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Ecological studies of aquatic moss pillars in Antarctic lakes 1. Macro structure and carbon, nitrogen and chlorophyll *a* contents

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Abstract: Structures of a typical 'moss pillar' submerged in Antarctic lakes were investigated to analyze the sizes, age distribution, and composition such as shoot density, dry weight, carbon, nitrogen and chlorophyll *a* using a sample collected from lake B-4 Ike in the Skarvsnes region, East Antarctica. The moss pillar was mainly composed of shoots of a moss species, *Leptobryum* sp. Most of the green shoots of the species were located at the top surface of the pillar, and brownish old shoots with prominent vegetative diaspores, so-called rhizoidal tubers, formed the internal body of the pillar. The internal core of the pillar was nearly empty, and seemed to be decomposed considerably. Dry weight, carbon, nitrogen and chlorophyll distributions in the pillar took heterogeneous patterns, that is, they were largely centered at apical parts. It is suggested that growth of the moss pillar occurred extensively at the apical part. The age was estimated *ca.* 250 years at *ca.* 20 cm below the apical top by the AMS method. The presence of the moss pillar in lake B-4 Ike indicates that a tremendous amount of biomass has been produced under the oligotrophic freshwater Antarctic lake environment over more than a quarter millennium.

key words: moss pillars, structure, C/N content, chlorophyll *a,* Antarctic lake

Introduction

Mosses are one of the most important primary producers among macro-size plants in the Antarctic terrestrial environment, the most severe habitat on Earth for plants. Even in the vicinity of Syowa Station in East Antarctica, one of the severest environments for the growth of terrestrial plants in the ice-free part of the Antarctic continent, there are some mosses colonizing limited areas, such as Yukidori Valley, in Langhovde, and some other ice-free areas; however, most of remaining terrestrial surface has almost no vegetation due to low temperature and low availabilities of water and nutrients (summarized by Kanda, 1982; Kanda *et al.*, 2002).

One of the recent topics in Antarctic moss ecology is the finding of luxuriant distribution of aquatic (benthic) moss communities, so-called 'moss pillars', in some freshwater lakes in the vicinity of Syowa Station where terrestrial vegetation is scarce

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Fig. 1. An underwater view in lake B-4 Ike. Well-developed moss pillars (*ca.* 50 cm height) distributed around >2.5 m depth. The photograph was captured from a digital videocassette record (Mini DV, Sony).

(Imura *et al.*, 1999). In their report, the moss pillars mainly distribute at 3–5 m depth where annual ice cover never reaches, and stand up from the lake bottom from more than 50 cm in height in some lakes of the Skarvsnes region (Fig. 1). After finding the moss pillars, a terrestrial biology group started research surveys under the Japanese Antarctic Research Expedition (JARE, hereafter) and summarized the moss pillar distribution at the bottoms of lakes along the Sôya Coast (Imura *et al.*, 2003).

It is probable that the moss forming such pillar-like growths appeared after the last glacial maximum (*ca.* 10000 years ago), because the glacier completely covered this region (Igarashi *et al.*, 1995). After the glacier disappeared, the moss community may have colonized, constructed its unique structure and produced biomass in freshwater lakes. Ecological studies are required for evaluation of the hypothesis.

In our papers, we have tried to describe some ecological features of the moss pillars. First, we report structural features of a typical moss pillar with some chemical compositions collected from the lake B-4 Ike, Skarvsness along the Sôya Coast of East Antarctica.

Materials and methods

Sampling

Moss pillars were sampled on 19 January 2000 with a joint research operation by the JARE-40 wintering party and JARE-41 summer party at lake B-4 Ike (69°28.7′S, 69°30.5′E), in the Skarvsnes ice-free area along the central Sôya Coast (Fig. 2). The

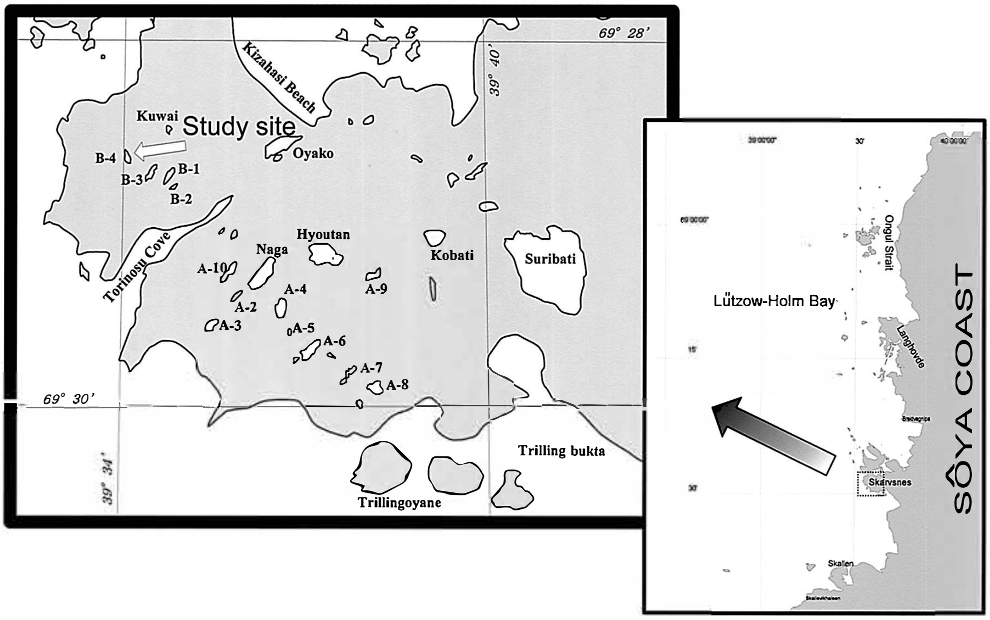

Fig. 2. A map of the study site.

lake is located at *ca.* 130 m in altitude, is surrounded by small hills and has no major inlet or outlet of water. There are no terrestrial plants or bird colonies in the catchment basin of water, and the lake water is clear so that one can see the deepest lake bottom (*ca.* 4.5 m) from the hills in an ice free summer, indicating that it is an oligotrophic lake. More information about water quality of the lake is described elsewhere by Kudoh *et al.* (2003).

Two samples of the moss pillar of >45 cm height, two of *ca.* 30 cm height and several of *ca.* 10 cm height were harvested gently from 2–3 m depth by SCUBA diving after visual observation with a submersible digital video recorder (Handycam with Marine-pack kit, Sony, Fig. 1). For sampling the large moss pillar, the apical part of the moss pillar was cut using a diving knife, transferred into a plastic bucket, and then the basal part was detached from the benthic algal mat carefully, and transferred into another plastic bucket (Fig. 3-1). Those buckets were submerged in advance, retrieved onto a rubber boat and the moss pillar sample was kept in tap water. The samples in buckets were immediately transferred to the laboratory on board the icebreaker *Shirase* by a helicopter, and stored in a freezer room (−20℃) until further analysis. Among the samples collected, a 47 cm height sample (apical section: 20 cm, basal section: 27 cm) was used for the present study.

Anatomical treatments of a moss pillar

The apical section (top to 20 cm) of the frozen moss pillar was vertically cut into 2 pieces using an ice saw at first (Fig. 3-2). A piece was then sliced into 4 equal-sized pieces along the vertical axis (1/8 size of the original moss pillar). Two of the sliced

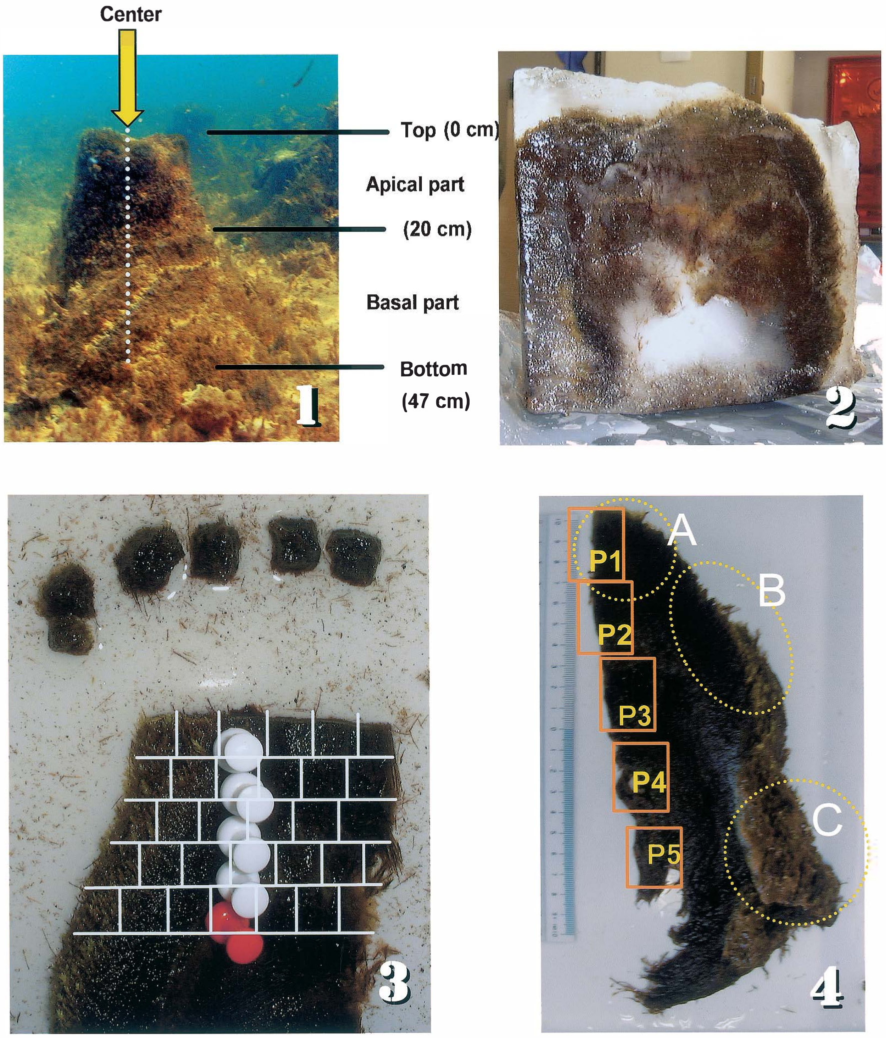

Fig. 3. Dissection of a moss pillar. 1: A photograph of a typical moss pillar sampled for the present study. The sample was cut through the apical section (top–20 cm below) at first, then the basal section (20–bottom) was removed from the lake bottom. 2: A vertical section of a frozen apical section sample. The white at the top and central bottom sections is ice 3: The way the sample was cut into cubes for dry weight, C/N and chlorophyll analyses. The sample was cut horizontally at 1 cm intervals, then cut vertically into *ca.* 1 cm^3 cubes as indicated by the white lines. The pins in the photograph are vertical marks at 1 cm intervals. 4: Samples for shoot and age analyses. Numbers and letters in the photograph correspond to Tables 1 and 2.

pieces were allowed to melt under room temperature (15–20℃) for one night, and the rest of the pieces were stored for further analysis in the future under −20℃.

A melted sample was cut horizontally at 1 cm intervals from top to bottom, and sectioned into *ca.* 1 cm^3 cubes from the surface toward the central part (Fig. 3-3) for measurements of dry weight, carbon/nitrogen contents and chlorophyll *a*. Another melted piece was cut into larger pieces for age estimation, visual and dissecting microscopic observations (Fig. 3-4).

The basal section of the frozen moss pillar (bottom to 27 cm height) was treated the same as the apical section.

Analytical procedures

Half of each cubed sample (wet weight) was separated and chlorophyll *a* was immediately extracted by the *N, N*-dimethylformamide method (Moran and Porath, 1980). The concentration of extracted chlorophyll *a* was fluorometrically determined using a fluorometer (Model 10-AU, Turner Design) against a pure chlorophyll *a* standard. The remainder of the cubed sample was dried in an oven at temperature 60 to 70℃ for a few days, and dry weight measured. Each dried sample was ground and a small amount of the powder was weighted again. Carbon and nitrogen contents were analyzed by an elemental analyzer (MT-5 CHN analyzer, Yanako) against an external antipyrine standard. Age was estimated using samples collected at 0, 5, 10, 15, and >15 cm from the apical top, corresponding to parts P1, P2, P3, P4 and P5 in Fig. 3-4 respectively, by AMS (Accelerator mass spectrometry) ^{14}C measurement.

A vertical profile of dry weight in the present moss pillar sample was simply calculated by summing over cubed samples collected from the same horizontal plane and multiplying by the dissection factor of 2×8. Carbon, nitrogen and chlorophyll *a* content were determined as fraction of the dry weight using the averaged data obtained from the same horizontal plane. Total contents of these parameters were obtained by vertically integrating the above values.

Results

Visual features of moss pillars

Many moss pillars were randomly distributed at the lake bottom at >1.7 m depth in lake B-4 Ike (Fig. 1). The moss pillar used for this study was harvested from 2–3 m depth of the lake (Tsuchiya, personal comm.). Green moss shoots that were sometimes elongated for a few cm from the tightly aggregated part were centered at the apical part of the pillars. The other parts of the pillars were brown in color (Fig. 3-1). When the sample was cut, some thin whitish layers were observed among thick blackish layers of moss shoots in the cross-section of moss pillars, like growth rings of trees. The internal (core) part of the large moss pillar was heavily black colored, and sometimes empty, especially in the lower part of the pillar (Fig. 3-2). When the sample was removed from the lake bottom to a sample bucket, black ooze, which probably derived from decomposed plant tissues, appeared from the core of the moss pillar. A strong smell like 'a rotten boiled egg', maybe a sulfur compound (H_2S or related chemical), was recognized from the ooze and the inside of the moss pillars when the sample was retrieved onto the rubber boat. This strongly suggests that the core part of the moss pillar was in an anaerobic environment due to decomposition of old moss tissues by microorganisms. The ooze, which appeared in the central core part of the moss pillars, could not be sampled quantitatively, so we could not include it in the later analyses.

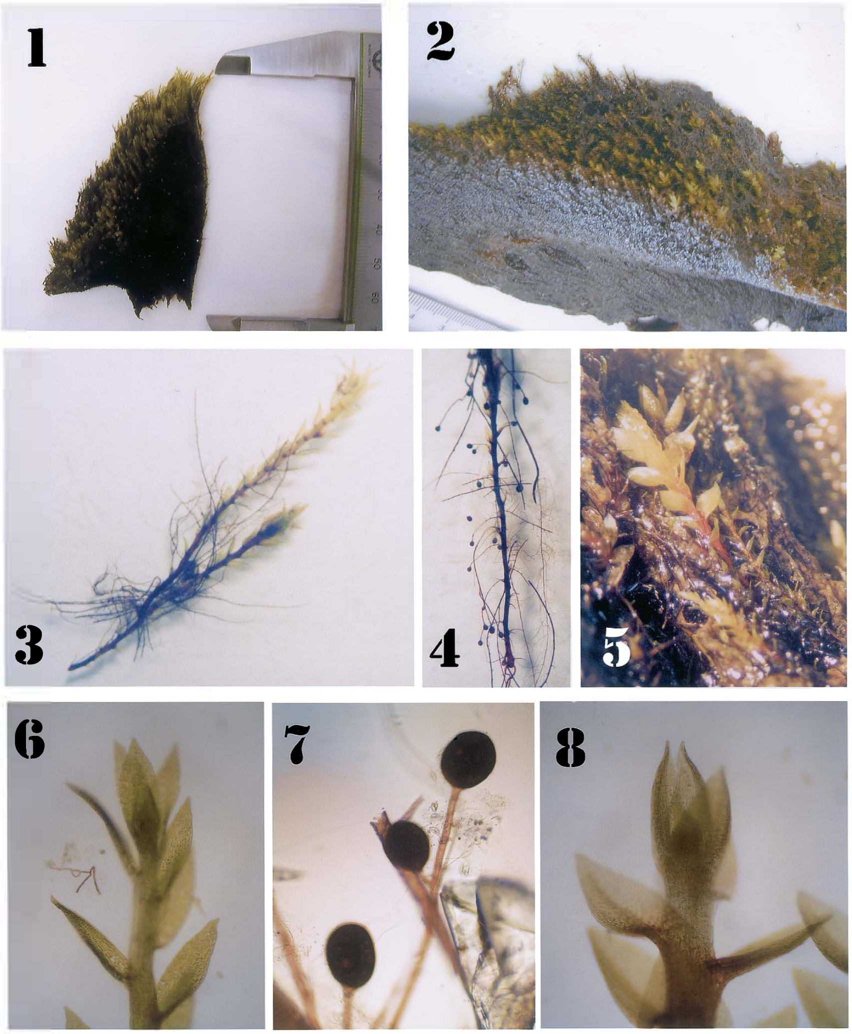

Fig. 4. Visual components of mosses. 1: An apical top sample. Many green shoots are observed. 2: Middle surface of the moss pillar. A few green shoots are distributed among brownish shoots. 3: Shoots of *Leptobryum* sp. collected from the top surface. 4. Rhizoidal system and vegetative diaspores of *Leptobryum* sp. found from P2 and P3 in Fig. 3-4. 5. *Bryum pseudotriquetrum* found on the middle surface of the pillar. 6: A bud of *Leptobryum* sp. 7: Vegetative diaspores of *Leptobryum* sp. 8: A bud of *Bryum pseudotriquetrum.*

Components of the moss pillar

As described by Imura *et al.* (1999), two species of aquatic moss, *Leptobryum* sp. and *Bryum pseudotriquetrum,* and a fine-filamentous green alga, *Oedogonium* sp., were found to consist of fibrous components of the pillar by visual and dissection microscope observations. Among them, *Leptobryum* sp. dominated and formed the main body (biomass) of the pillar. Small algae such as diatoms, green algae and cyanobacteria

Table 1. Shoot density and age estimated by the AMS method of moss at several parts of the apical moss pillar. Position marks in the table correspond to the same marks in Fig. 3-4.

Position mark	Distance from the apical top (cm)	Shoot density ($\times 10^3$ shoots/g-DW)	Age* (years)
1	0–3	25	111
2	3–6.5	16	119
3	7–10	11	118
4	11–15	15	140
5	>15	7.1	250

*Estimated ages have *ca.* 30% of uncertainty at most.

may exist in the pillar (Ohtani and Suyama, 2001; Ohtani *et al.*, 2001), though they could not be distinguished by the method of this study. An algal-mat-like compound coated the surface of the basal part of moss pillars. The compound was also seen among the moss shoots at the apical surface of the pillars, and it seemed to bundle or aggregate the moss shoots tightly.

At the top surface of the pillar, many shoots of *Leptobryum* sp. were easily recognized (Fig. 4-1, 3, 6, 7). Green leaves with reddish stem of *B. pseudotriquetrum* were observed among the shoots of *Leptobryum* sp., to a lesser extent, too (Fig. 4-2, 5, 8). Old stems and branched rhizoidal systems of both mosses were tightly aggregated in the inner part of the pillar.

Along the vertical axis of the apical section (Fig. 3-4), numbers of shoots in a unit dry weight (shoot density, hereafter) and age were checked (Table 1). At the top, dense shoots mainly composed of *Leptobryum* sp. were clearly recognized, though the shoot density gradually decreased downward, and reached nearly 1/3 to 1/4 of the top at the bottom part of the present measurement (P5), that is the part facing the empty core part. In parts P3–P5, no green shoot was observed; most shoots observed in these parts seemed to be decomposed to some extent. Well-branched rhizoids and prominent rhizoidal tubers (vegetative diaspores, Fig. 4-4, 7) of *Leptobryum* sp. were distributed densely in parts P1, P2 and P3, but the tubers were rarely seen in the rest of the parts. Estimated age was similar, *ca.* 100 years, in the upper and middle parts (P1–P3), which correlated the existence of the tubers. The lower parts (P4, P5) were nearly twice as old or more.

Numbers of green shoots of both mosses appearing on the surface of the pillar were counted from different parts of the apical section (A, B and C). Green shoots of *Leptobryum* sp. were 210 shoots/cm^2 at the apical top; the numbers were nearly half that at the middle surface (Table 2). No green shoot was observed at the bottom surface of the apical section in spite of the existence of many brown shoots of the species. On the other hand, many green shoots of *B. pseudotriquetrum* appeared in the middle part; few were in the other parts. At the basal section of the moss pillar almost no green shoot of either moss was observed, and decomposition of the old moss shoots seemed to be further advanced.

Table 2. Numbers of green shoots of *Leptobryum* sp. and *B. pseudotriquetrum* observed at the surface of the apical moss pillar (shoots cm^{-2}). Positions of A, B and C are the same as in Fig. 3-4.

Position / Species	A	B	C
Leptobryum sp.	210	102	0
Bryum pseudotriquetrum	7	41	5

Dry weight, C/N, chlorophyll profiles

The size of the present moss pillar sample, which was roughly estimated by outer size measurement, is summarized in Table 3 together with dry weight, carbon, nitrogen and chlorophyll contents, which will be discussed in detail later. The apical section of the sample was slender compared to the basal section. More precise vertical profiles of the size and dry weight of the apical section of the moss pillar are graphically shown in Fig. 5. The external diameter gradually increased from the top to 5 cm below the top, and a constant diameter of *ca.* 17 cm was measured in the lower part (Fig. 5, bottom). From the 7 cm section, empty space appeared in the central (core) part. The empty part gradually increased along the vertical axis to the bottom. The vertical profile of dry weight indicated that the heaviest part of the apical section was located below the top, *ca.* 7–12 cm from the top. The density index, the estimated dry weight/volume occupied by moss tissue, suggests that moss tissues were densely distributed from the top to *ca.* 10 cm below the top, 0.02–0.03 g/cm^3, and they then gradually decreased to 0.01 g/cm^3 in the lower part of the apical section. In the basal section, the density index was 0.016 g/cm^3 on average (Table 3).

Table 3. Size, volume, dry weight, carbon, nitrogen and chlorophyll *a* profiles of the present moss pillar.

		Apical part (top–20 cm below)	Basal part (20–47 cm)	Total
Diameter	(top, cm)	5.5	18	
	(bottom, cm)	18	40	
Volume	($\times 10^3$ cm^3)*1	4.0	24.4	28.4
Dry weight	(g)	79.0	392.8	471.8
Density index	(g/cm^3)*2	0.020	0.016	0.017
Carbon	(g)	29.6	84.8	114.4
Nitrogen	(g)	1.26	4.79	6.05
C/N	(wt/wt)	23.5	17.7	18.9
Chlorophyll *a*	(mg)	51.1	57.8	108.9
C/Chlorophyll *a*	(wt/wt)	579	1467	1051

*1 The values include the internal empty core.
*2 Estimated as dry weight/volume.

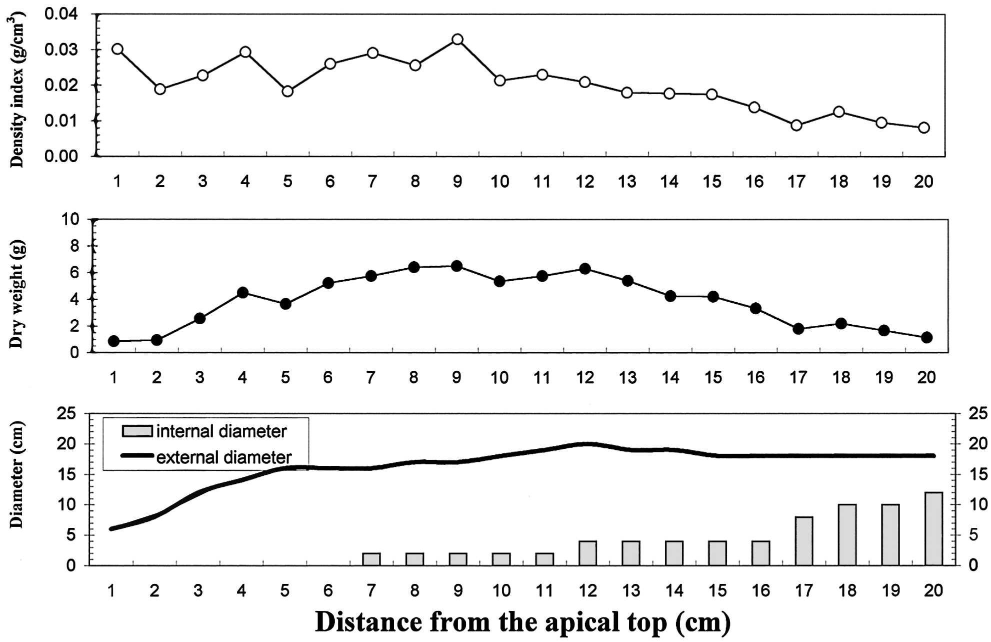

Fig. 5. Size, dry weight and density index profiles of the apical section of the present moss pillar.

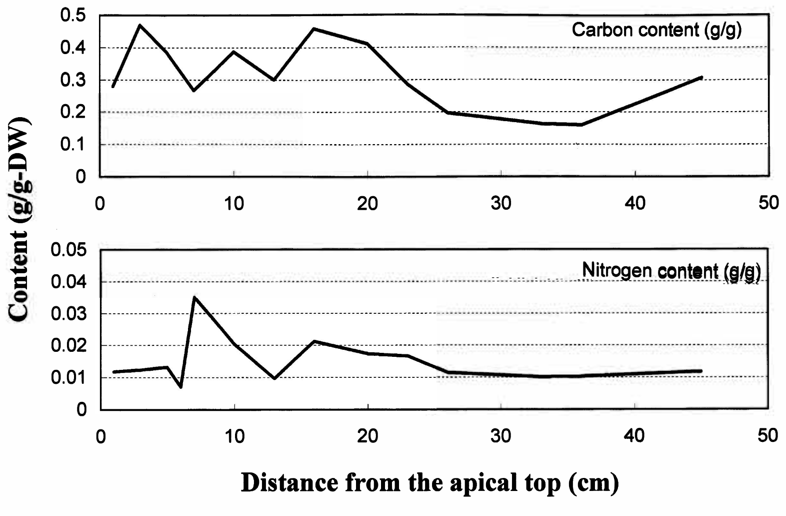

Fig. 6. Vertical profiles of carbon and nitrogen contents in the present moss pillar.

Carbon and nitrogen content along the vertical axis from top to bottom indicated higher content in the apical section than the basal section (Fig. 6). Carbon content of *ca.* 0.4 g/g-DW was distributed in the apical section; the content was about 1/2 in the basal section. Nitrogen content showed a little complex change. The content was not so high at the apical top (0.012 g/g-DW), but nearly 3 times the content was detected *ca.*

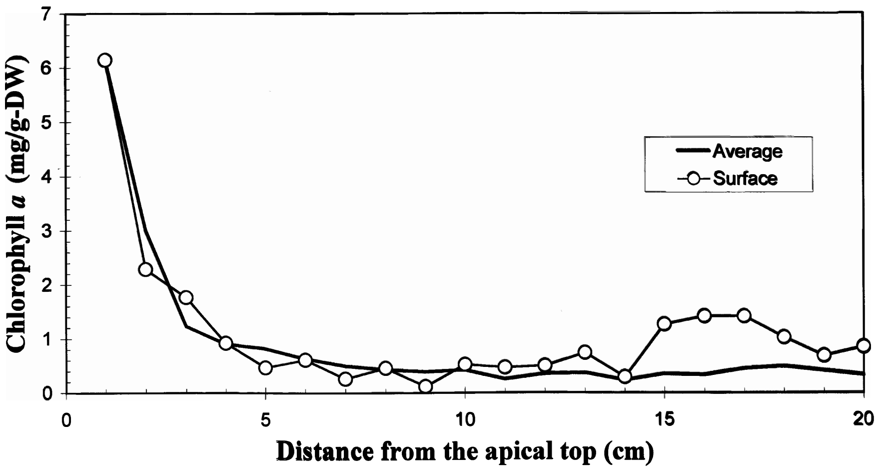

Fig. 7. Vertical profiles of chlorophyll *a* content in the apical section of the present moss pillar. Circles with thin lines indicate chlorophyll *a* content obtained in the outer surface cubes of each vertical position, and the thick line indicates the average content among the cubed samples positioned at the same horizontal plane.

7 cm below the top, and then the content showed *ca.* 0.02 and 0.01 g/g-DW in the rest apical and basal section, respectively. These suggested that the apical top had relatively carbon rich composition.

In spite of the total dry weight difference of nearly 5 times between the apical and basal sections, content of chlorophyll *a* pigment in both sections showed similar levels (Table 3). As in the visual observation, green shoots centered in the apical section contribute significantly to the heterogeneous distribution of chlorophyll *a*. Chlorophyll distribution along the vertical axis in the apical section clearly indicated that most of the pigment was detected from the surface of the apical top (Fig. 7). The chlorophyll content was quite low, <0.2 mg/g-DW on average, in the basal section.

Discussion

In the present study, a typical moss pillar which grew in the lake B-4 Ike was used in order to investigate structural profiles, such as shoot composition, dry weight, carbon, nitrogen and chlorophyll *a,* those are deeply related to the word 'biomass'. Dissection along the vertical axis, the classical idea method of drawing the production structure of a certain plant community (Monsi and Saeki, 1953) was adopted in the present analysis to study the questions "how the moss pillar creates a fertile world in Antarctic oligotrophic freshwater lakes" and "Why the moss constructs such a unique pillar or column at the lake bottoms".

Moss shoots, mainly *Leptobryum* sp., were distributed densely at the top surface of the pillar-like growth form and showed a gradual decrease toward the core (Table 1). The distribution of green shoots of the species also showed dynamic change; it was highest at the top surface and decreased to zero 20 cm below the top of the present moss pillar (Table 2). In addition, the empty core appeared inside from 7 cm below the top. These suggest that the main species of the present moss pillar, *Leptobryum* sp., grows actively at the top surface, and photosynthetic growth activity may decrease sharply downward. The vertical distribution of chlorophyll *a*, which showed rapid decrease downward (Fig. 7), supports this speculation. The concentrated occurrence of prominent tubers near the top surface may be the result of active growth of the species. Accordingly, the moss seems to have property that it grows upward more intensively than horizontally.

Age estimation revealed a similar age distribution from the top surface to *ca.* 10 cm inside, where the tubers were observed. Imura *et al.* (1999) reported that the shoot length of the *Leptobryum* sp. sometimes elongated nearly 10 cm long in some well-developed (>20 cm height) moss pillars. The shoot length was not accounted for in the present study, though if shoots appearing at the top surface reach *ca.* 10 cm inside, materials produced by the surface shoots could be transported inside the moss pillar through the living shoots. In addition, if new buds (shoots) from the tubers or branched shoots are produced occasionally at a certain depth inside, these might contribute to the homogenous distribution of age among the sub-surface structure. The estimated age of nearly 100 years may be the result of the mixed distribution of living and dead shoots even at the top surface.

We have not analyzed the ages of basal section and ooze released from the central core yet, though the oldest value, *ca.* 250 years, was detected near the central core, which was collected >15 cm below the top. This means that the present moss pillar grew and constructed a pillar for at least a quarter millennium. The biomass stored in the present moss pillar, which is summarized in Table 3, is the result of production spent more than a quarter millennium under an Antarctic lake environment. The slow decomposition rate due to low temperature (Ohyama *et al.*, 1990; Kudoh *et al.*, 2003) and lack of herbivorous macro consumers may have contributed considerably to the biomass gain without loosening the fixed organic materials, even though the production rate was not so fast.

The moss pillar is one of associations that is consisted of many plants and microorganisms; shoots and rhizoids of *Leptobryum* sp. composing the main frame of the pillar. An algal mat coated the surface. Shoots of *B. pseudotriquetrum* are living among them, and some epontic microalgae were living in the surface structure, a rather aerobic environment (Ohtani and Suyama, 2001; Ohtani *et al.*, 2001). Decomposition in the central core suggests the occurrence of microbial activity, and nutrient recycling may occur within the moss pillar. Such activities create a gradient of oxygen and nutrient availabilities from anaerobic central core to aerobic outer surface environment within the moss pillar. As a whole, the moss pillar forms an efficient ecosystem that can produce quite a large biomass in Antarctic oligotrophic lakes. Further studies are required to confirm the present working hypotheses. For this purpose, we are proceeding with the REGAL project (Research on Ecology and Geohistory of Antarctic Lakes), under the umbrella of the VIth 5-year research project in JARE (JARE-43 (2001) to JARE-47 (2006)).

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