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Research Article

Investigating geological records of tsunamis in Western Thailand with environmental DNA

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ABSTRACT

The identification of tsunami deposits in the geological record remains a challenge because the proxies availabilities are subject to the environment. The proxies may degrade over time and inherently inhibit the robustness of event interpretations. Multi-proxy methods, which leverage on each other's advantage/s and limitation/s, are employed to improve the identification of tsunami deposits from the geological record. Here, we assess the utility of environmental DNA (eDNA) for tsunami research by comparing and contrasting the eDNA collected from a sequence of well-documented palaeotsunami deposits spanning the past three millennia. We study swales in a coastal beach ridge sequences on Phra Thong Island, Thailand and test if eDNA can robustly discriminate the tsunami-deposited sand sheets that intercalate between the non-tsunami derived organic mud layers. Our results indicate that the 2004 Indian Ocean tsunami deposit and preceding tsunami deposits (approximately 550 to 700 years ago) contain microbial communities that differ significantly from the overlying and underlying organic mud layers (p-value = 0.0269) but the signal becomes restricted in the older sediment layers up to 2800-year-old that are constantly submerged in groundwater. This work demonstrates the potential for applying eDNA to study tsunami deposits over centennial time frames and perhaps longer.

1. Introduction

Tsunami deposits from recent, historical, and palaeo-events can provide information about the long-term tsunami hazard if the cause, source and age of these deposits are identified. Understanding the long-term tsunami hazard risk faced by coastal communities provides important information to support tsunami disaster risk assessment and disaster reduction efforts (e.g., Dominey-Howes, 2002; Peters et al., 2003; Dominey-Howes et al., 2006; Peterson et al., 2013). Tsunami disaster risk reduction efforts include tsunami early warning system(s), land-use zoning, building code standards and regulations, evacuation of buildings, and community education programs that promote disaster awareness and preparedness that can help to minimize the tsunami damage to coastal communities (Satake, 2014). For example, the 2004 Indian Ocean Tsunami (IOT) was triggered by an Mw 9.2 earthquake (Lay et al., 2005) that devastated the coastal zones of 14 countries and resulted in approximately 230,000 casualties (*International Tsunami Information Center*, 2016). Unfortunately, at the time of the event, most of the impacted countries lacked appropriate disaster preparedness protocols because the long-term tsunami hazard was unknown (Dominey-Howes et al., 2006; Suppasri et al., 2015; Rubin et al., 2017).

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1.1. Challenges in investigating palaeotsunami deposits

There are three mains challenges in palaeotsunami research. First, distinguishing whether the sediments in the geological record are attributable to tsunami or storm surge mechanisms (e.g., Nanayama et al., 2000). The characteristics of the preserved tsunami deposits are highly dependent on factors such as the number and size of the waves, types of sediment mobilized and deposited in the local onshore and offshore geomorphology, or post-depositional changes (Sugawara et al., 2008). The variety of characteristics mean that a wide range of indicators are needed to identify a sediment layer as a tsunami deposit, and doubts commonly remain about the unequivocal origins of a specific deposit. For example, the IOT alone left a variety of onshore sediment deposits such as sand (e.g., Jankaew et al., 2008), cobbles (e.g., Rajendran et al., 2013), and boulders (e.g., Goto et al., 2007; Etienne et al., 2011) in a diverse range of environments. Thus, effort is continually required to advance the toolkit of approaches to investigate palaeodeposits.

Second, palaeotsunami datasets from geological archives are, more often than not, incomplete because some tsunamis do not leave an imprint in the geological record or because their deposits were eroded or modified by subsequent events (Costa et al., 2021). As tsunamis are rare events, the study of modern tsunami and storm deposits provides much-needed modern analogs for comparison with the geological records (Nanayama et al., 2003; Goff et al., 2012). The combined study of sediment composition, texture, grading, stratification, thickness, geometry, geochemistry, faunal assemblages and geomorphology remains the primary strategy for deciphering tsunami histories from geological records (e.g., Engel et al., 2010; Chagué-Goff et al., 2017; Watanabe et al., 2022).

Third, where sedimentological evidence for potential tsunamis is present, since such sediments may be difficult to differentiate from the background sedimentation or from sediment deposited by other extreme-wave events including waves, storm surges, infragravity waves, and other floods (Costa and Andrade, 2020). Several studies have noted the considerable sedimentological similarities between tsunami and storm deposits (e.g., Nanayama et al., 2000; Tuttle et al., 2004; Kortekaas and Dawson, 2007; Morton et al., 2007; Switzer and Jones, 2008; May et al., 2017).

1.2. Multi-proxy methods to identify tsunami deposits in the geological record

Geoscientists have employed numerous techniques to improve their ability to identify a sedimentary unit as a tsunami deposit (Chagué-Goff et al., 2011). For instance, while geological techniques (grain size analysis, anisotropy of magnetic susceptibility, etc.) can accurately quantify the sedimentary physical properties and characteristics (Table 1), the properties of a tsunami deposit can be source-dependent and susceptible to erosion depending on the thickness of the deposit and mixing depth (e.g., Szczuciński, 2012; Gouramanis et al., 2017). Macro- and micropaleontological (e.g., shells, diatoms, foraminifera, ostracods, pollen) fossils are commonly used to estimate the provenance of the deposition, with the condition, the fossils are well preserved in undisturbed tsunami deposits at sites with sufficient accommodation space, and no potential carbonate dissolution (e.g., Mamo et al., 2009; Sawai et al., 2009; Pilarczyk et al., 2017; Gouramanis, 2020). Geochemical signals such as, total organic carbon, total nitrogen, salt, sulfur, chloride and heavy minerals (e.g., titanium and zirconium) are used to reconstruct the sediment transportation process, but these signals are sensitive to environmental changes such as vertical and horizontal groundwater movement (e.g., Pham et al., 2017), dilution by precipitation (e.g., Goff et al., 2012) or removal by microbial activities (e.g., Asano et al., 2020). As such, work is constantly required to refine and extend the range of methods used to increase our understanding and identification of tsunami and palaeotsunami deposits.

1.3. The potential of microbial molecular techniques

Environmental DNA (eDNA) refers to the genetic material present in a sample, which includes DNA from both living and dead organisms (Torti et al., 2015). DNA is a chemically stable molecule that can persist in the environment long after the death of the source organism in the form of dead biomass or preserved when absorbed to soil mineral surface (e.g., Torti et al., 2015). eDNA has the potential to be used as a new proxy to investigate tsunami deposits in the geological record (Szczuciński et al., 2016; Engel et al., 2021; Yap et al., 2021).

The characterisation of the identity and relative abundance of taxa within a sample (i.e., the microbial community) can be performed with a technique known as metabarcoding, which relies on millions of DNA sequences generated from high-throughput DNA sequencing technologies (Sinclair et al., 2015; Engel et al., 2021; Yap et al., 2021). Several studies have used eDNA approach to understand how microbial community respond to high-impact, short-duration environmental disturbances caused by natural hazards, such as flooding (Asano et al., 2020), forest fires (Dooley and Treseder, 2012; Taş et al., 2014), earthquakes (Kawagucci et al., 2012; Morimura et al., 2020), and various types of volcanic eruptions (Huber et al., 2003; Zeglin et al., 2016) and volcanic ashfalls (Tateno et al., 2019). There are only a handful of eDNA application used to investigate geological record of palaeotsunami deposits (e.g., Szczuciński et al., 2016; Yap et al., 2021).

1.4. Tsunami environmental DNA

Tsunami flooding causes abrupt disturbance to the coastal environment and changes the microbial communities in the sediments. Most studies that have attempted to examine the response of microbial community to tsunami flooding disturbance have focused on deposits from the 2004 Indian Ocean tsunami (e.g., Ramesh et al., 2006; Godson et al., 2014) and utilized the conventional culturing technique. The limitation is that only 5% of the naturally occurring microbial populations in the deposit can be cultivated using the current methods (Olsen et al., 1986; Handelsman, 2004). Yap et al. (2021) used metabarcoding sequencing approach and showed that microbial community signatures could be used to distinguish recent/modern overwash deposits (both storm and tsunami) from soils and non-overwash derived sediments at two locations in India and Thailand. Yap et al., 2021 also successfully discriminated known modern tsunami and storm deposited sediments at both locations. Asano et al. (2013, 2020) and Somboonna et al. (2014) were the first to use metabarcoding to examine changes in microbial communities in tsunami deposits. The Asano et al. (2013, 2020) work focused on the 2011 Tohoku tsunami in Japan while the Somboonna et al. (2014) study looked at the microbial communities in the 2004, 300- to 600-year-old, and >600-year-old tsunami deposits at Phra Thong Island, Thailand. All these investigations found distinct communities between tsunami deposits and non-tsunami sediments, showing that tsunami deposits may have distinct microbial signatures.

Here, we test the application of eDNA to a series of well-documented palaeotsunami deposit layers in the well-known Phra Thong Island site, Thailand to extend its application beyond modern deposits and go back in time (e.g., Jankaew et al., 2008; Gouramanis et al., 2017; Yap et al., 2021). We aim to identify tsunami and non-tsunami samples from the geological record and compare the microbial community's similarities and differences in known palaeotsunami deposits. In this study, we use a well-documented sequence of palaeotsunami deposits, representing a series of past tsunamis, that impacted Phra Thong Island, Thailand (Jankaew et al., 2008; Gouramanis et al., 2015) to test the feasibility of using eDNA to characterise 500- to 2800-year-old tsunami deposits. Specifically, we want to investigate (i) whether the microbial community approach can distinguish a series of tsunami-deposited sands from the background organic non-tsunami derived layers, (ii) what are the limitations of using eDNA to identify palaeotsunami deposits in a geological record, and (iii) whether the microbial tsunami indicator is

Table 1
Summary of multi-proxy methods/tools applied to identify overwash deposits from the geological records.

Proxies	Methodology	Proxies' description and/or diagnostic criteria	Remarks/limitations	Example References
Stratigraphy and sedimentary physical structure and texture	1. Characterise the grain size by sieving with calibrated meshes for coarse overwash deposits. 2. For clay to sand-sized sediments with a diameter $<$ 1 mm, the grain size is usually measured using a sieve and pipette or laser diffraction diffractometer.	1. Provides sedimentary description to characterise/interpret the hydrodynamics during overwash sediment deposition e.g., distinct upper and lower sub-units representing run-up and backwash. 2. Most tsunami deposits have fining upward and inland sediment profiles. 3. Presence of heavy mineral lamination within the sedimentary profile. 4. Deposits generally rise in altitude inland and can extend for several kilometers inland and 10's of kilometers alongshore. 5. Distinct upper and lower subunits representing run-up and backwash. 6. Lower contact is unconformable or erosional - infilling of microtopography is visible in more recent deposits. 7. Can contain intraclasts (rip-up clasts) of reworked material (natural and anthropogenic) material. 8. Often associated with loading structures at the base of the deposit	1. Each wave can form a distinct sedimentary unit and/or there may be laminated sub-units 2. Can be site-specific and source-dependent limited by local geological and geomorphological context. i.e., if the sediment source in offshore, nearshore and beach area is dominated by sand, then any overwash will be sand dominated too. 3. Subject to coastal environmental setting with sufficient accommodation space that allows for sediment to accumulate and preserve.	e.g., Atwater, 1987; Jankaew et al., 2008; Scicchitano et al., 2010.
The magnetic fabric of the tsunami deposits/ Anisotropy of Magnetic Susceptibility (AMS)	Characterising the paramagnetic versus ferromagnetic grains within the deposits based on magnetic susceptibility and magnetic anisotropy, to determine the magnetic fabric pattern and features of the sediments	1. Combined with grain size data can provide information to characterise the wave behavior i.e., hydrodynamic condition and energy, and analyze tsunami flow orientation, direction, and emplacement dynamics. 2. Magnetic ellipsoid shape factor (q) characterise flow speed fluctuation of bottom currents acting during the emplacement of tsunami deposit. 3. Magnetic lineation (L) and foliation (F) characterise the variation in current strength.	1. Useful when the sedimentary units are not distinctive. 2. Can be site-specific and source-dependent limited by local geological and geomorphological context. 3. Subject to coastal environmental setting with sufficient accommodation space that allows for sediment to accumulate and preserve. 4. Suitable for tsunami deposits trapped within a depression remote from a seashore. A local depression favors a better development of flow parallel magnetic fabrics. 5. Require dense sampling to gain precise information specially to constrain the mean K_{max} to determine the flow direction. This information can be affected by micro-topographic deflections of the tsunami flow and deposit.	Wassmer et al., 2010; Schneider et al., 2014.
Geochemical and mineralogical features (saltwater signature)	1. X-ray Diffraction (XRD) analysis using a diffractometer 2. X-ray Fluorescence (XRF) analysis using an energy dispersive polarization spectrometer 3. Petrographic microscope and scan electronic microscope (SEM) for heavy mineral composition	1. Characterise the sediment provenance, hydrodynamic processes i.e., erosion and deposition, and sediment transport reconstruction. 2. Increases in elemental concentration of sodium, sulfur, chlorine, calcium, strontium, magnesium (shell, shell hash, and coral), titanium, zirconium (associated with heavy mineral laminae if present) occur in tsunami deposits relative to background sediments layers indicating saltwater inundation and/or intrusion of high energy environment (heavy minerals) to low energy environment.	1. Often extend further inland than landward maximum extent of sedimentary deposits. 2. Can be site-specific and source-dependent limited by local geological and geomorphological context. 3. Subject to coastal environmental setting with sufficient accommodation space that allows for sediment to accumulate and preserve. 4. The concentration of the elements especially marine salt e.g., Na, S, and Cl may alter due to post-depositional change such as dilution or weathering processes. 5. Heavy minerals have variable density and are susceptible to sorting based on their density i.e., micas are commonly vertically distributed at the top of the deposits, but flake-shaped micas can be easily sorted. 6. Act as supporting evidence in	Switzer et al., 2005; Chagué-Goff et al., 2011; Jagodziński et al., 2012; Pham et al., 2017; Shinozaki, 2021

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Table 1 (*continued*)

Proxies	Methodology	Proxies' description and/or diagnostic criteria	Remarks/limitations	Example References
			conjunction with other more commonly used proxies	
Biomarker and organic anthropogenic markers (e.g., n-alkanes, isoprenoids, fatty acids, n-aldehydes, dinosterol, steroids etc.)	1. Extract anthropogenic markers (freely extractable compound) using overhead shaking solid-liquid extraction with organic solvents. 2. Extract biomarkers (bound compounds) using alkaline hydrolysis solid-liquid extraction. 3. The extracts are fractionated based on polarity differences 4. All samples' fractions were identified using gas chromatography (GC) such as GC-FID (flame ionization detector) or GC–MS (mass spectrometry).	1. Ratio differences between terrestrial and aquatic organic matter in the sediment.	1. Subject to location that have highly modified by human activities. 2. Subject to microbial alteration (i.e., microbes consuming the organic materials for metabolisms) 3. Preservation issue caused by post-depositional changes such as aeolian processes. 4. Can be diluted by weathering processes.	Alpar et al., 2012; Mathes-Schmidt et al., 2013; Shinozaki et al., 2015; Bellanova et al., 2020; Frenken et al., 2022
Environmental DNA	Extract genetic material directly from environmental samples and use next generation sequencing technology to obtain DNA sequences to reconstruct the microbial assemblages.	1. Microbial assemblages differed significantly between tsunami deposits and non-tsunami derived samples.	1. Can be site-specific and source-dependent depending on local geological and geomorphological context. 2. Limitation with reference database	Szczuciński et al., 2016; Yap et al., 2021;
Microfossil assemblages such as diatoms, foraminifera, pollen, shells, and others	Quantify microfossils using a microscope and perform assemblage identification by referring to a relevant standard reference.	1. Foraminifera species composition and taphonomy conditions (e.g., surface fragmentation, abrasion, corrosion) can be used to qualitatively estimate the origin of tsunami sediment, tsunami scouring depth, sediment transportation distance, and to identify tsunami deposits from background materials that have limited differences. 2. Allochthonous foraminifera marine assemblages within the terrestrial setting are indicative of a short-lived, abrupt marine incursion from a tsunami or storm. 3. Many broken diatom's valves are often observed in the deposits representing turbulent flows. 4. Variation in diatoms affinities often indicative of deposit provenance and magnitude of the event. 5. Lower pollen concentration observed in the overwash deposits as it may be diluted by marine inundation and/or presence of a high percentage of coastal pollen (e.g., mangrove) 6. Pollen above overwash deposits vary due to vegetation changes associated with saltwater flooding 7. Articulated and/or water-worn individual shells and shell-rich units are commonly found in overwash deposits. 8. Small, fragile shells and shellfish can be found near the upper surface of modern or more recent palaeotsunami deposits. 9. Vascular plant material and/or human/animal skeletal remains are buried in overwash deposits. 10. Less dense debris such as wood and shell can be found “rafted” near the top sequences of the overwash deposits.	1. Can be site-specific and source-dependent limited by local geological and geomorphological context. 2. Preservation issues caused by post-depositional changes such as acidic groundwater and potential carbonate dissolution 3. Ideal coastal environmental settings that are favorable to reconstruct microfossil assemblages are quiescent and contain sufficient accommodation space for sediment to accumulate and preserve.	Mamo et al., 2009; Sawai et al., 2009; Pilarczyk et al., 2017; Dura et al., 2016
Others such as geomorphological,		1. Geomorphology features such as modified dune geomorphology,	Depend on availability	Clark et al., 2019

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Table 1 (*continued*)

Proxies	Methodology	Proxies' description and/or diagnostic criteria	Remarks/limitations	Example References
archaeological, anthropological observations/evidence		evidence of either uplift or subsidence may indicate a tsunami inundation. 2. Written historical records and/or oral record of past tsunami event with associated archaeological remains such as seawall.		

consistently found in the tsunami sediments on Phra Thong Island.

2. Regional setting

Phra Thong Island is located in the Andaman Sea off the west coast of southern Thailand (Fig. 1). It is a barrier island with flat coastal plains, separated from the mainland by tidal channel (e.g., Brill et al., 2015). The island is located <500 km from and directly east of the Sumatran subduction zone. It was severely impacted by the 2004 Indian Ocean Tsunami (IOT) (e.g., Jankaew et al., 2008) when the subduction zone megathrust plate boundary ruptured. The IOT reached a maximum height of 19.6 m above mean sea level at Phra Thong Island (Tsuji et al., 2006), and inundated >2 km inland along the western coastline (Jankaew et al., 2008).

Phra Thong Island contains a series of shore-parallel beach ridges formed mainly by wave swash (Brill et al., 2015). The eastern portion of the island is formed during the last interglacial period (Scheffers et al., 2012) and the western portion prograde westward and formed as sea level regress from mid-Holocene (Brill et al., 2015). This beach ridges interspersed with organic-rich swales formed along the western part of the island and dense tidal mangroves on the island's eastern side (Brill et al., 2015). The swales form topographic lows that provide accommodation space where tsunami sediments can be deposited and preserved (Monecke, 2020). Accumulation of organic-rich marsh deposits often dominate in the swales, facilitating a clear distinction of coastal marsh sediments from marine washover deposition, which are frequently dominated by sand (e.g., Atwater et al., 2013; Gouramanis et al., 2017).

As the Phra Thong Island tsunami sequences have been well-documented, we adopt the stratigraphic labelling from Jankaew et al. (2008), for example, Swale X and Sandsheet A, for ease of reference. The stratigraphic sequence from top to bottom contains the 2004 IOT deposit (referred to as Sandsheet A) as well as three stratigraphically lower palaeotsunami deposits: Sandsheet B (dated 550 to 700 years ago), Sandsheet C (approximately 2200 years ago), and Sandsheet D (approximately 2800 years ago) (Jankaew et al., 2008; Prendergast et al., 2012). The study site geographic setting limits its exposure to intense storm surges (Jankaew et al., 2008; Gouramanis et al., 2017) and there is no historical nor geological records of intense storm inundation in this area (Brill et al., 2015) except one in year 2007 (Brill et al., 2015;

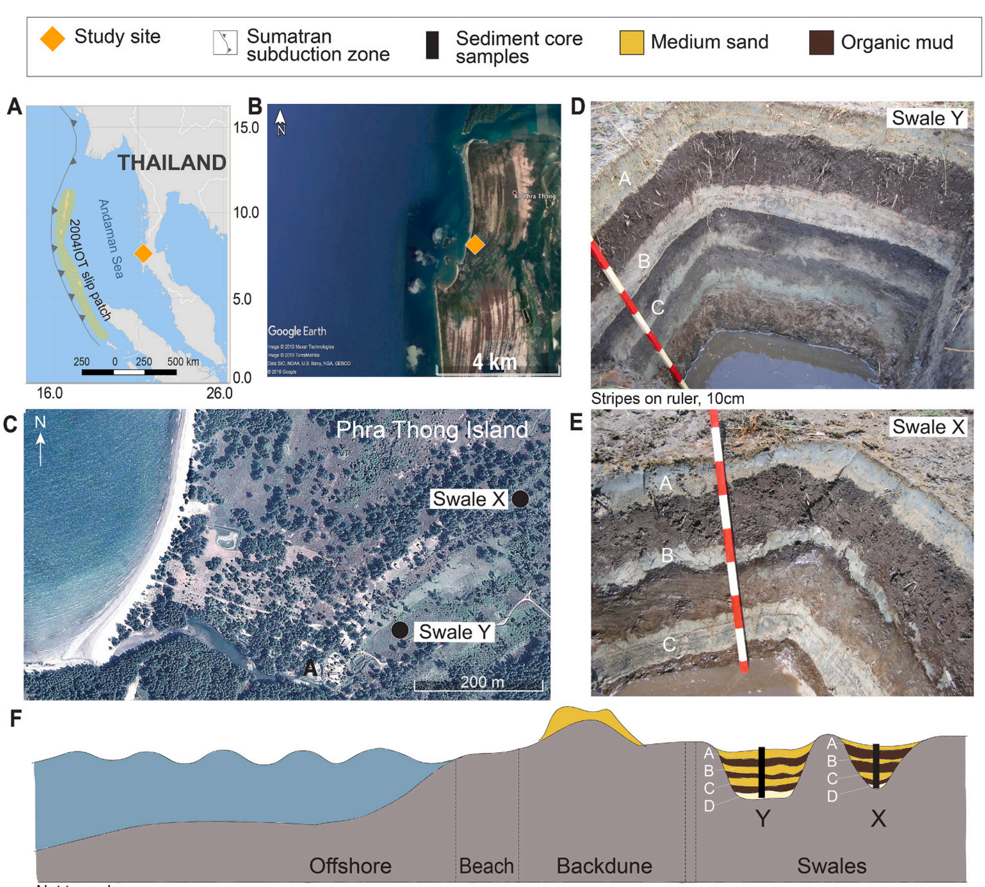

Fig. 1. (A) Study area on Phra Thong Island (orange diamond), Thailand. The gray line with triangles indicates the Sumatran subduction zone that triggered the 2004 Indian Ocean Tsunami (IOT). (B) A Google Earth image shows the northern beach ridge sequence on Phra Thong Island and the orange diamond highlights the study site. (C) The study site close-up image from Google Earth indicates the sediment core sampling site at Swale X and Swale Y (the black circles). The source map for (B) and (C) are from Google Earth. Pit photos at Swale Y (D) and Swale X (E) presenting the sand sheets alternating with dark organic mud layers taken after the 2004 IOT, photo by Jankaew, K. in March 2005. Sandsheet D is submerged in groundwater in (E). (F) A schematic stratigraphy of the study area with a cross-section focusing on Swale Y and Swale X.

Gouramanis et al., 2017). Gouramanis et al. (2017) reported that the 2007 storm deposited two overwash fans near the shoreline, located 490 m northwest and 380 m west of Swale Y.

3. Materials and methods

3.1. Sample collection

We obtained local research permission from Chulalongkorn University and sampling approval from the landowner before conducting the fieldwork.

We sampled the tsunami sequences from two adjacent swales located approximately 400 m (Swale X) and 550 m (Swale Y) east of the modern, western coastline of Phra Thong Island (Fig. 1) (e.g., Jankaew et al., 2008; Gouramanis et al., 2015; Pham et al., 2017; Yap et al., 2021). Our sampling sites are approximately 10 m (Swale X site, 9° 8.1742′ N, 98° 15.916′ E) and less than a 100 m away (Swale Y site, 9° 7.917′ N, 98° 15.744′ E) from Jankaew et al. (2008) sampling sites (Fig. 1).

We extruded a gouge-auger sediment core obtained from Swale X in July 2014, and one sediment core collected from each of Swale X and Swale Y in June 2015. We collected sediment core from two consecutive years to examine whether eDNA proxy is constant from one time period to another. We sub-sampled different layers in each core on-site using sterile 50 ml conical Falcon tubes, which were then immediately stored in a dry shipper filled with liquid nitrogen to prevent any form of biological degradation (Brow et al., 2010; Armbrecht et al., 2019). In order to prevent cross-contamination between samples, we pre-treated all equipment and tools used, such as the gouge-auger, hand trowel, hacksaw with a 20% bleach solution and air-dried between samples (Armbrecht et al., 2019). We wore powder-free surgical gloves while sampling to avoid modern DNA contamination. The samples were later transferred to the −80 °C ultra-low freezer facility housed in the Asian School of the Environment, Nanyang Technological University, Singapore. We collected a total of 26 sediment samples from Phra Thong Island in July 2014 (Swale X = 9 samples) and June 2015 (Swale X = 10 samples, Swale Y = 7 samples).

3.2. DNA extraction and construction of amplicon sequencing library

We extracted environmental DNA (eDNA) from 250 mg sediment samples using the DNeasy PowerSoil Kit (Qiagen, Hilden, Germany) following the method described in Yap et al. (2021). We performed polymerase chain reaction (PCR) on the extracted eDNA to amplify a targeted fragment using a short single-stranded DNA primer set. We used a universal primer that amplifies the 16S ribosomal ribonucleic acid (rRNA) genes hypervariable region of three domains of life (archaea, bacteria, and eukaryotes); this primer targets the hypervariable V6 to V8 region - 926wF (AAA CTY AAA KGA ATT GRC GG) and 1392R (ACG GGC GGT GTG TRC) (Wilkins et al., 2013; Allen and Cavicchioli, 2017). The PCR reactions contained 12.5 μl of 2× KAPA HiFi Hotstart Ready Mix (KAPA Biosystems, Cape Town, South Africa), 5 μl each of the forward and reverse primers (1 μM concentration) and 2.5 μl of genomic DNA (5 ng/ml concentration). We held the reactions at 95 °C for 3 min to denature the DNA, followed by 20 cycles of amplification at 95 °C for 30 s, 55 °C for 30 s, and 72 °C for 30 s. The amplification was ended with a 72 °C for 5 min extension to ensure complete amplification. We used a minimum amplification cycle of 20 to minimize potential bias of amplifying certain taxa more efficiently by PCR (Pedersen et al., 2015). Each sample was amplified in triplicate using a ThermoFisher SimpliAmp Thermal Cycler, pooled and purified using the Agencourt AMpure XP PCR purification system (Beckman Coulter, Singapore). All amplified PCR products were then sent to the sequencing facility at Macrogen Asia Pacific Pte. Ltd. where a sequencing library was prepared using the MiSeq Reagent Kit v3 chemistry and sequenced with an Illumina MiSeq machine (300 bp paired-end).

3.3. Sequence analysis

The raw sequences generated from Illumina MiSeq were first processed by removing primers from the sequences using cutadapt v. 2.10 (Martin, 2011). Next, we assessed the sequencing accuracy using quality score defined as a property that is logarithmically related to the base calling error probabilities, that is, a quality score of 20 is equivalent to the probability of an incorrect base call in every 100-sequencing read (Ewing and Green, 1998; Ewing et al., 1998). We removed sequences that were shorter than 280 in forward reads (R1), and 230 in reverse reads (R2), and those that had a quality score of equal to or less than two and have an expected error rate (sum (10ˆ (−quality score/10)) of higher than two (R1) and five (R2) using the filterAndTrim function in DADA2 package in R (Callahan et al., 2016). After removing low-quality sequences, we constructed a table of amplicon sequences variants (ASVs) by calculating the sequence error introduced during sequencing using the DADA2 algorithm (Callahan et al., 2016). As ASVs are formed based on DNA sequence differences, ASVs are used to infer the samples' true biological composition (Callahan et al., 2017). We performed taxonomy classification using Ribosomal Database Project (RDP) naïve Bayesian classifier as implemented in R DADA2 package with reference to the SILVA database v. 132 (Quast et al., 2012; Yilmaz et al., 2014). We removed ASVs with a bootstrap value below 90% at the supergroup/phylum level to ensure the ASVs table used for downstream statistical analysis have high confident level taxonomy classification and removed ASVs with lower than ten counts to remove noise from low abundant ASVs. The final ASVs table contains 34,440 ASVs generated from the 16S rRNA gene dataset.

3.4. Grain size analysis

We measured the grains size distribution of the sediment samples collected from Swale X (except organic mud E due to limited availability) and Swale Y using the Malvern Mastersizer 3000, which uses laser diffraction technique to measure the grain size and distribution, following the method described in Switzer and Pile, 2015. We pretreated the samples with 15% hydrogen peroxides (H_2O_2) to remove organic material and washed the samples with distilled water at least three times to remove the H_2O_2 before analysis (Switzer and Pile, 2015). We omitted the treatment using hydrochloric acid (HCl) as there was no visible carbonate present in the samples. We sonicated each sample for 1 min to further disaggregate the sediment particles before measurement (Switzer and Pile, 2015). Data generated from Malvern Mastersizer 3000 was processed using GRADISTAT v. 9.1 (Blott and Pye, 2001) which uses Folk and Ward and Method of Moments techniques to classify the sediments (Folk and Ward, 1957).

3.5. Geochemical analysis

We measured total organic carbon (TOC) and total nitrogen (TN) concentrations in the sediment samples collected from Swale X and Swale Y (except organic mud B due to limited availability) to characterise the tsunami deposits and the non-tsunami derived layers in the geological record using geochemical properties (e.g., Shinozaki, 2021). We also want to test the effects of geochemistry toward microbial community differences.

We measured TOC and TN at the Stable Isotope Laboratory at the University of Hong Kong. 30 mg of sediment sample was placed in 5 mm × 9 mm silver capsules (Sercon) and treated with 6 N of HCl to acidify any residual carbonate before analysis. All samples were dried overnight at 60 °C, then combusted and analyzed using an Elemental Analyzer attached to an Isotope Ratio Mass Spectrometer (EA-IRMS).

3.6. Statistical analysis

We performed six statistical analyses using the phyloseq package

(McMurdie and Holmes, 2013), vegan package (Oksanen et al., 2020) and DESeq2 package (Love et al., 2014) in the R software environment v. 3.6.1 (R Core Team, 2021). The number of reads in each sample was normalised using median sequencing depth to minimise biases toward the dominant community.

(1) An alpha-diversity analysis, using Shannon index (H′), to examine the microbial community differences within individual layers (e.g., sediment samples collected from Sandsheet A) in each sediment core (Sinclair et al., 2015; de Sá et al., 2018).
(2) A simple two-tailed *t*-test to examine if the microbial community within each sediment layer of a core have significant differences (Holmes and Huber, 2019).
(3) A beta-diversity analysis, using Bray-Curtis dissimilarity distance, to examine the microbial community differences between two sample types (i.e., tsunami-deposited sand layers and non-tsunami derived organic layers) (Sinclair et al., 2015; de Sá et al., 2018). We square-root transformed the ASVs data before calculating the Bray-Curtis dissimilarity distance to minimize the effect of the major abundant ASVs. The beta diversity analysis was visualised using distance-based redundancy analysis (dbRDA). The ellipses in the ordination plot were calculated with a 95% confidence interval.
(4) A hypothesis test, using permutational analysis of variance (PERMANOVA; Anderson, 2001) and permutational analysis of dispersion (PERMDISP; Anderson, 2006) available in the vegan package (Oksanen et al., 2020), to examine the significant value of the observed beta-diversity differences.
(5) A predictive analysis, using distance-based linear modelling analysis available in the vegan package (Oksanen et al., 2020), to investigate the relationship between the microbial community Bray-Curtis dissimilarity and the environmental variables such as TOC, TN, and sampling depth observed in the dbRDA ordination plot. The correlation results were indicated as arrows in the ordination plot, where the length of the arrows was scaled to their r^2 value. As TOC and TN correlate with sample type, a follow-up simple two-tailed t-test using the stats package (R Core Team, 2021) was calculated to examine any significant TOC and TN differences between sample types.
(6) A differential abundance analysis, using a negative binomial generalised linear model available in the DESeq2 package (Love et al., 2014), to determine the ASVs that contribute to the

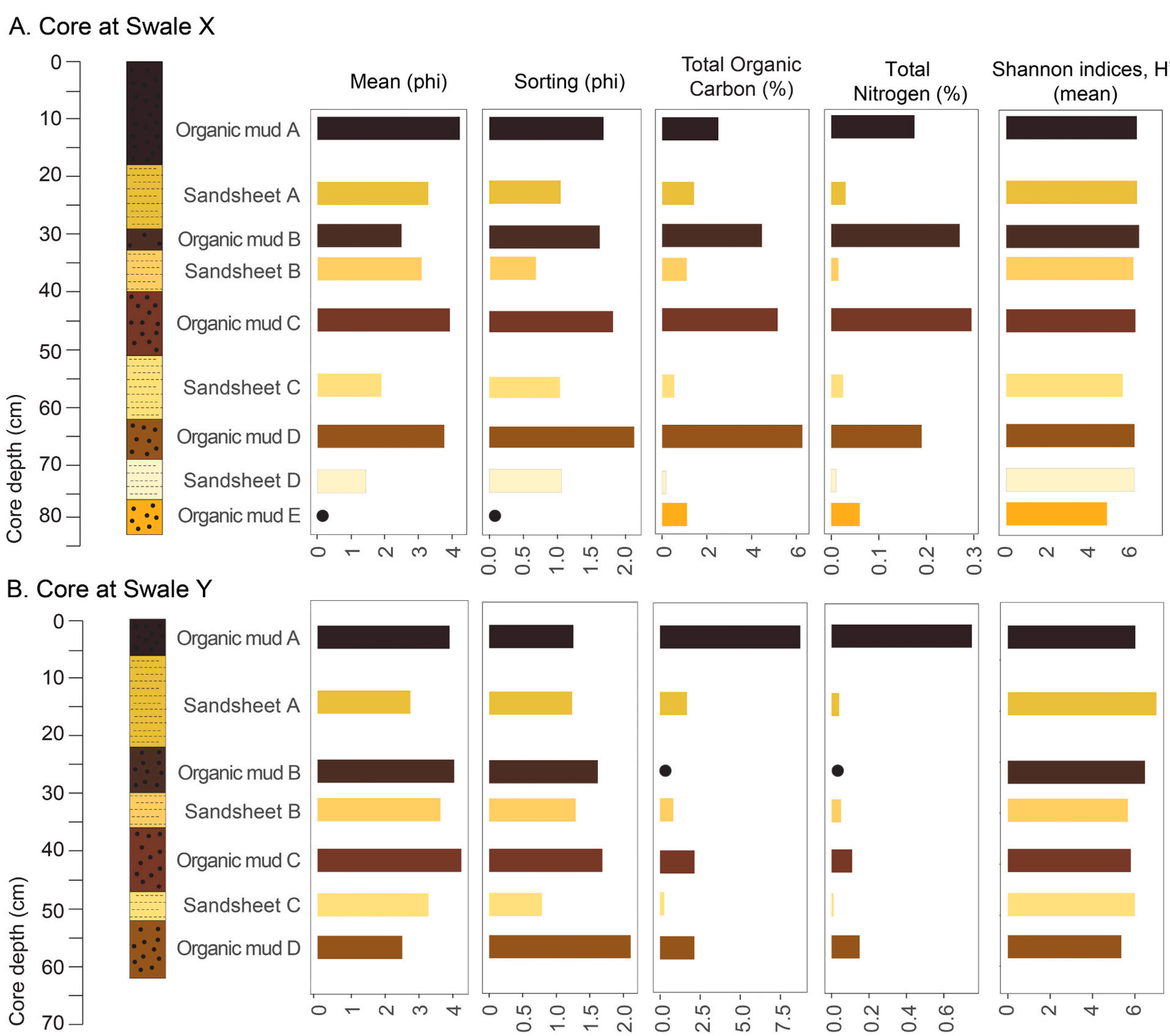

Fig. 2. Bar plots of grain size, chemical and molecular data analyses for representative sediment samples from each unit collected from Swale X (top row) and Swale Y (bottom row). The following are presented from left to right: Sediment core stratigraphy, grain size statistical data (mean and sorting), total organic carbon (TOC), total nitrogen (TN), and the microbial diversity within sediment samples are presented using Shannon's diversity indices (H′). Black circle indicates that the samples have limited quantity to perform the analysis.

differences between two sample types, (i.e., tsunami-deposited sand layers and the non-tsunami derived organic layers). The significant value of the result was calculated using the Wald test and adjusted based on the method described by Benjamini and Hochberg (1995) to correct the *p*-value for multiple hypothesis testing. ASVs with an adjusted *p*-values below 0.001 were selected, their abundances in each of the samples were presented in a bar plot.

4. Results

4.1. Microbial community differences in the geological records

The main objective of our work was to determine whether the microbial community in tsunami deposits could be differentiated from overlying and underlying non-tsunami sediments in the geological record.

The comparison between the tsunami-deposited sand sheets and the non-tsunami derived organic background sedimentation, using a pairwise comparison on the microbial alpha-diversity revealed no significant differences (t-test: Swale X, $t = 0.7045$, df = 7, p-value = 0.5039; Swale Y, $t = -0.6785$, df = 3.3936, p-value = 0.5408; Supplementary Table 1). This may suggest that the microbial community in the tsunami deposits were similar to the microbial communities preserved in the organic mud units. We observed that sediments in Swale X ($H' = 4.8578$ to 6.4104; Fig. 2) generally had a lower alpha-diversity in comparison to those found in Swale Y ($H' = 5.3572$ to 7.0136; Fig. 2).

The beta-diversity analysis (Supplementary Table 2C) also indicates the structure of the microbial community in the tsunami-deposited sand sheets and non-tsunami derived organic mud were similar. The constrained ordination using distance-based redundancy analysis (dbRDA) focused on the microbial community difference between the tsunami deposits and the organic- units (the distance between samples calculated based on microbial dissimilarity between samples). The first canonical axis (CAP1, responsible for 11.5% of the variance) in the dbRDA analysis (Fig. 3) revealed a distinct separation between the upper layers (Organic mud A, Sandsheet A, Organic mud B, and Sandsheet B) from the lower layers (Organic mud C, Sandsheet C, Organic mud D, Sandsheet D, and Organic mud E). Our results showed that the microbial community structure in the upper layers' samples was significantly different from those present in the lower layers' samples (PERMANOVA structure: pseudo-$F_{1,23} = 3.2205$, p-value = 0.0003 in Supplementary Table 2C). We observed a decrease in community dispersion of layers of comparable depth from the upper layers (average distance to median = 0.62) to the lower layers (average distance to median = 0.58).

The second canonical axis (CAP2, 5.7% of the variance; Fig. 3) in the dbRDA analysis, however, shows that among the upper layers (CAP1 > 0 in Fig. 3), Sandsheet A and Sandsheet B were clustered separately from Organic mud A, and Organic mud B (Fig. 3). There was a significant difference between the tsunami-deposited sand layers that intercalate with the background organic units in the upper layers (PERMANOVA: pseudo-$F_{1,11} = 1.4793$, p-value = 0.0269; Supplementary Table 2 A).

We observed that in the lower layers (CAP 1 < 0 in Fig. 3), Sandsheet C and Sandsheet D clustered more closely with Organic mud C, Organic mud D, and Organic mud E, and that this grouping is correlated strongly with their sampling depth (p-value = 0.002, Supplementary Table 3; Fig. 3). Our results showed that Sandsheet A (2004 IOT) and the youngest palaeotsunami deposit, Sandsheet B, have a microbial community structure that differs from non-tsunami samples. This is consistent with other studies summarized in Yap et al. (2021). However, the

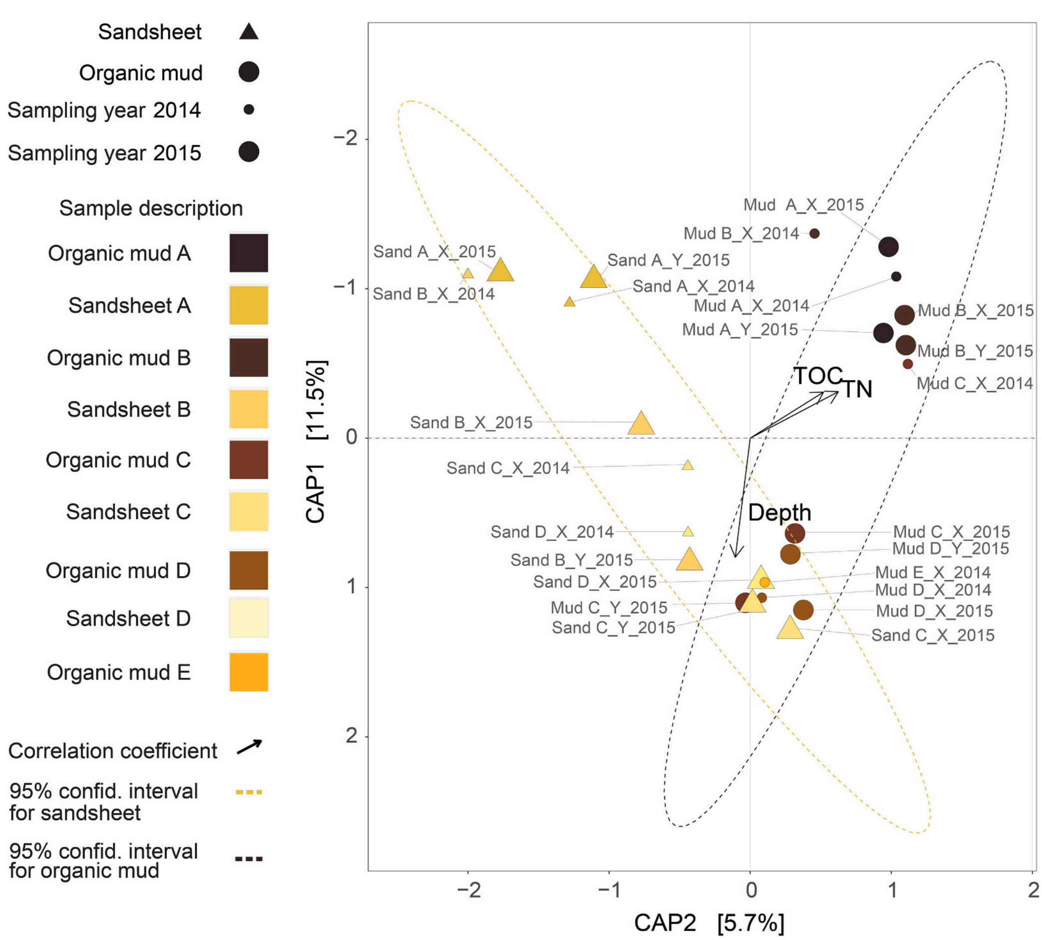

Fig. 3. Distance-based redundancy analysis (dbRDA) based on Bray-Curtis dissimilarity comparing the microbial community differences between two sample types, that is tsunami-deposited sand sheets (triangles) and organic non-tsunami derived layers (circles). The ellipses represent a 95% confidence interval within each sample type. The black arrows in the plot were environmental factors that had a significant (Supplementary Table 5, p-value < 0.05) influence on the microbial communities' difference between sample types. The environmental factors correlation coefficient scales the length of the arrows. The horizontal dashed line across the panel represents the separation between upper layers (Organic mud A, Sand A, Organic mud B, Sand B) and lower layers (Organic mud C, Sand C, Organic mud D, Sand D, Organic mud E) in the sediment core.

two oldest tsunami sands (Sandsheet C and Sandsheet D) do not have microbial communities that differ significantly from the background organic layers.

4.2. Understanding the relationship between grain size and geochemical properties toward microbial community differences

The sedimentary profile of Swale X shows that tsunami deposits were moderately sorted, medium to very fine sand (phi 1.44 - 3.13) whereas the background organic mud layers were poorly sorted, very fine sand (phi 3.05 - 4.12) (Fig. 2). Swale Y have similar sedimentary profile as Swale X with mean phi values increasing by 0.5 phi (Fig. 2). The geochemistry data showed that most of the organic mud layers in both swales contained higher TOC (0.63% - 8.74%; Fig. 2) and TN (0.03% - 0.75%; Fig. 2) as compared to sand layers (TOC: 0.03% - 2.01%, TN: 0.01% - 0.05%; Fig. 2). A simple two-tailed *t*-test revealed that the amounts of TOC and TN were significantly different between tsunami-deposited sand sheets and organic layers in Swale X (t-test: TOC, $t = 4.5121$, df $= 3.7424$, *p*-value $= 0.0124$; TN, $t = 7.1247$, df $= 3.1438$, *p*-value $= 0.0049$; Supplementary Table 4), but no statistical difference in Swale Y. Contrary to Swale X's chemical data, we observed that except for Organic mud A, there was minimal measurable chemical concentration in the organic mud layers in Swale Y.

Our result shows that there were no significant differences in TOC and TN between the upper and the lower layers in the geological records. We performed a distance-based linear modelling analysis to examine the relationship between each environmental variable with each ordination axis observed in Fig. 3 (Supplementary Table 3). Not surprisingly, the organic units (Organic mud A, and Organic mud B) are strongly correlated with TOC and TN (TOC: $r^2 = 0.6884$, *p*-value $=$ 0.005; TN: $r^2 = 0.6848$, *p*-value $= 0.005$; Supplementary Table 3; Fig. 3), whereas Sandsheet A and Sandsheet B, which were mostly devoid of organic material, were statistically independent with TOC and TN variables.

4.3. Searching for microbial tsunami indicators

We performed a differential abundance analysis using a negative binomial generalized linear model to identify potential microbial indicators of tsunami deposits. We identified a total of seven ASVs (representing the biological composition of the samples) (Fig. 4) abundant in the tsunami-deposited sand sheets but absent or present in low abundances in the organic mud layers. These ASVs were from the phylum: Acidobacteria, Nitrospirae, Calditrichaeota, Latescibacteria, Chloroflexi, Proteobacteria, and Crenarchaeota. We observed that these ASVs were mostly present in the upper tsunami-deposited sand layers (Sandsheet A and Sandsheet B in Swale X and Sandsheet A in Swale Y) but were absent in the lower tsunami-deposited sand layers (Sandsheet C and Sandsheet D in both swales; Fig. 4). Among these seven ASVs, we found that of the phylum: Nitrospirae, class: Thermodesulfovibrionia (ASVs assigned to this class were asv_015_00204 and asv_015_01747) were abundant in all the tsunami-deposited sand layers and absent in the organic mud layers at Swale X (Fig. 4). However, these two ASVs were only found in the modern tsunami-deposited sand layer (Sandsheet A) in Swale Y (Fig. 4). The phylum: Acidobacteria was the only taxon present in the lower tsunami-deposited sand layers in Swale X (not detected in

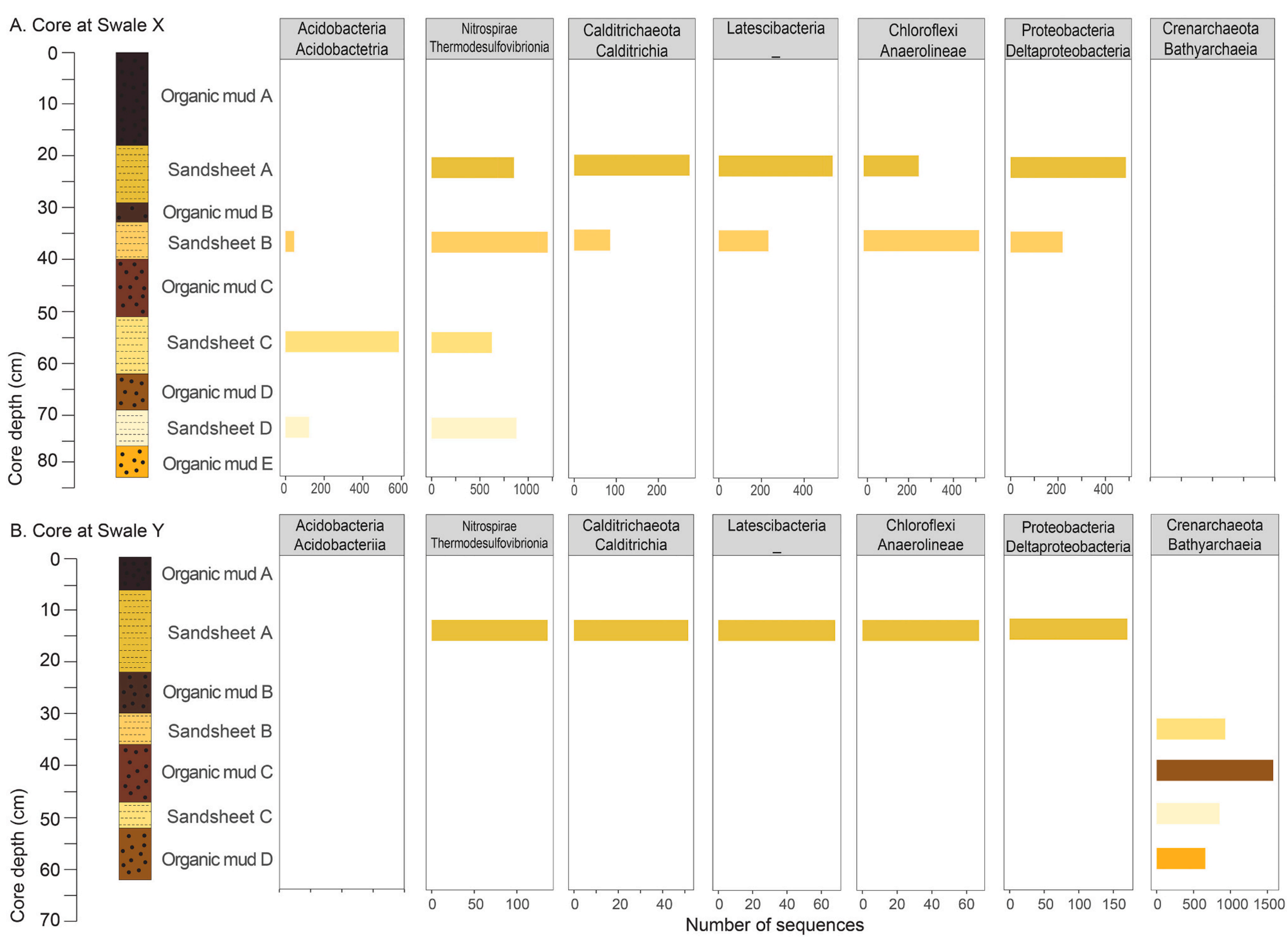

Fig. 4. Bar plots showing taxa that were present only in the tsunami-deposited sand layers and absent from the background organic mud in Swale X (top row) and Swale Y (bottom row). Each taxon is labelled with phylum-level followed by the assigned class-level.

Swale Y; Fig. 4).

5. Discussion

Yap et al. (2021) demonstrated that microbial community composition varies significantly between a modern overwash deposit formed by marine flooding events (either storm or tsunami deposits) and background soil and terrestrial sedimentation that are not affected by marine processes. They further highlighted that a microbial community-based approach could differentiate between the deposits of a modern tsunami and storm-surge flooding at two different locations (Yap et al., 2021).

5.1. Microbial community differ between tsunami-deposited sand sheets and organic mud layers in the geological record

Our results show that the microbial community in the tsunami-deposited Sandsheet A (the 2004 IOT deposits; Jankaew et al., 2008), and Sandsheet B, which formed approximately 700 years ago, differed significantly from the overlying and underlying non-tsunami derived organic mud units (Supplementary Table 2, Fig. 3). This microbial community differences correspond with the sedimentary profile and geochemical properties interpretation of the tsunami-deposited sand and the background mud unit in the geological record, which confirm the feasibility of utilizing eDNA to investigate palaeotsunami deposits. Despite at site where there are limited grain size and geochemical evidence, eDNA can still robustly differentiate overwash deposits from surrounding non-overwash derived sediments (Yap et al., 2021). We hypothesise that this microbial community differences are caused by a combination of three factors. One, the initial community is severely impacted and disturbed by tsunami flooding that result in local extinction of some taxa. Second, the altered environment (changes in pH, salt content and nutrient availability etc.) creates a new ecological niche. This, in combination with the third factor, where new taxa are transported and deposited to the environment, will result in a new community profile in the flooded layer.

The microbial community differences observed in this study are mostly independent from bioturbation and post-depositional changes. Jankaew et al. (2008) reported that there is no visible mixing by burrowing organisms and the island is largely unpopulated with limited infrastructure and human modification (Masaya et al., 2020). Our grain size results show that tsunami deposits are composed of medium to very fine sand with larger mean grain size as compared to the background organic mud layers (Fig. 2). The reported result is the same as the sedimentary profile described in pioneering 2004 IOT studies of the same site (Fujino et al., 2008; Jankaew et al., 2008; Fujino et al., 2009) even though the sediment cores of this work were obtained ten years after the event. Similarly, the geochemical properties of the swales remain distinctive between the sequences (Fig. 2) despite experiencing high seasonal rainfall over the past decade. The microbial community changes observed in this work are not an artifact of natural coastal processes.

5.2. Factors limiting the utility of eDNA to identify palaeotsunami deposits from a geological record

Our results show that the lower tsunami-deposited sand layers, that is, Sandsheet C, which formed 2200 years ago and Sandsheet D, the oldest palaeotsunami deposit that formed around 2800 years ago (Jankaew et al., 2008), had relatively similar microbial communities to the organic mud layers formed above and below the high energy marine deposits (Fig. 3, Supplementary Table 2 B). In addition, within the geological record, we noticed a microbial community differences between tsunami-deposited sand layers and organic mud layers below Sandsheet B (lower layers), and tsunami-deposited sand and organic mud layers above it (upper layers) (Supplementary Table 2C). The microbial community variance in the upper layers decreases in the lower layers indicating that the lower layers had a relatively homogenous microbial composition. The relatively similar microbial community in the lower layers is likely the product of multiple factors that reflects the influence of both time, sediment diversity and aquatic chemistry in the permanently saturated environment of the lower swale sequence.

We suspect that this limitation for differentiating tsunami-deposited sand layers and organic mud units in the lower sequence might be due to local environmental variables such as the annual groundwater fluctuation that is observed at our study site (Jankaew et al., 2008; Gouramanis et al., 2017; Pham et al., 2017). The swales on Phra Thong Island have groundwater levels that are influenced by tides and freshwater absorption into the sediment from monsoonal rainfall and the hot-dry intermonsoon periods (Choowaew, 2016). When the team visited Phra Thong Island in July 2014 and June 2015, we needed to use an auger to retrieve the sediment core instead of digging a pit at the swale as the groundwater table was high, whereas some studies report the collection of sediment samples from a pit or trench at the swale when the groundwater level was much lower (Jankaew et al., 2008; Gouramanis et al., 2017; Pham et al., 2017). The variability in the groundwater level may have resulted in a homogenous and resilient 'groundwater-adapted' microbial community that displaced some of the non-groundwater-adapted communities recovered from the upper layers. The constant changes in the environmental conditions and the intermixing of freshwater community and sediment community may result in much more stable microbial community in the sediment that is resistant to disturbance. Several experimental studies have demonstrated that repeated, small-magnitude pulse disturbances can lead to a more metastable community (Herren et al., 2016; Calderón et al., 2018; Jacquet and Altermatt, 2020). Future work may clarify this issue by looking at the groundwater microbial community, as well as the groundwater physiochemical parameters such as pH, electrical conductivity, dissolved organic carbon, and compare these against the sediment microbial community changes before and after the wet season to understand the effects of groundwater on the sediment microbial community in both the swales.

Interestingly, we observed that the microbial community in Swale X was significantly different from those in Swale Y (Supplementary Table 2). Based on the differential analysis result (Fig. 4), Calditrichia, Latescibacteria, Anaerolineae, Deltaproteobacteria were found in Sandsheet A and Sandsheet B in both swales. The primary differences between Swale X and Swale Y are noted in the lower layers where Thermodesulfovibrionia was present in all the tsunami-deposited sand layers in Swale X, and Swale Y Sandsheet A, but not in Swale Y deeper tsunami deposits. We suspect that these differences were due to a complicated sand emplacement mechanism in Swale Y, as discussed by Gouramanis et al. (2017). Gouramanis et al. (2017) highlighted that the sedimentation in Swale Y could be affected by tidal variations and swash bar sedimentation as he identified several previously unrecognized sand sheets compared to known tsunami sand sheets from closely spaced auger cores in Swale Y. The investigation of palaeotsunami deposits is often challenging and complicated because of limited knowledge of a site stratigraphy, for example, we could not determine whether the groundwater regime at Swale X and Swale Y is similar or different. Further studies at sites where there is limited facies variability or groundwater fluctuation will allow this hypothesis to be tested further, such as a coastal lake in Chile where nine tsunami deposits were found (Kempf et al., 2017), another coastal lake in Norway where three tsunami deposits were found (Bondevik et al., 2005), tidal marshes along the Cascadian margin where >60 sites contain potential or confirmed well-preserved tsunami deposits (Peters et al., 2007), or the coastal cave in Aceh, Indonesia where at least 11 prehistorical tsunami deposits were found (Rubin et al., 2017).

5.3. eDNA tsunami indicator identifying the tsunami sediments

On Phra Thong Island, seven ASVs were abundant in the tsunami-deposited sand sheets, while they were missing from the organic mud layers (Fig. 4). We wanted to examine if these ASVs are the potential eDNA tsunami indicator, that can be used to discriminate tsunami deposits from other types of deposition, and further analysed whether these ASVs were present in India, that was also impacted by the 2004 tsunami event, using Yap et al. (2021) dataset. Unfortunately, we found none in the Cuddalore, India's samples, implying that individual taxa detected using community analysis are insufficient to produce a robust eDNA tsunami indicator. Perhaps a taxa-specific analysis will have a higher sensitive to identify an eDNA tsunami indicator (Szczuciński et al., 2016). This study and Yap et al. (2021) both reinforce that the microbial tsunami indicator is site-specific, affected by geomorphological configuration, local topography and the nature of the sediment source. Besides sedimentary features, climatic and other environmental factors may also affect microbial tsunami indicator. Similar limitations are observed in almost all other proxies employed in tsunami geological study (Table 1), thus, integrating multi-proxy approach is essential to improve the accuracy of tsunami deposits interpretation in the geological record (Costa and Andrade, 2020).

6. Conclusions

Here, we investigated the changes in microbial community in response to a series of tsunami flooding disturbances on the western coast of Thailand in the past 2800 years. We found that microbial community differences can help distinguish the 2004 IOT and 700-year-old tsunami deposits from non-tsunami derived layers, however, the same approach did not adequately distinguish the 2200- and 2800-year-old tsunami deposits from overlying and underlying non-tsunami derived sediments. The investigation of the older tsunami layers was likely complicated by a variety of environmental factors at Phra Thong Island. Thus, it will be interesting to investigate historical and pre-historical tsunami deposits preserved in geologically stable environments such as caves, lakes, perpetually saturated ponds, marshes or wetlands to expand the application of environmental DNA in geological studies.

Identifying palaeotsunami deposits using eDNA expands the range of palaeotsunami proxies, thus improving the accuracy and efficiency of future palaeotsunami studies. Our study shows that the 2004 IOT deposit and preceding tsunami deposits contain microbial communities that differ from the overlying and underlying organic mud layers. The results from Phra Thong Island contribute to our understanding of soil microbial community dynamics in the context of a catastrophic natural disturbance, in this case a tsunami, and hence the interpretation of microbial community resistance and resilience post-disturbance. Our work represents a significant step forward in palaeotsunami research, as the geological record is critical for understanding what happened in the past beyond human knowledge, and for developing an accurate tsunami risk assessment for coastal communities.

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Data availability

Raw sequences data used in this study have been deposited in an open-source online data repository hosted by National Center for Biotechnology Information (NCBI) GenBank, readily accessible under the BioProject ID PRJNA343068. All statistical results are reported in https://github.com/wenshu-yap/Palaeotsunami-microbes.git

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.margeo.2023.106989.

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