



# Saltwater intrusion history shapes the response of bacterial communities upon rehydration



Tiffanie M. Nelson <sup>a,\*</sup>, Claire Streten <sup>a</sup>, Karen S. Gibb <sup>b</sup>, Anthony A. Chariton <sup>c</sup>

<sup>a</sup> Australian Institute of Marine Science, Sustainable Coastal Ecosystems and Industries in Tropical Australia, Arafura Timor Research Facility, 23 Ellengowan Drive, Casuarina, NT 0810 Australia

<sup>b</sup> Research Institute for the Environment and Livelihoods, Charles Darwin University, Darwin, NT, Australia

<sup>c</sup> CSIRO Land and Water, Locked Bag 2007, Kirrawee, NSW, Australia

## HIGHLIGHTS

- Soil microbes may be impacted by saltwater intrusion (SWI).
- We simulated a SWI event and documented changes in bacterial community composition.
- Sites with no history of SWI did not respond as they are not pre-conditioned to respond to saltwater.
- Sulfate-reducing bacteria increased following saltwater treatment at sites with a history of SWI.
- Saltwater impacts bacteria causing a shift in cycling of essential nutrients.

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## ABSTRACT

Saltwater intrusion (SWI) can result in the loss of dominant vegetation from freshwater habitats. In northern Australia, sea level is predicted to rise 17–50 cm by 2030–2070. This will exacerbate the impact of SWI, threatening Ramsar-listed habitats. Soil bacteria in these habitats play a significant role in biogeochemical cycling, regulating availability of essential nutrients such as nitrogen to vegetation. However, there is limited understanding as to how SWI will impact these soil bacteria. Floodplain soil samples were collected from the South Alligator River floodplain in Northern Australia from sites with contrasting histories of SWI. A SWI event was simulated over 7 days with treatments of saltwater and freshwater. Bacterial community composition before and after treatment were measured using next generation sequencing of bacterial DNA. Sites with no history of SWI showed no significant changes in community taxonomic composition following treatments, suggesting the community at these sites have broad functional capacity which may be due to their historic conditioning over many years. Sites with a history of SWI showed a significant response to both treatments. Following saltwater treatment, there was an increase in sulfate-reducing bacteria, which are known to have an impact on carbon and nitrogen cycling. We suggest that the impact of SWI causes a shift in the soil bacteria which alters the community to one which is more specialised, with implications for the cycling of essential elements and nutrients.

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## 1. Introduction

Soil bacterial communities are some of the most plentiful and diverse on the planet with an estimated  $2.6 \times 10^{29}$  cells (Whitman et al., 1998; Lozupone and Knight, 2007). In wetlands and freshwater habitats, soil bacteria contribute greatly to biogeochemical cycling of key nutrients, such as nitrogen, phosphorus, sulfur and methane, and are an important sink for carbon (Fuhrman, 2009). These nutrients are essential to plant growth, and the soil bacterial community has an important

role in regulating their availability. However, the composition and function of bacteria can be altered by abiotic changes, such as salinity (Horz et al., 2004; Lozupone and Knight, 2007; Jeffries et al., 2012). Under increased salinity regimes, bacterial communities display increases in carbon cycling and photosynthesis and decreases in phosphate and nitrogen cycling (Jackson and Vallaire, 2009; Jeffries et al., 2012; Cañedo-Argüelles et al., 2014).

Saltwater intrusion (SWI) has a significant effect on freshwater ecosystems (Mulrennan and Woodroffe, 1998; Long et al., 2012). The process involves saltwater moving into freshwater habitats due to a number of complex local features, including tidal influences, low altitude, sea-level rise, rainfall, boat traffic and the impact of feral animals (Mulrennan and Woodroffe, 1998; Petty et al., 2007; Hughes, 2010). This can result in the die-off of dominant vegetation and the loss of

Abbreviations: SWI, saltwater intrusion; FW, freshwater; SW, saltwater; PCR, polymerase chain reaction.

\* Corresponding author. Tel.: + 61 425 335 143.

E-mail address: [tiffanie.nelson@gmail.com](mailto:tiffanie.nelson@gmail.com) (T.M. Nelson).

suitable habitat for aquatic and terrestrial organisms (Winn et al., 2006; Bowman et al., 2010). Grasses such as *Pseudoraphis spinescens* and *Hymenachne acutigluma*, which are a major component of the vegetation on freshwater floodplains, and *Melaleuca* species will potentially be lost due to SWI (Finlayson, 1991). The debris of these grasses left at the end of the wet season and the leaf litter from the *Melaleuca* species is rich in nitrogen, phosphorous and potassium and they are important contributors to elemental cycling on the floodplains (Finlayson, 1991; Finlayson et al., 1993). Thus, a loss of these grass and *Melaleuca* species causes a decrease in available nutrients such as nitrogen. Freshwater vegetation species in Kakadu National Park are predicted to decline at  $\approx 3.7$  psu (practical salinity units) while mangroves in Northern Australia prefer a moderate salinity range of 16 to 50 psu (Ball, 1998). These findings suggest that following SWI, there will potentially be a period of low vegetation and a decrease in nutrient availability on the floodplains.

The Intergovernmental Panel on Climate Change predictions suggest increases of 17–50 cm by 2030–2070 (Stocker et al., 2013). This rise will amplify the occurrence of SWI in many areas, threatening the ecological function and maintenance of biodiversity in high-value wetlands. Because of the region's low topography, extensive areas in Northern Australia are susceptible to sea level rise (Hughes, 2010). Some areas have already undergone dramatic changes caused by SWI (Mulrennan and Woodroffe, 1998; Petty et al., 2007). On the Lower Mary River floodplains located adjacent to Kakadu National Park, more than 17,000 ha of freshwater habitat have been destroyed due to SWI (Mulrennan and Woodroffe, 1998; Bowman et al., 2010). This example provides a window into potential future impacts that predicted sea level rise scenarios could have on nearby World Heritage-listed Kakadu National Park and its extensive range of Ramsar-listed freshwater habitats.

To investigate the impact of SWI on the soil bacterial community of these wetland systems, we simulated a laboratory-based SWI event on floodplain soils collected from sites with contrasting histories of SWI. Changes in bacterial community function and biogeochemical cycling is often indicated by changes in bacterial community composition

(Reed and Martiny, 2013). Therefore, bacterial community composition was monitored before and after treatments with saltwater and freshwater.

## 2. Materials and methods

### 2.1. Study sites

The South Alligator River is located 220 km east of Darwin in the World Heritage-listed Kakadu National Park, Northern Territory, Australia (Fig. 1). It is a macro-tidal river 160 km in length with a tidal range of 5–6 m which extends 105 km up the river (Woodroffe et al., 1989). The floodplains flanking the river were previously covered with mangrove swamps up until 6000 year BP (before present) (Woodroffe et al., 1985, 1989). It was at this time that sea-level stabilised and the floodplains became the sedge and grass floodplain that exists today. The region is dominated by a tropical monsoonal climate, with a highly seasonal rainfall regime that defines two distinct seasons, the Dry and the Wet. Variation in rainfall, including rainfall intensity and the duration of the Wet season produces an immense change in the quantity of freshwater runoff transported across the catchment. The average annual rainfall of the region from Darwin to the Alligator Rivers is between 1300 and 1600 mm (Eliot et al., 2000). In contrast to this, very little rain falls during the Dry season months from May to September and this markedly affects the salinity structure of the river. The pronounced seasonality of the climate may be a significant factor in affecting regional vulnerability to saltwater intrusion (Woodroffe and Mulrennan, 1993).

Sites defined by Woodroffe et al. (1986) as lower floodplain were selected with different histories of SWI. Site 7 ( $12^{\circ}37'19.95''S$ ,  $132^{\circ}29'22.25''E$ ) had a history of SWI as indicated by tidal creek extension and mangrove encroachment around the site since the 1950s (Cobb et al., 2007). Soil salinity of replicates at this site was  $5.62 \pm 0.24$  psu. Site 10 ( $12^{\circ}33'5.57''S$ ,  $132^{\circ}27'29.23''E$ ) had no history of SWI and soil

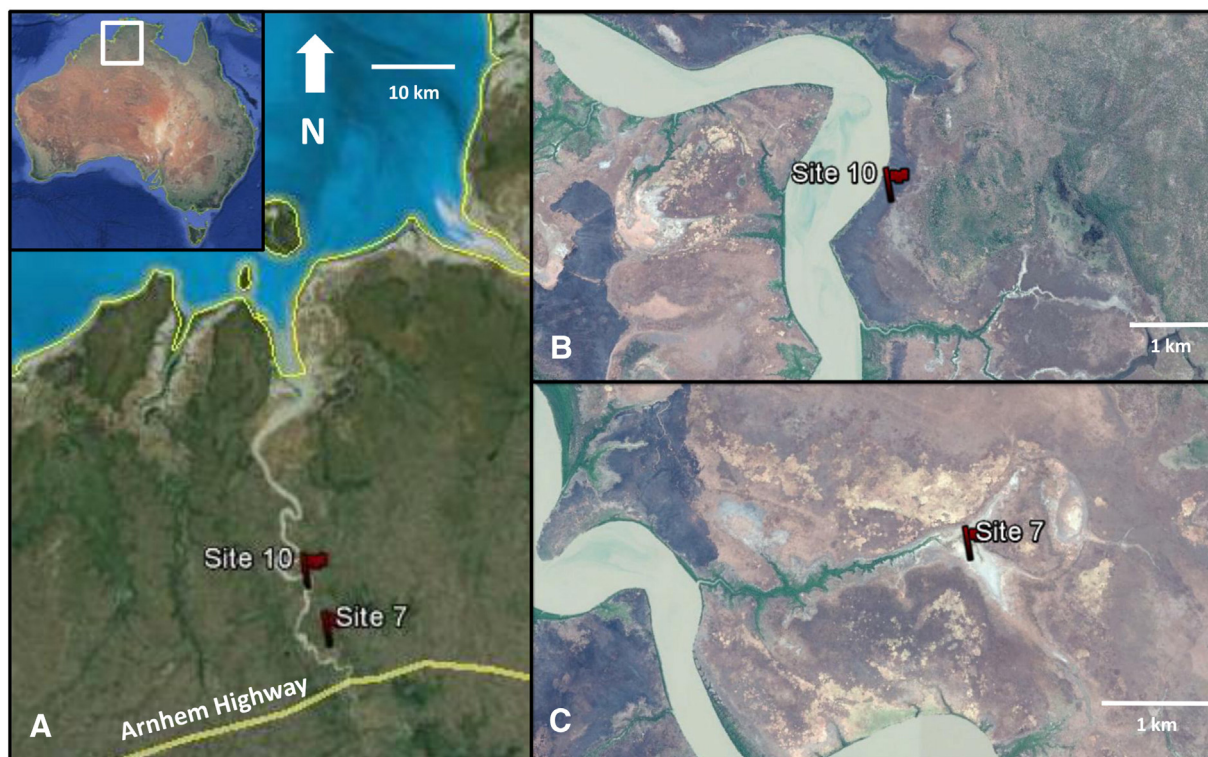


Fig. 1. Location of study site. Location of South Alligator River floodplain, Kakadu National Park (A) with location in reference to Northern Territory, Australia (inset). Sampling site with no history of saltwater intrusion (B) and sampling site with a history of saltwater intrusion (C) are shown enlarged.

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