Abstract

1. Detritivores need to up-cycle their food to increase its nutritional value. One method is to fragment detritus promoting the colonisation of nutrient-rich microbes, which consumers then ingest. This is known as microbial gardening. Observations and numerical models of the detritus-dominated ocean mesopelagic zone have suggested microbial gardening by zooplankton is fundamental process in the ocean organic carbon cycle, as it leads to increased respiration of carbon-rich detritus. However, no experimental evidence exists to prove microbial respiration is higher on smaller, fragmented detrital particles.
2. Using aquaria-reared Antarctic krill faecal pellets we showed fragmentation increased microbial particulate organic carbon (POC) turnover by 70 %, but only on brown faecal pellets of low nutritional value. Microbial POC turnover on un-and fragmented green faecal pellets of higher nutritional value was equal. Thus we find particle size alone is not enough to determine microbial activity, and the nutritional value and age of the particle are important.
3. We estimate mesopelagic zooplankton can potentially increase the proportion of essential nutrients (e.g. unsaturated fatty acids) in their food by at least 11 %. In addition we propose 'communal gardening' may occur whereby other mesopelagic organisms consume the particle and microbes gardened by a neighbouring detritivore.
4. Increases in microbial turnover of detrital POC reduces the sink of organic carbon in the ocean. Thus microbial gardening should be represented in models forecasting the future carbon cycle. Model parameterisations will require further understanding of the energetic gains to zooplankton communities, how microbial gardening influences other sinking particles such as detrital aggregates, and the relative importance of biological (i.e. particle lability, size and age) vs. physical (i.e. temperature and oxygen) constraints on gardening.

Keywords

Microbial gardening, zooplankton, mesopelagic zone, lability, carbon sink, faecal pellets.

Introduction

Sinking detrital particulate organic carbon (POC) in the ocean is a vital sink for atmospheric carbon, without which there would be 50 % more CO_2 in the atmosphere than there is today (Volk and Hoffert, 1985; Parekh *et al.*, 2005). Carbon settles to depth predominantly either as

phytodetrital aggregates made of dead/living phytoplankton cells, or as the faecal pellets of small pelagic crustaceans called zooplankton (Turner, 2015). Laboratory studies have shown crustaceous zooplankton often fragment and only partially consume detrital POC such as faecal pellets (Lampitt *et al.*, 1990), which at first appears a counter-intuitive feeding mechanism.

However, fragmentation can benefit zooplankton. Microbes rapidly colonise smaller particles (Kiørboe *et al.*, 2002; Kiørboe, 2003), and these microbes are rich in essential nutrients (e.g. unsaturated fatty acids) that are in low abundance in the mesopelagic zone (200 – 1000 m depth) (Cavan *et al.*, 2018), and the zooplankton cannot synthesise themselves (Okuyama *et al.*, 2008; De Carvalho and Caramujo, 2012; Moi *et al.*, 2018). This process of fragmentation to nurture then consume nutritionally-enriched food (i.e. microbes plus POC) is termed microbial gardening (Fenchel, 1970; Mayor *et al.*, 2014). There are three key steps in microbial gardening; 1) a particle is fragmented by zooplankton increasing particle surface area, 2) bacteria colonise the newly exposed organic material rapidly (0.1 – 1 mm^3 per hour (Kiørboe *et al.*, 2003)) increasing particle-attached bacterial abundances (also leading to increased remineralisation of POC), 3) zooplankton ingest the bacteria and POC, thus their food is more nutritious than the POC without the additional microbes (Anderson *et al.*, 2017). Fragmentation for microbial gardening is thus particularly beneficial in the mesopelagic zone where the dominant food source is detritus, which is low in essential fatty acids that the zooplankton require (Mayor *et al.*, 2014).

Whilst microbial gardening has been empirically proven in other ecosystems and habitats, for instance on detrital turtle grass fragmented by amphipods (Fenchel, 1970), the theory that fragmentation and declining particle size increases microbial remineralisation has not yet been empirically tested on sinking open-ocean biogenic particles. A biogeochemical modelling study of mesopelagic carbon stocks revealed this fragmentation process was essential to balance the carbon budget of the mesopelagic zone, by routing POC through the microbial loop (Giering *et al.*, 2014). In fact, physical (e.g. shear) or biological fragmentation of POC has been found to explain 50 % of the loss of sinking carbon with depth in the oceans (Briggs et al., 2020), although what proportion of this is directly linked to zooplankton particle fragmentation and microbial gardening is unknown. In addition, the lack of experimental research on this process means we do not fully understand its constraints and controls. Given microbial gardening is a prominent example of how ecological processes can

influence ocean biogeochemistry (Cavan *et al.*, 2019), it is essential we improve our mechanistic understanding of this process.

As producing smaller particles can indirectly result in a feedback to atmospheric CO_2 concentrations, by potentially increasing surface area for microbial remineralisation, ocean ecosystem models need to be able to parameterise the decline of POC flux with depth as a function of particle size. However, most empirical research on the influence of particle size in the mesopelagic zone focusses on how size influences particle sinking rates (Ploug *et al.*, 2008; Iversen and Ploug, 2010; Laurenceau-Cornec *et al.*, 2015), and not on microbial remineralisation rates, even though they have equal importance on the transfer efficiency of POC to depth (Boyd and Trull, 2007). Models also need to incorporate mesopelagic parameterizations of the energetic gains for zooplankton associated with microbial gardening, due to the increase in consumption of microbes with essential fatty acids (e.g. enhanced growth (Anderson *et al.*, 2017)). However, experiments are required first to test the hypothesis that smaller, fragmented particles are remineralised by microbes faster, and to determine in what scenario it is beneficial for zooplankton to invest in microbial gardening.

Here we use aquaria-maintained Antarctic krill (*Euphausia superba*) faecal pellets as a representative mesopelagic particle (Cavan *et al.*, 2015) and measure microbial POC turnover rates on artificially fragmented (small) and unfragmented (large) pellets. There were two types of pellets present in the krill aquaria (brown and green pellets) so the experiment was done separately on the different coloured pellets, as faecal colour indicates food source, age and lability of the pellet POC (Wilson *et al.*, 2008). The aim of this study is to experimentally test the first steps in microbial gardening, that fragmentation increases microbial remineralisation (POC turnover) to work toward parameterising POC turnover as a function of particle size in models. We hypothesise microbial POC turnover will be higher in fragmented pellets rather than unfragmented pellets, regardless of pellet colour. We also explore the ecological benefits of microbial gardening to mesopelagic communities.

Materials and Methods

Krill Research Aquaria

Faecal pellets from Antarctic krill (*Euphausia superba*) were collected from krill maintained in aquaria at the Australian Antarctic Division (AAD), Hobart, Tasmania. The krill are

maintained in large tanks at 0.5 ºC. They were collected during the Antarctic summer of 2017-2018 south of 60 ºS in the Indian Sector of the Southern Ocean, so had been in captivity for approximately a year at the time of the experiment in January 2019. The krill were a year older though during the separate *in situ* faecal production and fragmentation observation which was conducted in April 2020. The krill used were adults with an estimated age of 4 – 6 years. Approximately 2,000 krill were held in a 1.7 tonne circular tank. The lighting at the time of the experiment was set to a 'June' (Austral winter) irradiance regime. The krill are fed every morning, starting between 0800 and 0930 Australian Eastern Time (AET) with a mixture of live phytoplankton cultured at the AAD (the diatom *Phaeodactylum triconutum* and the flagellate *Pyramimonas gelidicola*, 10 L of each), 200 mL of 10 % instant algae shellfish diet diluted in seawater (Reed Mariculture) and FriPack FRESH prawn hatchery feed (2.5 g diluted into 200 mL of seawater). The aquaria is a recirculating system, which continuously circulates and filters the seawater in the system. Typically it takes about 1.5 h for a complete exchange of the water in the tank. The water flow in the tank was closed while feeding to maximise the chance for krill to feed on the added algal mixture. See Kawaguchi *et al.*, (2010) for more details on the aquaria set up.

Collection of Faecal pellets

For the main POC turnover experiment, faecal pellet collection occurred from 0930 – 1130 (AET) each morning, approximately 1.5 hours after the start of feeding when water flow was turned on. As part of the aquaria protocol pellets are emptied daily from the tank. Only pellets floating at the surface waters of the tank were used for this experiment. Pellets were collected using a plastic beaker with the base cut off and replaced with 100 µm mesh. As pellets were collected they were rinsed from the mesh with filtered seawater into a 1 L beaker. At least 300 pellets were collected per experiment over the 1.5 hour period and during this collection period both green and brown pellets were present. Pellets were gently separated into two beakers depending on their colour, green or brown. A sub-sample (~ 50 pellets) of each colour were then taken and stored in the fridge (4 ºC) for subsequent photography using light microscopy (Fig. 1), with the majority being used in the remineralisation experiment.

Pellet fragmentation and remineralisation experiment

The aim was to compare microbial POC turnover rates on faecal pellets that had and had not been fragmented, by physically breaking recently collected krill faecal pellets. Pellets from

the two beakers were put gently into four 20 mL respiratory vials; 2 vials with brown pellets and 2 vials with green pellets. One vial of each coloured pellet (1 x brown pellets and 1 x green pellets) was manually inverted once - quickly - to fragment the pellets. Fragmentation coloured the water in the vial revealing the release of dissolved organic material (DOM) from the faecal pellet (Fig. S1). Two other 20 mL vials were filled with water from the krill tank without pellets as control vials containing only free-living microbes. Thus, for each experiment (and therefore per day) there were 4 treatments (1 x whole brown pellets, 1 x brown fragmented pellets, 1 x whole green pellets, 1 x green fragmented pellets) and 2 x control vials. The tank water is filtered at 1 µm and therefore would contain some heterotrophic bacteria larger than this size that could attach to the pellets and decrease the oxygen concentration (Fig. S2). The treatment vials (with pellets) would also potentially contain microbes released from the krill gut already attached to the pellet (Hansen and Bech, 1996). The tank water is kept at 0.5 ºC, UV sterilised, with ammonia removed through bio-filtration to a level always below normal spectrophotometer detection limit (0.01 mg L^{-1}) and DOM was removed through a form fractionator which generates fine bubbles in a water column skimming organic materials. All vials were placed in a rack and submerged in a water bath within a fridge (5 ºC), representing the Southern Ocean south of the sub-Antarctic Front but north of 60 ºS (Govin *et al.,* 2009). The selected temperature was higher than that of the live krill (0.5 ºC) to ensure a measurable signal in microbial respiration (Brown *et al.*, 2004). We also present results with the oxygen uptake rates scaled to the *in situ* temperature of the krill at 0.5 ºC.

The experimental set-up and protocol follows Cavan and Boyd (2018). An initial dissolved oxygen reading was taken using a PreSens micro-electrode (limit of detection = 15 ppb, accuracy = 0.05 % O_2) as soon as the vials were placed in the fridge. The pellets were incubated in the dark and the dissolved oxygen concentration measured every 24 hours. The experiment was terminated after oxygen concentrations became < 100 µM so the pellets did not have anoxic centres which can stimulate anaerobic respiration (Ploug and Bergkvist, 2015). Termination occurred after 72 hours, resulting in 4 oxygen measurements per experiment. The vials were gently (so as not to further fragment pellets) manually inverted prior to oxygen measurements to mix the water.

The experiment was replicated 4 times, giving 4 replicates per experimental treatment. At the end of each experiment the contents of each vial were filtered through a pre-combusted

(overnight, 400 ºC) QMA-quartz filter, dried and then stored at room temperature. The QMA filters were then prepared and processed for POC analysis; the dried filters were placed into silver cups (Elemental Microanalysis) and 20 µL of 2NHCl Suprapur added to each cup. The cups were placed in a fuming bell overnight to remove inorganic carbon and then dried at 60 ºC for two days. Cups were pelleted and then C and N analysed on a CHN analyser (Thermo Finnigan EA 1112 Series Flash Elemental Analyser).

Microbial POC turnover rates

We measured the POC turnover rates of fragmented and unfragmented krill faecal pellets as a proxy for the possible increase in microbial activity associated with the fragmentation of sinking particles as suggested by microbial gardening. POC turnover rates were computed as in Cavan & Boyd (2018). Briefly, oxygen uptake rates were determined by computing the slope of the linear regression between oxygen concentration (µmol L^{-1}) and time (h) to give oxygen uptake (µmol L^{-1} h^{-1}). All slopes from vials containing pellets showed a significant ($p<0.05$) decrease in oxygen over 72-hours (Fig. S2). The slopes of the control vials (no pellets) were subtracted from the slopes of the treatment vials to account for any oxygen uptake by free-living bacteria using carbon from non-pellet sources. Oxygen uptake rates were converted from µmol L^{-1} h^{-1} to µmol h^{-1} by multiplying by the volume of the vials (0.02 L). Microbial POC turnover (k) was calculated as a rate per day:

$$k\ (d^{-1}) = \text{Oxygen uptake (µmol } h^{-1}) \text{ / POC mass (µmol)} * 24 \qquad \text{(Equation 1)}$$

Size of pellets

A Leica M205C dissecting stereo-microscope with a Leica DFC 450 camera and Leica LAS V4.0 software was used to image the pellets which had been kept aside in the fridge (4 ºC) in darkness. Images were taken using a magnification of 32x. ImageJ was used to analyse the photos by converting each image to 16-bit and subtracting the background with a rolling ball radius of 150 pixels. A threshold was applied and the background set to black with the pellets in white to automatically detect particles and measure their size (here perimeter), with a minimum detection limit of 0.01 mm. All errors given in this study are standard error of the mean.

Faecal pellet production and fragmentation *in situ*

To determine how faecal pellet colour and fragmentation change within the krill tank we ran a separate experiment observing the alteration of the size and colour of faecal pellets

produced as the amount of food changed with time since the start of the daily morning feed. Initially tank water was thoroughly inspected to ensure there were no faecal pellets existing prior to feeding. In this experiment, feeding started at 09:15. Water flow was closed between 09:30 - 12:30 to ensure food was not cleared out from the tank during this period. At 12:30 the water flow was turned on, decreasing the algal food supply available to the krill. Given that it takes 1.5 hours for the water to fully circulate no algal food remained in the tank by 14:00. Sampling of faecal pellets was undertaken at 10:15, 11:15, 12:35, 14:30, and 16:45. Faecal pellets floating in the water were gently siphoned out into a bucket prior to being photographed and analysed as above. 40 - 90 pellets were collected at each sampling time point, with collected pellets increasing in number with time due to fragmentation.

Results

Manual fragmentation of krill faecal pellets successfully created long and short pellets as a proxy of particles of different sizes with different surface area to volume ratio in the oceans (Fig. 1, Fig. 2a, Fig. S3). The mean perimeter length of unfragmented brown and green pellets was 7.28 ± 0.06 mm and 5.26 ± 0.03 mm respectively. Both types (brown and green) of unfragmented pellets were significantly longer than the short, manually fragmented pellets (t-test, $p<0.05$, Fig. 2a). The mean perimeter length of physically fragmented brown and green pellets were 2.40 ± 0.01 mm and 2.84 ± 0.01 mm respectively. There was no statistical difference in length between the brown and green fragmented pellets ($p>0.05$).

Linear regressions between oxygen concentration and time showed oxygen concentrations declined significantly ($p < 0.05$) over the 36 hour experiment in each microrespiration vial which contained pellets (Fig. S2). Oxygen concentrations approached 100 µM after 72 hours in 3 of the 4 replicate experiments. Microbial oxygen uptake rates ranged from 1.8 – 4.3 µmol L^{-1} h^{-1} with brown unfragmented pellets having the lowest and brown fragmented having the highest rates. Mean oxygen uptake rates per pellet treatment (colour and fragmentation, Fig. 2b) ranged from 2.0 – 3.0 µmol L^{-1} h^{-1} (Fig. 2b). We used a Q_{10} of 2.5 (equivalent to an activation energy of 0.65 eV) (Yvon-Durocher *et al.*, 2012) to also estimate the microbial oxygen uptake rates at 0.5 ºC, the temperature of the krill tank. This resulted in a range of oxygen uptake rates from 0.5 µmol L^{-1} h^{-1} to 3.4 µmol L^{-1} h^{-1}. For the remainder of the analysis we continue to use the raw rates computed at 5 ºC since the focus of this study is to explore the relative differences between treatments rather than absolute rates. Mean pellet

238 POC concentration ranged from 1.4 ± 0.2 mmol L^{-1} in the unfragmented green pellets to 2.4 ±
239 0.3 mmol L^{-1} in the unfragmented brown pellets (Fig. 2c).
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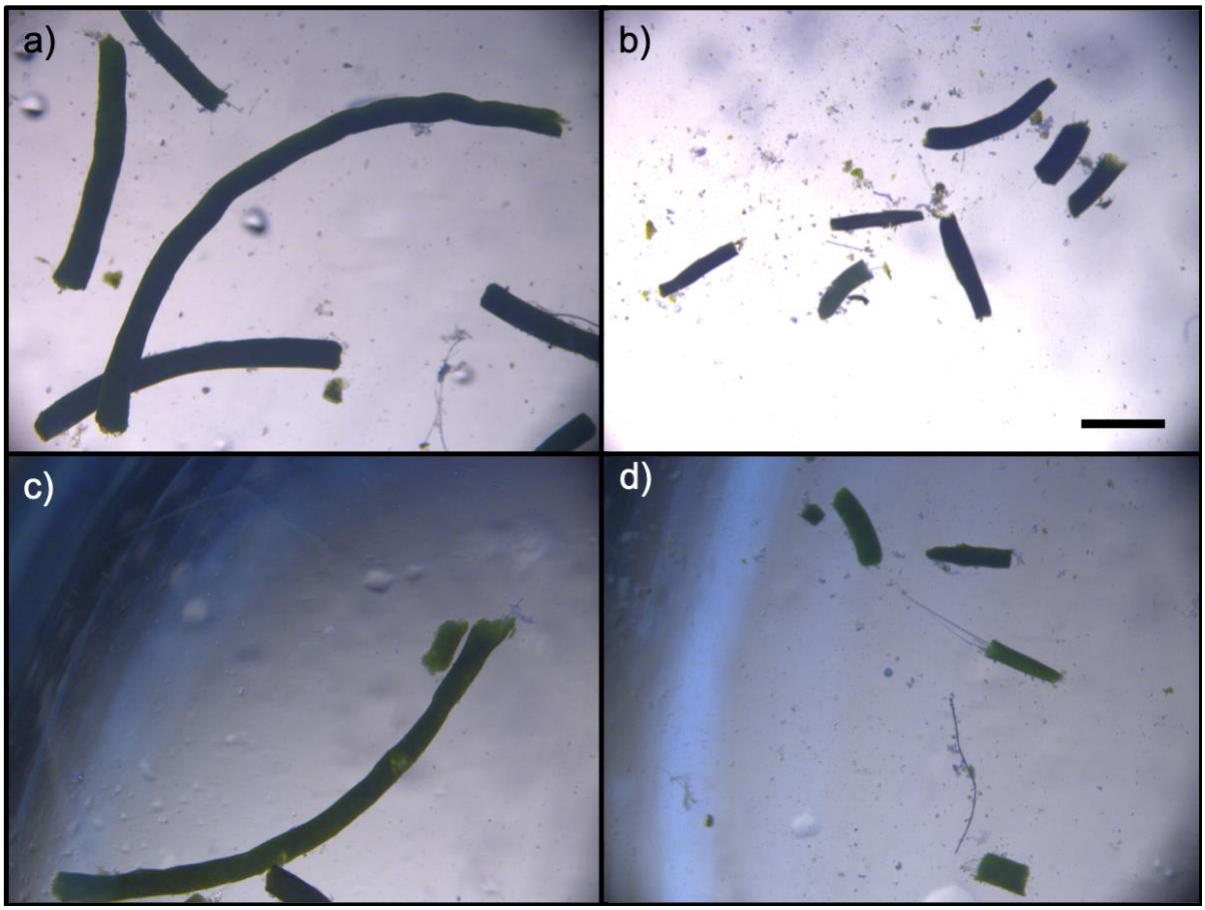

241
242 ***Figure 1.*** *Images of different colour and size faecal pellets; a) large brown unfragmented, b)*
243 *small brown fragmented, c) large green unfragmented and d) small green fragmented. The*
244 *horizontal scale bar in panel b) is 1 mm long.*
245
246 After normalisation of the oxygen uptake rate to faecal pellet POC, there was no overall
247 effect of pellet size ($p > 0.05$, Fig. 2d) on microbial POC turnover rate, as the POC turnover
248 was the same for un- and fragmented green pellets and fragmented brown pellets (~ 0.036 ±
249 0.01 d^{-1}). However, POC turnover in the fragmented brown pellets was significantly ($p <$
250 0.05) higher (70 %) than that of unfragmented brown pellets (0.021 ± 0.00 d^{-1}). Therefore,
251 microbial remineralisation did increase upon fragmentation but only for brown pellets and
252 not green pellets.

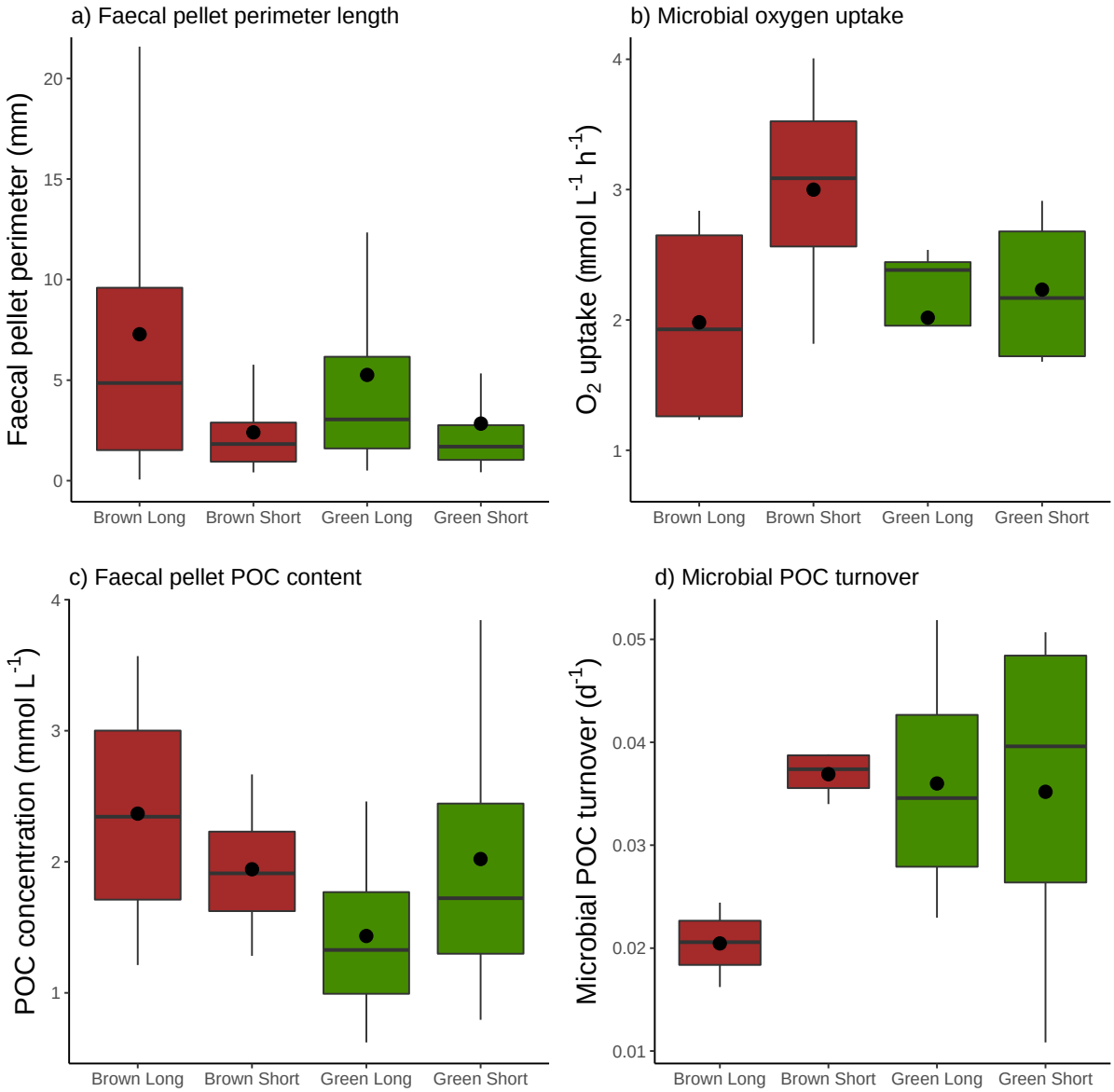

Figure 2. *Median differences in faecal pellet size, oxygen uptake, pellet POC and microbial POC turnover in un- (long) and fragmented (short) pellets of different colours (green and brown), a) perimeter of faecal pellets b) microbial oxygen uptake, c) pellet POC content, and d) microbial turnover of pellet POC. The solid black line in each box is the median, the upper and lower extent of the boxes (hinges) are the upper and lower quartiles respectively and the upper and lower whiskers are the highest and lowest data point. The mean is represented by the black point. The sample size is n = 4 for b), c) and d) and n = 130, 278, 191 and 408 respectively for each box in a).*

To find when green vs. brown pellets are produced by the krill within the tank and if *in situ* fragmentation occurred we ran a separate time-series experiment. Our results indicated that

whilst algal food was present in the tank (09:15 to 14:00, Fig. 3) pellets were predominantly green. Once the tank was empty of algal food (at 14:00) the pellets became browner in colour indicating the krill had begun to feed on the pellets (coprophagy) (Lampitt *et al.*, 1990), and the organic material had now passed through the krill gut at least twice (Fig. 3). We quantified the mean perimeter of these pellets, which was largest at the first time point and decreased continually throughout the day (Fig. 3) indicating that either brown pellets are smaller when egested or that krill preferentially fragment brown pellets whilst feeding. The number of pellets in each sample taken also increased with time, so there were more pellets that were also smaller toward the end of the day. This supports the latter theory, that krill were preferentially fragmenting the brown pellets. The results of this experiment provides evidence that the green pellets in this experiment had likely only passed through the krill gut once and therefore contained more labile organic matter than the brown pellets consistent with the findings of Wilson *et al.*, (2008). It also suggests Antarctic krill in the tank are undertaking microbial gardening of the more detrital pellets.

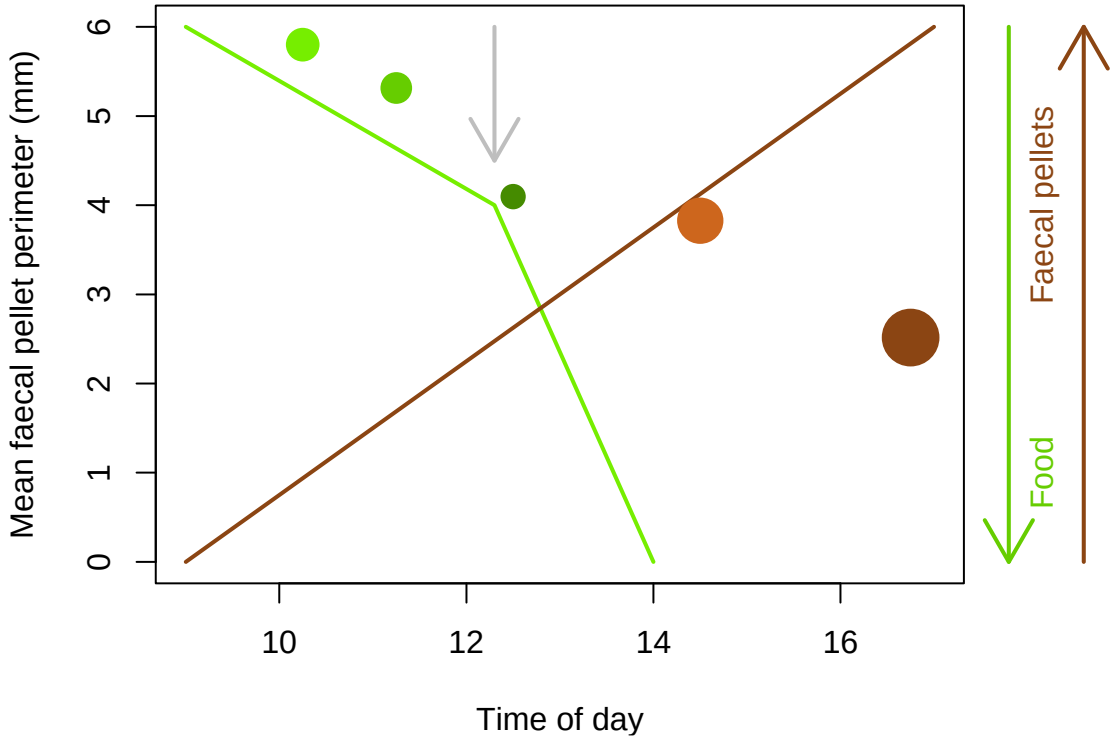

Fig. 3. *Results of the in-situ time-series experiment of faecal pellet size (perimeter) throughout the day post-feeding. Broad trends in algal food concentration and faecal pellet number are indicated to show when they increased and decreased respectively. In this experiment, feeding started at 09.15 in the morning and the water flow was turned on at*

12:30 (grey arrow), resulting in sharp decline in algal food which was completely removed from the tank by 14:00. Faecal pellets were present in the tank at all sampling times. The number of pellets in the sample collected (not normalised to volume) are indicated by the size of the points (40 – 91 pellets). The colours of faecal pellets within the points reflect the gradual change in dominance of green to brown pellets as observed by eye during the experiment. The mean faecal pellet perimeter (y-axis) were quantified showing pellet size decreased as algal food decreased indicating fragmentation of brown but not green pellets.

Our results are a step toward supporting the first tenet of the microbial gardening hypothesis as they confirm that fragmentation can enhance microbial remineralisation of POC, and thus potentially the nutritional value of food consumed by mesopelagic krill or zooplankton. However, given that pellet colour and therefore lability, influenced the experimental outcome the modelling microbial gardening may not be as straightforward as we hypothesised.

Discussion

Fragmentation and microbial gardening has been proposed by modelling studies (Giering *et al.*, 2014; Mayor *et al.*, 2014) as an important ecological process in the food-limited mesopelagic zone influencing the open-ocean carbon cycle. Gardening provides energy-rich food for zooplankton, whilst also having biogeochemical implications by increasing the remineralisation rates of sinking particulate carbon, ultimately reducing the ocean carbon sink. For the first time we experimentally tested the first steps in the microbial gardening hypothesis, that fragmentation of sinking particles increases microbial activity and the dissolution of POC associated with smaller particles, using krill faecal pellets as a representative detrital particle.

Our results showed that small pellets are subjected to higher remineralisation rates than larger particles, but only when the pellets were brown in colour, and thus particle size alone is not a clear descriptor of remineralisation rates. Brown pellets occur due to krill feeding on other pellets (coprophagy (Lampitt *et al.*, 1990)) when fresh algal concentrations are low (Fig. 3) e.g. in the mesopelagic (Fig. 4). Thus, brown pellets are indicative of highly recycled material that is refractory and of low nutritional value (Wilson *et al.*, 2008). Refractory material suggests most of the essential nutrients and labile organic carbon, such as unsaturated fatty acids and amino acids, have already been extracted leaving compounds that are less nutritious to consumers (Zimmerman and Canuel, 2001). In our *in situ* experiment green pellets were

produced from the krill feeding directly on phytoplankton cells (Fig. 3) and thus more indicative of euphotic zone food sources (Fig. 4). Both unfragmented and fragmented green pellets (with more labile, energy-rich organic carbon), were turned over at the same rate showing that if the carbon contains high energy compounds, the microbes will remineralise the carbon at the same rate regardless of particle size. As only the fragmentation of brown pellets increased POC turnover, we conclude that when the organic material is energy-poor fragmentation is needed to expose new material for microbes to remineralise.

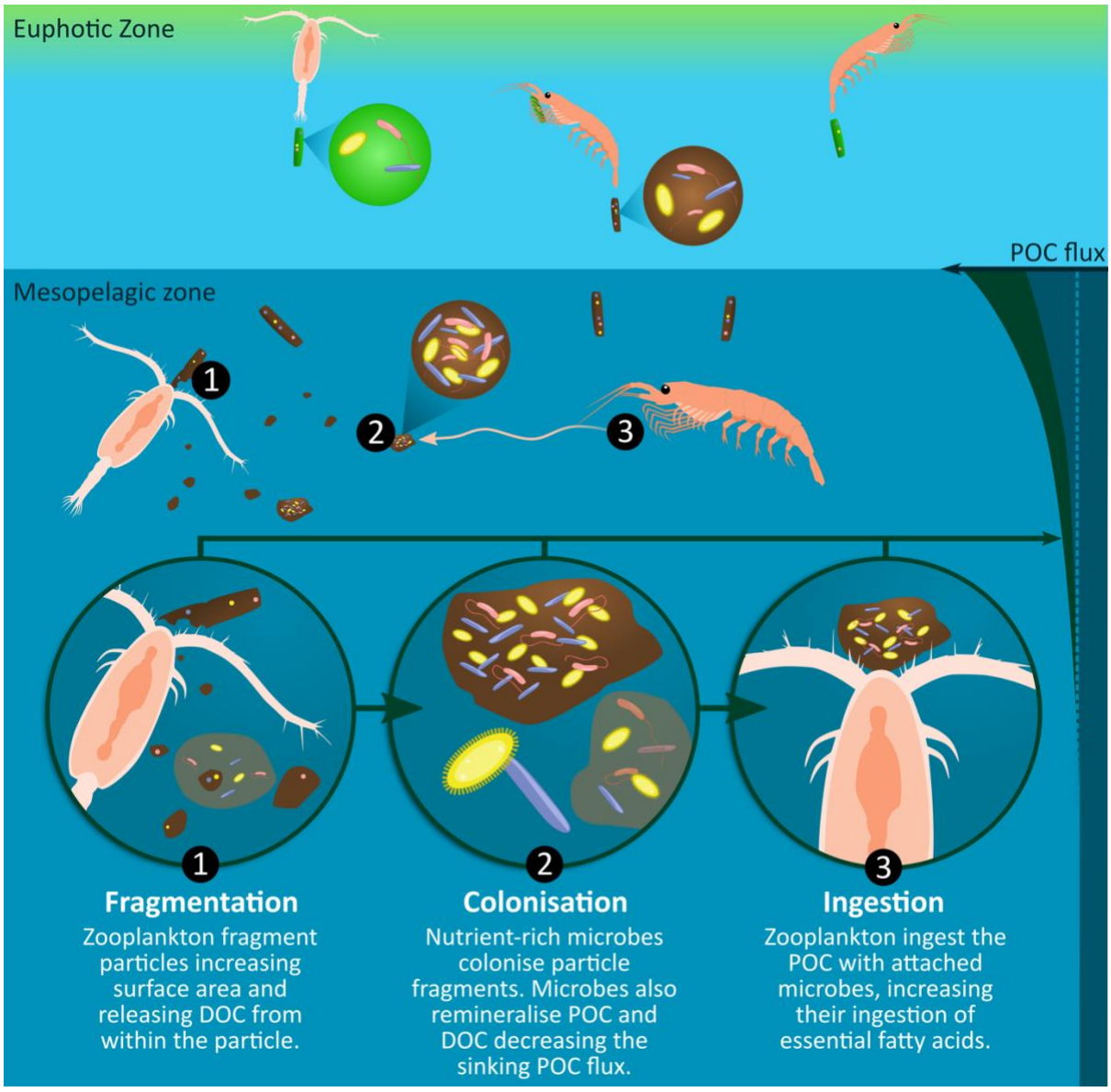

Fig. 4. *In the sunlit euphotic zone zooplankton and krill feed on phytoplankton cells, producing greener faecal pellets containing labile organic matter with some essential nutrients. As these pellets or particles sink and are consumed (Lampitt et al., 1990) the highly nutritious compounds are preferentially removed, so by the mesopelagic zone food is scarcer and less*

proteinaceous and labile, and pellets are brown in colour. To gain the essential nutrients mesopelagic resident or migratory zooplankton need, they fragment detrital food reducing the size of the particles and releasing dissolved organic carbon (DOC) (1), promoting the colonisation of microbes (2) that can synthesise nutrients essential to zooplankton (e.g. unsaturated lipids) and then the zooplankton ingest (3) both the particle and the attached microbes, which now contains the nutrients zooplankton need. Zooplankton may garden and consume the entire particle and many colonised bacteria, or move on leaving the particle and colonisers for other grazers, they themselves benefiting from other abandoned particles. The reduced size and therefore sinking rate of the fragmented particles, and the increased remineralisation by particle-attached (and free-living on any DOC released via fragmentation) microbes are responsible for large decreases in the sinking POC flux.

Particle fragmentation encourages free-living microbes to colonise and attach to the newly exposed surface area of the smaller particle. It is likely bacteria dominated the microbial community during our experiment, as bacteria outcompete other microbial groups during incubations of faecal pellets (Tamburini *et al.*, 2009). However, bacteria can occupy just 10 % of the surface area of faecal pellets after a similar incubation time (80 hours) to this study (Gowing and Silver, 1983). Thus even with fast microbe colonisation rates of 0.1 – 1 mm^3 per hour observed on sinking particles (Kiørboe *et al.*, 2003), the minute size, weight and carbon content of microbes means they do not increase the magnitude of food for zooplankton. We coarsely estimated using literature values of carbon content per cell for bacteria of 12 fg C $cell^{-1}$ (Boyd *et al.*, 2015) and for krill pellets of 0.39 µg C $pellet^{-1}$ (Gleiber *et al.*, 2012), and assuming a bacteria cell size of 2 µm diameter, and a krill pellet surface area of 5.5 mm^2 (converting a pellet from this study with a perimeter of 6 mm to a cuboid of dimensions 0.5 x 0.5 x 2.5 mm), that bacteria on 10 % of the pellet surface (Gowing and Silver, 1983) would only contribute to 0.54 % of pellet POC. This values assumes that bacteria marginally increase carbon stocks on pellets. In fact the presence of microbes actually decreases detrital POC by routing some POC through the microbial loop and multiple trophic levels (Anderson *et al.*, 2017) and completely remineralising some POC to CO_2. Instead, microbes increase the nutritional value of detrital food (Anderson *et al.*, 2017), which we now explore.

Bacteria are more nutritionally-rich and proteinaceous than detrital POC sinking into mesopelagic. The C:N of cultured marine bacteria is 4:1 (Vrede *et al.*, 2002), but for

mesopelagic (350 m depth) particulate organic matter (POM) is 10:1 (Kaiser and Benner, 2012) and for krill pellets 8:1 (Atkinson *et al.*, 2012). Thus most mesopelagic POM is formed of carbohydrates with sources of amino acids and lipids being low, having been produced by phytoplankton and then preferentially consumed in the surface ocean (Wakeham and Canuel, 1988; Wakeham and Lee, 1989; Close *et al.*, 2014; Mayzaud *et al.*, 2014; Cavan *et al.*, 2018). Colonisation of microbes can increase the amino acid and fatty acid content of the particles, as amino acids comprise just 2 % of mesopelagic total organic carbon at 500 m, but 64 % of organic carbon in marine bacteria (Kaiser and Benner, 2008). Marine bacteria are able to synthesise two essential polyunsaturated lipids, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), which make up 30 % of total fatty acids in bacteria (Okuyama *et al.*, 2008). This is much higher than the proportion these lipids make up of all the fatty acids in sinking mesopelagic POM (4 % for EPA and 10 % for DHA) (Mayzaud *et al.*, 2014). Instead mesopelagic POM fatty acids are comprised of more refractory saturated fatty acids (Cavan *et al.*, 2018). If 10 % of a pellet is colonised by bacteria (Gowing and Silver, 1983), then we estimate an 11 % increase in EPA and DHA compared to a pellet without bacteria. This is likely to be higher on fragmented pellets as more than 10 % of the pellet may be colonised by bacteria due to the increased surface area and exposure to 'new' labile surfaces previously inside the pellet. Zooplankton cannot synthesise lipids or amino acids so they must derive them from their food source, otherwise they may be susceptible to poor growth and fecundity and high mortality (Stoecker and Capuzzo, 1990). Unsaturated fatty acids are phospholipids, stable against reactive oxygen species (ROS) such as hydrogen peroxide, and form highly packed cell membranes preventing entry of ROS's compared to less unsaturated fatty acids (Okuyama *et al.*, 2008).

Microbial colonisation has been proven to enhance the nutritional value of detrital food for estuarine copepods, which grew poorly on a detritus-only diet, but grew well when the detritus was enriched with microbes (Heinle *et al.*, 1977). As we have shown in this study, microbial activity is increased when detrital particles (here brown krill faecal pellets) are fragmented (Fig. 2d, Fig. 4), but fragmenting the green pellets in this study (more similar to euphotic zone food (Fig. 4)) did not increase the microbial activity. Thus it would not be energetically beneficial for zooplankton to fragment more labile particles. Zooplankton may be able to detect the nutritional value of the particles they intercept (Friedman and Strickler, 1975; Kiørboe and Jackson, 2001) and actively chose whether to fragment them. Copepods can use chemo-detection to find particles (Jackson and Kiørboe, 2004), and zooplankton are

known to select food based on nutritional value (DeMott, 1990; Vanderploeg, 1994). Ultimately, how long the zooplankton remains in contact with the particles it has fragmented and its ability to affectively 'garden' them with microbes will be influenced by turbulence.

Turbulence is low in the mesopelagic compared to the surface ocean with the smallest turbulent eddies (10 x Kolmogrov length scale) being larger (10 mm flow length scale) (Siegel, 1998) than even unfragmented pellets in this study (< 6 mm mean perimeter length), laboratory produced aggregates (< 7 mm equivalent spherical diameter) (Iversen and Ploug, 2013) and natural sinking particles observed in the ocean (< 3 mm equivalent spherical diameter) (Cavan *et al.*, 2018). Fragmented particles are certainly to be smaller than mesopelagic turbulent eddies (Fig. 2a & 3). Thus it is reasonable to predict that in the less turbulent mesopelagic a zooplankton may remain within reach of its fragmented particle for some time, thus making it worthwhile to expend energy fragmenting particles and consuming the fragments and attaching microbes some time post-fragmentation. On the contrary, zooplankton may not choose to stay with the particle long enough, and benefit mostly from consuming contents inside the particle that were previously inaccessible and the first microbial colonisers. Thus perhaps 'communal gardening' occurs, whereby zooplankton partially consume labile material from their fragmented particle before moving onto other food sources, meanwhile the particle is continuously colonised by microbes which a neighbouring zooplankton may then ingest (Fig. 4). This relies on a certain level of altruism by zooplankton, knowing they will gain energy both from the particles they invest in fragmenting and those fragmented by others. This is particularly likely for krill who swarm in large numbers (trillions, 10^{12}) (Tarling and Fielding, 2016) and other zooplankton and fish living in deep scattering layers (Proud *et al.*, 2017) in close proximity to their neighbours' fragmented particles. Diel vertical migrators may have further advantage from accessing and grazing both phytoplankton in the euphotic zone and gardened particles down in mesopelagic zone. This could be a useful strategy for migrators and competitors for phytoplankton such as krill and salps (Loeb, 1997), if access to phytoplankton in the euphotic zone is highly competitive. The relationship between krill and salps has always been regarded as competitors, but if they are linked through communal gardening, our perspectives on their contribution to carbon transport, and how climate change may impact the ecosystem may need to be revisited.

As previously mentioned a side-effect of microbial gardening is increased remineralisation to CO_2 or solubilisation to dissolved organic carbon (DOC) of POC by particle- or pellet-attached bacteria. Increased remineralisation by free-living bacteria will also occur on any DOC released from inside a particle (Fig. 4 & Fig. S1). As the fragmented particles are smaller they will also sink more slowly (Iversen and Ploug, 2013; Laurenceau-Cornec *et al.*, 2019). The increased microbial activity and reduced sinking rates decreases the sink of POC in the ocean, reducing the capacity of deep ocean storage of atmospheric CO_2. Fragmentation of particles as they sink is responsible for 50 % of the decline in POC with depth (Briggs, 2020). This includes both physical fragmentation of more fragile aggregates and that biologically by zooplankton. Thus an ecological process to gain essential nutrients (microbial gardening) has wider implications on the carbon cycle and biogeochemistry. Linking ecological and biogeochemical processes are important to understand ecosystem feedbacks on climate (Schmitz *et al.*, 2013; Cavan *et al.*, 2019) including in modelling work. Here, we have shown that particle size alone is not a good enough descriptor of microbial activity and remineralisation, as particle food source, age and lability are important constraining factors. Thus, future models should strive to incorporate the time since production of the sinking particle in remineralisation parameterisations, although more research is needed to constrain these parameters. In ecosystem models remineralisation should decrease with particle age, and only then when the carbon is mostly refractory should a zooplankton-particle size effect be imposed on remineralisation rates.

To aid in model parameterisations future experiments should determine the extent of fragmentation in different food environments for zooplankton such as replete and depleted food sources and those of different labilities simulating the euphotic vs. mesopelagic zone. In addition concluding whether a particle remains within the predatory range of a zooplankton long enough in the mesopelagic for the zooplankton to effectively ‘garden’ the particle (microbe colonisation) or whether ‘communal gardening’ is more important. Whilst faecal pellets are an important component of particle flux globally, a similar empirical study to this is needed on phytodetrital aggregates, to show if lability influences the remineralisation rate of different sizes aggregates in the same way it does faecal pellets. Further research is also important to predict or forecast remineralisation rates with climate change. As most models parameterise remineralisation as a function of temperature or oxygen, research is needed on how the impacts of particle size and lability on remineralisation compare to these physical controls. It is likely that resident mesopelagic zooplankton (i.e. those not undergoing diel

vertical migration to feed at the surface) will benefit most from fragmenting particles they intercept, and hence euphotic zone models may need not incorporate microbial gardening processes. Mesopelagic models should allow an nutritional gain to zooplankton from microbial gardening.

Conclusions

We present experimental evidence towards refining the microbial gardening hypothesis by mesopelagic zooplankton. Previously modelling studies have shown that fragmentation of sinking organic particles by zooplankton can account for a large loss of the ocean carbon sink due to the increase in microbial remineralisation on the smaller particles. We suggest though that zooplankton may only fragment older and more refractory particles typically found in the mesopelagic zone, as only brown pellets in this study showed an increase in microbial activity when fragmented. Hence, size does not always matter, and the lability of the particle are important factors. It is likely that zooplankton can detect the nutritional value of the food they encounter and actively choose to fragment the sinking particle or not. The ecological benefit of fragmenting a particle rather than consuming it entirely is that bacteria composed of essential nutrients required by zooplankton colonise these smaller particles, which the zooplankton can then ingest. Whether zooplankton truly 'garden' the particle remaining within close proximity long enough to benefit from substantial bacterial colonisation, or abandon the fragments after initial consumption of labile compounds released is still up for debate. The latter hypothesis suggests a level of altruism amongst mesopelagic communities. Regardless, the increased abundance of microbes on smaller particles increases the remineralisation of organic carbon, declining the ocean carbon sink. Hence microbial gardening is an example of the impacts of ecology on biogeochemistry. It is clear from this and previous studies that microbial gardening is an important process in the mesopelagic zone, and one that is worthy of parameterisations in biogeochemical models. However, prior to that similar but extended experimental work is needed to investigate the controls and conditions of this process.

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