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published in
Soil Biology & Biochemistry
2013

DOI (link to publisher)
10.1016/j.soilbio.2012.08.007

document version
Peer reviewed version

Link to publication in KNAW Research Portal

citation for published version (APA)
Keuskamp, J. A., Schmitt, A., Laanbroek, H. J., Verhoeven, J. T. A., & Hefting, M. M. (2013). Nutrient amendment does not increase mineralisation of sequestered carbon during incubation of a nitrogen-limited mangrove soil. *Soil Biology & Biochemistry*, *57*, 822-829. https://doi.org/10.1016/j.soilbio.2012.08.007

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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

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Preprint submitted to Soil Biology and Biochemistry *July 11, 2012*

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.

Keywords:
Avicennia, *Rhizophora*, mangrove, fertilisation, microbial activity, SOC, nitrogen mining, decomposition, SIR, Saudi Arabia

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1 ## 1. Introduction

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

34 these are limiting. Likewise, nitrogen and phosphorus additions can be ex-
35 pected to stimulate decomposition when either of these elements is limiting
36 microbial activity. Feller et al. (1999) indeed found enhanced litter decom-
37 position after P addition in a P-limited mangrove. The effect of nitrogen
38 on decomposition rate is however rather complex, since SOC mineralisation
39 has been shown to be either increased, unaffected or decreased by addition
40 of N. Many studies have revealed that decomposition of recalcitrant litter
41 (Knorr et al., 2005; Berg and Laskowski, 2006) and SOC (Neff et al., 2002)
42 is inhibited by external nitrogen addition, while the decay of easily degrad-
43 able litter or labile organic carbon (LOC) is stimulated, as also predicted by
44 the nitrogen-mining theory (Moorhead and Sinsabaugh, 2006; Craine et al.,
45 2007). The net effect on total SOC is not always clear: Mack et al. (2004)
46 have demonstrated that long-term fertilisation in a tundra peatland leads to
47 a dramatic loss of soil carbon through increased SOC decomposition rates
48 while Shaver et al. (2006) find lower respiration rates in the same plots.

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

58 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 ± 0.5 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

108 C and N concentrations were corrected for weight changes due to the HCl
109 washing. Pore water was analysed for PO_4^-, NH_4^+, NO_3^-, dissolved organic
110 nitrogen (DON) and dissolved organic carbon DOC using a continuous flow
111 auto analyser (Skalar SA-40, Breda, The Netherlands).

112 *2.3. Incubation experiment*

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

131 To start the incubations, 0.1 ml treatment solution per gram soil fresh
132 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 (0.6 mg C g soil FW^{-1}). In the CNP treatment, an equal amount of carbon was added together with 0.06 mg g soil FW^{-1} nitrogen (as NH_4Cl) and 12 µg g soil FW^{-1} phosphorus (as Na_3HPO_4). Relative molar amounts of C, N and P were similar to that of aquatic microbial biomass ($C : N : P = 50 : 10 : 1$, Fagerbakke et al. (1996)) to ensure that microbial nutrient limitation was released. After amendment, soils were incubated for 6 days at 20 °C. During incubation, CO_2 production was measured at intervals of 130 minutes using a respirometer (Biometric Systems, Germany) equipped with optical CO_2 and O_2 sensors. The incubation flasks were flushed with fresh air whenever CO_2 levels exceeded 4.5 ml l^{-1} or O_2 levels decreased to less then 180 ml l^{-1}

Additional to these treatments, we also incubated the above-mentioned treatments with 7.5 mmol g soil FW^{-1} commercially available tannic acid to mimic the soluble tannin concentration of mangrove soils as reported by Alongi (1987), in order to assess whether these inhibit microbial activity.

2.4. Fumigation-extraction

In order to determine microbial biomass, 10 g of soil was subjected to a fumigation-extraction procedure according to Vance et al. (1987). We followed this procedure at the start and after 56 h of the incubation. In short, the procedure consisted of a 24 h treatment with an ethanol-free chloroform atmosphere followed by extraction with 50 mL of a 0.5 M K_2SO_4 solution.

After the extraction, DOC and DON concentrations were measured in fumigated and non-fumigated control soils using a continuous flow auto analyser (Skalar SA-40, Breda, The Netherlands). Following Vance et al. (1987),

158 microbial carbon was estimated by multiplying the amount of DOC liberated
159 by fumigation by an empirically derived factor of 2.64 reflecting the relative
160 amount of non-extractable to extractable carbon in microbial biomass. DON
161 was also measured to quantify nitrogen fluxes and calculate C/N ratios of
162 microorganisms. Following Brookes et al. (1985), we calculated microbial N
163 as the difference in extractable DON before and after fumigation divided by
164 0.54. Only the *Avicennia* soils were subjected to the fumigation-extraction
165 procedure as the necessary resampling for the *Rhizophora* soil was not logis-
166 tically possible.

167 *2.5. Data analysis & Statistics*

168 CO_2 production rates increased exponentially after amendment. The rate
169 of exponential increase was quantified using a logarithmic growth function
170 (Simkins and Alexander, 1984), by fitting the initial, rising part of the respi-
171 ration peak to an exponential growth function $R_{CO_2}(t)$ analogous to Colores
172 et al. (1996):

$$R_{\mathrm{CO}_2}(t) = p \cdot e^{\mu_{max}(t-b)} \tag{1}$$

173 with p being the initial respiration rate, b the delay (i.e. lag time) before
174 exponential growth starts and μ_{max} the relative growth rate. The fitting was
175 done using a least squares fitting procedure in Mathematica 7.0.0 (Wolfram
176 research, Champaign (IL), USA). For each plot, the initial respiration rate
177 p was determined by first fitting the CO_2 respiration rate of the control
178 treatment to a negative value for specific growth rate μ_{max}; the value found
179 for p then was used as a fixed parameter in the fitting procedure for the
180 amended samples originating from the same plot.

Other values used for further analysis were obtained from directly measured respiration rates or cumulative respiration as calculated by integrating respiration rates to time. Initial microbial biomass carbon (C_{micr}) was determined following the relationship given by Anderson and Domsch (1978) recalculated to standard units:

$$C_{micr} = 81.84 \cdot R_C + 3.7 \quad (2)$$

with C_{micr} in µg microbial C per g soil DW, and R_C the average carbon respiration over the first 4 hours in µg g CO_2-C h^{-1} g soil DW. We measured basal respiration (BR) as an averaged respiration rate in soils without amendment over the first 24 h after the pre-incubation. The metabolic quotient (q_{CO_2}), i.e. the relative respiration rate for microorganisms, was calculated as BR/C_{micr} (Anderson and Domsch, 1985). Respiration quotient q_C, the amount of carbon respired per unit available SOC, was calculated as $BR/SOC\ content$ (Anderson and Domsch, 1986). Microbial respiratory quotient RQ was measured as CO_2/O_2 in mol mol^{-1} (Dilly, 2001) for both amended and unamended soils.

All data were analysed using R version 2.12.1 (R Development Core Team, 2010). Some outliers occurred in the fumigation procedure and short-term CO_2 measurements. These were identified manually and removed upon confirmation by Grubbs test. Homogeneity of variances was confirmed using Levene's test. Normality was tested with the Shapiro-Wilk test. Treatment effects were tested using an ANOVA on a linear model with replicate as a random factor to account for soil heterogeneity. Post-hoc testing for group differences was done with Tukey HSD for group differences where applicable. Differences between two groups were judged using Welch's t-test in case of

205 normal distributions. The Mann-Whitney U test was used for group differ-
206 ences between non-parametric data such as redox conditions and temperature
207 in Table 1. Experimental data were tested on measured values per unit fresh
208 weight (FW) rather than units dry weight as we performed all experiments
209 keeping units fresh weight equal between treatments. Values after $\pm$ indicate
210 standard error.

211 3. Results

212 *3.1. Edaphic properties and initial nutrient status*

213 The *Avicennia marina* and the *Rhizophora mucronata* derived soils were com-
214 parable with respect to pH, redox, temperature and salinity (Table 1).The
215 black *Rhizophora* soil was a moderately organic silt loam, while the *Avicennia*
216 soil consisted of coarse calcareous sand and contained little organic material.
217 The difference between the soils was also reflected in the lower bulk density
218 and nutrient content of the *Avicennia* derived soil. The pore water phospho-
219 rus and ammonium concentrations were three to eight times higher in the
220 *Rhizophora* soil as compared to the *Avicennia* soil, as were the soil organic
221 carbon and nitrogen contents. The N:P ratio in the pore water of *Avicennia*
222 was 26, that of *Rhizophora* 12. In both the *Rhizophora* and *Avicennia* sys-
223 tems, surface water nutrient concentrations (*Rhizophora* : NO_3: $< 0.02\,mg\,l^{-1}$;
224 NH_4: $0.1\,mg\,l^{-1}$; PO_4: $0.1\,mg\,l^{-1}$, *Avicennia* : NO_3, NH_4, and PO_4: $<$
225 $0.02\,mg\,l^{-1}$) were lower than those of pore water (Table 1). Moisture content
226 at the time of incubation was close to field capacity with $0.32 \pm 0.01\,g\,g^{-1}$
227 and $0.60 \pm 0.03\,g\,g^{-1}$ for the *Avicennia* and the *Rhizophora* soil respectively.

Table 1: Edaphic and other site-specific properties as measured at two mangrove stands in Saudi Arabia (N=9). Significant (quantitative) differences between sites are indicated in the first column (* $p<0.05$, ** $p<0.01$ and *** $p<0.001$). Numbers after ± indicate standard errors.

Property	*Avicennia*	*Rhizophora*	Unit
location	Thuwal (SA)	Farazan (SA)	
soil type	Tidalic Gleyic Solonchaks	Tidalic Mollic Fluvisol (magniferric)	
texture	Sand	Silt	
tree height	3-4	3-4	m
pH	$6.9 \pm .1$	$7.0 \pm .1$	−
salinity*	50 ± 0	46 ± 1	$mg\,g^{-1}$
redox*	69 ± 53	-22 ± 20	mV
temperature*	$30.3 \pm .2$	$32.9 \pm .4$	°C
bulk density*	$1.15 \pm .11$	$.50 \pm .3$	$g\ soil\ DW\,cm^{-3}$
SOC***	18 ± 2	79 ± 6	$mg\,g\ soil\ DW^{-1}$
SON***	$1.4 \pm .1$	$4.7 \pm .2$	$mg\,g\ soil\ DW^{-1}$
DOC	32 ± 3	27 ± 1	$mg\,l^{-1}$
DON	$<.2$	$<.2$	$mg\,l^{-1}$
NO_3^-	<1	<1	$\mu mol\,l^{-1}$
NH_4^{+}**	13 ± 3	49 ± 9	$\mu mol\,l^{-1}$
PO_4^{3-}**	$.5 \pm .2$	4 ± 1	$\mu mol\,l^{-1}$

SOC: Soil Organic Carbon, DOC: Dissolved Organic Carbon SON: Soil Organic Nitrogen, DON: Dissolved organic nitrogen

228 *3.2. Initial microbial biomass and activity*

229 To compare microbial activity between the soils from the *Avicennia* and *Rhi-*
230 *zophora* sites at the start of the incubations, we determined microbial biomass
231 (C_{micr}), microbial respiration rate (BR), and metabolic quotient (q_{CO_2}) (Ta-
232 ble 2). Microbial respiration and SIR estimated microbial biomass per gram
233 soil were markedly higher for the *Rhizophora* than for the *Avicennia* soil (BR:
234 df=6.7, p<0.001, C_{micr}: df=5.1, p<0.05); note that these differences are not
235 significant when expressed per unit volume. The respiration per unit carbon
236 in the soil (q_C) was significantly lower for the more organic *Rhizophora* soil
237 (df=5.6, p<0.005), whereas microbial respiratory quotient (RQ) was for both
238 soil types well below 1, which is the RQ expected for aerobic consumption
239 of reduced carbon substrates without growth (Dilly, 2003).

240 *3.3. Respiration response to glucose and nutrient amendments*

241 Glucose additions induced a clear respiration peak between 10 and 40 h after
242 amendment (Figure 1: all treatments except Control and NP). The induced
243 peak initially follows a curve similar to that for logistic growth, with an
244 initial exponential growth phase followed by an exponential decrease of the
245 specific growth rate to zero. This first exponential phase showed a good
246 fit to the exponential growth rate function (eq. 1, $R^2 > 0.98$). μ_{max}, the
247 microbial specific growth rate in this equation describes change in time and
248 hence has unit h^{-1}. Unlike absolute measures such as respiration rate, μ_{max}
249 is suitable to compare microbial growth rates between systems as it does not
250 depend on weight or volume units and is therefore insensitive to differences in
251 moisture content or bulk mass. Overall, μ_{max} is higher in *Avicennia* than in
252 *Rhizophora* soil (F=67.0, p<0.001) (Figure 2a; Table 3). Because there was

Table 2: Microbial characteristics as measured in soils from two mangrove sites in the Red Sea dominated by either *Avicennia marina* or *Rhizophora mucronata* . Significant differences between sites are indicated in the first column (* p<0.05, *** p<0.001). Numbers after ± indicate standard errors.

Property	*Avicennia*	*Rhizophora*	Unit
BR^{***}	$1.7 \pm .1$	$4.0 \pm .3$	µg CO_2-C g soil DW^{-1} h^{-1}
$C_{micr}{}^{*}$	228 ± 54	559 ± 95	µg C_{mic} g soil DW^{-1}
q_{CO_2}	8 ± 2	9 ± 1	mg CO_2-C h^{-1} g $C_{mic}{}^{-1}$
$C_{mic} : C_{org}$	12 ± 4	7 ± 1	mg C_{mic} g SOC^{-1}
$q_C{}^{*}$	104 ± 16	52 ± 4	mg CO_2-C h^{-1} g SOC^{-1}
RQ	$.49 \pm .05$	$.47 \pm .04$	mol CO_2 mol $O_2{}^{-1}$

BR = basal respiration, C_{micr} = SIR determined microbial biomass, q_{CO_2} = metabolic quotient, $C_{mic} : C_{org}$ = mass ratio of (SIR determined) microbial biomass C and SOC, q_C= relative carbon use, RQ= microbial respiratory quotient.

Table 3: Experimental results of an incubation of soils from monospecific *Avicennia marina* and *Rhizophora mucronata* mangrove stands. Results are obtained during 150 h of incubation. The treatment consisted of a glucose addition (0.05 mmol C g soil FW^{-1}), with or without nitrogen (N) or phosphorus (P). Relative amounts of C:N:P were 50:10:1. Both soil types were analysed separately by ANOVA with treatment as a fixed factor, significant differences are indicated by letters. For μ_{max} all differences indicated are significant at $p<0.001$, for R_{cum} this is $p<0.01$.

Species	Treatment	μ_{max}		R_{cum}	
Avicennia	Control	$-.005 \pm .000$	a	$.18 \pm .01$	a
marina	NP	$-.005 \pm 001$	a	$.18 \pm .01$	a
	C	$1.2 \pm .01$	b	$.61 \pm .06$	bc
	CP	$1.3 \pm .01$	b	$.70 \pm .03$	b
	CN	$2.6 \pm .02$	c	$.59 \pm .03$	c
	CNP	$3.8 \pm .01$	d	$.63 \pm .03$	bc
Rhizophora	Control	$-.088 \pm .004$	A	$.45 \pm .03$	A
mucronata	C	$.6 \pm .1$	B	$1.15 \pm .07$	B
	CP	$.6 \pm .1$	B	$1.23 \pm .06$	B
	CN	$1.0 \pm .2$	B	$1.20 \pm .08$	B
	CPN	$2.8 \pm .3$	C	$1.25 \pm .11$	B

253 a significant interaction effect, we analysed the treatment effect separately
254 for the two soil types.

255 In the *Avicennia* soil, we tested if nitrogen plus phosphorus (NP) applica-
256 tion, without glucose addition, would induce a respiration peak, but this was
257 not the case; in fact, like in the control treatment, respiration just followed a
258 negative exponential curve after amendment (Fig 1: Control and NP treat-
259 ments). Addition of both nitrogen and phosphorus together with glucose
260 (CNP) increased μ_{max} significantly compared to glucose only (C) treatments
261 in both *Avicennia* and *Rhizophora* soil.

262 Lower stimulatory effects on glucose-induced growth were observed with
263 single nutrient additions (N or P) in both soils: phosphorus plus glucose
264 additions (CP) did not increase μ_{max} as compared to the C treatment, while
265 glucose plus nitrogen (CN) addition only increased μ_{max} in the *Avicennia*
266 soil (Figure 2a; Table 3).

267 After the exponential growth phase, respiration rates come to a maximum
268 rate R_{max} after which respiration declines again. R_{max} is equivalent to the
269 inflection point of a logistic growth curve, where a limitation starts to reduce
270 growth rates. The amount of CO_2 produced at R_{max} was about one-third
271 higher (df: 37.0, $p<0.001$) in *Rhizophora* (1.21 mg CO_2-C $\pm$ 0.06 mg CO_2-C)
272 than in *Avicennia* with 0.83 mg CO_2-C $\pm$ 0.04 mg CO_2-C, but did not differ
273 significantly between nutrient treatments.

274 In the CP treatment, a second respiration peak occurs consistently in both
275 *Rhizophora* and *Avicennia* derived soil, this second peak is small as compared
276 to the first one, but similar in shape (Figure 1: CP). Such a second respiration
277 peak is also observed in a number of *Avicennia* soil samples receiving the C

treatment. The total respiration is elevated by glucose ($p<0.001$). Within the glucose treatments, P has a stimulatory effect on total respiration ($F=7.3$, $p<0.05$).

The microbial respiratory quotient (RQ) over 150 hours was significantly lower for *Rhizophora* in comparison to *Avicennia* soil in all treatments (Fig. 2b; $F=157.1$, $p<0.001$). In the control treatment, RQ over the whole incubation was equal to initial values of RQ in *Rhizophora* soil, but it almost doubled to 0.83 ± 0.05 for *Avicennia* soil. Glucose addition elevated RQ significantly in both *Avicennia* ($F=11.9$, $p<0.01$) and *Rhizophora* ($F=75.0$, $p<0.001$) soil, but supplementary nitrogen or phosphorus amendment did not alter RQ significantly (Figure 2b). Tannic acid addition slightly increased total respiration with 0.10 mg CO_2-C g soil $FW^{-1} \pm 0.02$ mg CO_2-C g soil FW^{-1} in both *Avicennia* and *Rhizophora* soil ($F=28.3$, $p<0.001$), but did not modify μ_{max} in any of the treatments (data not shown).

3.4. Carbon and nitrogen budget

We accounted for all net carbon fluxes during incubation by measuring changes in DOC, C_{micr} and cumulative respiration losses over the incubation period (Figure 3a). Similarly, mineralisation of soil organic nitrogen can be quantified by measuring changes in nitrogen pools (Figure 3b) and correcting for nitrogen amendment where needed. We did not measure N_2O and N_2 efflux through denitrification, as earlier tests on these soils showed that gaseous nitrogen loss was negligible under the experimental conditions (data not shown). For both carbon and nitrogen the only unknown pool is soil organic matter, so that all changes in total pool can be attributed to either experimental addition or decomposition of soil organic matter.

Table 4: Microbial response to carbon and nutrient amendments in soils from an *Avicennia marina* dominated system. Results are obtained after 80 hours of incubation at 20 °C followed by fumigation-extraction. The treatments consisted glucose (C), nitrogen (N) and phosphorus (P) additions in various combinations. Glucose addition was 0.05 mmol C g soil FW^{-1}, relative amounts were 50:10:1 for C:N:P.

treatment	G_{eff}	C_{micr}	N_{micr}	C/N	SOC_{min}	SON_{min}
Initial	-	202 ± 28	17 ± 2	11 ± 1	-	-
Control	-	174 ± 51	15 ± 5	14 ± 5	69 ± 51	-1 ± 4
NP	-	216 ± 6	68 ± 17	3 ± 1	89 ± 51	5 ± 16
C	$.67 \pm 2$	621 ± 27	24 ± 5	22 ± 4	133 ± 66	6 ± 3
CP	$.49 \pm 5$	683 ± 114	37 ± 9	30 ± 7	233 ± 143	7 ± 4
CN	$.49 \pm 3$	617 ± 69	109 ± 7	6 ± 1	36 ± 76	34 ± 8
CNP	$.39 \pm 3$	508 ± 52	86 ± 12	6 ± 1	-28 ± 69	-3 ± 11

G_{eff} = growth efficiency (g C_{mic} g C consumed^{-1}), C_{micr} = microbial biomass C (µg C_{mic} g soil DW^{-1}), N_{micr} = microbial biomass N (µg N_{mic} g soil DW^{-1}), C/N = Microbial C/N ratio, SOC_{min} = mineralised SOC (µg C g soil DW^{-1}), SON_{min} = mineralised SON (µg N g soil DW^{-1}).

303 In the treatments where no glucose was added, C_{micr} slightly decreased
304 while DOC concentration remained the same. Respiration slightly exceeded
305 the loss of microbial carbon so that a net SOC mineralisation was observed
306 (Table 4). In the glucose-amended treatments (C, CP, CN, CNP), carbon
307 fluxes were markedly higher as a result of the added glucose: C_{micr} tripled
308 and respiration almost quintupled. The DOC pool did not change during in-
309 cubation with the exception of the C treatment where an increase in DOC in-
310 dicated an incomplete consumption of the added glucose (Figure 3a). To cal-
311 culate SOC decomposition in these treatments, the amended glucose-carbon
312 was subtracted from the total flux. In Figure 3a this is graphically shown by
313 the horizontal bar: the lower border of this bar represents the amendment
314 of glucose-carbon, the height of the bar indicates the SOC mineralisation in
315 the control treatment for comparison.

316 We tested the effects of carbon additions with a one-way ANOVA on the
317 control and the glucose treatment. Within each carbon treatment we tested
318 for the effect of nutrient addition with a two-way ANOVA with nitrogen and
319 phosphorus as main effects. Since these two comparisons are orthogonal,
320 ordinary F-tests without corrections were used. There were no significant
321 effects of P or C, but N did show a significant inhibition on SOC minerali-
322 sation ($F=7.9$, $p<0.05$) in the glucose-amended treatments. In Figure 4 we
323 summarised the effects of C and N on LOC and SOC decomposition.

324 Growth efficiency (G_{eff}) (microbial C/consumed C) indicates the amount
325 of carbon needed to produce a certain amount of microbial biomass. Phos-
326 phorus and nitrogen additions additively lowered growth efficiencies (nitro-
327 gen: $F=5.6$, $p<0.05$, phosphorus: $F=5.3$, $p<0.05$) so that the C treatment

328 had the highest and the CNP treatment the lowest growth efficiency (Table
329 4).

330 The nitrogen budget in Figure 3b shows changes in dissolved and micro-
331 bial nitrogen pools during the incubation. In the nitrogen amended treat-
332 ments DON + DIN as well as microbial N are clearly elevated indicating
333 partial consumption of the added N. The relative amount of immobilised
334 nitrogen (microbial N/(DIN + DON)) was increased by glucose addition
335 ($F=5.2$, $p<0.05$). Microbial C/N ratios in the control treatments did not
336 change significantly during incubation. The microbial C/N ratios in the
337 other treatments were strongly determined by the ammonium and glucose
338 additions and differed almost tenfold from 22-30 for samples receiving car-
339 bon without nitrogen to 3 for samples receiving nitrogen without carbon.

340 ## 4. Discussion

341 ### *4.1. Basal nutrient status and microbial activity*

342 The microorganisms in the studied mangrove soils are primarily energy-
343 limited, even though a substantial amount of organic carbon is locked in
344 the surrounding soil as SOC (*Avicennia* : 2 %, *Rhizophora* : 8 %). The high
345 metabolic quotient (q_{CO_2}) reveals low energy use efficiency as compared to
346 other terrestrial (Anderson and Domsch, 1993) or submerged soils (Torres
347 et al., 2011). At the same time, heterotrophic activity in the *Avicennia* and
348 *Rhizophora* dominated soils largely depends on the oxidation of refractory
349 compounds as shown by low *RQ* values (Dilly, 2001). We therefore hypoth-
350 esize that the microbial community of the studied soils are severely energy

351 stressed and largely composed of K-strategists: slowly growing microorgan-
352 isms oxidising mainly recalcitrant materials.

353 Differences in soil carbon dynamics were found between the two man-
354 grove genera, despite comparable edaphic properties. In the *Rhizophora* site,
355 mineralisation rates are twice as high as compared to the *Avicennia* soil,
356 while carbon storage is four times higher. Assuming that the carbon burial
357 rate is in steady state at the moment of sampling, the input of carbon to
358 the *Rhizophora* soil is therefore at least twice as high as encountered in the
359 *Avicennia* dominated system. Relative carbon use (q_C) and the microbial
360 biomass per unit carbon, were lower in the *Rhizophora* derived soil. This
361 translates to a higher average residence time of carbon in the *Rhizophora*
362 as compared to the *Avicennia* soil, and therefore a higher recalcitrance of
363 organic matter in the *Rhizophora* system. This finding is in accordance to
364 what is generally found for litter of these genera (Robertson, 1988; Sessegolo
365 and Lana, 1991; Middleton and McKee, 2001) and is often ascribed to the
366 higher tannin and lower nitrogen content of *Rhizophora* litter. Due to the
367 larger carbon pool and higher recalcitrance, priming effects of labile organic
368 carbon and inhibition of nitrogen on SOC decomposition are suspected to be
369 stronger in the *Rhizophora* site.

370 *4.2. Microbial growth rate, energy, and nutrient limitations*

371 The hypothesised energy limitation for heterotrophic microbial activity was
372 confirmed in the incubation experiment as adding labile organic carbon (LOC)
373 induced exponentially growing respiration rates, indicating microbial growth
374 at a constant rate μ_{max} . Nitrogen addition only increased respiration rates
375 when added in combination with LOC (in *Avicennia*) or in combination with

376 LOC and phosphate (in *Rhizophora*). This is in accordance to what could
377 have been expected based on pore water N:P ratios, when assuming a micro-
378 bial N:P of 10 as estimated for aquatic microorganisms by Fagerbakke et al.
379 (1996) or 6-16:1 by Vrede et al. (2002) for marine bacteria. In future assays
380 it would be worthwhile to assess if pore water nutrient concentrations are a
381 good predictor of nutrient limitation for heterotrophic growth.

382 In all treatments, including those with tannic acid amendment, a lower
383 μ_{max} and RQ was found in the *Rhizophora* soil as compared to the *Avicennia*
384 soil. This is not thought to be caused by differences in LOC or O_2 availability
385 between the two soils: μ_{max} does not decline with respiration rate, indicating
386 that carbon uptake rate is limited by uptake capacity and growth and not by
387 LOC availability or diffusion rates. The RQ is never larger than one, showing
388 that decomposition was aerobic. As the lower μ_{max} and RQ found for *Rhi-*
389 *zophora* was independent of nutrient status or soluble tannin concentrations,
390 the prevailing microbial communities must underlie this effect. The lower
391 microbial growth rate found for *Rhizophora* indicates an even larger domi-
392 nance of microbial K-strategists in these soils as compared to the *Avicennia*
393 derived soil.

394 A priming effect, a long-term change in decomposition rate due to a one-
395 time addition of a resource, could not be confirmed in any of the treatments,
396 as microbial activity did not reach equilibrium and still declined towards the
397 end of the incubation period of 180 hours. Nevertheless, from our glucose-
398 amended treatments it is clear that, when sufficient energy is supplied, ni-
399 trogen addition increases microbial growth rates and LOC mineralisation in
400 microbial communities of *Avicennia* and *Rhizophora* mangrove soils. Dur-

401 ing continuous loading with LOC and nitrogen, which would be a realistic
402 scenario for pollution by wastewater, we expect these dynamics to be the
403 same. Whether this has consequences for the SOC pool was assessed for the
404 *Avicennia* dominated soil where we measured changes in microbial biomass,
405 DOC and CO_2 efflux as well as soil nitrogen pool.

406 *4.3. Carbon and nitrogen budgets*

407 For the *Avicennia* derived soil, a carbon and nitrogen budget was constructed
408 by measuring microbial consumption, growth and respiration. In the glucose-
409 amended treatment not all glucose carbon was consumed, confirming that
410 heterotrophic activity was not energy-limited after amendment. Strikingly,
411 both nitrogen and phosphorus increased LOC use, while at the same time
412 decreasing growth efficiencies. The decreased energy efficiency suggests in-
413 creased synthesis of energy-rich substances. Possible mechanisms include
414 the synthesis of polyphosphate to store energy (Kortstee et al., 1994) in the
415 P-enriched soils and an increased internal enzyme production in the nitrogen-
416 enriched soils. A hint towards polyphosphate accumulation is the secondary
417 peak in respiration consistently observed not only in *Avicennia* but also in
418 *Rhizophora* derived soil after P addition (Fig. 1). This could be caused by
419 delayed synthesis of polyphosphate as an energy store, but the results do not
420 provide a definite answer as to which mechanisms are involved.

421 Added nitrogen was readily absorbed by microbial biomass, even in the
422 absence of growth, and the soil microorganisms proved quite plastic with
423 respect to their relative nitrogen content. Initially the microbial C/N ratio
424 was 11; after N addition, when carbon was limiting the C/N ratio decreased
425 to as low as 3. At that point the microbial C/N ratio is as high as that of

426 proteins, so that a higher N content is unlikely to occur (Fagerbakke et al., 427 1996). In the C and CP treatments, where nitrogen was made limiting, C/N 428 increased to 22-30, a value much higher than previously reported for marine 429 bacteria (Vrede et al., 2002), indicating internal carbon storage as glycogen 430 or some other polysaccharide.

431 While nitrogen in itself did not significantly stimulate or inhibit SOC 432 decomposition in these systems, nitrogen amendment clearly inhibited SOC 433 mineralisation when energy limitation was released. This finding is similar 434 to that of Yamasaki et al. (2011) in a terrestrial forest and fits the nitrogen- 435 mining theory (Craine et al., 2007), which states that under nitrogen limi- 436 tation, recalcitrant material is broken down to obtain nitrogen, even though 437 this is energetically not favourable.

438 *4.4. Effects of nutrient enrichment on mangrove carbon sequestration*

439 The microbial communities of mangrove soils form a large sink for carbon, 440 while enhancing nitrogen availability through decomposition of soil organic 441 matter. At the same time, the microbial community forms a large sink for 442 exogenous nitrogen and most likely also phosphorus when these are available 443 in higher concentrations. The size of this sink increases as long as there is 444 a concurrent input of labile organic carbon. Although nutrients stimulate 445 growth of microorganisms and increase the mineralisation rates of labile or- 446 ganic carbon, soil organic matter decay is not stimulated. It is therefore 447 not expected that increased nutrient exposure of the microbial communi- 448 ties in mangrove soils will deteriorate existing soil organic carbon pools in 449 mangroves.

450 Even more so, as primary production in mangroves is nutrient-limited

451 while decomposition is energy-limited, there is a differential limitation be-
452 tween carbon production and carbon decomposition. This suggests an in-
453 creased carbon sequestration rate of mangrove forests upon exposure to nu-
454 trients. From preliminary results (data not shown) it is clear that such an
455 increase in primary production upon nutrient enrichment takes place in the
456 *Avicennia* site from our research. Mangroves have been proposed to be us-
457 able as wastewater polishing facilities by a number of authors (Wong et al.,
458 1997; Tam et al., 2009). One could speculate that this could work without
459 losing carbon sequestration capacity: the nitrogen supplied increases primary
460 production thus increasing carbon input to the soil, while excess LOC is read-
461 ily decomposed by microorganisms. The extra microbial activity does not
462 lead to CO_2 release from SOC decomposition as long as sufficient nitrogen is
463 supplied.

464 Caution is required when extrapolating above conclusions up to the ecosys-
465 tem scale, as soil-plant feedbacks were not included in this study. Reef et al.
466 (2010) point out that nutrient enrichment potentially decreases primary pro-
467 duction. In addition, increasing nutrient availability decreases tannin pro-
468 duction (Lin et al., 2009) thereby potentially increasing decomposability.
469 Moreover, relative root production also decreases with rising nutrient avail-
470 ability (Naidoo, 2009) while mangrove roots decompose slowly and are im-
471 portant in peat formation (Middleton and McKee, 2001). On the long term
472 this may dramatically decrease the amount of sequestered carbon especially
473 in mangroves depending on soil accretion through accumulation of dead root
474 material.

5. Acknowledgements

We would like to thank Dr. Abdulazziz AlSuwailem, Dr. Zenon Batang, and Dr. Nabeel Abdulaziz of the CMOR Lab for their help in the field and Dr. Dennis Wigham and Dr. Ilka Feller at SERC for their ideas and suggestions on site selection and sampling.

This publication was supported by Award No KUK-C1-017-12, made available by King Abdullah University of Science and Technology (KAUST), Thuwal, Saudi Arabia.

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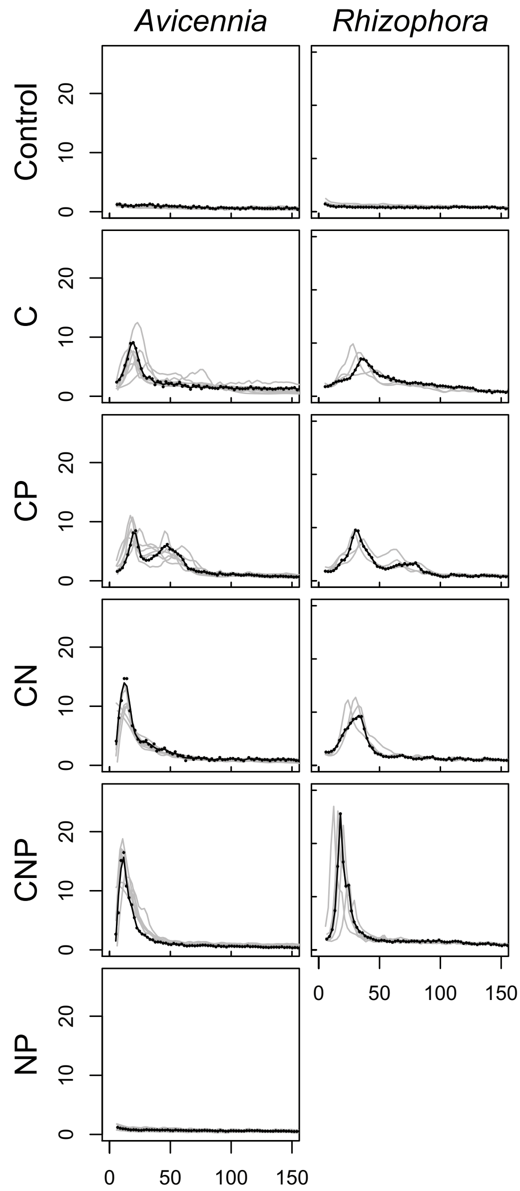

Figure 1: Respiration responses (µg CO_2-C h^{-1}) in time (h) of soils from *Avicennia marina* and *Rhizophora mucronata* dominated sites in the Red Sea after amendment of 0.6 mg glucose-C g soil FW^{-1} (C), ammonium (N) and/or phosphorus (P) at $t = 0$. CO_2 concentrations are measured every 2 hours. In each panel, one of the five to eight replicates is plotted in black with its measured values shown as black dots.

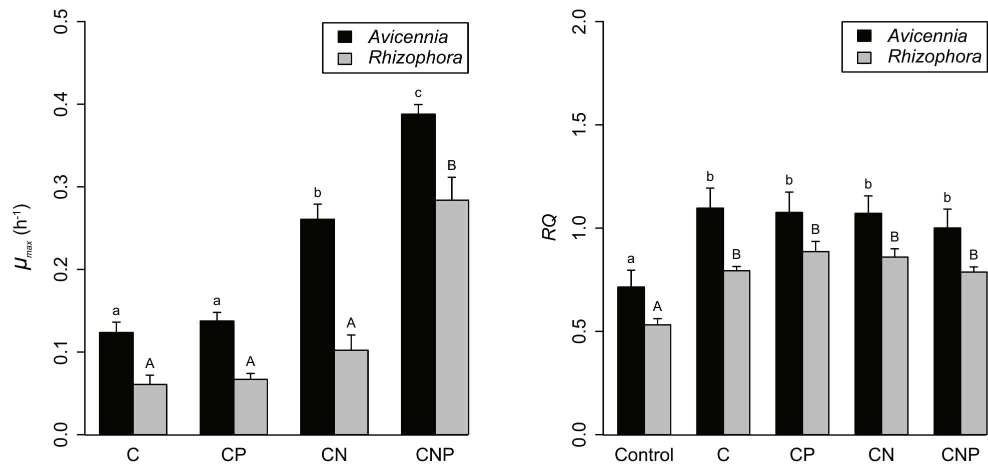

Figure 2: a) Microbial growth rate response and b) microbial respiratory quotient *RQ* (CO_2/O_2) of soils from *Avicennia marina* (black bars) and *Rhizophora mucronata* (grey bars) dominated sites in the Red Sea. Responses are measured after amendment with 0.6 mg glucose-C g soil FW^{-1} (C) in combination with ammonium (N) and/or phosphorus (P). Bars represent standard errors. Letters indicate differences between bars. All differences indicated are significant at $p<0.001$, differences between species are significant at $p<0.001$ in panel a and $p<0.05$ in panel b

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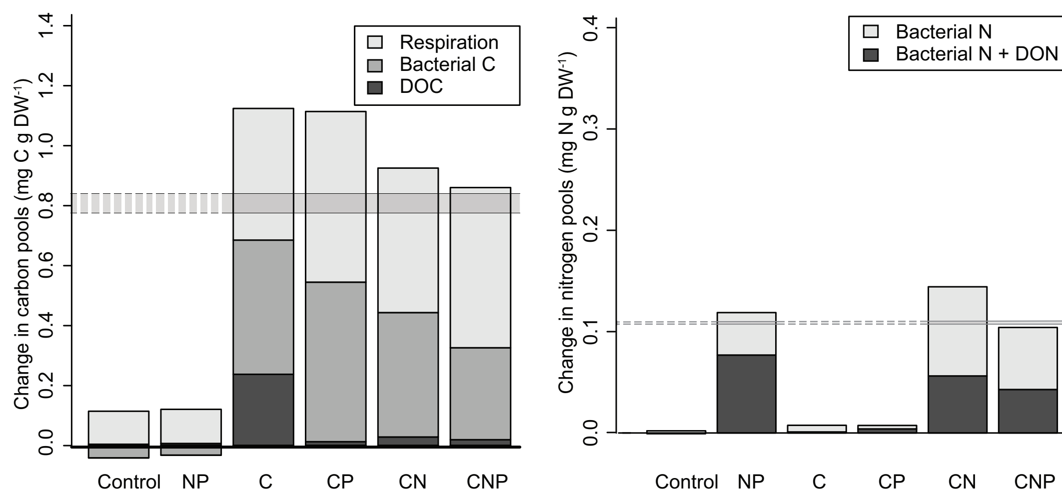

Figure 3: Total carbon (a) and nitrogen (b) budgets of soils from *Avicennia marina* dominated sites after 80 h of incubation. Soils were enriched with glucose (C), phosphorus (P) and ammonium (N) in various combinations. The horizontal bar shows the effect of amendment of C (a) or N (b) on the total budget. The lower border of this bar represents the direct effect of amendment; the height of the bar is the net budget change in the control treatment. N lowered total carbon mineralisation significantly compared with the C and CP treatments ($p<0.05$, panel a).

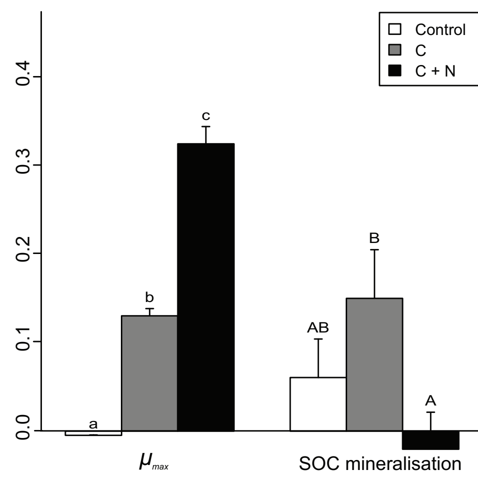

Figure 4: Relative microbial growth rates μ_{max} (h^{-1}) and net SOC mineralisation ($mg\,g\ soil\ DW^{-1}$) of soils from *Avicennia marina* dominated sites receiving $0.06\,mg\,g\ soil\ FW^{-1}$ of glucose in the C treatments and $0.06\,\mu g\,g\ soil\ FW^{-1}$ of nitrogen in the N treatments. Significance was tested using ANOVA for C and N within C, letters indicate significant differences at $p<0.05$.