

Article

Spatial Variations in Fine Root Turnover, Biomass, and Necromass of Two Vegetation Types in a Karst Ecosystem, Southwestern China

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Abstract: Revealing the patterns of fine root turnover traits can aid our understanding of the mechanisms of fine roots in adapting to soil nutrient changes. In a karst ecosystem of southwest China, the fine root turnover rate, production, biomass, necromass, biomass/necromass ratio, as well as the soil total and available nitrogen (N) and phosphorus (P) concentrations, and root carbon (C) and N concentrations were analyzed in upper, middle, and lower slope positions of two vegetation types (shrubland and forest). The results showed that the soil total and available N and P and fine root production, biomass, and necromass were significantly higher in upper slope positions than those in lower slope positions in both vegetation types. However, the fine root turnover rates were slightly higher in upper positions than those in lower positions. In addition, fine root necromass was significantly lower in shrubland than that in forest, while the biomass/necromass ratio was the opposite. Therefore, fine root production and biomass were significantly affected by slope position, while the fine root biomass/necromass ratio was significantly influenced by vegetation type. Additionally, fine root necromass was significantly influenced by the slope position and vegetation, but the turnover rate was slightly impacted by the two factors. It was also found that fine root production, biomass, and necromass had significant positive correlations with the soil total and available N and P and root C concentrations, and had significant negative correlations with root N concentrations. Moreover, the biomass/necromass ratio was positively and negatively related to the root N concentrations and C/N ratios, respectively. Thus, the variations in these five parameters of fine root turnover were mainly explained by fine root nutrients and the interactive effects between fine root and soil nutrients. The above results indicated that these variations in fine roots responding to soil and root nutrient changes might be an adaptive mechanism to enhance plant nutrient acquisition in nutrient-poor karst ecosystems.

Keywords: biomass; fine root turnover; necromass; production; karst ecosystem



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1. Introduction

Southwestern China features one of the world's largest karst landscapes with a large-area exposure of carbonate rocks, which encompasses approximately 540,000 km² [1]. The characteristics of this region include peak clusters, depressions, high slope gradients, a high ratio of rocky exposure, alkaline calcareous soils, and highly developed karst fissures [2]. Due to these unique topographical factors, water loss, and soil erosion are frequently high, and thus, soil nutrients are heterogeneous but generally poor. In the last few decades, unfortunately, the rocky desertification of the karst ecosystem had been accelerated because

of the increasing over-exploitation of natural resources and the ecological fragility [3]. Areas of many undisturbed or slightly disturbed forests have transformed into croplands, abandoned land, and grass-shrub lands, and soil nutrients have dramatically decreased during these land-use changes [4]. In recent years, people are pleasantly surprised to find that the worsening rocky desertification has been controlled through some ecological projects by the Chinese government [5–7]. Vegetation restoration is beneficial for halting the development of rocky desertification in this ecosystem [8]. However, it may be puzzling that the karst vegetations are dramatically limited by soil nitrogen (N) and phosphorus (P) [9,10].

Shrubland is widely distributed in the karst region of China and is a typical restoration stage between grassland and forest. This stage has been suggested as the first choice for vegetation restoration by planting supplementary shrubs in this region [9]. Although soil N and P contents and availabilities are increasing along with the vegetation restorations in this nutrient-poor ecosystem [2,11,12], shrubs in the shrub stage are co-limited by N and P, and trees in forest are mainly limited by P [10]. Therefore, increasing the nutrient supply capacity for plants may be very important in this ecosystem. To tolerate the nutrient-limited stresses, plants have developed a wide variety of nutrient acquisition strategies, such as enhancing their symbiosis of roots with arbuscular mycorrhizal fungi and N-fixing bacteria [8,13,14], rhizosphere priming by exudations [15,16], and specific root length and root tips [17]. Moreover, fine root turnover is another effective strategy for plants responding to soil nutrient limitations [18].

A large quantity of fine roots, which are less than 2 mm in diameter, can greatly benefit nutrient absorbability [19–22]. In nutrient-limited soils, new fine roots rapidly replace old ones to acquire enough nutrients for plant growth, because they have more vigor and plasticity [23,24]. The short-lived pattern of fine roots indicates that fine roots have high turnover rates. Generally, the annual fine root production is divided by the fine root biomass to acquire a fine root turnover rate [25–27], which is also dramatically impacted by necromass. Fine root biomass and necromass were the amounts of living roots and dead roots, respectively [28]. A low biomass/necromass ratio may be similar to a high fine root turnover rate [23]. The variations in the fine root turnover rate and related parameters may help successional vegetation adapt to changes in nutrient limitations. Thus, revealing the patterns of fine root turnover rate and related parameters can aid our understanding of the mechanisms of fine roots in adapting to soil nutrient changes. Although there has been great research interest in fine root turnover rate in other ecosystems in recent years [25–29], the spatial patterns of fine root turnover rates and the related parameters' responses to soil and root nutrients in different vegetation types in karst ecosystems remain unclear.

Generally, the fine root turnover rate and related parameters are changed by the variations in soil and root nutrients. Firstly, they are positively correlated with the increasing soil N, P, and organic matter in nutrient-poor conditions [30]. Soil nutrients generally occur in distinct topographical patterns and change with the growth and maturing of vegetation in natural ecosystems. Soil nutrients are spatially distributed because of topographical factors, such as slope position or others in terrestrial ecosystems [31–33]. For example, one previous study has shown that soil nutrient concentrations are lower in lower slope positions than in upper slope positions in Korshrinsk peashrub lands [34]. The spatial distribution of soil nutrients affects the fine root turnover rate and related parameters, which are higher on upper slopes than on lower slopes [31]. In addition, soil nutrient concentrations tend to increase from younger to older vegetation [35,36]. However, the fine root turnover rate and related parameter trends may differ in different vegetation types [37]. One study has shown that the fine root turnover rate is higher in later stages than in earlier stages [38]. Secondly, it is affected by plant physiological characteristics, e.g., fine root C and N concentrations [24]. A high fine root turnover rate is associated with a short lifespan [39,40]. In nutrients' limited ecosystems, plants will decrease the fine root lifespan [41] and will allocate mineral nutrients to roots rather than to aboveground parts [42]. This will lead to the fine roots having low N concentrations, resulting in high

C/N ratios. Broadly, the fine root turnover rate and related parameters are potentially more correlated with soil N, P, organic matter, and root C/N ratios or C concentrations, but are less correlated with root N concentrations at higher nutrient levels.

In this study, two vegetation types (shrubland and forest) were selected in a karst ecosystem in Guangxi province, southwestern China. Sequential coring and ingrowth cores were used in our present study [29]. The fine root turnover rate, production, biomass, necromass, biomass/necromass ratio, and C and N concentrations in both vegetation types were measured, as well as the soil total and available N and P. Our objectives were to (1) reveal the spatial patterns of fine root turnover rate, production, biomass, necromass, and the biomass/necromass ratio in different slope positions; (2) compare the differences of these five measured parameters between shrubland and forest; (3) and explore the response of these fine root characteristics to soil and fine root nutrients in two vegetation types in a karst ecosystem. We hypothesized that (1) the fine root turnover rate, production, biomass, and necromass would be higher, but the biomass/necromass ratio would be lower in the upper slope positions than in lower slope positions; (2) the fine root turnover rate, production, biomass, and necromass would be higher, but the biomass/necromass ratio would be lower in shrubland than in forest; (3) and the fine root turnover rate, production, biomass, and necromass would be positively correlated, however, the biomass/necromass ratio would be negatively correlated with increasing soil and root nutrients.

2. Material and Methods

2.1. Study Site

This study was conducted on two vegetation types (shrubland and forest) of a karst ecosystem, in Huanjiang county, Guangxi province, southwestern China (Figure 1). The karst ecosystem is located in the region of a typical subtropical monsoon climate, with a mean annual temperature and precipitation of approximately 19 °C and 1400–1500 mm, respectively [43]. Soil depth generally averages 10–30 cm on the hillslopes and soil pH ranges from 6.3 to 7.9 in the two study sites.

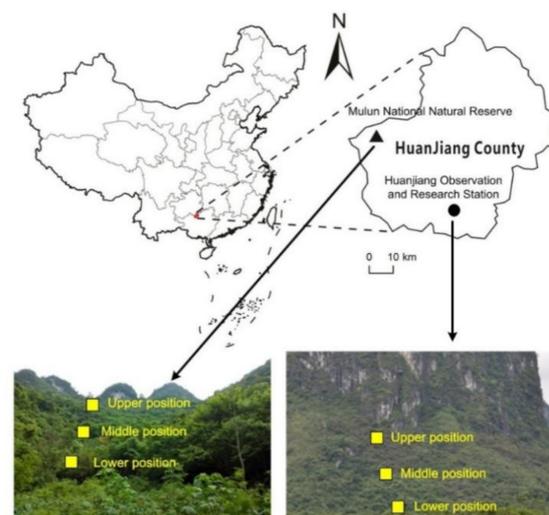


Figure 1. Map showing the Huanjiang Observation and Research Station (●) and Mulun National Natural Reserve (▲) in a karst ecosystem, southwestern China, and relative slope positions for sampling.

The shrubland plots were established in the Huanjiang Observation and Research Station ($24^{\circ}43'58.9''$ – $24^{\circ}44'48.8''$ N, $108^{\circ}18'56.9''$ – $108^{\circ}19'58.4''$ E) of the Chinese Academy of Sciences, and covers an area of 146.1 ha. This area experienced severe deforestation from 1958 to the mid-1980s and has been recovering through natural revegetation from the mid-1980s to the present day. Nowadays, approximately 70% of the hillslopes are dominated by shrubs [44]. The forest plots were established in the Mulun National Natural Reserve ($25^{\circ}06'09''$ – $25^{\circ}12'25''$ N, $107^{\circ}53'29''$ – $108^{\circ}05'42''$ E), which is approximately 35 km away

from the Huanjiang Observation and Research Station and covers an area of 108.6 km². It was established in 1991 to protect a remnant patch of undisturbed mixed evergreen and deciduous broadleaved forest in the karst region [11,45]. The forest at this site has not been disturbed for over 200 years.

2.2. Sampling

In May 2014, 15 plots (Table 1) were established in the Huanjiang Observation and Research Station and Mulun National Natural Reserve, respectively. Plots were designed in 10 × 10 m quadrats and were set up at greater than 50-m intervals along five sample lines in uniform vegetation on the whole slope at the two sites. Therefore, there were 5 plots in lower, middle, and upper slope positions (Figure 1), respectively.

Table 1. Plot characteristics of the two vegetations in a karst ecosystem in southwest China.

Vegetations	Number	Altitude (m)	Slope (°)	Community Height (m)	Dominant Species
Shrubland	15	280 ~ 420	23.2 ± 5.8	2.5 ± 0.2	<i>Vitex negundo</i> , <i>Croton lachnocarpus</i> , <i>Ligustrum sinense</i> , <i>Cipadessa cinerascens</i> , <i>Platycarya longipes</i>
Forest	15	320 ~ 480	35.3 ± 6.1	7.4 ± 0.5	<i>Pteroceltis tatarinowii</i> , <i>Rapanea kwangsiensis</i> , <i>Eurycorymbus cavaleriei</i> , <i>Murraya exotica</i> , <i>Cipadessa cinerascens</i>

Sequential coring and ingrowth core are generally used to estimate fine root turnover rates in different ecosystems [25,26]. In the karst ecosystem of southwestern China, the hillslopes are very steep, and the soil layer in the hillslopes is very shallow and contains a lot of gravel. For us, fine root samples were easily collected by a light soil auger in this region. Therefore, sequential coring and ingrowth cores were used to estimate fine root turnover rates in this study [29,46–49].

(1) The sequential coring

From May 2014 to May 2015, fine roots were sampled by sequential coring at bimonthly intervals in the lower, middle, and upper positions of each sample line. Each plot was divided into four subplots (5 m × 5 m). At the sampling sites, the soil layer is very shallow, with soil depths amounting to less than 20 cm, and there are many rock fragments in the soils on the middle and upper slopes, especially on the upper slopes [2,50]. In this study, in order to compare the differences in fine root parameters in the same soil depth among the three slope positions, 10 cm deep soil cores were taken as described in previous studies in this region [51,52]. Four soil cores per plot were bimonthly taken by an auger (10 cm diameter) and mixed to create a single sample. A total of 210 samples were collected, i.e., 2 sites × 15 plots × 7 times.

(2) The ingrowth cores

One mesh bag (10 cm diameter and 10 cm depth, mesh size 6 mm) was installed in each subplot, thus a total of 120 mesh bags were installed in the study area in May 2014. The ingrowth cores were collected in May 2015, and a total of 30 fine root samples were collected by mixing four soil cores with a single sample per plot.

Simultaneously, four soil cores (10 cm in depth) per plot were randomly taken and then mixed to create a single sample in May 2014. The mixed soil samples were air-dried and then ground to pass through a 2 mm mesh sieve for soil physicochemical analysis.

2.3. Laboratory Analyses

Soil samples taken by both methods were soaked in water for 24 h and then were washed free of soil. In the case of these collected roots, fine roots (with a diameter less than 2 mm) were distinguished and were divided into living roots and dead roots. The bright and resilient fine roots, which also have living stele, were defined as the living roots [26].

Each root sample was dried at 65 °C for at least 48 h and then was ground to pass through a 0.154 mm mesh sieve.

Fine root C concentrations (C_{root}) and N concentrations (N_{root}) were determined with an elemental analyzer (Vario MAX CN, Elementar, Hanau, Germany).

Soil total nitrogen (TN) was measured by a flow injection analyzer (FIAstar 5000, FOSS Company, Stockholm, Sweden) based on the Kjeldahl method; soil available nitrogen (AN) was extracted with a mixture of FeSO_4 and NaOH and then was titrated with a diluted solution of H_2SO_4 ; soil total phosphorus (TP) was digested in a solution of NaOH + H_2SO_4 , and available phosphorus (AP) was extracted with NaHCO_3 . The TP and AP were analyzed with phosphomolybdate blue [11].

2.4. Calculation of Turnover Rates

The fine root turnover rate for both the sequential cores and ingrowth cores was calculated as follows: firstly, the production (P) between two sampling dates was calculated using the decision matrix [46]; secondly, the annual fine root production (P_a) was summed by all the calculated production (P) from the first step; thirdly, the total biomass was divided by the sampling times per year to calculate the mean biomass (B_{mean}); finally, the P_a was divided by the B_{mean} to estimate the fine root turnover rate (yr^{-1}) [29].

2.5. Statistical Analyses

In this study, data were calculated using the R version 4.1.1 statistical software [53]. Before the statistical analysis, all data were checked for normality of distributions and homogeneity of variances. Firstly, the mixed model (lme4 package in R) was used to compare the soil total and available N and P properties, the fine root turnover rates, production, biomass, necromass, biomass/necromass ratios, and the C and N concentrations among upper, middle, and lower slope positions and between shrubland and forest (Figures 2 and 3 and Tables 2–4). The probability of significance was defined as $p < 0.05$. Secondly, the relationships between the fine root turnover parameters and soil and fine root nutrients were assessed using Pearson's correlation analysis (Tables 5 and 6) and redundancy analysis (RDA, Vegan package, Figure 4). Thirdly, the soil N (total and available N), soil P (total and available P) and fine root nutrient (C and N concentrations) variables were used to calculate the variances of the variations in fine root turnover parameters through variation partitioning analysis (Vegan package in R, Figure 5) [54].

Table 2. Soil nitrogen (N) and phosphorus (P) concentrations and availabilities in two vegetations of a karst ecosystem (mean \pm SE).

Slope Positions	Total Nitrogen (TN g kg ⁻¹)	Available Nitrogen (AN mg kg ⁻¹)	Total Phosphorus (TP g kg ⁻¹)	Available Phosphorus (AP mg kg ⁻¹)
Shrubland				
Upper	7.86 \pm 0.22 b	424.92 \pm 4.69 b	1.48 \pm 0.20 b	12.18 \pm 0.69 b
Middle	6.75 \pm 0.46 b	388.43 \pm 16.25 b	1.46 \pm 0.10 b	11.30 \pm 0.46 b
Lower	4.34 \pm 0.24 a	358.33 \pm 23.93 a	0.96 \pm 0.08 a	4.63 \pm 1.14 a
Overall	6.32 \pm 0.43 A	390.56 \pm 11.61 A	1.30 \pm 0.10 A	9.37 \pm 1.00 A
Forest				
Upper	13.54 \pm 1.39 b	584.23 \pm 63.21 b	1.64 \pm 0.07 b	10.50 \pm 1.42 b
Middle	9.63 \pm 2.43 b	508.15 \pm 90.28 b	1.66 \pm 0.21 b	9.52 \pm 1.67 b
Lower	4.70 \pm 0.17 a	314.68 \pm 9.14 a	1.14 \pm 0.10 a	4.32 \pm 0.32 a
Overall	9.29 \pm 1.30 B	469.02 \pm 45.65 B	1.48 \pm 0.10 A	8.11 \pm 1.00 A

Lowercase letters indicate significant differences ($p < 0.05$) among three slopes; capital letters indicate significant differences ($p < 0.05$) between shrubland and forest.

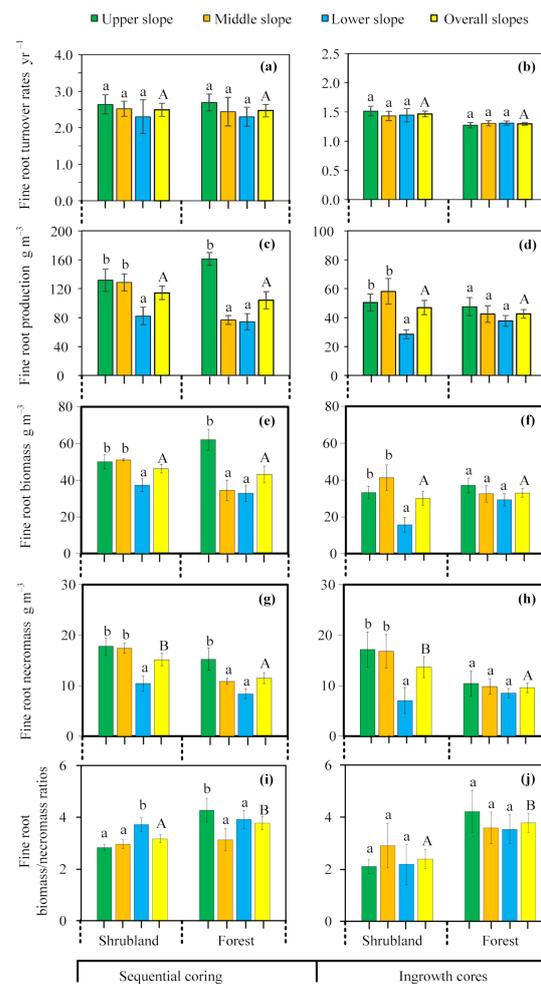


Figure 2. Fine root turnover rates, production, biomass, necromass, and biomass/necromass ratios in different slope positions and vegetations of a karst ecosystem. # Lowercase letters indicate significant differences ($p < 0.05$) among the three slope positions; capital letters indicate significant differences ($p < 0.05$) between the two vegetation types. (a,c,e,g,i) were the patterns of fine root turnover rates, production, biomass, necromass, and biomass/necromass ratios measured by sequential coring, respectively; (b,d,f,h,j) were the patterns of fine root turnover rates, production, biomass, necromass, and biomass/necromass ratios measured by ingrowth cores, respectively.

Table 3. Fine root turnover parameters responded to slope position and vegetation in a karst ecosystem.

Response Variable	Slope Position		Vegetation		Slope Position \times Vegetation	
	F Value	<i>p</i>	F Value	<i>p</i>	F Value	<i>p</i>
<i>Sequential coring</i>						
Fine root turnover rates	1.452	0.239	0.003	0.957	0.007	0.935
Fine root production	26.948	<0.001	0.888	0.355	2.009	0.168
Fine root biomass	19.675	<0.001	0.720	0.404	2.911	0.099
Fine root necromass	22.874	<0.001	10.248	0.004	0.076	0.786
Biomass/necromass ratios	0.473	0.498	4.540	0.042	3.182	0.086
<i>Ingrowth cores</i>						
Fine root turnover rates	0.129	0.722	9.811	0.004	0.633	0.434
Fine root production	5.094	0.033	0.496	0.488	0.637	0.432
Fine root biomass	4.147	0.052	0.074	0.788	0.215	0.647
Fine root necromass	4.038	0.055	5.733	0.025	1.465	0.237
Biomass/necromass ratios	0.001	0.977	5.280	0.030	1.087	0.307

C_{root} , fine root carbon concentration; N_{root} , fine root nitrogen concentration.

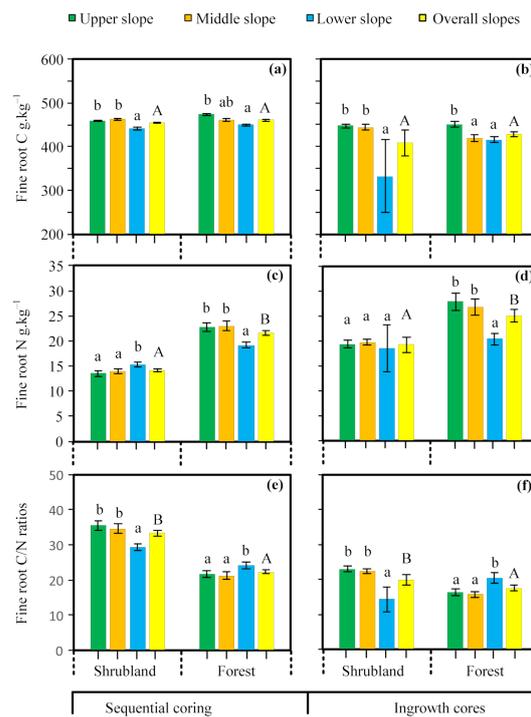


Figure 3. C_{root} and N_{root} of living fine roots in different slope positions and vegetations of a karst ecosystem. Lowercase letters indicate significant differences in living fine roots ($p < 0.05$) among the three slopes; capital letters indicate significant differences in living fine roots ($p < 0.05$) between shrubland and forest. (a,c,e) were the patterns of fine root C concentrations, N concentrations, and C/N ratios used by sequential coring, respectively; (b,d,f) were the patterns of fine root C concentrations, N concentrations, and C/N ratios used by ingrowth cores, respectively.

Table 4. Fine root nutrients responded to slope position and vegetation in a karst ecosystem.

Response Variable	Slope Position		Vegetation		Slope Position × Vegetation	
	F Value	p	F Value	p	F Value	p
<i>Sequential coring</i>						
Fine root C concentration	28.194	<0.001	0.238	0.626	2.923	0.056
Fine root N concentration	2.009	0.137	217.471	<0.001	11.936	<0.001
Fine root C:N ratios	1.639	0.197	175.577	<0.001	9.883	<0.001
<i>Ingrowth cores</i>						
Fine root C concentration	11.446	<0.001	1.666	0.210	2.525	0.102
Fine root N concentration	1.494	0.245	20.277	<0.001	11.764	<0.001
Fine root C:N ratios	0.190	0.828	22.35	<0.001	14.35	<0.001

Table 5. Relationships between fine root turnover and production, biomass, and necromass.

	Production	Biomass	Necromass	Biomass/Necromass Ratios
<i>Sequential coring</i>				
Turnover rates	0.492 **	−0.145	0.014	−0.233
Production	−	0.768 **	0.658 **	0.051
Biomass	−	−	0.739 **	0.230
Necromass	−	−	−	−0.454 *
<i>Ingrowth cores</i>				
Turnover rates	0.191	−0.141	0.716 **	−0.862 **
Production	−	0.941 **	0.806 **	−0.223
Biomass	−	−	0.558 **	0.063
Necromass	−	−	−	−0.659 **

** Correlation is significant at the 0.01 level (2-tailed); * Correlation is significant at the 0.05 level (two-tailed).

Table 6. Relationships between the characteristics of fine roots and soils.

	Soil TN	Soil AN	Soil TP	Soil AP	C _{root}	N _{root}	C/N Ratios
<i>Sequential coring</i>							
Turnover rates	−0.110	−0.230	0.290	0.109	0.182	0.024	−0.011
Production	0.439 *	0.263	0.344	0.530 **	0.712 **	−0.041	0.188
Biomass	0.612 **	0.512 **	0.393 *	0.542 **	0.684 **	−0.040	0.197
Necromass	0.318	0.173	0.315	0.590 **	0.570 **	−0.432 *	0.572 **
Biomass: Necromass ratios	0.283	0.336	0.068	−0.165	0.033	0.511 **	−0.504 **
<i>Ingrowth cores</i>							
Turnover rates	−0.028	−0.002	0.033	0.044	−0.095	−0.51 **	0.479 **
Production	0.381 *	0.252	0.376 *	0.506 **	0.503 **	−0.226	0.405 *
Biomass	0.468 **	0.324	0.392 *	0.503 **	0.540 **	−0.049	0.237
Necromass	0.120	0.052	0.250	0.381 *	0.290	−0.468 *	0.580 **
Biomass: Necromass ratios	0.340	0.336	0.186	0.045	0.020	0.514 **	−0.482 **

** Correlation is significant at the 0.01 level (two-tailed); * Correlation is significant at the 0.05 level (two-tailed). Soil TN, soil total nitrogen; Soil AN, soil available nitrogen; Soil TP, soil total phosphorus; Soil AP, soil available phosphorus; C_{root}, fine root carbon concentration; N_{root}, fine root nitrogen concentration; C/N, fine root C/N ratios.

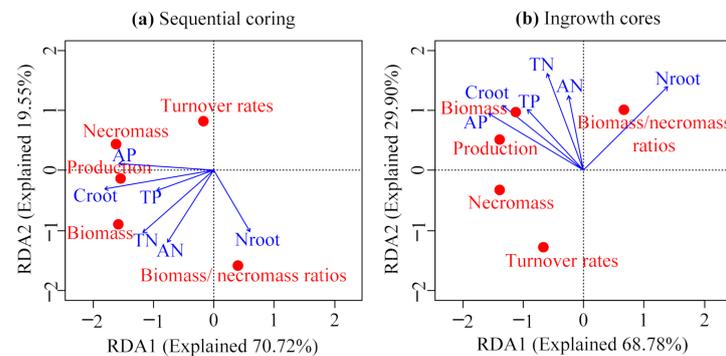


Figure 4. RDA results showed the effects of fine root and soil nutrients on fine root turnover parameters. TN, soil total nitrogen; TP, soil total phosphorus; AN, soil available nitrogen; AP, soil available phosphorus; C_{root}, fine root carbon concentration; N_{root}, fine root nitrogen concentration. Monte Carlo test of the RDA analysis ((a). F = 5.14, p = 0.001; (b). F = 2.42, p = 0.011).

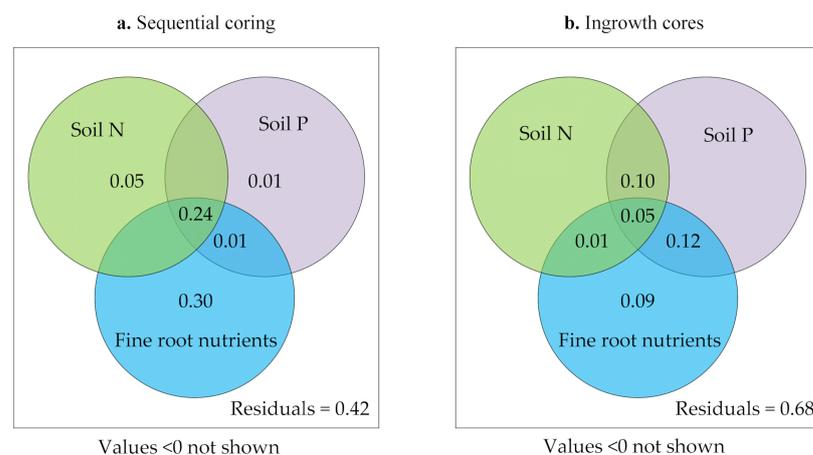


Figure 5. Venn diagram showing the variations in fine root turnover parameters contributed by soil N and P and fine root nutrients. Fine root turnover parameters included turnover rate, production, biomass, necromass, and biomass/necromass ratios; Soil N included total and available nitrogen concentrations; Soil P included total and available phosphorus concentrations; Fine root nutrients included fine root C and N concentrations.

3. Results

3.1. Characteristics of Soil Nutrients

Soil TN, AN, TP, and AP in upper and middle slope positions were all significantly higher than those in lower slope positions in shrubland and forest. Additionally, soil TN, AN, TP, and AP were highest in the upper positions in both vegetation types. Moreover, higher soil TN and AN were found in forest rather than in shrubland, while there were no differences in soil TP and AP between the two vegetation types. Therefore, soil TN and AN were affected by slope position and vegetation types, while soil TP and AP were mainly affected by slope position (Table 2).

3.2. Spatial Patterns in Fine Root Turnover Rate and Related Parameters

In our present study, the fine root turnover rate in shrubland and forest, when measured by sequential coring, was 2.49 yr^{-1} and 2.47 yr^{-1} , respectively (Figure 2a). However, the parameter in two vegetations, when measured by ingrowth cores, was 1.47 yr^{-1} and 1.30 yr^{-1} , respectively (Figure 2b).

Fine root turnover rates were slightly higher in the upper slope positions than in the other two positions in both vegetations (Figure 2a,b). Fine root production, biomass, and necromass were significantly higher in the upper slope positions than in the lower positions (Figure 2c–h). Fine root biomass/necromass ratios were lower on upper slopes in shrubland, while this was the opposite in forest (Figure 2i,j).

Fine root turnover rates, production, and biomass did not significantly differ between the two vegetation types (Figure 2a–f). The necromass was significantly higher in shrubland than in forest (Figure 2g,h), while the biomass/necromass ratios were opposite (Figure 2i,j).

Fine root turnover rates were insignificantly affected by the slope position and vegetation type. The production and biomass were slightly affected by the slope position. The necromass was significantly affected by vegetation and the slope position. The biomass/necromass ratios were significantly impacted by vegetation (Table 3).

3.3. Spatial Patterns of Living Fine Root Nutrients

The C concentrations of living fine roots were significantly higher in the upper slope positions than those in lower positions in both vegetations (Figure 3a,b). The N concentrations of living fine roots were significantly higher in the upper slope positions than in lower positions in forest, however, showed the opposite pattern in shrubland (Figure 3c,d). Additionally, the C:N ratios of living fine roots were significantly higher in the upper slope positions than in lower positions in shrubland, while this were opposite in forest (Figure 3e,f).

The variations in the C concentrations of living fine roots were significantly affected by slope position. The variations in the N concentrations and C:N ratios of living fine roots were significantly influenced by vegetation and the interactive effects between the slope position and vegetation (Table 4).

3.4. Relationships between Fine Roots and Soil Characteristics

Fine root turnover rates were positively correlated with production and necromass. The production was positively correlated with biomass and necromass. The necromass was negatively correlated with the biomass/necromass ratios (Table 5).

Fine root turnover rates were not significantly related to soil and fine root nutrients. The fine root production, biomass, and necromass were significantly related to soil N and P concentrations and availabilities and the fine root C concentration, however, they were negatively related to the fine root N concentration. In addition, the biomass/necromass ratios were positively related to the fine root N concentration but were negatively related to the fine root C:N ratios (Table 6 and Figure 4). The soil N, soil P, and fine root nutrient concentrations explained 90.27% and 98.68% of the variations in fine root turnover traits measured by sequential coring and ingrowth cores, respectively (Figure 5). Therefore,

the variations in fine root turnover traits were mainly explained by the interactive effects between the fine root and soil nutrients.

4. Discussion

4.1. Fine Root Turnover Rates in Karst Ecosystems

According to Gill and Jackson's study about the global fine root turnover rate of grasslands, shrublands, and forests [55], the average fine root turnover rate was from 1.4 yr^{-1} to 1.9 yr^{-1} . In Chinese forests, the rate was 1.19 yr^{-1} [56]. In this study, fine root turnover rates measured by sequential coring were higher in the studied karst region than in global or other Chinese regional patterns. This result indicated that a higher fine root turnover rate benefited terrestrial ecosystems by improving nutrient uptake, nutrient dynamics, and carbon sequestration [25,26]. Thus, this action may relieve the nutrient limitation, which is more restricted in karst areas than in other non-karst areas [4,17]. Therefore, increasing fine root turnover is an important strategy for plants to adapt to soil nutrient limitations in karst ecosystems.

In addition, fine root turnover rates determined by sequential coring were higher than those measured by ingrowth cores. There are two factors that can explain the phenomenon. Firstly, the soil's physical and chemical properties were changed by ingrowth cores. In order to remove the roots from studied soils, these soils must be dug out and mixed. These experimental processes will disturb soil bulk density, nutrient availability, microorganisms, and other environmental factors [57]. Thus, we must hypothesize that the growth of fine roots is not influenced by soil disturbance and root damage when we use the ingrowth cores to estimate the fine root turnover rate [51]. However, there is evidence refuting this hypothesis under natural conditions [58]. Therefore, after cutting off the root system, the growth rates and quantities of new fine roots are probably lower than those of old fine roots. Secondly, after cutting off the root system, the pressure of resource competition may decrease between roots, resulting in lower fine root turnover rates measured by ingrowth cores than that measured by sequential coring. Thus, fine root turnover rates measured using the sequential coring method may be more likely to approximate the true value in soils of karst ecosystems.

4.2. Variations in Fine Root Turnover with Changes in Slope Positions and Vegetation Types

In this study, higher fine root production, biomass, and necromass were found in upper slope positions than in lower slopes (Figure 2c–h). However, fine root turnover rates did not significantly differ among the three slope positions (Figure 2 and Table 2). Fine root turnover is a critical parameter for nutrient uptake, nutrient dynamics, and carbon sequestration in terrestrial ecosystems [22,25]. Generally, a high fine root turnover rate indicates that a new fine root system of plants will rapidly replace the old one, and plants can increase their nutrient absorption, even in plants reducing biomass allocation to below-ground portions [22,24]. The fine root biomass and necromass are the essential data to calculate the turnover rate [25–27]. If there is a high fine root production, biomass, and necromass in ecosystems, as well as low fine root biomass/necromass ratios, the fine root system of plants will be inclined to rapidly replace the old fine roots, such as the patterns in shrubland in the present study. In forest, fine roots had higher biomass/necromass ratios in the upper slope positions than those in lower slope positions, and they also had higher production, biomass, and necromass (Figure 2). It might suggest that the pattern of fine root turnover in forest was similar to that in shrubland. The above discussions indicated that the new fine root system of plants would still rapidly replace the old ones in the upper slope positions in both vegetation types. The differences in turnover parameters between the upper positions and the lower positions in both vegetation types supported the first hypothesis.

In natural ecosystems, fine root turnover rates, production, biomass, and necromass are generally influenced by vegetation types [59]. In the present study, fine root turnover rates, production, and biomass did not significantly differ between the two vegetation

types (Figure 2a,b and Table 2), but the three parameters were slightly higher in shrubland than in forest. Our results were consistent with the previous studies which found that the fine root biomass was higher in intermediate-aged stands than in younger and older stands [60,61]. Another study, which examined variations in fine root biomass in karst regions reported that it was higher in shrubland than in forests [62]. The phenomenon may be related to the adaptive mechanism for enhancing plant nutrient absorption efficiency in nutrient-poor soils. In karst ecosystems, plants are impacted by N and P co-limitation in shrubland and are mainly influenced by P limitation in forest [17]. In this nutrient-limited ecosystem, plants would have high fine root turnover rates to enhance nutrient absorption efficiency. It was found that fine root necromass was significantly higher in shrubland than in forest but the biomass/necromass ratio was the opposite (Figure 2g,h). These results also supported the second hypothesis.

Thus, the necromass was significantly affected by vegetation type and slope position. The production and biomass were significantly influenced by the slope position, and the biomass/necromass ratios were significantly impacted by the vegetation type. The turnover rates were slightly influenced by the vegetation type and slope position. The interactive effects of the slope position and vegetation type on these five parameters were not significant (Table 2).

4.3. Fine Root Turnover and Dynamics Related to Soil and Root Nutrients

The variations in fine root turnover rates, production, biomass, and necromass were affected by soil nutrient factors. Previous studies have indicated that fine root production, biomass, and turnover rates increase in nutrient-limited and heterogeneous soils, but decrease in fertile soils [63,64]. Our results were consistent with these previous findings that fine root production, biomass, and necromass were positively related to soil TN, TP, and AP (Table 4 and Figure 4). Firstly, in karst ecosystems, a higher slope gradient and surface cover of rock outcrops generally occur in upper slope positions rather than in lower slope positions [32], resulting in decreasing soil cover area and volume in the upper slope. However, large areas of rocky outcrops and high slope gradients are good at trapping litter to put back into the soil, and eventually enhance soil nutrient concentrations along the increasing gradients of slope position [65]. In the end, the distribution of soil nutrients reflected topographic heterogeneity [66], showing that the soil total and available N and P in upper and middle slope positions were higher than those in lower slope positions in two vegetation types (Table 1). In higher slope positions, the decreasing soil cover area and volume will increase the amounts of fine root per square/cubic meter of soil, as well as root diversity, complexity, and competition. Thus, fine roots may increase their nutrient uptake rates or turnover rates along the increasing gradients of slope position [67,68]. That offers support for our finding that fine root turnover rate, production, biomass, and necromass were higher on upper slopes than on lower slopes (Figure 2). Secondly, soil nutrients generally increased from younger to older vegetation. In this study, the soil total and available N in forest were higher than those in shrubland, however, the total and available P were not different between the two vegetation types (Table 1). Although the soil total and available N concentrations increased from shrubland to forest, soil nutrients were still poor in the karst region compared with non-karst regions in the same latitude [69].

The variations in fine root turnover rates, production, biomass, and necromass are also correlated with fine root nutrients [24]. Because fine root turnover is an active adaptation to soil environment changes, fine roots will have rapid decomposition and high nutrient absorption efficiency and cycling, higher C/N ratios of plant tissues, and higher degradation rates in terrestrial ecosystems [70,71]. In this study, the fine root C/N ratios were significantly higher in shrubland than those in forest (Figure 3). Thus, the fine root biomass/necromass ratios were significantly different between the two vegetation types, as were the C/N ratios. In addition, a high fine root turnover rate is associated with a low lifespan [39,40], to maintain high nutrient absorption efficiency and cycling. In nutrient-limited soils, N and P nutrients would not sequester in fine roots but cycle quickly [16,42]. This

might be consistent with the low N concentrations of fine roots in shrubland (Figure 3), due to N limitations in this vegetation. Additionally, fine root N and C/N ratios were higher in the upper slope positions than those in the lower positions in shrubland, but the opposite trends were seen in forest (Figure 3). The variations in the fine root N and C:N ratios might be mainly affected by N limitation in shrubland but not in forest. This would be supported by the significant influence of vegetation type, but not slope position on fine root N and C/N ratios (Table 4). According to the discussions, C will be good for fine root biomass communication, while N will be one important factor affecting fine root turnover. Thus, the relationships between fine root turnover rates, production, biomass, necromass and fine root C and C/N ratios were positive but the relationships between these four parameters and root N were negative, while the relationships between the biomass/necromass ratios and fine root C, N, and C/N ratios were opposite.

To sum up, our results supported the third hypothesis. The proportions of the variations in these five parameters were explained more by the fine root nutrients and the interactive effects between the fine root and soil nutrients than by individual soil N or P concentrations (Figure 5). Broadly, the patterns of high fine root turnover rates, production, biomass, and necromass in the upper slope positions and high necromass and low biomass/necromass ratios in shrubland were mainly affected by the fine root nutrients and their interaction with soil nutrients, suggesting that high fine root turnover rates may be a good adaptive mechanism for enhancing plant nutrient absorption efficiency in higher slope positions and younger vegetation in karst ecosystems. Future work on fine root turnover in heterogeneous nutrient-limited karst ecosystems would use multiple methods, such as controlled field experiments, isotopic tracing, and minirhizotron, to determine how soil and root nutrients influence the tradeoff between nutrient acquisition and resource investment for fine roots, fungi, and rhizosphere soils.

5. Conclusions

In this study, we provided a detailed insight into the spatial variations of fine root turnover rates and their related parameters in two vegetation types of a karst ecosystem. Fine root turnover rates did not vary significantly among upper, middle, and lower slopes and between shrubland and forest. Fine root necromass differ significantly among the three slope positions and between the two vegetation types. The production and biomass differed significantly among the three slope positions, and the biomass/necromass ratios differed significantly between the two vegetation types. In addition, soil N and P were distributed spatially according to topographic factors and N varied with the change in vegetation. The pattern of fine root turnover rate and related parameters changes is an adaptive mechanism of fine roots in response to soil nutrients, especially in nutrient-limited ecosystems. Fine root turnover rates were not significantly related to soil, but rather fine root nutrients. The production, biomass, and necromass were positively related to soil TN, TP, AP, and fine root C and C/N ratios. The necromass was negatively correlated with fine root N. The biomass/necromass ratios were positively and negatively correlated with fine root N and C/N ratios, respectively. The variations in these five parameters were mainly explained by the fine root nutrients and the interactive effects between fine root and soil nutrients. Thus, our study highlighted that plants enhanced the fine root turnover rate and related parameters to increase nutrient acquisition in spatial nutrient-limited soils in karst ecosystems.

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References

1. Yuan, D.X. On the karst ecosystem. *Acta Geol. Sin.-Engl. Ed.* **2001**, *75*, 336–338.
2. Pan, F.J.; Zhang, W.; Liang, Y.M.; Liu, S.J.; Wang, K.L. Increased associated effects of topography and litter and soil nutrients on soil enzyme activities and microbial biomass along vegetation successions in karst ecosystem, southwestern China. *Environ. Sci. Pollut. Res.* **2018**, *25*, 16979–16990. [[CrossRef](#)] [[PubMed](#)]
3. Wang, S.J.; Liu, Q.M.; Zhang, D.F. Karst rocky desertification in southwestern China: Geomorphology, landuse, impact and rehabilitation. *Land Degrad. Dev.* **2004**, *15*, 115–121. [[CrossRef](#)]
4. Chen, H.; Zhang, W.; Wang, K.; Hou, Y. Soil organic carbon and total nitrogen as affected by land use types in karst and non-karst areas of northwest Guangxi, China. *J. Sci. Food Agric.* **2012**, *92*, 1086–1093. [[CrossRef](#)]
5. Qi, X.; Wang, K.; Zhang, C. Effectiveness of ecological restoration projects in a karst region of southwest China assessed using vegetation succession mapping. *Ecol. Eng.* **2013**, *54*, 245–253. [[CrossRef](#)]
6. Jiang, Z.C.; Luo, W.Q.; Tong, L.Q.; Cheng, Y.; Yang, L.Y.; Wu, Z.Y. Evolution features of rocky desertification and influence factors in karst areas of southwest China in the 21st century. *Carsologica Sin.* **2016**, *35*, 461–468. (In Chinese)
7. Wang, K.L.; Yue, Y.M.; Chen, H.S.; Wu, X.B.; Xiao, J.; Qi, X.K.; Zhang, W.; Du, H. The comprehensive treatment of karst rocky desertification and its regional restoration effects. *Acta Ecol. Sin.* **2019**, *39*, 7432–7440. (In Chinese)
8. Liang, Y.; He, X.; Chen, C.; Feng, S.; Liu, L.; Chen, X.; Zhao, Z.; Su, Y. Influence of plant communities and soil properties during natural vegetation restoration on arbuscular mycorrhizal fungal communities in a karst region. *Ecol. Eng.* **2015**, *82*, 57–65. [[CrossRef](#)]
9. Pan, F.; Zhang, W.; Liu, S.; Li, D.; Wang, K. Leaf N:P stoichiometry across plant functional groups in the karst region of southwestern China. *Trees* **2015**, *29*, 883–892. [[CrossRef](#)]
10. Zhang, W.; Zhao, J.; Pan, F.; Li, D.; Chen, H.; Wang, K. Changes in nitrogen and phosphorus limitation during secondary succession in a karst region in southwest China. *Plant Soil* **2015**, *391*, 77–91. [[CrossRef](#)]
11. Liu, S.; Zhang, W.; Wang, K.; Pan, F.; Yang, S.; Shu, S. Factors controlling accumulation of soil organic carbon along vegetation succession in a typical karst region in Southwest China. *Sci. Total Environ.* **2015**, 521–522, 52–58. [[CrossRef](#)] [[PubMed](#)]
12. Xiao, K.; He, T.; Chen, H.; Peng, W.; Song, T.; Wang, K.; Li, D. Impacts of vegetation restoration strategies on soil organic carbon and nitrogen dynamics in a karst area, southwest China. *Ecol. Eng.* **2017**, *101*, 247–254. [[CrossRef](#)]
13. Thorley, R.M.S.; Taylor, L.; Banwart, S.; Leake, J.; Beerling, D. The role of forest trees and their mycorrhizal fungi in carbonate rock weathering and its significance for global carbon cycling. *Plant Cell Environ.* **2014**, *38*, 1947–1961. [[CrossRef](#)] [[PubMed](#)]
14. Liu, L.; He, X.; Wang, K.; Xie, Y.; Xie, Q.; O'Donnell, A.G.; Chen, C. The Bradyrhizobium-legume symbiosis is dominant in the shrubby ecosystem of the Karst region, Southwest China. *Eur. J. Soil Biol.* **2015**, *68*, 1–8. [[CrossRef](#)]
15. Clarholm, M.; Skjellberg, U.; Rosling, A. Organic acid induced release of nutrients from metal-stabilized soil organic matter—The unbutton model. *Soil Biol. Biochem.* **2015**, *84*, 168–176. [[CrossRef](#)]
16. Pan, F.; Liang, Y.; Zhang, W.; Zhao, J.; Wang, K. Enhanced Nitrogen Availability in Karst Ecosystems by Oxalic Acid Release in the Rhizosphere. *Front. Plant Sci.* **2016**, *7*, 687. [[CrossRef](#)]
17. Pan, F.; Liang, Y.; Wang, K.; Zhang, W. Responses of Fine Root Functional Traits to Soil Nutrient Limitations in a Karst Ecosystem of Southwest China. *Forests* **2018**, *9*, 743. [[CrossRef](#)]
18. Wurzburger, N.; Wright, S.J. Fine-root responses to fertilization reveal multiple nutrient limitation in a lowland tropical forest. *Ecology* **2015**, *96*, 2137–2146. [[CrossRef](#)]
19. Finér, L.; Helmisaari, H.S.; Löhmus, K.; Majdi, H.; Brunner, I.; Børja, I.; Eldhuset, T.; Godbold, D.; Grebenc, T.; Konôpka, B.; et al. Variation in fine root biomass of three European tree species: Beech (*Fagus sylvatica* L.), Norway spruce (*Picea abies* L. Karst.), and Scots pine (*Pinus sylvestris* L.). *Plant Biosyst.* **2007**, *141*, 394–405. [[CrossRef](#)]
20. Matamala, R.; Stover, D. Introduction to a Virtual Special Issue: Modeling the hidden half—the root of our problem. *New Phytol.* **2013**, *200*, 939–942. [[CrossRef](#)]
21. Artacho, P.; Bonomelli, C. Changes in fine-root production, phenology and spatial distribution in response to N application in irrigated sweet cherry trees. *Tree Physiol.* **2016**, *36*, 601–617. [[CrossRef](#)] [[PubMed](#)]
22. Vormstein, S.; Kaiser, M.; Piepho, H.P.; Joergensen, R.G.; Ludwig, B. Effects of fine root characteristics of beech on carbon turnover in the topsoil and subsoil of a sandy Cambisol. *Eur. J. Soil Sci.* **2017**, *68*, 177–188. [[CrossRef](#)]
23. Persson, H.Å.; Stadenberg, I. Spatial distribution of fine-roots in boreal forests in eastern Sweden. *Plant Soil* **2009**, *318*, 1–14. [[CrossRef](#)]
24. Bowsher, A.W.; Mason, C.M.; Goolsby, E.W.; Donovan, L.A. Fine root tradeoffs between nitrogen concentration and xylem vessel traits preclude unified whole-plant resource strategies in *Helianthus*. *Ecol. Evol.* **2016**, *6*, 1016–1031. [[CrossRef](#)] [[PubMed](#)]

25. Majdi, H.; Pregitzer, K.; Morén, A.S.; Nylund, J.E.; Ågren, G.I. Measuring Fine Root Turnover in Forest Ecosystems. *Plant Soil* **2005**, *276*, 1–8. [[CrossRef](#)]
26. Ostonen, I.; Lohmus, K.; Pajuste, K. Fine root biomass, production and its proportion of NPP in a fertile middle-aged Norway spruce forest: Comparison of soil core and ingrowth core methods. *For. Ecol. Manag.* **2005**, *212*, 264–277. [[CrossRef](#)]
27. Clemmensen, K.E.; Bahr, A.; Ovaskainen, O.; Dahlberg, A.; Ekblad, A.; Wallander, H.; Stenlid, J.; Finlay, R.D.; Wardle, D.A.; Lindahl, B.D. Roots and Associated Fungi Drive Long-Term Carbon Sequestration in Boreal Forest. *Science* **2013**, *339*, 1615–1618. [[CrossRef](#)]
28. Brunner, I.; Bakker, M.; Björk, R.; Hirano, Y.; Lukac, M.; Aranda, X.; Børja, I.; Eldhuset, T.D.; Helmisaari, H.S.; Jourdan, C.; et al. Fine-root turnover rates of European forests revisited: An analysis of data from sequential coring and ingrowth cores. *Plant Soil* **2013**, *362*, 357–372. [[CrossRef](#)]
29. Matamala, R.; González-Meler, M.A.; Jastrow, J.D.; Norby, R.J.; Schlesinger, W.H. Impacts of Fine Root Turnover on Forest NPP and Soil C Sequestration Potential. *Science* **2003**, *302*, 1385–1387. [[CrossRef](#)]
30. Quinto, H.; Caicedo, H.; Perez, M.T.; Moreno, F. Fine root dynamics and its relationship with soil fertility in tropical rainforests of Chocó. *Rev. Biol. Trop.* **2016**, *64*, 1709–1719.
31. Huang, G.; Zhao, X.Y.; Su, Y.G.; Zhao, H.L.; Zhang, T.H. Vertical distribution, biomass, production and turnover of fine roots along a topographical gradient in a sandy shrubland. *Plant Soil* **2008**, *308*, 201–212. [[CrossRef](#)]
32. Zhang, W.; Wang, K.; Chen, H.; He, X.; Zhang, J. Ancillary information improves kriging on soil organic carbon data for a typical karst peak cluster depression landscape. *J. Sci. Food Agric.* **2012**, *92*, 1094–1102. [[CrossRef](#)]
33. Liu, J.; Qiu, L.; Ao, G.; Zheng, B.; Wei, X.; Zhang, Y.; Gao, H.; Cheng, J. Effect of clipping on aboveground biomass and nutrients varies with slope position but not with slope aspect in a hilly semiarid restored grassland. *Ecol. Eng.* **2019**, *134*, 47–55. [[CrossRef](#)]
34. Wei, X.; Shao, M.; Fu, X.; Horton, R. Changes in soil organic carbon and total nitrogen after 28 years grassland afforestation: Effects of tree species, slope position, and soil order. *Plant Soil* **2010**, *331*, 165–179. [[CrossRef](#)]
35. Huang, L.; Zhang, P.; Hu, Y.; Zhao, Y. Vegetation succession and soil infiltration characteristics under different aged refuse dumps at the Heidaigou opencast coal mine. *Glob. Ecol. Conserv.* **2015**, *4*, 255–263. [[CrossRef](#)]
36. van Hall, R.L.; Cammeraat, E.; Keesstra, S.D.; Zorn, M. Impact of secondary vegetation succession on soil quality in a humid Mediterranean landscape. *Catena* **2017**, *149*, 836–843. [[CrossRef](#)]
37. Freschet, G.T.; Valverde-Barrantes, O.J.; Tucker, C.M.; Craine, J.M.; McCormack, M.L.; Violle, C.; Fort, F.; Blackwood, C.; Urban-Mead, K.R.; Iversen, C.M.; et al. Climate, soil and plant functional types as drivers of global fine-root trait variation. *J. Ecol.* **2017**, *105*, 1182–1196. [[CrossRef](#)]
38. Ma, Z.; Chen, H.Y. Positive species mixture effects on fine root turnover and mortality in natural boreal forests. *Soil Biol. Biochem.* **2018**, *121*, 130–137. [[CrossRef](#)]
39. Grassein, F.; Lemauviel-Lavenant, S.; Lavorel, S.; Bahn, M.; Bardgett, R.D.; Desclos-Theveniau, M.; Laine, P. Relationships between functional traits and inorganic nitrogen acquisition among eight contrasting European grass species. *Ann. Bot.* **2015**, *115*, 107–115. [[CrossRef](#)]
40. Valverde-Barrantes, O.J.; Smemo, K.A.; Blackwood, C. Fine root morphology is phylogenetically structured, but nitrogen is related to the plant economics spectrum in temperate trees. *Funct. Ecol.* **2015**, *29*, 796–807. [[CrossRef](#)]
41. Cornejo, N.S.; Hertel, D.; Becker, J.N.; Hemp, A.; Leuschner, C. Biomass, Morphology, and Dynamics of the Fine Root System Across a 3000-M Elevation Gradient on Mt. Kilimanjaro. *Front. Plant Sci.* **2020**, *11*, 13. [[CrossRef](#)] [[PubMed](#)]
42. Kulmatiski, A.; Beard, K.H.; Norton, J.M.; Heavilin, J.E.; Forero, L.E.; Grenzer, J. Live long and prosper: Plant-soil feedback, lifespan, and landscape abundance covary. *Ecology* **2017**, *98*, 3063–3073. [[CrossRef](#)] [[PubMed](#)]
43. Chen, H.; Zhang, W.; Wang, K.; Fu, W. Soil moisture dynamics under different land uses on karst hillslope in northwest Guangxi, China. *Environ. Earth Sci.* **2010**, *61*, 1105–1111. [[CrossRef](#)]
44. Nie, Y.P.; Chen, H.S.; Wang, K.L.; Yang, J. Water source utilization by woody plants growing on dolomite outcrops and nearby soils during dry seasons in karst region of Southwest China. *J. Hydrol.* **2012**, *420–421*, 264–274. [[CrossRef](#)]
45. Zongming, S.; Xiankun, L. The types of natural vegetation in karst region of Guangxi and its classified system. *Guangxi Zhiwu* **2003**, *23*, 289–293. (In Chinese)
46. Fairley, R.I.; Alexander, I.J. Methods of calculating fine root production in forests. In *Ecological Interactions in Soil: Plants, Microbes and Animals*; Fitter, A.H., Atkinson, D., Read, D.J., Eds.; Blackwell: Oxford, UK, 1985; pp. 37–42.
47. Burke, M.K.; Raynal, D.J. Fine root growth phenology, production, and turnover in a northern hardwood forest ecosystem. *Plant Soil* **1994**, *162*, 135–146. [[CrossRef](#)]
48. Montagnoli, A.; Terzaghi, M.; Di Iorio, A.; Scippa, G.; Chiatante, D. Fine-root seasonal pattern, production and turnover rate of European beech (*Fagus sylvatica* L.) stands in Italy Prealps: Possible implications of coppice conversion to high forest. *Plant Biosyst.-Int. J. Deal. All Asp. Plant Biol.* **2012**, *146*, 1012–1022. [[CrossRef](#)]
49. Chen, S.; Lin, S.; Reinsch, T.; Loges, R.; Hasler, M.; Taube, F. Comparison of ingrowth core and sequential soil core methods for estimating belowground net primary production in grass-clover swards. *Grass Forage Sci.* **2015**, *71*, 515–528. [[CrossRef](#)]
50. Fu, Z.; Chen, H.; Xu, Q.; Jia, J.; Wang, S.; Wang, K. Role of epikarst in near-surface hydrological processes in a soil mantled subtropical dolomite karst slope: Implications of field rainfall simulation experiments. *Hydrol. Process.* **2016**, *30*, 795–811. [[CrossRef](#)]

51. Song, M.; Peng, W.; Zeng, F.; Du, H.; Peng, Q.; Xu, Q.; Chen, L.; Zhang, F. Spatial Patterns and Drivers of Microbial Taxa in a Karst Broadleaf Forest. *Front. Microbiol.* **2018**, *9*, 1691. [[CrossRef](#)]
52. Peng, W.; Zhu, Y.; Song, M.; Du, H.; Song, T.; Zeng, F.; Zhang, F.; Wang, K.; Luo, Y.; Lan, X.; et al. The spatial distribution and drivers of soil microbial richness and diversity in a karst broadleaf forest. *For. Ecol. Manag.* **2019**, *449*, 117241. [[CrossRef](#)]
53. R Development Core Team. *R: A Language and Environment for Statistical Computing*; R Foundation for Statistical Computing: Vienna, Austria, 2013. Available online: <https://www.R-project.org/> (accessed on 20 January 2022).
54. Abbas, S.; Nichol, J.E.; Zhang, J.; Fischer, G.A. The accumulation of species and recovery of species composition along a 70 year succession in a tropical secondary forest. *Ecol. Indic.* **2019**, *106*, 105524. [[CrossRef](#)]
55. Gill, R.A.; Jackson, R.B. Global patterns of root turnover for terrestrial ecosystems. *New Phytol.* **2000**, *147*, 13–31. [[CrossRef](#)]
56. Wang, S.; Wang, Z.; Gu, J. Variation patterns of fine root biomass, production and turnover in Chinese forests. *J. For. Res.* **2017**, *28*, 1185–1194. [[CrossRef](#)]
57. Hendricks, J.J.; Hendrick, R.L.; Wilson, C.A.; Mitchell, R.J.; Pecot, S.D.; Guo, D. Assessing the patterns and controls of fine root dynamics: An empirical test and methodological review. *J. Ecol.* **2006**, *94*, 40–57. [[CrossRef](#)]
58. Steele, S.J.; Gower, S.T.; Vogel, J.G.; Norman, J.M. Root mass, net primary production and turnover in aspen, jack pine and black spruce forests in Saskatchewan and Manitoba, Canada. *Tree Physiol.* **1997**, *17*, 577–587. [[CrossRef](#)]
59. Hansson, K.; Helmisaari, H.S.; Sah, S.P.; Lange, H. Fine root production and turnover of tree and understorey vegetation in Scots pine, silver birch and Norway spruce stands in SW Sweden. *For. Ecol. Manag.* **2013**, *309*, 58–65. [[CrossRef](#)]
60. Yuan, Z.Y.; Chen, H.Y.H. Fine root dynamics with stand development in the boreal forest. *Funct. Ecol.* **2012**, *26*, 991–998. [[CrossRef](#)]
61. Sun, T.; Dong, L.; Mao, Z.; Li, Y. Fine root dynamics of trees and understorey vegetation in a chronosequence of *Betula platyphylla* stands. *For. Ecol. Manag.* **2015**, *346*, 1–9. [[CrossRef](#)]
62. Du, H.; Liu, L.; Su, L.; Zeng, F.; Wang, K.; Peng, W.; Zhang, H.; Song, T. Seasonal Changes and Vertical Distribution of Fine Root Biomass during Vegetation Restoration in a Karst Area, Southwest China. *Front. Plant Sci.* **2019**, *9*, 2001. [[CrossRef](#)]
63. Kochsiek, A.; Tan, S.; Russo, S.E. Fine root dynamics in relation to nutrients in oligotrophic Bornean rain forest soils. *Plant Ecol.* **2013**, *214*, 869–882. [[CrossRef](#)]
64. Pinno, B.D.; Wilson, S.D. Fine root response to soil resource heterogeneity differs between grassland and forest. *Plant Ecol.* **2013**, *214*, 821–829. [[CrossRef](#)]
65. Göransson, H.; Edwards, P.J.; Perreijn, K.; Smittenberg, R.; Venterink, H.O. Rocks create nitrogen hotspots and N:P heterogeneity by funnelling rain. *Biogeochemistry* **2014**, *121*, 329–338. [[CrossRef](#)]
66. Zhang, X.B.; Bai, X.; He, X.B. Soil creeping in the weathering crust of carbonate rocks and underground soil losses in the karst mountain areas of southwest china. *Carbonates Evaporites* **2011**, *26*, 149–153. [[CrossRef](#)]
67. Espeleta, J.F.; Donovan, L.A. Fine root demography and morphology in response to soil resources availability among xeric and mesicsandhill tree species. *Funct. Ecol.* **2002**, *16*, 113–121. [[CrossRef](#)]
68. Wang, G.; Xue, S.; Liu, F.; Liu, G. Nitrogen addition increases the production and turnover of the lower-order roots but not of the higher-order roots of *Bothriochloa ischaemum*. *Plant Soil* **2017**, *415*, 423–434. [[CrossRef](#)]
69. Hu, L.; Su, Y.; He, X.; Wu, J.; Zheng, H.; Li, Y.; Wang, A. Response of soil organic carbon mineralization in typical Karst soils following the addition of ¹⁴C-labeled rice straw and CaCO₃. *J. Sci. Food Agric.* **2012**, *92*, 1112–1118. [[CrossRef](#)]
70. Li, X.F.; Han, S.J.; Zhang, Y. Decomposition of leaf litter of quercus mongolica from four precipitation gradients in mongolian oak forest. *Chin. J. Appl. Ecol.* **2007**, *18*, 261–266. (In Chinese)
71. Pan, F.J.; Zhang, W.; Wang, K.L.; He, X.Y.; Liang, S.C.; Wei, G.F. Litter C:N:P ecological stoichiometry character of plant communities in typical karst peak-cluster depression. *Acta Ecol. Sin.* **2011**, *31*, 335–343. (In Chinese)