Soil Biology & Biochemistry 81 (2015) 38–47

Contents lists available at ScienceDirect

Soil Biology & Biochemistry

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

Short- and long-term effects of nutrient enrichment on microbial exoenzyme activity in mangrove peat

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ARTICLE INFO

Article history:
Received 10 September 2014
Received in revised form
31 October 2014
Accepted 3 November 2014
Available online 15 November 2014

Keywords:
Mangroves
Rhizophora
Peat
Microbial activity
SOC
Decomposition
Differential nutrient limitation
Microbial elemental stoichiometry

ABSTRACT

Mangroves receive increasing quantities of nutrients as a result of coastal development, which could lead to significant changes in carbon sequestration and soil subsidence. We hypothesised that mangrove-produced tannins induce a nitrogen (N) limitation on microbial decomposition even when plant growth is limited by phosphorus (P). As a result, increased N influx would lead to a net loss of sequestered carbon negating the ability to compensate for sea level rise in P-limited mangroves. To examine this, we quantified the short- and long-term effects of N and P enrichment on microbial biomass and decomposition-related enzyme activities in a *Rhizophora mangle*-dominated mangrove, which had been subjected to fertilisation treatments for a period of fifteen years. We compared microbial biomass, elemental stoichiometry and potential enzyme activity in dwarf and fringe-type *R. mangle*-dominated sites, where primary production is limited by P or N depending on the proximity to open water. Even in P-limited mangroves, microbial activity was N-limited as indicated by stoichiometry and an increase in enzymic activity upon N amendment. Nevertheless, microbial biomass increased upon field additions of P, indicating that the carbon supply played even a larger role. Furthermore, we found that P amendment suppressed phenol oxidase activity, while N amendment did not. The possible differential nutrient limitations of microbial decomposers versus primary producers implies that the direction of the effect of eutrophication on carbon sequestration is nutrient-specific. In addition, this study shows that phenol oxidase activities in this system decrease through P, possibly strengthening the enzymic latch effect of mangrove tannins. Furthermore, it is argued that the often used division between N-harvesting, P-harvesting, and carbon-harvesting exoenzymes needs to be reconsidered.

1. Introduction

Mangrove ecosystems are commonly found in tropical and subtropical coastal zones, where they are of major importance to local nutrient- and carbon-cycling (Alongi, 1996). In the absence of soil elevation, rising sea levels lead to dieback of mangroves resulting from increased inundation times. In systems where sediment input is not substantial, survival of mangroves depends mostly on the build-up of peat (McKee, 2011), resulting from the imbalance between primary production and decomposition. Nutrient enrichment in these systems can influence both of these processes. Eutrophication, therefore, may have unforeseen effects on mangrove stability (McKee et al., 2007), leading to habitat loss if soil accumulation is negatively affected.

Decomposition is catalysed by several key exoenzymes that allow for extracellular conversion of complex organic matter into simpler products, such as glucose, amino acids, and phosphate. Mangrove litter, especially from *Rhizophora* spp., contains large amounts of dissolvable and non-dissolvable tannins (Alongi, 1987; Maie et al., 2006; Zhang et al., 2010). As protein-binding phenolic compounds, tannins can inhibit microbial activity and lower nutrient mobilisation via substrate deprivation and enzyme inhibition (Schimel et al., 1996; Kraus et al., 2003; Joanisse et al., 2007). The immobilisation of exoenzymes in tannin-rich soils decreases

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http://dx.doi.org/10.1016/j.soilbio.2014.11.003
0038-0717/

their activity (Ximenes et al., 2011), resulting in reduced return of degradation products per unit of investment of nitrogen (N), carbon (C), and energy. This induces energy and/or N limitation in microorganisms producing these enzymes, thereby suppressing microbial decomposing activity.

We hypothesised that decomposer microorganisms in mangroves are ultimately N-limited, due to tannin-protein complexation. If primary production is phosphorus (P)-limited, as is common for mangroves in carbonate-rich environments, tannin-induced N limitation for microorganisms will lead to a differential nutrient limitation (DNL) for plants versus microbial decomposers. DNL therefore results in nutrient-specific biogenic controls of soil level in mangrove ecosystems. In such systems, enrichment in the plant-limiting nutrient (P) would result in net peat accumulation, whereas enrichment in the microbe-limiting nutrient (N) would result in net peat loss. The latter poses a large threat to mangrove systems on a global scale, because many coastal systems are experiencing increasing anthropogenic N inputs (Howarth and Marino, 2006).

Exoenzymes produced by microorganisms catalyse the rate-limiting step in nutrient-cycling and decomposition of the organic matter contained in peat (Sinsabaugh, 1994; Freeman et al., 2004), and their activities correlate with the rate of soil organic C decay and microbial nutrient demand (Sinsabaugh et al., 2009). Hydrolytic enzymes are specific in the reactions they catalyse and are frequently used to determine whether decomposition is limited by N or P (Sinsabaugh and Moorhead, 1994). These enzymes are not capable of breaking down tannins. The much less specific oxidative enzymes are capable of breaking down tannins and other polyphenolic compounds (Freeman et al., 2004; Limpens et al., 2008). The relative activities of hydrolytic and oxidative enzymes are a major determinant of peat formation (Freeman et al., 2001). A low relative activity of oxidative enzymes leads to enrichment with polyphenolic compounds which strongly promotes peat formation while a high relative oxidative enzyme activity depletes peat in polyphenolic compounds which can lead to rapid disappearance of historically formed peat (Freeman et al., 2001).

To gain insight into the effects of nutrient enrichment on enzyme-mediated decomposition processes in mangrove systems, we used two complementary approaches: i) tracking the short-term impacts of nutrient enrichments in laboratory incubations to assess the response to nutrient and C-enrichment without the confounding effects of plant–soil interactions, and ii) measuring the effects of long-term nutrient fertilisation in field experiments to examine the long-term, *in situ* consequences of nutrient enrichment on oxidative and hydrolytic enzyme activities in the context of nutrient competition between microorganisms and plants. In addition to quantifying potential enzyme activities, we compared microbial biomass, elemental stoichiometry, and metabolic activity between the different fertilisation treatments to test the hypothesis that tannin production creates an N limitation for microbial decomposers regardless of the limitations on primary production.

The present study was conducted at Twin Cays, a mangrove-dominated oceanic island group in the Caribbean Sea, 16 km off the coast of Dangriga, Belize. The islands consist of mangrove-derived peat that is up to 10 m thick (McKee et al., 2007). The dominant mangrove species on the island, *Rhizophora mangle*, shows a clear zonation with respect to growth form and nutrient limitation: Trees on the inland parts of the island show stunted growth and are strongly P-limited (dwarf zone), while trees near the fringes of the island are much taller, and are generally N-limited, depending on their proximity to open water (fringe zone) (Feller et al., 2002).

Earlier studies have attempted to quantify the effects of nutrient enrichment on peat decomposition in mangrove systems, using decomposition rates of roots (McKee et al., 2007) or tensile strength loss of cotton-strips (Feller et al., 2002) as indicators. These studies suggested that decomposition was either P-limited (Feller et al., 2002) or not sensitive to nutrient enrichment (McKee et al., 2007). However, McKee et al. (2007) observe peat decline upon N fertilisation, possibly due to increased decomposition rates (Lovelock et al., 2011). A mechanistic approach that employs the measurement of both hydrolytic and oxidative exoenzymes involved in the breakdown of organic matter provides a means to reconcile these seemingly contradictory results.

Following from our hypothesis, we expected microbial decomposition to be ultimately N-limited due to the effects of mangrove tannins. This would mean that the dwarf zone, where primary production is P-limited, has a DNL, while the fringe zone, where primary production is N-limited, does not. When microbial decomposers and primary producers are both limited by the same nutrient, the decomposition response comprises of direct effects and plant-mediated effects (i.e. through changes in tannin production or litter quality). The combined study of short- and long-term effects of nutrient amendments on enzyme production allows for differentiation between these direct and plant-mediated responses, indicating their relative importance. The results from this study can be used to evaluate the use of enzyme activities to assess microbial nutrient limitations (Sinsabaugh et al., 2008), and to qualify the potential consequences of eutrophication with respect to peat decomposition in mangroves.

2. Materials and methods

2.1. Study site and field experiment

Soil and water samples were collected at Twin Cays, Belize (16°49′N, 88°06′W). An extensive description of the hydrology, climate, primary production, and soil properties can be found in Feller (1995); Feller et al. (1999, 2002); McKee et al. (2007); Lee et al. (2008); Feller et al. (2009).

At the date of sampling, the *R. mangle*-dominated sites had been fertilised with either N or P for over 15 years using the method described in Feller (1995). In short, the fertiliser application consisted of the semiannual burial of two pieces of dialysis tubing filled with 150 m of either urea for N fertilisation or triple superphosphate for P fertilisation at opposing sides close to the base of each fertilised tree. This procedure led to a total annual amendment of 335 m NH_3 in the N treatment and 452 m PO_4 in the P treatment.

A detailed explanation of the lay-out of the long-term fertilisation experiment is described by Feller et al. (2002). Transects with a maximum length of 50 m perpendicular to the coastline were established at three randomly chosen positions (Feller, 1995). At each position, three parallel transects with a lateral distance of about 10 m were established and a fertilisation treatment (control, P fertilisation, and N fertilisation) was randomly assigned to each of these transects. Each transect consisted of a fringe zone where *R.*

Table 1
General properties as measured at *Rhizophora mangle*-dominated stands on a mangrove-covered island in the Caribbean sea near Belize ($N = 9$). Numbers following ± represent standard errors.

Property	Dwarf	Fringe	Unit
Tree height	0.99 ± 0.16	4.72 ± 0.36	m
Tree density	1.3 ± 0.1	1.0 ± 0.3	individuals m^{-2}
pH	6.7 ± 0.1	6.5 ± 0.1	–
Temperature	19.7 ± 0.6	16.4 ± 0.2	°C
Bulk density	0.92 ± 0.1	0.92 ± 0.1	g soil DW cm^{-3}