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Capacity of soil to protect organic carbon and biochemical characteristics of density fractions in Ziwulin Haplic Greyxems soil

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Abstract **Physical protection is one of the important ways to stabilize organic carbon in soils. In order to understand the role of soils as a carbon sink or source in global climatic change and carbon cycles and properly manage soils as a carbon sink, we ought to know how many organic carbon (OC) in a given soil could be protected. By a density fractionation approach and ultrasonic technique, each soil sample was divided into three fractions: free light fraction (free-LF), occluded fraction (occluded-LF) and heavy fraction (HF). The obtained fractions were analyzed for total OC content, carbohydrate content and recalcitrant OC content. The results showed: (i) In the whole soil profile, dominance of OC consistently decreased in the following order: HF, free-LF, occluded-LF. This suggested that OC in soils were mostly protected. From 0—10 to 60—80 cm horizons, the OC in free-LF decreased from 25.27% to 3.72%, while OC in HF they were increased from 72.57% to 95.39%. The OC in occluded-LF was between 2.16% and 0.89%. (ii) Organic carbon recalcitrance in free-LF was similar to that in HF, and was even higher than that in HF below the surface horizon. This suggested that free-LF was not always the most fresh and non-decomposed fraction. OM quality of HF was higher than that of free-LF in the surface 10 cm below, namely the protected OM had higher quality than free OM in these horizons.**

Keywords: Ziwulin, Haplic Greyxems soil, organic carbon, physical protection, density fractionation.

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In order to evaluate to what extent the increase in atmospheric CO_2 content could be compensated by current policies such as afforestation or by changing some current agriculture practices, people have paid great attention to the capacity of soils to accumulate and stabilize organic carbon (OC) in recent years. Most studies suggested that terrestrial ecosystems (particularly forest ecosystems) in northern middle-high latitudes act as sink of atmospheric CO_2[1—3]. However, the study of chronosequences suggested that the capacity of soils to stabilize OC in the long term is much lower than often assumed[4]. So, we are not clear about how long this can be maintained, or how many OC could be protected in a given soil.

In order to understand the function of soils in global climatic change and carbon cycles and properly manage soils as a carbon sink, we ought to understand first the reasons why the soil OC may become stable. There are three ways to stablize OC in soils: (i) Chemical processes, such as precipitation by Ca^{2+} and Fe^{3+}. (ii) Chemical recalcitrance. Because of inherent molecular structure of substrates, the content of organic fractions is difficult to be decomposed by micro flora, namely recalcitrant OC. (iii) Physical protection. OC and soil mineral particles (particularly clays and fine silts) form organ mineral complexes to make the OC partly unavailable for the micro flora, namely form the physical barriers between substrates and decomposer organisms[5]. Physical protection is one of the important ways to stabilize organic carbon in soils. Even though most studies suggested that the degree of physical protection and the degree of biochemical recalcitrance are related[6—8], we have also noticed that there is a substantial difference between both types of OC stabilization. While there is no apparent upper limit for the amount of recalcitrant OC that soils can accumulate, the amount of protected OC in a given soil must be finite, because the physical protection depends on the amount of mineral particles that are able to form organomineral complexes or microaggregates (fine silt, clays), and the amount of these particles in a given soil is finite. Thus, the question of how many OC a given soil could be physically protected has been addressed. The question was raised only some recent years[9].

Soil organic carbon was separated into OC in free light fraction (free OC), OC in occluded fraction (occluded OC) and OC in heavy fraction (HF OC) by physical density fractionations. Free OC included loose organic particles in the soil and particulate OC adhering to the exterior of secondary organomineral complexes. Occluded OC is the fraction of uncomplexed OM that is trapped and physically protected within secondary complexes (intra-aggregate OC). The distinction between free and occluded fractions of uncomplexed OC was based on experiment. Free OC is recovered from minimally dispersed samples in which aggregates remain intact, while occluded OC subsequently is isolated after dispersion of aggregates. HF OC was denser than free OC due to HF OC associated with plenty of mineral particles of different sizes[5].

Prevoius papers on the biochemical characterization are based solely on the whole soil sample, but papers on the obtained physical fractions (either by density or by

size) have become common in recent years. Solid-state ^{13}C-NMR[10] and pyrolysis[11] are widely applied, but both methods are sophisticated and expensive and therefore not useful for the large sets of samples commonly obtained in ecological studies. It is possible to apply conventional methods to the physical fractions, such as humus fractionation procedures, but there are few examples of this, probably because of the length of time needed for analysis. Recently, Leavitt et al.[12] and Rovira and Vallejo [13] overcame this problem by applying simpler chemical treatments to the physical fractions, such as the quantification of recalcitrant OC by means of acid hydrolysis. The non-hydrolyzable OC in each fraction was taken as recalcitrant OC, and recalcitrancy index was used as the index of organic matter quality.

In this paper, we use a density fractionation procedure to separate free-LF, occluded-LF and HF, and then use acid hydrolysis to quantify labile and recalcitrant fractions within them. We focus our attention on the three following questions through the above methods: (ⅰ) What is the distribution of OC among fractions? (ⅱ) What is relationship between the degree of physical protection and the chemical recalcitrance of the various fractions, and what changes would be made in physical protection and chemical recalcitrance depending on the position in the soil profile?

1 Materials and methods

(ⅰ) Site characteristics. We conducted this study at Lianjiabian plantation forest region of northern Ziwulin, Heshui County, Gansu Province. The site is located at latitude 35º03′—36º37′N, longitude 108º10′—109º08′E and at an altitude of about 1500 m above sea level, and belongs to foothill-gully zone of the Loess Plateau. The average annual precipitation was 587.6 mm, and the average annual air temperature was 7.4℃[14]. The soil of the study site was classified as calcareous cinnamon soil or forest Haplic Greyxems soil, which originates from primary or secondary loess of 50-100 m depths and the thickness of laterite below the loess is 80—100 m[15,16]. Pedogenic process is controlled by clayification and carbonate eluviation-illuviation. The typical soil profile consists of humus horizon, clayification horizon, calcic horizon and parent material horizon[17]. The proportion of soil particles is 63.44% of silts, 25.39% of clays and 9.76% of sands[1)]. The climatic climax vegetation is *Quercus liaotungensis* forest. The area of plantation forest has increased in Ziwulin in recent years. The *Pinus tabulaeformis* plantation forest occupies an area of about 53 thousand hm^2, and constitutes 81% of total area of plantation forest, which is dominated by *Pinus tabulaeformis*, and the most common companion species include *Quercus liaotungensis*, *Populus davidiana*, *Ostryopsis davidiana*, *Cotoneaster acutifolia* and *Lespedeza floribunda*.

(ⅱ) Sampling. Soils were collected from 12 sampling points of the 10—33 years old *Pinus tabulaeformis* forests at shady slopes in July, 2003. At each sampling point, the organic layer was removed, and soil samples were collected in 0—10, 10—20, 20—40, 40—60 and 60—80 cm layers. A total of 60 soil samples, from 12 soil profiles, were studied. The soil samples were air-dried, passed through a 2 mm mesh, and homogenized.

(ⅲ) Organic carbon fractionation. The method follows the concepts of Golchin et al.[10] and Roscoe et al.[18], which differentiates three degrees of physical protection for OC: non-protected (free light, extractable without sonication), occluded (extractable by sonication) and protected (retained in the heavy residue, after sonication). The differentiating procedure was as follows: (1) 20 g of sample was placed in a 200 mL centrifuge tube with 100 mL of NaI solution (d = 1.8 g/mL), gently shaken by hand, and left standing at room temperature overnight. After centrifugation for 15 min, 3500 r/min, the supernatant was filtered through a membrane filter (0.45 μm) into a millipore vacuum unit. The fraction recovered on the filter was washed with 0.01 mol/L $CaCl_2$ solution 100 mL and distilled water 200 mL, and then the fraction was transferred to a pre-weighted 50 mL beaker. The sediment was resuspended in 100 mL NaI centrifuged, and filtered as described above. The obtained fractions were added to the previous ones, left standing 24 h, dried at 60℃ for about 72 h and weighted, and was taken as free light fraction, free-LF. (2) The sediment was resuspended in 100 mL NaI, shaken and sonicated using a KQ-250D tank-type ultrasonic disintegrator for 15 min, at a power release of 100 W, and left standing 4 h. The centrifugation and filtration procedure was repeated two times as described above and the fraction recovered from the supernatant was referred to as Occluded fraction, occluded-LF. (3) The sediment was resuspended in 100 mL distilled water, shaken for 20 min and centrifuged 20 min at 4000 r/min. The sediment was washed with distilled water at least 3 times, then transferred to a pre-weighted beaker, dried at 60℃ to constant weight and weighted and was taken as heavy fraction, HF. All fractions were ground in an agatha mortar, through a 60 eyes mesh, and analyzed for OC content in a VarioEL elemental analyzer.

(ⅳ) Biochemical characteristics of free-LF and HF. To quantify OM quality in the fractions, an acid hydrolysis approach of Rovira and Vallejo[13] was applied. This analysis was carried out on all the fractions except the occulded-LF, because their quantity is too small for any further analysis. The experimental process was as follows: The ground, solid samples (500 mg for the HF, 50 mg for

1) Wu, T. Y., Comparison of Characteristics and dynamics of organic carbon in the typical agricultural soils from the loess plateau of China and Canadian Prairies, Lanzhou University, Ph D thesis, 2003, 50, 116—120.

the free-LF) were hydrolyzed with 20 mL of 2.5 mol/L H_2SO_4, 30 min at 100℃ in centrifugable, sealed Pyrex tubes, in a pot of water bath. In samples having carbonates, enough time was left to destroy them by excess acid before closing the tubes and placing them in the pot of water bath. The hydrolysate was recovered by centrifugation and siphonation. The residue was washed with 20 mL distilled water; the washing was recovered by centrifugation and added to the hydrolysate. This first hydrolysate was taken as Labile Pool Ⅰ (LP Ⅰ). The unhydrolyzed residue was dried at 60℃. Then, 2 mL of 13 mol/L H_2SO_4 was added to the tube. The tube was left overnight in an end-over-end shaker. Then, water was added to dilute the acid to 1 mol/L, and a second hydrolysis was done for 3 h at 100℃. This second hydrolysate was recovered as explained above, and taken as Labile Pool Ⅱ (LP Ⅱ). The residue was transferred to a pre-weighed crucible, dried at 60℃ to constant weight, and taken as recalcitrant fraction. The recalcitrant fraction was ground and analyzed for OC using a VarioEL elemental analyzer. Then, the 'recalcitrancy index' (RI) was calculated[13]

$$RI_c = (\text{Recalcitrant C/Total OC}) \times 100.$$

The Labile Pools Ⅰ and Ⅱ were analyzed for total carbohydrates, by the phenol–sulfuric method[19,20], using glucose as standard. To obtain the carbohydrate C, we assumed that the ratio weight/C of glucose to be 2.5[13]. In this paper, total carbohydrate is carbohydrate C. To avoid interferences with released Fe^{3+} ions[21], an aliquot of the hydrolysate was previously neutralized with anhydrous Na_2CO_3, and then centrifuged to precipitate iron oxides.

(v) Statistical analysis. The differences among layers for a given fraction were compared by standard ANOVA procedures. The differences between free-LF and HF for a given layer were compared by *t*-test procedures. The relationships between the OC in the fractions and OC in the total soil were analyzed with correlation and linear regression model. All statistical analyses were conducted in SPSS (version 11.0).

2 Results

(ⅰ) Distribution of OC among fractions. Distribution of the OC among fractions in Ziwuling forest Haplic Greyxems soil was as follows: The relative distribution of OC among the fractions was similar for all horizons. Dominance of the OC decreased in the following order: HF, free-LF, occluded-LF (Table 1). In addition, the importance of free-LF abruptly decreased while that of HF increased with depths. The importance of occluded-LF has no apparent change with depths. From 0—10 to 60—80 cm horizons, the OC in free-LF abruptly decreased from 25.27% to 3.72%, while OC in HF increased from 72.57% to 95.39%. The OC in occluded-LF accounted for 0.89%—2.16% of the total OC.

(ⅱ) Accumulation of OC in the fractions. In general, as the OC of total soil in the whole horizon accumulated, the amount of OC in each fraction increased, namely there was significant positive correlation between OC in total soil and OC in fractions (Fig. 1). But for some fractions the increase was inconsistent and data dispersion resulted in a lack of significance.

Table 1 Distribution pattern of organic carbon among the obtained fractions[a)]

Depths/cm	Free-LF/%	Occluded-LF/%	HF/%
0—10	25.27(10.11)	2.16 (0.83)	72.57(10.45)
10—20	20.16 (6.28)	1.96 (0.84)	75.38 11.58)
20—40	10.44 (4.96)	2.06 (1.51)	87.50 (5.48)
40—60	6.12 (2.72)	1.42 (0.75)	92.46 (2.81)
60—80	3.72 (1.80)	0.89 (0.40)	95.39 (1.76)

a) as % of the total OC. Data are averages of 12 samples; values in parentheses are standard deviations.

At 0—10 cm horizon, the correlation between OC in free-LF or HF and total OC was very significant, and the correlation between OC in occluded-LF and total OC was significant. This suggested that OC in all fractions affect the accumulation of total OC at 0—10 cm horizon. In addition, slope of lineal fit between total OC and OC in free-LF was the highest at 0—10 cm horizon, which suggested that accumulation of OC in free-LF was also very important for accumulation of total OC at this horizon. The 10—20 cm horizon, the correlation between OC in free-LF or HF and total OC were significant and slopes of lineal fit were the same between them. All this suggested that the effect of OC in free-LF to the accumulation of total OC was the same as OC in HF at this horizon. In addition, the correlation between OC in occluded-LF and total OC was not significant ($P>0.05$). At 20—40 cm horizon, correlation between OC in free-LF, HF and total OC was significant at $P<0.05$ level and at $P<0.01$ level respectively, and that between OC in occluded-LF and total OC was not significant ($P>0.05$). The same is the trend between 40—60 cm horizon and 60—80 cm horizon. The correlation was only significant between OC in HF and total OC, and there was no correlation between OC in free-LF, OC in occluded-LF and total OC. This suggested that the accumulation of OC in HF was very important for the accumulation of total OC while OC in free-LF and occluded-LF was less important for the accumulation of total OC.

In conclusion, in Ziwuling forest Haplic Greyxems soil the OC in HF was very important for the accumulation of total OC, and the importance of HF was more remarkable in deep horizon than in surface horizon. The accumulation of free-LF was also important and was one of the main causes of any net increase in the total OC at surface horizon. But OC in free-LF became less and less important with the depths. The correlations between OC in occluded-LF and total OC was not significant in the whole horizon except 0—10 cm horizon, and that illustrated the highly random incorporation of OC into this fraction.

(ⅲ) Biochemical characteristics of the fractions.

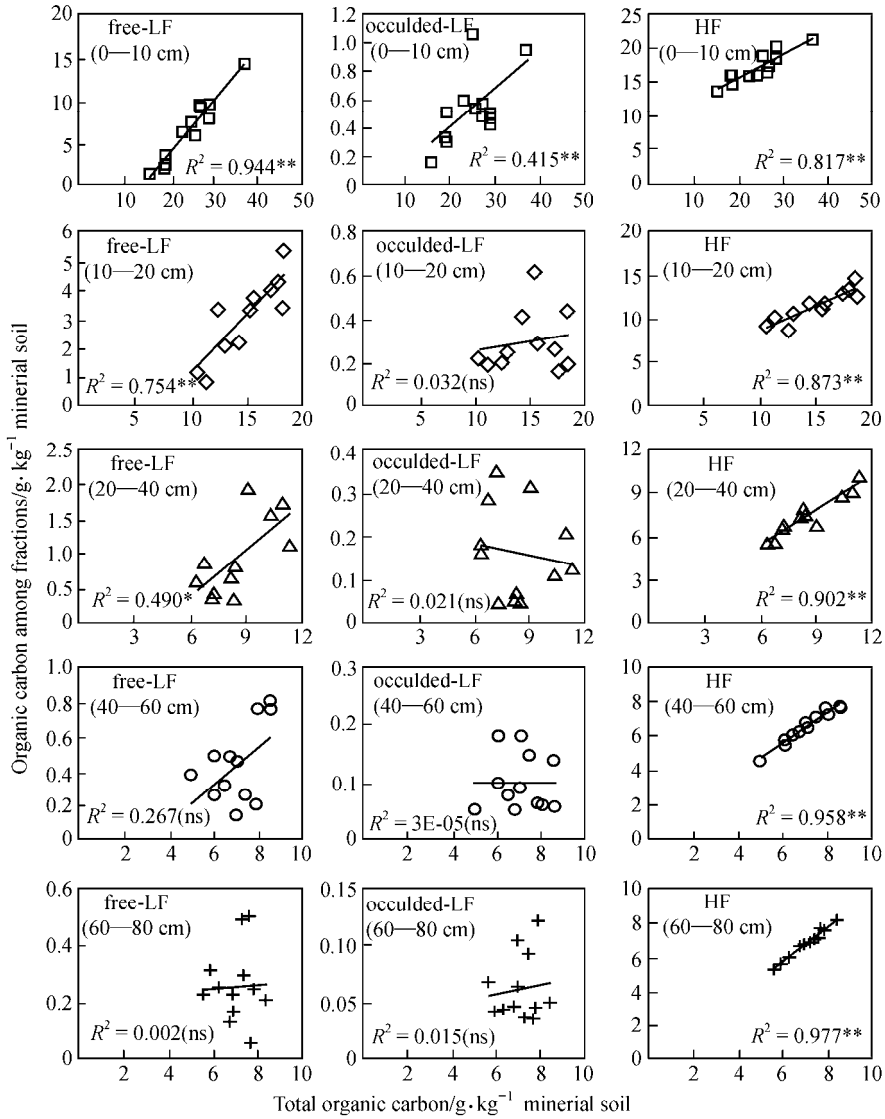

Fig. 1. Accumulation of OC in the various fractions vs. total OC in different horizon. Linear correlations are given. ns, not significant; *, significant at P=0.05; **, significant at P=0.01; n=12.

Total carbohydrate C content (mg/g) of each fraction was significantly higher in free-LF than in the HF. The distribution of total carbohydrate C content between fractions was similar for all horizons (Table 2).

As far as the ratio of carbohydrate C to total OC was concerned, total carbohydrate C accounted for 11.21%—22.75% of the total OC in the free-LF, and that accounted for 10.95%—18.07% of the total OC in the HF (Table 2). The ratio of carbohydrate C to total OC was significantly higher in free-LF than in HF in 0—10 cm horizon, and there was no significant difference between free-LF and HF in others horizons.

In short, when total carbohydrate C was considered per gram of the fraction, total carbohydrate C of free-LF was significantly higher than that of HF. When total carbohydrate C was considered per gram of fraction OC, total carbohydrate C of the free-LF and HF were similar in all horizons except 0—10 cm horizon. The difference came from difference of OC concentration between free-LF and HF.

When the percentage of recalcitrant OC in total OC in the fraction was considered (Table 3), there was no significant difference in RI_C values between free-LF and HF in 0—10 cm horizon. RI_C values were significantly higher in free-LF than in HF in 10—20, 20—40 and 60—80 cm horizons. RI_C value dispersion in HF resulted in a lack of significance in 40—60 cm horizon. RI_C values in free-LF and HF both increased with depths.

Comparison between fractions by t-test: data in the same row followed by the same letter do not differ at P<0.05; Comparison between horizon by ANOVA (LSD): data in the same column followed by the same number do

Table 2 Total carbohydrates C in the density fractions and total carbohydrate C / the total C in the fraction[a)]

Depths/cm	Total carbohydrates C in the fraction of the fraction/mg . g^{-1}		Total carbohydrates C / Total OC in the fraction %)	
	Free- LF	HF	Free- LF	HF
0—10	46.06 (5.29) a	3.51 (0.38) b	22.75 (3.59) a	18.07 (2.24) b
10—20	35.11 (4.55) a	1.83 (0.26) b	16.34 (1.89) a	14.99 (1.67) a
20—40	28.96 (5.58) a	1.07 (0.27) b	13.79 (1.06) a	14.47 (2.57) a
40—60	26.09 (2.60) a	0.92 (0.13) b	12.50 (1.57) a	13.61 (1.89) a
60—80	21.25 (3.27) a	0.76 (0.12) b	11.21 (1.51) a	10.95 (1.47) a

a) Data are averages of 12 samples; values in parentheses are standard deviations. Comparison between fractions by *t*-test: data in the same row followed by the same letter do not differ at $P<0.05$.

Table 3 RI_C value for the obtained density fractions[a)]

Depths/cm	Free- LF(%)	HF(%)
0—10	51.45(7.78) a1	45.74(4.67) a1
10—20	58.42(4.70) a1, 2	47.43(5.41) b1, 2
20—40	60.85(4.04) a2	47.01(7.64) b1, 2
40—60	60.39(2.41) a2	50.25(9.65) a1, 2
60—80	63.17(4.20) a2	52.88(7.13) b2

a) Data are averages of 12 samples; values in parentheses are standard deviations.

not differ.

We used RI_C value as an index of OM quality of the fractions in this paper. The lower the RI_C value, the higher the quality of OM. The OM quality of free-LF and HF was similar in 0—10 cm horizon. But OM quality of HF was higher than that of free-LF in the surface 10 cm below, namely the protected OM had higher quality of OM than free OM in these horizons in Ziwuling forest Haplic Greyxems soil.

3 Discussions

(ⅰ) Distribution of OC among fractions. In our study, dominance of the OC decreased in the following order: HF, free-LF, occluded-LF. The OC in free-LF and HF accounted for 25.27% and 72.57% of total OC respectively in 0—10 cm horizon while they accounted for 3.72% and 95.39% of total OC respectively in 60—80 cm horizon.

The data of Roscoe et al.[18] and Golchin et al.[10,22] were of special interest when compared with our data, since they were obtained using the same fractionation scheme. According to Roscoe, in native cerrado, the OC in free-LF decreased from 38% in 0—2 cm horizon to 1.86% in 67—110 cm horizon while OC in HF (sand+silt +clay) it increased from 61% to 98%. The OC in occluded-LF was about 1% of total OC. The percentage of OC in free-LF in this study is 12% higher than that in our study. This difference may partly be explained by difference in sampling method. They analyzed soil cores taken to a first depth of only 2 cm. In contrast, we take a surface soil core of 10 cm. So, the high dominance of free light fraction in the surface 2 cm horizon was diluted in the surface 10 cm horizon. It can also be explained by the difference in vegetation types and the climatic conditions. Both in our study and in the Roscoe' study[18] the OC in occluded-LF was the least, accounting only for 1%—2% of total OC. This suggested that light fraction OC consisted mainly of free OC. According to Golchin et al.[10,22], in forest soils the free light fraction, extractable without ultrasonic treatment, accounted for up to 31% of the total OC. It must be noted that, in most relative paper, the light fraction was recovered after dispersion (partial or total) of the structure, either by sonication or by a chemical dispersing agent. Christensen[23] summarized the proportion of light fraction at the upper 10—15 cm layer of 10 forested sites in temperate or cold temperate zone. The result showed that dry weight of light fractions accounted for 1.8%—14.7% of dry weight of total soil, and the OC in light fractions accounted for 17%—47% of total OC. In contrast to forest or plain protest, in plow layer of agriculture soil, the light fraction usually accounts for only 20% of total OC [24—26].

(ⅱ) Physical protection and biochemistry quality. In our study, as far as the rate of carbohydrate C to total OC or RI_C value was concerned, the recalcitrance of free-LF and HF was similar, even the recalcitrance of HF was higher than that of free-LF in the surface 10 cm below. This suggested that free-LF was not always the most fresh and non-decomposed fraction. Roscoe et al.[18] studied the dynamics of different physically separated SOM pools under the natural conditions and after 23 years of cultivated pasture via the replacement of the native C (C_3-derived) by pasture C (C_4-derived). The result showed that only in the first horizon the free-LF showed a higher replacement of organic C than the heavy fractions. But about 50% of the OC in the free-LF in the topsoil after 23 years was still from C_3 vegetation. Additionally, in the lower horizons, the free-LF showed replacement close to that of the HF and even lower. This suggests that not only in the first horizon higher turnover rates of light fraction are related to heavy fraction, but also recalcitrant material is present in free-LF fraction.

Higher turnover rates of light fraction in relation to heavy fraction were extensively reported[22,27], Wu[1)] concluded that native Haplic Greyxems soil lost 91% of the OC of light fraction and 60% of OC of heavy fraction after 42 years of cultivation. We considered that higher

1) See footnote 1) on page .

turnover rates of free-LF was not attributed to the chemical recalcitrance of free LF-C, but to the free-C more easily available for the microflora due to lack of physical protection, and the higher stability of HF was not the result of the recalcitrance of HF-C, but the result of the inaccessibility of HF-C.

Swanston et al.[28] measured the respiration of light fractions (LF), heavy fractions (HF) and recombined light and heavy fractions (RF) from seven forest soils in 300-day incubation experiment. They used CO_2 respiration as an index of substrate recalcitrance to study the difference in recalcitrance between LF and HF. The results showed that carbon concentration followed the pattern: LF>RF>HF, cumulative respiration that was considered per gram of substrate: LF>RF>HF. However, when expressed in per gram of initial C, the respiration of the LF was not significantly different from that of the HF. So they considered that the LF is the main source of heterotrophic soil respiration, but that contribution may be more related to the high C concentration of LF than to higher recalcitrance of the HF. The recalcitrance of HF is similar to that of LF and consequently, differences in their turnover rates may be due to microbial accessibility or physical protection. Our and Swanston's results were the same in essence though different methods were adopted. In addition, Golchin' studies[22,29] supported the viewpoint. They analyzed LF and HF from several different soil types and consistently found high level of carbohydrates and no phenolic compounds in the HF, and noted strong *O*-alkyl signals in the HF of an Oxisol. So they concluded that the HF-C was protected through complexation with clays, not by recalcitrance. They also reported that while HF-C appeared to be well protected in undisturbed soils; it rapidly declined with soil disturbance.

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