Dissolved organic carbon and its carbon isotope compositions in hill slope soils of the karst area of southwest China: Implications for carbon dynamics in limestone soil

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Dissolved organic carbon (DOC) can be considered fine indicators of soil quality that influence soil function in specific ways and are very sensitive to changes in soil management practices. In this study, the contents and isotopic compositions of dissolved organic carbon ($\delta^{13}C_{DOC}$) in soils were analyzed at different topographic positions on two typical slopes in a karst area to evaluate the source and fate of DOC in the soil from these regions. The results show that DOC contents of the two slopes were mainly affected by soil organic matter (SOM) input, topography, and soil quality. DOC contents at each topographic location on the two slopes decrease with increasing soil depth. The $\delta^{13}C_{DOC}$ of the shrubby slope surface soils varied from -15.1% to -22.1%, and are consistent with SOM $\delta^{13}C$ and local vegetation. The $\delta^{13}C_{DOC}$ of the grassy slope surface soils ranged from -20.0% to -20.9%, similar to isotopic compositions of soil organic carbon ($\delta^{13}C_{SOC}$), but are inconsistent with present-day vegetation. The $\delta^{13}C_{DOC}$ varied significantly with depth at each topographic location. Thus, the dependence of $\delta^{13}C_{DOC}$ in soil profiles on depth reflects the migration and transformation of DOC in soils.

Keywords: karst, slope land, soil organic carbon, dissolved organic carbon, δ^{13} C

INTRODUCTION

Peak-cluster depressions are representative of landscapes in karst regions, and are mainly distributed in southwest China. Karst peak-cluster depression regions generally comprise steep hill slopes and relatively flat depressions. This landscape is vulnerable to several ecological and environmental problems, including a fragile natural environment, slow soil formation, thin and unevenly distributed soil layers, sensitivity to human activities, especially changes in type of land use, and rocky desertification (Liu et al., 2006; Zhao et al., 2006). Irrational land use and development have led to forest degradation and serious soil erosion in this region, resulting in the reduction of soil quality and the degradation of the ecological environment (Wang et al., 2003; Li et al., 2004). In particular, numerous hill slopes have been severely eroded, yielding large amounts of exposed bedrock (Hu et al., 2004; Wang et al., 2003; Xu et al., 2003).

Dissolved organic carbon (DOC) in terrestrial ecosystems has been highlighted because of its ecological function in nutrient and element cycling (Michalzik *et al.*, 2001; Smith et al., 1998). DOC is operationally defined as the organic matter fraction in solution that passes a 0.45 μ m filter. It comprises only a small part of soil organic matter, but it affects many processes in soil and water, including the most serious environmental problems such as soil and water pollution, as well as global warming (Kalbitz and Kaiser, 2003; Bolan et al., 2004). Many studies have shown that DOC is a crucial environmental indicator for research on the carbon cycle and the environment (Don and Kalbitz, 2005; McDowell et al., 2006). During the last 30 years, land use and land cover changes have markedly increased DOC concentrations in UK rivers (Yallop and Clutterbuck, 2009). In short, determining the retention and turnover of DOC at global and regional scales is useful for characterizing and quantifying the C storage capacity of soils.

Stable carbon isotopes are a powerful tool for assessing the origin and dynamics of carbon in soils. Terrestrial plants can be divided into C3, C4, and CAM plants according to their photosynthetic pathways. C3 plants have δ^{13} C values ranging from -20% to -35% (mean *ca.* -27%), while C4 plants have δ^{13} C values ranging between -9% and -17% (mean *ca.* -13%). CAM plants use both C3 and C4 photosynthetic pathways, having δ^{13} C values between those of C3 and C4 plants (O'Leary, 1988; Farquhar *et al.*, 1989). Soil organic matter (SOM) has a

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stable carbon isotopic composition comparable to that of the source plant material, and changes in the proportion of C3 and C4 plants are bound to change the δ^{13} C value of SOM. Therefore, δ^{13} C values of SOM have been utilized to document the effects of land use practices on vegetation change (Ehleringer *et al.*, 2000; Dzurec *et al.*, 1985), and to quantify the soil organic matter turnover rates (Gregorich *et al.*, 1995; Hernandez-Ramirez *et al.*, 2011).

In this study, surface and profile soil samples were collected along two hill slopes with different land-use histories and vegetative covers in Huanjiang County, located in the central karst region of southwest China. The concentrations of DOC and the compositional characteristics of $\delta^{13}C_{DOC}$ in soils were studied in order to identify the sources and to characterize DOC in these soils, and eventually to clarify the carbon turnover in the soil of this karst region.

METHODOLOGY

Site description

The study site, which is located in Rencai Village, on the border of southern Huanjiang County, Guangxi Autonomous Region (Fig. 1), is a representative karst peakcluster depression. This area is a typical subtropical karst peak-cluster valley, and the altitudes range from 272.0 to 647.2 m. This area belongs to the middle subtropical monsoon humid climate, whose characteristics include simultaneously hot and humid seasons and long frost-free periods. The annual average temperatures range from 16.5°C to 19.9°C (Zhang et al., 2006). The annual average rainfall is 1389 mm, but the rainfall is unevenly distributed, with much of it concentrated in the rainy season from April to September, and little rain in the dry season from October to March. The soils in this area are mainly developed from limestone parent materials. Soil cover is very unevenly distributed, and the thicknesses of soil layers range from 10 to 160 cm. Bedrocks, especially those scattered in the summit, are widely exposed because of destruction by humans. In addition, the vegetation is seriously degraded, and the natural vegetation consists mainly of shrubs, herbs, and lianas.

Two typical slopes were selected in the study site, one of which was a shrubby slope. The shrubby slope, which had been destroyed by human activity and closed for over 20 years, was mainly covered by vegetation that included herbs and secondary shrubs. Except for the bedrocks at the top-slope, the slope is covered in soil. With the vegetation succession, the species gradually changed from herbs to a mix of shrubs and herbs. A grassy slope, which had been abandoned for approximately five years, was also selected. The vegetation on the grassy slope consisted mainly of herbs, and also included a small amount of *Vitex*



Fig. 1. Sampling locations in the study area.

negundo L. and ferns. Bedrocks beyond the shoulder-slope had already been exposed.

Soil sampling and analysis

In May 2007, surface soil samples of the two slopes were collected at a spatial interval of 20 m, from top to bottom. Soil profiles were dug at the shoulder-slope (data was missing for the grassy slope), back-slope, mid-slope, and foot-slope. Samples were collected at 5 cm intervals within the top 30 cm of each profile, and at 10 cm intervals below 30 cm depth. Litters and fresh leaves from the dominant plant species on the slopes were also collected (Fig. 2).

After soil sampling, visible stones and residues of plants and animals were removed. Soil pH values were determined in a 1:2.5 mix of soil and water, and soil water contents were determined by the drying method (Amelung et al., 1998). Some of the soil samples were air-dried, and soil particle contents were analyzed by a combined straw method of sieving and hydrostatic settlement. Meanwhile, the ground and sieved (0.15 mm sieve) air-dried soil samples were soaked in a 0.5 mol/L HCl solution for 24 h to remove the carbonates in soil, which were then washed to neutral with deionized water, dried at 60°C, and then ground. Soil organic carbon (SOC) contents were determined by a PE2400 elemental analyzer. Fresh soils were extracted with 0.5 mol/L K₂SO₄, oscillated in mixed water and soil at 1:4 for 1 h at room temperature, and then filtered through a 0.45 μ m filter membrane (Jones and Willett, 2006). A portion of the resulting products were utilized for the measurement of soil DOC contents using a 1030W Total Organic Carbon Analyzer (U.S. OI) (Bolan et al., 1996; Lu, 1999).



Fig. 2. Distances of landscape positions and locations of sampling points on the two slopes.



Fig. 3. Soil profile variations of DOC at different topographic positions. (a) The shrubby slope. (b) The grassy slope.

Meanwhile, fresh plant leaves were washed with deionized water, and dried and crushed at 65°C. Litters were dried and crushed at 65°C. Air-dried soil samples were ground and sieved and excessive 0.1 mol/L HCl was added to the samples to remove the carbonates after reacting for 24 h. The products were then washed to neutral and dried at 50°C. The extracted soil DOC obtained above was acidified to pH 2.0 with phosphoric acid, then freeze-dried and crushed. The δ^{13} C values of all the samples were then measured using a Continuous-Flow Isotope Ratio Mass Spectrometer (CF-IRMS) (Fry *et al.*, 1996; Lang *et al.*, 2007; Wang *et al.*, 2002) at the State Key Laboratory of Environmental Geochemistry in

Guiyang, China. Each sample was measured at least twice, and the data are reported as δ^{13} C values in reference to the V-PDB (Vienna Pee Dee Belemnite) standard. The analytical errors were less than $\pm 0.2\%$.

RESULTS AND DISCUSSION

DOC variations along soil depth and the hill slope

The content of soil DOC is a sensitive indicator of soil environment variations and plays an important role in the terrestrial ecosystem carbon cycling. Distributions of soil DOC contents in different topographic profiles of the shrubby slope were complicated. As shown in Fig. 3,

Land use type	No.*	pН	Water content in soil (%)	Clay (%)	SOC $(g \cdot kg^{-1})$	DOC (mg·kg ⁻¹)	$\delta^{13}C_{SOC}$ (%)	$\delta^{13}C_{DOC}$ (%)
Shrubby slope	1	7.12	14.5	6.3	152.45	150.55	-14.3	-15.1
	2	7.26	14.6	13.4	149.77	166.52	-16.2	-16.9
	3	7.43	16.9	24.7	132.68	183.0	-21	-20.6
	4	7.65	17.8	23.5	103.05	205.53	-21.7	-20.9
	5	7.62	19.0	29.7	79.6	201.18	-21.9	-22.1
	6	7.73	19.1	33.2	62.82	221.09	-20.1	-22.1
Grassy slope	1	6.71	15.9	36.9	51.72	134.6	-20.8	-20.3
	2	6.54	16.3	39.8	56.67	145.51	-20.7	-20.2
	3	6.65	19.1	36.6	51.83	174.5	-20.0	-20.3
	4	6.52	18.7	39.5	43.98	177.32	-21.4	-21.0
	5	6.23	21.3	46.1	73.64	234.51	-21.1	-21.5

Table 1. Surface soil (0–10 cm) properties of different slopes

*Soils were numbered along the descending slopes.

DOC distributions in the shoulder-slope and back-slope profiles differed from those in the downslope soil profiles. With increasing soil depth, DOC contents increased and then decreased, and those in the downslope mid-slope and foot-slope profiles decreased. The relatively new upslope soil profiles of the shrubby slope were formed mainly by the deposition of weathered rocks and plant residues. The resulting sands in the two soil profiles were subject to eluviation of DOC in the surface soil owing to the lack of protection by clays. Accordingly, the eluviated DOC was reabsorbed by the subsurface soil. Meanwhile, plant residues were further decomposed when ventilation was good. As a result, DOC contents in the subsurface soil increased. In contrast, soils in the two downslope profiles had been deposited for a longer time, and the resulting higher contents of clays prevent the fresh organic matter from permeating into the soil below the profiles. Therefore, DOC contents in the entire soil profile decreased with increasing soil depth. These results are consistent with previous reports in which the retaining time and effectiveness of SOC were verified to be prolonged and lowered with increasing soil depth (Ghidey and Alberts, 1993; Kalbitz, 2001; Kalbitz and Geyer, 2002). DOC contents in the soil profiles along the grassy slope all decreased with increasing soil depth, and the average DOC contents in the three profiles increase in the following order: back-slope (115.7 mg·kg⁻¹) < mid-slope $(148.2 \text{ mg}\cdot\text{kg}^{-1}) < \text{foot-slope} (195.7 \text{ mg}\cdot\text{kg}^{-1})$. All of the contents were lower than those in the corresponding profiles of the shrubby slope, which may be attributed to the reduced activity of SOC and less newly supplemented organic matter in the profiles due to the shorter abandonment time and soil compaction on the grassy slope.

Relationship between SOC and DOC

As shown in Table 1, the contents of SOC and DOC

in the surface soil of the shrubby slope were both higher than those of the grassy slope. The average contents of SOC and DOC of the shrubby slope were 113.4 $g \cdot kg^{-1}$ and 187.9 mg·kg⁻¹, and those of the grassy slope were 55.6 $g \cdot kg^{-1}$ and 173.3 $mg \cdot kg^{-1}$, respectively. The SOC contents of the two slopes apparently differed, whereas their DOC contents were similar. The results may be attributed to less human interference on the shrubby slope than on the grassy slope. The shrubby slope was covered with more vegetation that provided more fresh organic matter for the surface soil. Less organic matter was deposited on the grassy slope due to the shorter abandonment time, resulting in a significantly lower SOC than the shrubby slope. Although soil DOC was originated primarily from plant residues, it has been previously reported that DOC is mainly derived from dissolved organic matter and its decomposition by microorganisms other than the recent litter layers (McDowell, 1988). Thus, the supplement of DOC in the surface soil of the grassy slope derived from fresh organic matter was limited. Table 1 also shows that the contents of DOC and total organic carbon in the surface soil of the two slopes both increased and then decreased along the descending slope. Dependence of DOC/SOC on soil depth in the soil profiles of different slopes at different topographic positions is depicted in Fig. 4. Under various types of land use, different SOC components, formed by different plant litters, root exudates and microbial activities, apparently affected the DOC/SOC values. As shown in Fig. 4, minimal DOC/ SOC values are observed in the upslope soil profiles of the two slopes, and DOC/SOC values in the upslope soil profiles were all lower than those in the downslope ones. As discussed above, these results can primarily be attributed to more deposited organic matter in these profiles, as the main source of soil DOC is the eluviation and decomposition of SOM other than the recent litter layers.



Fig. 4. Dependence of DOC/SOC on soil depth. (a) The shrubby slope. (b) The grassy slope.

In addition, DOC/SOC values in the upslope soil profiles (20 cm) of the two slopes were lower than 0.2%. Accordingly, decomposition of SOM in these profiles was extremely low, which resulted in the SOM storage characteristics, including higher SOC content and lower DOC content. In contrast to previous results (Yu and Li, 2003), DOC content did not increase with increasing SOC content, which may be ascribed to the following reasons: (1) Upslope soil was newly deposited, which would lead to the insufficient decomposition of organic matter. (2) Clay content in the surface soil gradually increased in the downslope direction. The low content of clay in the upslope soil could not effectively retain DOC, leading to the easy eluviation of soil DOC. Conteh et al. (1998) compared the organic matter characteristics of five pairs of cultivated and uncultivated soils, and showed that the contents of active organic matter increased with decreasing aggregate particle sizes. Mo et al. (2006) reported that DOC of forest soil was 25% and 48% higher than those of grass and farmland soils, respectively, in Maolan, Guizhou.

Natural abundance of ¹³C in DOC and dominant plant species, litter layers, and surface soil

Fractionation of ¹³C differs in plants with different photosynthesis pathways (C3, C4). Accordingly, C3 and C4 plants have significantly different δ^{13} C values. Therefore, the δ^{13} C values of SOM can be used to measure the relative importance of C3 and C4 plants in the community. Considering that organic matter in the surface soil was originated from the litter layers of surface vegetation, δ^{13} C values of the SOM are mainly determined by the proportion of C3 and C4 plants growing on the surface soil with various carbon isotope ratios (Bernoux *et al.*, 1998; Boutton *et al.*, 1998).

Along the shrubby slope, there was a gradual change in vegetation from herbs at the shoulder-slope to mixed shrubs and herbs at the foot of the slope. The vegetation of the grassy slope mainly consisted of herbs, which were mixed with ferns, *Vitex negundo* L., etc. The δ^{13} C values of herbs, litter layers, ferns and *Vitex negundo* L. were determined to be -11.3% to -13.9%, -26.7% to -28.9%, -28.1%, and -28.5%, respectively.

Soil DOC is originated from both intrinsic and extraneous carbon sources. The latter includes humified organic matter, plant litters, root exudates, microbial biomass, and human interferences such as the application of organic fertilizers. Although the sources and variations of DOC in different soils and environmental conditions are still controversial, DOC has primarily been associated with the input of organic matter in soil (Delprata *et al.*, 1997). Kalbitz *et al.* (2000) reported that DOC mainly stemmed from the humus in recent plant litters and SOM. Thus, the sources and migration characteristics of DOC in soil were clarified by comparing the distribution of $\delta^{13}C_{DOC}$ in soil, as well as the $\delta^{13}C$ values of plant residues and SOM according to isotope tracing theory.

Surface soil $\delta^{13}C_{DOC}$ values of the shrubby slope fluctuated obviously from -15.1% to -22.1%. Table 1 shows



Fig. 5. $\delta^{13}C_{DOC}$ variations in the soil profiles at different topographic positions. (a) The shrubby slope. (b) The grassy slope.

that $\delta^{13}C_{DOC}$ of the surface soil (0–10 cm) gradually decreased in the downslope direction, which resembled the changing trend of $\delta^{13}C_{SOC}$. Meanwhile, the large variations of $\delta^{13}C_{DOC}$ were also closely related to the surface vegetation of the shrubby slope. Upslope vegetation of the shrubby slope mainly consisted of the C4 herbs, whereas the C3 shrubs gradually increased in the downslope vegetation. However, surface soil $\delta^{13}C_{DOC}$ values of the grassy slope ranged from -20.2% to -20.9‰, which are apparently smaller than those of the shrubby slope. The variations of $\delta^{13}C_{DOC}$ and $\delta^{13}C_{SOC}$ values were quite similar, and the differences between them were smaller than those of the shrubby slope. Moreover, the vegetation of the grassy slope was comprised mostly of herbs, with δ^{13} C values of -11.3% to -13.9%, much higher than the $\delta^{13}C_{DOC}$ values in the soil. In summary, variations of the surface soil $\delta^{13}C_{DOC}$ were similar to those of soil $\delta^{13}C_{SOC}$ in the two slopes, indicating that surface soil DOC of the two slopes were mainly derived from SOC. However, surface soil DOC of the shrubby slope was originated from the newly supplemented organic matter, whereas that of the grassy slope was originated from the original SOM.

Changes in the natural abundance of ¹³C in soil profiles

Carbon input by eluviation and deeply decomposed organic matter mainly contributes to the organic carbon in profile soils. It has been reported that δ^{13} C analysis is potentially valuable for exploring the dynamic migration of soil carbon. For instance, Del Galdo *et al.* (2003) found

that the δ^{13} C values of organic carbon in the soil profiles of forests or grass generally increased with increasing soil depth, and attributed this mainly to the isotope fractionation during litter decomposition and humification instead of the physical and chemical properties of soil. These results have also been ascribed to the tendency of microbes to utilize compounds containing ¹²C rather than ¹³C (Balesdent et al., 1993). The surface SOM is basically composed of newly supplemented unstable organic carbon. The particle sizes of SOM decrease with increasing soil depth, which enhances the decomposition procedure, leading to increasing carbon isotopes of soil organic matter ($\delta^{13}C_{SOC}$) towards higher ¹³C enriched values with depth. This is the consequence of the initial near-surface cycling of easily biodegradable material $({}^{12}C - {}^{12}C$ chemical bond) and the resulting progressive enrichment (¹²C $-^{13}$ C chemical bond) of less easily degradable to recalcitrant plant compounds with depth (Ehleringer et al., 2000; Guo et al., 2013). In addition, vegetation succession may also result in the variations of organic carbon δ^{13} C in the soil profiles. Therefore, δ^{13} C values of the organic matter in soil profiles are able to effectively record the changes of C3 and C4 vegetation systems in the ecosystem. Liu et al. (2002) effectively traced the content changes of SOM during the transformation of agriculture and forest ecosystem by employing the variations of organic carbon δ^{13} C in soil.

In this study, $\delta^{13}C_{DOC}$ values had different change trends at each topographic position of the shrubby slope. As shown in Fig. 5, at the shoulder-slope and back-slope

sites, $\delta^{13}C_{DOC}$ in the profile soils increased with increasing soil depth and both reached maxima at the bottom. The values ranging between -16.9%~-11.4% and -16.9%~-13.1%, respectively. This is thought to indicate the apparent enrichment processes of δ^{13} C, as well as active decomposition of soil organic matter. This was due mainly to two newly deposited profiles, which comprised plant residues and rock weathering products, and lack of clays. Thus, the ventilation and drainage of soil was favorable for continuous decomposition of SOM. However, soil $\delta^{13}C_{DOC}$ in the profiles at mid-slope and foot-slope increased and then decreased with increasing soil depth, with the maxima at about 20 cm. The changes ranged from 2.4% to 4.1%, and were stable before further decreasing at depths of 30 cm and 40 cm in the soil, respectively. In other words, organic matter in the top 20 cm of soil in the two profiles was intensively decomposed. Meanwhile, it has been demonstrated above that DOC content and δ^{13} C in the same soil layer also increased. Nevertheless, soil density and clay content in the soil below 20 cm increased, which effectively preserved and fixed the SOM (Jia et al., 2006), limiting the decomposition rate of organic matter to a certain extent. The increase of soil $\delta^{13} \mathrm{C}_{\mathrm{DOC}}$ at the bottom of the profile could probably be attributed to vegetation succession, which has also been reported previously (Boutton et al., 1998; Zhu and Liu, 2006).

Similarly, $\delta^{13}C_{DOC}$ in the top soil of each profile of the grassy slope all increased to various extents, but the increases were smaller than those of the corresponding topographic profiles of the shrubby slope. For instance, $\delta^{13}C_{DOC}$ values in the back-slope and mid-slope profiles only ranged within 0.8‰ and 1‰. Meanwhile, $\delta^{13}C_{DOC}$ values of the grassy slope rapidly increased compared to those of the shrubby slope after the input of fresh organic matter, demonstrating that DOC in the two profiles of the grassy slope was mainly derived from the original organic matter. In contrast, $\delta^{13}C_{DOC}$ in the top soil of the footslope profile ranged within 2.8‰, larger than those of the two profiles discussed above, and may be ascribed to the accumulation of SOM scoured from the upslope.

The profile distribution of soil $\delta^{13}C_{DOC}$ value in each profile of the two slopes helps to clarify the characteristics of DOC transport and transformation in soil. For the shrubby slope, better recovered vegetation provided more fresh organic matter for the soil profiles, which enriched $\delta^{13}C_{DOC}$ in soil during decomposition. Hence, soil $\delta^{13}C_{DOC}$ fluctuation was more evident at a certain depth. In contrast, past tillage practice led to soil hardening and less surface biomass in the grassy slope. As a result, less plant residue was absorbed by the surface soil, and could barely penetrate the profile soil layers, which also decreased the variations of soil $\delta^{13}C_{DOC}$.

CONCLUSIONS

Soil DOC is the most active organic carbon component in the vulnerable karst ecological region. Comparisons of the two differently recovered slopes show that the distribution and variation of DOC contents were subject to the influence of organic matter input, slope topography, and soil quality. Soil DOC contents of the shrubby slope were all higher than those of the grassy slope at each corresponding position, but DOC and SOC contents in the surface soil were all elevated along the descending slopes. DOC contents in the soil of major topographic profiles decreased with increasing soil depth, whereas those in the shoulder-slope and back-slope profiles of the shrubby slope increased and then decreased due to the more severe erosion and higher clay contents.

The $\delta^{13}C_{DOC}$ values in soil were closely associated with the type, quantity, and decomposition extent of the input SOM. Surface soil $\delta^{13}C_{DOC}$ variation ranges of the shrubby slope were higher than those of the grassy slope, which were closely related to the changes of $\delta^{13}C_{SOC}$ and vegetation. However, $\delta^{13}C_{DOC}$ values of the grassy slope, which are similar to $\delta^{13}C_{SOC}$ values, were not obviously associated with the vegetation conditions. Although increases in $\delta^{13}C_{DOC}$ were observed in the top soil of each profile, the $\delta^{13}C_{DOC}$ -depth variations differed significantly among the soil profiles examined, and the fluctuations of each profile of the shrubby slope were apparently higher than those of the corresponding profile of the grassy slope.

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