Dynamics of Newly Formed Humic Acid and Fulvic Acid in Aggregates After Addition of the ^{14}C-Labelled Wheat Straw in a Typic Hapludoll of Northeast China

Sen Dou, Song Guan, Guang Chen, and Gang Wang

Abstract This research chose the Typic Hapludoll in the northeast of China and studied dynamics of newly formed humic acid (HA) and fulvic acid (FA) in aggregates after addition of the ^{14}C-labelled wheat residues for 60, 180, and 360 days incubation using the aggregate classification combined with chemistry grouping of humic substances. Based on ^{14}C isotope tracer technology, the results showed that the sorting selection of >2,000-μm and 2,000–250-μm macroaggregates on newly formed ^{14}C-HA and ^{14}C-FA was higher than microaggregates. In the >2,000-μm and 2,000–250-μm macroaggregates, the ^{14}C-HA/^{14}C-FA ratio decreased with the increase of incubation time and the formation of ^{14}C-FA was faster than ^{14}C-HA, which humic substance formation supported lignin theory of Waksman. In the <53-μm silt and clay components, ^{14}C-HA/^{14}C-FA ratio increased with the increase of incubation days and the formation of ^{14}C-HA was faster than ^{14}C-FA, indicating that the formation of humic substances followed the polyphenol theory of Kononova. In conclusion, organic fertilization would facilitate the formation of macroaggregates and thereby result in an enhanced accumulation of humic substances in macroaggregates. Therefore, with more potential of carbon sequestration, the >2,000-μm and 2,000–250-μm macroaggregates may have important significance in the enhancement of soil carbon sequestration, fertility, and relief of elevated atmospheric CO_2 concentration.

Keywords C-labelled wheat straw • Soil aggregates • Humus formation • Dynamics • Humic acid (HA) • Fulvic acid (FA) • ^{14}C-HA/^{14}C-FA ratio

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Introduction

Humic substances are considered to be recalcitrant, rich in functional groups and interacting with mineral surfaces, for this reason, probably act as persistent binding agents (Lugato et al. 2010). Soil aggregates are the place where SOM is kept and the main place of humified. Complex and macromolecular humic substances are recalcitrant to microorganism, which can sequestrate carbon (Chaney and Swift 1986; Fortun et al. 1989; Piccolo et al. 1997; Albert et al. 2005). The research about the aggregates and inter-aggregate humic substances is one aspect of soil carbon sequestration mechanisms. But so far, the interaction between the aggregates and inter-aggregate humic substances is known a little.

We hypothesize two-way sorting selection occurred between humic substances and aggregates. On the one hand, sorting of aggregates on humic substances varies with aggregate size, which probably causes the difference in constitution and structure of humic substances in different aggregate sizes. On the other hand, the differences in properties of humic substances constituents possibly induce the formation of different aggregate sizes. The process of two-way sorting is certainly affected by agricultural managements such as fertilization. This research chose the Typic Hapludoll in the northeast of China and studied dynamics of newly formed humic acid (HA) and fulvic acid (FA) in different sizes of aggregates after the addition of ^{14}C-labelled wheat residues in Hapludoll by ^{14}C isotope tracer technology and using the method of aggregate classification combined with chemistry grouping of humic substances. Furthermore, we discussed sorting of different aggregate size fractions on humic substance constituents, which provides theory basis for enhancing soil fertility and potential of carbon sequestration.

Materials and Methods

Middle-level Hapludoll was taken from the top soil layer (0–20 cm) in Gongzhuling City, Jilin Province, northeast China (43°31′ N, 124°49′ E), in 2005. ^{14}C-labelled wheat residues were 3.7×10^3 kBq gC^{-1}. The soils were incubated in the dark at 25 °C for up to 360 days and sampled for size fractionation of aggregates at 60, 180, and 360 days with triplicate samples.

Separation of aggregates was done by wet sieving (Gryze et al. 2005). Humic substances were extracted with 0.1 mol L^{-1} NaOH + $Na_4P_2O_7$. Then 0.5 mol L^{-1} H_2SO_4 was added into extract for separating FA and HA. The ^{14}C activity (DPM) was determined by liquid scintillation counting (Tri-carb2800); radioactivity of residual ^{14}C in soil was obtained by the subtraction method.

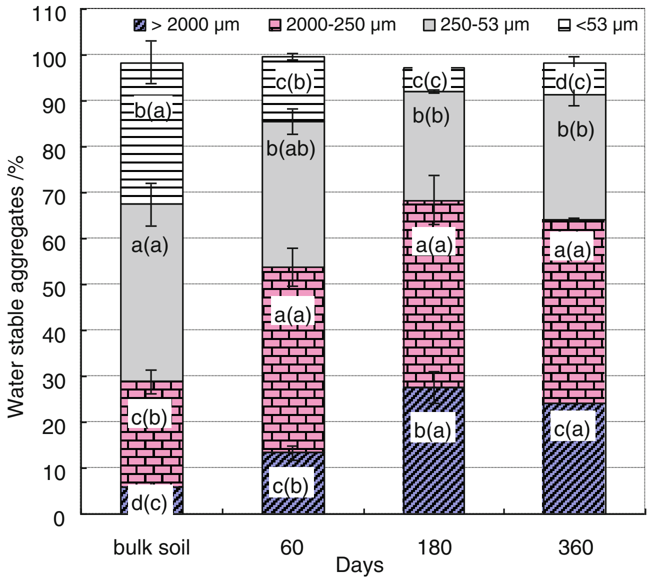

Fig. 1 Dynamic distribution of water stable aggregates with the addition of wheat residues in soil. Columns with a different letter are significantly different ($P < 0.05$) among aggregate size for the same incubation days. Columns with a different letter in the *bracket* are significantly different ($P < 0.05$) among incubation days for the same aggregate size. *Bars* represent the standard deviation

Results and Discussion

The amount of 250–53-μm microaggregates was the most in bulk soil. The 2,000–250-μm small macroaggregates became dominant aggregate size fraction, and OC mainly distributed in the >2,000-μm large macroaggregates with the addition of wheat residues in soil (Figs. 1 and 2).

Specific activity of residual ^{14}C declined gradually with increasing of incubation days in soil. Radioactivity of residual ^{14}C was 45% of added plant residue—^{14}C remained in soil after 360 days of incubation. Specific activity of newly formed ^{14}C-HA and ^{14}C-FA strengthened with prolongation of incubation days (Table 1), which showed that on the one hand, ^{14}C-wheat residues mineralized continually and on the other hand, humic substances were synthesized by decomposed ^{14}C-plant residues. 2.22–3.20% and 3.26–6.20% of ^{14}C-input were transformed into ^{14}C-HA and ^{14}C-FA, respectively.

With the addition of ^{14}C-wheat residues in soil, the sorting of >2,000-μm and 2,000–250-μm macroaggregates on newly formed ^{14}C-HA and ^{14}C-FA was higher than microaggregates. ^{14}C-HA and ^{14}C-FA mostly distributed in the >2,000-μm and 2,000–250-μm macroaggregates and enhanced with the increase of aggregate size. However, ^{14}C-HA decreased with the increase of aggregate size in the 250–53-μm and <53-μm microaggregates (Fig. 3).

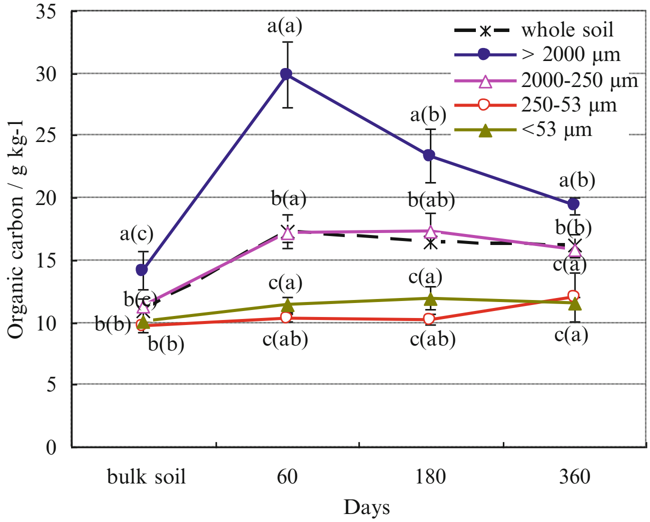

Fig. 2 Dynamics of OC in different aggregate size fractions with the addition of wheat residues in soil. Curves with a different letter are significantly different ($P < 0.05$) among aggregate size for the same incubation days. Curves with a different letter in the *bracket* are significantly different ($P < 0.05$) among incubation days for the same aggregate size. *Bars* represent the standard deviation

Table 1 Dynamics of residual ^{14}C and new ^{14}C-HA and ^{14}C-FA in soil

Days	Residual^{14}C/% of ^{14}C input	^{14}C-HA/% of ^{14}C input	^{14}C-FA/% of ^{14}C input
60	48.94 (0.89)a	2.22 (0.04)c	3.26 (0.19)b
180	46.01 (0.38)b	2.69(0.12) b	3.35 (0.29)b
360	45.02(1.12) b	3.20 (0.24)a	6.20 (0.62)a

Standard deviation of the means is shown in *brackets*
Columns with a different letter are significantly different ($P < 0.05$) among incubation days

Our research which showed dynamic formation and transformation between ^{14}C-HA and ^{14}C-FA was different with varied incubation days between the macroaggregates (>250 μm) and the microaggregates (<250 μm).

Radioactivity of ^{14}C-HA and ^{14}C-FA was measured in aggregates with the decomposition of ^{14}C-labelled wheat residues after 60-day incubation, which indicated a part of ^{14}C input was transformed into humic substances. For the macroaggregates (>2,000 μm and 2,000–250 μm), radioactivity of ^{14}C-HA was the highest and ^{14}C-HA/^{14}C-FA ratio $\geq$1.00 in 60 days of incubation. Then radioactivity of ^{14}C-HA weakened, and radioactivity of ^{14}C-FA enhanced, and ^{14}C-HA/^{14}C-FA ratio decreased with increasing incubation days from day 60 to day 360 ($P < 0.05$) (Fig. 4). These showed new ^{14}C-HA decomposed and transformed into ^{14}C-FA, which supported lignin theory of Waksman (1936). High molecular weight HA represents the first phase of humification and then is broken down into FA with microbial action in this theory. Lignin tissue is dominant

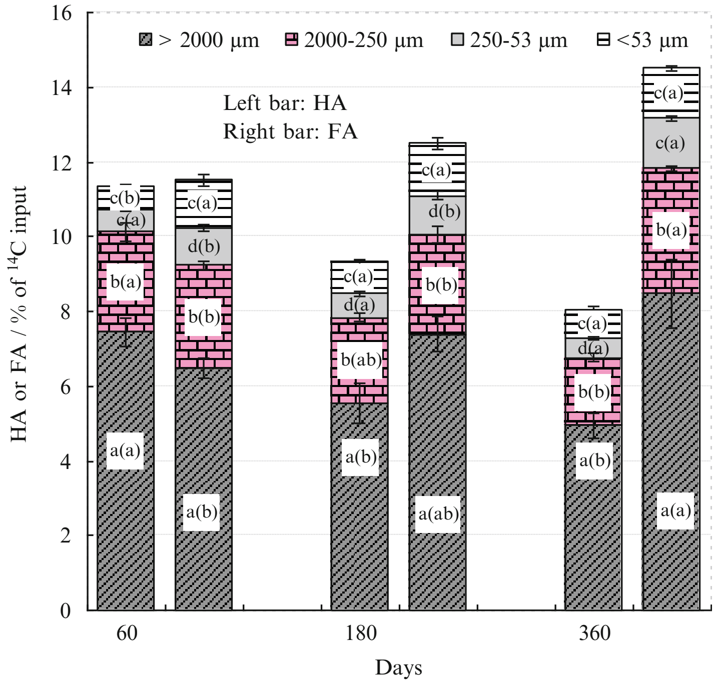

Fig. 3 Percent of ^{14}C-HA and ^{14}C-FA of ^{14}C input in different aggregate size fractions. Columns with a different letter are significantly different ($P < 0.05$) among aggregate size for the same incubation days. Columns with a different letter in the *bracket* are significantly different ($P < 0.05$) among incubation days for the same aggregate size. *Bars* represent the standard deviation

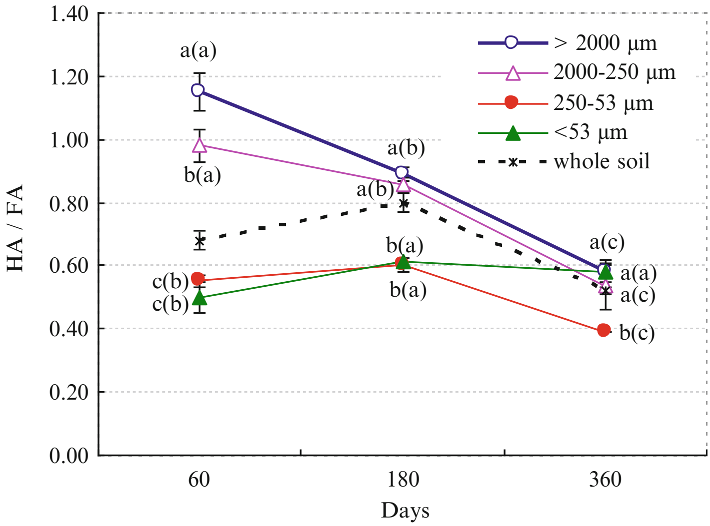

Fig. 4 The radio of ^{14}C-HA/^{14}C-FA in different aggregate size fractions and whole soil. Curves with a different letter are significantly different ($P < 0.05$) among aggregate size for the same incubation days. Curves with a different letter in the *bracket* are significantly different ($P < 0.05$) among incubation days for the same aggregate size. *Bars* represent the standard deviation

in chemical composition of organic residues, and the macroaggregates (>2,000 μm and 2,000–250 μm) were in favour of HA and FA formation by the accumulation of much organic residues in the above discussion, which proved humic substances formation conforming to lignin theory.

For the silt and clay fraction (<53 μm), ^{14}C-HA/^{14}C-FA ratio increased with increasing incubation days (Fig. 4), and the formation of ^{14}C-HA was faster than ^{14}C-FA, which demonstrated that humic substances formation was in support of Kononova (1966) polyphenol theory.

Conclusion

In conclusion, with organic fertilization, more macroaggregates and more humic substances in macroaggregates were formed, which showed the >2,000-μm and 2,000–250-μm macroaggregates had more potential of carbon sequestration and had important significance in the enhancement of soil carbon sequestration and fertility and relief of elevated atmospheric CO_2 concentration.

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