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Nutrient amendment does not increase mineralisation of sequestered carbon during incubation of a nitrogen limited mangrove soil

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ABSTRACT

Mangrove forests are sites of intense carbon and nutrient cycling, which result in soil carbon sequestration on a global scale. Currently, mangrove forests receive increasing quantities of exogenous nutrients due to coastal development. The present paper quantifies the effects of nutrient loading on microbial growth rates and the mineralisation of soil organic carbon (SOC) in two mangrove soils contrasting in carbon content. An increase in SOC mineralisation rates would lead to the loss of historically sequestered carbon and an enhanced CO_2 release from these mangrove soils.

In an incubation experiment we enriched soils from *Avicennia* and *Rhizophora* mangrove forests bordering the Red Sea with different combinations of nitrogen, phosphorus and glucose to mimic the effects of wastewater influx. We measured microbial growth rates as well as carbon mineralisation rates in the natural situation and after enrichment. The results show that microbial growth is energy limited in both soils, with nitrogen as a secondary limitation. Nitrogen amendment increased the rate at which labile organic carbon was decomposed, while it decreased SOC mineralisation rates. Such an inhibitory effect on SOC mineralisation was not found for phosphorus enrichment.

Our data confirm the negative effect of nitrogen enrichment on the mineralisation of recalcitrant carbon compounds found in other systems. Based on our results it is not to be expected that nutrient enrichment by itself will cause degradation of historically sequestered soil organic carbon in nitrogen limited mangrove forests.

1. Introduction

Mangroves are highly productive ecosystems, growing at the interface of land and sea along much of the tropical and subtropical coastlines, estuaries and river mouths. Their position between land and sea makes them critical in land-sea nutrient exchange. Due to their high primary productivity and biological activity they can be considered a hotspot for nutrient and carbon cycling so that changes in their functioning will have a substantial influence on coastal nutrient and carbon dynamics.

Mangrove soils are a large sink for carbon with estimated average carbon burial rates three to ten times higher than those of northern peatlands (Duarte et al., 2005; Bouillon et al., 2008; Limpens et al., 2008). Soil carbon content varies in mangroves, but many mangroves are peat-forming with peat layers up to several metres thick (Middleton and McKee, 2001; McKee et al., 2007) therefore containing significant amounts of carbon per unit of area.

During the past decades, nutrient influx to coastal systems has been increasing due to anthropogenic activity and could be considered a component of global change (Duarte, 2009; Nixon, 2009). These nutrient influxes affect several major processes in the mangrove carbon cycle, amongst which mangrove growth (Feller, 1995), peat build-up (McKee et al., 2007), and decomposition of leaf (Feller et al., 1999) and root litter (Huxham et al., 2010). Soil organic carbon (SOC) decomposition is potentially enhanced, changing mangroves from a carbon sink to a carbon source, especially if large amounts of historically sequestered carbon are mineralised. In peat-forming mangroves, this ultimately causes the system to collapse through elevational loss resulting in increasing inundation times and dieback of mangrove trees.

Nitrogen (N) and phosphorus (P) are the major limiting nutrients for mangrove tree growth (Reef et al., 2010) and their inflow

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rates to coastal waters have dramatically increased over the past decades (Seitzinger et al., 2010). The other macronutrients – potassium, calcium, magnesium and sulphur – are less likely to be limiting in a marine environment, as they are major constituents of seawater. We will therefore focus on nitrogen and phosphorus enrichment as a potentially moderating factor on SOC decomposition rates.

Additions of nitrogen and phosphorus stimulate plant growth if either of these are limiting. Likewise, nitrogen and phosphorus additions can be expected to stimulate decomposition when either of these elements is limiting microbial activity. Feller et al. (1999) indeed found enhanced litter decomposition after P addition in a P-limited mangrove. The effect of nitrogen on decomposition rate is however rather complex, since SOC mineralisation has been shown to be either increased, unaffected or decreased by addition of N. Many studies have revealed that decomposition of recalcitrant litter (Knorr et al., 2005; Berg and Laskowski, 2006) and SOC (Neff et al., 2002) is inhibited by external nitrogen addition, while the decay of easily degradable litter or labile organic carbon (LOC) is stimulated, as also predicted by the nitrogen-mining theory (Moorhead and Sinsabaugh, 2006; Craine et al., 2007). The net effect on total SOC is not always clear: Mack et al. (2004) have demonstrated that long-term fertilisation in a tundra peatland leads to a dramatic loss of soil carbon through increased SOC decomposition rates while Shaver et al. (2006) find lower respiration rates in the same plots.

To our knowledge, the direct effects of nutrient and LOC addition on microbial growth and mineralisation rates in mangrove ecosystems have not been elucidated yet. In our research we assessed the effects on microbial growth and activity by measuring microbial respiration rates in soils from two common mangrove genera *Avicennia* and *Rhizophora* after amendments of nutrients and glucose. We expect that nitrogen addition increases microbial growth rate and LOC mineralisation in both genera, but that overall microbial activity is lower in the *Rhizophora* soil due high content of tannins, known to inhibit decomposition (Robertson, 1988).

The effect of nutrient additions on SOC mineralisation rates was studied more detailed in the *Avicennia* soil by measuring change in microbial biomass and respiration upon nitrogen, phosphorus, and glucose amendment. Here we expect that the amended nutrients differentially modify LOC and SOC decomposition rates: nitrogen as well as phosphorus addition will stimulate decomposition of LOC, while nitrogen but not phosphorus will inhibit SOC mineralisation.

2. Material and methods

2.1. Study site features

The soils used for incubation were collected from mangrove stands dominated by either *Avicennia marina* or *Rhizophora mucronata* on Saudi Arabian islands in the Red Sea. The sampled *Avicennia* site was located on a small island just outside the campus of the King Abdullah University of Science and Technology (KAUST), near the village of Thuwal, Jeddah. The *Avicennia* covering the island varied in height, with tree sizes from ±.5 m in the dwarf zone up to 5 m in the fringe. The soil cores were taken in the western part of the island (22°19′52″N, 39°05′59″E) where tidal floods could freely enter. In this area, trees had an average height of 3.5 m.

The *Rhizophora* site was located at the Farazan Islands, an archipelago of coral islands in the southernmost part of Saudi Arabia. This group of islands probably supports the largest population of *Rhizophora* in the Red Sea (El-Demerdash, 1996). The soils were taken at the north-eastern part of Farazan Kebir, the largest of the Farazan Islands, at 16°47′24″N, 42°05′59″E. This site was protected from high-energy waves by a number of land-tongues. Average tree height in this stand was similar to that of the *Avicennia* site.

2.2. Soil collection and analysis

Within each study site, nine sampling locations were selected to account for small-scale variation. Soils were classified using the WRB soil classification system (IUSS Working Group WRB, 2007). At each location, eight 10 cm soil samples were taken using a stainless steel soil corer with a diameter of 9.6 cm. Directly after sampling, redox conditions were measured at 5 cm from the top of the core using a Sentix PtR electrode (WTW GmbH, Weilheim, Germany). Pore water was sampled using 10 cm long Rhizon soil moisture samplers (Eijkelkamp BV, Giesbeek, the Netherlands). On the day of sampling, pore water pH was measured using a Sentix 41 pH electrode (WTW GmbH, Weilheim, Germany) and salinity was determined using an optical refractometer with automatic temperature correction. Pore water and soil samples were sent to the Utrecht University, The Netherlands, and stored at 4 °C until further analysis and incubation. The *Rhizophora* soils were sampled in November 2009; the *Avicennia* soils were sampled one year later in November 2010. Incubations started within five weeks after sampling. Bulk density of the soil was calculated from the core weight and volume of the corer. It does therefore include the effect of crab holes and other tertiary structures. Soil moisture content was determined by weight loss after a 48 h drying period at 70 °C. C/N ratios were determined using an EA/110 CHNS-O analyser (Interscience BV, Breda, The Netherlands). In preparation for the C/N determination soils were homogenised and ground using an MM200 mixer mill (Retsch GmbH, Haan, Germany) at 20 RPS during two minutes. After grinding, the soils were washed with a 32% HCl solution to remove $CaCO_3$ and dried for 48 h at 70 °C to evaporate excess HCl. Measured C and N concentrations were corrected for weight changes due to the HCl washing. Pore water was analysed for PO_4^-, NH_4^+, NO_3^-, dissolved organic nitrogen (DON) and dissolved organic carbon DOC using a continuous flow auto analyser (Skalar SA-40, Breda, The Netherlands).

2.3. Incubation experiment

As mangrove soils are oxygenated with fresh air when the tide lowers, while mangrove roots oxygenate their surroundings during high tides, incubations were conducted in atmospheric circumstances as we believe this most closely matches the prevalent conditions in the sampled top layer. Before incubation, soils were allowed to drain with gravitation to mimic field conditions just after a flooding event. Collected soils were incubated to measure Substrate Induced Respiration (SIR) after Anderson and Domsch (1978) and subsequent growth respiration to calculate microbial growth rates. Respiration was measured after amendment with different combinations of glucose, ammonium, and phosphorus to alleviate energy and/or nutrient limitations. Soil samples of both *Avicennia* and *Rhizophora* stands were subjected to five treatments: Control, glucose (C), glucose plus ammonium (CN), glucose plus phosphorus (CP), and glucose plus ammonium plus phosphorus (CNP). The *Avicennia* soil additionally received an ammonium plus phosphorus (NP) treatment, without glucose. In preparation for the incubations, soil cores were manually cleared from roots and shells and homogenised. The homogenised soil was allowed to acclimatise for three days at 20 °C in a dark box covered with a moist cloth to minimise evaporation from the soil.

To start the incubations, .1 ml treatment solution per gram soil fresh weight (FW) was thoroughly mixed through the soil and 10 g of the amended soil was put in 600 ml flasks. D^+ glucose was added as a source of labile organic carbon in the C treatment (.6 mg C g soil