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Growth and Turnover of Microbial Biomass during the Decomposition of Organic Matter (*Polygonum cuspidatum*) *in vitro*

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Abstract

Air-dried fresh and dead specimens of *Polygonum cuspidatum* were incubated for 250 days in the laboratory, and the growth and turnover of microbial biomass-C in the organic matter were studied. The biomass-C in the fresh leaf and fresh stem attained maximum levels on day 14 and day 7, respectively, and then settled down to stable levels. In the dead leaf and dead stem, increase in biomass-C ceased by day 4 and the biomass-C levels did not change thereafter. The turnover time of the biomass-C was estimated from the amount of biomass-C and the release rate of CO_2-C. The turnover was rapid in the early period of incubation. Then the turnover time became longer and after incubation for 70 days the values approached those in natural soils (longer than 16 days). During the incubation period, nitrogen was not mineralized in any organic matter. In the dead leaf and dead stem, asymbiotic nitrogen fixation activity increased after incubation for about 40 days and disappeared by the end of the incubation period, whereas nitrogen fixation was hardly detected in the fresh leaf and fresh stem.

Key words: Asymbiotic nitrogen fixation; Decomposition *in vitro*; Microbial turnover; Nitrogen mineralization.

Introduction

In natural terrestrial ecosystems, consumers graze only about 10% of dry matter produced by plants and the remaining 90% falls to the ground as litter (Whittaker, 1970). In agricultural ecosystems, fresh plant materials, as well as dead materials, are plowed in as manure. Soil microbes form a central compartment of soil biomass (Jenkinson and Powlson, 1976; Anderson and Domsch, 1978) and utilize the supplied organic matter as an energy and nutrient source. They incorporate part of this organic matter into new biomass and mineralize the remainder. Hence growth and decay of microbial biomass on litter and fresh plant materials form the important process of energy flow and nutrient cycling in terrestrial communities. However, only a few authors have investigated the changes in soil microbial biomass occurring through organic matter decomposition (Jenkinson and Rayner, 1977; Ladd et al., 1981), although the decomposition rates of litter and fresh plant materials have been estimated in many field studies (Jenkinson, 1965; Smith and Douglas, 1971; Shields and Paul, 1973; Nyhan, 1975; Jenkinson and Ayanaba, 1977; Kawahara, 1977; Yoneda, 1986). One particularly neglected aspect has been the formation of soil microbial biomass at the early stage of decomposition, when a large amount of organic matter is lost.

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From an autecological viewpoint, the growth and decay of soil microbial biomass are also of interest. In the last few decades, studies have shown that the average turnover time of soil biomass, which is a useful indicator of the growth of individual microbes, is longer than a few months owing to energy deficiency (Babiuk and Paul, 1970; Jenkinson and Ladd, 1981), even though under favorable conditions the generation time of many microbial species is less than a few hours (Brock, 1966). Does this mean that soil microbes are unable to grow rapidly? One of the aims of the present research was to answer this question.

The present paper reports the growth and turnover of microbial biomass during the course of litter and fresh plant material decomposition *in vitro*. The organic matter was incubated in the laboratory in order to avoid fluctuation of environmental conditions. Since nitrogen often limits the growth of soil microbes at the early stage of organic matter decomposition and is the soil nutrient that plants require in greatest quantity (Alexander, 1977), mineralization and asymbiotic fixation of nitrogen were also measured throughout the incubation period.

Materials and Methods

Organic matter

Fresh leaves and stems, newly fallen dead leaves and standing dead stems of *Polygonum cuspidatum* Sieb. et Zucc. were collected on Mt. Fuji (1400 m altitude) in August 1984. The materials were air-dried at room temperature and crushed with a mill (<14 mesh). Carbon and nitrogen contents were determined with a Sumigraph NC-80 NC-Analyzer (Sumitomo Chemical). C/N ratio of the fresh leaf, fresh stem, dead leaf and dead stem were 24, 72, 48 and 110, respectively.

Soils

Soil contains organic matter that has been processed by the soil biomass for a long period. In order to compare the turnover time of microbial biomass in these organic materials with that in old materials, eleven organic soils were collected from the 0–5-cm layer in three

Table 1. Properties of the organic soils.

Soil No.	WHC[1] (%)	pH	C (%)	N (%)	Biomass-C (g C kg^{-1})	Turnover time of biomass-C	
						A[2] (day)	B[3] (day)
1	186	5.9	16.8	1.23	1.76	34	180
2	206	5.7	12.1	0.75	1.41	25	58
3	136	6.9	11.6	0.74	1.52	18	31
4	93	4.2	7.2	0.39	0.34	16	25
5	240	6.7	15.7	1.53	2.13	30	102
6	251	4.4	23.1	1.54	2.30	51	—[4]
7	241	4.3	21.1	1.62	1.89	30	114
8	189	4.5	18.1	1.06	1.24	41	1000
9	66	4.7	7.0	0.42	1.29	30	107
10	65	4.5	9.1	0.51	1.80	25	65
11	60	4.7	5.3	0.30	1.21	24	58

Soils 1–5 were from the campus of the University of Tokyo, soils 6–8 were from a coniferous forest on Mt. Fuji, and soils 9–11 were from sand dunes of Azigaura.
1) maximum water-holding capacity (dry weight basis). 2) m = 0.000 day^{-1} in Equation (2). 3) m = 0.024 day^{-1} in Equation (2). 4) turnover did not occur, because all energy was used for maintenance of cells.

areas: Three soil samples were taken from the sand dunes at Azigaura, three from a coniferous forest on Mt. Fuji and five from the University of Tokyo campus, in November 1984. The soils were stored at 4°C until the experiment. A proportion of each soil was air-dried and used for the determination of soil properties. Table 1 shows the properties of the soils.

Incubation procedure

Organic matter: Ten grams of wet soil (an even mixture of the eleven soils that were collected for comparison) was added to 500 ml of water and stirred for 10 min. After precipitation of the soil particles, 2.4 ml of the supernatant containing soil microbes was transferred to a glass vial (105 mm in length, 18 mm in diameter). Then, 600 mg of air-dried organic matter was added to the vial. After 1 h, the moistened organic matter was stuck on to the inside wall of the vial (about 2 mm in thickness, 40 mm in length) with a spatula to prevent the development of anaerobic conditions. Two hundred vials were prepared in this way for each sample of organic material, and the vials were loosely capped with sheets of aluminum foil. In order to reduce the effect of absence of microbial species on decomposition, and to distinguish any differences between the organic materials, I inoculated mixed microbes extracted from all the soils.

Soils: The soils were sieved (2 mm mesh) and the water content was adjusted to about 50% of the maximum water-holding capacity. Fifty grams of wet soil was then placed in a 100-ml polyethylene vial. Three vials were prepared for each soil sample and then wrapped with thin polyethylene film.

The organic matter and soils were incubated at 25°C in the dark. The moisture content was kept constant by adding water to the vials. After incubation for 70 days, the CO_2 evolution and biomass-C of the soils were determined.

Analytical methods

The dry weight of organic matter including dead and living microbial biomass was measured after drying at 80°C for one week in an oven. Thereafter these oven-dried samples were used for measurement of organic C and N. CO_2 evolution from the organic matter and soils was determined by the alkaline absorption method (Stotzky, 1965), after which the samples used were returned to the incubator. Duplicate and triplicate measurements were done for the dry weight and CO_2 evolution, respectively.

Microbial biomass-C was determined by the ATP method (Jenkinson and Oades, 1979). This method is based on the close link between the ATP content of soil and the presence of living organisms (Lee et al., 1971a, 1971b). Soil ATP and organic matter were extracted with trichloroacetic acid-phosphate-paraquat solution and 10% trichloroacetic acid, respectively. Wet soil (1 g) and organic material incubated in the glass vial was ultrasonicated for 1 min with 10 ml of each extractant. In order to correct for incomplete extraction of ATP, known amounts of authentic ATP was added to subsamples during extraction. The extracts were centrifuged (10,000 × g, 10 min), the precipitates discarded, and the supernatants were stored at −20°C. Within one week, ATP content was determined with a Chem-Glow J4-7441 Photometer (Aminco), following the method of Karl and Holm-Hansen (1978). FLE-50 firefly lantern extract (Sigma) was used for the luciferin-luciferase light-emission system. The recoveries of ATP added were 53.4–94.0% in the organic matter samples and 40.8–82.2% in the soils. Although the recoveries were incomplete, the reproducibility of the re-

covery was good. The biomass-C was estimated from 171×ATP (Tate and Jenkinson, 1982). The ATP contents of the organic matter samples and the soils were corrected using the ATP recovery measured at each sampling time. Triplicate samples were used for the measurement of both ATP content and ATP recovery.

Mineral nitrogen was extracted from the organic matter with 2 N KCl solution. The solution (10 ml) was added to the vial containing organic matter and vigorously stirred for 30 min. The suspension was centrifuged (5000×g, 10 min) and the supernatant filtered with a Whatman GF/F glass fiber filter. The precipitate was discarded. Ammonium, nitrite and nitrate in the supernatant were determined colorimetrically with a Technicon auto-analyzer (Technicon, 1971, 1976). Duplicate samples were used for mineral nitrogen extraction.

Nirogen fixation activity was estimated by the C_2H_2 reduction technique (Hardy et al., 1973). C_2H_2 (2 ml) was added to the glass vial, and after incubation for 4 h at 25°C in the dark, the concentration of C_2H_4 was measured. Subsamples were incubated without C_2H_2 in order to estimate endogenous C_2H_4 production. The concentration of C_2H_4 in 1-ml gas samples was measured with a Shimazu GC-9A gas chromatograph using a 2 m×2 mm Chromosorb 104 (80/100 mesh) column and a flame ionization detector. Duplicate samples and duplicate subsamples were used for the measurement. The samples incubated with C_2H_2 were discarded after the measurement, whereas the subsamples without C_2H_2 were returned to the incubator.

Calculation of turnover time

CO_2 evolution rate, R (mg CO_2-C day^{-1}), is expressed as the following equation:

$$R = mx + \left(\frac{1}{Y} - 1\right)\mu x \tag{1}$$

where x, m, Y, and μ are biomass-C (mg), specific maintenance rate (mg substrate-C mg biomass-C^{-1} day^{-1}), true growth yield (efficiency of growth, mg biomass-C mg substrate-C^{-1}), and specific growth rate (mg newly synthesized biomass-C mg biomass-C^{-1} day^{-1}), respectively. The microbes use the maintenance energy (mx) for protein and nucleotide turnover, osmoregulation, motility, etc. (Tempest and Neijssel, 1984). The substrate used for maintenance of cells is lost as CO_2. Part of the substrate used for synthesis of new cells is assimilated to new biomass (μx) and the remainder is lost as CO_2 ($(1/Y-1)\mu x$). By rearrangement, we obtain μ:

$$\mu = \left(\frac{Y}{1-Y}\right)\left(\frac{R}{x} - m\right) \tag{2}$$

We define the turnover time as μ^{-1}. In order to estimate the turnover time, the biomass-C and CO_2 evolution rate measured in the present experiment were used as x and R, respectively. Y is assumed to be 0.5, which is considered to be the maximum (Payne, 1970). The values, 0.000 and 0.024 day^{-1}, were used for m (Babiuk and Paul, 1970).

Results

By day 250, microbes had decomposed 28–39% of dry matter (Fig. 1-a). The decomposition rates were relatively high at the early stage, especially in the fresh leaf, and slowly decreased with time. The respiration rate was high in the early period, especially in the fresh

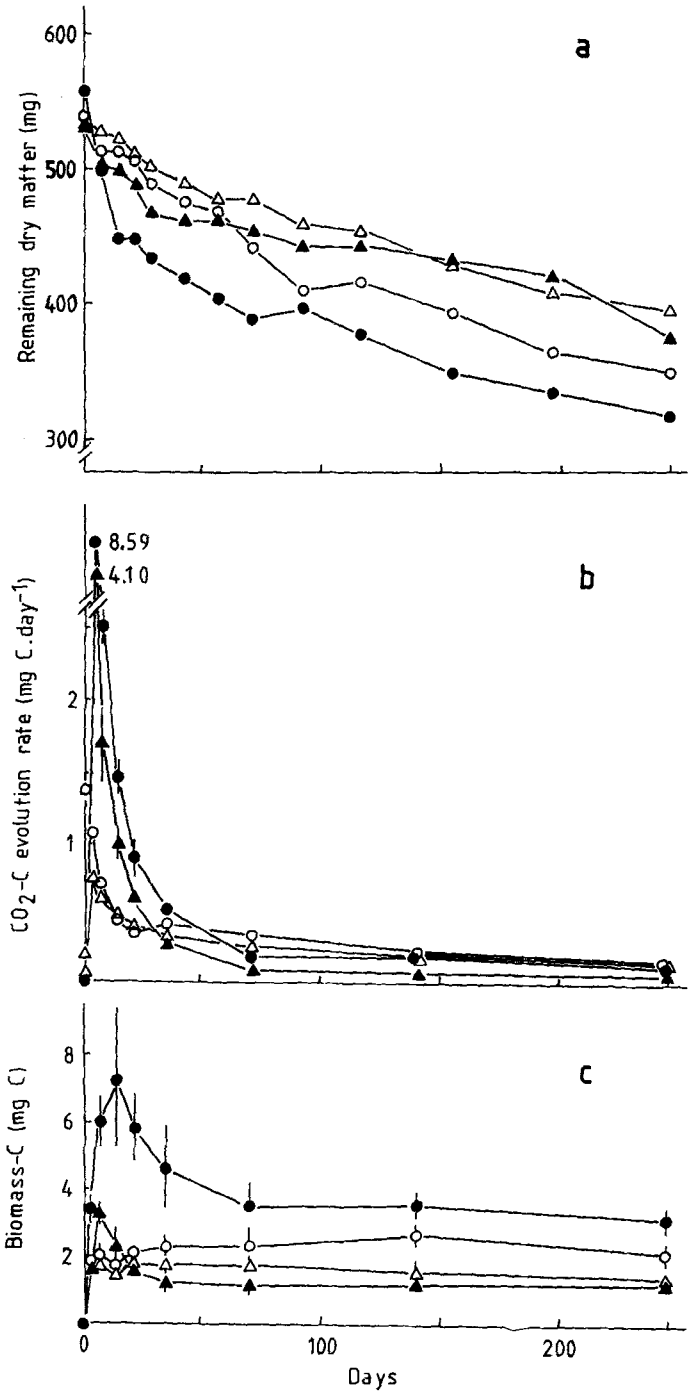

Fig. 1. (a) Remaining dry matter in a vial (mean of duplicate samples). (b) Changes in release rate of CO_2-C from a vial ($n = 3$, mean$\pm$SD). (c) Changes in biomass-C in a vial ($n = 3$, mean$\pm$SD). Symbols: ●, fresh leaf; ▲, fresh stem; ○, dead leaf; △, dead stem.

leaf and fresh stem (Fig. 1-b). Then the rate decreased and did not change remarkably from day 70 to day 250.

The microbial biomass-C increased and attained the maximum level on day 7 in the fresh stem and on day 14 in the fresh leaf (Fig. 1-c). At this stage, fungal hyphae covered the surface of the fresh leaf and fresh stem. Increase in the biomass-C in the dead leaf and dead stem ceased by day 4, and the hyphae showed no remarkable extension on them. The carbon in the plant material used by the microbes was estimated from the synthesized biomass-C plus the evolved CO_2-C. On the day when the increase in biomass-C ceased, 11.2%, 16.0%, 38.7% and 41.1% of utilized carbon remained in the biomass-C in the fresh leaf, fresh stem, dead leaf and dead stem, respectively. Thereafter the biomass-C settled down to stable levels in the fresh leaf and fresh stem and hardly changed in the dead leaf and dead stem.

Figure 2 shows the changes in turnover time of the microbial biomass during the course of organic matter decomposition. The turnover time in each soil is listed in Table 1. The shortest turnover times in the fresh leaf and fresh stem were 10 h and 11 h, respectively, on day 4 at $m = 0.000\ \text{day}^{-1}$. These values were similar to the generation time of many microbial species under favorable conditions (Brock, 1966). In the dead leaf and dead stem, the turnover times on day 4 were 1.8 and 2.5 days, respectively, at $m = 0.000\ \text{day}^{-1}$. Subsequently, the turnover time became longer. On day 250, the value was longer than 7 days when $m = 0.000\ \text{day}^{-1}$ and approached those in the natural soils (longer than 16 days at the same m value). This value of m gives the shortest time for turnover, because all energy is used for the synthesis of new cells. When $m = 0.024\ \text{day}^{-1}$, the time was longer than 8 days in the organic matter on day 250 and longer than 25 days in the natural soils. The longest turnover time, which is off the scale of the figure, was 75 days in the fresh leaf on day 250.

With respect to the turnover time in the soils, no considerable difference was observed

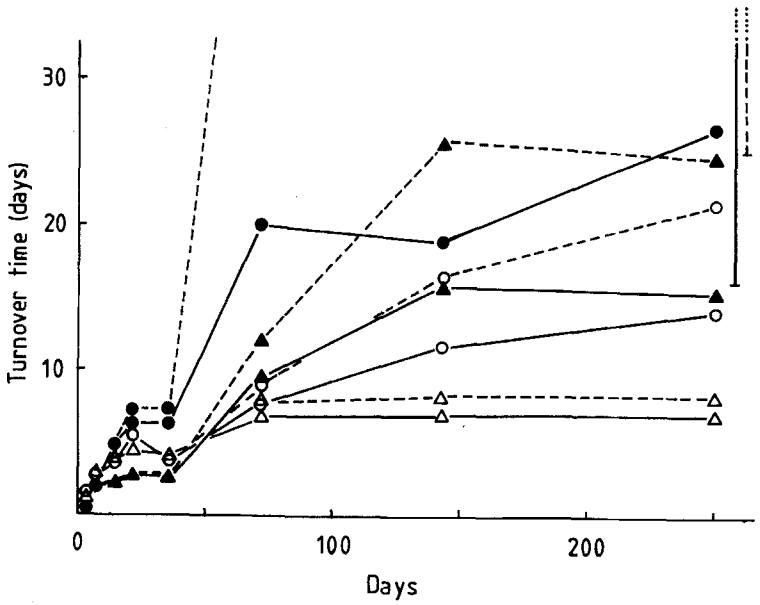

Fig. 2. Turnover time of microbes in the fresh leaf (●), fresh stem (▲), dead leaf (○), dead stem (△) and soils. Vertical bars on the right side of the figure represent the range of turnover time of the soils. Solid line and broken line represent the values when $m = 0.000\ \text{day}^{-1}$, and $m = 0.024\ \text{day}^{-1}$, respectively.

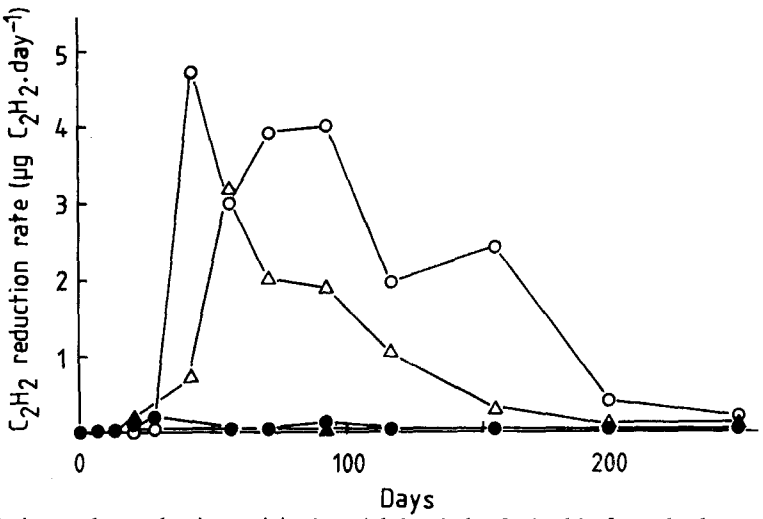

Fig. 3. Asymbiotic acetylene reduction activity in a vial. Symbols: ○, dead leaf; △, dead stem; ●, fresh leaf; ▲, fresh stem (mean of duplicate samples).

between the three areas where the soils had been sampled, although the values varied in each area. The growth yield Y, as well as the maintenance rate m, affects the turnover time estimated with this model (Chapman and Gray, 1986). When Y is smaller than 0.5, the turnover time is longer than that estimated at $Y = 0.5$, because Equation (3) represents a hyperbolic increase in the turnover time with the decrease of Y value under the same conditions, the other parameters being constant.

By day 4, the microbes had immobilized ammonium, which was initially present in small amounts, and did not produce ammonium thereafter. Nitrate and nitrite were not detected throughout the incubation period. In the dead leaf and dead stem, C_2H_2 reduction activity increased after a time lag of about 40 days and disappeared by day 250, whereas hardly any C_2H_2 was reduced by microbes in the fresh leaf and fresh stem (Fig. 3). Assuming that the C_2H_2:N_2 ratio was 3:1 (Baker and Attiwill, 1984), the amount of nitrogen fixed asymbiotically during the incubation period was estimated to be about 0.15 mg per vial in the dead leaf and 0.07 mg per vial in the dead stem. These values indicate that the nitrogen fixed during the incubation period was about 3% of the total in the dead leaf and dead stem. Owing to this inactivity of nitrogen mineralization and fixation, the organic nitrogen level did not change remarkably throughout the incubation period in any of the materials (Fig. 4).

Discussion

Survival strategy of soil microbes

In general, the soil microbial population is crowded and cannot grow actively owing to limitation of energy supply (Babiuk and Paul, 1970; Jenkinson and Ladd, 1981). Recent studies have focused on the physiological condition of the soil biomass and the interaction of soil biomass with soil organic matter. Several authors have shown that despite this energy deficiency, vegetative cells rather than resting spores are predominant in the soil, using a biochemical indicator, adenylate energy charge (Brookes et al., 1983, 1987: Tateno, 1985).

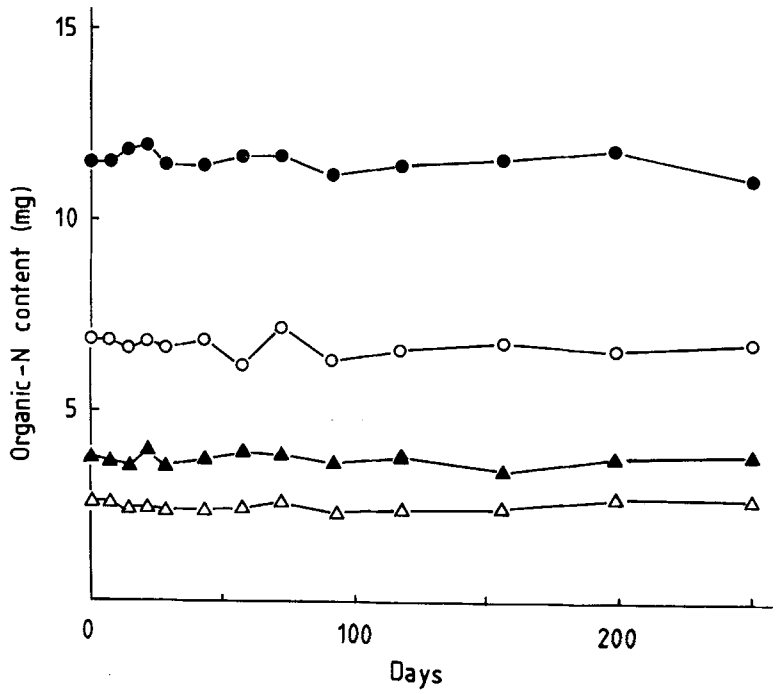

Fig. 4. Organic nitrogen content in a vial. Symbols: ●, fresh leaf; ▲, fresh stem; ○, dead leaf; △, dead stem (mean of duplicate samples).

Tateno (1988) also showed that soil microbes synthesize sufficient amounts of extracellular enzymes to degrade soil organic matter, and that a paucity of enzyme substrates causes the energy deficiency. These results suggest that most soil microbes obtain only small energy sources, and that these are barely sufficient to maintain their vegetative cells.

In the present experiment, the turnover time of the microbial biomass in eleven soils was longer than 16 days (Table 1). This indicates that the majority of microbes grow slowly in mature soils, roughly agreeing with previous reports (Babiuk and Paul, 1970; Jenkinson and Ladd, 1981). Also at the later stage of incubation, the turnover time in the four types of organic material became longer and approached that observed in the soils. We cannot conclude what the limiting factor of microbial growth was at this stage, because nitrogen as well as energy sources often limits the growth at this time (Alexander, 1977). In contrast with the soils and the organic materials at the later stage of the decomposition process, an increase in the biomass and a short turnover time were observed in the early period of decomposition, although the biomass in the fresh materials increased more rapidly than that in the dead materials and the turnover time in the former was shorter than that in the latter. The easily decomposable substrates contained in newly fallen litter and particularly those in fresh plant materials would enable soil microbes to grow rapidly at the early stage. The rapid consumption of this substrate in the fresh materials would lead to a decrease in the biomass at the succeeding stage. Thus, the ability to grow rapidly, a characteristic of many microbial species, can be exhibited only at the early stage of decomposition. It is thus probable that microbes with a large carrying capacity, rather than an improved growth rate, are selected in soil ecosystems except in the early stage of the decomposition process.

Nitrogen dynamics during decomposition

Nitogen assimilation and dissimilation are closely related to energy flow (Woldendrop, 1978). Under energy-rich and nitrogen-limited conditions, nitrogen is assimilated, while under the reverse conditions, nitrogen is dissimilated. When the nitrogen content of organic matter is low (C/N ratio higher than 25) microbes immobilize nitrogen, whereas they mineralize nitrogen when the nitrogen content is high (C/N ratio lower than 25) (Swift et al., 1979). However, mineral nitrogen did not accumulate even in the fresh leaf material, although the C/N ratio of the fresh leaf was 24 on day 0 and 15 on day 250. In contrast, Ohta and Kumada (1978) showed that nitrogen was mineralized when the C/N ratio was more than 25. Berg and Staff (1981) also showed that the critical C/N ratio for nitrogen mineralization was 167 in a Scots pine forest. These results indicate that the C/N ratio of whole organic matter is not the sole controlling factor of nitrogen assimilation and dissimilation.

One of the processes of nitrogen assimilation by soil microbes is asymbiotic nitrogen fixation. This has been detected in soil, especially in litter where nitrogen is often the limiting factor of microbial growth (Baker and Attiwill, 1984). Richard (1964) suggested the importance of asymbiotic nitrogen fixation in terrestrial nitrogen cycling. However, in the dead leaf and dead stem materials, only about 3% of the total nitrogen content was fixed asymbiotically during the incubation period, and in the fresh leaf and fresh stem material, hardly any such was detected. These results of the present study do not suggest that asymbiotic nitrogen fixation always contributes greatly to the terrestrial nitrogen cycle.

The variety observed in nitrogen dynamics would be partly attributable to the quality of organic matter available. Some substances might inhibit nitrogen fixation in the fresh leaf and fresh stem and mineralization in the fresh leaf, which has low C/N ratio. Furthermore, it is probable that the C/N ratio of the whole material does not always express the C/N ratio of the substrate directly utilized by the soil biomass. Even if the total C/N ratio is high, the nitrogen can be mineralized under conditions in which the C/N ratio of usable substrate is low. Conversely, if the C/N ratio of usable substrate is high, the nitrogen can not be mineralized from low-C/N materials. The C/N ratio of usable substrate would thus control the mineralization and immobilization of nitrogen. Furthermore, differences in microbial species would also affect the nitrogen dynamics. Cowling (1961) demonstrated a difference in cellulose depolymerization rate between two fungal species. A brown-rot fungus markedly decreased the degree of polymerization of sapwood cellulose, whereas a white-rot fungus produced a gradual decrease. The differences observed between the four materials used in the present experiment cannot be attributed to species, because the same extract was added in each case. However, the diversity of nitrogen dynamics between studies may reflect the differences among microbial flora from various separate regions. One of our next lines of study will be to clarify the effect of microbial species and substrate quality on nitrogen cycling in terrestrial ecosystems.

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