Combined deep sampling and mass-based approaches to assess soil carbon and nitrogen losses due to land-use changes in karst area of Southwestern China

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Abstract. The conversion of natural vegetation to managed ecosystems may negatively influence soil organic carbon (SOC) and total nitrogen (TN) stocks, particularly in the fragile ecosystems. The objective of present study was to assess SOC and TN stocks losses by combining deep sampling with mass-based calculations upon land-use changes in a typical karst area of Southwestern China. We quantified the changes from native forest to grassland, secondary shrub, eucalyptus plantation, sugarcane and corn fields (both defined as croplands), on the SOC and TN stocks down to 100 cm depth using fixed-depth (FD) and equivalent soil mass (ESM) approaches. The results showed that converting forest to cropland and other types significantly led to SOC and TN losses, although the effect magnitude...
partly depended on both sampling depths and soil mass considered. On average, the shifting from native forest to cropland led to SOC losses by 19.1%, 25.1%, 30.6%, 36.8% and 37.9% for the soil depths of 0–10, 0–20, 0–40, 0–60 and 0–100 cm, respectively, which highlighted that shallow sampling underestimated SOC losses. Moreover, the FD method underestimated SOC and TN losses for the upper 40 cm layer, but overestimated the losses in the deeper profiles. We suggest that the ESM together with deep sampling should be encouraged to detect the differences in SOC stocks. In conclusion, the conversion of forest to managed systems, in particular croplands significantly decreased in SOC and TN stocks, although the effect magnitude to some extent depended on sampling depth and calculation approach selected.

**Keywords:** Land-use change, karst area, soil carbon and nitrogen stocks, sampling depth, equivalent soil mass

1 **Introduction**

Land-use change, like deforestation has become a significant concern in terms of environmental degradation and global climate change (Harris et al., 2012; Mukhopadhyay et al., 2016; Wiesmeier et al., 2015). Globally, the large-scale conversions of natural ecosystems to croplands and other managed ecosystems have already resulted in historically large emissions of C into the atmosphere (as higher as 320 Pg C), since the dawn of settled agriculture (Lal, 2010). In turn, land degradation due to soil organic C (SOC) loss may damage ecosystem services and functions (Brevik et al., 2015; Costantini et al., 2016;
Foley et al., 2005), directly affecting the hydrological and biogeochemical cycles in the earth system (Brevik et al., 2015; García-Díaz et al., 2016; Sonneveld et al., 2016). Thus, the changes in quality and quantity of SOC may inevitably influence the soil degradation, agricultural productivity and food security (Carter, 2002; Janzen, 2015; Srinivasarao et al., 2014).

Many studies have shown serious decreases of soil organic matter (SOM) pools owing to human activities, such as cultivation of soils under forest or natural vegetation (Lal, 2009; Post and Kwon, 2000). However, most soil C studies have focused only on the surface layers (i.e., ≤ 30 cm) to clarify the SOC in response to land-use changes (Baker et al., 2007; Post and Kwon, 2000; Wei et al., 2014; West and Post, 2002). Because more than one third of roots and more than one half of soil C are stored below 20 cm depth (Jobbágy and Jackson, 2000), the conversion of land use change may well influence soil C in the subsoils. Therefore, the soil C stored in the deeper layers may be heavily neglected, but little is known about the quantity of SOC at depth and how it responds to land management (Guo and Gifford, 2002; Lozano-García and Parras-Alcántara, 2014; Wei et al., 2014). A more complete and accurate evaluation of management effects on SOC stocks should involve collecting deeper depths to identify the real changes (Baker et al., 2007; Olson and Al-Kaisi, 2015; Parras-Alcántara et al., 2015).

The SOC stock is widely quantified to a fixed-depth (FD) as the product of soil bulk density (BD), depth and concentration. This FD method, however, has been considered to introduce substantial errors when soil BD differs between treatments (VandenBygaart and Angers, 2006). Instead, equivalent soil...
mass (ESM) approach is required to correct the calculation (Ellert and Bettany, 1995; Lee et al., 2009). The ESM method, which can account for the differences in soil masses among treatments, is being increasingly employed (Don et al., 2011; Wiesmeier et al., 2015). As land-use conversions are often associated with the changes in soil BD, depth-based method would result in considerable differences in soil masses for the quantification of SOC stocks.

Karst is a distinctive topography, and the rocky desertification has become a serious environmental issue in this landscape of Guangxi Province, Southwestern China (Wang et al., 2004; Zhao et al., 2014). The typical karst areas are characterized by high fragility own to their natural settings and anthropogenic impacts (Xu and Zhang, 2014). This, in turn, has not only damaged eco-environment including soil erosion, lower biodiversity and decreased soil productivity, but also exacerbated the poverty level in the rural areas of the region (Hu et al., 2014; Liu et al., 2005; Wang et al., 2004). Thus, karst area is considered as small environment capacity, weak anti-disturbance, low stability and powerless self-adjustment.

Since the late 1990s, China initiated the Grain for Green project, and the karst region of Southwest China is one of the main regions involved in this project. Accordingly, the Guangxi governments had implemented an environmental resettlement program that transferred about 4 million people from this rocky region to the “in-migration” areas (Hu et al., 2008). The natural and land ecosystems in the in-migration areas exerted by ecological migrants might be negatively influenced. It is critically
important to evaluate the changes in SOM status, which are accompanied by changes in ecosystem services, processes, and functions (Carter, 2002; Smith et al., 2015). We hypothesized that the ecological resettlement program may deplete SOM stocks, and thus soil degradation. Recently, some researchers have paid more attention about the effect of land use change on the soil physicochemical properties and soil quality in this karst region (Fu et al., 2015; Hu et al., 2014; Xie et al., 2014; Xu et al., 2008). However, studies on soil C dynamics were mainly focused on the surface layers (e.g., ≤ 30 cm) and the FD method are utilized to assess SOC stocks (Chen et al., 2012; Fu et al., 2015; Liu et al., 2015b; Zhang et al., 2012). Very little effort has been devoted to the effect of land conversion on SOC stocks in the deeper soils. Thus, the present study was aimed to (1) quantify the changes in SOC and TN stocks down to 100 cm after conversion from native forest to croplands and other managed systems; (2) to examine whether the calculation methodology (i.e., equivalent soil mass vs. fixed depth) may change the interpretation of the results.

2 Materials and methods

2.1 Study area

This study was conducted at the Huanjiang County (107°51′–108°43′ E, 24°44′–25°33′ N), Guangxi Province, Southwestern China (Fig. 1). This county is surrounded by mountains, and has a total land area of 4 572 km² with elevation ranging from 149 to 1 693 m. The natural vegetation mainly consisted of shrubs, herbs and lianas. The region is characterized by a subtropical monsoon climate with a distinct
wet (from Apr to Sep) and dry season (from Oct to Mar). The annual mean temperature is 19.9 °C, and
the annual average precipitation is 1750 mm. The rainfall was uneven distributed and concentrated
during rainy seasons (about 70 %) from April to September. In this area, the soil is calcareous and has
been mainly developed from limestone parent materials.

Figure 1. Location of the study area and the distribution of soil sampling sites in northwest Guangxi, Southwestern
China
Since the 1999, the Huanjiang county had received about 70,000 environment migrants, and became the largest environmental resettlement region in the Southwestern karst area. In this region, we selected five ecosystems, i.e., native forest (NF), unused grassland (UG), secondary shrubland (SS), eucalyptus forest (EF) and croplands (including sugarcane field: SF and corn field: CF). The later four ecosystems are all originated from the native forest due to deforestation or land-use change. All the five ecosystems selected are adjacent to each other. Within the each land use type, five plots (about 0.067–0.10 ha) were chosen. All sites were located on the similar physiographical conditions (e.g., with slope about 15–25 °, and elevation) and the distance between sampling sites ranged about from 250 to 350 m. The detailed information for the land use types including vegetation, dominant plant species and management history are listed in Table 1.

Table 1. The general information of land-use types selected in this study

<table>
<thead>
<tr>
<th>Land-use types</th>
<th>Dominant species</th>
<th>Vegetation form</th>
<th>Management history</th>
</tr>
</thead>
<tbody>
<tr>
<td>Native forest (NF)</td>
<td>Platycarya longipes; Carpinus pubescen; Lithocarpus confines; Itea yunnanensis</td>
<td>Forest</td>
<td>Undisturbed natural mixed deciduous and evergreen broadleaved forest</td>
</tr>
<tr>
<td>Unused grassland, (UG)</td>
<td>Themeda japonica; Heteropogon ontortu</td>
<td>Grassland</td>
<td>Deforested and cultivated until 2005, natural recovery to grass</td>
</tr>
<tr>
<td>Secondary shrub, (SS)</td>
<td>Caesalpinia decapetala; Bauhinia championi; Pyracantha florineana</td>
<td>Shrub land</td>
<td>Deforested and cultivated until 2002, then naturally recovered to secondary forest</td>
</tr>
<tr>
<td>Eucalyptus forest, (EF)</td>
<td>Eucalyptus robusta Smith</td>
<td>Eucalyptus</td>
<td>Deforested and converted to eucalyptus forest in the 1990s</td>
</tr>
<tr>
<td>Sugarcane field</td>
<td>108 Saccharum</td>
<td>Sugarcane</td>
<td>Deforested and cultivated in the 1990s</td>
</tr>
<tr>
<td>------------------</td>
<td>---------------</td>
<td>-----------</td>
<td>---------------------------------------</td>
</tr>
<tr>
<td>(SF) Corn field</td>
<td>109 Zea mays</td>
<td>(CF) Corn</td>
<td></td>
</tr>
</tbody>
</table>

### 2.2 Soil samples collection and analysis

All samples were taken from July to August 2012. Before soil sampling, each plot was divided into five subplots and samples were collected using a auger (4.1 cm diameter) down to 100 cm (0–10, 10–20, 20–40, 40–60, 60–80, and 80–100 cm) after removing the litter layer if available. The five samples were taken randomly in an “S” form at each sampling subplot, and then these five samples were composited together to gain a representative sample at each depth. Soil bulk density (BD) was measured in all six layers by the core method using metal cylinders (5 cm high and 5 cm in diameter). The core samples were oven-dried at 105 °C for 24 h and then weighed. A total of 150 subplots were investigated, and 180 soil samples were collected. All samples were sieved through a 2-mm screen, and roots and other coarse debris fraction were removed. A portion of the samples were air-dried and stored at room temperature for soil physicochemical analysis. Another portion of samples for microbial biomass mass analyses was stored at 4 °C for no longer than one week before analyzing.

Soil organic C was analyzed with the Walkley and Black's dichromate oxidation method and total soil N was determined via the semi-micro Kjeldahl digestion procedure. Soil available phosphorus (AP) was extracted with 0.5 M NaHCO₃ at a pH of 8.5 and was analyzed with a colorimetric method. The above...
mentioned soil C, N and P analysis was measured according to (Bao, 2000). Soil texture analysis was determined by a pipette method and the soil particle was divided into three fractions according to USDA classification system: sand 2–0.05 mm, silt 0.05–0.002 mm and clay < 0.002 mm. The soil microbial biomass carbon (SMC) were conducted by chloroform fumigation–K₂SO₄ extraction carbon automatic analysis (Wu et al., 2006).

2.3 Soil organic C and total N calculations

We calculated SOC and TN stocks on both FD and ESM basis. The principle of ESM method is given by (Ellert and Bettany, 1995; Lee et al., 2009). When soils are sampled to the designated depth \((i = 1, \ldots, n)\), the soil mass is calculated as:

\[
M_i = BD_i \times Z_i \times 100 \quad (1)
\]

where \(M_i\) is dry soil mass (Mg ha\(^{-1}\)), \(BD_i\) is soil bulk density (g cm\(^{-3}\)), \(Z_i\) is the thickness of the \(i\)th soil layer (cm), and 100 is a unit conversion factor. The SOC stock in the fixed depth was calculated as:

\[
SO C_{i, fixed} = Con_i \times M_i \times 0.1 \quad (2)
\]

where \(SOC_{i, fixed}\) is the SOC mass to a fixed layer (Mg ha\(^{-1}\)) and \(Con_i\) is the SOC concentration (g kg\(^{-1}\)), 0.1 is a unit conversion factor. Hence, the equivalent C mass (Mg ha\(^{-1}\)) in a soil layer is calculated as:

\[
M_{i, ex} = M_i - M_{i, equiv} \quad (3)
\]
where $SOC_{i,\text{equiv}}$ is the equivalent SOC mass (Mg ha$^{-1}$), $M_{i,\text{equiv}}$ is the selected equivalent soil mass, $M_{i,\text{ex}}$ is the excess soil mass used to attain the ESM, $Con_{\text{bottom}}$ is SOC concentration in the deepest soil core segment (g C kg$^{-1}$ soil) (core segment = n), 1000 is a unit conversion factor. The TN stock was calculated using the same procedure as described above. We designated the lightest soil mass among the treatments for the specific layer as equivalent soil mass according to (Lee et al., 2009).

### 2.4 Statistical analysis

Analysis of variance was performed using the SPSS 11.0 software (SPSS, 2001) to compare the differences in SOC and TN concentrations and stocks, SMC, AP, and soil BD among different land use types or soil depths. The means were compared using Fisher’s protected least significant difference (LSD). Unless otherwise stated, all differences discussed are significant at the $P < 0.05$ probability level.

### 3 Results

#### 3.1 Selected soil physicochemical properties

For the 0–40 cm depth, the averaged SOC concentration was in the order of NF < UG < SS = EF, and SS > SF = CF, indicating deforestation significantly reduced SOC level, particularly converted into the croplands (Table 2).
Table 2. Selected soil physicochemical properties for the different land-use types (0–40 cm).

<table>
<thead>
<tr>
<th>Variable</th>
<th>NF</th>
<th>UG</th>
<th>SS</th>
<th>EF</th>
<th>SF</th>
<th>CF</th>
<th>F value</th>
</tr>
</thead>
<tbody>
<tr>
<td>SOC (g kg$^{-1}$)</td>
<td>15.76a</td>
<td>13.95b</td>
<td>12.40c</td>
<td>11.47cd</td>
<td>11.28d</td>
<td>10.99d</td>
<td>25.02**</td>
</tr>
<tr>
<td>TN (g kg$^{-1}$)</td>
<td>1.53a</td>
<td>1.25b</td>
<td>1.17b</td>
<td>1.02c</td>
<td>1.02c</td>
<td>0.98c</td>
<td>22.35**</td>
</tr>
<tr>
<td>AP (mg g$^{-1}$)</td>
<td>0.129</td>
<td>0.109a</td>
<td>0.091a</td>
<td>0.088a</td>
<td>0.104a</td>
<td>0.097a</td>
<td>2.46ns</td>
</tr>
<tr>
<td>SMC (mg kg$^{-1}$)</td>
<td>257a</td>
<td>134b</td>
<td>143b</td>
<td>111b</td>
<td>106b</td>
<td>119c</td>
<td>24.64*</td>
</tr>
<tr>
<td>BD (g cm$^{-3}$)</td>
<td>1.36a</td>
<td>1.41a</td>
<td>1.48a</td>
<td>1.42a</td>
<td>1.32a</td>
<td>1.48a</td>
<td>1.99ns</td>
</tr>
<tr>
<td>Clay (%)</td>
<td>21.3</td>
<td>21.9</td>
<td>24.8</td>
<td>25.5</td>
<td>31.0</td>
<td>30.4</td>
<td>–</td>
</tr>
<tr>
<td>Silt (%)</td>
<td>20.1</td>
<td>18.9</td>
<td>25.2</td>
<td>27.0</td>
<td>24.2</td>
<td>24.9</td>
<td>–</td>
</tr>
<tr>
<td>Sand (%)</td>
<td>58.6</td>
<td>59.2</td>
<td>50.0</td>
<td>47.5</td>
<td>44.8</td>
<td>44.7</td>
<td>–</td>
</tr>
<tr>
<td>Soil texture</td>
<td>Sandy Clay Loam</td>
<td>Sandy Clay Loam</td>
<td>Sandy Clay Loam</td>
<td>Sandy Clay Loam</td>
<td>Clay Loam</td>
<td>Clay Loam</td>
<td>–</td>
</tr>
</tbody>
</table>

Note: NF, native forest; UG, unused grassland; SS, secondary shrub; EF, eucalyptus forest; SF, sugarcane field; CF, corn field; SOC, soil organic carbon content; TN, total nitrogen; AP, available phosphorus; SMC, soil microbial biomass carbon concentration. BD, soil bulk density; Clay, soil clay (< 0.002 mm); silt, soil silt (0.05–0.002 mm); sand, soil sand (2.0–0.05 mm); Different letters following values indicate significance at $P = 0.05$; ns, *, **, no significant difference at $P = 0.05$, <0.05, <0.01, respectively.

Similarly, the highest TN concentration was found under NF, and followed by UG and SS, and the lowest was EF, SF and CF. The SMC concentration was also different among the six vegetation types ($P < 0.05$), and were significantly greater in the native forest than in the other land-use types. However, the averaged soil BD and available phosphorus (AP) under different ecosystems did not exhibit significantly differences (Table 2). The clay and silt contents slightly ranged from 21.3 % to 30.4 %, and...
18.9% to 27.0%, respectively among the six land-use types. Except for the two agricultural soils (SF and CF) which belonged to the clay loam soil, other four land-use types all were sandy clay loam soils.

### 3.2 Soil BD, SOC and TN concentrations across soil profile

Two-way ANOVA showed that land use types and soil depths significantly impacted soil BD, SOC and TN concentrations and stocks ($P < 0.001$; Table 3). We also observed their interactive effects for all the measured parameters ($P < 0.001$; only for soil BD, $P = 0.03$).

**Table 3.** Results ($P$-values) of two-way ANOVAs on the effects of land use types, soil depth and their interaction on soil bulk density (BD), soil organic C (SOC) and total nitrogen (TN) concentrations and stocks.

<table>
<thead>
<tr>
<th></th>
<th>BD</th>
<th>SOC concentration</th>
<th>TN concentration</th>
<th>SOC stock</th>
<th>TN Stock</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Land use</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SF</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>CF</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>SS</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>NF</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td><strong>Soil depth</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0–10 cm</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>10–20 cm</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>40–150 cm</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td><strong>Land use x soil depth</strong></td>
<td>0.03</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

Soil BD was higher under CF and SS than that of NF in 0–10 cm layer. In the 10–20 cm depth, the highest value was found in the CF soil, while the lowest occurred under SF. Below the 40 cm layer, limited differences were observed among different land use types (Fig. 2). Soil BD generally ranged from 1.22 to 1.69 g cm$^{-3}$ from surface to bottom layers, showing increasing tendency across soil profile irrespective of land use types (Fig. 2). Overall, soil BD was lower in upper 0–40 cm layers as compared with that in the deeper layers. The changes in soil BD across the soil profile might bias the
interpretation of SOC stocks calculated on the FD basis.

**Figure 2.** Changes in soil bulk density along soil profile under different land use types (NF, native forest; UG, unused grassland; SS, secondary shrub; EF, eucalyptus forest; SF, sugarcane field; CF, corn field). Error bars represent LSD ($P < 0.05$) for comparison among treatments at the same depth.
There was a significant interaction between land use types and depths on SOC and TN concentrations ($P < 0.001$; Table 3). Overall, SOC and TN concentrations generally decreased with depths across soil profile (Fig. 3).

Figure 3. Vertical distribution of soil organic C (a) and total N (b) concentrations as influenced by changes in land use types (NF, native forest; UG, unused grassland; SS, secondary shrub; EF, eucalyptus forest; SF, sugarcane field; CF, corn field). Error bars represent LSD ($P < 0.05$) for comparison among treatments at the same depth.

The highest value was observed in NF soil, while the differences among NF, UG and SS soils was not
significant ($P > 0.05$) in the 0–10 cm layer. Afterwards, a sharp and significant decline happened in the 10–20 cm layer (Fig. 3). In the 20–40 cm layer, SOC concentration under NF and UG were greater than those in other ecosystems, whereas no changes in SOC and N concentrations existed between SS, EF, SF and CF soils. Similarly, the TN concentration followed the order of NF > UG = SS > EF = SF = CF.

At the deeper layers (i.e., 40–60, 60–80, and 80–100 cm), both SOC and TN concentrations greatly decreased from native forest to other managed ecosystems (Fig. 3).

### 3.3 Soil organic C and TN stocks calculated on FD and ESM basis

The SOC and TN stocks calculated on FD and ESM basis are presented in Table 4. On the FD basis, no differences in SOC stock between land use types were found in the 0–10 cm layer ($P > 0.05$; Table 4), except for the SS, which had higher SOC stock than that of SF. In the 0–20 cm layer, NF had higher SOC stock by 31.8 % and 23.0 % than that of SF and CF treatments, respectively. Taken together the 0–40 cm depth, the SOC stock was in the order of NF = UG > SS = EF, and SS > SF = CF, and NF increased SOC by 50.2 % and 36.4 % than SF and CF respectively. Instead, for the 0– 60 cm profile, SOC stock showed a trend of NF > UG > SS > EF = SF = CF, and the SOC under NF was higher by 14.0 %, 25.5 %, 49.6 %, 64.4 % and 59.1 % than that of UG, SS, EF, SF and CF, respectively. Similar trend of SOC stock induced by land use conversion was found in the 0–100 cm profile. These data indicated that the SOC stocks changes heavily depended on the soil sampling depth considered. The TN
at different soil depths shares similar pattern with the SOC in these ecosystems.

**Table 4.** Soil organic C (SOC) and total N (TN) stocks calculated using fixed-depth (FD) and equivalent soil mass (ESM) methods in different soil depths under land-uses types (NF, native forest; UG, unused grassland; SS, secondary shrub; EF, eucalyptus forest; SF, sugarcane field; CF, corn field).

<table>
<thead>
<tr>
<th>Land use types</th>
<th>Soil depth cm</th>
<th>FD-based stock Mg ha⁻¹</th>
<th>ESM-based stock Mg ha⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>SOC</td>
<td>TN</td>
</tr>
<tr>
<td>NF†</td>
<td>0–10</td>
<td>23.48ab</td>
<td>2.18a</td>
</tr>
<tr>
<td>UG</td>
<td>22.55ab</td>
<td>2.00ab</td>
<td>1265ab</td>
</tr>
<tr>
<td>SS</td>
<td>24.39a</td>
<td>2.06ab</td>
<td>1362a</td>
</tr>
<tr>
<td>EF</td>
<td>20.27ab</td>
<td>1.76b</td>
<td>1346ab</td>
</tr>
<tr>
<td>SF</td>
<td>19.85b</td>
<td>1.70b</td>
<td>1267ab</td>
</tr>
<tr>
<td>CF</td>
<td>21.55ab</td>
<td>1.79b</td>
<td>1394a</td>
</tr>
<tr>
<td>NF</td>
<td>0–20</td>
<td>43.97a</td>
<td>4.25a</td>
</tr>
<tr>
<td>UG</td>
<td>40.21ab</td>
<td>3.58b</td>
<td>2695ab</td>
</tr>
<tr>
<td>SS</td>
<td>39.49bc</td>
<td>3.49bc</td>
<td>2864ab</td>
</tr>
<tr>
<td>EF</td>
<td>34.43d</td>
<td>3.02cd</td>
<td>2744ab</td>
</tr>
<tr>
<td>SF</td>
<td>33.37d</td>
<td>2.96d</td>
<td>2589b</td>
</tr>
<tr>
<td>CF</td>
<td>35.73d</td>
<td>3.13bcd</td>
<td>2924a</td>
</tr>
<tr>
<td>NF</td>
<td>0–40</td>
<td>83.00a</td>
<td>8.09a</td>
</tr>
<tr>
<td>UG</td>
<td>76.20a</td>
<td>6.83b</td>
<td>5771ab</td>
</tr>
<tr>
<td>SS</td>
<td>68.54b</td>
<td>6.81b</td>
<td>6035a</td>
</tr>
<tr>
<td>EF</td>
<td>62.23bc</td>
<td>5.60c</td>
<td>5801ab</td>
</tr>
<tr>
<td>SF</td>
<td>55.28c</td>
<td>5.09c</td>
<td>5357b</td>
</tr>
<tr>
<td>CF</td>
<td>60.84c</td>
<td>5.52c</td>
<td>5982a</td>
</tr>
<tr>
<td>NF</td>
<td>0–60</td>
<td>123.69a</td>
<td>11.76a</td>
</tr>
<tr>
<td>UG</td>
<td>108.46b</td>
<td>9.53b</td>
<td>8945ab</td>
</tr>
<tr>
<td>SS</td>
<td>98.29c</td>
<td>9.65b</td>
<td>9301a</td>
</tr>
<tr>
<td>EF</td>
<td>82.66d</td>
<td>7.47c</td>
<td>8974ab</td>
</tr>
<tr>
<td>SF</td>
<td>75.24d</td>
<td>6.88c</td>
<td>8304b</td>
</tr>
</tbody>
</table>
†Values followed by a different lowercase letter in the same row among tillage treatments are significantly different at $P < 0.05$. The bold form number for soil mass indicated the equivalent soil mass was chosen.

On the ESM basis, the highest SOC stock stored in the NF soil of the 0–10 cm layer ($\approx 1220$ Mg ha$^{-1}$). Compared with NF, the SOC stocks in this layer was reduced by 7.3 % under UG, 6.9 % under SS and 19.6 % under CF, though the differences between NF, UG and SS were not significant ($P > 0.05$) (Table 4). For the 0–20 cm depth ($\approx 2582$ Mg ha$^{-1}$), the SOC stock showed an order of NF > UG = SS, and UG > EF = SF = CF. Specifically, SOC stock was lower by 11.7 %, 16.7 % and 20.6 % following the shift from NF to UG, SS and CF respectively. Under the 0–40 cm profile selected ($\approx 5357$ Mg ha$^{-1}$), the averaged SOC stock under NF reduced by 10.8 % under UG, 22.1 % under SS and 30.4 % under CF soil. When deeper profile was considered ($\geq 60$ cm), for example, the SOC stored in the NF of 0–60 cm soil was reduced by 24.1 % under SS and 37.7 % under CF. For the whole 0–100 cm profile, the conversion from NF to SS and CF decreased SOC stocks by 26.8 % and 39.8 %, respectively. TN stocks followed similar patterns to those of SOC stocks. These data indicated that sampling depth considered may impact the interpretation of SOC losses upon the land use conversion.
4 Discussion

4.1 Sampling depth for assessing SOC and TN stocks

According to our study, the SOC and TN stocks markedly decreased following native forest conversion to managed ecosystems by human activities (Table 4; Fig. 3). These results are in line with findings that soil C and N stored in natural ecosystems are more than that in the converted lands (de Moraes Sá et al., 2015; Don et al., 2011; Fialho and Zinn, 2014; Guo and Gifford, 2002). In karst region of Southwestern China, similar results were also reported by other studies (Chen et al., 2012; Liu et al., 2015a).

Consequently, the depletion of SOM may be directly related to the loss of ecosystem services and land productivity.

It is worth noting that the effect magnitude of SOC and TN stocks losses by land-use change might partly depend on sampling depth considered. For example, the conversion from NF to croplands (including EF and CF) had led to SOC loss by 19.1 %, 25.1 % and 30.6 % in the 0–10, 0–20, and 0–40 cm layers, respectively (Table 4). Moreover, the decreased SOC stock loss under NF relative to CF was 36.8 % in the 0–60 cm depth and 37.9 % in the 0–100 cm whole profile. The data clearly suggest that the SOC loss due to land use changes generally increased with depth. We recommend that shallow sampling may bias the interpretation of soil C data, and should be paid more attention in further study. Our results are supported by other studies (Baker et al., 2007; Olson and Al-Kaisi, 2015). Otherwise, if
the shallow layers of soil profile are only collected, the SOC loss following land use changes might be underestimated to some extent.

In the karst area, most early reports have focused on the top soil layer (e.g., ≤ 30 cm) to assess the SOC stocks changes (Chen et al., 2012; Fu et al., 2015; Liu et al., 2015b; Zhang et al., 2012). These above mentioned works and other superficial studies on how land use change may alter SOM dynamics (Post and Kwon, 2000; West and Post, 2002), may lead to erroneous results. As a result, the whole soil profile, rather than the tilled layer, should be sampled to capture the possible differences in SOC stocks upon land use change. Lal (2009) considered that it is important to measure the land use induced changes in SOC to at least 1 m, preferably 2 m depth in forest systems where deep tap roots may transfer biomass C to deeper layers.

4.2 Changes in SOC and TN stocks calculated by FD and ESM approaches

In our study, the ESM method has been proposed in which soil C and N data for a fixed depth can be adjusted to normalize to a particular soil mass within specific layer (Ellert and Bettany, 1995). Overall, the patterns of SOC and TN stocks were better clarified by the ESM approach, especially in upper layers (e.g., ≤ 40 cm; Table 4). Specifically, using the FD method, we found that the SOC stock under NF soil was reduced by 11.8 %, 21.4 %, 30.0 %, 38.2 %, and 38.7 % in the 0–10, 0–20, 0–40, 0–60, and 0–100 cm layer, respectively, compared with the croplands. By contrast, these corresponding values
were 19.1 %, 25.1 %, 30.6 %, 36.8 %, and 37.9 %, when calculation on the ESM basis. These data indicate that FD method underestimated SOC loss in the 0–40 cm layer, but overestimated in the 0–60 and 0–100 cm profiles, although the two groups were not statistically different (Paired T-test, $P = 0.305$). The contrasting results are probably associated with the variations in soil BD across soil profile (Fig. 2). Thus, in studies which use an FD approach for the comparison of SOC stocks may misinterpret the SOC changes, which is supported by other studies (Don et al., 2011; Lee et al., 2009). Instead, ESM calculation appears to be more effective for detecting temporal trends in SOC and other nutrients stocks.

It should be noted that the calculation methods also influence SOC interpretation even in deeper profiles (e.g., 0–60 and 0–100 cm; Table 4). This case indicates that even if deeper soil profile (e.g., ≥ 60 cm) is collected, it is still necessary for ESM method to accurately assess SOC stock. Otherwise, the variations in soil BD, which usually occurs at surface soils, may obscure changes in profile SOC stock. In future study, we should reassess the archived soil C data using ESM instead of FD approach, which will help to improve our ability to detect the relatively small changes in SOM pool in response to land-use change.

4.3 Implications for future land-use management

Our study had shown that converting native forest towards agricultural ecosystems caused the highest SOM losses (as high as 39.8 % for SOC and 42.4 % for TN) down to 100 cm profile (Table 4; Fig. 2).
Historically, any land-use changes that disrupt the prior long-standing balance of input and decay processes will induce a shift in SOC stocks (Batjes, 2014; Janzen, 2015). Almost invariably, for example, converting forest to grassland or arable cropland resulted in loss of soil C and N (Don et al., 2011; Foley et al., 2005; Guo and Gifford, 2002; Wei et al., 2014), not only because disturbance stimulates SOM decay (Post and Kwon, 2000; Six et al., 2002), but also because inputs from aboveground and belowground are reduced (Guo and Gifford, 2002). Consequently, the declined SOM might drive land degradation that directly impairs on human society through loss of ecosystem goods and services (Carter, 2002; Jaiarree et al., 2014; Lal, 2010; Smith et al., 2015). Regretfully, we did not measure the aboveground biomass and crop yields in the present study and these knowledge should be determined for productivity analysis in future investigation.

In the karst regions of Southwestern China, the declined SOM had caused soil degradation, leading to the increased soil erosion and reduced land productivity (Hu et al., 2014; Liu et al., 2005; Xu et al., 2008). Xie et al. (2014) highlighted that the degradation trend of soil fertility was almost parallel to the aggravation of karst rocky desertification. Thus, how to conserve and restore the degraded karst areas is promoted by Chinese central and local governments (Hu et al., 2008; Zhao et al., 2014). Our results showed that the conversion of native forest to croplands had exported SOC from soils as higher as 39.8 % (Table 4). Instead, for the unused grassland (UG) and secondary shrub (SS) ecosystems, the SOC stock
was reduced by 12.8 % and 26.8 %, respectively, compared with the native forest (NF). Based on these results, we suggest that it is necessary to conduct some engineering programs (e.g., afforestation) to accelerate SOC recovery in the karst region. A recent study by Liu et al. (2015a) found that the soil microbial activities, texture, and litter fall played important roles in SOC accumulation along vegetation successions, and relevant strategies such as increased N import and less soil disturbances were proposed to enhance SOC sequestration, and thus ecological restoration in this vulnerable karst landscape. Thus, further understanding the mechanisms of SOM protection and recovery under land use change is heavily needed. This is particularly important in Southwestern China, because of the large area of native forests being increasingly conversed to agricultural land and other human managed ecosystems.

5 Conclusion

Our study demonstrates that the conversion of native forest into cropland and other managed systems led to SOC and TN losses. However, the effect magnitude of soil C and N stocks losses were dependent on sampling depths (i.e., surface vs. subsoil) and calculation approaches. The averaged SOC stocks on ESM basis in the depths of 0–10, 0–20, 0–40, 0–60 and 0–100 cm of croplands (including SF and CF) were 19.1 %, 25.1 %, 30.6 %, 36.8 % and 37.9 % lower, respectively, than those of NF soil. Generally, the FD based soil C and N stocks differed with ESM based ones, particularly in the surface soil where soil bulk density varies largely, suggesting it is crucial for soil mass correction for estimating SOC and TN stocks with land-use changes. Further study is needed to elucidate the mechanisms behind SOM
losses after the land-use conversions in the typical karst landscapes.

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