Deep-Sea Research Part I 148 (2019) 88–99

Contents lists available at ScienceDirect

Deep-Sea Research Part I

journal homepage: www.elsevier.com/locate/dsri

An environmental baseline for food webs at deep-sea hydrothermal vents in Manus Basin (Papua New Guinea)

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Keywords:
Hydrothermal vent
Deep-sea mining
Environmental management
Isotopic trait space
Food web
Trophic interactions

ABSTRACT

Food webs and trophic interactions provide a measure of ecosystem function and have been highlighted as an important element for environmental baselines and environmental impact assessments. Stable isotopes have long been used in the deep sea as a means to explore and summarize food webs, especially in chemosynthetic ecosystems, where there may be multiple sources of primary production with distinctive isotopic compositions. Hydrothermal vents in Manus Basin have been targeted for mining and, if mining proceeds, vent food webs will be altered. Here we describe a pre-mining baseline for the food-web state for 3 active vent fields. Manus vents support at least 9 feeding guilds, and both reverse TriCarboxylic Acid (rTCA) and Calvin-Benson-Bassham (CBB) carbon fixation pathways are inferred for symbionts of holobiont taxa. There are site differences in the C-N-S isotopic trait spaces (convex hulls) of Manus Basin vent food webs that likely reflect environmental variability. Isotopic diversity metrics (divergence, dispersion, evenness) are similar in Solwara 1, South Su, and PACMANUS vent food webs, but South Su has a higher degree of isolation in isotopic space (uniqueness). Because convex hull and isotopic diversity metrics are easy to calculate from multidimensional isotopic data, they may be useful food-web indicators for environmental management.

1. Introduction

Food webs have long been used as means to describe community structure and species' trophic relationships (Paine, 1980). Where strong interactions among food-web actors can be determined, impacts of human activities on those actors can lead to predictions of cascades to other elements of the ecosystem (Crowder and Norse, 2008). Such cascades may reveal functional characteristics within an ecosystem, such as bottom-up and top-down effects, trophic pyramids, and competition avoidance (Berg et al., 2015) that can be useful in developing tools for environmental management. Documentation of species' trophic positions and community trophic structure can also be used to generate a baseline against which the effects of natural disturbance or anthropogenic activities can be predicted, assessed, and mitigated, and thus contribute to an ecosystem-based approach to management (Giakoumi et al., 2015; Hussey et al., 2014). Descriptions of marine food webs are now recommended in a number of legal instruments adopted to protect the marine environment [including, for example, the Marine Science Framework Directive (European Commission, 2008)] as a means to represent the state and function of marine ecosystems (Crise et al., 2015). Identification of indicators that assess the health of food web structure and dynamics and acquisition of the scientific knowledge to develop indicators for ecosystem-based environmental management in diverse marine systems continue to be challenging (Rombouts et al., 2013b; Ruiter et al., 2010; Shephard et al., 2015).

For relatively inaccessible ecosystems such as chemosynthetic ecosystems in the deep sea, bulk stable isotope analyses together with feeding-guild assignments continue to be a powerful approach for descriptive and comparative food-web studies (Cordes et al., 2010; Gaudron et al., 2012; Lelièvre et al., 2018; Portail et al., 2018, 2016; Pruski et al., 2017; Reid et al., 2016, 2013; 2012; Shipley et al., 2017; Soto, 2009; Sweetman et al., 2013; Wang et al., 2018; Yamanaka et al., 2015). Contemporary food web studies at vents explore diverse topics, including the feeding ecology of species, relative contributions of

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Abbreviations: ISA, International Seabed Authority; rTCA, Tricarboxylic Acid; CBB, Calvin-Benson-Bassham; LTC, Legal and Technical Commission; PCR, Polymerase Chain Reaction; ROV, Remotely Operaterd Vehicle; IDiv, Isotopic Diversity; IDis, Isotopic Distinctiveness; ISim, Isotopic Similarity; INes, Isotopic Nestedness; IEve, Isotopic Evenness; IUni, Isotopic Uniqueness; SW1, Solwara 1; SSU, South Su; Pac, PACMANUS; ANOSIM, Analysis of Similarity; SIMPER, Similarity Percentage Analysis

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https://doi.org/10.1016/j.dsr.2019.04.018
Received 12 November 2018; Received in revised form 27 March 2019; Accepted 30 April 2019

photosynthetically derived versus chemosynthetic derived organic material, carbon fixation pathways in chemoautotrophic symbionts, and spatial and temporal variations in food-web structure.

Descriptive food-web approaches have also been recommended as a tool for assessment of seabed mining impacts (ISA, 2013, 2007). While arguments have been put forward for why mining at active hydrothermal vents may represent a threat of serious harm, vent protections are only in place in national waters for certain coastal states at present (Van Dover et al., 2018). Ecosystem-based management lies at the heart of the emergent practice of environmental management for the deep-sea mining sector (Anonymous, 2016) and attention currently focuses on documenting ecosystem structure, processes, function, and services through baseline studies. Should deep-sea mining activities be permitted at active vents in national waters or in areas beyond national jurisdictions, then when underway and during mine closure activities, indicators of foodweb attributes may need to be assessed through monitoring programs.

The first deep-sea environmental baseline study for a potential deep-sea mining operation at hydrothermal vents was undertaken by Nautilus Minerals, Ltd for mining leases in Manus Basin (Papua New Guinea; Coffey Natural Systems, 2008). Included in the baseline assessment was a study of food resources used by invertebrates colonizing peripheral hard substrata at the Solwara 1 prospect, compared to those used by invertebrates that are endemic to or associated with active vent sites (Erickson et al., 2009). This work relied on analysis of stable isotope compositions (δ^{13}C, δ^{15}N, δ^{34}S) of bulk tissues and assessment of feeding guilds based on the natural history of the taxa. More recently, carbon and nitrogen isotopic compositions of dominant taxa at the PACMANUS and Desmos Caldera vent sites within Manus Basin have been published (Wang et al., 2018). A 2008 field campaign in Manus Basin provided an opportunity to sample and analyze a greater diversity of vent invertebrates (31 taxa) from multiple vent fields (Solwara 1, South Su, PACMANUS; Fig. 1) to i) infer isotopic sources and trophic interactions, ii) add detail to the descriptive Galkin food-web model for Manus Basin vent ecosystems (Galkin, 1997), iii) test whether there may be environmentally influenced differences in isotopic niche space among sites, and iv) motivate discussion regarding indicators that can be used to summarize complex information and assess the status of food webs in chemoautotrophic (and other) deep-ses ecosystems.

Table 1
Manus Basin sampling site locations and depths. *Note*: Individuals were sampled at multiple points as indicated in this table, but they are referenced in the text simply as from Solwara 1, South Su, and PACMANUS. Coordinates: WGS84.

Site	Mound	Latitude (S)	Longitude (E)	Depth (m)
SOLWARA 1				
	sw1_active_1a	−3.789367	152.091417	1527
	sw1_active_2	−3.788438	152.094692	1501
	sw1_active_3	−3.787942	152.093624	1467
	sw1_hairy_snail	−3.789064	152.091096	1534
SOUTH SU				
	ssu_active_1	−3.804832	152.102789	1328
	ssu_active_2	−3.806738	152.104975	1324
	ssu_active_3	−3.807770	152.105227	1303
	ssu_active_4	−3.807739	152.104961	1253
	ssu_inactive_2	3.805613	152.1051072	1383
	ssu_inactive_5	3.806714	152.1031111	1304
PACMANUS				
Fenway	sw4e_active_1	−3.727949	151.672657	1677
Mimosa	sw8_active_1	−3.729134	151.673458	1705
Solwara 8	sw8_active_2	−3.730438	151.674272	1716
Solwara 6	sw6_active_1	−3.727739	151.681147	1708

2. Methods

2.1. *Collection of animals*

Animals were collected in July and August 2008 aboard the *Merchant Vessel NorSky* (S. Smith, Chief Scientist) from the Solwara 1 (formerly known as Suzette), South Su, and PACMANUS (Thal et al., 2014) hydrothermal vents fields (Table 1; Fig. 1). These sites lie along the Eastern Manus Volcanic Zone, a region of incipient rifting in felsic crust (Binns & Scott, 1993) and between the Djual and Weitin transform faults in Manus Basin. A quantitative description of the Solwara 1 and South Su vent invertebrate communities may be found in Collins et al.

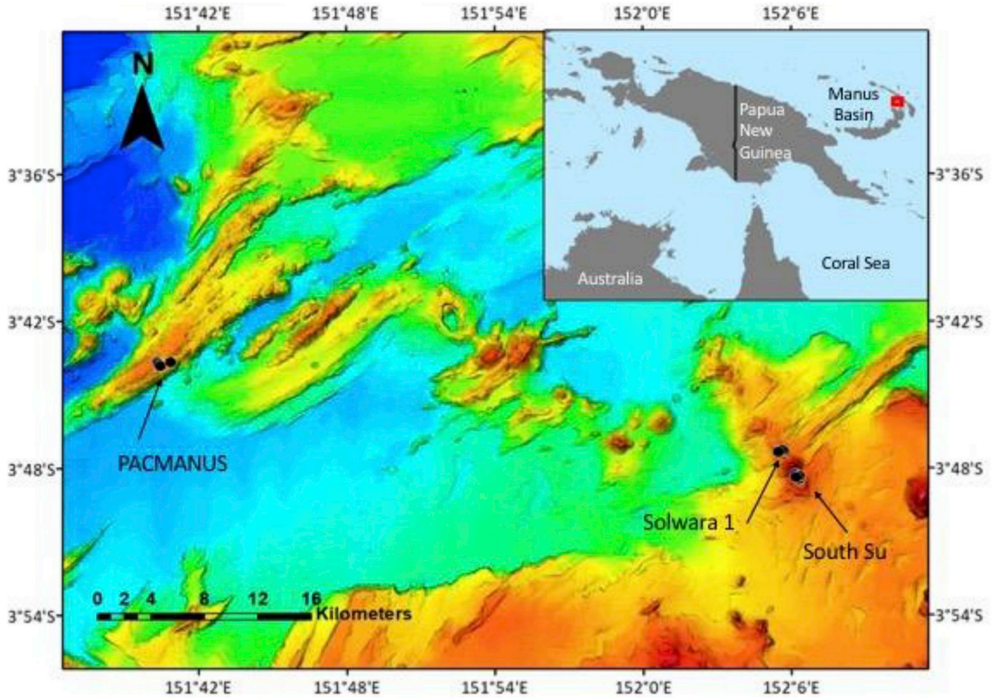

Fig. 1. Study Sites in Manus Basin: Solwara 1, South Su, PACMANUS. Black circles indicate sampling sites. Inset: Regional context. Basemap courtesy of Nautilus Minerals Ltd.

(2012). Within each vent field, the *ROV ST212* sampled areas that had signs of active venting, such as beds of mollusks (hairy snails, black snails, or mussels), live siboglinid tubeworms, sulfide chimneys, or shimmering water. Mollusk beds were sampled using scoops; other animals were collected via slurp gun or with the manipulators of the mechanical arm. Several eelpouts (*Pyrolycus manusanus*) that live among the vent invertebrates were inadvertently caught as bycatch. Tissue was removed from animals and dried for 48 h at 60 °C. Additional tissue was sampled from representatives of selected morphospecies and placed in non-denatured 95% ethanol for DNA sequencing and identification.

2.2. Identification of animals

Genomic DNA from selected taxa was extracted from muscle tissue using the Wizard SV Genomic DNA Purification System (Promega) following manufacturer's protocols. Polymerase chain reaction (PCR) was performed in a 20 µl reaction volume containing 2.5 µl template DNA, 1X PCR buffer (20 mM Tris, pH 8.8; 50 mM KCl; 0.01% Triton X-100, 0.02 mg/ml BSA), 2.5 mM $MgCl_2$, 0.125 mM dNTPs, 0.2 µl (1 unit) of Taq polymerase (Bioline), 0.375 µM of each direction of the primer pair. The primer pair LCO1490/HCO2198 (Folmer et al., 1994) was used to amplify *COI*. PCR was run on an Eppendorf thermocycler as follows: 94 °C/3 min, 35 x (94 °C/15 s, 48 °C/15 s, 72 °C/30 s), 72 °C/5 min. PCR product was purified using an ExoAP procedure and sequenced on a 3730xl DNA Analyzer using Big Dye terminator chemistry (Applied Biosystems). A consensus sequence was produced in Sequencher version 5.3 (Gene Codes).

Where *COI* did not amplify, or if a species-level match ($\geq$97% similarity) could not be found on GenBank and Barcode of Life Data Systems, then a region of the mitochondrial gene for 16S ribosomal RNA (*16S*) was sequenced, and phylogenetic analysis was performed to identify subfamily or genus. The primer pair 16 Sar/16Sbr (Palumbi, 1996) was used to amplify *16S*. Phylogenetic analysis was performed on either 16S sequences, or concatenated COI and 16S sequences. Sequences were aligned and all gaps, ambiguities, and missing data were removed using Sequencher v. 5.3 (Gene Codes).

2.3. Assignment of feeding guilds

Feeding guilds were assigned based on morphology, behavior, ecology (e.g., faunal associations), and literature accounts, as well as on the isotopic characteristics of each taxon, as discussed in Section 3.1.

2.4. Stable isotope analysis

Dried tissue was ground into powder using an agate mortar and pestle, after freezing with liquid nitrogen and fumed with 10% HCl to remove carbonates (carbon/nitrogen samples only), and submitted to either the Duke Environmental Stable Isotope Laboratory for paired carbon and nitrogen stable isotope analysis, or to the Washington State University Stable Isotope Core Facility for sulfur stable isotope analysis. Stable isotope ratios for each element are reported in per mil (‰):

$$\sigma X = (R_{sample}/R_{reference} - 1) \times 1000, \tag{1}$$

where X is C, N, or S. R is the molar ratio of heavy to light isotope ($^{13}C/^{12}C$, $^{15}N/^{14}N$, or $^{34}S/^{32}S$) in either sample material (R_{sample}) or the universal reference standards ($R_{reference}$) for carbon (Vienna Pee Dee Belemnite), nitrogen (air), or sulfur (Canyon Diablo Troilite). The standard deviation of replicate samples was ~0.1‰ for nitrogen/carbon and ~0.06‰ for sulfur. Bias due to mass spectrometer drift was < 0.1‰.

Carbon, nitrogen, and sulfur isotopic data reported for taxa from the active Solwara 1 vent field (hydroids, *Eosipho* sp., *Vulcanolepas parensis, Munidopsis lauensis, Austinograea alaysae, Eochionelasmus ohtai*, *Ifremeria nautilei*) by Erickson et al. (2009) are included in the isotope database analyzed here.

2.5. Statistical analyses

All data analyses were undertaken using R software (R Core Team, 2013). Mean and standard deviation of all carbon, nitrogen, and sulfur isotopic values for each taxon were calculated for each site.

2.5.1. Analysis of similarity (ANOSIM) and similarity percentage analysis (SIMPER)

Analysis of similarity (ANOSIM) among the isotopic compositions of taxa was undertaken at the site level for comparisons within feeding guilds. To eliminate potential biases from differences in the ranges of values of different stable isotopes, the range of each isotope was rescaled to between [0,1] for each site and calculated for each individual using formula (2).

$$\sigma X_{scaled} = [\sigma X - \min(\sigma X)]/[\max(\sigma X) - \min(\sigma X)]. \tag{2}$$

The Euclidean distance between each individual was calculated in the 3 isotopic dimensions and compiled as a similarity matrix. One-way analysis of similarities (999 permutations) between each species within feeding guilds were then performed for each site. This method yields an R value, where 1 highlights the most isotopically dissimilar species and 0 highlights the most isotopically similar species, and a p-value probability. For $p < 0.05$, the null hypothesis that the groups are similar can be rejected. Because this p-value is influenced by sample size and the R value is an absolute measure between two groups (Clarke and Gorley, 2006), the very small sample size of some species will trigger larger p values. The R value can thus indicate dissimilarity even if not significant. To identify which isotope contributed most to dissimilarities among groups, a similarity percentage analysis (SIMPER) was performed on the Euclidean distance matrix.

2.5.2. Community analyses

Convex hulls provide an *n*-dimensional measure of the volume of trait space (isotopic richness) occupied by species in a community (Cornwell et al., 2006) and were used to explore differences in site-specific stable isotope volume (isotopic trait space) among 6 species shared at 3 sites using non-scaled isotopic data. Two overlap metrics—isotopic similarity (ISim) and isotopic nestedness (INes)—were used for further comparison of isotopic trait spaces among sites. To compare the relative position of each species within the community, four additional isotopic diversity metrics [isotopic divergence (IDiv), dispersion (IDis), eveness (IEve), and uniqueness (IUni); Table S1] were used (Cucherousset and Villéger, 2015). For these 4 metrics, the isotopic ranges of the six taxa common to all 3 sites were rescaled to between 0 and 1 values using formula (2), as suggested by Cucherousset and Villéger (2015). The four metrics were then calculated using the mean isotopic value for each species to avoid bias triggered by an unbalanced sampling design (i.e., different number of replicates per species). These descriptive metrics were determined for $\delta^{13}C$ and $\delta^{15}N$, $\delta^{13}C$ and $\delta^{34}S$, and for $\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$ values for the 6 species that were shared among sites. A non-parametric bootstrap analysis (999 permutations) was performed and the credibility intervals were computed using the bias-corrected and accelerated method (Banjanovic and Osborne, 2016) at the 50%, 75% and 95% levels of confidence.

3. Results and interpretation

A combined total of 722 analyses for $\delta^{13}C$, $\delta^{15}N$, $\delta^{34}S$ across 3 vent fields was obtained from 31 taxa representing at least 9 feeding guilds (Table 2; see **Research Data** for the raw species-isotope spreadsheet). Taxonomic identification to species level for 11 of the taxa in this study were confirmed using gene sequences (Table S2); other taxa were identified to morphospecies. Although taxa for which isotopic data was obtained varied among the sites, nearly equal numbers of taxa were analyzed at each site (SW1: 17 taxa; SSu: 16 taxa; PACMANUS: 19 taxa; Table 2). For Solwara 1 and South Su, the taxa analyzed for isotopic

Table 2

Taxa collected from active hydrothermal vents in Manus Basin with interpreted feeding guilds based on morphology, behavior, ecology, and isotopic data (see results and interpretation); IDs (used in biplots, Fig. 3), location of samples (SW1: Solwara 1; SSu: South Su; PAC: PACMANUS), and number of individuals analyzed for C, N, and/or S; "–": no individuals analyzed from the site.

PHYLUM	TAXON	FEEDING GUILD	ID	SW1	SSu	PAC
Porifera	*Abyssocladia dominalba*	microcarnivore	Abd	–	4	–
Cnidaria						
Hydrozoa	Hydroids	microcarnivore	Hyd	1	–	–
Anthozoa	*Maractis* sp 1	omnivore	Ma1	–	–	5
Mollusca						
Gastropoda						
Lepetodrilidae	*Lepetodrilus schrolli*	bacteri(archae)ovore	Les	1	3	1
Chilodontidae	*Bathymargarites* sp.	bacteri(archae)ovore	Bmr	–	2	–
Phenacolepadidae	*Olgasolaris tollmanni*	bacteri(archae)ovore	Olg	5	5	–
Buccinidae	*Eosipho* sp.	omnivore	Eos	1	–	–
Provannidae	*Alviniconcha boucheti*	symbiotroph	Alb	5	20	5
	Alviniconcha kojimai	symbiotroph	Alk	2	–	–
	Ifremeria nautilei	symbiotroph	Ifn	20	14	25
Bivalvia						
Mytilidae	*Bathymodiolus manusensis*	symbiotroph	Bam	–	5	10
Pectinidae	cf *Sinepecten segonzaci*	suspension feeder	Sis	–	–	5
Annelida						
Polychaeta						
Nautiliniellidae	*Iheyomytilidicola lauensis*	mussel "commensal"	Ihl	–	–	8
Polynoidae	*Branchinotogluma segonzaci*	bacteri(archae)omnivore	Brs	–	–	1
	Branchinotogluminae sp.	bacteri(archae)omnivore	Br1	2	3	–
	Branchipolynoe pettiboneae	mussel "commensal"	Bpp	–	5	9
	Polynoidae sp. 1	omnivore	Po1	–	–	2
	Polynoidae sp. 2	omnivore	Po2	–	–	1
	Thermopolynoe branchiata	omnivore	Thb	1	2	4
Siboglinidae	*Arcovestia ivanovi*	symbiotroph	Ari	–	–	7
	Lamellibrachia sp.	symbiotroph	La1	–	2	–
Echiura	Echiurid sp.	deposit feeder	Ec1	1	–	–
Arthropoda						
Crustacea						
Cirripedia	*Vulcanolepas parensis*	suspension feeder	Vup	1	5	4
	Eochionelasmus ohtai	suspension feeder	Eoc	12	5	–
Decapoda						
Alvinocarididae	*Alvinocaris longirostris*	grazer/scavenger	All	–	1	3
	Alvinocaris kexueae	grazer/scavenger	Ake	–	4	2
	Chorocaris variabilis	grazer/scavenger	Chv	5	3	5
	Rimicaridinae sp.	grazer/scavenger	Ri1	–	2	–
Galatheidae	*Munidopsis lauensis*	omnivore	Mul	2	5	7
Bythograeidae	*Austinograea alayseae*	omnivore	Aua	10	–	3
Chordata	*Pyrolycus manusanus*	omnivore	Pym	2	–	10

compositions are a subset of the total species richness reported by Collins et al. (2012; Solwara 1 (33 taxa), South Su (39 taxa), and they include the most abundant holobiont and secondary consumer taxa as well as the taxa likely to be at highest trophic levels in the food web (decapod crustaceans, fish). Comparable species richness data for PACMANUS are not available.

A particular challenge in analyses of vent food webs is to distinguish between contributions of organic material derived from photosynthetic and chemosynthetic processes (Portail et al., 2018). Methane released from Manus vents is thermogenic, with $\delta^{13}C$ values in the range of $-15‰$ to $-8.6‰$, and available to methanotrophic bacteria. But methane concentrations in high-temperature vent fluids at Manus Basin vents are low (Reeves et al., 2014) and we found no report in the literature of methanotrophy in holobiont genera or species that occur at Manus vents, suggesting that symbiont-consumed methane is not a key carbon source in the food web. The two prevalent chemoautotrophic carbon fixation pathways associated with sulfide oxidation at vents yield isotopic values that are either depleted in ^{13}C [Calvin-Benson-Bassham (CBB) cycle: $\delta^{13}C$ typically $-36‰$ to $-30‰$] or enriched in ^{13}C [reverse TriCarboxylic Acid cycle (rTCA): $\delta^{13}C$ typically $-15‰$ to $-10‰$] (Portail et al., 2018). For non-symbiotroph consumers, mixed diets of CBB- and rTCA-derived carbon or methanotrophy would have intermediate isotopic compositions and would be undifferentiable from photoautotrophically derived carbon. In this study, given that the mean $\delta^{34}S$ value measured among all invertebrate samples was $-2.1‰$, that the $\delta^{34}S$ of vent sulfide is $< +5‰$ (Table S3), and that the $\delta^{34}S$ of seawater sulfate is $+18$ to $+22‰$ (Böttcher et al., 2007), we infer only a minor, if any, role of photosynthetically derived organic material in the Manus Basin vent food webs, with the exception of the diet of a carnivorous sponge (*Abyssocladia dominalba*), which we infer to feed on non-chemosynthetically derived food items. The sulfur isotopic compositions of vent invertebrates thus support inferrences by others that photosynthetically derived organic material does not play a major role in deep-water vent food webs (Portail et al., 2018 and papers cited therein), though it may be an important dietary element in shallow-water vent food webs (Comeault et al., 2010), be a key dietary resource for dispersive stages (Pond et al., 1997), or supply essential nutrients to vent invertebrates (Pond et al., 2002).

3.1. *Feeding guilds and trophic interactions*

Biplots of $\delta^{13}C/\delta^{15}N$ and $\delta^{13}C/\delta^{34}S$ compositions for each species sampled within each vent field (Fig. 2) allow for interpretation of feeding guilds and constrain trophic interactions. ANOSIM and SIMPER analyses (Tables S4–S9) are used to assess the similarity of isotopic compositions of taxa within feeding guilds.

Symbiotrophs. Six symbiotroph (holobiont) taxa were sampled at Manus vent fields, of which only the black snail *Ifremeria nautilei* and the hairy snail *Alviniconcha boucheti* were sampled from all three fields. *A. kojimai* is morphologically cryptic with *A. boucheti* and was only

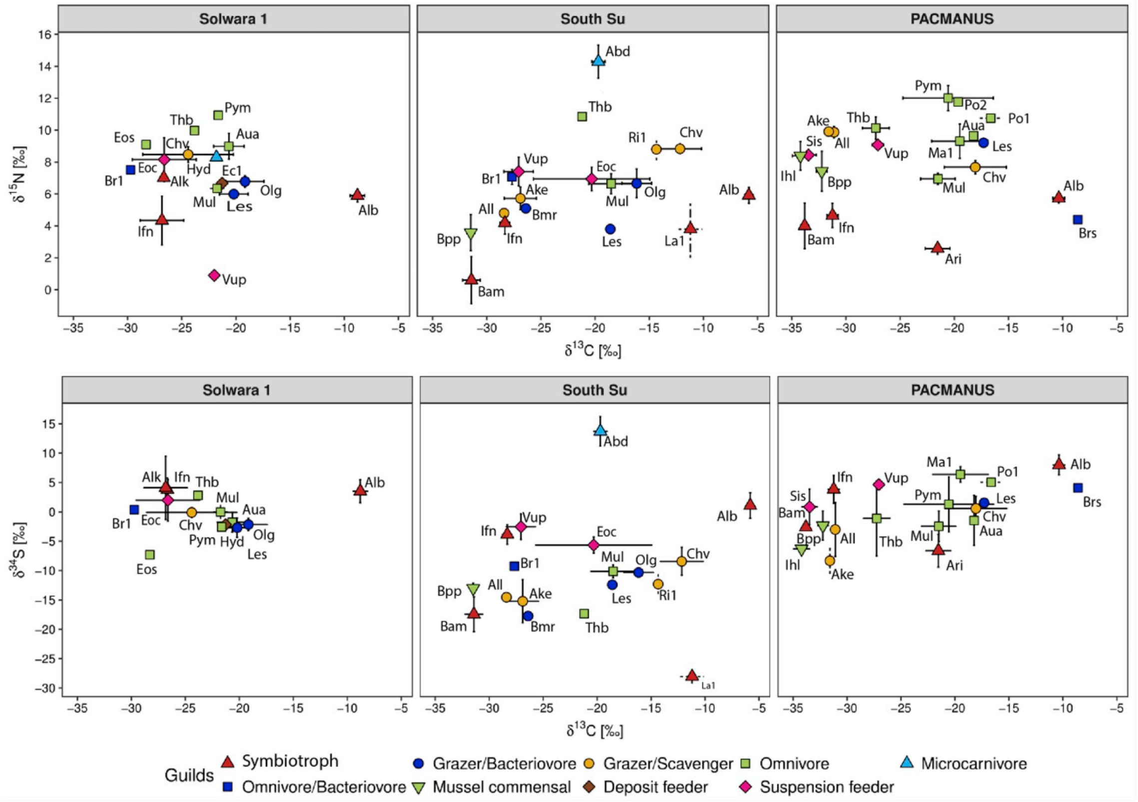

Fig. 2. Carbon-nitrogen and carbon-sulfur isotope biplots for invertebrate taxa (and one fish) from 3 vent fields (Solwara 1, South Su, PACMANUS) in Manus Basin, Papua New Guinea. Feeding guilds are indicated by symbol shape. Where n ≥ 3, values are means ± SD (solid lines); where n = 2, values are means ± ranges (dashed lines), where n = 1, values are points. Taxonomic abbreviations are listed in Table 2.

identified from Solwara 1. The mussel *Bathymodiolus manusensis* and the siboglinid tubeworms *Arcovestia ivanovi* (PACMANUS) and *Lamellibrachia* sp. (South Su) are so far not known from Solwara 1 (Collins et al., 2012).

Isotopic compositions of symbiotrophic taxa were dissimilar from one another, with the exception of *A. kojimai* and *I. nautilei,* which occupy similar isotopic niches (Table S4). δ^{13}C values for the symbiotrophs reflect the carbon fixation pathway of their symbionts. *Alviniconcha boucheti* had the most ^{13}C-enriched isotopic composition (δ^{13}C > -11‰), while its congener *A. kojimai* was depleted in ^{13}C (δ^{13}C < -27‰). This is consistent with environmental niche partitioning as described by (Beinart et al., 2012) for *Alviniconcha* congeners in Lau Basin, where ^{13}C-enriched values were observed in individuals hosting ε-proteobacteria (inferred rTCA pathway) and ^{13}C-depleted values were observed in individuals hosting thiotrophic γ-proteobacteria (inferred CBB pathway). The mussel *B. manusensis* and the black snail *I. nautilei* host γ-proteobacteria thiotrophic symbionts (Lorion et al., 2013; Nakagawa and Takai, 2008) and also had ^{13}C-depleted isotopic compositions (δ^{13}C < -26‰), consistent with CBB carbon fixation (Nakagawa and Takai, 2008). Siboglinid tubeworms associate only with γ-proteobacteria (Reveillaud et al., 2018), but the difference in δ^{13}C of ~10‰ for the symbiotrophic tubeworm taxa *Lamellibrachia* sp. (−11.2‰; South Su) and *A. ivanovi* (−21.5‰; PACMANUS) suggests that the two taxa occupy different microhabitats and rely on different carbon fixation pathways (Hugler and Sievert, 2011; Reveillaud et al., 2018), namely rTCA in *Lamellibrachia* sp. and RBB in *Arcovestia ivanovi*. Wang et al. (2018) report similar carbon isotopic values for *A. ivanovi* in Manus Basin. Symbiotrophic taxa were depleted in ^{15}N (δ^{15}N $<$ 8‰) relative to most other taxa analyzed in this study, a finding that is consistent with observations from many vent systems and which is attributed to strong N-isotope fractionation during chemosynthetic assimilation of ammonium or the local supply of ^{15}N-depleted ammonium (Bourbonnais et al., 2012).

Symbiotrophic invertebrates tended to be depleted in ^{34}S (δ^{34}S$_{\text{ave}} = -0.8 \pm 9.1$‰; δ^{34}S$_{\text{range}} = -29$ to $+14$‰) relative to modern seawater sulfate (δ^{34}S = 18 to +22‰; Böttcher et al., 2007), consistent with organic sulfur derived from dissolved inorganic sulfide in vent fluids (Table S3). *Lamellibrachia* sp. at South Su were remarkably depleted in ^{34}S (δ^{34}S < -20‰). This extreme depletion in ^{34}S in *Lamellibrachia* sp. is similar to that observed for a *Lamellibrachia* species at Gulf of Mexico seeps, where uptake of sulfide is from sediment porewaters by root tissue rather than at the sediment-water interface (Freytag et al., 2001; MacAvoy et al., 2005).

Mussel "commensals". The carbon and sulfur isotopic compositions of the mussel "commensal" polynoid polychaete (*Branchipolynoe pettibonei*) and the mussel host tissue are similar (Fig. 2), consistent with a commensal worm diet of mussel tissue. δ^{15}N contributes > 70% to the dissimilarity between host and *B. pettibonei* isotopic compositions (Table S5), which further supports a direct trophic relationship with the polychaete feeding on host-derived organic material. A consumer relationship for "commensal" polynoids feeding on organic material (tissues and/or mucus) derived from the host mussels has long been proposed, and has recently been supported by Portail et al. (2018) for the trophic relationship between *Branchipolynoe seepensis* and the host mussel *Bathymodiolus azoricus.* A second commensal species, *Iheyomytilidicola lauensis,* is a chrysopetalid polychaete that lives in the mantle cavity of *Bathymolodius manusensis* at PACMANUS. Like *B. pettibonei*, *I. lauensis* has isotopic compositions consistent with a diet of mussel tissue and(or) mucus (Fig. 2, Table S5).

Bacteri(Archae)ovores. Three gastropod taxa (*Lepetodrilus schrolli, Olgasolaris tollmani, Bathymargarites* sp.) are posited to be grazers on autotrophic microorganisms (Bacteria and/or Archaea) based on the nature of their rasping mouthparts and overlap of their δ^{15}N values with those of the symbiotrophs (i.e., δ^{15}N $<$ 8‰). *L. schrolli* at

PACMANUS is somewhat of an exception (δ^{15}N–9‰). These gastropods species have wide-ranging δ^{13}C values (∼-16‰ to −26‰), suggesting they rely on an isotopically diverse pool of microorganisms, as has been reported for bacteriovores elsewhere (Lelièvre et al., 2018). At Solwara 1, the isotopic composition of *O. tollmani* is similar to that of *L. schrolli*, but at South Su, the nitrogen isotopic composition contributes to dissimilarity in isotopic niche space (Table S6). *O. tollmanni* and *L. schrolli* occur in similar abundances among *Ifremeria nautilei* and *Alviniconcha* spp. assemblages at Solwara 1, but *L. schrolli* is at least an order of magnitude more abundant at South Su (Collins et al., 2012), suggesting an environment at South Su that favors *L. schrolli*. *Bathymargarites* sp. is isotopically dissimilar to both *L. schrolli* and *O. tollmani* (Table S6), due primarily to its more ^{13}C-depleted isotopic composition (δ^{13}C = ∼26‰) compared to that of *L. schrolli* and *O. tollmanni* (δ^{13}C = −14‰ to −21‰). The isotopic differentiation between *Bathymargarites* sp. and the other two species of bacteri(archae)ovores suggests there is microhabitat partitioning that results in selective microvory, similar to that inferred by Govenar et al. (2015) for limpets associated with aggregations of *Riftia pachyptila*.

Bacteri(Archae)omnivores. Two polynoid scaleworm polychaetes (*Branchinotogluma segonzaci*, Branchinotogluminae sp.), typically considered to be predators or omnivores capable of predatory and scavenging behavior due to their relatively large size and large jaws (Lelièvre et al., 2018), are posited here to feed on a mix of autotrophic microorganisms and on consumers of autotrophic microorganisms, given the relatively ^{15}N-depleted values (δ^{15}N < ∼8‰) of their tissues. Differences in their carbon isotopic composition (PACMANUS *Branchinotogluma segonzaci:* δ^{13}C = −8.6‰; Solwara 1 and South Su *Brachinotogluminae* sp 1: $\delta^{13}C_{\text{ave, sd}}$ = −28.5‰, 1.15) indicate these two morphologically similar polychaetes rely on different food resources.

Deposit Feeder. A single deposit-feeding echiurid was recovered and analyzed from Solwara 1. This taxon uses its proboscis to scoop up and ingest sediment. It occupies an isotopic position in the middle of the carbon and nitrogen isotopic space and, given its feeding mode, may be a good indicator of the isotopic composition of organic material in sediments near Manus vents (i.e., δ^{13}C = −21.8‰, δ^{15}N = 8.3‰, δ^{34}S = 2.2‰). The carbon isotopic composition of the echiurid is consistent with a mixed diet of microbially RBB- and rTCA-fixed organic carbon in the sediment and the low sulfur isotopic composition suggests autotrophic production depends of sulfide from vent fluids. Autotrophic CO_2 fixation is expected in microbial communities of sediments (Wang et al., 2017), but direct measurement of the isotopic composition of organic material in vent sediments is warranted, as is further study of the diet of the echiurid.

Grazers/Scavengers. Based on behavior and isotopic compositions, the shrimp taxa *Alvinocaris longirostris, A. kexueae, Chorocaris variabilis, and* Rimicaridinae sp. were *a priori* interpreted to feed on heterotrophic organisms, including juvenile invertebrates and detritus. The isotopic compositions of the two alvinocaridid species are similar, as are the isotopic compositions of the two rimicarid species. The alvinocarids and *Chorocaris*/Rimicaridinae are in different isotopic spaces (Table S7), due primarily to differences in their ^{13}C compositions ($\delta^{13}C_{\text{alvinocarids}}$ = −29.2 ± 2.4‰; $\delta^{13}C_{Chorocaris/\text{Rimicaridinae}}$ = −18.5 ± 3.1‰). Carbon isotopic values suggest that the alvinocarids occupy microhabitats dominated by organic carbon derived from the CBB pathway, while *Chorocaris*/Rimicaridinae occupy microhabitats dominated by organic carbon derived from the rTCA pathway. A plausible interpretation of the isotopic differences between these two groups of shrimp is that the *Chorocaris*/Rimicaridinae are symbiotrophs dependent primarily on episymbiotic autotrophic bacteria that use the rTCA pathway for their nutrition, as has been suggested for *Rimicaris exoculata* on the Mid-Atlantic Ridge (Hügler and Sievert, 2011)(), while the alvinocarids are more conventional grazers/scavengers. The smaller variance in δ^{15}N values for *Chorocaris*/Rimicaridinae (std dev = 0.07‰) is consistent with a limited dietary pool compared to the larger variance of the alvinocarids (std dev = 2.3‰), which is consistent with a mixed, grazing and scavenging dietary strategy.

Omnivores. Three large polynoid polychaetes (*Polynoidae* sp. 1 and sp. 2, *Thermopolynoe branchiata*), one snail (*Eosipho* sp.), the anemone (*Maractis* sp.), two crustaceans (*Munidopsis lauensis, Austinograea alyseae*) and a fish (*Pyrolycus manusanus*) were inferred *a priori* as having an omnivorous feeding behavior (i.e., predator, grazer, scavenger). Nitrogen isotopic compositions for these taxa were relatively enriched in ^{15}N, consistent with their roles as higher-trophic-level consumers in the Manus Basin food webs (Fig. 2), but they are not all in the same isotopic niche space (Table S8). The two polynoid taxa rely on different carbon sources ($\delta^{13}C_{T.\ branchiata}$ ∼ -26‰; $\delta^{13}C_{\text{Polynoid sp. 1}}$ ∼ −18‰) but have similar δ^{15}N values of ∼10.5‰ and are similar in nitrogen isotopic space to that of the fish *Pyrolycus manusanus*. *Eosipho* sp. is a carnivorous whelk that occupies an isotopic space distinct from that of other taxa in this feeding guild (Table S8). The rank order of mean δ^{15}N composition for this omnivorous feeding guild, and thus a suggestion of their relative trophic levels from lowest to highest, is *Eosipho* sp. (9.1‰) = *Austinograea alaysae* (9.1 ± 0.8‰) = *Maractis* sp.1 (9.1 ± 1.1‰) < *Munidopsis lauensis* (10.3 ± 0.5‰) = *Thermoplynoe branchiata* (10.3 ± 0.87‰) < Polynoid sp. 1 (10.7‰) < Polynoid sp. 2 (11.8‰) < *Pyrolycus manusanus* (11.8 ± 0.8‰). Stomach contents of *P. manusanus* are reported to "frequently" include shrimp body parts (Machida and Hashimoto, 2002). If shrimp are indeed a dominant dietary item of the fish (*Pyrolycus manusanus*: δ^{13}C = −20.7 ± 3.6‰; δ^{15}N = 11.8 ± 0.8‰), then consumption of rimicarid shrimp species (δ^{13}C = −18 ± 5.7‰; δ^{15}N = 8.3 ± 0.7‰) would be a good isotopic fit as prey items, better than the alvinocarid species (δ^{13}C = −29 ± 2.4‰; δ^{15}N = 7.7 ± 2.3‰).

The diet of the anemone *Maractis* sp. is challenging to interpret. Its congener at Atlantic vents is *Maractis rimicarivora,* a species that has been observed to consume *Rimicaris exoculata* (Fautin and Barber, 1999). At PACMANUS*, Maractis* sp. have δ^{13}C isotopic compositions ($\delta^{13}C_{\text{ave}}$ = −19.5‰) consistent with a diet of Rimicaridinae ($\delta^{13}C_{\text{ave}}$ = −18.1‰) and not alvinocarid ($\delta^{13}C_{\text{ave}}$ = −31.1‰) shrimp, but the large difference in δ^{34}S values (∼6‰) between the anemone and a putative diet of rimicarid shrimp suggests that the anemone diet is not restricted to Rimicaridinae shrimp.

Suspension feeders. The suspension-feeding guild is represented by the barnacles *Eochinelasmus ohtai* and *Vulcanolepas parensis,* and the bivalve *Sinepecten segonzaci*. This guild exhibits a very wide range of δ^{13}C (−29 to −14‰) and δ^{34}S values (−7.4 to +5.9‰) but occupies a relatively narrow δ^{15}N window [(δ^{15}N average 5.4–10.2‰, with the exception of one *V. parensis* individual from Solwara 1 (δ^{15}N = 0.9‰)]. The two barnacle species are isotopically similar (Table S9), consistent with their similar mode of cirral feeding, although they occupy different microhabitats at Manus vents. *Eochionelasmus ohtai* is a sessile barnacle found in high abundance close to *Ifremeria nautilei* and *Alviniconcha* spp. (Collins et al., 2012), while *Vulcanolepas parensis* is considered to be more of a peripheral species (Erickson et al., 2009). The cirri of *E. ohtai* are described as "delicate, with long, slender, multiarticulate rami clothed with fine setae, all adaptations for feeding on extremely fine particles" (Yamaguchi and Newman, 1990). Cirri of species in the genus *Vulcanolepas* are also described as long and delicate, with a diet of microorganisms that may have either chemo- or photosynthetically derived carbon isotopic compositions (Buckeridge et al., 2013). It has been suggested that *Vulcanolepas* species may feed on cirral episymbionts (Marsh et al., 2012). Scallops (*S. segonzaci*) at the PACMANUS site have carbon isotopic compositions that match those of mussels at the same site (i.e., mean δ^{13}C = −33‰), but *S. segonzaci* is more ^{15}N-enriched by about 4‰. Until there is evidence otherwise, we suggest this scallop is a mucociliary suspension feeder on microorganisms that use organic carbon derived from a CBB carbon fixation pathway.

Microcarnivores. Isotopic compositions for two microcarnivorous species—the cladorhizid sponge *Abyssocladia dominalba* from South Su and unidentified hydroids from Solwara 1—are reported in this study.

The sponge reportedly feeds on microcrustaceans (e.g., copepods) in the water column (Vacelet, 2006). *A. dominalba* is conspicuously enriched in ^{15}N, which is inferred to be a consequence of a predatory diet of allochthonous zooplankton. Moreover, *A. dominalba* is also enriched in ^{34}S ($\delta^{34}S$ = +11 to +17‰) compared to the rest of the South Su community; this is also consistent with an allochthonous source of organic sulfur derived from seawater sulfate ($\delta^{34}S$ = 21‰; Böttcher et al., 2007), rather than organic sulfur derived from hydrothermal fluid sulfides that are relatively depleted in ^{34}S. Carbon and nitrogen isotopic compositions of the hydroids fall within the range of those observed for the suspension-feeding guild that rely on microorganisms, despite belonging to the microcarnivore guild.

3.2. Manus Basin food web update

Galkin (1997) provided an early description of the Manus Basin food web based on the natural history of the dominant taxa (including zonation, behavior, and inferred feeding guilds). This preliminary food web included four guilds: symbiotrophs, grazers/deposit feeders, suspension feeders, and scavengers/carnivores). It also suggested a role for methanotrophic as well as sulfide-oxidizing bacteria and direct predation on symbiotrophs. With additional collections and natural history data, we resolve 9 feeding guilds (symbiotrophs, bacteri(archae)ovores, bacteri(archae)/omnivores, deposit feeders, suspension feeders, grazer/scavengers, omnivores, microcarnivores) (Fig. 3). The symbiotrophic guilds are further differentiated by the inferred carbon fixation pathway (CBB, rTCA, and a pathway yet to be determined) of their symbionts. Other consumer guilds are also subdivided into isotopically distinct groups (Fig. 3) that may represent trophic specializations (e.g., the possibility that rimicarid shrimp rely on their episymbionts for most of their nutrition while the chorocarid shrimp are more generalist grazers/scavengers) or other means of niche separation. The guild containing the microcarnivorous sponge is inferred to be isotopically (and thus nutritionally) independent of the vent food web. Apart from this microcarnivore guild, there is no isotopic evidence for a major role of photosynthetically derived organic material among the taxa analyzed. Nor is there evidence of an important role of thermogenic methane. The Manus Basin food web is not unlike that of other vent ecosystems where CO_2 (not methane) is the dominant carbon source (e.g., Levesque et al., 2006; Reid et al., 2013; Van Dover, 2008, 2002). As is typical of vent ecosystems, Manus Basin vents support multiple isotopically distinctive autotrophic carbon sources and multiple consumer guilds, but it is outside the scope of this report to undertake a comprehensive comparative study of vent food webs.

3.3. Isotopic trait space and diversity metrics

Only 6 species—*Lepetodrilus schrolli, Alviniconcha boucheti, Ifremeria nautilei, Thermopolynoe branchiata, Vulcanolepas parensis, Munidopsis lauensis*—were collected and analyzed from all 3 sites (Table 2). These taxa were used to compare isotopic trait spaces and diversity metrics among sites.

3.3.1. Isotopic trait spaces (convex hulls)

The C-N and C-S isotopic trait spaces for the 6 shared species at Solwara 1, South Su, and PACMANUS (Fig. 4) highlight differences among vent sites, especially in carbon and sulfur isotopic compositions. For example, the carbon isotopic compositions of *I. nautilei* progresses to lighter (more ^{13}C-depleted) values from Solwara 1 to South Su to PACMANUS, with little overlap across sites. The same pattern toward more ^{13}C-depleted carbon isotopic compositions in *Alviniconcha boucheti* from SW1 to SSu to PAC is also evident. Given that these two species are symbiotrophic taxa that fix inorganic carbon, this finding is consistent with a systematic depletion in ^{13}C in the source inorganic

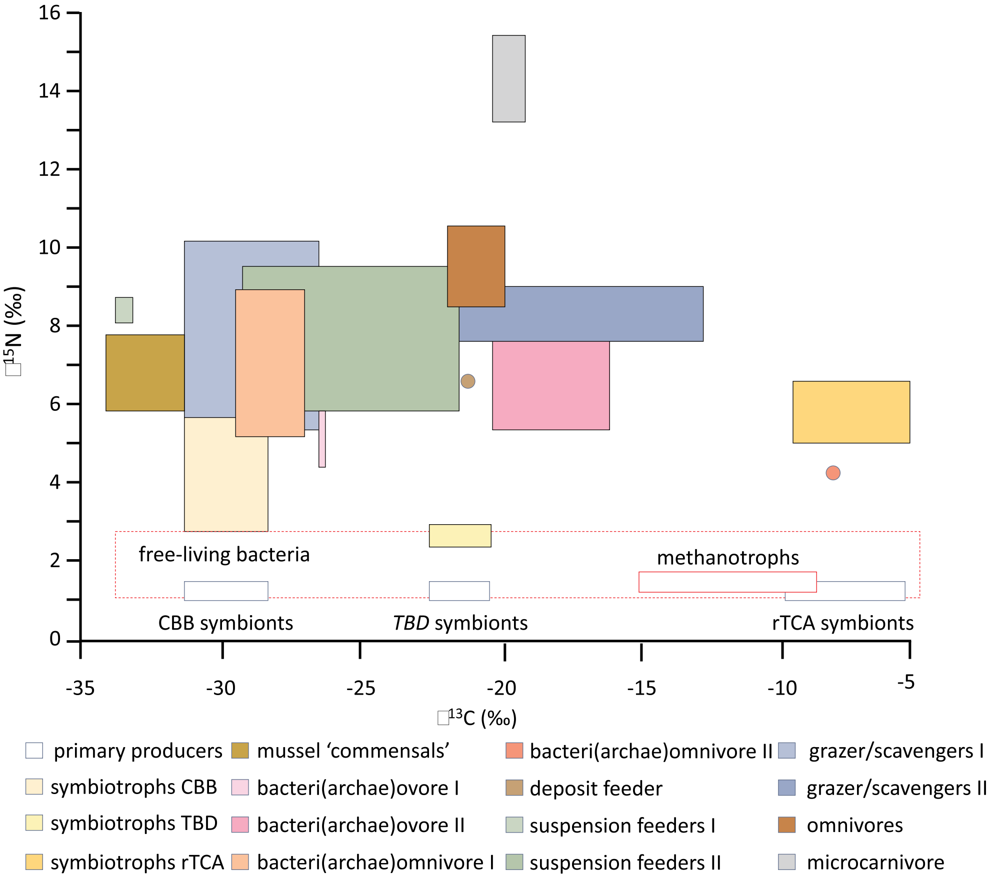

Fig. 3. Manus Basin (Solwara 1, South Su, PACMANUS) trophic guilds in carbon and nitrogen stable isotope space, with inferred values for microorganism types. Rectangles represent the standard deviation about the mean for each guild or subguild; circles represent values for a single specimen representing a guild. Note: rectangles for free-living and symbiotic bacteria are conceptual. **Symbiotroph CBB Guild**: *Alviniconcha kojima, Ifremeria nautilei, Bathymodiolus manusensis*; **Symbiotroph TBD (To Be Determined) Guild**: *Arcovestia ivanovi*; **Symbiotroph rTCA Guild**: *Alviniconcha boucheti, Lamellibrachia* sp.; **Mussel 'commensal' Guild:** *Branchipolynoe pettibonei, Iheyomytilidicola lauensis*; **Bacteri(Archae)ovore I Guild:** *Bathymargarites* sp.; **Bacteri(Archae)ovore II Guild:** *Lepetodrilus schrolli, Olgasolaris tollmani*; **Bacteri(Archae)Omnivore I Guild:** Branchinotogluminae; **Bacteri (Archae) Omnivore II Guild:** *Branchinotogluma segonzaci*; **Deposit-Feeder Guild:** Echiurid; **Suspension-Feeder I Guild:** cf *Sinepecten segonzaci*; **Suspension-Feeder II Guild:** *Vulcanolepas parensis, Eochionelasmus ohtai*; **Grazer/Scavenger I Guild:** *Alvinocaris longirostris, A. kexueae*; **Grazer/Scavenger II Guild:** *Chorocaris variabilis, Rimicaridinae* sp.; **Omnivore Guild:** *Pyrolycus manusanus*, Polynoidae sp. 2, Polynoidae sp. 1, *Thermopolynoe branchiate, Munidopsis lauensis, Maractis* sp., *Austinograea alaysae, Eosipho* sp.; **Microcarnivore Guild:** *Abyssocladia dominalba*, hydroid.

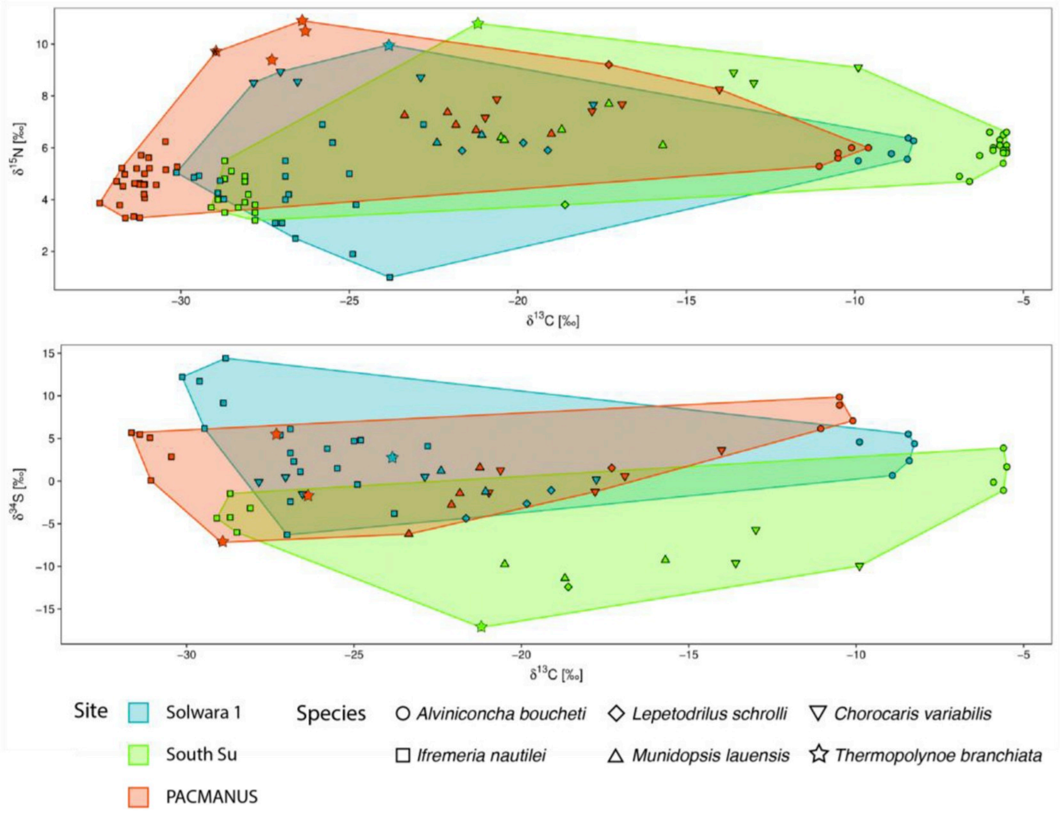

Fig. 4. Isotopic richness at the level of the vent field. Convex hulls for isotopic values for all individuals of 6 taxa occurring at all 3 vent sites (Solwara 1, South Su, PACMANUS) in $\delta^{13}C/\delta^{15}N$ and $\delta^{13}C/\delta^{34}S$ niche space.

Table 3
Pairwise site comparisons of isotopic similarity (*ISim*) and isotopic nestedness (*INes*) for all carbon, nitrogen, and sulfur isotopic compositions values for the 6 taxa shared at all three vent sites (*Alviniconcha boucheti, Ifremeria nautilei, Munidopsis lauensis, Lepetodrilus schrolli, Chorocaris variabilis, Thermopolynoe branchiata*).

Isotope Space	Sites	*ISim*	*INes*
$\delta^{13}C$ and $\delta^{15}N$	Sw1-SSu	0.5	0.7
	Sw1-PAC	0.6	0.8
	PAC-SSu	0.5	0.7
$\delta^{13}C$ and $\delta^{34}S$	Sw1-SSu	0.1	0.3
	Sw1-PAC	0.6	0.9
	PAC-SSu	0.4	0.7
$\delta^{13}C$, $\delta^{15}N$, $\delta^{34}S$	Sw1-SSu	0.0	0.1
	Sw1-PAC	0.3	0.6
	PAC-SSu	0.0	0.0

Table 4
Isotopic diversity metrics [divergence (IDiv), dispersion (IDis), evenness (IEve), and uniqueness (IUni)] for scaled, mean carbon, nitrogen, and sulfur isotope values for the 5 taxa (*Alviniconcha boucheti, Ifremeria nautilei, Lepetodrilus schrolli, Munidopsis lauensis, Chorocaris variabilis, Thermopolynoe branchiata*) shared at all three vent sites.

		Vent Field		
Isotope Space	Index	Solwara1	South Su	PACMANUS
$\delta^{13}C$ and $\delta^{15}N$	IDiv	0.68	0.73	0.67
	IDis	0.54	0.69	0.58
	IEve	0.74	0.94	0.76
	IUni	0.45	0.87	0.63
$\delta^{13}C$ and $\delta^{34}S$	IDiv	0.68	0.68	0.70
	IDis	0.41	0.52	0.56
	IEve	0.58	0.72	0.79
	IUni	0.35	0.53	0.51
$\delta^{13}C$, $\delta^{15}N$, $\delta^{34}S$	IDiv	0.70	0.72	0.69
	IDis	0.56	0.68	0.63
	IEve	0.75	0.87	0.77
	IUni	0.47	0.79	0.62

carbon from Solwara 1 to South Su to PACMANUS. The most conspicuous pattern for sulfur isotopic compositions among the three sites is a shift toward ^{34}S-depeted values in the South Su isotopic trait space relative to those of Solwara 1 and PACMANUS. The special isotopic character of South Su individuals is particularly evident in 3-dimensional isotopic trait space (using scaled data for the 6 shared species), where South Su is distinct from PACMANUS (ISim and INes = 0; Table 3) and has little overlap with Solwara 1 (ISim = 0.0; INes = 0.1). These site-specific differences call for caution in interpreting isotopic data from samples across sites in food-web studies and for the need to consider site-specific differences in vent fluids that modify isotopic trait spaces, as noted by De Busserolles et al. (2009).

3.3.2. *Other descriptive metrics*

A suite of additional descriptive metrics (IDiv, IDis, IEve, IUni) facilitate further comparisons of food webs across vent sites. The 3-dimensional (C,N,S) isotopic divergence (IDiv) metric (Table 4, Fig. 5) is > 0.6 for all sites (i.e., points tend to be closer to the edges of the convex hull than otherwise) and overlapping 95% confidence intervals for iDiv at Solwara 1, South Su, and PACMANUS suggest there is no difference in isotopic divergence among sites. Isotopic dispersion (IDis) is > 0.5 at all three sites, indicating differences among species in 3-D isotopic space within sites, but overlapping 95% confidence intervals suggest no difference in isotopic dispersion among sites (Fig. 5). Isotopic evenness (IEve) is also > 0.5 for all sites, suggesting species tend toward an even distribution in 3-D isotopic space; overlapping 95% confidence intervals suggest no difference in isotopic evenness among sites. Confidence intervals (95%) for isotopic uniqueness (IUni) in 3-D isotopic space do not overlap, with sites ordered toward increasing

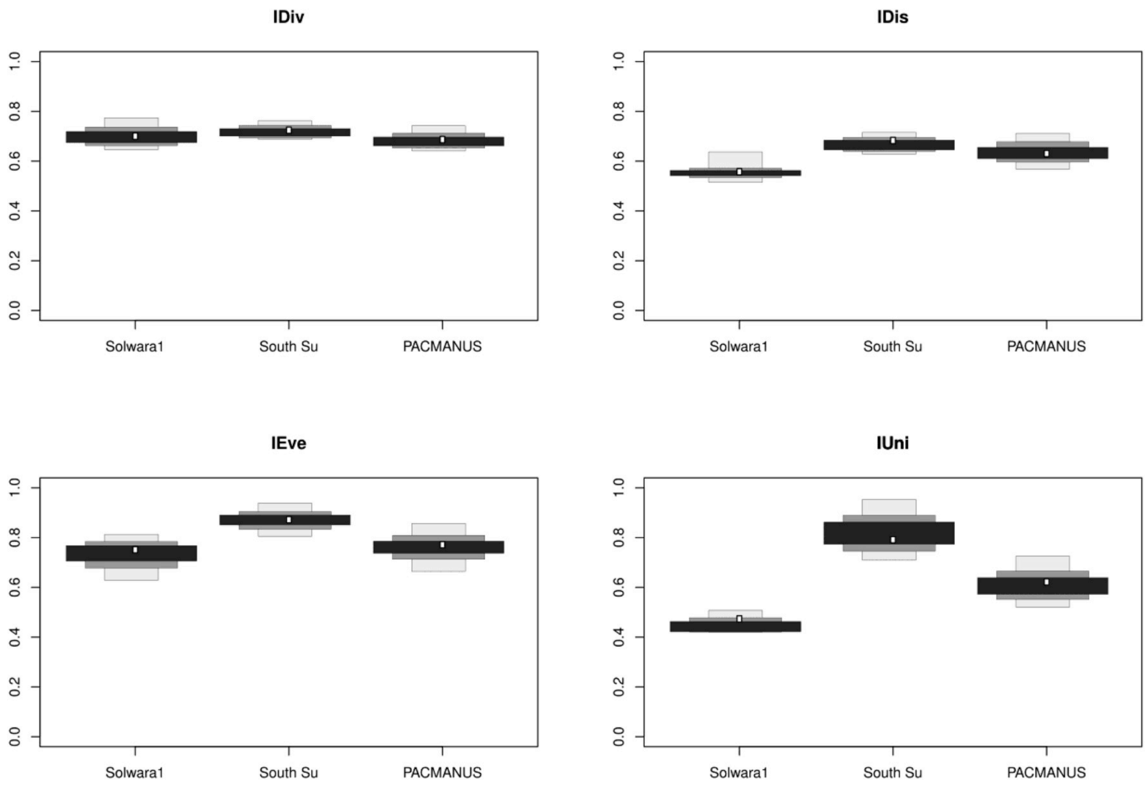

Fig. 5. δ^{13}C, δ^{15}N, δ^{34}S isotopic diversity metrics (see Table S2 for y-axis scaling) for each site, with rectangular bootstrapped credibility intervals (from wide to narrow: 50%, 75%, 95%).

redundancy as South Su < Solwara 1 < PACMANUS (Fig. 5).

4. Assessing impacts of environmental change on food webs of deep-sea vent ecosystems

While a case has been made that mining of active hydrothermal vents should not be permitted (Van Dover et al., 2018), such an outcome is far from guaranteed. As a consequence, it is important to consider how changes in food webs at vents may be assessed after mine closure. Stable isotope analyses have been key to understanding the trophic ecology of deep-sea vent systems since they were first discovered (Rau, 1981a, 1981b; Rau and Hedges, 1979), and they continue to be useful tools for unlocking trophic interactions and sources of organic material in chemosynthetic food webs, especially when used in conjunction with gut content analyses, fatty acid biomarkers, behavioral observations, and faunal distributions. Stable isotope analyses have also proven useful in other deep-sea environments to determine, for example, the relative role of pelagic and benthic food resources for benthopelagic fish (Preciado et al., 2017), the trophic positions of deep-sea fish (Stowasser et al., 2009), or the input of terrestrial organic material to deep-sea foodwebs in offshore canyons and elsewhere along continental margins (Pruski et al., 2017). Emerging work on functional diversity in vent ecosystems (Chapman et al., 2018; Portail et al., 2018) relies on trophic interactions interpreted from expert opinion and stable isotope data.

Environmental baselines in vent and other deep-sea ecosystems will likely continue to use carbon, nitrogen, and sulfur stable isotope analyses to understand trophic interactions. These baselines can inform the design of feasible and balanced sampling designs and selection of useful indicators to assess consequences of disturbance on trophic systems. Such indicators would need to be sensitive to ecologically relevant changes in a food web, be easy to measure and interpret, and facilitate ecosystem-based management decisions. Numerous food web indicators have already been proposed for marine ecosystems in general (Rolls et al., 2017; Tam et al., 2017), including ones associated with stable isotope profiles such as a nitrogen isotope 'trophometer' (Rombouts et al., 2013a).

Habitat modification during mining at and around vents has the potential to alter fluid flux and chemistry, with local loss of biodiversity and biomass. This would result in shifts in the occupancy and relative biomass of taxa as well as the nature of trophic interactions. An integrative suite of indicators that captures the extent of these changes and subsequent recovery at the ecosystem level seems critical. Metrics derived from convex hull areas (isotopic richness, isotopic nestedness, and isotopic similarity) together with isotopic diversity metrics (iDiv, iDis iEve, iUni), are readily calculated from multidimensional stable isotope data and summarize complex systems (Cucherousset and Villéger, 2015). These metrics may thus qualify as suitable indicators to monitor change in vent food webs. However, knowledge gaps persist in the use of any isotopic metric as a component of environmental management, particularly with regard to natural variability within vent food webs in space and time. A protocol for minimum requirements for collection of samples, for isotopic analyses, and for environmental measures (e.g., isotopic compositions of carbon, nitrogen, sulfur in the vent fluids, isotopic compositions of photosynthetically derived particulate organic material at the seabed) that can be used to assess changes in food webs (at vents or in other habitats that may be impacted by mining activities) before and after mining activities remains to be developed. Key questions include which taxa or feeding guilds are critical for assessment, which environmental measures are essential, how many replicates are needed over what spatial and temporal scales, and what changes in the food web would represent a threat of serious harm to the environment.

There is potential to explore underlying causes of change by examining variations in species' isotopic compositions, in the context of changes in community structure and habitat. Isotopic diversity metrics and other proposed indicators of trophic structure and function remain to be evaluated and prioritized by experts for deep-sea environmental management needs. Food web and other indicators alone are not a management panacea. There will still be a need for stakeholders and managers to determine what a biologically significant change is, its underlying cause, and the ability of the system to recover.

5. Conclusions

Additional collections and stable isotope data allow us to refine the Manus Basin food web first described by Galkin (1997). Nine feeding guilds are identified based primarily on natural history attributes and estimates of trophic levels based on nitrogen isotopic data. Several of these feeding guilds could be subdivided based on distinct groupings by carbon isotopic compositions. As in many other vent food webs, at least two symbiont carbon fixation pathways are inferred from carbon isotopic compositions (rTCA and CBB); the carbon-fixation pathway in a third group of symbiotrophs (the tubeworm *Arcovestia ivanovi*) at Manus Basin vents is not yet understood. Convex hull analysis shows that there are site-specific differences in the isotopic trait space of the Manus Basin vent fields, which we interpret to reflect primarily variations in environmental conditions. Isotopic diversity metrics suggest similarities in isotopic divergence, dispersion, and evenness. The PACMANUS food web has the greatest isotopic redundancy among species. Community-level analyses like the convex hull and diversity metrics may prove to be good indicators for environmental baselines and monitoring because they are easy to calculate from multidimensional isotopic data. There remains a need for experts to assess these and other indicators for their value in environmental management and to understand what changes in a food web represent serious harm to the environment.

Funding

This work was supported by a research contract from Nautilus Minerals Niugini Limited (CLVD), Duke University (CLVD), and the International Master of Science in Marine Biological Resources (IMBRSea; LVA, AFB). IMBRSea is a Joint Master's Degree under Erasmus Mundus coordinated by Ghent University (FPA 574482-EPP-1-2016-1-BE-EPPKA1-JMD-MOB).

Research data

Species-isotope ($\delta^{13}C$, $\delta^{15}N$, $\delta^{34}S$) database.

Declaration of interest

CLVD received a research contract from *Nautilus Minerals Niugini Ltd* in partial support of this research.

Acknowledgements.

This manuscript benefited from comments and insights offered by anonymous reviewers and the journal Editor-in-Chief, Imants Priede. The authors thank Dr. Samantha Smith of Nautilus Minerals, the captain and crew of the M/V NorSky andCanyon Offshore ROV teams, Andrew Thaler, William Saleu, Rebecca Jones, Ashley Sobel, and Pen-Yuan Hsing for assistance with field sampling, and David Honig for sample preparation and barcoding. Dr. Travis Washburn assisted with ANOSIM/SIMPER statistics. Organisms sampled from Manus Basin are on loan to Duke University from the Government of Papua New Guinea.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.dsr.2019.04.018.

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