



Water for a Healthy Country

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A pilot study

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Peter Gell², Steven Wakelin¹

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¹CSIRO Land and Water, PMB 2, Glen Osmond SA 5064, Australia.

² School of Earth and Environmental Sciences, University of Adelaide, Adelaide SA 5005, Australia

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Foreword

The environmental assets of the Coorong, Lower Lakes and Murray Mouth (CLLAMM) region are currently under threat as a result of ongoing changes in the hydrological regime of the River Murray. While a number of initiatives are underway to halt or reverse this environmental decline, such as the Murray-Darling Basin Commission's 'Living Murray', rehabilitation efforts are hampered by the lack of knowledge about the links between flows and ecological responses in the system.

The Coorong, Lower Lakes and Murray Mouth program is a collaborative research effort with the aim to produce a decision-support framework for environmental flow management for the CLLAMM region. This involves understanding the links between the key ecosystem drivers for the region (such as water level and salinity) and key ecological processes (generation of bird habitat, fish recruitment, etc). A second step will involve the development of tools to predict how ecological communities will respond to manipulations of the 'management levers' for environmental flows in the region. These include flow releases from upstream reservoirs, the Lower Lakes barrages, and the Upper South-East Drainage scheme, and dredging of the Murray Mouth. The framework will attempt to evaluate the social, economic and environmental trade-offs for different scenarios of manipulation of management levers, as well as different future climate scenarios for the Murray-Darling Basin.

One of the most challenging tasks in the development of the framework will be how to predict the response of ecological communities to future changes in environmental conditions in the CLLAMM region. The CLLAMMecology Research Cluster is a partnership between CSIRO, the University of Adelaide, Flinders University and SARDI Aquatic Sciences that is supported through CSIRO's Flagship Collaboration Fund. CLLAMMecology brings together a range of skills in theoretical and applied ecology with the aim to produce a new generation of ecological response models for the CLLAMM region.

This report is part of a series summarising the output from the CLLAMMecology Research Cluster. Previous reports and additional information about the program can be found at <http://www.csiro.au/partnerships/CLLAMMecologyCluster.html>

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Executive Summary

Estuaries are among the most biologically productive ecosystems on Earth because they receive organic matter inputs from a number of sources. This organic matter is either a source of nutrients to promote plant production within the estuary or can be used directly as an energy source by estuarine food-webs. The amount of organic matter delivered to or produced by an estuary ultimately sets the upper limit for how many higher organisms, such as fish and birds, the system can support. However, the form of organic matter produced or delivered also determines what kind of food-webs and organisms will be present. Thus, changes in the source or the cycling of organic matter in estuaries can have profound impacts on ecosystem function.

The Coorong (a set of Ramsar-listed coastal lagoons associated with the River Murray estuary, Australia) has degraded over the last 100 years as a result of regulation and diversions from the River Murray. Hypersalinisation and a shift in the origin of organic matter supporting the food-web are suspected to have contributed to the environmental decline. To evaluate if the sources of organic matter supporting the Coorong food-web have changed over the last ~150 years, sedimentary organic matter was characterised in cores spanning the Coorong salinity gradient at depths representative of pre- and post-European development of the region (as defined by the appearance of *Pinus* pollen in the cores).

A number of complementary techniques were used to characterise the sediment organic matter. Because there is a large range in the size of organic matter particles produced or delivered to estuaries, analyses were made on bulk sediment organic matter and three size-fractions (10–53 μm , 2–10 μm and <2 μm). Element concentrations (C, N and P), elemental ratios (C/N and C/P) and $\delta^{13}\text{C}$ were measured on all bulk samples and on some of the fractions. These are standard indicators to evaluate the sources of organic matter to estuaries. In addition, organic matter components (lipid, carbohydrates, etc) were also determined in the bulk sediment fraction by ^{13}C Nuclear Magnetic Resonance Spectroscopy (^{13}C -NMR) and the application of a molecular mixing model. Similar measurements were made on potential sources of organic matter to the Coorong (emergent macrophytes, macroalgae, phytoplankton, etc) to determine their characteristic signatures.

Spatial variability in organic matter composition in the Coorong

When comparing sediments spanning the length of the Coorong, there was a substantial variation in organic matter composition both prior to and following European development. From the North to the South Lagoon, sediments tended to have:

- higher N and P concentrations
- lower N/P and C/P ratios
- more enriched $\delta^{13}\text{C}$ signatures in some size fractions
- less lignin and more lipids.

The most important source of organic matter across the system appears to be locally produced algae. However, the North Lagoon also appears to receive other sources of organic matter as well. These variations in organic matter composition along the length of the Coorong are consistent with a strong salinity gradient currently present in the system (from fresh to marine at the northern end to hypersaline at the southern end). The salinity gradient would have been present in the past but is currently much stronger due to decreased freshwater inputs from the River Murray (at the northern end) and the Upper South East region (at the southern end), and a decreased tidal flushing due to a partially silted Murray Mouth. These findings indicate that the delivery to or the production of organic matter was and is different between the North and South Lagoon, potentially leading to different food-webs.

Changes in organic matter composition during pre- and post-European development

Substantial changes in organic matter composition were noted in sediments that were deposited prior to and after European settlement. Relative to pre-European sediments, recent sediments:

- contain a greater proportion of organic matter bound to fine sand and silt relative to clay
- have a stronger enrichment in N/P and C/P along the North – South axis
- are more ^{13}C -depleted
- have much less lignin in the upper North Lagoon.

The magnitude of the shift in $\delta^{13}\text{C}$ (up to 8.3‰) suggests that a significant change in biogeochemical cycling or in the origin of organic matter occurred following European development. Greater lignin content in the upper North Lagoon would be consistent with a greater input of organic matter from the River Murray in the past. However, the high $\delta^{13}\text{C}$ (–18.3‰) and low C/N (7.5) in lignin-rich sediments are inconsistent with a C-3 terrestrial organic matter source expected for terrestrial-derived organic matter transported by the River Murray. On the other hand, seagrasses such as *Ruppia megacarpa* (an aquatic plant, derived from angiosperms) also contain lignin and tend to have $\delta^{13}\text{C}$ and C/N signatures comparable to the ones observed in lignin-rich Coorong sediments. *Ruppia megacarpa*, an important food source for migratory waders, was formerly abundant in the North Lagoon but has been recently extirpated. Changes in the ecological and biogeochemical environment following European development rather than a diminished supply of terrestrially-derived organic matter by the River Murray appear responsible for the recent environmental decline of the Coorong.

1. Introduction

At the end of the Murray-Darling Basin, the Coorong (a set of coastal lagoons) hosts various environmental assets, as recognised by its listing along with the neighbouring Lake Albert and Lake Alexandrina under the Ramsar convention on wetlands (<http://www.ramsar.org/>) and a number of other international agreements (Phillips and Muller 2006). In particular, the region is one of the six most highly-frequented sites in Australia for migratory waterbirds (Paton 2000). The environmental assets of the region along with their associated social and economic values are currently under threat because of substantial water regime changes that have occurred since European development (Jensen et al. 2000; Murray-Darling Basin Commission 2005). Hypersalinisation and a shift in the origin of organic matter supporting the food-web are suspected to have contributed to the environmental decline of the Coorong. It is currently difficult to evaluate the significance of potential changes in the organic matter cycle in the estuary because conditions prior to European settlement are not well known.

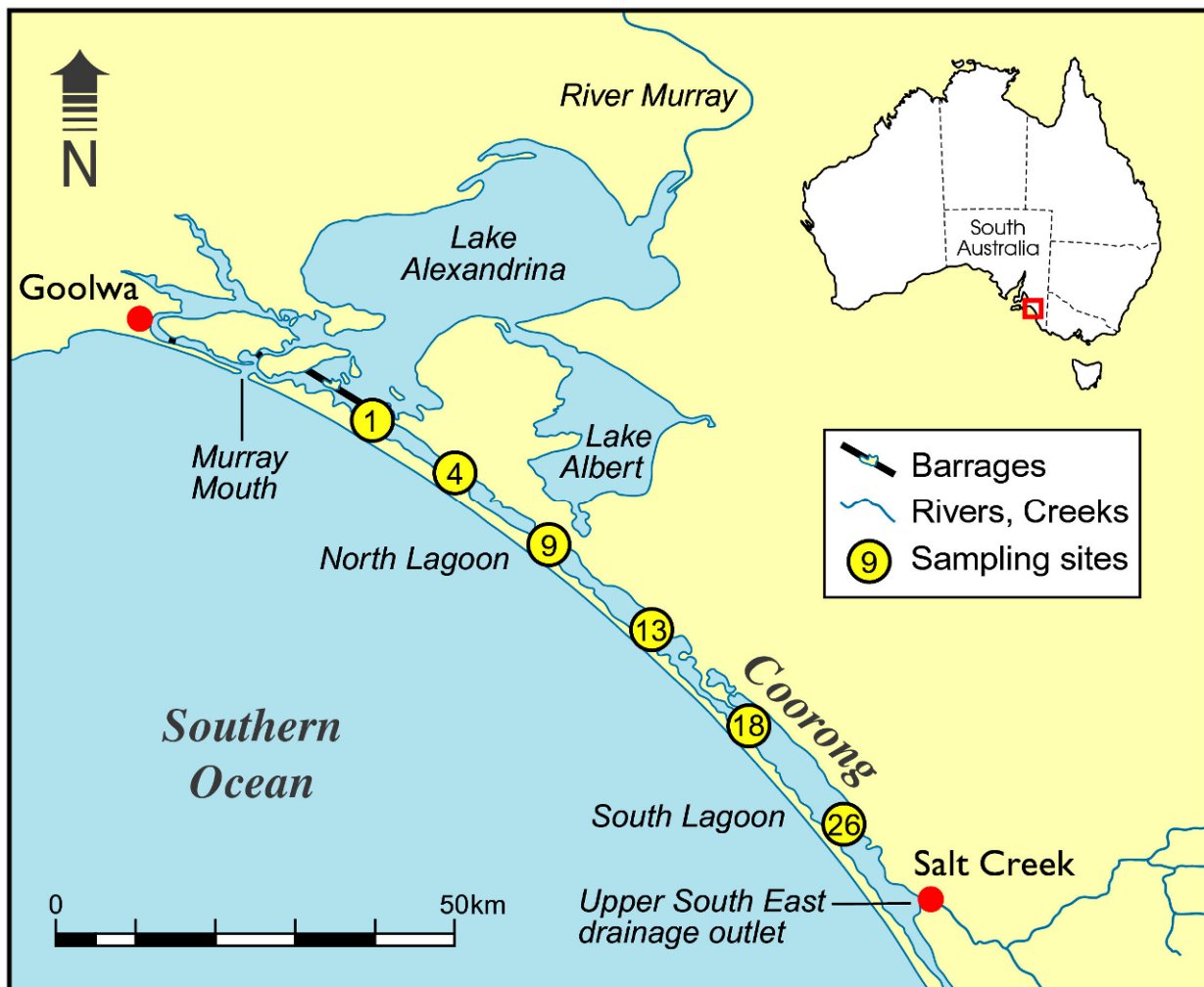


Figure 1. The Coorong lagoons, Lower Lakes and Murray mouth and the sampling sites of this study

Past analyses of sediment cores from the Lower Lakes and Coorong have indicated that the water quality of these systems was significantly different from what they are today. Unfortunately, many of these data are contained in unpublished reports or theses (e.g. Owen,

1993; von der Borch, 1994; Brereton, 1995; De Deckker, 1995; Tomlinson, 1996; Fluin, 2002; Gell and Haynes, 2005). The most comprehensive published data on the Coorong is summarized in articles by Bourman and Barnett (1995), Bourmann and Murray-Wallace (1991), Bourmann et al. (2000) and Cann et al. (2000).

Prior to the study by Gell and Haynes (2005) the only data available to assess pre-European conditions in the Coorong with regard to water quality are the ones by De Dekker (reported in Tomlinson, 1996). Fossil foraminifera and ostracode assemblages as well as ^{210}Pb data, taken from a core in the South Lagoon (near Salt Creek), were used to assess the changes in salinity before and after European settlement. Based on these data, the South Lagoon was interpreted as being a hydrologically more mixed system during pre-European times, with salinities ranging from marine, estuarine to relatively fresh water (approximately 3,000 to 35,000 mg^{-1}) on a 5-10 year basis. Salinities appeared to have changed after European settlement towards more consistently saline to hypersaline conditions in the lower North Lagoon and entire South Lagoon. Thus, De Deckker (1995) concluded that 'the Coorong before human impact did receive more water and thus was less saline overall near Salt Creek, and did remain more in direct contact ecologically speaking with the ocean'.

In a more detailed study, utilising paleolimnological data from cores collected across the entire length of the Coorong lagoons, Gell and Haynes (2005) reported the first changes in water quality in the early to mid-1900s. Later shifts were observed in the 1940s and 1980s, which might correlate with the construction of the barrages and the siltation of the mouth, respectively. Generally, however, their observations support the conclusions drawn by De Deckker. They found that before European settlement, the Coorong was a more open system compared with today. A stronger influence by the ocean (e.g. tidal currents) affected mainly the North Lagoon; however, estuarine conditions were also observed in the South Lagoon, possibly due to freshwater influx from the Upper South East. Gell and Haynes' (2005) assessments of salinity ranges in the pre-European Coorong are similar to those by De Deckker (1995) and may have varied between 5,000 and 35,000 mg^{-1} . Gell and Haynes (2005) found evidence that since European settlement salinity, sediment supply and turbidity increased in the Coorong lagoons. Influence of marine waters appears to be restricted to the area northwest of core C3, close to Mark Point (Fig. 1). Southeast of this site there is little evidence for regular flushing by marine waters today.

To evaluate if the sources of organic matter supporting the Coorong food-web have changed over the last ~150 years, sedimentary organic matter was characterised in cores spanning the Coorong salinity gradient at depths representative of pre- and post-European development of the region (as defined by the appearance of *Pinus* pollen in the cores). A number of complementary techniques were used to characterise the sediment organic matter. Because there is a large range in the size of organic matter particles produced or delivered to estuaries, analyses were made on bulk sediment organic matter and three size-fractions (<2 μm , 2–10 μm , and 10–53 μm). Element concentrations (C, N and P), elemental ratios (C/N and C/P), and $\delta^{13}\text{C}$ were measured on each fraction. These are standard indicators to evaluate the sources of organic matter to estuaries (see review below). In addition, Coorong sediments were characterised by ^{13}C -NMR spectroscopy to provide information on the major C-associated functional groups (alkyl, O-alkyl, aryl, O-aryl, carbonyl, ketones) present in the organic matter (e.g. Kogel-Knabner, 1997; Mathers et al. 2000). A more quantitative and detailed analysis of ^{13}C -NMR data was also achieved by applying a molecular mixing model (MMM), which was initially formulated by Nelson et al. (1999) and modified and improved by Baldock et al. (2004). The modelling approach is based on the assumptions that natural organic materials can be represented by a mixture of biomolecules (e.g. carbohydrate, lignin, protein, lipid, charcoal) and that each biomolecule can be assigned a representative distribution of ^{13}C -NMR signal intensity.

Similar characterisation measurements were made on potential sources of organic matter to the Coorong (emergent macrophytes, macroalgae, phytoplankton, etc) to determine their characteristic signatures. A complementary study investigating microbial diversity along the Coorong salinity gradient is also presented in Appendix 1. The analyses of Coorong sediment organic matter provides new information for how the ecosystem has changed following modification to its flow regime.

1.1. Organic matter in estuaries

Organic material discharged from rivers into the ocean is estimated to represent 50% of the organic carbon entering the river with the remaining material being oxidized within the system (25%) and stored as POC in the system as sediment (25%) (Hope et al., 1994). The source and chemistry (e.g. bioavailability) of organic matter in estuarine systems is an important factor in ecosystem processes. For example, sedimentary organic matter is an important substrate for epiphytes, algal mats as well as an important source of nutrients for microbial processes. Thus, changes in the type of organic matter can affect the base of the food-webs which in turn has consequences for higher trophic levels such as birds and fish. For example, the dramatic decline in bird and fish populations in the Coorong is likely to be associated with processes occurring at the base of the food-web and related sedimentary organic matter chemistry.

Thus, understanding the sources and characteristics of organic carbon transported to the ocean and stored in marine sediments or recycled in the marine food chain is crucial for assessing the effects of land-use changes, climatic and ecosystem changes and carbon burial dynamics. Estuaries form an important interface between marine and fluvial systems where large chemical changes, mixing, sedimentation and re-suspension occur and chemical and physical processes affect the transported substances (e.g. Burton and Liss, 1976).

Estuaries are highly dynamic environments in which geomorphic changes occur by deposition and erosion over timescales varying from hours to millennia. Sedimentary inputs are received and accumulate through fluvial, marine and aeolian processes. The origin of this organic matter can be quite diverse, including detritus from surrounding vegetation and soil, organic matter produced within the estuary (*'in situ'*) and anthropogenic sources (Ryan et al., 2003). Estuaries are some of the most productive ecosystems on Earth as fertile mixing zones are created where organic and mineral nutrients from the land and sea accumulate. In Australia, freshwater flows are often ephemeral and estuaries can have large ranges in salinity, varying from fresh to hypersaline. Furthermore, as a result of land management changes following European settlement, sediment transport from the land to coastal environments has increased five to ten-fold, with the highest fluxes occurring during drought-breaking floods (McCulloch et al., 2003). For example, Queensland east coast river catchments are estimated to deliver 14 million tonnes of sediment to estuaries and coastal marine environments each year (Turner et al., 2004).

1.1.1. Application of stable isotopes

Stable isotope analyses of C ($\delta^{13}\text{C}$), often with ratios of organic C to total N (C/N) as well as nitrogen isotopic ratios ($\delta^{15}\text{N}$), have been used frequently in studies aiming to assess the contribution of terrestrial versus marine-derived organic matter within the estuarine zone. The study by Emery et al. (1967) was among the first to use carbon isotopic analyses to distinguish saltmarsh sediments and freshwater peats in the coastal zone. Successive studies noted that $\delta^{13}\text{C}$ of organic carbon (OC) varied between a wide range of sources and the focus turned to identifying the respective sources of OC in coastal and marine environments. Fry and Sherr (1984) and Peterson and Fry (1987) pioneered the field of using isotopic data as source indicators in marine and freshwater ecosystems. They argued that the use of stable isotope analyses (and subsequent application in mixing models) to understand complex ecological processes is possible since the isotopic composition of sources is preserved downstream. They cautioned, though, that metabolic processes may change the isotopic signature of the sample and the associated fractionation needs to be corrected. As a result, estuarine systems in particular have become the focus of isotopic applications because of the complexity of multiple organic matter sources (e.g. Haines, 1976; Torgersen and Chivas, 1985; Ember et al., 1987; Chmura and Aharon, 1995; Middelburg and Nieuwenhuize, 1998).

Fry and Sherr (1984), however, stated that application of isotopic mixing models was warranted only in systems where two known sources contribute to the isotopic signature of the sample and

only one known point of influx occurs into the system. In estuarine systems, where multiple sources are present, they concluded that ‘carbon isotopic measurements are often of limited value in their usefulness for deciphering the complex carbon flows that occur in estuaries’. Notwithstanding, one of the most prominent areas of applying $\delta^{13}\text{C}$ to decipher organic matter sources can be found in the geological field, where $\delta^{13}\text{C}$ data of sediments are often utilised to reconstruct paleoenvironmental changes (summarized in Lamb et al., 2006). The prominent use of $\delta^{13}\text{C}$ analyses to reconstruct paleoenvironments lies in the apparent ease of distinguishing different organic matter sources through isotopic analyses (e.g. Wilson et al., 2005). However, if viewing an aquatic system (e.g. an estuary) as a continuum of terrestrial and aquatic influxes, including organic matter changes during transport, *in situ* production, decomposition and burial, a more complex picture emerges. Along this continuum, there are different hydrological (e.g. short-term storm events, high versus low flow, varying sedimentary loads) and biochemical controls (e.g. ‘hot-spots’ where terrestrial and aquatic habitats interface). Thus, there is an inherent complexity of organic matter processes that take place in riverine and estuarine systems that needs to be taken into account when using isotopes for reconstructing past environments.

1.1.2. Sources of organic matter

Coastal sediments receive organic material from autochthonous (i.e. *in situ* sources such as plants or phytoplankton) and allochthonous sources (i.e. organic material transported from elsewhere, e.g. soil, vegetation, sewage, plant materials in particulate or dissolved form). However, it is important to bear in mind that the distinction between ‘allochthonous’ and ‘autochthonous’ is habitat-dependent as freshwater plankton is considered autochthonous as long as it is within the river system but becomes an allochthonous source once it is transported to the estuarine system. All of the respective sources are characterised by a certain range of $\delta^{13}\text{C}$ and C/N ratios, and as a result, these differences are used in mixing models and mass-balance approaches to determine the proportion of organic matter sources. Figure 2 portrays some of the major groups of organic source materials and their respective isotopic value and C/N ratios, and Table 1 provides isotopic data about specific organisms and plants. Note that some sources display a significant variability, resulting in considerable overlap with other sources.

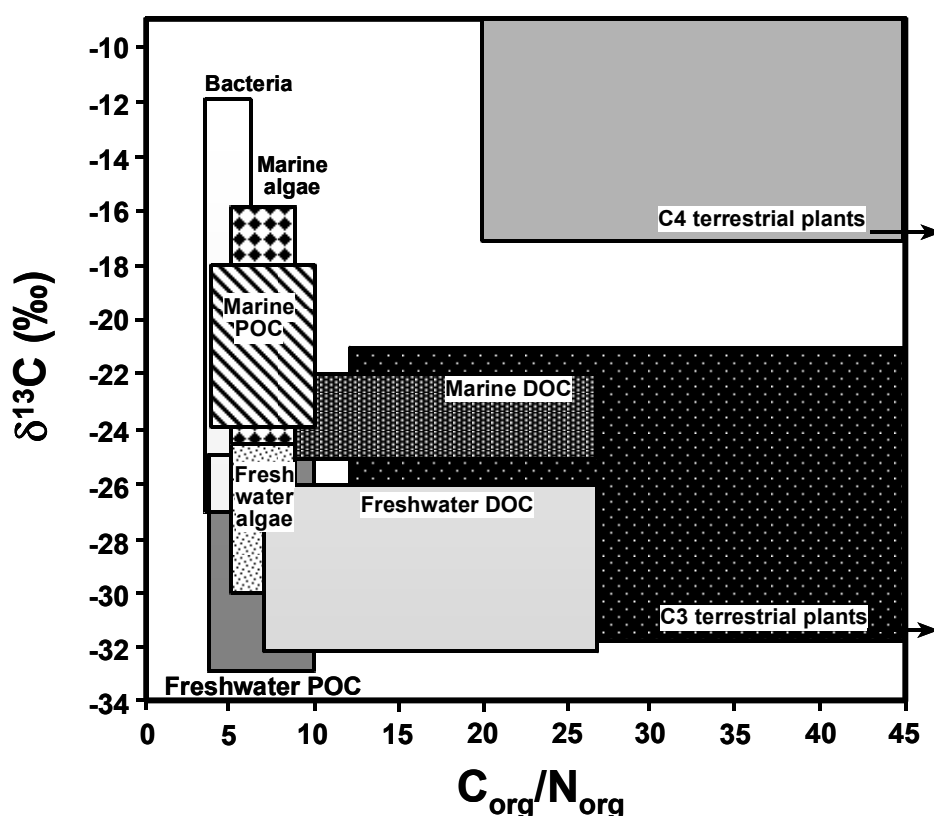


Figure 2. Common $\delta^{13}\text{C}$ and C/N ranges for organic sources, contributing to coastal environments (modified from Lamb et al., 2006)

Table 1. Examples of $\delta^{13}\text{C}$ values of different carbon sources

Source	$\delta^{13}\text{C}$ (‰)
Atmospheric CO_2	-8
Terrestrial C3 plants	-23 to -30
Terrestrial C4 plants	-10 to -14
CAM	-11 to -28
River seston (POC)	-25 to -27
Peat	-12 to -28
C3 marsh plants	-23 to -26
C4 marsh grasses	-12 to -14
Seagrasses	-3 to -15 (up to -24‰)
Macroalgae	-8 to -27
Unicellular benthic algae	-10 to -20
Microphytobenthos	-20 to -13
Temperate marine phytoplankton	-18 to -24
River estuarine phytoplankton	-24 to -30
Autotrophic S bacteria	-20 to -38
Methane-oxidizing bacteria	-62
POC (shelf + open estuarine)	-18 to -24
DOC	-22 to -32
DIC	-5 to -10 (fresh), 0‰ (marine)

As can be seen by the large fields and overlapping areas, there is a considerable range of possibilities of different source materials (Fig. 2). For example, marine macroalgae can vary by over 30‰, freshwater plants and seagrasses by over 10‰ and sometimes seagrasses can vary by over 4‰ within just one location (Fry and Sherr 1984). The reasons for these large variations among the primary producers are mainly due to the $\delta^{13}\text{C}$ of the dissolved inorganic carbon (DIC) pool, concentrations of CO_2 or HCO_3^- as well as degree of enzymatic fractionation. The $\delta^{13}\text{C}$ of DIC may vary between 0 and 10‰, depending on the environment being fresh, marine or brackish. The pH of the system will have an influence on the composition of DIC and accordingly, HCO_3^- will be the dominant species in the ocean, whereas more ^{13}C -depleted CO_2 constitutes the main DIC species in rivers and lakes. Enzymatic fractionation depends on the photosynthetic pathway with the RuBP enzyme of the C3 photosynthetic pathway fractionating the source C to a greater degree (between -23 and -41‰) compared with the PEP enzyme of the C4 photosynthetic pathway (between -0.5 and -3.6‰). However, due to the rate-limiting step of diffusion in the aquatic environment of CO_2 or HCO_3^- across the leaf boundary, actual $\delta^{13}\text{C}$ values of some seagrasses may be only between -10 to -14.5‰.

It is important to accentuate that the constituents presented in Figure 2 and Table 1 differ not only in isotopic value but also in size, and Figure 3 shows the respective size differences of various organic matter sources. Importantly, the different types of OM also vary widely in degradability due to chemical recalcitrance or protection by the minerals (e.g. Middelburg, 1989; Keil et al., 1994).

As can be seen in Figure 3, one of the major distinctions is the 0.45 μm boundary, which separates the dissolved from the non-dissolved ('particulate') fraction. Particulate OM that cannot be further identified may be separated into 'coarse' (>1000 μm), fine (1000-35 μm) and very fine (35-0.45 μm) particulate OM. Both phyto- and zooplankton vary in size from coarse to very fine particulate OM. Because of the difficulty in obtaining pure samples of phyto- or zooplankton in marine or estuarine systems, plankton is often measured as POC which is collected on filters of different mesh sizes. The problem with this approach is that, depending on the connectivity of the marine or estuarine environment to soil and riverine systems as well as

the filter size used, bacteria and allochthonous (e.g. plant litter, algal debris, soil, invertebrate fragments) material suspended in the water column is likely to be included in the analyses. Isotopic measurements of POC have been reported to increase from the river towards the estuary and ocean, which is commonly interpreted to be a reflection of a greater contribution of

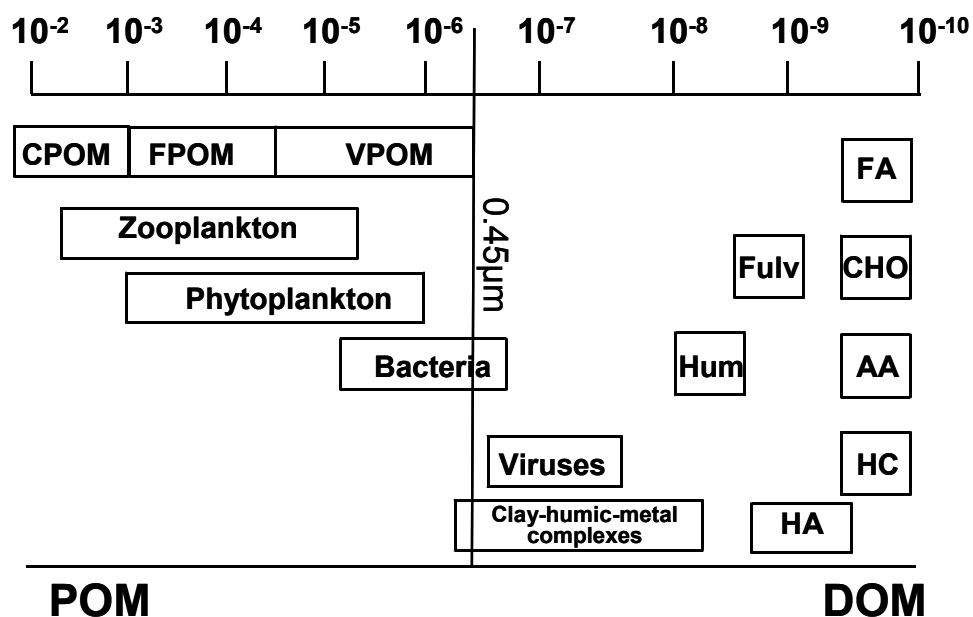


Figure 3. Size ranges of particulate and dissolved organic matter and carbon compounds. CPOM, FPOM and VPOM: coarse, fine and very fine particulate organic matter, respectively; FA: fatty acids; CHO: carbohydrates; AA: amino acids; HC: hydrocarbon; HA: hydrophilic acids (modified from Eatherall et al., 1998)

marine phytoplankton, which is isotopically ^{13}C -enriched compared to organic matter from terrestrial C_3 vegetation (e.g. Fichez et al., 1993; Middelburg and Nieuwenhuize, 1998; Wilson et al., 2005). Reported $\delta^{13}\text{C}$ of plankton (measured as POC) vary between -18 and -24‰ with lower values in the marine environment and warm waters compared with freshwater plankton and colder water temperatures. Because of the problematic overlap between different POC sources, C/N ratios have been used to differentiate between plankton-derived material (4-7) and terrestrial vegetation (>10). However, these assessments do not take into account that soil organic matter may have C/N ratios of 7 or 8. In addition, depending on the degree of burning and oxidation, the charcoal fraction of soil organic matter may have similarly low C/N ratios.

Due to the complexity of estuarine systems and the apparent overlap of $\delta^{13}\text{C}$ of potential sources, supplementation of the isotopic data by other analytical means such as ^{13}C -nuclear magnetic resonance spectroscopy (^{13}C -NMR) is advantageous. This analytical technique determines the functional groups present in organic matter (alkyl, o-alkyl, etc), which can also be used to identify the origin of organic matter in sediments.

2. Materials and Methods

2.1. Site description

The outlet of the Murray-Darling Basin consists of a set of terminal lakes (Lake Albert and Lake Alexandrina), a coastal lagoon (the Coorong), and a morphologically dynamic opening to the sea (the Murray mouth). The region was formed during the Holocene as a result of sea level

change and the formation of a coastal barrier dune system (Bourman 2000). Prior to European settlement, the Lower Lakes would have been mostly fresh with occasional saline intrusions (Sim and Muller 2004). While the Murray mouth could have occasionally closed in the past during droughts, the only recorded closure has occurred in 1981 (Newman 2000). The Coorong would have received freshwater input from the River Murray at the northern end and from the Upper South East Region at the southern end (Jensen et al. 2000; Murray-Darling Basin Commission 2005). Exchange between the Coorong and the ocean would have also been facilitated by constant river flows maintaining the Murray mouth open. The water level and salinity regimes in the Coorong are quite complex (see review in Webster 2005), giving rise to a wide diversity in wetland habitats (Phillips and Muller 2006).

In order to ensure a freshwater supply for irrigation and to improve water quality for metropolitan Adelaide, a system of barrages was completed in 1940 to maintain the Lower Lakes in a freshwater state. The rest of the River Murray is also highly regulated, with large quantities of water diverted for irrigation (Thoms and Walker, 1992). The role that the occasional flow events for the Upper South East region to the South Lagoon would have played is less well understood (Gell and Haynes 2005; Sims and Muller 2006). However, flood events from the Upper South-East are now less frequent or absent due to the diversions of flood waters to the sea via a system of drains engineered to by-pass the coastal barrier dune system (Lamontagne et al. 2004). Currently, median flows from the River Murray at the barrages are only 27% of natural conditions (Murray-Darling Basin Commission 2005) and the annual Spring flood events are either absent or subdued (Newman 2000). As a result, the Murray mouth has silted, the water level regime within the Coorong is subdued, and salinity levels have increased (Murray-Darling Basin Commission 2005; Webster 2005). The ecological impacts to Coorong ecosystems fostered by the water regime change have been summarised in Jensen et al. (2000), Phillips and Muller (2006), and Sims and Muller (2006).

2.2. Sample collection and processing

2.2.1. Core samples

Sediment samples were obtained from cores collected in 2005 by the Geographical & Environmental Studies group, Adelaide University, under the direction of Dr Peter Gell, a project commissioned by the Department of Water, Land and Biodiversity Conservation (DWL&BC) for the Upper South East (USE) Program. Thirty sites had been selected over the length of the Coorong to reveal trends of changing water quality conditions within and between both lagoons, using diatom analysis and dating techniques (Gell and Haynes, 2005). All the cores used in the current study were extracted using a field piston corer and 3 m lengths of 50 mm PVC pipe. They were sliced vertically using a circular saw and 'dustless' diamond blade, wrapped in multiple layers of cling film, and stored at the Adelaide University cold room at 4°C as a single core. Sediment samples were taken from one-half of the core in 7 cm intervals; sub-samples for diatom analyses were taken from the other half of the core at 0.5 - 1 cm intervals, and at 5cm intervals in the lower sections of all the longer cores; sub-samples for *Pinus* pollen analysis were collected from the top sediments over sections displaying obvious lithological change. Samples were kept cold during transfer from Adelaide University to CSIRO Land and Water and were immediately frozen upon arrival and subsequently freeze-dried for further analyses.

2.2.2. Laboratory analyses

LECO Carbon Analysis

Sediment samples from the Coorong cores were analysed on a LECO CR12 Carbon Analyser as described by Merry and Spouncer (1988). To remove carbonate each sample was pre-treated in ceramic boats containing a nickel liner, with sulphurous acid.

Acid pretreatment

Sediment samples were pre-treated with acid to remove any calcareous material for isotope and NMR analyses. Freeze dried sediment (2 x 3 g) was weighed into 50 ml centrifuge tubes and 1M HCl was added in 10 ml aliquots to each tube until the reaction had stopped and the HCl was in excess. The solution was centrifuged and the supernatant discarded. The remaining material was washed with deionised water (3 x 50 ml).

HF pretreatment

The sediments were treated with 2% hydrofluoric acid (HF) to remove paramagnetic materials and concentrate the organic fraction to remove interference and obtain better spectra on the NMR. 2% HF (~50 ml) was added to each tube containing the pre-treated sediment and the tubes were shaken end over end. After 1 h the tubes were centrifuged and the supernatant was discarded. This procedure was repeated and in total the samples were washed with 2% HF 5 x 1 h, 3 x overnight and 1 x 72 h. The HF treated sediment was then washed with deionised water (3 x 50 ml) and the remaining material freeze dried.

¹³C-NMR spectroscopy

All ¹³C CP/MAS NMR spectra were obtained at 50.309MHz on a Varian Unity 200 spectrometer with a 4.7 wide-bore Oxford superconducting magnet. Samples were spun at 5k Hz in 7mm diameter zirconia rotors with Kel-F caps in a Doty Scientific MAS probe. Spectra were accumulated with a 1-ms contact time, a 1-s recycle time, 50-Hz Lorentzian line broadening and a 0.01-s Gaussian broadening. Spectral distributions were calculated (Baldock and Smernik, 2002) by integrating the proportional contribution of the functional groups in the seven chemical shift regions (Wilson, 1987). The regions were: carbonyl (210-165 ppm), O-aromatic (165-145 ppm), aromatic (145-110 ppm), O₂-alkyl (110-95 ppm), O-alkyl (95-60 ppm), N-alkyl/methoxy (60-45 ppm) and alkyl (45 to -10 ppm).

Particle Size Separation of Sediments

Freeze dried core sediments (5 g) were acid pre-treated with 1M HCl to remove any calcareous material. They were then passed through a 53 µm sieve and any organic material was washed out of the >53 µm fraction, collected and oven dried. The remaining <53 µm fraction was further separated into the 10-53, 2-10 and <2 µm fractions.

The <53 µm fraction was made up to 500 mL, mixed and left to settle for the calculated time. The top 25 cm was removed and placed in a 1 litre measuring cylinder (<10 µm fraction). The cylinder was made up to 1 litre volume and the process repeated. The next <10 µm fraction removed was added to the corresponding 1 litre cylinder. The material remaining in the 500 ml cylinder was the 10-53 µm fraction. It was flocculated then freeze dried.

The <10 µm fraction in the 1 litre cylinder was made up to 1 litre volume, mixed and left to settle for the calculated time. The top 25 cm was removed and placed in a 2 litre beaker (<2 µm fraction). The 1 litre cylinder was again made up to 1 litre volume and the process repeated. The next <2 µm fraction removed was added to the corresponding beaker. The <2 µm fraction was flocculated then freeze dried.

The material remaining in the 1 litre cylinder was the 2-10 µm fraction. It was flocculated then freeze dried.

Total Metals By Acid Digestion

Total phosphorous and sulphur were determined by US EPA method 3051A (1998) microwave assisted acid digestion of sediments, sludges, soils and oils (US Environmental Protection Agency, Washington, DC). The finely ground sample was digested in a microwave oven using a mixture of nitric acid and hydrochloric acid. The solution was then analysed for S and P by inductively coupled plasma optical emission spectrometry (ICPOES).

Mass Spectroscopy

Each freeze dried fraction was analysed for $\delta^{13}\text{C}$ by weighing samples containing between 300 and 800 μg of carbon into ultra clean tin capsules. After sealing the capsules they were combusted and analysed on a 20-20 Europa Scientific Automated Nitrogen Carbon Analysis-Mass Spectrometer (ANCA-MS).

2.2.3. Field samples

Samples of aquatic and terrestrial plants, soil organic matter, surface sediments, phyto- and zooplankton were taken in March and November 2006. Plant materials were washed in de-ionized water, dried at 60°C and finely ground for isotopic and spectroscopic analyses. Soil material was dried at 40°C and size separated by wet-sieving into a >200, 53-200 and <53 μm fraction. Plankton and surface sediment materials were freeze dried and finely ground for isotopic and spectroscopic analyses.

3. Results and Discussion

3.1. Current sediment organic matter composition in the Coorong

3.1.1. Geochronology

An essential component of our analyses was the capability to identify pre- and post-European periods in the sedimentary record. We utilized a combination of data from geochemistry (radiogenic nuclides) and paleoecological analyses. These data were part of previous analyses undertaken by Adelaide University (Gell and Haynes, 2005) to assess sedimentation rates and to determine the chronology of core materials included ^{14}C AMS dating of basal parts of the cores to establish the extent of accumulation during the Holocene as well as optical stimulated luminescence (OSL) dating, ^{137}Cs and ^{210}Pb analyses of the upper parts of the cores.

Data showing ^{137}Cs activity (related to nuclear tests in the 1950s) and ^{210}Pb decay profiles (half life of 22.4 years) together with identification of the first arrival of *Pinus* pollen (an introduced species) were used to identify the beginning of European development. The first arrival of *Pinus* pollen has been estimated to provide a maximum age of c. 1850 A.D., although it can represent a later date, and may vary between 1913 and 1967 A.D. More detailed studies by Haynes et al. (2007) showed that in 6 cores, the *Pinus* line is approximated to occur around the 1950s (based on ^{210}Pb and ^{137}Cs analyses), coinciding with the commencement of the effects of major structural (barrages) and hydrological (restricted flows) changes in the Lower Lakes, Murray mouth and Coorong area (Fig. 4). Thus, the period just prior to or including the early beginnings of European development equates to sediments shortly below the first arrival of *Pinus*. In most cores *Pinus* did not occur above 10 cm and was usually found between 15 and 20 cm depth. Thus, surface sediments for this study taken from the uppermost 7 cm are considered to represent the biogeochemical status of the post-European condition of the Coorong. It is important to note that the analyses from these cores are a representation of the last few decades and cannot provide detail that allows reconstruction of annual events.

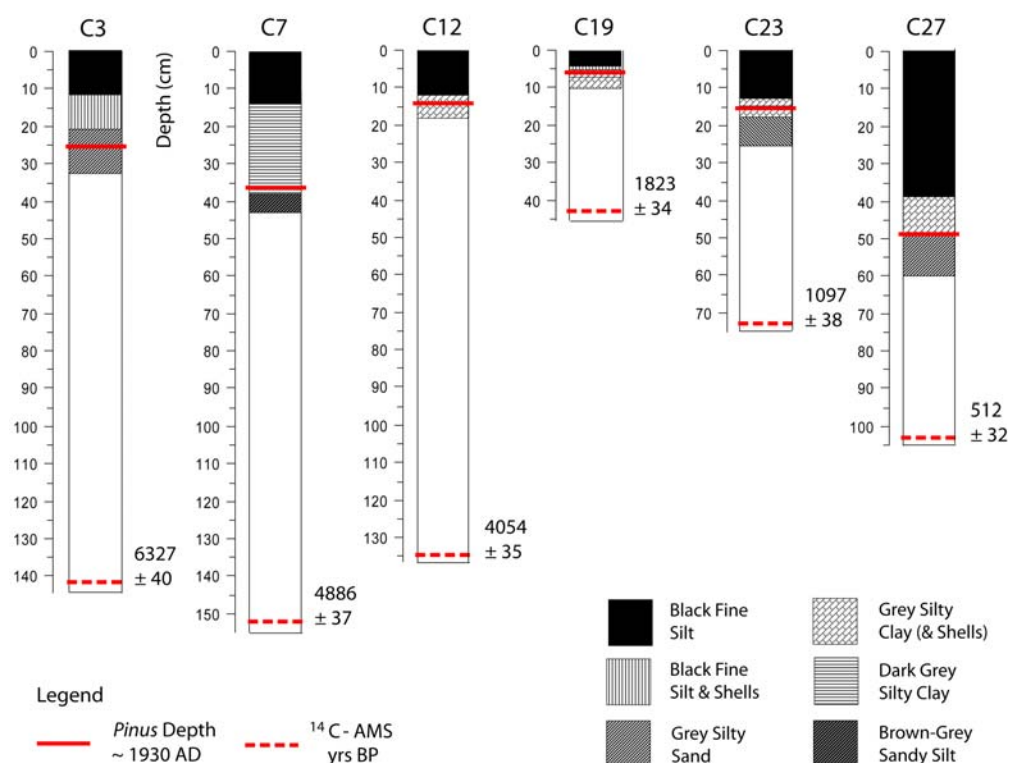


Figure 4 Selected cores on which detailed diatom analyses, dating and sediment accumulation rates were performed, showing the first occurrence of *Pinus*, approximate 1950 date (based on ^{210}Pb and ^{137}Cs modelling) and ^{14}C dating of deeper sediments (modified from Haynes et al., 2007).

3.1.2. Bulk geochemistry

Results from elemental analyses of surface sediments to characterize trends in nutrient and organic matter concentration show an increase in major nutrients (N and P) as well as total OC and carbonate concentrations within the North and from the North to the South Lagoon (Fig. 5). Similar trends of suspended and dissolved OM in the water column as well as chlorophyll *a* concentrations have been reported in Ford (2007).

These trends in water and sediment chemistry are similar to the ones in salinity within the North and South Lagoons. Figure 6 shows the increasing salinity level within the North Lagoon and the consistently hypersaline levels in the South Lagoon over the course of a year. The horizontal line represents the composition of sea water.

Recent sediments in the Coorong lagoons are characterised by a distinct dark, clay-rich zone, most likely a monosulfide horizon. Monosulfides are a family of FeS compounds commonly found in sediments where sulphate reduction takes place (Rickard and Morse, 2005). Sulphate reduction is a widespread biogeochemical process in marine sediments and probably always was a significant process in the Coorong due to elevated sulphate concentrations in both marine (Ford 2007) and River Murray waters (Lamontagne et al. 2006). The depth of the black zone varies between 5 to 39 cm, but is usually deeper in the South Lagoon when compared with the North Lagoon. The black horizon tends to be coincident with or above the level of the first appearance of *Pinus* (Gell and Haynes, 2005). However, monosulfides tend to be only meta-stable and can eventually be lost from sediment profiles during the formation of pyrite

(Rickard and Morse 2005). Thus, black zones as in the Coorong sediments cannot be strictly used as a stratigraphic marker but must be viewed as a geochemical horizon that is subject to changes.

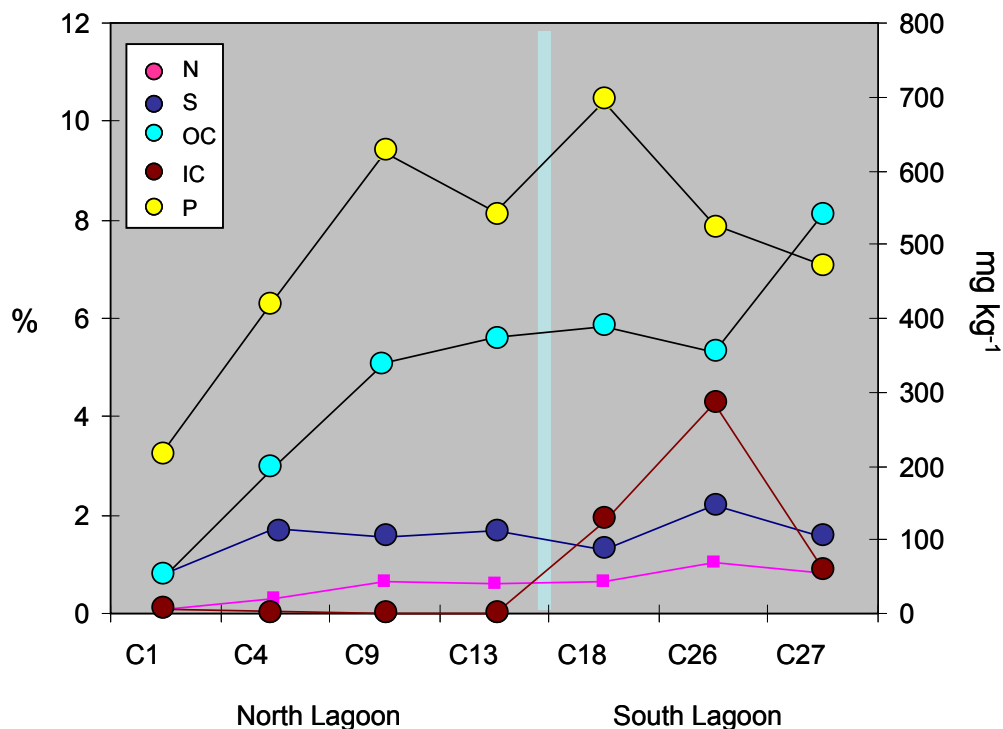


Figure 5 Total concentrations (in %) of nitrogen (N), sulphur (S), organic carbon (OC), inorganic carbon (IC) and phosphorous (P; in mg kg^{-1}) from bulk surface sediments from the North and South Lagoon. The vertical bar represents the boundary between the two lagoons.

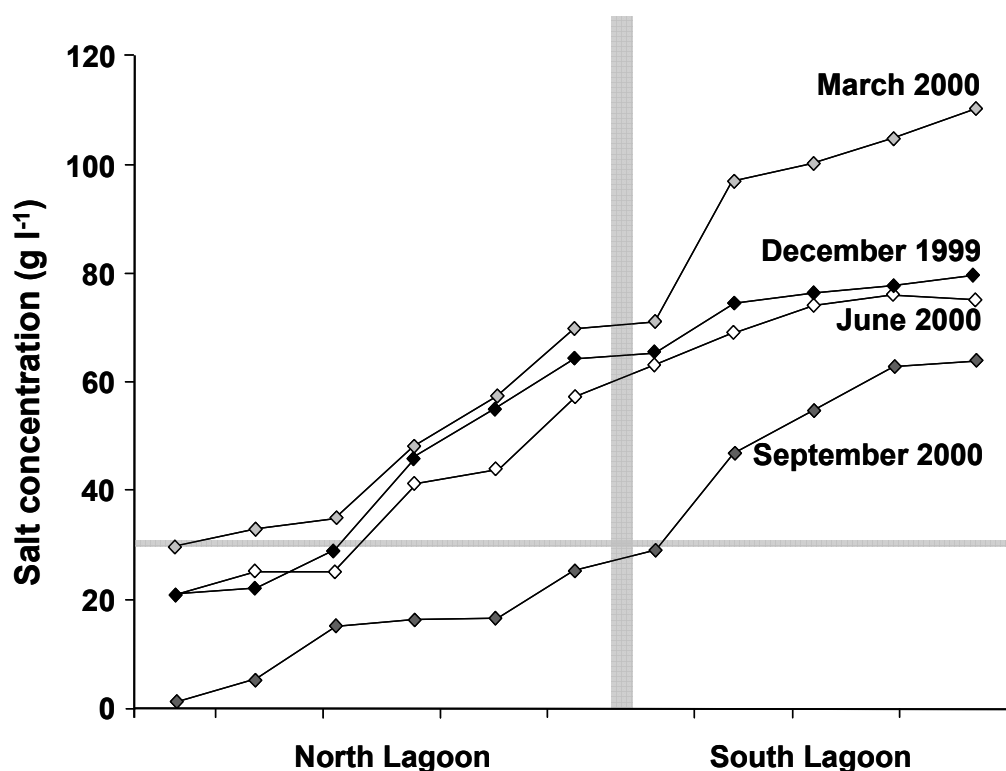


Figure 6: Salinity trends in the North and South Lagoon over the course of a year. The horizontal line represents the composition of seawater and the horizontal line represents the boundary between the two lagoons (David Paton, Adelaide University, unpubl. data).

3.1.3. C/N and $\delta^{13}\text{C}$ signatures in potential sources of organic matter

Isotopic data from the aquatic and terrestrial plants collected along the long axis of the North Lagoon can be broadly divided into two major categories, based on their habitat (aquatic versus terrestrial). Figure 7 illustrates the differences according to $\delta^{13}\text{C}$ and C/N ratios with aquatic plants being on average relatively ^{13}C -enriched and having generally lower C/N ratios compared with their terrestrial counterparts. All terrestrial plants sampled were employing the C3 photosynthetic pathway. The data of aquatic plants and biota had a much larger variability compared with the terrestrial vegetation, most likely owing to the differences in carbon fixation and habitat (e.g. submerged, emergent, planktonic, floating). The isotopic differences within species did not appear to be entirely random and both *Myriophyllum* and *Gracilaria* showed trends towards more ^{13}C -depleted isotopes when comparing the freshwater-influenced Goolwa barrage area with the saline Pelican Point (approximately near C1 in Figure 1). The isotopic differences between the Goolwa barrage area and Pelican point areas were between 3 and 4‰. Future studies will need to confirm whether this trend is robust and is indeed a measure of water quality or other environmental factors.

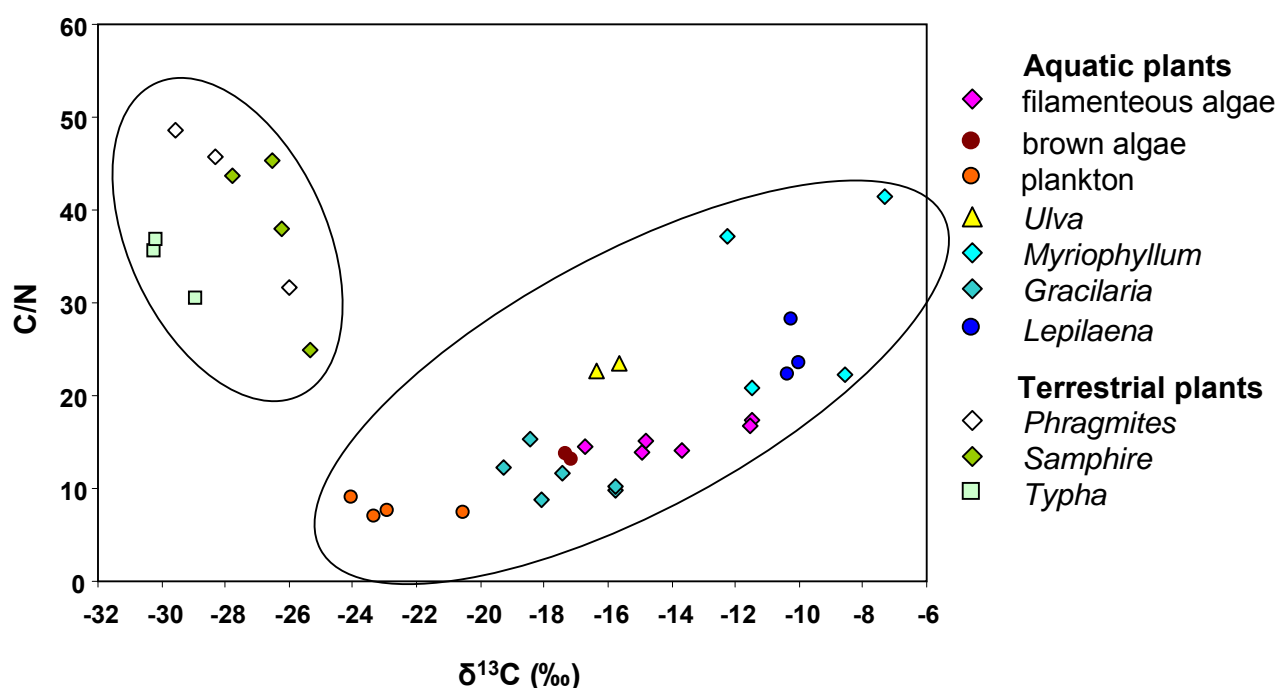


Figure 7 Crossplot of $\delta^{13}\text{C}$ versus C/N ratios of terrestrial and aquatic plants in the North Lagoon

3.1.4. Sedimentary organic matter: $\delta^{13}\text{C}$ and particle sizes

The $\delta^{13}\text{C}$ values of the recent sedimentary organic matter from surface sediments in the North and South lagoons show similar trend between the two lagoons (Fig. 8). Figure 8 shows the $\delta^{13}\text{C}$ for bulk organic matter as well as three size fractions. Apart from core C4, the bulk $\delta^{13}\text{C}$

value in both lagoons averaged -21.6‰ and the size fractions averaged $-22.1 \pm 0.1\text{‰}$. The isotopic data from bulk and size-separated material from core C4 are consistently more ^{13}C -depleted compared with the other cores. Preliminary analyses of diatom data from core C4 show a greater abundance of riverine taxa compared with the other cores (Haynes, pers.com.). The reason for the apparent influx of more freshwater at the core C4 location compared to the more proximal location of core C1, and this deviation is most likely due to freshwater influx, as suggested by a greater abundance of riverine diatom taxa (D. Haynes, unpublished data).

In comparison with the isotopic data, grain size variations (normalised to organic carbon content to illustrate associations with organic matter) showed a distinct difference between the northern part of the North Lagoon (cores C1 and C4) and its southern part (cores C9 and C13) (Fig. 9). While the northern part was dominated by the silt ($10\text{--}53\text{ }\mu\text{m}$) fraction, the southern part had a much greater proportion of fine silt and clay. This difference is most likely related to different depositional environments. The Coorong is characterised by significant wind-induced mixing events (Webster 2005), which would spatially influence the deposition and resuspension of sediments. A larger proportion of the silt size-fraction in the southern end of the North Lagoon suggests that wind-induced mixing is lesser there and that sedimentation rates are larger. This is also supported by sedimentation rates estimates by Gell and Haynes (2005). The South Lagoon displays a similar trend with silt-size fractions dominating its northern part and finer fractions becoming more abundant in the southern area.

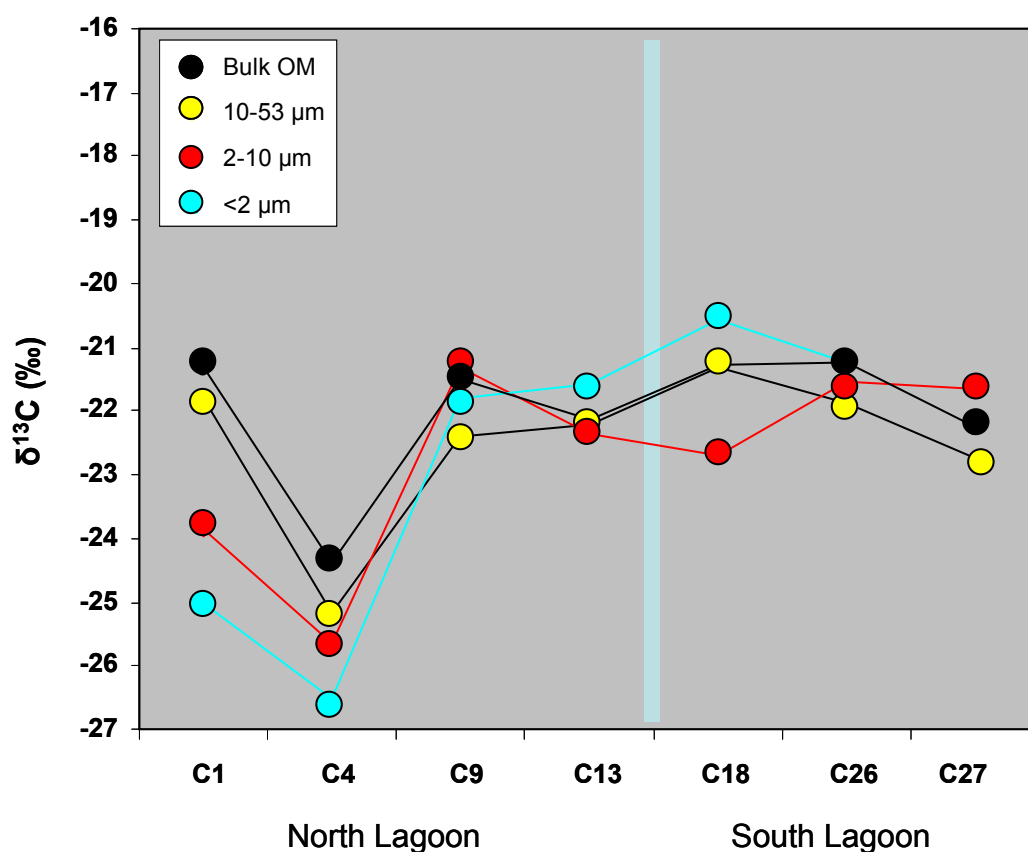


Figure 8 $\delta^{13}\text{C}$ values for the recent sedimentary organic matter in the North and South lagoons

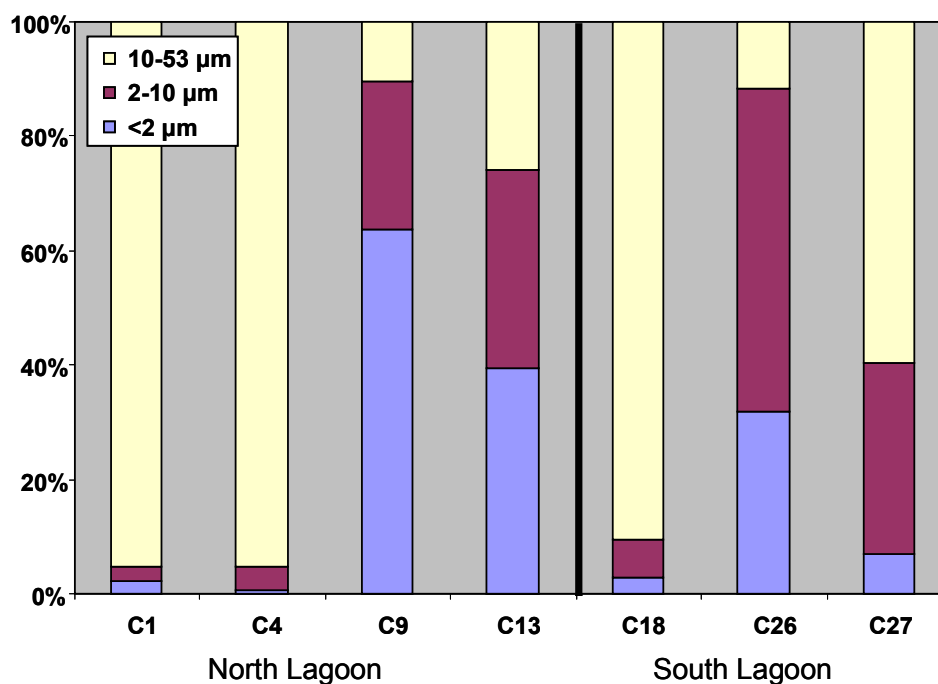


Figure 9 Size fractions of recent sediments (normalised for organic carbon content) in the Coorong lagoons. Note that these data do not include the >53 µm fraction.

3.1.5. ¹³C-NMR

Application of the molecular mixing model to ¹³C-NMR data from modern and pre-*Pinus* sediments allows a more detailed and quantitative analysis of the compositional organic matter changes than mere comparison of NMR spectra of different sources. Figure 10 shows the results of applying the MMM to NMR data obtained from surface sediments from the North and South Lagoon. The data show that organic matter in both lagoons is dominated by lipids, carbohydrate and protein. Only the northern part of the North Lagoon, which is closest to the Murray Mouth, contains a small amount of lignin. The relative contribution of lipid-containing organic matter increases in the southern portion of the North Lagoon and dominates the organic matter composition in the South Lagoon. In order to assess what the predominant contribution to the sedimentary organic matter may have been and how it changed along the long axis of the Lagoons, we compared these data with some of the organic matter sources that are currently present in the Coorong environment (e.g. macrophytic algae (*Gracilaria*, *Myriophyllum*) reed grasses (*Typha*, *Phragmites*), filamentous algae, halophytes (*Samphire*), soil organic matter and plankton). Figure 11 shows a comparison of NMR spectra of some of these sources with the spectra of surface sediment from the North Lagoon. While spectra from charcoal-containing soil organic matter, macrophytic algae (*Gracilaria*) and aquatic plants (*Lepilaena*) do not show a large resemblance to the sediment, degraded phytoplankton (NMR spectra from Hedges et al. [2002]) appeared to have the greatest similarity to the surface sediment organic matter.

This suggests that the current sedimentary organic matter is to a large degree plankton-derived with little contribution from other sources.

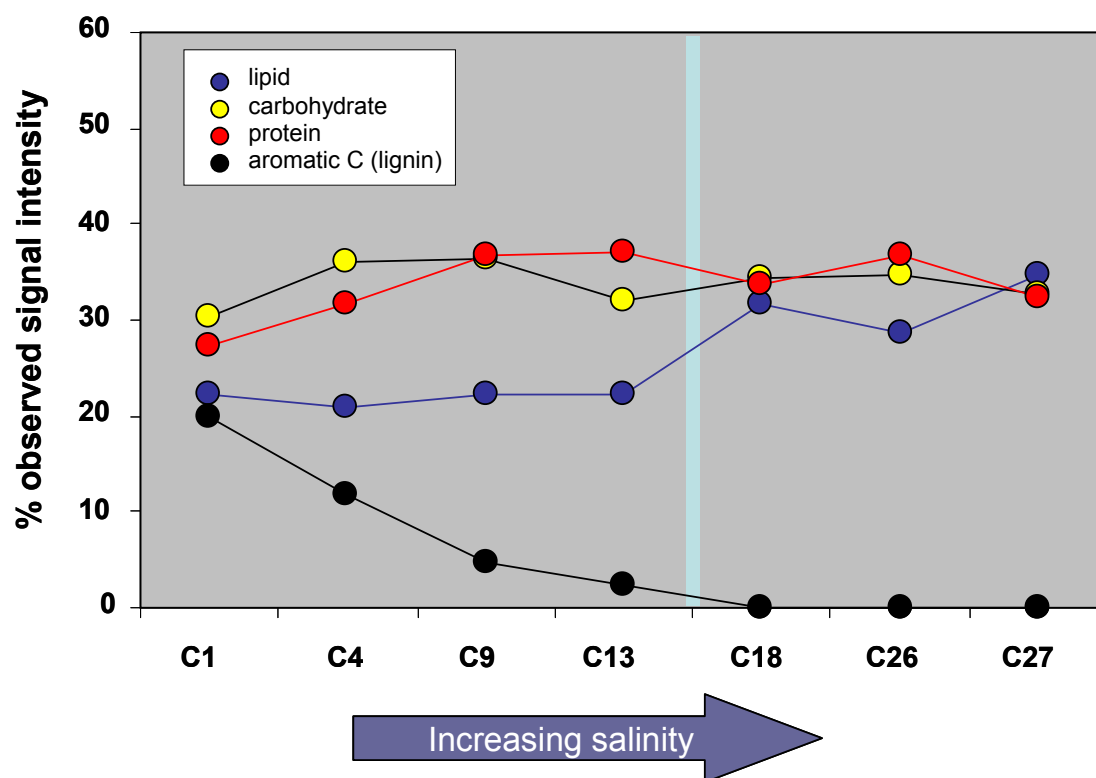


Figure 10 Relative abundance of major organic matter components of surface sediments from the North and South lagoons. The vertical line represents the approximate boundary between the northern and southern parts. Data are based on spectral intensity of NMR spectra and subsequent application of the molecular mixing model (Baldock et al., 2004).

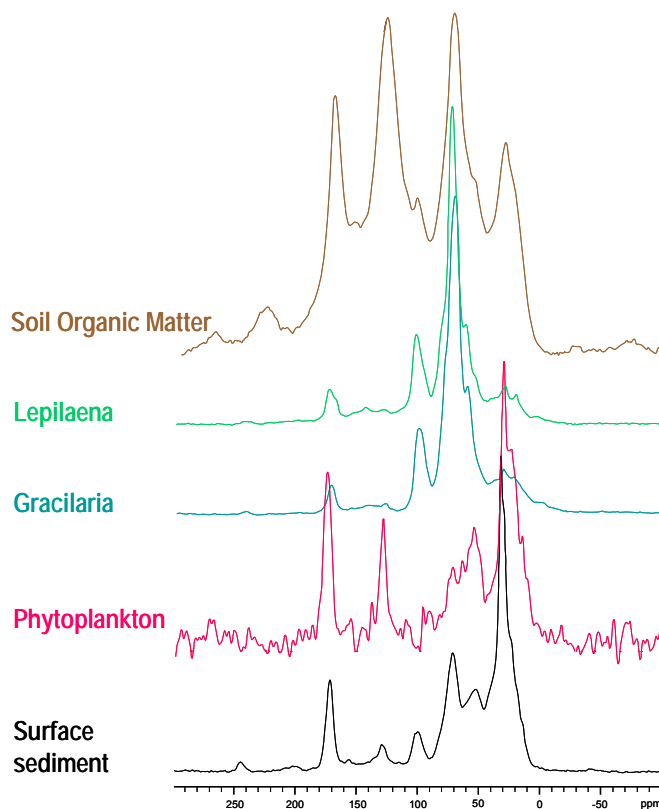


Figure 11 ^{13}C -NMR spectra of different sources of organic matter in comparison with sedimentary organic matter from the North Lagoon

The data shown in Figure 10 suggest a trend of changing OM composition with increasing salinity within the North Lagoon and between the North and South Lagoon. The model results indicate the presence of a small proportion of aromatic C in the cores proximal to the Murray Mouth and a declining (and eventually insignificant) proportion in more distal cores in the North and South lagoons. Similar trends, yet not as pronounced, are observed for the proportion of carbohydrates and proteins. The proportion of lipids, on the other hand, tends to increase with increasing salinity (with the exception of core C1) and is highest in the southern part of the North Lagoon at core C13. The increase in lipidic material may be a reflection of lipid-rich, plankton-derived organic matter becoming more predominant in the sedimentary OM. This restriction in ecological diversity within the long axis of the Coorong has been observed by an ecological assessment of macrobenthic abundance and diversity by Dittman et al. (2006). They documented much higher macrofaunal abundance in the North compared with the South Lagoon; in fact, parts of the South Lagoon were devoid of macrofauna. Within the North Lagoon, they observed a pronounced drop in species and diversity in the area of south of the location of core C9. During the summer, salinities in these areas north of core C9 varied between 35-40 ppt (normal marine) and increased to 100 ppt (hypersaline) in the area of core C13. Overall, the ^{13}C -NMR analysis of recent Coorong sediments suggest that a greater number of different sources of organic matter contribute to the North than the South Basin sediment pool.

3.2. Trends in sediment organic matter in pre-*Pinus* sediments

3.2.1. Bulk geochemistry

Elemental ratios (e.g. C/N, C/P and N/P) can provide information with regard to the source of organic matter to a sediment pool and can give some indications on the nutrients likely to limit primary production in the system. For example, sediments with higher C/N and C/P ratios are expected when terrestrial sediment influx are significant, and high N/P and C/P ratios may indicate that primary production is P limited (Ågren, 2004; Kähler and Koeve, 2001; Ruttenberg and Goni, 1997).

The geochemical trends of bulk sedimentary organic matter across the two lagoons mostly do not show significant variations between the present and the period immediately below the *Pinus* horizon (see discussion in 3.1 on timing of *Pinus*). The main differences occurred between the two lagoonal systems and within the long axis of the North Lagoon. C/N ratios were relatively constant within the North Lagoon and decreased in the South Lagoon (Fig. 12).

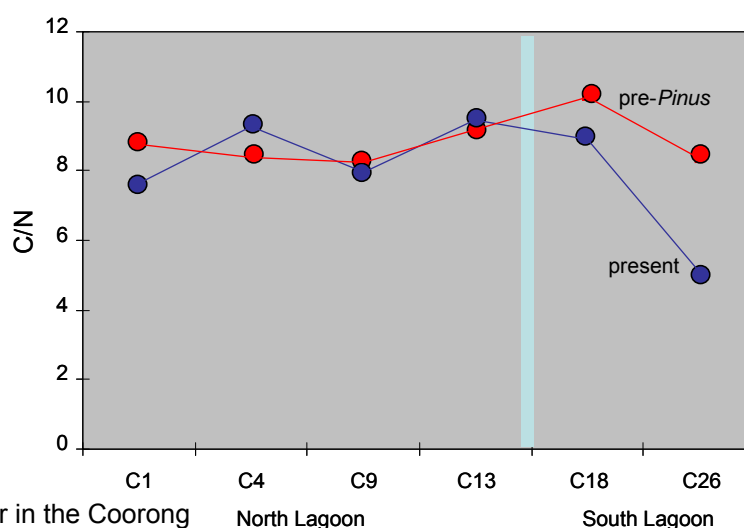


Figure 12 C/N ratios in organic matter from modern and pre-*Pinus* deposits

C/N ratios of living (i.e. undegraded) marine phytoplankton vary around 6.6 (Redfield et al, 1963) and the observed decrease in the South Lagoon, being more pronounced in present-day sediments compared to pre-*Pinus*, suggests greater contribution from planktonic organic matter compared with the North Lagoon. N/P and C/P ratios increased within the North Lagoon and remained high or increased further within the South Lagoon (Figs. 13 and 14).

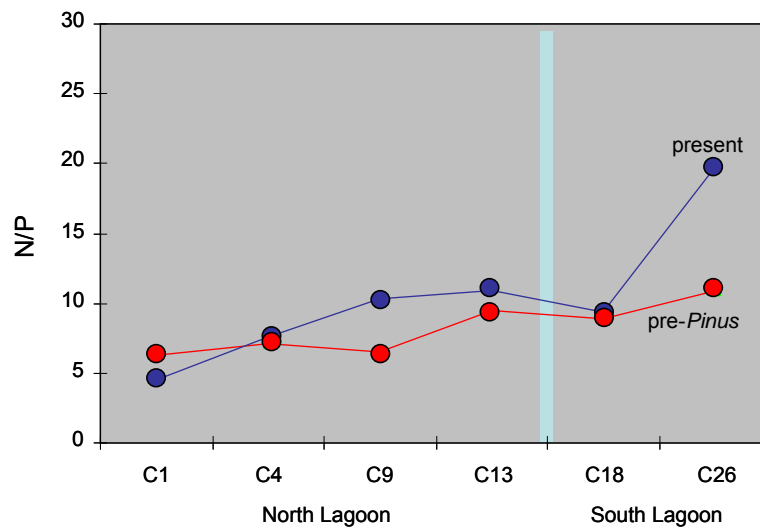


Figure 13 N/P ratios in organic matter from modern and pre-*Pinus* deposits

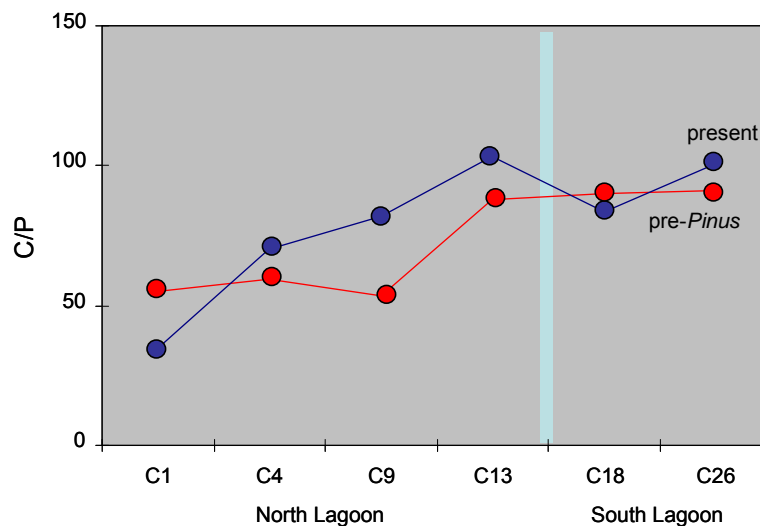


Figure 14 C/P ratios in organic matter from modern and pre-*Pinus* deposits

Overall, a greater rise was recorded in modern sediments compared to pre-*Pinus* intervals. These data suggest that the system may be becoming more P limited with increasing salinity within the long axis of the North Lagoon and the South Lagoon. Furthermore, while P-limitation appears to be more pronounced today, the trend towards greater P limitation in the South

compared with North Lagoon was also present prior to prominent changes to hydrology, thus appears to be – to a certain degree – inherent to the system.

With regard to C/N data, data from size-separated material proved to be more informative than the bulk data, which did not show significant changes within the North Lagoon in present compared with pre-*Pinus* conditions. The 10-53 μm and 2-10 μm fractions were similar to the bulk data, but the <2 μm fraction showed a significant decrease in ratios in the North Lagoon, with greater shifts observed in present compared with pre-*Pinus* sediments (Fig. 15). These data suggest that within the North Lagoon significant changes with regard to source organic matter have been occurring in the past and are occurring to an even greater degree today. The data suggest a trend towards more planktonic organic matter sources in the southern part of the North Lagoon, whereas the northern part appears to be more composed of a mixture of organic matter sources.

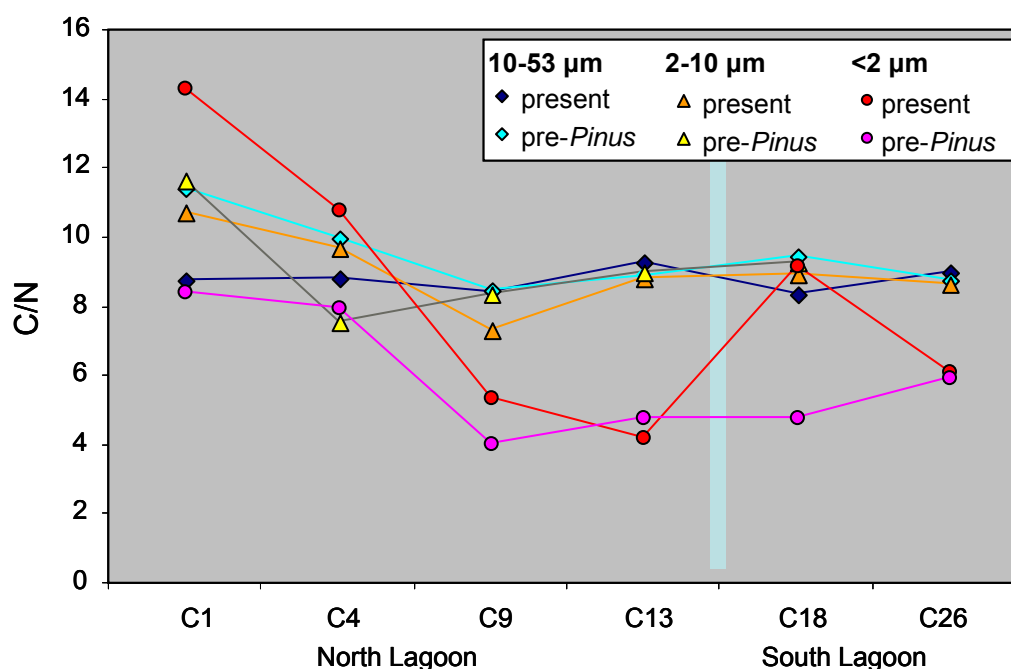


Figure 15 C/N in different organic size fractions from modern and pre-*Pinus* deposits

3.2.2. $\delta^{13}\text{C}$, particle sizes and ^{13}C -NMR

The $\delta^{13}\text{C}$ values of the organic matter deposited just below the first occurrence of *Pinus* differ significantly from those of the modern organic matter, with the pre-*Pinus* sediments being significantly ^{13}C -enriched (Fig. 16). Compared with the composition of the modern sediments (cf. Fig. 8), the differences between the individual fractions and between fractions and bulk organic matter are lower in the pre-*Pinus* sediments in the lower part of the North and all of the South Lagoon. However, a similarly ^{13}C -depleted isotopic value, as observed in core C4 in the modern sediments, is observed at core location C9 in the pre-*Pinus* sediments. Similar as discussed for the modern sediments (3.1.3), this excursion is most likely linked to influx from water sources with different (fresher) salinities. Alternatively, a different organic matter source or different composition of the community structure could produce the shift in isotopic values.

In the North Lagoon (excluding core C9), the average $\delta^{13}\text{C}$ value of bulk organic matter and size fractions was -19.7‰; in comparison, the average values in the South Lagoon were ^{13}C -

enriched by 1.2‰ (-18.1‰). Thus, the pre-*Pinus* sediments are not only overall ^{13}C -enriched compared with the modern sediments, but also indicate that a difference in organic matter sources or different chemo-physical constraints on organic matter fixation existed between the two lagoons.

Distribution of particle sizes in sediments occurring just below the *Pinus* line (Fig. 17) differs significantly from the distribution observed in recent sediments (cf. Fig. 9). Firstly, the distribution of size fractions is less variable across the two lagoons compared to the data of present-day sediments. Importantly, the distribution of size fractions, especially in the northern part of the North Lagoon is almost completely reversed with the $<2\ \mu\text{m}$ fraction dominating the sediment in C1 and the combined <2 and $2\text{--}10\ \mu\text{m}$ fractions making up the bulk of the sediment in the North and South Lagoons. Thus, these data suggest significant source and/or depositional changes as today's sediments in the northern North Lagoon are dominated by the coarser fraction. Sediments deposited prior to *Pinus* occurrence show a gradual trend of decreasing proportions of the clay-size ($<2\ \mu\text{m}$) fraction within the North as well as South Lagoon.

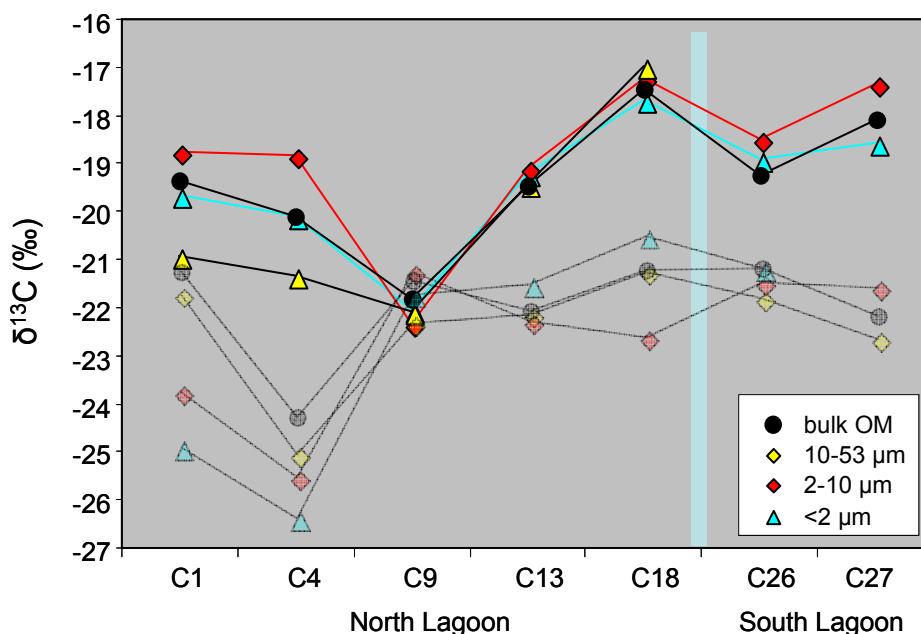


Figure 16 $\delta^{13}\text{C}$ values for the pre-*Pinus* sedimentary organic matter in the North and South lagoons. For reference, the data for the present-day organic matter (cf. Figure 9) are outlined in transparent colours.

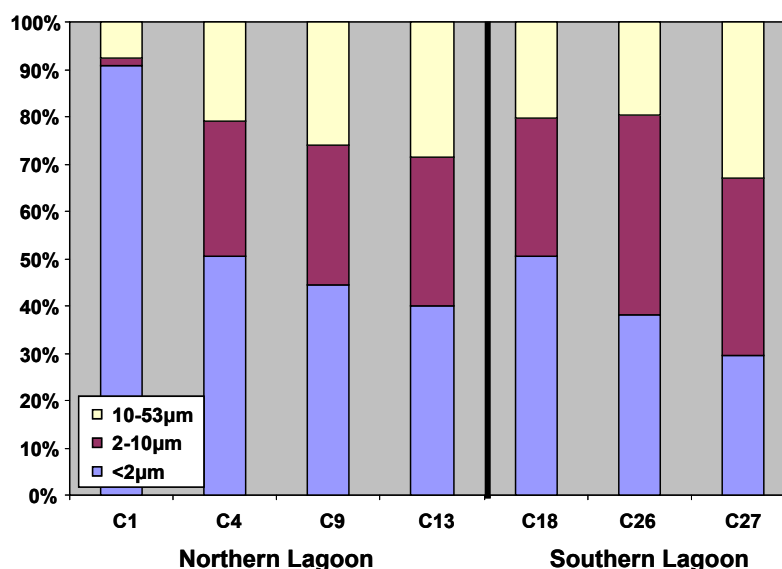


Figure 17 Size fractions of pre-*Pinus* sediments (normalised for organic carbon content) from the Coorong lagoons. Note that these data do not include the >53 μm fraction.

From these data, it is apparent that major biogeochemical and sedimentological changes have occurred in the Coorong prior to European development compared with today's conditions. However, the data do not provide information as to over what time period these isotopic and elemental changes were occurring and how gradual or abrupt they were. Figures 18 and 19 address these gaps by illustrating the isotopic changes occurring in the North and South lagoons in $\delta^{13}\text{C}$ shifts in bulk OM as well as in the size-separated fractions. The coloured horizontal lines indicate the first appearance of *Pinus* (Gell and Haynes, 2005 and Haynes, pers.com.) in the respective cores. The data show that shifts in $\delta^{13}\text{C}$ in bulk OM appears to coincide with the first appearance of *Pinus* in cores C4 and C13 in the North and in core C18 and possibly core C26 in the South Lagoon. For core C26, the position of the *Pinus* line could possibly be deeper than 16 cm and will have to await further analyses. In these cores, the first appearance of *Pinus* coincides with an isotopic shift from less to more ^{13}C -depleted values.

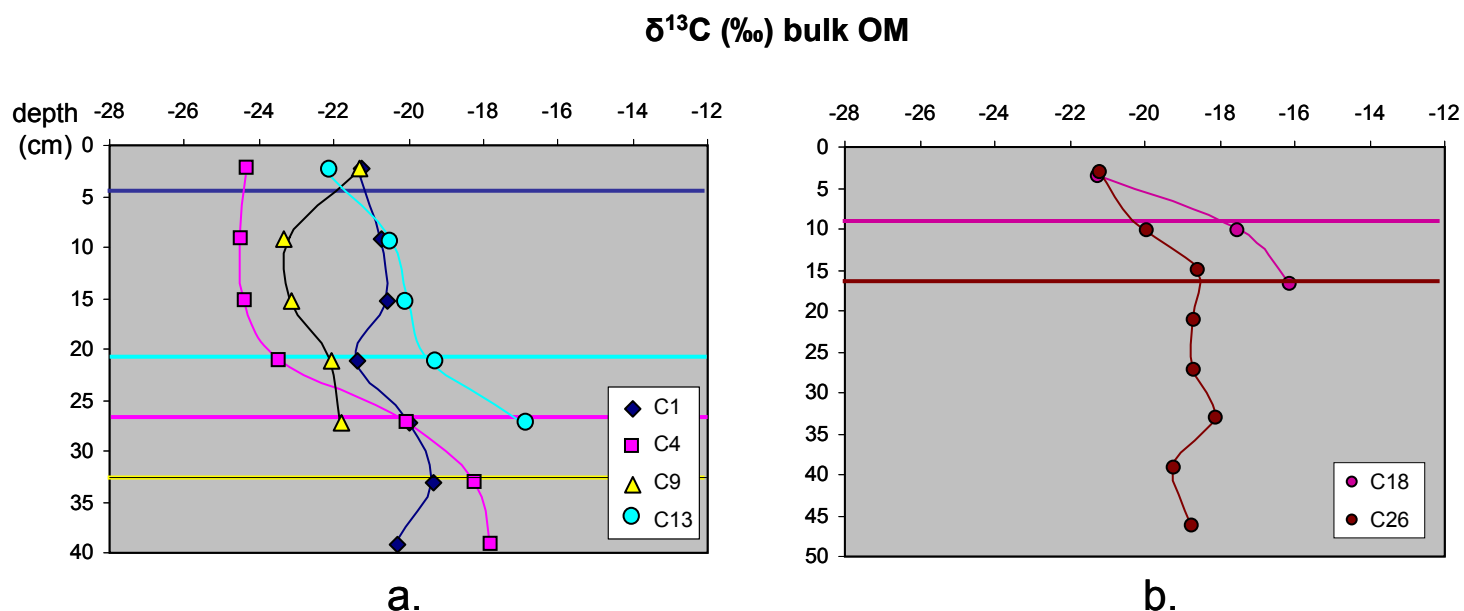


Figure 18 $\delta^{13}\text{C}$ depth trends in bulk OM in the North (a.) and South (b.) Lagoon

Further isotopic analyses of the individual size fractions proved to be more informative with regard to *Pinus* appearance and biochemical shifts. The data in figure 19a and b show that the isotopic shifts in cores C4 and C13 (where they had been observed in the bulk OM) were more pronounced in the fine silt (2-10 μm) and clay (<2 μm) fractions. Although the same fine fractions also show a significant isotopic shift in core C1 which did not show an unambiguous trend in the bulk OM, the shift occurs below the first occurrence of *Pinus*. Because *Pinus* occurrence can only approximate European development (not land management changes, such as barrage construction), these data need to be analysed in more detail by radionuclide analyses to assess the timing of the carbon isotopic shift. The first appearance of *Pinus* in core C9 occurs at 33 cm (Haynes, pers.com.), and even after $\delta^{13}\text{C}$ analysis of the size fractions, there does not appear to be a strong connection between palynological and isotopic data. Subsequent analyses of deeper parts of the core, however, revealed that the isotopic shift in core C9 is more gradual in comparison with the other cores from the North Lagoon (Figure 20)

$\delta^{13}\text{C}$ (‰) fractions

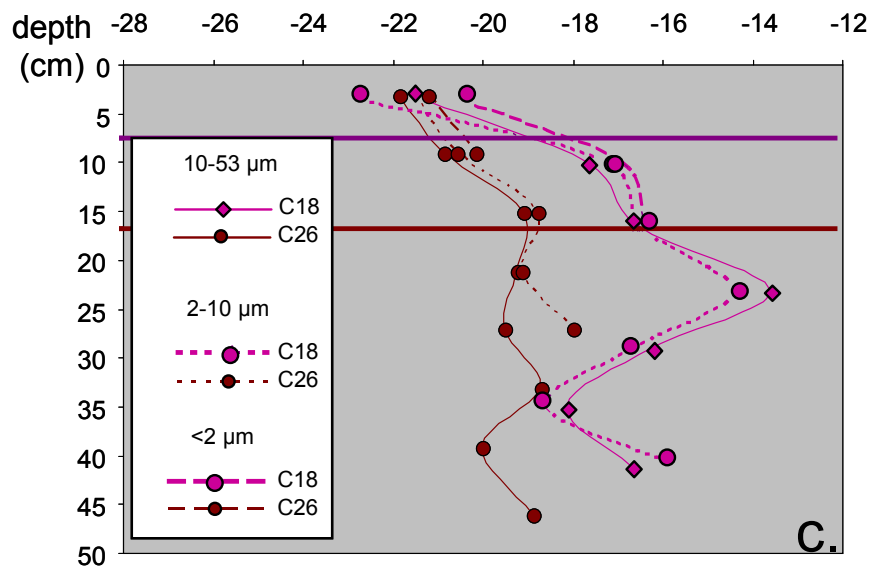
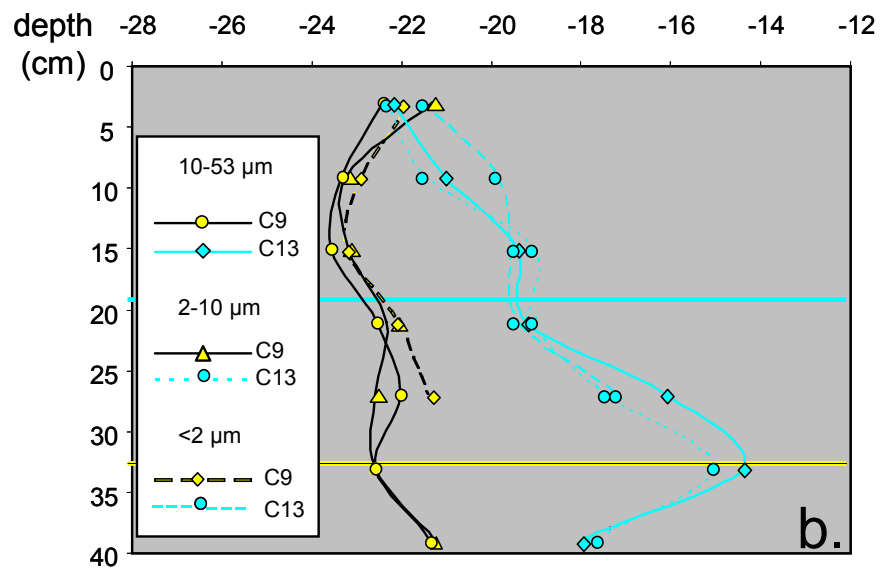
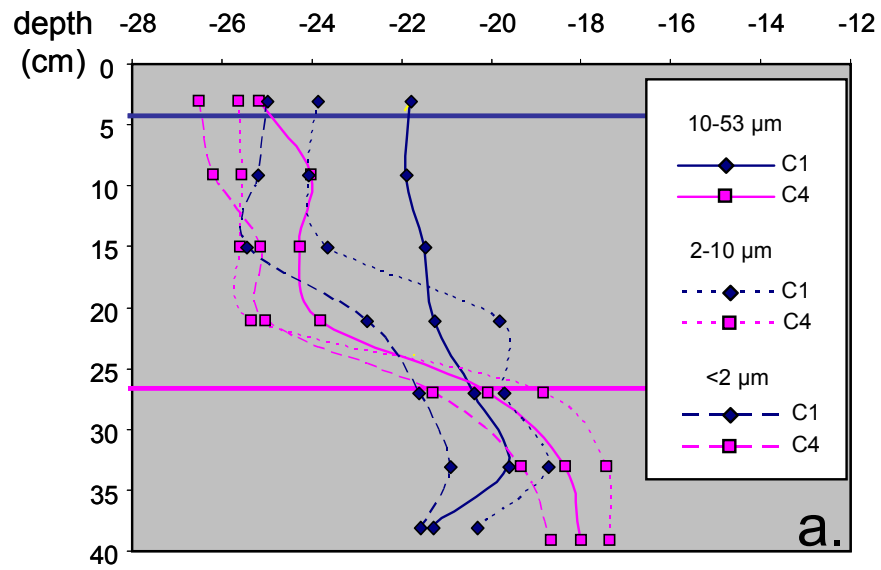


Figure 19 $\delta^{13}\text{C}$ depth trends in size fractions in C1 and C4 (a.), C9 and C13 (b) and C18 and C26 (c.)

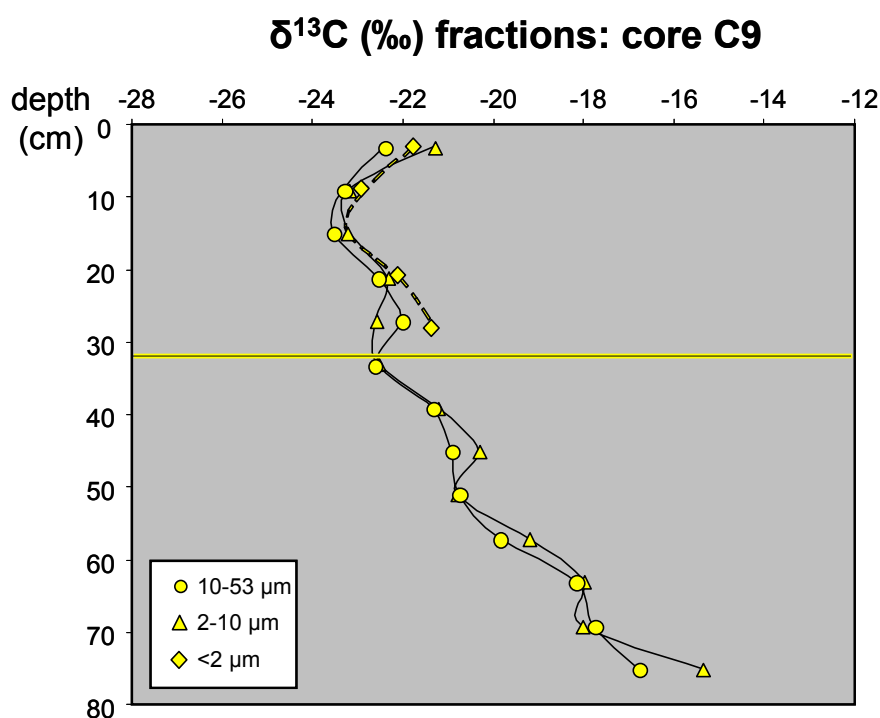


Figure 20 $\delta^{13}\text{C}$ depth trends in size fractions in deeper parts of core C9 (North Lagoon)

Hypothetically, it is possible that diagenesis may have been responsible for the shifts in isotopic values (and as such making the co-occurring *Pinus* purely coincidental) as burial and compaction may cause alterations in original isotopic values (e.g. Henrichs, 1992; Meyers and Ishiwatari, 1993; Spiker and Hatcher, 1984; Prahl et al., 1997 and Freudenthal et al., 2001; summarised in Fan et al., 2007). During burial, degradation of organic matter in sediments may lead to small changes in isotopic values, reported to be less than 2‰. During early diagenesis continued degradation of organic matter may result in a 0‰ to 4‰ decrease in the $\delta^{13}\text{C}$ values through the preferential removal of ^{13}C -enriched compounds. Lehmann et al. (2002) reported that in a lacustrine setting sediments are ^{13}C -depleted by about 1.5‰ in comparison with sinking organic matter. These isotopic changes are in agreement with a 1.6‰ decrease observed during a three months incubation-based diagenesis experiment (Lehmann et al., 2002). Thus, the negative isotopic shift from pre-*Pinus* to modern sediments is opposite to any isotopic changes observed during burial or diagenesis. Thus, we can be reasonably confident that the observed changes in isotopic values are genuine and not a result of post-burial alteration.

In order to determine the cause for the coinciding *Pinus* occurrence and ^{13}C -depletion in most of the cores of the Coorong, we examined the $\delta^{13}\text{C}$ and C/N ratios of potential sources and compared it with the isotopic data of the pre-*Pinus* and modern sediments (Fig. 21).

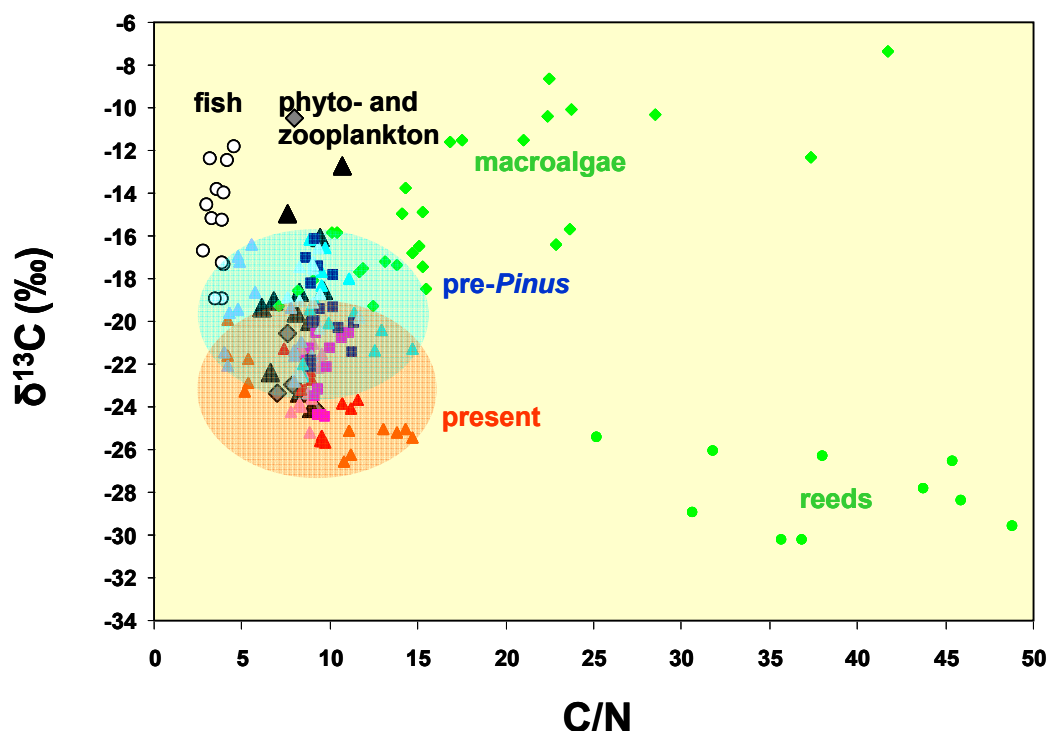


Figure 21 $\delta^{13}\text{C}$ and C/N data of modern and pre-*Pinus* sediments (size-separated and bulk) and potential organic matter sources. Blue and red squares indicate data from bulk sediments and blue and red triangles illustrate the finer sediment fractions. Black triangles are data from zooplankton and diamonds represent phytoplankton.

The results illustrated in Figure 21 do not point to an obvious cause for the isotopic shift. The greatest degree of overlap occurs between sediments and phytoplankton. However, given that shifts in C/N ratios from higher to lower values have been observed during burial and diagenesis (Meyers and Ishiwatari, 1993; Meyers, 1997 and Lehmann et al., 2002), it is difficult to determine how much reeds or macro-algae may have contributed especially to pre-*Pinus* sediments. As shown in Figure 11, based on ^{13}C -NMR data, the composition of recent sedimentary organic matter is dominated by planktonic sources.

A shift from ^{13}C -enriched to depleted values in aquatic sediments is commonly interpreted as a source change from less to more terrestrial-derived (^{13}C -depleted) organic matter. Thus, prior to the occurrence of *Pinus*, organic matter composition would be viewed as dominated by autochthonous sources and a shift towards ^{13}C -depleted values would indicate a greater proportion of allochthonous, possibly terrestrially-derived material. However, NMR data from recent sedimentary organic matter (Fig. 10) do not support the interpretation that modern sediment carries a significant proportion of terrestrially-derived material. Furthermore, as shown by the results in Figure 10, a terrestrial component would be represented by a significant portion of aromatic C (lignin), which only occurs in the northern half of the North Lagoon and even there, contributes only maximal 5% to the total OM composition. Thus, if the conventional interpretation of isotopic shifts within estuarine systems is correct, one would assume even lower amounts of aromatic C in pre-*Pinus* sediments and a greater proportion of lipids.

The results of the application of the MMM to sedimentary OM deposited just prior to the first occurrence of *Pinus* are summarised in Figure 22.

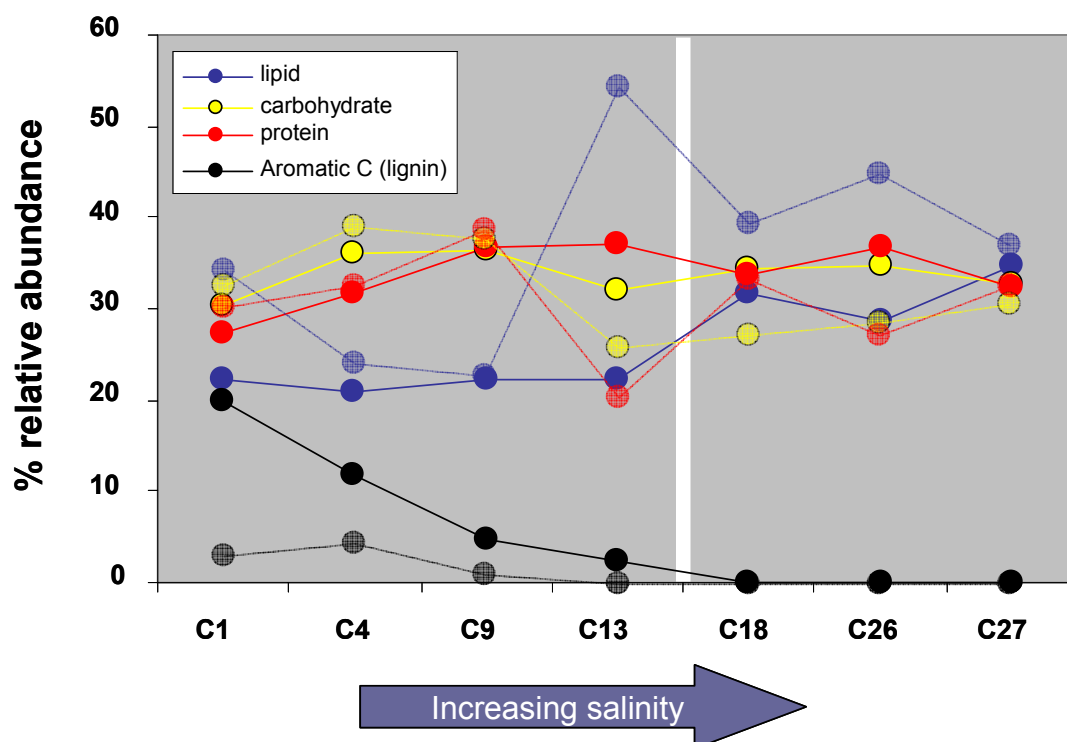


Figure 22 Bold colours represent the pre-*Pinus* OM composition (after application of the MMM to NMR data); transparent colours are the data shown in Figure 10, showing the composition of modern sediments in comparison

The results show a greater contribution (up to 20%) of aromatic C to sedimentary OM in the North Lagoon. Yet, the same declining trend with increasing salinity as observed today, can be seen in pre-*Pinus* sediments, and no contribution of aromatic C to sedimentary OM was detected in the South Lagoon. Along the two lagoons, trends in lipid, protein and carbohydrate abundance were more even compared with those observed in modern sediments. Still, there was a slight increase in lipid abundance in the South Lagoon; however, these levels are still below the ones observed today.

Thus, these findings appear to be at odds with the interpretation suggested by the isotopic results, implying a greater proportion of autochthonous material. Instead, the results from the NMR-based MMM suggest a greater contribution of aromatic C (lignin), which is customarily assumed to be terrestrial-derived and therefore relatively ^{13}C -depleted.

In order to resolve the reason and determine the possible source for more ^{13}C - and lignin-enriched OM in pre-*Pinus* sediments in the North Lagoon, additional autochthonous species (aquatic primary producers) as well as first-order consumers, besides the material shown in Figure 11, were obtained. These included the seagrass *Ruppia megacarpa*, which was once abundant in the Coorong but was recently extirpated.

The results of ^{13}C -NMR analyses and MMM application are shown in Figure 23.

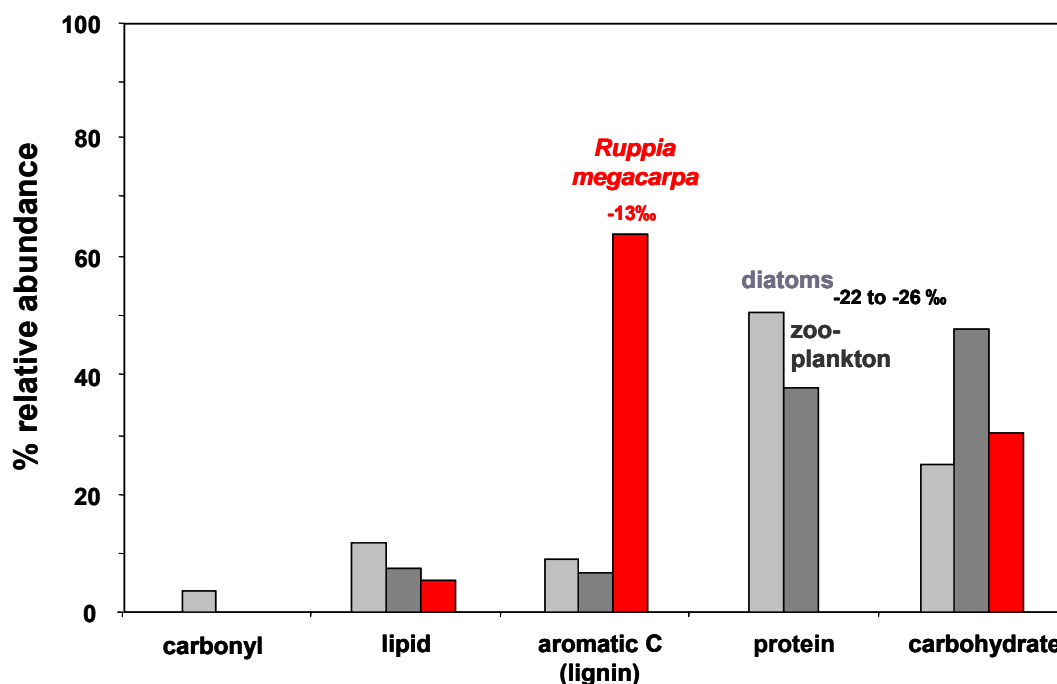


Figure 23 NMR-based MMM results of various aquatic primary producers, known to be or have been abundant in the Coorong

The data show that diatoms and zooplankton OM have low amounts of aromatic C. In addition, in these organisms the amount of aromaticity present is most likely associated with proteinaceous ring structures (e.g. as in DNA) rather than lignin. In comparison, aromatic C was the dominant constituent of *R. megacarpa*. Because *R. megacarpa* has evolved from terrestrial angiosperms, this seagrass species has maintained close to the original lignin content as observed in its terrestrial relatives. Thus, in this case, most of the aromatic C is indeed associated with lignin; however, due to the fact that photosynthetic carbon fixation is carried out in the aquatic environment, the $\delta^{13}\text{C}$ value of *R. megacarpa* is accordingly ^{13}C -enriched relative to terrestrial land plants (Fig. 23). By comparison, planktonic species such as diatoms and zooplankton tend to be relatively ^{13}C -depleted. Thus, the apparent conundrum of both lignin- and ^{13}C -enriched organic matter in pre-*Pinus* sediments can be explained by the larger contribution of seagrasses to Coorong organic matter. The possibility that seagrasses are the 'missing' source of carbon is plausible because it is clear from historical evidence that *R. megacarpa* and other seagrasses were once abundant in the Coorong (Phillips and Muller 2006).

3.3. Comparison with other palaeolimnological studies of the Coorong

The changes in the Coorong environment between the pre- and post-*Pinus* period observed in sediment organic matter are consistent with the inferences made using diatoms in other studies (Gell and Haynes, 2005; Haynes et al., 2007). A significant shift in sediment type is observed in all cores, relating to the change from dark grey or black clay (most likely due to anoxic conditions) to fine silt. This shift often coincides with the first occurrence of *Pinus* and occurs at

26 cm at core C4 – the same interval where the shift from ^{13}C -enriched to depleted isotopes occurs. Similarly, a significant shift in diatom assemblages is observed, with pre-*Pinus* assemblages being dominated by marine species; however, variable salinities are inferred by the fluctuating occurrences of *Campylodiscus* species. Following the occurrence of *Pinus*, the assemblage becomes dominated by a previously under-represented group (*Fragilariaceae*, represented by *Stauroseira* and *Staurosirella* species). The likely reason for the pronounced shift in diatom assemblage is not indicative of changes in salinity but increases in turbidity. The lack of significant amount of freshwater diatoms (*Aulacoseira*) suggests that the Coorong lagoons have always had a strong degree of marine influence and were probably never completely fresh.

As reported in Webster (2005, 2006), based on hydrological modelling, the biochemical data suggest that even prior to European development and management changes, the two lagoons were never connected to the degree that allowed thorough mixing of the two systems. Instead, even in the absence of human-induced hydrological changes, the North Lagoon appears to have had a greater degree of OM variability compared with the South Lagoon and probably a more diverse ecosystem.

4. Conclusion

The combined application of isotopic, elemental and spectroscopic analyses to Coorong sediment organic matter revealed that algae are the main primary producer in the system. However, the North Lagoon also appears to have a significant input of organic matter from other sources, possibly seagrasses such as *Ruppia megacarpa*. This additional source was making a larger contribution to the sediment organic matter pool in pre-European times, consistent with the extirpation of some *Ruppia* species from the Coorong observed during the last decades. There is little evidence for a terrestrial organic matter signal in Coorong sediments, suggesting that the River Murray or the Upper South-East region are not significant sources of organic matter to the Coorong. In the case of the River Murray, it is possible that most organic matter inputs during floods bypass the Coorong and are discharged to the sea. However, catchment inputs do influence what kind of organic matter is produced within the Coorong through their influence on the salinity regime (see review by Webster [2005] on the role of floods on the hydrodynamic regime of the Coorong). In addition, nutrient inputs (either in organic or inorganic forms) from the River Murray and the Upper South-East region could influence primary production rates and the form of primary production in the Coorong.

These results have significant implications for the management of the Coorong. They do support the hypothesis that the loss of food resources that has accompanied hypersalinisation is an important driver of the Coorong ecosystem decline. This is particularly true for waterbirds, which are not directly affected by elevated salinities but are indirectly impacted when their food resources are.

This study was a first attempt at characterising organic matter inputs to the Coorong. A number of recommendations can be made to further improve on the inferences made. These include:

- To use additional biomarkers (pigments, lipids, etc) to identify 1) what algal groups contribute to organic matter production in the Coorong and 2) to identify what functional forms are significant (that is, phytoplankton, macrophytobenthos, epiphytes, etc)
- When possible, to analyse cores at finer intervals to gain additional resolution in the temporal variability in organic matter inputs to the Coorong
- To repeat a similar analysis in the Lower Lakes to see if they trap a large part of organic matter inputs from the Murray-Darling Basin before they either get discharged to the Coorong or the sea.

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5. Appendix 1: Molecular genetic analyses

Introduction

Microorganisms, whether in soils, sediments or water, are intimately involved in the biogeochemical transformation of many key nutrients. Measuring process rates of these transformations can reveal a lot of information regarding the functioning of an ecosystem at a point in time. Moreover, assessing the diversity of the microorganisms involved in the process could give a good indication of the stability for the process to occur over time, and could potentially be used as an indicator for ecosystem deterioration. The 'insurance hypothesis' (Loreau et al., 2001) states that community diversity and species richness are linked to ecosystem stability and functioning through their buffering effect towards disturbances. These disturbances can be both natural and anthropogenic. Such concepts have been applied to soil microbial ecology at various levels, including concepts such as soil health (i.e. the ability of a soil to function) but likewise can be used in aquatic microbial ecology.

A good measure of the potential activity of a microbial ecosystem may be found in functional diversity (as opposed to broad phylogenetic diversity), particularly when assessed across a range of key functional groups. For example, the nitrification process (oxidation of ammonium to nitrate), a key process within the N cycle, can be studied in great detail using the *amoA* gene, encoding for a conserved subunit of the highly specific enzyme ammonia monooxygenase (AMO). Other molecular tools like Denaturing Gradient Gel Electrophoresis (DGGE) or Terminal Restriction Fragment Length Polymorphism (TRFLP) can be applied further to investigate the diversity of the microbial communities performing this particular function within the ecosystem. Recently, these techniques have been applied in aquatic systems and a loss of diversity of ammonia-oxidizing bacteria (AOB) was found with increasing salinity levels in an estuary system (Bernhard et al., 2005). In this particular study, the diversity was assessed using TRFLP.

In the current study, we want to explore this response in the Coorong ecosystem, where a clear gradient of salinity is present throughout the year moving from the North to the South lagoon. Therefore, DNA was extracted from several sediment samples along this salinity gradient and the diversity of the ammonia-oxidizing bacteria (AOB) was assessed using PCR to specifically amplify a portion of the *amoA* gene and DGGE analysis to explore diversity within the amplified fragments.

Methods

Sampling

Samples of sediments were taken from 5 locations along the North and South Lagoon of the Coorong in November 2006, respectively at Pelican Point, Mark Point, Long Point, Parnka Point and Jacks Point (see Figure 1). At each location, four replicate sediment samples were taken from 0 to 5 cm depth within a 100 m² area using a hand-held plastic coring device which could be closed under water. Sediment samples were all taken under a water column of 50 cm. Upon sampling, sediments were placed on dry ice until delivery to the laboratory within 6 hours whereupon samples were stored at 4°C prior to DNA extraction.

DNA extraction

DNA extractions were made in duplicate from each of the 4 replicate samples using the PowerSoil™ DNA extraction kit (MoBio Laboratories, CA). A FastPrep cell disruptor (Bio101) was used to enhance the extraction efficiency of DNA from the sediment samples. DNA from the duplicate extractions was pooled into a final volume of 200 µL in 10 mM Tris buffer. The isolated DNA was stored at -20°C.

PCR-DGGE

The community structures of the AOB in the Coorong sediment were analysed by PCR-DGGE profiling of the *amoA* gene. *AmoA*-specific PCR primers *amoA*-1F and *amoA*-2R* were used (Stephen et al., 1999). To avoid complete denaturing of the amplified fragments during DGGE, a 33-bp GC-clamp (5' CCG CCG CGC GGC GGG CGG GGC GGC GGC ACG GGG 3') was attached to the 5' end of the *amoA*-1F primer. PCR amplification was performed in a total reaction volume of 25 μ L, and used: primers at 10 mM each, dNTP's at 2.5 mM each, 1 U of Qiagen HotStar Taq DNA polymerase, 2.5 μ L of PCR buffer, 2.5 ng of BSA (bovine serum albumin), and 2 μ L of template DNA. The PCR cycle used a 15 min start at 94°C, followed 35 cycles of denaturation at 92°C for 1 min, annealing for 1 min at 50°C and extension at 70°C for 1 min. Confirmation of PCR was achieved by agarose gel (1.5%) electrophoresis of 4 μ L of each PCR mix, staining with ethidium bromide and visualising under UV light. The remaining reaction mix was used for DGGE analysis.

DGGE analysis of amplified bacterial *amoA* genes was performed on the Ingeny PhorU system (Ingeny International, The Netherlands). The polyacrylamide gels (8% w/v of acrylamide:bis-acrylamide at 37.5:1) contained a linear formamide/urea gradient ranging from 40% to 70% and were overlaid with a non-denaturing stacking gel. Each well contained 10 μ L of PCR product and 4 μ L of 2 \times loading buffer. Electrophoresis was conducted at 110V for 17h, after which the gels were stained with SYBR Gold (Molecular Probes) for 40 min, rinsed with H₂O and visualised on a Dark Reader (Clare Chemical Inc.). An Olympus E500 SLR digital camera was used to photograph stained gels.

Position and intensity data of bands on the DGGE gel were captured using TotalLab Version 2005 (Nonlinear Dynamics Ltd.). Band position (*amoA*-genotype) and intensity (abundance) data were collected. Band intensity data was 4th-root transformed and a resemblance matrix generated using the Bray-Curtis method using Primer5 software package (PrimerE Ltd.) with methods described and discussed by Clarke and Warwick (2001). Simpsons index was used to quantify diversity of bacterial *amoA* genotypes from each of the sampling locations.

Results and Discussion

The variation in the composition of the AOB communities was lower within sampling sites compared to across sites (Fig. A1). This clearly indicates the robustness of using the *amoA* gene as a functional molecular marker for environmental changes in the Coorong ecosystem. It must be noted that the diversity within sites increased with increasing salinity. This is indicated by the lower degree of similarity at both Parnka and Jacks Point with respectively 30% and 70% similarity, compared to the sampling sites of the North Lagoon (Pelican, Mark and Long Point) which all showed similarities of 90% or more within the sampling site (Fig. A1). The largest change in AOB community composition between sites occurred at the transition of the North to the South Lagoon (20% similarity, Fig. A2).

The diversity of the AOB communities (Simpson's diversity index) based on DGGE of *amoA* banding profiles differed significantly ($P < 0.001$) across sites (Fig. A2). The lowest diversity was found in Jacks Point (South Lagoon, highest salinity) and was significantly lower than all other sampling sites (Tukey's multiple comparison test, $P < 0.05$). This decrease in diversity coincided with an increase in salinity (Fig. 6) often even up to hyper saline conditions which are regularly found in the South Lagoon. Therefore, this group of bacteria with established ecosystem functions were directly impacted in both composition of species in the community and overall diversity.

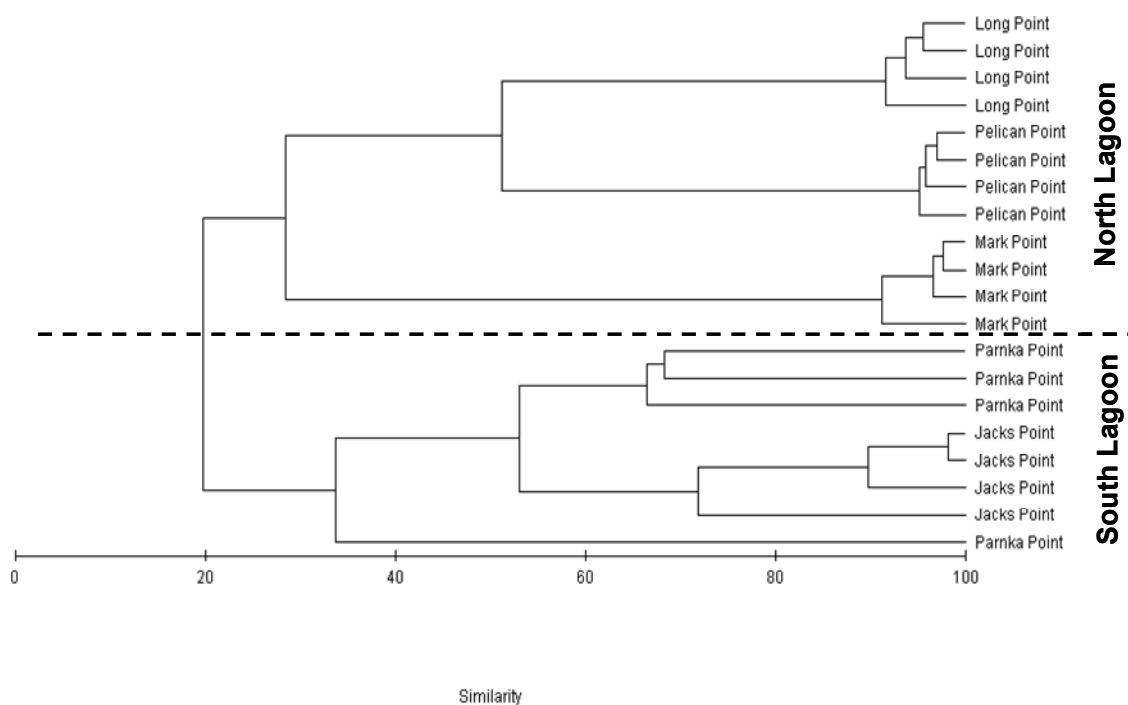


Figure A1 Cluster plot grouping of similarity between AOB community structures in the sediment from the Coorong. Community structures were assessed via PCR-DGGE of the *amoA* gene. Resemblance matrix was generated using the Bray-Curtis similarity algorithm on 4th-root transformed abundance (band intensity) data.

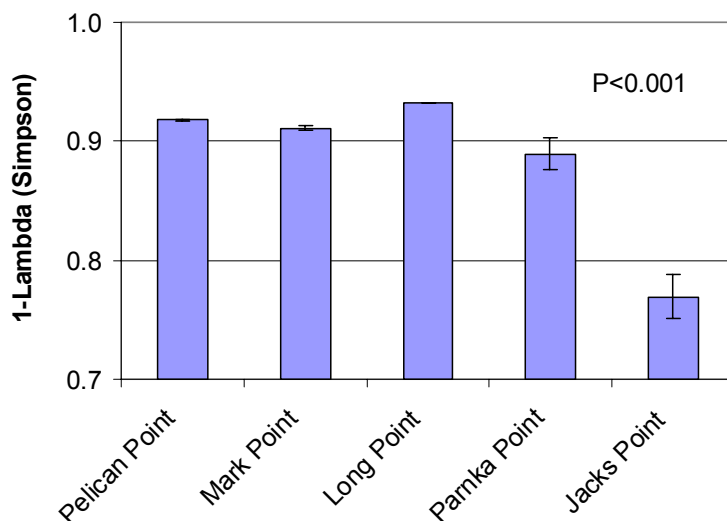


Figure A2 Simpson diversity index of the ammonia-oxidizing bacterial (AOB) community. Data based on *amoA* DGGE banding profiles. Error bars are representing standard errors of 4 replicate sediment samples.

In summary, our study supported the findings of Bernhard et al. (2005) who suggested that salinity is a strong environmental driver for AOB diversity. The stress caused by the increasing salinity clearly decreased the diversity within the AOB communities present. As such, this renders these communities more vulnerable to effects of additional stressors. These findings also support the findings by Dittmann et al. (2006), indicating that the trends in diversity and abundance, seen in bacterial communities, are transferred up the food chain.

