

# Combined Use of Carbon and Nitrogen Isotopes for Assessment of Soil Organic Matter Sources and Decomposition in a Typical Karst Area of Yunnan–Guizhou Plateau, Southwestern China

Howard Omar Beckford

USTB <https://orcid.org/0000-0002-9296-3044>

Changshun SONG

Chinese Academy of Sciences State Key Laboratory of Environmental Geochemistry

Cheng CHANG

University of Science and Technology Beijing

Hongbing JI (✉ [ji.hongbing@hotmail.com](mailto:ji.hongbing@hotmail.com))

University of Science and Technology Beijing

---

## Research Article

**Keywords:** Carbon and Nitrogen Isotopes, Soil Organic Matter, Karst

**Posted Date:** February 23rd, 2021

**DOI:** <https://doi.org/10.21203/rs.3.rs-225396/v1>

**License:** © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

---

# Abstract

Soil organic matter (SOM) has substantial influence on geochemical cycle, soil stability and global climate change, however total organic carbon sequestration mechanisms in karst soil remain poorly understood. For this study we assess, total organic content (TOC), total nitrogen (TN), C/N ratio and isotopes of carbon and nitrogen in four soil profiles over critical karst area to investigate organic matter source, mechanisms that influence fractionation and factors affecting SOM in Yunnan–Guizhou Plateau, Southwestern China. The results revealed that SOM comprised of mixed sources derived from both exogenous and endogenous materials. The soil profiles indicate intense vertical variation in  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  with an increase in both isotopes in the upper layers, decreased in  $\delta^{13}\text{C}$  below 20 cm and irregular fluctuation in  $\delta^{15}\text{N}$  with depth. Mechanisms such as mineralization and selective preservation influence isotopic fractionation in the upper soil surface, while translocation, nitrification and denitrification dominated the subsoil layers. Variation in TOC, TN and stable carbon and nitrogen isotopes were influence by vegetation cover, topography, soil water and external contribution. Moreover, the decrease in TOC and TN with depth were due to downward translocation of dissolved organic carbon and nitrogen caused by monsoon climate. Our results revealed that combination of TOC, TN, C/N,  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  can be used as proxy to decipher SOM source, external influence and stability of karst soils. Furthermore, the intense change in  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  throughout the soil profiles suggest that this karst soil is unstable which have implications for land management and carbon sequestration.

## 1. Introduction

In recent years, it has been recognized that sequestration of carbon and nitrogen dynamics in soil has impacted climate change (Durán et al. 2017; Laganière et al. 2010; Qiu et al. 2017). Soil is widely known to be a major reservoir of organic carbon and nitrogen sequestrations, which plays an integral role in reducing  $\text{CO}_2$  in the atmosphere, hence regulating the Earth's carbon cycle and climate system (Reichstein et al. 2013). In addition, with increase in global population greater demand on soil for food security and human development will increase. Therefore, it is important to fully understand the mechanisms and processes affecting carbon and nitrogen, especially within karst environment as these ecosystems are most vulnerable to human threat and environmental change. Carbon and nitrogen are stable isotopes that occur naturally in the environment, and are often used by researchers to determine origin of soil organic matter (Peterson and Fry 1987) as well as processes that govern cycling (Balesdent and Mariotti 1996; Ehleringer et al. 2000; Gill et al. 1999; Poage and Feng 2004). The isotopic compositions in soils surface are inherited from present vegetation cover, usually  $\delta^{13}\text{C}$  values for higher terrestrial C3 and C4 plant matter are considered to between -23‰ to -30‰ and -9‰ to -17‰ respectively (Boutton 1991). However, the fractionation by soil microorganisms during decomposition of soil organic matter (SOM) can complicates the contribution of C3 or C4 plants, and thus disturbs the accurate interpretation of SOM sources and estimation of its turnover (Krull and Skjemstad, 2003). Therefore, greater understanding of the  $^{13}\text{C}$  fractionation in SOM decomposition is important to accurately interpret source. Biological processes are the major cause of variations in carbon and nitrogen isotope, which often recorded the

largest isotopic fractionation. Most biological processes showed discrimination in isotopes, with preferential incorporation of the lighter isotopes (Menyailo et al. 2003), and leaving behind heavier isotopes in the substrate.

Basically, stable isotope ( $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ ) techniques and C/N ratios are robust tools that have been commonly used to determine sources, mixing and transformational activities in terrestrial, estuarine and coastal systems (Cai et al. 2015; Ramaswamy et al. 2008; Zhang et al. 2007). Carbon isotopes are widely used to explore the origins and the migratory principles of materials across several ecological environments, while nitrogen isotopes are extensively used in biological tracking. Therefore, both  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  isotopic composition along with C/N ratio can be used together to accurately determine the origin of organic matter (Cai et al. 2015; Middelburg and Herman 2007), processes and mechanisms in SOM decomposition. Since the magnitude and the direction of changes in SOM decomposition are still unclear in ecological fragile karst area, our study aims to use  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  along with C/N ratio to (1) investigate the source of soil organic matter, (2) identify the mechanisms that influence C and N isotopic fractionation and (3) determine the factors affecting SOM decomposition over typical karst area of Yunnan-Guizhou Plateau, southwestern China.

## 2. Materials And Methods

### 2.1 Study area

The study area is located in Shilin County, Yunnan Province ( $24^{\circ}30' - 25^{\circ}03'\text{N}$ ,  $103^{\circ}10' - 104^{\circ}40'\text{E}$ ) (Fig. 1a). The terrain is comprised of highlands in the east and low mountains in the west. The area experienced a typical subtropical climate with warm-humid conditions from May to October and cold-dry conditions from November to April. The original monsoonal climate divides the region into two distinct wet and dry seasons. The annual average temperature is  $16.3^{\circ}\text{C}$ , with relatively large temperature difference between day and night (Yuan 1992). The annual average precipitation approximately ranged between 800–850 mm. Shilin County is located in the karst area of the eastern part of the country. The typical characteristic feature of the landform is karst Mountains, with few exposed limestone rocks and deposit of thin soil. The soil in the area is susceptible to erosion with sparsely stunt vegetation cover as means of protection and are characterized as red soil and limestone soil (Can et al. 2008; Huang 2009; Liu 2015). The dominant lithology in the area are mainly Limestone of Permian period, Limestone and Dolomite of Middle-Lower Cambrian and Slate, with Limestone of Sinian period (Fig 1b).

### 2.2 Soil profile and sampling

The study sites were selected through indoor research and field surveys, according to soil type, soil affected by rocky desertification and human interference. Four distinct soil profiles were chosen, namely: Shilin Scenic Area 1 (SL1), Shilin Scenic Area 2 (SL2), Xinacun (XNC) and Dapo (DP) (Fig. 1c).

Shilin Scenic Area 1 (SL1) soil profile is located in the undeveloped section of Shilin near the Stone Forest scenic area at an elevation of 1770 m, with GPS coordinates of  $24^{\circ}48'\text{N}$ ;  $103^{\circ}18'\text{E}$  (Fig. 1b). The sampling

point is located at the top of the hill, with a relatively flat terrain and the immediate surrounding strata is bare. However, the surrounding vegetation cover is high and mainly consists of shrubs and herbaceous plants. Fresh soil samples were collected at an interval of 5 cm from top to bottom of the profile, numbered and labeled SL1-1 to SL1-10, respectively to a maximum depth of 50 cm (Fig. 1c). The dominant bedrock was Limestone of Permian period and sample was collected (SL1-0) at a depth of 100 cm. A total of 10 soil samples with soil density of 16.91 g/cm<sup>3</sup> were collected.

Shilin Scenic Area 2 (SL2) soil profile is located in the same general area below SL1 at altitude 1730 m, 24°48'N; 103°18'E (Fig. 1b). These soil profiles are naturally formed conical ridge on upland landscapes. The SL2 sampling point is located on the hillside steep slope. The surrounding area is comprised of bare rocks with relatively low vegetation cover consisting of predominantly shrubs. In this section the soil profile is naturally developed and without any visible sign of human disturbance. Fresh soil samples were collected at an interval of 12 cm from top to bottom of the profile. The samples numbered and labeled from SL2-1 to SL2-10, respectively to a maximum depth of 113 cm (Fig. 1c). Limestone bedrock sample was also collected (SL2-0) at 163 cm. A total of 10 soil samples with density of 17.10 g/cm<sup>3</sup> were collected.

The Xinaacun (XNC) soil profile is located at an elevation of 1720 m, with GPS coordinates of 24°49'6"N; 103°18'37"E (Fig. 1b), on a mountain slope in close distance to a road construction site. This sample point was a man-made vertical soil profile established by an excavator. The upper surface of this profile comprised of weeds, shrubs and tall trees with loose soil containing plant roots and black humus. No bedrock was present in this excavated soil profile, however the underlying bedrock was found in close proximity and identified as Limestone and Dolomite of Middle-Lower Cambrian. Fresh soil samples were collected starting at intervals of 10, 20, 40 to 50 cm from top to bottom of the profile, numbered and labeled XNC-1 to XNC-11, respectively to a maximum depth of 245 cm (Fig. 1c). A total of 11 soil samples were collected with a density of 19.30 g/cm<sup>3</sup>.

The Dapo (DP) soil profile is located at an elevation of 1920 m, with GPS coordinates of 24°50'21"N; 103°23'26"E (Fig. 1b) in the lower gully region of the upland landscapes. The area comprised of thatched grass, with few shrubs and a small number of coniferous trees. The underlying bedrock of this profile is Slate, Phyllite, Siltstone intercalated with Limestone of Sinian period. Fresh soil samples were collected at an interval of 10 cm from top to bottom of the profile, numbered and labeled DP-1 to DP-10, respectively to a maximum depth of 115 cm (Fig. 1c). Shale bedrock sample (DP-0) was also collected at 145 cm. A total of 10 soil samples, with density of 20.70 g/cm<sup>3</sup> were collected.

Soil organic matter content in the soil profile usually changed significantly from the soil surface to a depth of approximately 30 cm, and then slightly varied at greater depths below 30 cm (Han et al. 2017). Each studied soil profile has different depth vary in thickness ranging from 50-250 cm (Fig. 1c). In order to captured and represent these change soil sampling was done according to the genetic horizons of each profile; however, comparison was made over equidistant. Hence, soil samples were collected at varying intervals (5-20 cm), however all the soil profiles were chosen with a thickness of 50 cm and over

so as to compare the changes of TOC and TN contents and their stable isotope compositions with depth (Fig. 1c).

### 2.3 Soil preparation and analysis

All soil samples collected were naturally air dried at room temperature and impurities such as plant roots, gravel, and other debris were removed and soil then stored in a plastic bag. Soil samples were later ground to 200 mesh size and packaged in zip lock bags to determine Total organic carbon (TOC), Total nitrogen (TN), C/N ratio, nitrogen and carbon isotopes ( $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$ ).

The pH was determined by using the potentiometric method (Wang et al. 2007) whereby a small portion of air-dried soil sample that were pass through a 1 mm sieve placed in a liquid to soil ratio of 2.5:1 (Hash HQ40D) and measured using a pH meter. The powder soil sample was pretreated with 1mol/L HCl, and the carbonate minerals were removed (Midwood and Boutton 1998). The inorganic N were removed using 2 mol L<sup>-1</sup> potassium chloride (KCl) for 24 hours (Meng et al. 2005). The samples were washed with deionized water until the supernatant liquid pH value was neutral and then later dried at 60 °C. Approximately 100 mg of sieved dried soil sample was used for analysis of TOC, TN and stable isotopes contents. The TOC and TN contents were calibrated due to loss of carbonate and inorganic N respectively. The TOC and TN contents were determined by using an elemental analyzer (Elementar, Vario TOC cube, Germany) with a precision of C  $\pm$  0.1% and N  $\pm$  0.02%, monitored with standard samples. The stable carbon isotope ratio (<sup>13</sup>C/<sup>12</sup>C) and stable nitrogen isotope ratio (<sup>15</sup>N/<sup>14</sup>N) were determined by using the gas isotope ratio mass spectrometer (MAT-252, Germany). This testing was done by the State Key Laboratory of Environmental Geochemistry, Institute of Geochemistry. The measurements were normalized according to international standards material ( $\delta^{13}\text{C}_{\text{VPDB}}$ : 45.6‰  $\pm$  0.08‰;  $\delta^{15}\text{N}_{\text{Air}}$ : 0.24‰  $\pm$  0.13‰), and expressed as delta value ( $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ ) notation (‰) relative to Vienna Pee Dee Belemnite (VPDB) and atmospheric air, respectively, whereby:  $\delta^{13}\text{C}$  (‰) =  $[(^{13}\text{C}/^{12}\text{C})_{\text{sample}} - (^{13}\text{C}/^{12}\text{C})_{\text{standard}}] / (^{13}\text{C}/^{12}\text{C})_{\text{standard}} \times 1000$ ‰ and  $\delta^{15}\text{N}$  (‰) =  $[(^{15}\text{N}/^{14}\text{N})_{\text{sample}} - (^{15}\text{N}/^{14}\text{N})_{\text{standard}}] / (^{15}\text{N}/^{14}\text{N})_{\text{standard}} \times 1000$ ‰. Reproducibility was determined by replicate measurements that was better than 0.1‰ and 0.2‰ for  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  respectively.

## 3. Results

Table 1 illustrates depth distribution of pH, soil density, total organic carbon, total nitrogen, C/N ratios, and isotopic composition ( $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ ) of the soil profiles in Yunnan Plateau, Southwestern China.

### 3.1 Vertical distribution of soil pH, soil density, TOC, TN and C/N in soil profiles

As illustrated in figure 2 the pH values of the studied soil profiles vary from 4.05 to 6.09, which are slightly acidic in nature, however DP soil profile tend to be more acidic with average pH value of 4.23. There were slight fluctuations in pH values with increasing soil depth, with the upper soil surface being more acidic than rest of the profile except for SL1 and DP soil profiles (Fig. 2).

The soil density ranges from 1.13 g cm<sup>-3</sup> to 2.30 g cm<sup>-3</sup> with DP and SL2 recording the lowest and highest soil density respectively. Soil density varied with depth for the studied soil profiles with an overall general increase with depth (Fig. 2 and Table 2).

The TOC content within the studied soil profiles showed high variation with soil depth with a general decrease from upper soil surface to lower layers down to bedrock (Fig. 2 and Table 2).

Total Nitrogen (TN) tends to vary with soil depth for the studied soil profiles. Generally, there seem to be a decrease in TN with increasing soil depth with the upper soil surface having a higher TN value than the other lower soil profiles layer except for SL1 and XNC (Fig. 2 and Table 2). SL1 soil profile is significantly higher in TN content in comparison to the other profiles. The surrounding high vegetation cover which mainly consists of shrubs and herbaceous plants as well as the rearing of animals may account for the relatively high TN content in SL1 soil profile.

The studied soil profiles C/N values have high degree of variability, with values ranging from 1.5 (XNC) to 13.4 (SL1) with an average of  $6.60 \pm 4.02$ . The general trend indicates a gradual reduction in C/N values from the topsoil to the bottom of the soil profile layer (Fig. 3 and Table 2). There is a significant difference in the upper topsoil C/N ratio of all studied soil profiles (>10), which may indicate that plant residues or animals input contributes to the relatively high C/N value in the upper soil surface. There are also slight differences, which may be caused by variation in hydrothermal conditions, soil formation characteristics and soil morphology in different section of the profiles (Wang et al. 2012; Zhu 2006). The degree of human disturbance or interference may also influence the migration and transformation of nitrogen, resulting in C/N variation at different layers within the soil profiles.

### **3.2 The clay proportion and depth distribution of the soil profiles**

Table 1 also shows the clay proportion depth distributions of the four studied soil profiles. All soil samples at various depths were dominated by silt and sand (average 82.37%), while clay fractions accounted for only 7.90 to 36.63% in an increasing order of XNC, SL2, SL1 and DP. The highest clay content generally recorded in the mid-section of the profiles, while the lowest value recorded in the upper surface except for XNC and DP soil profiles.

### **3.3 Isotopic composition**

The  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  isotopic compositions of the four studied soil profiles from Yunnan Plateau, Southwestern China are presented in table 1 and figure 2. The overall  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values ranged from -15.64‰ to -25.82‰ and from 2.77‰ to 14.36‰ respectively and varied with soil depth. The general trend indicates that  $\delta^{13}\text{C}$  values increased with depth for the initial 0-20 cm for the soil profiles except for DP soil profile and the lower section of the profiles which indicate a decrease in  $\delta^{13}\text{C}$  values with depth (Fig. 2a, b, c and d) respectively. While  $\delta^{15}\text{N}$  values varied rapidly with depth with no obvious trend (Fig. 2). Additionally,  $\delta^{14}\text{C}$  radioisotope was used to determine the age of the soil profiles which ranged from

500 to 890 before present (BP), and 90 to 6537 BP for SL1 and SL2 respectively, no age determination data was available for XNC and DP soil profiles.

The  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values of organic matter in the SL1 soil profile ranges from -15.98‰ to -16.87‰ with an average of  $-16.40\text{‰} \pm 0.31\text{‰}$ ; and the  $\delta^{15}\text{N}$  value ranges from 10.43‰ to 14.36‰ with an average of  $11.89\text{‰} \pm 1.58\text{‰}$  (10 samples) respectively. The  $\delta^{13}\text{C}$  values of organic matter in the SL2 soil profile ranges from -18.10‰ to -21.69‰ with an average of  $-19.86\text{‰} \pm 1.27\text{‰}$  (10 samples); and the  $\delta^{15}\text{N}$  value ranges from 5.67‰ to 9.67‰ with an average of  $7.76\text{‰} \pm 1.47\text{‰}$  (10 samples). The  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values of organic matter in the XNC soil profile ranges from -15.64‰ to -25.82‰ with an average of  $-19.84\text{‰} \pm 2.98\text{‰}$  (11 samples); the  $\delta^{15}\text{N}$  value ranges from 2.77‰ to 13.22‰ with an average of  $6.37\text{‰} \pm 2.64\text{‰}$  (11 samples) (Fig. 2a, b and c) respectively. While the  $\delta^{13}\text{C}$  values of organic matter in the DP soil profile ranges from -21.18‰ to -24.75‰ with an average of  $-23.35\text{‰} \pm 1.09\text{‰}$  (12 samples); and the  $\delta^{15}\text{N}$  value ranges from 2.98‰ to 7.49‰ with an average of  $5.16\text{‰} \pm 1.56\text{‰}$  (12 samples). The  $\delta^{13}\text{C}$  values decreased (become more depleted) with increasing depth while  $\delta^{15}\text{N}$  values fluctuated rapidly with no regular pattern (Fig. 2d).

## 4. Discussion

### 4.1 Factors affecting TOC and TN vertical distribution during SOM decomposition

Land use significantly influenced the levels of the TOC and TN contents in soils, more so in the first 0 – 100 cm layer (Tuo et al. 2018) as well as soil bulk density (Chukwudi et al. 2018). Several studies reported that TOC and TN contents were much higher in cropland than in native forestlands (Li et al. 2010; Wang et al. 2010; Zhang et al. 2014), conversely however Tuo et al. (2018) and Liu et al. (2019) found that forestlands lands tend to have higher TOC and TN contents than other land use such as cropland, grassland and shrubland. Our studied soil profiles were located in forested area over karst which explain the relatively high TOC and TN contents in upper soil surface and the low carbon at depth. The decrease in TOC content below 20 cm depth for all studied soil profiles (Table 1; Fig. 2), were due to the relatively small amount of nutrients that are able to infiltrate into deeper soil. Similar decrease in TOC with depth pattern was reported by Liu et al. (2019) for different land use over karst area. However, for undisturbed natural forests, usually this decrease trend in TOC is largely determined by organic decomposition processes that result in mostly recalcitrant soil carbon at depth (Campbell et al. 2009; Paul et al. 1997). Similarly, other studies reported that the decrease in TOC with depth was due to the fact that few residues and biomass were introduced in deep soil layers (Jobbágy and Jackson 2000; Wiesmeier et al. 2012), and that the proportion of carbon matter in the fraction cycle decreased at deeper layers (Trumbore 2000). In addition, soil density and clay content seem to influence TOC and TN concentration in this karst soil as both indicate negative correlation for soil profiles SL2, XNC and DP, while positive correlation for SL1 soil profile (Fig. 2, 4 and 5). This negative correlation relationship has been exhibited in other studies (Njeru et al., 2017). This was due to the suppression of possibly the process of mineralization and nitrification in soils with high soil density values (De Neve and Hofman,

2000). Furthermore, soil with low density has the ability to store greater amount of TOC and TN content, as they can be mobilized through porous spaces within the soil profiles.

The climate in particular rainfall may also affect TOC and TN vertical distribution. In this study, carbon loss is mostly affected by the monsoonal climatic condition as the seasonal high and low precipitation impacted on the vertical translocation of DOC and anaerobic oxidation especially during the dry period e.g., as indicated by the sharp change in  $\delta^{13}\text{C}$  profile for all sites except for DP profile (Fig. 2). The observed pattern of  $\delta^{13}\text{C}$  for the soil profiles can be explained by the decomposition of TOC which directly affects isotopic composition by kinetic fractionation and preferential substrate decomposition (Guillaume et al. 2015). For all the studied soil profiles except for DP, the  $\delta^{13}\text{C}$  which is the heavier isotopes became enriched with increasing depth to approximately 20 cm. This clearly indicates kinetic fractionation whereby soil microorganisms during SOM decomposition preferentially removed the lighter  $\delta^{12}\text{C}$  isotopes leaving behind substrate that is isotopically heavy or enriched. On the other hand, at greater depth below 20 cm and for DP profile, the decrease in  $\delta^{13}\text{C}$  was due to the translocation of DOC from surface to deeper depth Kalbitz et al. 2005. The subsoil horizons with low carbon contents, may have DOM readily adsorbed to mineral surfaces, resulting in the reduction of  $\delta^{13}\text{C}$  in soil (Kalbitz et al. 2000; Kalbitz et al. 2005). Monsoonal climatic condition influence both decomposition and translocation processes which explain the carbon gain in the upper surface and carbon loss in subsoil. Zhu and Liu (2006) found similar result where  $\delta^{13}\text{C}$  declined with depth in karst soils.

Topography and vegetation also affect TOC and TN distribution within the karst soil profiles. The SL1 profile is the uppermost profile with an elevation of 1770 m, which explain having the lowest surface TOC content as a result of limited vegetation cover and its susceptibility to erosion and leaching. Xiong et al. (2018) demonstrated that this studied karst area is severely being affected by physical erosion and chemical weathering. Conversely, the SL2 upper surface soil has significantly higher TOC content ( $2.11 \text{ g kg}^{-1}$ ) than the other soil profiles (Fig. 2b). This is primarily due to its location being below SL1 at an elevation of 1730 m and so benefited from erosion and deposition of SOM from SL1. The other profiles surface soil TOC content relatively high ( $0.9 \text{ g kg}^{-1}$ ) and reflect the input of freshly decomposed matter from surrounding vegetation. The soil depth distribution trend of total organic carbon content is consistent with some other studies (Li et al. 2009; Liu et al. 2019; Wang et al. 2016; Zhu and Liu 2006) and is closely linked to the evolution of the soil profile (Chen et al. 2005) as well as the amount of activity from soil microbes (Zhu and Liu 2006). Both the TOC and TN concentrations are influenced by the erosion of carbon rich soil from upper surface layer (Don et al. 2011; Lal 2001) and by the subsequent degree of decomposition, depositional processes, delivery routes and amount of preservation (Tani et al. 2002). Guillaume et al. (2015) have indicated that the processes of erosion and decomposition can potentially contribute to the decrease of C content and stocks in soil. While erosion affect the concentration of TOC and TN, decomposition tends to affect the isotopic composition.

The C/N ratio in soil and litter may be an indicator of the rate of decomposition (Zhu and Liu 2006). For example, the lower the C/N value the higher the decomposition rate (Jafari et al. 2011). In comparison to

other studies for e.g., Liu et al. (2019) found C/N values range from 8.22 to 10.52, while our study C/N ratio were >10 for all the upper surface soils, except for SL1 profile (Table 2; Fig. 3). These findings indicate that the upper soil surface for the studied soil profiles, have a low rate of decomposition and low accumulation. The C/N ratio reflects carbon input, therefore the relatively high C/N values in the upper surface indicate the high C content in the surrounding shrubs and herbs vegetation (Fig. 3). Furthermore, the results also indicate that SL1 average C/N values fall in the range of cropland (disturbed) i.e., >10, while the other soil profiles average C/N values reflect typical undisturbed native land (<10) according to Zhang et al. 2014. The soil C/N ratio showed strong positive correlation with TOC ( $r^2 = 0.772, 0.822, 0.929$  and  $0.931$ ) and TN contents ( $r^2 = 0.694, 0.782$ ) for SL1 and SL2, however weak correlation for XNC and DP soil profiles respectively (Fig. 5a & b). This was directly due to influence of vegetation cover and water availability in the soil.

Stable carbon and nitrogen isotopes showed vertical variation in soils for this karst area with distinct characteristics. Factors such as land use, climate, topography and vegetation cover influenced TOC and TN distribution during SOM decomposition in this karst area.

#### 4.2 Source of Soil Organic Matter (SOM)

Soil is a direct sink for organic carbon and nitrogen with their stable isotopic signatures registered at any depth reflecting the dominant vegetation type. Higher terrestrial C3 plants have distinctly different  $\delta^{13}\text{C}$  value range from C4 plants as well as soil organic matter (Fig. 6). Many researchers used  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  isotopes along with C/N ratio to better identify the origins of soil organic matter (Cai et al. 2015). In using  $\delta^{13}\text{C}$  values, higher terrestrial C3 and C4 plant matter are considered to between  $-23\text{‰}$  to  $-30\text{‰}$  and  $-9\text{‰}$  to  $-17\text{‰}$  respectively (Boutton 1991), while that of soil organic matter ranges between  $-22\text{‰}$  to  $-25\text{‰}$  (Goni et al. 2003). Most non-fixing nitrogen plants have  $\delta^{15}\text{N}$  values in the range of about  $+5\text{‰} \pm 2\text{‰}$ , although plants with  $\delta^{15}\text{N}$  values less than  $-10\text{‰}$  or greater than  $+10\text{‰}$  are not unusual (Kendall et al. 2001) (Fig. 6). Our results indicated vertical variation of  $\delta^{13}\text{C}$  for each of the studied soil profiles (Table 1 and Fig. 2). The  $\delta^{13}\text{C}$  values for these studied soil profiles ranges between  $-15.64\text{‰}$  to  $-25.82\text{‰}$  with an average of  $-20.03\text{‰} \pm 3.02$  which lies outside the range of both C3 plants ( $-26.5$  to  $-28.0\text{‰}$ ), and C4 plants ( $-12.5$  to  $-14.0\text{‰}$ ) according to O'leary (1988) and Tieszen (1994) respectively (Fig. 6). However, some values conversely fall in the range of C3 plants based on Boutton 1991 ( $-23$  to  $-30\text{‰}$ ) and outside the C4 plants range ( $-9$  to  $-17\text{‰}$ ). Some of these studied profiles for e.g., XNC and DP have larger range of  $\delta^{13}\text{C}$  values which fall in the C3 plant and soil organic matter range according to Goni et al. 2003 and Thorp et al. 1998 (Fig. 6). The inconsistency in isotopic signature range was as a result of mixing of both C3 and C4 plants as well as the fractionation cause by influence of soil microbes. In the case of SL1 the soil organic matter is strongly influenced by other source (e.g., animal residues) especially since the  $\delta^{13}\text{C}$  values are relatively enriched (Fig. 6). The low value of  $\delta^{13}\text{C}$  in the upper surface of soil profiles implies that soil organic carbon mainly derived from decomposition of fresh plant matter, especially since surface horizons of soils reflect the C3 to C4 ratios of the present vegetation (Kelly et al. 1991). The  $\delta^{13}\text{C}$  values for soil profiles SL2, XNC and DP are closer to the C3 plant range while SL1 profile is significantly

different but closer to C4 plants (Fig. 6). The SL1 surrounding vegetation consist of shrubs and herbaceous plants which contributed to the  $\delta^{13}\text{C}$  values. The immediate surrounding vegetation of the other soil profiles comprised of shrubs, thatched grass, and coniferous trees that account for the high variation in isotopic signatures. Except for DP soil profile that recorded the most depleted ( $-21.18\text{‰}$ )  $\delta^{13}\text{C}$  value in the upper soil surface than the other profiles (Fig. 2d). This is because DP profile at the highest elevation would have the least contribution of soil deposition and accumulation from other landforms and so reflecting  $\delta^{13}\text{C}$  values close to the source.

In forested areas, the input of plant organic matter greatly affect the  $\delta^{15}\text{N}$  values in soil surface (Eissfeller et al. 2013). The  $\delta^{15}\text{N}$  values for plants are affected by the  $\delta^{15}\text{N}$  values of soil, resulting in a close relation between soils and plants nitrogen composition in the ecosystem (Nel et al. 2018). In addition, the influence of atmospheric deposition can also affect  $\delta^{15}\text{N}$  values in soils, for instance Southwest China wet deposition for  $\text{NO}_3^-$  is  $2\text{‰}$  and for  $\text{NH}_4^+$  is  $-12\text{‰}$  (Xiao and Liu 2002). In relation to  $\delta^{13}\text{C}$ , there are higher variation of  $\delta^{15}\text{N}$  values for the studied soil profiles with SL1 having a significantly higher average  $\delta^{15}\text{N}$  value than the other profiles (Table 1 and Fig. 2a). The  $\delta^{15}\text{N}$  values for SL1 soil profile ranges between  $10.43\text{‰}$  to  $14.85\text{‰}$  and are outside the range of both C3 and C4 plants  $1\text{‰}\pm 4$  or  $5\text{‰}$  and  $3\text{‰}\pm 4$  or  $5\text{‰}$  respectively, (Fig. 6). The input of plant organic matter and atmospheric deposition cannot explain the high  $\delta^{15}\text{N}$  values in SL1 profile. The presence of animal residue however could account for the relatively high  $\delta^{15}\text{N}$  value registered in SL1 soil profile. The other soil profiles SL2, XNC and DP,  $\delta^{15}\text{N}$  values fall within the range of C3 and C4 plants, except for one point in XNC soil profile (XNC-3 registered  $\delta^{15}\text{N} = 13.22$ ). The high  $\delta^{15}\text{N}$  value in XNC profile maybe due to soil water migration and transformation of nitrogen as a result of microbial activities. Stewart et al. 2014 reported that soil water processes affected redox environment, which control nitrification and denitrification. In addition, based on other literature Fogg et al. 1998 reported that the average  $\delta^{15}\text{N}$  values of soil from sites under animal waste, fertilizer, soil organic matter and sewer septic sources ranged from  $(+10$  to  $+25)$ ,  $(0$  to  $+5)$ ,  $(-3$  to  $+5)$ , and  $(+7$  to  $15\text{‰})$ , respectively. Our results suggest that animal waste could likely be a potential source for SL1 profile while the other profiles  $\delta^{15}\text{N}$  values fall in the range of soil organic matter with some amount of influence from other sources. This high variability of  $\delta^{15}\text{N}$  values within a single source and the overlapping of values may prove challenging in the ability to accurately distinguish one source from another. The  $\delta^{15}\text{N}$  in the soil organic layer can indicate the relative rate of soil N cycling due to significant correlation between  $\delta^{15}\text{N}$  and mineralization and nitrification rate (Templer et al. 2007). The relatively high  $\delta^{15}\text{N}$  values in the studied soil profiles which fluctuate with depth indicate microbial activities during mineralization, nitrification and denitrification. The enrichment of  $\delta^{15}\text{N}$  that occurred in the upper soil surface 0-20 cm of all the studied soil profiles were due to microbial activities present in karst soils. The high variability of  $\delta^{15}\text{N}$  in the subsoil was attributed to soil water processes and redox environment involving nitrification and denitrification (Stewart et al. 2014). The seasonal wet and dry conditions in this karst environment due to monsoonal climate facilitate intensive microbial activities in the upper surface as well as translocation of DON at depth resulting in high variability in  $\delta^{15}\text{N}$ .

In addition to  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  isotopic signatures, the C/N ratio can also be used to support the suggestion that the studied soil profiles are of mixed organic matter sourced. Studies have shown that the C/N ratio of higher terrestrial plants are usually  $>15$  (Kendall et al. 2001) and that C/N ratio of soil organic matter is usually between 10 and 13 (Parton et al. 1987; Tiessen et al. 1984). The C/N ratio of SL1 soil profile is between 10.3 and 13.4, with an average of  $11.75 \pm 2.3$  ( $n=10$ ) (Table 2), which suggest that the sources of organic matter are uniform and not mixed; in other words, the sources are in situ (indigenous) to the soil profile. However, the other soil profiles showed high variation in C/N ratio in the upper soil surfaces which are significantly different ( $>10$ ) from the lower layers (Table 2 and Fig. 3). This indicates that the sources of organic matter are mixed; reflecting influence of both exogenous and indigenous sources. Based on our findings it is evident that the source of organic matter within this karst area of Yunnan plateau, Southwestern China has mixed contribution of C3, C4 plants and microbes with animal residue influencing SL1 profile.

### **4.3 Mechanisms controlling $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ isotopes fractionation in karst soil profiles**

In ecological studies, difference in abundance of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  isotopes are important in the evaluation of changes under varying conditions of SOM decomposition and land use. Overall, our results indicate that  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values varied with soil depth with  $\delta^{15}\text{N}$  values showing greater irregular fluctuation (Fig. 2). Meanwhile, the general trend observed for  $\delta^{13}\text{C}$  values was enrichment with increasing depth to approximately 20 cm, followed by depletion with increasing depth (Fig. 2a, b & c). Except, however for DP profile which indicate a decrease in  $\delta^{13}\text{C}$  values with depth (Fig. 2d). This increased variation of  $\delta^{13}\text{C}$  with depth is a characteristic feature of forest soils (Chen et al. 2005; Powers and Schlesinger 2002a) and indicate microbial activities during SOM decomposition. Soils dominated by aerobic decomposition have a clear increase of  $\delta^{13}\text{C}$  values with depth. This is due to preferential release of the lighter  $\delta^{12}\text{C}$  isotopes during aerobic mineralization. The initial 0-20 cm increase supported the general trend expected for undisturbed soil where studies have shown that both isotopes become more enriched in the heavier isotopes with increasing soil depth (Broadbent et al. 1980; Campbell et al. 2018; Chen et al. 2005; Ehleringer et al. 2000; Garten et al. 2000; Gebauer and Schulze 1991; Ji-Suk and Hee-Myong 2018; Krull et al. 2006; Ledgard et al. 1984; Mariotti et al. 1980; Nadelhoffer and Fry 1988; Powers and Schlesinger 2002a,b; Steele et al. 1981). However, the increasing  $\delta^{13}\text{C}$  trend were only observed in the top surface layer for this karst soil. The increasing trend has been reported to be associated with a number of mechanisms which may include the Suess effect (i.e., decrease in  $\delta^{13}\text{C}$  in atmosphere due to combustion of fossil fuels), selective preservation, microbial turnover, microbial carbon mixing, and changes in plant community composition (Ehleringer et al. 2000; Krull and Skjemstad, 2003; Nadelhoffer and Fry 1988). In our study the observed enrichment trend of  $\delta^{13}\text{C}$  in surface soils were related to kinetic fractionation caused by microbes rather than Suess effect or changed in plant community composition. This is because Suess effect is likely to contribute to a vertical increase of  $\delta^{13}\text{C}$  throughout the profile (Garten et al. 2000), and this was not observed in our profiles.

In contrast soils that experience suppressed degradation due to anoxic conditions, coupled with DOC translocation usually indicate a decrease in  $\delta^{13}\text{C}$  values with depth. The lighter  $\delta^{13}\text{C}$  values is due to enrichment of recalcitrant organic substances during anaerobic mineralization, result in depletion of the heavier  $\delta^{13}\text{C}$ . Furthermore, subsoil horizons with low carbon contents, may have DOM readily adsorbed to mineral surfaces, resulting in the reduction of  $\delta^{13}\text{C}$  in soil (Kalbitz et al. 2000; 2005). At greater soil depth, selective preservation result in a decrease of  $\delta^{13}\text{C}$  rather than an increase (Wynn et al. 2006) as seen in figure 2a, b and c, usually after 20 cm depth and DP profile (Fig. 2d). This decrease is due to the preferential utilization of lighter  $\delta^{13}\text{C}$  from organic functional groups (e.g., simple carbohydrates) by microbial decomposers and selective preservation of plant lignin, which is depleted in  $^{13}\text{C}$  (McCorkle et al. 2016).

Soil  $\delta^{15}\text{N}$  values related to the nutrient input, humification and nitrogen transformation as influenced by land use and land cover change (Awiti et al. 2008). The  $\delta^{15}\text{N}$  values in this studied karst area forest soils (2.77 to 14.36‰) were significantly higher than those reported in other studies e.g., Liu et al. 2019 (2.9 to 4.3‰) indicating more rapid N cycling in these soils. The differences were due to influence of animal residues, as well as the differences in forest vegetation foliar N content, which depend on species, precipitation and temperature (Craine et al. 2010). The general trend naturally for  $\delta^{15}\text{N}$  in forest soils was the presence of two pools; top surface soil with low  $\delta^{15}\text{N}$  values and subsoil with high  $\delta^{15}\text{N}$  values (Nadelhoffer and Fry 1988). The vertical distribution of  $\delta^{15}\text{N}$  in this karst area showed high fluctuation which peak at depth for all the studied soil profiles (Fig. 2), except for XNC soil profile which peak at 25 cm (Fig. 2b). This was due to the spatial dynamics of soil water processes and the influenced on redox reaction associated with SOM nitrification and denitrification (Stewart et al. 2014). Water availability affect the cycling of carbon and nutrients and thereby regulating the growth and distribution of microbes. Similarly, strong fluctuation in  $\delta^{15}\text{N}$  with depth were reported by Liu et al. 2019 in karst watershed. The high variability pattern of  $\delta^{15}\text{N}$  distribution in these soil profiles is associated with fractionations that occur during the process of mineralization, nitrification and physical mixing. Similar findings were reported by Nadelhoffer and Fry 1994. The peak of  $\delta^{15}\text{N}$  in the deeper soil profile is common with other findings (Mariotti et al. 1980; Nadelhoffer and Fry 1988; Steele et al. 1981). This is because, at depth, microbial excretions of ammonium and nitrate become less depleted in  $\delta^{15}\text{N}$ , while the humus content of nitrogen becomes enriched, and the uptake of nitrogen by plant roots lower the  $\delta^{15}\text{N}$  abundance in the microbial reserve hence the total soil becomes enriched (Nadelhoffer and Fry 1994). In addition, further  $\delta^{15}\text{N}$  enrichment with depth may be because of N loss during high precipitation input. The high complexity of soils containing several isotopically different forms of nitrogen (Ledgard et al. 1984; Tiessen et al. 1984) resulting in N irregular behaviour, hence the notable high variation in  $\delta^{15}\text{N}$  values with depth for the studied soil profiles. The  $\delta^{15}\text{N}$  isotopic composition in soils and groundwater may not only be influenced by its source but also by microbial activities and physical processes such as ion exchange (Ostrom et al. 1998).

Our results in this karst environment illustrate a non-conservative behaviour in  $\delta^{15}\text{N}$  that is largely due to a combination of microbial activities and water availability, which affects redox reactions (Fig. 2a-d). The monsoonal climatic condition along with the topographical features of karst environment provide the uneven distribution of soil which result in the spatial and temporal disparity in soil water (Tokumoto et al. 2014). Essentially, the loss of nitrate through denitrification result in enrichment of  $\delta^{15}\text{N}$  content in the remaining substrate, while during nitrification process, the lighter  $\delta^{15}\text{N}$  isotope is preferentially incorporated into nitrate resulting in a decrease in  $\delta^{15}\text{N}$  (Ostrom et al. 1998). Therefore, the dominant mechanism affecting  $\delta^{15}\text{N}$  within karst area of Yunnan Plateau, Southwestern China are mineralization in the upper surface, while nitrification and denitrification in the subsoil.

#### **4.4 Interactions among $\delta^{13}\text{C}$ , $\delta^{15}\text{N}$ isotopes with TOC, TN and C/N ratio**

Vegetation is the primary source of TOC and TN which can significantly affect the quantity and quality of soil organic matter (Podwojewski et al. 2011). Our results indicated that the soil profiles TN contents were positively correlated with the TOC contents ( $r^2 = 0.982, 0.988$ ) for SL1 and SL2, however weak correlation for XNC and DP respectively. The result implied that majority of nitrogen were of similar organic origin for SL1, SL2 and that soil water affected N in XNC and DP profiles (Fig. 7). The predominant sources of nitrogen are from animal residue in SL1 profile, while leaf-litter, biological nitrogen fixation, and organic matter dominate the other profiles (Bai et al. 2001). This strong positive correlation between TOC and TN contents has also been found in other studies e.g., (Diwediga et al. 2017; Gelaw et al. 2014; Liu et al. 2019; Liu and Wang, 2009; Xiong et al. 2018).

The relationships between soil particulate  $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$  and C/N ratios are shown in figure 6 and 8. The results indicate mixture of end member particulates from terrestrial C3, C4 plants and soil organic matter, with SL1 significantly different from the other profiles. The high  $\delta^{15}\text{N}$  values were due to the input of animal residue as it comprised of similar isotopic range as reported by Fogg et al. 1998. Typically, higher terrestrial vascular plants usually have C/N ratios higher than 15 (Meyers, 1994). Overall, for these studied soil profiles the C/N ratios ranged between 1.5 to 13.4 (mean  $6.60 \pm 4.02$ ) indicating a mixture of terrestrial plants and other sources (Table 2; Fig. 8). A weak positive correlation was observed between the  $\delta^{13}\text{C}$  and C/N ratio for all the studied soil profiles, suggesting that to some extent both parameters are influenced by the same factors such as source of organic matter and decomposition rate of SOM (Fig. 8a).

This study revealed that both  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  of soil TOC and TN (Fig. 9a and b) reflect isotopic fractionation associated with litter fall decomposition, microbial activities, translocation and physical mixing processes. Forest soils are characterized by litter fall and root exudates that are constantly being decomposed and gradually mixed within the soil profile (Acton et al. 2013). The older more decomposed SOM found lower in the soil profile as a result of further decomposition, physical mixing, leaching and vertical mobilization (McCorkle et al. 2016; Nadelhoffer and Fry 1988). On the other hand, the newly decomposed or fresh C and N inputs found in the upper soil profile (Wang et al. 2018).

Figure 10 shows the relationship between  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  for the soil profiles which indicate weak negative correlation. This correlation was interpreted as the mixing of isotopically distinctive carbon and nitrogen source (Huon et al. 2002; Meyers and Takemura 1997), as well as microbial activities. The SL1 soil profile was significantly different from the other profiles, with intense enrichment in  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values, suggesting a higher degree decomposition and influence of animal residue (Fig. 2a and 7). Based on the C and N regression slope, the studied soil profiles can be placed in order of external influence and soil stability with undisturbed forest soil as baseline, followed by DP, XNC, SL2 and SL1 (Fig. 10). This provides support that  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  isotopic assessments can be used as a monitoring tool to determine source, external influence and soil stabilization.

## 5. Conclusions

This study provides insights into sources and distribution of soil organic matter, as well as the behaviour and mechanisms of carbon and nitrogen isotopes within karst soils in Yunnan Plateau, Southwestern China. The study indicated vertical variation in TOC and TN contents with soil depth which were largely influenced by climate, topography, vegetation cover and land use.

The joint end-member analysis of  $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$  and C/N ratios revealed organic matter of this karst soil have mixed sources which derived from both exogenous and endogenous materials. The external source being that of animal residues, and the internal source comprised of a mixture of terrestrial C3, C4 plants and soil microbes. In addition, the soil organic matter of these profiles was not dominated by any particular terrestrial plant group as they mostly fall outside the isotopic signature range and therefore showed characteristics of a mixing pool. We speculate that the ages of profiles are relatively young which were confirmed by  $\delta^{14}\text{C}$  radioisotope.

The carbon and nitrogen stable isotopes in these soil profiles revealed vertical variation and spatial differentiation, which reflected activities of SOM mineralization, translocation and leaching. We concluded that due to the variability and irregular pattern of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  isotopes fractionation, that the forest soil C and N contents are unstable over this karst environment. The dominant mechanisms that influenced  $\delta^{13}\text{C}$  variation within these soil profiles are mineralization in the upper surface while DOC translocation and selective preservation in the subsoil. Conversely, the high vertical variation in  $\delta^{15}\text{N}$  within these soil profiles were controlled by differences in the plant composition and fractionations that had occurred during the process of mineralization, nitrification and DON translocation.

The analysis of results further revealed strong positive correlation among  $\delta^{13}\text{C}$  versus TOC, TN and C/N, while negative correlation among  $\delta^{15}\text{N}$  versus TOC, TN and C/N for all the studied soil profiles within this karst area in Yunnan Plateau, Southwestern China. The negative correlation between  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  has been interpreted as the mixing of isotopically distinctive carbon and nitrogen source as well as microbial activities and translocation. This was due to the fact that  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  become enriched in the upper surface during decomposition of SOM, with the preferentially incorporation of the lighter isotopes by soil

microbes, leaving behind the heavier isotopes. Coupled with downward translocation of DOC and DON throughout the profiles result in a decrease in isotopes with depth. The findings suggest that within karst environment combination of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  isotopes along with other parameters can be used as a tracer proxy to determine soil organic matter source, external influence and soil stabilization.

## Declarations

### Acknowledgements

We are indebted to Professor Shijie Wang discussion and suggestions about this study. This work was jointly supported by the National Natural Science Foundation of China (NSFC) grants (Nos. 41473122, 41073096), National Key Basic Research Program of China (2013CB956702) and the Hundred Talents Program of the Chinese Academy of Sciences.

**Funding** (This work was jointly supported by the National Natural Science Foundation of China (NSFC) grants (Nos. 41473122, 41073096), National Key Basic Research Program of China (2013CB956702) and the Hundred Talents Program of the Chinese Academy of Sciences)

**Conflicts of interest/Competing interests** (We declared no conflict of interest exists in the submission of this manuscript)

**Availability of data and material** (All the data sets for this research is available in Table 1–2 of the manuscript. The data supporting figure 2–10 in the text is available in Table 1–2).

**Code availability** (Not applicable)

**Authors' contributions** (The authors have made equal contribution to this work)

**Ethics approval** (Not applicable)

**Consent to participate** (Not applicable)

**Consent for publication** (Not applicable)

## References

Acton, P., Fox, J., Campbell, E., Rowe, H., Wilkinson, M. 2013. Carbon isotope for estimating soil decomposition and physical mixing in well-drained forest soils. *Journal of Geophysical Research: Biogeosciences* 118, 1532–1545.

Awiti, A.O., Walsh, M.G., Kinyamario, J., 2008. Dynamics of topsoil carbon and nitrogen along a tropical forest cropland chronosequence: evidence from stable isotope analysis and spectroscopy. *Agriculture Ecosystems and Environment* 127, 265-272.

- Bai, J. H., Deng, W., Zhang, Y. X. 2001. Spatial distribution of nitrogen and phosphorus in soil of Momoge Wetland. *Journal of Soil and Water Conservation*, 15 (4): 79–81.
- Bai, J. H., Deng, W., Zhu, Y. M., et al. 2002. Comparative study on the distribution characteristics of soil organic matter and total nitrogen in wetlands-A case study of Xianghai and Horqin nature reserve. *Scientia Geographica Sinica*, 22 (2): 232–237.
- Balesdent, J., and Mariotti, A. 1996. Measurement of soil organic matter turnover using  $^{13}\text{C}$  natural abundance. *Mass spectrometry of soils*, T. Boutton and S. Yamasaki, eds., Marcel Dekker, New York, 83–111.
- Balesdent, J., Girardin, C., and Mariotti, A. 1993. Site-related  $\delta^{13}\text{C}$  of tree leaves and soil organic matter in a temperate forest. *Ecology* 74: 1713–1721.
- Boström, B., Comstedt, D., Ekblad, A. 2007. Isotope fractionation and  $^{13}\text{C}$  enrichment in soil profiles during the decomposition of soil organic matter. *Oecologia*, 153, 89–98.
- Boutton, T. W. 1991. Stable isotope ratios of natural materials: II. Atmospheric, terrestrial, marine, and freshwater environments. In: Coleman D C, Fry B, eds. *Carbon Isotope Techniques*. New York: Academic Press, 173–185.
- Cai, Y., Guo, L., Wang, X., Aiken, G., 2015. Abundance, stable isotopic composition, and export fluxes of DOC, POC, and DIC from the Lower Mississippi River during 2006-2008. *Journal of Geophysical Research: Biogeosciences*.
- Campbell, J.E.; James F. Fox, M.; Charles M. Davis, S.M.; Harold D. Rowe; and Nathan Thompson. 2009. Carbon and Nitrogen Isotopic Measurements from Southern Appalachian Soils: Assessing Soil Carbon Sequestration under Climate and Land-Use Variation. *Journal of Environmental Engineering*. DOI: 10.1061/(ASCE)EE.1943-7870.0000008.
- Can, J. H., Yuan, D. X., Tong, L. Q. 2008. Characteristics of karst ecosystem in southwest China and comprehensive control of rocky desertification. *Grassland Science*, 25(9): 40-50.
- Careddu, G., Costantini, M.L., Calizza, E., Carlino, P., Bentivoglio, F., Orlandi, L., Rossi, L., 2015. Effects of terrestrial input on macrobenthic food webs of coastal sea are detected by stable isotope analysis in Gaeta Gulf. *Estuarine coastal and shelf science journal* 154, 158-168.
- Chen, Q. Q., Shen, C. D., Sun, Y. M., Peng, S. L. 2005. Mechanism of distribution of soil organic matter with depth due to evolution of soil profiles at the Dinghushan Natural Reserve. *Acta. Pedol. Sin.* 42, (1):1–8.
- Chen, Q.Q., Shen, C.D., Sun, Y.M., Peng, S.L., Yi, W.X., Li, Z.A., Jiang, M.T. 2005. Spatial and temporal distribution of carbon isotopes in soil organic matter at the Dinghushan Biosphere Reserve, South China. *Plant Soil*, 273, 115–128.

Chukwudi, N., Okeke. O.J., Fashae, O., Nwankwoala, H. 2018. Soil organic carbon and total nitrogen stocks as affected by different land use in an Ultisol in Imo Watershed, southern Nigeria, *Chemistry and Ecology*, DOI:10.1080/02757540.2018.1508461.

Craine, J.M., Elmore, A.J., Aidar, M.P.M., Mercedes, B., Dawson, T.E., Hobbie, E.A., Ansgar, K., Mack, M.C., Mclauchlan, K.K., Anders, M. 2010. Global patterns of foliar nitrogen isotopes and their relationships with climate, mycorrhizal fungi, foliar nutrient concentrations, and nitrogen availability. *New Phytologist* 183, 980–992.

Davidson, E.A., Hart, S.C., Firestone, M.K. 1992. Internal cycling of nitrate in soils of a mature coniferous forest. *Ecology* 73, 1148–1156.

Dawson, T. E., Stefania Mambelli, Agneta H. Plamboeck, Pamela H. Templer, and Kevin P. Tu 2002. Stable Isotopes In Plant Ecology. *Annual Review of Ecology and Systematics*. 2002. 33:507–59 doi: 10.1146/annurev.ecolsys.33.020602.095451.

De Neve, S., Hofman, G. 2000. Influence of soil compaction on carbon and nitrogen mineralization of soil organic matter and crop residues. *Biology and Fertility of Soils*, 30(5–6): 544–549.

Diwediga, B., Le, Q. B., Agodzo, S., et al. 2017. Potential storages and drivers of soil organic carbon and total nitrogen across river basin landscape: the case of Mo River Basin (Togo) in West Africa. *Ecological Engineering* 99:298–309.

Don, A., Schumacher, J., Freibauer, A. 2011. Impact of tropical land-use change on soil organic carbon stocks – a meta-analysis. *Global Change Biology*, 17, 1658–1670.

Durán, J., Morse, J.L., Rodríguez, A., Campbell, J.L., Christenson, L.M., Driscoll, C.T., Fahey, T.J., Fisk, M.C., Mitchell, M.J., Templar, P.H., Groffman, P.M., 2017. Differential sensitivity to climate change of C and N cycling processes across soil horizons in a northern hardwood forest. *Soil Biology and Biochemistry* 107, 77–84.

Edmoned, R.L. 1991. Organic matter decomposition in western United States forests. USDA For. Ser. Gen. Tech. Rep. 280, 116–128.

Ehleringer, J. R., and R. K. Monson. 1993. Evolutionary and ecological aspects of photosynthetic pathway variation. *Annual Review of Ecology and Systematics* 24:411–439.

Ehleringer, J.R., Buchmann, N., and Flanagan, L.B. 2000. Carbon Isotope Ratios in Belowground Carbon Cycle Processes, *Ecological Applications*, vol. 10, pp. 412–422.

Eshetu, Z. and Högberg, P., 2000. Effects of land use on <sup>15</sup>N natural abundance of soils in Ethiopian highlands. *Plant and Soil*, 222: 109-117.

- Eissfeller, V., Beyer, F., Valtanen, K., Hertel, D., Maraun, M., Polle, A., Scheu, S. 2013. Incorporation of plant carbon and microbial nitrogen into the rhizosphere food web of beech and ash. *Soil Biology Biochemistry*, 62, 76–81.
- Farquhar, G.D.; Ehleringer, J.R.; Hubick, K.T. 1989. Carbon isotope discrimination and photosynthesis. *Annual Review Plant Biology* 40, 503–537.
- Fogg, G.E., Rolston, D.E., Decker, D.L, Louie, D.T, and Grismer, M.E., 1998. Spatial variation in nitrogen isotope values beneath nitrate contamination sources. *Groundwater*, Vol. 36, No.3, 418-426.
- Fox, J. F. 2005. Fingerprinting using biogeochemical tracers to investigate watershed processes. Ph.D. thesis, Univ. of Iowa, Iowa City, Iowa.
- Garten, C. T., Cooper, L. W., Post, W. M., III, and Hanson, P. J. 2000. Climate controls on forest soil C isotope ratios in the southern Appalachian Mountains. *Ecology*, 81, 1108–1119.
- Gebauer, G. and Meyer, M., 2003. N-15 and C-13 natural abundance of autotrophic and mycoheterotrophic orchids provides insight into nitrogen and carbon gain from fungal association. *New Phytologist*, 160(1): 209-223.
- Gelaw, A. M., Singh, B. R., Lal, R. 2014. Soil organic carbon and total nitrogen stocks under different land uses in a semi-arid watershed in Tigray, northern Ethiopia. *Agriculture Ecosystems and Environment* 188:256–263.
- Gill, R., Burke, I. C., Milchunas, D. G., and Lauenroth, W. K. 1999. Relationship between root biomass and soil organic matter pools in the short grass steppe of eastern Colorado. *Ecosystems*, 2, 226–236.
- Goni, M. A., Teixeira, M. J., Perkey, D. W. 2003. Sources and distribution of organic matter in a river dominated estuary (Winyah Bay, SC, USA). *Estuarine Coast Shelf Science* 57: 1023–1048.
- Gregory, A.S.; Dungait, J.A.J.; Watts, C.W.; Bol, R.; Dixon, E.R.; White, R.P.; Whitmore, A.P. 2016. Long-term management changes topsoil and subsoil organic carbon and nitrogen dynamics in a temperate agricultural system. *European Journal of Soil Science* 67, 421–430.
- Guillaume, T., Damris, M., Kuzyakov, K. 2015. Losses of soil carbon by converting tropical forest to plantations: erosion and decomposition estimated by  $\delta^{13}\text{C}$ . *Global Change Biology* 21, 3548–3560, doi: 10.1111/gcb.12907.
- Halaj, J., Peck, R. W., Niwa, C. G. 2005. Trophic structure of amacroarthropod litter food web in managed coniferous forest stands: A stable isotope analysis with  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$ . *Pedobiologia*, 49: 109–118.
- Han, G.L.; Li, F.S.; Tang, Y. 2017. Organic matter impact on distribution of rare earth elements in soil under different land uses. *Clean Soil Air Water*, 45, 1600235.

- Han, G.L., Tang, Y., Liu, M., Zwieten, L. V., Yang, X., Yu, C., Wang, H., Song, Z. 2020. Carbon-nitrogen isotope coupling of soil organic matter in a karst region under land use change, Southwest China. *Agriculture, Ecosystems and Environment*, 301. 107027.
- Harmeliv-vivien M, Loizeau V, Mellon C, et al. 2008. Comparison of C and N stable isotope ratios between surface particulate organic matter and micro phytoplankton in the Gulf of Lions (NW Mediterranean). *Continent Shelf Research*, 28: 1911–1919.
- Hogberg, P. 1997. Tansley review no. 95  $^{15}\text{N}$  natural abundance in soil-plant systems. *New Phytologist* 137:179–203.
- Huang, J. 2009. Yunnan stone forest karst mountain vegetation and soil degradation characteristics. Nanjing: Nanjing Forestry University.
- Huon, S., Grousset, F. E., Burdloff, D., Bardoux, G., Mariotti, A. 2002. Sources of fine-sized organic matter in North Atlantic Heinrich Layers:  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  tracers, *Geochimica et Cosmochimica Acta*. 66, 223–239.
- Jafari, M., Kohande, A., Baghbani, S., Tavili, A., Chahouki, M. A. Z. 2011. Comparison of chemical characteristics of shoot, root and litter in three range species of *Salsola rigida*, *Artemisia sieberi* and *Stipa barbata*. *Caspian Journal Environment Science*, 9, 37-46.
- Ji–Suk Park and Hee–Myong Ro. 2018. Temporal Variations in Soil Profile Carbon and Nitrogen during Three Consecutive Years of  $^{15}\text{N}$  Deposition in Temperate Oak and Pine Forest Stands. *Forests*, 9, 338; doi:10.3390/f9060338.
- Jobbágy, E. G., Jackson, R. B. 2000. The vertical distribution of soil organic carbon and its relation to climate and vegetation. *Ecological Applications*, 10, 423-436.
- Kalbitz, K., Schwesig, D., Rethemeyer, J., Matzner, E. 2005. Stabilization of dissolved organic matter by sorption to the mineral soil. *Soil Biology and Biochemistry*, 37, 1319-1331.
- Kalbitz, K., Schwesig, D., Rethemeyer, J., Matzner, E. 2000. Controls on the dynamic of dissolved organic matter in soils: a review. *Soil Science*, 165, 277-304.
- Kelly, E. F., Amundson, R.G., Marino, B.D., DeNiro, M. J. 1991. Stable carbon isotopic composition of carbonate in Holocene grassland soils. *Soil Science Society of American Journal* 55:1651–1658.
- Kendall, C., Silva, S. R., Kelly, V. J. 2001. Carbon and nitrogen isotopic compositions of Particulate organic matter in four large river systems across the United States. *Hydrological Processes*. 15: 1301–1346.
- Kohl, L., Laganière, J., Edwards, K. A., Billings, S. A., Morrill, P.L., Van Biesen, G., and Ziegler, S. E. 2015. Distinct fungal and bacterial  $\delta^{13}\text{C}$  signatures as potential drivers of increasing  $\delta^{13}\text{C}$  of soil organic matter with depth, *Biogeochemistry*, 124, 13–26.

- Krull, E.S., Bestland, E.A., Skjemstad, J.O., Par, J.F. 2006. Geochemistry ( $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$ ,  $^{13}\text{C}$  NMR) and residence times ( $^{14}\text{C}$  and OSL) of soil organic matter from red-brown earths of South Australia: Implications for soil genesis. *Geoderma* 132, 344–360.
- Krull, E.S., Skjemstad, J.O. 2003.  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  profiles in  $^{14}\text{C}$ -dated Oxisol and Vertisols as a function of soil chemistry and mineralogy. *Geoderma* 112, 1 – 29.
- Laganière, J., Pare, D., Bradley, R.L. 2010. How does a tree species influence litter decomposition? Separating the relative contribution of litter quality, litter mixing, and forest floor conditions. *Canadian Journal of Forest Research* 40, 465–475.
- Lal, R. 2001. Soil degradation by erosion. *Land Degradation & Development*, 12, 519–539
- Ledgard, S., Freney, J., and Simpson, J. 1984. Variations in natural enrichment of  $^{15}\text{N}$  in the profiles of some Australian pasture soils. *Australian Journal of Soil Research*, 22, 155–164.
- Li, C., Li, Y., Tang, L. 2010. Soil organic carbon stock and carbon efflux in deep soils of desert and oasis. *Environmental Earth Sciences*, 60, 549-557.
- Li, X.J., Ogrinc, N., Hamilton, S. K., Szramek, K., Kanduc, T., Walter, L. M. 2009. Inorganic carbon isotope systematics in soil profiles undergoing silicate and carbonate weathering (Southern Michigan, USA). *Chemical Geology* 264:139–153.
- Liu, C. Q. 2009. *Biogeochemical Process and Surface Material Cycle*. Beijing: Science Press, 318–348.
- Liu, J. 2015. *Research on Karst area forest vegetation climate characteristics*. Kunming: Yunnan Normal University.
- Liu, M., Han, G., Qian Zhang, Q., Song, Z. 2019. Variations and Indications of  $\delta^{13}\text{C}_{\text{SOC}}$  and  $\delta^{15}\text{N}_{\text{SON}}$  in Soil Profiles in Karst Critical Zone Observatory (CZO), Southwest China. *Sustainability*, 11, 2144; doi:10.3390/su11072144.
- Liu, W., Yang, H., Cao, Y., Ning, Y., Li, L., Zhou, J. and An, Z., 2005c. Did an extensive forest ever develop on the Chinese Loess Plateau during the past 130 ka?: a test using soil carbon isotopic signatures. *Applied Geochemistry*, 20: 519-527.
- Liu, W.G., Wang, Z. 2009. Nitrogen isotopic composition of plant-soil in the Loess Plateau and its responding to environmental change. *Chinese Science Bulletin* 54, 272–279.
- Mariotti, A., Pierre, D., and Vedy, J. C. 1980. The abundance of natural nitrogen 15 in the organic matter of soils along an altitudinal gradient. *Catena*, 7, 293–300.
- McCorkle, E.P., Asmeret Asefaw Berhe, Carolyn T. Hunsaker, Dale, W. Johnson, Karis J. McFarlane, Marilyn L. Fogel, Stephen C. Hart. 2016. Tracing the source of soil organic matter eroded from temperate

forest catchments using carbon and nitrogen isotopes. *Chemical Geology* 445, 172–184.

Meng, L., Ding, W., Cai, Z. 2005. Long-term application of organic manure and nitrogen fertilizer on N<sub>2</sub>O emissions, soil quality and crop production in a sandy loam soil. *Soil Biology Biochemistry*, 37, 2037–2045.

Menyailo, O.V., B. A. Hungate, J. Kehmann, et al. 2003. *Isotopes Environ. Health Stud.* 39, 41.

Meyers, P. A., and Takemura, K. 1997. Quaternary changes in delivery and accumulation of organic matter to sediments of Lake Biwa, Japan, *Journal of Paleolimnology* 18, 211–218.

Meyers, P.A., 1994. Preservation of elemental and isotopic source identification of sedimentary organic matter. *Chemical Geology* 114, 289-302.

Middelburg, J.J., Herman, P.M.J., 2007. Organic matter processing in tidal estuaries. *Marine Chemistry* 106, 127-147.

Midwood, A.J.; Boutton, T.W. 1998. Soil carbonate decomposition by acid has little effect on  $\delta^{13}\text{C}$  of organic matter. *Soil Biology and Biochemistry* 30, 1301–1307.

Motavalli, P. P., Palm, C. A, Parton, W. J., et al. 1995. Soil pH and organic C dynamics in tropical forest soils: evidence from laboratory and simulation studies. *Soil Biology and Biochemistry*, 27(12): 1589–1599.

Nadelhoffer, K. J., and Fry, B. 1994. Nitrogen isotope studies in forest ecosystems. *Stable isotopes in ecology and environmental science*, K. Lajtha and R. Michener, eds., Blackwell, Cambridge, U.K., 22–44.

Nadelhoffer, K.J., and Fry, B. 1988. Controls on natural Nitrogen 15 and Carbon 13 abundances in forest soil organic matter. *Soil Science Society of American Journal* 52, 1633–1640.

Narayan, C., and Anshumali. 2016. Elemental composition of Sal forest soils around Chota Nagpur Plateau, India. *Chemical Ecology* 32 (6): 533-549.

Nel, J.A., Craine, J.M., Cramer, M.D. 2018. Correspondence between  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  in soils suggests coordinated fractionation processes for soil C and N. *Plant Soil*, 423, 1–15.

Njeru, C. M., Ekesi, S., Mohamed, S. A., et al. 2017. Assessing stock and thresholds detection of soil organic carbon and nitrogen along an altitude gradient in an east Africa mountain ecosystem. *Geoderma Regional*, 10: 29–38.

O’Leary, M. 1981. Carbon isotope fractionation in plants. *Phytochemistry*, 20, 553–567.

O’Leary, M.H., 1988. Carbon isotopes in photosynthesis, *Bioscience* 38. 328–336.

- Ostrom, N. E., Keith, E., Knoke, Lars O. Hedin, G. Philip Robertson, Alvin J.M Smucker. 1998. Temporal trends in nitrogen isotope values of nitrate leaching from an agricultural soil. *Chemical Geology* 146. 219–227.
- Ou, Y., Rousseau, A. N., Wang, L. X., et al. 2017. Spatio-temporal patterns of soil organic carbon and pH in relation to environmental factors—A case study of the Black Soil Region of Northeastern China. *Agriculture, Ecosystems & Environment*, 245: 22–31.
- Parton, W. J., Schimel, D. S., Cole, C. V. et al. 1987. Analysis of factors controlling soil organic matter levels in Great Plains grasslands. *Soil Science Society of American Journal* 51: 1173–1179.
- Paul, E. A., Follett, R. F., Leavitt, S. W., Halvorson, A., Peterson, G. A., and Lyon, D. J. 1997. Radiocarbon dating for determination of soil organic matter pool sizes and dynamics. *Soil Science Society of American Journal*, 61: (4) 1058–1067.
- Perakis, S.S.; Compton, J.E.; Hedin, L.O. 2005. Nitrogen retention across a gradient of  $^{15}\text{N}$  additions to an unpolluted temperate forest soil in Chile. *Ecology* 86, 96–105.
- Peterson, B.J., and Fry, B. 1987. Stable isotopes in ecosystem studies. *Annual Review of Ecology and Systematics* 18, 293-320.
- Poage, M. A., and Feng, X. H. 2004. A theoretical analysis of steady state  $\delta^{13}\text{C}$  profiles of soil organic matter. *Global Biogeochemical Cycles*, 18 (2), GB2016.
- Podwojewski, P., Poulencard, J., Nguyet, M. L et al. 2011. Climate and vegetation determine soil organic matter status in an alpine inner-tropical soil catena in the Fan Si Pan Mountain, Vietnam. *Catena*, 87(2): 226–239.
- Powers, J. S., and Schlesinger, W. H. 2002a. Geographic and vertical patterns of stable carbon isotopes in tropical rain forest soils of Costa Rica. *Geoderma*, 109 (1–2), 141–160.
- Powers, J. S., and Schlesinger, W. H. 2002b. Relationships among soil carbon distributions and biophysical factors at nested spatial scales in rain forests of northeastern Costa Rica. *Geoderma*, 109 (3–4), 165–190.
- Qiu, L., Hao, M., Wu, Y., 2017. Potential impacts of climate change on carbon dynamics in a rain-fed agroecosystem on the Loess Plateau of China. *Science Total Environment* 577, 267–278.
- Ramaswamy, V., Gaye, B., Shirodkar, P.V., Rao, P.S., Chivas, A.R., Wheeler, D., Thwin, S., 2008. Distribution and sources of organic carbon, nitrogen and their isotopic signatures in sediments from the Ayeyarwady (Irrawaddy) continental shelf, northern Andaman Sea. *Marine Chemistry* 111, 137e150.
- Reichstein, M., Bahn, M., Ciais, P., Frank, D., Mahecha, M. D., Seneviratne, S. I., Zscheischler, J., Beer, C., Buchmann, N., Frank, D. C., Papale, D., Rammig, A., Smith, P., Thonicke, K., van der Velde, M., Vicca, S.,

- Walz, A., and Wattenbach, M. 2013. Climate extremes and the carbon cycle, *Nature*, 500, 287–295.
- Robinson, D. 2001.  $^{15}\text{N}$  as an integrator of the nitrogen cycle. *Trends in Ecology and Evolution* 16:153–62.
- Schulze, E.-D., Chapin III, F.S. and Gebauer, G., 1994. Nitrogen nutrition and isotope differences among life forms at the northern tree line of Alaska. *Oecologia*, 100: 406-412.
- Staelens, J., Rütting, T., Huygens, D., De Schrijver, A., Müller, C., Verheyen, K., Boeckx, P. 2012. In situ gross nitrogen transformations differ between temperate deciduous and coniferous forest soils. *Biogeochemistry* 108, 259–277.
- Steele, K., Wilson, A., and Saunders, W. 1981. Nitrogen isotope ratios in surface and subsurface horizons of New Zealand improved grassland soils. *New Zealand Journal of Agricultural Research*, 24, 167–170.
- Stewart, K.J., Grogan, P., Coxson, D.S., Siciliano, S.D. 2014. Topography as a key factor driving atmospheric nitrogen exchanges in arctic terrestrial ecosystems. *Soil Biology and Biochemistry*, 70, 96–112.
- Talbot, M.R., 2001. Nitrogen Isotopes in Palaeolimnology. In: J.P. Smol (Editor), *Tracking Environmental Change Using Lake Sediments*. Kluwer Academic Publishers, Dordrecht, The Netherlands, pp. 401-439.
- Templer, P.H.; Arthur, M.A.; Lovett, G.M.; Weathers, K.C. 2007. Plant and soil natural abundance  $\delta^{15}\text{N}$ : Indicators of relative rates of nitrogen cycling in temperate forest ecosystems. *Oecologia*, 153, 399–406.
- Thorp, J. H., Delong, M. D., Greenwood, K. S., et al. 1998. Isotopic analysis of three food web theories in constricted and floodplain regions of a large river. *Oecologia*, 117: 551–563.
- Tiessen, J., Stewart, J. W., Hunt, H. W. 1984. Concepts of soil organic matter transformation in relation to organo-mineral particle size fractions. *Plant Soil*, 76: 287–295.
- Tieszen, L. L. 1991. Natural variations in the carbon isotope values of plants: implications for archaeology, ecology, and paleoecology. *Journal of Archaeological Science* 18:227– 248.
- Tieszen, L.L. 1994. Stable isotopes in the Great Plains: vegetation analyses and diet determinations. Pages 261–282 in D. W. Owsley and R. L. Jantz, editors. *Skeletal biology in the Great Plains: a multidisciplinary view*. Smithsonian Institution Press, Washington, D.C., USA.
- Tietema, A., Wessel, W.W. 1992. Gross nitrogen transformations in the organic layer of acid forest ecosystems subjected to increased atmospheric nitrogen input. *Soil Biology and Biochemistry*, 24, 943–950.
- Tokumoto, I., Heilman, J.L., Schwinning, S., McInnes, K.J., Litvak, M.E., Morgan, C.L.S., Kamps, R.H. 2014. Small-scale variability in water storage and plant available water in shallow, rocky soils. *Plant Soil*, 385,

193–204.

Trumbore, S.E., 2000. Age of soil organic matter and soil respiration: radiocarbon constraints on belowground C dynamics. *Ecological Applications*, 10 (2), 399–411.

Tuo, D. A, Guangyao Gao, Ruiying Chang, Zongshan Li, Ying Ma, Shuai Wang, Cong Wang, Bojie Fu 2018. Effects of revegetation and precipitation gradient on soil carbon and nitrogen variations in deep profiles on the Loess Plateau of China. *Science of the Total Environment* 626; 399–411.

Turner, G.L., Bergersen, F.J., Tantala, H. 1983. Natural enrichment of  $^{15}\text{N}$  during decomposition of plant material in soil. *Soil Biology and Biochemistry*, 15, 495–497.

Wang, C., Lu, X., Mori, T., Mao, Q., Zhou, K., Zhou, G., Nie, Y., Mo, J. 2018. Responses of soil microbial community to continuous experimental nitrogen additions for 13 years in a nitrogen-rich tropical forest. *Soil Biology and Biochemistry*, 121, 103–112.

Wang, M., Nan, C. B., Wang, Z. H., et al. 2007. Determination of pH in soil. Beijing: China Agriculture.

Wang, T., Kang, F., Cheng, X., et al. 2016. Soil organic carbon and total nitrogen stocks under different land uses in a hilly ecological restoration area of north China. *Soil and Tillage Research*, 163:176–184.

Wang, Y., Li, Y., Ye, X., Chu, Y., Wang, X. 2010. Profile storage of organic/inorganic carbon in soil: From forest to desert. *Science of the Total Environment*, 408, 1925-1931.

Wiesmeier, M., Spörlein, P., Geuss, U., et al. 2012. Soil organic carbon stocks in southeast Germany (Bavaria) as affected by land use, soil type and sampling depth. *Global Change Biology*, 18:2233–2245.

Wynn, J. G., Harden, J. W., and Fries, T. L. 2006. Stable carbon isotope depth profiles and soil organic carbon dynamics in the lower Mississippi Basin, *Geoderma*, 131, 89–109.

Xiao, H.Y., Liu, C.Q. 2002. Sources of nitrogen and sulfur in wet deposition at Guiyang, Southwest China. *Atmos. Environ.*, 36, 5121–5130.

Xiong, K., Yin, C., Ji, H.B. 2018. Soil erosion and chemical weathering in a region with typical karst topography. *Environmental Earth Sciences*, 77:500; doi.org/10.1007/s12665-018-7675-0.

Yu, F.L., Zong, Y.Q., Lloyd, J.M., Huang, G.Q., Leng, M.J., Kendrick, C., Lamb, A.L., Yim, W.W.S., 2010. Bulk organic  $\delta^{13}\text{C}$  and C/N as indicators for sediment sources in the Pearl River delta and estuary, southern China. *Estuarine, Coastal and Shelf Science*, 87, 618-630.

Yuan, D. X. 1992. Karst in southwestern China and its comparison with karst in North China *Quaternary Research*, (4): 352-361.

- Zhang, C., Liu, G. B., Xue, S., et al. 2013. Soil organic carbon and total nitrogen storage as affected by land use in a small watershed of the Loess Plateau, China. *European Journal of Soil Biology*, 54: 16–24.
- Zhang, J., Wang Xiu-jun, Wang Jia-ping and Wang Wei-xia. 2014. Carbon and Nitrogen Contents in Typical Plants and Soil Profiles in Yanqi Basin of Northwest China *Journal of Integrative Agriculture* 13(3): 648-656.
- Zhang, J., Wu, Y., Jennerjahn, T.C., Ittekkot, V., He, Q., 2007. Distribution of organic matter in the Chang jiang (Yangtze River) Estuary and their stable carbon and nitrogen isotopic ratios: implications for source discrimination and sedimentary dynamics. *Marine Chemistry*, 106, 111-126.
- Zhang, L., Chen, F. R., Yang, Y. Q., et al. 2008. Distinguish of sources of organic matter in sediment in Pearl Estuary and adjacent water. *Marine Environmental Science*, 27: 447–451.
- Zhong, Z., Chen, Z., Xu, Y., Ren, C., Yang, G., Han, X., Ren, G., and Feng, Y. 2018. Relationship between Soil Organic Carbon Stocks and Clay Content under Different Climatic Conditions in Central China. *Forests*, 9, 598; DOI:10.3390/f9100598.
- Zhu, S. F., Liu, C.Q. 2006. Vertical patterns of stable carbon isotope in soils and particle-size fractions of karst areas, Southwest China. *Environmental Geology*, 50:1119–1127.

## Tables

**Table 1 showing depth distribution with pH, soil density, TOC, TN, C/N, N, C and Clay proportion for the four studied soil profiles (SL1, SL2, XNC and DP) from karst area in Yunnan-Guizhou Plateau of southwestern China.**

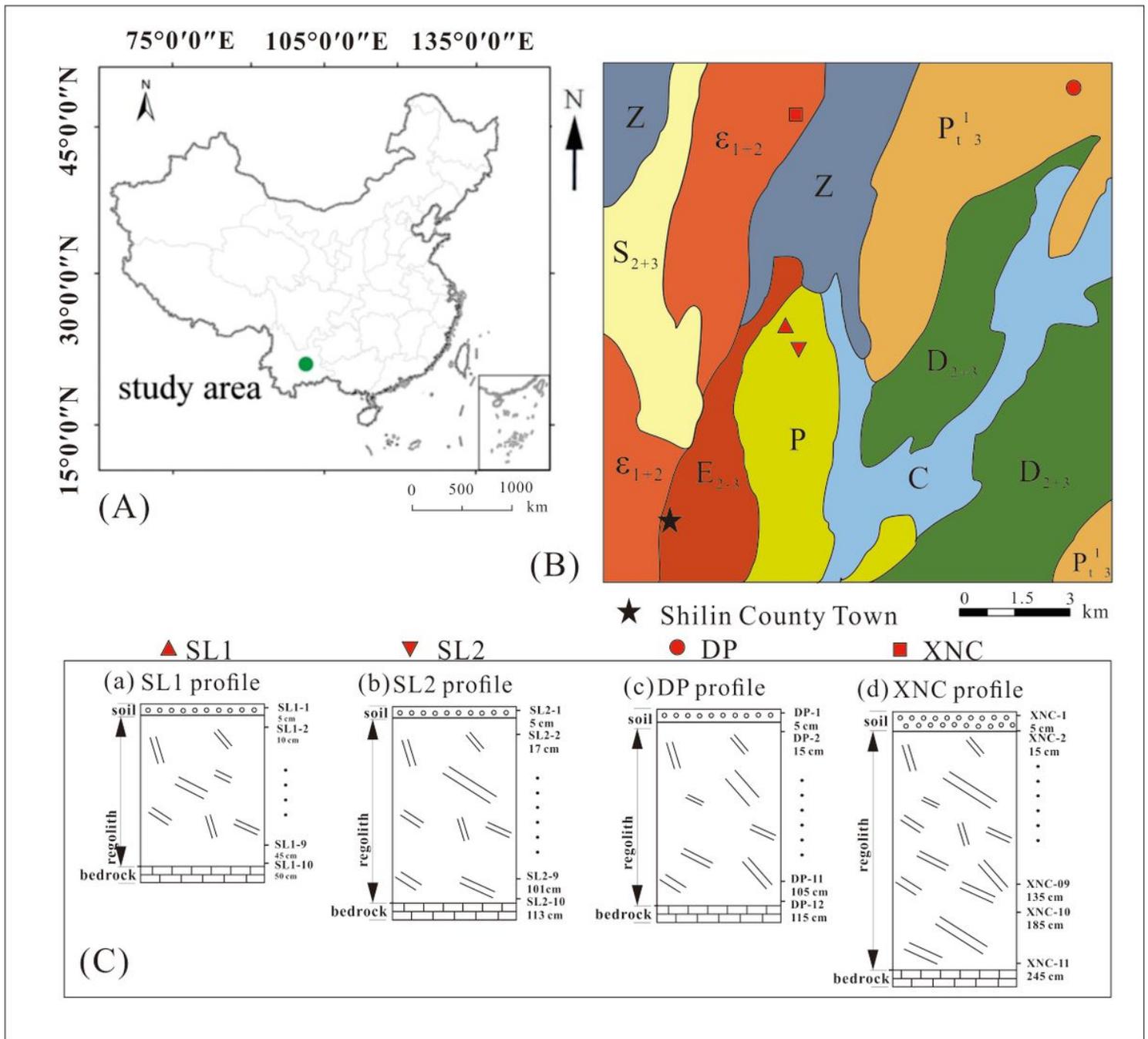
Soil profiles	Depth (cm)	pH	Density g/cm <sup>3</sup>	TOC /g·kg <sup>-1</sup>	TN /g·kg <sup>-1</sup>	C/N	δ <sup>15</sup> N	δ <sup>13</sup> C	Clay (%)
SL1-1	5	5.99	1.14	0.286	0.05	5.7	11.718	-16.831	8.02
SL1-2	10	6.03	1.83	2.255	0.168	13.4	14.369	-16.517	11.16
SL1-3	15	6.09	1.75	2.051	0.154	13.3	10.523	-16.008	12.37
SL1-4	20	6.08	1.89	1.676	0.142	11.8	10.757	-15.984	13.85
SL1-5	25	6.02	1.32	1.764	0.132	13.4	11.335	-16.204	15.88
SL1-6	30	6.02	2.01	1.56	0.122	12.8	10.438	-16.367	16.91
SL1-7	35	6.06	2.04	1.372	0.113	12.1	11.138	-16.219	19.66
SL1-8	40	6.04	1.61	1.41	0.111	12.7	14.855	-16.345	17.07
SL1-9	45	6	1.74	1.157	0.102	11.3	10.977	-16.683	12.75
SL1-10	50	5.95	1.58	0.953	0.087	11	12.791	-16.874	16.67
SL2-1	5	5.02	1.65	2.118	0.16	13.3	5.67	-20.698	6.68
SL2-2	17	5.07	1.16	0.729	0.072	10.2	6.128	-18.345	9
SL2-3	29	5.11	1.64	0.413	0.058	7.1	8.975	-18.103	8.26
SL2-4	41	5.22	1.76	0.351	0.051	6.9	9.074	-18.34	7.76
SL2-5	53	5.05	1.62	0.24	0.045	5.4	6.334	-19.307	10.91
SL2-6	65	5.09	1.68	0.153	0.037	4.2	9.676	-20.001	10.65
SL2-7	77	5.45	2.3	0.154	0.032	4.8	7.734	-20.314	9.1
SL2-8	89	5.58	1.42	0.158	0.038	4.1	9.415	-21.001	9.72
SL2-9	101	5.26	2.04	0.16	0.046	3.5	7.784	-20.83	7.78
SL2-10	113	5.17	1.83	0.145	0.043	3.3	6.821	-21.696	7.59
XNC-1	5	5.01	1.52	0.942	0.089	10.6	5.192	-20.268	7.24
XNC-2	15	5.36	1.75	0.712	0.08	8.9	6.2	-18.534	7.35
XNC-3	25	5.44	1.59	0.799	0.081	9.8	13.22	-15.642	8.14
XNC-4	35	5.77	1.4	0.257	0.049	5.2	5.098	-17.609	8.16
XNC-5	45	5.93	1.78	0.286	0.049	5.8	7.221	-16.573	7.91
XNC-6	55	6.07	1.84	0.185	0.046	4	6.182	-18.11	8.72
XNC-7	75	6.07	1.55	0.121	0.044	2.7	2.779	-21.626	8.67

XNC-8	95	6.06	1.94	0.154	0.047	3.3	6.116	-19.555	9.6
XNC-9	135	6.08	1.91	0.189	0.103	1.8	8.052	-21.894	7.8
XNC-10	185	5.63	1.91	0.118	0.047	2.5	5.218	-22.629	9.2
XNC-11	245	5.54	2.11	0.058	0.038	1.5	4.88	-25.824	4.12
DP-1	5	4.285	1.29	0.919	0.089	10.4	4.95	-21.181	39.5
DP-2	15	4.18	1.13	0.288	0.061	4.7	6.113	-21.837	36.72
DP-3	25	4.23	1.81	0.217	0.061	3.6	3.271	-22.533	40.93
DP-4	35	4.27	1.93	0.179	0.058	3.1	6.679	-23.047	41.25
DP-5	45	4.16	1.95	0.189	0.056	3.4	4.195	-23.162	40.58
DP-6	55	4.235	1.86	0.189	0.053	3.5	4.091	-24.439	41.76
DP-7	65	4.055	1.64	0.177	0.065	2.7	6.992	-24.29	50.63
DP-8	75	4.26	1.64	0.184	0.063	2.9	6.757	-24.754	31.77
DP-9	85	4.24	1.25	0.143	0.067	2.1	7.497	-23.437	36.6
DP-10	95	4.285	2.06	0.126	0.056	2.2	4.494	-23.64	26.28
DP-11	105	4.365	2.08	0.15	0.05	3	2.982	-24.434	28.23
DP-12	115	5.16	2.07	0.286	0.05	5.7	4	-23.561	25.3

**Table 2 showing average density, TOC, TN and C/N for the four studied soil profiles (SL1, SL2, XNC and DP) from karst area in Yunnan-Guizhou Plateau of southwestern China.**

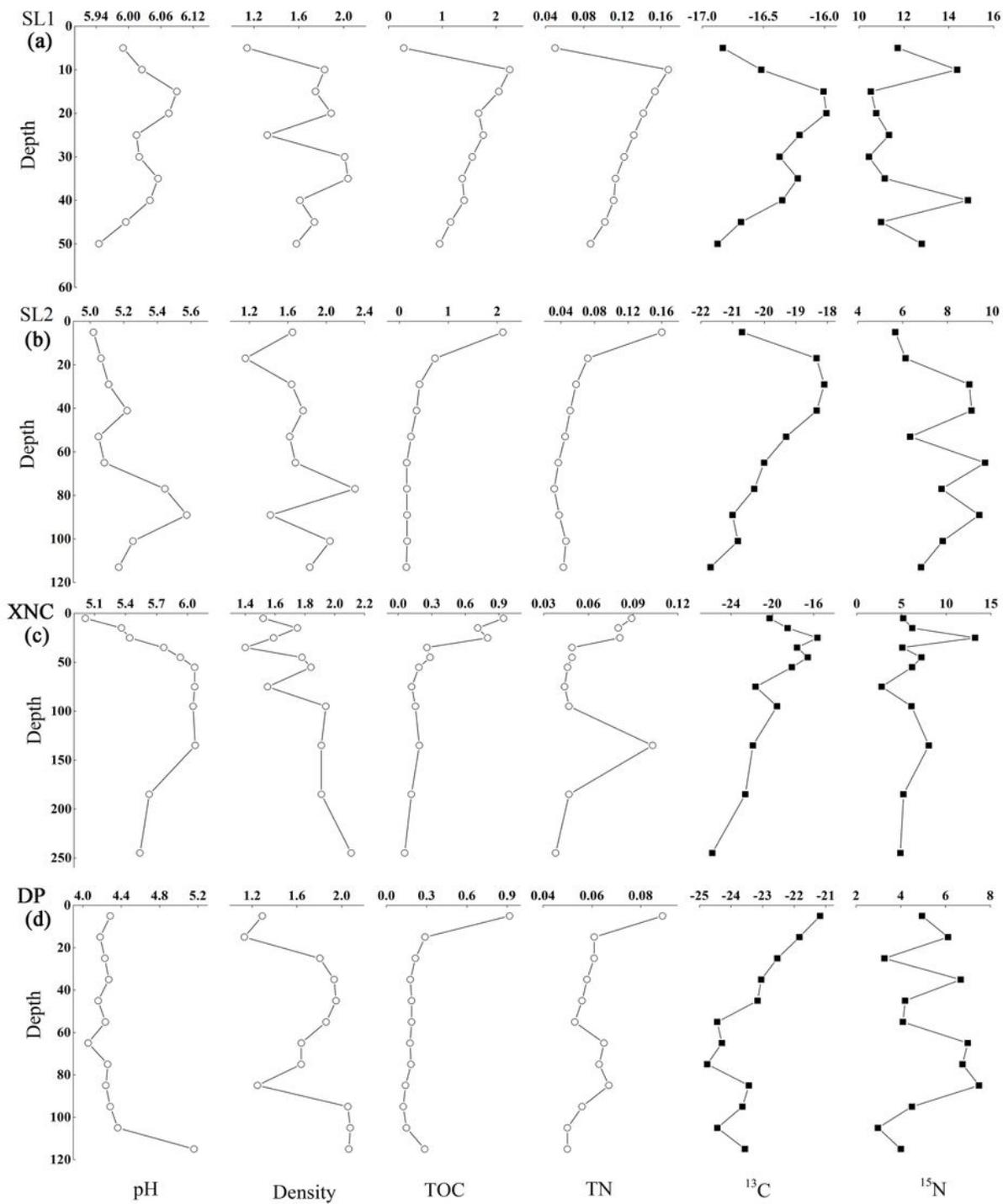
Soil profiles	Avg. density (g/cm <sup>3</sup> )	Avg. TOC (/g·kg <sup>-1</sup> )	Avg. TN (/g·kg <sup>-1</sup> )	Avg. C/N (/g·kg <sup>-1</sup> )
SL1	1.69±0.28 (n=10)	1.45±0.56 (n=10)	0.12±0.03 (n=10)	11.75±2.3 (n=10)
SL2	1.71±0.31 (n=10)	0.46±0.61 (n=10)	0.06±0.04 (n=10)	6.3±3.21 (n=10)
XNC	1.75±0.22 (n=11)	0.35±0.31 (n=11)	0.06±0.02 (n=11)	5.1±3.28 (n=11)
DP	1.72±0.34 (n=12)	0.25±0.21 (n=12)	0.06±0.01 (n=12)	4.0±2.26 (n=12)

## Figures



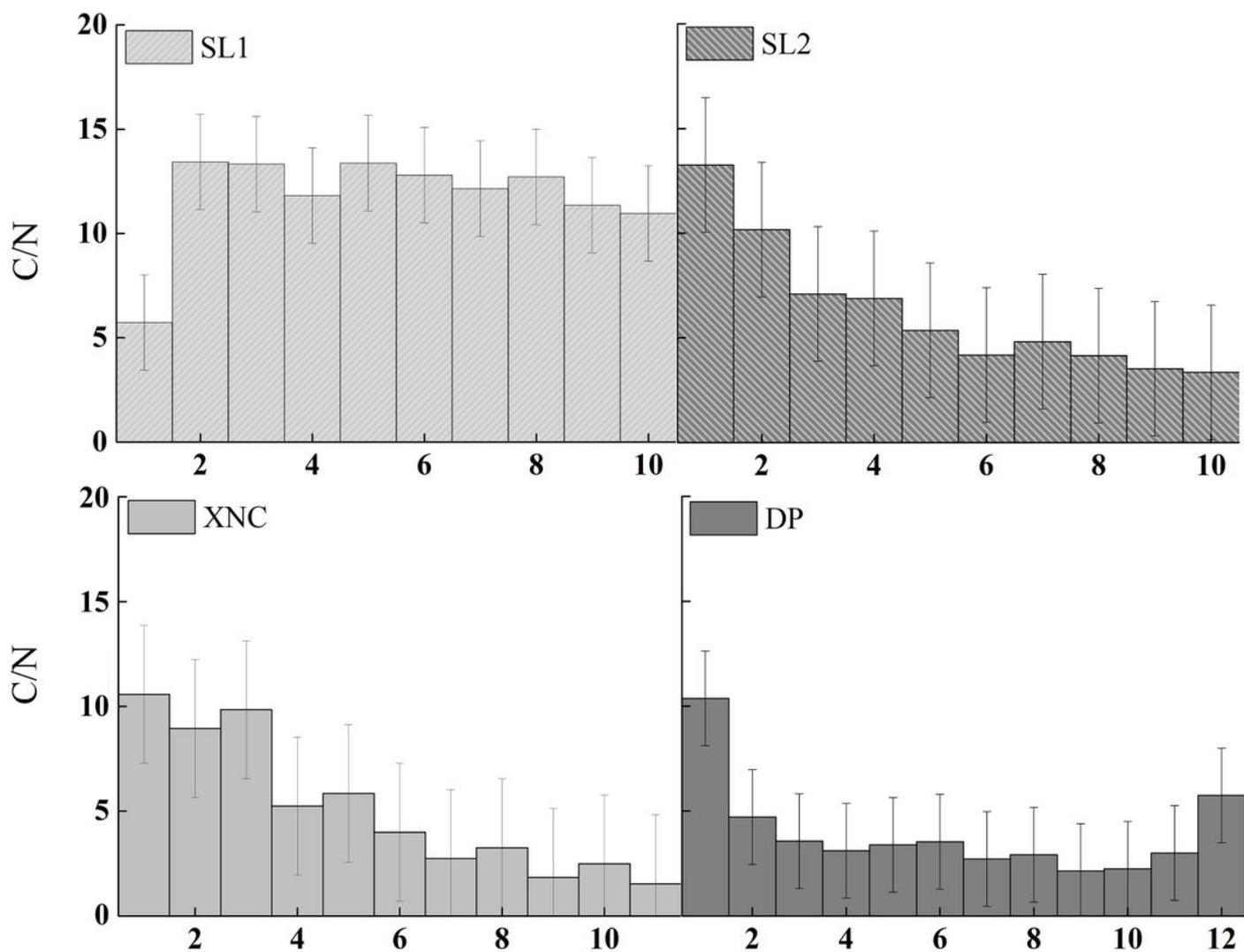
**Figure 1**

(a) Map showing location of study area and (b) lithology of sample profiles and (c) the schematic drawings of sample profiles at Yunnan-Guizhou Plateau, Southwestern China. Note: The designations employed and the presentation of the material on this map do not imply the expression of any opinion whatsoever on the part of Research Square concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. This map has been provided by the authors.



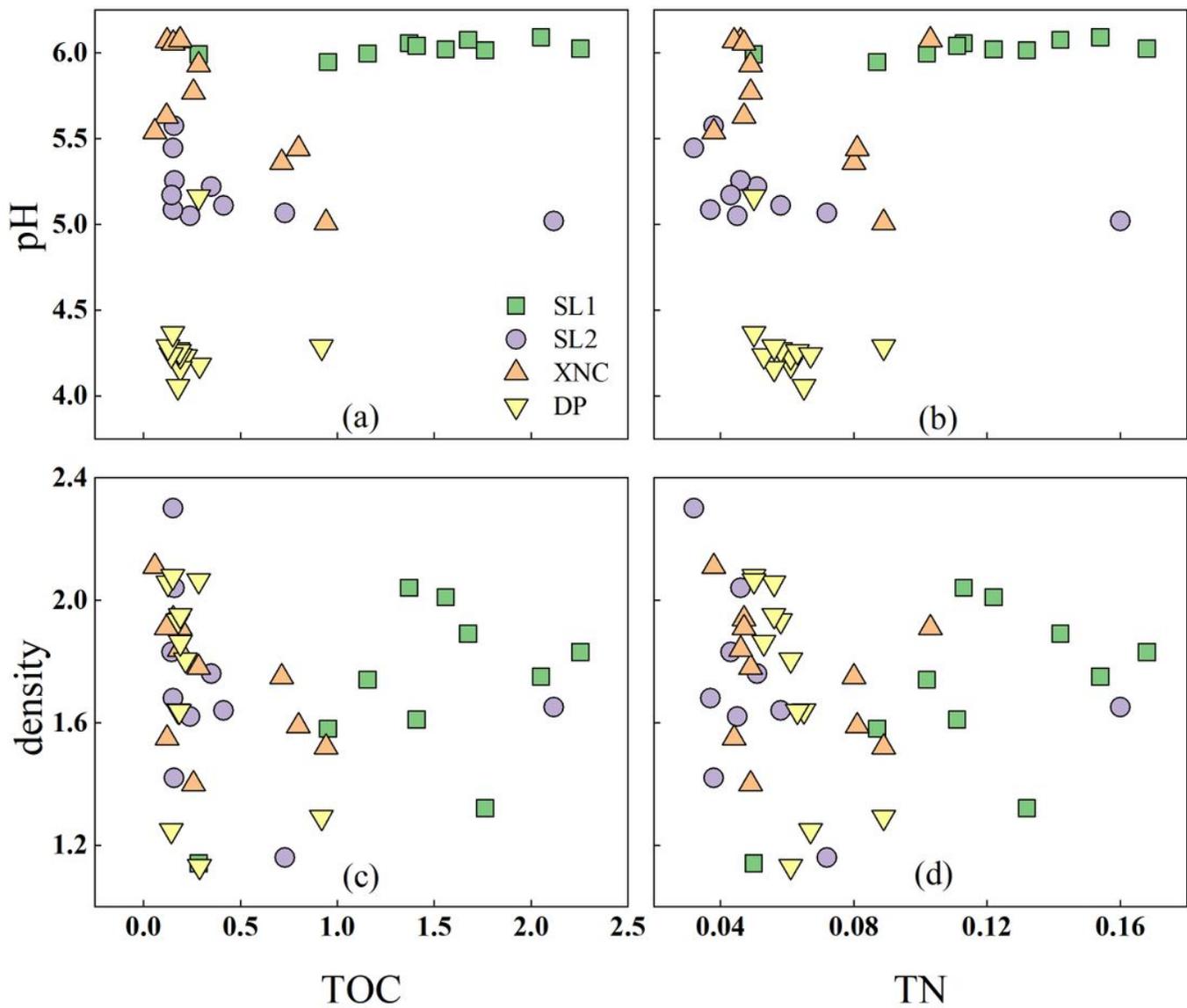
**Figure 2**

Depth profiles characteristics of pH, soil density, TOC, TN,  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  of (a) SL1, (b) SL2, (c) XNC and (d) DP soil profiles respectively, from karst area in Yunnan-Guizhou Plateau of southwestern China.



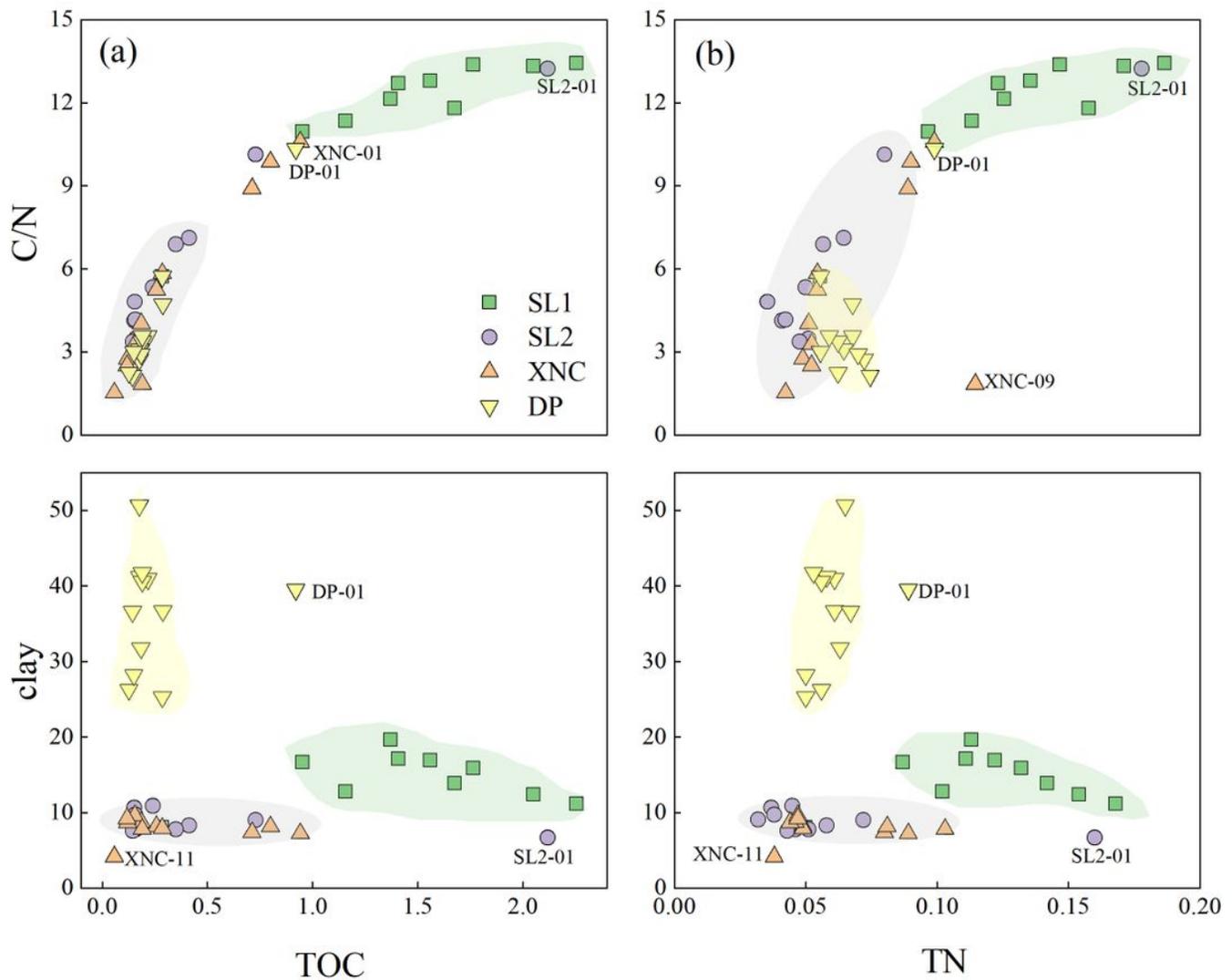
**Figure 3**

C/N ratio depth distribution of the four studied soil profiles (SL1, SL2, XNC and DP) from karst area in Yunnan-Guizhou Plateau of southwestern China.



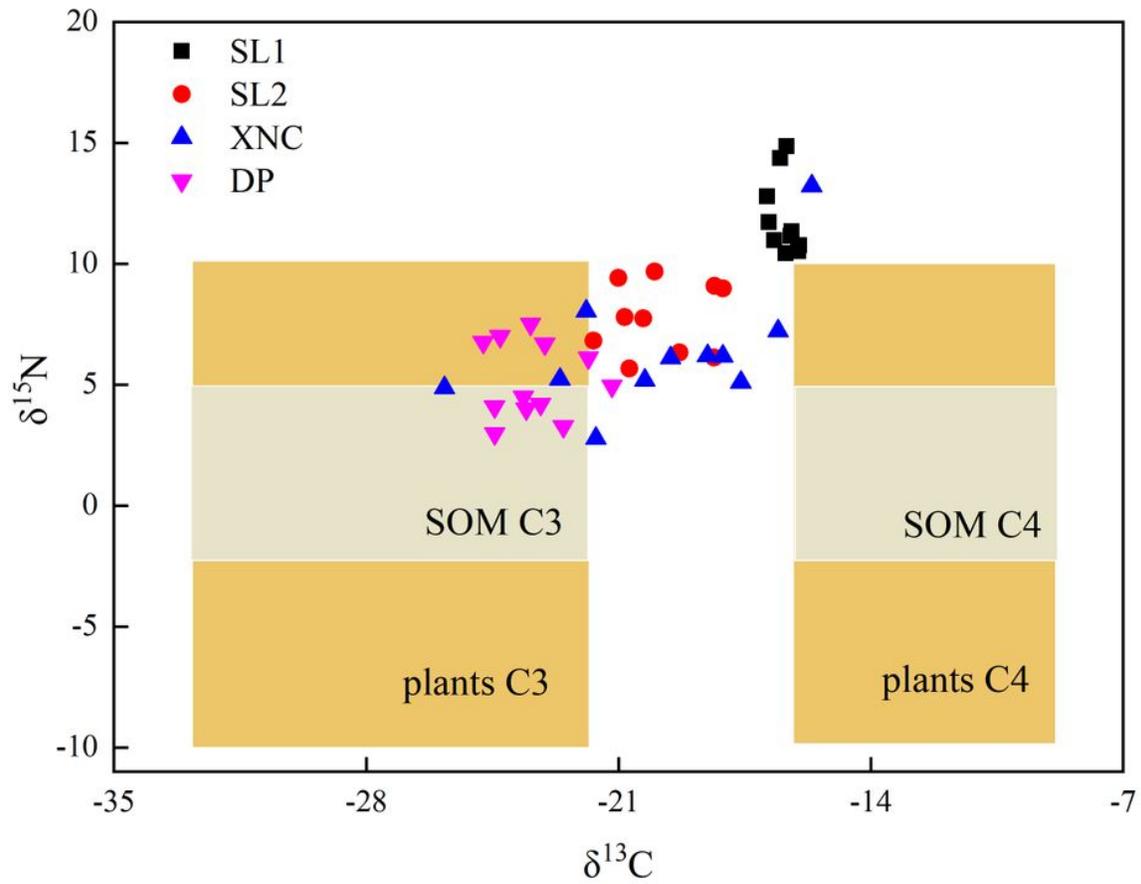
**Figure 4**

Relationship of soil pH with (a) TOC (b) TN and soil density with (c) TOC and (d) TN for the four studied soil profiles (SL1, SL2, XNC and DP) from karst area in Yunnan-Guizhou Plateau of southwestern China.



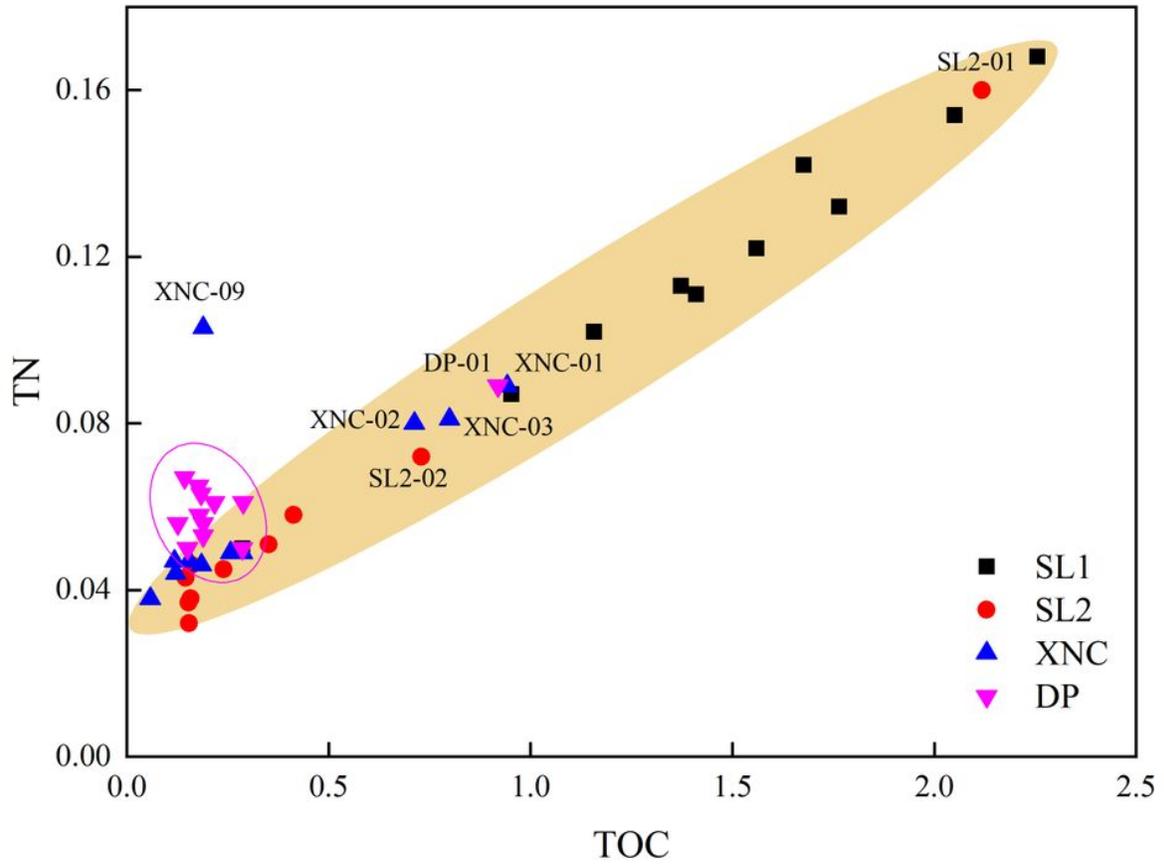
**Figure 5**

Relationship of C/N with (a) TOC and (b) TN also clay relationship with (c) TOC and (d) TN for the four studied soil profiles (SL1, SL2, XNC and DP) from karst area in Yunnan-Guizhou Plateau of southwestern China.



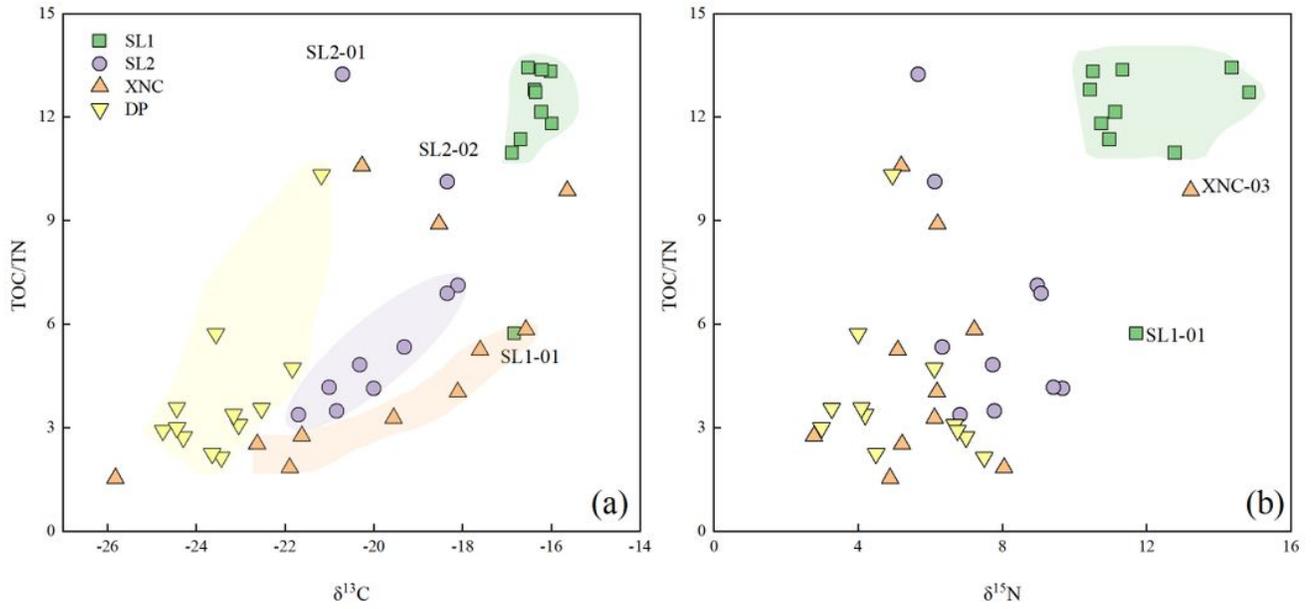
**Figure 6**

Relationship of  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  for the four studied soil profiles (SL1, SL2, XNC and DP) from karst area in Yunnan-Guizhou Plateau of southwestern China. Illustrating isotopic signature ranges of potential sources: terrestrial C3, C4 plants and soil organic matter, data taken from Kendall et al. 2001.



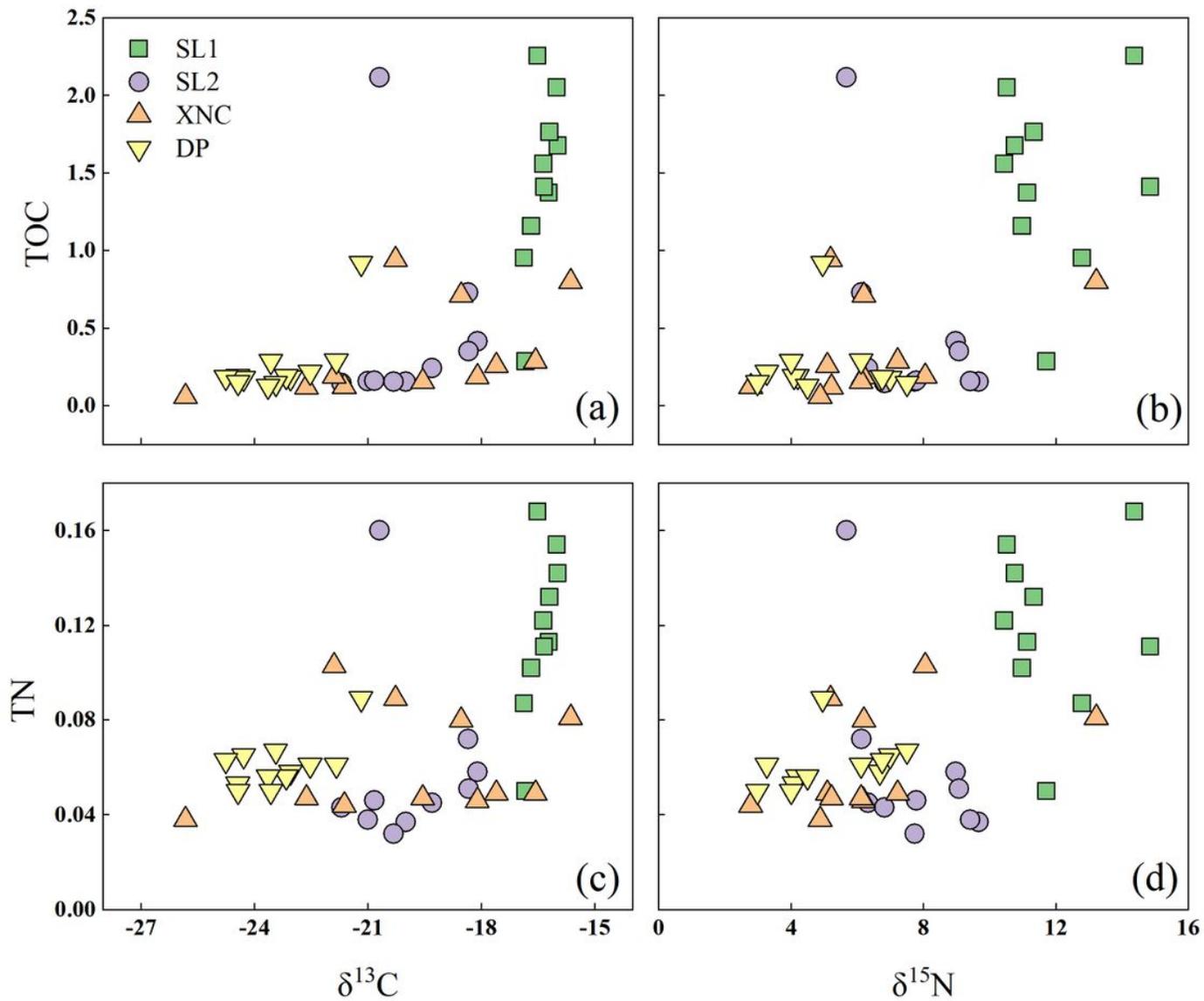
**Figure 7**

Relationship between TN and TOC for the four studied soil profiles (SL1, SL2, XNC and DP) from karst area in Yunnan-Guizhou Plateau of southwestern China.



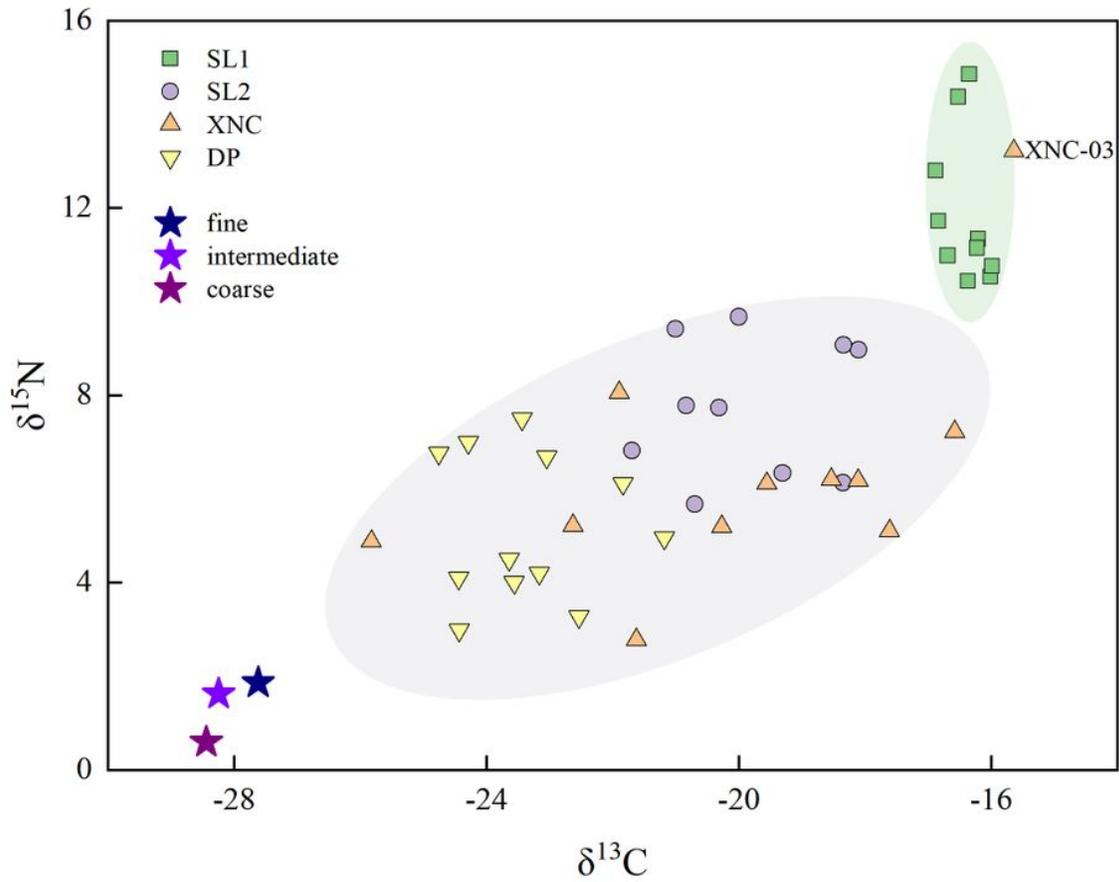
**Figure 8**

Relationship of TOC/TN (C/N) with (a)  $\delta^{13}\text{C}$  and (b)  $\delta^{15}\text{N}$  for the four studied soil profiles (SL1, SL2, XNC and DP) from karst area in Yunnan-Guizhou Plateau of southwestern China.



**Figure 9**

Relationship of TOC with (a)  $\delta^{13}\text{C}$  (b)  $\delta^{15}\text{N}$  and TN with (c)  $\delta^{13}\text{C}$  and (d)  $\delta^{15}\text{N}$  for the four studied soil profiles (SL1, SL2, XNC and DP) from karst area in Yunnan-Guizhou Plateau of southwestern China.



**Figure 10**

Relationship of  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  for the four studied soil profiles (SL1, SL2, XNC and DP) from karst area in Yunnan-Guizhou Plateau of southwestern China. Also showing natural undisturbed forest soil baseline data for  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ , with fine, intermediate and coarse grain compare with studied soil profiles indicating intensity of disturbance, additional forest soil data taken from Campbell et al. 2009.