



Royal Netherlands Academy of Arts and Sciences (KNAW) KONINKLIJKE NEDERLANDSE AKADEMIE VAN WETENSCHAPPEN

Potential of sulfate reduction in mangrove forest soils: Comparison between two dominant species of the Americas

Balk, M.; Keuskamp, Joost; Laanbroek, H.J.

published in

Frontiers in Microbiology
2016

DOI (link to publisher)

[10.3389/fmicb.2016.01855](https://doi.org/10.3389/fmicb.2016.01855)

document version

Peer reviewed version

[Link to publication in KNAW Research Portal](#)

citation for published version (APA)

Balk, M., Keuskamp, J., & Laanbroek, H. J. (2016). Potential of sulfate reduction in mangrove forest soils: Comparison between two dominant species of the Americas. *Frontiers in Microbiology, in press*, Article 2016.01855. <https://doi.org/10.3389/fmicb.2016.01855>

General rights

Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from the KNAW public portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain.
- You may freely distribute the URL identifying the publication in the KNAW public portal.

Take down policy

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

E-mail address:

pure@knav.nl

1 Potential for sulfate reduction in mangrove forest soils:
2 Comparison between two dominant species of the Americas

3
4
5 Melike Balk^{1,2}, Joost A. Keuskamp^{1,3}, Hendrikus J. Laanbroek^{1,3*}

6
7
8 ¹Department of Microbial Ecology, Netherlands Institute of Ecology (NIOO-KNAW),
9 Droevendaalsesteeg 10, 6708 PB Wageningen, The Netherlands

10 ² Faculty of Geosciences, Utrecht University, Budapestlaan 4, 3584 CD Utrecht, The
11 Netherlands

12 ³ Ecology and Biodiversity Group, Department of Biology, Utrecht University, Padualaan
13 8, 3584 CH Utrecht, The Netherlands

14
15
16
17
18
19
20
21
22 *** Correspondence**

23 Hendrikus J. Laanbroek

24 r.laanbroek@nioo.knaw.nl

25
26 **Running title:** Potential for sulfate reduction in mangrove soils

27 **Keywords:**

28 Sulfate reduction, mangroves, *Avicennia germinans*, *Rhizophora mangle*, *dsrB* gene

29 **[Summary]**

30 *Avicennia* and *Rhizophora* are globally occurring mangrove genera with different
31 traits that place them in different parts of the intertidal zone. It is generally accepted
32 that the oxidizing capacity of *Avicennia* roots is larger than that of *Rhizophora* roots,
33 which initiates more reduced conditions in the soil below the latter genus. We
34 hypothesize that the more reduced conditions beneath *Rhizophora* stands lead to more
35 active sulfate-reducing microbial communities compared to *Avicennia* stands.

36 To test this hypothesis, we measured sulfate reduction traits in soil samples collected
37 from neighboring *Avicennia germinans* and *Rhizophora mangle* stands at three
38 different locations in southern Florida. The traits measured were sulfate reduction
39 rates in flow-through reactors (FTR's) containing undisturbed soil layers in the
40 absence and presence of easily degradable carbon compounds, copy numbers of the
41 *dsrB* gene, which is specific for sulfate-reducing microorganisms, and numbers of
42 sulfate-reducing cells that are able to grow in liquid medium on a mixture of acetate,
43 propionate and lactate as electron donors.

44 At the tidal locations Port of the Islands and South Hutchinson Islands, steady state
45 sulfate reduction rates, *dsrB* gene copy numbers and numbers of culturable cells were
46 significantly higher at the *A. germinans* than at the *R. mangle* stands, although not
47 significantly for the numbers at Port of the Islands. At the non-tidal location North
48 Hutchinson Island, results are mixed with respect to these sulfate reduction traits. At
49 all locations, the fraction of culturable cells were significantly higher at the *R. mangle*
50 than at the *A. germinans* stands. The dynamics of the initial sulfate reduction rates
51 implied a more *in situ* active sulfate-reducing community at the intertidal *R. mangle*
52 stands. It was concluded that in agreement with our hypothesis *R. mangle* stands
53 accommodate a more active sulfate-reducing community than *A. germinans* stands,
54 but only at the tidal locations. The differences between *R. mangle* and *A. germinans*
55 stands were absent at the non-tidal, impounded location.

56

57 **INTRODUCTION**

58 Mangrove species inhabiting tropical and subtropical coastal zones are adapted to
59 tidal influences on soil temperature, water content and salt concentration, and to
60 varying degrees of anoxia (Alongi, 2008). Species-specific adaptations of propagule

61 dispersal, salt tolerance physiology, and root zone aeration often lead to zonation of
62 species perpendicular to the shoreline. *Avicennia* and *Rhizophora*, both mangrove
63 genera with a global distribution, developed different mechanisms to adapt to stress
64 factors imposed by the prevailing tidal regime. Due to these differences, *Avicennia*
65 and *Rhizophora* generally form mono-specific stands at distinct positions in the tidal
66 zone. In Florida, for example, *Rhizophora mangle* usually occurs lower in the
67 intertidal zone than *Avicennia germinans* that can be found more at in-land sites
68 where tidal inundation is less frequent (McKee, 1993).

69 In most regions, mangrove forests are characterized by high primary production
70 (Bouillon et al., 2008; Kristensen et al., 2008). It was estimated that on average 40%
71 of the mangrove production is decomposed in the soil, 30% is exported from the
72 forest, 10% is stored in the soil and 9% is consumed by herbivores (Duarte and
73 Cebrian, 1996). Hence, a major part of primary mangrove production is decomposed
74 locally. Microbial decomposition will depend on soil environmental conditions,
75 which in the case of mangroves are largely governed by the prevailing tidal
76 regime (Keuskamp et al., 2015). Aerobic microbial processes, which are
77 thermodynamically most favorable (Laanbroek, 1990), are restricted to the first few
78 oxygen-containing mm's of the soil as has been shown for soils covered by *Avicennia*
79 *marina* and *Rhizophora apiculata* (Andersen and Kristensen, 1988; Kristensen et al.,
80 1988; Kristensen et al., 1992; Kristensen et al., 1994). Through the presence of
81 aerenchyma in specialized root structures, both *Avicennia* spp. and *Rhizophora* spp.
82 are able to transfer oxygen from the air to their roots in anoxic sediments (Scholander
83 et al., 1955) facilitating aerobic decomposition at deeper soil layers. Several
84 observations suggest that *Avicennia* species generally maintain a more oxidized root
85 zone compared to *Rhizophora* species (Nickerson and Thibodeau, 1985; Thibodeau
86 and Nickerson, 1986; Alongi et al., 2000). Since the redox status in the root zone is
87 determined by the balance between oxygen-producing and -consuming processes, the
88 more oxidized zones with *Avicennia* are likely due to a higher oxygen to labile carbon
89 ratio released from the roots of this mangrove species compared to *Rhizophora*
90 species.

91 Sulfate reduction, which is accomplished by strictly anaerobic microorganisms, is
92 globally the second most important respiratory process after aerobic respiration
93 involved in the decomposition of mangrove-derived soil organic matter (Kristensen et

94 al., 2008). An earlier study by Balk et al (Balk et al., 2015) suggests an effect of depth
95 on traits of the sulfate-reducing microbial community in mangrove forest soils.
96 Potential sulfate reduction rates, copy numbers of the *dsrB* gene, which is specific for
97 sulfate-reducing microbes, and numbers of sulfate-reducing cells that were able to
98 grow on a mixture of acetate, propionate and lactate were all significantly higher in
99 samples from the reduced sub-surface layers (4-6 cm depth) than in samples from the
100 more oxidized surface layers (0-2 cm depth). Hence, traits of the sulfate-reducing
101 community are strongly influenced by the redox status of the soil. Assuming more
102 reduced conditions below *Rhizophora* than below *Avicennia* stands, we hypothesize
103 that the soil from beneath *Rhizophora* has higher potential sulfate reduction rates and
104 higher numbers of sulfate-reducing microorganisms than soil from underneath
105 *Avicennia*. To test this hypothesis, we collected the upper 2 cm of soil from beneath
106 *A. germinans* and *R. mangle* stands on three locations in South Florida that differ
107 mutually in tidal regimes. At each location, both species were present in separated
108 zones. Since oxygen penetrates generally only a few mm's into the soil (Andersen and
109 Kristensen, 1988; Kristensen et al., 1988; Kristensen et al., 1992; Kristensen et al.,
110 1994), we assumed that differences among species in the upper 2 cm of the soil can
111 largely be ascribed to differences in the balance between oxygen and carbon released
112 by the plant roots.

113

114 MATERIALS AND METHODS

115

116 STUDY SITES

117 In March 2009, soil samples were collected from monospecific stands of *Avicennia*
118 *germinans* and *Rhizophora mangle* at three mangrove forest locations in southern
119 Florida that differ mutually in total organic carbon content (Table 1). One sampling
120 location was situated at the Port of the Islands (25°56' N and 81°30' W) in Collier
121 County on the east coast of the Gulf of Mexico, one location near Jensen Beach
122 (27°17' N and 80°13' W) in Martin County on South Hutchinson Island, and one
123 location near Fort Pierce (27°28' N and 80°18' W) in St. Lucie County on North
124 Hutchinson Island. North and South Hutchinson Islands are part of a range of barrier
125 islands sheltering the Indian River lagoon from the North Atlantic Ocean. Both

126 locations are situated on the land side of the islands along the lagoon. At the moment
127 of sampling, the mangrove forest at North Hutchinson Island was impounded and
128 tidal influences were restricted (Verhoeven et al., 2014). The absence of tide at this
129 location affected mostly the stands of *A. germinans*. At the tidal locations Port of rthe
130 Islands and South Hutchinson Island, intertidal soils from *R. mangle* stands were
131 collected from the seaward fringes, while supratidal soils from *A. germinans* were
132 sampled more from the interior of the forests. In all cases, soils were collected from
133 mature stands with an average tree height of more than 2 m. The soil samples were
134 taken close to the aerial roots of the trees. Replicate samples from 0-2 cm depth were
135 collected within an area of 1 square meter and transported to the laboratory on ice.

136

137 DETERMINATION OF SULFATE REDUCTION RATES

138 Sulfate reduction rates were determined in the flow-through reactors (FTRs) as
139 described before (Balk et al., 2015). Shortly, the incubation of intact soil layers ran in
140 these reactors at a constant rate of 1 ml h⁻¹ in a temperature-controlled room at 20-22
141 °C. To maintain anoxic conditions throughout the experiments, inflow solutions and
142 connecting tubing were purged with argon and the incubations were performed in the
143 dark to avoid oxygen production by photosynthesis. Before the start of the incubation
144 experiments, reactors were flushed with a salt solution containing various
145 concentrations of NaCl that match the salt concentrations measured at the sampling
146 sites (Table 1). Flushing with salt solutions made it also possible to establish the time
147 it takes a solution to move from the bottom to the top of the soil in the reactor. These
148 so-called breakthrough times for Port of the Islands, South Hutchinson Island and
149 North Hutchinson Island were 65, 61 and 52 hours for the *Avicennia* soils,
150 respectively, and 69, 58 and 52 hours for the corresponding *Rhizophora* soils. These
151 breakthrough times correlated well ($R^2=0.765$) with the total carbon contents
152 presented in Table 1. During the first 90 hours of the incubation experiment, the
153 inflow solutions contained 4 mM sodium sulfate, defined concentrations of NaCl and
154 no electron donor. In order to determine the effect of easily available organic carbon
155 on sulfate reduction rates, both sodium acetate (10 mM) and sodium lactate (10 mM)
156 were supplied after 90 hours, while sodium sulfate was increased to 8 mM to fulfill
157 the demand for extra oxidant capacity. Sulfate concentrations were measured by ion
158 chromatography using a Dionex DX120 (Water, Milford, MA, USA) with an IonPac

159 ICE-AS6 column and Anion-ICE Micro Membrane II suppressor. Steady-state sulfate
160 reduction rates (SRR) were calculated as described before (Balk et al., 2015).

161

162 DNA EXTRACTION

163 Chromosomal DNA was isolated from the soils by a modification of the DNA
164 isolation procedure as described previously by Zhou et al. (Zhou et al., 1996). DNA
165 was further purified using the DNA Clean & Concentrator kit (Zymo Research,
166 Orange, CA). The quantity and quality of the extracted DNA were analyzed by
167 spectrophotometry using a ND-1000 spectrophotometer (NanoDrop Technologies,
168 Wilmington, DE) and by agarose gel electrophoresis. The genomic DNA was stored
169 at -20°C for further analyses.

170

171 QPCR AMPLIFICATION

172 qPCR amplification for sulfate-reducing prokaryotes targeting on the *dsrB* gene was
173 performed in a total volume of 20 µl with the primer pair DSRp2060F and DSR4R
174 (Geets et al., 2006) on a Rotor-Gene 3000 (Corbett Research, QIAGEN, Valencia,
175 CA). Each PCR mixture was made using a CAS-1200 pipetting robot (Corbett
176 Research, QIAGEN, Valencia, CA) and contained 3 µl diluted template, 10 µl
177 Absolute™ QPCR SYBR Green Mix (Thermo Scientific, Epsom, UK), 0.4 µl of
178 each primer (10 µM) and 1µl Bovine Serum Albumin (BSA) (20 mM). The qPCR
179 procedure was as follows: 10 s at 95 °C for initial denaturation, 45 cycles of 20 s at 95
180 °C, 30 s at 56 °C and 45 s at 72 °C. At each cycle, fluorescence was obtained at 84 °C.
181 A melting curve was performed from 55 °C to 99 °C to confirm PCR product
182 specificity. Purified PCR products from extracted DNA originating from the same soil
183 samples, generated with the same primer set and cloned into the pGEM-T Easy
184 Vector (Promega, Madison, WI) were used for the production of the standard curve.
185 In order to get specific products and avoid inhibition, dilution series were made of the
186 soil DNA solution to test for inhibition and set a 100-fold dilution as the final
187 template. The amplification efficiency ranged from 98-104% with R² values greater
188 than 0.99.

189

190 ENUMERATION OF SULFATE-REDUCING MICROORGANISMS ABLE TO
191 GROW ON A MIXTURE OF ACETATE, PROPIONATE AND LACTATE

192 A Most Probable Number (MPN) assay was applied for enumeration of sulfate-
193 reducing microorganisms able to grow on a mixture of acetate, propionate and lactate
194 (APL medium). Briefly, soil was re-suspended in phosphate-buffered saline (pH 7.4)
195 in a soil to buffer ratio of 1:6 and shaken for 2 hours. Subsequently, 10-fold dilutions
196 were made in microtiter plates containing a minimal salt medium (Widdel and Bak,
197 1992) supplemented with a mix of acetate, propionate and lactate (15 mM each) as
198 electron donors. Sodium sulfate (20 mM) was provided as the electron acceptor.
199 Sodium thioglycolate (0.5 mM) was added as reducing agent and iron sulfate (0.2
200 mM) as indicator for the occurrence of sulfate reduction. The formation of black iron
201 sulfide precipitates was indicative for sulfate reduction. The microtiter plates were
202 incubated for 3 months at 25 °C in anaerobic incubation bags (Anaerocult® A mini,
203 Merck, Darmstadt, Germany). Based on the number of positive wells, the most
204 probable numbers and related confidence limits were calculated using standard tables
205 (Rowe et al., 1977).

206

207 STATISTICS

208 The obtained data were fitted to (mixed) linear models in R 3.2.3 (R Core Team,
209 2015) with site as a random factor where appropriate. Normality and
210 homoscedasticity of residuals were visually assessed and confirmed by Shapiro-Wilk
211 and Levene's tests, respectively. Where necessary, log transformations were applied
212 prior to analysis. Overall effects were tested for significance using ANOVA with type
213 II sum of squares using *car* 2.0-22 (Fox and Weisberg 2011), while pairwise
214 comparisons were conducted using Tukey's HSD as implemented in the *multcomp*
215 1.4-6 package (Hothorn et al., 2008). Due to the commonly observed interactive
216 effect of plant species and locations, the effects of both fixed variables on the sulfate
217 reduction traits were tested separately.

218

219 **RESULTS**

220

221 POTENTIAL SULFATE REDUCTION RATES

222 Differences between sulfate concentrations in- and outflowing the FTRs filled with
223 undisturbed mangrove soils reflect the activity of sulfate-reducing microorganisms in
224 these soils. Figure 1 presents the sulfate reduction rates measured in the FTRs. The
225 increase in rates after 144 hours was due to the amendment of easily degradable
226 carbon sources (*i.e.* acetate and lactate) to the inflowing medium. These carbon
227 sources were added 90 hours after the start of the incubation. Delay between
228 amendment and observation of its effects was determined by the breakthrough time,
229 which is the time needed for the solution to pass the entire reactor. In the non-
230 amended incubations of soils samples from Port of the Islands and South Hutchinson
231 Island, the reactors reached steady state sulfate reduction rates within 96 and 48
232 hours, respectively, for *R. mangle* soils, while soils from *A. germinans* required 120
233 hours to reach steady state sulfate reduction rates. Such a difference between *A.*
234 *germinans* or *R. mangle* stands was not observed in North Hutchinson Island: Soils
235 from both species required 96 hours to reach steady state rates.

236 For the calculation of average steady state sulfate reduction rates two periods were
237 used: One from 96 – 144 hours measured in the absence of added carbon sources, and
238 one from 160 – 198 hours determined in the presence of added acetate plus lactate.
239 The results are presented in Table 2. When only the natural carbon sources were
240 available, steady state sulfate reduction rates were significantly higher in soil samples
241 from *A. germinans* stands than in soil samples from *R. mangle* stands for the locations
242 Port of the Islands and South Hutchinson Island ($p < 0.001$ and $p < 0.01$, respectively),
243 while in samples from North Hutchinson Island no significant differences were
244 observed between stands from both plant species (Supplemental Table S1). Sampling
245 location had a significant effect on measured steady state sulfate reduction rates
246 ($p < 0.001$). Due to interactive effects of plant species and sampling locations, the
247 effect of location on steady state sulfate reduction rates was tested separately for each
248 species (Supplementary Table S2). In *R. mangle* stands, no significant differences in
249 sulfate reduction rates were observed between Port of the Islands and South
250 Hutchinson Island, although both showed significantly higher steady state rates
251 compared to soil from North Hutchinson Island. In soils from *A. germinans* stands,
252 steady state sulfate reduction rates in the absence of added organic compounds
253 differed significantly between locations: North Hutchinson Island $<$ South Hutchinson

254 Island < Port of the Islands. Hence with both mangrove species, steady state sulfate
255 reduction rates were significantly lower in samples from North Hutchinson Island.

256 The rate differences between *A. germinans*- and *R. mangle*-derived soils became more
257 pronounced by addition of extra electron donors (Figure 2, Table 2). Steady state
258 sulfate reduction rates were consistently higher in soil samples from *A. germinans*
259 stands than in samples from *R. mangle* stands (Supplementary Table S1). Sampling
260 location had also a significant effect on these rates ($p < 0.001$). For soils from *R.*
261 *mangle* and *A. germinans* stands, steady state sulfate reduction rates increased
262 significantly between locations in the same order: Port of the Islands < South
263 Hutchinson Island < North Hutchinson Island (Supplementary Table S2).

264

265 NUMBERS OF SULFATE-REDUCING MICROORGANISMS

266 Numbers of sulfate-reducing microorganisms in the mangrove soil were enumerated
267 in two ways, *i.e.* by determining the copy numbers of the *dsrB* gene and by
268 establishing the number of cells able to grow in APL medium by an MPN method.
269 Numbers of these latter cells were on average 96000 times lower than the copy
270 numbers of the *dsrB* gene.

271 Copy numbers of the *dsrB* gene were on average higher in soil samples from *A.*
272 *germinans* stands than from *R. mangle* stands (Figure 2a). However, this was only
273 significant for samples collected at South Hutchinson Island (Supplementary Table
274 S1). Due to the interactive effects of plant species and sampling locations, the effect
275 of sampling location on *dsrB* gene copy numbers was tested separately for both
276 mangrove species. For *R. mangle* stands, gene copy numbers were significantly
277 higher in samples from North Hutchinson Island than in samples from Port of the
278 Islands and South Hutchinson Island that did not significantly differ from each other
279 (Supplementary Table 2). For *A. germinans* stands, gene copy numbers were
280 significantly lower in samples from Port of the Islands than in samples from South
281 and North Hutchinson Island that did not significantly differ from each other.

282 The effect of numbers of cells that grew in APL medium was also dependent on
283 sampling location (Figure 2b). Only in soil samples from South Hutchinson Island,
284 significantly higher cell numbers were observed in samples from *A. germinans* stands
285 than in samples from *R. mangle* stands (Supplementary Table S1). At the other

286 locations, numbers of cells able to grow on a mixture of acetate, propionate and
287 lactate did not differ significantly between *A. germinans* and *R. mangle* stands. For *A.*
288 *germinans* stands sampling location had no significant effect on the number of such
289 cells, but for *R. mangle* stands these numbers were significantly higher at North
290 Hutchinson Island than at Port of the Islands and South Hutchinson Island, which did
291 not differ significantly from each other (Supplementary Table S2).

292 Both plant species and sampling location had a significant effect on the fraction of
293 *dsrB*-containing cells that were able to grow in APL medium (Supplementary Table
294 S3). This fraction was higher in the *R. mangle* stand than in the *A. germinans* stands,
295 and higher at Port of the Islands than at the other locations (Figure 2c).

296

297 **DISCUSSION**

298 Based on the generally accepted idea that *Rhizophora* soils are more reduced than
299 *Avicennia* soils, we hypothesized that sulfate reduction traits such as community size
300 and activity would be more manifest in soils from *Rhizophora* stands than in soils
301 from *Avicennia* stands. A number of observations seemed to disprove the hypothesis.
302 Potential steady state reduction rates, copy numbers of the *dsrB* gene and numbers of
303 culturable cells were all significantly higher values in samples obtained from *A.*
304 *germinans* stands than in samples from *R. mangle* stands, at least at the tidal locations
305 Port of the Islands and South Hutchinson Island. Then the question arises what causes
306 the unexpected findings of higher sulfate reduction traits in *A. germinans* stands than
307 in *R. mangle* stands, which are assumed to be more reduced than the *Avicennia*
308 locations?

309 Was the original assumption correct that *Avicennia* is able to maintain a more
310 oxidized root zone than *Rhizophora*? By a series of gas exchange experiments
311 Scholander and colleagues showed that both *Avicennia nitida* (synonymous with *A.*
312 *germinans*) and *R. mangle* are able to supply their aerenchymatous roots with oxygen
313 from the atmosphere, albeit with different temporal cycles (Scholander et al., 1955).
314 Both *A. nitida* and *R. mangle* showed diurnal cycles with the highest oxygen
315 concentrations at day time. However, *A. nitida* growing in the tidal zone revealed also
316 tidal cycles with the highest oxygen concentrations during low tide. Thibodeau and
317 Nickerson measured a decreasing redox potential in the soil with an increasing

318 distance to *A. germinans* trees, whereas the opposite, *i.e.* increasing redox potential
319 with increasing distance to the trees, was observed with *R. mangle* (Thibodeau and
320 Nickerson, 1986). As shown in another publication by these authors (Nickerson and
321 Thibodeau 1985), the root zone-oxidizing capacity of *A. germinans* may lead to
322 growth of this species in more sulfide-rich soils than *R. mangle*. In contrast to *A.*
323 *germinans*, *R. mangle* is not able to lower the sulfide concentration of the surrounding
324 soil. In a study on below-ground decomposition of organic matter in mangrove forest
325 dominated by *Avicennia marina* or *Rhizophora stylosa*, Alongi and colleagues (2000)
326 observed lower redox potential in the upper soil layers of *R. stylosa* stands than in the
327 upper soil layers of *A. marina* stands, even when both species grew in the same tidal
328 zone. In only one case studied by Alongi and colleagues, a lower redox potential was
329 observed in the upper layers of an *A. marina* stand. The authors suggested that such a
330 difference in behavior between the species may be due to differential exposure to
331 wave action and perhaps to differences in flooding and drainage cycles. So in general,
332 we have no reason to assume that our *R. mangle* stands are less reduced than our *A.*
333 *germinans* stands. The more so because the intertidal *R. mangle* stands at the tidal
334 locations Port of the Islands and South Hutchinson Island were flooded twice daily,
335 whereas the supratidal *A. germinans* stands were flooded seldom. With respect to the
336 non-tidal stands in the impounded mangrove forest of North Hutchinson Island, the *R.*
337 *mangle* stands were always wetter than the *A. germinans* stands. Another indication of
338 more oxidized conditions at the *A. germinans* stands in our study may be the higher
339 salinities measured pair-wise in the *A. germinans* and *R. mangle* stands at the three
340 locations. A higher salinity reflects a higher degree of evapotranspiration and
341 consequently a higher degree of air drawn into the soil.

342 Might the lower *dsrB* gene copy numbers observed at the tidal *R. mangle* stands be
343 explained by inhibition of the qPCR procedure by the presence of inhibiting
344 compounds? A generally low nutrient content combined with a relatively high content
345 of condensed tannins and phenolic compounds, lead generally to a lower carbon
346 decomposition rate and consequently to more organic carbon accumulation at
347 *Rhizophora* stands than at *Avicennia* stands (Robertson, 1988; McKee and Faulkner,
348 2000; Middleton and McKee, 2001; Erickson et al., 2004; Kristensen et al., 2008;
349 Galeano et al., 2010; Lima and Colpo, 2014). The *A. germinans* and *R. mangle* stands
350 have been selected pair-wise at three locations in mutually remote regions. These

351 pair-wise stands showed always a higher total carbon content at the *A. germinans* than
352 at the *R. mangle* stands, except at the impounded, non-tidal location of North
353 Hutchinson. However, we cannot exclude that certain carbon compounds specifically
354 present at *R. mangle* stands have affected the enumeration of *dsrB* gene copies by
355 qPCR negatively. An argument against a possible inhibition of the qPCR enumeration
356 by organic compounds present at the *R. mangle* stands, might be the distribution of
357 culturable cell numbers at the different locations that show the same trends as
358 observed with *dsrB* gene copies. Finally, the trend of higher numbers at *A. germinans*
359 stands compared to *R. mangle* stands, reflects the higher potential sulfate reduction
360 rates measured in samples from *A. germinans* stands.

361 Were the levels of *dsrB* gene copy numbers very different from numbers determined
362 in other studies? Irrespective of *A. germinans* or *R. mangle*, the median abundance of
363 the *dsrB* gene we observed at the different stands varied between 7.5×10^7 and $4.4 \times$
364 10^9 copies per cm^3 , which is slightly above the range we found in the carbon-poor *A.*
365 *marina* soils at the Red Sea coast (Balk et al. 2015). At the upper 5 cm of an oil-
366 contaminated, Brazilian mangrove soil, Andrade and colleagues observed 3.6×10^8
367 *dsrB* gene copies per g soil (Andrade et al., 2012). In other contaminated mangrove
368 soils in Brazil, the *dsrB* gene copy numbers varied from 7.9×10^4 to 2.0×10^5 per g
369 soil (Varon-Lopez et al., 2014). These last numbers are lower than the other numbers
370 from mangrove soils, but Varon-Lopez and colleagues mixed the upper 30 cm of the
371 soil before they started their analyses. From the work of Andrade and colleagues it is
372 known that *dsrB* gene copy numbers decline with depth (Andrade et al., 2012). The
373 numbers of *dsrB* gene copies found in our study were at the same range as found for
374 example in estuarine sediments (Kondo et al., 2004) and in paddy soil (Liu et al.,
375 2009).

376 A striking observation was the significantly larger fraction of culturable cells in
377 samples from the *R. mangle* stands than in samples from the *A. germinans* stands,
378 although absolute numbers of *dsrB*-containing and growing cells were larger at the *A.*
379 *germinans* stands. A larger fraction of culturable cells might be explained by
380 differences in community composition, but this was not determined in this study. In
381 our former study on sulfate-reducing communities at the Red Sea coast we found a
382 higher fraction of culturable cells in the deeper soil layers than in the surface layers,
383 but we were not able to relate this to differences in genus diversity and to differences

384 in functional diversity (Balk et al 2015). So most likely, the higher fraction of
385 culturable cells mimicked more active sulfate-reducing communities at the intertidal
386 *R. mangle* stands.

387 The presence of a more active sulfate-reducing community present at intertidal *R.*
388 *mangle* stands than at supratidal *A. germinans* stands may also be inferred from the
389 differences in time required to obtain steady state rates as was observed during the
390 measurements of potential sulfate-reducing activities. At the tidal locations, samples
391 from *R. mangle* stands collected at Port of the Islands and South Hutchinson Island
392 required 96 and 48 hours, respectively, before steady state rates were obtained, while
393 the corresponding time for samples from *A. germinans* stands amounted to 120 hours
394 for both locations. No difference with respect to reaching steady state sulfate
395 reduction rates was observed between *R. mangle* and *A. germinans* samples collected
396 at the impounded, non-tidal location of North Hutchinson Island. The time required to
397 reactivate the cells from the more oxidized stands may be due to the establishment of
398 anoxic conditions upon starting the measurements in the FTR's. Sulfate-reducing
399 microorganisms have been observed in oxic parts of estuarine and lake sediments
400 before (Laanbroek and Pfennig, 1981; Sass et al., 1997). Sulfate-reducing strains
401 isolated from oxic sediment layers revealed a higher oxygen tolerance and capacity of
402 oxygen respiration than strains originating from anoxic sediment layers (Sass et al.,
403 1997). In this latter study, no sulfate reduction was observed under oxic conditions
404 because oxygen was preferentially reduced. All strains of a diverse group of sulfate-
405 reducing isolates were able to oxidize lactate and even to oxidize sulfide in the
406 presence of oxygen thereby creating again anoxic conditions (Sass et al., 2002).

407 The impounded, non-tidal location at North Hutchinson Island reacted also more
408 strong than the tidal stations to the addition of acetate and lactate as electron donors to
409 the FTR's used for determining potential sulfate reduction rates. This may be
410 explained by a different community present in the non-tidal stands or on repression of
411 sulfate reduction by organic compounds present in the tidal stands. With respect to
412 this latter explanation: The supratidal *A. germinans* stands at the tidal locations
413 showed a more intense effect in the FTR's upon the addition of acetate and lactate
414 than the intertidal *R. mangle* stands, while these tidal stands contained more organic
415 carbon than the samples from the non-tidal North Hutchinson Island location. Hence,
416 it seems that more oxidized habitats at the non-tidal stations and at the supratidal *A.*

417 *germinans* stands at the tidal stations contain a community able to react more intense
418 of the addition of acetate and lactate once they have been activated by creating anoxic
419 conditions in the reactors.

420

421 *Conclusions*

422 Firstly, when potential sulfate-reduction rates and total numbers of *dsrB* gene copies
423 and of culturable cells are not the most reliable indicators for active sulfate-reducing
424 communities, but when the dynamics of sulfate reduction rates measured during the
425 initial period in the FTR's and the size of the fraction of culturable cells are, then the
426 tidal stands of *R. mangle* accommodate a more active sulfate-reducing community
427 than the stands of *A. germinans*, and we cannot disprove our hypothesis.

428 Secondly, the supratidal *A. germinans* stands at the tidal locations and the *A.*
429 *germinans* and *R. mangle* stands at the impounded, non-tidal location accommodate
430 apparently a sulfate-reducing community that is able to survive oxidized conditions.

431

432 ACKNOWLEDGEMENTS

433 We would like to acknowledge Drs. Mariet Hefting, Jos Verhoeven and Dennis
434 Whigham for their assistance during the collection of the samples. We also want to
435 acknowledge the help we obtained from Dr. Valery Paul, Mr. Woody Lee, and staff of
436 the Smithsonian Marine Station at Fort Pierce. The research has been funded by the
437 King Abdullah University of Science and Technology (KAUST) (www.kaust.edu.sa)
438 Center-in-Development Award to Utrecht University via the KAUST Global
439 Research Partnership and by the Netherlands Organization for Scientific Research
440 (NWO)/User Support Program Space Research (Project no. ALW-GO/12-43). This is
441 publication number XXXX of the Netherlands Institute of Ecology and publication
442 number YYYY of the Smithsonian Marine Station.

443

444 Legend to the figures:

445

446 **Figure 1:** Sulfate reduction rates measured in through-flow reactors containing the
447 top 2 cm of intact soil samples collected from *Avicennia germinans* (gray dots) and
448 *Rhizophora mangle* stands (orange dots) at Port of the Islands, South Hutchinson
449 Island and North Hutchinson Island. Note the split Y-axes for the points presented
450 after 144 hours.

451

452 **Figure 2:** Numbers of *dsrB* gene copies (a), numbers of sulfate-reducing cells able to
453 grow on liquid medium with acetate, propionate, lactate (APL medium) (b) and the
454 fraction of *dsrB* gene-containing cells able to grow on APL medium (c) in soil
455 samples collected from *Avicennia germinans* (gray boxplots) and *Rhizophora mangle*
456 *stands* (orange boxplots) at Port of the Islands, South Hutchinson Island and North
457 Hutchinson Island.

458

459 Tables

460

461 **Table 1:** Characteristics of mangrove forest soil samples collected from Florida, USA

462

Mangrove species	<i>Avicennia germinans</i>			<i>Rhizophora mangle</i>		
Location	Port of the Islands	South Hutchinson Island	North Hutchinson Island	Port of the Islands	South Hutchinson Island	North Hutchinson Island
Pore water salinity (g/L)	69.0	41.0	94.5	38.5	37.0	57.0
Pore water pH	7.7	5.7	7.5	7.4	7.4	6.7
Mean particle size (DV50 ^a)	83.9	91.5	156.7	86.5	115.8	291.7
Total organic carbon (% dw)	26.2	26.0	3.5	22.3	13.0	6.1

463 ^a as volume-based mean diameter

464

465 **Table 2:** Steady-state sulfate reduction rates (nMol S cm⁻³ h⁻¹) measured in non-
 466 carbon-amended and in carbon-amended flow-through reactors containing the upper 2
 467 cm of soils collected from *Avicennia germinans* or *Rhizophora mangle* stands.

	Non-carbon amended reduction rates			Carbon-amended reduction rates		
	Port of the Islands	South Hutchinson Island	North Hutchinson Island	Port of the Islands	South Hutchinson Island	North Hutchinson Island
<i>Avicennia germinans</i>	97	83	49	336	361	471
<i>Rhizophora mangle</i>	68	73	52	217	257	359

468

469

470 References

- 471 Alongi, D.M. (2008). Mangrove forests: Resilience, protection from tsunamis, and
 472 responses to global climate change. *Estuarine Coastal and Shelf Science*
 473 76(1), 1-13. doi: 10.1016/j.ecss.2007.08.024.
- 474 Alongi, D.M., Tirendi, E., and Clough, B.F. (2000). Below-ground decomposition of
 475 organic matter in forests of the mangroves *Rhizophora stylosa* and *Avicennia*
 476 *marina* along the arid coast of Western Australia. *Aquatic Botany* 68(2), 97-
 477 122.
- 478 Andersen, F.O., and Kristensen, E. (1988). Oxygen microgradients in the rhizosphere
 479 of the mangrove *Avicennia marina*. *Marine Ecology Progress Series* 44(2),
 480 201-204. doi: 10.3354/meps044201.
- 481 Andrade, L.L., Leite, D.C.A., Ferreira, E.M., Ferreira, L.Q., Paula, G.R., Maguire,
 482 M.J., et al. (2012). Microbial diversity and anaerobic hydrocarbon degradation
 483 potential in an oil-contaminated mangrove sediment. *Bmc Microbiology* 12.
 484 doi: 10.1186/1471-2180-12-186.
- 485 Balk, M., Keuskamp, J.A., and Laanbroek, H.J. (2015). Potential Activity, Size, and
 486 Structure of Sulfate-Reducing Microbial Communities in an Exposed, Grazed
 487 and a Sheltered, Non-Grazed Mangrove Stand at the Red Sea Coast. *Frontiers*
 488 *in Microbiology* 6. doi: 10.3389/fmicb.2015.01475.
- 489 Bouillon, S., Borges, A.V., Castaneda-Moya, E., Diele, K., Dittmar, T., Kristensen,
 490 E., et al. 2008. Mangrove production and carbon sinks: A revision of global
 491 budget estimates. *Global Biochemical Cycles* [Online], 22(2).
- 492 Duarte, C.M., and Cebrian, J. (1996). The fate of marine autotrophic production.
 493 *Limnology and Oceanography* 41(8), 1758-1766.
- 494 Erickson, A.A., Bell, S.S., and Dawes, C.J. (2004). Does mangrove leaf chemistry
 495 help explain crab herbivory patterns? *Biotropica* 36(3), 333-343.
- 496 Fox, J., and Weisberg, S. (2011). *An {R} Companion to Applied Regression* [Online].
 497 Thousand Oaks, CA. Available:
 498 <http://socserv.socsci.mcmaster.ca/jfox/Books/Companion> [Accessed].
- 499 Galeano, E.G., Pineda, J.E.M., and Calderon, J.H.M. (2010). Effect of the substrate
 500 on the litter decomposition in three mangrove species in the Biosphere
 501 Reserve Seaflower, Colombian Caribbean. *Caldasia* 32(2), 411-424.
- 502 Geets, J., Borrernans, B., Diels, L., Springael, D., Vangronsveld, J., van der Lelie, D.,
 503 et al. (2006). DsrB gene-based DGGE for community and diversity surveys of
 504 sulfate-reducing bacteria. *Journal of Microbiological Methods* 66(2), 194-205.
- 505 Hothorn, T., Bretz, F., and Westfall, P. (2008). Simultaneous Inference in General
 506 Parametric Models. *Biometrical Journal* 50(3), 346-363.
- 507 Keuskamp, J.A., Hefting, M.M., Dingemans, B.J.J., Verhoeven, J.T.A., and Feller,
 508 I.C. (2015). Effects of nutrient enrichment on mangrove leaf litter
 509 decomposition. *Science of the Total Environment* 508, 402-410. doi:
 510 10.1016/j.scitotenv.2014.11.092.
- 511 Kondo, R., Nedwell, D.B., Purdy, K.J., and Silva, S.D. (2004). Detection and
 512 enumeration of sulphate-reducing bacteria in estuarine sediments by
 513 competitive PCR. *Geomicrobiology Journal* 21(3), 145-157. doi:
 514 10.1080/01490450490275307.
- 515 Kristensen, E., Andersen, F.O., and Kofoed, L.H. (1988). Preliminary assessment of
 516 benthic community metabolism in a southeast Asian mangrove swamp.
 517 *Marine Ecology Progress Series* 48(2), 137-145. doi: 10.3354/meps048137.

- 518 Kristensen, E., Bouillon, S., Dittmar, T., and Marchand, C. (2008). Organic carbon
519 dynamics in mangrove ecosystems: A review. *Aquatic Botany* 89(2), 201-219.
520 doi: 10.1016/j.aquabot.2007.12.005.
- 521 Kristensen, E., Devol, A.H., Ahmed, S.I., and Saleem, M. (1992). Preliminary-study
522 of benthic metabolism and sulfate reduction in a mangrove swamp of the
523 Indus delta, Pakistan. *Marine Ecology Progress Series* 90(3), 287-297. doi:
524 10.3354/meps090287.
- 525 Kristensen, E., King, G.M., Holmer, M., Banta, G.T., Jensen, M.H., Hansen, K., et al.
526 (1994). Sulfate reduction, acetate turnover and carbon metabolism in
527 sediments of the Ao Nam Bor mangrove, Phuket, Thailand. *Marine Ecology*
528 *Progress Series* 109(2-3), 245-255. doi: 10.3354/meps109245.
- 529 Laanbroek, H.J. (1990). Bacterial Cycling of Minerals That Affect Plant-Growth in
530 Waterlogged Soils - a Review. *Aquatic Botany* 38(1), 109-125.
- 531 Laanbroek, H.J., and Pfennig, N. (1981). Oxidation of short-chain fatty-acids by
532 sulfate-reducing bacteria in fresh-water and in marine-sediments. *Archives of*
533 *Microbiology* 128(3), 330-335.
- 534 Lima, R.G., and Colpo, K.D. (2014). Leaf-litter decomposition of the mangrove
535 species *Avicennia schaueriana*, *Laguncularia racemosa* and *Rhizophora*
536 *mangle*. *Journal of the Marine Biological Association of the United Kingdom*
537 94(2), 233-239. doi: 10.1017/s0025315413001574.
- 538 Liu, X.Z., Zhang, L.M., Prosser, J.I., and He, J.Z. (2009). Abundance and community
539 structure of sulfate reducing prokaryotes in a paddy soil of southern China
540 under different fertilization regimes. *Soil Biology & Biochemistry* 41(4), 687-
541 694. doi: 10.1016/j.soilbio.2009.01.001.
- 542 McKee, K.L. (1993). Soil physicochemical patterns and mangrove species
543 distribution - reciprocal effects. *Journal of Ecology* 81(3), 477-487. doi:
544 10.2307/2261526.
- 545 McKee, K.L., and Faulkner, P.L. (2000). Restoration of biogeochemical function in
546 mangrove forests. *Restoration Ecology* 8(3), 247-259. doi: 10.1046/j.1526-
547 100x.2000.80036.x.
- 548 Middleton, B.A., and McKee, K.L. (2001). Degradation of mangrove tissues and
549 implications for peat formation in Belizean island forests. *Journal of Ecology*
550 89(5), 818-828. doi: 10.1046/j.0022-0477.2001.00602.x.
- 551 Nickerson, N.H., and Thibodeau, F.R. (1985). Association between pore water sulfide
552 concentrations and the distribution of mangroves. *Biogeochemistry* 1(2), 183-
553 192. doi: 10.1007/bf02185041.
- 554 Robertson, A.I. (1988). Decomposition of mangrove leaf litter in tropical Australia.
555 *Journal of Experimental Marine Biology and Ecology* 116(3), 235-247. doi:
556 10.1016/0022-0981(88)90029-9.
- 557 Rowe, R., Todd, R., and Waide, J. (1977). Microtechnique for most-probable-number
558 analysis. *Applied and Environmental Microbiology* 33(3), 675-680.
- 559 Sass, A.M., Eschemann, A., Kuhl, M., Thar, R., Sass, H., and Cypionka, H. (2002).
560 Growth and chemosensory behavior of sulfate-reducing bacteria in oxygen-
561 sulfide gradients. *Fems Microbiology Ecology* 40(1), 47-54. doi:
562 10.1111/j.1574-6941.2002.tb00935.x.
- 563 Sass, H., Cypionka, H., and Babenzien, H.D. (1997). Vertical distribution of sulfate-
564 reducing bacteria at the oxic-anoxic interface in sediments of the oligotrophic
565 Lake Stechlin. *Fems Microbiology Ecology* 22(3), 245-255. doi:
566 10.1111/j.1574-6941.1997.tb00377.x.

567 Scholander, P.F., Vandam, L., and Scholander, S.I. (1955). Gas exchange in the roots
568 of mangroves. *American Journal of Botany* 42(1), 92-98. doi:
569 10.2307/2438597.

570 Team, R.C. 2015. R: A language and environment for statistical computing. R
571 Foundation for Statistical Computing. Available: <https://www.R-project.org/>.

572 Thibodeau, F.R., and Nickerson, N.H. (1986). Differential oxidation of mangrove
573 substrate by *Avicennia germinans* and *Rhizophora mangle*. *American Journal*
574 *of Botany* 73(4), 512-516. doi: 10.2307/2444255.

575 Varon-Lopez, M., Dias, A.C.F., Fasanella, C.C., Durrer, A., Melo, I.S., Kuramae,
576 E.E., et al. (2014). Sulphur-oxidizing and sulphate-reducing communities in
577 Brazilian mangrove sediments. *Environmental Microbiology* 16(3), 845-855.
578 doi: 10.1111/1462-2920.12237.

579 Verhoeven, J.T.A., Laanbroek, H.J., Rains, M.C., and Whigham, D.F. (2014). Effects
580 of increased summer flooding on nitrogen dynamics in impounded mangroves.
581 *Journal of Environmental Management* 139, 217-226. doi:
582 10.1016/j.jenvman.2014.02.035.

583 Widdel, F., and Bak, F. (1992). "Gram-negative mesophilic sulfate-reducing
584 bacteria," in *The Prokaryotes*, eds. A. Balows, H.G. Trüper, M. Dworkin, W.
585 Harder & K.H. Schleifer. (New York: Springer), 3352-3378.

586 Zhou, J.Z., Bruns, M.A., and Tiedje, J.M. (1996). DNA recovery from soils of diverse
587 composition. *Applied And Environmental Microbiology* 62(2), 316-322.

588