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Evidence for Microfloral Succession on Allochthonous Plant Litter in Apalachicola Bay, Florida, USA

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Abstract

Changes in hydrolytic, respiratory, catabolic and lipid biosynthetic activities depend at least in part on successional changes in the microfloral populations of allochthonous plant litter incubated in a semi-tropical estuary. Initial colonization is by populations which have a high content of muramic acid relative to the adenosine triphosphate (ATP) and which are progressively displaced by a microflora with a lower ratio of muramic acid to ATP. Scanning electron micrography of the plant-litter microflora shows a succession of forms, with an initial bacterial colonization and its progressive displacement by more complex forms. Estimates of the microbial mass and the rates of phospholipid synthesis suggest that the detrital microflora has a relatively slow growth rate compared to its growth potential.

Introduction

To accurately study microbial population dynamics, the symbiotic associations and the advantages of surfaces must be maintained (Alexander, 1964; Jannasch and Pritchard, 1972). Enumeration of microorganisms by cultural methods characteristically results in underestimates 1 to 2 orders of magnitude below direct counts (Jannasch and Jones, 1959; Gray *et al.*, 1968) or biomass estimates based on adenosine triphosphate (ATP) levels (Holm-Hansen, 1973), and the selectivity in cultural media distorts the *in situ* microbial activities. Increasingly, measurements of the metabolic activities of microbial populations have been utilized.

Rates of lipid synthesis by detrital microflora have been shown to correlate with enzyme activities, respiratory activities, and the ATP content of the biomass (White *et al.*, in press). In the present study, the colonization of allochthonous plant litter incubated in a semi-tropical estuary will be reported. Changes in enzymatic activities, respiratory activities, and rates of phospholipid and neutral lipid biosynthesis will be compared to the active biomass of the detrital microflora as estimated by the total ATP. ATP has been shown to represent an accurate measure of the biomass in soil and litter (Ausmus, 1973). Muramic acid is a hydrolytic product from the muramyl peptide polymers in the cell walls and is found exclusively in bacteria and blue-green algae (Salton, 1960). Comparison of the muramic acid to ATP content of the litter microflora suggests a succession from a primary bacterial colonization toward a mixed population of bacteria, fungi and algae, that can be confirmed by the sequential morphological changes in the detrital microflora determined by scanning electron microscopy. It will be suggested that at least a part of the changes in metabolic activity represents succession in the micropopulations.

Fungal succession has been clearly documented on decaying fern brackens (Frankland, 1966), senescent mangrove leaves (Fell and Master, 1973), red mangrove seedlings (Newell, 1973), leaf surfaces during senescence and fall (Hudson, 1971) and in soil (Garrett, 1963). Successions involving bacteria have been described in soils and plant litter (Alexander, 1964; Gyllenberg and Eklund, 1974), in the formation of dental plaques in vertebrates (Gibbons and Van Houte, 1973), in the relatively late activation of bacillary spores in soils (Mishustin and Mirsoeva, 1968), in the colonization of glass capillaries in soils and sediments (Perfil'ev and Gabe, 1969), on glass slides incubated in seawater (Marshall

et al., 1971) and on electron microscope grids immersed in lake water (Jordan and Staley, 1976).

Materials and Methods

Sampling

Dried leaves from an isolated live oak (*Quercus virginiana*) on the Florida State University campus, and needles from a slash pine (*Pinus elliottii*) stand in Tallahassee, Florida, were gathered after winter leaf fall and stored in plastic bags. Approximately 200 g of plant material were placed in cubical baskets made of 2.54 cm mesh neoprene-coated hardware cloth, 30.5 cm on each side, lined with 1.3 mm mesh fiberglass screen on the sides and bottom and 6.4 mm screen on the top. These baskets were placed at Station 4A in East Bay, in the Apalachicola Bay system, Florida (Fig. 1) for up to 6 weeks during the period 17 June to 29 July, 1975. The characteristics of this relatively unpolluted estuary have been described by Livingston *et al.* (1974). The water quality data for this station during the sampling period are given in Table 1. Baskets were removed at weekly intervals and transported to the laboratory at ambient temperature in water mixed and aerated to maintain an oxygen tension of 6 to 7 ppm. Analyses were begun immediately on return to the laboratory.

Oak leaves were cut into 6.5 mm diameter discs using a standard paper punch. Pine needles were cut into 15 mm lengths. All discs or segments needed for all analyses for one sampling point were placed in a large container in aerated estuarine water; leaf discs or needle segments for each procedure were drawn from the same pool.

The weights of equivalent areas of plant litter for each sampling time were determined by drying 10 sets of 10 leaf discs or 10 needle segments at 80°C *in vacuo* to a constant weight and determining the mean sample weight. Assay values are expressed per gram of plant litter.

Analytical Methods

Enzyme Activities

Activities of β-D-glucosidase, α-D-mannosidase, β-D-galactosidase, alkaline phosphatase, and phosphodiesterase were measured as the increase in absorbance at 410 nm due to the release of covalently bonded p-nitrophenol from the chromogenic substrates, p-nitrophenyl-β-D-glucoside, p-nitrophenyl-α-D-mannoside, p-nitrophenyl-β-D-galactoside, p-nitrophenyl phosphate disodium and bis p-nitrophenylphosphate (Sigma Chemical Co., St. Louis, Missouri), respectively.

Ten randomly selected leaf discs or segments from the large sample pool described above were used for each sample. The initial substrate concentration was 5 mM; the suspending medium was 25 mM $NaHCO_3$ buffer, pH 9; the temperature was maintained at 25°C. The reaction was stopped at 30 min by rapid freezing in ethanol-dry ice. Samples were thawed and immediately filtered through a 0.45 µ Millipore filter. The color was developed further by the addition of 4 ml of 50 mM $NaHCO_3$ buffer, and samples were read in a Gilford 2400-S Spectrometer (Gilford Instrument Laboratories, Oberlin, Ohio).

Respiration

Respiration was measured as the rate of oxygen utilization detected with a Clark oxygen electrode and YSI model 57 sensor (Yellow Springs Instrument Co.). The electrode was inserted in a sealed chamber containing 10 leaf discs or needle segments and 10 ml of 0.05 M bicarbonate buffer, pH 9.0, at 25°C and monitored for 20 min. Chemical oxygen utilization, estimated after addition of 10% trichloroacetic acid to be less than 10% of the respiratory rate, was subtracted from the experimental determinations.

Heterotrophic Potential

Heterotrophic activity was determined after the methods of Parsons and Strickland (1962), Wright and Hobbie (1965), and Harrison *et al.* (1971). Five leaf discs or needle segments were incubated aerobically in 2 ml of filter-sterilized estuarine water with glucose-^{14}C(U) (New England Nuclear, Boston, Massachusetts), in successive concentrations of 1.74, 0.82, 0.44, 0.19, 0.12 and 0.06 µg/l. Each sterile 25 ml incubation flask was sealed with a serum stopper from which was suspended a plastic cup containing a 25 mm x 50 mm accordion-folded piece of Whatman No. 1 filter paper and incubated in the dark for 1 h at 30°C. At the end of the incubation period, 0.2 ml β-phenethylamine (Eastman Kodak Company, Rochester, New York) was injected through the stopper onto the filter paper to serve as a CO_2 trap. The reaction was terminated, and dissolved $^{14}CO_2$ was liberated by the injection of 0.2 ml 2N H_2SO_4. Flasks were shaken for 5 to

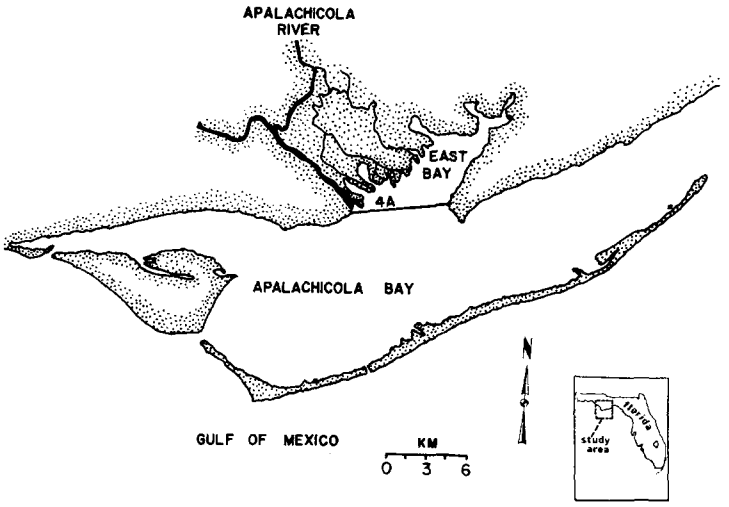

Fig. 1. Study area, Apalachicola Bay, Florida, USA

Table 1. Water quality data, East Bay of Apalachicola Bay System, Station 4A, 17 June - 29 July, 1975. nd: No data

Week	Date (1975)	Temperature (°C)		Salinity (‰)		Dissolved oxygen (ppm)		pH		Depth (m)
		Surface	Bottom	Surface	Bottom	Surface	Bottom	Surface	Bottom	
0	17 June	28.0	27.5	4.3	5.8	6.9	6.8	nd	nd	1.6
1	24 June	27.5	27.0	1.3	1.3	6.4	6.4	6.7	6.7	1.5
2	1 July	28.0	27.5	4.4	5.0	7.2	7.0	6.8	6.5	1.3
3	8 July	27.5	27.5	5.0	5.1	6.6	6.6	8.4	7.1	1.6
4	15 July	25.0	26.0	0.5	1.5	6.4	6.3	7.8	7.6	1.5
5	22 July	27.5	27.5	0.4	5.2	5.5	5.5	6.5	6.7	1.6
6	29 July	26.0	26.0	0.5	0.5	7.2	7.0	6.6	6.6	1.7

6 h and the filter papers were counted in 4.0 ml Aquasol (New England Nuclear, Boston, Massachusetts) in a scintillation spectrometer (Packard Tri-Carb Model 2425, Packard Instrument Co., Inc., Downers Grove, Illinois). Counting efficiencies were corrected by channels-ratio. Data is given as V_{max} in terms of ng CO_2 collected per liter per g dry weight per hour.

Adenosine Triphosphate (ATP) Extraction and Analysis

Adenosine triphosphate (ATP) was extracted by a procedure based on the method of Karl and LaRock (1975). Six replicates of 20 leaf discs or needle segments were withdrawn from the same large pools also used for other analyses. The plant litter was extracted in 5 ml 0.6 N cold H_2SO_4; ethylenediaminetetraacetic acid was added to give a final concentration of 4.8 mM; the solution was neutralized to pH 7.8 and brought to volume (10 ml) with 25 mM Tris(hydroxymethyl)amino methane buffer (TRIS); the samples were frozen until analyzed. The ATP in solution was assayed by the luciferin-luciferase technique (Holm-Hansen, 1973) using firefly lantern extract (Sigma Chemical Co.), prepared as described by Karl and LaRock (1975). 0.5 ml enzyme solution was injected into a 1 ml sample plus 0.5 ml TRIS buffer and luminescence was measured by a scintillation spectrometer (Packard Tri-Carb Model 2425) in the noncoincidence mode.

Lipid Synthesis

Incorporation of $H_3{}^{32}PO_4$ and sodium acetate-1-^{14}C into lipid was measured by

incubating plant material with 10 µCi $H_3{}^{32}PO_4$ and 4 µCi sodium acetate-1-^{14}C (New England Nuclear) in 2 ml estuarine water for 2 h at 25°C. Sixteen oak leaf discs or 15 pine needle segments were randomly selected from the sample pools for each of 15 replicates for each sample point.

The lipids were extracted from the leaf discs or pine needles by a modification of the Bligh and Dyer (1959) method. Detritus pieces were removed from the incubation mixture with forceps, washed twice in water and placed in 30 ml separatory funnels containing 10 ml absolute methanol, 5 ml chloroform, 4 ml water and 0.08 ml concentrated HCl. After mixing and extracting at room temperature (ca. 22°C) for 2 h, 5 ml $CHCl_3$ and 5 ml water were added; the mixture was shaken vigorously and allowed to separate into 2 phases. The lower phase was collected; $CHCl_3$ was removed by evaporation under a stream of nitrogen. Lipids were transferred in $CHCl_3$ to scintillation vials, evaporated to dryness and dissolved in a scintillation fluid of 9.28 mM 2,5-bis-[tert-butyl benzoxazolyl-(2')]-thiopene in toluene (White and Tucker, 1969). Samples were counted in a Packard Tri-Carb liquid scintillation spectrometer Model 2425.

Muramic Acid

Leaf material was hydrolyzed in 6 N HCl in sealed glass tubes for 4.5 h at 105°C with a ratio of acid to detritus of 20 to 1. After cooling and filtration through coarse glass filters, the HCl was removed by evaporation under reduced pressure, the sample was redissolved in acetone:0.1 N HCl, 9:1, and applied in a strip to a thick-layer plate (1 mm x 20 cm x 20 cm) of microcrystalline cellulose powder. After drying, the plates were developed 4 times with a solvent of acetone:glacial acetic acid:water, 9:1:1 (v/v). After drying, the band with mobility R_f 0.5 to 0.57 was removed, the muramic acid was eluted with methanol: water, 7:3 (v/v), and the lactate was liberated from the muramic acid by mild alkali (Tipper, 1968) and measured colorimetrically (Hadzja, 1974).

Scanning Electron Microscopy

Samples were prepared for scanning electron microscopy (SEM) immediately upon removal from estuarine water. Plant litter segments were fixed in 0.175% glutaraldehyde (Ladd Research Industries, Burlington, Vermont) in Krebs-Ringer solution (118 mM NaCl; 9.5 mM KCl; 2.5 mM $CaCl_2$; 1.2 mM KH_2PO_4; 1.2 mM $MgSO_4$; 12.3 mM dextrose; 23.8 mM $NaHCO_3$; pH 7.4) at 4°C for 1 h, placed in 3.3% glutaraldehyde in Krebs-Ringer solution at 4°C for 1 h, washed 4 times in Krebs-Ringer solution, fixed in 1.33% osmium tetroxide (Electron Microscopy Sciences, Fort Washington, Pennsylvania) in 0.2 M *sym*-collidine buffer (Ladd Research Industries, Burlington, Vermont), pH 7.4, at 4°C for 1 h, and washed 4 times in distilled water. The samples were dehydrated in graded ethyl alcohol concentrations of 20, 40, 50, 70, 80, 90% and three times in 100%, for 10 min each. Samples were dried in a custom-made critical-point dryer using liquid CO_2 (Anderson, 1951; Cohen, 1974), mounted on pedestals and coated with gold:palladium (60:40, w/w) to a thickness of approximately 100 Å using a Denton DV-502 Rotary Vacuum Evaporator. Samples were observed in a Cambridge Stereoscan S4-10 microscope (Cambridge Instrument Co., Ossining, New York) and photographs were taken using a Polaroid Series 125 camera and Polaroid type 105 film.

Results

Microbial Activity Measurements versus Time of Incubation in the Bay

Fig. 2 shows changes in the specific activities of several esterases, heterotrophic potential, and oxygen utilization of the detrital microflora on oak leaves during incubation in the estuary.

The activities follow three patterns. β-D-galactosidase progressively increases in activity throughout the 6-week incubation period and is closely paralleled, except for a slight drop in the sixth week, by β-D-glucosidase (Fig. 2A). The second type of response, shown by heterotrophic potential measured as rate of glucose mineralization and by alkaline phosphatase activity, reaches a maximum in the third to fourth weeks (Fig. 2B). The third type of response shows an early increase in activity to a level that stays relatively constant for the remainder of the 6-week period. This is true of the rate of respiration, the phosphodiesterase activity and the α-D-mannosidase activity (Fig. 2C). The rapid rise occurs during the first week for the respiratory activity, the second week for the phosphodiesterase and the third week for the α-D-mannosidase activity.

Changes in Rates of Detrital Microflora Lipid Biosynthesis

The rates of phospholipid and total lipid biosynthesis by the microflora of

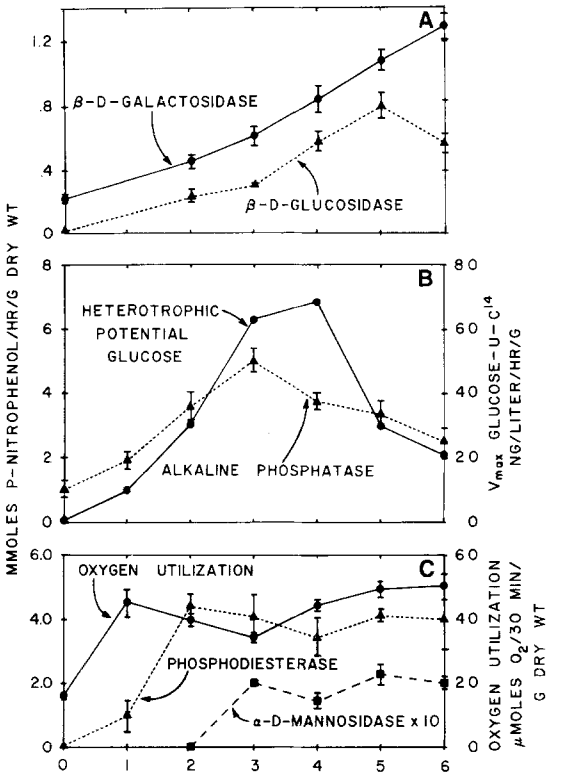

Fig. 2. Changes in specific activities of esterases (A, B and C), heterotrophic potential (B) and respiratory activity (C) of detrital microflora on allochthonous oak (*Quercus virginiana*) leaves incubated in Apalachicola Bay for 6 weeks (abscissae)

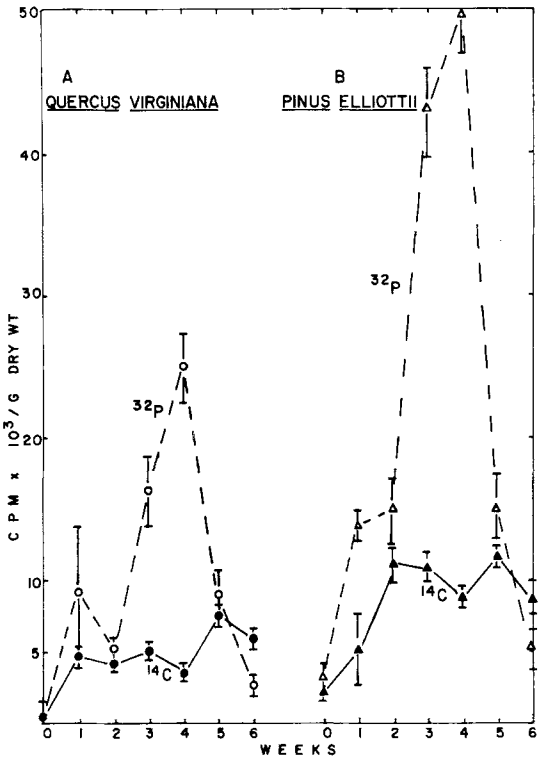

Fig. 3. Incorporation of $H_3{}^{32}PO_4$ (open symbols) and sodium acetate-1-^{14}C (closed symbols) in allochthonous oak leaves (A, circles) and pine needles (B, triangles) by detrital microflora after incubation in Apalachicola Bay

both pine needles and oak leaves show a sharp increase in the rate of incorporation of ^{32}P into phospholipids in the third and fourth weeks that is not reflected in the rates of incorporation of acetate-1-^{14}C into the total lipid fraction (Fig. 3). The relative magnitudes of the incorporation data depend upon the specific activities of the precursor pools. The specific activity of the added sodium acetate-1-^{14}C was 1.71 x 10^8 cpm/µmole and that of the $H_3{}^{32}PO_4$ was 9.16 x 10^{10} cpm/µmole. If one assumes that acetate is a reasonable representative of the various precursors of the total lipid components, then the actual rates of total lipid biosynthesis must have been several-fold greater than the rates of phospholipid biosynthesis to produce the data illustrated in Fig. 3.

Figs. 2 and 3 document that changes in activities per gram dry weight of detritus occur as incubation proceeds. Does this exclusively represent changes in the physiological responses of a relatively fixed population, or is species succession also taking place?

Relative Measures of Bacterial and Total Detrital Biomass

The data of Fig. 4 show the proportion of ATP to muramic acid in the detrital microflora of the oak leaves (A) and pine needles (B) incubated in the estuary. Muramic acid was derived by acid hydrolysis from the muramyl peptide of the detrital microflora. The only organisms known to contain this muramyl-peptide polymer are bacteria and blue-green algae (Salton, 1960). ATP has been utilized as a measure of the functional biomass of many planktonic or detrital species (Holm-Hansen and Paerl, 1972; Ausmus, 1973). During the 6-week incubation, the oak litter lost approximately 19% of its dry mass, and the pine needles lost 40% of their dry mass.

All data was normalized to the dry weight of the detritus. Replotting of the data in terms of the total estimated surface area gave essentially similar results.

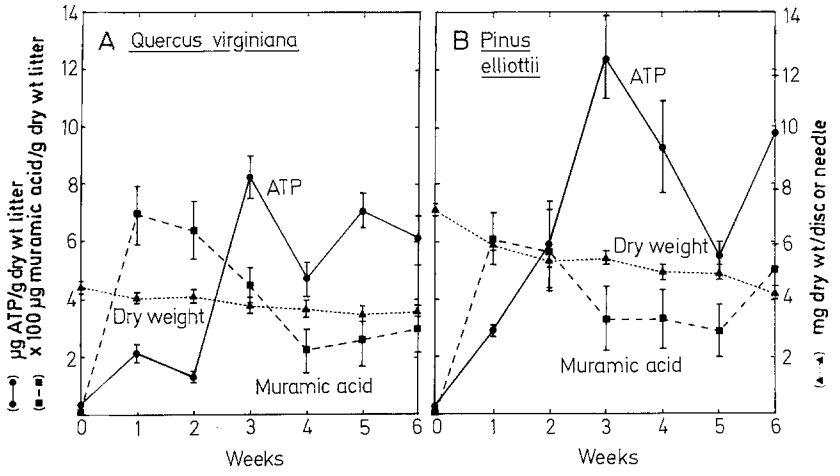

Fig. 4. Muramic acid (squares), extractable ATP (circles), and dry weight (triangles) of allochthonous oak leaves (A) and pine needles (B) incubated in Apalachicola Bay

Scanning Electron Microscopy

The progressive colonization of both the dorsal and ventral surfaces of the *Quercus virginiana* leaves is illustrated in the scanning electron micrographs of Fig. 5. Higher magnification micrographs of a variety of specific organisms are shown in Fig. 6. The two surfaces differed significantly in the degree and rate of accumulation of microflora and debris, but neither had a substantial microfloral population at the outset.

The dorsal surface, shown at low magnification at Weeks 0, 2, 4 and 6 in Fig. 5A, B, C and D, respectively, is colonized slowly and in patches. Newly fallen leaves, not yet exposed to estuarine water, have a sparse distribution of debris particles, unidentifiable even at higher magnification (Week 0, Fig. 5A). Through Week 2 (Fig. 5B), the dorsal leaf surface is covered by patches of microorganisms and debris, but much of the surface remains uncolonized, with little evidence of fungal growth. Several bacterial forms were seen on the dorsal leaf surface during the early stages of colonization, including the debris-covered organisms in Fig. 6A, which are attached by commonly observed filaments; bacilli, also with distinct attachment appendages (Fig. 6B and 6C); smooth-surfaced coccoid bacteria, including dividing forms (Fig. 6D); and a few filamentous forms (Fig. 6D). By Week 4 (Fig. 5C) the surface is more densely covered and filamentous organisms, probably fungi, are much in evidence. The dorsal surface is not fully colonized until the fifth and sixth weeks (Fig. 5D).

The ventral surface of the live oak leaf (Weeks 0, 1, 5, 6 – Fig. 5E, F, G, H, respectively) is also relatively free from debris and microflora prior to incubation in the estuary. This surface is covered with leaf structures known as stellate hairs, which conceal most of the leaf stomata (Week 0, Fig. 5E). The surface is colonized rapidly and debris is trapped, as shown at Week 1 in Fig. 5F, in which the stellate hairs are still visible but have become heavily covered by particles. Bacterial forms on the ventral surface are often seen (Fig. 6E), frequently barely visible among the mass of debris. Other distinct forms are observed commonly on the ventral surface, including both intact and fragmented diatoms (Fig. 6F and G), fungal hyphae (Fig. 6G), algal or fungal filaments (Fig. 6H), and spirochete-like organisms (Fig. 6F). As in the case of the dorsal surface, while fungal filaments may be observed occasionally early in the incubation period, they become much more abundant in the latter stages. At Weeks 4 and 5 (Fig. 5G), dense filamentous mats, probably responsible for macroscopic white patches seen on the leaf surface, may be observed in localized areas, although they do not typically dominate the entire ventral surface, as shown by the representative micrograph of a 6-week sample (Fig. 5H).

Discussion

Changes in Microfloral Activities

During the colonization of oak-leaf litter surfaces, three patterns of activ-

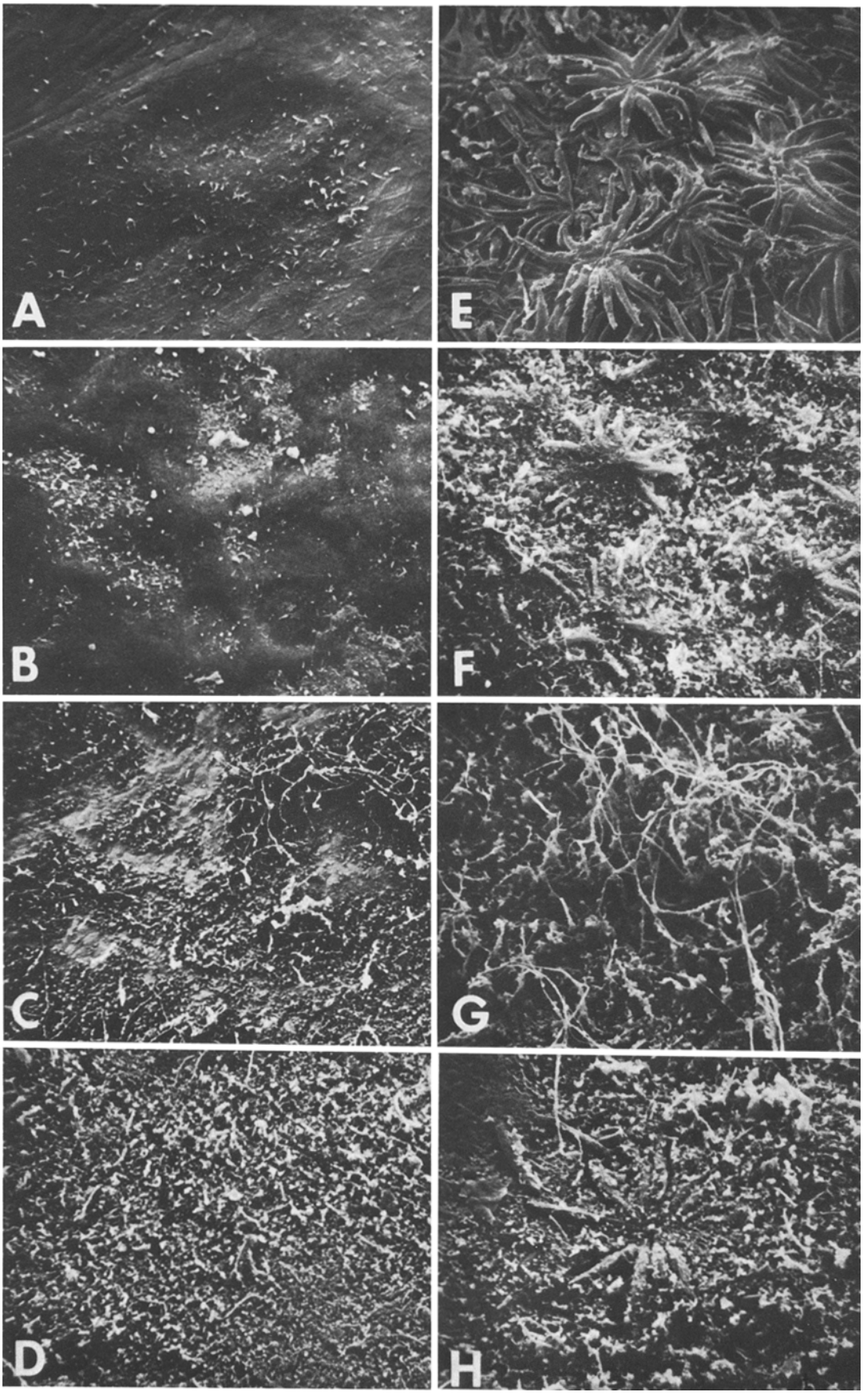

Fig. 5. Scanning electron micrographs of colonization of *Quercus virginiana* leaves during incubation for various lengths of time in estuarine water. (A) Dorsal surface, Week 0, 230X, 10Kv; (B) dorsal surface, Week 2, 200X, 30Kv; (C) dorsal surface, Week 4, 140X, 5Kv; (D) dorsal surface, Week 6, 210X, 10Kv; (E) ventral surface, Week 0, 210X, 10Kv; (F) ventral surface, Week 1, 160X, 30Kv; (G) ventral surface, Week 5, 230X, 10Kv; (H) ventral surface, Week 6, 290X, 10Kv

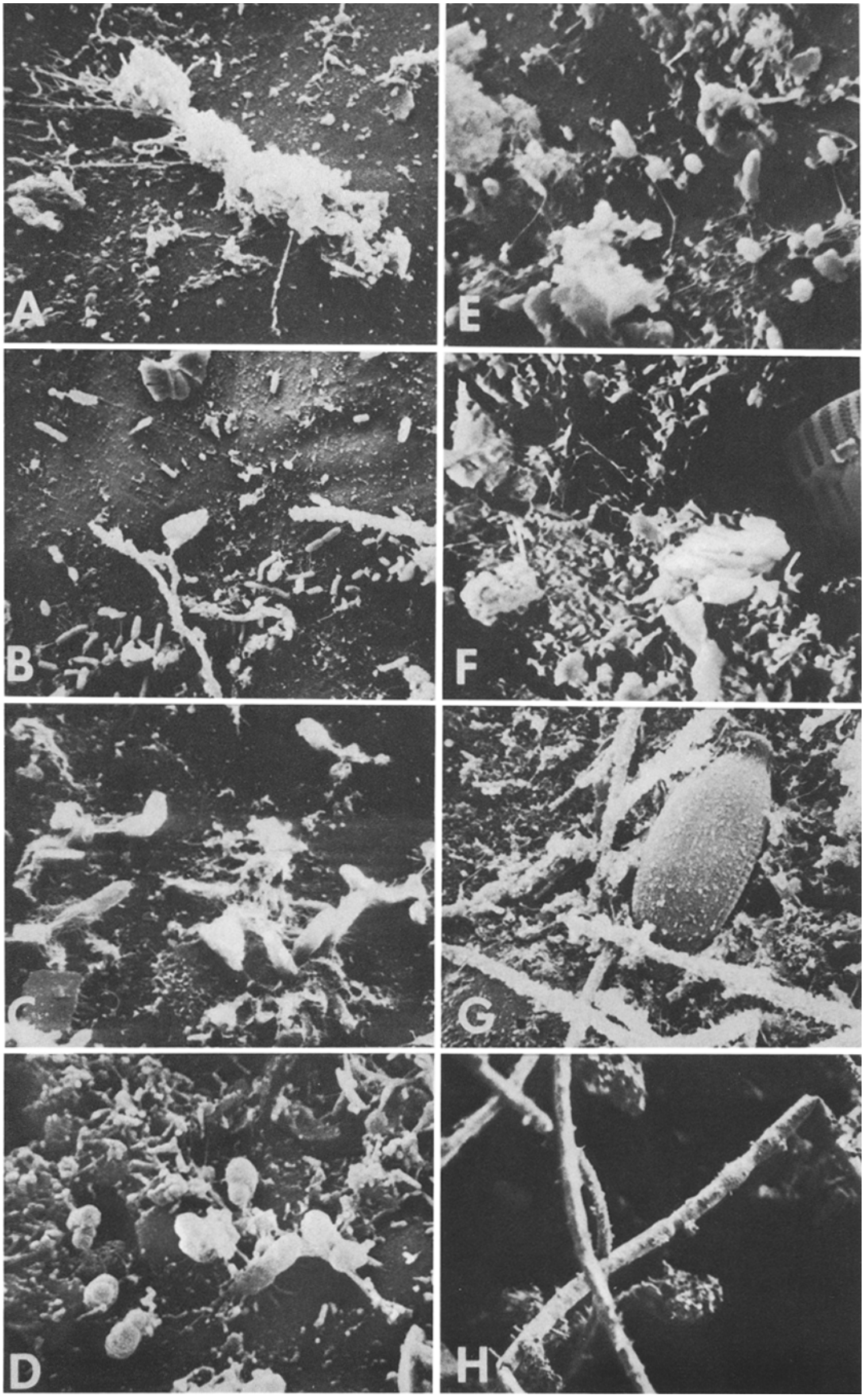

Fig. 6. Scanning electron micrographs of organisms observed on surface of *Quercus virginiana* leaves colonized in estuarine water. (A) Dorsal surface, Week 1, 553OX, 3OKv; (B) dorsal surface, Week 1, 26OOX, 3OKv; (C) dorsal surface, Week 2, 532OX, 3OKv; (D) dorsal surface, Week 3, 842OX, 3OKv; (E) ventral surface, Week 5, 54OOX, 1OKv; (F) ventral surface, Week 1, 6O9OX, 3OKv; (G) ventral surface, Week 3, 169OX, 3OKv; (H) ventral surface, Week 4, 144OX, 5Kv

ities appear: (1) continual increases with time of incubation (Fig. 2A), (2) increases to a maximum in 3 to 4 weeks followed by a rapid decline (Fig. 2B), and (3) a rapid initial rise to a plateau of activity that is maintained throughout the 6-week period (Fig. 2C). On both pine and oak litter, rates of ^{14}C accumulation into the total lipid rise initially and are then maintained, while phospholipid biosynthesis shows maximum activity in the third and fourth weeks. These patterns of respiratory activity, heterotrophic activity, esterase activities, total lipid and phospholipid biosynthesis were repeated on litter incubated in a similar experiment one month later.

Succession

By examining a more specific parameter, i.e., muramic acid, a succession of organisms can be shown to be responsible for at least part of the changes in enzyme activities of the detrital microflora. The muramyl peptide polymer which yields muramic acid on acid hydrolysis is found in the cell walls of only bacteria and blue-green algae. There apparently is little blue-green photosynthetic activity in the detritus, since light or 0.1 mM 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU) plus light have little effect on oxygen utilization by the microflora. Therefore, the muramic acid reflects primarily bacteria in the detrital microflora. The ATP level accurately reflects the total biomass of microflora of sediment or leaf litter (Ausmus, 1973). From the data of Fig. 4, the initial colonizers of the oak leaves and pine needles were bacterial, as indicated by high ratios of muramic acid to ATP. With longer incubation in the estuary, the ATP values rose and the muramic acid decreased. One would expect the initial surface microflora to be predominantly bacterial and to be diluted in time by fungi, algae and other more complex organisms which contain ATP but not muramic acid. This is indeed the morphological sequence depicted in Figs. 5 and 6 by the scanning electron micrographs of the leaf-surface microorganisms.

Data were normalized to grams dry weight of litter. During this experiment, oak leaves lost 19% and pine needles 40% of their mass (Fig. 4). This is the resultant net change due to both increases in detrital biomass and decreases in leaf mass. Normalization of data of leaf area, based on number of oak-leaf discs, each 6.5 mm in diameter, produced similar patterns and ratios. The surfaces of oak leaves colonized by microflora are clearly not simple planar surfaces, as shown by the micrographs of Fig. 5. Estimations based on areas of pine needles were more difficult because of changes in diameter of needles with length but, again, similar patterns were observed. Dry weight is a practical, albeit imperfect, basis for comparisons of activities, and would be the only practical way to correlate the activities on these detrital substrates with those of wood fragments or sediments which also are important in this estuary.

Initial colonization by bacteria of new surfaces introduced to environments has been reported for soils after biocidal treatments (Powlson, 1975), chitin strips or glass slides incubated in soils (Gray *et al.*, 1968), the rhizoplane in soils (Alexander, 1964), stone surfaces in trickling filters (Mack *et al.*, 1975), various surfaces exposed to the sea (Skerman, 1956; Sladeckova, 1966; Marshall *et al.*, 1971; Sieburth, 1975), or detritus recolonized after passage through an amphipod gut (Fenchel, 1970).

The traditional succession reported for microflora on plant litter introduced into an aquatic environment has been a primary fungal colonization followed by a broader microbial community. Studies of mangrove leaves (Newell, 1973), beech and oak leaves (Suberkropp and Klug, 1974), driftwood (Brooks *et al.*, 1972) or *Spartina alterniflora* internodes (Gessner *et al.*, 1972) possibly relied more on the impressive microscopic morphology of the fungi and their relative ease of cultivation. Kaushik and Hynes (1968) combined cultural techniques on selective media with antibiotic treatments to show the dominance of fungi. However, failures of antibiotics for specific inhibition in nature and the inapplicability of cultural methods to assess bacterial activity in ecological associations have been well documented (Skinner *et al.*, 1952; Jannasch and Jones, 1959; Yetka and Wiebe, 1974). The requirement for fungal metabolism for the growth of some soil bacteria is also well documented (Gray *et al.*, 1968; Mishustin and Mirsoeva, 1968). From our results, oak leaves introduced into the estuary are initially colonized by a microflora rich in bacteria.

Calculation of Microbial Mass

Calculation, from the muramic acid data (Fig. 4A), using conversion factors of 4 µg muramic acid/mg dry weight Gram-negative bacteria or 10 µg muramic acid/

mg dry weight Gram-positive bacteria (Millar and Casida, 1970), indicate that there are between 20 and 170 mg dry weight bacteria/g dry weight oak leaf during the 6-week period. Conversion from oxygen consumption rates (Fig. 2C) based on a Q_{O_2} of 100 µl O_2/h/g dry weight bacteria, a reasonable value for bacteria (Doetsch and Cook, 1973), to bacterial mass gives values of 14 to 22 mg dry weight bacteria/g oak leaf. The bacterial biomass, based on the ATP levels (Fig. 4A) and an average value of 1 mg ATP/g dry weight bacteria for exponentially growing cells (Ausmus, 1973), ranges from 1.3 to 8.3 mg dry weight bacteria/g dry weight leaf. In the more complex microorganisms, which were shown by scanning electron microscopy to comprise part of the detrital microflora (Figs. 5 and 6), the oxygen utilization rates and ATP content per organism are both lower. The presence of these organisms increases the estimation of dry weight biomass based on oxygen utilization and ATP.

Metabolic State of Detrital Microflora

The bacterial portion of the detrital microflora would have the highest proportion of phospholipid relative to total lipids (Kates, 1964). The levels of ^{32}P incorporated into lipid should reflect primarily bacterial growth in the short-time incubations. The maximum rate of phospholipid synthesis for oak, based on the data of Fig. 3A (Week 4), was 2.7 x 10^{-4} nmoles lipid-phosphate per 2 h per gram leaf, since the specific activity of ^{32}P was 9.16 x 10^{7} cpm/nmole inorganic phosphate. If the bacteria contain 50 nmoles lipid-phosphate/mg dry weight cells, as documented for monocultures of several bacteria (Lennarz, 1970), there was an increase of 5.4 x 10^{-3} µg dry weight bacteria/g oak in 2 h. This is an increase of 0.024 to 0.0096% of the biomass based on total muramic acid, or 0.11% of the biomass based on ATP in 2 h, indicating a very slow growth rate for detrital microflora.

Bacterial doubling times below optimum have been observed for filamentous organisms in streams on glass slides (Bott, 1975) and on the surface of seaweeds (Brock, 1967). Prolonged doubling times between 9 and 20 days in soils have been documented by Gray *et al.* (1974), Siala and Gray (1974), and Gray (1976) and doubling times from 10 to 281 h were observed in lacustrine sediments (Gambaryan, 1966). Even in the relatively rich and warm vertebrate gastrointestinal system, doubling rates of 0.5 to 1.4 divisions/day were measured by Gibbons and Kapsimalis (1967), and 1.92 doublings/day in the bovine rumen were calculated by El-Shazly and Hungate (1965).

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