Dissolved and colloidal organic matter in a tropical lagoon-estuary system surrounded by sugar cane plantations

Dissertation

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Zusammenfassung

Brasilien ist weltweit der Hauptproduzent von Zuckerrohr mit einem steigenden Bedarf an Land für die Ausweitung des Zuckerrohranbaus. Der Einfluss auf die Umwelt ist dabei vielfältig, von den Emissionen in die Atmosphäre, Bodenerosion und Bodenverarmung, bis zu Eutrophierung und Kontamination von Gewässersystemen durch den Austrag von Düngemitteln und organischen Bioziden. Diese Arbeit wurde im Rahmen des brasilianisch/ deutschen Projektes POLCAMAR durchgeführt, welches den Einfluss der Zuckerrohr-monokultur auf Ästuar- und Küstengewässer Nordostbrasiliens untersuchte. In dem hier beschriebenen Teilprojekt wurden drei Expeditionen zum tropischen Manguaba Lagunen-Ästuar-System durchgeführt, dessen Einzugsgebiet durch ausgedehnten Zuckerrohranbau im Hinterland geprägt ist. Ziel des Teilprojektes war die Quantifizierung des organischen Materials (OM) mit Zuckerrohrursprung auf dem Weg vom Fluss zu den Küstengewässern, unter besonderer Berücksichtigung der Quellen und Senken gelöster organischer Substanz.

Proben wurden auf DOC, DON, $\delta^{13}C$ und UV-Absorption analysiert. Zusätzlich wurde $\delta^{13}C$ in partikulärem organischem Kohlenstoff in Wasser, Boden und terrestrischen Pflanzen-materialien bestimmt. Ferner wurde das DOM, mit einer in dieser Arbeit etablierten und verifizierten tangentialen Flussfiltration, in kleine, mittlere und große Molekular-gewichtsfraktionen getrennt, die ebenfalls elementar- und isotopenanalytisch bestimmt wurden.

Das in allen Proben angereicherte $\delta^{13}C$ Signal von DOM belegt, dass Zuckerrohrkohlenstoff in den Boden und das Wassersystem aufgenommen wurde. Bodenprozesse, einschließlich der Ausfällung von Kolloiden, bestimmen die Größenverteilung des DOM im Frisch- und Brack-wassersystem, wobei fast das gesamte DOM in der kleinen Molekulargewichtsfraktion (< 1 kDa, 92%) vorliegt. Die hochmolekulare Kolloidfraktion (> 50 kDa) unterscheidet sich - anders als die niedermolekulare Phase - in C/N und $\delta^{13}C$ nicht von der partikulären Phase. Einträge des DOM in Flüsse und Lagune stammen aus Grundwasser, Feldabfluss, sowie aus Einleitungen von Zuckerrohrfabriken. Durchschnittlich ein Drittel des DOM in Flüssen und Lagune hat einen Zuckerrohrursprung.
Während starker Niederschläge wurde im Übergang von Fluss- zu Brackwasser ein Nettoverlust von DOM durch Flockung und Sedimentierung beobachtet. Ein Nettogewinn hingegen wurde durch die Zugabe von photolysiertem, resuspendiertem OM aus dem Sediment in der Trockenzeit (Grundwasserabfluss) hervorgerufen.
Substanzen, die in die Lagune gelangen, verweilen hier für mehrere Wochen. Das DOM kann währenddessen bakteriell und photolytisch umgesetzt werden. Fast das gesamte zuckerrohrbürtige OM wird in der Lagune abgebaut. Der verbleibende refraktäre Anteil mischt sich konservativ durch das Ästuar in den Atlantischen Ozean. Eine grobe Abschätzung des jährlichen DOM-Exportes mit Zuckerrohrursprung ergibt 150 t pro Jahr aus der Manguaba Lagune und 1.500 - 15.000 t pro Jahr von den brasilianischen Zuckerrohrfeldern der Atlantikküste.

Abstract

Brazil is the major sugar cane producer in the world with an increasing demand of land due to the expansion of sugar cane cultivation. The impact on the environment thereby is manifold: from emissions to the atmosphere, soil erosion and impoverishment, to eutrophication and contamination of aquatic systems by the application of fertilizers and agro-toxic organic pollutants. This work was carried out in the framework of the Brazilian/ German POLCAMAR (Pollution from sugar Cane in Marine systems) project, which aimed to assess the environmental impacts of sugar cane monocultures on estuarine and coastal waters of Northeast-Brazil. In this subproject, three expeditions were conducted to the tropical Manguaba lagoon-estuary-system, whose catchment is characterised by extensive sugar cane cultivation in its hinterland. The aim of this subproject was the quantification of the sugar cane derived amount of organic matter (OM) along its way from riverine to coastal waters with particular emphasis on sources and sinks of dissolved organic matter.

Collected samples were analysed for DOC, DON, $\delta^{13}C$ and UV absorption. In addition, $\delta^{13}C$ of particulate organic carbon was determined in water, soil and terrestrial plant samples. Bulk DOM was further separated into low, high and very high molecular components using a tangential flow filtration method established and verified in this thesis. These size fractions were also determined analytically for their elemental and isotopic composition.

The enriched $\delta^{13}C$ signal in OM of all samples proves that sugar cane carbon is incorporated into the soil and water system. Soil processes, including colloid precipitation, control the size distribution of DOM in the fresh and brackish water system, whereby the LMW fraction (< 1kDa, 92%) represents almost all of the DOM pool. The very high molecular weight fraction (> 50 kDa) is not distinguishable from the particulate phase, in contrast to the low molecular weight phase. DOM fluxes into the river and lagoon originate from baseflow, field runoff and sugar cane factory effluents. On average, one third of riverine and lagoonal DOM has a sugar cane source.

During heavy rainfall a net loss of DOM from flocculation and sedimentation was observed in the transition zone from riverine to brackish waters. A net gain of DOM from addition of photolysed resuspended sedimentary OM, in turn, was observed during the dry situation (baseflow).

Substances introduced into the lagoon will remain there for several weeks. In this time, DOM can be transformed by heterotrophic bacteria and by photolytic processes. Almost all of the sugar cane derived OM is degraded within the lagoon. The residual refractory remains are mixed conservatively through the estuary into the Atlantic Ocean. A back-of-the-envelop estimation resulted in an annual export of sugar cane derived DOM of 150 t yr^{-1} for the Manguaba lagoon and of 1,500 - 15,000 t yr^{-1} for all brazilian sugar cane fields of the Atlantic coast.

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1. Introduction

This study was part of the Brazilian/ German project POLCAMAR (Pollution from sugar Cane in Marine systems) which intends to assess the impact of pollutants from sugar cane monocultures on estuaries and coastal waters of Northeast-Brazil in order to understand pollutant transport and fate as well as to develop sustainable management strategies. The focus of this thesis is on biochemical processes in the Manguaba lagoon-estuary system with the emphasis on characterisation and transport behaviour of organic matter. As extensive sugar cane monoculture cultivation and production is present in the lagoon hinterland the following chapter provides an insight into sugar cane monocultures in Brazil as well as on the biogeochemical processes concerning organic matter pathways.

1.1 Sugar cane monocultures in Brazil

Monoculture production is a widespread agronomical strategy in tropical countries, for which sugar cane is a good example in Brazil. Brazil is with about 40% the major sugar cane producer in the world (FAOSTAT, 2008), providing approximately one million direct jobs (Goldemberg et al., 2008). To date, this industry (2010/2011) crushes 660 million metric tons of sugar cane from a harvested area of 9.8×10^4 km^2 in Brazil (Barros, 2010).

The increasing demand of land, owing to the expansion of the agricultural activities and the installations of new industrial plants, leads to serious impacts on the environment. Monocultural cultivation of sugar cane suffers from the long-term exploitation of always the same mineral resources leading to impoverishment of soil quality and land erosion (Martinelli and Filoso, 2008). It requires permanent fertilization and, from a lack of natural enemies for the various weeds and pests, large amounts of specific agrochemicals. As a consequence of improper agricultural management, particularly during the recultivation period, eutrophication and contamination of recipient aquatic systems (i.e. rivers, lakes, aquifers, estuaries and coastal waters) and its biota occur from the application of fertilizers and agro-toxic organic pollutants. Emissions of particles (soot), hydrocarbons and trace gases to the atmosphere from crop burning practices result in atmospheric pollution (Fig. 1.1). From the atmosphere, nitrogen partly returns to the earth surface via wet and dry deposition and hence,

acid rain becomes another problem associated with burning of sugar cane.

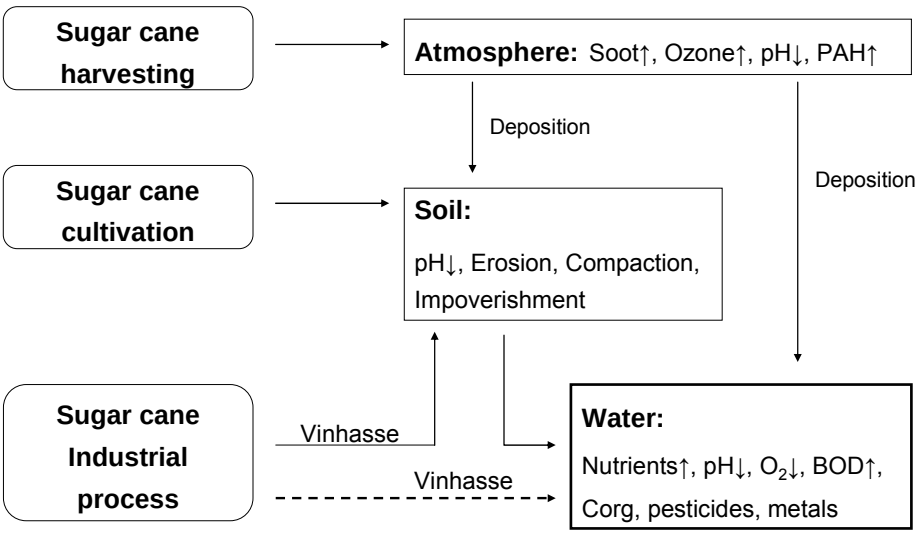

Fig. 1.1 *Main environmental impacts of the sugar cane agro industry (modified according to Martinelli and Filoso, 2008)*

For the harvest season 2010/2011, the main final products of the sugar cane industry are crystalline sugar and bio ethanol, with a destined production from total sucrose of 44.6 and 55.4%, respectively (Barros, 2010). The latter is obtained from fermentation and distillation of sugar cane juice and molasses. Bagasse, the solid residue from sugar cane juice extraction, and stillage (vinhasse), the liquid waste effluent from the distillation process, are the most significant by-products of a sugar cane plant. Sugar cane plants with alcohol distilleries generally release an average of 156 L of stillage and 250 kg of bagasse per 1,000 kg of cane (Gunkel et al., 2007). While bagasse is used as combustible fuel for energy production, vinhasse is mainly disposed to fields via fertigation, a combination of fertilisation and irrigation. Plants dispose vinhasse also directly into rivers. The high concentration of potassium in vinhasse can accumulate at toxic levels in the soil. The fertigation fluid contains organic acids decreasing the pH of soils and waters (Gunkel et al., 2007). The rivers' main ecological problem is the high content of organic matter in vinhasse causing oxygen depletion by enhanced biological oxygen demand through heterotrophic biodegradation.

1.2 Sugar cane monoculture in the northeast coastal zone

Sugar cane is located mainly in the south-central and north-eastern regions of Brazil accounting for 89% and 11% of production, respectively. In contrast to the south-central region (mainly the state of São Paulo) where the agribusiness is primarily situated inland, the remainders of the states exert their sugar cane production (26.8% produced on ~ 2 x 10^4 km^2) along the comparatively narrow coastal (Atlantic) strip that extends from the state of Rio Grande do Norte to Rio de Janeiro in the south (Fig. 1.2)(UNICA, 2010). Except for the large Sao Francisco catchment, this coastal strip is characterized by a series of small to medium size catchments that discharge into the Atlantic Ocean directly or indirectly, via lagoons.

The north and northeastern states of Sergipe, Alagoas and Pernambuco contribute about 8.9% to Brazil's total sugar cane production. Due to the regions historical development and the introduction of the Brazilian bioalcohol program (PROALCOÓl) in 1976, sugar cane is a significant agricultural and industrial factor in the northeastern region of Brazil (Fig. 1.2). Therefore, land use can have an immediate impact on local and regional land-ocean material fluxes.

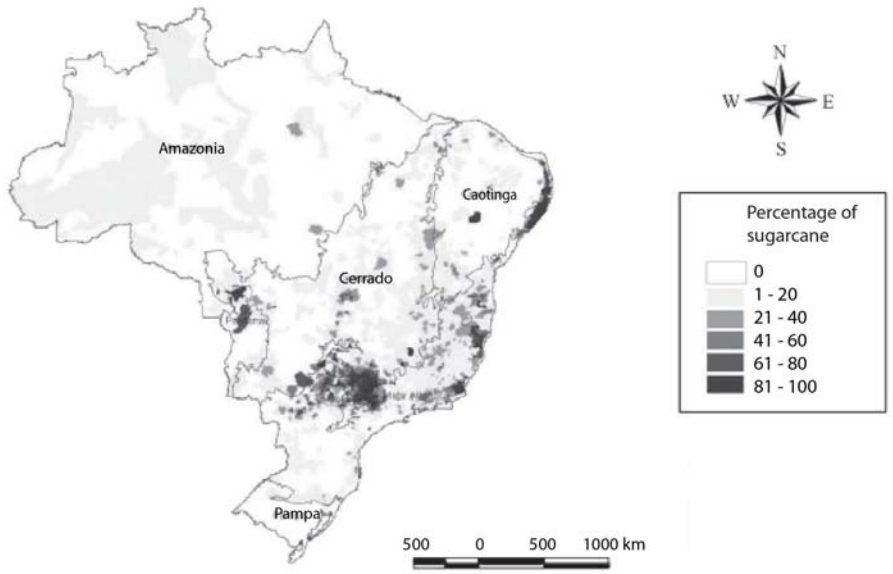

Fig. 1.2 *Percentage of sugar cane crops in Brazil. (Source: Goldemberg et al., 2008)*

The state of Alagoas produces the main fraction of sugar cane in the northeast. It further comprises the largest number of lakes, coastal lagoons and estuaries surrounded and affected directly by the sugar cane plantations. One of the largest, the Manguaba lagoon, is a "choked lagoon" solely connected with a single channel to the coastal Atlantic Ocean. Limited water exchange and hence, long water residence times make it particularly vulnerable to the environmental impacts of sugar cane cultivation (Knoppers et al., 1991).

1.3 Organic matter characterisation

Dissolved organic matter (DOM) consists of different organic substances which may have a complex structure and manifold interactions with e.g. minerals and suspended matter. It has been widely accepted that DOM is defined as those organic substances of TOC (total organic carbon) in aqueous solution that pass through a membrane filter with a nominal pore size of 0.45 µm (Thurman, 1985; Bianchi, 2007). The analytical method to determine concentrations of DOM and TOC is the analysis of the carbon content and hence the concentration is either given as dissolved organic carbon (DOC) or TOC. The concentration of DOM in aquatic systems covers a broad range from < 1 mg/l DOC for ground- and seawater, up to 25 mg/l in wetlands and up to several hundred mg/l DOC in soil pore water in the upper layer of the soil horizons (Thurman, 1985). The content of riverine organic matter depends on the nature of soils and the land use in the watershed (Cauwet, 2002).

Two basic approaches have been used for the chemical characterisation of DOM: (a) direct analysis, with the challenge of low concentration of organic compounds with high salt content in brackish or marine water samples and (b) analysis of concentrated DOM (Benner, 2002). A variety of concentration and isolation techniques have been applied to natural waters, whereby solid-phase extraction and ultrafiltration are the most frequently used ones (Benner, 2002). Solid-phase extractions rely primarily on chemical properties of DOM to partition between the aqueous and sorbent phase. This method is often used to isolate humic substances (Thurman, 1985). Ultrafiltration techniques are primarily based on physical properties to separate size fractions of DOM, as organic matter in natural waters covers a broad range of molecular sizes. The dissolved fraction excludes particulate material and bacteria (Guéguen et al., 2002) and can be further subdivided into a colloidal fraction with high molecular weight (HMW) in the size range from 0.001 to 1 µm and the 'truly' dissolved fraction with low molecular weight (LMW) organic compounds. Thorough contaminant transport modelling requires that colloids are considered as a third, separate phase, distinct

from the 'dissolved' and 'particulate' phases (Gschwend and Wu, 1985), as aquatic colloids are entities with supramolecular structure and properties, but small enough to remain in suspension (Amon and Benner, 1996; Buffle et al., 1998). Thus, types of organic colloids present in aquatic systems include macromolecular organic matter, microorganisms, viruses, biocolloids, aggregates of exudates and nanoparticles such as clay minerals and oxides of iron, aluminium and manganese coated with or sorbed on organic matter (Guo and Santschi, 2006). Examples of the size spectrum of chemical species in aquatic systems are given in Figure 1.3. In natural waters organic colloids are composed of humin (40-50%), carbohydrates (30-40%), hydrolysable amino acids (10-20%), and lipids (1-2%) (Galimov, 2006; Repeta et al., 2002).

The size-based distinction of chemical species between particulate, colloidal and dissolved phases is important to better understand the biogeochemical cycles of organic and inorganic components that serve as carriers for trace elements and contaminants. Aquatic colloids have been recognised as important intermediates in the removal of trace elements by coagulation enabling contamination transport, organic carbon cycling, and micronutrient bioavailability (Benner, 2002, Guo and Santschi, 2006). Studies on the distribution or partitioning between HMW and LMW DOM are quite useful in order to understand sources, transformation and transport of chemical species.

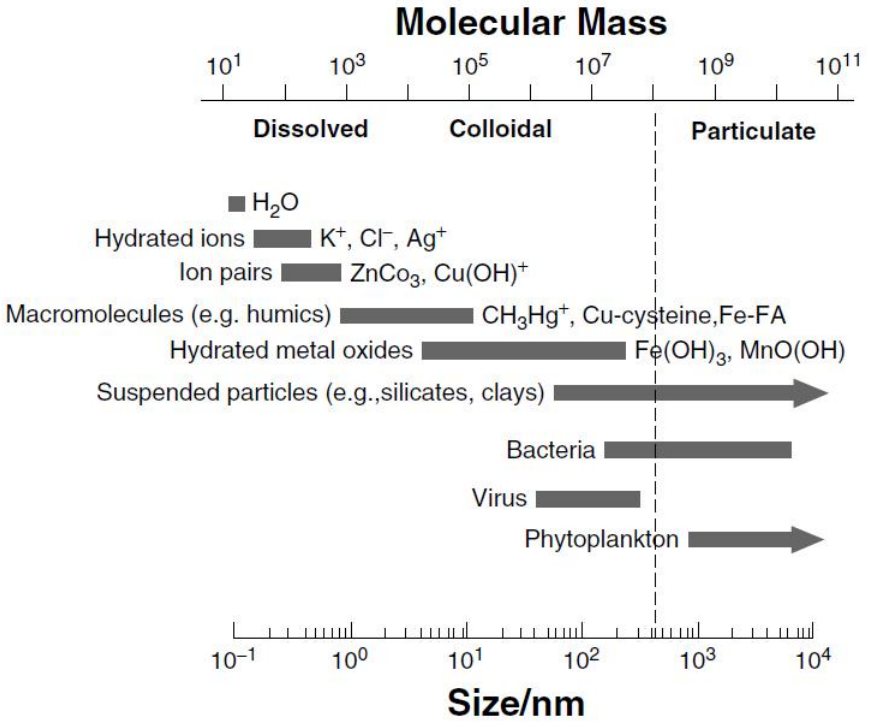

Fig. 1.3 *Size spectrum of chemical species in aquatic systems (Source: Guo and Santschi, 2006)*

1.4 Transport pathways in coastal waters

DOM dynamics in the coastal zone are very high due to river inputs and the intense physical-chemical and biological activities in coastal waters (Cauwet, 2002). River borne materials introduced to the estuary undergo particle-water transformations due to the sudden changes in salinity, pH, turbidity, respiration and primary production, whereby some components are retained by sedimentation and others are bypassed to the sea without reaction (Amon and Benner, 1996; Turner and Millward, 2002). The physical-chemical processes such as adsorption, desorption, aggregation, flocculation and deflocculation as well as photooxidation (Fig. 1.4), affect the structure and biodegradability of organic matter, whereby the lower salinity regions in estuaries appear to be important sinks for DOM (Sholkovitz et al, 1978; Bianchi, 2007). DOM can also accumulate in estuaries due to autochthonous production through released DOM of natural excretion from living cells and lysis of planktonic cells (Fig. 1.4) (Cauwet, 2002). Recent studies identified the photochemical production of DOM from resuspended sediments as an additional internal and significant source of DOM in estuaries (Kieber et al., 2006; Mayer et al., 2006; Riggsbee et al., 2008).

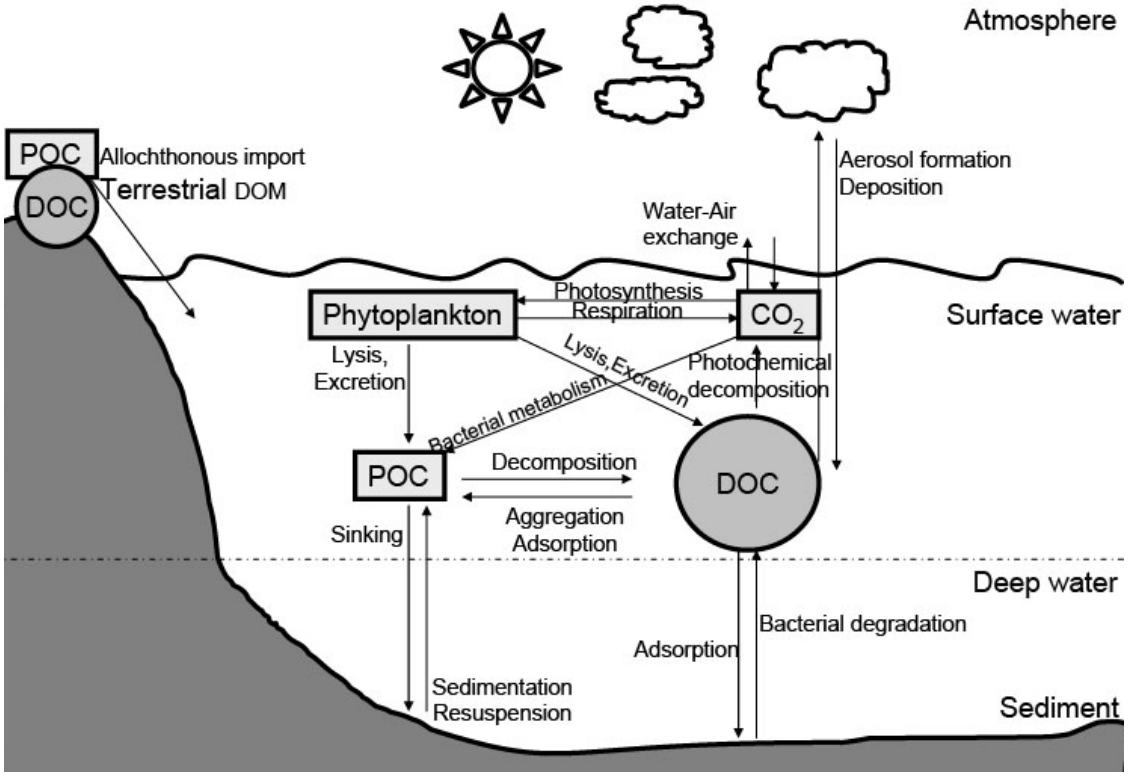

Fig. 1.4 *Pathways of organic matter in water. Modified according to Wangersky, 1972.*

Whether DOM mixes conservatively or non-conservatively in an estuary depends on the specific physicochemical and biological characteristics of each estuary and may vary

seasonally (Miller, 1999; Uher et al., 2001). The degree to which materials are exported to the sea thereby also depends upon the flushing time of the aquatic system, the magnitude of material input and the degree of exchange between fresh and marine waters. Coastal lagoons are able to retain and recycle materials while delta estuaries release more materials to the sea (Knoppers, 1994).

In order to determine the residence time, degradation potential and fate of sugar cane derived organic matter in a tropical lagoon-estuary system these compounds must be followed through several size fractions of particulate, colloidal and dissolved matter. Although the three organic matter fractions are presumably apt to have a common or at least a dominant source, they have contrasting compositions and fates in the water, due to differences in biodegradation stage and sorptive partitioning of molecules onto minerals (Krusche et al., 2002).

Sugar cane organic matter in the water system result from soil flushing from cane fields and from sugar factory effluents (Brockmeyer and Spitzy, 2011) and is composed of fibers, coarse particles, colloids and dissolved fractions. These compounds may be enriched in nitrogen due to usage of fertilizers and hence, more easily adsorb with inorganic clay particles of rivers and estuaries. Thus, composition and transformation kinetics within and between fractions of these materials may change along the course of the river and as it passes through the estuary.

1.5 Tracing organic matter cycling

To evaluate the ultimate size fraction through which transport of sugar cane derived organic matter occurs, this material must be distinguished and quantified from other sources of organic matter. Basic approaches often used to discriminate between different sources of organic matter are: (i) bulk compositional indicators such as environmental isotopes, elemental composition and spectral properties, (ii) molecular tracers such as lipids and structural biomacromolecules (e.g. lignin and hemicellulose), (iii) chemical composition of isolated fractions of DOM such as amino acids and carbohydrates and (iv) structure of molecules (Hedges et al., 1997; Benner, 2002).

In this study bulk chemical indicators (stable carbon isotopes, carbon and nitrogen ratios) were used to distinguish between the different sources of organic matter in a sugar cane impacted tropical estuary. Vascular plants exhibit many bulk chemical and isotopic properties that distinguish them from marine organisms. Higher plant tissues have a predominance of nitrogen-free macromolecules compared to protein structures (C/N ~ 3-4) which makes them

characteristically carbon rich (C/N 20-500) versus plankton (C/N ~ 7) or bacteria (C/N ~ 4) (Hedges et al., 1997). The sugar cane plant, with a high amount of carbon rich carbohydrates, has a C/N ratio of ~ 99 (Ilokur and Oluka, 1995). The bulk C/N ratios for dissolved humic substances range from 36 to 57, indicating that humic substances comprise a highly N-depleted fraction of DOM as they are derived from plant tissue (Benner, 2002).

All natural reactions (especially enzymatic reactions) and chemical reactions are known to fractionate isotopes, thus leading to distinct isotopic compositions in the long term. Isotope fractionation during photosynthesis consists of several diffusive kinetics and equilibrium effects, including diffusion from the atmosphere, CO_2 uptake by chloroplasts, enzymatic carboxylation, photorespiration and respiration (Galimov, 2006; Still and Powell, 2010).

Among the terrestrial plants two major isotopic categories can be distinguished according to their mode of CO_2 fixation. The more common C3 plants incorporate CO_2 by ribulose biphosphate carboxylation (Calvin cycle). C4 plants, which are represented, for example, by corn, sugar cane and often by savanna and desert vegetation, fix CO_2 through phosphoenol pyruvate carboxylation (Hatch–Slack cycle). There is also the relatively small group of the crassulacean acid metabolism (CAM) plants. They are represented by succulents and have isotopic compositions intermediate between C3 and C4 plants. The average carbon isotope fractionation during photosynthetic assimilation of CO_2 is about -18 to -20 ‰ for C3 plants and about -4 to -5 ‰ for C4 plants (Galimov, 2006). Because metabolic fractionation of organic compounds is minimal (≤ 1 ‰ per trophic level), the carbon isotopic label of locally predominant plants has a characteristic imprint on food webs and in organic remains (Hedges et al., 1997).

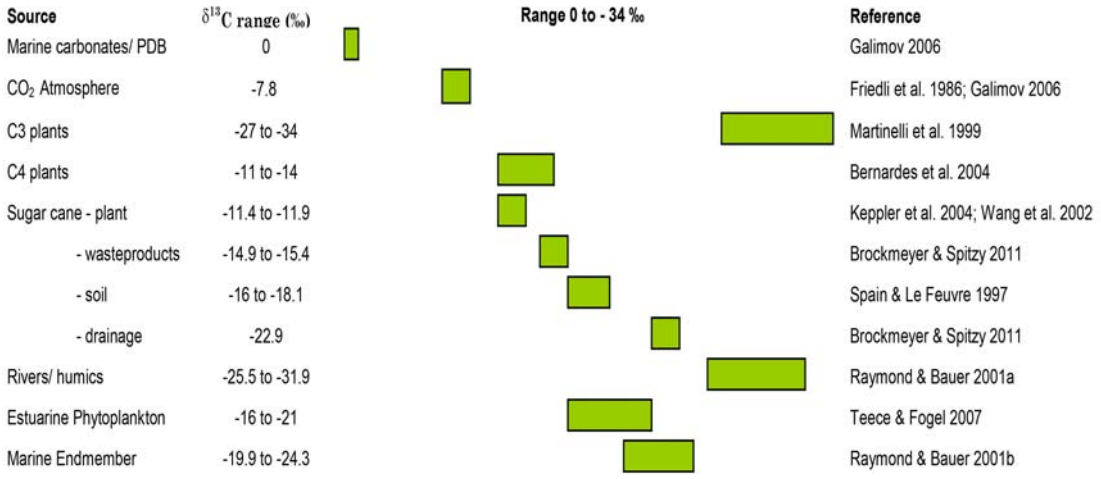

Fig. 1.5 *Isotope signature of selected C pools (CO_2, deep ocean water, pristine rivers, C3 and C4 plants, phytoplankton)*

As a consequence, each C-pool has a specific isotopic signature (Fig. 1.5), resulting from the balance of source and sink terms and their isotopic fractionation.
Since the $\delta^{13}C$ value of sugar cane, is typically ~ -12 ‰, it allows to distinguish it from C3 plant derived organic matter ($\delta^{13}C$ ~ -27 ‰). This, for example, could be used to trace the input of sugar cane and other C4 plants to soil and riverine organic matter (Martinelli et al, 1999; Krusche et al., 2002; Bernardes et al., 2004; Dalzell et al., 2005; Dalzell et al., 2007) as the $\delta^{13}C$ signatures of the likely sources are sufficiently different from each other and well constraint for each individual source. Under these conditions, the $\delta^{13}C$ signatures of organic matter can be used to identify sources of carbon and their contributions (relative and absolute) to a given organic matter pool, using simple two or three end member mass balance calculations (Bauer, 2002).

1.6 Aims of the study (objectives)

The overall objective of this study was to quantify the amount of sugar cane derived organic matter in a sugar cane impacted lagoon-estuary system along its way from riverine to coastal waters. Therefore, the focus was on the following questions:

1. What are the main sources and sinks of organic matter in such an impacted lagoon system? What are the main transport pathways of organic matter?
2. Are the microbial communities adapted to the sugar cane cultivation in the hinterland of the lagoon system in order to degrade this specific organic matter source?
3. Which essential differences exist between low- and high molecular weight DOM? Which conclusions can be drawn from the molecular weight distribution of organic matter with regard to DOM dynamics?

For the separation of the individual molecular weight fractions for further source identification, a tangential flow filtration method had to be investigated for its performance on natural dissolved organic matter.

1.7 Thesis outline

This thesis is subdivided into 7 chapters. After this introduction (Chapter 1), Chapter 2 focuses on the spatial distribution and fate of the bulk dissolved organic matter composition in a tropical sugar cane impacted lagoon-estuary system. In Chapter 3, distribution patterns of microorganisms were compared with environmental variables in the same lagoon estuary system as in chapter 2. Chapter 4 presents a method for separation of natural colloidal organic matter from the dissolved water phase by tangential flow filtration to enable a better understanding of processes between "truly" dissolved, colloidal and particulate phase in the natural environment. In chapter 5, the method developed in chapter 4 was used for a detailed analysis on the molecular weight distribution within the organic matter pool of the studied lagoon estuary system. In chapter 6, an estimation of the DOM export, especially of the sugar cane derived fraction, to the coastal ocean was conducted. Chapter 7 presents the general conclusions and the outlook of this study.
The chapters 2, 3, 4 and 5 are based on published or submitted manuscripts in peer-reviewed scientific journals:

Chapter 2
Brockmeyer, B., Spitzy, A., 2011: **Effects of sugar cane monocultures on origin and characteristics of dissolved organic matter in the Manguaba lagoon in northeast Brazil.** *Organic Geochemistry, 42, 74-83*

Chapter 3
Wolf, L., Schwalger, B., Knoppers, B.A., Ferreira da Silva, L.A., Petter Medeiros, P.R., Pollehne, F., 2010: **Distribution of prokaryotic organisms in a tropical estuary influenced by sugar cane agriculture in northeast Brazil.**
Brazilian Journal of Microbiology 41, 890-898.

My contributions to chapter 3 (Wolf et al., Braz. J. Microbiol.):
- water sampling
- analysis and interpretation of DOC concentrations (part of the data set).
- Co-work in discussion and manuscript preparation

Chapter 4

Schwalger, B., Spitzy, A., 2009: **Separation of natural organic colloids with a PALL tangential flow filtration system.**

Water Science and Technology, Water Supply, 9.5, 583-590.

Chapter 5

Brockmeyer, B., Spitzy, A., Petter Mederios, P. R., Knoppers, B.A.: **Composition of dissolved and colloidal organic matter in the sugar cane impacted Manguaba estuarine-lagoon, NE-Brazil**

Submitted to: *Biogeochemistry*

2 Effects of sugar cane monocultures on origin and characteristics of dissolved organic matter in the Manguaba lagoon in Northeast-Brazil

Berit Brockmeyer and Alejandro Spitzy

Organic Geochemistry (2011) 42: 74-83

ABSTRACT

Brazil has extensive sugar cane monocultures, which significantly alter hydrogeochemical material fluxes. We studied dissolved organic matter (OM) fluxes in the Manguaba lagoon-estuary system, which drains a sugar cane monoculture-dominated hinterland and discharges into the Atlantic coastal ocean. The OM fluxes into the lagoon originate from baseflow, field runoff and sugar cane factory effluents. In this study, dissolved organic carbon concentration, $\delta^{13}C$ DOC and UV absorbance were analysed along a freshwater-seawater salinity gradient that encompasses river (DOC 9-11 mgl^{-1}; $\delta^{13}C$ -22.2‰ to -25.5‰), lagoon (4-11 mgl^{-1}; -20.5‰ to -24.8‰), estuary (3-9 mgl^{-1}; -22.6‰ to -25.3‰) and coastal waters (1.6 mgl^{-1}; -21‰) with different intra-seasonal runoff conditions. We used the carbon isotope data to quantify the sugar cane derived DOC. Where river water meets brackish lagoon water, substantial loss of DOC occurs during rainy conditions, when suspended sediment from eroded fields in the river is very high. During dry weather, at much lower suspension levels, DOC increases, however, presumably from addition of photolysed resuspended sedimentary OM. In the estuary, mixing of DOC is strictly conservative. Ca. 1/3 of riverine DOM discharged into the lagoon has a sugar cane source. Within the lagoon on avg. 20% of the bulk DOM is comprised of sugar cane DOM whereas during heavy rainfall the amount increases to 31%, due to intensified drainage flow and soil erosion. In the estuary, 14 to 26% are of sugar cane origin. The sugar cane-derived component follows the mixing patterns of bulk DOM.

2.1 Introduction

Estuaries and coastal systems are sites where large amounts of organic matter (OM) in dissolved (DOM) and particulate (POM) form are both removed and added by biotic and abiotic processes (Raymond and Bauer, 2001a). These land-margin ecosystems receive OM laterally advected from both land and sea, as well as locally produced from *in situ* primary production (Peterson et al., 1994). Photochemical production of DOM from resuspended sedimentary POM has been recently identified as a further potential source of DOM in estuaries (Kieber et al., 2006; Mayer et al., 2006; Riggsbee et al., 2008).

We studied origin and fluxes of DOM in the Manguaba lagoon-estuary system, where major river inputs of allochthonous OM derive from sugar cane cultivation and processing. In rivers, DOM usually has minor amounts of biodegradable plant, phytoplankton and bacterial residues, which are being rapidly recycled and major amounts of biologically refractory residues. The latter are comprised of substituted aromatic structures, and branched cyclic aliphatic structures, which are partially oxidized (Spitzy and Leenheer, 1991). Sequential photochemical degradation of DOM and subsequent microbial uptake of degradation products occurs in both, limnic and oceanic systems (Wetzel et al., 1995; Mopper and Kieber, 2002).

Among variables that determine the fate and reactivity of OM in estuaries are the hydraulic residence time, river discharge, resuspension events and light availability (influenced by chromophoric and suspended OM) (Bianchi, 2007 and references therein). In the lower salinity regions in estuaries fractions of DOM are removed due to flocculation (Sholkovitz et al., 1978) or sorptive reactions (Uher et al., 2001).

The physicochemical and biological controls on conservative or non-conservative mixing of DOM in an estuary may vary seasonally (Miller, 1999; Uher et al., 2001; Cauwet, 2002) and, in addition, affect different subunits of DOM in different ways. Obviously, some OM components may undergo minimal degradation, while others cycle rapidly (Cole et al., 2007; del Giorgio and Pace, 2008). Chromophoric DOM (CDOM) can be used as a tracer of terrigenous/soil OM, as terrigenous DOM has a much greater absorbance per unit carbon than marine autochthonous DOM, or either microbially or photochemically degraded DOM (Stedmon et al, 2006; Spencer et al., 2007). Thus, the mixing behaviour of CDOM can be differentiated from that of bulk DOM. Within the CDOM fraction, differential mixing behaviour can occur, e.g., Uher et al. (2001) observed that a substantial CDOM fraction mixed non-conservatively, while the residual CDOM fraction behaved conservatively.

In systems, where potential DOM sources have distinct carbon isotopic signatures (expressed as $\delta^{13}C$), the carbon isotope ratio in DOC ($\delta^{13}C$ DOC) can be applied as a quantitative source

indicator (Raymond and Bauer, 2001a). Since the $\delta^{13}C$ of C4 plants, e.g., sugar cane, is typically ca. -12‰, it allows to discriminate against C3-plant-derived OM ($\delta^{13}C$ ca. -27‰) and, for example could be used to trace the input of sugar cane and other C4 plants to soil and riverine OM (Martinelli et al, 1999; Krusche et al., 2002; Bernardes et al., 2004; Dalzell et al., 2005, 2007). Likewise, in the agricultural area of the Everglades, Wang et al. (2002) concluded from $\delta^{13}C$-analysis that 23% of the DOM pool was derived from sugar cane.

The coastal area of northeastern Brazil, where ~10% of Brazil's sugar cane production (Brazilian total in the harvest season 2008/2009: 570 x 10^6 t from a total harvested area of 8 x 10^6 ha; UNICA, 2010) occurs, is characterized by numerous lagoons of varying size and shallow water depth (Oliveira and Kjerfve, 1993). One of the largest, the Manguaba lagoon, is a "choked lagoon" connected only with a single channel to the coastal Atlantic ocean. Limited water exchange and hence, long water residence times makes it particularly vulnerable to the environmental impacts of sugar cane cultivation (Knoppers et al., 1991). Among them are (i) atmospheric fall-out from pre-harvest sugar cane burning, (ii) soil erosion, (iii) flushing of fertilizer and pesticide from the fields, and (iv) discharge of organic matter rich effluents from sugar cane processing plants (Cortez and Perez, 1997; Cheesman, 2004; Gunkel et al., 2007; Martinelli and Filoso, 2008). Furthermore, carbohydrate rich OM is leached and eroded from the soils that accumulate sugar cane carbon (Wang et al., 2002).

In view of the rapidly expanding sugar cane production along tropical and subtropical coastal zones, the Manguaba lagoon-estuary system may serve as a model system for the study of related impacts on the marine environment. Here, we focused on DOM fluxes and composition along a salinity gradient from freshwater to the coastal ocean by analysing bulk DOC, its chromophoric fraction and $\delta^{13}C$ DOC. Our objective was to establish the overall DOM balance in this system, identify the relevant sources and sinks, and, in particular, quantify the sugar cane derived fraction of DOM.

2.2 Sampling site

The Mundaú-Manguaba shallow tropical coastal lagoon complex is located in the state of Alagoas, northeast of Brazil and consists of the Lagoa Manguaba (43 km^2) and the Lagoa Mundaú (24 km^2) (Oliveira and Kjerfve, 1993). The two lagoons, have an average depth of 2 m, and are connected to the Atlantic via a network of mainly mangrove-lined narrow channels and terminate in two ocean inlets. Each inlet is about 200 m wide and separated by a barrier of several hundred metres width (Fig. 2.1). Several small drainages run into the lagoon during the

wet season, and contain no water in the dry season.

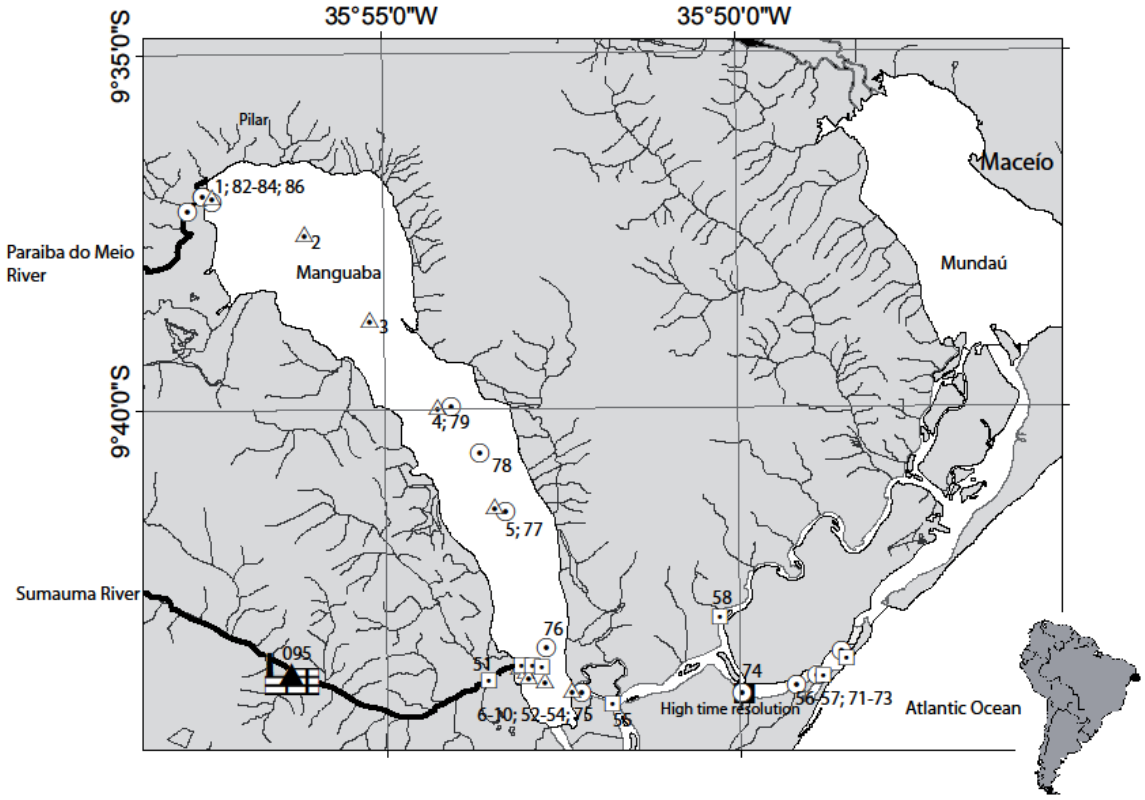

Fig. 2.1 *Study site (Mundaú-Manguaba coastal lagoon system) with location of samples collected during three transects in March and during the high time resolution sampling in October 2007. Triangles symbolise samples of transect A, squares of transect B and cycles of transect C.*

The climate is tropical (annual precipitation in 2007, 1633 mm) and semi-humid, with well defined wet (April - August) and dry (September - March) seasons, leading to highly variable discharge from the main tributaries Paraiba do Meio (river basin 3299 km^2), Sumauma (river basin 372 km^2) and Mundaú (river basin 2135 km^2). For the Manguaba lagoon, the combined daily river discharge of the Paraiba do Meio and Sumauma rivers varies between a minimum of 1 m^3s^{-1} and a maximum 604 m^3s^{-1}, with an annual average of 28 m^3s^{-1} (1963 - 1974; Oliveira and Kjerfve, 1993).

In both lagoons the main freshwater input is river runoff. The flux of salt water from the coastal ocean into the lagoon is strongly dampened by the channel system, where tidal cycles determine the direction and magnitude of currents and hence salinity. The tidal amplitudes vary biweekly as a result of a distinct spring neap cycle. During the dry season, when river runoff is low and

water depths shallow, salinity in the lagoon is slightly higher (avg. 3) than during the wet season (avg. 1), when substantial freshwater runoff predominantly determines water exchange (Oliveira and Kjerfve, 1993).

In the river catchment area, sugar cane is the dominant land cover, with minor fragments of pristine vegetation, including Atlantic rainforest, mangrove and flood plains. In addition to the anthropogenic impacts of sugar cane cultivation and processing, the Mundaú lagoon is further impacted by urban effluents from the city of Maceío (population ca. 1 million). Major sources of pollution for the Manguaba lagoon are cultivation of sugar cane and waste effluents from the sugar and alcohol industries along the rivers Paraiba do Meio and Sumauma (ANA, 2005). We selected the Manguaba lagoon-estuary-system as a type locality, where we could focus on sugar cane impacts, avoiding additional influences from urban waste waters.

Pollution of the system during the harvest period/dry season is characterized by point sources of OM inputs from sugar cane processing industries that discharge fructose-rich wash water and waste products. During this period, the main primary producers in the lagoon shift from diatoms to cyanobacteria, mainly *Anabaena spiroides* and *Microcystis aeruginosa* (de Souza et al., 2002). Pollution of the system during the wet season is characterized by diffuse inputs of agrochemicals, metals and soil OM eroded, leached and flushed via drainage and rivers into the lagoon.

2.3 Methods

2.3.1 Sample collection and storage

In March of 2007, at the end of the dry season and harvest period, we did extensive water sampling across three transects (Fig. 2.1). The first (A) (5th March) was a longitudinal transect from the river mouth (sal = 2) of the Paraiba do Meio (PdM) through the brackish Manguaba lagoon to the freshwater end-member of the Sumauma river. The second (B) (8th March) covered the salinity gradient from the freshwater end-member of the Sumauma river through the lagoon into the estuary. The third (C) (9th March) covered the salinity gradient from the freshwater end-member of the PdM river towards the estuary. Further samples were taken (i) in the Sumauma river (10th March) 8 km upstream of its mouth, close to a sugar cane factory, (ii) from the drainage of a nearby sugar cane field and (iii) from marine end-member waters between 2 and 6 km off the coastline. On October 1st of 2007 at the beginning of the dry season and harvest period, we performed a high time resolution (1 sample h^{-1}) 14 h sampling at a fixed

point within the estuary (Fig. 2.1), covering a full tidal cycle.

While sampling within the late dry season around early March of 2007, occasional heavy rainfall occurred with precipitation intensities comparable to wet season values (J.L. de Souza, unpublished daily precipitation data of Maceío). These few extreme events (28th February, 3rd March, 9th March) with precipitation in the range of 36 - 67 mm day^{-1} (monthly avg. February 2007, 5.0 mm day^{-1}; March 2007, 5.5 mm day^{-1}) influenced sampling of transects A and C. Low precipitation with a rate <1 mm day^{-1} mimicked the typical dry situation during transect B as well as during the high resolution sampling time in October 2007.

Water samples were filtered through precombusted 47.2 mm glass fibre filters with a nominal pore size of 0.7 µm (Whatman (GFF)) to remove POM. All samples for DOC and δ^{13}C DOC measurements were acidified to pH ca. 2 with 85% H_3PO_4 and frozen (-20 °C) prior to analysis. Filtered fresh and low salinity samples from transect C were measured immediately for the UV absorption of their CDOM.

Solid matter samples included fresh mangrove leaves (*Laguncularia racemosa*), a sugar cane plant (*Saccharum officinarum)* and surface soil samples from sugar cane fields in different cultivation states from 3 month old (0.3 m height) to ripe sugar cane (3 m height). Vinhasse and molasses were obtained from a sugar cane factory. Vinhasse, the substance remaining after sugar cane alcohol distillation, contains a high OM content and is used for fertirrigation of sugar cane fields or disposed into rivers (Cortez and Pérez, 1997; Gunkel et al., 2007). Molasses is a by-product of the sugar crystallisation process.

Finally, plankton was collected with a phytoplankton net (25 µm) during the October campaign in a bloom patch. The content of the plankton net was filtered onto a GFF filter, directly frozen to avoid contamination by fungi, and oven dried prior to analysis.

2.3.2 Analysis

2.3.2.1 Salinity

Salinity was measured *in situ* with a portable conductivity/salinity probe (WTW model LF330 - Tetracon 325).

2.3.2.2 Chromophoric DOM absorbance

The absorption spectrum of CDOM typically has high absorption in the UV and blue wavelength interval and almost no absorption in the red and infrared regions. CDOM

absorbance was determined at 340, 360, 380, 412 and 442 nm with a UV-VIS spectrophotometer (Micronal B582). These reference wavelengths were chosen to enable CDOM absorption measurements at lower concentrations, with the 442 nm wavelength representing the blue-green chlorophyll maximum (Blough and del Vecchio, 2002). All samples were analysed within 3 h of collection in 10 mm cuvettes. All spectra were referenced to blank distilled water. CDOM absorbance coefficients values $\alpha(\lambda)$ were calculated from:

$$\alpha(\lambda) = 2.303\ A(\lambda) / l \qquad \text{Eq. 2.1}$$

where $A(\lambda)$ is the absorbance, λ the wavelength and the optical pathlength in m (Green and Blough, 1994). All absorbance data are expressed as $\alpha(\lambda)$ in units of m^{-1} (Spencer et al., 2007).

2.3.2.3 DOC, $\delta^{13}C_{org}$ and $\delta^{13}C$ DOC

DOC concentrations of all water samples were measured using a high temperature catalytic oxidation analyser (Shimadzu TOC 5050) with a Pt catalyst at 680 °C. Acidified samples were purged for 5 min to remove inorganic carbon prior to analysis. Synthetic air was used as carrier gas in the TOC analyser. Standards (potassium hydrogen phthalate) were analysed immediately prior to and after analysis of 10 samples and were prepared with water from a Millipore Q-Pod system. The detection limit was found at 0.02 mg l^{-1}. Accuracy was tested with CRM-seawater standards (Hansell, 2005). All samples were analysed in triplicate. Precision, in terms of the relative standard deviation, was better than 2%.

$\delta^{13}C_{org}$ of solid samples (soil, filter and plant material) was determined according to Ertl and Spitzy (2004), involving sealed tube combustion of the sample, cryogenic trapping of CO_2, and isotope ratio mass spectrometry by a Finnigan Mat 252 – dual-inlet system.

$\delta^{13}C$ DOC of liquid samples was determined analogously as concerns cryogenic trapping (coupled on-line) and isotope ratio measurement (MAT 252 – dual-inlet). Before trapping, a 20 ml sample was combusted by continuous injection (0.85 ml min^{-1}) in a Helium stream into a self assembled high temperature catalytic oxidation unit, consisting of a furnace heated to 950 °C and a quartz glass column filled with copper oxide and cerium oxide. Combustion gases were dried by Peltier coolers and a magnesia perchlorate trap.

$\delta^{13}C$ values were obtained from at least duplicate analyses and referenced to the Vienna Peedee Belemnite (V-PDB) standard:

$$\delta^{13}C\ (‰) = [{}^{13}C/{}^{12}C_{sample} / {}^{13}C/{}^{12}C_{standard} - 1] \times 1000 \qquad \text{Eq. 2.2}$$

The standard deviation of both methods was better than 0.5‰.

2.3.3 Mixing experiment

The laboratory mixing experiment was designed to simulate the behaviour of pure sugar cane OM (molasses from the factory) and of water extracted soil OM while mixing with brackish lagoon water. This is important as soil OM is flushed into rivers via drainage systems and sugar cane OM is discharged into rivers by factories. Both bulk DOC concentration and CDOM absorbance were measured to differentiate the specific mixing behaviour of DOM subunits.
Sugar cane OM was diluted with Millipore purified water, such that a DOC concentration of 21 mg l^{-1} was obtained, comparable to riverine DOC measured near a sugar cane factory. For the soil OM, an aqueous extract was prepared from soil from a sugar cane field with plants of 0.5 m stem height. The material was dried at 50 °C and mixed (1:2) with Millipore purified water. The mixture was occasionally stirred during a period of 24 h and filtered (GFF, 0.7 µm). The aqueous filtrate of the soil OM was further diluted (1:4) with Millipore purified water to obtain concentrations comparable to those of molasses in the experiment. Molasses and the aqueous soil extract were each mixed with artificial seawater (35 g l^{-1}; Tropic Marin, Germany) containing 0.2 mg l^{-1} DOC to salinities in the range 1-10, 20 and 30. All mixtures were stirred and filtered through precombusted GFF filters after 30 min. The filtrates were immediately measured for DOC concentration and CDOM absorbance.

2.4 Results

2.4.1 Salinity

The salinity in the lagoon reflects the balance between riverine fresh and marine salt water inputs. Freshwater input depends on a seasonal wet-dry cycle; the salt water source fluctuates with the tides. In March 2007, salinity within the lagoon increased from 3 to 5 in the upper and from 5 to 7.9 in the lower part of the lagoon (avg. 4.5). These values are higher than those reported by Oliveira and Kjerfve (1993) because of changes in size and position of the ocean inlet and a concurrent shortening of the flow path between the lagoon and the coast that has occurred since. In the channel system, salinity increased to 26 at high tide and decreased to lagoon values at low tide. The sharp salinity gradients at the mouths of the PdM and the Sumauma rivers, as shown in Figure 2.2, allowed sampling of the freshwater-brackish water transition within a very restricted spatial area.

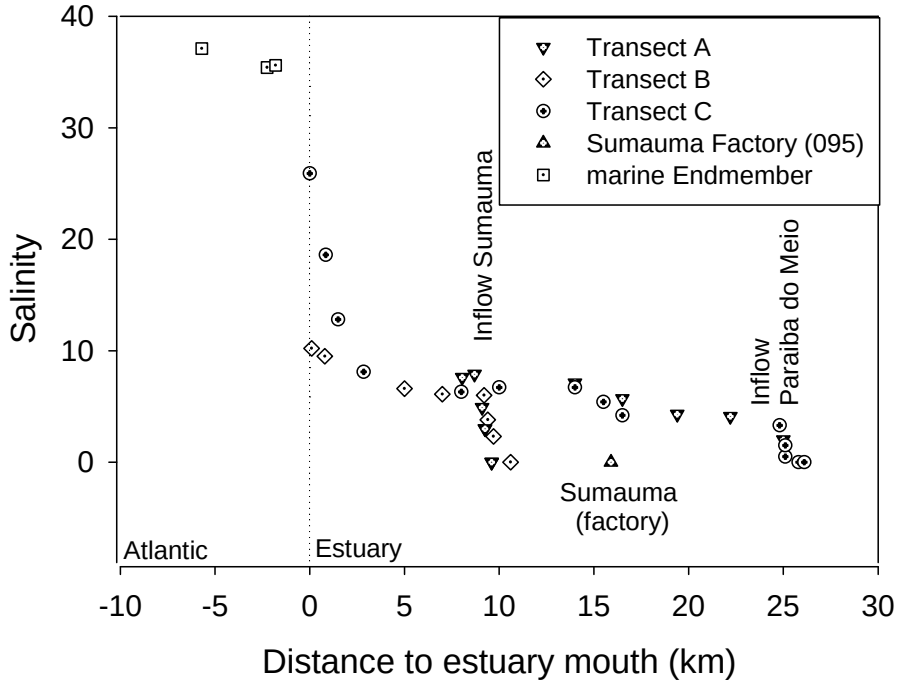

Fig. 2.2 *Salinity as function of distance to estuary mouth. Each symbol represents a different sampling transect.*

2.4.2 DOC and δ^{13}C DOC

2.4.2.1 Sources

Excluding negligible inputs of domestic and municipal waste, DOC in the lagoon system is derived from the following four principal sources: (1) river DOC from leaching of soil OC and surface runoff; (2) OC discharged from sugar cane processing plants; (3) plankton production within the brackish lagoon; and (4) marine DOC. Each of these OM sources has a distinct stable carbon isotope fingerprint.

River DOC in the study area is a mix of 'pristine' terrestrial DOC and a sugar cane-derived component. As a surrogate to 'pristine', we determined the carbon isotopic ratio of DOC in a river of the same climate zone as the Manguaba lagoon but without sugar cane impact: the Serrano river, 3°S and 6°W of the Manguaba lagoon in the Chapada Diamantina National Park (12°35.254S, 41°23.230W). Its DOC had a δ^{13}C value of -28.6‰. In comparison, DOC in the riverine end-member of the Manguaba lagoon was significantly ^{13}C enriched, with δ^{13}C DOC of

-22.2 to -23‰ in rainy and -25.5‰ in dry weather conditions. Soil samples from sugar cane fields in different states of cultivation around the lagoon showed $\delta^{13}C_{org}$ values of -18.1 ± 1.5‰. The equivalent field runoff sampled in drainage had a $\delta^{13}C$ DOC value of -22.9‰. The aqueous soil extract from sugar cane soil used in the laboratory mixing experiment had a $\delta^{13}C$ DOC value of -21.8‰.

The sugar cane plant (*Saccharum officinarum*) had a $\delta^{13}C$ value of -11.8‰. Sugar cane molasses and vinhasse from the factory in which we measured a DOC of ca. 13,000 mgl^{-1}, had values of -14.9‰ and -15.4‰, respectively, showing depletion during processing to sugar and alcohol.

The autochthonously produced OM was determined for filtered phytoplankton samples. The filtered biomass, dominated by the cyanobacteria, *Anabaena spiroides* and *Microcystis aeruginosa*, and the diatom *Cyclotella meneghiana* (Melo-Magalhaes et al., 2009), had a $\delta^{13}C_{org}$ value of -17.1‰.

The average marine end-member (salinity 36) from our coastal shelf samples had a $\delta^{13}C$ DOC value of -21 ± 0.7‰ (n = 3) with a DOC concentration of 1.64 ± 0.15 mg l^{-1}.

2.4.2.2 Rivers and lagoon

The freshwater end-member DOC in the PdM and Sumauma rivers was quite similar: 9 - 10 in the Sumauma and ca. 11 mg l^{-1} in the PdM. The corresponding $\delta^{13}C$ DOC values were -23 to -25.5‰ in the Sumauma and -22.2‰ in the PdM river. At the end of the sampling campaign, we sampled the Sumauma river 8 km upstream from the river mouth close to a sugar cane factory. Here, DOC was 21 mg l^{-1}, i.e. twice as high as during sampling near the river mouth 5 days earlier, and $\delta^{13}C$ DOC was -17.9‰.

DOC of both rivers, although at comparable levels initially, behaved in distinct ways upon mixing into the brackish lagoon at their river mouths. We observed a sharp decrease in DOC in the PdM river transition from fresh river water (salinity 0) to brackish lagoon water (salinity 4), that deviated significantly from a conservative mixing line (Fig. 2.3; transect C). In contrast, we observed a slight increase in DOC with increasing salinity in the Sumauma river mouth (Fig. 2.3; transect B). In the river mouth of both the PdM and the Sumauma river, mixing of riverine freshwater into brackish lagoon water leads to enrichment in ^{13}C compared to the conservative mixing line, as can be seen from Figure 2.5.

Within the lagoon, DOC ranged from 4.1 to 11.1 mg l^{-1} (avg. 8.2 mg l^{-1}; n = 19) and did not correlate with salinity (R^2 0.013 for all lagoon samples). $\delta^{13}C$ DOC ranged widely (from -20.5 to -24.8‰; Fig. 2.5) and was not correlated with salinity. The overall mean chlorophyll *a* within

the lagoon is 35 mg m^{-3} (Wolf et al., 2010) and avg. Secchi depth is 0.5 m.

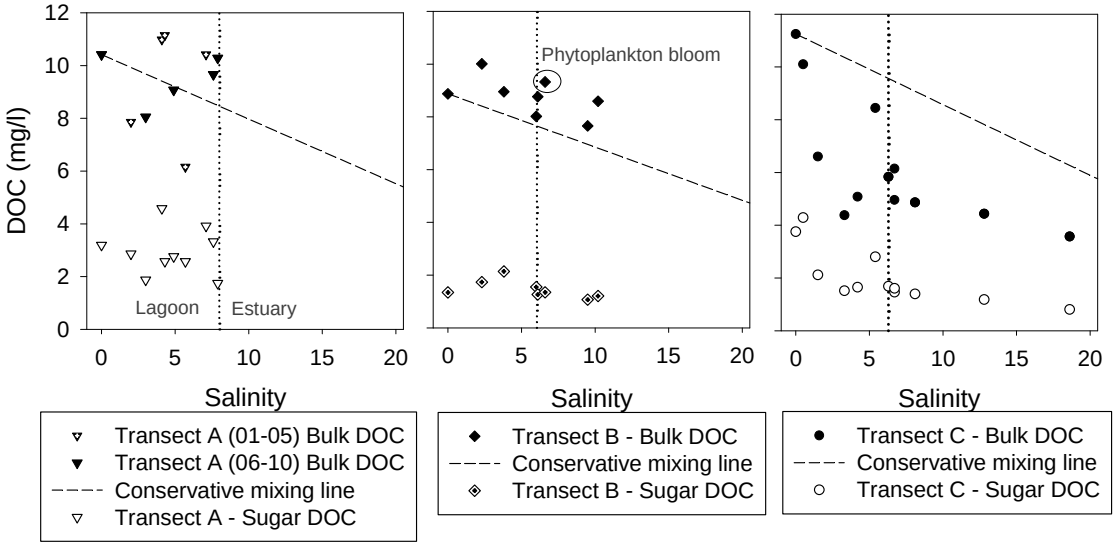

Fig. 2.3 *DOC vs. salinity and sugar cane derived DOC vs. salinity for three transects from rivers Paraiba do Meio and Sumauma to the estuary in March 2007. The conservative mixing line is plotted using the marine end-member data.*

2.4.2.3 Estuary

The estuary of the Manguaba complex is a channel of ca. 8 km length, connecting the brackish lagoon with the coastal ocean.

At low tide (transect B), salinity in the estuary ranged from 6.1 to 10.2, and DOC from 7.7 to 9.3 mg l^{-1} - comparable to average lagoon DOC. The highest value (9.3 mg l^{-1}) coincided with a local phytoplankton bloom (Fig. 2.3).

At an intermediate tide situation (transect C), salinity ranged between 6.6 and 18.6 and DOC between 3.6 and 5.8 mg l^{-1} (Fig. 2.3), both correlating linearly (R^2= 0.97). As one moves from the outlet of the lagoon towards the coastal ocean through the estuary (transects B and C), isotopic values become more homogeneous, with $\delta^{13}C$ DOC values -23.7 ± 0.3‰ for transect B and -22.6 ± 0.7‰ for transect C (Fig. 2.5).

During the high resolution sampling in October at one fixed point in the estuary over a tidal cycle (14 h), we covered salinities from 2.5 to 26.9. DOC was in the range of 6.3 -2.9 mg l^{-1} and correlated linearly with salinity (R^2 = 0.97) (Fig. 2.6). The $\delta^{13}C$ DOC values ranged from -24.1 to -25.3‰ (Fig. 2.6).

2.4.2.4 Chromophoric DOM and mixing experiment

In the field (transect C), a strong non-conservative decline of CDOM within the salinity range 0 - 5, where α_{CDOM} (340) dropped from 91 to 17m^{-1} (Fig. 2.4A), indicates ca. 80% loss of coloured material concurrent with the observed 50% loss of DOC.

In a laboratory mixing experiment with artificial sea water, within the same salinity range, bulk DOC as well as CDOM from an aqueous soil extract from a sugar cane field decreased significantly up to a salinity of 7 (Fig. 2.4B_1 and B_2) with a non-conservative loss of 50% DOC and 90% of absorbance α_{CDOM} (340). In contrast, the parallel experiment with sugar-rich molasses showed a conservative mixing in DOC and CDOM (Fig. 2.4B_1 and B_2).

The collected absorbance data of other wavelengths than 340 nm showed a similar pattern and thus are not further presented.

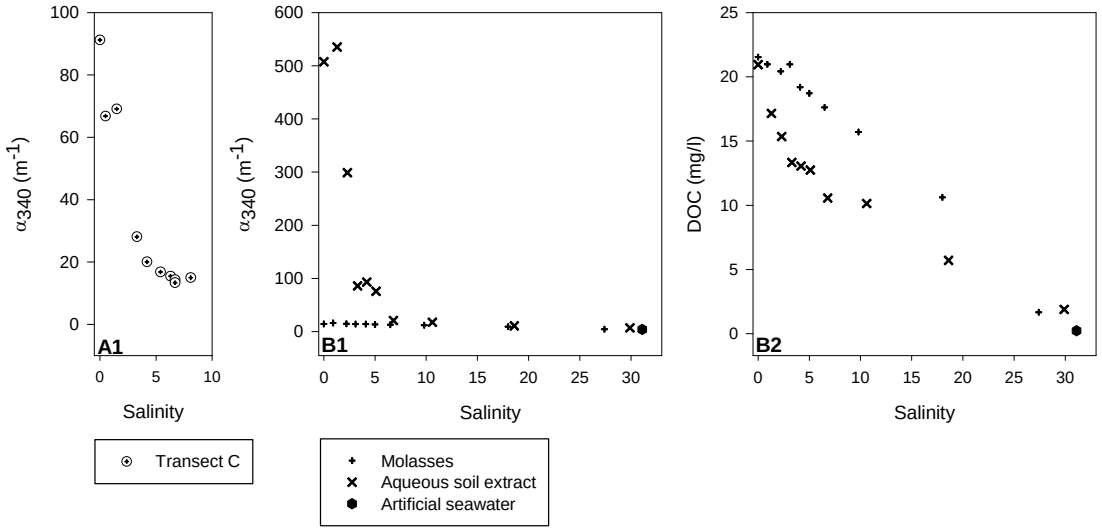

Fig. 2.4 *A: UV absorbance in m^{-1} (λ = 340 nm) vs. salinity from transect C. B: DOC (mg l^{-1}) and UV absorbance (m^{-1}) (λ = 340 nm) vs. salinity in laboratory mixing experiment - artificial seawater was mixed with molasses and in the same way with an aqueous soil extract (soil from a sugar cane field). NB, different UV absorbance scale.*

2.5 Discussion

2.5.1 Bulk DOM

The PdM and Sumauma rivers receive most of their DOC from the surrounding sugar cane fields by baseflow during dry and by surface runoff, interflow and drainage of top soil during rainy conditions, with additional intermittent inputs from effluents of sugar cane factories that operate during harvest in the dry season. Consequently, these rivers' DOC is enriched in $\delta^{13}C$ (-23.6‰) compared to pristine tropical rivers like the Serrano (-28.6‰: own measurement) or the Amazon (-28‰; Raymond and Bauer, 2001b). A similar enrichment (e.g., $\delta^{13}C$ ca. -23‰) was reported for DOC in the Piracicaba River in south-eastern Brazil by Krusche et al. (2002) due to contributions of pasture C4 plant material.

While the enriched $\delta^{13}C$ DOC values at the mouths of the PdM and Sumauma rivers (transects A and C; exceptionally high discharge after heavy rain prior to and during sampling) compare well with those of the drainage sample, the single most depleted δ^{13} DOC value was found on transect B, when sampling occurred during dry weather with no drainage and field runoff.

In small rivers, generally, seasonal variations in DOM composition are strongly coupled with the shift from groundwater inputs during baseflow to surface soil inputs during local rainfall events (Duan et al., 2007), with ^{13}C in organic matter leached from different soil horizons potentially varying as well. For example, Spain and Le Feuvre (1997) observed $\delta^{13}C$ depletion of river OM during dry spells, when baseflow flushes deeper, carbohydrate and ^{13}C depleted soil horizons. Similarly, Dalzell et al. (2005, 2007) observed $\delta^{13}C$ depletion of river OM during baseflow as compared to flood conditions in a catchment (Indiana, USA) where corn (a C4 plant) residues significantly contribute to allochthonous river OM.

In the mixing zone of riverine fresh and brackish lagoon waters, plots of DOC (and CDOM in the case of the PdM) versus salinity deviate significantly from a conservative mixing line. Although DOC behaves non-conservatively at low salinities in both, the PdM and Sumauma river mouths, there is a net loss of DOC (- 50%) and CDOM (- 80%) in the PdM while in the Sumauma we observed a net gain of DOC (+ 12%).

The PdM had – due to heavy rainfall – a very high suspended sediment load, and as these sediments settled in the river mouth, they could promote DOM removal by scavenging flocculated and adsorbed DOM.

In the Sumauma river mouth a different balance between DOM sources and sinks is indicated. Here, rainfall was absent and suspended load in the river significantly lower. As a consequence, (i) the products of salinity driven flocculation were less efficiently scavenged by settling

sediments and (ii) deeper light penetration in the river mouth would not only promote *in situ* production of phytoplankton DOC but also photolytic production of DOC from POC (Kieber et al., 2006; Mayer et al., 2006; Riggsbee et al., 2008).

Diffusive DOC flux from sediments (Burdige and Homstead, 1994; Argyrou et al., 1997; Bianchi, 2007) would accumulate in the water column in the Sumauma case, but become scavenged by massive sediment settling in the case of the PdM.

In the laboratory, purely sugar cane derived material (molasses) did not flocculate when mixed with artificial seawater (R^2 = 0.99) while aqueous soil extract did with a flocculation loss of 50% for DOC and 90% for CDOM. These losses are as high as in the field, in spite of the fact that no suspension was present in the experiment. Since it is coloured, high molecular weight humic acids that flocculate (Sholkovitz et al, 1978; Uher et al., 2001) these materials must form a substantial fraction of the OM that is leached from the soils to the receiving waters. Together with the fact that the CDOM values in the PdM are at the high end of reported data for river and low salinity waters (Uher et al., 2001; Blough and del Vecchio, 2002), this points to a high degree of humification.

Conservative mixing lines for $\delta^{13}C$ DOC along salinity gradients were computed with the simple two component (freshwater – seawater) mixing model of Strain and Tan (1979):

$$\delta^{13}C = (K_1 S - K_2) / (K_3 S - K_4) \quad \text{Eq. 2.3,}$$

with $K_1 = C_a * \delta^{13}C_a - C_b * \delta^{13}C_b$; $K_2 = S_b * C_a * \delta^{13}C_a - S_a * C_b * \delta^{13}C_b$

$K_3 = C_a - C_b$; $K_4 = S_b * C_a - S_a * C_b$; $\delta^{13}C$ = isotope ratios of DOC; C = concentration of DOC; S = salinity; a,b = indicators referring to the riverine (a) and marine (b) end-members. Note that in conservative mixing, bulk DOC depends linearly on salinity, while $\delta^{13}C$ DOC is a non-linear function of salinity, because the distinct end-member isotopic distributions are concentration-weighted (Fry, 2002).

In the river mouths of the PdM and Sumauma rivers $\delta^{13}C$ DOC behaves non-conservatively, with an enrichment in both cases (Fig. 2.5). This unidirectional $\delta^{13}C$ DOC trend in both rivers, showing opposite sign in the DOC balance, is consistent with the fact that *in situ* phytoplankton DOC ($\delta^{13}C$ = -17‰) and DOC from photolysis of POC from advected river sediment as well as from resuspended lagoon surface sediment (C_{org} = 4.8 ± 0.96%; $\delta^{13}C_{org}$ = -20.23‰ ±1.2 (*n* = 4); Spörl and Jennerjahn, 2009) shift $\delta^{13}C$ DOC to heavier values. Furthermore, the non-flocculation of pure sugar cane derived material rich in ^{13}C seen in the laboratory mixing experiment would imply preferential removal of the relatively more ^{13}C depleted component derived from soil leaching.

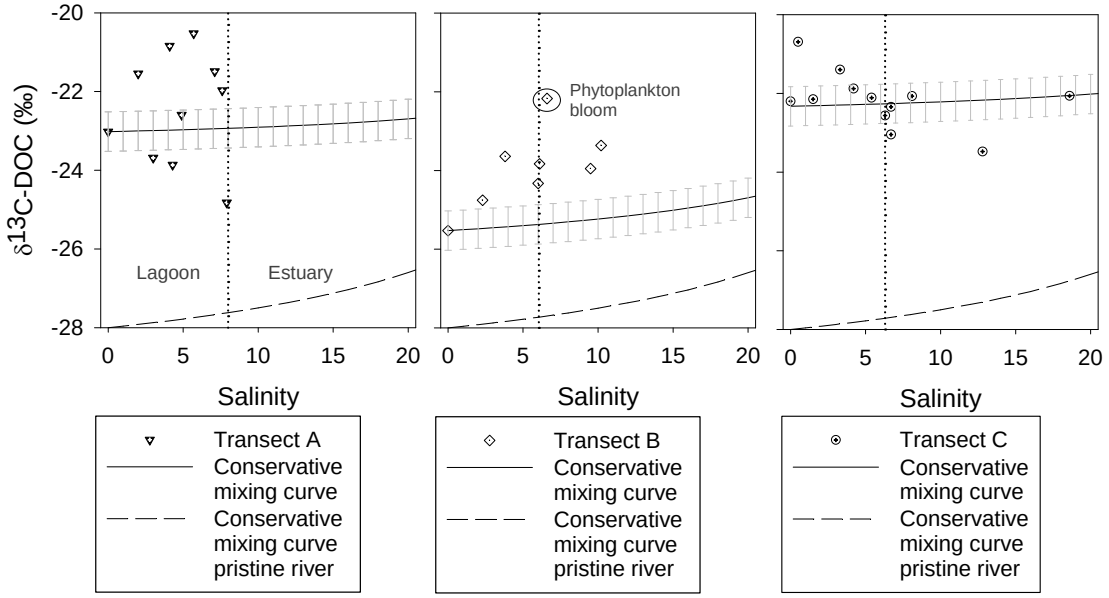

Fig. 2.5 *δ^{13}C DOC vs. salinity for samples from transects A, B and C in the Manguaba lagoon system. The solid line represents theoretical conservative mixing for each transect including error bars (grey) for isotopic analysis (0.5‰) For comparison, dashed line represents conservative mixing of pristine river (e.g. from the Amazon) with assumed values of -28‰ for freshwater and -21‰ for marine end-member.*

Within the lagoon we have a pronounced variability of DOM, which results from small scale patchiness of phytoplankton blooms, variable river inputs and water mass inhomogeneity resulting from non steady-state mixing of fresh and brackish lagoon waters.

We roughly estimate phytoplankton DOC production from the available Chl*a* data (avg. 35 mg m^{-3}) assuming one cell division per day (Goldman and Carpenter, 1974) with DOC = Chlα * F * ER (extracellular release), where F denotes the cellular carbon-to chlorophyll ratio which may vary with light, temperature, nutrients and trophic state between 10 and 100 (Strickland, 1960; Banse, 1977, Geider et al, 1997). Thus, Chlα*F = POC, whose fraction ER is exudated as DOC. ER has been reported to vary within the range of 0.1 - 0.3 (Baines and Pace, 1991; Biddanda and Benner, 1997; Nagata, 2000). Chosing F = 50, this range translates into a daily production of 0.2 - 0.6 mg l^{-1} autochthonous DOC, equivalent to 2 - 9 % average lagoon DOC. Although some of the exudated DOC may form refractory DOC (Brophy and Carlson, 1989; Tranvik and Kokalj, 1998), we assume that the bulk is quickly recycled microbially and photolytically and hence does not accumulate.

Our phytoplankton δ^{13}C value (-17.1‰) is in the range of data reported by Teece and Fogel

(2007), who report $\delta^{13}C$ values of -16‰ for *Anabaena* and -21‰ for an estuarine phytoplankton consisting mainly of diatoms. Sample No. 58 from within a massive cyanobacterial bloom, was significantly enriched in ^{13}C (+2‰) versus the samples from outside the bloom patch (Fig. 2.5; transect B).

The estuarine samples obtained in transects (B and C) represent brackish lagoon water that is slightly mixed with seawater. They show conservative mixing of DOC during transit through the estuary, in which our coastal marine end-member samples had DOC and $\delta^{13}C$ DOC values that are typical for coastal waters (Thurman, 1985; Raymond and Bauer, 2001a and references therein; Cauwet, 2002).

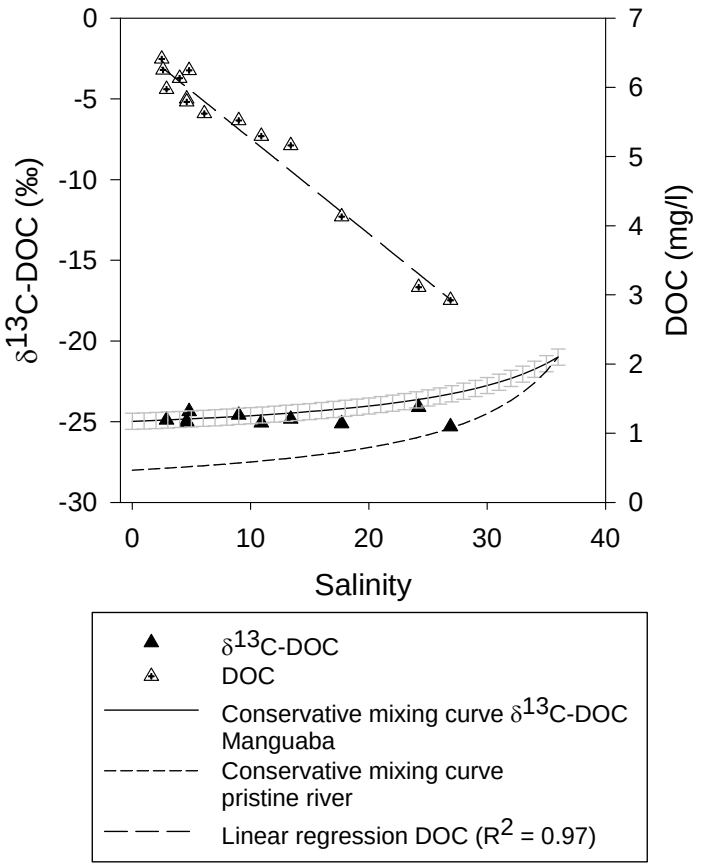

Fig. 2.6 *$\delta^{13}C$ DOC (‰) and DOC (mg l^{-1}) vs. salinity for a 14 h time series sampling during a tidal cycle at a fixed station in the estuary. Long dashed line is a linear regression of the measured DOC data. Solid line for $\delta^{13}C$ DOC is theoretical conservative mixing curve, including error bars (grey) for isotopic analysis (0.5‰), assuming -25‰ for the river end-member and -21‰ for the marine end-member. For comparison, the short dashed line would result if the river end-member were -28‰, as is typical for pristine rivers such as the Amazon.*

This conservative mixing behaviour was substantiated during the high resolution sampling in October, when both, DOC and $\delta^{13}C$ DOC followed a conservative mixing pattern, with a slight deviation towards more depleted $\delta^{13}C$ DOC at high salinities (Fig. 2.6). This deviation could be due to inputs of DOC released during high tide (high salinity) flushing of intertidal mangroves ($\delta^{13}C_{org}$ mangrove leaves = -29.5‰). $\delta^{13}C$ DOC was depleted versus samples from March, because dry weather conditions prevented drainage inflow and field runoff into the lagoon system.

2.5.2 Sugar cane derived DOM

The known sugar cane end-member signature allows us to calculate the percentage of organic matter of sugar cane origin in river samples from the following mixing model:

$$\delta^{13}C_{Sample} * DOC_{Sample} = \delta^{13}C_X * DOC_X + \delta^{13}C_A * (DOC_{sample} - DOC_X) \quad \text{Eq. 2.4 ,}$$

where A represents the pristine riverine end-member having a constant organic carbon isotope ratio ($\delta^{13}C_A$) of -28‰ and X the fraction of sugar cane origin having a constant $\delta^{13}C_X$ of -11.8‰. The -11.8‰ value for the sugar cane plant in our study is close to the 11.9 ± 0.1‰ and -11.4 ± 0.4‰ reported by Keppler et al. (2004) and Wang et al. (2002), respectively. No additional components were included in the mixing model for the rivers, since production of DOC by algae in the river and the river mouth should be insignificant given the low chlorophyll *a* (avg. ~4 mg m^{-3}) values as reported in Wolf et al. (2010), as an effect of light limitation in the highly turbid waters (Secchi transparency 0.1 – 0.4 m).

In comparison with results from the Everglades, where $\delta^{13}C$ DOC ranged from -24 to -28‰ (less than 23% of sugar cane derived DOC) (Wang et al., 2002), the rivers flowing into the Manguaba lagoon carry a higher amount of sugar cane DOC during rainy sampling, with 31±1.2% and 35±0.9% (Sumauma; PdM) and a comparable amount, 15±2.2%, during dry sampling (Sumauma).

The exceptionally high DOC at the sugar cane factory corresponds to 62±3% of sugar cane derived OM and obviously was from discharged vinhasse, in which we measured a DOC of ca. 13,000 mg l^{-1}, consistent with Benke et al, 1999. If the input from the factory were continuous for longer than the transit time of the water between factory and river mouth (ca. 4 h at flow rates less than 1 m s^{-1} over a distance of 8 km) this signal would arrive at the lagoon practically undiluted by longitudinal turbulent mixing. From a simple one-dimensional model (J. Pätsch, person. comm.) we can estimate that, if the factory were releasing one litre of vinhasse per second over a time span of half an hour into a river discharging 1m^3 s^{-1} at a flow rate of 0.5 m s^{-1}

and physically mixing with a turbulent coefficient D = 5.7 $m^2 s^{-1}$, then, the peak concentration of the tracer cloud would be still 9.7 mg l^{-1} (initially 13 mg l^{-1}) at the time of arrival at the river mouth 4.5 h later. Bacterial degradation downstream the factory is 0.7 mg C l^{-1} d^{-1} (Wolf et al., in prep.) and therefore would remove only ~0.1 mg l^{-1}. Photochemical loss due to DIC production from photobleached DOC would be even an order of magnitude lower than the bacterial degradation loss if we applied the average production rate of 0.25 µmol $l^{-1}hr^{-1}$ reported by Miller and Zepp (1995) from various fresh- and coastal water samples. Hence, the discharge of vinhasse from the factory must be intermittent, in unknown quantity and intervals.

Within the lagoon we have to add a phytoplankton DOC source term to the mixing model. Assuming that phytoplankton DOC is isotopically identical with algal POC (-17.1‰) (Williams and Gordon, 1970), the sugar cane component of lagoonal DOM amounts on avg. to 20% during dry weather, whereas during heavy rainfall the amount increases to 31% as a result of intensified drainage flow and soil erosion.

The amount of sugar cane derived organic matter still present within the estuary can be determined from the amount of riverine DOC in our estuarine samples, which we obtain by subtracting phytoplankton DOC and marine DOC from the sample's bulk DOC. The phytoplankton DOC production for the estuary is estimated from the available Chlα data (30 mg m^{-3})(Wolf et al., 2010) choosing the calculation factors as described above, resulting in 0.1 - 0.5 mg l^{-1} autochthonous DOC production. Marine DOC = (salinity sample / salinity marine)*1.65 mg l^{-1}, according to our coastal shelf samples. Thus, depending on salinity, 62 - 96% of the DOC is of riverine origin in the estuary. Assuming that all sugar cane DOC is in the riverine part of DOC we can then estimate the percentage of sugar cane carbon in estuarine samples with Eq. 4, using the $\delta^{13}C$ values of each transect (-25,5‰; -22,2‰) as sample value. As a result, the sugar cane component of estuarine DOM is on avg. 14% during dry weather and 26% in rainy conditions.

From river to lagoon and through the estuary the sugar cane derived DOM fraction follows the same non-conservative and conservative mixing patterns as observed for bulk DOM (Fig. 2.3). Apparently, recycling within the lagoon involves primarily the autochthonous DOM. Here, the microbial community is an autogenically balanced production-decomposition system with the microbes being probably more adapted to intrinsic conditions than to quantity and quality of external input variables (Wolf et al., 2010). Thus, the sugar cane-derived components of DOM should undergo most of their biological degradation already within the soil, drainages and rivers

by specialised bacteria and reach the lagoon in a relatively refractory state.

2.6 Summary

We have used DOC concentrations and $\delta^{13}C$ DOC values to trace the flux of sugar cane derived DOM in a tropical lagoon-estuary system which drains a catchment marked by extensive sugar cane cultivation and processing. Rivers discharging into the brackish lagoon have negligible *in situ* production of DOM as a result of limited light availability in the highly turbid waters, but continuously receive DOM from the surrounding fields from baseflow, drainage and field runoff – depending on rainfall patterns. Occasional inputs of OM-rich vinhasse effluent from sugar cane processing factories are limited to the harvest/dry season and can locally drive up the sugar component of riverine DOC to 62%.

Quantity and quality of riverine DOM differ between baseflow and rainfall-runoff conditions: deeper, carbohydrate impoverished soil horizons, are flushed during dry spells by baseflow whereas heavy rainfall mobilised larger amounts of OM from the surface layers rich in sugar cane material. Hence enriched riverine $\delta^{13}C$ DOC values were observed during rainy conditions, and the isotopically derived sugar component of riverine DOC varied between 15 and 35%. This hydrologically driven variability was also reflected in the contribution of sugar cane to bulk DOM within the lagoon, which was on avg. 20% in dry conditions and 31% during heavy rainfall. Autochthonous DOM from in situ phytoplankton production is a minor contribution (2 - 9%) to overall lagoon DOM.

The fate of riverine DOC during mixing with brackish lagoon water was not only affected by the salinity gradient, but also hydrologically driven via the precipitation dependent load of suspended sediment in the river. Bulk DOC, UV absorbance and $\delta^{13}C$ DOC consistently reflect non-conservative mixing of DOC, with a net loss of DOC for the rainfall-runoff and a net gain of DOC for the dry/baseflow situation. The loss resulted from flocculation of humic rich soil DOC and subsequent scavenging by settling sediments. Laboratory mixing experiments showed that pure sugar cane molasses does not participate in flocculation – unlike the aqueous soil extract, whose DOM showed a pronounced non-conservative mixing related to significant loss. The gain can be related to the low riverine suspension load with deeper light penetration, resulting in enhanced in situ primary production and photochemical transformation of POC from resuspended sediments into DOC.

The long water residence times in the lagoon enable efficient transformation and degradation of labile autochthonous DOM, while the more refractory remains are mixed conservatively through

the estuary, as substantiated by both, DOC concentrations and $\delta^{13}C$ DOC during longitudinal transects, as well as during a high resolution time series at a fixed point in the estuary. The sugar cane-derived components of DOM represent a relatively refractory part of DOM as microbial degradation of these components is probably accomplished to a large extend already within the soil-drainage-river-system.

In conclusion, lagoon-estuary size and geometry combined with river flow conditions significantly influence the quality of DOM that ultimately reaches the marine environment. In the state of Alagoas this applies to its 49 lagoons of varying size along a coastline of 230 km (Espino et al., 1994).

Our conclusions derive from data for dry seasons with intermittent, occasional rainfall. Further studies should focus on the fully developed rainy season proper, when salinity in the lagoon is almost zero, water residence times in the lagoon are much shorter, sugar cane factory effluent is absent and continuous flushing of organics and nutrients from the fields promotes massive phytoplankton blooms. From a coastal water perspective, we should expect a much heavier load of relatively undegraded, sugar cane rich OM reaching the marine environment in the wet season.

3 Distribution of prokaryotic organisms in a tropical estuary influenced by sugar cane agriculture in northeast Brazil

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Abstract

In a joint Brazilian-German case study, distribution patterns of microorganisms were compared with environmental variables in the tropical coastal Manguaba lagoon in northeast Brazil, which is situated downstream of several sugar cane processing plants. 16S rDNA and 16S rRNA single strand conformation polymorphism (SSCP) gene fingerprinting were used to follow the composition and distribution of microorganisms throughout the salinity gradient of the lagoon. Potentially abundant microorganisms were identified by sequencing representative SSCP bands. It could be demonstrated that the distribution of microbes was in close relation to the physicochemical environmental settings and followed a common scheme. In the in- and outlet areas of the lagoon rather transient microbial communities were found, whereas in the central part a stable, diverse community was encountered, that due to the long residence time of the water, had ample time for development and adaptation.

3. 1 Introduction

In the mid-seventies, Brazil implemented the "Pro-Alcohol Program" to boost the production of ethanol as an alternative energy source (Unica, 2006). Meanwhile, the production of agro-fuels has become a prime issue since the worldwide demand for alternative energy sources increased (Farrel et al., 2006). More than 60 % of Brazil's sugar cane production is sustained by the State of São Paulo in the Southeast, and about 20 % along the coastal zone of the Northeast in the States of Alagoas, Pernambuco and Sergipe.

Sugar cane monoculture practices induce environmental impacts, as they involve the application of fertilizers and biocides, alter the composition and water retention capacity of soils, enhance land erosion and affect the quality and balance of groundwater (Lacerda et al., 1983). The materials and associated pollutants are introduced into aquatic systems by diffuse wash-out from the drainage basin and point-source emissions from industrial processing plants into rivers. The emission of sooth particles and PAH´s to the atmosphere from crop burning adds to environmental problems.

Most studies in Brazil addressing these problems focused on the limnic system, investigating the alterations of the biological oxygen demand generated by the introduction of a waste product of sugar cane processing, the nutrient water quality, the impacts upon the sustenance of commercially relevant organisms, as well as air pollution effects (Azevedo and Carmouze, 1994; Bernhard et al., 2005). However, the degree to which the multiple impacts from sugar cane practices affect Brazilian estuaries and coastal waters has as yet to be discerned. Impacts on limnic and estuarine systems may differ considerably, as land-borne materials and associated pollutants undergo complex physico-chemical particle-water reactions during estuarine mixing due to changes of pH, salinity, biological production, and degradation processes (Means and Wuayaratne, 1982; Turner and Millward, 2002; Ben-Hur et al., 2003).

Microbial communities play a key role in the transformation of inorganic and organic constituents, including pollutants. The microbial utilization of dissolved and particulate biogenic matter and a variety of pollutants is governed by the size of the molecules and particles, the nature of particle coatings and the reactivity and age of the materials (Amon and Benner, 1996). The composition and activity of microbial communities also differs between limnic, brackish and marine systems, as well as the composition of allochthonous material input and the systems trophic state itself (Gocke et al, 2003). Tropical estuarine-coastal lagoons characterized by a high degree of enclosure and residence time of water, efficiently retain and transform river-borne and autochthonous produced matter and exhibit large spatial variations of microbial communities (Knoppers, 1994). When affected by multiple pollutant

sources they serve as ideal sites for studies related to alterations of the composition and activity responses of microbial communities. The state of Alagoas provides an excellent model to investigate effects of monocultural land-use on estuarine transport patterns. This area is exemplary for the coastal region in the northeast of Brazil, where lagoons connect terrestrial and marine aquatic system.

This study is part of the joint Brazilian/ German POLCAMAR Project, which focuses on transport and impact of pollutants from sugar cane monoculture to the coastal sea. It addresses the distributional patterns of microbial communities in its relation to changes of the physical-chemical environment along estuarine gradients of the tropical Manguaba lagoon system. By employing molecular biological approaches it attempts to describe and map the potentially abundant microbial community of this specific tropical estuarine system.

3.2 Material and Methods

3.2.1 Study site

The estuarine lagoon system of Mundaú-Manguaba, state of Alagoas, NE-Brazil (latitude 9.58° and 9.77°S and longitude 35.73° and 35.97°W) corresponds to a shallow (in average 2 m) choked lagoon and comprises three main sub systems: one being Mundaú lagoon (A = 24 km^2) towards the north, Manguaba lagoon (A = 43 km^2) towards the south and the mangrove dominated canal system (A = 12 km^2), which links both lagoons with the sea. The system is affected by its mesotides up to 2.7 m but 85% of the tidal energy dissipates within the canals which results in an average tidal range of 0.20 m in Mundaú and 0.03 m in Manguaba.

The longest average water residence time is attained with up to 6 weeks in Manguaba (Oliveira and Kjerfve, 1993). The climate of the system is tropical, semi-humid with well defined dry (October - March) and rainy seasons (May-August). The average annual rainfall is around 1,600 mm. The main rivers are the Paraibo do Meio in the upper compartment and the smaller Sumauma River in the lower compartment of Manguaba (Fig 3.1). The total daily average discharge freshwater is 28 m^3 sec^{-1} (Mendes et al, 2007). The basins of both rivers are almost entirely covered by sugar cane monocultures and several processing plants use the rivers for extracting and discharging process water.

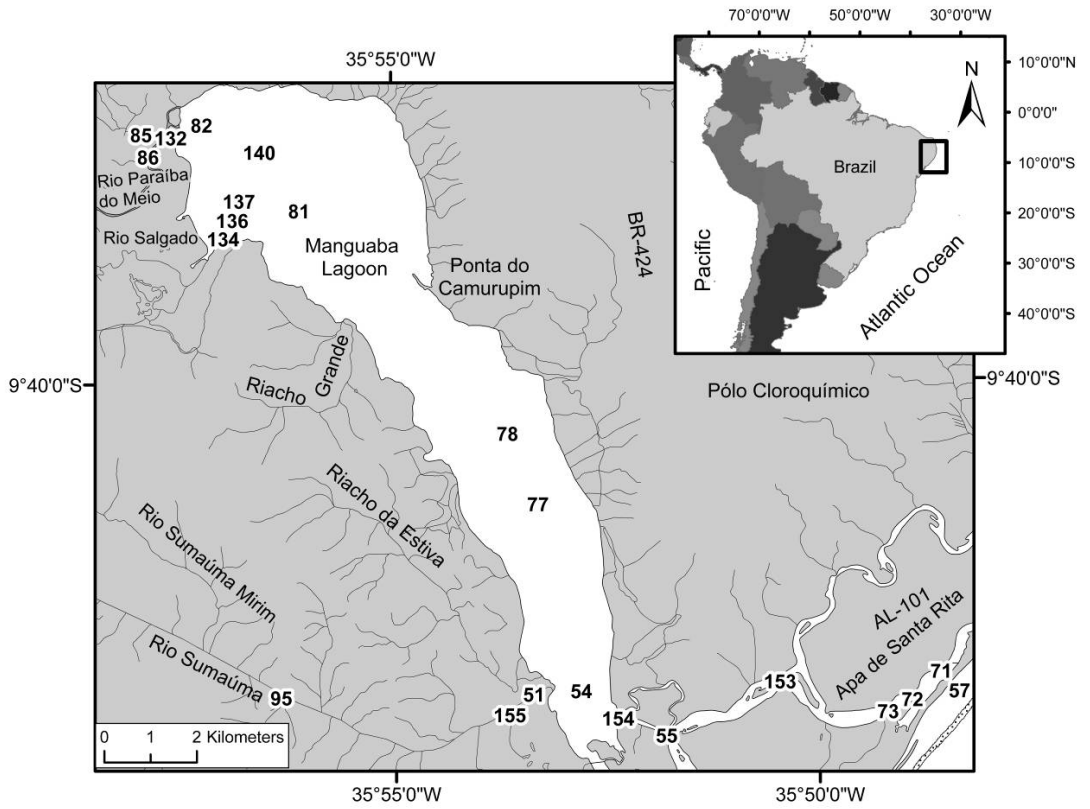

Fig. 3.1 *Sampling positions in the Manguaba lagoon as well as the supporting rivers and connected inlet canals. Sample positions 90 and 92 are not displayed; these samples were taken in drainage canals northeast of the lagoon. The position of the state Alagoas in Brazil is displayed in the right upper corner.*

3.2.2 Sampling

Sampling was performed in March 2007 in the Manguaba lagoon system along a salinity gradient between 0 and 24 PSU between the outflow of the Sumauma river in the lower lagoon and the outlet of the lagoon into the Atlantic Ocean. The sampling stations are displayed in Figure 3.1. Surface water samples for physico-chemical parameters were collected from the boat directly into 2 l bottles and processed further in the laboratory. Samples for DOC measurements were filtered onto precombusted GFF filter (Whatman) and the filtrates were acididfied to pH 2 with 85 % H_3PO_4 and kept frozen until analysis. Samples for nucleic acid extraction were sampled directly from the surface water into a syringe, filtered immediately onto Nuclepore filters (pore size 0.2 µm) and cooled on ice. After return to the laboratory they were stored frozen at –20 °C for later analysis.

Temperature, conductivity and oxygen were measured with WTW probes (WTW GmbH, Germany) and colorimetric nutrient analyses (NO_3, NO_2, NH_4, PO_4) where performed after filtration according to Grasshoff et al. (1983). Chlorophyll *a* and total suspended solids (TSS) were analyzed according to Strickland and Parsons (1972). The DOC concentrations were measured using a high temperature combustion analyzer (Shimadzu TOC 5050) with a Platinum catalyst at 680°C. **C**anonical **C**orrespondence **A**nalyses (CCA) (ter Braak, 1986) were performed for salinity, Secci depth and DOC content.

3.2.3 Nucleic acid extraction and ribosomal complementary DNA (rcDNA) synthesis

Nucleic acid extraction and quantification from the frozen filters was performed by parallel extraction of RNA and DNA using a phenol extraction protocol described by Weinbauer et al. (2002). Prior to RT-PCR, RNA extracts were purified from DNA by incubation with DNase I (DNA-free-Kit, Ambion) for 30 min at 37°C and their concentrations were determined using a NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies). To retrieve 16S rcDNA 200 ng of template RNA were reverse transcribed at 42°C using the iScript cDNA synthesis kit (Bio-Rad). In addition to hexamers provided in the kit, the universal reverse primer 1492R (5'-GGTTACCTTGTTACGACTT-3') (Lane, 1991) was also applied. In each reverse transcription reaction, some RNA samples used as controls in the PCR were not supplemented with reverse transcriptase, in order to rule out DNA contamination.

3.2.4 16S rRNA fingerprint analysis

16S rcDNA and 16S rRNA genes were analyzed by single strand conformation polymorphism (SSCP). Bacterial Com-primers (amplifying positions 519 to 926 of *E. coli* numbering of 16S rRNA gene) (Schwieger and Tebbe, 1998) were used for gene amplification. Thermocycling started with an initial denaturation for 5 min at 94°C. A total of 25 cycles (30 s at 94°C, 30 s at 55°C, 2 min at 72°C) were followed by a final elongation step of 10 min at 72°C. 16S rRNA genes were amplified analogously with a total of 35 cycles. Generation and purification of single-stranded DNA (ssDNA) and SSCP analysis were performed according to Schwieger and Tebbe (1998). Re-amplification of individual bands excised from the SSCP gels was performed as described by Pöhler et al. (2002). PCR products were purified using the NucleoSpin® Extract 2 (Macherey & Nagel) as described by

the manufacturer and were sequenced by Qiagen (Hilden, Germany). Forward and reverse sequences of all samples were checked for accuracy using the SeqMan software (DNASTAR).

3.2.5 Phylogenetic analyses

Phylogenetic affiliations of the partial 16S rRNA sequences were estimated using the basic local alignment search tool BLAST (Altschult et al., 1997). The 16S rRNA gene sequences determined in this study were deposited in the GenBank database under accession numbers GU88510 to GU088529.

3.3 Results

3.3.1 Environmental parameters

Means of environmental variables measured at specific sections within the different estuarine provinces coincided largely with the geographical division (Tab. 3.1) of the lagoon system, which was also affirmed by CCA statistic analyses:
(I) the limnic part of the estuarine gradient, the river itself and the areas close to the river mouth were characterized by turbid, low saline water with high concentrations of phosphate, nitrate and silicate (Tab. 3.1). This limnic section was sampled in drainage channels and the rivers Paraibo do Meio and Sumauma as suppliers to the lagoon.
(II) The central Manguaba lagoon was a brackish water body with distinct endemic features. Due to the sedimentation of the riverine mineral particles to the bottom of the lagoon, light penetrated far deeper into the water and promoted intensive phototrophic activity (Tab. 3.1). At a salinity of about 4 PSU chlorophyll *a* values were an order of magnitude higher than in the supplying rivers with associated lower values of inorganic nutrients and higher oxygen concentrations. Light microscopic inspection of the phytoplankton community showed a mixture of green algae, diatoms and colonial and filamentous cyanobacteria as dominant groups (data not shown). DOC concentrations showed a patchy distribution within the central Manguaba lagoon.

Tab. 3.1 Physical and chemical parameters of the sampling stations. (ND, not determined)

Station [Polca]	Latitude [S]	Longitude [W]	Temperature [°C]	Conductivity [ms]	Salinity [PSU]	O_2 [µM]	Secchi depth [m]	Chlorophyll *a* [mg m^3]	Pigment [mg m^3]	TSS [mg l^{-1}]	Nitrate [µM]	Nitrite [µM]	Ammonia [µM]	Phosphate total [µM]	Phosphate diss. [µM]	DOC [mg l^{-1}]
Paraibo do Maio river , the Sumauma river and drainage canals (I)																
Mean			29.09	0.21	0	150.53	0.00	3.91	5.09	65.42	15.23	1.13	2.03	3.44	1.60	9.86
standard deviation $^{+}/_{-}$			1.55	0.07	0	122.03	0.00	1.63	3.69	44.29	18.85	0.72	2.56	1.57	1.26	6.09
155	-9.73	-35.89	28.50	0.13	0	17.12	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
095	-9.73	-35.94	28.70	0.14	0	254.41	ND	2.81	5.49	32.00	8.40	1.09	0.79	2.76	1.24	20.96
051	-9.73	-35.89	28.70	0.15	0	34.25	0.00	5.82	0.00	18.00	0.75	0.43	0.33	2.33	0.57	8.87
092	-9.57	-35.87	32.80	0.18	0	319.59	ND	5.23	3.74	145.50	ND	ND	ND	ND	ND	3.03
090	-9.55	-35.85	27.70	0.20	0	296.35	ND	1.92	3.74	62.50	36.55	1.86	4.98	5.24	3.00	8.50
086	-9.62	-35.96	28.70	0.26	0	73.39	0.00	5.00	11.12	70.00	ND	ND	ND	ND	ND	11.25
085	-9.62	-35.96	29.00	0.29	0	72.10	0.00	2.69	6.47	64.50	ND	ND	ND	ND	ND	6.55
132	-9.62	-35.96	28.60	0.32	0	136.99	0.00	ND	ND	ND	ND	ND	ND	ND	ND	ND
Manguaba lagoon upper and lower compartment (II)																
Mean			29.88	8.55	4.25	180.29	0.48	34.89	86.90	38.50	13.43	0.61	2.82	4.07	1.08	7.40
Standard deviation $^{+}/_{-}$			0.45	2.29	1.36	51.14	0.20	14.03	36.68	12.67	14.22	0.29	1.90	3.00	0.70	1.58
082	-9.62	-35.96	30.30	5.99	3	137.08	0.40	24.39	59.61	47.50	42.90	1.14	6.06	8.81	1.43	4.38
140	-9.62	-35.94	30.30	6.31	3	171.93	0.50	37.73	88.53	41.00	7.42	0.64	2.41	2.19	1.19	8.99
081	-9.63	-35.93	29.50	6.65	3	244.50	0.00	ND	ND	ND	ND	ND	ND	ND	ND	ND
137	-9.63	-35.95	30.30	6.68	3	153.35	0.50	22.14	57.52	41.50	7.96	0.64	4.56	2.62	1.38	8.76
134	-9.64	-35.95	30.20	6.71	3	146.38	0.35	30.95	78.41	26.06	10.03	0.61	0.06	4.67	1.38	6.79
136	-9.63	-35.95	30.30	6.76	3	160.76	0.60	31.41	78.33	30.40	6.16	0.56	4.25	3.19	1.14	6.29
154	-9.73	-35.87	30.00	9.81	5	208.09	ND	28.80	71.74	25.17	8.71	0.24	1.60	1.81	0.33	ND
153	-9.73	-35.84	29.70	9.21	5	140.29	ND	41.03	105.70	41.14	0.97	0.37	1.60	1.76	0.29	ND
078	-9.68	-35.89	29.40	9.49	5	299.93	0.65	13.62	28.16	68.00	28.72	1.09	4.48	10.81	2.62	8.44
054	-9.73	-35.88	29.90	11.65	6	151.13	0.55	57.12	141.54	27.25	1.49	0.43	1.14	3.10	0.38	8.02
055	-9.74	-35.86	29.80	11.89	6	137.22	0.55	60.08	155.17	30.50	1.71	0.27	1.02	3.81	0.43	8.76
077	-9.69	-35.89	28.90	11.50	6	212.81	0.70	36.57	91.24	45.00	31.64	0.72	3.79	2.05	1.33	6.14
inlet canal between Manguaba and the Atlantic ocean (III)																
Mean			29.10	27.60	15.75	110.52	0.83	29.64	72.21	38.51	22.62	0.55	4.82	3.63	0.46	5.48
Standard deviation $^{+}/_{-}$			1.24	3.21	1.50	82.24	0.21	13.96	35.97	43.21	17.25	0.26	2.61	3.03	0.11	2.19
057	-9.73	-35.81	30.20	19.11	10	0.00	ND	58.43	143.01	46.00	2.24	0.40	2.02	4.38	0.33	8.59
073	-9.73	-35.82	28.90	21.20	12	130.42	0.85	24.39	60.30	28.20	33.67	0.66	8.25	5.14	0.52	4.43
072	-9.73	-35.82	28.80	29.80	17	146.87	0.80	21.67	52.02	45.12	26.47	0.69	4.79	2.90	0.62	3.57
071	-9.72	-35.81	28.50	40.30	24	164.80	ND	14.05	33.49	34.71	28.11	0.45	4.22	2.10	0.38	5.32

(III) The outlet channels of the lagoon system are the connection to the Atlantic Ocean, where brackish lagoon and coastal marine waters mix. The mixing gradient was, however, not continuous, but strongly influenced by tides. Due to the generally lower DOC concentration of coastal ocean water, DOC concentrations in the channels were also lower, compared to the central lagoon, as a result of mixing with coastal waters (Tab. 3.1). Chlorophyll *a* values were still high but lower oxygen concentrations pointed towards lower phototrophic activity and increased heterotrophic processes.

3.3.2 Microbial distribution and identification

For the whole estuarine gradient 20 microorganisms could be allocated to *Proteobacteri*a, *Cyanobacteria*, *Bacteroidetes*, or *Actinobacteria* (Fig. 3.2). The quality of this allocation was dependent on the match between extracted and deposited sequences and in the range from 91 to 100 % (Fig. 3.2). The distribution patterns of prokaryotic microorganisms in general followed the scheme of environmental parameters described above:

(I) In the limnic part of the estuarine gradient, the river itself and the areas close to the river outlet, different *Beta*-, *Epsilon*-, and *Gammaproteobacteri*a were abundant in the fingerprint analyses.

(II) Several of the organisms detected in the limnic part of the lagoon were also found in the central lagoon with its brackish water body, but in general the detected microbial diversity increased with more organisms belonging to the *Gammaproteobacteria* - especially *Acinetobacter* - *Cyanobacteria*, *Bacteriodetes*, and *Actinobacteria*. *Cyanobacteria* were mostly represented by *Synechococcu*s spp. The fraction of *Betaproteobacteria* decreased and was nearly undetectable on rRNA level. No *Epsilonproteobacteria* were detected anymore.

(III) The outlet channels of the lagoon system were characterized by microbial assemblages comparable to the ones of the central lagoon system. However, no 16S rRNA of *Betaproteobacteria* could be detected in this system anymore.

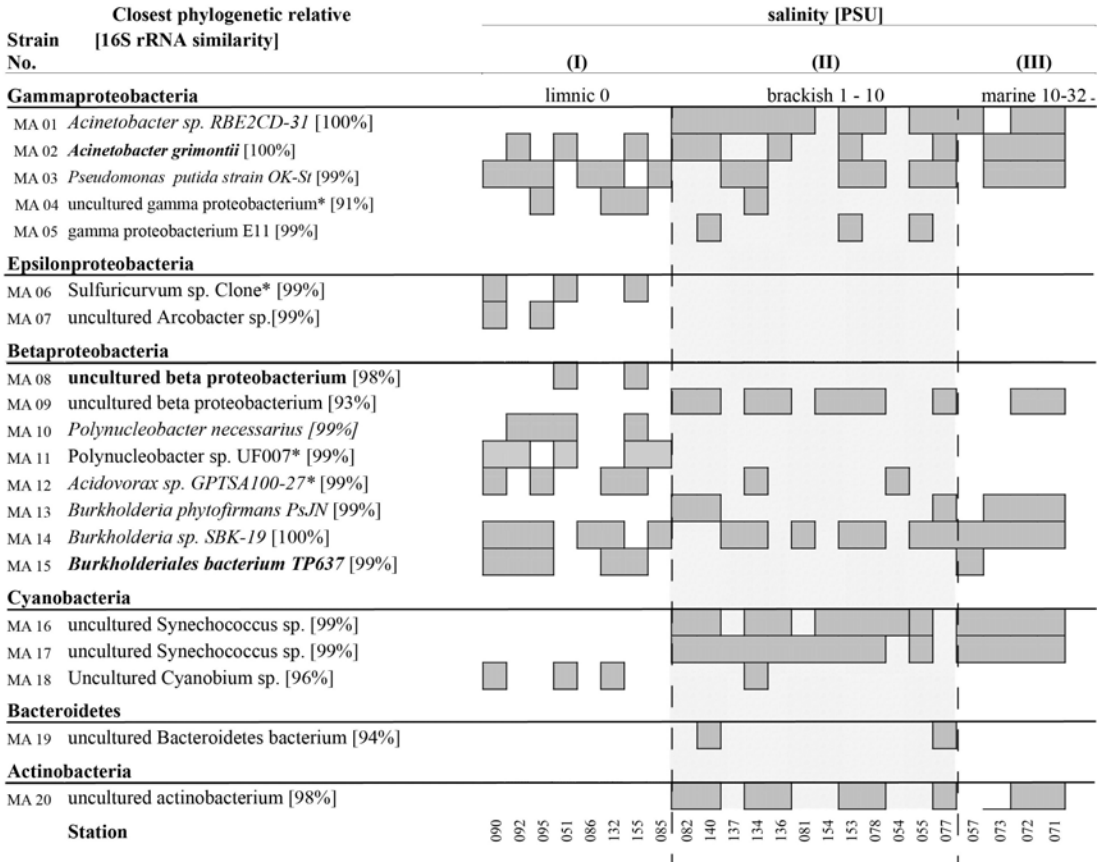

Fig 3.2 *Horizontal distribution of identified taxa based on 16S rRNA gene or 16S rRNA SSCP fingerprinting. Bacteria identified on DNA as well as on RNA level are indicated in bold. Bacteria identified only on RNA level are marked by asterisks.*

3.4 Discussion

The results both from environmental measurements and analyses of the microbial communities classify the Manguaba lagoon as an estuary with three different subsystems with an overall coherence between environmental indicators and microbial abundance patterns:

(I) Limnic system: The first subsystem, comprising of rivers feeding the lagoon and regions at the river inlets, was characterized by physicochemical and biological features which were indicative for a non-steady state situation. In our study period large amounts of nutrients, organic matter, and microorganisms have quite recently been washed into the river systems along the cane fields. Although the rainfall in March 2007 with 169 mm/month was only slightly above the statistical average monthly precipitation for March, the rain was concentrated on a few extreme events in the range from 40 mm to 60 mm per day. This led to heavy local erosion of soil, organisms and elevated input of dissolved organic and inorganic

compounds. High DOC values are supposed to derive from the same source as well as from direct input of organic constituents from the sugar processing factories. *In situ* production of DOC by algae was unlikely due to the fact that chlorophyll *a* and pigment values were low as an effect of light limitation in the highly turbid waters. All this was reflected in the chemical and microbial composition within the river water with a bacterial community typical for soils and freshwater (Fig. 3.2). Especially *Betaproteobacteria* related 16S rRNA bands MA 15 or MA 8 were indicative for soils or associated with terrestrial plants roots, respectively (Bollmann et al, 2007; Vandenkoornhuyse et al, 2007). MA 14 related sequences belonging to *Burkholderia* were isolated from tropical soils which had been influenced by fires (Otsuka et al, 2008). The species *Burkholderia phytofirmas* PsJN, highly related to sequence MA 13, was described in a study on endophytic bacteria in sugar cane which show beneficial effects on plant growth, but may also be associated with putative opportunistic human pathogenic bacteria (Mendes et al, 2007). Other sequences found in this environment (MA 11, 10, 12) were related to typical members of bacterial fresh water plankton like *Polynucleobacter*-, or *Acidovorax*-related sequences. The potential abundance of *Betaproteobacteria* in this part of the gradient was expected, as this group is known as a major member of the limnic compartment of estuarine systems (del Giorgio and Bouvier, 2002; Bernhard et al, 2005). *Gammaproteobacteria* as the next abundant group is an ubiquitous class of bacteria found in most known habitats and so their presence could as well be anticipated.

To summarize, high concentrations of DOC and inorganic nutrients (Tab. 3.1) fostered potential heterotrophic organisms and activity. Photoautotrophic processes and their contribution to DOC production were low due to high turbidity and therefore the system can be considered to be heterotrophic. Both substrate and decomposers were probably to a large degree imported from soil and due to the short residence time in the aquatic environment can not be assumed to be in decomposition equilibrium.

(II) Brackish system: The above described scenario changed, once the water entered the second subsystem – the central lagoon. The turnover time of water in the central lagoon is in the range of 36 days (Oliveira and Kjerfve, 1993) and therefore supports many generation times of unicellular organisms which form a self sustained microbial system. As the introduced mineral matter quickly sank out of the water at the river inlets due to a reduction of turbulence in the open lagoon, light penetrated deeper into the water and enabled photoautotrophic production. This in turn provided organic matter which can be decomposed and mineralized right away. An autogenic balanced production-decomposition system was established in the lagoon, where the microbes were probably more adapted to intrinsic conditions than to quantity and quality of external input variables. This was reflected in

chlorophyll increase, nutrient decrease (Tab. 3.1) and as well in the community composition, with different autotrophic cyanobacteria, mostly related to the picocyanobacterium *Synechococcus*, and several heterotrophs (Fig. 3.2). Elevated concentrations of ammonia indicated a high turnover of organic substrates and a functioning cycle of matter between autotrophic and heterotrophic agents. High productivity in lakes and lagoons usually means an increased carbon flux towards the sediments with associated oxygen deficiencies in the bottom water in spite of a well oxygenated surface layer. In general, the higher relative abundance of *Gammproteobacteria*-related sequences corresponded to the findings of Bernhard et al. (2005) who also studied the distribution of 16S rRNA genes in estuarine systems. Within this group, *Acinetobacter* was abundant in the brackish system. From these, phylogenetic relatives of SSCP bands MA 1 or MA 4 have been found to degrade persistent organic pollutants. Thus, within the natural microbial assemblage of the lagoon a high potential for resistance against xenobiotic substances or even for detoxification could exist.
Introduced substances will remain here for a considerable time span and will meet successive environmental conditions of extreme diversity. Water and dissolved substances are transported repeatedly through considerable environmental gradients, which allow specialized organisms to attack these substrates. In these diverse environments bacteria can potentially be found with a high variety of different metabolic pathways and the ability to withstand and degrade complex organic substances. In the central lagoon we found a system which had the potential, both in environmental background conditions and in terms of microbial diversity, to efficiently modify introduced substances. Considering the presence of specialized bacteria, which were able to cope with organic pollutants, this point towards a certain decontamination potential.

(III) Outlet channels: At the outlet channel turbidity as well as oxygen concentration was lower than in the brackish water (Tab. 3.1). The banks of this channel were grown over with mangrove stands, removing a high amount of particulate matter from the water. In addition to the mixing with clear water from the coastal ocean this may lead to a further reduction of turbidity as compared to the central lagoon and an increase in nitrate and ammonia values, whereas orthophosphate seemed to be kept within the sediments. No betaproteobacteria were detectable on 16S rRNA level anymore, indicating that this limnic group was inactive at more saline conditions. The remaining detected microbial diversity was comparable to the brackish system; however, increased turbulence, reduced water residence times in specific environments and the tidal mixing generated a strong salinity gradient (ranging from 10 to 24 PSU). In contrary to the central lagoon, these conditions demanded high adaptation efforts within a short time span, probably resulting in a decrease of decontamination efficiency.

The general conclusion of this study is that the Manguaba lagoon in its present condition has a diverse and well adapted microbial community. A system like this is generally adapted to cope with additional external loads of natural substances, as it operates on an elevated decompositional level due to the high internal substrate turnover. In Lagoa Manguaba this is based on the morphology of the lagoon and the long residence time of water as well as on the presence of a variety of adapted microbial organisms. Drastic difference in functional properties of this system could probably be expected if the water residence times are changed by technical measures or major changes in the drainage patterns.

4 Separation of natural organic colloids with a PALL tangential flow filtration system

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Abstract

The applicability of a PALL tangential flow filtration (TFF) system for size fractionation of natural dissolved organic matter was investigated. The performance of polyethersulfone membranes with nominal molecular weight cut-off of 1 kDa, 5 kDa and 50 kDa was examined for isolation of low and high molecular weight compounds in fresh and estuarine waters with diverse physico-chemical properties. Detailed protocols for operating the TFF-system and for membrane cleaning are proposed. The ultrafiltration membranes can be efficiently cleaned to provide low carbon blanks (< 0.09 mg/l). Standard colloid tests confirmed that the higher molecular weight compounds were isolated in the retentate and the lower molecular weight compounds remain in the permeate. Mass balance of fractionated natural samples showed good recoveries for dissolved organic carbon (DOC) (99 ± 13 % (1 kDa); 103 ± 20 % (5 kDa); 94 ± 14 % (50 kDa) (n = 9). Moreover, high ionic strength or high DOC content did enhance neither fouling nor contamination of the membrane. These findings demonstrate that the PALL TFF system is reliable for the fractionation of natural organic colloids in aquatic systems.

4. 1 Introduction

Organic Matter (OM) has traditionally been subdivided into dissolved and particulate fractions, separated by filtration in the cut-off range of 0.2 – 1.0 µm (Gustafsson et al., 1996). The dissolved fraction excludes particulate material and bacteria (Guéguen et al., 2002) and can be further subdivided into a colloidal fraction with high molecular weight (HMW) and the 'truly' dissolved fraction with low molecular weight (LMW) organic compounds. Realistic contaminant transport modelling requires that colloids are considered as a third, separate phase, distinct from the 'dissolved' and 'particulate' phases (Gschwend and Wu, 1985). Aquatic colloids are entities with supramolecular structure and properties, but small enough to remain in suspension (Amon and Benner, 1996; Buffle et al., 1998) and they are widespread in the aquatic environment. The boundary between the LMW and colloidal phase is defined operationally as a given membrane's molecular weight cut-off (MWCO) in an ultrafiltration (UF) / tangential flow filtration (TFF) system (Guo and Santschi, 2006). Typically, membranes used for natural organic matter separations have MWCOs in the range from 1.000 – 10.000 Dalton, and the 1.000 Dalton size exclusion is widely used to separate 'dissolved' from 'colloidal' matter (Guo et al., 1994; Buesseler et al., 1996 and references therein).

OM is the most important component of natural colloids in aquatic systems (Dai and Benitez-Nelson, 2001; Wilding et al., 2005). Colloids comprise 10 - 40 % of the marine organic carbon pool (Guo et al., 1994; Dai and Benitez-Nelson, 2001) and show a considerably increasing abundance in estuaries (4 - 45 %), rivers (36 - 86 %) and lakes (~ 50 %) (Guéguen et al., 2002). However, ~50 % of colloidal organic matter (COM) is still poorly characterized (Guo and Santschi, 1996). Relatively little is known about the molecular size distribution and chemical composition within the COM fraction. Electron microscopy images showed three morphologies in colloids: fibrils, globules and amorphous matter (Buffel and Leppard, 1995; Wilkinson et al., 1999); suggesting that COM is composed of many different components.

In recent years, TFF has become one of the most commonly used techniques for fractionating freshwater and marine colloids. TFF can process greater volumes per unit area of membrane surface compared to standard filtration. It allows preparative isolation of the desired amount of colloids for further studies, despite their low concentration (Doucet et al., 2004). The crossflow prevents build up of molecules at the membrane surface avoiding fouling as the sample solution flows through the feed channel, tangentially to the surface of the membrane as well as through the membrane. In spite of the increased use of TFF systems, just a few controlled laboratory studies aiming at the implementation of stringent experimental

protocols and operational procedures during TFF fractionation have been done (Guo and Santschi, 2006). Size class separations of natural fresh and marine water dissolved OM have been performed using varying types and properties of ultrafiltration membranes (e.g. Chin and Gschwend, 1991; Amon and Benner, 1996; Guo and Santschi, 1996) and may explain disagreements between reported data.

Also, few detailed analyses of molecular weight distribution within the COM fraction have been performed upon natural samples with different ionic strengths and organic matter contents (see review by Guo and Santschi 2006), because using more than one ultrafiltration membrane is time consuming as each membrane must be checked for its performance. Furthermore, larger volumes of initial samples are required for the necessary conditioning of each membrane.

In our study we tested the PALL Centramate TFF system with three low protein binding Omega membranes with different MWCO for colloid characteristic in fresh- and estuarine water environments. This system has mainly been used in lab scale process development and production applications (Schwartz and Seeley, 2009), but also in environmental colloid studies (Fine et al., 2002; Minor et al., 2002; Powell et al., 2005; Waiser and Roberts, 2005). However, no sufficient details on the TFF operational protocol and the membrane characteristics were given by these authors to allow direct comparison of their colloid data. The TFF system, originally developed for industrial and biochemical purposes, necessitates additional and specific TFF integrity studies before environmental application, because of the stark contrast between industrial fluids and the dilute and heterogeneous colloid suspension of natural waters. Therefore, we not only tested the applicability of the PALL Centramate system to fractionate and concentrate natural dissolved OM samples with different properties, but also checked the membrane retention capacity and the TFF system blank of each membrane. We further established an efficient cleaning procedure for the application on natural samples. The resulting information should prove useful for further studies on the qualitative and quantitative characteristics of molecular weight size fractionated natural COM.

4. 2 Materials and methods

4.2.1 The TFF system

The TFF system consists of a PALL Centramate membrane holder with an Omega membrane, a cogwheel pump with control unit (Gather), tubing, valves, clamps, two pressure gauges, and a sample reservoir and permeate flask. The membranes with an area of 0.09 m^2 are made of low protein-binding modified polyethersulfone (PES). The membrane holders, luer fitting, tie rods and washers are made of stainless steel. The O-ring for the luer fitting consisted of EPDM.

The sample reservoir is made of a conical bottomed glass flask (2.5 l) with a centred outlet and a sidewise coming permeate inlet 5 cm above the outlet, while permeate is collected in a glass flask. Pump, reservoir, permeate flask and membrane holder are connected with tygon tubings (R 3603). Pressure gauges are installed at the feed and retentate ports, to monitor and control the pressure for more consistent results.

In all experiments three Centramate Omega PES cassette membranes with different nominal cut-off sizes: 1 kDa, 5 kDa and 50 kDa, were used individually to separate very high molecular weight (VHMW) (> 50 kDa), from HMW (> 5 or >1 kDa) and LMW organic compounds (< 1 kDa).

4.2.2 Fractionation by TFF

The following steps are necessary to operate the Centramate system:

1. Rinsing the TFF system before use to remove the storage agent.
2. Conditioning the system with the sample buffer. This step helps to remove air from the system, to adjust the system temperature and to prevent possible precipitation or denaturation of biomolecules resulting from contact with flushing solution.
3. Sample processing (concentration / fractionation).
4. System cleaning and determining the cleaning efficiency.
5. Storing TFF membranes.

The terms permeate and retentate used in this text are defined as followed: permeate is the fraction passing through TFF membranes while retentate is defined as the fraction retained by TFF membranes.

TFF can be carried out in two modes of operation: recirculation and concentration.
During recirculation mode, both, permeate and retentate flow were directed back into the reservoir flask and thus the reservoir sample volume remained constant. This mode is used for cleaning the membrane and for preconditioning it with natural sample. In the concentration mode, however, the permeate flow is collected in the permeate flask, while the retentate flow is recycled back into the reservoir. The concentration of colloids in the sample reservoir increases with time in direct proportion to the decrease in sample volume and enables its use for colloid isolation and concentration (Larsson et al., 2002; Wilding et al., 2004).
A parallel filtration scheme as used by Guo et al. (1994) and Amon and Benner (1996) was applied, to avoid carryover of contamination or sample losses from former membranes.

4.2.3 TFF process variables

Important variables involved in TFF are transmembrane pressure (TMP) and crossflow velocity (CFV) (Guo and Santschi, 2006). The TMP is the force that drives fluid through the membrane, carrying along the permeable molecules. The CFV is the rate of the solution flow through the feed channel and across the membrane. The crossflow sweeps away larger molecules and aggregates that are retained on the surface of the membrane, preventing the formation of a concentrated biomolecule layer on the membrane surface that can foul or plug the membrane.
Samples flowing through the narrow feed channel create a pressure drop between the feed and retentate ports. This pressure, which is applied to the membrane, can be further increased by increasing the CFV or by restricting the tubing at the retentate port valve. Using TFF effectively means to regulate both the TMP and the CFV to prevent membrane fouling and restriction of the filtrate flow, thus allowing a greater volume of product to be processed in the least possible time.
Earlier studies indicate the importance of the cross flow ratio (CFR) for colloid recovery, as higher CFR resulted in increasing recoveries (Gustafsson et al., 1996; Larsson et al., 2002). The CFR is the retentate to permeate flow ratio, calculated as:

$$CFR = \psi_{Ret} / \psi_{Perm} \quad \text{Eq. 4.1}$$

where ψ_{Ret} and ψ_{Perm} are the flow rate (ml min^{-1}) of the retentate and permeate, respectively. A CFR > 15 appears necessary to obtain good colloid recoveries (Larsson et al., 2002). The CFR were hence adjusted to > 25 for the 1 kDa and > 15 for the 5 kDa membrane, whereby

flux restrictions on the retentate tubing were applied. The restriction influenced the feed pressure in the TFF system. Membranes with high MWCO have high transmembrane fluxes and hence CFR ≥ 15 cannot be reached with the 50 kDa membrane (Larsson et al., 2002; Kottelat et al., 2008).

The maximum operating pressure of Omega membranes is rated at 5 bar (500 kPa, 75 psi). The Centramate system was operated with a TMP of 1.1 - 1.8 Bar (depending on the sample flow) yielding a permeate flow rate of 10 - 25 ml min^{-1} (1 kDa), 40 - 80 ml min^{-1} (5 kDa), 350 - 700 ml min^{-1} (50 kDa) and a retentate flow rate/ CFV ranging from 900 - 1000 (1 kDa), 850 - 1050 (5 kDa) and 200 - 700 ml min^{-1} (50 kDa).

The concentration factor (cf), defined as the ratio of the initial sample volume to the retentate volume, was 20. The same operating conditions were used in all experiments, as consistent cf and CFR are critical in order to ensure reproducible and comparable colloid data. High cf (10 – 20) have been widely used in recent studies to minimise retention of LMW molecules that would otherwise lead to overestimation of the concentration in the colloidal fraction (Larsson et al., 2002; Wilding et al., 2004; Guo and Santschi, 2006). Further increasing the cf could possibly cause breakthrough of HMW compounds into the permeate, even if this is reported to be minimal during ultrafiltration (Guo and Santschi, 2006).

4.2.4 Cleaning procedure and sample concentration

Before each use, the membranes were cleaned with distilled water until neutral pH was reached in permeate and retentate flux to remove NaOH from the system. 0.1 N NaOH was used as membrane storage solution to avoid bio fouling and crystallisation on the membrane surface. The pH decreased linearly (0.97) with increasing volume of distilled water for cleaning; until neutral pH. After 2 l of flushing with distilled water, permeate and retentate were free of noticeable organic carbon residues. The cleaning step was followed by preconditioning of the membrane with natural prefiltered sample (500 - 1000 ml) in the recirculation mode. Thus, to reduce contamination from the system and to minimize sorptive losses to the membrane and other surfaces (Buesseler et al., 1996). At the end the sample was discarded. The sample (2000 ml) for colloid isolation was then run in the concentration mode until ~100 ml were left in the retentate reservoir.

The established cleaning procedure after TFF of a sample was the same for each membrane:

1. 1 - 2 l of distilled water was passed in concentration mode to remove the sample from the system.
2. To remove inorganic salts especially iron from the membrane surface, the system was flushed with 4 % citric acid and recirculated for 15 minutes afterwards.
3. Distilled water was passed in concentration mode to remove the citric acid from the system (until neutral pH).
4. 0.2 N NaOH was used in the recirculation mode for at least 15 minutes to remove for instance biomolecules, fats, proteins, starches, polysaccharides, and organic colloids from the membrane surface.
5. NaOH was discarded and the system flushed with clean 0.1 N NaOH to prevent bio fouling during storage.
6. Membranes were stored in 0.1 N NaOH at 4°C in an air and water tight box at manufacturers' recommendation.

4.2.5 Membrane retention test / size cut-off

The choice of membrane is usually guided by its nominal molecular weight cut-off (NMWCO), which is typically defined as the equivalent molecular weight of the smallest molecule that would exhibit 90 % rejection (Guo and Santschi, 2006).

In order to know the retention performance of the used membranes, we examined the ability to retain standard molecules of known MW. The retention coefficient (RC) can be derived from

$$RC = 1 - (C_{Perm} / C_{Ret}) \quad \text{Eq. 4.2,}$$

where C_{Perm} and C_{Ret} are the concentrations of a standard molecule in permeate and retentate, respectively.

A range of standard organic colloids were used to assess the membrane retention: Polyethylene glycol (600, 1000, 1500, 3000, 4000, 6000, 10000, 35000, 40000, 116000 Da; Merck, Germany); alpha-D-raffinose, sucrose, α-cyclodextrin (Serva Feinbiochemica, Germany); L-glutamic acid (Merck, Germany); aspartic acid (Aldrich-Chemicals, Germany).

All used standard organic colloids have shown wide stability and applicability in various size exclusion studies (Gustafsson et al., 1996; Guo and Santschi, 2006)

The membrane retention tests were carried out in the recirculation mode under sample operating conditions. Diluted standard molecules had a concentration of 20 mgC/l. These

solutions were processed for 1 hour to establish steady-state conditions in both permeate and retentate. At the end retentate and permeate samples were collected for further dissolved organic carbon (DOC) measurements.

4.2.6 Recovery of organic carbon in natural samples

To address losses of substances during TFF, organic carbon recovery (Recov) was calculated as:

$$\% \text{ Recov} = 100 \times (C_{Ret} + C_{Perm}) / C_{PFW} \quad \text{Eq. 4.3,}$$

where C_{Perm} and C_{Ret} are the organic carbon concentrations in permeate and retentate, respectively and C_{PFW} is the organic carbon concentration of the prefiltered water which was used as sample feed.

This approach uses the OM in natural water itself and provides a useful initial indicator of gross contamination.

4.2.7 Sample collection and storage

Water samples from fresh to coastal waters were collected in the tropical Manguaba lagoon complex (Maceió, NE-Brazil) and from a pond in the temperate zone (Hamburg, N-Germany). The samples had a broad range of salinities (0 - 35) and DOC contents (Tab. 4.1) and their dissolved OM sources were manifold (freshwater from river and pond, brackish phytoplankton and marine dissolved OM).

The pond sample is characterised as a freshwater sample with very high DOC (22.5 mg/l). Lagoon fresh and low salinity water samples ranged from low to high DOC (3.6 - 11.5 mg/l). To obtain a sample with medium salinity and high DOC, pond water was mixed 1:1 with low DOC Arctic water. Medium and high salinity water samples with low DOC (1.5 - 4.1 mg/l) were obtained in the estuary of the lagoon complex.

The water was filled into 20 l carboys and directly brought to the laboratory for immediate filtration through a precombusted GFF filter (Whatman). To minimise contamination the first 3 l of the filtrate were discarded. The filtrated samples were analysed for bulk DOC and further processed using TFF. All aqueous samples were acidified to pH ~ 2 with 85 % H_3PO_4 and frozen during storage.

4.2.8 DOC measurements

The DOC concentrations of all water samples were measured using a high temperature combustion analyzer (Shimadzu TOC 5050) with a Pt catalyst at 680 °C. Samples were sparged for 5 min immediately prior to analysis with the same ultra-high purity synthetic air that was used as a carrier gas in the TOC analyzer. Standards (potassium hydrogen phthalate) were analyzed immediately prior and after analysis of samples. Water for the standards was prepared by Millipore Q-Pod system (total organic carbon < 5ppb), producing a total DOC blank including water and instrument of approximately 0.02 mg/l.
All samples were analysed in triplicate. Precision, in terms of the relative standard deviation was ≤ 2 %.

4.3 Results and discussion

4.3.1 Integrity of the TFF membranes

In order to check the integrity of each membrane, different standard molecules that spanned the colloidal size range were used. As expected, RC values increased with the size of the standard colloids. Since a broad range of standard colloid sizes was used, the obtained data could be applied to calibrate the cut-off size of the TFF membranes.

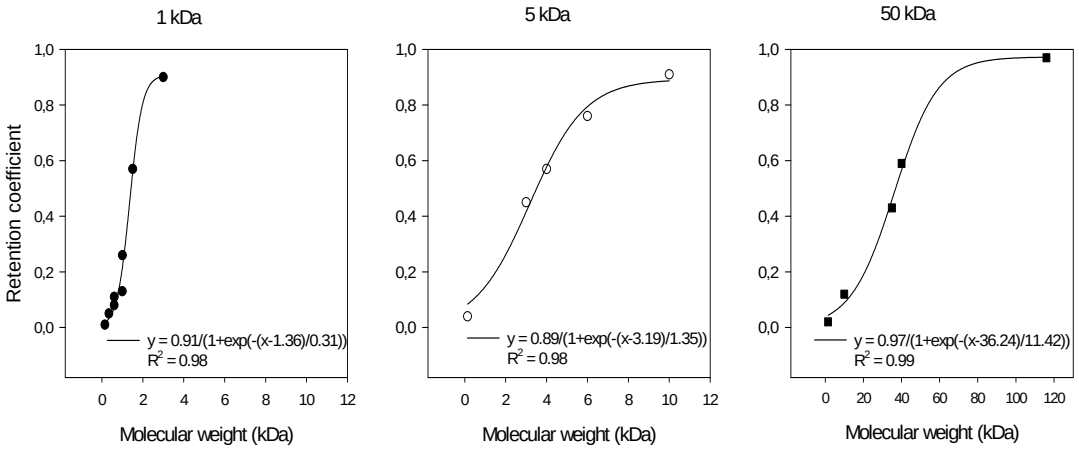

Fig. 4.1 *Molecular weight of standard molecules (kilo Dalton) and their retention coefficients for the determination of the actual molecular weight cut-off of manufacturers specified 1 kDa, 5 kDa and 50 kDa Omega membranes.*

The rejection rate is not necessarily the same for other molecules having the same MW but different molecular properties and configurations (Guo and Santschi, 2006). The MWCO, defined as the majority of pores that retain 90 % of the molecules was deduced by fitting a sigmoidal equation to the retention profiles (Fig. 4.1). The calibrated cut-off of the different TFF membranes was found to be 1.4 kDa (1 kDa), 3.2 kDa (5 kDa) and 35.2 kDa (50 kDa); manufacturers specification in parentheses. We have to keep in mind that a small portion of HMW molecules may pass through the membrane and a portion of LMW molecules could be retained by the membrane and thus the determined MWCO is not static. In general the actual cut-offs agreed well with the cut-offs specified by the manufacturers. The rejection rate for the 1 kDa membrane shows a slightly lower retention than specified, while 5 kDa and 50 kDa membranes retain more molecules relative to manufacturers' specification, when using globular molecules and polysaccharides. These small differences can occur because the cut-off characteristic of the membrane is affected by tertiary shape, electrostatic attraction or repulsion and other physico-chemical interactions of the compound in solution with the membrane and so retention is not simply a function of molecular weight (Buffle et al., 1998; Buesseler et al., 1996).

4.3.2 Blank of fractionation system

TFF fractionation of natural colloids requires correspondingly low blanks of the system, thus construction material must minimally affect OC of the sample. Blank tests are also essential checks of the performance of the used ultrafiltration system, as the membrane can be a major source of contamination.

The system blank was determined treating distilled water strictly the same way as a sample. Permeate and retentate were analysed for DOC. The cleanliness of the membranes and the TFF system was judged according to the differences in DOC levels between blank distilled water and permeate / retentate. The DOC concentration in permeate was the same as in distilled water before TFF process. In the retentate blank an equal amount of DOC (as in the distilled water) was found in the 1 kDa system and 0.06 - 0.09 mg/l were found in the 5 and 50 kDa systems. These results show that the used cleaning procedure can be applied to obtain negligible DOC levels in permeate and retentate and that the membranes are not a source of contamination. Moreover these blanks were negligible compared to natural contents. Nevertheless, at low DOC contents in natural samples, cleaning procedure must be performed with great care.

4.3.3 Mass balance of DOC in TFF experiments with natural samples

Recoveries of < 100 % show that losses are greater than contamination, whereas for recoveries of >100 % contamination is greater than losses to the system (Gustafsson et al., 1996). Organic carbon recoveries of natural samples using the Centramate TFF system were 70 to 132 % for all experiments (Tab. 4.1). The recoveries of all samples independent of ionic strength or DOC content differ on average by less than ± 15 % when using 50 or 1 kDa membrane and by ± 20 % using 5 kDa membrane. The mass balance in other TFF studies showed a similar range of recoveries (Guéguen et al., 2002; Wilding et al., 2005; Kottelat et al., 2008).

The 50 kDa membrane showed a positive linear correlation between recovery and salinity (R^2 = 0.94). There was no linear correlation between recovery and organic carbon content of the sample (R^2 = 0.07). The recovery of different freshwater and low salinity samples showed that the higher the DOC content the better the OC recovery. All these samples had OC recoveries < 100 %. Samples with high salinity instead had OC recoveries > 100 %. High salinity samples with low DOC showed higher recoveries than high salinity samples with high DOC (R^2 = 0.63). Membrane interaction was obviously depending on the ionic strength of the sample, whereby samples with high ionic strength (estuarine samples) showed poorer efficiencies, as also found in the study by Guéguen et al. (2002).

The 5 kDa membrane also showed a clear correlation between recovery and salinity in the high salinity samples (R^2 = 0.94). DOC and OC recoveries of high salinity samples correlated (R^2 = 0.71) similarly to the 50 kDa system. In fresh and low salinity water samples, OC recoveries ranged from 70 to 130 %. Fresh and low salinity water samples with low DOC content were very close to optimal recovery whereas samples with more than 10 mg/l caused important losses of organic carbon to the system. Low salinity and high DOC waters enhance material adsorption to TFF membrane. One exception is the freshwater sample with a DOC of 6.1 mg/l with a very high OC recovery, just explainable by contamination of the system.

5 and 1 kDa membranes retained the natural colloidal OC in a similar manner, whereas the overall performance of the 1 kDa was better than the 5 kDa membrane. The 1 kDa membrane showed a great range of OC recoveries from 77 to 108 % in the fresh and low salinity water samples and from 83 to 118 % in the high salinity samples.

In principle, losses of colloids to the TFF system could occur either through a hydrodynamic effect, such as concentration polarisation, or through chemical interaction between the macromolecule and the membrane surface (sorptive losses). Thus, a high CFR is a preferable mode of operation to enable a substantial tangential flow ‘self-cleaning’, as higher CFR

imply lower linear permeate flow velocity against which colloids needs to diffuse (Larsson et al., 2002). Even though the CFR was much lower than 15 when using the 50 kDa system, recoveries were acceptable.

Tab. 4.1 *Different types of natural samples (Salinity, initial DOC concentration of prefiltered water) and their mass balance (Recovery) when fractionated with a Centramate tangential flow filtration system with Omega membranes of different nominal molecular weight cut-offs: 50 kDa, 5 kDa and 1 kDa.*

Sample	Sal	DOC (mg/l)	Recov (%) with NMWCO		
		PFW	50 kDa	5 kDa	1 kDa
Pond	0	22.5	89.9	80.0	108.3
River	0	6.1	81.0	129.8	106.3
River	0	3.6	78.4	100.7	76.6
Estuary	1.1	4.1	83.9	107.4	103.0
Estuary	2.4	11.5	82.9	70.2	97.4
Mix pond & SW	18	10.6	103.5	93.6	83.1
Estuary	23.2	3.8	105.6	111.2	117.5
Estuary	23.5	4.1	108.5	102.0	91.6
Estuary	35.4	1.5	115.6	132.2	107.5

NMWCO = Nominal molecular weight cut-off; PFW = prefiltered water; SW = seawater

Membrane fouling and cake formation occur, especially at high colloid concentration and high cf, when retained particles build up on the membrane surface and pores clog. Indicators are a relation between recovery and organic carbon in the retentate fractions and a decreasing permeate flow. In our experiments with natural samples, the permeate flow was relatively constant during TFF processing, suggesting the proper functioning of the Centramate membranes. Hence, the established TFF process variables and cleaning protocol for the Centramate system (though time consuming - 45 minutes for the 50 kDa, 75 min for the 5 and 1 kDa membranes) are very efficient (low blank) and provide good separation and low contamination.

4.4 Conclusions

It is difficult to directly compare results on colloid fractions between different studies because of the absence of standardised operating conditions. In this study we successfully established an operating protocol to enable comparable studies of colloids in aquatic environments in the future.

Therefore, the integrity of TFF using a PALL Centramate system for sampling natural organic colloids has been assessed. The performance of TFF membranes was tested on natural samples with a large range of DOC (1.5 – 22.5 mg/l) and ionic strength (salinity 0 - 35). These physico-chemical parameters can alter recoveries in TFF. The used TFF protocol (cleaning, conditioning, concentration factor, cross flow rate) is reliable and efficient for separating natural organic colloids from fresh and estuarine waters.

System cleaning requires time and large volumes of reagents and clean water in order to prevent cross-contamination. This has to be considered before application in the field, especially when more than one membrane for size fractionation per sample should be used. Membranes should further be periodically verified for integrity and cut-off with standard colloids as the actual membrane MWCO does not necessarily confirm the nominal cut-off provided by manufacturers.

5 Composition of dissolved and colloidal organic matter in the sugar cane impacted Manguaba estuarine-lagoon, NE-Brazil

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Biogeochemistry (submitted)

Abstract

We studied the dry season's elemental (DOC/DON) and carbon isotopic (δ^{13}C-DOC) composition of size fractionated DOM in the brackish (Sal 0-10) Manguaba lagoon, which drains a sugar-cane monoculture-dominated hinterland in tropical Northeast Brazil, adjacent to the West Atlantic ocean. DOM in the lagoon's freshwater endmember is terrestrial/allochthonous. Based on its δ^{13}C, 23% of DOC are sugar-cane (C4-plant) carbon. A heavy rain flood event within the dry season multiplied DOC fluxes and shifted δ^{13}C-DOC to heavier values, reflecting addition of inputs from shallower, sugar cane enriched soil horizons to the baseflow that characterizes the dry season. From the δ^{13}C and C/N data, the elevated background DOM in the lagoon can be viewed as the sum of inputs from continuous baseflow, intermittent floods and DOM from resuspended sediments. The molecular weight distribution of DOM, changed neither with hydrology nor with salinity. The persistent pattern is ~90% low-molecular-weight (<1kDa) "truly dissolved" and ~10% high-molecular-weight (>1kDa) "colloidal" DOM, the latter almost completely as very-high-molecular-weight (>50 kDa). This pattern seems to be fixed already within the kaolinite-, Fe- and Al- rich acidic soil environment, that favours both, low colloid formation as well as colloid precipitation. In the lagoon, which has a water residence time of weeks to months, freshly produced labile colloidal DOM is presumably degraded on shorter time scales and therefore does not accumulate. The >50kDa DOM closely resembles POM (>0.45µm) in C/N and δ^{13}C and therefore is most likely a decomposition product of POM rather than an aggregate of low-molecular-weight DOM.

5.1 Introduction

Brazil is the world's major (about 40%) sugar cane producer (FAOSTAT, 2008). During the harvest season of 2010/2011, 660 million metric tons of sugar cane were produced from an agricultural area of 9.8 x 10^4 km^2 (Barros, 2010). The country's sugar cane production is split between its south-central and north-eastern regions, accounting for 89% and 11% of total production, respectively. While the cultivated watersheds of the south-central region drain into the Parana river thousands of km upstream of its delta (La Plata river mouth), sugar cane monocultures from the northeastern region can have an immediate impact on local and regional land-ocean organic matter (OM) fluxes – the subject of this study - as their watersheds drain directly into the Atlantic Ocean over comparatively short distances. In this region, where sugar cane covers more than 80% of agricultural areas (Goldemberg et al., 2008), many rivers discharge to the sea via shallow coastal lagoons, which are particularly vulnerable to natural and anthropogenic impacts, because of limited water exchange and long water residence times (Knoppers, 1994). There are 18 littoral lagoons along the Atlantic coast of the State of Alagoas (Lanza-Espino et al., 1994), where this study was done.

The manifold environmental impacts of sugar cane production have been reviewed in detail by Martinelli and Filoso (2008). Downstream sugar cane processing plants, marked physico-chemical changes of river water (Gunkel et al., 2007) and elevated DOC (Brockmeyer and Spitzy, 2011) occur. The erosion risk in a sugar cane field is quite high as sugar cane is burned and harvested each year and each 5-6 years the plants are removed and re-cultivated, exposing bare soils for months (Krusche et al., 2002). Thus, soil material is easily flushed from the fields into the water system.

The $\delta^{13}C$ signature of sugar cane (C4 plant with ~ -12 ‰) is very different from pristine vegetation (C3 plant with ~ -28 ‰) (Bernardes et al., 2004; Krusche et al., 2002; Wang et al., 2002). Soil OM $\delta^{13}C$ closely resembles the $\delta^{13}C$ of the vegetation from which it was derived, because the fractionation during decomposition is small relative to the original fractionation during C fixation (Bernoux et al., 1998). A soil survey made in the Piracicaba river basin, São Paulo state, SE-Brazil, has shown by means of $\delta^{13}C$-analysis, that after 12 and 50 yr of landcover change from pristine to sugar cane cultivation, the surface soil had ~15% and ~39% of sugar cane carbon, respectively (Vitorello et al., 1989). Similarly, Flessa et al. (2000) deduced from $\delta^{13}C$-data that after 37 years of continuous maize (a C4 plant) cropping in Halle (Germany), 15% of the total soil organic carbon in the top soil originated from maize carbon. Incorporation of C4 plant material into the aquatic system, specifically its DOM is

evidenced by an enrichment of DOM´s δ^{13}C-values (Bernardes et al., 2004; Brockmeyer and Spitzy, 2011; Wang et al., 2002).

The pool of aquatic organic matter is commonly divided into *particulate* (> 0.45 µm) and *dissolved* (< 0.45 µm) OM (Thurman, 1985; Leenheer and Croué, 2003). The dissolved pool can be further partitioned into *low molecular weight* (< 1kDa) or "truly dissolved" and *high molecular weight* (> 1 kDa) or "colloidal" (Gustafsson and Gschwend, 1997). This partitioning within DOM is relevant in terms of the DOM´s bioavailability (Amon and Benner, 1996) and its pollutant transport potential (Wilding et al., 2005). The relative abundance of colloids within the DOM pool varies widely (10 - 80%) among various aquatic environments (Dai and Benitez-Nelson, 2001; Gueguen et al., 2002; Guo et al., 1994; Guo and Santschi, 1996), with freshwaters generally having higher abundances than marine waters. Within the freshwater domain, abundances are highly variable as well. For example, colloids were on average 76 % of the total DOM in the pristine tropical Amazon River system and its tributaries (Benner and Hedges, 1993), while only 20 % on average in watersheds of the Amazon basin that were transformed from pristine forest to pasture (Bernardes et al., 2004).

In this study, we investigated elemental (DOC, DON) and carbon isotopic (δ^{13}C-DOC) composition of bulk and molecular weight fractionated DOM in the river water - brackish lagoon water transition zone of the Manguaba lagoon system (NE-Brazil). This lagoon is surrounded by extensive sugar cane fields and therefore was expected to display compositional features of DOM specific for this kind of expanding land use. Our principal aim was to establish the dissolved versus colloid partitioning of DOM and to quantitatively trace the incorporation of sugar cane carbon into DOM and its fractions in this specific aquatic environment. The work was part of the bilateral joint Brazilian/ German research project POLCAMAR, dealing with the impact of sugar cane monoculture upon adjacent estuarine systems.

5.2 Materials and methods

5.2.1 Study site

The Manguaba lagoon (A = 43 km^2) is a shallow (Z_m = 2.1 m, Z_{max} = 3.9 m), tropical, oligohaline (Sal 0 – 10), coastal lagoon in the State of Alagoas, NE-Brazil (Lat. 9^o 35' S, Long. 35^o 44' W, Fig. 5.1). Its watershed is comprised of the catchments of the Paraiba do

Meio (PdM; A_B = 3300 km^2) and Sumauma (A_B = 372 km^2) rivers whose average annual fresh water discharge into the lagoon is 17.6 m^3s^{-1} and 5 m^3s^{-1}, respectively. This lagoon has a long water residence time of several weeks and discharges to the sea through a mangrove lined channel system which dissipates 98 % of the semi-diurnal tide's energy, thereby strongly dampening the flux of salt water from the coastal ocean into the lagoon (Oliveira and Kjerfve, 1993; de Souza et al., 2002).

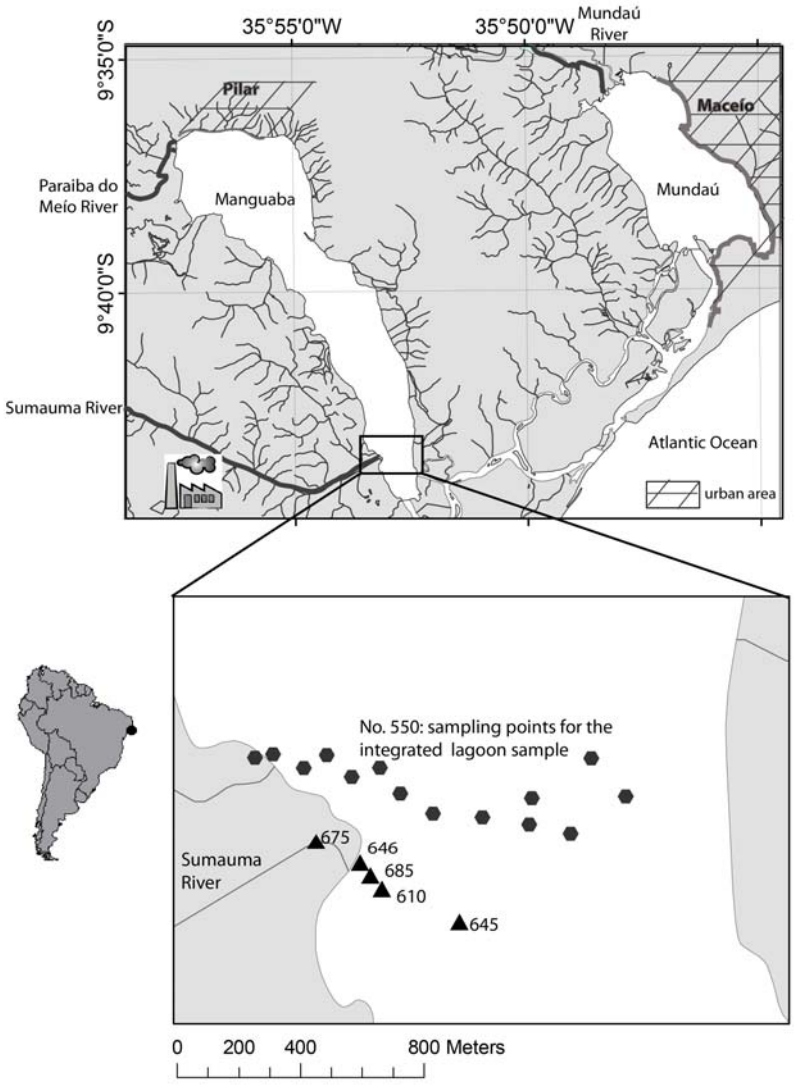

Fig. 5.1 *Study site (Mundaú-Manguaba coastal lagoon system) with location of samples collected in February 2008.*

The upper PdM subbasin (75% of the PdM catchment's total area) is hilly/ mountainous, with a maximum altitude of 1000 m at its head in the State of Pernambuco. Adjacent to the lagoon

are the lower, seaward subbasin of the PdM (25% of the PdM catchment's total area) and the catchment of the Sumauma (100%). They are part of an extended coastal plain - a late Tertiary plateau ('Barreiras Formation') of 50 to 100 m in height, covered with Fe- and Al-rich dark-yellow Latossols (Boulet et al., 1998, Dematte et al., 1996).

The watershed's climate is governed by a marked spatial and temporal variability. Its upper basin is semi-arid (Köppen Type Bhw), with an annual average precipitation of 800 mm, and the lower basin is tropical humid (Köppen Type As'), with a precipitation (P) of 1654 mm and distinct dry summer (November to March, P_{max} = 34 mm) and wet winter (May to August, P_{max} = 254 mm) conditions. The variability in climatic forcing between the rainy and dry periods leads to a ratio of maximum to minimum freshwater discharge to the lagoons that exceeds 100. The maximum monthly average water temperature of the lagoons is 31^{o}C in the dry season and 25^{o}C in the wet season (Oliveira and Kjerfve, 1993).

The major sources of surface water pollution are linked to the cultivation of sugar cane fields and to the waste effluents from the sugar cane processing plants along the rivers Paraiba do Meio and Sumauma (ANA, 2005). The sugar cane fields of the lower PdM subbasin (sugar cane on 36% of total area) and the Sumauma catchment (sugar cane on 46% of total area) taken together represent 95% of the area cultivated with sugar cane in the watershed of the lagoon (Sos, unpublished).

Water pollution during the harvest season (December to March) is characterized by irregular events of point source organic matter inputs from the sugar cane processing industries, discharging fructose rich wash water and waste products (Vinasse). Water pollution during the wet season is characterized by diffuse inputs of agrochemicals, metals and eroded organic matter from the soils that are flushed into the lagoon via drainages and rivers (Costa et al., 2011; Maioli et al., 2010).

5.2.2 Sample collection and isolation of colloid fractions

Water samples were collected in February 2008, at the end of the dry/ harvest season (Fig. 5.1). Five individual samples were taken along a transect at the river mouth of Sumauma river discharging into the Manguaba lagoon. The lagoon background was represented by an integrated sample covering a transect across the Manguaba lagoon outside the immediate influence of the Sumauma inflow. All samples, except sample no. 685, were taken on dates when there had been no precipitation for at least the past 4 days. Sample no. 685 was taken

towards the end of a heavy rain and storm event (20.6 mm/day). The time consuming process of ultrafiltration limited sampling to one sample per day.

Water samples were filled into 20 l carboys (rinsed with ambient water before final sample was collected) and transferred to the laboratory for filtration and ultrafiltration. Filtration of particles was done with a 20 l stainless steel pressure vessel (Sartorius) connected to a stainless steel disc filter holder (S&S Jürgens) equipped with a precombusted GFF filter of 0.45µm pore size and 14.2 cm diameter (Whatman). To minimise contaminations the first 3 l of the filtrate were discarded. Filters were frozen (-20 °C). Filtrates were subsequently ultrafiltered under natural pH. Filtrates for DOC, TDN and carbon isotope analysis were acidified to pH ~ 2 with 85 % H_3PO_4 and frozen (-20 °C). Water samples for dissolved inorganic nitrogen (NO_2^-, NO_3^-, NH_4^+) were filtered through single use membrane filters (0.45 µm pore size) into prewashed PE bottles and fixed with $HgCl_2$ solution.

Ultrafiltration of samples was carried out with two Centramate Omega PES cassette membranes (PALL) of cut-off sizes 1 kDa and 50 kDa. We used a parallel filtration scheme to avoid accumulation of sorptive losses to membranes and carryover of contamination. The used ultrafiltration system and process protocols are described in detail in Schwalger and Spitzy (2009). Briefly, the recirculation mode, where both, permeate and retentate flow back into the reservoir, was used for system cleaning (ultrapure water, 4% citric acid and 0.1 N NaOH) and membrane preconditioning with natural sample. The concentration mode, where permeate is collected while retentate is recycled, was used for colloid isolation and concentration. The concentration factor, defined as the ratio of the initial sample volume to the retentate volume, was 20.

5.2.3 Sample definitions/terminology

Unfractionated and fractionated samples are defined as follows:

Bulk, DOC (dissolved organic carbon) = GFF filtrate (< 0.45µm)

Colloidal, COM (colloidal organic matter) = Retentate 1 kDa (1 kDa < colloidal < 0.45µm)

LMW (low molecular weight) = Permeate 1 kDa (< 1 kDa)

VHMW (very high molecular weight) = Retentate 50 kDa (50 kDa < VHMW < 0.45µm)

HMW (high molecular weight) = 1 kDa < HMW < 50 kDa

POC (particulate organic carbon) = > 0.45µm

TOC (total organic carbon) = Bulk DOC + POC

Data for bulk, colloidal, LMW and VHMW samples were obtained by analysis of filtrate, retentate and permeate as defined above.
Data for the HMW fraction were computed: HMW = Colloidal -VHMW

5.2.4 Sample analysis

DOC and TDN concentrations of all water samples were measured using a high temperature catalytic oxidation analyser (Shimadzu TOC-V) with a Pt catalyst at 730 °C, coupled with a TNM-1 total nitrogen measuring unit. Acidified samples were purged for 5 min to remove inorganic carbon prior to analysis. Synthetic air was used as carrier gas in the TOC-TDN analyser. Standards (potassium hydrogen phthalate, potassium nitrate) were analysed immediately prior to and after analysis of 10 samples and were prepared with ultrapure water from a Microlab-Genpure system (TKA, Germany). The detection limit was found at 0.02 mg l^{-1}. All samples were analysed in triplicate. Precision, in terms of the relative standard deviation, was better than 2%.
Dissolved inorganic nitrogen was analysed using a continuous flow analysing system (Skalar SAN++System). Nitrate + Nitrite (NOx-) were detected spectrophotometrically and ammonium (NH_4^+) fluorometrically as coloured / fluorescence dye (Grasshoff et al., 1999). Dissolved inorganic nitrogen (DIN) is the sum of NOx- and NH_4^+. Determination limits were 0.08 µM for NOx- and 0.05 µM for NH_4^+ according to Deutsches Institut für Normung (DIN 32645). The coefficient of variation of the procedure was <3.4%. DIN measurements from each sample were substracted from TDN measurements to determine DON content.
For POC measurements (excluding samples 550 and 675) the filter material was dried at 40 °C, acidified with 1 N HCL to remove inorganic carbon and dried again at 40 °C. Total carbon was subsequent analysed in a Carlo Erba NA 2100 elemental analyser, where the samples were oxidized at 1100 °C and the oxidation products were transported by a carrier gas (He). After removing water and halogens, the evolving CO_2 was quantified by a thermal conductivity detector. The relative standard deviation for the method was ± 4.5 %.
The stable carbon isotope ratio of particulate organic carbon (δ^{13}C-POC) was determined according to Ertl and Spitzy (2004), involving sealed tube combustion of the sample, cryogenic trapping of CO_2, and isotope ratio mass spectrometry by a Finnigan Mat 252 – dual-inlet system.
δ^{13}C-DOC of aqueous samples was determined analogously as concerns cryogenic trapping and isotope ratio measurement (MAT 252 – dual-inlet). Before trapping, a 20 ml sample was

combusted by continuous injection (0.85 ml min^{-1}) in a Helium stream into a self assembled high temperature catalytic oxidation unit, consisting of a furnace heated to 950 °C and a quartz glass column filled with copper oxide and cerium oxide. Combustion gases were dried by Peltier coolers and a magnesia perchlorate trap. This unit was coupled on-line to the cryogenic trap.

$\delta^{13}C$ values were obtained from at least duplicate analyses and referenced to the Vienna Peedee Belemnite (V-PDB) standard:

$$^{13}C\ (‰) = [^{13}C/^{12}C_{(sample)} / {}^{13}C/^{12}C_{(standard)} - 1] * 1000 \qquad \text{Eq. 5.1}$$

The standard deviation of both methods was better than 0.5 ‰.

5.3 Results

5.3.1 Bulk organic matter

Freshwater DOC in the Sumauma river was 3.6 mg/l, with a $\delta^{13}C$-DOC of -24.2 ‰. At dry sampling days, DOC increased linearly with salinity ($R^2 = 0.92$) up to a lagoon value of 10.2 mg/l (Sal 7.4) (Fig. 5.2; Tab. 5.1).

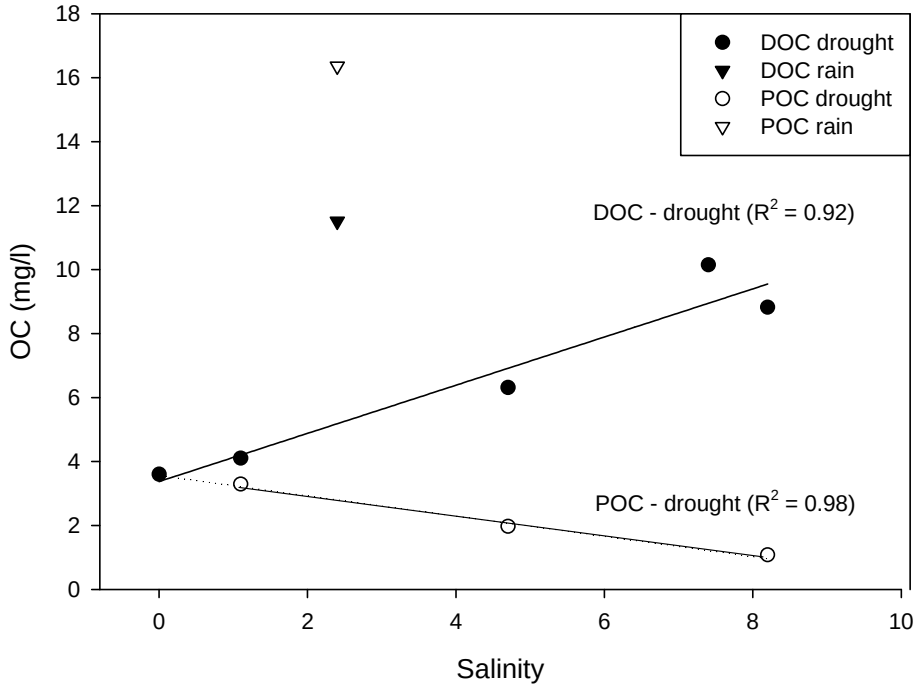

Fig. 5.2 *POC (white) and DOC (black) in samples of different salinities and hydrological conditions.*

Tab. 5.1 *Dissolved nitrogen and C/N-molar ratio of dissolved organic matter of bulk and fractionated samples. Dissolved organic nitrogen (DON) is computed as total dissolved nitrogen (TDN) minus dissolved inorganic nitrogen (DIN). In fractions > 1kDa (colloidal, HMW, VHMW), DIN is assumed to be zero and therefore DON = TDN.*

Sample		DIN (mg/l)	TDN (mg/l)			DON (mg/l)		DOC (mg/l)				DOC/DON (molar ratio)			
		Bulk	Bulk	Coll	VHMW	Bulk	LMW	Bulk	Coll	LMW	VHMW	Bulk	Coll	LMW	VHMW
Polca 675		0.03	0.10	0.018	0.018	0.07	0.047	3.6	0.20	2.6	0.2	64.6	13.2	64.2	13.1
Polca 646		0.05	0.27	0.017	0.017	0.22	0.201	4.1	0.26	4.0	0.2	21.9	17.8	23.0	11.8
Polca 610	dry	0.02	0.26	0.074	0.028	0.24	0.164	6.3	0.34	4.9	0.3	31.0	5.3	34.9	13.9
Polca 550		0.07	0.46	0.068	0.064	0.39	0.327	10.2	0.82	5.6	0.7	29.9	14.1	19.9	12.4
Polca 645		0.11	0.40	0.034	0.052	0.29	0.249	8.8	0.41	5.0	0.4	36.3	14.1	23.5	9.0
Polca 685	rain	0.04	0.39	0.046	0.055	0.35	0.303	11.5	1.01	10.2	0.4	38.4	25.6	39.2	9.5

Bulk = < 0.45µm

LMW (Low molecular weight) = < 1kDa

COM (Colloidal organic matter) = 1 kDa – 0.45 µm

VHMW (Very high molecular weight) = 50 kDa – 0.45 µm

DON LMW = DON bulk – TDNcolloidal

δ^{13}C-DOC was enriched in brackish samples (avg -22.7 ‰), but showed no trend with salinity (Tab. 5.3). Sample 685 (Sal 2.4), taken after heavy rain, is an exception, having the highest and most ^{13}C-enriched DOC (11.5 mg/l; -19.6 ‰) of all samples.
POC concentrations decreased linearly with salinity (R^2 = 0.98) and varied from 1.1 to 3.3 mg/l, comprising 11 - 45% (average 26 ± 17%) of TOC. An exception is sample 685, which had 16.4 mg/l of POC (Fig. 5.2; Tab. 5.2), comprising 59% of TOC. ^{13}C-POC ranged from -21.4 to -24 ‰ (Tab. 5.3) and correlates neither with salinity nor POC. The sample most enriched in ^{13}C-POC was the integrated lagoon sample (550).
The C/N ratio of the dissolved organic compounds (DOC/DON) was 65 for the Sumauma river and 30 ± 6 for the lagoon samples (Fig. 5.3; Tab. 5.1). The C/N ratio of POM was 11.8 for the Sumauma river and between 7.7 – 10.6 for the lagoon samples. C/N of surface sediments was 9.9 ± 1.2 and for soils 19.4 ± 4.

5.3.2 Partitioning of dissolved organic matter

Organic carbon recoveries (as percentage of the initial DOC) during ultrafiltration were between 77 and 106% for the 1 kDa membrane and between 72 and 84% for the 50 kDa membrane. An exception was sample 550, with a recovery of 63% for the 1 kDa and 61 % for the 50 kDa. Apparently, lagoon samples with high DOC and higher amount of phytoplankton exudates are susceptible to material losses to the membrane. Similar mass balances and ranges of recovery were reported in e.g. Guéguen et al. (2002) and Wilding et al. (2005).
Within bulk DOC, VHMW-DOC comprised between 4.0 - 10.5% (avg 6.6%) and HMW-DOC between 0 - 5% (avg. 1.6%). The LMW-DOC accounted for the remaining 87.3 - 94.5% (avg = 92.1%) (Fig. 5.4; Tab. 5.2). The colloid fraction consisted mainly of VHMW-DOC (66 to 100%, except for sample 685 with 44%). The percentages of colloidal and LMW ("truly dissolved") material remained relatively constant, in spite of the pronounced variability in DOC and salinities (Fig. 5.4).

Tab. 5.2 *Carbon content of particulate, bulk dissolved and fractionated dissolved organic matter.*

Sample		Sal	POC (mg/l)	C/N molar ratio Particulates	% in DOC Coll	LMW	HMW	VHMW
Polca 675		0.0	n.d.	n.d.	7.3	93.8	0.0	7.3
Polca 646		1.1	3.3	9.4	6.2	93.8	2.1	4.1
Polca 610	dry	4.7	2.0	9.1	6.5	94.5	0.0	6.5
Polca 550		7.4	n.d.	8.5	12.8	87.2	2.3	10.5
Polca 645		8.2	1.1	n.d	7.6	92.4	0.1	7.4
Polca 685	rain	2.4	16.4	n.d.	9.0	91.0	5.0	4.0

n.d. = not determined

POC (Particulate organic carbon) = > 0.45 µm

DOC (Dissolved organic carbon) = < 0.45µm

Coll (colloidal) = 1 kDa – 0.45 µm

LMW (Low molecular weight) = < 1kDa

HMW (High molecular weight) = 1 kDa – 50 kDa

VHMW (Very high molecular weight) = 50 kDa – 0.45 µm

5.3.3 $\delta^{13}C$ and C/N in size fractionated organic matter

During drought, $\delta^{13}C$ values ranged from -21.1 to -24.6 ‰ in LMW, -22.8 to -24.8 ‰ in COM, -20.8 to -24.0 ‰ in VHMW and -21.4 to -24.0 ‰ in POC (Tab. 5.3). The single most depleted value was found in the colloidal fraction of the freshwater end member sample (675) of the Sumauma river (-24.8 ‰). The sample collected after a heavy rain (685) had the single most enriched $\delta^{13}C$ value (-18.8 ‰) in the LMW fraction. In this sample POM had a $\delta^{13}C$ value of -22.8‰ (Tab. 5.3), very close to the value of VHMW-DOM (-23.4 ‰). While $\delta^{13}C$ of bulk DOC and its LMW-fraction are shifted to heavier values in the rain event, this is not the case in $\delta^{13}C$ of POM and VHMW-DOM (Fig. 5.3).

In general, DOC/DON in the LMW fraction was higher compared to the colloidal fraction (Tab. 5.1). In LMW it decreased from river (64) to lagoon (20 -35) in the dry situation (Fig. 5.3; Tab. 5.1), while sample 685 from the rain event had an elevated DOC/DON of 39. DOC/DON of the VHMW fraction, representing most of the colloidal fraction, ranged between 9 and 14 (including the sample after storm) and showed no trend in DOC/DON with

changing salinity. DOC/DON of the VHMW fraction and C/N of POM (Fig. 5.3) did not respond to the rain event.

Tab.5.3 $\delta^{13}C$ *of particulate, colloidal and dissolved organic matter*

Sample		Sal	$\delta^{13}C$-POC (‰)	$\delta^{13}C$-DOM (‰)				
				Bulk	LMW	Coll	VHMW	comments
Polca 675		0	-23.6	-24.2	-24.6	-24.8	-24.0	river, dry
Polca 646		1.1	-22.2	-22.2	-21.1	-22.2	-23.4	river plume, dry
Polca 610	dry	4.7	-24.0	-23.5	-24.1	-22.5	-23.4	river plume, dry
Polca 550		7.4	-21.4	-22.5	-22.8	-23.3	-20.8	lagoon, dry
Polca 645		8.2	-22.1	-22.7	-24.2	-22.1	-22.6	lagoon, dry
Polca 685	rain	2.4	-22.8	-19.6	-18.8	-19.6	-23.4	river plume, rain

POC = Particulate organic carbon
Bulk = Total dissolved organic matter
LMW (Low molecular weight) = < 1kDa
Coll (Colloidal) = 1 kDa – 0.45 µm
VHMW (Very high molecular weight) = 50 kDa – 0.45 µm

5.4 Discussion

DOM in the lagoon system is composed of material that is: i) advected from the sea, ii) advected by rivers, iii) added locally from phytoplankton and iv) added locally from photolysed OM from resuspended bottom sediment. In rivers, phytoplankton production is negligible (chlorophyll a ~ 4mg m^{-3}) (Wolf et al., 2010) and DOM hence derived from soil, whose OM is a mix of material derived from pristine vegetation and from sugar cane. In a previous work (Brockmeyer and Spitzy, 2011), the lagoon phytoplankton production has been estimated to contribute to less than 10% of DOC and the following end member $\delta^{13}C$ values have been identified:

- Sugar cane: $\delta^{13}C$ = -11.8 ‰
- marine DOM: $\delta^{13}C$ = -21.0 ‰
- lagoon bottom sedimentary OM: $\delta^{13}C$ = -20.2 ‰
- phytoplankton: $\delta^{13}C$ = -17.1 ‰

- sugar field surface soil OM: $\delta^{13}C$ = -14.2 to -19.6 ‰ (avg = -17.1 ‰)
- drainage: $\delta^{13}C$ = -22.9 ‰
- pristine surface soil OM/ vegetation: $\delta^{13}C$ = - 28 ‰

Due to the ~16 ‰ spread in $\delta^{13}C$ between pristine soil/ vegetation and sugar cane, the sugar cane derived carbon in OM of soil and river samples can be quantitatively assessed by:

$$\delta^{13}C_{Sample} = \delta^{13}C_{pristine} * (1-X) + \delta^{13}C_{sugar\ cane} * X \quad \text{Eq. 5.2,}$$

where X is the fraction of organic C from sugar cane. A one per mil shift from the pristine value of -28 ‰ to heavier values corresponds to a 6.25% addition of sugar cane carbon, with e.g. a 50:50 mix having a $\delta^{13}C$ value of -20 ‰.

5.4.1 Bulk organic matter

5.4.1.1 Freshwater endmember

The riverine freshwater end member was sampled once (sample 675), after an uninterrupted dry spell of 10 days (no rain event sample available from within the river for comparison). It has low DOC, depleted $\delta^{13}C$ and high C/N (Tab. 5.1, Tab. 5.3, Fig. 5.3), all consistent with exclusively baseflow feeding the river. Baseflow flushes DOC-impoverished deep soil horizons. Within the soil, sugar cane residues become depleted in $\delta^{13}C$ during decomposition, as a result of lignin enrichment, discrimination against ^{13}C by microorganism and dilution of the residues with admixed soil materials (Spain and le Feuvre, 1997). In sugar cane fields, substantial depletion of $\delta^{13}C$ (from -16 to -24 ‰) within the soil profiles first meter has been observed by Spain and le Feuvre (1997). Accordingly, Krusche et al. (2002) found the most depleted $\delta^{13}C$ values in riverine samples during drought when DOC percolates through the entire soil column, before entering the river. The observed $\delta^{13}C$ value in our study translates into 23% contribution of sugar cane C to bulk DOC (Eq. 2), which is within the range of data (15 - 35%) from a previous year (Brockmeyer and Spitzy, 2011) and at the upper limit of data (23%) from the Florida Everglades (Wang et al., 2002).

The C/N of sugar cane, local soil OM and humic substances dissolved in groundwater is 100 (Ilokur and Oluka, 1995), 19 (this study) and 66 (Thurman, 1985), respectively. The latter compares well with the DOC/DON = 65 of the freshwater end member sample 675 (Tab. 5.1; Fig. 5.3).

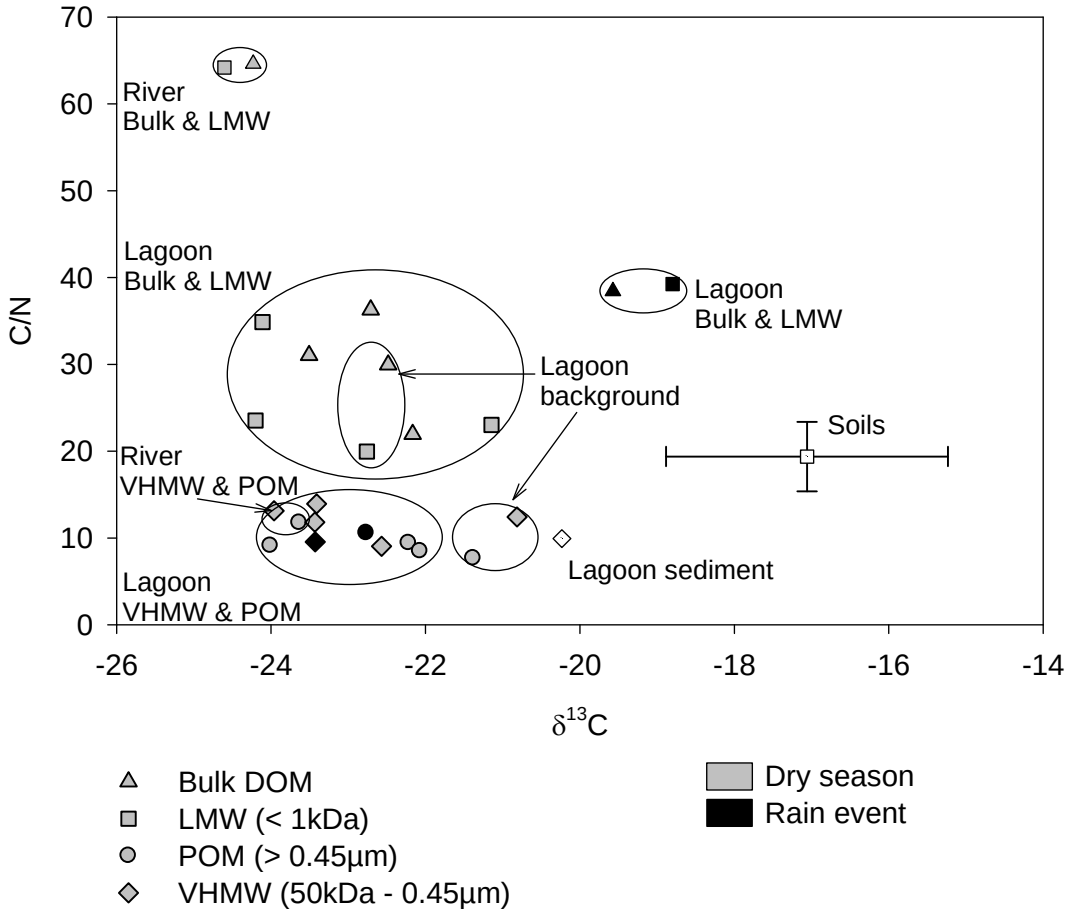

Fig. 5.3 *C/N ratio versus $\delta^{13}C$ of bulk dissolved organic (triangel), low molecular weight (< 1kDa, square), very high molecular weight (50kDa – 0.45µm, diamond) and particulate/ suspended organic matter (>0.45µm, circle). Samples taken in the dry season are symbolized by grey colour and samples taken in a rain event in black.*

The $\delta^{13}C$ and C/N of the particulate phase of this sample fall within the range of data from the brackish water samples (see below). The relatively low C/N value (11.8) compared to soil Corg (C/N ~ 18) would suggest substantial phytoplankton input, which, however is unlikely given the low Chl-a values. Alternative sources of nitrogen might be degradation of bacteria (C/N ≥ 4.8) attached to particles, or untreated domestic effluents of the small settlements along the river.

5.4.1.2 Brackish water

As the freshwater from the river end member mixes into the brackish lagoon, DOC increases significantly (Fig. 5.2), due to conservative mixing with high DOC of the lagoon background represented by the integrated sample 550. Within the lagoon, DOC and δ^{13}C-DOC are consistent with average lagoon values of 2007 (Brockmeyer and Spitzy, 2011). δ^{13}C-POC is in the same range as δ^{13}C-DOC, with the exception of sample 550 where it is enriched by added phytoplankton material.

Dry conditions are generally characterised by a ratio of DOC/POC >1. This ratio is reversed in the rainy storm event (POC = 59% of TOC). The 3-fold DOC and 5-fold POC concentrations during this event result from overland flow added to enhanced baseflow. The additional input from OM- and δ^{13}C- enriched horizons, is evident in the observed δ^{13}C-DOM increase by 2 - 3 ‰. Similar patterns of variation in OM-δ^{13}C linked to varying hydrological conditions (high water → δ^{13}C enrichment, low water → δ^{13}C depletion) have been reported from brazilian sugar cane impacted catchments by Martinelli et al. (1999) and Krusche et al. (2002). The hydrological driven enrichment in δ^{13}C was also observed by Dalzell et al. (2005) for corn residues. However, POC changed neither in δ^{13}C nor in C/N, indicating a uniform erosion source of river suspension in dry and rainy situations, with suspended material going through cycles of sedimentation and resuspension in the drainage and river channels. If the area specific erosion rate were uniform over the entire catchment area, than, from the 13% proportion of sugar cane plantation and the δ^{13}C soil data, an integrated river POC δ^{13}C of -26.7% would result. The heavier values observed, in fact, reflect higher area specific erosion rates for the cultivated terrain.

5.4.2 Fractionated organic matter

5.4.2.1 Molecular weight distribution

In our study the molecular weight distribution was relatively uniform, independent of changes in DOC, hydrology and salinity (Fig. 5.4). Similarly uniform molecular weight spectra of DOM under varying hydrological conditions and salinities have been reported previously (Dalzell et al., 2005; Argyrou et al., 1997). In pristine, podzolisation areas, such as the Amazon river basin, DOC is mainly composed of HMW (> 1 kDa) compounds (Benner and Hedges, 1993; Eyrolle et al., 1996). In other tropical environments, e.g. the Paraná area,

where rainforest was replaced by pasture, sugar-cane, coffee or secondary forests, the major part of DOC is also concentrated in the lowest MW fraction (Eyrolle et al., 1996). Similarly, LMW-DOC accounts for more than 50 % of total DOC in the Everglades, where sugar cane carbon represents a significant fraction of DOC (Wang et al., 2002). LMW-DOC enrichment by agriculture was also reported by Cronan et al. (1999) for a Maine river basin (USA), consistent with Wilson and Xenopoulos (2009), who found a decrease in structural complexity of DOM as a result of agricultural land use in Ontario (Canada). The characteristic feature of the DOM molecular weight spectra in our study is the surprisingly small contribution of colloids to DOM: less than 10% on average, leaving more than 90% as "truly dissolved". The colloids, in turn, are dominated by the VHMW fraction (on average 90%).

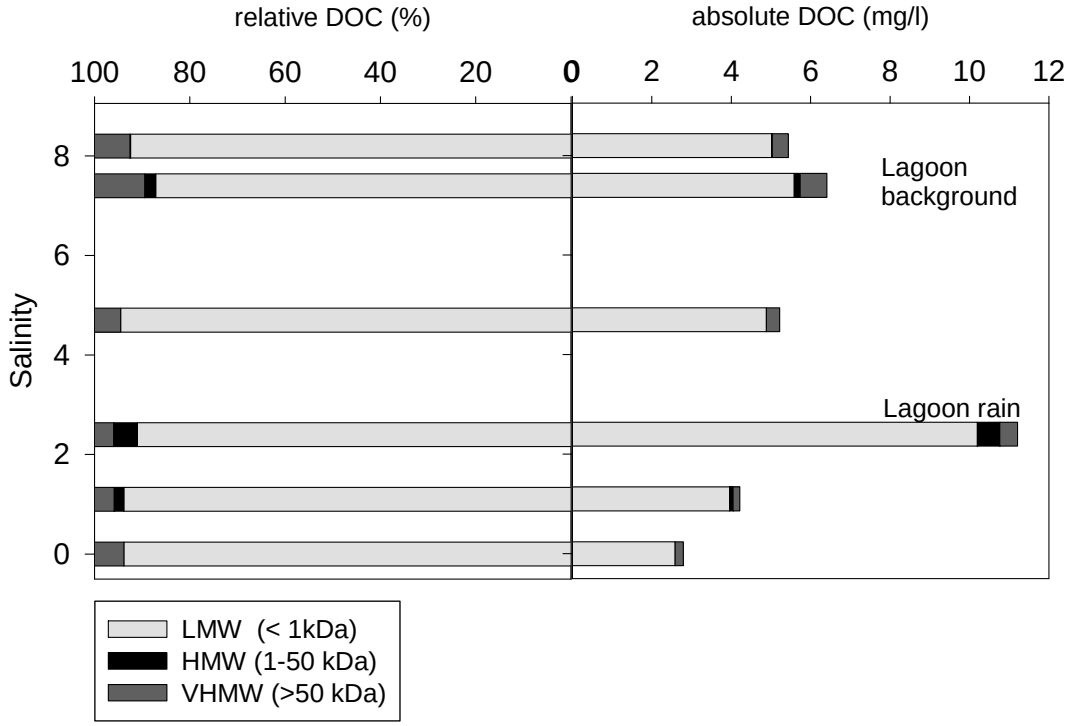

Fig. 5.4 *Dissolved-colloidal partitioning of DOC in the Manguaba river-lagoon-system, according to low molecular weight LMW (< 1 kDa), high molecular HMW* (1 - 50 kDa) and very high molecular weight VHMW (50 kDa - 0.45 µm). *HMW is calculated by subtracting VHMW (50kDa-0.45µm) from COM (1kDa-0.45µm).*

Autochthonous, phytoplankton derived DOM colloids from the lagoon proper decompose within days (Amon and Benner, 1996) and do not accumulate. DOM in the lagoon therefore is predominantly allochthonous. Consequently, its size distribution is already set within the freshwater system. Rivers receive their waters from baseflow, interflow and surface runoff.

Hence, during low flow conditions, which apply for the end of the dry season, baseflow feeds the river with groundwater whose DOM concentration and quality is controlled by processes within the soil profile, discussed in the following paragraph.

5.4.2.2 Soil processes

Within the soil, the DOM balance results from diverse processes such as dissolution of OM from the solid matrix, adsorption to mineral surfaces (clay minerals, aluminium and iron hydroxides) and degradation by microbes (Kaiser and Guggenberger, 2000; Thurman, 1985). DOM is also able to bind a variety of compounds ranging from small charged compounds such as metals to e.g. lager hydrophobic substances such as pesticides and polyaromatic hydrocarbons (Nierop et al., 2002). Previous work has demonstrated that HMW-DOM preferentially binds to mineral surfaces (Guggenberger and Kaiser, 2003; Maurice et al., 2002), while LMW-DOM seeps through the soil and can be further exported by subsurface drains to streams and rivers (Guo and Chorover, 2003). Furthermore, the molecular weight distribution is influenced by soil physical parameters such as pH, ionic strength and concentrations of polyvalent ions, which even change within the soil, when soil waters pass through different horizons (Nierop et al., 2002; Riise et al., 2000; Scheel et al., 2007).
The soil type of the Manguaba lagoon hinterland is dark yellow Latosol, also called Ferrisol, which is an acidic soil (pH 4.5 - 5.3) known for its saturation with Al, Fe and their oxides (Dematte et al., 1996; Oliveira et al., 2004). The kaolinite developed from the Barreiras Group of the northeastern coastal plateaus of Brazil (Boulet et al., 1998) is a low activity clay with low cation exchange capacity and small surface area and hence only forms little soil colloids (Brady and Weil, 2007). Organic colloids in these acidic soils are instead formed by chelation of organic matter with aluminium (Al) and iron (Fe), as these are the most prominent interacting metals (Nierop et al., 2002). When the soil is saturated with these metals, colloids not only coagulate but also (co)precipitate upon increased aggregation (Riise et al., 2000; Scheel et al., 2007). The formation of precipitates is very likely in acidic soils as this depends on pH (Scheel et al., 2007). Up to 90% of the initial DOM can be removed within the soil with these processes (Nierop et al., 2002; Scheel et al., 2007). One reason for precipitation of DOM is charge neutralisation by Al, resulting in a reduced solubility of Al-OM complexes. The formation of $Al(OH)_3$ controls the solubility of Al at pH>4,2 (Scheel et al., 2007), rather than Al^{3+} at lower pH.

The low colloid concentration in allochtonous DOM of this study therefore is a consequence of the soil characteristic specific to this area. The similarity of the molecular weight spectra in baseflow vs. rainy situations can be explained by colloid precipitation taking place already in the upper soil, from where additional soil pore water drained into the river during rain events.

5.4.3 $\delta^{13}C$ and C/N within different size fractions

The enrichment of $\delta^{13}C$ by sugar cane carbon in riverine organic matter is evident across the complete molecular weight spectrum of DOM. In the lagoon LMW-DOM, making up most of the bulk DOM, becomes enriched in $\delta^{13}C$ and N. Given the weak phytoplankton source (see above), we assume that the added N is from photolysed, resuspended lagoon sediments (Kieber et al., 2006), which has a $\delta^{13}C$ of -20.2‰ and a C/N of 9.9. Accumulation of $\delta^{13}C$ rich DOM from sporadic flood events is a further potential factor for the shift to heavier $\delta^{13}C$ values, as bulk and LMW DOM responded to the short term rain flood event with a marked shift of $\delta^{13}C$ towards heavier values. As for the COM fraction, the data compare well with the -23.8 ‰ obtained by Krusche et al. (2002) in the Piracicaba basin, which is dominated by C4 plants. COM in the pristine Amazon has a $\delta^{13}C$ = -29.2 ‰ (Hedges et al., 1994). The $\delta^{13}C$ values of POM from the Manguaba freshwater inflow are within the range reported for other freshwater systems in which C4 vegetation dominates the drainage basin (Hedges et al., 1994; Krusche et al., 2002; Martinelli et al., 1999).

POM and COM consistently showed uniform $\delta^{13}C$ and C/N patterns, which do not change with hydrological situations and salinities and are distinct from those of LMW and bulk DOM (Tab. 5.3; Fig. 5.3). Hence, VHMW has a common source with POM and is most likely disintegrated POM rather than aggregated LMW-DOM. Unlike for LMW-DOM, there is evidence of phytoplankton derived OM in the POM and VHMW DOM fraction of the integrated lagoon sample (~2 ‰ more enriched $\delta^{13}C$) as compared to the other samples (Tab. 5.3).

5.5 Conclusions

OM in the Manguaba lagoon system showed a uniform molecular weight distribution independent of hydrology and salinity. Its outstanding feature is the <10% contribution of colloidal DOM, which is concentrated in the >50kDa fraction and in terms of C/N and $\delta^{13}C$

behaves like POM. These two fractions can therefore be viewed as one phase. This molecular weight pattern is set by soil processes that drive the balance of colloid formation and precipitation. Further research of these soil type dependent processes would help to predict the amount of colloidal DOM formed within the soil.

The $\delta^{13}C$ data showed that sugar cane cultivation leaves a substantial imprint on OM of the soil as well as of the DOM flushed from it, which is further exported to the lagoon system. DOM accumulating in the lagoon derives from baseflow, flood flow and from resuspension of sedimentary OM. $\delta^{13}C$ and C/N of POM and VHMW-DOM points to a common source and suggest that the VHMW fraction is a decomposition product of POM rather than an aggregate of LMW-DOM. Short term flood events changed the composition of DOM but not of POM.

6 Estimation of DOM export from a tropical estuary

6.1 Introduction

The coastal ocean covers approximately 7% (26 x 10^6 km^2) of the global ocean and the coastline extends over about 350,000 km worldwide (Bianchi, 2007). Coastal oceans represent an important link in the global carbon cycle as DOM is transported from streams, down rivers into the world´s oceans. Coastal margins receive terrestrial inputs either from numerous estuaries with little direct effects from rivers, or by large direct inputs from rivers, such as deltaic regions. These differences will have serious consequences for the amount of terrestrial material entering the coastal zone, as well as how these materials (particulate and dissolved) will be transported offshore (Bianchi, 2007).

On a global scale, riverine inputs of DOM represent about 0.25 – 0.36 Gt C per year (Spitzy and Leenheer, 1991). Due to intense physical, chemical and biological activities, the mixing zones between rivers and ocean are one of the most dynamic parts in terms of DOM transport (Cauwet, 2002). Riverine DOM is modified by these activities, such as flocculation, photooxidation and bacterial uptake, influencing the fate of DOM in the coastal zone. In addition to riverine and marine DOM, autochthonous DOM production by heterotrophic bacteria is a significant source of DOM in estuaries (Cauwet, 2002). A relatively large fraction of DOM might be degraded, with turnover times ranging from days to years. The turnover of DOM thereby is in part controlled by its residence time, which depends on the rate of riverine input and the exchange of estuarine water with the coastal ocean.

The tropical lagoon estuary system studied in this thesis, the Manguaba lagoon, has a water residence time of several months affecting the fate of DOM and sugar cane derived DOC (sDOC). To assess the importance of DOM export from sugar cane impacted coastal catchments, this chapter estimates the export of sDOC.

6.2 Estimation of DOM and sDOC export from the Manguaba lagoon

DOC, $\delta^{13}C$ and salinity data were used to calculate the fractions of OM derived from marine, riverine and sugar cane sources. Each end member was given a characteristic $\delta^{13}C$ and salinity value as described in chapter 2.

To estimate the amount of DOM that is recycled and degraded within the lagoon, a comparison between lagoon DOM and estuarine DOM from the outlet of the lagoon-estuary system was conducted. Both samples were taken in the same sampling campaign at the end of the dry season in 2008, assuming that they represent the same water masses. The integrated lagoon background sample (Chapter 5, Fig. 5.1) thereby represents the total lagoon DOM, with a DOC concentration of 10.1 mg/l at a salinity of 7.4 (Fig. 6.1). In the estuary, conservative mixing was observed in samples during a 25 hours time series at a fixed station in the outlet of the estuary, covering two tidal cycles. Extrapolating the mixing line to a salinity of 7.4 (Fig. 6.1) only 6.9 mg/l DOC are estimated. As a result a reduction from 10.1 to 6.9 mg/l was found. Thus, about one third of the DOM is degraded within the lagoon.

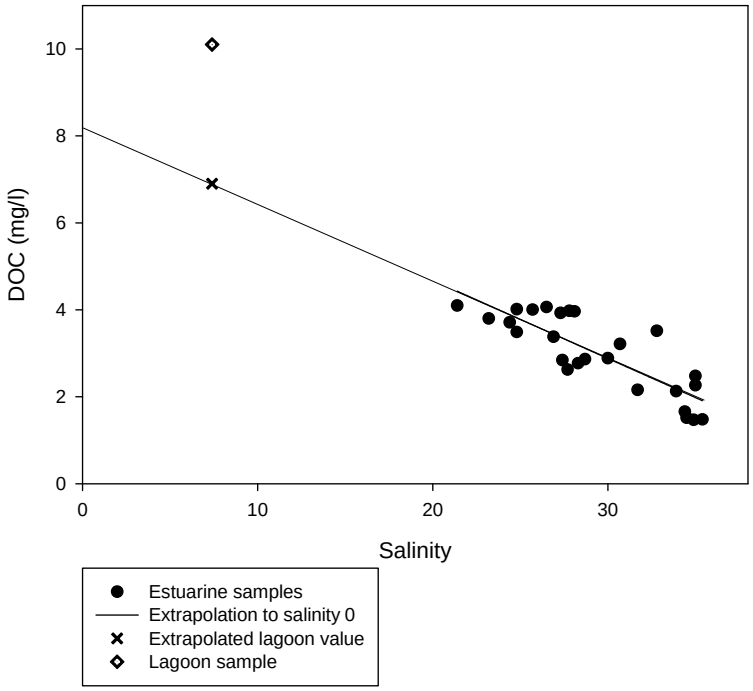

Fig. 6.1 *DOC vs. salinity from a 25 hour time series at a fixed point in the Manguaba estuary outlet and from the lagoon background samples of February 2008. The straight line represents the extrapolation of the 25 hour sampling to salinity zero. The x represents the extrapolated DOC concentration at the lagoon background sample´s salinity 7.4.*

DOC in the estuary has a riverine, phytoplankton and marine component. To derive sDOC, one has to estimate first the riverine component, as sDOC is contained only in this fraction. With other DOC components given, the riverine DOC component still present in the estuary can be calculated from the mass balance:

$$DOC_{Estuary} = \mathbf{DOC_{River}} + DOC_{Phyto} + (DOC_{Marine} * Salinity_{Estuary}/36) \qquad \text{Eq. 6.1}$$

The marine component is marine DOC at salinity 36 (1.6 mg/l), normalised to the salinity in situ. DOC_{phyto}, derived from chlorophyll α data (see Chapter 2), is in the range of 0.1 – 0.5 mg/l. From those high salinity samples for which δ^{13}C-DOC data are available (salinity 23.2, 23.5 and 35.4), a mean estuarine sample with 3.1 mg/l DOC and a salinity of 27.4 was computed. The calculated riverine fraction of DOC in the estuarine sample therefore ranges between 1.4 and 1.8 mg/l, depending on the value chosen for DOC_{phyto}.
In order to calculate the amount of sugar cane carbon within the fraction of river water that was present in the estuary, the next step involves calculation of the carbon isotope value of this river water masses DOC, using the following equation:

$$\delta^{13}C_{Sample} * DOC_{Sample} = \mathbf{\delta^{13}C_{River}} * DOC_{River} + \\ + \delta^{13}C_{Phyto} * DOC_{Phyto} + \delta^{13}C_{Marine} * DOC_{Marine} \qquad \text{Eq. 6.2}$$

The mean estuarine sample (see above) has a δ^{13}C-DOC of -22.2 ‰. $\delta^{13}C\text{-}DOC_{phyto}$ is -17.1 ‰, and $\delta^{13}C\text{-}DOC_{marine}$ is -21 ‰. DOC values are those used in equation 6.1 (see above). The resulting $\delta^{13}C\text{-}DOC_{river}$ ranges from -23.3 to -25.1 ‰, depending again on the value chosen for DOC_{phyto}.
Having obtained δ^{13}C and DOC of the estuarine samples´ riverine fraction, sDOC can be calculated from equation 2.4 (Chapter 2):

$$\delta^{13}C_{riverine\ fraction} * DOC_{riverine\ fraction} = \delta^{13}C_X * DOC_X + \\ + \delta^{13}C_A * (DOC_{riverine\ fraction} - DOC_X) \qquad \text{Eq. 2.4 ,}$$

where A represents the pristine riverine end member having a constant organic carbon isotope ratio ($\delta^{13}C_A$) of -28 ‰ and X represents the fraction of sugar cane origin having a constant $\delta^{13}C_X$ of -11.8 ‰.
As a result, DOC_{river} in the estuary consists of 18 - 29% sDOC (0.3 - 0.5 mg/l sDOC), the range depending on the phytoplankton values. Analogously, sugar cane carbon in the lagoon background sample accounts for 29 - 32% of DOC_{river} in the lagoon (equivalent to 2.6 - 3.2

mg/l sDOC, depending on a broader range of DOC_{phyto} 0.1 - 1 mg/l). The sDOC relative component of DOC_{river} is obviously very similar in river, lagoon and estuary.

The sDOC obtained numerically for an estuarine sample with a salinity of 27.4 can – assuming conservative mixing – be extrapolated to salinity 7.4, yielding a lagoonal sDOC ranging from 0.7 – 1.2 mg/l. This is ~2 mg/l lower than the actual value obtained in the lagoon. The same approach was used for DOC_{river}, yielding a DOC_{river} loss of 53 - 67% within the lagoon. Thus, a considerable fraction of DOC_{river} and hence sDOC (58 – 78%) is removed within the lagoon. The overall estimation for DOC_{river} and sDOC export from the lagoon to the estuary in the dry season is presented in Figure 6.2.

In addition to the integrated lagoon background sample, we calculated sDOC for all lagoon samples (n=6 with average Sal 6.8, DOC 8.3 mg/l and δ^{13}C-DOC -22.5‰) of the same sampling campaign (February 2008). The phytoplankton contribution to total DOC was set to a mean value of 5% resulting in sDOC of 2.4 mg/l. This value was compared with the sample at a salinity of 6.8 extrapolated from the times series, resulting in sDOC concentration of 1.7 mg/l. We also extrapolated the time series to a salinity of 0 to obtain the amount of DOC and sDOC that reaches the ocean per litre of freshwater. Each litre of freshwater that reaches the ocean thus contains 8.3 mg/l DOC and 2.3 mg/l sDOC.

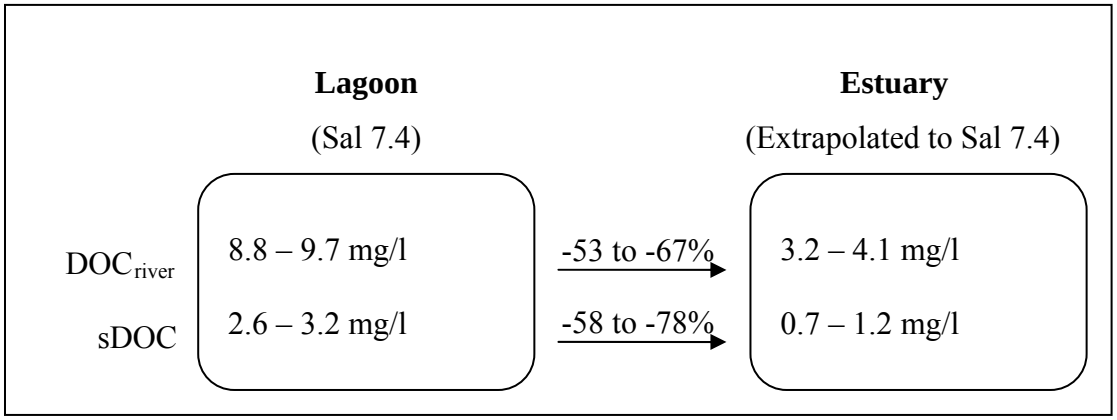

Fig. 6.2 *Estimates of lagoonal and extrapolated estuarine DOC to salinity 7.4 for the dry season: absolute (mg/l) amounts of DOC_{river} and sugar cane carbon sDOC in DOC and its relative loss from lagoon to estuary (%).*

The lagoon has a high capacity to recycle and degrade the riverine fraction of DOC, which constantly contains about one third sDOC. The removal of both, DOC_{river} and sDOC occurs predominantly during the passage of water through the lagoon. The water residence time was calculated to be 272 days, resulting from the volume of the lagoon (85.3 x 10^6 m^3) in the dry season and the average river discharge for February (3 m^3s^{-1} for the Paraiba do Meio

river)(LOICZ, 2002). As the integrated lagoon background sample was located already near the outlet of the lagoon and hence merely had to pass 1/10 of the total lagoon water volume, the water residence time for our estimations is less than one month, including the 0.7 m^3 s^{-1} from the Sumauma river. In conclusion, we observed the removal of more than half of DOC_{river} and sDOC within the lagoon within about 10% of the total lagoons residence time.

6.3 DOC export from sugar cane fields in Brazil

The DOC export from sugar cane fields in Brazil to the coastal Atlantic is roughly estimated here, as an accurate calculation of the land to ocean flux of DOC from sugar cane plantations in Brazil is hampered by the lack of data. The non-coastal catchments, which account for 73.2% of Brazil's sugar harvesting area, drain to the vast Parana-Uruguay catchment (Goldemberg et al, 2008; UNICA, 2010). From there, waters move thousands of kilometres, through a series of large reservoirs, until they finally discharge into the Atlantic Ocean via the Rio de La Plata delta, where the sugar cane derived DOM is already completely recycled. The potential sugar cane impacts on land-sea fluxes are limited to the remaining 26.8%, i.e. 20,000 km^2 harvested area (UNICA, 2010). Assuming that sugar cane fields occupy on average 1/2 of their catchment's total area, 40,000 km^2 of the coastal catchment area is affected by sugar cane. The Paraiba do Meio and Sumauma catchments (3,672 km^2) studied here would hence represent ~10% of coastal and ~1% of total Brazilian sugar cane impacted catchments.

The annual flux of DOC from the Sumauma and the Paraiba do Meio into the lagoon is not known, since only the dry season was sampled. Whether DOC concentrations are equal, higher or lower during the rainy season, when river discharge is enhanced by more than a factor of 5 (LOICZ, 2002) is further not known. However, a "flushing" (DOC up) scenario would seem more likely than a "dilution" (DOC down) scenario, as there was a strong DOC increase during the few extreme rain events in the dry season (Chapter 2 and 5).

A minimum estimate would mean that ~0.5 mg/l sDOC is constantly exported to the ocean throughout the year. The annual water export of the Manguaba lagoon system is 3 x 10^8 m^3 yr^{-1} and thus the annual sDOC export would be 150 t yr^{-1} or 24.5 t km^{-2} yr^{-1}. Extrapolation to the 100% of harvested area in the coastal regions thus would yield a sDOC flux from coastal Atlantic Brazilian sugar fields of 1,500 t yr^{-1}.

In reality, however, a several fold additional flux of sDOC is superimposed on this value during the rainy season, since DOC concentration raises with discharge. The magnitude of this effect depends on how the DOC scales with discharge. A further limitation in this

estimate is the fact, that this is based on data from a system containing a lagoon that acts as a purifying reactor before the water reaches the sea. This implies an underestimate of not only bulk DOC fluxes but also of its labile fraction. Taking these points into consideration we estimate a 2 - 10 fold sDOC flux of 3,000 – 15,000 t yr^{-1} to reach the coastal ocean, with a substantial labile DOC component. From a coastal water quality perspective, therefore, the rainy season should be of particular concern.

While these estimate gives a total DOC flux to the ocean, estimates of the riverine DOC flux at a range of watershed sizes gives comparable results with regard to vegetation types, as DOM source, export and quality are affected by terrestrial vegetation sources (Aitkenhead and McDowell, 2000). For that reason we also calculated the area-normalised DOC and sDOC export for sugar cane fields in 2 steps. In the first step we calculated the annual export of DOC and sDOC in the freshwater with 2500 and 690 t yr^{-1}, respectively, by multiplying the extrapolated values for DOC and sDOC (8.3 and 2.3 mg/l, respectively) with the annual water export of the Manguaba lagoon system. In a second step the annual freshwater DOC and sDOC export was normalized to the area planted with sugar cane in the catchment of the Manguaba lagoon. According to IBGE (2009) 45,000 ha in the catchment of the Manguaba lagoon is cultivated with sugar cane. Thus, the area-normalised DOC and sDOC export is 55 and 15 kg C ha^{-1} yr^{-1}, respectively.

These findings are well in the range of rivers with tropical forest (30-139 kg C ha^{-1} yr^{-1}) or warm conifers (18-53 kg C ha^{-1} yr^{-1}) in its catchment (Richey et al., 1990; Aitkenhead and McDowell, 2000). Compared to croplands in the U.S. (mainly soybeans and maize) with an area-normalised DOC export of 4.4 – 10.4 kg C ha^{-1} yr^{-1} (Dalzell et al., 2011) our results are higher, while our results are in a similar range of croplands across Europe 41 ± 13 kg C ha^{-1} yr^{-1} (Kindler et al., 2011).

7. General conclusions

7.1 Syntheses

In this thesis, riverine and estuarine sources of organic carbon in DOM and POM have been identified by means of the stable isotope composition of carbon and by the molecular mass ratio of carbon-to-nitrogen. The generally enriched $\delta^{13}C$ signal of organic carbon in the studied Manguaba lagoon system confirms that sugar cane, which is the dominant plant in the lagoons´ catchment, substantially contributes to riverine organic matter. During drought DOM is low and most depleted in $\delta^{13}C$ because baseflow flushes C_{org} poor and ^{13}C depleted soil profiles. During flood, in turn, flushing from shallower C_{org} and ^{13}C enriched soil strata is reflected in heavier $\delta^{13}C$ DOC values and a several fold increase in DOC. POM during flood is high due to soil erosion and resuspension of river bottom sediment. The molecular weight distribution of organic matter instead was independent of hydrological conditions, but controlled by soil processes. Occasional inputs of OM-rich vinhasse effluent from sugar cane processing factories are limited to the harvest/dry season and can locally enhance the sugar component of riverine DOC to more than 60%.

On average, one third of riverine DOM discharged into the lagoon has a sugar cane source. In the lagoon the sugar cane derived DOM is degraded. The lagoon therefore is an effective system for the purification of sugar cane derived DOM in the dry season when the water residence time is much longer than in the rainy season. From a coastal water quality perspective, one should expect a much greater load of relatively undegraded, sugar cane rich OM reaching the marine environment in the wet season, when continuous flushing of organics and nutrients from the fields promotes massive phytoplankton blooms and the water residence time is short. A rough overall estimate of the annual sugar cane derived DOC flux from coastal Atlantic Brazilian sugar fields yields 1,500 - 15,000 t yr^{-1}.

Size fractions of DOM were obtained by a tangential flow filtration system according to a newly established operating protocol (Chapter 4). The used TFF protocol (cleaning, conditioning, concentration factor, cross flow rate) proved reliable and efficient for separating natural organic colloids from fresh and estuarine waters with a large range of DOM concentration (1.5 – 22.5 mg/l) and ionic strength (salinity 0 – 35 psu).

The study focussed on the following questions:

1. What are the main sources and sinks of organic matter in such an impacted lagoon system? What are the main transport pathways of organic matter?

From $\delta^{13}C$ data we can conclude that the sugar cane cultivation in the hinterland of the Manguaba lagoon left an imprint on the origin of OM sampled from soils and fluvial systems. Organic matter fluxes into the river and lagoon originate from baseflow, field runoff and sugar cane factory effluents and their properties depend on rainfall patterns. While rivers have negligible *in situ* production of DOM as a result of limited light availability in the highly turbid waters, DOM in the lagoon is greatly affected by internal sources such as resuspension of sedimentary OM and autochthonous DOM production.
In the transition zone from riverine to brackish waters DOM mixes non-conservatively, with a net loss of DOM during rainfall and a net gain of DOM during the dry/baseflow situation. A substantial loss of DOM resulted from flocculation of humic rich soil DOM and subsequent scavenging by settling sediments, when the contribution of suspended sediment from eroded fields in the river is very high. At much lower suspension levels DOM increases, however, presumably from addition of photolysed resuspended sedimentary OM. Pure sugar cane organic matter, as released as vinhasse from the factories, is not influenced by processes within the mixing zone and thus, these components reach the lagoon unaffected.
Autochthonous DOM is a minor contribution (2 - 9%) to overall lagoon DOM, because OM from photoautotrophic production is mineralised immediately. The long water residence times in the lagoon also enable efficient degradation of sugar cane derived DOM: about 50% of sugar cane derived carbon is lost from the DOM pool within the lagoon, while the rest is mixed conservatively through the estuary.

2. Are the microbial communities adapted to the sugar cane cultivation in the hinterland of the lagoon system in order to degrade this specific organic matter source?

The Manguaba lagoon in its present condition has a diverse and well adapted microbial community. It could be demonstrated that the distribution of microbes was in close relation to the physicochemical environmental settings such as organic matter concentration and salinity. The riverine system showed a heterotrophic bacterial community typical for soils (*Betaproteobacteria*), as soil organisms were flushed into the river due to local soil erosion, and freshwaters (*Polynucleobacter*-, and *Acidovorax*-related species), as well as for sugar

cane (*Burkholderia phytofirmas PsJN*). Microbial degradation of sugar cane derived components of DOM is probably accomplished to a large extend already within the soil-drainage-system. Studies downstream a sugar cane factory showed a bacterial degradation potential of 0.7 mg C l^{-1} d^{-1} (Wolf et al., in prep.), which is low considering the short residence time of water in the river.

Substances introduced to the lagoon will remain there for a considerable time span and allow specialized organisms to attack these substrates and hence to efficiently modify complex organic substances. Especially the presence of specialized bacteria (*Acinetobacter),* that were able to cope with organic pollutants associated with sugar cane cultivation, showed that the microbial community is well adapted to degrade the specific substrate from a sugar cane environment.

3. Which essential differences exist between low- and high molecular weight DOM? Which conclusions can be drawn from the molecular size distribution of organic matter with regard to DOM dynamics?

While DOM concentrations in the Manguaba lagoon system were greatly affected by hydrology and local source/ sink processes, the molecular size distribution of DOM remained constant, with the LMW fraction (< 1kDa, 92%) representing almost all of the DOM. Colloids as intermediary between "truly" dissolved and particulate OM represent only about 10% of the DOM pool, with the VHMW DOM (50 kDa – 0.45 µm) representing the main fraction within the colloids. Soil processes, including colloid precipitation, mainly controlled the size distribution of OM in the freshwater system. From the similarly low colloid concentration in the entire Manguaba lagoon system, it can be concluded that autochthonous DOM is cycled on short time scales, because this fresh OM is decomposed quickly by heterotrophic bacteria. VHMW DOM is similar to POM rather than DOM in its C/N and $\delta^{13}C$ and therefore is unlikely an aggregate of LMW DOM.

7.2 Outlook

While studies performed within this Ph.D.-thesis gave many fascinating results and some answers, they also pose new questions and suggest further research and different approaches: One, of course, is the degradation potential of DOM by microbes in the Manguaba lagoon-estuary system. In the light of the strong UV radiation in tropical estuaries in the dry season, the enhanced primary production and the degradation potential of photochemical reactions should not be underestimated. Future research should determine not only the potential to degrade organic matter but also to produce DOM, e.g. from resuspended sediments. Therefore, the molecular weight distribution, especially its impact on the degradation efficiency should be further investigated. In this context, the size-reactivity-continuum model explaining DOM degradation in the ocean (Amon and Benner, 2002), should be tested for applicability on fresh- and brackish water systems.

The results of this thesis enable a better understanding of the complex processes of organic matter cycling in a tropical estuary impacted by sugar cane. However, the interpretation of carbon isotope ratios was limited as individual sources of DOM have similar signatures and are hence hardly distinguishable in the mix of different DOM sources. Biomarkers such as lignin could additionally aid to identify sources of DOM. The determination of $\delta^{14}C$ in OM would help to further investigate the environmental consequences of sugar cane monoculture cultivation on the organic matter cycle, as increased soil leaching and the aerobic decomposition of carbon from soil in consequence of intensive drainage lead to soil erosion. This may finally result in land cover change and therefore has to be quantified in the future.

To predict the transport of colloidal-associated pollutants (metals, pesticides) to the ocean, the amount of colloidal OM must be determined. Thereby further research should focus on the influence of soil processes on colloid formation and pollutant bonding as well as on colloid precipitation, as this is dependent on the soil type (pH and concentration of polyvalent ions such as iron and aluminium).

In order to quantify the export of sugar cane derived DOM to the oceans for all sugar cane regions in coastal catchments more precisely, a next logical step would be the determination of directly draining systems and of estuarine systems during the rainy season, when the water residence times are much shorter than in a semi-enclosed water body in the dry season. As a follow-up to this study, in which the fate of DOM was studied within the estuarine system,

the fate of the specific DOM from sugar cane cultivation should be tracked in the ocean.

Figure captions

Fig. 1.1 Main environmental impacts of the sugar cane agro industry (modified according to Martinelli and Filoso, 2008)

Fig. 1.2 Percentage of sugar cane crops in Brazil. (Source: Goldemberg et al., 2008)

Fig. 1.3 Size spectrum of chemical species in aquatic systems (Source: Guo and Santschi, 2006)

Fig. 1.4 Pathways of organic matter in water. Modified according to Wangersky, 1972.

Fig. 1.5 Isotope signature of selected C pools (CO_2, deep ocean water, pristine rivers, C3 and C4 plants, phytoplankton)

Fig. 2.1 Study site (Mundaú-Manguaba coastal lagoon system) with location of samples collected during three transects in March and during the high time resolution sampling in October 2007. Triangles symbolise samples of transect A, squares of transect B and cycles of transect C.

Fig. 2.2 Salinity as function of distance to estuary mouth. Each symbol represents a different sampling transect

Fig. 2.3 DOC vs. salinity and sugar cane derived DOC vs. salinity for three transects from rivers Paraiba do Meio and Sumauma to the estuary in March 2007. The conservative mixing line is plotted using the marine end-member data.

Fig. 2.4 A: UV absorbance in m^{-1} (λ = 340 nm) vs. salinity from transect C. B: DOC (mg l^{-1}) and UV absorbance (m^{-1}) (λ = 340 nm) vs. salinity in laboratory mixing experiment - artificial seawater was mixed with molasses and in the same way with an aqueous soil extract (soil from a sugar cane field). NB, different UV absorbance scale.

Fig. 2.5 $\delta^{13}C$ DOC vs. salinity for samples from transects A, B and C in the Manguaba lagoon system. The solid line represents theoretical conservative mixing for each transect including error bars (grey) for isotopic analysis (0.5‰) For comparison, dashed line represents conservative mixing of pristine river (e.g. from the Amazon) with assumed values of -28‰ for freshwater and -21‰ for marine end-member.

Fig. 2.6 $\delta^{13}C$ DOC (‰) and DOC (mg l^{-1}) vs. salinity for a 14 h time series sampling during a tidal cycle at a fixed station in the estuary. Long dashed line is a linear regression of the measured DOC data. Solid line for $\delta^{13}C$ DOC is theoretical conservative mixing curve, including error bars (grey) for isotopic analysis (0.5‰), assuming -25‰ for the river end-member and -21‰ for the marine end-member. For comparison, the short dashed line would result if the river end-member were -28‰, as is typical for pristine rivers such as the Amazon.

Fig. 3.1 Sampling positions in the Manguaba lagoon as well as the supporting rivers and connected inlet canals. Sample positions 90 and 92 are not displayed; these samples were taken in drainage canals northeast of the lagoon. The position of the state Alagoas in Brazil is displayed in the right upper corner.

Fig 3.2 Horizontal distribution of identified taxa based on 16S rRNA gene or 16S rRNA SSCP fingerprinting. Bacteria identified on DNA as well as on RNA level are indicated in bold. Bacteria identified only on RNA level are marked by asterisks.

Fig. 4.1 Molecular weight of standard molecules (kilo Dalton) and their retention coefficients for the determination of the actual molecular weight cut-off of manufacturers specified 1 kDa, 5 kDa and 50 kDa Omega membranes.

Fig. 5.1 Study site (Mundaú-Manguaba coastal lagoon system) with location of samples collected in February 2008.

Fig. 5.2 POC (white) and DOC (black) in samples of different salinities and hydrological conditions.

Fig. 5.3 C/N ratio versus $\delta^{13}C$ of bulk dissolved organic (triangel), low molecular weight (< 1kDa, square), very high molecular weight (50kDa – 0.45µm, diamond) and particulate/ suspended organic matter (>0.45µm, circle). Samples taken in the dry season are symbolized by grey colour and samples taken in a rain event in black.

Fig. 5.4 Dissolved-colloidal partitioning of DOC in the Manguaba river-lagoon-system, according to low molecular weight LMW (< 1 kDa), high molecular HMW* (1 - 50 kDa) and very high molecular weight VHMW (50 kDa - 0.45 µm). *HMW is calculated by subtracting VHMW (50kDa-0.45µm) from COM (1kDa-0.45µm).

Fig. 6.1 DOC vs. salinity from a 25 hour time series at a fixed point in the Manguaba estuary outlet and from the lagoon background samples of February 2008. The straight line represents the extrapolation of the 25 hour sampling to salinity zero. The x represents the extrapolated DOC concentration at the lagoon background sample´s salinity 7.4.

Fig. 6.2 Estimates of lagoon and extrapolated estuarine DOC to salinity 7.4 for the dry season: absolute (mg/l) amounts of DOC_{river} and sugar cane carbon sDOC in DOC and its relative loss from lagoon to estuary (%).

Table captions

List of abbreviations

CCA	Canonical correspondance analysis
C/N	Carbon-to-Nitrogen ratio is the ratio of the molecular mass of carbon to the mass of nitrogen in a substance
Chl	Chlorophyll
CDOM	Chromophoric dissolved organic matter
COC	Colloidal organic carbon
COM	Colloidal organic matter
cDNA	complementary Desoxyribonucleic acid
cf	concentration factor used with tangential flow filtration
CAM	Crassulacean acid metabolism
CFR	Cross flow ratio
CFV	Cross flow velocity
$\delta^{13}C$	isotope ratio of R = $^{13}C/^{12}C$
DIN	Dissolved inorganic nitrogen
DOC	Dissolved organic carbon
DOM	Dissolved organic matter
DON	Dissolved organic nitrogen
GFF	Glass fibre filter
HMW	High molecular weight in the range of 1-50 kilo Dalton
IR-MS	Isotope ratio mass spectrometry
LMW	Low molecular weight in the range of < 1kilo Dalton, also called "truly" dissolved
MWCO	Molecular weight cut-off
NMWCO	Nominal molecular weight cut-off
OC	organic carbon
Recov	organic carbon recovery
OM	Organic matter
PdM	Paraiba do Meio river
POC	Particulated organic carbon
POM	Particulated organic matter
POLCAMAR	Pollution from sugar cane in marine systems
PAH	Polyaromatic hydrocarbon
PES	Polyethersulfone

psu	practical salinity unit
PFW	Prefiltered water
RT-PCR	Real-time polymerase chain reaction
rcDNA	relaxed circular Desoxyribonucleic acid
RC	retention coefficient
rDNA	ribosomal Desoxyribonucleic acid
rRNA	ribosomal Ribonucleic acid
SSCP	Single strand confirmation polymorphism gene fingerprinting
ssDNA	Single-stranded Desoxyribonucleic acid
sDOC	sugar cane derived dissolved organic carbon
TFF	Tangential flow filtration
TDN	Total dissolved nitrogen
TOC	Total organic carbon
TSS	Total suspended solids
TMP	Transmembrane pressure
UF	Ultrafiltration
UV	Ultraviolet
VHMW	Very high molecular weight in the range of 50 kilo Dalton - 0.45 µm
V-PDB	Vienna Peedee Belemnite

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