Reports

Nitrogen Source for a Detritivore: Detritus Substrate Versus Associated Microbes

Abstract. *Detritivores have been thought to derive nutrition from microbes associated with detrital particles and not from the nonliving plant substrate. The polychaete* Capitella capitata, *however, was found to derive a major portion of its nitrogen from the plant substrate when it feeds on detritus derived from seaweed. Microbes play a role as a nitrogen source for this detritivore when it feeds on detritus derived from marsh grass.*

Nitrogen availability limits the growth of many aquatic detritivores (*1*) and therefore may be important in regulating benthic production. It is generally believed, from indirect evidence, that the major source of nitrogen for consumers are the microbes growing on the nonliving plant substrate (*2*). The idea of a detritus → microbe → detritivore food chain has become entrenched, probably because most research has focused on relatively refractory types of detritus such as marine vascular plants and deciduous leaves (*3*). In contrast, for seaweed-derived detritus, nitrogen in the plant material itself may be available to consumers (*3*). The issue is whether the detritus substrate, depending on its source or biochemical composition, may be directly available to detritivores.

To investigate the possibility that the nitrogen source for detritivores may be a nonliving plant substrate, we used ^{15}N as a tracer to determine whether microbe or substrate is the more important nitrogen source for the polychaete *Capitella capitata*, which was fed detritus derived from seaweed and marsh grass.

The red seaweed *Gracilaria foliifera* was cultured for 4 days in an outdoor tank receiving ^{15}N-labeled NH_4Cl (0.3 g per day; 95 atom percent); *G. foliifera* was also grown under similar conditions except ^{14}N-labeled NH_4Cl was added instead of ^{15}N label. Individual seedlings of the marsh grass *Spartina alterniflora* were transplanted from the field into 15 plastic pots, which were placed in outdoor tanks with standing, aerated seawater. Labeled NH_4Cl (1.3 mg of ^{15}N) was added to each pot weekly for 3 months. *Spartina* was collected from the field for use as nonlabeled material (*4*). All plants from a treatment were combined, ground to pass through a 250-μm mesh, washed with seawater to remove unincorporated ^{15}N, and then freeze-dried. On two separate occasions, these stocks of plant material were aged and fed to the *C. capitata*.

Three different aging treatments were used to generate detritus: either substrate and microbes both contained ^{15}N (Fig. 1A), only substrate contained ^{15}N (Fig. 1B), or only microbes contained ^{15}N (Fig. 1C). Plant material was aged for 3 days in ^{15}N- or ^{14}N-enriched f/2 medium (*5*). Location of the label was not unequivocal. For example, microbes may incorporate ^{15}N from the ^{15}N-labeled substrate in treatment B. Tracer studies involving labile substances such as nitrogen almost always suffer from recycling of label (*6*), but comparison of the ^{15}N content of detritus after the three aging treatments provides information on the location of the label.

Analysis of the ^{15}N content of marsh grass and seaweed showed good enrichment of plants grown in the presence of ^{15}N and low background concentrations of unlabeled plants (Fig. 2). The relatively lower ^{15}N enrichment of marsh grass was probably because the $^{15}NH_4Cl$ added to the sediment was diluted by ammonia in the interstitial water.

Analysis of the ^{15}N content of detritus after the three aging treatments indicates that the availability of ^{15}N in the aging medium markedly affects the ^{15}N content of the substrate-microbe complex. Unlabeled marsh grass and seaweed increased in ^{15}N enrichment after aging in the presence of ^{15}N-labeled NH_4Cl (Fig. 2). Presumably, this increase represented growth of microbes that incorporated ^{15}N from the medium or turnover of microbial nitrogen, or both. In treatments A and B, ^{15}N released from the plant material was another source of ^{15}N for the microbes, but this pathway appeared to be relatively unimportant. If it were a significant pathway, ^{15}N-labeled plant material aged in the presence of ^{14}N (treatment B) would show identical ^{15}N enrichment before and after aging. In fact, excess ^{15}N decreased slightly for both seaweed- and marsh grass–derived detritus (Fig. 2); this result is consistent with dilution of ^{15}N in the substrate-microbe complex by microbial incorporation of ^{14}N from the medium. These findings indicate that our treatments labeled the intended component and that ^{15}N probably was not translocated between substrate and microbes to a great extent.

The large increase in ^{15}N content of ^{15}N-labeled marsh grass after aging in ^{15}N medium (treatment A) was probably due to microbial incorporation of ^{15}N from the medium. In contrast, ^{15}N-labeled seaweed had the same ^{15}N content after aging in ^{15}N- or ^{14}N-labeled medium. This suggests that microbial nitrogen was a smaller proportion of total nitrogen for seaweed detritus (initial N, 1.7 percent of dry weight) than for marsh grass detritus (initial N, 0.44 percent).

Spartina alterniflora and *G. foliifera* detritus, after the aging treatments, were

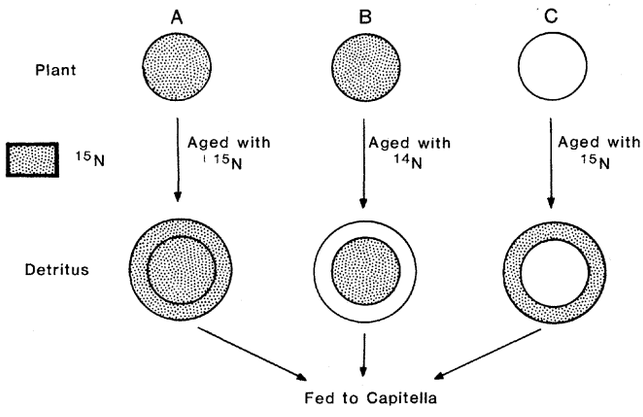

Fig. 1. Three aging treatments used to incorporate ^{15}N label: (A) both substrate and microbes contain ^{15}N, (B) only the substrate contains ^{15}N, and (C) only the microbes contain ^{15}N. Inner circles represent nonliving detrital substrate, and outer circles represent associated microbes. The diagram is not drawn to scale.

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fed to *C. capitata* in flow-through chambers for 4 days (*7*). The amount of food represented a nonlimiting nitrogen source for this polychaete (*1*), which was wet-weighed before and after feeding to measure growth (*7*). Before the experiment, representative worms were collected for total nitrogen (Perkin-Elmer CHN Analyzer) and ^{15}N analysis (*8*). Initial total nitrogen and ^{15}N content of each aged detritus was also measured. Incorporation of ^{15}N by the polychaetes was calculated from the initial and final worm weights with their respective total nitrogen and ^{15}N content (*9*).

Growth rates of *C. capitata* were roughly 15 percent per day when fed marsh grass detritus and about 25 percent per day when fed seaweed detritus (*10*). Growth rates were similar for all aging treatments for a given detritus.

Overall, ^{15}N enrichment in *C. capitata* (Fig. 2) reflected the ^{15}N content of the food. In all cases, the polychaete probably derived nitrogen from both substrate and microbes but comparison among treatments allows identification of the major nitrogen source. The three treatments yielded significant differences in ^{15}N incorporation rates for marsh grass and seaweed detritus (*11*). When *C. capitata* was fed marsh grass detritus, there was greater enrichment from treatments in which the microbes contained ^{15}N (Fig. 2), whereas *C. capitata* fed seaweed detritus showed the greatest enrichment in the treatments in which the substrate contained ^{15}N (Fig. 2). These results suggest that when fed seaweed detritus, the polychaete derived most of its nitrogen from the plant substrate but, for marsh grass detritus, microbes were a relatively more important nitrogen source.

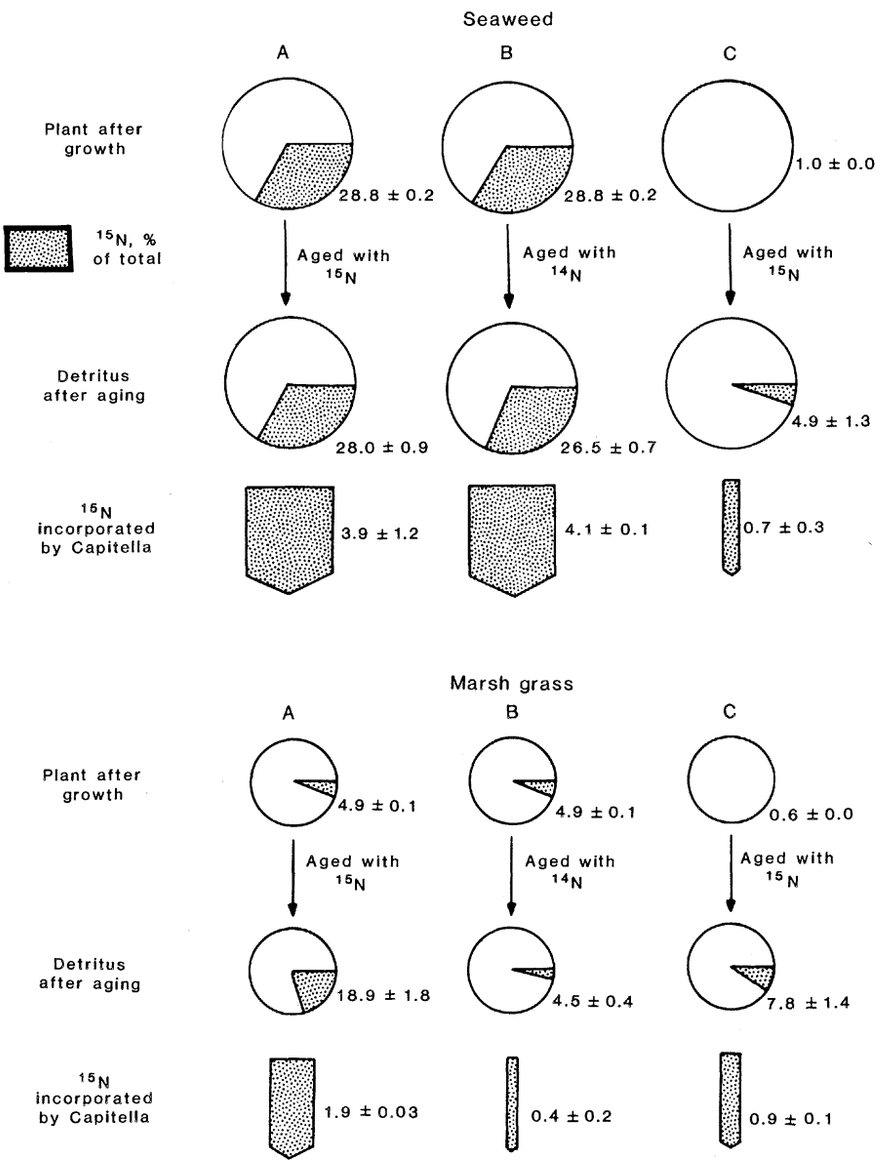

Fig. 2. The ^{15}N content of marsh grass and seaweed plant material and aged detritus and rate of ^{15}N incorporation by *C. capitata*. The area of the circle represents total nitrogen, and the stippled area represents ^{15}N content. Treatments A, B, and C as described in the legend to Fig. 1. Width of stippled arrows represent ^{15}N incorporation by *C. capitata*. Values of ^{15}N incorporation [micrograms per milligram of worm (dry weight) per day] are means ± 1 standard deviation of the mean (*9*).

The idea that only microbes provide nutrition for detritivores has arisen due to the preponderance of research on refractory types of detritus (*12*). Microbes on the substrate or in the gut facilitate detritus assimilation in the case where substrate nitrogen is in some unavailable form such as complexes, chitin, or humic acids (*12*) although these may also be available directly (*13*). However, mass budgets of standing stocks of nonliving organic matter and bacteria, with their respective assimilation efficiencies, indicate that bacteria alone can not support the energetic requirements of deposit-feeders (*14*).

Our results provide direct evidence that, at least for detritus derived from *S. alterniflora* and *G. foliifera*, the availability of plant substrate to consumers is related to detrital source. We do not intend to generalize from two types of detritus, but these results indicate that the role of microbes in detritivore nutrition may vary and depend on the source of detritus. Apparently a variety of factors can regulate the transfer of detritus to consumers, and these factors can vary for detritus derived from different sources.

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4. Entire seaweed plants and aboveground portion of marsh grass were harvested. See B. E. Lapointe [*J. Phycol.* **17**, 90 (1981)] for description of seaweed culture system. *G. foliifera* growth rate (10 percent per day) and nitrogen content (1.7 percent, dry weight) of ^{15}N-labeled and unlabeled plants were within the range for plants grown under similar conditions. *S. alterniflora* growth (150 percent per month) and nitrogen content (0.44 percent, dry weight) were similar to values for high-marsh *S. alterniflora* [J. L. Gallagher, R. Reimold, R. Linthurst, W. Pfeiffer, *Ecology* **61**, 303 (1980)].
5. The f/2 medium was prepared as described by R. Guillard and J. Ryther [*Can. J. Microbiol.* **8**, 229 (1962)] but with twice the amount of nitrogen.
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7. Detritus (250 mg, dry weight) in six 30-cm^2 chambers (two detrital types and three aging treatments) were fed on each of two separate occasions. Growth measures are described by

K. R. Tenore and R. B. Hanson [*Limnol. Oceanogr.* **25**, 553 (1980)].
8. The ^{15}N content was determined for two subsamples of plant material and a subsample of aged detritus from each of two replicate tests. All worms from a chamber on each occasion were ground together and a subsample taken for ^{15}N analysis. The ^{15}N analysis itself is reproducible to within ± 2 percent [H. Paerl and P. Bland, *Appl. Environ. Microbiol.* **43**, 218 (1982)].
9. Incorporation of ^{15}N [micrograms of ^{15}N per milligram of worm (dry weight) per day] = growth (per day) × nitrogen content [micrograms of nitrogen per milligram of worm (dry weight)] × ^{15}N content (micrograms of ^{15}N per microgram of nitrogen, in the worms).
10. *C. capitata* is a rapidly growing, opportunistic species which can grow 10 to 30 percent per day when fed seaweed detritus and 3 to 12 percent when fed marsh grass detritus (K. Tenore, unpublished data).
11. Three treatments with two observations per treatment, $P = .01$, $N = 6$, Kruskal-Wallis test for both marsh grass and seaweed detritus.
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15. We thank Drs. D. Menzel, J. Meyer, L. Pomeroy, and B. Wallace for helping improve early drafts, D. Lehsau for drafting the figures, and B. Christiansen for typing. This work was supported by NSF grant OCE82-00385 to K.R.T.
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5 March 1982; revised 8 June 1982

Clustering of Leukocyte and Fibroblast Interferon Genes on Human Chromosome 9

Abstract. *At least ten leukocyte interferon genes and the single known fibroblast interferon gene have been localized on the pter→q12 region of human chromosome 9. Gene mapping was accomplished by blot hybridizations of cloned interferon complementary DNA to DNA from human-mouse cell hybrids with a translocation involving human chromosome 9. Supporting evidence suggests these genes are clustered.*

The interferons are a family of proteins that convey viral resistance in target cells, and also affect cell proliferation and the immune response (*1*). Three distinct forms of human interferon have been classified according to their biochemical and antigenic properties: IFN-α (leukocyte), IFN-β (fibroblast), and IFN-γ (immune) (*2*). Molecular cloning studies have led to the identification of more than ten distinct genes coding for IFN-α (*3*, *4*), and a single gene each for IFN-β (*5*) and IFN-γ (*6*). DNA sequence determinations indicate that the genes for IFN-α are closely related (80 to 95 percent nucleotide sequence homology), and the gene for IFN-β contains 40 to 50 percent sequence homology to the genes for IFN-α. The genes for IFN-α and IFN-β do not contain introns. By contrast, the IFN-γ has no significant DNA sequence homology with the IFN-α and IFN-β genes, and the coding region of the gene is interrupted by three introns (*6*).

These observations suggest that genes for IFN-α and the gene for IFN-β are more closely related evolutionarily than either is to the gene for IFN-γ [for gene mapping nomenclature, see (*7*)]. Because of these similar properties, it is of interest to determine whether the several leukocyte and the fibroblast interferon genes are clustered on the same chromosome. Clustering of the genes might be an important aspect of the control of their expression and of the evolutionary origin of the interferon genes. We had previously assigned the genes for IFN-α and IFN-β to chromosome 9 by using cloned interferon genes to examine human-mouse somatic cell hybrid DNA by the Southern hybridization technique (*8*). The chromosome 9 assignment of the gene for IFN-β has been confirmed (*9*, *10*). In addition, analysis of recombinant DNA clones established that several human IFN-α genes are located within several thousand nucleotides of one another (*4*). We now report the localization of leukocyte and fibroblast genes on the pter→q12 region of chromosome 9, suggesting that virtually all of the genes for IFN-α and perhaps the gene for IFN-β may be clustered.

Human fibroblasts (GM2836) with a reciprocal translocation [46,XY,t(9;17)(q12;p11)] involving chromosomes 9 and 17 (Fig. 1) were fused to mouse LM/TK$^-$ (thymidine kinase–deficient) cells, and cell hybrids were isolated (*11*). The translocation chromosomes 9/17 (9pter→9q12::17p11→17pter) and 17/9 (17qter→17p11::9q12→9qter) separate chromosome 9 into two regions with the breakpoint at 9q12 (see Fig. 1). The leukocyte and fibroblast interferon genes encoded on chromosome 9 can be localized on either of these two translocation chromosomes by using Southern blot analysis of DNA from somatic cell hybrids (*8*, *12*) individually retaining these rearranged chromosomes.

After cell fusion with polyethylene glycol (*13*), cell hybrids were isolated and cloned in the hypoxanthine-aminopterin-thymidine (HAT) selection medium (*11*) and examined for human chromosomes (*14*) and enzyme markers (*12*) assigned to each chromosome. The TK selectable marker located in the q21→q22 region of chromosome 17 [see (*15*)] was utilized with the HAT system to selectively retain one of the transloca-

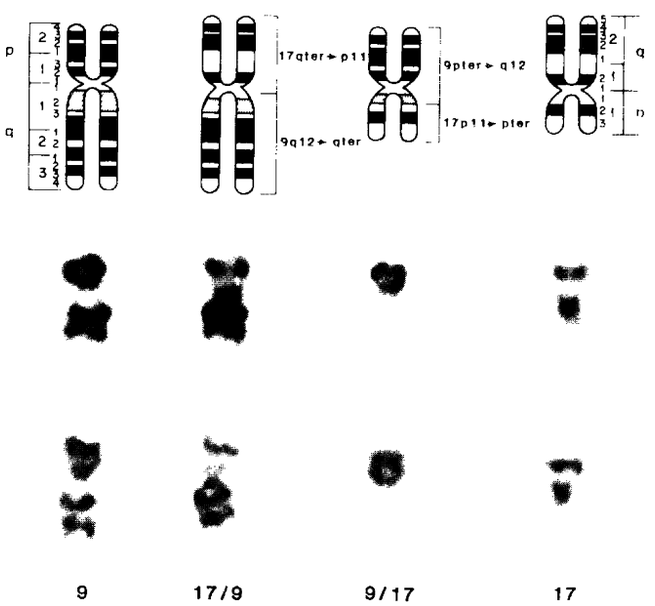

Fig. 1. The human fibroblast line GM2836, with a reciprocal translocation between chromosomes 9 and 17, was examined karyotypically by the Giemsa-trypsin procedure (*14*). A partial karyotype featuring the chromosomes involved is presented. All other chromosomes had a normal banding pattern. Nomenclature for the banding pattern follows the Paris Conference (*18*). The translocation has been designated [46, XY,t(9;17)(q12;p11)] and involves a 17/9 chromosome (17qter→17p11::9q12→9qter) and a 9/17 chromosome (9pter→9q12::17p11→17pter). These chromosomes are diagrammed (top) and examples from two different cells (bottom) are included for comparison. The fibroblasts (GM2836) were obtained from the Human Genetic Mutant Cell Repository, Camden, New Jersey.

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