

The fate of nitrogen in the *Orbulina universa* foraminifera–symbiont system determined by nitrogen isotope analyses of shell-bound organic matter

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Abstract

To trace the flow of nitrogen through the foraminifera–symbiont system, juvenile specimens of the symbiont-bearing planktonic foraminifera, *Orbulina universa* were collected by SCUBA divers off the coast of Santa Catalina Island, California, and grown in filtered seawater solutions spiked with ¹⁵N-labeled nitrate. Our work isolated each pathway of this symbiotic system to determine the amount of nitrogen translocated to the foraminifera from its endosymbionts and its captured diet. Our model results show that when the nitrate uptake by the symbionts is at a maximum, between 50 and 57% of the foraminiferal nitrogen was translocated from the symbionts and the remainder was derived from the captured diet. In nitrate-deficient environments, when the symbiont nitrate uptake was at a minimum, ~90–100% of the nitrogen was transferred to the foraminifer by the symbionts from the recycled nitrogen (NH₄⁺) pool. In nitrate-deficient conditions, the primary role of the captured diet may be to provide the system with phosphorus. Highly efficient nutrient use is particularly important to the foraminifera–symbiont system, especially when nutrient concentrations are low. The results indicate a limited correlation between the isotopic composition of the NO₃⁻ in the culture solutions and the δ¹⁵N values of the foraminifera. However, the δ¹⁵N values of the foraminifera much more strongly reflect the δ¹⁵N values of the NH₄⁺ recycled through the system. It appears that recycled nitrogen (NH₄⁺) is a more important source of nitrogen to the symbionts and the foraminifera than nitrate; therefore, the δ¹⁵N values of the foraminifera may not reflect the isotopic composition of the surface-water nutrients. Understanding the nitrogen flow within modern foraminifera–symbiont associations is also important to ancient marine systems, because symbiont-bearing foraminifera are ubiquitous in the fossil record. This study shows that analysis of individual, symbiont-bearing foraminifera species is a necessary first step toward the development of more reliable use of nitrogen isotopes for paleoceanographic reconstructions.

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Algal–invertebrate symbiosis often occurs between dinoflagellates (zooxanthellae) and a variety of hosts, including foraminifera, reef corals, anemones, and tridacnid clams (e.g., Muscatine and Porter 1977; Anderson and Burris 1987; ter Kuile and Erez 1987; Sutton and Hoegh-Guldberg 1990). These organisms are able to subsist phototrophically (Muscatine and Porter 1977) and to use and conserve inorganic nutrients in oligotrophic marine environments where the level of these dissolved nutrients is very low (D'Elia and Webb 1977; Muscatine 1980; D'Elia 1988). In these symbiotic associations, the zooxanthellae mediate the uptake of inorganic nutrients, assimilate these nutrients into organic compounds, and translocate some of these compounds to their host (Trench 1979; Muscatine 1980).

Work on nitrogen cycling in symbiotic systems has iden-

tified several pathways for nitrogen uptake and metabolism that allows the host and its endosymbionts to effectively use the nitrogen from the surrounding environment (Webb and Wiebe 1978; Risk and Muller 1983; Muscatine and Kaplan 1994; Snidvongs and Kinzie 1994). These pathways include (1) the uptake of dissolved inorganic nitrogen (DIN: NO_3^- , NO_2^- , or NH_4^+) from surface waters; (2) the uptake of NH_4^+ , which is typically excreted by the host organism; and (3) the translocation of nitrogenous organic compounds to the host. In addition, the host may play a significant role in the health of the algal population by providing a continuous supply of nitrogen in the form of NH_4^+ (Burriss 1983; Parry 1985; Anderson and Burriss 1987).

Despite the fact that certain species of planktonic foraminifera contain dinoflagellate symbionts within their spines, only a few studies focused on symbiotic associations with planktonic foraminifera as the host organism (Lee and McEnergy 1984; ter Kuile and Erez 1987). The majority of these studies were concerned with the flow of carbon within the foraminifera–symbiont system, with little work on the flow of nitrogen within algal–foraminifera symbiosis (Uhle et al. 1995, 1997). Work on symbiont-bearing foraminifera has shown that organic matter (proteins) is bound in the calcite matrix (tests) of the foraminifera and that this material is comprised of compounds from both the symbionts and the diet (e.g., Langer et al. 1993; Stathoplos and Tuross 1994; Uhle et al. 1997). Because the growth of the zooxanthellae and the prey is dependent on nutrient use, there is an indirect link between surface-water nutrients and shell-bound organic matter in the foraminifera (Uhle et al. 1997).

Stable isotope analysis is well suited to trace the flow of nitrogen through complex systems. The $\delta^{15}\text{N}$ values record the biochemical history of the organic matter, and the isotopic signatures reflect evidence of the fractionation between the organic matter and its sources (Macko et al. 1987; Hare et al. 1991). The $\delta^{15}\text{N}$ value of the shell-bound organic matter (protein matrix) in foraminifera should reflect the $\delta^{15}\text{N}$ value of the nitrogen used by the symbionts, provided that nitrogen is indeed translocated from the zooxanthellae to the host. To understand how nitrogen is cycled through the foraminifera–symbiont system, all the sources of nitrogen to the foraminifera and their symbionts must be identified, as well as the pathways along which nitrogen is transferred between these organisms. In addition, the isotopic fractionations associated with the translocation of nitrogen at each branching point in the system must be quantified.

As with the other symbiotic associations (e.g., Wilkerson and Muscatine 1984; Anderson and Burriss 1987; Snidvongs and Kinzie 1994), we assume that nitrogen is derived from three distinct sources and is transferred throughout the foraminifera–symbiont system along pathways identified in Fig. 1. Inorganic nitrogen is incorporated by symbionts (pathway 1) and fixed into organic compounds that are then transferred to the foraminifera (pathway 2). Additional nitrogen is acquired from the diet (pathway 3). The nitrogen derived from these sources is used for different biosynthetic processes, such as amino acid and protein synthesis, and is ultimately incorporated into the shell-bound organic matrix of the foraminifera. Excess nitrogen from foraminiferal metabolism is excreted in the form of relatively ^{15}N -depleted ammonium

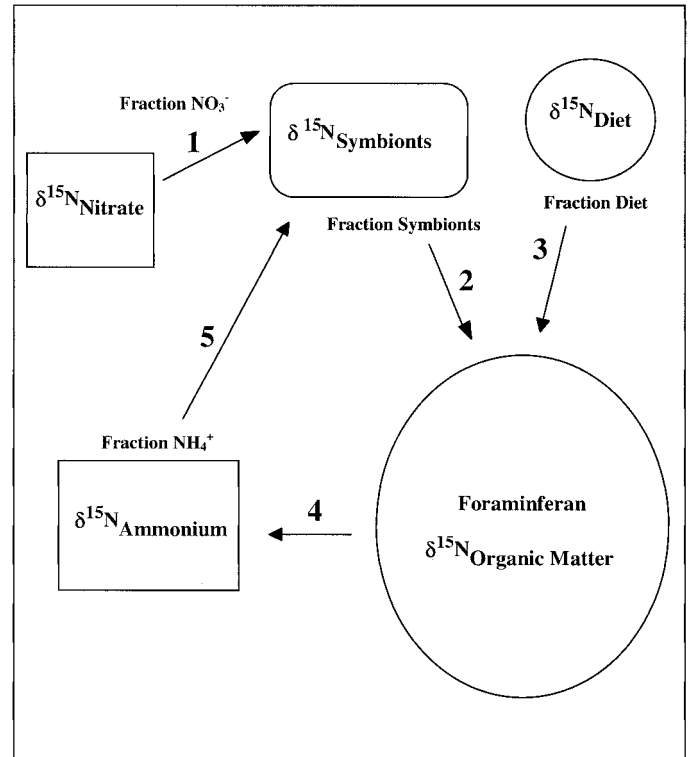


Fig. 1. Components of the foraminifera–symbiont system. Inorganic nutrients are taken up by the symbiont and transferred to the foraminifera. Additional nitrogen is supplied by the captured diet (*A. salina*), and recycled nitrogen (NH_4^+) can be reused by the symbionts. Components shown in bold are measured values. The values of the components enclosed in shaded boxes are calculated from mass balance equations.

(pathway 4), where it is reused by the symbionts (pathway 5). Although the foraminifera–symbiont system appears to be a direct link between the foraminifera and dissolved inorganic nitrogen in seawater, the bulk isotopic composition of these organisms may not directly reflect the $\delta^{15}\text{N}$ of the surface-water nutrients. Isotopic fractionations associated with each pathway could alter the final isotopic composition of the organic nitrogen.

Here, we describe the results of experiments in which modern symbiont-bearing foraminifera were grown under controlled laboratory conditions to quantify the amount of nitrogen translocated to the foraminifera from the symbionts and the diet and to model the flow of nitrogen through this foraminifera–symbiont system.

Analytical methods

Controlled Growth of Orbulina universa—Juvenile specimens of *O. universa* were collected by SCUBA divers 1–2 km north of Santa Catalina Island in the waters of the San Pedro Basin (Southern California Bight). Each individual foraminifera was grown under controlled laboratory conditions at the Wrigley Institute for Environmental Science in a glass jar containing $0.8 \mu\text{m}$ filtered sea water spiked with ^{15}N -labeled nitrate ($\delta^{15}\text{N} = 110$ or 119%). Light conditions

ranged from dark (12 h) to ambient (12 h). Ambient light conditions were maintained by indirect sunlight illumination ($150\text{--}250 \mu\text{E m}^{-2} \text{s}^{-1}$), and culture vessels were stored in flowing seawater tanks at $21 \pm 1.5^\circ\text{C}$. Each foraminifera was fed a single *Artemia salina* nauplius (Great Salt Lake strain 1178; Spero et al. 1993) each day, which was the sole dietary source of nitrogen. The ontogenetic stages of each specimen were determined daily.

The life cycle of *O. universa* consists of a juvenile stage with a multichambered calcite shell that is filled with cytoplasm, followed by an adult stage in which *O. universa* secretes a spherical chamber that surrounds the juvenile, multichambered form (Hemleben et al. 1989). This sphere is supported by a protein matrix that, in our study, was grown under laboratory conditions. In the last stage of the life cycle, the organism releases its gametes eliminating virtually all its cytoplasm from within the sphere. Approximately 300 juvenile specimens of *O. universa* were placed into culture and then removed after reproduction 7–13 d later. The specimens were archived after reproducing to ensure that only shell-bound organic matter was recovered from the organisms. All 300 specimens analyzed had matured from the juvenile stage (average size of $135 \mu\text{m}$) to the adult stage (average size of $780 \mu\text{m}$). Therefore the organic material isolated from the foraminifera reflects that which was grown under controlled laboratory conditions, and the material associated with growth prior to culture is negligible.

Extraction of total organic matter—Two 6-mg samples of *O. universa* tests containing only shell-bound organic matter were used for bulk stable isotope analyses. To eliminate any cytoplasm or adsorbed organic matter from the surface of the tests, all foraminifera were treated with a 0.25% sodium hypochlorite solution for 20 to 30 min, followed by three distilled water washes (Spero et al. 1991). After cleaning, the carbonate tests in each 6-mg sample were acidified with 1N HCl (Ultrex, Sigma Chemical). The shell-bound organic matter from the *O. universa* was then collected by filtration onto a $0.4 \mu\text{m}$ glass-fiber filter (Whatman GF/C, pre-ashed at 550°C for 1 h).

To isolate the organic matter of the internal tissues from the chitinous exoskeletal material of *A. salina* (brine shrimp), we placed ~ 2.5 mg (dry wt.) of brine shrimp in 100 ml of distilled water for 24 h and disrupted the organisms with a teflon-coated rotary tool. The suspension was then filtered through a $0.4\text{-}\mu\text{m}$ glass-fiber filter (Whatman GF/C, pre-ashed at 550°C for 1 h), followed by drying of the filtrate and the filters at 60°C .

Isolation of ammonium and nitrate from culture solutions—Ammonium and nitrate were extracted by steam distillation from 1-liter samples of the initial and final culture solutions according to the method described by Velinsky et al. (1989). First, the ammonium was converted to ammonia by the addition of ~ 1 ml of 5N NaOH (resultant pH ≥ 10). The ammonia was then distilled from the culture solutions using a Labconco Rapid Kjeldahl System (RapidStill II) and collected in 0.03N HCl that converted the ammonia back to ammonium. In order to collect the ammonium, ~ 120 mg of a molecular zeolite sieve were added to the culture solutions

and the pH was adjusted between 5.5 and 6.0. The samples were stirred for 1 h to ensure optimal binding of the ammonium to the zeolite. Two bindings were completed for each sample to facilitate complete recovery of the ammonium.

After filtering each solution, the molecular zeolite was dried overnight at 67°C . The remaining nitrate was collected from the samples by reduction to ammonium with 1.5 grams of Devarda's alloy (50% Cu, 45% Al, 5% Zn). The resultant ammonium was converted to ammonia, distilled, bound to the zeolite molecular sieve, filtered, and dried. The zeolite from both bindings of each sample was removed from the filters and combined.

Determination of phosphate concentrations in culture solutions—The concentration of phosphate in the culture solutions was determined by colorimetry (Hitachi Model 100 Spectrophotometer) following a modified version of the procedure described by Strickland and Parsons (1968). Briefly, 1 ml of combined reagent (consisting of ammonium molybdate, sulfuric acid, ascorbic acid, and potassium antimonyl tartrate) was added to a 10-ml aliquot of filtered ($0.2 \mu\text{m}$ membrane filters) culture solution. The solution was shaken and the color was allowed to develop for 20 min. The absorbances (at 885 nm) of several standard solutions (anhydrous potassium dihydrogen phosphate and deionized water) were used to generate a standard curve using a 1 cm path length cuvette. The phosphate concentrations in the sample solutions were determined from the standard curve.

Stable nitrogen isotope measurements—The ammonium and nitrate (converted to ammonium) recovered from the culture solutions, as well as the total organic matter isolated from *O. universa*, and the *Artemia* nauplii were converted to N_2 for isotope analyses by a modified Dumas sealed-tube combustion method (Macko 1981). The materials were mixed with purified, coarsely ground cupric oxide and pure granular copper, placed in a quartz tube, sealed under vacuum, combusted for 1 h at 850°C , and allowed to gradually cool to room temperature. The generated N_2 was isolated cryogenically and analyzed with a Fisons triple collector PRISM stable isotope ratio mass spectrometer.

The stable isotopic ratios are reported as the per mil deviation from a standard given by the relationship

$$\delta^{15}\text{N} = [(R_{\text{sample}}/R_{\text{standard}}) - 1] \times 10^3, \quad (1)$$

where R is the ratio of $^{15}\text{N} : ^{14}\text{N}$. The $\delta^{15}\text{N}$ values are reported relative to the atmospheric N_2 standard. For replicate analyses, secondary laboratory standard of pure nitrogen gas was used as the standard for $\delta^{15}\text{N}$. Each sample was run in duplicate and the reproducibility of the measurements was $\pm 0.05\text{‰}$ or better.

Concentrations of ammonium and nitrate—To determine the concentrations of ammonium and nitrate in the culture solutions, the generated N_2 from each sample was cryogenically isolated and introduced into the mass spectrometer ion source. The concentrations of NH_4^+ and NO_3^- in each sample were determined by comparing the major beam (m/z 28) intensities to a standard curve (Fig. 2). The standard curve

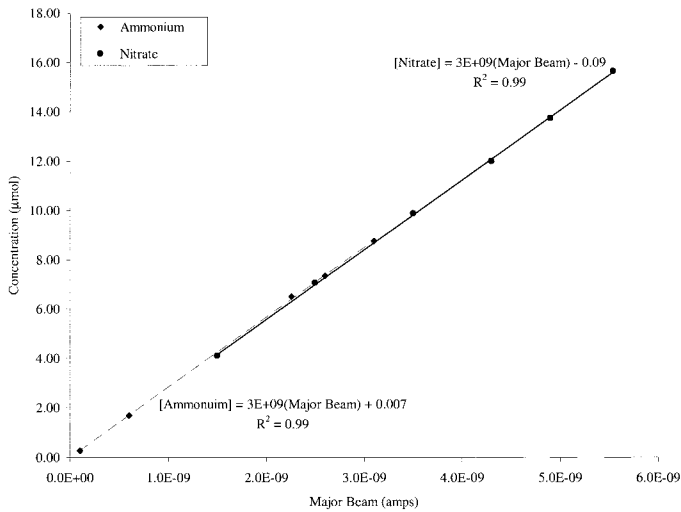


Fig. 2. Standard curve to determine concentrations of NH_4^+ and NO_3^- in culture solutions from mass spectrometer major ion beam intensity.

was generated by plotting the resultant major ion beam intensities versus the concentrations of nitrogen salts (NH_4Cl and KNO_3 ; Sigma Chemical). The ion beam method has been compared to other standard wet chemical methods (Strickland and Parsons 1968) to determine the ammonium and nitrate concentrations in rain water (Russell 1997 unpubl. data). The comparisons yielded best fit lines with slopes of 0.998 for the NH_4^+ determination and 1.067 for the NO_3^- determination between 1 and 10 μmol (Russell 1997 unpubl. data). The relationship for NO_3^- was not as well constrained at concentrations $>10 \mu\text{mol}$, probably owing to an incomplete conversion of NO_3^- to NH_4^+ . In general, for the concentrations in this study, these results show that the ion beam method provides reliable estimates of the NO_3^- and NH_4^+ concentrations in culture solutions.

Results

The flow of nitrogen through the foraminifera–symbiont system can be quantified by a series of mass balance equations. Application of these equations requires that several parameters be quantified. These parameters are the amount of nitrogen translocated from each pool, the $\delta^{15}\text{N}$ values of each component in the system, and the isotopic fractionations associated with both the transfer of nitrogen between the pools and trophic interactions (Fig. 1).

Measured $\delta^{15}\text{N}$ values of model components—The isotopic composition of the shell-bound organic matter was measured for two groups, each containing ~ 150 individual tests of *O. universa* (Table 1). The internal tissues of the *Artemia* nauplii were 7.3‰ enriched in ^{15}N relative to the carapace of the organism. This difference is consistent with results from other studies (Macko et al. 1990, 1991; Schimmel 1995), showing that crustacean exoskeletons can be depleted in ^{15}N by up to 12‰ relative to the entire organism. The $\delta^{15}\text{N}$ value of the internal tissues more accurately reflects the isotopic composition of the diet as foraminifera ingest the internal

Table 1. Measured $\delta^{15}\text{N}$ values of model components.

Component	$\delta^{15}\text{N}$ Values ($\pm 0.05\%$)	
	Group 1	Group 2
Foraminifera tests	37.4	35.7
NO_3^- added to culture solutions	119.0	110.7
NH_4^+ recovered from culture solutions	25.5	16.8
<i>Artemia</i> (carapace)	6.1	6.1
<i>Artemia</i> (internal tissue) –1	13.4	13.4

tissue of their prey and discard the carapace (Hemleben et al. 1989). Based on these data, the $\delta^{15}\text{N}$ value of the diet used in the model is 13.4‰.

Model constraints and results

The $\delta^{15}\text{N}$ values of all the model components (Fig. 1) were measured by conventional isotope ratio mass spectrometry (IRMS) except for the $\delta^{15}\text{N}$ values of the endosymbionts. Direct isotope analysis of these dinoflagellate algae is not possible because the symbionts are digested or discarded by the foraminifera prior to gametogenesis (reproduction) (Bé et al. 1983). However, these values can be estimated via mass balance relationships where the $\delta^{15}\text{N}$ value of the symbionts is related to the amount and isotopic composition of the inorganic nutrients incorporated by the endosymbionts. The amounts of nitrogen derived from the NO_3^- and NH_4^+ (recycled nitrogen) pools must be estimated from diffusion fluxes of these nutrients under our culture conditions. In addition, the $\delta^{15}\text{N}$ values of the symbionts must be corrected for the isotope fractionation associated with NO_3^- and NH_4^+ (recycled nitrogen) uptake, and the $\delta^{15}\text{N}$ values of the foraminifera must be corrected for trophic level effects. These factors are addressed in the following discussion.

Diffusion flux of nutrients—The total amount of nitrogen incorporated into the endosymbionts is limited by the quantity of NH_4^+ and NO_3^- that can be assimilated by the symbionts under the culture conditions. The assimilation of NO_3^- and NH_4^+ is dependent on the diffusion of these nutrients through the culture medium to the symbionts and ultimately across their cell membranes (Jørgensen et al. 1985). The flux of the nutrients to the symbionts is expressed by

$$F = 4\pi DRS, \quad (2)$$

where F is the diffusion flux of the nutrient, D is the molecular diffusion coefficient, R is the radius of the sphere of photosynthesis, and S is the concentration of the nutrient in the culture solution (Jørgensen et al. 1985). The radius of the sphere of photosynthesis (R) is an average value determined for adult *O. universa* by measuring the distance from the surface of the test to the tip of the spines (Spero et al. 1991) in ~ 300 adult *O. universa*. This parameter is related to the actual spherical area of photosynthesis where nutrients are used by the endosymbionts. The measured values varied by $\sim 30 \mu\text{m}$. We chose to use the average value because the calculated fluxes were not significantly affected by the variation in our measurements ($\pm 0.02 \text{ nmol N h}^{-1}$).

Table 2. Nutrient flux parameters under culture conditions.

Parameter	Nitrate values	Ammonium values	Phosphate values
Temperature (°C)	20.0 (±1.5)	20.0 (±1.5)	20.0 (±1.5)
Molecular diffusion coefficient (D ; mm ² h ⁻¹)	6.24	6.51	2.42
Radius of photosynthetic sphere (R ; mm)	0.51	0.51	0.51
Nutrient concentration (S ; pmol mm ⁻³)	4.85	2.80	0.52*

* Data from Li and Gregory (1974).

We employed diffusion theory because measurements of the actual nitrate and ammonium concentrations surrounding the foraminifera and symbionts were not possible. Microelectrode studies (e.g., Jørgensen et al. 1985; Rink et al. 1998) may provide better estimates, but until these techniques are developed for nitrate and ammonium, we must calculate nutrient assimilation values based on diffusion fluxes.

Diffusion flux of nitrate—Assuming that the concentration of NO₃⁻ surrounding the symbionts is zero or that the uptake of NO₃⁻ is at a maximum, then under the culture conditions (Table 2), the diffusion of nitrate to the endosymbiont cells is 0.19 nmol N h⁻¹. This value represents the maximum amount of nitrogen that can be incorporated into the symbionts by nitrate assimilation. The symbionts are assumed to be the principal nitrate sink in our experiments. The δ¹⁵N values of the nitrate in the culture solutions did not substantially change between the beginning and the end of the experiments (initial δ¹⁵N value of +119 versus resultant value of +120). It is possible that denitrification may occur in anoxic microenvironments within the foraminifera; however, it does not appear that this is a significant sink for nitrate in our experiments. To obtain the fraction of nitrogen derived from the NO₃⁻ pool, this value must be compared to the total amount of nitrogen needed to sustain the growth of the symbionts under the culture conditions.

Nitrogen requirements for balanced growth—The amount of nitrogen required for the balanced growth of the endosymbionts can be calculated from the net carbon assimilation for the *O. universa* symbiont population assuming a photosynthetic quotient (photosynthesis : respiration) of 1 and a C : N ratio of 106 : 16, which is typical of phytoplankton (Redfield et al. 1963). The net carbon assimilation rate for the symbionts (at 20°C) was estimated to be 10.3 nmol C h⁻¹ at photosynthetic maximum light levels (>386 μE m⁻² s⁻¹) (Spero et al. 1991). Light levels in our experiments were ~200 μE m⁻² s⁻¹. Using a photosynthesis-irradiance relationship for *O. universa* (Spero and Parker 1985), the net carbon assimilation under our culture conditions is ~4.79 nmol C h⁻¹ per individual adult foraminiferan. Assuming Redfield ratios for C : N and a photosynthetic quotient of 1, ~0.72 nmol N h⁻¹ (4.79/(106/16)) are required to maintain balanced growth of the endosymbionts under the culture conditions. We chose to use the original Redfield ratios be-

cause more recent estimates (e.g., Broecker and Peng 1982; Takahashi et al. 1985) do not substantially affect our results (3% difference). The diffusion flux of nitrate to the symbionts can be used in conjunction with the amount of nitrogen required for balanced growth to determine the fraction of nitrogen in the symbionts that is associated with nitrate assimilation.

Fractions of nitrogen derived from the inorganic nutrients—The fraction of foraminiferal nitrogen that is derived from the nitrate pool is 0.27 (0.19/0.72 nmol N h⁻¹), which suggests that the diffusion of nitrate to the endosymbionts (at maximum efficiency with an ambient nitrate concentration of zero) can account for only 27% of the nitrogen required for balanced growth (Fig. 1, pathway 1). The remainder of the required nitrogen must be derived from the NH₄⁺ (recycled nitrogen) pool, as this is the only other source of nitrogen to the symbionts. Consequently, the NH₄⁺ pool should contribute 0.53 nmol N h⁻¹, or ~73%, of the nitrogen required for balanced growth (Fig. 1, pathway 5). The question now becomes, is the concentration of NH₄⁺ in the culture solution sufficient to sustain the NH₄⁺ flux required for an uptake of 0.53 nmol N h⁻¹?

Diffusion flux of ammonium to the endosymbionts—The concentration of ammonium needed to yield an ammonium flux of 0.53 nmol N h⁻¹ can be calculated by solving Eq. 2 for S :

$$S = F(4\pi DR)^{-1} \quad (3)$$

and using the molecular diffusion coefficient of NH₄⁺ (Table 2). Assuming the most efficient uptake of NH₄⁺ (ambient ammonium concentration of zero) under our culture conditions (Table 2), the concentration of NH₄⁺ surrounding the endosymbionts would have to be 12.7 pmol mm⁻³, or 2.6 times greater than the NH₄⁺ concentration measured in the culture solutions (Table 2). Elevated NH₄⁺ concentrations near the endosymbionts would most probably reflect NH₄⁺ excretion by the foraminiferan.

From foregoing calculations, it seems reasonable to propose that the NH₄⁺ (recycled nitrogen) and NO₃⁻ pools contribute 73% and 27% respectively of the nitrogen required for balanced growth of the symbionts. Before these data can be used to calculate the δ¹⁵N of the symbionts, the nitrogen isotopic fractionations during the assimilation of NO₃⁻ and NH₄⁺ must be considered.

Nitrogen isotopic fractionation during the assimilation of nitrate—Studies have shown that the extent of nitrogen fractionation during the uptake of NO₃⁻ by phytoplankton at micromolar concentrations varies from 0‰ to -10‰ (Table 3). The widest range in values, 0‰ to -24‰, was observed at millimolar concentrations (Wada and Hattori 1978; Mariotti et al. 1982). The isotopic fractionation was a function of the concentration of NO₃⁻ in the culture solution (Wada and Hattori 1978; Mariotti et al. 1982; Macko et al. 1987). However, no clear relationship was observed from algal cells grown at lower NO₃⁻ concentrations.

Active transport of nitrogen has been reported in different species of marine diatoms (Falkowski 1975; Packard 1979).

Table 3. Isotopic fractionation for nitrogen assimilation.

Reaction	Fractionation (‰)
NO₃⁻ Assimilation	
Cultures	
mmol concentrations	0 to 24
μmol concentrations	-10
Field observations	
μmol concentrations	-4 to -5
NH₄⁺ assimilation	
Cultures	
mmol concentrations	0 to -15
μmol concentrations	-3 to -27
Field observations	
μmol concentrations	-10

After Fogel and Cifuentes (1993).

This transport was controlled by a membrane-bound enzyme, which appeared to be activated by the presence of nitrate. In terms of isotope discrimination, evidence from higher plants shows that there is no fractionation for the transport of NO₃⁻ across cell membranes (Mariotti et al. 1982). However, isotopic fractionation would occur if, under natural conditions, the NO₃⁻ were in sufficient supply to saturate the nitrate reductase (Fogel and Cifuentes 1993).

These studies show that the isotopic fractionation associated with the uptake of NO₃⁻ varies from zero per mil with diffusive transport to ca. -10 per mil with active transport. Active transport mechanisms discriminate against the heavy isotope ¹⁵N, resulting in a negative fractionation factor (i.e., -10‰). We assume that diffusion is the primary mechanism by which nitrate is provided to the symbionts (Jørgensen et al. 1985); however, it may be possible that active transport of some nitrate across the symbiont membranes does occur. To account for possible active transport mechanisms, calculations involving values associated with the process of NO₃⁻ uptake by endosymbionts were considered at the 0‰ (diffusive transport), the -5‰, and the -10‰ (active transport) fractionation levels.

Nitrogen isotopic fractionation during the assimilation of ammonium—Isotopic fractionations associated with the assimilation of ammonium by phytoplankton range between 0‰ and -27‰ (Table 3). Some of the smallest values were observed at mmol NH₄⁺ concentrations, whereas at μmol concentrations, the fractionations were as large as -27‰ (Table 3).

Active transport of ammonium has been reported in both prokaryotic and eukaryotic organisms (Fogel and Cifuentes 1993, and references therein) at concentrations below 100 μmol. Fogel and Cifuentes (1993) developed a model to predict the fractionation of nitrogen during the assimilation of NH₄⁺ due to active transport of ammonium across the cell membrane. This model predicts that the isotope fractionation is dependent on the concentrations of ammonium and organic nitrogen both inside and outside the cell. At slightly higher external concentrations (>100 μmol), the fraction-

Table 4. Calculated δ¹⁵N values of the endosymbionts.

Uptake efficiency and isotopic fractionation	δ ¹⁵ N _{symbionts} (‰)	
	Group 1	Group 2
Maximum uptake efficiency		
0‰ fractionation	50.7	49.8
-5‰ fractionation	49.4	48.5
-10‰ fractionation	48.0	47.1
Minimum uptake efficiency		
0‰ fractionation	34.4	33.1
-5‰ fractionation	33.9	32.8
-10‰ fractionation	33.4	32.4

ation would be the greatest, whereas at the lowest concentrations (<10 μmol) the amount of nitrogen able to leak from the cells before it is assimilated is essentially zero. Therefore, the fractionation between inorganic nitrogen and particulate nitrogen is extremely small (Fogel and Cifuentes 1993).

These results were confirmed by laboratory experiments in which the largest fractionations (up to -27‰) were associated with NH₄⁺ concentrations between 10 and 100 μmol (Pennock et al. 1988). Active transport was considered to be induced owing to the higher concentrations of NH₄⁺ inside than outside the cell, indicating that there was an excess of nitrogen for assimilation (Fogel and Cifuentes 1993). In addition, other laboratory results indicated that smaller nitrogen fractionations are associated with active NH₄⁺ transport (Fogel and Cifuentes 1993, and references therein). From model calculations, at concentrations of NH₄⁺ < 20 μmol, the system would be NH₄⁺ limited, which would result in isotopic fractionation of approximately -2‰ (Fogel and Cifuentes 1993).

The concentration of NH₄⁺ in the culture solutions was 4.8 μmol, which is substantially <20 μmol. Although the NH₄⁺ concentration surrounding the foraminiferan and its symbionts may actually be as high as 12.7 μmol, owing to NH₄⁺ excretion by the foraminiferan, this is still less than the threshold value cited by Fogel and Cifuentes (1993). These data indicate that the system is NH₄⁺ limited, and the isotopic fractionation due to NH₄⁺ by the endosymbionts is approximately -2‰. The resultant calculations involving values derived from the uptake of ammonium by the symbionts are corrected for the -2‰ fractionation.

δ¹⁵N values of the endosymbionts—Accounting for the fractionation of nitrogen during nitrate and ammonium assimilation, the δ¹⁵N of the symbionts can be calculated such that

$$\delta^{15}\text{N}_{\text{sym}} = f_{\text{NO}_3^-} \times (\delta^{15}\text{N}_{\Delta\text{NO}_3^-}) + f_{\text{recycled N}} \times (\delta^{15}\text{N}_{\Delta\text{NH}_4^+}) \quad (4)$$

$$f_{\text{NO}_3^-} + f_{\text{NH}_4^+} = 1 \quad (5)$$

where f_x is the fraction of nitrate or recycled nitrogen and δ¹⁵N_{ΔNO₃⁻}, and δ¹⁵N_{ΔNH₄⁺} and δ¹⁵N_{sym} are the isotopic compo-

sitions of the nitrate and recycled nitrogen (NH_4^+) corrected for fractionation and the symbionts, respectively.

The calculated $\delta^{15}\text{N}$ values of the symbionts for two nitrogen-uptake scenarios (maximum and minimum) are shown in Table 4. The difference between the $\delta^{15}\text{N}$ values of the symbionts calculated at maximum and minimum nitrate assimilation is 16‰. This deviation reflects nitrogen uptake from different source pools in each scheme. At maximum nitrogen uptake, the concentration of nitrate is sufficient to allow the endosymbionts to use all available nitrate so that the ambient nitrate concentration surrounding the symbionts is zero. Minimum nitrogen uptake is synonymous with nitrate-deficient environments in which the majority of foraminiferal nitrogen is derived from the recycled nitrogen (NH_4^+) pool. The $\delta^{15}\text{N}$ values calculated for specific fractionation levels vary by <1.3‰ between the two experimental groups (Table 4). In addition, the total range in $\delta^{15}\text{N}$ values calculated for all fractionation levels under maximum nitrate assimilation is 3.6‰, and the range for minimum nitrate assimilation is 2.0‰ (Table 4).

The next step in the model is to determine the relative fractions of foraminiferal nitrogen derived from the symbionts and the captured diet. This is accomplished by using the $\delta^{15}\text{N}$ values of the symbionts, the diet, and the foraminifera. The $\delta^{15}\text{N}$ values of the foraminifera must be corrected for the isotopic fractionation associated with trophic interactions.

Trophic level shifts in foraminifera—Isotope fractionation associated with trophic level shifts is defined as the difference between the $\delta^{15}\text{N}$ values of a whole organism minus its diet (DeNiro and Epstein 1981). The organisms used in this study were grown in seawater spiked with ^{15}N nitrate; therefore, simply calculating the difference between the $\delta^{15}\text{N}$ values of the foraminifera and its diet will give erroneous isotope values that do not reflect the trophic level shift. However, the isotope fractionation associated with trophic interactions for *O. universa* has been previously determined (Uhle et al. 1997). This value is applicable to the present study because the diets used by the foraminifera in both studies were identical (*A. salina*—Great Salt Lake strain 1178; Spero et al. 1993).

The $\delta^{15}\text{N}$ values of organic nitrogen in the tests of *O. universa* and *Globigerina bulloides* were previously determined to be 15.6‰ and 17.5‰, respectively (Uhle et al. 1995). Thus the symbiont-bearing species, *O. universa*, is 2.2‰ enriched in ^{15}N relative to its brine shrimp diet (13.4‰), whereas the symbiont-free species, *G. bulloides*, is 4.1‰ enriched relative to its captured diet (13.4‰). The translocation of nitrogen from the symbionts to *O. universa* most likely obscures the true trophic shift; therefore, the isotopic fractionation associated with trophic interactions in foraminifera is probably 4.1‰, which is close to the 3‰ enrichment observed for other organisms (DeNiro and Epstein 1981). This 4.1‰ fractionation is used to correct the $\delta^{15}\text{N}$ values of the foraminifera for trophic enrichment so that the fractions of nitrogen derived from the symbionts and the diet can be assessed from mass balance equations.

Table 5. Fractions of nitrogen in *O. universa* derived from the endosymbionts and the captured diet.

Isotopic fractionation	$f_{\text{symbionts}}$ (%)	f_{diet} (%)
Maximum uptake efficiency		
Group 1		
0‰ fractionation	53	47
−5‰ fractionation	55	45
−10‰ fractionation	57	43
Group 2		
0‰ fractionation	50	50
−5‰ fractionation	52	48
−10‰ fractionation	54	46
Minimum uptake efficiency		
Group 1		
0‰ fractionation	95	5
−5‰ fractionation	97	3
−10‰ fractionation	99	1
Group 2		
0‰ fractionation	92	8
−5‰ fractionation	94	6
−10‰ fractionation	96	4

Fraction of nitrogen in O. universa derived from the endosymbionts and the diet—The fraction of nitrogen derived from the endosymbionts and the diet can be calculated such that

$$\delta^{15}\text{N}_{(\Delta\text{foram})} = f_{\text{sym}}(\delta^{15}\text{N}_{\Delta\text{sym}}) + f_{\text{diet}}(\delta^{15}\text{N}_{\text{diet}}) \quad (6)$$

$$f_{\text{sym}} + f_{\text{diet}} = 1 \quad (7)$$

where f_x is the fraction of nitrogen from the symbionts or the diet, $\delta^{15}\text{N}_x$ is the isotopic composition of the diet (*A. salina*), and the *O. universa* and the symbionts corrected for trophic effects and nutrient assimilation, respectively.

Assuming that nitrate uptake by the endosymbionts is at maximum, from Eqs. 6 and 7, ~50–57% of the nitrogen bound in the foraminifera is derived from the symbionts, whereas the diet contributes the remaining 43–50% of the nitrogen (Table 5). At minimum NO_3^- uptake in nitrate-deficient environments, the symbionts would translocate 92–99% of the nitrogen sequestered in the foraminifera from the recycled nitrogen (NH_4^+) pool. The dietary nitrogen comprises the remaining 1–8% of the total nitrogen in *O. universa* (Table 5). The results from the two experimental groups are consistent. The total range in values determined for each nitrogen source (symbionts and diet) in both experimental groups varies by ~7‰. This variation is consistent regardless of nitrate-uptake efficiency (Table 5). In addition, the total range in percentages calculated at each nitrate fractionation level (0‰, −5‰, or −10‰) is 4‰ (Table 5). These data show that the fractionation-induced error that is propagated through the model calculations does not produce highly variable results.

Discussion

The model calculations have shown that when nitrate uptake by the symbionts is at a maximum, only 27% of the

nitrogen required for balanced endosymbiont growth can be supplied by NO_3^- diffusion. The remaining (73%) nitrogen must be derived from the recycled nitrogen pool, which is comprised of NH_4^+ excreted by the foraminifera. Using these data and the $\delta^{15}\text{N}$ values measured for the model components (Table 1), the $\delta^{15}\text{N}$ values of the symbionts ranged between 47 and 50‰, reflecting nitrogen input from the ^{15}N -enriched NO_3^- pool. At less efficient nitrate uptake in nitrate-deficient environments, the $\delta^{15}\text{N}$ values of the symbionts were 32–34‰, which is consistent with a higher percentage of nitrogen from the NH_4^+ pool (Table 4). The model results have also shown that when nitrate uptake by the endosymbionts is at a maximum, the percent of nitrogen transferred from the symbionts is approximately equal to the amount of nitrogen incorporated from the diet N (Table 5).

However, when nitrate uptake is less efficient, the symbionts can contribute almost all the nitrogen sequestered in the foraminifera (Table 5). Under these conditions, the $\delta^{15}\text{N}$ values of the shell-bound organic matter in the foraminifera do not seem to be greatly influenced by the nitrogen derived from the diet. This may suggest that when the nitrate levels are low, the nitrogen derived from the diet is not completely incorporated by the foraminifera. This nitrogen may be excreted as NH_4^+ , and therefore recycled by the symbionts so that both organisms may subsist.

If over 90% of the total nitrogen in the foraminifera is derived from the symbionts when nitrate concentrations can support <10% of the required nitrogen, then we must ask, what is the primary function of the diet? Results from culture experiments, in which symbiont-bearing foraminifera were grown in the dark, suggest that the organisms will not grow unless a diet is provided (Spero pers. comm.). Therefore, the diet must supply critical nutrients to sustain and promote growth of the symbionts and the foraminifera. If the phosphate concentration of the culture solutions is too low to sustain growth of the symbionts and the foraminifera, then the diet may compensate for the phosphate deficiency.

Phosphorous requirements for balanced growth—The amount of phosphorous required for the balanced growth of the symbionts can be calculated from the net carbon assimilation rate for *O. universa* determined for our culture conditions (4.79 nmol C h⁻¹). Using the Redfield C:P ratio of 106:1 and a photosynthetic quotient of 1, ~45 pmol P h⁻¹ (4.79/(106/1)) would be required to maintain balanced growth in our experiments.

The phosphorous concentrations in the culture solutions were 0.05 ppm, or 0.5 pmol PO_4^{3-} mm⁻³. The amount of PO_4^{3-} that can be assimilated by the symbionts is dependent on the diffusion of PO_4^{3-} to the cell membranes of the endosymbionts under the culture conditions. Applying Eq. 2 to the culture conditions (Table 2), and assuming the most efficient uptake of PO_4^{3-} , the diffusion flux of nitrate to the endosymbiont is 0.78 pmol P h⁻¹. Consequently, diffusion of PO_4^{3-} to the endosymbionts can account for only 2% (0.78/45) of the PO_4^{3-} required for balanced growth. The remaining 98% of the required PO_4^{3-} must be derived from the diet.

Thus, in terms of PO_4^{3-} , the foraminifera–symbiont system may be diffusion limited, and prey capture may provide the

necessary PO_4^{3-} to sustain growth. Despite the fact that the algal symbionts can produce excess organic carbon (Jørgensen et al. 1985) and nitrogen, perhaps growth can only be maintained if enough phosphorous is obtained through the diet. The captured prey supplies the foraminiferan and its symbionts with essential nutrient packages with C:N:P ratios similar to the biomass that must be developed (Jørgensen et al. 1985). The overall benefit of the symbiotic relationship is that the foraminifera would not have to respire the energy derived from its prey to cover its metabolism (Jørgensen et al. 1985). The extra energy necessary for metabolism could be derived from the algal photosynthates, which could allow the foraminifera to efficiently retain and internally recycle nutrients from its prey (Jørgensen et al. 1985; Snidvongs and Kinzie 1994). This is particularly important, as these organisms are typically found in nutrient-poor, oligotrophic environments (Jørgensen et al. 1985; Spero and Parker 1985; Spero et al. 1991).

Conclusion

The experiments conducted here have isolated each pathway of the symbiotic system to quantify the amount of nitrogen translocated to the foraminifera from its symbionts and its captured diet, and to model the flow of nitrogen through the foraminifera–symbiont system. We assume that the nutrient uptake by the endosymbionts is diffusion limited, which is consistent with other interpretations of the flux of nutrients in these types of symbiotic relationships (e.g., Wilkerson and Muscatine 1984; Jørgensen et al. 1985). Based on the present work, we suggest that under highly efficient uptake of nitrate by the symbionts, the endosymbiont contribution of nitrogen to the foraminifer ranges between 50 and 57% and the remaining nitrogen is derived from the diet. At less efficient nitrate uptake, 90 to 100% of the nitrogen in the foraminifera could be obtained from the symbionts from the recycled ammonium pool. The diffusion of PO_4^{3-} to the symbionts can provide only 2 to 36% of the phosphorous necessary for balanced growth, which suggests that the majority of the phosphorous must be obtained from the diet. These data demonstrate the importance of recycled nutrients in facilitating growth of these planktonic organisms. Assuming that these relationships can be applied to oligotrophic areas of the open ocean, efficient nutrient recycling in the foraminifera–symbiont system is critical in maintaining growth and hence survival of these organisms in these environments where nutrients are highly limited.

The data presented here show that there is a limited correlation between the isotopic composition of the NO_3^- in the culture solutions and the $\delta^{15}\text{N}$ values of the foraminifera. It appears that recycled nitrogen (NH_4^+) is a more important source of nitrogen to the symbionts and the foraminifera than nitrate. Understanding the sources and pathways that control nitrogen flow within the foraminifera–symbiont system is important not only to modern marine environments, but also to ancient marine systems, because foraminifera–symbiont associations have a unique application to the fossil record.

In light of these findings, we suggest that caution be used when interpreting the $\delta^{15}\text{N}$ values of shell-bound organic

matter in fossil symbiont-bearing foraminifera. These values most likely reflect the $\delta^{15}\text{N}$ values of the NH_4^+ recycled through the system, and therefore may not reflect the $\delta^{15}\text{N}$ values of surface-water nutrients. While interpretations of the fossil records can be obtained from the $\delta^{15}\text{N}$ values of shell-bound organic matter in fossil foraminifera, much more work is needed if absolute determinations of the $\delta^{15}\text{N}$ values of surface-water nutrients in ancient oceans are to be made.

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