Baja California Sur mangrove deep peat microbial communities cycle nitrogen but do not affect old carbon pool

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ABSTRACT: Mangroves provide important ecosystem services, including storing carbon belowground for millennia. Mangrove carbon storage relies in part on high primary productivity, but essential to the long-lived nature of this storage is the slow rate of microbial decomposition of peat. In this study, we (1) examined how carbon and nitrogen densities and microbial community composition vary with peat age and (2) describe the formation of peat deposits over time. At 4 mangrove sites near La Paz, Baja California Sur, Mexico, we cored the sediments until rejection and obtained 5 cm samples at 20 cm intervals. In these samples, we measured organic carbon (C_{org}), total nitrogen, $\delta^{13}C$, $\delta^{15}N$, and radiocarbon (^{14}C) age. We observed peat carbon densities of $3.4 \times 10^{-2} \pm 0.2 \times 10^{-2}$ g cm^{-3}, C_{org}:N ratios of 42 ± 3, and inter-site variation in C_{org}:N that reflects differing preservation conditions. Recalcitrant organic matter sources and anaerobic conditions leave a strong imprint on peat microbial communities. Microbial community composition and diversity were driven by depth and sediment characteristics, including C_{org}:N ratio and ^{14}C age. Carbon dating allowed us to reconstruct the accumulation of organic matter over the last 5029 ± 85 yr. Even over this long time scale, though microbes have evidently continuously cycled the peat nitrogen pool, peat carbon density remains effectively unchanged.

KEY WORDS: Mangrove · Sediment · Peat · Microbiome · Blue carbon · Nitrogen

1. INTRODUCTION

Mangrove forests export organic matter and juvenile animals (Alongi 1990, Nagelkerken et al. 2008), as well as acting as sinks for carbon (Twilley et al. 1992). Though leaf litter and propagules are consumed by detritivores or exported (Odum & Heald 1975), woody material including roots can remain unconsumed for long periods of time (Middleton & McKee 2001). Some of this production is buried in accumulating peat deposits, where anoxic conditions and recalcitrance help preserve organic matter for millennia (McKee et al. 2007). As a result, mangroves and other coastal wetlands exhibit high rates of carbon sequestration (Chmura et al. 2003), and soil carbon stocks in these systems surpass those in other forest types several times over (Donato et al. 2011). Recognition in the last decade of the disproportionately high contribution of mangroves, salt marshes, and seagrass beds to carbon storage has added a new prong to the study of their ecosystem services: the quantification of 'blue carbon' (Nellemann et al. 2009). This research area has taken on special urgency due to an alarming 2% global loss of mangrove area annually from 1980–2000 (Valiela et al. 2001), threatening the carbon sequestration and other eco-

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system services provided by these wetlands (Costanza et al. 1997). Fortunately, deforestation rates have slowed in recent years (Goldberg et al. 2020), and the annual net loss of mangrove area may become a net gain if the use of carbon stock estimates continues to drive economic investment in their conservation and restoration to meet greenhouse gas mitigation goals (Thomas 2014). Though researchers have quantified mangrove carbon stocks around the world (Donato et al. 2011, Adame et al. 2013, Alongi et al. 2016), understanding of the processes that control these stocks is far from complete.

Decades of research on estuarine carbon cycling has explored the role of microorganisms in these ecosystems. Early work focused on microbial breakdown of mangrove primary production, moving it up the food chain to be consumed by animals. Fell et al. (1975) cultured phycomycete fungi from mangrove leaf litter and tested their impact on its degradation, which increased nitrogen to carbon ratios and thus facilitated its consumption by invertebrates. In a temperate salt marsh/seagrass system, microbial abundance varied with depth and to a lesser extent with physical sediment characteristics (Ferguson & Murdoch 1975). Odum & Heald (1975) discussed the role that microbes' partial consumption of and attachment to leaf litter play in the detritus-based mangrove food web of North River, Florida. Sediment bacterial densities and productivity rates exhibit high spatial and seasonal variability in mangroves of northeastern Australia, where a large part of ecosystem production passes through the bacterial community (Alongi 1988). Bacterial densities also vary among forest types, with greater densities in large deltaic systems than in smaller fringe or riverine forests, and bacterial productivity is correlated with the availability of dissolved organic nitrogen and carbon (DON and DOC) (Alongi & Sasekumar 1992). Though sediment microbes and mangrove trees interact biogeochemically, bacterial densities and growth rates vary with edaphic conditions rather than the species of nearby trees (Alongi et al. 1993).

More recent research has shifted attention toward the functional role of microbes in mangrove ecosystems. Sediment microbes fix nitrogen and immobilize it from the dissolved pool, consume and transform organic carbon (C_{org}), and solubilize phosphorus, making it available for nutrient-limited mangrove growth (Holguin et al. 2001). Balanced methane production by microbes and subsequent oxidation in the sediment (Giani et al. 1996), as well as microbial solubilization of mineral phosphate (Vazquez et al. 2000), have both been measured in a mangrove lagoon in

Baja California Sur, Mexico, with implications for the carbon balance and productivity of these forests. More recently, researchers have begun the study of microbial diversity in mangrove sediments using genetic sequencing methods (Andreote et al. 2012), making it possible to map out the biogeochemical potential of mangrove soil communities and their potentially useful functions, such as the mitigation of pollutants (Tiralerdpanich et al. 2018).

We examined variation in carbon, nitrogen, and the microbial community with depth and age of sediment. We hypothesized (1) that the microbial community varies with sediment type, reflecting a specialized community associated with peat, as opposed to calcite, clay, or sand; (2) that deeper, older peat contains lower C_{org} density and higher stable isotopic ratios of carbon and nitrogen ($\delta^{13}C$ and $\delta^{15}N$) due to carbon and nitrogen remineralization by these microbes over time; and (3) that with increasing peat age the microbial community shifts toward lower richness and increased dominance by specialized taxa that can consume refractory organic matter under anoxia.

2. MATERIALS AND METHODS

2.1. Field sites

The Bahia de la Paz on the east coast of Baja California Sur experiences arid, subtropical conditions, with <200 mm yr^{-1} of precipitation (Rebman & Roberts 2012). In addition to mangroves, the coastal plant community in this region contains many drought-tolerant members of the Caryophyllales order (e.g. Amaranthaceae and Cactaceae; Rebman & Roberts 2012), known to employ C4 and CAM photosynthetic pathways. These pathways are associated with higher biomass $\delta^{13}C$ (in the range of −10 to −20‰) than is common in C3 plants such as mangroves (in the range of −20 to −35‰) (Andrews et al. 1984). Despite relatively low productivity in this extreme habitat (López-Medellín & Ezcurra 2012), some of these mangroves still store large carbon stocks in peat deposits that are millennia old (Ezcurra et al. 2016). We sampled these deposits to probe the role of microbes in the slow decomposition of buried organic matter. We chose 4 sites expected from previous sampling to contain deep peat deposits (Fig. 1). Only one core was taken at each site. Thus, this study does not focus on intersite comparisons, but rather on the exploration of variation in peat biogeochemistry and microbial community with depth. In the large mangrove at the southern tip of Isla San José, we cored at 2 sites: San José A,

Fig. 1. The 4 sites sampled in this study in the Bay of La Paz area of the Gulf of California: El Mérito (24.301°, −110.327°), San Gabriel (24.432°, −110.347°), and San José (containing 2 sites — A: 24.880°, −110.567°; B: 24.869°, −110.575°). Beneath each site name is a qualitative description of the site's location in the tidal frame (low or high) and the dominant species present (*AG*: *Avicennia germinans*; *LR*: *Laguncularia racemosa*; *RM*: *Rhizophora mangle*)

near a tidal creek in a low intertidal area dominated by *Rhizophora mangle*, and San José B, in an area where the high intertidal steeply abuts a supratidal berm and where all 3 local mangrove species (*Avicennia germinans*, *Laguncularia racemosa*, and *R. mangle*) are present. The San Gabriel core was taken in an area dominated by *L. racemosa* and *R. mangle* in a sandy coastal lagoon on Isla Espíritu Santo. The core at El Mérito was taken at the high intertidal edge of a small, geomorphically constrained bay, in a zone currently inhabited by dwarf *A. germinans* trees.

2.2. Field methods

We sampled mangrove sediments using a Russian peat corer (Aquatic Research Instruments), taking semi-cylindrical sections of sediment 5 cm in dia-

meter and up to 50 cm in length. By adding extension rods, we then returned to the same hole to obtain successively deeper 50 cm sections of sediment, making sure to insert the corer with the blade in the exact same alignment so that the corer tip was horizontally offset from the sampled area and thus did not disturb the next core segment. We photographed and inspected each core segment upon retrieval to confirm complete recovery without physical disturbance. We repeated this process to rejection (i.e. when the corer tip hit hard substratum), capturing the entire sediment column. Each core segment was subsampled every 20 cm with depth (0–5, 20–25 cm, etc.) and at the maximum depth, using a knife and a measuring tape to obtain samples of 5 cm vertical extent. Each horizon was visually identified as one of the following sediment types: calcite, clay, peat, or sand. Peat, defined as sediment of high organic matter content, with thresholds of ≥50% and 65% organic matter used in the literature (Andriesse 1988), is functionally applied in this study to samples visibly composed mainly of fibers of plant matter. Samples for carbon analysis were taken adjacent in the core to samples for microbial analysis. Microbial samples were taken from the core using tools sterilized with a 1:1 solution of bleach and ethanol (Nilsson et al. 2022).

2.3. Carbon and nitrogen analysis

We placed each sample for sediment analysis in a drying oven at 60°C until dry (≥24 h). When it was not possible to dry the samples immediately, we kept them on ice until they could be dried. We weighed the dried samples and then homogenized them using a ball mill and mortar and pestle until they passed through a 500 μm sieve. To remove $CaCO_3$, the samples were HCl-fumigated, following Ramnarine et al. (2011), before analysis, so that the only carbon remaining was organic. From each sample, 6–9 mg was precisely weighed into a tin envelope and analyzed by GC-IRMS (Carlo Erba NA 1500 elemental analyzer), yielding percent carbon and nitrogen by mass as well as $\delta^{13}C$ and $\delta^{15}N$. Percent carbon (or nitrogen) multiplied by the measured bulk density of the sample gives the mass of carbon (or nitrogen) per unit volume.

Individual pieces of plant tissue were picked out of the dried samples for radiocarbon (^{14}C) analysis at the KCCAMS ^{14}C facility at the University of California Irvine, following standard procedures. Radiocarbon estimates of the fraction of modern carbon in each sample are corrected for isotopic fractionation

using δ^{13}C measurements at the KCCAMS facility and calibrated using the OxCal tools to estimate calendar age ranges (Ramsey 2008), given 95% confidence intervals (CIs).

To construct carbon and nitrogen budgets for the deep peat deposit at El Mérito, we combined the data from those peat samples with those from 2 cores taken within 10 m of that coring location in an expedition in 2014 in which nitrogen and C$_{org}$ were measured with depth (carbon data in Ezcurra et al. 2016). Using the linear relationship found between depth and ^{14}C age in the peat from El Mérito in this study (linear regression, slope = 0.43 ± 0.05 mm yr^{-1}, intercept = 171 ± 192 mm, t = 8.6, p < 0.001), we ascertained the age of the peat samples from the nearby cores as a function of their depths. Combining these data provided a sample size of 19, rather than just the 10 from the core in this study. Assuming that the loss of nitrogen or carbon with age from peat would follow a simple exponential decay, we plotted the natural log of these sediment constituents against age and tested for a negative linear relationship.

Data analysis was conducted using R version 3.6.1 (R Core Team 2019).

2.4. DNA extraction, quantification, and barcoded amplicon sequencing

The 16S ribosomal RNA gene (16S rRNA) was analyzed to classify the diversity and community composition of archaea and bacteria present in the sediment samples. To do so, sediment from each sampling point in each core underwent DNA extraction, and the 16S genes from these DNA extracts were amplified using PCR, purified, and then sequenced.

Samples for molecular analysis were stored at −20°C and transferred in dry ice to UC Riverside, where they were analyzed. Microbial DNA was extracted using a PowerSoil DNA Isolation kit (MO BIO Laboratories) following the manufacturer's instructions and a PowerLyzer$^{®}$ 24 bench-top bead-based homogenizer (MO BIO Laboratories). A NanoDrop 2000/2000c UV-Vis spectrophotometer (Thermo Fisher Scientific) was used to quantify the DNA in soil extracts. PCR for bacteria and archaea was performed using primers that target the 16S V3 and V4 regions (S-D-Bact-0341-b-S-17 and S-D-Bact-0785-a-A-21; Klindworth et al. 2013) of the 16S rRNA gene. Microbial genomic DNA (2.5 µl) was combined with forward and reverse primer (5 µl each) and 2× KAPA HiFi HotStart ReadyMix (KAPA Biosystems) (12.5 µl). A Bio-Rad MJ Research PTC 200 Thermocycler was used to amplify all samples at one time with the following program: 95°C for 3 min; 25 cycles of 95°C for 30 s, 55°C for 30 s, and 72°C for 5 min; and hold at 4°C. AMPure XP beads (Beckman Coulter Genomics) were used to purify the 16S amplicon of primers and primer dimers. Dual indices and Illumina sequencing adapters were attached to the amplicon using the Nextera XT Index Kit (Illumina). Amplicon DNA (5 µl) was combined with 2× KAPA HiFi HotStart ReadyMix (25 µl), Index 1 and 2 primers (5 µl each), and PCR-grade water (10 µl). The same thermocycler was used with the following program: 95°C for 3 min; 8 cycles of 95°C for 30 s, 55°C for 30 s, 72°C for 30 s, and 72°C for 5 min; and hold at 4°C. A second bead cleanup was used to purify the final library before quantification. The samples were verified with gel electrophoresis after every step and then quantified in duplicate using the Quant-iT PicoGreen dsDNA assay kit (Life Technologies). All samples were pooled in equimolar concentrations and then sequenced with an Illumina MiSeq instrument.

2.5. Sequence analysis

Quantitative Insights into Microbial Ecology (QI-IME; Kuczynski et al. 2012) was used to quality filter the sequences and determine taxonomic identity against the Greengenes reference databases using 97% similarity. Using QIIME, we performed alpha diversity analyses and generated a list of microbial taxa (operational taxonomic units, OTUs) that compose the core microbiome shared across all samples or across the subset of samples consisting of peat.

Analysis of archaeal and bacterial community composition was conducted in R using the 'vegan' package (Oksanen et al. 2012, R Core Team 2019). Biodiversity of the microbial community was quantified using the form of Simpson's diversity index equal to $1 - \Sigma_{i=1}^{R} p_i^2$, where R is the total number of OTUs sequenced in a sample and p_i is the proportional abundance of the ith OTU. This index increases with the richness or evenness of the abundance distribution of OTUs.

To examine microbial community variation and to determine the relationships between community composition and sediment variables, we used a PERM-ANOVA of microbial community data using Unifrac dissimilarity and the 'adonis' function in the 'vegan' package of R (Anderson et al. 2008, Oksanen et al. 2012). First, we investigated the relationships between microbial community composition and both site and a set of sediment variables. We included depth, bulk

density, C_{org} and N densities, $\delta^{13}C_{org}$, $\delta^{15}N$, and ^{14}C age in our PERMANOVA analyses to examine whether variation in these environmental variables was associated with the variation observed in microbial community composition across samples. Next, to remove the effect of variation across sediment types (calcite, clay, peat, and sand), we restricted the PERMANOVA to test only against permutations of data from within each of the sediment types separately. Then, to remove the effect of inter-site variation across cores, we restricted the PERMANOVA to test only against permutations of data from within each of the sites separately.

We used non-metric multidimensional scaling with Unifrac to visualize microbial taxonomic variation with the sediment variables. Observing significant shifts in community composition with depth or age of peat would support the hypothesis that a particular microbial community is associated with deep, long-preserved carbon in these peat deposits.

To explore differences in community by identifying which OTUs were significantly over- or under-represented across binary groupings of the samples, the 'DESeq2' function in R was used. 'DESeq2' uses a negative binomial generalized linear model to test whether each OTU is over- or under-represented in 2 groupings of the data set (Love et al. 2014). A threshold of p = 0.05 was used to determine whether an OTU was significantly over- or under-represented.

3. RESULTS

3.1. Core description

The cores obtained from each site were qualitatively distinct from one another (Fig. 2). San José A, the *Rhizophora mangle*-dominated, low intertidal fringe core, transitioned at 94 cm depth from peat to coarse sand, with peat fragments to a maximum depth of 119 cm. In contrast, the high intertidal core at San José B was composed of

peat from the surface to the bottom at 191 cm, with the section from 131–191 cm depth also containing some clay. The San Gabriel core contained sand from the surface to 30 cm, peat from 30–54 cm, and sand from 54 to the core bottom at 59 cm. At El Mérito, the top
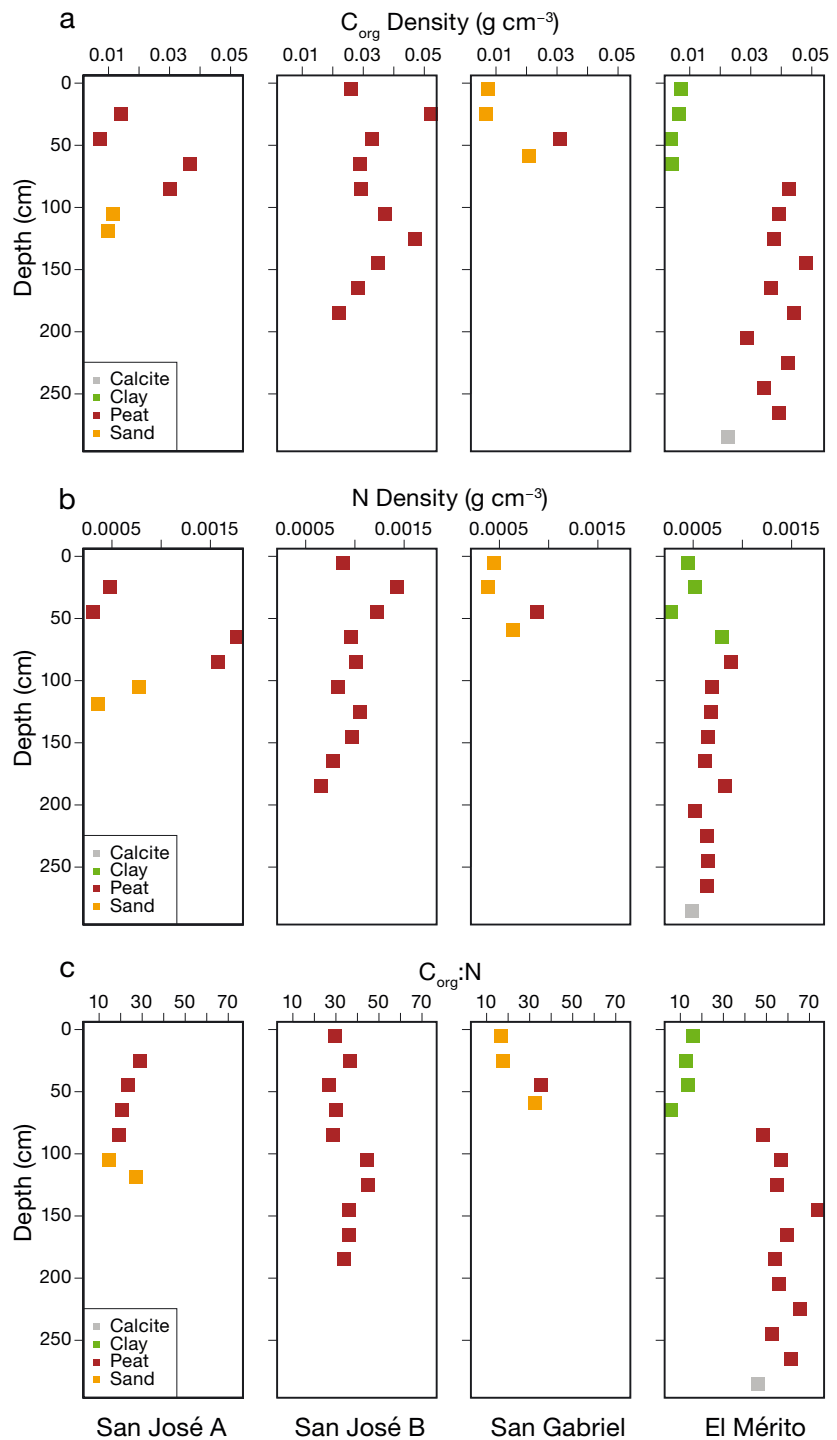

Fig. 2. Depth profiles of (a) organic carbon (C_{org}) density, (b) nitrogen density, and (c) C_{org}:N ratios from the 4 study sites. Sediment type is marked with color (legend at bottom-left)

70 cm of the core comprised a layer of gray-green clay with occasional *Avicennia germinans* roots, followed by a layer of peat from 70–278 cm, beneath which was a basement layer of limestone gravel with occasional root fragments.

3.2. C_{org}

Peat samples had higher C_{org} density (mean ± SE: $3.4 ± 0.2 × 10^{-2}$ gC_{org} cm^{-3}) than all other sediment types ($1.0 ± 0.2 × 10^{-2}$ gC_{org} cm^{-3}). The subsequent analyses of carbon and nitrogen are specifically on the peat samples. Peat C_{org} density did not vary consistently with depth across all sites (Fig. 2a), though inter-site differences in peat stratigraphy complicate comparison. For instance, peat extended from 30–54 cm depth at San Gabriel, non-overlapping with the 70–278 cm peat deposit at El Mérito. Patterns with depth and among sites, however, can still be described, especially in the 2 cores with vertically extensive peat deposits: San José B and El Mérito. Neither of these sites' peat deposits varied in C_{org} density with depth, and there was no significant difference between the C_{org} densities of the 2 sites (Welch's *t*-test, $t = -1.6$, p > 0.1).

3.3. Nitrogen

The nitrogen density of the peat averaged $8.6 ± 0.7 × 10^{-4}$ g cm^{-3}; the other sediment types averaged $5.1 ± 0.5 × 10^{-4}$ g cm^{-3}. At San José B, peat nitrogen density appeared by visual inspection to decrease with depth (least-squares fit: slope = $-2.3 × 10^{-6}$ g cm^{-4}, intercept = $1.2 × 10^{-3}$ g cm^{-3}; Fig. 2b). The nitrogen density of El Mérito's peat samples did not appear to decrease with depth as did those from San José B, and their mean value of $6.8 ± 0.3 × 10^{-4}$ g cm^{-3} was lower than that from San José B, at $9.8 ± 0.7 × 10^{-4}$ g cm^{-3} (Welch's *t*-test, $t = 3.9$, p < 0.002). One of the most notable differences among the sites' profiles was in the ratio of C_{org} to nitrogen (Fig. 2c). C_{org}:N ratios varied by site between San José B and El Mérito (Welch's *t*-test, $t = -7.7$, p < 0.001), with average ratios of 35 ± 2 at San José B and 58 ± 2 at El Mérito.

3.4. $\delta^{13}C$ and $\delta^{15}N$

The average $\delta^{13}C$ of peat deposits across all sites was $-25.1 ± 0.2‰$ (Fig. S1 in the Supplement at www.int-res.com/articles/suppl/m695p015_supp.pdf).

Those from the 2 major peat deposits at San José B and El Mérito did not appear to vary consistently with depth. However, the sample from 40–45 cm depth in San José B had an anomalously high $\delta^{13}C$ of $-22.1‰$ (>1.5× the interquartile range from the median value). San José B's peat samples had an average $\delta^{13}C$ of $-24.6 ± 0.3‰$, significantly greater than that of El Mérito at $-25.6 ± 0.1‰$ (Welch's *t*-test, $t = 3.2$, p < 0.008), regardless of whether this outlier was included.

Peat $\delta^{15}N$ averaged $5.0 ± 0.2‰$ across sites and appeared to increase with depth when data from across all sites were combined though, again, there were inter-site differences (Fig. 3a).

3.5. Radiocarbon dating

As peat accumulates vertically over time, the age of the tissues making up the peat deposits increases with depth. Inter-site differences are noteworthy (Fig. 3b). At San José A and B, the peat layer beginning from the surface is of consistently modern age (from since 1950 CE) extending downward at least 85 cm at San José A and 45 cm at San José B. Beyond these depths, peat increases in age with depth, indicating successive accumulation over time. San Gabriel's shallow subsurface peat deposit from 30–54 cm depth is not modern, but dates to 754 ± 18 yr before present (BP). At El Mérito, the surface clay layer that extends down to 70 cm is consistently modern down to the 60–65 cm sample, the age of which is 5243 ± 122 yr BP, matching that of the calcite beneath the peat deposit, 5263 ± 124 yr BP.

3.6. Microbial communities

The microbial community varied with depth (PERMANOVA, p < 0.001), site (p < 0.001; Fig. 4a), and sediment type (p < 0.001; Fig. 4b). Simpson's diversity index decreased with age in peat samples (linear regression, slope = $-2.10 ± 0.27 × 10^{-6}$ yr^{-1}, intercept = 0.998, $t = -7.7$, $R^2 = 0.72$, p < 0.001; Fig. 5). To reveal the roles of factors that may covary with sediment type, we removed the effect of sediment type from the PERMANOVA and found that depth (p < 0.001), ^{14}C age (p < 0.001), C_{org}:N ratio (p < 0.001), and $\delta^{15}N$ (p = 0.006) were significant factors predicting variation in the microbial community. Similarly, when we removed the site effect from the PERMANOVA, we found that depth (p < 0.001), ^{14}C age (p < 0.001), C_{org}:N ratio (p < 0.001), and $\delta^{15}N$ (p = 0.008) were
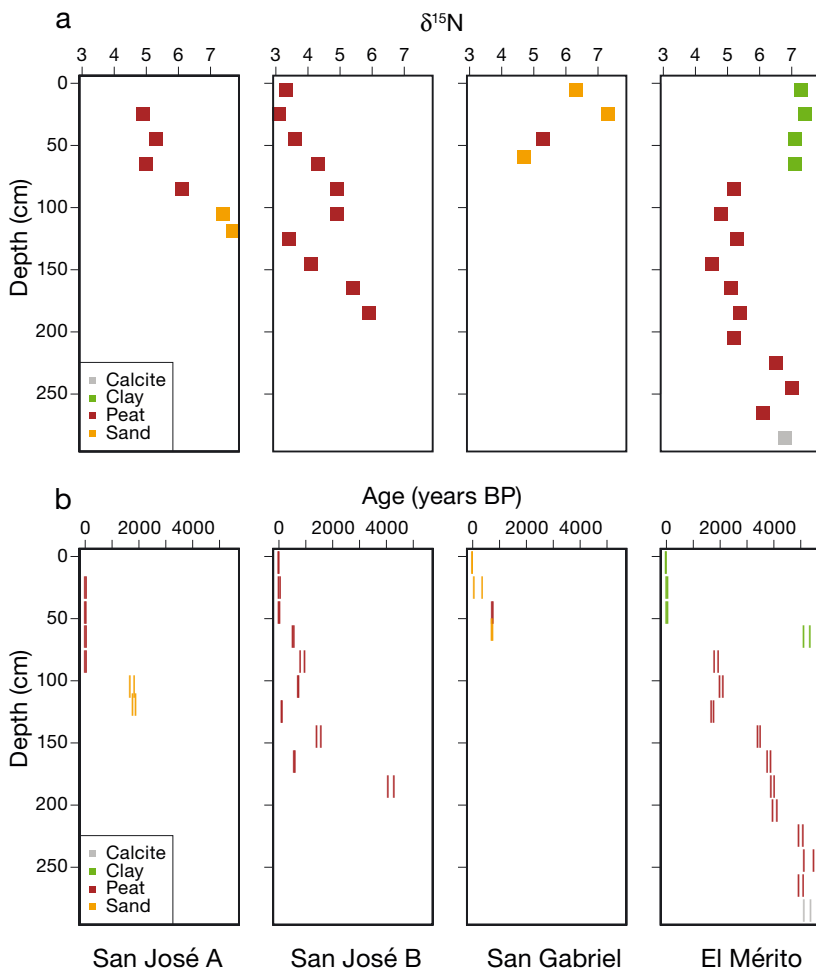
Fig. 3. Depth profiles of (a) $\delta^{15}N$ and (b) calendar age in years before present (BP) from the 4 study sites. Sediment type is marked with color (legend at bottom-left). Age ranges represent 95% CIs

again significant factors; in addition, bulk density (p < 0.001) and C_{org} density (p = 0.025) were also significant factors, suggesting that these factors underlie the effect of site on the microbial community.

3.7. Microbial community description

Across all samples, 65 521 OTUs were obtained in the study. The 10 most abundant OTUs counted, averaged across all samples, are given in Table 1. All OTUs identified in this study to the phylum level came from 74 phyla, and the 4 most abundant phyla (in decreasing order) across samples were *Chloroflexi*, *Protobacteria*, *Planctomycetes*, and *Crenarchaeota*. The distribution of these major microbial phyla shows variation across all samples grouped by sediment type (Fig. 6a), and within peat samples only, grouped by ^{14}C age bins (Fig. 6b).

In a DESeq2 analysis of microbial taxa over- or under-represented in peat vs. non-peat samples, 1141 OTUs were overrepresented in peat samples and 132 underrepresented (Table S1). In an analysis of peat samples only, grouped into bins of samples from <1000 yr BP (n = 13) and those from
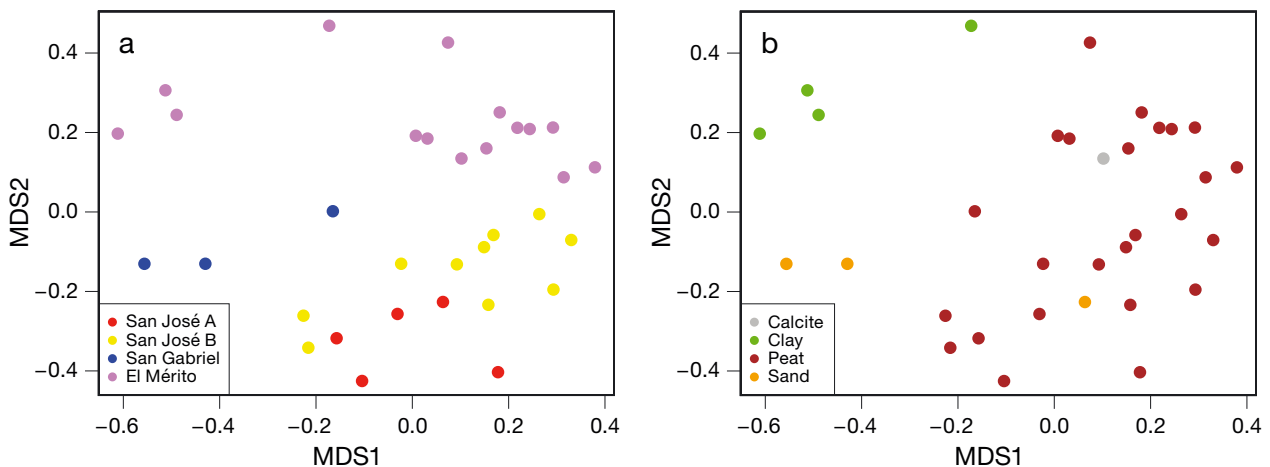

Fig. 4. Nonmetric multidimensional scaling (NMDS) plot of 16S sequencing results from all samples, showing bacterial and archaeal community variation across (a) sites and (b) sediment types. Point colors indicate sites in (a) and sediment types in (b); more closely clustered points indicate greater similarity among the samples' communities
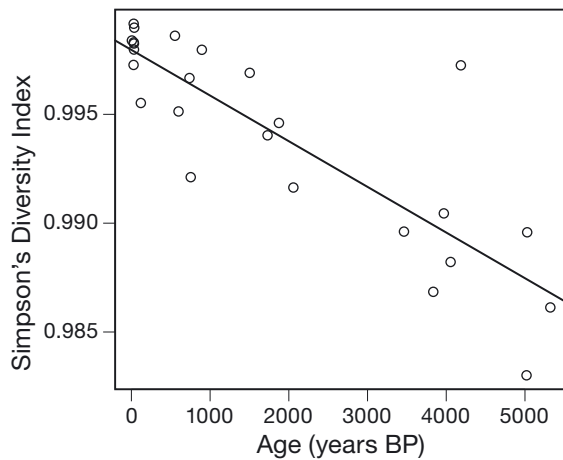
Fig. 5. In peat samples, Simpson's diversity index — a measure of bacterial and archaeal operational taxonomic unit richness and evenness — plotted against increasing ^{14}C age (linear regression, slope = $-2.10 \pm 0.27 \times 10^{-6}$ yr^{-1}, intercept = 0.998, $t = -7.7$, $R^2 = 0.72$, p < 0.001)

≥ 1000 yr BP (n = 12), 1724 OTUs were overrepresented in the younger bin and 323 were underrepresented (Table S2).

4. DISCUSSION

4.1. Peat formation at each mangrove site

Inter-site variability in this study revealed how environmental setting influences the formation of mangrove peat deposits. Though rarely acknowledged in the literature, variation in peat deposits within and among local estuaries appears to be the norm rather than the exception, at least in the mangroves of Baja California Sur (Costa 2014). The variation distinguishing the 4 sites in this study, separated by only a few 10s of km, underscores this point.

Table 1. The 10 most abundant operational taxonomic units (OTUs), in terms of average counts across all samples are shown. Taxonomic ranks are shown to family (some taxonomic ranks are left blank because not all OTUs were identified past the phylum level)

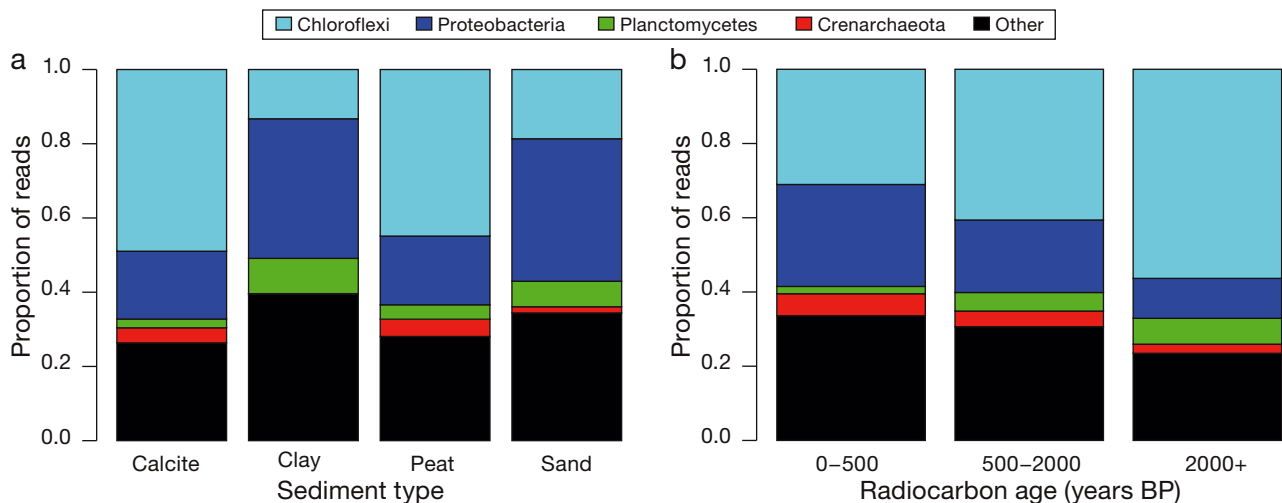| OTU ID	Kingdom	Phylum	Class	Order	Family	Average OTU count
550479	*Bacteria*	*Chloroflexi*	*Dehalococcoidetes*	GIF9		2249
784233	*Bacteria*	*Chloroflexi*	*Dehalococcoidetes*	*Dehalococcoidales*	*Dehalococcoidaceae*	1895
New.ReferenceOTU1484	*Bacteria*	*Chloroflexi*	*Dehalococcoidetes*	*Dehalococcoidales*	*Dehalococcoidaceae*	1590
4296752	*Bacteria*	*Chloroflexi*	*Dehalococcoidetes*	*Dehalococcoidales*	*Dehalococcoidaceae*	928
274098	*Bacteria*	*Chloroflexi*	*Anaerolineae*			827
133584	*Bacteria*	CD12				769
New.ReferenceOTU1710	*Bacteria*	*Chloroflexi*	*Dehalococcoidetes*	*Dehalococcoidales*	*Dehalococcoidaceae*	715
New.ReferenceOTU1067	*Bacteria*	*Actinobacteria*	*Actinobacteria*	WCHB1-81	At425_EubF1	712
4360500	*Bacteria*	*Chloroflexi*	*Dehalococcoidetes*	*Dehalococcoidales*	*Dehalococcoidaceae*	625
199002	*Bacteria*	*Proteobacteria*	*Deltaproteobacteria*			551

Fig. 6. Proportion of operational taxonomic units (OTUs) from (a) each of the 4 most abundant phyla in all samples, grouped by sediment type (n = 33), and (b) from those phyla in peat samples only, grouped by age bins of 0–500, 500–2000, and 2000+ yr before present (BP) (n = 25)

San José A, on a low-intertidal creek fringe dominated by *Rhizophora mangle*, seems to represent a young stage of mangrove deposit development, with about 1 m of modern peat overlying an older sandy layer (Fig. 3b). The fact that almost the entire surface meter of peat was of 20th century age highlights an important point for the interpretation of these data. Peat deposits formed from mangrove roots are not 'sediment'; i.e. their source material does not settle in layers above marginally older material. As a result, the records of organic matter age in mangrove peat deposits can be vertically mixed over an interval equivalent to the depth range within which mangrove roots grow. In *R. mangle*, most fine roots are distributed within a few cm of the surface, preventing the passage of surface detritus into the belowground zone where peat is formed (Covington & Raymond 1989). Ezcurra et al. (2016) discussed the role that species differences in root growth patterns play in the belowground formation of peat. In this study, the 45–85 cm of surface peat of modern age seen at both San José sites (followed at the older B site by an increase, with 2 anomalously young samples, in age with depth), supports the idea that there is a near-surface peat formation zone in which live roots grow among dead detritus.

Mangrove sedimentary deposits have shown a history of accretion in accommodation of sea-level rise (SLR) over the Holocene Epoch (Toscano & Macintyre 2003, McKee et al. 2007). This accretion moves the sediment surface upward, along with the depth range of root growth, adding material from the bottom of this zone to the peat deposit, forming a temporal sequence now visible in the ^{14}C record. Much of the physical compaction of the peat likely occurs at this early stage of deposition, as the mixed, actively growing surface peat can be much less consolidated than the deeper part of the peat deposit. For instance, the 2 most shallow samples from the San José A core have much lower C_{org} density values than the deeper peat samples from that core (Fig. 2a) due to their much lower bulk densities (mean bulk densities: 0.055 and 0.553 g cm^{-3} respectively). These observations on the surface zone of uncompacted, still-forming peat with some roots penetrating 10s of cm deep can inform the use of mangrove peat records for the reconstruction of SLR or other environmental change, indicating the importance of focusing on deeper deposits and dating thoroughly with depth.

The deep peat deposit at El Mérito presents a relatively simple case of peat accumulation, without any anomalously young peat ^{14}C dates. In fact, the age of the deepest peat sample in this core, 5029 ± 85 yr BP, is the oldest mangrove peat core measured in the region. This site possesses the interesting feature of a capping layer of clay above the peat, from which it is distinctly demarcated. The 5243 ± 122 yr BP age of the bottom of this clay layer suggests that it has lain atop the growing peat deposit for millennia, further supporting the conception of these peats as forming fully belowground rather than accreting from the surface (see Ezcurra et al. 2016). In this site, it appears that clay sediment has gradually been added at the surface, while belowground the mangrove peat deposit expanded upward. The 1875 ± 72 yr BP age of the top of the peat deposit suggests that at this location, peat stopped accumulating almost 2 millennia ago. The dwarf *Avicennia germinans* trees currently growing at this location apparently are contributing to the minor fraction of organic matter in the clay layer, as their roots reach about 0.5 m down into the clay, seen in the modern organic matter dates from the clay samples from the surface to 40–45 cm depth (Fig. 3b), but they apparently do not reach through the bottom of the clay into the established peat deposit, of much greater age.

4.2. Carbon and nitrogen pool characterization

Carbon densities do not decrease with depth. It was hypothesized that deeper, older peat samples exposed longer to microbial decomposition would possess decreased carbon densities. This interpretation assumes that the C_{org} and nitrogen of the source material has not changed over time, supported by the highly stable δ^{13}C of peat over time. An additional assumption is that changes in C_{org} density are due to loss of organic matter to decomposition. A decrease in C_{org} density due to decomposition could theoretically be counterbalanced by an increase in bulk density due to compaction. The lack, however, of a change in slope in the depth–age relationship in the peat deposit at El Mérito, the most vertically extensive in this study (Fig. 3b), suggests that there is not significant compaction after initial peat formation in this environment. Thus, assuming peat compaction to be insignificant, the lack of a pattern of C_{org} density with age suggests that organic matter loss is so slow as to be undetectable. There is, however, a negative relationship between peat deposit nitrogen density and depth, especially at San José B, suggesting that peat nitrogen may be more sensitive to microbial processes than carbon, but within-site replication is required to confirm this trend.

The most salient difference among sites can be seen in the ratio of C_{org} to nitrogen in the 2 sites with peat deposits of significant depth, San José B and El Mérito (Fig. 2c), whose ratios are 35 ± 2 and 58 ± 2 respectively. The markedly higher peat C_{org}:N ratio at El Mérito than at the other sites might suggest a difference in the stoichiometry of the source. Mangrove detritus can vary in C:N ratio due to variation in nutrient limitation status (Feller et al. 2002) or by species, with *R. mangle* detritus tending to have higher C:N ratios than that of *A. germinans* (Twilley et al. 1996). El Mérito is (at least today) dominated by *A. germinans*, whereas *R. mangle* is more prevalent at the other sites, which would (if anything) decrease, rather than increase, the C_{org}:N ratio at this site if source drove this variation. In addition, the $\delta^{13}C$ value of the peat from San José B and El Mérito are −24.6 ± 0.3 and −25.6 ± 0.1 respectively, not signaling a drastically different source. The higher C_{org}:N ratio of the El Mérito peat may instead indicate preservation conditions distinctive to this site. The presence at El Mérito of a layer of clay above the peat for the entirety of its history sets this core apart. The physical characteristics of this cap of clay may affect the availability of electron acceptors for microbial respiration, shifting the balance of nitrogen cycling processes including denitrification and annamox (Cornwell et al. 1999), resulting in greater nitrogen loss during peat formation than at other sites. These results suggest that conditions near the surface may influence the material that passes into long-term storage beneath the zone of peat formation. It should be noted that acid fumigation, used in this study, has been observed to affect measurements of C:N and $\delta^{15}N$ (+1 to 2 for C:N, Brodie et al. 2011; +0.1‰ for $\delta^{15}N$, Harris et al. 2001), but the artificial effects are an order of magnitude lower than the variation uncovered in this study (Figs. 2a & 3c). While caution should be taken in interpreting the results, the major trends are not due to methodological artifacts.

The $\delta^{13}C$ of peat samples were consistent across sites and did not appear to vary with depth. The −25.1 ± 0.2 average value is within the range of $\delta^{13}C$ values observed in C3 plants, including the local mangrove taxa (Andrews et al. 1984). The anomalously high $\delta^{13}C$ value of −22.1 at 40–45 cm depth at San José B may indicate that the organic matter in this section of the peat record contained a significant component of algal biomass or other coastal plants of the region, typically higher in $\delta^{13}C$. There is no evidence of a shift toward higher $\delta^{13}C$ with depth due to preferential remineralization of isotopically light carbon, contrary to our hypothesis.

The cycling of nitrogen between organic and mineral forms is central to the maintenance of productivity in mangrove ecosystems (Alongi et al. 1992). It is likely for this reason that the $\delta^{15}N$ record seems to be more sensitive to processes of microbial turnover than that of $\delta^{13}C$. Combining the samples from the 2 cores with significantly deep peat deposits, $\delta^{15}N$ has a negative linear relationship with age (linear regression, slope = 4.6 ± 0.7 × 10^{-4}‰ yr^{-1}, intercept = 3.9 ± 0.2‰, t = 6.2, R^2 = 0.68, p < 0.001; Fig. 7), supporting our hypothesis. The y-intercept of this fit, indicating the $\delta^{15}N$ at the time of burial, is 3.9 ± 0.2‰. Theoretically, $\delta^{15}N$ could shift over the record due to a shift in source material, and mangrove biomass $\delta^{15}N$ has been shown to drop under nitrogen limitation (McKee et al. 2002). Research in the Gulf of California, however, suggests that the region's mangroves are generally phosphorus-limited (Vazquez et al. 2000, Sánchez-Carrillo et al. 2009). In addition, a source $\delta^{15}N$ of 3.9 ± 0.2‰ is at the high end of reported $\delta^{15}N$ values for mangrove sediment (Reis et al. 2017), so the values >6.0‰ for the older peat samples are unlikely to be due to variation in source. Furthermore, the unchanging $\delta^{13}C$ values with age suggest an unchanging source. Further research could use other biomarkers to explore whether a shift from a marine upwelling to an N-fixation source of nitrogen could alternatively explain the change in $\delta^{15}N$ observed. Assuming that this trend is diagenetic, the increase in peat $\delta^{15}N$ is likely due to microbial activity. Bacterial growth on mangrove detritus enriches it in bacterial nitrogen, which tends to have higher $\delta^{15}N$ than that in plant biomass (Alongi et al. 1992). In addition, nitrogen is isotopically fractionated when it
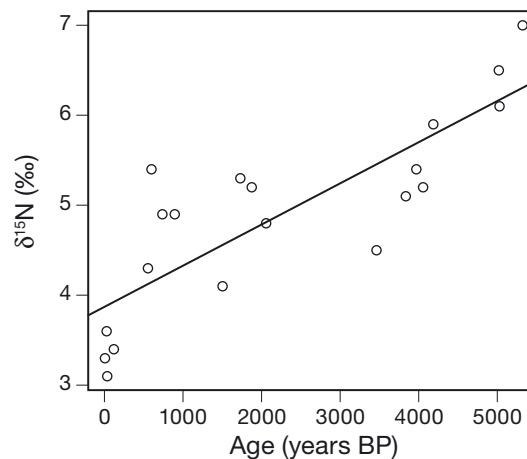

Fig. 7. $\delta^{15}N$ vs. calendar age (in years before present) of peat samples from San José B and El Mérito, fit with a linear regression (slope = 4.6 ± 0.7 × 10^{-4}‰ yr^{-1}, intercept = 3.9 ± 0.2‰, t = 6.2, R^2 = 0.68, p < 0.001)

undergoes microbial processes including anammox and denitrification, resulting in the preferential release of ^{14}N as gas and retention of ^{15}N in organic matter (Reis et al. 2017). Thus, while C_{org} seems unchanged over millennia, microbial activity may be affecting the nitrogen pool.

4.3. Microbial community composition

Carbon and nitrogen cycling in mangrove sediments are largely microbial processes, so mapping the microbial communities in this study helps illuminate the dominant biogeochemical processes at work in this carbon-sequestering environment. Across all samples, 6 of the 10 most abundant OTUs belonged to the class *Dehalococcoidetes*, in the phylum *Chloroflexi* (Table S1). The *Dehalococcoidetes* contain taxa capable of sulfate reduction and of anaerobic dehalogenation of aliphatic organic compounds (Kaster et al. 2014). These and other refractory compounds in woody mangrove detritus comprise much of the peat organic matter pool available for microbial heterotrophy. Poor substrate quality and anoxia create an environment suitable for a microbial assemblage that is rare in other settings, where more efficient metabolic pathways allow other taxa to dominate. The other OTUs among the 10 most abundant included another *Chloroflexi* class, *Anaerolineae*, known from marine sediments and extreme, anoxic environments (McIlroy et al. 2017); a member of the CD12 or '*Aerophobetes*' phylum, associated with anoxic marine sediments (Wang et al. 2016b); an actinobacter known from gas hydrates in the Gulf of Mexico (Lanoil et al. 2001); and a deltaproteobacter.

Microbial community variation across sediment types reveals adaptation to the metabolism of recalcitrant peat under anoxia, particularly in more ancient peat samples. The distribution of microbial phyla varied markedly by sediment type (Fig. 6a). While surface samples of sand or clay were dominated by *Proteobacteria*, peat samples and calcite (below the peat deposit at El Mérito) possessed greater abundance of the Phylum *Chloroflexi*, which contains the *Dehalococcoidetes*. The *Crenarchaeota* were also more well represented in peat and calcite samples than surface samples. Within peat samples, a trend of increasing representation of *Chloroflexi* and *Crenarchaeota* was seen with increasing age (Fig. 6b), suggesting that this community is associated with ancient, recalcitrant organic matter in well-preserved peat deposits.

Of the 1141 OTUs overrepresented in peat samples, the most common phylum was *Chloroflexi*, and

the most common family was the *Chloroflexi* family *Dehalococcoidaceae*. Not only do peat samples harbor a distinct community, but this community becomes further specialized as peat deposits age, as evidenced by the fact that 2047 OTUs were significantly over- or under-represented in peat samples less than 1000 yr old vs. older samples (Table S2). The *Chloroflexi*, associated with peat samples in general, was also the most common overrepresented microbial phylum in peat samples ≥1000 yr old. The traits of *Chloroflexi*, and especially *Dehalococcoidaceae*, of sulfate reduction and dehalogenation of aromatic compounds support their potential role as consumers of recalcitrant, ancient peat organic matter in these anoxic sediments. Archaeans were overrepresented in peat samples as well, with 36 OTUs belonging to the Phylum *Crenarchaeota*, mostly classified in the MCG, or Miscellaneous *Crenarchaeota* Group, a little-understood group known from anoxic sediments which may include taxa capable of breaking down aromatic compounds (Fillol et al. 2016). The relative dominance of these taxa in these peats indicates the importance to their biogeochemistry of normally sidelined metabolic pathways of recalcitrant organic matter degradation.

The most common phylum among OTUs underrepresented in peat samples was *Proteobacteria*, including many families of bacteria, but the single most common family among OTUs underrepresented in peat samples was the *Pirellulaceae*, from the *Planctomycetes* phylum. Known for ammonium oxidation in marine environments (Mohamed et al. 2010), members of the *Pirellulaceae* may benefit from the less-reducing conditions in near-surface sand and clay deposits to utilize the ammonium released from the metabolism of sediment organic matter.

4.4. Microbial community variability

We uncovered measurable structure in the bacterial and archaeal community in the mangrove sediments. Community composition varied significantly with depth and by site and sediment type (Figs. 4 & 5). This result supports the hypothesis that different sediment materials vary in their microbial communities in these mangroves (Haldar & Nazareth 2018, Behera et al. 2019). Given the particular cores taken in this study, some sediment types are exclusively or predominantly found at only certain sites, requiring further analysis to disentangle the statistical effects of these factors. In a PERMANOVA in which the effect of sediment type was removed, the C_{org}:N ratio

emerged as a significant predictor of community composition. As discussed above, C_{org}:N was significantly different between the large peat deposits of San José B and El Mérito, so this factor captures important differences between sites. The effects of sediment stoichiometric factors such as C_{org}:N on the microbial community are likely the proximate drivers of the detected site effects. Sediment type, however, can influence microbial community directly via physical and chemical conditions. In a PERMANOVA removing the site effect, bulk density and C_{org} density emerged as predictors. These 2 factors were among the most obvious differences between peat and the other sediment types, with peat having lower bulk density and higher carbon content. Other effects of sediment type on microbial communities may be important, as, for instance, sand has greater relative pore volume than clay, affecting habitat characteristics and the diffusion of oxygen, organic matter, and nutrients.

Depth is also associated with variation in the microbial community, along with ^{14}C age and δ^{15}N. Age increases with depth in peat, and δ^{15}N increases with peat age (Fig. 7), so all 3 of these factors show collinear behavior in this data set. Thus, these results do not reveal separate effects of depth, age, and δ^{15}N on the microbial community. Rather, the latter 2 variables reflect physical changes to the peat over time, for which depth is a proxy. Depth has been shown to structure mangrove sediment microbial activity, with depth variation in dehalogenation and methane oxidation (Pan et al. 2017, Zhang et al. 2018). Specifically, we hypothesize that the microbial community shifts with peat age in response to the gradual change in the character of the peat organic matter substrate. Peat δ^{15}N increases with age, reflecting microbial nitrogen processing in which nitrogen is repeatedly broken down and reassimilated into microbial biomass, with the remineralization and loss processes incurring an isotopic fractionation in favor of ^{14}N. The decrease in Simpson's diversity index with age reflects a decrease in richness and evenness as peat ages. In still-forming, modern peat near the surface, as seen at San José, we hypothesize that there exists a broader array of microbial functional groups capable of consuming organic matter over a range of lability in a more spatially complex and bioturbated environment with some patchy availability of oxygen and inorganic nutrients. Deeper in the peat, only a community of specialists, including slow-growing sulfate-reducers and consumers of recalcitrant organic matter, are able to dominate, reducing microbial diversity. These hypotheses could be further tested by measurements of the activities of specific microbial metabolic processes across the age-associated gradients in peat deposits (Zhang et al. 2018).

4.5. Carbon and nitrogen pool dynamics

Attempts to estimate budgets of carbon or nitrogen into or out of mangrove ecosystems have generally used proxies of mangrove productivity such as leaf litterfall rate and derived estimates of ecosystem burial efficiency (Duarte & Cebrián 1996, Bouillon et al. 2008); direct measurements of fluxes of gasses or solutes across sediment, water, and air boundaries (Alongi et al. 2004); and measurements of soil composition and surface sediment accretion measured by sedimentary radioisotopes (Chmura et al. 2003). These approaches face several limitations. The biology of mangrove root growth, exudation, and turnover is an active area of research with much still unknown (Bouillon et al. 2008, Adame et al. 2017), but these processes are of central importance given the fact that mangrove peat deposits are mainly composed of dead fine roots (McKee et al. 2007, Xiong et al. 2017) rather than accumulated leaf litter. In addition, gas and tidal flux measurements, while valuable for providing current estimates of elemental fluxes, are necessarily snapshots of growth and decomposition processes that play out over centuries and likely miss the spatio-temporal patchiness of mangrove gas fluxes due, for instance, to the hotspots of respiration associated with mangrove aerial roots and crab burrows (Kristensen et al. 2008, Wang et al. 2016a, Martin et al. 2020). Finally, studies of carbon burial based on near-surface carbon densities and accretion estimated from the decay of radioisotopes such as ^{137}Cs and ^{210}Pb generally assume a surface source of carbon that passes into storage as it moves past the sediment surface, when in fact mangrove roots add biomass to the soil and height to the sediment column (McKee et al. 2007). The fact that the top 45−85 cm of peat in the San José cores from this study were well mixed with regard to C_{org} age implies that measuring carbon burial and sediment accretion from the surface down would lead to an underestimate of carbon accumulation rate by decreasing the slope of the age−depth relationship. Long-term burial of carbon begins at the bottom margin of this zone, deeper than the depths to which many sedimentation studies core. In contrast, measuring the composition and ^{14}C profile of the sediment to its maximum depth allows for the estimation of fluxes of material into and out of the peat deposit. Given that long-term storage on the

centuries- to millennia-scale is what sets apart blue carbon ecosystems (Mitra et al. 2005), creating budgets of carbon, nitrogen, etc. for their deep peat pools is of great relevance to the study of their role as carbon sinks.

In the peat deposit at El Mérito, there was a statistically significant negative relationship between ln(N density) and age (linear regression, slope = −7.13 ± 3.04 × 10^{-5}, intercept = −6.95 ± 0.11, t = −2.3, R^2 = 0.24, p = 0.031; Fig. 8). Exponentiating the intercept gives the inferred density of nitrogen in sediment at the time of burial, 9.6 × 10^{-4} gN cm^{-3} (95% CI: 8.6 × 10^{-4}–1.07 × 10^{-3} gN cm^{-3}). This value, multiplied by the linear accretion rate of peat measured at this site of 0.474 mm yr^{-1}, gives the rate of nitrogen burial over the course of this deposit's formation, 4.1 ×

10^{-5} gN cm^{-2} yr^{-1}, equal to 4.1 × 10^{-3} MgN ha^{-1} yr^{-1} (95% CI: 3.7 × 10^{-3}–4.6 × 10^{-3} MgN ha^{-1} yr^{-1}). The exponential decay equation $N = N_0(1 - e^{d/t})$ gives the amount of nitrogen lost from the peat deposit per year, where N_0 is the depth-integrated nitrogen pool of the deposit, d is the exponential decay rate, and t equals 1 yr. Integrating nitrogen density with depth across the peat samples at El Mérito from this study gives a value of $N_0 = 0.0312$ gN cm^{-2}, and the slope of the regression of ln(N density) against age gives a value of $d = -7.13 \pm 3.04 \times 10^{-5}$ yr^{-1}. Using these values in the exponential decay equation gives an annual loss of nitrogen from the deposit of 2.2 × 10^{-6} gN cm^{-2} yr^{-1}, equal to 2.2 × 10^{-4} MgN ha^{-1} yr^{-1} (95% CI: 1.3 × 10^{-4}–3.2 × 10^{-4} MgN ha^{-1} yr^{-1}), roughly 5% of the estimated annual burial rate of 4.1 × 10^{-3} MgN ha^{-1} yr^{-1} (95% CI: 3.7 × 10^{-3}–4.6 × 10^{-3} MgN ha^{-1} yr^{-1}), resulting in a positive net burial rate, representing the long-term balance of many nitrogen loss, gain, and transformation processes happening in the ecosystem (rates of various processes reviewed in Table 1 of Reis et al. 2017). This slow loss of nitrogen and increase in $\delta^{15}N$ with age (Fig. 7) suggests that microbial cycling of nitrogen derived from organic matter in these peat deposits results in the gradual release of remineralized nitrogen or DON, with an isotopic preference for the retention of ^{15}N.

Unlike for nitrogen, ln(C_{org} density) shows no significant linear relationship with age (linear regression, t = −0.12, p > 0.1; Fig. 8b). Though we observed a significant exponential decay in peat nitrogen but not in carbon with age, this decoupling is extremely slow, as can be seen from the apparent stability of the C_{org}:N ratio of the peat deposits at San José B and El Mérito with depth (Fig. 2c). The lack of a detectable change in peat composition inferred from these stable C_{org}:N ratios even after millennia reinforces the conception of mangrove peats as highly effective carbon sinks, where decomposition is slowed virtually to zero. With the loss of C_{org} from this system apparently negligible, we can only estimate the annual gain of carbon via burial. This burial rate is estimated by the product of the mean C_{org} density of the peat, 3.93 ± 0.17 × 10^{-2} gC_{org} cm^{-3}, and the accretion rate, 0.427 mm yr^{-1}, equal to 1.7 ± 0.1 × 10^{-3} gC_{org} cm^{-2} yr^{-1}, or 1.7 ± 0.1 × 10^{-1} MgC_{org} ha^{-1} yr^{-1}. This long-term sequestration rate is an order of magnitude lower than the average in Mcleod et al. (2011) based on studies estimating carbon accumulation in near-surface sediments, suggesting the importance of carbon cycling and loss processes during initial peat formation. Because this deposit is no longer forming peat (see Fig. 3b, where the youngest peat age is

Fig. 8. (a) In peat samples from El Mérito, the ln of sediment nitrogen density (g cm^{-3}) is plotted against calendar age of peat (linear regression, R^2 = 0.24, slope = −7.13 ± 3.04 × 10^{-5}, intercept = −6.95 ± 0.11, t = −2.3, R^2 = 0.24, p = 0.031). The dotted line indicates the extrapolation to the intercept, when age = 0 yr before present (BP), to infer the density of N of sediment at the time of burial. (b) The ln of sediment carbon density is plotted against peat age

1875 ± 72 yr BP), this rate refers to the burial during the period when peat was forming, from 5029 ± 85 to 1875 ± 72 yr BP.

As with other methods of estimating fluxes of carbon or nitrogen into or out of mangrove systems, this approach has limitations, the most important of which is that it assumes constant carbon and nitrogen composition and static rates of peat accumulation and decomposition over time. There is no evidence that the species or general climatic conditions of mangroves in this region have deviated significantly over the late Holocene, so large changes in the composition of peat material buried are not expected. The assumption of static accumulation is supported by steady rates of SLR during recent millennia reported in Toscano & Macintyre (2003) and Ezcurra et al. (2016), in addition to the rate estimated in this study. Given the apparent recalcitrance of the organic matter, slow course of change in the nitrogen pool and microbial community with age, and apparent isolation from surface processes of this peat, it is reasonable to assume that decomposition rates do not fluctuate considerably in this kind of steadily accumulating deposit.

We note that the long-term estimate of accretion rate and thus inferred SLR measured in this study at El Mérito of 0.43 ± 0.05 mm yr^{-1} does not agree with that of 0.70 ± 0.07 mm yr^{-1} estimated in Ezcurra et al. (2016), even though these values were estimated from mangrove peat deposits in the same region. This pattern may reflect the El Mérito core coming from higher in the tidal frame than those used to estimate the rate in Ezcurra et al. (2016). It is difficult to draw further conclusions with the present data, as these accretion rate estimates all reflect average rates of accretion over many centuries, during which a given location may have shifted its place in the tidal frame, as for instance the El Mérito core seems to show progression from subtidal, high-energy gravel bottom to *R. mangle* swamp to high intertidal mudflat partially colonized by *A. germinans*. Further work to measure accretion rates in relation to tidal elevation in other coastal environments on the Baja Peninsula would provide insight into whether sedimentation has generally tracked relative SLR in a consistent manner in this tectonically active region.

4.6. Implications and future work

We have shown that sediment microbial communities vary with depth and sediment characteristics in peat deposits, with diversity trends reflecting a community response to organic matter age. These descriptions are only the beginning. Further work will capture the role of other microbial groups, including fungi, which are known in mangroves to contribute to leaf litter enrichment (Fell et al. 1975) and phosphorus solubilization (Vazquez et al. 2000), while their role in mangrove carbon cycling is an area of ongoing research (Alongi & Sasekumar 1992, Luis et al. 2019). The increasing availability of molecular tools to examine patterns of microbial diversity in mangroves and other ecosystems brings with it opportunities for process-based studies estimating rates of bacterial heterotrophic production, nitrogen and carbon remineralization, and other processes. Though performing experimental manipulations of these microbial processes in mangrove ecosystems and detecting their effects poses challenges, results from such studies could provide the mechanistic understanding necessary to use this information for the protection and management of mangrove ecosystems (Allard et al. 2020).

Microbe–plant–soil interactions have been recognized for their influence on fluxes of carbon and nutrients in mangroves (Alongi et al. 1993, Spivak et al. 2019), and the goal of elucidating these mechanisms takes on clear value in light of the urgent need to maintain or to increase natural carbon sinks under climate change. Beyond carbon sequestration, capturing the functional role of microbes in mangrove ecosystems has implications for other important functions of these forests. The outwelling of mangrove leaf litter has long been considered a major source of carbon for coastal food webs (Odum & Heald 1975), while recent research suggests that mangrove POC and DOC may be much more important in that role (Kristensen et al. 2008, Santos et al. 2021). Microbial metabolism strongly affects the nutritional quality of litter exported and is a major conduit whereby mangrove carbon passes into the particulate and dissolved pools (Odum & Heald 1975, Holguin et al. 2001). In addition to their long-recognized food web roles, mangrove sediment microbes have now been shown to break down microplastics in the environment (Auta et al. 2018), a topic of growing concern in marine ecosystems.

A complex interplay of environmental dynamics, microbial community development, and plant growth, death, and decay set the conditions for carbon burial in mangrove peat deposits. This work shed light on several of the phenomena at play in this process. The ^{14}C profiles at San José demonstrated that actively forming peat deposits are well-mixed with regard to carbon accumulation (the quantity of interest for blue

carbon) in the top 45–85 cm due to active root growth. It is only below this point that 'dead' peat accumulates successively beneath the root zone, left behind as the live roots shift upward with vertical peat accumulation. The role of subsurface root growth in influencing elevational changes in mangroves is further demonstrated at El Mérito, where ^{14}C dating of the peat and overlying clay suggest that belowground root and peat layers had expanded vertically independent of surface fine sediment accretion for millennia until mangrove peat formation ceased. These observations demonstrate that realistic carbon accumulation rates can be calculated based on carbon and age measurements specifically over the range of depths over which peat age increases with depth, not simply starting from the surface. Alternative approaches to estimating the budget of carbon, nitrogen, or other constituents of wetland sediments can be constructed from deep, high-resolution sampling as done in this study. In this case, we detected no loss of carbon from the peat over more than 3000 yr, underscoring the value of these blue carbon ecosystems for mitigating carbon emissions, but did estimate a slow loss of nitrogen, 2.2×10^{-4} MgN ha^{-1} yr^{-1} (95% CI: 1.3×10^{-4}–3.2×10^{-4} MgN ha^{-1} yr^{-1}). These biogeochemical processes are carried out by a microbial community structured by sediment properties and peat age, with assemblages in ancient peats that seem to be specialized for sulfate reduction and the breakdown of recalcitrant organic matter under anoxic conditions. More detailed investigation into the metabolic activity of these microbial groups will provide more insight into mangrove element cycling, with the possibility of developing methods to manage these ecosystems to increase their carbon sink capacity. Thus, integrative analysis of peat biogeochemistry and microbial ecology with depth in mangrove sediments offers valuable opportunities for understanding the ecology and ecosystem services of these wetlands.

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