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The Selection Exerted by Oil Contamination on Mangrove Fungal Communities

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Abstract Mangrove ecosystems are tropical environments that are characterized by the interaction between the land and the sea. As such, this ecosystem is vulnerable to oil spills. Here, we show a culture-independent survey of fungal communities that are found in the sediments of the following two mangroves that are located on the coast of Sao Paulo State (Brazil): (1) an oil-spill-affected mangrove and (2) a nearby unaffected mangrove. Samples were collected from each mangrove forest at three distinct locations (transect from sea to land), and the samples were analyzed by quantitative PCR and internal transcribed spacer (ITS)-based PCR-DGGE analysis. The abundance of fungi was found to be higher in the oil-affected mangrove. Visual observation and correspondence analysis (CA) of the ITS-based PCR-DGGE profiles revealed differences in the fungal communities between the sampled areas. Remarkably, the oil-spilled area was quite distinct from the unaffected sampling areas. On the basis of the ITS sequences, fungi that are associated with the *Basidiomycota* and

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Ascomycota taxa were most common and belonged primarily to the genera *Epicoccum*, *Nigrospora*, and *Cladosporium*. Moreover, the *Nigrospora* fungal species were shown to be sensitive to oil, whereas a group that was described as "uncultured Basidiomycota" was found more frequently in oil-contaminated areas. Our results showed an increase in fungal abundance in the oil-polluted mangrove regions, and these data indicated potential fungal candidates for remediation of the oil-affected mangroves.

Keywords Tropical microbiology · Fungi · ITS · PCR-DGGE · Oil spill · Bioremediation

1 Introduction

Mangroves are typically found in tropical and subtropical regions, located immediately on the border of the land and sea. They are subjected to periodic flooding by high tides (Holguin et al. 2001; Kathiresan and Bingham 2001). The sediments present in mangroves differ from common soils because of the water saturation that is present in the mangrove ecosystem, which leads to a low availability of oxygen and high salinity levels (Ferreira et al. 2010). Highly reducing redox potentials result in poor organic matter degradation, thus limiting the availability of nutrients in these environments (Ferreira et al. 2010; Holguin et al. 2001).

Mangrove ecosystems have been characterized as hotspots of microbial diversity (Holguin et al. 2001; Dias et al. 2010, 2011). In these ecosystems, microbial communities play a pivotal role in the functioning of the whole system, such as playing a role in the maintenance of its productivity, its conservation and its recovery from stressed or perturbed conditions (Alongi 1988; Santos et al. 2011). The microbiota are often directly involved in key processes, such as the transformation of nutrients, photosynthesis, nitrogen fixation, methanogenesis, phosphate solubilization, sulfate reduction and the production of antibiotics and enzymes (Holguin et al. 2001; Alongi 2002; Das et al. 2006). Recent studies on mangrove sediments have been focused on inventorying the bacterial (Dias et al. 2010; Santos et al. 2011) and archaeal diversity, community composition and functionality (Yan et al. 2006; Dias et al. 2011). However, the fungal communities living in mangrove sediments have not been studied to the same extent, with only a few studies focusing on the culturable fungal species in the mangrove ecosystem (Ananda and Sridhar 2004). Recently, Arfi et al. (2012) described the specificity of fungi colonization in plant species living in this ecosystem (*Avicennia marina* and *Rhizophora stylosa*) using a pyrosequencing approach. This indicated that similar culture-independent approaches may lead to an increased understanding of the fungi that reside in the mangrove ecosystem.

Additionally, besides being reservoirs of microbial diversity, mangrove ecosystems should be regarded as important sources of products that could be of biotechnological interest, such as enzymes (Aniszewski et al. 2010). Dias et al. (2009) described the production of several enzymes by bacterial isolates that were retrieved from mangrove sediments. Therefore, the application of these enzymes for bioremediation in contaminated mangrove areas constitutes a promising new approach. Organisms that have adapted to the mangrove conditions can be used to remove contaminants after perturbation of the ecosystem (Santos et al. 2011). Recently, Santos et al. (2011) inventoried the major contaminants that are present in mangroves and identified oil spills as the major effector of global mangrove deforestations. Therefore, understanding and remediating the effects of oil spills on the mangrove ecosystem represents a key issue that must be resolved to preserve these ecosystems.

The changes in bacterial communities after oil spills in mangroves have been described. The differential compositions of nitrogen-fixing organisms (Taketani et al. 2010) and increases in the presence of bacteria from the genera *Marinobacterium*, *Marinobacter* and *Cycloclasticus* have all been found to result after oil spills, albeit identified using in vitro conditions (Santos et al. 2011). However, these studies have not addressed the effect of an oil spill on the fungal communities of mangroves. In addition to inventorying the fungi that inhabit mangroves, the role of fungus in this environment might be further explored, i.e., whether these organisms are involved in key processes such as bioremediation.

Here, we address these issues by assessing the fungal diversity of mangrove regions that were either subjected to an oil spill or not (each region was divided into three sub-regions). Thus, the composition of the fungal communities in relation to the selective pressure exerted by the oil contamination was analyzed. Besides representing the first culture-independent analysis of fungi in mangrove sediments, our data will pinpoint fungal organisms that adapt to oil contamination and that can be further explored for bioremediation.

2 Materials and Methods

2.1 Mangrove locations

Two distinct mangroves, located approximately 2 km apart near the city of Bertioga, on the coast of the São Paulo state (Brazil), were sampled. The samplings were performed in July 2008 (average temperature 18°C), and expeditions were performed during periods of time when the tide was low. These two mangroves were affected by different sources of contamination. Whereas one was affected by oil contamination that was the result of a spill (OilMgv; 23 °53″49′ S, 46 °12″28′ W), the other was under anthropogenic pressure due to its close proximity to Bertioga (AntMgv; 23 °54″06′ S, 45 °15″ 03′ W). The OilMgv has a history of contamination, specifically 35 million liters of oil was spilled into the mangrove area in 1983. Approximately 29 years after the spill, the presence of oil is visible, mainly in the undersurface layers (30 to 50 cm depth), and the native vegetation is still recovering from the spill (mainly dominated by one single species, *Laguncularia racemosa*). These two mangrove areas were selected because of the localized effect of the oil spill, and a series of studies analyzing the mangroves has already been performed (Dias et al. 2010, 2011).

2.2 Sample Characteristics

Sediment samples were collected from the two mangrove ecosystems, following a transect path from the sea to the land. Along this transect, three sub-regions were defined as follows: (1) close proximity to the sea (point 1), (2) the middle of the mangrove (point 2) and (3) the most proximal to land (point 3). The average distance between points 1, 2 and 3 was approximately 100 m. In each of these three sub-regions, samples were collected with a cylindrical steel sampler (7 cm diameter×30 cm depth), and triplicate samples were collected (separated by approximately 50 m). In total, 18 sediments samples were taken, i.e., two mangroves×three sub-regions×three replicates. For the OilMgv site, sampling points 2 and 3 were observed to have increased evidence of oil contamination compared to the unaffected mangrove, based on the observation of the vegetation state (more diverse in the unaffected mangrove) and the quantity of crabs at the sampling points (small crabs were present in higher numbers in the oil-affected area). Sampling point 1 in the oil-contaminated mangrove was less contaminated and had similar characteristics to all of the sampling points of AntMgv. The distinction between the sub-regions of the OilMgv is heightened by a small river that crosses the mangrove, effectively limiting oil drainage toward point 1.

In addition to the sediment cores extractions, in each sampled area, some environmental conditions were estimated and physicochemical analyses of the sediments were performed. These data were already published by our group (Dias et al. 2011) (Electronic Supplementary Material (ESM) Table S1).

2.3 DNA Extraction from the Mangrove Sediment Samples

All samples were thoroughly homogenized before any subsamples were removed. Subsequently, a 0.3-g subsample of each of the sediment samples was subjected to DNA extraction using the Power Soil DNA Isolation kit (MoBio, USA), which resulted in similar amounts of DNA being obtained from all of the samples and an average of 30 ng μl^{-1} (100 µL total). The quantification of the extracted DNA was performed by estimating the concentration retained in the gel following electrophoresis and measurements using a NanoDrop (Thermo Scientific, USA).

2.4 Quantification of the Fungal Community Abundance by Quantitative PCR

Quantification of the fungal community abundance (density) was carried out on the basis of internal transcribed spacer (ITS) regions. The quantification was performed twice for each of the sediment replicates using an ABI Prism 7300 Cycler (Applied Biosystems, Germany). The specificity of the amplification products was confirmed by a melting curve analysis where a unique peak was observed. The size of the amplicons was also checked using a 1.5 % agarose gel that was stained with ethidium bromide. Standard curves were obtained by serially diluting a cloned ITS fungal fragment that was isolated from *Rhizoctonia solani.* The standard was diluted from 10^2 to 10^7 gene copies per microliter. The potential inhibitory effects that could have been exerted by co-extracted humid compounds were checked by spiking the samples with a standard concentration of gene copies and amplifying the resulting mixes. The primer set that was selected was described by Fierer et al. (2005), who have analyzed the efficiency of these primers to quantify fungi in environmental samples with high specificity and low bias. Primers 5.8 S forward (5′-CGC TGC GTT CTT CAT CG-3) and ITS1f reverse (5′-TCC GTA GGT GAA CCT GCG G-3′) were used, which generated amplicons of 300 bp. All quantitative PCR (qPCR) reactions were carried out in a 25-μl PCR mixture that contained 12.5 μl of ABsolute qPCR Master Mix (ABgene), 1.25 μl of each primer (10 μM; Invitrogen), 2.5 μl bovine serum albumin (10 mg ml^{-1}; Invitrogen) and 1 μl (approximately 30 ng) of environmental DNA. The PCR reactions were carried out using cycling times of 5 min at 95°C, followed by 40 cycles of 95°C for 1 min, 53°C for 30 s for the annealing temperature, and 72°C for 1 min. Only samples with specific melting curves and the expected amplicon sizes that were analyzed by electrophoresis in agarose gels were used for the quantification of the target group. The results were compared by an ANOVA and the Tukey test, at a significance level of 0.05.

2.5 Analysis of the Fungal Communities by PCR-DGGE

Fungal amplicons from the ITS region were obtained as described by Anderson et al. (2003a), using primers EF4 (GGA AGG GRT GTA TTT ATT AG) and ITS4 (TCC TCC GCT TAT TGA TAT GC) in the first reaction, followed by a nested amplification using primers ITS1 F (5′-GC clamp – CTT GGT CAT TTA GAG GAA GTA A) and ITS2 (GCT GCG TTC TTC ATC GAT GC). Prior to the DGGE analysis, the resulting amplicons were verified by electrophoresis in agarose gels to determine their sizes and to determine the specificity of the reactions. DGGE analysis was performed as previously described (Anderson et al. 2003b) using the Ingeny PhorU2 apparatus (Ingeny International, Goes, The Netherlands). The PCR amplicons were loaded into 8 % polyacrylamide gels, in 0.5× TAE buffer (20 mM Tris–acetate, 1 mM EDTA, pH 8.0). The polyacrylamide gels were made with denaturing gradients that ranged from 20 to 55 % (the 100 % denaturant fraction contained 7 M urea and 40 % formamide). The gels were run at 100 V for 16 h at 60°C. Subsequently, the gels were soaked for 1 h in SYBR Gold nucleic acid staining solution (Invitrogen, Brazil) before they were photographed under UV light.

The DGGE profiles were normalized and compared using the GelComparII software (Applied Maths, Sint Martens Latem, Belgium). The resulting bands were identified, and a matrix that comprised the relative area of each band in each sample was generated and used for further analyses as described by Andreote et al. (2009). First, the matrix was used for a detrended correlation analysis (DCA), where a normal distribution of these data was observed (first gradient length was 4.644). This analysis indicated the use of correspondence analysis (CA) as the most suitable model to visualize the sample clustering, which was performed using the software Canoco 4.5 for Windows (Ter Braak and Smilauer 2002).

2.6 Construction and Analysis of Clone Libraries for the Targeted Genes

To construct the clone libraries, the PCR amplified solutions from the 18 samples were pooled based on our major objectives and the qPCR and DGGE results, which generated the following three distinct libraries: one from point 1 from OilMgv (OilMgv1), another from points 2 and 3 from OilMgv (OilMgv2–3), and a third from points 1, 2, and 3 from AntMgv.

For the clone libraries, amplicons were generated using the primer sets described above, except that the primer ITS1 F was used without the GC clamp. Amplicons from the same areas were mixed, and the resulting amplicon pools were purified and cloned into the pGEM-T Easy cloning vector (Promega, Madison, WI, USA). The resulting plasmids were transformed into *Escherichia coli* Top10 (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's protocol. Plasmid DNA with the insert was extracted by alkaline lysis, and DNA sequencing was performed using plasmid DNA, the SP6 primer and a DYEnamic ET Terminator Cycle Sequencing kit (GE Healthcare, Little Chalfont, Buckinghamshire, UK) according to the manufacturer's specifications. The purified reaction was resuspended in HiDi formamide (Applied Biosystems, Foster City, CA, USA), and the samples were analyzed in an ABI PRISM 3100 Genetic Analyzer (Applied Biosystems).

A total of 180 clones with inserts were obtained. Sequence quality was assessed, and vector sequences were removed using the Lucy program, available from the Ribosomal Data Project pipeline. Moreover, MOTHUR (Schloss et al. 2009) was used to identify operational taxonomic units (OTUs) at 95 % of similarity and was used to generate rarefaction curves. Coverage values were determined using Good's estimative. A 95 % of similarity was selected to ensure the possibility to use all of the OTUs in the resulting phylogenetic trees.

Each representative OTU sequence was compared to those available in the GenBank (nr/nt) by BlastN analysis (Altschul et al. 1997). The best-matched sequences were retrieved and added to the alignment for phylogenetic reconstruction. Cluster analysis of the sequences that were generated compared to those retrieved from the database was performed by the neighbor-joining method (Saito and Nei 1987) and the Kimura-2 parameter (Kimura 1980) using MEGA 5.0 software (Tamura et al. 2011). A bootstrap consensus tree, identified from 1,000 replicates, represented the evolutionary history of the taxa that were analyzed. Branches that corresponded to partitions that were reproduced in less than 40 % bootstrap (Felsenstein 1985) replicates were collapsed.

Sequences presented in this study were submitted to GenBank and are available using the accession numbers JQ038242 to JQ038371.

3 Results and Discussion

The microbial communities that inhabit the soil/sediment systems are often very abundant and complex. Mangrove sediments have very unique characteristics, where bacteria, archaea, and fungi have been suggested to interact closely with each other and with the specific plants and animals that inhabit this ecosystem (Holguin et al. 2001). Here, we analyze the fungal communities in two selected mangrove systems using culture-independent approaches, given that all recent studies have focused on the bacterial and archaeal communities of mangroves (Das et al. 2006; Yan et al. 2006; Liang et al. 2007; Dias et al. 2010, 2011). Assuming that fungi are less responsive than bacteria to important ecological factors such as pH (Rouske et al. 2010), any shifts that are observed in the fungal community structure might indicate strong selective pressure. Furthermore, particular characteristics of fungi, such as their ability to form extended mycelial networks, the low specificity of their catabolic enzymes and their ability to use different pollutants as growth substrates, make these organisms potentially beneficial bioremediation agents (Harms et al. 2011). Moreover, fungi have been recently shown to possess a range of cellular and genomic adaptations that enable them to persist in anoxic environments (Embley 2006).

3.1 Quantification of the Fungal Communities in Mangrove Sediments

The quantification of fungal ITS in the mangrove samples was performed with quantitative PCR. The size of the amplified regions was determined to be 300 bp, and the regions amplified with an average efficiency of 85.5 % and an R^2 value of 0.99. This amplification system had a detection limit of 100 copies of the target region per reaction.

Concerning the numbers of fungal ITS copies that were identified in the mangrove sediment samples, a trend for an increased fungal abundance in the OilMgv samples, as compared to the AntMgv samples, was observed (Fig. 1). Sampling points 2 and 3 in OilMgv were observed to have higher numbers of fungal ITS compared to all other sampled points ($p<0.05$). In points 2 and 3 for the OilMgv samples, values of 9.18 and 8.53 logs of fungal ITS per gram of sediment were identified, respectively. At point 1 in the same mangrove, 7.83 logs of fungal ITS were identified. In AntMgv, the values were similar across all of the three sampled regions, consistently lower than the OilMgv, and ranged from 7.51 to 7.63 logs per gram of sediment (Fig. 1). These results indicate that there is an increase in the fungal abundance that is related to a history of mangrove

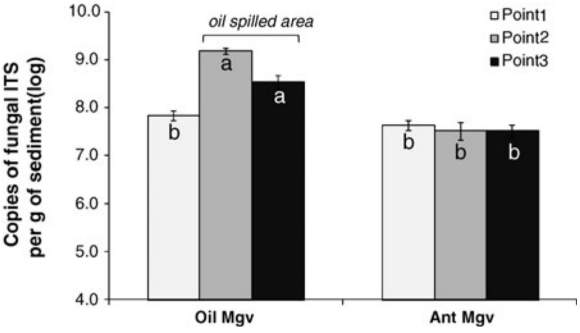

Fig. 1 Quantification of fungi ITS in mangrove samples by qPCR. Values indicate the average of three biological replications, and bars represent standard errors of the mean. Similar *letters in bars* indicate the absence of a statistical difference by the Tukey test at a significance value of 0.05

contamination with oil. Although there may be confounding or indirect factors that affect these communities, this potentially indicates that certain members of the fungal communities are adaptive to oil. This provides an opportunity to identify these fungi for future mangrove bioremediation. Additionally, comparing the values that were obtained in the current study with those from the literature (Rouske et al. 2010; Hussain et al. 2011), an abundant fungal community was found, especially in the oil-affected sediment samples. Most studies that have quantified fungi in environmental samples have been performed in soil samples with obtained values of approximately 2×10^3 fungal ITS per gram dry soil (Rouske et al. 2010). Additionally, Hussain et al. (2011) quantified the fungal abundance in the rhizosphere of rice in flooded soils, and observed a higher fungal abundance than those that were described for soils. The fungal abundance in the rhizosphere ranged from 10^4 to 10^5 copies of the target gene per gram of dry soil, according to the plant genotype. The lower fungal abundances in the soil compared to the levels found in this study in the mangrove sediments appear to signify the importance of fungal communities in the mangrove sediments. They appear highly abundant in the less-contaminated mangrove, and an increase in response to oil contamination was observed. Therefore, these organisms may be applicable for distinct biotechnological applications. Moreover, the high abundance of organic matter (sugars and derivatives) and other environmental conditions may lead to the presence of yeasts, which are possibly responsible for some of the fermentative processes in this environment.

3.2 Differential Composition of the Fungal Communities in the Analyzed Mangroves

PCR-DGGE was used to provide an overview of the dominant fungal phylotypes (ITS-based) that were present in the mangroves. The first article describing the use of PCR-DGGE to assess the composition of the fungal community in soils was performed in soils under the *Pinus sylvestris* pine species (Anderson et al. 2003a). The authors described an effect that the gradient moorland-forest had on the structure of the soil fungal communities. In the same year, these authors tested the efficiency and specificity of the primers that were used in the study, and they showed a rather low bias for non-specific fungal DNA amplification (Anderson et al. 2003b).

In the current study, we used the PCR-DGGE system to highlight the differences in the fungal communities of the two mangrove ecosystem sediments. A variable band profile was initially observed between samples that were collected in the same region (Fig. 2). Nevertheless, besides the occurrence of "erratic" bands, the presence of specific bands between distinct areas was observed, with a special remark for bands that were only found in samples collected in the oil-spilled location (OilMgv points 2 and 3). These bands are indicated in Fig. 2a (arrows). Erratic bands are not observed for other members of the microbial community. Dias et al. (2011) assessed the archaeal communities in the same samples and observed very similar band fingerprinting in the same replicates. This may suggest that the fungal communities are assembled in a more complex fashion, which may lead to the reduced repeatability of fingerprinting that was observed in some of the analyzed areas in the current study.

The analysis of the PCR-DGGE profiles using CA supported the differences that were observed in the fungal communities between each of the sampled mangroves. Due to the variability in the fungal profiling among replicates, the resolution of such an analysis was not able to clearly distinguish each of the mangrove areas. Combining the visualization of the first three axes (in total, 36.8 % of variance explained), the two mangroves were not able to be fully distinguished. A few samples, mostly from point 2 from the OilMgv, clustered together, while two samples from point 3 in the OilMgv also clustered near the samples from point 2. These observations support the differential selection of fungi in the oil-affected area. Comparing the clusters that were observed for fungi with those clusters that were previously obtained in the same area for Archaea (Dias et al. 2011), it is clear that the fungal communities are more responsive to the effects of the oil spill. Furthermore, comparing the physicochemical characteristics of the sediments that were previously described by Dias et al. 2011 (ESM Table S1), large differences were not observed between the studied areas, which suggests that the fungal community may be responsive to other parameters that were not considered, such as the proximity of the fungus to plant roots (Gomes et al. 2010).

Combining the results of the fungal PCR-DGGE and considering the objective of this study, we inferred that the two mangroves might be divided into three distinct areas that relate to the composition of the

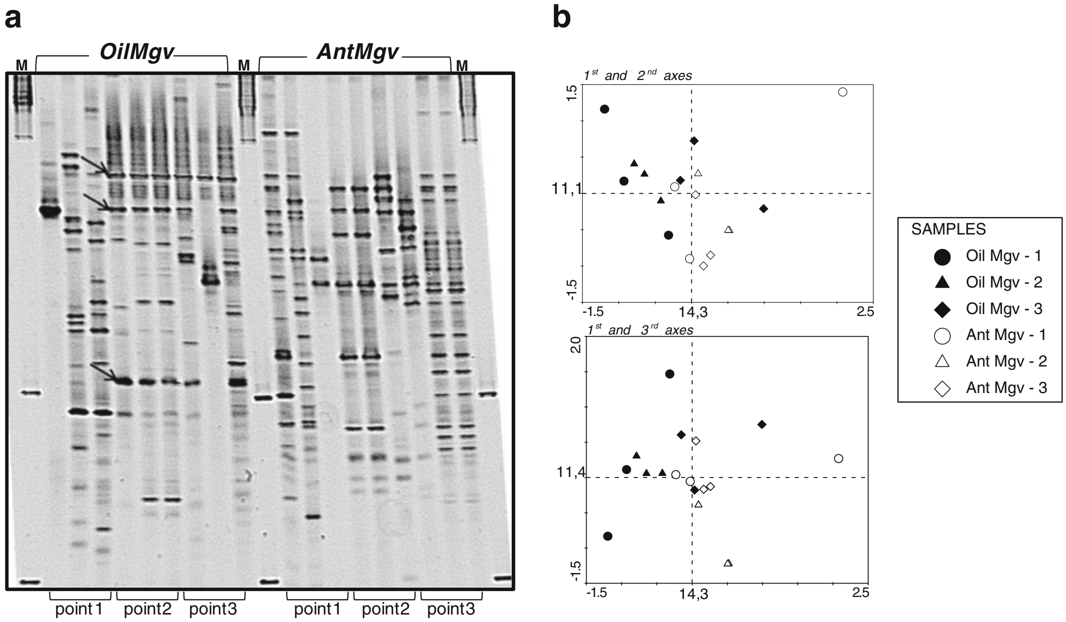

Fig. 2 Fungal communities in mangrove sediments assessed by PCR-DGGE based on ITS of fungi (**a**), where *M* indicates the marker for electrophoresis, and correspondence analysis (**b**), showing the first three axes, where a total value of 36.8 % of the variance is explained. *Arrows* in **a** indicate the specific bands that were found in the area with higher effects from the oil spill

fungal communities. The first area was composed of point 1 from the OilMgv, the second area encompassed points 2 and 3 from the OilMgv and the third area consisted of samples from the AntMgv. This division supports the fungal quantification data because the area denoted OilMgv2–3 was observed to have the most abundant fungal community. The pooling of all samples from the AntMgv was performed because the main focus of this work is to determine the role of the oil spill and not natural variations in a mangrove transect. Given this division, the three areas were used for further sequencing and phylogenetic reconstruction of the ITS-based clone libraries.

3.3 Sequence Analysis of the Fungal Groups in the Mangrove Sediments

Based on the foregoing, three clone libraries were constructed and analyzed. After quality analysis, a total of 180 sequences were selected and further analyzed as follows: 77 from OilMgv1, 48 from OilMgv2–3 (encompassing points 2 and 3 from this mangrove), and 55 from AntMgv.

Blast-N analysis revealed the occurrence of common fungal groups in the three mangrove areas (Fig. 3a). Thus, sequences that were affiliated with the *Nigrospora* spp., the *Cladosporium* spp., the *Epicoccum* spp., uncultured Basidiomycota and uncultured fungi were determined to be the most common matches (78 % of sequences). These groups occurred in distinct proportions between the three libraries that were analyzed, with a remarkably higher occurrence of the uncultured Basidiomycota sequences identified in the samples from OilMgv2–3 (40 % of total sequences). In other areas, the presence of this group was lower, with 10 and 8 % in OilMgv1 and AntMgv, respectively (Fig. 3a). Conversely, the library from the OilMgv2–3 area revealed a lower frequency of sequences with similarities to *Cladosporium* and *Nigrospora* spp. compared to OilMgv1 and a lower frequency of uncultured fungi compared to AntMgv (Fig. 3a). This indicates that these fungal groups may be more sensitive to the environmental conditions in the mangrove sediment that were established by the oil spill.

These fungal groups have been previously associated with mangroves. *Cladosporium* is a fungal genus that is described as primary saprotrophs and has been recovered from mangrove leaves where they produce enzymes that play a vital role in the breakdown of recalcitrant plant material (Raghukumar 2004). The complex group of “white rot fungi” is described to also have access to poorly available substrates because these fungi secrete extracellular enzymes that are

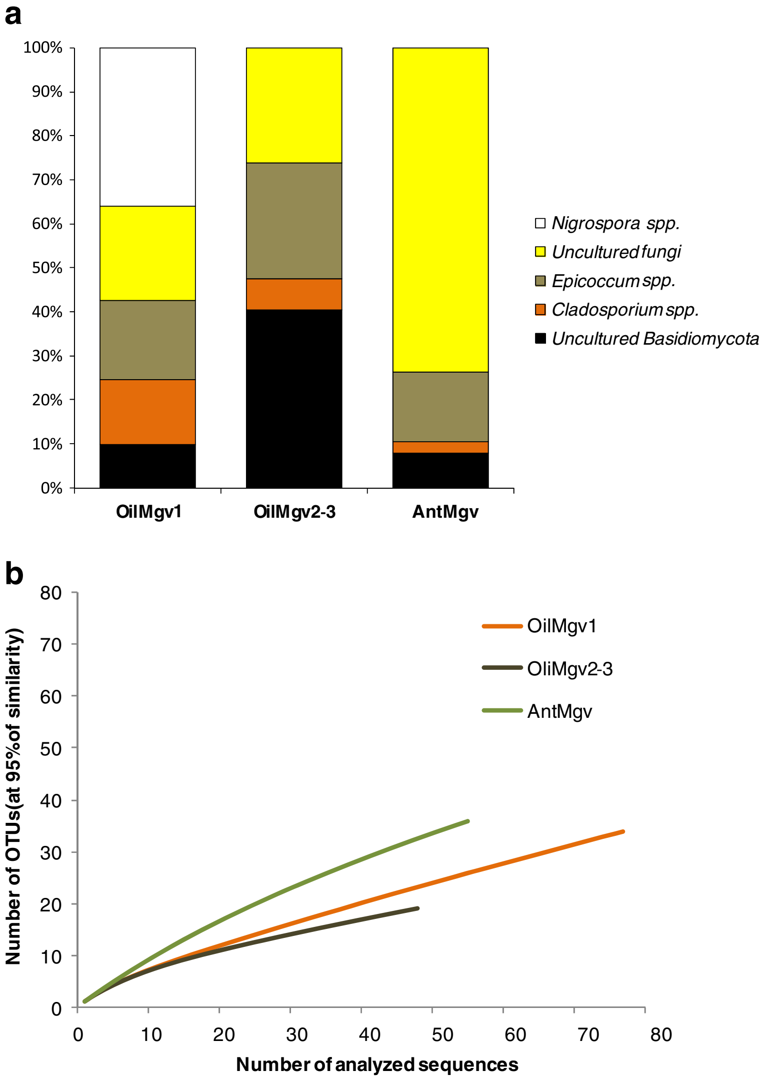

Fig. 3 Sequence analyses by the proportion of taxonomic affiliation that were determined by BlastN analysis against the GenBank nr/nt database (**a**), and rarefaction analyses for libraries of ITS regions that were found in the mangrove samples (**b**). In rarefaction, distinct fungal groups are represented at 95 % similarity to OTUs

involved in the oxidation of complex aromatic compounds, such as lignins and hazardous pollutants (Field et al. 1992; Barr and Aust 1994). The "white rot fungi" group contains both diverse Ascomycota and Basidiomycota. Therefore, one can hypothesize that some of the fungi that are found in higher frequencies after oil exposure may be members of the "white rot fungi" group.

Concerning those sequences that were not classified into one of these five major groups, we observed the presence of sequences that were associated with yeasts that belonged to the genera *Bullera*, *Candida*, *Cryptococcus*, *Metschnikowia*, and *Saccharomyces*. However, due to the low amount of these sequences, a comparison of the frequency of occurrences is limited using our approach.

To validate the observed differences, we determined the rarefaction curves of our datasets (Fig. 3b). The saturation level was clearly closer for the library that was constructed with the DNA from OilMgv2–3, which reinforces the hypothesis that the oil spill provided a selective pressure on the fungal community (Fig. 3b). Coverage values were also determined at 95 % of similarity, which resulted in values of 54.5, 64.6, and 40.0 percent for the OilMgv1, OilMgv2–3 and AntMgv libraries, respectively. These coverage values indicate that our analysis assessed roughly half of the fungal groups that comprised the fungal communities in the mangrove sediments. Moreover, these values suggest a shift in the fungal communities in the area that were affected by the oil spill.

To analyze the taxonomical affiliation of the fungal sequences that were identified from the mangroves, cluster analyses were carried out separately for each of the five most frequently found fungal groups (Fig. 4 and ESM Fig. S1). The first three groups that were analyzed were very concise, and they encompassed the genera *Cladosporium* (13 OTUs), *Epicoccum* (24 OTUs) and *Nigrospora* (21 OTUs). Sequences that were affiliated with *Cladosporium* and *Epicoccum* were similar between all of the mangrove samples, which suggested that these groups represent common inhabitants of all of the mangrove sediments (Fig. 4). Conversely, sequences that were affiliated with *Nigrospora* were only obtained from the OilMgv1 area (Fig. 4) that remained free from oil contamination. This potentially indicates that this fungal group is sensitive to oil. Therefore, this species could be used as a possible bioindicator for oil contamination.

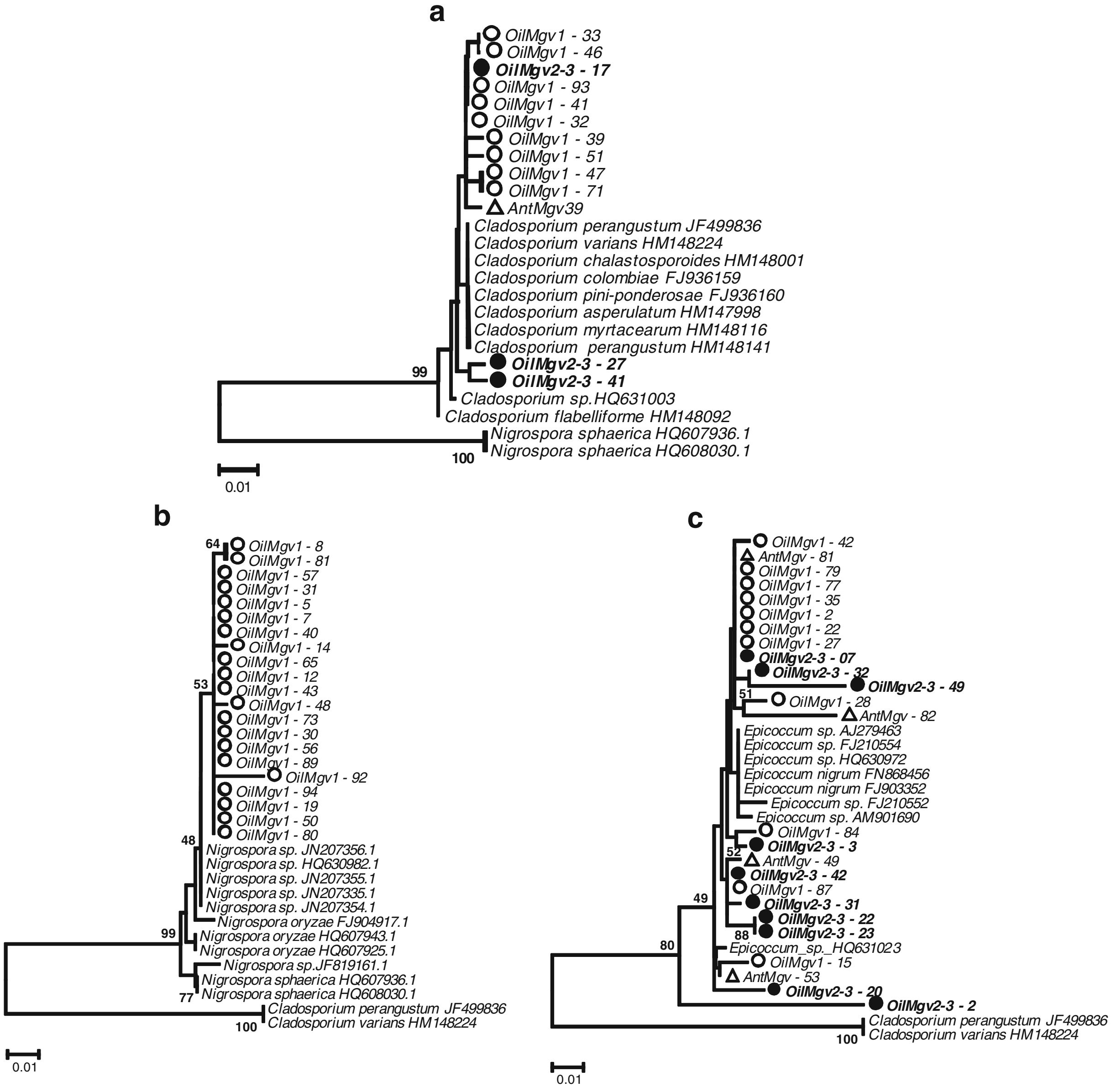

Fig. 4 Neighbor-joining tree of sequences affiliated with the genera *Cladosporium* (**a**), *Nigrospora* (**b**) and *Epicoccum* (**c**). Sequences represent distinct OTUs determined at 95 % similarity to ITS sequences retrieved from the mangrove sediments. *Bold sequences* were retrieved from the OilMgv2–3

Concerning the other two groups, the sequences were most commonly affiliated with "uncultured fungi" (43 OTUs) and "uncultured Basidiomycota" (24 OTUs). Detailed analyses of these sequences with respect to their affiliation with known genera or species were not possible. However, a comparison based on sequence similarities showed remarkable clustering between the mangrove areas (ESM Fig. S1). We observed a separation of sequences from the oil-exposed area compared to the other regions, indicating that this exposure to oil may have exerted a selection pressure on the local fungal communities. This pressure could have promoted the increase in specific populations of fungi that were not present in the mangrove areas that were not exposed to oil. Additionally, most of the sequences that were exclusively found in the OilMgv2–3 were associated with "uncultured Basidiomycota" (ESM Fig. S1). This makes this fungal group an important cluster that is possibly involved in oil consumption and, consequently, has the potential for mangrove bioremediation. Moreover, the possible presence of yeasts (not identified and allocated into the uncultured Basidiomycota) might indicate that they are an important group that needs to be further explored in mangroves.

4 Conclusions

In summary, we described fungal communities that resided in mangrove sediments, some of which likely colonized in response to environmental conditions that were the result of oil contamination. Selective pressure from the oil contamination may have resulted in the increased abundance of certain groups, thus shifting the composition of the fungal community. This shift may have resulted in a higher efficiency of oil consumption and could be playing a role in the bioremediation of the mangrove ecosystem. Future studies should focus on methods that favor the development of these fungal communities to achieve bioremediation after an oil spill by either providing conditions that favor the natural development of the fungal communities or by deliberately adding these organisms to the mangrove ecosystem.

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