Biogeosciences Discuss., https://doi.org/10.5194/bg-2018-231
Manuscript under review for journal Biogeosciences
Discussion started: 28 May 2018

The distinct roles of two intertidal foraminiferal species in phytodetrital carbon and nitrogen fluxes - results from laboratory feeding experiments

Julia Wukovits[1], Max Oberrauch[1], Annekatrin J. Enge[1], Petra Heinz[1]

[1] University of Vienna, Department of Palaeontology, Althanstrasse 14, 1090 Vienna, Austria

Correspondence to: Julia Wukovits (julia.wukovits@univie.ac.at)

Abstract. Benthic foraminifera play a major role as primary consumers and detrivores redistributing organic carbon and nitrogen in intertidal environments. Here we compared the differences of phytodetrital carbon and nitrogen intake and turnover of two dominant intertidal foraminifera, *Ammonia tepida* and *Haynesina germanica*. Their lifestyles in relation to feeding behaviour (feeding preferences, intake and turnover of phytodetrital carbon and nitrogen) and temperature adaptations were compared to obtain a closer definition of their specific roles in intertidal organic matter processing. For this comparison, we carried out a series of short-term laboratory incubations with stable isotope labelled (^{13}C & ^{15}N) detritus as food source. We compared the response of the two species to diatom detritus at three different temperatures (15°C, 20°C, 25°C). The quite low metabolic nitrogen turnover in *H. germanica* was higher than the carbon turnover, and in contrast to the latter, not affected by temperature. This might be related with the chloroplast husbandry in this species and lower demands of food derived nitrogen sources. In contrast, *A. tepida* showed a very high, temperature-influenced intake and turnover rates with more excessive carbon turnover. Further, the latter species prefers a soft chlorophyte food source over diatom detritus, which is harder to break down. In conclusion, *H. germanica* is a highly specialized species with low rates of carbon and nitrogen budgeting. In contrast, *A. tepida* shows a generalist behaviour that links with high fluxes of organic matter (OM). Due to its high rates of OM processing and abundances, we conclude that *A. tepida* is an important key-player in intertidal carbon and nitrogen turnover, specifically in the short-term processing of OM and the mediation of dissolved nutrients to associated microbes and primary producers.

1 Introduction

Benthic foraminifera are ubiquitous marine protists and highly abundant in coastal sediments (Lei et al., 2014; Mojtahid et al., 2016; Murray and Alve, 2000). Coastal sediments represent the largest pool of marine particulate organic matter (OM), despite their rather small area (less than 10% of the ocean floor), and play an essential role in global carbon and nitrogen cycles (Jahnke, 2004). Benthic foraminifera feed on various sources of labile particulate OM, including microalgae and detritus, providing a pivotal link between primary production and higher trophic levels (Bradshaw, 1961; Goldstein and Corliss, 1994; Heinz et al., 2001; Lee et al., 1966; Lee and Muller, 1973; Nomaki et al., 2005b, 2006, 2009, 2011). Certain key species in

foraminiferal communities contribute with a major extant to the OM processing in extensive, highly productive marine environments (Enge et al., 2014, 2016, Moodley et al., 2000, 2002, Nomaki et al., 2005a, 2008; Witte et al., 2003; Wukovits et al., 2018). Therefore, the quantification of foraminiferal carbon and nitrogen processing derived from OM and food selectivity in foraminiferal communities, and the identification of key species in this process is essential to understand marine OM fluxes.

Environmental conditions of temperate tidal flats are physiologically challenging (high fluctuations of physical and chemical parameters, e.g. temperature or OM quality) and therefore host very few, highly adapted foraminifera species. Monospecific or near monospecific foraminiferal communities are characteristic for temperate, estuarine regions (e.g. Alve & Murray 1994, 2001, Hayward 2014, Martins et al. 2015, Saad & Wade 2017). *Ammonia tepida* and *Haynesina germanica* are typical representatives of these communities and their standing crop can reach more than 150 individuals per cm^3 (Alve and Murray, 2001; Mojtahid et al., 2016; Wukovits et al., 2018). Despite the high availability of OM and food sources, food related resource partitioning, or different feeding niches, can be expected, due to high spatial competition. In exposed, muddy intertidal sediments, foraminiferal habitats are often restrained to the uppermost millimeters of the sediment, where dense populations of foraminifera communities of low diversity can be found (Alve and Murray, 2001; Cesbron et al., 2016). Therefore, spatial as well as temporal competition could be circumvented by different feeding preferences and different feeding behaviour.

Temperature has a strong impact on metabolic rates and can therefore play another major role in niche separation or in species-specific adaptations in the consumer community. Benthic foraminifera show strong metabolic responses to temperature fluctuations (Bradshaw, 1961; Cesbron et al., 2016; Heinz et al., 2012). Species specific responses can have a strong impact on community compositions, with regard to seasonal temperature fluctuation, but also due to human induced global warming. Early experimental investigations and monitoring studies suggest feeding preferences or selective feeding. However, these studies rely on indirect observations from environmental monitoring (Hohenegger et al., 1989; Papaspyrou et al., 2013) or from a laboratory study focusing on the more diverse saltmarsh communities (Lee and Muller, 1973). The latter study revealed, that foraminiferal salt marsh communities are characterized by highly specialized feeding strategies. Analogically, the close spatial coexistence of *A. tepida* and *H. germanica* is also likely based on different feeding strategies and different preferences of other environmental variables. Therefore, the aim of this study was to obtain a closer definition of the ecological feeding niches of *A. tepida* and *H. germanica* in relation to intertidal fluxes of OM and OM processing at different temperatures. Additionally, this study offers the first estimates for the release of OM derived carbon and nitrogen in foraminifera.

To reach our aim, we carried out laboratory feeding experiments with stable isotope labelled (^{13}C & ^{15}N) food sources (chlorophyte detritus: *Dunaliella tertiolecta*, diatom detritus: *Phaeodactylum tricornutum*). Both food sources were offered simultaneously to *A. tepida* to identify feeding preferences of this species. Further, we compared diatom detritus intake and retention of food-derived carbon (pC) and nitrogen (pN) of *A. tepida* and *H. germanica* at three different temperatures (15°C, 20°C, 25°C). The evaluation of the metabolic costs of pC and pN during a 24 hour starvation period can further help to explain species specific OM processing due to metabolic nutrient budgets.

Biogeosciences Discuss., https://doi.org/10.5194/bg-2018-231
Manuscript under review for journal Biogeosciences
Discussion started: 28 May 2018

Finally, we collected quantitative data of the abundances of both species in the sampling area to estimate species specific contributions to intertidal fluxes of OM derived carbon and nitrogen.

2 Material and Methods

2.1 Sampling area & sample preparation

The sampling area is located at the Elbe river estuary in the German Wadden Sea. Samples were collected at low tide in April 2016, close to the shoreline. Three sediment cores (4.5 cm diameter) were taken in random spacing within an area of ~ 4 m^2. The uppermost centimetre of the cores was fixed in a mixture of ethanol and Rose Bengal to stain the cytoplasm of live foraminifera. At the University of Vienna, the sediment core material was sieved to obtain size fractions of 125 – 250 µm, 250 – 355 µm and < 355 µm. Brightly stained (living) foraminifera were identified and counted to calculate abundances (individuals per m2) to estimate the relevance of *A. tepida* and *H. germanica* in intertidal OM fluxes.

For the laboratory experiments, sediment was collected at low tide from the uppermost sediment layer and sieved in the field over 125 µm and 500 µm to remove larger meiofauna and organic components. The sediment was filled into plastic containers with seawater and transported back to the University of Vienna. Individuals were picked from the sediment in sufficient and collected in crystallizing dishes, containing a layer of North Sea sediment (< 63 µm) and filtered North Sea seawater. They were fed with a mixture of live *Dunaliella tertiolecta* and *Phaeodactylum tricornutum* once to twice a week until the beginning of the experiments. Live individuals were identified by showing bright and intensive cytoplasm colour, cyst formation (in case of *A. tepida*), material gathered around the aperture, and movement tracks in the sediment. The experiments started after accumulation of sufficient foraminiferal material three weeks after the field sampling.

2.2 Production of artificial phytodetritus

Labelled food was produced by growing *D. tertiolecta* and *P. tricornutum* (SAG 1090-1a) in stable isotope-enriched growth medium. Algae were cultured in sterile 5 L Erlenmeyer bottles, containing F1/2 growth medium (Guillard, 1975; Guillard and Ryther, 1962) enriched with aliquots of 98 atom% NaH13CO3 and 98atom% Na15NO3 (SigmaAldrich). The algae cultures were incubated at 20°C (type ST 2 POL-ECO Aparatura incubation chambers) at a 18 hrs:6 hrs light:dark cycle and bubbled with ambient air. Cultures were harvested at stationary growth (after 14-16 days) by centrifugation, washed three times in sterile, carbon and nitrogen free artificial seawater, shock frozen with liquid nitrogen, and lyophilized to get ^{13}C and ^{15}N-labeled phytodetritus (c.f. Wukovits et al. 2017). Three batches of algae were produced. Final isotopic concentrations were: *P. tricornutum* 7 atom%^{13}C and 15 atom% ^{15}N (experiment 1) and *D. tertiolecta* 22 atom%^{13}C (experiment 1), *P. tricornutum* 14 atom%^{15}N (experiment 2).

2.3 Experiment 1: Nutrient demand and temperature response of *A. tepida* and *H. germanica*

Fifty specimens of *A. tepida* and 50 specimens of *H. germanica* of the size fraction 250 – 355 µm were distributed into separate wells on a 6 well plate, containing North Sea seawater (12 mL per well, salinity: 28 PSU, practical salinity units). In total, triplicate samples were prepared. The food source, *P. tricornutum* (1.5 g dry weight m^{-2}) was added into each well. Wells were then covered with a headspace to prevent evaporation and incubated at 15°C, 20°C or 25°C (Table 1).The specimens were incubated at a 12 hrs : 12 hrs light:dark cycle, starting the incubation with the light cycle. Two equal setups were prepared for incubation. The first setup was terminated after a 24 hour incubation period, to determine the intake of *P. tricornutum* detritus per species and temperature ('24 hrs fed'). The specimens were removed from the wells, transferred to Eppendorf© tubes and frozen at -20°C. The specimens of the second setup were washed three times in carbon and nitrogen free artificial seawater and transferred to crystallizing dishes (9 cm diameter), containing 150 mL filtered North Sea seawater and covered with parafilm. Subsequently, the dishes were incubated for another 24 hours (15°C, 20°C, 25°C; 12 hours light, 12 hours dark, starting with the light cycle). These samples were analysed to determine the remaining phytodetrital carbon and nitrogen after a 24 hour starvation period ('24 hrs starved'). The temperature effect on phytodetrital carbon (pC) and phytodetrital nitrogen (pN) within the foraminiferal cytoplasm, and pC:pN was tested using permutation tests and pairwise permutation tests for post-hoc testing (r package rcompanion). Homogeneity of variances was tested using Fligner Killeen test.

Relationships of pC and pN after feeding and starvation were explored using linear regression for both species, to observe if pC and pN processing are coupled processes in the two species. Finally, the relative amount of food source-derived carbon and nitrogen after 24 hours starvation was evaluated, to compare the carbon and nitrogen costs of the two species during the period without food.

2.4 Experiment 2: Feeding preferences of *A. tepida*

Ammonia tepida individuals were incubated at 20°C within 6 well plates (55 individuals per triplicate/well, size fraction 250 – 355 µm). Each well was filled with 12 mL North Sea seawater. After acclimation of the individuals within the plates, three different dietary setups were established (Table 1). The first diet consisted of chlorophyte derived detritus, uniformly ^{13}C labeled (*D. tertiolecta*, 1.5 g dry weight cm^{-2}), the second was diatom detritus (*P. tricornutum*, 1.5 g dry weight cm-2), uniformly ^{15}N labelled, and the third consisted of a homogenized mixture of both food sources (0.73 g cm^{-2} each). The differential labelling approach allows calculation of nutrient uptake for the distinct phytodetritus source after determination of respective algal carbon and nitrogen composition. Triplicate samples were taken after 1 hour, 3 hours, 6 hours, 12 hours, and 24 hours, and specimens were frozen at -20°C for subsequent isotope ($^{13}C/^{12}C$ and $^{15}N/^{14}N$) and elemental analysis (total organic carbon TOC and total nitrogen TN). Similarly as in experiment 1, plates were incubated at a 12 hrs : 12 hrs light:dark cycle, starting the incubation with the light cycle.

Biogeosciences Discuss., https://doi.org/10.5194/bg-2018-231
Manuscript under review for journal Biogeosciences
Discussion started: 28 May 2018

The algal C:N ratio was used to calculate the pN aliquot for pC of the ^{13}C labelled chlorophyte and pC for the ^{15}N labelled diatom food source, for a better visual comparison of the food intake (this serves as a rough estimate of equivalent pC or pN intake at the two diets).

To describe and compare uptake dynamics for the different diets, Michaelis Menten curves were applied on pC and pN data. The models were tested by applying the lack-of fit method (R package drc). To compare pC and pN values for both diets, pN was calculated from pC for *D. tertiolecta*, and pC from pN for *P. tricornutum*. Hereby acquired estimates for pC, pN might be underestimated or overestimated respectively, due to possible differences in the ratios of carbon:nitrogen excretion or remineralisation respectively.

2.5 Sediment core data and foraminiferal abundances

Sediment core samples (uppermost cm) were sieved to fractionate size classes (125 – 250 µm, 250 – 355 µm, < 355 µm). Rose Bengal-stained individuals were counted for each size fraction to obtain abundance data for the live foraminiferal community at the sampling date. The sediment core data, together with the data from the laboratory experiments, were used to estimate total foraminiferal biomass (TOC, TN) and foraminiferal carbon and nitrogen processing. In case of *H. germanica*, these contributions were only estimated for the 250 – 355 µm fraction (as used in laboratory experiments). For *A. tepida*, the 125 – 250 µm fraction was included to the estimation, using size fraction and feeding relationships from Wukovits et al. 2018). Further, the abundances of *A. tepida*, as derived by the latter study, were compared with the recent study.

2.6 Sample preparation and isotope analysis

Prior to isotope analysis, foraminifera were carefully cleaned from adhering particles in carbon and nitrogen free artificial seawater, rinsed with ultrapure water in a last cleaning step to remove salts, transferred to tin capsules, and dried at 50°C for several hours. Subsequently, the foraminifera were decalcified with 10 – 15 µL 4 % HCl, and kept at 50°C for three days in a final drying step. The optimum range for isotope and elemental analysis was 0.7 – 1.0 mg cytoplasmic dry weight. In the 250 µm size fraction, 30 – 40 individuals met this criterion. Tools for preparation (hairbrush, needles, tin capsules, tweezers) were rinsed with dichloromethane (CH_2Cl_2) and methanol (CH_4O) (1:1, v:v). Glassware for microscopy was combusted at 500°C for 5h. The samples were analysed at the Large-Instrument Facility for Advanced Isotope Research at the University of Vienna (SILVER). Ratios of $^{13}C/^{12}C$, $^{15}N/^{14}N$ and the content of organic carbon and nitrogen were analysed with an Isotope Ratio Mass Spectrometer (IRMS; DeltaPLUS, Thermo Finnigan) coupled with an interface (ConFlo III, Thermo Finnigan) to an elemental analyzer (EA 1110, CE Instruments). Isotope ratio data, the Vienna Pee Dee Belemnite standard for C (RVPDB = 0.0112372) and the standard for atmospheric nitrogen for N ($R_{atm}N$ = 0.0036765) were used to calculate atom% of the samples, where X is ^{13}C or ^{15}N:

$$\text{atom\%}X = \frac{100 \times R_{standard} \times \left(\frac{\delta X_{sample}}{1000} + 1\right)}{1 + R_{standard} \times \left(\frac{\delta X_{sample}}{1000} + 1\right)}, \quad (1)$$

Intake of phytodetrital carbon and nitrogen into foraminiferal cytoplasm was calculated by determining the excess (*E*) of isotope content within the samples using natural abundance data and data of enriched samples (Middelburg et al., 2000):

$$E = \frac{(atomX_{sample} - atomX_{background})}{100}, \quad (2)$$

where *X* is ^{13}C or ^{15}N. Excess and content of total organic carbon and nitrogen (TOC and TN per individual) were used to calculate incorporated isotopes derived from the food source I_{iso} [µg mg-1] or [µg ind-1] = E x C (N) [µg ind-1]. The amount of phytodetrital carbon (pC [µg ind-1]) and nitrogen (pN [µg ind-1]) within foraminiferal cytoplasm was calculated as follows (Hunter et al., 2012):

$$pX = \frac{I_{iso}}{\left(\frac{atom\%X_{phyto}}{100}\right)}, \quad (3)$$

3 Results

3.1 Experiment 1: Nutrient demand and temperature response of *A. tepida* and *H. germanica*

Phytodetrital pC and pN levels derived from *P. tricornutum* detritus was 2 – 5 times higher in *A. tepida* compared to *H. germanica* (Fig. 1 a, b). Different incubation temperatures resulted in significant effects on pC levels after 24 hours feeding and 24 hours starvation in both species. *Ammonia tepida* showed a significantly lowered pC content when feeding at 25°C (Fig. 1 a, *A. tepida*, 24 hrs fed, $p < 0.05$). The 24 hour incubation period with no food resulted in significantly lowered pC levels at 20°C and 25°C (Fig. 1 a, *A. tepida*, 24 hrs starved, $p < 0.05$). In *H. germanica*, the 24 hours feeding period had as similar effect like on *A. tepida*, resulting in significantly lowered pC levels at 25°C (Fig. 1 a, *H. germanica*, 24 hrs fed, $p < 0.05$). A strong effect of increased temperature after the starvation period was present at 25°C (Fig. 1 a, *H. germanica*, 24 hrs starved, $p < 0.05$).

The pN levels in *A. tepida* were considerably affected by temperature after feeding and starvation, whereas there was no apparent effect on *H. germanica*s pN levels, neither after feeding, nor after incubation without food (Fig. 1 b). *Ammonia tepida* reacted with simultaneously lowered pN and pC levels at 25°C after feeding and starvation (Fig. 1 b, *A. tepida*, $p < 0.05$).

The ratios of pC:pN were affected by temperature in both species during feeding and starvation (Fig. 1 c, $p < 0.05$). Increased temperatures promoted a drop of pC:pN ratios in *A. tepida* during the starvation period (Fig. 1 c, *A. tepida*, $p < 0.05$). In contrast, temperature specific pC:pN ratios in *H. germanica* showed no change between the incubations with food (24 hrs fed), and the starvation period (24 hrs starved; Fig. 1 c, *H. germanica*). Ratios of C:N show significant temperature related changes in *H. germanica* ($p < 0.05$), but not in *A. tepida* (Fig. 1 d).

The higher relative pN content in *A. tepida* also shows in a steeper relationship of cytoplasmic pN and pC, compared to *H. germanica* (Fig. 2 a). Further, there is a far higher metabolic turnover of pC and pN in *A. tepida*, specifically at 20°C (Fig. 2 b).

3.2 Experiment 2: Feeding preferences of *A. tepida*

Michaelis Menten curves fitted with no significant deviation of variance within the sample replicates. Enrichment of algal nutrients in foraminiferal cytoplasm were highest when a single diet of *D. tertiolecta* was available (Fig. 3 a). Here, saturation levels (max. 180 ng C ind-1) were already reached within three hours after detritus introduction and half saturation with pC in *A. tepida* was reached after 0.6 hours (Table 2.). In contrast, a single *P. tricornutum* diet resulted in a slower food intake (Fig. 3 b), with a half saturation of pN levels after 1.4 hours (Table 2). Further, diatom phytodetritus intake resulted in lower levels of pC (max. ~ 80 ng C ind-1). In the mixed feeding approach, half saturation of chlorophyte pC was reached after 1.4 hours and diatom pN half saturation was already reached after 0.1 hours. Further, the maximum pC levels of the chlorophyte diet still reached ~ 70 % of those in the single chlorophyte diet, whereas the pN levels of the diatom diet only reached about 30 % of those in the single diatom diet (Fig. 3, Table 2). Chlorophyte intake was faster and higher, both in the single and mixed diet, and diatom pN stagnated already after less than 1 hour in the mixed diet, but after this time period, chlorophyte detritus intake in the mixed diet had continued with increasing pC levels, saturating between 6 and 10 hours (Fig. 3 a & b).

3.3 Relevance of *A. tepida* and *H. germanica* in intertidal OM fluxes

Data for the live foraminiferal community from the three stained sediment cores showed a typical, low biodiversity mudflat community consisting of *A. tepida*, *H. germanica* and very low abundances of *Elphidium williamsonii* (< 1258 ind. m^{-2}, all size fractions). Abundances of *A. tepida* and *H. germanica* were equal within all three size fractions, and decreased with increasing size fraction, with very high abundances in the 125 – 250 µm fraction. The biomass of live foraminifera in units of TOC would be max. ~ 120 mg C m^{-2} (both species, all size fraction, Table 3). From combining in situ abundances and pC estimates (15°C), this foraminiferal community has the potential to take min. 4 ~ mg C m^{-2} d^{-1}, when taking only diatom detritus into account. The contribution of *H. germanica* to this OM processing is only at about 15 %. In comparison, the abundance and biomass (~ 390 mg C m^{-2}) of live *A. tepida* in early May 2015 in the same sampling area was far higher than in the sampling period (late April 2016) for this study (see Table 3).

4 Discussion

Different ecologic lifestyles or adaptations to environmental parameters are important organismic attributes to avoid inter- and intra-specific competition. Further, different metabolic adaptations result in species specific rates of organic matter turnover. Our results clearly demonstrate, that food resource partitioning and different temperature adaptations contribute to the fluctuating, temporal distribution and abundance of *A. tepida* and *H. germanica*. Due to these specific adaptations, both species play different roles in intertidal organic matter fluxes. There are, however, limitations for the interpretation of results derived from laboratory incubations. A laboratory setup cannot accurately readjust natural conditions completely. Therefore, the foraminiferal responses might deviate slightly from their natural behavior. To enable a compatible comparison, we incubated freshly sampled individuals at stable, near natural conditions. Both tested food sources are considered as good food sources

for intertidal foraminifera (Lee et al., 1966). *Dunaliella tertiolecta* is commonly used in feeding experiments with foraminifera. Phaedactylum tricornutum, which represents a more stable (due to the silicate frustule) source of OM, is a common food source of intertidal foraminifera (Murray, 1963). Additional tested food sources would give a more comprehensive picture, but there were limitations in time and material. In the following sections, our results are discussed with respect to these restrictions.

4.1 Experiment 1: Nutrient demand and temperature response of *A. tepida* and *H. germanica*

Experiment 1 shows clear differences in the amount of phytodetritus intake and different carbon and nitrogen budgeting between the two species (Fig. 1, Fig. 2). *Ammonia tepida* has a higher affinity to the diatom detritus food source with a three times higher intake of diatoms at the two lower temperatures, compared to *H. germanica*. This lower food intake in *H. germanica* could be explained by the mixotrophic lifestyle of this species. *Haynesina germanica* is known to host kleptoplasts, exploiting the photosynthetic activity of ingested chloroplasts as an energy source (Lopez, 1979; Pillet et al., 2011). This species might therefore utilize nutrients (carbohydrates) derived from the photosynthetic activity of incorporated chloroplasts (Cesbron et al., 2017). This lifestyle could cause a lower demand for and lower turnover of OM as food source (Cesbron et al., 2017). Highly specialized sea slugs use plastids as energy reservoirs at times of low food availability (e.g. Hinde & Smith 1972, Marín & Ros 1993, Cartaxana et al. 2017), where carbon supply from chloroplasts can cover 60% of total carbon input (Raven et al., 2001). In our study, the pC intake in *H. germanica* was ~ 67% lower than that of *A. tepida* (Fig. 1). In kleptoplast hosting sea slugs, free NH_4^+ from the seawater is a primary source for the generation of amino acids via kleptoplast metabolism within the slug (Teugels et al., 2008). A similar mechanism in *H. germanica* might explain the high relative turnover of pN (Fig. 2b). Phytodetrital nitrogen might therefore be disposed at a higher rate in a relatively temperature independent process, probably in the form of dissolved organic nitrogen, further causing higher pC:pN ratio in the cytoplasm of *H. germanica* (Fig. 1).

In addition to the higher rates of phytodetritus intake, *A. tepida* shows a considerably higher metabolic turnover of pC and pN than *H. germanica* (Fig. 2b). According to Cesbron et al. 2016), respiration rates (normalized to pmol mm^{-3} d^{-1}) are about 2 – 12 times higher in *A. tepida* specimens than in *H. germanica* specimens from the same location. In this study, a 4 - 7 times higher release of TOC per individual and day (size fraction 250 – 355 µm) was observed in *A. tepida*. Interestingly, this study shows similar reactions of both species in carbon loss due to increased temperature. An earlier study on the temperature effect on *D. tertiolecta* detritus intake of the two species showed a higher sensitivity to increased temperatures in *H. germanica*, and far lower rates of chlorophyte detritus intake compared to this study (Wukovits et al., 2017). In contrast *Ammonia tepida* seems to be more tolerant to higher temperatures when feeding on chlorophyte detritus. The results of Experiment 1 suggest a niche separation of the two species with respect to phytodetritus or OM availability and temperature.

4.2 Experiment 2: Feeding preferences of *A. tepida*

The findings of Experiment 2 suggest that *A. tepida* might prefers OM food sources, which are easy to exploit and to break down. The high intake values in the *D. tertiolecta* mono-diet one hour after incubation and the saturation of cytoplasmic pC

levels after three hours indicate a high affinity to chlorophyte detritus (Fig. 3, Table 2). Earlier studies also observed quick and high ingestion rates of chlorophyte detritus (*Chlorella* sp.) by the genus *Ammonia* (Linshy et al., 2014; Wukovits et al., 2017, 2018). The fast saturation with diatom detritus after one hour in the mixed diet and the advanced and high intake of *D. tertiolecta* could even indicate an avoidance of *P. tricornutum* and selective feeding on *D. tertiolecta*. Probably, the soft cell of chlorophytes enables a faster and easier metabolic processing of this food source compared to the harder diatom frustules. The recognition of such food sources could be achieved by chemosensory behaviour of the foraminifera (comp. Langer & Gehring 1993) and the attraction to specific substances attached to, or leaking from the food particles, similar to some other protists, which react to food-specific amino acids (Almagor et al., n.d.; Levandowsky et al., 1984).

Microalgal communities in tidal sediments typically consist of microphytobenthic diatoms, which are considered to be the main food source for intertidal foraminifera. An isotope labelling study has shown that diatoms (*Navicula salinicola*) are taken up by *A. tepida* at high rates, but the complete release of the content of the diatom frustules can take several days (LeKieffre et al., 2017). This might not fit the nutrient demands of *A. tepida* at times of high metabolic activity. Therefore, a shift from microphytobenthos to particulate OM from riverine or tidal transport might be a feeding strategy in *A. tepida*. Specifically at higher temperatures, when more energy is needed to maintain metabolic activities.

In general, food sources of *A. tepida* include microalgae, phytodetritus, bacteria and sometimes metazoans (Bradshaw, 1961; Dupuy et al., 2010; Moodley et al., 2000; Pascal et al., 2008). Bacteria are considered to play a minor role in the diet of *A. tepida* (Pascal et al., 2008), and reports on metazoan feeding in *A. tepida* are restricted to a single observation (Dupuy et al., 2010). In contrast to *A. tepida*, *H. germanica* does actively ingest bacteria and they can occasionally be preferred over diatoms (Brouwer et al., 2016). Diatoms are reportedly taken up by *H. germanica*, and conical test structures serve as tools to crack diatom frustules open (Austin et al., 2005; Ward et al., 2003). These chloroplasts derived from diatoms remain as functional kleptoplasts, as mentioned above, within the cytoplasm of *H. germanica*.

4.3 Relevance of *A. tepida* and *H. germanica* in intertidal OM fluxes

Data of foraminiferal abundances or foraminiferal biomass are important variables to estimate foraminiferal nutrient fluxes. In this section, we discuss the relevance of *A. tepida* or *H. germanica* in intertidal fluxes of phytodetrital carbon and nitrogen as estimated from sediment core data in combination with the laboratory feeding experiments of this study. The biomass of the two species at the sampling area ranges between ~ 116 and > 380 mg C m^{-2} (Tab. 3) at the sampling dates in late April/early May in two consecutive years. However, our biomass data is in the range of biomass estimations for hard-shelled foraminifera (TOC max. ~ 160 - 750 mg C m^{-2}) in other areas of the Wadden Sea (van Oevelen et al., 2006a, 2006b).

Our study shows high rates of OM carbon and nitrogen turnover in the foraminiferal community, specifically in populations of *A. tepida* (Table 3). The process of carbon and nitrogen regeneration by OM remineralisation plays an important role in marine biogeochemical cycling. Carbon loss, e.g. due to organismic respiration or OM remineralisation to CO_2, reduces the availability of organic carbon sources in the heterotrophic food web. Whereas, dissolved organic carbon sources from organismic excretion can serve as an important nutrient source for bacteria (e.g. Zweifel et al. 1993, Snyder & Hoch 1996,

Kahler et al. 1997). Therefore, the fast processing of OM in *A. tepida* might be an important link in intertidal carbon and nitrogen fluxes. According to this study, maximum pC flux through *A. tepida* can reach values of ~ 36 mg C m^{-2} d^{-1} when feeding on chlorophytes at 20°C (estimated from Experiment 2, Fig. 2 relative release, and max. abundances). Therefore, *A. tepida* could contribute up to 10% of the turnover of OM derived from gross particulate phytoplankton production at the sampling date in April/May 2016, with a gross particulate primary production between ~ 230 – 1500 mg C m^{-2} d^{-1} (Tillmann et al., 2000). This is comparable with the study of Moodley et al. 2000, which showed a fast and high intake of chlorophyte detritus (~7 %), in sediment core incubated feeding experiments.

Planktonic protozoa are the primary regenerators of marine nitrogen, transforming OM-nitrogen to their primary N-excretion product, NH_4^+ (Glibert, 1997). The excretion of ammonia by marine protists can contribute to a large part to the nutritional demands of marine primary productivity (Ferrierpages and Rassoulzadegan, 1994; Ota and Taniguchi, 2003; Verity, 1985). Nitrogen regeneration by protozoa was supposed to play a far higher role than bacterial nitrogen regeneration in the marine microbial food chain (Goldman and Caron, 1985). Indeed, excreted nitrogen can serve as important nutrient sources for microbes (Wheeler and Kirchman, 1986). The release of dissolved organic nitrogen and ammonium by e.g. copepods , can be a major driver for marine microbial production (Valdés et al., 2018). Here, foraminiferal nitrogen excretion values are in the range of estimations for weight-specific NH_4^+ excretion in marine protozoa according to Dolan 1997 (data for foraminiferal weight, comp. supplementary Fig. 1). Due to high abundances, nitrogen release by e.g. *A. tepida* as observed in this study could reach 2.5 mg N m^{-2} d^{-1} or ~ 73 nmol N dm^{-2} h^{-1}, respectively, at 15°C and high diatom availability (comp. Table 3). As a rough estimate for *A. tepida* feeding at high abundances and high availability of chlorophyte detritus at 20°C, these values could increase to ~ 22 mg N m^{-2} d^{-1} or ~ 0.6 µmol N dm^{-2} h^{-1} (Fig.1 & Table 3). Therefore, foraminiferal nitrogen release could cover a considerable amount of the nutritional demand for NH_4^+ or amino acid-N for marine bacteria (comp. Wheeler & Kirchman 1986) and supply high nutrient input for microphytobenthos growth.

Vice versa, the labile dissolved organic matter derived from bacterial decomposition of refractory organic matter provides a valuable food source for some benthic foraminifera, and they are indispensable for the reproduction of some foraminifera species (Jorissen et al., 1998; Muller and Lee, 1969; Nomaki et al., 2011). This study supports the consideration of a strong interplay of intertidal foraminiferal and bacterial population dynamics and constitute an important link in the food web complex of primary consumers and decomposers. This consideration can also relate to the fact, that deep sea benthic foraminifera play a significant role in the short-time processing of phytodetrital carbon, while bacteria dominate the long term carbon processing (Moodley et al., 2002). Foraminifera can play a significant role in the mediation of dissolved carbon and nitrogen sources from particulate OM for primary productivity or bacterial production. Further, the seasonal, specific dominance of a mixotrophic species like *H. germanica* or a heterotrophic species like *A. tepida* might relate to differences in seasonal availability of OM sources and carbon and nitrogen fluxes. Seasonal cyclicity of the standing stock of intertidal foraminifera communities as observed e.g. in Murray & Alve 2000 and temperature related foraminiferal carbon and nitrogen budgets could include a strongly tied dynamic interplay with microbial population fluctuations.

5 Conclusions

This study compares differences in the feeding behavior, nutrient demand and OM flux of two intertidal foraminiferal species. Our results clearly show, that *A. tepida* has a higher impact on the fluxes of phytodetrital carbon and nitrogen in intertidal sediments than *H. germanica*. This can partly be explained by their different lifestyles. Differences in temperature acclimatization or preferences to different food sources can serve as strategies to avoid spatial and temporal competition, resulting in a niche separation of the two species with respect to phytodetritus or OM availability and temperature. Accordingly, *H. germanica* could be associated with environmental conditions with at least moderate availability of microphytobenthos and lower temperatures, as given prior to the diatom spring bloom. Whereas *A. tepida* could take advantage of seasons characterized by higher input of allochthonous OM. Further, temperature fluctuations in combination with allochtonous OM availability have less effect on the carbon and nitrogen processing in *A. tepida*. These differentiations in their metabolic OM processing and lifestyles suggest a far higher relevance of *A. tepida* in the mediation of the fluxes of intertidal carbon and nitrogen. Interactions between foraminifera and bacteria might play a significant role in foraminiferal population dynamics, partly due to the high rates of OM carbon and nitrogen turnover in *A. tepida*.

References

Almagor, M., Ron, A. and Bar-Tana, J.: Chemotaxis in *Tetrahymena thermophila*, Cell Motil., 1(2), 261–268, doi:10.1002/cm.970010208, n.d.

Alve, E. and Murray, J. W.: Ecology and taphonomy of benthic foraminifera in a temperate mesotidal inlet, J. Foraminifer. Res., 24(1), 18–27, 1994.

Alve, E. and Murray, J. W.: Temporal variability in vertical distributions of live (stained) intertidal foraminifera, southern England, J. Foraminifer. Res., 31(1), 12–24, doi:10.2113\0310012, 2001.

Austin, H. A., Austin, W. E. N. and Paterson, D. M.: Extracellular cracking and content removal of the benthic diatom *Pleurosigma angulatum* (Quekett) by the benthic foraminifera *Haynesina germanica* (Ehrenberg), Mar. Micropaleontol., 57(3–4), 68–73, doi:10.1016/j.marmicro.2005.07.002, 2005.

Bradshaw, J. S.: Laboratory experiments on the ecology of foraminifera, Contrib. Cushman Found. Foraminifer. Res., 7(3), 87–106, 1961.

Brouwer, G. M., Duijnstee, I. A. P., Hazeleger, J. H., Rossi, F., Lourens, L. J., Middelburg, J. J. and Wolthers, M.: Diet shifts and population dynamics of estuarine foraminifera during ecosystem recovery after experimentally induced hypoxia crises, Estuar. Coast. Shelf Sci., 170(Supplement C), 20–33, doi:10.1016/j.ecss.2015.12.015, 2016.

Cartaxana, P., Trampe, E., Kühl, M. and Cruz, S.: Kleptoplast photosynthesis is nutritionally relevant in the sea slug *Elysia viridis*, Sci. Rep., 7(1), 7714, doi:10.1038/s41598-017-08002-0, 2017.

Cesbron, F., Geslin, E., Jorissen, F. J., Delgard, M. L., Charrieau, L., Deflandre, B., Jézéquel, D., Anschutz, P. and Metzger, E.: Vertical distribution and respiration rates of benthic foraminifera: Contribution to aerobic remineralization in intertidal mudflats covered by Zostera noltei meadows, Estuar. Coast. Shelf Sci., doi:10.1016/j.ecss.2015.12.005, 2016.

Cesbron, F., Geslin, E., Kieffre, C. L., Jauffrais, T., Nardelli, M. P., Langlet, D., Mabilleau, G., Jorissen, F. J., Jézéquel, D. and Metzger, E.: Sequestered Chloroplasts in the Benthic Foraminifer *Haynesina germanica*: Cellular Organization, Oxygen Fluxes and Potential Ecological Implications, J. Foraminifer. Res., 47(3), 268–278, doi:10.2113/gsjfr.47.3.268, 2017.

Dolan, J. R.: Phosphorus and ammonia excretion by planktonic protists, Mar. Geol., 139(1), 109–122, doi:10.1016/S0025-3227(96)00106-5, 1997.

Dupuy, C., Rossignol, L., Geslin, E. and Pascal, P.-Y.: Predation of Mudflat Meio-Macrofaunal Metazoans by a Calcareous Foraminifer, *Ammonia tepida* (cushman, 1926), J. Foraminifer. Res., 40(4), 305–312, doi:10.2113/gsjfr.40.4.305, 2010.

Enge, A. J., Witte, U., Kucera, M. and Heinz, P.: Uptake of phytodetritus by benthic foraminifera under oxygen depletion at the Indian Margin (Arabian Sea), Biogeosciences, 11, 2017–2026, doi:doi:10.5194\bgd-10-15305-2013, 2014.

Enge, A. J., Wukovits, J., Wanek, W., Watzka, M., Witte, U. F. M., Hunter, W. R. and Heinz, P.: Carbon and Nitrogen Uptake of Calcareous Benthic Foraminifera along a Depth-Related Oxygen Gradient in the OMZ of the Arabian Sea, Aquat. Microbiol., 71, doi:10.3389/fmicb.2016.00071, 2016.

Ferrierpages, C. and Rassoulzadegan, F.: N-Remineralization in Planktonic Protozoa, Limnol. Oceanogr., 39(2), 411–418, doi:10.4319/lo.1994.39.2.0411, 1994.

Glibert, P. M.: Interactions of top-down and bottom-up control in planktonic nitrogen cycling, Hydrobiologia, 363(1–3), 1–12, doi:10.1023/A:1003125805822, 1997.

Goldman, J. C. and Caron, D. A.: Experimental studies on an omnivorous microflagellate: implications for grazing and nutrient regeneration in the marine microbial food chain, Deep Sea Res. Part Oceanogr. Res. Pap., 32(8), 899–915, doi:10.1016/0198-0149(85)90035-4, 1985.

Goldstein, S. T. and Corliss, B. H.: Deposit feeding in selected deep-sea and shallow-water benthic foraminifera, Deep Sea Res. Part Oceanogr. Res. Pap., 41(2), 229–241, doi:http:\\dx.doi.org\10.1016\0967-0637(94)90001-9, 1994.

Guillard, R. R. L.: Culture of phytoplankton for feeding marine invertebrates, in Culture of Marine Invertebrate Animals, edited by W. L. Smith, M. H. Chanley, and M. H. Chanley, pp. 29–60, Plenum Press, New York, USA. [online] Available from: :\\BCI:BCI197612023356, 1975.

Guillard, R. R. L. and Ryther, J. H.: Studies of marine planktonic diatoms. I. *Cyclotella nana* Hustedt and *Detonula confervacea* Cleve, Can. J. Microbiol., 8(2), 229–239, 1962.

Hayward, B. W.: "Monospecific" and near-monospecific benthic foraminiferal faunas, New Zealand, J. Foraminifer. Res., 44(3), 300–315, 2014.

Heinz, P., Kitazato, H., Schmiedl, G. and Hemleben, C.: Response of deep-sea benthic foraminifera from the Mediterranean Sea to simulated phytoplankton pulses under laboratory conditions, J. Foraminifer. Res., 31(3), 210–227, doi:10.2113\31.3.210, 2001.

Heinz, P., Marten, R. A., Linshy, V. N., Haap, T., Geslin, E. and Kohler, H. R.: 70 kD stress protein (Hsp70) analysis in living shallow-water benthic foraminifera, Mar. Biol. Res., 8(7), 677–681, doi:10.1080\17451000.2011.650177, 2012.

Hinde, R. and Smith, D. C.: Persistence of Functional Chloroplasts in *Elysia viridis* (Opisthobranchia, Sacoglossa), Nature. New Biol., 239(88), 30–31, doi:10.1038/newbio239030a0, 1972.

Hohenegger, J., Piller, W. and Baal, C.: Reasons for spatial microdistributions of foraminifers in an intertidal pool (Northern Adriatic Sea), Mar. Ecol., 10(1), 43–78, doi:10.1111\j.1439-0485.1989.tb00065.x, 1989.

Hunter, W. R., Veuger, B. and Witte, U.: Macrofauna regulate heterotrophic bacterial carbon and nitrogen incorporation in low-oxygen sediments, ISME J., 6(11), 2140–2151, doi:10.1038/ismej.2012.44, 2012.

Jahnke, R. A.: Transport processes and organic matter cycling in coastal sediments, edited by A. R. Robinson and K. H. Brink, pp. 163–192., 2004.

Jorissen, F. J., Wittling, I., Peypouquet, J. P., Rabouille, C. and Relexans, J. C.: Live benthic foraminiferal faunas off Cape Blanc, NW-Africa: Community structure and microhabitats, Deep Sea Res. Part Oceanogr. Res. Pap., 45(12), 2157–2188, doi:10.1016/S0967-0637(98)00056-9, 1998.

Kahler, P., Bjornsen, P. K., Lochte, K. and Antia, A.: Dissolved organic matter and its utilization by bacteria during spring in the Southern Ocean, Deep-Sea Res. Part Ii-Top. Stud. Oceanogr., 44(1–2), 341–353, doi:10.1016/S0967-0645(96)00071-9, 1997.

Langer, M. and Gehring, C.: Bacteria Farming - a Possible Feeding Strategy of Some Smaller, Motile Foraminifera, J. Foraminifer. Res., 23(1), 40–46, doi:10.2113/gsjfr.23.1.40, 1993.

Lee, J. J. and Muller, W. A.: Trophic dynamics and niches of salt marsh foraminifera, Am. Zool., 13(1), 215–223, doi:10.2307/3881901, 1973.

Lee, J. J., McEnery, M., Pierce, S., Freudent.Hd and Muller, W. A.: Tracer experiments in feeding littoral foraminifera, J. Protozool., 13(4), 659–670, doi:10.1111/j.1550-7408.1966.tb01978.x, 1966.

Lei, Y.-L., Stumm, K., Wickham, S. A. and Berninger, U.-G.: Distributions and biomass of benthic ciliates, foraminifera and amoeboid protists in marine, brackish, and freshwater sediments, J. Eukaryot. Microbiol., 61(5), 493–508, doi:10.1111\jeu.12129, 2014.

LeKieffre, C., Bernhard, J. M., Mabilleau, G., Filipsson, H. L., Meibom, A. and Geslin, E.: An overview of cellular ultrastructure in benthic foraminifera: New observations of rotalid species in the context of existing literature, Mar. Micropaleontol., doi:10.1016/j.marmicro.2017.10.005, 2017.

Levandowsky, M., Cheng, T., Kehr, A., Kim, J., Gardner, L., Silvern, L., Tsang, L., Lai, G., Chung, C. and Prakash, E.: Chemosensory Responses to Amino-Acids and Certain Amines by the Ciliate *Tetrahymena* - a Flat Capillary Assay, Biol. Bull., 167(2), 322–330, doi:10.2307/1541279, 1984.

Linshy, V. N., Nigam, R. and Heinz, P.: Response of shallow water benthic foraminifera to a 13C-labeled food pulse in the laboratory, in Approaches to Study Living Foraminifera. Collection, Maintenance and Experimentation, edited by H. Kitazato, J. M. Bernhard, U. Förstner, and W. Salomons, Springer, Japan., 2014.

Lopez, E.: Algal chloroplasts in the protoplasm of 3 species of benthic foraminifera - taxonomic affinity, viability and persistence, Mar. Biol., 53(3), 201–211, doi:10.1007\bf00952427, 1979.

Marín, A. and Ros, J.: Ultrastructural and ecological aspects of the development of chloroplast retention in the sacoglossan gastropod *Elysia timida*, J. Molluscan Stud., 59(1), 95–104, doi:10.1093/mollus/59.1.95, 1993.

Martins, M. V. A., Silva, F., Laut, L. L. M., Frontalini, F., Clemente, I. M. M. M., Miranda, P., Figueira, R., Sousa, S. H. M. and Dias, J. M. A.: Response of Benthic Foraminifera to Organic Matter Quantity and Quality and Bioavailable Concentrations of Metals in Aveiro Lagoon (Portugal), PLoS ONE, 10(2), doi:10.1371/journal.pone.0118077, 2015.

Middelburg, J. J., Barranguet, C., Boschker, H. T. S., Herman, P. M. J., Moens, T. and Heip, C. H. R.: The fate of intertidal microphytobenthos carbon: An in situ C-13-labeling study, Limnol. Oceanogr., 45(6), 1224–1234, 2000.

Mojtahid, M., Geslin, E., Coynel, A., Gorse, L., Vella, C., Davranche, A., Zozzolo, L., Blanchet, L., Bénéteau, E. and Maillet, G.: Spatial distribution of living (Rose Bengal stained) benthic foraminifera in the Loire estuary (western France), J. Sea Res., 118, 1–16, doi:10.1016/j.seares.2016.02.003, 2016.

Moodley, L., Boschker, H. T. S., Middelburg, J. J., Pel, R., Herman, P. M. J., de Deckere, E. and Heip, C. H. R.: Ecological significance of benthic foraminifera: 13C labelling experiments, Mar. Ecol. Prog. Ser., 202, 289–295, doi:10.3354/meps202289, 2000.

Moodley, L., Middelburg, J. J., Boschker, H. T. S., Duineveld, G. C. A., Pel, R., Herman, P. M. J. and Heip, C. H. R.: Bacteria and Foraminifera: key players in a short-term deep-sea benthic response to phytodetritus, Mar. Ecol. Prog. Ser., 236, 23–29, 2002.

Muller, W. A. and Lee, J. J.: Apparent indispensability of bacteria in foraminiferan nutrition, J. Protozool., 16(3), 471–478, doi:10.1111/j.1550-7408.1969.tb02303.x, 1969.

Murray, J. W.: Ecological experiments on Foraminifera, J. Mar. Biol. Assoc. UK, 43(3), 621–642, doi:10.1017/S0025315400025571, 1963.

Murray, J. W. and Alve, E.: Major aspects of foraminiferal variability (standing crop and biomass) on a monthly scale in an intertidal zone, J. Foraminifer. Res., 30(3), 177–191, doi:10.2113\0300177, 2000.

Nomaki, H., Heinz, P., Hemleben, C. and Kitazato, H.: Behavior and response of deep-sea benthic foraminifera to freshly supplied organic matter: A laboratory feeding experiment in microcosm environments, J. Foraminifer. Res., 35(2), 103–113, doi:10.2113/35.2.103, 2005a.

Nomaki, H., Heinz, P., Nakatsuka, T., Shimanaga, M. and Kitazato, H.: Species-specific ingestion of organic carbon by deep-sea benthic foraminifera and meiobenthos: In situ tracer experiments, Limnol. Oceanogr., 50(1), 134–146, 2005b.

Nomaki, H., Heinz, P., Nakatsuka, T., Shimanaga, M., Ohkouchi, N., Ogawa, N. O., Kogure, K., Ikemoto, E. and Kitazato, H.: Different ingestion patterns of 13C-labeled bacteria and algae by deep-sea benthic foraminifera, Mar. Ecol. Prog. Ser., 310, 95–108, doi:10.3354\meps310095, 2006.

Nomaki, H., Ogawa, N. O., Ohkouchi, N., Suga, H., Toyofuku, T., Shimanaga, M., Nakatsuka, T. and Kitazato, H.: Benthic foraminifera as trophic links between phytodetritus and benthic metazoans: carbon and nitrogen isotopic evidence, Mar. Ecol. Prog. Ser., 357, 153–164, doi:10.3354\meps07309, 2008.

Nomaki, H., Ohkouchi, N., Heinz, P., Suga, H., Chikaraishi, Y., Ogawa, N. O., Matsumoto, K. and Kitazato, H.: Degradation of algal lipids by deep-sea benthic foraminifera: An in situ tracer experiment, Deep-Sea Res. Part -Oceanogr. Res. Pap., 56(9), 1488–1503, doi:10.1016\j.dsr.2009.04.013, 2009.

Nomaki, H., Ogawa, N. O., Takano, Y., Suga, H., Ohkouchi, N. and Kitazato, H.: Differing utilization of glucose and algal particulate organic matter by deep-sea benthic organisms of Sagami Bay, Japan, Mar. Ecol. Prog. Ser., 431, 11–24, doi:10.3354/meps09144, 2011.

van Oevelen, D., Soetaert, K., Middelburg, J. J., Herman, P. M. J., Moodley, L., Hamels, I., Moens, T. and Heip, C. H. R.: Carbon flows through a benthic food web: Integrating biomass, isotope and tracer data, J. Mar. Res., 64(3), 453–482, doi:10.1357/002224006778189581, 2006a.

van Oevelen, D., Moodley, L., Soetaert, K. and Middelburg, J. J.: The trophic significance of bacterial carbon in a marine intertidal sediment: Results of an in situ stable isotope labeling study, Limnol. Oceanogr., 51(5), 2349–2359, 2006b.

Ota, T. and Taniguchi, A.: Standing crop of planktonic ciliates in the East China Sea and their potential grazing impact and contribution to nutrient regeneration, Deep-Sea Res. Part Ii-Top. Stud. Oceanogr., 50(2), 423–442, doi:10.1016/S0967-0645(02)00461-7, 2003.

Papaspyrou, S., Diz, P., Garcia-Robledo, E., Corzo, A. and Jimenez-Arias, J.-L.: Benthic foraminiferal community changes and their relationship to environmental dynamics in intertidal muddy sediments (Bay of Cadiz, SW Spain), Mar. Ecol. Prog. Ser., 490, 121–135, doi:10.3354/meps10447, 2013.

Pascal, P.-Y., Dupuy, C., Richard, P. and Niquil, N.: Bacterivory in the common foraminifer *Ammonia tepida*: Isotope tracer experiment and the controlling factors, J. Exp. Mar. Biol. Ecol., 359(1), 55–61, doi:http:\\dx.doi.org\10.1016\j.jembe.2008.02.018, 2008.

Pillet, L., de Vargas, C. and Pawlowski, J.: Molecular Identification of Sequestered Diatom Chloroplasts and Kleptoplastidy in Foraminifera, Protist, 162(3), 394–404, doi:10.1016\j.protis.2010.10.001, 2011.

Raven, J. A., Walker, D. I., Jensen, K. R., Handley, L. L., Scrimgeour, C. M. and McInroy, S. G.: What fraction of the organic carbon in sacoglossans is obtained from photosynthesis by kleptoplastids? An investigation using the natural abundance of stable carbon isotopes, Mar. Biol., 138(3), 537–545, doi:10.1007/s002270000488, 2001.

Saad, S. A. and Wade, C. M.: Seasonal and Spatial Variations of Saltmarsh Benthic Foraminiferal Communities from North Norfolk, England, Microb. Ecol., 73(3), 539–555, doi:10.1007/s00248-016-0895-5, 2017.

Snyder, R. A. and Hoch, M. P.: Consequences of protist-stimulated bacterial production for estimating protist growth efficiencies, Hydrobiologia, 341(2), 113–123, doi:10.1007/BF00018115, 1996.

Teugels, B., Bouillon, S., Veuger, B., Middelburg, J. J. and Koedam, N.: Kleptoplasts mediate nitrogen acquisition in the sea slug *Elysia viridis*, Aquat. Biol., 4(1), 15–21, doi:10.3354/ab00092, 2008.

Tillmann, U., Hesse, K.-J. and Colijn, F.: Planktonic primary production in the German Wadden Sea, J. Plankton Res., 22(7), 1253–1276, doi:10.1093/plankt/22.7.1253, 2000.

Valdés, V., Fernandez, C., Molina, V. and Escribano, R.: Nitrogen excretion by copepods and its effect on ammonia-oxidizing communities from a coastal upwelling zone, Limnol. Oceanogr., 63(1), 278–294, doi:10.1002/lno.10629, 2018.

Verity, P. G.: Grazing, respiration, excretion, and growth rates of tintinnids, Limnol. Oceanogr., 30(6), 1268–1282, doi:10.4319/lo.1985.30.6.1268, 1985.

Ward, J. N., Pond, D. W. and Murray, J. W.: Feeding of benthic foraminifera on diatoms and sewage-derived organic matter: an experimental application of lipid biomarker techniques, Mar. Environ. Res., 56(4), 515–530, doi:10.1016/S0141-1136(03)00040-0, 2003.

Wheeler, P. A. and Kirchman, D. L.: Utilization of inorganic and organic nitrogen by bacteria in marine systems1, Limnol. Oceanogr., 31(5), 998–1009, doi:10.4319/lo.1986.31.5.0998, 1986.

Witte, U., Wenzhofer, F., Sommer, S., Boetius, A., Heinz, P., Aberle, N., Sand, M., Cremer, A., Abraham, W. R., Jorgensen, B. B. and Pfannkuche, O.: In situ experimental evidence of the fate of a phytodetritus pulse at the abyssal sea floor, Nature, 424(6950), 763–766, 2003.

Wukovits, J., Enge, A. J., Wanek, W., Watzka, M. and Heinz, P.: Increased temperature causes different carbon and nitrogen processing patterns in two common intertidal foraminifera (*Ammonia tepida* and *Haynesina germanica*), Biogeosciences, 14(11), 2815–2829, doi:10.5194/bg-14-2815-2017, 2017.

Wukovits, J., Bukenberger, P., Enge, A. J., Gerg, M., Wanek, W., Watzka, M. and Heinz, P.: Food supply and size class depending variations in phytodetritus intake in the benthic foraminifer *Ammonia tepida*, Biol. Open, bio.030056, doi:10.1242/bio.030056, 2018.

Zweifel, U., Norrman, B. and Hagstrom, A.: Consumption of Dissolved Organic-Carbon by Marine-Bacteria and Demand for Inorganic Nutrients, Mar. Ecol. Prog. Ser., 101(1–2), 23–32, doi:10.3354/meps101023, 1993.

Table 1. Experimental setup and conditions

	species	Individuals per replicate	Sampling intervals [h]	T [°C]	food source	amount of food added [mgC m^{-2}]	amount of food added [mgN m^{-2}]
Exp. 1	*A. tepida*	50 - 55	24 / fed 24 / starved	15, 20, 25	Diatom	540	100
	H. germanica	50 - 55	24 / fed 24 / starved	15, 20, 25	Diatom	540	100
Exp. 2	*A. tepida*	55	1, 3, 6, 12, 24	20	Chlorophyte	410	71
	A. tepida	55	1, 3, 6, 12, 24	20	Diatom	647	21
	A. tepida	55	1, 3, 6, 12, 24	20	Chlorophyte + Diatom	206 + 324	35 + 10

Biogeosciences Discuss., https://doi.org/10.5194/bg-2018-231
Manuscript under review for journal Biogeosciences
Discussion started: 28 May 2018

Table 2. Michaelis Menten parameters of curves for pC and pN intake in Figure 4 (bold font = data from measured values, regular font = data from calculated values).

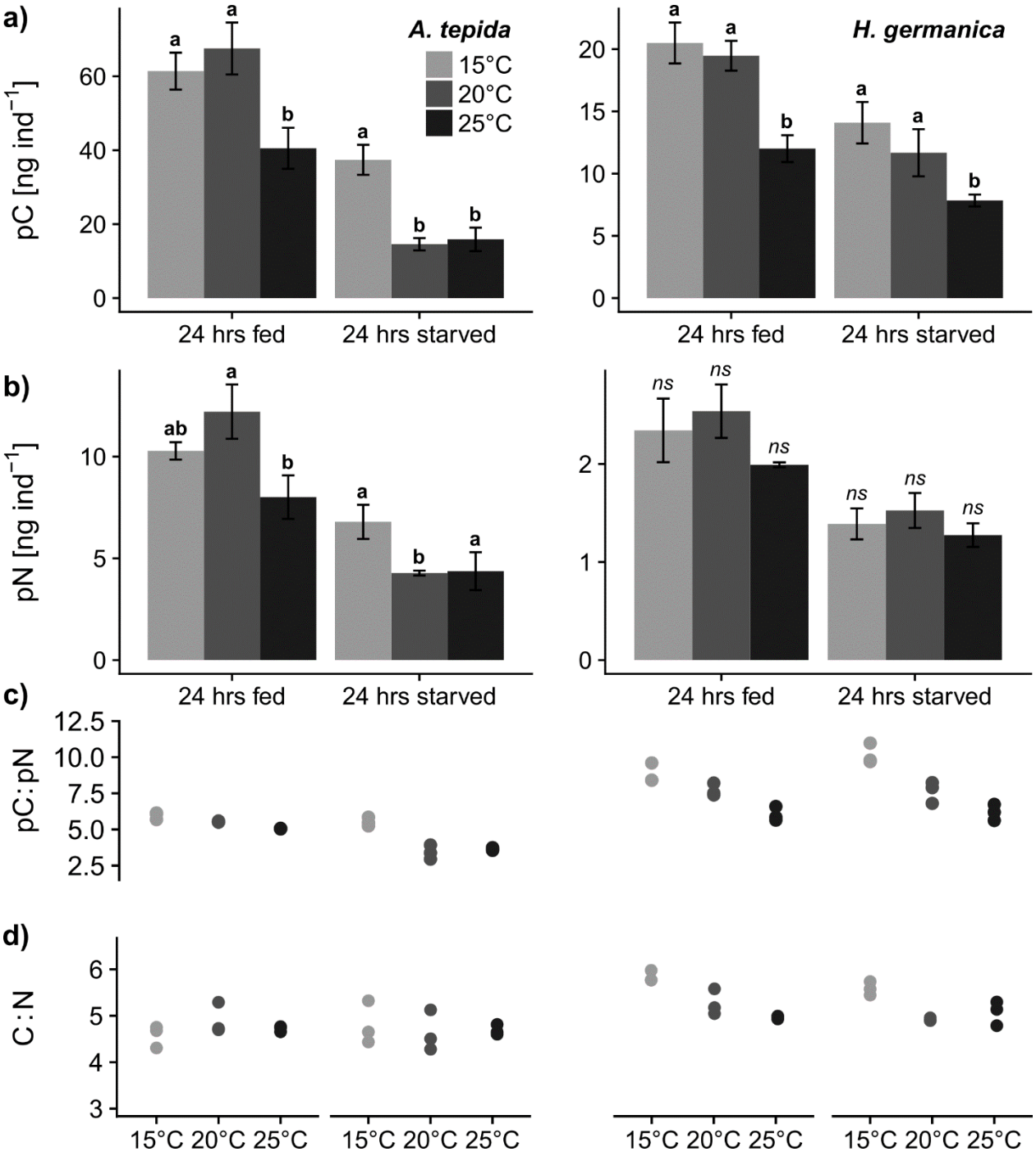
		Vmax	Km	Res. SE	DF
pC	**Chlorophyte mono diet**	**179.875**	**0.611**	**20.745**	**16**
	Chlorophyte mixed diet	**124.196**	**1.359**	**11.918**	**15**
	Diatom mono diet	80.191	1.374	9.290	16
	Diatom mixed diet	24.000	0.098	2.983	16
pN	Chlorophyte mono diet	30.860	0.611	3.559	16
	Chlorophyte mixed diet	21.307	1.359	2.286	12
	Diatom mono diet	**10.912**	**1.374**	**1.264**	**12**
	Diatom mixed diet	**3.267**	**0.100**	**0.410**	**16**

Table 3. Mean abundances of live *A. tepida* and *H. germanica* (0-1 cm sediment depth), TOC, TN, and carbon and nitrogen flux calculated from sediment cores (early May 2015* n = 1, late April 2016 n = 3). Data for 15°C of Experiment 1 were used to estimate carbon and nitrogen fluxes (n.d. = not determinded).

	size fraction [µm]	abundance [ind m^{-2}]	TOC [mg m^{-2}]	TN [mg m^{-2}]	pC_{intake} [mg C m^{-2} d^{-1}]	$pC_{release}$ [mg C m^{-2} d^{-1}]	pN_{intake} [mg N m^{-2} d^{-1}]	$pN_{release}$ [mg N m^{-2} d^{-1}]
A. tepida[1]	125 - 250	1166979	226.516	77.322	20.937	8.375	5.333	1.813
2015	250 - 355	186742	163.428	35.817	11.467	4.480	1.919	0.651
	>355	3773	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
H. germanica	125 - 250	109823 (±54078)	25.717	6.867	n.d.	n.d.	n.d.	n.d.
2016	250 - 355	29342 (±12768)	30.978	5.311	0.601	0.188	0.069	0.028
	>355	3773 (±2741)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
A. tepida	125 - 250	97248 (±2741)	21.317	7.277	1.745	0.698	0.444	0.151
2016	250 - 355	43594 (±11041)	38.152	8.361	1.802	0.704	0.302	0.102
	>355	4401 (±12786)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

[1] Data from Wukovits et al. 2018

Biogeosciences Discuss., https://doi.org/10.5194/bg-2018-231
Manuscript under review for journal Biogeosciences
Discussion started: 28 May 2018



510 **Figure 1 a – d). Comparison of pC and pN from diatom feeding in *A. tepida* and *H. germanica* after a 24 hours feeding period (24 hrs fed) and 24 hours without food (24 hrs starved) at 15°C, 20°C, and 25°C. Letters show significant differences of a) cytoplasmic pC and b) pN between incubation temperatures within the 24 hours feeding period/24 hrs fed and the 24 hours incubation without food/24 hrs starved; $p < 0.05$, pairwise permutation tests). c) pC : pN ratio (n=3, in all cases). d) ratios of foraminiferal cytoplasmic C:N ratios.**

Biogeosciences Discuss., https://doi.org/10.5194/bg-2018-231
Manuscript under review for journal Biogeosciences
Discussion started: 28 May 2018

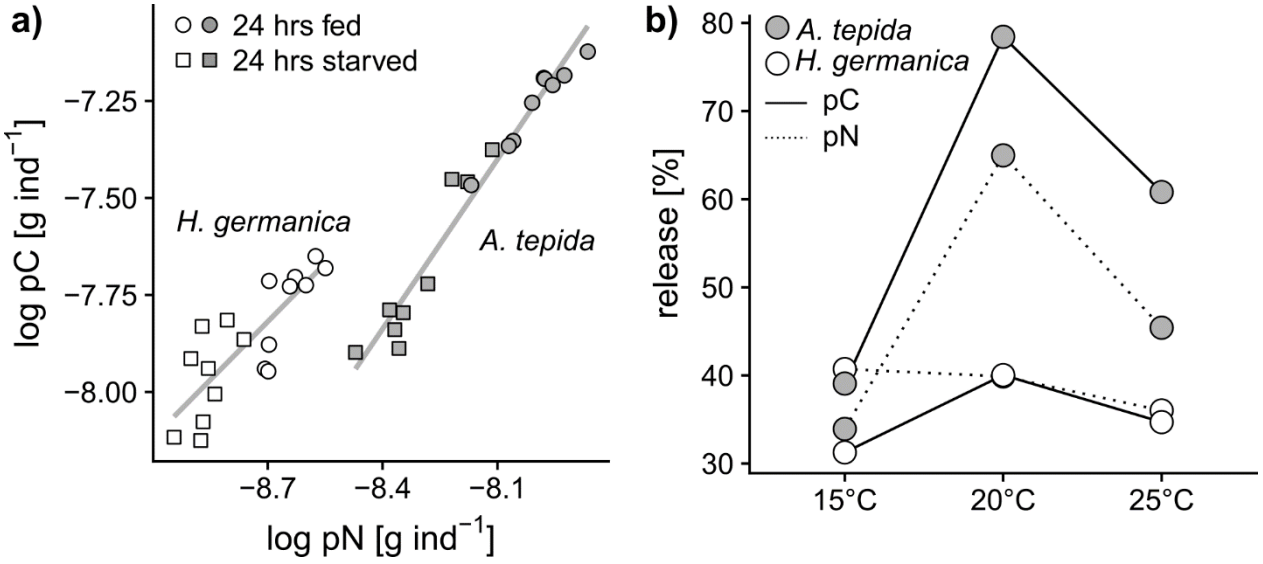


Figure 2 a-b. a) Relationship of pC and pN in *A. tepida* and *H. germanica* (*A. tepida* $R^2 = 0.96$, $p < 0.01$; *H. germanica* $R^2 = 0.64$, $p = 0.011$) and b) phytodetrital carbon and nitrogen turnover as percent release (of total intake of pC or pN per day, respectively).

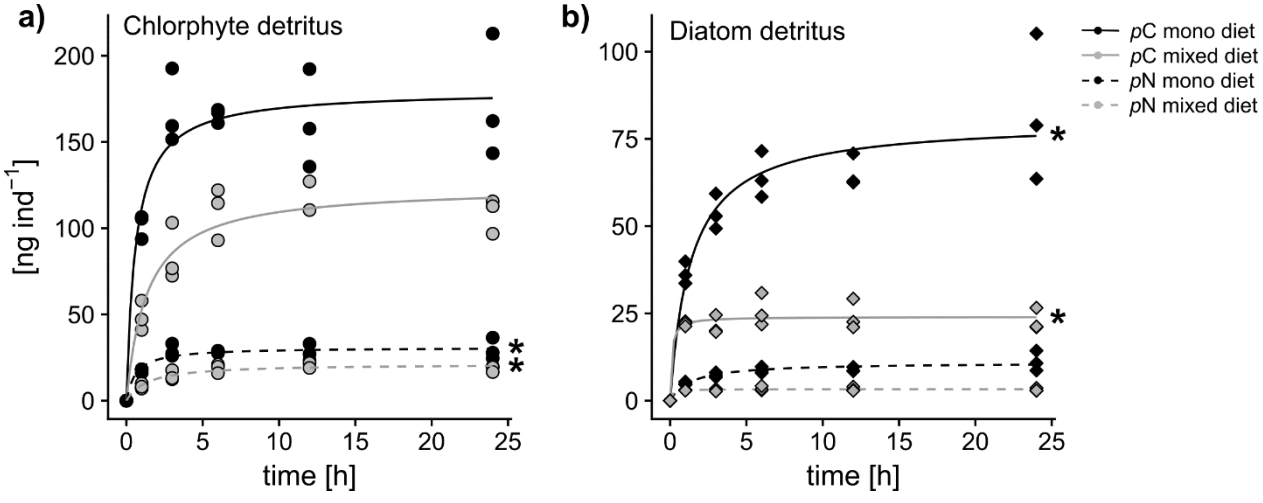


Figure 3 a-b. Comparison of chlorophyte and diatom phytodetritus feeding in *A. tepida* for 24 hours, presenting feeding dynamics for a) chlorophyte detritus and b) diatom detritus. Curves show Michaelis Menten Fits through triplicates for each approach (stars * indicate calculated values for pC or pN).