Changes in Fatty Acid Composition with Age and Environment in Different Types of Peat Profiles in Japan

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Fatty acid composition of the crude lipid fraction of peat was investigated using several typical peat profiles in Japan. Fatty acid composition varied with the peat layers accumulated in a peat profile since 32,000 years BP. Deposition of long-range transported volcanic ash tephras also affected the composition remarkably due to the acceleration of decomposition. Fatty acid composition differed among high moor, transitional moor, and low moor peat profiles sampled in several locations in Hokkaido, Japan. The difference in the plants involved in the formation of peat was considered to be a very important factor determining the fatty acid composition. For example, arachidic acid was considered to be a good indicator for the presence of reed, while the percentage of stearic acid tended to be high in the high moor peat profiles dominated by sphagnum. As the fatty acids with a longer chain length were more stable than those with a shorter chain length, the percentage of longer fatty acids (C24 and C26) tended to increase and that of shorter fatty acids (C14 and C16) to decrease in the lower peat profiles. The drying of peatland also exerted a similar effect on the fatty acid composition.

Key Words: **fatty acid composition, peat, peat-forming plant, tephra, wetland.**

We are investigating the organic matter composition of several peat profiles, in order to determine how it reflects the process of peat formation, including the changes in environments as well as in vegetation. In the previous papers, we reported the presence of lignin-degradation products, lipids, and opal phytholiths in a peat profile accumulated since 32,000 years B.P. (Tsutsuki et al. 1993), lignin-derived phenolic compounds in another profile in the same peatland as above (Tsutsuki et al. 1994), and in different types of peat profiles in Hokkaido, Japan (Tsutsuki and Kondo 1995). We have also investigated the composition of phenolic compounds, fatty acids, sterols, and saccharides in several important peatland plants in Japan (Tsutsuki and Kondo 1997a, b). Soil lipids belong to a highly refractory organic fraction in soil and account at most for 20-30% of the total organic carbon, especially in peat soils which are formed under an anaerobic environment (Stevenson 1970; Dinel et al. 1990). However, there are few analytical data on peat lipids, including their fatty acid composition. In this paper we report on the fatty acid composition of the peat samples which have been used in our previous studies, assuming that soil lipids, including fatty acids, may reflect the changes in the topographic status of wetlands, climates, and plants which were involved in the formation of peat (Barber 1993).

MATERIALS AND METHODS

Peat samples. Peat samples used in this study were collected in 4 cm thick blocks from Ohnohara wetland 86-1 profile in Tsukude-village, Aichi, Japan (Tsutsuki et al. 1994), and from four peat profiles in different types of peatlands in Hokkaido, Japan (Tsutsuki and Kondo 1995). Description of the profiles and data of carbon and nitrogen contents, degree of humification, as well as the composition of lignin-derived phenolic compounds were presented in the previous papers.

Analysis of fatty acids in a crude lipid fraction of peat. One gram of a fine powder of air-dried peat sample was weighed accurately to the order of 0.1 mg into a glass centrifuge tube with a ground glass stopper. Thirty milliliters of chloroform-methanol (2 : 1) mixed solvent was added and treated with ultrasonics for 90 s at 350 W. The mixture was centrifuged at 2,000 rpm for 10 min. The supernatant was decanted into a round bottom flask. The residue was extracted two more times by the same procedure, and the supernatant was collected. The combined extracted solution was evaporated to dryness with a rotary evaporator. The dried residue was dissolved again into 20 mL of chloroform-methanol mixed solvent, transferred into a glass test tube with a teflon lined screw cap, and stored in a freezer until capillary gas chromatographic analysis.

One milliliter of the stock solution was evaporated to dryness in a glass screw test tube, 1 mL of 1.3 M HCl in methanol was added, the tube was stoppered tightly with a teflon-lined screw cap, and the sample was heated at 90°C for 2 h. After heating, 1 mL of distilled water was added, and the methylated product was extracted with 5 mL of hexane 3 times. Hexane solution was combined, washed with 4 mL of sodium bicarbonate solution (20 g L^{-1}), dehydrated with 5 g of anhydrous sodium sulfate, and finally evaporated to dryness. The dried residue was solubilized again in 1 mL of hexane. One microliter was taken from this solution and injected into a capillary GC, Shimadzu GC-14A in a split mode. The Ulbon HR-SS-10 column (50 m, 0.25 mm i.d., Shinwa Chemical Industries, Ltd.) was used. The carrier gas was helium, set at 2.5 kg cm^{-2} at the injection port. The oven temperature increased from 150°C to 220°C at 3°C min^{-1}. The injector temperature and FID detector temperature were both 250°C. Identification of the fatty acid peaks was performed by comparing the retention times with those of authentic fatty acids (analytical grade reagents purchased from Wako Pure Chemical Industries and Tokyo Kasei Organic Chemicals Ltd.).

RESULTS AND DISCUSSION

Fundamental characteristics of the peat profiles

In the Tsukude 86-1 peat profile, the carbon contents showed minimum values when volcanic ash tephras, such as Kikai-Akahoya (K-Ah: 6,300 yBP), Utsuryo-Oki (U-Ok: 9,300 yBP), Daisen(DMs, DHg, and DSs: 17,000-18,000 yBP), and Aira (AT: 22,000-25,000 yBP) were deposited on this peatland. The changes in the carbon content related to these quaternary events as well as climatic changes were analyzed by Tsutsuki et al. (1994).

For the peat profiles of Oikamanai, Sarobetsu high moor, Sarobetsu low moor, and Bibai, the characteristics of the profiles, kinds of peat forming plants, carbon and nitrogen contents, as well as the parameters related to the degree of humification were described by

Tsutsuki and Kondo (1995).

Oikamanai low moor peat was formed on the shore of a lagoon under an eutrophic environment. The vegetation consisted of bush of *Alnus japonica* and *Phragmites communis*. The ash content was rather high in this peatland due to the inflow of mineral materials. Though the layers Oa_3 and Oa_5 were excluded from the categories of peat due to their low carbon content, they were included in the samples for comparison.

Sarobetsu high moor peat was formed from sphagnum moss, sedges (*Carex*), and bog cranberry (*Vaccinium*) up to a 70 cm depth (SH1-SH4), which corresponds to a highmoor layer. The lower layers (Oa_1 and Oa_2; SH6, SH7, and SH8) of this peat profile consisted of low moor peat formed from *Phragmites*. Whole layers of the Sarobetsu low moor peat profile were formed mainly from *Phragmites*.

In the Bibai peat profile, the succession from low moor peat, via transitional peat, to high moor peat was observed. The major peat-forming plants in the uppermost high moor peat layers were sphagnum moss and *Carex*. The uppermost layer of this peat profile underwent organic matter decomposition due to the drying of peatland. The major peat-forming plant in the transitional peat layer (Oe_2) was *Moliniopsis japonica*, while the major peat-forming plants in the low-moor peat layer (Oe_3) were *Phragmites communis* and *Alnus japonica*. Inflow of mineral materials was inferred in layers around B78 based on the high ash content.

Total fatty acid content in different peat profiles (Fig. 1)

In the Tsukude 86-1 peat profile, the yield of fatty acids reflected more remarkably the deposition of various volcanic ash tephras than the yield of phenolic compounds (Tsutsuki

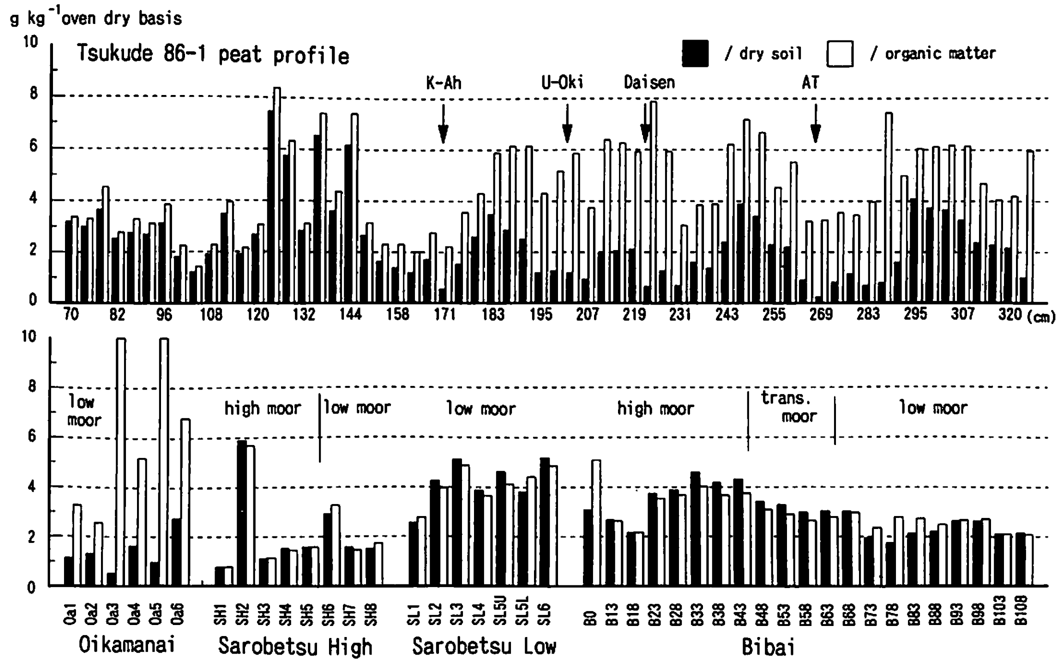

Fig. 1. Yield (g kg^{-1} dry matter basis and organic matter basis) of total fatty acids in various peat profiles.

et al. 1994). The total yield of fatty acids expressed on a dry soil basis and on an organic matter basis both showed minimum values at a depth of around 269 cm (AT tephra), 223 cm (Daisen tephra), 203 cm (U-Oki tephra), and 169 cm (K-Ah tephra).

In the layers below the K-Ah tephra, the yield of total fatty acids on an organic matter basis was remarkably higher than that on a dry soil basis. This phenomenon was ascribed to the lower carbon (or organic matter) content in the layers below the K-Ah tephra as shown by Tsutsuki et al. (1994). Arai et al. (1989) determined the bulk densities of several representative layers from this profile, and reported values of 0.99, 1.00, 1.21, 1.04, 1.30, 1.06, 1.04, 1.21, 1.18, and 0.94 g cm^{-3} at the depths of 100, 150, 174, 181, 205, 235, 255, 270, 285, and 310 cm, respectively. These data also indicate that the bulk density was higher in the layers below the K-Ah tephra. Generally in peat soils, the degree of decomposition expressed by the fiber content is well correlated with the ash content or bulk density of samples (Kaila 1956).

Arai (1989) also determined the proportion of the peat fraction finer than 2 mm using different peat profiles located very near to this profile in the same wetland. They reported that the proportion of the fraction <2 mm increased in the deeper peat layer which corresponded to the layers older than the K-Ah tephra.

It is well known that the deposition of volcanic ash affects the formation of peat layers by supplying mineral nutrition (Sakaguchi 1974). Therefore, it is reasonable to assume that the inflow of mineral materials as well as the deposition of volcanic ash enhanced the decomposition of peat in the lower layers of this peat profile.

However, as shown in Fig. 1, the total fatty acid contents on an organic matter basis were larger in the layers below the K-Ah tephra than the contents in most of the upper layers. This observation suggests that the lipid fraction was selectively preserved in the organic matter of peat, because it was more resistant against decomposition than the other carbohydrate or phenolic components in peat.

In the Oikamanai profile, the yield of total fatty acids on an organic matter basis was much higher than that on a dry soil basis, presumably due to the selective preservation of fatty acids in peat along with the decomposition of more easily decomposable constituents.

In the Sarobetsu high moor and low moor profiles, yields on an organic matter basis and dry soil basis were almost the same, reflecting the low ash content of the peat. The yield of fatty acids was considerably higher in the Sarobetsu low moor peat profile than in the high moor peat profile, except for the second and sixth layers of the Sarobetsu high moor peat profile. In the second layer, a sedge (*Carex middendorfii*) was the dominant peat-forming plant, and in the sixth layer, reed (*Phragmites communis*) was dominant. In the Sarobetsu low moor peat profile, reed was the dominant peat-forming plant throughout the profile (Tsutsuki and Kondo 1995). Therefore, the high yields of fatty acids may be attributed to the presence of reed.

In the Bibai peat profile, the yield of fatty acids on an organic matter basis was considerably larger than that on a dry soil basis in the topmost layer which exhibits a drying tendency and in the layers around 78 cm depth where the inflow of mineral materials was assumed. Since both the aerobic environment and the eutrophic environment associated with the mineral materials are conducive to the decomposition of peat, selective preservation of fatty acids was inferred in these layers, as in the lower layers of the Tsukude profile and in the Oikamanai profile.

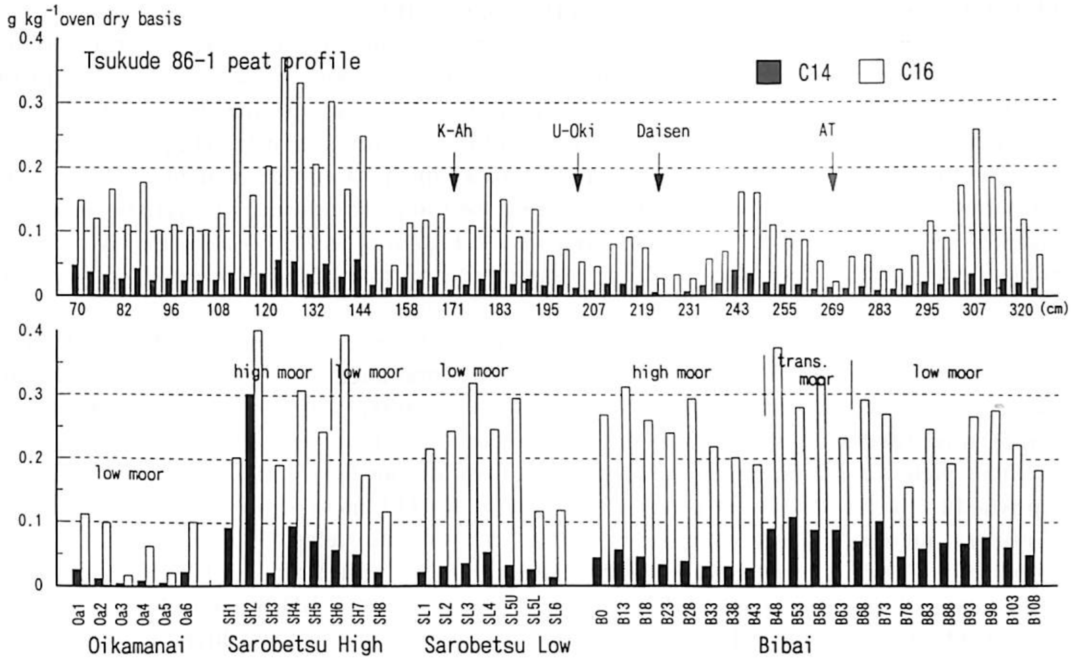

Fig. 2. Yields of myristic acid and palmitic acid in various peat profiles.

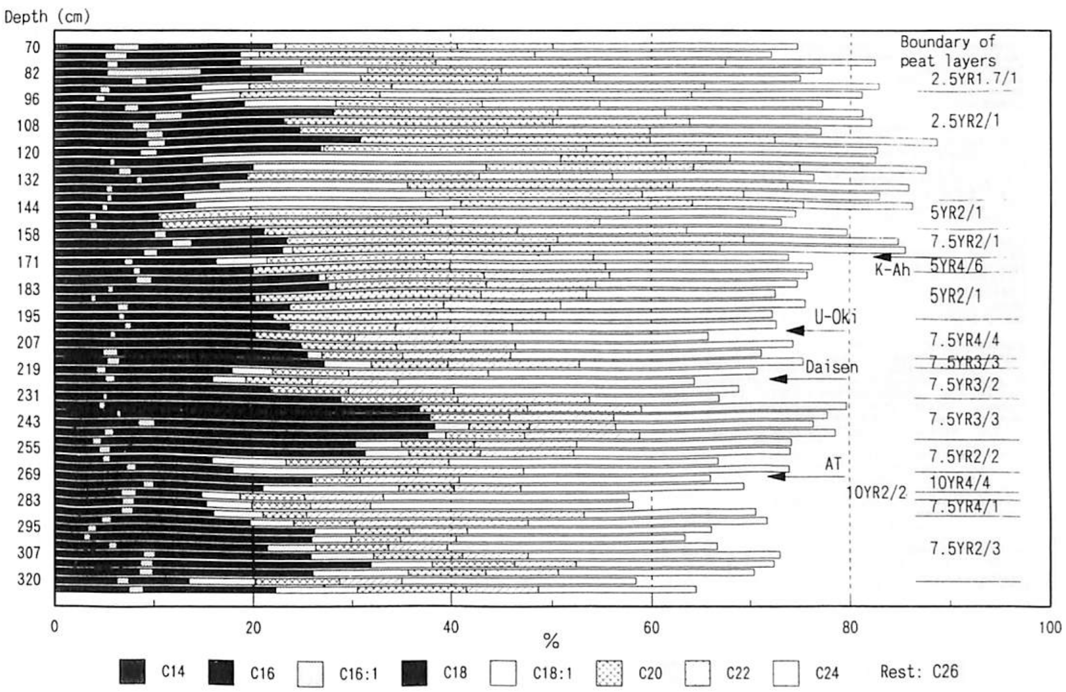

Fig. 3. Relative percentages of fatty acids constituting the crude fat fraction of layerwise soil samples obtained from Tsukude 86-1 peat profile.

Yields of myristic acid (C14) and palmitic acid (C16)

Myristic acid and palmitic acid are very common, and occur in plants, animals, and microbes. Although their origin cannot be specified, plants may be the main source in the case of peat samples. Although the yield of palmitic acid was 2-5 times higher than that of myristic acid, the behavior of these two fatty acids was almost similar (Fig. 2).

The yield of myristic acid was relatively lower in the Tsukude peat profile compared with the Sarobetsu high moor and Bibai peat profiles. The levels of myristic acid and palmitic acid increased and remained high in the Tsukude peat profile in the layers where stable wetland conditions persisted for a long period of time (such as layers shallower than 144 cm, layers around 183, 243, and 307 cm).

The yield of myristic acid was extremely high in the second layer of the Sarobetsu high moor peat where a sedge (*Carex middendorfii*) predominated as a peat-forming plant, and also very high in the transitional moor layers of the Bibai peat profile (48-68 cm), where purple grass (*Moliniopsis japonica*) predominated (Fig. 2).

The yield of palmitic acid tended to be low in the layers where volcanic ash was deposited in the Tsukude peat profile, as well as in the Oikamanai peat profile where inflow of mineral materials was observed. On the other hand, the yield of palmitic acid was high in the second and sixth layers of the Sarobetsu high moor peat profile and in the transitional moor layers of the Bibai peat profile, where grass vegetation predominated. In the Tsukude peat profile, the yield of palmitic acid, like that of myristic acid, was high in layers where stable wetland conditions prevailed (Fig. 2).

The relative percentage of each fatty acid is shown in Figs. 3 and 4. In the peat profiles

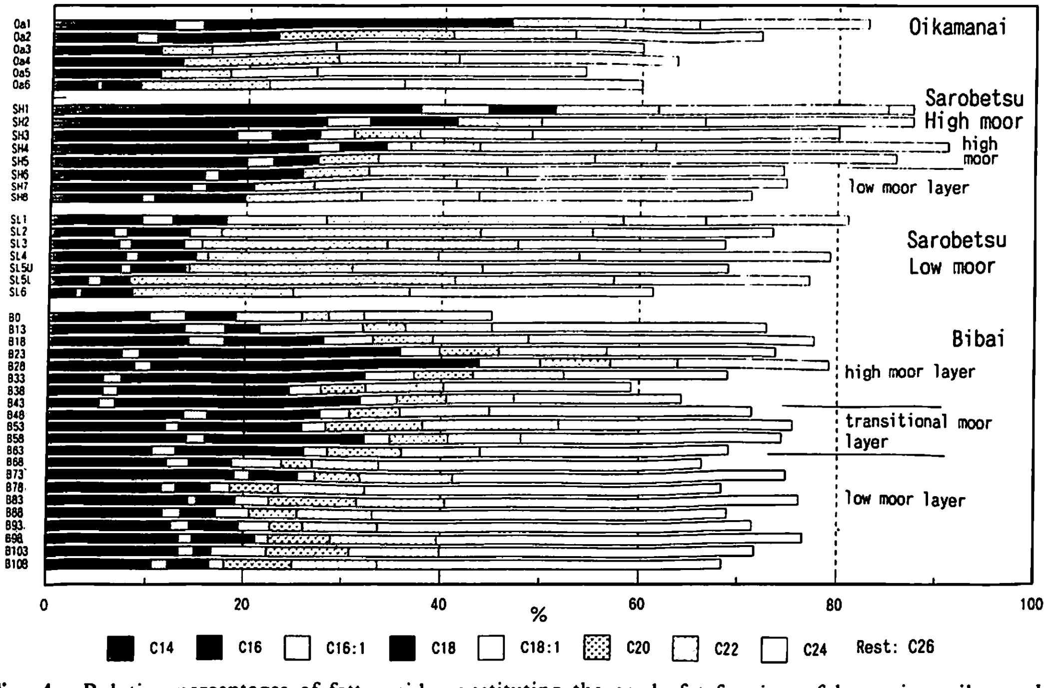

Fig. 4. Relative percentages of fatty acids constituting the crude fat fraction of layerwise soil samples obtained from various types of peat profiles in Hokkaido.

of the Oikamanai, Sarobetsu high moor, Sarobetsu low moor, and Bibai high moor (Fig. 4), the percentages of myristic acid and palmitic acid decreased conspicuously from the top layer to the lower layer. This observation suggests that myristic acid and palmitic acid are relatively easily decomposed among the long chain fatty acids, and that their percentages decrease with age. In the top layer of the Bibai high moor peat profile, the percentages of these fatty acids decreased again, presumably due to the acceleration of decomposition of peat under the drying tendency of the peatland.

The percentages of myristic acid and palmitic acid differed considerably among the peat profiles. The percentage was largest in the Sarobetsu high moor peat, and lowest in the Oikamanai low moor peat followed by the Sarobetsu low moor peat. The percentages of myristic acid and palmitic acid were also remarkably different between the high moor layers and transitional moor layers of the Bibai peat profile. Abrupt changes in the myristic acid and palmitic acid percentages were also observed in the Tsukude 86-1 peat profile at the depths of 100, 158, 228, 269, and 303 cm (Fig. 3). These changes all corresponded to the changes in the color and materials of the peat layers observed in the field. Therefore, it is considered that the percentages of myristic acid and palmitic acid reflect accurately the peatland conditions and the composition of peat-forming plants.

Yields of stearic acid (C18) and arachidic acid (C20) (Fig. 5)

Although the yield of stearic acid (C18) was much higher than that of palmitic acid (C16), the behavior of both fatty acids was similar in the Tsukude peat profile. In the layers with volcanic ash deposition, the yield of stearic acid was very low, suggesting that stearic acid is relatively easily decomposed among the lipid constituents. In the Tsukude profile, stearic acid showed also remarkably high yields in the very old layers for example between

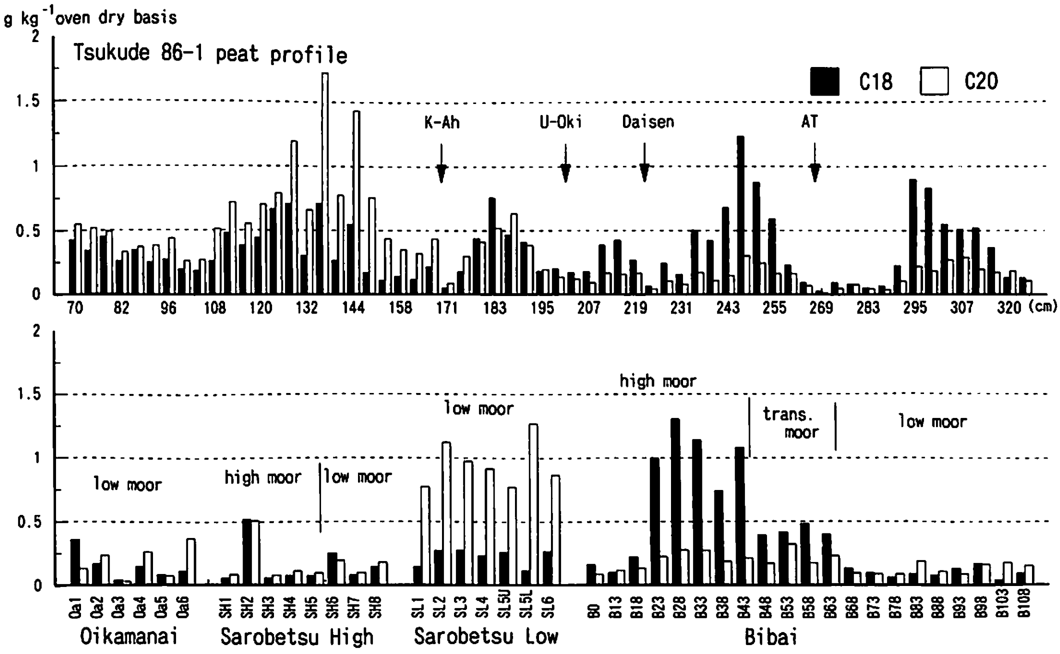

Fig. 5. Yields of stearic acid and arachidic acid in various peat profiles.

the deposition of the Daisen tephra (17,000-18,000 yBP) and Aira tephra (22,000-25,000 yBP) peaking at 247 cm depth, and those formed long before the deposition of the Aira tephra peaking at 295 cm depth. Taking into consideration the fact that these layers were formed during the coldest period of the last glacial maximum era, sphagnum moss was the dominant peat-forming plant in these layers.

The relative percentage of stearic acid showed a remarkable change at a 179 cm depth in the Tsukude 86-1 profile, where the Kikai-Akahoya tephra was deposited (Fig. 3). Abrupt changes in the percentages were also observed at depths of 223, 259, 279 cm where the Aira tephra was deposited, and 320 cm corresponding to the boundary between peat and clay layers, which all reflected the remarkable changes in the characteristics of peat layers. The percentage of stearic acid was higher in the layers below the deposition of the Akahoya tephra.

In the Bibai peat profile, the yield of stearic acid was highest in the lower part (23-48 cm) of the high moor peat layers (Fig. 5). The low yield in the 0-23 cm part may be due to the drying of peatland. The major peat-forming plants in this high moor layer were sphagnum moss and *Carex*. However, in the high moor peat layers (SH1-SH5) of the Sarobetsu high moor peat profile, the yield of stearic acid was not as high as that in the high moor layers of the Bibai profile, for unknown reasons.

In the analysis of peatland plants (Tsutsuki and Kondo 1997a, b), arachidic acid occurred in large amounts from reed (*Phragmites communis*). As a result, the yield of arachidic acid (Fig. 5), as well as the percentage of arachidic acid to total fatty acids (Fig. 3), tended to increase after the deposition of the Utsuryo-Oki tephra (9,300 yBP) in the Tsukude profile. Thereafter, the climate of Japan became warmer and wetter. The prevalence of reed was also inferred based on the analysis of opal phytoliths using another profile (Shirasu profile) formed at the same time in this wetland (Tsutsuki et al. 1993).

The yield and percentage of arachidic acid were also very high in the Sarobetsu low moor peat profile (Figs. 4 and 5), where reed was the dominant peat-forming plant. In the low moor peat layers of the Bibai profile, the yield of arachidic acid was not appreciably high, presumably because the dominant peat-forming plant was not reed but alder tree (*Alnus japonica*) in this layer.

Yields of lignoceric acid (C24) and cerotic acid (C26) (Fig. 6)

Lignoceric acid and cerotic acid are components of wax and resin which are relatively refractive lipid components. The fact that the stability of fatty acids in the soil environment increases with the increase in the chain length was also reported by Jambu et al. (1995).

The behavior of these two fatty acids was very similar in the Tsukude 86-1 peat profile. The percentage of cerotic acid to total fatty acids tended to increase in the lower layers (Fig. 3). Due to its refractiveness, it is considered that the relative percentage of this fatty acid had increased over a long period of time.

The behavior of these two fatty acids was also similar in the Oikamanai, Sarobetsu High, and Sarobetsu Low profiles. The yield of these two fatty acids was highest in the Sarobetsu low moor peat profile, reflecting the large plant biomass production of reed. Only in the Bibai profiles did these two fatty acids behave slightly differently. The yield of cerotic acid tended to decrease in the transitional moor and low moor layers, while that of lignoceric acid remained almost constant throughout the profile. Although the changes in the composition of peat-forming plants may account for this phenomenon, fundamental data of plant fatty acid analysis are required.

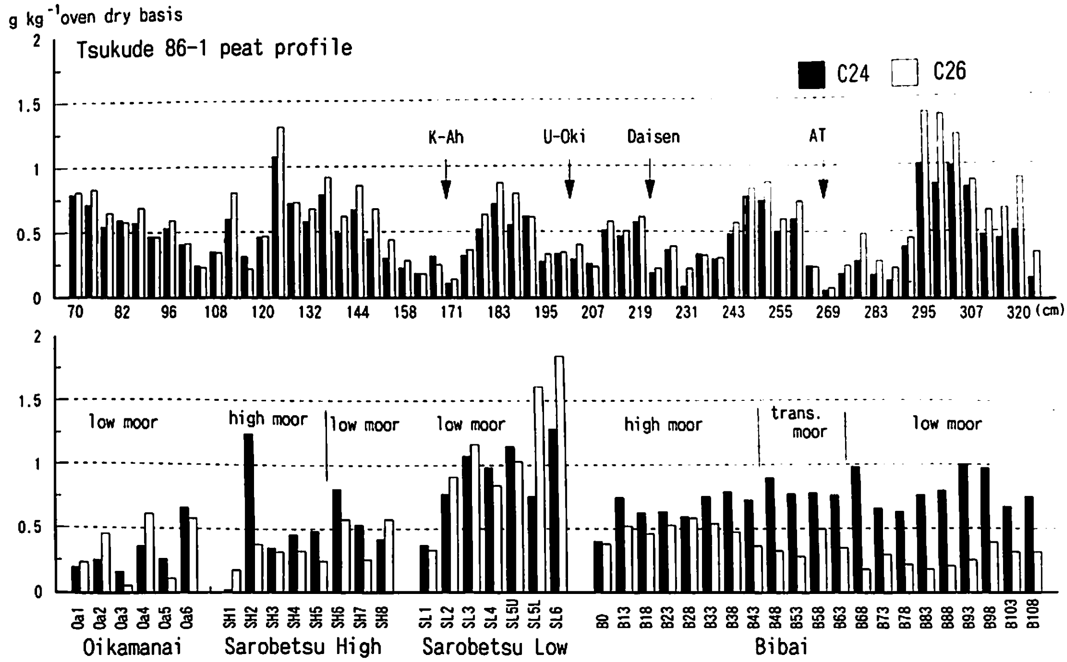

Fig. 6. Yields of lignoceric acid and cerotic acid in various peat profiles.

The percentages of lignoceric acid and cerotic acid tended to increase in the lower layers of the Oikamanai, Sarobetsu high moor, Sarobetsu low moor, and Bibai peat profiles (Fig. 4), reflecting the higher stability of the longer chain fatty acids. It also tended to increase in the surface two layers (B0 and B13) of the Bibai profile, because the decomposition of shorter chain fatty acids was accelerated by the drying of peatland.

Yields of unsaturated fatty acids

Among the long chain unsaturated fatty acids, oleic acid (C18 : 1) was formed in largest amounts followed by palmitoleic acid (C16 : 1). Though linoleic acid (C18 : 2), linolenic acid (C18 : 3) and erucinic acid (C22 : 1) were also observed in the chromatogram, their yields were very low. Therefore, they were not examined in this paper.

The yield of oleic acid varied considerably among the peat layers. In the Tsukude 86-1 peat profile (Fig. 3), it was largest in the 124-148 cm layers and occurred found in relatively large amounts in layers between 70 and 100 cm and in those below 243 cm.

Oleic acid was hardly detected in the Oikamanai and Sarobetsu high moor profiles (Fig. 4). In the Sarobetsu low moor peat profile, oleic acid was detected in considerable amounts in the uppermost layer but its content decreased steeply with depth (Fig. 4). In the Bibai peat profile, oleic acid occurred in largest amounts in the high moor layers. In the transitional moor and low moor layers the yield of oleic acid was lower than that in the highmoor layers, but oleic acid still occurred in larger amounts compared with the Oikamanai and Sarobetsu low moor peat profiles.

Thus, the yield of oleic acid did not show a uniform pattern among the types of peat layers. As oleic acid is more readily decomposed than other saturated fatty acids, it is suggested that the yield of oleic acid is high in layers maintained under continuously anaerobic conditions.

Though the yield was much lower than that of oleic acid, palmitoleic acid (C16 : 1) was detected in almost all the peat layers except for the mineral layers of Oikamanai (Fig. 3). Therefore, palmitoleic acid is considered to be more resistant to decomposition than oleic acid.

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