Material and Method

Studying sites

　　From 2014 to 2020, the upper GPSC and GS were repeatedly visited by the National Taiwan University's R/V Ocean Researcher 1 and New Ocean Researcher 1 (Fig. 1). Corresponding to previous studies (i.e. Liao et al., 2017; 2020), the two shallowest stations were chosen in this study and were abbreviated as GC1 and GS1, respectively. At each visit, CTD/rosette cast and UNSEL box corer (Hessler and Jumars, 1974) or OSIL megacorer were deployed. The hydrocasts of temperature and salinity were measured with a CTD recorder (Sea-Bird SBE 911). For the box core operation, five transparent polycarbonate tubes (i.d. = 67 mm) were inserted into the sediments to take subsamples. For the megacorer operations, a maximum of 12 polycarbonate tubes (i.d. = 105 mm) were recovered as the replicate subsamples. The detail of cruise, sampling sites, and sampling gears are included in Table. 1.

Sediment carbon budget

　　In the deep-sea sediments, the total inventory of OC can be divided into the living and the non-living components. The living component of OC is mainly made up of the biomass of prokaryotes (mostly bacteria), protozoan (mostly foraminifera), meiofauna (> 40 μm in length), and macrofauna (> 1 mm in length) (Burnett, 1979; Mare,1942; Rowe 1983), whereas the non-living component of OC comprises labile (e.g. neutral sugars and amino acids, support rapid microbial production turnover with time scales of minutes to days; Hansell & Carlso, 2001), semi-labile (i.e. polysaccharides, cycles with intermediate time scales of weeks to years; Benner et al., 1992; Ogura 1972), and refractory OC (e.g. humic and fulvic acids, structural carbohydrates and “black” carbon, with very low degradation rates; Danovaro, 2009). The source of OC is mostly supplied by the rain of particulate organic carbon (POC) from the euphotic zone, and the lateral advection of POC from terrestrial or marine organics. On the other hand, the loss of OC balances the input through biological utilization of labile OC, predation on living components of OC, carbon remineralization, the long-term burial of refractory OC, and down-slope export (e.g. by turbidity currents).

Sampling procedures of living component of OC

Prokaryote biomass

　　A cutoff 10 ml sterile syringe (i.d. 15 mm) was used to take the subsample from the top 1 cm of sediment within a core tube. The syringe plunger was held fixed at the sediment surface as the operation of a piston core, and then the barrel was pushed into the sediment to take 2 mL of the subsample. Then, the subsample was added to a 15 mL polyethylene centrifuge tube which contained 2 mL of pre-filtered PBS solution. Later, 0.3 mL of 16% formaldehyde was also added to the centrifuge tube until the sample reached a concentration of 2% formalin, and then it was stored in a 4˚C fridge. In the lab, the sediment samples were 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 and Carpenter, 2008; Kallmeyer et al., 2008). The prokaryote abundance was counted with epifluorescence microscopy, and the cellular dimensions in each slide were estimated from an image taken by a CCD digital camera. By assuming a conversion factor of 10 fg C per cell (Deming & Capenter 2008), the stock of bacterium OC was calculated as,

and finally converted to the unit of mg C/m2.

Meiofauna biomass

　　A cutoff 10 ml sterile syringe (i.d. 15 mm) was used to take the subsample from the top 5 cm of sediment as suggested by Montagna et al (2017). When taking bacterium samples, the syringe plunger was held fixed at the sediment surface, and then the barrel was 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 Na2B4O7, and 1 g L-1 Rose Bengal) is added to the sediment sample to make a concentration of 5% formalin. Later in the lab, the sediment samples were wet sieved through a 1000 µm sieve with a 40-µm sieve underneath, and then transferred to 70% ethanol. The meiobenthos specimens were extracted from the sediments using Ludox HS40 solution (gravity = 1.18 g cm-3) after centrifuging at 8,000 rpm for 10 min with 3-times repeat (Danovaro, 2009; Montagna et al., 2017). Then the meiofauna was enumerated into major taxonomic groups under a high power stereomicroscope (Olympus® SZX16; 0.7-11.5 X zoom). The body volume of meiofauna specimens was calculated with the formula:

where V was the volume, L was the length, W was the width, and C is the taxon-specific conversion factors (Warwick and Gee, 1984). The biovolume was converted into wet weight by assuming a specific gravity of 1.13 (Warwick and Gee, 1984), and converted into OC using the conversion factor of 12% (Baguley et al., 2004). Finally, the meiofauna OC stock was divided by the sampling area and then converted to the unit of mg C/m2.

Macrofauna biomass

Once the sediment cores were recovered, the supernatant water above the sediment surface was siphoned carefully through a 300-µm sieve. Then, the top 10 cm of the sediments were extruded by an extruder and washed with 5 µm filtered seawater through the same 300-µm sieve as suggested by Montagna et al (2017). Later, the remaining was kept in a 250 mL specimen jar with an equal volume of 10% buffered formalin (with borax, sodium tetraborate Na2B4O7, and 1 g L-1 Rose Bengal) to fix the samples for at least 24 hours. Macrobenthos samples were sorted and enumerated into major taxonomic groups using a stereo sorting microscope (Olympus® SZ61; 0.67-4.5X zoom) and then permanently-preserved in 70% ethanol. The body volume of macrofauna specimens will be estimated by the same length-width relationship as eq.2.

For the common taxa such as polychaetes and nematodes, the conversion factor was from previous study (i.e. Warwick and Gee, 1984), whereas for the taxa whose conversion factors were not available, the biovolume was calculated from length and width using the nearest geometric shapes (e.g., cone shape: scaphopods; cylinder shape: aplacophorans, sipunculans, and nemerteans; ellipsoid shape: ophiuroids and asteroids.) The biovolume was also converted into wet weight by assuming a specific gravity of 1.13 (Warwick and Gee, 1984), and then multiplied by 4.3 % to obtain the organic carbon content (Rowe, 1983). Finally, the OC stock of macrofauna was divided by the sampling area and then converted to the unit of mg C/m2.

Sampling procedures of non-living component of OC

Detrital organic carbon

Surface sediment were taken and stored in 50-ml centrifuge tube in -20°C freezer. In the lab, the sediment samples were freeze-dried for 3 to 5 days to measure wet weight (before freeze-drying), dry weight (after freeze-drying), water content, and porosity. An aliquot of freeze-drying sediment (~0.4 g) was 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).

To calculate the stock of detritus OC, the core area (m2) was first converted to volume (m3) multiplying by the sampling depth (i.e. 0.1 m). The volume was converted into mass by assuming the sediment bulk density of 2.65 (g/cm3) (Eleftheriou, 2013), and then multiplied by the analyzed TOC content. Finally, the OC stock was divided by the sampling area and then converted to the unit of mg C/m2.

Sediment community oxygen consumption (SCOC)

　　Three core tubes recovered from a megacorer assigned to measure SCOC were incubated in the dark within a temperature-controlled water bath onboard. If the supernatant water was not enough to fill the core tube, the sediment core was carefully filled with bottom water collected from the CTD rosette. The tube was closed hermetically with a custom-built HDPE lid, and air bubbles were carefully removed. A magnetically driven impeller (60–80 rpm) attached to the core lid gently circulated the water during the incubation. Then, the sediments were acclimated for approximately 6 hours until the flocculent materials settled and the overlying water was clear. The dissolved oxygen concentration was measured every 8 hours with a miniature oxygen optode (i.d. 2 mm) through a sampling port on the core lid (PreSens® Microx 4). According to Glud (2008), the dissolved oxygen concentration was measured until decreased by 15% of the initial concentration to prevent hypoxic stress. The fluxes of oxygen into or out of the sediments will be calculated as:

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where V was the volume of overlying water.

　　After the shipboard incubation, three oxygen microelectrode (100 µm tip size) were inserted simultaneously into sediments at 100 µm increments using Unisense® Field Microprofiling System. The diffusive oxygen fluxes through the sediment-water interface were calculated using Unisense® Profile software according to Fick’s first law of diffusion-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) was determined by the depth where dissolved oxygen concentration was < 5 µmol L-1. The diffusive carbon remineralization was calculated by the flux of oxygen in moles multiplying a respiratory quotient of 0.85.

In general, the sediment oxygen profile concentration measures the diffusive oxygen utilization (DOU) mainly contributed by aerobic respiration of microorganisms through slow diffusion of oxygen molecules. In contrast, the sediment incubation experiment on shipboard measures the total oxygen utilization (TOU, also referred to as SCOC), which not only accounts for TOU but also for benthos' respiration and the benthos-mediated oxygen utilization (BMU) through their bioirrigation and bioturbation activities (Glud, 2008; Lichtschlag et al., 2015; Wenzhöfer and Glud, 2004). Therefore, the difference between the TOU and DOU is the benthos-mediated oxygen utilization (BMU), characterizing benthos' contribution to sediment oxygen dynamics.

Rain of POC

To understand the ocean’s biological carbon cycle, accurate estimates of the sinking particle flux, or POC, are important. In other words, without the accurate quantification of the carbon export, it is impossible to balance the ocean carbon budget. The sediment trap was widely used in oceanographic studies to capture vertically sinking materials (Giering et al., 2014; Steinberg et al., 2008), however, there were several biases caused by this tool. For example, the local conditions such as hydrodynamic variables, and the characteristics of the sinking particles would have an impact on sample bias (Baker et al., 2020). In Liu & Lin (2004) and Liu et al. (2006), they reported that the estimated mass flux collected by sediment traps exceeded 700 g m-2 d-1 in the GPSC during spring tide. But a lower value (c.a. 200 g m-2 d-1) was observed before spring tide passing. If we multiply this value with the TOC content (c.a. 0.4-0.6 %) reported in Liu et al. (2006), the POC flux would be 800 to 4200 mg C m-2 d-1. Considering the huge range of variation of POC flux, the quantity of this input was left to be determined by the model. On the other hand, we could also compare the model results with the measurements obtained from previous studies.

Burial efficiency of organic carbon

The sedimentation rates of the Gaoping continental shelf and canyon system were studied by Huh et al. (2009) and Hsu et al. (2014), therefore, the sedimentation rates were converted and the burial efficiency was directly used as *in situ* data in our model. For GS1, the average sedimentation rate in the upper slope region ranging from 200 to 600 m water depth was reported as 0.43 g cm-2 yr-1. In contrast, a higher mass accumulation rate (> 1.0 g cm-2 yr-1) was calculated in the GPSC at a water depth of 300 m (GC1). With this information, we converted the accumulation rate into the unit of mg m-2 d-1 and then multiplied by TOC content (%) measured in 2 areas respectively. Finally, flow rates with a unit of mg C m-2 d-1 were combined into our model.

Linear inverse model formulation

Structure

Linear inverse modeling started with choosing relevant abiotic and biotic compartments and specifying the links between them (Fig. 14). We assumed that the influx of POC was a complex assemblage of organic matters derived from the water column with a portion of energy flowing out of the sedimentary system through the process of burial and/or export (orange flows). Then, the general idea was that the predators of each size class preyed on organisms of the same and smaller size classes, that was, the black flows assumed that bacteria fed only on detrital OC; meiofauna fed on bacteria and detrital OC; macrofauna fed on meiofauna, bacteria, and detrital OC. The grey flows indicated that carbon was lost as feces and was consumed by benthopelagic/pelagic predators (Fig. 14). In this LIM model, the compartments with orange color were part of the food web model, whereas the compartments with blue color were only considered carbon influx or efflux but are not directly modeled.

Mass balances

A broadly accepted physical constraint is that mass is conserved for each chemical element, and this mass balance principle is the backbone of the food web model. First of all, the mass balance could be written in the general form:

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indicating that the temporal mass change of a compartment(X) was equal to the difference between the incoming( and outgoing( flows. Therefore, if was larger than , X would increase in time. Based on this principle, we could derive the mass balance equations of all the compartments with the assumption that all the compartments were invariant in time:

Furthermore, this mass balance principle could also be applied to organisms’ physiological behaviors. For example, when organisms ingest food, only part of the food is assimilated, and the rest is expelled as feces. The assimilated food is used to maintain its basal metabolism, growth, and reproduction. For heterotrophic organisms, the energy needed for growth and maintenance was paid by respiration. Thus, we could write this process as:

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Where was the biomass of the organism, and was its growth rate. This mass-balance equation stated that the biomass changes to the difference between feeding and loss terms. Note that the balance of all food web compartments was tightly linked. For instance, if species A feeds on species B, not only does an increased flux flow into A, but a loss of the same magnitude of flux flows out of B. As a result, the direction of the flows matters, and we could take the mass-balanced equation as sums and subtractions of these unknown quantities of flows. We classified this linear mass balance equation as the “equality equation”. It could be expressed with matrix notation in the general form:, in which x was a vector consisting of unknown flows, b was a vector consisting of changed rates of the component, and the flows are non-negative quantities, .

Constraints

On the other hand, the physiology and behavior of organisms imposed a limitation on their feeding and growth rates with upper bound and lower bound. For example, when organisms searched for food, not only the encounter rate but the external handling time determined the maximal foraging capacity (Holling, 1966). Also, physiological and digestive constraints regulated the process of assimilation of ingested food. Therefore, animals can only process a finite amount of food per unit per time. These maximum rates imposed an upper bound on ingestion flows, providing important constraints on the magnitudes of the grazing flows in the model. Similarly, respiration flows were restricted by allometric rules (e.g., Mahaut et al., 1995). The minimal basal respiration rate required to sustain metabolism was imposed as lower bounds. Other physiological constraints restricted the relationships between flows. For example, growth efficiency was defined as the ratio of secondary production to assimilated food, which is suggested to be 60-80% (Calow,1977; Schroeder, 1981). These constraints can also be transformed into a matrix equation with inequality:, in which x was still an unknown-flows vector, h was a vector comprising constraints. Most of these constraints could be extracted from literature.

Here, we applied the four most used constraints in LIM studies (van Oevelen et al., 2006; Stratmann et al., 2018) to our model, including respiration (R), assimilation efficiency (AE), production (P), and net growth efficiency (NGE).

For meiofauna and macrofauna, R was defined as the sum of maintenance respiration (biomass-specific respiration, MR) and growth respiration (associated with growth processes, e.g. synthesis of new structures in growth, GR). The maintenance respiration was taken proportional to 1% at 20℃ of the biomass per day (Fenchel, 1982; Nielsen et al., 1995), and then corrected with a temperature correction factor: *Tlim*, which could be calculated as,

where Q10 = 2, T was the bottom water temperature for each site. Q10 is a measure of temperature dependence based on the process or reaction. For most biological systems, this value is ~2 to 3. Therefore, the relationships of respiration could be expressed as:

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AE was calculated as,

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where I was the ingested food and F was the feces (Crisp,1971). The minium-maximum range was set from 0.456 to 0.699 for meiofauna (Conover 1966) and from 0.6 to 0.7 for macrofauna (Loo & Rosenberg 1996).

The secondary production (P) was calculated as,

The for meiofauna was set between 0.0009 and 0.0493 (Fenchel, 1982; Fleeger and Palmer, 1982), while for macrofauna is set between 0.0008 and 0.0048 (Stratmann et al., unpublished). On the other hand, the bacterial growth efficiency (BGE) was defined as the amount of new bacterial biomass produced per unit of assimilated OC, and it could be used to relate the production and respiration of bacteria (del Giorgio & Cole,1998). The range of BGE was set from 0.02 to 0.61.

Finally, NGE was calculated as (Clausen and Riisgård, 1996),

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The minimum-maximum range was set from 0.3 to 0.5 for meiofauna (Herman & Heip, 1985; Banse & Mosher, 1980; Herman et al., 1983; 1984), and from 0.6 to 0.72 for macrofauna (Navarro et al., 1994, Nielsen et al., 1995).

The data types mentioned above are derived from the general principles applied to most ecosystem; however, the in situ data is still necessary to deal with a food web model of a specific location. Because of the valuable information from site-specific measurements, this type of data is generally implemented as equality equations: , where f represents the vector that contains *in situ* data.

In our model, besides the estimated biomass were the stocks of organism compartments, the SCOC data served as directly measured flows. As suggested in Mahaut et al. (1995), the value of bacterial carbon mineralization (DOU; the flow of bacteria to DIC in our model) represented about 30% of the TOU.

Model Solution: Likelihood method

To achieve the final model, we combined three types of data, including mass balance equations, physiological constraints, and in situ data. The solution of this model was a set of flow values (**x**). Depending on the number of equations and the numbers of the unknowns, different methods of solution were used. Ideally, the equations lead to only one set of solutions that perfectly fits the data when the number of equations equals the numbers of the unknowns. However, the most commonly encountered situation is that the number of equations is far less than the number of unknowns. As a result, there is no unique solution set, whereas an infinite number of valid solution sets exist, creating a multidimensional solution space. Earlier modeling studies usually selected one solution from this solution space. The principle of parsimony, the flow set with the minimal sum of squared value, had often been applied as the selection criterion (Vézina and Platt, 1988). However, the parsimonious food web model usually takes extreme values to meet the criterion (Difendorfer et al., 2001; Kones et al., 2006). Alternatively, a likelihood approach based on Markov Chain Monte Carlo (MCMC) algorithm has been developed, which calculates the mean values and standard deviations of the flows from the possible solution sets (Kones et al., 2006). We used *LIM* package (Soetaert and Herman, 2009; van Oevelen et al., 2010) in R (R Development Core Team 2020) to set up and solve the conceptual food-web model (Fig. 14) using MCMC and likelihood approach.

Network indices of ecosystems

After *LIM* solved the food web model, we conducted the network analysis to better understand the structural properties and energy transformations in the ecosystem.

Several network indices were calculated from the outputs of LIM to examine the food-web functioning with uncertainty estimation (Kones et al., 2009). Network indices were robust estimators of food web function despite the inherent uncertainty (i.e. uncontrollability and unpredictability) in the exact value of food web flows.

In Kones et al. (2009), they divided 25 different network indices into 6 categories. Considering our simplified model, we only used 3 types of network indices which were widely calculated in deep-sea LIM food web studies, including Total system throughput , Total system throughflow , and Total system cycled throughflow of general indices; Finn’s cycling index of pathway analysis; and Average mutual information of network uncertainty.

was a measure of the growth and size of the system, obtained by summing all flow magnitudes, while the total system throughflow () was the sum of compartmental throughflows. These two indices inferred the general properties of the food web system. That was, the more material or energy flowed through the system, the larger the value of and would be.

The cycled portion of the total system through flow () was the sum of cycled flow in all through flows (Finn, 1976). The proportion of and was referred to as the Finn cycling index (), which summarized the fraction of the material/energy that was generated by the recycling process (Allesina and Ulanowicz, 2004). also denoted how much further a unit of inflow traveled compared to a straight-through flow during a cycling process (Finn, 1976). For example, if the straight pathway was 10 and , meaning that an average unit of inflow travels 15 because it cycled through the system. helped understand the stability, stress, and structural difference in different systems, the more cycling, the more efficiently input matter/energy were distributed in the system. For a valid comparison between systems, the systems needed to have the same structure and level of organization. (Finn, 1976)

Finally, the index average mutual information () based on information theory measured the average amount of constraint placed on a single unit of flow anywhere in the network (Ulanowicz, 1997). To put it simply, is a measure of uncertainty regarding the energy/material exchange in the network. For example, if the chances of energy/material flow from any particular compartment to any potential compartment are equal, the uncertainty of flows in the network maximize. While if all energy/material from a particular component flow to only one recipient, the uncertainty of the source will no longer exist. Ulanowicz (1980, 1986, 1997) hypothesized that an ecosystem form greater mutual constraints as autocatalytic loops during development, which resulted in a higher value in a trophic specialization or climax community (Ulanowicz, 2004). More details on the derivation and calculation could be found in Latham & Scully (2002), Ulanowicz (2004), and Kones et al. (2009), but the summary of nomenclature (Table. ) and calculation algorithms (table.) were included. All the network indices will be directly calculated in R using R-package NetIndices (Kones et al., 2009).