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Microbial decomposition of reed (*Phragmites communis*) leaves in a saline lake

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Abstract

Microbial colonization and its relation to the decomposition of reed (*Phragmites communis*) leaf litter were studied in the littoral area of a saline lake from autumn to summer using litter bag method. There was considerable fungal population on the leaves at the beginning of submergence. These fungi were probably terrestrial in origin. The fungal population rapidly disappeared few days after submergence, when bacteria, including cellulolytic and xylanolytic types, proliferated. Associated with this rapid colonization of bacteria, decomposition rates of cellulose and xylan increased. The rates declined from day 39 to day 100 with decreasing water temperature, though cellulolytic and xylanolytic bacteria maintained a sizeable population until day 150. A community of cellulolytic and xylanolytic fungi increased steeply after day 150. It coincided with a second increase in decomposition rate. These results suggest that the principal decomposers of reed leaf litter were bacteria in the initial phase and fungi in the later phase of the experiment.

Introduction

In shallow littoral environments, the majority of carbon fixed by aquatic vascular plants enters the pool of dead organic materials in the form of litter (Berrie, 1976; Fenchel & Jorgensen, 1977). The importance of microorganisms as a link between primary and secondary production in detritus-based food webs has long been recognized (Fenchel & Jorgensen, 1977; Odum & de la Cruz, 1967; Teal, 1962). The major portion of organic matter in litter consists of the polymers cellulose, hemicellulose and lignin. The former two are hydrolyzed by the extra-cellular enzymes cellulase and hemicellulase produced by cellulolytic and hemicellulolytic microorganisms.

There are many studies about the succession of microorganisms on litter (Bälocher & Kendrick, 1974; Suberkropp & Klug, 1974 and 1976; Morrison *et al.*, 1977; Federle & Vestal, 1980 and 1982), and the chemical change of litter (Kaushik & Hynes, 1971; Mason & Bryant, 1975; Hodkinson, 1975; Polunin, 1982) during decomposition in various aquatic ecosystems. However, there are few researchers who observed the dynamics of structural polymer decomposers and the changes in chemical composition of litter simultaneously.

The present study examines the dynamics of microorganisms and the change in the chemical composition of leaves during decomposition of reed (*Phragmites communis*) leaf litter submerged in a saline lake during one year. Special attention was paid to the relation between the dynamics of cellulolytic and xylanolytic microorganisms and the decomposition rates of cellulose and xylan, and to the relative contribution of bacteria and fungi in the hydrolysis of cellulose and xylan.

The study site

A litter bag experiment was performed near the shore of Lake Shinhama (35° 40′ N, 39° 56′ E), a man-made saline lake which is located near the coast of Tokyo Bay, Japan. Its surface area is 0.3 km^2 and the maximum depth is 6 m. The lake water salinity almost equals that of seawater, because the lake is connected to Tokyo Bay by two channels. The lake water is eutrophic (Furota, 1980) with a transparency of less than 2 m. The shoreline and shallows are dominated by *P. communis*.

Materials and methods

Dead, brown leaves of *P. communis* were collected in October 1980 from dead shoots standing in the margin of Lake Shinhama. One hundred pieces of air-dried, and weighed leaves were enclosed in each litter bag (30 × 20 × 20 cm, 2 mm mesh size), which was made of two hard plastic baskets. Seven litter bags were submerged at about 0.5 m below the water surface near the shore on 31 October, 1980. Sessile organisms on the outer surface of the bags were frequently scrubbed off to allow water circulation in the bags. One bag was recovered at each sampling time. Water temperature at recovery was measured by a mercury-in-glass thermometer. Maximum and minimum water temperature during successive two recovering periods was measured by a maximum and minimum thermometer. The leaves in the bag were transferred to a polyethylene bag containing sterile seawater and transported to a laboratory. The leaves were gently rinsed with sterile seawater to remove mud, and subjected to chemical and microbiological analyses.

At first, several wet leaves were ground up with a motor mill, and the pH of the suspension was measured. Next, seventy pieces of the leaves were air-dried, powdered with a motor mill and used for the determination of carbon and nitrogen and for the proximate analysis of several litter constituents. Carbon and nitrogen were analyzed with a CHN corder (Model MT-2, Yanagimoto Co. Ltd.). To determine ash content, the leaf powder was combusted in a muffle furnace for 1 hour at 450 °C and the residue was weighed. Cellulose, xylan (hemicellulose) and crude fat were analyzed by the method of Allen *et al.* (1974). For the determination of cellulose and xylan, the leaf powder was delignified with sodium chlorite in dilute acetic acid at 75 °C, and then treated with 24% potasium hydroxide. The alkaline extract was neutralized after filtration, allowed to stand overnight, and mixed with excess alcohol. The resultant precipitate was recovered and weighed as xylan. The residue of alkaline extraction was weighed as cellulose. For determination of crude fat, the leaf powder was extracted with ether in a Soxhlet apparatus for 6 hours. Next, ether in the flask was evaporated off under a stream of oxygen-free N_2. Then, the residue in the flask was weighed as crude fat after drying in a vacuum oven at 40 °C for 30 minutes. Hot-water soluble matter was determined after Waksman & Tenney (1927).

Bacteria and fungi were counted using leaf homogenate prepared as follows. Wet leaves were ground with a sterile motor mill and homogenized with a sterile Potter glass homogenizer after addition of sterile seawater (final volume 400 ml). Total bacterial cells were counted by an acridine-orange epifluorescence direct-count technique (Tanaka & Tezuka, 1982) after fixation with glutaraldehyde (final concentration 0.5%). A count of total viable heterotrophic bacteria in the leaf homogenate was made by a spread plate method with ZoBell 2216E agar medium

(Oppenheimer & ZoBell, 1952). The inoculated plates were incubated at 20 °C for 14 days before counting. Cellulolytic bacteria were counted by a pour plate method (Kadota, 1956), except that precipitated cellulose was replaced by Avicel SF cellulose powder (Asahi Chemical Industry Co. Ltd.) (5 g l^{-1}, final). A preliminary test showed that Avicel SF provided clearer halos and higher counts than precipitated cellulose. Incubation was performed for 30 days at 20 °C. Xylanolytic bacteria were counted by the method of Fujisawa *et al.* (1967), using the xylan extracted from reed shoots by the method described above. The inoculated plates were incubated for 7 days at 20 °C. A preliminary test showed that the count declined with prolonged incubation over 7 days because of overlapping halos. Fungi were counted by a dilution plate method with a glucose-yeast extract agar medium (glucose 19 g l^{-1}, yeast extract 1 g l^{-1}) described by Tubaki (1974). Streptomycin sulfate and Na-penicillin G were added to this medium at the concentration described by Aaronson (1970) (500 mg l^{-1} each) to inhibit bacterial growth. Serially diluted samples were spread over the surface of dried agar plates. According to Park (1972), the spread plate method gives more uniform colony development than the pour plate method. Fungal colonies were counted after incubation of 7 days at 20 °C, and then each colony was picked up and purified by isolating single hyphae. Capabilities of cellulolysis and xylanolysis of the purified isolates were tested by inoculating them on dried agar plates of the Avicel medium and of a Sørensen's xylan medium (Sørensen, 1957) (4 g l^{-1} xylan, Tokyo Chemical Industry Co., Ltd.). Numbers of cellulolytic and xylanolytic fungi were estimated by these results. All media for bacterial and fungal counts were prepared with aged seawater filtered through glass fiber filters (Whatmann GF/C).

Length of fungal hyphae on the surface of the leaves was determined as follows. Ten pieces of the leaf discs (1 cm diameter) were punched out from the leaves with a cork borer. Then, they were stained with lactophenol-cotton blue solution and safranin solution successively, and observed under a bright field microscope (× 400). Number of the intersections of hyphae and graticules on an ocular micrometer (250 × 250 μm, 100 mesh) was counted at 20 microscopic fields for each side of the leaf disc. The length of fungal hyphae was calculated from this count by the method 5 of Olson (1950).

To survey the microflora on dead leaves before submergence, microbiological analyses were made for the aerial dead leaves which were collected in November 1981 from dead standing shoots of reed. The leaves were homogenized with a Polytron homogenizer in distilled water (0.05 g ml^{-1}) and used for analyses. ZoBell 2216E medium was used for the count of total viable heterotrophic bacteria and fungi, the Avicell medium for the cellulolytic bacteria and fungi, and the Fujisawa's xylan medium for the xylanolytic bacteria and fungi. No antibacterial or antifungal agents were added to the media. Distilled water instead of seawater was used for preparation of the media.

Results

Figure 1 shows changes in water temperature (A) and pH of seawater (B) during the litter bag experiment. The water temperature declined to 5 °C about day 100, and then increased steadily to 30 °C on day 200. The pH of seawater was about 7.5 until day 200, and then rose to more than 8.5, probably due to an algal bloom in summer. As shown in Fig. 2, leaf pH was about 6 throughout the whole experimental period, except for a lower value in the beginning of the experiment.

Figure 3 shows ash content of the litter. It declined to less than 10% until day 145 and then increased rapidly to more than 50%. Carbon and nitrogen in ash free dry weight (AFDW) are shown in Fig. 4. Carbon content was constant around 50 to 60% throughout the experimental period, but nitrogen increased after day 145 from 1 to 3%. As a result, the ratio of carbon to nitrogen (C/N ratio) declined from 60 to 20. Figure 5 shows the changes in the contents of cellulose, xylan, hot-water soluble matter and crude fat in

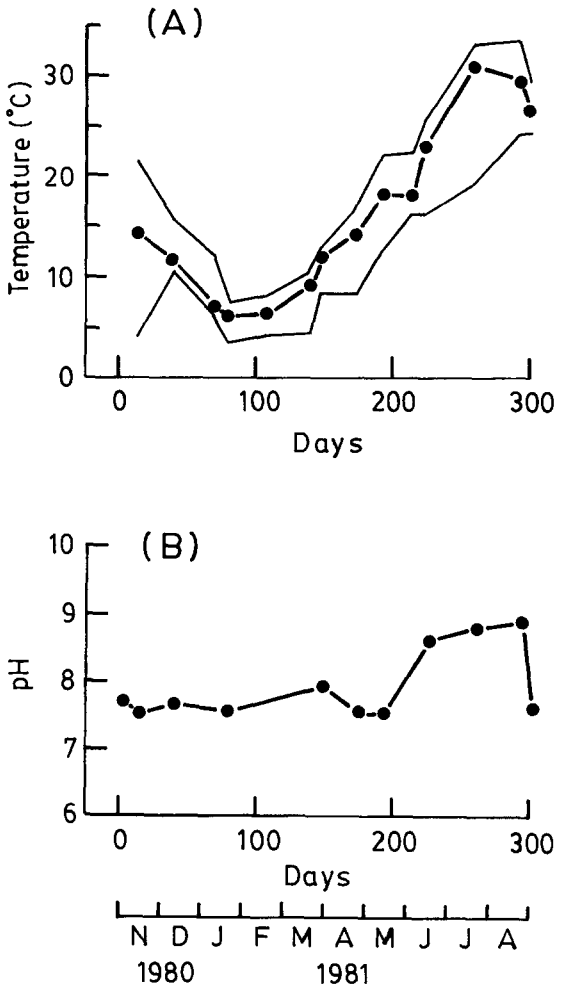

Fig. 1. Changes in ambient water temperatures (A) and pH (B) at times of recovering the litter bags. (Thin two lines in (A) represent maximum and minimum water temperatures during recovering period.)

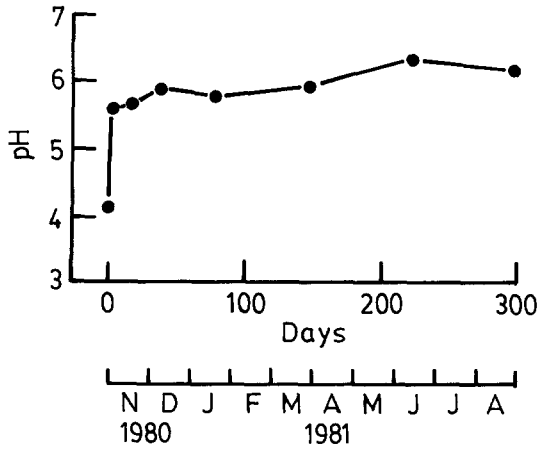

Fig. 2. Change in pH of *Phragmites* leaf litter during the experimental period.

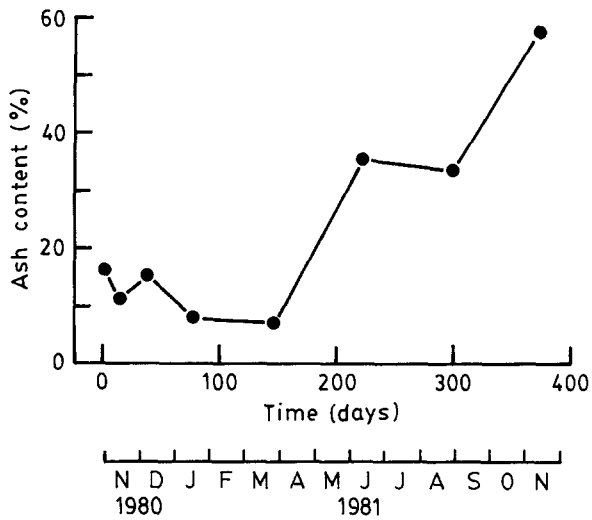

Fig. 3. Change in ash content per dry weight of *Phragmites* leaf litter during the experimental period.

AFDW. The contents of cellulose and xylan were about 25 and 20%, respectively, at the initial stage, and decreased gradually afterward. The content of hot-water soluble matter decreased until day 39 and then steadily increased. The initial drop must have been caused by leaching and/or microbial decomposition, and the later increase appears to be due to accumulation of intermediate and/or end products of degradation of structural carbohydrate. Accumulation of cellular matters of microorganisms which proliferated steadily with time seems to be also one of the causes of the later increase. The crude fat content was constant throughout.

Figure 6 shows the changes in AFDW (A), cellulose and xylan (B) and the other constituents (C) in percentages of initial quantities. Time required for 50% breakdown of AFDW was about 190 days. This value is close to those reported by Mason & Bryant (1975) for *P. communis*, and by Polunin (1982) for *P. australis* leaves submerged in freshwater. The

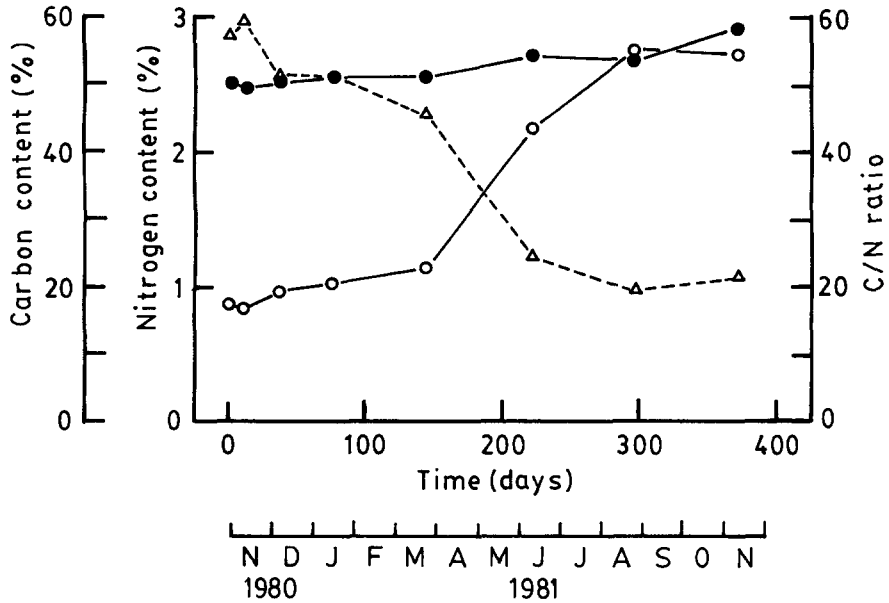

Fig. 4. Changes in carbon and nitrogen contents per ash-free dry weight and carbon/nitrogen ratio of *Phragmites* leaf litter during the litter bag experiment. ●, carbon; ○, nitrogen; △, C/N ratio.

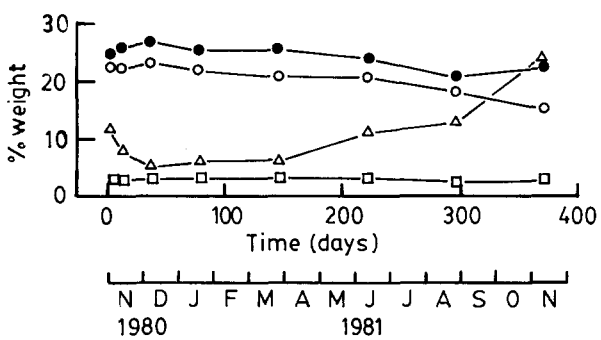

Fig. 5. Change in chemical composition of *Phragmites* litter as percentages of ash-free dry weight during the experimental period. ●, cellulose; ○, xylan; □, crude fat; △, hot-water soluble matter.

decay rates for cellulose and xylan were similar to that of AFDW. Hot-water soluble matter and ash decreased rapidly during the initial month. Ash, however, increased markedly after about 150 days. Attachment of debris including sand or clay on the surface of the leaf litter could have caused this increase, because observation with a scanning electron microscope showed that the litter surface became densely covered with many particles in the later period. Crude fat decreased steadily throughout the whole period.

Specific decomposition rate was estimated for cellulose and xylan from the changes in percentages of initial quantities.

The rate is defined as:

$$R = \Delta W/(\Delta t \times W)$$

where R is the specific decomposition rate, ΔW is the difference in the percentage of weight remaining during successive sampling periods, Δt is the time interval of two successive samplings, W is the percentage of weight remaining at the former sampling period.

As shown on Fig. 7, the rates rose steeply during the initial 30 days, dropped from day 30 to day 100, and then rose again. The changes in the rates after day 30 were paralleled by a change in water temperature.

Figure 8 shows the changes in bacterial numbers per gram AFDW during the experimental period. Direct and viable counts of bacteria increased rapidly in the initial 39 days, then

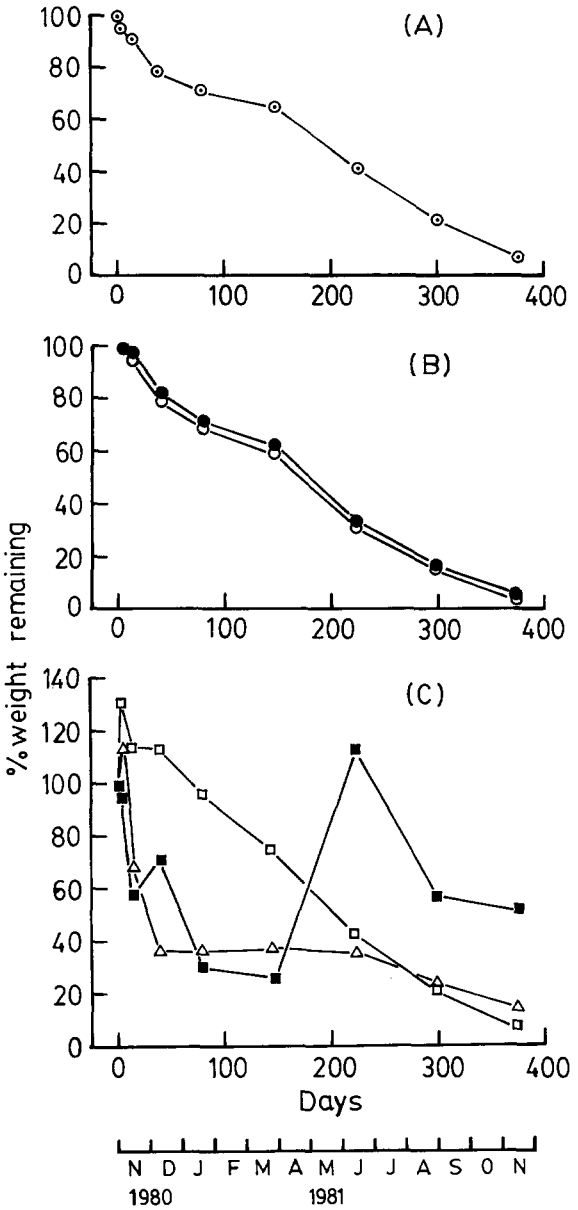

Fig. 6. Changes in (A) ash-free dry weight, (B) structural polymer, and (C) other constituents of *Phragmites* litter in percentages of the starting quantities during the experimental period. ⊙, ash-free dry weight; ●, cellulose; ○, xylan; △, hot-water soluble matter; □, crude fat; ■, ash.

became stable, and increased again, gradually, after day 145. Cellulolytic and xylanolytic bacteria also increased rapidly during the initial 39 days, but decreased after about day 145. As shown in Fig. 9, conspicuous peaks were observed at day

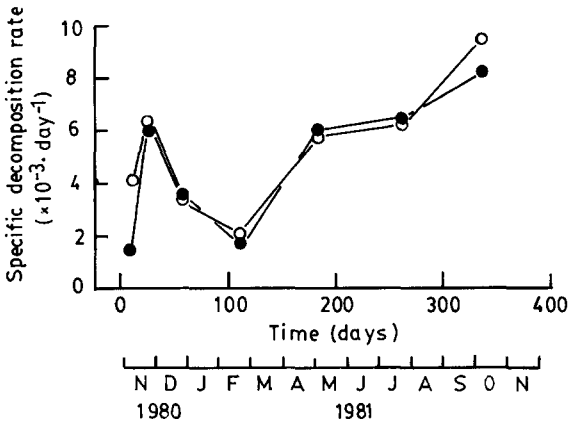

Fig. 7. Changes in specific decomposition rates of structural polymers of *Phragmites* litter during the experimental period. ●, cellulose; ○, xylan.

39 in the percentages of cellulolytic and xylanolytic bacteria versus total heterotrophic bacteria. These peaks coincide with initial peaks of the specific decomposition rate of cellulose and xylan (Fig. 7).

Changes in the counts of fungi and the length of fungal hyphae are shown in Fig. 10. Though a fairly high fungal biomass was detected immediately after submergence, it decreased steeply after few days, and gradually increased again after about 50 days. Counts of cellulolytic and xylanolytic fungi were low during the initial 100 days but increased steadily afterwards in accordance with the increase in specific decomposition rate of cellulose and xylan.

Figure 11 shows that there was a considerable fungal population on the aerial dead leaves. Counts of total, cellulolytic and xylanolytic fungi on the aerial leaves were higher than the maximum counts of those fungi on the submerged leaves. A hyphal length also longer than the maximum length on the submerged leaves. On the contrary, bacterial counts on the aerial dead leaves were considerably lower than the minimum counts of bacteria on the submerged leaves. Fungi might be more important than bacteria for the decomposition of the aerial dead leaves. Yeast-like microorganisms, which possessed a xylanolytic ability, were detected on the aerial dead leaves. Apinis *et al.* (1972) also observed that yeasts and

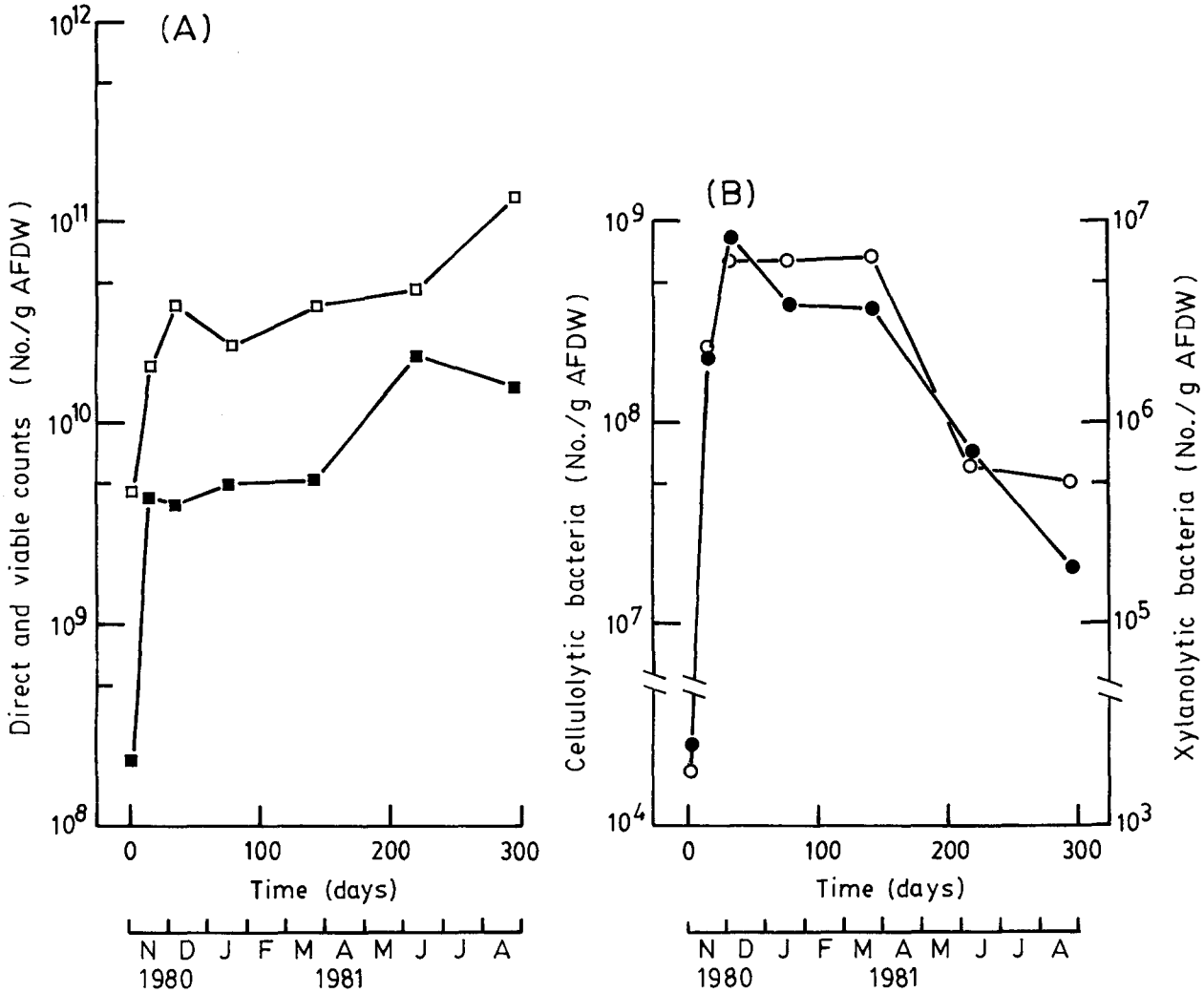

Fig. 8. Changes in (A) viable and direct counts of bacteria and (B) numbers of cellulolytic and xylanolytic bacteria associated with *Phragmites* litter during the experimental period. □, direct count; ■, viable count; ●, cellulolytic bacteria; ○, xylanolytic bacteria.

yeast-like fungi colonized on young and green leaves of *P. communis*.

Discussion

The decomposition process of *P. communis* leaf litter can be divided into three stages:

Stage I, from day 0 to day 50 (autumn)
Stage II, from day 50 to day 150 (winter)
Stage III, from day 150 to day 300 (spring, summer)

In stage I, the total heterotrophic bacteria increased rapidly (Fig. 8). Rapid initial colonization of bacteria on submerged litter has been observed by many investigators (Federle & Vestal, 1982; Suberkropp & Klug, 1976; Oláh, 1972). Oláh (1972) suggested that intense initial colonization of bacteria on *Phragmites* litter occurred by utilization of dissolved organic matter in the leaves. Rapid decrease of hot-water soluble matter in the present study supports this suggestion. In addition to total heterotrophic bacteria, cellulolytic and xylanolytic bacteria also

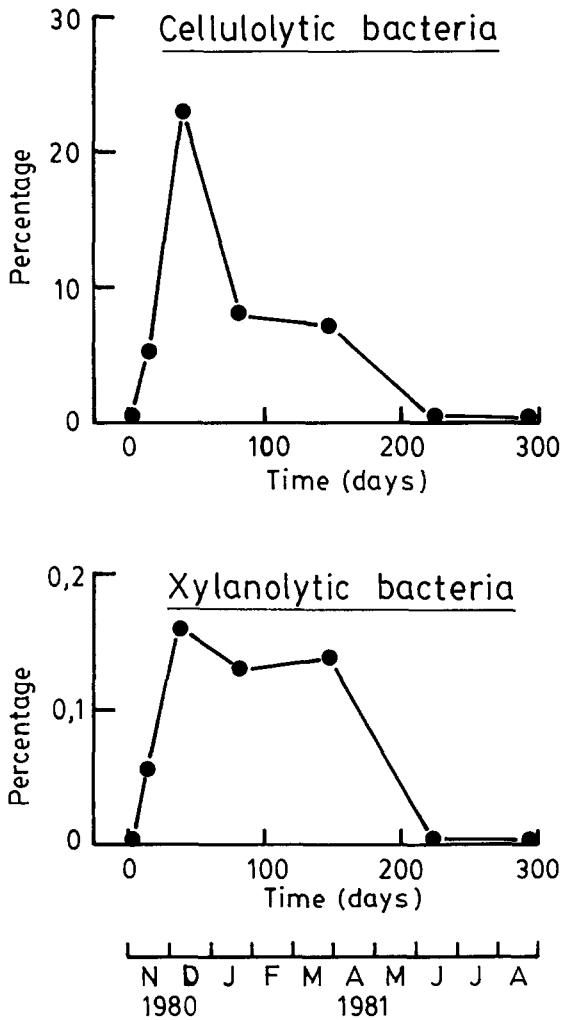

Fig. 9. Changes in cellulolytic and xylanolytic bacteria as percentages of viable counts.

increased rapidly (Fig. 8), in parallel with decomposition rates of cellulose and xylan (Fig. 7). The ratio of cellulolytic and xylanolytic bacteria to total heterotrophic bacteria also increased markedly (Fig. 9). In contrast to the increase of the bacteria, fungi decreased rapidly after submergence (Fig. 10). In conclusion, it appears that bacteria were the main decomposer at this stage.

Analysis of the microbial flora on the aerial dead leaves showed that there were large numbers of fungi on the leaves, and suggested that the fungal population observed in the beginning of submergence was of terrestrial origin. The rapid initial decrease in fungal biomass may be due to death of this terrestrial mycoflora in a seawater environment. Apinis *et al.* (1972) observed a fungal colonization on young and green *P. communis* leaves, and Taligoola *et al.* (1972) reported that the aerial parts of *P. communis* after being submerged, lost their original aerial mycoflora. These support the above suggestion.

At stage II, the decomposition rate dropped with water temperature while large populations of cellulolytic and xylanolytic bacteria were still present. Low water temperature should became limiting factor for decomposition by microorganisms.

At stage III, fungal populations including cellulolytic and xylanolytic species increased steadily with decomposition rate, while cellulolytic and xylanolytic bacteria decreased conspicuously. Biomass and activity are often not directly related, because biomass of microorganisms is a reflection of both growth rate and removal rate. Thus, a very active and rapidly growing microbial population may not reach a large biomass when grazing animals are also active. In the present experiments, however, the decrease of cellulolytic and xylanolytic bacteria at stage III was not caused by grazing, because both total cell numbers and viable counts of bacteria steadily increased throughout the period (Fig. 8). Therefore, the decrease of the cellulolytic and xylanolytic bacteria at stage III should indicate a decline in growth rate and thus in cellulolytic and xylanolytic activities of the bacterial community. In conclusion, it is presumed that main decomposer changed from bacteria to fungi at stage III.

Reese (1977) pointed out that bacteria are generally less adapted than fungi for penetrating a complex structure of plant tissue. In the present study, cellulolytic and xylanolytic bacteria may have exhausted cellulose and xylan on the litter surface by the middle of the experimental period, and disappeared afterward due to their inability for utilizing the plant tissue itself. Inhibitory effects of the fungi, which increased markedly, seem to be another cause of the decrease in cellulolytic and xylanolytic bacteria. On the other hand, the increase of water temperature after

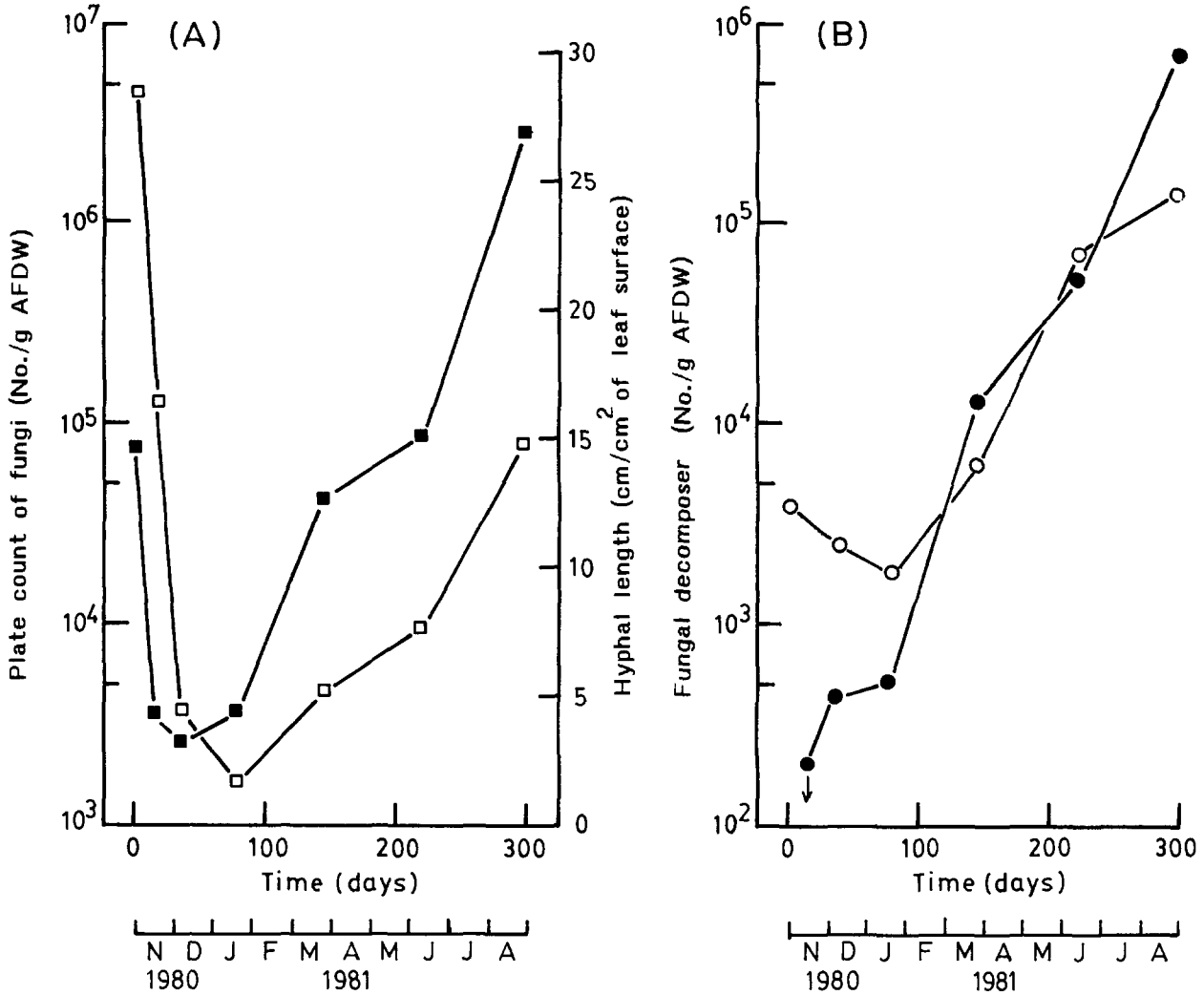

Fig. 10. Changes in (A) plate count and hyphal length of fungi and (B) numbers of cellulolytic and xylanolytic fungi associated with *Phragmites* litter during the experimental period. ■, plate count; □, hyphal length; ●, cellulolytic fungi; ○, xylanolytic fungi (↓ indicates less than that value).

about day 100 may have been one of the causes of fungal increase at stage III.

To further clarify the relationships between microbial dynamics and chemical change of the litter, a determination of decomposing activities of microorganisms is needed. Cellulose and xylan in the litter are hydrolyzed by enzymes (cellulases and xylanases) derived from decomposer microorganisms. Therefore, decomposing activity may be determined by measuring enzyme activities on the litter. Furthermore, since characteristics of the enzymes vary with the species of microorganism producing them, analyses about the characteristics of the enzymes associated with the litter will provide valuable information about the species of important decomposer microorganism. In the present litter bag experiment, enzymological analyses were made simultaneously with chemical and microbiological ones. The results of the enzymological analyses will be described separately.

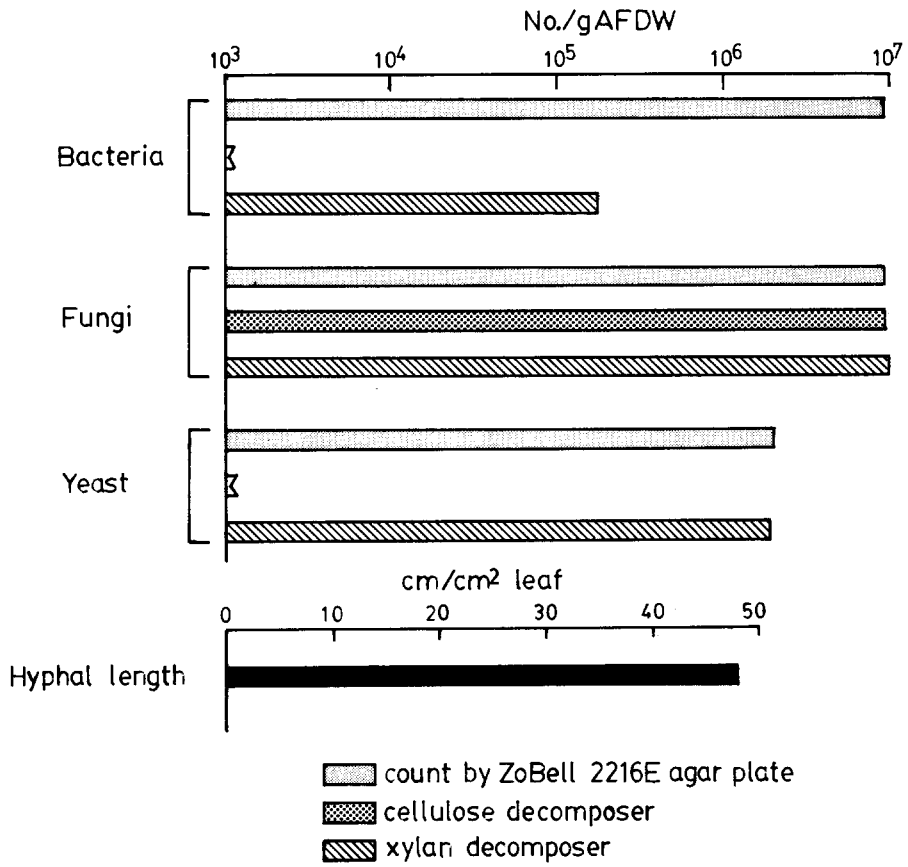

Fig. 11. Microbial flora on aerial dead leaves of *Phragmites* collected at November 1981. (< indicates less than that value).

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References

Aaronson, S., 1970. Experimental microbial ecology. Academic Press, New York. 236 pp.

Allen, S. E., H. M. Grimshaw, J. A. Parkinson & C. Quarmby, 1974. Chemical analysis of ecological materials. Blackwell Scientific Publications, Oxford. 565 pp.

Apinis, A. E., C. G. C. Chesters & H. K. Taligoola, 1972. Colonisation of *Phragmites communis* leaves by fungi. Nova Hedwigia 23: 113–124.

Bärlocher, F. & B. Kendrick, 1974. Dynamics of the fungal population on leaves in a stream. J. Ecol. 62: 761–791.

Berrie, A. D., 1976. Detritus, microorganisms and animals in fresh-water. In J. M. Anderon & A. McFayden (eds.), The role of terrestrial and aquatic organisms in the decompositional processes. Blackwell Scientific Publications, Oxford: 323–338.

Federle, T. W. & J. R. Vestal, 1980. Microbial colonization and decomposition of *Carex* litter in an arctic lake. Appl. Environ. Microbiol. 39: 888–893.

Federle, T. W. & J. R. Vestal, 1982. Evidence of microbial succession on decaying leaf litter in an arctic lake. Can. J. Microbiol. 28: 686–695.

Fenchel, T. M. & B. B. Jorgensen, 1977. Detritus food chains of aquatic ecosystems: the role of bacteria. Adv. Microb. Ecol. 1: 1–57.

Fujisawa, H., M. Murakami, M. Hamada & H. Sera, 1967. Studies on xylan-decomposing bacteria in the marine

environment-I. Methods of isolation and enumeration of aerobic β-1,4′-xylan-decomposing bacteria. (in Japanese) Bull. Jap. Soc. Sci. Fish. 33: 438–447.

Furota, T., 1980. Seasonal variation of phytoplankton standing stocks in temperate embayments. (in Japanese) Bull. Plank. Soc. Jap. 27: 63–73.

Hodkinson, I. D., 1975. Dry weight loss and chemical changes in vascular plant litter of terrestrial origin, occurring in a beaver pond ecosystem. J. Ecol. 63: 131–142.

Kadota, H., 1956. A study on the marine aerobic cellulose-decomposing bacteria. Mem. Coll. Agr. Kyoto Univ. 74: 1–128.

Kaushik, N. K. & H. B. N. Hynes, 1971. The fate of the dead leaves that fall into streams. Arch. Hydrobiol. 68: 465–515.

Mason, C. F. & R. J. Bryant, 1975. Production, nutrient content and decomposition of *Phragmites communis* Trin. and *Typha angustifolia* L. J. Ecol. 63: 71–95.

Morrison, S. J., J. D. King, R. J. Bobbie, R. E. Bechtold & D. C. White, 1977. Evidence for microfloral succession on allochthonous plant litter in Apalachicola Bay, Florida, USA. Mar. Biol. 41: 229–240.

Odum, E. P. & A. A. de la Cruz, 1967. Particulate organic detritus in a Georgia salt-marsh-estuarine ecosystem. In G. H. Lauff (ed.), Estuaries. American Association for the Advancement of Science, Washington, D.C.: 383–388.

Oláh, J., 1972. Leaching, colonization and stabilization during detritus formation. In U. Melchiorri-Santolini & J. W. Hopton. (eds.) Mem. Ist. Ital. Idrobiol. 29 Suppl.: 105–127.

Olson, F. C. W., 1950. Quantitative estimates of filamentous algae. Trans. am. Micr. Soc. 59: 272–279.

Oppenheimer, C. H. & C. E. ZoBell, 1952. The growth and viability of sixty-three species of marine bacteria as influenced by hydrostatic pressure. J. mar. Res. 11: 10–18.

Park, D., 1972. Methods of detecting fungi in organic detritus in water. Trans. Br. mycol. Soc. 58: 281–290.

Polunin, N. V. C., 1982. Processes contributing to the decay of reed (*Phragmites australis*) litter in fresh water. Arch. Hydrobiol. 94: 182–209.

Reese, E. T., 1977. Degradation of polymeric carbohydrates by microbial enzymes. Rec. Adv. Phytochem. 11: 311–365.

Sørensen, H., 1957. Microbial decomposition of xylan. Acta Agric. Scand. Suppl. 1: 1–86.

Suberkropp, K. F. & M. J. Klug, 1974. Decomposition of deciduous leaf litter in a woodland stream I. A scanning electron microscopic study. Microb. Ecol. 1: 96–103.

Suberkropp, K. & M. J. Klug, 1976. Fungi and bacteria associated with leaves during processing in a woodland stream. Ecology 57: 707–719.

Taligoola, T. K., A. E. Apinis & C. G. C. Chesters, 1972. Microfungi colonizing collapsed aerial parts of *Phragmites communis*. Trin. in water. Nova Hedwigia 23: 465–472.

Tanaka, Y. & Y. Tezuka, 1982. Dynamics of detritus-attached and free-living bacteria during decomposition of *Phragmites communis* powder in seawater. Jap. J. Ecol. 32: 151–158.

Teal, J. M., 1962. Energy flow in the salt marsh ecosystem of Georgia. Ecology 43: 614–624.

Tubaki, K., 1974. Taxonomy and distribution of marine fungi. (in Japanese) In N. Taga (ed.), Marine Microorganisms. University of Tokyo Press, Tokyo: 65–81.

Waksman, S. A. & F. G. Tenney, 1927. The composition of natural organic materials and their decomposition in the soil: I. Methods of quantitative analysis of plant materials. Soil Sci. 24: 275–283.