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ORIGINAL ARTICLE

Initial insights into bacterial succession during human decomposition

Embriette R. Hyde · Daniel P. Haarmann · Joseph F. Petrosino · Aaron M. Lynne · Sibyl R. Bucheli

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Abstract Decomposition is a dynamic ecological process dependent upon many factors such as environment, climate, and bacterial, insect, and vertebrate activity in addition to intrinsic properties inherent to individual cadavers. Although largely attributed to microbial metabolism, very little is known about the bacterial basis of human decomposition. To assess the change in bacterial community structure through time, bacterial samples were collected from several sites across two cadavers placed outdoors to decompose and analyzed through 454 pyrosequencing and analysis of variable regions 3–5 of the bacterial 16S ribosomal RNA (16S rRNA) gene. Each cadaver was characterized by a change in bacterial community structure for all sites sampled as time, and decomposition, progressed. Bacteria community structure is variable at placement and before purge for all body sites. At bloat and purge and until tissues began to dehydrate or were removed, bacteria associated with flies, such as *Ignatzschineria* and *Wohlfahrtimonas*, were common. After dehydration and skeletonization, bacteria associated with soil, such as *Acinetobacter*, were common at most body sites sampled. However, more cadavers sampled through multiple seasons are necessary to assess major trends in bacterial succession.

Keywords Decomposition · Human cadavers · Bacterial succession · Metagenomics

Introduction

Decomposition of cadavers is a dynamic ecological process dependent upon the environment, climate, insect, and vertebrate scavenging activity, microbial activity, and intrinsic properties of the individual antemortem [1]. Alteration of the ecosystem, such as exclusion of insects or burial, may lead to a unique trajectory for decomposition and potentially anomalous results [2–4]; therefore, it is critical to forensics that the interplay of ecological factors be understood. However, despite possible variation, certain patterns exist. The rate of decomposition, dependent upon ambient temperature, is measured in accumulated heat units (degree hours or days). Accumulation of heat units is a function of total time spent between a thermal minimum and maximum below or above which metabolic functions of bacteria, insects, and chemical reactions are slowed or stopped. Rate may be accelerated or decelerated as temperatures increase or decrease, respectively [1, 5–7]. In general, as a body decomposes, it passes through roughly five stages of change discernable by gross morphological appearances [8]. Although continuous, in most instances, these changes can be assessed visually [5, 9, 10]. The early stages of decomposition are wet and marked by discoloration of the flesh and the onset and cessation of bacterially induced bloat. During decay, intrinsic bacteria are active from the inside out digesting away surrounding tissues [11]. During putrefaction, bacteria undergo anaerobic respiration producing gaseous by-products [12]. The buildup of

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E. R. Hyde · J. F. Petrosino
Alkek Center for Metagenomics and Microbiome Research, Baylor College of Medicine, Houston, TX, USA

E. R. Hyde
Integrative Molecular and Biomedical Sciences Training Program, Baylor College of Medicine, Houston, TX, USA

D. P. Haarmann · A. M. Lynne · S. R. Bucheli (✉)
Department of Biological Sciences, Sam Houston State University, Huntsville, TX, USA
e-mail: bucheli@shsu.edu

J. F. Petrosino
Department of Molecular Virology and Microbiology, Baylor College of Medicine, Houston, TX, USA

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resulting gas creates pressure, inflating the cadaver, and eventually forcing fluids out [11]. This purging event marks the shift from early decomposition to late decomposition. Purge is associated with an opening of the abdominal cavity to the environment [11], at which point, the rate of decay may greatly increase as larval flies remove large portions of tissues [2, 13–16]. The final stages of decomposition last through to complete skeletonization and are the driest stages [2, 3, 9, 17].

Bacteria are credited as a major driving force for the process of decomposition with numerous studies attempting to catalog the microbiome of decomposition [3, 11, 12, 17–26]. However, most of these studies have relied on culture-dependent techniques, which can limit the number of microorganisms documented. Recently, researchers have begun to investigate bacterial community structure of carrion and cadavers using high-throughput sequencing techniques [1, 27, 28]. Using pigs as surrogates for human cadavers, Pechal et al. [27] demonstrated that skin and mouth bacterial communities change during decomposition while Metcalf et al. [28], using a mouse model, showed that microbial community changes are dramatic, measurable, and repeatable. Both Pechal et al. [27] and Metcalf et al. [28] were able to use their data to generate a model to estimate the postmortem interval based on microbial sequence patterns. Hyde et al. [1] conducted one of the first exploratory investigations into the internal microbiome of cadavers placed outdoors to decompose under natural conditions and were able to produce an initial catalog via metagenomic techniques of bacterial species associated with the bloat stage of human decomposition. Overall, the study showed a shift in aerobic to anaerobic bacteria between the onset and forced-end of the bloat stage. While no model to estimate the postmortem interval was published, they recorded biodiversity for an important landmark of decomposition that may serve as a point of comparison for future work.

Despite these recent advances, still, little is known regarding potential sources of colonizing bacteria that may help drive decomposition and how these communities change through time. Bacteria are an intrinsic factor of the corpse, both internally and externally, but may also be introduced from a variety of sources including scavengers (vertebrate and invertebrate) and the ecosystem (soil, air, water). Investigation of patterns of microbial community succession may lead to additional tools available for forensic researchers. Organisms that interact with cadavers are a product of factors such as geographic region, season, weather, and conditions of the cadaver [3, 8, 9]. A comprehensive database of organisms associated with cadavers in conjunction with seasonality and accumulated degree days to which a cadaver has been exposed for a particular geographical area can be vital information for criminal investigations if these organisms can be shown to colonize cadavers in a predictable fashion (as with insects and cadavers [2, 13–16]). Here, using next-generation sequencing techniques, we document diversity of surface bacterial communities of human cadavers left to decompose outdoors under natural conditions in the Pineywoods ecoregion of east Texas. These data are the first documentation of succession of bacterial species during human decomposition and begin to assess how these bacterial communities change through time.

Materials and methods

Placement of cadavers This research used cadavers donated to the Southeast Texas Applied Forensic Science (STAFS) Facility at the Center for Biological Field Studies (CBFS) at Sam Houston State University (SHSU), which is a willed body donation facility designed for decomposition studies and training (the SHSU Institutional Review Board, the Protection of Human Subjects Committee, has determined that IRB approval is not required for use of human cadavers since no personal or identifiable information is collected). The STAFS facility at CBFS is approximately 5 km north of Huntsville, Texas (Walker County), and is an outdoor, 2-ac area fenced off within the larger 247 ac of CBFS located in the Pineywoods ecoregion. This ecoregion is characterized by a humid, subtropical climate and a sparse forest covering of pine trees with a ground covering of herbaceous plants. The soil is a fine, friable sand that is moderately to strongly acidic and is moderately well drained with medium available water content and slow permeability and runoff [29].

For this experiment, two cadavers were placed in tandem in the outdoor facility to decompose under natural conditions including weather, insects, and vertebrate scavengers. STAFS 2012-021 and STAFS 2012-023 were sampled from 15 August until 8 September 2012. Due to the nature of the donation program, it is difficult to acquire cadavers that are similar in physical, medical, cause of death, or postmortem conditions, but efforts to reduce variables are made to the best of our abilities. Neither cadaver had been autopsied; each was assessed to be in early decomposition according to the Megyesi et al. [5] total body scoring (TBS) system. Each was placed supine without clothing. STAFS 2012-021 was an approximately 85-year-old female, approximately 1.6 m tall and 48 kg with an unknown medical history and unknown cause of death. STAFS 2012-023 was an approximately 55-year-old male, approximately 1.8 m tall and 72.5 kg with a vague medical history and unknown cause of death. STAFS 2012-021 was stored frozen for 22 days (−17 to −12 °C) with 0 days in the cooler (3.33 °C). STAFS 2012-023 was stored frozen for 14 days (−17 to −12 °C) with 0 days in the cooler (3.33 °C).

Average daily temperature and humidity conditions and total rainfall for August and September 2012 (Fig. 1a, b, c) were generated using data from an on-site HOBO weather monitoring station. Accumulated degree days above a base of

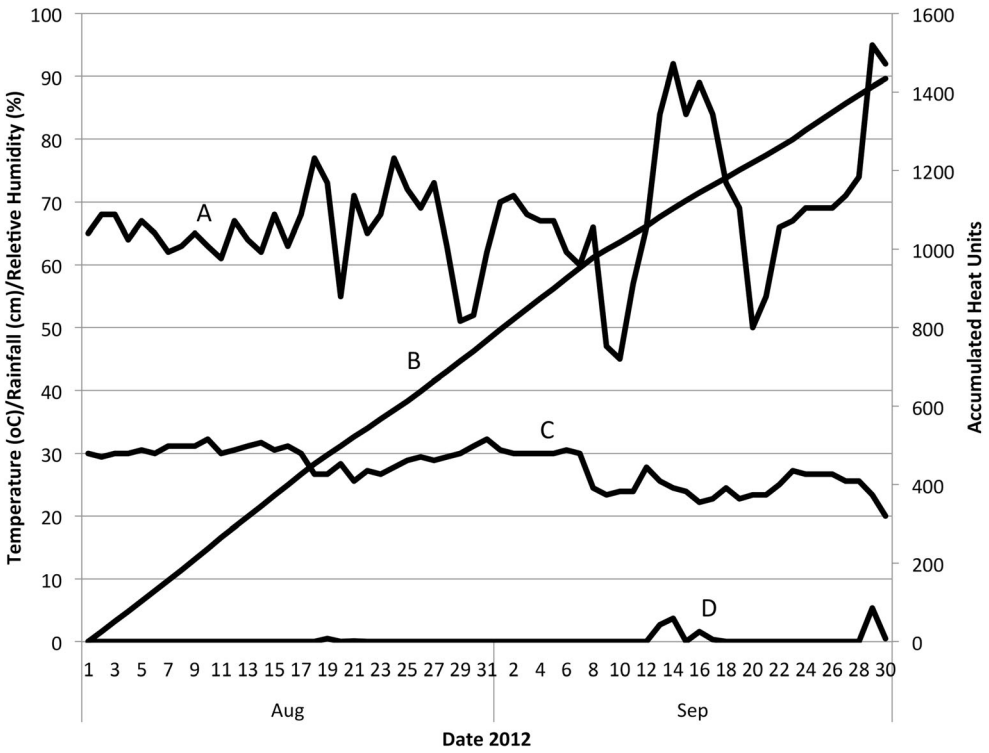

Fig. 1 Average temperature, °C (*a*); humidity , % (*b*); rainfall, cm (*c*); and accumulated degree day (ADD, *d*) heat units calculated at a base of 4 °C for August and September 2012. Degree day (DD) = ((maximum temperature + minimum temperature)/2)−base temperature; ADD = ($DD_x + DD_{x+1}$)

4 °C (Fig. 1d) for each cadaver were calculated using the method of Micozzi et al. [3].

Decomposition is a continuous, dynamic process with no clear demarcation between the end of one stage and the beginning of another. Since there is a significant temporal component to this project, it would be difficult to assign specific stages of decomposition as described by Pinheiro [8] to each of the time points sampled. Therefore, rather than to refer to the five standard stages of decomposition, we instead choose to refer to phases of decomposition relative to bloat and purge, which are easily discernible landmarks of decomposition and mark the shift between early and late decomposition [1].

Collection of bacterial samples Bacterial swab samples for each cadaver were taken from the mouth (internal left buccal region), external left/right cheeks (zygomatic arch region), external left/right bicep region, torso (above sternum), and fecal (rectal) on a daily or every other day basis as dictated by temperature, weather patterns, and TBS assessment [5]. Sample locations are justified as follows: mouth and fecal are representative of the internal sites, which cannot be easily accessed without invasive sampling [1]; the face, the torso, and the arms decompose at variable rates and selected based on this variability. Sample time points (a) day of placement, (b) pre-bloat, (c) bloat, (d) purge, (e) putrefaction, and (f) skeletonization were determined post hoc by photographs rather than anticipating when the stage would be entered.

Due to variable rates of decomposition between cadavers, STAFS 2012-023 reached skeletonization of certain sites more rapidly than STAFS 2012-021, which resulted in unequal sample events between cadavers. Each site was sampled with a sterile cotton tipped applicator (Puritan Medical, Guilford, ME). The cotton tip was removed and placed in a collection tube containing 0.5 ml sterile phosphate-buffered saline, pH 7.4, and stored at −80 °C until processing for bacterial genomic DNA. Standard personal protection equipment (PPE) was worn including full-body Tyvek suits, rubber boots, nitrile gloves, face masks, and face shields.

Sample processing, 16S rRNA gene amplification, and 454 pyrosequencing Sample processing, 16S ribosomal RNA (16S rRNA) gene amplification, and 454 pyrosequencing were performed following protocols benchmarked as part of the Human Microbiome Project [30, 31]. These protocols have been validated to reduce errors in the data analysis pipeline. By following these benchmarked protocols, we have generated a dataset that is comparable to the Human Microbiome Project and the American Gut Project. As larger datasets are generated from cadavers, the need to compare these datasets to other established datasets (HMP, etc.) requires the use of standardized protocols. Bacterial genomic DNA was extracted from samples using the PowerSoil DNA Isolation Kit (MOBiO, Carlsbad, CA). The V3-V5 regions of the 16S rRNA gene were amplified from genomic DNA using primer 357F (5′-CCTACGGGAGGCAGCAG-3′) and barcoded primer 926R (5′-CCGTCAATTCMTTTRAGT-3′).

Both primers were modified with the addition of 454 FLX Titanium adaptor sequences. Ten microliters of DNA and 2 μL of 4-μM paired primer stock were used in a 20-μL PCR reaction with 0.15 μL AccuPrime High Fidelity Taq polymerase and 5.85 μL PCR water. Reactions were heated for 2 min at 95 °C followed by 30 cycles of 20 s at 95 °C, 45 s at 50 °C, and 5 min at 72 °C. Amplicons were sequenced on a multiplexed 454 FLX Titanium pyrosequencing run at the Human Genome Sequencing center at Baylor College of Medicine. Raw sequence data are deposited in the Sequence Read Archive (BioSample accession #SAMN02808403-SAM02808667).

Data analysis with QIIME 16S rRNA gene sequencing data were processed and analyzed using QIIME version 1.6.0 [32]. Reads were de-multiplexed and quality-filtered and trimmed according to the following parameters: minimum/maximum sequences length of 200/1000 bp, no ambiguous bases, no homopolymer longer than 6 bp, no more than two primer mismatches, and no more than one barcode mismatch. Additionally, an average quality score of 25 over a 50 base pair sliding window was required; sequences were trimmed at the first base of the low quality score window, and any sequences less than 200 nucleotides in length after truncation were removed. Quality trimming yielded 1,149,806 high-quality reads, with a minimum/maximum/average sequence length of 200/570/352 bp. Four samples had 0 reads associated with them and were thus removed from the dataset, yielding 223 total samples used (out of 227 collected) in subsequent analyses. Sequences were binned into operational taxonomic units (OTUs) using uclust with a sequence identity of 97 %, and taxonomy was assigned using RDP Classifier (version 2.2) trained to the GreenGenes database (October 2012 release). After removal of singleton reads (0.2 % of reads), the OTU table was subsampled so that each sample had 1009 reads associated with it (the smallest number of reads associated with any one sample), and this normalized OTU table was used for alpha and beta diversity analyses. The number of observed species and the Shannon diversity index were calculated on OTU tables randomly rarified five times from 10 to 910 sequences per sample in steps of 100 sequences. Non-parametric *t* tests using 999 Monte Carlo permutations were used to determine significant differences in alpha diversity (number of observed species and Shannon diversity index) between sample categories (cadaver, body site, sample type). Bacterial communities were cross-compared using unweighted UniFrac to construct a distance matrix that was subsequently visualized on a principal coordinates analysis plot constructed using Emperor (v 0.9.3). Using the OTU table, bar charts were also constructed depicting the relative abundances of taxa present across individual samples and across groups of samples (body site, body site through time). All genera present at a total abundance of less than 0.1 % across all samples were lumped into one category, denoted as "Low Abundance Genera," for ease of visualizing both the chart and the legend.

Results and discussion

Temperatures during August 2012 were warmer on average than in September 2012, with lower relative humidity levels in August increasing in September. Rainfall was negligible (Fig. 1). By 8 September 2012, approximately 979 degree hours had accumulated (Fig. 1). In both STAFS 2012-021 and 2012-023, decomposition progressed rather rapidly with bloat and purge (marking the shift from early decay to late decay) estimated to have occurred 3 days after placement on 18 August 2012 between the scheduled sample dates of 17 August 2012 and 19 August 2012. In STAFS 2012-021, after purge occurred around 18 August 2012, the majority of wet biomass was lost around 23 August 2012. Thereafter, the rate of soft tissue loss and skeletal tissue exposure increased; however, soft tissues dehydrated and persisted after sampling ceased. In STAFS 2012-023, after purge occurred around 18 August 2012, the majority of wet biomass was lost around 21 August 2012. Thereafter, the rate of soft tissue loss and skeletal tissue exposure increased until the body was mostly skeletonized by 24 August 2012. STAFS 2012-023 decomposed and reached skeletonization more quickly that STAFS 2012-021 likely due to four factors: STAFS 2012-23 was stored frozen for fewer days than STAFS 2012-021; STAFS 2012-023 was slightly more decomposed than STAFS 2012-021 at the time of placement; STAFS 2012-023 had less body fat than STAFS 2012-021; and STAFS 2012-023 had more vertebrate scavenging activity than STAFS 2012-021. However, STAFS 2012-021 had more maggot activity than STAFS 2012-023. STAFS 2012-021 and STAFS 2012-023 were similar in terms of bacterial community richness and diversity for skin and fecal samples (pre- and post-bloat samples included); however, the mouth samples (pre- and post-bloat samples included) of STAFS 2012-023 were significantly richer and more diverse than those of STAFS 2012-021 (Table 1). As decomposition progressed, the microbial communities of all samples (mouth, skin, and fecal) from both bodies became more similar, as seen on an unweighted UniFrac-based PCoA plot with days since placement as the *x*-axis (Fig. 2). Initially, there is wide separation between samples on PC1, but as decomposition progressed (left to right across the *x*-axis), the sample began to cluster closer together.

Both cadavers were characterized by similar changes in the relative abundance of phyla present through time for all body sites (Fig. 3). Initially, four of five skin sites sampled (right cheek, left and right biceps, and torso) were dominated by *Proteobacteria*, which comprised from 60 to over 80 % of the

Table 1 Differences in richness and diversity of mouth, skin, and fecal samples between cadavers

STAFS 2012-021 mouth observed species ± s.d.	STAFS 2012-023 mouth observed species ± s.d.	*p* value	STAFS 2012-021 mouth Shannon index ± s.d.	STAFS 2012-023 mouth Shannon index ± s.d.	*p* value
57.4±27.8	84.9±31.7	0.04	2.59±1.37	3.61±1.03	0.05
STAFS 2012-021 skin observed species ± s.d.	STAFS 2012-023 skin observed species ± s.d.	*p* value	STAFS 2012-021 skin Shannon index ± s.d.	STAFS 2012-023 skin Shannon index ± s.d.	*p* value
111.6±80.1	106.2±40.8	n.s.	3.57±1.88	3.88±0.96	n.s.
STAFS 2012-021 fecal observed species ± s.d.	STAFS 2012-023 fecal observed species ± s.d.	*p* value	STAFS 2012-021 fecal Shannon index ± s.d.	STAFS 2012-023 fecal Shannon index ± s.d.	*p* value
114±72.1	96.2±22.5	n.s.	3.96±1.79	3.60±0.81	n.s.

The average (±standard deviation) number of observed species and Shannon diversity index are listed for each body for each sample type. *p* values indicate significant differences in richness and diversity. Skin includes skin, bicep, and torso samples

s.d. standard deviation, *n.s.* not significant

biomass during the first 2 days of decomposition (before purge), depending on sample site. *Firmicutes* increased in abundance on these sites during the later phases of decomposition. *Actinobacteria*, although at a lower relative abundance, also increased in the later stages of decomposition, comprising roughly 5–20 % of the bacterial community during the last five sampling events. This pattern is slightly different for the left cheek, which remained dominated by *Proteobacteria* at all sample times except the last time point, 8 September 2012, at which point the *Actinobacteria* comprised nearly 70 % of the microbial community (only 2012-021 is reported as STAFS 2012-023 lost this skin sample site due to decomposition). The microbial community of the mouth sample sites also followed this general pattern of high abundance of *Proteobacteria* followed by an increased abundance of *Firmicutes*. In contrast, *Firmicutes* and *Bacteroides* were the most abundant phyla in the fecal samples before purge (roughly 70 and 20 % relative abundance, respectively) with *Proteobacteria* dominating the most after purge until the drier phases when the *Firmicutes* again increased in abundance. *Actinobacteria* also increased in the drier phases of decomposition, comprising 10–12 % relative abundance.

Some variation exists between the microbial communities associated with each cadaver at the generic level and warrants discussion; however, we recognize that the sample size was low and consequently makes no claims regarding trends relative to cadaver individuality, such as gender, age, or weight. In general, bacteria community composition is variable at placement and before purge for all body sites likely owing to inherent variation in the individual microbiome [30, 31]. It is important to remember that each cadaver was stored frozen and this likely had an effect on the initial microbiome. Across all body sites (all time points inclusive, Fig. 4), STAFS 2012-021 was dominated by *Ignatzschineria* (20–55 % relative abundance) and *Actinetobacter* (5–15 % relative abundance, while STAFS 2012-023 was dominated by *Clostridium* (10–40 % relative abundance) and *Acinetobacter* (5–20 % relative abundance). These taxa are of particular interest due

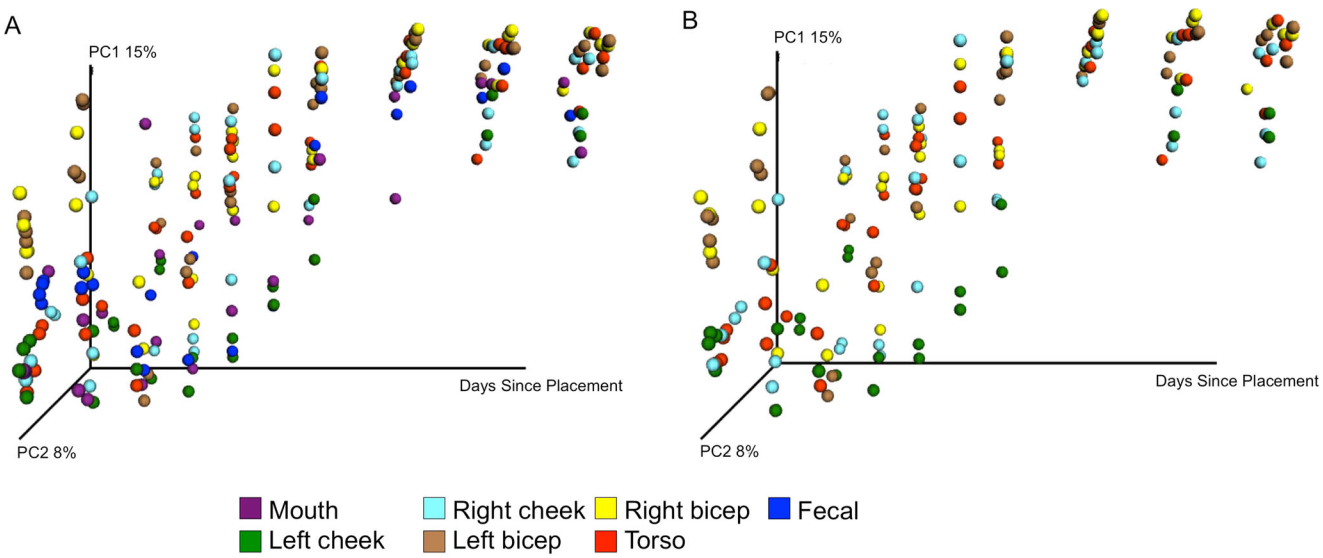

Fig. 2 Unweighted UniFrac-based PCoA plot for **a** all samples and **b** only skin samples (right and left cheek, right and left bicep, torso). The *x*-axis is defined by days since placement

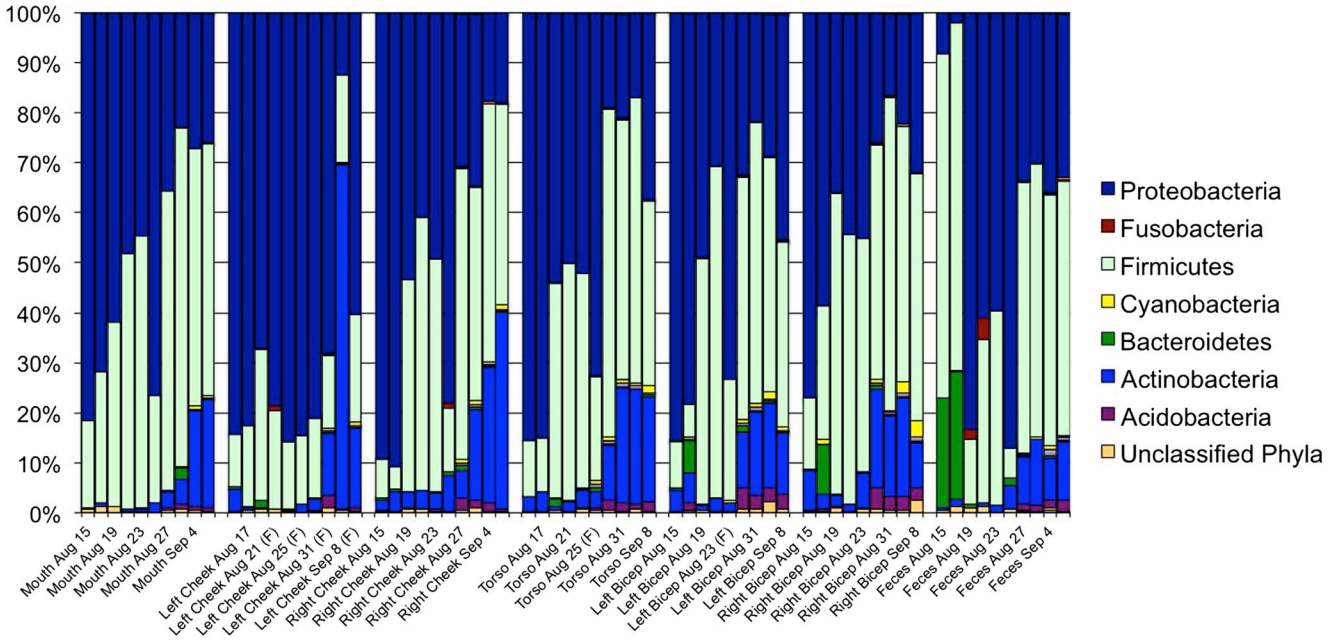

Fig. 3 Relative abundances of phyla present in each body site through time. The percent abundance of each phylum was determined for each body site for each body at each sampling date. The percent abundances for the two bodies were then averaged to obtain the mean relative abundance of phyla present at each body site at each sampling date. (*F*) denotes samples that were only obtained from the female cadaver (STAFS 2012-021) due to accelerated decomposition at the site in the male cadaver (STAFS 2012-023)

to their association with insects and/or their ubiquitous nature in the environment as well as their identification in other recently described decomposition studies [28, 27].

Ignatzschineria is a bacterial genus of the *Xanthomonadaceae* family and associated with myiasis by fly larvae in the Sarcophagidae family [33–37]. As described in detail below, this genus dominated the bacterial communities of all body sites during the wettest phases of decomposition for STAFS 2012-021, decreasing in abundance as time progressed and the body dehydrated and was skeletonized. In STAFS 2012-023, although present, *Ignatzschineria* was less abundant and less persistent; in contrast, *Wohlfahrtiimonas*, a genus in the same bacterial family as *Ignatzschineria* and also associated with myiasis by fly larvae of the Sarcophagidae family [33–37], was detected, albeit in low abundance. Metcalf et al. [28] found that the family *Xanthomonadaceae* increased on mouse cadaver skin and grave soil through time, and Pechal et al. [27] recorded the family *Xanthomonadaceae* in low amounts on pig corpses, suggesting that the *Xanthomonadaceae* may be key contributors to the general process of decomposition, regardless of host type.

Acinetobacter (family *Moraxellaceae*, class *Gammaproteobacteria*) is a bacterial genus commonly found in soil [38], which likely explains its presence on both cadavers during the late stages of decomposition. Metcalf et al. [28] demonstrated significant levels of *Gammaproteobacteria* both in the soil and on the skin of mouse corpses and found the family *Pseudomonadaceae*, not *Moraxellaceae*, as a significant member of the skin microbial community. Conversely, Pechal et al. [27] demonstrated significant levels of *Moraxellaceae* on fresh pig corpses through 24 h of decomposition, with negligible levels of this family detected thereafter. Again, these results suggest that the *Moraxellaceae* family may be an important group of decomposers across host type.

Clostridium is a widely variable oxygen-tolerant anaerobic genus found in soil, freshwater, marine sediments, animal and human feces, and human clinical isolates such as blood, wounds, and abscesses [39]. It is this ubiquitous nature that likely explains the presence of *Clostridium* and *Clostridiaceae* on both cadavers in this study. Metcalf et al. [28] did not report *Clostridium* in or on mouse corpses or grave soils but did report the presence of *Clostridiaceae* in the mouse corpse. Likewise, Pechal et al. [27] did not report *Clostridium* but did report the presence of *Clostridiaceae* on pig corpses. As with the *Xanthomonadaceae* and *Moraxellaceae*, the *Clostridiaceae* family could be another key contributor the general decomposition process. However, specific statistical analyses will need to be done on larger cohorts of mouse, pig, and human cadaver cohorts to determine whether these three bacterial groups can indeed be considered general decomposers. In the current study, the relative abundances of these three bacterial taxonomic groups varied, sometimes drastically, throughout decomposition, as discussed in detail below.

The fecal samples from cadaver STAFS 2012-021 were comprised of roughly 55 % unclassified genera within family *Clostridiaceae* in the first two sample dates (before purge),

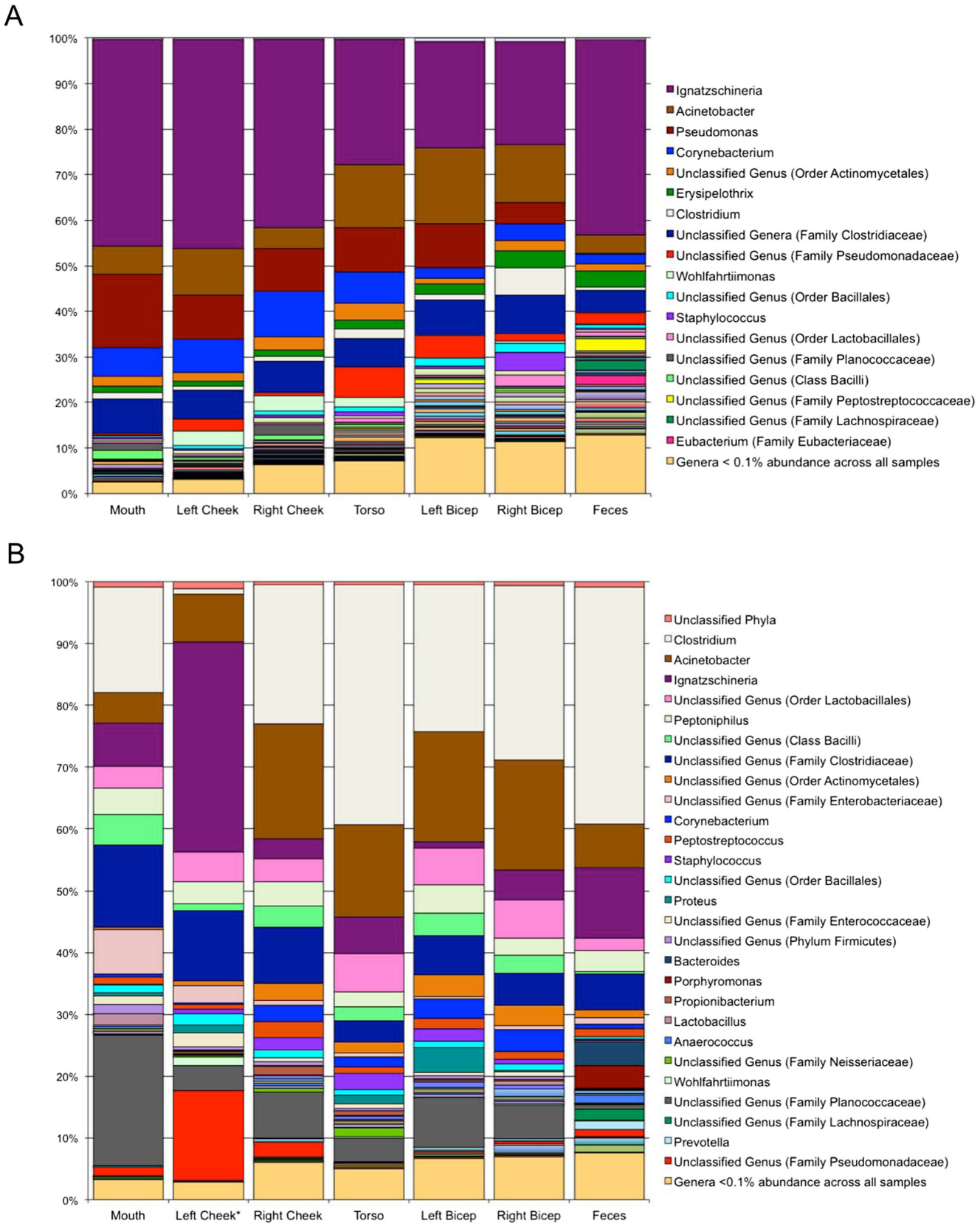

Fig. 4 Mean relative abundances of genera present, all sampling dates inclusive, of body sites for **a** STAFS 2012-021 and **b** STAFS 2012-023

while *Ignatzschineria* increased in abundance after purge to a maximum relative abundance of nearly 90 %, decreasing again to about 6 % relative abundance as wet biomass was lost and tissues began to dry out. This change in abundance was concurrent with the increase in insect activity during the wet stage of decomposition and a subsequent reduction in insect activity during the dry stage of decomposition. At this point during decomposition, the *Clostridiaceae* were again the most abundant, comprising 64 % of the bacterial community. In the mouth, *Pseudomonas* dominated the community structure before purge, comprising 92 and 68 % of the bacterial community on days 1 and 2 of the study, respectively. As with the fecal samples, *Ignatzschineria* increased in abundance after purge (again occurring during higher levels of insect activity), to a high of 95 % relative abundance, and decreased to about 7 % relative abundance as wet biomass was lost. *Corynebacterium* was the most abundant after most wet biomass was lost, increasing to 32 % relative abundance at the last time point sampled. For all skin samples (torso, right and left cheek, and right and left bicep), *Pseudomonas* was dominant before purge (40–97 % relative abundance, depending on body site and sampling date), and *Ignatzschineria* increased in abundance after purge (15–90 %, depending on body site and sampling date), decreasing again as wet biomass was lost (0.5–3 %, depending on body site and sampling date). On the biceps, *Acinetobacter* (up to 54 % relative abundance) replaced *Ignatzschineria* as the most abundant during the drier phases of decomposition, while on the cheek, *Corynebacterium* (up to 53 % relative abundance) and *Acinetobacter* (up to 32 % relative abundance) replaced *Ignatzschineria* as the most abundant during the drier phases of decomposition. (see Supplementary Table S1 for a list of relative abundances through time of genera discussed above.)

The fecal samples collected from STAFS 2012-023 were comprised mainly of *Bacteroides* (33 % relative abundance) and *Porphyromonas* (32 % relative abundance) on August 15 and August 17, respectively (before purge). *Ignatzschineria* increased in abundance after purge to a maximum relative abundance of 59 % and decreased in abundance to negligible amounts as wet biomass was lost and tissues began to dry out. *Clostridium* became the most abundant genus present in the bacterial community after most wet biomass was lost (roughly 70 % relative abundance). In the mouth community, unclassified genera of the family *Enterococcaceae* dominated before purge (44 and 16 % relative abundance on August 15 and August 17, respectively). After purge, unclassified *Planococcaceae* dominated in the mouth, increasing to 45 % relative abundance, and then dropped off as *Clostridium* increased to 47 % relative abundance. For all the skin samples (torso, right and left cheek, and right and left bicep), *Acinetobacter* dominated the bacterial community before purge (15–78 % relative abundance, depending on body site and sampling date), while *Clostridium* dominated in the later phases of decomposition (22–73 % relative abundance, depending on body site and sampling date; see Supplementary Table S2 for a list of relative abundances through time of genera discussed above).

In both cadavers, differences in the diversity and relative abundances of taxa present on the left and right sides of the body were observed (Figs. 5 and 6). The diversity of bicep samples did not differ significantly between right and left sides of the body on either cadaver, either before or after purge (Supplementary Figs. S1 and S2), but there were differences in the relative abundances of taxa present between the right and left biceps (Fig. 5a, c). The difference in bacterial community diversity and composition was much more drastic in the cheek samples (Figs. 5b, d and 6). On STAFS 2012-021, the microbial community of the right cheek was significantly more diverse than that of the left, and this difference was more significant when only post-purge samples were analyzed (Fig. 6). The increased diversity of the right cheek was likely due to a "bloom" of low-abundance genera detected on the right but not the left cheek at drier phases of decomposition (see Fig. 5b). Tilting of the head to the left side that occurred during decomposition likely altered the microhabitat of the site and may explain this variation. On STAFS 2012-023, the relative abundances of specific taxa differed between the right and left checks both before and after purge (see Fig. 5d); however, due to biomass loss after day 6, we could not thoroughly assess the differences in the cheek communities after purge.

The HMP has established a core community for healthy adults between the ages of 18 and 40 years. Our research cadavers are very different than the targeted HMP cohort: They are beyond the age of 40 years, they have unknown or vague medical histories, and they have died of unknown or vague causes. Additionally, our study is a long-term longitudinal study exploring bacterial succession during decomposition. However, we can make a very tentative comparison to the HMP results where samples were taken from comparable locations (fecal and oral) [1]. Overall, we see a similar trend in the initial fecal and oral samples from both cadavers compared to HMP fecal and oral samples.

These results are intriguing given that decomposition in general is not uniform. Not all body sites bloat at the same time or for the same duration of time [5, 10, 40], and we have observed that the head tends to decompose more quickly than the rest of the body, which is consistent with other controlled studies [5]. Our current results suggest that even small spatial differences owing to intrinsic properties of the cadavers and to abiotic influences, such as solar irradiance and ambient temperatures [41], on the biotic components of the ecosystem may have an effect on decomposition and the resulting microbial community. As mentioned, one limitation of our study was that the left cheek of STAFS 2012-023 decomposed more quickly than the right cheek, and thus, we were unable to

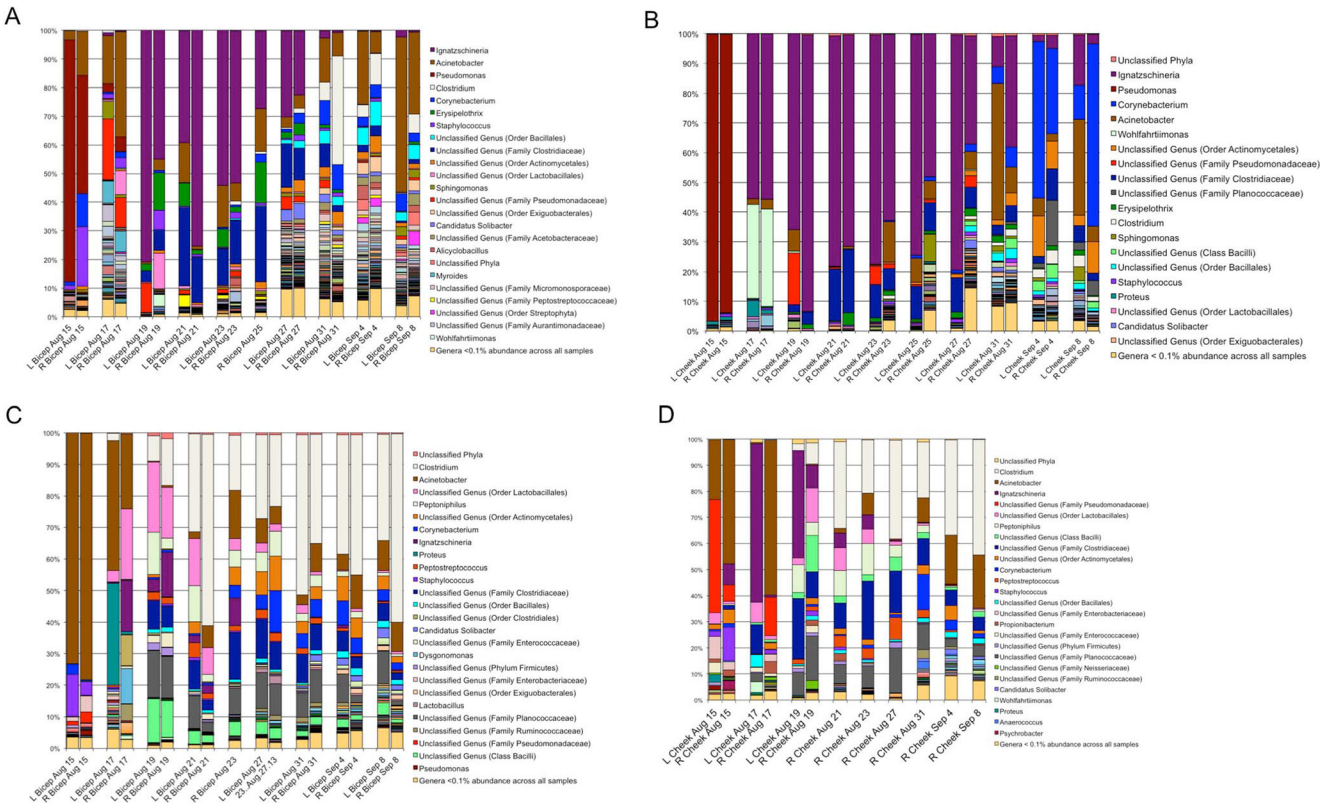

Fig. 5 Relative abundances of genera present at each sampling date for **a** right and left bicep, STAFS 2012-021; **b** right and left cheek, STAFS 2012-023; **c** right and left bicep, STAFS 2012-023; and **d** right and left cheek, STAFS 2012-023

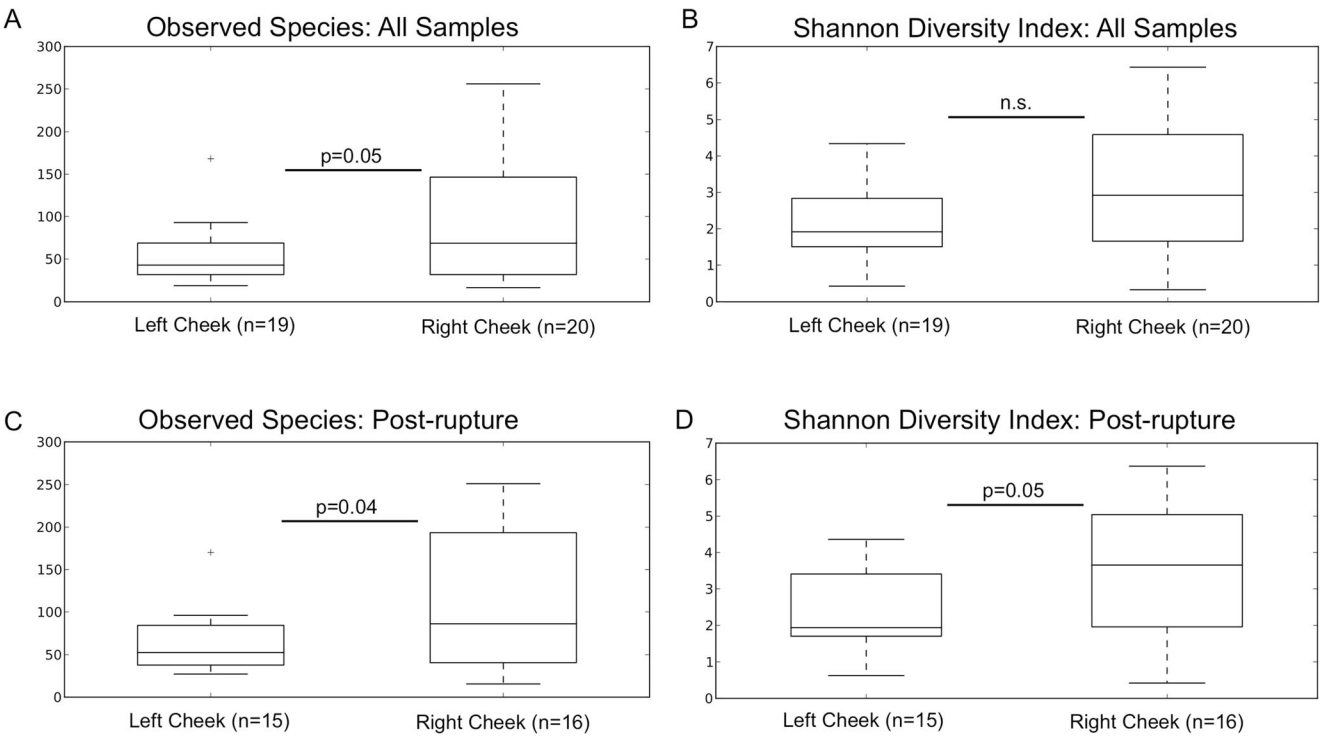

Fig. 6 Box and whisker plots illustrating the median, minimum, maximum, first quartile, and third quartile for **a** the number of observed species and **b** the Shannon diversity index of right and left cheek samples collected from STAFS 2012-021 collected pre- and post-rupture and the **c** number of species and **d** Shannon diversity index of right and left cheek samples collected after rupture. Alpha diversity metrics were calculated on a sequencing depth of 910 sequences per sample. Statistics were calculated using a non-parametric *t* test with 999 permutations

collect samples from both cheeks from 6 days after placement onward. Without more samples from additional cadavers, it is difficult to conclude whether the head itself is more variable spatially or whether position or abiotic components of the head have greater influence on the microbial community as decomposition progresses.

Conclusion

This is the first study to describe successive bacterial community changes associated with human cadavers. Because this study is limited in that only two human cadavers were cataloged, it should not be used to make inferences about human decomposition in general (i.e., across genders, geographic regions, seasons, etc.) or to assign specific bacterial taxa to specific stages of decomposition, but should be viewed as an initial insight into microbial succession associated with human decomposition. While each cadaver showed a change in community structure for all sites sampled through time, no conclusions regarding a global pattern of community succession can be made at this point due to the limited sample size. However, shifts in community structure were recorded and can be associated with major taphonomic events such as purge, the shift from early to late decomposition, loss of wet biomass, and skeletonization. To firmly establish successive change, more studies building on this study including cadavers of different ages, antemortem conditions, and gender, placed at different seasons across different geographical regions, should be done.

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