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The Vertical Differences in the Change Rates and Controlling Factors of Soil Organic Carbon and Total Nitrogen along Vegetation Restoration in a Subtropical Area of China

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Abstract: The study was to investigate the change patterns of soil organic carbon (SOC), total nitrogen (TN), and soil C/N (C/N) in each soil sublayer along vegetation restoration in subtropical China. We collected soil samples in four typical plant communities along a restoration chronosequence. The soil physicochemical properties, fine root, and litter biomass were measured. Our results showed the proportion of SOC stocks (Cs) and TN stocks (Ns) in 20–30 and 30–40 cm soil layers increased, whereas that in 0–10 and 10–20 cm soil layers decreased. Different but well-constrained C/N was found among four restoration stages in each soil sublayer. The effect of soil factors was greater on the deep soil than the surface soil, while the effect of vegetation factors was just the opposite. Our study indicated that vegetation restoration promoted the uniform distribution of SOC and TN on the soil profile. The C/N was relatively stable along vegetation restoration in each soil layer. The accumulation of SOC and TN in the surface soil layer was controlled more by vegetation factors, while that in the lower layer was controlled by both vegetation factors and soil factors.

Keywords: content and stock; increase rates; C/N; fine root biomass; vegetation-soil interaction

1. Introduction

The global carbon (C) and nitrogen (N) cycles have attracted much attention recently because the rapid emission of the oxides of C and N (often the greenhouse gas) has aggravated global climate change [1]. The cycle and interaction of C and N play a key role in ecosystem productivity and stability [2]. Soil is the greatest C and N pool in the terrestrial ecosystem [3]. The small varieties in the soil C and N pools will dramatically affect the atmosphere. Soil organic carbon (SOC) and total nitrogen (TN) contents are regarded as good indicators of soil fertility. The C and N have a good coupling relationship in response to environmental factors and spatial distribution [4]. The C sequestration in terrestrial ecosystem is largely restricted by the ability of N supply in soil [5]. The soil C/N ratio (C/N) is a vital indicator to measure this coupling relationship and is worth further study. Studying the dynamics of SOC, TN, and C/N will help us to understand the accumulation process of soil C and N, the process of soil quality change and the C-N coupling relationship [6], which is important for sustainable land use [7].

Vegetation type change could significantly affect the amount and distribution of SOC and TN. The potential explanations could be that the changes in vegetation type may alter plant species and community composition, leading to changes in the litter [6], the architecture and exudates of the root [8], and the soil properties [9]. The above changes will finally affect the amount and spatial distribution of SOC, TN, and C/N. The subtropical area of China is characterized by superior hydrothermal conditions, diverse forest type, and extremely high productivity, which contributes much to the CO_2 sequestration and climate change mitigation [10]. However, the frequent human disturbance, changeable topography, and complex climate have had a great effect on subtropical forest ecosystems, which has made climax forests uncommon here [11]. In the past twenty years, China's central government has carried out lots of forestry ecological engineering projects to restore the destroyed forest ecosystem. These engineering projects included the program of natural forests protection, the Grain to Green program, and the vegetation restoration along the Yangtze River. In our study site, the local government mainly implemented the natural forest protection plan. Consequently, the ecological environment has greatly improved and lots of secondary vegetation communities at different restoration stages have been formed here [12]. With the implementation of these projects, studies about the restoration and evolution of forest ecosystem service function have become one of the most important tasks in the subtropical forest ecosystem positioning study and more and more research efforts have been focused on this topic. For example, there have been many studies about the effects of vegetation restoration on SOC and TN. Some researchers have shown that SOC and TN contents increase significantly along vegetation restoration [13], and both stand characteristics and soil properties have important impacts on soil nutrients [14]. The effect of vegetation restoration on C stocks (Cs) and N stocks (Ns) has also been demonstrated in recent years [15,16]. However, research efforts have largely focused on the changes of SOC and TN contents and stocks in the whole soil layer along vegetation restoration. The change rates of SOC and TN in different soil sublayers were ignored. Due to the uneven distribution of litter and roots (especially fine roots) in the soil profile, the change patterns of SOC and TN vary with soil depths. How do the change rates of SOC and TN contents differ in each sublayer? Will the storage capacity of SOC and TN in different soil layers change along vegetation restoration? Will the effects of vegetation and soil factors on SOC and TN contents change with soil depth? In order to solve the above questions and improve our understanding of the SOC and TN dynamics after vegetation restoration, it is urgent to compare the difference of SOC and TN dynamics in different soil layers along vegetation restoration.

The changes in soil stoichiometry along vegetation restoration have been well studied recently. However, the conclusions about the C/N dynamics are not consistent. Some studies have showed that the C/N either increases [15,17] or stays stable [18] along vegetation restoration. Some studies found the C/N first decreased and then increased [19] along vegetation restoration. However, the change pattern of C/N along vegetation restoration in subtropical area of China has not been well recognized.

In our study, four typical vegetation communities are selected considering the restoration process of subtropical forest communities: 4–5 years scrub-grass-land (SG), 10–12 years shrubbery (Shrub), 45–46 years coniferous-broad leaved mixed forest (CF), and more than 90 years old evergreen broad-leaved forest (EF) [20]. We sample in these four vegetation communities. Soil physicochemical properties and vegetation features are then investigated. We aim to study the change pattern of SOC, TN, and C/N in each sublayer of 0–40 cm soil depth along vegetation restoration, and explore the complex influence of soil factors and vegetation factors on them. Here are the two hypotheses: (1) with vegetation restoration, the contents and stocks of SOC and TN would increase significantly, but the increase rates of SOC and TN contents would decrease with soil depth, while the storage capacity in the deep soil layer would enhance; and (2) the C/N would change but only fluctuate in a small range. In addition, the synthetically effects of vegetation factors and soil factors on SOC and TN contents in each sublayer are studied.

2. Materials and Methods

2.1. Study Site and Plant Community Description

As shown in Figure 1, we conducted the study at Changsha County (28°23′–28°24′ N, 113°17′–113°27′ E) in Hunan Province, China. The topography is characterized as a typical low hilly landscape. The soil is mainly composed of Acrisol (FAO-UNESCO), which is clay-rich, acidic, and well-drained. The area has a humid mid-subtropical monsoon climate with high temperature and abundant rainfall. The mean annual air temperature is 17.3 °C, while the annual average precipitation is 1416.4 mm (mainly from April to August). The natural vegetation used to undergo severe human interference. However, the programs of natural forest protection have been implemented in the region since 1998, thus many vegetation communities have restored rapidly, resulting in a vegetation restoration gradient [20].

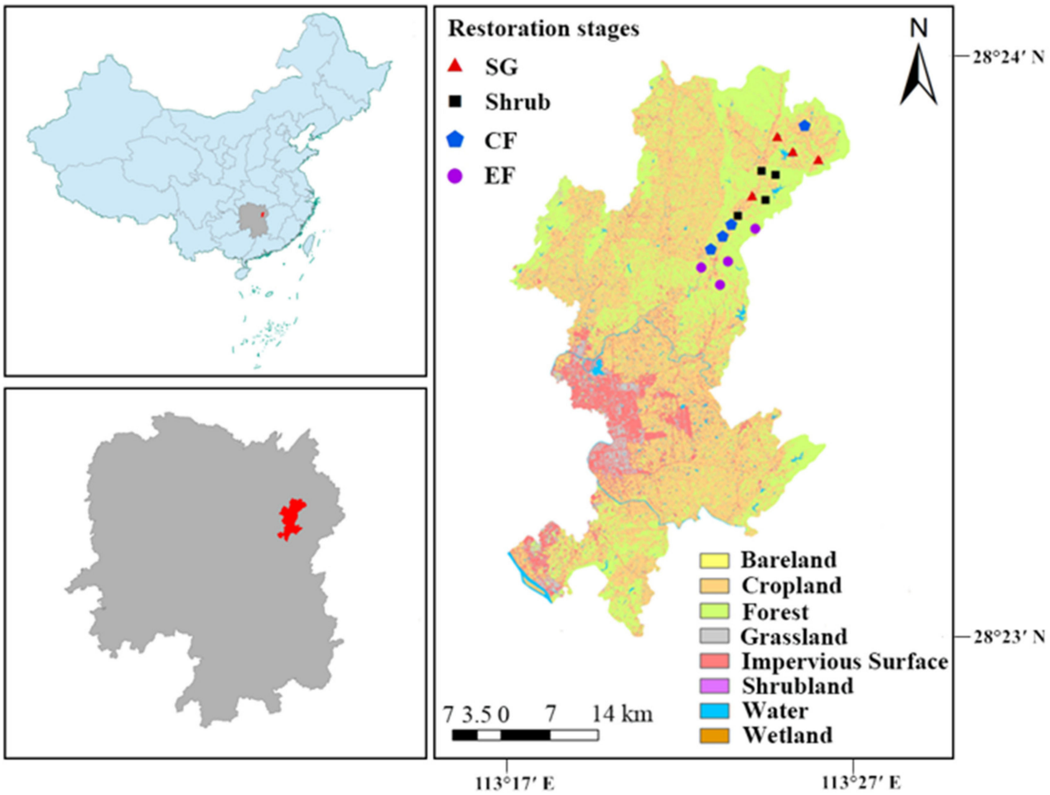

Figure 1. Location and plots distribution of the study area. SG is the scrub-grassland (4–5 years). Shrub is shrubs (10–12 years), CF is the coniferous and broadleaved mixed forest (45–46 years), and EF is the evergreen broadleaved forest (more than 90 years).

In October 2015, four different and adjacent vegetation communities along a restoration chronosequence were selected. The basic information of these communities is listed below:

(1) 4–5 years scrub-grassland (SG): In 1965, the natural evergreen broadleaved forest experienced controlled burns and site preparation. Lots of Pinus massoniana plantations were then planted here, and 24 years later (1990), these mature plantations were all felled to meet the living needs of the local people. Later, the woodlands here were repeatedly harvested, and vegetation restoration did not actually begin until 2012. When we sampled here in 2016, the community was mainly composed of herbs and some dwarf shrubs, which was often the typical community composition of early vegetation restoration stage in subtropical area;

(2) 10–12 years shrubbery (Shrub): In 1965, the local evergreen broadleaved forest was burned and destroyed, and then the Cunninghamia lanceolata plantations were planted here, and 23 years later (1989), these plantations were all felled. Later, the woodlands here were cut every 3–5 years. After 2004, vegetation restoration began and a shrub community was gradually formed. When we sampled here in 2016, the community was composed of well-grown shrubs and some scattered herbs;

(3) 45–50 years coniferous and broadleaved mixed forest (CF): In the early 1970s, the locals felled the native evergreen broadleaved forest. Then, this place was abandoned and began secondary succession. After 45 to 50 years, a coniferous and broadleaved mixed forest gradually formed. Lots of seedlings and saplings were found in this forest, while a few trees with relatively large breast diameter were found at the same time;

(4) More than 90 years old growth evergreen broadleaved forest (EF): Because there was little human disturbance, some native evergreen broadleaved forests were kept in the area. These communities had relatively stable structures and functions. Through communication with local farmers, we found that the old growth forest was over 90 years old.

We established four fixed plots in each vegetation community in the same year. All these plots had almost the same topography (elevation, slope, and aspect). In each community, four plots were randomly located in the place where there was little human interference and the plants were evenly distributed. Among four fixed plots in each vegetation community, the necessary space distance was kept (more than 1000 m) to obtain statistically independent samples. In the SG and Shrub communities, we set up four 20 m × 20 m plots. However, in the CF and EF communities, we established four wider 30 m × 30 m plots, because the community composition and structure of CF and EF communities were much more complex than SG and Shrub communities (Figure 1). Then, every plot was subdivided into several subplots (10 m × 10 m) to conduct a typical community investigation. At each subplot, the total height, height to the lowest live branch, crown width, and diameter at breast height (DBH) were measured for each tree with DBH ≥ 1 cm. The Shannon Index (*SI*) was used in our study with Equation (1):

$$SI = -\sum_{i=1}^{s} P_i \ln P_i. \tag{1}$$

where S = the total number of species in the community; P_i = relative frequency of species i in the community [21].

The detailed description of each site is shown on Table S1.

2.2. Litter and Fine Root Biomass Measurement

For the measurement of litter biomass (LB), we collected all litter in a 1 m × 1 m quadrat in the center of each 10 m × 10 m subplot. All the litter was then oven-dried at 75 °C for 72 h. The LB of each restoration stage was shown in Table 1.

The standing fine root biomass (FB) was then measured. We referred to the method used by Liu et al. [8]. We first randomly selected three points at different slope positions in each plot. Then, near these three points, we used a root drill with an inner diameter of 10 cm to take fine root soil samples at four different directions and at four soil depths of 0–10, 10–20, 20–30, and 30–40 cm. These four directions were east, west, south, north. Thus, there were 48 root samples in each permanent plot, 192 in each plant community, and 768 in total. All the samples were then quickly put into refrigeration (4 °C) before fine root separation. When we started to process the fine root, we first soaked the samples in water for 2 h and then spun the samples under faint running water in a 0.25-mm mesh to separate the roots from the soil. After that, residues sticking to the roots were carefully removed by tweezers. The clean root without any soil was divided into two classes (i.e., diameter ≤2 mm or

>2 mm). Fine roots (≤2 mm) were selected to oven-dry at 75 °C for 48 h and their dry mass was measured. We calculated the fine root biomass (*FB*) as follows (Equation (2)):

$$FB\left(\text{kg}\cdot\text{ha}^{-1}\right) = \frac{FBM \times 10^5}{\pi \times (d_s/2)^2} \tag{2}$$

where *FBM* and *ds* represent, respectively, the fine root dry mass per steel auger (g) and the inner diameter of the root drill (cm).

The FB in four different vegetation restoration stages is presented in Table 1.

Table 1. Soil physicochemical properties, fine root, and litter biomass for four restoration stages.

Variables	Soil Depth (cm)	SG	Shrub	CF	EF
BD ($g\ cm^{-3}$)	0–10	1.35 ± 0.12Aa	1.44 ± 0.18 Aa	1.26 ± 0.12 Aa	1.28 ± 0.06 Aa
	10–20	1.47 ± 0.07 ABab	1.55 ± 0.03 Aab	1.37 ± 0.10 Bab	1.44 ± 0.01 ABb
	20–30	1.46 ± 0.09 Aab	1.54 ± 0.03 Aab	1.45 ± 0.05 Ab	1.46 ± 0.03 Ab
	30–40	1.54 ± 0.06 Ab	1.64 ± 0.03 Bb	1.42 ± 0.05 Cb	1.46 ± 0.05 ACb
	0–40	1.45 ± 0.04 AB	1.54 ± 0.04 A	1.37 ± 0.07 B	1.41 ± 0.02 B
Sand (%)	0–10	37.42 ± 2.60 Aab	68.71 ± 1.71 Ba	44.60 ± 20.39 Aa	22.09 ± 8.03 Aa
	10–20	34.55 ± 2.61 Aa	60.51 ± 1.38 Bb	40.26 ± 20.96 ABa	22.24 ± 1.19 Aa
	20–30	34.96 ± 3.77 Aab	62.47 ± 5.42 Bb	41.49 ± 19.99 ABa	20.65 ± 2.42 Ba
	30–40	40.23 ± 4.83 ABb	63.07 ± 3.11 Ab	39.83 ± 22.21 ABa	21.91 ± 3.52 Ba
	0–40	36.79 ± 3.26 A	63.69 ± 1.67 B	41.55 ± 20.82 AB	21.72 ± 1.51 A
Silt (%)	0–10	50.33 ± 4.80 Aa	20.29 ± 0.10 Ba	41.71 ± 17.03 ABa	62.16 ± 11.96 Aa
	10–20	63.17 ± 2.58 Ab	26.98 ± 3.17 Bb	53.12 ± 22.99 Aa	59.89 ± 4.21 Aa
	20–30	62.51 ± 3.41 Ab	26.70 ± 3.26 Bb	51.22 ± 23.87 ABa	64.30 ± 3.14 Aa
	30–40	57.24 ± 5.32 Ab	25.92 ± 1.43 Bb	55.42 ± 24.94 Aa	67.00 ± 4.66 Aa
	0–40	58.31 ± 1.94 A	24.97 ± 0.57 B	50.37 ± 22.21 A	63.34 ± 1.65 A
Clay (%)	0–10	12.25 ± 6.97 Aa	11.00 ± 1.66 Aa	13.68 ± 3.63 Aa	15.76 ± 4.38 Aa
	10–20	2.28 ± 0.52 Ab	13.18 ± 3.23 BCb	6.62 ± 2.20 ABa	17.87 ± 4.85 Ca
	20–30	2.52 ± 0.71 Ab	10.83 ± 2.38 BCb	7.29 ± 5.30 ABa	15.05 ± 3.93 Ca
	30–40	2.54 ± 0.55 Ab	11.01 ± 1.91 Bb	4.75 ± 3.52 Aa	11.09 ± 2.29 Ba
	0–40	4.90 ± 1.84 A	11.50 ± 1.46 BC	8.09 ± 1.86 AB	14.94 ± 2.92 C
pH	0–10	4.39 ± 0.12 Aa	4.71 ± 0.10 Ba	4.19 ± 0.13 ACa	3.99 ± 0.05 Ca
	10–20	4.67 ± 0.14 Ab	4.88 ± 0.12 Aab	4.35 ± 0.11 Bb	4.29 ± 0.09 Bb
	20–30	4.83 ± 0.07 Ab	5.02 ± 0.13 Bbc	4.40 ± 0.05 Cb	4.30 ± 0.11 Cb
	30–40	5.09 ± 0.11 Ac	5.08 ± 0.16 Ac	4.49 ± 0.08 Bb	4.33 ± 0.20 Bb
	0–40	4.74 ± 0.09 A	4.92 ± 0.07 B	4.36 ± 0.09 C	4.23 ± 0.10 C
FB ($kg\ ha^{-1}$)	0–10	304.67 ± 68.92 Aa	677.70 ± 153.16 Aa	1745.23 ± 326.26 Ba	3026.41 ± 371.47 Ca
	10–20	152.24 ± 41.39 Ab	474.95 ±67.33 Bb	1236.22 ± 282.69 Cb	2100.15 ±284.41 Db
	20–30	30.45 ± 8.71 Ac	135.56 ± 39.49 Bc	436.31 ± 88.40 Cc	679.47 ± 94.42 Dc
	30–40	20.30 ± 5.20 Ac	67.76 ± 15.79 Ac	218.16 ± 38.64 Bc	370.62 ± 74.75 Cc
	0–40	507.65 ±114.39 A	1355.97 ± 73.96 B	3635.91 ± 622.91 C	6176.65 ± 696.40 D
LB ($t\ ha^{-1}$)		1.57 ± 0.93 A	6.28 ± 0.70 B	7.09 ± 1.43 BC	7.84 ± 0.75 C

Note: BD is bulk density; Sand is the sand content (soil particle size fraction > 0.05 mm); Silt is the silt content (soil particle size fraction 0.05–0.002 mm); Clay is the clay content (soil particle size fraction < 0.002 mm); FB is fine root biomass; LB is litter biomass. Different capital letters indicate statistically significant differences ($p < 0.05$) among different vegetation restoration stages in the same soil layer. Different lower letters indicate statistically significant differences ($p < 0.05$) among different soil layers at the same vegetation restoration stage.

2.3. Soil Sampling and Analysis

Soil samples were collected in October 2016 from three points at different slope positions in each plot. We collected from the soil profiles at 10 cm intervals from 0 to 40 cm after removing the litter on the topsoil. At the same time, to determine the soil bulk density (BD), undisturbed soil cores were taken using the steel corer (7 cm in diameter, 5.2 cm in height) by the volumetric ring method.

In every plot, the samples at the same depth were pooled together, while plant residues and gravel were excluded. These soil samples were divided into several parts. A part of that was used to measure soil particle size fractionation (through 2-mm mesh), while another part was used to measure soil pH (through 1-mm mesh) and SOC and TN contents (through 0.25-mm mesh).

SOC was determined following the $K_2Cr_2O_7$-H_2SO_4 digestion method, while TN was determined using the Kjeldahl method. After mixing soil and deionized water according to 1–2.5 ratios, soil pH was measured using a pH meter (FE20, Mettler Toledo, Switzerland) [20]. The soil particle size fractionation was measured using a Mastersizer 3000 (Malvern). The soil physicochemical properties are shown in Table 1.

2.4. Statistical Analysis

The Cs and Ns (all in $\mathrm{Mg{\cdot}ha^{-1}}$) were calculated as follows (Equations (3) and (4)) [15]:

$$C_S\left(\mathrm{Mg{\cdot}ha^{-1}}\right) = \mathrm{SOC} \times \mathrm{BD} \times \mathrm{D} \times 10^{-1} \quad (3)$$

$$N_S\left(\mathrm{Mg{\cdot}ha^{-1}}\right) = \mathrm{TN} \times \mathrm{BD} \times \mathrm{D} \times 10^{-1} \quad (4)$$

where SOC = soil organic content ($\mathrm{g{\cdot}kg^{-1}}$); TN = total nitrogen content ($\mathrm{g{\cdot}kg^{-1}}$); BD = soil bulk density ($\mathrm{g{\cdot}cm^{-3}}$) and D = the soil thickness (cm). In our study, we calculated Cs and Ns for each depth of soil.

Furthermore, to probe if there was an isometric relationship between SOC and TN concentrations along vegetation restoration in each soil depth, we examined the relationship between SOC and TN contents using the reduced major axis regression (RMA) with R package 'lmodel2' v1.7–3 [6,22]. The proportional changes between SOC and TN concentrations will be better presented in this regression method. We calculated as follows (Equation (5)):

$$\log y = a + b(\log x) \quad (5)$$

where y = TN concentration ($\mathrm{g{\cdot}kg^{-1}}$), x = SOC concentration ($\mathrm{g{\cdot}kg^{-1}}$), 'a' is the intercept, and 'b' is the scaling slope.

If 'b' was not significantly different from 1.0 (i.e., the 95% of confidence interval of 'b' covered 1.0), we concluded that there was an isometric relationship between SOC and TN concentrations.

We conducted all statistical analyses with R (3.6.1) [23]. The results in this paper were reported as means ± standard deviation (SD) for four replicates. All the C/N appearing in the paper was converted into mass ratios. We used ANOVA and Two-way ANOVAs at a 5% level of significance to examine the effects of vegetation restoration stages, soil depths, and their interaction on SOC, TN, Cs, Ns, and C/N. We studied the relationships among SOC, TN, and environmental factors using the Pearson linear correlation coefficients analysis. We examined the change rates of SOC and TN at different soil depths using the linear regression method. In order to investigate the contribution of different environmental factors to the variation of SOC and TN along vegetation restoration, we first used the Partial Least Squares Regression (PLSR) to select important factors (R package 'pls' v2.7–2). Then, after using the forward selection (R package 'packfor' v0.0–8) to screen important vegetation and soil factors with two stopping criteria suggested by Blanchet et al. [24], we used the Variance Partitioning Analysis (VPA) with the R package 'vegan' v2.3–3 to examine the joint and independent contributions of vegetation and soil factors to SOC and TN in each soil layer.

3. Results

3.1. SOC and TN Content

Vegetation restoration stage, soil depth, and their interactions significantly affected contents of SOC and TN (Table S2). As shown in Table 2, in a given soil layer, the contents of SOC and TN all increased along vegetation restoration. In 0–10 and 10–20 cm soil layers, a significant increase in SOC contents was found when entering the CF stage, while in the same soil layer, a significant increase in TN contents was found when entering Shrub stage. However, SOC and TN contents in the 20–30 and 30–40 cm soil layers did not significantly increase until entering the EF and CF stages ($p < 0.05$), respectively. This indicates that SOC and TN contents of the surface soil significantly changed at an earlier stage than the deeper soil. Furthermore, the SOC and TN contents decreased with increasing soil depth at every restoration stage, in particular, a significant decrease was found from 0–10 to 10–20 cm soil depth ($p < 0.05$).

Table 2. Soil organic carbon and total nitrogen contents at different soil depths for different restoration stages ($n = 4$).

Soil Depth (cm)	SOC Concentrations (g·kg^{-1})			
	SG	Shrub	CF	EF
0–10	12.22 ± 3.08 Aa	19.49 ± 0.77 ABa	25.65 ± 7.52 Ba	47.58 ± 5.88 Ca
10–20	4.49 ± 2.62 Ab	7.67 ± 0.62 ABb	10.89 ± 2.94 Bb	18.22 ± 3.52 Cb
20–30	2.95 ± 1.71 Ab	5.32 ± 0.83 Ac	7.01 ± 2.41 Ab	14.89 ± 4.69 Bb
30–40	1.67 ± 0.70 Ab	3.01 ± 0.43 Ad	5.07 ± 1.47 Ab	13.51 ± 4.46 Bb
0–40	5.33 ± 1.87 A	8.87 ± 0.49 AB	12.16 ± 3.51 B	23.55 ± 4.53 C

Soil Depth (cm)	TN Concentrations (g·kg^{-1})			
	SG	Shrub	CF	EF
0–10	0.67 ± 0.14 Aa	1.30 ± 0.19 Ba	1.38 ± 0.34 Ba	3.05 ± 0.33 Ca
10–20	0.27 ± 0.08 Ab	0.53 ± 0.04 Bb	0.65 ± 0.18 Bb	1.34 ± 0.22 Cb
20–30	0.18 ± 0.08 Abc	0.39 ± 0.07 ABbc	0.47 ± 0.13 Bb	1.19 ± 0.27 Cb
30–40	0.13 ± 0.03 Ac	0.29 ± 0.02 ABc	0.40 ± 0.09 Bb	1.12 ± 0.27 Cb
0–40	0.31 ± 0.08 A	0.63 ± 0.07 AB	0.73 ± 0.18 B	1.67 ± 0.25 C

The results of linear regression showed that contents of SOC and TN were correlated with restoration stage at high significance levels ($R^2 > 0.65$, $p < 0.05$) for all soil layers (Figure 2a,b). However, the k value (the increase rate) generally decreased with soil depth, showing that the SOC and TN contents of different sublayers had different change patterns. Furthermore, in the same soil layer, the k value of TN was less than that of SOC, showing that the growth rate of TN content along vegetation restoration was slower than that of SOC.

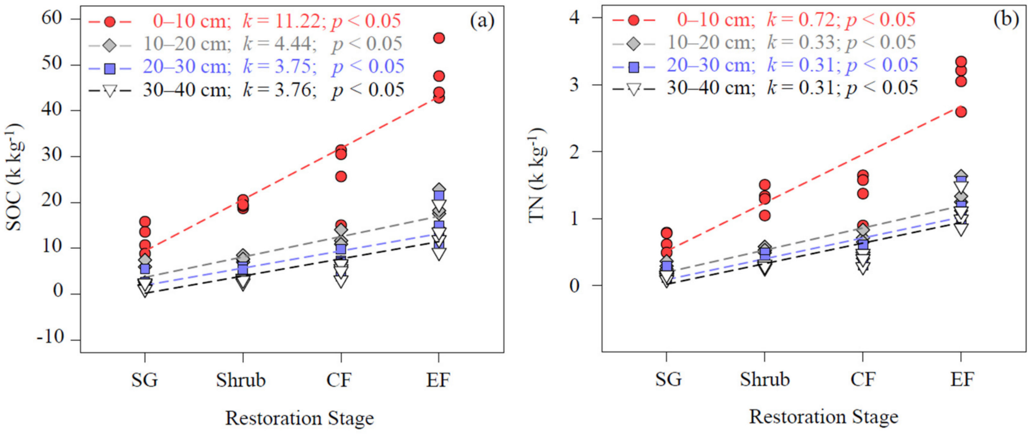

Figure 2. The soil organic carbon (**a**); and soil total nitrogen (**b**) contents at different soil layers along vegetation restoration. Note: Linear regressions were fitted for individual soil layers. k was the slope of the regression equation and p was the significant level.

3.2. SOC and TN Storage

Vegetation restoration stage, soil depth, and their interactions significantly affected Cs and Ns (Table S2). In a given soil layer, Cs and Ns all increased during the restoration process (Figure 3a,b). However, similar to the change patterns of SOC and TN contents, Cs and Ns of the surface soil significantly changed at an earlier stage than the deeper soil. This indicates that the deeper the soil layer, the more slowly the Cs and Ns increased along vegetation restoration. Furthermore, nearly all the significant increase of Ns was found at earlier restoration stages compared to Cs. In addition, at a given restoration stage, the vertical decrease patterns of Cs and Ns was similar to that of SOC and TN contents.

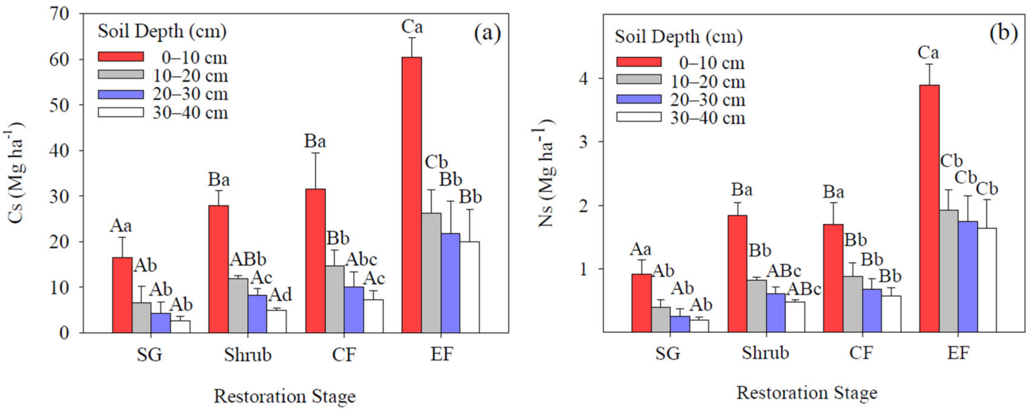

Figure 3. Soil organic carbon (**a**); and total nitrogen (**b**) stocks at different soil layers along vegetation restoration.

The proportion of Cs and Ns in each soil depth to 0–40 cm soil layer varied with soil depth and restoration stage, but the change patterns of the proportion were different at different soil depths (Figure 4a,b). In the 0–10 cm soil layer, the proportion of Cs and Ns significantly decreased along vegetation restoration ($p < 0.05$). In the 10–20 cm soil layer, the proportion was not significantly different. In the 20–30 cm soil layer, the proportion of Cs and Ns increased slowly along vegetation restoration, and in the 30–40 cm soil layer, the proportion of Cs and Ns significantly increased along vegetation restoration ($p < 0.05$), indicating that the storage capacity of SOC and TN in the deep soil layer enhanced along with vegetation restoration.

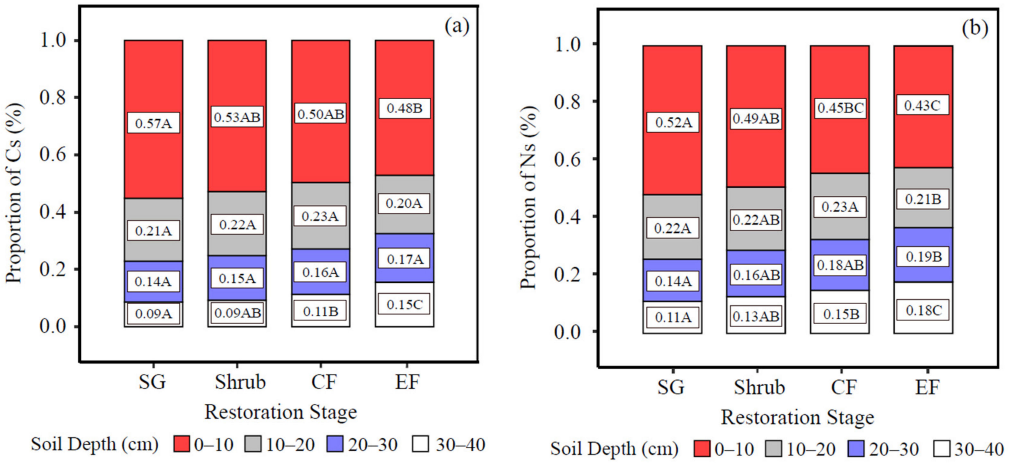

Figure 4. The proportion of Cs (**a**) and Ns (**b**) in each soil depth at different restoration stage. Note: The proportion of Cs in each soil depth (Cs in each soil layer divided by Cs in 0–40 cm soil layer) at different restoration stage (**a**); The proportion of Ns in each soil depth (Ns in each soil layer divided by Ns in 0–40 cm soil layer) at different restoration stage (**b**); Different capital letters indicate statistically significant differences ($p < 0.05$) among different vegetation restoration stages in the same soil layer.

3.3. Soil Carbon/Nitrogen Ratio (C/N)

Vegetation restoration stage and soil depth significantly affected soil C/N ($p < 0.05$) (Table S2). In all soil samples, the C/N decreased from SG stage to Shrub stage, increased from Shrub stage to CF stage, and finally decreased from CF stage to EF stage. In general, the C/N of SG and CF stages was higher than that of Shrub and EF stages in each soil layer (Table 3). However, in the 10–20 and 30–40 cm soil layers, there was no significant difference in C/N among four stages ($p > 0.05$). Additionally, in the 0–40 cm soil layer, the C/N was significantly different among the four restoration stages ($p < 0.05$). The RMA analysis indicated that the slope (*k* value) of the log-transformed C-N stoichiometric relationship was, respectively 1.07, 0.96 1.08, and 0.97 at the 0–10, 10–20, 20–30, and 30–40 cm soil layers, which were not statistically different from 1.0 ($p > 0.05$), indicating that SOC and TN contents in each soil layer exhibited an isometric pattern, and there was relatively stable C/N in each soil layer along vegetation restoration (Figure 5, Table 4).

Table 3. Soil C/N at different soil depths for different restoration stages ($n = 4$).

Soil Depth (cm)	**C/N**			
	SG	**Shrub**	**CF**	**EF**
0–10	18.07 ± 1.24 Aa	15.20 ± 1.84 Ba	18.43 ± 1.19 Aa	15.62 ± 1.37 Ba
10–20	15.59 ± 5.43 Aa	14.41 ± 1.26 Aa	16.70 ± 0.31 Ab	13.59 ± 0.60 Ab
20–30	16.07 ± 2.47 Aa	13.80 ± 2.75 ABa	14.60 ± 1.35 ABc	12.36 ± 1.05 Bbc
30–40	13.07 ± 4.44 Aa	10.39 ± 1.95 Ab	12.40 ± 1.15 Ad	11.93 ± 1.07 Ac
0–40	16.75 ± 2.33 A	14.25 ± 1.79 BC	16.58 ± 0.91 AB	14.02 ± 0.76 C

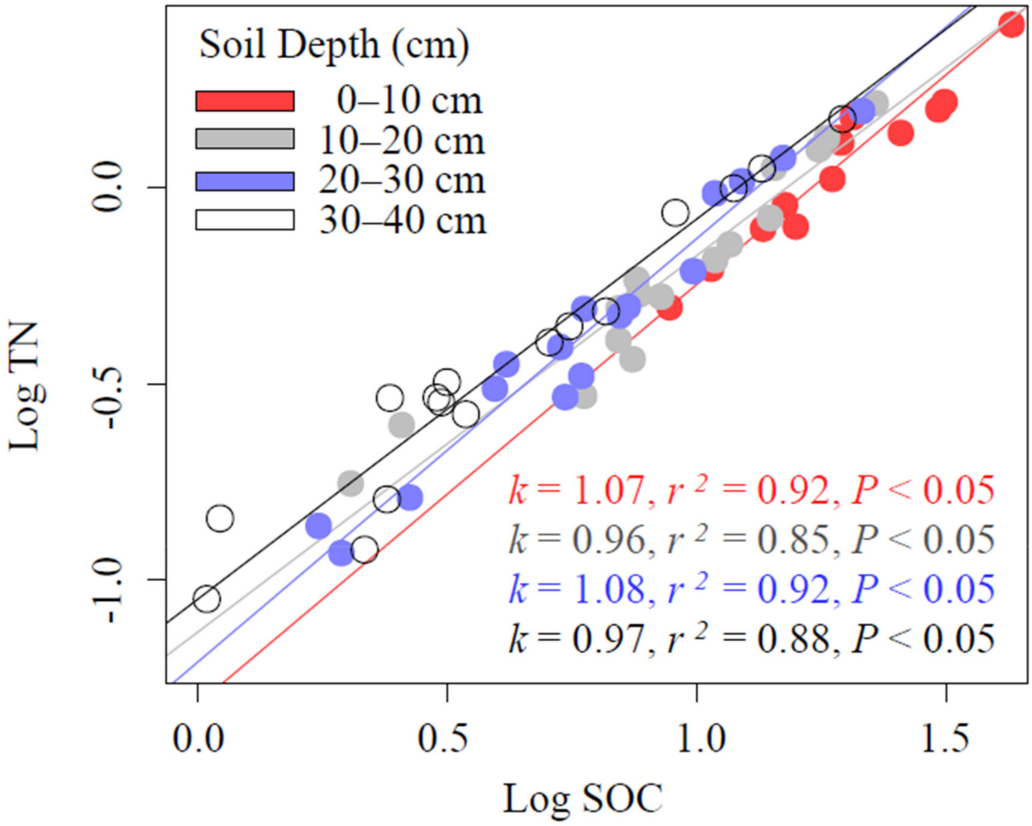

Figure 5. C-N stoichiometric relationships in each soil layer.

Table 4. Summary of reduced major axis analysis of the log-transformed C-N stoichiometric relationship for different soil depths.

Soil Depth (cm)	Slope	95% CI of Slope	r^2	p	n
0–10	1.07	0.95, 1.20	0.92	<0.05	16
10–20	0.96	0.82, 1.12	0.85	<0.05	16
20–30	1.08	0.96, 1.21	0.92	<0.05	16
30–40	0.97	0.84, 1.11	0.88	<0.05	16

Note: The title of "95% CI of slope" means the 95% of confidence interval of the slope.

As shown in Table 3, at a given restoration stage, the C/N decreased with increasing soil depth, except the SG stage. There was a neutral effect of soil layers on C/N in the SG stage ($p > 0.05$), while there was a significantly higher C/N at the surface soil than the deeper soil in the Shrub, CF, and EF stages ($p < 0.05$).

3.4. Environmental Factors Affecting SOC and TN Contents

To compare different environmental factors affecting SOC and TN, the Pearson correlation analysis and PLSR were conducted and the results are shown in the supporting information (Tables S3 and S4). For further details, please see Supplementary Materials. The VPA was also conducted to study the joint and independent contributions of vegetation and soil factors to SOC and TN in each soil layer (Figure 6). In order to better understand this part, we will discuss it in detail in Section 4.3.

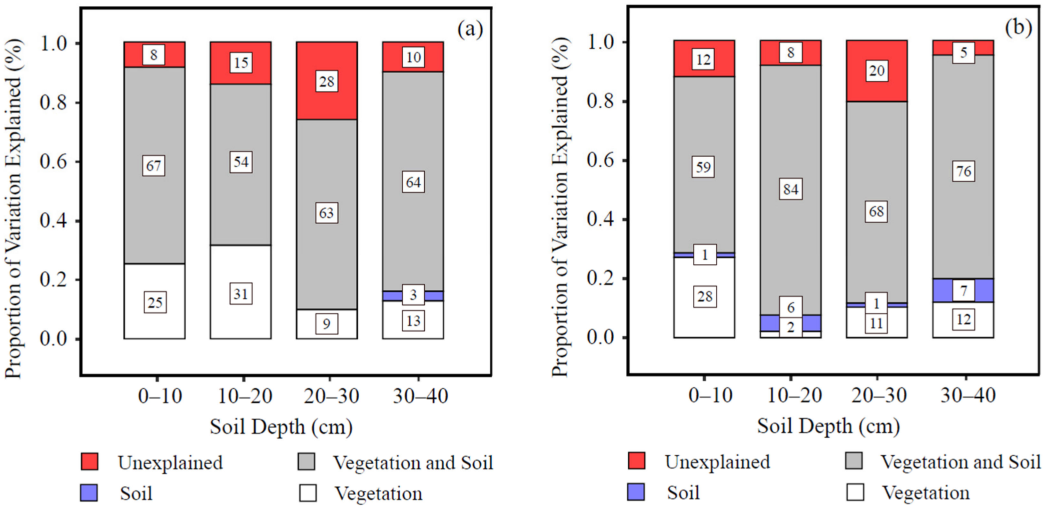

Figure 6. The variation partition analysis. Note: The Variation Partition Analysis (VPA) of the contributions of vegetation and soil factors to the soil organic carbon (**a**) and total nitrogen (**b**) changes. The numbers in each part stand for the percentage of variation in SOC or TN that can be explained by different factors: vegetation factors (white), soil factors (blue), the interaction between vegetation and soil factors (gray), as well as the unexplained factors (red).

4. Discussions

4.1. Changes of SOC, TN Contents, and Stocks along Vegetation Restoration

In this study, SOC and TN contents and their stocks in each layer were found to increase significantly along vegetation restoration (Table 2, Figure 3). However, the increase rates decreased as soil depth increased (Figure 2). Furthermore, the storage capacity of SOC and TN in deep soil layer enhanced along vegetation restoration (Figure 4). Our results support the first hypothesis and indicate that the vegetation restoration could promote SOC and TN sequestration, and improve the soil holding capacity of C and N by improving the storage capacity of C and N in the deep soil layer.

Our results showed that vegetation restoration significantly promoted the SOC contents. Previous studies have explained this phenomenon. As far as we know, Gu et al. studied the variation of SOC along vegetation restoration in the same study site and found that the rapid increase of SOC can be mainly attributed to the impact of the plant biomass and soil nutrients [20]. Furthermore, our results showed the increase rates of SOC content in the topsoil layer were much greater than those in the deep soil layer (Figure 2). This was consistent with the results of Wei et al. and Li [25,26], who also found that the SOC content of the surface soil increased more rapidly than that of deep soil along vegetation restoration. These results can be attributed to the following four reasons. First, this can be attributed to the differences in the amount of C input associated with litter and root in different soil layers [25]. Plant litter, root exudates, and fine root turnover were considered to be the main sources of SOC [27]. There are more litter and plant roots on the soil surface, which will provide more carbon input for the surface soil than the deeper soil [26]. Second, the FB, the dominant factor affecting SOC in our study (Table S4), was found to have a clear downward trend as the soil layer deepened (Table 1), which may also explain the increase rate difference. Third, previous studies have found that surface soil has a better humic process than the deeper soil, which will promote the decomposition process of the plant litter and roots [28]. The accelerated decomposition process will speed up the C input. Fourth, the uneven distribution of Clay may be one of the reasons. Soil texture has been proven to be an important factor affecting SOC [29]. In this study, the Clay was found to be significantly positively correlated with SOC content (Table S3) and the PLSR also found that Clay was a dominant factor affecting SOC content (Table S4). This indicates that Clay may have a great effect on SOC. There have been some studies explaining how the clay particles affect the SOC. For example, some studies have explained that soil clay particles could effectively protect SOC from microbial and soil enzymes and ultimately promote SOC accumulation [30,31]. Kulmatiski et al. also found that there was greater fine root biomass on clay than on sand soil because the clay soil was more nutritious [32]. More biomass means more C input. This indicates that soil clay particles may also promote SOC accumulation by increasing the C input. The high correlation among the Clay, FB, and LB in our study supports the above explanation (Table S3). In Table 1, generally, the Clay was found to decrease with soil depth. Thus, we conjectured that the uneven distribution of the Clay in 0–40 cm soil depth may result in the different increase rates of SOC content in each soil layer, which was our fourth explanation.

The change pattern of TN content was consistent with SOC content (Table 2). The vegetation restoration significantly promoted the TN content and the increase rates of TN content in the topsoil layer were much greater than those in the deep soil layer. The same result was also found by Hernández et al. and Cheng et al. [33,34]. The mechanism affecting the increase rate of SOC and TN is different [35], but the plant biomass change may be the common reason which can both explain the change of SOC and TN. Both TN and SOC are derived from the decomposition of litter and fine roots. Thus, the increase of LB and FB along vegetation restoration will also increase the TN content. Furthermore, the uneven distribution of LB and FB with soil depth may also result in the different increase rate of TN content with soil depth.

In addition, in our study, the increase rate of SOC was higher than that of TN, which agreed with the research results of Li et al. and Yang et al. [36,37]. It is well-known that the SOC and TN are mainly derived from decomposition of litter and fine roots. Since the contents of SOC in leaves and fine roots are higher than those of TN [38,39], when they decompose, the amount of SOC produced per unit of time is greater than that of TN, i.e., the increase rate of SOC is higher. In addition, it may also be related to the increase of soil N absorption along vegetation restoration. The C in plants mainly comes from the atmosphere, while the N in plants mainly comes from the soil. Along vegetation restoration, the increase of biomass [28] makes plants absorb more N-rich substances (such as enzymes, transport proteins, and amino acids) to support the metabolism activities, which leads to the increase of the plant N consumption [40]. The increase of the plant N consumption will restrain the soil N accumulation.

In this study, the Cs and Ns increased along vegetation restoration (Figure 3a,b), which agreed with Wei et al., Li, and Deng et al. [25,31]. Furthermore, our study has revealed that the proportion of deeper soil Cs and Ns increased ($p < 0.05$), while the proportion of surface soil Cs and Ns decreased along vegetation restoration ($p < 0.05$) (Figure 4a,b), indicating that deep soil contributes much to the growth of Cs and Ns along vegetation restoration. Similar findings were reported on the Loess Plateau of China, which suggested that the proportion of 20–40 cm soil Cs gradually increased during a natural restoration process [15]. These results can be due to the following reasons. First, afforestation affects Cs and Ns both in surface and deep soil [17]. These effects in deep soil mainly include the plant roots and root exudates, dissolved organic matter, and bioturbation [41]. Second, Jobbágy et al. also mentioned that there was decreasing SOC turnover with depth, which was more conducive to carbon accumulation in deep soil layers [41].

4.2. Changes of C/N along Vegetation Restoration

In the present study, the C/N varied along vegetation restoration, especially in 0–10, 20–30, and 0–40 cm soil depths (Table 3). These results partially supported our second hypothesis and were similar to previous studies which showed that the C/N would change with the land use types [16,27,42]. The preliminary reason why the C/N fluctuates along vegetation restoration could be that the growth rate of SOC and TN is not consistent. In the present study, from SG to Shrub stage, the increase range of SOC content in each soil layer (59–80%) was lower than that of TN content (94–123%), resulting in a decreasing C/N. From Shrub to CF stage, the increase range of SOC content in each soil layer (32–68%) was higher than that of TN content (6–38%), resulting in an increasing C/N. From CF to EF stage, the increase range of SOC content in each soil layer (86–166%) was lower than that of TN content (106–180%), resulting in a decreasing C/N again. The inconsistent increase rate resulted in the different C/N among different restoration stages. In addition, there are more specific reasons: plants changed C, N, and *p* ratios by absorbing or releasing them from or to soil [27]. Different plants in different restoration stages have different nutrient requirements and different capacities for releasing C and N into the soil [43], resulting in different C/N. For example, the highest C/N (18.4 in mass ratio) was found at the 0–10 cm depth for the CF stage (Table 3). This higher value could be explained by different N use efficiencies in coniferous forests, resulting in strong N deficiency and eventually leading to higher C/N [6].

Furthermore, a well-constrained C/N was found at all soil layers because SOC and TN scaled isometrically in each soil layer (Table 4, Figure 5). Although the C/N in some soil layers varied significantly among different stages (Table 3), this fluctuation was actually constrained around a fixed value (Table 4, Figure 5). These relatively fixed elemental ratios are called "Redfield-like" ratios, which were found in the marine ecosystem first [44]. In terrestrial ecosystems, the same "Redfield-like" ratios were found by Cleveland and Liptzin [4], who indicated that there was a global well-balanced C/N of 12.3 in mass ratio in 0–10 cm soil layer. This isometric pattern of SOC and TN was also found by Tian et al., Yang et al., Yang and Luo, and Li et al. [5,6,45,46]. This phenomenon could be induced by the fact that the formation of organic matter requires a certain amount of N and other nutrients in a relatively fixed ratio with C [46].

Furthermore, the C/N was found to decease as the soil layer deepened (Table 3). This result has supported earlier findings [47,48]. This phenomenon can be due to the fact that as the soil depth increased, the SOC content decreased faster than TN content, because TN was more stable [48]. More specifically, compared with surface soil, there is more decomposed humus in the deep soil. As the decomposition progresses, the easily decomposable substances are consumed quickly, while the N is immobilized in microbial biomass and decay products, resulting in difficult-to-decompose substances with lower C/N in the bottom layer [6]. In addition, rainfall could promote the migration of N to deep soil, resulting in N accumulation in deeper soil. This is also one reason why the C/N will decrease for deeper intervals [49].

4.3. Key Factors Affecting SOC and TN of Each Sublayer in 0–40 cm Soil Layer

Soil properties and stands characteristics may affect SOC and TN [47,50]. Both SOC and TN contents were found to be significantly positively correlated with the Clay, LB, and FB, while significantly negatively correlated with BD, pH, and the sand content (except that SOC was not significantly correlated with Sand) (Table S3). Using the method of PLSR, we found the key factor in each soil layer (Table S4). In Table S4, the higher value of the standard partial regression coefficient means that the predictor variable has greater effect on the response variable. FB, with the highest standard partial regression coefficient in each soil layer, was the key factor affecting SOC in all soil layers. Furthermore, we found that FB was the key factor affecting TN change in the 0–10 cm soil layer, and then Clay replaced FB as the most important when entering the deeper soil. The mechanism by which FB and Clay affect SOC and TN contents has been discussed before. In addition, our VPA showed that the vegetation factors alone explained 25%, 31%, 9%, and 13% of this variation of SOC in the 0–10, 10–20, 20–30, and 30–40 cm soil layers, respectively, while the soil factors alone explained 3% of this variation of SOC in the 30–40 cm soil layer (Figure 6). Furthermore, we found that the vegetation factors alone explained 28%, 2%, 11%, and 12% of this variation of TN in the 0–10, 10–20, 20–30, and 30–40 cm soil layers, respectively, while the soil factors alone explained 1%, 6%, 1%, and 7% of this variation of TN in each soil layer, respectively. These results have suggested that vegetation factors have a greater effect on surface soil than deep soil, while soil factors have a greater effect on deep soil than surface soil. These results can be explained by the following reasons. First, Deng et al. found that plant roots were very important for the SOC increase in the topsoil [51], which can be explained that FB or LB decreased as soil layers deepened (Table 1). The similar root distribution was also found by Jackson et al. [52]. Thus, the impact of vegetation factors becomes weaker when entering deeper soil. Second, Jobbágy et al. found that the correlation between SOC and Clay was highest in deep soil layers [41], which suggested that Clay may have a great effect on SOC content in deep soil. As the influence of vegetation factors decreased with soil depth, the neglected soil factors such as Clay began to dominate the C and N changes in the deep soil. Soil clay particles could promote the plant biomass input [32] and protect soil organic matter from decomposition, which may contribute to SOC and TN sequestration [30,31].

The VPA quantitatively studied the relative contributions of different factors and their interaction to soil SOC and TN variation (Figure 6). We found that vegetation factors contributed more to SOC and TN variation than soil factors in each soil layer in most cases. This result indicates that vegetation factors had a greater impact on SOC and TN than soil factors along vegetation restoration.

Additionally, we found that the interaction of vegetation factors and soil factors explained most of the variation in SOC, which was 67%, 54%, 63%, and 64% in 0–10, 10–20, 20–30, and 30–40 cm soil layers, respectively. We also found that their interaction explained most of the variation in TN, which was 59%, 84%, 68%, and 76% in each soil layer, respectively. This indicates that the interaction of vegetation and soil factors was a dominant factor for the change of SOC and TN with vegetation restoration. Similar results have been found by Liu et al. [53], whose study found the accumulation of nutrients and organic matter in the surface soil causes complex interactions between biotic processes that are regulated by plants and soil biota, as well as by abiotic processes driven by environmental factors. Although the mechanism of the interactions between vegetation factors and the soil factors on SOC and TN accumulation is not clear now, past research can still give us some inspiration. For example, on the one hand, the soil factor will affect the vegetable factor. Some studies found that there was greater fine root biomass on clay than sand soils [32]. One potential explanation can be that clay soil with more nutrients is better for the growth of plants, resulting in greater fine-root biomass [32]. On the other hand, the vegetable factor will also affect the soil factor. As mentioned before, the LB will increase with the restoration stage (Table 1). Thus, the exposed surface area will decrease, which will facilitate the formation of fine soil particles (silt and clay particles). Past studies have showed that fine soil particles (silt and clay particles) are more conducive to SOC and TN sequestration [54].

Although soil and vegetation factors have explained most of the variation, there is still some variation in SOC and TN that cannot be explained. This indicates that there are still some important environmental factors that are not found. Some studies have found that microbial biomass may greatly affect SOC accumulation along vegetation restoration [53], which may be the direction for further research.

5. Conclusions

Our study suggested that vegetation restoration significantly promoted SOC and TN sequestration in a subtropical area of southern China, and the SOC and TN of the surface soil increased faster than the deep soil. Furthermore, vegetation restoration promoted the uniform distribution of SOC and TN on the soil profile by increasing the proportion of Cs and Ns in the deeper soil. Different but well-constrained C/N was found at both top and deep soil. There were different factors controlling the accumulation of C and N in different soil layers. The surface soil was found to be mainly affected by the vegetation factors, while the deeper soil was controlled by both vegetation factors and soil factors. With the increasing afforestation area in China, it is critical to pay more attention to the mechanisms of C and N dynamics after vegetation restoration, which will strengthen our ability to understand C and N pool dynamics and predict the Cs and Ns changes after vegetation restoration.

Supplementary Materials: The following are available online at http://www.mdpi.com/2071-1050/12/16/6443/s1, Table S1: Vegetation community characteristics and site factors at different restoration stages, Table S2: The result of two-way ANOVA tests, Table S3: The result of Pearson's correlation analysis, Table S4: Results of partial least squares regression (PLSR) in each soil layer.

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