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Soil aggregate formation and stability induced by starch and cellulose

Katsutoshi Mizuta [1], Satoru Taguchi, Shinjiro Sato*

Department of Environmental Engineering for Symbiosis, Soka University, Tokyo, 192-8577, Japan

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ABSTRACT

Soil aggregate (SA) can be formed and stabilized when soil organic matter (SOM) is decomposed in the soil. However, the relationships between the SA dynamics and SOM with different decomposition rates have not been clarified. Therefore, this study examined the effects of the addition of polysaccharides to soil on SA formation and stability. A Japanese tropical soil was incubated for 99 d at 30 °C in a dark environment following the addition of 0.5% (w/w) starch or cellulose. The decomposition rates of the amendments, and SA formation and stability were evaluated by measuring soil respiration rates, and distribution fractions of soil aggregate sizes and mean weight diameter (MWD) of SA, respectively. The cumulative soil respirations with all treatments rapidly increased until Day 12 of the incubation. The initial slope of the cumulative soil respiration in the soil with starch was significantly higher than that in the soil with cellulose. In either soil with starch or cellulose, the fractions of macro-aggregates (>1000 μm in diameter) significantly increased, respectively, compared with control soil. However, the fractions of meso-aggregates (250–1000 μm) and nano-aggregate (<20 μm) in the soil with starch significantly decreased, while those fractions in the soil with cellulose fluctuated until Day 6. The MWDs reached the maximum on Day 6, indicating the SA formation in the soils with starch or cellulose. The increasing rate of the SA formation in the starch-amended soil was greatly higher than that in the cellulose-amended soil. After Day 6, the MWDs in the soils with either polysaccharide decreased with similar trends with no significant differences between treatments, indicating similar stability of the SA in both treatments. This study showed that the different decomposability of the organic amendments might influence the SA formation differently, but not the SA stability.

1. Introduction

Soil aggregate (SA) structure plays a significant role in carbon storage in terrestrial regions (Stockmann et al., 2013) because of storage capability within the structure and physical characteristics that regulate microbial decomposition rates (McCarthy et al., 2008). The SA structure can be classified in a hierarchical model based on the size (Tisdall and Oades, 1982). Nano-aggregates (<20 μm in diameter) are formed mainly by combining soil clay particles; aggregates measuring 20–250 μm and >250 μm in diameter are classified as micro- and macro-aggregates, respectively (Edwards and Bremner, 1967; Oades and Waters, 1991). Some studies have separated aggregate sizes into different categories depending on study designs (Alberts et al., 1983; Jastrow, 1996; Plante and McGill, 2002). However, it appears appropriate to define macro-aggregates (>250 μm) into the specific classes of meso-aggregates (250–1000 μm) and macro-aggregates (>1000 μm) because of the fraction movements within size fractions (Chang et al., 2013; Yoo et al., 2014).

The SA structure can be formed in the soil with binding agents such as decomposed dead organisms and the excretion of living organisms which contain saccharides (Guggenberger et al., 1999). These hydrocarbon compounds in the soil, known as soil organic matter (SOM), may bind the soil particles together with cohesive force, thus creating resistance against outside physical forces such as percolation water (Nimmo and Perkins, 2002). The dynamics of soil aggregation and structural stability induced by SOM may differ depending on the decomposition rates of the SOM present in the system (Bronick and Lal, 2005). Monnier proposed a conceptual scheme for soil aggregate stability using different SOM inputs (translated by Abiven et al., 2009). According to this model, easily decomposable inputs such as green manure would have strong but transient effects on aggregate stability (Kiem and Kandeler, 1997;

Abbreviation: SA, soil aggregate; SOM, soil organic matter; SR, soil respiration; MWD, mean weight diameter; RMSE, root mean square error.

* Corresponding author. Tel.: +81 42 691 9490.
E-mail address: ssato@soka.ac.jp (S. Sato).
[1] Current affiliation: Soil and Water Science Department, University of Florida, Gainesville, FL 32611, USA.

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Liu et al., 2005), while more recalcitrant inputs such as decomposed manures would show weak but long-term effects (Celik et al., 2004).

However, there are some inconsistencies in the collected data on the relationship between the decomposition rates of SOM and soil aggregate stability (Abiven et al., 2009). For example, straw was classified as having fast and transient, intermediate, and recalcitrant agents (Martin and Waksman, 1940; McCalla, 1945; Sonnleitner et al., 2003). The humic substances in the soil were also found in multiple groups (Fortun et al., 1989). Possible reasons for this inconsistency may be related to the methodology and experimental design of the studies such as time scales (Stolt and Bakken, 2014). However, the organic materials may, in fact, contain both easily decomposable and recalcitrant substances; therefore different decomposability of organic materials may make the dynamics of SA formation and stabilization extremely complex.

The measurement of SOM decomposition is not clearly defined. The C:N ratio or molecular weight has been applied as an indicator of decomposition rates (Rousk and Bååth, 2007; Wickland and Neff, 2008). However, those measurements might be unable to clearly distinguish easily decomposable and recalcitrant parts of the SOM and to identify differences in the decomposition among different pure polysaccharides such as starch and cellulose since these compounds contain no nitrogen atoms and structurally have the same molecular weight.

In this study, the effects of organic matter with different decomposition rates on soil aggregate formation and stability over time were investigated by measuring soil aggregate size distribution and mean weight diameters of the SA that was formed. This study focused on two different polysaccharides because they are classified as transient agent factors for soil aggregation (Harris et al., 1966). The two specific objectives were to 1) explore the decomposition rates of the polysaccharides after being applied to soil based on soil respiration measurement and 2) clarify the relationship between the decomposition rates of the polysaccharides and soil aggregate behaviors including formation and stabilization.

2. Materials and methods

2.1. Soil sample preparation

The soil used in this study was collected from a fallow field located in Okinawa Prefecture, Japan, in September 2013, and some basic characteristics for the soils were examined (Table 1). The collected soil was dried in an oven at 45 °C for 24 h and passed through a 2 mm sieve.

2.2. Soil respiration experiment

Nine (9) 125 mL plastic bottles were prepared for the soil respiration experiment. Fifty grams of the soil, in triplicate, were weighed in each bottle, which was placed inside an 1100 mL plastic bottle. The soil was pre-incubated to stabilize soil microbial activity under the dark condition in an incubator at 30 ± 2 °C for 11 d. At the end of the pre-incubation, 0.5% (w/w) of starch or cellulose dissolved in distilled water was added to the soil. Control treatment without any amendments, in triplicate, was included. All bottles were incubated for 99 d under the same conditions as the pre-incubation. Distilled water was applied to the soil to maintain 50% of the water holding capacity of the soil every 1 d or 7 d throughout the incubation period depending on amounts of water loss. The amounts of water applied to the soil were equivalent to differences of the bottle weights compared to those at the beginning of the pre-incubation. Day 0 was defined as the day before each polysaccharide was added to the soil.

Soil respiration (SR), carbon flux rates from the soil, was measured by using the closed static chamber method of alkali trapping during the main incubation (Nordgren, 1988). The daily amount of CO_2 released from the soil was trapped in a 20 mL of 1 mol L^{-1} NaOH solution inside a glass vial that was also placed inside each 1100 mL bottle. Every day during the main incubation period, the glass vials were removed from the 1100 mL bottles and replaced by new 20 mL of 1 mol L^{-1} NaOH solutions.

The alkali solutions were titrated using 0.2 mol L^{-1} HCl (V_{sample}) and its titer (F, 8.7 × 10^{-5} mol CO_2 mL^{-1}) to eliminate the inaccuracy caused by unreacted solutions of HCl and NaOH during the titration. In addition, a 1100 mL bottle containing the NaOH solution without the bottle with soil was prepared (V_{blank}). The amount of CO_2 emitted from the soil (SR_{total}, mol CO_2 kg^{-1} soil d^{-1}) was calculated using the following equation (Luo and Zhou, 2006):

$$SR_{total} = \left(V_{sample} \times F\right) - (V_{blank} \times F) \quad (1)$$

After the calculation, the time required for the cumulative soil respiration to reach half of the maximum respiration was calculated based on the cumulative soil respiration generalized by the following logistic equation:

$$Y = a/\{b + \exp(-cX)\} \quad (2)$$

where a, b, and c are empirical constants, and X and Y are time and the SR rates, respectively (Rodeghiero and Cescatti, 2005; Aanderud et al., 2013). The equation can indicate that the steeper the initial slope of the cumulative respiration is from the soil after the amendment addition, the more easily decomposable the amendment is. The accuracy for the equation fitting with the obtained data was performed using coefficient of determination (R^2) and root mean square error (RMSE) (Williams, 1987).

2.3. Soil aggregation experiment

Sixty three (63) 125 mL bottles were prepared for the soil aggregation experiment. Fifty grams of the soil, in triplicate, were weighed in each bottle. The conditions of the pre-incubation and main incubation including periods, temperature, soil water content, and treatments were same as in the soil respiration experiment.

Table 1
Basic analysis results for physical and chemical properties in the soil.

pH[a]	WHC[b] g kg^{-1}	Sand[c] %	Silt[c] %	Clay[c] %	Total carbon[d] g kg^{-1}	Total nitrogen[d] g kg^{-1}	$CaCO_3$ equivalent[e] %
5.7	363	69	10	21	4.5	1.0	25

[a] Distilled water at 1:2.5 soil:solution ratio.
[b] Modified available water-holding capacity (Cassel and Nielsen, 1986).
[c] Hydrometer method for soil particle size distribution (Gee and Bauder, 1986).
[d] Dumas dry oxidation method measured with CHN recorder (Nielsen and Sommers, 1996).
[e] Acetic acid dissolution method (Loeppert and Suarez, 1996).

Both soil respiration and soil aggregation experiments started at the same time.

On 0, 1, 3, 6, 18, 42, and 99 d of the main incubation period, 9 bottles (3 from each treatment) were removed from the incubator. The size distribution fractions of the water-stable soil aggregates were determined destructively using 50 g of the soil from each bottle and a water sieving technique measuring soil weights passed through different mesh sieve sizes (20, 100, 250, 500, 1000, and 2000 μm) after mechanical shaking in distilled water at 30 cycles min^{-1} for 5 min (Yoder, 1936; Nimmo and Perkins, 2002). The fraction of the smallest size of soil (<20 μm) was calculated using the difference between the initial soil weight and the sum of the weights of the remaining fractions.

The soil aggregate stability was expressed by the mean weight diameter (MWD) based on the proportion of soil weights in each size fraction. The MWD values were calculated as follows:

$$MWD = \sum X_i \times W_i \quad (3)$$

where X_i and W_i indicate the mean diameter of each size fraction and the proportion of sample weights in the corresponding size fraction, respectively. The summation was carried out over all size fractions (van Bavel, 1949). The soil aggregate stability was defined as the temporal length for which the MWD was greater compared to that at the beginning of the main incubation (Day 0).

2.4. Statistical analyses

The mean and standard deviation were reported where appropriate. Two sample t-test was performed on initial slopes of the cumulative soil respiration by using R software (Ver. 3.1.2). Non-repeated measures of the ANOVA (Tukey comparison tests) were performed on distribution fractions for each soil aggregate size and the MWD of the SA for each treatment for each sampling date by using Statistica 6 (StatSoft. Inc., USA).

3. Results

3.1. Soil respiration

The soil respiration at the beginning of the main incubation (Day 0) averaged 0.1 mg CO_2 kg^{-1} soil. The SR in all treatments rapidly increased during the initial phase of the incubation up to Day 12, and reached stable phases after Day 42 (Fig. 1). The cumulative soil respiration curves were fitted well to the logistic equation (eq. (2)) with R^2 being 0.94, 0.97, and 0.99 for control, starch, and cellulose treatments, respectively. The initial slopes of the cumulative soil respiration curves at the time required to reach half of the maximum respiration were 6.1, 36.3 and 7.0 mg CO_2 kg^{-1} soil d^{-1} with control, starch, and cellulose treatments, respectively (Table 2). The initial slope and maximum cumulative respiration of the soil respiration curve with starch treatment were significantly greater compared with those with control and cellulose treatments, respectively, among which they were not significantly different (Table 2).

3.2. Soil aggregate size distribution

On Day 0, before the amendment addition, the proportions of 250–500 and 500–1000 μm fractions were predominant for all treatments, followed by the proportions of >1000 μm, <20 μm, and 20–250 μm fractions, respectively (Fig. 2).

For the control soil, significant decreases in the size fraction were observed with 1000–2000 μm fraction on Days 42 and 99, 500–1000 μm fraction on Day 42, and <20 μm fraction on Day 6 of the incubation (Fig. 2a; Table 3).

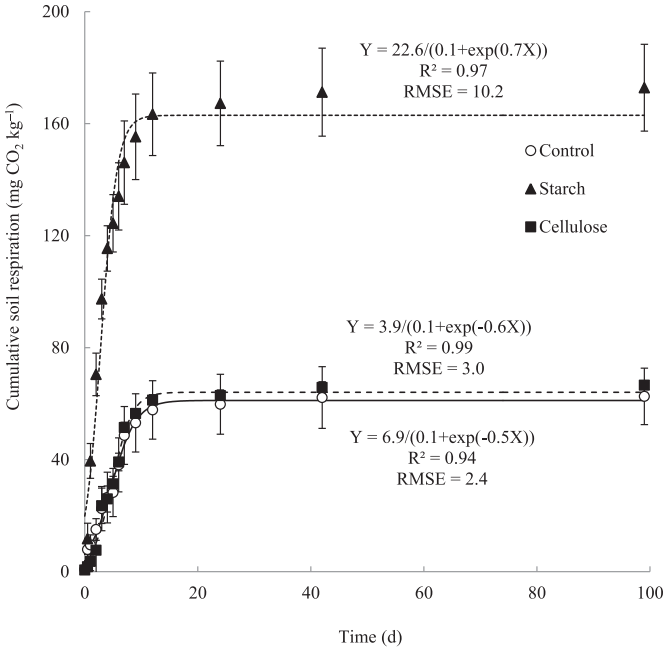

Fig. 1. Cumulative soil respiration from the soils during the incubation. Fitted equations and lines are based on the logistic equation. Coefficient of determination (R^2) and root mean square error (RMSE) were used as uncertainty indicators. Solid, small-dashed, and long-dashed lines represent fitted lines for control, starch, and cellulose treatments, respectively.

In the soil amended with starch, the proportions of >2000 μm and 1000–2000 μm fractions significantly increased from Day 0–6, whereas those of 500–1000 μm and <20 μm fractions significantly decreased during that time (Fig. 2b; Table 3). The proportions of >2000 μm and 1000–2000 μm fractions on Day 99 decreased to similar proportions to those on Day 0. On the other hand, the proportions of 250–1000 μm and <106 μm fractions on Day 99 increased to higher proportions than those on Day 0.

In the soil amended with cellulose, the proportions of all fractions >250 μm widely fluctuated during the incubation (Fig. 2c; Table 3). On Day 99, the proportions of >1000 μm fractions decreased to less than those before the cellulose addition, whereas those 106–500 μm fractions significantly increased.

3.3. Soil aggregate stability

The changes of MWD of the soil aggregates varied among the treatments during the incubation (Fig. 3). The MWD in the control soil significantly decreased throughout the incubation, reaching smaller size on Day 99 than that on Day 0. The MWD in the soil with starch reached the maximum of 498 μm on Day 6, which was significantly higher than that before the addition (Table 4). The positive linear regression line for the MWD with time was well correlated, and a significant correlation was observed in the soil with starch during the first 6 d of the incubation ($R^2 = 0.55$, $p < 0.01$). After 99 d of incubation, the MWD significantly decreased to 240 μm which was even lower than that on Day 0.

The MWD with the cellulose treatment, however, varied during the first 6 d of the incubation, but significantly increased to 445 μm on Day 6. The linear regression line for the MWD with time was not well correlated ($R^2 = 0.13$, $p = 0.24$). At the end of the incubation, the MWD significantly decreased to a smaller size than that before the amendment.

Table 2
Initial slope and time required to reach half of the maximum cumulative respiration during the soil respiration experiment.

Treatment	Initial slope	Maximum cumulative respiration	Time required to reach half of the maximum respiration[a]
	mg CO_2 kg^{-1} d^{-1}	mg CO_2 kg^{-1}	d
Control	6.1 (2.0)[b]b[c]	62.6 (10.1) b	4.7
Starch	36.3 (4.0) a	172.8 (15.5) a	2.9
Cellulose	7.0 (1.5) b	66.6 (2.6) b	4.9

[a] The time was calculated for each treatment based on the fitting models, respectively.
[b] The standard deviation of the mean is shown in parentheses (n = 3).
[c] Different letters assigned in columns denote significant differences ($p < 0.01$) among treatments.

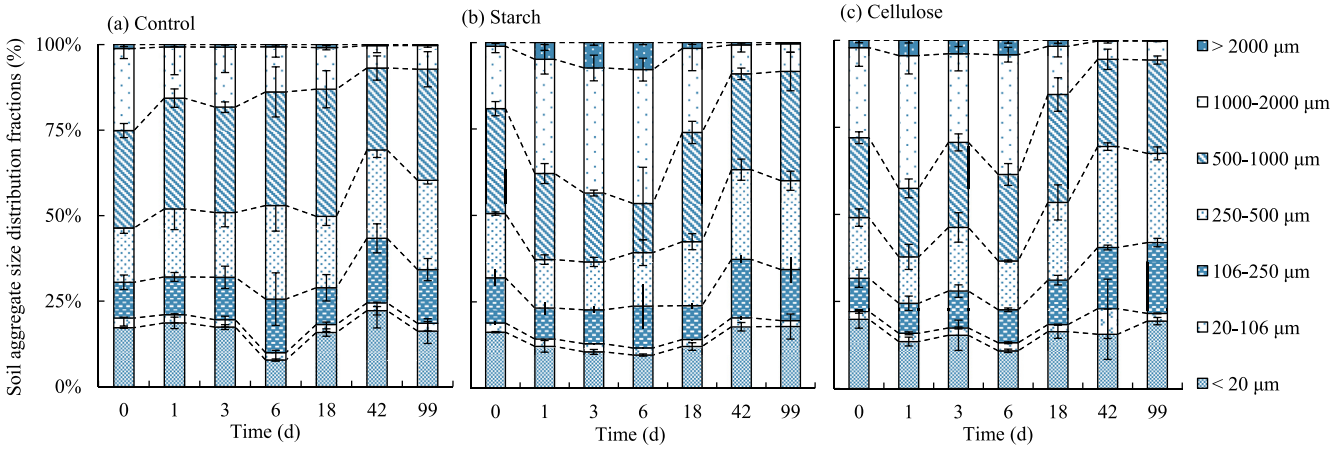

Fig. 2. Soil aggregate size distribution fractions in (a) control, (b) starch, and (c) cellulose treatments during the incubation.

4. Discussion

4.1. Polysaccharides decomposition

The cumulative soil respiration from the control soil was unexpectedly high being comparable with that from the soil with cellulose (Fig. 1). Possible explanations may include CO_2 released from the chemical reaction of calcium carbonate in the moderately acidic soil (Table 1) and CO_2 released due to continued microbial stabilization (Setia et al., 2010). Similar soil respirations were observed from the similar soils as in our study, although the actual respiration rates were highly variable depending on soil characteristics and incubation conditions (Wang et al., 2003; Kolar et al., 2007). Another explanation may be speculated that cellulose may have not been decomposed in the soil enough to make differences in the soil respiration compared with control.

Nevertheless, the difference in the cumulative soil respiration between amended treatments was obvious indicating that cellulose was more recalcitrant to decomposition than starch after application to soil based on the results of the longer time required to reach half of the maximum cumulative respiration and lower initial slope (Table 2). The recalcitrant characteristic of cellulose has been also documented in previous studies (Miltner and Zech, 1998; Boer et al., 2005), although studies on comparisons between starch and cellulose are scarce. The difference in decomposability between starch and cellulose can be explained by the higher energy required to break down cellulose than starch into consumable substances such as glucose (Boswell, 1941).

The decomposition behavior of organic substances in the soil can vary depending on the type of substance and the soil involved because the microbial activity can be regulated by the molecular complexity of the substance and the soil environmental factors such as pH and nutritious status (Craine et al., 2007; Blagodatskaya and Kuzyakov, 2008).

Table 3
Summary of Tukey comparison results by non-repeated measures of the ANOVA test on the soil aggregate size distribution fractions for each soil aggregate size during the soil aggregation experiment.

Treatment	Aggregate size (μm)	Incubation period (d)						
		0	1	3	6	18	42	99
Control	>2000	a[a]	a	a	a	a	a	a
	1000–2000	a	ab	ab	ab	ab	b	b
	500–1000	ab	ab	ab	ab	a	b	ab
	250–500	a	a	a	a	a	a	a
	106–250	a	a	a	a	a	a	a
	20–106	a	a	a	a	a	a	a
	<20	ab	a	ab	b	ab	a	ab
Starch	>2000	c	b	ab	a	c	c	c
	1000–2000	cd	ab	a	a	bc	d	d
	500–1000	a	ab	ab	b	a	ab	a
	250–500	b	b	b	b	b	a	a
	106–250	a	a	a	a	a	a	a
	20–106	b	ab	ab	ab	ab	ab	a
	<20	ab	bc	c	c	bc	a	a
Cellulose	>2000	abc	a	ab	ab	bc	c	c
	1000–2000	b	a	b	ab	c	c	c
	500–1000	ab	b	ab	ab	a	ab	ab
	250–500	bcd	d	bcd	cd	abc	a	ab
	106–250	b	b	b	b	b	a	a
	20–106	a	a	a	a	a	a	a
	<20	a	a	a	a	a	a	a

[a] Different letters assigned within each aggregate size for each treatment denote significant differences ($p < 0.05$) among different incubation periods.

4.2. Soil aggregate formation

Compared with the soil aggregate size distributions and the MWD in the control soil, it was clearly demonstrated that the application of either polysaccharide significantly contributed to the formation of macro-aggregates in soil, particularly in the beginning

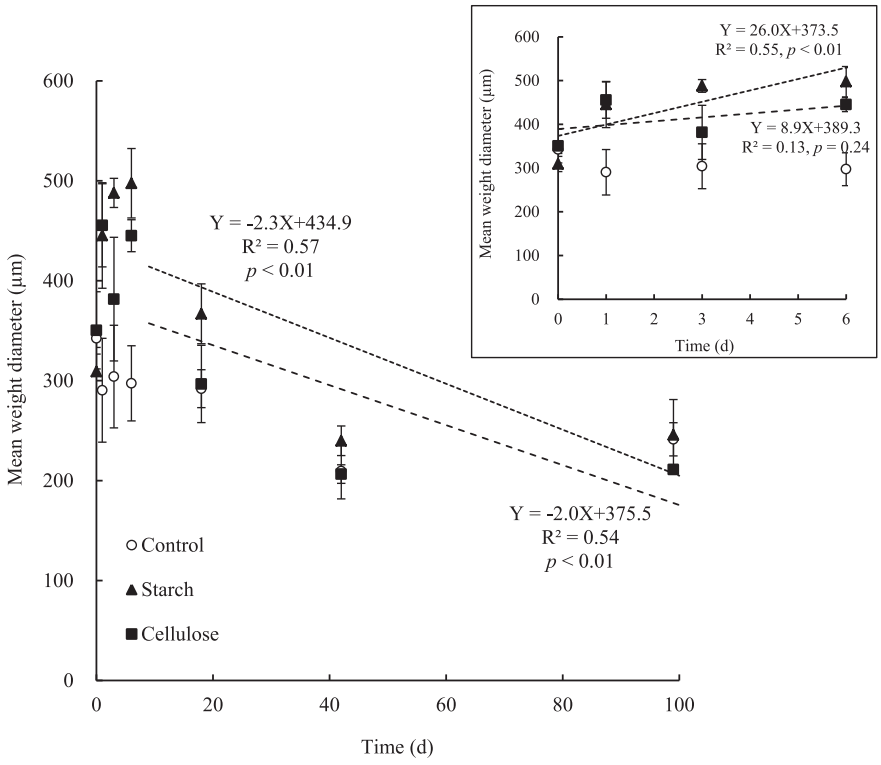

Fig. 3. Mean weight diameter (MWD) of soil aggregates formed in the soils during the incubation. The MWDs during Day 0–6 of the incubation are shown in the box. The positive and negative linear regressions were fitted to the MWDs in the soils with polysaccharides during Day 0–6 and Day 6–99 of the incubation, respectively. Small-dashed and long-dashed lines represent fitted lines for starch and cellulose treatments, respectively.

Table 4
Summary of Tukey comparison results by non-repeated measures of the ANOVA test on the mean weight diameters during the soil aggregation experiment.

Treatment	Incubation period (d)						
	0	1	3	6	18	42	99
Control	a[a]	abc	ab	abc	abc	c	bc
Starch	cd	ab	a	a	bc	d	d
Cellulose	bc	a	abc	ab	cd	d	d

[a] Different letters assigned within each treatment denote significant differences ($p < 0.05$) among different incubation periods.

of the incubation up to Day 6 (Figs. 2 and 3). The initial increase of the macro-aggregates fractions and MWD seemed to coincide with the rapid increase of soil respiration with either polysaccharides application (Fig. 1), which was similar with the previous review by Abiven et al. (2009). The microbial decomposition of SOM can convert complex organic substances into degradable simple substances such as polysaccharides which can contribute to the formation of the soil aggregates (Morel et al., 1987). It is also known that root mucilage and secretion of soil microbes, mainly composed of polysaccharides, may enhance the development and stabilization of soil structures due to their physical cohesiveness (Martens, 2000). The rapidly increasing fraction of the macro-aggregates, which consequently increased the MWD in the soils amended with starch and cellulose observed in our study, was likely due to the increased cohesive interaction caused by the increased soil microbial activities induced by the polysaccharides (Tisdall, 1995). In particular, starch which was more easily decomposable than cellulose enhanced more the macro-aggregate formation, which was consistent with the previous finding (Griffiths and Jones, 1965).

However, it appears that different size fractions of SA contributed to the formation of the macro-aggregates with the application of different polysaccharides in our study (Fig. 2; Table 3). In the soil amended with starch, some fractions of the nano-aggregates and meso-aggregates (500–1000 μm) apparently decreased to form the macro-aggregates during the initial incubation period. Meanwhile, the macro-aggregates in the soil amended with cellulose seemed to be formed by combining different sizes within the meso-aggregate fraction. The differences in the aggregate size fractions for the macro-aggregate formation may be explained by different microbial activities in the soils caused by different polysaccharides (Møller et al., 1999). More easily decomposable substances such as starch might increase bacteria biomass (Guggenberger et al., 1999), while more recalcitrant substances such as cellulose might enhance fungi biomass in the soil (Rousk and Bååth, 2007), both which could contribute to the formation of the macro-aggregates (Meidute et al., 2008). Therefore, the enhanced growth of the different microorganisms in the soil may have contributed to the formation of the macro-aggregates by combining the different size fractions after the addition of different polysaccharides. However, it appears contradictory that the soil respiration from the control soil was relatively comparable with that from the cellulose-amended soil, if the cellulose addition ought to enhance fungi biomass in the soil. Another possible explanation to the formation of the macro-aggregates caused by the cellulose addition could be speculated to be a physical adhesiveness of cellulose when in contact with soil water. Relationships between different soil microorganisms and macro-aggregate formation in soil need further clarification.

4.3. Soil aggregate stability

The MWD increased from Day 0 through 6 of the incubation with both polysaccharides application appeared to have been disintegrated into smaller size fractions than the macro-aggregates with similar trends from Day 18 through 99 (Fig. 3). This result

indicates that the organic amendments with different decomposability structurally affected the SA formation but not so much for the SA disintegration (stability). This phenomenon was also observed in a previous study (Schlecht-Pietsch et al., 1994), however only a few studies on the SA stability have been conducted, with reports of some soil biochemical factors being related to the SA stability (Abiven et al., 2009). For example, soil nutrient scarcity and pH changes regulate microbial activities and root behaviors, which lead to an abundance of biochemical mucilage in SA structures (Chan and Heenan, 1999; Güsewell and Gessner, 2009). Other studies stated that mucilage types (or chemical saccharides compositions) and amounts secreted from different plant species, as well as soil moisture levels and soil fauna also influenced SA structures (Degens and Sparling, 1996; Six et al., 2004; Bossuyt et al., 2005). However, what and how soil properties affect the stabilization of SA are not well understood yet. Since the formation and stabilization processes of SA structures can affect the carbon dynamics in the terrestrial regions, further investigations on SA structures, especially the stabilization mechanism related to soil organic matter application are needed.

5. Conclusions

The application of polysaccharides such as starch and cellulose may accelerate the soil macro-aggregate formation. However, the decomposition of the amendments may influence only the formation process of the soil aggregate or aggregation, but not the disintegration process or aggregate stabilization. The effect of adhesive powers polysaccharides have in solution on the aggregate formation and stabilization must be investigated as a fundamental study for the further investigations such as modeling.

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