Biogeosciences, 14, 2815–2829, 2017
https://doi.org/10.5194/bg-14-2815-2017


Biogeosciences
Open Access
EGU

# Increased temperature causes different carbon and nitrogen processing patterns in two common intertidal foraminifera (*Ammonia tepida* and *Haynesina germanica*)

**Julia Wukovits[1], Annekatrin Julie Enge[1], Wolfgang Wanek[2], Margarete Watzka[2], and Petra Heinz[1]**

[1]University of Vienna, Department of Palaeontology, Vienna, Austria
[2]University of Vienna, Department of Microbiology and Ecosystem Science, Terrestrial Ecosystem Research, Vienna, Austria

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

Received: 23 November 2016 – Discussion started: 28 November 2016
Revised: 28 April 2017 – Accepted: 11 May 2017 – Published: 9 June 2017

**Abstract.** Benthic foraminifera are highly abundant heterotrophic protists in marine sediments, but future environmental changes will challenge the tolerance limits of intertidal species. Metabolic rates and physiological processes in foraminifera are strongly dependent on environmental temperatures. Temperature-related stress could therefore impact foraminiferal food source processing efficiency and might result in altered nutrient fluxes through the intertidal food web. In this study, we performed a laboratory feeding experiment on *Ammonia tepida* and *Haynesina germanica*, two dominant foraminiferal species of the German Wadden Sea/Friedrichskoog, to test the effect of temperature on phytodetritus retention. The specimens were fed with $^{13}C$ and $^{15}N$ labelled freeze-dried *Dunaliella tertiolecta* (green algae) at the start of the experiment and were incubated at 20, 25 and 30 °C respectively. Dual labelling was applied to observe potential temperature effects on the relation of phytodetrital carbon and nitrogen retention. Samples were taken over a period of 2 weeks. Foraminiferal cytoplasm was isotopically analysed to investigate differences in carbon and nitrogen uptake derived from the food source. Both species showed a positive response to the provided food source, but carbon uptake rates of *A. tepida* were 10-fold higher compared to those of *H. germanica*. Increased temperatures had a far stronger impact on the carbon uptake of *H. germanica* than on *A. tepida*. A distinct increase in the levels of phytodetrital-derived nitrogen (compared to more steady carbon levels) could be observed over the course of the experiment in both species. The results suggest that higher temperatures have a significant negative effect on the carbon exploitation of *H. germanica*. For *A. tepida*, higher carbon uptake rates and the enhanced tolerance range for higher temperatures could outline an advantage in warmer periods if the main food source consists of chlorophyte phytodetritus. These conditions are likely to impact nutrient fluxes in *A. tepida*/*H. germanica* associations.

## 1 Introduction

The intertidal zone is an extreme environment, exposed to intense seasonal and diurnal fluctuations in temperature, challenging the physiological limits of benthic organisms, for instance foraminifera. Foraminifera are marine heterotrophic protists with a common worldwide occurrence in extant and fossil communities. Future environmental changes are expected to affect coastal foraminiferal communities and assemblage structures (Schafer et al., 1996; Culver and Buzas, 1995) since some intertidal species exhibit a fast response to rapid changes in their environment (e.g. warming). Temperature affects physiological performances, resulting in a lack of fitness, altering community structures and leading to shifts in nutrient fluxes and ecosystem balance (Brown et al., 2004; Allen et al., 2005; Petchey et al., 2010; O'Connor et al., 2009; Yvon-Durocher et al., 2010).

In intertidal mudflats, smaller benthic foraminifera contribute up to 80 % of the protist biomass (Lei et al., 2014) and are an important component of the food web (Lipps and Valentine, 1970; Buzas, 1978; Buzas and Carle, 1979; Nomaki et al., 2008). However, the allocation of their

trophic role still lacks defined considerations. Due to their high abundance and substantial carbon incorporation, it is assumed that they play a major role in the carbon cycle of these environments (Moodley et al., 2000). *Haynesina germanica* and *Ammonia tepida* often co-occur with high abundance in intertidal sediments of the temperate zone. The dominance of these two species shows seasonal fluctuations and can be location specific (Murray, 2014; Alve and Murray, 1994; Debenay et al., 2006; du Chatelet et al., 2009). Factors controlling these community shifts still need to be specified and might include differential sensitivity to general disturbances, differences in food preferences or variations in physiological limits to physical stress. Temperature has been proven to play a major role in reproduction, growth and respiration rates of intertidal foraminifera (Bradshaw, 1955, 1957, 1961; Lee and Muller, 1973; Haynert and Schönfeld, 2014; Cesbron et al., 2016). Elevated temperatures around 35 °C were reported to increase the expression of stress proteins in *A. tepida* (Heinz et al., 2012) and represent the range of minimum respiratory activity, which subsequently drops to the lethal point of 45 °C (Bradshaw, 1961). There is still a lack of data concerning physiological effects of temperature on *H. germanica*, though there is evidence about lower environmental temperatures (12 °C) being most supportive for its reproduction. (Goldstein and Alve, 2011). However, possible restraints or advances of temperature effects on phytodetritus uptake have not been evaluated yet. Foraminiferal food sources include microalgae, phytodetritus or bacteria (Muller and Lee, 1969; Lee et al., 1966; Goldstein and Corliss, 1994), which are prevalent elements of the intertidal particulate organic matter (POM) pool. Distributions of POM sources or microalgae groups are often used to correlate foraminiferal abundance and to relate the availability of different POM sources with population dynamics or food preferences (Alve and Murray, 1994, 2001; du Chatelet et al., 2009; Hayward et al., 1996; Murray and Alve, 2000; Ward et al., 2003; Topping et al., 2006; de Nooijer, 2007; Diz and Francés, 2008; Goineau et al., 2012; Papaspyrou et al., 2013; Melis and Covelli, 2013). Microalgae are proposed to be the preferred food source of *A. tepida*, in particular over bacteria (Pascal et al., 2008b), while *H. germanica* possesses a mechanism to efficiently feed on diatoms (Austin et al., 2005). Foraminifera accumulate their food with a pseudopodial network to ingest and transport food particles to the endoplasm (Goldstein and Corliss, 1994; Bowser et al., 1992), which is often protected by an inorganic (e.g. calcareous) shell. Their ingestion rates of algae or phytodetritus are comparable to bacterial assimilation rates of detrital carbon (Moodley et al., 2000, 2002).

Although abundant data sources exist on food-derived carbon in foraminiferal cytoplasm, information on nitrogen remains scarce. Research on the coupling of food-derived carbon and nitrogen in foraminifera is limited to a few in situ studies in the bathyal of the Arabian Sea, where foraminifera play an important role in benthic carbon fluxes (Enge et al., 2014, 2016). Tidal flats function as both an important source and sink for nutrients (Joye et al., 2009) and represent essential ecological components of the earth's marine systems. The abundant intertidal primary producers like microalgae or diatoms control C and N flows of the sediments (Cook et al., 2004), with foraminifera being an important consumer of their biomass. Regarding the high abundance of foraminifera in benthic communities, significant effects of temperature on their food processing could potentially influence intertidal nutrient fluxes, in particular if they relate to foraminiferal carbon and nitrogen coupling. This study aims to investigate the effects of temperature on the food exploitation and on C and N cycling of *H. germanica* and *A. tepida*. The response to an artificially produced phytodetrital food source (*Dunaliella tertiolecta*, Chlorophyta) was tested under three temperature regimes. *Dunaliella tertiolecta* has been identified as a valuable food source for *A. tepida* (Pascal et al., 2008a), but has not been tested on *H. germanica* so far. This study also tests which species shows a more efficient response to chlorophyte detritus in a mesocosm setting. The laboratory feeding experiment was performed in incubation chambers, with the temperature being adjusted to 20, 25 and 30 °C. To obtain direct estimates of the uptake of carbon and nitrogen, phytodetritus was labelled with stable isotopes ($^{13}C$, $^{15}N$). This method has provided important data on the in situ feeding behaviour of foraminifera in various environments (Moodley et al., 2002; Enge et al., 2016; Moodley et al., 2000; Middelburg et al., 2000; Witte et al., 2003; Nomaki et al., 2005, 2009, 2011; Sweetman et al., 2009; Enge et al., 2011; Jeffreys et al., 2013). The temperatures chosen for this experiment correspond to experimentally determined values that cover optimum or tolerance ranges of physiological processes in intertidal foraminifera (Bradshaw, 1957, 1961; Lee and Muller, 1973). Further, they lie in the range of seasonal and diurnal temperature amplitudes measured on intertidal surface sediments close to the sampling area (Al-Raei et al., 2009). Simulated variations in temperature were assumed to influence the food uptake efficiency of the species due to potential temperature stress. The amount of phytodetrital carbon (pC) and nitrogen (pN) uptake should reveal information about the nutrient processing potential of the two species. Simultaneous detection of both stable isotopes allows us to determine the ratio in which pC relative to pN is retained in bulk foraminiferal cytoplasm over time. This helps to interpret phytodetrital uptake in relation to foraminiferal carbon and nitrogen coupling (Enge et al., 2016; Evrard et al., 2010; Hunter et al., 2012) and temperature influences might show imbalances of these ratios between treatments. Benthic foraminifera are used in the assessment of (palaeo-) environmental data. Studies aiming to develop new foraminiferal proxies (e.g. for organic matter accumulation or physical parameters) generally apply statistical analysis to field surveys. There is, however, a comparably small amount of biological or ecological data available. This study offers an unique data set on the effect of an altered environmental condition (temperature) on food

resource exploitation on the level of the cytoplasmic balance of food-derived carbon and nitrogen. The aim is to support definitions of the role of benthic foraminifera in intertidal carbon and nitrogen fluxes with respect to warming events and changes in POM availability.

## 2 Material and methods

### 2.1 Sampling site and material collection

Surface sediment was taken on 24 April 2014 during low tide in the intertidal mudflat of the German Wadden Sea near Friedrichskoog (Germany). Water temperature and salinity at the sampling site were 19.9 °C and 31 (practical salinity units). Sediment was collected and sieved at the sampling site through 500 and 63 µm meshes to remove larger meiofauna and organic particles. In the laboratory, samples were sieved again to obtain foraminiferal specimen in the size fraction of 125–355 µm. The sediment contained high abundances of *Ammonia tepida* and *Haynesina germanica* individuals, which were picked and collected for further processing. Living individuals were identified under the microscope regarding intact protoplasma and particle accumulation around the aperture (Moodley et al., 2002; Nomaki et al., 2005; Moodley et al., 1997; Nomaki et al., 2006). Care was taken to achieve a homogenous size distribution within specimens in each replicate. Each species was kept separately in crystallising dishes and fed regularly with living *Dunaliella tertiolecta* up to the start of the experiment.

#### 2.1.1 Production of $^{13}C$ and $^{15}N$-enriched phytodetritus

At the start of the experiment, lyophilised powder of *Dunaliella tertiolecta* was used to simulate a phytodetritus pulse. The chlorophyte was grown in f/2 medium (Guillard and Ryther, 1962; Guillard, 1975) enriched with 98 at. % $^{13}C$ ($NaH^{13}CO_3$, Sigma-Aldrich) and 98 at. % $^{15}N$ ($Na^{15}NO_3$, Sigma-Aldrich) to final concentrations of 1.5 mmol $L^{-1}$ $NaH^{13}CO_3$ and 0.44 mmol $L^{-1}$ $Na^{15}NO_3$. Algal cultures were kept within incubators ($T = 20$ °C; dark: light = 16 : 8). After 20 days, they were harvested by centrifugation (800 G; 10 min) and rinsed three times in simplified artificial seawater (ASW, compare preparation in Enge et al., 2011) to remove an unassimilated isotope tracer. The thereby obtained algal slurry was flash frozen with liquid nitrogen and lyophilised at −55 °C and 0.180 mbar for 6 days.

#### 2.1.2 Experimental set-up

Separate time series for *A. tepida* and *H. germanica* were performed at three temperatures (20, 25, 30 °C) in triplicate. Additionally, background samples of untreated specimens were taken to obtain natural abundance values for $^{13}C$ / $^{12}C$ and $^{15}N$ / $^{14}N$ and initial cytoplasmic total organic carbon (C) and nitrogen (N) content. Specimens were transferred into 72 experimental dishes (150 individuals of *A. tepida*/170 individuals of *H. germanica* per dish), each containing 280 mL of modified synthetic seawater without sediment (SSW; Kester et al., 1967; Dickson and Goyet, 1994), adjusted to a pH of 8.10 and a salinity of 32. The dishes were incubated at a light–dark = 12 : 12 h cycle (type ST 2 POL-ECO Aparatura incubation chambers). After 3 days of acclimation, the labelled algal diet was added ($396.36 \pm 45.57$ mg C $m^{-2}$; $40.26 \pm 9.43$ mg N $m^2$). No further feed amendment was provided throughout the experiment. Algal particles were visible as a green layer on the bottom of the experimental vessels by the end of the experiment. Oxygen, salinity and pH were kept constant at optimum levels during incubation. Airtight experimental dishes were sealed and opened after 2 days to avoid hypoxia (measured $O_2$ at sampling days was 5–8 mg $L^{-1}$). Both species were subsampled to obtain data of phytodetritus processing on the 2nd, 4th, 7th and 14th days of the experiment.

#### 2.1.3 Sample preparation

Foraminifera were removed from the experimental dishes and frozen at −20 °C to stop metabolic activities. The foraminifera were cleaned from adhering particles with a hairbrush. Further on, the organisms were carefully washed in simplified ASW. An amount of 50 (*A. tepida*) or 60 (*H. germanica*) individuals met the optimum range of 0.7–1.0 mg cytoplasmic dry weight, necessary for isotope and elemental analysis. Only foraminifera meeting the criteria for live specimens as described above were prepared for analysis. Figure 1 shows relative amounts of cytoplasm conditions and analysed individuals on the respective sampling days. Specimens were transferred to tin capsules, dried at 50 °C, decalcified and dried in a final drying step for 3 days. All glassware used for preparation was combusted at 500 °C for 5 h, picking tools and tin capsules were cleaned in a solution of dichloromethane ($CH_2Cl_2$) and methanol ($CH_4O$) (1 : 1, $v : v$).

After opening the experimental dishes on the second day, water samples (aliquots of 50 mL) were taken from the *H. germanica* series. They were transferred to 50 mL headspace vials. Some drops of $HgCl_2$ were added to stop respiratory activities and biological production of $CO_2$ (Kroopnick et al., 1972). The vials were sealed and stored at 4 °C. To determine $^{13}C$ of the dissolved inorganic carbon (DIC) in the samples, 12 mL vials were flushed with He, filled with 0.5 mL 85 % $H_3PO_4$ and 2 mL of these water samples, sealed airtight and stored to equilibrate for 48 h (Li et al., 2007; Taipale and Sonninen, 2009).

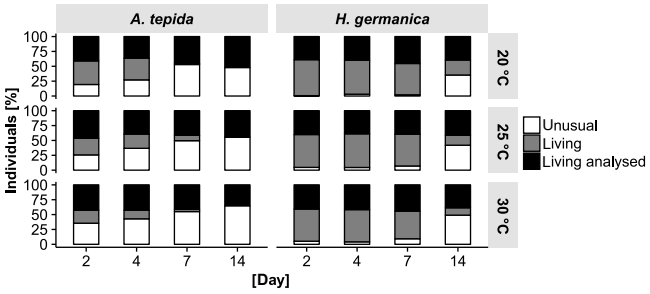


**Figure 1.** Relative amounts of individuals per sampling day and temperature treatment. The terms "unusual" and "intact" refer to the cytoplasmic appearance of the specimen; additionally the fraction of analysed specimen is shown. Unusual cytoplasm includes individuals with patchy distribution of cytoplasm within the test or an unusual coloration. Individuals with intact cytoplasm include former live specimens as described in the text.

### 2.1.4 Sample analyses

Foraminiferal samples and water samples were analysed at the Stable Isotope Laboratory at the University of Vienna for Environmental Research (SILVER). Phytodetrital (dry weight) and foraminiferal content of organic carbon or nitrogen and ratios of $^{13}C\,/\,^{12}C$ and $^{15}N\,/\,^{14}N$ were determined with an isotope ratio mass spectrometer (IRMS; DeltaPLUS, Thermo Finnigan) coupled with an interface (ConFlo III, Thermo Finnigan) to an elemental analyser (EA 1110, CE Instruments). $\delta^{13}C$ of dissolved inorganic carbon (DIC-$\delta^{13}C$) was measured after release as $CO_2$ by $H_3PO_4$ addition in the headspace (headspace gas sampler: GasBench II, Thermo Fisher) of the prepared samples using an IRMS (Delta Advantage V, Thermo Fisher). Atoms of the samples were derived from isotope ratio data and were calculated using the Vienna PeeDee Belemnite standard for C (RVPDB = 0.0112372) and atmospheric nitrogen for N ($R$atmN = 0.0036765), where $X$ is $^{13}C$ or $^{15}N$:

$$\text{at.}\,\% = \frac{100 \times R_{\text{standard}} \times (\frac{\delta X_{\text{sample}}}{1000} + 1)}{1 + R_{\text{standard}} \times (\frac{\delta X_{\text{sample}}}{1000} + 1)}. \quad (1)$$

Net uptake (uptake into the foraminiferal cell, excluding released amounts, hereafter referred to as "uptake") of phytodetrital carbon and nitrogen in foraminiferal cytoplasm was calculated by determining the excess ($E$) of isotope content within the samples against the natural abundance of the isotopes in the foraminiferal cytoplasm (Middelburg et al., 2000):

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

where $X$ is $^{13}C$ or $^{15}N$. Excess and total organic carbon and nitrogen (C, N, biomass normalised, per mg sample weight or per individual) were used to calculate the amount of incorporated isotope $I_{\text{iso}}$ µg mg$^{-1}$ or µg ind$^{-1}$ $= E \times C(N)$ µg mg$^{-1}$ or µg ind$^{-1}$, to obtain the amount of phytodetrital carbon (pC µg mg$^{-1}$ or µg ind$^{-1}$) and nitrogen pN (µg mg$^{-1}$ or µg ind$^{-1}$) within the foraminiferal cytoplasm (Hunter et al., 2012):

$$pX = \frac{I_{\text{iso}}}{\frac{\text{at.}\,\%X_{\text{phyto}}}{100}}. \quad (3)$$

For a better comparison of the variation in uptake dynamics between the two species, the uptake of labelled particles was time normalised to obtain uptake rates of phytodetrital carbon and nitrogen (ng mg$^{-1}$ h$^{-1}$). Exponential decay functions ($y = a(-b \times x)$) were applied with a least squares global curve fitting for carbon uptake rates. The steepness of the decrease $b$ (high $b$ value = fast decrease) indicates whether there is a fast or slow drop in the uptake rates within the related samples over time. An $F$ test was carried out with mean values of treatment curves to compare the nonlinear regression models (exponential decrease) for uptake rates of phytodetrital carbon and evaluate whether a single model can be fitted for the two species at 20 °C. The same procedure was used to prove that uptake rates of phytodetrital carbon vary within species between the three temperature treatments.

Homogeneity of variances was tested using Fligner–Killeen's test which can be applied when population means are not known (Conover et al., 1981) and can overcome some problems with small sample sizes (Wasserstein and Boyer Jr., 1991). Additionally, a graphical exploration of variance homogeneity was carried out by plotting the residuals for pC, pN, TOC and TN (Zuur et al., 2010); for details see Fig. S1 in the Supplement. Welch's $t$ test was used to detect differences of C : N ratios, pC : pN ratios and cytoplasmic C and N content between species. This test is recommended for sample sizes < 10, which are robust against heteroscedasticity within the data (Moser and Stevens, 1992; Ruxton, 2006; McDonald, 2014) and can still be applied in case of unequal variances. To compare within species differences, a two-way ANOVA was carried out with time and temperature as independent factors and pC, pN, C and N as dependent variables. The statistical analysis applied here were meant to emphasise the visualisations (graphical depictions) of the findings of this study. The applied tests were chosen to suit and represent the data in the most appropriate way (to retain robustness with respect to the design of the study). Graphs and data analysis were produced using R (R Development Core Team, 2008) via RStudio (RStudio Team, 2015) and the packages ggplot 2 (Wickham, 2009) nlstools, (Florent Baty et al., 2015) and plyr (Wickham, 2011).

**Table 1.** Delta values for carbon and nitrogen isotopes. Natural $\delta^{13}$C and $\delta^{13}$N values of unlabelled background samples (BG), isotopically enriched samples of food (*D. tertiolecta*) and foraminifera and DIC-$\delta^{13}$C within water samples on the second day of the incubation period (standard deviation in parenthesis; letters denote Tukey Grouping of significant differences in DIC-$\delta^{13}$C between temperatures; n.d. is no data).

| | | $\delta^{13}$C‰ | $\delta^{15}$N‰ | DIC $\delta^{13}$C |
|---|---|---|---|---|
| *D. tertiolecta* | BG | −18.3 | 16.2 | n.d. |
| | labelled | 30267 | 213298 | n.d. |
| *A. tepida* | BG | −13.9 (±0.2) | 13.4 (±0.6) | n.d. |
| | 20 °C | 1742 (±216) | 7277 (±1203) | n.d. |
| | 25 °C | 1269 (±318) | 5758 (±1426) | n.d. |
| | 30 °C | 707 (±122) | 4109 (±320) | n.d. |
| *H. germanica* | BG | −13.7 (±0.7) | 11.7 (±0.4) | n.d. |
| | 20 °C | 248 (±26) | 1087 (±71) | 1845 (±76) a |
| | 25 °C | 118 (±20) | 784 (±86) | 2186 (±60) b |
| | 30 °C | 116 (±5) | 879 (±29) | 2214 (±66) b |

## 3 Results

### 3.1 Carbon processing

Elevated $^{13}$C values of the foraminiferal samples demonstrated a strong response to the labelled food source (Table 1). Temperature had a significant impact on pC levels in *H. germanica* (Table 2 and Fig. 2). An interactive effect of time and temperature caused a different time-related processing of pC at 20 °C compared to 25 and 30 °C (Table 2 and Fig. 2). The content of pC in *H. germanica* at 20 °C was significantly higher than at 25 and 30 °C on each day of data collection ($p < 0.05$; no significant difference in 25 and 30 °C samples). Additionally, values for DIC-$^{13}$C measured in water samples (day 2) at elevated temperatures were significantly higher than those from the 20 °C approach (Table 1). Time-related variations in cytoplasmic C (biomass normalised) in *H. germanica* specimens were also temperature dependent (Table 2). In contrast, there was no relation between time and temperature concerning the pC content of *A. tepida*. Levels of pC in *A. tepida* were significantly increased at 25 °C (25–20 °C: p = 0.015, 25–30 °C: $p < 0.001$, pairwise *t* testing with pooled SDs, since no significance of time effects), revealing a higher optimum grazing temperature for *A. tepida* compared to *H. germanica* (pC, Fig. 2). Temperature had no effect on biomass C in *A. tepida*, while there were time-dependent variations in biomass C (Table 2). These appeared at the start and end of the experiment but there was no significant influence by temperature treatments on the other days. Individual cytoplasmic C content µg ind$^{-1}$ differed between the two species and proportions of individual uptake of pC to individual C content showed similar proportions to biomass-related carbon values (Fig. 3). For detailed information about individual pC, pN, TOC and TN content, see Supplement Table S1.

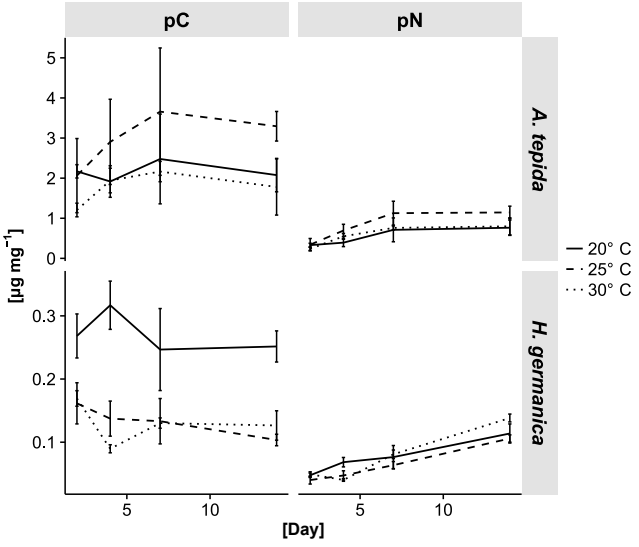


**Figure 2.** Phytodetrital-derived carbon and nitrogen in foraminiferal cytoplasm. Biomass normalised pC and pN of *A. tepida* and *H. germanica* at 20, 25 and 30 °C. Error bars denote standard deviation.

Carbon uptake rates showed an exponential decrease with time for both species (Fig. 4), with a very good fit of curves except for *A. tepida* at 25 °C where a relatively low determination coefficient $R^2$ was observed (Table 3). Mean uptake rates display a highly significant difference between species ($F$ test: $F = 33.74$, $p = 0.029$). *H. germanica* showed a much lower efficiency in the uptake of phytodetritus derived from *D. tertiolecta* compared to *A. tepida*. Uptake rates in *A. tepida* can be pooled within a single function ($F$ test; $F = 7.664$, $p = 0.062$). Temperature causes a strong variation in rates for *H. germanica* ($F$ test; $F = 24.97$, $p = 0.012$). The rate of *A. tepida* showed a slightly faster decrease at 20 °C

**Table 2.** Effects of time and temperature. Results of a two-way ANOVA displaying the main effects of time and temperature on the dependent variables pC, pN, C, N in *A.tepida* and *H. germanica* and their interactions. Significant $p$ values are printed in bold letters.

| | | | Df | SM | $F$ value | $p$ value |
|---|---|---|---|---|---|---|
| *A. tepida* | pC | temperature | 2 | 17.341 | 7.898 | **0.002** |
| | | day | 3 | 5.298 | 2.413 | 0.092 |
| | | temperature × day | 6 | 1.299 | 0.592 | 0.734 |
| | | error | 24 | 2.196 | | |
| | pN | temperature | 2 | 0.728 | 9.284 | **0.001** |
| | | day | 3 | 1.939 | 24.716 | < **0.001** |
| | | temperature × day | 6 | 0.084 | 1.073 | 0.406 |
| | | error | 24 | 0.079 | | |
| | C | temperature | 2 | 81.230 | 2.373 | 0.110 |
| | | day | 4 | 445.060 | 12.998 | < **0.001** |
| | | temperature × day | 8 | 42.650 | 1.246 | 0.308 |
| | | error | 30 | 34.240 | | |
| | N | temperature | 2 | 2.112 | 2.848 | 0.074 |
| | | day | 4 | 4.221 | 5.690 | **0.001** |
| | | temperature × day | 8 | 0.601 | 0.810 | 0.600 |
| | | error | 30 | 0.742 | | |
| *H. germanica* | pC | temperature | 2 | 0.296 | 81.300 | < **0.001** |
| | | day | 3 | 0.010 | 2.654 | 0.071 |
| | | temperature × day | 6 | 0.010 | 2.826 | **0.032** |
| | | error | 24 | 0.004 | | |
| | pN | temperature | 2 | 0.002 | 8.524 | **0.002** |
| | | day | 3 | 0.027 | 133.994 | < **0.001** |
| | | temperature × day | 6 | 0.001 | 5.260 | **0.001** |
| | | error | 24 | < 0.000 | | |
| | C | temperature | 2 | 14.973 | 3.630 | **0.039** |
| | | day | 4 | 40.819 | 9.898 | < **0.001** |
| | | temperature × day | 8 | 17.057 | 9.898 | **0.002** |
| | | error | 30 | 4.124 | | |
| | N | temperature | 2 | 2.240 | 13.722 | < **0.001** |
| | | day | 4 | 2.978 | 18.248 | <**0.001** |
| | | temperature × day | 8 | 0.275 | 1.685 | 0.143 |
| | | error | 30 | 0.163 | | |

**Table 3.** Carbon uptake rates. Regression variables, correlation coefficient $R^2$, standard error of estimate $S$ and decay $b$ the exponential decrease of carbon uptake rates of *H. germanica* and *A. tepida* versus time.

| | | $R^2$ | $S$ | $b$ |
|---|---|---|---|---|
| *A. tepida* | 20 °C | 0.873 | 11.382 | −0.262 (±0.048) |
| | 25 °C | 0.595 | 21.247 | −0.130 (±0.047) |
| | 30 °C | 0.918 | 4.797 | −0.130 (±0.017) |
| | all $T$ | 0.628 | 17.048 | −0.162 (±0.030) |
| *H. germanica* | 20 °C | 0.945 | 0.957 | −0.247 (±0.030) |
| | 25 °C | 0.904 | 0.797 | −0.343 (±0.058) |
| | 30 °C | 0.912 | 0.789 | −0.523 (±0.090) |
| | all $T$ | 0.732 | 1.680 | −0.316 (±0.053) |

relative to the higher temperatures and the highest deviation ($S$) of 21.247 ng mg$^{-1}$ h$^{-1}$ from average uptake rates at 25 °C. In general, *H. germanica* exhibited a faster drop in the uptake rates at higher temperatures (see $b$, Table 3).

### 3.2 Nitrogen processing

The $\delta^{15}$N signatures of all foraminiferal cytoplasm samples showed a strong increase after addition of labelled algae (Table 1). Like in pC, time and temperature showed interactive effects on the pN content in *H. germanica* (Table 2). *Haynesina germanica* showed a temperature-related variation on days 4 and 14. Cytoplasmic N showed less variation with temperature in *A. tepida* than in *H. germanica*. In contrast to the pC values, progressing time caused significant increases in pN content in both species (Fig. 2, pN). N changes in *A. tepida* were time dependent and increased at the start of the 25 and 30 °C approach. Analogous to cytoplasmic C, individ-

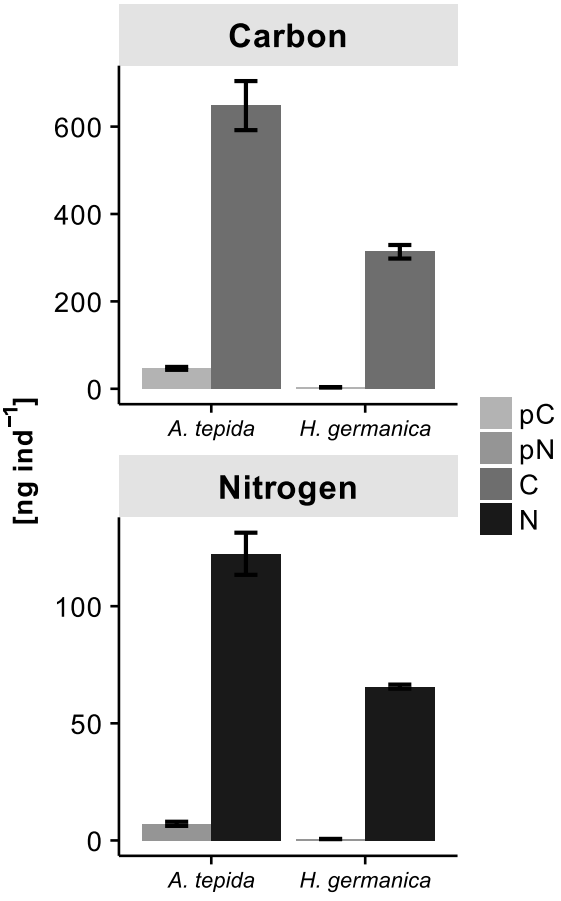


**Figure 3.** Cytoplasmic carbon (C) and nitrogen (N) content and pC or pN uptake per foraminiferal individual. Data represent values for day 2 of the 20 °C approach. Error bars denote standard deviation.

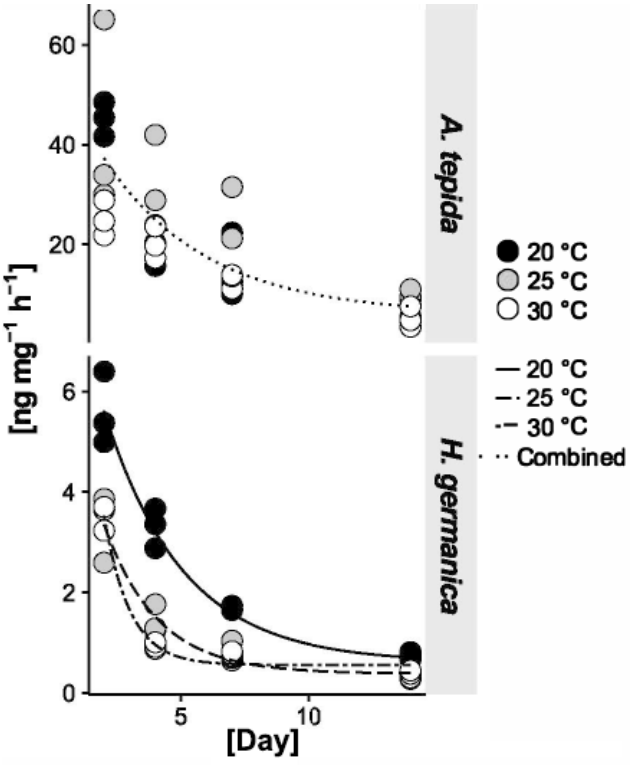


**Figure 4.** Carbon uptake rates. Uptake rates of phytodetrital carbon of *A. tepida* and *H.germanica* at three temperatures. Dots represent the calculated uptake rates derived from isotope data per time, curves show functions of exponential decrease (curves are combined for *A. tepida* due to statistical similarity).

ual N and pN are proportional to total biomass-related values (Fig. 3).

In general, nitrogen uptake rates of *H. germanica* showed a steeper decline over time and at all temperatures in contrast to *A. tepida* (Fig. 5). While *H. germanica* showed the highest uptake rates at 20 °C, uptake rates for *A. tepida* were highest at 25 °C. At 30 °C, uptake rates of phytodetrital nitrogen showed fluctuating patterns for both species. In *Ammonia* samples, rates at 25 °C and 30 °C dropped after the fourth day. The steepest decrease at 20 °C can be found between day 2 and day 4. Uptake rates of nitrogen were about 10-fold higher in *A. tepida* compared to *H. germanica*.

There was a noticeable peak in N uptake on the fourth day at 30 °C in both species. In *A. tepida*, this peak describes an increase of the uptake rate followed by a linear decrease. In contrast, all uptake rates showed a rapid drop in *H. germanica*, especially at the higher temperatures, with a recognisable negative peak at 30 °C on day 4 and a flattening of nitrogen uptake rates at 25 and 30 °C on day 4.

### 3.3 Relationship of cytoplasmic and phytodetrital-derived carbon and nitrogen content

*Haynesina germanica* showed a pN enrichment in contrast to pC loss or relatively stable pC over time (Fig. 2). This enrichment of pN was detectable in both species, but was stronger in *H. germanica* with an almost equal increase at all temperatures by the end of the experiment. In contrast, the pN rise in *A. tepida* was restricted to the first week. Despite the constant enrichment of pN in *H. germanica*, pC levels were lower at higher temperatures. This faster loss of pC at 25 and 30 °C at similar levels of phytodetritus uptake results in lower pC : pN ratios at higher temperatures (Fig. 6). The interactive effect of temperature and time on the food uptake of *H. germanica* caused a decoupling of pC and pN (Table 2 and Fig. 7). This is visualised by a lack of correlation between phytodetrital carbon and nitrogen content in the cytoplasm of *H. germanica* over the course of the experiment (Fig. 7). In contrast, pC and pN were strongly coupled in *A. tepida* across the entire time series (Fig. 7). Cytoplasmic C : N ratios were generally similar in both species, with no significant impact of temperature (Table 4).

**Table 4.** C : N ratios of algae and foraminiferal cytoplasm. Brackets contain standard deviation. Ratios in bold letters denote differences of C : N between *A. tepida* and *H. germanica*. Welch's *t* test, $p < 0.050$.

| | | 20 °C | 25 °C | 30 °C |
|---|---|---|---|---|
| *D. tertiolecta* | | 6.08 | – | – |
| *A. tepida* | 0 days | 4.20 (±0.42) | 4.20 (±0.42) | 4.20 (±0.42) |
| | 2 days | 5.32 (±0.59) | 5.32 (±0.20) | 5.79 (±0.90) |
| | 4 days | 5.02 (±0.21) | 4.97 (±0.07) | **5.70** (±0.36) |
| | 7 days | 5.30 (±0.49) | **5.60** (±0.30) | 4.99 (±0.05) |
| | 14 days | 4.29 (±0.87) | 4.04 (±0.44) | 4.41 (±0.35) |
| *H. germanica* | 0 days | 5.07 (±0.31) | 5.07 (±0.31) | 5.07 (±0.31 ) |
| | 2 days | 4.77 (±0.14) | 4.85 (±0.21) | 4.68 (±0.15) |
| | 4 days | 4.63 (±0.43) | 4.39 (±0.32) | **3.65** (±0.38) |
| | 7 days | 4.57 (±0.42) | **4.34** (±0.17) | 4.67 (±0.36) |
| | 14 days | 5.04 (±0.09) | 4.32 (±0.32) | 4.51 (±0.18) |

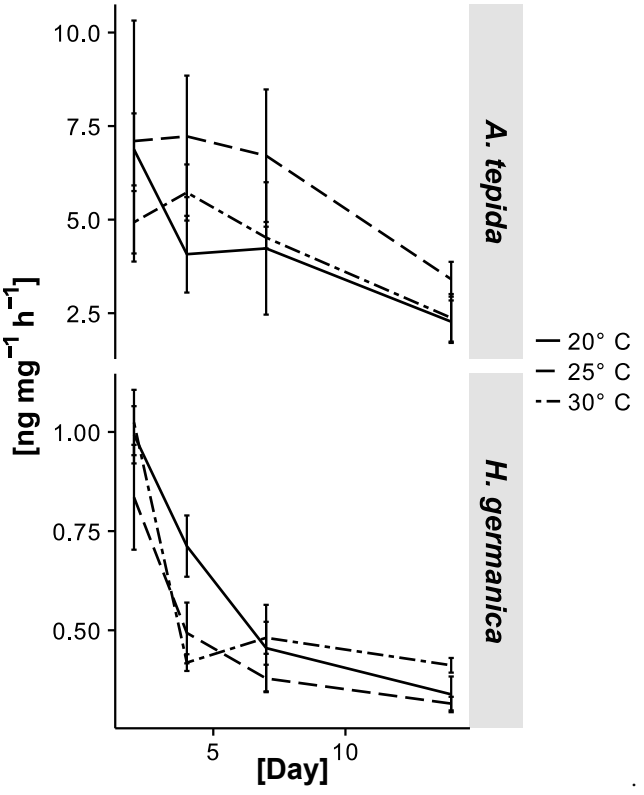


**Figure 5.** Nitrogen uptake rates. Uptake rates of phytodetrital nitrogen by *A. tepida* and *H.germanica* at 20, 25 and 30 °C. Error bars denote standard deviation

## 4 Discussion

### 4.1 Effect of temperature on carbon and nitrogen uptake

The metabolism of *H. germanica* was significantly affected by elevated temperatures. Higher temperatures reduced the amount of pC in the foraminiferal cytoplasm, while a slighter effect on pN indicates a lower impact of temperature on the general uptake of algal phytodetritus. In contrast, pC processing in *A. tepida* was favoured at the intermediate experimental temperature level of 25 °C, demonstrating different optimum phytodetritus processing temperatures in the two species. The loss of pC (Fig. 2) in *H. germanica* in the warmer environment or the faster decline of uptake rates at 25 and 30 °C (Fig. 4 and Table 3) was most likely caused by elevated respiration rates. Accordingly, increased DIC-$^{13}$C content within the SSW at elevated temperatures suggests higher respiratory activities (Table 1). Elevated temperatures have been reported to increase respiration and food consumption in aquatic herbivores, detritivores and foraminifera (Bradshaw, 1961; Carr and Bruno, 2013), to lower grazing rates with simultaneously increased oxygen consumption (Ferreira et al., 2010) or to raise respiration to cover the costs of maintenance of protein metabolism levels (Whiteley and Faulkner, 2005).

In general, DIC-$^{13}$C values in this experiment were very high compared to foraminiferal $^{13}$C. It cannot be excluded that the foraminiferal cultures contained bacteria. Thus, a considerable amount of DIC could originate from phytodetritus remineralization by microbial activity. Several perturbation and long-term experiments on marine bacteria have also reported increased respiration due to warming (Vázouez-Domínguez et al., 2007; Hoppe et al., 2008; Wohlers et al., 2009).

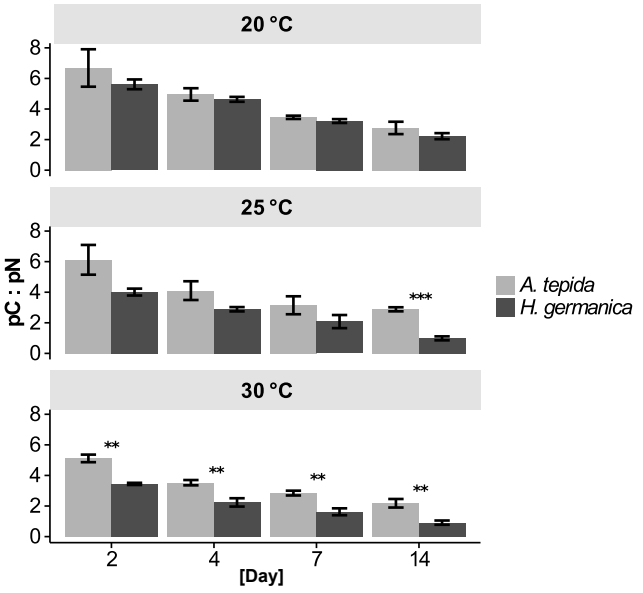


**Figure 6.** Ratios of cytoplasmic pC : pN ratios. Biomass normalised pC : pN within foraminiferal cytoplasm. Error bars denote standard deviation, stars indicate significant differences of pC : pN ratios between the two species on the respective days (Welch's $t$ test; $*p < 0.050$, $**p < 0.010$; $***p < 0.001$).

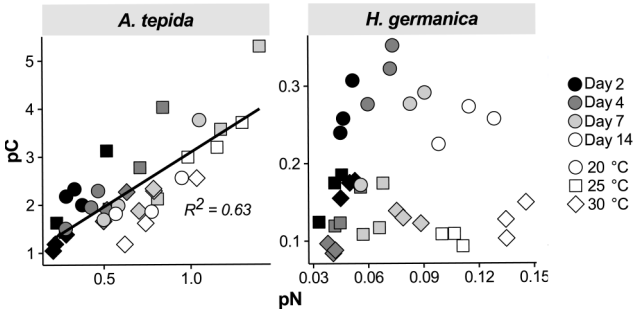


**Figure 7.** Carbon and nitrogen coupling. Phytodetrital-derived nitrogen (pN) vs. carbon (pC) of *A. tepida* and *H. germanica* at 20 °C (circles), 25 °C (squares) and 30 °C with linear regression for *A. tepida*.

However, the higher temperatures trigger noticeable stress in *H. germanica*, which impacts food uptake efficiency. The interactive time/temperature effect supports this argument: the higher temperatures caused convergence of pC and pN content compared to the 20 °C results, particularly up to day 7. This indicates an offset of nutritional ingestion performance in the two warmer approaches. It further suggests a general preference of lower environmental temperatures. Evidently, lower temperatures are more supportive when triggering reproduction in *H. germanica* (Goldstein and Alve, 2011). Interestingly, there is no significant temperature effect on pC between 25 and 30 °C. This implies a critical threshold for this species between 20 and 25 °C. Above this level, the mineralisation of carbon increases, as the DIC-$^{13}$C rises at 25 and 30 °C as a result of elevated respiratory activity. The decoupling of pC and pN in *H. germanica* over time supports this observation.

In contrast, *A. tepida* showed a trend for optimum uptake of carbon at 25 °C and the steadiest carbon uptake rates at 30 °C, while the pC and pN values showed similar patterns (Figs. 2 and 4). Laboratory experiments on *A. tepida* specimens collected at the Brouage mudflat (France) revealed an optimum temperature of grazing on bacteria at 30 °C (Pascal et al., 2008b). This indicates that strains of *A. tepida* feature adaptations to the higher average sea surface temperatures of the Brouage mudflat. In other laboratory experiments (Bradshaw, 1957, 1961) warm temperatures between 25 and 30 °C offered optimum conditions for reproduction, resulted in higher growth and reproductive rates and a decrease in generation time in laboratory experiments with *A. tepida* specimen. Accordingly, higher temperatures are likely to offer competitive advantages for *A. tepida* over *H. germanica* in terms of food uptake. Considering the high expression of stress proteins at 35 °C in *A. tepida* (Heinz et al., 2012) in relation to the results presented here, a critical temperature limit affecting physiologic performances is likely to be found between 30 and 35 °C in this species.

Interestingly, the higher temperature treatments caused an increase of biomass C and N in *H. germanica* samples on the second sampling day, followed by a quick decrease on the fourth day. A similar trend was observed in *A. tepida*. This effect is difficult to interpret and more detailed information about initial food uptake between day 0 and day 2 would be necessary. In summary, continued food C and N uptake appears to be a key factor in the strategies of *A. tepida*, according to the strong response to phytodetritus and the low effect of temperature on its uptake. Simultaneously, a high viability of the specimen in culture implies specific environmental adaptations and a strong response and sensitivity to disturbances caused by the transfer to the laboratory (Fig. 2). In contrast, *H. germanica* showed a comparably high robustness and overall vitality, but there was clearly a strong impact of temperature on mechanisms involved in carbon processing. This implies investment of physiological resources for survival through the cost of increased metabolic activity.

### 4.2 Relationships between carbon and nitrogen uptake and cytoplasmic C : N ratios

In both species, the relation of pC to pN (pC : pN ratio) within foraminiferal cytoplasm decreased compared to their phytodetrital C : N ratio of $\sim 10$ (Table 4) as time progressed. In theory, a comparably high ratio of detrital-derived pC : pN would approach or undercut somatic consumer C : N ratio to retain homeostasis or ensure appropriate nutrition (Cross et al., 2005; Frost et al., 2005; Sterner and Elser, 2002). The growing content of pN within foraminiferal cytoplasm over

time with a remarkable increase of $^{15}$N values (Table 1 and Fig. 2) could be explained by the integration of a high amount of food-derived nitrogen into amino acids and metabolic consumption of pC. Benthic foraminifera inhabiting Antarctic sediments have been reported to use a large amount of algae-derived carbon sources to synthesise nitrogen-rich proteins in relation to a lower generation of other products with a lower nitrogen content (Rivkin and DeLaca, 1990). Such effects could be responsible for the relatively slight decrease in nitrogen uptake rates over time compared to the strong decrease in carbon uptake rates (Figs. 4 and 5). A strong decline of continued food uptake or degradation-induced change of phytodetrital C : N could likewise be responsible for this effect.

Both tested species showed similar demands for food-derived carbon and nitrogen, since at moderate temperatures (20 °C) pC : pN ratios showed a similar trend over time (Fig. 6). Again, minor temperature influences were demonstrated within the overall linear relationship of pC to pN in *A. tepida* across the samples (Fig. 7). In contrast, the rising variation and highly significant difference in pC : pN (lower ratios at higher temperatures, Fig. 6), resulting in the diffuse relationship of pC : pN (Fig. 7) in *H. germanica*, reflect the thermal stress on the level of increased carbon expenditure at steady feeding progress (barely influenced pN levels between treatments). Therefore, sediments with a high dominance of *H. germanica* like river inlets or estuaries (Debenay et al., 2006; Murray and Alve, 2000) could possibly experience additional temperature induced nutrient flux variations, boosting C losses while triggering N retention.

An in situ experiment, monitoring population shifts of near-shore benthic foraminifera to artificially heated sediments, proposed migrations to deeper (colder) regions as a response to elevated environmental temperatures (Schafer et al., 1996). Marine species are known to migrate depending on temperature changes (Parmesan, 2006). To maintain an optimum energy budget, expenses of metabolic maintenance could therefore be compensated by a shift of *H. germanica* populations towards the subtidal zone. Foraminiferal C : N ratios are known from bathyal foraminifera of the Sagami Bay and from oxygen minimum zones of the Arabian Sea, where they range from 2.6 to 6.4 (Nomaki et al., 2008, 2011; Enge et al., 2016). In our study, both species showed similar C : N ratios and natural $^{13}$C and $^{15}$N signatures (Tables 1 and 4), indicating equivalent trophic levels and similar nutritional demands. During the course of the experiment, the temperature stress in *H. germanica* was even noticeable on the level of cytoplasmic stoichiometry. The remarkable drop of the C : N ratio on the fourth day at 30 °C (Table 4) and the subsequent recovery and rapid increase of pC and pN after the fourth day (Fig. 2) could denote a metabolic adaptation process. However, the strong temperature influence on carbon uptake persisted.

In *A. tepida*, the occasionally significant (at 25 °C) fluctuations in the C : N ratio can be related to the high uptake of phytodetritus containing high C : N ratios and it is therefore an influence of food source C : N on the grazer C : N (Fig. 3 and Table 4). Generally, considerations of C : N ratios and intracellular pC / pN coupling also reflect the lower effects of high temperature exposure on *A. tepida* and the high impact on the feeding behaviour of *H. germanica*. These observations reflect the different strategies of the two coexisting intertidal foraminiferal species on the level of nutrient balance. It further implies shifts in population distributions of *A. tepida* and *H. germanica* at events of persisting food source and/or temperature changes. This study verifies a strong involvement of foraminifera in the turnover of intertidal POM. Depending on the POM source or dominant foraminiferal species, this turnover can be strongly influenced by increased temperature, causing a decoupling of carbon and nitrogen cycling. However, when relating these findings to natural environments, considerations of cascading effects of temperature change, e.g. switches in trophic conditions and related shifts in nutrient coupling of primary producers, have to be included.

### 4.3 Species-specific food uptake

The very high uptake rates and levels of phytodetrital carbon and nitrogen in *Ammonia tepida* (day 2 $\sim$ 14% pC : C; $\sim$ 10 % pN : N, comp. Figs. 3 and 4) indicate a strong response to the food source, in contrast to *H. germanica* which showed a clear, but much lower uptake (day 2: $\sim$ 2% pC : C; $\sim$ 1.5 % pN : N). The observed decline of carbon uptake rates over time in both species suggests the highest uptake within the first 2 days of the experiment and indicates a fast processing of the phytodetritus source. During an in situ experiment, Moodley et al. (2000) also observed a rapid response of *Ammonia* sp. to green algae (*Chlorella* sp.) within hours, together with the much weaker ingestion of such a food source by *Haynesina* sp.

The quick slowdown of food uptake rates could indicate levels of saturation especially after a strong response following the food pulse. In addition, a cellular release of the label is likely to exceed uptake as time progresses. Other reasons for the decrease in food uptake with increasing time (reflected in decreasing uptake rates of C and N as well as in pC and pN) could be the stage of phytodetrital decay or decreasing food availability on the subsequent sampling days. Aging or phytodetrital degeneration and its change in quality in marine benthic environments is a result of bacterial colonisation and subsequent transformation and mineralisation of the algal material (Middelburg et al., 2000; Moodley et al., 2002; Buhring et al., 2006; Gihring et al., 2009). Through bacterial mineralisation, the degrading detritus decreases in quality with an increase in C : nutrient ratios. However, microorganisms colonising patches of degrading detritus are likely to be incorporated simultaneously with foraminiferal phytodetritus grazing. Therefore, a fraction of the isotope label within the foraminiferal cytoplasm could be a result of

indirect label intake with phytodetritus-associated microorganisms, especially towards the end of the experiment. However, a visible layer of phytodetritus particles persisted until the end of the experiment. The influence of food availability on feeding, where high food concentrations support increased feeding rates, have been reported for foraminifera and also in other organismic groups such as macrofauna or bacteria (Pascal et al., 2008b, a; Quijón et al., 2008; Mayor et al., 2012). Fast incorporation of food pulses seems to be necessary to cover the high energy demand for reproduction and growth, since food concentrations (*Dunaliella*) sp. of less than 112 cells $mm^2$ (0.40 µg C $cm^{-2}$; Heinz et al. (2002)) do not permit growth or reproduction in *A. tepida*, while growth rate and reproductive activity increase with additional food (Bradshaw, 1955, 1957). Lee et al. (1966) stated that *Ammonia beccarii* specimen preferred to feed on "new" (Fig. 1. 10 days old) cultures of living *Chlorococcum* sp. over "old" (up to 40 days old) cultures. Following these observations, food limitation resulting from the aging of the phytodetrital food source could be present in this study. This proceeding degradation of phytodetritus appears to be particularly problematic to *A. tepida* specimen, which showed an increasing fraction of dead individuals with time (Fig. 1). On the other hand, the health condition of the specimens could be a response to the conditions of the laboratory incubation. Compared to a previous feeding experiment by Linshy et al. (2014) with *A. tepida* fed with *D. tertiolecta* phytodetritus (614 mg C $m^{-2}$), uptake rates of carbon of 1899 pg $ind^{-1}$ $h^{-1}$ were much higher in this study after 48 h than in the other feeding experiment (149 pg $ind^{-1}$ $h^{-1}$). This might be related to general differences in feeding of the tested strains.

The low affinity of *H. germanica* to *D. tertiolecta* phytodetritus could be explained by a generally lower carbon demand or, more likely, a specialisation on other food sources, for instance diatoms or secondary products of microphytobenthic biofilms (e.g. extracellular polymeric substances). The latter assumption corresponds to the preference of a diatom diet over sewage-derived POM (Ward et al., 2003), the presence of a diatom cracking mechanism (Austin et al., 2005), the sequestration of chloroplasts derived exclusively from diatoms (Knight and Mantoura, 1985; Pillet et al., 2011; Cevasco et al., 2015) or the correlation of the distribution of *H. germanica* populations with a high abundance of *Nitzschia* sp. (Hohenegger et al., 1989). This coherence with food availability or organic matter accumulation respectively, was used to explain population distributions of a *H. germanica*/*A. beccarii* assemblages in muddy sediments of Spain (Papaspyrou et al., 2013; Diz et al., 2012). The different patterns of pC accumulation over time in the two species also reflect a different feeding behaviour. The onset of declining health conditions in *H. germanica* specimen at the end of the experiment (Fig. 1) was likely caused by starvation due to the absence of an appropriate food source. In general, the quality of organic carbon or foraminiferal food sources oscillates throughout the year and includes allochthonous detritus (Heip et al., 1995), while the main source of primary production in intertidal environments are microphytobenthic diatoms, accompanied by chlorophytes or other autotrophic microorganism, which seasonally suppress diatom dominance (Scholz and Liebezeit, 2012). In this experiment, *D. tertiolecta* was used as a food source, because this species is easy to maintain in culture, was previously used in several feeding experiments with benthic foraminifera and serves as a representative for allochthonous detrital carbon sources. Ratios of *A. tepida* and *H. germanica* abundances are considered to be related to organic matter quality or environmental variability due to their different feeding specialisations or environmental adaptations (Scholz and Liebezeit, 2012). High amounts of pC (max. 30 % pC to C) in *A. tepida* compared to *H. germanica* (max. 2.6 % pC to C), together with the low influence of temperature on the feeding behaviour of *A. tepida*, prove an opportunistic feeding behaviour and generalist temperature adaptations in *A. tepida*. These findings will help to interpret oscillations in the abundances of these two common intertidal foraminiferal species in relation to organic carbon quality and ambient temperatures. These data report varying strategies in phytodetritus feeding by means of source exploitation and time dependence. However, laboratory studies do not always reflect natural behaviour accurately and might be biased regarding the suppression or alteration of natural behaviours in the investigated organisms. This work is meant to help solve ecological problems and the patterns presented here of foraminiferal feeding behaviour can be useful for the careful interpretation or explanation of successions in species dominance by means of food source availability, and it complements data from field surveys.

## 5 Conclusions

According to the results of this study, *H. germanica* exhibited a more vulnerable carbon retention behaviour to variations in temperature and a relatively low affinity to *D. tertiolecta* phytodetritus, suggesting other food preferences. *Ammonia tepida* showed a broader tolerance range of temperature concerning carbon and nitrogen uptake and a highly effective food exploitation. The different responses of the two species to the applied treatments implies different strategies and environmental adaptations. Consequently, a temperature-related shift in abundances of the two species could alter carbon and nitrogen fluxes in intertidal sediments, with respect to high chlorophyte detritus uptake of *A. tepida* (*A. tepida*: pC max. 90 ng $ind^{-1}$, pN max. 20 ng $ind^{-1}$ *H. germanica*: pC max. 5 ng $ind^{-1}$, pN max. 2 ng $ind^{-1}$) and temperature sensitivity of *H. germanica*. In contrast to food-derived carbon, the increasing accumulation of nitrogen was barely affected by temperature in either species. This raises the hypothesis that rapid temperature increases in *H. germanica*-dominated sediments could cause a shift in organic carbon and nitrogen cycling towards enhanced nitrogen retention and carbon

losses. A general enrichment in cytoplasmic nitrogen in both species prove that nitrogen has a higher potential to be retained within the foraminiferal consumer. Further, increasing time had a prolonged negative influence on the food uptake rates from a single food pulse. A reduced availability of fresh phytodetritus sources is likely to cause such effects and appeared to be particularly limiting for *A. tepida*.

*Data availability.* Datasets of EA/IRMS and GC/IRMS for this study are provided in the Supplement.

**The Supplement related to this article is available online at https://doi.org/10.5194/bg-14-2815-2017-supplement.**

*Author contributions.* A. J. Enge, P. Heinz and J. Wukovits planned the study. A. J. Enge and J. Wukovits conducted the fieldwork and processed the samples in the lab. M. Watzka performed elemental and isotope analysis and adapted the method for the foraminiferal samples. J. Wukovits performed data analysis and wrote the manuscript. A. J. Enge, P. Heinz and W. Wanek contributed by discussing results and critically revising the manuscript drafts.

*Competing interests.* The authors declare that they have no conflict of interest.

*Acknowledgements.* Gerhard Schmiedl (Universität Hamburg) thankfully provided equipment for field sampling. Yvonne Milker (Universität Leipzig) and Katharina Müller-Navarrra (Universität Hamburg) supported us at the sampling location. Patrick Bukenberger helped with lab work and microscoping at the University of Vienna. This article was supported by the Open Access Publishing Fund of the University of Vienna.

Edited by: H. Kitazato
Reviewed by: W. R. Hunter and T. Toyofuku

## References

Al-Raei, A. M., Bosselmann, K., Böttcher, M. E., Hespenheide, B., and Tauber, F.: Seasonal dynamics of microbial sulfate reduction in temperate intertidal surface sediments: controls by temperature and organic matter, Ocean Dynam., 59, 351–370, 2009.

Allen, A., Gillooly, J., and Brown, J.: Linking the global carbon cycle to individual metabolism, Funct. Ecol., 19, 202–213, 2005.

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

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

Austin, H. A., Austin, W. E., 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, 68–73, 2005.

Bowser, S. S., Alexander, S. P., Stockton, W. L., and Delaca, T. E.: Extracellular matrix augments mechanical properties of pseudopodia in the carnivorous foraminiferan Astrammina rara: role in prey capture, J. Protozool., 39, 724–732, 1992.

Bradshaw, J. S.: Preliminary laboratory experiments on ecology of foraminiferal populations, Micropaleontology, 1, 351–358, 1955.

Bradshaw, J. S.: Laboratory Studies on the Rate of Growth of the Foraminifer, Streblus beccarii (Linné) var. tepida (Cushman), J. Paleontol., 39, 1138–1147, 1957.

Bradshaw, J. S.: Laboratory experiments on the ecology of foraminifera, Contributions from the Cushman Foundation for foraminiferal research, 7, 87–106, 1961.

Brown, J. H., Gillooly, J. F., Allen, A. P., Savage, V. M., and West, G. B.: Toward a metabolic theory of ecology, Ecology, 85, 1771–1789, 2004.

Buhring, S., Lampadariou, N., Moodley, L., Tselepides, A., and Witte, U.: Benthic microbial and whole-community responses to different amounts of 1 3C-enriched algae: In situ experiments in the deep Cretan Sea (Eastern Mediterranean), Limnol. Oceanogr., 51, 157–165, https://doi.org/10.4319/lo.2006.51.1.0157, 2006.

Buzas, M. A.: Foraminifera as prey for benthic deposit feeders: results of predator exclusion experiments, J. Mar. Res., 36, 617–625, 1978.

Buzas, M. A. and Carle, K. J.: Predators of foraminifera in the Indian River, Florida, J. Foramin. Res., 9, 336–340, 1979.

Carr, L. A. and Bruno, J. F.: Warming increases the top-down effects and metabolism of a subtidal herbivore, PeerJ, 1, e109, https://doi.org/10.7717/peerj.109, 2013.

Cesbron, F., Geslin, E., Jorissen, F., Delgard, M., 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 S., 179, 23–28, https://doi.org/10.1016/j.ecss.2015.12.005, 2016.

Cevasco, M. E., Lechliter, S. M., Mosier, A. E., and Perez, J.: Initial Observations of Kleptoplasty in the Foraminifera of Coastal South Carolina, Southeast. Nat., 14, 361–372, 2015.

Conover, W. J., Johnson, M. E., and Johnson, M. M.: A comparative study of tests for homogeneity of variances, with applications to the outer continental shelf bidding data, Technometrics, 23, 351–361, 1981.

Cook, P. L. M., Revill, A. T., Clementson, L. A., and Volkman, J. K.: Carbon and nitrogen cycling on intertidal mudflats of a temperate Australian estuary. III. Sources of organic matter, Mar. Ecol.-Prog. Ser., 280, 55–72, https://doi.org/10.3354/meps280055, 2004.

Cross, W. F., Benstead, J. P., Frost, P. C., and Thomas, S. A.: Ecological stoichiometry in freshwater benthic systems: recent progress and perspectives, Freshwater Biol., 50, 1895–1912, 2005.

Culver, S. and Buzas, M.: The effects of anthropogenic habitat disturbance, habitat destruction, and global warming on shallow marine benthic foraminifera, The Journal of Foraminiferal Research, 25, 204–211, 1995.

de Nooijer, L. J.: Shallow water benthic foraminifera as proxy for natural versus human-induced environmental change, vol. 272, Utrecht University, 2007.

Debenay, J.-P., Bicchi, E., Goubert, E., and du Châtelet, E. A.: Spatio-temporal distribution of benthic foraminifera in relation to estuarine dynamics (Vie estuary, Vendée, W France), Estuar. Coast. Shelf S., 67, 181–197, 2006.

Dickson, A. G. and Goyet, C.: Handbook of methods for the analysis of the various parameters of the carbon dioxide system in sea water, publisher not identified, 1994.

Diz, P. and Francés, G.: Distribution of live benthic foraminifera in the Ría de Vigo (NW Spain), Marine Micropaleontology, 66, 165–191, 2008.

Diz, P., Barras, C., Geslin, E., Reichart, G.-J., Metzger, E., Jorissen, F., and Bijma, J.: Incorporation of Mg and Sr and oxygen and carbon stable isotope fractionation in cultured Ammonia tepida, Mar. Micropaleontol., 92, 16–28, 2012.

du Chatelet, E. A., Degre, D., Sauriau, P.-G., and Debenay, J.-P.: Distribution of living benthic foraminifera in relation with environmental variables within the Aiguillon cove (Atlantic coast, France): improving knowledge for paleoecological interpretation, Bulletin de la Société Géologique de France, 180, 131–144, 2009.

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, https://doi.org/10.5194/bg-11-2017-2014, 2014.

Enge, A. J., Nomaki, H., Ogawa, N. O., Witte, U., Moeseneder, M. M., Lavik, G., Ohkouchi, N., Kitazato, H., Kučera, M., and Heinz, P.: Response of the benthic foraminiferal community to a simulated short-term phytodetritus pulse in the abyssal North Pacific, Mar. Ecol.-Prog. Ser., 438, 129–142, 2011.

Enge, A. J., Wukovits, J., Wanek, W., Watzka, M., Witte, U. F., 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, Front. Microbiol., 7, https://doi.org/10.3389/fmicb.2016.00071, 2016.

Evrard, V., Soetaert, K., Heip, C. H., Huettel, M., Xenopoulos, M. A., and Middelburg, J. J.: Carbon and nitrogen flows through the benthic food web of a photic subtidal sandy sediment, Mar. Ecol.-Prog. Ser., 416, 1–16, 2010.

Ferreira, V., Goncalves, A. L., Godbold, D. L., and Canhoto, C.: Effect of increased atmospheric $CO_2$ on the performance of an aquatic detritivore through changes in water temperature and litter quality, Glob. Change Biol., 16, 3284–3296, 2010.

Florent Baty, Christian Ritz, Sandrine Charles, Martin Brutsche, Jean-Pierre Flandrois, and Marie-Laure Delignette-Muller: A Toolbox for Nonlinear Regression in R: The Package nlstools, J. Stat. Softw., 66, 1–21, 2015.

Frost, P. C., Evans-White, M. A., Finkel, Z. V., Jensen, T. C., and Matzek, V.: Are you what you eat? Physiological constraints on organismal stoichiometry in an elementally imbalanced world, Oikos, 109, 18–28, 2005.

Gihring, T. M., Humphrys, M., Mills, H. J., Huette, M., and Kostka, J. E.: Identification of phytodetritus-degrading microbial communities in sublittoral Gulf of Mexico sands, Limnol. Oceanogr., 54, 1073–1083, 2009.

Goineau, A., Fontanier, C., Jorissen, F., Buscail, R., Kerhervé, P., Cathalot, C., Pruski, A. M., Lantoine, F., Bourgeois, S., Metzger, E., Legrand, E., and Rabouille, C.: Temporal variability of live (stained) benthic foraminiferal faunas in a river-dominated shelf – Faunal response to rapid changes of the river influence (Rhône prodelta, NW Mediterranean), Biogeosciences, 9, 1367–1388, https://doi.org/10.5194/bg-9-1367-2012, 2012.

Goldstein, S. T. and Alve, E.: Experimental assembly of foraminiferal communities from coastal propagule banks, Mar. Ecol.-Prog. Ser., 437, 1–11, 2011.

Goldstein, S. T. and Corliss, B. H.: Deposit feeding in selected deep-sea and shallow-water benthic foraminifera, Deep Sea Res. Pt. I, 41, 229–241, 1994.

Guillard, R. R.: Culture of phytoplankton for feeding marine invertebrates, in: Culture of marine invertebrate animals, Springer, 29–60, 1975.

Guillard, R. R. and Ryther, J. H.: Studies of marine planktonic diatoms: I. Cyclotella Nana Hustedt, and Detonula Confervacea (CLEVE) Gran, Can. J. Microbiol., 8, 229–239, 1962.

Haynert, K. and Schönfeld, J.: Impact of changing carbonate chemistry, temperature, and salinity on growth and test degradation of the benthic foraminifer Ammonia aomoriensis, J. Foramin. Res., 44, 76–89, 2014.

Hayward, B. W., Grenfell, H., Cairns, G., and Smith, A.: Environmental controls on benthic foraminiferal and thecamoebian associations in a New Zealand tidal inlet, J. Foramin. Res., 26, 150–171, 1996.

Heinz, P., Hemleben, C., and Kitazato, H.: Time-response of cultured deep-sea benthic foraminifera to different algal diets, Deep Sea Res. Pt. I, 49, 517–537, 2002.

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

Heip, C., Goosen, N., Herman, P., Kromkamp, J., Middelburg, J., and Soetaert, K.: Production and consumption of biological particles in temperate tidal estuaries, Oceanography and Marine Biology: an annual review, 33, 1–149, 1995.

Hohenegger, J., Piller, W., and Baal, C.: Reasons for spatial microdistributions of foraminifers in an intertidal pool (northern Adriatic Sea), Mar. Ecol., 10, 43–78, 1989.

Hoppe, H.-G., Breithaupt, P., Walther, K., Koppe, R., Bleck, S., Sommer, U., and Jürgens, K.: Climate warming in winter affects the coupling between phytoplankton and bacteria during the spring bloom: a mesocosm study, Aquat. Microb. Ecol., 51, 105–115, 2008.

Hunter, W. R., Levin, L. A., Kitazato, H., and Witte, U.: Macrobenthic assemblage structure and organismal stoichiometry control faunal processing of particulate organic carbon and nitrogen in oxygen minimum zone sediments, Biogeosciences, 9, 993–1006, https://doi.org/10.5194/bg-9-993-2012, 2012.

Jeffreys, R. M., Burke, C., Jamieson, A. J., Narayanaswamy, B. E., Ruhl, H. A., Smith Jr, K. L., and Witte, U.: Feeding preferences of abyssal macrofauna inferred from in situ pulse chase experiments, PloS one, 8, e80510, https://doi.org/10.1371/journal.pone.0080510, 2013.

Joye, S. B., De Beer, D., and Cook, P. L. M.: Biogeochemical dynamics of coastal tidal flats, in: Coastal wetlands: an ecosystem integrated approach, edited by: Perillo, G. M. E., Wolanski, E., Cahoon, D. R., and Brinson, M. M., Elsevier, 345–374, 2009.

Kester, D. R., Duedall, I. W., Connors, D. N., and Pytkowicz, R. M.: Preparation of artificial seawater, Limnol. Oceanogr., 12, 176–179, 1967.

Knight, R. and Mantoura, R. F. C.: Chlorophyll and carotenoid pigments in Foraminifera and their symbiotic algae: analysis by high performance liquid chromatography, Mar. Ecol.-Prog. Ser., 23, 241–249, 1985.

Kroopnick, P., Weiss, R., and Craig, H.: Total CO2, 13C, and dissolved oxygen-18O at Geosecs II in the North Atlantic, Earth Planet. Sci. Lett., 16, 103–110, 1972.

Lee, J. J. and Muller, W. A.: Trophic dynamics and niches of salt marsh foraminifera, Am. Zool., 13, 215–223, https://doi.org/10.1093/icb/13.1.215, 1973.

Lee, J. J., McEnery, M., Pierce, S., Freudenthal, H., and Muller, W.: Tracer experiments in feeding littoral foraminifera, J. Protozool., 13, 659–670, 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, 493–508, 2014.

Li, Z.-P., Tao, M.-X., Li, L.-W., Wang, Z.-D., Du, L., and Zhang, M.-F.: Determination of isotope composition of dissolved inorganic carbon by gas chromatography-conventional isotope-ratio mass spectrometry, Chinese J. Anal. Chem., 35, 1455–1458, 2007.

Linshy, V., 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, 115–131, 2014.

Lipps, J. H. and Valentine, J. W.: The role of foraminifera in the trophic structure of marine communities, Lethaia, 3, 279–286, 1970.

Mayor, D. J., Thornton, B., and Zuur, A. F.: Resource quantity affects benthic microbial community structure and growth efficiency in a temperate intertidal mudflat, PLoS One, 7, e38582, https://doi.org/10.1371/journal.pone.0038582, 2012.

McDonald, J.: Multiple comparisons: Controlling the false discovery rate: Benjamini–Hochberg procedure, Handbook of Biological Statistics, Baltimore, Maryland: Sparkly House Publishing, 254–60, 2014.

Melis, R. and Covelli, S.: Distribution and morphological abnormalities of recent foraminifera in the Marano and Grado Lagoon (North Adriatic Sea, Italy), Mediterranean Marine Science, 14, 432–450, 2013.

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

Moodley, L., Van der Zwaan, G., Herman, P., Kempers, L., and Van Breugel, P.: Differential response of benthic meiofauna to anoxia with special reference to Foraminifera (Protista: Sarcodina), Mar. Ecol.-Prog. Ser., 158, 151–163, 1997.

Moodley, L., Boschker, H., Middelburg, J., Pel, R., Herman, P., De Deckere, E., and Heip, C.: Ecological significance of benthic foraminifera: 13C labelling experiments, Mar. Ecol.-Prog. Ser., 202, 289–295, 2000.

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

Moser, B. K. and Stevens, G. R.: Homogeneity of variance in the two-sample means test, Am. Stat., 46, 19–21, 1992.

Muller, W. A. and Lee, J. J.: Apparent indispensability of bacteria in foraminiferan nutrition, J. Protozool., 16, 471–478, 1969.

Murray, J. W.: Ecology and palaeoecology of benthic foraminifera, Routledge, 2014.

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

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, 134–146, 2005.

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, 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, 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. Pt. I, 56, 1488–1503, 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, 2011.

O'Connor, M. I., Piehler, M. F., Leech, D. M., Anton, A., and Bruno, J. F.: Warming and resource availability shift food web structure and metabolism, PLoS Biol, 7, e1000178, https://doi.org/10.1371/journal.pbio.1000178, 2009.

Papaspyrou, S., Diz, P., García-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 Cádiz, SW Spain), Mar. Ecol.-Prog. Ser., 490, 121–135, 2013.

Parmesan, C.: Ecological and evolutionary responses to recent climate change, AAnnu. Rev. Ecol. Evol. S., 37, 637–669, 2006.

Pascal, P.-Y., Dupuy, C., Mallet, C., Richard, P., and Niquil, N.: Bacterivory by benthic organisms in sediment: Quantification using 15 N-enriched bacteria, J. Exp. Mar. Biol. Ecol., 355, 18–26, 2008a.

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, 55–61, 2008b.

Petchey, O. L., Brose, U., and Rall, B. C.: Predicting the effects of temperature on food web connectance, Philos. T. R. Soc. Lond. B, 365, 2081–2091, 2010.

Pillet, L., de Vargas, C., and Pawlowski, J.: Molecular identification of sequestered diatom chloroplasts and kleptoplastidy in foraminifera, Protist, 162, 394–404, 2011.

Quijón, P. A., Kelly, M. C., and Snelgrove, P. V.: The role of sinking phytodetritus in structuring shallow-water benthic communities, J. Exp. Mar. Biol. Ecol., 366, 134–145, 2008.

R Development Core Team: A language and environment for statistical computing, R Foundation for Statistical Computing, Vienna, Austria, 2008.

Rivkin, R. and DeLaca, T.: Trophic dynamics in Antarctic benthic communities. I. In situ ingestion of microalgae by Foraminifera and metazoan meiofauna, Marine Ecol.-Prog. Ser., 64, 129–136, 1990.

RStudio Team: RStudio: Integrated Development Environment for R, RStudio, Inc., Boston, MA, available at: http://www.rstudio.com/ (last access: 2015), 2015.

Ruxton, G. D.: The unequal variance t-test is an underused alternative to Student's t-test and the Mann–Whitney U test, Behav. Ecol., 17, 688–690, 2006.

Schafer, C., Cole, F., Frobel, D., Rice, N., and Buzas, M.: An in situ experiment on temperature sensitivity of nearshore temperate benthic foraminifera, Oceanographic Literature Review, 9, 913, 1996.

Scholz, B. and Liebezeit, G.: Microphytobenthic dynamics in a Wadden Sea intertidal flat–Part II: Seasonal and spatial variability of non-diatom community components in relation to abiotic parameters, Eur. J. Phycol., 47, 120–137, 2012.

Sterner, R. W. and Elser, J. J.: Ecological stoichiometry: the biology of elements from molecules to the biosphere, Princeton University Press, 2002.

Sweetman, A. K., Sommer, S., Pfannkuche, O., and Witte, U.: Retarded response by macrofauna-size foraminifera to phytodetritus in a deep Norwegian fjord, J. Foramin. Res., 39, 15–22, 2009.

Taipale, S. J. and Sonninen, E.: The influence of preservation method and time on the $\delta$13C value of dissolved inorganic carbon in water samples, Rapid Commun. Mass Sp., 23, 2507–2510, 2009.

Topping, J., Murray, J., and Pond, D.: Sewage effects on the food sources and diet of benthic foraminifera living in oxic sediment: a microcosm experiment, J. Exp. Mar. Biol. Ecol., 329, 239–250, 2006.

Vázouez-Domínguez, E., Vaque, D., and Gasol, J. M.: Ocean warming enhances respiration and carbon demand of coastal microbial plankton, Glob. Change Biol., 13, 1327–1334, 2007.

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, 515–530, 2003.

Wasserstein, R. L. and Boyer Jr., J. E.: Bounds on the power of linear rank tests for scale parameters, Am. Stat., 45, 10–13, 1991.

Whiteley, N. and Faulkner, L.: Temperature influences whole-animal rates of metabolism but not protein synthesis in a temperate intertidal isopod, Physiol. Biochem. Zool., 78, 227–238, 2005.

Wickham, H.: ggplot2: Elegant Graphics for Data Analysis, Springer-Verlag New York, available at: http://ggplot2.org (last access: 2016), 2009.

Wickham, H.: The Split-Apply-Combine Strategy for Data Analysis, J. Stat. Softw., 40, 1–29, 2011.

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

Wohlers, J., Engel, A., Zöllner, E., Breithaupt, P., Jürgens, K., Hoppe, H.-G., Sommer, U., and Riebesell, U.: Changes in biogenic carbon flow in response to sea surface warming, P. Natl. Acad. Sci. USA, 106, 7067–7072, 2009.

Yvon-Durocher, G., Jones, J. I., Trimmer, M., Woodward, G., and Montoya, J. M.: Warming alters the metabolic balance of ecosystems, Philos. T. R. Soc. Lond. B, 365, 2117–2126, 2010.

Zuur, A. F., Ieno, E. N., and Elphick, C. S.: A protocol for data exploration to avoid common statistical problems, Methods in Ecology and Evolution, 1, 3–14, 2010.