GEOGRAPHICAL REPORTS
OF TOKYO METROPOLITAN UNIVERSITY 46 (2011) 63-72

DISTRIBUTIONAL OPTIMUM OF SCLEROTIA, RESTING BODIES OF *CENOCOCCUM GEOPHILUM* IN FOREST SOILS

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Abstract *Cenococcum geophilum* (*Cg*) is known for its vast habitat range in temperate and arctic-alpine climatic zone. The resting bodies of *Cg*, which reveal their persistence for a long term as a structural organic component in soils, are studied from their geographical aspect in this paper. The objective of this study is to understand the distributional optimum of sclerotia as soil organic component by examinations along altitudinal gradient and its seasonal variance in central and northern Japan. Distributional properties of sclerotia were examined in soil in terms of weight density (mg g^{-1} soil), count density (no. g^{-1} soil), and mean weight per grain (mg $no.^{-1}$ sclerotium). Density of sclerotia showed an optimum distribution in cool-temperate and subalpine vegetation zones with a distinct peak at the boundary of these two zones, while soil T-C did not show such apparent peaks. Furthermore, sclerotia content showed larger seasonal variance compared to T-C, presumably due to primary activities of micro-organisms. Altitudinal distribution and seasonal variance of sclerotia of *Cg* in Japanese forest soils, highlighted sclerotia as more biotic soil organic component compared to soil humus, supposedly caused not only by germination but also by still unknown biological implication of sclerotia remaining in soil. Regarding all samples studied in this study, C/N ratio of soil behaved as one of the regulating factors of formation size of sclerotia. Although the contribution of sclerotial carbon to total soil carbon is small (<0.96%), it was suggested that sclerotia may have an important role as sink and/or source of soil carbon in cool-alpine to alpine climatic zone, aside from seasonal turnover.

Key words: *Cenococcum geophilum*, Forest soil, Sclerotia, Soil organic component, Warmth Index

1. Introduction

Cenococcum geophilum Fr. (*Cg*) is known for its vast habitat range (LoBuglio 1999). Ectomycorrhiza of *Cg* have a worldwide distribution from temperate to arctic-alpine climatic zones, and have even been observed above the Arctic Circle in Alaska and Canadian High Arctic (75°33’N, 84°40’W) and as an important symbiont of trees at timber line in the Washington and Oregon Cascade mountain range (Trappe 1964; Haselwandter and Read 1982; Trappe 1988; Bledsoe *et al*. 1989). Such wide distribution of *Cg* suggests the adaptability of it against severe environment. The experiment, which performed that *Cg* still increased in soils under exposure of simulated rain at pH 2.5 (Meier *et al*. 1989), suggests one of the excel ability in soil ecosystem.

Sclerotia are fungal resting structures, or persistent propagules which can germinate to produce mycelia, asexual spores of sporocarps such as apothecia in which sexual spores are borne (Coley-Smith and Cooke 1971). According to Trappe (1969), sclerotia of *Cg* tend to be particularly abundant near *Cenococcum* mycorrhizae. It is also well known that mycorrhizal root tips and sclerotia have their maximum production in autumn (e.g. Vogt *et al*. 1981, 1982; Lussenhop and Fogel 1999). Sclerotia of *Cg* distribute in acidic soils, and tend to form larger grains in soils with higher content of exchangeable aluminum which is potentially phyto-toxic for plant roots (e.g. Watanabe *et al*. 2004). The ^{14}C ages of sclerotia collected from buried A horizons of Fulvic Andosol in Mt. Myoko, central Japan were reported as ca. 300~1,200 yr BP (Watanabe *et al*. 2007b) and thus they exhibited its persistence as a structural organic component in soils. From studies on spatial distribution of *Cg* sclerotia, floor vegetation and micro-topography in a single stand of *Picea abies* forest in Germany, severe conditions such as low pH and high Al^{3+} content were recognized as regulating factors for sclerotia accumulation (Sakagami 2009).

The objective of this study is to obtain further knowledge on the function of sclerotia as soil organic component by examinations on geographical distribution of sclerotia along altitudinal gradient in temperate and arctic-alpine climatic zones in central and northern Japan. In addition, seasonal change of sclerotia distribution was monitored at two experimental sites in *Fagus* forest of Mt. Mito, Tokyo.

2. Materials and Methods

Examination was carried out at Mt. Iwaki (Aomori Prefecture) and Mt. Ontake (Gifu Prefecture) along altitudinal gradient and at Mt. Mito (Tokyo) for seasonal monitoring (Fig. 1). Soil samples were collected from surface A horizon (approximately 0~5cm in depth). Twenty two points were examined

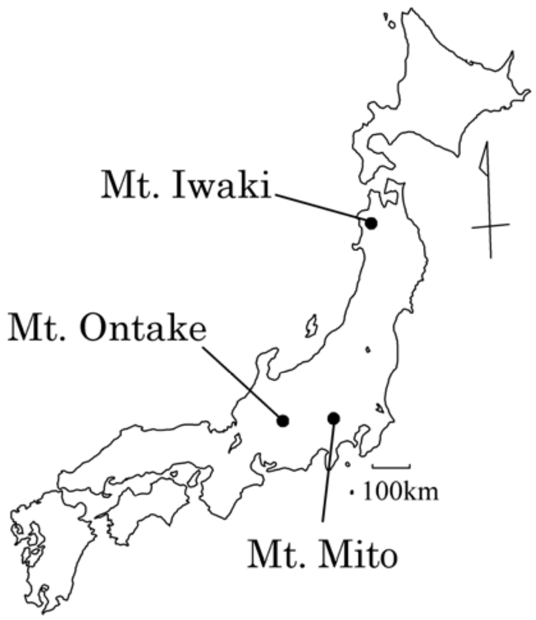

Fig. 1 Study area in central and northern Japan.

at Mt Iwaki along the driveway and the trail: line 1 (Mt. Iwaki 1), from point 1 (40°37’41”N, 140°15’15”E, 400m a.s.l.) to point 10 (40°39’14”N, 140°18’02”E, 1480m a.s.l.); line 2 (Mt. Iwaki 2), from point 11 (40°37’56”N, 140°16’04”E, 484m a.s.l.) to point 22 (40°39’07”N, 140°17’26”E, 1156m a.s.l.) on July and September 2006 (Fig. 2a). The vegetation of the pediment area of Mt. Iwaki was secondary forest (mainly *Quercus serrata*, Fig. 2b) and points 4~7 and 14~21 were *Fagus* forest (Fig. 2c), and higher points were occupied with *Betula ermanii*, *Alnus maximowiczii* or *Sasa kurilensis* (Fig. 2d). As for Mt. Ontake, soil samples were taken from 16 points (35°55’29”N, 137°26’41”E, 1715m a.s.l. ~ 35°54’37”N, 137°28’59”E, 2770m a.s.l.) along trail on June 2006 (Fig. 3a). The main vegetations were *B. ermanii* at point 1, and *Abies veitchii* or *Tsuga diversifolia* at points 2~11 (Fig. 3b, c). The forest line was around point 12, and higher regions were covered with *Pinus pumila* (Fig. 3d). In addition, Warmth Index (WI) for all points was calculated according to Kira (1976), using the Mesh Climatic Data 2000 (Japan Meteorological Agency 2002). Seasonal change of sclerotia was monitored in *Fagus* forest of Mt. Mito from April 2007 to June 2008. Three replicate samples were taken from two sites (site A: 35°44’12”N, 139°00’46”E, 1480 m a.s.l.; site B: 35°44’28”N, 139°01’29”E, 1250m a.s.l.).

Figure 4 shows assembly of sclerotia separated from Mt. Iwaki, Mt. Ontake and Mt. Mito soils according to the following procedure. 10~30 g of air-dried soil were stirred after adding 300 ml of distilled water, and all sclerotia were picked up from the float of the suspension using tweezers. Sclerotia samples were then kept air-dried for two weeks. The collected sclerotia were weighed using a micro balance and then counted to obtain their weight density (mg g^{-1} soil) and count density (no. g^{-1}) in soil. Mean weight per grain (mg $no.^{-1}$ sclerotium) was calculated by weight

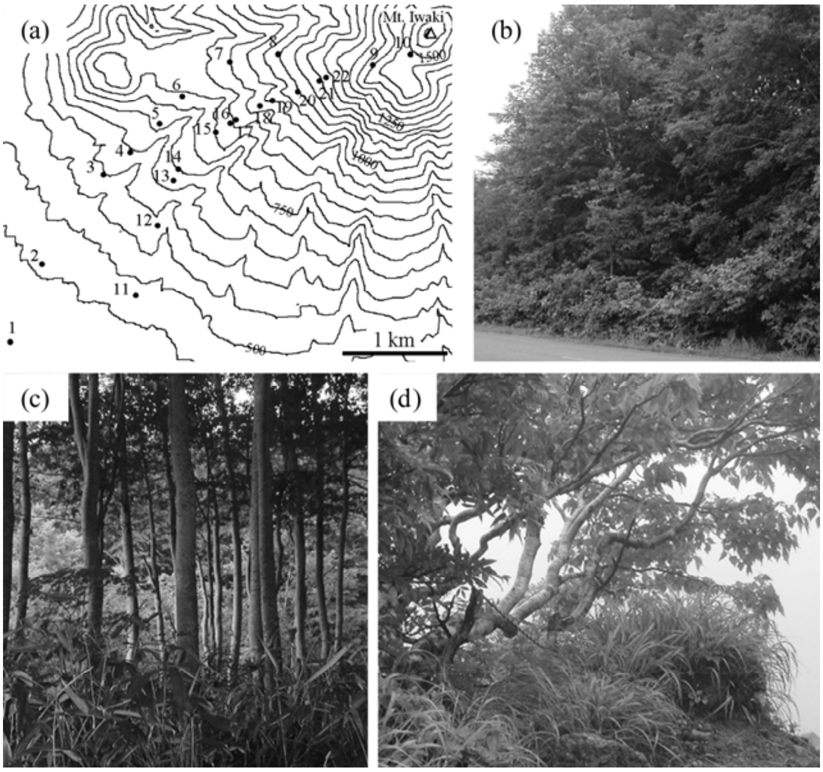

Fig. 2 Sampling area at Mt. Iwaki, a) sampling points and b-d) landscapes.

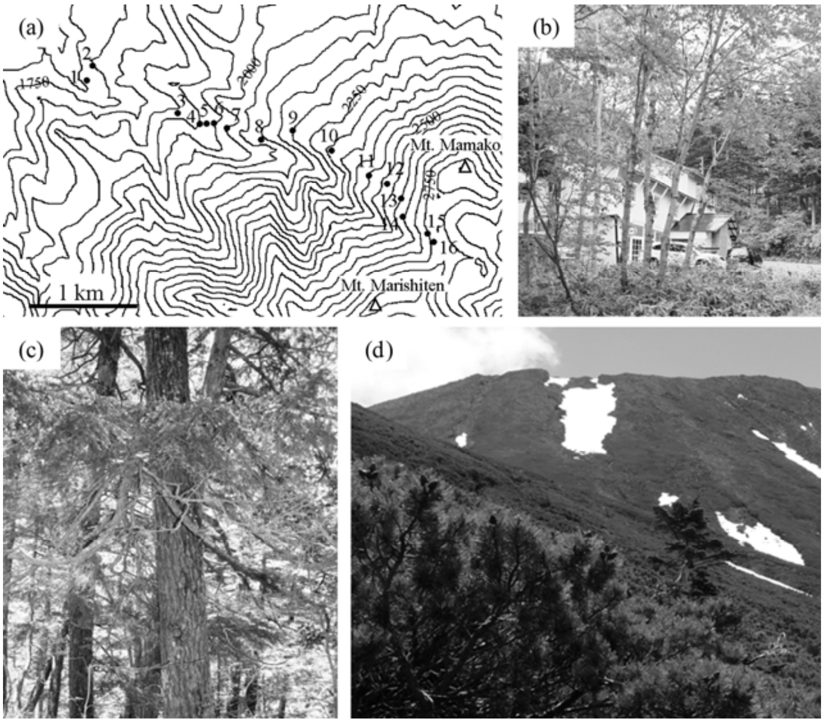

Fig. 3 Sampling area at Mt. Ontake, a) sampling points and b-d) landscapes.

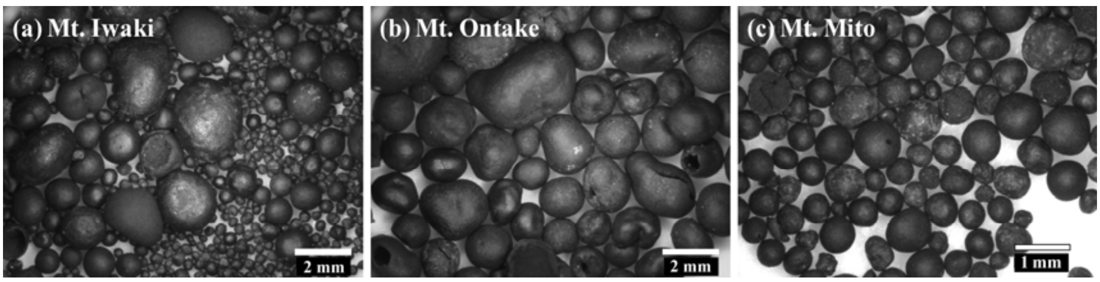

Fig. 4 Sclerotia separated from forest soils at a) Mt. Iwaki, b) Mt. Ontake and c) Mt. Mito.

and count densities. Soil pH (soil:1M KCl = 1:2.5) was measured using a pH-meter (TPX-90i, Toko Chemical Laboratories Co. Ltd., Tokyo). Total carbon (T-C) and nitrogen (T-N) contents of soils were measured by dry combustion method using an NC-analyzer (NC-22A, Sumica Chemical Analysis Service Ltd., Tokyo). Soil samples were prepared air-dried and sieved under a 2 mm for the above two measurements.

3. Results and Discussions

Altitudinal distribution of sclerotia

The distribution of sclerotia at Mt. Iwaki and Mt. Ontake are summarized in Fig. 5. The averages of weight density of sclerotia for Mt. Iwaki and Mt. Ontake were 0.80 mg g^{-1} and 0.81 mg g^{-1},

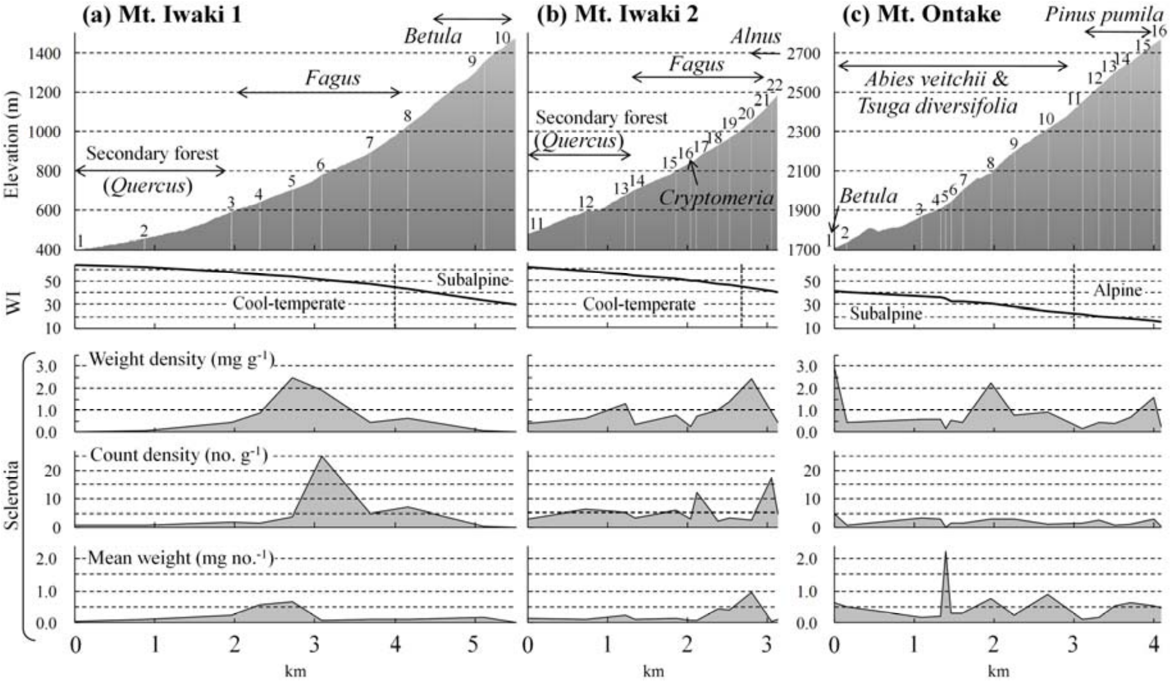

Fig. 5 Topography, vegetation, WI (vegetation zone) and sclerotia distribution (weight density, count density and mean weight) along altitudinal gradient at Mt. Iwaki (line 1, 2) and Mt. Ontake.

respectively. There was a clear peak of sclerotia content at point 5 for the weight density (2.5 mg g^{-1}) and at point 6 for the count density (25 no. g^{-1}) in *Fagus* forest of Mt. Iwaki 1. As for Mt. Iwaki 2, two peaks of weight density were observed around the boundary area of *Fagus* forest (points 13, 14 and 20, 21). A relatively low weight and low count of sclerotia was observed at point 16, where *Cryptomeria japonica* locally dominated, comparing to the around area. Since *C. japonica* is known as non-ectomycorrhizal tree species, those sclerotia were most likely originated from the extended *Cg* of the surrounding *Fagus* trees. Otherwise, they may be the remains associated to ectomycorrhizal trees in past. Sclerotia of Mt. Ontake tended to be large comparing to them of Mt. Iwaki. Three distributional peaks were recognized at point 1, 8 and 15 in Mt. Ontake and extremely large values for both weight (2.9 g kg^{-1}) and count (4.7 no. g^{-1}) were observed at point 1. As point 1 was occupied with *B. ermanii*, planted as a part of development of lodging area in Nigorigo-Onsen village, sclerotia of this point might be formed under anthropogenic condition.

Figure 6 illustrates the relationship between pH (KCl) and T-C under different WI values. Besides several exceptions, there was a notable tendency that pH (KCl) descended and T-C ascended when WI shifted from 64 to 46 at Mt. Iwaki (WI>45 area, Fig. 6a). On the contrary, pH increased and T-C decreased when WI shifted from 44 to 30 (Fig. 6b). As the same, pH decreased and T-C increased when WI changed from 42 to 35 (Fig. 6c), and opposite trend for points with WI from 33 to 15 (Fig. 6d) at Mt. Ontake. The weight density of sclerotia was high in area of pH(KCl) < 3.5 and T-C > 200, which is represented by dotted circle in Fig. 6.

Figure 7 shows the strong positive relationship between C/N ratio and mean weight of sclerotia. According to Littke *et al*. (1984) and LoBuglio (1999), *Cg* was observed to grow more rapidly at low

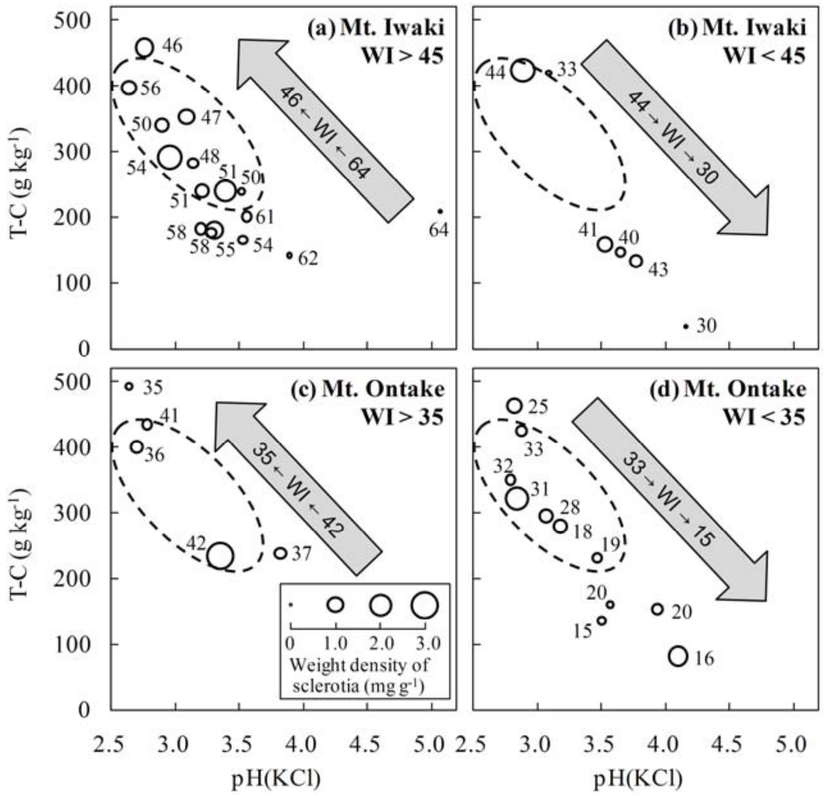

Fig. 6 The relationship between pH(KCl) and T-C under different WI values. Plot size indicates weight density of sclerotia.

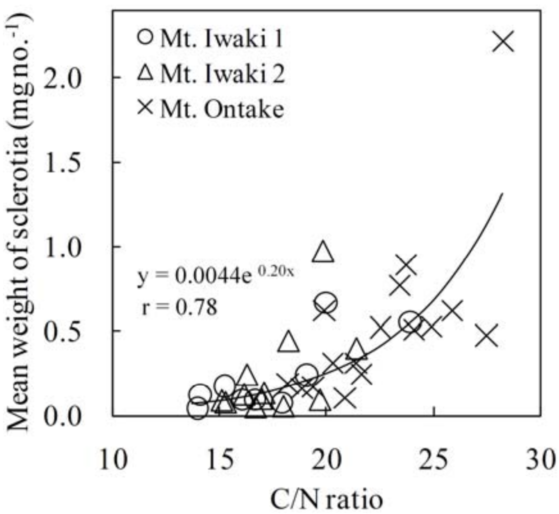

Fig. 7 The relationship between C/N ratio and mean weight of sclerotia (mg $no.^{-1}$) at Mt. Iwaki and Mt. Ontake.

N concentrations, but at the expense of biomass production. It is suggested that sclerotia may enlarge their sizes in soil of low N concentrations with association of preservation of their species.

As it is known that approximately 50 wt% of sclerotia consists of C (Watanabe *et al.* 2007a), the contribution of carbon in sclerotia to soil T-C can be estimated from the following equation;

Sclerotial C contribution (%) = (Weight density of sclerotia× 0.5) / (T-C) ×100

The average of their contribution in the studied area was 0.17% and the maximum contribution was 0.96% in mineral soil under *P. pumila* at Mt. Ontake (Point 15).

Seasonal distribution of sclerotia

Table 1 and Fig. 8 show the monitoring results on weight density, count density, mean weight of sclerotia, and T-C content in Mt. Mito. The weight densities of sclerotia were 0.42~0.84 mg g^{-1} and 0.08~0.29 mg g^{-1} in sites A and B, respectively. The count densities in sites A and B were 3.6~8.8 no. g^{-1} and 1.5~3.9 no. g^{-1}, respectively. The averages of weight and count were 0.63 mg g^{-1}, 5.7 no. g^{-1} in site A, and 0.13 mg g^{-1}, 1.9 no. g^{-1} in site B. The averages of mean weight of sclerotia were 0.11 mg $no.^{-1}$ in site A, and 0.06 mg $no.^{-1}$ in site B. Site A had larger values in every parameters compared to site B. Such results may be explained by the difference of WI, where values for site A (52) is smaller than B (59). Site A may have more preferable condition for *Cg* to form sclerotia than site B. The seasonal change of sclerotia was observed by a gradual decrease in site A from April to August 2007. This decrease could be understood as decomposition of sclerotia by activity of other micro-organisms and/or *Cg* itself, supposedly including germination of fungal mycelia. Then sclerotia in site A gradually increased in autumn 2007 and showed maximum distribution in spring 2008. Similar to site A, sclerotia in site B decreased from April to May 2007, but no content increase of sclerotia were

Table 1 Properties of sclerotia distribution and soil carbon at Mt. Mito, observed from April 2007 to June 2008

	Sclerotia			T-C	T-N	C/N ratio
	Weight density	Count density	Mean weight			
	mg g^{-1}	no. g^{-1}	mg $no.^{-1}$	g kg^{-1}		
Site A (n=13)						
Average	0.63	5.7	0.11	271	15.3	17.7
Min.	0.42	3.6	0.081	247	13.8	17.3
Max.	0.84	8.8	0.14	314	18.0	18.1
SD	0.11	1.7	0.018	17.7	1.07	0.237
CV (%)	19	29	16	6.55	7.00	1.34
Site B (n=13)						
Average	0.13	1.9	0.06	86.5	6.60	13.1
Min.	0.08	1.5	0.049	76.7	5.95	12.8
Max.	0.29	3.9	0.071	116	8.56	13.6
SD	0.053	0.61	0.006	10.1	0.675	0.187
CV (%)	43	31	10	11.7	10.2	1.43

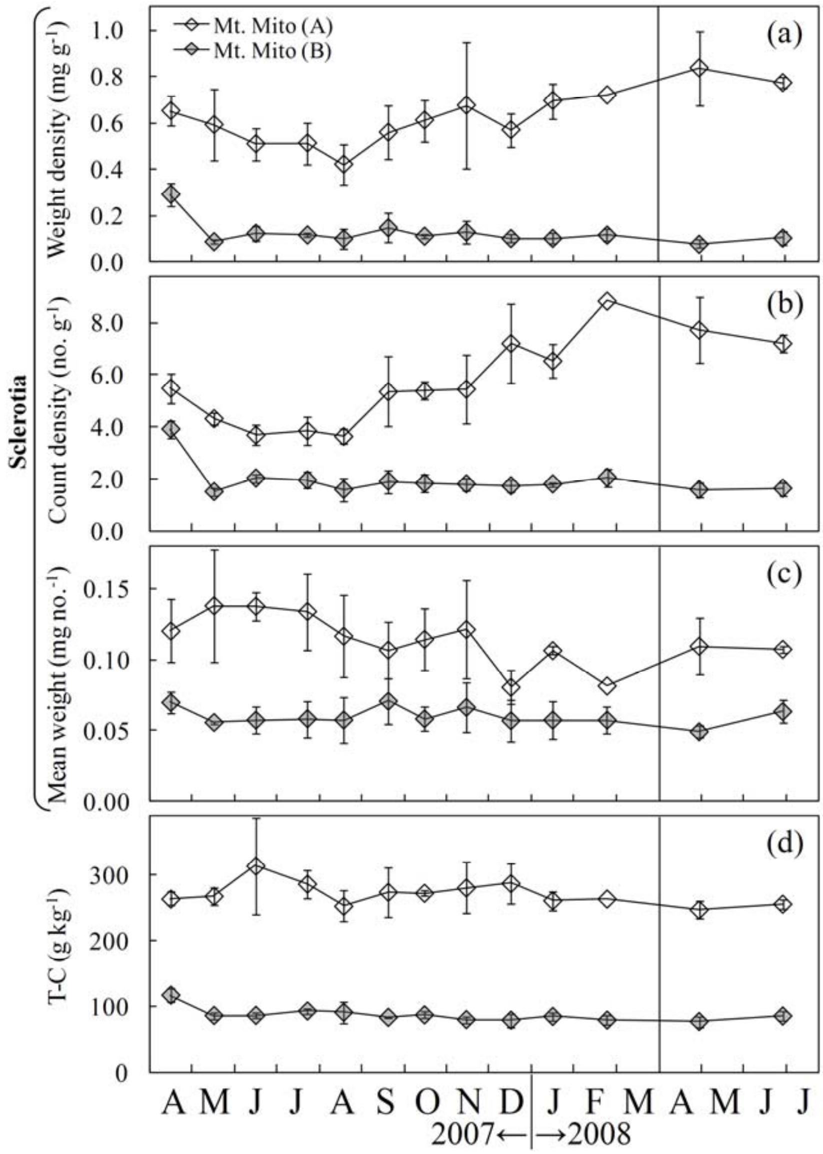

Fig. 8 Seasonal change of properties of sclerotia and soil, a) weight density, b) count density, c) mean weight of sclerotia, and d) T-C in surface soils at Mt. Mito.

recognized in autumn. Although the results obtained from the two sites is not yet enough to discuss in precise, it could be assumed as followed:
1) Seasonal change in sclerotia formation does not occur homogenously in surface of forest soils.
2) Seasonal change in sclerotia formation at site A is likely to follow the fact reported by Vogt *et al*. (1981, 1982) and others as previously mentioned in this paper.
As far as we refer to the results obtained from site A alone, the seasonal variance for formation size of sclerotia showed a clearly countered trend with the decrease and increase of sclerotia weight and count. This fact suggests that relatively small sclerotia are more likely exposed to the annual cycle of decomposition and formation. Figure 8d shows seasonal change of T-C of surface soils at Mt. Mito. As represented in Table 1, coefficients of variation (CV) for weight density of sclerotia were 0.19 and

0.43 for site A and B, respectively. On the contrary, CV for T-C were 0.07 and 0.12 for each site, respectively. Consequently, T-C showed a small seasonal change compared with sclerotia content in either site. Sclerotia of *Cg* seem to have an attribute of biotic variance as soil organic component.

4. Conclusion

Sclerotia had an optimum distribution in cool-temperate and subalpine vegetation zones with a distinct peak at boundary of these two zones. Sclerotia content showed larger seasonal variance compared to T-C, presumably due to activities of micro-organisms. Soils of which large amount of sclerotia were accumulated, such as Mt. Iwaki and Mt. Ontake, are likely to be resulted from lower decomposing rate of soil organics due to the lower temperature.

Although the contribution of sclerotial carbon to total soil carbon (T-C) is small (<0.96%), sclerotia should have important role as sink and/or source of soil carbon in cool-alpine to alpine zones, aside from seasonal turnover.

A classical model proposed by Mohr and Van Baren (1954) explained humus accumulation in temperature gradient (i.e. altitudinal gradient) by the balance of humus production and humus destruction. Similarly, distributional optimum of sclerotia can be regulated by a balance of sclerotia formation and decomposition. Formation of sclerotia may have a close relationship to the activities of *Cg* (i.e. formation of its mycorrhizae) and thus it would be strongly affected by dominant plants. Decomposition of sclerotia may be regulated by heat and water conditions and activities of micro-organisms or soil animals that feed on *Cg*.

Further examination on sclerotia accumulation in soil, such as study on food web related to *Cg* in forest soils, is recommended to understand implication of sclerotia formation in such low pH forest soils.

Acknowledgement

The author is grateful to Prof. K. Sakurai (Kochi Univ.) and Associate Prof. K. Narisawa (Ibaraki Univ.) for their valuable advices to promote this study.

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