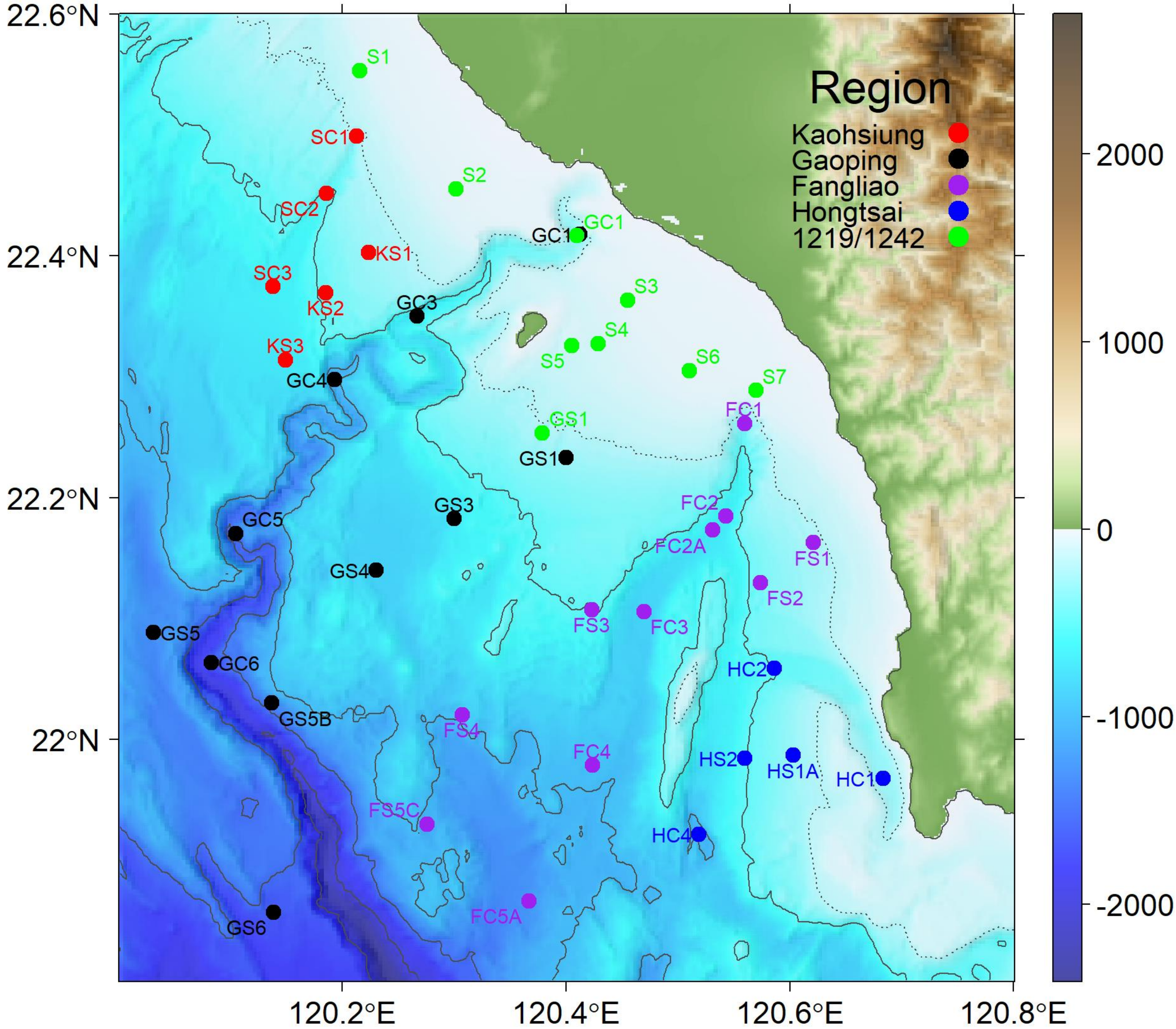

Fig. 3. OR1-1219、1242 及過去沉積物採樣及培養站位。

3. 延續性研究計畫背景

Submarine canyons are effective conduits of river-borne and marine sediment into the deep sea (Harris & Whiteway 2011). The delivery, transport, and trapping of organic materials in the canyon may provide essential energy to the generally food-scarce deep ocean (Rex et al. 2006, Wei et al. 2010). Nevertheless, mass wasting events (Coleman & Prior 1988, Okey 1997, Puig et al. 2004, Canals et al. 2006) in the canyon also cause severe disturbance and thus the local extinction or alteration of benthic community structure (Liao et al. Submitted, 2017, Okey 1997). In the Gaoping Submarine Canyon (GPSC) off the SW Taiwan, sediment mass flux exceeding 700-800 g m^{-2} day^{-1} and substantial organic carbon accumulation has been documented by near-bottom sediment traps and ^{210}Pb activities in the down-core sediment (Liu & Lin 2004, Liu et al. 2006, 2009, Kao et al. 2006, Huh et al. 2009, Hsu et al. 2014). Meanwhile, strong bottom currents driven by internal tides (Wang et al. 2008, Lee et al. 2009a b, Chiou et al. 2011) and gravity flows triggered by storm surges, river flooding, and earthquakes (Hsu et al. 2008, Su et al. 2012, Liu et al. 2013, 2016) also frequently occur in the GPSC. Due to these unique physical conditions, GPSC has been considered as a natural laboratory for studying sediment dynamics (Liu et al. 2013, 2016) and environmental control on benthic communities (Liao et al. Submitted, 2017).

In the past FATES project, we found that the benthic communities in the upper GPSC were mainly a subset of the adjacent slope assemblages (Liao et al. 2017). Several abundant taxa (e.g., ostracods, peracarid crustaceans, and mollusks) on the slope were greatly reduced or disappeared from the canyon (Liao et al. 2017). Among the taxa occurred in both habitats, the canyon nematodes were dominated by non-selective deposit feeders, but the slope assemblages were comprised of diverse functional groups, including non-selective deposit feeders, epigrowth feeders, and predators/omnivores (Liao et al. Submitted). Also, the slope polychaetes were mostly habitat engineers, discretely motile/sessile suspension feeders, and omnivores/predators (Chen 2018). In contrast, the canyon polychaetes were dominated by burrowing, motile subsurface deposit feeders (Chen 2018). By linking the biological observations to modeled internal tides (Jan et al. 2008, Chiou et al. 2011), we found that internal tide energy negatively affected and food availability positively affected the abundance, taxonomic diversity and functional diversity of benthic communities (Liao et al. Submitted, 2017, Chen 2018). Nevertheless, the internal tides also negatively influenced the food supplies through sorting for low-organic contents, coarse sediments. The benefit of enhanced organic matter delivery to the canyon floor (Liu & Lin 2004, Liu et al. 2006, 2009, Kao et al. 2006, Huh et al. 2009) was likely overwhelmed by its physical disturbances through erosion and resuspension.

Among the well-studied river-canyon systems, for example, the Hudson Canyon (Rowe et al. 1982), Mississippi Canyon (Baguley et al. 2008, Wei et al. 2012) and Kaikoura Canyon (De Leo et al. 2010), the benthic standing stocks were usually elevated along the canyons axis and near the canyon head. Even many canyons without the riverine influence, such as Whittard (Amaro et al. 2016), Nazaré (Tyler et al. 2009) and Scripps, and La Jolla Canyons (Vetter & Dayton 1998), were hotspots of benthic abundance and biomass. In contrast, the GPSC was a cold spot of benthic abundance and diversity (Liao et al. Submitted, 2017, Chen 2018). The scale of biological responses to physical disturbance was unprecedented, suggesting that the GPSC may be a new paradigm in the study of submarine canyon ecology. An intriguing question remained is how the benthic communities in the GPSC compared to that in the other canyon systems off the SW Taiwan? Whether the environmental controls on the benthic communities may differ between the SMR-fed canyon (e.g., river connected and shelf-incising Gaoping Canyon) and the blind canyons (e.g., slope-incising Kaohsiung, Fangliao and Hongchai canyons)? Through the FATES project, we have continued to explore the canyon-slope systems of different origins to better understand how the complex seafloor morphology may contribute to the benthic diversity of the continental margin (Fig. 3).

Between the GPSC and adjacent slope, the internal tide flushing and episodic submarine geohazards (e.g., turbidity currents and debris flows) are the main drivers for variation in benthic community structure (Liao et al. Submitted, 2017). The sediment erosions by bottom currents sync with the tidal cycles and likely have long-term, recurrence, and pressing effects on seafloor communities (Okey 1997, Harris 2014). In contrast, the mass wasting events occur on longer timescales (e.g., seasonal, annual or decadal) but likely have devastating effects (Hsu et al. 2008, Huh et al. 2009, Su et al. 2012, Liu et al. 2012, 2013). Such contrasting environmental and biological properties provide a unique opportunity to study the cumulative impacts and recovery of benthic communities, and more importantly, the impact and recovery of their ecosystem functioning (Thrush & Lohrer 2012, Snelgrove et al. 2014). By textbook definition, the ecosystem functioning is the flow of matter and energy transferring within or between trophic levels or ecosystems (Danovaro et al. 2008, Loreau 2008). For example, sediment reworking and irrigation by burrowing infauna and epifauna may affect microbial carbon remineralization, sediment oxygen penetration, carbon storage, and nutrient regenerations (Lohrer et al. 2004). The feeding, predation, growth, and mortality of the benthos also directly affect the productivity, carbon sequestration, nutrient cycling and organic matter decomposition on the seafloor (Snelgrove et al. 2014). Off the SW Taiwan, the GPSC received 14-49 MT of sediments from the Gaoping Rivers each year (Hsu et al. 2014). On average, more than 27 g m^{-2} d^{-1} of sediments are accumulated on the upper GPSC seafloor, which is approximately 4 to 270 times less than the mass flux measured from the sediment traps (Huh et al. 2009, Hsu et al. 2014). Assuming the equal amount of organic carbon (OC) in both estimates, the majority of the OC is likely exported down the GPSC and buried in the deep South China Sea (Liu et al. 2013, 2016, Kao et al. 2014, Hsu et al. 2014). However, this view completely ignores the role of the sediment benthos, which likely remineralizes the OC through their feeding, burrowing, respiration and predation activates, and may lead to erroneous estimates of the OC cycling on the seafloor (Snelgrove et al. 2017).

In this continued project, we will measure and contrast benthic carbon stocks and carbon cycling of the heterogeneous seafloor off SW Taiwan. The carbon flows entering the system, between the stocks and leaving the system, will be measured by the *in-situ* lander and ship-board sediment incubation, derived from literature or model to evaluate the carbon cycling (i.e., one of the most crucial seafloor ecosystem functions) among the heterogeneous seafloor habitats. To the best of our knowledge, only two studies so far attempt to construct comprehensive benthic carbon food web in the submarine canyons. In the northern Gulf of Mexico, the carbon food webs were contrasted between the head of Mississippi Canyon and the adjacent mid-slope (Rowe et al. 2008). In another study, the carbon food web was contrasted within three sections of the Nazaré Canyon off the coast of Portugal (van Oevelen et al. 2011). In the Gulf of Mexico, extremely high abundance (> 20,000 individual m^{-1}) and biomass (> 10 g m^{-1}) of macrofauna were found at the head of the Mississippi Canyon (Wei et al. 2012). As a result, the relative role of bacteria and meiofauna in the total OC remineralization was reduced (comparing to the slope environment) and almost 40% of rain of POC was exported down the canyon (Rowe et al. 2008). In the Nazaré Canyon, the prokaryotic uptake of DOC and its respiration to DIC, nonselective feeding by meiofauna, and predation and scavenging by macrofauna dominated the carbon cycling in the upper canyon. In contrast, the megafauna deposit-feeders dominated the carbon cycling in the mid-canyon and all carbon flows diminished in the lower canyon (van Oevelen et al. 2011). Nevertheless, we expected that carbon cycling in the submarine canyons off the SW Taiwan would be drastically different from that of the Mississippi or Nazaré Canyons (Rowe et al. 2008, van Oevelen et al. 2011) due to their high energy setting (Liu et al. 2013, 2016) peculiar biology (Liao et al. Submitted, 2017, Chen

2018).

Given the technical problems of the previous lander experiments, in this continued project, we will mainly derive the carbon stocks and flows from ship-board measurements or literature when applicable. For this proposed project, the main task will be to complete the meiofauna sorting and body size measurements from the previous field surveys (i.e., macrofauna were mostly completed). We will continue to push for *in-situ* lander experiments, but fully recognized the risk of not getting enough *in-situ* observations for the carbon budget model. Therefore, we will focus on the technical aspect of this continued project more on the development of lander technology for future long-term, deep ocean monitoring. The data generated from *in-situ* observation will mainly use to validated the ship-board incubation experiment. More importantly, in the past year, we have encountered, overcame, but also recognized many technical problems during the first two sea trails of the benthic lander, which made us to modified the operation procedures and reconsidered the lander design to better fit to our research purpose and the environment in Taiwan. These step-by-step adjustments and gradual capacity building are necessary given the novelty and risk involved in the benthic lander technology. Based on these experiences, in the new proposed project, we plan to re-design and construct a lightweight, self-sustained benthic chamber. The new chamber will be made from plexiglass and is light enough to be deployed by SCUBA diver, remotely operated vehicle (ROV), or benthic lander. By replacing the stainless-steel boxcorer with an plexiglass chamber, we will transform the lander to a more lightweight and simplified system. The major difference is that the plexiglass chamber cannot recover sediment and is about 130 kg lighter than the stainless-steel boxcorer and thus greatly reduced the risk of anchoring effect during the lander recovery. The lightweight and simplified system will also give us more confidence in the initial autonomous lander deployment in this continued project. With more success under our belt, we can switch the lander configuration between sampling or non-sampling depending on the scientific purposes. Ultimately, in the future, we hope to operate the benthic lander regularly down to its maximum depth at 6000-m water depth.

（二）研究方法、進行步驟及執行進度。請分年列述：1.本計畫採用之研究方法與原因及其創新性。2.預計可能遭遇之困難及解決途徑。3.重要儀器之配合使用情形。4.如為須赴國外或大陸地區研究，請詳述其必要性以及預期效益等。

1. 本計畫採用之研究方法與原因及其創新性。

1.1. Experimental design

Sediment carbon budget: In the deep-sea sediments, the total inventory of organic carbon (OC) can be partitioned into the living and non-living components (Fig. 4). The living component of OC is mainly contributed by the biomass of prokaryotes (mainly bacteria), protozoan (mainly foraminifera), meiofauna (> 0.04 mm in length) and macrofauna (> 0.3 mm in length) (Rowe 1983, Rex et al. 2006, Wei et al. 2010). The non-living component of OC includes labile (i.e., fresh phytodetritus or chlorophyll-a contents), semi-labile (i.e., lipid, protein, and carbohydrate), and refractory OC (i.e., humic and fulvic acids, structural carbohydrates, and “black” carbon) (Danovaro 2010). The source of OC is mainly supplied by the slow rain of particulate organic carbon (POC) from the euphotic zone or lateral advection of POC from terrestrial or marine organics.

The loss of OC balances the source through carbon remineralization (SCOC), biological utilization of labile OC, the predation among living components of OC, long-term burial of the refractory OC and down-slope OC export (i.e., turbidity currents) (Fig. 4).

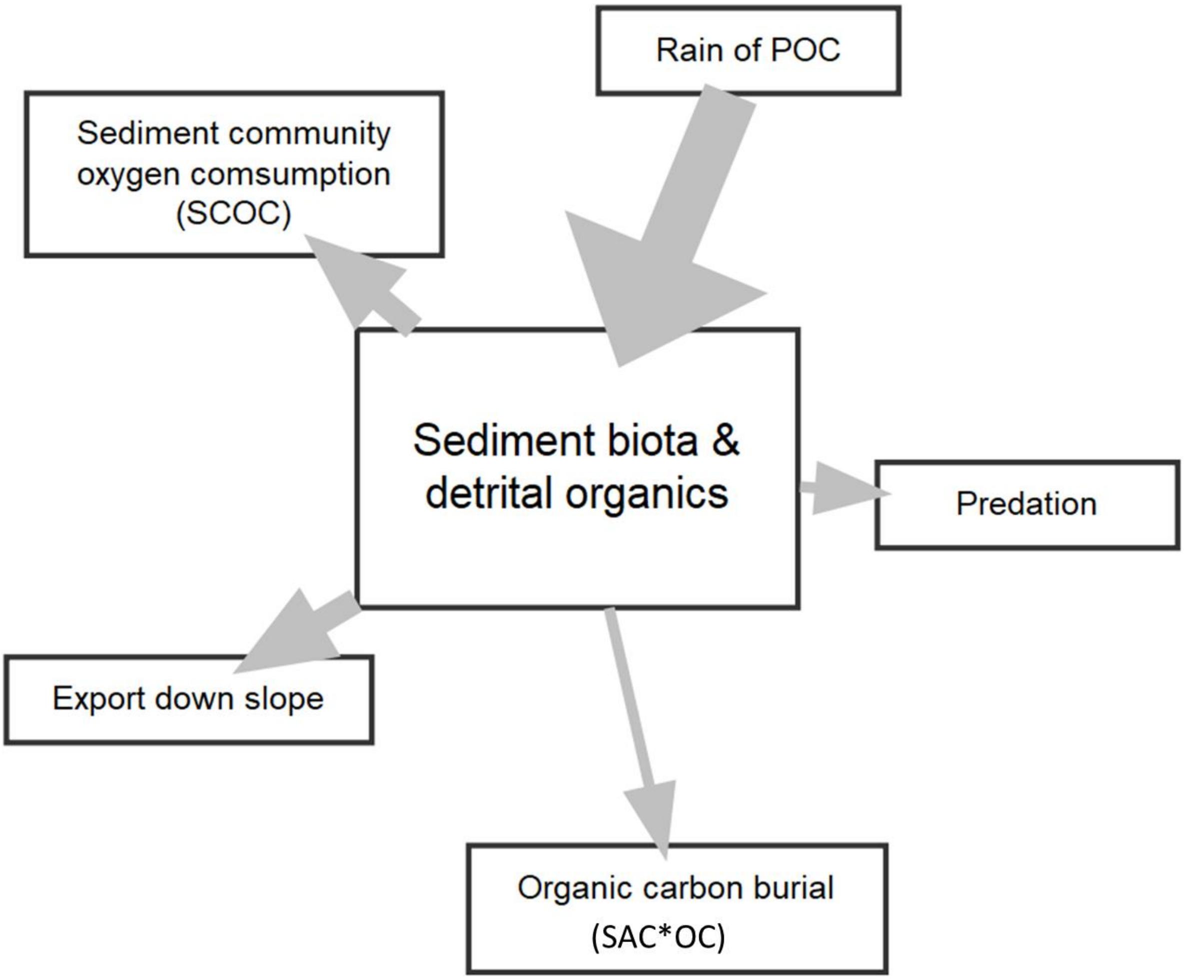

Fig. 4. Carbon budget and cycling in the deep-sea sediment. The conceptual model, from Rowe et al. (2008). The mass flux at the head of GPSC likely exceeds 700-800 g m^{-2} d^{-1}, which is equivalent to 3200 mg C m^{-2} d^{-1}, assuming 0.4% organic carbon (Liu & Lin 2004, Liu et al. 2006, 2009). The export POC flux on the shelf break of the northern South China Sea was estimated to be 48 ± 6 mg C m^{-2} d^{-1} (Hung & Gong 2010). The contrasted POC input and physical condition between two the canyon and slope habitats likely contribute to distinctively different carbon budgets and cycling.

Field sampling: Field sampling was conducted in four submarine canyon systems off the SW Taiwan (Fig. 3). 1) The Gaoping Submarine Canyon (GPSC) connects to a small mountain river and subjects to strong physical disturbances, including strong bottom currents and frequent submarine geohazards. 2) Kaohsiung Submarine Canyon (north of the GPSC) and Fangliao Submarine Canyon (south of the GPSC) are initially caused by the slope failure along the shelf edge and further evolved due to subsequent sediment sliding and slumping (Yu & Lu 1995, Yu & Chiang 1997). 3) Further south, the Hongtsai Submarine Canyon originates from both the tectonic and submarine erosional processes and develops into a U-shape channel west of the Hengchun Peninsula (Yu & Chiang 1995). Depending on the canyon morphology, these submarine canyons can be classified into river-connected canyon (or Type 1 canyon), such as the Gaoping Canyon, the shelf-incising canyon (or Type 2 canyon), including Kaohsiung and Fangliao Canyons, and the blind canyon (confine to slope or Type 3 canyon), such as Hongtsai Canyon (Harris & Whiteway 2011, Chiang & Yu 2017). Samples from the adjacent slope of these major canyon systems were taken for comparisons. In 2019, we also extended our sampling to the continental shelf from Kaohsiung to Pingtung (OR1 1219 & 1242), representing the potential organic deposition area of the Gaoping River. The variations in the origin, evolution, morphology, sediment transports and hydrodynamic regimes of the submarine canyons, slope, and shelf, contribute to the habitat heterogeneity of continental margin off SW Taiwan and we attempt to reveal how the topographic complexity may affect the carbon cycling processes on the seafloor. At each location, we deployed a megacorer (Fig. 5a). Each deployment recovered 12 sediment tubes (i.d. 11.5 cm). Among the recovered sediment samples, three tubes were used for porewater oxygen profiling and collecting for bacteria, meiofauna, and foraminifera samples. The other three tubes were used for ship-board core incubation (Fig. 5b), porewater oxygen profiling (Fig. 5c) and then the sediments were retained for macrofauna samples. One separated tube were used for grain size, sediment TOC/TN, chlorophyll-a, phaeopigment measurements, and PAH. All the remaining tubes (if available) were retained for macrofauna PAH. In addition to the existing samples, we will propose to deploy a pair of dark and light chambers from a benthic lander (or from a remotely operated vehicle, ROV Triton XLX) to measure sediment community oxygen consumption (SCOC) at a depth of approximately 200 m (Fig. 3, Station GS1). Detailed procedures are described in the following sections. An megacore will be depolyed at the same station and the same sediment samples will be taken. If the ROV Triton XLX is available, push cores will be taken from a manipulator instead. The SCOC from ship-board core incubation will be compared with the in-situ SCOC from the chambers.

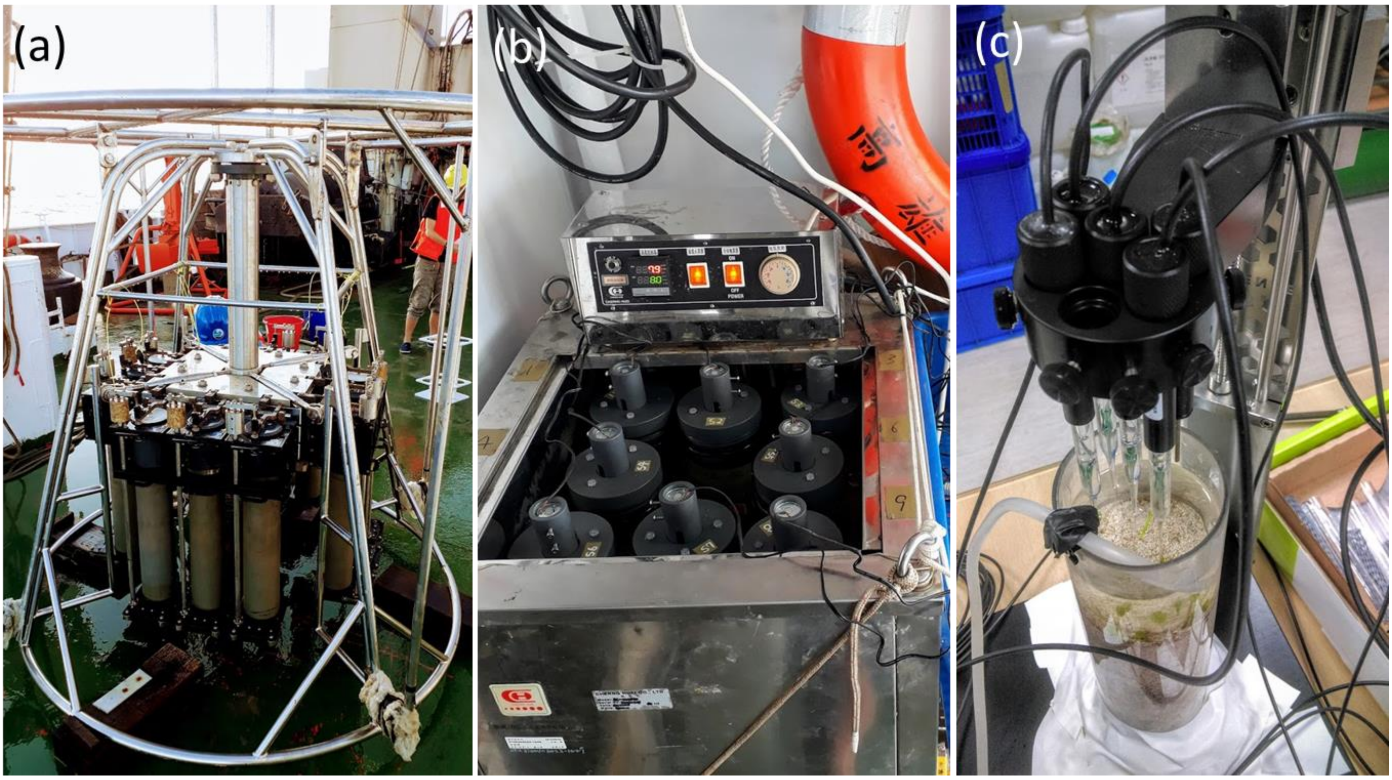

Fig. 5. Biological and geochemical samples from (a) megacorer , (b) ship-board sediment incubation, and (c)

sediment microprofiling .

1.2. Sediment biota and detrital organic

Prokaryote biomass: Sediment subsamples will be taken by a cutoff 10-ml sterile syringe (i.d. 15 mm) and collected at depths of 0-1 cm from the extruded sediments within a polycarbonate core. Similar to the operation of a piston core, the syringe plunger will be held fixed at the sediment surface, and then the barrel will be pushed into the sediment to take 2 mL of the sample. The syringe sample will be added to a 15-mL polyethylene centrifuge tube containing 2 mL of pre-filtered PBS solution. Approximately 0.3 mL 16% formaldehyde will also be added to the centrifuge tube until the sample reaching a final concentration of 2% formalin and then stored the sample at 4˚C. In the lab, the sediment samples will be further diluted by 500- or 1000-fold in PBS solution depending on the number of potentially interfering particles, treated with Triton-X detergent to loosen attached or aggregated cells, centrifuged through Nycodenz® and then placed on a 0.2-µm pore size filter, stained with SYBR Green and DAPI stains, and mounted on a slide for enumeration (Deming & Carpenter 2008, Kallmeyer et al. 2008). The prokaryote abundance will be determined by epifluorescence microscopy and the cellular dimensions in each slide will be estimated from an image taken by a CCD digital camera. The mean biovolume of cell sizes in each sample will be calculated assuming each cell is a sphere [(4/3) r3× π]. The biomass will be converted from biovolume using a conversion factor of 310 fg C µm−3 (Fry, 1990). The prokaryote biomass will be conducted by postdoc 廖健翔 (IONTU) applying through this grant application in collaboration with 林立虹 at Department of Geoscience, NTU.

Foraminifera biomass: Surface 1-cm of sediment will be retained due to most of the living foraminifera inhabiting near the surface (Rathburn & Corliss 1994). An equal volume of 10% buffered formalin (with borax, sodium tetraborate $Na^2B^4O^7$) and rose bengal (1 g L^{-1}) will be added to the water or sediment samples to make a final concentration of 5% formalin. The sample will be allowed to stain for a least one week. In the lab, the sediment samples will be first freeze-drying and wet sieved through a 63-µm and 150-µm sieve. Only individuals are intensively pink, or red-stained is counted (individuals g^{-1} dry sediment), picked out and sorted for identification. The organic carbon biomass will be converted from maximum diameter and length using species-specific linear equations from Altenbach (1985) and Kurbjeweit et al. (2000). The foraminifera biomass determination will be collaborated with FATES co-PI 林慧玲 (NSYSU).

Meiofauna biomass: Sediment subsamples will be taken by a cutoff 10-mL plastic syringe (i.d. 25 mm) and collected the surface 5 cm of the extruded sediments as suggested by Montagna (2017). The syringe plunger will be held fixed at the sediment surface, and then the barrel will be pushed into the sediments to create vacuum suction to draw the sediment samples into a 250-ml specimen jar. An equal volume of 10% buffered formalin (with borax, sodium tetraborate $Na^2B^4O^7$) will be added to the water or sediment samples to make a final concentration of 5% formalin. Upon returning to the lab, the sediment samples will be wet sieved through a 1000-μm sieve with a 40-μm sieve underneath and then add rose Bengal for a least one week before transferring to 70% ethanol. The meiobenthos specimens will be extracted from the sediments using Ludox (colloidal silica) flotation method (Danovaro 2010, Montagna et al. 2017) and enumerated to major taxonomic groups under a high power stereomicroscope (Olympus® SZX16; 0.7-11.5 X zoom). The body volume of meiofauna specimens (100 randomly selected nematodes & all harpacticoids) will be estimated by its geometric shapes (i.e., cylinders or ellipsoid) based on images taken by a digital camera and analyzed by image analysis software ImageJ. The volume will be converted into wet weight, assuming a specific gravity of 1.13 (Warwick & Gee 1984) and organic carbon using the conversion factor of 12% (Baguley et al. 2004). The meiofauna biomass and nematode identification will be conducted by postdoc 廖健翔 (IONTU) and graduate/undergraduate students applying through this grant application.

Macrofauna biomass: Once the sample is recovered, the supernatant water above the sediment surface will be siphoned carefully through a 300-μm sieve. The top 10 cm of the sediments will be extruded (by an extruder) and washed with filter seawater (with a 5-μm filter) through the same 300-μm sieve as suggested by Montagna (2017). In our previous surveys, we also found that most of the macrofauna were in the top 10 cm of the sediments (Liao et al. 2017). The remaining sediments will be kept in a 250-mL specimen jar. An equal volume of 10% buffered formalin (with borax, sodium tetraborate $Na_2B_4O_7$ and Rose Bengal) will be added into the sampling jars to fix the samples for at least 24 hours (yielding a final 5% of formalin solution) and then transfer to 70% ethanol for permanent preservation. Macrobenthos samples will be sorted and enumerated into major taxonomic groups and polychaete genus using a stereo sorting microscope (Olympus® SZ61; 0.67-4.5X zoom) and permanently preserved in 70% ethanol. The body volume of macrofauna specimens will be estimated by its geometric shapes (i.e. cylinders or ellipsoid) based on images taken by a digital camera and analyzed by image analysis software ImageJ. The volume will be converted into wet weight assuming a specific gravity of 1.13 (Warwick & Gee 1984) and then multiplied by 4.3 % to obtain the organic carbon content (Rowe 1983). The macrofauna sorting and biomass measurements will be conducted by research assistant 劉妍莉 (IONTU) and graduate/undergraduate students applying through this grant application. The macrofauna PAHs measurement will collaborate with FATES co-PI 李宗霖 (NSYSU)

Detrital organic carbon: Surface sediment will be taken and stored in 50-ml centrifuge tube in -20°C freezer. In the lab, an aliquot of frozen sediment (up to 2 g) will be extracted (12 h at 4 °C in the dark) with 8 ml of 90% acetone. A fluorometer will measure the chlorophyll a and phaeopigments in the extract (after acidification with HCl). The chlorophyll a concentration will be converted to carbon, assuming a carbon: chlorophyll-a ratio of 40 in phytoplankton (Stephens et al. 1997). The sediment samples will be freeze-dried for 3 to 5 days to measure wet weight (before freeze-drying), dry weight (after freeze-drying), water content, and porosity, assuming the sediment bulk density of 2.65 g cm^{-3} (Eleftheriou, 2013). An aliquot of freeze-drying sediment (~0.3 g) will be centrifuged (4500rpm / 5min) with distilled water to remove the salt.

The cement and carbonates will be removed by adding 10% of 12N hydrochloride (HCl), and the organic matter will be removed by adding 15% hydrogen peroxide (H_2O_2) for 1-2 days. Sodium hexametaphosphate ($Na(PO_3)_6$) will be added to deflocculate and disperse sediment particles, and then analyze for grain size with laser diffraction particle size analyzer (Beckman Coulter LS13 320). Another aliquot of freeze-drying sediment (~0.4 g) will be acidified with HCl to remove calcium carbonate, combusted at 1000°C with pure oxygen, and analyzed with a Flash EA 1112 elemental analyzer for total organic carbon (TOC) and total nitrogen (TN). The measurement of detrital organic carbon will collaborate with FATES's co-PI 林玉詩 (NSYSU). Extensive data on sedimentary chlorophyll-a, phaeopigment, and TOC in the proximity of our sampling site will be provided by FATES co-PIs 林慧玲 and 蘇志杰 (IONTU) or extracted from published values for comparison (Kao et al. 2006, Chen 2012, Hsu et al. 2014).

1.3. **Sediment community oxygen consumption (SCOC)**

In-situ incubation: We propose to construct a pair of plexiglass dark and light chambers based on the design by Rowe et al. (1994) and Warnken et al. (2000) (Fig. 6a & b). The *in-situ* paired chambers enclose a volume of 9 liters of water in each covered an area of 0.09 m^2, with a collar on the outside of each to assure precise penetration depth into the sediments (Fig. 6b). A one-way valve on the lid of each plexiglass chamber releases the excess of water, caused by the insertion of the chamber in the sediment, ensuring a gentle placement on the seafloor. The chambers will be continuously stirred by a pair of inductively driven magnetic stirs, directing flow from near the sediment-water interface out over the top of the chambers. This general flow pattern has been confirmed with the use of dyes (Rowe 1994) and the chamber design has participated in an extensive inter-calibration with chambers of other designs (Tengberg et al. 2004). We have installed a pair of proto-type chambers on the lander to simulate the implantation of the chambers after the lander reaching the bottom (Fig. 6a) during OR1 1242 cruise.

The oxygen sensing system will be composed of two Aanderaa oxygen optode 4330. A custom-built data acquisition computer (for oxygen optodes), a stirring motor controller, and a lithium battery pack are developing by 張宏毅 (IONTU). Initially, we will use high-density polyethylene (HDPE) housings to enclose the stirring motor or electronics, allowing them to be submerged to 500-m water depth. In the future, the HDPE housing can be replaced by titanium housings to allow the chamber to be submerged down to 6000-m water depth. The new chamber system will be self-sustained and lightweight enough to be deployed by SCUBA Diver or remotely operated vehicle (ROV). In this proposed project, we will deploy the newly designed chamber from an autonomous benthic lander or with an ROV Triton XLX manipulator (if available) to measure in-situ SCOC (Fig. 6a). The lander used is constructed by combining two square 1.4 x 1.4 m stainless-steel frames. It carries: (1) acoustically controlled disposable anchors; (2) 12 floatation spheres providing 300 kg of buoyancy; (3) primary and redundant acoustically commanded release mechanism to control anchor release; (4) electronic timed-release system to implant (or release) chambers to the bottom; (5) power supply; (6) time-lapse video camera and deep-sea light source (Fig. 6c & d); (7) two oxygen optodes and data acquisition system; and (8) additional environmental sensor (Aanderaa Seaguard II) to record basic hydrographic properties outside the chambers over time. The time-lapse video camera and deep-sea light source will be custom-built by the OR1 instrumentation center. The Aanderaa Seaguard II data logger will equip with Aanderaa oxygen (4330), turbidity (4112), pressure (4117), and conductivity (4319) sensors. The fluxes of oxygen into or out of the sediments will be calculated as Flux = [Change in concentration x Volume of overlying water] / [Chamber area x Time]. The dissolved inorganic carbon produced by the respiration will be calculated by the flux of oxygen in moles multiplying a respiratory quotient of 0.85.

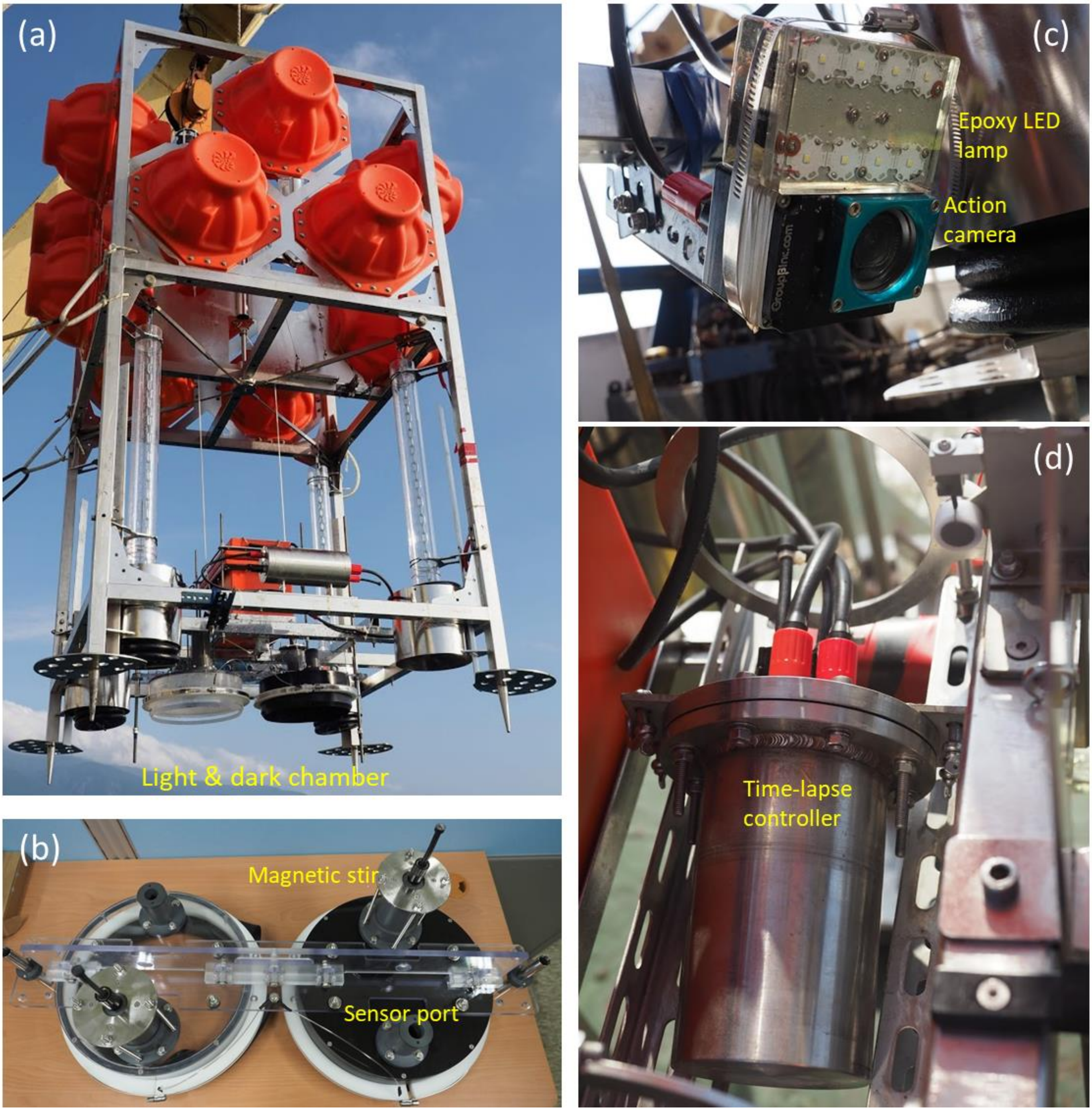

Fig. 6. (a) Benthic lander (1.4 x 1.4 x 3 m) with 12 glass flotations providing 300-kg of positive buoyancy. The lander is equipped with two acoustic releases and burn wires to provide redundancy for releasing anchor weights. (b) Paired light and dark benthic chamber (9 litters of volume and 0.09 m^2 of the area). (c) Task camera and epoxy encapsulated LED lamp for the lander. The deep-sea light source follows the design by

Oguri (2015). (d) Time-lapse controller for the video camera and light source.

Ship-board incubation: Three core tubes from megacorer (assigned to measure SCOC) will be first incubated in the dark at *in-situ* temperature (based on previously recorded temperature) in a temperature-controlled water bath (Fig. 7b). If the original supernatant water is not enough to fill the core tube, the sediment core will be carefully topped with bottom water collected from the CTD rosette. The sediments will be allowed to acclimate for approximately 6 hours until the flocculent materials settle, and the overlying water is clear. After the acclimation, the polycarbonate tube will be closed hermetically with customized polycarbonate lid, and any air bubbles will be removed. During the incubation, a magnetically driven impeller (60–80 rpm) attached to the core lid will gently circulate the water to prevent stratification. The dissolved oxygen concentration will be measured for every 8 hours with a miniature oxygen optode (i.d. 2 mm) through a sampling port on the core lid (PreSens® Microx 4). Dissolved oxygen will be measured until the concentrations decreased by 15% of the initial concentration according to Glud (2008) to prevent hypoxic stress. The fluxes of oxygen into or out of the sediments will be calculated as Flux = [Change in concentration x Volume of overlying water] / [Core area x Time]. The dissolved inorganic carbon produced by the respiration will be calculated by the flux of oxygen in moles multiplying a respiratory quotient of 0.85.

Sediment oxygen profiling: After the ship-board incubation, three oxygen microelectrode (100- μm tip size) will be inserted simultaneously into sediments at 100-μm increments using Unisense® Field Microprofiling System (Fig. 7c). The diffusive oxygen fluxes through the sediment–water interface will be calculated using Unisense® Profile software according to the Fick's first law based on oxygen porewater profile concentration, sediment porosity, and initial concentrations in the overlying water and oxygen diffusion coefficient corrected by temperature (Berg et al. 1998, Glud 2008). The oxygen penetration depth (OPD) will be determined by the depth where dissolved oxygen concentration < 5 μmol L^{-1}. The diffusive carbon remineralization will be calculated by the flux of oxygen in moles multiplying a respiratory quotient of 0.85. In general, the sediment oxygen profile concentrations measure the diffusive oxygen utilization (DOU) mainly contributed by aerobic respiration of microorganisms, whereas the sediment incubation experiment measures the total oxygen utilization (or SCOC). Therefore, in addition to DOU, the SCOC also accounts for the respiration of benthos, as well as the benthos-mediated oxygen utilization through their bioirrigation and bioturbation activities (Wenzhöfer & Glud 2004, Glud 2008, Lichtschlag et al. 2015). The difference between the SCOC and DOU is then the benthos-mediated oxygen utilization (BMU), characterizing the effects of benthos activities on sediment oxygen dynamics. The measurement of DOU will collaborate with FATES's co-PI 林玉詩 (NSYSU).

1.4. Organic carbon burial

The rate of organic carbon burial will be estimated by multiplying sediment accumulation rate (SAR) with total organic carbon (TOC) (Jahnke 1996). Extensive SAR data and empirical relationships between SAR and depth in our study are available from Hsu et al. (2014), Huh et al. (2009) and Kao et al. (2006).

1.5. Rain of POC

The rain of POC will be estimated in two different ways. First, the mass flux of sediment or export POC flux will be derived from the previous sediment trap measurements from the FATES project (Liu & Lin 2004, Liu et al. 2006) or other published data (Chung et al. 2004, Hung & Gong 2010, Wei et al. 2017). Second, if we consider the seafloor as an ultimate sediment trap, the rain of POC will equal to the sum of organic carbon burial and sediment community oxygen consumption (SCOC) (Jahnke 1996, 2001). The sediment trap data will be provided by FATES's PI 劉祖乾 (NSYSU).

1.6. Food web modeling

We assume that bacteria feed on detrital OC; meiofauna feeds on bacteria and detrital OC; macrofauna feeds on meiofauna, bacteria, and detrital OC; megafauna invertebrate feeds on macrofauna, meiofauna, bacteria, and detrital OC; fish feeds on megafauna and macrofauna invertebrates; consumption of detrital OC is equal to the inputs (Fig. 7). The growth efficiency for each compartment is set to 10%, and the secondary production is proportionally partitioned among the higher trophic levels according to their biomass. Sediment community oxygen consumption (SCOC) will be partitioned in three different ways to test the model sensitivity. (1) The respiration of bacteria, meiofauna and macrofauna will be partitioned allometrically by their biomass (Rowe et al. 2008, Leduc et al. 2016). (2) SCOC is partitioned into diffusive oxygen utilization (DOU) and benthos-mediated oxygen utilization (BMU) (Wenzhöfer & Glud 2004, Glud 2008, Lichtschlag et al. 2015, Leduc & Pilditch 2017). The microbial respiration will be set to the DOU and BMU is partitioned allometrically among the meiofauna and macrofauna biomass. (3) The respiration of meiofauna and macrofauna will be calculated based on body size and temperature (Mahaut et al. 1995, Moodley et al. 2008). The respiration of bacteria will then be estimated by subtracting the integrated meiofauna and macrofauna respiration from the SCOC. The respiration of megafauna invertebrate and fish will be calculated based on body mass and temperature (Mahaut et al. 1995, Clarke & Johnston 1999, Killen et al. 2010, Ruhl et al. 2013). The unknown predator-prey relationships or carbon flows (arrows in Fig. 8) will be solved by a linear inverse model (LIM) based on the mass balance of each stock (box in Fig. 8) and the upper and lower bounds of carbon flow from the available literature. The *LIM* package (Soetaert & Herman 2009, van Oevelen et al. 2010) in R (R Development Core Team 2017) will be used to set up and solved the conceptual model in Fig. 8. Based on a likelihood approach, an infinite number of solutions may be reached by LIM given the large numbers of unknown carbon flows. In such a case, the modeled carbon flows will be randomly re-sampled to calculate the mean and standard deviation of the solutions.

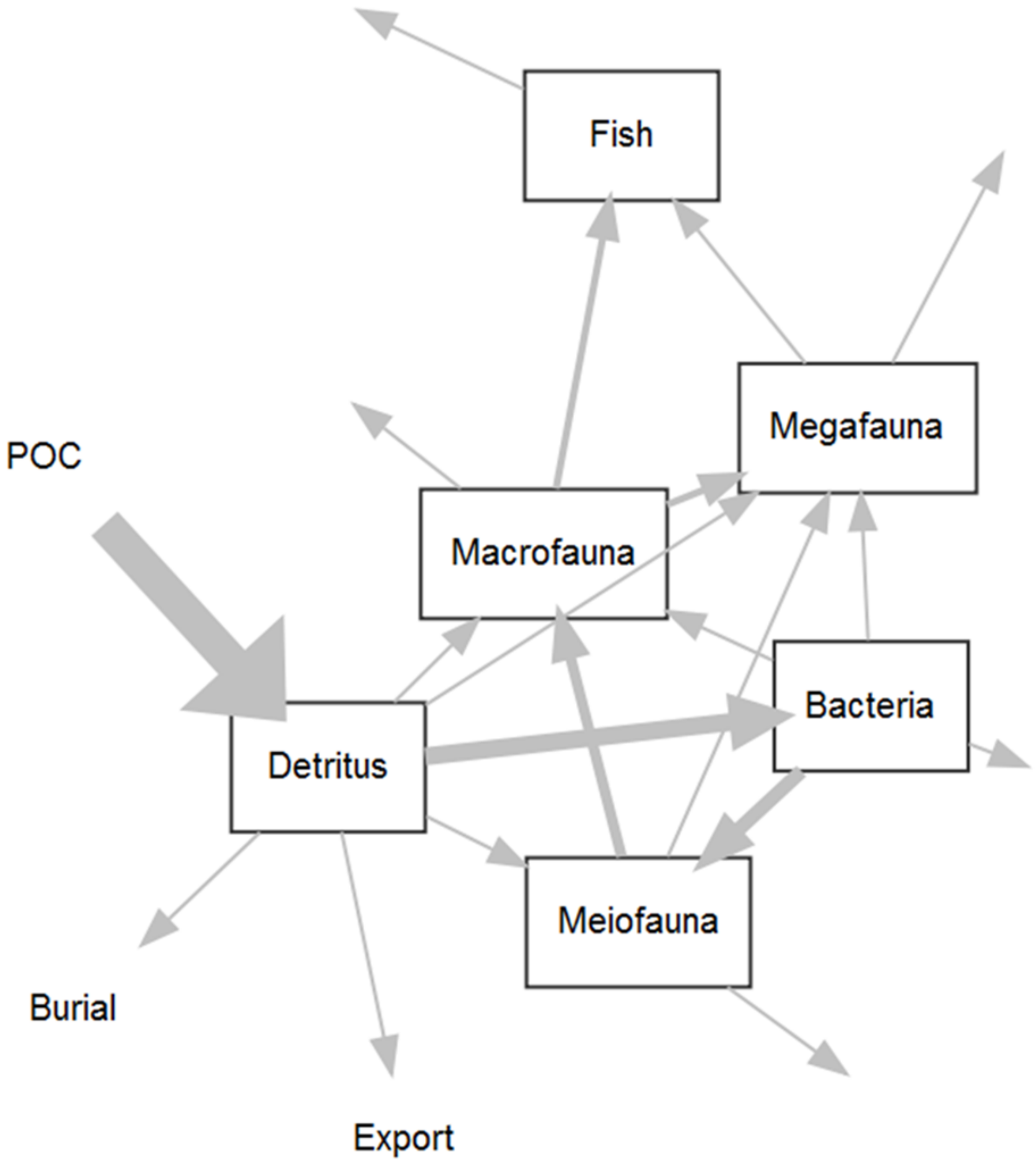

Fig. 7. Conceptual benthic food web model off SW Taiwan

1.7. Statistical analysis

A mixed-effect permutational multivariate analysis of variance (PERMANOVA) will be performed on both univariate (i.e., abundance, biomass, diversity, oxygen utilization, or environmental factor) and multivariate data (i.e., assemblage composition) to detect possible spatial variability between canyon and slope. The variabilities of assemblage composition between canyon and slope will be examined by a distance-based permutational test for homogeneity of multivariate dispersion (PERMDISP, Anderson et al. 2008).

Linear mixed effect models (LME) with abiotic variables as fixed factors will be used to identify potential drivers of community structure (i.e., abundance, biomass, and diversity) and function (i.e., carbon remineralization). Distance-based linear modeling (DistLM) will be used to fit multivariate compositional data with environmental variables. The best subset of environmental variables will be selected by the smallest Akaike information criterion corrected by small sample size (AICc). The bio-environmental relationships of the best DistLM will be visualized by distance-based redundancy analysis (dbRDA). Variance partition based on partial RDA will be conducted to examine the relative contributions of environmental factors and taxon composition on the multi-functionality of the benthic community (e.g., carbon remineralization). The univariate and multivariate analysis will use PRIMER 7 & PERMANOVA (Anderson et al. 2008, Clarke & Gorley 2015) and *vegan* and *nlme* packages in R (R Development Core Team 2017).

1.8. The novelty of the study methods

In this study, we will develop a novel benthic lander platform capable of autonomously conducting *in-situ* experiment from the deep sea. Although these techniques have been widely used in deep-sea explorations for decades (especially in the developed countries), this will be the first attempt to develop and apply such *in-situ* technologies in Taiwan's deep-sea ecosystem. We will also develop a new self-sustained benthic chamber system, which can be deployed by SCUBA diver, remotely operated vehicle (ROV), or benthic lander, and a time-lapse deep-sea camera system, which can also be used for environmental monitoring. Over the past decade, the FATES team has contributed significantly to the understanding of the source-to-sink dynamic of sediment and carbon transports off the SW Taiwan. In this study, we will attempt to integrate the geochemical data collected from the past FATES activities (e.g., flux measurement for sediment traps, dating, and organic carbon chemistry from sediment core) and the new information on the standing stocks of sediment benthos by a state of art modeling method.

2. 預計可能遭遇之困難及解決途徑。

Benthic lander: Although the OR1 marine instrument center has over 20 years of experience in deploying and recovery customized mooring of all kinds, this will be the first time to develop techniques in handling benthic lander. In the past year, we have successfully assembled the lander parts (lander legs, acoustic releases, chamber, sensors, logger, camera, lights, glass flotations, radio beacon, and flash beacon). Due to its height (almost 3.5 meter tall when fully installed), we have to burrow an off-campus factory with bridge cranes for our initial assembling, as there is still no space and facility to handle such large instruments at NTU. Besides its height, the weight of the lander (>500 kg in the air) and the anchoring effect of the chamber during sediment sampling also present considerable risks for instrument recovery. Different from the ordinary mooring instrument, the benthic lander measure processes at the sediment-water interface and thus require access to the seafloor. As a result, the ballast weights have to shift to the corners of the lander frame and the release mechanism is more complicated and more parts to break than the traditional mooring. All of these factors need to carefully considered to ensure the successful deploy and recovery of the lander. In the previous project, we attached a surface flotation array to the lander frame to ensure that we can recover the lander, even when the release system fails. After several tests, we found that the release mechanisms are quite reliable, and we never had to use the spare acoustic release. Nevertheless, this strategy has limited our deployment on the continental shelf. In the proposed project, we plan to conduct the autonomous lander deployment in the deeper water (~ 200 m), which will prevent the use of surface floats. To add more security, we will adopt the ropeless fishing method and attach a bottom stowed release bag to the top of the lander (Fig. 8). The bag holds coiled rope within a hard plastic mesh material. The top is held shut by a release cord, which is held in place by the lever of a fishing acoustic release (Desert Star ARC-1XD). Upon triggering the acoustic release, the release cord will lose tension. Positive buoyancy from the hard floats within the bag will cause the top to open and release the floats to the surface. The method has been widely used for commercial crab fishery in Australia and New Zealand to prevent whale entanglement with the rope and surface floats. Together with the primary, redundant acoustic release, and the burnwire timed-release, the bottom stowed release bag will provide the 4th option to recover the lander. Also, the new lightweight custom-built chambers will greatly reduce the weight of the lander system; therefore, the lander can be deployed alone without extra glass floats to provide positive buoyancy during recovery.

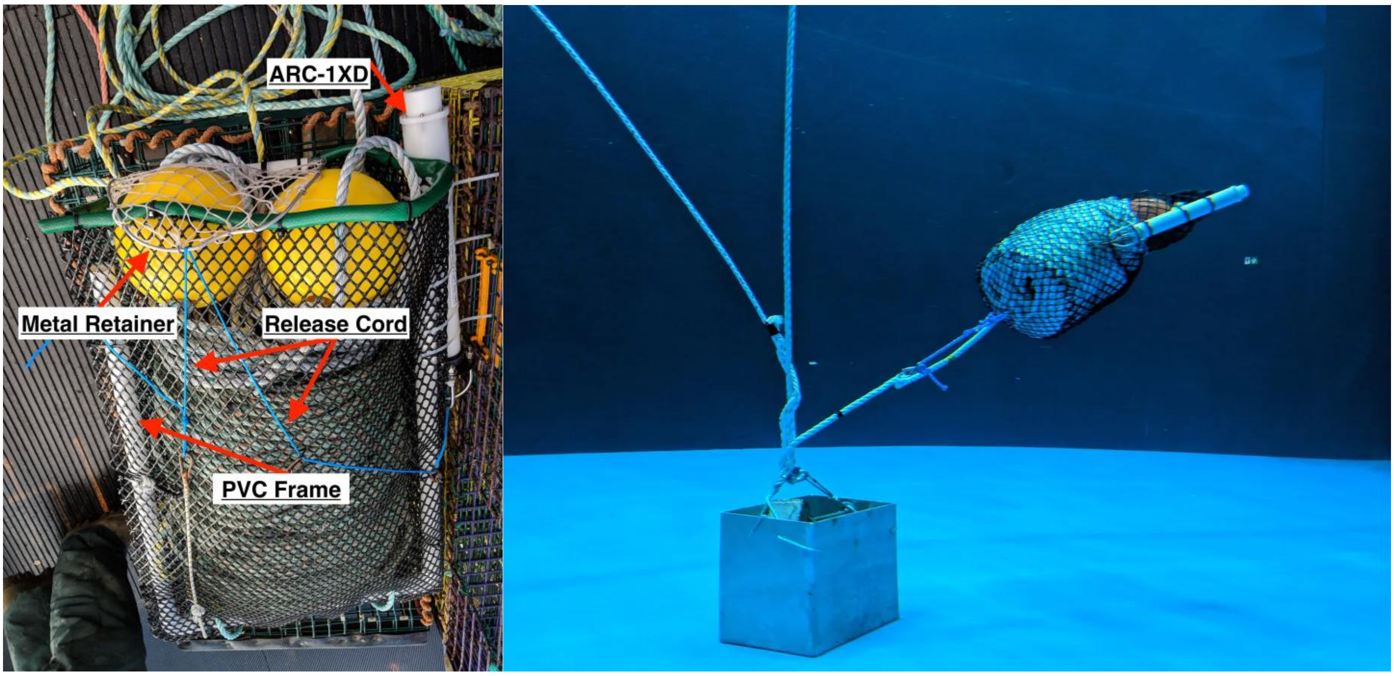

Fig. 8. Configuration of release bag (left) and testing the release bag in a pool (image credit: Desert Star, CBC news).

Megacorer: After four years and half of the operations, the crews and technicians have been familiar with the deployment and recovery of the megacorer. The current SOP is to lower the megacorer at ~0.5 m/s and then with a slower descent (~0.2 m/s) from 30 m above the maximum depth of the seafloor to prevent disturbance of sediments. Once arriving at the bottom, we continue to feed the slack wire for 5 m at 0.2 m/s and then let the megacorer sit on the seafloor for 60 seconds to allow the core head to penetrate the sediments (at 1 cm/s). If the steel cable connected to the megacorer is straight and not twisted, the customized stainless steel cage will protect the megacorer from entangling by the excess slack wire. This SOP often works in the calm sea. However, the success rate remains low in rough weather. This is because the surface vessel often drags the corer off the bottom when the vessel drifts faster than the rate of paying out the slack wire. The sampling is especially problematic in the deepwater (> 1600-m depth) with coarse sediment. Regardless, it is difficult to know the exact reason with our current observing technology. A seabed camera will be required to observe and improve sampling efficiency in the future. In the short term, we can adjust the hydrostatic damping system to increase the speed of core head penetration (up to 12 cm/s). A faster penetration rate likely generates bow waves to disturb the sediment, but the sampling efficiency may be improved by reducing the contact time between the megacorer and seabed, especially when the vessel cannot be kept stationary. Currently, successful deployment and recovery of megacorer will require good weather, superb handling of the research vessel, good communication between the technician and bridge, and careful selections of coring location. Obtaining sediment samples from the GPSC axis remains challenging. Such a problem may be alleviated by a new research vessel with a dynamic position system.

Taxonomy expertise: Understanding biodiversity is a major challenge for all deep-sea investigation. The small body size of the deep-sea infauna also makes the taxonomic identification difficult and time-consuming. It is, therefore, the postdoc candidate, 廖健翔, will bring his important expertise in nematode taxonomy and meiobenthos ecology to this project. Meiofauna is the most abundant metazoan invertebrates in the deep-sea sediments and responsible for the majority of secondary production. It is also one of the most difficult taxonomic group to work on but also represent a novel research field in Taiwan's oceanography. Dr. Liao has previously worked with us through NTU's "Aim for Top University Project" and then move to JAMSTEC to continue his training under Katsunori Fujikura. He will be the core of this project. Honestly, without him, we will miss an essential component of the benthic food web and will not able to conduct carbon budget analysis. Moreover, the macrofauna polychaete and peracarid crustaceans are equally difficult invertebrate groups in the deep-sea. We have sought collaboration with 薛攀文 from NCHU. He will help us to train the research assistant, 劉妍莉, to focus on polychaete identification. In the meantime, we will also seek international collaborations to expand our capability to assess the biodiversity off the SW Taiwan. For example, 馬林 from IOCAS will help us identifying meiofauna harpacticoids, another highly diverse and challenging invertebrate group. The full assessment of biodiversity, however, is not likely to be completed within a year. A future project will be proposed to target the biodiversity issue.

3. 重要儀器之配合使用情形。

Benthic lander, chamber, megacorer, and sediment microprofiling system will be provided by OR1 marine instrument center. PreSens® Microx 4 optical oxygen meter, and all microscopes and digital cameras required for fauna sorting and size measurements are available in the applicant's laboratory. Customized shipboard incubation chambers and two temperature-controlled water baths for sediment whole core incubations are also available in the applicant's laboratory. An epifluorescence microscope will be provided by 林立虹 (Geology, NTU). Laser particle analyzer (Beckman Coulter LS13 320) for sediment grain sizes will be provided by 蘇志杰 (IONTU). The element analyzer (Flash EA 1112) will be provided by 王珮玲 (IONTU).

4. 如為須赴國外或大陸地區研究，請詳述其必要性以及預期效益等。

（三）預期完成之工作項目及成果。請分年列述：1.預期完成之工作項目。2.對於參與之工作人員，預期可獲之訓練。3.預期完成之研究成果（如實務應用績效、期刊論文、研討會論文、專書、技術報告、專利或技術移轉等質與量之預期成果）。4.學術研究、國家發展及其他應用方面預期之貢獻。

1. 預期完成之工作項目。

<table>
<tr><th colspan="13">Current proposal</th></tr>
<tr><td>Year</td><td colspan="5">2019</td><td colspan="7">2020</td></tr>
<tr><td>Month</td><td>Aug</td><td>Sep</td><td>Oct</td><td>Nov</td><td>Dec</td><td>Jan</td><td>Feb</td><td>Mar</td><td>Apr</td><td>May</td><td>Jun</td><td>Jul</td></tr>
<tr><td>Field and Lab Work</td><td colspan="2">Assembling lander</td><td>OR1 cruise</td><td colspan="9">Sediment and biological sample processing, design and construct the benthic chamber</td></tr>
<tr><td>Scientific Exchanges</td><td colspan="12">data analysis & manuscript preparation</td></tr>
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<tr><th colspan="13">This proposal</th></tr>
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<tr><td>Field and Lab Work</td><td colspan="6">Sediment and biological sample processing, design and construct the benthic chamber</td><td>Preparing lander</td><td>planed cruise</td><td colspan="4">Sediment and biological sample processing</td></tr>
<tr><td>Scientific Exchanges</td><td colspan="12">data analysis & manuscript preparation</td></tr>
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2. 對於參與之工作人員，預期可獲之訓練。

We will train one postdoctoral researcher, 廖健翔, to work on nematode diversity and ecosystem functioning relationships. Marine nematodes are among the most abundant metazoan invertebrates, usually accounting for over 90% of the abundance in meiobenthos. The high abundance, biomass, and secondary production mean that the nematodes process a significant amount of organic carbon on the seafloor and is a critical link to transfer microbial carbon up the food chain. Despite their importance, nematode ecology remains unexplored in Taiwan. Through this project, we will create a new research field in Taiwan’s marine ecology. We will also train the postdoc to develop proxies for seafloor ecosystem functions, such as microbial biomass, microbial trophic efficiency, and organic carbon remineralization, which may be affected by the activities of nematodes. Moreover, the postdoc will have the first-hand opportunity to participate in the development of the new autonomous benthic lander, chamber, shipboard experiments and cruise activity coordination. More importantly, the postdoc will have the opportunity to conduct independent research related to the main proposal, as well as gaining experience in statistical and food web modeling, preparing a manuscript and eventually publish scientific results. We will train one research assistant, 劉妍莉, on the identification of polychaetes. Polychaete is the most important macrofauna group for environmental monitoring. It is also the only infauna group consistently occurring in the GPSC. For the past four years, Yen-Li has developed the skill to identified polychaete to genus levels. It is essential to maintain taxonomic capability to conduct long-term ecological researches. Especially such capacity has been declining in Taiwan. We will continue to train Yen-Li to focus on polychaete to conduct long-term, consistent genus-level identification. We will train three student workers for meiofauna and macrofauna sorting and body size measurements. We will train four graduate students to be familiar with benthic sample collections, shipboard experiments, operations of megacorer and sediment mciroprofiler, and sample sorting in the laboratory. Furthermore, graduate students will learn the statistical skills and R programing language by analyzing the biological and environmental data. Most importantly, we will train the graduate students to observe nature, find research questions and implement independent research.

3. 預期完成之研究成果（如實務應用績效、期刊論文、研討會論文、專書、技術報告、專利或技術移轉等質與量之預期成果）。

We plan to generate two manuscripts from this project. One will focus on the body sizes spectrum, biomass and trophic efficiency of metazoan infauna between upper GPSC and adjacent slope. The other one will focus on spatial variability on the abundance and composition of nematodes, as well as their relationship with environmental factors in the upper GPSC and adjacent slope. We also plan to write a paper based on shipboard sediment incubation, profiling, and lander incubation data to examine sediment oxygen dynamics between submarine canyons and adjacent slope. The biomass paper and oxygen dynamic paper will be the foundation of the food web simulation. There may be many spin-off papers depending on the interests of graduate students and collaborators; for example, to examine the relative contributions of environmental drivers and biodiversity on ecosystem functioning and whether this is a link between the diversity and ecosystem functioning. One may also examine the composition of major benthic taxonomic groups (i.e., polychaetes, harpacticoids, or paracarid crustaceans). Since no such data is available in SMRs associated submarine canyons, we will significantly contribute to the understanding of this understudied deep-sea ecosystems.

4. 學術研究、國家發展及其他應用方面預期之貢獻。

Deep-sea benthos is poorly studied in the western Pacific and the South China Sea. A recent compilation of all available information of annelid polychaete in the South China Sea suggested that little to no data are available past several hundred meters of water depth (Glasby et al., 2016). Our preliminary identification of merely 256 crustacean tanaid individuals collected from the upper Gaoping slope found six tentative species. All of which are potentially new to science (personal communication with 薛攀文, NCHU). In 2015 alone, we recovered 121 polychaete genera/families (Chen 2018) and 163 nematode species (Liao et al. Submitted). These are just a few examples showing that our sampling off the SW Taiwan is unraveling the hidden biodiversity and will contribute to the scarce ecological information in the deep South China Sea.

Moreover, of the more than 5900 large submarine canyons worldwide, only ~3% are connected to rivers. Of which, even fewer percentages are connected to a mountain river like GPSC, which is characterized by high sediment load, high tectonic activities, and thus frequent submarine geohazards in the system. Also, the

GPSC is situated near where the world's largest internal solitary waves. As a result, the GPSC is subjected to internal tide energy 3-7 times higher than the well-known Monterey Submarine Canyon (MSC) with similar shape and size off central California. Over the past 30 years, the Monterey Bay Research Institute (MBRI) has conducted hundreds of studies and developed cutting-edge undersea technology in the MSC to make it a poster child in deep-sea research. The GPSC, our gateway to the deep South China Sea, is more physically dynamic than the MSC and an equally if not more exciting location to study the deep-sea ecology. Over the past decade, FATES has made a name for GPSC in the geoscience community. This proposed project, however, will extend the FATES efforts into the field of ecology. Perhaps one day we can make the GPSC recognized as not only a classic example of source-sink sediment dynamics, but also a natural laboratory of excellent deep-sea biology researches.

In the wake of the global climate changes, the changes in storm and precipitation patterns may lead to changes in the frequency and intensity of submarine geohazards in the Gaoping Canyon and adjacent slope. Understanding how the large-scale disturbance shapes the ecological assemblage and the associated carbon cycling processes (e.g., internal tide and turbidity currents) will enhance our ability to predict the potential impacts of climate changes on the submarine canyon ecosystems.

（四）整合型研究計畫說明。如為整合型研究計畫請就以上各點分別說明與其他子計畫之相關性。

Because of their small size, high abundance and diversity, fast turnover, and sedentary lifestyle, benthic metazoan invertebrates (i.e., macrofauna and meiofauna) are often used as bioindicators for the environmental changes. They are also essential components of aquatic food webs and thus affecting the transport and cycling of nutrients and toxicants in the marine environment. However, among the major benthic taxa, foraminiferans are often ignored by benthic ecologists, whereas the foraminferan researchers also do not consider the metazoan benthos. It is thus this proposed project, and the sub-project 2 (by 林彗玲) will complement each other to cover the major components of living biomass in the sediments (both the invertebrates and foraminiferans). Given the strong connections of benthos to their surrounding environment, this proposed project is naturally linked to many sub-projects of FATES, which characterize the living habitats of the benthic communities. For instance, the sediment dynamics and the river plumes deliver essential food and energy to deep-sea benthos but also smoother the seafloor, clog their feeding apparatus, and bury and suffocate the living communities. The data from sediment traps and detailed characterization of sediment cores by sub-project 1 (劉祖乾) will provide us critical information to understand the benthic habitats and the source of energy. Moreover, the sub-project 7 (林玉詩) will examined the relationship between biological activities (assessed via oxygen utility), physical environments, and OM bulk properties in the sediment. These are also essential variables to determine the biomass and distribution of sediment benthos. In-situ observations of internal tides (sub-project 9 by 李逸環) will help us understand the amplitude and frequency of the tidal flushing disturbance on benthos. Organic matters in the sediments are an important food source for benthic infauna, but grossly polluted sediments often lead to a decline in biodiversity, the dominance of opportunistic species, and a decrease in ecosystem function and service. The small metazoan invertebrates are the intermediates for persistent organic pollutants (POPs) to enter the marine food web. We will work with sub-project 3 (by 李宗霖) to measure polycyclic aromatic hydrocarbons (PAHs) in the sediments and the tissues of macrobenthos. The PAHs concentration in biotic and abiotic samples will help us to construct a chemical fate model, which will be coupled with the benthic carbon food web model. We will also work with sub-project 5 (by 張懿) to extract high-resolution ocean color data to model the slow sinking of phytodetritus, which will be an important parameter in our benthic carbon food web. In short, our proposed project represents an import component in FATES to link the integrated researches from the geosphere to the hydrosphere and then to the biosphere. The results will answer the critical question of the biological responses to the source-to-sink dynamics in the SMRs associated submarine canyon and deep-sea environments.

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