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Effects of Plum Plantation Ages on Soil Organic Carbon Mineralization in The Karst Rocky Desertification Ecosystem of Southwest China

Hui Yang[a,b], Biqin Mo[a,b], Mengxia Zhou[a,b], Tongbin Zhu[a,b]* and Jianhua Cao[a,b]*

[a] Institute of Karst Geology, CAGS, Karst Dynamics Laboratory, MNR and Guangxi, Guilin 541004, China
[b] International Research Centre on Karst, Under the Auspices of UNESCO, Guilin 541004, China
* Correspondence:Corresponding author 1: Tongbin Zhu, E-mail address: zhutongbin@gmail.com
Address: Institute of Karst Geology, CAGS, Karst Dynamics Laboratory, MLR and Guangxi, Guilin 541004, China Tel. +86 773 5837840, Fax. +86 773 5837845
* Correspondence:orresponding author 2: Jianhua Cao, E-mail address: jhcaogl@karst.ac.cn
Address: Institute of Karst Geology, CAGS, Karst Dynamics Laboratory, MLR and Guangxi, Guilin 541004, China Tel. +86 773 7796626, Fax. +86 773 5837845

Abstract: Soil organic carbon (SOC) mineralization is closely related to carbon source or sink of terrestrial ecosystem. Understanding soil organic carbon (SOC) mineralization under plum plantation is essential for improving our understanding of SOC responses to land-use change in karst rocky desertification ecosystem. In this study, 2-y, 5-y and 20-y plum plantations and adjacent woodland were sampled and a 90-day incubation experiment was conducted to investigate the effect of plum plantation with different years on SOC mineralization in subtropical China. Results showed that: (1) there was no significant difference in SOC content between different planting years, but there were significant differences in accumulative SOC mineralization (C_t) and potential SOC mineralization (C_0); (2) the dynamics of the SOC mineralization was a good fit to a first-order kinetic model. Both C_0 and C_t in calcareous soil of this study was several to ten folds lower than that in other soils, indicating that SOC in karst region has higher stability. (3) Correlation analysis revealed that both C_t and C_0 was significantly correlated with soil calcium (Ca) and C/N, indicating the important role of Ca and C/N in SOC mineralization in karst rocky desertification area.

Keywords: calcareous soil; plantation ages; organic carbon mineralization; fitting parameters; organic carbon accumulation

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1. Introduction

Due to the fragile geological and ecological conditions the rocky desertification widely occurs in the southwest karst region of China [1], which is characterized by serious soil erosion, devoid of vegetation and soil [2]. To effectively prevent rocky desertification, a series of ecological restorations have been carried out to increase the forest cover and to mitigate soil erosion by the Chinese government [3]. Subsequently, various land uses including undisturbed (e.g., grassland and shrub) and man-made (e.g., corn, woodland and fruit crop) ecosystems have been formed in karst rocky desertification region. These ecological restoration measures have tremendously affected the physical, chemical and microbiological properties in soils [4]. Plum plantation is one of the sustainable development models of characteristic agriculture in karst rocky desertification area, which can not only effectively restore the ecological environment in karst rocky desertification area, but also significantly increase farmer' income in local. In recent years, the planting area of plum trees has been increasing continuously in the process of controlling rocky desertification.

Dynamic change of soil organic carbon (SOC) is of great significance to global C cycle and current climate change. The quantity and intensity of carbon dioxide (CO_2, an important greenhouse gas) released by SOC mineralization through microbial decomposition can reflect the quality of soil and evaluate soil carbon emissions into the atmosphere [5, 6]. In addition, SOC mineralization is

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closely related to the maintenance of soil nutrients and the formation of CO_2 [7]. The CO_2 emission rate and its dynamic change process are also important indicators reflecting the change of soil quality. Furthermore, SOC concentration at a particular time is controlled by the balance between C input from litter and C output from SOC mineralization [8]. Investigating SOC mineralization process is the most effective methods to evaluate C loss or stability [9].

Vegetation type influences the rate of accumulation and mineralization of organic matter in forest soil [10]. Some research proved that C loss from soil respiration depends on stand age. It is low in young, high in intermediate, and low again in old stands [11, 12]. Plum is one of the principal tree species in rocky desertification restoration area in the National Sustainable Development Experiment and Demonstration Zone in Gongcheng county. However, it is less study about SOC storage and SOC mineralization in plum forests in rocky desertification restoration area. In particular, it is not clear whether plum plantation age is the key factor controlling SOC stability and how other factors influence SOC decomposition dynamics. In addition, Calcareous soil developed on carbonate rock is characterized by high pH and Ca materials in a karst region [1], which may lead to the obvious differences in the SOC mineralization compared to other soil types. The lack of knowledge regarding the SOC mineralization in plum forests of karst rocky desertification restoration limits the ability to predict how this ecosystem will respond to climate change.

Therefore, we measured the distribution and mineralization of organic C in soils collected from plum fields with different plantation years (2-y, 5-y and 20-y) and adjacent abandoned land in the karst rocky desertification region of subtropical China. The main objectives of this study were to (1) estimate SOC contents and SOC mineralization in plum forest and soil nutrients under different stand age in the study area; (2) evaluate the relative importance of soil properties affecting SOC and SOC mineralization; (3) assess potentially mineralization carbon and decomposition rates.

2. Materials and Methods

2.1. Study Area

The study site is the National Sustainable Development Experiment and Demonstration Zone and also a key area of national rocky desertification control, was located in Gongcheng county, Guilin, Guangxi Zhuang Autonomous Region (110°47′4″E, 24°54′35′′N) (Figure 1). The area is a subtropical monsoon climate, with an annual average temperature of 19.7°C and an annual average precipitation of 1438mm.

The study area is a hilly and middle-low mountain landform. Its parent material is Carboniferous limestone. Karst soil is sparse and drought-prone. Because of long-term human activities, natural vegetation is destroyed and large areas of steep slopes are reclaimed, resulting in surface rock bareness, coupled with thin soil layer, shallow bedrock exposure, storm erosion, and a large number of rock gradually exposed after soil erosion. Severe rocky desertification occurs (Figure S1).

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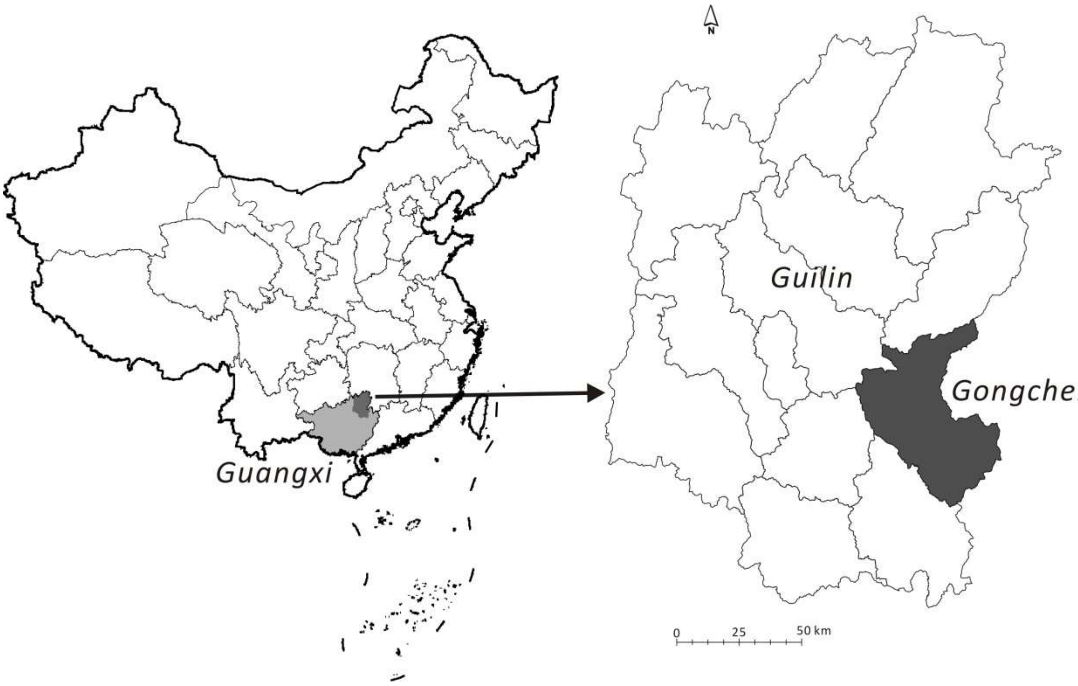

Figure 1. The location of the study area.

2.2. Soil Sampling and preparation

Soil samples were collected from three plum fields with 2-y, 5-y and 20-y plantation ages, and abandoned land was used as control. The understory of the plum plantation was dominated by *the meda villosa (Poir.) A. Camus* and *Digitaria sanguinalis (Linn.) Scop.*. The dominated plants of the abandoned land were herbs, dominated by *Miscanthus* with a small amount of *Conyza canadensis (Linn.) Cronq*. Fertilizer was applied four times a year, including three times of chemical fertilizer and one time of organic fertilizer. The chemical fertilizer was compound fertilizer (including N 18 %, P_2O_5 18%, K_2O 18%). The organic fertilizer was cattle manure (including C 413.8 g kg^{-1}, N 2.7 g kg^{-1}, P_2O_5 1.3 g kg^{-1}, K_2O 6.0 g kg^{-1}). Under each plum tree, an average of 2kg of chemical fertilizer and 20kg of organic fertilizer were applied each year.

In each plum plantation, three 20 m × 20 m plots with uniform body and similar growth among plum trees of the same age, separated at least 40 m from each other, were randomly selected. Soils of the top 0–10 cm were collected from five quadrats (1 m × 1 m) in each plot, one on each corner and one in the center were mixed to form a composite sample in November 2015.

After removing animal and plant debris and stones, the collected samples were air-dried at room temperature. One sample was passed through a 2-mm sieve and homogenized for SOC mineralization experiment and the other was passed through a 0.25-mm sieve and homogenized for soil physical and chemical properties determination.

2.3. Experiment design

Laboratory incubation experiment was carried out to determine SOC mineralization. Soil samples were incubated with a constant temperature regime at 20°C, which was close to the annual mean temperature (19.7 °C) in sampled area. The incubation temperatures were controlled by digital biochemical incubators (SPX-70B, Hangzhou Julai Instrument Co., Ltd.).

Each soil sample (100 g dry) including three repeats was placed in a 1000 ml incubation jar (Figure S2). The soil moisture content was adjusted to 60% of field capacity prior to incubation. All samples were pre-incubated at 20 °C for seven days to minimize the burst of respiration due to wetting the dry soils [9]. Then, a 50 ml beaker containing 10 ml of 0.1 mol/L NaOH solution was placed at the bottom of the incubation jar, sealed and capped, and incubated in a 20°C thermostat in darkness. Three blank controls were set up at the same time. A total of 15 soil samples were used for

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the incubation experiment. Deionized water was added to the soil twice a week to keep the loss of soil water within 2% [13]. The 50 ml beaker containing 10 ml of 0.1 mol/L NaOH solution was replaced at days 2, 5, 8, 14, 20, 26, 32, 38, 44, 62, 74, and 90. The amount of CO_2 released during incubation can be calculated by titrating residual NaOH with 0.1 mol/L HCl solution.

2.4. Methods

Soil pH was determined at a 1:2.5 (w:v) soil:water ratio by a DMP-2 mV/pH detector (Quark Ltd., Nanjing, China); SOC was determined using the $K_2Cr_2O_7$-H_2SO_4 volumetric dilution heating method; total nitrogen was determined using the Kjeldahl procedure [14]; total potassium (TK) concentration was determined with the HF-$HClO_4$ flame photometric method; and total phosphorus (TP) was measured using $HClO_4$–H_2SO_4 digestion followed by a Mo–Sb colorimetric assay [1]; soil calcium (Ca) was extracted by HNO_3-HF-$HClO_4$ and analyzed by Inductively Coupled Plasma-Atomic Emission Spectrometry (ICP-AES). Three replicates were performed for each soil sample.

The mineralization of SOC was calculated by the following formula (1).

$$C_m=C_{HCl}\times(V_0-V_1)\times 22/0.1 \qquad (1)$$

Where, C_m was the amount of CO_2 release (mg CO_2/kg soil); C_{HCl} was the concentration of hydrochloric acid (mol/l); V_0 was the volume of hydrochloric acid consumed in blank titration (ml); V_1 was the volume of hydrochloric acid consumed in titration of samples (ml); 22 was half of the Molar mass of CO_2 (mol/kg); 0.1 was soil weight (kg).

2.5. Data Analysis

Variance analysis and Duncan multiple comparisons were conducted to compare the significant differences between different planting years at the level of $p<0.05$. The data was fitted in exponential model to obtain kinetics of SOC mineralization [15](2).

$$C_t=C_0\,(1-e^{-kt}) \qquad (2)$$

Where, C_t was the cumulative mineralization of SOC after t days; C_0 was amount of potential mineralizable SOC (mg/kg); k for constant of mineralization rate of SOC (/d).

The half turnover period was calculated by the formula (3).

$$T_{1/2}=\ln 2/k \qquad (3)$$

Where, $T_{1/2}$ was half turnover period (d).

3. Results

3.1. Soil Properties under Different Plantation Age

Compared to abandoned land (CK), plum plantation significantly decreased pH and the contents of SOC and TN ($p<0.05$), but there was no significant difference among plum plantation with different years ($p>0.05$). Although C:N ratios in soils under plum plantation is lower than that under abandoned land, the significant difference was only found between abandoned land and 5-y plum plantation. There was no significant difference in TP contents among abandoned land and three plum plantations ($p>0.05$). The highest TK contents were found in soils under 5-y plum plantation and abandoned land, which were significantly higher than 2-y and 20-y plum plantations. The Ca contents decreased significantly from CK to different plantation ages ($p<0.05$).

Table 1. Soil properties under plum plantation with different ages.

	pH	SOC (g/kg)	TN (g/kg)	C:N	TP (g/kg)	TK (g/kg)	Ca (%)
CK	6.60±0.10 a	22.38±0.53 a	1.01±0.04 a	25.97±1.62 a	0.49±0.08 a	18.07±0.05 a	0.97±0.13 a
2-yr	5.31±0.32 b	11.87±3.18 b	0.61±0.18 b	22.83±2.82 ab	0.55±0.25 a	14.56±0.55 b	0.54±0.23 b
5-yr	5.91±0.51 c	10.17±3.28 b	0.74±0.20 b	17.96±8.61 b	0.44±0.21 a	19.47±1.68 a	0.27±0.10 c
20-yr	5.17±0.28 b	11.70±5.27 b	0.66±0.27 b	21.34±6.50 ab	0.63±0.31 a	15.06±1.25 b	0.40±0.16 bc

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Note: CK represents abandoned land; 2-yr represents plum forests plantation for 2 years; 5-yr represents plum forests plantation for 5 years; 20-yr represents plum forests plantation for 20 years. SOC represents soil organic carbon; TN represents total nitrogen; C:N represents the molar ratio of SOC:TN; TP represents total phosphorus; TK represents total potassium. Ca represents calcium. Identical letters indicate no significant differences in the average values among soils under different plantation age at the 0.05 level.

3.2. Mineralization Rate of Soil Organic C under Plantation with Different Ages

Soil organic carbon mineralization rate decreased with incubation time (Figure 2), and accorded with logarithmic function y=a+bln(x) (Table 2), indicating that SOC mineralization rate would change b% absolute value when incubation time changed by 1% unit.

Table 2. Regression equation of SOC mineralization rate relative to plantation age.

Treatment	Regression equation	R^2
CK	y=8.221-2.101ln(x)	0.734**
2-yr	y=4.499-1.078ln(x)	0.835**
5-yr	y=4.736-1.085ln(x)	0.831**
20-yr	y=4.040-0.971ln(x)	0.853**

Note: y represents CO_2 production rate; x represents incubation day; ** means significant correlation at 0.01 level.

According to the decline rate of SOC mineralization (Figure 2), it can be divided into three stages. The first stage (2-14 days) was the early stage of the incubation. The rate of CO_2 production decreased rapidly from the peak (2 days) and changed greatly. There was no significant difference in mineralization rate of SOC among three planting years, but it was significantly lower than CK. The second stage (14-62 days) was the medium stage of the incubation, and the rate of CO_2 production was in a slow decline to a stable stage. The SOC mineralization rate of CK is higher than that in the three planting years. At the last stage (62-90 days), the SOC mineralization rate of CK began to be lower than that of soils with different planting years and the difference was significant.

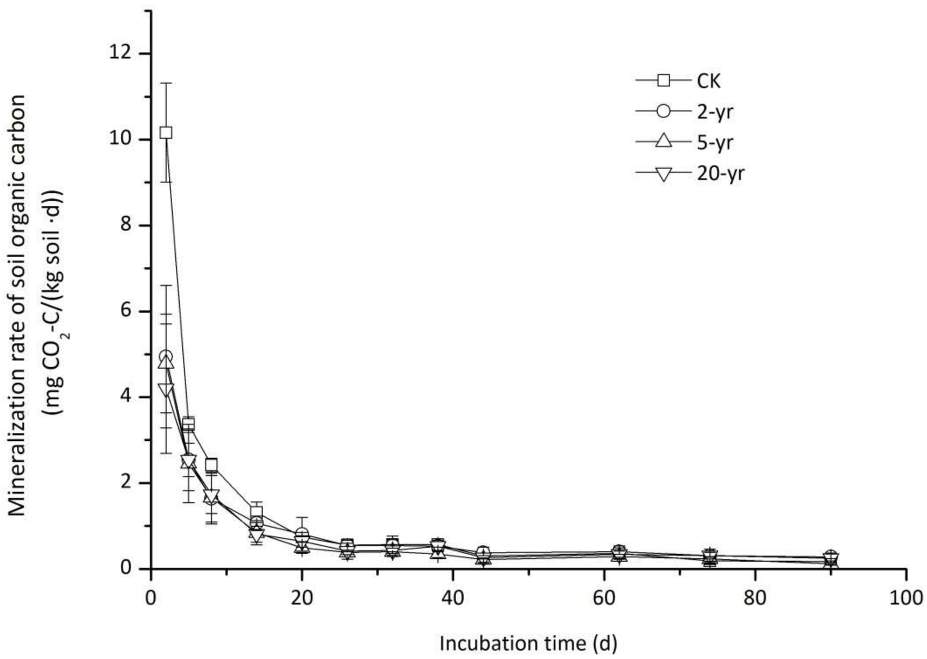

Figure 2. Daily mineralization rate of soil organic carbon.

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3.3. Cumulative Mineralization of Soil Organic Carbon under Different Plantation Age

Carbon mineralization showed a curvilinear relationship with time over the incubation period (starting from 0 to day 90) (Figure 3). Across different plantation ages, cumulative CO_2-C emission varied from 2.29 mg CO_2-C/kg soil (day 2) to 61.17 mg CO_2-C/kg soil (day 90).

The cumulative release of CO_2 increased with incubation time, but the cumulative release intensity gradually slowed down. During the whole incubation, the cumulative release of CO_2 was higher in CK than that in other three plantation ages. Among the three soils from different plantation ages, the order of cumulative release of CO_2 from high to low is 2-yr, 20yr and 5-yr.

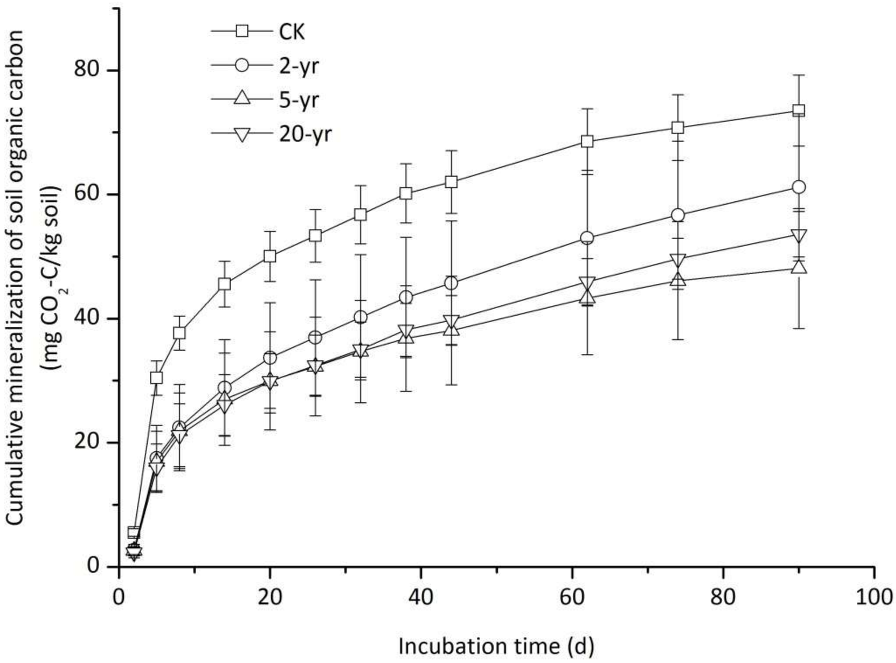

Figure 3. Cumulative mineralization of SOC relative to plantation age.

3.4. Parameters of Soil Organic Carbon Mineralization Kinetic Equations under Different Plantation Age

The first-order kinetic equation was used to fit the cumulative mineralization of SOC under plum plantation with different years, and the fitting results were good (R^2>0.90). The potentially mineralization carbon (C_0) and constant for SOC mineralization rate (*k*) estimated from the first-order kinetic equation are showed in Table 3. The values of C_0 ranged from 44.13 mg/kg (5-y) to 67.10 mg/kg (CK), and it was significant higher in CK than that in other three plantation ages ($P<0.05$). The CO_2-C release from mineralization of soil potential organic carbon, i.e. the turnover rate (*k*) of bioactive organic carbon pool, ranged from 0.043 (2-y) to 0.079 d^{-1} (CK), and the half-turnover period is 8.80 (CK) to 16.1 d (2-y) (Table 3). The values of *k* for different soil depths showed the same trend with C_0. With plum plantation ages, soil potential mineralized carbon pool decreased, but soil potential mineralized carbon pool increased slightly after 20 years of restoration, but the difference was not significant between 5-y and 20-y plum plantations ($p>0.05$).

Table 3. Cumulative mineralization of SOC after the 90 days of incubation and parameters of its kinetic equations.

Treatment	**C_t (mg/kg)**	**C_0 (mg/kg)**	***k* (/d)**	**$T_{1/2}$ (d)**	**C_0/SOC (%)**	**R^2**
CK	73.52±8.43 a	67.10±7.56 a	0.079±0.003 a	8.80	0.30	0.93
2-yr	61.17±5.56 b	57.92±1.33 b	0.043±0.001 b	16.1	0.49	0.96
5-yr	48.09±3.27 c	44.13±5.71 c	0.060±0.001 c	11.6	0.43	0.93

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20-yr	53.60±4.11 c	49.85±2.55 c	0.046±0.002 b	15.1	0.43	0.94

Note: C_t represents cumulative mineralization of SOC; C_0 represents amount of potential mineralizable SOC; k represents constant of mineralization rate of SOC; $T_{1/2}$ represents half turnover period; C_0/SOC represents ratio of potential mineralizable organic carbon to total organic carbon in soil. Values followed by different letters in the same column mean significance at 0.05 level.

4. Discussion

4.1. Effects of plantation age on soil organic carbon and SOC mineralization

Plantation age was the key driver of variation in soil properties, and SOC decomposition. With the increase of plum plantation years, SOC content decreased gradually, which was nearly 50% lower than CK, indicating that SOC contents under long-term plum plantation was deficient. The reason is that the source of SOC mainly depends on the residual litter of plum trees, which is not enough to counterbalance the decomposition of organic matter through mineralization consumption, even applying the large organic fertilizer. However, SOC content increased under 20-y plum plantation. This might be attributed to that the plum trees planted for 20 years gradually entered a recession period. In order to increase yield, the application rate of chemical fertilizer increased. Therefore, SOC content increased again under 20-y plum plantation. Compared with abandoned land, TP content in soil under plum plantation did not significantly change, which was consistent with previous study that TP was mainly derived from the weathering release of soil minerals, rather than from the short-term biological cycle in karst rocky desertification area [1]. Oppositely, potassium sources in soil are mainly derived from potassium minerals and fertilization. With the increase of planting years, the content of Ca in soil decreased obviously, which may be because plum trees absorb a lot of Ca as one of nutrient elements in the process of growth.

4.2. SOC Mineralization and Affecting Factors

In this study, CO_2 production rate is faster in the initial stage of incubation, possibly owing to the priming effect [16]. After pre-incubation, a large number of active organic substances such as sugars and proteins can be effectively decomposed by microorganisms in the initial stage of mineralization [17]. Greater SOC mineralization in abandoned land than plum plantations was often attributed to greater total SOC [18] or greater labile SOC (e.g., DOC and MBC) in abandoned land, which could stimulate the abundance and activity of microorganisms and subsequently accelerate SOC mineralization [19, 20]. With the prolongation of incubation time, the mineralization rate of SOC gradually decreased with the decrease of decomposible organic matter. At the later stage of incubation, the organic matter in soil was mainly composed by cellulose and lignin [16], which were difficult to decompose, and could not be utilized by microorganisms thus, the mineralization rate of organic carbon declined. The CO_2 emission pattern in this study showed a similar trend with many research results [7, 16, 21]. In addition, the relationship between SOC mineralization rate and incubation time was logarithmic function, which was consistent with previous research results [7, 22, 23].

Table 4. Correlations between the carbon parameters and soil property factors.

	pH	SOC	TN	C/N	TP	TK	Ca
C_t	0.593	0.923**	0.690	0.974**	-0.035	-0.079	0.959**
C_0	0.524	0.883*	0.621	0.981**	0.014	-0.159	0.931**
k	0.986**	0.823*	0.983**	0.423	-0.627	0.741	0.733

*represents significant correlation (p<0.05); ** represents extremely significant correlation (p<0.01).

Soil organic carbon content was positively correlated with C_t and C_0. Therefore, the difference of SOC mineralization in plum plantations with different years was mainly due to the difference in SOC content. The proportion of active organic carbon varies with the content of SOC. Among the many factors affecting the mineralization intensity of SOC, the Ca content and C/N ratio significantly

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affect SOC mineralization (Table 4). The soil mineralization rate is the highest in the abandoned land with the highest Ca content and C/N ratio.

In this study, the cumulative mineralization and mineralization rate of SOC under plum plantation with different years were lower than abandoned land. The reason may be that the long-term application of chemical fertilizer in plum-planting soils is not conducive to the formation of soil aggregates, making the microbial growth environment worse, resulting in the decrease of soil microbial biomass [24]. It may also be that nitrogen in fertilizer combines with lignin in soil to form more stable organic compounds [25], which then inhibits the mineralization of SOC. The mechanism of soil characteristics affecting microbial species and activities in plum forests of different years in karst mountainous areas remains to be further studied.

4.3. Carbon pool stability and factors that potentially affect SOC decomposition

Both cumulative CO_2-C emission and potential mineralizable SOC in calcareous soil of this study was several to ten folds lower than that in other soils [7, 12, 16], similar to the results in soils of karst region [26], indicating that SOC in karst region has higher stability. Both Ca and C/N were important factors affecting the SOC mineralization in this study. Soil C/N affects the number, activity and community composition of microorganisms [27]. Calcium is the necessary metabolic component of microbial growth, and fungal and bacterial heterotrophs may access and accumulate root Ca to form oxalates, which can be used to maintain microbial metabolism under unfavorable soil conditions. Therefore, it has an important influence on the decomposition of SOC.

5. Conclusions

The SOC mineralization in plum forests of different ages was evaluated in an incubation experiment and fitted with a first order kinetic equation. The results indicated that plantation age is a critical factor affecting SOC mineralization. Both C_t and C_0 in calcareous soil of this study was several to ten folds lower than that in other soils, indicating that SOC in karst region has higher stability. The factors affecting SOC mineralization are complex. The mineralization of SOC in plum forest in karst area is significantly correlated with soil Ca and C/N, indicating the important role of Ca in SOC mineralization in rocky desertification area.

Supplementary Materials: Figure S1: Rocky desertification in the study area, Figure S2: The sketch of incubation device.

Author Contributions: Conceptualization, J.C.; writing-original draft preparation, H.Y.; investigation, B.M. and M.Z.; Writing-review and editing, T.Z.

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Conflicts of Interest: The authors declare no conflict of interest.

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