Soil Biology & Biochemistry 115 (2017) 73–81

Contents lists available at ScienceDirect

Soil Biology & Biochemistry

journal homepage: www.elsevier.com/locate/soilbio

# Short-term microbial respiration in an arid zone mangrove soil is limited by availability of gallic acid, phosphorus and ammonium

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ARTICLE INFO

*Article history:*
Received 12 September 2016
Received in revised form
7 August 2017
Accepted 11 August 2017
Available online 18 August 2017

*Keywords:*
Microbial respiration
Nutrient and carbon limitation
Exmouth Gulf
*Avicennia marina*

ABSTRACT

Microbial activity in soils of oligotrophic, arid zone mangroves is likely strongly limited by carbon (C) and nutrient availability, where even small changes in microbial activity could result in significant shifts in ecosystem functioning. We hypothesised that microbial respiration in arid mangrove ecosystems is primarily limited by supply of labile C sources. We measured short-term respiration responses to addition of glucose, citric acid, gallic acid, phosphate, ammonium ($NH_4^+$), nitrate ($NO_3^-$), urea and glutamic acid to mangrove soils from different tidal positions and soil depths in the laboratory using an adaptation of the MicroResp™ procedure (μg $CO_2$-C g $soil^{-1}$ $hr^{-1}$). We also measured short-term respiration responses to added glucose, gallic acid, phosphate, ammonium and urea in the field using an infrared gas analyser (g $CO_2$ $m^{-2}$ $hr^{-1}$). Both laboratory and field measurements indicated microbial communities were limited by gallic acid, phosphate and ammonium. Respiration rates were enhanced in both the laboratory and the field after addition of gallic acid but not glucose, suggesting adaptations of the arid microbial community to more complex C sources. Addition of phosphate alone and in combination with other substrates enhanced respiration more than ten-fold in the laboratory. In the field, nutrient addition (ammonium and/or phosphate) generally induced greater respiration responses in the surface soils (0–1 cm) compared to subsurface (>1 cm), which we attribute to more nutrient-limited autotrophic microbes in the surface soils. Addition of phosphate also induced slightly higher activity in the low intertidal fringing forests compared to high intertidal scrub forests. Our results contrast with studies of more productive tropical mangrove systems and demonstrate the critical role of microorganisms in maintaining organic matter turnover and nutrient supply in a relatively pristine and water-limited environment.



## 1. Introduction

Mangrove ecosystems are generally considered as highly productive, especially in the wet tropics (Bouillon et al., 2008; Kristensen et al., 2008). Mangrove soils thus potentially play an important role in global carbon (C) storage (Alongi et al., 2003). However, around a quarter of the global mangrove coastline occurs in arid and semi-arid climates (Spalding et al., 2010). The main controls of organic matter (OM) turnover and thus C storage of arid zone mangrove ecosystems are largely unknown. Mangroves in the arid tropics are less productive than other tropical forests as a consequence of low rainfall and high salinities (Cintron et al., 1978; Saenger and Snedaker, 1993; Semeniuk, 1993), which likely reduces the amount of available C to the soil microbial community. Mangrove ecosystems in more arid environments are also often naturally oligotrophic, which is likely to further limit microbial activity (Reef et al., 2010). Consequently, arid conditions may create more severe C and nutrient limitations for soil microorganisms compared to their wet tropical counterparts.

Mangrove soils show vertical stratification of the structure of the microbial community over relatively small scales (mm to cm scales; Leopold et al., 2013; Tam, 1998). Autotrophic microorganisms (such as diatoms and cyanobacteria) are often abundant and form a surface layer in soils of both tropical and temperate mangrove forests (Alongi et al., 2001; Leopold et al., 2013; Lovelock,

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http://dx.doi.org/10.1016/j.soilbio.2017.08.010
0038-0717/

2008). Conceptually, this layer might be considered somewhat analogous to the biological soil crusts in deserts (Belnap and Lange, 2001; Elliott et al., 2014), and often include a high abundance also of nitrogen -fixing diazotrophs (Holguin et al., 2001). Autotrophs are likely to be nutrient rather than C-limited as they fix $CO_2$ during daylight hours. For example, benthic microalgae in wet tropical mangrove soils have been described as either phosphorus (P) or nitrogen (N) limited (Alongi et al., 1993; Lee and Joye, 2006). The canopies of arid zone mangroves are more open than tropical forests and thus surface microbes are generally not light limited. We might thus expect that autotrophic microbes in surface soils to respond to nutrient additions. In deeper soil, where heterotrophic microorganisms dominate (Tam, 1998), microbial metabolism may also be more limited by the availability of labile carbon substrates.

In mangrove ecosystems, intertidal hydrology can further impact microbial processes due to tidal influence on biophysical factors such as supply of allochthonous C and nutrients, as well as the quality and supply of OM (Kristensen et al., 2008; Lee and Joye, 2006; Lovelock, 2008). Generally, the low intertidal soils receive more allochthonous OM inputs; fine roots of trees fringing the water edge may also have higher nutrient contents compared to those higher in the intertidal zone (Adame et al., 2010; Lovelock et al., 2006). Arid mangrove ecosystems are likely to rely more upon tidal inputs of C and nutrients for maintenance of productivity (Alongi et al., 2003; Lovelock et al., 2011), whereas tropical ecosystems may also rely upon riverine inputs (Twilley et al., 1992). In the arid zone, hypersalinity occurs in many higher intertidal positions due to low rates of freshwater inputs and less frequent inundation coupled with high rates of evaporation, which in turn may result in a shorter 'scrub' forest. Long-term studies of mangrove soils have demonstrated soil respiration and/or microbial biomass increases more in response to long-term fertilisation of trees (or scrub) high in the intertidal zone compared with areas that are more frequently inundated by tides (Keuskamp et al., 2015; Lovelock et al., 2014). However, short-term experiments are required to better understand potential microbial metabolic responses to differences in both forms and amounts of C, and nutrient availability, that can arise from lower rates of OM production and inputs at higher compared to lower intertidal positions.

Mangrove soils in the Exmouth Gulf of Western Australia are low in C and nutrients (Lovelock et al., 2011) compared to those in the wet tropics, owing to extreme aridity, ancient weathered parent materials (Beard, 1990) and isolation from human developments (Brunskill et al., 2001). Here, we sought to determine the C and/or nutrient substrates that promote microbial respiration in a low productivity mangrove ecosystem. We hypothesised that (i) microbial respiration in arid mangrove soils is primarily limited by availability of labile C and secondarily by N and P; (ii) microbial respiration is more limited by P and N in surface soils but by labile C in subsurface soils; and (iii) C, P and N limit microbial respiration more in high intertidal scrub forest soils compared to low intertidal fringe forest soils.

## 2. Methods

### 2.1. Study site description and field sampling

The study site was at Giralia Bay, on the oligotrophic Exmouth Gulf of arid Western Australia (22.53°S, 114.3°E). Mean annual rainfall is 260 mm and daytime maximum air temperatures range between 24 °C in July and 38 °C in January (Australian Bureau of Meteorology, 2016). Samples were collected and measurements taken from mangrove soils beneath *Avicennia marina* (Forsk.) Vierh. stands at the low intertidal position (fringe) and from scrub forest at higher elevations near an inland saltpan where tidal influence is reduced (scrub). For a full description of the study site see Lovelock et al. (2011).

For laboratory incubations we collected five replicate soil cores (10 cm deep and 6 cm diameter) at each of the low and high intertidal soils in early August 2013. Porewater was collected from water that infiltrated the core holes at approximately 10 cm depth, which was then filtered to 0.2 μm and stored at 4 °C until analysis within two weeks. Cores were collected within 1 m of the base of each of five trees using PVC pipe and capped. Cores were stored intact in the dark at 4 °C for approximately three months. Prior to commencement of the experiment, all soil cores were brought to room temperature and then incubated at 25 °C in the dark for one week. Surface (0–1 cm) and sub-surface samples (1–4 cm) were then separated before measurement of response to C and nutrient additions.

### 2.2. Laboratory carbon and nutrient additions

In order to assess the potential limitations of C and nutrients to soil microbial activity we used an adaption of the MicroResp™ method (Campbell et al., 2003), which we adjusted for wetter intertidal soils. To our knowledge this is the first time the MicroResp™ method has been applied to mangrove soils; we also tested nutrient substrates in addition to C substrates. The MicroResp™ method consists of two microtitre plates. One plate is a deep-well plate that holds soil samples with added substrates. Another plate sits on top of the soil plate and detects the evolved $CO_2$ via colour change. The plate that detected colour change had modified reactant concentrations for the indicator gel as per Lalor et al. (2007). Approximately 4 $mm^3$ of fresh soil was sub-sampled from each soil depth (surface or subsurface) using a 1 mL syringe with the tip cut off and placed into a deep well in a 96-well microtitre deep-well plate. The syringe volume measure of 4 $mm^3$ approximated 0.38 g ± 0.02 SD (standard deviation) dry weight in the fringe and 0.45 g ± 0.06 SD dry weight in the scrub soils. Soil was dispensed into wells as quickly as possible and wells were plugged with an airtight seal to maintain soil moisture until substrates were added.

A range of substrates was selected to test microbial community responses to labile C and nutrient limitations, with a Milli-Q (MQ) water control (Table 2). Substrates were either C-based (glucose, citric acid, gallic acid), phosphorus-based ($NaH_2PO_4$), or nitrogen-based ($(NH_4)_2SO_4$, $NaNO_3$, urea and glutamic acid). Representative C (glucose and gallic acid), P (phosphate), and N ($NH_4^+$) substrates were also combined in a pairwise manner to test for any secondary limiting substrates. We focused on combinations of C with N that were expected to produce the largest response based on published studies of mangrove soils (see Table 1); amounts of each substrate added were also consistent with those used in previous studies of Western Australian soils (Cookson et al., 2008; Lalor et al., 2007). Combined phosphate and gallic acid was not tested under laboratory conditions owing to limited surface soil collected, and a small time window in which to pipette soil to wells and add substrates without soils drying out. The same molar amount of each substrate was added to each well in their separate and combined form.

Preliminary tests (data not shown) were undertaken to confirm the concentrations of substrate added to the soil samples were in excess of what the microbial community could use within a two hour incubation period (i.e. no significant decrease in respiration rates over the two hour period). Because the mangrove soils had a higher moisture content (approx. 30–40% w/w) than most soils under field conditions, we reduced the concentrations of substrate added in Lalor et al. (2007) by approximately a third. Therefore, most substrates were applied at a rate of 22 mg substrate g soil