CHARACTERISATION OF ORGANIC NITROGEN COMPOUNDS IN SEDIMENT AND LEAVES OF A MANGROVE ECOSYSTEM IN NORTH BRAZIL

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LIST OF ABBREVIATIONS

N	nitrogen
TN	total nitrogen
C	carbon
TOC	total organic carbon
AA-N/TN	amino acid nitrogen from total nitrogen
AA- /TOC	amino acid carbon from total organic carbon
THAA	total hydrolysable amino acid
P	phosphorous
Eh	redox potential
SEM	Scanning Electron Microscopy
TBC	Total Bacterial Counts
AO	acridine orange
HPLC	High Performance Liquid Chromatography
$HgCl_2$	mercuric chloride
min	minutes
N_2	atmospheric nitrogen
dw	dry weight
ww	wet weight
w/w	absolute weight to weight ratio
w/v	weight/volume
cal yrs BP	calibrated years before present
TAE	tannic acid equivalents
No	number
Avi	*Avicennia geminans*
Rhi	*Rhizophora mangle*
Ses	*Sesuvium portulacastrum*
Ses roots	*Sesuvium portulacastrum* roots
Spo	*Sporobulus virginicus*
Spo roots	*Sporobulus virginicus* roots
Bat	*Batis maritima*
Bat roots	*Batis maritima* roots

asp	aspartic acid
glu	glutamic acid
asn	asparagine
ser	serine
gln	glutamine
thr	threonine
his	histidine
gly	glycine
arg	arginine
ala	alanine
tyr	tyrosine
val	valine
phe	phenylalanine
ile	isoleucine
leu	leucine
alx	aspartic acid and asparagine
glx	glutamic acid and glutamine

ABSTRACT

Mangrove forests are comprised of halophytic plants and are an important vegetation type along tropical coasts. Besides physiological constraints due to environmental stresses, the mangrove plants also experience restrictions in nutrient uptake through the immobilisation of nitrogen (N) into refractory complexes. Knowledge about the relationships between plants, sediments and hydrology in the mangrove ecosystem is crucial to the understanding of nutrient dynamics. The present work deals with N turnover in different compartments of this ecosystem and considers various organic N compounds and their availabilities as nutrient sources in order to understand the biotic and abiotic driving forces of N dynamics.

Research was conducted on the Bragança peninsula in North Brazil, where two vegetation units with contrasting inundation regimes were selected. Transect 1 was situated in a mixed forest (mainly *Rhizophora mangle* and *Avicennia geminans*) with tree heights up to 15 m and a semi-diurnal flooding regime, whereas transect 2 was set in a young *Avicennia* stand on a dry area, with low inundation frequency and high salt stress. In the driest part of transect 2 the succulents *Sesuvium portulacastrum* and *Batis maritima* and the salt tolerant grass *Sporobolus virginicus* are associated with *A. germinans*.

Sediment and leaf samples from field sampling and decomposition experiments were analysed for total nitrogen (TN) and total organic carbon (TOC) content in order to generally characterise the mangrove organic matter. $\delta^{15}N$ and $\delta^{13}C$ were assessed to trace sources of sedimentary organic matter and as indicators of environmental stress in plant material. Amino acid isomer concentrations (D- and L-enantiomers) were determined as markers for microbial transformation and diagenesis, and total phenols were analysed as representative of refractory substances.

Leaf degradation processes were assessed in a field and a laboratory experiment. The degradation of leaves in various states of senescence was followed in a mangrove creek under “natural” conditions and in the laboratory, where bactericides and fungicides were added to assess the importance of different microorganisms in the degradation of organic N compounds. In a separate experiment the ability of the mangrove crab *Ucides cordatus* to digest refractory substances such as tannins was evaluated.

The patterns of TN, TOC, isotopic ratios and tannins illustrated that sediments were divided into two pools of organic matter: one on the sediment surface, strongly influenced

by the current vegetation, and the second in the subsurface sediments, mainly characterised by the decomposers and the palaeoenvironment. In transect 2 the influence of the vegetation was seen clearly by the impact of the succulent *S. portulacastrum,* which induced high TN and TOC values in the surface sediments as compared to the forest without *Sesuvium* cover. Further analysis of the roots and rhizosphere of this species showed an association with mycorrhizal fungi and N_2-fixing bacteria. Evidence strongly suggests that there is a link between the occurrence of *S. portulacastrum*, the associated microorganisms and the accumulation of TN and TOC in the surface sediments.

The strong impact of environmental stress factors such as salinity and inundation frequency on plants was reflected in a positive correlation between $\delta^{13}C$ values and sediment salinity, i.e. with increasing stress, the effectiveness of the photorespiratory system to discriminate towards the lighter ^{12}C isotope diminished. Total amino acid concentrations as well as individual amino acids reacted differently depending on mechanisms of salt resistance (such as salt-excretion in *A. germinans* and salt-exclusion in *R. mangle*) and species-specific differences in osmoregulatory processes. The salt threshold, at which osmoregulation is initiated, differed between species, as did the compounds that would be used as cytoplasmic osmoregulators. Tannins generally increased with increasing salt stress and during the dry season.

During decomposition, tannins were lost rapidly from the leaves. Although leaching was partly responsible for this loss of tannins, the main actors in tannin decomposition were identified as the fungi, while bacteria were important in terms of N accumulation and immobilisation. The decomposition experiments clarified the source of TOC and TN accumulation in decaying plant material as being the bacterial colonisation, which increased TOC and TN partly through new synthesis of amino acids.

Concerning the main actors in terms of microbial litter decomposition, bacteria were shown to be dominant on decaying yellow and brown leaves, while black leaves were mainly colonised and decomposed by fungi. The amino acid D-alanine could be confirmed as a reliable biomarker for bacteria.

For the macrobenthos, the mangrove crab *Ucides cordatus* has been shown to be responsible for the breakdown of at least 67 % of total leaf litter (Schories*, et al.*, 2003). Decomposition experiments in the present study demonstrated that *U. cordatus* is able to digest hydrolysable tannins, thus proving the crabs to be an even more important link between plant litter and nutrient availability than has been previously thought.

In conclusion the importance of crabs and fungi in the breakdown of tannins and thus recycling of nutrients within an ecosystem could be demonstrated. The probable input of N into the system through N_2-fixation is achieved through the bacterial population, which, in the case of *S. portulacastrum*, was associated directly with the plant roots. The findings of this work give important information about N cycling within mangrove ecosystems and identified first clues for the possible restoration of wetland habitats with *S. portulacastrum*.

ZUSAMMENFASSUNG

Mangrovenwälder bestehen aus Halophyten und sind ein wichtiger Vegetationstyp an tropischen Küsten. Neben den physiologischen Beschränkungen aufgrund von umweltbedingtem Stress schränkt die Immobilisierung von Stickstoff (N) in refraktäre Komplexe die Nährstoffaufnahme der Mangrovenpflanzen zusätzlich ein. Die vorliegende Arbeit beschäftigt sich mit dem Stickstoffumsatz in unterschiedlichen Teilen dieses Ökosystems und berücksichtigt verschiedene Stickstoffverbindungen sowie ihre Verfügbarkeit als Nährstoffquellen, um die biotischen und abiotischen Einflußgrößen auf die Stickstoffdynamik zu verstehen.

Die Untersuchungen wurden auf der Halbinsel Bragança im Norden Brasiliens durchgeführt. Um die Zufuhr und die Persistenz von N sowie die Beziehung zwischen Pflanzen, Sediment und Hydrologie im Ökosystem Mangrove zu untersuchen, wurden zwei Vegetationseinheiten mit unterschiedlicher Überflutungsrate ausgewählt. Transekt 1 wurde in einem Mischwald (hauptsächlich *Rhizophora mangle* und *Avicennia geminans*) mit einer Baumhöhe von bis zu 15 m und semidiurnalem Überflutungspuls angelegt. Transekt 2 befindet sich in einem trockenen Gebiet mit jungem *Avicennia*-Bestand, einer niedrigen Überflutungsfrequenz und hohem Salzstress. Im trockensten Bereich des zweiten Transektes waren die Sukkulenten *Sesuvium portulacastrum* und *Batis maritima* sowie das salztolerante Gras *Sporobolus virginicus* mit *Avicennia* vergesellschaftet.

Sediment- und Blattproben von Feldstudien und Abbauversuchen wurden auf den Gehalt von Gesamtstickstoff (TN) und Gesamtkohlenstoff (TOC), als generelle Charakterisierung der organischen Substanzen in der Mangrove, untersucht. $\delta^{15}N$- und $\delta^{13}C$-Isotope wurden bestimmt, um die Quelle sedimentärer, organischer Substanzen ausfindig zu machen und um die Auswirkung von umweltbedingtem Stress auf das Verhältnis der Isotope in den Pflanzen abschätzen zu können. Aminosäure-Isomere (D- und L- Enantiomere) wurden als diagenetische Indikatoren und Indikatoren der mikrobiellen Umwandlung ermittelt. Gesamtphenol wurde als repräsentative refraktionäre Substanz analysiert.

Der Blattabbau wurde in einem Feld- und einem Laborexperiment untersucht. Der Abbau von Blättern in unterschiedlichen Seneszenzstadien wurde *in situ* in einem Mangrovenpriel verfolgt, während im Labor die Blätter mit Bakteriziden und Fungiziden behandelt wurden, um die Rolle, die diese beiden Organismengruppen beim Abbau organischer N-Verbindungen spielen, zu beurteilen. In einem separaten Experiment

wurde die Fähigkeit der Mangrovenkrabbe *Ucides cordatus* bewertet, Tannine zu verdauen.

Die Verteilung von TN, TOC, Isotopenverhältnissen und Tannin im Boden zeigten, dass die organischen Substanzen in zwei Pools aufgeteilt werden können. Einen an der Sedimentoberfläche, der stark von der aktuellen Vegetation beeinflusst wird und einen weiteren in der darunter liegenden Sedimentschicht, die hauptsächliche von den Destruenten und der Palaeovegetation gekennzeichnet ist. Im Transekt 2 zeigt sich der Einfluss der Vegetation deutlich an Hand der Sukkulenten *S. portulacastrum*. Verglichen mit dem Wald ohne *Sesuvium*-Decke) sind die TN- und TOC-Werte an der Sedimentoberfläche signifikant höher. Weitere Analysen der Wurzeln und der Rhizosphäre dieser Art zeigten eine Assoziation mit einem Mykorrhiza-Pilz und N_2-fixierenden Bakterien. Dieses weist stark darauf hin, dass eine Verbindung zwischen dem Auftreten von *S. portulacastrum,* den assoziierten Mikroorgansimen und der Akkumulation von TN und TOC besteht.

Die Auswirkungen der Stressfaktoren wie Salzgehalt und Überflutungsfrequenz auf die Pflanzen, spiegelte sich in der positiven Korrelation zwischen $\delta^{13}C$-Verhältnis und dem Salzgehalt des Sediments wider, d.h., mit steigendem Umweltstress sinkt die Effektivität des photorespiratorischen Systems, nach dem leichteren ^{12}C Isotop zu diskriminieren. Veränderungen in der Konzentration der Gesamtaminosäuren sowie der einzelnen Aminosäuren erfolgten in Abhängigkeit von den verschiedenen Salzresistenzmechanismen der Pflanzen (wie z.B. Salzexkretion bei *A. germinans* und Salzexklusion bei *R. mangle*) und von den artspezifischen Unterschieden in bei den osmoregulatorischen Prozessen. Der Grenzwert, bei dem Osmoregulation einsetzte, war wie auch die Osmolyten bei allen untersuchten Arten unterschiedlich. Der Tanningehalt stieg mit steigendem Stress und während der Trockenzeit.

Während des Blattabbaus wurde ein rapider Verlust der Tannine beobachtet. Obwohl Auswaschung auch zum Teil verantwortlich war, konnten Pilze als die wichtigsten mikrobiellen Akteure im Tanninabbau identifiziert werden. Der bakterielle Bewuchs von zerfallendem Pflanzenmaterial hingegen konnte als Quelle der TN-Akkumulation und Immobilisation identifiziert werden. Bakterien erhöhten den TOC- und TN-Gehalt teilweise durch Neu-Synthese von Aminosäuren.

Bakterien sind die Hauptakteure bezüglich des mikrobiellen Abbaus von gelben und braunen Blättern, während die schwarzen Blätter hauptsächlich von Pilzen kolonisiert und

abgebaut werden. Die Aminosäure D-Alanin konnte in dieser Studie als verlässlicher Bioindikator für die Anwesenheit von Bakterien bestätigt werden.

Die Mangrovenkrabbe *Ucides cordatus* ist nachweislich für den Abbau von mindestens 67 % des anfallenden Streumaterials verantwortlich (Schories *et al.,* 2003). In der vorliegenden Arbeit konnte an Hand von Abbauexperimenten gezeigt werden, dass *U. cordatus* hydrolysierbares Tannin verdauen kann und damit eine wichtigere Rolle als Bindeglied zwischen Streumaterial und Nahrungsverfügbarkeit einnimmt als bisher angenommen.

Somit konnte in der vorliegenden Arbeit erstmals die Bedeutung von Krabben und Pilzen im Abbauprozess von Tannin und damit der Regenerierung von Nährstoffen innerhalb des Ökosystems Mangrove aufgezeigt werden. Der mutmaßliche Eintrag von N in das System wird über die N_2-Fixierung der Bakterienpopulation geleistet. Diese Bakterien waren im Fall von *S. portulacastrum* direkt mit den Wurzeln der Pflanze assoziiert. Die Ergebnisse dieser Arbeit liefern wichtige Informationen zur Zirkulation von N innerhalb eines Mangrovenökosystems und erste Anhaltspunkte für eine mögliche Renaturierung tropischer und subtropischer Feuchtgebiete mit *S. portulacastrum* als Pionierpflanze.

RESUMO

Manguezais, os quais são dominados por plantas halófilas, são um tipo de vegetação importannte em áreas costeiras tropicais. Além de restrições fisiológicas devido a estresses ambientais, os manguezais também experimentam limitações na aquisição de nutrientes, dada a imobilização do Nitrogênio (N) em complexos refratários. Ressalta-se que o Conhecimento sobre as relaçoes entre Plantas, Sedimentos e Hidrologia de um sistema de mangue é essencial para o entendimento das dinamicas dos Nutrientes. O presente trabalho investiga a reciclagem de N em diferentes compartimentos deste ecossistema e considera as várias formas orgânicas de N e suas disponibilidades como fontes de nutrientes afim de entender as forças propultivas bioticas e abioticas das dinamicas de N.

A presente Pesquisa foi conduzido na península de Bragança, no Norte do Brasil, onde duas unidades de vegatação com regimes de inundanção contrastante foram selecionadas. O primeiro Transeto localisou-se em uma floresta mixta (principalmente *Rhizophora mangle* e *Avicennia geminans*) com arvores ate 15 m de altura e um regime de inundação semi-diurnal, enquanto que o segundo Transeto encontrava-se em uma floresta de jovens *Avicennia* em uma area seca com baixa frequencia de inundação e alto nivel de estresse de sal. Na área mais seca do segundo Transeto os halofitos *Sesuvium portulacastrum* e *Batis maritima* e a erva gramínea tolerante ao sal *Sporobolus virginicus* foram associadas com *A. germinans*.

Sedimento e folhas obtidas através de amostragens em campo e experimentos de decomposição foram analisados no que se refere ao Nitrogênio Total (TN) e conteúdo de carbono total (TOC) afim de obter uma caracterização geral da matéria orgânica derivada do manguezal. Os isótopos $\delta^{15}N$ e $\delta^{13}C$ foram analizados para traçar fontes de matéria orgânica sedimentar e como indicadores para o estresse ambiental refletido nas plantas. Isômeros de aminoácidos (enantiômeros D e L) foram determinados como marcadores para a transformação microbial e a diagenese. Fenóis totais foram analisados como representantes de substâncias refratárias.

Os processos de degradação das folhas em vários estádios de senescência foram investigdos em experimentos de campo (em um canal de maré) e em Laboratório. No processo de análise Laboratorial, bactericidas e fungicidas foram adicionadas para investigar a importancia de diferentes microorganismos no processo de degradação das formas orgânicas de N. Em um experimento adicional foi analisada a abilidade do

Caranguejo *Ucides cordatus* em digerir substâncias refratárias como por exemplo taninos.

O padrão de TN, TOC, proporção isotópica e taninos ilustraram que os sedimentos foram divididos em dois grupos de matéria orgânica: um sobre sedimentos superficiais, fortemente influenciados pela vegetação presente e o segundo nos sedimentos sub-superficiais, principalmente caracterizados por decompositores e pelo paleo-ambiente. No segundo Transecto, a influência da vegetação foi vista, claramente, pelo impacto da suculenta *S. portulacastrum*, a qual induziu altos valores de TN e TOC nos sedimentos superficiais, em comparação com florestas sem cobertura de *Sesuvium*. Análises adicionais das raízes e da rizoesfera desta espécie mostrou associação com fungos micorrísicos e bactérias fixadoras de N_2. Evidências sugerem fortemente que existe uma conexão entre a ocorrência de *S. portulacastrum*, os microorganismos associados e a acumulação de TN e TOC nos sedimentos superficiais.

Os fatores de estresse ambiental como salinidade e freqüência de inundação exercem forte influência sobre as plantas. Isto esteve refletido em uma correlação positiva entre o $\delta^{13}C$ e salinidade do sedimento, significando que com o aumento do estresse ambiental, a eficiencia do sistema fotorespiratorio de escolher pelo isótopo mais leve ^{12}C diminuiu. As concentrações totais de aminoácidos, bem como aminoácidos individuais reagiram diferentemente dependendo dos mecanismos de resistência ao sal (por exemplo excreção de sal em *A. germinans* e exlusão de sal em *R. mangle*) e das diferencas nos processos osmo-regulatorios das espécies. Os limites para iniciar a osmo-regulação do sal diferenciam-se entre as espécies, bem como os componentes que poderiam ser utilizados como osmoreguladores Citoplasmáticos. Taninos geralmente aumentaram com o estresse de sal e durante o período seco.

Durante a decomposição as Folhas perderam rapidamente taninos. O lixiviação poderia ser responsável parcialmente para a perda de taninos, no entanto o causador principal para a decomposição de taninos puderam ser identificado como Fungo enquanto que as Bacterias foram importante para a accumulação e a immobilisação de N. Os experimentos de decomposição demostraram as fontes de acumulação de TOC e TN na decomposição do material vegetal como sendo a colonização bacteriana, as quais aumentaram TOC e TN através da biossíntese de aminoácidos.

Bactérias foram os principais responsáveis pela decomposição de folhas amarelas e marrons, enquanto que folhas pretas foram principalmente colonizadas e decompostas

por fungos. O aminoácido D-alanin pôde ser confirmado como um biomarcador seguro para as Bacterias.

Schories *et al.* (2003) demonstraram que o caranguejo *Ucides cordatus* é responsável para a redução de pelo menos 67% do total da desfolhagem. Experimentos decompositórios da presente pesquisa demonstraram que *U. cordatus* é capaz de digerir taninos hidrolisaveis. Com isso se comprova que os carangueijos são uma conexão bem mais importante entre a desfolhagem e a disponibilidade nutritiva do que pensado anteriormente.

Conluye-se a importância dos Caranguejos e dos Fungos na redução de taninos e com isso na reciclagem de nutrientes no ecosistema. O provável input de N no sistema por fixação de N_2 foi alcancado pela população bacteriana que no caso de *S. portulacastrum* foram diretamente associados com as raizes das plantas. Os resultados deste trabalho são fonte de importante informação a respeito da reciclagem de N dentro de um ecosistema de Mangue bem como sobre futuras possibilidades de processos de restauração de Zonas Úmidas.

1 INTRODUCTION

Mangrove forests are the characteristic and predominant intertidal plant formations of sheltered tropical and subtropical coastlines, spreading from approximately 32°N to 38°S (Saenger, 2002). About 60-75 % of the tropical shores are fringed by this unique ecosystem (Spalding*, et al.*, 1997), however the ongoing destruction due to human activity has been estimated to have reduced the world's area of mangrove forests by about 35 % between the years 1980 and 2000 (Valiela*, et al.*, 2001). In the wet tropics where climatic conditions are favourable for rapid plant growth, mangroves are usually highly productive and play a major role in supporting coastal food webs and nutrient cycles (Dittmar, 1999; Alongi*, et al.*, 2000). They are probably one of the driving forces for near-shore primary and secondary production, transporting inorganic and organic nutrients by means of tides and currents to adjacent environments.

In the 19th century Charles Darwin compared the mangroves forests to the grass in a churchyard, "both [are] nourished by putrid exhalations" (Darwin, 1839), and until late into the 20th century such negative perceptions of mangroves as unhealthy, rotting environments prevailed. However, with increasing knowledge of mangroves, their interest and values became more apparent. The ecological and economical values of mangrove forests are manifold, including shoreline protection through their unique root architectures, timber and charcoal production and fisheries resources (Saenger, 2002). Mangroves are an important habitat for the intrinsic fish and invertebrate species, but also serve as nursery for many pelagic species (Krumme*, et al.*, 2004). After considerable destruction of this ecosystem, mangroves are increasingly being protected and managed today. Restoration of destroyed habitats, however, remains a process that is still poorly understood and not always successful (Zedler, 2000; Zedler*, et al.*, 2003).

The definition of what does and does not constitute a 'true' mangrove plant is not settled in the literature. The difficulty arises because mangrove communities are an ecological assemblage rather than a taxonomic or morphological grouping (Saenger, 2002). For example, most mangrove trees are not obligate halophytes. Some mangrove species are able to survive in other habitats, although they are generally slow-growing species and therefore do not compete well with faster-growing species unless those species are suppressed by salt. Nevertheless 24-26 families have been recognised throughout the world as being mangroves (Tomlinson, 1986; Saenger, 2002). Most species have developed sophisticated above- and below-ground root systems which can absorb wave

energy, regulate sedimentation and erosion and increase nutrient and water retention in the sediments.

Sesuvium portulacastrum and *Sporobolus virginicus* are conspicuous representatives of salt marsh flora worldwide and can grow at substrate salinities similar to or higher than sea water (Tomlinson, 1986; Marcum and Murdoch, 1992; Boorman, 1999). They are indicators of change of inundation dynamics and salinity oscillations, are used for stabilisation of dunes, for seawater horticulture or landscape shaping and can play a role as pioneer plant in hypersaline or arid regions (Bush*, et al.*, 1999). Interactions between species as well as between species and sediments must be better understood, including the effect of environmental gradients and stress factors such as high salinities and hypoxia.

Mangrove ecosystems demonstrate close links between the vegetation assemblage and geomorphologically-defined habitats (Woodroffe and Grindrod, 1991), but up to today there is conflicting evidence of the effects of different mangrove species on interstitial water chemistry, nutrient and trace element chemistry, redox potential, decomposition rates and pathways of sedimentary organic matter; and - vice versa - of the effects of the sediment and habitat conditions on the vegetation (Alongi*, et al.*, 1998). A key question that remains is whether the reported biochemical differences found in plants and sediments are a function of physical setting (intertidal position, geographical setting) or whether it is rather the capacity of different species to alter sediment conditions that influences the physical conditions?

The physico-chemical circumstances can influence the vegetation in various manners. Even subtle increases in the soil salinity caused by changes in rainfall or tidal flooding regimes can produce significant ecotone shifts in coastal regions. The understanding of such ecotone displacements at local and regional scale requires a deep knowledge of mangrove dynamics in relation to the influence of inundation regime and topography on sediment porewater salinity (Slavich*, et al.*, 1999).

At this point it must be considered to what extent the effect of substrate porewater salinity on vegetation structure can be separated from the influence of the flooding regime itself. Both variables are highly correlated, however the inundation frequency affects not only salinity but also the redox conditions in the sediment and, through this, the nutrient availability and forest structure (Feller*, et al.*, 2003a; Feller*, et al.*, 2003b). In previously investigated high-resolution topographic gradients in the Bragança Peninsula, North

Brazil, the inundation frequency was the main factor influencing leaf phosphorus, tree height and available phosphorus in the sediment substrate (Silva and Sampaio, 1998; Cordeiro*, et al.*, 2003).

The frequent hypoxic conditions in flooded soils also have a negative effect on nitrogen (N) uptake and turnover (Koch*, et al.*, 1990). Decreased aeration affects ammonium uptake in *Spartina alterniflora* and *Panicum hemitomon*. In coastal wetlands with abundant sulphate from marine water, hypoxia leads to bacterial sulphate reduction to sulphide. An important negative effect of sulphide on plant growth is an inhibition of N uptake, which decreases with increasing sulphide concentration and is reflected e.g. in a decreased leaf elongation. Under these conditions, it is particularly relevant in which way in-situ remineralisation of plant detritus is integrated into N turnover. In general, organic matter turnover is slow under hypoxia.

In addition to the effects of hypoxia on nutrient dynamics, salt can affect growth through the action of sodium as a competitive inhibitor of NH_4^+ uptake (Odum, 1988). The activity of nitrate reductase (NRA), the enzyme which reduces nitrate to ammonium in N uptake of plants, often decreases under salt stress (Flores*, et al.*, 2000). Decreases in NRA activity and in total nitrogen and nitrate uptake have been reported in leaves of the mangrove species *Bruguiera parviflora* (Parida and Das, 2003; Parida*, et al.*, 2004). The activity and transcript abundance of ferredoxin-dependent glutamate synthase which is the key enzyme of N assimilation and biosynthesis of amino acids, decreases in leaves in response to salt stress (Popova*, et al.*, 2002).

Interestingly, halophytes react to salt stress which impedes N uptake by the production of nitrogenous compounds such as proline or glycine betaine to compensate osmotic pressure. In *S. portulacastrum* and *Batis maritima* levels of proline increased considerably in the dry season (Luttge*, et al.*, 1989). From a physiological point of view, vascular plants in salt water invest more energy than do freshwater plants to exclude or extrude salts and sulphides; energy that might be otherwise stored as net primary production.

While there are several studies on phosphorous dynamics in coastal wetlands in dependency of flooding regimes (Cohen*, et al.*, 2004), less is known on its effect on N dynamics along gradients of flood-dependent parameters in wetland sediments such as salinity and redox potential. Organic N is of particular importance as a direct nutrient source for bacteria or as substrate for regeneration of inorganic nutrients.

This PhD thesis deals with nitrogen turnover in different compartments of an ecosystem dominated by halophytic or halotolerant vegetation, and considers various organic nitrogen compounds and their availability as nutrient source (*Figure 1.1*). Key questions include: Which part of the nitrogen input into the ecosystem (e.g. through leaf litter) is available to organisms as nutrient source and how much is bound to refractory substances such as tannin or tannin-associated proteins. How long do refractory substances such as tannin persist in the ecosystem without being available as a nutrient source and are there organisms which can degrade these so-called non-degradable substances?

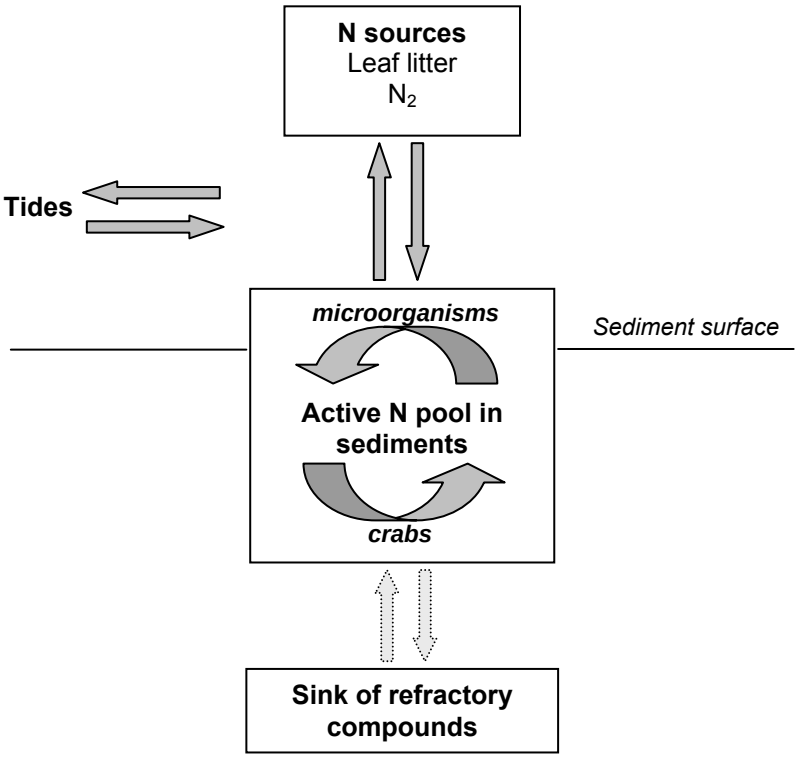


Figure 1.1: Diagram of the nitrogen cycle in mangrove sediments

Nitrogen input into the ecosystem occurs mainly through leaf litter and atmospheric N_2-fixation. On the Bragança peninsula leaf litter from the mangrove trees has been identified as one of the most important sources of nutrients to the sediment and waters in the mangrove. Mehlig (2001) measured a mean total litter fall rate of 3.5 g m^{-2} d^{-1}. Approximately 70 % of this total litter could be attributed to leaves of *Rhizophora mangle* and *Avicennia germinans*. Calculations and data from Nordhaus *et al.* (2006) and Schories *et al*. (2003) estimated that between 70 and 80 % of the annual litter fall remains within the system and is decomposed by benthic organisms, mainly the crab *Ucides cordatus*.

Atmospheric N_2-fixation as process of N input into the system has often been assumed to be widespread in mangroves (Holguin*, et al.*, 1992), but reported rates vary substantially with species and location (Pelegri and Twilley, 1998; Ravikumar*, et al.*, 2004) and has not been measured for the Bragança pensinsula. Better understanding of the mechanisms of N_2-fixation in mangrove ecosystems is essential for determining N budgets and dynamics. Ravikumar *et al.* (2004) investigated the role of N_2-fixing bacteria from a mangrove habitat and their utility as marine biofertilizers for wetland regeneration. The question of the main actors in the process of N_2-fixation – a bacterial community, fungal association (arbuscular mycorrhizal root associations) or maybe both – has become a central issue on this field in recent years. In general, studies about the association of N_2-fixing microorganisms with halophytic plants are scarce, particularly in relation to a changing salinity medium (Bekki*, et al.*, 1987; Soussi*, et al.*, 1999; Serraz*, et al.*, 2001; Ravikumar*, et al.*, 2004).

In the sediments, organic N compounds can be incorporated into refractory or slowly degradable macromolecules in the course of humification of organic matter, and compounds otherwise considered easily available for heterotrophic organisms, such as peptides and amino acids, could be trapped in macromolecules and selectively protected from enzymatic attack (Lara*, et al.*, 1993). Thus, the remineralisation or direct uptake of this fraction of the total N pool is retarded or even impeded. In consequence this part of the N pool is withdrawn from the active turnover fraction and hence its assignment to the labile fraction of N compounds solely based on amino acid quantification leads to an overestimation of the nutritional status of the substrate.

Tannins are polyphenolics which are known to contribute to the refractory substances in sediments. Historically, interest in tannins stems from its ability to bind proteins in the process of tanning leather. Biologically, tannins have been known as feeding repellents (Zucker, 1983) or protectors of solar radiation (Lovelock*, et al.*, 1992). Geochemically, potential nitrogen binding and nitrogen immobilisation by tannins are of great interest and represent processes that are still poorly understood. The insoluble fraction of tannins can be broken down through some very slow microbial decomposition to soluble polyphenols and both fractions, soluble and insoluble, can form polyphenol-protein complexes. The complexes can originate from senescent plant tissue when polyphenols stored in the central vacuole come into contact with cytoplasmic proteins, or in the sediment substrate when they complex with proteins originating from leaf litter and/or microorganisms (Hattenschwiler and Vitousek, 2000). These complexes are resistant to most decomposers. However, resistance varies with quality of polyphenolics. Knicker and Hatcher (1997) found that nitrogenous organic matter was protected from degradation by

encapsulation within a macromolecular matrix which was not affected by hydrolysis in 6N HCl and survived more than 4000 yrs in sediments.

Unlike carbohydrates, lipids, amino acids and pigments which are ubiquitous in organic matter and can have both marine and terrestrial sources, tannins (along with lignin and cutin) are uniquely terrestrial, thus could provide important source information. Tannins in vascular plants occur as two types, condensed and hydrolysable (*Figure 1.2*). Condensed tannins (also referred to as proanthocyanidins) are the most abundant polyphenols in woody plants, but are usually absent in herbaceous plants. Hydrolysable tannins have a more restricted occurrence, and have been reported in only 15 of the 40 orders of dicotyledons, which can produce both condensed and hydrolysable tannins (Hattenschwiler and Vitousek, 2000; Kraus*, et al.*, 2004). Total tannins can comprise as much as 20 % of leaf tissue of the mangrove species *Rhizophora mangle* (Benner*, et al.*, 1990b).

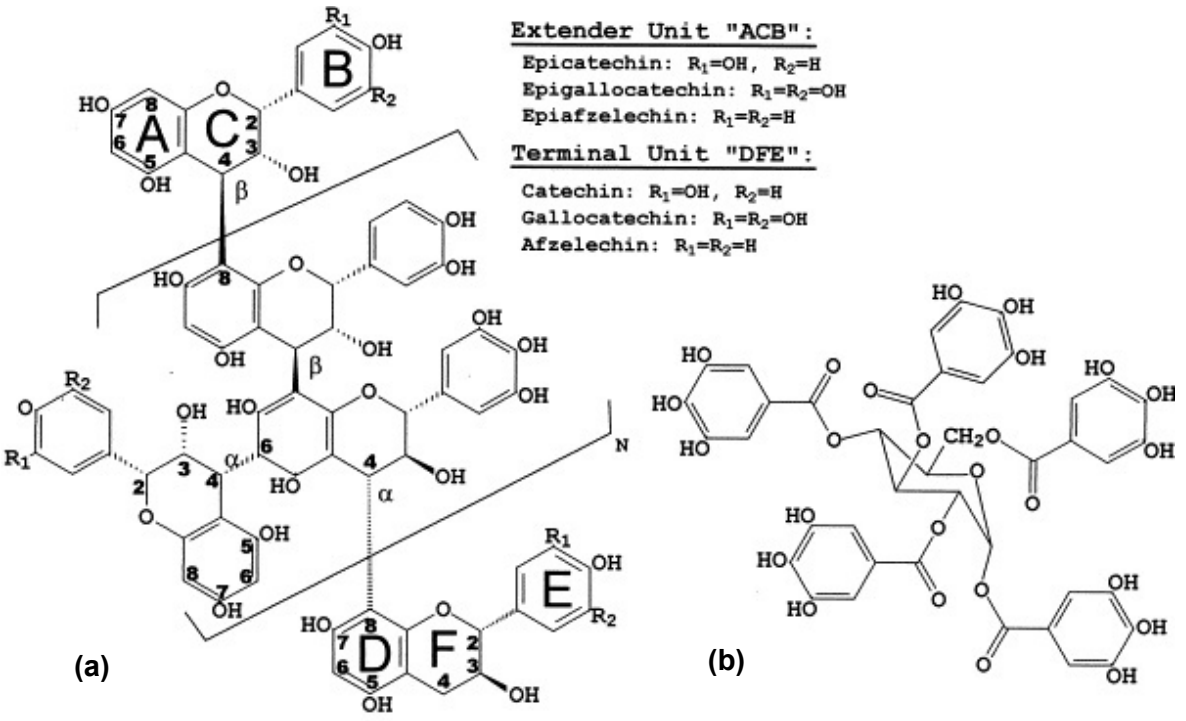


Figure 1.2: Structures of typical condensed (a) and hydrolysable tannins (b) (from Hernes *et al.*, 2001)

Amino acids are structural components of proteins and are the most abundant nitrogen-bearing compound in most organisms. Moreover, together with carbohydrates they quantitatively dominate organic carbon in living organisms (Wakeham*, et al.*, 1997; Unger*, et al.*, 2005b).

Thus amino acids are ubiquitously found in most environments and their analysis provides a useful tool to evaluate the reactivity of organic matter. Hubberten *et al.* (1994) for example found evidence for an association between amino acids and recalcitrant organic matter in the marine environment. Hence, in combination with the aforementioned tannin analysis, the amino acid data can offer clues about the diagenetic status of organic matter.

Amino acids exist in two isomers with either left- or right-handed symmetry, designated the L- and the D-forms, respectively. The amino acid residues in protein molecules are almost exclusively L-stereoisomers. D-amino acids have been found only in a few peptides, such as peptidoglycan in bacterial cell walls or certain antibiotics (Campbell*, et al.*, 1999; Nelson and Cox, 2000). Particular amino acids can be used to trace the source of organic matter; specifically the presence and abundance of microbial derived organic matter can be traced on the basis of the D-enantiomers of amino acids. D/L-ratios of individual amino acids have been frequently used as indicators for microbial activity or microbial transformation of organic matter (Engel and Macko, 1993; Dauwe and Middelburg, 1998)

The differentiation of labile and refractory or slowly degradable forms of nitrogen is central to the question of nutrient availability for coastal vegetation, particularly in suboxic environments. The usual determination of total N does not allow a deep insight into substrate fertility and its influence on vegetation structure and dynamics, since the N uptake under suboxic conditions is a complex, energetically disfavoured process with significant isotopic fractionation. Hence, a potential primary source of N like the fixation of atmospheric N_2 and a potential temporary “sink” such as the formation of slowly degradable substances, are essential targets in the study of N cycling in halophytic communities.

The MADAM project

This work was performed in the frame of the MADAM project (Mangrove Dynamics and Management) which was launched in 1995 and ended in 2005. MADAM was a cooperation between the Federal University of the State of Pará (UFPa) located in Belém,

Brazil, and the Centre for Tropical Marine Ecology (ZMT) in Bremen, Germany. The project was based on an integrated approach to mangrove dynamics and management (Berger*, et al.*, 1998) and comprised the scientific fields of biology, biogeochemistry, socio-economics, geography and meteorology. This study was integrated into the working group 'Internal cycles' of Dr. R.J. Lara which comprised works on nutrient and organic matter dynamics, reconstruction of mangrove palaeoenvironment and coastal vulnerability.

2 STUDY AREA

All sampling was carried out in a mangrove ecosystem in Northern Brazil near the city of Bragança (01°03'S, 46°45'W). The research area is located on a peninsula of about 160 km² on the Atlantic coast of Brazil, approximately 300 km southeast of the Amazon delta and 200 km east-north-east of Belém, the capital of the federal state Pará (*Figure 2.1*).

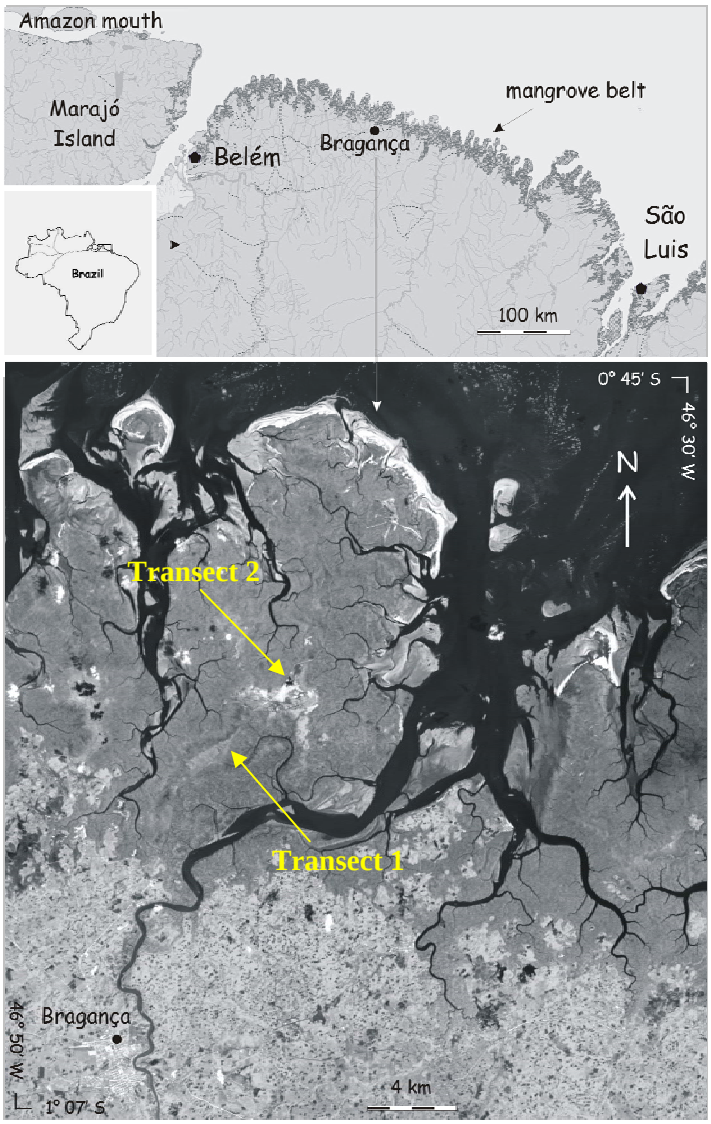


Figure 2.1: Study area. Rio caeté estuary and peninsula of Bragança, maps modified after Lara (2003).

The peninsula is part of a 8,900 km² coastal mangrove area which forms one of the world's largest mangrove ecosystems (Kjerve and Lacerda, 1993) and is bordered by the Caeté River estuary on the east side and the Maiaú Bay in the west. The area is crossed

by numerous tidal creeks and channels. About 87 % of the peninsula is covered by mangrove forests, dominated by the three tree species *Rhizophora mangle* (red mangrove), *Avicennia germinans* (black mangrove) and *Laguncularia racemosa* (white mangrove), the latter being the least dominant. Due to varying elevation and periods of inundation, different forest types have formed. Low *Avicennia* forests with individuals of less than 1 m occur next to mixed forests with tree heights of 25 m and more. The high mangrove forest is inundated only fortnightly during spring tides, whereas the lower stands are subjected to diurnal or semi-diurnal flooding. Previous studies showed that growth rates often correlated with salinity (Menezes*, et al.*, 2003). *R. mangle* stands are found closer to the banks of the river or tidal channels, whereas *A. germinans* is found in the drier parts of the peninsula.

Total litter fall contributes approximately 3.5 g dry matter m^{-2} d^{-1} (Mehlig, 2001) and represents an important source for the organic carbon and nitrogen pool in the research area. The principle pathway for nutrient and organic matter export from the mangrove to the estuary is porewater flow to the tidal creek and subsequently to the estuary. In areas with low inundation, leaf litter is mixed with sediment during the tides and remains in the sediment body during dry periods (Dittmar, 1999).

The peninsula is crossed by a paved road, which was built during the 1970's and connects the city of Bragança with the northern beach of Ajuruteua. In consequence of the modified hydrological conditions, large areas of mangrove forest died and left several degraded areas.

At the centre of the peninsula the elevation is approximately 2.5 m above mean sea level. Here an extensive marsh area dominated by halophytic herbs such as *Sporobulus virginicus* and *Eleocharis geniculata* has formed. On some small unflooded patches within the marsh region so-called "terrafirme forests" have developed: a dense vegetation of palms, cashew trees and other non-halophytic species.

The most conspicuous species of the benthos is the mangrove crab *Urcides cordatus* which represents about 84 % of the total benthic biomass of the study area. The species constitutes the main income source of more than 50 % of the local rural households hence is one of the most heavily exploited resources of the Bragantinian mangrove forests. *U. cordatus* is a key species which retains and processes large amounts of leaf litter and hence organic matter within the system (Diele, 2000; Schories*, et al.*, 2003; Nordhaus, 2004).

The climate in the Bragantine region is characterised by a marked seasonality with a rainy season between January and May and a dry season between June and December. Mean monthly precipitation rates and air temperatures for the years 2003 and 2004 are shown in *Figure 2.2*. The mean annual air temperature was 27 ± 0.6 °C. Temperature data for October-December 2004 as well as precipitation data for December 2003 are missing due to technical problems at the weather station.

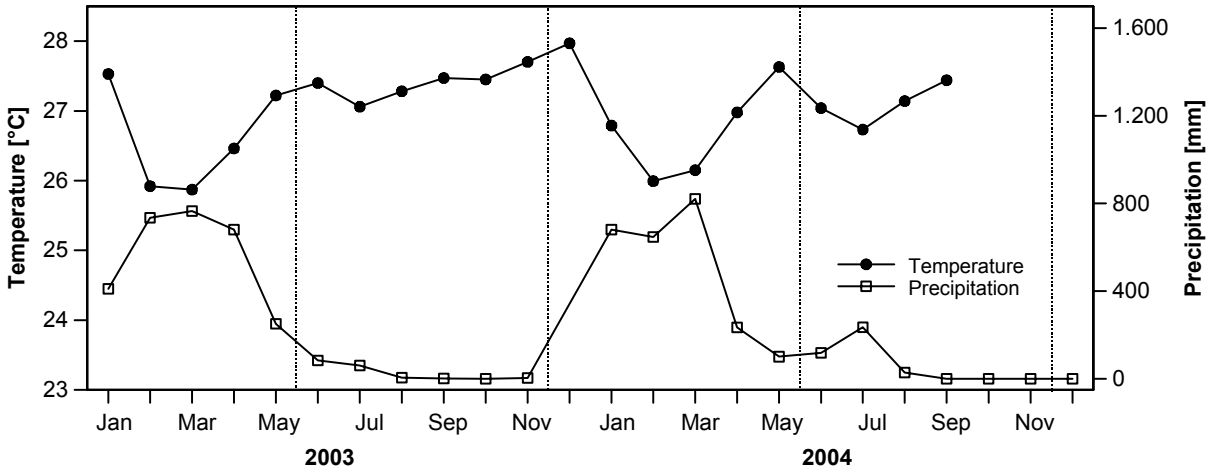


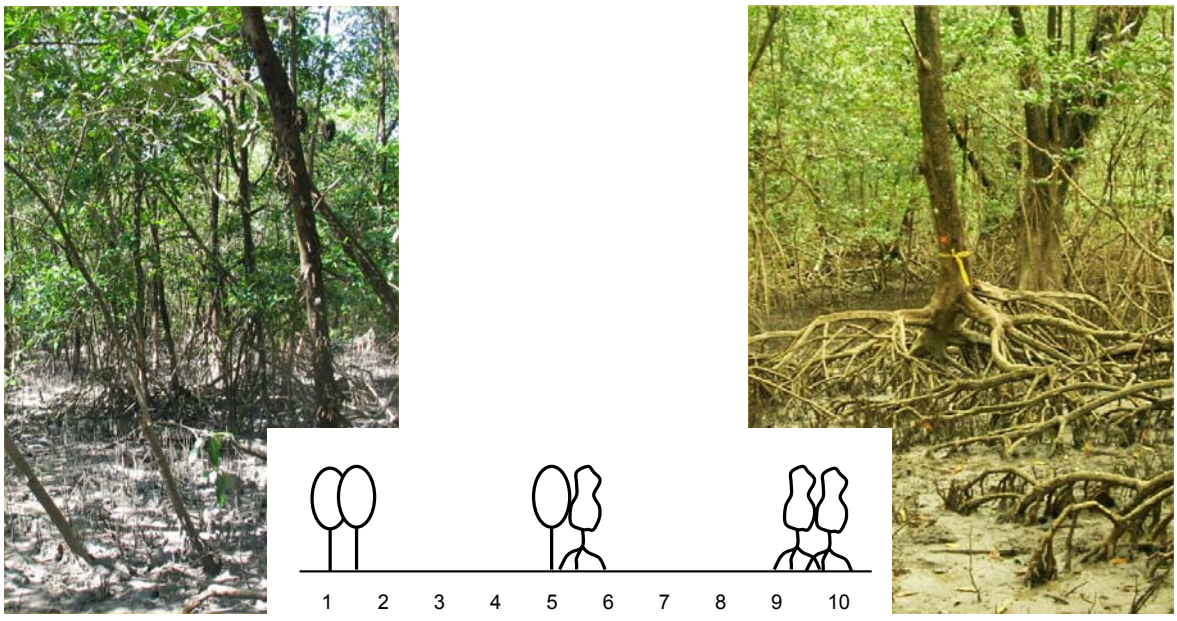
Figure 2.2: Precipitation and air temperatures for the years 2003 and 2004, data from the automatic weather station at Furo Grande (0°50'02"S, 46°38'27"W) operated by the UFPa. Vertical lines indicate division between rainy and dry season.

Sampling areas

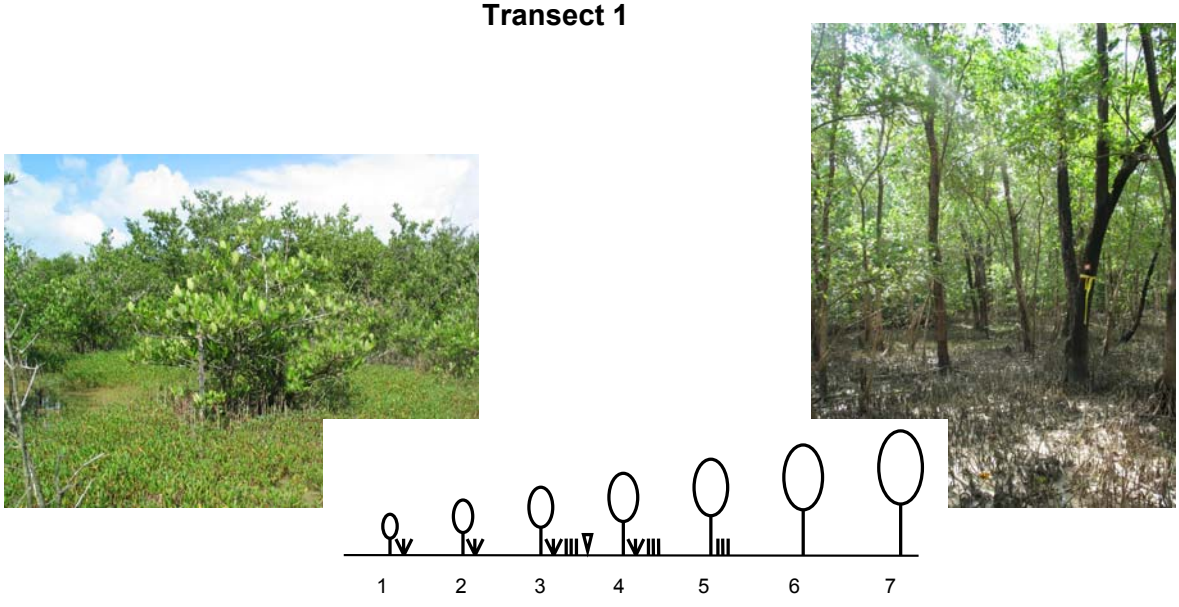
To assess input and persistence of nitrogen in the mangrove ecosystem, two vegetation units with contrasting inundation regime were selected (*Figure 2.3*). Transect 1 was set in a mixed forest (*Rhizophora mangle* and *Avicennia geminans*) with tree heights up to 15 m and a semi-diurnal flooding regime. 10 stations were established with station 1 being nearest to the road and station 10 being nearest to the tidal channel. The distance between stations ranged from 58 to109 m (on average 77.6 ± 20.5 m). Station 1 was nearest to the road, point 10 nearest to the tidal channel. Stations 1 to 4 were situated in the *Avicennia*-dominated part of the forest, whereas stations 7 to 10 describe the *Rhizophora*-dominated region. Stations 5 and 6 were located in the transition zone, where both species occur in approximately equal numbers.

Transect 2 was located in an *Avicennia germinans* stand on a dry area, with low inundation frequency and high salt stress. Tree heights ranged from 1.2 m to 10 m. In the driest part of this area the two halophytes *Sesuvium portulacastrum* and *Batis maritima*

and the salt tolerant grass *Sporobolus virginicus* were associated with *Avicennia germinans*. Here samples were taken at 7 stations, with station 1 being in the dry part of the transect and station 7 nearer to a tidal channel. The distance between stations ranged from 30 to 56 m (on average 41.2 ± 12.5 m). Point 1 was nearest to the road where only small *Avicennia* trees (minimum height of 1.2 m) grow into a former salt marsh dominated by *Sesuvium portulacastrum*, point 7 was situated furthest into the older mangrove forest, where no herbaceous plants grow and trees reach a height of 10 m.



Transect 1



Transect 2

Figure 2.3: Description of the sampling areas Transect 1 and 2, numbers are station numbers.

3 MATERIAL AND METHODS

3.1 Field sampling

Samples were collected during April/May 2003 and 2004 for the rainy season and in September/October 2003 and 2004 for the dry season.

3.1.1 Sediment material (Surface sediment and sediment cores)

Surface sediment was taken with a clean spatula directly into precombusted glass vials, sealed and stored in the freezer as quickly as possible. Sediment cores were drilled up to 1.5 m with a Russian Sampler. The cores (three replicate cores per station) were taken whole to the laboratory and stored in a freezer. Surface samples and samples from different depths (generally 10 and 50 cm) taken from the cores were freeze-dried, ground and stored in the freezer until further analysis of amino acids and tannin. In transect 10 cores were taken from the surroundings of *Avicennia* and *Rhizophora* trees separately to monitor whether the different tree species affect the sediment differently.

For Total Bacterial Counts (TBC) sediment samples were stored in a 4 % formalin solution, whereas the samples for Scanning Electron Microscopy (SEM) were fixed in 3 % glutaraldehyde in 0.065 M phosphate buffer (pH 7.4).

3.1.2 Plant material

Leaves from *R. mangle* and *A. germinans* were collected in different mangrove areas from trees of varying heights. Leaf and root samples from the halophytic herb *S. virginicus* and the succulents *S. portulacastrum* and *B. maritima* were collected from the salt marsh area. Epiphyta covering mangrove roots were scraped off with a sterile spatula and stored in a glass vial. All plant samples were washed with distilled water. For amino acid and tannin analysis the samples were freeze-dried, ground and stored in clean precombusted glass vials in a freezer at -20°C for further analysis.

As for sediments, leaf and root samples for TBC were stored in a 4 % formalin solution, whereas the samples for Scanning Electron Microscopy (SEM) were fixed in 3 % glutaraldehyde in 0.065 M phosphate buffer (pH 7.4).

3.2 Physico-chemical parameters: pH, salinity, grain size

3.2.1 Sediment pH

For pH measurements a standard method (DIN ISO 10390) was applied. 5 g sediment was mixed with 20 ml 0.0125M $CaCl_2$. The sediment was left to settle for 10 min and the pH was measured in the supernatant with a WTW pH-Meter.

3.2.2 Sediment salinity

Salinity was measured and calculated according to a method of Schwendenmann (1998). After homogenising the sediment samples, one sub-sample was taken for determination of water content and another one for sediment extract preparation. To determine sediment humidity, samples were weighed, oven-dried to constant weight at 104°C and weighed again. The weight difference is equivalent to the water content. The second sub-sample was mixed with distilled water (1:5 w/v) and stored for 24 hrs in a closed container. After 24 hrs the conductivity and salinity was measured in the sediment water extract with a WTW conductivity meter.
Sediment salinity (*Ss*) was calculated as follows

$$Ss = \frac{Vs + Ve}{Vs}$$

Where *Se* = salinity measured in sediment water extract, *Vs* = water content of sample used for sediment extract in ml and *Ve* = water volume used for sediment-water extract in ml.

3.2.3 Granulometry

Grain size was determined according to the methodology described by (Suguio, 1973). Sediment samples were washed repeatedly with distilled water to free them from any salt that might disturb the granulometry. To make sure that all salts were washed out, a drop of $AgNO_3$ was added to the wash water. If a white precipitate appeared, salts were still present and the wash procedure had to be repeated. When sediments were salt free, the samples were dried at 50°C. 10 g of the dried sediment samples were sieved through a 62 µm sieve to separate the sand fraction from the silt and clay fraction. The coarser material that was retained in the sieve was dried at 80°C and weighed to determine the percentage of silt/clay in the sample.

3.3 Biochemical analysis

All solutions were prepared using deionised or distilled water. Glassware was either precombusted at 450°C for at least 3 hrs or acid-washed.

Due to complexities of some analytic procedures, time restraints or restricted access to analytic equipment some analyses were not completed for all stations or all replicate cores. Generally at least one replicate of one sample of a minimum of 5 stations was evaluated and random samples were analysed with all replicates to test for consistency of the values. Samples which showed extreme values were always repeated and all replicates were analysed.

3.3.1 Elemental analysis

Carbon (C) and nitrogen (N) content was quantified with an elemental analyser (Fisons, NA 2100). 1-30 mg of dried sediment or plant material was weighed to the nearest 0.001 mg into tin cups and combusted in an oxygen flow at 1050°C in the elemental analyser. The combustion products CO_2, NO_x and H_2O were passed over a copper reduction tube to convert the oxides of N to molecular N_2. Water was removed from the system through a water trap. The resulting gas mixture was released to a thermal conductivity cell where C was detected as CO_2 and N as N_2.

LECO soil standard (for sediment samples) and Standard Reference Material (SRM) 1515 (for plant material) were used for a 15 point calibration and as a quality standard after every fifth sample. To assess reproducibility selected samples were analysed 3-5 times.

For sediment samples organic C was determined by removing inorganic C through acidification with 1 N HCl. The samples were covered with 1 N HCl and kept at 40°C until the evaporation was completed. HCl converts inorganic carbonate in the samples to water vapour, CO_2 and calcium chloride. The samples were then analysed for particulate C. The resultant data represented the organic C content. Inorganic carbon content in plant material was extremely low, hence samples were not acidified and consequently total carbon was considered to be equivalent to total organic carbon.

TN and TOC are expressed in weight% and C/N is calculated on w/w basis – as is common in soil sciences – not on an atomic basis. The latter can be obtained by multiplying C/N (w/w) values by 1.17 C/N values (mol/mol).

3.3.2 Determination of stable C and N isotopes in solid samples

Sediment and plant samples were analysed for stable C and N isotopes by Continuous Flow Isotope Ratio Mass Spectroscopoy (CF-IRMS) (Finnigan, Delta Plus). After sample combustion and generation of N_2 and CO_2 in an elemental analyser (for details see above) the separated gases are introduced through a continuous flow device into the mass spectrometer. Here the gases are ionised by electrons under high vacuum and the resulting ions are accelerated and focussed to ion beams by electrostatic lenses. According to the different masses (28, 29 and 30 for N_2 and 44, 45 and 46 for CO_2) the ion beams are deflected in a magnetic field and split into 3 beams each. The single beams are quantified into three collector cups and measured against a standard gas. Isotope ratios are calculated by comparison with international reference standards and given in ‰ deviation from the standard value.

$$\delta \text{ in } ‰ = \left(\frac{Rs}{Rstd} - 1 \right) \text{ x } 1000$$

Where *Rs* = the measured isotope ratio of the sample and *Rstd* = the measured isotope ratio of the standard.

3.3.3 Tannin analysis

The abundant, but often controversial literature on tannins is probably largely related to the analytical challenges in measuring tannin. Even within the two groups of condensed and hydrolysable tannins, differences between individual forms arise from variations in chain length, position of intermonomer linkages, stereochemistry, branching extent and substitution pattern (Kraus*, et al.*, 2004). The major limitations on all methods of tannin analysis are the different responses given by the different phenolics and the difficulty of procuring an appropriate standard (Hagerman, 2002).

Methods for total phenolics have been frequently used and yield fairly constant results for similar species (Hattenschwiler and Vitousek, 2000). These measurements such as the Prussian Blue Assay used in the present study rely on functional groups instead of the whole molecule, any non-tannin compound with similar functionalities will register as tannin. However, comparison with other methods and analysis of other phenolic compounds such as lignin in plant material give confidence that measuring total phenolics does not greatly overestimate total tannin concentrations (Hernes*, et al.*, 2001). Water may be a good physiological extractant for ecological work and has been used in the present work, although organic solvents have been reported to be more efficient (Mole

and Waterman, 1987). 70% acetone has been found to be the better extractant, but was found to interfere with the colorimetric assay for total phenolics.

The Prussian Blue Assay relies on direct interaction of ferric ions with phenols which produce a coloured complex. The production of coloured complexes between phenolics and iron chloride solutions are well-known and their differing colours may be used as a rough guide to the types of phenolics involved. Typically they are green for condensed tannins and blue for hydrolysable tannins (Mole and Waterman, 1987). In the present study the tendency of the sediment samples was to light green (*Figure 3.3.1*), plant samples turned blue-green. Thus we possibly measured condensed tannins as well as hydrolysable tannins. This assay was standardised with the simple phenolic tannic acid, but complex polyphenolics may have a different response on a molar or mass basis than the simple standard. Thus the results are expressed in Tannic Acid Equivalents (TAE). For convenience the measured substances will be called 'tannins'.

Figure 3.3.1: Extract of sediment sample after addition of ferric ions.

As various preliminary test analyses showed that tannin analysis was more stable when performed with un-dried, frozen sediments, tannin content was determined with wet sediments. Dry weight was defined for each sample separately. For plant sample no significant difference could be detected between freeze-dried and fresh samples, hence the analysis was performed with dried plant material.

Tannin was determined with a Prussian Blue Assay (Price and Butler, 1977) that was modified to fit our purposes. 100 mg of ground sediment or 10 mg of ground plant material were shaken constantly for 24 hrs in 50 ml of deionised water. The mixture was centrifuged at 3000*g* for 5 min. 2 ml 0.008M $FeCl_3$ (Merck) in 0.008M HCl were added to 1 ml of the supernatant, followed by timed addition of 10 ml of 0.0015M $K_3Fe(CN)_6$ (Merck). Optical density was read after an incubation time of exactly 15 min in the dark at 720 nm on a UVIKON spectrophotometer - 941 PLUS (in Brazil) or a Perkin Elmer 552 Spectrophotometer (in Germany) which had been zeroed with water.

The $FeCl_3/K_3Fe(CN)_6$ system provides a sensitive method for quantitative determination of total polyphenolics. This method can be coupled with the use of an insoluble matrix like polyvinyl polypyrrolidone (PVPP) that binds tannin-phenolics (Hagerman, 2002). 100 mg PVPP were weighed into test tubes.1 ml of each, distilled water and supernatant (see

above) were added and mixed well. The tubes were kept at 4°C for 15 minutes, mixed again and centrifuged at 3000*g* for 10 min. The supernatant was analysed for phenolics according to the method described above. As PVPP binds tannins, the latter would have been precipitated along with the PVPP, hence the phenolics measured in the supernatant after PVPP treatment represent only non-tannin phenolics. Tannin concentration would then be calculated by substracting the concentration of non-tannin-phenolics from the concentration of total phenolics. As results with or without PVPP addition did not differ much, it was assumed that either the concentration of non-tannin phenolics was neglectable or the non-tannin phenolics present in our samples were also bound by PVPP. Thus, the PVPP method was omitted and results refer to the data from the Prussian Blue Assay only. As the calibration curve was generated with tannic acid (Sigma) the results are presented as Tannic Acid Equivalents (TAE).

3.3.4 Amino acid analysis (Stereospecific separation of amino acids)

The determination of amino acids enantiomers was adapted from Fitznar (1999).

Acidic hydrolysis/ Sample preparation

To quantify amino acids, it is necessary to split them into monomers before analysis since they occur as free and bound species in plant and sediment material. As a standard method acidic hydrolysis with 6M HCl at 110°C for 24 hrs under nitrogen atmosphere was applied. 10 mg of ground sediment or leaf material was dissolved in 10 ml of distilled water and 10 ml of HCl (32 %) were added to achieve the acid concentration for hydrolysis. Ascorbic acid (to reduce the possible oxidation of amino acids through nitrate) was not added as it produced a brown precipitate in many samples. Samples with and without ascorbic acid were analysed and no significant difference was detected. Hence all further samples were hydrolysed without adding ascorbic acid.

High Performance Liquid Chromatography

After cooling, 9.8 ml of a borate buffer (30.91 g boric acid dissolved in 1l 32 % NaOH) were added and the mixture was adjusted to pH 8.5 by adding concentrated HCl or NaOH.

Glycine (gly) and the D- and L-enantiomers of the individual amino acids listed in *Table 3.3.1* were determined in the hydrolysates with high-performance liquid chromatography (HPLC) with fluorescence detection after precolumn derivatisation with *o*-phthaldialdehyde (OPA) and *N*-isobutyryl-D/L-cysteine (IBDC/IBLC). 800 µl of the sample was injected into a reaction vial, to which 80 µl OPA and 20 µl IBLC/IBDC were added for derivatisation

(*Figure 3.3.2*). After a reaction time of 120 s, 5 µl of the derivatised sample were injected onto the column. Eluent gradient profiles are depicted in *Figure 3.3.3*. Detection was performed with a fluorescence detector, system parameter settings are shown in *Table 3.3.2*. Calibration was performed with a Pierce standard (Amino Acid Standard H, No. 20088) and a mixture of single standards (Aldrich). The system (Merck Hitachi, LaChrom) was allowed to equilibrate for at least half an hour before the start of the analysis. To prevent evaporation and deterioration of samples, the sample tray in the autosampler was cooled to 10°C.

OPA used for derivatisation does not react with secondary amino acids, hence proline (pro) could not be detected with this method. Lysine (lys) forms unstable OPA-IBLC-derivates and was not accounted for with this method. Aspartic acid (asp) and glutamic acid (glu) were formed quantitatively from asparagine (asn) and glutamine (gln) during hydrolysis. Consequently asp/asn and glu/gln will be summed and designated as asx and glx for the evaluation. Additionally, hydrolysis led to complete degradation of tryptophane (trp) and to partial oxidation of methionine (met). Hence, both amino acids could not be quantified. Recovery rates along with the common abbreviations for each amino acid (henceforth used in the text) are given in *Table 3.3.1.*

Percentage of D-amino acids from total hydrolysable amino acids (THAA), as well as the D/L ratio of the biomarker amino acid alanine (ala), percentage of amino acid nitrogen of total nitrogen (AA-N/TN) and amino acid carbon of total organic carbon (AA-C/TOC) were calculated from absolute amounts (mg/g dw). Concentrations are mainly given in mass, some mean values will be given in mole as well as mass. The distribution of individual amino acids is shown in mol% in the Results chapter and in absolute amounts (mg/g dw) in the Appendix.

Table 3.3.1: Abbreviations, recovery rates and elution times of individual amino acids

Amino acid	Abbreviation	Elution time [min]	Recovery rate [%]
L-Aspartic acid	L-asp	12.3	104
D-Aspartic acid	D-asp	13.8	104
L-Glutamic acid	L-glu	20.2	98
D-Glutamic acid	D-glu	22.6	86
L-Asparagine	L-asn	26.1	0
L-Serine	L-ser	27.8	86
D-Asparagine	D-asn	29.0	0
D-Serine	D-ser	30.5	101
L-Glutamine	L-gln	32.4	7
L-Threonine	L-thr	35.1	84
L-Histidine	L-his	36.8	67
Glycine	gly	37.6	111
D-Histidine	D-his	38.8	97
L-Arginine	L-arg	42.9	88
L-Alanine	L-ala	44.1	85
D-Arginine	D-arg	45.6	88
D-Alanine	D-ala	47.9	85
L-Tyrosine	L-tyr	51.3	85
D-Tyrosine	D-tyr	54.7	85
L-Valine	L-val	58.7	93
D-Valine	D-val	64.9	99
L-Phenylalanine	L-phe	65.6	92
L-Isoleucine	L-ile	66.4	86
D-Phenylalanine	D-phe	68.7	91
L-Leucine	L-leu	71.2	92
D-Isoleucine	D-ile	72.4	85
D-Leucine	D-leu	74.8	93

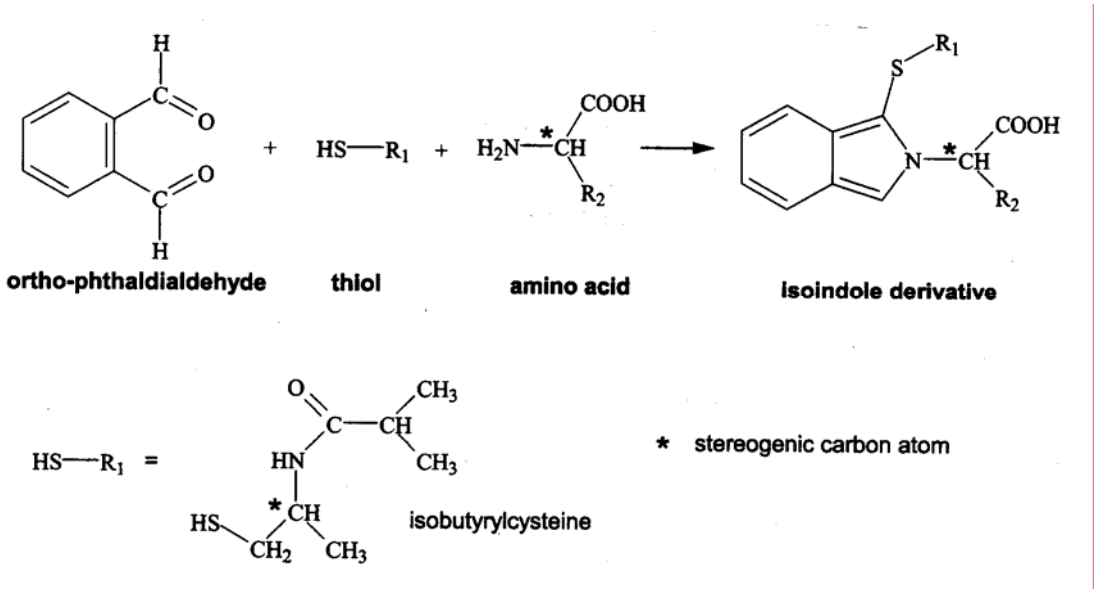


Figure 3.3.2: Amino acid derivatisation with *o*-phthaldialdehyde (OPA) and *N*-isobutyryl-D/L-cysteine (IBDC/IBLC).

Table 3.3.2: System parameter settings for HPLC

HPLC Merck LaChrom	
Pump L-7100	Flow rate: 0.8 ml min^{-1} Pressure limits: min. 5 bar, max. 320 bar
Autosampler L-7250P	Injection volume
Column oven	21°C
Fluorescence detector L-7480	Ex 340nm, Em 445nm
Precolumn	Merck Superspher 100 RP18 LiChrochart E-4
Column	Merck Superspher 100 RP18 LiChrochart 125-4
Eluents	A: 25mM sodium acetate pH 7.0 B: 25mM sodium acetate pH 5.3 C: Methanol
Cooling	10°C

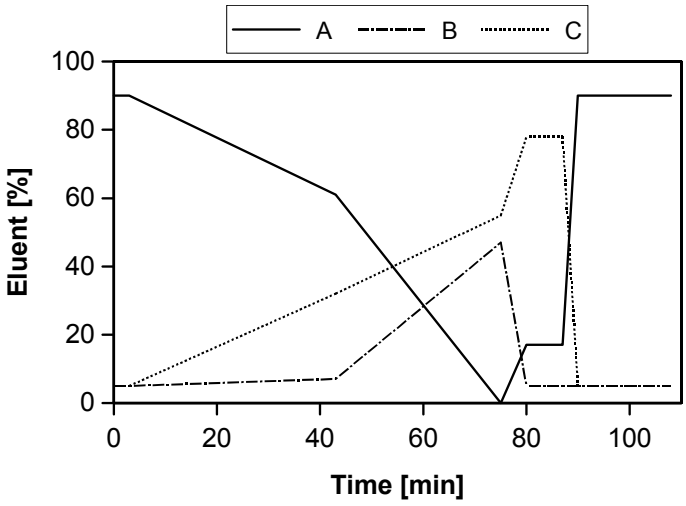


Figure 3.3.3: Gradient profiles for the mobile phase of the amino acid analysis by HPLC

3.4 Microbiological and microscopical analysis

3.4.1 Analysis of N_2-fixation, acetylene reduction assay

The N_2-fixing complex nitrogenase also reduces acetylene to ethylene, thus the rate of acetylene reduction may be used as an index of the rate for N_2-fixation. Stewart *et al.* (1967) introduced a simple method for measuring acetylene reduction as an index of N_2-fixation in the field. Experiments were carried out in gas-tight 50 ml serum bottles with rubber stoppers. 10 ml of filtered creek water (2 µm) and 5 ml of acetylene were added with a syringe to the sediment or root sample that was to be analysed for N_2-fixation. Gas samples of 1 ml were taken directly after adding acetylene (time point 0) and after 6, 12, 18, 24 and 48 hrs and kept in 1.5 ml gas-tight vials until they were analysed for ethylene on a Varian CP-3800 gas chromatograph.

3.4.2 Total Bacterial Counts (TBC)

Bacterial numbers were estimated according to the acridine orange epifluorescence technique of Hobbie *et al.* (1977). In short, all samples were fixed with formalin (4 % final concentration) and stored at 4°C (or in a refrigerator) prior to enumeration. Water samples were diluted 1:5 with filtered (0.2 µm pore size filter) water. Sediment samples were diluted 1:10, homogenised by sonication for 5 x 1 min and afterwards further diluted 1:10 with filtered water. The samples were stained with 0.01 % (final concentration) acridine orange (AO) for 3 min and filtered on black polycarbonate filters (Nucleopore, 0.2 µm pore size). To give a better distribution of cells a cellulose-nitrate filter (0.45 µm) was placed beneath the Nucleopore filter. Sediment samples were rinsed with 3 ml of Na-Citrate-

buffer (pH 4.0) to reduce background-staining. The filter membranes were mounted on microscopic slides with one drop of Cargille oil. Bacteria were counted by means of an epifluorescence microscope (Zeiss Axioplan 2, filter set 09, excitation BP 450- 490, beamsplitter FT 510, emission LP 515). A minimum of 1000 cells were counted in at least 20 microscopic fields that were chosen randomly. Photos of the selected microscopic fields were taken with a connected digital camera (Sensicam, PCO Computer Optics, Kelheim, Germany).

3.4.3 Scanning Electron Microscopy (SEM)

The Scanning Electron Microscope serves to characterise and describe leaf and root surfaces. The method is based upon the interaction of electrons and a metal surface that has been applied to the sample.

Fixed samples (3 % glutaraldehyde in 0.065M phosphate buffer (pH 7.4)) were washed with tap water and cut into small pieces under the binocular. Subsequently the water in the samples were successively replaced with 15 %, 30 %, 40 %, 60 %, 70 %, 80 %, 90 % and finally 100 % acetone (as the 'intermediate and dehydration fluid' being miscible with liquid CO_2 which will be used in the following step) to prepare the specimen for the critical point drying. Critical point drying is a method of drying tissue without collapsing or deforming the structure of wet, fragile specimens.

A critical point, also called a critical state, specifies the conditions (temperature and pressure) at which the liquid state of the matter ceases to exist. At this point, the phases are no longer distinguishable. The transition from liquid to gas at the critical point takes place without an interface because the densities of liquid and gas are equal at this point. In the apparatus (BAL-TEC, CPD 030) for critical point drying the sample is totally immersed in liquid CO_2 below its critical point. When the CO_2 is then taken to a temperature and pressure above the critical point the sample is then immersed in gas (i. e. dried) without being exposed to the damaging surface tension forces.

After drying the samples were mounted on small aluminium plates and coated with a thin gold layer (Sputter Coater: BALZERS UNION, SCD 040) and subsequently analysed under an ISI 100B Scanning Electron Microscope. Photos were taken with a reflex camera.

3.5 Degradation experiments

3.5.1 Experiments on microbial degradation of mangrove leaves

To describe the influence of bacterial and fungal degradation on leaf degradation, mangrove leaves of *R. mangle* and *A. germinans* were collected near Furo do Meio. For the *field experiment* leaves in various states of senescence (yellow, brown and black collected from the ground or in channels at low tide) were sewn into net bags and left in a mangrove creek with semidiurnal inundation pattern for 42 days (*Figure 3.5.1*). Every week samples were taken, processed and stored for further analysis of total C and N, $\delta^{15}N$ and $\delta^{13}C$ isotopes, tannins and amino acids.

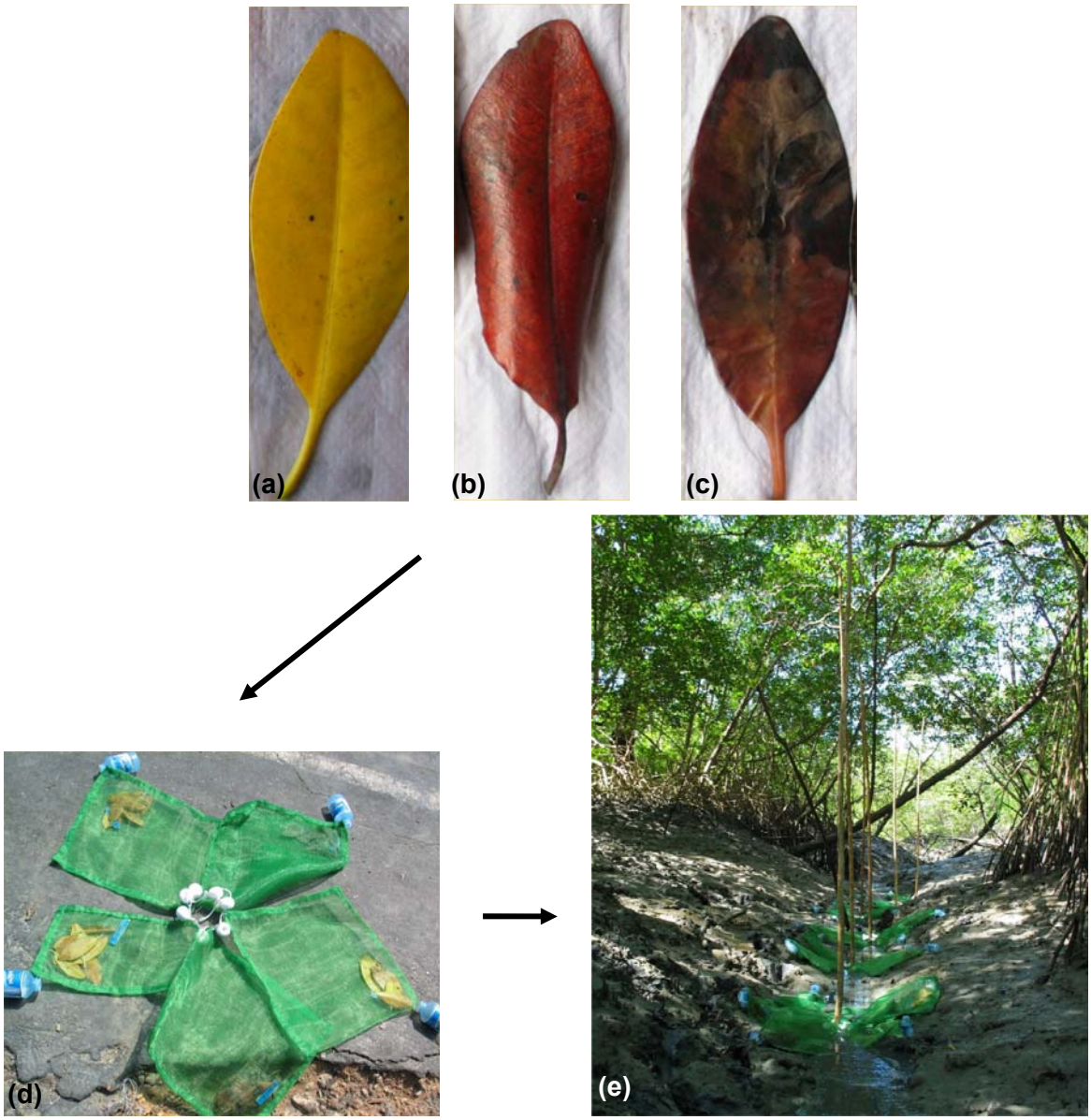


Figure 3.5.1: The degradation experiment in the field, samples of (a) yellow, (b) brown and (c) black leaves on day 0, (d) net bags, (e) experimental setup.

In the *laboratory*, yellow leaves of *R. mangle* and *A. germinans* (collected from the ground or in channels at low tide) were subjected to different treatments with bactericides and fungicides to asses which organisms play a major role in the degradation of the nitrogen compounds mentioned above. For details of the experimental setup see *Table 3.5.1* and *Figure 3.5.2*. The water used for all solutions was taken from Furo do Meio at high tide and filtered through a sterile 1µm filter. Leaf samples for analysis of total C and N and $\delta^{15}N$ and $\delta^{13}C$ isotopes, tannins, amino acids and TBC were taken each week and stored as described above. Total experimental time was 42 days.

Figure 3.5.2: The degradation experiment in the laboratory

Table 3.5.1: Setup of treatments of yellow leaves of *R. mangle* and *A. germinans* in the laboratory experiment. Conc. = concentration

Treatment	Volume	Added chemicals	Conc.	Species	Initial no. of leaves	Total no. of flasks
"Natural reference"	1l	None		*R.mangle, A. germinans*	18 in each flask	4
"Control"	1l	$HgCl_2$	110mg/l	*R.mangle, A. germinans*	18 in each flask	4
Fungicide 1	1l	Benomyl	20mg/l	*R.mangle, A. germinans*	18 in each flask	4
Fungicide 2	1l	Benomyl Cycloheximid	20mg/l 3mg/l	*R.mangle, A. germinans*	18 in each flask	4
Bactericide	1l	Chloramphenicol Ampicillin Rifampicin	20mg/l 50mg/l 3mg/l	*R.mangle, A. germinans*	18 in each flask	4

No chemicals were added to the “natural reference”, the leaves of this treatment were submerged in filtered water from the tidal channel “Furo do Meio”. Throughout the thesis it will be referred to as the “reference” treatment. In the “control” treatment, poisoning with mercuric chloride was achieved successfully to suppress microbial growth (Kattner, 1999). It will be referred to as $HgCl_2$-treatment. Of the two fungicide treatments, treatment 2 proved more effective in terms of fungal growth constraints, thus results and discussion will refer to the second treatment called fungicide-treatment or ‘Bacteria’, as only bacteria grew in these flasks (Chiocchio*, et al.*, 2000; Chen*, et al.*, 2001a, 2001b). The last treatment with bactericides repressed bacterial growth and allowed fungi to thrive, thus will be referred to as bactericide-treatment or ‘Fungi’ (Helmke, pers. comm.; Helmke and Weyland, 1995).

3.5.2 Determination of tannase activity in crab intestine

Tannin acyl hydrolase commonly called tannase is produced by a number of microorganisms and catalyses the hydrolysis of ester linkages in hydrolysable tannins like tannic acid. A colorimetric assay (Mondal*, et al.*, 2001) was used to determine tannase activity in crab intestines. The specimen of the mangrove crab *Ucides cordatus* used for this experiment were collected some hours prior to the experiment and kept in small tanks. The animals were killed and dissected immediately before the start of the experiment, the gut content was weighed and analysed as described below.

The reaction mixture consisting of 1 ml substrate tannic acid (5 mg/ml) and 2 g crab gut content mixed with 8 ml of filtered and autoclaved mangrove creek water was incubated at 60°C for a defined period. Blanks with 8 ml filtered and autoclaved mangrove creek water and 8 ml distilled water were treated the same way. At determined time points (0 hrs, 6 hrs and 24 hrs after start of the experiment) an aliquot of 1.5 ml of the reaction mixture was removed to a centrifugation tube and the enzymatic reaction was stopped by the addition of 3 ml BSA solution (1 mg/ml Bovine Serume Albumin (Sigma) was prepared with 0.17M sodium chloride in 0.2M acetate buffer (pH 5.0)), which also precipitated the remaining tannic acid. The tubes were centrifuged at 5000*g* for 5 min and the resulting precipitate was dissolved in 3 ml SDS-triethanolamine solution (1 % (w/v) Sodium Dodecyl Sulfate (Sigma) solution containing 5 % (v/v) triethanolamine (Sigma) in distilled water). Finally 1 ml of a 0.01M $FeCl_3$ solution in 0.01N HCl was added and the mixture was kept in the dark for 15 min for stabilisation of the colour. The absorbencies were measured at 530 nm with a UVIKON spectrophotometer - 941 PLUS. A calibration curve was generated with tannic acid.

3.6 Statistical analyses

Data were tested for normality with the Shapiro-Wilk's W test. Normally distributed data were tested for significant differences with the independent t-test. When the normality test failed, non-parametric statistics were applied. For comparison between two independent groups the Mann-Whitney-U-test was used. All tests were considered significant when $p<0.05$.

The Pearson r Correlation was applied to investigate possible relationships between two variables. Correlations were assumed significant when $p<0.05$.

Multiple regression analysis was used to analyse differences between relationships of two variables. Differences were considered significant at $p<0.05$.

4 RESULTS

4.1 Physico-chemical parameters

4.1.1 Transect 1: Inundation frequency and topography

The inundation frequency – here shown in days/year (*Figure 4.1.1*) – increased steadily throughout the transect with the lowest frequency of 41 days/year at station 1 and the highest value of 148 days/year at station 12. It showed a negative relationship to topography (Cordeiro*, et al.*, 2003; calculated after Cohen*, et al.*, 2004).

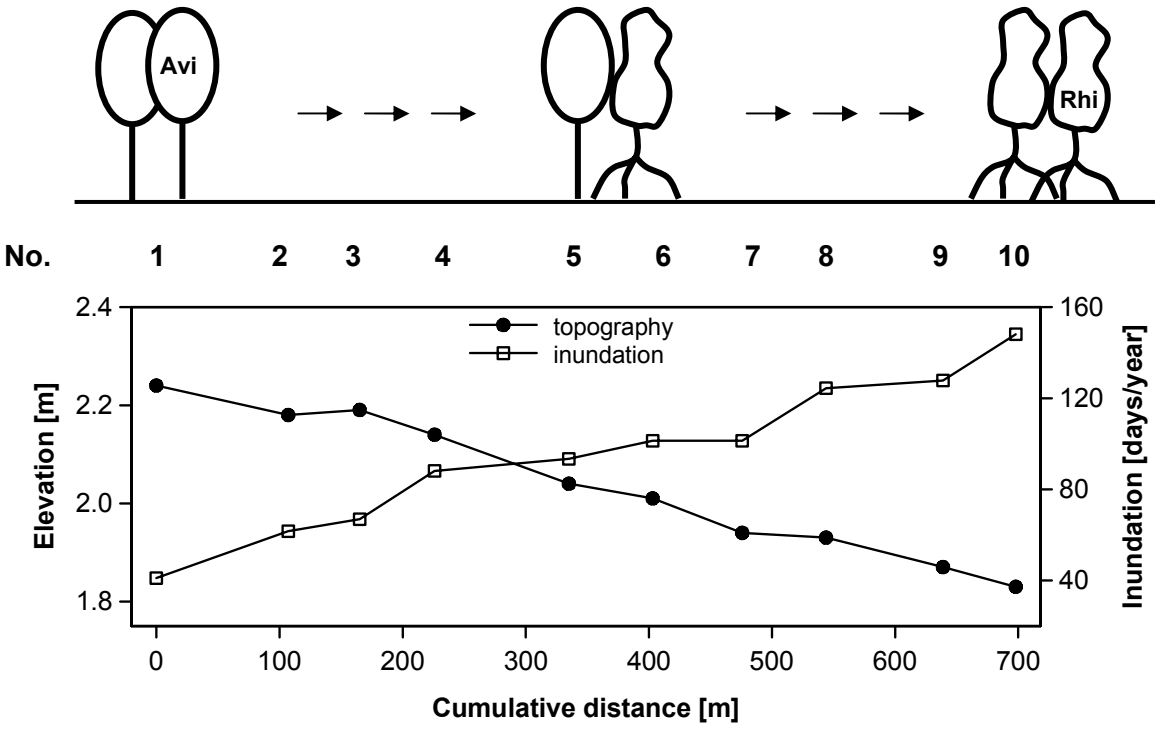


Figure 4.1.1: Inundation frequency and topography (elevation above mean sea level) for the 10 stations of transect 1. The dominating tree species and their distribution are depicted above the station numbers (No.).

4.1.2 Transect 1: Sediment salinity

The sediment salinity was recorded during the rainy and the dry season at all stations and at 4 different depths (0, 5, 10 and 50 cm). The depth of 5 cm was used instead of surface values as salt incrustations on the sediment surface lead to unstable values which exceeded 150 during the dry season. The 5 cm sample has greater significance for the plant as root uptake or exclusion of salts does not take place at the sediment surface. Values for surface sediments are shown in Appendix A1. *Figure 4.1.2* shows the salinity

(mean of *Avicennia*- and *Rhizophora*-sediments) at 5, 10 and 50 cm depth for the rainy (a) and the dry season (b).

In general there is a trend from significantly higher salinities at station 1-4 to lower salinities in the more humid and *Rhizophora* dominated part of the forest. During the rainy season this trend is more pronounced in the deeper sediment layers (50 cm), here salinities are significantly higher in the depth (41.3 ± 6.3, n=20) than at 5 and 10 cm (21.0 ± 6.3, n=32) throughout the transect.

During the dry season, salinities show nearly identical values at the three different depths at each station, but differ significantly throughout the transect. Values in the dry area of the transect (stations 1-4, 51.1 ± 6.0, n=20) are significantly higher ($p<0.001$) than salinities in the humid region (stations 7-10, 28.7 ± 4.0, n=20).

4.1.3 Transect 1: Sediment pH

The sediment pH was similar throughout the transect and only varied with depth ($p<0.005$) but not significantly with season (*Figure 4.1.2*).

The sediment pH varied significantly with depth ($p<0.005$) and also slightly with season or station number. Generally the pH augmented with depth from a minimum of 5.5 at the surface during rainy season near a *Rhizophora* tree to a maximum of 8.1 at 50 cm depth during the dry season near an *Avicennia* tree. The *Avicennia*- and *Rhizophora*-sediments mainly showed similar values, except for station 2 where they could differ up to a whole pH unit (especially during the dry season).

4.1.4 Transect 1: Grain size

Percentage of the silt/clay fraction (grain size ≤ 63µm) was determined at three stations in surface and 50 cm sediment samples during both seasons (*Figure 4.1.2*). A clear trend towards coarser sediments in the *Rhizophora*-dominated region can be seen in both seasons, being more pronounced in surface samples and in the rainy season. The maximum value of 99.7 % silt/clay was found at station 2 during the dry season at 50cm depth, whereas the minimum value of 94.0 % silt/clay occurred in surface sediments of station 8 during the rainy season.

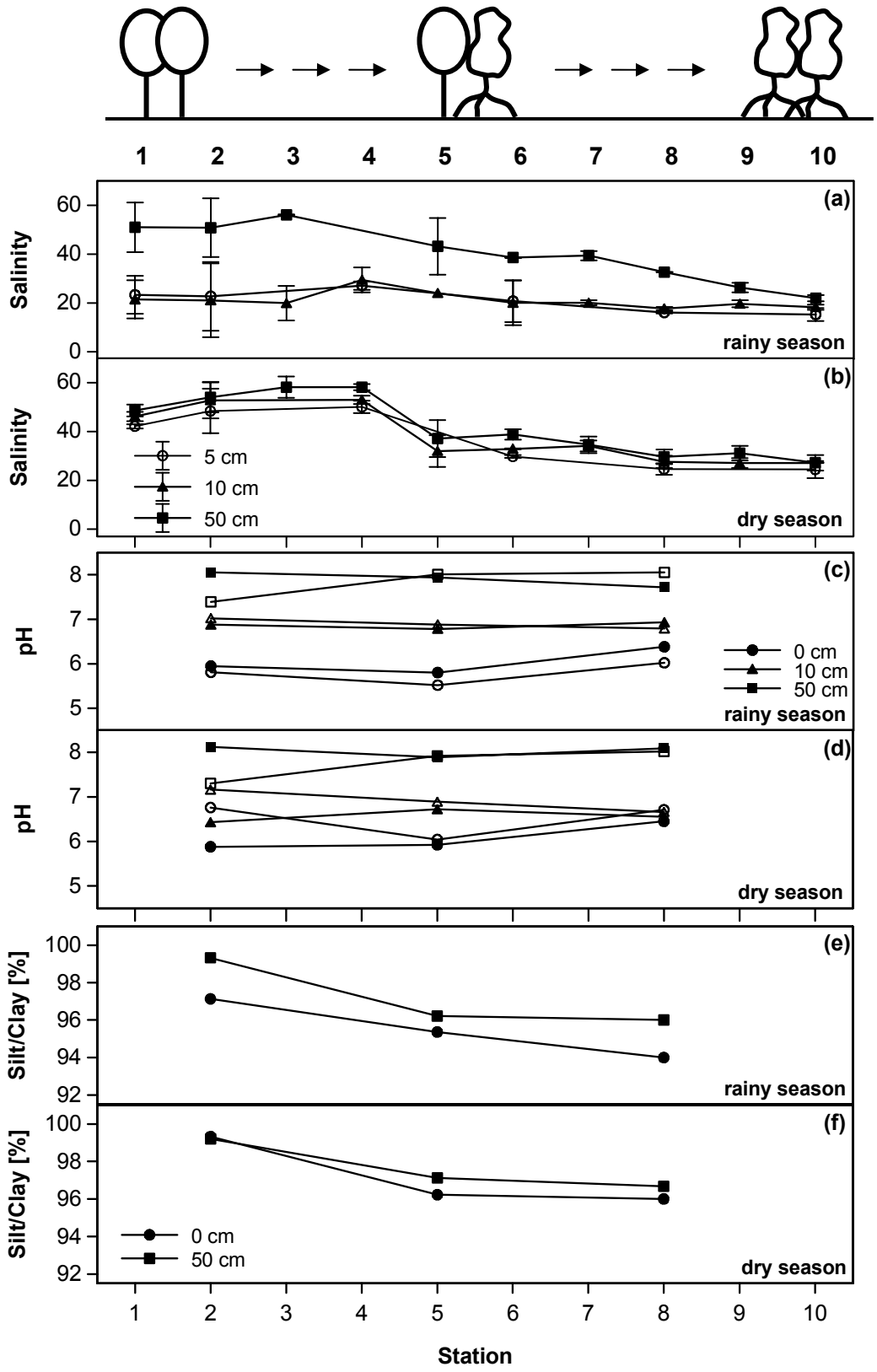


Figure 4.1.2: Physico-chemical parameters during rainy and dry season for transect 1. For salinity, mean ± SD for *Avicennia* (Avi)- and *Rhizophora* (Rhi)-dominated sediments are shown; for pH, filled markers are Avi and open markers are Rhi; for silt/clay, Avi and Rhi samples were pooled before analysis.

4.1.5 Transect 2: Inundation frequency and topography

The inundation frequency – here shown in days/year (*Figure 4.1.3*) – increased steadily throughout the transect from station 2 (35 days/year) to the highest value of 138 days/year at station 7. At station 1 a depression increased the inundation frequency in comparison to station 2 to 50 days/year (Cordeiro*, et al.*, 2003; calculated after Cohen*, et al.*, 2004).

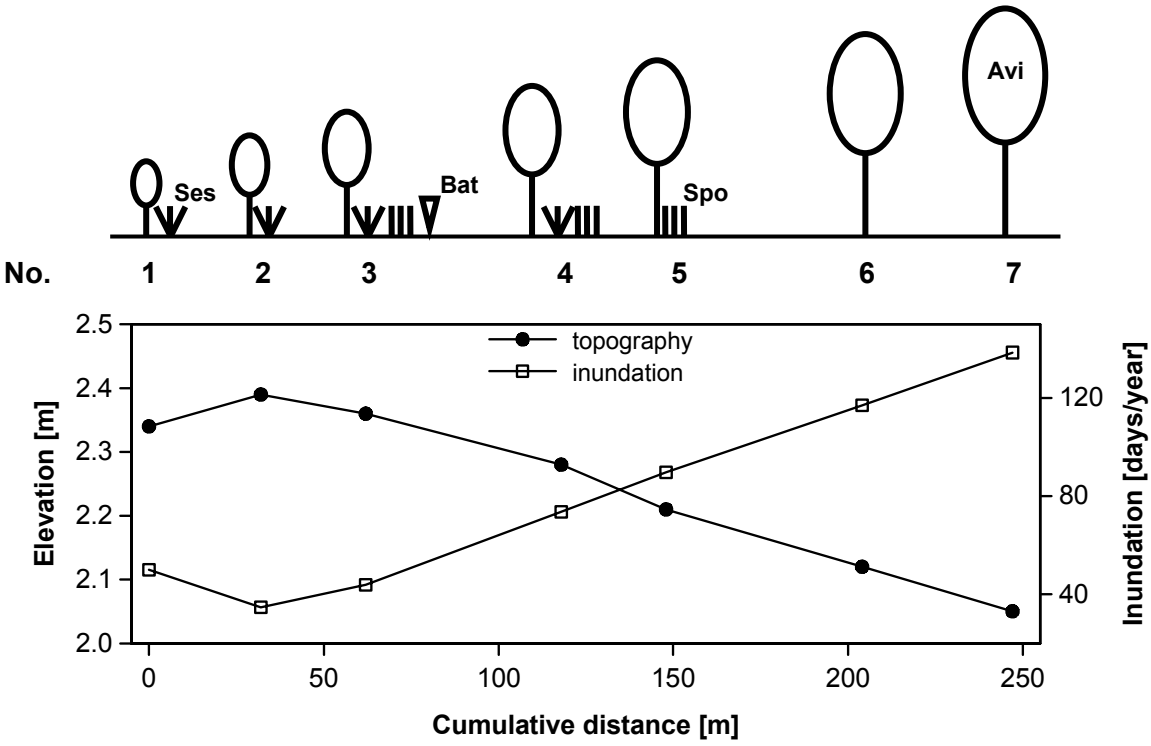


Figure 4.1.3: Inundation frequency and topography (elevation above mean sea level) for the 7 stations of transect 2. The dominating plant species *A. germinans* (Avi), *S. portulacastrum* (Ses), *S. virginicus* (Spo), *B. maritima* (Bat) and their distribution are depicted above the station numbers (No.).

4.1.6 Transect 2: Sediment salinity

As for transect 1 the depth of 5 cm was used instead of surface values as salt incrustations on the sediment surface lead to unstable values which exceeded 150 during the dry season.

Figure 4.1.4 shows the salinity at 5, 10 and 50 cm during the rainy and the dry season, values for surface sediments are shown in Appendix A2. Salinities during the rainy season ranged from 3.9 to 37.0 (18.4 ± 10.7) and were significantly lower ($p<0.001$, $n=21$) than dry season values with a minimum of 42.4 and a maximum of 91.2 (68.6 ± 14.4). There

was no significant trend or difference between different depths in both seasons. In the dry season however a significant decrease ($p<0.001$) of salinity could be observed when comparing stations 1-5 (mean value of 76.2 ± 7.9, n=15) to stations 6 and 7 (49.5 ± 6.5, n=6).

4.1.7 Transect 2: Sediment pH

Sediment pH values showed no significant difference between seasons (*Figure 4.1.4*). However, a difference between the stations and depth could be observed. At stations 1 and 4 the pH decreased from 6.3 ± 0.2/6.8 ± 0.04 (rainy/dry season) at the surface to 4.7 ± 0.4/4.7 ± 0.2 (rainy/dry season) at 50cm, whereas at station 7 the pH values increased from 6.4/6.8 (rainy/dry season) at the surface to 7.4/7.1 (rainy/dry season) in the deep layers.

4.1.8 Transect 2: Grain size

Percentage of the silt and clay fraction (grain size ≤ 63µm) was determined at all stations for surface, 10 cm and 50 cm sediment layers and during both seasons. (*Figure 4.1.4*).

During the rainy season only small variations between 96.6 % and 99.6 % silt/clay was observed for the surface and 10cm values. However, the 50 cm depth layer showed a significant difference ($p<0.005$) between coarser sediments at stations 1 and 2 (93.9 ± 0.1 % silt/clay, n=4) and finer sediments at stations 3-7 (98.6 ± 0.7 % silt/clay, n=10). Dry season samples were stable for 10 cm sediment layers throughout the transect, whereas the surface values presented an inverse u-shaped form with the maximum of 98.6 % at station 4. Values for 50 cm depth were highly variable and ranged from 90.9 % for the silt/ clay fraction at station 2 to a maximum of 99.6 % at station 3.

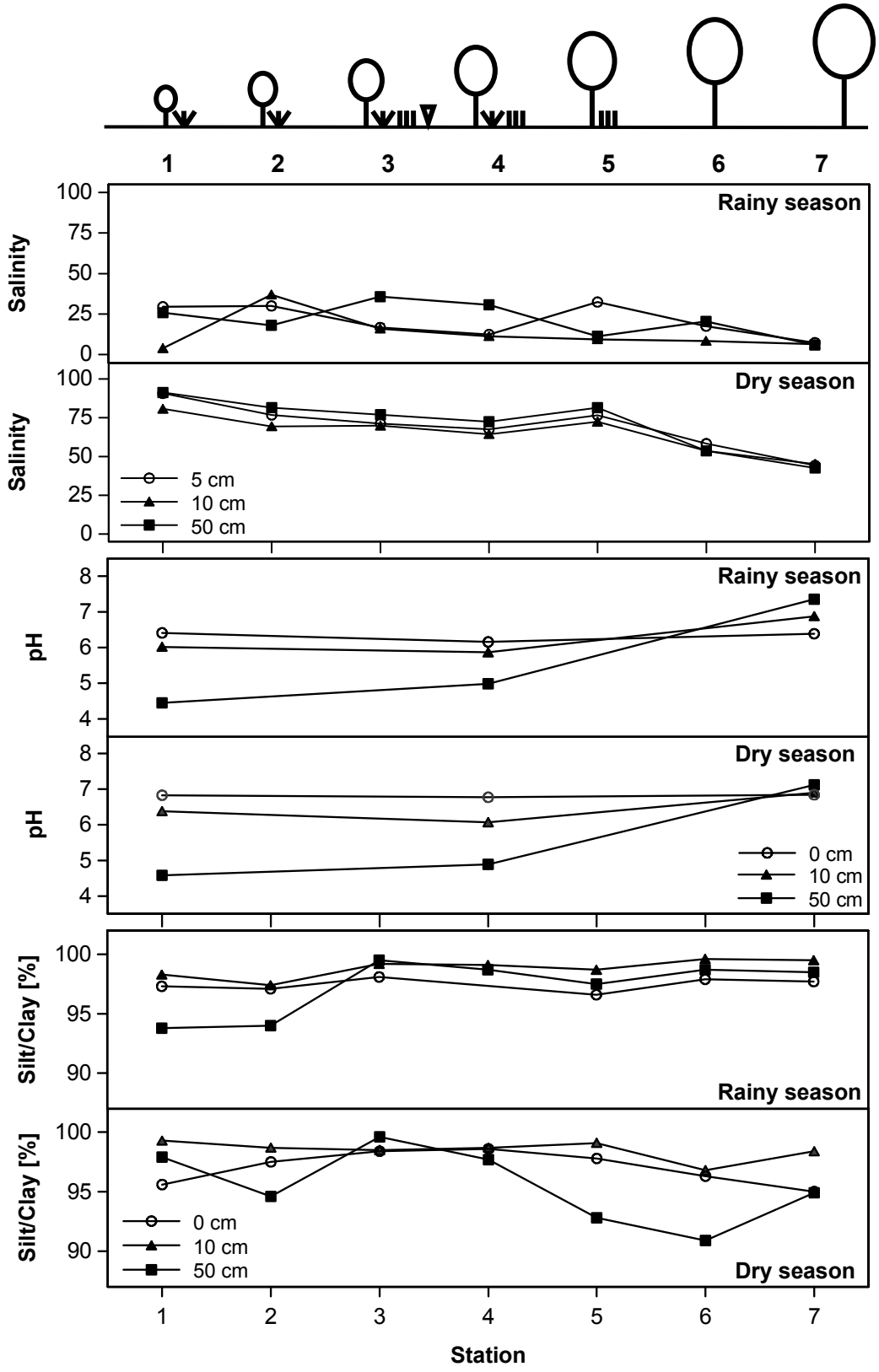


Figure 4.1.4: Physico-chemical parameters during rainy and dry season for transect 2.

4.2 The field studies

4.2.1 Elemental and isotopic composition

4.2.1.1 Transect 1: Elemental and isotopic composition of sediments

Elemental composition

The Total Organic Carbon (TOC) and Total N (TN) contents of the sediment at different depths and for both seasons are shown in weight % of dry weight (*Figure 4.2.1*).

TOC was highly variable ranging from 0.84 % to 4.18 %. Here both, the minimum and the maximum value were measured at 50 cm depth. Mean TOC contents were 2.96 ± 0.47 %, 2.21 ± 0.59 % and 1.94 ± 0.70 %TOC for surface, 10 cm and 50 cm sediments respectively. TN developed mainly parallel to TOC content ranging from a minimum of 0.7 % at 50 cm to 0.27 % at the surface. Mean TN contents were 0.2 ± 0.02 %, 0.16 ± 0.04 % and 0.13 ± 0.03 % TN for surface, 10 cm and 50 cm sediments respectively.

Generally the cores taken near *Avicennia* trees showed a stronger stratification of both TOC and TN during both seasons especially in the dryer, *Avicennia*-dominated region (stations 1-4) with decreasing values from the surface to 50 cm depth. A statistical difference ($p<0.001$, $n=16$) between the values of TOC and TN at all measured depths could be shown for this part of the forest, whereas in the *Rhizophora*-dominated area only the surface sediments with higher values differed significantly from the deeper substrate. There was no significant difference for both parameters between the rainy and the dry season or between the *Avicennia*- and *Rhizophora*-sediments. For the *Rhizophora*-sediments station 5 showed elevated values for all depths during the dry season, for surface sediments only during the rainy season.

C/N ratios (*Figure 4.2.1*) were also highly variable, ranging from 9.38 to 18.95. During the dry season values increased throughout the transect, showing a significant difference between low ratios at station 1 (11.5 ± 2.0 and 11.8 ± 0.8 for *Avicennia* and *Rhizophora* sediments respectively) and high ratios at station 10 (15.0 ± 1.1 for *Avicennia* and 16.6 ± 1.3 for *Rhizophora* sediments) ($p<0.005$, $n=18$). Rainy season samples showed a similar but not significant trend.

Isotopic composition

$\delta^{13}C$ and $\delta^{15}N$ values of surface, 10 cm and 50 cm sediments at 3 stations are shown in *Figures 4.2.3* and *4.2.4.*

The isotopic composition was generally stable at all measured stations and between seasons with two exceptions. For the $\delta^{13}C$ values a significant difference between stations 1 and 10 during the dry season in both *Avicennia*- and *Rhizophora*-substrate was calculated (*Figures 4.2.3c* and *d*) ($p<0.05$, n=6). The *Avicennia*-sediments during the dry season (*Figure 4.2.4c*) showed a conspicuous decrease for the $\delta^{15}N$ values in surface and 10 cm substrates. The values ranged from -25.5 ‰ to -27.3 ‰ and from 3.9 ‰ to 6.7 ‰ for $\delta^{13}C$ and $\delta^{15}N$ respectively, with mean values of -26.1 ± 0.7 ‰ $\delta^{13}C$ and 5.0 ± 0.7 ‰ $\delta^{15}N$.

4.2.1.2 Transect 1: Elemental and isotopic composition of plants

Elemental composition

In this transect the plant community consisted only of *A. germinans* and *R. mangle* and the green leaves were collected directly from the trees as a mix of sun-exposed leaves in the upper canopy and shaded leaves in the lower canopy.

R. mangle contained significantly more ($p<0.001$, n=30) organic carbon (45.1 ± 1.0 %) than *A. germinans* (42.3 ± 1.9 %). TOC values varied between 39.5 % (*A. germinans*, dry season) and a maximum of 47.0 % (*R. mangle*, rainy season). For both species there was a significant difference ($p<0.001$, n=15) between the rainy and the dry season, where rainy season TOC content were generally higher than dry season values (*Figure 4.1.5*).

TN content varied between 1.4 % in *R. mangle* leaves and 3.9 % in *A. germinans* leaves (*Figure 4.1.6*). As for TOC, there was a significant difference ($p<0.001$, n=30) between the two species, with *A. germinans* containing significantly more nitrogen (3.0 ± 0.4 %) than *R. mangle* (1.8 ± 0.1 %). For TN of all stations no statistical difference between seasons could be revealed. However, *A. germinans* leaves showed an upward trend throughout the transect in the rainy season with significantly higher values at stations 8-10 than at stations 1-4, and a downward trend during the dry season where TN values were significantly lower at stations 8-10 than at stations 1-4. TN in *R. mangle* leaves was stable throughout the transect.

Values for C/N ratios (*Figure 4.1.7*) of *A. germinans* reflected the significant differences between stations 8-10 and stations 1-4 in the opposite direction with a downward trend in the rainy season and an upward trend during the dry season. Generally the C/N ratios for *R. mangle* were much higher (25.8 ± 2.1) than for *A. germinans* (14.2 ± 2.0) ($p<0.001$, n=30).

Isotopic composition

$\delta^{13}C$ and $\delta^{15}N$ in the green leaves were measured for both species at 4 stations throughout the transect and in both seasons (*Figure 4.1.8* and *4.1.9*).

Generally $\delta^{13}C$ values showed an downward trend throughout the transect with a maximum of -25.1 ‰ $\delta^{13}C$ in *R. mangle* leaves at station 2 during the dry season and a minimum of -32.3 ‰ $\delta^{13}C$ in *R. mangle* at station 10 in the rainy season. $\delta^{15}N$ was more variable with a maximum value of 7.42 ‰ in *A. germinans* leaves at station 2 in the rainy season and a minimum of 4.94 ‰ in *R. mangle* leaves at station 8 during dry season.

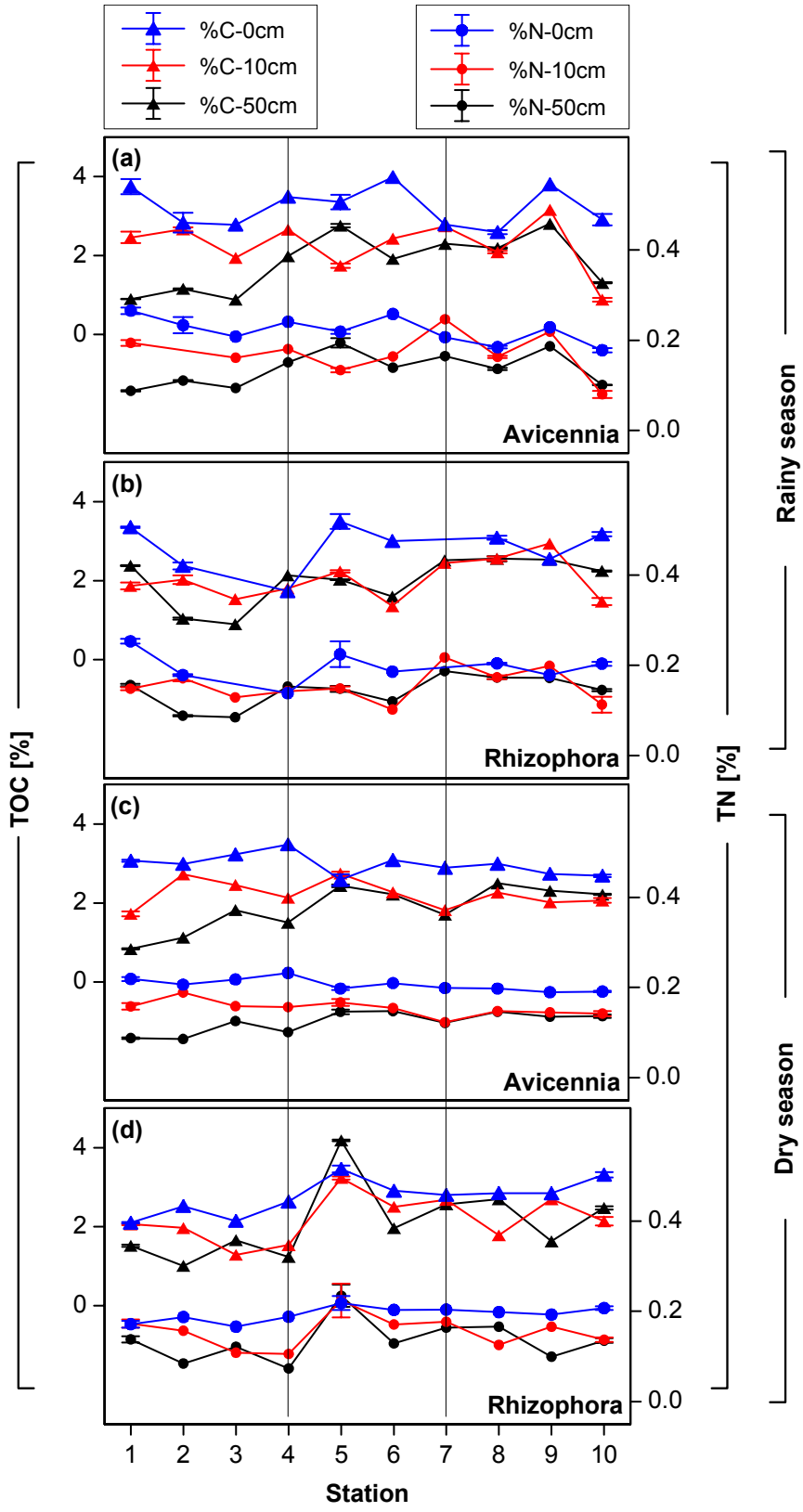


Figure 4.2.1: Mean ± SD of total organic carbon (TOC) and total nitrogen (TN) content in sediment samples near *A. germinans* (a, c) and *R. mangle* trees (b, d) for transect 1 during rainy and dry season. Vertical lines indicate the *Avicennia*- (left) and *Rhizophora*-dominated areas (right) and the transition zone (middle).

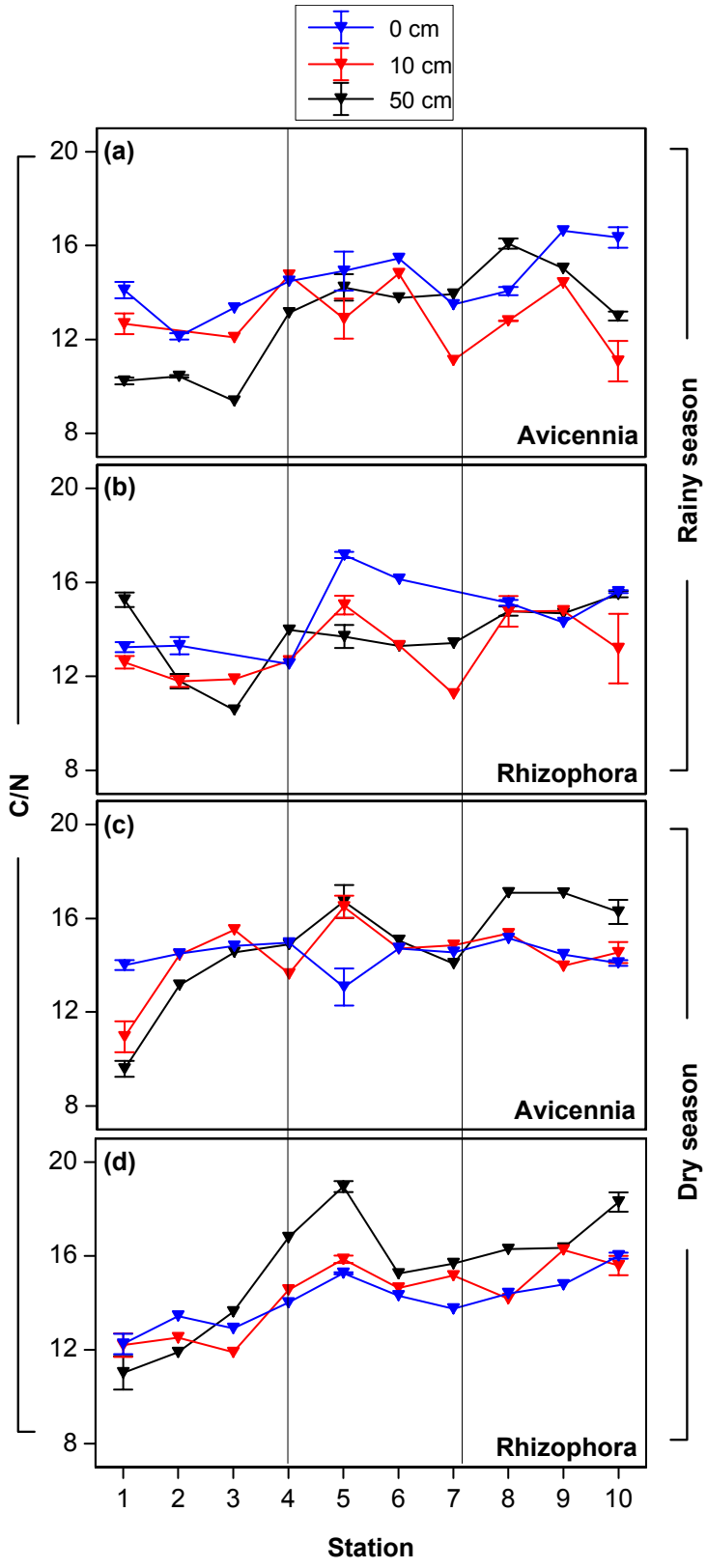


Figure 4.2.2: Mean ± SD of C/N ratios of sediment samples near *A. germinans* (a, c) and *R. mangle* trees (b, d) for transect 1 during rainy and dry season. Vertical lines indicate the *Avicennia*- (left) and *Rhizophora*-dominated areas (right) and the transition zone (middle).

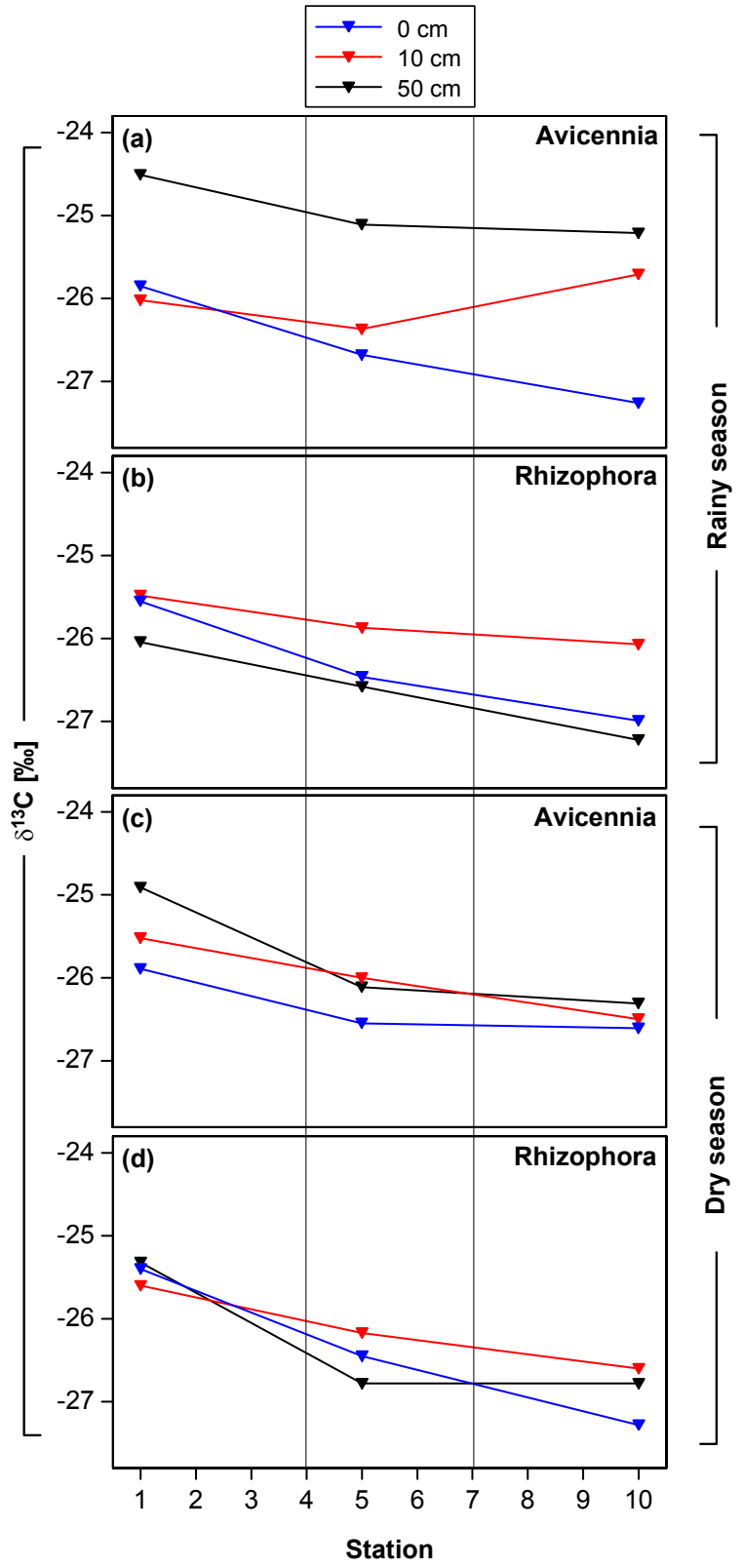


Figure 4.2.3: $\delta^{13}C$ values of sediment samples near *A. germinans* (a, c) and *R. mangle* trees (b, d) for transect 1 during rainy and dry season. Vertical lines indicate the *Avicennia*- (left) and *Rhizophora*-dominated areas (right) and the transition zone (middle).

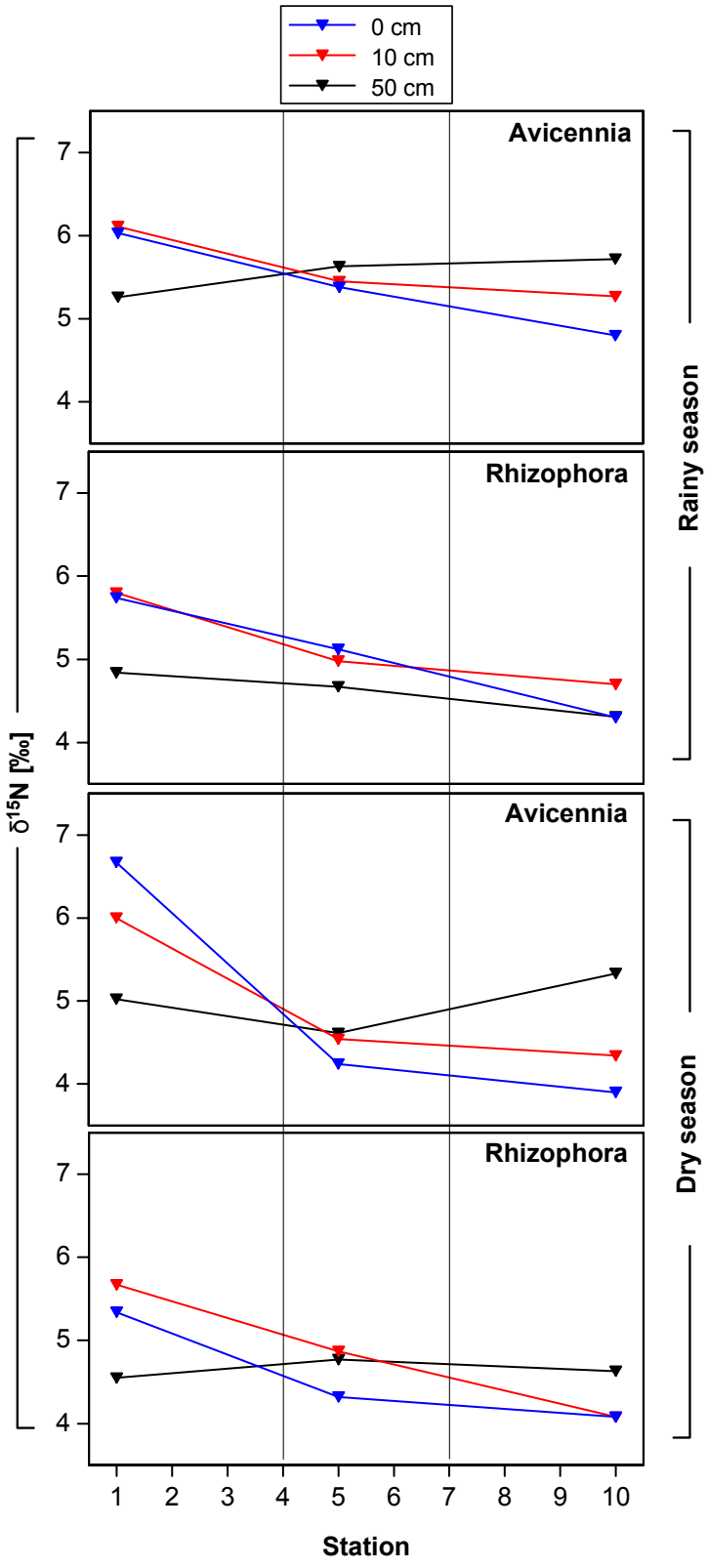


Figure 4.2.4: $\delta^{15}N$ values of sediment samples near *A. germinans* (a, c) and *R. mangle* trees (b, d) for transect 1 during rainy and dry season. Vertical lines indicate the *Avicennia*- (left) and *Rhizophora*-dominated areas (right) and the transition zone (middle).

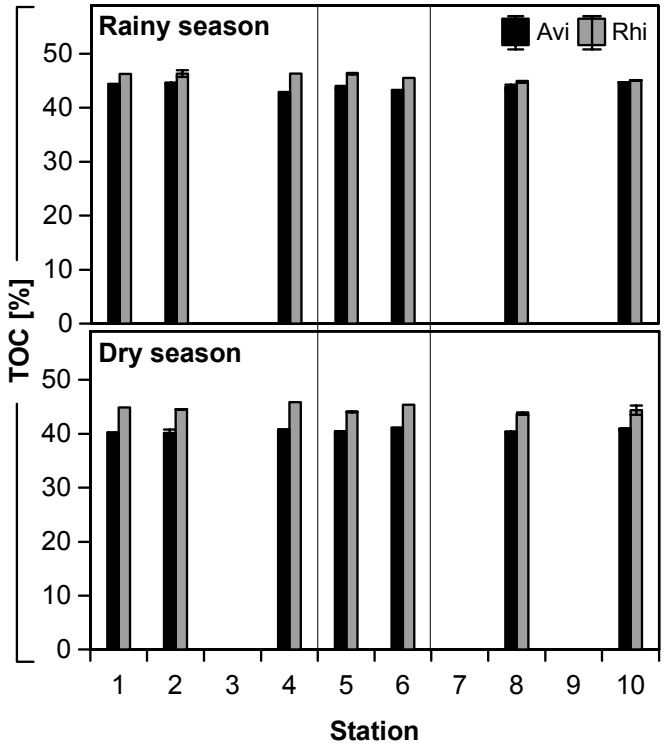


Figure 4.2.5: Mean ± SD of total organic carbon (TOC) content in leaf samples of *A. germinans* (Avi) and *R. mangle* trees (Rhi) for transect 1 during rainy (a) and dry season (b). Vertical lines indicate the *Avicennia*- (left) and *Rhizophora*-dominated areas (right) and the transition zone (middle).

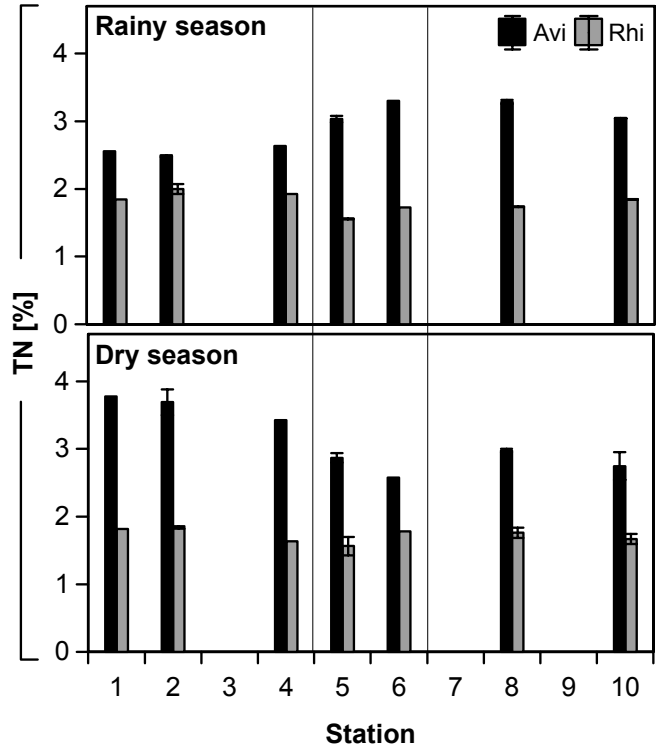


*Figure 4.2.6: M*ean ± SD of total nitrogen (TN) content in leaf samples of *A. germinans* (Avi) and *R. mangle* trees (Rhi) for transect 1 during rainy (a) and dry season (b). Vertical lines indicate the *Avicennia*- (left) and *Rhizophora*-dominated areas (right) and the transition zone (middle).

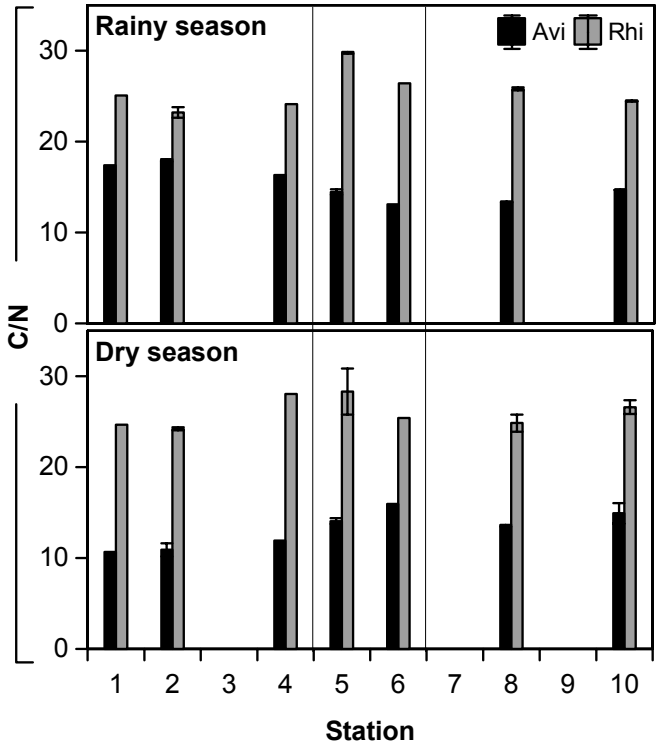


Figure 4.2.7: Mean ± SD of C/N ratios in leaf samples of *A. germinans* (Avi) and *R. mangle* trees (Rhi) for transect 1 during rainy (a) and dry season (b). Vertical lines indicate the *Avicennia*- (left) and *Rhizophora*-dominated areas (right) and the transition zone (middle).

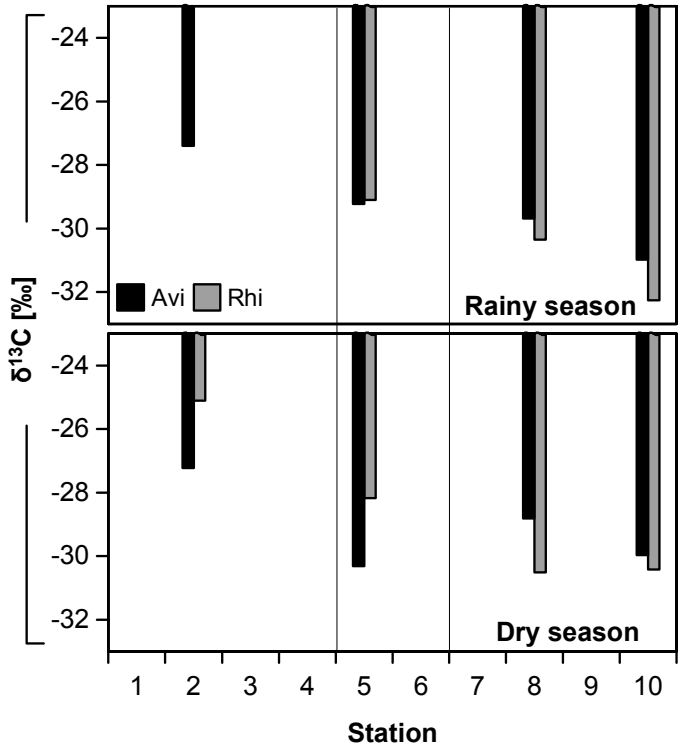


Figure 4.2.8: $\delta^{13}C$ values of leaf samples of *A. germinans* (Avi) and *R. mangle* trees (Rhi) for transect 1 during rainy (a) and dry season (b). Vertical lines indicate the *Avicennia*- (left) and *Rhizophora*-dominated areas (right) and the transition zone (middle).

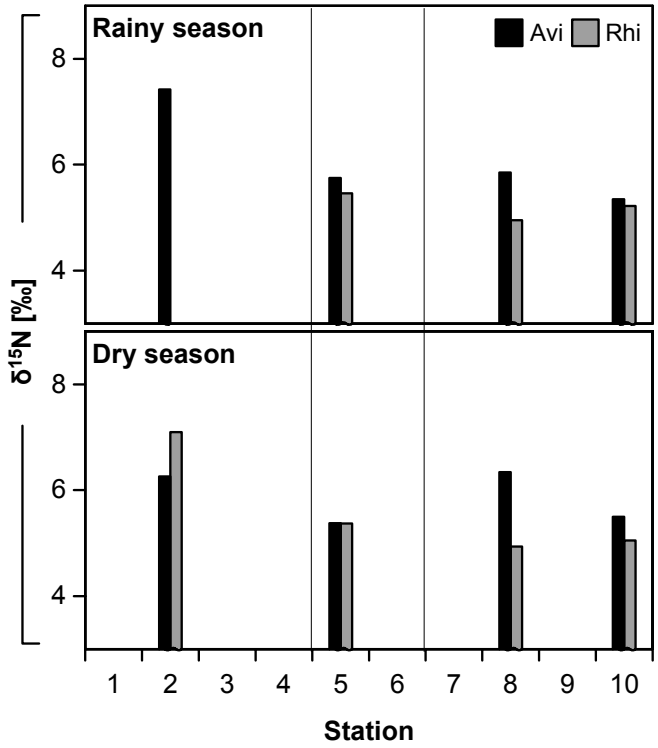


Figure 4.2.9: $\delta^{15}N$ values of leaf samples of *A. germinans* (Avi) and *R. mangle* trees (Rhi) for transect 1 during rainy (a) and dry season (b). Vertical lines indicate the *Avicennia*- (left) and *Rhizophora*-dominated areas (right) and the transition zone (middle).

4.2.1.3 Transect 2: Elemental and isotopic composition of sediments

Elemental composition

The total organic carbon (TOC) and total nitrogen (TN) content of the sediment at different depths and for both seasons are shown in weight% of dry weight (*Figure 4.2.10*).

TOC and TN content followed the same patterns in both rainy and dry season and at all measured depths. For the rainy season (*Figure 4.2.10a*) the most striking feature is a conspicuous and significant ($p<0.001$) step of high values (6.5 ± 0.5 % and 0.43 ± 0.01 % for TOC and TN respectively, n=11) in surface sediments of stations 1-4 to much lower values (2.9 ± 0.1 % TOC and 0.2 ± 0.01 % TN, n=7) at stations 5-7. This step can also be observed during the dry season, although it is not as pronounced here due to very low TOC and TN content at stations 1 and 2. During the rainy season values decreased continuously and differed significantly ($p<0.001$) from 5.2 ± 1.8 % TOC and 0.35 ± 0.11 % TN in surface sediments (n=18) to 1.7 ± 0.9 %TOC and 0.13 ± 0.05%TN at 10cm (n=16) and finally 0.56 ± 0.2%TOC and 0.05 ± 0.01%TN at 50cm depth (n=16). For the dry season the trend and values are very similar although the surface TOC and TN content with 3.8 ± 1.5 % TOC and 0.26 ± 0.09 % TN is significantly lower ($p<0.005$, n=18) than during the rainy season.

C/N ratios differed significantly between seasons ($p<0.005$) with mean values of 12.8 ± 2.2 (n=49) in the rainy season and 14.2 ± 2.2 during dry season (n=46) (*Figure 4.2.11a-b*). During the rainy season a trend could be seen for surface and 10cm sediments from significantly higher values at stations 1-4 (15.0 ± 0.6 and 14.0 ± 1.4 for surface and 10cm respectively, n=11) to lower values at stations 5-7 (13.7 ± 0.5 and 11.2 ± 1.9, n=7). The same downward trend could be seen during the dry season, but only for 10cm depth. In contrast to the TOC and TN content of the substrate this difference between the stations was not a sudden downward step, but followed a rather continuous downward slope.

Isotopic composition

$\delta^{13}C$ composition of the substrate showed a similar pattern during both seasons, being more pronounced during the rainy season (*Figure 4.2.12a-b*). Values varied between a maximum of -21.83 ‰ and a minimum of -26.52 ‰ (both during the rainy season at station 2), whereby the values of the surface and the 10 cm layers display an exactly mirror each other especially at the first 4 stations.

$\delta^{15}N$ values were highly variable especially during the dry season (*Figure 4.2.12c-d*). During the rainy season a stratification between sediment layers could be seen with surface sediment values (5.9 ± 0.7 ‰) being significantly lower ($p<0.05$, n=7) than both 10 cm and 50 cm samples (6.3 ± 0.8 ‰ and 6.4 ± 0.6 ‰ respectively). For dry season samples no significant difference between the various depths could be detected, but both 10 cm and 50 cm sediment layers showed two pronounced minima at station 2 and 6.

4.2.1.4 Transect 2: Elemental and isotopic composition of plants

Elemental composition

Leaves from all plant species present in this transect and roots from the herbaceous species were analysed for TOC and TN (*Figures 4.2.13* and *4.2.14*, mean values and sample sizes for each plant are presented in *Table 4.2.1*).

TOC content in *S. portulacastrum* and *B. maritima* leaves was significantly lower ($p<0.001$) than in *S.virginicus* and *A. germinans* during both rainy and dry season (*Figure 4.2.13*). Mean values ranged from 42.5 ± 0.9% and 43.2 ± 0.8% for *A. germinans* and *S. virginicus* to 35.7 ± 2.3 for *S. portulacastrum* and 27.7 ± 0.1% TOC for *B. maritima*. Both succulent species also contained significantly less TOC ($p<0.05$) during the dry season (30.0 ± 1.8% and 20.9 ± 1.0% for *S. portulacastrum* and *B. maritima* respectively) compared to rainy season values. Between stations only a slight downward trend in TOC values for *S. portulacastrum* during the dry season could be observed. TOC content in roots was usually higher than in leaves for *S. portulacastrum* (except at station 1 during the rainy season), did not differ much for in *S.virginicus*, and was always significantly higher for *B. maritima*.

TN values were significantly different between all plant species during the rainy season ($p<0.01$) (*Figure 4.2.14*). *A. germinans* contained the highest amount of TN (2.2 ± 0.2 %), followed by *B. maritima* with 1.8 ± 0.04 %, *S. portulacastrum* with 0.9 ± 0.1 % and *S. virginicus* with 0.7 ± 0.1% TN. During the dry season *A. germinans* and *B. maritima* reached the same level with 1.9 ± 0.1 % and 1.7 ± 0.01 % TN respectively. Compared with these the two other herbaceous species again showed lower values with 1.3 ± 0.05 % for *S. portulacastrum* and 0.8 ± 0.1% for *S. virginicus*. All species except *S. virginicus* had significantly lower values during the dry season than during the rainy season ($p<0.05$). In contrast to TOC values, TN values in *S. portulacastrum* roots were generally lower than in leaves with the exception of station 1 during the rainy season. For *S. virginicus* TN values were usually higher in the roots (except for station 5 during the rainy

season), whereas *B. maritima* showed a much lower value for the roots during the dry season, but not during the rainy season.

During the rainy season C/N ratios were lowest for *A. germinans* (19.2 ± 1.6) and *B. maritima* (15.1 ± 0.3) and highest for *S. virginicus* (65.4 ± 13.2) (*Figure 4.2.15*). Ratios of *S. portulacastrum* (42.7 ± 5.8) were significantly higher than of *A. germinans* ($p<0.001$), but significantly lower than the values for *S. virginicus* ($p<0.001$), whereas during the dry season its values dropped to the same level as those of *A. germinans*. For the dry season samples C/N ratios differed significantly between *S. virginicus* (50.2 ± 4.6) and both *S. portulacastrum* (23.4 ± 1.1) and *A. germinans* (22.6 ± 1.5) ($p<0.001$). Values for *B. maritima* (12.0 ± 0.6) were significantly lower than those for all other species ($p<0.001$). Values for all species except *S. virginicus* were significantly different between seasons ($p<0.05$). For the roots of *S. portulacastrum* C/N values were always higher than C/N values in leaf material with the exception of station 1 during the rainy season. C/N values for *S. virginicus* roots were always lower than values in leaves except at station 5 during the rainy season were the root value was higher. *B. maritima* roots always had higher C/N ratios than leaves.

Isotopic composition

$\delta^{13}C$ and $\delta^{15}N$ in green leaves were measured for all species within the transect and in both seasons. Mean values are shown below (*Table 4.2.1*). Only one sample was analysed for *B. maritima*, hence it was not included in the statistical analysis.

Only the grass *S. virginicus* differed significantly ($p<0.001$) from all other plants in its low $\delta^{13}C$ values (-15.0 ± 1.0 ‰ and -15.9 ± 0.03 ‰ for rainy and dry season respectively), whereas *S. portulacastrum* had significantly lower ($p<0.001$) $\delta^{15}N$ values in both seasons (3.9 ± 0.6‰ for the rainy and 3.1 ± 0.3‰ for the dry season). *S. virginicus* also showed low $\delta^{15}N$ values during the dry season (4.4 ± 0.6 ‰), but the difference was not significant.

The values for the roots of the herbaceous species were not significantly different from the $\delta^{13}C$ and $\delta^{15}N$ values in the leaves with the exception of *S. portulacastrum* roots during the dry season were values for $\delta^{15}N$ were significantly higher (5.0 ± 0.7 ‰) than for the leaves (3.1 ± 0.3) ($p<0.05$).

Table 4.2.1: Mean values ± SD for total organic carbon (TOC), total nitrogen (TN), C/N values and isotopic composition of leaves of the 4 plant species of transect 2 (values for $\delta^{13}C$ and $\delta^{15}N$ of roots of herbaceous species in brackets), *only one sample was measured.

Sample	n	TOC [%]	TN [%]	C/N	$\delta^{13}C$ [‰]	$\delta^{15}N$ [‰]
A. germinans						
rainy season	19	42.5 ± 1.0	2.2 ± 0.2	19.2 ± 1.6	-27.5 ± 1.6	6.0 ± 1.0
dry season	15	42.0 ± 0.9	1.9 ± 0.1	22.6 ± 1.6	-27.3 ± 1.3	6.6 ± 0.7
S. portulacastrum						
rainy season	10 (4)	35.7 ± 2.3	0.9 ± 0.1	42.7 ± 5.8	-28.7 ± 0.3 (-28.3 ± 1.1)	3.9 ± 0.6 (4.2 ± 0.5)
dry season	8 (4)	30.0 ± 1.8	1.3 ± 0.05	23.4 ± 1.1	-28.1 ± 0.5 (-28.8 ± 1.7)	3.1 ± 0.3 (5.0 ± 0.7)
S. virginicus						
rainy season	7 (3)	43.2 ± 0.8	0.7 ± 0.1	65.4 ± 13.2	-15.0 ± 1.0 (-14.9 ± 1.2)	6.0 ± 0.6 (5.7 ± 0.2)
dry season	4 (2)	42.3 ± 0.1	0.8 ± 0.1	50.2 ± 4.6	-15.9 ± 0.03 (-14.4 ± 0.2)	4.4 ± 0.6 (4.4 ± 0.6)
B. maritima						
rainy season	3 (2)	27.7 ± 0.2	1.8 ± 0.04	15.1 ± 0.3	-28.7* (-26.1*)	5.8* (5.4*)
dry season	3 (2)	20.9 ± 1.0	1.7 ± 0.01	12.0 ± 0.6	-26.6* (-26.5*)	3.9* (4.5*)

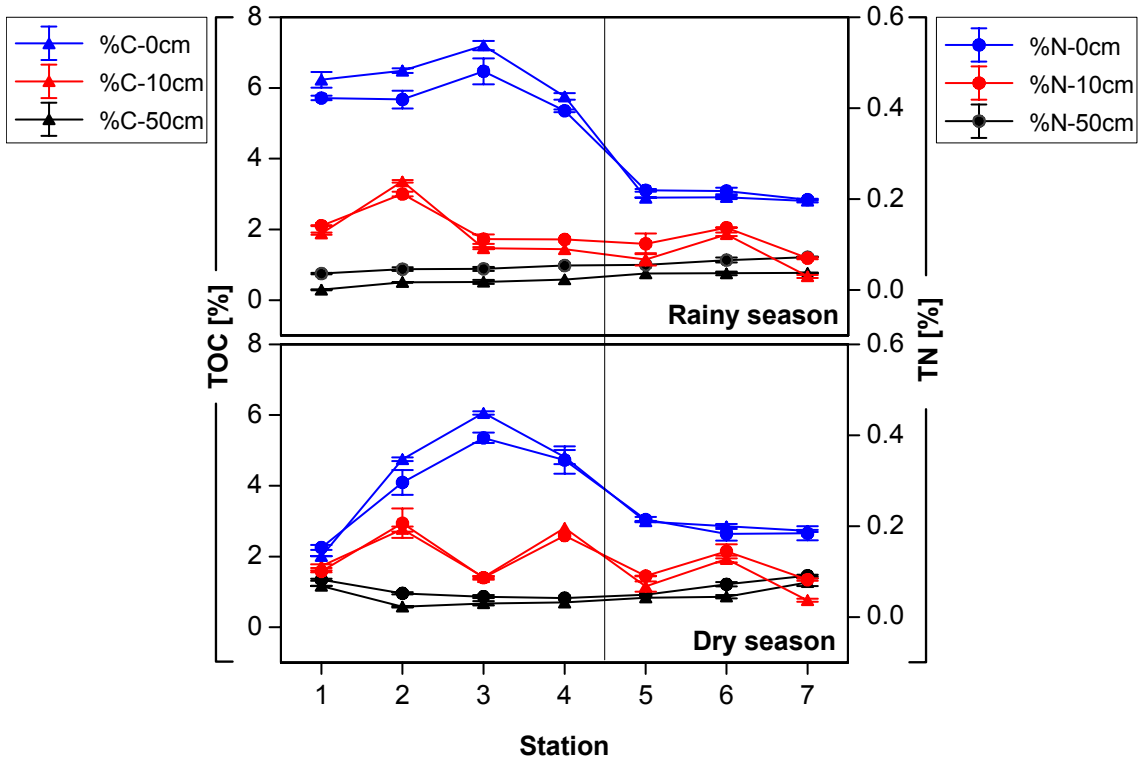


Figure 4.2.10: Mean ± SD of total organic carbon (TOC) and total nitrogen (TN) content in sediment samples of transect 2 during rainy and dry season. Vertical line indicates area with (left) and without (right) herbaceous vegetation.

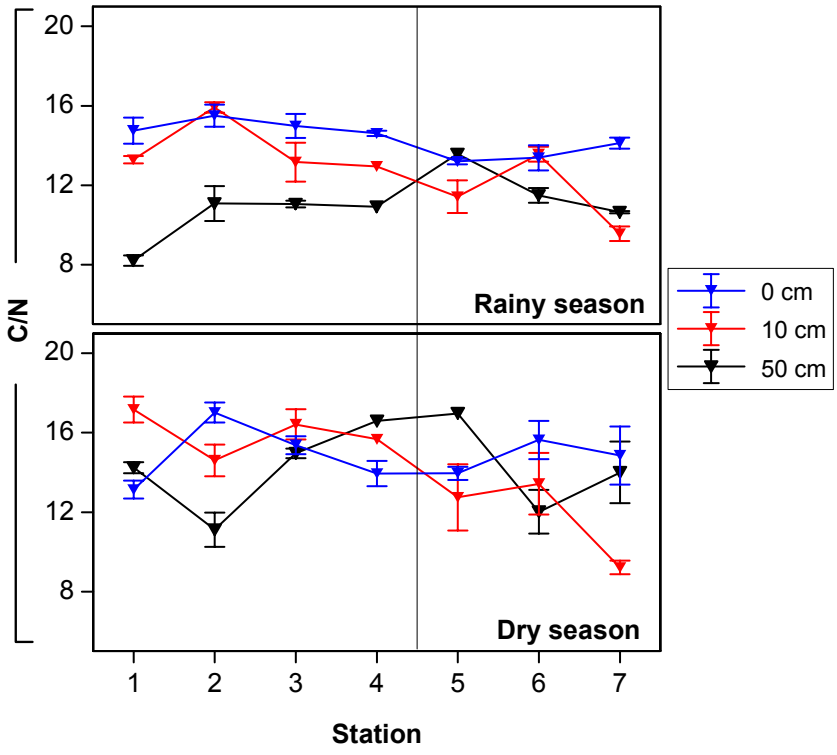


Figure 4.2.11: Mean ± SD of C/N values of sediment samples of transect 2 during rainy and dry season. Vertical line indicates area with (left) and without (right) herbaceous vegetation.

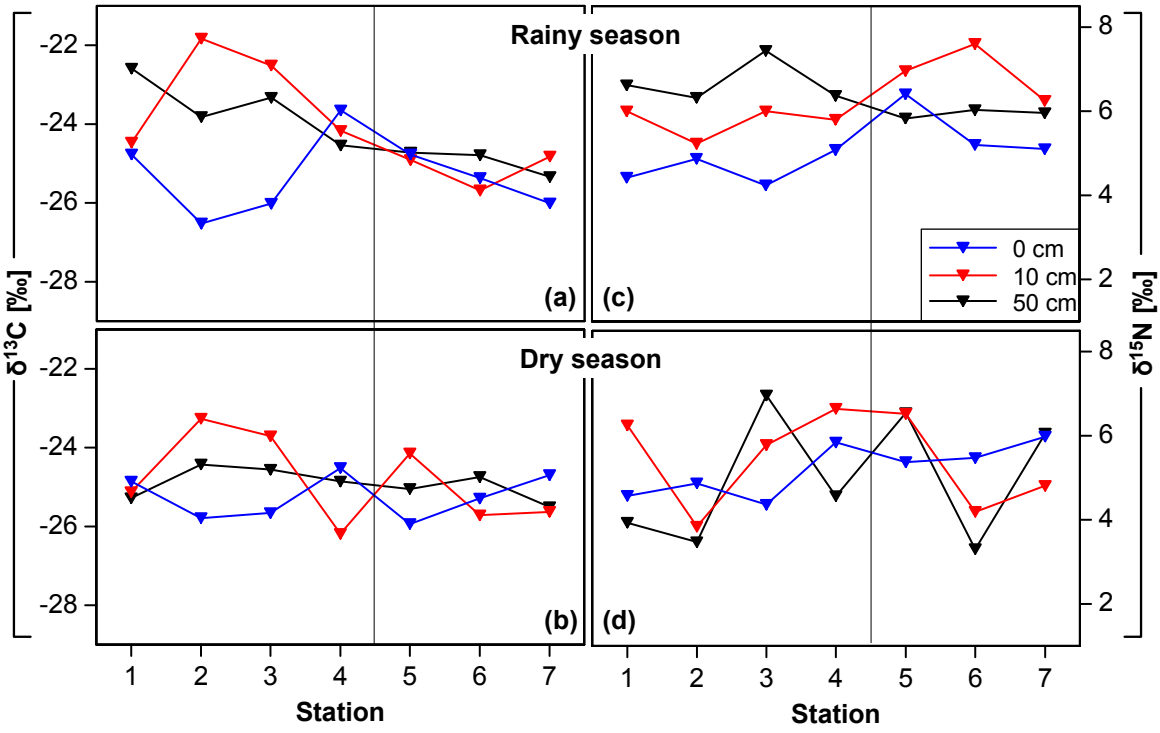


Figure 4.2.12: $\delta^{13}C$ (a, b) and $\delta^{15}N$ (c, d) values in sediment samples of transect 2 during rainy and dry season. Vertical line indicates area with (left) and without (right) herbaceous vegetation.

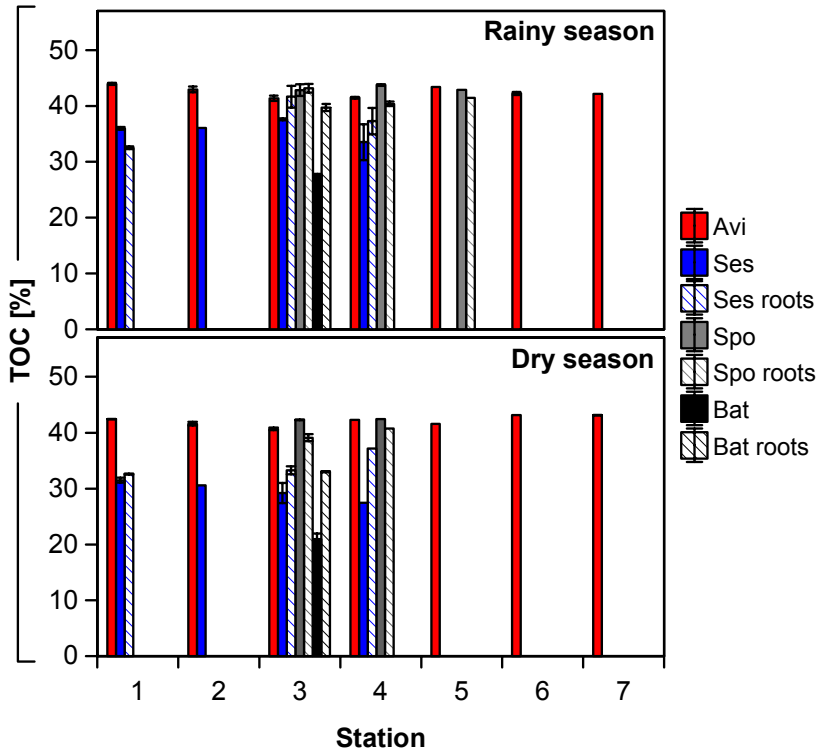


Figure 4.2.13: Mean ± SD of total organic carbon (TOC) content in leaf samples of *A. germinans* (Avi) and in leaf and root samples of *S. portulacastrum* (Ses), *S. virginicus* (Spo) and *Batis maritima* (Bat) for transect 2 during rainy and dry season. No bars = no data.

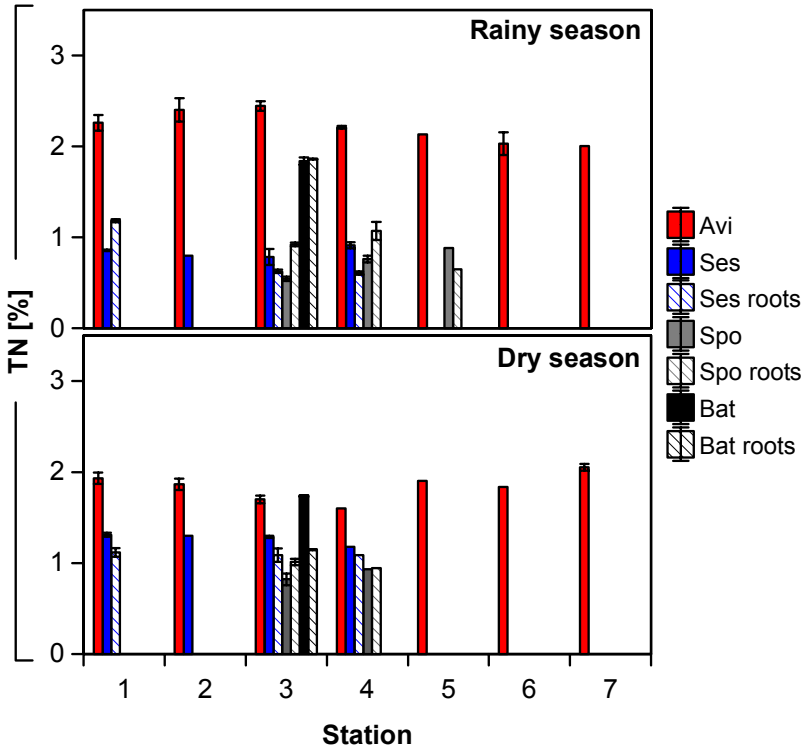


Figure 4.2.14: Mean ± SD of nitrogen (TN) content in leaf samples of *A. germinans* (Avi) and in leaf and root samples of *S. portulacastrum* (Ses), *S. virginicus* (Spo) and *Batis maritima* (Bat) for transect 2 during rainy and dry season. No bars = no data.

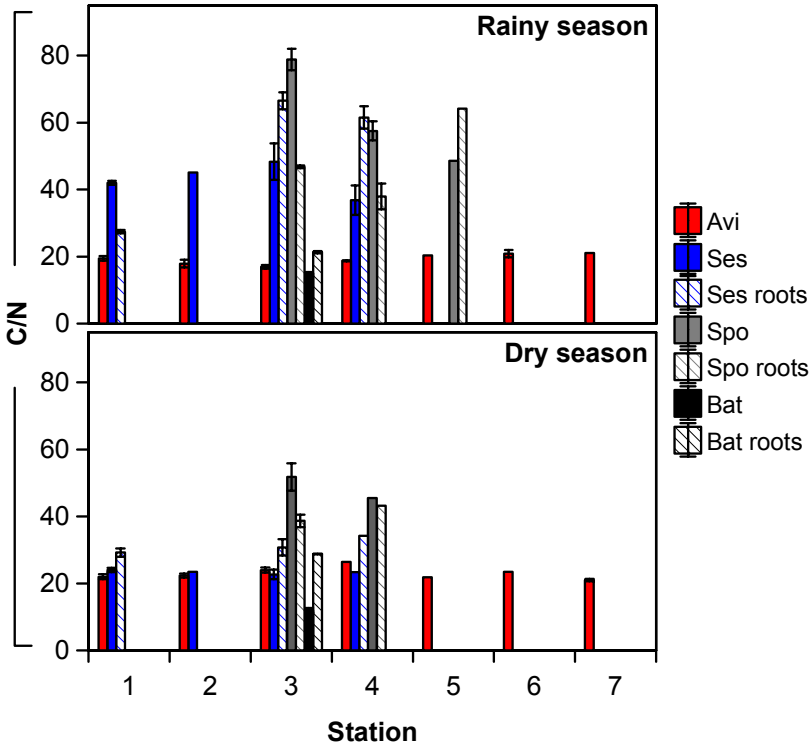


Figure 4.2.15: Mean ± SD of C/N values in leaf samples of *A. germinans* (Avi) and in leaf and root samples of *S. portulacastrum* (Ses), *S. virginicus* (Spo) and *Batis maritima* (Bat) for transect 2 during rainy and dry season. No bars = no data.

4.2.2 Tannins

4.2.2.1 Transect 1: Tannins in sediment material

Tannin concentrations are expressed in Tannic Acid Equivalents (TAE) per g dry weight.

Values for surface and 10 cm samples ranged from 0.18 to 1.33 mg TAE/g dw and 0.16 to 0.90 mg TAE/g dw respectively throughout the transect and seasons (*Figure 4.1.16*). Especially during the rainy season a u-shaped trend was observed with high values at station 1, declining towards station 3 or 4 and increasing again towards the end of the transect near the tidal channel. During the dry season this trend was not as pronounced for both sediment layers, but appears mainly in 10 cm depth.

In contrast the concentrations at a depth of 50 cm showed a clear upward trend from the *Avicennia*- to the *Rhizophora*-dominated area of the forest during both seasons and in all cores. The values of stations 1-4 (0.17 ± 0.08 mg TAE/g dw, with a minimum of 0.09 and a max of 0.37 mg TAE/g dw, n=24) differed significantly ($p<0.001$) from the tannin concentrations at stations 7-10 (1.0 ± 0.29 mg TAE/g dw, with a minimum of 0.44 and a maximum of 1.6 mg TAE/g dw, n=20).

4.2.2.2 Transect 1: Tannins in plant material

Tannins – expressed in Tannic Acid Equivalents (TAE) per g dry weight – differed significantly between species (*Figure 4.1.17*), with *R. mangle* containing a more than 4-fold concentration of tannin (83.7 ± 23.4 mg TAE/g dw) than *A. germinans* leaves (18.3 ± 5.3 mg TAE/g dw) ($p<0.001$, n=38). The highest value of 120.1 mg TAE/g dw was found in *R. mangle* leaves during the dry season, whereas the minimum value occurred in *A. germinans* leaves in the rainy season. For *R. mangle* leaves there was a significant increase of tannin content from rainy to dry season ($p<0.001$, n=19), whereas tannin concentration in *A. germinans* leaves did not differ significantly with seasons.

In both seasons and for both species a significant decrease ($p<0.05$) in tannin concentration could be observed throughout the transect with highest values at stations 1-4 (17.4 ± 3.9 mg TAE/g dw for *A. germinans* (n=14) and 73.4 ± 7.6 mg TAE/g dw for *R. mangle* (n=14)) and lowest values in the *Rhizophora*-dominated area at stations 8-10 (12.8 ± 1.1 mg TAE/g dw for *A. germinans* (n=12) and 44.2 ± 10.3 mg TAE/g dw for *R. mangle* (n=12)).

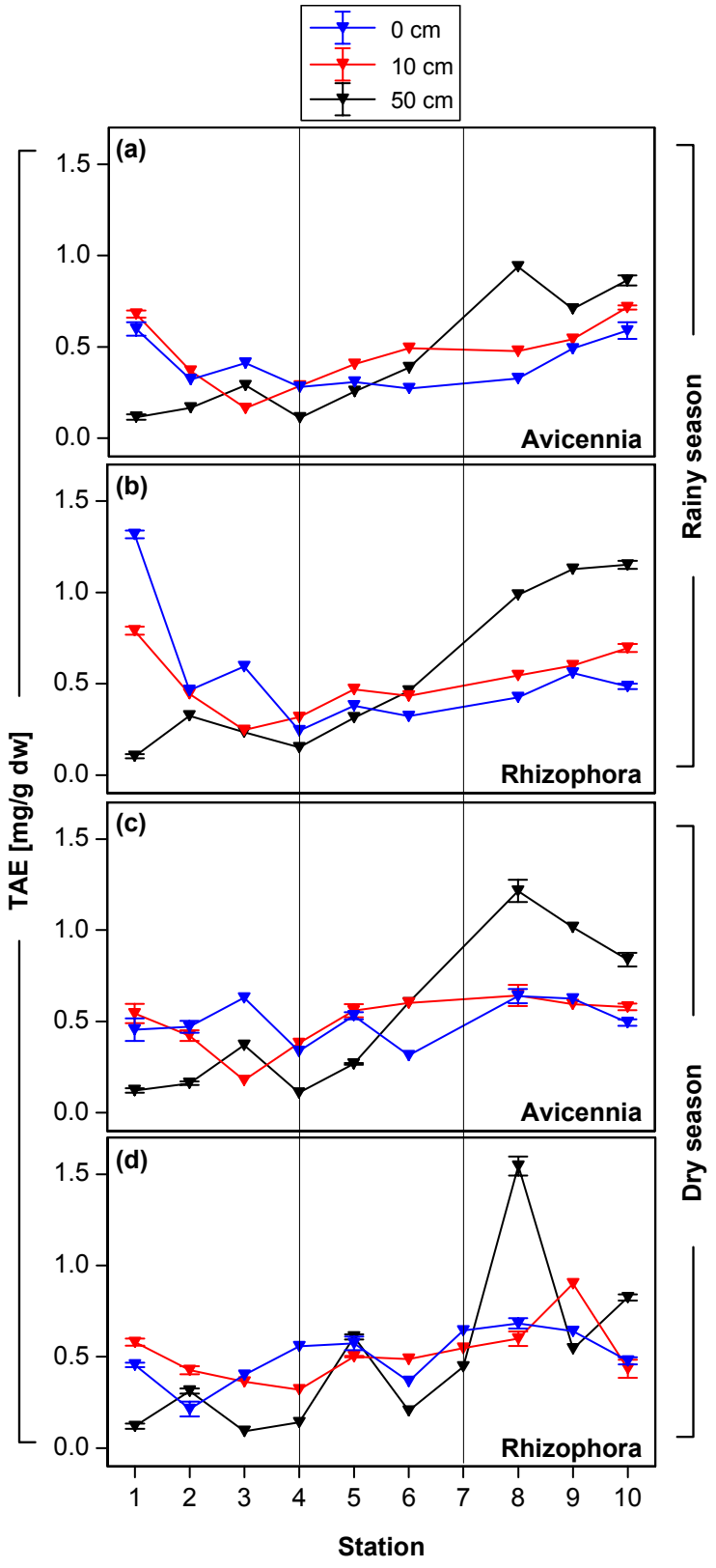


Figure 4.2.16: Mean ± SD of tannin concentrations (as TAE) in sediment samples near *A. germinans* (a, c) and *R. mangle* trees (b, d) for transect 1 during rainy and dry season. Vertical lines indicate the *Avicennia*- (left) and *Rhizophora*-dominated areas (right) and the transition zone (middle).

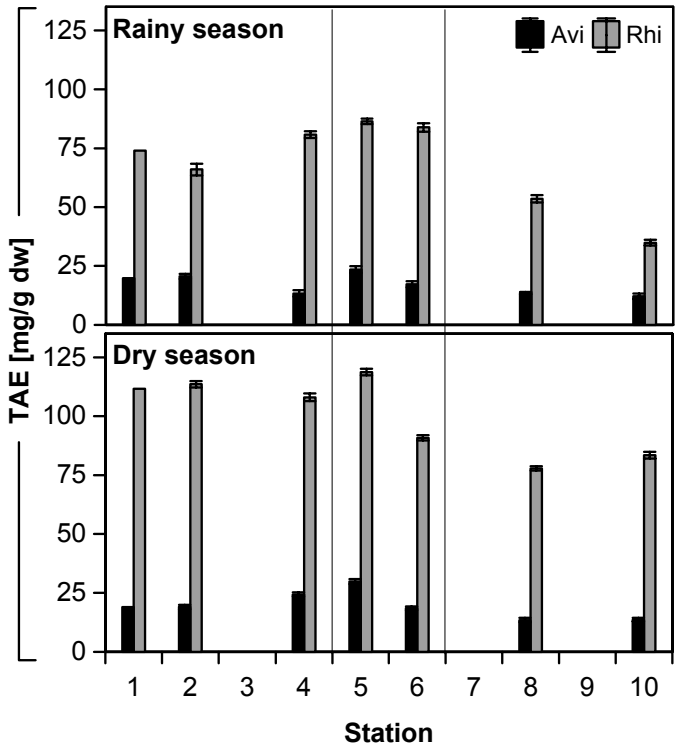


Figure 4.2.17: Mean ± SD of tannin concentrations (as TAE) in leaf samples of *A. germinans* (Avi) and *R. mangle* trees (Rhi) for transect 1 during rainy (a) and dry season (b). Vertical lines indicate the *Avicennia*- (left) and *Rhizophora*-dominated areas (right) and the transition zone (middle).

4.2.2.3 Transect 2: Tannins in sediment material

Tannin concentrations are presented in Tannic Acid Equivalents (TAE) per g dry weight.

During the rainy season a significant difference ($p<0.05$) between high tannin content at stations 1-4 and low values at stations 5-7 could be observed for surface and 10cm substrate layers (*Figure 4.2.18*). For the first 4 stations values of 0.9 ± 0.2 mg TAE/g dw for surface and 0.9 ± 0.1 mg TAE/g dw for 10 cm sediments were found (n=12), whereas substrate samples from stations 5-7 contained only 0.7 ± 0.1 mg TAE/g dw and 0.6 ± 0.1 mg TAE/g dw for surface and 10cm sediments respectively (n=9). At all stations tannin content in the 50 cm samples (0.2 ± 0.2 mg TAE/g dw) was significantly lower and more stable than in both 10 cm and surface sediments ($p<0.001$, n=21). At 50 cm depth values were very low at all stations (0.1 ± 0.03 mg TAE/g dw) except for station 7 where tannin content was raised suddenly to 0.5 ± 0.03 mg TAE/g dw.

Values for the dry season were significantly lower ($p<0.001$, n=63) than for the rainy season and did not show such a clear pattern. The 50 cm values however, showed the same tannin content as during the rainy season (raising from 0.1 ± 0.03 mg TAE/g dw to 0.6 ± 0.03 mg TAE/g dw at station 7) and were also significantly lower than in surface and 10 cm sediment layers ($p<0.005$, n=21). Here tannin content in surface sediments showed a minimum at station 3 (0.2 mg TAE/g dw) and a maximum at station 7 (0.6 mg TAE/g dw), whereas for values of the 10 cm layers two minima at station 1 and 5 and two maxima at station 2 and 7 were observed.

4.2.2.4 Transect: Tannins in plant material

Tannin concentrations – shown in Tannic Acid Equivalents per g dw – differed significantly between seasons and species (*Figure 4.2.19*). For all species tannin content was significantly higher during the dry season ($p<0.001$, n=42). For both seasons *A. germinans* had significantly higher tannin content (15.1 ± 3.0 mg TAE/g dw and 30.1 ± 10.2 mg TAE/g dw, n=21) than the herbaceous species. Among the latter the difference was significant only between the two succulents and *S. virginicus* during the rainy season ($p<0.05$). During dry season, tannin values of *S. portulacastrum* were significantly higher (22.4 ± 6.4 mg TAE/g dw, n=12) than tannin content of *B. maritima* (11.1 ± 1.0 mg TAE/g dw, n=3) or *S. virginicus* (12.3 ± 2.9 mg TAE/g dw, n=6) ($p<0.01$).

Within the transect no conspicuous trend could be observed during the rainy season, whereas in the dry season *A. germinans* showed increased content for stations 1-5 compared to stations 6 and 7. For *S. portulacastrum* tannin values increased continuously from a minimum of 14.2 mg TAE/g dw at station 1 to a maximum of 31.4 mg TAE/g dw at station 4.

Tannin values for roots differed significantly from leaf values only for *S. portulacastrum* ($p<0.05$, n=12 for leaves and n=9 for roots), being much higher at all stations and both seasons other than station 1 during the rainy season. The difference was more pronounced during the dry season.

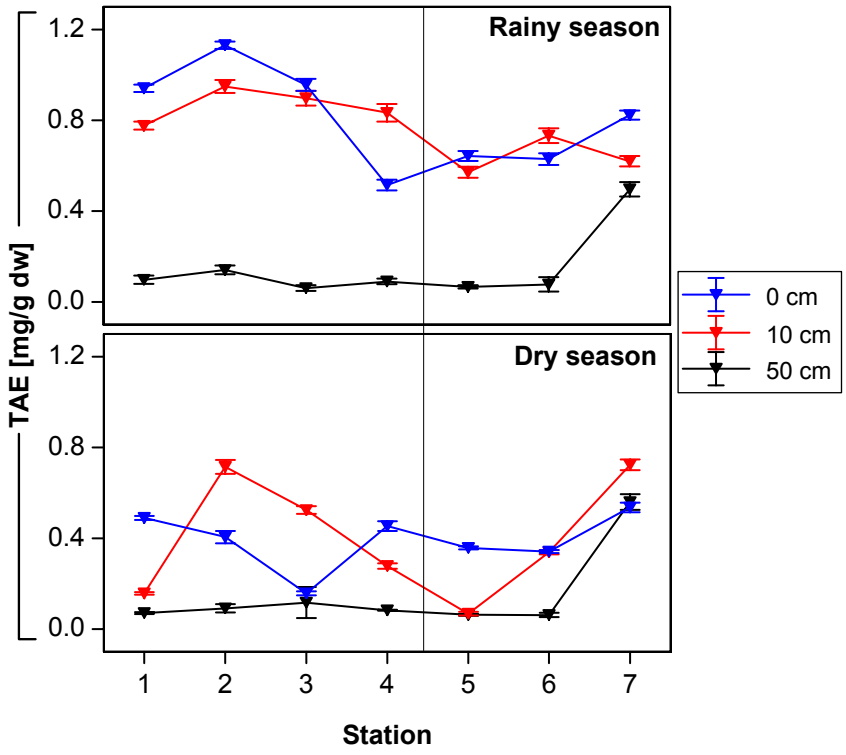


Figure 4.2.18: Mean ± SD of tannin concentrations (as TAE) in sediment samples of transect 2 during rainy and dry season. Vertical line indicates area with (left) and without (right) herbaceous vegetation.

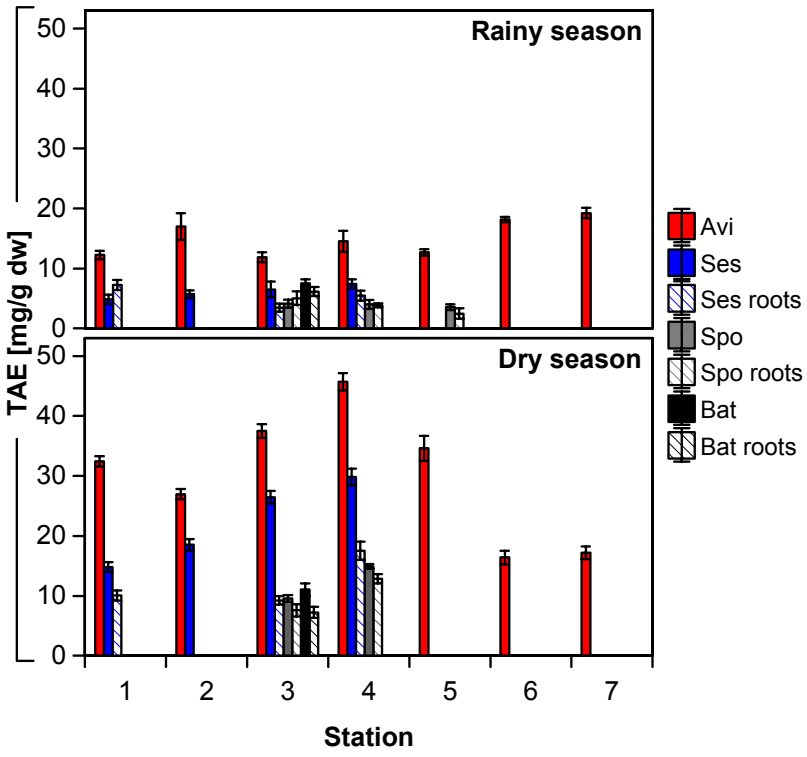


Figure 4.2.19: Mean ± SD of tannin concentrations (as TAE) in leaf samples of *A. germinans* (Avi) and in leaf and root samples of *S. portulacastrum* (Ses), *S. virginicus* (Spo) and *Batis maritima* (Bat) for transect 2 during rainy and dry season. No bars = no data.

4.2.3 Total Hydrolysable Amino Acids (THAA)

4.2.3.1 Transect 1: Total Hydrolysable Amino Acids in sediment material

The data of the amino acid analysis are presented in *Figures 4.2.20 -4.2.27*, five stations were analysed at three different depths. For the data set of *Avicennia*-sediments from the dry season, samples from station 1 are missing. Data are given in mg/g dw, corresponding values for THAA in mol will be given in brackets.

In all cores - except the sediment samples near *Rhizophora* in the dry season (*Figure 4.2.20d*) and station 5 in all cores – the total hydrolysable amino acids (THAA) showed significantly higher concentrations at the surface compared to the deeper sediment layers ($p<0.05$, $n=12$). The mean values for all cores were 8.4 ± 1.4 mg THAA/g dw (68.3 ± 11.5 µmol/g dw), 5.5 ± 1.4 mg THAA/g dw (44.7 ± 11.4 µmol/g dw) and 4.9 ± 1.9 mg THAA/g dw (40.0 ± 15.0 µmol/g dw) for surface, 10 cm and 50 cm substrates respectively. The minimum concentration of 2.08 mg THAA/g dw (16.5 µmol/g dw) was recorded at 50 cm in the dry season (*Figure 4.2.20d*), whereas the maximum value of 11.16 mg THAA/g dw (91.4 µmol/g dw) occurred at the surface during the rainy season (*Figure 4.2.20b*).

Figure 4.2.21 shows the proportion of amino acid carbon (AA-C) and nitrogen (AA-N) of TOC and TN in weight %. Both dry season samplings and the rainy season *Avicennia*-sediments showed stable values with no significant differences for both measurements at all measured depths with mean values of 11.6 ± 2.2 % AA-C/TOC and 49.6 ± 8.9 % AA-N/TN. Only the rainy season samples of the *Rhizophora*-sediments showed a downward trend from the dry to the humid part of the transect. Samples at all depths had significantly higher values for both AA-C/TOC and AA-N/TN at stations 1 and 2 (17.1 ± 4.8 % AA-C/TOC and 64.4 ± 13.8 % AA-N/TN) than at stations 8 and 10 (9.3 ± 1.8 % AA-C/TOC and 40.0 ± 7.1 % AA-N/TN) ($p<0.005$, $n=6$).

The percentage of D-amino acids of THAA varied with depth and station between 4.0 % and 14.6 % but showed no consistent trend or difference throughout the transect (*Figure 4.2.22*). Only the dry season samples of the *Avicennia*-sediments showed a pronounced stratification with a significant difference between the low surface values of 5.6 ± 1.6 % and the higher values of 9.7 ± 1.7 % and 8.5 ± 1.6 % at 10 cm and 50 cm depths respectively. Rainy season samples displayed an increase at station 2, followed by a subsequent decrease at station 5 in both *Avicennia*- and *Rhizophora*-cores, whereas dry season samples from both cores showed an increase from station 8 to 10.

The ratio between D- to L-alanine differed with depth only in the rainy season, where the values were significantly lower in the surface sediments (0.12 ± 0.01 for both *Avicennia*- and *Rhizophora*-sediments versus a mean of 0.19 ± 0.06 for deeper substrates), hence displaying an increase in total D-alanine with depth (*Figure 4.2.23*).

Composition of amino acid monomers in mol% is shown for surface, 10 and 50 cm sediments for station 1 and 10 (*Figures 4.2.24* and *4.2.25*). The dominant L-amino acids in all cores at all depths and in both seasons were L-asx, L-glx, gly and L-ala. D-amino acids were generally less abundant (< 4 mol%) and showed more variation than the L-enantiomers. At station 1 D-ile, D-asx and D-ala were dominating during the rainy season, whereas D-asx prevailed in the dry season. At station 10 only D-asx and D-ala were dominant during the rainy season especially at 10 and 50 cm. During the dry season the composition was similar to station 1 with D-asx being the only dominant D-amino acid.

The absolute amounts for indicidual amino acid composition in mg/g dw are shown in Appendix A3 and A4.

4.2.3.2 Transect 1: Total Hydrolysable Amino Acids in plant material

Total hydrolysable amino acids (THAA) were analysed in leaf mixtures at 4 stations throughout the transect, during both seasons and for both species. As no significant trend or difference could be observed between stations, mean values for THAA, proportion of amino acid carbon and nitrogen of TOC and TN respectively (AA-C/TOC and AA-N/TN, values given in weight% of dry weight), percentage of D-amino acids (%D-AA) and D/L-alanine ratios, are presented in *Table 4.2.2*. For THAA values in mol are given in brackets.

No general trend for all parameters was apparent. For THAA *R. mangle* leaves showed significantly lower values during the rainy season, as was the case for AA-C/TOC. During the dry season *R. mangle* leaves had a significantly higher proportion of amino acid nitrogen than *A. germinans* leaves ($p<0.05$, $n=4$). Percentage of D-amino acids showed no difference between species or season, whereas the D/L-alanine ratio was significantly higher in *R. mangle* leaves ($p<0.05$, $n=8$).

Table 4.2.2: Mean values ± SD for total hydrolysable amino acids (THAA), proportion of amino acid carbon and nitrogen of TOC and TN respectively (AA-C and AA-N, values given in weight% of dry weight), percentage of D-amino acids (%D-AA) and D/L-alanine ratios in leaves of *R. mangle* and *A. germinans* during rainy and dry season. (*) indicates significant difference.

Sample	THAA [mg/g dw] (µmol/g dw)	AA-C/TOC [%]	AA-N/TN [%]	D-AA [%]	D/L-alanine
A. germinans					
rainy season	86.7 ± 6.2 (676 ± 46)	9.0 ± 0.7	41.7 ± 3.2	1.3 ± 0.3	0.02 ± 0.007
dry season	78.9 ± 11.5 (626 ± 91)	9.0 ± 1.3	36.3 ± 5.2	1.1 ± 0.2	0.02 ± 0.007
R. mangle					
rainy season	59.0 ± 7.1* (466 ± 45)	5.9 ± 0.7*	46.1 ± 2.6	1.3 ± 0.2	0.03 ± 0.01*
dry season	79.4 ± 14.7 (625 ± 111)	8.3 ± 1.6	63.5 ± 8.8*	1.5 ± 0.3	0.04 ± 0.01*

Amino acid composition of D- and L-enantiomers (in mol%) in leaves did not differ much between stations, but showed distinct variation between seasons (*Figures 4.2.26* and *4.2.27*). Generally L-asx, L-glx, L-ser, gly, L-ala and L-leu were the dominant L-amino acids. The most conspicuous change occurred in gly percentage that doubled in both species and at all measured stations during the dry season. D-asx dominated the pool of D-enantiomers followed by D-glx and D-leu. At station 10 *Rhizophora* leaves showed a D-ala peak during the dry season.

Absolute amounts of individual amino acids in mg/g dw of individual amino acids is shown in Appendix A5 and A6.

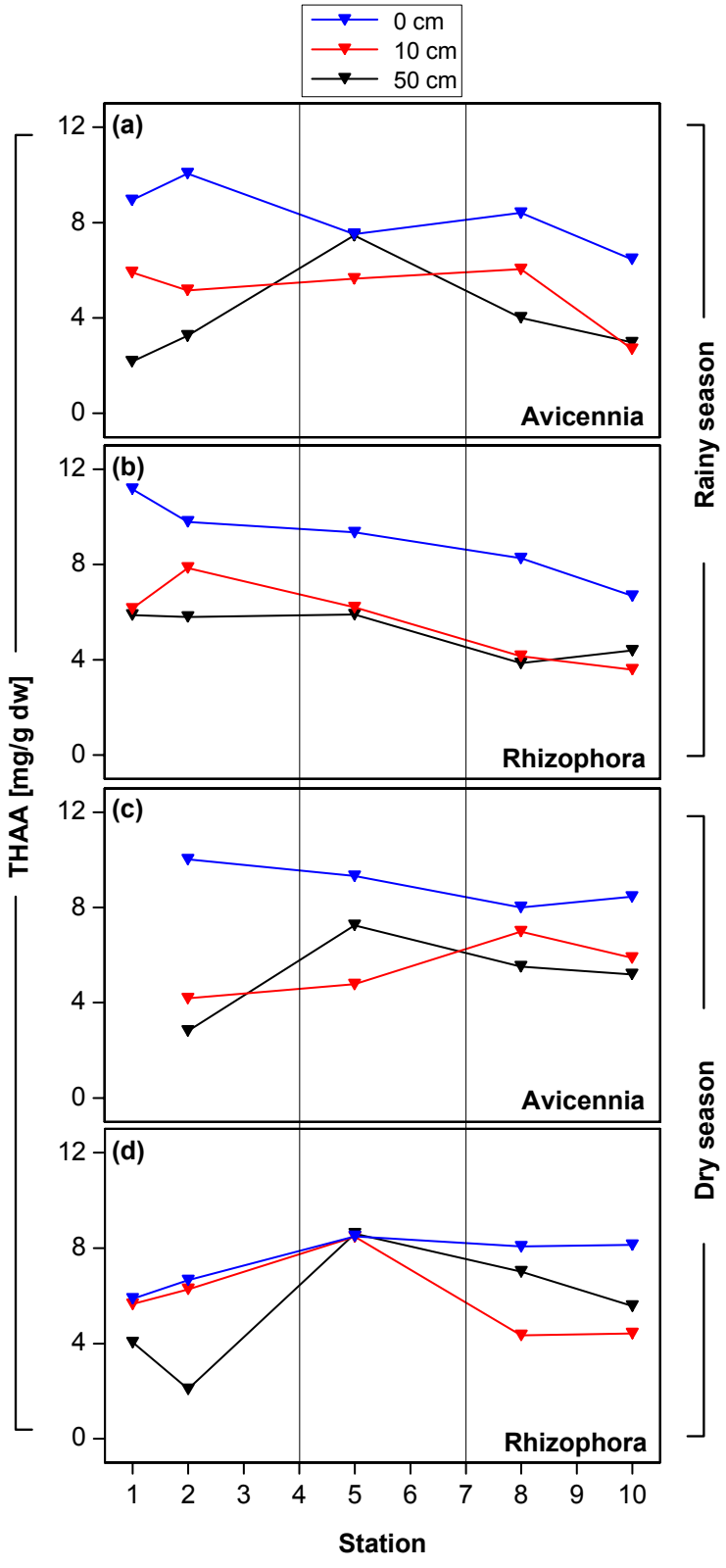


Figure 4.2.20: Concentration of total hydrolysable amino acids (THAA) in sediment samples near *A. germinans* (a, c) and *R. mangle* trees (b, d) for transect 1 during rainy and dry season. Vertical lines indicate the *Avicennia*- (left) and *Rhizophora*-dominated areas (right) and the transition zone (middle).

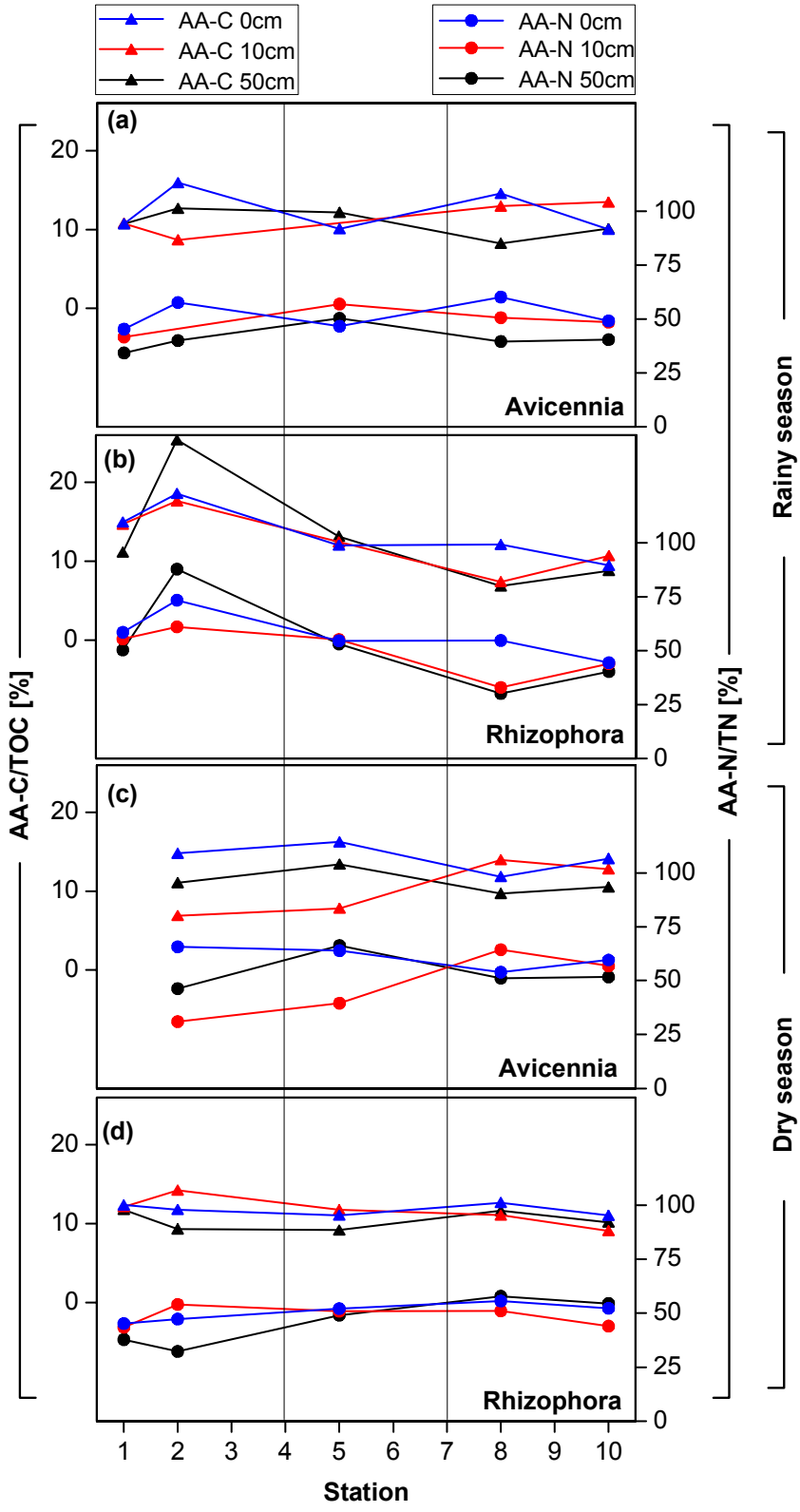


Figure 4.2.21: Proportion of amino acid C and N from TOC and TN, respectively, in sediment samples near *A. germinans* (a, c) and *R. mangle* trees (b, d) for transect 1 during rainy and dry season. Vertical lines indicate the *Avicennia*- (left) and *Rhizophora*-dominated areas (right) and the transition zone (middle).

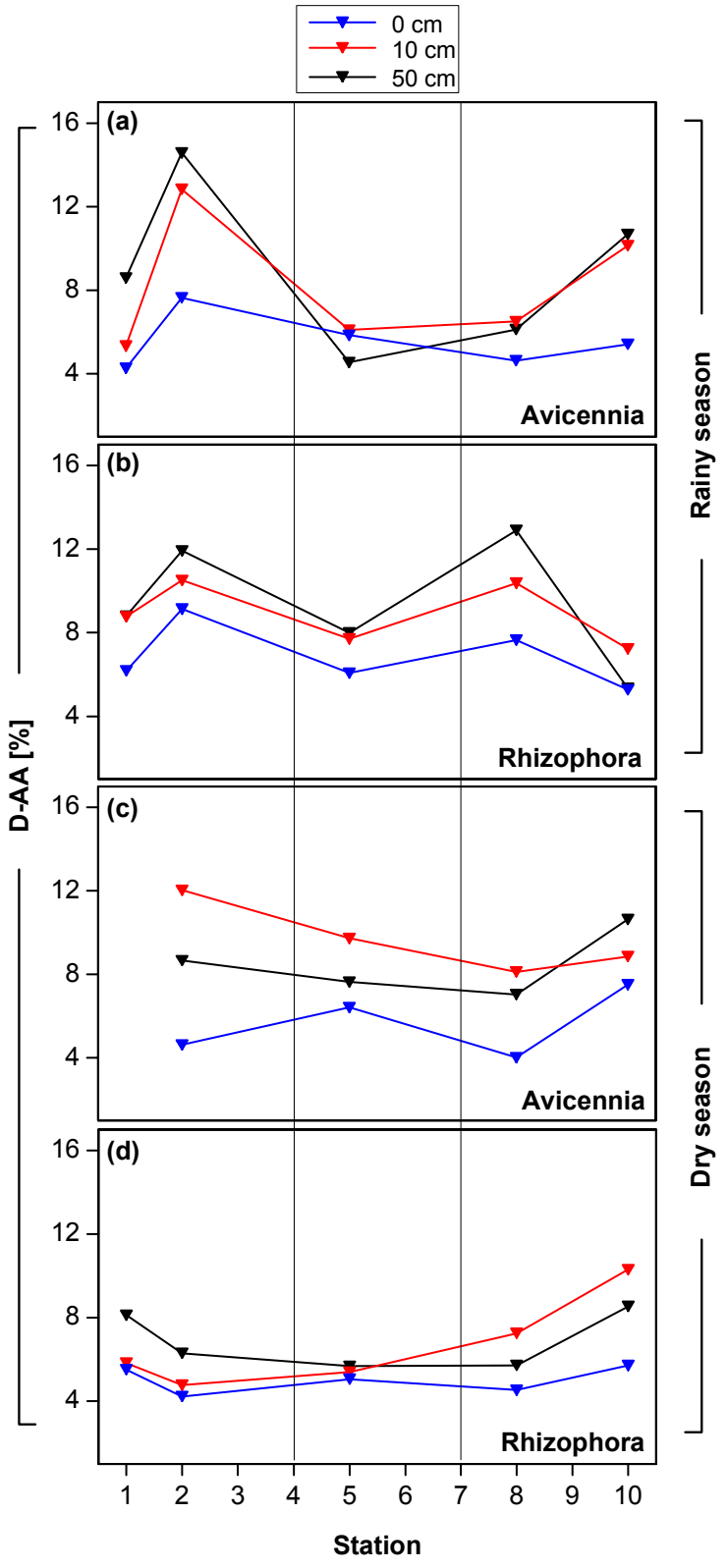


Figure 4.2.22: Proportion of D-amino acids from THAA, in sediment samples near *A. germinans* (a, c) and *R. mangle* trees (b, d) for transect 1 during rainy and dry season. Vertical lines indicate the *Avicennia*- (left) and *Rhizophora*-dominated areas (right) and the transition zone (middle).

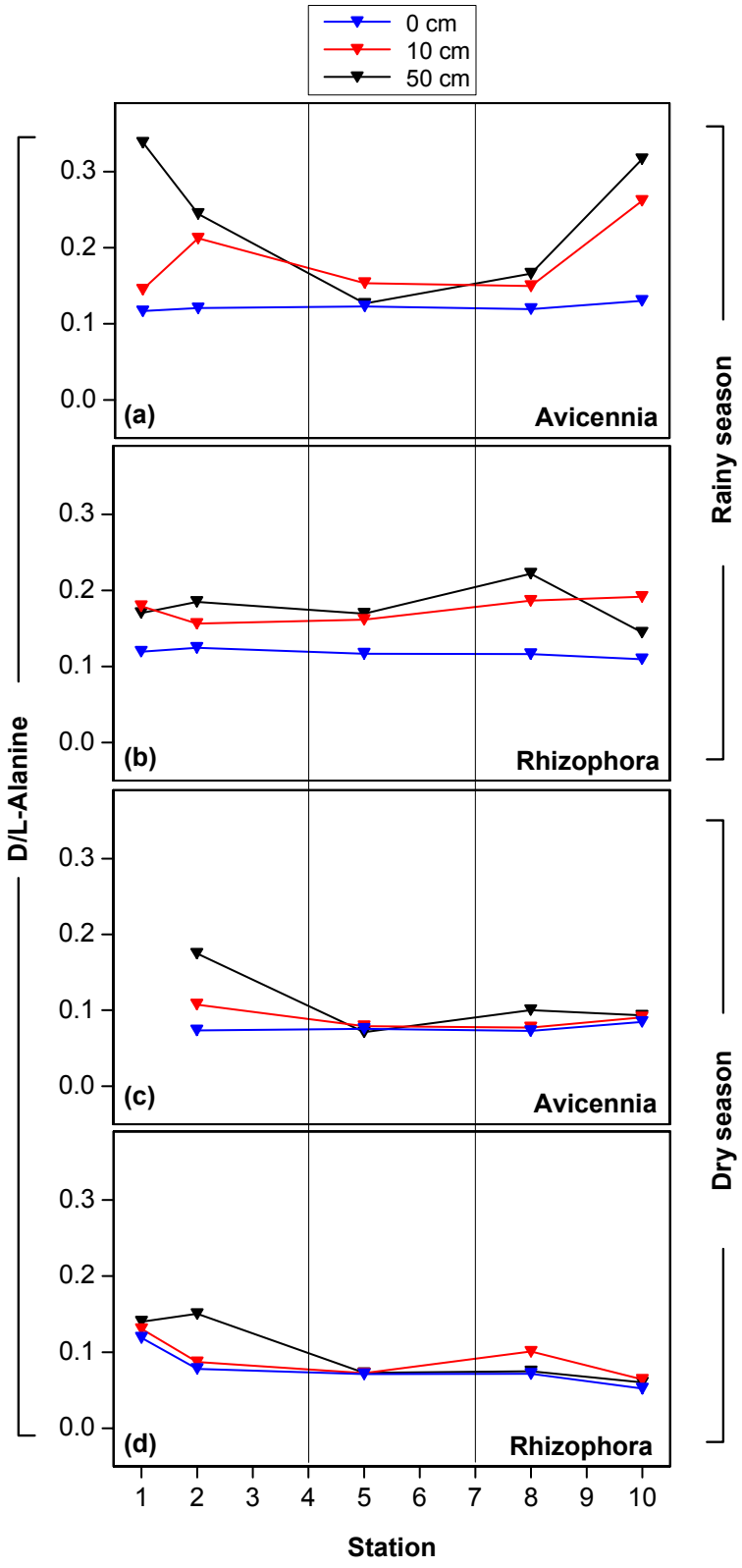


Figure 4.2.23: D/L-alanine ratio in sediment samples near *A. germinans* (a, c) and *R. mangle* trees (b, d) for transect 1 during rainy and dry season. Vertical lines indicate the *Avicennia*- (left) and *Rhizophora*-dominated areas (right) and the transition zone (middle).

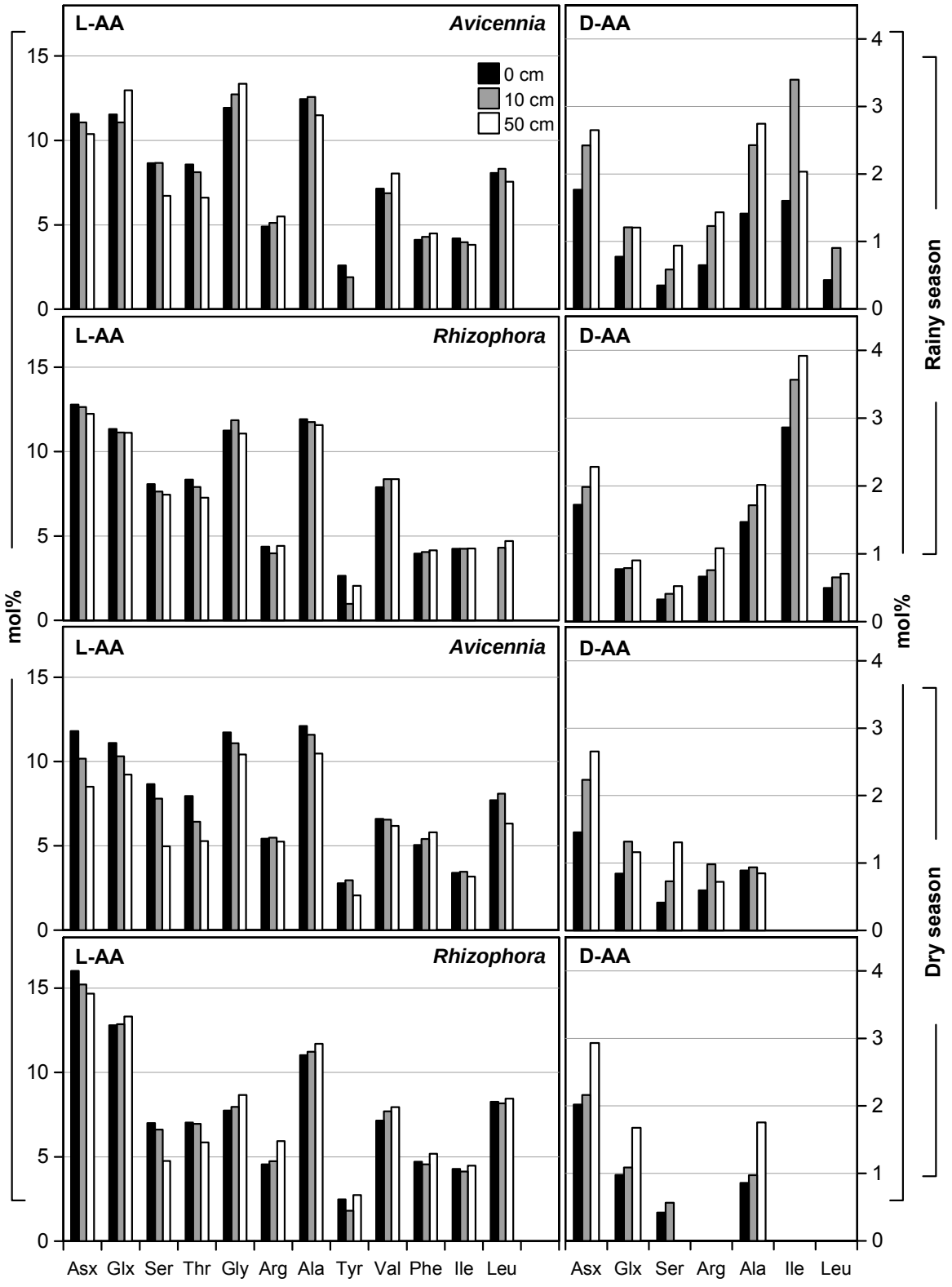


Figure 4.2.24 Composition of individual L- and D-amino acids (in mol%) at station 1 of transect 1 for surface, 10 cm and 50 cm sediment layers near *A. germinans* (*Avicennia*) and *R. mangle* (*Rhizophora*) trees during the rainy and the dry season.

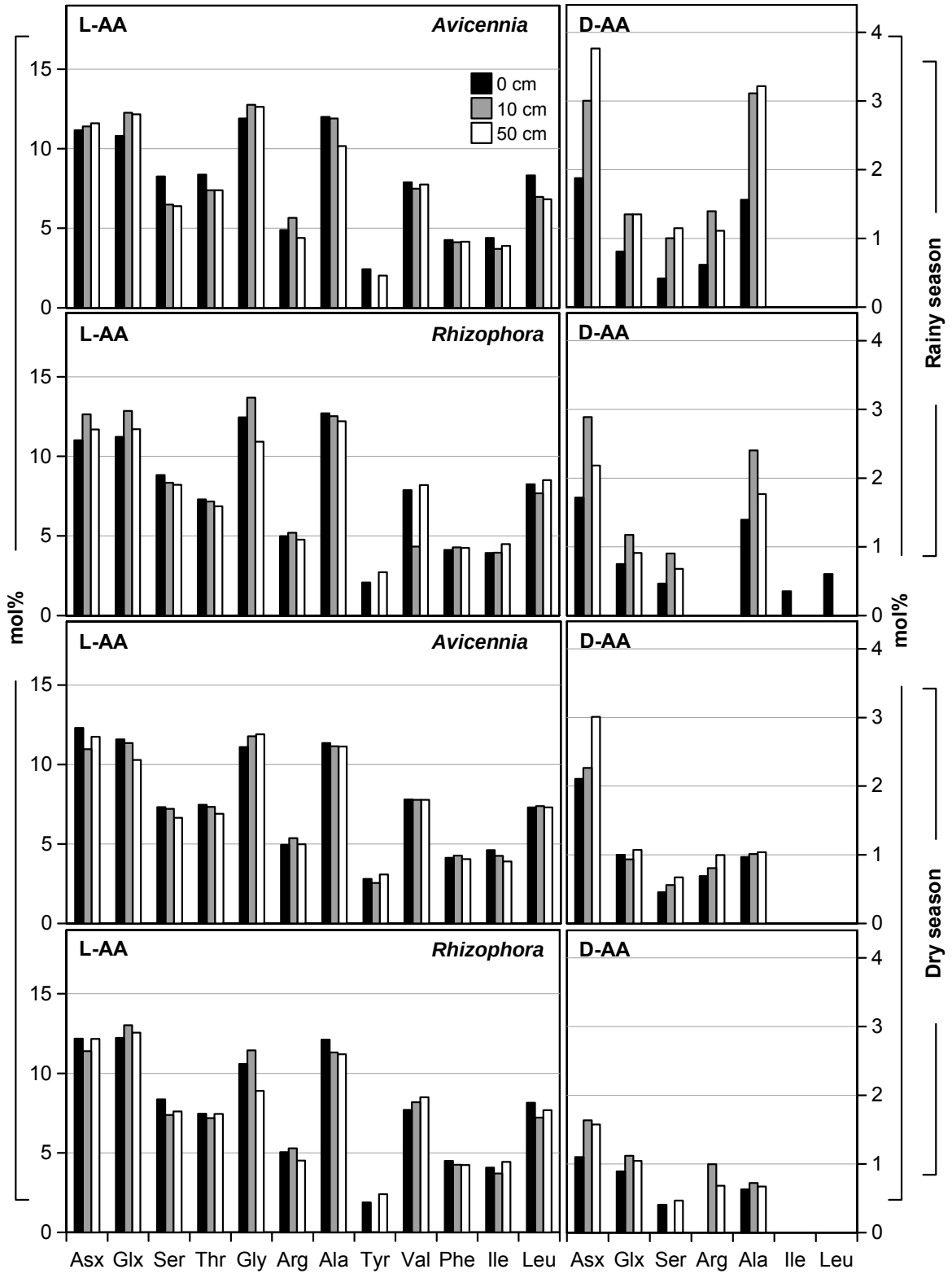


Figure 4.2.25: Composition of individual L- and D-amino acids (in mol%) at station 10 of transect 1 for surface, 10 cm and 50 cm sediment layers near *A. germinans* (*Avicennia*) and *R. mangle* (*Rhizophora*) trees during the rainy and the dry season.

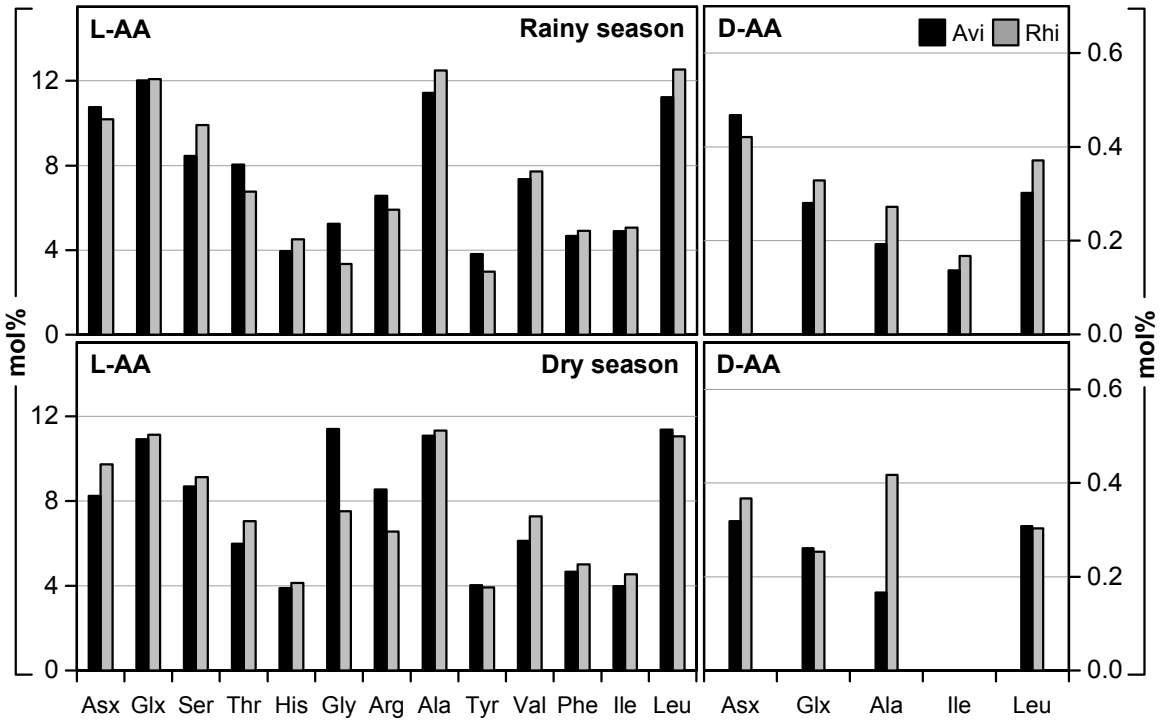


Figure 4.2.26: Composition of individual L- and D-amino acids (in mol%) at station 1 of transect 1 for leaves of *A. germinans* (Avi) and *R. mangle* (Rhi) during the rainy and the dry season.

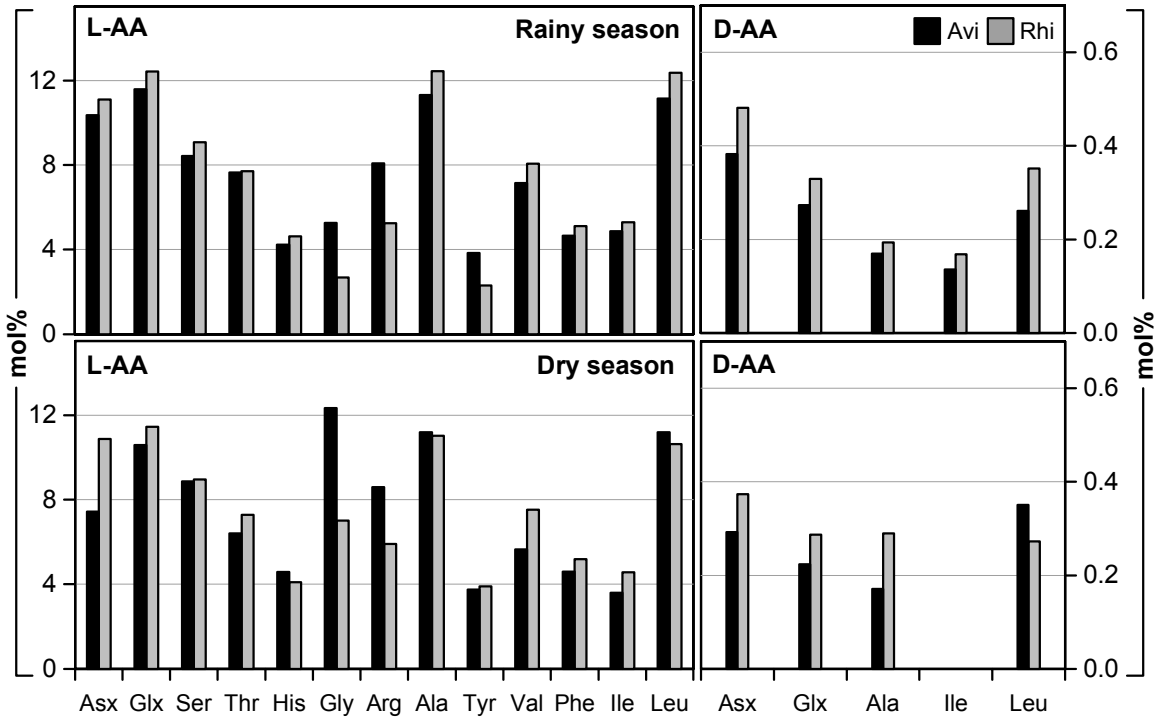


Figure 4.2.27: Composition of individual L- and D-amino acids (in mol%) at station 10 of transect 1 for leaves of *A. germinans* (Avi) and *R. mangle* (Rhi) during the rainy and the dry season.

4.2.3.3 Transect 2: Total hydrolysable amino acids in sediment material

The data of the amino acid analysis are presented in *Figure 4.2.28-35*, all stations were analysed at three different depths.

Total hydrolysable amino acids (THAA, presented in mg/g dw, corresponding values in mol are given in brackets) showed a similar trend as TOC, TN and tannin content during the rainy season (*Figure 4.2.28a*). The values for surface sediments were significantly higher ($p<0.05$, $n=7$) than values from both 10 cm and 50 cm samples and showed the pronounced downward step after station 4. Surface sediments contained 19.2 ± 3.5 mg THAA/g dw (181 ± 4 µmol/g dw) at stations 1-4, compared to 6.4 ± 3.9 mg THAA/g dw (52 ± 32 µmol/g dw) at stations 5-7. 10 cm substrate samples had an average of 3.8 ± 1.9 mg THAA/g dw (31 ± 16 µmol/g dw), whereas the deepest measured sediment layers contained only 1.3 ± 0.4 mg THAA/g dw (10 ± 4 µmol/g dw). The minimum value of 0.8 mg THAA/g dw (6.3 µmol/g dw) occurred at 50 cm depth, whereas the highest value was encountered at the surface of station 3.

The dry season data showed similar mean values (*Figure 4.2.28b*) and no significant difference to the rainy season was detected. The surface values however exhibited a completely different pattern compared to the rainy season surface data with two maxima at station 3 (17.4 mg THAA/g dw or 141 µmol/g dw) and 5 (15.5 mg THAA/g dw or 125 µmol/g dw).

Figure 4.2.29 shows the proportion of amino acid carbon (AA-C) and nitrogen (AA-N) of TOC and TN in weight%. During the rainy season the values for surface substrates were significantly higher ($p<0.005$, $n=7$) than the values for 10 cm or 50 cm sediments for both AA-C/TOC and AA-N/TN. Mean values ranged from 13.5 ± 1.0 % AA-C/TOC and 58.3 ± 6.5 % AA-N/TN at the surface to 11.5 ± 2.4 % AA-C/TOC and 35.7 ± 3.0 % AA-N/TN at 50 cm depth. At station 6 both AA-C/TOC and AA-N/TN values fell to a minimum of 4.6 % and 20.7 % respectively.

Values for samples from the dry season were less clearly stratified, but showed a significant difference ($p<0.05$, $n=7$) between the substrates at 50 cm (10.0 ± 3.0 % and 47.7 ± 8.3 % for AA-C/TOC and AA-N/TN respectively) and the samples of surface or 10 cm sediments (11.8 ± 2.3/12.0 ± 3.2 % AA-C/TOC and 52.4 ± 8.7/49.5 ± 2.1 % AA-N/TN for surface/10cm substrates). Values did not differ significantly between seasons for any depths.

Percentage of D-amino acids (D-AA) of THAA (*Figure 4.2.30*) was lowest at the surface and highest at 50 cm depth during both seasons. Statistically the difference was significant between 50 cm substrates and both surface and 10 cm sediments ($p<0.005$, $n=7$) for the rainy season and between 50 cm and surface sediments only for the dry season ($p<0.01$, $n=7$). Mean values for the rainy season were 5.6 ± 2.2 %, 8.0 ± 2.3 % and 13.4 ± 3.5 % for surface, 10 cm and 50 cm respectively. The proportion of D-AA of THAA during the dry season did not differ significantly from these values (6.0 ± 1.4 %, 11.1 ± 4.9 % and 16.1 ± 8.2 % for surface, 10 cm and 50 cm respectively).

The ratio between D- and L-alanine showed conspicuous peaks for the deep sediment layer (50 cm) for both seasons (*Figure 4.2.31*). During the rainy season two peaks could be observed at stations 1 (0.79) and 3 (0.54), whereas stations 2 and 4 showed very low values (0.20 and 0.22). This pattern is repeated in a less conspicuous manner in the 10 cm substrate samples. During the dry season values increased towards a peak at station 4 (0.74) and decreased again towards the end of the transect. In this case the pattern is not repeated in the 10 cm substrates, which showed a "zig-zag" pattern, oscillating between higher values at stations 1, 3, 5 and 7 (values between 12.3 and 18.9 %) and lower values at stations 2, 4 and 6 (between 6.0 and 6.8 %).

Generally the composition of L-amino acids (in mol%) was relatively invariant between seasons and stations especially for surface and 10 cm sediments where L-asx, L-glx, gly and L-ala were dominant (*Figures 4.2.32* and *4.2.33*). The L-amino acid composition showed more variation at 50 cm depth where L-glx, gly and L-arg were the predominant monomers during the rainy season at station 1, whereas during the dry season the proportion of all three amino acids diminished leaving only L-glx and L-leu dominating the L-amino acid pool. At station 7 the amino acid composition at 50 cm was more consistent resembling the proportions at the surface and 10 cm with L-asx, L-glx, gly and L-ala dominating in both seasons. The D-amino acids showed a very variable composition depending on station and depth. At station 1 D-asx, D-glx and especially D-ala were abundant in the 50 cm sediment layer during the rainy season. During the dry season however D-asx disappeared at 50 cm, D-glx was nearly unchanged and D-ala decreased significantly compared to the rainy season. At station 7 the D-amino acid composition also differs between seasons. During the rainy season D-asx and D-ala predominated especially at 10 and 50 cm depth. During the dry season D-leu which was not measured at all during the rainy season accounts for nearly 5 mol% of total amino acids in 10 and 50 cm substrate.

Absolute amounts of individual amino acids in mg/g dw of individual amino acids is shown in Appendix A7 and A8.

4.2.3.4 Transect 2: Total hydrolysable amino acids in plant material

Total hydrolysable amino acids (THAA) were analysed in 1-5 samples (depending on occurrence of plant species) during both seasons and for all species. As no significant trend or difference could be observed between stations, mean values for THAA, proportion of amino acid carbon and nitrogen of TOC and TN respectively (AA-C/TOC and AA-N/TN, values given in weight% of dry weight), percentage of D-amino acids (%D-AA) and D/L-alanine ratios, are presented in *Table 4.2.3*. For *B. maritima* which only occurred at one station, only one sample was analysed, hence values were not incorporated into the statistical analysis.

A significant difference ($p<0.005$) in total hydrolysable amino acids was observed between plant species, with highest amounts for *A. germinans* (63.5 ± 9.2 mg/g dw (499 ± 85 µmol/g dw) for both seasons, n=9), followed by *S. portulacastrum* with 42.6 ± 4.9 mg/g dw (333 ± 39 µmol/g dw) (mean of both seasons, n=6) and lowest values for *S. virginicus* (27.1 ± 4.5 mg/g dw (213 ± 53 µmol/g dw) for both seasons, n=4). *B. maritima* ranged with a concentration of 64.0 mg/g dw THAA (496 µmol/g dw) within the values for *A. germinans* (data for the rainy season sample of *B. maritima* are missing). For all species there was no significant difference between rainy and dry season.

AA-C/TOC was similar in *A. germinans* (6.7 ± 1.0 %) and *S. portulacastrum* leaves (6.1 ± 0.4 %), but was significantly lower ($p<0.05$) in *S. virginicus* (2.9 ± 0.5 %, n=4), values are means of both seasons. Values for AA-N/TN varied substantially during rainy season with *S. portulacastrum* concentrating ~77 % of TN in amino acid nitrogen, whereas both *A. germinans* and *S. virginicus* showed AA-N/TN values of ~40 and ~49 %, respectively. For both herbaceous species values diminished during the dry season, whereas *A. germinans* showed a slight increase from rainy to dry season samples.

The percentage of D-amino acids (D-AA) was constant in all species and seasons with the exception of *A. germinans* leaves during the dry season. Here the value of 1.3 ± 0.1 % (n=4) was significantly lower ($p<0.05$) than for all other species or for *A. germinans* leaves during the rainy season.

Ratios of D/L-alanine differed during both seasons. In samples from the rainy season the value for *S. portulacastrum* leaves (0.8 ± 0.02, n=3) was twice as high as values in both *A. germinans* and *S. virginicus*. During dry season values increased significantly ($p<0.05$) from 0.01 ± 0.002 (n=4) in *A. germinans* leaves to 0.03 ± 0.004 (n=3) in *S. portulacastrum* and 0.07 ± 0.01 (n=3) in *S. virginicus*.

The roots of the herbaceous species generally showed lower values than the corresponding leaves, especially for THAA, AA-C/TOC and AA-N/TN.

Table 4.2.3: Mean values ± SD for total hydrolysable amino acids (THAA), proportion of amino acid carbon and nitrogen of TOC and TN respectively (AA-C and AA-N, values given in weight% of dry weight), percentage of D-amino acids (%D-AA, values given in weight%) and D/L-alanine ratios for leaves of the 4 plant species of transect 2 (values for roots of herbaceous species in brackets), *only one sample was measured, n.m.: not measured

Sample	THAA [mg/g dw]	AA-C/TOC [%]	AA-N/TN [%]	D-AA [%]	D/L-alanine
A. germinans					
rainy season	63.2 ± 11.3	6.8 ± 1.1	39.7 ± 6.4	2.0 ± 0.5	0.04 ± 0.01
dry season	64.2 ± 6.1	6.6 ± 0.8	45.3 ± 2.8	1.3 ± 0.1	0.01 ± 0.002
S. portulacastrum					
rainy season	46.5 ± 1.0 (29.6*)	6.0 ± 0.4 (3.6*)	76.9 ± 6.9 (71.5*)	1.7 ± 0.1 (1.5*)	0.08 ± 0.02 (0.06*)
dry season	40.0 ± 4.7 (29.6*)	6.2 ± 0.5 (3.6*)	46.9 ± 2.9 (39.8)	1.9 ± 0.1 (2.7*)	0.03 ± 0.004 (0.07*)
S. virginicus					
rainy season	30.5 ± 2.6 (20.5*)	3.3 ± 0.2 (2.2*)	48.6 ± 4.3 (42.3*)	1.7 ± 0.1 (2.0*)	0.04 ± 0.003 (0.05*)
dry season	23.8 ± 2.8 (27.7*)	2.5 ± 0.3 (3.1*)	38.8 ± 1.0 (38.0*)	1.9 ± 0.1 (2.2*)	0.07 ± 0.01 (0.09*)
B. maritima					
rainy season	n.m.	n.m.	n.m.	n.m.	n.m.
dry season	64.0* (41.9*)	13.9* (5.7*)	52.0* (51.8*)	1.2* (1.4*)	0.02* (0.03*)

Distribution of L- and D-amino acid enantiomers in leaves of *A. germinans* as well as leaves and roots of *S. portulacastrum* and *S. virginicus* for station 1 is shown in *Figure 4.2.34* (in mol%). As *S. virginicus* did not grow at station 1, the data from station 3 was used for this species. Data for *B. maritima* is not shown. For station 7 only data for *A. germinans* is shown as the herbaceous species were not found in this part of the transect

(*Figure 4.2.35*). The composition of L-amino acid monomers was generally dominated by L-asx, L-glx, L-ser, gly and L-ala for all species, although gly was reduced at station 1 during the rainy season. Concerning the D-enantiomers D-asx and D-ala prevailed especially in plant material of the herbaceous species. In *A. germinans* the proportion of D-amino acids generally decreased during the dry season at both stations.

Absolute amounts of individual amino acids in mg/g dw of individual amino acids is shown in Appendix A9 and A10.

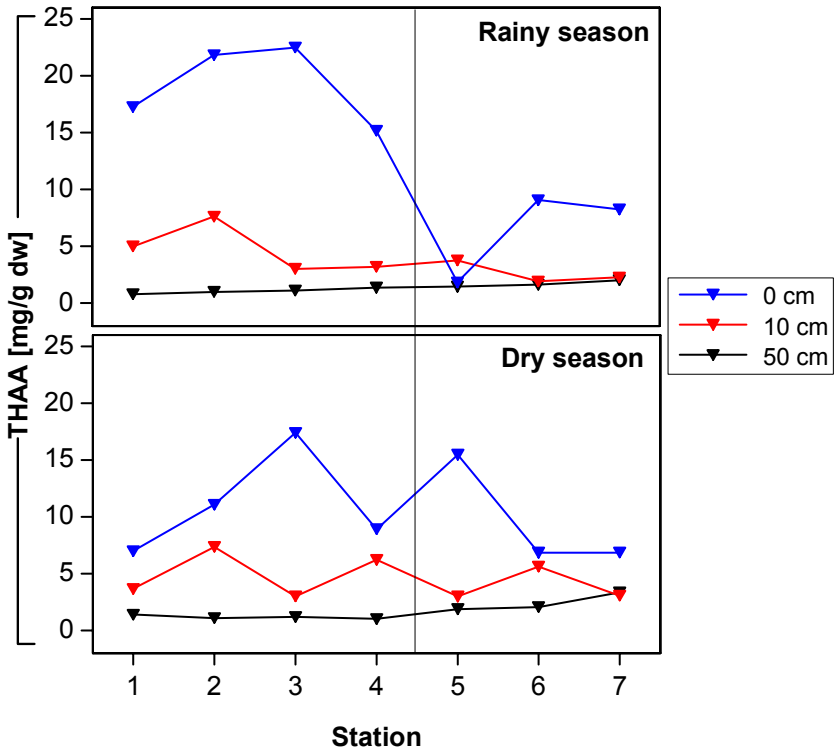


Figure 4.3.28: Concentration of total hydrolysable amino acids (THAA) in sediment samples of transect 2 during rainy and dry season. Vertical line indicates area with (left) and without (right) herbaceous vegetation.

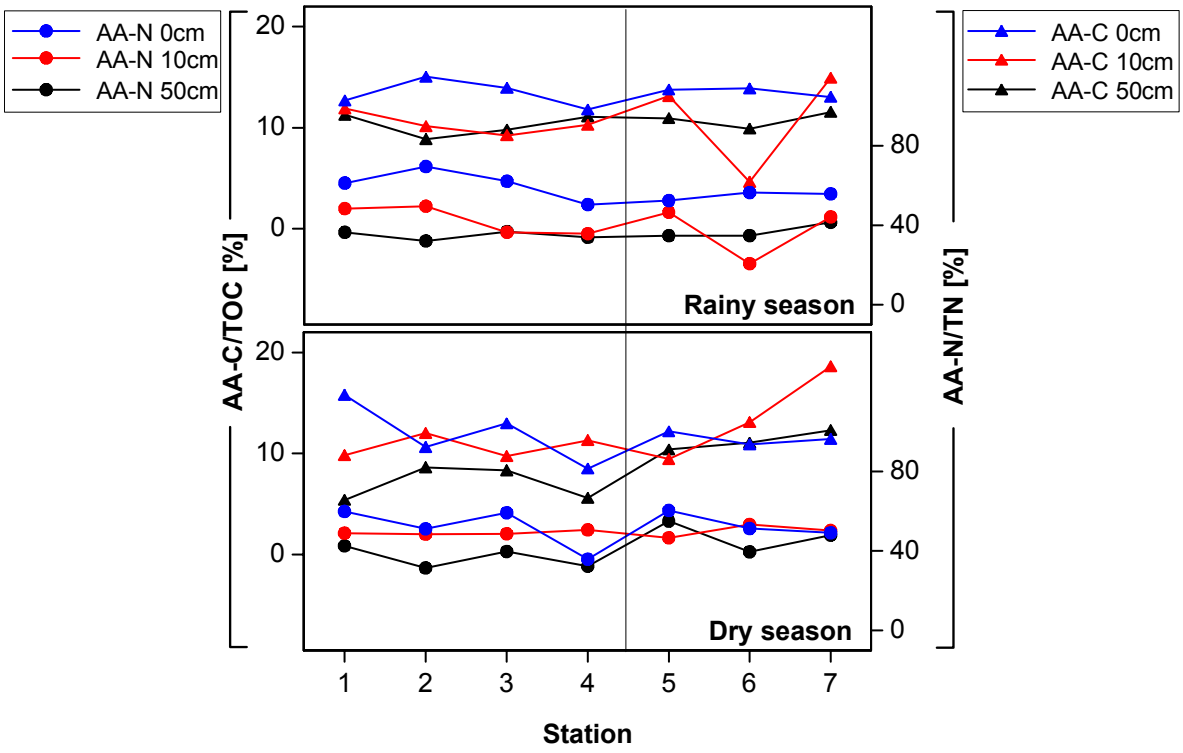


Figure 4.2.29: Proportion of amino acid C and N from TOC and TN, respectively, in sediment samples of transect 2 during rainy and dry season. Vertical line indicates area with (left) and without (right) herbaceous vegetation.

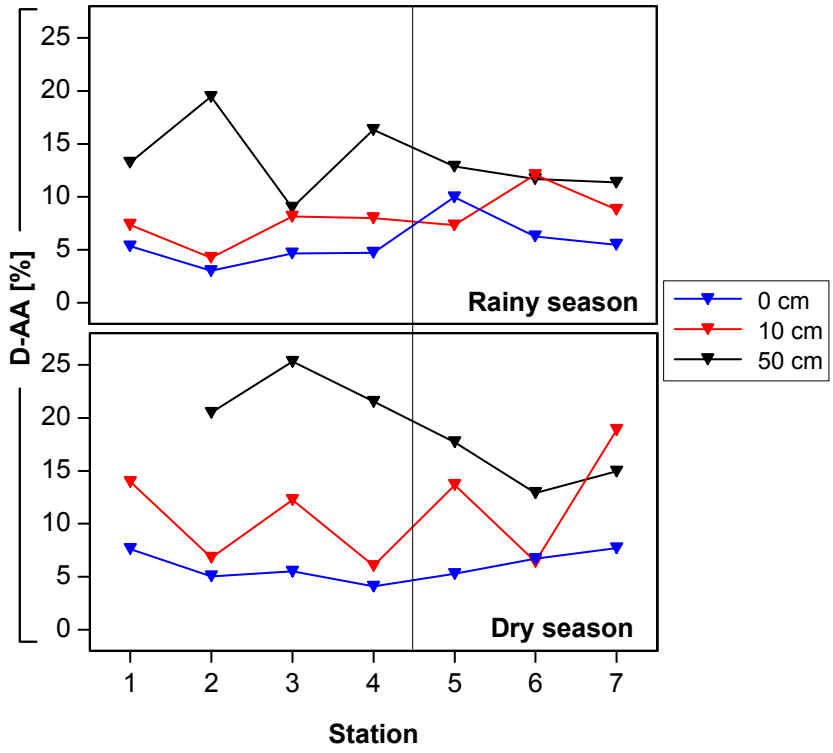


Figure 4.2.30: Proportion of D-amino acids from THAA, in sediment samples of transect 2 during rainy and dry season. Vertical line indicates area with (left) and without (right) herbaceous vegetation.

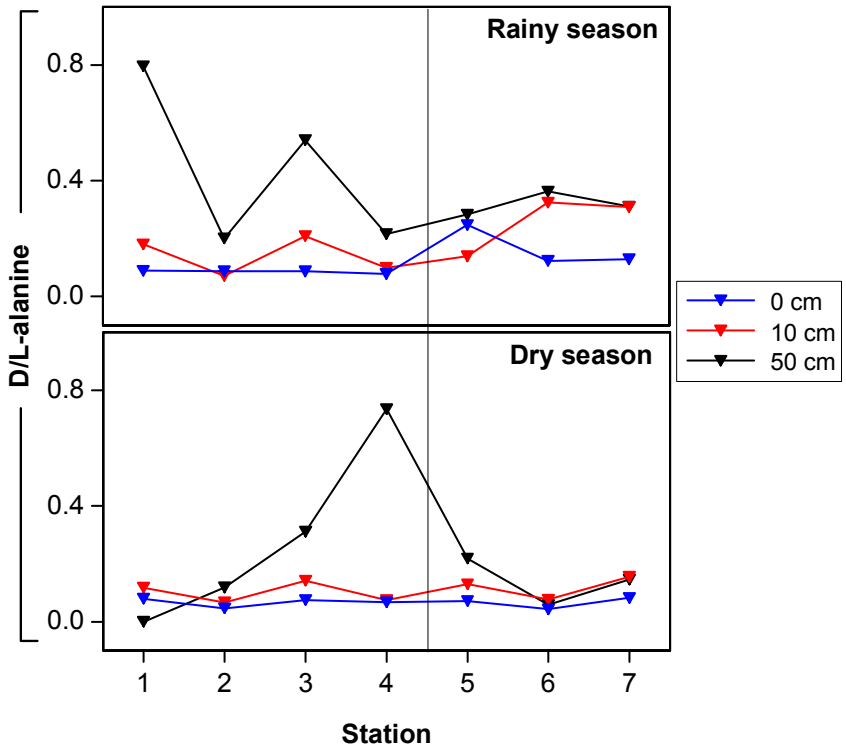


Figure 4.2.31: D/L-alanine ratio in sediment samples of transect 2 during rainy and dry season. Vertical line indicates area with (left) and without (right) herbaceous vegetation.

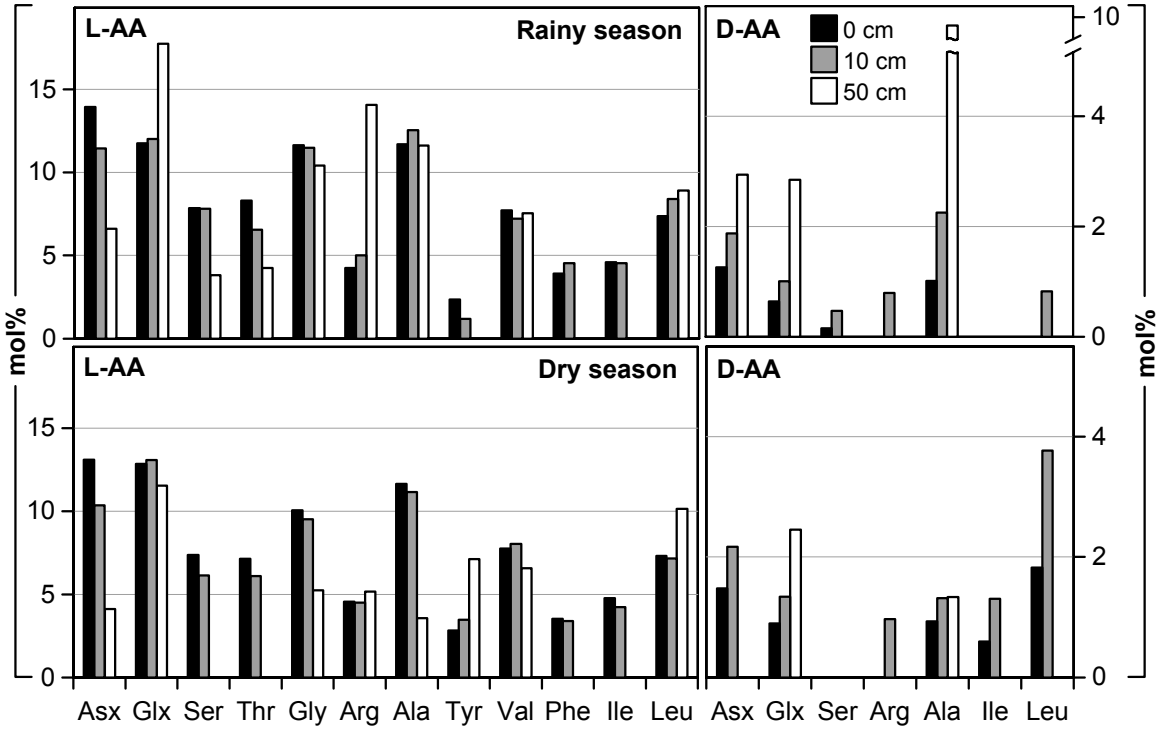


Figure 4.2.32: Composition of individual L- and D-amino acids (in mol%) at station 1 of transect 2 for surface, 10 cm and 50 cm sediment layers during the rainy and the dry season.

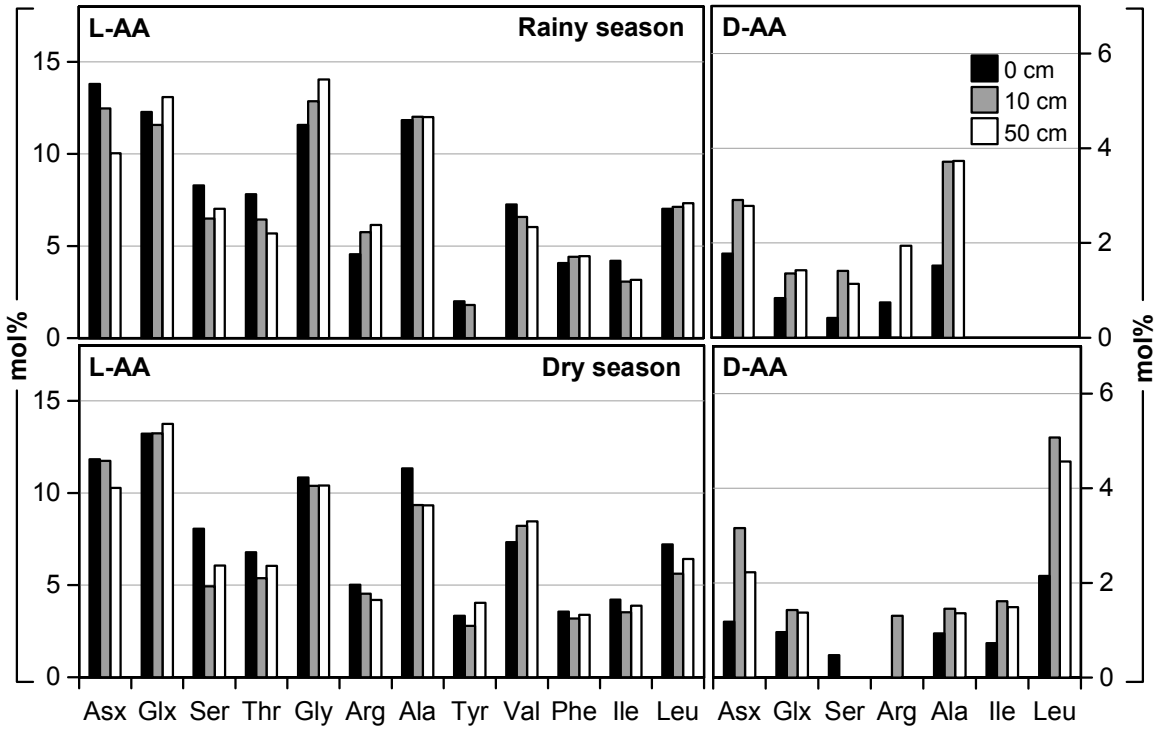


Figure 4.2.33: Composition of individual L- and D-amino acids (in mol%) at station 7 of transect 2 for surface, 10 cm and 50 cm sediment layers during the rainy and the dry season.

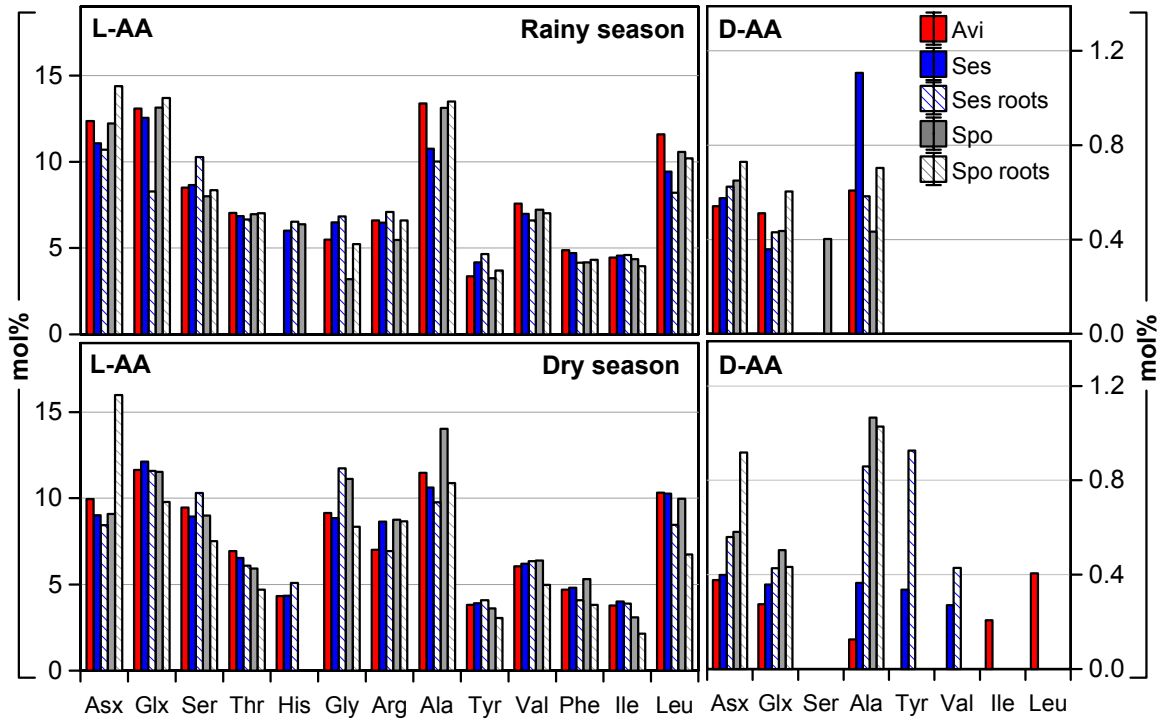


Figure 4.2.34: Composition of individual L- and D-amino acids (in mol%) at station 1 of transect 2 in leaf samples of *A. germinans* (Avi) and in leaf and root samples of *S. portulacastrum* (Ses), *S. virginicus* (Spo) and *Batis maritima* (Bat) during rainy and dry season.

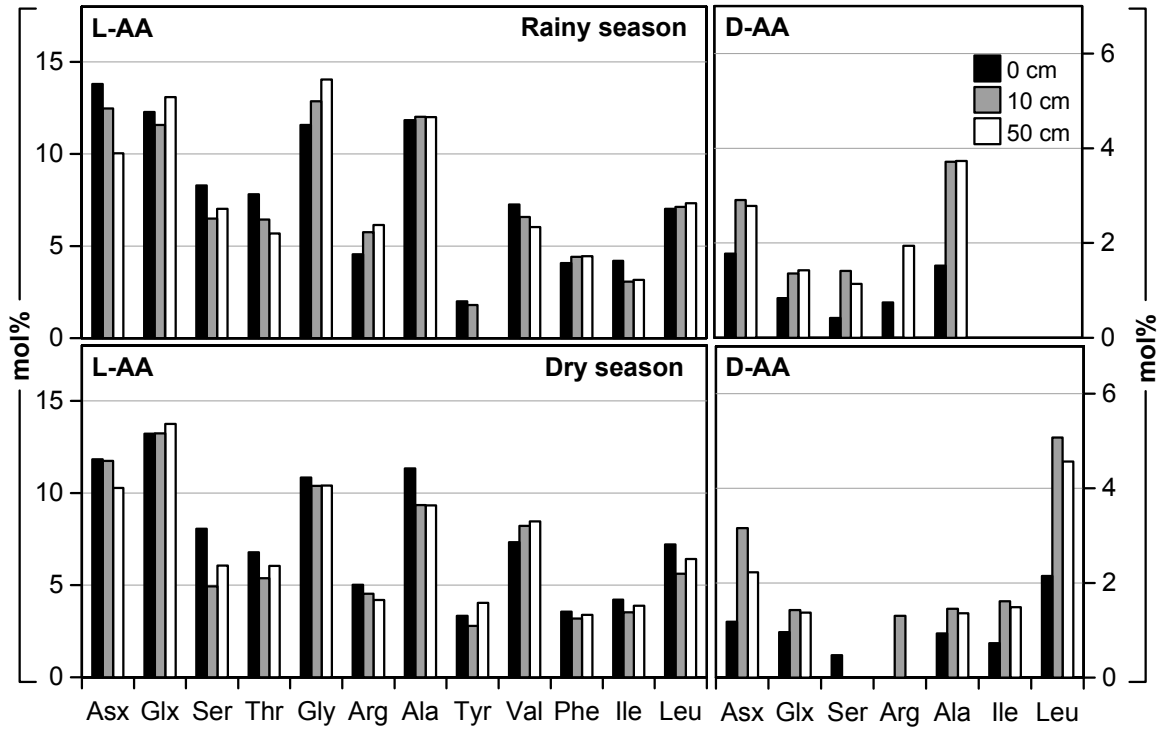


Figure 4.2.35: Composition of individual L- and D-amino acids (in mol%) at station 7 of transect 2 in leaf samples of *A. germinans* during rainy and dry season.

4.2.4 Further analyses of *Sesuvium portulacastrum*, its rhizosphere and adjacent sediment

To investigate possible impacts or influences of *S. portulacastrum* on the surrounding sediment, further analyses were conducted.

4.2.4.1 Atmospheric nitrogen fixation

The activity of N_2-fixing bacteria in surface sediments of transect 2 was analysed with the Acetylene Reduction Assay for substrate not directly influenced by plants (i.e. not covered by any of the herbaceous species or algal mats and in the gaps of *A. germinans* roots), for epiphytes growing on the *A. germinans* pneumatophores, and for *S. portulacastrum* roots (not washed to include the rhizosphere in immediate vicinity of the roots). Results (in µg Ethylene/mg dw) are shown in *Figure 4.2.36* for an experimental design over 48 hrs.

Fixation of atmospheric nitrogen was greatest in roots/rhizosphere sample of *S. portulacastrum*, followed by the non-defined epiphytes. Surface sediment samples showed no measurable N_2-fixing ability.

4.2.4.2 Total bacterial counts

Total bacterial cell numbers were counted in *Avicennia*-influenced surface sediments (surrounded by *A. germinans* pneumatophores), *Sesuvium*-influenced surface substrate (within 10 cm of *S. portulacastrum* plants) and in the rhizosphere of *S. portulacastrum* (substrate in the immediate vicinity of the roots). Results are presented in *Figure 4.2.37*.

Numbers increased significantly ($p<0.001$, $n=10$) from very low numbers in *Avicennia*-influenced sediments ($5.4 \pm 1.5 \times 10^6$ cells/mg dw), followed by $22.4 \pm 4.6 \times 10^6$ cells/mg dw in *Sesuvium*-influenced surface substrate to highest numbers in the rhizosphere surrounding *S. portulacastrum* roots ($105.5 \pm 16.9 \times 10^6$ cells/mg dw).

4.2.4.3 Scanning Electron Microscopy

Scanning Electron Microscope (SEM) photographs of the roots of *S. portulacastrum* gave evidence of a mycorrhiza, a mutualistic symbiosis between a plant and a fungus usually localised in the roots or root-like structures (*Figure 4.2.38*). 60 % of the analysed roots showed this phenomenon pictured below.

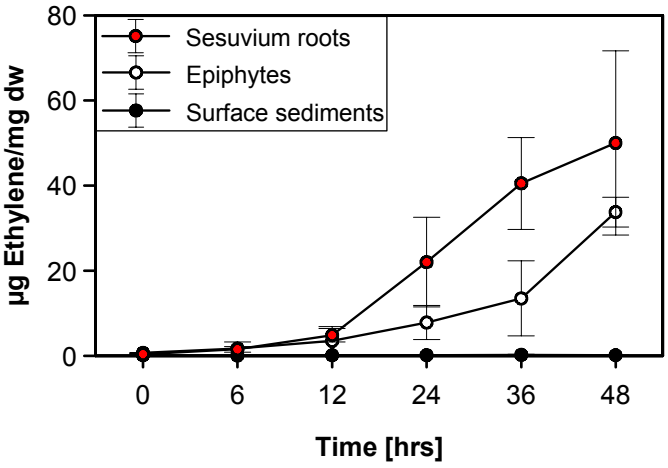


Figure 4.2.36: Fixation of atmospheric nitrogen (measured as reduction of acetylene to ethylene) by roots and rhizosphere of *Sesuvium portulacastrum*, epiphytes growing on pneumatophores of *A. germinans*, and surface sediments.

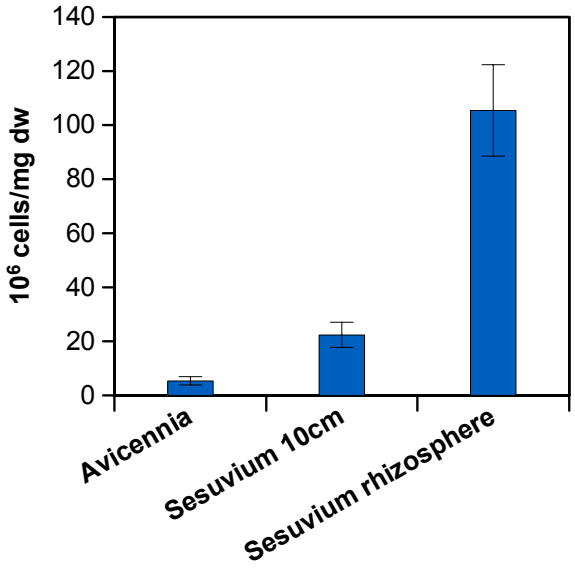


Figure 4.2.37: Total bacterial cell numbers in *Avicennia*-influenced surface sediments, *Sesuvium*-influenced surface sediments (within 10 cm of the plants) and rhizosphere of *Sesuvium portulacastrum*.

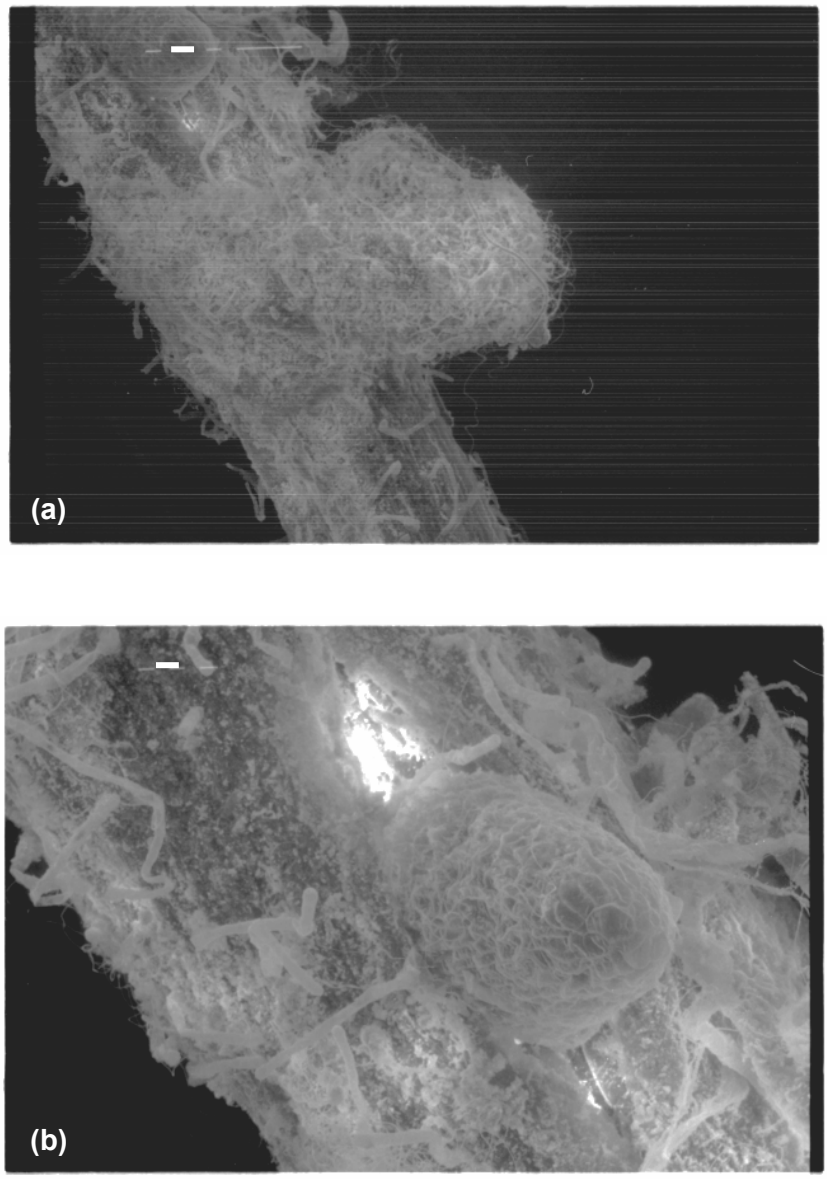


Figure 4.2.38: SEM photographs of a root tip of *S. portulacastrum* (a) with and (b) without mycorrhiza, white bar = 100µm.

4.3 Decomposition experiments

4.3.1 Field experiment

Most net bags with leaves of *A. germinans* were lost during the experiment, due to strong currents which broke the plastic rings by which the bags were attached to the wooden poles. Thus only data for *R. mangle* will be presented below (*Figures 4.3.1* and *4.3.2*). Mean values for leaves on day 0, i.e. for time point of leaf collection, before the experiment began and for day 42, i.e. after 6 weeks of incubation are depicted for all measured parameters. Values for the time series, precisely the values for samples collected weekly are shown in detail in Appendix A13-15.

4.3.1.1 Total C and N

TOC content at the beginning of the experiment was highest in brown leaves (48.5 ± 0.1 %, n=3) and lowest in black leaves (45.3 ± 0.05 %, n=3). During the 6 weeks of incubation TOC increased in all three groups, with the highest change occurring in yellow leaves where TOC was raised to 54.3 ± 0.3 % (n=3). TN was highest in black leaves in week 0 (1.2 ± 0.02 %, n=3) and nearly equal in yellow and brown leaves (0.5 ± 0.04 and 0.6 ± 0.02 % respectively, n=3). TN content increased in all three leaf stages, being doubled in yellow and brown leaves. Concomitant with the changes in TN, C/N values decreased from yellow to black leaves and during incubation.

Yellow leaves attached to tree differed in their TOC and TN content from the yellow leaves that were collected from the ground for the experiments. Attached yellow leaves contained 0.65 % TN and 42.7 % TOC (C/N was 65.7). Data is not shown in the graphs.

4.3.1.2 Isotopic composition

Black leaves were slightly depleted in ^{13}C at the beginning of the experiment (-28.4 ‰) in comparison with both yellow and brown leaves. After incubation values had hanged towards more negative values in all three leaf colours. $\delta^{15}N$ values were highest in yellow leaves (5.6 ‰) and lowest in black leaves (4.4 ‰) and decreased by ~2 ‰ in yellow and ~1 ‰ in brown and black leaves during decomposition.

Yellow leaves still attached to the tree were depleted in ^{13}C (-30.7 ‰) and ^{15}N (2.6 ‰) in comparison to green leaves.

4.3.1.3 Tannins

Tannin concentrations were highest in yellow leaves (145.4 mg TAE/g dw) and lowest in black leaves (56.8 mg TAE/g dw) at the start of the experiment. After 6 weeks of incubation tannin level in all three leaf groups had declined substantially to 24.3 mg TAE/g dw, 38.6 mg TAE/g dw and 30.6 mg TAE/g dw for yellow, brown and black leaves respectively.

The yellow leaves attached to the tree had higher concentrations of tannins than green leaves (110.4 mg TAE/g dw as opposed to 98.7 ± 17.0 mg TAE/g dw in green *R. mangle* leaves during the dry season).

4.3.1.4 Total hydrolysable amino acids (THAA)

Concentrations of THAA were lowest in yellow leaves (14.7 mg/g dw / 119.6 µmol/g dw) and highest in black leaves (53.4 mg/g dw / 428.4 µmol/g dw) when the experiment was started. During the incubation THAA increased in all three groups with the highest raise in yellow leaves to 55.1 mg/g dw (409.7 µmol/g dw). Percentage of amino acid carbon of total carbon (AA-C/TOC) followed the same pattern. AA-N/TN however displayed a different trend with highest values in the brown leaves (67.2 %) and lowest percentage in the yellow leaves (40.0 %) at the beginning of the experiment. After 42 days, values in yellow leaves had risen by a factor of ~1.6 to 66.0 %, whereas AA-N/TN in brown and black leaves had declined substantially to 47.8 % and 50.7 % respectively.

Proportion of D-amino acids of THAA was nearly unchanged after the experiment although some variation had occurred throughout the weeks (see Appendix). Values were highest for yellow and brown leaves (1.6 % for both on day 0) and lowest in black leaves (1.3 % in week 0). D/L-alanine ratios were conspicuously higher in yellow leaves (0.1) than in brown and black leaves as well as green leaves on the tree (0.04 ± 0.01). During incubation the value nearly halved for yellow leaves whereas ratios in brown leaves did not change at all and the value for black leaves decreased only by 0.01.

The composition of amino acids (*Figure 4.3.3*, in mol%) showed the high D-alanine percentage which caused the high D/L-alanine ratios in yellow leaves on day 0. The L-amino acids showed a similar distribution as has been reported for green leaves before with L-asx, L-glx, L-ser, gly, L-ala and L-leu being the dominant monomers. After incubation only one conspicuous variation had occurred, namely for gly in yellow leaves which rose from 12.3 mol% to 17.0 mol%.

THAA was not measured in yellow leaves from the trees.

4.3.2 Laboratory experiment

Yellow leaves from *R. mangle* and *A. germinans* were collected from the ground in a mixed mangrove forest at low tide and subjected to 4 treatments in the laboratory. Results of the measurements of various parameters at the beginning of the experiment (day 0) and after 6 weeks of incubation (day 42) are shown in *Figures 4.3.4* and *4.3.5*. Values for the time series, precisely the values for samples collected weekly are shown in detail in Appendix X. No chemicals were added to the reference ('Reference'), mercuric chloride killed all microorganisms in the second treatment ('$HgCl_2$'), addition of the fungicide Benomyl created an environment with mainly bacteria as microbial decomposers ('Bacteria') and a cocktail of bactericides favoured the fungi in treatment 4 ('Fungi').

4.3.2.1 Total C and N

TOC content was 45.8 ± 0.2 % in yellow *R. mangle* leaves at the beginning of the experiment and had increased to 47.6 ± 1.7 % and 46.7 ± 0.8 % in the reference and fungicide treatment respectively after 42 days of incubation (n=4). The mercuric chloride and bactericide treatments showed slight downward trends, but neither the increase nor the decrease were statistically significant as standard deviations were high. Yellow *A. germinans* leaves had higher TOC content than yellow *R. mangle* leaves (47.2 ± 0.1 %, n=4), in contrast to green leaves, where *R. mangle* contained more total carbon than *A. germinans*. During decomposition a significant increase of TOC values was observed for all treatments except the mercuric chloride approach. TOC content decreased significantly in the mercuric chloride treatment.

TN showed very similar dynamics for both species. *A. germinans* generally had higher TN (0.6 ± 0.01 %) content than *R. mangle* (0.4 ± 0.002 %), confirming the observations in green leaves (see transect 1), but both showed significant increases of TN in the 'Reference' and the 'Bacteria' leaves, whereas TN in leaves of treatment 2 ('$HgCl_2$') decreased and values for the treatment with bactericides did not change significantly.

C/N values essentially mirrored TN dynamics, showing higher values for *R. mangle* on day 0 (106.7 ± 0.5 and 80.6 ± 0.9 for *R. mangle* and *A. germinans* respectively, n=4) and a significant decrease in reference and fungicide treatments after 6 weeks. C/N values for

leaves of the mercuric chloride treatment increased for both species, whereas values for the 'Fungi' leaves decreased in *R. mangle* and increased in *A. germinans*.

4.3.2.2 Isotopic composition

R. mangle leaves were depleted in ^{13}C (-27.7 ‰) compared to *A. germinans* tissues (-26.7 ‰). *Rhizophora* leaves of all treatments showed more negative $\delta^{13}C$ values, except for the reference group; however standard deviations between replicates of day 42 were high. In *Avicennia* leaves ^{13}C depletion occurred in all treatments, but showed great variations for the 'Bacteria' and the 'Fungi' groups.

$\delta^{15}N$ values were higher in *Avicennia* (7.0) than in *Rhizophora* leaves (5.0). In *R. mangle* values increased significantly in the mercuric chloride ('$HgCl_2$') and bactericide treatment ('Fungi'), no changes were observed for leaves of the two other treatments. In *A. germinans* only the control group decreased substantially after 6 weeks of incubation.

4.3.2.3 Tannins

As for green leaves, yellow *Rhizophora* leaves had much higher tannin content (149.2 ± 6.6 mg TAE/g dw) than *Avicennia* leaves (58.9 ± 4.3 mg TAE/g dw). The decomposition trends however were similar with a significant decrease of tannins in all treatments and the highest remaining tannin values after 6 weeks in the leaves of the mercuric chloride treatments. In *R. mangle* leaves tannin concentrations were significantly lower in the 'Fungi' and the 'Reference' group than in the 'Bacteria' group at the end of the decomposition experiment.

4.3.2.4 Total hydrolysable amino acids (THAA)

THAA dynamics were similar for both species. The values of yellow leaves on day 0 were 19.1 mg/g dw (150.4 µmol/g dw) and 22.1 mg/g dw (174.6 µmol/g dw) for *R. mangle* and *A. germinans* respectively. After incubation THAA content nearly doubled in leaves of both species in the reference and fungicide treatment ('Bacteria'). The two remaining treatments did not show any effect on THAA concentrations in *R. mangle* leaves, whereas for *A. germinans* values in leaves treated with mercuric chloride decreased by 40 %.

AA-C/TOC essentially followed the trend given by THAA, whereas AA-N/TN showed different dynamics. AA-N/TN was higher in *R. mangle* leaves (58.2 %), where it increased substantially in the '$HgCl_2$' and the 'Bacteria' group. Values for the reference leaves

however decreased. Standard deviations were high in all groups. *A. germinans* leaves had lower values at the beginning of the experiment (50.2 %) and values increased within the 42 days of incubation in all but the 'Fungi' group.

D-amino acid content and behaviour also differed between species. Percentage of D-amino acids in yellow leaves of *R. mangle* was 1.7 % in week 0. After 6 weeks values had risen to up to three times that value in all treatments. The highest increase was found in the 'Fungi' leaves. *A. germinans* leaves had slightly higher values at the beginning of the experiment (2.2 %), but showed hardly any variation after 6 weeks. D/L-alanine ratios were lower in *R. mangle* (0.03) and did not show differing values after incubation, although variations during the experimental time did occur (see time series data). Leaves of *A. germinans* had higher D/L-alanine ratios on day 0 (0.04), and exhibited a decrease in leaves of all 4 treatments.

The composition of the monomers (in mol%) is shown for one sample for each *Rhizophora* and *Avicennia* on day 0 and 42 (*Figure 4.3.6*). In *R. mangle* leaves the L-amino acids showed a similar distribution as has been reported before (see section 4.1 and 4.3.1) with L-asx, L-glx, L-ser, gly, L-ala and L-leu being the dominant monomers. However, in comparison to yellow leaves that were used for the field experiment gly was reduced and L-leu was increased on day 0 of the laboratory experiment. Only three D-amino acids were detected (D-asx, D-glx and D-ala). After 6 weeks of incubation L-leu had decreased from the initial 17 mol% to ~10 mol% in all treatments. L-glx and L-ala decreased in all treatments, but not in the reference and L- asx decreased in the treatments with mercuric chloride ('$HgCl_2$') and fungicide ('Bacteria'). Additional D-amino acids were detected at the end of the experiment, especially in the treatments with fungicide ('Bacteria') and bactericide ('Fungi'). *A. germinans* leaves showed a similar distribution at the beginning of the experiment, although L-leu was less dominant and D-ser appeared as additional D-amino acid. After 6 weeks L-asx had decreased and gly had increased in both fungicide and bactericide treatment. Additional D-amino acids were detected at the end of the experiment, especially in the treatments with mercuric chloride ('$HgCl_2$') and the reference group.

4.3.2.5 Total Bacterial Counts and Scanning Electron Microscopy

To assess whether treatments had been successful, total bacterial counts (TBC) were conducted for the water of all treatments after 42 days and leaves for SEM were collected weekly.

For TBC, highest numbers of cells (77.7 ± 2.2 *10^6 cells/ml, n=10) were found in *Rhizophora* samples treated with fungicide ('Bacteria') (*Figure 4.3.7*). The *Avicennia* samples of the same treatment showed lower cell counts staying within the range of the reference samples. Samples treated with bactericide ('Fungi') yielded low cell counts (4.9 ± 2.3 *10^6 cells/ml and 11.9 ± 3.0*10^6 cells/ml for *Rhizophora* and *Avicennia* samples respectively, n=10), but showed an accumulation of hyphal structures under the microscope (*Figure 4.3.8*). Treatment with mercuric chloride resulted in a significant reduction of cells (0.02 ± 0.01 * 10^6 cells/ml and 0.05 ± 0.03 * 10^6 cells/ml for *Rhizophora* and *Avicennia* samples, respectively).

SEM photos of leaves after the first week of incubation are shown in *Figure 4.3.9*. As for TBC is can be observed, that treatments had been successful, with fungi growing into the stomata of the 'Fungi' leaves, bacteria on the 'Bacteria' leaves and no colonisation on the '$HgCl_2$' leaves.

4.3.3 Decomposition of Tannins in crab intestine

The experiment with crab intestine yielded reduced tannin concentrations after 24 hrs of exposure to enzymes present in crab intestines (*Figure 4.3.10*). Reduction was between 43 and 98 %.

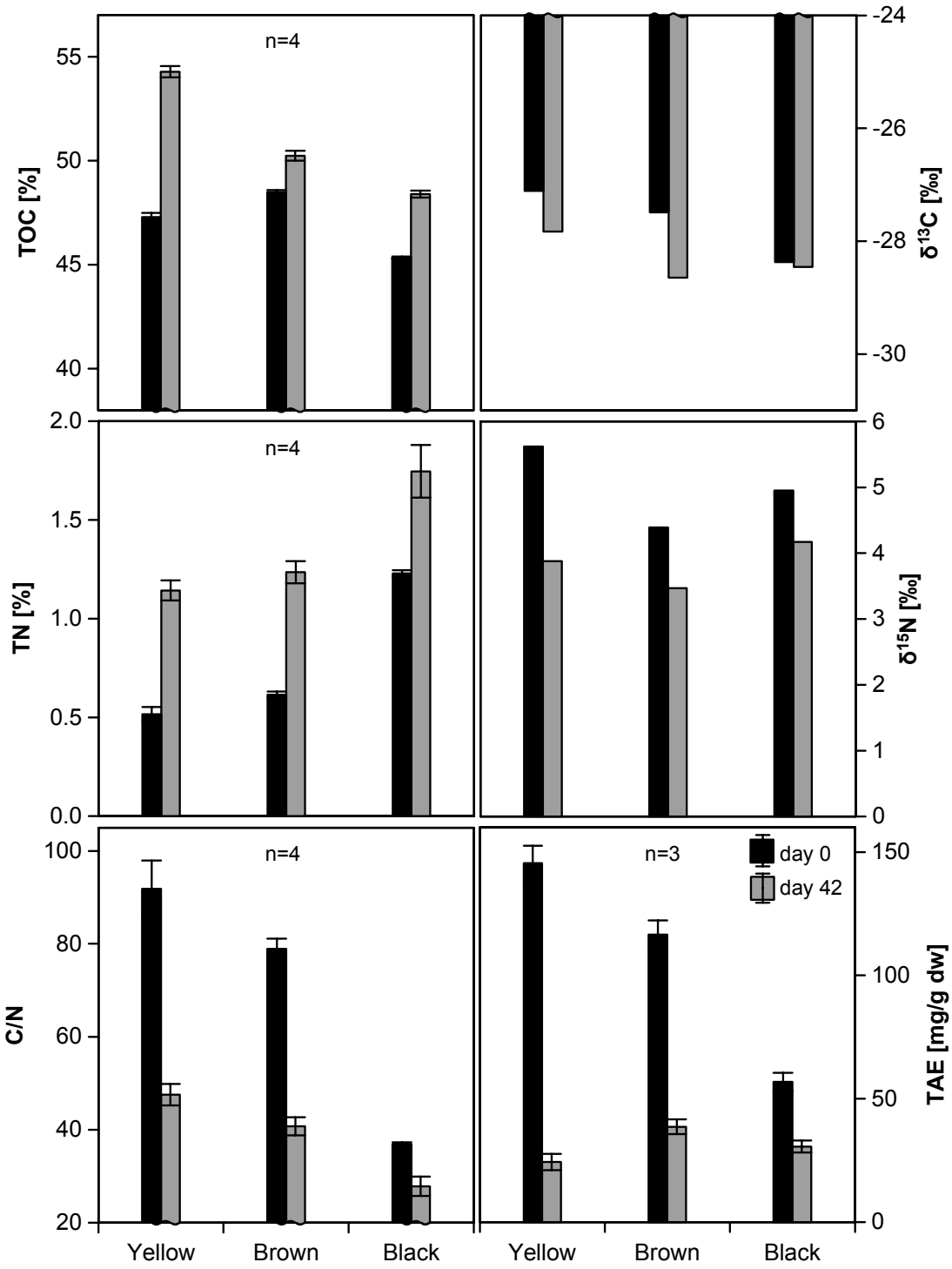


Figure 4.3.1: Elemental and isotopic composition as well as tannin concentrations (as TAE) in leaves of *R. mangle* during the field experiment. The values for yellow, brown and black leaves are presented for the start (day 0) and the end of the experiment (day 42). For TOC, TN, C/N and tannin the mean value ± SD are shown; n is given in graphs; for isotopes only one sample was measured for each value.

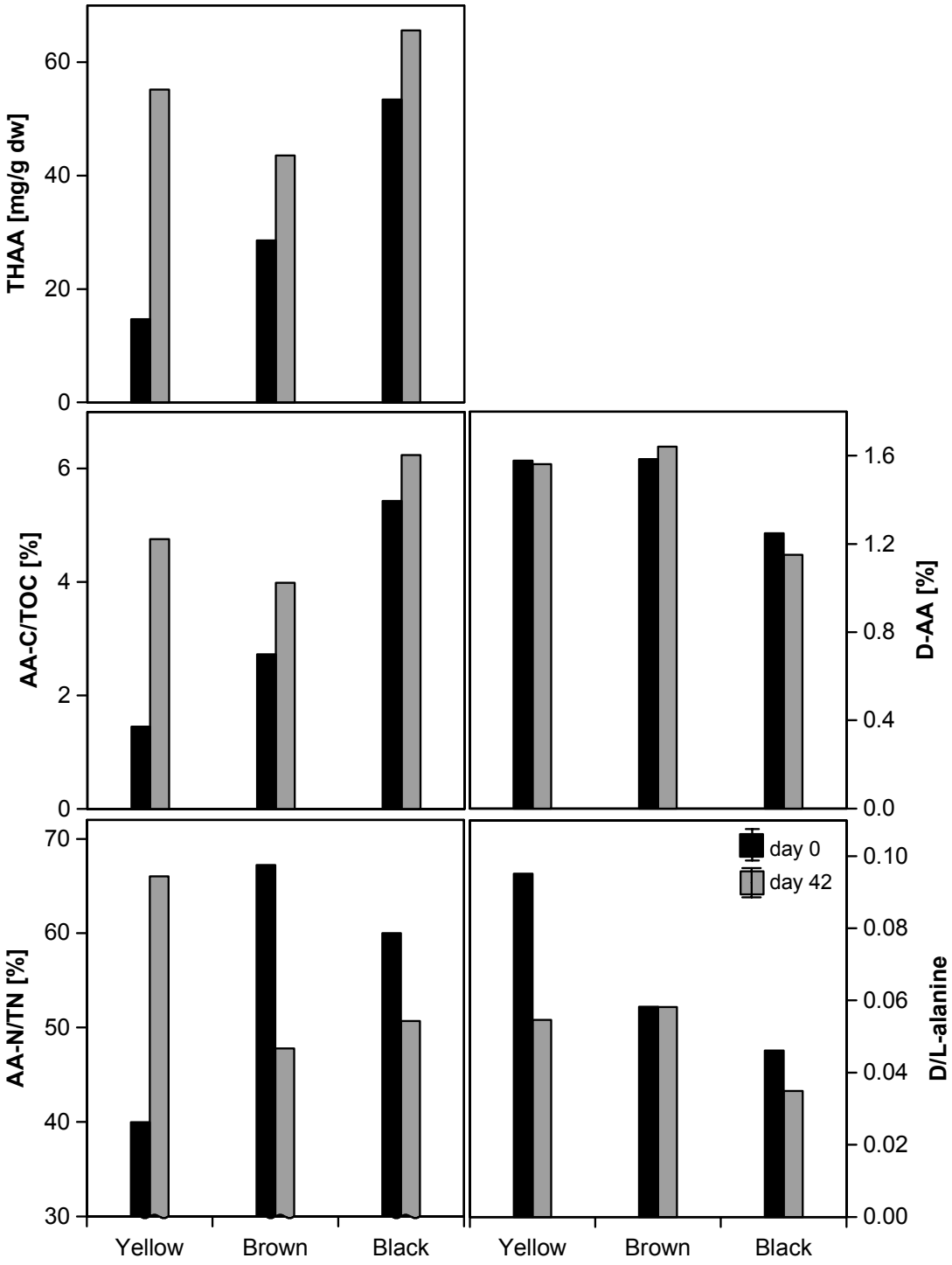


Figure 4.3.2: THAA, AA-C/TOC, AA-N/TN, %D-amino acids of THAA (D-AA) and D/ L-alanine ratios for leaves of *R. mangle* during the field experiment. The values for yellow, brown and black leaves are presented for the start (day 0) and the end of the experiment (day 42).

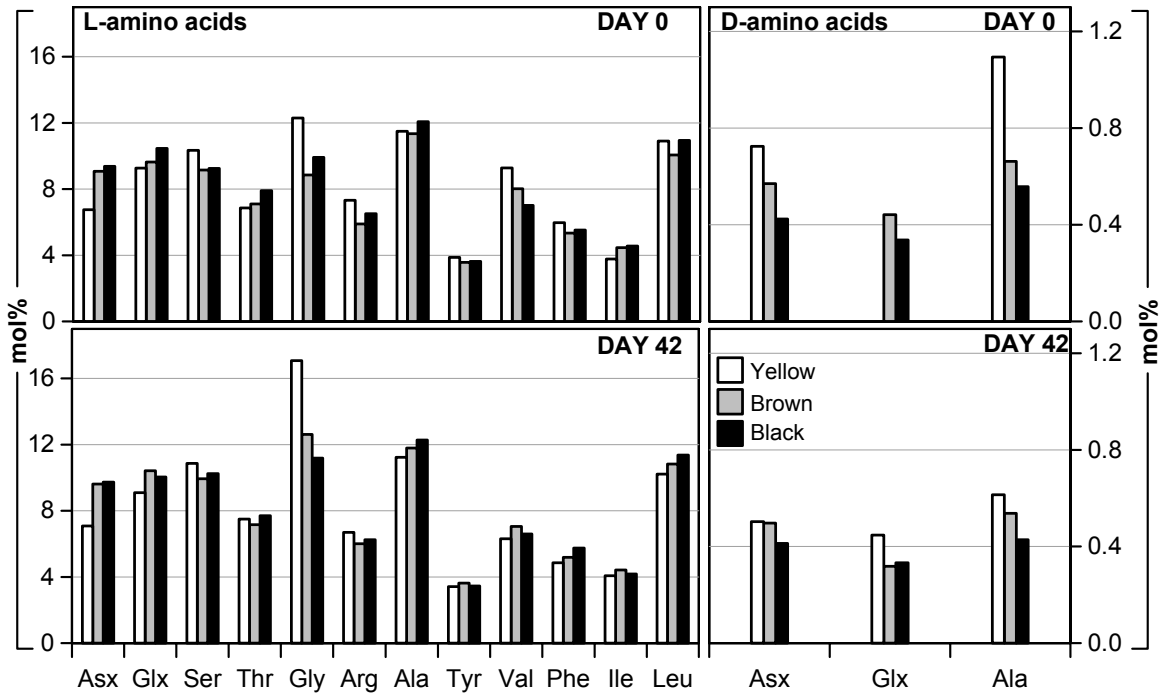


Figure 4.3.3: Composition of individual L- and D-amino acids in leaves of *R. mangle* during the field experiment. The values for yellow, brown and black leaves are presented for the first day (day 0) and the last day of the experiment (day 42).

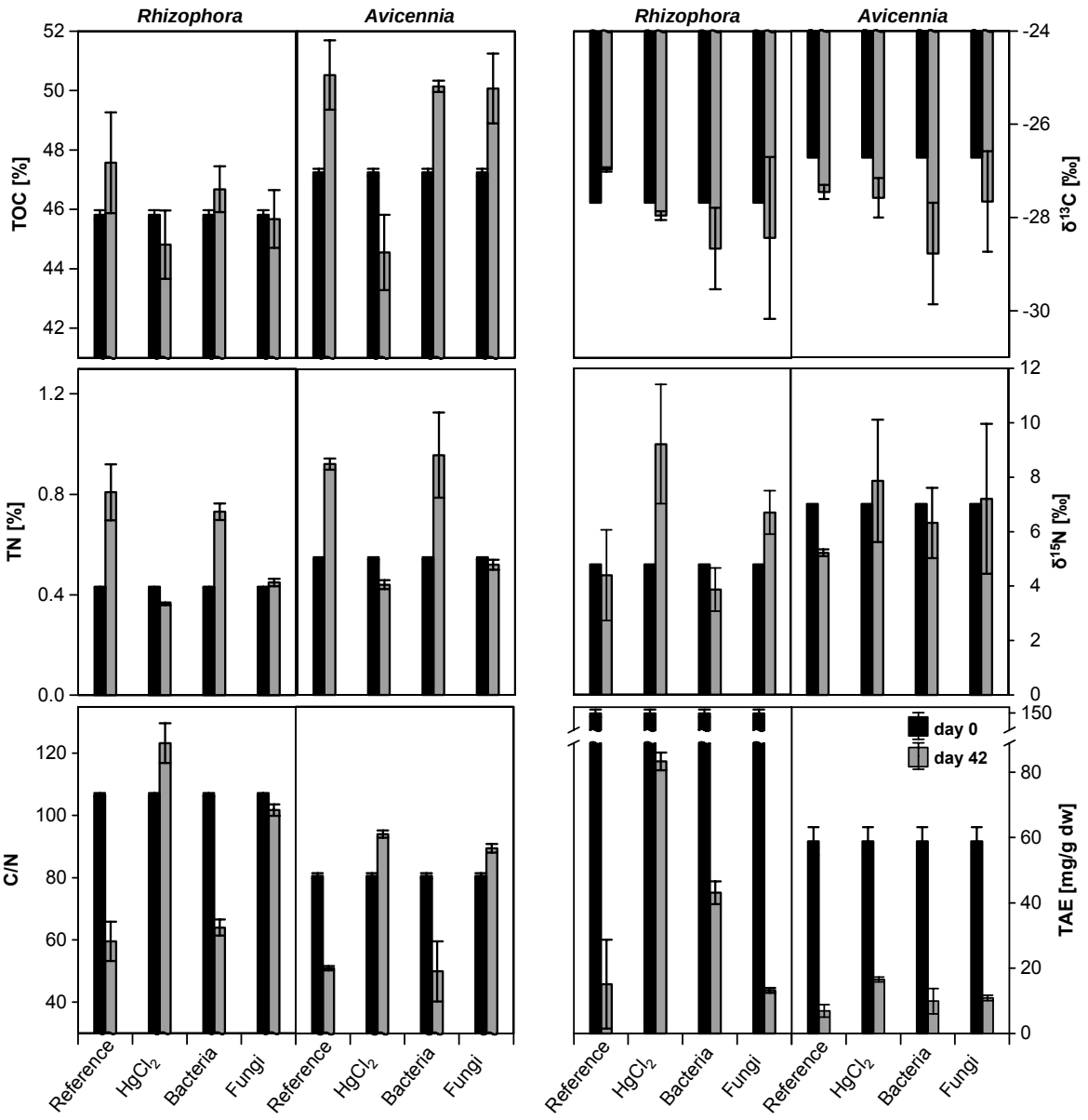


Figure 4.3.4: Elemental and isotopic composition as well as tannin concentrations (as TAE) in leaves of *R. mangle* (*Rhizophora*) and *A. germinans* (*Avicennia*) during the laboratory experiment. The values for the reference group (Reference) and the three different treatments with mercuric chloride ($HgCl_2$), fungicide (Bacteria) and bactericide (Fungi) are presented for the first day (day 0) and the last day of the experiment (day 42). Mean value ± SD are shown, n=4.

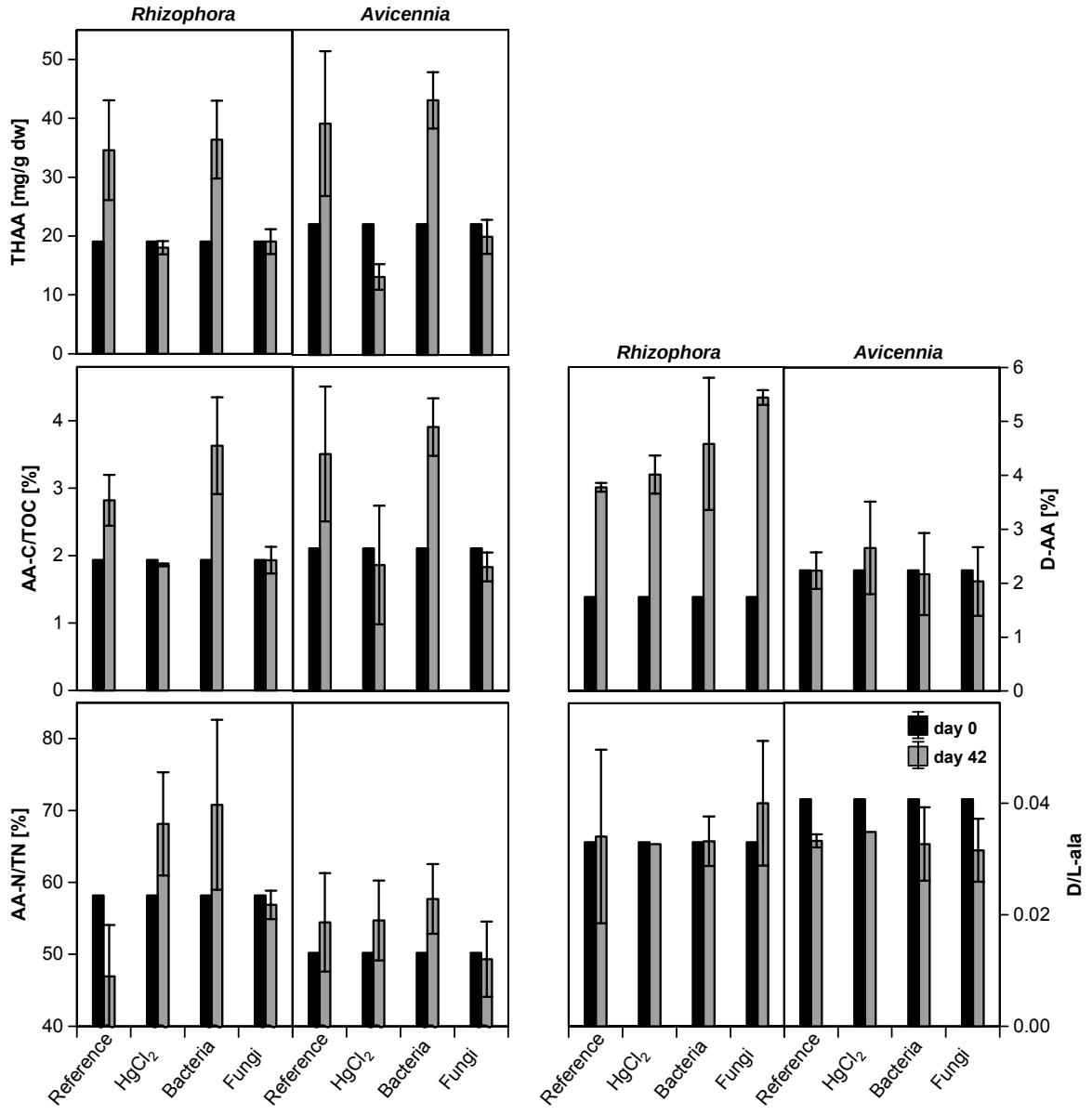


Figure 4.3.5: THAA, AA-C/TOC, AA-N/TN, %D-amino acids of THAA (D-AA) and D/ L-alanine ratios for leaves of *R. mangle* (*Rhizophora*) and *A. germinans* (*Avicennia*) during the laboratory experiment. The values for the reference group (Reference) and the three different treatments with mercuric chloride ($HgCl_2$), fungicide (Bacteria) and bactericide (Fungi) are presented for the first day (day 0) and the last day of the experiment (day 42). Mean value ± SD are shown, n=3.

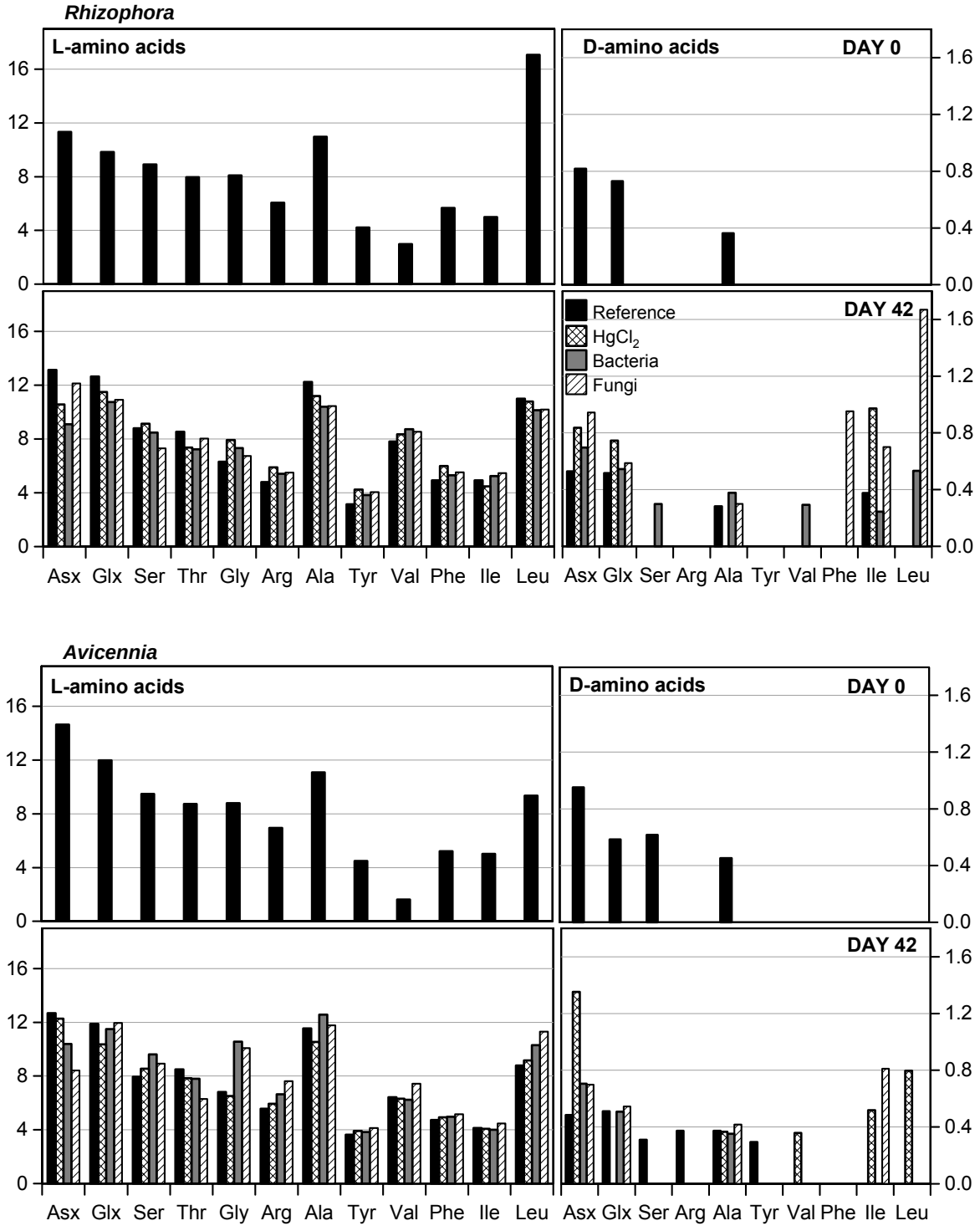


Figure 4.3.6: Composition of individual L- and D-amino acids in leaves of *R. mangle* (*Rhizophora*) and *A. germinans* (*Avicennia*) during the laboratory experiment. The values for the reference group (Reference) and the three different treatments with mercuric chloride ($HgCl_2$), fungicide (Bacteria) and bactericide (Fungi) are presented for the first day (day 0) and the last day of the experiment (day 42).

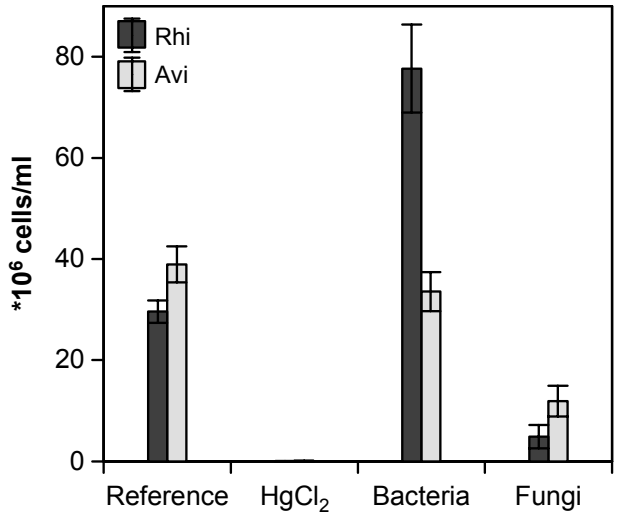


Figure 4.3.7: Total bacterial counts for the water of the reference group (Reference) and the three different treatments with mercuric chloride ($HgCl_2$), fungicide (Bacteria) and bactericide (Fungi). Mean values ± SD are presented for *R. mangle* (Rhi) and *A. germinans* (Avi) on the last day of the experiment (day 42).

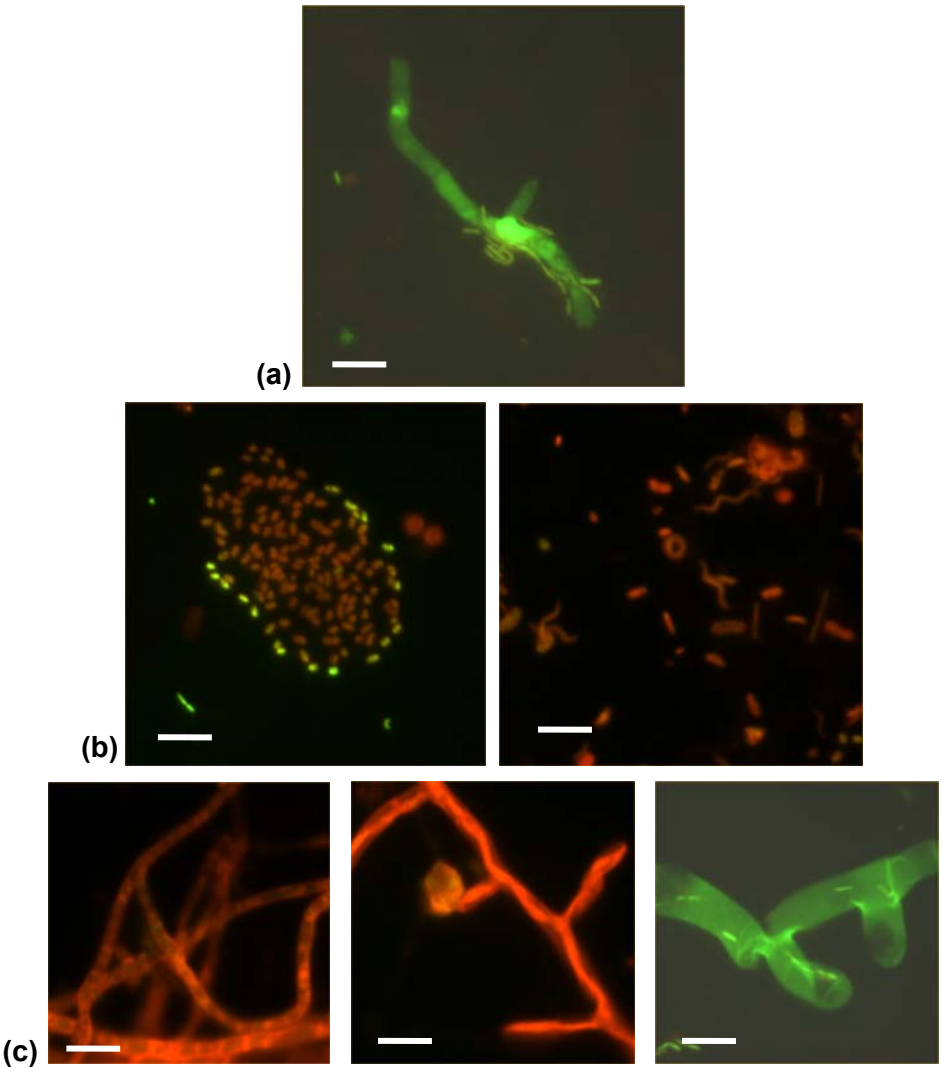


Figure 4.3.8: Photographs of dyed bacterial cells and hyphal structures of fungi in the water of (a) the reference group showing bacteria attached to a fungus, (b) the 'Bacteria' group showing different species of bacteria and (c) the 'Fungi' group with different hyphal structures of fungi. White bar = 10 µm

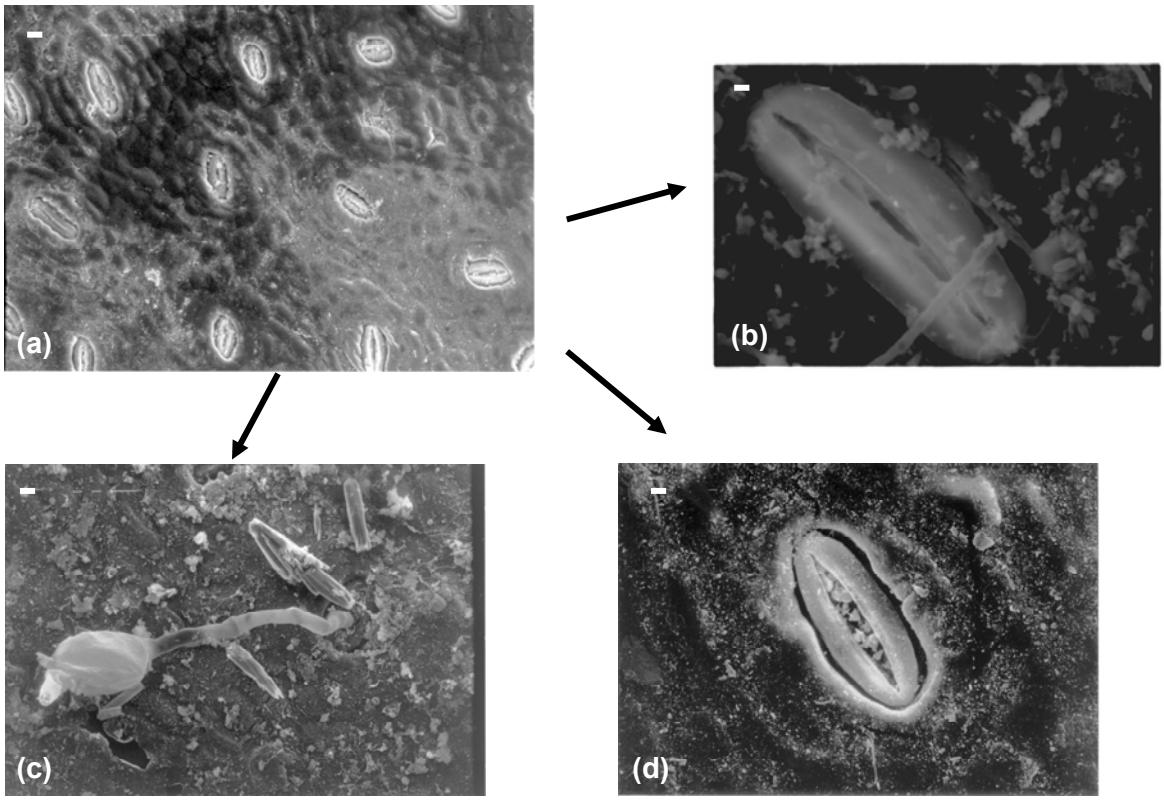


Figure 4.3.8: SEM photographs of leaves of *R. mangle* during the laboratory incubation. (a) Overview of a leaf underside on day 0, white bar = 100 µm (b) stomata of a leaf of the 'Bacteria' group after 1 week of incubation, white bar = 1 µm (c) stomata of a leaf of the 'Fungi' group after 1 week of incubation, white bar = 10 µm (d) stomata of a leaf of the '$HgCl_2$' group after 1 week of incubation, white bar = 1 µm.

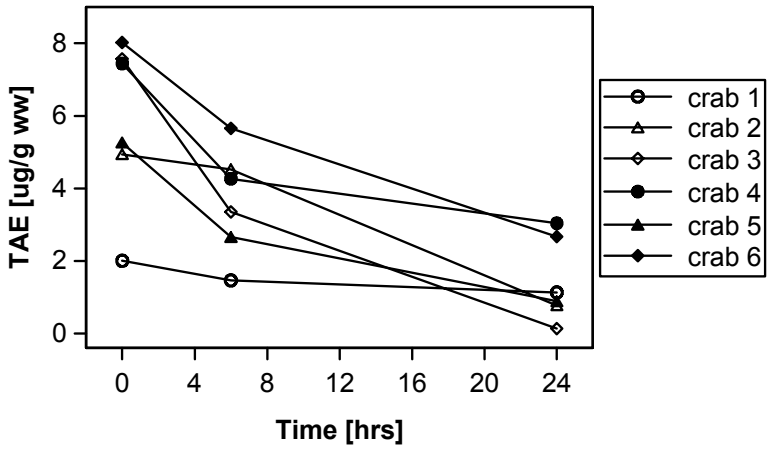


Figure 4.3.9: Degradation of commercial tannic acid by contents of crab (*Ucides cordatus*) intestines.

5 DISCUSSION

Mangrove ecosystems are highly productive and support adjacent marine communities and ecosystems including seagrass beds and coral reefs (Mumby*, et al.*, 2004). For the Bragança peninsula Dittmar (1999) found that the mangroves play a key role for the nutrient cycles of the adjacent coastal and marine ecosystems. They are probably one of the driving forces for near-shore primary and secondary production, transporting inorganic and organic nutrients by the means of tides and currents to adjacent environments. However, regarding the ever increasing amount of literature addressing the topic, there is still little agreement on a general model or on the main driving forces behind the processes that regulate nutrient dynamics within and between ecosystems. Especially for mangrove ecosystems, the literature is manifold, but the results remain site-specific (Feller*, et al.*, 1999; Dittmar and Lara, 2001a, 2001b; Dittmar*, et al.*, 2001; Feller*, et al.*, 2003b). Mangrove trees and their sediments seem to have a complicated relationship.

The few mangroves where fertilisation experiments have been conducted were either phosphorous-limited (P) (Feller, 1995) or differentially N- and P- limited along a tidal gradient (Feller*, et al.*, 2003a; Feller*, et al.*, 2003b). These studies demonstrated that patterns of nutrient uptake and limitation, as well as plant-sediment interactions are complex in mangrove ecosystems and that not all processes react similarly to the same nutrient. Different habitats are not always limited by the same nutrient when different mangrove forests are compared. The co-occurrence of other stressors such as salinity suggests that the nutrient limitation in mangroves especially in landward positions is often secondary to other growth-limiting factors (Mendelssohn, 1979 for saltmarshes; McKee*, et al.*, 2002).

In order to allow a more detailed description of the nitrogen dynamics in the studied forest and to look for the driving forces behind the nitrogen dynamics, the reactions of the forest on a molecular level were studied in relation to the natural stressors. Within two transects differing in tree height and age, inundation frequency and species composition, amino acids and potentially complexing compounds such as tannins (total phenolics as tannic acid equivalents), as well as physico-chemical characteristics, total organic carbon (TOC) and total nitrogen (TN) content in leaves and sediments were assessed.

5.1 The field studies

5.1.1 Elemental and isotopic composition of sediments and plants

5.1.1.1 Transect 1: Elemental and isotopic composition

Carbon and nitrogen

The TOC and TN content in sediments and leaves of *R. mangle* and *A. germinans* was generally within the range of several other studies on mangrove trees and sediments (Lacerda*, et al.*, 1995; Rivera-Monroy*, et al.*, 1995; Jennerjahn and Ittekkot, 1997; Fry*, et al.*, 2000; Koch, 2002; Feller*, et al.*, 2003b; Nordhaus, 2004). The mean TOC values measured for sediments at the surface, 10 cm and 50 cm depth were - in that order - 2.96 ± 0.47 %, 2.21 ± 0.59 % and 1.94 ± 0.70 % TOC, the mean TN contents were 0.2 ± 0.02 %, 0.16 ± 0.04 % and 0.13 ± 0.03 % N. C/N ratios for sediments were highly variable, ranging from 9.38 to 18.95 (*Figure 4.2.1* and *4.2.2*). *R. mangle* leaves contained significantly more ($p<0.001$) organic carbon (45.1 ± 1.0 %) than *A. germinans* (42.3 ± 1.9%), wheras *A. germinans* had significantly higher values for TN (3.0 ± 0.4 %) than *R. mangle* (1.8 ± 0.1%). Generally the C/N ratios for *R. mangle* were much higher (25.8 ± 2.1) than for *A. germinans* (14.2 ± 2.0) ($p<0.001$) (*Figures 4.2.5 - 4.2.7*).

Sediments generally showed a stronger stratification for both TOC and TN in the *Avicennia*-dominated region of the forest near *Avicennia* trees with decreasing values from the surface to 50 cm layers (see *Figure 4.2.1*). *Rhizophora*-dominated sediments did not show such a strong difference with depth. The stratification in the *Avicennia*-dominated part of the forest could be related to the different rooting systems of *Avicennia* spp. and *Rhizophora* spp. *Avicennia* trees have a cable root system that stretches parallel to the surface with pneumatophores arising to the sediment surface and extending upward into the air, whereas *Rhizophora* species have stilt roots that grow from the trunk deep into the substrate (Tomlinson, 1986; Saenger, 2002).

A significant difference was found in this study between the subsurface samples of *Rhizophora*- versus *Avicennia*-near sediment samples. At 50 cm depth TOC values near *Rhizophora* roots were about 20 % higher than values near *Avicennia* roots (2.2 ± 0.4 % for *Rhizophora* versus 1.7 ± 0.4 % for *Avicennia* sediments). Thus, the stratification of the TOC and TN values could originate in the root type which in the case of *Rhizophora* is more likely to influence the sediment layers uniformly than is the cable root system of *A. germinans*. Boto *et al*. (1989) reported a subsurface peak of organic carbon coinciding with the main rooting zone of the *Rhizophora* trees in Australia suggesting that organic matter was passed out from the roots to the sediments where it was available to microbial

degradation. The organic carbon peak measured in their study as well as the results of the present work could also result from exudation or degradation of fine roots or root hairs with short life times. The higher bioturbation rates through the mangrove crab Ucides cordatus in the *Rhizophora*-dominated area of the transect could also account for a more consistent distribution of compounds throughout the sediment layers. Mendoza (unpublished data) found that in the studied transect 1, redox values (Eh) were always higher around *R. mangle* trees than in sediments near *A. germinans* trees, when Eh was measured in areas with the same inundation frequency. These higher redox values, i.e. greater oxidation of the sediments, are probably related to the crab burrows which are more frequently found near *R. mangle* trees (Diele, 2000; Nordhaus, 2004).

Lacerda *et al.* (1995) observed a similar phenomenon of stratification in mangrove sediments under *R. mangle* and *A. schaueriana* in south-east Brazil where TOC and TN content decreased in *Avicennia*-sediments but not in *Rhizophora*-soils from 0 to 15 cm depth. Preservation of nitrogen in *Rhizophora*-sediments has been suggested by these authors as a consequence of higher tannin content in *Rhizophora* leaves. Tannin content is much higher in *Rhizophora* than in *Avicennia* plants (this study (see below) and Lacerda*, et al.*, 1986) and these substances have been reported to decrease the activity of benthic and microbial organisms (Boto*, et al.*, 1989). In our study area we do not find less macrobenthic activity. On the contrary, the most conspicuous member of the benthos, the mangrove crab *U. cordatus* is generally found in higher numbers in the *Rhizophora*-dominated forests (Wessels, 1999). However, this fact is probably related to the softness of the mud and the abundance of food in the *Rhizophora* forest. As will be discussed below *U. cordatus* is able to digest tannin-like molecules. A preservation of nitrogen through tannins and a subsequent uniformity of nitrogen distribution would be reflected in a similar distribution of nitrogen and tannins. Tannin values did indeed increase in *Rhizophora* sediments, but mainly in the 50 cm sediment layers. The possible and probable interactions between tannins and other nitrogen compounds are discussed further below, but they can not account for the uniform distribution of TOC and TN in the *Rhizophora*-influenced soils. Likewise, the percentage of D-amino acids in the lower sediment layers, where tannins are abundant, do not point to reduced microbenthic activity in the *Rhizophora*-dominated forest.

C/N ratios of sediment samples were rising simultaneously at all depths especially during the dry season from the *Avicennia*-dominated to the *Rhizophora*-dominated stations, suggesting a proportionally higher increase in TOC than in TN values towards the *Rhizophora*-dominated area. This behaviour was reflected in the plant leaves with much

higher C/N ratios in *R. mangle* (25.8 ± 2.1) than in *A. germinans* (14.2 ± 2.0). Lallier-Verges et al (1998) compared sediments of various vegetation units in a mangrove swamp in the French West Indies and also found highest C/N ratios under *R. mangle* and *Laguncularia racemosa* stands. The lowest values were found in the landward sites under *Pterocarpus officinalis* (*R. mangle* was growing in the seaward sites), confirming the results of the present study. An increase of C/N values in the sediments could reflect the degradation of proteins and the partial or total loss of the corresponding nitrogen. However THAA values of sediments do not show such a consistent trend as the C/N values (*Figure 4.2.20*). THAA values only drop steadily from station 1 to station 10 during the rainy season and in sediments near *Rhizophora* trees, suggesting a more complex story that may be more dependent on physico-chemical parameters of the environment than on the source of the organic matter. Lallier-Verges (1998) also described a mangrove swamp where variations in geochemical composition of organic matter may be related more to preservation conditions than to variations in source organisms.

Differences in grain size have also been reported as a reason for C/N ratio variation. With increasing clay fraction, decreases of values for C/N ratios have been observed due to preferential C loss while N is fixed in the finer sediments e.g. by adsorption of NH_4^+ onto clay surfaces (Müller, 1977; Lallier-Verges and Alberic, 1990). However, although we found decreasing values for the silt/clay fraction when comparing the *Avicennia*-dominated area with the *Rhizophora*-dominated part of the forest in this study, all stations had values greater than 90 % for the silt/clay fraction thus the differences might not be significant enough to influence the C and N dynamics.

As TOC and TN values decreased simultaneously with depth, C/N ratios did not differ significantly between the surface, 10 cm and 50 cm sediments, indicating no preferential (or a similar) degradation or preservation pattern in terms of total amounts of nitrogen or carbon. Other studies equally found little variation of C/N values with depth (e.g. Rivera-Monroy*, et al.*, 1995).

Isotopic composition

Analyses of stable carbon and nitrogen isotopic composition of ecosystem components (e.g. leaves and sediments) can be used to assess the interplay of physical and chemical parameters and their relationship to plant growth and nutrient dynamics.

Carbon stable isotopic variation in plants is largely determined by the photosynthetic mode of plants (Bender, 1971; Hoefs, 2004). The $\delta^{13}C$ values measured in both species

(-29.2 ± 1.3 ‰ for *A. germinans*, -28.8 ± 2.8 ‰ for *R. mangle*) are typical for C_3 land plants (Bender, 1971; Lallier-Verges*, et al.*, 1998; Lamb*, et al.*, 2005). In most plants, initial fixation of carbon occurs via rubisco, the Calvin cycle enzyme that adds CO_2 to ribulose bisphosphate. Such plants are called C_3 plants, because the first organic product of carbon fixation is a three-carbon compound, 3-phosphoglycerate. Lamb (2005) described C_3-plants as plants with a C/N value greater than 12 and $\delta^{13}C$ values between -32 and -21 ‰, a description which can fully be applied to the plants in this transect (*Figure 5.1.1*). The two other possible pathways of CO_2 fixation (Crassulacean Acid Metabolism (CAM) and C_4 plants) will be discussed in detail in section 5.1.1.2.

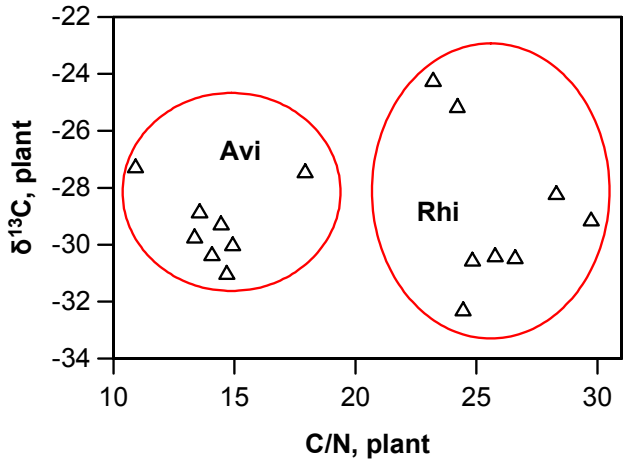


Figure 5.1.1: C/N versus $\delta^{13}C$ values for *A. germinans* (Avi) and *R. mangle* (Rhi) leaves in transect 1, data pooled from rainy and dry season.

There is however a variability of carbon isotope ratios within C_3 plants, which in its strongest implication could be caused by a switch of photosynthetic mode (i.e from C_3 to C_4 or to CAM (Popp, 1995; Parida*, et al.*, 2004). However, only some halophytes have been found to perform such an alteration of the photosynthetic pathway, but a variation of $\delta^{13}C$ values connected to varying environmental factors has been reported for several species (Di Martino*, et al.*, 2003; Hoefs, 2004). Plotting $\delta^{13}C$ values in plant tissues against the sediment salinity at all three measured depths results in a positive correlation for both species, although the relationship is stronger for *Rhizophora* (*Figure 5.1.2*). An important factor determining this variation is the CO_2 concentration of the internal leaf space (C_i). Lower C_i will increase the $\delta^{13}C$ values of photosynthates as discrimination against ^{13}C decreases. As C_i in C_3 leaves is largely determined by the stomatal conductance, environmental factors which decrease stomatal conductance (thus decreasing internal CO_2 concentrations) will increase $\delta^{13}C$ values during photosynthesis as less fractionation occurs. One relevant environmental factor is sediment or porewater

salinity and several studies have reported that an increase in salinity decreases stomatal conductance and consequently increases $\delta^{13}C$ values in the leaves of *R. mangle*, *A. germinans* and other mangrove species (Farquhar*, et al.*, 1982a; Farquhar*, et al.*, 1982b; Ish-Shalom-Gordon*, et al.*, 1992).

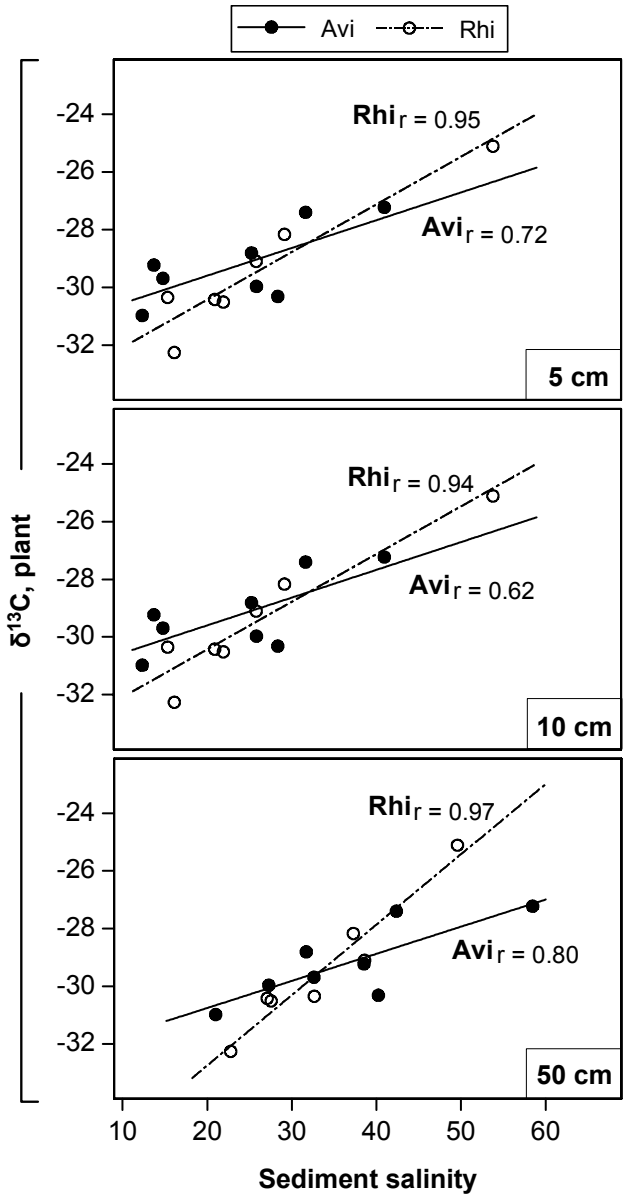


Figure 5.1.2: Correlations between $\delta^{13}C$ values of leaves from *A. germinans* (Avi, n=8) and *R. mangle* (Rhi, n=7) versus sediment salinity in 5 cm, 10 cm and 50 cm sediments in transect 1, data pooled from rainy and dry. Correlations are significant ($p<0.05$) for al $r>0.60$.

The $\delta^{13}C$ also correlated with another environmental factor. A negative correlation occurred between inundation frequency and $\delta^{13}C$ values in both plants and sediments (*Figure 5.1.3*). Both low and high inundation frequency can be interpreted as stress factors for plants. The frequent hypoxic conditions (i.e. low redox potentials) in frequently

flooded soils have a negative effect on nitrogen uptake and turnover (Saenger, 2002). Decreased aeration affects ammonium uptake in *Spartina alterniflora* and *Panicum hemitomon*. In coastal wetlands with abundant sulphate from marine water, hypoxia leads to bacterial sulphate reduction to sulphide. An important negative effect of sulphide on plant growth is an inhibition of N uptake, which decreases with increasing sulphide concentration and is reflected e.g. in a decreased leaf elongation (Koch*, et al.*, 1990). On the other hand, low redox potentials in anaerobic soils have been found to increase phosphorous (P) availability, thus reducing another potential stress factor as P has been frequently reported to be a limiting factor in mangrove ecosystems (Feller*, et al.*, 2003a). In the present study $\delta^{13}C$ values in plant leaves correlated positively with the stress factor salinity and negatively with inundation frequency. If we deduce that higher $\delta^{13}C$ values are related to higher stress, it can be concluded that despite the lower redox conditions, the higher inundation frequencies induce less stress on the plant than the lower inundation frequencies which are coupled to higher salinities and lower P availability.

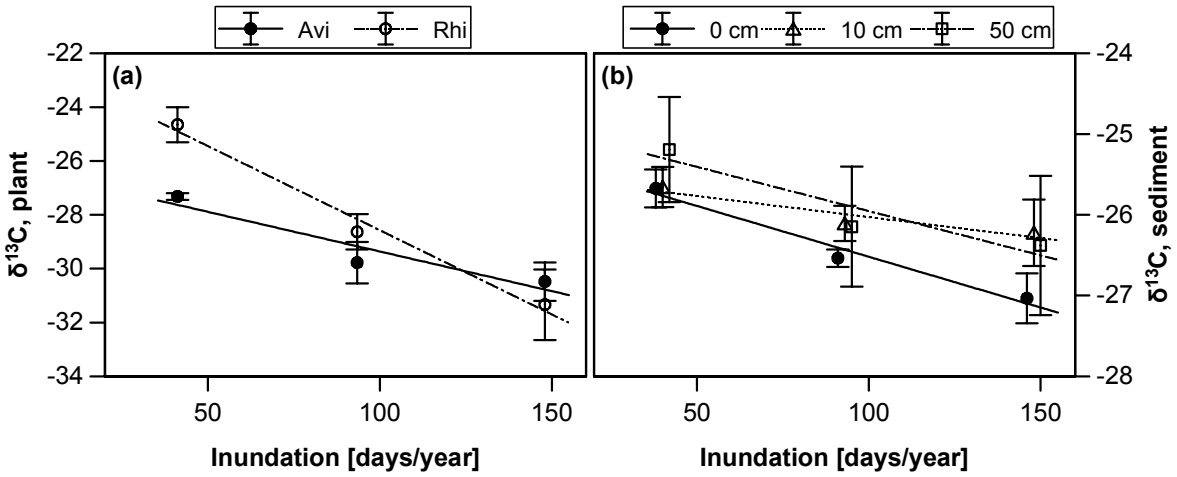


Figure 5.1.3: Correlation between (a) $\delta^{13}C$ values of leaves of *A. germinans* (Avi) and *R. mangle* (Rhi) and (b) $\delta^{13}C$ values of surface, 10 cm and 50 cm sediments versus inundation frequency in transect 1, data pooled from both seasons. For plants n=6, for sediments n=12, all are significant at $p<0.05$.

There was, however, no relationship between the TN or C/N of sediments and $\delta^{13}C$ in leaves. Yet, it has been suggested that there is a negative relationship between salinity and nutrient availability (Medina and Francisco, 1996; Sengupta and Chaudhuri, 2002) or nutrient uptake (Odum, 1988; Flores*, et al.*, 2000). Sodium can act as a competitive inhibitor of NH_4^+ uptake and the activity of nitrate reductase which reduces nitrate to ammonium often decreases under salt stress. Total nitrogen or total C/N ratios only give us a description of how much nitrogen and carbon is present, but not how much is available for plant uptake or how much the plant is able to take up. It is therefore possible,

that an impact of N limitation through salt stress exists, but does not become apparent when the total amount of nitrogen is considered.

For C/N values of sediments we find a negative correlation with $\delta^{13}C$ values in sediments (*Figure 5.1.4*). The lower the C/N values, the higher (more positive) the $\delta^{13}C$ values of the sediments. During biodegradation, generally the lighter carbon isotope ^{12}C is depleted leading to increased $\delta^{13}C$ values (Fry and Sherr, 1984). Low C/N values could result from rapid C loss of carbon from litter due to mineralisation or leaching, or from N contribution through N_2-fixation. The latter would take place mainly at the sediment surface. Low C/N ratios and ^{13}C enrichment were found in the *Avicennia*-dominated part of the transect (*Figures 4.2.2* and *4.2.3*). Thus, sediments at the first stations may show a higher degree of degradation.

The $\delta^{13}C$ values in sediments were less negative than in the corresponding plants where plant $\delta^{13}C$ showed a mean of -29.0 ± 2.1 ‰ (ranging from -32.3 ‰ to -24.2 ‰) whereas sediments ranged from -26.4 ± 0.6 ‰ to -25.9 ± 0.9 ‰ for surface and 50 cm sediments respectively. This observation was confirmed in several other studies (Benner*, et al.*, 1991; Lallier-Verges*, et al.*, 1998). The enrichment of ^{13}C in the sediments can have several causes. It can be explained by microbial activity which can alter the isotopic composition during the decomposition processes through fractionation, leaving degradation products which are enriched in ^{13}C. It could also originate from the algal or bacterial communities themselves (marine algae and bacteria have $\delta^{13}C$ values ranging between -12 and -27 ‰ (Lamb*, et al.*, 2005)), i.e. organisms other than higher plants being the source of differing $\delta^{13}C$ values, not only their degradation products.

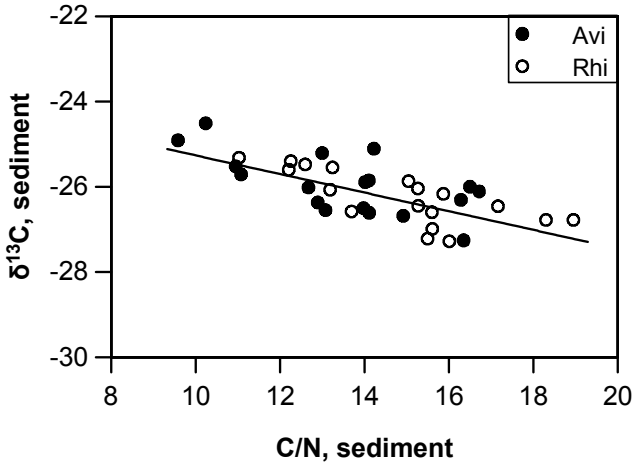


Figure 5.1.4: C/N versus $\delta^{13}C$ values of sediments near *A. germinans* and *R. mangle* trees in transect 1, regression line for pooled data of both species, n = 36, r = -0.72, p<0.001

For leaf $\delta^{15}N$ the range measured at various stations throughout the transect was approximately 2.5 ‰ for both species which is wider than has been reported for other terrestrial plant species across greater spatial differences (Nadelhoffer*, et al.*, 1996; McKee*, et al.*, 2002). The simplest explanation for changes in isotopic values in leaves is variation in $\delta^{15}N$ of nitrogenous nutrients. A weak, but significant ($p<0.001$) positive correlation ($r = 0.55$) can be distinguished between $\delta^{15}N$ values of the three sediment layers and plant $\delta^{15}N$ at 3 different stations (*Figure 5.1.5*). It shows however, that the variation in the sediments - although generally lower than in plants - (3.9 ‰ to 6.7 ‰) is similar to the differences within plant material (4.9 ‰ to 7.4 ‰).

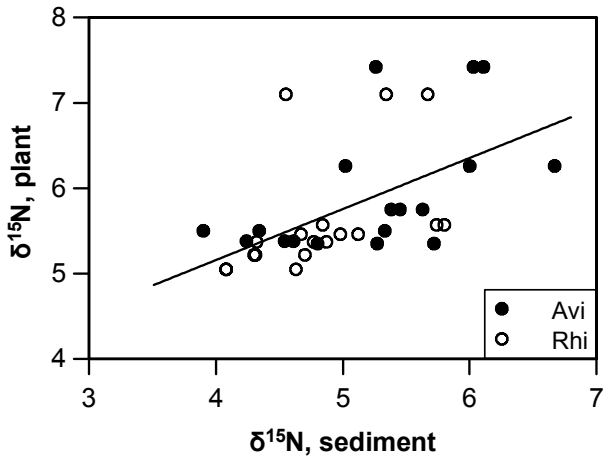


Figure 5.1.5: $\delta^{15}N$ values of sediment versus plant material for *A germinans* (Avi) and *R. mangle* (Rhi) in transect 1, regression line for pooled data of both species, $n = 36$, $r = 0.55$, $p<0.001$

Two models have been proposed by Fry et al (2000) to explain differences in source and plant $\delta^{15}N$. The first model explains a high source $\delta^{15}N$ with lower plant $\delta^{15}N$ due to plant isotopic fractionation during N uptake. The second model describes low source $\delta^{15}N$ value with higher plant $\delta^{15}N$ due to microbial isotopic fractionation during nitrification and denitrification. In the first model the plant uptake is an important factor for the isotopic fractionation resulting in lower $\delta^{15}N$ for the plant. In the present study, the ranges of values for sediments and plants were similar; hence the model does not give a satisfactory explanation for the situation. The second model emphasises the importance of microbial $\delta^{15}N$ fractionation during degradation processes. Where N is limiting, all N is typically used regardless of isotope, resulting in higher $\delta^{15}N$ values in the leaves (Fry*, et al.*, 2000; McKee*, et al.*, 2002). TN (%) of sediments does not show a consistent relationship to $\delta^{15}N$ values of the leaves (*Figure 5.1.6a-c*). For the surface sediments the *Avicennia* samples show a positive, the *Rhizophora* samples show a negative trend, at 10

cm both species have a positive, but very weak relationship and at 50 cm the correlation is negative. All correlations, except for *Avicennia* leaves versus TN in surface sediments, are not significant. Hence, in this study site, the trees either do not react strongly to N limitation in terms of $\delta^{15}N$ variances or – as was suggested earlier in this chapter) – TN is not a suitable measure for N availability or N uptake efficiency of the plants. However, when $\delta^{15}N$ values of the plants are shown in relation to salinity, which can, as mentioned before have a negative influence on N availability or N uptake efficiency, we find some strong positive correlations; especially for *R. mangle* leaves (*Figure 5.1.6d-f*). Thus, the higher the salt stress, the higher the $\delta^{15}N$ values.

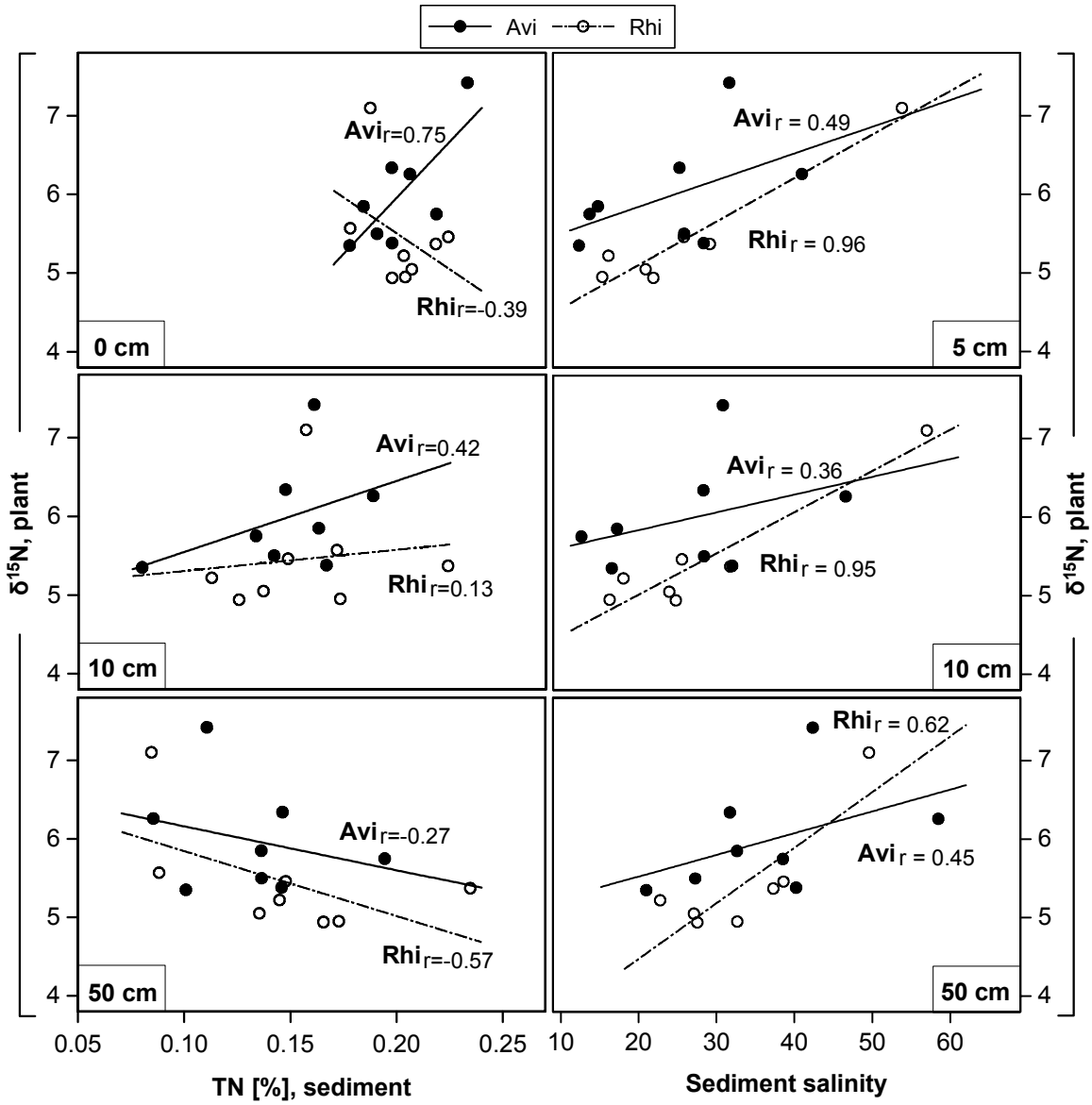


Figure 5.1.6: Correlation between (a-c) TN in surface, 10 cm and 50 cm sediments versus $\delta^{15}N$ composition in leaves of *A germinans* (Avi) and *R. mangle* (Rhi), and (d-e) sediment salinity at 5, 10 and 50 cm depth versus $\delta^{15}N$ composition in leaves of *A. germinans* (Avi) and *R. mangle* (Rhi) in transect 1, n=8, correlations are only significant ($p<0.01$) at $r>0.6$.

For inundation frequency correlation with $\delta^{15}N$ values in *A. germinans* leaves and in sediment samples was negative, but not significant (*Figure 5.1.7*). As discussed earlier in this section, both low and high inundation frequency can be interpreted as stress factors for plants. In the present study $\delta^{15}N$ values in plant leaves and sediments correlated positively with the stress factor salinity and negatively with inundation frequency. Thus, complementing the results of the $\delta^{13}C$ values, higher $\delta^{15}N$ values can be related to higher stress. Lower inundation frequencies coupled to higher salinities and lower P availability induce higher stress on the plant than high inundation frequencies combined with hypoxia, which is reflected in higher $\delta^{13}C$ and $\delta^{15}N$ values.

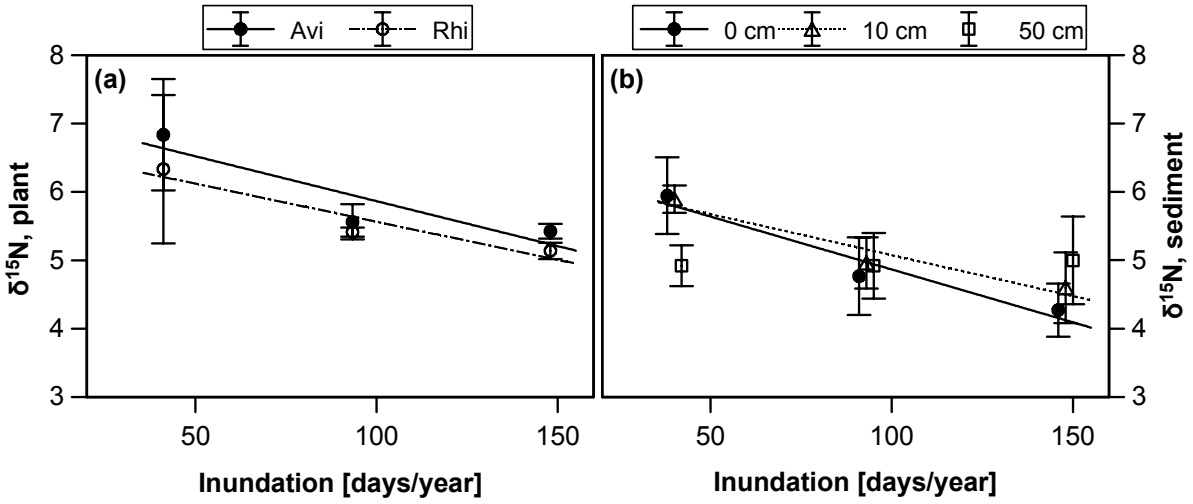


Figure 5.1.7: Correlation between (a) $\delta^{15}N$ values of leaves of *A. germinans* (Avi) and *R. mangle* (Rhi) and (b) $\delta^{15}N$ values of surface, 10 cm and 50 cm sediments versus inundation frequency in transect 1, data pooled from both seasons. For plants n=6, for sediments n=12, all shown regression lines have a correlation coefficient of r<-0.7, but only the correlation between $\delta^{15}N$ values of surface and 10 cm sediments and inundation frequency are significant.

The relationship between $\delta^{13}C$ values against $\delta^{15}N$ values of the plants therefore givs us a good measure of the stress level to which the plant is subjected (*Figure 5.1.8*)

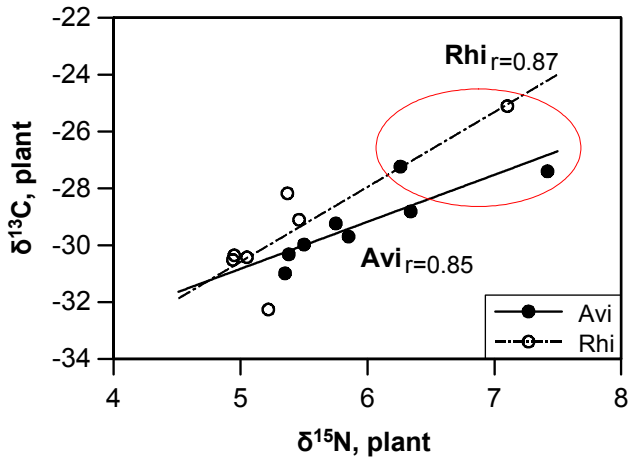


Figure 5.1.8: $\delta^{15}N$ versus $\delta^{13}C$ values for leaves of *A. germinans* (Avi, n=8) and *R. mangle* (Rhi, n=7) in transect 1. The red ellipse comprises the samples from the driest part of the transect. Both correlations are significant at $p<0.01$.

5.1.1.2 Transect 2: Elemental and isotopic composition of soils and plants

Plant material of *Avicennia germinans*, *Sesuvium portulacastrum*, *Sporobulus virginicus* and *Batis maritima* were collected and analysed in this transect. However, this discussion will mainly deal with the first three species as *B. maritima* only occurred at one station in low numbers.

Carbon and nitrogen

Both TOC and TN values in sediment samples showed a pattern with some striking features within the transect. For both parameters a conspicuous and significant downward step was measured in surface sediments from high values (6.5 ± 0.5 % TOC and 0.43 ± 0.01 % TN) at stations 1-4 to much lower values (2.9 ± 0.1 % TOC and 0.2 ± 0.01 % TN) at stations 5-7. This step could also be observed during the dry season, although it is not as pronounced as during the rainy season due to lower TOC and TN content at station 1 and 2. TOC and TN values decreased with depth and showed a more stable distribution at 10 and 50 cm depth (*Figure 4.2.10*). The physico-chemical parameters salinity, pH and silt clay content did not follow this same pattern for the surface sediments, hence can not be the cause of this sudden downward step. However the step coincided with the herbaceous vegetation, more specifically with *Sesuvium portulacastrum* which grows at exactly the stations with high TOC and TN content.

To investigate this coincidence *S. portulacastrum* was examined in more detail. The Acetylene Reduction Assay yielded the highest amount of N_2-fixation in roots with adjacent rhizosphere of *Sesuvium*. The unidentified epiphytes growing on the *Avicennia*

roots did also show some N_2-fixation, which has been reported in earlier studies (Sheridan, 1991). Higher plants can not fix atmospheric nitrogen themselves, but are frequently found to undergo symbiosis with N_2-fixing microorganisms. Many plant families include species which form symbiontic relationships with N_2-fixing bacteria representing a built-in source of nitrogen for assimilation into organic compounds. Most of the research has been done on the family Leguminoseae. Species of this family have swellings on their roots called 'nodules' composed of plant cells that contain N_2-fixing bacteria of the genus *Rhizobium*.

In the present work SEM photos of *S. portulacastrum* roots however showed no such conspicuous structures, but rather gave evidence of another kind of mutualistic symbiosis: 60 % of the analyzed roots had a mycorrhizal association (*Figure 4.2.38*), a symbiosis between a plant and a fungus localised in the root or root-like structure*. S. portulacastrum* has been reported to be a facultative mycorrhizal species by Sengupta et al (2002) who suggested that environmental stress factors may predispose the otherwise independent plants to mycorrhizal colonisation. Mycorrhizae are well-known to influence the plant nutrition and soil environment. They increase nutrient and water absorption and can degrade complex mineral and organic substances in soils and make the essential elements available to the host. Mycorrhizal fungi also offer protection against pathogens and contribute significantly to organic matter turnover and nutrient cycling in forest ecosystems. The association with a mycorrhizal fungus has been known to increase stress tolerance of various plants (Gupta*, et al.*, 2000).

Total bacterial cell counts showed that bacterial cells accumulated in the *Sesuvium* rhizosphere (*Figure 4.2.37*) and $\delta^{15}N$ measurements of rhizosphere sediment gave values between 1.4 and 3.8 ‰. Fry *et al.* (2000) stated for marine systems that N_2-fixation leads to values near -1 ‰ in planktonic material, while higher values (usually 4-7 ‰) occur in systems where deep-water nitrate is important (see also Hoefs, 2004). Values above 10 ‰ probably indicate anthropogenic input. The values measured in *Sesuvium* leaves, roots and especially in the rhizosphere do not reach negative values, but they are substantially lower than what has been observed in all other measured plant materials in the studied ecosystem, giving further evidence of possible N_2-fixation associated with this species. There is also some evidence of an association of mycorrhizal fungi with N_2-fixating bacteria (Azcón-Aguilar and Barea, 1992; Newell, 1996) which would explain the capacity of the *Sesuvium* roots and/or rhizosphere in the present study to fix atmospheric nitrogen.

Accumulation of C and N could thus be due to enhanced N accumulation through fixation of atmospheric N and associated elevated C dynamics. It must be taken in account though, that these measures of TN and TOC do not give information about the form in which the elements are present. It is not clear yet to which extent these elevated N levels are also available to the plants as nutrients. It could also be a measure of microbial and fungal biomass or even benthic algae, which caused the values of TN and TOC to rise. Total bacterial cell counts however decreased with distance to *Sesuvium* (*Figure 4.2.37*). At a 10 cm distance of the plant the amount of cells was reduced to a fifth of the number of cells found directly in the rhizosphere. Comparing TOC, TN and $\delta^{15}N$ values of the rhizosphere with the surrounding sediments gives a further indication that the bacterial biomass might be confined to a very narrow space around the *Sesuvium* roots. TOC and TN values were both higher in the rhizosphere (8.7 ± 2.4 % and 0.54 ± 0.1 % respectively) than in surrounding sediments (6.5 ± 0.5 % and 0.43 ± 0.01 %) indicating an accumulation of biomass. The $\delta^{15}N$ values of the rhizosphere reached values as low as 1.4 ‰ which represents a clear connection to N_2-fixing bacteria (see above), whereas the sediment values of surface and 10 cm sediments never fell below 3.8 ‰. Conspicuous mats of benthic algae only accumulated at station 1 after spring tides. Due to a topographic depression this station remained flooded for several days after the tide. At stations 2-4 algal mats were not recognised visually.

The lower values during the dry season at stations 1 and 2 could be linked to the very harsh conditions in this part of the transect. Both are very dry and get inundated only at spring tides. Salt accumulated especially on the sediment surface where it could form encrustations mainly at station 2. At station 1, where the warm brackish water remained after the tide, the sediment conditions became very anoxic even at the sediment surface until the water evaporated and the soil turned into a hard crust exposed to the sun with only little shade as the *A. germinans* trees are merely small shrubs at this station. The plant cover of *S. portulacastrum* was reduced during the dry season at station 1, linking the C and N dynamics again to the dynamics of this plant (*Figure 5.1.9*).

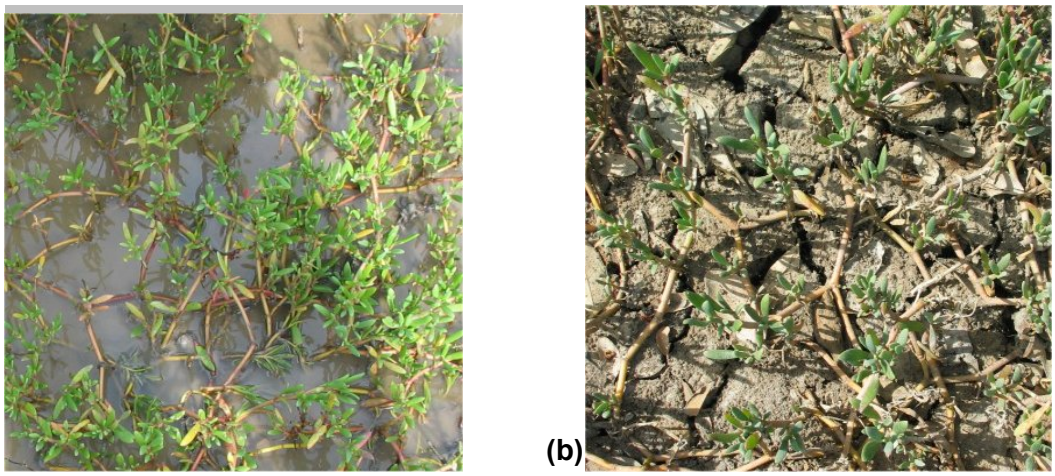


Figure 5.1.9: Abundance of *Sesuvium portulacastrum* in (a) rainy and (b) dry season at station 1 of transect 2.

Sesuvium plants themselves, however, did not show particularly high levels of TOC and TN. *A. germinans* and *S. virginicus* leaves had the highest TOC contents (42.5 ± 0.9 % and 43.2 ± 0.8 % TOC, respectively), *S. portulacastrum* followed with 35.7 ± 2.3 % TOC and *B. maritima* had the lowest values (27.7 ± 0.1 % TOC) during the rainy season. Dry season values were slightly lower (*Figure 4.2.13*). TN contents showed a different distribution: *A. germinans* contained the highest amount (2.2 ± 0.2 % TN), followed by *B. maritima* with 1.8 ± 0.04 %, *S. portulacastrum* with 0.9 ± 0.1 % and *S. virginicus* with 0.7 ± 0.1% TN (*Figure 4.2.14*). These values however do not give indications on nutrient limitations, uptake or dynamics, thus do not necessarily represent a contradiction to what has been discussed before. It was postulated earlier in this chapter that *S. portulacastrum* has found means to insure greater nutrient uptake or availability through symbiosis with mycorrhizal fungi and possibly N_2-fixing bacteria. This does not imply necessarily that TN and TOC levels in the plant should be higher than in others. On the contrary it could underline the ability of *S. portulacastrum* to deal with inhospitable conditions by being able to thrive with low nutrient and high stress levels.

Halophytes such as *S. portulacastrum* could play an important role in coastal or arid regions for agriculture adaptation to freshwater scarcity, coastal protection through substrate stabilisation (e.g. dunes) and also in regeneration and creation of wetlands. *S. portulacastrum* is a fast growing species which can cover barren soil in short times (Slama et al 2005). Its mat-forming growth habit holds and traps sediment and characterises this species as an important pioneer and colonising species in the tropics and subtropics (Lonard and Judd, 1997). The results of the current study indicate that by improving substrate conditions *Sesuvium portulacastrum* could possibly act as a pioneer plant in the

process of wetland restoration, an undertaking which is still poorly understood (Zedler, 2000; Zedler*, et al.*, 2003).

Isotopic composition

As for transect 1, the stable carbon and nitrogen isotopic composition of ecosystem components (i.e. leaves and sediments) was used to assess the interplay of physical and chemical parameters and their relationship to plant growth and nutrient dynamics. The relevance of $\delta^{15}N$ to understand possible N_2-fixing mechanisms was described above.

The importance of the photosynthetic mode of plants and its relation with carbon stable isotopic variation was described for C_3 plants in the previous section about transect 1 (5.1.1.1). In most plants, initial fixation of CO_2 occurs via a three-carbon compound, thus they are termed C_3 plants. C_4 plants are so named because they preface the Calvin cycle with an alternate mode of carbon fixation that forms a four-carbon compound as its first product. A unique leaf anatomy is correlated with the C_4 photosynthesis which enables the plant to fix CO_2 more effectively in hot and/or dry weather when the stomata are partially closed. Consequently C_4 plants evolve and thrive mostly in hot regions with intense sunlight. A second photosynthetic adaptation to arid conditions has evolved in succulent plants. These plants open their stomata during the night and close them during the day (just the reverse of how other plants behave) to prevent excessive water loss during the day. This mode of carbon fixation is called Crassulacean Acid Metabolism (CAM) after the succulent plant family Crassulaceae. The difference between CAM and C_4 plants is that the latter have the initial steps of CO_2 fixation structurally separated from the Calvin cycle, whereas in CAM plants the separation is through time, i.e. the CO_2 is fixed into organic acids at night and consequently released to the Calvin cycle during the day (Campbell*, et al.*, 1999) (*Figure 5.1.10*).

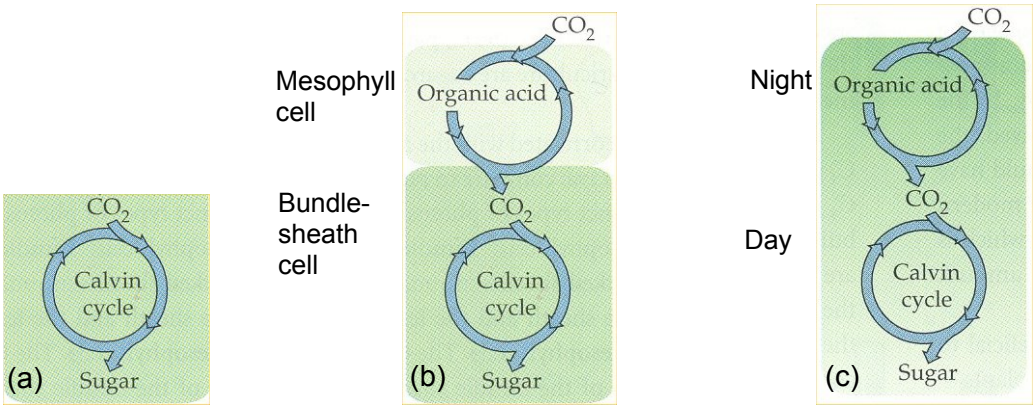


Figure 5.1.10: Diagram of the incorporation of CO_2 into the Calvin cycle in (a) C_3, (b) C_4 and (c) CAM plants. Modified after Campbell *et al. (1999).*

The $\delta^{13}C$ values measured in *A. germinans*, *S. portulacastrum* and *B. maritima* (-27.4 ± 1.3, -28.4 ± 0.5 and -27.6 ± 1.4 ‰ respectively) are typical for C_3 land plants (or CAM plants as C_3 and CAM plants can not be distinguished by means of their $\delta^{13}C$ values). *S. virginicus*, however, shows the distinctive characteristics of a C_4 metabolism (-15.4 ± 0.8 ‰), a typical feature of monocotyledonous plants such as grasses (*Figure 5.1.11*). The isotopic values in roots did not differ significantly from the leaves.

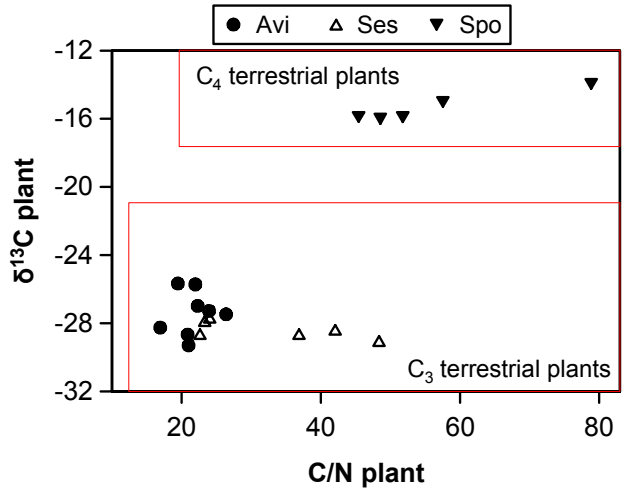


Figure 5.1.11: C/N versus $\delta^{13}C$ for *A. germinans* (Avi), *S. portulacastrum* (Ses) and *S. virginicus* (Spo) leaves in transect 2, red fields indicate classification into C_3 and C_4 terrestrial plants after Lamb *et al.* (2005). Data pooled from both seasons.

The correlation of $\delta^{13}C$ values with environmental factors such as soil salinity (*Figure 5.1.12*) and inundation frequency (*Figure 5.1.13*) showed a relationship only for *A. germinans*. With regard to salinity this correlation only occurred during the dry season. The influence of environmental stresses on stomatal conductance and consequently on $\delta^{13}C$ values has been introduced in section 5.1.1. For *S. virginicus* and *S. portulacastrum* there was no significant relationship between $\delta^{13}C$ values and both environmental factors (only data for *Sesuvium* is shown in the figure). *S. virginicus* uses the C_4 modus for photosynthesis (indicated by the high $\delta^{13}C$ values) and *S. portulacastrum* can possibly switch to the CAM pathway, which is common to many succulent plants. The photosynthetic adaptations of these two plant species may explain why their CO_2 uptake and consequently their $\delta^{13}C$ composition are less affected by environmental factors than it is the case for *A. germinans*. The C/N values of the sediment at all depths had no influence on $\delta^{13}C$ values in plants of this transect.

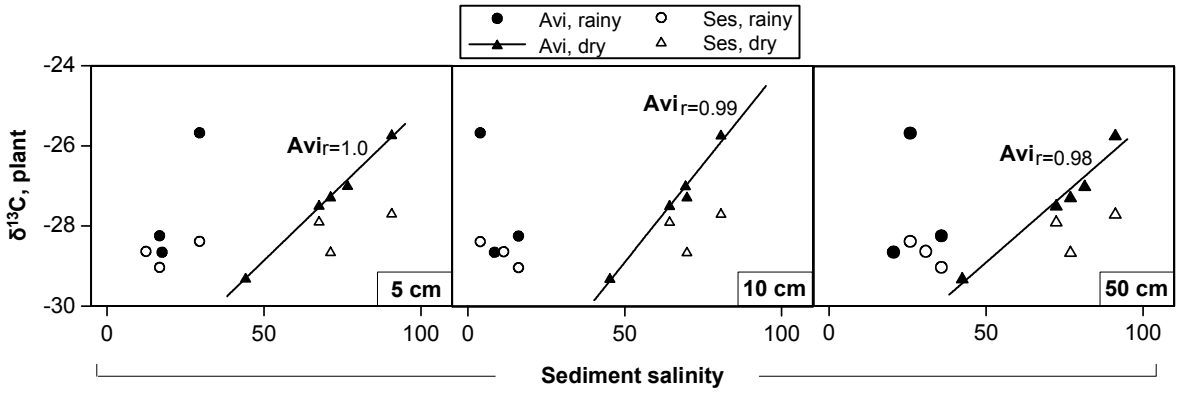


Figure 5.1.12: Relationship between $\delta^{13}C$ isotopic composition of leaves from *A. germinans* (Avi) and *S. portulacastrum* (Ses) versus sediment salinity in 5 cm, 10 cm and 50 cm depth during rainy (circles) and dry season (triangles) in transect 2, regression line and correlation coefficient only shown for *A. germinans* during dry season, n=5, p<0.001.

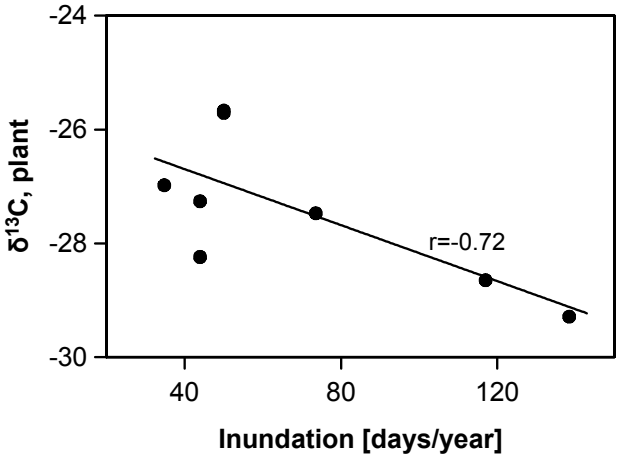


Figure 5.1.13: Correlation between $\delta^{13}C$ values of leaves of *A. germinans* and inundation frequency in transect 2, n=7, p<0.05.

As was described for transect 1, the $\delta^{13}C$ values in sediments ranging from -26.5 to -21.8 ‰ were less negative than in the corresponding plants (with the exception of *S. virginicus*) with values from -29.3 to -25.7 ‰ (*Figure 4.2.12*). The curves for $\delta^{13}C$ values in surface and 10 cm sediments showed an interesting feature for stations 1-4 where they are mirroring each other in both seasons, the values for 10 cm going up and then down and the surface values showing the opposite trend. The surface sediments have the highest peak at station 4 where the grass *S. virginicus* was most abundant. *S. virginicus* differs in its $\delta^{13}C$ from the other plants in the transect (see above) and an influence on the surface sediments is likely. Thus a trend towards more positive values could be expected from the surface sediments at stations 3-5 coinciding with the growth of *S. virginicus*. However the surface sediments only showed a conspicuous peak at station 4. At station 3 the occurrence of all three other plant species might mask the influence of *S. virginicus* and at

station 5 a greater influence of the tide may lead to a greater wash-out. The peak was greater during the rainy season than during the dry season, suggesting that the heavy tropical rains lead to greater leaching from the plants and plant litter.

The fact that the curves for $\delta^{13}C$ values at 10 and 50 cm differ from the surface sediments leads to the assumption that there may be two different C pools in the sediment: one at the surface with strong influence from the present vegetation and one in the deeper sediment layers representing more refractory and/or decomposed organic matter. The TOC and TN data discussed above emphasise this theory. TN and TOC values in the surface sediments were strongly influenced by the presence of *S. portulacastrum*.

For $\delta^{15}N$ the values measured in plant tissue varied greatly ranging from 2.8 ‰ in *S. portulacastrum* to 7.5 ‰ in *A. germinans*. As has been proposed before for transect 1, $\delta^{15}N$ variations can be linked to variation in source $\delta^{15}N$. In the case of *S. portulacastrum*, the option of a different nitrogen source through associated N_2-fixing bacteria has been discussed earlier in this section. The low $\delta^{15}N$ values in the rhizosphere of the plant (1.4 to 3.8 ‰) possibly represent the bacterial, N_2-fixing link between the generally higher values in the surrounding sediments (5.9 ± 0.7 ‰, 6.3 ± 0.8 ‰ and 6.4 ± 0.6 ‰ for surface 10 cm and 50 cm sediments respectively) and the plant (2.7 to 4.6 ‰).

In contrast to findings in transect 1, sediment salinity in transect 2 had no apparent effect on $\delta^{15}N$ composition of plant material, whereas inundation frequency seemed to influence the isotopic N composition in *Avicennia* leaves in a non-linear fashion (*Figure 5.1.14*). In transect 1, the correlation was negative, i.e. lower inundation frequencies (higher stress) resulted in higher $\delta^{15}N$ values. For transect 1 it was postulated that when stress is high, i.e. the plant has additional energetic costs due to e.g. salinity, drought or nutrient limitation all N is typically used regardless of isotope, resulting in higher $\delta^{15}N$ values in the leaves. It is possible that the presence of the herbaceous vegetation in transect 2, namely of *S. portulacastrum* at stations 1-4 improves the sediment quality and thus counteracts some of the implications of high salinities and low inundation frequencies.

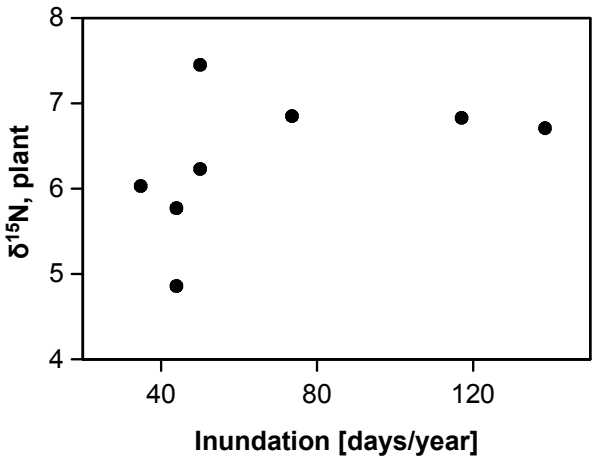


Figure 5.1.14: Influence of inundation frequency on $\delta^{15}N$ values of *A. germinans* leaves in transect 2, n=8.

5.1.2 Tannins in soils and plants

5.1.2.1 Transect 1: Tannins

Leaves of *R. mangle* contained a more than 4-fold concentration of tannin (83.7 ± 23.4 TAE/g dw) than *A. germinans* (18.3 ± 5.3 mg TAE/g dw). The highest TAE value of 12 % of dry weight was found in *R. mangle* leaves during the dry season (*Figure 4.2.17*). Such significant differences between the two mangrove tree species are reported by other authors even when using different analysis methods hence possibly determining a different pool of polyphenolics. McKee (1995) for example found much higher levels of total phenolics (measured as Gallic Acid Equivalents) and of condensed tannins in *R. mangle* and *Laguncularia racemosa* compared to *A. germinans* in a mangrove forest in Belize. Condensed tannins were not detectable in *A. germinans*.

One characteristic determining plant success is related to how well its tissues are protected against herbivores. Loss of even small amounts of tissue can have large effects on growth, survival and reproduction of plants (McKee, 1995). Phenolic compounds such as tannins, predominate as anti-herbivore protection in plants growing in nitrogen-limited environments (McKee, 1995). They have been shown to inhibit growth and survival of many herbivores, therefore acting as a feeding repellent (Zucker, 1983), its major defensive power being attributed to its ability to bind proteins (see also below in sediments). A negative effect of phenolic compounds on mangrove herbivores is still discussed controversially in the literature (Kraus*, et al.*, 2003), but observations in the study area confirmed the hypothesis. In March 2003 and 2004 the larvae of the moth *Hyblaea puera* efficiently defoliated large areas of *A. germinans* forest (*Figure 5.1.15*), but

was not found to feed on *R. mangle* leaves in direct vicinity of the infested *Avicennia* trees. The high concentration of tannins in the *R. mangle* leaves is a probable explanation for the behaviour of the larvae. Mehlig (2001) observed the same phenomenon in 1998.

Figure 5.1.15: Defoliated *A. germinans* trees after an attack of larvae of the moth *Hyblaea puera* in transect 1.

Tannins are well-known to be toxic and bacteriostatic undergoing non-reversible reactions with proteins (Scalbert, 1991) and have been found to inhibit the activity of enzymes in rumen microbes. In monogastric animals hydrolysable tannins such as tannic acid can cause kidney or liver necrosis. Nevertheless, some bacteria may degrade phenolic compounds through aerobic and anaerobic pathways (Bhat*, et al.*, 1998). Some herbivores (insects and ruminants) have developed mechanisms to overcome the adverse effects of tannins by harbouring a specific gastro-intestinal microflora of tannin-tolerant and -degrading microorganisms. The enzyme tannase has been described as early as 1913 (Bhat*, et al.*, 1998). The mangrove crab *Ucides cordatus* which represents about 84 % of the benthic biomass in the study area, has been reported to show a strong food preference for the N-poor and tannin-rich *R. mangle* leaves (Nordhaus). In a separate experiment (see section 4.3.3 in Results) it was demonstrated that the gut flora of *U. cordatus* is able to break down hydrolysable tannins such as commercial tannic acid.

In addition to their potential role in anti-herbivore defence, phenolic compounds may also protect leaves from high levels of solar radiation (Lovelock*, et al.*, 1992). Ultraviolet (UV) radiation has been shown to be destructive to plants. Exposure to enhanced UV-B radiation inhibits plant growth and depresses photosynthesis (Caldwell*, et al.*, 1989). In the tropics where UV-radiation is high, this might be an important factor influencing the synthesis of phenolic compounds in mangrove trees. Lovelock *et al.* (1992) observed higher levels of soluble phenolic compounds in sun leaves compared to shade leaves in

three different mangrove species. Although it would neither explain the increasing trend from the humid to the dry part of the transect nor the differences between the two species, it could however coincide with the increasing values in the dry season, as the precipitation rates and therefore the cloud cover are much higher in the rainy season.

Tannin content in *R. mangle* and *A. germinans* correlated positively in both seasons (*Figure 5.1.16*). With a correlation coefficient of r=0.83 the relationship was stronger during the dry season. The higher axis intercept for the dry season curve depicts the significant increase of tannin content in *R. mangle* especially in the landward part of the transect. *A. germinans* showed a slight, but not significant increase at some stations. However, both species show a positive correlation with soil salinity at all depths, the relationships being strongest at 5 and 10 cm (*Figure 5.1.17a-b*). High soil salinities result in highly conservative use of water in mangroves, which depresses photosynthesis rates during periods of high solar radiation and high leaf temperatures. The $\delta^{13}C$ composition of *R. mangle* and *A. germinans* leaves in this study gave evidence of a significant impact of sediment salinity on photosynthesis metabolism. Other authors have reported that under additional environmental stress such as drought or salinity, photosynthesis rates were less affected when UV-absorbing pigments in the leaves were high (Lovelock*, et al.*, 1992). Tannin content also shows a negative relationship to inundation frequency, which is another potential stress factor, although correlations were not significant (*Figure 5.1.17c-d*).

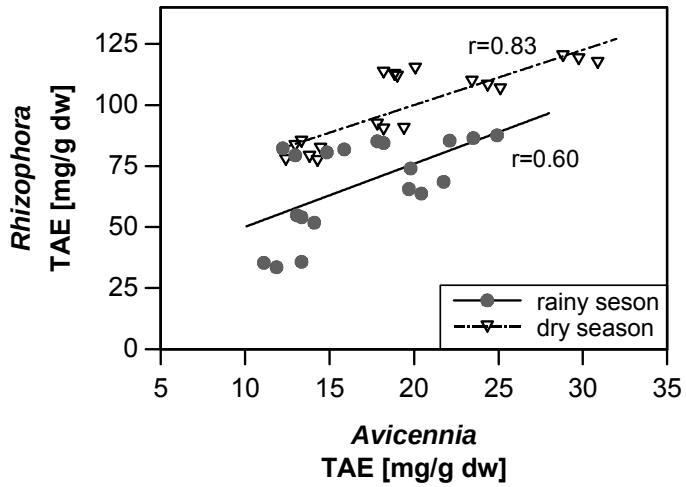


Figure 5.1.16: Tannin concentrations (as TAE) of *A. germinans* versus tannin concentrations of *R. mangle* leaves in transect 1, n=19, correlations are significant at p<0.01.

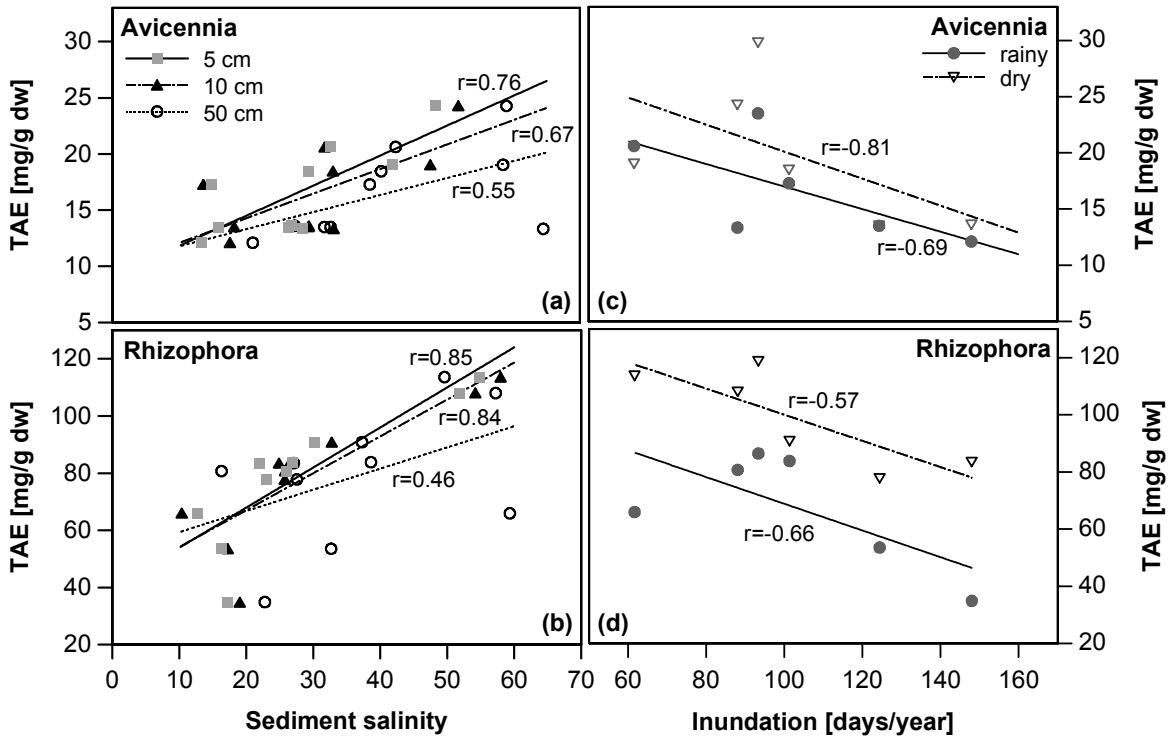


Figure 5.1.17: Correlation between tannin concentrations (as TAE) in leaves of *A. germinans* and *R. mangle* versus (a, b) sediment salinity at 5, 10 and 50 cm depth and (c, d) inundation frequency in transect 1, n=10, correlations were significant when r>0.60; or (c, d) inundation frequency, n=6, correlations were not significant.

Of both measured stress factors (sediment salinity and inundation frequency) the correlation was strongest for salinity. As was demonstrated with the isotopic compositions of C and N (section 5.1.1.1), lower inundation frequencies represent higher stress levels, in this case refelechted in higher tannin content. It is however likely that not one factor alone is the sole cause for changes in tannin concentrations. The biochemical composition of the plant tissues characteristic for each species determines the basic levels (low in *Avicennia*, high in *Rhizophora*), but an interplay of environmental stress factors such as sediment salinity, inundation frequency, possibly UV-radiation and herbivory are controlling the fluctuations in tannin levels.

The two species show distinct characteristics in terms of TN, TOC, C/N in relation to tannin content, but do not show any correlation between these factors (*Figure 5.1.18*).

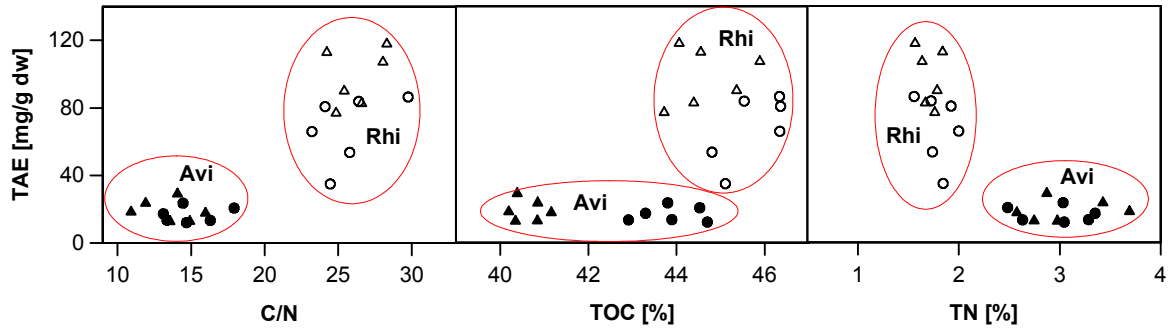


Figure 5.1.18: Tannin content (as TAE) versus TN, TOC and C/N in leaves of *A. germinans* (Avi) and *R. mangle* (Rhi) during rainy (circles) and dry season (triangles) in transect 1.

Polyphenols from plants can (passively) enter the soil by two pathways: through the leachate from plant parts (above- and belowground) and through plant litter, the latter probably being the larger input (Hattenschwiler and Vitousek, 2000) although little is known about the relative contributions of both pathways. The insoluble fraction of condensed tannins might predominate by the time the polyphenols reach the sediments as the hydrolysable fraction undergoes a higher turnover in plant tissue. Condensed tannins are considered to be metabolic end products with minimal turnover and a tendency to accumulate in ageing tissues (Maie*, et al.*, 2003). Increases in protein-binding capacity of condensed tannins in senescent tissues compared to green leaves have also been observed (Hattenschwiler and Vitousek, 2000). In the present work the values of the tannins measured with the Prussian Blue Assay in green and yellow leaves on *R. mangle* trees in a different forest suggest no resorption of the measured tannins before leaf detachment or the synthesis of other polyphenolics such as the pigment group of anthocyanins (see *Figure 5.2.1* and Discussion in 5.2.2). On the contrary the tannin concentrations even increased in the yellow leaves compared to the green leaves attached to the tree. This phenomenon will be discussed in detail in relation to the decomposition experiments.

In the present study sediment values of tannin at 50 cm depth showed a clear upward trend towards the *Rhizophora*-dominated part of the forest (*Figure 4.2.16*), possibly giving an indication on the species of woody plants which have been dominating this area. Behling et al (2001) estimated by pollen analysis and radiocarbon dating that mangrove vegetation at the present study site started to develop approximately 2100 ^{14}C yr BP. The depth of 50 cm corresponds to approximately 400-600 cal yr BP (Cohen*, et al.*, 2005a; Cohen*, et al.*, 2005b). It would be possible that the species pattern 400-600 yrs ago was similar to the present situation, thus explaining the accumulation of refractory polyphenol-

protein complexes or of condensed tannins in the deeper sediments of the *Rhizophora*-dominated area.

Boto *et al.* (1989) explained tannin accumulation at 60 to 70 cm depth through diffusion along a concentration gradient both towards the surface and towards the deeper sediments from the point of expected tannin production, which in their study was the rooting zone at 5 to 30 cm. The tannin which diffuses towards the surface would be rapidly utilised by bacteria in this zone. This theory would however not explain the tannin peaks at the surface and subsurface sediments in the *Avicennia*-dominated part of the forest especially during the rainy season. Tannin concentrations at the surface and in 10 cm depth displayed a u-shaped distribution with highest values at the beginning and at the end of the transect. The first stations of the transect (located in the *Avicennia*-dominated area) have the lowest inundation frequency. In transect 2 (see next section) we also find high tannin concentration in surface sediments of stations with the lowest inundation frequencies during the rainy season. These values might be a function of both high precipitation rates during the rainy season inducing stronger leaching from the plants, and the intertidal position, i.e. less export of organic matter through the tides.

Polyphenolics can influence litter quality and decomposition rates by various effects on the activity of soil organisms. Different types of phenolics can have varying effects on bacteria and mycorrizal fungi; they can inhibit as well as stimulate (Kuiters, 1990). The abundant but often contradictory data on this topic must be treated with precaution as most data are from laboratory or artificial experiments. In the decomposition experiments of the present study we found higher bacterial counts on *R. mangle* than on *A. germinans* leaves although the latter have less tannin content. In both the laboratory and the field experiment, tannins were lost from the leaves very quickly, partly through leaching, but also through microbial degradation. Hence the bacterial community found on decaying mangrove leaves were not inhibited by the present polyphenolics.

The inducible enzyme tannase which hydrolyses the ester linkages of tannic acid has been described in a variety of bacteria, yeasts and filamentous fungi (Iacazio*, et al.*, 2000). It has an activity optimum at pH 5-6 and has been found unstable above pH 6 or 8. In deeper sediments (10 and 50 cm) of this transect pH values were always above 6.4 hence the concentration of polyphenolics in the deeper sediments could not only be due to the refractory nature of the substances, but could also be related to the activity ranges of the enzyme although this would only be relevant for hydrolysable tannins.

Physico-chemical properties of the sediments such as pH or Eh not only influence enzyme activities, but are also likely to be related to concentrations of phenolic substances and their implications for plant growth. Acidic soil conditions for example have been shown to prolong the life-time of phenolic acids in morhumus sites, whereas in calcareous soils most phenolics were rapidly metabolised or immobilised e.g. through adsorption to clay minerals or complexation with Aluminium (Al) or Iron (Fe) ions (Kuiters, 1990). Generally pH and Eh of sediments are related to inundation frequency, the higher the inundation frequency, the higher the pH as the influence of sea water is greater and the lower the Eh as anaerobic conditions are prevalent (Saenger, 2002). In this transect pH varied rather with depth than with station or inundation frequency (*Figure 4.1.2*), but the redox potential was negatively correlated with inundation frequency (Mendoza, unpublished data). Thus the tannin concentrations in the sediments are possibly not only related to the past and present vegetation but are also a function of the inundation dynamics.

The data of the decomposition experiments showed that *R. mangle* trees efficiently resorbed more than 60 % of the TN present in green leaves before detachment of the senescent yellow leaves, but contrarily increased the concentration of tannins (or possibly phenolic pigments) in senescent leaves compared to green leaves (see below *Figure 5.2.1*). Northup *et al.* (1995a; 1995b; 1998) and other authors (Kuiters, 1990; Hattenschwiler and Vitousek, 2000) suggested an active role of the plants and the polyphenols in the soil nitrogen dynamics for terrestrial forests on acidic and infertile soils. The impact of plant polyphenolics on N cycling is interpreted by these authors as a plant adaptation to nitrogen limitation that effectively monopolises the N contained in litter by immobilising it into a form (polyphenolics or polyphenol-protein-complexes) for which the plant's associated mycorrhizae have been shown to have a competitive acquisition advantage. The possible applicability of this theory on mangrove ecosystems will be discussed further below (section 5.1.3.1). The occurrence of mycorrhizae in mangrove species has been demonstrated by various authors (Sengupta and Chaudhuri, 2002) and has been described in transect 2 of the present study, but up to today the question if mangrove ecosystems are to be classified as nutrient-rich or -poor has still not been settled. However, in the present work $\delta^{13}C$ values in *R. mangle* and *A. germinans* leaves gave evidence of a possible reduction of N availability or of N uptake efficiency linked to salinity stress .

It has also been suggested that polyphenolics can enhance P availability by desorbing previously fixed phosphate and lowering Al, Fe and Mn activities (Northup*, et al.*, 1998; Georgantas and Grigoropoulou, 2006). In transect 1 of the present study plant available P

increases towards station 10 in sediments down to 50 cm, which is generally attributed to lower Eh and the consequent liberation of the available P from iron (III) hydroxide (Mendoza, unpub. data). However, the synchronous development of tannin concentrations with available P may imply another link between the availability of P and the presence of tannins.

5.1.2.2 Transect 2: Tannins

Tannin in surface and 10 cm sediments of transect 1 showed a similar pattern to TOC and TN content in surface sediments especially in the rainy season, where values for the first stations were much higher than in the *Avicennia*-dominated area of the transect (*Figure 4.1.18*). Tannin has mainly been described in woody plants and has not been associated frequently with herbaceous plants such as *Sesuvium*, to which the TOC and TN increases have been attributed. However, *S. portulacastrum* plants contained about 40 % the amount of tannin of *A. germinans* leaves during the rainy season (6.1 ± 1.2 mg TAE/g dw in *S. portulacastrum* and 15.1 ± 3.9 mg TAE/g dw in *A. germinans*) and showed even higher values during dry season (22.4 ± 6.4 mg TAE/g dw in *S. portulacastrum* versus 30.1 ± 10.2 mg TAE g/dw in *A. germinans*). The content in the roots was generally lower than in the leaves. These results underline the assumption that both hydrolysable and condensed tannins are extracted and measured with the Prussian Blue Assay. Condensed tannins are most abundant in woody species, but often absent in herbaceous plants (Hättenschwiler 2000). Thus in *A. germinans* and *R. mangle* we probably measured condensed and hydrolysable, whereas in *S. portulacastrum* the response can be mainly attributed to hydrolysable tannins.

Kuiters (1990) and Northup *et al.* (1995a; 1995b; 1998) suggested an active role of the plants in tannin dynamics. The review proposed short-term conservation of nitrogen through polyphenol-protein complexes. This so-called ‘tanning’ process has been interpreted as a type of storage mechanism of nitrogen in leaves (polyphenol-protein complexes during senescence) in temperate zones (see also 5.1.3), the intention being to create a pool of N in the sediment that is released slowly and not so easily lost from the system. Mycorrhizal fungi have frequently been reported as being able to utilise refractory material such as proteinaceous source materials that have been precipitated with tannin or other recalcitrant polymers as a nitrogen source (Finlay and Söderström, 1992; Read, 1992). Thus we might be looking at a complicated picture where nutrient dynamics are not only influenced by physico-chemical parameters, decay and bacterial degradation, but also by controlled interactions between the plant, the mycorrhizal fungi and the surrounding substrate. Without any further information about the nature of the mycorrhiza,

the enzymes present and the actual tannin and nitrogen flow, the intrinsic tannin dynamics will remain a matter of speculation. However, the direction in which the data of the present study point, reveal another piece in the puzzle about the high TOC and TN contents in the surface sediments of these stations.

The high tannin values in surface sediments could also be linked to leaching from the canopy of *A. germinans* and from the herbaceous plants or from leaf litter during the rainy season. As has been discussed before for the $\delta^{13}C$ values, the surface sediments might have a stronger relationship to the vegetation's biochemical signature than the deeper sediments. The difference between the first few and the last few stations may come from the difference in inundation frequency. The first stations are less frequently subjected to tidal flooding, thus the litter or leachates remain for a longer time on the sediment surface having greater opportunity to imprint their signature.

In transect 1 tannin in plant leaves was linked to stress factors such as salinity and inundation frequency. In transect 2 we do not find a significant relationship between tannin content in leaves and inundation frequency, but there was a significant increase of tannin content in all species during the dry season (*Figure 4.2.19*). Within the dry season *A. germinans* and *S. portulacastrum* leaves showed a positive relationship between leaf tannin concentration and salinity at 10 cm depth (*Figure 5.1.19*).

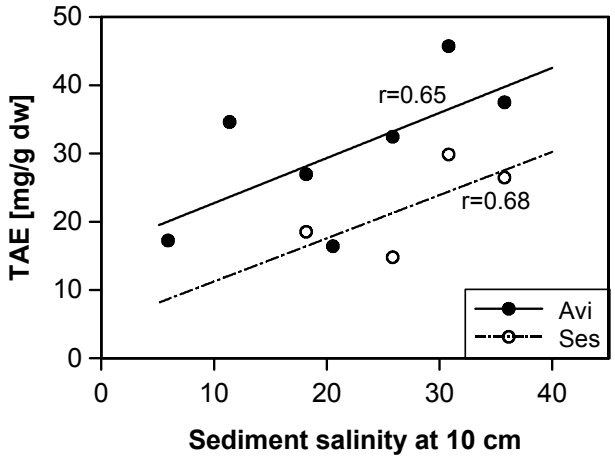


Figure 5.1.19: Relationship between tannin content (as TAE) in leaves of *A. germinans* (Avi) and *S. portulacastrum* (Ses) and sediment salinity at 10 cm depth during the dry season in transect 2, correlations are not significant.

There was a significant difference between the tannin concentrations in *A. germinans* leaves in the two transects. In the dry season, leaves of *A. germinans* had significantly lower tannin concentration at the driest stations of transect 1 (23.8 ± 4.8 mg TAE/g dw,

stations 1-5) than at the driest stations of transect 2 (35.4 ± 6.5, stations 1-5), confirming the frequently reported observation of a convergent evolution of tannin-rich plant communities on highly stressed soils (Northup*, et al.*, 1995b). If tannins are acting as defence against herbivores as has been discussed for transect 1, then tannin increase in relation to stress may indicate that the plant is allocating more energy into the defences as it cannot afford to loose leaves when stress is high.

It has been suggested in the last section that, phenolic compounds in their function as protectors against UV-radiation might increase in more arid and saline sites (Lovelock*, et al.*, 1992). High sediment salinities result in highly conservative use of water in mangroves, which depresses photosynthesis rates during periods of high solar radiation and high leaf temperatures. The $\delta^{13}C$ composition of *A. germinans* leaves in this transect gave evidence of a significant impact of sediment salinity on photosynthesis metabolism during the dry season (*Figure 5.1.12*). It has been reported that under additional environmental stress such as drought or salinity, photosynthesis rates were less affected when UV-absorbing pigments in the leaves were high (Lovelock*, et al.*, 1992). Thus, the increase of tannins for all species during the dry season and the correlation of leaf tannin content with salinity during the dry season may be connected to seasonally increased stress level in terms of water uptake, salinity and UV-radiation.

However, tannin content was highest at station 4 for all species. Thus there must be more factors involved in the control of tannin content in plant tissues as the stress factors considered in this work increased towards station 1 and 2 and did not peak at station 4.

At 50 cm depth tannin content was very low at stations 1-6 (0.1 ± 0.03 mg TAE/g dw) and was suddenly raised to 0.5 ± 0.03 mg TAE/g dw at station 7 in both seasons (*Figure 4.2.18*). An increase of tannin content was also determined in surface substrate at that station. Station 7 has the highest tree heights and is the only station of the transect that has had a longer history of woody plants (Cohen*, et al.*, 2005a; Cohen*, et al.*, 2005b). As has been discussed for transect 1 (previous section) tannins can form refractory polyphenol-protein-complexes which can survive for many 1000 years and hence could function as ‘paleo-indicators’, giving a hint on the time span for which woody vegetation has been dominating the area. When discussing subsurface samples of vertical profiles it must be kept in mind, that sediment parameters are not only influenced by present-day dynamics, but are also a function of the paleo-vegetation which populated the area hundreds of years ago. Pollen analysis and radiocarbon dating for the area of transect 2 relates sediments at 50 cm depth to an age of 400-600 cal yr BP and reveals a history of

hypersaline tidal flats, i.e. barren land for all stations except station 7 (Cohen*, et al.*, 2005a; Cohen*, et al.*, 2005b). At station 7, however, mangrove pollen at 50 cm depth indicates that this area was populated by mangroves approximately 400-600 ^{14}C yr BP as was postulated above from the tannin profiles at that depth.

The sediment pH of these deeper sediment layers may also be responsible for the difference in tannin concentration between stations. At stations 1 and 4 where tannin content is low, the pH ranges between 4.5 and 5.0, whereas at station 7 where tannin concentration is greatest sediment pH rises to 7.2 ± 0.2. As discussed in the previous section, the enzyme tannase has an activity optimum at 5-6 and has been found unstable above pH 6 (ref) or 8 (ref). Thus, if higher tannin concentrations had been present at stations 1-6, the low tannin concentrations could also be related to the activity of microbial decomposers producing tannase although this would only be relevant for hydrolysable tannins and not for the stable condensed tannins or tannin-protein-complexes. As was concluded for transect 1, the tannin concentrations in the sediments are therefore not only a function of the past and present vegetation but also of the inundation dynamics.

5.1.3 Total hydrolysable amino acids (THAA) in soils and plants

5.1.3.1 Transect 1: Total hydrolysable amino acids

Amino acids are the most abundant nitrogen-bearing compound in most organisms and total amount as well as the distribution of single amino acids and their enantiomeric ratios can provide a powerful tool for characterising source and reactivity of nitrogenous material (McCarthy*, et al.*, 1998).

The concentration of THAA in sediments in the present study ranged from 2.1 mg/g dw in the deeper sediment layers to 11.2 mg/g dw at the surface, which is consistent with the results of other authors working with marine and coastal sediments (Stanley*, et al.*, 1987; Jennerjahn and Ittekkot, 1997; Colombo*, et al.*, 1998; Dauwe*, et al.*, 1999a; Dauwe*, et al.*, 1999b; Unger*, et al.*, 2005a; Unger*, et al.*, 2005b). At most stations a drop of THAA levels was found towards the deeper sediments.

In the previous sections a theory was introduced suggesting an active role of the plants and the polyphenols in the sediment nitrogen dynamics (Kuiters, 1990; Northup*, et al.*, 1995a; Northup*, et al.*, 1995b; Northup*, et al.*, 1998; Hattenschwiler and Vitousek, 2000). The impact of plant polyphenolics on nitrogen cycling is interpreted by these authors as a

plant adaptation to nitrogen limitation that effectively monopolises the N contained in litter by immobilising it into a form (polyphenolics or polyphenol-protein-complexes) which is not so easily lost to the ecosystem through leaching. This strategy of adaptation and N immobilisation however has been proposed for terrestrial forests. In terrestrial ecosystems the significance of such a strategy is clear as leaf litter remains within the system. In mangrove ecosystems the situation is different as up to 97 % of the leaf litter is either exported by the tides or consumed by crabs (see Schories*, et al.*, 2003 for the Bragança peninsula), while in terrestrial forests, leaf litter decays mainly on the sediment surface. The consumption by crabs implies a faster breakdown of the decaying material. The mechanical breakdown of the leaves makes the organic matter more susceptible to subsequent microbial breakdown. It has been shown in this study that crabs can digest hydrolysable tannins, but there is no information yet about the digestibility and utilisation of condensed tannins or stable tannin-protein-complexes. Thus, although 67-81 % of the leaf litter is consumed and broken up by crabs on the Bragança peninsula (Schories*, et al.*, 2003; Nordhaus, 2004), the immobilised tannins may still remain in the system.

To assess a possible cycle from the plant to the sediment, THAA and tannin content of the yellow leaves shortly before detachment would be of importance. As leaves of this stage were not measured in this study, our assumptions will be based on values of green leaves and sediments.

By creating a THAA/Tannin (w/w) ratio some information about possible N-immobilisation through tannins can be obtained for this transect (*Figures 5.1.20* and *5.1.21*). *Avicennia* leaves had THAA/Tannin values above 3.0, i.e. high THAA and low tannin content, whereas values for green leaves of *R. mangle* never exceeded 1.3 (high tannin and lower THAA content). For the sediments two conclusions can be drawn from the THAA/Tannin ratios of surface, 10 cm and 50 cm sediment layers. First, surface values are generally linked to the vegetation characteristics with higher values in the *Avicennia*-dominated area and close to *A. germinans* trees, whereas lower THAA/Tannin values dominated in the *Rhizophora*-dominated region and near *Rhizophora* trees. Second, at 50 cm depth the tannins gain importance in relation to the THAA values (i.e. ratios decrease) towards station 10, highlighting the increase of tannin and decrease of THAA concentrations in the *Rhizophora*-dominated area. Thus from the present data set we can not deduce a close or consistent relationship between THAA and tannin content in the sediment. However, as will be shown below, THAA measured in sediments do not only originate from the decomposing leaf litter, but have other sources such as microbial biomass which might be using external N sources such as N_2-fixation, whereas tannin is probably introduced to the

system only by decaying plant material. Hence the different origin of the two parameters may be influencing the results. High THAA/Tannin ratios at the surface could also be ascribed to benthic algae, although unattached microalgae are generally low in tropical sediments including mangroves. A rich macroalgal flora attached to pneumatophores, stilt roots and bases of mangrove trees are more frequently reported, but would not influence the surface sediments (Alongi and Robertson, 1992).

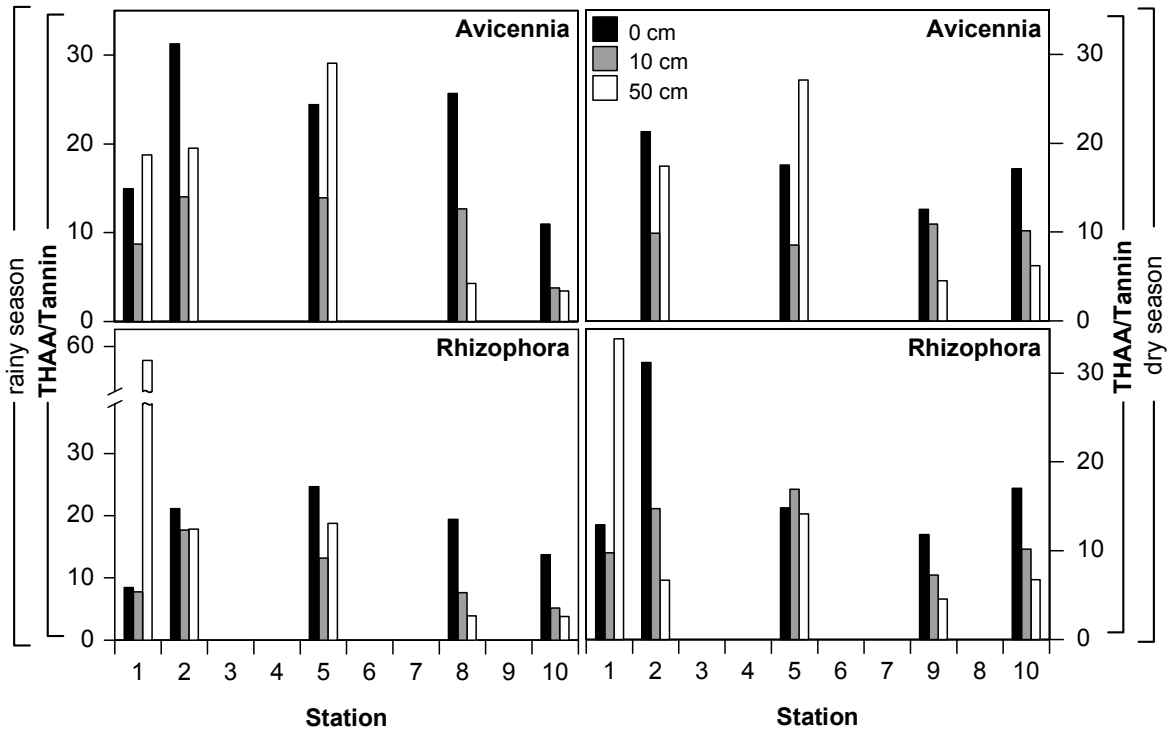


Figure 5.1.20: THAA/Tannin (w/w) ratios for sediment samples near *Avicennia*- and *Rhizophora*-trees, during rainy and dry season for transect 1.

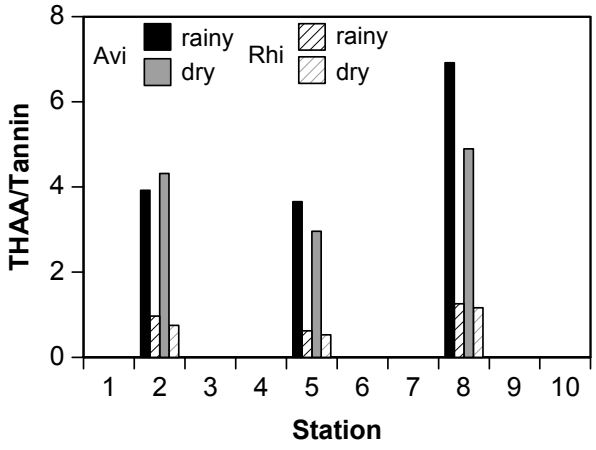


*Figure 5.1.21:*THAA/Tannin (w/w) ratios for leaf samples of *A. germinans* (Avi) and *R. mangle* (Rhi) during rainy and dry season for transect 1.

The proportional distribution (in mol%) of individual amino acids was relatively invariant between seasons, stations and *Rhizophora*- and *Avicennia*-sediments with L-asx, L-glx, gly and L-ala being the dominating L-amino acids, and D-asx, D-ala, and in some samples D-ile, prevailing for the D-enantiomers (*Figures 4.2.24* and *4.2.25*). Similar amino acid compositions have been found in various organisms and sediments (Jennerjahn and Ittekkot, 1997; Unger*, et al.*, 2005a; Unger*, et al.*, 2005b). Variations in monomeric composition have been attributed to the alterations occurring during decomposition due to different reactivities of bonds between individual monomers (Dauwe*, et al.*, 1999a; Dauwe*, et al.*, 1999b; Unger*, et al.*, 2005b). A preservation of gly that has been reported by several authors for marine sediments and the open ocean (Hubberten*, et al.*, 1994; Unger*, et al.*, 2005a) is found only at some stations and not always in the deeper sediments.

L-asx often decreased with depth at station 1, but does not show this consistent tendency in the *Rhizophora*-dominated area of the forest at station 10. L-ala, L-ser and L-thr are generally depleted with depth. The literature on this topic is very abundant but does not always reach the same conclusions. Dauwe *et al.* (1999) link the resulting shifts in biochemical composition to the degradation state in sinking particles and in deep-sea sediments by comparing enzymatically hydrolysable amino acids with THAA in unaltered source organisms (phytoplankton, bacteria, zooplankton). They assign preferential accumulation of gly and thr to their concentration in diatom cell walls that are preserved during sinking and decomposition, whereas amino acids that are concentrated in the cytoplasma such as tyr, phe and glu tend to be depleted during degradation (see also Hubberten et al 1995 in sea water). Unger *et al.* (2005b) found an enrichment of gly, asp, ser and thr relative to fresh organic matter in shallow trap particles, while relative abundance of glu and ala decreased.

However, most of these references deal with environments such as open ocean and deep-sea marine sediments. The situation in mangrove sediments might be a different one and so far not much literature has been published on the distribution of individual amino acids in the mangrove environment. The results published by Jennerjahn and Ittekkot (1997) and Stanley *et al.* (1987) correspond well with the findings in this study with asp, glu, gly and ala dominating the spectra. However, this composition of L-amino acids does not differ essentially from the results found in other environments, with the exception of gly which is usually dominating in marine sediments. Gly has been associated to diatom cell walls (see above), which are a major primary source of organic matter in oceanic

environments, but may not play such an important role in the mangrove ecosystem. Here the amino acid composition is more likely related to (1) the mangrove vegetation, i.e. decomposing plant material and exudates from roots and (2) the microbial decomposers in the sediment. Amino acid concentrations in water overlying sediments at high tide were found to be 10 times lower than in the sediment pore water (Stanley *et al.*, 1987), thus have not been attributed a significant import function to mangrove sediments. Gly is a ubiquitous small amino acid which is used in most proteins, thus its presence can not be exclusively linked to diatom walls. The L-amino acid spectra of *R. mangle* and *A. germinans* were essentially similar to the composition in the sediments (*Figures 4.2.26* and *4.2.27*), although in leaves the proportion of L-leu was more prominent and gly showed a significant proportional increase during the dry season. Amino acid composition in leaves will be discussed further below.

In terms of absolute amounts (mg AA/g dw, see Appendix xy), L-asx and L-glx were still dominating, followed by L-leu and L-ala, whereas gly became less important. The distribution was comparable to the absolute amounts found in the leaves of both tree species (see below), especially in the surface sediments and in some cores also in the 10 cm layer. At a depth of 50 cm, absolute amounts of L-amino acids were moving towards a more uniform distribution especially at station 1. Thus the L-amino acid dynamics corroborate the postulated assumption that the surface sediments are linked to the vegetation whereas the deeper layers are influenced stronger by the decomposers (see section 5.1.1.2).

The distribution of D-amino acids coincided with general trends described in the literature (Amon*, et al.*, 2001). Mol% of D-asx, D-ala and D-ser increased substantially with depth (*Figures 4.2.24* and *4.2.25)*. All three enantiomers have been reported in bacterial peptidoglycan and in some bacterial antibiotics (Tremblay and Benner, 2006). D-ala in particular has been used as a microbial and diagenetic biomarker in several earlier studies (Pelz*, et al.*, 1998). The increase with depth of these biomarkers is probably due to an increase in bacterial biomass as D-enantiomers of amino acids are only prevalent in bacterial cell walls and have not been found in higher plants. The biological occurrence of D-amino acids is restricted to only a few specific functions such as D-ala and D-glu in the cross-linking of peptidoglycan, which builds bacterial cell walls. Tremblay and Benner (2006) found that D-ala was probably more representative of bacterial C dynamics than other biomarkers. Although amino acids are considered relatively reactive compared to bulk C (Cowie and Hedges, 1992) peptides containing D-enantiomers, mostly peptidoglycan, appear to be more resistant to decomposition than those containing L-

enantiomers (Tremblay and Benner, 2006). Hence the accumulation of those D-amino acids might not only be due to bacterial activity, but could also reflect accumulation of resistant biomolecules in deeper sediment layers. The preservation of these resistant peptides may not only be a function of the slower decomposition of these compounds, but could be part of the refractory polyphenol-protein-complexes described in the previous section about tannins (5.1.2.1).

The condensation reactions whereby small reactive organic molecules combine to form larger and more complex organic compounds is often termed 'humification', although there is some ambiguity in the definition of 'humic substances' (Hansell and Carlson, 2002). Classical models of humification processes describe slow changes in the organic matter composition due to condensation reactions among low-molecular-weight decomposition products. However, the amino acid data from the present study rather stands in agreement with a new theory suggested by Tremblay and Benner (2006) where detrital N acquires a biochemical composition similar to that of bacteria, the primary decomposers, and the rate of acquisition is directly related to the rate of microbial decomposition. Ishiwatari (1992) proposed a similar model where decaying organic material is attacked by bacteria and more than 90 % of the organic constituents of phytoplankton are degraded and lost by this bacterial attack, i.e. would acquire the biochemical composition of the microorganisms. During these bacterial attacks, reactions take place among refractory biopolymers and other molecules, leading to the formation of humic substances.

The observation that bacterial organic matter becomes prevalent during decomposition could give the impression that some components of bacterial cells are highly resistant to decomposition. However the concentration of D-amino acids in decomposing matter might not only be related to the greater structures in which they are bound. The actual breakdown of D-amino acids is more energy consuming as the first step needs to be either racemisation or deamination. O'Dowd *et al.* (1997) observed that substrate-induced respiration (SIR) was significantly less in soils amended with D-amino acids than with L-amino acids. The authors suggest that the difference between D- and L-amino acid SIR seen in soils is not due to gross differences in community composition, but rather due to an overall slower rate of D-amino acid compared with L-amino acid metabolism (maybe due to an additional metabolic step, see above). Ogawa *et al.* (2001) proposed a direct link of microbial decomposition and organic matter preservation. The study reported that, within 2 days, bacteria in a laboratory experiment produced a major component of

refractory uncharacterised (but N-poor) organic matter which was resistant for more than 1 year from labile substrates.

THAA content in leaves did not show a correlation with stations, but varied significantly with season especially for *R. mangle* leaves. In *R. mangle* THAA concentration increased by about a third from rainy to dry season as did AA-N/TN and AA-C/TOC. The strongest increase occurred in L-ile (2-fold) in the *Rhizophora*-dominated area and for gly (3-fold) in the *Avicennia*-dominated part of the mangrove forest. *A. germinans* leaves however showed a decrease of THAA content and AA-N/TN in the dry season, while AA-C/TOC did not change. Nevertheless, the absolute amount of gly also increased in *A. germinans* by a factor of 2.2 at the dry stations of the transect (for absolute amounts see Appendix A5 and A6).

Similar trends have been reported for *R. mangle* and *A. germinans* seedlings, where amino N increased in *Rhizophora*, but declined in *Avicennia* seedlings with increasing salt concentration (Saenger, 2002). The changes with season recorded in the present study are most likely related to the augmented salinity stress during the dry season and the differences between the two species correspond to the different mechanisms by which salt tolerance is achieved. *Avicennia* species are highly efficient salt-extruders, i.e. they excrete salt through specialized salt glands in their leaves, whereas *Rhizophora* species are known to exclude salts through an effective mechanism in their roots where water is taken up and salt is largely excluded. However, despite this effective strategy which can exclude 80-95 % of the salt, the regulation of the 5-20 % that enters the roots has still to be considered. Storage of salts in senescent tissues and/or in the vacuole as well as dilution of the accumulated salts by increasing leaf water content (succulence) are common strategies in non-secreting species (Popp, 1995). To avoid shrinking or swelling of the cells, the osmolarity increase in the vacuole can be balanced through an accumulation of organic solutes in the cytoplasm and in other intracellular compartments. It is however not clearly documented how efficient the salt extrusion is in *A. germinans*. The conspicuous differences in THAA budget measured in the present study lead to the assumption that the two species do not only differ in their mechanisms of excluding salt from the plant tissue, but also in the means by which they deal with intracellular salinity challenges. *A. germinans* may use different, non-amino acid osmolytes or amino acids that were not measured in this study, whereas *R. mangle* increases the total amino acids pool.

The different THAA dynamics could also illustrate the different levels of salt tolerance of the two species, as Hartzendorf and Rolletschek (2001) proved in *Phragmites australis* where the secondary amino acid proline only increased when the 'critical salinity level' was reached. This level can be different for every plant or even for every clone of the same species. When substrate salt concentrations exceed the critical level, the plant will start accumulating osmolytes in high amounts. The higher the critical salinity level, the higher the salt tolerance of the species. Thus, the differences in THAA concentrations might also reflect the different critical salinity levels of *R. mangle* and *A. germinans*. In *A. germinans* the salinities in transect 1 may not have reached the 'critical level', whereas *R. mangle* which has been found to be limited to salinities below 65 ‰ in the field (Saenger, 2002) has much lower critical salinity levels. *A. germinans* has been found at salinities up to 100 ‰, but often becomes dwarfed when soil salinities approach 60-80 ‰ (Saenger, 2002).

As for THAA the general pattern of individual amino acid composition in leaves of *R. mangle* and *A. germinans* did not differ significantly between stations, but showed distinct variation between seasons (*Figures 4.2.26* and *4.2.27*). The most conspicuous change occurred in gly percentage that doubled in both species and at all measured stations in the dry season (absolute amounts also increased substantially (see above), but mainly at the dryer stations, see Appendix A5 and A6). Absolute amounts of gly in leaves of both species correlated positively with salinity at 5 and 10 cm, but showed no significant relationship to sediment salinity at 50 cm (*Figure 5.1.22*). Glycine has been reported to accumulate with increased salinity in plants and invertebrates. Along with D- and L-ala it has also been reported in crab muscle tissue where it contributes to the muscle osmoregulation during downstream migration from the river to the sea of the Japanese mitten crab (Abe*, et al.*, 1999). Martino *et al.* (2003) found that spinach leaves reacted to mild salt stress with an increase in gly and ser. As both amino acids are strongly involved in the photorespiratory cycle their accumulation suggests that one of the early effects of salinity is an increase in photorespiration rate. Photosynthesis and stomatal conductance were reduced, even if no significant change in Rubisco activity and content as well as chlorophyll content occurred, supporting the idea that photorespiration increased. A decrease in stomatal conductance with increasing salinity and decreasing inundation frequency in our study area was postulated before in relation to ^{13}C enrichment in *R. mangle* and *A. germinans* leaves (see 5.1.1 of Discussion). Martino *et al.* (2003) also observed a reduction of glu which could be explained with its use as substrate in the synthesis of gly. In this study, the proportion of glx decreased only slightly. This might be a result of the method which does not allow a separate analysis of glu and gln. Several

studies report reduction of glu, but increase of gln (Hartzendorf and Rolletschek, 2001) hence the slight reduction of glx in the present study could reflect the two processes, where the decrease of glu outweighs the increase of gln by a small percentage.

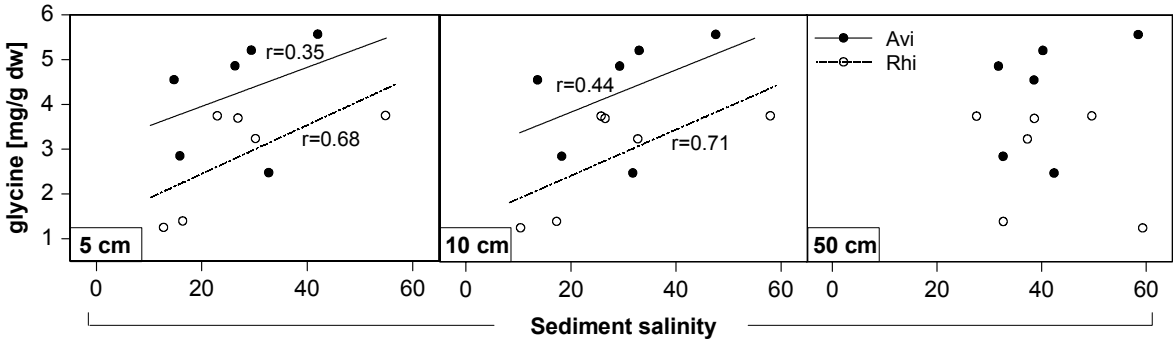


Figure 5.1.22: Correlation between sediment salinity at different depths and glycine content in leaves of *A. germinans* (Avi) and *R. mangle* (Rhi) in transect 1, n=6, correlations are not significant.

Absolute amounts of glx (mg/g dw) however increased in sediments as well as leaves. The role of the glutamate pathway in proline accumulation under stress conditions is well established (Slama*, et al.*, 2006) and proline has been discussed as osmolyte for various mangrove species (Popp*, et al.*, 1985; Ashihara*, et al.*, 1997; Datta and Ghose, 2003). Proline could not be measured with the methods in this study, but absolute glx accumulation with increasing salinity stress could be linked to proline dynamics in *R. mangle* and *A. germinans*.

Severe salt stress is often associated with stronger inhibition of the biochemical and photochemical capacities of the leaf. Glycine betaine is frequently reported to substitute gly and ser as osmolytes when salinity increases (Summers and Weretilnyk, 1993; Boncompagni*, et al.*, 1999; Di Martino*, et al.*, 2003). Glycine betaine is synthesised by oxidation of choline that in turn derives from serine (Summers and Weretilnyk, 1993). In the present study, glycine betaine was not determined, but there was no effect of salt stress on proportion of ser in this transect. Absolute concentrations of L-ser only increased during the dry season in *R. mangle* leaves in the humid part of the transect, where salinity stress is low. Proline has been reported frequently in relation with osmoregulation in plants (Hartzendorf and Rolletschek, 2001), but as it does not react with the derivatisation reagent OPA (see Materials and Methods) it could not be determined in this study.

The proportion of D-amino acids in leaves was generally low (< 0.5 mol %) and showed very similar patterns at the different stations, only differing between seasons. So far D-

amino acids, especially D-ala have mainly been associated with bacterial cell walls and microbial degradation. A few studies have reported D-amino acids in tissues of crustaceans and mollusks (Abe*, et al.*, 1999) and in marine macroalgae (Nagahisi*, et al.*, 1995), but higher land plants usually lack D-enantiomers entirely. The origin of the small amounts of D-amino acids reported here might result from bacteria that have grown into the leaf epidermis or stomata.

Racemisation from an L- to a D-form of an amino acid occurs in sediments and fossils and has been used as a mean of relative age determination (Engel and Macko, 1993) However, the values measured in the present study were either too low - as is the case for D/L-leu – or too high (D/L-ala) to fit into the presented equations for deep sea sediment or fossil samples, suggesting that other dynamics account for the D/L-amino acid ratios in mangrove sediments and mask the racemisation.

5.1.3.2 Transect 2: THAA

The concentration of THAA in sediments in this transect ranged from 0.8 mg/g dw in the deeper sediment layers to 22.5 mg/g dw at the surface, varying over a much greater range than in sediments of transect 1 and reaching a maximum value double the highest value in surface sediments of transect 1 (*Figure 4.2.28*). During the rainy season the THAA pattern exhibited a similar trend to TOC, TN and tannin content where surface sediments showed a conspicuous drop from high values at stations 1-4 (19.2 ± 3.5 mg THAA/g dw) to low values at stations 5-7 (6.4 ± 3.9 mg THAA/g dw). The dry season values for surface sediments follow a different pattern with two maxima at station 3 and 5. As for TOC and TN content THAA values at 10 and 50 cm were significantly lower than in surface sediments. THAA values at 50 cm were close to 0 at all stations except station 7 where they increased very slightly mainly during the dry season. A similar trend has been observed for tannins (see section 5.1.2.2) and has been linked to palynological data of the region, suggesting that at station 7, remains of an old mangrove forest can be found at 50 cm depth, whereas stations 1-6 have a younger history of colonisation by woody plants (Cohen*, et al.*, 2005a; Cohen*, et al.*, 2005b).

Linking amino acid fate with tannin dynamics, a theory was presented in before suggesting an active role of the plants in tannin dynamics. Plants would actively be creating a slowly degradable pool of N by accumulating polyphenol-protein-complexes in their leaves before detachment. As we have no data of THAA in yellow leaves before detachment we can not make assumptions on the processes that occur within the plant.

However by creating a THAA/Tannin ratio for sediment and plant samples, some information about the relationship between these compounds can be deduced (*Figures 5.1.23* and *5.1.24*). Ratios for sediments ranged between 2.6 and 110.3, while all values for THAA/Tannin of green plant material were below 10 in the rainy and below 5 in the dry season, thus THAA increased versus tannin in the sediment – with some exceptions - when it is compared to the fresh leaf material. A cause for these higher THAA values is probably the microbial biomass present in the sediments. For the samples from the rainy season, a link between surface sediments and vegetation characteristics can be observed again, as has been postulated before: At stations 1-4 the THAA/Tannin ratio is greatest. At these stations *S. portulacastrum* has been associated with higher TN and TOC values and more importantly with higher total bacterial counts, which would also be reflected in higher amino acid concentrations. Moreover, AA-N/TN values are higher at stations 1-3 during the rainy season, indicating that amino acids hold a higher share of TN at these stations. Conspicuous algal mats that have been observed at station 1 after spring tides may also have some influence on surface sediment properties and THAA concentrations at this particular station.

The high THAA/Tannin ratios at 50 cm of stations 1-6 are a function of the very low tannin concentrations which increased suddenly at station 7 (where a low THAA/Tannin ratio was found, see above). For the dry season, no conspicuous pattern can be observed. A substantial increase of leaf litter shed from *A. germinans* during this season (Mehlig, 2001) and considerable decline of *S. portulacastrum* during the dry months (personal observation, see *Figure 5.1.9*) coupled with less leaching of tannin from the leaves (see section 5.1.2.2) could account for the difference in THAA/Tannin ratios between the seasons.

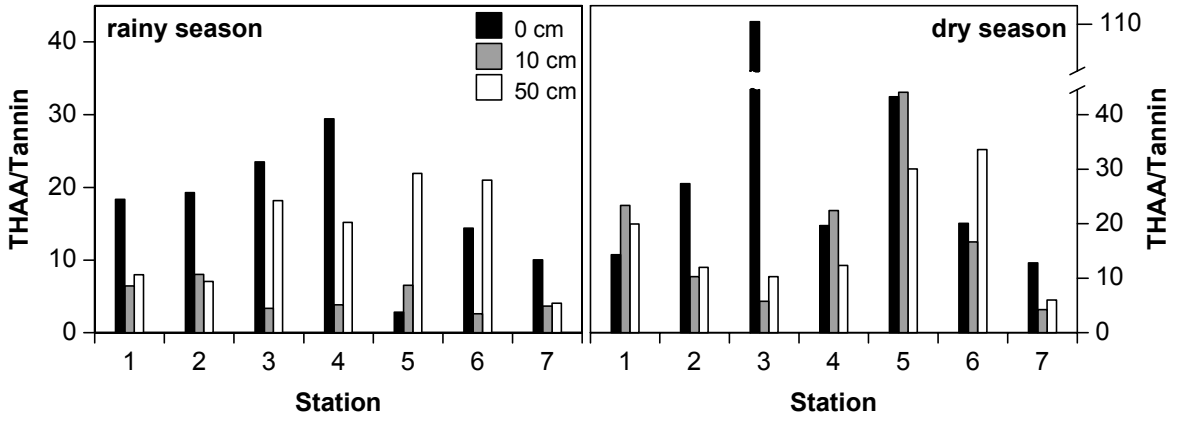


Figure 5.1.23: THAA/Tannin ratios for sediments during rainy and dry season for transect 2.

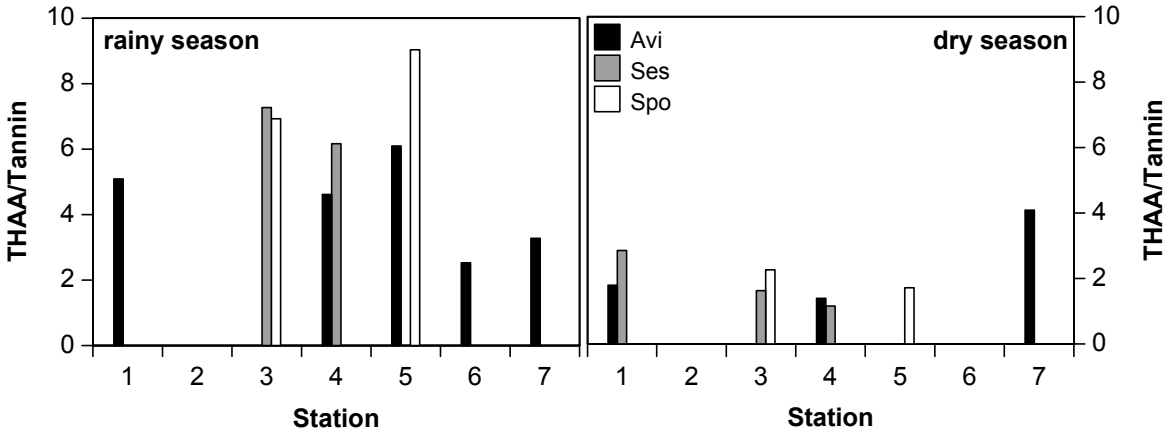


Figure 5.1.24: THAA/Tannin ratios for leaf samples of *A. germinans* (Avi), *S. portulacastrum* (Ses) and *S. virginicus* (Spo) during rainy and dry season for transect 2.

The distribution of the individual L-amino acids (mol%) was relatively invariant between seasons and stations especially for surface and 10 cm sediments where L-asx, L-glx, gly and L-ala were dominant. The L-amino acid composition showed more variation at 50 cm depth both between stations and between seasons. At station 1 L-glx, gly and L-arg were the predominant monomers at 50 cm during the rainy season, whereas during the dry season the proportion of all three amino acids diminished, leaving only L-glx and L-leu dominating the L-amino acid pool. At station 7, the amino acid composition at 50 cm was more consistent, resembling the proportions at the surface and 10 cm with L-asx, L-glx, gly and L-ala dominating in both seasons. This fact is probably linked to the paleo-vegetation of the area. Point 7 has been a mangrove forest for at least ~400 yrs (Cohen*, et al.*, 2005a; Cohen*, et al.*, 2005b). Here *A. germinans* trees have a height of 10 m and the root expansion will have a higher ability to retain water and nutrients than the roots of the small trees and herbaceous vegetation at station 1. Generally the proportion of gly, L-ser and L-asx is lower at station 1 than at station 7 especially in the 50 cm sediment layer. Gly could also simply be a marker for higher biomass as it is integrated in most proteins. Thus the low abundance of these biomarkers for vegetation at station 1 may relate to a history of salt marsh or even unvegetated area. Pollen analysis indicates a paleo-environment of hypersaline tidal flats in a core taken near station 1 of transect 2 of the present study (Cohen*, et al.*, 2005a; Cohen*, et al.*, 2005b).

In absolute amounts (mg/g dw, see Appendix), L-asx and L-glx were also the most abundant amino acids in the sediments, followed by L-leu and L-ala. The distribution, especially in the surface sediments, was comparable to the composition found in all plant

species in the transect, a feature that has been observed in transect 1 as well. The concentrations of the single amino acids are highest in surface sediments at station 1 during the rainy season, displaying values up to 3 times higher than at station 7 or any of the measured stations of transect 1. This fact underlines the assumption postulated earlier that the biochemical composition of the surface sediments has a closer link to the present vegetation. At station 1 the absolute amounts of amino acids drop by up to 82 % in the case of L-asx between concentrations at the surface and at 10 cm during the rainy season. As this coincides with a drop in salinity at this station (see *Figure 4.1.4*), the low values at this depth could be explained with a wash-out through heavy rains as the root expansion at this station is very low and may not have a strong ability to retain nutrients and decomposition products.

Although absolute amounts of L- and also of D-amino acids decreased with depth, the proportion of D-amino acids within the THAA pool increased from the surface to deeper sediment layers at both stations. As has been illustrated before, D-amino acids have been used as a microbial and diagenetic biomarker in several earlier studies (Pelz*, et al.*, 1998; Tremblay and Benner, 2006). Thus, the high proportions of these amino acids at depth could indicate (1) high bacterial activity in this sediment layer or (2) simple preservation and accumulation of slowly degradable material such as bacterial cell walls. It is very likely that both assumptions participate in the dynamics which build the great picture with varying weightings depending on physico-chemical characteristics of the sediments and higher plant cover. The influence of the vegetation can be seen, by comparing proportional and absolute distributions of individual amino acid at station 1 and 7. The distribution is more even and stable at station 7, where tree and forest age have been influencing the sediments uniformly for a longer time period than is the case at station 1. The preservation and even distribution of resistant peptides may also be part of the refractory polyphenol-protein-complexes described in the previous section about tannins.

In plant samples of transect 2, THAA content in general did not increase during the dry season, in contrast to what has been measured in plants of transect 1. In *S. portulacastrum* and *S. virginicus* THAA concentrations decreased from rainy to dry season, whereas in *A. germinans* they remained unchanged. AA-C/TOC stayed fairly constant with values between 6 and 7 % for *A. germinans* and *S. portulacastrum*, whereas the grass *S. virginicus* showed lower values (3.3 ± 0.2 % during the rainy season, decreasing to 2.5 ± 0.3 % in the dry season). AA-N/TN increased in *A. germinans* during the dry season to 45.3 ± 2.8 % compared to 39.7 ± 6.4 % (rainy season), whereas the herbaceous species showed decreasing values, *S. portulacastrum* from a very high value

of 76.9 ± 6.9 % (rainy season) to 46.9 ± 2.9 % (dry season) and *S. virginicus* from 48.6 ± 4.3 % to 38.8 ± 1.0 %. The changes with season recorded in the present area may be linked to the higher salinity stress during the dry season and the differences between the species relate to the different mechanisms by which salt tolerance is achieved. *S. virginicus* and *A. germinans* actively secrete salt whereas *S. portulacastrum* stores salt in the vacuole and increases leaf succulence (Tomlinson, 1986; Marcum and Murdoch, 1992; Saenger, 2002). It is therefore likely, that the plants also differ in the type of osmolytes which are used to compensate the higher concentration of salts in the cells. Osmolytes are often synthesised from amino acids, which explains the reduction of the latter in the dry season when salt stress increases.

Opposite trends were found for *A. germinans* in transect 1 where amino acid nitrogen decreased during dry season. However as has been mentioned in the discussion of transect 1 the different reactions of this species may be due to the different salinities in the two transect. Salinities in transect 1 (all < 60) might still lie under the critical salinity level of *A. germinans*, whereas salinity levels in transect 2 were above 60 at most stations during the dry season. When the critical salinity for one species is reached, it possibly switches to different mechanisms of osmoregulation. With higher salinities the difficulty of taking up N from the sediments also increases, thus influencing the plants nitrogen metabolism. In *Spartina alterniflora*, for example, salt affected growth through the action of sodium as a competitive inhibitor of ammonium uptake (Odum, 1988). Thus, *A. germinans* may have a critical salinity level above 60 at which point it switches to non-amino acid osmolytes (or to amino acids which were not measured in this study). As *Avicennia* species have been found at salinities up to 100 (Saenger, 2002), the assumption of a critical salinity of 60 is well within the bounds of possibility.

The high percentage of AA-N/TN in *S. portulacastrum* during the dry season corresponds to higher mol% of N-rich amino acids such as L-his or L-gln. As glu and gln were measured together in this study the actual percentage of gln can not be assessed. During the dry season THAA and AA-N/TN decreased significantly in *Sesuvium* leaves. Succulents like *S. portulacastrum* have commonly been described to store the salts in their leaves (Saenger, 2002). Proline has been discussed as osmolyte for *S. portulacastrum* and *S. virginicus* (Marcum and Murdoch, 1992; Slama*, et al.*, 2006), but could not be measured with the current methods in this study. Proline is synthesised from either glutamate or ornithine. *Sesuvium* is considered a highly proline accumulating species and the role of the glutamate pathway in proline accumulation under stress conditions is well established (Slama*, et al.*, 2006). In *S. virginicus* - and to a smaller

degree in *S. portulacastrum*- both proportional and absolute amounts of L-glx decreased during the dry season. Hence glu could be accumulating during times when stress is reduced and would be synthesised to proline when salinity increases.

Pitcairn *et al.* (2003) demonstrated that when N availability increases, N may be taken up by plants in surplus and accumulated rather than being used in biomass production. At a cellular level if protein is not synthesised, NH_4^+ is assimilated into specific N metabolites of which amino acids such as asp and arg are particularly important. Thus the greater efficiency of N uptake in plants when salinity is lower could lead to storage of amino acids such as asp and asn which were found in higher proportion (mol%) in *S. portulacastrum* during the rainy season (measured as asx). Hartzendorf and Rolletscheck (2001) found that in leaves of *Phragmites australis* amino sugars became more important than amino acids when salt stress increased thus equally displaying a negative relationship between amino acid content and salinity levels.

Generally gly and L-ser proportion (mol%) increased in all species during the dry season whereas glx was reduced in all plant tissues except *Sesuvium* roots (*Figure 4.2.34*). Gly and ser have been reported to accumulate with increased salinity in plants and invertebrates (Abe*, et al.*, 1999; Di Martino*, et al.*, 2003). Both amino acids are strongly involved in the photorespiratory cycle and their accumulation suggests that the early effect of salt is an increase in photorespiration rate (see also 5.1.1.2). A decrease in stomatal conductance with increasing salinity and decreasing inundation frequency in our study area was related to ^{13}C enrichment only for *A. germinans*. As gly and L-ser also increased in the herbaceous species where an influence of salinity on the photorespiratory cycle has not been shown, they probably also have a function as osmolyte. Gly has frequently been reported as osmolyte when salt stress was mild, being then substituted by glycine betaine or proline when salinity increases (Summers and Weretilnyk, 1993; Boncompagni*, et al.*, 1999; Di Martino*, et al.*, 2003).

D-amino acids (as percentage of THAA) did not show significant differences between the species (*Table 4.2.3*). The composition of the individual D-amino acids however varied with species and season (*Figure 4.2.34*). D-ala has mainly been associated with bacterial cell walls and microbial metabolism, but a few studies have reported D-amino acids in tissues of crustaceans and mollusks (Abe*, et al.*, 1999) and in marine macroalgae (Nagahisi*, et al.*, 1995). The occurrence of D-amino acids in the roots of *Sesuvium* may result from the bacteria that have been found associated with the mycorrhiza. The capacity of *S. portulacastrum* rhizosphere to fix atmospheric N_2 and the associated low

$\delta^{15}N$ values led to the assumption that the mycorrhiza detected on *Sesuvium* roots live in association with N_2-fixing bacteria. In leaves of *A. germinans* a small amount of D-amino acids was determined, being generally reduced during dry season. Presumably in the case of *Avicennia,* the D-amino acids originated from bacteria that have grown into the leaf epidermis or stomata.

5.2 Decomposition experiments

In this study, field and laboratory experiments were conducted simultaneously to obtain information about the applicability of data from laboratory experiments. Unfortunately most samples of *A. germinans* were lost to the tide during the field experiment (possibly due to strong currents), but the leaves of *R. mangle* were all retrieved. The yellow leaves used in the field and the laboratory experiment were of the same degradation stage, i.e. yellow leaves collected from the ground. Several studies have been published on decomposition experiments. Some were conducted in the field in buried net bags, others in laboratories with ground plant material or whole leaves, roots or rhizomes (Benner*, et al.*, 1990a; Benner*, et al.*, 1990b; Constantinides and Fownes, 1994; Albers*, et al.*, 2004). Because of these differences of experimental setup, it is difficult to compare the data. Nevertheless general trends can be observed even for different plant material, details of which will be discussed below.

5.2.1 Decomposition experiments: Elemental and isotopic composition

The three degradation stages used for the field experiment (yellow, brown and black) each had a different chemical composition. Initial TOC content varied between 45.3 ± 0.1 and 48.5 ± 0.1 % being highest in brown leaves. During incubation in the net bags, TOC values increased in all colour groups, with the greatest increase in yellow leaves (from 47.3 ± 0.2 initial TOC to 54.3 ± 0.3 % after 6 weeks of incubation in the field). TN doubled in yellow and brown leaves from 0.5 ± 0.03 and 0.6 ± 0.02 % to 1.1 ± 0.1 and 1.2 ± 0.1 % respectively. Concomitant with the increase in nitrogen during the experiment, the black stages had much higher TN content than yellow and brown leaves and also increased further during the 42 days of incubation in the net bags. As a consequence of the slight increases in TOC but strong increases in TN, C/N values decreased with decomposition of the *R. mangle* leaves (*Figure 4.3.1*). From yellow leaves still attached to the tree to the black leaves collected in tidal channels, N content nearly doubled from 0.65 % TN in yellow to 1.23 % TN in black leaves. However, compared to the green leaves, the yellow leaves attached to the tree had already lost ~60 % of their nitrogen content (Figure 5.2.1, own data represented in an adaptation of a figure from Hernes*, et al.*, 2001). This loss is probably due to resorption, a process frequently occurring in plants to recover nutrients before abscission of senescent tissues, where essential elements are shunted to storage tissues in the stem or into the newly developing leaves before leaf detachment (Campbell*, et al.*, 1999). The great majority of foliar N in the green leaf is locked up in the photosynthetic apparatus that is associated with chlorophyll (Cornelissen*, et al.*, 2000).

Chlorophyll is broken down in senescent leaves and although most products of its breakdown still remain unidentified it is likely that these molecules are returned to the plant for recycling (Hendry*, et al.*, 1987; Cornelisson, 1996; Cornelissen*, et al.*, 2000).

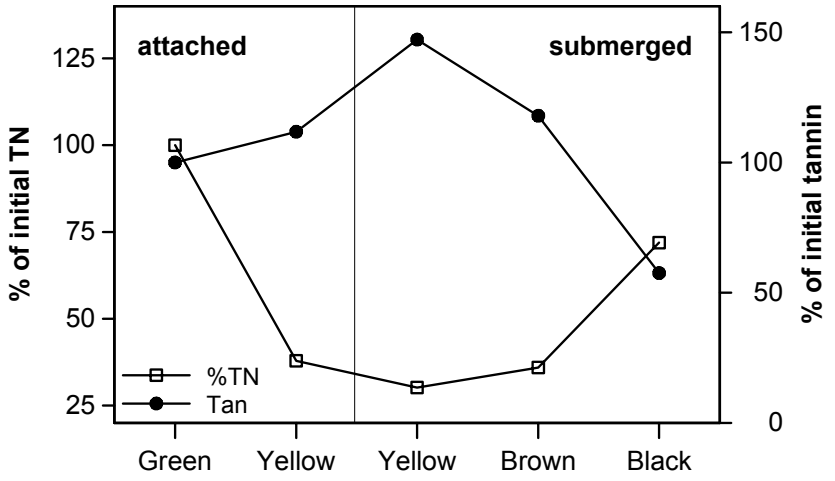


Figure 5.2.1: Percent of initial total nitrogen (TN) and tannin (Tan) in green leaves of *R. mangle* and their senescent and submerged counterparts.

The accumulation of nitrogen in plant litter has been observed frequently during decay of various species (Rice, 1982; Wilson*, et al.*, 1986; Benner*, et al.*, 1990a; Benner*, et al.*, 1991; Tremblay and Benner, 2006), but neither the exogenous sources nor the mechanisms of nitrogen accumulation have been fully understood. Whereas originally most investigators advocated a stimulation of microbial activity for the immobilized N, direct molecular or isotopic evidence for this origin has not been presented. Some investigators state that microbial mass can account directly for only a few percent of the N that accumulates in decaying tissue (Wilson*, et al.*, 1986). Benner *et al.* (1991) however reported that N immobilisation and accumulation ultimately results from utilisation of exogenous N sources by the attached population of decomposer microorganisms.

The laboratory experiments in the present study also suggested that bacteria play a major role in N accumulation. The control treatment as well as the treatment with fungicide where bacterial cell counts were highest, displayed the highest increase of TN (*Figure 4.3.4*). In other studies of submerged *R. mangle* leaves, bacteria and mycelial oomycotes also played a more important role than fungi (Raghukumar*, et al.*, 1994; Newell, 1996). The changes in the isotopic composition of the leaves in the field and the laboratory experiment indicated selective uptake or selective degradation of C and N isotopes during decay. Although the variation between initial values of leaves of different colours in the

field experiment and changes after the incubation were only ~1 ‰ for both isotopes some trends could be detected. Values for $\delta^{13}C$ decreased with decomposition time and age of the leaves, whereas $\delta^{15}N$ was highest in yellow leaves (5.6 ‰), lowest in brown leaves (4.4 ‰) and increased again in black leaves (5.0 ‰). During the 42 days of incubation the $\delta^{15}N$ values decreased by 30, 20 and 16 % in yellow, brown and black leaves respectively. Zieman (1984) observed similar trends in a 6 weeks decomposition experiment with *R. mangle* leaves. Raghukumar *et al.* (1994) observed distinct sequences of colonisation by fungal species and attainment of peak fungal and bacterial biomass by 21 and 35 days, respectively. As fungal and bacterial biomass were not monitored throughout the whole experiment, but only accounted for by total bacterial cell counts after 42 days in the laboratory study, no definite statements can be made about a possible succession of different microbial actors in the course of decomposition. However, the information drawn from the biochemical analyses discussed below will give some important clues even without a microbiological study.

In the laboratory experiment, decreases of $\delta^{15}N$ values were linked to bacteria (although values were not significant) whereas the $HgCl_2$ and bactericide treatment showed significant increases of $\delta^{15}N$. The result of the bactericide treatment emphasises the assumption that differences in isotopic composition could result from variations in microbial colonisation and utilisation of the decaying material. Black leaves of the field experiment for example showed a higher $\delta^{15}N$ values compared to the brown, but lower values than the yellow leaves on day 0. Although differences and sample size were only small, it could be assumed that black leaves are colonised by a higher proportion of fungi than brown leaves. This assumption and implications of high values of yellow leaves will be discussed further below.

The increase of $\delta^{15}N$ in the $HgCl_2$ treatment rather leads to the assumption that selective removal through leaching is also involved in the process. Benner *et al*. (1991) suggested a model where the processes controlling the isotopic composition of carbon in detrital material are the selective removal or degradation of components with distinct isotopic signatures, thus altering the overall isotopic composition. Amino acids for example are typically enriched in ^{13}C relative to total organic C in cells.

5.2.2 Decomposition experiments: Tannins

Tannin contents (expressed as TAE) was lowest in black leaves (56.8 mg TAE/g dw) and highest in yellow leaves (145.4 mg TAE/g dw), showing higher values for yellow than for

green leaves attached to the tree (mean value of 98.7 mg TAE/g dw during the dry season, see *Figure 5.2.1*). This same pattern with an initial increase in the yellow leaves followed by a rapid drop in the brown and black leaves was found by other investigators using different methods to measure tannin (Benner et al 1990b, Hernes et al 2001). As discussed in the previous sections an explanation for this increase in tannins has been proposed by several authors (Kuiters, 1990; Northup*, et al.*, 1995a; Northup*, et al.*, 1995b; Northup*, et al.*, 1998; Hattenschwiler and Vitousek, 2000), suggesting that plants have adapted to possible N limitation by immobilising N in senescent leaves in the form of polyphenolics or polyphenol-protein complexes which will be slowly released into the sediment after abscission and during decomposition of the leaf.

However, the fast decrease in tannin content during both experiments (*Figures 4.3.1* and *4.3.4*) suggests that not all tannin present is linked to refractory complexes. The dynamics measured in the experiments could also be linked to other polyphenols, as the Prussian Blue Assay probably detects total polyphenols. For deciduous trees in temperate zones it has been shown that the concentration of a different kind of polyphenolics, the anthocyanins, also increase in leaves shortly before abscission and after leaf fall, with a subsequent decrease during decomposition of the leaf (Sanger, 1971). Anthocyanins are pigments and are usually associated with bronze, reds and purple in nature. In ageing leaves they are mainly responsible for the red coloration which occurs in some species after or at the same time as the yellow colour (Sanger, 1971; Strasburger, 2002). Sanger (1971) found that in oak leaves, anthocyanins form as soon as chlorophyll, which is responsible for the green coloration during the summer, begins to decompose. The concentration of anthocyanins rises abruptly to a maximum and thereafter declines throughout the winter. In leaves of *R. mangle* a stage of orange colour could be found in the degradation/colour scale of decomposing leaves, which could be described as intermediate between yellow and brown leaves (personal observation; 1990a; Hernes*, et al.*, 2001).

In the present study, orange leaves were not incorporated into the experiments, but it is likely that if anthocyanins were present, they would be measurable in yellow as well as orange leaves. The yellow colour is mainly due to carotenoids, various polyterpenes present in the leaves throughout the green phase, but usually masked by the bright green colour of the chlorophylls. In autumn leaves of the temperate zones carotenoids are degraded quickly after leaf fall (Sanger, 1971; Hendry*, et al.*, 1987; Cornelisson, 1996).

The brown colour of degrading leaves represents mostly phenolic compounds such as lignin and tannin (Cornelisson, 1996), whereas the cause behind the colour of black leaves has not yet been researched on in detail. Melanins represent a group of high molecular weight pigments of brown or black colour formed by oxidative polymerisation of polyphenols. They are formed by many soil and pathogenic fungi, but have also been reported in black tea leaves, where they are thought to have formed during the green tea processing (Sava*, et al.*, 2001; Langfelder*, et al.*, 2002; Zavgorodnyaya*, et al.*, 2002). Thus the black colouring of *R. mangle* leaves could be due to internal chemical processes or to the colonising fungi or both. The variation in isotopic composition, although not significant, gave an indication of differences in the microbial colonisation of the different leaf groups (see above), linking the black stages rather to fungi than bacteria.

In consequence the successive pigmentation would be due to chlorophylls in the green, carotenoids in the yellow, anthocyanins in orange, tannins and lignins in brown and melanins in black leaves. Cornelissen (1996) found that significant heterogeneity in relative weight loss during leaf decomposition of 125 vascular plants in Britain could be explained by variations in leaf coloration. In this chapter a link between leaf colour, variations in decomposition pathways and microbial colonisation will be presented.

The increase in polyphenolics measured with the Prussian Blue Assay would thus only be partly due to the tannin and the tannin-protein-complexes, but would also include anthocyanins. The rapid decrease of polyphenolics within the 42 days may be mainly due to the degradation or loss of anthocyanins, whereas the more refractory polyphenolics such as tannins or tannin-protein-complexes would remain. These results confirm the hypothesis that with the colorimetric assay of total phenols, both hydrolysable and condensed tannins are detected. Other studies have found a similar decrease of extractable tannins measured by colorimetric methods in *R. mangle* leaves, whereas the remaining tannins measured by ^{13}C-NMR appeared to be highly resistant to decomposition (Benner *et al.*, 1990b). The data of the laboratory experiment in the present study suggest that microorganisms are involved in the process of loss or alteration of the labile polyphenolics. In the treatment with mercuric chloride, where microorganism numbers were near zero, the amount of tannins present after 42 days was higher than in all other treatments where a microbial population was active. The degradation of polyphenolics was closely linked to fungal growth showing the greatest decrease in the reference and the bactericide treatment where fungi were allowed to grow. However, the decrease of polyphenolics in the $HgCl_2$ treatment indicated the simultaneous occurrence of leaching.

5.2.3 Decomposition experiments: Total hydrolysable amino acids

As polyphenolics decreased with time and age of the leaves, they can not be responsible for the TN increase observed in both experiments. Total hydrolysable amino acids (THAA) and the composition of L- and D-amino acids could give an indication of the source of the immobilised and accumulated N and/or of possible selective mechanisms during uptake or degradation. In the present field study an increase of THAA was found with age of the leaves and incubation time (*Figure 4.3.2*). However the proportion of amino acid N of TN (AA-N/TN) did not show such a clear pattern. In yellow leaves AA-N/TN increased substantially within the 42 days of incubation from 40.0 to 66.0 %. Brown and black leaves on the other hand decreased from high values of 67.2 and 60.0 % AA-N/TN respectively by ~30 % for brown and ~17 % for black leaves to 47.8 and 50.7 % AA-N/TN. Hence in brown and black leaves the accumulation of N can not entirely be assigned to an increase in amino acids as a measure of protein, whereas in yellow leaves TN may be more closely linked to THAA. AA-C/TOC increased in all leaf colour groups with the greatest increase in yellow leaves were values had tripled after 42 days of incubation.

The THAA values of *Rhizophora* leaves in the laboratory experiment on day 0 were 19.1 mg/g dw (*Figure 4.3.5*). After 42 days THAA content had nearly doubled in the reference and fungicide treatment ('Bacteria'). The two remaining treatments did not show any effect on THAA concentrations in *R. mangle* leaves. AA-C/TOC essentially followed the trend given by THAA, whereas AA-N/TN showed different dynamics with very high standard deviations. The results for AA-C/TOC linked the increase of THAA closely to bacterial biomass as values for both parameters only rose in the reference and the fungicide treatment, where bacterial cell numbers were high. Thus the substantial increase of AA-C/TOC in the yellow leaves of the field experiment may indicate increased colonisation.

The percentage of D-amino acids and the ratio of D/L-ala, which are often used as a measure of bacterial presence and/or activity, decreased in yellow and brown leaves only in the first weeks, and rose again in week 6 (see whole time series in Appendix A13), thus showing nearly unchanged levels after 42 days. Interestingly yellow leaves showed values for D/L-ala which were nearly 3-fold higher than in green leaves attached to the tree, suggesting considerable bacterial colonisation at the time of leaf collection. During incubation however, the value decreased by ~40 % in yellow leaves after also having decreased in the first weeks and augmented slightly by week 6. In black leaves % D-amino acids and the ratio of D/L-ala behaved in the opposite way by first increasing up to week 4 and falling again in week 6. Joining this information with what was deduced from

the THAA and AA-C/TOC values, it can be assumed that in yellow and possibly also in brown leaves bacterial colonisation was predominant at the time of collection. However, bacterial activity might have been reduced during the first weeks of the experiment in favour of fungal decomposers (on yellow leaves $\delta^{15}N$ values gave an indication of fungal presence on day 0), a suggestion that is supported by the decreasing values of D-amino acids and D/L-ala. After a few weeks of incubation, bacterial activity increased again resulting in the higher THAA and AA-C/TOC values discussed above. Although % D-amino acids rose in the first few weeks of incubation in black leaves, both % D-amino acids and D/L-ala values were substantially smaller than in yellow and brown leaves at the beginning and at the end of the experiment, supporting the hypothesis of fungal colonisation on black leaves suggested in relation to melanin content earlier in this chapter.

In the laboratory experiment % D-amino acids in leaves of *R. mangle* was 1.7 % on day 0 (*Figure 4.3.5*). After 42 days values had risen to up to three times that value in all treatments. On day 0 only the three amino acids D-asx, D-glx and D-ala were detected in the *R. mangle* leaves (*Figure 4.3.6*), which corresponded to the composition found in all leaves of the field experiment throughout the incubation time (*Figure 4.3.3*).

After 42 days, however, some D-amino acids had increased and several new D-amino acids had appeared on leaves of R. mangle, especially in the 'Bacteria' and the 'Fungi' group. Of particular importance was D-ala. Its origin becomes clear by looking at the development of the absolute concentrations in mg/g dw during the 42 days of the incubation (*Figure 5.2.2*). The development was similar on both *R. mangle* and *A. germinans* leaves. After some oscillations, the values stabilise towards the end of the experiment, showing highest concentrations in the samples where bacteria are present, the 'Reference' and 'Bacteria'. In the Fungi samples, values stay constant, while in the mercuric chloride treatment, D-ala concentrations decrease. It can therefore be clearly deduced that D-ala finds its source in bacteria.

The increase of D-ala concentrations in the 'Bacteria' and 'Reference' leaves underlines the assumption stated in an earlier section (5.2.1), that the accumulation of TN is linked to bacterial colonisation.

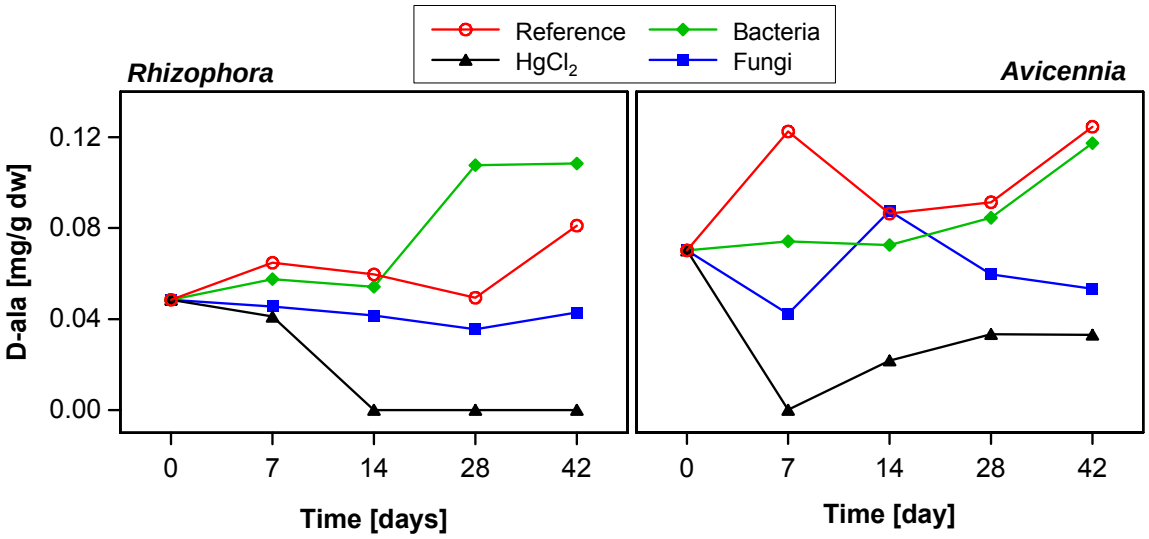


Figure 5.2.2: Absolute concentrations [mg/g dw] of D-alanine (D-ala), in leaves of *R. mangle* (*Rhizophora*) and *A. germinans* (*Avicennia*) during the 42 days of incubation in the laboratory experiment. Values for the reference group (Reference) and the three different treatments with mercuric chloride ($HgCl_2$), fungicide (Bacteria) and bactericide (Fungi) are presented.

Absolute amounts of L-tyr and L-phe increased steadily from yellow to black leaves. Both amino acids are known to be precursors of melanin (Karlson*, et al.*, 1994), which has been suggested as being responsible for the black colour of this leaf group.

5.2.4 Decomposition experiments: Synthesis

The joint results from the field and laboratory experiments imply the importance of microbial activities at least in the early stages of diagenesis, suggesting that in addition to some leaching of soluble compounds, the different microbial species contribute differently to the decomposition process both in time and specialisation of the compounds to be degraded. As mentioned above Raghukumar *et al.* (1994) observed distinct sequences of colonisation by first fungal and then bacterial species. Møller *et al.* (1999) suggested antagonistic interaction of fungi and bacteria during decomposition and confirmed the pivotal role of fungi in the degradation of recalcitrant litter C sources and yet another study hypothesised a difference in colonisation of submerged leaves and those that might periodically dry out (Newell, 1996). Eumycotic (i.e. true fungal) activity would be greater on leaves which are not in permanent contact with water, whereas for submerged mangrove leaves, oomycotic activity (mycelial protists) was found of greater importance in the decomposition process. These differences in colonisation may also explain some of the differences between the dynamics of yellow leaves in the laboratory and the field

experiment (e.g. values for D-amino acids or AA-N/TN), as laboratory leaves were constantly submerged whereas leaves of the field experiment fell dry with every low tide.

Thus it can be concluded that microbial colonisation may explain N accumulation, but not yet the mechanisms of N preservation. Most investigators suggest that humification reactions are involved due to the fact that humic substances are enriched in N relative to vascular plant tissue (Mayer, 2004). An indication of humification reactions would be inverse correlation between tannin and N in leaves (Hernes*, et al.*, 2001). Interestingly the data from the field experiment display a significant inverse correlation for yellow and brown, but not for black leaves (*Figure 5.2.3a*), indicating as has been implied before, that decomposition processes and dynamics change throughout the different stages of diagenesis. In the yellow and brown leaves, where bacterial activities may dominate fungal colonisation after 42 days of incubation, the correlation between polyphenolics and TN indicate humification reactions. It was demonstrated earlier in this chapter that part of the measured polyphenolics in yellow leaves may also be anthocyanins. As the latter have been known to be degraded quickly in decaying leaves (Hoch*, et al.*, 2003), the fragments of these polyphenols might be involved in the humification process as much as the actual tannins. In black leaves the possible pigment group of melanins does not interfere with the Prussian Blue Assay as in this group of compounds the phenolics have been oxidised to chinones (Karlson*, et al.*, 1994). Zavgorodnyaya *et al.* (2002) reported that although showing some differences in functional group content, spectral parameters and molecular weights, fungal melanins can be very similar to humic acids in terms of their chemical properties. Hence the assessment of humification processes in black leaves may not be possible along the same lines as in yellow and black leaves, explaining the differences for the Tannin/TN correlation results.

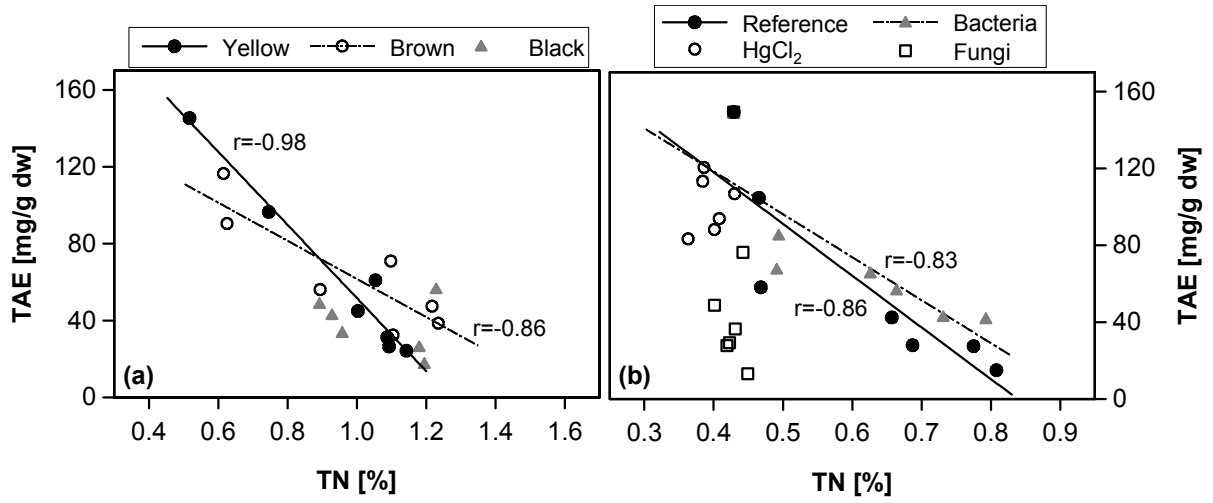


Figure 5.2.3: Correlation between tannin (as TAE) and total nitrogen (TN) content in leaves of *R. mangle* during the field (a) and the laboratory experiment (b). Correlation coefficient r is given next to the regression line, n=7, $p<0.05$.

The correlation between tannin and TN for the laboratory experiment yielded some interesting results as well. The control and the fungicide treatment, both hosting bacteria, showed significant negative correlations, whereas no relationship appeared for the mercuric chloride and the bactericide treatment (*Figure 5.2.3b*). This emphasises the possible link between bacteria and the humification process, supporting another possible mechanism explaining nitrogen increase in decaying plant material brought forward by Ogawa *et al.* (2001). The authors had observed the rapid production of refractory organic matter during bacterial growth on simple organic substrates in a laboratory experiment and speculated that promiscuous or alternative enzymatic activities altered the structure and reactivity of biomolecules in a few days. This mechanism directly links the processes of microbial decomposition and organic matter preservation and suggests that microbial contributions to non-living organic matter and its preservation are greater than previously suspected.

Other authors suggest that microbial enzymes are only responsible for the preliminary steps of depolymerisation of the detritus substrate producing reactive carbohydrates, phenols, small peptides and amino acids. The carbohydrates and phenols condense with polypeptides, amino acids and exoenzymes exuded by the microorganisms to produce nitrogenous geopolymers. As this humification process proceeds, detritus N is transformed from relatively labile compounds into recalcitrant heterocyclic aromatic forms typical of mature sedimentary humic material (Rice, 1982; Wilson*, et al.*, 1986).

Both mechanisms can be postulated for the current work, as the results from the field and the laboratory experiment show a connection between bacteria and humification processes. It could however not be deduced at which stages of the process the bacterial activity is involved.

For *R. mangle* the dynamics in the control treatment of the laboratory experiment generally moved alongside the decomposition processes of the yellow leaves in the field experiment, strengthening the confidence over the validity of the laboratory data. The use of bactericides and fungicides gave important insights on the significance of microbial decomposition in early diagenesis and its close link to the accumulation and preservation of organic matter. In the special circumstances of a mangrove environment, leaf decomposition is likely to occur in two settings: in the water of the tidal creeks and channels or during inundation of the forest and on the sediment surface during low tide and in the parts of the forest that are not frequently inundated. In the present study, the leaves of the field experiment were left in a tidal creek which would be inundated semi-diurnally, thus allowing the bags to fall dry for several hours per day. This might explain the lack of a leaching phase for TN often reported in decomposition experiments conducted in big seawater tanks where the leaves are constantly submerged. While leaves in the laboratory experiment were always immersed in a small quantity of water, the time between the tides may have given the decomposing microorganisms ample opportunity to grow on the leaves of the field experiment, thus counterbalancing the loss of organic compounds through leaching.

The comparison between the behaviour of *R. mangle* and *A. germinans* leaves can only be conducted for the laboratory experiment. A slight depletion in ^{13}C, higher $\delta^{15}N$ values, a fast decrease of tannin concentrations and discrepancies in amino acid data such as a higher percentage in D-amino acids, higher D/L-alanine ratios and a generally lower percentage of amino acid nitrogen in *Avicennia* compared to *Rhizophora* leaves suggest that the dynamics during early decomposition are not entirely the same for both species. In general the decay of *Avicennia* leaves has been found to be significantly faster than of *Rhizophora* leaves (Schories*, et al.*, 2003). Differences in structural components, such as thickness of cuticula and scleromorphic structures, probably affect cell lysis and leaching. This fact is reflected in the data of the mercuric chloride treatment in the laboratory experiment, where tannins as well as THAA concentrations showed a greater and in the case of tannins also a faster decline in *A. germinans* leaves compared to *R. mangle* tissues (see time series data in Appendix A14 and A15). In *R. mangle* tannins decreased by 45 % within 42 days in the mercuric chloride treatment, whereas for *A. germinans* the

decline was of 83 %. In an experiment on the effects of mangrove leaf decomposition in shrimp ponds, Hai and Yakupitiyage (2005) observed that ponds with *Avicennia* leaves had the highest tannin concentrations, indicating stronger leaching for leaves of this species.

The rapid decrease of extractable tannins in *A. germinans* leaves within the first week in the three other treatments indicates that microbial species may find the access to the compounds easier in *Avicennia* than in *Rhizophora* leaves. *Avicennia* leaves have a dense covering of hairs on the lower surface of the leaves and thus have more surface area on which bacteria could settle (personal observation; Tomlinson, 1986; personal observation; Roth, 1992). The salt glands which are an additional opening in the epidermis of *Avicennia* leaves may also give the microbial decomposers more opportunities to enter the dead leaves, than in *Rhizophora* where only the stomata are present. The SEM pictures of *Rhizophora* leaves (*Figure 4.3.9*) showed a rapid colonisation of the leaf surface with fungi and bacteria frequently growing into the stomata. The lower concentrations of tannins, which have been shown to be toxic for some bacteria (Bhat*, et al.*, 1998), in *A. germinans* compared to *R. mangle* may be another reason for the quick degradation of these compounds in *A. germinans*. However the total bacterial counts revealed a more than 2-fold population of bacteria in the water of submerged *R. mangle* leaves compared to the *Avicennia* samples (*Figure 4.3.7*). Hence the difference in microbial colonisation and utilisation of the two species may not be found in the differing microbial biomass or cell numbers, but rather in the difference in the accessibility of the organic matter for the microbes.

In *A. germinans* leaves no correlation occurred between tannin and TN content, indicating low levels of humification at this early stage of diagenesis or different mechanisms acting towards organic matter preservation in this species.

The comparison between the field and the laboratory experiment as well as the evaluation of the differences between *A. germinans* and *R. mangle* leaves within the laboratory study have shown some distinct mechanisms which occur during mangrove leaf decomposition. While these mechanisms may hold true for leaves that will decompose within the mangrove, they have to be considered in the setting of the whole ecosystem. According to Schories *et al.* (2003) only about 2-3 % of the total annual litter fall is decomposed within the mangrove ecosystem. They calculated that 67 % was removed by the mangrove crab *Ucides cordatus* and the remaining 30 % were exported with the tide. Nordhaus *et al.* (2006) reported a mean of 81 % as estimated litter removal by *Ucides*. The leaf

decomposition experiments showed the importance of tannins and possibly other polyphenolics in N preservation, while the experiment conducted with crab intestines confirmed the theory from Nordhaus (2004) that *U. cordatus* can digest these refractory substances. The mangrove crab *U. cordatus* was established as an important link between intact leaves and microbial degradation to organic matter as its gut flora showed the ability to digest hydrolysable tannins (*Figure 4.3.10*). The crabs may remove the leaves from the sediment surface, but they do not remove the organic matter from the system as tidal export does. Mechanical processing of leaf litter by crabs enhances rates of nutrient cycle as crabs break down litter at >75 the rate of microbial decay alone (Robertson and Daniel, 1989), thereby facilitating high rates of productivity by sedimentary bacteria.

6 CONCLUSIONS

In conclusion, the patterns of TN, TOC, isotopic composition, tannins and THAA illustrated that sediments were divided into two pools of organic matter. The first one was located at the sediment surface and was strongly characterised by the current vegetation, while the second pool comprised the subsurface sediments and showed a stronger relationship to the signature of the anaerobic decomposers and the palaeoenvironment. The comparison between the two transects and also within the transects showed that the lower the inundation frequency, the higher the influence of the plants, as the litter or leachate remained for longer time periods on the sediment surface. The seasons also had an impact on the relationship between plants and sediments, as stronger rains caused stronger leaching.

The two forests showed different N dynamics, partly related to the difference in inundation frequency and sediment salinity, but also strongly influenced by the different species and stand age of the mangrove trees. Although some stratification could be recognised in the sediment depth profiles of transect 1, the uniform influence of the “older” forest was apparent, especially through the distribution of stable tannins in the sediment layer at 50 cm depth which had been dated to an age of 400-600 cal yrs BP by a previous study of Cohen *et al*. (2005a and b). The correspondence with pollen analysis and ^{14}C dating underlined the use of stable tannins or tannin-complexes as palaeo-indicators for woody vegetation.

In transect 2 the impact of the present herbaceous vegetation and a recent past with tidal flats without any vegetation was evident in terms of the TN and TOC values. These parameters as well as tannins and THAA were high in surface sediments, which was attributed to the presence of the succulent *S. portulacastrum*. It also confirmed the strong stratification between surface and subsurface sediments in this younger and dryer part of the forest. *S. portulacastrum* was found to undergo a mycorrhizal symbiosis and showed considerable ability of N_2-fixation in terms of acetylene reduction. Total bacterial counts and low $\delta^{15}N$ values of the rhizosphere confirmed the assumption of *S. portulacastrum* being able to improve nutrient conditions in the substrate through additional fixing of atmospheric N_2. This succulent could therefore have potential as a pioneer species in the process of wetland restoration.

The “older” part of the transect with forest stands originating in a past of possibly up to 400-600 cal yrs BP, showed the influence of the more expanded root structure of *A. germinans* through the uniform distribution of the measured substances. All measured parameters showed less stratification between the different sediment layers than was the case in the younger part of the transect. Higher tannin concentrations in the sediment coupled with pollen analysis of Cohen *et al.* (2005a and b) indicated that woody mangrove vegetation had been populating the area for at least 400 yrs.

Not only did the vegetation have an influence on the sediments, but environmental stress in terms of high salinity and low inundation frequency also had a strong influence on several compounds in the plants. The impact on the photorespiratory processes became apparent through shifts in the ^{13}C isotopic composition of leaves, i.e. higher stress induced and enrichment of the heavier isotope ^{13}C as discrimination in favour of the light isotope ^{12}C diminished. The reaction of total hydrolysable amino acids to salt stress clearly showed the salt resistance strategies used by the different species, such as salt-excretion through salt glands in *A. germinans* and salt-exclusion at the roots in *R. mangle*. The thresholds at which osmoregulation would be initiated also differed between species as did the compounds possibly used as osmolytes. For *A. germinans* for example a critical salinity of about 60 was postulated, above which the plant switched to non-amino acid osmolytes.

Tannins in plants increased with stress, but the exact cause could not be defined. They acted as successful defence against herbivory of the moth larvae *Hyblaea puera* in *R. mangle* and were possibly actively used by the plant as a form of storage mechanism of N in plant litter. The tannins or tannin-protein-complexes may be slowly released to the environment through leaching and decay or may be made accessible to the plant through mycorrhizae which have been known to break down recalcitrant polyphenolics. However the increase of tannins in yellow leaves before abscission that has been observed in the present and in other studies may in fact be related to the production of anthocyanins, a phenolic pigment reported in senescent leaves. As total phenolics were measured, it is possible that the increase and subsequent fast loss of “tannins” was due to an increase in anthocyanins.

Although leaching of phenolics was partly responsible for the loss of phenolics from the leaves, the main actors in tannin decomposition could be identified as being the fungi, while bacteria were important in terms of nitrogen accumulation and immobilisation. The decomposition experiments clarified the source of TN accumulation in decaying plant

material, which has been discussed controversially in the literature, as being the bacterial colonisation, which increased TOC and TN partly through new synthesis of amino acids.

Concerning the main actors in terms of microbial litter decomposition, bacteria were shown to be dominant on decaying yellow and brown leaves, while black leaves were mainly colonised and decomposed by fungi. The laboratory experiment confirmed that D-ala is a reliable biomarker for bacteria.

The mangrove crab *U. cordatus* has been shown to be responsible for the breakdown of at least 67 % of total leaf litter (Schories *et al.,* 2003), with preferential consumption being *R. mangle* leaves (Nordhaus, 2004). In the present study it could be shown that *U. cordatus* is able to digest hydrolysable tannins, thus possibly providing an explanation for the crabs' preference of the tannin-rich *Rhizophora* leaves to the tannin-poor *Avicennia* leaves. The crabs are therefore possibly an even more important link between plant litter and nutrient availability than has been previously thought.

Several insights and new perspectives arose from this work. The improvement of substrate conditions through the succulent *S. portulacastrum* opens perspectives for its possible use as pioneer plant in wetland restoration. The probable input of N into the system through N_2-fixation is achieved through a bacterial population which is directly associated with the roots of *S. portulacastrum*. However, more research, such as planting field experiments, is needed to confirm the practicability of this assumption.

Crabs and fungi have been shown to play a major role in breakdown of tannins and thus recycling of nutrients, while bacteria were responsible for the frequently reported accumulation of N on leaf litter. As a challenge for future research, it remains to be proven what the inorganic N source of the bacteria is: atmospheric N_2 or ammonium.

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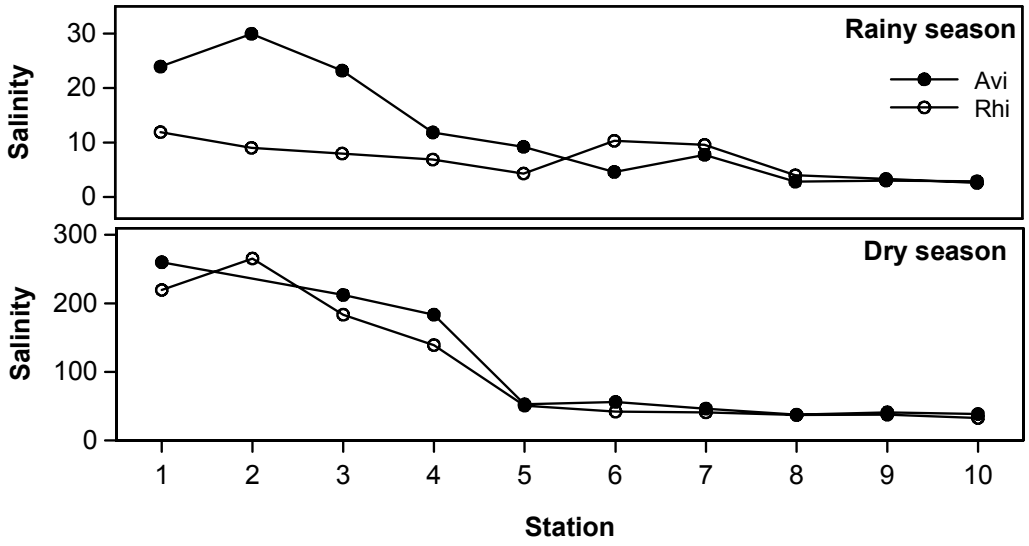
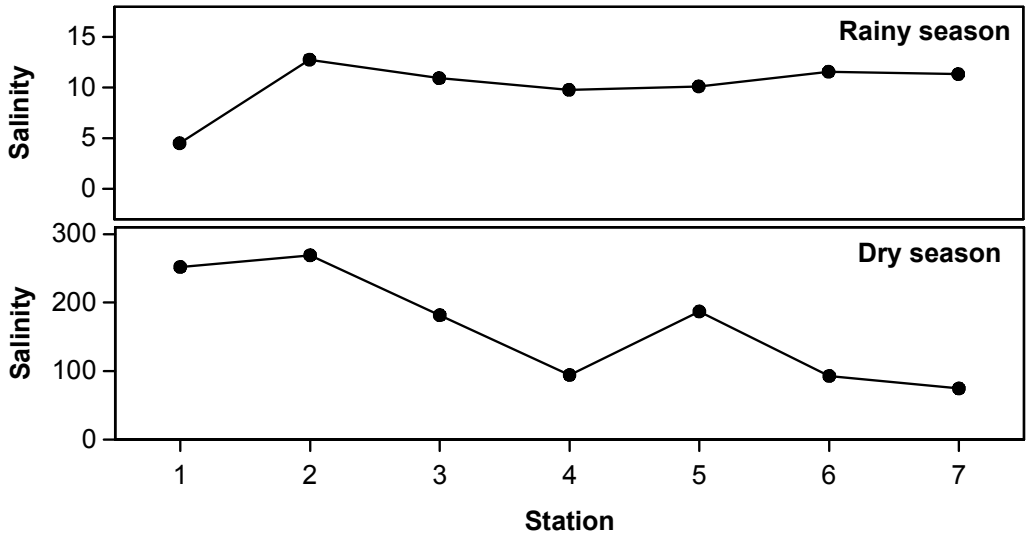
10 APPENDIX



A1: Salinity of surface sediments near *A. germinans* (Avi) and *R. mangle* (Rhi) trees for transect 1 during rainy and dry season.



A2: Salinity of surface sediments for transect 2 during rainy and dry season

Season	Rainy season						Dry season					
Species	*Avicennia*			*Rhizophora*			*Avicennia*			*Rhizophora*		
Depth [cm]	**0**	**10**	**50**	**0**	**10**	**50**	**0**	**10**	**50**	**0**	**10**	**50**
L-asx	1.1	0.7	0.3	1.6	0.9	0.8	1.6	0.7	0.3	1.1	1.0	0.3
L-glx	1.3	0.8	0.4	1.5	0.8	0.8	1.6	0.8	0.3	1.0	1.0	0.3
L-ser	0.7	0.5	0.1	0.8	0.4	0.4	0.9	0.4	0.1	0.4	0.4	0.08
L-thr	0.8	0.5	0.1	0.9	0.5	0.4	1.0	0.4	0.2	0.4	0.4	0.1
gly	0.7	0.5	0.2	0.8	0.5	0.4	0.9	0.4	0.2	0.3	0.3	0.1
L-arg	0.6	0.4	0.2	0.7	0.4	0.4	0.9	0.5	0.2	0.4	0.4	0.2
L-ala	0.8	0.6	0.2	1.0	0.5	0.5	1.1	0.5	0.2	0.5	0.5	0.2
L-tyr	0.4	0.2	n.d.	0.4	0.1	0.2	0.5	0.3	0.1	0.2	0.2	0.08
L-val	0.6	0.4	0.2	0.9	0.5	0.5	0.8	0.4	0.2	0.4	0.5	0.2
L-phe	0.5	0.4	0.1	0.6	0.3	0.3	0.8	0.4	0.2	0.4	0.4	0.1
L-ile	0.4	0.3	0.1	0.5	0.3	0.3	0.4	0.2	0.1	0.3	0.3	0.1
L-leu	0.8	0.5	0.2	0.9	0.5	0.5	1.0	0.5	0.2	0.6	0.5	0.2
D-asx	0.2	0.1	0.1	0.2	0.2	0.1	0.2	0.1	0.08	0.1	0.1	0.06
D-glx	0.1	0.08	0.05	0.09	0.07	0.06	0.1	0.07	0.04	0.08	0.08	0.04
D-ser	0.03	0.03	0.03	0.03	0.03	0.03	0.04	0.03	0.03	0.02	0.03	n.d.
D-arg	0.09	0.09	0.07	0.09	0.08	0.09	0.1	0.06	0.05	n.d.	n.d.	n.d.
D-ala	0.1	0.09	0.07	0.1	0.1	0.09	0.07	0.04	0.04	0.04	0.04	0.03
D-ile	0.8	0.2	0.2	0.3	0.3	0.2	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
D-leu	0.05	0.05	n.d.	0.05	0.06	0.04	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

A3: Composition of individual L- and D-amino acids in mg/g dw at station 1 of transect 1 for surface, 10 cm and 50 cm sediment layers near *A. germinans* (*Avicennia*) and *R. mangle* (*Rhizophora*) trees during the rainy and the dry season.

Season	Rainy season						Dry season					
Species	*Avicennia*			*Rhizophora*			*Avicennia*			*Rhizophora*		
Depth [cm]	**0**	**10**	**50**	**0**	**10**	**50**	**0**	**10**	**50**	**0**	**10**	**50**
L-asx	0.8	0.3	0.4	0.8	0.5	0.6	1.1	0.7	0.7	1.1	0.5	0.7
L-glx	0.9	0.4	0.4	0.9	0.6	0.6	n.d.	n.d.	n.d.	1.2	0.7	0.8
L-ser	0.5	0.2	0.2	0.5	0.3	0.3	0.5	0.4	0.3	0.6	0.3	0.4
L-thr	0.5	0.2	0.2	0.5	0.3	0.3	0.6	0.4	0.4	0.6	0.3	0.4
gly	0.5	0.2	0.2	0.5	0.3	0.3	0.6	0.4	0.4	0.5	0.3	0.3
L-arg	0.5	0.2	0.2	0.5	0.3	0.3	0.6	0.5	0.4	0.6	0.3	0.4
L-ala	0.6	0.2	0.2	0.6	0.3	0.4	0.7	0.5	0.4	0.7	0.4	0.5
L-tyr	0.2	n.d.	0.09	0.2	n.d.	0.2	0.4	0.2	0.2	0.2	n.d.	0.2
L-val	0.5	0.2	0.2	0.5	0.2	0.4	0.6	0.4	0.4	0.6	0.3	0.5
L-phe	0.4	0.2	0.2	0.4	0.2	0.3	0.5	0.3	0.3	0.5	0.3	0.3
L-ile	0.3	0.1	0.1	0.3	0.2	0.2	0.4	0.3	0.2	0.4	0.2	0.3
L-leu	0.6	0.2	0.2	0.6	0.3	0.4	0.7	0.5	0.4	0.7	0.3	0.5
D-asx	0.1	0.09	0.1	0.1	0.1	0.1	0.1	0.2	0.1	0.1	0.08	0.09
D-glx	0.06	0.04	0.05	0.06	0.05	0.05	0.1	0.07	0.07	0.09	0.06	0.07
D-ser	0.02	0.02	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	n.d.	0.02
D-arg	0.06	0.05	0.05	n.d.	n.d.	n.d.	0.08	0.07	0.07	n.d.	0.06	0.05
D-ala	0.07	0.06	0.07	0.07	0.06	0.06	0.06	0.04	0.04	0.04	0.02	0.03
D-ile	n.d.	n.d.	n.d.	0.03	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
D-leu	n.d.	n.d.	n.d.	0.04	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

A4: Composition of individual L- and D-amino acids in mg/g dw at station 10 of transect 1 for surface, 10 cm and 50 cm sediment layers near *A. germinans* (*Avicennia*) and *R. mangle* (*Rhizophora*) trees during the rainy and the dry season.

Season	Rainy season		Dry season	
Species	***Avicennia***	***Rhizophora***	***Avicennia***	***Rhizophora***
L-asx	9	6.8	7.1	8.6
L-glx	11.1	8.9	10.4	10.9
L-ser	5.6	5.2	6	6.4
L-thr	6	4	4.6	5.6
L-his	3.9	3.5	3.9	4.3
gly	2.5	1.3	5.6	3.8
L-arg	7.2	5.1	9.7	7.6
L-ala	6.4	5.5	6.4	6.7
L-tyr	4.4	2.7	4.8	4.7
L-val	5.4	4.5	4.7	5.7
L-phe	4.9	4.1	5	5.5
L-ile	4	3.3	3.4	4
L-leu	9.3	8.2	9.7	9.7
D-asx	0.4	0.3	0.3	0.3
D-glx	0.3	0.2	0.3	0.3
D-ser	0.1	0.1	0.1	0.3
D-arg	n.d.	n.d.	n.d.	0.2
D-ala	0.2	n.d.	n.d.	n.d.
D-ile	0.1	0,1	n.d.	n.d.
D-leu	0.3	0.2	0.3	0.3

A5: Composition of individual L- and D-amino acids in mg/g dw at station 1 of transect 1 for leaves of *A. germinans* (Avi) and *R. mangle* (Rhi) during the rainy and the dry season.

Season	Rainy season		Dry season	
Species	*Avicennia*	*Rhizophora*	*Avicennia*	*Rhizophora*
L-asx	9.9	5.1	5.2	6.3
L-glx	12.3	7.1	8.2	12
L-ser	6.4	4.4	4.9	6.7
L-thr	6.6	3.4	4	6.2
L-his	4.7	n.d.	3.7	4.5
gly	2.9	3.7	4.9	3.7
L-arg	10.2	5.7	7.9	7.3
L-ala	7.3	4.6	5.2	7
L-tyr	5	2.9	3.6	5
L-val	6	3.8	3.5	6.3
L-phe	5.5	4	4	6.1
L-ile	4.6	2.1	2.5	4.3
L-leu	10.6	6.7	7.7	9.9
D-asx	0.4	0.07	0.2	0.4
D-glx	0.3	0.05	0.2	0.3
D-ala	0.1	0.02	0.08	0.2
D-tyr	n.d.	n.d.	0.2	0.2
D-phe	n.d.	n.d.	n.d.	0.2
D-ile	0.1	0.02	n.d.	n.d.
D-leu	0.3	0.05	0.2	0.3

A6: Composition of individual L- and D-amino acids in mg/g dw at station 10 of transect 1 for leaves of *A. germinans* (Avi) and *R. mangle* (Rhi) during the rainy and the dry season.

Season	Rainy season			Dry season		
Depth [cm]	0	10	50	0	10	50
L-asx	3.3	0.6	0.06	1	0.4	0.05
L-glx	3.1	0.7	0.2	1.1	0.6	0.2
L-ser	1.5	0.3	0.03	0.4	0.2	n.d.
L-thr	1.8	0.3	0.3	0.5	0.2	n.d.
gly	1.6	0.4	0.05	0.4	0.2	0.04
L-arg	1.3	0.4	0.2	0.5	0.2	0.8
L-ala	1.9	0.5	0.07	0.6	0.3	0.03
L-tyr	0.8	0.09	n.d.	0.3	0.2	0.1
L-val	1.6	0.4	0.06	0.5	0.3	0.07
L-phe	1.2	0.3	n.d.	0.3	0.2	n.d.
L-ile	1.1	0.3	n.d.	0.4	0.2	n.d.
L-leu	1.7	0.5	0.07	0.5	0.3	0.1
D-asx	0.3	0.1	0.03	0.1	0.08	n.d.
D-glx	0.2	0.06	0.03	0.08	0.06	n.d.
D-ser	0.03	0.02	n.d.	n.d.	n.d.	n.d.
D-arg	n.d.	0.06	n.d.	n.d.	0.05	n.d.
D-ala	0.2	0.08	0.05	0.05	0.03	n.d.
D-ile	n.d.	n.d.	n.d.	0.04	0.05	n.d.
D-leu	n.d.	0.04	n.d.	0.1	0.1	n.d.

A7: Composition of individual L- and D-amino acids in mg/g dw at station 1 of transect 2 for surface, 10 cm and 50 cm sediment layers during the rainy and the dry season.

Season	Rainy season			Dry season		
Depth [cm]	**0**	**10**	**50**	**0**	**10**	**50**
L-asx	1.2	0.3	0.2	0.9	0.4	0.4
L-glx	1.2	0.3	0.3	1.1	0.5	0.5
L-ser	0.6	0.1	0.1	0.5	0.1	0.2
L-thr	0.6	0.1	0.1	0.5	0.2	0.2
gly	0.6	0.2	0.2	0.5	0.2	0.2
L-arg	0.5	0.2	0.2	0.5	0.2	0.2
L-ala	0.7	0.2	0.2	0.6	0.2	0.2
L-tyr	0.2	0.06	n.d.	0.3	0.1	0.2
L-val	0.6	0.2	0.1	0.5	0.2	0.3
L-phe	0.5	0.1	0.1	0.3	0.1	0.2
L-ile	0.4	0.08	0.07	0.3	0.1	0.1
L-leu	0.6	0.2	0.2	0.5	0.2	0.2
D-asx	0.2	0.07	0.06	0.09	0.1	0.08
D-glx	0.08	0.04	0.04	0.08	0.05	0.05
D-ser	0.03	0.03	0.02	0.03	n.d.	n.d.
D-arg	0.09	n.d.	0.06	n.d.	0.06	n.d.
D-ala	0.09	0.06	0.06	0.05	0.03	0.03
D-ile	n.d.	n.d.	n.d.	0.05	0.05	0.05
D-leu	n.d.	n.d.	n.d.	0.2	0.2	0.2

A8: Composition of individual L- and D-amino acids in mg/g dw at station 7 of transect 2 for surface, 10 cm and 50 cm sediment layers during the rainy and the dry season.

Season	Rainy season					Dry season				
Species	*Avi*	*Ses*	*Ses roots*	*Spo*	*Spo roots*	*Avi*	*Ses*	*Ses roots*	*Spo*	*Spo roots*
L-asx	4.8	5.2	3.3	4.1	3.1	6.1	4.04	2.7	2.1	4.8
L-glx	5.6	6.6	4.1	4.9	3.3	7.9	6	4.1	3	3.1
L-ser	2.6	3.2	2.5	2.1	1.4	4.6	3.2	2.6	1.7	1.7
L-thr	2.5	2.9	1.8	2.1	1.4	3.7	2.6	1.8	1.2	1.2
L-his	n.d.	3.3	2.3	2.5	n.d.	3.1	2.3	1.9	n.d.	n.d.
gly	1.2	1.7	1.2	0.6	0.6	3.2	2.3	2.1	1.5	1.4
L-arg	3.4	4	2.8	2.4	1.9	5.6	5.1	2.9	2.7	3.3
L-ala	3.5	3.4	2.1	2.9	1.9	4.7	3.2	2.1	2.2	2.1
L-tyr	1.8	2.7	1.9	1.5	1.1	3.2	2.4	1.8	1.2	1.2
L-val	2.6	2.9	1.8	2.1	1.4	3.3	2.4	1.8	1.3	1.3
L-phe	2.4	2.8	1.6	1.7	1.2	3.6	2.7	1.6	1.5	1.4
L-ile	1.7	2.1	1.4	1.4	0.8	2.3	1.8	1.2	0.7	0.6
L-leu	4.5	4.4	2.5	3.5	2.2	6.2	4.5	2.7	2.3	1.9
D-asx	0.2	0.3	0.2	0.2	0.2	0.2	0.2	0.2	0.1	0.3
D-glx	0.2	0.2	0.2	0.2	0.1	0.2	0.2	0.2	0.1	0.1
D-ser	n.d.	n.d.	n.d.	0.1	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
D-ala	0.2	0.1	0.1	0.1	0.1	0.05	0.1	0.2	0.2	0.2
D-tyr	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.2	0.4	n.d.	n.d.
D-val	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.1	0.1	n.d.	n.d.
D-ile	n.d.	n.d.	n.d.	n.d.	n.d.	0.1	n.d.	n.d.	n.d.	n.d.
D-leu	n.d.	n.d.	n.d.	n.d.	n.d.	0.2	n.d.	n.d.	n.d.	n.d.

A9: Composition of individual L- and D-amino acids in mg/g dw at station 1 of transect 2 in leaf samples of *A. germinans* (Avi) and in leaf and root samples of *S. portulacastrum* (Ses), *S. virginicus* (Spo) and *Batis maritima* (Bat) during rainy and dry season.

Season	**Rainy season**	**Dry season**
L-asx	6.8	7.9
L-glx	8.3	9.3
L-ser	4.5	5.2
L-thr	4.5	5.1
L-his	n.d.	3.4
gly	3.2	3.4
L-arg	4.6	6.5
L-ala	5.7	5.6
L-tyr	3.6	4
L-val	5	4.1
L-phe	4.5	4.5
L-ile	3.3	2.9
L-leu	7.5	7.6
D-asx	0.4	0.3
D-glx	0.3	0.2
D-ser	n.d.	n.d.
D-ala	0.3	0.05
D-tyr	n.d.	0.2
D-val	n.d.	n.d.
D-ile	0.3	0.1
D-leu	0.5	0.3

A10: Composition of individual L- and D-amino acids in mg/g dw at station 7 of transect 2 in leaf samples of *A. germinans* during rainy and dry season.

Time	Day 0			Day 42		
	Yellow	**Brown**	**Black**	**Yellow**	**Brown**	**Black**
L-asx	1.1	2.7	5.3	2.2	4.5	6.9
L-glx	1.6	3.2	6.6	3.2	5.4	7.9
L-ser	1.3	2.2	4.2	2.7	3.7	5.7
L-thr	1	1.9	4	2.1	3	4.9
L-his	n.d.	2	1	n.d.	n.d.	n.d.
gly	1.1	1.5	3.2	3	3.4	4.5
L-arg	1.5	2.3	4.9	2.7	3.7	5.8
L-ala	1.2	2.3	4.6	2.4	3.7	5.8
L-tyr	0.8	1.5	2.8	1.5	2.3	3.3
L-val	1.3	2.1	3.5	1.8	2.9	4.1
L-phe	1.2	2	2.4	1.9	3	5
L-ile	0.6	1.3	2.6	1.3	2.1	2.9
L-leu	1.7	3	6.1	3.2	5	7.9
D-asx	0.1	0.2	0.2	0.2	0.2	0.3
D-glx	n.d.	0.2	0.2	0.2	0.2	0.3
D-ala	0.1	0.1	0.2	0.1	0.2	0.2

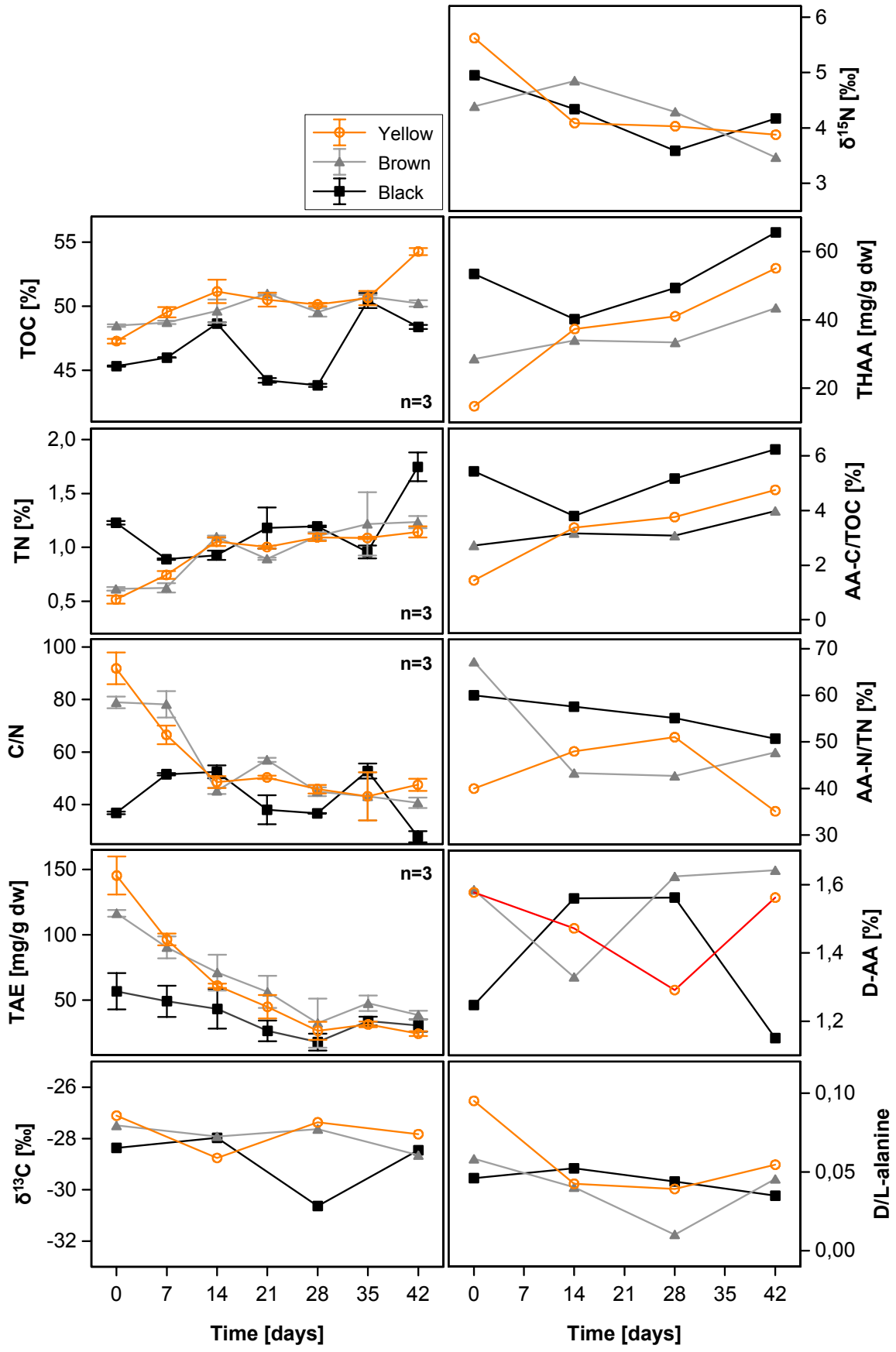
A11: Composition of individual L- and D-amino acids in mg/g dw in leaves of *R. mangle* during the field experiment. The values for yellow, brown and black leaves are presented for the first day (day 0) and the last day of the experiment (day 42).

Species	*Rhizophora*				
Time	**Day 0**	**Day 42**			
Treatment		**Reference**	**$HgCl_2$**	**Bacteria**	**Fungi**
L-asx	2.3	5.7	1.9	3.9	2.6
L-glx	2.2	6	2.3	5.1	2.6
L-ser	1.4	3	1.3	2.9	1.2
L-thr	1.4	3.3	1.2	2.8	1.5
L-his	n.d.	n.d.	n.d.	2.5	n.d.
gly	0.9	1.5	0.8	1.8	0.8
L-arg	1.6	2.7	1.4	3.03	1.5
L-ala	1.5	3.5	1.4	3	1.5
L-tyr	1.2	1.8	1	2.2	1.2
L-val	0.5	3	1.3	3.3	1.6
L-phe	1.4	2.6	1	2.8	1.5
L-ile	1	2.1	0.8	2.2	1.2
L-leu	3.4	4.7	2	4.3	2.2
D-asx	0.2	0.2	0.2	0.3	0.2
D-glx	0.2	0.3	0.2	0.3	0.1
D-ser	n.d.	n.d.	n.d.	0.1	n.d.
D-arg	n.d.	n.d.	n.d.	n.d.	n.d.
D-ala	0.05	0.08	n.d.	0,1	0.04
D-tyr	n.d.	n.d.	n.d.	n.d.	n.d.
D-val	n.d.	n.d.	n.d.	0.1	n.d.
D-phe	n.d.	n.d.	n.d.	n.d.	0.3
D-ile	n.d.	0.2	0.2	0.1	0.2
D-leu	n.d.	n.d.	n.d.	0,2	0.4

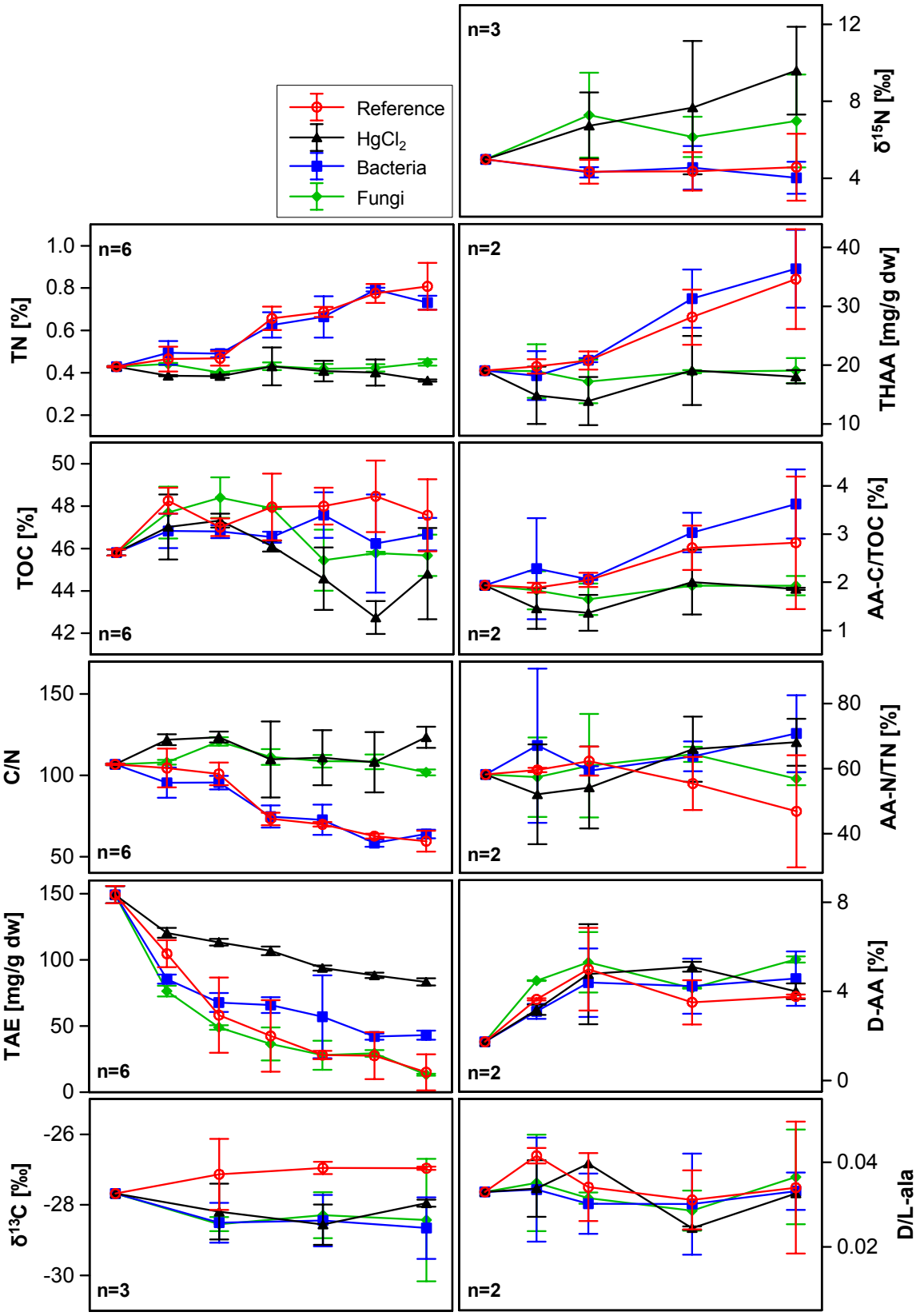
A12: Composition of individual L- and D-amino acids in mg/g dw in leaves of *R. mangle* (*Rhizophora*) during the laboratory experiment. The values for the reference group (Reference) and the three different treatments with mercuric chloride ($HgCl_2$), fungicide (Bacteria) and bactericide (Fungi) are presented for the first day (day 0) and the last day of the experiment (day 42).

Species	*Avicennia*				
Time	**Day 0**	**Day 42**			
Treatment		**Reference**	**HgCl2**	**Bacteria**	**Fungi**
L-asx	3.4	6.3	3.1	5.2	1.6
L-glx	3.1	6.5	2.9	6.3	2.5
L-ser	1.7	3.1	1.7	3.8	1.3
L-thr	1.8	3.8	1.8	3.5	1.1
L-his	n.d.	2.9	1.8	n.d.	n.d.
gly	1.2	1.9	0.9	3	1.08
L-arg	2.1	3.6	2	4.3	1.9
L-ala	1.7	3.8	1.8	4.2	1.5
L-tyr	1.4	2.5	1.4	2.6	1.07
L-val	0.3	2.8	1.4	2.7	1.2
L-phe	1.5	2.9	1.6	3.1	1.2
L-ile	1.2	2.03	1.03	2	0.8
L-leu	2.1	4.3	2.3	5.06	2.1
D-asx	0.2	0.2	0.4	0.4	0.1
D-glx	0.2	0.3	n.d.	0.3	0.1
D-ser	0.1	0.1	n.d.	n.d.	n.d.
D-arg	n.d.	2.4	n.d.	n.d.	n.d.
D-ala	0.07	0.1	0.06	0.1	0.05
D-tyr	n.d.	0.2	n.d.	n.d.	n.d.
D-val	n.d.	n.d.	0.08	n.d.	n.d.
D-phe	n.d.	n.d.	n.d.	n.d.	n.d.
D-ile	n.d.	n.d.	0.1	n.d.	0.2
D-leu	n.d.	n.d.	0.2	n.d.	n.d.

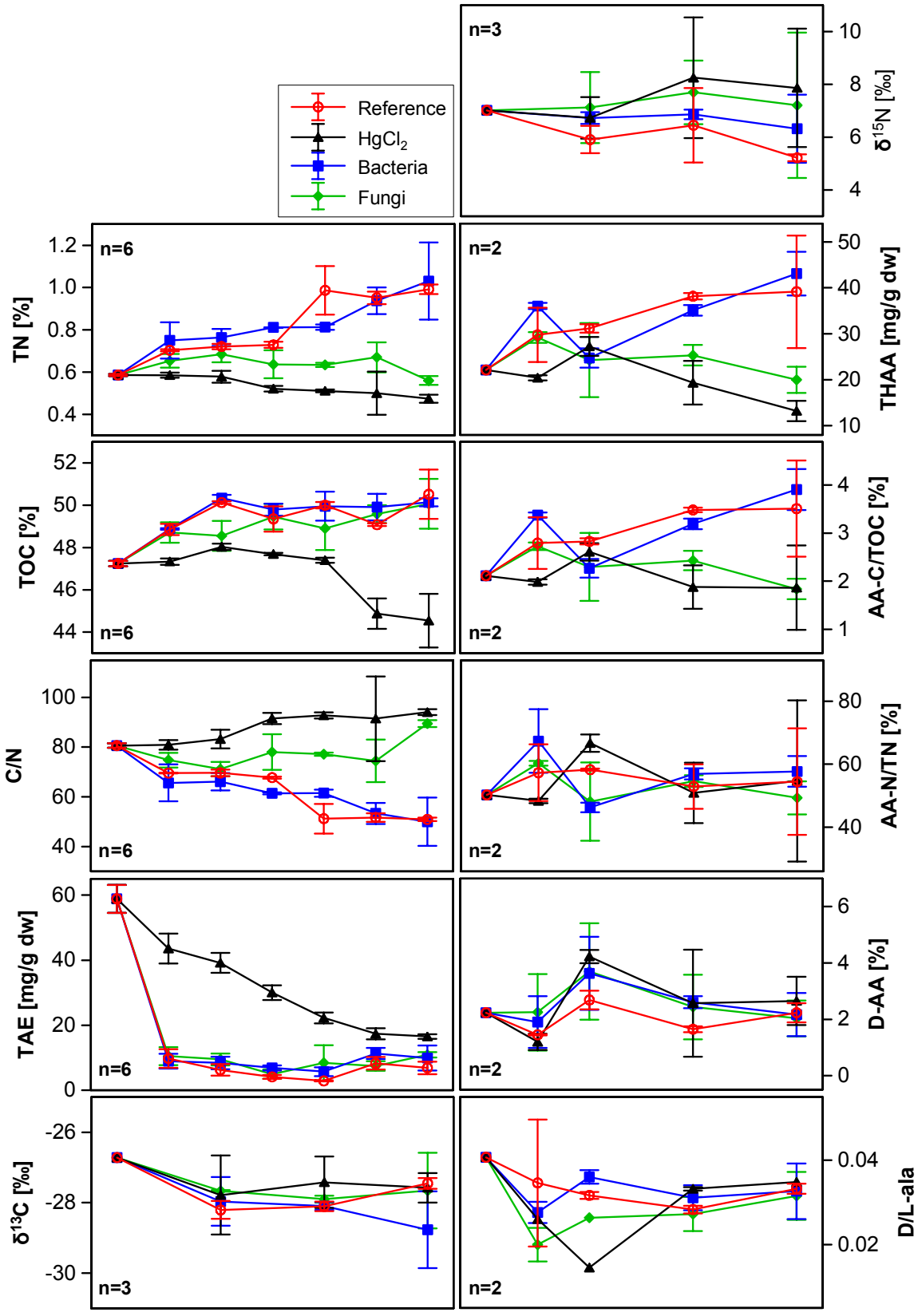
A13: Composition of individual L- and D-amino acids in mg/g dw in leaves of *A. germinans* (*Avicennia*) during the laboratory experiment. The values for the reference group (Reference) and the three different treatments with mercuric chloride ($HgCl_2$), fungicide (Bacteria) and bactericide (Fungi) are presented for the first day (day 0) and the last day of the experiment (day 42).



A13: Values for all measured parameters of weekly samples of yellow, brown and black *R. mangle* leaves taken during the field experiment, mean ± SD, n is given in graph.



A14: Values for all measured parameters of weekly samples of *R. mangle* leaves taken during the laboratory experiment. Values for the reference group (Reference) and the three different treatments with mercuric chloride ($HgCl_2$), fungicide (Bacteria) and bactericide (Fungi) are presented, mean ± SD, n is given in graph.



A15: Values for all measured parameters of weekly samples of *A. germinans* leaves taken during the laboratory experiment. Values for the reference group (Reference) and the three different treatments with mercuric chloride ($HgCl_2$), fungicide (Bacteria) and bactericide (Fungi) are presented, mean ± SD, n is given in graph.

Erklärung:

Hiermit erkläre ich, dass ich die vorliegende Dissertationsschrift selbständig verfasst und keine anderen als die angegebenen Hilfsmittel verwendet habe.

Bremen, den 06.06.2006

Bettina B. Schmitt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b r e m e n


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