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Nutritional environment of soil and roots in and around mycelial blocks of an ectomycorrhizal fungus *Tricholoma bakamatsutake* in an evergreen Fagaceae forest

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In the evergreen Fagaceae forests of Japan, an ectomycorrhizal fungus *Tricholoma bakamatsutake* forms "shiros" or developing mycelial blocks. To determine the physiological characteristics of the mycelial blocks, organic acids of the soil and major nutrient elements of the soil and roots were compared at three types of sites: presently colonized mycelial blocks, previously colonized sites behind the blocks, and uncolonized sites in front of the blocks. The upper part of the mycelial blocks showed the following features compared with the uncolonized site: lower pH (5.1), higher concentrations of oxalic and gluconic acids, lower content of total nitrogen, a similar amount of total carbon, reduced total and available phosphorus, higher content of total calcium and lower content of exchangeable calcium. These findings suggested that the activity of the fungus led to soil acidification by the organic acids, an increase in C/N ratio, depletion of phosphorus and accumulation of calcium.

Key Words——ectomycohhrizal fungus; mat-forming fungus; mycelial block; soil nutrient; *Tricholoma bakamatsutake*.

Several ectomycorrhizal fungi form visible mycelial mats in the upper soil layer. Such mycelial mats have certainly modified the biotic and abiotic environment of the soil (Fisher, 1972; Cromack et al., 1979). In coniferous forests of the United States Pacific Northwest, organic acid production, nutrient accumulation, microbial biomass and nursing plant seedlings in the mat soil formed by ectomycorrhizal fungi, *Hysterangium* and *Gautieria*, greatly differed from those in the adjacent non-mat soil (Griffiths et al., 1990; Griffiths et al., 1991a, b; Entry et al., 1992; Griffiths and Caldwell, 1992; Griffiths et al., 1992). This strongly suggested that these mat-forming fungi had certain functions in the ecosystem of the Pacific Northwest.

In the evergreen Fagaceae forests of Japan, an ectomycorrhizal fungus *Tricholoma bakamatsutake* Hongo forms "shiros," which are distinctly colonized subterraneous spaces developed as a result of mycelial growth in the humus layer and shallow mineral soil (Terashima et al., 1993). Forefronts of these "shiros" are composed of several discernible mycelial blocks. Some allied species of this fungus have also been observed to form "shiros" in either broad-leaved or coniferous forests (Ogawa, 1978). It is presumed that some kinds of environmental modifications also occurred in this condensed biosphere in a manner similar to the mat soils in the Pacific Northwest. However, relatively little information about the environment of "shiros" has been reported, except for the study of microbial communities in and around "shiros" of *Tricholoma matsutake* (S. Ito & Imai) Sing., an allied species of *T. bakamatsutake*, in *Pinus* forests (Ogawa, 1977).

In this paper, we selected three kinds of sites: the mycelial blocks of *T. bakamatsutake*, sites behind these blocks inside the "shiros," and sites in front of the blocks outside the "shiros." The mycelial blocks were presently colonized by the fungus; the backward sites had ceased to be colonized; and the forward sites were yet to be colonized. The object here was to examine the physiological properties of the biosphere of the concentrated mycelia of *T. bakamatsutake* in a Fagaceae forest. We report differences between the three kinds of sites in terms of acidification by oxalic and gluconic acids and accumulation or depletion of major total nutritional elements in the soil and roots by the fungus. Oxalic acid had an important effect on weathering soils in the Pacific Northwest (Fisher, 1972; Cromack et al., 1979; Griffiths and Caldwell, 1992, Griffiths et al., 1992); and gluconic acid secreted by the cultured mycelia of *T. bakamatsutake* was specific to this fungus compared with some of its allied species (Iwase, 1992).

Materials and Methods

Sampling Study was conducted at two connecting "shiros" in a mixed forest of *Pasania edulis* Makino and *Castanopsis cuspidata* (Thunb.) Schotty var. *sieboldii* (Mak.) Nakai in Chiba Prefecture (Terashima et al., 1993). In May 1993, ten 100-cm^3 soil-root core samples were excavated into stainless tubes (50 mm in diam and 51 mm tall) from the soil surface after removing the rough litter (Fig. 1).

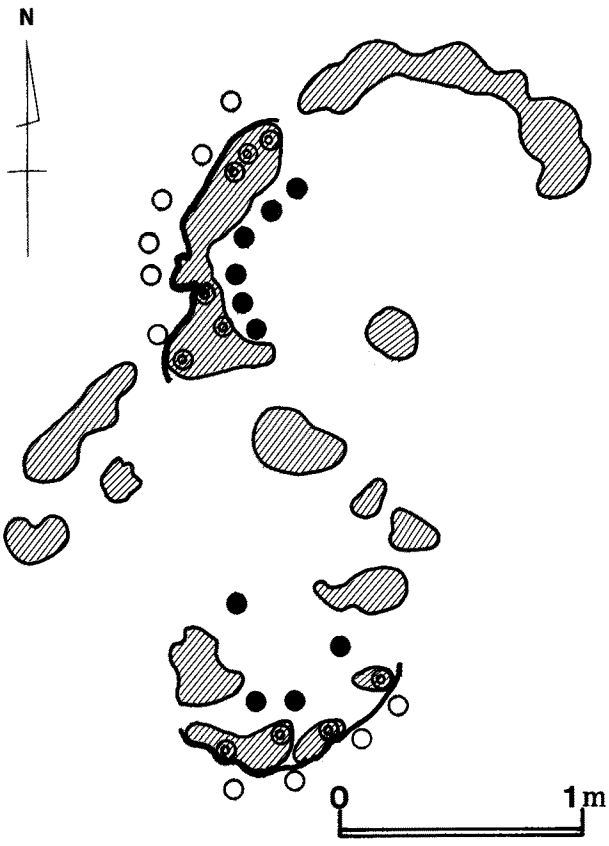

Fig. 1. Sampling locations at "shiros" of *T. bakamatsutake* in a horizontal plane.
▨Mycelial blocks: bold lines indicate the estimated forefronts of "shiros". Sampling locations: ○ uncolonized site in front of mycelial blocks, ◎ mycelial blocks and ● previously colonized site behind mycelial blocks.

Nutrient analysis The water content of the core samples was measured after drying at 60°C for 48 h. Core samples taken from the mycelial blocks were separated into two layers: the upper fungal layer which contained mycelia combined with soil, and the lower soil layer which consisted of only soil. The mycelia-soil complex and the soil were used as different soil samples. The samples were gently dispersed in a tray and roots were carefully removed. The remaining soil was passed through a 0.5-mm mesh sieve to remove the rough detritus. The roots were finely ground.

The pH of soil samples was measured using a pH meter (Twin pH B-112, Horiba Co., Ltd.) after adding ten times the weight of distilled water. The soil contents of total carbon and total nitrogen were measured using a CN-Corder (MT-600, Yanagimoto Co., Ltd.). The cation exchange capacity and contents of exchangeable potassium, calcium, magnesium and sodium were measured by the Peech method (Peech et al., 1947). Available phosphorus in soil was determined by the bicarbonate extraction method (Olsen et al., 1954). After digestion of the soil samples with nitric acid and perchloric acid, total phosphorus content was determined colorimetrically (molybdenum blue method) with a spectrophotometer (200-20, Hitachi Co., Ltd.). The total contents of four elements (potassium, calcium, magnesium and sodium) were determined using an atomic absorption spectrophotometer (Z-6000, Hitachi Co., Ltd.). After extracting the organic acids from the soil samples with four times the weight of distilled water (Takijima, 1961), the contents of gluconic acid and oxalic acid were determined by UV absorption using an F-kit (Boehringer Mannheim, Yamanouchi Co., Ltd.) with a fluorescence spectrophotometer (UV-150-02, Shimadzu, Co., Ltd.).

The contents of phosphorus and the previously mentioned four elements in the root samples were determined after acidic digestion of the samples in the same manner as the soil samples.

Statistics The soil and root samples dried at 60°C were dried again at 105°C for 24 h to obtain their oven-dry weights. All values determined for samples dried at 60°C were converted on the basis of the oven-dry weights and compared using Duncan's multiple range test at the 5% level.

Results

Habit of roots and mycorrhiza Mycorrhiza were formed by *T. bakamatsutake* on all the terminal roots in the fungal layer of all the core samples of the presently colonized mycelial blocks. In six core samples out of ten from the previously colonized sites, mycorrhiza and very sparse mycelia of this fungus attached to them were found only on a few terminal roots.

Water content and soil pH The mycelial block was the driest among the three kinds of sites. Water content was 39% in the mycelial blocks, whereas it was 42% in the previously colonized sites and 47% in the un-

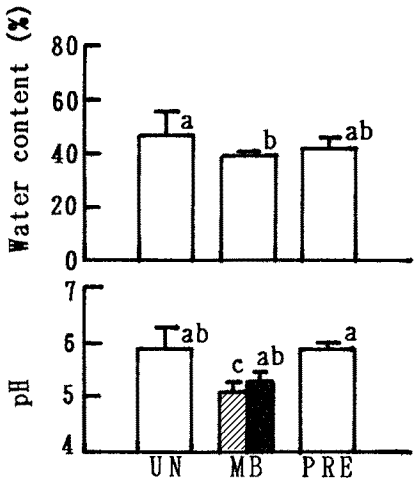

Fig. 2. Water content and pH of soil samples in and around mycelial blocks of *T. bakamatsutake*.
UN: Uncolonized site in front of mycelial blocks, MB: mycelial blocks, which consist of ▨ mycelial and ■ soil layers, and PRE: previously colonized site behind mycelial blocks. The bars indicate standard deviations. The same letters on the shoulders of the histograms indicate no significant difference at the 5% level.

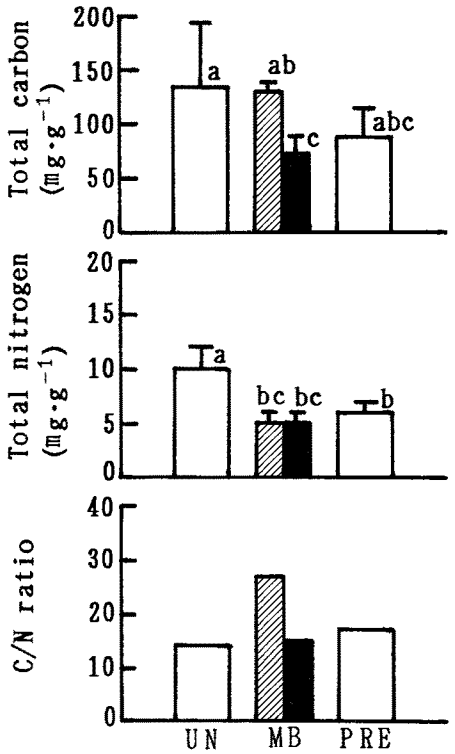

Fig. 3. Contents of total carbon and nitrogen, and C/N ratio of soil samples in and around mycelial blocks of *T. bakamatsutake*.
Legend is the same as in Fig. 2.

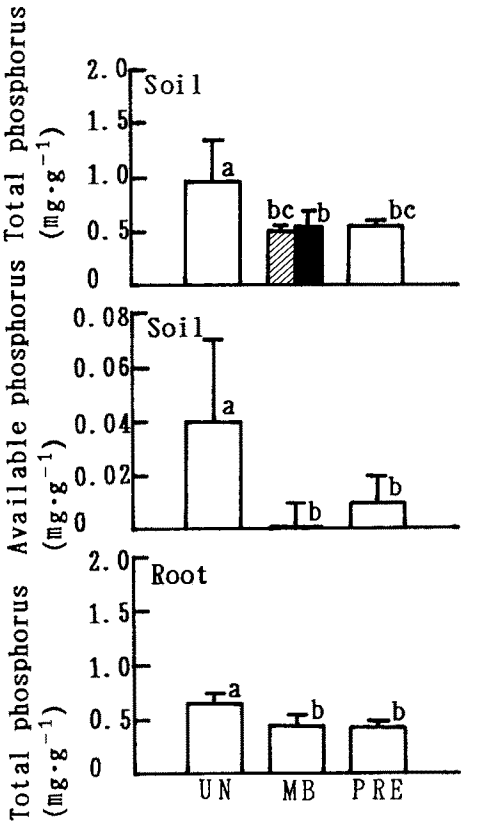

Fig. 4. Contents of total and available phosphorus of soil samples and total phosphorus of root samples in and around mycelial blocks of *T. bakamatsutake*.
Legend is the same as in Fig. 2.

colonized sites (Fig. 2).

The fungal layer of the mycelial blocks showed higher acidity than the other samples. The soil pH was significantly the lowest in the fungal layer of the mycelial blocks (5.1) followed by the soil layer in the same sites (5.8). The pHs of the previously colonized and uncolonized sites were both 5.9.

Carbon and nitrogen As shown in Fig. 3, the total carbon and nitrogen of the soil samples were less in the presently and previously colonized sites than in the uncolonized site. In the mycelial blocks, the total nitrogen in the fungal layer was about the same as that in the soil layer, but the total carbon in the fungal layer was a significantly higher than in the soil layer and was at the same level as that in the uncolonized sites. Thus, the fungal layer of the mycelial blocks showed the highest ratio of carbon content to nitrogen content (C/N) among the samples.

Phosphorus A low content of total phosphorus and an extremely low content of available phosphorus were particular to the mycelial blocks (Fig. 4). The total phosphorus of the soil samples was lower in both the presently and previously colonized sites than in the uncolonized sites. The available phosphorus of the soil samples was extremely low in the mycelial blocks, followed by the previously colonized sites. The total phosphorus of the root samples showed the same tendency as the available phosphorus in the soil samples.

Calcium A high concentration of total calcium was detected in the soil samples from the fungal layer of the mycelial blocks (Fig. 5). The exchangeable calcium of the soil samples was lower in the presently and previously colonized sites than in the uncolonized sites. The total calcium of the root samples showed a similar tendency to the exchangeable calcium of the soil samples.

Potassium, magnesium and sodium As shown in Table 1, the total magnesium and sodium of the soil samples were slightly lower in the presently and previously colonized sites than in the uncolonized sites. But the contents of these two elements did not show the apparent correlation with the presence of the fungus that was seen with total phosphorus and calcium. Among the other samples, the largest amount of total potassium was found in the fungal layer.

The cation exchange capacity and the exchangeable potassium, magnesium and sodium of the soil samples were slightly lower in the presently and previously colonized sites than in the uncolonized sites (Table 2). The contents of total magnesium and sodium in the root samples showed the same tendency as the above exchangeable elements in the soil samples. Only the exchangeable potassium acted differently. A higher total potassium was found in the root samples from the uncolonized sites than in the other two kinds of sites.

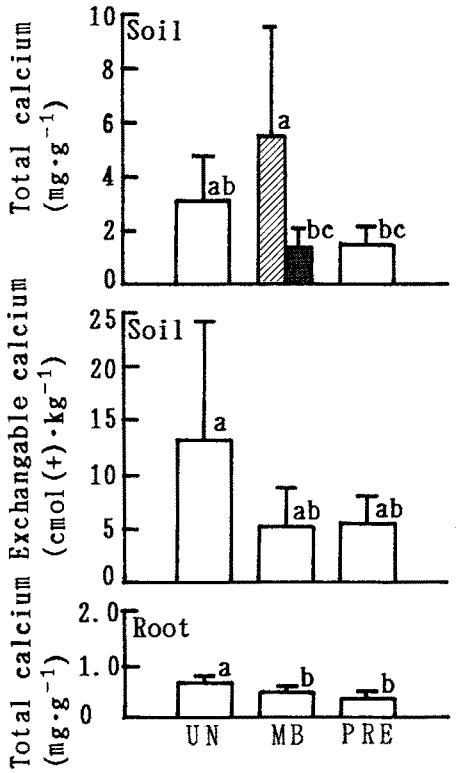

Fig. 5. Contents of total and exchangeable calcium of soil samples and total calcium of root samples in and around mycelial blocks of *T. bakamatsutake*.
Legend is the same as in Fig. 2.

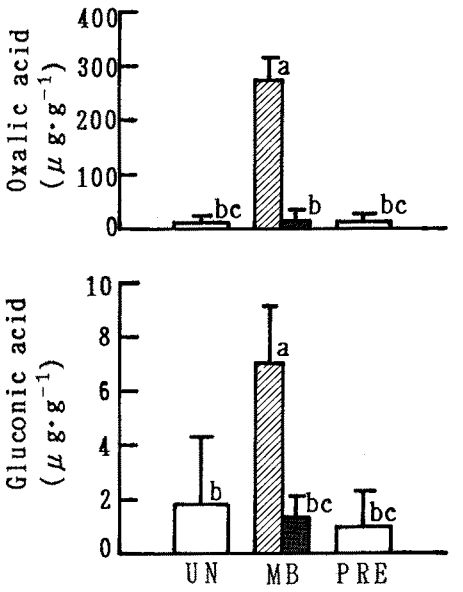

Fig. 6. Contents of oxalic and gluconic acid of soil samples in and around mycelial blocks of *T. bakamatsutake*.
Legend is the same as in Fig. 2.

Organic acids Figure 6 shows that the oxalic and gluconic acid contents in the fungal layer of the mycelial blocks were respectively 27 times and 4 times higher than in the uncolonized sites.

Discussion

The low pH and high contents of oxalic and gluconic acids in the fungal layer of *T. bakamatsutake* strongly indicated that this fungus lowered the soil pH by producing and secreting organic acids. Oxalic acid secretion by ectomycorrhizal fungi was confirmed in vitro for *Paxillus involutus* (Lapeyrie et al., 1987; Duchesne et al., 1989), and in the field for *Hysterangium crassum* (Cromack et al., 1979) and *Rhizopogon luteolus* (Malajczuk and Cromack, 1982). The pH in the mat soil of *Gautieria* and *Hysterangium* in coniferous forests was found to be significantly lower than that in the non-mat soil due to the secretion of oxalic acid by the fungi (Cromack et al., 1979; Griffiths et al., 1992). Gluconic acid secretion in vitro has been reported to be a distinguishing feature of *T. bakamatsutake* compared with some of its allied species (Iwase, 1992). Although gluconic acid was detected in lower amounts than oxalic acid, this fungus definitely secreted gluconic acid not only in vitro but also in the field.

The high concentration of total calcium and low concentration of exchangeable calcium in the mycelial blocks indicated that the activity of the fungus might lead to depletion of soluble calcium and accumulation of insoluble calcium. Accumulation of calcium oxalate crystals was observed in the mantle of the mycorrhiza of *H. crassum* (Cromack, 1979) and *Rhizopogon lutedus* (Malajcsuk and Cromack, 1982), and in both cases the concentration of calcium was significantly higher in the mat soils

Table 1. Contents of total magnesium, potassium and sodium of soil and root samples in and around mycelial blocks of *T. bakamatsutake*.

Sampling location	Soil (mg·g⁻¹, Mean±S.D.)			Roots (mg·g⁻¹, Mean±S.D.)		
	Mg	K	Na	Mg	K	Na
Uncolonized site	2.09±0.26a	0.66±0.21ab	0.85±0.15a	0.56±0.04a	0.27±0.08ab	0.48±0.30a
Mycelial blocks						
Fungal layer	1.87±0.13abc	0.72±0.22a	0.69±0.10bc	0.48±0.07ab	0.36±0.07ab	0.18±0.18ab
Soil layer	1.87±0.21abc	0.53±0.07abc	0.73±0.05ab			
Previously colonized site	1.89±0.14ab	0.56±0.15abc	0.66±0.12bc	0.50±0.06ab	0.40±0.14a	0.41±0.37ab

The same letters in each column indicate no significant difference at the 5% level.

Table 2. Cation exchangeable capacity and contents of exchangeable magnesium, potassium and sodium of soil samples in and around mycelial blocks of *T. bakamatsutake*.

Sampling location	CEC (cmol(+)·kg^{-1}, Mean±S.D.)	Exchangable element (cmol(+)·kg^{-1}, Mean±S.D.)		
		Mg	K	Na
Uncolonized site	44.69±11.44a	1.55±0.85a	0.53±0.24a	0.69±0.28a
Mycelial blocks	34.64±3.97ab	0.94±0.54ab	0.24±0.10b	0.57±0.19ab
Previously colonized site	33.53±5.53ab	0.99±0.40ab	0.33±0.11ab	0.62±0.18ab

The same letters in each column indicate no significant difference at the 5% level.

than in the non-mat soil.

The low contents of total and available phosphorus in the mycelial blocks suggested that phosphorus might be extracted and mobilized, and nearly all available phosphorus might be depleted due to the fungus. The organic acids secreted by *T. bakamatsutake* might have an effect on the weathering of the soil, although the mechanism is unknown. In the mat soil of *Hydnellum ferrugineum* in *Pinus* forests, the total and extractive phosphorus were also considerably less than that in the control soil, and it was assumed that exuded organic acids could accomplish the mobilization of ions (Fisher, 1972).

If the major nutrient elements are depleted by *T. bakamatsutake*, the values determined should show a gradation according to the density of the fungul mycelia, from lower values in the presently colonized mycelial blocks, through those in the previously colonized backward sites, to higher values in the uncolonized forward sites; or at least the values in the two kinds of colonized sites should be lower than those in the uncolonized sites. From this point of view, nitrogen, phosphorus, magnesium and sodium were depleted because of the fungus. Lower contents of nitrogen, phosphorus and potassium have been reported in mats of *H. ferrugineum* than in controlled soil in Finland (Hintikka and Naykki, 1967), and lower nitrogen, phosphorus and magnesium in mats of *Hysterangium scleropodium* in Canada (Fisher, 1972). On the contrary, in the United States Pacific Northwest, the contents of nitrogen, phosphorus, magnesium and potassium were higher in the mat soil of *Hysterangium setchllii* than in the non-mat soil (Entray et al., 1992). The apparent solubilization of phosphorus by ectomycorrhizal fungi was commonly observed, but that of nitrogen, magnesium and potassium might differ according to fungal species. In vitro, the solubilization of phosphorus and non-solubilization of potassium by an ectomycorrhizal fungus *Boletus felleus* (Rosendahl, 1943) and the solubilization of phosphorus, magnesium and potassium by *Laccaria laccata* (Leyval and Berthelin, 1989) have been reported.

The existence of the visibly concentrated mycelia itself, the numerous secretions of the organic acids due to the mycelia and the low nitrogen content in the fungal layer of *T. bakamatsutake* were obviously related to the high C/N ratio. Elevated C/N ratios have been observed in the mat soil of *Gautieria* and *Hysterangium* in coniferous forests, for which the dense concentration rate of fungi themselves (Griffiths et al., 1990; Griffiths et al., 1991b), organic acid released by the fungi (Griffiths et al., 1992) and selective removal of dissolved organic nitrogen by the fungi (Griffiths and Caldwell, 1992) have been advanced as explanations. Because densely colonized mycelia of *T. bakamatsutake*, organic acid secretion and low total nitrogen were observed in the mycelial layer of this fungus, these three reasons seem to apply to the high C/N ratio of this fungus in the Fagaceae forest.

The major nutrient elements of the root samples, phosphorus, calcium and magnesium, showed similar tendencies to the exchangeable or available elements of the soil samples, which were detected at lower levels in the presently and previously colonized sites than in the uncolonized sites. However, more potassium was found in the root samples from the two kinds of colonized sites than in those from the uncolonized sites. How these elements moved between the fungus and host plants cannot be speculated, because the mechanism for absorption and translocation of these elements by the host plants is unknown.

The mycelial blocks in the "shiros" of *T. bakamatsutake* in the broad-leaved Fagaceae forest of Japan were of similar appearance to the visible mycelial mats in the Pacific Northwest coniferous forests. The properties of the mycelial blocks of *T. bakamatsutake*, soil acidification due to organic acids, high C/N ratio, depletion of phosphorus and accumulation of calcium were identical with those described for the mat-forming fungi.

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