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MICROBIAL CONTRIBUTIONS TO THE GROWTH AND DEGRADATION OF TROPICAL SEAGRASSES

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ABSTRACT

Seagrass ecosystems are an important link in the marine food web. These submergent vascular plants are found in both eutrophic and oligotrophic waters and their nutritional interactions with bacteria appear to be obligatory and complex. Bacterial contributions to the growth of seagrasses seem even more important in the nutrient poor waters and carbonate sediments of the Bahamas.

Results presented in this communication indicate that seagrass roots become colonized by water column bacteria. This colonization is plant species specific and is maintained by root exudation of fixed carbon and allelopathic compounds. In turn, the microflora protect roots by producing a mucopolysaccharide covering. Within this matrix, the microflora provide available nutrients for plant growth. For example, nitrogen fixation occurs on seagrass roots and leaves, and phosphate solubilization has been suggested in the seagrass rhizosphere.

Seagrass tissue degrades relatively quickly compared with other plants. Degradation is also mediated by the microflora. During the breakdown of seagrass tissue, a succession of bacterial abundance and populations appears to take place. Because of seagrass-bacterial interactions, an overall increase in nutrient status, diversity and stabilization of the ecosystem results.

INTRODUCTION

The importance of seagrasses in the overall ecology of coastal marine environments has been well documented (Phillips, 1978; Zieman, 1982; Thayer and others, 1984). Seagrasses not only form the basis for complex food webs in the water column (Penhale, 1977, McRoy and Helfferich, 1977) but also form the structural and nutritional basis of complex communities in the sediment (Orth, 1973; Penhale and Wetzel, 1983).

Bacteria may be the organisms most closely associated with seagrasses. They have been observed as leaf epiphytes (Kirchman and others, 1980, 1984) where they appear to utilized excreted compounds (Penhale and Smith, 1977; Penhale and Thayer, 1980) and contribute toward the nutrition of the plants by fixing nitrogen (Capone and Taylor, 1977; 1980; Capone and others, 1979; Smith and Hayasaka, 1982a; 1982b). Similar, but perhaps more complex, relationships between seagrass root-rhizome systems and bacteria have also been reported. Overall microbial activity appears relatively high in the seagrass rhizosphere (Moriarty and Pollard, 1981; 1982; Smith and Hayasaka, 1986). In addition, reported microbial nutrient transformations include nitrogen fixation (Capone, 1982; 1983; Capone and Budin, 1982; Patriquin and Knowles, 1972; Smith and Hayasaka, 1982a; 1982b) nitrification (Boon and others, 1986a; Iizumi and others, 1980) ammonium generation (Boon and others, 1986b, Iizuni and others, 1982; Smith and others, 1984) and phosphate solubilization (Craven and Hayasaka, 1982). These nutritional interrelationships indicate a close association between seagrasses and their microflora.

This communication reports results of experiments designed to help understand both structural and nutritional aspects of the seagrass-microflora consortium. From this and other studies, a conceptual model of microbial colonization and establishment on seagrass roots is proposed.

MATERIALS AND METHODS

Sampling Methods

Seagrass samples were obtained from North Carolina (*Zostera marina* and *Halodule wrightii*), Florida, and San Salvador, Bahamas

(*Thalassia*, *Halodule* and *Syringodium*). Seagrass samples were removed from the sediment with corers of various dimensions. All samples requiring the isolation of microflora and nutrient transformations were taken with 9.8 x 12 cm corers and removed as aseptically as possible (Fig. 1). Bacterial isolates were grown on a glycerol-artificial seawater medium as described by Smith and others, 1982). Scanning electron microscopy was performed on root-rhizome segments or freeze-fractured roots as described by Smith and others (1979) and Kenworthy and others (1987).

Acetylene Reduction

Nitrogenase activity was measured using the acetylene reduction technique (Hardy and others, 1968; Steward and others, 1967). Root or leaf samples were placed into 50 cc syringes from which 5 cc of air were removed and replaced with acetylene. Syringes were incubated at 25°C. After incubation, 0.5 ml of gas mixture were removed from each syringe and injected into a Varian Aerograph 912 gas chromatograph fitted with a stainless steel column (10' x 1/8") packed with Poropak-R (100-120 mesh). The chromatograph was equipped with a flame ionization de-detector and operated under the following conditions: N2 carrier gas flow rate, 30 ml/min; H2 flow rate, 30 ml/min; air flow rate, 90 ml/min; injector, detector, and column temperatures were maintained at 100°, 100° and 50°C, respectively. Ethylene peak heights were compared with known standard concentrations.

Seagrass leaves were incubated either under fluorescent lights or in the dark. Root samples were incubated either directly after washing in a stream of sterile 3.5% Rila salts (Rila Products Co., Teaneck, NJ) or after surface sterilizing. Roots were surface sterilized for 15 seconds (or until bleaching occurred) in a solution of 1.05% hypochlorite in artificial seawater (Rila Salts Mixture). Surface sterilization was checked by spreading washed, sterilized root sections on agar plates and incubating for three weeks. No growth was detected on plates containing surface sterilized root sections. Plates with unsterilized root surface sections showed abundant growth within two days. After incubation, plant tissues were removed from the syringes and dried to constant weight in an 80° hot air oven. All determinations were reported as means of triplicate samples.

Ammonification Assay

Ammonification rates were determined for leaf and root-rhizome tissue from San Salvador seagrasses. Either leaf or root segments (2.0 cm) were placed into 60 ml serum bottles containing 25.0 ml of sterile seawater. Half of the serum bottles also contained glutamic acid (final concentration 10-3M) to determine potential ammonification rates. Bottles without the glutamate were used to measure actual ammonification rates. Subsamples were removed from the serum bottles 0, 24 and 48 hours after addition of plant material and assayed for ammonium using the Solorzano method as described by Strickland and Parsons (1972). The means of triplicate assays were reported.

RESULTS AND DISCUSSION

Seagrasses form extensive root hairs (Figures 2 and 3). This root hair network provides a large surface area for nutrient absorption and microbial colonization (Smith and others, 1979; Smith and Thayer, 1987). Morphological and biochemical characteristics of microbial isolates indicated that those colonizing the root surface were more similar to water column microflora than to sediment microflora. Figure 4 shows that the surface of the roots are covered with an amorphous substance containing inorganic particles as well as microbial cells. Pure culture studies of the root microflora indicated that all rhizoplane isolates were capable of producing extensive mucopolysaccaride capsules. Although plants can also exude polysaccarides along their roots (Rovira, 1965), it appears that most of this "mucigel" was of microbial origin.

The close physical association between seagrass roots and their rhizoplane microflora, in this mucopolysaccaride matrix, provides an environment which could be beneficial to both microbes and plants. For example, Smith and others (1982) suggested that both plant and bacterial components of the rhizoplane may be protected from toxic effects of heavy metals, often found in anoxic sediments, by the establishment of the mycopolysaccaride matrix. The matrix would retarde the diffussion of reduced

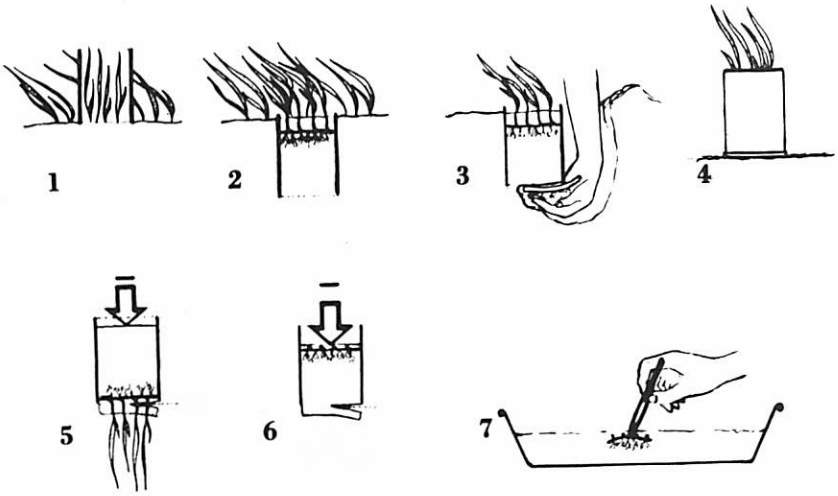

Fig. 1. Isolation technique for obtaining rhizoplane microflora from seagrass roots. 1) Leaves in coring tube, 2) Corer inserted into sediment, 3) Corer capped from below, 4) Core removed to lab, 5) Top 2 cm removed with sterile knife, 6) bottom 2cm removed, 7) Adhering sediment removed in sterile artificial seawater.

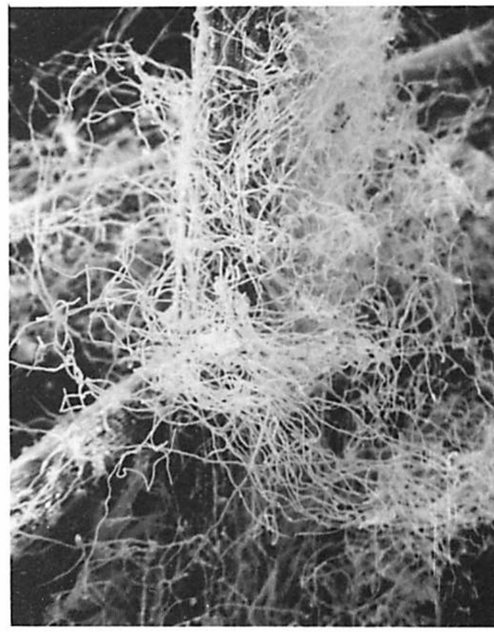

Fig. 2. Scanning electron micrograph of extensive root hairs on *Zostera marina* (40x).

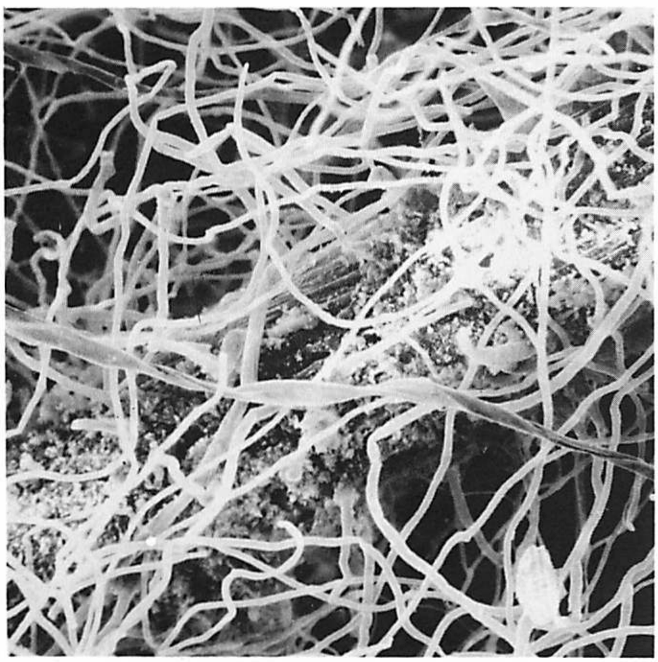

Fig. 3. SEM of *Zostera marina* root hairs and root surface (150x).

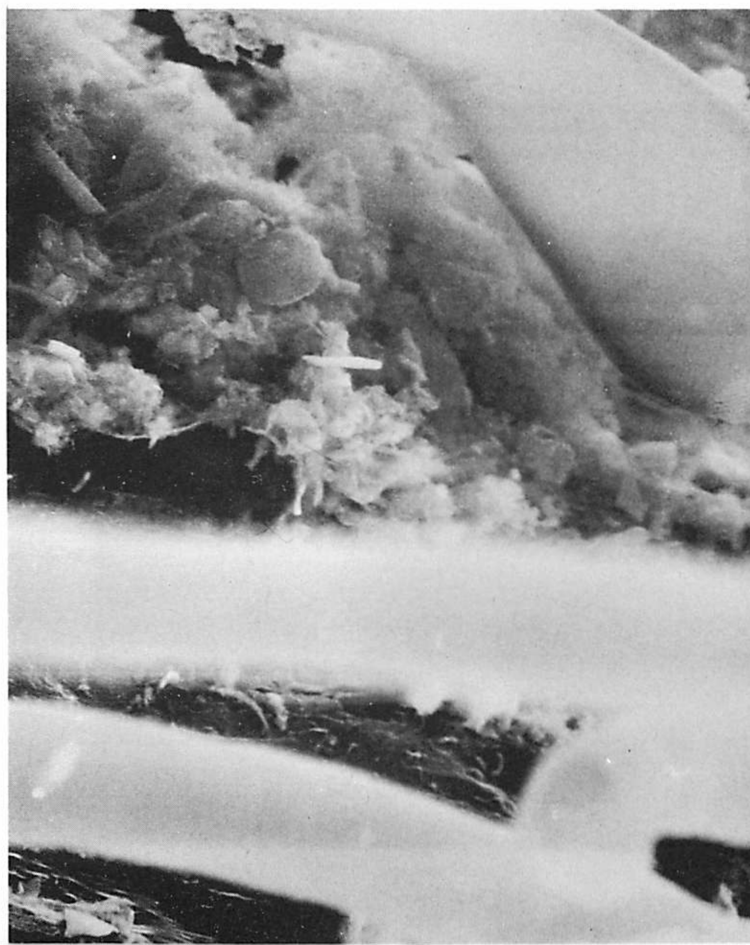

Fig. 4. SEM of seagrass root surface showing mucopolysaccaride Matrix (4800x).

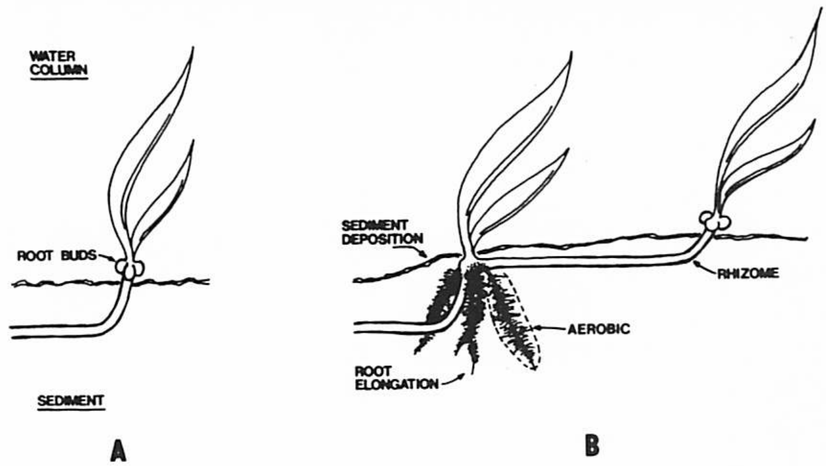

Fig. 5. Microbial colonization of seagrass roots. (A) Initial colonization of root buds. (B) Establishment of rhizoplane population during root elongation.

Fig. 6-8. Freeze-fractured SEM of bacteria localized in cortical tissue of *Halodule wrightii.*

Fig. 6. (5200x).

Fig. 7. (14000x).

Fig. 8. (8000x).

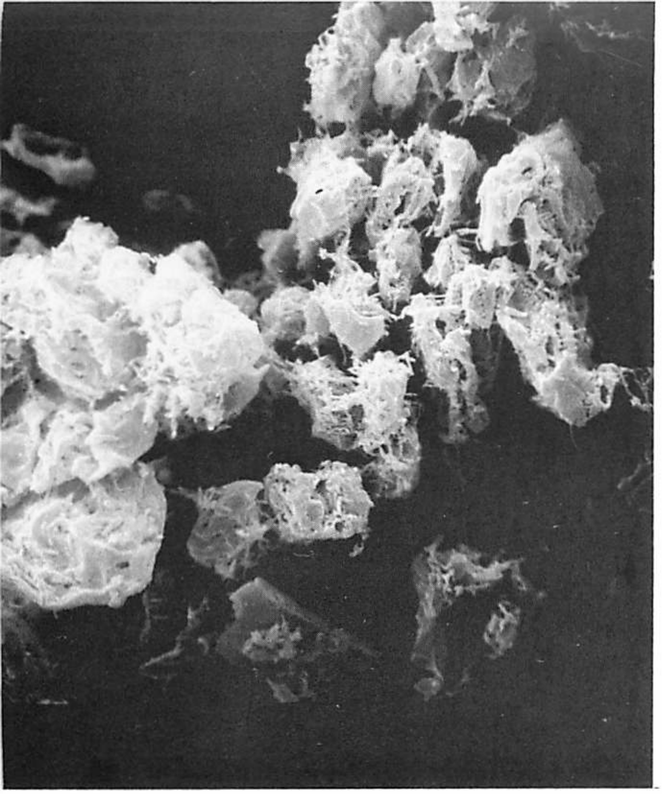

Fig. 9. SEM of degrading seagrass tissue (400x).

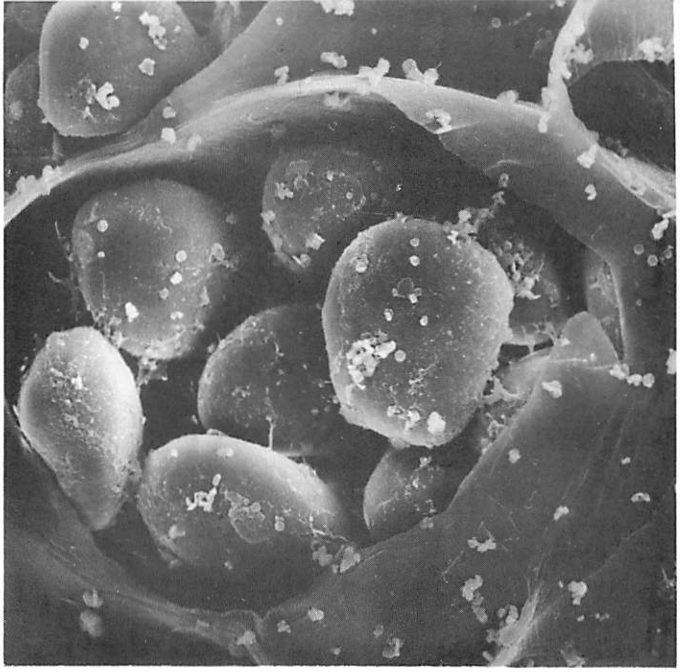

Fig. 10. Starch granules in degrading rhizome tissue showing microbial colonization (800x).

Fig. 11-12. Advanced degradation of seagrass rhizomes showing complex assemblages of bacteria.

Fig. 11. (2400x).

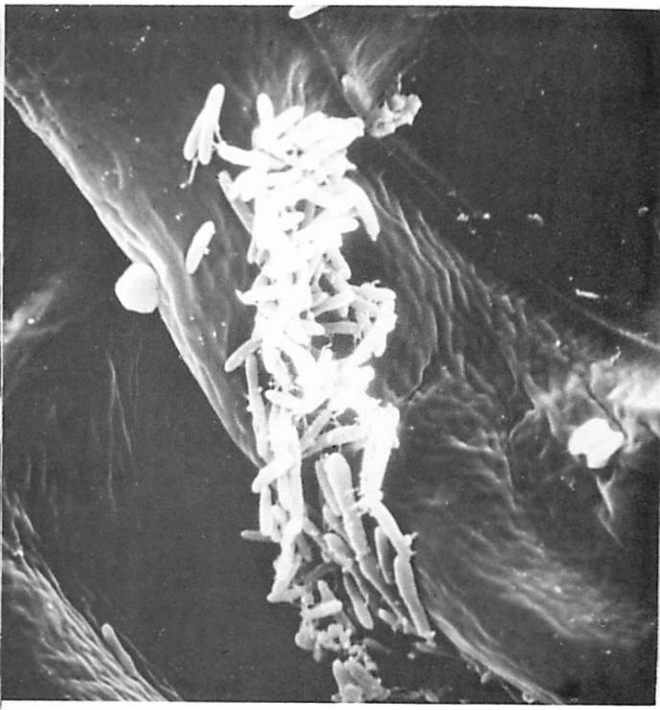

Fig. 12. (3400x).

metal ions until they were oxidized and precipitated by the microflora. Oxygen could be provided to the microbes by internal transport through the plants' lacunal system (Oremland and Taylor, 1977). This is supported by the observation that some of the seagrass rhizoplane isolates were strict aerobes.

The fact that strict aerobic bacteria were isolated from the seagrass rhizoplane, growing in anoxic sediment, also indicates that the origin of the microflora may be the water column. Figure 5 suggests a possible mechanism of microbial colonization of the seagrass rhizosphere. Because of the morphological and biochemical similarities of the rhizoplane and water column isolates, and because of the aerobic nature of some of the rhizoplane isolates, it is believed that initial colonization takes place on root buds in the water column (Fig. 5A). There is evidence that chemotaxis toward root exuded amino acids (Wood and Hayasaka, 1981) and allelopathy (unpublished) may also play a role in initial colonization. Once root buds have been colonized and covered with sediment (Fonseca and others, 1983), the rhizoplane microflora replicate during root elongation (Fig. 5B). The interrelationship between the seagrass roots and microflora would be maintained by a variety of nutrient interactions.

Among the various nutrient interactions between seagrasses and their microflora, nitrogen transformations may be the most important in many environments (Kenworthy and others, 1982; Short, 1983a, 1983b). Among the nitrogen transformations, nitrogen fixation has been most extensively studied (Capone, 1983). Data in Table 1 indicate that (except for *Syringodium*) rates of nitrogen fixation (acetylene reduction) increase when leaf samples are incubated in the light. This light induced increase in rates probably is due to photosynthetic stimulation of cyanobacterial leaf epiphytes (Goering and Parker, 1972).

Rhizosphere nitrogen fixation rates can be much higher than phyllospheric rates (Table 2). In addition, eliminating the surface microflora by hypochlorite treatment indicates the possible presence of nitrogen-fixing root endophytes. Figures 6-8 show root cortical cells from *Halodule* which are colonized by bacteria. Smith and Hayasaka (1982b) identified nitrogen-fixing *Klebsiella* isolated from surface-sterilized *Halodule* roots. The presence of these bacteria inside the roots was later confirmed by fluorescent antibody studies (Schmidt and Hayasaka, 1985). Data in Table 2 indicate that a similar relationship may exist with *Syringodium*.

Deamination of amino acids and peptides that occur as root exudates (Wood and Hayasaka, 1981) or as detritus (Kenworthy and others, 1982) may be another significant role that the microflora play in nitrogen

Table 1. Acetylene Reduction in the Phyllosphere of Selected Seagrasses.

Sample	Incubation Conditions	nmoles C_2H_4/gdwt/d
Thalassia	Light	Trace
	Dark	Trace
Halodule (FL)	Light	48 + 29
	Dark	Trace
Syringodium	Light	141 + 108
	Dark	205 + 43
Zostera	Light	877 + 432
	Dark	382 + 178
Halodule (NC)	Light	584 + 181
	Dark	292 + 86

Table 2. Rhizosphere Acetylene Reduction Rates of Selected Seagrasses.

Sample	Hypochlorite Treatment	nmoles C_2H_4/gdwt/d
*Thalassia**	-	Trace
	+	Trace
Halodule (Florida)	-	2799 + 1333
	+	1470 + 1324
Syringodium	-	2838 + 635
	+	2084 + 586
Zostera	-	524 + 246
	+	Trace
Halodule	-	2675 + 1525
	+	1595 + 909

* Although these particular *Thalassia* samples had very low rates of acetylene reduction, other reports have observed much higher rates. (see Capone, 1983).

cycling associated with seagrass roots. This is particularly likely because ammonium is the preferred form of nitrogen available to seagrass roots (Short and McRoy, 1984). At least some of the nitrogen available to living seagrasses is formed in place by the degradation of root rhizome systems (Kenworthy and others, 1987). Figures 9 and 10 show starch granules in *Zostera* detritus becoming colonized by bacteria. Bacteria also colonize other detrital plant cells (Figures 11 and 12). At least some of these bacteria appear to be nitrogen fixers (Kenworthy and others, 1987). Table 3 suggest different nitrogen transformations associated with the seagrass rhizosphere and the probable locations of each process.

Table 3: Nitrogen Transformations in the Seagrass Rhizosphere

Outer Rhizosphere	Rhizoplane	Root
Anaerobic N2-Fixation	Aerobic N2-Fixation	N2-Fixation (*Halodule*)
Denitrification	Nitrification	N-Uptake
Ammonification	Ammonification	Exudation Amination Transamination

Nitrogen, however, is not the only nutrient that is made available to seagrasses through microbial transformations. In fact, there is evidence that phosphorous is the limiting seagrass nutrient in the carbonate sediments of the Bahamas (Short and others, 1985). Rhizosphere bacteria can provide phosphate to seagrass roots by solubilizing minerals (Craven and Hayasaka, 1982), but much more work will have to be performed before the functional significance of this process is understood.

In summary, observations presented in this and other reports point out the nutritional importance of the microflora to seagrass growth. Most of the work, to date, has dealt with nitrogen nutrition. It is probable that other seagrass nutrients are also subject to microbial transformations but mechanisms and significance awaits further research.

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