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ORIGINAL ARTICLE

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Vertical patterns of stable carbon isotope in soils and particle-size fractions of karst areas, Southwest China

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Abstract Taking limestone soil and yellow soil, the two major soil types in karst areas as examples, analyzing stable carbon isotope composition (δ^{13}C value) of soil organic matter (SOM) in bulk soils and particle-size fractions of four soil profiles under three vegetable forms, the following results are reached: in the limestone soil profile, soil organic carbon contents are all above 1.0%, the highest value is 7.1% in the surface soil; however, they are between 0.3% and 4.6% in the three yellow soil profiles. From the surface to the bottom of the soil profiles, the variation of δ^{13}C value of soil organic carbon for limestone soil profile is only between −24.1‰ and −23.0‰, however, it's between −24.8‰ and −21.1‰ for yellow soil profiles. The variation range of δ^{13}C value of soil organic carbon associated with particle-size separates is slight for limestone soil but is considerable for yellow soil. The contrast research indicates that the changes between the contents and the δ^{13}C value of soil organic carbon with depth are complex. The vertical patterns of stable carbon isotope in soil organic matter have a distinct regional characteristic in karst areas.

Keywords Karst areas · Soil organic matter · Stable carbon isotope · Particle-size fractionation · Southwest China

Introduction

Regional land rock desertification is a serious ecological problem in the subtropical karst regions of southwest China and it leads to progressive impoverishment of the local residents (Yuan 1997a). It is now recognized that rock desertification is a process of land degradation resulting from the comprehensive action due to the overprint of irrational anthropological activities on the karst fragile eco-geo-environment background (Yuan 1997b). Soil degradation is the hard core of land degradation (Raich and Potter 1995). In the process of soil degradation and global carbon cycle, as an important composition of soil and on behalf of a major carbon pool of the terrestrial carbon reserves (Lugo and Brown 1993; Raich and Potter 1995), soil organic matter (SOM) plays a key role in ecosystems. Besides the impacts of SOM on soil quality, the accelerated decomposition of SOM due to agriculture and the resulting loss of carbon to the atmosphere and its contribution to the greenhouse effect is a serious global problem.

A better understanding of how SOM affects soil quality, ecosystem functioning, and atmospheric CO_2 concentrations would be gained through knowledge of its dynamics. The stable carbon isotope composition (δ^{13}C values) and particle-size fractionations have proved to be a useful tool for studying SOM dynamics and investigating the biogeochemical processes occurring in soil (Malhi et al. 1999; Canadell et al. 2000; Schimel et al. 2001; Chen et al. 2002). Stable carbon isotope measurements have been widely used to address the origin of carbon in SOM (Balesdent et al. 1987; Gregorich et al. 2000), to document vegetation change, and to reconstruct the history of climate changes

(Cerling et al. 1989; Boutton et al. 1994, 1998). Knowledge of the natural variability of the carbon isotope composition of soils is critical for interpreting the above three kinds of studies.

The data are reported on the depth profiles of $\delta^{13}C$ in soil organic carbon (SOC) in four plots under different soil types and vegetation forms in karst areas of southwest China. The aim of this study is to characterize the vertical patterns of stable carbon isotope in soils and particle size fractions of karst areas.

Materials and methods

Study area

Sites studied are in the southwest area of Guiyang city (106°49′18″E, 26°28′19″N), Guizhou Province, China (Fig. 1), at an altitude of 1,300 m. The climate of the region is subtropical, monsoonal and humid with a mean annual temperature of 14.8°C and a mean annual precipitation of 1,100 mm. Within 1 km^2 area, four soil profiles were chosen for sampling, among which one is limestone soil, the others are yellow soil from quaternary red clay. The visible characteristics of the studied profiles are summarized in Table 1. The dominant species of YSS, YSF and LSF profiles are C_3 plants. *Miscanthus sinensis*, an herbaceous plant with C_4-photosynthesis growing on the YSM profile, its degree of coverage is about 70% and the $\delta^{13}C$ value of leaves is $-11.8‰$.

Soil collection

Soil samples were taken at the soil surface (0–10 cm depth), followed by samples from the walls of soil pits at 10 cm increments to a depth of 210 cm in profile of yellow soil in shrub (YSS), 200 cm in profile of yellow soil in meadow (YSM), 180 cm in profile of yellow soil in forest (YSF) and profile of limestone soil in forest (LSF), respectively. Soil samples were air dried at ambient conditions and ground to pass through a 2 mm sieve.

Soil samples were treated with 0.5 mol L^{-1} HCl at 25°C for 24 h to remove carbonates (Midwood and Boutton 1998), washed to neutrality with distilled water, centrifuged and dried at 60°C, then pulverized, and saved for carbon and isotopic analysis.

Soil clay content, soil pH, organic carbon and nitrogen content analyses

Soil clay content was determined by the pipette method (Conway 1978). Soil pH was measured with a glass electrode in a 1:2.5 soil to water suspension. Organic

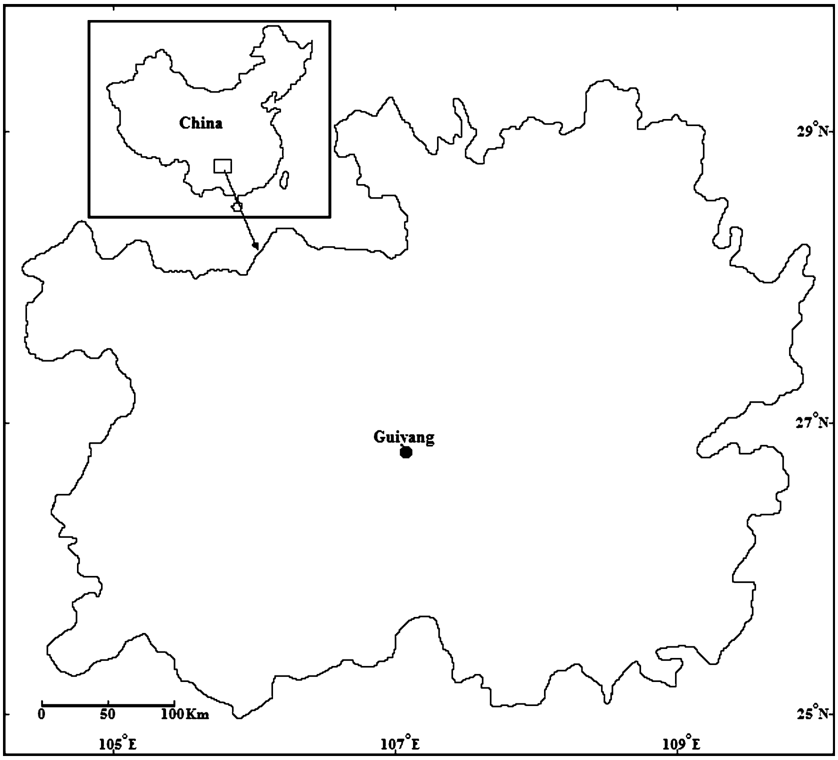

Fig. 1 Location of the study area

Table 1 Visible characteristics of the four soil profiles

Profile	Dominant species	Depth (cm)	Visible characteristics
Profile of yellow soil in shrub (YSS)	*Betula luminifera*	0–20	Grayish yellow, humus layer, granular structure, loose, porous, abundant plant roots
	Q. aliena var. acuteserrata		
	Castanea sequinii		
	Rhus chinensis	20–56	Yellow, medium amount of plant roots, tight
	C. sinensis	56–122	Yellow, no rootlet, tight
	L. davidii	122–210	Yellowish brown, merges to weathered crust below
Profile of yellow soil in meadow (YSM)	*Miscanthus sinensis*	0–38	Brown, humus layer, granular structure, loose, abundant plant debris and grass roots
	Pterdium aquilinum var. latiusculum		
	Lycopodium japonicum		
	Leontopodium leontopodioides	38–68	Yellow, block structure, tight, no rootlet
		68–103	Yellow, visible block, tight
		103–200	Orange, more tight, merges to weathered crust below
Profile of yellow soil in forest (YSF)	*Pinus massoniana*	0–8	Brown, humus layer, loose, abundant plant roots
	Betula luminifera		
	Aralia chinensis	8–30	Yellow, massive structure, more tight, less rootlets
		30–76	Yellow orange, clayey, tight
		76–101	Yellow, tight, no roots
		101–180	Yellow, tight, merges to weathered crust below
Profile of limestone soil in forest (LSF)	*Quercus acutissima*	0–26	Dark brown, humus layer, loose, abundant plant roots
	Cunninghamia lanceolata		
	Prunus davidiana	26–62	Black brown, clayey, visible plant roots
	Broussonetia papyrifera		
		62–104	Yellow brown, more clayey
		104–180	Brown, more clayey, visible blocks of carbonate rock

carbon and nitrogen content was analyzed by combustion in an Elemental Analyzer (PE2400, Perkin Elmer, USA). The precision of analysis was $\leq$ 0.1%.

Particle-size fractionation

Particle-size fractionation was done on < 2 mm material (bulk soil) according to Amelung et al. (1998). After removing visible root remnants, 30 g of soil was treated ultrasonically at 60 $\mathrm{JmL^{-1}}$ with a probe type sonicator (Branson-450, Branson, USA) in a soil:water ratio of 1:5 (w/v) to disperse microaggregates. The probe tip was placed in the center of the sample and was submerged 1.5 cm into the solution. Sand size fraction (2,000–53 μm) was isolated by wet sieving. To completely disperse the remaining material in the < 53 μm suspension, ultrasound was again applied with an energy input of 440 J $\mathrm{mL^{-1}}$ in a soil:water ratio of 1:10 (w/v). An ice bath was used during ultrasonic dispersion. Centrifugation was used to separate the silt size fraction (53–2 μm) from the clay size fraction (< 2 μm). All particle size fractions were dried at 60°C before grinding them for chemical analysis.

Stable carbon isotope analyses

For stable isotopic analyses, a sample mass yielding 0.5 mg carbon was placed in a quartz tube with CuO and Pt wire, the sample tube was then evacuated and flame-sealed. Organic carbon in the sample was oxidized to CO_2 at 850°C for 5 h. CO_2 was collected and purified cryogenically in a vacuum extraction line, with the quantity of CO_2 measured anometrically before collection into a break-seal tube for subsequent mass spectrometric analysis (Boutton et al. 1983). Stable carbon isotope ratios ($^{13}C/^{12}C$) were measured on a mass spectrometer (MAT-252, Finnigan MAT, USA) and expressed in standard δ notation (‰ = per mil) relative to the Pee Dee Belemnite standard. Analytical precision, determined as the standard deviation obtained on 35 replications of laboratory reference IAEA C_3 cellulose, is ± 0.1‰.

Results

Soil organic carbon content, C/N ratio, pH and clay content

The yellow soils are characterized by relatively low SOC contents, which are 3.3%, 4.6% and 4.4% for surface soil of the three yellow soil profiles, respectively (Fig. 2). The SOC content of limestone soil is distinctly different from that of yellow soil. Among all the soil horizons of limestone soil profile, SOC contents are all above 1.0%, with the highest value being 7.1% in the surface soil. The decreasing rate of SOC content with depth in YSS and YSF profiles is faster than that in YSM and LSF profiles below the surface soil.

C/N ratios for surface soil of YSS, YSF and LSF profiles are rather close to each other and lower than that for the surface soil of YSM profile (Fig. 3). Except for YSF profile, C/N ratios for surface soil of the other three profiles increase slightly and then decrease with the increment of depth.

Yellow soil's pH value is obviously lower than that of limestone soil (Fig. 4). The soil pH value of the three yellow soil profiles is below 5.1. Limestone soil has a high pH value ranging from 6.2 at the surface to 6.9 at the bottom.

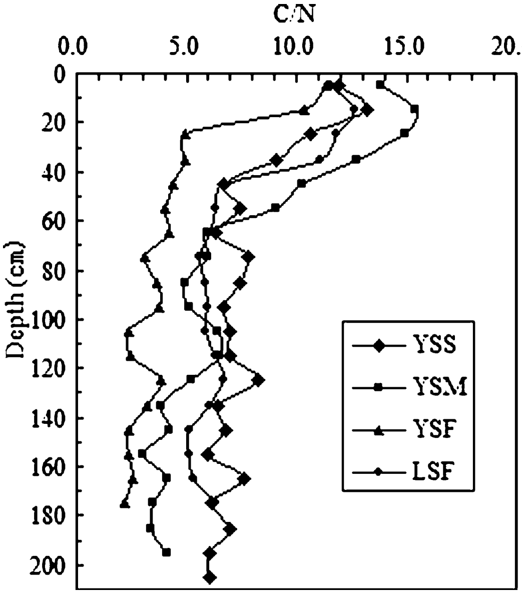

Fig. 3 Changes in soil C/N ratios with depth of the four soil profiles

The clay content is lower in YSS profile than the other three profiles, with an average value of 37.9%, and changes slightly with depth (Fig. 5). The clay content is higher in YSM and YSF profiles than in YSS profile, and changes considerably with depth. In the four profiles, the highest clay content is in the LSF profile and the average is 51.6%. Clay exhibits obvious enrichment from surface to bottom of the LSF profile.

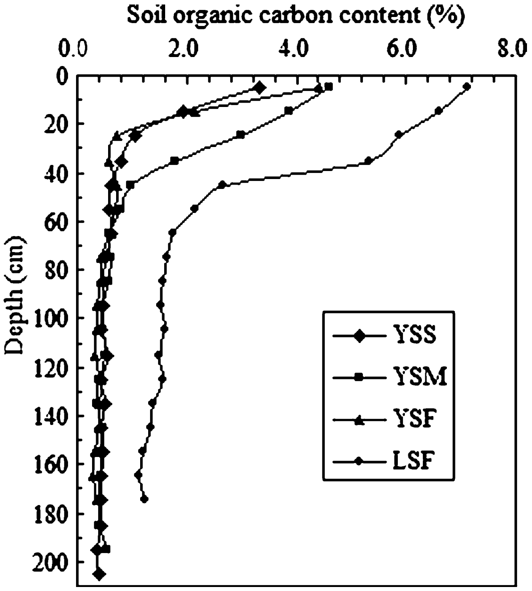

Fig. 2 Changes in soil organic carbon with depth of the four soil profiles

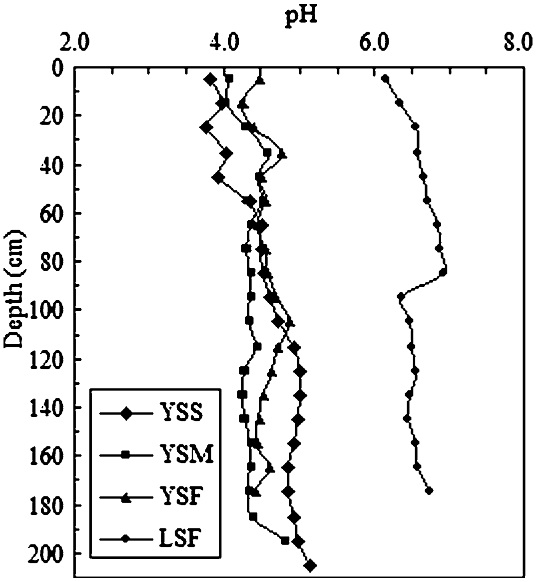

Fig. 4 Changes in soil pH with depth of the four soil profiles

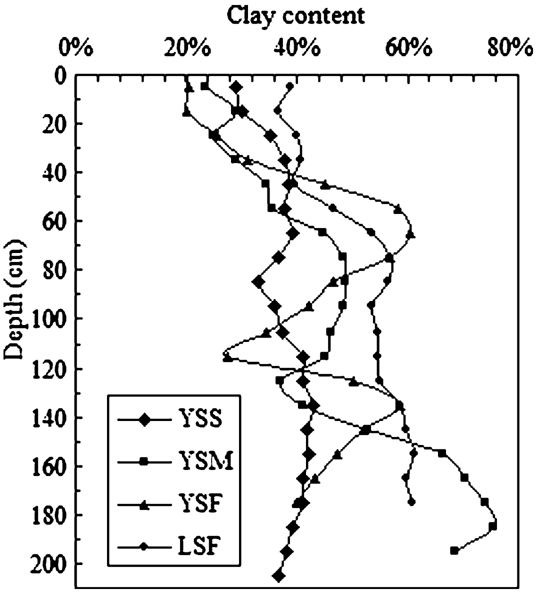

Fig. 5 Changes in soil clay content with depth of the four soil profiles

SOM δ^{13}C values analyses

The δ^{13}C values of topsoil SOM of YSS, YSF and LSF profiles are −24.4‰ (Fig. 6), −24.8‰ (Fig. 8) and −23.8‰ (Fig. 9), respectively. The growth of *Miscanthus sinensis*, a grass with C_4 photosynthesis on the YSM profile, makes the δ^{13}C values of topsoil SOM (−22.7‰) evidently higher than that of the other three profiles (Fig. 7).

The δ^{13}C value of SOM in bulk soil of the YSS profile increases from −24.4‰ at the surface to −22.5‰ at 25 cm (Fig. 6). The bulk soil is enriched in ^{13}C as compared with the coarser fraction (sand + silt), while the clay is the most enriched in the heavier isotope. The δ^{13}C value increased with decreasing particle size. The δ^{13}C value of SOM in bulk soil and associated with the clay and silt fractions continues to decrease gradually below 25 cm, but with some erratic changes of δ^{13}C value in sand fractions.

From the surface to 25 cm, the δ^{13}C values of SOM in bulk soil and all size fractions of YSM profile increases about 1.5‰, then decreases dramatically from 25 cm to 45 cm (Fig. 7). The δ^{13}C value increases with decreasing particle size above 45 cm. The δ^{13}C values of SOM in bulk soil and clay fraction show a trend to decrease below 45 cm and that associated with sand cannot be measured because the sand content is very low, below 130 cm.

The δ^{13}C values of SOM in bulk soil and all size fractions of YSF profile exhibit a rapid rise of about 3‰ from the topsoil to 45 cm, followed by a slower decrease with increasing depth except the sand fraction, which has erratic changes of δ^{13}C values (Fig. 8) .

A dramatic difference in SOM δ^{13}C value in bulk soil and all size fractions is found in LSF profile compared with the yellow soil profiles (Fig. 9). δ^{13}C values of SOM in bulk soil change only 1.1‰ and that associated with silt and clay fractions is 1.5‰ from the topsoil to the

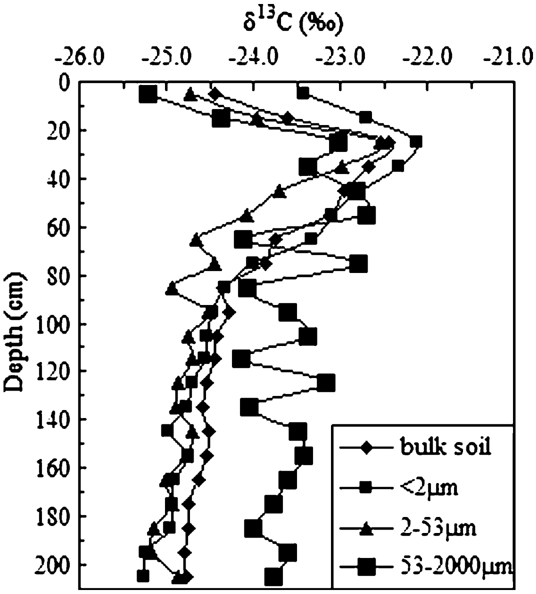

Fig. 6 Vertical pattern of stable carbon isotope in bulk soil and all size fractions of YSS profile

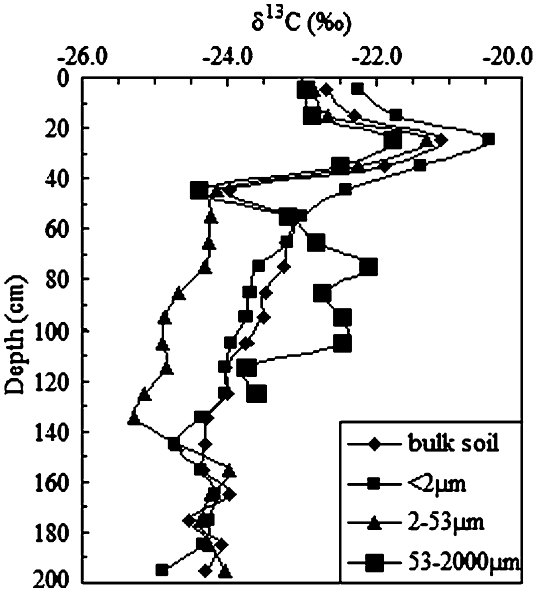

Fig. 7 Vertical pattern of stable carbon isotope in bulk soil and all size fractions of YSM profile

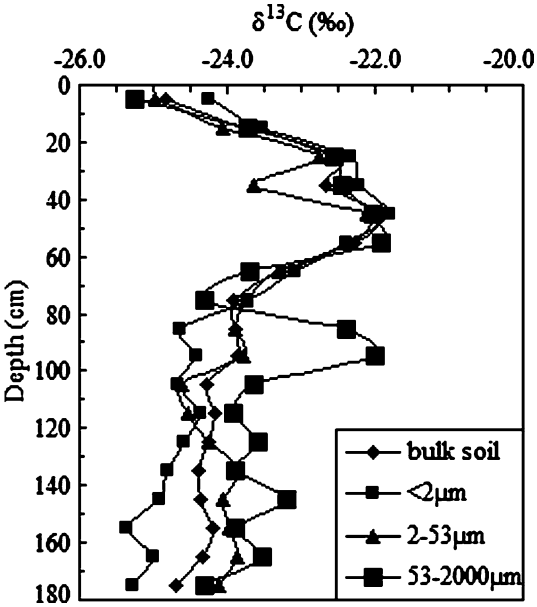

Fig. 8 Vertical pattern of stable carbon isotope in bulk soil and all size fractions of YSF profile

bottom. Compared with the bulk soil, silt and clay fractions, the change of δ^{13}C values in SOM associated with sand fraction is greater but only between −24.9‰ and −22.8‰.

Discussion

Depth distribution of SOM content

The change of SOC content with depth can be divided into three soil layers in each of the four soil profiles (Fig. 2). From the maximal value of the topsoil, SOC content first decreases exponentially with depth, then reduces slightly below certain depth, and becomes stable more or less in deep soils. But the decreasing rate and thickness of surface soil layer are different from each other in the four profiles. C/N ratios are commonly used as an indicator of decomposition of SOM and tend to decrease with increased decomposition. Figure 3 shows this regular change, but because of the difference in vegetation forms on the four profiles, C/N ratios of litter inputting into the soil is different.

The distribution of SOC with depth is closely related to the evolution of soil profiles (Chen et al. 2005). From topsoil to subsurface soil till deep soil, the source of organic matter reduces gradually with the time prolongation of soil formation, but the losses due to decomposition increase continuously. As a result, SOC content reduces with the increase of profiles depth. The SOM of topsoil has a rapid decomposition and turnover rate because there is a great deal of microbes in topsoil. SOC content decreases exponentially with depth from the maximal value of the topsoil. The amount of microbe decreases continuously and the turnover rates of SOM reduce with increase in depth, so SOC content decreases slowly with depth. Despite the low SOC content at deep depths, the SOM comprises mainly stable components and SOC content becomes stable.

The content of SOC is the balance between plant material input and losses of organic carbon caused mainly by heterotrophic decomposition (Li and Zhao 2001). Under the subtropical monsoonal humid climate, the high temperature and abundant rainfall environment is propitious to the vegetation's fast growth, so there is considerable plant debris input annually into the soil. In the condition of calcium-abundant limestone soil, the activity of microorganism, such as bacteria and actinomycetes, is extraordinarily active, so as to make organic matter decompose continuously and form humus. Humus combined with calcium ion produces polymeric and stable humus calcium that makes the limestone soil acquire humus calcium accumulation (Office of General Survey on Soil 1998). This is one of the important reasons why the SOC of LSF profile maintains higher content than other profiles. In the yellow soil with pH value below 5.1, more calcium and magnesium leaching loss from soil and weak calcium enrichment by organism lead to an evidently lower SOM content than limestone soil.

There is a positive correlation between SOC content and soil clay content (Ladd et al. 1996; Bird et al. 2003). This kind of phenomenon is distinct in deep soil, which

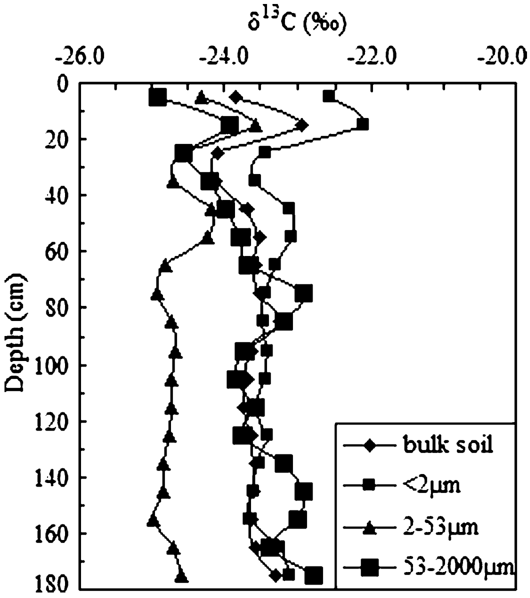

Fig. 9 Vertical pattern of stable carbon isotope in bulk soil and all size fractions of LSF profile

may be due to the relative proportion of slow and passive SOC pools increasing gradually with depth. LSF and YSM profiles have higher clay content and clearly display clay enrichment from topsoil to the bottom (Fig. 5). Clay minerals have a high specific surface area and carry a charge, enabling them to bind, and thereby chemically stabilize, organic matter. Clay aggregates also provide micropores for the physical protection of SOM (Wattel-Koekkoek et al. 2001). These two kinds of protection effects may be another important reason that not only makes the LSF profile soil maintain a high content of SOC but also makes the decreasing rate of SOC contents with depth slow down from the surface of the LSF and YSM profiles.

For the same climate, there is a direct correlation between SOC content and aboveground vegetation form, which is a significant determinant of the vertical distribution of SOC (Jobbagy and Jackson 2000). Plant debris and root exudation is a major source of SOM under normal native vegetation. For the same yellow soil type, SOC content of topsoil is higher in YSM profile and decreases gradually from YSM to YSF and YSS in order (Fig. 2). It may be because *Miscanthus sinensis* is the dominant species and the high biomass production (especially roots) of it is responsible for maintaining higher SOC contents in the uppermost, 45 cm of YSM profile (Table 1). The net primary productivity of forest vegetation is usually higher than shrub, so the organic matter input into the soil reduces from YSM to YSF and YSS in order. On the other hand, the aboveground vegetation form affects soil texture. The topsoil of YSM profile has high SOC content and the soil texture is loose so that SOM is easy to transfer downward. As a result, the above layer with high SOC content of YSM profile is thicker than that of the other two profiles.

Depth distribution of SOM stable carbon isotope composition

Under the same yellow soil type, the topsoil SOM δ^{13}C value of YSM profile is distinctly different from that of YSS and YSF profiles because of the diversity of vegetation forms. The aboveground vegetation litter is a major source of soil SOM and there exists a direct correlation between δ^{13}C value of litter and the vegetation forms. So the C_3 plants and C_4 plants with distinct carbon isotope ratios, which have been growing on the profiles, become the major factor deciding the difference of δ^{13}C value of SOM. The growth of *Miscanthus sinensis*, a grass with C_4 photosynthesis on the YSM profile, makes the δ^{13}C values of topsoil SOM evidently higher than that of the other three profiles and the increase range of SOM δ^{13}C value less than the YSS and YSF profiles from topsoil to 25 cm.

Compared with the research results of other regions, SOM δ^{13}C values do not increase constantly with depth but instead reach a maximal value at a certain depth, then reduce gradually, and tend to remain almost stable. From topsoil to certain depth (25 cm for YSS, YSM and YSF profiles, 15 cm for LSF profile), the δ^{13}C values of SOM increase by 1–3‰ with depth. Many authors have discussed the possible factors leading to the observed enrichment of ^{13}C with depth (Balesdent and Mariotti 1996; Boutton et al. 1998; Ehleringer et al. 2000). (1) Influence of atmospheric change—the δ^{13}C values of atmospheric CO_2 have been decreasing by 1.3‰ since the dawn of the Industrial Revolution because of the combustion of ^{13}C-depleted fossil fuels. Compared with the newly-formed SOM of topsoil, SOM in these deeper soil layers that have originated at a time when the δ^{13}C value of atmospheric CO_2 were more positive has higher δ^{13}C value. (2) Microbial fractionation during litter decomposition—in the process of metabolic reactions associated with litter decomposition and SOM oxidation, microorganism preferentially use the carbon sources of depleted ^{13}C, and then the residual SOM should become progressively more positive in its δ^{13}C values. (3) Microbial and fungal residues become SOM and the δ^{13}C values of microbes and fungi should be higher than their food source as a result of carboxylation reactions. This is because whenever a carboxylation reaction is involved in catabolism, that CO_2 molecule is likely to have originated from the soil atmosphere, which is ^{13}C heavier than the ^{13}C content of the organic materials being decomposed. (4) Belowground biomass (roots) is enriched in ^{13}C compared with aboveground biomass (leaves). Below 25 cm, δ^{13}C values of SOM in yellow soil decrease with depth and this change is usually ascribed to the effects of microbial degradation. Microbial population preferentially metabolizes nutrient and energy-rich high-^{13}C compounds such as polysaccharides, leaving the remaining SOM enriched in low-^{13}C and recalcitrant compounds such as lignin (Benner et al. 1987).

A greater difference of SOM δ^{13}C value is found in LSF profile compared with the yellow soil profiles. The variation range of the δ^{13}C value of SOM is considerable from topsoil to the base of yellow soil profiles—2.3‰ in YSS profile (Fig. 6), 3.4‰ in YSM profile (Fig. 7) and 2.9‰ in YSF profile (Fig. 8), but it is only 1.1‰ in LSF profile (Fig. 9). The difference in variation range of SOM δ^{13}C values between the yellow soil and limestone soil may be attributed to three factors. (1) The pH value of yellow soil is distinctly lower than that of limestone soil. There exists a direct correlation between soil pH and the content of different components in SOM. Low pH promotes hydrolytic reactions in soil and acid hydrolysis is known to remove proteins, nucleic acids and polysaccharides (Smith 1987; Ostle et al. 1999; Zang et al. 2000), all of which are enriched in ^{13}C. Guggenberger et al. (1994, 1995) also observed a significant lignin contribution in soils with a $pH < 5$, whereas soils with $pH > 5$ showed little evidence of lignin. (2) More calcium and magnesium elements have leached out from yellow soil; however, their contents are abundant in limestone soil. Humus originated from the quick decomposition of topsoil organic matter combines with calcium ion and forms over-polymerized stable humus calcium then accumulates gradually in LSF profile. Therefore, the SOM turnover rate decreases and results in small carbon isotope fractionation below topsoil. (3) The LSF profile has high clay content and clearly displays clay enrichment from topsoil to the bottom. Clay content is a major factor influencing the change of SOM $\delta^{13}C$ with depth in a profile (Bird et al. 2003). On the one hand, the chemical and/or physical protection brought by clay minerals reduces the decomposition rates of SOM and results in small carbon isotope fractionation. At the same time, high clay content in soil reduces oxygen supply for the deep soil and most aerobe decomposing SOM exist only in topsoil, so the corresponding depth with the maximal value of $\delta^{13}C$ is distinctly thin in LSF profile.

The $\delta^{13}C$ value of SOM associated with various size fractions increase with decreasing particle size from topsoil to 25 cm, the highest being observed in SOM bound to the clay. The humification degree of SOM associated with various size fractions is different and increases gradually with decreasing particle size, which could involve (1) the preferential loss of ^{12}C during decomposition of organic carbon; (2) the increasing contents of ^{13}C enriched organic components such as microbial tissues, which are less available for microbial digestion (Roscoe et al. 2000; Gerzabek et al. 2001); and (3) the decreasing contents of ^{13}C depleted organic substrates such as lignin in the finer size separates (Benner et al. 1987). Below 25 cm, the changes of $\delta^{13}C$ value of SOM associated with silt and clay is similar to that of the bulk soil, but it shows erratic changes in $\delta^{13}C$ value in sand fractions. The organic matter associated with sand fraction is called particulate organic matter (POM). POM is thought to represent partly decomposed plant material at an early stage of decomposition and isotope fractionation due to decomposition can be assumed to be small. The source plant of SOM determines POM $\delta^{13}C$ value but the carbon isotope composition of litter and roots input to soil are different, such as below-ground biomass (roots) is enriched in ^{13}C compared with above-ground biomass (leaves). This may explain the erratic changes in $\delta^{13}C$ value of SOM associated with sand fractions.

Conclusions

The $\delta^{13}C$ value of SOM in bulk soils and particle size fractions of four soil profiles under three vegetation forms in karst areas of southwest China is measured. There exists a distinct difference in vertical patterns of the stable carbon isotope composition of SOM between yellow soil and limestone soil. It is speculated that this difference reflects in site-specific factors, such as soil type, vegetation forms, soil pH values, and clay content, etc., which control contents of different organic components comprising SOM and soil carbon turnover rates in the profiles. These findings have implications for studies of soil carbon dynamics that employ stable isotopic tracers. Compared with the research results of other regions, the vertical patterns of stable carbon isotope composition in soils and particle-size fractions have a distinct regional characteristic in karst areas. An in-depth study on those will contribute to the perfect of biogeochemistry cycle module of terrestrial ecosystem SOM.

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