Increasing SOC sequestration and closing N cycle during post-agricultural restoration in karst region, Southwest China

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Abstract

Purpose

Post-agricultural restoration affects soil organic carbon (SOC) sequestration and ecosystem nitrogen (N) cycle. However, the control mechanism of SOC sequestration and alteration of ecosystem N status following post-agricultural restoration are not well understood in karst regions.

Methods

Croplands, abandoned croplands, and native vegetation forests were selected to represent three stages following post-agricultural restoration using a space for time substitution approach in a karst critical zone in Guizhou province, Southwest China. The variations of soil aggregate associated SOC and relationships between soil Ca and SOC were analyzed to identify SOC sequestration potential. Foliar $\delta^{15}\text{N}$ composition and soil to plant ^{15}N enrichment factor ($EF = \delta^{15}\text{N}_{\text{litter}} - \delta^{15}\text{N}_{\text{soil}}$) were analyzed to determine ecosystem N status.

Results

Macro-aggregate proportions and their SOC concentrations significantly increased following post-agricultural restoration. Soil Ca concentrations non-linearly increased with increasing SOC concentrations of bulk soils and aggregates. Foliar $\delta^{15}\text{N}$ values and EF values significantly decreased following post-agricultural restoration, mainly attributed to the increasing plant uptake of ^{15}N -depleted inorganic N, which was produced from soil organic nitrogen (SON) mineralization and nitrification. During post-agricultural restoration, the increasing plant biomass and slow SON mineralization led to more inorganic N uptake and less N loss, i.e., a more closed N cycle.

Conclusion

Soil aggregates and Ca play important roles in promoting SOC sequestration, and ecosystem N cycles are towards closed during post-agricultural restoration in the karst ecosystem.

1. Introduction

The soil organic carbon (SOC) pool (1550 Gt) is approximately double the size of the atmospheric C pool (760 Gt) and triple the size of the pant C pool (560 Gt) (Lal 2004). The dynamics of the SOC pool likely cause significant influences on atmospheric CO_2 concentration and global climate change (Yang et al. 2016; Liu and Han 2020, 2021). Nitrogen (N) is an essential bio-element for vegetation growth, which generally restricts the primary productivity in many terrestrial ecosystems at middle and high latitudes

(Vitousek and Howarth 1991; Davidson et al. 2007; Song et al. 2021), and C storage in plants and soils (Luo et al. 2004; Xia et al. 2020). Post-agricultural restoration, as one type of land use change, has been widely recognized to affect SOC dynamics (Clark and Johnson 2011; Yang et al. 2016; Kalinina et al. 2019; Bell et al. 2020, 2021; Djuma et al. 2020). Poeplau et al. (2011) found that SOC storage decreased with the conversion from natural lands to croplands in several decades, but it could be recovered during post-agricultural restoration. In many studies, SOC accumulation during post-agricultural restoration is closely linked to the increased litterfall and root input with increasing vegetation biomass (Ghafoor et al. 2017; Scott et al. 2021). Generally, SOC storage is primarily determined by the balance between organic debris input and microbial decomposition loss (Jobbagy and Jackson 2000; Yang et al. 2016; Juhos et al. 2021). However, only the accumulation of SOC pool which can undergo long-term stabilization is meaningful for global climate because short residence time (several days to several months) of labile SOC pool determines the negligible influence for the balance between soil C pool and atmospheric C pool. SOC sequestration mainly depends on the accumulation of the stable SOC which is not easily mineralized in the soil environment (Fan et al. 2020). Thus, identifying SOC stabilization mechanism is key to understanding the SOC sequestration potential following post-agricultural restoration (Li et al. 2012). The three mechanisms of SOC stabilization have been widely proposed (Sollins et al. 1996; Six et al. 2002; John et al. 2005; Wright and Hons 2005; Schmidt et al. 2011) are: (1) biochemical stabilization depended on recalcitrant organic compounds; (2) chemical stabilization through association with clay particles; and (3) physical stabilization via the protection of soil aggregates. Generally, the primary SOC stabilization approach differs between sites due to the discrepancies in land use management types and soil physicochemical properties (Fan et al. 2020). Thus, it is necessary to confirm the main SOC stabilization approach following post-agricultural restoration at a specific site.

In terrestrial ecosystems, SOC accumulation significantly affects soil N processes because both of them are existing mainly as organic complexes. The ^{15}N stable isotope ratio ($\delta^{15}\text{N}$) has been widely considered to evaluate N processes and sources, including N fertilizer application (Choi et al. 2017), symbiotic N uptake by mycorrhiza (Hobbie and Ouimette 2009; Taylor et al. 2019), N_2 fixation of N_2 -fixing plants (Hogberg 1997); atmospheric N deposition (Currie et al. 2004; Liu et al. 2006), plant N uptake and microbial N assimilation (Fowler et al. 2013), SON mineralization (Zhang et al. 2015; Liu et al. 2017), nitrification and denitrification (Robinson 2001; Galloway et al. 2008), and ammonia volatilization (Choi et al. 2017). In croplands, crop N is mainly derived from chemical N fertilizer, some leguminous plants utilize N through N_2 fixation (Hogberg 1997). Thus, the $\delta^{15}\text{N}$ composition of crops is significantly affected by the ^{15}N -abundance of synthetic fertilizer (mean $\delta^{15}\text{N}$: $0.3 \pm 0.2\text{‰}$, Choi et al. 2017) and atmospheric N_2 (0‰). After agricultural abandonment, plant N is mainly derived from the available inorganic N in soils, such as NH_4^+ via SON mineralization and NO_3^- via nitrification (Hobbie and Ouimette 2009). Generally, SON mineralization causes ^{15}N enrichment in organic residues and produces ^{15}N -depleted NH_4^+ (Corre et al. 2007). Subsequently, nitrification causes ^{15}N enrichment in NH_4^+ and produces ^{15}N -depleted NO_3^- (Lim et al. 2015). Thus, the available inorganic N is generally ^{15}N -depleted and is easily lost compared to SON. For the leaky N cycle, too much ^{15}N -depleted inorganic N is lost,

resulting in ^{15}N enrichment of the whole soil—plant system. For the closed N cycle, the ^{15}N -depleted inorganic N is mainly absorbed by plants, resulting in foliar ^{15}N depletion. Thus, the natural ^{15}N -abundance of foliage had been widely used to indicate forest ecosystem N status (Koopmans et al. 1997; Boeckx et al. 2005). However, the absolute value of foliar ^{15}N -abundance is unsuitable for use in the comparison of N statuses at different sites (Ross et al. 2004; Liu et al. 2006). Foliar ^{15}N -abundance can be affected by soil $\delta^{15}\text{N}$ composition (Hobbie and Ouimette 2009), which are generally different at the different sites (Taylor et al. 2019). Subsequently, the $\delta^{15}\text{N}$ value of the surface soils is used to calculate foliar ^{15}N enrichment at a specific site, which is expressed as the soil to plant ^{15}N enrichment factor ($EF = \delta^{15}\text{N}_{\text{litter}} - \delta^{15}\text{N}_{\text{soil}}$) (Pardo et al. 2007). In the present study, foliar $\delta^{15}\text{N}$ values and soil to plant ^{15}N EF values were innovatively used to indicate ecosystem N status following post-agricultural restoration. A closed N cycle means less soil inorganic N loss and larger plant N uptake (Li et al. 2017), which are closely associated with soil N availability and ecosystem primary productivity. Thus, identifying N status can predict ecosystem evolution.

In the karst region of southwestern China, many croplands have been abandoned and naturally restored since the promulgation of the Grain for Green Project (GGP) program (Wang et al. 2017). The dynamics of SOC and soil N following post-agricultural restoration have been widely reported in the karst ecosystem (Wen et al. 2016; Xiao et al. 2018; Han et al. 2020). In the karst region, Liu et al. (2020) found that macro-aggregate played an important role in sequestering SOC. Li et al. (2017) suggested that Ca was the major factor determining soil organic matter (SOM) stability and soil SOC and N level based on the classification and regression tree (CART) analysis. Ca^{2+} is the most major polyvalent cation in neutral and alkaline soils of limestone region, and SOM interacted with mineral surfaces via polyvalent cations results in more stable SOM (Lützow et al. 2006). Furthermore, OM- Ca^{2+} -mineral complexation can affect micro-aggregate formation and subsequent SOC dynamics (Six et al. 1998, 2000, 2002). According to these findings, we hypothesized that soil aggregates and Ca are the major factors determining SOC sequestration during post-agricultural restoration in the karst region (H1). Additionally, Li et al. (2021) suggested that post-agricultural restoration significantly enhanced soil N availability based on the increased gross N mineralization rate. The increment of gross N mineralization is mainly attributed to the increased gross microbial biomass carbon C and liable SON. However, the proportion of SON which can easily mineralize decreases during post-agricultural restoration, due to increased SON stabilization via OM- Ca^{2+} -mineral complexes and soil aggregates. Moreover, vegetation restoration enhances plant N uptake (Yang et al. 2017). Thus, we hypothesized ecosystem N cycles are towards closed during post-agricultural restoration in the karst region (H2). The research objectives were to: (1) determine the control mechanism of SOC sequestration during post-agricultural restoration by analyzing the relationships of SOC with soil aggregates and Ca; (2) determine the alteration of ecosystem N cycle during post-agricultural restoration by analyzing plant and soil $\delta^{15}\text{N}$ composition and EF value; and (3) establish a conceptual pattern of SOC sequestration and ecosystem N cycle during post-agricultural restoration in the karst ecosystem. SOC sequestration and ecosystem N cycle are closely linked to greenhouse gas release, ecosystem primary productivity, soil fertility (Robinson 2001; Lal 2004). The GGP program is no

longer to only seek a better social and ecological environment for residents, possibly it is of great importance to global climate change, ecosystem development, and soil health based on our study objects.

2. Materials And Methods

2.1. Study area

The study area is located in the Chenqi catchment (26°15.779'–26°16.710'N, 105°46.053'–105°46.839'E), a karst critical zone (KCZ) in Guizhou province, Southwest China. The climate is sub-tropical monsoonal with a MAT of 15.1°C and a MAP of 1315 mm (Zhao et al. 2010). Over 80% of total annual precipitation concentrates in the period from May to October (Yue et al. 2020). The typical karst hoodoo depression physiognomy in the catchment is characteristic of many peak clusters and valleys (Liu et al. 2020). The altitude of this catchment ranges from 1310 m to 1524 m (average: 1350 m) above sea level. The thin calcareous soils (generally 30 ~ 50 cm) on the hillslopes, which belong to Mollic Inceptisols based on the soil taxonomy of USDA (Soil Survey Staff 2014), are mainly developed from the limestone of the Guanling Formation of the Middle Triassic (Zhao et al. 2010). The thick (over 70 cm) quaternary deposits in the valley floor mainly originate from the calcareous soils on surrounding hillslopes (Green et al. 2019).

In the second half of the 20th century, intensive and unreasonable agricultural production activities had caused serious soil degradation in the karst region (Liu et al. 2020; Zeng and Han 2020). To alleviate the ecological problems, the Chinese government carried out the GGP, which encouraged farmers to give up low-yield sloping croplands (Wang et al. 2017). Under this background, a series of abandoned croplands with different abandonment ages are widely distributed in the KCZ (Liu et al. 2020). In the catchment, three stages of agricultural abandonment were selected to provide a chronosequence of vegetation structure and soil ecosystem recovery following post-agricultural restoration by the space for time substitution approach (Blois et al. 2013). These were: (1) croplands with continuous cultivation or during the fallow period; (2) abandoned croplands within 3 ~ 8 years; and (3) native vegetation lands without agricultural disturbance for over 50 years (Liu et al. 2020). The croplands are mainly cultivated with maize and other minor crops such as oilseed rape, sweet potato, and soybean in rotation. Urea and compound N-P-K fertilizer provide 225 ~ 375 kg ha⁻¹ yr⁻¹ N, 69 ~ 102 kg ha⁻¹ yr⁻¹ P, and 5 ~ 7 kg ha⁻¹ yr⁻¹ K into agricultural soils (Li et al. 2018), whereas manures are applied at only sowing period. Generally, plow tillage (about 20 cm depth) and sowing seeds are conducted in March; crop harvest is conducted in October. After harvest, a part of croplands enter into the fallow period without cultivation and fertilization. The dominant vegetation species at the three stages following post-agricultural restoration are given in Table S1 and photographs of the three stages are shown in Fig. 1.

2.2. Soil and leaf sampling

In June 2016, a total of 18 soil sampling sites from croplands (CL, $n = 8$), abandoned croplands (AL, $n = 5$), and native vegetation lands (NV, $n = 5$) were selected. Information about topography and cropping history at each soil site is exhibited in Table 1. The sampling sites at the same stage of post-agricultural restoration spaced 100 m apart. A 1 m $\times$ 1 m square was set up at each sampling site, and three duplicate subsites were selected from the corners of the square. A 0.3 m $\times$ 0.3 m plot with 0.5 m depth at each subsite was dug to collect soil samples. Soil samples were collected from the layer at the 0 ~ 10, 10 ~ 20, and 20 ~ 30 cm depth, orderly. The three duplicate soil samples at the same depth were mixed to be one sample.

Table 1
Location, topography, and cropping history at each soil site

Sampling site	longitude and latitude	Altitude	Topography	Cropping history
Cropland				
CL1	26°15.797'N, 105°46.468'E	1319 m	Flat terrace, slope < 3°	Long-term cultivation and fertilization, mainly maize
CL2	26°15.817'N, 105°46.267'E	1320 m	Flat terrace, slope < 3°	Long-term cultivation and fertilization, mainly oilseed rape
CL3	26°15.806'N, 105°46.295'E	1320 m	Flat terrace, slope < 3°	Long-term cultivation and fertilization, mainly peanut
CL4	26°16.010'N, 105°46.433'E	1333 m	Flat terrace, slope < 3°	Long-term cultivation and fertilization, mainly spanish potato and scallions
CL5	26°15.805'N, 105°46.278'E	1334 m	Flat terrace, slope < 3°	Long-term cultivation and fertilization, mainly soybeans
CL6	26°16.010'N, 105°46.433'E	1334 m	Flat terrace, slope < 3°	Cropland during the fallow period, without cultivation and fertilization for 1 y
CL7	26°15.872'N, 105°46.278'E	1335 m	Flat terrace, slope < 3°	Cropland during the fallow period, without cultivation and fertilization for 2 y
CL8	26°16.019'N, 105°46.839'E	1335 m	Flat terrace, slope < 3°	Cropland during the fallow period, without cultivation and fertilization for 2 y
Abandoned cropland				
AL1	26°15.893'N, 105°46.490'E	1348 m	Converted from flat terrace, slope < 5°	Without cultivation and fertilization for 3 y, covered by goose grasses
AL2	26°15.870'N, 105°46.495'E	1350 m	Converted from flat terrace, slope < 5°	Without cultivation and fertilization for 5 y, covered by goose grasses
AL3	26°15.811'N, 105°46.291'E	1365 m	Converted from flat terrace, slope < 5°	Without cultivation and fertilization for 5 y, covered by goose grasses
AL4	26°16.046'N, 105°46.543'E	1370 m	Converted from flat terrace, slope < 5°	Without cultivation and fertilization for 7 y, covered by goose grasses
AL5	26°16.021'N, 105°46.564'E	1376 m	Converted from flat terrace, slope < 5°	Without cultivation and fertilization for 8 y, covered by goose grasses
Native vegetation land				

Sampling site	longitude and latitude	Altitude	Topography	Cropping history
NV1	26°16.091'N, 105°46.589'E	1401 m	Relatively flat ridge, slope 3°~5°	Without cultivation and fertilization > 50 y, shrub-grass land at present
NV2	26°15.984'N, 105°46.569'E	1404 m	Relatively flat ridge, slope 3°~5°	Without cultivation and fertilization > 50 y, shrub-grass land at present
NV3	26°15.980'N, 105°46.597'E	1425 m	Relatively flat ridge, slope 3°~5°	Without cultivation and fertilization > 80 y, shrub land at present
NV4	26°15.779'N, 105°46.264'E	1442 m	Relatively flat ridge, slope 3°~5°	Without cultivation and fertilization > 80 y, shrub land at present
NV5	26°16.125'N, 105°46.544'E	1466 m	Relatively flat ridge, slope 3°~5°	Without cultivation and fertilization > 100 y, shrub-arbor land at present

The dominant vegetation species under the three land use types were identified in the field. Leaf samples from at least five plants with same species were selected at the same stage of post-agricultural restoration. The mature leaves of the dominant vegetation species were collected at the high, middle, and low tree height. The leaves from each tree height and same species were mixed to be one sample. Litter samples were collected within a 1 m × 1 m square, in which the center of the square corresponds to one of the soil sampling sites.

2.3. Sample analysis

After washing the dust on the leaf surface with pure water for 3 times, the leaf and litter samples were dried at - 40°C in a freezer dryer, then ground into powder by an attritor (Liu et al. 2020). Soil samples were air-dried at room temperature (25°C) for at least 20 days after removing big gravel and roots. One part of the dried soil samples was conserved as the bulk soils after passing through a 2 mm sifter. Other parts of the dried soil samples, which were not crushed, were used to separate different-sized aggregates physically by the modified wet sieving method (Six et al. 1998). Concretely, the dried soil samples were slowly wetted via capillary water absorption, then naturally disintegrated into a series of different-sized aggregates. In pure water, these different-sized aggregates were passed through a 2000 µm, 250 µm, and 53 µm sifter in that order. The aggregates over 2000 µm in diameter were crushed by a tweezer, to make all aggregates passing through the 2000 µm sifter. Macro-aggregates (250 ~ 2000 µm) and micro-aggregates (53 ~ 250 µm) were collected after passing through the 250 µm and 53 µm sifters, respectively. The silt + clay sized fractions (< 53 µm) were extracted from the residual mixed liquid by centrifugation. The moist soil aggregates were dried in an oven at 55°C until constant weight, then weighed to calculate the mass-proportions of the different-sized aggregates.

The samples of bulk soils and different-sized aggregates were ground into powder by an agate mortar and then passed through a 149 µm sifter. The pulverized samples (< 149 µm) were treated using 0.5 mol L⁻¹ HCl solution to remove carbonates (Midwood and Boutton 1998; Liu et al. 2021b) and using 2 mol·L⁻¹ KCl solution to remove inorganic N (Meng et al. 2005). The moist samples were washed and centrifuged

repeatedly until the neutrality of the supernatant liquid, then were dried and ground into powder (< 149 μm). The SOC and SON concentrations in the pulverized samples were measured using a multi-elemental analyzer (Elementar, Vario TOC, Germany) in the Laboratory of Surficial Environment Hydrogeochemistry, China University of Geosciences (Beijing). The precision was greater than $\pm 0.01\%$ for C and better than $\pm 0.02\%$ for N. Actual SOC and SON concentrations in the original bulk soils or aggregates can be calibrated based on the loss of carbonates and inorganic N, according to the method by Liu et al. (2020). In brief, the measured values are multiplied by the ratio of sample mass after treating to before treating to obtain the actual SON and SOC concentrations in different-sized aggregates and bulk soils. The foliar C and N concentrations were also analyzed using the multi-elemental analyzer.

The N stable isotope ratio ($^{15}\text{N}/^{14}\text{N}$) of SON in the treated bulk soils and aggregates were determined by an isotope mass spectrometer (Thermo, MAT-253, USA) in the Central Laboratory for Physical and Chemical Analysis, Institute of Geographic Sciences and Natural Resources Research, Chinese Academy of Sciences. The measurements are expressed in standard δ notation (‰) to indicate the differences between the $^{15}\text{N}/^{14}\text{N}$ ratio of the samples and accepted standard materials (atmospheric N_2), where:

$$\delta^{15}\text{N}_{\text{sample}} (\text{‰}) = [(R_{\text{sample}} - R_{\text{air}})/R_{\text{air}}] \times 1000, R = ^{15}\text{N}/^{14}\text{N}.$$

Reference material GBW04494 ($\delta^{15}\text{N}_{\text{Air}}$: $0.24\text{‰} \pm 0.13\text{‰}$) was monitored by repeatedly measuring to determine the precision (greater than $\pm 0.1\text{‰}$). The $\delta^{15}\text{N}$ values of leaf and litter samples were also analyzed using the isotope mass spectrometer.

The samples of bulk soils were ground using an agate mortar and passed through a 200-mesh (< 74 μm) sifter to analyze Al and Ca concentrations. The 50 mg pulverized samples were digested with 3 mL concentrated HNO_3 , 3 mL concentrated HF, and 1 mL concentrated HClO_4 solution in a Teflon crucible at 120°C for 72 h (Li et al. 2020). The digested samples were reconstituted to 25 mL with dilute HNO_3 , then analyzed Al and Ca concentrations (mg kg^{-1}) in solution by an inductively coupled plasma-optical emission spectrometry (ICP-OES, Optima 5300DV, Perkin Elmer, USA). Blank samples and standard substance (limestone soil, No. GBW07404) were treated and measured in the same way, to monitor the precision and accuracy of test data. Al and Ca concentrations in solution were converted into the concentration (mg kg^{-1}) in soils (Liu et al. 2021a).

2.5. Statistical analysis

One-way ANOVA with the least significant difference (LSD) test was conducted to identify the significant differences in the SOC and SON concentrations of bulk soils, the proportions of different-sized aggregates, the aggregate-SOC and SON concentrations in bulk soils, soil Ca concentrations, soil Ca/Al ratios, the $\delta^{15}\text{N}$ values of SON in bulk soils and different-sized aggregates, and the *EF* values at the three stages following post-agricultural restoration, at the significance level of $P < 0.05$. Linear and non-linear regression analyses were used to determine the variations of foliar N concentrations, $\delta^{15}\text{N}$ values, and C/N ratios following post-agricultural restoration. Based on the linear and non-linear regression analyses,

the equations of best-fit lines were determined, and the coefficients of r and P -value showed the fitting degree and validity, respectively. Pearson correlation and Spearman's rank correlation analyses were performed to determine the linear relationships and rank order relationships of SOC and SON concentrations in bulk soils and different-sized aggregates with soil Ca concentrations and soil Ca/Al ratios, respectively. Statistical analyses were performed by the SPSS 18.0 software (SPSS Inc., Chicago, IL, USA). All figures were done by the SigmaPlot 12.5 software (Systat Software GmbH, Erkrath, Germany) and Adobe Illustrator CS2 software (Adobe Systems Inc., California, USA).

3. Results

3.1. Variations of foliar N concentrations, $\delta^{15}\text{N}$ values, and C/N ratios

Regression analyses were used to determine the variations of foliar N concentrations, $\delta^{15}\text{N}$ values, and C/N ratios following post-agricultural restoration (Fig. 2). Although their variations did not strictly correlate with the restoration time, significantly decreasing foliar N concentrations and $\delta^{15}\text{N}$ values and increasing foliar C/N ratios following post-agricultural restoration were still observed. Additionally, foliar N concentrations of C_4 vegetation were lower than those of C_3 vegetation (Fig. 2a), while foliar C concentrations between them were not significantly different (Table S1). Thus, foliar C/N ratios of C_4 vegetation were higher than those of C_3 vegetation (Fig. 2c). However, foliar $\delta^{15}\text{N}$ values between C_3 vegetation and C_4 vegetation were not significantly different (Fig. 2b).

3.2. SOC and SON concentrations and aggregate distribution

The SOC and SON concentrations of native vegetation lands were significantly higher than those of croplands and abandoned croplands at the 0 ~ 10 and 10 ~ 20 cm depth, while there were no significant differences between them at the 20 ~ 30 cm depth (Fig. 3a and 3b). These results indicate that SOC and SON concentrations increased following post-agricultural restoration but only in the surface soils (0 ~ 20 cm depth). Macro-aggregates accounted for the largest proportion (> 60%) of total aggregates in the calcareous soils (Fig. 3c). In the soils at the 0 ~ 10 cm depth, macro-aggregate proportions in croplands were significantly lower than those in abandoned croplands and native vegetation lands, while the proportions of micro-aggregates and silt + clay sized fractions were significantly higher. Silt + clay sized fraction proportions at the 10 ~ 30 cm depth and macro-aggregate proportions at the 20 ~ 30 cm depth were not significantly different between the three land use types. These results indicated that macro-aggregates increased, while micro-aggregates and silt + clay sized fractions decreased following post-agricultural restoration. Furthermore, these variations were affected by soil depth. Additionally, compared to the croplands, macro-aggregate proportions in the 3 ~ 8 years abandoned croplands significantly

increased, while the SOC and SON concentrations did not. The results indicated that the response of soil aggregates to post-agricultural restoration was more rapid than the response of SOC and SON.

3.3. Aggregate-SOC and SON concentrations in bulk soils

In the soil layers of the 0 ~ 10 and 10 ~ 20 cm depth, macro-aggregate-SOC and SON concentrations in bulk soils under native vegetation lands were significantly higher than those under croplands (Fig. 4). These concentrations under abandoned cropland were intermediary and did not show significant differences with those under cropland (lowest) and native vegetation land (highest). However, these concentrations under the three land use types were not significantly different at the 20 ~ 30 cm depth. Additionally, the SOC and SON concentrations in micro-aggregates, silt + clay sized fractions, and bulk soils were not significantly different between the three land use types at the all soil depths. These results indicated that the macro-aggregates played the most important role in increasing SOC and SON storage during post-agricultural restoration.

3.4. Soil Ca concentrations and Ca/Al ratios

Soil Ca concentrations under native vegetation lands were significantly higher than those under croplands and abandoned croplands at the 0 ~ 10 and 10 ~ 20 cm depth; while there were no significant differences at the 20 ~ 30 cm depth (Fig. 5a). However, Ca belongs to an easily migrated element in the natural soil environment. The lower or higher Ca concentrations at different soil sites with the same bedrock can be caused by, in addition to the depletion or enrichment of Ca with land use change, the different degrees of chemical weathering (Li and Han 2021). Al is difficult to migrate during chemical weathering, thus soil Ca/Al ratios can eliminate the effects of chemical weathering on soil Ca concentrations and indicate the degree of soil Ca depletion or enrichment only caused by land use change (Balls et al. 1997). In the present study, soil Ca/Al ratios under native vegetation land were also significantly higher than those under croplands and abandoned croplands at the 0 ~ 10 and 10 ~ 20 cm depth (Fig. 5b). The result indicated that soil Ca in native vegetation land was indeed enriched compared to that in croplands and abandoned croplands at the 0 ~ 20 cm depth.

3.5. Soil $\delta^{15}\text{N}$ of SON in bulk soils and aggregates and soil to plant ^{15}N EF values

In the bulk soils and different-sized aggregates at the 0 ~ 10 cm depth, the $\delta^{15}\text{N}$ values of SON under croplands were significantly higher than those under abandoned croplands and native vegetation lands; while there were no significant differences at the 10 ~ 20 and 20 ~ 30 cm depth (Fig. 6). These results indicated that the SON of bulk soils and aggregates gradually enriched ^{15}N following post-agricultural restoration. Additionally, in the soils at all depths under the three land use types, the $\delta^{15}\text{N}$ values of aggregate-associated SON followed the order: micro-aggregates < macro-aggregates < silt + clay sized fractions. Although these differences of $\delta^{15}\text{N}$ values between different-sized aggregates were not statistically significant, the same trends that occurred in all soil samples indicated that the discrepancies of $\delta^{15}\text{N}$ values in different-sized aggregates is relevant.

The values of soil to plant ^{15}N enrichment factor (EF) under abandoned croplands (mean = -8.5‰) were significantly more negative than those under croplands (mean = -3.2‰), while significantly more positive than those under native vegetation lands (mean = -10.4‰) (Fig. 7).

4. Discussion

4.1. Increasing SOC sequestration during post-agricultural restoration

In the present study, SOC concentrations increased during post-agricultural restoration in surface soils (Fig. 3a), which corresponds to previous reports in the karst region (Yang et al. 2016; Li et al. 2017; Liu et al. 2020). Generally, increased SOC sequestration is mainly attributed to enhanced SOC storage (or the concentration in soils) and decreased SOC turnover rate (Lal 2004; Cotrufo et al. 2019). Soil aggregates play an important role in affecting SOC dynamics under different land use management systems (Huang et al. 2010; Zeng et al. 2018). Macro-aggregates increased, whereas micro-aggregates and silt + clay sized fractions decreased during post-agricultural restoration (Fig. 3c). These results indicated that soil aggregation gradually intensifies with the cease of agricultural disturbances. The increased soil aggregates will provide more physical protection for SOC (Oades and Waters 1991), which is conducive to enhancing SOC accumulation and stabilization in the soil environment (Six and Paustian 2014). Furthermore, only macro-aggregate associated SOC concentration in bulk soils significantly increased during post-agricultural restoration (Fig. 4a). This result indicates that macro-aggregates mainly affect SOC storage after cropland abandonment, relative to micro-aggregates or silt + clay sized fractions. Generally, macro-aggregates are more sensitive to land use management than other small-sized aggregates (Franzluebbers and Arshad 1997), which is determined by the physical and chemical stability of different-sized aggregates (Six et al. 2004). It suggests that tillage activities are more likely to destroy macro-aggregates rather than small-sized aggregates, and macro-aggregates can be more rapidly restored after stopping cultivation. Moreover, the SOC within macro-aggregates accounts for 60% ~ 80% of the total SOC in bulk soils (Liu et al. 2020). Thus, the variations of macro-aggregates are closely associated with SOC dynamics during post-agricultural restoration.

The calcareous soils developed from limestones (mainly CaCO_3) contain abundant Ca^{2+} , which can easily absorb clay minerals and organic matter to form stable OM- Ca^{2+} -mineral complexes (Li et al. 2017). As a form of physical protection, the organic-inorganic complexes can enhance SOC stabilization to resist microbial decomposition (Schmidt et al. 2011). Thus, soil Ca concentrations generally show a positive effect on SOC sequestration. Soil Ca in native vegetation lands were enriched compared to that in croplands and abandoned croplands (Fig. 5). This result is likely attributed to the strong loss of Ca via leaching along with nitrate and harvesting under long-term cultivation (Guo et al. 2010), while soil Ca is lost only slightly under the native vegetation. As the main source of soil Ca, rock weathering should continue for several hundred years at least, which can cause a significant increase in soil Ca concentration. Theoretically, soil Ca concentration decreases slowly during several decades of post-

agricultural restoration, while soil Ca accumulation is almost impossible. However, soil Ca accumulated in the limestone region after a relatively short-term post-agricultural restoration (Fig. 5). On the one hand, the rapid limestone weathering rate can replenish depleted soil Ca pool after cropland abandonment (Li et al. 2017). On the other hand, the formation of OM-Ca²⁺-mineral complexes in the SOC-rich soils also reduces Ca²⁺ leaching with increasing SOM. The rapid replenishment of soil Ca is closely associated with increased SOC sequestration following post-agricultural restoration in the karst catchment. Li et al. (2017) reported that soil Ca played an important role in improving SOC and N storage after agricultural abandonment. Pearson correlation coefficients showed that there were no linear relationships between soil Ca concentration and SOC concentration of bulk soils and different sized aggregates (Table 2). However, a significant non-relationship ($P < 0.01$) between them was present at all soil depths according to Spearman's rank correlation coefficients. These results verified the close relationships between soil Ca replenishment and SOC sequestration in the karst region, and underscored a non-linear increase between them.

Table 2

Pearson and Spearman's rank correlation relationships between SOC concentration, SON concentration, Ca concentration, and Ca/Al ratio

	Soil depth	SOC concentration				SON concentration			
	(cm)	BS	MA	MI	SC	BS	MA	MI	SC
Pearson correlation coefficient (<i>r</i>)									
Ca concentration	0 ~ 10	0.12	0.11	0.12	0.21	0.22	0.22	0.23	0.32
	10 ~ 20	0.12	0.10	0.14	0.15	0.27	0.26	0.28	0.38
	20 ~ 30	0.36	0.35	0.42	0.36	0.57*	0.58*	0.61**	0.59*
Ca/Al ratio	0 ~ 10	0.13	0.12	0.13	0.22	0.23	0.23	0.24	0.33
	10 ~ 20	0.09	0.07	0.12	0.13	0.25	0.24	0.26	0.36
	20 ~ 30	0.35	0.34	0.41	0.36	0.56*	0.57*	0.60**	0.58*
* <i>P</i> < 0.05; ** <i>P</i> < 0.01									
Spearman's rank correlation coefficient (<i>ρ</i>)									
Ca concentration	0 ~ 10	0.67**	0.77**	0.80**	0.74**	0.76**	0.85**	0.83**	0.79**
	10 ~ 20	0.78**	0.78**	0.80**	0.79**	0.90**	0.88**	0.91**	0.88**
	20 ~ 30	0.74**	0.75**	0.77**	0.74**	0.80**	0.85**	0.84**	0.84**
Ca/Al ratio	0 ~ 10	0.71**	0.81**	0.82**	0.79**	0.79**	0.87**	0.86**	0.83**
	10 ~ 20	0.70**	0.71**	0.72**	0.70**	0.86**	0.86**	0.88**	0.85**
	20 ~ 30	0.73**	0.74**	0.75**	0.74**	0.77**	0.81**	0.80**	0.83**
** <i>P</i> < 0.01									
BS, bulk soil; MA, macro-aggregate; MI, micro-aggregate; SC, silt + clay sized fraction									

4.2. Towards closed N cycles during post-agricultural restoration

Similar to SOC, SON was protected by soil aggregates and OM-Ca²⁺-mineral complexes due to the extensive homology (SOM) between them, as shown in Fig. 4b. In addition to increased SON concentration (Fig. 3b), these protection mechanisms also enhance SON stabilization following post-agricultural restoration (Six et al. 1998, 2000, 2002). The increased SON stabilization likely restricts N mineralization and subsequent nitrification to produce available N, resulting in decreasing soil N availability. There were two pieces of evidence to verify the decreased soil N availability during post-agricultural restoration. Firstly, foliar N concentrations decreased and C/N ratios increased after cropland abandonment (Fig. 2a and 2b), which could affect litter N concentrations and C/N ratios. Generally, the decomposition of litter with a low N concentration and high C/N ratio will be restrained at the initial stage of litter decomposition (Xia et al. 2020). Thus, the N mineralization rate of litter is also slower under native vegetation lands than that under croplands. Secondly, the $\delta^{15}\text{N}$ values of aggregate-associated SON followed the order: micro-aggregates < macro-aggregates < silt + clay sized fractions (Fig. 6), which was associated with the different degrees of SON mineralization. Generally, SON mineralization produces ¹⁵N-depleted NH₄⁺ and ¹⁵N-enriched organic residue remains (Robinson 2001; Hobbie and Ouimette 2009). Thus, the SON within micro-aggregates was fresher than within macro-aggregates and silt + clay sized fractions, which was similar to the situation of SOC associated with different sized aggregates (Liu et al. 2020). Aggregate hierarchy suggests that micro-aggregates are formed within macro-aggregates (Six et al. 2000). The SON within micro-aggregates is more stable compared to within macro-aggregates due to the stronger stabilization of micro-aggregates and the protection provided by macro-aggregates (Beare et al. 1994). During post-agricultural restoration, decreased macro-aggregate turnover with the cease of cultivation activities is conducive for the formation and stabilization of micro-aggregates inside (Six et al. 2000). Accordingly, fresh SON within micro-aggregates can be physically protected as well. Thus, the N mineralization rate of fresh SON is also slower under native vegetation than that under cropland, due to increases in soil aggregation.

During post-agricultural restoration, increased plant biomass enhances available N uptake, while decreases in soil N availability slow down the supply of available N via SON mineralization. After cropland abandonment, the slow SON mineralization and faster plant N uptake lead to less inorganic N loss, i.e., tending towards closed N cycles. The more negative foliar ¹⁵N-abundance (Fig. 2b) and soil to plant ¹⁵N *EF* values (Fig. 7) indicates the alteration from leaky to closed N cycles following post-agricultural restoration (Xiao et al. 2018). Generally, SON mineralization causes ¹⁵N enrichment in organic residues and produces ¹⁵N-depleted NH₄⁺ (Baggs et al. 2003; Corre et al. 2007). Subsequently, nitrification causes ¹⁵N enrichment in NH₄⁺ and produces ¹⁵N-depleted NO₃⁻ (Lim et al. 2015). For the leaky N cycle ecosystem, the long-term loss of ¹⁵N-depleted NO₃⁻ will cause the ¹⁵N enrichment of the soil and plant N pool (Yang et al. 2017). Thus, higher foliar $\delta^{15}\text{N}$ and *EF* values are closely associated with a larger importance of net nitrification and more NO₃⁻ loss (Xiao et al. 2018). During post-agricultural restoration, slow SON mineralization restricts NH₄⁺ production; subsequently, the low NH₄⁺ concentration restricts nitrification to slow down NO₃⁻ produces (Koopmans et al. 1997; Robinson 2001). Furthermore, the ¹⁵N-

depleted NO_3^- is more easily absorbed completely with increasing plant biomass. Thus, more closed N cycle means larger plant N uptake of ^{15}N -depleted NO_3^- , which corresponds to lower foliar $\delta^{15}\text{N}$ and EF values. The results underscore that the karst ecosystem N cycle tends to close following post-agricultural restoration.

4.3. A conceptual model of post-agricultural restoration in the karst ecosystem

In the present study, we propose a conceptual model about the increasing SOC sequestration and closing N cycles following post-agricultural restoration in the karst ecosystem (Fig. 8). During post-agricultural restoration, increased plant biomass enhances organic matter input into soils via litter. Increased fresh plant debris and the cease of cultivation activities promote macro-aggregate formation and stabilization, and slow its turnover rate. Macro-aggregates can protect fresh SOC to increase its storage and stabilization. Slow macro-aggregate turnover is conducive to the formation and stabilization of micro-aggregates inside, which provide further protection for fresh SOC. Silt + clay sized fractions mainly protect old SOC. Limestone weathering brings abundant Ca^{2+} into soils, which can easily absorb clay minerals and organic matter to form stable OM- Ca^{2+} -mineral complexes. On the one hand, the OM- Ca^{2+} -mineral complexes reduce Ca^{2+} loss, which is conducive to soil Ca accumulation. On the other hand, the complexes increase SOC storage and stabilization. Thus, SOC sequestration increases via the protection from soil aggregates and OM- Ca^{2+} -mineral complexes following post-agricultural restoration. Soil aggregates and OM- Ca^{2+} -mineral complexes also enhance SON stabilization, resulting in slowed SON mineralization. Moreover, the decreased foliar N concentrations and increased C/N ratios restrain N mineralization at the initial stage of litter decomposition. Soil N availability decreases with the slow N mineralization, resulting in slowed production of ^{15}N -depleted inorganic N. During post-agricultural restoration, increased plant biomass enhances plant absorption of ^{15}N -depleted inorganic N, which causes the decreases in foliar $\delta^{15}\text{N}$ and EF values. More plant uptake for inorganic N means less soil N loss, i.e., the ecosystem N cycle tends to close.

The conceptual model verifies the positive implications of the GGP program to global climate change and ecosystem development. As the wide progress of the GGP program in China, the conceptual model can provide basic science guidance for policymakers in the karst region, and even in the non- karst region. However, considering the differences in soil type, climate, vegetation restoration approach (natural restoration without disturbance, forest plantation, grass plantation, and so on), the main mechanism of SOC sequestration and soil N transformation processes and magnitude differ between the GGP programs at different region. For future research, the conceptual model should be adjusted according to the local situation.

5. Conclusions

Foliar N concentrations, $\delta^{15}\text{N}$ values, and C/N ratios, soil aggregate distribution, SOC and SON concentrations, and the $\delta^{15}\text{N}$ values of SON in bulk soils and different sized aggregate were analyzed to identify SOC sequestration potential and ecosystem N status following post-agricultural restoration in the KCZ in Guizhou province, Southwest China. During post-agricultural restoration, macro-aggregate proportions and the SOC concentrations in bulk soils significantly increased. Soil Ca concentrations non-linearly increased with increasing SOC concentrations. Soil macro-aggregates and Ca^{2+} played important roles in enhancing SOC storage and stabilization, which was critical to SOC sequestration. Foliar $\delta^{15}\text{N}$ values and *EF* values were more negative following post-agricultural restoration, which was directly associated with the plant uptake of ^{15}N -depleted inorganic N produced from SON mineralization and nitrification. During post-agricultural restoration, increased plant biomass and decreased SON mineralization led to the more sufficient absorption of the produced inorganic N, less NO_3^- loss, and a more closed N cycle. These results underscore the increasing SOC sequestration potential and the closing of N cycles following post-agricultural restoration in the karst region.

Declarations

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Conflicts of interest/Competing interests

The authors declare that they have no conflict of interest.

Availability of data and material

The data that support the findings of this study are available on request from the corresponding author.

Code availability

Not applicable

Authors' contributions

Man Liu and Guilin Han designed the experiment. Man Liu performed the experiment. Man Liu and Guilin Han wrote the manuscript. Man Liu and Qian Zhang contributed to fieldwork and sample processing and data analysis.

Ethics approval

Not applicable

Consent to participate

Not applicable

Consent for publication

Not applicable

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Supplementary Information

Table S1 Foliar N and C concentration, C/N ratio, $\delta^{15}\text{N}$, and $\delta^{13}\text{C}$ of dominant vegetation species at the three stages following post-agricultural restoration.

Plant Latin name	C ₃ /C ₄ type	N concentration (%)	C concentration (%)	C/N	δ ¹⁵ N (‰)	δ ¹³ C (‰)
Cropland (CL)						
<i>Solanum tuberosum</i>	C ₃	4.12	53.63	13.01	7.76	– 27.84
<i>Arachis hypogaea</i>	C ₃	4.01	59.37	14.82	0.17	– 27.03
<i>Artemisia argyi</i>	C ₃	3.24	56.61	17.46	2.88	– 28.98
<i>Helianthus annuus</i>	C ₃	2.88	50.82	17.67	5.22	– 29.03
<i>Catalpa ovata</i>	C ₃	2.57	56.32	21.92	2.35	– 29.83
<i>Colocasia esculenta</i>	C ₃	2.17	54.19	24.92	3.95	– 27.53
<i>Zea mays</i> (leaf)	C ₄	1.96	57.97	29.55	5.41	– 12.01
<i>Zea mays</i> (stem)	C ₄	1.66	56.24	33.92	3.54	– 11.14
<i>Zea mays</i> (root)	C ₄	1.48	56.27	38.05	3.47	– 11.93
Abandoned cropland (AL)						
<i>Chrysanthemum indicum</i>	C ₃	2.47	50.04	20.27	–2.40	– 28.88
<i>Conyza canadensis</i>	C ₃	2.33	57.14	24.47	–2.94	– 30.41
<i>Photinia serratifolia</i>	C ₃	2.30	59.28	25.78	0.10	– 27.17
<i>Padus racemosa</i>	C ₃	2.24	59.17	26.37	–2.33	– 28.17
<i>Pyracantha fortuneana</i>	C ₃	1.98	59.20	29.84	–2.19	– 28.52
<i>Coriaria nepalensis</i>	C ₃	1.60	51.70	32.34	–2.96	– 25.32
<i>Quercus fabri</i>	C ₃	1.52	58.01	38.08	–2.60	– 28.15
<i>Berchemia sinica</i>	C ₃	1.52	52.39	34.43	–2.71	–

						30.44
<i>Ilex macrocarpa</i>	C ₃	1.45	56.93	39.33	−0.83	− 26.75
<i>Iris tectorum</i>	C ₃	1.34	52.50	39.17	−3.25	− 25.30
<i>Imperata cylindrica</i>	C ₄	0.96	52.05	54.06	−4.99	− 12.65
<i>Setaria viridis</i>	C ₄	0.89	52.85	59.12	−0.43	− 12.24
<i>Miscanthus sinensis</i>	C ₄	0.84	52.81	63.18	−4.06	− 12.71
Native vegetation land (NV)						
<i>Padus racemosa</i>	C ₃	2.32	55.82	24.08	−4.19	− 28.35
<i>Litsea pungens</i>	C ₃	2.00	58.62	29.38	−8.20	− 28.90
<i>Rhus chinensis</i>	C ₃	1.83	58.41	31.85	−5.86	− 29.76
<i>Cyclocarya paliurus</i>	C ₃	1.66	58.69	35.44	−7.15	− 29.34
<i>Rosa acicularis</i>	C ₃	1.55	56.97	36.80	− 10.48	− 28.85
<i>Pteridium aquilinum</i>	C ₃	1.52	55.71	36.63	−5.32	− 28.81
<i>Pinus tabuliformis</i>	C ₃	1.48	58.56	39.47	−6.91	− 27.59
<i>Rhamnus parvifolia</i>	C ₃	1.44	57.14	39.58	−5.72	− 28.72
<i>Cinnamomum camphora</i>	C ₃	1.42	59.45	41.73	−4.23	− 30.12
<i>Camellia japonica</i>	C ₃	1.39	56.80	40.95	−8.84	− 27.89
<i>Rhamnus davurica</i>	C ₃	1.19	52.31	43.98	−5.20	− 28.28
<i>Ilex crenata</i>	C ₃	1.19	58.09	48.91	−5.20	− 27.50
<i>Ilex chinensis</i>	C ₃	1.04	50.33	48.61	− 12.17	− 29.11
<i>Arthraxon hispidus</i>	C ₄	0.69	48.39	69.97	−6.31	−

						12.01
<i>Eleusine indica</i>	C ₄	0.66	55.78	83.90	–	–
					10.10	12.22

The data of foliar $\delta^{13}\text{C}$ values were cited by [Liu et al. \(2020\)](#).

Figures

Figure 1

Location of karst critical zone (KCZ) and photographs of the three stages following post-agricultural restoration in Guizhou province, Southwest China.

Figure 2

Foliar N concentration (a), $\delta^{15}N$ value (b), and C/N ratio (c) at the three stages following post-agricultural restoration. CL, cropland; AL, abandoned cropland; NV, native vegetation land

Figure 3

The concentrations of SOC (a) and SON (b) and the proportions of different sized aggregates (c) in the bulk soils at the three stages following post-agricultural restoration. Error bar is standard error (SE) of mean. Different lowercase letters indicate significant differences in SOC concentration, SON concentration, and the proportion of different sized aggregates in the same depth of soils between the

three stages following post-agricultural restoration, based on the one-way ANOVA with LSD test at the level of $P < 0.05$. CL, cropland; AL, abandoned cropland; NV, native vegetation land

Figure 4

The concentrations of aggregate-SOC (a) and SON (b) in the bulk soils at the three stages following post-agricultural restoration. Error bar is standard error (SE) of mean. Different lowercase letters indicate significant differences in aggregate-SOC and SON concentrations in the same depth of soils between the

three stages following post-agricultural restoration, based on the one-way ANOVA with LSD test at the level of $P < 0.05$. CL, cropland; AL, abandoned cropland; NV, native vegetation land

Figure 5

Soil Ca concentrations (a) and Ca/Al ratio (b) in the bulk soils at the three stages following post-agricultural restoration. Error bar is standard error (SE) of mean. Different lowercase letters indicate significant differences in Ca concentration and Ca/Al ratio in the same depth of soils between the three stages following post-agricultural restoration, based on the one-way ANOVA with LSD test at the level of $P < 0.05$. CL, cropland; AL, abandoned cropland; NV, native vegetation land

Figure 6

Soil $\delta^{15}\text{N}$ values of SON in bulk soils and different sized aggregates in different depth soils at the three stages following post-agricultural restoration. Error bar is standard error (SE) of mean. The $\delta^{15}\text{N}$ values in the soils at the depth of the 0~10 cm (a), 10~20 cm (b), and 20~30 cm (c). Different lowercase letters indicate significant differences in soil $\delta^{15}\text{N}$ values of SON between bulk soils and different sized aggregates at the same stage of post-agricultural restoration; different uppercase letters indicate significant differences in soil $\delta^{15}\text{N}$ values of SON in the bulk soils or different sized aggregates between the three stages following post-agricultural restoration, based on the one-way ANOVA with LSD test at the level of $P < 0.05$. CL, cropland; AL, abandoned cropland; NV, native vegetation land

Figure 7

Soil to plant ^{15}N Enrich factors (EF) at the three stages following post-agricultural restoration. EF = $\delta^{15}\text{N}_{\text{litter}} - \delta^{15}\text{N}_{\text{soil}}$; the $\delta^{15}\text{N}_{\text{soil}}$ was the $\delta^{15}\text{N}$ value of SON in the soil at the 0~10 cm depth; the $\delta^{15}\text{N}_{\text{litter}}$ was the $\delta^{15}\text{N}$ value of litter at the soil site. Error bar is standard error (SE) of mean. Different uppercase letters indicate significant differences in EF values between the three stages following post-agricultural restoration, based on the one-way ANOVA with LSD test at the level of $P < 0.05$. CL, cropland; AL, abandoned cropland; NV, native vegetation land

Figure 8

A conceptual model about the increasing SOC sequestration and closing N cycle following post-agricultural restoration in the karst ecosystem. The green arrows indicate the positive processes; the red arrows indicate the negative processes.