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Research Article

RESEARCH ARTICLE

Tannins from senescent *Rhizophora mangle* mangrove leaves have a distinctive effect on prokaryotic and eukaryotic communities in a *Distichlis spicata* salt marsh soil

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One sentence summary: This study has shown that migration of tannin-rich *Rhizophora mangle* into tannin-poor soils will lead to changes in the prokaryotic and eukaryotic communities, with the greatest effects on the latter.

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ABSTRACT

Due to climate warming, tannin-rich *Rhizophora mangle* migrates into tannin-poor salt marshes, where the tannins interfere with the biogeochemistry in the soil. Changes in biogeochemistry are likely associated with changes in microbial communities. This was studied in microcosms filled with salt marsh soil and amended with leaf powder, crude condensed tannins, purified condensed tannins (PCT), all from senescent *R. mangle* leaves, or with tannic acid. Size and composition of the microbial communities were determined by denaturing gradient gel electrophoresis, high-throughput sequencing and real-time PCR based on the 16S and 18S rRNA genes. Compared with the control, the 16S rRNA gene abundance was lowered by PCT, while the 18S rRNA gene abundance was enhanced by all treatments. The treatments also affected the composition of the 16S rRNA and 18S rRNA gene assemblies, but the effects on the 18S rRNA gene were greater. The composition of the 18S rRNA gene, but not of the 16S rRNA gene, was significantly correlated with the mineralization of carbon, nitrogen and phosphorus. Distinctive microbial groups emerged during the different treatments. This study revealed that migration of mangroves may affect both the prokaryotic and the eukaryotic communities in salt marsh soils, but that the effects on the eukaryotes will likely be greater.

Keywords: *Rhizophora mangle*; senescent leaves; condensed tannins; salt marsh soils; prokaryotic community; eukaryotic community

INTRODUCTION

Mangroves inhabiting tropical and subtropical coasts are among the most carbon-rich forests (Donato *et al.* 2011). A highly productive and diverse microbial community living in mangrove ecosystems continuously transforms nutrients from dead material into sources of nitrogen, phosphorus and other

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nutrients that can be used by the plants (Cundell *et al.* 1979; Benner, Peele and Hodson 1986; Holguin and Bashan 2001). Rich bacterial and fungal microbiota have been observed along with slowly degrading structural material of senescent *Rhizophora mangle* leaves, with leaching of tannins after about one month (28–49 days) (Cundell *et al.* 1979). Mangroves, and in particular species from the Rhizophoraceae, contain large amounts of tannins (Basak, Das and Das 1999; Hernes *et al.* 2001; Hernes and Hedges 2004). Senescent mangrove leaf material is an important source of carbon, nitrogen and other nutrients for coastal food webs (Hernes *et al.* 2001), but it may interfere with biogeochemical cycling because of its high amounts of tannins (Hättenschwiler and Vitousek 2000; Arnold and Targett 2002). Low-molecular-weight tannins can be used as a carbon source for microbes (Kanerva *et al.* 2006), but higher molecular weight tannins may be toxic for them (Fierer *et al.* 2001). However, toxic tannin oligomers that have been built from non-toxic tannin monomers can be detoxified again by further polymerization to higher molecular weight compounds (Field and Lettinga 1992). Several mechanisms underlie the toxicity of tannins for microorganisms. First, tannins can be directly toxic to soil microbes due to hydrogen bonding to vital proteins such as enzymes. Second, polyphenols, and especially the tannin fractions, are expected to affect the availability of nitrogen to plants during their growing season, mainly through complexation (Fierer *et al.* 2001; Kraus, Dahlgren and Zasoski 2003; Nierop *et al.* 2006). Third, tannins can form complexes with protein-containing organic matter through which it becomes resistant to decomposition. The uptake, transformation and/or metabolism of tannin–protein complexes by soil organisms might be a major link between tannins and nutrient cycling. Tannins extracted from mangrove leaves include hydrolyzable and condensed tannins (Hernes *et al.* 2001), of which the latter could be divided into extractable, protein-bound and fiber-attached condensed tannins (Lin *et al.* 2006). Because of the wide diversity in tannin chemistry (e.g. condensed vs hydrolyzable tannins, procyanidin vs prodelphinidin, stereochemistry, hydroxylation pattern, substitutions and chain length), specific microorganisms were found after the addition of different tannins or polyphenols to soils (Fierer *et al.* 2001; Schmidt *et al.* 2013).

As a consequence of global warming, mangroves migrate in polar direction thereby entering salt marshes (Bianchi *et al.* 2013; Saintilan *et al.* 2014). In contrast to mangroves, tannins in salt marsh grasses are unlikely to be a significant component of humic substances (Alberts *et al.* 1988). We hypothesized that senescent tannins-rich leaves of *R. mangle* will interfere with the mineralization of carbon, nitrogen and phosphorus in pristine salt marsh soils. In a 4-year field experiment, in which seedlings and senescent leaves of *R. mangle* were transferred to a *Distichlis spicata*-dominated salt marsh, we demonstrated that the senescent leaves stimulated the growth of the seedlings, but retarded the decomposition of *D. spicata* litter (Laanbroek *et al.* 2018). Stimulation of seedling growth by annual replacement of *D. spicata* litter by senescent *R. mangle* leaves indicated an increase in nutrient availability. However, repression of *D. spicata* litter decomposition suggested inhibition by the tannins from the mangrove leaves. In a follow-up experiment, samples from senescent *R. mangle* leaves containing increasingly more purified condensed tannins were mixed with the *D. spicata* salt marsh soil in microcosms and incubated for 42 days (Zhang and Laanbroek 2018). With increasingly more purified condensed tannins, i.e. from leaf powder (LP), via crude to purified condensed tannins, CO_2 emission decreased significantly when measured over the whole incubation period. The microcosms with complete, but ground *R. mangle* leaves showed immobilization of mineral nitrogen and phosphorus, while the microcosms with less or more purified condensed tannins revealed net nitrogen and phosphorus mineralization. The observed differences in response between the treatments can be explained by differences in the relative amounts of carbon applied, but also by differences in the quality of the organic fractions in the additions. With increasing purification of condensed tannins from the leaves, the amounts of other, often smaller organic compounds will decrease. For example, the microcosms with only purified condensed tannins will lack compounds such as readily leachable and low-molecular carbohydrates, organic acids, proteins, phenols, cyclitol and lignin (Cundell *et al.* 1979; Benner and Hodson 1985; Benner, Weliky and Hedges 1990; Kandil *et al.* 2004). Many bacterial taxa in soils prefer to use labile and low-molecular organic compounds often coming from fungal degradation of biopolymers that are otherwise inaccessible for most bacteria (De Boer *et al.* 2005; Zinger *et al.* 2011; Štursová *et al.* 2012; Urbanová, Šnajdr and Baldrian 2015). Hence, bacteria and fungi have each a distinct role in the degradation of leaves as has been shown for other systems (Mutabaruka, Hairiah and Cadisch 2007; Urbanová, Šnajdr and Baldrian 2015; Purahong *et al.* 2016; Sun *et al.* 2017).

Differences in the quality of compounds extracted from senescent *R. mangle* leaves with increasingly more purified condensed tannins will be reflected in the composition of emerging bacterial and fungal communities. In the present experiment, we aimed to study the specific roles of bacteria and fungi in carbon degradation in the treatments with these *R. mangle*-derived tannin compounds. Microcosms filled with *D. spicata* salt marsh soil are an appropriate system to use for such a study, because the microorganisms in this soil never encountered leaves from *R. mangle* before. As the same microcosm experiment has been used to study the effect of condensed tannins on biogeochemical cycles in the salt marsh soil (Zhang and Laanbroek 2018), the outcome of the molecular analyses can be compared with the results obtained on the cycling of nutrients. The genetic characteristics of the bacterial and fungal communities were established by a combination of different molecular methods, i.e. PCR-DGGE, real-time PCR and high-throughput sequencing (HTS), all based on the 16S and 18S ribosomal RNA (rRNA) genes. In particular, we addressed the following questions: (i) How does organic matter, especially tannins, from senescent *R. mangle* leaves affect the abundance and composition of the bacterial and fungal communities in the soil after 42 days of incubation? and (ii) Are specific populations of microorganisms enriched in the different treatments that represent a range of decreasing complexity with respect to the composition of added organic material?

MATERIALS AND METHODS

Origin and characteristics of salt marsh soil and senescent *R. mangle* leaves

The origin and characteristics of salt marsh soil and senescent *R. mangle* leaves have been reported before (Laanbroek *et al.* 2018; Zhang and Laanbroek 2018).

Condensed tannins extraction and purification

Extraction and purification of condensed tannins from senescent *R. mangle* leaves have been described in detail before (Zhang and Laanbroek 2018). Briefly, the ground, dried leaf litter was

extracted with acetone:water (70:30), the extract was filtered through a Buchner funnel and concentrated by vacuum-rotary evaporation. The aqueous phase containing condensed tannins was extracted three times with *n*-hexane and then freeze-dried to obtain the crude condensed tannins (CCT) extract. Part of CCT was loaded on a Sephadex LH-20 column (Amersham Biosciences, Uppsala, Sweden) and removed low-molecular-weight phenolics and flavonoid compounds first using 50% methanol:water, and then eluted with an acetone:water (70:30) solution to remove the bonded tannin fraction. Finally, purified condensed tannins (PCT) were obtained by vacuum-rotary evaporation and freeze-drying.

Soil microcosms

Details on the application of the soil microcosms have been described before (Zhang and Laanbroek 2018). In short, the soil microcosms consisted of stored soil samples at the equivalent of 5 g dry soil contained in 500 mL screw-cap bottles that can be closed with a silicon rubber inlay. At the start of the incubation period, the soil samples were enriched with LP, CCT, PCT, all from *R. mangle* or with hydrolyzable tannins (TA, tannic acid, Sigma-Aldrich Co., St Louis, MO, USA, Molecular Weight 1701.20, $C_{76}H_{52}O_{46}$). One part of the soil microcosms served as control (C) and did not receive a source of *R. mangle*-derived compounds or tannic acid. By adding sodium chloride, the salinity of the pore water was adjusted to 28 PSU, which resembles the average salinity measured in the field over the years (Laanbroek *et al.* 2018). Each treatment was replicated five times. So, the incubation experiment included 25 microcosms in total. All soil samples were pre-incubated for 7 days at room temperature to allow the microbial activity to stabilize before the experiment started. A content of 10 mg tannins g^{-1} dry soil was considered to be ideal to explore its ecological effects (Kraus *et al.* 2004). Since the weight percentage of PCT in the CCT extracts and in the dried *R. mangle* leaf litter was 30.4 and 13.40%, respectively, the 5 g dry soil in the pre-incubated microcosms was thoroughly mixed with 373, 164, 50 or 50 mg of *R. mangle* leaf powder, crude condensed tannins, purified condensed tannins and tannic acid, respectively, to reach the 10 mg tannins g^{-1} dry soil. The microcosms were then incubated for 42 days in a dark room at a temperature of 25°C and 80% moisture content. Bottles were loosely closed with screw caps to prevent suboxic conditions and large fluctuations in moisture content. Every week, the bottles were opened for 24 h and soil moisture content was measured by weighting and when necessary corrected by adding sterilized and deionized water.

Soil sample preparation and properties determination

On the 42nd day of the incubation, the soil in the bottles was collected and split into three portions after finishing gas collection. The first portion was stored at −80°C for DNA extraction, the second portion was freeze-dried for analyses of total phenolics, total tannins and hydrolyzable tannins, and the last portion was used for determination of soil physicochemical properties. The chemical analyses have been described before (Laanbroek *et al.* 2018; Zhang and Laanbroek 2018).

DNA extraction

From three out of five randomly selected soil samples of each treatment, total genomic DNA was extracted from 0.2 grams of freeze-dried material that was homogenized in 1 mL cetyltrimethylammonium bromide (CTAB) buffer in MP Lysing Matrix tubes (MP Biomedicals, Santa Ana, California, USA). The extraction protocol was according to our previous study (Laanbroek *et al.* 2018). Quantity and quality of soil DNA were established by a Nanodrop ND-1000 Spectrophotometer (Thermo Scientific, Waltham, EUA). The DNA was stored at −80°C for subsequent qPCR, PCR-DGGE and HTS of the 16S rRNA and the 18S rRNA genes.

Real-time PCR assays on the 16S and 18S rRNA genes

Real-time PCR (qPCR) reactions of the 16S rRNA and the 18S rRNA genes were performed on a StepOne Plus real-time PCR system (Applied Biosystems, CA, USA). All samples were analyzed in triplicate. The qPCR conditions were as listed in Table 1. The 16S rRNA and the 18S rRNA genes were determined with the primer sets 338F/806R (Huws *et al.* 2007; Huhe *et al.* 2017) and 817F/1196R (Rousk *et al.* 2010), respectively. The polymerase chain reaction (PCR) reactions were performed in 25 μL volumes containing 12.5 μL Premix Ex taq (Takara, Dalian), 2 μL of a 100 times diluted extract of soil DNA, 0.5 μL of bovine serum albumin (BSA, 10 mg·mL^{-1}) and 1 μL of each primer (0.25 μM each). High amplification efficiencies of 95–103% were obtained with R^2 values of 0.99–1.00 and slopes from −3.0 to −3.4. No template DNA was included in a qPCR assay as negative control. Product specificity was confirmed by melting curve analysis and visualization in 1.0% agarose gels. No significant inhibitors were found in the DNA extracts.

PCR-DGGE analysis

In order to estimate the approximate difference in community composition among treatments before high-throughput (MiSeq) sequencing, the dominant prokaryotic and eukaryotic taxa were revealed by PCR-DGGE analysis based on the 16S and 18S rRNA genes, respectively. Conditions of the PCR-DGGE were as listed in Table 1. The PCR–DGGE method was according to previous studies using the primer set 341F-GC/534R for the 16S rRNA gene (Muyzer, De Waal and Uitterlinden 1993) and the primer set F1427-GC/R1616 for the 18S rRNA gene (Hannen *et al.* 1998; Yu, Yan and Feng 2008). PCR amplification was performed in a 50 μL reaction mixture comprising 1 × PCR buffer, 400 μM of each deoxy-ribonucleoside triphosphate (dNTP), 2.5 U Taq DNA polymerase (Takara, Dalian), 0.2 mg mL^{-1} bovine serum albumin (BSA) plus 0.2 mM of each primer, and 2 μL of the 100 and 20 times diluted genomic DNA as individual templates for the 16S and 18S rRNA genes, respectively. Twelve microliters of the PCR products was loaded into polyacrylamide gel wells. After the runs, the gels were stained with 1:10 000 SYBRGreen I (Sigma, Taufkirchen, Germany) for 30 min and scanned with a GelDoc XR scanner (BIO-RAD, Hercules, CA).

High-throughput sequencing

The methods of sequencing of the prokaryotic and eukaryotic communities were similar as published before in a study on grassland soils from the Loess Plateau in China (Huhe *et al.* 2017). PCR was performed with a GeneAmp 9700 PCR system (Applied Biosystems, Foster City, CA, USA). The primer sets 338F/806R (Huws *et al.* 2007; Huhe *et al.* 2017) and 817F/1196R (Rousk *et al.* 2010), both with attached barcode sequences, were applied for the PCR amplification of the 16S rRNA and 18S rRNA genes, respectively. The PCR conditions were as listed in Table 1. PCR

Table 1. Conditions applied for qPCR, PCR-DGGE and HTS in the present study.

Target gene	Primer	Sequence (5′–3′)	Amplicon length (bp)	Thermal profile for real-time PCR (qPCR)	Thermal profile for PCR	DGGE conditions	Reference
16S rRNA	341F-GC	CCTACGGGAGGCAGCAGCC GCCGCGCGGCGGGCGGGGC GGGGGC ACGGGG	193		2 min at 93°C, followed by 28 cycles of 30 s at 94°C, 30 s at 54°C and 1 min at 72°C, and a final extension at 72°C for 20 min	8 (w/v) polyacrylamide gels containing denaturing gradients of 43–65%. Electrophoreses were run at 90 V for 14 h.	(Muyzer *et al.* 1993)
	534R	ATTACCGCGGCTGCTGG					
	338F	ACTCCTACGGGAGGCAGCAG	435	2 min at 95°C, followed by 40 cycles of 30 s at 95°C and 30 s at 55°C. Fluorescence was read during each cycle at 82°C.	2 min at 95°C, followed by 30 cycles of 30 s at 95°C, 30 s at 55°C and 30 s at 72°C, and a final extension at 72°C for 5 min		(Huws *et al.* 2007)
	806R	GGACTACHVGGGTWTCTAAT					
18S rRNA	F1427-GC	TCTGTGATGCCCTTAGATGT TCTGGGCGCCCGCCGCGCCC CGCGCCCGGCCCGCCGCCCC CGCCC	210		2 min at 93°C, followed by 33 cycles of 30 s at 94°C, 30 s at 54°C and 60 s at 72°C, and a final extension at 72°C for 20 min	8 (w/v) polyacrylamide gels containing denaturing gradients of 13–58%. Electrophoreses were run at 95 V for 13 h.	(Hannen *et al.* 1998; Yu *et al.* 2008)
	R1616	GCGGTGTGTACAAAGGGCAGGG					
	817F	TTAGCATGGAATAATRRAAT AGGA	399	4 min at 95°C, followed by 40 cycles of 30 s at 95°C and 30 s at 55°C. Fluorescence was read during each cycle at 82°C.	3 min at 95°C, followed by 35 cycles of 30 s at 95°C, 30 s at 55°C and 45 s 72°C, and final extension at 72°C for 10 min		(Rousk *et al.* 2010)
	1196R	TCTGGACCTGGTGAGTTTCC					

reactions were performed in triplicate in 20 μL mixtures containing 0.4 μL of FastPfu polymerase, 4 μL of 5 × FastPfu buffer, 0.8 μL (5 μM) of each primer, 2 μL of 2.5 mM dNTPs and 10 ng of template. PCR products were quantified using the QuantiFluor-ST Fluorometer (Promega Biotech, Beijing, China). The sequencing of the DNA samples with three replicates for each of the five treatments was carried out by Shanghai Majorbio Bio-pharm Technology (Shanghai, China), using an Illumina MiSeq PE300 platform (Illumina, USA).

16S and 18S rRNA genes sequence analysis

The raw sequences analysis of both the 16S rRNA and the 18S rRNA genes from the HTS was with minor modifications similar to a method used in previous studies (Huhe *et al*. 2017; Fang *et al*. 2018). The data were analyzed on the free online Majorbio I-Sanger Cloud Platform (www.i-sanger.com). Raw sequences obtained from the 15 salt marsh soil samples comprising the control and the LP, CCT, PCT and TA treatments were demultiplexed and quality-filtered by Trimmomatic (Bolger, Lohse and Usadel 2014) and merged by FLASH (Magoč and Salzberg 2011) using the criteria from an earlier study (Guo *et al*. 2019). Sequences with chimera were also removed. Then, the optimized sequences were clustered into operational taxonomic units (OTUs) with similarities of 97% or greater using UPARSE 7.1 (Edgar 2013). To compare all samples within the five treatments at the same sequencing level, the minimum sequence number read within the data sets was used to subsample those data sets that returned a greater number of reads. Subsequently, effective sequences were aligned against the SILVA database (Quast *et al*. 2013) and then identified to phylum, class, order, family and genus levels using the Ribosomal Database Project (Wang *et al*. 2007) with the Bayesian classifier at 70% threshold. The raw fastq format sequencing data were deposited at the NCBI GenBank under BioProject ID PRJNA573297 (16S rRNA gene) and PRJNA573299 (18S rRNA gene), and under SRA accession numbers SRP222982 (16S rRNA gene) and SRP22298 (18S rRNA gene).

Statistical analysis

The similarity of denaturing gradient gel electrophoresis (DGGE) profiles of both the 16S and 18S rRNA genes was analyzed by CLIQS 1D Pro v1.0 of the TotalLab software package (Nonlinear dynamics, Newcastle upon Tyne, England). Based on OTU information, alpha-diversity indices including microbial community diversity (Shannon and Simpson), community richness (Sob and Chao) and sequencing depth (Coverage) were determined with the Mothur software package (Schloss *et al*. 2009). The VennDiagram package of the R was used to show the overlap in OTUs in soils between the treatments. For comparison of communities per treatment, principal coordinate analyses (PCoA) were performed based on Bray–Curtis distances created by Qiime 1.7.0 (Caporaso *et al*. 2010) combined with permutational multivariate analyses of variance (PERMANOVA) (Anderson 2005). The relationships between microbial community structure and environmental factors were analyzed by redundancy analyses (RDA) using the software package of Canoco 4.5. To detect significantly different taxa between the treatments, the linear discriminant analysis (LDA) effect size (LEfSe) method was used based on a normalized relative abundance matrix (Segata *et al*. 2011). An LDA threshold score of 3.5 and a significant α of 0.05 were applied to detect distinctive taxa with a comparison of all-against-all styles.

All other statistical analyses were performed with the SPSS software package version 23 (IBM Corp. Armonk, NY). Spearman rank-order correlation analysis was done between diversity indices based on the 16S rRNA or the 18S rRNA genes and the physicochemical soil properties and total CO_2 emission ($CO_2{}^T$). One-way ANOVA followed by Duncan tests was applied for pH, Total C, Total N, C/N ratio, Total S, $NH_4{}^+$, N_{MIN}, total phenolic and total tannins, $CO_2{}^T$. For gene diversity indices, significant differences at $P < 0.05$ level between the treatments were established according to Tukey's test, except for the Simpson index of the 16S rRNA gene where a non-parametric Kruskal–Wallis test for pairwise comparisons was used. A non-parametric Kruskal–Wallis test for pairwise comparisons was also applied to $NO_3{}^-$, $PO_4{}^{3-}$, P_{MIN} and hydrolyzable tannins due to the absence of a normal distribution.

RESULTS

Soil properties and CO_2 emission

Soil properties and total CO_2 emission for the different treatments have been published in Zhang and Laanbroek (2018). For the convenience of the reader, we summarized the data for the control, and the treatments with LP, CCT, PCT and commercially available tannic acid (TA) in Table S1 (Supporting Information).

The impact of treatments on 16S rRNA and 18S rRNA gene abundances

Soil microcosms amended with LP, CCT, PCT or TA affected the abundances of the 16S and 18S rRNA genes differently over an incubation period of 42 days (Fig. 1). Compared with the numbers in the control without any addition, the 16S rRNA gene abundance was significantly reduced by PCT, while no significant effects were found by the other treatments (Fig. 1). Correlating the 16S rRNA gene abundance with a variety of soil factors as measured earlier (Zhang and Laanbroek 2018) and that are summarized in Table S1 (Supporting Information) showed only a significant correlation with total phenolics (Table S2, Supporting Information).

The highest copy numbers of the 18S rRNA gene were found in the treatments with CCT and LP followed by the other treatments and the control in the order of CCT = LP > TA = PCT > C (Fig. 1). The 18S rRNA gene abundance was significantly and positively correlated with the C/N ratio, total tannins and the total CO_2 emission, but negatively with $NO_3{}^-$, $NH_4{}^+$, N mineralization, $PO_4{}^{3-}$ and P mineralization (Table S2, Supporting Information).

Prokaryotic and eukaryotic community composition

The DGGE banding patterns observed with the 16S and 18S rRNA genes in soils sampled 42 days after the addition of the organic compounds also revealed differences between the treatments. The 16S rRNA gene-based DGGE profiles showed that the compositions of prokaryotic communities clustered into two groups at the level of 59% mutual similarity with one subgroup shaped by the control and the treatments with leaf powder and crude condensed tannins, and another by the treatments with purified condensed tannins and tannic acid (Figure S1A, Supporting Information). Furthermore, the replicates per treatment clustered also together according to the DGGE profiles. The DGGE patterns of the 18S rRNA gene revealed that the treatments clustered into two subgroups at the level of 38% mutual similarity,

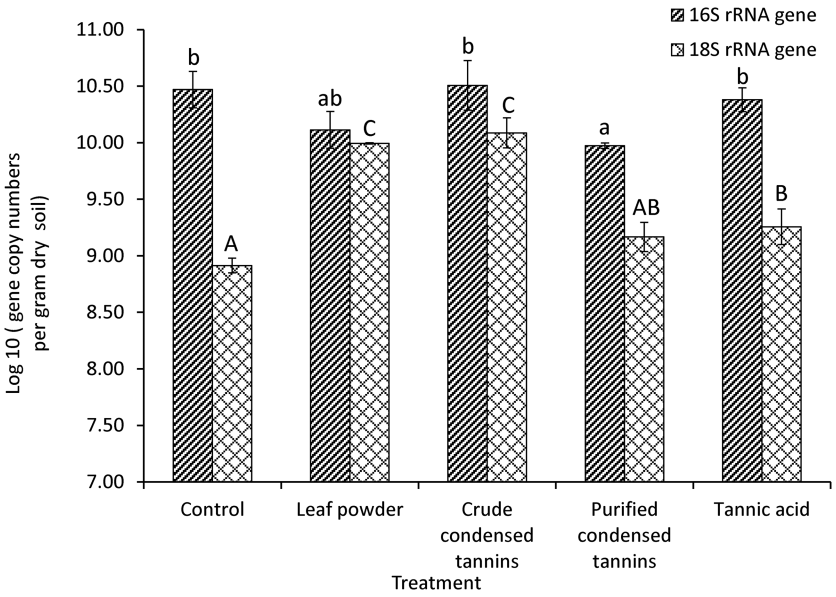

Figure 1. Abundances of the 16S and 18S rRNA genes in salt marsh soils 42 days after the start of the treatments. Error bars indicate standard deviations ($n = 3$). Within the same series of prokaryotic 16S rRNA gene and eukaryotic 18S rRNA gene copy numbers, numbers followed by the same letter are not significantly different at $P < 0.05$.

one included the control and the treatment with purified condensed tannins, and another contained the treatments with leaf powder, crude condensed tannins and tannic acid (Figure S1B, Supporting Information). The replicates belonging to the treatments with leaf powder and crude condensed tannins did not completely separate from each other.

The untreated 16S rRNA gene sequence reads, which appeared from HTS analysis, ranged from 16 307 to 34 500 sequencing reads. The lowest number of 16 307 was applied to the other 14 samples for comparing all samples at the same sequencing depth. The detected taxa all belonged to the bacterial domain; no archaeal taxa were found. Based on the 16S rRNA gene, bacterial phyla with >1% relative abundance were the phyla of Proteobacteria, Chloroflexi, Actinobacteria, Bacteroidetes, Gemmatimonadetes, Ignavibacteriae, Acidobacteria, Planctomycetes, Spirochaetae, Verrucomicrobia, Saccharibacteria and Parcubacteria, and Proteobacteria included Deltaproteobacteria, Gammaproteobacteria, Alphaproteobacteria, Betaproteobacteria, Epsilonproteobacteria and Proteobacteria_Incertae_Sedis at the class level (Figure S2A, Supporting Information). Of these phyla, the phylum Proteobacteria was most abundant across all samples with the highest relative abundance in the TA treatment (51 ± 6%) followed by the treatments with PCT (50 ± 5%), CCT (50 ± 4%) and LP (42 ± 1%). The Proteobacteria in the control amounted to 43 ± 3% of the total prokaryotic community.

The untreated 18S rRNA gene sequence reads ranged from 32 024 to 41 087 sequencing reads. The lowest number of 32 024 was applied to the other samples for comparing all samples at the same sequencing level. Due to the lack of sufficient replications, the results of the TA treatment were excluded from further analyses of the 18S rRNA gene results. Among the detected taxa, only the relative abundances of members of the kingdoms of Fungi and Alveolata were higher than 1%. The contribution of the Fungi to the total eukaryotic community amounted to 89 ± 3%, 93 ± 2%, 83 ± 6% and 90 ± 9% in the control and in the treatments with LP, CCT and PCT, respectively. The part of Alveolata in the total eukaryotic community was 4 ± 2%, 6 ± 2%, 12 ± 6%, and 11 ± 11% in the control soils and in the soils of the treatments with C, LP, CCT and PCT, respectively. Based on the 18S rRNA gene, phyla with >1% relative abundance were the fungal phyla of Ascomycota, Basidiomycota, Glomeromycota and Chytridiomycota, the protist phyla of Ciliophora and Choanoflagellida, and the animal phylum Nematoda (Figure S2B, Supporting Information). The variation in the relative abundances of the different phyla observed between triplicates and treatments was larger for the 18S gene than for the 16S rRNA gene, and the variation in results observed in the HTS analyses was higher than those detected in PCR-DGGE (Figure S1A and B, Supporting Information).

Microbial taxa with statistically significant differences between treatments

After clustering and alignment of the 16S rRNA genes, we found 1741 different OTUs based on a 0.97% similarity threshold across all samples. Among the 1741 different OTUs, some were exclusively detected in the control ($n = 26$; 1.5% of the total) or in the treatments with LP ($n = 41$; 2.4% of the total), CCT ($n = 1$), PCT ($n = 4$) or TA ($n = 7$). However, most of the OTUs ($n = 1186$, 68.12%) were shared by the samples (Figure S3A, Supporting Information).

After clustering and alignment of the 18S rRNA genes, we found 176 different OTUs based on a 0.97% similarity threshold across all samples with a range of 39 to 93 OTUs for the individual samples. Among the 176 different OTUs, a large number of OTUs ($n = 85$; 48% of the total) were shared by all samples, whereas a number was exclusively detected in the control (4 unique OTUs) and in the LP (3 unique OTUs), CCT (12 unique OTUs) and PCT (16 unique OTUs) treatments (Figure S3B, Supporting Information).

To determine the prokaryotic and eukaryotic taxa that were significantly more present in one treatment compared with the other treatments, so-called distinctive taxa, we used the linear discriminant analysis (LDA) effect size (LEfSe) method on the taxa as determined by HTS. The taxa with LDA threshold values

of 3.5 or larger as confirmed by the LEfSe method are presented in Tables 2 and 3. A large part of the 25 distinctive prokaryotic taxa were affiliated with the classes Gammaproteobacteria (33%) and Alphaproteobacteria (25%). Other bacterial classes were less represented. The larger part of the 13 distinctive eukaryotic taxa belonged to the kingdom Fungi (85%), the rest fitted to the kingdom Alveolata. Most fungal taxa belonged to the phylum Ascomycota (54% of the total eukaryotic taxa). The distinctive lineages are also shown in cladograms (Figures 4A and B, Supporting Information). The distinctive taxa were significantly correlated with one or more soil properties and with the cumulative CO_2 production (Tables S3 and S4, Supporting Information). Most distinctive eukaryotic taxa that emerged in the treatments correlated significantly (positively or negatively) with the mineralization of carbon (i.e. cumulative CO_2 production), nitrogen and phosphorus. This was also true for the enriched prokaryotic taxa although to a lesser percentage. The number of distinctive taxa that correlated significantly with total phenolics, total tannins or hydrolyzable tannins in the soil was low, even lower for the eukaryotes than for the prokaryotes (Tables S3 and S4, Supporting Information).

The impact of treatments on microbial diversity

For the 16S rRNA gene, the alpha diversity indices of Shannon, Simpson, Sobs and Chao1 varied among the treatments (Table 4). The coverage was not significantly affected by treatments and averaged 98.08% for all samples, suggesting that sequencing depth was sufficient to cover most of the prokaryota, including a portion of rare species. Based on the 16S rRNA gene sequences, the Shannon diversity index dropped significantly in the PCT and TA treatments compared with the control, but not in the LP and the CCT treatments. Compared with the control, the Sobs index decreased significantly in the treatment with TA, but not in the other treatments. The Simpson and the Chao1 indices were not significantly affected by the treatments. The results of Spearman rank-order correlation analyses (Table S2, Supporting Information) showed that the Shannon and Sobs indices were both positively and significantly correlated with pH, but negatively with total sulfur, total tannins and hydrolyzable tannins. The Simpson index was positively related with hydrolyzable tannins, but negatively with pH. Finally, the Chao1 index was negatively correlated with total carbon.

For the 18S rRNA gene, there were no significant differences in the diversity indices of Sobs and Chao1 between the treatments (Table 4). The coverage was also not affected by treatment and averaged 99.97%. Compared with the control, the Shannon diversity index increased significantly in the treatments with CCT and PCT, but was not significantly affected by the treatments with LP. On the contrary, the Simpson index increased only significantly in the treatment with LP. The results of Spearman rank-order correlation analyses (Table S2, Supporting Information) showed that Shannon's index correlated significantly and positively with total carbon, total nitrogen and total sulfur, but negatively to pH, while the Simpson index correlated negatively with total carbon, total nitrogen and total sulfur. The Sobs index correlated significantly and positively with hydrolyzable tannins, but negatively with pH.

The results of a PCoA grouped clearly the prokaryotic OTU assemblies according to the five treatments (Fig. 2A). The first two axes together explained 49.4% of the total variance among treatments. The eukaryotic OTU assemblies also grouped according to the treatments (Fig. 2B). The first two axes explained together 71.3% of the total variance observed between the treatments. According to a PERMANOVA analysis, both bacterial ($R^2 = 0.669$; $P = 0.001$) and fungal ($R^2 = 0.724$; $P = 0.002$) community compositions were significantly affected by the different treatments with *R. mangle*-derived organic matter.

Relationship between microbial community structure and environmental factors

Addition of *R. mangle*-derived organic matter and tannic acid changed the soil microbial community structures. However, the impact of addition of organic substances on microbial communities may mainly be due to changes in the physicochemical characteristics of the soils caused by the additions. Redundancy analysis (RDA) revealed that the prokaryotic and eukaryotic community structures were affected by several environmental factors including pH, total carbon, total nitrogen, C/N ratio, total sulfur, nitrate, ammonia, nitrogen mineralization, phosphate, phosphate mineralization, total phenolics, total tannins, hydrolyzable tannins and total CO_2 emission (Fig. 3A and B). Of the cumulative variance observed in the RDA on 16S rRNA genes, 71.7% is explained by the first two axes of the RDA biplot. The prokaryotic communities observed in the PCT and the TA treatments were affected differently from the communities in the control soil and in the soils of the LP and CCT treatments by a number of environmental factors (Fig. 3A). However, of the environmental factors tested, only soil pH and total phenolics were significantly correlated with the prokaryotic community structure (Table S5, Supporting Information).

With respect to the assemblies of 18S rRNA genes, the communities in the LP and in the PCT treatments were affected differently from the communities in the control and in the CCT treatment by a number of environmental factors, although the control and the CCT treatment behaved patchier (Fig. 3B). Of the cumulative variance observed in the RDA on 18S rRNA genes, 80.4% is explained by the first two axes of the RDA biplot. Of the environmental factors determined, soil pH, C/N ratio, total sulfur, nitrogen mineralization, phosphate, phosphorus mineralization and the total amount of CO_2 emission were all significantly correlated with the eukaryotic community structure (Table S5, Supporting Information). None of the phenolics and tannins fractions were significantly correlated with eukaryotic community composition.

DISCUSSION

Compared with the control, the microbial abundance and composition of *D. spicata* salt marsh soil were altered with the application of different organic fractions from senescent *R. mangle* leaves during 42-day incubation. The duration of the incubation experiment was comparable with the time that new microbial populations had been observed by scanning electron microscopy after 28–49 days of immersion of senescent *R. mangle* leaves collected from Virginia Key, Florida (Cundell *et al.* 1979). Our results showed that eukaryotes were more sensitive to the different treatments than prokaryotes. This is the first time in which the unique microbial community in salt marsh soil as selected by senescent *R. mangle* leaves and their condensed tannins is described in detail.

Although a number of studies have been done to study the effects of tannins from different sources on microorganisms (none of them including *R. mangle*), these studies often relied on more conventional tools, such as culture plate counting (Lewis and Starkey 1969; Grant 1976; Blum and Shafer 1988), chloroform

Table 2. Prokaryotic genera that discriminate between treatments at a log score of at least 3.50 as determined by LDA.

Treatment	Taxon ID	Taxonomy					Log LDA score	Percentage distribution				
		Phylum	Class	Order	Family	Genus		C	LP	CCT	PCT	TA
Control	C1	Proteobacteria	Gammaproteobacteria	Thiotrichales	Thiotrichaceae	No rank	3.69	1.67	0.58	0.98	1.34	1.01
	C2	Proteobacteria	Gammaproteobacteria	Unclassified	Unclassified	Unclassified	4.28	6.75	3.16	5.23	5.61	5.49
	C3	Proteobacteria	Betaproteobacteria				4.28	2.26	0.75	1.04	1.13	1.25
	C4	Proteobacteria	Deltaproteobacteria	Desulfobacterales	Desulfobacteraceae		3.60	2.08	1.54	1.51	1.91	1.74
	C5	Proteobacteria	Deltaproteobacteria	Myxococcales			3.59	1.78	1.02	1.17	1.27	1.49
	C6	Actinobacteria	Acidimicrobiia	Acidimicrobiales	OM1_clade	No rank	3.97	4.20	2.74	3.38	3.31	3.51
	C7	Gemmatimonadetes	Gemmatimonadetes	Gemmatimonadaceae	Gemmatimonadaceae	No rank	3.56	1.24	0.64	0.73	0.92	0.91
	C8	Gemmatimonadetes	Gemmatimonadetes	No rank	No rank	No rank	4.01	4.09	2.40	3.35	3.74	3.68
LP	LP1	Bacteroidetes	Bacteroidia	Bacteroidales	Prolixibacteraceae	*Prolixibacter*	3.81	0.02	1.18	0.11	0.05	0.01
	LP2	Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae		4.12	3.00	5.37	4.08	4.59	2.72
	LP3	Bacteroidetes	SB_5	No rank	No rank	No rank	3.61	0.49	1.53	0.46	0.37	0.38
	LP4	Proteobacteria	Epsilonproteobacteria	Campylobacterales			3.57	0.08	0.98	0.09	0.09	0.06
CCT	CCT1	Proteobacteria	Gammaproteobacteria	Oceanospirillales	Oceanospirillaceae	*Marinomonas*	3.54	0.10	0.35	0.78	0.13	0.17
	CCT2	Proteobacteria	Gammaproteobacteria	34P16	No rank	No rank	4.24	0.09	1.78	3.43	0.04	0.02
	CCT3	Proteobacteria	Alphaproteobacteria	Sphingomonadales			3.64	0.22	0.49	0.97	0.12	0.74
	CCT4	Proteobacteria	Alphaproteobacteria	Rhizobiales	Phyllobacteriaceae	*Parvibaculum*	3.72	0.04	0.49	1.17	0.03	0.04
PCT	PCT1	Proteobacteria	Gammaproteobacteria				4.74	16.23	14.08	20.69	24.42	20.75
	PCT2	Proteobacteria	Gammaproteobacteria	Oceanospirillales			4.72	0.96	2.67	4.55	11.61	0.70
TA	TA1	Proteobacteria	Gammaproteobacteria	Alteromonadales	Alteromonadales	*Marinobacter*	4.56	0.38	1.06	1.16	0.40	7.60
	TA2	Proteobacteria	Gammaproteobacteria	Xanthomonadales			3.86	1.82	1.17	1.35	1.37	2.50
	TA3	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Erythrobacteraceae	No rank	3.51	0.04	0.05	0.09	0.03	0.64
	TA4	Proteobacteria	Alphaproteobacteria	Rhizobiales	Xanthobacteraceae	No rank	3.85	2.46	1.58	2.02	2.56	2.92
	TA5	Proteobacteria	Alphaproteobacteria	Rhizobiales	Unclassified	Unclassified	3.95	1.27	1.39	1.89	1.65	3.17
	TA6	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	*Labrenzia*	3.88	0.02	0.87	0.56	0.10	1.55

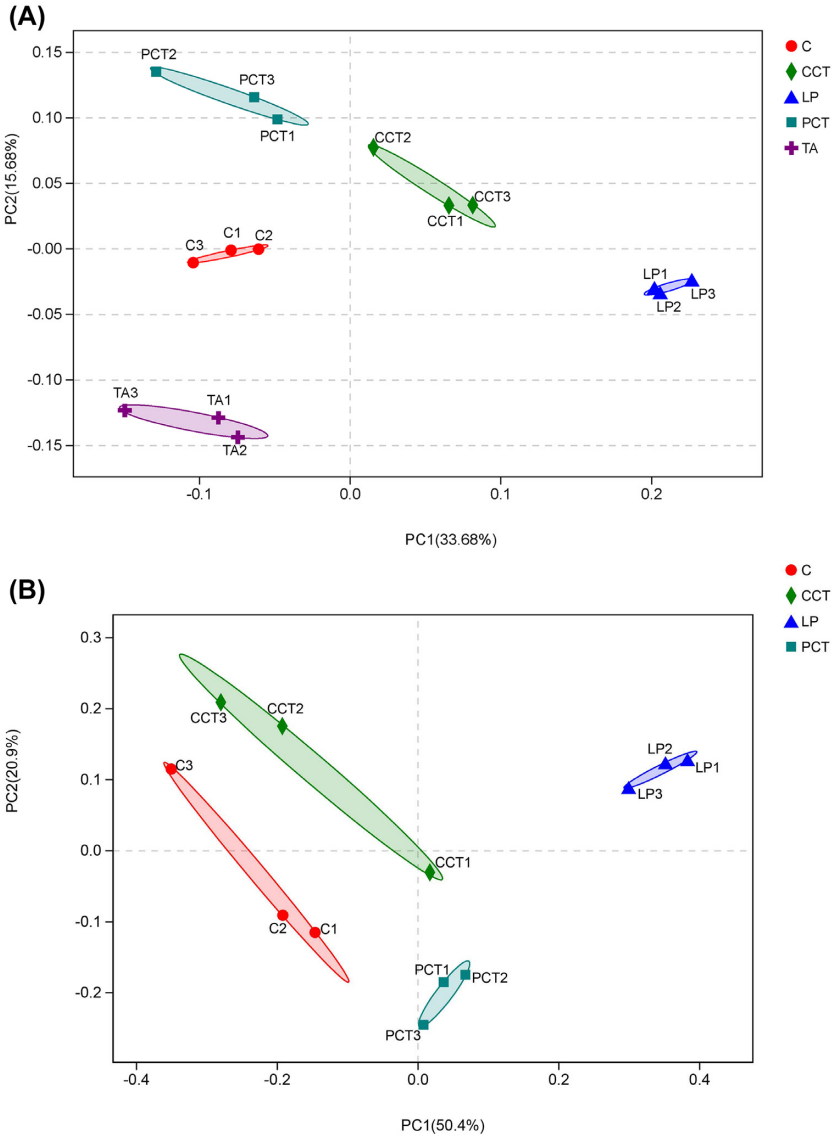

Figure 2. Biplots of the results of the PCoA of OTUs based on the 16S rRNA gene **(A)** and the 18S rRNA gene **(B)** observed in salt marsh soils characterized by the different treatments. The results of triplicate samples are shown for each treatment.

fumigation extraction (Bradley, Titus and Preston 2000; Fierer *et al.* 2001), community level physiological profiling (Schmidt *et al.* 2013), Biolog GN micro-plating (Bradley, Titus and Preston 2000), phospholipid fatty acid analysis (PLFA; Mutabaruka, Hairiah and Cadisch 2007), DGGE (Schmidt *et al.* 2013) and single-strand conformation polymorphism (Baptist *et al.* 2008). A more complete picture of microbial diversity could not be offered by these methods, while the HTS method as used in our study provides a more complete analysis of prokaryotic and eukaryotic communities with a high resolution of microbial taxa from the phylum to even the genus level.

Effects of tannins and other leaf-derived compounds on microbial abundances

Our results indicate a lower prokaryotic abundance in soils treated with PCT than in soils with other treatments including the control, while no differences were observed among the control and the other treatments. Assuming no significant mortality among the prokaryotic community during the incubation period of 42 days, the higher numbers of 16S rRNA genes in the control soils than in the soil of the PCT treatment implies that prokaryotic growth occurred on the organic compounds already present in the salt marsh soil and that purified condensed tannins repressed this growth. Cause for such a repression has been reported before, i.e. binding of tannins to vital proteins such as enzymes and complexation of organic nitrogen compounds making these compounds less degradable (Field and Lettinga 1992; Adamczyk *et al.* 2017). In contrast to the prokaryotic abundance, the eukaryotic numbers increased by organic additions in all treatments compared with the control, although not significantly in the treatment with PCT. Hence, purified condensed tannins did not stimulate growth of eukaryotes significantly. Since the 18S rRNA gene abundance in the control soil is significantly lower than in soils of the treatments (except PCT), it cannot be concluded that organic material existing in pristine salt marsh soil sustained growth of eukaryotic microorganisms like it did in the case of prokaryotes.

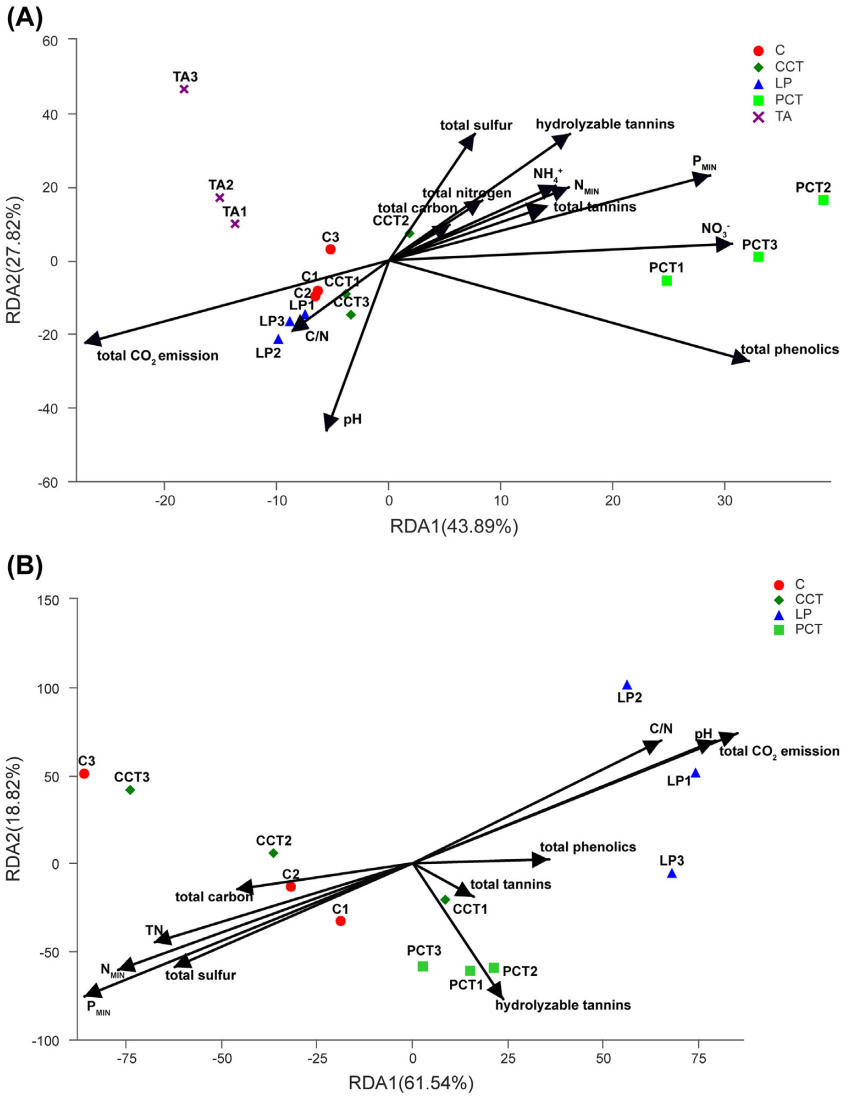

Figure 3. Biplots of redundancy analyses (RDA) between prokaryotic **(A)** or eukaryotic **(B)** communities (at the genus level) and environmental properties.

For all treatments, prokaryotic abundance was higher than that of the eukaryotes. Others also observed a higher bacterial to fungal ratio in agricultural soils with a pH gradient ranging from 4.0 to 8.3 (Rousk *et al.* 2010) and in reclaimed mine soils of a forest ecosystem (Sun *et al.* 2017). However, the ratio of the 16S rRNA gene to the 18S rRNA gene abundance in our study declined from 37.7 in the control to 1.4, 2.8, 6.3 and 1.3 in the treatments with leaf powder, crude condensed tannins, purified condensed tannins and tannic acid, respectively. It was remarkable that the prokaryotic to eukaryotic abundances decreased the least in the treatment with purified condensed tannins, which is in line with the statements on these compounds made in the former paragraph. The decrease in the ratio of prokaryotic to eukaryotic abundance by purified condensed tannins compared with the control, which agreed with an earlier study (Mutabaruka, Hairiah and Cadisch 2007), suggests that eukaryotes gained more from the organic fractions from senescent *R. mangle* leaves than the prokaryotes, which can be expected from the ability of fungi to degrade more complex organic matter due to the filamentous growth form of most taxa (de Boer *et al.* 2005). The eukaryotic gene abundance was significantly related to several environmental factors such as C/N ratio, NO_3^-, NH_4^+, N_{MIN}, PO_4^{3-}, P_{MIN}, total tannins and total CO_2 emission (Table S4, Supporting Information). In contrast, the 16S rRNA gene was only significantly related to total phenolics in the soils. This suggests that the eukaryotes will use the added organic matter as a C source to a larger extent than the prokaryotes, and that the eukaryotic abundance was more sensitive to environmental factors than the prokaryotic abundance. The latter might be expected from growing populations.

Effects of tannins and other leaf-derived compounds on microbial community structures

The mechanisms of microbial community formation will largely depend on the characteristics of the available organic compounds (Prescott and Grayston 2013) and on the initial colonizers of the soil (Cline and Zak 2015). Therefore, the structural differences among the carbon pools present in the different fractions of organic matter derived from senescent *R. mangle* leaves will affect the abundance and composition of soil microbial communities to various extents. Compared with the control, clear

Table 3. Eukaryotic genera that discriminate between treatments at a log score of at least 3.50 as determined by LDA.

Treatment[a]		Taxonomy					Log LDA score	Percentage distribution			
		Phylum	Class	Order	Family	Genus		C	LP	CCT	PCT
C	C1	Ascomycota[b]	Eurotiomycetes	Eurotiales	Trichocomaceae	unclassified	5.08	26.38	0.11	21.44	0.40
LP	LP1	Ascomycota[b]	Sordariomycetes	Lulworthiales			5.37	0.00	19.72	0.03	0.02
CCT	CCT1	Ascomycota[b]	Ascomycetes	Pleosporales	Pleosporaceae	*Cochliobolus*	4.25	0.00	0.02	0.76	0.00
	CCT2	Ascomycota[b]	Eurotiomycetes	Eurotiales	Unclassified	Unclassified	3.95	0.05	0.01	0.06	0.04
	CCT3	Ciliophora[c]	Oligohymenophorea	Conthreep	Oligohymenophorea	*Pseudocohnilembus*	4.64	1.60	4.67	8.94	2.93
	CCT4	Chytridiomycota[b]	No rank	No rank	No rank	No rank	4.32	0.38	0.71	4.02	0.21
PCT	PCT1	Ascomycota[b]	unclassified	Unclassified	Unclassified	Unclassified	4.14	0.48	0.42	0.75	2.53
	PCT2	Ascomycota[b]	Sordariomycetes	Diaporthales	Unclassified	Unclassified	4.14	0.47	0.03	0.32	0.52
	PCT3	Ascomycota[b]	Sordariomycetes	Unclassified	Unclassified	Unclassified	4.14	0.39	0.81	0.98	2.11
	PCT4	Basidiomycota[b]	Agaricomycetes	Unclassified	Unclassified	Unclassified	4.73	12.09	2.62	6.14	13.79
	PCT5	unclassified	unclassified	Unclassified	Unclassified	Unclassified	4.22	1.03	0.54	1.29	3.72
	PCT6	Choanoflagellida[c]	Craspedida	Codonosigidae	Codonosigidae	*Monosiga*	4.29	2.46	0.24	1.13	3.06
	PCT7	Glomeromycota[b]	No rank	No rank	No rank	No rank	4.09	2.10	0.18	0.89	2.41

[a]The treatment with tannic acid (TA) is missing due to lack of sufficient replications; [b]belonging to the kingdom of Fungi; [c]belonging to the kingdom of Alveolata.

Table 4. Effect of treatments on the average alpha diversity indices of prokaryotes and eukaryotes observed in the soils 42 days after the start of the treatments.

Treatment	Alpha diversity indices based on the 16S rRNA gene					Alpha diversity indices based on the 18S rRNA gene				
	Shannon	Simpson	Sobs	Chao1	Coverage	Shannon	Simpson	Sobs	Chao1	Coverage
Control (C)	5.96 ± 0.01 b	0.006 ± 0.000 a	1233 ± 35 b	1453.58 ± 79.35 a	0.9820 ± 0.0020 a	2.34 ± 0.42 b	0.182 ± 0.069 a	72 ± 33 a	77.29 ± 34.93 a	0.9998 ± 0.0001 a
Leaf powder (LP)	5.96 ± 0.06 b	0.006 ± 0.001 a	1254 ± 12 b	1522.55 ± 43.13 a	0.9800 ± 0.0020 a	1.65 ± 0.08 a	0.370 ± 0.079 b	83 ± 7 a	89.00 ± 7.83 a	0.9996 ± 0.0001 a
Crude condensed tannins (CCT)	5.88 ± 0.02 b	0.006 ± 0.000 a	1199 ± 20 ab	1442.41 ± 54.33 a	0.9810 ± 0.0010 a	2.61 ± 0.21 b	0.153 ± 0.045 a	102 ± 2 a	106.97 ± 2.90 a	0.9997 ± 0.0001 a
Purified condensed tannins (PCT)	5.62 ± 0.08 a	0.012 ± 0.002 a	1185 ± 25 ab	1453.89 ± 18.85 a	0.9800 ± 0.0000 a	2.42 ± 0.07 b	0.178 ± 0.031 a	102 ± 5 a	104.31 ± 5.34 a	0.9998 ± 0.0001 a
Tannic acid (TA)	5.68 ± 0.13 a	0.011 ± 0.005 a	1160 ± 36 a	1425.48 ± 32.91 a	0.9810 ± 0.0000 a					

The values are means of replicates ($n = 3$) ± (SE). Different letters within a column indicate significant differences ($P < 0.05$) between the treatments according to Tukey test except of the Simpson index of the 16S rRNA gene using a non-parametric Kruskal–Wallis test for pairwise comparisons.

shifts in the prokaryotic and eukaryotic communities from the phylum to the genus level were observed in the soils after addition of different fractions of organic matter derived from senescent *R. mangle* leaves. Moreover, the specific responses of microbial communities to different fractions of organic matter and to hydrolyzable tannin were confirmed by combining intuitive and high-resolution patterns as obtained by the DGGE profiles and HTS, respectively. Both techniques showed a larger effect of the organic additions on the eukaryotic than on the prokaryotic community.

The different treatments with organic fractions from senescent *R. mangle* leaves or with tannic acid had different effects on prokaryotic and eukaryotic diversity. Looking at the prokaryotic communities, the treatment with purified condensed tannins had only a significant and negative effect on the diversity (Shannon index), while the treatment with tannic acid had the same effect on richness (Sobs index). Hence, purified condensed tannins and tannic acid lowered the diversity and richness of prokaryotic communities. The other treatments had no effect on diversity indices (Table 4). We showed that the Shannon and the Sobs indices were significantly correlated with soil pH, total sulfur, and total and hydrolyzable tannins. Looking at the eukaryotic communities, the treatment with leaf powder had a significant and negative effect on the Shannon diversity index and a significant but positive effect on the Simpson similarity index. The other diversity indices were not significantly affected by treatments. The Shannon index and the Simpson index were significantly correlated with soil pH (only Shannon), total carbon, total nitrogen, C/N ratio (only Simpson) and total sulfur. Between the replicates, the prokaryotic communities were more similar and less variable than the eukaryotic communities. The prokaryotic diversity was higher than the eukaryotic diversity over all the treatments, which is consistent with an earlier study on forest litter and soil (Urbanová, Šnajdr and Baldrian 2015)

Whereas the total microbial community structure was related to different environmental factors, the eukaryotic community composition was most impacted as shown by RDA (Table S5, Supporting Information). This was similar as found in other studies (Zinger *et al.* 2011; Kaisermann *et al.* 2015; Urbanová, Šnajdr and Baldrian 2015; Schlemper, van Veen and Kuramae 2017; Sun *et al.* 2017). Several factors such as a microbial priority effect and concentrations of nutrients and levels of carbon, nitrogen and phosphorus may contribute to the contrasting effects of added organic compounds on prokaryotes and eukaryotic communities (Schmidt *et al.* 2014). Fungal hyphae are, for example, better adapted than bacteria to cross nutrient-poor spots in soil in searching for heterogeneously distributed nutrient resources (De Boer *et al.* 2005; Urbanová, Šnajdr and Baldrian 2015). Different fractions of phenolic compounds from senescent mangrove leaves will likely have different effects on the microbial composition during the 42 days of incubation. Leaf litter of *R. mangle* is mostly composed of recalcitrant biopolymers as represented by polysaccharides and polyphenols, which are utilized more easily by saprotrophic fungi than by most bacteria. Due to the filamentous form of most taxa, fungi are often considered better suited for and consequently more involved in the decomposition of polymeric compounds (De Boer *et al.* 2005), while many bacterial taxa preferentially utilize low-molecular-mass organic compounds, and therefore depend on the products of fungal biopolymer decomposition for nutrition (Štursová *et al.* 2012). As a consequence, the litter traits should have stronger effects on the community of the saprotrophic fungi than on bacteria (Kubartova *et al.* 2009).

Effects of tannins and other leaf-derived compounds on the emergence of distinctive taxa

The different treatments led to the emergence of distinctive prokaryotic and eukaryotic taxa. The emergence of distinctive microorganisms in the presence of different organic fractions derived from senescent *R. mangle* leaves may present an insight in the ecological role of such microorganisms. Since the additions in the form of freeze-dried leaves, processed and purified condensed tannins and commercial tannic acid were most likely axenic due to their pre-treatment, the emerged distinctive taxa must have originated from the *D. spicata* salt marsh soil. As discussed before (Zhang and Laanbroek 2018), the microorganisms in the control soil could only use the original organic matter of pristine soils as carbon source, while the microbes in the treatments with added organic compounds will additionally be supported by these compounds. The simplest addition was the addition of tannic acid. Unfortunately, LDA did not yield distinctive eukaryotes because of the lack of sufficient replicates. The LDA on prokaryotes, however, generated six distinctive taxa: two were classified at the level of order (Xanthomonadales and Rhizobiales), two at the level of family (Erythrobacteraceae and Xanthobacteraceae) and two at the level of genus (*Marinobacter* and *Labrenzia*). Metabolic characteristics can best be predicted at the genus level. Families and higher taxonomic levels will generally comprise more metabolic types than genera. Members of the marine genus *Marinobacter* (family Oceanospirillaceae, class Gammaproteobacteria) are aerobic, with a non-fermentative metabolism and they are able to denitrify with N_2 production (Gauthier *et al.* 1992). They grow on a range of carbon compounds but not on carbohydrates and amino acids (except L-proline and L-glutamate). They can degrade a large variety of aliphatic or aromatic hydrocarbons. *Marinobacter* was found before in mangrove sediment that was contaminated with oil (Santos *et al.* 2011). This suggests that members of this genus are able to use aromatic rings as they appear in oil and likely in tannic acid. Members of the marine genus *Labrenzia* (family Rhodobacteraceae, class Alphaproteobacteria) are strictly aerobic and utilized a wide range of organic carbon sources, including fatty acids, tricarboxylic acid cycle intermediates and sugars (Biebl *et al.* 2007). They do not reduce nitrate. *Labrenzia* has been detected before in mangroves at the coast of the Bay of Bengal (Ghosh and Bhadury 2018).

The addition of PCT to the salt marsh soil yielded two distinctive prokaryotic taxa and seven distinctive eukaryotic taxa. The prokaryotic taxa could only be classified at the level of phylum (Gammaproteobacteria) and class (Oceanospirillales), respectively. One of the distinctive eukaryotic taxa could not be classified, two were classified at the phylum level (Ascomycota and Glomeromycota), two at the level of class (Sordariomycetes and Agaricomycetes), one at the level of order (Diaporthales) and one of the level of genus (*Monosiga*). Members of the *Monosiga* belong to the phylum Choanoflagellates, which are ubiquitously found Protists in marine environments; they bear an apical flagellum surrounded by a distinctive collar of actin-filled microvilli, with which choanoflagellates trap bacteria and detritus, thereby linking bacteria to higher trophic levels and thus having a critical role in carbon cycling and in the microbial food web (Sherr *et al.*1982; Boenigk and Arndt 2002; King *et al.* 2008).

In addition to condensed tannins, distinctive microorganisms in the treatment with crude condensed tannins will have used low-molecular-weight phenols, flavonoid monomers, sesquiterpenes and diterpenes (Kanerva *et al.* 2006). The treatment with crude condensed tannins added yielded four

distinctive prokaryotic taxa and four distinctive eukaryotic taxa. Of the prokaryotic taxa, two were classified at the order level (Sphingomonadales and 34P16) and two at the genus level (*Marinomonas* and *Parvibaculum*). The lineages belonging to these genera, which were discriminatory for CCT-treated soils, appeared also in relatively large numbers in the LP-treated soils, but not in the soils treated with purified condensed tannins (Table 3). This suggests that these lineages took advantage of organic compounds from senescent *R. mangle* leaves other than purified condensed tannins. Members of the genus *Marinomonas* (family Oceanospirillaceae) have an aerobic, strictly respiratory type of metabolism and utilize a range of more simple organic substrates (Sanchez-Amat and Solano 2015). *Marinomonas* sp. strain D104, which has been isolated from sediment of the Arctic Ocean, contained the potential to degrade aromatic rings (Dong *et al.* 2014). The type species of the genus *Parvibaculum*, i.e. *Parvibaculum lavamentivorans* (Phyllobacteriaceae), is a strictly aerobic organism with a limited range of carbon substrates (Schleheck *et al.* 2011).

Of the distinctive eukaryotic taxa that emerged in the CCT-treatment, one was identified at the phylum level (Chytridiomycota), one at the order level (Eurotiales) and two at the genus level (*Cochliobolus* and *Pseudocohnilembus*). Members of the genus *Cochliobolus* (phylum Ascomycota) comprise many destructive plant pathogens that cause severe crop losses worldwide (Manamgoda *et al.* 2011). Some of the species are endophytic, while others are saprobic. Members of the cysts-containing genus *Pseudocohnilembus* (kingdom of Alveolata) are able to adapt to hard conditions of salinity, pH, desiccation and anoxia (Olendzenski 1999) as well as to circumstances of abundant nitrogen and low temperature (Jones *et al.* 2010).

In addition to the carbon compounds that are available in the treatments with crude and purified tannins, the microorganism in the soil of the treatment with leaf powder will have used additional compounds such as readily leachable and low-molecular carbohydrates, organic acids, proteins, phenols, cyclitol and lignin (Cundell *et al.* 1979; Benner and Hodson 1985; Benner *et al.* 1990). The treatment with leaf powder yielded four distinctive prokaryotic taxa and one distinctive eukaryotic taxon. Of the four prokaryotic taxa, one was classified at the class level (SB_5, phylum Bacteroidetes), one at the order level (Campylobacterales), one at the family level (Flavobacteriaceae) and one at the genus level (*Prolixibacter*). Actually, two distinctive lineages belonged to the genus *Proxilibacter* (family Flavobacteriaceae), i.e. the species *Prolixibacter bellariivorans* and *Prolixibacter denitrificans*. Both *Proxilibacter* species are facultative anaerobic, heterotrophic organisms (Holmes *et al.* 2007; Iino *et al.* 2015). *Prolixibacter bellariivorans* strain F2^T has been isolated from a marine sediment of Boston Harbor (MA, USA) contaminated with polycyclic aromatic hydrocarbons (Bond *et al.* 2002; Holmes *et al.* 2004, 2007). *Prolixibacter denitrificans* strain MIC1–1^T has been isolated from a crude oil sample collected at an oil well in Akita Prefecture, Japan. It plays an important role in biogeochemical cycles since oxygen as well as nitrate could be used as alternative electron acceptor, with nitrite as the end product of nitrate reduction (Holmes *et al.* 2007; Iino *et al.* 2015). Most but not all members of the family Flavobacteriaceae are aerobic with a primarily respiratory metabolism. Utilization of macromolecules such as polysaccharides and proteins is a common feature of many members of the family Flavobacteriaceae (McBride 2014). The only distinctive eukaryotic taxon that emerged on the treatment with leaf powder was affiliated with the order Lulworthiales (marine Ascomycetes). Finally, eight distinctive prokaryotic taxa and one distinctive eukaryotic taxon came to the fore in the control treatment. Hence, these taxa were stimulated by incubation of salt marsh soil without any organic additions. Of the eight prokaryotic taxa, three were identified at the class level (Betaproteobacteria, Gammaproteobacteria and Gemmatimonadetes), one at the order level (Myxococcales) and four at the family level (Thiotrichaceae, Desulfobacteraceae, OM1_clade and Gemmatimonadaceae). The only distinctive eukaryotic taxon belonged to the family Trichocomaceae. These taxa that emerged in the control were apparently overgrown by other taxa in the treatments that are mentioned above.

CONCLUSION

Our research revealed in detail the effects of tannins and other non-identified organic compounds from senescent *R. mangle* leaves on the abundance and diversity of prokaryotic and eukaryotic microorganism in *D. spicata* salt marsh soil. Organic matter derived from the senescent leaves affected the abundance and community composition of bacteria less than the numbers and community composition of fungi and protists, although the bacteria were more abundant than the eukaryotes. Hence, eukaryotic communities will likely become more prominent in relation to prokaryotic communities when mangroves migrate into salt marsh soils. In addition, our results give also some insight into the ecological role of prokaryotes and eukaryotic microbial communities with respect to the degradation of different organic fractions derived from *R. mangle* in the transition zone between terrestrial and marine ecosystems. Finally, some distinctive microorganisms as emerging from different additions of condensed tannins and tannic acid, which both are types of polyphenolic compounds, might have potential functions in the bioremediation of environmental pollution caused by oil or aromatic compounds.

SUPPLEMENTARY DATA

Supplementary data are available at *FEMSEC* online.

Compliance with Ethical Standards Not Applicable

Ethical Approval: This article does not contain any studies with human participants or animals performed by any of the authors.

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Conflict of interest. None declared.

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