Effect of Nutrients and Salinity on Growth of Temperate Mangroves (*Avicennia marina* var *australasica*) in Northern New Zealand

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Abstract

Estuarine environmental conditions in New Zealand have changed greatly due to human catchment activity (e.g., deforestation, intensive agricultural activity, and urbanisation). These factors have led to additional accretion of sediment (sedimentation) and nutrients (eutrophication) throughout New Zealand waterways and coast waters. In recent years, New Zealand mangroves (*Avicennia marina* var *australasica*) have shifted their distribution within estuaries and rapidly spread into areas where they have never been found before. Many local communities and councils are worried that mangroves have replaced sandy bare tidal flats and other estuarine habitats (i.e., seagrass beds, marshlands) and will turn them into muddy sites. Researchers have proposed several reasons for the spread of temperate mangroves, including estuary infilling, increased nutrient inputs, climate warming, changes in sea level and a combination of some of these factors. Indeed, it has been shown that increased sedimentation correlates well with rapid mangrove accretion in New Zealand through the emergence of additional mangrove habitat space, but the effect of the nutrient uploads has received less attention. Additionally, there is almost no information on the salinity levels that are characteristic for temperate New Zealand mangrove ecosystems. Indeed, it is well documented that salinity is an important controlling factor for mangrove growth in tropical mangrove ecosystems, but there is almost no such studies in temperate mangrove areas.

Current research is highly relevant to on-going controversial discussions regarding management *versus* conservation of New Zealand mangroves, because it provides the review and experimental work on the cycling of nutrients in temperate mangrove and ecosystems as well as how salinity levels affect mangrove growth. This study presents previously missing information on the sources of nutrients in New Zealand estuarine ecosystems, as well as how these nutrients are conserved and stored in below ground biomass of *A. marina*. Field fertilisation experiments were conducted to describe nutrient availability as a primary driver for the difference in growth forms of mangrove plants (tall plants at the edge and stunted inland) in temperate New Zealand conditions. Controlled laboratory experiments were conducted to demonstrate how nutrient availability changes metabolite profiles of individual mangrove plants. The present research also provides novel information on how seasonal changes in salinity distribution patterns across the intertidal gradient in temperate mangrove ecosystems affect sodium composition of mangrove leaves. Results of the growth trial also suggest that moderate salinity has beneficial effects on *A. marina* seedling growth.

Overall, results suggest that a unique combination of factors can increase growth and spread of temperate mangroves in estuarine and coastal territories in northern New Zealand. One of the most important factors is the cooler and wetter New Zealand climate, which is, due to high precipitation rate and low temperature. These conditions result in lower salinity levels, which are beneficial for *A. marina* growth. Another factor impinging on these mangroves is the natural nutrient deficiency state of these coastal ecosystems, and anthropogenic influences. These anthropogenic influences are mainly due to the increasing nutrient input over the past 100 years, originating from fertilisation, livestock urine runoff from dairy and meat farming, and human sewage inputs, which promotes growth and survival of mangrove seedlings. In addition, it can be concluded that the presence of mangrove plants at the interface between anthropogenically affected terrestrial lands and coastal ecosystems may mangrove habitats may act as nutrient sinks, thus mitigating coastal and marine eutrophication.

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Attestation of authorship

I hereby declare that this submission is my own work and that, to the best of my knowledge and belief, it contains no material previously published by another person (except where explicitly defined), nor material which to a substantial extent has been submitted for the award of any other degree or diploma of a university or other institution of higher learning.

Iana Gritcan

Co-authored works

Chapter 2. Dynamic nutrient partitioning of temperate *Avicennia marina* var *australasica* mangrove species in New Zealand (Tran *et al.*, 2016, New Zealand Journal of Marine and Freshwater Research)

Author	**Contribution**	**Total, %**	**Signature of authors**
Phan Tran	Experimental design Data collection and field work Statistical analysis Data interpretation Writing	60	
Iana Gritcan	Data collection and field work Statistical analysis Data interpretation Writing	20	
Jarrod Cusens	Data collection and field work Writing Review/edit	3	
Andrea C. Alfaro	Writing Review/edit	3	
Sebastian Leuzinger	Data collection and field work Data interpretation Writing Review/edit	14	

Chapter 3. Effect of anthropogenically derived nutrients on temperate mangrove nutrient status among three New Zealand harbours with contrasting human activities (Gritcan *et al.*, 2016, Frontiers in Plant Science)

Author	**Contribution**	**Total, %**	**Signature of authors**
Iana Gritcan	Experimental design Data collection and field work Statistical analysis Data interpretation Writing	80	
Mark Duxbury	Experimental design Data collection and field work Data interpretation Writing Review/edit	10	
Andrea C. Alfaro	Experimental design Review/edit	5	
Sebastian Leuzinger	Experimental design Review/edit	5	

Chapter 5. Salinity and nutrient levels in temperate New Zealand mangroves under natural conditions and effect of nutrient addition on mangrove growth in the field (Gritcan *et al.*, 2018, *in submission*)

Author	**Contribution**	**Total, %**	**Signature of authors**
Iana Gritcan	Experimental design Data collection and field work Statistical analysis Data interpretation Writing	80	
Mark Duxbury	Experimental design Data interpretation Review/edit	6	
Martin K.-F. Bader	Statistical analysis Data interpretation Review/edit	6	
Andrea C. Alfaro	Experimental design Review/edit	4	
Sebastian Leuzinger	Experimental design Review/edit	4	

Publications and conference presentations

Publications

Phan Tran, Iana Gritcan, Jarrod Cusens, Andrea C. Alfaro & Sebastian Leuzinger (2016). Biomass and nutrient composition of temperate mangroves (*Avicennia marina* var. *australasica*) in New Zealand, *New Zealand Journal of Marine and Freshwater Research*, doi:10.1080/00288330.2016.1260604

Iana Gritcan, Mark Duxbury, Sebastian Leuzinger and Andrea C. Alfaro (2016). Leaf stable isotope and nutrient status of temperate mangroves as ecological indicators to assess anthropogenic activity and recovery from eutrophication, *Frontiers in Plant Science*, doi:10.3389/fpls.2016.01922

Iana Gritcan, Mark Duxbury, Martin K.-F. Bader, Sebastian Leuzinger and Andrea C. Alfaro (2018). Nutrient availability, not salinity, limits growth of *Avicennia marina* var. *australasica* mangroves in temperate estuaries. *In submission.*

Conference presentations

November, 2013. **EcoTas'13, Student day, Auckland, New Zealand.**
Presenter.
Effect of nutrients on growth, productivity, and the potential spread of temperate mangroves in Mangawhai Harbour Estuary, northern New Zealand.
Gritcan I., Andrea C. Alfaro, Leuzinger S.

November, 2014. **New Zealand Coastal Society Conference, Raglan, New Zealand**
Poster.
Effect of salinity and nutrients on growth, productivity, and spread of temperate mangroves in Mangawhai Harbour Estuary, northern New Zealand
Iana Gritcan, Andrea C. Alfaro, Sebastian Leuzinger
Institute for Applied Ecology New Zealand, School of Applied Sciences, AUT University

December, 2015. **Herrenhausen Symposium "Sustainable Development Goals and the Role of Science: A Focus on Coastal Regions", Hannover, Germany**
The travel grant recipient, poster and presentation.
Effect of anthropogenic pollution on the nutrient status of coastal mangroves in northern New Zealand.

Iana Gritcan, Mark Duxbury, Andrea C. Alfaro, Sebastian Leuzinger, Institute for Applied Ecology New Zealand, School of Applied Sciences, AUT University

February, 2016. **School of Applied Sciences Showcase (SASS 2016), AUT, Auckland, New Zealand**

Presenter, winner of the best PhD presentation.

Effect of an anthropogenic activity on the nutrient status of temperate mangroves in northern New Zealand.

Iana Gritcan, Mark Duxbury, Andrea C. Alfaro, Sebastian Leuzinger

July, 2016. **New Zealand Marine Sciences Society Conference (NZMSS 2016), Wellington, New Zealand**

Presenter.

Effect of anthropogenic activity on the nutrient status of temperate mangroves in northern New Zealand.

Iana Gritcan, Mark Duxbury, Andrea C. Alfaro, Sebastian Leuzinger

August, 2016. **3 Minute Thesis Competition, AUT, Auckland, New Zealand**

Winner of the best PhD presentation.

Effect of nutrients and salinity on mangrove growth in northern New Zealand.

Iana Gritcan

September, 2016. **3 Minute Thesis Competition Asia-Pacific, UQ, Brisbane, Australia**

Semi-finalist.

Effect of nutrients and salinity on mangrove growth in northern New Zealand.

Iana Gritcan

Other publications generated during candidature

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Chapter 1. General introduction and literature review

1.1 Factors that affect plant growth

The biomass of green plants on Earth accounts for more than 50% of total live biomass. Plants have various forms from woody trees to mosses and seaweeds, but they are generally multicellular organisms, which carry out photosynthesis. Scientists view plants as "small biofactories" because of their ability to convert inorganic carbon in the form of CO_2 during the course of photosynthesis, inorganic nutrients (mainly nitrogen in the form of NO_3^- and NH_4^+) and phosphorus (PO_4^{3-}), and other microelements into organic compounds. Such transformations happen during the growth and development of a plant and consist of multiple biochemical reactions. The efficiency, speed, and presence of growth processes per se are determined by various environmental factors.

1.1.1 Factors that affect plant growth

Plant growth is defined as the process of accumulating dry mass, volume, length or area (Lambers, Chapin III, & Pons, 2008). It results from two main processes: carbon assimilation and carbon expenditure. Carbon assimilation happens via photosynthesis (carbon source activity), and plants invest this carbon into biomass via various growth processes (carbon sink activity). There are environmental and edaphic factors that control or limit biomass accumulation by affecting both carbon source and carbon sink activities (e.g., light, space, CO_2 availability, temperature, water and nutrient availability, stress conditions; Figure 1.1).

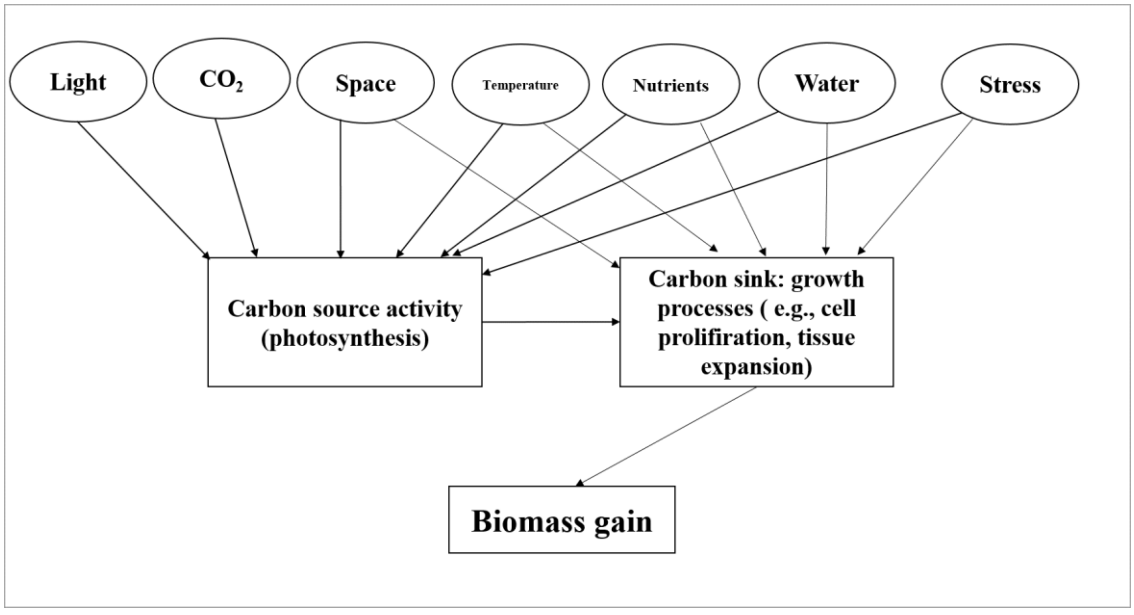


Figure 1.1. Factors that can affect plant growth.

However, it has been acknowledged that these factors do not play an equal role in plant growth and may not all act at the same time. In fact, it is usually the case that at any given time only one factor can control plant growth, namely the most limiting one. This mode of action was first introduced in 1840 by Justus von Liebig (reviewed in Körner, 2015). He pointed out that crop growth is controlled not by the total amount of resources available, but by the dominant factor that limits resource acquisition. For instance, vegetation in a desert is mainly controlled by water limitation. However, the identification of dominant factors that limit plant growth for any given plant tissue at a given time is difficult, as both available resources and environmental conditions vary greatly at very short spatiotemporal scales. For example, within the very same plant, simultaneously, the upper canopy can be water limited and not light limited while the opposite may be the case in lower leaves. Thus, it is necessary to evaluate the priority of growth limiting factor/s for each type of vegetation in detail and over longer periods of time.

1.1.2 Carbon source activity generally does not limit plant growth

Carbon source activity in plants occurs via photosynthesis. In order to understand how this process is linked to plant growth and what factors limit it, it is essential to describe this process as it is experienced by the plant. When green leaves are exposed to solar radiation (light; namely photosynthetically active radiation, spectral region between 400 and 700 nm), biochemical reactions are triggered in chloroplast cells. As a result, CO_2 is trapped in the form of photosynthates (carbohydrate molecules) that can later either be invested into biomass production or accumulated as polysaccharides molecules (starch) for use in future light limited periods. Substrates for photosynthesis are carbon dioxide and water, thus, the rate of photosynthesis depends on the presence of light, CO_2, and water (Monson, 2014).

In order for plants to gain carbon, there are requirements for space, which allow the plant to have access to light and underground resources (e.g., water, nutrients, microelements). Nutrients are essential, as they are vital for the formation of photosynthetic enzymes (e.g., RuBisCo) and cell components. Despite the photosynthetic rate being dependent on various physical and environmental factors, it has been hypothesised that carbon source activity (carbon assimilation processes) itself does not limit biomass production (Fatichi, Leuzinger, & Körner, 2013; Körner, 2013; Körner, 2015). This hypothesis has received confirmation from multiple experimental field and laboratory trials. It was demonstrated that plants are able to accumulate excess amounts of photosynthetic products

(nonstructural carbohydrates, NSC), and in some cases the concentrations of NSCs are several times higher than what the plant requires for the sole biomass accumulation purposes (Mäenpää *et al.*, 2001; Li, Hoch, & Körner, 2002; Hoch & Körner, 2003, as cited in Körner, 2003). Another piece of evidence that carbon assimilation activity is not limiting for biomass production was derived from experiments where plants were grown under elevated CO_2 concentrations, in both laboratory trials and field settings (Poorter & Nagel, 2000; Ellsworth *et al.*, 2017). These authors showed that plants growing under elevated CO_2 conditions do not accumulate more biomass compared to control plants, unless they are supplemented with nutrients and water. Thus, CO_2 addition alone did not result in higher biomass accumulation. Furthermore, photosynthesis occurs even during conditions when growth processes are suppressed. For instance, at air temperatures of 5-6°C, photosynthesis is still active (Körner, 2012), while cell expansion and cell division activities (actual growth processes) are almost halted (as summarised in Körner, 1999).

Thus, the amount of CO_2 and light are factors that generally do not affect growth and productivity of plants, as they do not directly affect carbon sink processes (Figure 1.2).

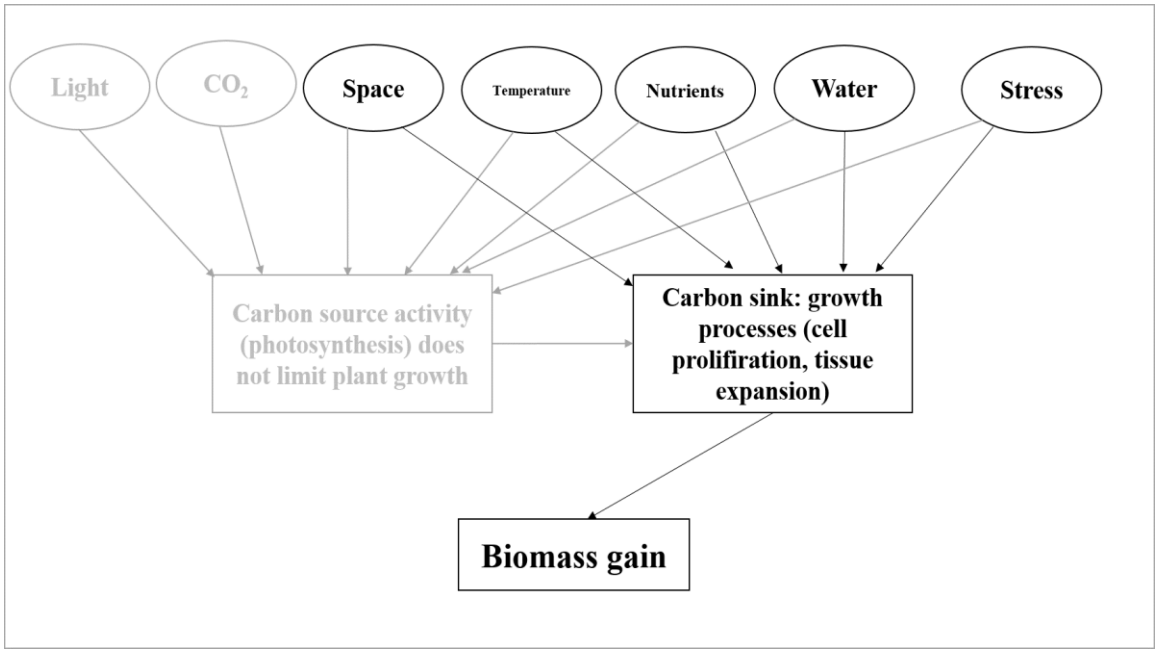


Figure 1.2. Factors that affect plant growth. Grey boxes and arrows indicate factors that affect only carbon source activity, and, thus, rarely limit plant growth. Black borders and arrows specify factors that strongly affect plant growth and biomass gain.

1.1.3 Factors that affect carbon sink (growth) processes

Unlike carbon source activity, where photosynthesis is the main biochemically process that results in carbon accumulation, plant growth *per se* is a series of cellular biochemical

reactions, such as cell division, cell expansion, cell enlargement, cell differentiation, and the synthesis of energy molecules, such as adenosine triphosphate (ATP). These plant cell growth processes or carbon sink activities are more sensitive to the presence of growth limiting factors, such as temperature, water and nutrient availability, and the presence of stress conditions (e.g. salinity, heavy metals or pathogens) and, thus, should be considered as the main factors affecting plant growth in general (Figure 1.2).

All plants, with some minor exception, (e.g., epiphytes) need to occupy some space (e.g., a patch of soil) to exist. While this patch of soil may provide the necessary resources for the plant (e.g., access to light, water, and nutrients; Prusinkiewicz & DeReuille, 2010). The question as to how space affects growth and productivity of the plant is not clearly understood. Space does not directly affect any growth processes in plants, but space limitation prohibits access to resources and affects plant performance in general. If there is no available space, seedling establishment will not happen, as well as juvenile progress into mature plants. A shortage of space also causes resource-mediated competition among plants occupying the same ecological niche (Stoll & Weiner, 2000). Because the space that plants occupy provides resources for both carbon source and carbon sink processes, it has an indirect limitng effect on both these activities.

Plant growth processes (carbon sink activities) are basically a series of biochemical reactions, which have a typical optimal temperature range. From a biochemical perspective, all growth processes, such as cell doubling or mitosis, are a series of enzyme driven reactions, and enzyme activity, which are directly affected by temperature (Figure 1.3). At certain low temperatures, these growth processes will be almost halted. For example, at 0°C cell doubling and duration of mitosis approaches infinity (Körner, 2003). On a global scale, biomass productivity decreases at both latitudinal and altitudinal scales, and it is believed that the main driver for the variations in biomass accumulation is temperature (Körner, 2003).

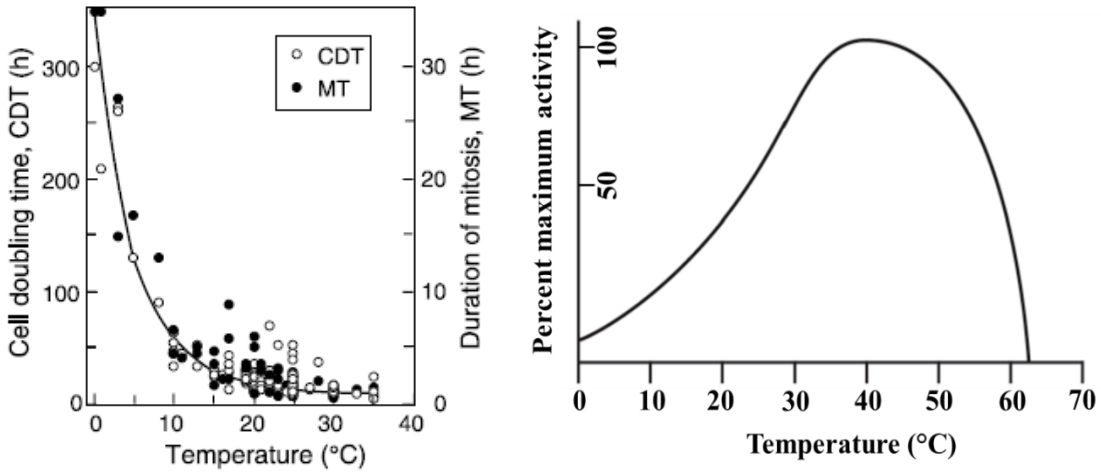


Figure 1.3. On the right, information from the literature survey of the dependency of cell division on temperature expressed as either cell doubling time or mitotic time (70 references, Körner, 1999); reproduced from Körner, 2003. On the left, the general diagram of the effect of temperature on the enzyme activity.

Low-temperature stress not only inhibits biochemical processes, but freezing temperatures also can damage cell structure due to formation of ice crystals. Despite plants having multiple adaptations to tolerate frost, some plants are still more adapted than others. Among the strategies that plants use for frost tolerance, the less efficient one is to avoid frost exposure. For example, some alpine plants are shorter, so in winter they can be covered by snow and avoid exposure to the freezing temperatures (Körner, 2003). Another strategy is to avoid freezing via solute accumulation or by avoiding nucleation (Larcher, 2003). Some true freezing tolerant plants allow freezing in the extracellular space (apoplast-water) and keep intercellular and cell-membrane in the liquid state and are also able to repair and replace damaged cells (Körner, 1995).

Plant growth is also limited when the water availability is low. Water has various functions in plant cells. The most important funstion of water in plant cells is to regulate stomatal opening, and, thus, allow the photosynthesis to take place. It also plays a role in the plant's transport system as all photosynthetic products, and macro- and micronutrients are distributed throughout the plant body in aqueous solution. Additionally, water is a necessary component of cell elongation or enlargement processes (Heyn, 1940; Lambers, Chapin III, & Pons, 2008; Monson, 2014). In many experiments, plants in drought conditions are less productive and have poorer growth rates compared to those that are adequately watered (Nagel, Konings, & Lambers,1994; Lambers, Chapin III, & Pons, 2008).

Poor nutrient availability, among other factors, has the strongest effect on plant biomass increase (Poorter & Nagel, 2000). Essential nutrients are nitrogen (in the form of nitrate or ammonium ion) and phosphorus (in the form of phosphate ions) compounds, and they are vital for the synthesis of enzymes, amino acids, and nucleic acids (DNA, RNA). Nutrient resources are highly variable in different ecosystems and their availability also declines over time. For example, low nitrogen availability is a major growth limiting factor for many terrestrial ecosystems, and phosphorus availability also decreases during forest ageing (Lambers, Chapin III, & Pons, 2008). It is also a common agricultural practice to add fertiliser (in the form of nitrogen and phosphorus compounds) to improve plant performance and crop yield. Low micronutrient (trace element) availability also plays a role in limiting plant growth (Welch & Shuman, 1995).

Additionally, various stress conditions, such as high salinity, heavy metals, herbicide presence, and various pathogens in many cases suppress the growth of plants. Soil salinization is currently a global issue, since much agricultural land is affected by increased salt content which results from poor land management, some agricultural practices (e.g., over fertilisation, irrigation etc.), and climate change. Accumulation of salts makes agricultural land unsuitable for crop production since many crop plants cannot survive or sustain high productivity under elevated soil salinity (Parida & Das, 2004; Rengasamy, 2010; Roy, Negrão, & Tester, 2014).

Overall, carbon source processes do not limit plant growth, rather carbon sink activities (plant growth processes) are sensitive to climatic and edaphic conditions.

1.2 Factors that affect mangrove plant growth

1.2.1 Mangrove plants

Mangroves are a group of halophytes or salt-tolerant plants that occupy intertidal habitats within coastal areas and estuaries. This group of plants is uniquely adapted to grow under constant salinity stress conditions, as well as constant waterlogging of sediment (Tomlinson, 1986). There are approximately 70 species of mangroves within 19 families (Morrisey, Beard, Morrison, Craggs, & Lowe, 2007). Despite the great diversity of mangrove species, they share some general characteristics. Most mangrove species have aerial roots, are viviparous, have adapted physiological mechanisms to tolerate high salt concentrations, lack distinctive annual growth rings, and have highly efficient mechanisms for nutrient retention (Tomlinson, 1986; Alongi, 2009).

Globally, mangrove ecosystems have great ecological value. They support high species diversity, provide breeding and nursery habitats for a range of different terrestrial and marine species, including bacteria, fungi, algae, invertebrates (e.g., snails, oysters, mussels) and vertebrates (e.g., fish, birds, mammals). It also has been suggested that tropical and subtropical mangrove ecosystems are highly productive (Bouillon *et al.*, 2008). They export particulate and dissolved organic carbon to adjacent coastal waters (Alongi, 2009, Table 1.1). Jennerjahn & Ittekot (2002) indicated that while mangrove forests cover only 0.1% of the Earth's continental surface, they are responsible for 11% of the total input of terrestrial carbon into the ocean. However, later studies have argued that this number is likely to be underestimated since the annual litterfall commonly used as an index of exported carbon is only a third of the total tree biomass (Kristensen, Bouillon, Dittmar, & Marchand, 2008). Additionally, mangrove ecosystems play an important role in the physical functioning within estuaries. They trap suspended sediments, which maintain the water clarity, and they help stabilise the shoreline and prevent erosion by reducing the wave energy (Morrisey *et al.*, 2007).

Table 1.1. Export of particulate organic carbon (mol C m^{-2} $year^{-1}$) from different mangrove stands, reproduced from Alongi, 2009.

Location	Export	Reference
Rookery Bay, Florida	5.3	Twilley (1985)
South Florida	15.5	Twilley (1985)
Tuff Crater, New Zealand	9.3	Woodroffe *et al.* (1985a, b)
Darwin Harbour, Australia	26.7	Woodroffe, Bardsley, Ward, & Hanley (1988), Burford, Alongi, McKinnon, & Trott (2008)
Matang, Malaysia	19.1	Gong & Ong (1990), Alongi *et al.* (2004)
Klong Ngao, Thailand	0.1	Wattayakorn, Wolanski, & Kjerfve (1990)
Itacuruca, Brazil	18.3	Lacerda (1992)
Fly River, Papua New Guinea	23.8	Robertson & Phillips (1994)
Missionary Bay, Australia	27.7	Alongi (1998)
Hinchinbrook Channel, Australia	10.4	Ayukai, Miller, Wolanski, & Spagnol (1998)
Sawi Bay, Thailand	5.9	Alongi *et al.* (2000)
Caetè estuary, Brazil	16.1	Dittmar, Lara, & Kattner (2001)

The latest data on mangrove distributions indicate that they are found between latitude 30° N and 30° S (Giri *et al.*, 2011; Figure 1.4). These mangrove ecosystems are commonly associated with tropical and subtropical areas. However, there are several species that extend into the range of cooler warm-temperate climates typical for South

Africa, Japan, southern Australia, southern North America, and northern New Zealand (Morrisey *et al.*, 2007).

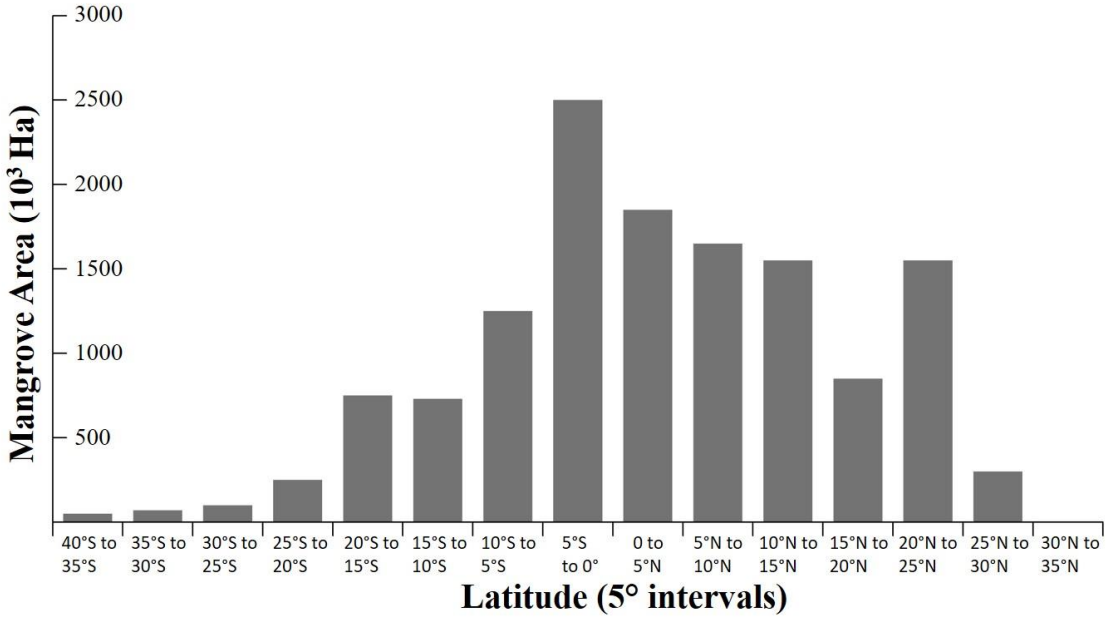


Figure 1.4. Latitudinal distribution of mangrove forest of the world, from Giri *et al.* (2011).

As these plants exist in the intertidal areas, growth and productivity of mangroves are heavily affected by salinity stress adaptations, as many resources (namely nutrients) are required for protecting carbon gain and growth processes from the harmful presence of salt (NaCl). Thus, photosynthesis and plant growth processes in mangroves are affected by temperature, water (freshwater), nutrient availability, and salinity stress (Alongi, 2009; Day, Crump, Kemp, & Yanez-Arancibia, 2013; Morrisey *et al.*, 2007).

1.2.2 Carbon assimilation processes in mangrove plants

As was mentioned previously, carbon source processes (namely photosynthesis) are not primary growth limitation factor for plants, and this is also true for mangroves. One of the main pieces of evidence for this can be derived from the fact that mangroves, similarly to other plants, allocate biomass to have access to the most limiting resources (Poorter & Nagel, 2000; Valentine & Mäkelä, 2012; McMurtrie & Dewar, 2013, as presented in Gill & Finzi, 2016). Indeed, it has been found that up to 80-90% of mangrove biomass is allocated below ground in the form of live and dead roots, which may suggest that mangrove growth is limited mostly by the low availability of below ground resources (such as freshwater and nutrients) (Alongi, Clough, Dixon, & Tirendi, 2003; Komiyama, Ong, & Poungparn, 2008; Tran, Gritcan, Cusens, Alfaro, & Leuzinger 2016).

As reviewed above, high levels of non-structural carbohydrates (NSC) in higher plant tissue can be considered as another indication that carbon assimilation does not limit plant growth. In fact, mangroves accumulate NSC in the same manner as other terrestrial plants (review by Gil *et al.*, 2013). The author also noted that extra NSC plays an additional role as osmolytes and may help mangrove plants to mitigate salt stress. Indeed, carbohydrate levels in salt-tolerant plants have been shown to increase with increasing salinity levels (Doddema, Eddin, & Mahasneh, 1986; Murakeözy, Smirnoff, Nagy, & Tuba, 2002; Murakeözy, Nagy, Duhaze, Bouchereau, & Tuba, 2003).

Further evidence that CO_2 assimilation processes do not limit mangrove growth is that CO_2 fertilisation alone does not cause biomass increase, while nutrient fertilisation has a strong effect on biomass accumulation. Indeed, some studies demonstrated this trend in mangrove plants (Farnsworth, Ellison, & Gong, 1996; Ball, Cochrane, & Rawson, 1997; McKee & Rooth, 2008; Reef, Markham, Santini, & Lovelock, 2015; Reef *et al.*, 2017). In addition, numerous laboratory and field nutrient fertilisation experiments strongly suggest that nutrient availability alone plays the major limitation role for carbon assimilation processes in mangroves (Boto, Saffigna, & Cloughl, 1985; Naidoo, 1987; Feller, 1995; McKee, 1996; Yates, Ashwath, Midmore, 2002; Lovelock, Feller, McKee, & Thompson, 2005; Naidoo, 2009).

Additionally, mangrove plants are uniquely adapted to grow under salinity stress. In order to maintain growth under high salinity conditions in the sediment, mangrove plants accumulate salt in leaves. While this adaptation helps plants to overcome osmotic differences for water uptake, it adversely affects enzyme activity. Indeed, it has been shown that mangrove plants that grow in hypersaline conditions demonstrated low photosynthesis levels (Ball & Farquhar, 1984; Ball, 1988; Santiago, Lau, Melcher, Steele, & Goldstein, 1999; Theuri, Kinyamario, & Speybroeck, 1999; Parida, Das, & Das, 2002; Reef *et al.*, 2015). Thus, carbon assimilation in mangrove plants does not limit mangrove growth directly, rather climatic and edaphic conditions limit the rate of the photosynthesis.

1.2.3 Space effect on mangrove growth

Mangroves, like other plants, need to occupy some space to have access to underground and aboveground resources, and mangroves have unique adaptations to grow in conditions where other terrestrial vegetation cannot (Tomlinson, 1986). They occupy a

very limited space of the intertidal zone, so, they can avoid competition with other terrestrial vegetation. However, the effect of space limitation is the strongest for seedling growth, compared to mature plants that are already established. When more suitable space is available, mangrove stands and/or forests can spread, and this has been observed in poleward and inland spreading of temperate mangroves caused by rising temperature and sea level rise (Lovelock, Sorrell, Hancock, Hua, & Swales, 2010).

1.2.4 Temperature effect on mangrove growth

The main factor that restricts mangrove distribution in high latitudes is temperature (Woodroffe & Grindrod 1991; Duke, Ball, & Ellison, 1998; Quisthoudt *et al.*, 2012; Hutchison, Manica, Swetnam, Balmford, & Spalding, 2014). Mangroves, like other tropical plants, are poorly adapted to tolerate stress imposed by freezing temperatures and generally classified as 'tender' (cannot tolerate temperatures below 0°C) or 'slightly hardy' (can tolerate freezing temperatures up to -5°C; Levitt, 1980 [as cited in Beard, 2007]). At the ecosystem level, with increasing latitudes mangrove plants exhibit a reduction in both tree size and species diversity compared to tropical mangroves. Low temperatures in temperate conditions cause a decline in net primary productivity (Saenger & Snedaker, 1993) and in annual growth increments of trees (Morrisey *et al.*, 2010), and success in reproduction (Duke, 1990).

At the plant level, mangrove growth is affected by low temperature, in the same way as in terrestrial plants, for example, by affecting enzyme activity of carbon assimilation and/or growth processes (Beard, 2006). They are also affected by direct damage of cellular structures of leaves, branches and reproductive tissues (Saintilan, Rogers, & McKee, 2009). Additionally, low temperatures impose anatomical constraints on water transport in temperate mangroves, Stuart, Choat, Martin, Holbrook, & Ball (2007) showed that in high latitudinal mangrove species xylem vessel diameters are smaller.

However, some mangrove species seem to be more frost tolerant than others. It was found that, *Avicennia marina* var *australasica* that occurs in temperate New Zealand conditions (at latitudes greater than 30°; Morrisey *et al.*, 2007; Morrisey *et al.*, 2010). Moreover, mangrove plants of the same species, but from different latitudes can be variously adapted to frost stress. McMillan (1974) and Markley, McMillan, & Thompson (1982) demonstrated that *Avicennia germinans* (L.) L. from Belize (17° 31` N) was more damaged by frost treatment than those from Harbor Island, Texas (27° 50` N).

1.2.5 Salinity effect on mangrove growth

Water availability for mangrove plant uptake is inseparably linked to the salinity of the sediment and these two factors should be reviewed together. In general, the presence of a high amount of salt in the sediment causes osmotic imbalance and prevents plant water uptake, creating a water shortage. This limits mangrove carbon and nutrient uptake, affecting growth and productivity (Ball, 2002). Thus, it is necessary to consider the mechanism of salt stress on plant performance in general.

Previous studies have shown that salt stress and water shortage (dehydration stress) have very similar physiological effects and often synergistic effects on osmotic stress, toxic effects, cell damage, and cell death (Mahalingam, 2015). In the early stages of salt exposure, plants cannot uptake water because of the osmotic difference between the high salt environment and the low salt concentration in leaves. However, if the salt stress progresses, plants accumulate sodium ions in leaf tissues to overcome the osmotic difference and take up water. Such ion accumulation leads to toxic effects, for example, ion imbalance between cell compartments and accumulation of reactive oxygen species. In severe cases, both accumulation of sodium ions in plant tissues and rising osmotic stress causes damage to cell organelles, impeding interior and exterior cellular biochemical reactions, and in severe cases can trigger programmed cell death (Gupta & Huang, 2014).

Unlike glycophytes (non-salt tolerant species), mangroves are well-adapted to be productive under constant salinity stress because of numerous physiological and biochemical adjustments. They can overcome osmotic stress by accumulating salt in leaves and decoupling water from ion uptake when covered by seawater (Stuart *et al.*, 2007; Reef & Lovelock, 2015). Under mild to mid-salinity conditions (salinity of seawater or below) physiological mechanisms are of greatest importance for mangrove growth and productivity. Two of the most important ones are root-exclusion and leaf-secretion mechanisms. Krishnamurthy *et al.* (2014) showed that roots of mangrove plants filter the salt out and prevent absorption of toxic ions in plant tissue. Duarte, Sleimi, & Cacador (2014) demonstrated that leaves of mangroves can secrete salt to protect leaf metabolic processes from damaging by excessive salt concentration. However, under hypersaline conditions, when the salt concentration is several times higher than the concentration of seawater, it has been shown that mangrove growth is severely suppressed

by both water shortage and metabolic effects of salt toxicity (Ball, 1988; Martin *et al.*, 2010).

As an example of salinity being one of the most significant growth controlling factors for mangrove plants growth and productivity, Ball (2002) proposed that levels of salt concentrations in the sediment 'dictate' the species and height zonation in tropical mangrove forests. The author found that with increasing distance from the sea or channel edge, sediment and pore water salt concentration increases, which suppresses mangrove growth and productivity. Thus, freshwater availability does not directly affect mangrove growth, rather mangrove growth is affected and controlled by the complex equilibrium between freshwater availability and amount of salt in the sediment.

1.2.6 Nutrient effect on mangrove growth

Mangrove ecosystems are also nutrient limited, and mangrove plants can persist in low nutrient environments (Day *et al.*, 2013). Nutrients, such as nitrogen and phosphorus compounds, are the most important mineral nutrients for all forms of life (Campbell & Farrell, 2006). Nitrogen is important for the synthesis of proteins, and it facilitates enzymatic activity, whereas phosphorus is the essential element for energy transfer and storage in cells in the form of adenosine triphosphate (ATP). Moreover, both these elements are contained in DNA (deoxyribonucleic acids).

The importance of nutrient availability in growth and productivity of mangroves has been demonstrated in numerous laboratory (Alongi, 2011; Boto *et al.*, 1985; Naidoo, 1987; Yates *et al.*, 2002) and field fertilisation studies (Boto & Wellington, 1983; Feller, 1995; Lovelock *et al.*, 2007b; Naidoo, 2009). In these experiments, scientists have demonstrated that mangrove plant growth and biomass production respond with great sensitivity to variations in nutrient concentrations. There are two main conclusions that have been reached based on such fertilisation studies. Firstly, mangroves normally exist in nutrient-limited conditions, which means that nutrient availability plays an important role in mangrove growth and, secondly, mangroves can be N-limited, P-limited or co-limited in both main nutrients. The type of nutrient limitation may severely affect mangrove metabolism and growth adaptation strategies.

1.3 Factors that affect temperate mangrove growth

Mangroves have been characterised as tropical and subtropical plants with additional limited distribution in temperate areas. For example, in the southern hemisphere above 30°C latitude temperate mangroves occur in New Zealand, Australia, South America, and South Africa (Duke *et al.,* 1998; Giri *et al.,* 2011). Temperate New Zealand mangroves are native plants, which grow along some coastlines and estuaries in the northern North Island. There is only one mangrove species in New Zealand: *Avicennia marina* var *australasica*. This species exists at the extreme southern limit of mangrove distribution and exhibits several significant distinctions from tropical mangroves (Morrisey *et al.,* 2007).

In temperate mangroves, plant growth rate, productivity, the number of species, and biological diversity of mangrove ecosystems decline with increasing latitudes (Alongi, 2009; Morrisey *et al.,* 2007; Morrisey *et al.,* 2010). Plant productivity (the process of accumulation of biomass), in mangroves, is tightly correlated with mean annual temperature and, thus, the growth rate is lower in temperate mangroves than that of tropical counterparts. Saenger & Snedaker in 1993 showed that mangrove biomass ranges from 5.7–43.6 kg m^{-2} in the tropics (between 23°N to 23°S), to 0.8–16.4 kg m^{-2} in the subtropics and more temperate regions (between 23 and 30° N and S). Furthermore, under cool temperate conditions in New Zealand only one mangrove species occurs, while in the tropical Indo-Malaysian region researchers found 48 mangrove species (Giri *et al.,* 2011). Also, recently it has been shown that temperate mangrove ecosystems do not maintain high species diversity compared to tropical counterparts (Alfaro, 2010). Faunal species (e.g., large crabs, large snails, rodents, monkeys) that normally feed on mangrove matter in the tropics, consume only a minor amount of mangrove biomass in temperate areas. In New Zealand, mud crabs (*Helice crassa*) and grazing snails consume small amounts of mangrove leaves, and they mainly feed on microalgae within biofilms on mangrove surfaces (Alfaro, 2010; Alfaro, Thomas, Sergent, & Duxbury, 2006).

According to geographical latitudinal zonation, New Zealand is situated in the temperate climate zone. Although, the northern part of the country (where mangroves occur) has a sub-tropical climate with typical summer (December to February) daytime air temperatures (22 to 26°C) and (12°C to 17°C) in winter (June to August) with occasional frosts at night time during the coldest month (July). Mean annual precipitation is around 1200 mm with the highest precipitation rate in winter (100 to 170 mm per month) and

less in summer (70 to 100 mm per month) with autumn and spring precipitation being around 100 mm and average temperature around 15°C (https://www.niwa.co.nz). These climatic differences can affect factors that modulate mangrove growth and productivity in temperate areas.

1.3.1 Mangrove growth under New Zealand temperate climatic conditions

How carbon source capabilities of temperate mangroves (namely photosynthesis) is affected by the colder New Zealand climate was studied in detail by Beard in 2006. The author found that photosynthesis rate had a seasonal trend, whereby it reached its maximum in summer and was substantially reduced during cold winter days. The author also found that the main reason for reduced photosynthetic activity was low temperature, which affects the activity of photosynthetic enzyme Ribulose 1,5-bisphosphate (RuBP). While these findings can explain why temperate mangrove plants have lower growth rates compared to tropical mangroves, they cannot explain why temperate mangroves that grow at the same location often exhibit significant tree form variations along the stand gradient (taller trees at the edge and lower trees and sometimes dwarfed trees in the interior of the mangrove stand).

Additionally, as previously discussed, low temperature reduces the rate of plant growth processes (such as cell division, cell expansion, cell enlargement, cell differentiation, and the synthesis of ATPs) before it affects photosynthesis. Once more, temperature effects can be one of the reasons why temperate mangroves are less productive than tropical counterparts, but cannot be linked to tree form variation between the edge and interior mangroves along the stand gradient.

Another growth limiting factor for temperate mangroves, namely space availability, has received a lot of attention in New Zealand. As was mentioned earlier, if more space becomes available (e.g., estuary infilling, sea level rise, or global warming) mangrove plants occupy these new areas. Indeed, it has been shown that temperate New Zealand mangroves are rapidly spreading into areas where they have never been found before (Green *et al.*, 2003; Schwarz, 2003; Stokes, Healy, & Cooke, 2010). This has caused on-going controversial discussions regarding management *versus* conservation of New Zealand mangroves. There is not only scientific evidence of that fact, but also many local communities and councils are worried that mangroves have replaced sandy bare tidal flats and other estuarine habitats (i.e., seagrass beds, marshlands) and will turn them into

muddy sites. Such concerns have resulted in legal and illegal management programmes, which often include removal of mangroves (Alfaro, 2010). Such disagreement among residents and local councils has sometimes culminated in expensive environmental lawsuits, where the outcomes have always pointed to the lack of scientific knowledge of these complex ecosystems.

However, this accretion of mangrove habitats is not unique to New Zealand alone. There are studies that have shown that, although throughout the tropics mangrove plants are endangered species, under threat and their territories are declining, temperate mangrove stands in south eastern Australia (Bird, 1986; Saintilan & Williams, 1999), in South and East Africa (Di Nitto *et al.*, 2014), and in the USA (Cavanaugh *et al.*, 2014) have been accreting. Researchers proposed that the main reasons for temperate mangrove spread could be global climate change and local human-derived changes. Generally, researchers have shown that global warming and sea level rise correlate well with mangrove landward and poleward expansion (Cavanaugh *et al.*, 2014; Di Nitto *et al.*, 2014; Godoy & De Lacerda, 2015; Coldren & Proffitt, 2017).

Additionally, changes at the local scale due to human activity could be a factor promoting mangrove growth and spread (e.g., estuary infilling, extensive agricultural activity with runoff high in nutrients, and anthropogenic sewage inputs). For example, it has been shown that sedimentation rate and nutrient concentrations in estuarine and coastal areas in New Zealand have changed greatly due to human catchment activity during last 100 years (Cooper & Thomsen, 1983; Vant, 1997; Hart, Quin, & Nguyen, 2004; Heggie & Savage, 2009; Thrush *et al.*, 2013; Walker & Vaughan, 2013). Lovelock and colleagues in 2007b also showed that estuary infilling promotes temperate mangrove stand expansion in two New Zealand estuaries and, although nutrient addition did not appear to have a direct effect on mangrove spread rate, it might play a complimentary role.

Overall, lower photosynthesis rate during cold seasons and negative temperature effects on plant growth processes (e.g., the majority of enzymatic biochemical reactions) have been found to limit plant biomass accretion in temperate mangroves compared to tropical ones (Beard, 2006). However, these growth limiting factors do not explain tree form differences along temperate mangrove stand gradients (edge vs interior). While spreading of New Zealand mangroves associated with increasing space availability due to human-derived estuary infilling processes (e.g., deforestation and sedimentation) has received

some attention, there are still substantial gaps in understanding what effect human-derived nutrient inputs can have on temperate mangrove growth and productivity. Moreover, nutrient and salinity levels that temperate New Zealand mangroves are exposed to have not been studied previously.

1.4 Salinity effect on temperate mangrove growth

Since environmental salinity plays a vital role in controlling mangrove plant growth, it is important to study salinity levels in temperate New Zealand mangrove coastal and estuarine ecosystems and to evaluate its potential effect on mangrove growth, which has not been done previously. Salinity or presence of sodium cations and chloride anions in the environment is toxic for most terrestrial plant species. Sodium cations are toxic because they negatively affect potassium uptake and cytosolic enzyme activity, which ultimately affects photosynthesis and metabolism (Tuffers, Naidoo, & Willert, 2001; Parida & Jha, 2010).

Moreover, scientists are unsure whether salinity is a necessary condition for mangrove species successful performance. Indeed, several years ago, there was a debate on whether mangroves are facultative or obligate halophytes (Wang *et al.*, 2011; Krauss & Ball, 2012). Wang *et al.*, (2011) argued that mangroves are truly obligatory halophytes and cannot grow and develop under solely freshwater conditions. Another opinion is that mangroves are facultative halophytes. Indeed, Krauss & Ball (2012) suggested that mangrove plants are just adapted to growth in the environments where the salt is present. However, both groups agreed that more salinity-related studies on mangroves are needed to develop a better understanding of the halophytic nature of mangrove plants.

To deeply understand the effects of salinity on mangrove growth, it is necessary to describe types of adaptations mangrove plants have developed to combat salinity presence in the environment. The most striking feature of mangrove plants, as halophytes is that unlike other terrestrial vegetation types they have unique adaptations to tolerate harsh saline environmental conditions of coastal marine settings (Flowers & Colmer, 2008). The main strategy of mangrove plants to tolerate high salinity in the sediment and/or pore water is a range of physiological mechanisms. For instance, root-exclusion and/or selective (fresh vs saline) water uptake and leaf-secretion was found to be particularly important (Rozema, Gude, & Pollak, 1981; Waisel, Eshel, & Agami, 1986; Takemura *et al.*, 2000; Duarte *et al.*, 2014; Krishnamurthy *et al.*, 2014; Shabala, Bose, &

Hedrich, 2014; Reef *et al.*, 2015; Santini, Reef, Lockington, & Lovelock, 2015). Another set of mechanisms, which some of the most salt tolerant mangrove species have developed, is accumulation of Na^+ and Cl^- ions in specialised leaf cell compartments (vacuoles). This adaptation also helps to overcome the osmotic differences between salt-rich sediment and salt-poor cytoplasm and extracellular fluid to uptake water (Aziz & Khan, 2001). As the salinity of the sediment increases, these plants accumulate more salt in leaf cells and higher total sodium concentration in leaves. Indeed, it has been described that in hypersaline environments, sodium concentration and sodium/potassium (Na/K) ratio in mangrove leaves is higher than in moderate or low saline conditions (Ball, Chow, & Anderson, 1987; Ye, Tam, Lu, & Wong, 2005). Thus, measuring and comparing this stoichiometric parameter in mangrove leaves in single stands and/or between different stands could provide important information about the average salinity levels in the field.

Therefore, mangroves are halophyte plants that can survive in seawater because they have physiological and biological adaptations (as salt excretion, salt accumulation, and salt secretion; etc.), which allow them to actively regulate and handle harmful amounts of salt (Tomlinson, 1986; Alongi, 2009; Parida & Jha, 2010). Despite extensive available information on the effects of high salt concentration on the growth of mangrove plants and productivity, there is little available information on mangrove strategies to tolerate a low concentration or absence of salt that presumably is common under temperate New Zealand conditions.

1.4.1 Salinity in tropical *vs* temperate mangrove environments

While salinity levels have been shown and recognised by scientists as a major reason for species and productivity gradients in tropical mangrove forests, there is only limited information on salinity levels in temperate mangrove ecosystems, and there is almost no information on seasonal variations of salinity in mangrove environments under temperate New Zealand climatic conditions (Ball, 1996; Ball, 2002). However, salinity is a highly variable parameter, both spatially and seasonally. Salinity effects also depend on the stand morphology, location, and proximity to freshwater sources nearby. Based on available published information, the comparison table below was prepared to illustrate these potential salinity differences (Table 1.2).

Table 1.2. Table comparing sediment and/or pore water salinity among mangrove sites at different latitudes.

Salinity, PSU (min-max)	**Species composition**	**Geographical location and coordinates**	**Reference**
56.8±2.4	*Avicennia marina* (dwarf)	Richards Bay Harbour, (28°48'S, 32°05'E), South Africa	Naidoo (2006), (2009)
40 – 53	*Avicennia marina* var *australasica*	Clyde River at Batemans Bay, (32°42'S, 150°12'E), southeastern Australia	Martin *et al.* (2010)
34.9–38.8 (±0.3)	*Rhizophora mangle*	Twin Cays, (16°41'N, 88°11'W), Belize	Feller (1995) McKee, Feller, Popp, & Wanek (2002)
34 – 40	*Avicennia germinas*		
51 – 53	*Laguncularia racemosa*		
57 – 60 (±2)			Lovelock, Feller, Ball, Engelbrecht, & Ewe (2006)
36.9 (±1.2)			Feller, Lovelock & McKee (2007) Feller, Lovelock & Piou (2009)
34 – 50(±15)	*Rhizophoracae* family	Hinchinbrook Island, (18°20'S, 146°13'E), Australia	Boto & Wellington (1984)
33 – 54	*Rhizophora mangle*	North Hutchinson Island, (27°33'N, 80°20'W), St. Lucie County, Florida	Lovelock & Feller (2003)
45 – 55			
49 – 55(±1)	*Avicennia germinas* *Laguncularia racemosa*		Lovelock *et al.* (2006) Feller, Lovelock & McKee (2007)
20.6 – 37.0	NA	Darwin Harbour, (12°46'S, 130°86'E), Australia	Smith *et al.* (2012)
35-38	*Avicennia marina* and *Bruguiera gymnorrhiza* (L.) Lam.	Durban Bay, (29°53'S, 31°00'E), South Africa	Tuffers et al (2001)
5.8-11.4	*Avicennia marina* and *Bruguiera gymnorrhiza* (L.) Lam.	Beachwood, (29°53'S, 31°00'E), South Africa	
32.4 (±0.5) – 32.7 (±0.7)	*Rhizophora mangle, Laguncularia racemose* and *Avicennia germinans*	Indian River Lagoon, (27°33'N, 80°13'W), Florida	Feller *et al.* (2009)
33.3 (±1.9) – 39.4 (±1.2)	*Rhizophora mangle*	Bocas del Toro, (9°09'N, 82°15'W), Panamá	

37 – 40.6	*Avicennia germinans*	Bahı´a de Lobos, (27°18'N; 110°27'W), North-western Mexico	Sa´nchez-Carrillo *et al.* (2009)
22.2 – 29.8 (edge area) 19.6 – 22.8 (dwarf area)	*Avicennia marina* var *australasica*	Waikopua (36°56'S, 174°57'E) and Whangapoua (36°43'S, 175°37'E), New Zealand	Lovelock *et al.* (2007b)
28	*Avicennia marina* var *australasica*	Firth of Thames, (37°21'S, 175°48'E), New Zealand	Lovelock *et al.* (2010)

As can be seen, salinity levels significantly decrease with increasing latitudes, but information for latitudes higher than 30°S is very limited. Therefore, it is necessary to investigate salinity variations at seasonal and spatial scales for northern New Zealand mangrove stands.

1.4.2 Effects of low salinity on temperate mangrove growth

In general, scientists consider mangroves to be facultative halophytes (Krauss & Ball, 2012). However, recently it was hypothesised that some mangrove species could have obligatory salinity requirements (Wang *et al.*, 2011; Nguyen, Stanton, Schmitz, Farquhar, & Ball, 2015; Naidoo, 2016). Some mangrove species may have developed strong adaptation to high salinity levels, so they could occupy their own salinity zonation niche within land-sea interface environments. Thus, it is possible that some mangrove species are so dependent on the salinity in their growth-related processes that they perform very poorly under freshwater conditions and/or very low salinity levels. It is also possible that those "halophilic" mangrove species have developed and mastered such strong physiological, biochemical, and biological mechanisms to tolerate hypersaline environments that they have opted out of their ability to grow and be productive under exclusively freshwater conditions. As a response to that hypothesis, recent researchers have started studying growth patterns and physiological responses of different mangrove species under contrasting salinity levels in pot trials (Nguyen *et al.*, 2015). However, biochemical mechanisms of mangrove plant adjustments to contrasting salinity levels, specifically what metabolites are involved in such adaptations receive less attention in the literature.

The mangrove species, A*vicennia marina* var *australasica*, has shown to be extremely salt tolerant and, overall, very stress-resistant (Lovelock, Krauss, Osland, Reef, & Ball,

2016). Indeed, it has been described that this species in tropical conditions occurs in areas with hypersaline sediment (Lovelock & Feller, 2003; Krauss & Ball, 2012). This species also has many physiological (Nguyen *et al.*, 2015), biological (Popp, 1984; Aziz & Khan, 2001; Wang *et al.*, 2002), genetic (Mehta, Sivaprakash, Parani, Venkataraman, & Parida, 2005), and metabolic (Tuffers *et al.*, 2001; Parida & Jha, 2010) adaptations to high salinity in the field. Additionally, laboratory growth trials demonstrated that this species has poor growth rate at low and/or absence of salinity (Downton, 1982; Clough, 1984; Yan, Wang & Tang, 2007). Conversely, there is little information on mangrove strategies to tolerate a low concentration or absence of salt. Additionally, it has been indicated that further research is needed to better understand how low salinity conditions affect mangrove growth and productivity (Tuffers *et al.*, 2001; Nguyen *et al.*, 2015).

Some earlier studies have found that that long-term exposure of A*vicennia marina* var *australasica* to pure seawater conditions, as well as the presence of only freshwater, tend to decrease mangrove growth and productivity (Downton, 1982; Figure 1.5). In the early stages of germination and growth, freshwater conditions are favourable for mangroves, but at later stages, pure freshwater slows down growth, and after 6-8 months plants treated with 0 and 100% seawater show similar stunted growth.

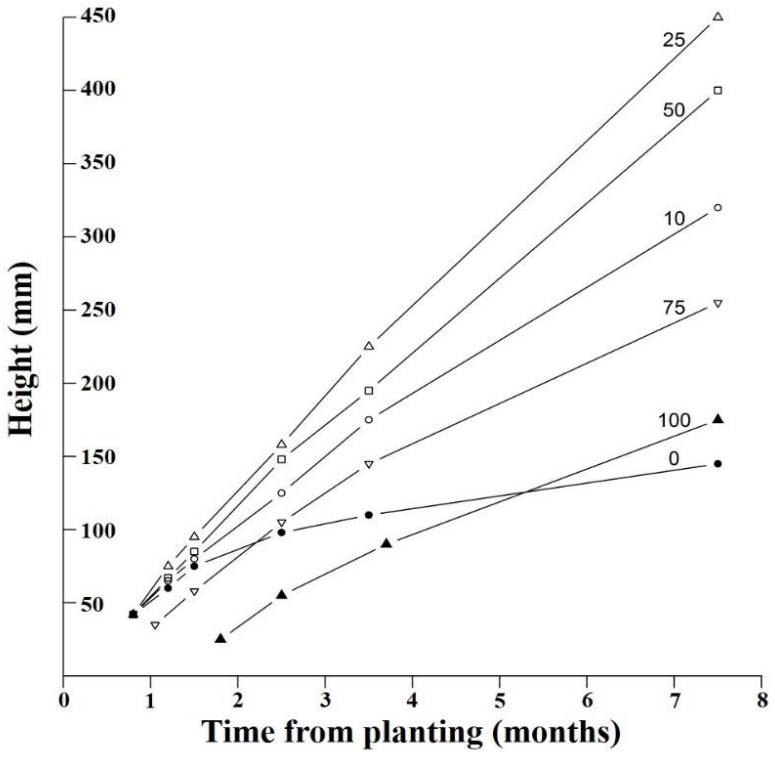


Figure 1.5. The growth rate of *Avicennia marina* var *australasica* in long term exposure to different salinities, retrieved from Downton (1982).

In the tropics, interior mangroves are constantly exposed to higher salinity than their edge neighbours as a result of evapotranspiration, high temperatures, and diurnal tidal fluctuations (Ball, 1996; Ball, 2002). Although temperate New Zealand mangroves also exhibit height gradients between edge and interior zones, the distribution of mangrove trees along salinity gradients may be opposite to that observed in the tropics due to lower temperatures limiting evaporation (Naidoo, 2009) and larger freshwater inputs. These salinity trends may be linked to mangrove growth and productivity parameters that are yet to be investigated and explained for New Zealand stand morphology.

Based on that information available in the literature, I hypothesised that in some cases stand morphology and growth of A*vicennia marina* var *australasica* in New Zealand is affected not by hypersalinity conditions, but on the contrary by a hyposalinity conditions. Growth and productivity suppression probably occur and can be detected at the biochemical level, since the biochemistry of high salinity stress in mangrove plants are well addressed in the literature.

1.4.3 Effect of salinity on mangrove biochemical processes

Mangrove leaves and fine roots are the most affected parts of the plant by the presence of salt. To overcome the negative osmotic potential, mangrove accumulate salt in leaves. Then salt is either excreted to the leaf surface or accumulated in specialised cell compartments (vacuoles) within the leaf cells (Clough, 1982; Alongi, 2009). However, with higher salinity in the sediment more salt is required in leaves to maintain osmotic balance and allow water uptake (Aziz & Khan, 2001). When very large quantities of Na^+ ions are accumulated in the cells of the leaves, secondary oxidative stress takes place and leads to the accumulation of reactive oxygen species (ROS), and biochemical response is triggered. The main purpose of that biochemical adjustment is to "protect" the photosynthetic machinery, such as PSII and PSI complexes (Sengupta & Majumder, 2009), the electron transport chain, and other cellular processes from chemical damage from highly reactive ROS. Examples of biochemical adjustments under salinity stress conditions, include osmolytes accumulation (e.g., quaternary ammonia compounds, proline, mannitol, etc), accumulation of nitrogen reserve in form of amino acids (namely asparagine), and synthesis of ROS scavenging agents (e.g., proline; Popp, Larher & Weigel, 1985; Munns, 2002; Sharma & Dietz, 2006; Planchet, Rannou, Boutet-Mercey, Maia-Grondard, & Limami, 2011).

Sodium concentration in mangrove tissues

It is well described that salinity in the mangrove sediment and/or pore water is intrinsically linked to the concentration of sodium (Na) in mangrove leaves *via* the adaptations that those plants possess for overcoming osmotic differences in water uptake (Aziz & Khan, 2001). Indeed, it has been described that in hypersaline environments, the sodium concentration and sodium/potassium (Na/K) ratio in mangrove leaves is higher than in moderate or low saline conditions (Downton, 1982; Ball *et al.*, 1987; Flowers & Colmer, 2008; Canalejo, Martinez-Dominguez, Cordoba, & Torronteras, 2014; Chen & Ye, 2014; Duarte *et al.*, 2014; Ye at al., 2005; Table 1.3).

Table 1.3. Ionic composition of leaves from *Avicennia marina* exposed to different levels of salinity and potassium treatments, from Ball, Chow & Anderson, 1987.

	Low salinity		**High salinity**	
	High K^+	**Low K^+**	**High K^+**	**Low K^+**
Salt content (mmol kg^{-1} dry wt)				
Na^+	640±91	1015±61	2071±64	1935±116
K^+	1102±50	639±40	512±71	320±40
Cl^-	1220±76	1326±70	2194±104	2074±117
(mol m^{-3} leaf water)				
Na^+	210±17	388±12	673±21	728±25
K^+	379±34	248±25	167±24	103±15
Cl^-	415±28	511±36	718±39	747±24
Na^+/ K^+	0.60±0.11	1.62±0.14	4.40±0.11	6.03±0.11
% dry wt K^+	4.30±0.2	2.50±0.2	2.00±0.3	1.50±0.3

Although, assessment of salinity levels in mangrove environments traditionally is performed by measuring the salinity of the pore water, measuring Na concentration in mangrove leaves may be more accurate. For example, it has been shown that mangrove roots are able to access multiple sediment layers containing different water types (fresh and saline) simultaneously and will prefer to uptake fresh over saline water (Wei, Lockington, Poh, Gasparon, & Lovelock, 2013; Reef *et al.*, 2015; Santini *et al.*, 2015). Thus, measurements of Na concentration in mangrove leaves gives an "averaged" information on all water sources available to the plant. This information can be used to assess the exact salinity that plants experience.

In contrast to the tropics, there is no information available on salinity levels in New Zealand mangroves, especially with respect to its effect on the foliar elemental (Na and K) concentrations. There are several studies that have reported salinity measurements of mangrove sediments in New Zealand (Lovelock *et al.*, 2007b; Lovelock *et al.*, 2010; Yang, Gao, Cheung, Schwendenmann, & Costello, 2013), but these values were not related to the Na concentration in mangrove leaves. Hence, little is known to date about what possible range of salinity concentrations temperate mangrove plants are exposed to, as well as what possible effects these salinity levels might have on mangrove growth in northern New Zealand.

ROS levels in mangrove leaves

Reactive oxygen species (ROS) in plants are common metabolites (Mittler, 2002, Foyer & Noctor, 2005). They are products and intermediate compounds in many biochemical pathways and under normal conditions produced in a controlled manner. For example, ROS are formed in mitochondria during respiration, in chloroplasts during photosynthetic photosystem PSI and PSII reactions, and during fatty acid β-oxidation (Asada & Takahashi, 1987; Baker & Graham, 2002; Asada, 2006 as summarised in Bose, Rodrigo-Moreno, & Shabala, 2013). However, under stress conditions, such as the presence of high concentrations of Na^+, many of these biochemical processes may become out of balance. Reactive oxygen species, thus, may be produced in excess, accumulate, and in severe cases eventually could cause cell death through oxidative damage to lipids, proteins and nucleic acids (Parida et al. 2004b; Bose, Rodrigo-Moreno & Shabala, 2013).

Halophyte plants, such as mangroves, appear to have developed unique biochemical mechanisms to "protect" important biological growth-related processes under salinity stress and, hence, under elevated ROS levels. These strategies are highly variable and generally are classified as physiological (e.g., salt excursion, salt accumulation, salt secretion) and biological (e.g., accumulation of compatible solutes, induction of antioxidative enzymes), as summarised in Parida & Jha (2010). These mechanisms vary between mangrove species as well. A*vicennia marina* var *australasica* in New Zealand are among the most stress-resistant mangroves, and they are well adapted to grow under elevated salinity as well as oxidative stress conditions (Naidoo, Hiralal, & Naidoo, 2011; Dasgupta, Chowdhury, & Das, 2015; Nguyen *et al.*, 2015). Moreover, *Avicennia marina* growth and growth-related processes have higher rates under moderate salinity conditions, thus at 25% of standard seawater mangrove seedlings growth was the highest

(Downton, 1982; Clough, 1984; Ball, 1988; Burchett, Clarke, Field, & Pulkownik, 1989; Ball & Pidsley 1995). Despite observations, that hypersaline conditions trigger ROS accumulation in mangrove plants, information on how exposure to low salinity or freshwater conditions may affect ROS levels in leaves of very salt-tolerant *Avicennia marina* species are largely unknown.

Osmolyte compounds in mangrove tissues

One of the main ways for halophytes to protect cellular processes from salt, accumulated in vacuoles is to accumulate compounds known as osmolytes or compatible solutes (Bose, Rodrigo-Moreno, & Shabala, 2013; Slama, Abdelly, Bouchereau, Flowers, & Savoure, 2015). Moreover, these compounds can counteract both osmotic differences between vacuoles and inter- and intracellular liquid and harmful ROS activity (Flowers, Troke & Yeo, 1977; Flowers & Lauchli 1983; Muns & Tester, 2008; Parida & Jha, 2010). Common osmolytes that were found under high salinity environments in A*vicennia marina* leaves are mainly three classes of biochemical compounds: amino acids, quaternary ammonium compounds, and carbohydrates (Hibino *et al.,* 2001; Waditee *et al.,* 2002; Wang, Ke, Tam, & Wong, 2002; Parida & Jha, 2010; Patel, Gupta & Pandey, 2010; Dasgupta *et al.,* 2015; Khan, Adnan & Aziz, 2016).

Among amino acids, mainly proline is viewed as an osmolyte, which helps to tolerate salinity stress, and associated with low water potential stress (Szabados & Savouré, 2010; Verslues & Sharna, 2010). Quaternary ammonium compounds (QACs) such as glycine betaine and choline are also related to osmoregulation in some mangrove species (as reviewed in Slama *et al.,* 2015). For example, salt stress induces accumulation of glycine betaine in roots and leaves of *Avicennia marina* (Waditee *et al.,* 2002). One more group of osmolytes is carbohydrates (e.g., glucose, raffinose, sucrose) and their derivatives (pinitol, mannitol, sorbitol, etc.; Slama *et al.,* 2015). Parida & Jha (2010) in their review summarised information on particular osmolytes which characterise different mangrove plants and associates (Table 1.4).

Table 1.4. Osmolytes in mangroves and mangrove associates, from Parida & Jha (2010).

Osmolytes	Mangrove species	References
Pinitol	*Kandelia candel, Rhizophora stylosa, Bruguiera gymnorrhiza, Avicennia marina*	Hibino *et al.* (2001)
	Ceriops tagal	Popp *et al.* (1985)

Mannitol	*Kandelia candel, Rhizophora stylosa, Bruguiera gymnorrhiza*	Hibino *et al.* (2001)
	Sonneratia alba	Yasumoto *et al.* (1999);
	Lumnitzera racemosa	Ashihara *et al.* (2003)
Proline	*Kandelia candel, Rhizophora stylosa, Bruguiera gymnorrhiza*	Hibino *et al.* (2001)
	Bruguiera parviflora	Parida *et al.* (2002)
	Aegiceras corniculatum	Fu *et al.* (2005)
	Bruguiera sexangula, Avicennia alba, Xylocarpus granatum	Datta & Ghosh (2003)
	Acanthus ilicifolius, Hibiscus tiliaceus	Datta & Ghosh (2003)
	Avicennia marina	Datta & Ghosh (2003); Hibino *et al.* (2001)
	Ceriops roxburghiana	Rajesh *et al.* (1999)
	Ceriops tagal	Aziz & Khan (2001)
Betaine	*Avicennia marina*	Hibino *et al.* (2001); Ashihara *et al.* (1997); Popp *et al.* (1985)
	Ceriops roxburghiana	Rajesh *et al.* (1999)
	Hibiscus tiliaceus	Popp *et al.* (1985)
Aspartic acid	*Ceriops roxburghiana*	Suarez & Medina (2006)

Besides this information on the concentrations of osmolytes under hypersaline stress, there are few studies, which address osmolyte content in A*vicennia marina* leaves and/or roots under low or zero salinity conditions (e.g., Suzuki, Yasumoto, Baba, & Ashihara, 2003). This information is needed because compounds that are traditionally viewed as osmolytes can have another protective function. For instance, accumulation of betaine was found to improve low temperature tolerance in several plant species (Alia, Hayashi, Chen, & Murata, 1998; Holmström, Somersalo, Mandal, Palva, & Welin, 2000; Xing & Rajashekar, 2001). Thus, A*vicennia marina* var *australasica* species that grow in New Zealand, at the southernmost border of global mangrove distribution, may accumulate these compounds not as osmolytes, especially if salinity levels might be not that high, but for the frost protection.

1.5 Effect of nutrient availability on temperate mangrove growth

In addition to salinity, low nutrient availability is another factor that affects mangrove growth. The most important macro-nutrients are nitrogen compounds (in the form of nitrate, ammonium etc.) and phosphorus compounds (mainly phosphate ions). Nitrogen is important for the synthesis of proteins and it facilitates enzymatic activity (e.g., Rubisco, an enzyme that is involved in the carbon fixation), whereas phosphorus is an essential element for energy transfer and storage in cells (adenosine triphosphate [ATP]).

Moreover, both these elements are contained in DNA (deoxyribonucleic acids; Tester & Jorgensen, 2014).

1.5.1 Nutrient concentrations in temperate mangroves

Mangrove plants occupy estuarine and coastline ecosystems where nutrient dynamics is complex due to effects of freshwater flow, tidal activity, and anthropogenic factors (Alongi, 2002; Bianchi, 2007). Estuaries themselves serve as a sink for nutrients, sediment and other chemicals that come from catchments, through either groundwater or surface runoff (Day *et al.*, 2013). Traditionally, the amount of nutrients passing through estuarine ecosystems is low, because the majority of terrestrial nutrients are recycled within terrestrial ecosystems, and only a fraction of the nutrient pool can be carried out by the surface and/or ground water flow (Bianchi, 2007; Day *et al.*, 2013). On the other hand, seawater is low in nutrient content, too (Paytan & McLaughlin, 2007; Gruber, 2008).

Since mangroves occur in estuarine and coastal settings that *per se* are nutrient-limited, growth of mangroves is strongly affected by this low nutrient availability (as reviewed in Reis, Nardoto, Rochelle, Vieira, & Olivera, 2017). This fact has received confirmation in numerous field mangrove fertilisation studies as well as in numerous laboratory growth trials (Boto & Wellington, 1983; Boto *et al.*, 1985; Naidoo, 1987; Feller, 1995; Yates *et al.*, 2002; Lovelock *et al.*, 2007b; Naidoo, 2009; Alongi, 2011). In these experiments, mangrove plant growth and biomass production responded with great sensitivity to variations in nutrient concentrations. These field studies also have shown that mangrove plants in natural habitats the can be N-limited, P-limited or co-limited in both main nutrients, depending on geographical location and field position (e.g., at the edge of the mangrove stand, or at the interior; Feller, 1995; Lovelock, Feller, Ball, Ellis, & Sorrell, 2007a; Alongi, 2009; Reef, Feller, & Lovelock, 2010). However, nitrogen limitation occurred more often than phosphorus, as reviewed in Reef *et al.* (2010).

Since nutrient limitation severely affects mangrove growth processes, these plants have developed various nutrient conservation strategies (Alongi, 2003; Reef *et al.*, 2010). They allow plants to be highly productive in a low nutrient environment as well as to cope with salinity stress. The main nutrient conservation strategy is allocation of the majority of biomass below ground (Alongi *et al.* 2003). Firstly, higher root biomass simply increases nutrient uptake (Reef *et al.* 2010). Secondly, it has been shown that root proliferation in

mangroves under nutrient limited conditions mostly occurs in decaying old root channels, which allow growing roots to recapture leaching nutrients (McKee, 2001). Indeed, some controlled studies have demonstrated the dependence of root/shoot ratios on nutrient availability, indicating that mangrove seedlings invest more in roots in nutrient poor conditions (McKee 1995; Naidoo 2009).

Nutrient availability in mangrove environments may be assessed through measuring total nitrogen (%N) and total phosphorus (%P) concentrations in mangrove leaves (Aerts & Chapin III, 1999; Güsewell, 2004). An exact value for the level of nitrogen and phosphorus at which growth limitation of *Avicennia marina* occurs has not been determined in natural ecosystems, and many variables may impact on growth (Alongi, 2011). However, studies under controlled conditions may be relevant to indicate potential mangrove leaf concentrations, below which nutrient limitation can occur. With respect to this, Alongi (2011) studied the effect of nitrogen and phosphorus on the growth of *Avicennia marina* and five other mangrove species under controlled tidal hydroponic growth conditions. Plants were grown in seawater with a range of nitrogen concentrations and the growth rate and leaf nutrient content was determined. It was observed that the leaf nitrogen contents of *Avicennia marina* var *australasica* increased with increasing concentrations of nitrogen in the seawater solution, and ranged from a low of 1.13% at low nitrogen supplementation rates to a high of 3.40% total leaf nitrogen at very high supplementation rates. *Avicennia marina* displayed an S shaped nitrogen dependent growth curve which plateaued at a nitrogen supply rate of 10 mmol m^{-2} d^{-1}, which gave a measured average leaf content of 2.06% nitrogen. Thus, under optimal conditions, the growth of *Avicennia marina* may be nitrogen limited up to a leaf content of 2.06% N. However, in the case of phosphorus fertilisation leaf phosphorus concentration was increasing, plant growth rate did not correlate with these values.

Overall, dynamic nutrient partitioning (e.g., the allocation of nitrogen and phosphorus to various tree organs) represents a nutrient conserving strategy in mangrove trees (Aerts & Chapin III, 1999). While we know that tropical mangroves store substantially larger amounts of nutrients per unit surface area below ground than temperate mangroves (Alongi *et al.*, 2003), nutrient partitioning in temperate New Zealand mangroves has not been described previously. Quantification of nutrient concentrations in leaves also allows for the calculation of N:P ratios and, thus, helps to determine possible patterns of nutrient limitation for plant growth (Aerts & Chapin III, 1999, Tran *et al.*, 2016).

In addition, determination of the nutrient limitation (nitrogen- and/or phosphorus-) type in the field can reveal what nutrient limits mangrove growth and productivity in the particular geographical location (Reef *et al.*, 2010). While in the tropics, studies that investigate types of nutrient limitation are abundant, there are few studies that assess nutrient limitation type in New Zealand mangroves. Two of them reported that temperate mangroves in general are nitrogen limited (Lovelock *et al.*, 2007b; Lovelock *et al.*, 2010). These authors tried to link recently discovered mangrove expansion in New Zealand with nutrient availability in temperate estuaries. They found that nutrient availability was not the primary reason, rather factors such as high sedimentation rate and tidal activity determined succession of mangrove plants. Moreover, these and some other authors agreed that more fertilisation studies could reveal other nutrient limitation patterns in temperate mangrove areas (Alongi, 2009; Morrisey *et al.*, 2007; Reef *et al.*, 2010).

1.5.2 Nutrient sources in temperate mangrove ecosystems (anthropogenic vs natural)

Nutrients in mangrove ecosystems (in estuaries and harbours) can originate from natural processes such as weathering and leaching in the adjacent terrestrial soils, as well as from anthropogenic activity (e.g., agricultural, urban, and rural wastewater, industrial discharge, storm water, and overflow discharges; Bianchi, 2007). It is well known that extensive human activity in coastal settings can greatly influence nutrient loads in aquatic ecosystems, mainly from being nutrient deficient to nutrient enriched (eutrophied).

Ecological consequences of eutrophication are directly associated with nutrient concentrations in the tissues of organisms present in these ecosystems. Aquatic coastal and estuarine primary producers (e.g., phytoplankton, macroalgae, seagrasses, and mangroves) serve as 'coastal filters' and are able to absorb and bind nutrients into plant biomass (Costanzo *et al.*, 2001; McGlathery *et al.*, 2007; Nixon, Buckley, Granger, & Bintz, 2001). Some of these studies demonstrated that mangrove plants from eutrophied environments have been affected by the presence of elevated nutrients as well and have accumulated more nutrients in their leaves.

Additionally, along with elevated nutrient concentrations, it has been shown that nitrogen stable isotope values ($\delta^{15}N$) of coastal plants can provide reliable information about the source (anthropogenic *vs* natural) of nutrients within coastal areas (Costanzo *et al.*, 2001; Fry, 2006; Fry, Bern, Ross, & Meeder, 2000; Lindau, Delaune, Patrick, & Lambremont,

1989; McClelland & Valiela, 1998; Rogers, 2003). These studies demonstrate that different sources of nutrients have characteristic $\delta^{15}N$ values: 0‰ is characteristic of agricultural fertiliser inputs, such as urea; +4-+6‰ values are characteristic of organic matter mineralisation processes in the soils; and +10‰ is attributed to human and animal sewage, which is generally correlated with the presence of excess nutrients (eutrophication). Thus, measuring nitrogen stable isotope values ($\delta^{15}N$) in mangrove leaves can be used as a tool to monitor anthropogenic nutrient pollution in a given environment.

Indeed, in a model study, Costanzo *et al.* (2001) showed that different dominant plant groups (e.g., seagrasses, macroalgae and mangroves) within a habitat could be used as anthropogenic activity indicators, such as high sewage input *vs* pristine environments (Figure 1.6). Of particular importance are mangrove plants, which can grow extensively and form stable stands within many coastal regions of the tropics and subtropics (Figure 1.6).

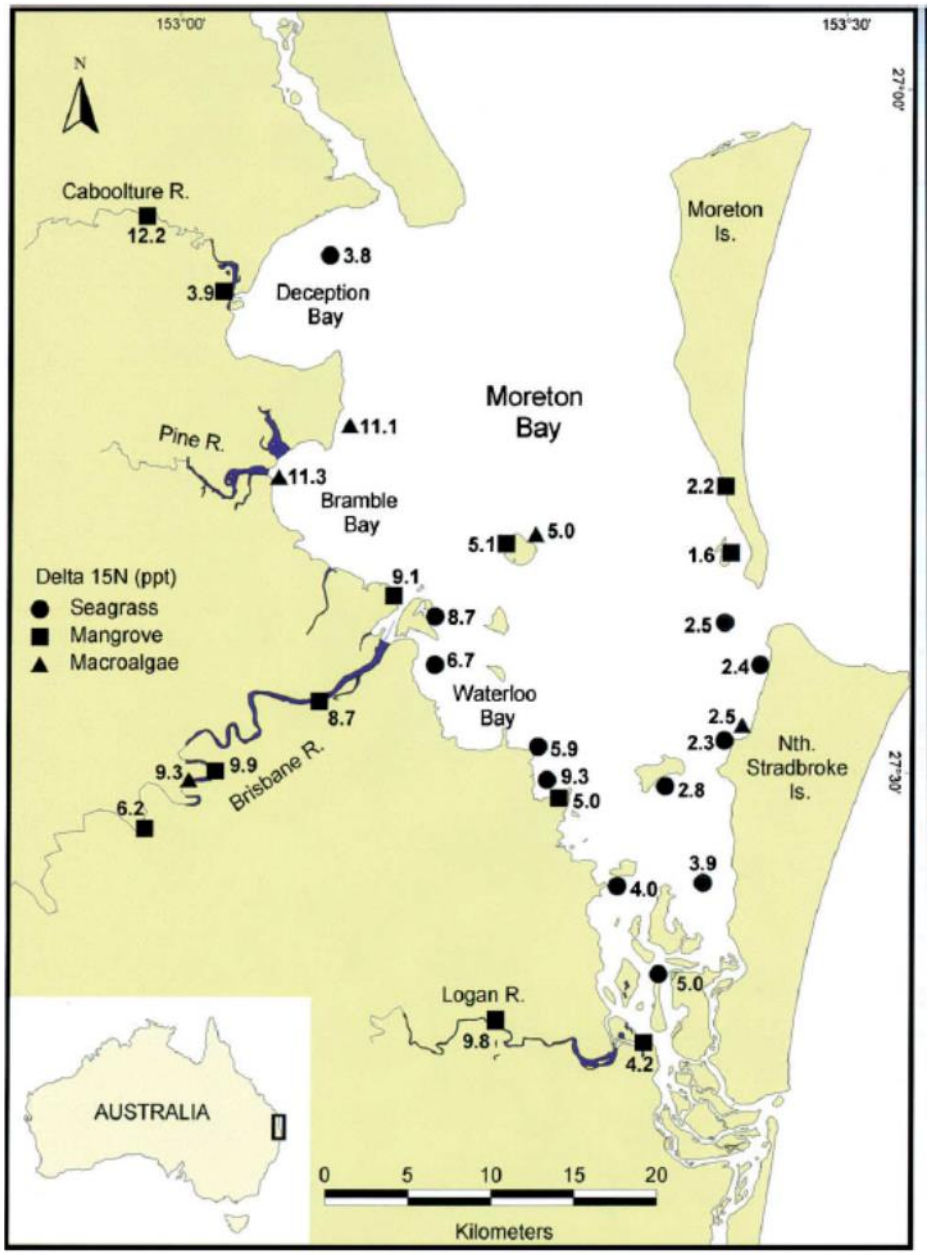


Figure 1.6. Sewage distribution mapped by applying $\delta^{15}N$ ratio measurements in aquatic organisms (macroalgae, seagrass, and mangroves) across Moreton Bay, Australia, retrieved from Costanzo *et al.*, 2001.

As for New Zealand, nutrient concentrations in New Zealand coastal waters have changed greatly due to various anthropogenic activities (Cooper & Thomsen, 1983; Hart *et al.*, 2004; Heggie & Savage, 2009; Thrush *et al.*, 2013; Vant, 1997). In the recent Marine Water Quality Annual Report issued by Auckland Council in 2013, some areas around highly populated Auckland regions were described as having 'poor' water quality (Walker & Vaughan, 2014). However, the effect of such changes on the nutrient status of temperate mangroves has been poorly studied. Moreover, mangrove stands have been expanding within many northern New Zealand estuaries (Green *et al.*, 2003; Schwarz,

2003; Stokes *et al.*, 2010) as the result of increasing catchment-derived sediment inputs (Lovelock *et al.*, 2007b; Morrisey *et al.*, 2010). However, the role of nutrient input from sewage, agriculture practices and livestock production on mangrove expansion has not been clearly demonstrated yet.

1.5.3 Nitrogen metabolism in mangrove plants

Low nitorgen availability is a limiting factor for mangrove growth in field studies and in laboratory growth trials (Boto & Wellington, 1983; Feller, 1995; Yates *et al.*, 2002; Lovelock *et al.*, 2007b; Naidoo, 2009; Alongi, 2011). On the biochemical level nitrogen shortage affects the concentration of nitrogen compounds required for both plant growth, for example amino acids, and for salinity tolerance, e.g., glycine betaine (Khamis, Lamaze, Lemoine, & Foyer, 1990; Foyer *et al.*, 1994; Keller, Kiene, Matrai, & Bellows, 1999).

Amino acids, which essential for growth

The pool of free amino acids holds a central place in the nitrogen cycle in plants (D'Mello, 2015). Amino acids are a group of diverse organic molecules, grouped by the general formula: R-CH2-(NH2)-COOH, where R is a group (or a side chain) specific to each amino acid (Barrett & Elmore, 1998). The primary biochemical role for amino acids is as units of proteins and enzymes that control and act as substrate in growth-related biochemical reactions (Liu, Liu, & Song, 2011). Nitrogen uptake in mangrove plants occurs via reaction of non-organic nitrogen (in the form of ammonium cation HN_4^+) with glutamic acids (Glu), and transformation into glutamine (Gln; Lea &Azevedo, 2006; Osuji & Madu, 2012; Osuji & Madu, 2015; Figure 1.7). Gln is also a starting compound for the synthesis of all other amino acids that then make up proteins and enzymes in a plant cell (Swarbreck, Defoin-Platel, Hindle, Saqi, & Habash, 2010). Redistribution and nitrogen storage occur in a form of asparagine (Asn) as it is chemically inert and has a higher N:C ratio (Lea & Miflin, 1980; Sieciechowicz, Joy, & Ireland, 1988; Fischer *et al.*, 1998, as summarised in Duff, 2015).

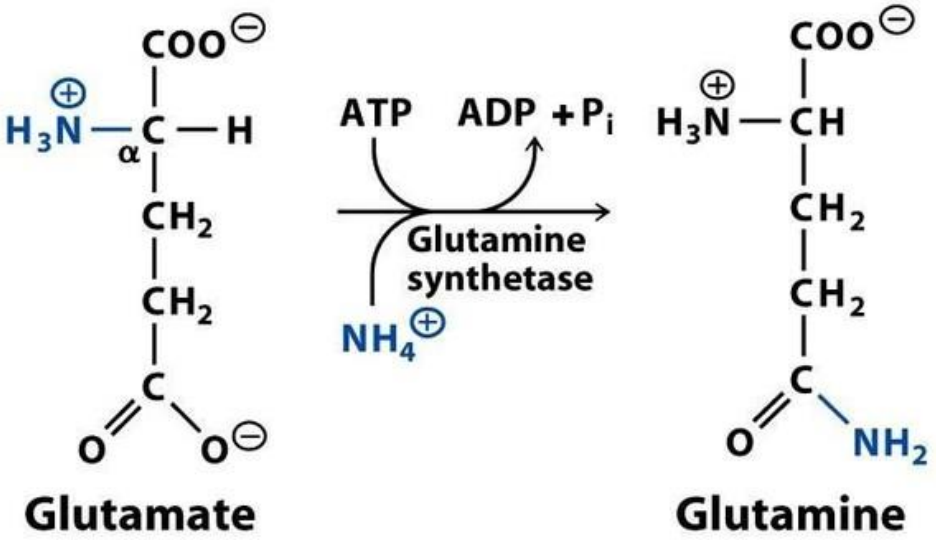


Figure 1.7. Glutamate-glutamine ammonium absorption biochemical pathway characteristic for mangrove plants.

Some other amino acids essential for growth include serine (Ser), which is involved in synthesis of growth and development enzymes, for example, Chao *et al.* (2011) demonstrated that serine induced synthesis of sphingolipids affects root ability to maintain ion homeostasis, required for growth. Serine level also regulates folate metabolism, which in turn regulates root development and photorespiration (Collacova *et al.*, 2008; Srivastava *et al.*, 2011, as reviewed in Ros, Munoz-Bertomeu, & Krueger, 2014). Glycine (Gly) is interconvertible to serine, and both are major sources of one-carbon units (e.g., in methyl transfer reactions; Schirch, 1984; Bourguignon, Neuburger, & Douce, 1988; Mouillon *et al.*, 1999). One more amino acid that is important for nitrogen metabolism in plants is alanine (Ala). Indeed, Ala is viewed as an important link between nitrogen and carbon metabolism in plant cells because of its ability to mediate glycolysis and the tricarboxylic acid cycle (Miyashita, Dolferus, Ismind, & Good, 2007; Rocha *et al.*, 2010; Raychaudhuri, 2015). Lastly, phenylalanine (Phe) is required for synthesis of flavonoids and lignins, which, in turn, are essential for pigment and wood cell wall formation (Swain & Williams, 1970; Cooper & Nicola, 2015).

However, there is only one study, to my knowledge, where amino acid pool in *Avicennia marina* mangrove species was investigated (Ashihara *et al.*, 1997). However, these authors did not investigate how the free amino acid pool in mangrove plants is affected by nitrogen and salinity levels. This information might provide insights into the role of the aforementioned amino acids in mangrove plant growth.

Amino acids as osmolytes

Although the main role of amino acids is constituent in growth related biochemical processes, the very same amino acids can play the role of osmolytes, primarily because of their chemical properties (e.g., existence as a zwitterion at neutral pH). Moreover, there is a lot of research that illustrates accumulation of these amino acids under stress conditions in glycophytes and halophytes, which has a synergetic effect on overall plant performance (Mansour, 2000; Planchet *et al.*, 2011; Ahmad & Prasad, 2012; Planchet & Limami, 2015).

Growth under saline conditions means growth under nitrogen limitation. In order to grow succesfully under salinity stress, plants, especially mangroves, limit water uptake, and, thus, nutrient uptake (Flowers, 2004; Kumari, Das, Parida, & Agarwal, 2015). Mangrove plants as halophytes are able to adjust their physiological and biochemical processes to counteract salinity effects (Yu *et al.*, 2011). A central role of these biochemical changes is related to adjustment in the free amino acid pool and/or concentrations of specific amino acids. For example, Brosche *et al.* (2015) found that the halophyte *Populus euphratica* up-regulates the level of free amino acids under salinity stress, as well as many mangrove species accumulate proline, tyrosine, alanine, cysteine, arginine, glycine, glutamine, and asparagine (Mansour, 2000; Ahmad & Prasad, 2012).

In fact, nutrient addition, namely nitrogen, under high salinity conditions in mangroves can stimulate growth, through affecting plant nitrogen metabolism. For instance, Martin *et al.* (2010) demonstrated in vivo that mangroves from hypersaline areas after N fertilisation improved primary growth parameters, and water use efficiency, but the authors did not perform a metabolomic analysis. However, through the metabolic studies this effect can be explained. Nitrogen addition may increase concertation of free amino acids, which, in turn, enhances speed of growth processes, with extra nitrogen supply mangroves might be able to synthesise more osmolytes (protective compounds). For example, in glycophytes, Foyer *et al.* (1994) and Khamis *et al.* (1990) observed increases in concentrations of free amino acids in maize (*Zea mays* L. cv. Contessa) leaves after additional nitrogen supply.

The most common free amino acid accumulated under stress conditions is proline (Pro). Proline accumulation as a response to various environmental stresses (including salinity and oxidative stress) was observed in glycophytes, as well as in halophytes (Singh,

Aspinall, & Paleg, 1972; Munns, 2002; Parida & Jha, 2010; Gupta & Huang, 2014; Planchet & Limami, 2015). In Table 1.4 (part 1.4.3) it is shown that a lot of mangrove species indeed accumulate proline as an osmoprotectant. *Avicennia marina* also has proline in its leaves, but in smaller quantities, than, for example, glycine betaine, which is believed to be the main osmoprotectant for this species (Parida & Jha, 2010). However, information on how the pool of osmolytes in mangroves differ under low vs high salinity has not been studied previously. This study can highlight the importance of certain osmolytes as well as provide information on how mangroves change theirs metabolomics depending on environmental stress.

1.6 Research aims and rationale

The main focus of the present dissertation is to study how growth of temperate mangrove plants is affected by nutrient and salinity levels in New Zealand conditions. Although, nutrient and salinity interactions take place in mangrove sediments, the outcome of these interactions can be assessed through analysis of the aboveground biomass (Figure 1.8).

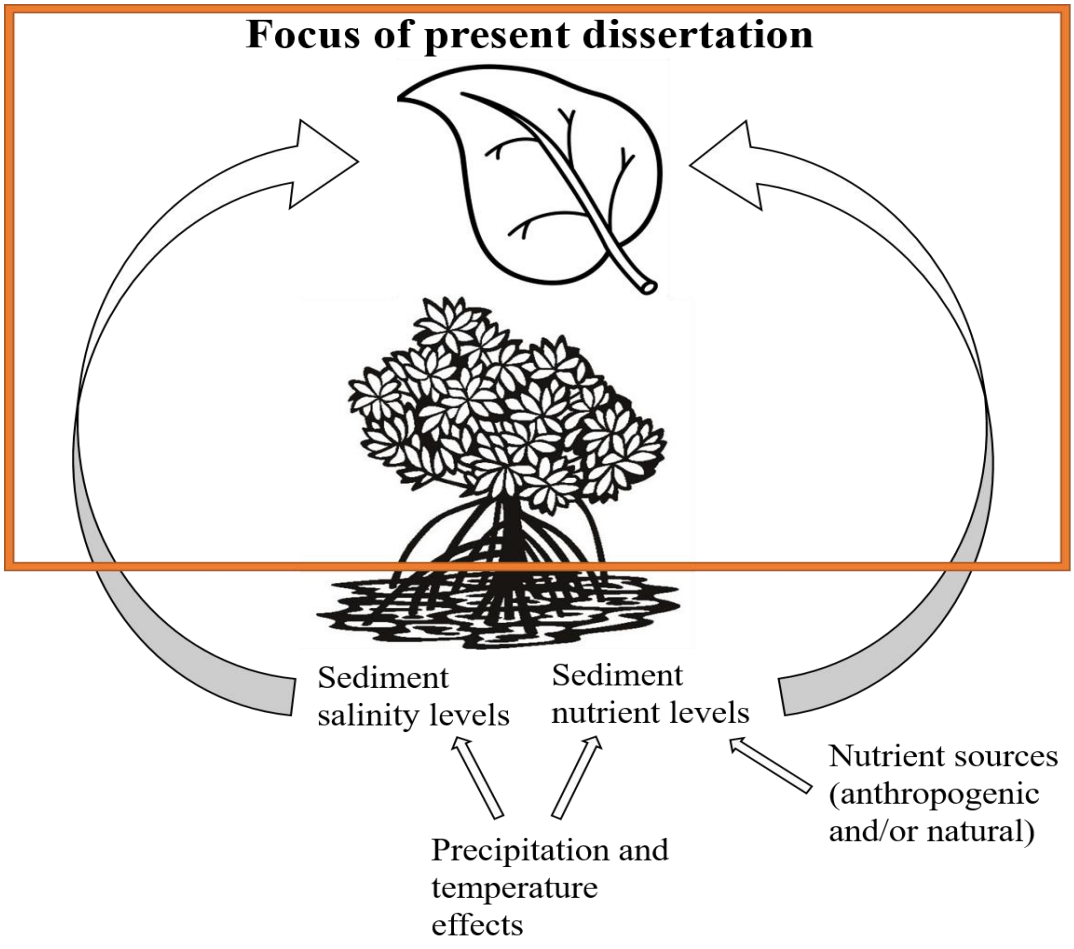


Figure 1.8. Diagram of the main focus of the present dissertation, assessing effect of salinity and nutrients on mangrove growth through analysis of the aboveground biomass.

Temperate New Zealand mangroves have received less attention since they are estimated to only form a small fraction of the global mangrove area. However, recent public concerns about mangrove spreading problems have drawn interest to what might trigger unwanted growth and increases in plant productivity. From the biological point of view, low salinity levels due to lower temperature regimes and higher precipitation rate and increased nutrient loads from human and animal sewage and agricultural runoff might affect growth and productivity of temperate mangroves in New Zealand coastal and estuarine environments. Thus, four aims were formulated, according to knowledge gaps on effects of low temperate salinities and nutrient concentrations on temperate mangrove plant growth and productivity in controlled laboratory conditions as well as in field settings.

Firstly, there is a need to determine the pattern of dynamic nutrient partitioning (e.g., the allocation of nitrogen and phosphorus to various tree organs) within temperate mangrove ecosystems in New Zealand estuaries. This work has not been conducted before, but such information is crucial for understanding how *Avicennia marina* var *australasica* plants conserve nutrients under temperate New Zealand conditions. This research question will be addressed in Chapter 2.

Secondly, the effect of anthropogenically derived nutrients was investigated on temperate mangrove nutrient status among three New Zealand harbours with contrasting human activities. Human activity has changed nutrient concentrations in New Zealand coastal waters, but an effect of additional nutrient input on mangrove growth has not been clearly demonstrated yet. This is especially important since there is rapid spreading of mangrove stands in some New Zealand estuaries, where the presence of additional nutrients may play a complementary role along with increased sedimentation. This research question will be investigated in the Chapter 3.

Thirdly, it is necessary to understand the effect of contrasting salinity levels and nutrient concentrations on growth and metabolism of temperate New Zealand mangroves in controlled laboratory conditions. Biochemical changes (ROS levels, osmolyte concentrations and changes in amino acids compositions) were assessed in A*vicennia marina* var *australasica* leaves and roots, as well as biomass accumulation patterns under contrasting salinity and nutrient levels. Results of a laboratory growth trial, which was conducted to investigate this question, are presented in Chapter 4.

Finally, the effect of nutrient addition on temperate mangrove growth and nutrient status was investigated at Mangawhai Harbour Estuary in northern New Zealand. Due to a lack of scientific information on nutrient and salinity levels, which are characteristic to temperate New Zealand mangroves, it is largely unknown whether salinity levels affect mangrove growth in the same manner as in tropical settings. A study addressing this question is described in Chapter 5.

1.7 Materials and methods

1.7.1 Study sites

The main study objective of the present dissertation is the investigation of temperate mangrove ecosystems. The study sites were located across northern New Zealand (Figure 1.9).

Figure 1.9. Present study site locations in northern New Zealand.

1.7.2 Methods to study temperate mangroves dynamic nutrient partitioning

A collaborative study was performed to access the nutrient allocation of temperate mangroves. Nutrient allocation can be assessed only after accurate biomass estimation, and because biomass estimation of temperate mangrove plants did not fit into the scope of the present dissertation, a collaborative experiment was performed. Plant nutrient

analysis include analysis of total nitrogen (TN), total phosphorus (TP), and total carbon (TC) concentration in leaf, stem, and root mangrove plant material. Quantification of TP included acid digestion of plants material, followed by quantification using an inductively coupled plasma spectrometer (ICP-AES, Varian Liberty AX Series II, USA). Total nitrogen (TN) and total carbon (TC) measurements were obtained using an element analyser (CE 440, Exeter Analytical, North Chelmsford, MA, USA).

1.7.2 Methods to study nutrient sources in temperate mangrove ecosystems

Leaf nutrient composition survey was used to study the possible sources of nutrients in temperate mangrove ecosystems. For this survey three locations with contrasting human activity were chosen. Two locations that are situated within the city of Auckland, Waitemata Harbour to the north-east and the Manukau Harbour to the south-west, and the third with less human presence one will be chosen at Mangawhai Harbour. Waitemata and Manukau harbours are located within New Zealand's largest city with a population of 1.4 million within the wider Auckland region (Census 2013). The main difference between these locations is that the main Auckland sewage treatment plant is situated in Manukau Harbour. Additionally, Manukau Harbour exhibits the greater proportion of farmland and is highly urbanised, compared to the Waitemata Harbour. The third main site sampled was Mangawhai harbour located 81km north of Auckland. The main source of anthropogenic nitrogen at Mangawhai is likely to be from farm animals and since Mangawhai has a relatively small human population of 1,329 this site is considered to be more pristine (Census 2013).

Measurements of total nitrogen and total phosphorus concertation, and stable nitrogen isotope values ($\delta^{15}N$) of *A. marina* leaves were the main method used to characterise the source of nutrients (namely nitrogen). Analysis for total phosphorus was conducted using acid digestion, followed by quantification using an inductively coupled plasma atomic emission spectrometer (ICP-AES, Varian Liberty AX Series II, USA). Total nitrogen and stable isotope ($\delta^{15}N$) analyses were conducted externally at the Waikato University Stable Isotope Unit.

Nitrogen stable isotope ratio ($\delta^{15}N$) is representative for animal and human nitrogen discharge *versus* natural source, and, hence, can be used to identify the nitrogen source. This ratio originates from differences in the kinetics of chemical reactions of simultaneously occurring light (^{14}N) and heavy (^{15}N) isotopes. $\delta^{15}N$ is higher when

abundance of nitrogen is present, so heavy isotopes are accumulated in the tissue of primary producers (e.g., algae, seagrass, and mangroves). Indeed, some earlier studies in tropical mangroves demonstrated that mangrove foliar composition can inherit these specific values derived from anthropogenic sewage discharge (Costanzo *et al.*, 2001).

1.7.3 Methods to study how salinity and nutrients affect the mangrove growth in the field

To assess the effect of salinity levels and nutrients on growth of mangrove plants in the field under temperate New Zealand conditions one-year growth observational field study was conducted followed by one-year fertilisation experiment. During the first year of the experiment, growth rate (shoot growth and new leaf gain), levels of nutrients (total nitrogen and total phosphorus concentrations in the leaves) were measured to assess the level of nutrients present in the environment. Sodium and potassium concentrations in mangrove leaves were used to estimate the levels of the environmental salinity. After addition of the fertiliser, plant growth measurements were continued along with measurements of nutrient composition in leaves. Nitrogen stable isotope ratio ($\delta^{15}N$) was analysed to control the level of fertiliser absorption. Quantification of elements in mangrove leaves was conducted using the aforementioned methods.

1.7.4 Methods to study how salinity and nutrients affect nitrogenous metabolomics in mangrove seedlings

The greenhouse growth trial was used to determine the exact effect of nutrient and salinity levels on growth and metabolomics of mangrove plants. Mangrove propagules were collected and grown in the laboratory under different nutrient and salinity treatments for 6 months. After 6 months, plants growing at different conditions were treated with the same amount of labelled $^{15}NH_4Cl$ for estimation of the speed of nitrogen uptake. One leaf from each seedling was collected before, after 3 hours, and after 2 days of labelled treatment to characterise the nitrogenous metabolomics of mangrove seedlings. At the end of the growing trial, the total wet and afterwards dry biomass was estimated, using the destructive harvest method.

The analytical analysis of nitrogenous metabolomics was based on characterisation of amino acids profile, using the Liquid Chromatography –Mass Spectrometry (LC-MS) instrument. Nitrogen uptake levels was studied using the nitrogen stable isotope ratio ($\delta^{15}N$) values and total nitrogen concentration in mangrove leaves. Quantification of these

parameters was performed using the aforementioned methods. Additionally, concentration of osmolyte compounds (glycine betaine, choline) and level of Reactive Oxygen Species (ROS) was estimated to determine the effect of salinity treatments on the biochemical processes in mangrove plants.

1.8 Thesis structure

The overall objective for the dissertation was to investigate how climatic conditions characteristic for New Zealand as well as human activity affect growth of temperate mangrove plants through nutrients and salinity levels in estuarine ecosystems. To achieve this objective, three major studies were conducted as well as one collaboration work. Chapters 2 to 5 were written as original research papers. The general introduction (Chapter 1) discusses the context of existent knowledge and specifies scientific gaps that present research will fulfil. The general discussion (Chapter 6) brings together generated new information and critically discusses what contribution it makes to the existent knowledge on temperate New Zealand mangrove growth.

Chapter 2. Dynamic nutrient partitioning of temperate *Avicennia marina* var. *australasica* mangrove species in New Zealand

The current chapter presents a study of dynamic nutrient allocation in mangroves *A. marina* within different biomass pools (leaf, stem, roots) under temperate New Zealand conditions. These findings contribute to the main aim of the dissertation to investigate nutrient cycling in temperate mangrove ecosystems. The content and experimental results of this chapter were included in a published article "Biomass and nutrient composition of temperate mangroves (*Avicennia marina* var. *australasica*) in New Zealand", authored by Phan Tran, Iana Gritcan, Jarrod Cusens, Andrea C. Alfaro, Sebastian Leuzinger. The first author of the paper planned the experiments, led the fieldwork, collected and analysed the tree biomass components of temperate mangroves (*Avicennia marina* var. *australasica*), and was in charge of submission processes. My contribution to this work involved field assistance, sample preparation, chemical analysis of plant biomass, and data processing of nutrient allocation and presentation of the results for publication purposes. I also contributed to the writing of the article with regards to the nutrient analysis of mangrove biomass. I was also involved in the revision processes of the submitted manuscript. Herein, I present only the research components that I was responsible for and which constitute to my thesis.

2.1 Abstract

Accurate estimates of biomass pools and their nutrient contents are key for assessing the potential of vegetation to mitigate anthropogenic carbon emissions. In this study, we used the harvest method to estimate above- and below-ground biomass (AGB and BGB) and nutrient content of the New Zealand mangrove *Avicennia marina* var. *australasica* at Mangawhai Harbour, northern New Zealand. AGB of *A. marina* was estimated at 5.7 ± 1.79 kg m^{-2} and BGB at 13.15 ± 1.55 kg m^{-2} (mean ± SE). The root-shoot ratio at this site was 1.73. Fine roots contributed most to the total biomass (37%) followed by woody biomass (32%), coarse roots (27%), leaves (3%), and pneumatophores and seedlings (1%). Allocation of total carbon (TC) followed similar proportions as total biomass with was 64% in the roots, 33% in wood, and 3% in the leaves (by dry mass). Roots contained 64% of total nitrogen (TN) and 53% of total phosphorus (TP) (by dry mass). In leaves, TN was 5% and TP was 6% with the remaining nutrients in woody biomass. The foliar N:P ratio was 8.2, suggesting nitrogen limitation at this site. Based on these results, we estimate that New Zealand mangroves store a total of 0.2–1.1
Mt carbon (C) above ground and 1.06–1.72 Mt C below ground.

2.2 Introduction

Analysis of how plants allocate biomass complimented by nutrient partitioning information gives information on where plants invest photosynthetically fixed carbon (Alongi, 2009). When analysis is performed on the same species, but in different climatic conditions, assessment of stress levels can be gained, as well as improving our understanding of plant adaptive strategies. For example, Poorter & Nagel (2000) performed a quantitative review which showed that plants growing in nutrient limited conditions have higher ratios of below to above ground biomass. They concluded that it is a common plant adaptation strategy to invest fixed carbon to access the most limiting resource.

Mangroves, as with other plants, have similar conservation strategies that enable them to cope with environmental stress (e.g., temperature, nitrogen and/or phosphorus shortage, and high salinity) and thus allow them to be highly productive in challenging or extreme environments. The allocation of below-ground biomass has been described as a main nutrient conservation strategy for mangrove plants (Alongi *et al.*, 2003). In the first instance, higher root biomass simply increases nutrient uptake (Reef *et al.*, 2010). Additionally, it has been shown that root proliferation in mangroves under nutrient limited conditions mostly occurs in decaying old root channels, which allow growing roots to recapture leaching nutrients (McKee, 2001). Furthermore, some controlled studies have demonstrated the dependency of root/shoot ratios on nutrient availability, indicating that mangrove seedlings invest more in roots in nutrient poor conditions (McKee, 1995; Naidoo, 2009).

Dynamic nutrient partitioning (e.g. the allocation of nitrogen and phosphorus to various tree organs) reflects another nutrient conservation strategy in trees (Aerts & Chapin III, 1999). While we know that tropical mangroves store substantially larger amounts of nutrients per unit surface area below ground than temperate mangroves (Alongi *et al.*, 2003), nutrient partitioning in temperate New Zealand mangroves has not been described previously. Quantification of nutrient concentrations in leaves also allows for the calculation of N:P ratios and thus helps determine possible patterns of nutrient limitations for plant growth (Aerts & Chapin III, 1999).

2.3 Materials and methods

Study site

Mangawhai Harbour Estuary (36° 07' 00" S, 174° 36' 00" E) is located about 100 km north of Auckland, New Zealand. It is characterised as a relatively sandy estuary with two main streams (Tara Creek and Bob Creek) and a variety of wetlands, including salt marshes, sand/mud flats, and about 87 ha of mangroves (Figure 2.1).

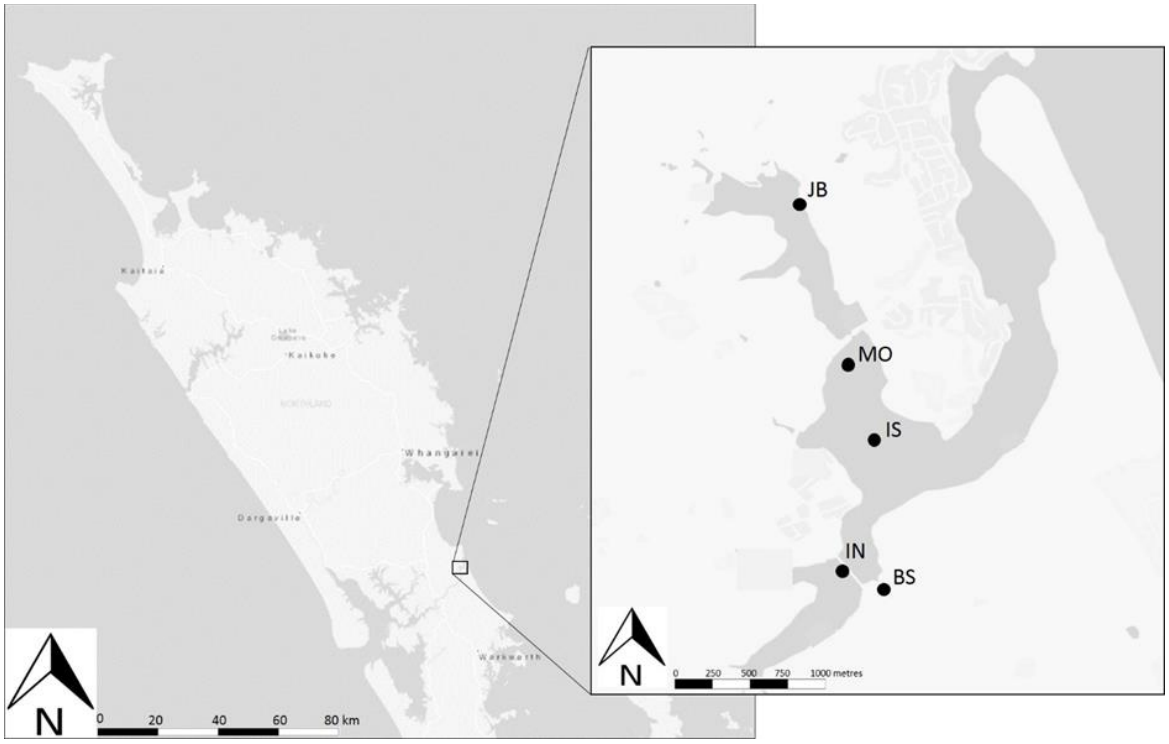


Figure 2.1. Map of Mangawhai Heads, northern New Zealand with five selected sites: Jack Boyd (JB), Molesworth (MO), Island (IS), Insley (IN) and Black Swamp (BS).

Nutrient content analysis

Five out of the 10 trees harvested from the BS site were used to measure nutrient content and estimation of nutrient partitioning in different mangrove components (leaves, wood, fine roots and coarse roots). Three replicate samples from each component per tree were used for these analyses. Roots were washed to remove all sediment. All samples were oven-dried to constant weight at 65°C. The dried material was then ground to fine powder with a ball mill (PM 100, Retsch, © Retsch GmbH). Quantification of total phosphorus (TP) was conducted using Kjeldahl digestion in HNO_3 and $HClO_4$, with a modified method from McQuaker, Brown, & Kluckner (1979), followed by quantification using an inductively coupled plasma spectrometer (ICP-AES, Varian Liberty AX Series II, USA). Total nitrogen (TN) and Total carbon (TC) measurements were obtained using an element

analyser (CE 440, Exeter Analytical, North Chelmsford, MA, USA). A National Institute of Standards & Technology (NIST) Peach Leaf standard reference material (SRM1547) was used as a quality control in all analytical analyses and was run together with mangrove samples. The experimental deviation from this standardised material was less than 5%.

Estimation of nutrient partitioning

Nutrient partitioning (n=5 harvested trees, BS site) for each biomass pool (wood, fine roots, coarse roots, pneumatophores, seedlings, and leaf) was calculated as the mean concentration for each nutrient parameter (TC, TN, TP) multiplied by the percent biomass estimated for each component (Figure 2.2). These values were standardised to 100% for the total tree biomass.

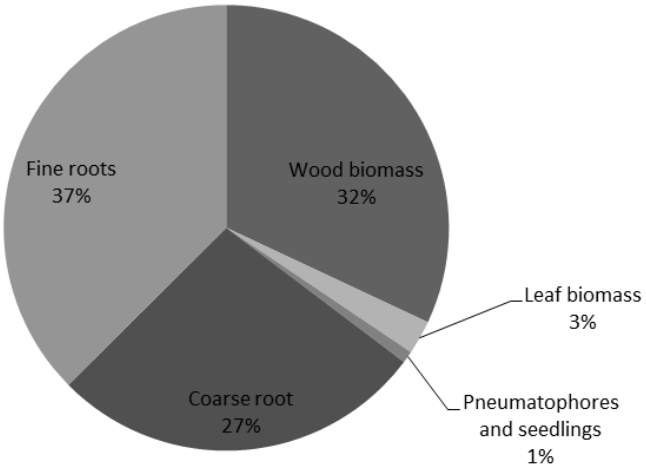


Figure 2.2. Biomass allocation (%) per square meter estimated for each biomass pool of Mangawhai mangroves. Presented are the mean values of two sites (n=2) Jack Boyd (JB) and Molesworth (MO). Standard error values were ±2.3, ±2.1, ±4.9, ±0.4, and ±0.1 for wood, fine roots, coarse roots, pneumatophores and seedlings, and leaf biomass, respectively.

2.4 Results

Total carbon content was almost the same in the three mangrove components (leaf, wood and root), including 44.10±0.14% of dry weight in wood, 43.43±0.25% in leaf material and 41.91±2.19% in roots. Total nitrogen content of leaf material was almost two times higher than in wood and roots (2.10±0.05%, 1.04±0.05% and 1.28±0.13%, respectively). The highest total phosphorus per dry weight was found in leaves (0.25±0.03%), while

wood and root biomass was estimated at 0.15±0.01% and 0.12±0.01%, respectively (Figure 2.3). The foliar N:P ratio for the harvested trees at the BS site was calculated to be 8.2. Nutrient allocated lowest in leaf with 3% TC, 5% TN, and 6% TP. The wood had an estimated 33% TC, 27% TN and 37% TP, while roots contained 64% TC, 68% TN and 57% TP (Figure 2.4).

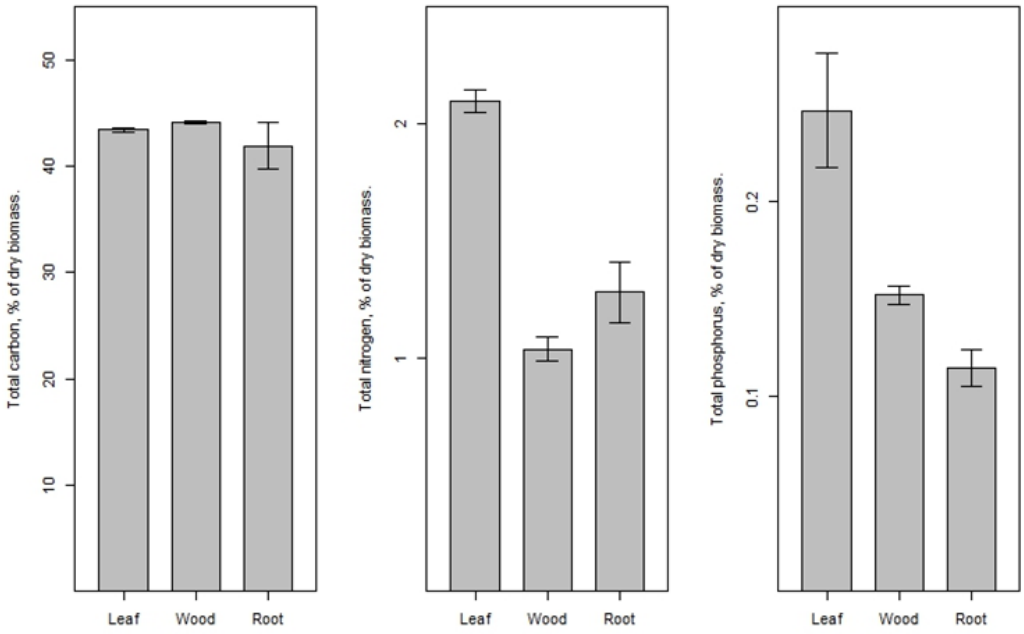


Figure 2.3. Mean (±SE, n = 5 harvested trees) element concentrations in different compartments (leaf, wood and roots) of *A. marina* trees at Mangawhai Harbour, BS site.

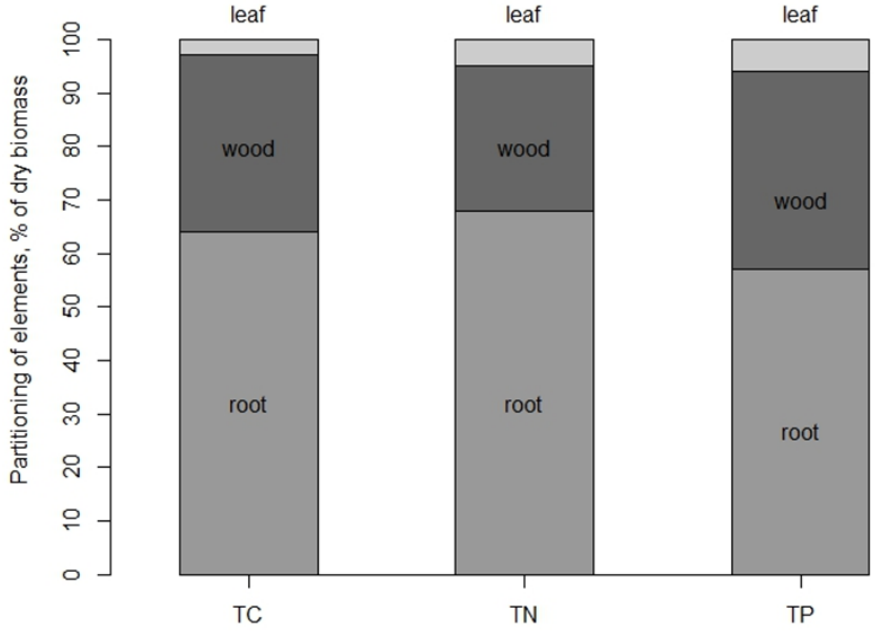


Figure 2.4. Partitioning of total nitrogen (TN), total phosphorus (TP), and total carbon (TC) (% of dry biomass) in *A. marina* var *australasica* trees within different biomass pools (roots, wood, and leaves, n=5 harvested trees) at Mangawhai Harbour, BS site.

2.5 Discussion

While the highest concentration of nutrients was expected to be in the leaves, since they are the site for carbon fixation (requiring high concentration of enzymes, biochemical complexes, and other metabolites), the largest estimated plant nutrient pool was found below ground (68% TN and 57% TP). These results suggest that mangroves at the study site accumulate significant amounts of nutrients below ground in the form of fine and coarse roots. Alongi *et al.*, (2003) proposed that this type of nutrient allocation in BGB could serve as a nutrient conservation mechanism, and these nutrients could then be made available for the rest of the plant during nutrient shortages via organic matter decomposition and recapturing processes. While we did not measure nutrient concentrations in the sediment, previous studies suggest that when estimating below-ground biomass (BGB) and sediment, nutrient allocations to the below ground pool increase significantly, due to the added dead plant material and inorganic forms of nutrients. Thus, up to 88 ± 3 and 99 ± 0.4% of TC and TN may be stored in mangrove ecosystems in below-ground and sediment pools and potentially could be utilised by the plant (Bulmer, Lundquist, & Schwendenmann, 2016). To gain further insights on nutrient partitioning, especially phosphorus, in mangrove ecosystems, future studies should investigate the relative contribution of nutrients within the plant and the surrounding sediment.

The foliar total nitrogen:phosphorus ratio (N:P ratio) calculated for mangroves at this particular location was 8.2, which suggests nitrogen limitation according to Aerts & Chapin III, (1999). This is in line with the previously suggested notion that temperate mangroves are nitrogen limited (Lovelock *et al.*, 2007b; Morrisey *et al.*, 2010). However, observed N:P ratios for many mangrove ecosystems in New Zealand average 12.96±0.42, and mangroves in this location might be unusually low in nitrogen, due to their close proximity to pastoral lands that provide additional phosphorus inputs (Hart *et al.*, 2004). To address the type of nutrient limitation in New Zealand mangrove ecosystems, more studies should be conducted to assess the nutrient ratios of surrounding lands and their effects on mangrove stands. Mangrove fertilisation experiments also could be conducted to specifically identify the type of nutrient limitation (if any), as well as providing insights on human-introduced nutrient effects on mangrove ecosystems.

Chapter 3. Effect of anthropogenically derived nutrients on temperate mangrove nutrient status among three New Zealand harbours with contrasting human activities

This chapter presents results of temperate mangrove leaf nutrient content surveys in three New Zealand harbours with high, medium, and low anthropogenic nutrients input. Mangrove leaf nutrient concentration is inseparably linked to the nutrient concentration in the environment, and, moreover, reflects the potential sources of nutrients (anthropogenic vs natural). It was hypothesised that human derived nutrients might cause nutrient enrichment in temperate estuarine ecosystems, which are naturally low in nutrients. If so, mangrove plants might be provided with additional nutrients, which might contribute to the spread of temperate mangrove stands in northern New Zealand. The content of this chapter contributes to the main dissertation question by providing the previously missing information on the presence of different nutrient sources in temperate mangrove ecosystems, as well as its effect on anthropogenically derived nutrients (namely nitrogen) in mangrove leaf composition. Content and findings of this chapter was published as a paper "Leaf stable isotope and nutrient status of temperate mangroves as ecological indicators to assess anthropogenic activity and recovery from eutrophication" in Frontiers in Plant Science, on 23rd December, 2016.

3.1 Abstract

We measured nitrogen stable isotope values ($\delta^{15}N$), and total phosphorus (%P) and total nitrogen (%N) contents in leaves of the temperate mangrove (*Avicennia marina* var *australasica*) from three coastal ecosystems exposed to various levels of human impact (Manukau, high; Mangawhai, low; and Waitemata, intermediate) in northern New Zealand. We measured $\delta^{15}N$ values around 10‰ in environments where the major terrestrial water inputs are sewage. The highest average total nitrogen contents and $\delta^{15}N$ values were found in the Auckland city region (Manukau Harbour) at 2.2%N and 9.9‰, respectively. The lowest values were found in Mangawhai Harbour, situated about 80 km north of Auckland city, at 2.0%N and 5.2‰, respectively. In the Waitemata Harbour, also located in Auckland city but with less exposure to human derived sewage inputs, both parameters were intermediate, at 2.1%N and 6.4‰. Total phosphorus contents did not vary significantly. Additionally, analysis of historical mangrove leaf herbarium samples obtained from the Auckland War Memorial Museum indicated that a reduction in both leaf total nitrogen and $\delta^{15}N$ content has occurred over the past 100 years in Auckland's harbours. Collectively, these results suggest that anthropogenically derived nitrogen has had a significant impact on mangrove nutrient status in Auckland harbours over the last 100 years. The observed decrease in nitrogenous nutrients probably occurred due to sewage system improvements. We suggest that mangrove plant physiological response to

nutrient excess could be used as an indicator of long-term eutrophication trends. Monitoring leaf nutrient status in mangroves can be used to assess environmental stress (sewage, eutrophication) on coastal ecosystems heavily impacted by human activities. Moreover, nitrogen and phosphorus leaf contents can be used to assess levels of available nutrients in the surrounding environments.

3.2 Introduction

Human activities continue to affect coastal ecosystems throughout the world at alarming rates. Habitat destruction (e.g., deforestation, urbanization) and eutrophication (e.g., agricultural runoff, sewage inputs) have been identified as major factors that affect water quality in aquatic ecosystems within coastal areas (Heaton, 1986; Valiela *et al.*, 1992; Nixon *et al.*, 1996; Valiela *et al.*, 1997; McClelland & Valiela, 1998; Verhoeven, Arheimer, Yin, & Hefting, 2006). Indeed, excessive nutrient inputs (eutrophication) from intensive agricultural activity and growing populations within coastal regions usually result in rapid degradation of water quality and modifications of ecological features (Heisler *et al.*, 2008; McGlathery *et al.*, 2007; Schindler, 2006; Smith & Schindler, 2009).

Ecological consequences of eutrophication are directly associated with nutrient contents (e.g., nitrogen, phosphorus) in the tissues of organisms present in these ecosystems. Previous studies have shown that plant tissue nutrient composition is linked to the nutrient availability in the surrounding environment (Aerts & Chapin III, 1999; Güsewell, 2004), making the nutrient status of those organisms susceptible to any nutrient excess. Aquatic coastal and estuarine primary producers (e.g., phytoplankton, macroalgae, and seagrasses) serve as 'coastal filters' and are able to absorb and sequester nutrients into plant biomass (Costanzo *et al.*, 2001; McGlathery *et al.*, 2007; Nixon *et al.*, 2001).

Mangrove stands could play an important role in mitigating eutrophication in coastal settings as they have been described as nutrient limited ecosystems with a generally positive physiological response to nutrient addition (Alongi, 2009; Reef *et al.*, 2010). For example, previous laboratory (Alongi, 2011; Boto *et al.*, 1985; Naidoo, 1987; Yates *et al.*, 2002) and field studies (Boto & Wellington, 1983; Feller, 1995; Lovelock *et al.*, 2007b; Naidoo, 2009) have shown that mangrove leaf nutrient status (nitrogen and phosphorus contents) correlate well with levels of nutrient addition and/or natural variability in nutrient concentrations within the environment. Mangrove plants, which can

grow extensively and form stable stands within most protected coastal regions of the tropics and subtropics could be of particular importance for remediation.

To study the ability of organisms in taking up nutrients derived from anthropogenic sources, nitrogen stable isotope values ($\delta^{15}N$) can be used as they provide reliable information about the source (anthropogenic *vs* natural) of nutrients within coastal areas (Costanzo *et al.,* 2001; Fry, 2006; Fry *et al.,* 2000; Lindau *et al.,* 1989; McClelland & Valiela, 1998; Thimdee, Deein, Sangrungruan, & Matsunaga, 2002; Rogers, 2003). $\delta^{15}N$ values of around +10‰ are attributable to human and animal sewage, which is generally correlated with the presence of excess nutrients (eutrophication) (Costanzo *et al.,* 2001; Fry *et al.*, 2000). Elevated $\delta^{15}N$ values arise due to the presence of excess nutrients in the environment allowing increased isotope fractionation via increased volatilisation of ammonia and /or increased microbial processing. Indeed, Rogers (2003) used nitrogen stable isotope values within mussel and limpet samples to show how fast the aquatic system recovered from sewage outfall closures. Also, Costanzo *et al.* (2001) showed that different dominant plant groups (e.g., seagrasses, macroalgae and mangroves) could be used as anthropogenic activity indicators (sewage input *vs* pristine environments) within a habitat.

The mangrove genus *Avicennia* is widely distributed across the tropical and sub-tropical regions, including Australia, the Arabian Peninsula, Brazil, China, India, Indonesia, Japan, Malaysia, the Phillipines, Pakistan, the southern United States and Central America, South Africa and New Zealand (Duke *et al.,* 1998). *Avicennia*'s characteristic occurrence in the intertidal ecotone makes it an ideal indicator genus for monitoring eutrophication in coastal habitats in large areas of the world. Indeed, population growth in coastal areas where *Avicennia* is present, makes the implementation of eutrophication monitoring methods increasingly important, since population growth is generally linked to increased environmental eutrophication (Ansari & Gill, 2014). The methods described in the current research are easily applied and widely applicable to environmental monitoring in the tropical and subtropical coastal regions, where a large proportion of the world's population resides.

It is well documented that nutrient concentrations in New Zealand coastal waters have increased greatly in the past 100 years due to various anthropogenic activities (Cooper & Thomsen, 1983; Hart *et al.,* 2004; Heggie & Savage, 2009; Thrush *et al.,* 2013; Vant,

1997), and some areas around the highly populated Auckland region have been described as having 'poor' water quality (Walker & Vaughan, 2014). However, the effect of such changes on the nutrient status of the endemic mangrove *Avicennia marina* var *australasica* is understudied, especially with regards to the role of nutrient inputs from sewage, agriculture practices and livestock production. Additionally, mangrove stands have been expanding within most northern New Zealand estuaries (Green *et al.*, 2003; Schwarz, 2003; Stokes *et al.*, 2010) as the result of increasing catchment-derived sediment inputs (Lovelock *et al.*, 2007b; Morrisey *et al.*, 2010), with undocumented effects on the nutrient status of these coastal ecosystems. In addition to contemporary sampling from northern New Zealand estuaries, Auckland Museum (AM) specimen collections are also available to investigate long-term variations in mangrove nutrient status using herbarium samples of the past 100 years.

Thus, the aim of this research is to quantify foliar nutrient parameters (total nitrogen, total phosphorus, and nitrogen stable isotope ratio values in temperate mangroves of northern New Zealand and relate these measurements to sources and magnitudes of human activity (e.g., sewage input, agricultural runoff). A further aim is to investigate the potential use of mangrove plants as indicators of eutrophication in long term monitoring of aquatic ecosystems.

3.3 Materials and methods

Study sites

This study was conducted in northern New Zealand. The city of Auckland is situated on an isthmus between two inlets, the Waitemata Harbour to the north-east and the Manukau Harbour to the south-west. Even though these two inlets are only about 1.6 km apart at their closest point on the isthmus, there is no direct connection between the two, with the Waitemata opening to the Hauraki Gulf and the Manukau to the Tasman Sea. The third main site sampled was Mangawhai harbour located 81km north of Auckland. The sites were selected because they are in close proximity to anthropogenic activity hot spots (e.g., water treatment plant) and they covered a wide demographic and geographic region. Also, in the present study, an attempt was made to locate mangroves growing in relatively pristine conditions, by obtaining samples from nature reserves on Great Barrier and Motu Kaikoura Islands, located approximately 90 km offshore from central Auckland.

Contemporary leaf collections

Initially, mangrove leaf samples were collected in 2013 from 18 sites throughout Manukau Harbour, Auckland and 9 sites in the Mangawhai Harbour Estuary, Mangawhai. In 2014, leaves were collected from 12 sites in Waitemata Harbour, Auckland. A larger collection was made in 2015, with 30 sites throughout Manukau Harbour, 29 sites in Waitemata Harbour, 10 sites in Mangawhai Harbour and 5 sites in the Great Barrier Island archipelago. In all years, collections were made during the winter season (April-September) so that the leaves would be mature following the peak summer growing season. Sampling points were recorded with geographic coordinates and are listed in Supplementary Table 3.1. A total of 10 leaves per tree were sampled from 10 trees spaced approximately 10 meters apart at each sampling site. For consistency, the leaves were selected to be fully mature, but not senescent. All leaves were oven dried at 65°C for 3 days.

Auckland Museum herbarium samples

To compare contemporary and past leaf nutrients levels, historical herbarium mangrove leaves were obtained from the Auckland Museum specimen collections and compared to contemporary samples from the same locations. When no samples from the same locations could be obtained or exact information about the herbarium sample location was missing, the closest geographical location was sampled or the mean value of the entire site was used. These leaves were mainly from Waitemata Harbour (18 samples), Manukau Harbour (3 samples), and one sample from Great Barrier Island (Supplementary Table 3.2). The collection dates for the herbarium material ranged from 1863 to 1990. The leaves were analysed for nitrogen content and nitrogen stable isotope ratio. Total phosphorus contents were not measured in herbarium mangrove leaves, since the amount of leaf sample available from the museum was limited.

Total nitrogen, total phosphorous and stable isotope analyses

Dried composite leaf samples were ground to a fine powder with a ball mill (PM 100, Retsch, © Retsch GmbH) and sifted through a 200 µm pore size sieve. Analysis for total phosphorus (%P) was conducted using wet digestion in concentrated HNO_3 (McQuaker *et al.*, 1979), followed by quantification using an inductively coupled plasma atomic emission spectrometer (ICP-AES, Varian Liberty AX Series II, USA). A National Institute of Standards & Technology (NIST) Peach Leaf standard reference material (SRM1547) was used as a quality control in all analytical batches. A total of 0.1-0.5 grams

(g) of each composite leaf sample was sent to the Waikato University Stable Isotope Unit for total nitrogen (%N) and stable isotope ($\delta^{15}N$) analyses.

Herbarium samples from the Auckland Museum were brought to the laboratory and ground to a fine powder in the presence of liquid nitrogen. These samples were analysed using the same procedures as mentioned above. Only one leaf per herbarium voucher could be analysed, which could have caused some additional variation.

Statistical analyses

Leaf %N, %P, N/P ratio and $\delta^{15}N$ values are reported as mean ± standard error (SE). To test for differences between polluted and less polluted harbours using contemporary leaves, ANOVA were used to compare %N, %P, N/P ratio and $\delta^{15}N$ values between the three harbour locations in the main trial year (2015). Residuals of ANOVA were normal and homogeneous so that no transformations were considered necessary. ANOVAs were followed by Tukey's HSD tests when significant differences were detected. To test for changes in leaf nutrients over time by comparing contemporary and historical samples, %N and $\delta^{15}N$ of Herbarium and inter-annual variability samples were tested for normality and T-test or Wilcoxon rank-sum test were used accordingly. Inter-annual variability was assessed using T-test, since all variables were normal. For all statistical analyses and plotting, the R software version 3.2.1 was used (R development core team, 2015).

3.4 Results

For the main sampling year (2015), the highest leaf total nitrogen contents and nitrogen stable isotope ($\delta^{15}N$) values were found at Manukau (2.2 ±0.1%N, 9.9 ±0.4‰, respectively) and the lowest at Mangawhai (2.0 ±0.1%N, 5.2 ±0.4‰, respectively; Figures 3.1 and 3.2). The differences in both %N and $\delta^{15}N$ ratios were highly significant ($p<0.01$, Tukey's HSD test). In addition, the lowest $\delta^{15}N$ value observed at Manukau (7.1‰) was higher than all the $\delta^{15}N$ values at Mangawhai, i.e. there was no overlap in $\delta^{15}N$ values between the two harbours. The %N contents and $\delta^{15}N$ values for the Waitemata mangrove leaves (Figures 3.1 and 3.2) were intermediate in value between those for Manukau and Mangawhai (2.1 ±0.1% %N and 6.4 ±0.2‰). In contrast to the nitrogen values, the leaf phosphorus contents were not significantly different among the three sites, with 0.18 ±0.01% at Mangawhai, 0.19 ±0.01% at Waitemata and 0.20 ±0.01% at Manukau (Figures 3.1 and 3.3). Leaf nitrogen-phosphorus ratios also did not show any significant trend among sites (Figures 3.1 and 3.3).

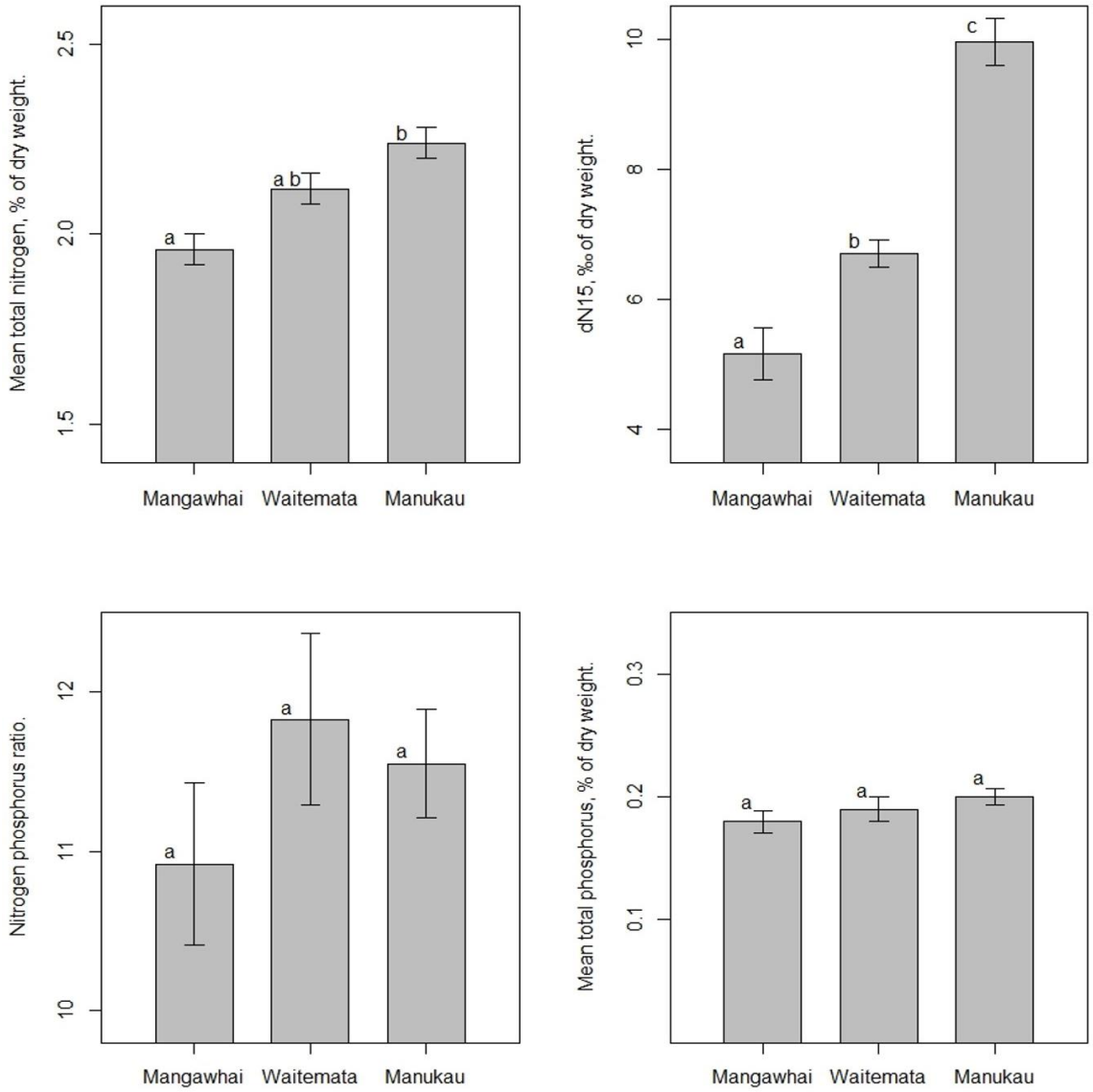


Figure 3.1. Mean (±SE) nutrient parameters (Mean total nitrogen, $\delta^{15}N$, Nitrogen phosphorus ratio, Mean total phosphorus) in mangrove (*Avicennia marina* var *australasica*) leaves within three harbours (Mangawhai n=10, Waitemata n=29, Manukau n= 30) during the main sample collection in winter 2015. ANOVAs were followed by Tukey's HSD tests when significant differences were detected. Values with different letters are significantly different at $p<0.01$.

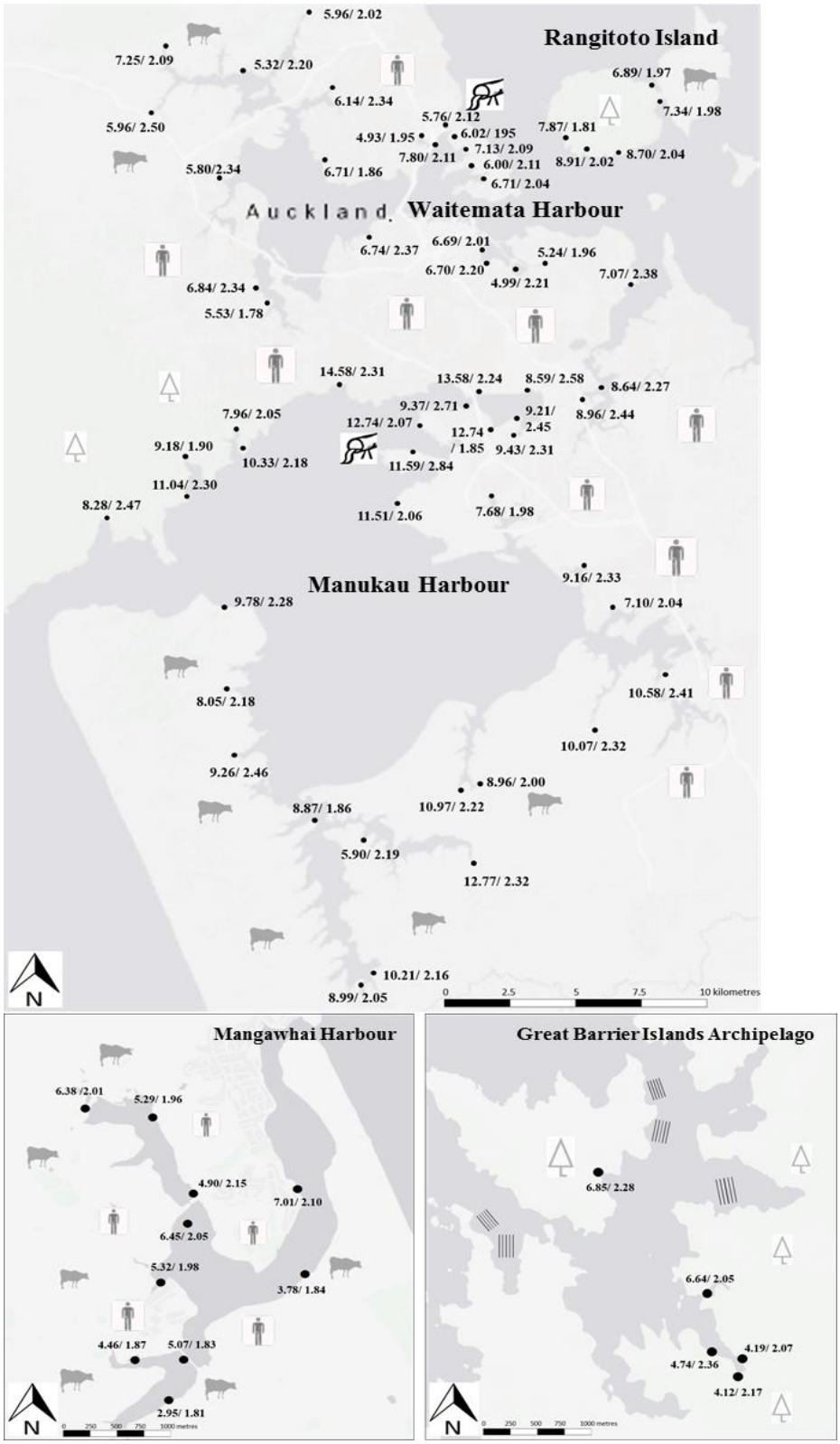


Figure 3.2. Nitrogen stable isotope ratios (‰)/total nitrogen (%N), of dry weights in mangrove (*Avicennia marina* var *australasica*) leaves at individual sampling sites around Auckland city (top map), Mangawhai Harbour and Great Barrier Island Archipelago during the main sample collections in winter 2015.

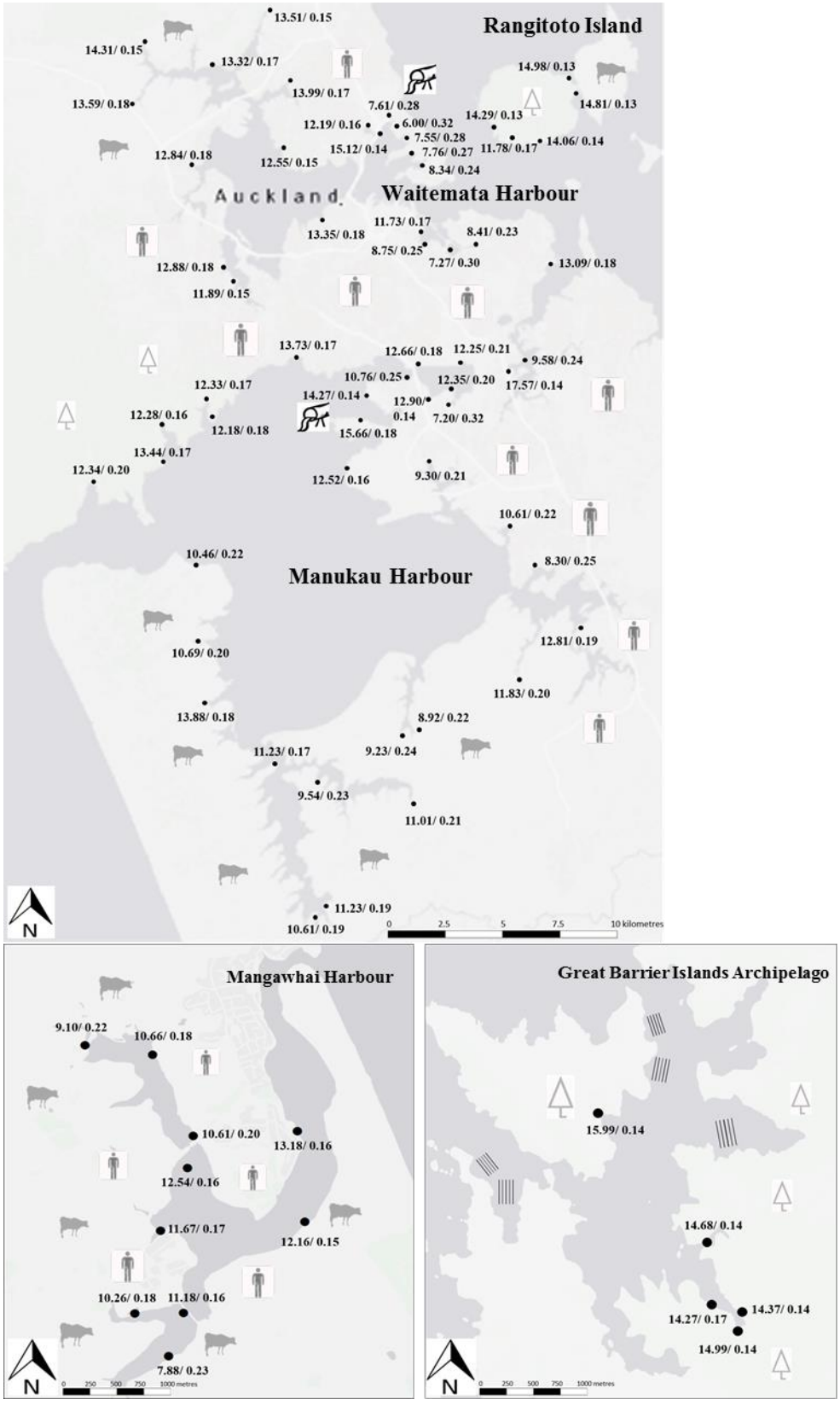


Figure 3.3. Nitrogen phosphorus ratio/total phosphorus (%P), of dry weights in mangrove (*Avicennia marina* var *australasica*) leaves at individual sampling sites around Auckland city (top map), Mangawhai Harbour and Great Barrier Island Archipelago during the main sample collections in winter 2015.

The %N contents and $\delta^{15}N$ ratios at Great Barrier Island were 2.2 ±0.1%, and 5.3 ±0.6‰, respectively. Great Barrier Island mangroves had the lowest total phosphorus contents in their leaves (0.15 ±0.01%) and the highest nitrogen to phosphorus ratios (14.9 ±0.3) of any sampled site (Figures 3.2 and 3.3).

Inter-annual variability

When comparing parameters measured during the preliminary study years (2013, 2014) with the data from the same locations gathered in the main sampling year (2015), measurements did not differ greatly, except for the nitrogen stable isotope ($\delta^{15}N$) ratios between Manukau samples (p-value < 0.05, T-test or Wilcoxon test, Table 3.1).

Table 3.1. Comparison of data from the preliminary and main sampling collections.

Sites	**Preliminary sampling (2013 and 2014)**		**Main sampling (2015)**	
	TN, % dry mass	**$\delta^{15}N$, ‰ dry mass**	**TN, % dry mass**	**$\delta^{15}N$, ‰ dry mass**
Manukau (2013,n=18)	2.4 ±0.1	10.4 ±0.5	2.2 ±0.1	9.8 ±0.4
Waitemata (2014,n=12)	2.2 ±0.1	6.3 ±0.2	2.1 ±0.1	6.5 ±0.3*
Mangawhai (2013, n=9)	2.2 ±0.1	5.3 ±0.3	2.0 ±0.1	5.4 ±0.3

*p-value < 0.05, T-test or Wilcoxon test

Museum samples

The highest nitrogen stable isotope value in the historical museum samples was found in the Manukau Harbour at Ihumatao Street point (18.2‰) in the mid-1980s. The highest total nitrogen content was found in Purewa Bush point (3.1%) in the mid-1950s.

In general, nutrient parameters of mangrove leaves found in the present study were lower than historical values, except for two sampling points at the Manukau site (Figure 3.4). The mean total nitrogen content of herbarium samples was significantly higher at 2.6 ±0.1%, compared to 2.1 ±0.1% in the 2015 samples (p-value<0.001, T-test). The stable nitrogen isotope ratio in the herbarium samples were also significantly higher (7.9 ±0.4‰) in comparison with values found in the 2015 samples (6.2 ±0.2‰) (p-value=0.001, Wilcoxon test).

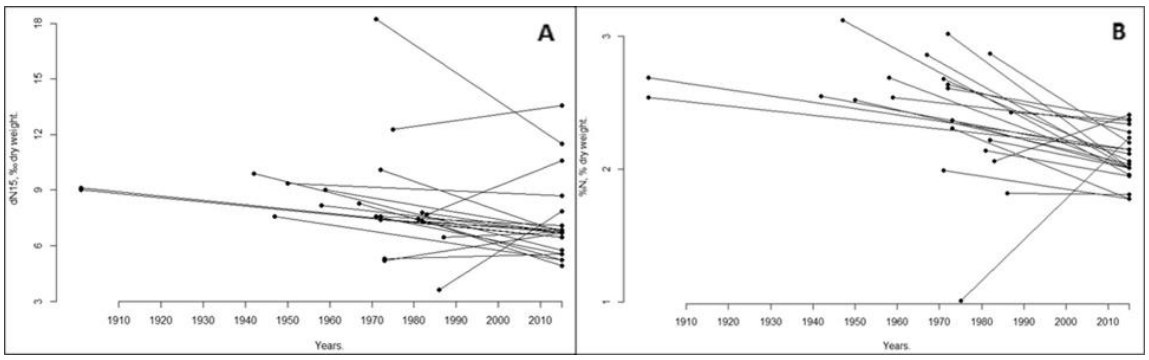


Figure 3.4. General trend between nitrogen stable isotope ratios, ‰ (A) and total nitrogen, % (B) in historical Auckland Museum herbarium samples and samples from the present study conducted in winter 2015. In both diagrams, the lines link historical herbarium samples collected at various dates to leaf samples from the same site in 2015.

3.5 Discussion

Leaf $\delta^{15}N$ values at the three contemporary harbour sites

In general for a variety of plants growing under pristine natural conditions in various areas of the world, the $\delta^{15}N$ of tree leaves ranges from -8 to +3‰ (Peterson & Fry, 1987) and leaf values lying within this range have been observed in *Avicennia marina* mangroves (1.6‰, 2.2‰) growing on an off-shore island in Australia (Costanzo *et al.*, 2001). In contrast, in the present study, the three studied sites in northern New Zealand had mean $\delta^{15}N$ values ranging from +5 to +10‰, and no single site had a $\delta^{15}N$ mean value within the natural range.

Two potential sources of the nitrogen which may have caused these enhanced $\delta^{15}N$ values are agricultural practices and human sewage. With respect to agriculture, New Zealand has the third highest use of nitrogenous fertiliser in the OECD countries after South Korea and Japan at 27kg/ha (OECD, 2015). Urea, the main nitrogen fertiliser used in New Zealand, has a $\delta^{15}N$ value of 0‰ when manufactured (Lindau *et al.*, 1989) and, thus, direct run-off of fertiliser from land into waterways will not be directly detected as an increase in $\delta^{15}N$. However, increased urea use on agricultural land leads to increased grass growth, which allows for higher animal stocking rates. This, in turn, leads to increased urea production from farm animals and excretion in the form of urine. Excess deposition of urea on land from either fertilisation or by livestock urination results in an increased probability of kinetic isotope fractionation either via ammonia volatilisation or by microbial denitrification due to system "leakiness" associated with the excess nitrogen (Heaton 1986, Fry, Gace, & McClelland, 2003). Thus, both in New Zealand and

internationally, high levels of dairy and animal farming are associated with high levels of $\delta^{15}N$ in nitrate produced by microbial processes (Harrington, Kennedy, Chamberlain, Blum, & Folt, 1998; Komor & Anderson, 1993). Plants, in turn, absorb this ^{15}N enriched nitrate, leading to elevated $\delta^{15}N$ values in plant tissues. For example, in a study of $\delta^{15}N$ and % N in *Rhizophora* mangrove leaves in Florida, the highest $\delta^{15}N$ and %N values were observed where canals draining agricultural lands deliver high-nitrate waters to fringing mangrove marshes. Mangroves growing adjacent to agricultural canals had $\delta^{15}N$ values in the range +11‰ to +16‰, whereas mangroves growing at a more pristine site had values that ranged from -5‰ to +2‰ (Fry *et al.*, 2000). A similar elevation in $\delta^{15}N$ values has been associated with proximity to human sewage sources in both aquatic plants in general (McClelland & Valiela, 1998) and *Avicennia marina* mangrove stands in particular (Costanzo *et al.,* 2001). While direct measurements of the ^{15}N signature of water running into the harbours is technically feasible there would be a high intra-day and inter-day variability in such data due to changes in temporal events such as rainfall and land use. The advantage of using mangroves as ecological indicators is that the mangrove acts as a continuous sampler, integrating and storing ^{15}N over the lifetime of the leaf. In addition, mangrove leaves are more easily accessible than other potential indicators (such as macroalgae) and the trees themselves have long lives, potentially allowing sampling from the same plant over several decades.

Based on the $\delta^{15}N$ values observed at the three sites, it appears that Mangawhai at 5.2‰ is receiving mildly elevated inputs of anthropogenic nitrogen at +2‰ above the upper natural background level of +3‰ (Peterson & Fry, 1987), while the Manukau at +9.9 ‰ is receiving strong anthropogenic inputs at almost +7‰ above the natural background level. The Waitemata Harbour lies between these two values at +6.4‰. The main source of anthropogenic nitrogen at Mangawhai is likely to be from farm animals since Mangawhai has a relatively small human population of 1,329 (Census 2013). In contrast, the city of Auckland, where the Waitemata and Manukau harbours are located, is New Zealand's largest city with a population of 1.4 million within the wider Auckland region (Census 2013). The highly significant difference in $\delta^{15}N$ values between the two Auckland sites (6.4 *vs* 9.9‰) is, therefore, a potential indicator that the Manukau Harbour receives greater anthropogenic nitrogen inputs.

The city of Auckland is situated on an isthmus between two inlets, the Waitemata Harbour to the Northeast and the Manukau Harbour to the South-West. Even though these two

inlets are only about 1.6 km apart at their closest point on the isthmus, there is no direct connection between the two, with the Waitemata opening to the Hauraki Gulf and the Manukau to the Tasman Sea. The fact that there is a highly significant difference in δ15N values between these two sites (6.71 vs 9.95‰) is, therefore, a potential indicator that the Manukau Harbour receives greater anthropogenic nitrogen inputs. The two most likely sources for this difference are the location of the main Auckland sewage treatment plant (with an effluent output of c. 120,000,000 m3 of treated sewage per year and an average total nitrogen content of 8.3 gm-3 in winter and 6.8 gm-3 in summer; Watercare GRI Report 2013). This sewage treatment plant is on the Manukau Harbour and the greater proportion of farmland on the Manukau Harbour is highly urbanised, compared to the Waitemata Harbour. In addition to the main sewage treatment plant at Mangere, there are three smaller plants located on the southern shore of the Manukau Harbour (Waiuku, Clarks Beach, and Kingseat) that discharge a total of around c. 1,000,000 m^3y^{-1} of treated sewage.

Total leaf nitrogen and phosphorus values at the three contemporary harbour sites

The Waitemata and Manukau Harbours have elevated leaf total nitrogen contents compared to Mangawhai Harbour, which supports the $\delta^{15}N$ observations that indicate higher anthropogenic nitrogen inputs at these two sites compared to Mangawhai. The mangrove leaf N data from the present study also correlate with Auckland water quality monitoring data. According to the 2013 Auckland Council Marine Water Quality Annual Report, calculated total inorganic nitrogen concentrations of Manukau Harbour surface water were significantly higher than in the Waitemata Harbour (0.15 and 0.05 mgL^{-1} respectively) (Walker & Vaughan, 2014).

The majority of studies in natural ecosystems have found that mangrove productivity is primarily limited by nitrogen and occasionally by phosphorus (Reef *et al.*, 2010). An exact value for the level of nitrogen and phosphorus at which growth limitation of *Avicennia marina* occurs has not been determined in natural ecosystems since many variables may impact on growth (Reef *et al.*, 2010; Alongi, 2011). However, studies under controlled conditions may be relevant with respect to indicating potential effects of anthropogenically derived nutrients on mangrove productivity. For example, Alongi (2011) studied the effect of nitrogen and phosphorus on the growth of *Avicennia marina* and five other mangrove species under controlled tidal hydroponic conditions. Plants were grown in seawater with a range of nitrogen concentrations, and the growth rates and

leaf nutrient contents were determined. Results from those measurements indicated that the leaf nitrogen contents of *A. marina* increase with increasing concentrations of nitrogen in the seawater solution, and ranged from a low of 1.13% at low nitrogen supplementation rates to a high of 3.40% total leaf nitrogen at very high supplementation rates. *A. marina* displayed an S-shaped nitrogen dependent growth curve, which plateaued at a nitrogen supply rate of 10 mmol m^{-2} d^{-1}, which resulted in a measured average leaf content of 2.06% nitrogen.

Thus, under optimal conditions, the growth of *A. marina* may be nitrogen limited up to a leaf content of 2.06% N. With respect to the present study, it is noteworthy that the average *A. marina* leaf nitrogen contents from the two Auckland sites exceed this 2.06% value, implying that the natural nitrogen limitation on growth would be removed from these mangroves (under optimum salinity conditions). Alongi (2011) commented that the nitrogen solution concentration that gave this supply rate was likely to be the maximum that would be obtained under natural environmental conditions, but might be exceeded in polluted ecosystems, which gives further credence to the δ15N observations that indicate anthropogenic nitrogen inputs into the mangroves of the two sites close to Auckland.

Leaf N/P ratios at the three contemporary harbour sites

Generally, nitrogen or phosphorus limitation in plants has been reported as an N/P ratio of less than 10 or greater than 20, respectively (Güsewell, 2004). Furthermore, an absolute value of less than 0.1% phosphorus in leaves is also generally indicative of phosphorus limitation (Güsewell, 2004). Based on these criteria none of the mangrove sites in the three contemporary harbour sites surveyed in the current study showed clear phosphorus limitation. However, while the mean N/P values for the three sites indicate that nitrogen limitation is unlikely to be present overall, several plots at all three sites may be nitrogen limited since 17 of the 69 plots surveyed had N/P ratios of less than 10.

Herbarium leaf analyses

The general trends for the herbarium samples strongly suggest that there has been a decline in nitrogen stable isotope ratios and total nitrogen content in mangrove leaves over the past 100 years in Auckland's estuaries. This finding is consistent with other herbarium studies that have observed a decline in leaf %N and $\delta^{15}N$ in a variety of plant species over the last century in other parts of the world (McLauchlan, Ferguson, Wilson, Ocheltree, & Craine, 2010; Peñuelas & Estiarte, 1997; Peñuelas & Filella, 2001; Peñuelas

& Matamala, 1990). The authors in these other herbarium studies ascribed the decline in leaf %N and $\delta^{15}N$ to either increased absorption of elevated atmospheric CO_2 related to anthropogenic activities over the last century, or to increased absorption of anthropogenically derived atmospheric nitrogen species depleted in ^{15}N. However, in the present study, the decline is more likely to correlate with the known history of the Auckland sewage treatment system, which has generally improved over the past 100 years (Fitzmaurice, 2009), potentially leading to reduced inputs of $\delta^{15}N$ and %N. Initially, the major input of poorly treated sewage took place in the Waitemata Harbour at Orakei Basin. Sewage treatment was later moved to the Manukau Harbour at the present day Watercare plant, where the treatment process has undergone significant improvements since the mid-2000s.

In contrast to the present study, other herbarium studies used plant samples largely obtained from relatively pristine ecosystems where nitrogen was likely limiting. Under these conditions, elevated CO_2 levels have been shown to cause reductions in plant leaf nitrogen content (McGuire, Melillo, & Joyce, 1995). However, under elevated CO_2 conditions and ample nitrogen availability, both reductions and increases in leaf and plant %N have been observed (McGuire, Melillo, & Joyce, 1995). Likewise, in the herbarium study of Peñuelas & Filella (2001), low (-10-0‰) leaf $\delta^{15}N$ was observed in samples from relatively pristine areas in the Mediterranean, where precipitation derived anthropogenic nitrogen species depleted in ^{15}N are likely to make a large contribution against a low natural background. Atmospheric nitrogen supply has been calculated to represent a high proportion (36-53%) of the N incorporated into biomass in Mediterranean systems due to low soil moisture, in contrast to more temperate ecosystems where nitrogen species, such as ammonium or nitrate, are derived from groundwater (Peñuelas & Filella, 2001). Therefore, in the present study, where consistently high $\delta^{15}N$ values were present, the major source of nitrogen is likely to be ammonium or nitrate from microbially processed urea in water derived from sewage or farm runoff, which is typically enriched in $\delta^{15}N$ (rather than depleted in ^{15}N as is atmospheric ammonium or nitrate). The observed historic decline in leaf $\delta^{15}N$ and %N is hence more likely to be related to a reduction in sewage impact rather than to atmospherically derived nitrogen or to elevated CO_2 levels. However, some effects due to elevated CO_2 levels over the last century cannot be completely excluded and the observed decline in leaf %N and $\delta^{15}N$ may well be due to a combination of both elevated CO_2 levels and improvements in the Auckland sewage system.

Pitt, Connolly, & Maxwell (2009) found that mangrove plants, unlike algae and crabs, had no detectable response in $\delta^{15}N$ values to an improvement in sewage outfall quality in the Moreton Bay catchment, Australia, up to 2 years after an upgrade had been completed. They ascribed this lag to factors such as that the growth rate and turnover time of nitrogen in mangroves being likely to be much slower than for algae. Mangroves source most of their N via their roots from sediments that can accumulate large quantities of N and are likely to store N for an extended period and that, unlike algae, mangroves recycle a proportion of their N internally since they resorb up to 64% of N from senescing leaves prior to abscission. Here, we see no or minimal changes occurring over short time periods (1-2 years), but significant changes occurring over longer time periods (decades) as indicated by the herbarium samples. Thus, algae may be more suitable for monitoring short term trends in eutrophication (Pitt, Connolly, & Maxwell, 2009) whereas mangroves are long lived and they integrate nitrogen signatures across large spatiotemporal scales, making them more suitable for monitoring long term trends.

Great Barrier Island mangroves

None of the mangroves in the 69 locations sampled in the three sites had $\delta^{15}N$ values in the -8 to +3‰ range, which is expected under pristine natural conditions (Fry, 2006). Costanzo *et al.* (2001) observed high $\delta^{15}N$ values in mangrove leaves associated with sewage outfalls, but also observed low values (1.6-2.2‰) on an offshore island located approximately 20 km away from the main site. In the present study, an attempt was made to locate mangroves in similar, relatively pristine conditions, by obtaining samples from nature reserves on Great Barrier and Motu Kaikoura Islands, located approximately 90 km offshore from central Auckland. However, the leaf $\delta^{15}N$ and %N values from these sites were relatively high, with means of 5.3‰ and 2.18%. Conversely, the %P values were relatively low with a mean of 0.15% P. This resulted in high N/P ratios, with a mean of 14.9. Indeed, this 14.9 N/P ratio approaches the values of 15-18 seen in old growth New Zealand pristine forests, which are typically phosphorus limited (Parfitt *et al.*, 2005; Richardson, Peltzer, Allen, McGlone, & Parfitt, 2004). Thus, the phosphorus limitation results at this site suggest that the area might be pristine, while conversely the nitrogen results do not.

However, it was noted that while there were few potential terrestrial anthropogenic sources of nitrogen in the area sampled, there were two potential marine sources of anthropogenic nitrogen. Firstly, there were three oyster farms in the area, and aquaculture

in general (e.g. shrimp farming) has been associated with elevated $\delta^{15}N$ values in mangroves (Costanzo, O'Donohue, & W. C. Dennison, 2004; Thimdee *et al.*, 2002). This source of potential anthropogenic nitrogen is supported by the observation that the lowest leaf $\delta^{15}N$ and %N values in the sampled area were at Kiwiriki Bay, which is most distant from the oyster farms and potential yacht anchorages.

It is notable that a similar high N, low P effect appears to be occurring on Rangitoto Island, located at the entrance to the Waitemata Harbour, which is also a nature reserve. The mangroves on this island have low leaf phosphorus values compared to the mangroves in the main inlet, but high leaf $\delta^{15}N$ and %N values. Rangitoto Island is adjacent to the Rosedale Rd sewage outfall pipe, which extends approximately 3 km into the ocean. This outfall discharges c. 20,215,000 m^3 of treated sewage per year with an average total nitrogen content of 12g/m^3 (Kelly, 2013). In addition, farming occurs on the adjacent Motu Tapu Island, with typical livestock numbers of 3500 sheep and 1000 cattle. Thus, in the case of both island nature reserves, terrestrial pristine conditions appear to be impacted by the discharge of localised marine pollution. The fact that the mangrove nitrogen status is affected, but not phosphorus, by localised marine pollution is likely due to the much greater mobility of nitrogen chemical species in water than phosphorus. Phosphorus is more likely to bind to form refractory sediments and precipitate, thus becoming unavailable, especially when salinity is low (Jordan, Cornwell, Boynton, & Anderson, 2008).

Contribution of mangroves to the nitrogen dynamics of the harbours

The presence of mangroves at the three main sites may provide positive benefits with respect to their role in the nitrogen dynamics of these coastal areas. Nitrogenous inputs from anthropogenic sources are often associated with harmful algal blooms. Since the nitrogen limitation, which characterises large areas of the world's oceans, coastal and estuarine waters are removed allowing excessive algal growth (Paerl, 1997). The presence of mangroves at the three sites may ameliorate this nitrogenous input both by directly storing nitrogen within the mangrove plant itself and by providing a sediment and microbial habitat associated with the mangrove roots and leaf litter able to both immobilise and denitrify anthropogenic nitrogen (Lambs, Leopold, Zeller, Herteman, & Fromard, 2011; Rivera-Monroy & Twilley, 1996).

Increased mangrove productivity due to the removal of the natural nitrogen limitation to growth typically found in pristine environments may have a beneficial effect on carbon sequestration, particularly in the Waitemata and Manukau harbours, in which mangroves appear to have leaf nitrogen contents higher than the 2.06% at which nitrogen growth limitation no longer occurs (Alongi 2011). The removal of a natural growth limitation typically found in pristine natural environments may potentially allow the mangroves in the two harbours to grow more vigorously, thus trapping more carbon. Mangrove habitats around the world are rapidly being destroyed (Polidoro *et al.*, 2010), leading to a decrease in carbon sequestration, so the potential increased productivity of New Zealand mangroves associated with increased nitrogen input via eutrophication may be viewed paradoxically as an environmentally beneficial effect if it leads to more carbon trapping. However, there are other limiting factors which might potentially constrain this growth, such as local site salinity (Downton 1982), which need to be further investigated in the harbours.

3.6 Conclusion

Mangroves can absorb nutrients in coastal waters, which is reflected in their tissue nutrient status. Anthropogenically derived discharges into coastal waters, such as animal and human sewage, and agricultural runoff could provide additional nutrients to mangrove plants, which may in turn affect growth, since under natural conditions mangrove productivity is often nitrogen limited. In the present study, we observed contrasts in both leaf nitrogen contents and stable isotope ratios in *Avicennia* mangroves which correspond with human activities such as sewage discharge and farming on both spatial and temporal scales. The *Avicennia* mangrove genus is widely distributed across the tropical and sub-tropical regions of the world. Our finding that *Avicennia marina* leaves can provide a robust medium-term record of changes in anthropogenic N discharge, indicates that it is a useful indicator species for monitoring of eutrophication in coastal habitats where population growth is likely to exacerbate eutrophication. Implementation of eutrophication monitoring methods such as those employed in the present study will become increasingly important in monitoring of anthropogenic impacts on ecological systems internationally.

3.7 Supplementary tables

Supplementary Table 3.1. Nutrient parameters at individual sampling locations during the main mangrove nutrient trial from April-October 2015 in Waitemata, Manukau, and Mangawhai harbours in northern New Zealand.

Date collected	Street location	Marine location	Coordinates	TP, % dry weight	TN, % dry weight	$\delta^{15}N$, ‰
Waitemata Harbour						
7/05/2015	St Peters Street	Tuff Crater (Dwarf)	-36.801377, 174.751402	0.16	4.93	1.95
7/05/2015	Exmouth Road	Tuff Crater (Tall)	-36.804405, 174.759107	0.14	7.8	2.11
7/05/2015	Balmain Road	Soldiers Bay, Birkenhead	-36.813342, 174.698193	0.15	6.71	1.86
7/05/2015	Manuka Rd	Oruamo Creek, Glenfield	-36.777850, 174.698435	0.17	6.14	2.34
7/05/2015	Wharf Rd	Lucas Creek, Albany	-36.732961, 174.687831	0.15	5.96	2.02
7/05/2015	Chatham Avenue	Chatham Reserve, Poremoremo	-36.765793, 174.648290	0.17	5.32	2.20
7/05/2015	Wharf Rd	Rangitopuni Stream, Riverhead	-36.756720, 174.598452	0.15	7.25	2.09
7/05/2015	Dale Road	Brigham Creek, Whenuapai	-36.786027, 174.598837	0.18	5.96	2.50
14/05/2015	Parawai Crescent	Coxs Creek	-36.850657, 174.727947	0.18	6.74	2.37
14/05/2015	Glen Marine Parade	Whau River, Glendene (Dwarf)	-36.881097, 174.660162	0.15	5.53	1.78
14/05/2015	Glen Marine Parade	Whau River, Glendene (Tall)	-36.880886, 174.658507	0.18	6.84	2.34
14/05/2015	Moire Rd	Henderson Creek,Henderson	-36.824154, 174.635069	0.18	5.80	2.34
14/05/2015	Shore Road	Hobson Bay, Remuera	-36.863276, 174.789393	0.17	6.69	2.01
14/05/2015	West Tamaki Road	Tahuna Torea Reserve, Wai o Taiki Bay, Glendowie	-36.872494, 174.885754	0.18	7.07	2.38
14/05/2015	Princes Street (East)	Seaside Park, Otahuhu	-36.931732, 174.865515	0.14	8.96	2.44
16/08/2015	Greydene Place, Takapuna	Auburn Reserve walkway	-36.791201, 174.766965	0.28	5.76	2.12
16/08/2015	Harley Close, Takapuna	Creek	-36.797126, 174.772594	0.32	6.02	1.95
16/08/2015	Francis street, Takapuna	Shoal Bay	-36.803183, 174.780547	0.28	7.13	2.09
16/08/2015	Kawerau Avenue, Devonport	Ngataringa Bay	-36.815459, 174.785910	0.27	6.00	2.11

16/08/2015	Ngataringa Park, Devonport	Lake Road Bridge	-36.817955, 174.794182	0.24	6.71	2.04
7/09/2015	Church street, Otahuhu	Tamaki Estuary	-36.935997, 174.845149	0.24	8.64	2.27
26/09/2015	Waitaramoa Reserve	Hobson Bay, Remuera	-36.865751, 174.794834	0.25	6.70	2.20
26/09/2015	Palmers in Remuera, walkway	Hobson Bay, Remuera	-36.865044, 174.807779	0.30	4.99	2.21
26/09/2015	Kepa Bush Reserve, Purewa	Purewa	-36.863954, 174.827796	0.23	5.24	1.96
27/09/2015	Islington Bay	Rangitoto Island	-36.775703, 174.897284	0.13	7.34	1.98
27/09/2015	Rangitoto Warf	Rangitoto Island	-36.806625, 174.862581	0.14	8.70	2.04
27/09/2015	Mangrove Bridge	Rangitoto Island	-36.805938, 174.851080	0.17	8.91	2.02
27/09/2015	Coast Guard Bay	Rangitoto Island	-36.790776, 174.831076	0.13	7.87	1.81
27/09/2015	Gardiner Gap	Rangitoto Island	-36.770939, 174.893522	0.13	6.89	1.97
Manukau Harbour						
14/05/2015	Norana Avenue	Harania Creek, Mangere	-36.945102, 174.816248	0.20	9.21	2.45
14/05/2015	Norana Avenue	Mangere-Seashore	-36.943872, 174.811313	0.14	12.74	1.85
14/05/2015	Hugo Johnstone Drive	Pikes Point-Onehunga	-36.929091, 174.820272	0.21	8.59	2.58
9/06/2015	Arapito Road	Little Muddy Creek, Laingholm	-36.959014, 174.646605	0.18	10.33	2.18
9/06/2015	Landing Road	Little Muddy Creek, Laingholm	-36.950275, 174.646272	0.17	7.96	2.05
9/06/2015	Huia Road	Big Muddy Creek (Nihotapu Dam)	-36.966978, 174.615878	0.16	9.18	1.90
9/06/2015	Armour Road	Big Muddy Creek (Armour Bay)	-36.973610, 174.619152	0.17	11.04	2.30
9/06/2015	Huia Dam Road	Huia Stream	-36.997863, 174.566588	0.20	8.28	2.47
9/06/2105	Ambury Road	Ambury Farm Park Seashore	-36.948857, 174.756389	0.14	12.74	2.07
9/06/2015	Island Road	Puketutu Island, Mangere	-36.961788, 174.755108	0.18	11.59	2.84
9/07/2015	Linwood Road, Glasson Bridge	Whangamaire Stream, Karaka	-37.101615, 174.864016	0.20	10.07	2.32
9/07/2015	Capriole Crescent	Clarks Creek (Eastern arm), Kingseat	-37.126285, 174.788238	0.22	8.96	2.00
9/07/2015	McKenzie Road	Clarks Creek (Western arm),Kingseat	-37.135613, 174.782305	0.24	10.97	2.22
9/07/2015	Racecourse Road	Waiuku River (Eastern Waiuku Estuary)	-37.235014, 174.730393	0.19	10.21	2.16
9/07/2015	Rangiwhea Road	Waiuku River (Western Waiuku Estuary)	-37.234623, 174.728397	0.19	8.99	2.05

9/07/2015	Featon Avenue	Awhitu Park Creek, Awhitu	-37.086042, 174.646441	0.20	8.05	2.18
9/07/2015	Poaka Road	Matakawau Creek (Bay)	-37.116289, 174.656715	0.18	9.26	2.46
9/07/2015	Big Bay Road	Big Bay Creek	-37.044112, 174.638830	0.22	9.78	2.28
10/07/2015	Lewis Street	Blockhouse Bay	-36.926531, 174.706971	0.17	14.58	2.31
10/07/2015	Alfred Street	Mangere Inlet, Onehunga	-36.931112, 174.795638	0.18	13.58	2.24
10/07/2015	Coronation Road	Mangere Inlet, Mangere Bridge	-36.937349, 174.786838	0.25	9.37	2.71
10/07/2015	Peninsula Road	Pukaki Creek, Mangere	-36.982885, 174.799222	0.21	7.68	1.98
10/07/2015	Ihumatao Road	Ihumatao seashore	-36.990991, 174.743286	0.16	11.51	2.06
10/07/2015	Hanford Place	Puhinui Creek	-37.020841, 174.858409	0.22	9.16	2.33
10/07/2015	Sandwick Drive	Pahurehure Inlet, Manurewa	-37.040344, 174.875970	0.25	7.10	2.04
7/09/2015	Mary Place, Favona	Harania Creek, Mangere	-36.951673, 174.812821	0.19	10.58	2.41
7/09/2015	Dunsmuir Road	Taihiki River	-37.161984, 174.725717	0.32	9.43	2.31
7/09/2015	Te Toro point	Taihiki River, Harbour	-37.150691, 174.697052	0.23	5.90	2.19
7/09/2015	Mauku Bridge, Manukau	Taihiki River, upper catchment	-37.173125, 174.794499	0.17	8.87	1.86
Mangawhai Harbour Estuary						
16/07/2015	Cove Road	Tara Creek, (West)	-36.094944, 174.562831	0.22	6.38	2.01
16/07/2015	Jack Boyd	Tara Creek, (East)	-36.095966, 174.573002	0.18	5.29	1.96
16/07/2015	Molesworth Drive	Tara Creek, Molesworth Bridge (North)	-36.108047, 174.579077	0.20	4.90	2.15
16/07/2015	Molesworth Drive	Tara Creek, Molesworth Bridge (South)	-36.109912, 174.579699	0.16	6.45	2.05
16/07/2015	Pearson Street	Mangawhai Harbour	-36.120458, 174.575078	0.17	5.32	1.98
16/07/2015	Kedge Drive	Insley Channel (North arm)	-36.131841, 174.572365	0.18	4.46	1.87
16/07/2015	Insley Street	Insley Bridge	-36.131849, 174.579462	0.16	5.07	1.83
16/07/2015	Clarke Road	Insley Channel (South arm)	-36.137232, 174.576693	0.23	2.95	1.81
16/07/2015	Tern Point	Mangawhai Harbour	-36.117808, 174.586707	0.15	3.78	1.84
16/07/2015	Lincoln Street	Mangawhai Harbour	-36.107866, 174.596599	0.16	7.01	2.10
Great Barrier Islands						
12/04/2015	Motu Kaikoura Island	Port Fitzroy	-36.180500, 175.333370	0.14	6.85	2.28
12/04/2015	Great Barrier	Kiwiriki Bay	-36.206894, 175.354304	0.14	6.64	2.05
12/04/2015	Great Barrier	Kiwiriki Bay	-36.207946, 175.357988	0.17	4.74	2.36

12/04/2015	Great Barrier, old trees	Kiwiriki Bay Estuary	-36.208788, 175.358441	0.14	4.12	2.17
12/04/2015	Great Barrier, young trees	Kiwiriki Bay Estuary	-36.208798, 175.358624	0.14	4.19	2.07

Supplementary Table 3.2. Historical Auckland Museum (AM) mangrove leaf total nitrogen (TN) and nitrogen stable isotope ($\delta^{15}N$) ratios.

Catalogue number	Date collected	Location	TN, % dry weight	$\delta^{15}N$, ‰ dry weight
Waitemata Harbour				
11603	1863-1873	NA	2.54	9.01
264607	1885	Waitemata Harbour	2.69	9.12
132293	6/09/1942	Takapuna,tidal estuary	2.55	9.90
40247	3/09/1947	Purewa Bush	3.12	7.57
264609	/06/1958	Hobson Bay	2.69	8.16
264610	/06/1959	Rosebank Creek, Avondale	2.54	9.01
116494/5	16/06/1967	Hobson Bay	2.86	8.29
261846	10/03/1971	Pollen Island	1.99	7.56
130391	10/08/1972	Hobson Bay, mudflats	3.02	10.10
131931	8/05/1973	Shore Road, Hobson bay	2.37	5.19
217901	10/06/1973	Pollen Island	2.31	5.31
270916	29/04/1981	Tuff Crater	2.14	7.43
259324	18/03/1982	Shore Road, Hobson Bay	2.22	7.78
220693	26/03/1982	Waitaramoa Reserve, Hobson Bay	2.87	7.33
276242	3/05/1987	Cox's Creek	2.43	6.47
279002	15/04/1990	Meola Creek	2.27	8.25
31838	NA	Rangitoto	2.52	9.36
175670	11/05/1986	north from White Beach, Rangitoto	1.82	3.61
130398	21/08/1972	Glenn Innes Domain	2.61	7.59
Manukau Harbour				
129240	16/11/1971	Ihumatao street	2.68	18.23
181001	4/02/1975	Onehunga waterfront	1.01	12.26
273891	17/07/1983	Bottle Top Bay	2.06	7.67
Great Barrier Island				
130515	22/08/1972	Whangaparapara (Inlet)	2.64	7.41

Chapter 4. Combined effects of salinity and nutrient levels on growth and nitrogenous metabolism of temperate mangrove seedlings (*Avicennia marina* var *australasica*)

This chapter reports on a study on the effects of nutrient and salinity levels on growth, nitrogen uptake, and nitrogenous metabolisms of temperate mangrove seedlings under controlled laboratory conditions. This study provides previously missing information on the speed of nitrogen uptake by mangrove seedlings and what nitrogenous compounds (namely amino acids) are synthesised by plants growing under different nutrient and salinity levels. This information directly contributes to the main research question, as it presents an experimental confirmation of the effects of nutrients and salinity on temperate mangrove growth. The content and results featured in this chapter for part of a manuscript that is being prepared for submission to the Journal of Experimental Biology.

4.1Abstract

Previous research demonstrated that *Avicennia marina* is a mangrove species exhibited stunted growth under 0% standard seawater salinity treatment, while 25-50% standard seawater salinity (9-17 PSU) conditions are optimum, and at salinities over 75% seawater mangroves are stunted again. Nutrients are known to be involved in mitigating the stress posed by high salinity, but whether nutrients can improve growth of mangroves at low salinity is unknown. A mangrove growth trial was carried out with low and medium salinity treatments and contrasting nutrient levels for 6 months. Then, labelled $^{15}NH_4Cl$ was supplied and mangrove leaves were sampled for changes in $\delta^{15}N$ before, after 3 hours and after 2 days. Roots were also sampled for $\delta^{15}N$, but only after 2 days. Growth, nitrogen uptake, nitrogenous metabolomics (amino acids), ROS, and osmolyte concentrations were measured. We found that despite nitrogen uptake being faster in zero salinity conditions, as measured by uptake of ^{15}N into leaves, mangroves at 25% standard seawater (9 PSU) salinity and high nutrient concentration exhibited the highest biomass accumulation. An amino acid profile did not provide insight into biomass accumulation differences between zero and 25% standard seawater salinity (9 PSU) conditions, since the concentrations of major amino acids were affected by nutrient availability.

4.2 Introduction

Mangrove plants are a group of halophytes that are adapted to grow under saline conditions in nutrient-limited coastal and estuarine settings (Tomlinson, 1986; Alongi, 2009). Salinity and scarcity of nutrients pose a major challenge for growth of mangrove plants (Duarte *et al.*, 1998; Ball, 2002; Feller *et al.*, 2007; Naidoo, 2009; Reef *et al.*, 2010). Salinity directly affects mangrove growth on physiological and biochemical levels.

For example, it was found that under high salinity conditions the activity of enzymes, water uptake, and growth in general was suppressed in mangrove plants (Ball *et al.*, 1987; Naidoo, 1987; Ball, 2002). Under high salinity, sodium ions are accumulated in leaf tissues to overcome the negative osmotic potential for water uptake (Clough *et al.*, 1982; Aziz & Khan, 2001; Alongi, 2009). This can lead to secondary oxidative stress and accumulation of reactive oxygen species (ROS; Parida & Jha, 2010).

Mangrove plants can adjust their biochemical mechanisms to "protect" their tissues and components, such as PSII and PSI complexes in the photosynthetic machinery (Sengupta & Majumder, 2009). The biochemical adjustments under salinity stress conditions include osmolyte accumulation (e.g., quaternary ammonia compounds, proline, mannitol), accumulation of nitrogen reserves in the form of amino acids (namely asparagine), and synthesis of ROS scavenging agents (e.g., proline; Popp *et al.*, 1985; Munns, 2002; Sharma & Dietz, 2006; Parida & Das, 2010; Planchet *et al.*, 2011). Surprisingly, low salinity (less than 5% of standard seawater salinity) also negatively affects growth of some mangrove species, such as *Avicennia marina* var. *australasica* (Downton, 1982; Tuffers *et al.*, 2001; Nguyen *et al.*, 2014). However, the biochemical changes that can be associated with this growth phenomenon are less known.

With respect to nutrients, such as nitrogen (N) and phosphorus (P), mangroves in pristine environments are often nutrient limited, and have developed nutrient conservation mechanisms, such as the accumulation of biomass below ground (Alongi *et al.*, 2003). At the biochemical level, nitrogen shortage in mangroves has been less studied, but in other plants it is known that it primarily affects the concentration of nitrogenous compounds required for both plant growth (e.g., amino acids), and for salinity tolerance (e.g., glycine betaine; Khamis *et al.*, 1990; Foyer *et al.*, 1994; Keller *et al.*, 1999). However, in one field study Martin *et al.* (2010) demonstrated that after N fertilisation, mangroves from hypersaline areas improved primary growth parameters and water use efficiency, but the authors did not perform a metabolomic analysis, which could explain the mechanism of these changes. Additionally, it is largely unknown how fast mangroves accumulate nitrogenous nutrients, and how the combined effects of simultaneously varying levels of salinity and nutrient availability affect nutrient uptake and allocation of nutrients to different metabolites (e.g., growth metabolites *vs* osmolytes). Therefore, the objective of the current study is to quantify the effects on nitrogen uptake and nitrogenous metabolites

of supplying mangrove seedlings with either low or optimal nitrogen and phosphorus levels under optimal and zero salinity conditions.

4.3 Materials and methods

Glasshouse growth trial

For the growth trial, *Avicennia marina* var *australasica* propagules were collected in March 2015. The propagules were washed with a 1% solution of sodium hypochlorite and graded by weight. Propagules of medium weight (7-10 grams) were placed in 1.5 litre pots with autoclaved river sand as a sediment. Pots were put in trays, 6 pots in every tray (Figure 4.1).

For germination three propagules per pot were placed on the top of the wet sand. During the germination time, propagules were watered with fresh water only, the water being added to the tray in three litre portions so that 2/3 of the pot height was covered with water. After the first water addition, the water level was marked and maintained at that level on a weekly basis when it had evaporated.

Figure 4.1. The schematic organisation of pots per treatment during the growth trial. The picture of a tray in the greenhouse with two-month seedlings at the end of the germination stage.

Over a two-month period, when plants had established, the plants were thinned so that one plant per pot was left with its cotyledons removed and different treatment levels were introduced (Table 4.1). Nutrient and salinity treatments were imposed in a randomized block design using 12 replicates per treatment. Thus, there were four treatment groups 0SLN (zero salinity low nutrients), 0SHN (zero salinity high nutrients), 25SLN (25% standard seawater salinity low nutrients), and 25SHN (25% standard seawater salinity

high nutrients). The saline solution was made by diluting artificial sea salts (Sigma-Aldrich Co. LLC, New Zealand) to the required concentration. Nutrients were added as a solution of N and P salts. Since artificial seawater salts has high level of potassium (K), additional potassium was supplied to the zero salinity plants to the match 25 mM concentration of K in the higher salinity groups. This was done to avoid K deficiency in the zero salinity group.

During the southern hemisphere winter season (June-August 2015) the mangrove seedlings were kept in a glasshouse, and nutrient and salt solutions were re-applied monthly. From September 2015 until the end of the growing trial in late November 2015, the position of individual trays and pots between trays with the same treatment level were randomised weekly by using a random number generator in R software version 3.2.1 (R Development Core Team, 2017; www.r-project.org).

Table 4.1. Treatment levels in the growth trial.

Nutrient/salinity	**0% Seawater**	**25% Seawater (9 PSU)**
Low N (0.5 mM) and P (0.25 mM)	n=12 (2 trays)	n=12 (2 trays)
High N (5 mM) and P (2.5 mM)	n=12 (2 trays)	n=12 (2 trays)

Stable isotope (^{15}N) treatment and harvest of plant material

At the end of the growing trial plant height, leaf number, and leaf traits (length, width, and chlorophyll levels in leaves) were measured. At the beginning of the labelling trial one leaf from each plant was sampled as control, then 75 ml of 5 mmol $^{15}NH_4Cl$ solution per plant was added at 10 minutes intervals to allow time for harvesting plant material in a timely manner. One leaf per plant was harvested after 3 hours of ^{15}N label addition and after 2 days one more leaf and a piece of root material was collected. Plant material was snap frozen directly after detaching from the plant and after weighing. Wet leaf, stem and root biomass was recorded directly after plant harvesting at the end of the labelling trial. Later plant biomass was oven-dried for two days at 60°C, then dry biomass was recorded. Snap frozen material was brought to the laboratory and was stored at -80°C prior for further analysis. Relative chlorophyll content of green leaf was measured by atLEAF CHL Plus Chl meter (FT Green LLC, Wilmington, DE) on five fully expanded leaves of each mangrove seedling.

Reactive Oxygen Species (ROS) analysis

100 mg of snap frozen plant and root material were homogenised in 1 ml of Tris-HCl buffer at pH 7.2, and following reaction with 2´,7´-Dichlorofluorescein diacetate, changes in fluorescence values was measured by fluorometer (FLUOstar Omega, BMG LABTECH Pty. Ltd., Germany; Sunkar, 2010). These results were standardised by the known concentration of peroxide and expressed as hydrogen peroxide equivalent.

Analysis of total QACs

Quaternary ammonium compounds (QACs) namely betaine and/or choline were precipitated as the periodide complex at low pH. Total QACs was measured spectrophotometrically as amount of the complex in dichloroethane (as described in Grieve & Grattan, 1983). Standard choline and betaine solutions were used to quantify measured values.

Nuclear Magnetic Resonance (NMR) determination of glycine betaine and choline

Extractions of betaine and choline ware prepared by shaking dry ground plant material (0.25 g) with deionised water (10 ml) over 24 h. 0.5 ml of filtered extracts were cooled to 0°C and 0.1 ml of KI-I_2 solution was added to form a periodide complex. Water was removed and the complex dissolved in [^{2}H] methanol. Specific levels of glycine betaine and choline were determined by ^{1}H-NMR spectrometry as described in Hayashi *et al.* (1997).

LC-MRM-MS analysis of targeted amino acids

Fifty milligrams of snap frozen root and leaf material were placed in 2 ml tubes with 2mm ceramic beads (Lysing matrix D, MP Biomedicals, USA). A 990µL volume of 100 % ice cold methanol and 10 µl of 40 µM 2,3,3,3-[^{4}H] alanine was added. Samples were homogenised for 1 min at 6000 rpm using a tissue homogenizer (FastPrep-24, MP Biomedicals, USA), followed by centrifugation for 5 min at 12 000 rpm (Z216MK, HERMLE Labortechnik GmbH, Germany). A 10 µl volume of extract was used to perform pre-column derivatisation with 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate (AccQ-Tag), following the method reported in Salazar *et al.* (2012).

The LC-MS was an Agilent 1260 Series liquid chromatograph comprising a G1311B quaternary pump, G1329B thermostatted autosampler and a G1330B thermostatted column compartment (Agilent Technologies, Santa Clara, USA). Mobile phase A was

0.6% formic acid in ultrapure water, mobile phase B was 0.1% formic acid in acetonitrile, the injection volume was 5 µL. The column was a Phenomenex Kinetex EVO C18 column, measuring 2.1x150 mm with 1.7 µm diameter packing material, maintained at 30°C. The chromatographic gradient started at 2% B, ramped to 15% B at 20 minutes, 90% B at 21 minutes, and 97% B at 21.01 minutes, and held at 97% B for 1 minute and then returned to 2% B at 23.50 minutes. The total run time was 35 minutes. For detection an Agilent 6420 triple quadrupole mass spectrometer fitted with an Agilent Multimode Ionisation source was operated in positive electrospray mode using Multiple Reaction Monitoring [MRM]. MRM transitions were established using Agilent MassHunter Optimiser B06.00 software and are presented in Supplementary Figure 1. Amino acids were quantified by reference to a dilution series of external standards (mixture of all targeted amino acids derivatised in the same manner) at concentrations of 1, 10, 20, 30, 40, 50 µM. Data was collected using Agilent MassHunter Quantitative Analysis B06.00 software and amino acids were quantified by normalising to recovery of the internal standard.

4.4 Results

Biomass characterisation

Plants in the 25SHN group had the highest total dry biomass value (6.00 ± 0.78 g; Fig 4.2a). The lowest biomass values were observed in the 0SLN and 25SLN groups (2.35 ± 0.18 g and 2.49 ± 0.16 g, respectively). In the 0SHN group plants (4.35 ± 0.28 g) had significantly lower values compared to 25SHN and significantly higher values than 0SLN and 25SLN plants.

Plants from the 0SLN and 25SLN groups did not differ significantly in biomass allocation patterns (Fig 4.2d). They had a significantly larger below ground (root) pool (51.71 ± 1.31% and 53.24 ± 0.94%, respectively). The 0SHN plants (35.04 ± 1.26%) had significantly higher biomass allocated to roots compared to the 25SHN plants (30.02 ± 1.11%). Seedlings from all treatment groups had a similar percent of biomass allocated to stems at around 23%. The highest leaf percent was observed in the 25SHN mangroves (47.59 ± 1.05%); the second highest percent of leaf biomass was observed in 0SHN plants (42.45 ± 1.25%). The lowest leaf biomass was in the 0SLN and 25SLN groups (25.16 ± 1.15% and 23.60 ± 0.70%, respectively).

Leaf number, leaf chlorophyll, and leaf length parameters had the same trend, significantly higher in 0SHN and 25SHN plants, and lower in 0SLN and 25SLN plants (Fig 4.2b, c, e, f). The width of leaves was the highest in 25SHN plants (3.44 ± 0.19 cm), which was significantly higher than in 0SHN plants (2.97 ± 0.10 cm). However, the values in 0SLN and 25SLN did not differ significantly from each other and were the lowest (1.91 ± 0.08 cm and 2.01 ± 0.08 cm, respectively).

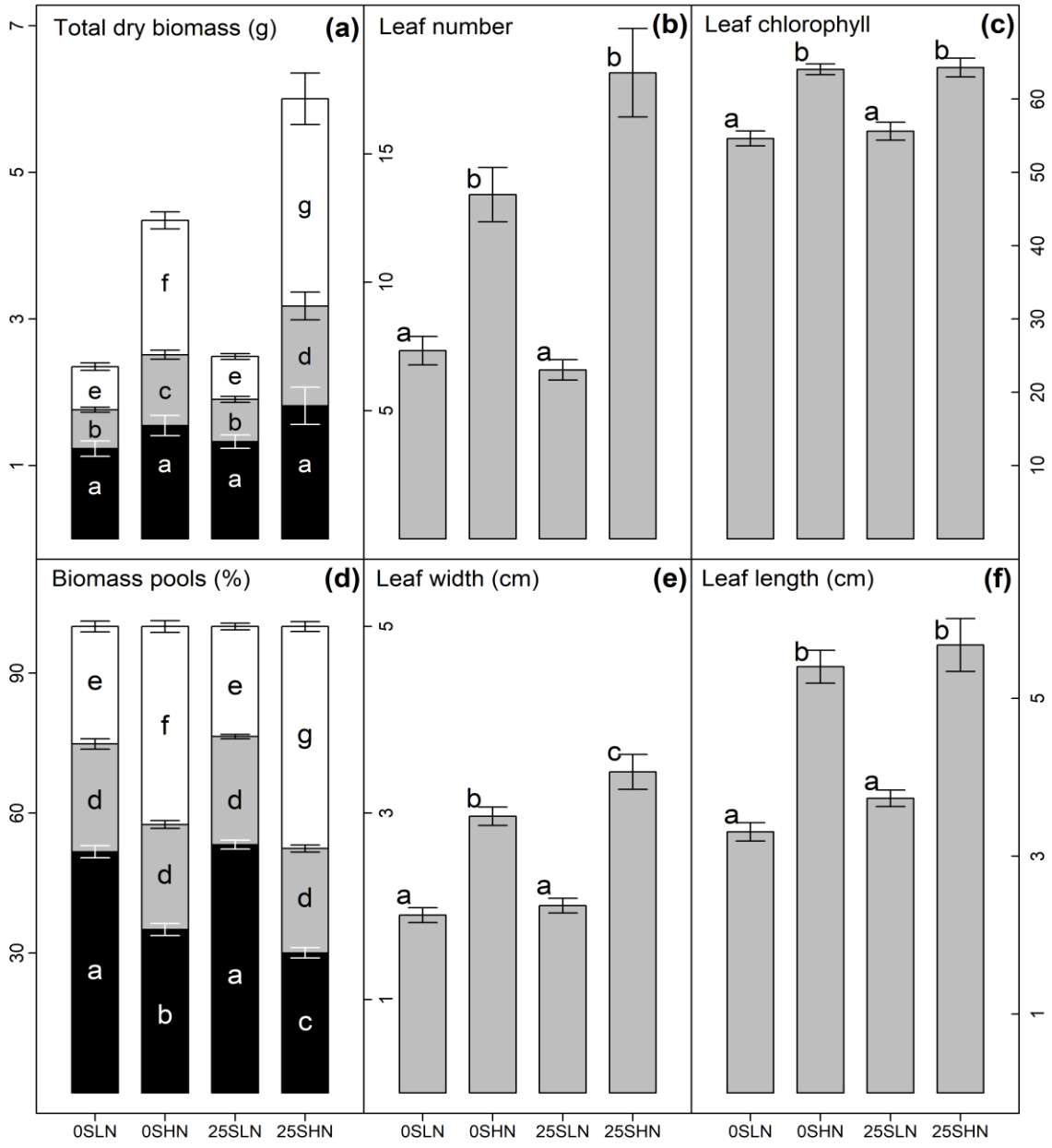


Figure 4.2. Mean (*n* = 12 ± SE) total dry weight biomass (black – root, grey – stem, white – leaf ; g; a), mean (n = 12 ± SE) biomass allocation to plant pools (black – root, grey – stem, white – leaf; d; %), mean (n = 12 ± SE) leaf number (b), mean (n = 12 ± SE) leaf chlorophyll (arbitrary; c), mean (n = 12 ± SE) leaf width (cm; e), and mean (n = 12 ± SE) leaf length (cm; f) of *Avicennia marina* var *australasica* 6-months old seedlings among

four treatment levels (0S = 0% seawater, 25S = 25% seawater, LN = low nutrients, HN = high nutrients). Data labelled with different letters are significantly different at $P < 0.05$ (one-way ANOVA followed by Tukey's HSD test).

Leaf total nitrogen concentration showed the same trend before and after addition of labelled nitrogen: it was higher in 0SHN and 25SHN plants at around 3% of dry weight than in 0SLN and 25SLN plants which was below 2% of leaf dry weight, except for 0SLN plants after 2 days of labelled nitrogen addition (2.17 ± 0.15 % of dry weight; Fig 4.3a).

Before ^{15}N addition, the leaf nitrogen stable isotope ratio ($\delta^{15}N$) was highest in the 0SHN (9.40 ± 0.57‰) and lowest in 25SHN (6.91 ± 0.40‰) groups, while the values in the 0SLN and 25SLN groups were intermediate (8.01 ± 0.29‰ and 8.25 ± 0.29‰, respectively; Fig 4.3b). After 3 hours following addition, $\delta^{15}N$ values did not differ among all groups. After 2 days, the highest $\delta^{15}N$ values was found in 0SLN (12 108.97 ± 1 000.01‰) group, which was significantly higher than in 25SLN (7 988.23 ± 1 369.27‰) group. Values in 0SHN and 25SHN plants were the lowest and did not differ significantly (2 722.10 ± 335.90 ‰ and 1 138.30 ± 191.94‰, respectively).

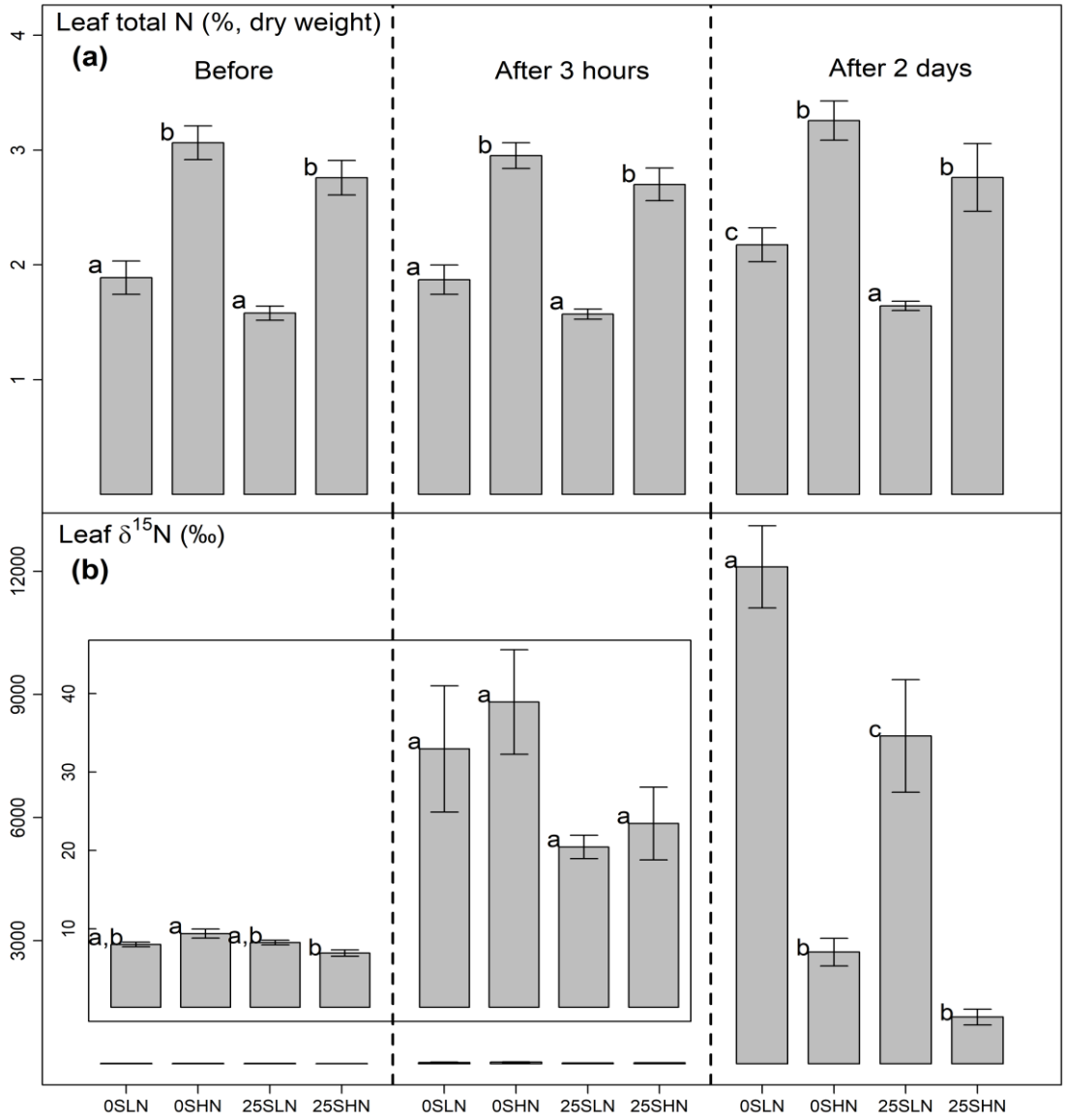


Figure 4.3. Mean (n = 12 ± SE) leaf total nitrogen (%N; a) and mean (n = 12 ± SE) leaf nitrogen stable isotope ratio ($\delta^{15}N$; b) of *Avicennia marina* var *australasica* 6-months old seedling among four treatment levels (0S = 0% seawater, 25S = 25% seawater, LN = low nutrients, HN = high nutrients). Data labelled with different letters are significantly different at $P < 0.05$ (one-way ANOVA followed by Tukey's HSD test).

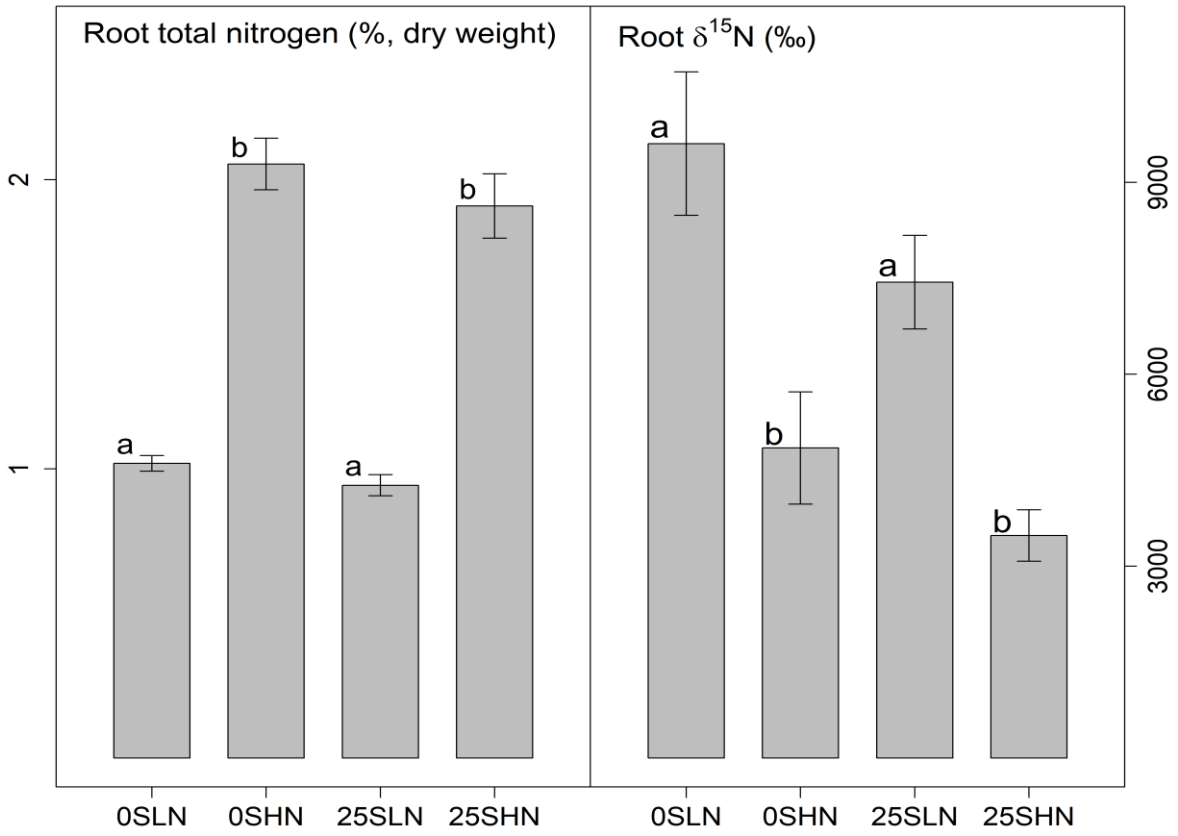


Figure 4.4. Mean (n = 12 ± SE) root total nitrogen (%N) and mean (*n* = 12 ± SE) stable nitrogen isotope ratio ($\delta^{15}N$) of *Avicennia marina* var *australasica* 6-months old seedling among four treatment levels (0S = 0% seawater, 25S = 25% seawater, LN = low nutrients, HN = high nutrients). Data labelled with different letters are significantly different at $P < 0.05$ (one-way ANOVA followed by Tukey's HSD test).

Total nitrogen concentration in roots was higher in 0SHN and 25SHN plants with 2.05 ± 0.09% and 1.91 ± 0.11% of dry weight, respectively (Fig 4.4). Lowest values were found in 0SLN and 25SLN plants (1.02 ± 0.03 % and 0.94 ± 0.04 % of dry weight, respectively). The stable nitrogen isotope ratio ($\delta^{15}N$) was higher in 0SLN and 25SLN plant roots (9 603.78 ± 1 121.08 ‰ and 7 438.55 ± 731.70 ‰, respectively) and lower in 0SHN and 25SHN plants (4 846.78 ± 876.56 ‰ and 3 478.35 ± 402.06 ‰, respectively).

Metabolite analysis

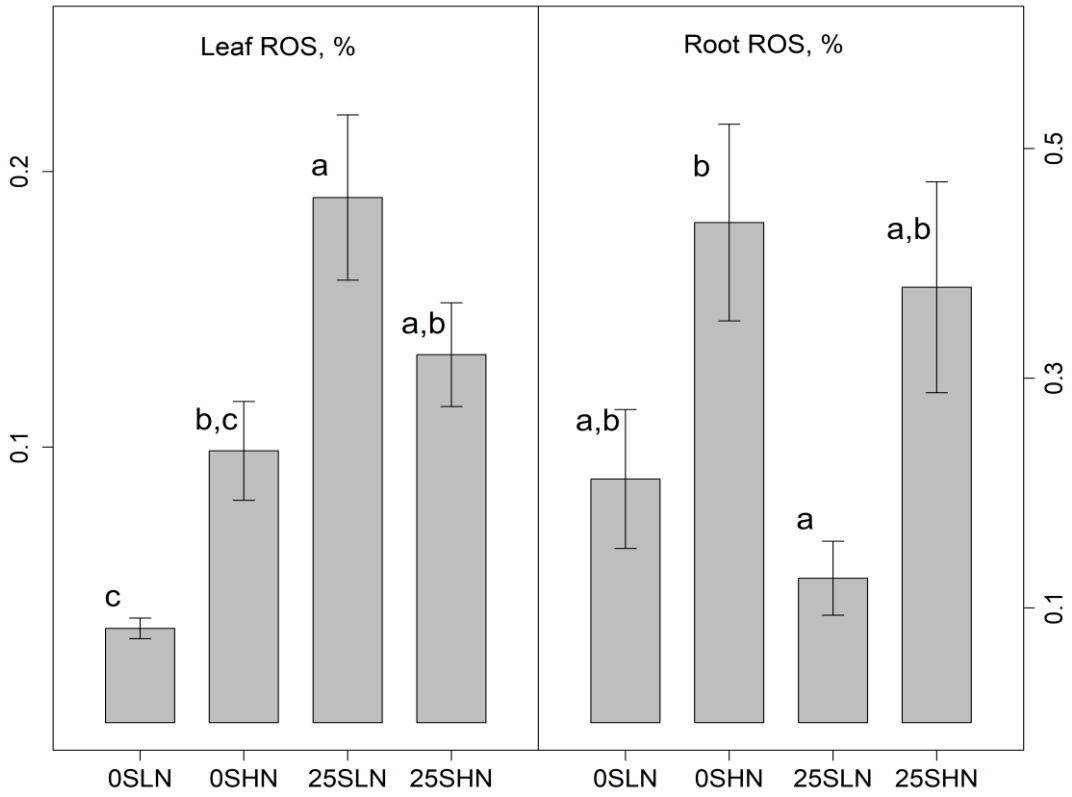


Figure 4.5. Mean (n = 12 ± SE) reactive oxygen species (ROS; %) activity of *Avicennia marina* var *australasica* 6-months old seedling leaves and roots among four treatment levels (0S = 0% seawater, 25S = 25% seawater, LN = low nutrients, HN = high nutrients). Data labelled with different letters are significantly different at $P < 0.05$ (one-way ANOVA followed by Tukey's HSD test).

The leaf level of reactive oxygen species was lowest in 0SLN plants (0.034 ± 0.004%) and highest in 25SLN plants (0.191 ± 0.030%), while in 0SHN and 25SHN plants it was intermediate (0.099± 0.018% and 0.134± 0.019%; respectively; Fig 4.5). In roots, the lowest level was in 25SLN plants (0.126 ± 0.032%) and highest was in 0SHN plants (0.436 ± 0.086%), plants from 0SLN and 25SHN groups ROS concentrations were intermediate (0.212± 0.061% and 0.379± 0.092%; respectively).

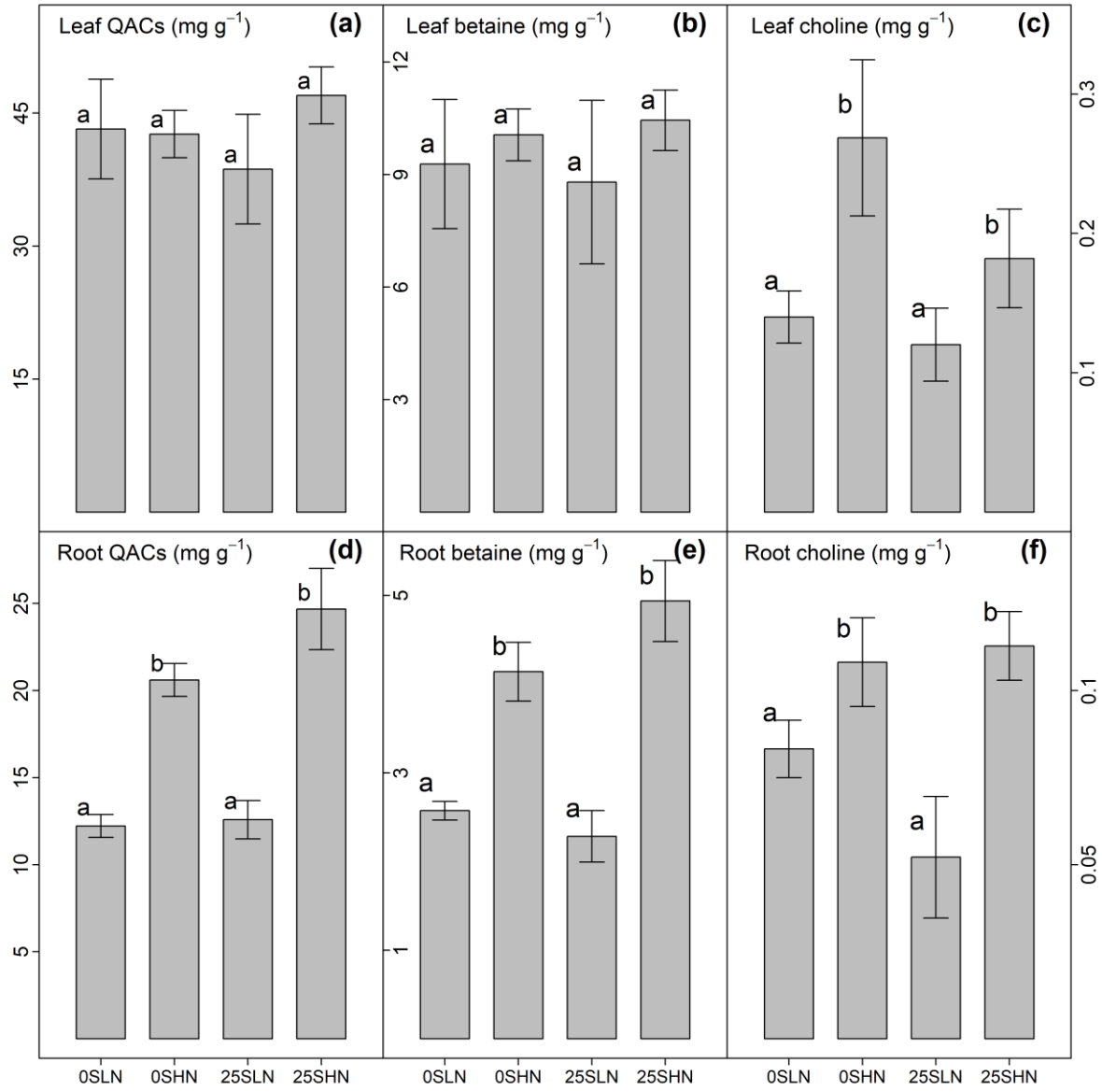


Figure 4.6. Mean (n = 12 ± SE) total quaternary ammonia compounds in leaves (QACs; a; mg/g), mean (n = 12 ± SE) QACs in roots (mg/g; d), mean (n = 12 ± SE) of glycine betaine (mg/g; b) and choline (mg/g; e) in leaves, and mean (n = 12 ± SE) of glycine betaine (mg/g; c) and choline (mg/g; f) in roots of *Avicennia marina* var *australasica* 6-months old seedling among four treatment levels (0S = 0% seawater, 25S = 25% seawater, LN = low nutrients, HN = high nutrients). Data labelled with different letters are significantly different at $P < 0.05$ (one-way ANOVA followed by Tukey's HSD test).

The concentration of total quaternary ammonia compounds (QACs) in leaves did not differ significantly among treatment groups (Fig 4.6). In 0SLN and 25SLN plant roots concentrations were lower (12.22 ± 0.66 mg/g and 12.58 ± 1.10 mg/g, respectively), compared to 0SHN and 25SHN plants (20.60 ± 0.95 mg/g and 24.67 ± 2.34 mg/g, respectively).

The overall concentration of betaine was higher in leaves and roots than the concentration of choline. Betaine concentration in leaves was the same among all treatments. Leaf choline concentration in 0SLN and 25SLN plants was lower compared to plants from 0SHN and 25SHN groups. The same pattern was observed in root betaine and root choline concentrations.

Amino acid profile of roots and leaves

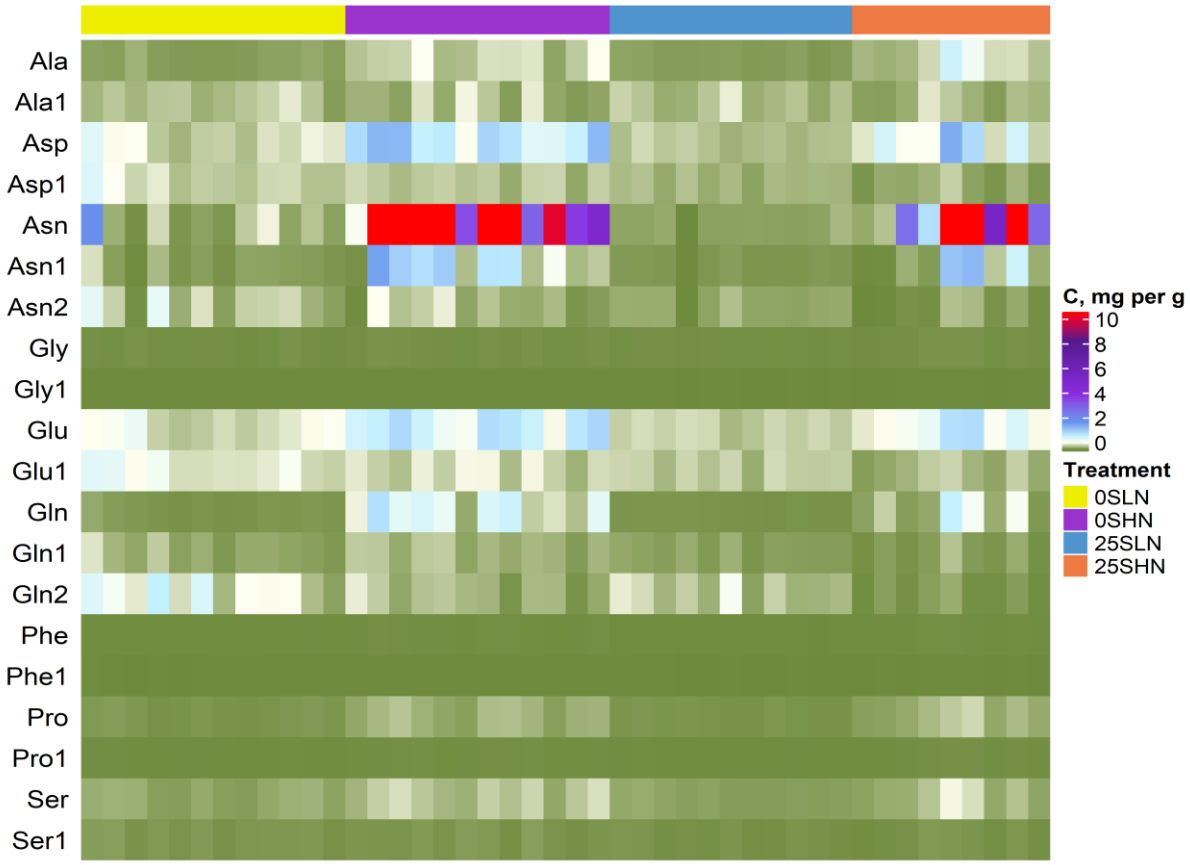


Figure 4.7. Concentration of amino acids in roots (mg/ g) after two days of ^{15}N treatment of *Avicennia marina* var *australasica* 6-month old seedling among four treatment levels (0S = 0% seawater, 25S = 25% seawater, LN = low nutrients, HN = high nutrients).

The concentration of Ala1, Gly, and Ser1 in roots was not affected by either nutrient or salinity treatments (the number following the standard three letter amino acid abbreviation e.g. Ala1 refers to the heavy isotope version of the amino acid observed in mass spectrometry, i.e. the ^{13}C,^{15}N version of the amino acid; Fig 4.7). However, the levels of Ala, Asn, Glu, Phe, Phe1, in the roots of mangrove seedlings were affected by nutrient treatments, but not affected by salinity. Another group of amino acids, whose concentration was affected by salinity, but was not affected by nutrient treatment, included Asp1, Glu1, Glu2, Pro, Pro1, and Ser. For both Asp and Gln there was no

significant difference among the three groups 0SLN, 25SLN, and 25SHN, but 0SHN plants had the highest concentration. Other amino acids had other patterns, Asp1 concentration was lower in 0SLN and 25SLN, and higher but not significantly different between 0SHN and 25SHN. Asp2 was lower in 0SHN and 25SHN plants, and higher in 0SLN and 25SLN. Gly1 was higher in 0SLN and 0SHN plant roots and lower in 25SLN and 25SHN. Glu1 had highest concentration in 0SLN plants and lowest in 25SHN, otherwise there was no significant difference among other treatment groups. The concentration of Asn in roots was the highest among all amino acids.

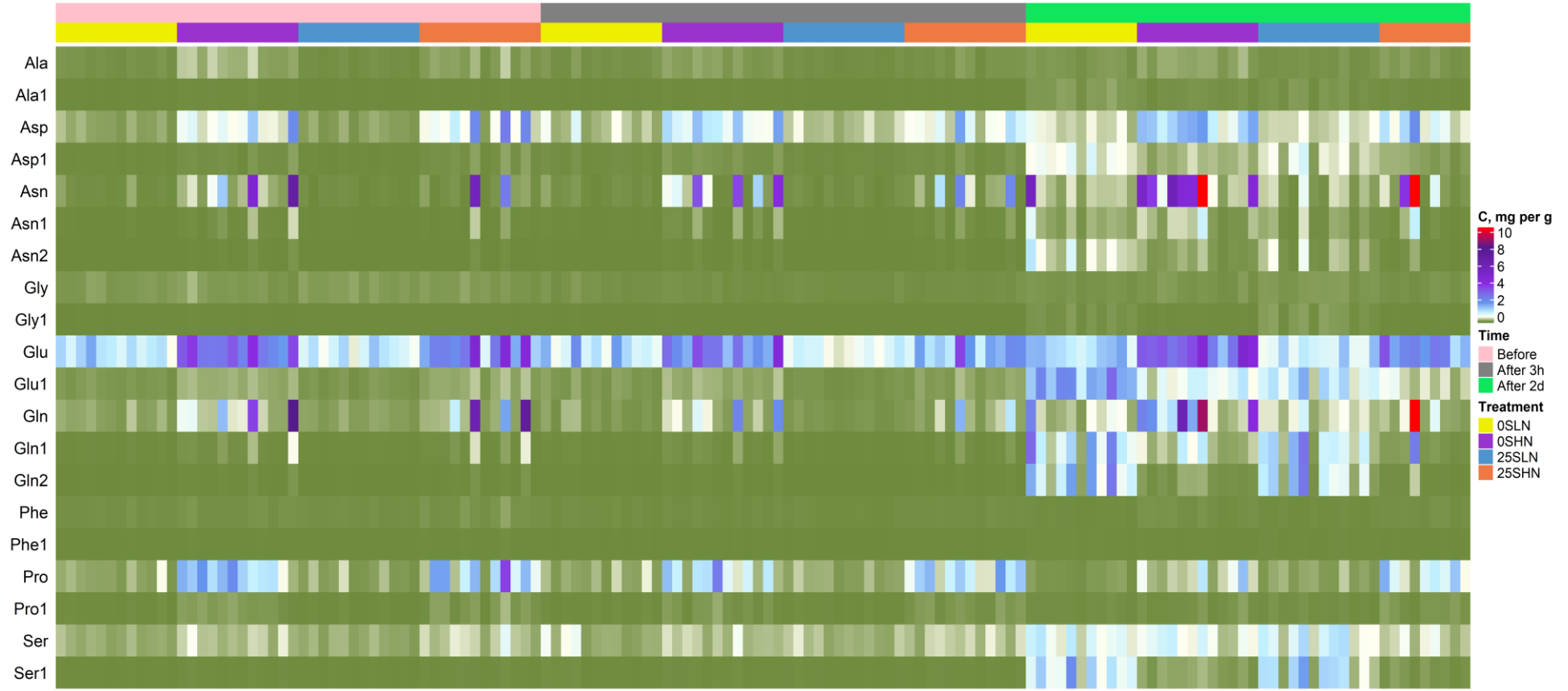


Figure 4.8. Concentration of amino acids in leaves (mg per g) of *Avicennia marina* var *australasica* 6-month old seedling among four treatment levels (0S = 0% seawater, 25S = 25% seawater, LN = low nutrients, HN = high nutrients), and across different treatment time: before of ^{15}N label addition, after 3 hours, and after 2 days.

Concentration of Gln in leaves was not affected by either nutrient and salinity treatments or time of exposure to the ^{15}N labelled nitrogen fertiliser (Fig 4.8). Its concentration along with concentration of Glu and Asn was the highest in the leaves among all measured amino acids. Pro1 concentration was affected only by the nutrient treatment: higher in 0SHN and 25SHN and lower in 0SLN and 25SLN. Levels of Ser, Phe1, and Gly in leaves did not differ among different nutrient and salinity treatment levels. Concentrations of Pro, Asn, Asn1, Asp, and Glu were affected by treatment levels (significantly higher levels in high nutrient plants 0SHN and 25SHN) and time (concentration increased after 2 days in both 0SHN and 25SHN and 0SLN and 25SLN plants). However, the statistical model suggested no interaction between treatment and time for these amino acids. Levels of Ser, Phe, Gln2, Gln1, Glu, Gly1, Asn2, Asp1, Ala1, Ala were similarly affected by time and treatment, but there was also a significant interaction between those two factors.

4.5 Discussion

Biomass trends

As expected, our results demonstrated that nutrient levels play a major role in *Avicennia marina* species biomass accumulation, since mangrove seedlings in high nutrient groups (0SHN and 25SHN) had higher total dry biomass values than low nutrient plants (0SLN and 25SLN). Such trends were observed in previous laboratory studies (Boto *et al.*, 1985; Naidoo, 1987; Yates *et al.*, 2002; Alongi, 2011). We found that mangrove seedlings in higher nutrients at 25% salinity had the highest total dry biomass among all treatment groups. However, under nutrient limitation, salinity levels did not affect biomass accumulation, as there was no significant difference among plants from the 0% and 25% salinity groups. Surprisingly, although there were significant differences in overall biomass between groups, the absolute biomass of roots did not differ significantly and was around 1.5g of dry root mass among all treatment groups.

The highest above ground biomass was observed in the 25SHN group. Under high nutrient conditions more than 65% of total dry biomass was accumulated in stems and leaves. However, under low nutrient conditions, more than 70% of dry biomass was located belowground. Such biomass allocation was also observed in previous laboratory studies (McKee, 1995; Naidoo 2009). In pristine natural conditions mangrove plants often face low nutrient availability, and field biomass estimation studies demonstrated that more than 60% of total tree biomass is allocated below ground (Tran *et al.*, 2016). The common explanation is that plants generally, and mangroves in particular, allocate

biomass below ground to get access to more limiting resources, namely nutrients (Chapin, 1980; McKee, 2001). Another explanation in the case of mangroves may be that the plant initially prioritises root growth over stem growth in order to achieve a firm anchor against wave impact action, then once a minimum density of roots have accumulated, a shift of nutrient allocation to above ground (stem and leaf) growth takes place. The two hypotheses (nutrient accumulation *versus* anchoring) are not mutually exclusive, and it may be advantageous for the mangrove in terms of both nutrient accumulation and anchoring to prioritise root growth. However, the results of the present study indicate that the ability to absorb nitrogen was relatively independent of root biomass, with plants with similar root biomass having dramatically different ^{15}N uptake ability. Thus, the present data support the latter (anchoring) hypothesis over the former (nutrient accumulation) hypothesis, as it appears that mangroves can compensate for low nutrient concentrations by actively up-taking additional nitrogen as observed in the low nutrient groups.

All other physiological leaf parameters (leaf number, leaf chlorophyll, and leaf length) were affected only by nutrient treatment (higher under high nutrient treatment and lower under nutrient limitation), and not by the salinity treatment. However, leaves were significantly wider in 25SHN plants compared to 0SHN.

Nitrogen dynamics

Total nitrogen (%N) concentration in mangrove leaves and roots reflected nitrogen levels supplied in the sediment. %N in seedlings that grow under high nutrient conditions (in both 0SHN and 25SHN groups) was *ca* 30% higher in leaves and 50% higher in roots compared to plants from low nutrient groups (0SLN and 25SLN). Three hours after addition of labelled nitrogen fertiliser, total nitrogen concentration was not affected significantly. However, after two days of labelled nitrogen addition, plants from 0SLN group accumulated significantly more nitrogen (% N) than plants from 25SLN group.

Thus, our results suggest that when nitrogen is limiting, *Avicennia marina* var *australasica* takes up nitrogen faster under 0% salinity conditions than under the 25% salinity conditions, which are generally regarded as optimal for *Avicennia* growth. This trend has been observed in non-halophyte plants. For example, Dersch *et al.* (2016) found that rice (*Oryza sativa*) plants under elevated salinity conditions had decreased nitrogen uptake. Other mangrove species also exhibited a negative correlation between high salinity in the sediment and nitrogen uptake, for example in *Kandelia candel* species

(Shiau, Lee, Chen, Tian, & Chiu, 2016), *Bruguiera parviflora* (Parida & Das, 2014), as well as in *Avicennia marina* (Naidoo, 1987).

These results were further confirmed by dynamics of labelled nitrogen. The $\delta^{15}N$ ratio represents relative abundance of heavy (^{15}N) isotopes over light (^{14}N) ones (Fry, 2006). This ratio under natural conditions depends on the nitrogen availability and source of nitrogen (Lindau *et al.*, 1989; Costanzo *et al.*, 2001). Treatment with labelled nitrogen fertiliser (e.g., $^{15}NH_4Cl$) is a common practise for studying nitrogen dynamics in plants. Three hours after addition of $^{15}NH_4Cl$, plants from 0SLN and 0SHN had twice as high leaf $\delta^{15}N$ ratios, which indicated faster nitrogen uptake. After two days, plants from the 0SLN group had the highest nitrogen stable isotope ratio, followed by plants from the 25SLN group, and the lowest leaf $\delta^{15}N$ ratio was observed in 0SHN and 25SHN. These results suggest that at 0% salinity mangrove seedlings can take up nitrogen faster, and if mangrove plants grow under nutrient limitation they can incorporate nitrogen at faster than mangroves from high nutrient environments.

The observations that plants in the 0% salinity group took up nitrogen faster than the 25% "optimal" salinity group when nutrients were limiting may be partly related to the fact that *A. marina* actively favours uptake of fresh water over saline water (Reef *et al.*, 2015). However, this would not explain the significantly reduced ^{15}N uptake in the 0SHN group *versus* the 0SLN group. This may indicate that some type of active N transport may be involved in addition to a preference for fresh water over saline water.

Surprisingly, even though mangrove seedlings at 0% salinity conditions took up nitrogen faster than at 25% salinity, plants in 25SHN exhibited the highest biomass accumulation. In a growth trial across different salinity levels study, Downton (1982) observed that 0% salinity *Avicennia* plants initially had the highest growth rates when compared to plants grown in a range of salinities from low to high, but eventually the growth rate of the 0% salinity plants plateaued and the 0% salinity plants became stunted relative to the optimal salinity plants (which initially grew more slowly, but the growth was not supressed later). The results of the present study indicate that the faster growth rate observed in Downtown's study may be partly related to higher nitrogen uptake in 0% salinity plants. Furthermore, the stunting observed in both the present study and Downtown's study does not appear to be related to 0% salinity inhibiting the ability of *Avicennia* to uptake nitrogen.

Metabolites of growth processes

Amino acid dynamics

Concentrations of all amino acids before addition of labelled ammonium chloride ($^{15}NH_4Cl$) in the low nutrient groups (0SLN and 25SLN) were significantly lower than in leaves of plants from the high nutrient groups (0SHN and 25SHN). After addition of labelled nitrogen fertiliser plants from the low nutrient group started converting extra nitrogen into amino acids, which was demonstrated by higher levels of ^{15}N labelled amino acids in leaves and roots.

Amino acids in leaves

The most common amino acids in leaves were glutamic acid (Glu) and glutamine (Gln; around 50% in low nutrient groups and 30% in high nutrient; Supplementary Figure 2). The level of glutamine in leaves was not affected by nutrient and salinity treatments, which indicates that plants under nutrient limitation produced that amino acid in the same quantity as plants that were not nutrient-limited. In higher plants the glutamate/glutamine pathway is a primary ammonium assimilation (NH_4^+) pathway through the actions of glutamine synthase (GS) and glutamate synthase (GOGAT; Lea & Azevedo, 2006). As mangrove plants prefer to assimilate ammonia over nitrate from the sediment (reviewed in Reef *et al.*, 2010), Glu and/or Gln might play a role as the primary source of nitrogen in *A. marina* leaves and as substrates for transamination reactions which are commonly mediated through these two amino acids. Glutamic acid is commonly converted into other amino acids (e.g., aspartic acid and asparagine) and/or amines as well as serving as a protective osmolyte *per se* in higher plants (Forde & Lea, 2007 as reported in Pfautsch, Bell, & Gessler, 2015).

However, such a principal role of glutamate/glutamine as a nitrogen source in mangrove leaves was not reported previously. Two earlier studies reported that arginine, alanine and asparagine had the highest concentrations in mangrove leaves (Popp *et al.*, 1985; Ashihara *et al.*, 1997). Two days after the addition of ^{15}N, we found that concentration of labelled glutamine (Gln1 and Gln2) in nutrient limited plants (0SLN and 25SLN) increased from around 1 mg/g up to *ca* 3 mg/g after but it did not change in high nutrient plants. These results further confirm that *A. marina* accumulates glutamine as a nitrogen source in the leaves where it can be used for further growth needs. Other amino acids that nutrient limited plants accumulated after ^{15}N addition were labelled Glu1, Asp1, Asn1, and Asn2, whose concentration significantly increased after 2 days of exposure.

Amino acids in roots

In contrast to the results from leaves, the amino acid found in highest concentration in roots was asparagine (Asn) in the high nutrient plants (red in the heat map in Figure 7 at around 70% of the measured amino acids). Ashihara *et al.* (1997) also found that in *Avicennia marina* asparagine was the major amino acid present in roots from plants grown in a greenhouse for two years. However, the nutrient and salinity conditions were not specified in this experiment, so direct comparison is difficult with the present results as the plant age, nutrient content and salinity content may all be different. Two alternative hypotheses are available for the high asparagine content in roots: the first is that it is acting as an osmolyte to protect the plant from the effects of salinity, the second is that it is acting in a nitrogen storage and transport role (reviewed in Pfautsch, Gessler, Adams, & Rennenberg, 2009). These two hypotheses are not mutually exclusive because asparagine may be acting both as an osmolyte and as a nitrogen storage/transport role. For example, Ashihara *et al.* (1997) suggested that asparagine might be acting as a compatible solute (osmolyte) in *Avicennia*, because it was previously found that under NaCl treatment *Arabidopsis thaliana* species was able to tolerate this stress when asparagine was provided externally.

In contrast to Ashihara *et al.*'s experiment, the experimental conditions in the present study allow to separate the effect of salinity from those of nutrients (nitrogen storage and transport). Our results show that changes in salinity had little effect on the asparagine content in roots, whereas changes in nitrogen availability had a dramatic effect. Thus, the results in present experiment indicate that the osmolyte hypothesis is likely to be incorrect and that the primary role for asparagine is in nitrogen transport and storage. This is consistent with its known role in other plant species (Pfautsch *et al.*, 2009). As noted by Duff (2015), that asparagine has one of the highest nitrogen to carbon ratio among 20 essential amino acids, it has relatively neutral charge, and it can be a substrate for only a small number of highly specific enzymes. These biochemical properties make asparagine an ideal candidate for the transport and temporary storage of nitrogen. Tsuchiya *et al.* (2013) also found that asparagine was most abundant amino acid in cotyledon protoplasts of mangrove *Avicennia marina.*

It has been suggested that mangroves accumulate below ground biomass as a nutrient preservation strategy (Alongi *et al.*, 2002; Alongi, 2009). Thus, it is feasible to hypothesise that asparagine accumulation in mangrove roots is the main short-term nitrogen storage mechanism when nitrogen is in excess. Especially, with the view that

mangroves often utilise old root channels enriched with the decaying root material to proliferate new root hairs (McKee, 2001). Duff (2015) suggested that due to its high carbon to nitrogen ratio, asparagine is likely to be the main amino acid of many storage proteins. Thus, it is likely possible that much of the enhanced nitrogen content in the high nutrient plants roots may reside in this form in mangroves, which have the main nutrient storage below ground.

However, in low nutrient plants (0SLN and 25SLN) the most abundant amino acids were labelled Gln2 (around 15-20%), followed by Glu and Glu1, Ala1, Asp, Asn, Asn1 and Asn2 (*ca* 10% each). These findings indicate that an accumulation of the glutamate family amino acids (Glu and Gln) may indeed serve *Avicennia marina* mangroves as nitrogen assimilation mechanism as well as important nitrogen source in both leaves and roots. Glutamate and glutamine serve a central role in nitrogen assimilation (the so called GS/GOGAT cycle) and amino acid metabolism via transamination reactions and also in the synthesis of aspartate and asparagine (Pfautsch *et al.*, 2009). The differences in the main amino acids reported in leaves between the present study and the earlier studies may be related to the age of the plants. Seedlings may prioritise growth over storage and not store asparagine in leaves, but use it to transport N to the leaves from the roots, then reconvert it to the primary amino acids involved in transamination reactions, glutamate and glutamine.

One more interesting trend was observed in levels of serine. Concentrations of labelled serine (Ser1) increased significantly after 2 days of ^{15}N fertiliser addition in leaves of nutrient deprived mangrove seedlings (0SLN and 25SLN). Serine was found to be associated with the synthesis of growth and development enzymes and promotes synthesis of sphingolipids (Chao *et al.*, 2011). Serine level also regulates folate metabolism, which in turn regulates root development and photorespiration (Collacova *et al.*, 2008; Srivastava *et al.*, 2011, as reviewed in Ros *et al.*, 2014). Accumulation of serine, thus, can be related with growth induction in nutrient limited plants after nitrogen addition.

Osmolytes

In addition to nitrogen assimilation and nitrogen transport, amino acids can act as osmolytes, because of their ability to form a zwitter-ion. Indeed, some researchers associate accumulation of glutamate with an osmoprotection function (Planchet &

Limami, 2015). However, the main view on the free amino acid pool is that plants accumulate them as a precursor for further targeted osmolyte synthesis. For example, glutamic acid can be converted into proline, accumulation of which was shown to be associated with abiotic stress, especially salinity, tolerance in plants (Munns, 2002; Sharma & Dietz, 2006; Planchet *et al.*, 2011).

However, in the present experiment, proline concentrations were affected only by nutrient treatment, and not by salinity. Furthermore, addition of nitrogen in the labelled ^{15}N plants did not produce more proline even under nutrient deficient conditions, which suggests that proline in *Avicennia marina* seedlings under experimental conditions was not required as an osmolyte. In the review of Parida & Jha, 2010 proline was reported to be the main osmolyte in some mangrove species, but not in *Avicennia marina* species. Instead, glycine betaine, a quaternary ammonia compound, was reported to serve as compatible solute, which protects photosynthetic machinery (Popp *et al.*, 1985; Ashihara *et al.*, 1997; Hibino *et al.*, 2001).

We found that glycine betaine concentrations in leaves of *Avicennia marina* var *australasica* were not affected by either salinity or nutrient levels. However, the precursor for glycine betaine, choline, was affected by nutrient levels, and its level was higher in high nutrients treatments (0SHN and 25SHN). This trend was observed in both roots and leaves, as well as in root betaine concentrations. Both glycine betaine and choline can counteract osmotic differences between vacuoles (where salt is stored) and inter- and intracellular liquid as well as scavenge reactive oxygen species (ROS; Flowers *et al.*, 1977; Flowers & Lauchli 1983; Muns & Tester, 2008; Parida & Jha, 2010). Thus, our results suggest that under treatment conditions such as 0% and 25% salinity, *Avicennia marina* var *australasica* did not require significant accumulation of osmolytes, which seems reasonable as the salinity range we used is low to optimum for growth of *A. marina*, whereas osmolytes are likely to be produced under high salinity conditions.

Reactive oxygen species (ROS) are commonly used as an indicator of stress in plants (Parida & Jha, 2010) and it might have been expected that the seedlings that displayed the most stunted growth, namely 0SLN and 25SLN, would have the highest ROS levels. Although, in our study we observed the highest ROS levels in the leaves of 25SLN and the lowest in 0SLN plants. Overall, leaf ROS was higher under elevated salinity

conditions and lower at zero salinity, which was previously described for mangrove plants as an indication of salinity related stress (Bose *et al.*, 2013).

However, in roots, ROS levels were higher in high nutrient groups (0SHN and 25SHN) and lower in low nutrient groups (0SLN and 25SLN). The high ROS concentrations in the high nutrient groups may be an indicator of growth associated processes. For example, ROS are formed in mitochondria in the course of respiration, and/or during fatty acid β-oxidation (Bose *et al.* 2013). An alternative explanation may be that the primary root function is to uptake and deliver nutrients and water to aboveground parts of the plant, and this difference in ROS can be explained by the relatively smaller root biomass in the higher nutrient group (30-35%) in contrast to root biomass of the low nutrient plants (51-53%). The smaller relative root biomass in the high nutrient groups provides resources to the larger aboveground parts (stem and leaves), so concentrations of the biochemically active compounds (including ROS) in root tissue may be higher as a result of this increased need.

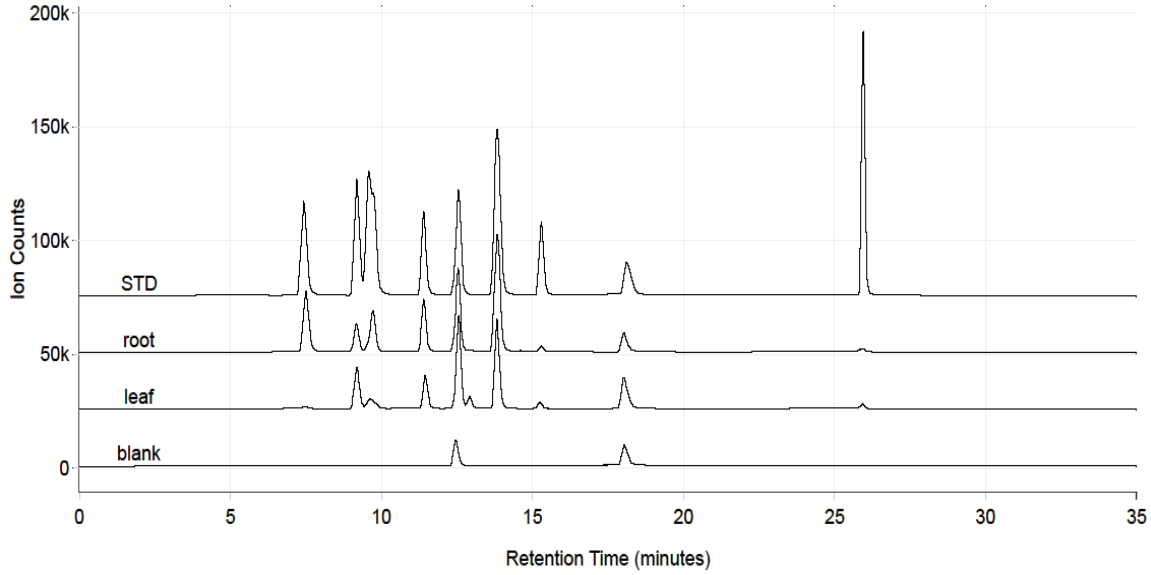
4.6 Conclusion

Overall, our results suggest that *A. marina* growing in zero salinity conditions and optimal salinity conditions (around 25% of seawater) do not suffer stress related to reactive oxygen species, nor does salinity have a negative effect on nitrogen absorption, but that instead, zero salinity enhances absorption of nitrogen. It has been debated in the past whether some mangrove species can be obligate halophytes (Wang *et al.*, 2011; Krauss & Ball, 2012). The study of Nguyen *et al.*, 2014 revealed that saline conditions benefit the growth of *Avicennia marina*, especially the formation of stem and leaf hydraulic systems, and, hence, water uptake. As salinity benefits water uptake, it also should benefit nitrogen uptake. However, we observed the opposite trend, 0% salinity plants assimilated nitrogen faster than 25% salinity plants. This may be related to the know preference for fresh water over saline water sources in *Avicennia* (Reef *et al.*, 2015).

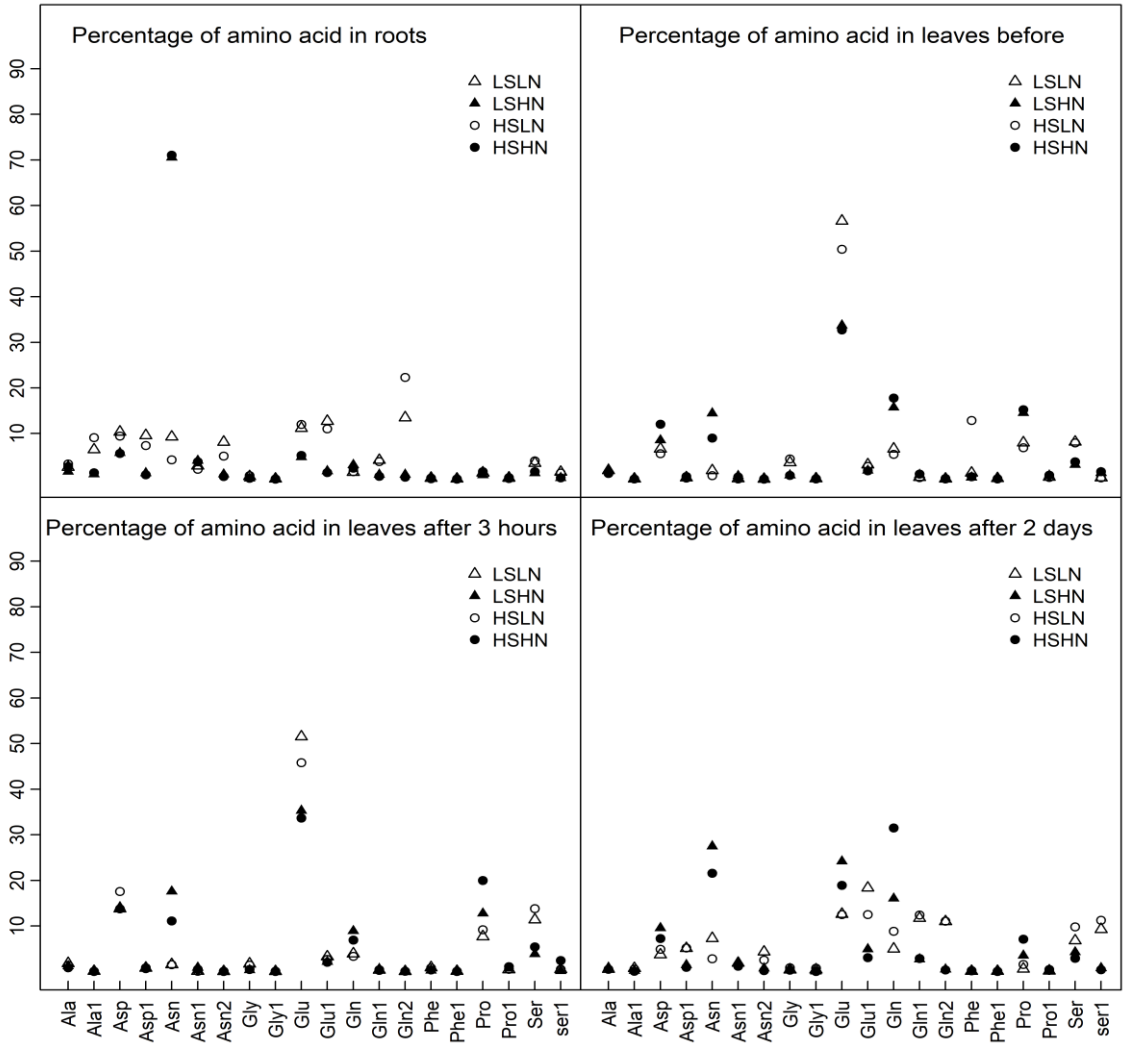
For future work, we suggest incorporating *Avicennia marina* species from different climatic areas in one experiment, as it has been found that maternal origin of seedlings affects adaptation to specific salinity levels (Alam, Mahmood, & Rahman, 2018). Such a design can help to identify how mangrove plants can change their physiological and biochemical traits in response to environmental salinity. Also, measurements of amino acid concentrations in the field are beneficial for understanding general pathways of N

uptake for various mangrove species and form different climatic zones. Additionally, untargeted metabolomic and genomic analyses can reveal specific genes and mechanisms involved in salinity tolerance and growth biochemical processes, which are important for survival strategies under salinity stress in mangrove plants.

4.7 Supplementary material



Supplementary Figure 4.1. TIC chromatograms of LC-MRM-MS analysis of amino acids in the blank, mixed standard (STD), root, and leaf tissues of *Avicennia marina* var *australasica* 6 months old seedlings in a 35-minute run.



Supplementary Figure 4.2. Percent of amino acids in roots (%), leaves before labelled ^{15}N addition (%), leaves after 3 hours (%), and leaves after 2 days (%) of *Avicennia marina* var *australasica* 6-months old seedlings among four treatment levels (0S = 0% seawater [triangle symbols], 25S = 25% seawater [circle symbols], LN = low nutrients [empty symbols], HN = high nutrients [filled symbols]).

Chapter 5. Salinity and nutrient levels in temperate New Zealand mangroves under natural conditions and effect of nutrient addition on mangrove growth in the field

In this chapter, nutrient and salinity levels in temperate mangrove ecosystems were investigated. *Avicennia marina* var. *australasica* leaf nutrient composition was monitored to measure available nutrients and salinity levels for two years. Primary growth parameters were also measured and correlated with nutrients and salinity availability. A one-year fertilisation experiment was conducted to establish the nutrient limitation patterns in temperate mangroves. Results of these experiments contribute to the general understanding of the nutrient patterns along temperate mangrove stand gradient and seasonal nutrient and salinity trends in temperate New Zealand estuaries. The content and results featured in this chapter are part of a manuscript being prepared for submission in the journal Ecosystems.

5.1 Abstract

Mangrove ecosystems contain salt-tolerant plant communities, which display complex interactions between tree physiognomy and sediment conditions, such as nutrient and salinity levels. Over two years, we measured mangrove growth (shoot increments and new leaf gains) and leaf nutrient concentrations (total nitrogen (%N), total phosphorus (%P), sodium (Na) and potassium (K) concentrations) in temperate mangroves (tall trees near channel edges and short trees inside mangrove stands) before and after nitrogen (N) and phosphorus (P) fertiliser addition. Results indicate that tree growth correlated well with %P concentration in leaves as well as with Na:K ratios. N fertilisation significantly improved growth of mangroves at most sites, while P addition improved growth only at one site. Additionally, we found strong seasonal variations in %P and Na:K ratios in mangrove leaves. Results from Na and K measurements showed that salinity distribution patterns commonly found in tropical settings (low salinity at low intertidal edges of mangrove stands and hypersaline conditions in upper intertidal inland areas) are not the trend for temperate conditions. At Mangawhai Estuary, salinity levels were either equal or lower inside mangrove stands compared to edges (near waterways). Hence, we suggest that salinity may not be responsible for the temperate tree height gradient (taller trees at the edge and short trees inside the stand). Instead, low nutrient availability may determine tree growth differences in New Zealand's temperate mangrove ecosystems.

5.2 Introduction

Mangrove ecosystems are formed by salt-tolerant plant communities, which dominate tropical and subtropical coastlines and estuaries worldwide (Tomlinson, 1986). They reach greatest heights (> 20 m) and species-richness near the equator, and form low,

species-depauperate stands towards their northern and southern distribution limits (Alongi, 2009; Giri et al., 2011). General trend is thatThe primary growth (e.g., tree height, shoot elongation, and number of leaves) of mangroves decline with increasing latitudes, primarily due to lower temperatures. For example, the southernmost mangroves occur in New Zealand and show stunted growth compared to tropical counterparts (Beard, 2006; Morrisey et al., 2007).

However, in both tropical and temperate regions, growth and thus mangrove physiognomy may vary substantially along small-scale environmental gradients. Spatial variability in nutrients and salinity of the surrounding sediments may be key drivers for plant growth (Duarte and others, 1998; Ball, 2002; 2007; Naidoo, 2009). For example, in situ N and/or P fertilisation experiments have shown higher growth stimulation in inland mangroves, compared to edge plants, which suggests the presence of an interaction between local salinity and nutrient levels (Boto & Wellington, 1983; Lovelock et al., 2006; Feller et al., 2007). Also, spatially varying salt concentrations may cause local changes in community composition and tree growth patterns in tropical mangrove forests where high salt concentrations cause stunting in some mangrove species that grow further inland (Ball, 2002; Naidoo, 2006).

Foliar elemental composition strongly reflects nutrient availability in the surrounding environment (Aerts & Chapin, 1999; Güsewell, 2004). For instance, in a greenhouse experiment, Alongi (2011) showed that N and nitrogen:phosphorus (N:P) ratios in mangrove leaves increased in treatments with higher N addition. Some field fertilisation trials have also demonstrated that nutrient concentration in mangrove leaves is related to the interstitial nutrient concentration in the surrounding sediments (Duarte et al., 1998; Feller, 1995). Similarly, Na concentrations and sodium/potassium (Na:K) ratios in mangrove leaves are consistently higher in hypersaline conditions rather than in moderate or low saline environments (Downton, 1982; Ball et al., 1987; Flowers & Colmer, 2008; Chen & Ye, 2014; Duarte et al., 2014). Moreover, the salinity of the mangrove sediments can be variable, because it is influenced by tidal regime and freshwater inputs from the surface and groundwater sources (Bianchi, 2007), especially in the temperate climates where seasonal difference of these parameters is more pronounced. Thus, measurements of salinity in the porewater can be less informative when it is applied to assessing the salinity effect on mangrove plant growth. Recently a split-root study demonstrated that mangrove plants can increase water uptake from freshwater patches and avoid less

favourable saline water sources (Reef et al., 2015). This finding was also demonstrated in field studies, where some mangroves preferentially utilised fresh groundwater over surface saline sources (Ewe et al., 2007; Lovelock, Reef, & Ball, 2017). Thus, measuring salinity as Na and Na:K ratios directly in mangrove leaf tissues seems to provide an alternative method to assess environmental salinity, and represents an integrated average of all water sources available to the plant and is less likely to be confounded by mangrove uptake selectivity.

A common method to investigate the effect of nutrients on mangrove growth is through fertilisation experiments (e.g., Feller, 1995). In such studies, researchers measure initial nutrient concentrations (%N and %P) in mangrove leaves prior to the fertiliser application, and then monitor leaf traits of treatment and control trees. However, this approach does not consider possible seasonal nutrient fluctuations, which in the case of temperate mangroves, can be significant. In order to provide quantitative data which accurately links growth changes due to the fertilisation treatment beyond that which is normally measured in standard growth enhancement trials, we chose to use nitrogen stable isotope ($\delta^{15}N$) measurements as an indicator of fertiliser (urea) absorption by the plant. Urea has a $\delta^{15}N$ value of 0‰, because during urea manufacturing, carbon dioxide and N are isolated from the atmosphere and $\delta^{15}N$ of air is equal to zero (Lindau, Delaune, Patrick, & Lambremont, 1989; Fry, 2006). As $\delta^{15}N$ values in New Zealand mangroves are normally in the 5-10‰ (Gritcan et al., 2016), if mangroves absorb urea directly, $\delta^{15}N$ values in the leaves should decline.

Climatic differences (e.g., temperature and rainfall) between tropical and temperate zones can influence nutrient and salinity dynamics in estuarine ecosystems (Bianchi, 2007), which, in turn, may strongly affect mangrove growth in temperate areas. While the overall decline of mangrove forest productivity from tropical to temperate latitudes is clearly driven by temperature (Beard, 2006; Quisthoudt et al., 2012), regional and local differences in growth are not well understood. New Zealand mangroves occur in temperate climates, which are characterised by mild, wet winters and moderately warm summers with less rainfall (Morrisey et al., 2007). These seasonal differences in temperature and rainfall have a strong effect on nutrient availability. For example, soluble reactive phosphorus (SRP) availability drops with decreasing temperature and freshwater inputs (Bianchi, 2007). Lovelock and others (2007, 2010) showed that nutrient inputs from agricultural runoffs play an important role in the spread of mangroves in New

Zealand. However, ability of mangroves to absorb these nutrients can vary depending on the mangrove stand's proximity to the fertilised land and seasonal fluctuations of temperature and rainfall. Here, we aim to investigate New Zealand temperate estuarine mangroves to: (1) characterise the seasonal variations in growth, nutrient status, and salinity conditions; and (2) determine whether growth in mangrove stands is N- and/or P-limited using a nutrient addition approach complemented by nitrogen stable isotope analysis.

5.3 Materials and methods

Study site

The study area at Mangawhai Harbour Estuary (36° 07' 00" S, 174° 36' 00" E) is situated 100 km north of Auckland in northern New Zealand. Within *Avicennia marina* var. *australasica* stands, two study sites were selected (upper and middle estuary). At each site, mangroves were sampled within low intertidal zone (near the channel) and upper intertidal (away from the channel) areas (Fig. 5.1). Mangrove trees at the low intertidal zone were situated within 0-40 meters from the channel and upper intertidal zone trees were approximately 50-100 meters away from the channel. On average, trees were taller but with sparse canopy at study site 1 compared to the site 2, and tree height declined from low intertidal to the upper intertidal zone at each spot (Tran, 2014).

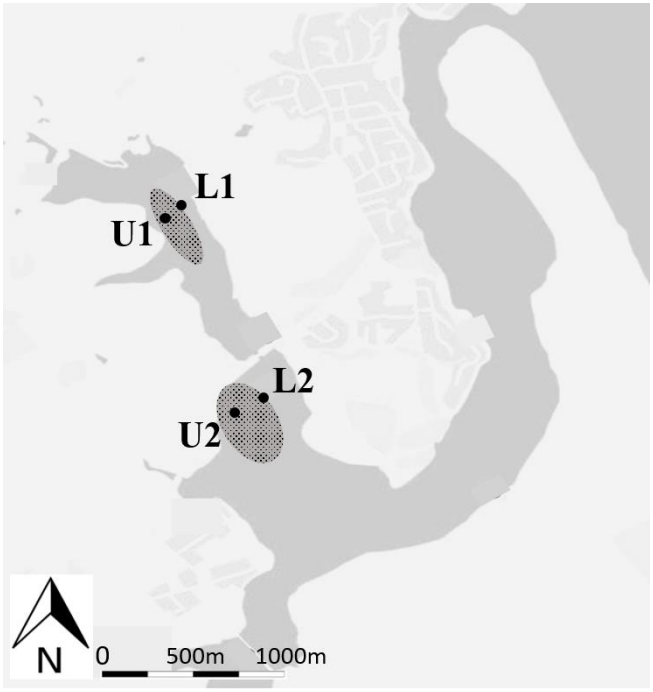


Figure 5.1. Map of Mangawhai Harbour Estuary with darker areas of two study sites, where site 1 is located upper estuarine and site 2 is mid estuarine with lower intertidal zone (L) and upper intertidal zone (U).

Pre-treatment year

Baseline data on growth and foliar nutrient composition within mangrove trees was collected for one year (pre-treatment year) before the fertilisation experiments were conducted. Seven trees (at least 5 meters apart) were selected at each of the four locations for growth measurements every 2 months. Shoot length and the number of leaves on that shoot were recorded for all sampled trees. During each sampling event, ten top canopy leaves (fully matured, but not senescent) were collected from each tree and brought to the laboratory for elemental analysis. In addition, the length of five shoots and their number of leaves were recorded. Those shoots were marked with flagging tape for repeated measurements.

Fertilisation year

The fertilisation experiment was conducted from September 2015 to September 2016, following the methods of Feller (1995). At each site, three types of fertilisation treatment (unfertilised control, N-fertilised, P-fertilised) were allocated to 15 trees (5 trees per treatment level). The common agricultural fertiliser urea (CH_4N_2O; 45:0:0) was used as a N fertiliser and triple superphosphate ($Ca(H_2PO_4)_2•H_2O$; 0:45:0) was used as a P fertiliser (Ravensdown Fertiliser Trading Company, Auckland, New Zealand). The fertiliser doses (300 g per tree) were placed in dialysis tubing with a 14000 molecular weight membrane (MEMBRA-CEL, MD34, USA) in 150-gram portions to ensure the slow release of the fertiliser. Two nutrient tubes, each containing one of the two dry fertilisers, were buried 30 cm below the ground and 30 cm away from each selected tree. The same procedure using empty dialysis tubing was applied to the unfertilised control trees. The fertiliser was reapplied in the same manner after six months. Following the addition of the fertiliser, shoot growth measurements and leaf samples were taken every two months.

Leaf nutrient analyses

Ten leaves were collected per tree, oven dried at 65°C for 3 days, mixed together and ground to a fine powder with a ball mill (PM 100, Retsch Ltd., Haan, Germany) and sifted through a 200 µm pore size sieve. Then leaf material from 5 replica trees was additionally pooled per treatment. Total phosphorus (% P), sodium (% Na), and potassium (% K) concentrations were analysed using wet digestion in concentrated HNO_3 (McQuaker *et al*., 1979), followed by quantification using an inductively coupled plasma atomic emission spectrometer (ICP-AES, Varian Liberty AX Series II, USA). A National

Institute of Standards & Technology (NIST) Peach Leaf standard reference material (SRM1547) was used as a quality control in all analytical batches. A total of 0.1-0.5 g of each composite leaf sample was sent to the Waikato University Stable Isotope Unit (University of Waikato, Waikato, New Zealand) for total nitrogen (% N) and stable isotope ($\delta^{15}N$) analyses.

Meteorological data

Daily rainfall and temperature data were downloaded from the Mangawhai Heads Weather Station web page (http://www.mangawhaiweather.co.nz), less than 10 km away from the experimental sites.

Statistical analyses

A generalised additive mixed model (GAMM, R package *mgcv*; Wood, 2011) was used to model patterns of plant growth (shoot and leaf gain) from the pre-treatment year and new leaf gain from the fertilisation year; N:P ratio; and to explore the relationship between the Na:K ratio and rainfall. Model selection was based on Akaike's Information Criterion (AIC; Burnham & Anderson, 2016). For each response variable, we fitted a GAMM with separate smoothers and intercepts for each site, thus allowing for a time × site interaction (or rainfall × site interaction). Using a likelihood ratio test, this full model was compared to a restricted model with varying intercepts for each site, but one common smoother across all sites (no interaction model). In the case of a significant interaction, *post-hoc* testing was performed by pooling sites using all possible grouping combinations and running the corresponding GAMMs, which were then compared to the full model via likelihood ratio tests. This site grouping approach allowed us to test the null hypothesis that pooled sites have similar temporal trends (or trends in rainfall). The resulting *P*-values were corrected for multiplicity using the Benjamini & Hochberg (1995) method.

Nonlinear trends of shoot growth rates during the fertilisation year were modelled by using generalised nonlinear least square models (GNLS) with the restricted maximum likelihood (R package *nlme*, Pinheiro and others, 2017). We applied a similar approach as described above, comparing a full model allowing separate parameter estimates for each site to a restricted model with common parameter estimates across sites. Then, the same *post-hoc* procedure and multiplicity adjustment as described above was applied to determine which sites differed in shoot growth. A simple ANOVA model was used to test

for the effect of N addition on $\delta^{15}N$ in mangrove leaves followed by a multiple comparison procedure using Tukey contrasts (R package *lsmeans*; Lenth, 2017).

Model assumptions (normality and homogeneity of residuals) were verified using quantile–quantile plots, histograms, and residuals vs fitted values plots. All models were considered for the repeated measurement values. In some GNLS models we detected variance heterogeneity, which was accounted for by incorporating an exponential or power variance function (*varExp*, *varPower*). For all statistical analyses and figures, the R software version 3.2.1 was used (R Development Core Team, 2017; www.r-project.org).

5.4 Results

Growth trends

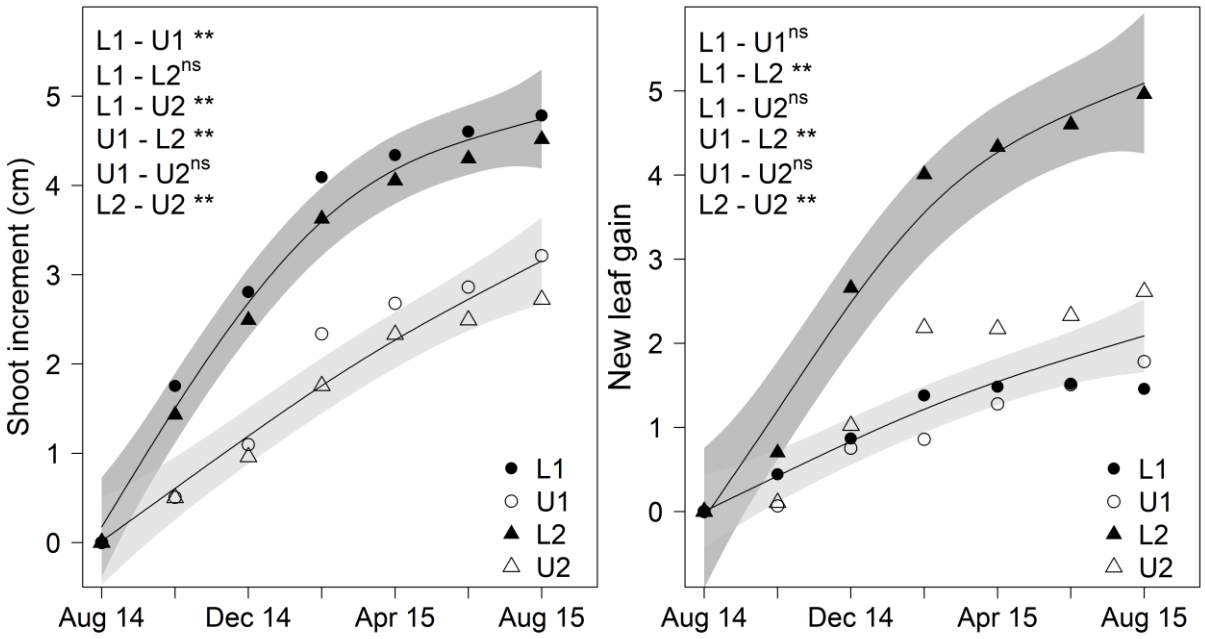


Figure 5.2. Shoot increment (cm; left panel) and new leaf gain (right panel) of *Avicennia* var. *marina australasica* trees during the pre-treatment year at two sites (1 = site 1, 2 = site 2, U = upper intertidal zone, L = lower intertidal zone). Lines represent GAMM smoothers. Grey areas around model fits indicate the 95% confidence intervals. Symbols represent means (n = 7). Table insets show the results of site comparisons, followed by *post-hoc* procedure and multiplicity adjustment, where 'ns' indicates not significant, ** $P < 0.01$. Non-significant differences allowed the pooling of certain sites reflected in a joint model fit.

Model fits for shoot increment and gain of new leaves resulted in significant site × time interactions, indicating that plants at the edge sites grew and produced leaves faster than at the interior sites (Fig. 5.2). At the end of the pre-treatment year, mangrove plants from edge sites (L1 and L2) had longer shoots by 1.5 cm than those from interior sites (U1 and U2). Leaf accretion was significantly higher only at one site (L2) compared to the other sites, and trees at L2 had 1.5 times more leaves at the end of the trial than those at the other sites (L1, U1 and U2).

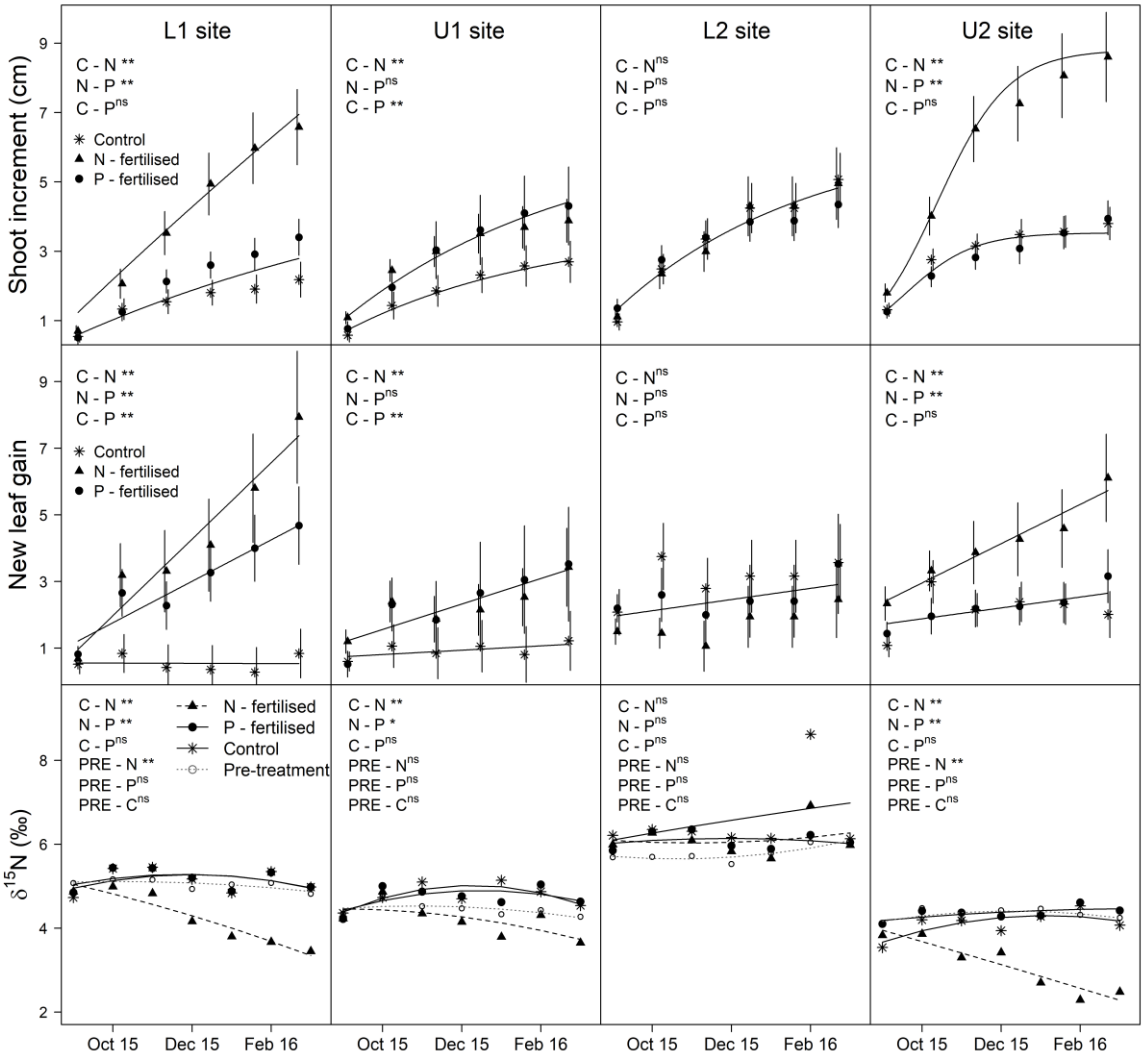


Figure 5.3. Shoot increment (cm; top row), new leaf gain (middle row), and leaf nitrogen stable isotope ratio ($\delta^{15}N$; ‰; bottom row) of *Avicennia marina* var. *australasica* control (C) and fertilised trees (N = nitrogen, P = phosphorus, PRE = pre-treatment year) at two study sites (1 = site 1, 2 = site 2, U = upper intertidal zone, L = lower intertidal zone). Symbols represent means (n = 5 ± SE). Lines represent GNLS, GAMM smoothers, and linear regression fits, respectively. Table insets show the results of site comparisons, followed by post-hoc procedure and multiplicity adjustment, where 'ns' indicates not

significant, * $P < 0.05$, ** $P < 0.01$. Non-significant differences allowed the pooling of certain fertilisation treatment levels in a joint model fit.

In three out of four locations (L1, U1, and U2), there were significant time × fertiliser interactions, suggesting different slopes for shoot increment rates across nutrient treatments (Fig. 5.3), and reflecting the actual nutrient uptake (see below). However, at the L2 site, no treatment-related effect on shoot growth was found (Fig. 5.3). At the two upper estuary sites (L1 and U1), N addition resulted in shoots that were 3 and 2 times longer than those of control trees. At the L1 site, growth in P-fertilised trees did not differ from the control trees, whereas at the U1 site there was no difference between P-fertilised and N-fertilised trees, but both differed significantly from the control. At the U2 site, all trees showed a logistic shoot growth rate, which was similar in the control and P-treated trees, but showed nearly 3-fold higher plateau values in the N-treated trees (Fig. 5.3).

Similar to shoot growth rates, a significant time × fertiliser interaction in leaf accretion suggests different slopes among fertiliser treatment levels at three sites (Fig. 5.3). However, there was no treatment-related effect on leaf accretion at the L2 site. Control trees at the two upper estuarine sites formed few new leaves. However, fertiliser addition (both N and P) at the U1 site significantly boosted leaf formation, resulting in an average of 2 more leaves per shoot at the end of the fertilisation trial. At L1 site, treatments have significant effect on new leaf gain, N-fertilised trees had on average 2 more leaves per shoot than the P-fertilised trees, which in turn, had around 3 more leaves per shoot than control trees. At the U2 site, N-fertilised trees produced significantly more leaves, ca. 4 more leaves, at the end of the fertilisation period compared to P-fertilised and control trees (Fig. 5.3).

At all sites, apart from L2, foliar $\delta^{15}N$ steadily declined in N-fertilised trees throughout the fertilisation year, while pre-treatment $\delta^{15}N$ values and those of control and P-fertilised trees remained the same and did not differ significantly from each other. At the U1 site, $\delta^{15}N$ values during pre-treatment year were found to be intermediate between fertilisation year values found in P-treated and control trees and N-fertilised trees (Fig. 5.3).

Nutrient and salinity dynamics

An AIC-based model selection procedure favoured the GAMM with two smoothers for site pairs U1 + L2 and L1 + U2. We plotted N:P ratio trends found in mangrove leaves

during the pre-treatment year and during the fertilisation year in control trees with no fertilisation added (Fig. 5.4a). At the U1 and L2 sites, N:P ratio dramatically decreased to a minimum of 9 in late autumn during both years, which indicates that N-limitation (N:P < 10, Güsewell, 2004) occurred during winter and spring periods in (2014: August to December and 2015: July to November). Mangrove trees at the U1 and U2 sites were N-limited throughout the 2014 monitoring period and well into 2015, only exceeding the threshold value of 10 in November 2016. N:P values rose to a peak of 12 at the end of the summer 2016 (February/March) and then declined in a linear fashion falling below 10 again in August 2016. From September 2015 to the end of the monitored period, the difference between the two groups gradually disappeared. Neither N nor P fertilisation significantly affected N:P ratios in mangrove leaves, allowing pooling of the control and fertilisation treatments.

All sites shared a similar nonlinear temporal Na:K ratio pattern, as suggested by an AIC-based model comparison, favouring the GAMM with one common smoother. However, a Tukey post-hoc comparison applied to the intercepts (i.e. the site-specific vertical shifts of the smoothing fit) showed that the L2 site had a significantly larger intercept than the remaining sites, which shared similar intercepts. The Na:K ratio for all sites peaked in February 2015 and 2016 (Fig. 5.4d). The lowest ratios for all sites occurred in September 2015 and 2016.

There were significant linear relationships between shoot growth and new leaf gain and phosphorus concentration (%P; Fig. 5.4b,c, respectively), as well as between Na:K ratio and new leaf gain (Fig. 5.4e).

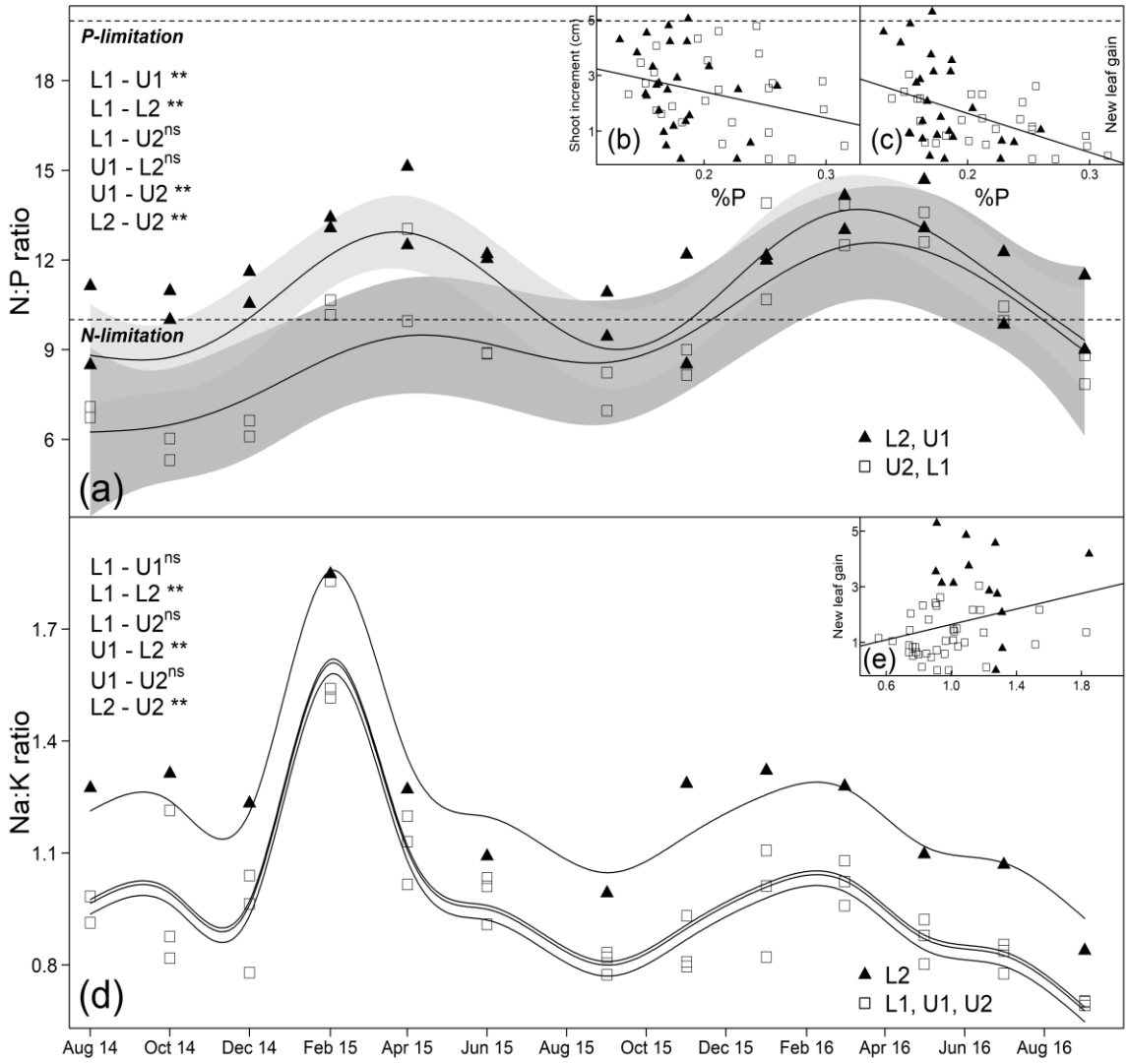


Figure 5.4. Temporal variation in the N:P (a) and in the Na:K (d) ratio in leaves of A*vicennia marina* var. *australasica* during both pre-treatment and treatment years at two sites at Mangawhai Harbour. Relationship between total phosphorus (%P) and shoot increment (cm; b); and total phosphorus (%P) and new leaf gain (c); relationship between Na:K ratio and and new leaf gain (e).Symbols represent means (n = 5; 1 = site 1, 2 = site 2, U = upper intertidal zone, L = lower intertidal zone). Table insets show the results of site comparisons, followed by post-hoc procedure and multiplicity adjustment, where 'ns' indicates not significant, ** $P < 0.01$. Non-significant differences allowed the pooling of certain site temporal trends in a joint model fit. Dashed lines indicate N and P limitation (Güsewell, 2004). Lines represent GAMM smoothers and linear model fit in as a relationship model. Grey areas around N:P ratio model fits represent 95% confidence intervals.

5.5 Discussion

Growth trends

During the pre-treatment year, shoot elongation was higher at the low intertidal zones of both study sites (L1 and L2), but the new leaf formation was higher only at one low intertidal zone situated in the middle estuary site (L2). These results are in agreement with previously observed growth trends in tropical mangroves, where plants growing in low intertidal areas accumulate biomass at higher rates than their upper intertidal counterparts (McKee *et al*., 2002; Lovelock Feller, McKee, Engelbrecht, & Ball, 2004; Rodriguez & Feller, 2004; Naidoo, 2009; Alongi, 2009). In the present study, the fertilisation experiment also showed greater nutrient limitation at upper intertidal zones (U1 and U2), as tree growth responded positively to N addition in these areas in contrast to low intertidal zones, where plant growth had increased after fertilisation only at the first site (L1).

Nitrogen dynamics

The most common driver of a growth gradient in mangrove trees within a stand or a forest is nutrient availability (Feller, Whinham, McKee, & Lovelock, 2003; Lovelock *et al*., 2004; Krauss *et al*., 2008; Reef, *et al*., 2010). We found that plants in the upper estuary responded to nitrogen fertiliser at both locations (L1 and U1) and, hence, are more N-limited than in the middle estuary (only at U2). Thus, our study supports early findings that mangrove stands within an estuary can have various nutrient distribution patterns (McKee, 1993; Feller *et al*., 2002). In our case, the amount of water passing through the ecosystem, and, thus, the amount of nutrients that are transported across the mangrove stands appear to be of critical importance in the nutrient dynamics of the system. Other edaphic conditions, such as the oxidation-reduction status of soil and sulphide concentration may also play a role in these dynamics.

We found no significant correlation between total nitrogen concentration in mangrove leaves and plant growth (data not shown). However, we observed strong inter-annual fluctuations in N concentrations in mangrove leaves (Supplementary figure 1). All trees, including those in the controls, displayed an increase in leaf N concentration during the treatment year, which may be associated with increased precipitation. The total amount of rain during the pre-treatment year (September 2014 – September 2015) was 973.3 mm, and increased to 1429.2 mm during the fertilisation period (October 2015 – October 2016). Because freshwater influx is thought to be the primary source of N in estuarine

ecosystems (Nixon *et al*., 1996; Seitzinger, Sanders, & Styles, 2002; Bianchi, 2007), more nitrogen could have been sourced from either terrestrial nutrient cycling or anthropogenic activities (e.g., fertilisation of croplands or animal farming runoff).

In our study, leaf N in mangrove trees at all study sites remained unaffected by N addition (not significantly different from the control trees), suggesting that the extra N for the fertilised trees was completely converted into new plant tissues (e.g., fine roots, shoots or leaves). This contradicts most mangrove fertilisation studies conducted in the tropics (e.g., Feller, 1995; Lovelock *et al*., 2004), but this trend has been observed in temperate mangroves before (e.g., Lovelock *et al*., 2010). The observed pattern of N investment can be further supported by the previously described mechanism of N accumulation in mangrove ecosystems. It suggests that mangroves do not accumulate large amounts of nutrients in the live biomass, but rather store nutrients in a below-ground pool of dead roots (Alongi, 2003; Alongi, 2009; Bulmer *et al*., 2016; Tran *et al*., 2016).

Nitrogen stable isotope ($\delta^{15}N$) ratios

During the pre-treatment year, $\delta^{15}N$ values were similar within mangroves in low or upper intertidal zones sites between study sites, and varied significantly between low and upper intertidal zones within the same study site. $\delta^{15}N$ values did not have pronounced seasonal trends within an individual site. However, during the fertilisation year, $\delta^{15}N$ values decreased steadily in N-fertilised trees at three sites. Variations in isotope compositions of mangrove leaves can either originate from the source of nutrients or microbial mediated biochemical processes occurring in the surrounding sediment. Microbial processes, such as nitrification, denitrification, ammonification, and de-ammonification can also enrich or deplete nitrogen sediment pools in ^{15}N. These microbial-mediated reactions are temperature dependent with optima between 35-50°C (Myers, 1974; Bowden, Castro, Steudler, & Aber, 1994; Davidson & Swank, 1986; Maag & Vinther, 1996; Vouve´ *et al*., 2000). However, there are no data available on microbial activity in cooler temperate New Zealand mangrove sediments. It has been estimated that in tropical conditions, 4-12% of the total nitrogen in the sediment is denitrified, and around 15% undergoes nitrification (Alongi, 2009). As temperature regimes in New Zealand (20°C in summer and 10-12°C in winter) are substantially cooler, we assume smaller effects of these microbial processes on $\delta^{15}N$ in mangrove leaves. Another reason for variations in $\delta^{15}N$ values may be the source of nutrients (anthropogenic vs natural). It has been suggested that $\delta^{15}N$ values around +3‰ represent organic matter mineralisation in the soil, values close to +6‰ are

attributed to mild human and animal sewage, and values of +10‰ and higher indicate anthropogenic N pollution in the area (Lindau *et al*., 1989). In New Zealand mangroves, foliar $\delta^{15}N$ values are closely linked to human-derived N sources. This was shown by Gritcan *et al*. (2016), who found decreasing $\delta^{15}N$ values in mangrove leaves along an anthropogenic N deposition gradient ranging from around 10‰ in a region receiving sewage inputs from a large wastewater treatment plant to about 5‰ in a sparsely populated area with modest anthropogenic N-inputs.

Results from the pre-treatment year showed that mangroves at low intertidal zones had higher foliar $\delta^{15}N$ (L2 – 5.8‰, L1 – 5.1‰) compared to those at upper intertidal (4.5‰ and 4.3‰ at U1 and U2, respectively). We propose that plants at the edge of a mangrove stand receive larger amounts of N compounds from anthropogenic sources than mangroves inland. This assumption is corroborated by other studies showing that mangrove roots act as a very efficient "coastal filter" able to trap and hold particulate and dissolved nutrients and to make them readily available for plant uptake (Chapman, 1940; Gill & Tomlinson, 1976; McKee, Mendelssohn, & Hester, 1988).

We hypothesised that N-limited mangrove systems will absorb urea directly, which should be reflected in decreasing $\delta^{15}N$ values. Our study demonstrated that changes in mangrove leaf $\delta^{15}N$ values can indeed be used as a tool to provide evidence for nitrogen fertiliser absorption. After urea fertilisation, mangrove leaf $\delta^{15}N$ values declined gradually, while at the same time primary growth rates (shoot elongation and leaf production) increased, indicating a direct link between N uptake and growth improvements. Previously, the same pattern was described in field crops, where for example, wheat grains and straw $\delta^{15}N$ values decreased as a result of urea addition in a N-deficient system (Serret, Ortiz-Monasterio, Pardo, & Araus, 2008). Recently, differences in $\delta^{15}N$ values between synthetic and organic fertilisers have come into use to scrutinize organic farming practices as it may indicate whether plants have been grown exclusively under organic conditions (Bateman & Kelly, 2007).

An interesting trend was observed at the L2 site, where plants did not improve their growth after fertilisation and their leaf $\delta^{15}N$ values did not decrease either, which suggests that urea was not absorbed by the mangrove plants at this site. One explanation for this observation might be that the fertiliser was lost due to leaching. Indeed, this site is situated at the bank of the very narrow channel at the middle of the Mangawhai Harbour Estuary

and water movement is possibly very intense in this location. This shows the utility of the $\delta^{15}N$ urea technique adopted in the present study, since in a conventional nitrogen fertiliser study this result might have been erroneously ascribed as indicating that the mangroves at the site were not nitrogen limited since no growth increase occurred on fertilisation. The present observation is a strong argument for incorporation of $\delta^{15}N$ measurements as a routine quality control measure in fertilisation studies.

Phosphorus dynamics

Total phosphorus concentrations in mangrove leaves had a negative linear correlation with both shoot growth and new leaf gain. We also measured a pronounced late autumn drop and a spring-summer rise of P concentrations in mangrove leaves at three of our sites (L1, U1, and U2). This fluctuation of phosphorus concentration in mangrove leaves is likely linked to the fluctuations of the reactive phosphorus in the environment, where P availability is strongly controlled by temperature and salinity (Bianchi, 2007)

Commonly, reactive P release occurs via microbial processes which are typically highest in summer months due to higher temperatures. Estuarine sediments are generally more reducing in summer and oxidising in late autumn and winter. During reducing conditions, iron-bond P is released as phosphate (PO_4^{3-}), whereas under the oxidising periods much of the P is bound to iron (III) oxides (Rozan *et al*., 2002). Additionally, primary production in temperate estuaries is highest in summer and all biota require P, which can further deplete P availability in midsummer-autumn (end of the growing season).

Another factor influencing reactive P availability in estuarine ecosystems is the proportion of freshwater. When waters are more saline (e.g., in summer), iron reducing aerobic bacteria convert amorphous iron (III) into iron (II) releasing iron associated phosphates and this process does not occur under freshwater conditions (e.g., in winter; Roden & Edmonds, 1997). As a result, reactive P is immobilised in freshwater sediments making many estuaries P-limited at times of high precipitation, such as spring and autumn (Conley, 1999).

An interesting observation was made that at the L2 site, foliar P concentrations in mangrove leaves were the lowest and showed no noticeable seasonal trend (Supplementary figure 2). We also observed that the overall leaf salt concentrations at this site were the highest with the lowest seasonal variability. Since P availability is

largely controlled by temperature and/or salinity, we concluded that salinity (or amount of freshwater) is a primary mediator for the reactive P availability in temperate New Zealand mangrove ecosystems. This hypothesis is underpinned by the significant negative correlation between N:P ratio in mangrove leaves and rainfall observed in this study (Supplementary figure 3).

Indeed, Jensen, Mortensen, Andersen, Rasmussen, & Jensen (1995) described complex salinity trends (decreasing in autumn and winter; and rising in spring and summer) in mangrove sediments that affects seasonal storage of sediment-bound P in winter and spring. The authors also stated that temperature-controlled release of P in summer can account for most of the temporal variability observed in estuarine systems. However, studies in tropical mangrove ecosystems demonstrated no seasonal changes in mangrove growth and nutrient composition of sediment in mangrove ecosystems (Boto & Wellington, 1984, 1988; Chen & Twilley, 1999; Krauss, Doyle, Twilley, Rivera-Monroy, & Sullivan, 2006). The present study provides evidence of seasonal trends through mangrove leaf P concentrations, which reflects the seasonal nutrient cycle in temperate mangrove ecosystems.

Salinity dynamics

Along with nutrient availability, salinity is viewed as one of the main factors affecting mangrove growth and productivity (McKee, 2001; Feller *et al.*, 2003; Naidoo, 2006; Alongi, 2009; Morrisey *et al*., 2010; Reef *et al*., 2010). Tropical mangroves show a strong salinity gradient from the low intertidal to upper intertidal zones, where reduced tidal activity and higher evapotranspiration rates lead to a build-up of salt in the sediment (Ball, 2002). However, we found that leaf Na and Na:K ratio which we used as a tool to measure salinity indicates that salinity gradients in temperate New Zealand conditions are absent or reversed, indicating that high salinity does not necessarily cause dwarfism in mangrove plants under temperate New Zealand conditions.

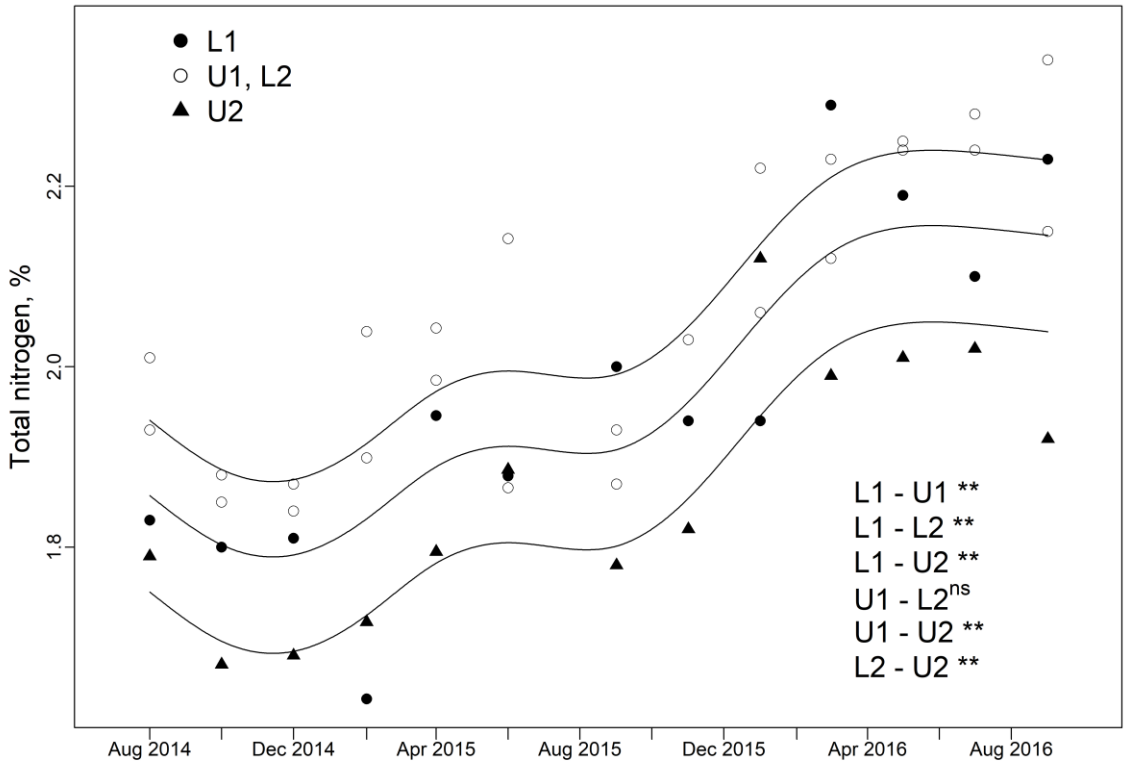
Similar salinity gradients have previously been reported for two New Zealand estuarine sediments by Lovelock *et al.* (2007b). They found higher salinity values at the edge of two estuarine mangrove stands (29.8 ± 0.3 and 22.2 ± 1.2 PSU) and lower salinity in the interior where dwarf mangroves occurred (22.8 ± 1.0 and 19.6 ± 0.9 PSU, respectively). Another study conducted in New Zealand by Yang *et al*. (2013), demonstrated similar trends in average concentrations of total dissolved salts (TDS) in porewater (22.1 ± 1.0

mg L^{-1} on the edge and 18.3 ± 1.6 mg L^{-1} in the mangrove interior). Only once, during summer, did they find the highest TDS concentrations (45 mg L^{-1}) in the interior mangrove zone, highlighting the strong seasonal variation in TDS.

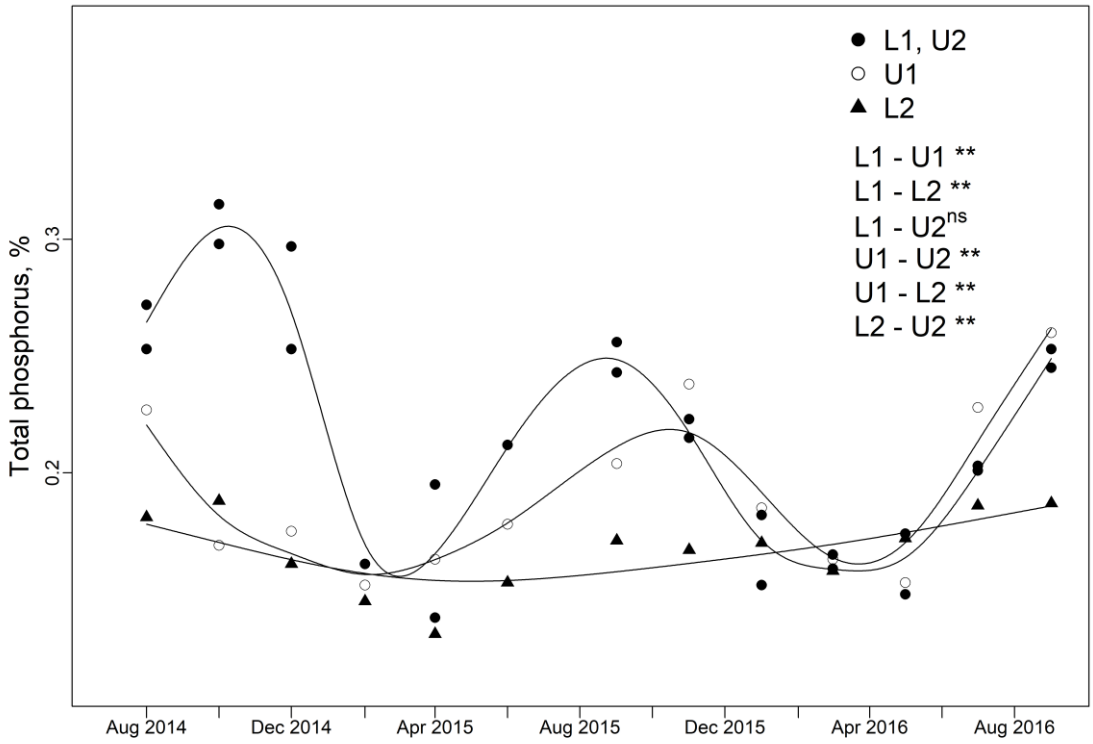
Surprisingly, new leaf gain had a positive linear correlation with Na:K ratio. The reason might be that salinity was higher during summer months when the growth of plants was the highest as well. Indeed, Na:K ratio measurements positively correlated with the temperature (Supplementary figure 3). Thus, our findings suggest that salinity trends in temperate mangrove ecosystems are opposite to those observed in tropical mangrove forests. This type of reversed salinity gradient may be characteristic for New Zealand estuaries, especially those adjacent to hilly landscapes, where mangroves are likely to receive larger freshwater inputs due to greater surface run-off from the elevated surroundings, especially during winter months.

Overall, our results suggest salinity concentrations in temperate mangrove ecosystems are lower than those in tropical mangrove forests, and high salinity concentrations may not result in differences in tree stature between low and upper intertidal zones of a temperate mangrove stand. The cooler and wetter New Zealand climate results in opposite trends to tropical salinity distribution between low and upper intertidal zones of temperate mangrove stands. However, we found that nutrient availability (especially nitrogen) can improve growth of stunted mangrove trees at the upper intertidal areas of mangrove stands. The increasing nutrient input over the past 100 years (mainly originating from fertilisation and livestock urine runoff from dairy and meat farming) may be responsible for the expansion of mangrove areas that has been observed in New Zealand over the last few decades. This is strongly supported by our finding that mangroves in New Zealand are mostly N-limited. Therefore, mangrove ecosystems may act as N sinks, thus mitigating coastal and marine eutrophication.

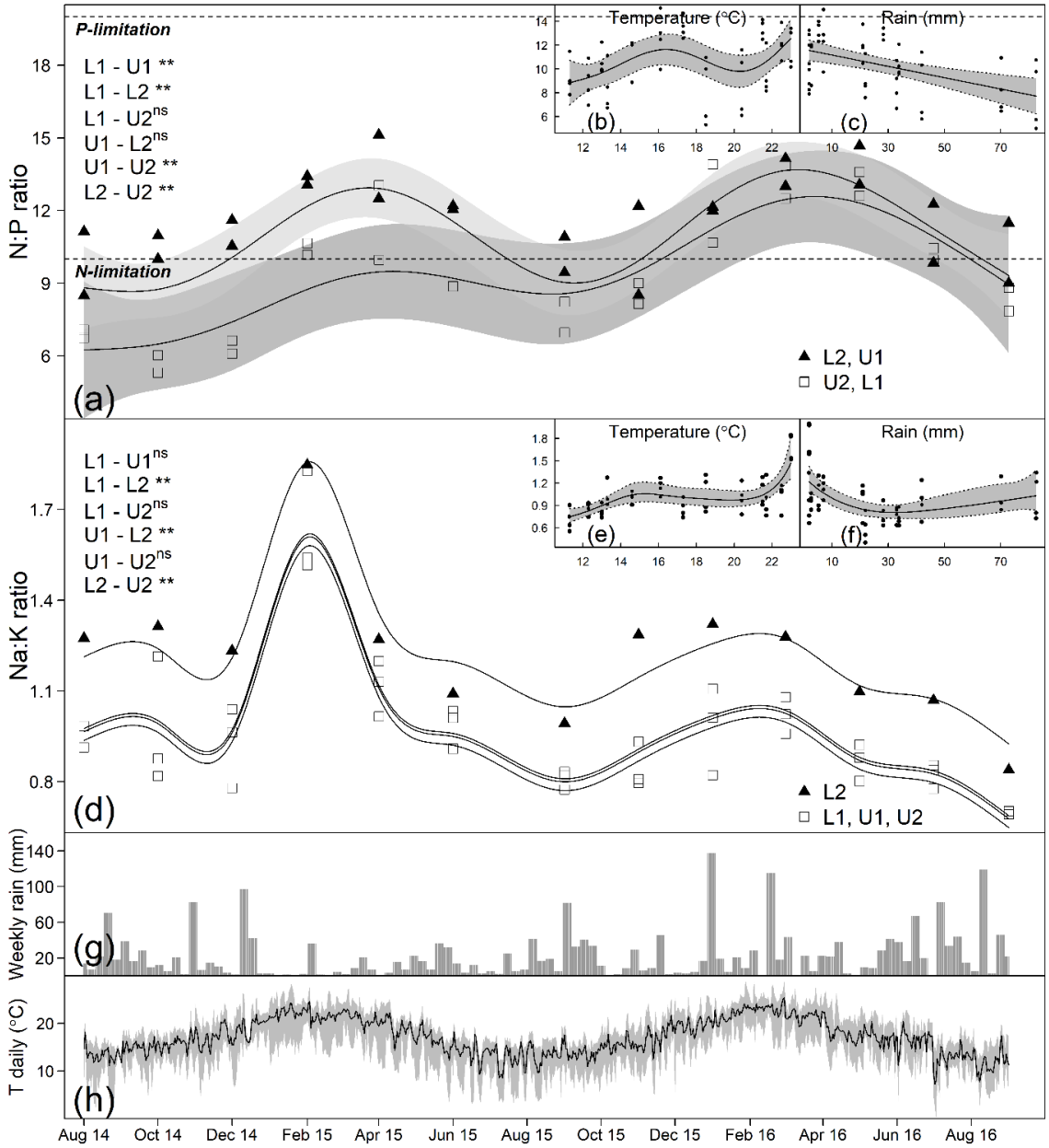
5.6 Supplementary material



Supplementary figure 5.1. Temporal variation in total nitrogen concentration (%, dry weight) in leaves of A*vicennia marina* var. *australasica* during both pre-treatment and fertilisation years at two sites at Mangawhai Harbour. Symbols represent means (n = 5; 1 = site 1, 2 = site 2, U = upper intertidal zone, L = lower intertidal zone). Table insets show the results of site comparisons, followed by post-hoc procedure and multiplicity adjustment, where 'ns' indicates not significant, ** $P < 0.01$. Non-significant differences allowed the pooling of certain site temporal trends in a joint model fit. Lines represent GAMM smoothers.



Supplementary figure 5.2. Temporal variation in total phosphorus concentration (%, dry weight) in leaves of A*vicennia marina* var. *australasica* during both pre-treatment and fertilisation years at two sites at Mangawhai Harbour. Symbols represent means (n = 5; 1 = site 1, 2 = site 2, U = upper intertidal zone, L = lower intertidal zone). Table insets show the results of site comparisons, followed by post-hoc procedure and multiplicity adjustment, where 'ns' indicates not significant, ** $P < 0.01$. Non-significant differences allowed the pooling of certain site temporal trends in a joint model fit. Lines represent GAMM smoothers.



Supplementary figure 5.3. Temporal variation in the N:P (a) and in the Na:K (d) ratio in leaves of A*vicennia marina* var. *australasica* during both pre-treatment and treatment years at two sites at Mangawhai Harbour. Also shown are weekly precipitation (mm; g), and daily mean, minimum and maximum temperatures (°C; h); the black line indicates the daily mean temperature and the grey area delineates the daily minima and maxima. Relationship between N:P ratios and Temperature (°C; b), and Rain (mm; c); relationship between Na:K ratio and Temperature (°C; e), and Rain (mm; f). Data for rainfall and temperature were retrieved from the Mangawhai Heads Weather Station (http://www.mangawhaiweather.co.nz). Symbols represent means (n = 5; 1 = site 1, 2 = site 2, U = upper intertidal zone, L = lower intertidal zone). Table insets show the results of site comparisons, followed by post-hoc procedure and multiplicity adjustment, where 'ns' indicates not significant, ** $P < 0.01$. Non-significant differences allowed the

pooling of certain site temporal trends in a joint model fit. Dashed lines indicate N and P limitation (Güsewell, 2004). Lines represent GAMM smoothers. Grey areas around N:P ratio model fits represent 95% confidence intervals and around average temperature line represent the daily minimum and maximum values.

Chapter 6. General discussion, limitations, future work, and conclusion

The discussion in this chapter summaries some of the main findings and key points reported in the previous chapters, and gives an overview of the relevance of the research. For more details on research questions and specific discussions, the reader should refer to the individual discussions in Chapters 2, 3, 4 and 5 (Figure 6.1).

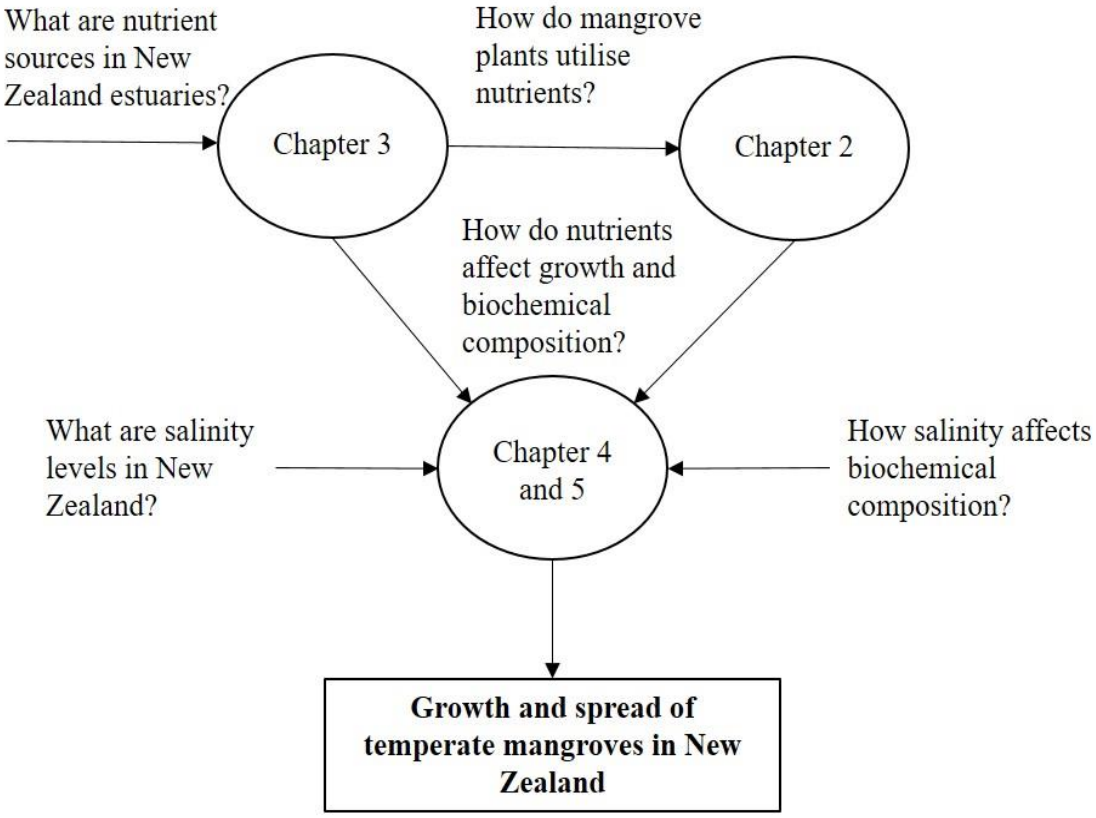


Figure 6.1. Flowchart of how content of thesis chapters contributes to the main research question.

“Things are not always what they seem; the first appearance deceives many; the intelligence of a few perceives what has been carefully hidden.”

— Phaedrus, philosopher.

Avicennia marina var *australasica* is a truly intricate mangrove plant. Firstly, it was described that this species unlike other mangroves does not grow well under freshwater conditions. Secondly, they are the only mangrove species that occur at the extreme southern limit of mangrove distribution (e.g., in New Zealand) and, hence, can tolerate occasional frosts. Thirdly, these plants have been spreading in many New Zealand estuaries in contrast to the worldwide mangrove distribution decline. This intricate nature of temperate *A. marina* has been acknowledged in two major reviews on New Zealand mangroves (Morrisey *et al.*, 2007; Morrisey *et al.*, 2010). Authors have also highlighted that there are some general knowledge gaps of what ecological, biological and environmental factors allow *A. marina* mangroves to occur in temperate New Zealand conditions. Such information would be valuable not only for understanding the temperate mangroves, but also will provide some generic insights into the biological and biochemical mechanisms that are involved in plasticity of mangrove species adaptations to extreme conditions.

6.1 Salinity levels and temperate mangrove growth

One of the most heavily debated question in the past was whether mangroves plants are obligatory or facultative halophytes (Wang *et al.*, 2011; Krauss & Ball, 2012). Despite arguments for the facultative nature of mangroves at that time were more sound, my study along with few recent findings provide evidence that the halophytic nature of mangrove species might be more complicated than it was previously considered. I think that some mangrove species can be facultative halophytes and exhibit the highest plant growth in freshwater conditions, while other mangrove species can be obligatory halophytes and presence of salt is a necessary condition for their growth.

There are several arguments to support the idea that *A. marina* species can indeed be an obligatory halophyte. For example, in several laboratory growth trials it was found that *A. marina* mangroves are amongst the few who grow poorly at 0% salinity compared to 25-50% salinity conditions (Downton, 1982; Clough, 1984; Yan *et al.*, 2007; Chapter 4 of the present dissertation). A recent study by Nguyen *et al.* (2014) revealed that salinity

conditions (25-50% seawater) benefits growth of *Avicennia marina*, especially in the formation of stem and leaf hydraulic systems, and, hence, water uptake. My study also provides some confirmation of this hypothesis, as I was able to demonstrate that stunted mangrove plants that grow inside the temperate mangrove stand have lower sodium concentration (Na) in leaves compared to the seaward mangroves. Na leaf concentrations also had seasonal fluctuations (high in summer and low in winter). Such a trend was observed in the mangrove sediment previously (Lovelock *et al.*, 2007b; Yang *et al.*, 2013), but the authors did not measure the Na in mangrove leaves. Collectively, the hypothesis of the obligatory salinity requirements for *A. marina* growth and my results provide early evidence that growth of mangrove *A. marina* might be negatively affected by low salinity in temperate New Zealand estuaries.

6.2 Effect of salinity on nutrient uptake

If *A. marina* species has some salinity requirements for optimum growth, there should be some biochemical processes, which can be involved in such unique salinity requirements. I hypothesised that nitrogenous metabolomic analysis may reveal some understanding of this phenomenon. I expected that level of nitrogen and primary nitrogen metabolites (as amino acids) may be lower in 0% salinity conditions, which can negatively affect growth. However, I found no significant difference in amino acid content of *A. marina* seedlings that grow at different salinity levels. Moreover, speed of nitrogen uptake had the opposite pattern, plants at 0% salinity conditions assimilated nitrogen faster than plants at 25% salinity, but for some reason this assimilated nitrogen was not invested into the biomass production. Indeed, mangrove seedlings at 25% salinity (which was described as optimum) produce significantly more above ground biomass in case of the high nutrient supply; even the speed of nitrogen uptake was lower. This result leads to the contradictory argument that *A. marina* nitrogen uptake is negatively affected by salinity, but the growth of mangrove plants improves at 25-50% salinity of seawater.

Additionally, the nitrogen uptake data acquired in my greenhouse study may partially explain the observation made by Downton (1982). Author observed that *A. marina* mangrove seedlings in fresh water grew faster initially than seedlings in 25% salinity, but after 2 months growth of 0% salinity seedlings slowed down, while plants at 25% salinity exhibited a steady growth. My nitrogen uptake data does not explain why 0% mangroves stop growing and become stunted, but my nitrogen uptake findings does indicate that this stunting may not be due to nutrient limitation, but may be related to some other, as yet

unknown physiological factor. Downton (1982) in his greenhouse study observed that stunted mangroves growing in fresh water were able to produce shoots and leaves, but that these new growths then died back due to necrosis. A similar observation was made by Woodroffe (1985a, 1985b) in stunted mangroves growing at Tuff Crater in New Zealand, where he observed that dieback occurred without apparent explanation. In both cases, it would appear that the plants obtained sufficient nutrients to produce growth, but that some unknown stress factor caused death of this newly created tissue. Reactive oxygen species (ROS) are generally regarded as a marker of stress in plants. However, the ROS levels in the present study did not indicate the presence of a salinity related stress effect. Nguyen *et al.* (2014) also noted that necrotic dieback occurred in mangroves grown initially in 50% seawater, then transferred to freshwater and observed changes in shoot hydraulic systems in the seedlings, suggesting that water transport was impaired. If such a process occurred in the present study, it did not have any apparent effect on nitrogen uptake, although one difference was that in the present study the seedlings were grown from the outset in fresh water.

6.3 Nutrients and temperate mangrove growth

Nutrient levels were demonstrated to have a generic positive effect on growth of *A. marina* mangroves in previous studies (as reviewed in Reef *et al.*, 2010), but this information was missing for temperate mangrove ecosystems. My study provides a comprehensive investigation of nutrient status of temperate mangroves, which includes description of nutrient sources in temperate estuaries, nutrient allocation patterns of *A. marina* in temperate New Zealand conditions, and speed and biochemical mechanisms of *A. marina* nutrient uptake. This information was missing in the literature as well. As I mentioned earlier, mangroves have a contradictory reputation in New Zealand. On the one hand, locals consider mangroves invasive and aggressive species that spread into the intertidal areas where they have never been found before. On another hand, environmentalists think that human activity have greatly changed environmental conditions (e.g., level of nutrients) in the estuarine settings in northern New Zealand and excessive growth of estuarine vegetation, including mangroves, is merely a reflection of that fact. Thus, it was hypothesised that nutrient load, originating from human activities can trigger mangrove growth and cause spread of mangroves in New Zealand estuaries (Lovelock *et al.*, 2007b), but this hypothesis is still lacking the experimental confirmation. Indeed, in New Zealand due to extensive agricultural activities and urban development, levels of nutrients have increased over the last 100 years (Hume & Dahm, 1992; Swales,

Hume, & Green, 1997). Mangrove stands are uniquely located between affected terrestrial ecosystems and aquatic and/or coastal waterways they can be some of the first recipients of this excessive nutrient load.

There are several types of anthropogenic nutrient sources, which can be present in New Zealand: agricultural runoffs from the fertilised croplands and animal and human sewage. Animal and human nitrogen discharge has a specific nitrogen stable isotope ratio ($\delta^{15}N$) signature, which can be used to identify the nitrogen source. This ratio originates from differences in the kinetics of chemical reactions of simultaneously occurring light (^{14}N) and heavy (^{15}N) isotopes. $\delta^{15}N$ is higher when abundance of nitrogen is present, so heavy isotopes are accumulated in the tissue of primary producers (e.g., algae, seagrass, and mangroves). Indeed, some earlier studies in tropical mangroves demonstrated that mangrove foliar composition can inherit these specific values derived from anthropogenic sewage discharge (Costanzo *et al.*, 2001). My study demonstrates that New Zealand mangroves that grow in close proximity to sewage discharge hotspots and in highly urbanised areas exhibit higher foliar $\delta^{15}N$ and absorb significantly more nitrogen, compared to less affected areas. I was able to extend these findings through analysis of mangrove herbarium samples. In this experiment, I demonstrated that mangrove leaf content is dynamic over time and changes when nutrient sources change, which can be a valuable tool for long-term environment monitoring programs. Incorporating monitoring of nitrogen concentration in mangrove leaves was suggested to improve water quality monitoring schemes for tracing the effect of wastewater discharge into Manukau Harbour, Auckland, New Zealand in Vopel, Gritcan, & Laverock (2017).

Nutrient allocation patterns of temperate *A. marina* were found to be similar to those of their tropical counterparts (Chapter 2). My study shows that temperate mangroves allocate significant amounts of nutrients below ground (between 60 and 70% of total carbon, total nitrogen and total phosphorus). These conclusions are supported by another study in temperate New Zealand mangrove ecosystems, whose authors argue that temperate mangrove plants are able to trap a significant amount of carbon and nutrients (namely nitrogen) and store them below ground (Bulmer *et al.*, 2016). An interesting conclusion originates from the findings that the presence of mangrove stands in estuarine and coastal settings in New Zealand may act as a nutrient sink and sequester human-derived nutrient loads.

This study also highlights the fact that temperate mangroves provide the important environmental services, such as sequestering carbon from the atmosphere (namely CO_2) more efficiently than some terrestrial ecosystems (Bulmer et al., 2016). Additionally, another recent study of Reef et al., (2016) demonstrated that growth of mangrove seedlings under elevated CO_2 conditions had a synergic effect on plant growth, when complimented with nutrient addition. Taking into consideration these studies and the present findings that temperate mangroves are nitrogen limited and take additional nitrogen quickly, the ongoing presence of mangrove ecosystems in New Zealand will be even more beneficial in the future. Global trends of rising level of CO_2 in the atmosphere will allow New Zealand mangroves to incorporate nutrient loads faster and in larger quantities. Thus, it is likely that temperate mangroves will become even more efficient N sinks. Eventually, with rising temperatures and CO_2 concentrations it can be expected that the relative contribution of carbon stocks and nitrogen assimilation efficiency in temperate mangrove ecosystems will increase at the global scale.

Several findings of the present research were relevant for mangrove plants in general and not specifically to the temperate mangroves. Firstly, I demonstrated that mangrove seedlings accumulate nitrogen within hours. This information demonstrates that mangrove plants indeed are very efficient in sequestering nitrogen from the environment. Secondly, my field fertilisation experiment demonstrated that mangrove plants can invest extra nutrients into the growth of shoots and new leaf gain. Indeed, the nitrogen that was added in the fertilisation treatment got transferred into the leaves, which was reflected in the nutrient status. Lastly, I also conducted the long-term observational study of temperate mangrove leaf nutrient status, which provides an understanding of the nutrient cycling in the temperate estuaries in comparison to tropical conditions. I found that significant seasonal fluctuation of nutrients in mangrove leaves (namely phosphorus) are likely linked to the fluctuations of nutrients in the environment. In comparison to studies in tropical ecosystems where no seasonal changes in mangrove growth and nutrient composition of mangrove sediment were observed (Boto & Wellington, 1984,1988; Chen & Twilley, 1999; Krauss *et al.*, 2006), my study provides evidence that growth of mangroves is mobile and under temperate conditions, it depends on the nutrient availability in the environment.

6.4 Biochemical utilisation of nutrients by mangrove plants

In my study, I found that *A. marina* mangrove plants, similarly to glycophytes accumulate more amino acids under nitrogen excess conditions. The laboratory growth trial demonstrated that the main amino acid in the roots under high nutrient conditions was asparagine (Asn), similarly to what Ashihara *et al.* (1997) found in an earlier study. However, the results of my study clearly indicate that asparagine was acting in a nitrogen storage and transport capacity, rather than as an osmolyte, as suggested by Ashihara *et al.* (1997). Indeed, it was described that the common function of asparagine in roots is to transport nitrogen from roots to leaves and/or plants also can accumulate asparagine as a nitrogen storage (Pfautsch *et al.*, 2009; Planchet *et al.*, 2011). Hence, it can be further hypothesised that this amino acid may play a role of nitrogen storage in roots, and that mangrove plants can accumulate asparagine when nitrogen is abundant, and use it when nitrogen levels are scarce. In contrast to roots, the most common amino acid in leaves was glutamic acid together with glutamine, which differs from the results of Ashihara *et al.* (1997). Another surprising result is that the initial concentration of Glu and Gln was not affected by nutrient or salinity conditions (nutrient-limited plants had the same quantity as plants under high nutrient supply). This phenomenon can be explained by the fact that in glycophyte plants, these amino acids were found to be essential sources of nitrogen, as all other amino acids can be synthesised from them. The importance of glutamic acid and glutamine for growth in mangroves can be further confirmed by the fact that when nitrogen deficient plants were provided with extra nitrogen, levels of these amino acids increased in leaves. My study, therefore, was one of the first attempts to characterise the nitrogenous metabolomics in mangrove plants, and it seems feasible to conclude that glutamic acid and glutamine are essential amino acids for *A. marina* seedling growth biochemical processes in leaves. Asparagine is important for storage and transport of nitrogen in roots, but is probably not functioning as an osmolyte, since its level was not affected by the salinity treatment.

The current study also found that availability of nitrogen not only affects the accumulation of metabolites involved in growth processes, but it also increases levels of osmolytes. Osmolytes are necessary for mangrove plant survival under salinity stress. Experimental results further confirm that the main osmolyte for *A. marina* mangrove plants is glycine betaine rather than proline, as was reviewed in Parida & Jha, 2010. The role of this osmolyte, though, may also be the reason why *A. marina* species occur in temperate New Zealand conditions, but not other mangrove species. For example, in terrestrial plants one

of the strategies that allow plants to be frost tolerant is to avoid freezing. One way (the least effective, though) to do this is to accumulate solutes. For example, alpine plants accumulate sugars and quaternary ammonium compounds, such as glycine betaine, in response to decreasing temperatures (Körner, 2003). Indeed, it was shown that transformed *Arabidopsis thaliana* plants, which accumulate high levels of enzyme that converts choline to glycine betaine, have enhanced their low temperature tolerance (Alia *et al.*, 1998). Since some mangroves species (e.g., *A. marina*) accumulate the same compounds to tolerate salinity, simultaneously, they become more frost resistant. Solute accumulation, however, is the least effective strategy, which does not allow mangroves to be fully frost tolerant.

6.5 Limitations and future work

The main limitations of the present study were the omissions of nutrient and salinity measurements in mangrove sediments and the sole focus on foliar N, P, and Na concentrations. Indeed in many cases sediment measurements would have strengthen the conclusions. For example, in Chapter 2 sediment N% and P% measurements could have revealed information on the source of nutrients and provide more insights into mangrove sediment belowground C, N and P storage. Also, it is possible that in Chapter 5 Na:K ratios in leaves cannot be called salinity measurements because conventional salinity is measured in mangrove sediment porewater.

However, there are several reasons why sediment measurements can be replaced with leaf elemental characterisation. From the early 1990's the correlation between nutrient concertation in soil and plant leaves has been revealed (Aerts & Chapin III, 1999) and later widely accepted (Güsewell, 2004). Such correlation was also demonstrated for mangrove plants and scientists have pointed out that, for example, foliar N:P ratio can be used as a measure to assess nutrient availability in the surrounding sediment (Reef et al., 2010; Alongi, 2011).

Another reason to favour leaf characterisation (if time and resources do not allow to measure both) originates from recent research. Scientists have demonstrated that mangrove root biomass in the field conditions is often higher than 60% of the total plant biomass (e.g., Phan et al., 2016) and mangrove roots can sprawl from the tree for 5-10 meters (personal observation). This information poses a difficulty of where exactly mangrove sediment should be sampled in order to be representative of the nutrient or

salinity levels. To complicate this question even further, especially for salinity measurements, recently scientists have demonstrated that mangrove plants can actively uptake fresh water over saline water if there are various water sources available (Lovelock et al., 2016; Reef et al., 2015). Thus, leaf sodium concentration represents the average information on the freshwater *vs* saline water sources available for plant uptake.

After analysing this available literature, I concluded that sediment nutrient measurements and porewater salinity is less informative than leaf N, P, and Na characterisation for my research aims. Leaf element concentration provides a better understanding of how mangrove plants experience environmental nutrient and salinity levels. This information is more valuable for understanding the effect of nutrient availability and salinity levels on mangrove growth rather than absolute nutrient and salinity values in the sediment.

One of the major limitations is that measurements of nitrogen uptake (it was faster at 0% salinity, compared to the 25% salinity conditions) contradicts the growth results (mangroves grow better in the moderate salinity conditions). Although my nitrogenous metabolomics analysis could not provide any explanations for the observed trend, I can suggest how to improve the future work design to solve these contradictions. I suggest performing an untargeted metabolomics and proteomic analyses and including higher salinity treatments. Untargeted metabolomics, genomics, and proteomics can provide some insights into cycles of amines, proteins, and carbohydrates. Carbohydrates and amines were shown to be involved in salinity tolerance of mangrove plants, and may help plants grow better in the unfavourable conditions. Genomics and proteomics may reveal specific enzymes and genes responsible for the improved growth at presence of moderate salinity. Initially, I also planned to have three salinity levels (0%, 25%, and 75% seawater salinity), because it was described in the literature, that salinity over 75% seawater severely affects growth of *A. marina* species and I wanted to investigate whether the speed of nitrogen uptake under these conditions as well. The first attempt to perform a greenhouse study, where I included three salinity levels failed. Sixty percent of seedlings in the high salinity group (75% seawater strength) died out and I ended up not having enough replicates for statistical analysis. Thus, I decided to focus my study on growth of mangrove seedlings at low (0% seawater) and optimum (25%) salinity levels as for temperate mangroves these high salinity levels might not be as common as in the tropics, and I decided to have higher replication to increase the statistical power. For more advanced understanding on how nutrients and salinity affect mangrove metabolomics, I

also suggest incorporating *Avicennia marina* species from different climatic areas in a single experimental setup, as it has been found that the maternal origin of seedlings affects adaptation to specific salinity levels (Alam *et al.*, 2018). Such a design can help identify how mangrove plants can change their physiological and biochemical traits in response to environmental variations in nutrients and salinity.

Another limitation is that my study did not provide enough scientific data to support my hypothesis that growth of mangrove plants is affected by lower than tropical salinity levels in field conditions. I think the reason for this is that mangroves at my study site were still affected by the seawater input. Fortunately, in New Zealand there are some locations where mangrove plants exhibit very stunted growth in the interior stand areas. One particular location of interest is called Tuff Crater, Auckland, New Zealand. Mangroves at this location were previously characterised in several papers (Woodroffe, 1985a, 1985b). The authors characterised mangrove plants as approximately 30-40 cm in height, and they still are the same height at present (personal observation). A study at this site might provide further information on what causes stunted growth in temperate mangrove ecosystems. I think that low salinity levels might be the major contributor to the mangrove growth restriction at this site, as the tidal activity is restrained by the natural and artificial obstructions (e.g., crater shape with a single seawater channel and construction of the motorway).

6.6 Conclusion

The main aim of this study was to fill some of the gaps in the scientific knowledge of how nutrient availability and salinity affects the growth and spread of New Zealand temperate mangroves. Scientific explanations of this phenomenon are critically needed in the on-going controversial public discussions regarding management *versus* conservation of New Zealand mangroves. Overall, my results suggest that a unique combination of factors have resulted in increased growth and spread of temperate mangroves in estuarine and coastal areas in northern New Zealand. First, is the cooler and wetter New Zealand climate, which, due to high precipitation rate and low evapotranspiration, results in lower salinity levels, optimal for *A. marina* species. Another factor is the natural nutrient deficiency state of temperate mangrove ecosystems, which allows mangrove plants to absorb additional nutrients. One more factor is anthropogenic influence, mainly the increasing nutrient input over the past 100 years, originating from fertilisation, livestock urine runoff from dairy and meat farming, and human sewage inputs. The present study

also demonstrates that the presence of mangrove plants at the interface between the land surface and underground water runoffs and coastal ecosystems may act as buffers, since mangroves may as actively sequester nutrients, thus mitigating coastal and marine eutrophication.

Chapter 7. Reference

Aerts, R., & Chapin III, F. S. (1999). The Mineral Nutrition of Wild Plants Revisited: A Re-evaluation of Processes and Patterns. *Advances in Ecological Research, 30*, 1-67. doi:10.1016/S0065-2504(08)60016-1

Ahmad, P., & Prasad, M. N. V. (2012). *Environmental Adaptations and Stress Tolerance in Plants in the Era of Climate Change.* NewYork, NY: Springer Science Business Media, LLC. doi:10.1007/978-1-4614-0815-4

Alam, M. R., Mahmood, H., & Rahman, M. M. (2018). Maternal origins induced plasticity in salt adaptability of *Avicennia officinalis L*. seedlings in the Sundarbans of Bangladesh. *Hydrobiologia, 820*(1), 227-244. doi:10.1007/s10750-018-3659-z

Alfaro, A. C. (2010). Effects of mangrove removal on benthic communities and sediment characteristics at Mangawhai Harbour, northern New Zealand. *ICES Journal of Marine Science, 67*, 1087–1104. doi:10.1093/icesjms/fsq034

Alfaro, A. C., Thomas, F., Sergent, L., & Duxbury, M. (2006). Identification of trophic interactions within an estuarine food web (northern New Zealand) using fatty acid biomarkers and stable isotopes. *Estuarine, Coastal and Shelf Science, 70*, 271-286.

Alia, Hayashi, H., Chen, T. H. H., & Murata, N. (1998). Transformation with a gene for choline oxidase enhances the cold tolerance of *Arabidopsis* during germination and early growth. *Plant, Cell and Environment, 21*, 232-239.

Alongi, D.M. (1998). *Coastal ecosystem processes.* Boca Raton, FL: CRC Press.

Alongi, D. (2002). Present state and future of the world's mangrove forests. *Environmental Conservation, 29*(3), 331-349.

Alongi, D. M. (2009). *The energetics of mangrove forests*. Townsville, Australia: Springer Science + Business Media B.V.

Alongi, D. M. (2011). Early growth responses of mangroves to different rates of nitrogen and phosphorus supply. *Journal of Experimental Marine Biology and Ecology, 397*(2), 85-93. doi:10.1016/j.jembe.2010.11.021

Alongi, D.M., Wattayakorn, G., Ayukai, T., Clough, B.F., Wolanski, E., & Brunskill, G.J. (2000). An organic carbon budget for mangrove-fringed Sawi Bay, southern Thailand. *Phuket Mar Biol Cent Spec Publ, 22*, 79–85

Alongi, D.M., Clough, B.F., Dixon, P., Tirendi, F. (2003). Nutrient partitioning and storage in arid-zone forests of the mangroves *Rhizophora stylosa* and *Avicennia marina*. *Trees, 17*, 51-60. doi:10.1007/s00468-002-0206-2

Alongi, D.M., Sasekumar, A., Chong, V.C., Pfitzner, J., Trott, L.A., Tirendi, F., Dixon, P., & Brunskill, G.J. (2004). Sediment accumulation and organic material flux in a managed mangrove ecosystem: Estimates of land-ocean-atmosphere exchange in peninsular Malaysia. *Mar Geol, 208*, 383–402

Ansari, A. A., & Gill, S. S. (2014). *Eutrophication, Causes, Consequences and Control*. Townsville, QLD: Springer Science + Business Media

Asada, K. (2006). Production and scavenging of reactive oxygen species in chloroplasts and their functions. *Plant Physiology, 141*, 391–396.

Asada, K., & Takahashi, M. (1987). Production and scavenging of active oxygen in chloroplasts. In *Photoinhibition*. Elsevier, Amsterdam.

Ashihara, H., Adachi, K., Otawa, M., Yasumoto, E., Fukushima, Y., Kato, M., . . . Baba, S. (1997). Compatible Solutes and Inorganic Ions in the Mangrove Plant *Avicennia marina* and Their Effects on the Activities of Enzymes. *Z. Naturforsch., 52*(c).

Ashihara, H., Wakahara, S., Suzuki, M., Kato, A., Sasamoto, H., & Baba, S. (2003). Comparison of adenosine metabolism in leaves of several mangrove plants and a

poplar species. *Plant Physiology and Biochemistry, 41*(2), 133-139. doi:10.1016/s0981-9428(02)00019-0

Ayukai, T., Miller, D., Wolanski, E., Spagnol, S. (1998). Fluxes of nutrients and dissolved and particulate organic carbon in two mangrove creeks in northeastern Australia. *Mangr Salt Marsh, 2*, 223–230

Aziz, I., & Khan, M.A. (2001). Experimental Assessment of Salinity Tolerance of *Ceriops tagal* Seedlings and Saplings from the Indus Delta, Pakistan. *Aquatic Botany, 70*, 259-268.

Baker, A., & Graham, I. (2002). *Plant peroxisomes: biochemistry, cell biology and biotechnological applications*. Kluwer Academic Publishers, Dordrecht, The Netherlands.

Ball, M.C. (1988). Salinity tolerance in the mangroves, *Aegiceras corniculatum* and *Avicennia marina*. I. Water use in relation to growth, carbon partitioning and salt balance. *Austr J. Plant Physiol, 15*, 447-464.

Ball, M.C. (1996). Comparative ecophysiology of mangrove forest and tropical lowland moist rainforest. In *Tropical forest plant ecophysiology*. New York: Chapman and Hall.

Ball, M. C. (2002). Interactive effects of salinity and irradiance on growth: implications for mangrove forest structure along salinity gradients. *Trees, 16(2-3),* 126-139. doi:10.1007/s00468-002-0169-3

Ball, M.C., & Farquhar, G.D. (1984). Photosynthetic and stomatal responses of two mangrove species, *Aegiceras corniculatum* and *Avicennia marina*, to long-term salinity and humidity conditions. *Plant Physiol, 74*, 1-6.

Ball, M. C., Chow, W. S., & Anderson, J. M. (1987). Salinity-induced potassium deficiency causes loss of functionl photosystem II in leaves of the grey mangrove, *Avicennia marina*, through depletion of the atrazine-binding polypeptide. *Australian Journal of Plant Physiology, 14*, 351-361.

Ball, M.C., Cochrane, M.J., & Rawson, H.M. (1997). Growth and water use of the mangroves *Rhizophora apiculata* and *R. stylosa* in response to salinity and humidity under ambient and elevated concentrations of atmospheric CO_2. *Plant Cell Environ, 20*, 1158–1166.

Ball, M. C., & Pidsley, S. M. (1995). Growth Responses to Salinity in Relation to Distribution of Two Mangrove Species, *Sonneratia alba* and *S. lanceolata*, in Northern Australia. *Functional Ecology, 9*(1), 77-85.

Barrett, G. C., & Elmore, D. T. (1998). *Amino acids and peptides*. Cambridge, UK: Cambridge University Press.

Bateman, A. S., & Kelly, S. D. (2007). Fertilizer nitrogen isotope signatures. *Isotopes Environmental Health Studies, 43(3)*, 237-247. doi:10.1080/10256010701550732

Beard, C. (2006). Physiological constraints on the latitudinal distribution of the mangrove *Avicennia marina* (Forsk.) Vierh. subsp. *australasica* (Walp.) J. *Everett in New Zealand*. University of Waikato, New Zealand.

Benjamini, Y., & Hochberg, Y. (1995). Controlling the false discovery rate: a practical and powerful approach to multiple testing. *Journal of the Royal Statistical Society, 57(1)*, 289-300.

Bianchi, T. S. (2007). *Biogeochemistry of estuaries*. Department of Oceanography, Texas A&M University: Oxford university press.

Bird, E.C.F. (1986). Mangroves and intertidal morphology in Westernport Bay, Victoria, Australia. *Marine Geology, 69*, 251–271.

Bose, J., Rodrigo-Moreno, A., & Shabala, S. (2013). ROS homeostasis in halophytes in the context of salinity stress tolerance. *J Exp Bot, 65*(5), 1241-1257. doi:10.1093/jxb/ert430

Boto, K., Saffigna, P., & Cloughl, B. (1985). Role of nitrate in nitrogen nutrition of the mangrove *Avicennia marina*. *21*, 259-265.

Boto, K., & Wellington, J. T. (1983). Phosphorus and nitrogen nutritional status of a northern Australian mangrove forest. *Marine Ecology - Progress series, 11*, 63-69.

Boto, K., & Wellington, J. T. (1984). Soil characteristics and nutrient status in a northern australian mangrove forest. *Estuaries, 7(1)*, 61-69.

Boto, K., Saffigna, P., & Cloughl, B. (1985). Role of nitrate in nitrogen nutrition of the mangrove *Avicennia marina*. *Marine ecology – progress series, 21*, 259-265.

Bouillon, S., Borges, A. V., Castaneda-Moya, E., Diele, K., Dittmar, T., Duke, N. C., ... Twilley, R. R. (2008). Mangrove production and carbon sinks: A revision of global budget estimates. *Global Biogeochem. Cycles, 22*, GB2013. doi:10.1029/2007GB003052

Bourguignon, J., Neuburger, M., & Douce, R. (1988). Resolution and characterisation of the glycine-cleavage reaction in pea leaf mitochondria. Properties of the forward reaction catalysed by glycine decarboxylase and serine hydroxymethyltransferase. *Biochem. J., 255*, 169-178.

Bowden, R. D., Castro, M. S., J.M., M., Steudler, P. A., & Aber, J. D. (1993). Fluxes of greenhouse gases between soils and the atmosphere in a temperate forest following a simulated hurricane blowdown. *Biogeochemistry, 21(2)*, 61-71.

Brosche, M., Vinocur, B., Alatalo, E. R., Lamminmaki, A., Teichmann, T., Ottow, E. A., . . . Kangasjarvi, J. (2005). Gene expression and metabolite profiling of *Populus euphratica* growing in the Negev desert. *Genome Biol., 6*, 17.

Bulmer, R.H., Schwendenmann, L., & Lundquist, C.J. (2016). Carbon and nitrogen stocks and below-ground allometry in temperate mangroves. *Front Mar Sci, 3*, 1-11. doi:10.3389/fmars.2016.00150

Burchett, M. D., Clarke, C. J., Field, C., D., & Pulkownik, A. (1989). Growth and respirtaion in two mangrove species at a range of salinities. *Physiologia Plantarum, 75*, 299-303.

Burford, M.A., Alongi, D.M., McKinnon, A.D., & Trott, L.A. (2008). Primary production and nutrients in a tropical macrotidal estuary, Darwin Harbour, Australia. *Est Coast Shelf Sci, 79*,440–448

Burnham, K. P., & Anderson, D. R. (2016). Multimodel Inference. *Sociological Methods & Research, 33(2)*, 261-304. doi:10.1177/0049124104268644

Campbell, M. K., & Farrell, S. O. (2012). *Biochemistry* (7th ed.). Belmont, California: Brooks/Cole Cengage Learning.

Canalejo, A., Martínez-Domínguez, D., Córdoba, F., & Torronteras, R. (2014). Salt tolerance is related to a specific antioxidant response in the halophyte cordgrass, Spartina densiflora. *Estuarine, Coastal and Shelf Science, 146*, 68-75. doi:10.1016/j.ecss.2014.05.017

Cavanaugh, K. C., Kellner, J. R., Forde, A. J., Gruner, D. S., Parker, J. D., Rodriguez, W., & Feller, I. C. (2014). Poleward expansion of mangroves is a threshold response to decreased frequency of extreme cold events. *Proc Natl Acad Sci U S A, 111*(2), 723-727. doi:10.1073/pnas.1315800111

Chapin, F.S. III (1980). The mineral nutrition of wild plants. *Annu. Rev. Ecol. Syst., 11*, 233-260.

Chapman, V. J. (1940). The functions of the pneumatophores of *Avicennia nitida Jacq*. *Proceedings of the Limnology Society of London, 152*, 228-233.

Chao, D. Y., Gable, K., Chen, M., Baxter, I., Dietrich, C. R., Cahoon, E. B., . . . Salt, D. E. (2011). Sphingolipids in the root play an important role in regulating the leaf ionome in *Arabidopsis thaliana. Plant Cell, 23*(3), 1061-1081. doi:10.1105/tpc.110.079095

Chen, R., & Twilley, R. R. (1999). A simulation model of organic matter and nutrient accumulation in mangrove wetland soils. *Biogeochemistry, 44*, 93-118.
Chen, Y., & Ye, Y. (2014). Effects of Salinity and Nutrient Addition on Mangrove *Excoecaria agallocha. PLoS One, 9(4)*, 1-11. doi:10.1371/
Clough, B. F. (1982). *Mangrove ecosystems in Australia*. Canberra, Australia, London, England and Miami, Fla., USA: ANU Press.
Clough, B. F. (1984). Growth and salt balance of the mangroves *Avicennia marina* (Forsk.) Vierh. and *Rhizophora stylosa Griff.* in relation to salinity. *Australian Journal of Plant Physiology, 11*, 419–430.
Coldren, G. A., & Proffitt, C. E. (2017). Mangrove seedling freeze tolerance depends on salt marsh presence, species, salinity, and age. *Hydrobiologia, 803*(1), 159-171. doi:10.1007/s10750-017-3175-6
Collakova, E., Goyer, A., Naponelli, V., Krassovskaya, I., Gregory, J. F., 3rd, Hanson, A. D., & Shachar-Hill, Y. (2008). Arabidopsis 10-formyl tetrahydrofolate deformylases are essential for photorespiration. *Plant Cell, 20*(7), 1818-1832. doi:10.1105/tpc.108.058701
Conley, D. J. (2000). Biogeochemical nutrient cycles and nutrient management strategies. *Hydrobilogia, 410*, 87-96.
Cooper, B. A., & Thomsen, C. E. (1983). Nitrogen and phosphorus in streamwaters from adjacent pasture, pine, and native forest catchments. *New Zealand journal of marine and freshwater research, 22*(2), 279-291. doi:10.1080/00288330.1988.9516300
Cooper, R., & Nicola, G. (2015). Natural Products Chemistry: Sources, Separations, and Structures. NW, USA: Taylor & Francis Group.
Costanzo, S. D., O'Donohue, M. J., Dennison, W. C., Loneragan, N. R., & Thomas, M. (2001). A new approach for detecting and mapping sewage impacts. *Marine pollution bulletin, 42*(2), 149-156.
Costanzo, S. D., O'Donohue, M. J., & W. C. Dennison, W. C. (2004). Assessing the influence and distribution of shrimp pond effluent in a tidal mangrove creek in north-east Australia. *Mar Pollut Bull, 48*(5-6), 514-525. doi:10.1016/j.marpolbul.2003.09.006
Dasgupta, N., Chowdhury, P., & Das, S. (2015). Comparative Adaptability Assessment of Two Mangroves from Indian Sundarbans: Some Biochemical Appearances. *Natural Science, 07*(12), 519-534. doi:10.4236/ns.2015.712053
Datta, P. N., & Ghosh, M. (2003). Estimation of osmotic potential and free amino acids in some mangroves of the Sundarbans, India. *Acta Bot Croast, 62*, 37-45.
Davidson, E. A., & Swank, W. T. (1986). Environmental parameters regulating gaseous nitrogen losses from two lorested ecosystems via nitrification and denitrification. *Applied and Environmental Microbiology, 52(6)*, 1287-1292.
Day, J. W. J., Crump, B. C., Kemp, W. M., & Yanez-Arancibia, A. (2013). *Estuarine ecology* (2nd ed.). Hoboken, New Jersey: John Wiley & Sons, Inc.
Dersch, L. M., Beckers, V., Rasch, D., Melzer, G., Bolten, C., Kiep, K., . . . Wittmann, C. (2016). Novel Approach for High-Throughput Metabolic Screening of Whole Plants by Stable Isotopes. *Plant Physiol, 171*(1), 25-41. doi:10.1104/pp.15.01217
Di Nitto, D., Neukermans, G., Koedam, N., Defever, H., Pattyn, F., Kairo, J. G., & Dahdouh-Guebas, F. (2014). Mangroves facing climate change: landward migration potential in response to projected scenarios of sea level rise. *Biogeosciences, 11*(3), 857-871. doi:10.5194/bg-11-857-2014
Dittmar, T., Lara, R.J., & Kattner, G. (2001). River or mangrove? Tracing major organic matter sources in tropical Brazilian coastal waters. *Mar Chem, 73*, 253–271.

D'Mello, J. P. F. (2015). *Amino acids in higher plants*. Croydon, UK: CAB International.

Doddema, H., Eddin, R. S., & Mahasneh, A. (1986). Effects of seasonal changes of soil salinity and soil nitrogen on the N-metabolism of the halophyte Arthrocnemum fruticosum (L.) Moq. *Plant and Soil, 92*(2), 279-293.

Downton, W. J. S. (1982). Growth and osmotic relations of the mangrove *Avicennia marina*, as influenced by salinity. *Australian Journal of Plant Physiology, 9*, 519-528.

Duarte, B., Sleimi, N., & Cacador, I. (2014). Biophysical and biochemical constraints imposed by salt stress: learning from halophytes. *Frontiers in Plant Science, 5*, 746. doi:10.3389/fpls.2014.00746

Duarte, C. M., -Hansen, O. G., Thampanya, U., Terrados, J., Fortes, M. D., Kamp-Nielsen, L., . . . Boromthanarath, S. (1998). Relationship between sediment conditions and mangrove *Rhizophora apiculata* seedling growth and nutrient status. *Marine Ecology Progress Series, 175*, 277-283.

Duff, S. M. G. (2015). Asparagine synthetase. In J. P. F. D'Mello (Ed.), *Amino acids in higher plants*. Oxford, UK: CAB International.

Duke, N. (1990). Phenological trends with latitude in the mangrove tree *Avicennia marina. Journal of Ecology, 78*, 113-133.

Duke, N., Ball, M. C., & Ellison, J. C. (1998). Factors influencing biodiversity and distributional gradientsin mangroves. *Global Ecology and Biogeography Letters, 7*, 27-47.

Ellsworth, David S., Anderson, Ian C., Crous, Kristine Y., Cooke, J., Drake, John E., Gherlenda, Andrew N., . . . Reich, Peter B. (2017). Elevated CO_2 does not increase eucalypt forest productivity on a low-phosphorus soil. *Nature Climate Change, 7*(4), 279-282. doi:10.1038/nclimate3235

Farnsworth, E. J., Ellison, A. M., & Gong, W. K. (1996). Elevated CO2 alters anatomy, physiology, growth, and reproduction of red mangrove (*Rhizophora mangle* L.). *Oecologia, 108*(4), 599-609.

Fatichi, S., Leuzinger, S., & Körner, C. (2014). Moving beyond photosynthesis: from carbon source to sink-driven vegetation modelling. *New Phytologist, 201*, 1086 - 1095.

Feller, I. C. (1995). Effects of nutrient enrichment on growth and herbivory of dwarf red mangrove (*Rhizophora Mangle*). *Ecological Monographs, 65*(4), 477-505.

Feller, I. C., Lovelock, C. E., & McKee, K. L. (2007). Nutrient Addition Differentially Affects Ecological Processes of *Avicennia germinans* in Nitrogen versus Phosphorus Limited Mangrove Ecosystems. *Ecosystems, 10(3),* 347-359. doi:10.1007/s10021-007-9025-z

Feller, I. C., Lovelock, C. E., & Piou, C. (2009). Growth and Nutrient Conservation in *Rhizophora mangle* in Response to Fertilization along Latitudinal and Tidal Gradients. *Smithsonian contributions to the marine sciences, 38,* 345-358.

Feller, I. C., McKee, K. L., Whigham, D. F., & John, O. N. (2002). Nitrogen vs. phosphorus limitation across an ecotonal gradient in a mangrove forest. *Biogeochemistry, 62,* 145-175.

Feller, I. C., Whigham, D. F., McKee, K. L., & Lovelock, C. E. (2003). Nitrogen limitation of growth and nutrient dynamics in a disturbed mangrove forest, Indian River Lagoon, Florida. *Oecologia, 134*, 405-414. doi:10.1007/s00442-002-1117-z

Fischer, W.-N., André, B., Rentsch, D., Krolkiewicz, S., Tegeder, M., Breitkreuz, B., & Frommer, W. B. (1998). Amino acid transport in plants. *Trends in plant science, 3*, 188-195.

Fitzmaurice, J. R. (2009). *History of Auckland Wastewater and Mangere Wastewater Treatment Plant.* presented at the meeting of the 3rd Australasian Engineering Heritage Conference., Australia.

Flowers, T. J. (2004). Improving crop salt tolerance. *J. Exp.Bot., 55*, 307-319. doi:10.1093/jxb/erh00

Flowers, T. J., & Colmer, T. D. (2008). Salinity tolerance in halophytes. *New Phytologist, 179(4)*, 945-963. doi:10.1111/j.1469-8137.2008.02531.x

Flowers, T. J., & Lauchli, A. (1983). Sodium versus potassium: substitution and compartmentation. *Inorganic plant nutrition*, 651-681.

Flowers, T., Troke, P. F., & Yeo, A. R. (1977). The mechanism of salt tolerance in halophytes. *Annual Review of plant physiology, 28*, 89-121.

Forde, B. G., & Lea, P. J. (2007). Glutamate in plants: metabolism, regulation, and signalling. *Journal of experimental Botany, 58*, 2339-2358.

Foyer, C. H., Noctor, G., Lelandais, M., Lescure, J. C., Valadier, M. H., Boutin, J. P., & Horton, P. (1994). Short-term effects of nitrate, nitrite and ammonium assimilation on photosynthesis, carbon partitioning and protein phosphorylation in maize. *Planta, 192*, 211-220.

Foyer, H. C., & Noctor, G. (2005). Oxidant and antioxidant signalling in plants: a re-evaluation of the concept of oxidative stress in a physiological context. *Plant, Cell and Environment, 28*, 1056-1071.

Fry, B. (2006). *Stable Isotope Ecology*. New York, NY 10013, USA: Springer Science+Business Media,LLC. 40-75

Fry, B., Bern, A. L., Ross, M. S., & Meeder, J. F. (2000). ^{15}N studies of nitrogen use by the red mangrove, *Rhizophora mangle L*. in South Florida. *Estuarine, Coastal and Shelf Science, 50*, 291-296. doi:10.1006/ecss.1999.0558

Fry, B., Gace, A., & McClelland, J. W. (2003). Chemical Indicators of Anthropogenic Nitrogen-Loading in Four Pacific Estuaries. *Pacific Science, 57*(1), 77-101. doi:10.1353/psc.2003.0004

Fu, X., Huang, Y., Deng, S., Zhou, R., Yang, G., Ni, X., . . . Shi, S. (2005). Construction of a SSH library of *Aegiceras corniculatum* under salt stress and expression analysis of four transcripts. *Plant Science, 169*(1), 147-154. doi:10.1016/j.plantsci.2005.03.009

Gil, R., Boscaiu, M., Lull, C., Bautista, I., Lidon, A., & Vicente, O. (2013). Are soluble carbohydrates ecologically relevant for salt tolerance in halophytes? *Functional Plant Biology, 40*, 805–818. doi:10.1071/fp12359

Gill, A. M., & Tomlinson, P. B. (1975). Studies on the growth of red mangrove (*Rhizophora mangle L.*), 4. The adult root system. *Biotropica, 9*, 145-155.

Gill, A. L., & Finzi, A. C. (2016). Belowground carbon flux links biogeochemical cycles and resource-use efficiency at the global scale. *Ecol Lett, 19*(12), 1419-1428. doi:10.1111/ele.12690

Giri, C., Ochieng, E., Tieszen, L. L., Zhu, Z., Singh, A., Loveland, T., . . . Duke, N. (2011). Status and distribution of mangrove forests of the world using earth observation satellite data. *Global Ecology and Biogeography, 20*, 154-159. doi:10.1111/j.1466-8238.2010.00584.x

Godoy, M. D., & de Lacerda, L. D. (2015). Mangroves Response to Climate Change: A Review of Recent Findings on Mangrove Extension and Distribution. *An Acad Bras Cienc, 87*(2), 651-667. doi:10.1590/0001-3765201520150055

Gong, W.K., & Ong, J.E. (1990). Plant biomass and nutrient flux in a managed mangrove forest in Malaysia. *Est Coast Shelf Sci, 31*, 516–530

Green, M., Ellis, J., Schwarz, A. M., Green, N., Lind, D., & Bluck, B. (2003). For and against mangrove control. *NIWA Information Series N31*.

Grieve, C. M., & Grattan, S. R. (1983). Rapid assay for determination of water soluble quaternary ammonium compounds. *Plant and Soil, 70*, 303-307.
Gritcan, I., Duxbury, M., Leuzinger, S., & Alfaro, A. C. (2016). Leaf Stable Isotope and Nutrient Status of Temperate Mangroves As Ecological Indicators to Assess Anthropogenic Activity and Recovery from Eutrophication. *Frontiers in Plant Science, 7*, 1922. doi:10.3389/fpls.2016.01922
Gruber, N. (2008). The Marine Nitrogen Cycle: Overview and Challenges. In *Nitrogen in the Marine Environment*: Elsevier Inc.
Gupta, B., & Huang, B. (2014). Mechanism of salinity tolerance in plants: physiological, biochemical, and molecular characterization. *Int J Genomics, 2014*, ID 701596. doi:10.1155/2014/701596
Güsewell, S. (2004). N : P ratios in terrestrial plants: variation and functional significance. *New Phytologist, 164*(2), 243-266. doi:10.1111/j.1469-8137.2004.01192.x
Harrington, R. R., Kennedy, B. P., Chamberlain, C. P., Blum, J. D., & Folt, C. L. (1998). ^{15}N enrichment in agricultural catchments: field patterns and applications to tracking Atlantic salmon *(Salmo salar*). *Chemical Geology, 147*, 281-294. doi:0009-2541/98/$19.00
Hart, M. R., Quin, B. F., & Nguyen, M. L. (2004). Phosphorus runoff from agricultural land and direct fertilizer effects: A review. *Journal of Environmental Quality, 33*(6), 1954-1972.
Hayashi, H., Alia, Mustardy, L., Deshnium, P., Ida, M., & Murata, N. (1997). Transformation of *Arabidopsis thaliana* with the codA gene for choline oxidase; accumulation of glycinebetaine and enhanced tolerance to salt and cold stress. *The Plant Journal, 12*(1), 133-142.
Heaton, T. H. E. (1986). Isotopic studies of nitrogen pollution in the hydrosphere and atmosphere: a review. *Chemical Geology, 59*, 87-102.
Heggie, K., & Savage, C. (2009). Nitrogen yields from New Zealand coastal catchments to receiving estuaries. *New Zealand journal of marine and freshwater research, 43*, 1039-1052. doi:10.1080/00288330.2009.9626527
Heisler, J., Glibert, P. M., Burkholder, J. M., Anderson, D. M., Cochlan, W., Dennison, W. C., . . . Suddleson, M. (2008). Eutrophication and harmful algal blooms: A scientific consensus. *Harmful Algae, 8*(1), 3-13. doi:10.1016/j.hal.2008.08.006
Heyn, A. N. J. (1940). The physiology of cell elongation. *Botanical Review, 6*(10), 515-574.
Hibino, T., Meng, Y.-L., Kawamitsu, Y., Uehara, N., Matsuda, N., Tanaka, Y., Ishikawa, H., Baba, S., Takabe, T., Wada, K., Ishii, T., & Takabe, T. (2001). Molecular Cloning and Functional Characterization of Two Kinds of Betaine-Aldehyde Dehydrogenase in Betaine-Accumulating Mangrove *Avicennia marina* (Forsk.) *Vierh. Plant Molecular Biology, 45*, 353-363.
Hoch, G., & Körner, C. (2003). The carbon charging of pines at the climatic treeline: a global comparison. *Oecologia, 135*(1), 10-21.
Holmström, K.-O., Somersalo, S., Mandal, A., Palva, T. E., & Welin, B. (2000). Improved tolerance to salinity and low temperature in transgenic tobacco producing glycine betaine. *Journal of Experimental Botany, 51*(343), 177-185.
Hume, T.M., & Dahm, J. (1992). *An investigation of the effects of Polynesian and European land use on sedimentation in Coromandel estuaries*. DSIR Water Quality Centre, Hamilton, Consultancy Report No. 6104, prepared for the Department of Conservation, Hamilton Office, February 1992, 55 p.
Hutchison, J., Manica, A., Swetnam, R., Balmford, A., & Spalding, M. (2014). Predicting Global Patterns in Mangrove Forest Biomass. *Conservation Letters, 7*(3), 233-240. doi:10.1111/conl.12060

Jensen, H. S., Mortensen, P. B., Andersen, F. O., Rasmussen, E., & Jensen, A. (1995). Phosphorus cycling in a coastal marine sediment, Aarhus Bay, Denmark. *Limnology and Oceanography, 40(5),* 908-917.

Jennerjahn, T. C., & Ittekkot, V. (2002). Relevance of Mangroves for the Production and Deposition of Organic Matter along Tropical Continental Margins. *Naturwissenschaften, 89,* 23-30. doi:10.1007/s00114-001-0283-x

Jordan, T., Cornwell, C., Boynton, W. R., & Anderson, J. (2008). Changes in phosphorus biogeochemistry along an estuarine salinity gradient: The iron conveyer belt. *Limnol. Oceanogr., 53*(1), 172-184.

Keller, M. D., Kiene, R. P., Matrai, P. A., & Bellows, W. K. (1999). Production of glycine betaine and dimethylsulfoniopropionate in marine phytoplankton. II. N-limited chemostat cultures. *Marine Biology, 135,* 249-257.

Kelly, S. (2013). *Harbour Monitoring 2013-14*. Mangere Wastewater Treatment Plant.

Khan, F., Adnan, M. Y., & Aziz, I. (2016). Metabolic implications of salt induced osmolyte accumulation in *Avicennia marina. Pak. J. Bot., 48*(1), 29-36.

Khamis, S., Lamaze, T., Lemoine, Y., & Foyer, C. (1990). Adaptation of the photosynthetic apparatus in Maize leaves as a result of nitrogen limitation. Plant Physiology, 94, 1436-1443.

Komiyama, A., Ong, J. E., & Poungparn, S. (2008). Allometry, biomass, and productivity of mangrove forests: A review. *Aquatic Botany, 89*(2), 128-137. doi:10.1016/j.aquabot.2007.12.006

Komor, S. C., & H. W. Anderson, J. (1993). Nitrogen isotopes as indicators of nitrate sources in Minnesota Sand-Plain aquifers. *Ground water, 31*(2), 260-270.

Körner C. (1995) Alpine Plant Diversity: A Global Survey and Functional Interpretations. In: Chapin F.S., Körner C. (eds) *Arctic and Alpine Biodiversity: Patterns, Causes and Ecosystem Consequences.* Ecological Studies (Analysis and Synthesis), vol 113. Springer, Berlin, Heidelberg

Körner, C. (1999). *Alpine Plant Life*. Berlin, Germany: Springer-Verlag Berlin Heidelberg. doi:10.1007/978-3-642-18970-8

Körner, C. (2003). Carbon limitation in trees. *Journal of Ecology, 91,* 4-17.

Körner, C. (2012). *Alpine treelines: functional ecology of the global high elevation tree limits.* Basel, Switzerland: Springer.

Körner, C. (2013). Growth controls photosynthesis – mostly. Nova Acta Leopoldina, 114(391), 273 – 283.

Körner, C. (2015). Paradigm shift in plant growth control. *Curr Opin Plant Biol, 25,* 107-114. doi:10.1016/j.pbi.2015.05.003

Krauss, K. W., & Ball, M. C. (2012). On the halophytic nature of mangroves. *Trees, 27*(1), 7-11. doi:10.1007/s00468-012-0767-7

Krauss, K. W., Doyle, T. W., Twilley, R. R., Rivera-Monroy, V. H., & Sullivan, J. K. (2006). Evaluating the relative contributions of hydroperiod and soil fertility on growth of south Florida mangroves. *Hydrobiologia, 569(1),* 311-324. doi:10.1007/s10750-006-0139-7

Krauss, K. W., Lovelock, C. E., McKee, K. L., López-Hoffman, L., Ewe, S. M. L., & Sousa, W. P. (2008). Environmental drivers in mangrove establishment and early development: A review. *Aquatic Botany, 89(2),* 105-127. doi:10.1016/j.aquabot.2007.12.014

Krishnamurthy, P., Jyothi-Prakash, P. A., Qin, L., He, J., Lin, Q., Loh, C. S., & Kumar, P. P. (2014). Role of root hydrophobic barriers in salt exclusion of a mangrove plant *Avicennia officinalis. Plant Cell Environ, 37*(7), 1656-1671. doi:10.1111/pce.12272

Kristensen, E., Bouillon, S., Dittmar, T., & Marchand, C. (2008). Organic carbon dynamics in mangrove ecosystems: A review. *Aquatic Botany, 89*, 201-219. doi:10.1016/j.aquabot.2007.12.005

Kumari, A., Das, P., Parida, A. K., & Agarwal, P. K. (2015). Proteomics, metabolomics, and ionomics perspectives of salinity tolerance in halophytes. *Front Plant Sci, 6*, 537. doi:10.3389/fpls.2015.00537

Lacerda, L. D. (1992). Carbon burial in mangrove sediments, a potential source of carbon to the sea during events of sea-level change. In L. D. Lacerda, B. Turcq, B. Knoppers, & B. Kjerfve (Eds.), *Paleoclimatic changes and the carbon cycle.* (pp. 107–114). Rio de Janeiro, Brazil: Soc Bras Geoquim.

Lambers, H., Chapin III, F. S., Pons, T. L. (2008). *Plant physiological ecology.* New York, USA: Springer-Verlag New York. doi: 10.1007/978-0-387-78341-3

Lambs, L., Leopold, A., Zeller, B., Herteman, M., & Fromard, F. (2011). Tracing sewage water by 15N in a mangrove ecosystem to test its bioremediation ability. *Rapid Commun Mass Spectrom, 25*(19), 2777-2784. doi:10.1002/rcm.5120

Larcher, W. (2003). *Physiological plant ecology*. Berlin, Germany: Springer-Verlag Berlin Heidelberg.

Lea, P. J., & Miflin, B. J. (1980). Transport and metabolism of asparagine and other nitrogen compounds within the plant. In B. J. Miflin (Ed.), *The Biochemistry of Plants*. New York: Academic Press.

Lea, P. J., & Azevedo, R. A. (2006). Nitrogen use efficiency. 1. Uptake of nitrogen from soil. *Annals of Applied Biology, 149*, 243-247.

Lenth, R., & Love, J. (2017). Package 'lsmeans'. Repository CRAN: R-core.

Levitt, J. (1980). Chilling injury and resistance. In *Responces of plants to environmental stresses. Vol 1: Chilling, freezing and high temperatures.*

Li, M., Hoch, G., & Körner, C. (2002). Source/sink removal affects mobile carbohydrates in *Pinus cembra* at the Swiss treeline. *Trees, 16*, 331-337.

Lindau, C. W., Delaune, R. D., W.H. Patrick, J., & Lambremont, E. N. (1989). Assessment of stable nitrogen isotopes in fingerprinting surface water inorganic nitrogen sources. *Water, Air and Soil Poluution, 48*, 489-496.

Liu, H., Liu, H., & Song, Y. (2011). Absorption and translocation of nitrogen in rhizomes of Leymus chinensis. *Rapid Commun Mass Spectrom, 25*(5), 665-671. doi:10.1002/rcm.4897

Lovelock, C. E., & Feller, I. C. (2003). Photosynthetic performance and resource utilization of two mangrove species coexisting in a hypersaline scrub forest. *Oecologia, 134*, 455-462. doi:10.1007/s00442-002-1118-y

Lovelock, C. E., Feller, I. C., McKee, K. L., Engelbrecht, B. M. J., & Ball, M. C. (2004). The effect of nutrient enrichment on growth, photosynthesis and hydraulic conductance of dwarf mangroves in Panama. *Functional Ecology, 18*, 25-33.

Lovelock, C. E., Feller, I. C., McKee, K. L., & Thompson, R. (2005). Variation in mangrove forest structure and sediment characteristics in Bocas del Toro, Panama. *Caribbean Journal of Science, 41*(3), 456-464.

Lovelock, C. E., Feller, I. C., Ball, M. C., Engelbrecht, B. M., & Ewe, M. L. (2006). Differences in plant function in phosphorus- and nitrogen-limited mangrove ecosystems. *New Phytologist, 172(3)*, 514-522. doi:10.1111/j.1469-8137.2006.01851.x

Lovelock, C. E., Feller, I. C., Ball, M. C., Ellis, J., & Sorrell, B. (2007a). Testing the growth rate vs. geochemical hypothesis for latitudinal variation in plant nutrients. *Ecol Lett, 10*(12), 1154-1163. doi:10.1111/j.1461-0248.2007.01112.x

Lovelock, C. E., Feller, I. C., Ellis, J., Schwarz, A. M., Hancock, N., Nichols, P., & Sorrell, B. (2007b). Mangrove growth in New Zealand estuaries: the role of

nutrient enrichment at sites with contrasting rates of sedimentation. *Oecologia, 153*, 633-641. doi:10.1007/s00442-007-0750-y

Lovelock, C. E., Sorrell, B. K., Hancock, N., Hua, Q., & Swales, A. (2010). Mangrove Forest and Soil Development on a Rapidly Accreting Shore in New Zealand. *Ecosystems, 13(3)*, 437-451. doi:10.1007/s10021-010-9329-2

Lovelock, C. E., Krauss, K. W., Osland, M. J., Reef, R., & Ball, M. C. (2016). The Physiology of Mangrove Trees with Changing Climate. In *Tropical Tree Physiology* Switzerland: Springer Nature Switzerland.

Lovelock, C. E., Reef, R., & & Ball, M. C. (2017). Isotopic signatures of stem water reveal differences in water sources accessed by mangrove tree species. *Hydrobiologia*. doi: 10.1007/s10750-017-3149-8

Maag, F. P., & Vinther, F. P. (1996). Nitrous oxide emission by nitrification and denitrification in different soil types and at different soil moisture contents and temperatures. *Applied Soil Ecology, 4*, 5-14.

Mäenpää, E., Skre, O., Malila, E., Partanen, R., Wielgolaski, F. E., & Laine, K. (2001). Carbon economy in birch dominated ecosystem species in northern Fennoscandia. *Nordic mountain birch ecosystems. Man and the biosphere series, 27*, 93–114.

Mahalingam, R. (2015). *Combined Stresses in Plants*. Switzerland: Springer International Publishing. doi:10.1007/978-3-319-07899-1_5

Mansour, M. M. F. (2000). Nitrogen containing compounds and adaptation of plants to salinity stress. *Biol.Plant., 43*, 491-500. doi:10.1023/A:1002873531707

Markley, J., McMillan, C., & Thompson, G. J. (1982). Latitudinal differentiation in responce to chilling temperatures among populations of three mangroves, *Avicennia germinans, Laguncularia racemosa* and *Rhizophora mangle*, from the western tropical Atlantic and Pacific Panama . *Canadian Journal of Botany, 60*, 2704-2715.

Martin, K. C., Bruhn, D., Lovelock, C. E., Feller, I. C., Evans, J. R., & Ball, M. C. (2010). Nitrogen fertilization enhances water-use efficiency in a saline environment. *Plant, Cell and Environment, 33*, 344-357. doi:10.1111/j.1365-3040.2009.02072.x

McClelland, J. W., & Valiela, I. (1998). Linking nitrogen in estuarine producers to land-derived sources. *Limnol. Oceanogr., 43*(4), 577-585.

McGlathery, K. J., Sundbäck, K., & Anderson, I. C. (2007). Eutrophication in shallow coastal bays and lagoons: the role of plants in the coastal filter. *Marine Ecology Progress Series, 348*, 1-18. doi:10.3354/meps07132

McGuire, A. D., Melillo, J. M., & Joyce, L. A. (1995). The role of nitrogen in the response of forest net primary productuion to elevated armospheric carbon dioxide. *Annu. Rev. Syst., 26*(473-503).

McKee, K. L. (1993). Soil physicochemical patterns and mangrove species distribution - reciprocal effects? *Journal of Ecology, 81*, 477-487.

McKee, K.L. (1995). Interspecific variation in growth, biomass partitioning, and defensive characteristics of neotropical mangrove seedlings: response to light and nutrient availability. *American Journal of Botani, 82*, 299–307.

McKee, K.L. (1996) Growth and physiological responses of mangrove seedlings to root zone anoxia. *Tree Physiology,16*, 883–889.

McKee, K.L. (2001). Root proliferation in decaying roots and old root channels: a nutrient conservation mechanism in oligotrophic mangrove forests? *Journal of Ecology, 89*, 876–887. doi:10.1046/j.0022-0477.2001.00606.x

McKee, K. L., Feller, I. C., Popp, M., & Wanek, M. (2002). Mangrove isotopic ($\delta^{15}N$ and $\delta^{13}C$) fractionation across nitrogen *vs.* phosphorus limitation gradient. *Ecology, 83(4)*, 1065-1075.

McKee, K. L., Mendelssohn, I. A., & Hester, M. W. (1988). Reexamination of pore water sulfide concentrations and redox potentials near the aerial roots of *Rhizophora mangle* and *Avicennia germinans*. *American Journal of Botany, 75*, 1352-1359.

McKee, K.L., & Rooth, J.E. (2008). Where temperate meets tropical: multifactorial effects of elevated CO_2, nitrogen enrichment, and competition on a mangrove-salt marsh community. *Glob Change Biol, 14*(5), 971–984. doi:10.1111/j.1365-486.2008.01547.x

McLauchlan, K. K., Ferguson, C. J., Wilson, I. E., Ocheltree, T. W., & Craine, J. M. (2010). Thirteen decades of foliar isotopes indicate declining nitrogen availability in central North American grasslands. *New Phytol, 187*(4), 1135-1145. doi:10.1111/j.1469-8137.2010.03322.x

McMillan, C. (1974). *Adaptive differatiation to chilling in mangrove populations*. Presented at the Proceedings of the International Symposium on Biology and management of mangroves, East-West Center, Honolulu, Hawaii.

McMurtrie, R. E., & Dewar, R. C. (2013). New insights into carbon allocation by trees from the hypothesis that annual wood production is maximized. *New Phytol, 199*(4), 981-990. doi:10.1111/nph.12344

McQuaker, N. R., Brown, D., & Kluckner, P. D. (1979). Digestion of environmental materials for analysis by inductively coupled plasma-atomic emission spectrometry. *Analytical chemistry, 51(7)*, 1082-1084.

Mehta, P. A., Sivaprakash, K., Parani, M., Venkataraman, G., & Parida, A. K. (2005). Generation and analysis of expressed sequence tags from the salt-tolerant mangrove species *Avicennia marina* (Forsk) Vierh. *Theor Appl Genet, 110*(3), 416-424. doi:10.1007/s00122-004-1801-y

Miyashita, Y., Dolferus, R., Ismind, K. P., & Good, A. G. (2007). Alanine aminotransferase catalyses the breakdown of alanine after hypoxia in Arabidopsis thaliana. *The Plant Journal, 49*, 1108-1112.

Mittler, R. (2002). Oxidative stress, antioxidants and stress tolerance. *Trends in plant science, 7*(9), 405-410.

Monson, R. K. (2014). Ecology and the environment. New York, USA: Springer Science+Business Media New York 2014.

Morrisey, D., Beard, C., Morrison, M., Craggs, R., & Lowe, M. (2007). The New Zealand mangrove: review of the current state of knowledge. Auckland Regional Council Technical Number 325.

Morrisey, D., Swales, A., Dittmann, S., Morrison, M. A., Lovelock, C. E., & Beard, C. M. (2010). The ecology and management of temperate mangroves.

Mouillon, J.-M., Aubert, S., Bourguignon, J., Gout, E., Douce, R., & Rebeille, F. (1999). Glycine and serine catabolism in non-photosynthetic higher plant cells: their role in C1 metabolism. *The Plant Journal, 20*(2), 197-205.

Munns, R. (2002). Comparative Physiology of Salt and Water Stress. *Plant, Cell & Environment, 25*, 239-250. doi: 10.1046/j.0016-8025.2001.00808.x

Munns, R., & Tester, M. (2008). Mechanisms of salinity tolerance. *Annu Rev Plant Biol, 59*, 651-681. doi:10.1146/annurev.arplant.59.032607.092911

Murakeözy, E.P., Smirnoff, N., Nagy, Z., & Tuba, Z. (2002). Seasonal accumulation pattern of pinitol and other carbohydrates in Limonium gmelinii subsp. hungarica. *Journal of Plant Physiology, 159*, 485-490.

Murakeözy, E.P., Nagy, Z., Duhaze, C., Bouchereau, A., & Tuba, Z. (2003). Seasonal changes in the levels of compatible osmolytes in three halophytic species of inland saline vegetation in Hungary. *Journal of Plant Physiology, 160*, 395-401.

Myers, R. J. K. (1975). Temperature effects on ammonification and nitrification in a tropical soil. *Soil Biology and Biochemistry, 7*, 83-86.

Nagel, O. W., Konings, H., & Lambers, H. (1994). Growth rate, plant development and water relations of the ABA-deficient tomato mutant sitiens. *Physiologia Plantarum, 92*, 102-108.
Naidoo, G. (1987). Effects of salinity and nitrogen on growth and water relations in the mangrove, *Avicennia marina* (FORSK.). *New Phytol., 107*, 317-325.
Naidoo, G. (2006). Factors contributing to dwarfing in the mangrove *Avicennia marina*. *Annals of Botany, 97*, 1095-1101. doi:10.1093/aob/mcl064
Naidoo, G. (2009). Differential effects of nitrogen and phosphorus enrichment on growth of dwarf *Avicennia marina* mangroves. *Aquatic Botany, 90*(2), 184-190. doi:10.1016/j.aquabot.2008.10.001
Naidoo, G. (2016). The mangroves of South Africa: An ecophysiological review. *South African Journal of Botany, 107*, 101-113. doi:10.1016/j.sajb.2016.04.014
Naidoo, G., Hiralal, O., & Naidoo, Y. (2011). Hypersalinity effects on leaf ultrastructure and physiology in the mangrove *Avicennia marina*. *Flora - Morphology, Distribution, Functional Ecology of Plants, 206*(9), 814-820. doi:10.1016/j.flora.2011.04.009
Nguyen, H. T., Stanton, D. E., Schmitz, N., Farquhar, G. D., & Ball, M. C. (2015). Growth responses of the mangrove *Avicennia marina* to salinity: development and function of shoot hydraulic systems require saline conditions. *Ann Bot, 115*(3), 397-407. doi:10.1093/aob/mcu257
Nixon, S., Ammerman, J. M., Atkinson, L. P., Berounsky, V. M., Billen, G., Boicourt, W. C., . . . Seitzinger, S. P. (1996). The fate of nitrogen and phosphorus at the land-sea margin of the North Atlantic Ocean. *Biogeochemistry, 35*, 141-180.
Nixon, S., Buckley, B., Granger, S., & Bintz, J. (2001). Responses of Very Shallow Marine Ecosystems to Nutrient Enrichment. *Human and Ecological Risk Assessment: An International Journal, 7*(5), 1457-1481. doi:10.1080/20018091095131
OECD. (2015). *Environment at a Glance 2015: OECD Indicators*. Paris: OECD Publishing. doi:10.1787/19964064
Osuji, G. O., & Madu, W. C. (2012). Using the RNA synthetic activity of glutamate dehydrogenase to illuminate the natural role of the enzyme. *Advances in Biological Chemistry, 3*, 379-389.
Osuji, G. O., & Madu, W. C. (2015). Glutamate Dehydrogenase. In J. P. F. D'Mello (Ed.), *Amino Acids in Higher Plants*. Oxford, UK: CABI Publishers.
Paerl, H. W. (1997). Coastal eutrophication and harmful algal blooms: Importance of atmospheric deposition and groundwater as "new" nitrogen and other nutrient sources. *Limnol. Oceanogr., 42*(5, part 2), 1154-1165.
Parfitt, R. L., Ross, D. J., Coomes, D. A., Richardson, S. J., Smale, M. C., & Dahlgren, R. A. (2005). N and P in New Zealand Soil Chronosequences and Relationships with Foliar N and P. *Biogeochemistry, 75*(2), 305-328. doi:10.1007/s10533-004-7790-8
Parida, A. K., & Das, A. B. (2004). Effects of NaCl stress on nitrogen and phosphorous metabolism in a true mangrove *Bruguiera parviflora* grown under hydroponic culture. *Journal of Plant Physiology, 161,* 921-928. doi:10.1016/j.jplph.2003.11.006
Parida, A. K., Das, A. B., & Das, P. (2002). NaCI stress causes changes in photosynthetic pigments, proteins, and other metabolic components in the leaves of a true mangrove, *Bruguiera parviflora*, in hydroponic cultures. *Journal of Plant Biology, 45*(1), 28-36.
Parida, A. K., & Jha, B. (2010). Salt tolerance mechanisms in mangroves: a review. *Trees, 24*, 199-217. doi:10.1007/s00468-010-0417-x

Patel, N. T., Gupta, A., & Pandey, A. N. (2010). Strong positive growth responses to salinity by Ceriops tagal, a commonly occurring mangrove of the Gujarat coast of India. *AoB Plants, 2010*, plq011. doi:10.1093/aobpla/plq011

Paytan, A., & McLaughlin, K. (2007). The Oceanic Phosphorus Cycle. *Chem. Rev., 107*, 563-576.

Peñuelas, J., & Estiarte, M. (1997). Trends in plant carbon concentration and plant demand for N throughout this century. *Oecologia, 106*(69-73).

Peñuelas, J., & Filella, I. (2001). Herbaria century record of increasing eutrophication in Spanish terrestrial ecosystems. *Global Change Biology, 7*, 427-433.

Peñuelas, J., & Matamala, R. (1990). Changes in N and S leaf content, stomatal density and specific leaf area of 14 plant species during the last three centuries of $C0_2$ increase. *Journal of Experimental Botany, 41*(230), 1119-1124.

Peterson, B. J., & Fry, B. (1987). Stable isotopes in ecosystem studies. *Annual Review of Ecology and Systematics, 18*, 293-320. doi:0066-4162/87/1120-0293$02.00

Pfautsch, S., Gessler, A., Adams, M. A., & Rennenberg, H. (2009). Using amino-nitrogen pools and fluxes to identify contributions of understory *Acacia* spp. to overstorey *Eucaliptus regnans* and stand nitrogen uptake in temperate Australia. *New Phytologist, 183*, 1097-1113.

Pfautsch, S., Bell, T. L., & Gessler, A. (2015). Uptake, transport and redistribution of amino nitrogen in woody plants. In *Amino Acids in Higher Plants*. Croydon, UK: CAB International.

Pinheiro, J. (2017). *Package 'nlme'*. Repository CRAN: R-core.

Pitt, K. A., Connolly, R. M., & Maxwell, P. (2009). Redistribution of sewage-nitrogen in estuarine food webs following sewage treatment upgrades. *Mar Pollut Bull, 58*(4), 573-580. doi:10.1016/j.marpolbul.2008.11.016

Planchet, E., & Limami, A. M. (2015). Amino Acid Synthesis Under Abiotoc Stress. In J. P. F. D'Mello (Ed.), *Amino Acids in Higher Plants*. UK: CAB International.

Planchet, E., Rannou, O., Boutet-Mercey, S., Maia-Grondard, A., & Limami, A. M. (2011). Nitrogen metabolism responses to water deficit act through both abscisic acid (ABA)-dependent and independent pathways in *Medicago truncatula* during post-germination. *Journal of Experimental Botany, 62*, 605-615.

Polidoro, B. A., Carpenter, K. E., Collins, L., Duke, N. C., Ellison, A. M., Ellison, J. C., .Yong, J. W. (2010). The loss of species: mangrove extinction risk and geographic areas of global concern. *PLoS One, 5*(4), e10095. doi:10.1371/journal.pone.0010095

Poorter, H., & Nagel, O. (2000) The role of biomass allocation in the growth response of plants to different levels of light, CO_2, nutrients and water: A Quantitative Review. *Australian Journal of Plant Physiology, 27*, 595-607. doi.org/10.1071/PP99173_CO

Popp, M. (1984). Chemical Composition of Australian Mangroves II. Low Molecular Weight Carbohydrates. *Zeitschrift für Pflanzenphysiologie, 113*(5), 411-421. doi:10.1016/S0044-328X(84)80097-5

Popp, M., Larher, F., & Weigel, P. (1985). Osmotic adaptation in Australian mangroves. In W. G. Beeftink, J. Rozema, & A. H. L. Huiskes. Symposium conducted at the meeting of the *Ecology of coastal vegetation*, Haamstede.

Prusinkiewicz, P., & DeReuille, P. (2010). Constraints of space in plant development. *J Exp Bot, 61*(8), 2117-2129. doi:10.1093/jxb/erq081

Quisthoudt, K., Schmitz, N., Randin, C. F., Dahdouh-Guebas, F., Robert, E. M. R., & Koedam, N. (2012). Temperature variation among mangrove latitudinal range limits worldwide. *Trees, 26(6)*, 1919-1931. doi:10.1007/s00468-012-0760-1

Rajesh, A., Arumugam, R., & Venkatesalu, V. (1999). Responses of Ceriops roxburghiana to NaCl stress. *Biol Plant, 42*, 143-148.

Raychaudhuri, A. (2015). Alanine Aminotransferase. In J. P. F. D'Mello (Ed.), *Amino Acids in Higher Plants*. Oxford, UK: CAB International.

Reef, R., Feller, I. C., & Lovelock, C. E. (2010). Nutrition of mangroves. *Tree Physiology, 30*, 1148-1160. doi:10.1093/treephys/tpq048

Reef, R., & Lovelock, C. E. (2015). Regulation of water balance in mangroves. *Annals of Botany, 115*(3), 385-395. doi:10.1093/aob/mcu174

Reef, R., Markham, H. L., Santini, N. S., & Lovelock, C. E. (2015). The response of the mangrove *Avicennia marina* to heterogeneous salinity measured using a split-root approach. *Plant and Soil, 393(1-2),* 297-305. doi:10.1007/s11104-015-2489-2

Reef, R., Spencer, T., Mller, I., Lovelock, C. E., Christie, E. K., McIvor, A. L., . . . Tempest, J. A. (2017). The effects of elevated CO_2 and eutrophication on surface elevation gain in a European salt marsh. *Glob Chang Biol, 23*(2), 881-890. doi:10.1111/gcb.13396

Reis, C. R., Nardoto, G. B., Rochelle, A. L., Vieira, S. A., & Oliveira, R. S. (2017). Nitrogen dynamics in subtropical fringe and basin mangrove forests inferred from stable isotopes. *Oecologia, 183*(3), 841-848. doi:10.1007/s00442-016-3789-9

Rengasamy, P. (2010). Soil processes affecting crop production in salt-affected soils. *Functional Plant Biology, 37*(7), 613-620. doi:10.1071/FP09249

Richardson, S. J., Peltzer, D. A., Allen, R. B., McGlone, M. S., & Parfitt, R. L. (2004). Rapid development of phosphorus limitation in temperate rainforest along the Franz Josef soil chronosequence. *Oecologia, 139*(2), 267-276. doi:10.1007/s00442-004-1501-y

Rivera-Monroy, V. H., & Twilley, R. R. (1996). The relative role of denitrification and immobilization in the fate of inorganic nitrogen in mangrove sediments (Terminos Lagoon, Mexico). *Limnol. Oceanogr., 41*(2), 284-296.

Robertson, A.I., Phillips, M.J. (1995). Mangroves as filters of shrimp pond effluent: predictions and biogeochemical research needs. *Hydrobiologia, 295*, 311–321

Rocha, M., Licausi, F., Araújo, W. L., Nunes-Nesi, A., Sodek, L., Fernie, A. R., & van Dongen, J. T. (2010). Glycolysis and the tricarboxylic acid cycle are linked by alanine aminotransferase during hypoxia induced by waterlogging of *Lotus japonicus. Plant Physiology, 152*, 1501-1513.

Roden, E. E., & Edmonds, J. W. (1997). Phosphate mobilizationin iron-rich anaerobic sediments: Microbial Fe(III) oxide re-duction versus iron-sulfide formation. *Archive for Hydrobiology, 139(3),* 347-378.

Rodriguez, W., & Feller, I. C. (2004). Mangrove landscape characterization and change in Twin Cays, Belize using aerial photography and IKONOS satellite data. *Atoll Research Bulletin, 513*, 1-22.

Rogers, K. (2003). Stable carbon and nitrogen isotope signatures indicate recovery of marine biota from sewage pollution at Moa Point, New Zealand. *Marine pollution bulletin, 46*(7), 821-827. doi:10.1016/s0025-326x(03)00097-3

Ros, R., Munoz-Bertomeu, J., & Krueger, S. (2014). Serine in plants: biosynthesis, metabolism, and functions. *Trends Plant Sci, 19*(9), 564-569. doi:10.1016/j.tplants.2014.06.003

Rozan, T. F., Taillefert, M., Trouwborst, R. E., Glazer, B. T., Ma, S., Herszage, J., . . . Luther III, G. W. (2002). Iron–sulfur–phosphorus cycling in the sediments of a shallow coastal bay: Implications for sediment nutrient release and benthic macroalgal blooms. *Limnology and Oceanography, 47(5),* 1346–1354.

Rozema, J., Gude, H., & Pollak, G. (1981). An ecophysiological study of the salt secretion of four halophytes. *New Phytologist, 89*, 201-217.

Roy, S. J., Negrao, S., & Tester, M. (2014). Salt resistant crop plants. *Curr Opin Biotechnol, 26*, 115-124. doi:10.1016/j.copbio.2013.12.004

R Core Team (2017). R: A Language and Environment for Statistical Computing. Vienna: R Foundation for Statistical Computing. Available at: https://www.r-project.org/

Saenger, P., & Snedaker, S. C. (1993). Pantropical trends in mangrove above-ground biomass and annual litterfall. *Oecologia, 96*(3), 293-299.

Saintilan, N., Rogers, K., & McKee, K. L. (2009). Salt marsh-mangrove interactions in Australasia and the Americas. In G. M. E. Perillo et al (Eds.), *Coastal Wetlands-An Integrated Ecosystem Approach.* Amsterdam: Elsevier.

Saintilan, N., & Williams, R. J. (1999). Mangrove transgression into saltmarsh environments in south-east Australia. *Global Ecology and Biogeography 8*, 117−124.

Salazar, C., Armenta, J. M., & Shulaev, V. (2012). An UPLC-ESI-MS/MS Assay Using 6-Aminoquinolyl-N-Hydroxysuccinimidyl Carbamate Derivatization for Targeted Amino Acid Analysis: Application to Screening of *Arabidopsis thaliana* Mutants. *Metabolites, 2*(3), 398-428. doi:10.3390/metabo2030398

Sánchez-Carrillo, S., Sánchez-Andrés, R., Alatorre, L. C., Angeler, D. G., Álvarez-Cobelas, M., & Arreola-Lizárraga, J. A. (2009). Nutrient fluxes in a semi-arid microtidal mangrove wetland in the Gulf of California. Estuarine, *Coastal and Shelf Science, 82*(4), 654-662. doi:10.1016/j.ecss.2009.03.002

Santiago, L.S., Lau, T.S., Melcher, P.J., Steele, O.C., & Goldstein, G. (2000). Morphological and physiological responses of Hawaiian *Hibiscus tiliaceus* population to light and salinity. *Intl J Plant Sci, 161*, 99-106

Santini, N. S., Reef, R., Lockington, D. A., & Lovelock, C. E. (2015). The use of fresh and saline water sources by the mangrove *Avicennia marina. Hydrobiologia, 745(1)*, 59-68. doi:10.1007/s10750-014-2091-2

Schindler, D. W. (2006). Resent advances in the understanding and management of eutrophication. *Limnol. Oceanogr., 51*(1, part 2), 356-363.

Schirch, L. V. (1984). *Folates in glycine and serine metabolism*. New York: Wiley Interscience.

Schwarz, A. M. (2003). Spreading mangroves: a New Zealand phenomenon or a global trend? *Water & Atmosphere, 1*(11), 8-10.

Seitzinger, S. P., Sanders, R. W., & Styles, R. (2002). Bioavailability of DON from natural and anthropogenic sources to estuarine plankton. *Limnology and Oceanography, 47(2)*, 353-366.

Sengupta, S., & Majumder, A. L. (2009). Insight into the salt tolerance factors of a wild halophytic rice, *Porteresia coarctata*: a physiological and proteomic approach. *Planta, 229*(4), 911-929. doi:10.1007/s00425-008-0878-y

Serret, M. D., Ortiz-Monasterio, I., Pardo, A., & Araus, J. L. (2008). The effects of urea fertilisation and genotype on yield, nitrogen use efficiency, δ15N and δ13C in wheat. *Annals of Applied Biology, 1*. doi:10.1111/j.1744-7348.2008.00259.x

Shabala, S., Bose, J., & Hedrich, R. (2014). Salt bladders: do they matter? *Trends Plant Sci, 19*(11), 687-691. doi:10.1016/j.tplants.2014.09.001

Sharma, S. S., & Dietz, K. J. (2006). The significance of amino acids and amino acid-derived molecules in plant responses and adaptation to heavy metal stress. *J Exp Bot, 57*(4), 711-726. doi:10.1093/jxb/erj073

Shiau, Y.-J., Lee, S.-C., Chen, T.-H., Tian, G., & Chiu, C.-Y. (2017). Water salinity effects on growth and nitrogen assimilation rate of mangrove (*Kandelia candel*) seedlings. *Aquatic Botany, 137*, 50-55. doi:10.1016/j.aquabot.2016.11.008

Sieciechowicz, K. A., Joy, K. W., & Ireland, R. J. (1988). The metabolism of asparagine in plants. *Phytochemistry, 27*, 663-671.

Singh, T. N., Aspinall, D., & Paleg, L. G. (1972). Proline accumulation and varietal adaptability to drought in barley; a potential metabolic measure of drought resistance. *Nature, 286*, 188-190.
Slama, I., Abdelly, C., Bouchereau, A., Flowers, T., & Savoure, A. (2015). Diversity, distribution and roles of osmoprotective compounds accumulated in halophytes under abiotic stress. *Ann Bot, 115*(3), 433-447. doi:10.1093/aob/mcu239
Smith, V. H., & Schindler, D. W. (2009). Eutrophication science: where do we go from here? *Trends Ecol Evol, 24*(4), 201-207. doi:10.1016/j.tree.2008.11.009
Smith, J., Burford, M. A., Revill, A. T., Haese, R. R., & Fortune, J. (2012). Effect of nutrient loading on biogeochemical processes in tropical tidal creeks. *Biogeochemistry, 108*(1-3), 359-380. doi:10.1007/s10533-011-9605-z
Srivastava, A. C., Ramos-Parra, P. A., Bedair, M., Robledo-Hernandez, A. L., Tang, Y., Sumner, L. W., . . . Blancaflor, E. B. (2011). The folylpolyglutamate synthetase plastidial isoform is required for postembryonic root development in *Arabidopsis. Plant Physiol, 155*(3), 1237-1251. doi:10.1104/pp.110.168278
Stokes, D. J., Healy, T. R., & Cooke, P. J. (2010). Expansion dynamics of monospecific, temperate mangroves and sedimentation in two embayments of a barrier-enclosed lagoon, Tauranga Harbour, New Zealand. *Journal of Coastal Research, 26*(1), 113-122. doi:10.2112/08-1043
Stoll, P., & Weiner, J. (2000). A Neighborhood View of Interactions among Individual Plants. *The Geometry of Ecological Interactions: Simplifying Spatial Complexity.* Cambridge, UK: Cambridge University Press. International Institute for Applied Systems Analysis.
Stuart, S. A., Choat, B., Martin, K. C., Holbrook, N. M., & Ball, M. C. (2007). The role of freezing in setting the latitudinal limits of mangrove forests. *New Phytol, 173*(3), 576-583. doi:10.1111/j.1469-8137.2006.01938.x
Suarez, N., & Medina, E. (2006). Influence of salinity on Na and K accumulation, and gas exchange in *Avicennia germinans. Photosynthetica, 44*, 268-274.
Sunkar, R. (2010). *Plant stress tolerance*. New York, USA: Springer Science+Business Media, LLC.
Suzuki, M., Yasumoto, E., Baba, S., & Ashihara, H. (2003). Effect of salt stress on the metabolism of ethanolamine and choline in leaves of the betaine-producing mangrove species *Avicennia marina. Phytochemistry, 64*(5), 941-948. doi:10.1016/s0031-9422(03)00445-x
Swain, T., & Williams, A. (1970). The role of the phenylalanine in flavonoid biosynthesis. *Phytochemistry, 9*, 2115-2122.
Swales, A., Hume, T., & Green, M., (1997). Holocene sedimentation and recent human impacts on infilling in a drowned valley estuary. In: *13th Australasian Coastal and Ocean Engineering Conference and the 6th Australasian Port and Harbour Conference Proceedings*. Christchurch, New Zealand, Centre for Advanced Engineering, University of Canterbury, pp. 895-900
Swarbreck, S. M., Defoin-Platel, M., Hindle, M., Saqi, M., & Habash, D. Z. (2011). New perspectives on glutamine synthetase in grasses. *J Exp Bot, 62*(4), 1511-1522. doi:10.1093/jxb/erq356
Szabados, L., & Savoure, A. (2010). Proline: a multifunctional amino acid. *Trends Plant Sci, 15*(2), 89-97. doi:10.1016/j.tplants.2009.11.009
Takemura, T., Hanagata, N., Sugihara, K., Baba, S., Karube, I., & Dubinsky, Z. (2000). Physiological and Biochemical Responses to Salt Stress in the Mangrove, *Bruguiera gymnorrhiza*. *Aquatic Botany, 68*, 15-28.
Tester, M., & Jorgensen, R. (2014). *The Plant Sciences*. New York: Springer Science+Business Media.

Theuri, M.M., Kinyamario, J.I., van Speybroeck, D. (1999). Photosynthesis and related physiological process in two mangrove species, *Rhizophora mucronata* and *Ceriops tagal*, at Gazi Bay, Kenya. *Afr J Eco, 137*, 180-193.

Thimdee, W., Deein, G., Thimdee, W., Sangrungruan, C., & Matsunaga, K. (2002). High %N and $d^{15}N$ Values in mangrove leaves and sediments of a mangrove-fringed estuary, Thailand. Effects of shrimp pond effluents. *Bulletin of the Society of Sea Water Science, Japan, 56*, 166-173.

Thrush, S. F., Townsend, M., Hewitt, J. E., Davies, K., AM, A. M. L., Lundquist, C., & Cartner, K. (2013). The many uses and values of estuarine ecosystems.: Manaaki Whenua Press, Lincoln, New Zealand.

Tomlinson, B. P. (1986). *The botany of mangroves.* Cambridge, England: Cambridge University Press.

Tran, P., Gritcan, I., Cusens, J., Alfaro, A. C., & Leuzinger, S. (2016). Biomass and nutrient composition of temperate mangroves (*Avicennia marina* var. *australasica*) in New Zealand. *New Zealand journal of marine and freshwater research, 51(3),* 427-442. doi:10.1080/00288330.2016.1260604

Tsuchiya, S., Ogita, S., Kawana, Y., Oyanagi, T., Hasegawa, A., & Sasamoto, H. (2013). Relation between Amino Acids Profiles and Recalcitrancy of Cell Growth or Salt Tolerance in Tissue and Protoplast Cultures of Three Mangrove Species, *Avicennia alba, Bruguiera sexangula*, and *Sonneratia alba*. *American Journal of Plant Sciences, 04*(07), 1366-1374. doi:10.4236/ajps.2013.47167

Tuffers, A., Naidoo, G., & von Willert, D. J. (2001). Low salinities adversely affect photosynthetic performance of the mangrove, *Avicennia marina. Wetlands Ecology and Management, 9*, 225-232.

Twilley, R.R. (1985). The exchange of organic carbon in basin mangrove forests in a southwest Florida estuary. *Est Coast Shelf Sci, 20*, 543–557

Valentine, H. T., & Makela, A. (2012). Modeling forest stand dynamics from optimal balances of carbon and nitrogen. *New Phytol, 194*(4), 961-971. doi:10.1111/j.1469-8137.2012.04123.x

Valiela, I., Foreman, K., LaMontagne, M., Hersh, D., Costa, J., Peckol, P., . . . Lajta, K. (1992). Coupling of watersheds and coastal waters: sources and concequences of nutrient enrichment in Waquoit Bay, Massachusetts. *Estuaries, 15*(4), 443-457. doi:0160-8347/92/040443-15$01.50/0

Valiela, I., McClelland, J., Hauxwell, J., Behr, P. J., Hersh, D., & Foreman, K. (1997). Macroalgal blooms in shallow estuaries: Controls and ecophysiological and ecosystem consequences. *Limnol. Oceanogr., 42*(5, part 2), 1105-1118.

Vant, B. (1997). Nutrient loads and nuisance phytoplankton blooms in estuaries. *Water & Atmosphere, 5*(2), 25-27.

Verhoeven, J. T., Arheimer, B., Yin, C., & Hefting, M. M. (2006). Regional and global concerns over wetlands and water quality. *Trends Ecol Evol, 21*(2), 96-103. doi:10.1016/j.tree.2005.11.015

Verslues, P. E., & Sharma, S. (2010). Proline metabolism and its implications for plant-environment interaction. *Arabidopsis Book, 8*, e0140. doi:10.1199/tab.0140

Vopel, K., Gritcan, I., & Laverock, B. (2017). *Ecological effects of the Mangere Wastewater Treatment Plant: directions for Manukau Harbour monitoring*. AUTEL Client report: WSL 20179.

Vouve, F., Guiraud, G., Marol, C., Girard, M., Richard, P., & Laima, M. J. C. (2000). NH4+ turnover in intertidal sediments of Marennes-Oléron Bay (France): effect of sediment temperature. *Oceanologica Acta, 23(5),* 575-584.

Waditee, R., Hibino, T., Tanaka, Y., Nakamura, T., Incharoensakdi, A., Hayakawa, S., . . . Takabe, T. (2002). Functional characterization of betaine/proline transporters

in betaine-accumulating mangrove. *J Biol Chem, 277*(21), 18373-18382. doi:10.1074/jbc.M112012200

Waisel, Y., Eshel, A., & Agami, M. (1986). Salt balance of leaves of the mangrove *Avicennia marina*. *Physiologia Plantarum, 67*, 67-72.

Walker, J., & Vaughan, M. (2014). *Marine water quality report 2013.* Auckland Council technical report, TR2014/030.

Wang, W., Yan, Z., You, S., Zhang, Y., Chen, L., & Lin, G. (2011). Mangroves: obligate or facultative halophytes? A review. *Trees, 25*(6), 953-963. doi:10.1007/s00468-011-0570-x

Wang, W. Q., Ke, L., Tam, N., & Wong, Y. S. (2002). Changes in the main osmotica during the development of Kandelia candel hypocotyls and after mature hypocotyls were transplanted in solutions with different salinities. *Marine Biology, 141*(6), 1029-1034. doi:10.1007/s00227-002-0951-1

Wattayakorn, G., Wolanski, E., & Kjerfve, B. (1990). Mixing, trapping and outwelling in the Klong Ngao mangrove swamp, Thailand. *Est Coast Shelf Sci, 31,* 667–688

Wei, L., Lockington, D. A., Poh, S. C., Gasparon, M., & Lovelock, C. E. (2013). Water use patterns of estuarine vegetation in a tidal creek system. *Oecologia, 172(2),* 485-494. doi:10.1007/s00442-012-2495-5

Welch, R. M., & Shuman, L. (1995). Micronutrient Nutrition of Plants. *Critical Reviews in Plant Sciences, 14*(1), 49-82. doi:10.1080/07352689509701922

Wood, S. N. (2011). Fast stable restricted maximum likelihood and marginal likelihood estimation of semiparametric generalized linear models. *Journal of the Royal Statistical Society, 73(1),* 3-36.

Woodroffe, C.D. (1985a). Studies of mangrove basin, Tuff Crater, New Zealand. I. Mangrove biomass.and production of detritus. *Est Coast Shelf Sci 20,* 265–280

Woodroffe, C.D. (1985b). Studies of mangrove basin, Tuff Crater, New Zealand. II. Comparison of volumetric and velocity-area methods of estimating tidal flux. *Est Coast Shelf Sci, 20,* 431–445

Woodroffe, C. D., & Grindrod, J. (1991). Mangrove biogeography: the role of Quaternary environmental and sea-level change. *Journal of Biogeography, 18,* 479-492.

Woodroffe, C.D., Bardsley, K.N., Ward, P.J., & Hanley, J.R. (1988). Production of mangrove litter in a macrotidal embayment, Darwin Harbour, N.T., Australia. *Est Coast Shelf Sci 26,* 581–598

Xing, W., & Rajashekar, C. B. (2001). Glycine betaine involvement in freezing tolerance and water stress in *Arabidopsis thaliana. Environmental and Experimental Botany, 46*, 21-28.

Yan, Z., Wang, W., & Tang, D. (2007). Effect of different time of salt stress on growth and some physiological processes of *Avicennia marina* seedlings. *Marine Biology, 152*, 581–587.

Yang, J., Gao, J., Cheung, A., Liu, B., Schwendenmann, L., & Costello, M. J. (2013). Vegetation and sediment characteristics in an expanding mangrove forest in New Zealand. *Estuarine, Coastal and Shelf Science, 134,* 11-18. doi:10.1016/j.ecss.2013.09.017

Yasumoto, E., Adachi, K., Kato, M., Sano, H., Sasamoto, H., Baba, S., & Ashihara, H. (1999). Uptake of inorganic ions and compatible solutes in cultured mangrove cells during salt stress. *In Vitro Cell. Dev. Biol.-Plant, 35*, 82-85.

Yates, E. J., Ashwath, N., & Midmore, D. J. (2002). Responses to nitrogen, phosphorus, potassium and sodium chloride by three mangrove species in pot culture. *Trees, 16*, 120-125. doi:10.1007/s00468-001-0145-3

Ye, Y., Tam, N. F.-Y., Lu, C.-Y., & Wong, Y.-S. (2005). Effects of salinity on germination, seedling growth and physiology of three salt-secreting mangrove species. *Aquatic Botany, 83*(3), 193-205. doi:10.1016/j.aquabot.2005.06.006

Yu, J., Chen, S., Zhao, Q., Wang, T., Yang, C., & Diaz, C. (2011). Physiological and proteomic analysis of salinity tolerance in *Puccinellia tenuiflora. J. Proteome Res., 10*, 3852-3870. doi:10.1021/pr101102p