Effect of runoff from acid-sulfate soils on pneumatophores of the grey mangrove, *Avicennia marina*

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Abstract. Runoff from acid-sulfate soils (ASS) is increasingly threatening the structure and function of estuarine ecosystems worldwide. Along the eastern coast of Australia, sulfuric acid is known to affect the growth and survival of mangrove saplings; however, impacts of ASS runoff on the structure and function of established mangrove trees are unclear. Pneumatophores, the aerial roots produced by some species of mangrove, are critical sites of gas exchange, allowing these species to persist in waterlogged soils. They also provide physical structure in estuarine sediments, facilitating communities of algae, invertebrates and, at high tide, fish. We tested the hypotheses that *Avicennia marina* (Forsk.) Vierh. pneumatophores would be less abundant, shorter, thinner and weaker close to major ASS outflow drains. Sampling at sites close to and away from drains within each of two estuaries of New South Wales, Australia, showed no effect of exposure to runoff on pneumatophore density or thickness. Pneumatophores were, however, shorter (\textless{} 2 cm) and weaker (up to two-fold) at acidified sites than reference sites. Although the reduced length and strength of pneumatophores at acidified sites may limit the number of epifaunal molluscs they can support, the persistence of dense pneumatophores indicates that the capacity to benefit invertebrates and fish remains.

Additional keywords: acidity, estuaries, Instron, mangrove roots, pH, waterlogged soils.

Introduction

Mangrove ecosystems provide complex habitat that supports a wide variety of marine flora and fauna (e.g. Skilleter and Warren 2000; Kathiresan and Bingham 2001; Bishop \textit{et al}. 2009). Mangrove trees are autogenic ecosystem engineers (Jones \textit{et al}. 1994) that modify the environment by shading the substrate below and by introducing hard structure to sedimentary environments. Notably, mangrove trees of the genera *Avicennia*, *Sonneratia* and *Lumnitzera* develop conspicuous aerial roots, known as pneumatophores, which are important sources of habitat complexity. Pneumatophores emerge and stand erect on the mid-shore, above the mud surface or water, and typically provide the only attachment point for many benthic organisms in the more seaward zone that would otherwise consist of a matrix of bare mud (Kathiresan and Bingham 2001; Hogarth 2007). They can modify the efficacy of benthic-feeding predators (Primavera 1997; Macia \textit{et al}. 2003), and inhibit the burrowing activities of crabs (Kelachet \textit{et al}. 1998; Lim and Rosiah 2007). Processes that modify the structure and morphology of pneumatophores are therefore likely to have large flow-on effects to estuarine ecosystems.

Acid sulfate soil-induced acidification is among the growing threats to mangrove ecosystems. Acid-sulfate soils (ASS) occur when iron sulfide-rich soils are waterlogged (Dent 1986). Drainage, excavation, droughts and other disturbances expose ASS to atmospheric oxygen and oxidise sulfide-rich sediments into large volumes of sulfuric acid (Dent and Pons 1995; Russell and Helmke 2002; Dove and Sammut 2007\textit{b}). The resulting acid mobilises metals by dissolving sulfides and metal-bearing aluminosilicates, which are transported into estuarine waters during periods of rainfall (Dent and Pons 1995). Following rainfall events, the pH of estuarine waters can drop below 3 in the vicinity of drains that channel water from the land (Dent and Pons 1995; Sammut \textit{et al}. 1996; NSW DPI 2007). Estuarine acidification from ASS outflow is typically most pronounced following major rainfall events, but can persist through time if waters have long residence-times, or if acidification events are sufficiently frequent, prolonged or severe (Dent 1986; Sammut \textit{et al}. 1996; Dove 2003; Green \textit{et al}. 2006).

It is unclear to what extent ASS might affect the structure and function of mangroves at sites adjacent to drains that experience persistently low pH from recurring runoff events. Pneumatophore-bearing trees, such as *Avicennia* spp., are adapted to the waterlogged soils of the mid–low shore (Nickerson and Thibodeau 1985; McKee 1993). Pneumatophores enable oxygen diffusion to the underground root system, have photosynthetic capacity and provide tree anchorage in the more muddy and fluid seaward soils (Kathiresan and Bingham 2001; Kitaya \textit{et al}. 2009).
2007). Nevertheless, several studies have indicated that the production and accumulation of sulfuric acid in waterlogged soils can lead to reduced growth, and even death of mangrove saplings (McKee 1993; Kryger and Lee 1996; Youssef and Saenger 1998). It is not known how established trees may be affected by ASS runoff or whether it influences the abundance, morphology and strength of pneumatophores. Research on the ecological impacts of ASS-induced acidification has primarily focussed on impacts to molluscs (Dove and Sammut 2007a, 2007b; Amaral et al. 2011) and fish (Brown et al. 1983; Sammut et al. 1995; Russell and Helmke 2002), with little consideration of impacts to aquatic vegetation.

Here, we assess the effect of repeated exposure to runoff from ASS on the structure of A. marina pneumatophores in estuaries of New South Wales (NSW), Australia, at sites where negative impacts of ASS runoff on benthic invertebrate populations have previously been detected (Amaral et al. 2011). Specifically, we tested for differences in (1) the abundance, (2) morphology and (3) strength of pneumatophores, between areas close to and away from major ASS outflow drains. We hypothesised that in areas close to drains, where pH is permanently depressed, pneumatophores would be less abundant, thinner, shorter and weaker than those at unaffected reference sites. Understanding how estuarine acidification influences the structural complexity of mangrove habitats is essential for the management of this important ecosystem.

Materials and methods

Sampling sites

We examined the effects of ASS outflows on the density, morphology and strength of Avicennia marina pneumatophores within two estuaries of NSW, Australia. Each of the estuaries, the Hunter River (32.915S, 151.801E) and Port Stephens (32.708S, 152.196E), contained areas of high ASS risk (Naylor et al. 1998; NSW DECCW 2010) and were characterised by a subtropical to temperate climate, with hot summers and rainfall throughout the year (Stern et al. 2000).

Within each estuary, we sampled two mangrove forests within the vicinity (<900 m) of major ASS outflow drains with floodgates, at sites previously documented to be affected by ongoing acidification (hereafter, acidified sites), and two mangrove forests at least 2400 m away from drains, and classified as low ASS runoff risk (hereafter, reference sites; Naylor et al. 1998; NSW DECCW 2010).

Measurements of water temperature, salinity and pH were taken at all sites with a multi-parameter, hand-held, water-quality meter (Eutech CyberScan PCD 650, Eutech Instruments Pte Ltd, Singapore) on eight randomly selected dates, including four dates in April–May 2009 and another four in January–February 2010. Over this period, the acidified sites had a pH of 6.52–6.98. The reference sites had a pH of 7.88–7.93. All sites were of similar water temperature (22–23°C), and sites adjacent to drains were of slightly lower (~1) salinity. The acidified sites, at Fullerton Cove and Tomago Wetland in the Hunter River and the entrance and middle of Fenningshams Island (Tilligerry Creek) in Port Stephens, had previously been recorded as having pH values as low as 2–5 (NSW DPI 2006, 2008, 2009).

Sampling

We sampled pneumatophores on the mid-intertidal shore (mean low water ~0.5–0.7 m) of each site in April 2010. We estimated the density of pneumatophores at each site by counting the number within seven randomly positioned 0.5 × 0.5-m quadrats, each separated by at least 5 m. Pneumatophore morphology and strength were assessed from 45–60 randomly selected pneumatophores, each at least 50 mm long, per site. Pneumatophores were collected by cutting each at the sediment–air interface with clippers. Pneumatophores were transported moist and kept refrigerated until strength testing, within 7 days of collection. We measured the height of each pneumatophore, from base to apex, and its diameter (up to 0.01-mm precision) at the base, apex and halfway between the base and apex (hereafter, mid) with Vernier calipers.

The force (N) required to break pneumatophores was determined by three-point bending tests on an Instron Universal testing system (Instron Corporation, Canton, Massachusetts, USA). Each pneumatophore was tested wet. We positioned each pneumatophore horizontally over two 10-mm wide supports, such that at either end the pneumatophore over-hung these by exactly 5 mm. The distance between the two supports consequently varied according to pneumatophore height. A load was applied to the middle of each pneumatophore by a perpendicular metal probe (2 mm diameter) moving downwards at 1.7 mm s⁻¹ until the specimen failed. The maximum load on each pneumatophore immediately before failure was taken as a measure of strength and was correlated with the thickness of the pneumatophore at the middle point at which the force was applied. Although we acknowledge that short-term storage of pneumatophores may influence their absolute strength, our study was more concerned with relative differences between acidified and reference sites.

Statistical analysis

Hypotheses about the influence of estuarine acidification on the total abundance, height and thickness of pneumatophores were tested using analyses of variance (ANOVA). The mixed-model ANOVAs had three factors, namely estuary (2 levels, random), treatment (acidified v. reference, orthogonal to estuary, fixed) and site (2 levels, nested in treatment and estuary, random). Prior to each ANOVA, assumptions of homoscedasticity of variances and normality were confirmed. Where ANOVA detected significant differences, post hoc Tukey’s honestly significant difference (HSD) tests were used to identify sources of differences.

Within each estuary, we compared the relationship between pneumatophore strength and mid-pneumatophore thickness between acidified and reference sites. Sites within treatments of each estuary were pooled where two-tailed Student’s t-tests indicated that the regression slopes between pneumatophore strength and mid-pneumatophore thickness did not differ between these. The regression slopes obtained for each treatment within an estuary were then compared with two-tailed Student’s t-tests (Zar 1984). The null hypothesis was that the regression slopes would not significantly differ.
Results and discussion

Pneumatophore abundance and morphology

Contrary to expectations, neither the density nor the basal- or mid-root thickness of pneumatophores differed between sites close to and those away from drains channelling ASS runoff (Table 1, Fig. 1). Of the morphological variables quantified, only the height and apical thickness of pneumatophores differed between reference and acidified areas (Table 1, Fig. 1). Within each of the two estuaries, pneumatophores were, on average, 20 mm shorter at acidified than at reference sites (Fig. 1).

At acidified sites, the density and basal- and mid-root thicknesses of pneumatophores were within ranges previously reported for mangrove habitats of estuaries in NSW without ASS runoff (Underwood and Barrett 1990; Kelaher et al. 1998; Bishop et al. 2007). Instead, these variables displayed significant variability among sites, irrespective of pH. The absence of an effect of proximity to ASS drains on pneumatophore density or thickness suggests that either pneumatophores that are damaged during severe acidification events can recover during intervening drier periods, or that ASS runoff is unimportant in influencing pneumatophore abundance and thickness relative to other natural and anthropogenic factors.

Our study was conducted following a relatively dry period in which the average rainfall was 1.7 and 1.3 mm day\(^{-1}\) in the Port Stephens and Hunter River estuaries, respectively (calculated based on the preceding 10 weeks; Bureau of Meteorology, Australian Government), and the difference in pH between acidified and reference sites was 1. During wetter periods, the difference in pH between acidified and reference sites can be as high as 6 (NSW DPI 2006, 2008, 2009), and it is possible that larger impacts may be seen at these times. Furthermore, our study could not control for stand age that may directly, and indirectly through environmental modification, influence mangrove morphology (Nickerson and Thibodeau 1985; McKee 1993; Kryger and Lee 1996). Nevertheless, we did not observe differences in the succession and development of mangrove forests among sites, nor have reason to expect significant differences in stand age among sites. Hence, we do not expect that stand age was a significant source of variability.

Although our study was descriptive and not experimental, we have strong reason to believe that differences in pneumatophore height between sites close to and away from drains were primarily due to acidification. Although drains may also conceivably influence salinity, sedimentation and supply of limiting nutrients such as nitrogen and phosphorus, each of which can

<table>
<thead>
<tr>
<th>Source</th>
<th>Density</th>
<th>Height</th>
<th>Basal thickness</th>
<th>Mid-thickness</th>
<th>Apex thickness</th>
</tr>
</thead>
<tbody>
<tr>
<td>d.f.</td>
<td>MS</td>
<td>F</td>
<td>P</td>
<td>d.f.</td>
<td>MS</td>
</tr>
<tr>
<td>Trt</td>
<td>(1 \times 10^4)</td>
<td>(5 \times 10^{-2})</td>
<td>0.854</td>
<td>1</td>
<td>(4 \times 10^4)</td>
</tr>
<tr>
<td>Est</td>
<td>(2 \times 10^5)</td>
<td>(2 \times 10^2)</td>
<td>0.907</td>
<td>1</td>
<td>(4 \times 10^4)</td>
</tr>
<tr>
<td>Site (Trt × Est)</td>
<td>(4 \times 10^5)</td>
<td>4.9</td>
<td>(0.002)</td>
<td>4</td>
<td>(1 \times 10^5)</td>
</tr>
<tr>
<td>Trt × Est</td>
<td>(1 \times 10^5)</td>
<td>1.3</td>
<td>0.313</td>
<td>1</td>
<td>26</td>
</tr>
<tr>
<td>Residual</td>
<td>(1 \times 10^6)</td>
<td></td>
<td>401</td>
<td>636</td>
<td></td>
</tr>
</tbody>
</table>

Fig. 1. Mean (a) density and (b) height of *Avicennia marina* pneumatophores at acidified and reference sites within each of two estuaries, the Hunter River and Port Stephens. Bars that do not share letters are significantly different at \(P = 0.05\) (Tukey’s HSD test). Within each estuary, differences between sites within treatments (i.e. acidified or reference) were non-significant (Table 1), enabling comparison of pneumatophore heights across treatments. Assessments of pneumatophore density were based on \(n = 10\) quadrats per site. For pneumatophore height, \(n = 89–125\) pneumatophores were considered per treatment, within each estuary. Error bars represent s.d.
influence mangrove structure and function (e.g. Kathiresan and Bingham 2001; Ellis et al. 2004; Lovelock et al. 2006), our data are not consistent with an effect of any of these variables. First, the difference of 1 in salinity detected between sites close to and away from drains was negligible compared with the variation in salinity that estuarine sites typically experience across a tidal cycle. Second, the pattern of greater pneumatophore height close to than away from drains was opposite to what would be expected if drains were influencing pneumatophore growth by enhancing the supply of growth-limiting nutrients such as nitrogen or phosphorous. Third, the similar density of pneumatophores at acidified and reference sites suggests that sedimentation, which tends to increase pneumatophore density (Ellis et al. 2004), was not a contributing factor. Instead, the observation of shorter pneumatophores adjacent to drains is consistent with previous observations that in areas where sulfuric acid has accumulated in mangrove soil, *Avicennia* spp. pneumatophores are abnormally short (Kryger and Lee 1996). High sulfide concentrations can damage the membranes of mangrove root cells, causing stomatal closure and inhibiting photosynthetic gas exchange (Youssef and Saenger 1998).

**Pneumatophore strength**

Whereas mid-pneumatophore thickness did not differ between acidified and reference sites, pneumatophores were up to two-fold weaker close to drains (Table 2, Fig. 2). Previous studies

### Table 2. Results of two-tailed Student’s *t*-tests comparing slopes of linear regressions describing the relation among force and pneumatophore thickness within acidified (A1, A2) and reference (R1, R2) sites within the Hunter River (H) and Port Stephens (P) estuaries

<table>
<thead>
<tr>
<th>Comparison</th>
<th><em>t</em></th>
<th>d.f.</th>
<th><em>P</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>HA1 v. HA2</td>
<td>0.69</td>
<td>83</td>
<td>0.507</td>
</tr>
<tr>
<td>HR1 v. HR2</td>
<td>1.66</td>
<td>77</td>
<td>0.159</td>
</tr>
<tr>
<td>PA1 v. PA2</td>
<td>0.39</td>
<td>82</td>
<td>0.712</td>
</tr>
<tr>
<td>PR1 v. PR2</td>
<td>0.74</td>
<td>83</td>
<td>0.490</td>
</tr>
<tr>
<td>HA v. HR</td>
<td>4.21</td>
<td>169</td>
<td><strong>0.004</strong></td>
</tr>
<tr>
<td>PA v. PR</td>
<td>5.29</td>
<td>164</td>
<td><strong>0.002</strong></td>
</tr>
</tbody>
</table>

Values significant at *a* = 0.05 are highlighted in bold. All data were analysed untransformed.
have shown that acidification of water weakens the strength of the carbonate structures of marine shell-forming organisms, such as corals, bivalves and gastropods, by decreasing the saturation state of seawater for carbonate ions (Kathiresan and Bingham 2001; Feely et al. 2004). The corrosive capacity of the sulfuric acid produced from ASS is also able to reduce the strength of various materials, ranging from plastic to concrete (Dent and Pons 1995). Given that pneumatophores are not calcified structures and we found no signs of corrosion, these mechanisms do not, however, appear to apply here. Instead, the accumulation of sulfides in the root system of mangrove trees (Kryger and Lee 1996; Youssef and Saenger 1998), responsible for shorter pneumatophores at ASS-affected areas, may also be involved in the loss of pneumatophore strength at these locations. Because no previous studies exist on pneumatophore load resistance, future research is required to ascertain whether the impact of sulfide accumulation is via a change in the wood density, the elastic properties and/or other features of pneumatophores.

Implications of findings

Overall, the effects of ASS runoff on pneumatophores observed in the present study were of lower magnitude than expected from previous studies that have documented large impacts of this disturbance on naïve molluscs (Dove and Sammut 2007a, 2007b) and mobile fish (Brown et al. 1983; Russell and Helmke 2002). Coupled with another recent study, which documented minimal effects of acidification on wild molluscs (Amaral et al. 2011), our results suggest that estuarine systems might possess some natural capacity to resist ASS disturbances. Our study was not, however, designed to fully capture spatial and temporal variability in the impact of ASS runoff on the structure and strength of pneumatophores. The duration, intensity and frequency of acidification events are likely to contribute to environmental impacts (e.g. Sammut et al. 1996) and may in turn be dictated by floodplain drainage, inundation, geomorphology and floodgate management (Dent 1986; Russell and Helmke 2002; Johnston et al. 2009).

Our observation of shorter and weaker pneumatophores close to than away from ASS runoff drains raises the possibility of cascading effects to dense and diverse mangrove fauna, the distribution and abundance of which is strongly tied to pneumatophore traits. Taller pneumatophores offer greater protection from predators, provide a greater surface area for attachment of sessile organisms and may be more effective at trapping wrack and other organic matter than are shorter pneumatophores (Skilleter and Warren 2000; Chapman et al. 2005; Bishop et al. 2009). Longer pneumatophores also decrease the erosive force of tides and waves, leading to more sediment deposition, potentially increasing nutrients and food, and reducing fluid stress on organisms (Gwyther and Fairweather 2002; Ellis et al. 2004; Kathiresan and Qaim 2005). Hence, at acidified sites, weakened and stunted pneumatophores may be able to support fewer molluscs, such as sessile oysters and mussels, before collapse. Nevertheless, the persistence of dense pneumatophores at acidified sites suggests that the capacity for pneumatophores to facilitate other invertebrates and fish communities may remain. More studies focusing on functional elements of mangrove forest communities are necessary for better understanding of the effects of estuarine acidification on these highly productive and valuable ecosystems.

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