly taller trees.

Soil level calculations

At each site, the difference between soil and water level was measured using a ruler, and the time of measurement was denoted. Soil elevation relative to Mean Lower Low Water (MLLW) was calculated using the TideCal 10 (Kaleberg

Table 3.1: Basic properties as measured at *Rhizophora mangle*-dominated stands on a mangrove-covered island in the Caribbean sea near Belize (N=9). Numbers after ± represent standard errors.

Property	Dwarf	Fringe	Unit
tree height	99 ± 16	472 ± 36	cm
tree density	1.3 ± 0.1	1.0 ± 0.3	ind. m^{-2}
pH	6.7 ± 0.1	6.5 ± 0.1	–
temperature	19.7 ± 0.6	16.4 ± 0.2	℃
bulk density	0.92 ± 0.1	0.92 ± 0.1	g soil DW cm^{-3}

Symbionts, 2010) OS X port of Xtide v 2.10 (Flater, 2008) with the tide_db v1.07 harmonics file (Depner, 2003) using the Belize City measurement station as a reference.

Sample collection and processing
In spring 2012, average tree height and density were measured in a 2·2 m quadrant with the fertilised tree as the centre. Soil and porewater samples were taken approximately 10 cm from the base of the tree. Porewater was collected using 10 cm long rhizons (Eijkelkamp BV, Giesbeek, The Netherlands). Soil samples were taken by extracting cores with a depth of 10 cm using a stainless steel corer with a diameter of 9.6 cm. All samples taken in the field were transported to the lab within 12 hours and stored at 3 °C until further processing. After determination of soil bulk density, roots larger than 2 mm were removed and all samples were manually homogenised before further processing. Gravimetric soil moisture was determined based on moisture loss upon 48 h of oven drying at 70 °C. Soil was freeze-dried and ground at 20 RPM using an MM200 mixer mill (Retsch GmbH, Haan, Germany). Total organic C and total N were determined using an EA/110 elemental CN analyser (InterScience BV, Breda, The Netherlands) after washing with a 32 % HCl solution to remove calcium carbonates.

Tannin content was determined using the Folin-Ciocalteu reagens for phenolic compounds following the recommendations of Cicco and Lattanzio (2011) to avoid formation of precipitations. Tannins were extracted by shaking 15 mg freeze-dried soil with 2 ml extraction fluid at 40 °C for 1 h at 20 rpm, using deionised water as a solvent for soluble tannins and a 50%-50% methanol-water mixture as a solvent for total tannin extraction. After determination of concentrations of total and soluble phenolic compounds, the insoluble tannin concentration was calculated as the total concentration minus the soluble phenolic compounds. PO_4^{3-}, NH_4^+, NO_3^-, dissolved organic N, and dissolved organic C (DOC) measurements of porewater was conducted using a continuous flow auto analyser (Skalar SA-40, Breda, The Netherlands).

Microbial biomass and stoichiometry
Microbial biomass and stoichiometry was determined using a fumigation-extraction procedure as also described in Chapter 2. After extraction with a 0.5 M K_2SO_4 solution, we measured the amount of extracted N, P and C compounds that were liberated by a 24h fumigation with ethanol-free chloroform. To estimate microbial content of C, N, and P we divided the liberated amounts of these substances by their respective extractable fractions: 0.38 for C (Vance et al., 1987), 0.54 for N (Brookes et al., 1985) and 0.40 for P (Brookes et al., 1982).

Short-term incubation experiment
Analogue to Chapter 2, soil cores from non-fertilised dwarf sites were incubated for 7 days at 20 °C following amendment with all possible combinations of labile organic C (as glucose), N (as NH_4Cl), and P (as Na_3HPO_4) leading to a total of eight different treatments. The amount of carbon added was 5 nmol g^{-1} of

fresh soil with molar C:N:P concentrations 50:10:1 as to mimic microbial cellular stoichiometry (Fagerbakke et al., 1996).

Potential enzyme activities
Potential enzyme activity assays of six hydrolytic and two oxidative enzymes were conducted on fresh soil from the various treatments in the dwarf and fringe zones. In addition, potential activities of hydrolytic enzymes were measured in soil from the non-fertilised control treatment in the dwarf zone after 96 h of laboratory incubation with various nutrient amendments. Following (Sinsabaugh et al., 2008, 2009), the measured hydrolytic enzymes can be grouped by their function in acquiring P, N, or C and energy. Alkaline (Acid) phosphatase (AP) liberates phosphate through the breakdown of organic phosphate compounds. Leucine aminopeptidase (LAP) and glycine aminopeptidase (GAP) are involved in the acquisition of N-containing compounds. LAP catalyses the hydrolysis of leucine residues from peptides and proteins and GAP preferentially hydrolyses the terminal N-bond of glycine and alanine. Cellobiohydrolase (CBH) and β-1,4-glucosidase (BG) are subsequently involved in the depolymerisation of cellulose, resulting in free glucose for use as a carbon and energy source. β-N-acetylglucosaminidase (NAG) cleaves N-acetylglucosamine from the fungal and bacterial cell wall components chitin and murein, liberating both C and N. The oxidative enzymes examined, potential phenol oxidase (POX) and peroxidase (POD), are non-specific and attack C bonds in complex structures such as tannins and lignin.

Potential enzyme activities were measured based on absorbance measurements in 96-well micro plates, following a protocol modified from (Allison and Vitousek, 2004). An enzyme-specific substrate is added to a soil extract to create substrate saturating conditions. In the enzymic reaction pNP (in case of AP, CBH, BG and NAG) or pNA (in case of GAP and LAP) is formed or L-Dopa (in case of POX and POD) is broken down. Soil extracts were prepared by suspending 2 g soil in 20 ml TRIS buffered MBL artificial seawater (Cavanaugh, 1956). To measure enzyme activity, eight wells were filled with 150 µl of the suspension and 50 µl of TRIS-MBL with or without substrate to serve as reaction and reference wells, respectively. The plates were incubated in the dark at 20 ℃ for durations ranging from 30 min to 3 h, depending on the enzyme of which the activity was assayed. During the incubation, the plates were shaken at 600 RPM to keep soil particles in suspension. After incubation, soils were allowed to precipitate and 100 µl of particle-free solution was transferred to a new plate for measurement of the absorption at 405 nm for pNP- and pNA-containing substrates and at 450 nm for L-Dopa. Absorbance was measured using a SPECTROstar nano photospectrometer (BMG LABTECH, Offenburg, Germany). Product formation was calculated using the difference in absorbance between reaction and reference wells, corrected for change of absorbance through substrate consumption:

$$\Delta[P] = \frac{(\Delta ABS) - \alpha_s[S]_{t0}}{\alpha_p - \alpha_s} \tag{3.1}$$

with α_s and α_p absorption coefficients of enzyme substrate and product respectively. ΔABS was calculated as the difference of the median absorbances measured in the reaction and the reference well. Finally, potential enzyme activity was expressed as ΔP g soil $DW^{-1}\,h^{-1}$.

Data analysis & Statistics
Results were analysed using R (R Core Team, 2012) and the R packages *nlme* (Pinheiro et al., 2012) for linear-mixed effect modeling and *car* (Fox and Weisberg, 2011) for type II sum of squares. Treatment effects on porewater nutrient concentrations were tested using the non-parametric Wilcoxon Rank Sum test due to the strong heteroscedacity. The other data were fitted to linear mixed-effects models with nutrient treatment or zone as fixed factor with transect and sampling location as random factors where appropriate. Homoscedasticity was confirmed by Levene's test and normality was checked using Shapiro's test. In the enzyme analysis, outliers were identified as being more than two times the interquartile range from the first or the third quartiles resulting in the removal of 2 % of data points. Treatment effects for random models were tested using Walds χ^2 test with type II sum of squares.

3.3 Results

Soil and mangrove characteristics
Fringe and dwarf zones differed with respect to porewater nutrient and DOC concentrations (Tab. 3.2): In the dwarf zone, DOC and NH_4^+ concentrations were higher than in the fringe zone, while the fringe zone contained more PO_4^{3-}. Soil organic nitrogen (SON) content of the porewater was slightly lower in the fringe zone, while soil organic carbon (SOC) content, did not differ between the two zones.

At the time of sampling, nutrient levels in the porewater clearly reflected the fertilisation treatments in the dwarf zone, while this was much less so in the fringe zone (Tab. 3.2). The N fertilisation led to a higher NH_4^+ porewater concentration in the dwarf, but not in the fringe zone. The P fertilisation increased porewater PO_4^{3-} concentrations in both zones, but this increase was six times higher in the dwarf zone as compared to the fringe zone.

Phosphorus fertilisation significantly increased tree height in the dwarf, but not in the fringe sites, while N fertilisation did not affect tree height in either of the zones (Fig. 3.1a, Tab. 3.3). The same was true for relative surface level, which in the dwarf zone was 11 cm higher in the P treatment as compared to the control (Fig. 3.1b, Tab. 3.3) and for concentrations of soluble phenolic compounds, which were also higher in the P-fertilised dwarf plots (Fig. 3.1c, Tab. 3.3). Insoluble phenolic compounds showed a different pattern: P-fertilised sites had higher concentrations of insoluble phenolic compounds in both the dwarf and fringe sites as compared to the control treatment, but there was again no effect of N fertilisation (Fig. 3.1d, Tab. 3.3).

Table 3.2: Measured nutrient and carbon contents in porewater and soil from nitrogen- (N) and phosphorus- (P) fertilised and unfertilised (C) plots of fringe and dwarf *Rhizophora mangle*-dominated sites on Twin Cays, Belize. Symbols denote significance levels ($^{***}p < 0.001$, $^{**}p < 0.01$, $^{*}p < 0.05$, $^{\cdot}p < 0.1$) of dwarf/fringe effect under C, and treatment effect under N and P. Significance levels result from either the non-parametric Wilcoxon Rank Sum test (W) for mineral nutrients or Wald's χ^2 ANOVA analysis of a mixed effects model with transect as a random factor and N and P or Zone as fixed factors for the organic nutrients.

	Dwarf			**Fringe**			
	C	**N**	**P**	**C**	**N**	**P**	
NH_4^+	0.03^{**}	0.86^{***}	0.02	0.01^{**}	$0.06^{\cdot}$	n.d.	mmol l^{-1}
PO_4^{3-}	n.d.**	n.d.	0.30^{***}	0.002^{**}	n.d.	0.05^{***}	mmol l^{-1}
NO_3^-	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	mmol l^{-1}
SOC	336^{***}	$304^{\cdot}$	335	359^{***}	379	344	mg C g soil DW^{-1}
SON	14^{*}	13	14	16^{*}	15	12^{*}	mg N g soil DW^{-1}
DOC	3.3^{**}	2.8	4.2	1.9^{**}	2.8	1.8	mmol C l^{-1}
DON	n.d. *	n.d.	0.02	0.04^{*}	n.d	0.05	mmol N l^{-1}

SOC: soil organic carbon, DOC: dissolved organic carbon, SON: soil organic nitrogen, DON: dissolved organic nitrogen, n.d = non-detectable

Soil microbial characteristics
Field fertilisation modified both microbial biomass as well as their elemental stoichiometry (Fig. 3.2, Tab. 3.3). In the control, microbial biomass was much lower in the dwarf zone as compared to the fringe zone. Also, the relative C content of microbial biomass, was low in the dwarf zone as reflected in the lower C:N and C:P ratios. Microbial N:P did not differ between the two zones. Compared to the average ratios for marine microbial biomass as given by (Fagerbakke et al., 1996), the relative N content was low in both zones, while the relative content of C is somewhat below average for the dwarf, and above average for the fringe zones.

In the dwarf zone, N fertilisation decreased microbial biomass, C:N , and C:P , while N:P remained unaffected. P fertilisation quintupled microbial biomass, while it increased C:N marginally. C:P and N:P were not significantly different from the control treatment.

Within the fringe zone, N and P fertilisation had similar effects such that microbial biomass was lower in both the N and the P treatments. Mean C:N was not significantly different between control and treatments, while C:P was significantly depressed in both the N as well as the P treatments. In both zones, microbial N:P remained unaffected by either fertilisation treatment.

Enzyme activities in the field
Potential activities of hydrolytic enzymes involved in N, P, and C acquisition as well as potential activities of the oxidative POX enzyme were measured in fresh soil collected from both the fringe and the dwarf zone (Fig. 3.3 and Tab. 3.4). Between dwarf and fringe plots only the potential AP activity differed significantly, with a somewhat higher activity in the control dwarf plot. N-fertilisation led to a higher potential AP activity in the fringe, but not in the dwarf zone. P-fertilisation caused an increased potential GAP and a decreased potential POX activity in both the dwarf and the fringe zone, while it increased potential activities of CBH and LAP in the fringe zone only. No POD activities are shown, because no POD activity was detectable in either of the sites.

Enzyme activities in the short-term incubation experiment
Responses of potential enzyme activities to short-term, nutrient amendments in laboratory incubations of dwarf control soils were generally much stronger than those observed for long-term nutrient fertilisation in the field (Fig. 3.4, Tab. 3.5). Glucose amendment increased potential activities of BG, LAP, and AP. Without glucose, N amendment had the strongest effect with significant increases in potential BG, LAP and AP activities as compared to the control while P amendment only increased potential LAP activity. Within the glucose treatment, potential BG, NAG, LAP and AP activities were stimulated by N addition. The amendment of P stimulated potential BG activity while it decreased potential AP activity. Both with and without glucose amendment there was a negative interaction effect between P and N for potential activities of LAP and AP, because simultaneous amendment led to lower potential activities of these enzymes as compared to N amendments only.

Table 3.3: ANOVA-table with Walds χ^2 values and significances of N,P and Zone in a mixed effects model of site, soil and microbial properties in fringe and dwarf *Rhizophora mangle*-dominated sites with transect as a random factor. Symbols denote significance levels ($^{***}p < 0.001$, $^{**}p < 0.01$, $^{*}p < 0.05$, $^{\cdot}p < 0.1$)

		tree height	soil elevation	soluble phenolics	insoluble phenolics	Microbial Biomass	Microbial C:N	Microbial C:P	Microbial N:P
Zone		182.9 ***	8.11	42.10 ***	0.77	71.86 ***	8.60 **	14.75 ***	0.73
Dwarf	**N**	0.05	0.37	1.43	0.11	7.48 **	8.52 **	5.02 *	1.66
	P	25.28 ***	24.89 ***	34.42 ***	112.4 ***	53.20 ***	3.29 ·	1.14	0.11
Fringe	**N**	0.12	0.65	0.80	2.01	9.46 **	3.21 ·	6.10 *	0.08
	P	0.00	0.42	0.06	9.15 **	7.37 **	3.40 ·	12.80 ***	0.26

CBH and GAP were not significantly affected by any of the nutrient treatments. This result is in contrast to the observed responses to P enrichment in the long-term fertilisation experiment in the dwarf zone. Conversely, the high sensitivity of AP to short-term N and glucose enrichment is contrary to the relatively weak responses observed in the long-term treatments.

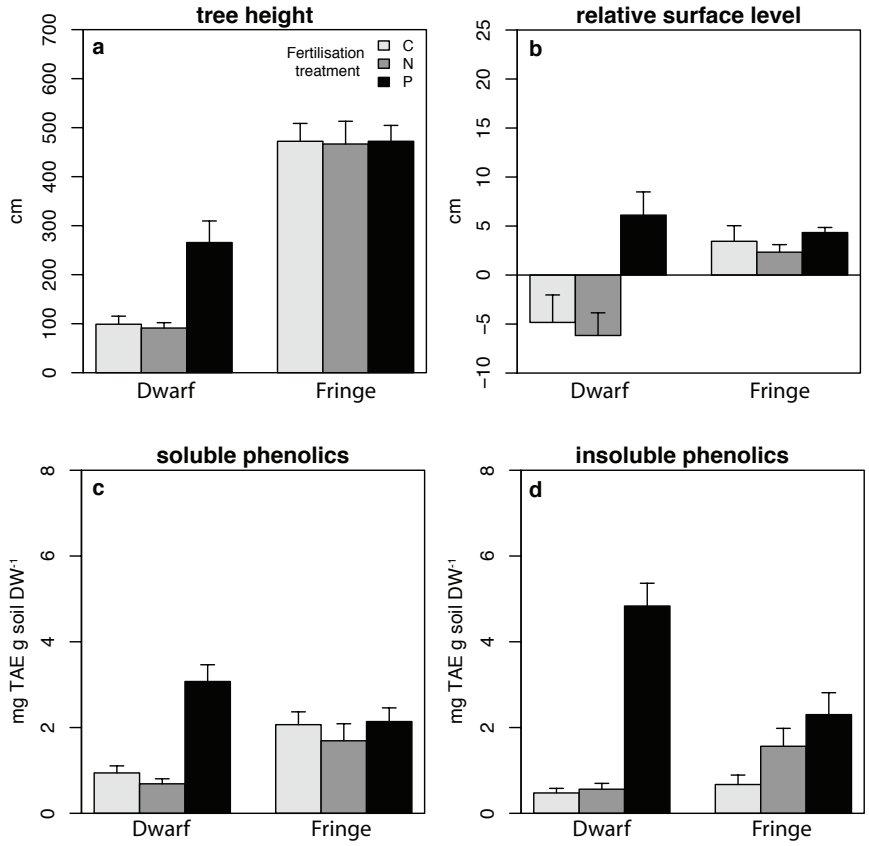

Figure 3.1: Site characteristics in *Rhizophora mangle*-dominated sites at Twin Cays, Belize. The inner 'dwarf' zone showed stunted growth of *Rhizophora* trees, while the outer 'fringe' zone showed normal growth. Sites were fertilised with nitrogen (grey bars) or phosphorus (black bars). Relative surface level is defined against the Mean Lower Low Water (MLLW) level.

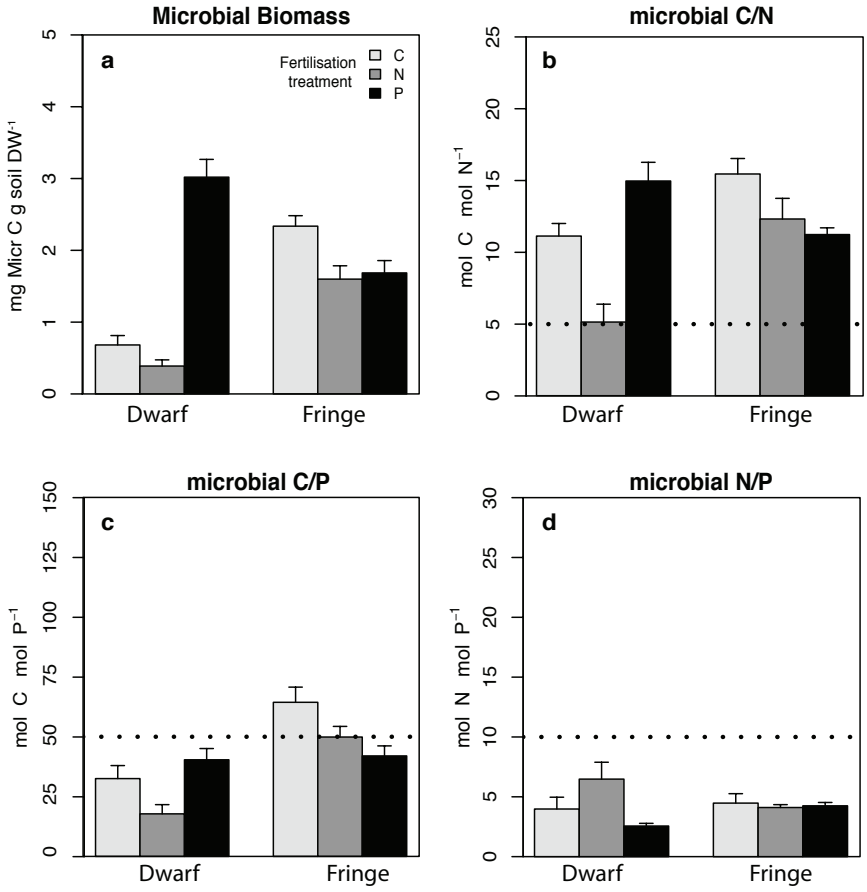

Figure 3.2: Soil microbial characteristics in in *Rhizophora mangle*-dominated sites at Twin Cays, Belize. Two types of *Rhizophora mangle*-dominated sites are distinguished: The inner 'dwarf' zone with stunted growth of *Rhizophora mangle* trees, and the outer 'fringe' zone where *Rhizophora mangle* trees have a much taller growth form. Plots in each zone were left unfertilised (light-grey bars), nitrogen-fertilised (dark-grey bars) and phosphorus-fertilised (black bars). The horizontal lines indicate average value for marine bacteria as given by Fagerbakke et al. (1996)

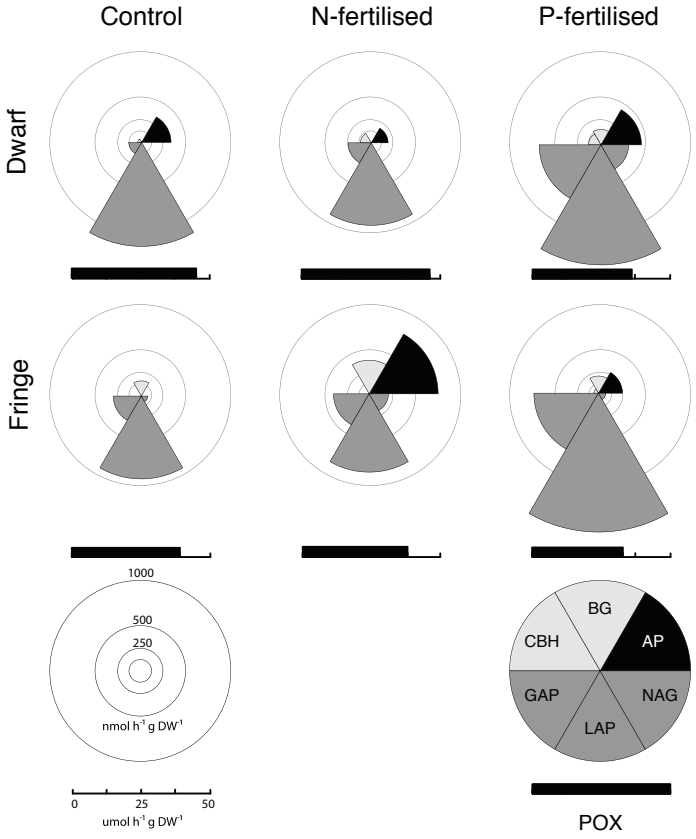

Figure 3.3: Potential activity of oxidative (bars) and hydrolytic microbial exoenzymes (segments) involved in acquiring carbon (light-grey), nitrogen (dark-grey) and phosphorus (black) in soils dominated by *Rhizophora mangle* with a dwarf (top series) or fringe growth form (middle series). Sites from both zones were subject to long term fertilisation with either nitrogen (N) or phosphorus (P). Hydrolytic enzymes are plotted as circle segments with radius indicating potential activity (AP = phosphatase, NAG = β-N-acetylglucosaminidase, LAP = leucine aminopeptidase, GAP = glycine aminopeptidase, CBH = cellobiohydrolase, BG = β-1,4-glucosidase). Potential POX (*phenol oxidase*) activity is plotted on the bars below the circles.

Table 3.4: ANOVA table with Walds χ^2 test values of a mixed effects model of the potential enzymatic activity in *Rhizophora mangle*-dominated sites in the dwarf and fringe zone with transect as a random factor and fixed factors printed in boldface. Nutrient effects were separately tested for the dwarf and the fringe zone, the effect of zone was tested on the control treatment only. Symbols denote significance levels ($^{***}p < 0.001$, $^{**}p < 0.01$, $^{*}p < 0.05$, $^{\cdot}p < 0.1$)

		BG	CBH	NAG	LAP	GAP	AP	POX
Zone		1.86	1.51	1.30	2.11	2.59	7.81 **	3.79 ·
Dwarf	**N**	0.00	1.65	2.38	1.62	0.43	0.12	0.43
	P	3.81 ·	2.38	1.82	0.62	8.63 **	0.01	6.99 **
Fringe	**N**	3.67 ·	0.66	0.98	0.15	0.23	10.5 **	0.11
	P	0.13	4.63 *	0.02	23.5 ***	8.60 **	5.36 *	5.89 *

AP = phosphatase, NAG = β-N-acetylglucosaminidase, LAP = leucine aminopeptidase, GAP = glycine aminopeptidase, CBH = cellobiohydrolase, BG = β-1,4-glucosidase, POX = phenol oxidase

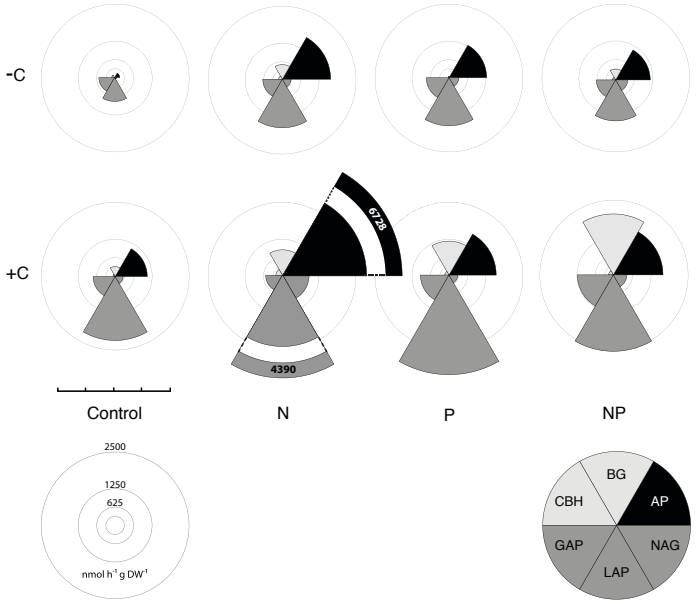

Figure 3.4: Potential activity of oxidative (bars) and hydrolytic microbial exoenzymes (segments) involved in acquiring carbon (light-grey), nitrogen (dark-grey) and phosphorus (black) in soils dominated by stunted 'dwarf' *Rhizophora mangle* after four days of incubation with amendment of various combinations of ammonium (N), phosphate (P) and glucose (C). Hydrolytic exoenzymes are plotted as circle segments with radius indicating potential activity (AP = phosphatase, NAG = β-N-acetylglucosaminidase, LAP = leucine aminopeptidase, GAP = glycine aminopeptidase, CBH = cellobiohydrolase, BG = β-1,4-glucosidase).

Table 3.5: ANOVA table with Walds χ^2 test values of a mixed effects model of the potential enzymatic activity in stunted 'dwarf' *Rhizophora mangle*-dominated sites sample location as a random factor, and amendments as fixed-factors (N = nitrogen, P = phosphorus, C = glucose). Nutrient effects were separately tested incubations with and without C amendment, the effect of C-amendment was tested on control incubations only. Symbols denote significance levels ($^{***}p < 0.001$, $^{**}p < 0.01$, $^{*}p < 0.05$, $^{\cdot}p < 0.1$)

		BG	CBH	NAG	LAP	GAP	AP
C		5.17 *	0.03	1.16	7.03 **	3.06 ·	5.66 *
-C	**N**	7.96 **	1.20	3.23·	7.30 **	0.03	4.87 *
	P	0.01	1.15	3.66·	7.08 **	0.10	0.58
	N*P	0.45	0.37	0.61	9.02 **	0.15	4.41 *
+C	**N**	7.27**	2.38	9.19 **	4.59 *	0.01	19.4 ***
	P	22.2***	0.01	0.70	0.06	0.89	6.06 *
	N*P	2.11	1.57	2.59	11.2 ***	0.51	13.4 ***

AP = phosphatase, NAG = β-N-acetylglucosaminidase, LAP = leucine aminopeptidase, GAP = glycine aminopeptidase, CBH = cellobiohydrolase, BG = β-1,4-glucosidase

3.4 Discussion

Stoichiometry

We hypothesised that decomposer microorganisms were N-limited in Twin Cays due to the protein binding capacity of mangrove related tannin compounds, even in the dwarf zone where plant growth is P-limited. The elemental stoichiometry of the microbial community in the field supports this hypothesis. Without fertilisation, microbial N:P ratios were below average, and C:N ratios were three to four times above the average for aquatic microorganisms (Fagerbakke et al., 1996; Cleveland and Liptzin, 2007). In the dwarf zone, N fertilisation restored the C:N ratios to average global values, but the low N:P ratios decreased even further. This indicates that microorganisms were able to accumulate substantial amounts of P, even in the sites where P is strongly limiting primary production.

Nevertheless, P enrichment quintupled microbial biomass in the dwarf zone, while N amendment did not have an effect. This is seemingly contradictory to the stoichiometric response to fertilisation. This either means that the soil microbial community is P-limited, like the primary producers, or that microbial growth is somehow indirectly stimulated by higher primary production. Available evidence suggests the latter. In the dwarf zone, P fertilisation increased primary production (Feller et al., 2002) and C input to the soil (McKee et al., 2007). At the same time, the microbial C:P ratio increased in these plots. In other words, relative microbial P concentrations decreased despite increased availability of P. The observed increase in microbial biomass upon P enrichment in the dwarf zone thus appears to be the result of increased C input, rather than increased P availability.

Enzyme activities in incubation experiments
Enzyme activity responses to amendments of nutrients and carbon were measured in short-term laboratory incubations, to isolate the direct responses of soil matrix enrichment from plant-mediated responses. Two types of responses were observed in the incubation experiment: i) A change in overall exoenzyme activity, without an apparent shift in relative enzyme activities. We will interpret this as increased overall enzyme production, as a consequence of alleviation of a general limitation to microbial activity; ii) A change in relative enzyme activity. Relative activities of C- and nutrient-acquiring exoenzymes are often applied as an indicator for the nature of the nutrient limitations of decomposer microorganisms, under the assumption that microbial communities optimise their allocation of resources to maximise their productivity (Sinsabaugh and Moorhead, 1994; Sinsabaugh, 1994; Sinsabaugh et al., 2010).

Single amendments of N, P, and labile organic C all rendered similar increases in overall hydrolytic enzyme activity, without markedly changing the relative activities (Fig. 3.4, upper half). From this we conclude that the microorganisms producing these exoenzymes are co-limited by each of those elements. This result confirms the observation that Liebig's law of the minimum does not apply to microbial decomposers (Kaspari et al., 2008) possibly resulting from the large amount of simultaneously active species (Danger et al., 2008). Amendment of either N or P did not have an effect, but if C was added concurrently, their effect was super-additive (i.e. with a positive interaction). From this we conclude that labile organic C was the most limiting for exo-enzyme production. Within the C-amended treatments, the effect of N on enzyme activity was considerably larger than the effect of P. This confirms the finding from stoichiometry that N is more limiting to microbial activity than P.

Stimulation of specific exoenzymes in response to short-term amendments was also observed, although not all responses were easy to interpret. Upon N amendment, potential activity of the P-acquiring AP enzyme increased, which matches the expectations based on optimal resource allocation. Activity of the N-harvesting LAP enzyme could however not be explained from this principle as its activity increased with all amendments, including that of N. Similarly, potential NAG activity increased upon N addition if also glucose was given. Although generally considered as strictly N-harvesting (Sinsabaugh et al., 2009), their end products, leucine ($C_6H_{13}NO_2$) and N-acetylglucosamine ($C_8H_{15}NO_6$) respectively, contain considerably more C than N. This means that their activity contributes more to energy and C uptake than to N uptake, which may explain the unexpected increase in activity even if enough mineral N is available. The same is true for the end product of GAP (galanine - $C_2H_5NO_2$)), but in our study, its activity did not respond to any of the given amendments.

Enzyme activity in the field fertilisation experiment
In the field fertilisation experiment, responses of enzyme activities to nutrient amendments were much weaker as compared to the laboratory incubations, but they roughly followed the same direction. In contrast to the short-term response,

potential LAP, BG and NAG activities were not significantly stimulated by N amendment. The increased potential activities of AP in the N fertilised dwarf and fringe zones indicate that P became the limiting nutrient, while it was not before. Conversely, P fertilisation increased the potential activity of N acquiring enzymes LAP and GAP, indicating that N had become limiting, where it was not before. The latter is not in accordance with the elemental stoichiometry of the microbial biomass, which suggests that N is also limiting in the control. Similar to the short-term incubations, the largest increase in activity of nitrogen acquiring enzymes is observed in LAP. This strengthens the suspicion that that this enzyme is less specific than previously thought, but could also result from a deepening of nitrogen depletion in the rhizosphere resulting from increased plant production. The potential activity of the oxidative POX enzyme decreased after P fertilisation, while N did not have an effect. This is in contrast to other studies, which report a decline of POX activity upon N amendment (Carreiro et al., 2000; Saiya-Cork et al., 2002; DeForest et al., 2004) and a neutral effect of P amendment (Wright and Reddy, 2001).

Despite the substantially higher microbial biomass in the P fertilisation treatment of the dwarf zone, the potential enzyme activities did not increase, indicating that the enzyme production per unit microbial biomass decreased in these treatments. This is consistent with the hypothesis that tannins specifically inhibit microorganisms capable of producing-exoenzymes. P enrichment consistently decreased POX activity and therefore inhibits decomposition of recalcitrant organic matter. Thus, as an indirect effect of P amendment, microbial production and turnover of freshly produced labile carbon compounds increased, as indicated by higher microbial biomass. This higher C turnover and higher microbial biomass did not lead to a positive priming effect on the breakdown of recalcitrant organic matter, as shown by the similar potential activities of hydrolytic enzymes and the lower POX activity in comparison to the non-fertilised sites.

Conclusions

Potential exoenzyme activities have been used to predict microbial decomposer nutrient limitation (Sinsabaugh et al., 1993). We showed that in mangroves, responses of potential exoenzyme activities upon short-term nutrient amendment proved to be similar to long term responses. Short-term incubations are therefore a useful tool to predict microbial enzyme responses, without the confounding effects of plant responses to nutrient enrichment. Not all responses in exoenzyme activities could however be directly linked to microbial nutrient limitation. The end products of the N-harvesting enzymes GAP, LAP and NAG do not only contain N but also significant amounts of C and could therefore well serve as sources of C and energy. A similar finding was done by Steenbergh et al. (2011) for the 'P-harvesting' enzyme AP, which was shown to relieve microbial carbon limitation in a marine soil. The presumed distinction in 'P-harvesting', N-harvesting' and C-harvesting' enzymes is therefore not as clear-cut as often suggested (in e.g. Sinsabaugh et al. (2009)) and needs to be reconsidered.

The microbial stoichiometry was consistent with the hypothesised N limitation in the mangrove soils studied. The expected increase in peat decay has not been confirmed, as enzyme production is above all C-limited. Overall, the effects of P enrichment were much larger than that of N, despite the fact that microbial decomposers are N- limited. In the P-limited dwarf site, P increased the turnover of labile C compounds, as indicated by the threefold increase in microbial biomass but decreased the turnover of recalcitrant compounds as indicated by the lower POX activity. This result has been reported for inorganic N (e.g. Sinsabaugh (2010); Hobbie et al. (2012)), but not for P enrichment. As most terrestrial ecosystems are N-limited, it is well possible that the effects found are not specific to the element N or P but rather to the element limiting primary production. Whether this is the case, requires more explicit comparison of results from nutrient enrichment in P- versus N- limited systems.

3.5 Acknowledgements

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Chapter 4

Tea Bag Index: A novel approach to collect uniform decomposition data across ecosystems

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Abstract Changes in the balance between soil carbon storage and release can significantly amplify or attenuate global warming. Although a lot of progress has been made in determining potential drivers of carbon release through large-scale decomposition experiments, climate predictions are still hampered by data limitation at a global scale as a result of high effort and measurement costs of comparative litter decomposition studies.

We introduce an innovative, cost-effective, well-standardised method to gather data on decomposition rate and litter stabilisation using commercially available teabags as standardised test kits. By using two tea types with contrasting decomposability we can construct a decomposition curve using a single measurement in time. The acquired Tea Bag Index (TBI) consists of two parameters describing decomposition rate (k) and litter stabilisation (S).

The method was tested for its sensitivity and robustness in contrasting ecosystems and biomes, confirming that the Tea Bag Index is sensitive enough to discriminate between these systems. Within an ecosystem, TBI is responsive to differences in abiotic circumstances such as soil temperature and moisture content. The collected k and S values are in accordance to expectations based on decomposition process literature. They are therefore interpretable within the current knowledge framework.

TBI is a unique, multi-functional method requiring few resources and minimal prior knowledge. The standardisation and simplicity of the method make it possible to collect comparable, globally-distributed data through crowdsourcing. TBI can further provide an excellent decomposition reference and has the potential to increase reliability of soil carbon flux estimates based on extrapolations of decomposition data.

4.1 Introduction

Ecosystem carbon emissions are fundamentally driven by the balance between primary production and respiration, much of which is derived from decomposition of plant litter. The regulating factors of these processes are relatively well studied, but it remains a challenge to separate effects of environmental factors on decomposition from litter quality and litter trait effects. Global climate models generally estimate terrestrial soil respiration on the basis of relationships between climate and map-based soil quality data (Sanchez et al., 2009). This method leaves large uncertainties due to the diverse interactions between decomposition and climate driven by changes in CO_2 concentration and temperature. These uncertainties can only be resolved by a more process-based evaluation of decomposition and the related carbon efflux from soils (Heimann and Reichstein, 2008).

Earlier efforts to obtain standardised global scale decomposition data made use of different cellulose objects such as cotton strips (Harrison et al., 1988; Correll et al., 1997; Slocum et al., 2009). The relation to litter decomposition can be weak as these methods do not account for the complex chemical composition of plant litter, ignoring interactions among the decay of cellulose and other plant-constituents (Tiegs et al., 2007; Fritz et al., 2011).

Only a handful of studies have used plant litter to test decomposition on a global scale (Berg et al., 1993; Trofymow et al., 2002; Parton et al., 2007; Stadler et al., 2010). They show that the combination of temperature and moisture can explain 50 to 70 % of the variation in decomposition. These studies used coarse grids, sampling 20 to 39 locations in 1 to 7 biomes, often not spanning the whole North to South gradient or lacking extreme environments (Berg et al., 1993; Trofymow et al., 2002; Parton et al., 2007; Stadler et al., 2010). Testing the current generation of climate models with the litter decomposition data obtained from these studies (Bonan et al., 2012) revealed that there is a strong need for higher resolution measurements with a global coverage to increase the predictive power of such models (Bonan et al., 2012; Stockmann et al., 2013).

The approach described here uses a standardised plant-litter to measure decomposition and stabilisation at a scale and resolution not previously possible. The key component of the approach is the use of commercially available teabags (Fig. 4.1) as highly standardised test kits containing tea as representative dead plant material. Uniquely, this method enables the generation of a global database with the participation of volunteers worldwide. The gathered data can be used to compute a tea bag index (TBI) that provides process-driven information on soil functions at local, regional and global scales. TBI is determined through a simplified litter bag experiment (Wieder and Lang, 1982) which involves burial of green and rooibos tea bags, followed by measurement of mass loss after a period of time.

The TBI has two primary applications. First, it is an attainable way to increase the resolution of decomposition measurements. Secondly, TBI is a useful reference alongside decomposition studies to disentangle litter quality aspects from the full set of environmental conditions constituting the 'decomposition matrix'. The use

of TBI as a reference facilitates data comparison between biomes, ecosystems and soil types.

4.2 Material and Methods

Tea material

A simplified litter bag experiment was carried out with commercially available tetrahedron-shaped synthetic tea bags with sides of 5 cm containing approximately 2 g of green tea or rooibos tea (Lipton, Unilever) (Fig. 4.1). The green tea consisted of 89 % green tea and the rooibos tea consisted of 93 % rooibos, both were supplemented with natural flavouring. Mesh size of 0.25 mm allowed microorganisms and mesofauna to enter the bags, but excluded macrofauna (Setälä et al., 1996).

Chemical analyses

Green tea and rooibos tea were analysed for carbon fractions using a sequential extraction technique (Ryan et al., 1990; Hobbie, 2000; Shaver et al., 2006; Prescott, 2010). Four fractions were determined by sequential extraction: nonpolar extractives (NPE), water-solubles (WS), acid-solubles (AS), and acid-insolubles (AIS). The NPE (e.g. fats and waxes) and WS (e.g. simple sugars and phenolics) fractions were continuously extracted for 24 h using a Soxhlet apparatus with dichloromethane followed by deionised water as solvents. Sulphuric acid (72 %) was used to extract the AS (e.g. cellulose) fraction. The remaining material (AIS (e.g. lignin) and ash) was ignited at 550 °C to determine the ash content. The hydrolysable fraction H is defined as the sum of the NPE, WS and AS fractions (Tab. 4.1). H is assumed to be rapidly decomposable in contrast with the recalcitrant non-hydrolysable fraction (AIS and ash).

Total carbon and nitrogen was measured on the oven-dried (70 °C) ground tea with a CHN-analyser (EA NA 1110, Carlo Erba, Milan, Italy).

In vitro incubation

To determine decomposition of green and rooibos tea over time, we incubated the tea bags *in vitro* in incubators at 15 °C and 25 °C ($n = 6$), which are well within the expected range of summer soil temperatures. Soil for incubation was collected in spring in a deciduous broadleaf alluvial forest in Landgoed Rhijnauwen, The Netherlands (52°4′11″N, 5°10′35″E). The tea bags were incubated in the dark in covered boxes on a layer of the collected soil underlain by saturated sand to prevent the soil from drying out. After 0, 4, 7, 14, 30, 68 & 130 days of incubation the bags were retrieved, dried (48 h, 70 °C) and weighed. We used the remaining mass to fit exponential decay functions (Eqn. 4.3) for both tea types at both 15 °C and 25 °C.

Field application

We tested our method in different ecosystems using the protocol described in Box 4.1. Green and rooibos tea bags were buried pairwise at a depth of 8 cm

Table 4.1: Results from ANOVAs of quality parameters and weights of four batches of green tea and rooibos tea with different production numbers (n = 3).

		Green Tea			Rooibos Tea		
		mean ± sd	$F(3,8)$	P	mean ± sd	$F(3,8)$	P
Non-polar extractable fraction	$g\,g^{-1}$	0.066 ± 0.003	5.062	0.030 *	0.049 ± 0.013	12.950	0.002 **
Water soluble fraction	$g\,g^{-1}$	0.493 ± 0.021	0.975	0.451	0.215 ± 0.009	0.418	0.745
Acid soluble fraction	$g\,g^{-1}$	0.283 ± 0.017	0.625	0.618	0.289 ± 0.040	2.149	0.172
Acid insoluble fraction	$g\,g^{-1}$	0.156 ± 0.009	0.356	0.787	0.444 ± 0.040	1.166	0.381
Mineral fraction	$g\,g^{-1}$	0.002 ± 0.0009	7.084	0.012 *	0.004 ± 0.0006	3.158	0.086
Hydrolysable fraction (H)	$g\,g^{-1}$	0.842 ± 0.023	0.295	0.828	0.552 ± 0.050	1.189	0.374
Total carbon	%	49.055 ± 0.109	0.243	0.864	50.511 ± 0.286	2.769	0.111
Total nitrogen	%	4.019 ± 0.049	0.151	0.926	1.185 ± 0.048	0.727	0.564
C:N ratio		12.229 ± 0.129	0.145	0.930	42.870 ± 1.841	0.774	0.541
Total tea bag weight	g	2.019 ± 0.026	1.260	0.351	2.152 ± 0.013	0.848	0.506
Empty bag weight	g	0.246 ± 0.001	2.058	0.184	0.245 ± 0.001	0.487	0.701

Figure 4.1: Tetrahedron-shaped synthetic tea bag used for TBI experiments.

and retrieved after approximately 90 days (see table 4.2 for location details). We buried between 5 and 32 pairs of tea bags per location. The bags were oven-dried for at least 48 h at 70 °C, and weighed after removal of adhered soil particles. Burial depth of 8 cm prevented loss or displacement of the bags yet allowed that they were still located in the active soil layer (Schenk and Jackson, 2002; Laio et al., 2006). Moreover, environmental influences, such as temperature and moisture content, are more stable in the soil than under the litter layer. The mesh size did allow ingrowth of fine roots, but they were easily removable by hand. We did not observe substantial accumulation of roots and fungal biomass in our incubations.

TBI parameters

In litter bag studies, decomposition is measured by weight loss of plant material in time. A decomposition curve is often estimated by fitting this weight loss to an exponential decay function with decomposition rate constant k. This approximation assumes that half-life of litter is constant in time. The problem with this assumption is that, as decomposition progresses in time, easily degradable compounds in plant litter will be rapidly decomposed, while more recalcitrant compounds will be lost at relatively lower rates. As a result, k is no longer constant as it decreases with time due to the increasing proportion of recalcitrant material.

A simple, but relatively accurate approximation of this process is reached when grouping labile and recalcitrant compounds and estimating k separately for those two groups (Wieder and Lang, 1982):

$$W(t) = ae^{-k_1 t} + (1-a)e^{-k_2 t} \tag{4.1}$$

where $W(t)$ is the relative weight of the substrate after incubation time t, a is the labile and $1-a$ is the recalcitrant fraction of the litter. The decomposition rate constants of the labile and recalcitrant fractions are described by k_1 and k_2,

Box 4.1: TBI protocol

1. Use one bag of Lipton Green Tea (EAN: 87 22700 05552 5) and one Lipton rooibos tea (EAN: 87 22700 18843 8) per replicate.
2. Take a green tea and rooibos tea bag per replicate. To obtain better estimates of TBI, bury more replicates per site.
3. Measure the initial weight of the tea bag and subtract the weight of an empty bag (see also Tab. 4.1) to determine the initial weight of the tea.
4. Mark the tea bags on the white side of the label with a permanent black marker.
5. Bury the teabags in 8 cm-deep, separate holes while keeping the labels visible above the soil.
6. Mark the burial site with a stick.
7. Note the date of burial, geographical position, ecotype and experimental conditions of the site.
8. Recover the tea bags after approximately 90 days
9. Remove adhered soil particles and dry in a stove for 48 h at 70 °C (not warmer!).
10. Remove what is left of the label but leave the string, weigh the bags and subtract the weight of an empty bag without the label to determine the weight after incubation.
    a. To get a more precise estimation, open the bag and weigh its content; ignite the content at 700 °C and subtract what is left from the content weight.
11. Calculate stabilisation factor S and decomposition rate k using eqn 4.2-4.4.

More (facultative) instructions and tips on how to incorporate the TBI in scientific experiments can be found on our website: http://www.decolab.org/tbi/protocol.html

Table 4.2: Descriptions of incubation sites with coordinates and estimated k and S with standard deviations. numbers correspond with the legend of Figure 4.3.

No.	Country Code	Ecosystem	Subtypes	Coordinates	# pairs	$k \cdot 10^3$	S
1	US-FL	Mangrove	dwarf	28°22′31″N, 80°36′47″W	10	9.9 ± 1.5	0.27 ± 0.09
2			fringe		8	22.5 ± 6.0	0.19 ± 0.06
3	IE	Oceanic raised bog	disturbed	53°19′18″N, 7°37′38″W	5	9.4 ± 0.9	0.23 ± 0.05
4			undisturbed		5	10.2 ± 0.7	0.2 ± 0.01
5	IS	Geothermal wet grassland	warmed	64°2′20″N, 21°11′43″W	28	18.7 ± 5.9	0.04 ± 0.11
6			ambient		30	20.4 ± 9.7	0.15 ± 0.1
7	CN	Semi-arid desert	sandy	39°29′37″N, 110°11′29″E	5	10.4 ± 3.9	0.31 ± 0.1
8			loamy		5	3.5 ± 3.4	0.54 ± 0.15
9	NL	Forest		52°4′12″N, 5°16′28″E	4	20.4 ± 3.7	0.14 ± 0.04
10		Wet forest		52°8′10″N, 5°6′58″E	4	13.4 ± 1.1	0.13 ± 0.01
11		Pasture		52°7′48″N, 5°8′59″E	4	11.9 ± 0.7	0.14 ± 0.02
12		Floating fen		52°9′54″N, 5°7′17″E	4	10.1 ± 1.4	0.23 ± 0.02
13	PA	Lowland tropical forest		9°13′12″N, 79°44′24″W	18	39.2 [1]	0.06 ± 0.09
14	AU	Mixed forest		48°35′28″N, 15°39′29″E	7	26.2 ± 3.9	0.26 ± 0.11
15		Birch forest		48°35′28″N, 15°39′29″E	12	20.4 ± 4.9	0.29 ± 0.07

[1] Could not be calculated due to overdispersion

respectively. During the first phase, the labile fraction is rapidly broken down and the weight loss of the litter is mainly determined by k_1. When all labile material is gone, weight loss is determined by k_2. By definition, k_2 is very low, so that it can only be estimated on very long time scales. To calculate the TBI, we assumed that during short field incubations the weight loss of the recalcitrant fraction is negligible. As a consequence k_2 equals zero, and a becomes the decomposable fraction. This reduces equation 4.1 to:

$$W(t) = ae^{-kt} + (1 - a) \tag{4.2}$$

Decomposition rate constant k can only be estimated from the early stages of decomposition, while decomposable fraction a, which is conceptually equal to the limit value (Berg and Meentemeyer, 2002), is only estimable most of the labile material is gone.

Estimating both k and a would require time series, when only one litter type is used. Instead, we use two litter types with different decomposition rates. The decomposition rate of rooibos tea is low in comparison to green tea. Consequently, decomposition of labile material still continues in rooibos tea after all labile material in green tea has already been consumed. The difference between these litter types allows us to estimate the decomposable fraction from green tea (a_g) and decomposition rate constant k from rooibos tea at a single point in time.

To solve equation 4.2, estimation of the decomposable fraction of rooibos tea (a_r) is needed. We do so by making use of the relation between decomposable fraction a as measured in the field and hydrolysable fraction H, the chemically expected labile fraction. a_r can be estimated from a_g, when assuming that the relation between H and a only depends on environmental conditions.

During decomposition, parts of the labile compounds stabilise and become recalcitrant (Prescott, 2010). This stabilisation depends on environmental factors (Berg and Meentemeyer, 2002) and results in a deviation of the actual decomposed fraction (i.e. limit value) a from the hydrolysable (i.e. chemically labile) fraction H. This deviation can therefore be interpreted as the inhibiting effect of environmental conditions on the decomposition of the labile fraction, and will be referred to as stabilisation factor S:

$$S = 1 - \frac{a_g}{H_g} \tag{4.3}$$

where a_g is the decomposable fraction and H_g is the hydrolysable fraction of green tea.

The decomposable fraction of rooibos tea (a_r) is calculated from the hydrolysable fraction of rooibos tea (H_r) (Tab. 4.1) and the stabilisation factor S:

$$a_r = H_r(1 - S) \tag{4.4}$$

With $W_r(t)$ and a_r known, k is calculated using the exponential decay function given in equation 4.2.

The implicit assumption in Eqn. 4.4 is that S is equal for both tea types, i.e. that the environmental stabilisation of labile material is independent of the relative size and composition of the hydrolysable fraction. To test to what extent the obtained results depend on this assumption, all statistical analyses were repeated under the alternative assumption that stabilisation of hydrolysable rooibos material does not occur, so that S is always zero and $a_r = H_r$. None of the reported relations changed in significance or direction, confirming that the results obtained with the TBI are robust for deviations from the intuitive assumption made in Eqn 4.4 (data not shown).

Relating TBI parameters to environmental factors
We related the calculated k and S values obtained from our field sites to temperature and precipitation, which are key environmental factors for decomposition (Prescott, 2010). The relation of k and S with temperature was explicitly tested on data from Iceland, where temperature varied considerably ($\sim$12 °C) on very short distances due to geothermal activity. S values calculated for the field dataset were correlated with classes of carbon sequestration suitability based on soil, climate, moisture and land cover conditions as defined by FAO (2000). Mean annual temperature (MAT) and mean annual precipitation (MAP) were obtained from weather stations closest to the incubation sites (Cantymedia, 2013).

In our dataset, the MAT and MAP correlated strongly ($r = 0.84$, $n = 17$, $p < 0.001$) so that we decided to construct a joint climate factor calculated by averaging relative values of MAP and MAT. The relation of k and S with this climate factor and other environmental factors were analysed using ANOVA on linear mixed models with location as a random factor (R package: lme{nlme} (Pinheiro et al., 2012)). The Icelandic sites were excluded from these analyses as their decomposition largely depended on local geothermal conditions. Within the Icelandic site, the relation of k and S with soil temperature was analysed using a linear model. Levene's test was used to test for homogeneity of variance and Shapiro-Wilk test to confirm normality of residuals. All statistical tests were conducted using the R statistical package (R Core Team, 2012)

4.3 Results and Discussion

Laboratory incubation
Decomposition dynamics of rooibos and green tea were monitored in a laboratory incubation with multiple harvests. Initial decomposition of green tea was very fast, and began to level off after 40–60 days (Fig. 4.2). Decomposition was much slower in rooibos tea, only starting to level off towards the end of the lab incubation experiment. For a large proportion of the incubation time, green tea had already reached its limit value, allowing estimation of S, while the labile fraction of rooibos tea was still actively decomposing, allowing estimation of k. Based on this result, the duration of TBI field incubations was set to 90 days. This period is expected to be sufficiently long to determine stabilisation (S) by measuring the weight loss of the green tea, while short enough to determine initial

decomposition rate (k) of the rooibos tea under a wide range of environmental conditions.

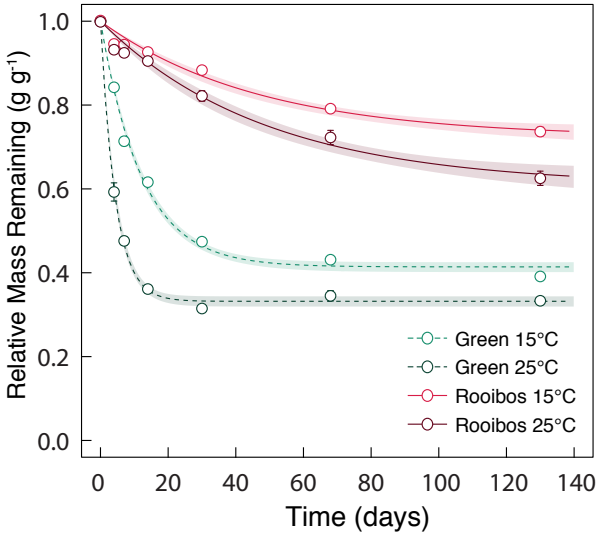

Figure 4.2: Relative mass remaining of rooibos and green Tea as measured in laboratory incubations on temperate forest soil at 15 °C and 25 °C. The tea bags were incubated in the dark in covered boxes with moist soil on top of saturated sand and retrieved after 0, 4, 7, 14, 30, 68, and 130 days of incubation ($n = 6$). Lines show fitting to exponential decay function (Eqn. 4.3) with 95 % confidence intervals. Vertical bars represent standard errors.

Global application

Field application of the TBI found a clear discrimination of both k and S between ecosystems after an incubation period of approximately 90 days (Fig. 4.3). Calculated k values increased with mean annual temperature and precipitation ($\chi^2 = 6.0$, $p < 0.05$) in accordance with general expectations of litter decomposition rates (Parton et al., 2007; Zhang et al., 2008). k was expected to be higher in geothermally warmed Icelandic plots than in ambient plots, but no significant difference was found.

S values decreased with mean annual temperature and precipitation ($\chi^2 = 6.7$, $p < 0.01$). We expected S to increase with terrestrial soil carbon sequestration potential (as defined by FAO (2000)). Indeed this relation was significant ($\chi^2 = 46.2$, $p < 0.001$): S was low in tropical rainforest (site 13 in Fig. 4.3), intermediate in forest on humic soils (sites 9 and 10) and high in carbon-accumulating peatlands (sites 3, 4 and 12). A comparison of S between warmed and ambient Icelandic grassland plots (sites 5 and 6) showed that S was lower for warmed plots ($F_{1,52} = 35.9$, $p < 0.001$). This indicates a positive feedback between dimin-

ishing carbon storage and increased temperature, as suggested by Davidson and Janssens (2006).

The results presented here show that the TBI decomposition parameters are sensitive to ecosystem specific differences, and at the same time follow general climatic trends at a global scale. While this dataset suffices to validate the method, a much larger dataset is required to unravel the exact nature of the relationships between decomposition and environmental factors. We therefore encourage people to collaborate in expanding the dataset, leading to robust global information about decomposition. This effort will also help to evaluate the assumptions made in calculating k and S.

In addition to the results shown, we performed pilot studies with an incubation period of one year. In many systems however, substantial amounts of labile material in rooibos tea had been decomposed after a year, leading to inaccurate estimations of k. In the field experiments the duration of 90 days proved to be sufficient for most sites. However, low microbial activity, such as in Chinese loamy arid soils (Fig. 4.3, site 8), made the calculation of S unreliable within the set incubation time, so that this site was excluded from statistical analyses. At the other end of the scale, three months proved to be the absolute maximum incubation time in the most active site (tropical forest – Fig. 4.3, site 13), as mass loss of rooibos tea approached its entire labile fraction.

The incubation time in such extreme sites can be adjusted to facilitate calculation of S and k. Incubation time in sites with extremely high k values (e.g. sites with high temperature and precipitation like site 13 in Fig. 4.3) can be reduced without influencing the result or the comparability of the TBI parameters. In fact, a reduced incubation time is recommended in cases where the weight loss of rooibos tea approaches the limit value, because this may lead to an underestimation of k. Equally, incubation time can be extended in sites with extremely low k values, as in these cases it is not certain that green tea has reached its limit value, leading to an overestimation of S. Therefore, we recommend to extend the incubation time in sites with low k values in combination with a high S value (e.g. Fig. 4.3, site 8). Adjustments of the incubation period facilitate the use of the TBI in extreme environments, generating meaningful parameters in extreme cases.

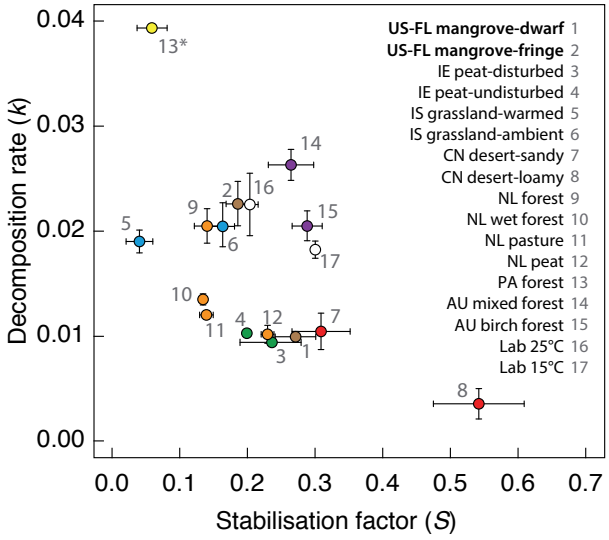

Figure 4.3: In situ initial decomposition rate k and stabilisation factor S for different sites showing the discriminatory potential of TBI between and within ecosystems. k represents short term dynamics of new input and S is indicative for long term carbon storage. Calculations were based on a single incubation time between 66 and 90 days. Labels indicate country (US-Florida (US-FL; n = 10), China (CN; n = 5), Panama (PA; n = 20), The Netherlands (NL; n = 4), Austria (AU; n = 10), Ireland (IE; n = 5) and Iceland (IS; n = 32)) followed by ecosystem and either soil type or temperature (Table 4.2). Mangrove ecosystems are printed in bold. The lab incubations shown in Fig. 4.2 were also included (16-17; n = 6). Error bars are standard errors.

∗ Error bars missing due to overdispersion.

4.4 Conclusions

While this method cannot substitute the thoroughness and precision of conventional litter bag methods, TBI considerably reduces the effort necessary to fingerprint local decomposition. The parameters comprising the TBI, k and S, are meaningful integrative estimators to characterise and compare carbon decomposition dynamics between different biomes, ecosystems and soil types. We foresee a broad application for TBI:

i) By applying it alongside field decomposition experiments as a reference, TBI can provide a contribution in comparing decomposition rates between field experiments in different biomes and ecosystems leading to new insights in global climate effects on decomposition.

ii) The simplicity and cost-effectiveness of the method also makes it suitable for educational purposes. By involving citizen scientists and schools, the method can increase awareness of a living soil while simultaneously generating numerous data points.

iii) Crowdsourcing with the help of social media and research networks will provide decomposition data with a higher resolution and at a larger scale than previously attainable, improving extrapolations of long-term studies over larger areas. We foresee that, with a wide geographic distribution, a validated global soil decomposition map could be assembled.

4.5 Acknowledgments

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Chapter 5

Effects of nutrient enrichment on mangrove leaf litter decomposition

Submitted

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Abstract Nutrient enrichment of mangroves, a common phenomenon along densely populated coastlines, may strongly affect carbon and nutrient recycling and negatively affect carbon sequestration in the mangrove system.

This study describes effects of nutrient enrichment on litter, decomposition rates, and mineralisation/immobilisation patterns of decomposing mangrove leaf litter. Measurements were done in two contrasting mangrove sites, which were subject to at least five years of experimental nitrogen and phosphorus fertilisation.

By making use of reciprocal litter exchange experiments in addition to in-situ incubations of litter, it is tested if nutrient addition primarily acts on the primary producers (i.e. changes in litter quality) or microbial decomposers (i.e. changes in nutrient limitation for decomposition).

Our results have shown that decomposing microorganisms can be N-limited even in sites were primary production is P-limited. Relieving the nutrient limitation of the primary producer, increased decomposition rates while alleviating the decomposer nutrient limitation did not. This result emphasises the importance of plant-mediated fertilisation effects for changes in decomposition rates and carbon sequestration.

5.1 Introduction

Mangroves are amongst the most carbon-rich forests in the tropics (Donato et al., 2011), due to high primary production and slow litter decomposition (Cebrian, 1999). The latter process governs important ecosystem services as soil carbon sequestration and nutrient removal from through-flowing water. Microbial decomposers are a key functional group processing most of the carbon and nutrient fluxes in these coastal systems (Holguin et al., 2001). Leaf litter is a primary resource for decomposers, and its quality and quantity are important drivers of decomposer activity (Couteaux et al., 1995; Strickland et al., 2009). Through mineralisation and immobilisation, decomposers have a major impact on nutrient availability for the vegetation (Cherif and Loreau, 2009). As a change in nutrient availability affects litter production and chemical quality, changes in decomposition dynamics have a strong potential feedback on the decomposer community composition, nutrient availability, and primary production (Holguin et al., 2001; Hessen et al., 2004; Norris et al., 2012).

The rate of decomposition generally correlates positively with nutrient availability in terrestrial and wetland ecosystems (Cebrián et al., 1998). Anthropogenic nutrient enrichment might therefore have a negative effect on carbon storage (Hessen et al., 2004). Nutrient enrichment may change litter decomposition in two different ways, i.e. directly, via increased nutrient availability in the soil matrix (Norris et al., 2012), and indirectly, via changes in litter chemistry after fertilisation effects on the vegetation (Bryant et al., 1983; Prescott, 2010; Hobbie et al., 2012). Indirect effects of nutrient enrichment include an increase in nutrient content (Hobbie et al., 2012) and a decrease in carbon rich secondary compounds such as lignin (Bryant et al., 1983), both of which decrease litter recalcitrance.

Direct effects of nutrient enrichment on decomposition are quite complex, as both retarding and stimulating effects of nutrient amendment have been reported. While decomposition of labile litter compounds is mostly stimulated by nutrient addition, the effect on more recalcitrant litter compounds is element specific, as nitrogen generally retards decomposition rates of recalcitrant litter (O'Connell, 1993; Knorr et al., 2005; Craine et al., 2007), while phosphorus also stimulates breakdown of recalcitrant litter compounds (O'Connell, 1993).

Nutrient enrichment thus affects decomposer activity, litter chemistry and their interaction. Nutrient availability (O'Connell, 1993) and litter chemistry (Osono and Takeda, 2004) also change patterns of nutrient immobilisation and mineralisation during decomposition. As these processes are tightly coupled to nutrient uptake by mangrove trees (Holguin et al., 2001), changes in nutrient dynamics of decomposing leaves may have important consequences for primary production. Nutrient enrichment therefore could result in complex interactions culminating in net accumulation or net loss of litter and soil organic matter. For example, enrichment in one nutrient may decrease availability of another nutrient through sequestration (immobilisation) or leaching (mineralisation). This, in turn, may slow down primary production, decomposition, or both.

In the present study, we investigated the effects of nitrogen (N) and phos-

phorus (P) enrichment on patterns of litter decomposition and nutrient mineralisation/immobilisation in two mangrove forests on Twin Cays in Belize and on North Hutchinson Island in Florida. Within these mangroves plots have been subject to several years of fertilisation with N or P amendments as described in Feller et al. (2009). Both stands have similar vegetation, dominated by *Avicennia germinans* and *Rhizophora mangle*, but contrast in nutrient limitation and soil carbon content. Both locations show a clear zonation in tree height with trees fringing the coastline being much taller than trees growing further inland. In this very nutrient-limited inner zone, mangrove trees are stunted and growth is limited by phosphorus on Twin Cays and by nitrogen on North Hutchinson Island. Earlier experiments in Twin Cays showed that not only primary productivity, but also decomposition was lower in the inner zones as compared to the fringe zone (Feller et al., 2002). Whether the lower decomposition results from nutrient limitation or other site-specific properties is not known.

Manipulation of nutrient limitations by the long-term fertilisation treatments applied in these locations allows for testing effects of nutrient availability on decomposition without the confounding effects of location and plant species composition. Long-term amendment of nutrients alleviated nutrient limitations at both locations, while control plots are limited by either N or P, depending on zone and location. By comparing decomposition dynamics of litter in the different treatments we can assess i) whether leaf litter decomposition rates are predominantly dictated by nutrient status or other site-specific circumstances, ii) whether the effect of enrichment with a limiting nutrient is element-specific (i.e. whether N- or P-limited systems respond differently) and iii) whether nutrient mineralisation and immobilisation dynamics during the decomposition process are influenced by nutrient enrichment.

We hypothesise that nutrient enrichment affects leaf litter decomposition both directly, via changing the decomposition environment, and indirectly, via changing litter chemistry. We also hypothesise that the effect of nutrient amendment is not nutrient-specific but merely depends on the alleviation of nutrient limitations for primary production and decomposition. Finally, we hypothesise that immobilisation and mineralisation rates of both limiting and non-limiting nutrients are altered if the soil matrix is enriched with a nutrient limiting decomposition.

In order to test these hypotheses, we performed a set of three litterbag experiments at both locations. First, litter was incubated at the exact site where it was produced (Native Litter experiment); second, litter from an unfertilised control plot was incubated in each fertilisation treatment (Common Litter experiment) and third, litter from each treatment plot was incubated at one unfertilised control plot (Common Site experiment). While soil matrix effects are reflected in the Common Litter experiment, litter-mediated effects can be detected in the Common Site experiment and both litter and soil matrix effects with their interactions can be observed in the Native Litter experiment. If a response depends on alleviating a microbial nutrient limitation, the effects of soil and litter enrichment should be identical and non-additive. If a relation depends on litter properties

other than available nutrients, the effect of soil- and litter-enrichment are not expected to be identical and should be additive.

5.2 Materials and Methods

This study was conducted at two mangrove-dominated sites: Twin Cays, Belize, and an impounded mangrove (Mosquito Impoundment 23) on North Hutchinson Island, Indian River Lagoon, Florida, USA. Twin Cays is a relatively pristine, peat-based, 92 hectare archipelago, 12 km offshore (16°50′N, 88°06′W) where it receives no terrigenous freshwater or sediment inputs (McKee et al., 2007). Mosquito impoundment 23 is a 122 hectare abandoned impoundment, located on the lagoon side of North Hutchinson Island, Indian River County, Florida, USA (27°33′N, 80°20′W). Both sites have been the focus of long-term research (e.g. (Feller et al., 2007, 2009)).

On North Hutchinson Island, the soil is highly disturbed with little structure, composed primarily of sand and shell fragments that were deposited when the impoundment was built (Feller et al., 2002, 2003). In contrast, Twin Cays is underlain by deep deposits of mangrove peat, 8 – 12 m thick (Macintyre et al., 2004b; McKee et al., 2007). In spite of their contrasting edaphic properties, vegetation structure is similar at both sites.

Twin Cays and North Hutchinson Island are primarily dominated by Red mangrove (*Rhizophora mangle*) and Black mangrove (*Avicennia germinans*) intermingled with White mangrove (*Laguncularia racemosa*) (Feller et al., 2009). Both sites showed a clear zonation perpendicular to the shoreline. The outer zone of 5 – 20 m consists of a narrow stand of uniformly tall (5 – 6 m) *R. mangle* trees. Further from the shoreline, trees rapidly decrease in height (2 – 4 m) and form a transition zone that varies in width from 5 – 30 m. This transition zone is still dominated by *R. mangle* on Twin Cays and by *A. germinans* on North Hutchinson Island. Further inland, both locations are dominated by stunted trees of low stature ($\leq$ 1.5 m). This so called dwarf zone is dominated by *R. mangle* on Twin Cays and by *A. germinans* on North Hutchinson Island.

Experimental design

The experimental designs are described in detail in Feller et al. (2002, 2003). Briefly, trees for experimental manipulation were chosen at Twin Cays in 1995 and at North Hutchinson Island in 1997. Per location, three transect blocks, 25 – 50 m long, were oriented perpendicular to the shoreline (Fig. 5.1). Each transect block consisted of three parallel transects separated by 10 m buffer zones to exclude effects of possible lateral migration of fertilisers. Transects were subdivided into fringe, transition, and dwarf zones based on tree height as described above. In each zone, three trees per transect were selected to be treated and were fertilised with N, P or were left unfertilised so that in total eighty-one trees per site, nine per transect, were selected to accommodate the experimental treatments. In addition to the above, a reference plot outside the influence of the nutrient enrichment was established in the fringe of both locations. These plots were used for incubation

of litter from other sites and as a source of reference litter. The reference plot on Twin Cays was established in a *Rhizophora*-dominated fringe; the reference plot on North Hutchinson Island was located in an *Avicennia*-dominated fringe site.

The fertilisation treatments (N, P, Control) were randomly assigned to the three transects within each transect block (Feller et al., 2002, 2003). Fertilisation consisted of the semiannual burial of 300 g of urea ($CO(NH_2)_2$) or triple superphosphate ($Ca(H_2PO_4)2{\cdot}H_2O$), leading to the application of 335 g year^{-1} of NH_3 and 452 g year^{-1} of PO_4 respectively.

To deliver the fertiliser to the soil matrix, two 30 cm deep holes, 4 cm in diameter, were made at opposing sides of each tree, beneath the outermost margin of the canopy and approximately 30 cm from a root of the target tree (Fig. 5.1). Twice a year, these holes were filled with the fertiliser contained in dialysis tubing (Spectrapor Membrane Tubing, 50 mm diameter, 6000-8000 MWCO).

Each hole was sealed with soil substrate. For control trees, holes were cored and sealed but no fertiliser was added. This method was based on previous studies (Feller, 1995), in which plant growth dramatically increased in response to addition of the limiting nutrient and small patches of fertilised trees immediately around the target tree were created.

Litter collection and incubation

To quantify the effects of direct, indirect, and interactive effects of soil nutrients and litter quality, three incubation experiments were conducted: i) Native Litter (NL), where litter was incubated at its site of origin; ii) Common site (CS), where litter harvested from different treatments was incubated at a reference plot; iii) Common litter (CL), where litter harvested at the reference plot was incubated in all treatments plots. (See also Table 5.1).

Litter was collected by picking fully senescent leaves directly from the target trees at the experimental sites. Litter from different transects but originating from the same zone, treatment and location, was combined and thoroughly mixed, so that nine different litter types were collected. In addition, litter was collected from the reference plot at each location.

After collection, litter was carefully rinsed and dried 70 °C for 48 h. Approximately 8 – 10 g soil DW leaf litter was included in 1 mm^2 mesh bags of $10 \cdot 10$ cm. Replicate sets of seven bags were deployed randomly among the three replicates per zone at each site where they were collected. To amass enough leaf litter to set up these experiments, senescent leaves were collected over a 6-month period in 2000 and the experiments were deployed in October 2000. These bags were retrieved after 0, 2, 4, 8, 24 and 36 weeks of incubation to determine remaining mass, carbon (C), N, and P. In addition, lignin concentration was determined on the litter from zero-day incubations

Chemical analysis and mass loss determination

After incubation, litterbags were carefully rinsed and dried at 70 °C for 48 h before further processing. Initial weights were corrected for handling losses and initial moisture content to determine initial litter mass. These correction factors were

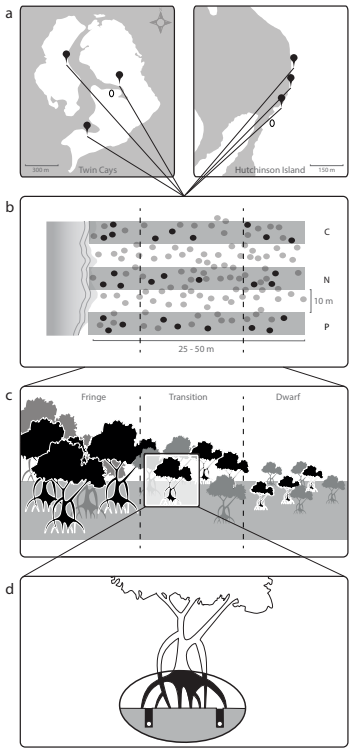


Figure 5.1: a) The setup of the mangrove fertilisation experiment in Twin Cays, Belize and North Hutchinson Island, Florida, USA. Per location, three transect blocks, 25 m to 50 m long, were oriented perpendicular to the shoreline. In addition, a reference plot was established at each location (open circles). b) Each transect block consisted of three parallel transects separated by 10 m buffer zones against possible lateral migration of fertilisers. All transects randomly received one of three fertilisation treatments: Phosphorus (P), Nitrogen (N), and Control (C). c) Transects were subdivided into fringe, transition, and dwarf zones based on tree height. In each transect, three trees per zone were selected for treatment. d) The fertilisation was applied to specific trees by burying fertiliser-filled dialysis tubing near the roots and plugging the hole with soil substrate.

Table 5.1: Experimental setup of litter incubation in mangrove stands at Twin Cays, Belize and North Hutchinson Island, Florida. The setup is repeated for each of the three zones, in each of three transect blocks, at both locations. Within a site, all zones and all transects share a single reference plot.

experiment	origin		incubation	treatment effect
Native Litter (NL)	C N P	$\longrightarrow$ $\longrightarrow$ $\longrightarrow$	C N P	soil nutrients, litter quality
Common Site (CS)	C N P	$\longrightarrow$ $\longrightarrow$ $\longrightarrow$	reference	litter quality
Common Litter (CL)	reference	$\longrightarrow$ $\longrightarrow$ $\longrightarrow$	C N P	soil nutrients

determined on litter from bags that were taken into the field, but not incubated (0 weeks). Litter mass loss was determined by weighing litter after incubation and subtracting the corrected initial weight.

Total C, N, P and lignin concentrations were measured after grinding the litter in a Wiley Mill to pass through a 0.38 mm screen. Concentrations of C and N were determined with a Model 440 CHN Elemental Analyser (Exeter Analytical, North Chelmsford, Mass., USA) at the Smithsonian Environmental Research Center, Edgewater, MD. Concentrations of P were determined using an inductively coupled plasma spectrophotometer (ICP) by Analytical Services, Pennsylvania State University, Pa. Lignin analysis was done at Northern Arizona University, based on methods outlined by Iiyama and Wallis (1990) as modified by Chapman et al. (2003). Briefly, 10 ml of deionised water was added to 10—15 mg of dry, ground litter samples in test tubes. The tubes were then placed in a dry block, heated at 60 °C for 1 h, and stirred at 10 min intervals. Samples were then filtered through GF/A glass fibre filters and rinsed three times with each of the following sequence of solutions: water, ethanol, acetone and diethyl ether. After drying overnight at 70 °C, samples were digested in 25 % acetyl bromide in acetic acid and perchloric acid for 40 min at 70 °C, cooled, diluted with 10 ml of 2 mol l^{-1} NaOH, then brought to 50 ml volume with glacial acetic acid. Samples were filtered and absorbances were read on a spectrophotometer at 280 nm (Spectromax Plus 184 Molecular Devices, Sunnyvale, California, USA). National Institute of Standards and Technology pine standard (NIST no. 1575) was used for calibration.

Statistical Analysis

Results were analysed using R (R Core Team, 2012) and the R packages *nlme* (Pinheiro et al., 2012) for linear-mixed effect modeling, *car* (Fox and Weisberg, 2011) for type II sum of squares and *mgcv* (Wood, 2011) for generalised additive

mixed modeling (GAMM). Hypothesis testing for linear models was done using Wald's χ^2 test with type II sum of squares. Homoscedasticity was confirmed by Levene's test and normality was checked using Shapiro's test.

Treatment effects for GAMM models were tested using a Wald's F test for general additive models. Lignin, N, and P concentrations of fresh litter were analysed using a linear model with treatment, zone and their interactions as fixed effects and transect as a random effect. Changes in absolute N and P content during decomposition were tested separately at the two locations using a GAMM with a cubic regression splined smooth for N and P content, factorial splines and parametric terms for each fertilisation and zone within transect as random effect.

5.3 Results

Litter Quality

Overall, the litter quality on Twin Cays is lower as compared to North Hutchinson Island, with lower N and P concentrations and higher lignin contents (Fig. 5.2, Tab. 5.2). This is also true when comparing the fringe zones, which are dominated by *R. mangle* in both Twin Cays and North Hutchinson Island.

At both locations, the chemical composition of leaf litter significantly depends on zone and nutrient treatments (Tab. 5.2). While fertilisation had a larger effect on litter quality on Twin Cays as compared to North Hutchinson Island, the zonation effect is relatively stronger at North Hutchinson Island.

Within Twin Cays, the initial N response is highly variable with multiple interactions between zone and fertilisation treatment. Nevertheless, initial leaf litter N concentration is clearly higher in the N-fertilised treatments (Fig. 5.2). In the P treatments, the initial N concentration is lower, but this is most apparent in the dwarf zone. On North Hutchinson Island, N concentration is also higher in the N-fertilised plots, but in this location, the effect of zone is stronger. On Twin Cays, P-fertilised trees produced litter with a higher P concentration as compared to other treatments, while on North Hutchinson Island there is no effect of P-fertilisation on litter chemistry. In this location, there is only an effect of zone, with senescent leaves in the transition zone having a higher P concentration.

Decomposition rates

Leaf litter decomposition rates are generally lower on Twin Cays as compared to North Hutchinson Island (Fig. 5.3). However, in the Native Litter experiment (NL), decomposition rates of fringe *R. mangle* litter do not differ between Twin Cays and North Hutchinson Island, while in the common site experiment, *R. mangle* litter decomposes even more rapidly on Twin Cays as compared to North Hutchinson Island (Fig. 5.3, Tab. 5.3).

In each of the three litter-incubation experiments, i.e. with Native Litter, Common Site and Common Litter, decomposition rates significantly differ between fertilisation treatments, zones, or both. On Twin Cays, P-fertilisation is the most important modifier in all three experiments, while on North Hutchinson Island

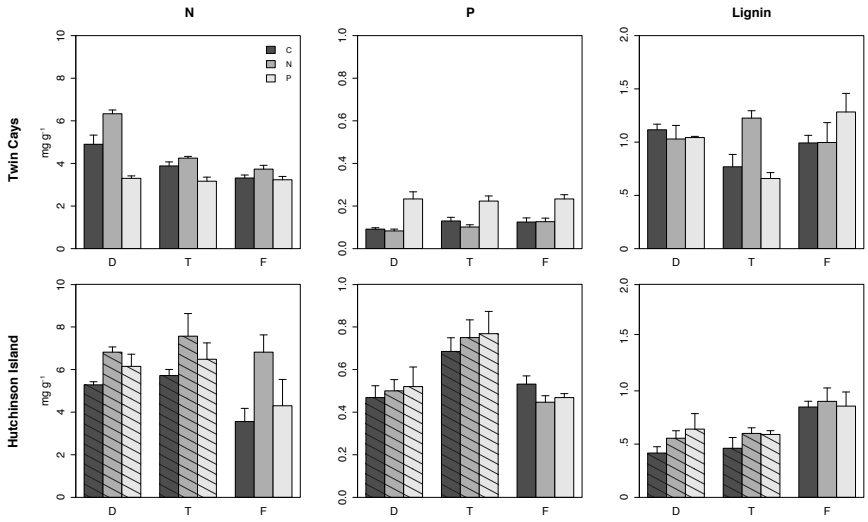


Figure 5.2: Relative nitrogen (N), phosphorus (P), and lignin content of senescent leaves from *Avicennia germinans* (hatched bars) and *Rhizophora mangle* (solid bars) mangrove trees at Twin Cays, Belize and North Hutchinson Island, Florida, USA. Values were measured in control, nitrogen- (N) and phosphorus-fertilised (P) plots in dwarf (D), transition (T) and fringe zones (F).

Table 5.2: Summary of the results of Wald's χ^2 test on linear mixed-effects models examining the initial concentration of nitrogen ([N]), phosphorus ([P]) and lignin of senescent leaves in Twin Cays, Belize (TC) and North Hutchinson Island, Florida, USA (HI). Effects of fertilisation with nitrogen (N) and phosphorus (P) between and within locations are examined. Between locations, only fringe trees were compared, as they were the same species. Within locations, the effect of zonation and its interaction with N or P fertilisation is also examined. Symbols denote significance levels ($^{***}p < 0.001$, $^{**}p < 0.01$, $^{*}p < 0.05$)

		[N]			[P]		lignin	
		Df	χ^2	p	χ^2	p	χ^2	p
Between	location	1	4.12	*	87.91	***	4.49	*
	N:location	2	11.69	**	7.80	*	0.19	
	P:location	2	0.38		17.00	***	2.69	
Within								
TC	N	1	19.62	***	0.81		1.79	
	P	1	23.00	***	77.53	***	0.20	
	zone	2	78.68	***	3.93		5.72	.
	N:zone	2	8.68	*	0.92		6.52	*
	P:zone	2	13.90	***	2.41		3.67	
HI	N	1	13.03	***	0.01		1.65	
	P	1	1.71		0.24		2.54	
	zone	2	8.06	*	33.38	***	21.24	***
	N:zone	2	1.37		1.65		0.23	
	P:zone	2	0.01		1.72		1.39	

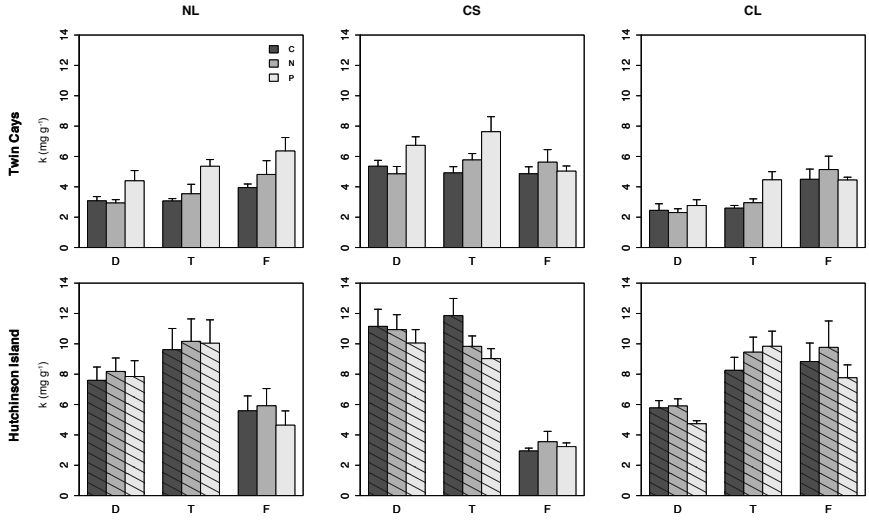


Figure 5.3: Decomposition rate constants (k) of leaf litter produced by *Avicennia germinans* (hatched bars) and *Rhizophora mangle* (solid bars) at Twin Cays, Belize and North Hutchinson Island, Florida, USA. k values were calculated from mass loss at four sampling moments between 64 and 400 days, using a single pool exponential decay model $W_t = W_0 \cdot e^{-kt}$. Both stands showed a distinct zonation perpendicular to the waterfront, differing in hydrology and primary production: fringe (F), transition (T) and dwarf (D) zones. In each zone, plots were fertilised with nitrogen (N), with phosphorus (P) or not fertilised (C). In each location, litter was incubated in its own treatment (Native Litter - NL), in a reference location (Common Site - CS), or litter from the reference location was incubated in the treatment sites (Common Litter - CL).

Table 5.3: Summary of the results of Wald's χ^2 test on a linear mixed-effects model examining the relationship of decomposition rate constant k with nitrogen (N) and phosphorus (P) enrichment in Twin Cays, Belize (TC) and North Hutchinson Island, Florida, USA (HI). Effects of fertilisation with nitrogen (N) and phosphorus (P) between and within locations are examined separately for the three incubation experiments. Between locations, only fringe trees were compared, as they were the same species. Within locations, the effect of zonation and its interaction with N or P fertilisation is also examined.

		Native litter			Common site		Common litter	
		Df	χ^2	p	χ^2	p	χ^2	p
Between	location	1	0.00		25.43	***	6.62	*
	N:location	2	0.71		0.70		0.35	
	P:location	2	7.08	*	0.45		1.09	
Within								
TC	N	1	0.05		0.58		0.30	
	P	1	22.43	***	8.87	**	5.71	*
	zone	2	16.01	***	4.08		41.62	***
	N:zone	2	0.10		2.55		0.77	
	P:zone	2	0.86		3.49		2.71	
HI	N	1	0.83		0.30		1.34	
	P	1	0.71		1.95		0.09	
	zone	2	109.92	***	398.09	***	53.24	***
	N:zone	2	0.06		4.28		0.48	
	P:zone	2	2.85		1.85		4.44	

differences in decomposition rate are only explained by zone. Nitrogen does not significantly control decomposition rates in any of the experiments (Tab. 5.3).

Results from the Native Litter (NL) experiment incorporate both litter quality and environmental influences on decomposition rate. On Twin Cays, decomposition rate is significantly higher in the P-fertilised sites as compared to the other treatments, and also in the fringe zone decomposition rates are significantly higher in comparison to the dwarf and transition zone (Tab. 5.3, Fig. 5.3). On North Hutchinson Island, however, the decomposition rates are markedly lower in the *R. mangle*-dominated fringe, as compared to the *A. germinans*-dominated transition and dwarf zones. Here, fertilisation did not have any significant effect.

The Common Site (CS) experiment investigates the effects of litter quality differences on decomposition rates without the effects of soil conditions, as litter from all treatments is incubated in the same reference plot. On Twin Cays, the effects of fertilisation are similar to those in the NL experiment, i.e. a significant stimulation by the P treatment, except for the fringe zone (Fig. 5.3). On North Hutchinson Island, results from NL and CS show less similarity. Fertilisation did not have a significant effect on decomposition rates (Tab. 5.3), while the decomposition rate constant of fringe-derived (*R. mangle*) litter is significantly lower than that of the other zones (Fig. 5.3).

The Common Litter (CL) experiment tests only environmental effects on decomposition rates, as the incubated litter in all treatment plots is derived from the same reference plot. On Twin Cays, there is a significant zone effect, as litter decomposes faster in the fringe as compared to the other zones. Also, P-availability increases decomposition rate significantly, most notably in the transition zone. On North Hutchinson Island there is again only an effect of zone with the decomposition rate constant of CL incubated in the dwarf zone being notably lower as compared to the other zones.

Nutrient dynamics during decomposition

The absolute amounts of N and P in the decomposing material comprised of plant litter plus microbes in the course of the decomposition process are shown in Figs. 5.4 and 5.5 for Twin Cays and North Hutchinson Island, respectively. Fertilisation treatments are plotted separately, and the significance of treatment effects is given in Table 5.4. Between experiments, overall patterns of N and P dynamics during decomposition are quite similar, whereas between both locations the observed patterns are clearly distinct. On Twin Cays, the absolute amount of N increases during the initial phase of decomposition until almost half of the initial litter weight is lost (Fig. 5.4). At this point, 25 to 50 percent of the N in the litter results from net immobilisation. After this immobilisation phase, N amounts start to decrease. Phosphorus amounts do not increase, but stay more or less constant except for a slight decline after the start of N decrease.

On North Hutchinson Island, there is generally a much less pronounced increase in N content during the initial decomposition phase, but its decline starts around the same stage of proportional weight loss as on Twin Cays. Phosphorus is initially mineralised rapidly on North Hutchinson Island, but this is followed by a

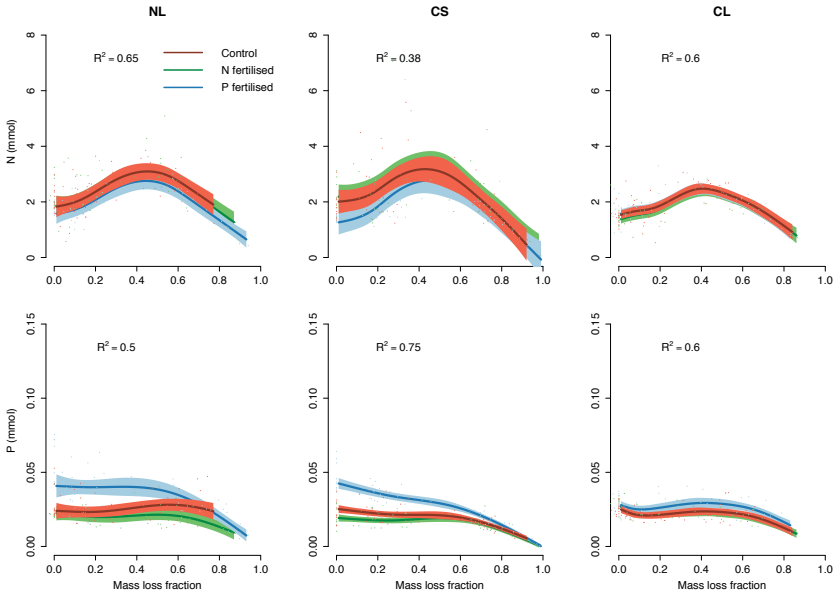


Figure 5.4: Absolute amount of nitrogen (N) and phosphorus content (P) (mmol) against mass loss of mangrove leaf litter during decomposition in litter bags on Twin Cays, Belize. Litter was incubated in its own treatment (Native Litter - NL), in a reference location (Common Site - CS), or litter from the reference location was incubated in the treatment sites (Common Litter - CL). Lines and shaded areas are splined fits with 95 % confidence intervals of the non-parametric term in a model with linear effects of nutrient treatments and zonation. R^2 indicates the goodness of fit.

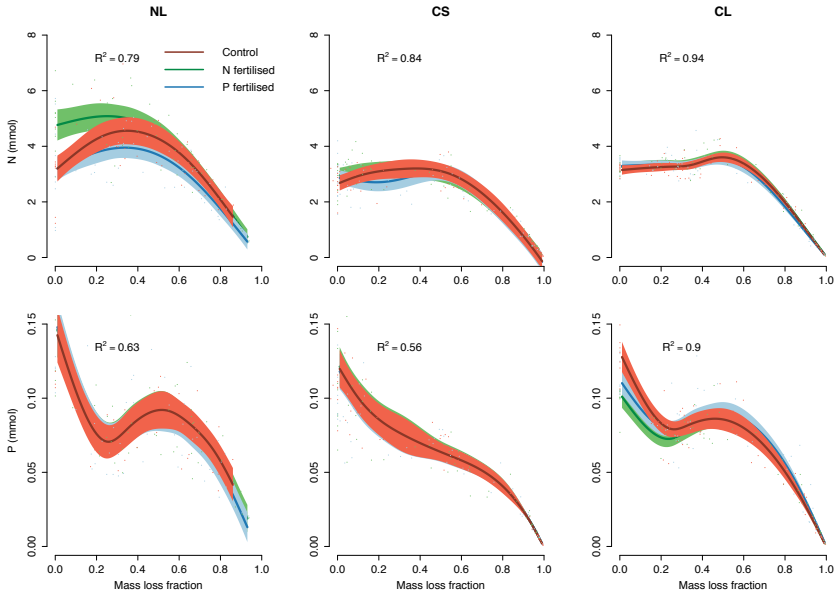


Figure 5.5: Absolute amount of nitrogen (N) and phosphorus (P) (mmol) against mass loss of mangrove leaf litter during decomposition in litterbags on North Hutchinson Island, Florida, USA. Litter from the treatment sites was incubated in its original location (Native Litter - NL), in a reference location (Common Site - CS), or litter from a reference location was incubated in the treatment sites (Common Litter - CL). Lines and shaded areas are splined fits with 95 % confidence intervals of the non-parametric term in a model with linear effects of nutrient treatments and zonation. R^2 indicates the goodness of fit.

net immobilisation phase relatively late in the decomposition process after about one-third of the initial mass is lost. When about half the initial litter mass is lost, both P and N amounts start decreasing (Fig. 5.5).

Within each site, there is little difference in the pattern of immobilisation and mineralisation between fertilisation treatments (Figs. 5.3 and 5.4, Tab. 5.4). In fact, there was only one instance where nutrient dynamics during decomposition significantly deviated between fertilisation treatments: In the CL experiment on North Hutchinson Island both fertilisation treatments showed smaller initial loss of P as compared to the control treatment.

5.4 Discussion

Our study confirms the importance of species identity and location-specific conditions for leaf litter decomposition rates, while it shows that soil nutrient availability and nutrient concentrations of litter are of less importance than expected. We did find an increase in decomposition rates after P-fertilisation at P-limited sites, but such effects were not found for N-fertilisation at N-limited sites.

In accordance with earlier incubation studies at Twin Cays (Middleton and McKee, 2001), we found strong effects of within-stand zonation on decomposition rates at both locations. Based on results from a belowground decomposition experiment (Feller et al., 2002), we expected that the differences between zones were in part driven by differences in nutrient limitation. This was not confirmed by our results. The effects of zonation were not modified by nutrient treatments at either Twin Cays or at North Hutchinson Island, despite the mutual differences in nutrient limitation. This clearly shows that within-stand differences in decomposition rates are driven primarily by environmental factors such as hydrology, soil texture and temperature rather than by differences in nutrient availability.

Nutrient amendments influenced litter chemistry at both sites, but the effect on decomposition rates was rather limited. Despite its strong influence on litter N concentration, N enrichment did not affect litter decomposition rates at either of the locations, while P-addition increased decomposition rates on Twin Cays only.

This result is striking, since litter initial N concentration and N:lignin ratios are often proposed as major factors controlling decomposability of litter (Melillo et al., 1982; Prescott, 2010) and since litter-mediated effects of N enrichment are commonly found to increase decomposition rates in mangroves (Mfilinge et al., 2002; Kristensen et al., 2008). At our sites, we could not confirm these observations. On the contrary, the highest increase in decomposition rates were found in litter produced in response to P enrichment of P-limited sites, which, incidentally, exposed the lowest nitrogen concentrations. Although the phosphorus-induced stimulation of decomposition as found in the present study is less commonly seen, it has been reported in e.g. a P-limited freshwater wetland (Rejmánková and Houdková, 2006) and an Eucalyptus forest (O'Connell, 1993).

The litter-mediated effect of P enrichment was restricted to sites where primary

Table 5.4: Spline fitting results of a GAMM model describing the amounts of nitrogen and phosphorus in mangrove leaf litter during the decomposition process. The fitted model consists of parametric terms for fertilisation treatment, and non-parametric cubic splined smoothing terms, describing the change of absolute nitrogen and phosphorus amounts during the decomposition process. Within the smoothing terms, an overall relation, and deviations for each treatment were fitted: control (C), nitrogen- (N) and phosphorus-fertilised (P). For each line the estimated degrees of freedom, the F-value and p-level is shown. Separate models were fitted for each combination of experimental setup (Native Litter, Common Site and Common Litter) and location (Twin Cays, Belize (TC) and Hutchinson, Florida, USA (HI)). Symbols denote significance levels ($^{***}p < 0.001$, $^{**}p < 0.01$, $^{*}p < 0.05$)

			Native litter			Common site			Common litter		
		Term	*Df*	*F*	*p*	*Df*	*F*	*p*	*Df*	*F*	*p*
TC	Nitrogen	N	1.00	0.12		1.00	1.90		1.00	2.20	
		P	1.00	12.33	***	1.00	9.22	*	1.00	0.00	
		mass loss	3.32	39.85	***	3.30	29.41	***	3.69	45.37	***
		C:mass loss	1.00	0.00		1.00	0.05		1.00	0.00	
		N:mass loss	1.00	0.02		1.00	0.05		1.00	0.00	
		P:mass loss	1.00	0.02		1.00	0.02		1.20	0.01	
	Phosphate	N	1.00	17.41	***	1.00	20.84	***	1.00	0.34	
		P	1.00	41.73	***	1.00	97.56	***	1.00	19.25	***
		mass loss	2.39	8.81	***	3.11	14.17	***	3.65	9.93	***
		C:mass loss	1.00	0.00		1.00	0.35		1.01	0.02	
		N:mass loss	1.00	0.07		2.1	0.21		1.69	0.30	
		P:mass loss	2.19	2.00		1.00	1.33		1.73	0.55	
HI	Nitrogen	N	1.00	7.71	**	1.00	1.34		1.00	1.88	
		P	1.00	6.66	*	1.00	0.59		1.00	0.01	
		mass loss	2.79	18.26	***	3.41	66.95	***	3.54	120.45	***
		C:mass loss	2.08	1.39		2.07	2.16		1.75	0.31	
		N:mass loss	1.00	0.48		1.00	0.55		1.00	0.32	
		P:mass loss	1.00	0.22		3.66	1.44		2.71	1.18	
	Phosphate	N	1.00	0.76		1.00	0.05		1.00	7.37	**
		P	1.00	0.86		1.00	0.04		1.00	0.14	
		mass loss	4.25	22.76	***	4.06	4.22	*	4.46	38.41	***
		C:mass loss	1.00	0.05		1.07	0.26		3.03	2.75	*
		N:mass loss	1.00	0.06		1.06	0.28		1.00	0.17	
		P:mass loss	1.00	0.07		1.08	0.32		1.00	0.21	

production was P-limited before the experiment started as described in Feller et al. (2003). No such effects were found after P amendment in N-limited zones. This shows that the litter-mediated effect of P enrichment depends on alleviation of a nutrient limitation for primary producers. This does not necessarily mean that microbial decomposers are limited by phosphorus. In fact, the results from the CL experiment at Twin Cays suggest that this is not the case. Here, P enrichment of the strongly P-limited dwarf zone did not increase CL decomposition rates. If the increase in decomposition rates found in the NL and CS experiments would have been driven by microbial nutrient limitations, the effects of soil- and litter-enrichment should have been similar, provided that microorganisms had access to both sources.

An alternative explanation for the increased decomposition rates after P amendment is that it results from changes in litter C chemistry induced by alleviation of a nutrient limitation for primary producers (Waring et al., 1985; Entry et al., 1998). In *Sphagnum*, for example, it has been shown that increased N availability reduces polyphenol content (Bragazza and Freeman, 2007), which then accelerated decay.

We did not find direct evidence for such a change in leaf litter C chemistry after fertilisation with a nutrient limiting primary production. Lignin content, which is an important determinant of litter recalcitrance (Berg and Laskowski, 2005), did not change with nutrient treatments. The idea of nutrient-induced C chemistry change is, however, supported by the results from the CS experiment at Twin Cays. In this experiment, where litter from different sources decomposed in a reference plot, litter originating from P-fertilised trees in P-limited zones decomposed faster as compared to other litter types, despite the fact that primary production in the reference plot is N- and not P-limited.

The limitation for microbial decomposers can be derived from nutrient content of the litter-decomposer complex during the decomposition process. These changes are brought about by microbial growth and their need for nutrient and or energy: A nutrient in excess is promptly leached from this complex (mineralisation), whereas nutrients limiting decomposers are kept in the complex, or even absorbed from the environment (immobilisation). These patterns generally show a shift from nutrient limitation where nutrients are immobilised to a phase of energy limitation where nutrients are mineralised. Hence, an increase in immobilisation rate and an earlier onset of mineralisation is expected if more nutrients are available. To facilitate comparability between nutrient dynamics of litter with different decomposition rates, we have expressed the N and P content of the leaf-decomposer complex as a function of mass loss, rather than as a function of time.

Strikingly, mineralisation and immobilisation patterns of nutrients were hardly modified by external nutrient amendment. Phosphorus was not immobilised in the initial phase of decomposition, and higher initial amounts in litter from P-treated sites converged to those of untreated sites. To our surprise, the location (Twin Cays or North Hutchinson Island) where decomposition took place was

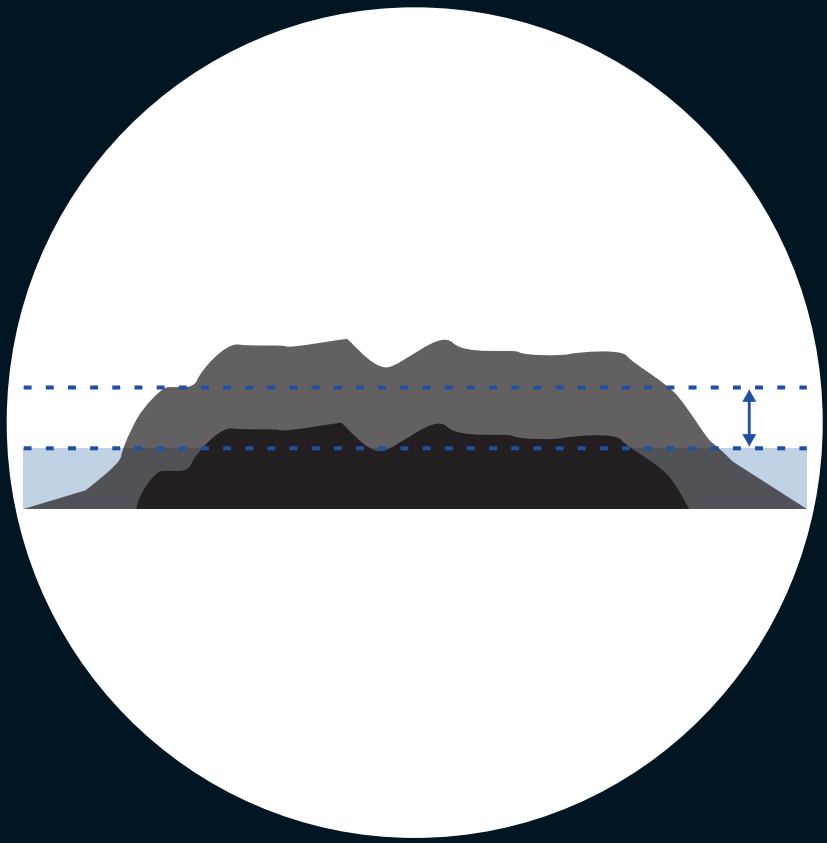
a much better predictor of the observed mineralisation/immobilisation patterns. While on Twin Cays, decomposition started with a clear immobilisation phase of N, with N uptake from the environment. On North Hutchinson Island, P was immobilised from the environment somewhat later in the decomposition process. As this pattern is fairly constant between nutrient treatments, it is unlikely that microbial activity was simply limited by nutrient availability.

Still, the consistent initial build-up of N on Twin Cays indicates that here N, and not P, is limiting microbial decomposition. The observed increase of decomposition rate of litter produced by P-amended trees in P-limited zones therefore must have been driven by changes in C quality rather than by microbial nutrient availability. At North Hutchinson Island, the build-up of P was accompanied by an increase in iron (Fe) content (data not shown). This leads us to believe that a chemical rather than a biological process has driven this late immobilisation of P. The sites at North Hutchinson Island are irregularly flooded, so it is well possible that such a flooding event caused precipitation of Fe-P complexes on litter.

Summarising, we can conclude that the consequences of eutrophication on litter decomposition are fairly limited. Nutrient dynamics during decomposition are not modified by nutrient enrichment. Increased decomposition rates of leaf material in mangrove ecosystems only occurs in P-limited mangroves and is controlled by changes in litter quality rather than by making more P available to microbial decomposers. In addition, our result show that decomposing microorganisms can be N-limited even in sites were primary production is P-limited.

5.5 Acknowledgements

The technicians of the Smithsonian Environmental Research Centre, who put great effort in collecting and processing the leaf material used in this study. We would also like to thank Annick Spoelstra for the designing and drawing Fig. 5.1 of this chapter.



Chapter 6

Increased decomposition rate below *Avicennia* stands as compared to *Rhizophora* stands: A mechanism for cyclic succession in peat-forming mangroves

J.A. Keuskamp, I.C. Feller, B.J.J. Dingemans, H.J. Laanbroek, J.T.A. Verhoeven and M.M. Hefting

Abstract Mangrove trees typically grow in monospecific stands, giving rise to forests with a well-defined spatial structure. In the western hemisphere, *Avicennia* spp. are typically dominant in the higher and *Rhizophora* spp. in the lower intertidal zone. Paleo-ecological evidence shows that species dominance is dynamic in time, which is often attributed to catastrophic events. In this paper, we present an alternative autogenic mechanism for cyclic succession patterns in peat-based mangrove systems driven by negative reciprocal effects of species dominance induced by differences in peat decomposition rates. The presented mechanism implies that *Avicennia*-derived peat decomposes more rapidly than *Rhizophora*-derived peat. To test this, we measured peat recalcitrance, exoenzyme activity, microbial biomass and decomposition rates in peat soils underlying *Avicennia germinans* and *Rhizophora mangle* stands on Twin Cays, a group of peat-forming islands in Belize. We confirmed that peat underlying *Avicennia* stands is chemically more labile than peat underlying *Rhizophora* stands and decomposes more rapidly. The proposed mechanism is therefore supported by our measurements and matches field observations of mangrove successional patterns. Cyclic succession may play an important role in peat-building and shaping community structure of mangroves.

6.1 Introduction

Mangroves comprise a heterogeneous group of woody plants that are able to grow in the saline, waterlogged conditions of the intertidal zone throughout most of the tropics. Typically, mangrove forests consist of more or less monospecific stands forming banded vegetation zones parallel to the shoreline (Estrada et al., 2013). A number of different mechanisms responsible for this zonation have been proposed, such as species-specific differences in propagule predation (Smith III, 1987), dispersal and seedling establishment (Jiménez and Sauter, 1991; Sousa et al., 2007), salinity tolerance (Ball, 1988), nutrient efficiency (Chen and Twilley, 1999; Feller et al., 2003) and flooding tolerance (McKee, 1993). The relative importance of those mechanisms is still under debate, resulting from the fact that many of these factors covary in the zonation. Nevertheless, flooding tolerance and soil elevation are generally considered to be critical factors in determining species dominance (Krauss et al., 2008; Crase et al., 2013).

In the western hemisphere, zonation is characterised by the dominance of *Avicennia* spp. in the higher intertidal zone and *Rhizophora* spp. in the lower intertidal zone, where inundation exerts a stronger influence. *Rhizophora* produces more recalcitrant litter with higher amounts of tannins as compared to *Avicennia* (Erickson et al., 2004; Fernando and Bandeira, 2009; Barroso Matos et al., 2012). As a result, decomposition rate of soil organic matter (SOM) underlying *Rhizophora* stands is expected to be substantially lower than that of peat underlying *Avicennia* stands. In carbonate environments, mangroves keep up with sea level rise through peat formation (Woodroffe, 1990), which is the net result of mangrove litter production, erosion and decomposition (McKee and Faulkner, 2000b; McKee et al., 2007). This implies that an increase in decomposition rates slows down accretion rates or even leads to subsidence if historically formed peat is broken down.

If *Rhizophora* builds up peat, this changes the environment in a direction where inundation time and height decline through time, so that eventually *Avicennia* becomes the stronger competitor, replacing *Rhizophora*. The higher decomposition rates in the presence of *Avicennia* in turn lead to soil subsidence relative to the sea level, accompanied by increasing flooding towards a point where this species does not survive. The disappearance of vegetation even accelerates subsidence rates (Cahoon et al., 2003). This leads to increased inundation, eventually facilitating *Rhizophora* recruitment. Thus, reciprocal effects of these two genera on relative soil level may lead to a cyclic succession (Fig. 6.1).

The concept of cyclic succession was first introduced by Watt (1947), who argues that cyclic succession occurs when the species involved are self-inhibitory and at some point develop under conditions imposed by their neighbours. In other words, cyclic succession does not depend on external disturbances but is driven by internal processes. In the context of mangroves, cyclic succession has often been associated with repeated catastrophic events such as hurricanes (Lugo, 1980), which because of its dependence on external disturbance, is an example of repeated secondary succesion rather than autogenic cyclic succession.

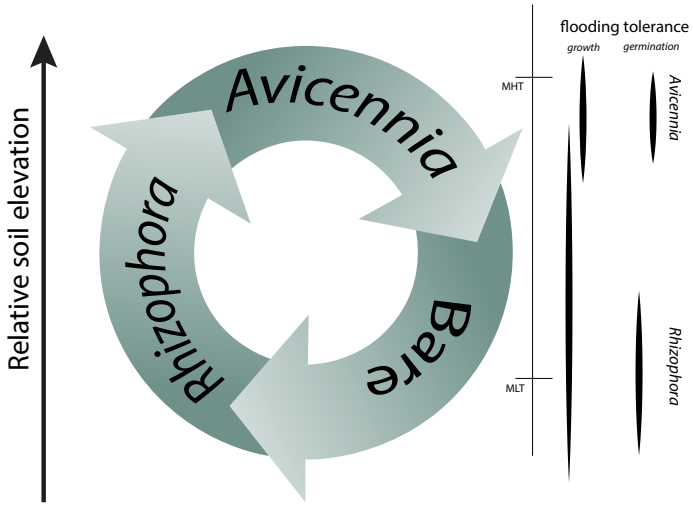


Figure 6.1: Cyclic succession in peat-based mangrove systems driven by autogenous processes. The lower decomposition rates of peat underlying *Rhizophora mangle* stands as compared to *Avicennia germinans* stands, lead to changes in relative soil elevation until the point where the inundation pattern is outside the tolerance level for growth and germination of the dominant species. MHT = Mean High Tide, MLT = Mean Low Tide

Based on paleo-ecological and field studies, McKee and Faulkner (2000a) already suggested the occurrence of repetitive succession patterns of *Avicennia germinans* and *Rhizophora mangle* in peat-based mangrove islands of the Caribbean, and attributed that to the buildup of toxic compounds in the soil. In this paper, we present an alternative, autogenic mechanism for cyclic succession patterns in peat-based mangrove systems driven by negative reciprocal effects of species dominance on peat decomposition rates.

This mechanism would imply that decomposition rate is higher for peat underlying *Avicennia* vegetation than for peat underlying *Rhizophora* vegetation with a comparable production rate. In this study we tested this hypothesis by measuring chemical characteristics and indicators of microbial decomposition activity (i.e., microbial respiration and biomass, enzyme activities) in peat underlying *Avicennia germinans*- and *Rhizophora mangle*-dominated sites on Twin Cays, typical examples of oceanic peat-based mangrove islands without terrigenous inputs as found in the Caribbean sea (Macintyre et al., 2004a).

6.2 Material and Methods

Site description

The study was conducted at Twin Cays, an archipelago of peat-based islands located at 16 km west off the Belizean coast. Twin Cays does not receive terrigenous inputs and its underground entirely consists of mangrove-formed peat (Macintyre

et al., 2004a). The vegetation of the island mainly consists of *A. germinans* and *R. mangle* mangrove trees. Although the fringe is mostly dominated by *R. mangle*, the interior consists of a patchy distribution of monospecific stands of either species. *R. mangle* stands show a clear internal structure with declining heights with distance from the coast, while such a pattern was absent in the *A. germinans* stands. Detailed information with respect to zonation, hydrology, edaphic properties and climate can be found in McKee (1995b), Feller et al. (1999, 2002, 2009), Feller et al. (2002), Macintyre et al. (2004a), Rodriguez and Feller (2004), McKee et al. (2007) and Lee et al. (2008).

To characterize the mangrove forests and the chemical, structural, and microbial properties of mangrove soil at Twin Cays, six rectangular plots, three for each species, with an approximate length of 10 m were laid out in the *A. germinans* and *R. mangle* stands at least 30 m from the coast. In each plot, three equidistant trees were selected resulting in 18 sub-plots, formed around nine trees of either species. To compare forest structure in the two types of stands we measured average tree height and density were measured in a $2 \cdot 2$ m quadrant surrounding each plot. These parameters were subsequently used to estimate total biomass per m^2 using the allometric equations in (Smith III and Whelan, 2006).

Soil and pore water sampling

In March 2013, samples were taken within a 10 cm wide circle around a tree. Porewater and soil from the top 10 cm were collected using 10 cm long rhizons (Eijkelkamp BV, Giesbeek, The Netherlands) and a stainless steel corer respectively. Samples were stored at 3 °C within 12 h. Relative soil elevation was determined using actual and modelled inundation depths as described in Chapter 3 of this thesis.

Edaphic properties and chemical analyses

Intact cores were weighed to determine bulk density, while all other measurements followed homogenisation and removal of roots exceeding a diameter of 2 mm. Gravimetric soil moisture was calculated from weight loss after 48 h of oven drying at 70 °C. Prior to further chemical and elemental analyses, soils were freeze-dried and ground using an MM200 mixer mill (Retsch GmbH, Haan, Germany).

Soil organic C (SOC) and soil organic N (SON) were determined with an EA/110 elemental CN analyser (InterScience BV, Breda, The Netherlands), following removal of calcium carbonate with a 32 % HCl solution. Total and water-soluble phenolic compounds were extracted with a 50%-50% methanol-water solution and deionised water, respectively. After extraction, phenolic compounds were measured using the Folin-Ciocalteu reagent for phenolic compounds as described in Cicco and Lattanzio (2011). Concentrations of pore water nutrients and dissolved organic carbon and nitrogen (DOC/DON) were measured on a continuous flow auto analyser (Skalar SA-40, Breda, The Netherlands).

Peat recalcitrance was determined using a sequential extraction (Ryan et al., 1990; Shaver et al., 2006) on freeze-dried soil samples. The procedure divides peat in five fractions: non-polar extractives (NPE), water-solubles (WS), acid-solubles (AS), acid-insolubles (AIS) and the mineral fraction (MF). The NPE (e.g. fats

and waxes) and WS (e.g. simple sugars and phenolic compounds) fractions were sequentially extracted for 24 h with a Soxhlet apparatus, using dichloromethane and deionised water as solvents, respectively. Sulphuric acid (72%) was used to extract the AS (e.g. cellulose) fraction. The remaining material was heated to 550 °C to determine (AIS (e.g. lignin) and MF). The carbon fractions were corrected for the ash content.

Soil incubation and respiration measurement
To quantify soil respiration rates, soil cores from *A. germinans*- and *R. mangle*-dominated sites were incubated at 20 °C either without amendment, or with amendment of 5 nmol g^{-1} C as glucose. During the 150 −hour incubation period, CO_2 production and O_2 consumption were measured at intervals of 130 minutes using a respirometer (Biometric Systems, Germany) equipped with optical CO_2 and O_2 sensors.

Basal respiration (BR) was quantified as the average respiration rate over 24 h in the incubation without glucose amendment. As disturbance effects led to elevated respiration during the first day of incubation, BR was calculated based on respiration rates measured on the second day of incubation. The metabolic quotient (q_{CO_2}), was calculated as BR/C_{micr} with C_{micr} microbial carbon per gram soil(Anderson and Domsch, 1985). Respiration quotient q_C was calculated as $BR/SOCcontent$ (Anderson and Domsch, 1986). Microbial respiratory quotient (RQ) was measured as (CO_2 consumed /O_2 produced) in mol mol^{-1} (Dilly, 2001).

Microbial biomass and stoichiometry
To quantify differences in microbial communities in *A. germinans* vs. *R. mangle* peat, microbial biomass and stoichiometry were determined using a fumigation-extraction procedure adapted from (Vance et al., 1987) as also described in Chapters 1 & 2. In short, microbial nitrogen (N), phosphorus (P), and carbon (C) was calculated from extracted C, N and P after chloroform fumigation correcting for the extractability of microbial compounds according to Brookes et al. (1982, 1985); Vance et al. (1987).

Potential enzyme activities
To quantify the role of enzyme activities in peat breakdown in the *A. germinans* and *R. mangle* plots, potential activities of six hydrolytic enzymes were measured on fresh soil samples and again after 96 h of incubation. The enzymes measured and the substrates used are listed in Table 6.1. Measurements were conducted following the protocol modified from (Allison and Vitousek, 2004) as described in Chapter 3 of this thesis.

Data analysis & Statistics
Results were analysed using R (R Core Team, 2013) and the R packages *nlme* (Pinheiro et al., 2012) for linear-mixed effect modeling and *car* (Fox and Weisberg, 2011) for type II sum of squares. Data were fitted to linear mixed-effects models with species as fixed factor and plot as random factor where appropriate. Levene's test was used to confirm homoscedacity. Normality of residuals was checked using

Table 6.1: Enzymes considered in this study, with the abbreviations as used in the text, enzyme commission (EC) numbers, and the substrates that were employed to measure their potential activity.

name	abbr.	EC	substrate
β-1,4-glucosidase	BG	3.2.1.14	pNP-β-glucopyrasonide
alkaline (acid) phosphatase	AP	3.1.3.1	pNP-phosphate
cellobiohydrolase	CBH	3.2.1.91	pNP-cellobioside
glycine aminopeptidase	GAP	3.2.1.14	glycine p-nitroanilide
leucine aminopeptidase	LAP	3.4.11.1	leucine p-nitoranilide
β-N-acetylglucosaminidase	NAG	3.2.1.52	pNP-β-N-acetylglucosaminide

Shapiro's test. In the enzyme analysis, values deviating more than two times the interquartile range from the first or the third quartiles were removed to avoid strong biases due to outliers. Species effects for random models were tested using Walds χ^2 test with type II sum of squares.

6.3 Results

Vegetation, edaphic properties and nutrient status

Avicennia germinans trees were higher and had a lower density as compared to *Rhizophora mangle* trees, but both density and height varied considerably within species (Tab. 6.2). Estimated total biomass per □m did not differ significantly between the two species ($\chi^2 = 0.41$), but within species variation was significant for both *A. germinans* ($F = 16.7$, $p < 0.01$) and *R. mangle* ($F = 8.4$, $p < 0.05$). As expected, *A. germinans* trees were taller than *Rhizophora* trees ($\chi^2 = 4.44$, $p < 0.05$), but again within species variation was large and significant (*A. germinans* ($F = 21.5$, $p < 0.01$), *R. mangle* ($F = 7.7$, $p < 0.05$)).

Despite the large difference in relative soil elevation, pore water nutrient and phenolic compound concentrations were surprisingly similar between the *R. mangle*- and the *A. germinans*-dominated sites with no significant differences detected. However, the relative density and N content of the peat underlying *A. germinans* was significantly higher than the peat underlying *R. mangle* stands (Tab. 6.3). Phenolic compound (i.e. tannins) concentrations are substantially higher in *Rhizophora* than in *Avicennia*, although this is not significant due to

Table 6.2: Site properties of *Avicennia germinans*- and *Rhizophora mangle*-dominated stands on Twin Cays, a peat-based mangrove island in the Caribbean Sea, 14km off the coast of Belize ($n = 9$). Relative surface elevation is defined against the Mean Lower Low Water (MLLW) level.

Property	*Avicennia*	*Rhizophora*	Unit
stem height	1.8 – 2.8	0.4 – 1.0	m
tree density	0.8 – 1.5	1.0 – 1.7	ind. m^{-2}
aboveground biomass	0.1 – 1.3	0.1 – 0.8	kg DW m^{-2}
relative surface elevation	1 – 10	-14 – 2	cm

high variance. Also the organic content of the peat was quite similar, although the peat underlying *A. germinans* stands had significantly higher WS ($\chi^2 = 4.1$, $p < 0.05$) and AS fractions ($\chi^2 = 7.4$, $p < 0.001$). The larger NPE fraction of *R. mangle* borders significance with $\chi^2 = 3.2$, $p < 0.1$ (Fig. 6.2).

Microbial biomass and stoichiometry
Microbial biomass under *A. germinans* is four times as high as compared to *R. mangle* stands (Fig. 6.3a). Relative microbial N content was not significantly different between *R. mangle* and *A. germinans* plots. In both stands, the microbial biomass had an N content lower than average for marine microorganisms (Fagerbakke et al., 1996) indicating N limitation (Chapter 2) (Fig. 6.3b). In the *A. germinans* stands, relative microbial P content was about average for marine microorganisms (Fagerbakke et al., 1996), while in the *R. mangle* stands relative P content of microbial organisms was higher than average. The relative microbial P content in the *A. germinans* stands was significantly higher than in the *R. mangle* stands ($\chi^2 = 17.7$, $p < 0.001$). Consequently, microbial N/P ratio was significantly lower in the *A. germinans* stands ($\chi^2 = 23.98$, $p < 0.001$) (Fig. 6.3d).

Microbial Respiration
Cumulative microbial respiration during four days of incubation was higher in *A. germinans* as compared to *R. mangle* soil samples ($\chi^2 = 7.68$, $p < 0.01$). Respiration was substantially higher in the glucose-amended incubations, but the species effect was no longer significant ($\chi^2 = 0.12$, $p = 0.72$) (Fig. 6.4a). Glucose amendment did not provoke a respiration peak but rather increased respiration over the total duration of the incubation (data not shown). The respiration quotient (RQ) was higher in peat underlying *Avicennia* plots than in the peat from *Rhizophora* plots ($\chi^2 = 15.11$, $p < 0.001$) (Fig. 6.4b, Tab. 6.4). RQ was substantially higher in the glucose amendment treatments as can be expected when glucose is consumed, but the RQ was still larger in peat underlying *A. germinans* stands as compared to the *R. mangle* stands ($\chi^2 = 10.44$, $p < 0.01$).

In the *A. germinans* stands, microbial respiration per unit of microbial biomass (q_{CO_2}) was low compared to the *R. mangle* stands. However, microbial biomass and respiration relative to soil organic carbon ($C_{mic} : C_{org}$ and q_C respectively) were significantly higher in the *A. germinans* stands (Tab. 6.4). The higher q_C of *A. germinans* translates to an expected half life of approximately 9 years for peat below *A. germinans* and 13 years for peat below *R. mangle*.

Exoenzyme activities
Potential enzyme activities of AP and GAP were higher in *A. germinans* stands than in *R. mangle* stands indicating a higher metabolic effort to acquire N and P from peat (Fig. 6.5, Tab. 6.5), while potential activities of CBH, BG, NAG and LAP did not show a significant difference between the tree species. Glucose stimulates AP in both *A. germinans*- and *R. mangle*-dominated sites, but the effect of glucose on the other enzymes was not significant.

Table 6.3: Edaphic properties of *Avicennia germinans*- and *Rhizophora mangle*-dominated stands on Twin Cays, Belize (n = 9) with standard errors. Significant difference between sites are indicated in the last column. ($^{***}p < 0.001$, $^{**}p < 0.01$, $^{*}p < 0.05$, $^{\cdot}p < 0.1$)

Property	*Avicennia*	*Rhizophora*		Unit
pH	6.3 ± 0.2	6.7 ± 0.1	-	
bulk density	0.20 ± 0	0.16 ± 1	***	g soil DW cm^{-3}
water content	0.78 ± 0	0.84 ± 0	***	$g\,g^{-1}$
NH_4^+	82 ± 8	50 ± 7	·	$\mu mol\,l^{-1}$
NO_3^-	<1	<1		$\mu mol\,l^{-1}$
PO_4^{3-}	<1	<1		$\mu mol\,l^{-1}$
DOC	26 ± 4	40 ± 6		$mg\,C\,l^{-1}$
DON	<0.2	<0.2		$mg\,N\,l^{-1}$
soluble phenolics	6.9 ± 1.0	9.4 ± 2.0		μg TAE g soil DW^{-1}
insoluble phenolics	2.0 ± 1.5	4.7 ± 1.3		μg TAE g soil DW^{-1}
C	353 ± 10	341 ± 4		mg g soil DW^{-1}
N	18 ± 1	14 ± 0	***	mg g soil DW^{-1}
CN	25.9 ± 0.7	33.1 ± 0.4	*	$mol\,mol^{-1}$

SOC: Soil Organic Carbon, DOC: Dissolved Organic Carbon SON: Soil Organic Nitrogen, DON: Dissolved organic nitrogen, TAE: Tannic acid equivalents, DW: Dry weight

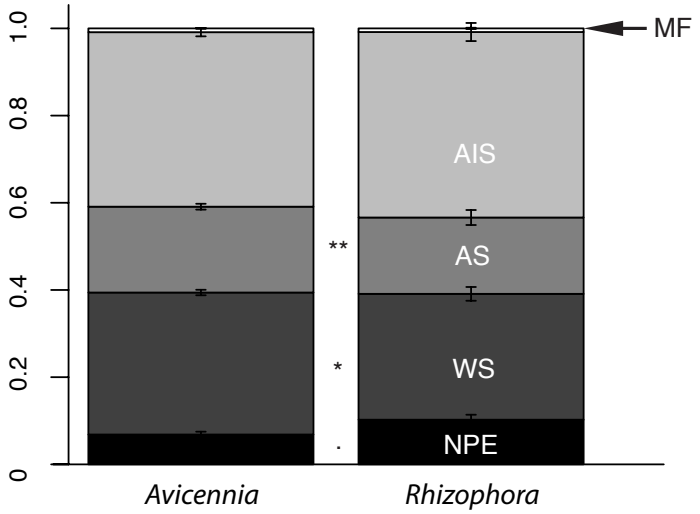


Figure 6.2: Fractionation of *Avicennia germinans* and *Rhizophora mangle* as determined by sequential carbon extraction (Ryan et al., 1990). NPE = Non-Polar Extractives, WS = Water Solubles, AS = Acid Solubles, AIS = Acid Insolubles, MF = Mineral Fraction. Significant differences are indicated between bars ($^{***}p < 0.001$, $^{**}p < 0.01$, $^{*}p < 0.05$, $^{\cdot}p < 0.1$).

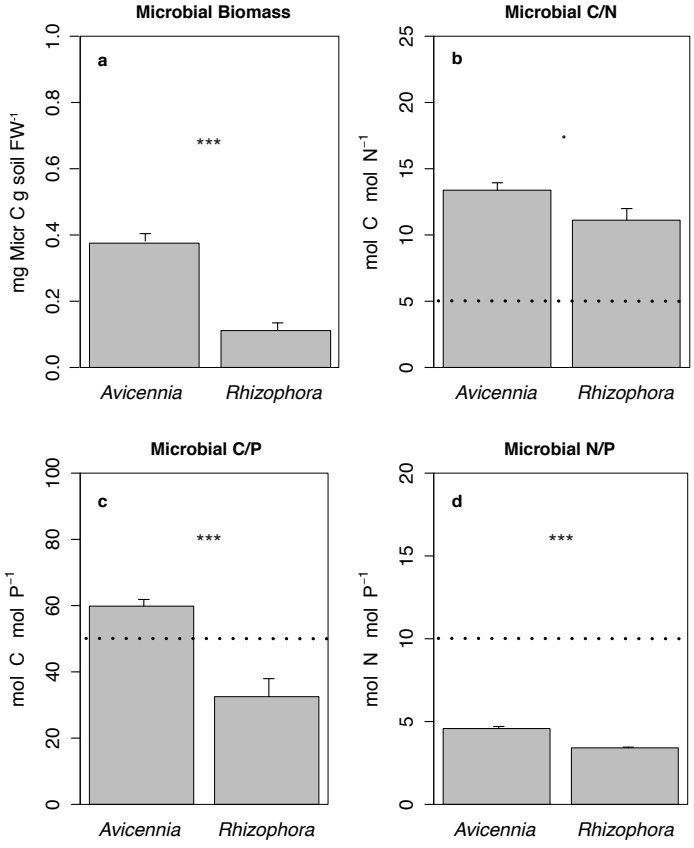


Figure 6.3: Soil microbial characteristics in *Avicennia germinans*- and *Rhizophora mangle*-dominated sites at Twin Cays, Belize. The horizontal lines indicate average values for marine bacteria as provided in (Fagerbakke et al., 1996). Symbols denote significance of difference between *Avicennia*- and *Rhizophora*-dominated sites ($^{***}p < 0.001$, $^{**}p < 0.01$, $^{*}p < 0.05$, $^{\cdot}p < 0.1$).

Table 6.4: Microbial characteristics as measured in peat below *Avicennia germinans* and *Rhizophora mangle* stands on Twin Cays, Belize. Respiration rates are measured as CO_2-C. Significant differences between species are indicated using symbols ($^{***}p < 0.001$, $^{**}p < 0.01$, $^{*}p < 0.05$, $^{\cdot}p < 0.1$).

Property	*Avicennia*	*Rhizophora*		Unit
BR	4.9 ± 0.4	3.3 ± 0.4	**	µg C h^{-1} g soil DW^{-1}
C_{micr}	1.7 ± 0.1	0.7 ± 0.1	***	mg C_{mic} g soil DW^{-1}
q_{CO_2}	3.0 ± 0.3	6.4 ± 1.2	*	mg C h^{-1} g ${C_{mic}}^{-1}$
$C_{mic} : C_{org}$	5.7 ± 0.3	2.0 ± 0.4	***	mg C_{mic} g SOC^{-1}
q_C	13 ± 1	9 ± 1	*	µg C h^{-1} g SOC^{-1}
RQ	0.66 ± 0.05	0.45 ± 0.06	***	mol CO_2 mol ${O_2}^{-1}$

BR = Basal respiration, C_{micr} = microbial biomass, q_{CO_2} = metabolic quotient, $C_{mic} : C_{org}$ = mass ratio of microbial biomass C and SOC, q_C = relative carbon use, RQ = microbial respiratory quotient

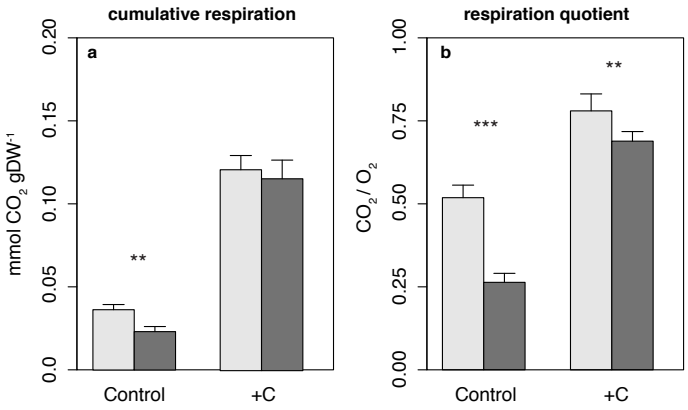


Figure 6.4: a) Cumulative microbial respiration and b) respiration quotient (RQ) of incubated soils from sites dominated by *Avicennia germinans* (light bars) and *Rhizophora mangle* (dark bars) at Twin Cays, Belize. Incubations lasted for 4 days at 20 °C with or without amendment of glucose (C). Significant differences between soils are indicated by symbols ($^{***}p < 0.001$, $^{**}p < 0.01$, $^{*}p < 0.05$, $^{\cdot}p < 0.1$).

Table 6.5: ANOVA-table with χ^2 values and significance levels in a mixed effects model of potential enzyme activities in *Avicennia germinans*- and *Rhizophora mangle*-dominated sites on Twin Cays, Belize. Species dominance and glucose amendment were modelled as fixed factors, with site as a random factor. Symbols denote significance levels ($^{***}p < 0.001$, $^{**}p < 0.01$, $^{*}p < 0.05$, $^{\cdot}p < 0.1$).

	CBH	BG	AP	NAG	LAP	GAP
Species	1.65	2.04	29.24 ***	0.67	0.91	34.93 ***
Glucose	0.13	2.49	36.28 ***	0.14	3.78 ·	3.19 ·
Glucose · Species	0.03	0.40	0.00	0.21	6.28 *	3.46 ·

AP = phosphatase, NAG = β-N-acetylglucosaminidase, LAP = leucine aminopeptidase, GAP = glycine aminopeptidase, CBH = cellobiohydrolase, BG = β-1,4-glucosidase

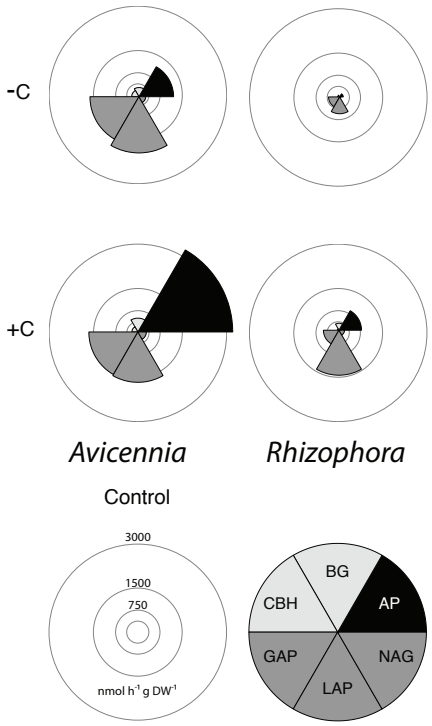


Figure 6.5: Potential activities of microbial hydrolytic exoenzymes involved in acquiring carbon (light-grey), nitrogen (dark-grey) and phosphorus (black) in soils dominated by *Avicennia germinans* (left panel) or *Rhizophora mangle* (right panel). Soils were incubated for 4 days with or without amendment of glucose (+C and -C, respectively). Enzymes are plotted as circle segments with radius indicating potential activity (AP = phosphatase, NAG = β-N-acetylglucosaminidase, LAP = leucine aminopeptidase, GAP = glycine aminopeptidase, CBH = cellobiohydrolase, BG = β-1,4-glucosidase).

6.4 Discussion

The soil of Twin Cays largely consists of mangrove-formed peat, on top of which *A. germinans* and *R. mangle* trees grow in a patchy distribution of monospecific stands. Despite the differing chemical quality of the litter produced, the chemical composition of the peat underlying stands of either species was surprisingly similar. Still, there are various important differences. Peat underlying *A. germinans* stands does not contain as much phenolics (i.e. tannins) and has a higher N content and a larger water-soluble and hydrolysable fraction. All of these indicate a higher decomposability of peat underlying *A. germinans* stands as compared to *R. mangle* stands.

Our results on microbial activity indicate that the rate of peat decomposition is indeed higher in *A. germinans* stands. The microbial biomass and respirations associated with the *A. germinans* stands are more than twice as high as compared to *R. mangle*-dominated stands. As a result, the expected half life of C in the peat underlying *A. germinans* stands is 30 % lower as compared to peat underlying *R. mangle* stands. The relative oxygen consumption (RQ) of peat in *A. germinans* stands is higher than that of *R. mangle* stands, which indicates oxidation of less refractory compounds (Dilly, 2001), and thus confirms the higher decomposability of *A. germinans* peat. The higher potential enzyme activities further support the observation that decomposition rates of peat are higher in *A. germinans* stands. Both P- and N- acquiring enzymes were more active in peat underlying *A. germinans* than in peat underlying *R. mangle*, which also shows that under *A. germinans* stands non-soluble, structural elements of peat are broken down at a higher rate.

The higher decomposition rate in peat underlying *A. germinans* as compared to peat underlying *R. mangle* stands in itself does not prove that this drives cyclic succession in peat-forming mangroves. It does provide, however, a sound and falsifiable mechanism explaining field observations of succession patterns on various peat-forming mangrove islands in the Caribbean. The succession of *Rhizophora* spp. by *Avicennia* spp., followed by dieback of *Avicennia* and recolonisation by *Rhizophora*, has already been described by Cameron and Palmer (1995) and McKee and Faulkner (2000a). It has also been noted that in many cases, the peat underlying *Avicennia* stands is often *Rhizophora*-derived (Woodroffe, 1981; McKee and Faulkner, 2000a), an indication that vertical accretion mainly took place during dominance of *Rhizophora*. Furthermore, the difference in decomposition rates fits the observation that paleo-ecological studies of mangrove-derived peat layers in the Caribbean went through cyclic periods of fast and slow vertical accretion (Cameron and Palmer, 1995).

Based on our results, a cyclic succession pattern driven by changes in relative soil elevation implies that dominance duration is modified by changes in sea water level. Thus, during periods of relatively rapid sea level rise, the succession may be arrested in the phase where *Rhizophora* spp. are dominant, while *Avicennia* spp. dominance may be favoured during periods of relatively slow sea level rise. But even without such a cyclic pattern, the observed differences in peat decomposition

rates have important consequences for the resilience of mangroves to long-term changes in sea water level which is critical for stability of peat-based mangrove ecosystems and their conservation as a carbon sink in the face of a changing climate.

6.5 Acknowledgements

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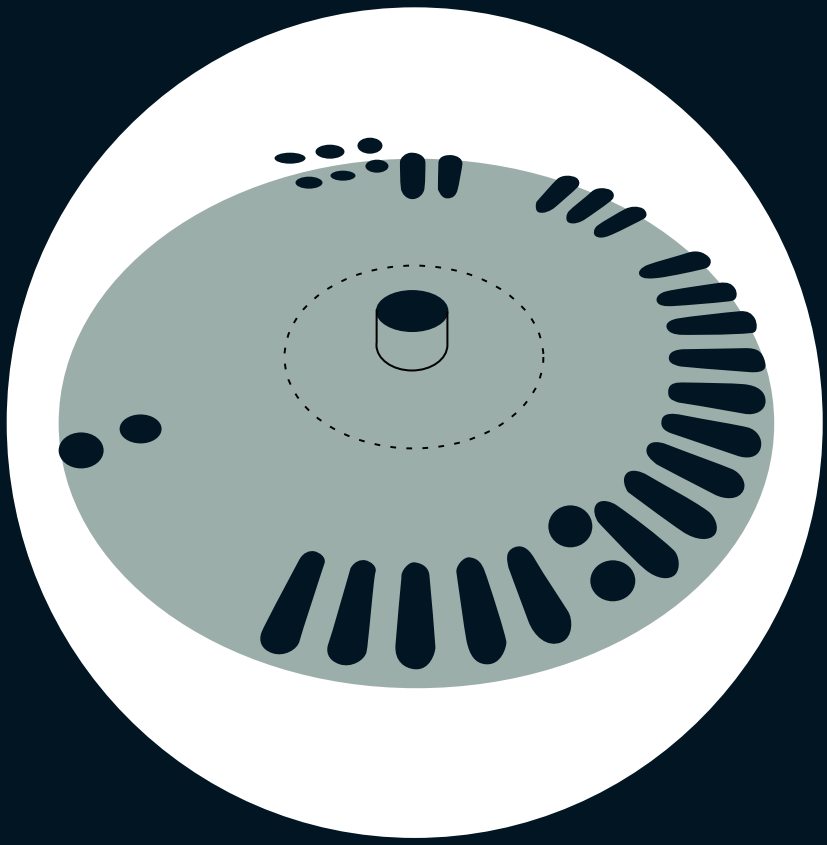

Chapter 7

Synthesis

In this thesis I have investigated the relation between mangrove forest eutrophication and heterotrophic decomposition of mangrove litter and soil organic matter (SOM) with the aim to get a more fundamental insight in the processes involved. The synthesis summarises the most relevant observations and discusses the main conclusions that can lead us to a better understanding of the potential impacts of nutrient and labile organic carbon (LOC) enrichment on the decomposition dynamics in mangrove ecosystems.

The direct and indirect responses of the decomposer microbial community to nutrient enrichment will be discussed in the first two sections of this synthesis. In the third section, the mechanisms and processes underlying the observed responses will be discussed in somewhat more detail. Some methodological aspects of this study will be discussed in section four, with a focus on the most promising, but also the most problematic issues that I encountered during this study. Finally, I will end this synthesis by discussing the role of mangrove forests in carbon sequestration and the effects of their nutrient enrichment in a broader context and suggest directions for further research.

7.1 Direct effects of labile organic carbon and nutrient enrichments on decomposition

The experiments described in this thesis revealed that the activity of the microbial community increases when a limiting substance is added (Chapter 2, Chapter 3). This in itself is not so surprising, but led to two observations which are of importance when predicting the effects of nutrient enrichment on the carbon balance of mangrove forests.

1. Primary producers and the heterotrophic community may experience different nutrient limitations

Within tolerance limits of salinity and moisture, mangrove primary production is generally limited by nutrient availability (Reef et al., 2010). Microbial activity can either be primarily energy-limited, as was the case in the *Avicennia marina* and *Rhizophora mucronata* forests of Saudi-Arabia (Chapter 2) or primarily nutrient-limited, as observed in the *Avicennia germinans*- and *Rhizophora mangle*-dominated peat-forming mangroves in Belize (Chapter 3). Moreover, in the Belizean case microbial biomass was primarily N-limited, while the vegetation was P-limited. Differential limitation means that drivers of autotrophic and heterotrophic activities are uncoupled and that nutrient enrichment might increase decomposer activity without increasing primary production and vice-versa. This makes the effects of exogenous amendments much less predictable as compared to situations where increased decomposition rates are accompanied by increased primary production.

2. Microbial biomass can experience several simultaneous limitations

Liebig's Law of the minimum, which states that the productivity of a plant depends on the most limiting nutrient (von Liebig, 1841; Hooker, 1917), does

not seem to apply to microbial growth and activity in some of our experiments. Chapter 3 shows that single amendments of N, P, and LOC each increased exoenzyme production by the soil microbiota in a similar fashion. Growth was only induced when N, P, and LOC where amended concurrently. Such behaviour is possibly attributable to the large number of simultaneously active microbial taxa (Danger et al., 2008), individually experiencing different limitations, but could also result from adaptive changes in response to nutrient availability such as shifting relative investments in enzyme production, cell growth, and overflow respiration (Sinsabaugh et al., 2013). This implies that a) different amendments may equally increase decomposer activity and b) the effect of amendments may be non-additive.

7.2 Indirect effects of labile organic carbon and nutrient enrichment on decomposition

Nutrients not only modify decomposer activity directly through their availability in the soil matrix, but perhaps even more profoundly, also indirectly through modification of the elemental composition and chemistry of newly produced organic matter by primary producers. The latter aspect of nutrient enrichment was tested in Chapters 3 and 5. In Chapter 3, heterotrophic activity was measured in soil from mangrove stands that had received long-term fertilisation in Belize. In Chapter 5, weight loss and nutrient dynamics of litter that had been produced in long-term fertilised sites was decomposed in control sites and vice-versa. This experiment was conducted in two mangrove forests which contrasted in their nutrient limitation and SOM content.

In Chapter 3, several indirect effects of long-term nutrient enrichment on soil microbial activity were encountered. Microbial stoichiometry and exoenzyme response indicated that the microbial biomass was predominantly N-limited, but lifting the P limitation of the primary producers quintupled microbial biomass. The limited increase in exoenzyme activity that accompanied the increase in microbial biomass suggests a community shift towards r-strategists benefiting from the larger input of labile organic carbon resulting from the higher primary productivity. In addition, the increased primary production had resulted in higher tannin inputs, while the activity of the tannin-degrading enzyme phenol oxidase (POX) was suppressed, which resulted in a higher tannin content of the soil. Overall, P enrichment increased the turnover of labile C compounds as indicated by the higher microbial biomass, but decreased the turnover of recalcitrant compounds as indicated by the lower POX activity. The increased tannin content of the soil is expected to slow down decomposition of SOM as compared to the non-enriched sites even further.

The effect of eutrophication on litter chemistry and litter decomposition rate was tested in Chapter 5. The experimental design allowed for a separation of the effects of litter chemistry and external nutrient availability on decomposing microorganisms. From the stoichiometric decomposition theory (Hessen et al.,

2004), it was expected that external nutrient enrichment would facilitate microbial growth, leading to rapid buildup of microbial biomass and increased decomposition. Such an effect was not confirmed in my experiment: neither litter stoichiometry nor external nutrient availability did affect decomposition rates. This was quite surprising, as especially initial N concentrations generally show strong positive correlations with decomposition rates of leaf litter (Melillo et al., 1982; Prescott, 2010). Still, I found that lifting a P limitation for mangrove tree growth increased the decomposition rate of newly produced litter, which I attributed to yet unknown change in litter chemistry.

Above observations raise the question whether the stimulating and inhibiting effects of N enrichment on decomposition rates found in field studies (Sinsabaugh, 2010; Hobbie et al., 2012) are caused by limitations for primary production, rather than by relieving limitations for heterotrophic microorganisms as stated by these authors. The P amendments in the dwarf zone of *R. mangle* as presented in Chapter 3 were expected to amplify the N limitation of microorganisms and therefore to lead to enhanced SOM decomposition by stimulation of the production of exoenzymes by K-strategists. This was however not confirmed by my observations on exoenzyme production. On the contrary, N amendment seemed to increase exoenzyme production even more than P amendent. A similar observation was done in Chapter 5 where litter produced in P-fertilised sites showed the highest decomposition rate, in spite of its low N content. Rejmánková and Houdková (2006) encountered a similar stimulating effect of direct and indirect P enrichment on decomposition in an oligotrophic P-limited wetland.

Based on these as well as my own observations I expect that the responses of the microbial community to nutrient enrichment are in large part caused by changes in litter chemistry resulting from lifting a limitation for primary production. This means that some of the effects specifically attributed to N enrichment may in fact also apply to P, if that is limiting autotrophic production.

7.3 Eutrophication and decomposition - processes and mechanisms

Priming effects: r- vs K-strategists and exoenzyme activity

Low respiration quotient (RQ) values (i.e. CO_2/O_2 ratios) indicated dominance of K-strategists respiring recalcitrant carbon (Dilly, 2003) in the SOM-poor soils of Saudi-Arabia (Chapter 2) as well as in the SOM-rich soils of Belize (Chapter 3). While in Saudi-Arabia amendments of labile organic carbon (LOC) induced growth of r-strategists, especially in the *Avicennia* soil, similar amendments of LOC did not induce a growth response at all in the mangrove soil in Belize (Chapter 3), indicating the absence of r-strategists that were able to use the available energy source.

The short-term enrichment experiments of Chapters 2 and 3 showed that certain combinations of labile organic carbon and nutrient enrichment in short-

term incubations increased microbial growth rate while they decreased respiration efficiency, SOM decomposition (Chapter 2), and exoenzyme activity (Chapter 3). Combined amendments of labile C and N strongly increased exoenzyme activities, while they did not induce microbial growth (Chapter 3). Concurrent amendments of labile C, N, and P, in contrast, stimulated microbial growth while there was little response in terms of exoenzyme activity. Such a pattern of growth and exoenzyme production dynamics suggests that the microbial activity was N-limited and that the K-strategists dominated the active microbial community. The combined amendment of C and N induced a P limitation, and r-strategists were only able to grow if also P is amended. The results from the short-term amendments confirm the observation of Chena et al. (2013) that priming effects can be predicted within a framework of competition for nutrient and energy sources between r- and K-strategists. In the case of the mangrove in Belize N-amendment is expected to induce positive priming as K-strategists are stimulated, while in the case of the Saudi-Arabian mangrove, N-amendment is shown to induce negative priming, as r-strategists are stimulated.

While N enrichment is generally found to decrease phenol oxidase activity (Carreiro et al., 2000; Saiya-Cork et al., 2002; DeForest et al., 2004; Sinsabaugh, 2010) as also predicted from the nitrogen-mining theory (Moorhead and Sinsabaugh, 2006; Craine et al., 2007; Chena et al., 2013), this was not the case in the long-term fertilisation experiment in the Belizean mangrove stands. Instead, P fertilisation inhibited phenol oxidase, even though microbial decomposers were primarily N-limited. Whether this is an effect of increased labile organic carbon input, increased tannin input or a direct effect of P availability remains unclear. Although preliminary results from lab incubations suggest the latter, this requires further study.

Tannins

Tannins play a central role in plant-litter-soil interactions (Kraus et al., 2003), as their leaching to the soil matrix can exert significant influence on ecosystem-scale carbon- and nitrogen-cycling (Schimel et al., 1996, 1998). Tannins decrease SOM decomposition and nitrogen mineralisation by deactivating exoenzymes and inhibiting the K-strategists producing them. It has often been argued that the release of tannins to the environment is an adaptation to reduce leaching of nitrogen via the inhibition of mineralisation processes in mangrove stands (Lin et al., 2009) and other systems (Northup et al., 1995; Kraus et al., 2003). Indeed, Maie et al. (2007) showed that tannins produced by *Rhizophora mangle* mangroves precipitate DON, thereby strongly limiting nutrient loss through leaching. Whether mangroves are able to make use of this accumulated N stock, still remains to be explored. Whether the precipitated nitrogen-tannin complexes are broken down by mangrove-associated arbuscular mycorrhiza is still under debate (Maie et al., 2007; Reef et al., 2010), but even without mycorrhiza, mineralisation of nitrogen is likely enhanced in the oxygenated root zone, where increased oxygen availability can enhance the breakdown of phenolics. Nitrogen is also mobilised through photo-degradation of nitrogen-tannin complexes (Maie et al., 2007). This

may contribute to the breakdown of DON-tannin complexes in the dwarf zone where relatively large amounts of solar radiation reach the forest floor due to its sparse vegetation. This in part, may help explain the N-limitation of the denser vegetation at the fringes of those systems.

The production of tannin can be expected to be influenced by eutrophication. The carbon-nutrient balance hypothesis, (Bryant et al., 1983) predicts a decrease in tannin production when a nutrient limitation is alleviated, irrespective of the nature of this limitation. If mangroves invest in tannin production as part of an N-conservation strategy, as suggested by Lin et al. (2009), one could expect tannin concentrations to be lower in P-limited systems as compared to N-limited systems as there is less pressure to retain N in the system. In addition, an increased tannin concentration in litter and soil could be expected when an N limitation is induced (e.g. through P enrichment), while a decreased tannin concentration could be expected when an N limitation is alleviated. This thesis confirms the latter: I did indeed find an increased soil tannin concentration after inducing N-limitation in a *Rhizophora mangle* stand. As leaf litter concentrations of tannins were not measured, however, I cannot tell with certainty whether the increased tannin concentration has resulted from changes in the nature of the nutrient limitation for plant growth or from increased primary production itself. In Chapter 3 I found that decomposer microorganisms were N-limited, even if plant growth was P-limited. I also found a shift towards producing less enzymes per unit biomass when tannin content increased.

What do these findings imply for the effects of tannins on the microbial community? From the work of Kraus et al. (2003) and Schimel et al. (1996) one would expect a negative correlation between tannin concentrations and K-strategist dominance or exoenzyme activity, and a positive correlation between tannin concentration and microbial N-limitation. This would also mean that the generally positive relation between microbial N-stress and success of K-strategists (Craine et al., 2007) is modified by the production of tannins. The results of Chapter 3, where lifting an N limitation for microorganisms in a tannin-rich soil activated enzyme production but did not stimulate microbial growth, and hence promoted K- but not r-strategists, indeed hints to that direction of thinking. This means that in similar mangrove systems, N amendment can be expected to increase decomposition in SOM as the mineral N amendment relieves the tannin-induced N-stress experienced by K-strategist microbial decomposers.

Peat formation in mangroves

A question that remains unanswered is what underlies the large differences in soil organic carbon content of mangroves, ranging from 1 to 36 % (This study, Breithaupt et al. (2012)). Large part of this variation results from differences in sedimentation rates and from differences in the particle composition of the sediment (mineral versus organic) (Breithaupt et al., 2012). This study shows large differences in SOM-processing between mangroves receiving little sediment. In some mangroves, litter decomposition does hardly occur resulting in the formation of peat (as shown in Chapter 3 and 6), whereas in others organic material largely

consists of microbial-processed material (like the *Rhizophora* forest in Chapter 2). I have found indications that tannins play a major role in inactivating exoenzymes that would otherwise catalyse SOM decomposition.

The enzymic latch theory of Freeman et al. (2001) states that carbon sequestration in peatlands depends on the inhibition of phenol oxidase by anoxic conditions, which in turn leads to the inhibition of hydrolytic enzymes by phenolic compounds. Would such an enzymic latch also explain the differences in peat formation by mangroves? The enzymic latch theory predicts that peat formation takes place as long as tannin input rate exceeds the sum of tannin export and decay rates. Since most tannins are soluble (Maie et al., 2007), tidal flushing has the potential to remove large quantities of tannins. Tannins are, however, sorbed by peat (Maie et al., 2007), so that they are retained in the system. As this in turn inhibits breakdown of peat, this may be a self-reinforcing mechanism. As the decay rates of tannins are limited by oxygen availability, factors increasing oxygenation like large tidal amplitude, high bioturbation and soil oxygenation by roots are expected to decrease the effect of an enzymic latch.

Differences between *Avicennia* and *Rhizophora*

Chapters 2, 5 and 6 explored the differences between *Avicennia* and *Rhizophora* mangrove forests, both on mineral (Chapter 2 and 5) and on peat (Chapter 6) soils. In both soil types, average SOM residence time was higher in *Rhizophora* spp. than in *Avicennia* spp., even though edaphic properties were similar between the two stands. Chapter 2 shows that both relative carbon use (q_C) and the microbial biomass per unit carbon were lower in the *Rhizophora*-derived soil. A higher recalcitrance and residence time of carbon in *Rhizophora* soil was also indicated by a larger fraction of K-strategists as indicated by a lower μ_{max} upon LOC amendment. This was confirmed in Chapter 6, where lower tannin concentrations and more labile organic matter suggested higher decomposition rates in *Avicennia* stands in otherwise rather similar peat soils underlying species of the two genera. From respiration measurements, the half-life of carbon was estimated to be 50 % longer in peat underlying *Rhizophora* as compared to peat underlying *Avicennia*. In Chapter 5, it was shown that fresh *Avicennia* leaf litter also decomposed faster than fresh *Rhizophora* leaf litter.

This difference in soil carbon half life brought me to the hypothesis that in peat-forming mangroves of the Americas, *A. germinans* and *R. mangle* experience cyclic succession: *R. mangle* dominates and peat is formed until the soil surface is sufficiently high above the waterline for *A. germinans* to settle. This turns the forest into a source of carbon, until *R. mangle* takes over again. This hypothesis is supported by the fact that peat underlying *Avicennia* stands is often *Rhizophora*-derived (Woodroffe, 1981; McKee and Faulkner, 2000b), an indication that vertical accretion mainly took place during dominance of *Rhizophora*. The proposed mechanism implies that species dominance is modified by changes in sea water level such that in periods of relatively rapid sea level rise, the succession may be arrested in the phase where peat-building *Rhizophora* are dominant, while slower peat-building species are favoured during periods of relatively slow sea

level rise. This succession pattern has important consequences for the resilience of mangroves to sea level rise and is critical for stability of peat-based mangrove ecosystems and their conservation as a carbon sink in the face of a changing climate.

7.4 Methodologies

Scale

One of the goals of this study was to gain a mechanistic understanding of microbial decomposer dynamics, in order to predict the effects of eutrophication on organic matter decomposition in mangrove soils. A methodological choice was made to study decomposition dynamics at the mesocosm scale, rather than on the micro- (physiological unit) or macro- (ecosystem) scale. While this has the advantage that the activity of the entire decomposing community with all its interactions is observed, it also holds the danger that scaling-up is not possible, as not all parameters that are influencing the system's behaviour are known. This is a common problem in ecological studies, and also global estimates of mangrove carbon sequestration are hampered by shortage of data, as also emphasised by Bouillon et al. (2008b) and Breithaupt et al. (2012).

Comparability

A second goal of this thesis was therefore to facilitate comparison of decomposition dynamics between mangroves and other ecosystems, by using relatively simple and cheap methods rendering ecologically relevant results. The tea bag index (TBI) presented in Chapter 4 was exclusively developed with this idea in mind. The use of this method allows inter-comparable data collection at a scale not attainable with more complex methods. Also the use of short-term incubation studies to measure microbial decomposer ecophysiological parameters like relative carbon use, potential growth rate and potential enzyme activity allow for large scale comparisons between different mangroves. To facilitate comparability between nutrient dynamics of litter with different decomposition rates, I have expressed the N and P content of the leaf-decomposer complex as a function of mass loss, rather than as a function of time (Chapter 5).

Redox conditions

The results presented in this thesis are derived from measurements of fresh, non-disturbed soil and of soil incubations conducted under controlled conditions. In both cases the redox conditions were not explicitly included in our reasoning. While one can safely assume that the non-disturbed soils are representative for the field, this is not a reasonable assumption for the laboratory incubations. Thorough mixing exposed the soil to an oxic atmosphere prior to incubation, while during incubation oxygen diffusion was severely restricted due to the lack of roots, tidal action and bioturbation. By keeping the laboratory incubations as short as possible, it was attempted to minimise the disturbing effects. Enzyme activities of fresh soil, and of soil that was incubated for seven days following

acclimation were similar. Basal respiration and microbial biomass decreased slightly towards the end of the incubations, but the effect was small: CO_2/O_2 did not decrease, indicating no change in consumed substrate.

Potential enzyme activities
Chapter 3 showed that responses of potential exoenzyme activities upon short-term nutrient amendment proved to be rather similar to long-term responses. Short-term incubations are useful to measure microbial exoenzyme responses, without the confounding effects of plant responses to nutrient enrichment. By comparing microbial response to nutrient enrichment in short-term incubations to the response to in long-term field fertilisation experiments I could separate the direct and indirect (primary producer-mediated) effects of nutrient-enrichment. I used relative microbial investment in exoenzyme activity as a proxy for shifts between r- and K-strategists dominance, where I defined r-strategists as microorganisms primarily investing in growth and K-strategists as microorganisms primarily investing in exoenzymes.

Several authors have used the relative activities of C-, N- and P-acquiring exoenzymes as an indicator for the nature of the nutrient limitations of decomposer microorganisms. The underlying assumption is that microbial communities optimise their allocation of resources to maximise their productivity (Sinsabaugh and Moorhead, 1994; Sinsabaugh et al., 2002, 2010). In the incubations and fertilisation experiments presented in this thesis the availability of nutrients was manipulated, provoking a change in nutrient limitation towards microorganisms. For soils from Belize this did not alway lead to changes in exoenzyme production, especially not with exoenzymes classified as 'N-acquiring' (Chapter 3). Instead, I mostly found non-specific overall changes in exoenzyme activity. The strict distinction in C-, N- and P- acquiring enzymes is however questionable. The end products of the N-harvesting enzymes leucine aminopeptidase (LAP) and N-acetylglucosaminidase (NAG) (Sinsabaugh et al., 2008), i.e. leucine ($C_6H_{13}NO_2$) and N-acetylglucosamine ($C_8H_{15}NO_6$) respectively, contain considerably more C than N. Also the 'P-acquiring' phosphatase (AP) sometimes relieves microbial carbon limitation rather than limitation of phosphorus as shown by Steenbergh et al. (2011). The strict distinction between 'P-harvesting', N-harvesting' and 'C-harvesting' enzymes has indeed been recently reconsidered in (Sinsabaugh et al., 2013).

7.5 Conclusion and Outlook

The central question addressed in this thesis was whether the on-going eutrophication of coastal zones could be expected to modify carbon sequestration rate in mangrove forests. From my results it came forward that the effects of LOC and nutrient enrichment on decomposition rate of SOM and litter are either positive or negative, as dependent on the limitations of primary producers and microbial decomposers. It was also shown that the effect of external enrichment depends on how the competition between r- and K-strategist microbial decomposers is affected. Depending on the nature of the system the same nutrient can either stimulate or inhibit SOM mineralisation. If both energy and nutrients are available, r-strategists are stimulated and decomposition rate of SOM is inhibited. If there are multiple limiting elements, enrichment with one of them stimulates K-strategists, leading to increased losses of historically sequestered SOM. Especially in case of differential nutrient limitation between primary producers and decomposers the effect on carbon sequestration in mangroves can be significant, as K-strategists can be stimulated while primary production is not and vice-versa.

I also showed the importance of species identity for carbon turnover times, and that shifts between those species may form a stabilising factor in the resilience of oceanic islands against sea level rise. From those results one may speculate that settlement of *Avicennia* spp. increases carbon loss, while settlement of *Rhizophora* spp. increases carbon storage.

Until now, there has been a limited amount of research on the consequences of nutrient enrichment for carbon sequestration in mangrove ecosystems. In their elaborate reviews on the importance on mangrove sequestration, Bouillon et al. (2008a) and Breithaupt et al. (2012) discuss the consequences of mangrove disappearance, but they do not address the potential consequences of nutrient enrichment. This is surprising as nutrient inputs to coastal systems are projected to increase in large parts of the mangrove distribution area. At the same time, sea level rise threatens oceanic islands, and the rate of peat accretion is a critical factor in the resilience of those islands against climate change. Results from this thesis showed the importance of explicitly considering nutrient inputs when estimating current and future rates of carbon sequestration and the potential risk of drowning of peat-based mangrove systems.

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Abstract

The sequestration and release of carbon from soil organic matter (SOM) plays an important role in determining atmospheric carbondioxide (CO_2) concentrations, so that changes in the balance between these two processes have the potential to amplify or attenuate global warming. Mangrove forests cover large parts of the tropical and subtropical coasts where they sequester substantial amounts of carbon as SOM. This high net sequestration rate is of special importance for oceanic mangrove systems, as the build-up of peat is essential to avoid inundation due to sea level rise. In many parts of the world, mangroves are being exposed to increasing quantities of external nutrients and labile organic compounds from agricultural runoff and wastewater. The central question addressed in this thesis was how the ongoing eutrophication of coastal zones could be expected to modify growth and functioning of decomposing micro-organisms in mangrove soils and if this has the potential to lead to major changes in carbon sequestration potential of mangroves. De research is conducted in stands formed by mangroves from the genera *Avicennia* and *Rhizophora*. Both genera are dominant throughout the entire mangrove distribution.

After the general introduction of the study in Chapter 1, Chapter 2 explores the short-term effects of mimicked wastewater enrichment on microbial stoichiometry, biomass, and respiration rates in *Avicennia marina* and *Rhizophora mucronata*-dominated mangrove forests of Saudi-Arabia. The use of short-term incubations allowed me to study the direct effects of nutrients and organic material on the microbial community, without the interference of indirect effects such as alterations in litter production by primary producers. The results show that microbial growth is energy-limited in both the *Avicennia marina* and the *Rhizophora mucronata* de-

rived soils, with nitrogen as a secondary limitation. Phosphorus amendment did not affect decomposition rates of organic material. Nitrogen amendment, in contrast, increased the rate at which labile organic carbon was decomposed, while it decreased SOM mineralisation rates. Due to this inhibitory effect I do not expect that nutrient enrichment causes degradation of historically sequestered SOM in nitrogen-limited mangrove forests.

Chapter 3 focuses on long-term effects of nutrient amendments and the role of microbial exoenzymes. I conducted this study in a highly organic soil from an oceanic mangrove island in Belize, where the quality rather than the quantity of soil organic carbon determines microbial activity. The site was subject to long-term experimental fertilisation with nitrogen or phosphorus. This facilitated the detection of long-term effects of nutrient input on decomposition dynamics driven by changes in input quality. Microbial limitation is detected by assessing exoenzyme activity rather than growth in order to specifically study functional changes not associated with rapid growth and microbial community change. In this study I show that tannins induce a nitrogen limitation on microbial decomposition even when plant growth is limited by phosphorus. Such a differential nutrient-limitation between microbial decomposers and primary producers implies that the impact of eutrophication on carbon sequestration is nutrient-specific. In addition, this chapter shows that the oxidative enzyme phenol oxidase, which is involved in peat decomposition, is inhibited by P, but not by N enrichment. Furthermore I argue that the often used division between N-harvesting, P-harvesting, and C-harvesting exoenzymes needs to be reconsidered.

Decomposition in a broader context is the focus of Chapter 4. This chapter describes a novel low-cost method to measure decomposition at a high resolution using commercially available teabags as standardised test kits. Besides being applied in mangroves, this method is tested in a large number of ecosystems of different types. The outcome of this experiment gives an indication of decomposition rates in mangroves as compared to other terrestrial ecosystems. By using two tea types with contrasting decomposability I can construct a decomposition curve using a single measurement in time. The acquired Tea Bag Index (TBI) consists of two parameters describing decomposition rate (k) and litter stabilisation (S). Trials in contrasting ecosystems and biomes, confirmed that the Tea Bag Index is sensitive enough to discriminate between these systems. Within an ecosystem, TBI is responsive to differences in abiotic circumstances such as soil temperature and moisture content. The acquired k and S values are in accordance to expectations based on decomposition process literature. They are therefore interpretable within the current knowledge framework. TBI

provides an excellent decomposition reference suitable for crowd-sourcing and educational purposes. It has the potential to increase reliability of soil carbon flux estimates based on extrapolations of decomposition data.

Chapter 5 deals with the nutrient dynamics of litter decomposition in mangroves of Belize and Florida. It assesses whether chemistry of newly produced litter is altered through external nutrient enrichment, and whether the impact of such indirect, litter-mediated enrichment on decomposition differs from that of direct nutrient additions to the microbial community. Litter decomposition rates were measured in two contrasting mangrove sites, which were subject to at least five years of experimental nitrogen and phosphorus fertilisation. By making use of reciprocal litter exchange experiments in addition to in-situ incubations of litter, I tested if nutrient addition primarily acts on the primary producers or on the microbial decomposers. The results show that decomposing microorganisms can be N-limited even in sites where primary production is P-limited. Relieving the nutrient limitation of the primary producer, increased decomposition rates while alleviating the decomposer nutrient limitation did not. This result emphasises the importance of plant-mediated fertilisation effects for changes in decomposition rates and carbon sequestration.

Another aspect of carbon sequestration in mangroves is explored in Chapter 6. In this chapter I propose an autogenic mechanism for cyclic succession patterns in peat-based mangrove systems driven by negative reciprocal effects of species dominance induced by differences in peat decomposition rates. The proposed mechanism implies that *Avicennia germinans*-derived peat decomposes more rapidly than *Rhizophora mangle*-derived peat. To test this, I measured peat recalcitrance, microbial exoenzyme activity, microbial biomass and decomposition rates in peat soils underlying *Avicennia germinans* and *Rhizophora mangle* stands on Twin Cays, a group of peat-forming islands in Belize. I confirmed that peat underlying *Avicennia germinans* stands is chemically more labile than peat underlying *Rhizophora mangle* stands and decomposes more rapidly. The proposed mechanism is therefore supported by our measurements and matches field observations of mangrove successional patterns. Cyclic succession may play an important role in building peat and shaping community structure of mangroves.

In Chapter 7 I conclude that the effects of labile organic carbon and nutrient enrichment on decomposition rate of soil organic matter and litter are either positive or negative, as dependent on the limitations of primary producers and microbial decomposers. As (recalcitrant) SOM is mineralised by K- and not by r-strategist microbial decomposers, the effect of external enrichment is determined by its effect on the competition between those

two groups. Depending on the nature of the system the same nutrient can either stimulate or inhibit SOM mineralisation. If both energy and nutrients are available, r-strategists are stimulated and decomposition rate of SOM is inhibited. If there are multiple limiting elements, enrichment with one of them stimulates K-strategists, leading to increased losses of historically sequestered SOM. Especially in case of differential nutrient limitation between primary producers and decomposers the effect on carbon sequestration in mangroves can be significant, as K-strategists can be stimulated while primary production is not and vice-versa. I also showed the importance of species identity for carbon turnover times, and that shifts between those species may form a stabilising factor in the resilience of oceanic islands against sea level rise. The lower decomposition rates in *Rhizophora* spp. stands as compared to *Avicennia* spp. suggest that settlement of *Avicennia* spp. generally increases carbon loss, while settlement of *Rhizophora* spp. increases carbon storage.

Until now, there has been a limited amount of research on the consequences of nutrient enrichment for carbon sequestration in mangrove ecosystems. Results from this thesis showed the importance of explicitly considering nutrient inputs when estimating current and future rates of carbon sequestration and the potential risk of drowning of peat-based mangrove systems.

ملخص

يلعب عزل وإطلاق الكربون من المادة العضوية الترابية (SOM) دورا هاما في تحديد تركيزات أكسيد الكربون في الغلاف الجوي (CO_2)، حيث أن التغيرات في التوازن بين هاتين العمليتين يمكن أن يعمل على تضخيم أو تخفيف ظاهرة الاحترار العالمي. وتغطي غابات المنغروف أجزاء كبيرة من السواحل الاستوائية وشبه الاستوائية حيث عزل كميات كبيرة من الكربون مثل المادة العضوية الترابية. ويكتسب ذلك المعدل المرتفع الصافي أهمية خاصة لنظم المنغروف المحيطية، حيث يعتبر تراكم الخُث أمرا ضروريا لتجنب الفيضان بسبب ارتفاع مستوى سطح البحر. وفي أجزاء كثيرة من العالم، يتعرض المنغروف إلى تزايد كميات المواد المغذية والمركبات العضوية المتغيرة الخارجية من مياه الصرف الزراعي والصرف الصحي. وكان السؤال المركزي الذي تدور حوله هذه الأطروحة هو كيف يمكن أن يتوقع من المغذيات المستمرة للمناطق الساحلية أن تعدل نمو وعمل الكائنات الحية الدقيقة في التربة المتحللة بالمنغروف، وما إذا كان يمكن أن يؤدي ذلك إلى تغييرات كبيرة في إمكانات عزل الكربون من أشجار المنغروف. وتجري البحوث على مجموعات شكلتها أشجار المنغروف من أنواع الآفيسينيا (*Avicennia*) والريزوفورا (*Rhizophora*)، وهما سائدان في المنغروف.

وبعد عرض مقدمة عامة للدراسة في الفصل (١)، يغوص الفصل (٢) في استكشاف الآثار على المدى القصير من تحاكي التخصيب مياه الصرف الصحي على مستوى حساب العناصر المتفاعلة الميكروبية والكتلة الحيوية ومعدلات التنفس في غابات المنغروف التي ينتشر

ملخص

بها الآفيسينيا المرينا (*Rhizophora mucronata*) والريزوفورا الموكروناتا (*Avicennia marina*) بالمملكة العربية السعودية. كما أن استخدام حضانات قصيرة الأجل سمح في دراسة الآثار المباشرة للمواد الغذائية والمواد العضوية على المجتمع الميكروبي، دون تدخل من الآثار غير المباشرة مثل التغيرات في إنتاج القمامة من قبل المنتجين الأساسيين. وقد أظهرت النتائج أن نمو الجراثيم محدود الطاقة في ترب مستمدة من كل من آفيسينيا مارينا وريزوفورا موكروناتا ويكون النيتروجين محدودا ثانيا. ولم يؤثر تعديل الفوسفور على معدلات تحلل المواد العضوية. وقد زاد تعديل النيتروجين في المقابل من المعدل الذي تحلل فيه الكربون العضوي المتغيرة، مع انخفاض معدلات تحويل المادة العضوية الترابية إلى معدن. ونتيجة لهذا التأثير المثبط لا أتوقع أن يسبب تخصيب المغذيات تدهورا للمادة العضوية الترابية المحتبسة تاريخيا في غابات المنغروف المحدودة بالنيتروجين.

ويركز الفصل (٣) على الآثار طويلة الأجل لتعديلات المغذيات ودور الإنزيمات الخارجية الميكروبية. وأجريت هذه الدراسة في التربة العضوية المرتفعة من جزيرة المنغروف المحيطية في بليز، حيث نوعية الكربون العضوي في التربة أكثر من كميته هو ما يحدد من طرف تقييم نشاط الإنزيمات الخارجية. وكان يخضع هذا الموقع للإخصاب التجريبي طويل الأمد مع النيتروجين أو الفوسفور. وذلك مما سهل الكشف عن الآثار طويلة الأجل لإدخال المواد الغذائية على ديناميات التحلل مدفوعة بالتغيرات في نوعية المدخلات. وقد تم الكشف عن الحد الميكروبي لنشاط العدوى بدلا من النمو من أجل دراسة التغييرات الوظيفية التي لا ترتبط بالنمو السريع والتغير في المجتمع الميكروبي. وفي هذه الدراسة أظهر أن أحماض التنيك تحث حدود النيتروجين على التحلل الميكروبي حتى عندما يحدد نمو النبات بسبب الفوسفور. كما أن مثل هذا الحد في المغذيات المختلفة بين المحللات الميكروبية ومنتجي المواد الأولية يعني أن تأثير زيادة المغذيات في عزل الكربون هو محدد المغذيات. وبالإضافة إلى ذلك، يبين هذا الفصل أن الفينول الأوكسيديز وهو انزيم ينتج الأكسيد، الذي يشارك في تحلل الخُث، يكبحه تخصيب الفسفور وليس النيتروجين. وعلاوة على ذلك أزعم أن التقسيم المستخدم غالبا بين الإنزيمات الخارجية حصاد النيتروجين وحصاد الفسفور وحصاد الكربون يحتاج إلى إعادة نظر.

كما أن التحلل في سياق أوسع يكون محور الفصل (٤). ويصف هذا الفصل طريقة جديدة منخفضة التكلفة لقياس التحلل بدقة عالية باستخدام أكياس الشاي المتوفرة تجاريا باسم مجموعات الاختبار الموحدة. وإلى جانب التطبيق في أشجار المنغروف، يتم اختبار هذه الطريقة في عدد كبير من النظم الإيكولوجية من أنواع مختلفة. وتعطي نتائج هذه التجربة مؤشرا على معدلات التحلل في أشجار المنغروف بالمقارنة مع النظم الإيكولوجية الأرضية الأخرى. وباستخدام نوعي الشاي مع تناقض قدرتهما في التحلل يمكنني بناء منحنى للتحلل باستخدام قياس واحد في الوقت المناسب. وهو مؤشر كيس شاي المكتسب (TBI).

يتكون مؤشر كيس شاي (TBI) من معلمين يوضحا معدل التحلل (k) واستقرار البقايا (S). كما أن المحاولات في تباين النظم الإيكولوجية والمناطق الإحيائية يؤكد أن مؤشر كيس الشاي حساس بما يكفي للتمييز بين هذه النظم. وداخل النظام الإيكولوجي، يستجيب TBI لاختلافات في الظروف غير الحيوية مثل درجة حرارة التربة ومحتوى الرطوبة. كما أن قيم k وS المكتسبة تكون وفقا للتوقعات على أساس عملية التحلل الموصوفة في مؤلفات حول الموضوع. ولذلك فهي مازالت قابلة للتأويل في إطار المعرفة الحالية. ويقدم TBI إشارة تحلل ممتازة مناسبة لأغراض التعليم والمصادر. ومن ثم يحتوي على القدرة على زيادة موثوقية تقديرات تدفق الكربون في التربة بناء على استقراء بيانات التحلل.

ويتناول الفصل (٥) ديناميات المغذيات من تحلل القمامة في غابات المنغروف ببليز وفلوريدا. وهي تقيم ما إذا كان التغيير الكيميائي في القمامة المنتجة حديثا من خلال تخصيب المواد الغذائية الخارجية، وعما إذا كان أثر ذلك غير المباشر من قمامة التخصيب على التحلل وهو ما يختلف عن ذلك من حيث الإضافات المغذيات المباشرة للمجتمع الميكروبي. وتم قياس معدلات التحلل في القمامة على موقعين متناقضين بالمنغروف، وهو ما يخضع لخمس سنوات على الأقل من التسميد التجريبي بالنيتروجين والفوسفور. ومن خلال الاستفادة من التجارب المتبادلة لصرف القمامة بالإضافة إلى حضانات من القمامة في الموقع، فإنني اختبرت ما إذا كان تأثر المغذيات المضافة أساسيا على المنتجين الأساسين أو على المحللات الميكروبية. وتبين النتائج أن الكائنات

الدقيقة المحللة يمكن أن تكون محدودة النيتروجين حتى في المواقع حيث يكون الإنتاج الرئيسي محدود الفسفور. كما أن تخفيف قيود المغذيات للمنتج الأساسي يزيد من معدلات التحلل حين لم يحدث تخفيف قيود تحلل المغذيات. وتؤكد هذه النتيجة على أهمية آثار الإخصاب من خلال النبتات على التغيرات في معدلات التحلل وعزل الكربون.

ويتم التعرف على استكشاف جانب آخر من عزل الكربون في غابات المنغروف في الفصل (٦). وفي هذا الفصل أقترح آلية انعكاسية لأنماط تتابع دورية في نظم المنغروف على أساس الخُث مدفوعة بآثار سلبية متبادلة من هيمنة الأنواع الناجمة عن الاختلافات في معدلات تحلل الخُث. وتتضمن الآلية المقترحة أن الخُث المشتق من آفيسينيا يتحلل بسرعة أكبر من الخُث المستمد من الريزوفورا. ولاختبار هذا، قمت بقياس تعنت الخُث، والنشاط الميكروبي لإنزيم خارجي، والكتلة الحيوية الميكروبية ومعدلات التحلل في تربة الخث الكامنة في أشجار الآفيسينيا والريزوفورا في توين كايز، وهي مجموعة من الجزر التي تشكل الخُث في بليز. وأكد أن الخُث الكامن في أشجار الآفيسينيا يكون كيميائيا أكثر قابل للتغيير من الخُث الكامن في أشجار الريزوفورا ويتحلل بسرعة أكبر. وبالتالي يتم اعتماد الآلية المقترحة اعتمادا على قياساتنا والملاحظات الميدانية لأنماط تعاقب المنغروف. ويمكن أن يلعب ذلك التتابع دورا هاما في بناء وتشكيل الخث داخل أشجار المنغروف.

وفي الفصل (٧) أورد تلخيصا لآثار الكربون العضوي المتغير والتخصيب المواد الغذائية على معدل التحلل للمواد العضوية في التربة والقمامة يكون إيجابيا أو سلبيا، اعتماداً على القيود المفروضة على منتجي المواد الأولية والمحللات الميكروبية. كما أن المادة العضوي في التربة (SOM) المتمرد يكون معدنيا من قبل كاند وليس من محللات ميكروبية استراتيجية r، ويتم تحديد أثر التخصيب الخارجي عن طريق تأثيرها على المنافسة بين هاتين المجموعتين. واعتمادا على طبيعة النظام يمكن لنفس المغذيات أن تحفز أو تثبط تحول المادة العضوي في التربة إلى معدن. وفي حالة توافر كل من الطاقة والمواد المغذية، يتم تحفيز استراتيجيات r وتحلل معدل تثبيط المادة العضوية الترابية.

وإذا كانت هناك عناصر متعددة الحد، فإن الإثراء مع واحد منهم يحفز استراتيجيات K، مما أدى إلى زيادة خسارة المادة العضوي في التربة المنعزلة تاريخيا. خصوصا في حالة تحديد المغذيات المختلفة بين المنتجين الأوليين والمحللات فإن التأثير على امتصاص الكربون في غابات المنغروف يمكن أن تكون كبيرة، حيث أن استراتيجيات K يمكن أن تحفز بخلاف الإنتاج الأولي والعكس بالعكس. وقد أشير أيضا إلى أهمية تحديد الأنواع لمرات دوران الكربون، وأن التحولات بين هذه الأنواع قد يشكل عامل استقرار في مرونة الجزر المحيطية عند ارتفاع مستوى سطح البحر. ويوحي انخفاض معدلات التحلل في ريزوفورا بالمقارنة مع آفيسينيا إلى أن مجموعات آفيسينيا تزيد عموما من فقد الكربون، بينما تزيد مجموعات ريزوفورا من تخزين الكربون.

وحتى الآن، كانت هناك كمية محدودة من البحوث حول النتائج المترتبة على تخصيب المغذيات لعزل الكربون في نظام بيئي منعروف. وأظهرت نتائج هذه الأطروحة أهمية النظر صراحة إلى مدخلات المغذيات عند تقدير المعدلات الحالية والمستقبلية من عزل الكربون والمخاطر المحتملة لغرق نظم المنغروف على أساس الحُث.

Samenvatting

Het vastleggen en weer vrijkomen van koolstof uit bodemorganische stof heeft een substantiële invloed op de hoeveelheid koolstofdioxide (CO_2) in de atmosfeer. Hierdoor kunnen veranderingen in de balans tussen productie en afbraak van bodemorganische stof de opwarming van de aarde versnellen of juist remmen. Mangrovebossen bestrijken grote delen van tropische en subtropische kusten en riviermondingen en leggen aanzienlijke hoeveelheden organische stof vast in de bodem. Dit is van bijzonder belang voor het voortbestaan van oceanische mangrove-eilanden, omdat deze meestijgen met de zeespiegel door het vastleggen van organische stof in veenlagen. Als gevolg van kustontwikkeling worden mangroven in toenemende mate blootgesteld aan externe nutriënten, die samen met makkelijk afbreekbare organische stof worden aangevoerd door afstroming uit landbouwgebieden en door afvalwater. De centrale vraag in dit proefschrift is of de eutrofiëring van kustgebieden zorgt voor veranderingen in de groei en het functioneren van reducerende micro-organismen en wat de effecten hiervan zijn op de afbraak van (bodem)organische stof in mangrovebossen. Het onderzoek is uitgevoerd in bodem onder standplaatsen van mangroven uit de genera *Avicennia* en *Rhizophora*. Beide zijn dominant in vrijwel het gehele verspreidingsgebied van mangroven.

Na de algemene inleiding van de studie in hoofdstuk 1, wordt in hoofdstuk 2 onderzocht wat de kortetermijneffecten zijn van eutrofiëring op de activiteit, biomassa en stoichiometrie van reducerende micro-organismen in bodems van door *Avicennia marina* en *Rhizophora mucronata* gedomineerde mangrovebossen in Saoedi-Arabië. Het gebruik van kortetermijnincubaties maakt de directe effecten van nutriënten en makkelijk afbreekbare organische stof op de microbiële gemeenschap zichtbaar, zonder verstoring

door indirecte effecten zoals veranderingen in de productie van dood organisch materiaal. De resultaten van dit experiment laten zien dat microbiële groei in de bodems van zowel door *Avicennia marina* als door *Rhizophora mucronata* gedomineerde bossen beperkt is door energie, en dat stikstof een secundaire limitatie vormt. Verrijking van de bodem met fosfor had geen effect op de afbraak. Stikstofverrijking daarentegen verhoogde de snelheid waarmee makkelijk afbreekbare organische stof werd afgebroken, maar verlaagde de afbraaksnelheid van de bodemorganische stof. Door dit remmende effect verwacht ik dat, in stikstofbeperkte mangrovebossen, eutrofiëring weinig effect heeft op de afbraak van historisch vastgelegde bodemorganische stof.

Hoofdstuk 3 richt zich, naast de korte-, ook op de langetermijneffecten van eutrofiëring op de afbraak van bodemorganische stof en de rol van microbiële exo-enzymen hierin. De in dit hoofdstuk beschreven studie is uitgevoerd in een zeer organische (veen)bodem van een mangrove-eiland in de Caribische Zee. Doordat de bodem vrijwel geheel bestaat uit organische stof wordt de microbiële activiteit hier niet bepaald door de beschikbaarheid, maar door de afbreekbaarheid van bodemorganische stof. Voorafgaand aan het experiment is deze site meer dan tien jaar lang bemest met stikstof of fosfor. Hierdoor kunnen ook langetermijneffecten van nutriënten op afbraak worden gemeten, die bijvoorbeeld worden veroorzaakt door veranderingen in de samenstelling van nieuw geproduceerd dood organisch materiaal. In dit experiment is de door micro-organismen ondervonden limitatie gemeten door te kijken naar de activiteit van specifieke exo-enzymen, zodat ook de effecten van bemesting die niet zijn geassocieerd met groei en veranderingen in de microbiële gemeenschap kunnen worden bestudeerd. Deze studie laat zien dat door de mangroven geproduceerde tannines leiden tot een microbiëel stikstoftekort waardoor de afbraak wordt geremd. Dit stikstoftekort ontstaat ook in de gevallen waar de plantengroei juist wordt beperkt door fosfor. Een dergelijk verschil in nutriëntenlimitatie tussen microbiële reducenten en primaire producenten betekent dat stikstofverrijking van fosforverrijking tegengestelde effecten op de koolstofopslag hebben. Bovendien toont dit hoofdstuk aan dat het oxiderende enzym fenol-oxidase, dat een belangrijke rol speelt bij de afbraak van veen, wordt geremd door fosfor-, maar niet door stikstofverrijking. Verder wordt aangetoond dat de vaak gebruikte voorspelling van microbiële (nutriënten)-limitatie uit relatieve activiteiten van exo-enzymen niet zonder meer geldig is.

Afbraak van organisch materiaal wordt in hoofdstuk 4 in een bredere context geplaatst. Dit hoofdstuk beschrijft een nieuwe en goedkope methode om afbraak van organisch materiaal met een hoge dichtheid en grote reikwijdte te meten, door in de winkel beschikbare theezakjes te gebruiken

als standaard testmateriaal. Naast in mangroven, wordt de door mij ontwikkelde methode getest in een groot aantal verschillende ecosystemen. Het experiment geeft een indruk van de afbraaksnelheden van bodemorganische stof in mangroven in vergelijking met andere terrestrische ecosystemen. Door het gebruik van twee soorten thee met contrasterende afbreekbaarheid kan de afbraak in de tijd worden geschat uit een eenmalige meting. De zogenaamde Tea Bag Index (TBI) bestaat uit twee parameters die afbraaksnelheid (k) en stabilisatie van organisch materiaal beschrijven (S). Proeven in contrasterende ecosystemen en biomen bevestigen dat de TBI gevoelig genoeg is om verschillen in afbraaksnelheid tussen deze systemen meetbaar te maken. Ook binnen een ecosysteem kunnen verschillen in afbraak van organisch materiaal door een afwijkende bodemtemperatuur of vochtgehalte worden aangetoond. De verzamelde k en S waarden zijn in overeenstemming met de verwachtingen op basis van literatuur en zijn daarmee dus interpreteerbaar binnen het huidige kader. De TBI biedt een uitstekende afbraakmeting geschikt voor crowd-sourcing en educatieve doeleinden. Het heeft de potentie om de betrouwbaarheid van de bodem koolstofflux-schattingen op basis van extrapolaties van afbraakgegevens te verhogen.

Hoofdstuk 5 behandelt de nutriëntendynamiek van afbraak van bladstrooisel in mangrovebossen in Belize en Florida. Er wordt gekeken of de samenstelling van nieuw geproduceerd bladstrooisel verandert als gevolg van eutrofiëring en of het effect van dergelijke indirecte eutrofiëringseffecten verschilt van directe eutrofiëringseffecten op de microbiële gemeenschap. Strooiselafbraak werd gemeten in twee contrasterende mangrove sites, die gedurende ten minste vijf jaar waren bemest met stikstof of fosfor. Door gebruik te maken van strooiseluitwisselingsexperimenten is getest of nutriënten hoofdzakelijk een direct effect hebben op de microbiële reducenten of op de primaire producenten. De resultaten laten zien dat microbiële reducenten N-beperkt kunnen zijn, zelfs op plekken waar primaire productie P-beperkt is. Het opheffen van de nutriëntenlimitatie voor de primaire producent verhoogt de afbraaksnelheid, terwijl het opheffen van de nutriëntenlimitatie voor de microbiële reducenten geen effect heeft. Dit resultaat benadrukt het belang van eutrofiëringseffecten op de plant als oorzaak voor veranderingen in de afbraaksnelheid van organisch materiaal.

Een ander aspect van koolstofvastlegging in mangroven wordt onderzocht in hoofdstuk 6. In dit hoofdstuk stel ik een autogeen mechanisme voor voor cyclische successiepatronen in Caribische veenvormende mangrovesystemen. Het is bekend dat *Avicennia germinans* in deze systemen op hogere plekken groeit dan *Rhizophora mangle*. Mijn hypothese is dat er een cyclisch patroon ontstaat doordat veen onder *Avicennia germinans* stand-

plaatsen sneller afbreekt dan veen onder *Rhizophora mangle* standplaatsen. Dit leidt tot negatieve wederkerige en positieve wederzijdse effecten op de dominantie van *Avicennia germinans* and *Rhizophora mangle*. De standplaats van beide soorten verandert zo, dat deze op de lange duur wordt ingenomen door de andere soort. Om dit te valideren heb ik metingen gedaan in veenvormende mangrove-eilanden in de Caribische Zee. Hier heb ik naast veensamenstelling en -afbraaksnelheid ook exo-enzymactiviteit en microbiële biomassa gemeten. Ik kon bevestigen dat veen onder *Avicennia germinans* beter afbreekbaar is en ook sneller afbreekt dan veen onder *Rhizophora mangle*. Het voorgestelde mechanisme wordt ondersteund door mijn metingen en past in veldwaarnemingen van mangrovesuccessiepatronen en historische veenopbouw. Cyclische successie kan een belangrijke rol spelen in veenopbouw en het kan bepalend zijn voor het ontstaan van soortdominantiepatronen in veenvormende mangrovebossen.

In hoofdstuk 7 concludeer ik dat verrijking met makkelijk afbreekbare organische stof nutriënten de afbraak van bodemorganische stof zowel kunnen versnellen als vertragen, en dat dit afhankelijk is van de ondervonden limitatie voor primaire producenten en microbiële reducenten. Het effect van eutrofiëring wordt voor een groot deel bepaald door haar invloed op de competitie tussen reducerende micro-organismen met een r- of een K-strategie. K-strategen zijn in staat om bodemorganische stof af te breken, terwijl r-strategen dat nauwelijks doen. Of een bepaald nutriënt de afbraak van bodemorganische stof stimuleert of remt hangt af van de aard van het systeem: Als na verrijking zowel energie als nutriënten beschikbaar zijn, worden de r-strategen gestimuleerd en wordt de afbraaksnelheid van SOM geremd. Als er meerdere tekorten zijn stimuleert opheffing van één ervan de K-strategen, waardoor historisch vastgelegde voorraden bodemorganische stof versneld worden afgebroken. Vooral in het geval van gedifferentieerde nutriëntenlimitatie tussen primaire producenten en reducenten kunnen de effecten op de koolstofopslag in mangroven aanzienlijk zijn doordat decompositie kan worden gestimuleerd zonder dat de primaire productie afneemt en vice versa. Ook het effect van de dominante mangrovesoort op afbraaksnelheid is belicht. Verschuivingen tussen *Avicennia* spp. en *Rhizophora* spp. kunnen een stabiliserende factor vormen in de resistentie van oceanische eilanden tegen zeespiegelstijging. Aan de hand van mijn resultaten kan men vermoeden dat uitbreiding van *Avicennia* spp. leidt tot een versneld verlies van bodemorganisch materiaal, terwijl ontwikkeling van *Rhizophora* spp.-gedomineerde bossen de koolstofopslag verhoogt.

Dit proefschrift laat zien hoe eutrofiëring de afbraak van bodemorganische stof beïnvloedt en wat de mogelijke gevolgen zijn voor koolstofopslag in mangrovesystemen. De resultaten laten zien dat men expliciet rekening

moet houden met de toenemende aanvoer van nutriënten bij het schatten van de huidige en toekomstige snelheid waarmee koolstof wordt vastgelegd in mangroven. Deze kennis kan ook worden gebruikt om het risico van het wegzinken van mangrove-eilanden als gevolg van eutrofiëring van de zee beter in te schatten.

Dankwoord

Dit proefschrift was er niet gekomen zonder de vele hulp die ik heb gehad van collega's, vrienden, familie en natuurlijk Annemieke. Heel veel dank hiervoor!

Hier namen noemen zou tekort doen aan de waardering die ik heb voor ieder van jullie. In plaats daarvan kies ik er voor om zoveel mogelijk mensen persoonlijk te bedanken.

Als je promoveert is het niet altijd makkelijk om niet in een wereld terecht te komen waarin er buiten het onderwerp van onderzoek niet veel meer lijkt te zijn. Speciaal voor hen is het gedicht van de Argentijnse dichter Jorge Louis Borges op de volgende pagina's.

Joost

Otro Poema de los Dones

Gracias quiero dar al divino
laberinto de los efectos y de las causas
por la diversidad de las criaturas
que forman este singular universo,
por la razón, que no cesará de soñar
con un plano del laberinto,
por el rostro de Elena y la perseverancia de Ulises,
por el amor, que nos deja ver a los otros
como los ve la divinidad,
por el firme diamante y el agua suelta,
por el álgebra, palacio de precisos cristales,
por las místicas monedas de Ángel Silesio,
por Schopenhauer,
que acaso descifró el universo,
por el fulgor del fuego,
que ningún ser humano puede mirar sin un asombro
antiguo,
por la caoba, el cedro y el sándalo,
por el pan y la sal,
por el misterio de la rosa,
que prodiga color y que no lo ve,
por ciertas vísperas y días de 1955,
por los duros troperos que en la llanura
arrean los animales y el alba,
por la mañana en Montevideo,
por el arte de la amistad,
por el último día de Sócrates,
por las palabras que en un crepúsculo se dijeron
de una cruz a otra cruz,
por aquel sueño del Islam que abarcó
Mil Noches y Una Noche,
por aquel otro sueño del infierno,
de la torre del fuego que purifica
y de las esferas gloriosas,
por Swedenborg,
que conversaba con los ángeles en las calles de
Londres,
por los ríos secretos e inmemoriales
que convergen en mí,
por el idioma que, hace siglos, hablé en Nortumbria,
por la espada y el arpa de los sajones,
por el mar, que es un desierto resplandeciente
y una cifra de cosas que no sabemos,

Nog een gedicht van de gaven

Dank wil ik brengen aan het goddelijk
labyrinth van oorzaak en gevolg,
voor de diversiteit van de schepselen
die dit unieke universum vormen,
voor de rede, die niet zal ophouden te dromen
over een plattegrond van het labyrinth,
voor het gezicht van Helena en de volharding van Odysseus,
voor de liefde, die ons de anderen laat zien
zoals God hen ziet,
voor de vaste diamant en het losse water,
voor de algebra, paleis van nauwkeurig kristallen,
voor de mystieke munten van Angelus Silesius,
voor Schopenhauer,
die wellicht het universum heeft ontraadseld,
voor de gloed van het vuur
waarnaar geen enkele menselijk wezen kijken kan zonder
oeroude verbazing,
voor het mahoniehout, het cederhout, het sandelhout,
voor het brood en het zout,
voor het mysterie van de roos
die kwistig is met kleur en het niet ziet,
enkele beladen avonden en dagen in 1955,
voor de geharde gaucho's die op de vlakte
de dieren en de dageraad voortdrijven,
voor de ochtend in Montevideo,
voor de kunst van de vriendschap,
voor de laatste dag van Socrates,
voor de woorden die in een schemering werden uitgesproken
van het ene naar het andere kruis,
voor die droom van de islam met een omvang
van *Duizend-en-één-nacht*,
voor die andere droom van de hel,
voor die toren van louterend vuur
en voor de glorieuze hemelsferen,
voor Swedenborg,
die in de straten van Londen praatte met de engelen,
voor de geheime en onheuglijke rivieren
die in mij samenvloeien,
voor de taal die ik, eeuwen geleden, sprak in
Northumberland,
voor het zwaard en de lier van de Saksen,
voor de zee, die een glinsterende woestijn is
en een code van dingen die we niet weten,

por la música verbal de Inglaterra,
por la música verbal de Alemania,
por el oro, que relumbra en los versos,
por el épico invierno,
por el nombre de un libro que no he leído:
Gesta Dei per Francos,
por Verlaine, inocente como los pájaros,
por el prisma de cristal y la pesa de bronce,
por las rayas del tigre,
por las altas torres de San Francisco y de la isla de
Manhattan,
por la mañana en Texas,
por aquel sevillano que redactó la Epístola Moral
y cuyo nombre, como él hubiera preferido,
ignoramos,
por Séneca y Lucano, de Córdoba
que antes del español escribieron
toda la literatura española,
por el geométrico y bizarro ajedrez
por la tortuga de Zenón y el mapa de Royce,
por el olor medicinal de los eucaliptos,
por el lenguaje, que puede simular la sabiduría,
por el olvido, que anula o modifica el pasado,
por la costumbre,
que nos repite y nos confirma como un espejo,
por la mañana, que nos depara la ilusión
de un principio,
por la noche, su tiniebla y su astronomía,
por el valor y la felicidad de los otros,
por la patria, sentida in los jazmines
o en una vieja espada,
por Whitman y Francisco de Asís,
que ya escribieron el poema,
por el hecho de que el poema es inagotable
y se confunde con la suma de las criaturas
y no llegará jamás al último verso
y varía según los hombres,
por Frances Haslam, que pidió perdón
a sus hijos
por morir tan despacio,
por los minutos que preceden al sueño,
por el sueño y la muerte,
esos dos tesoros ocultos,
por los íntimos dones que no enumero,
por la música, misteriosa forma del tiempo.

J.L. Borges

voor de verbale muziek van Engeland,
voor de verbale muziek van Duitsland,
voor het goud dat fonkelt in de verzen,
voor de epische winter,
voor de naam van een boek dat ik niet heb gelezen:
Gesta Dei per Francos,
voor Verlaine, onschuldig als de vogels,
voor het glazen prisma en het bronzen gewicht,
voor de strepen van de tijger,
voor de hoge torens van San Francisco en op het
eiland Manhattan,
voor de ochtend in Texas,
voor de Sevillaan die de *Épístula Moral* schreef
en wiens naam wij, zoals hij zou hebben verkozen,
niet kennen,
voor Seneca en Lucanus, uit Córdoba,
die vóór de Spaanse taal
de hele Spaanse literatuur al schreven,
voor het meetkundig, bizarre schaakspel,
voor de schildpad van Zeno en de kaart van Royce,
voor de medicinale geur van eucalyptus,
voor de taal, die wijsheid kan veinzen,
voor de vergetelheid, die het verleden opheft of wijzigt,
voor de gewoonte,
die ons herhaalt en ons bevestigt als een spiegel,
voor de ochtend, die ons een illusie
van een begin verschaft,
voor de nacht, zijn duisternis en zijn astronomie,
voor de moed en het geluk van de anderen,
voor het vaderland, gevoeld in de jasmijn
of in een oud zwaard,
voor Whitman en Fransciscus van Assisi,
die het gedicht al schreven,
vanwege het feit dat een gedicht onuitputtelijk is
en versmelt met alle schepselen
en nooit zal komen bij het laatste vers
en naargelang de mensen varieert,
voor Frances Haslam, die haar kinderen
vergeving vroeg
voor haar langzame dood,
voor de minuten doe voorafgaan aan de slaap,
voor de slaap en voor de dood,
die twee verborgen schatten,
voor de intieme gaven die ik niet opsom,
voor de muziek, geheimzinnige gedaante van de tijd.

Uit: Jorge Louis Borges — Alle gedichten.
Vertaald door *Barber van de Pol en Maarten Steenmeijer*
Uitgeverij De Bezige Bij, Amsterdam

Curriculum Vitæ

Joost Aleid Keuskamp was born in Amsterdam, The Netherlands, on the 13^{th} of September 1977. He started his studies in biology in 1998. After an intermission in which his focus shifted towards sustainable development in Latin America, Joost finished his Biology studies with theses on root competition and self/non-self recognition of clonal plants, thermodynamics and nitrous oxide formation in decomposition, and organic carbon storage in naturally warmed soils of Iceland. After his studies, Joost joined the Netherlands Environmental Assessment Agency as a researcher before starting his PhD research of which this thesis is the result. Since January 2014 Joost holds a temporary position as a junior assistant professor in the Ecology & Biodiversity group in the Biology department of the Utrecht University, The Netherlands.