Journal of Environmental Management 252 (2019) 109694

Contents lists available at ScienceDirect

Journal of Environmental Management

journal homepage: http://www.elsevier.com/locate/jenvman

Research article

Soil and fine roots ecological stoichiometry in different vegetation restoration stages in a karst area, southwest China

Liang Su[a,b,c,e,1], Hu Du[b,c,1], Fuping Zeng[b,c], Wanxia Peng[b,c], Muhammad Rizwan[e], Avelino Núñez-Delgado[f], Yaoyu Zhou[d], Tongqing Song[b,c,**], Hua Wang[a,*]

[a] College of Bioscience and Biotechnology, Hunan Agricultural University, Changsha, 410128, Hunan, China
[b] Key Laboratory of Agro-ecological Processes in Subtropical Region, Institute of Subtropical Agriculture, Chinese Academy of Sciences, Changsha, 410125, Hunan, China
[c] Huanjiang Observation and Research Station of Karst Ecosystem, Chinese Academy of Sciences, Huanjiang, 547100, Guangxi, China
[d] College of Resources and Environment, Hunan Agricultural University, Changsha, 410128, Hunan, China
[e] Microelement Research Center, College of Resources and Environment, Huazhong Agricultural University, Wuhan, 430070, Hubei, China
[f] Department of Soil Science and Agricultural Chemistry, Engineering Polytechnic School, Campus Univ., 27002, Lugo, Univ. Santiago de Compostela, Spain

ARTICLE INFO

Keywords:
Carbon
Nitrogen
Phosphorus
Vegetation type
Soil layer
Karst ecosystem

ABSTRACT

The cyclic process of carbon (C), nitrogen (N), and phosphorus (P) elements is an important factor affecting the function of the forest ecosystem. However, the relation between soil and root stoichiometric ratios, especially in karst areas with extremely fragile geology and intensive human disturbance has rarely been investigated. In the current study the concentrations of C, N, and P and their stoichiometric characteristics were investigated using sequential soil coring under different stages of vegetation restoration (primary forest, secondary forest, shrubland and grassland) and soil layer (0–10 cm, 10–20 cm, 20–30 cm) in fine root and soil samples. The results showed that total C concentration had no significant change in all four vegetation types and three soils layer in the fine root, whereas total N and P concentration reached the maximum value in secondary forest and the minimum in grassland. In addition, soil organic C (SOC) and total N increased continuously with natural succession and decreased with soil depth. Secondary forest showed the largest total P concentration in soil, with the smallest corresponding to grassland. Furthermore, both vegetation type and soil layer significantly affected soil C, N, and P stoichiometric ratios. There was a positive correlation among C, N and P in the fine roots, as well as in the soil. While fine root C:N and C:P ratios were negatively related to soil C:N and C:P, fine root N:P was significantly related to soil N:P. This study can provide a scientific basis for the restoration of fragile ecosystem vegetation and for comprehensive treatment of rocky desertification in karst.

1. Introduction

Ecological stoichiometry, which combines the basic principles of ecology and stoichiometry, focuses on the relation between ecological energy equilibrium and the balance of multiple chemical elements, and it is a scientific theory to analyze the influence of the quality balance of elements on the ecological interaction (Reich et al., 2006, W and Guirui, 2008). At present, ecological stoichiometry mainly focuses on the stoichiometry relations of carbon (C), nitrogen (N) and phosphorus (P), because they are important elements for life, and play important roles in material circulation and energy flow (Vitousek et al., 2002; Sistla and Schimel, 2012; Ruttenberg, 2013). Meanwhile, Study the balance of C, N and P is of great significance for understanding the carbon sink potential of ecosystems and how ecosystems respond to future warming (Davidson et al., 2000). According to ecological stoichiometry, organisms can control many characteristics of themselves, including nutrient balance, pH stability, etc., so that the internal environment does not change violently with the change of external environment (Cheng et al., 2010). Therefore, the theory of mass balance restriction can be applied to understand the flow of energy and matter between organism and

---

* Corresponding author.

** Corresponding author. Key Laboratory of Agro-ecological Processes in Subtropical Region, Institute of Subtropical Agriculture, Chinese Academy of Sciences, Changsha, 410125, China.

E-mail addresses: songtongq@isa.ac.cn (T. Song), wangchina926@hunau.edu.cn (H. Wang).

[1] These authors contributed equally to this work.

https://doi.org/10.1016/j.jenvman.2019.109694
Received 5 June 2019; Received in revised form 6 October 2019; Accepted 7 October 2019

environment (Elser et al., 2000; Michaels, 2003). Moreover, ecological stoichiometry is closely related to scale size, and the characteristics of different levels of ecological stoichiometry are also different (Sardans et al., 2012).

In recent years, a large number of studies have been conducted on the relation between aboveground leaves and soil. For example, Heyburn et al. (2017) found that increases in plant C:N ratios do not predict long-term increases in soil C content and C pools. Han et al. (2010) analyzed the leaves of 753 species of Chinese plants and concluded that the P limit of Chinese plants was higher than that of other regions. However, there are few studies on underground parts of plants and their relations with the environment. The root system, as an important link between the aboveground and underground parts, plays an important role in the underground ecological process and the maintenance of aboveground productivity (Eshel and Beeckman, 2013). Furthermore, fine roots (<2 mm) have a large surface area and undertake most of the physiological activities of the root system. There are studies showing that the growth, death, and turnover of fine roots are influenced by soil factors in addition to the genetic control of the tree species (Berhongaray et al., 2013, Gregory, 2014). In the process of growth and development, plants can also improve some physical and chemical properties of soil through root exudates and litters, thus affecting the recovery of vegetation (Copley, 2000). Like leaves, fine roots were considered to be sensitive organs in response to changes in environmental nutrients. Lili et al. (2018) found that the age distribution of C, N and C:N in fine roots were similar to those in leaves, and there were a close coupling between the fine root and soil nutrients in the Loess Plateau of China. Zhou et al. (2018) found that different communities have different stoichiometric ratios, and C, N, and P in soil were changed with the restoration of vegetation, and large spatial heterogeneities were found for soil C:P and N:P ratios in both horizontal and vertical planes. Furthermore, Yang et al. (2018) found strong links among the C:N:P stoichiometry in leaves, roots, litter, and soil in regions affected by desertification in China. Therefore, it is really important to study nutrients and its stoichiometry in underground root systems.

Southwest China is the region with the most extensive karst landscape distribution in the world, which belongs to the subtropical monsoon climate zone, with abundant rain and heat resources, but uneven distribution, high rock exposure rate, shallow and discontinuous soil layer, obvious binary structure, and special karst drought in humid climate is severe (Chen et al., 2013). The climax plants community is mixed evergreen-deciduous broadleaf forest. Due to the intense human disturbance, the coexistence of different vegetation types: grassland, shrub, secondary forest and primary forest (Du et al. 2015). Previous works showed clear explanation regarding the soil, leaf, litter and microbiology ecological stoichiometric at different restoration stages in karst region (Liu et al., 2018; Pan et al., 2015; Wang et al., 2018; Zeng et al. 2016, 2018). However, eventual relations between root and soil stoichiometric ratios have not been found.

Herein, in this study, four different restoration stages in depressions between karst hills were taken as the objects to explore the relations between the stoichiometric characteristics of fine roots with soil in different restoration stages, in the hope of further understanding the underground growth process of karst vegetation. The main objectives of this study were to (i) explore the stoichiometric characteristics of fine roots in different restoration stages, (ii) explore the stoichiometric characteristics of soil in different restoration stages, (iii) analyze the relationship between fine roots and soil stoichiometric characteristics.

2. Materials and methods

2.1. Study site

The research was conducted at karst region of Huanjiang County (Fig. 1). The highest elevation in the study area is 1028.0 m, with annual average temperature being 15.7 °C, January average temperature 10.1 °C, July average temperature 28 °C, minimum temperature of 5.2 °C, frost-free period 290 days, the average annual sunshine hours 1451, annual rainfall of 1389.1 mm, with precipitation from April to September accounting for 70% of annual rainfall, average evaporation being 1571.1 mm, and average relative humidity of 70% (Du et al., 2017). The geomorphic type is depressions between karst hills. The soil is mainly dark or brown calcareous soil developed over carbonate rocks, which is non-zonal soil with shallow soil and high rock exposure rate, soil pH varied from 7.06 to 7.68. (Du et al., 2019).

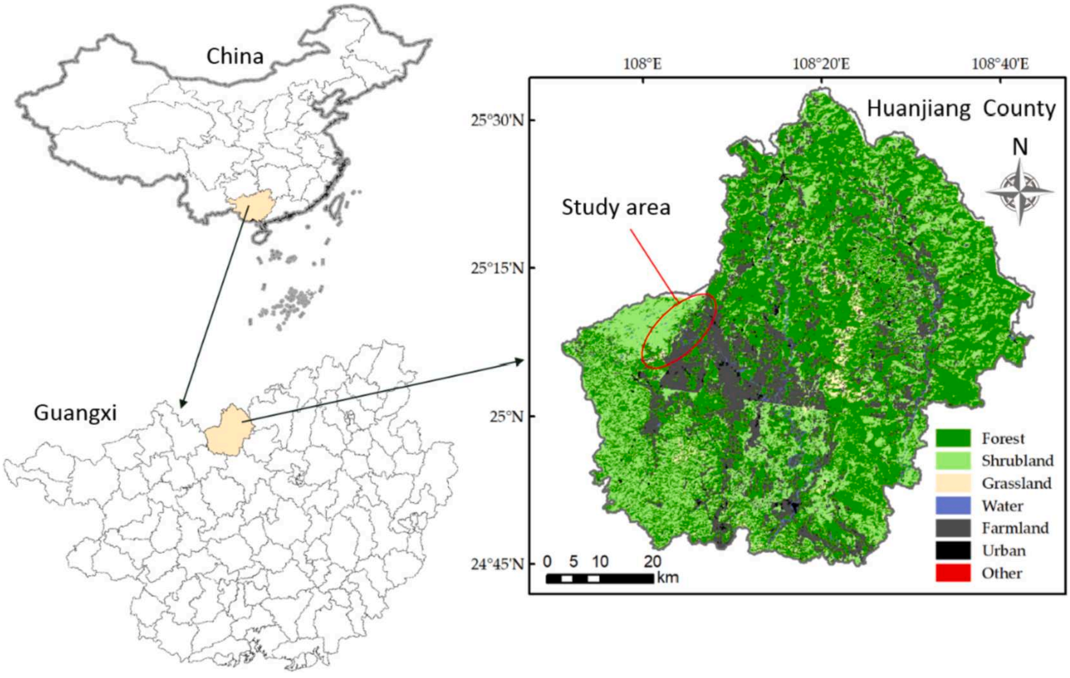

Fig. 1. Vegetation types in the study area located in the karst area of southwest China.

2.2. Experimental design

A post-agriculture succession sequence including four vegetation types, i.e., grassland, shrubland, secondary forest and primary forest was selected based on a space-for-time substitution approach. Three plots of size 20 m × 20 m were established for each vegetation type (Du et al., 2019). The major species found in grassland included *Saccharum arundinaceum*, *Imperata cylindrica*, *Microstegium fasciculatum*, *Ischaemum ciliare*, *Arthraxon hispidus* and *Bidens Pilosa*. The major species found in shrub included *Boehmeria dolichostachya*, *Vitex negundo*, *Alchornea trewioides, Mallotus barbatus* and *Pyracantha fortuneana*. The major species found in secondary forest included *Alangium chinense, Cryptocarya austrokweichouensis* and *Bauhinia brachycarpa*. The major species found in primary forest included *Handeliodendron bodinieri*, *Cleidion bracteosum*, *Cyclobalanopsis glauca*, *Choerospondias axillaris*, *Machilus pauhoi*, *Eurycorymbus cavaleriei*, *Platycarya strobilacea*, *Acer laevigatum*, *Platycladus orientalis*, *Quercus phillyraeoides*, *Sinosideroxylon pedunculatum*, *Calocedrusmacrolepis* and *Carpinus luochengensis* (Du et al., 2015).

2.3. Fine root and soil sampling and processing

Fine roots thrive in autumn and winter (López et al., 2001), so we sampled in September 2016. We randomly selected 8 points in various places and adopted a soil drill with an internal diameter of 10 cm, and collected fine root and soil samples in 0–10, 10–20 and 20–30 cm soil layers (Makkonen and Helmisaari, 1999). There were 36 samples in total (4 vegetation types × 3 replicate plots × 3 soil depths) at each sampling time. In the laboratory, fine root samples (<2 mm) were oven dried at 75 °C to constant weight after 24 h. Retsch's MM200 ball mill was then used for grinding. Meanwhile, the soil samples were dried in a cool place, and then were passed through a 2 mm sieve. Thereafter, total carbon and nitrogen in fine root (FRC and FRN) were measured by means of a German Elementar vario MAX CN analyzer. The organic carbon concentration of soil (SC) was measured by wet oxidation using the dichromate redox colorimetric method (Nelson et al., 1996). The total nitrogen of soil (SN) was determined according to the Kjeldahl method (Gallaher et al., 1976) using the flow injection apparatus (AA3). Total phosphorus in fine root (FRP) and soil (SP) was measured using an ultraviolet spectrophotometer based on the Mo–Sb colorimetric method (Ru-kun 1999).

2.4. Statistical analysis

R (X64 3.5.2) software (University of Auckland, Auckland, New Zealand) was used to analyze all the data (R Core Team, 2018). We used two-way ANOVAs to analyze the effects of vegetation, soil layer and their interactions on fine root and soil stoichiometric characteristics. One-way ANOVA with least significant difference (LSD) test at the $P < 0.05$ level of significance was used for differences among vegetation types and soil layers. We implemented the Pearson correlation of fine root and soil regarding stoichiometric characteristics using the Performance Analytics package (Peterson et al. 2014). OriginPro 9.0 (Originlab Inc., USA) for Windows were used for drawing figures.

3. Results

3.1. Fine root C, N and P stoichiometry characteristics

Two-way ANOVA indicated that carbon content in fine roots (FRC) was not significantly affected by vegetation type, soil layer and their interaction. The contents of nitrogen (FRN) and phosphorus (FRP) in fine roots were only significantly affected by vegetation type, while the soil layer and interaction between soil layer with vegetation type was not significant (Fig. 2A). In addition, multiple comparisons showed that the FRN content of secondary forest was the highest in each soil layer, and the lowest FRN content of grassland was found in the 0–10 cm soil

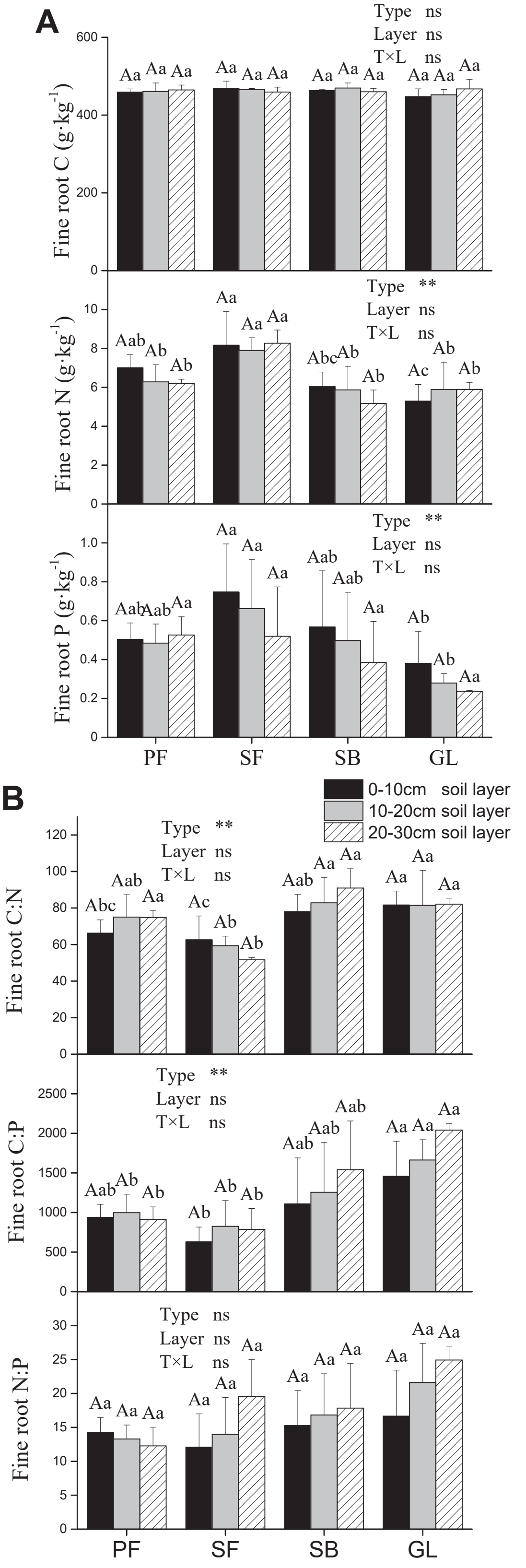

Fig. 2. Distributions of C, N, P content (A) and C:N, C:P, N:P (B) in fine root under different vegetation restoration stages. **Note:** PF, SF, SB and GL represent Primary forest, Secondary forest, Shrub and grassland. Values are the means ± SE of three plots. Different capital letters above the bars indicate significant differences among soil layers, and different small letters above the bars indicate significant differences among vegetation types ($p < 0.05$). $^{*}P < 0.05$; $^{**}P < 0.01$; ns, no significant.

layer. The highest FRP content corresponded to secondary forest, while the lowest was found in grassland for each soil layer.

Two way ANOVA indicated that the ratio of fine root C:N (FRC:N) and C:P (FRC:P) was significantly affected by vegetation type, but the soil layer and interaction between vegetation type with soil layer was not significant. The fine root N:P ratios (FRN:P) were not significantly affected by vegetation type, soil layer and their interaction (Fig. 2B). The multiple comparisons tests showed that the FRC:N values of secondary forest were the highest in all three soil layers, and FRC:P was greater in grassland than in primary forest and secondary forest at all three soil layers.

3.2. Soil C, N and P stoichiometry characteristics

Two-way ANOVA indicated that the content of organic carbon (SC) and nitrogen (SN) in soil was significantly affected by vegetation type and soil layer, but not by their interaction. The content of phosphorus (SP) in soil was only significantly affected by vegetation type (Fig. 3A). The multiple comparisons showed that, in terms of vegetation types, the SC presented a changing trend of primary forest > secondary forest > shrub > grassland in the 0–10 cm and 10–20 cm soil layers; whereas the SN of primary forest was highest and shrub was lowest in 10–20 cm and 20–30 cm; and the SP of secondary forest was highest only in the 10–20 cm soil layer. In terms of soil, SC and SN decreased with the depth of the soil layer in all vegetation types except grassland.

Two-way ANOVAs indicated that both vegetation type and soil layer significantly affected soil C:N (SC:N), C:P (SC:P) and N:P (SN:P). Interactions between vegetation type and soil layer did not affect any ratio in a significant manner. In terms of vegetation types, the multiple comparisons tests showed that in the soil layer of 0–10 cm, the SC:N and SC:P increased (or tended to increase) with vegetation restoration, but did not differ in the 10–20 cm and 20–30 cm soil layers. Meanwhile, the SN:P value was lowest in the 10–20 cm soil layer (Fig. 3B). In terms of soil layer, the SC:N and SC:P of primary forest and secondary forest both decreased with the depth, while SN:P only decreased with depth in secondary forest.

3.3. Relations between fine root and soil stoichiometry

As shown in Fig. 4, the SC was positively related to FRN and SN ($P < 0.01$), and SP was positively correlated to FRP ($P < 0.05$). As shown in Fig. 5, the FRC:N was negatively correlated to SC:N and SC:P. The FRC:P was positively correlated to FRN:P and SN:P, while it was negatively correlated to SC:N and SC:P. In addition, SN:P was positively correlated to SC:P, whereas SC:N was positively correlated to SC:P.

4. Discussion

4.1. Fine root and soil C:N:P characteristics in four vegetation types

As a structural element, C is ubiquitous in plants and has little variability, so in the current study, the fine root C was not significantly affected by vegetation type, soil layer and its interaction. However, fine root N and P concentrations were significantly different between secondary forest and the other three types, which contributed to establish differences in N, P, C:N and C:P in vegetation types. This is consistent with previous studies showing that plants C:N and C:P ratios differ in vegetation types (Mcgroddy et al., 2004). Meanwhile, C:N and C:P of plants reflect the utilization efficiency of nutrients and the growth rate of these plants to a certain extent (Vitousek, 1982; Gray, 1983, David A et al., 2004). In this study, the fine root N and P concentrations in secondary forest were higher than in the other three types, because the nutrients in the community are concentrated in the tree layer, while the natural succession of the primary forest, shrub and grassland is relatively complex, and it should be taken into account that competition between species is fierce and the nutrient distribution pattern of the

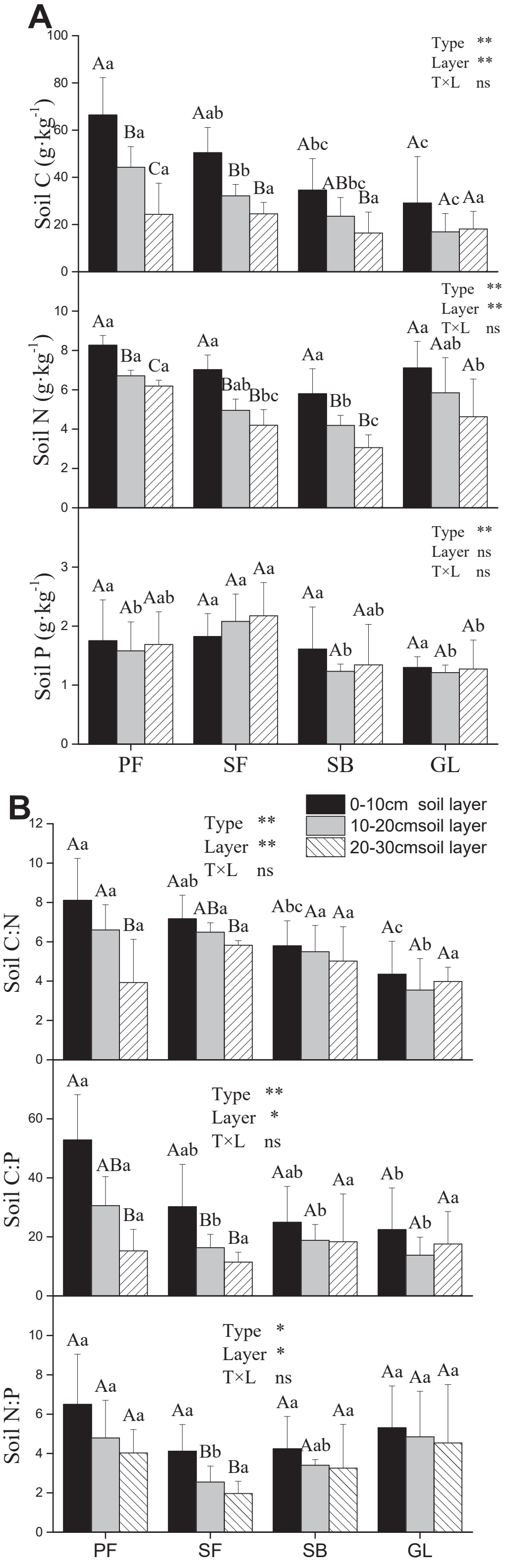

Fig. 3. Distributions of C, N, P content (A) and C:N, C:P, N:P (B) in soil under different vegetation restoration stages. **Note:** PF, SF, SB and GL represent Primary forest, secondary forest, shrub, grassland. Values are the means ± SE of three plots. Different capital letters above the bars indicate significant differences among vegetation types, and different small letters above the bars indicate significant differences among soil layers ($p < 0.05$). *$P < 0.05$; **$P < 0.01$; ns, no significant.

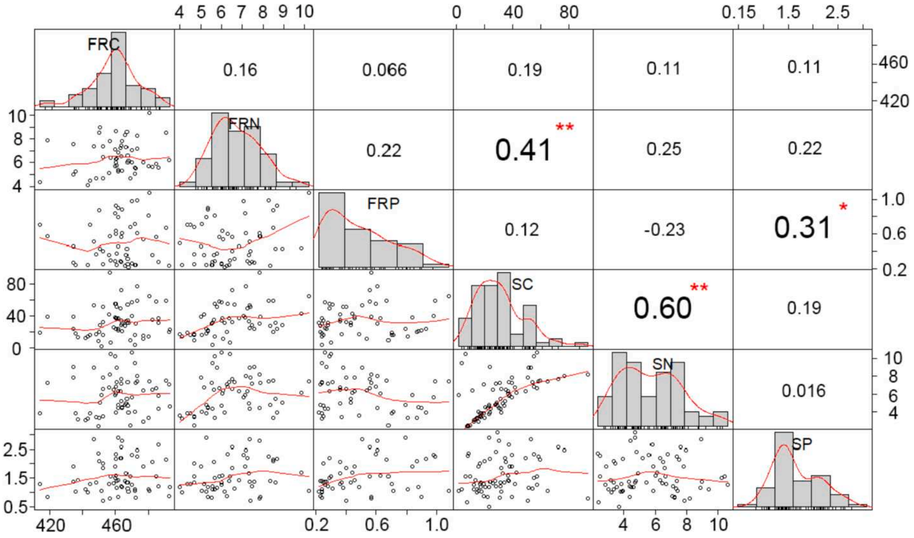

Fig. 4. Correlation coefficients of fine root and soil in C, N and P (n = 72). **Note:** FRC represents fine root total carbon; FRN represents fine root total nitrogen; FRP represents fine root total phosphorus; SC represents soil organ carbon; SN represents soil total nitrogen; SP represents soil total phosphorus; *P < 0.05; **P < 0.01; significant correlations are indicated in bold.

community is different. In addition, compared with average global C:N in fine roots (48.25) (Jackson et al., 1997), our results in each succession stage were higher than that. The results showed that the root system of karst ecosystem had higher element utilization efficiency. Previous studies have shown that N:P ratios <14 suggest N limitation, and N:P ratios >16 suggest P limitation (Dj, 2003; Reich, 2010). In the current study, the average N:P ratios increased from 13.26 in the primary forest to 21.06 in the grassland, which also suggested an increase in P limitation. This is consistent with most subtropical studies (Liu et al., 2010; Fan et al., 2015). On the other hand, there was no difference in fine root nutrients at different depths of soil in the current study, but some other studies found that the effect of different root branch order on nutrient was significant (Pregitzer et al., 1997; Jl et al., 2002; Pregitzer, 2002). As a consequence, further future studies on different root orders in karst ecosystems are needed.

Changes in soil nutrients due to differ land use and depth are well known (Powers, 2004; Gamboa and Galicia, 2011). For example, Liu et al. (2017) reported that soil nutrients were significantly lower in most soil layers of cropland than those in natural land in the Ili River Valley. Song and co-workers also found that SOC in natural grassland and shrub was significantly higher than that in cropland and orchard (Song et al., 2012). In addition, our previous results from karst areas with poor soil and high heterogeneity indicate that the soil nutrients content was increased with vegetation succession and decreased with soil depth, regardless of different scale (Chen et al., 2012; Du et al. 2015; Li et al., 2017; Wang et al., 2018). In this work, the changing trend of SOC showed a consistent pattern: Primary forest > Secondary forest > Shrub > Grassland. This is because the input of recalcitrant litters and tissues increased with the vegetation succession, which can promote the accumulation of SOC (Paul et al., 2003). In previous studies, the variation in soil C:N ratio was small for different vegetation types (Mcgroddy et al., 2004). In our study, with the exception of grassland, there was no difference in soil C:N among vegetation types, indicating that SOC and soil N are relatively stable in karst forests. Furthermore, the lowest soil N concentration was found in shrubs rather than in grassland (in the early stage of succession), which perhaps could be attributed to the different N absorption rates of plant species, and to the existence of grazing in the plots (U and U 2005; Pei et al., 2008).

Our results showed that soil P concentration was significantly lower than the global average (2.8 mg g^{-1}), which is consistent with the low levels of soil P in subtropical areas (Benjamin et al., 2008). Soil C:P is an indicator of phosphorus availability (Hobbie and Vitousek, 2000; Hessen et al., 2004), and we found that this ratio was significantly affected by vegetation type, giving that primary and secondary forests were larger than shrubs and grasses in surface soil layer. It can be seen that the soil P availability of shrubs and grasslands was higher, attribute to

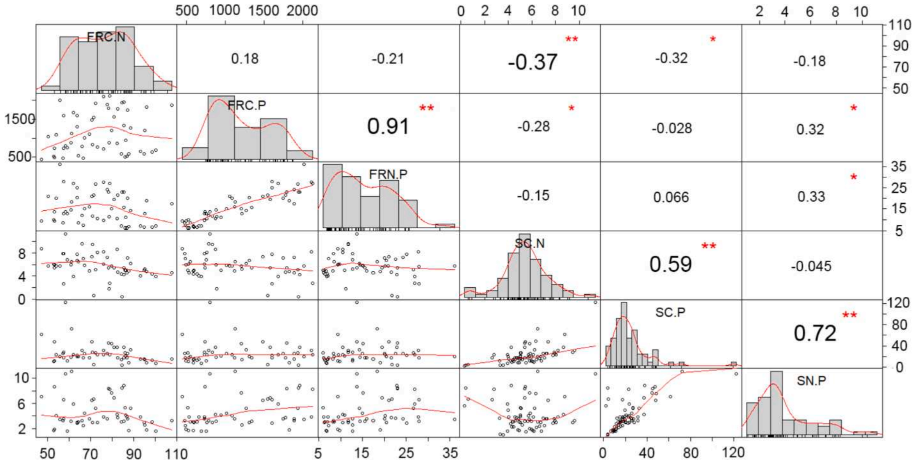

Fig. 5. Correlation coefficients of fine root and soil in C:N, C:P, and N:P (n = 72). **Note:** FRC:N represents the ratio of total carbon with total nitrogen in fine root; FRC:P represents the ratio of total carbon with total phosphorus in fine root; FRN:P represents the ratio of total nitrogen with total phosphorus in fine root; SC:N represents the ratio of organic carbon with total nitrogen in soil; SC:P represents the ratio of organic carbon with total phosphorus in soil; SN:P represents the ratio of total nitrogen with total phosphorus in soil; *P < 0.05; **P < 0.01; significant correlations are indicated in bold.

fertilization, that has influence on soil in early succession stages (Niu et al., 2011). Moreover, soil N:P, which can be used as prediction index of nutrient restriction type, reaches the maximum value in the primary forest, indicating that soil N accumulated, thus showing a comparatively stable condition in primary forest.

4.2. Relations between fine root and soil C:N:P characteristics in four vegetation types

The cycle of C, N, and P in the ecosystem results in translocation between plants, litters and soil. The stability of plant C:N:P ratio will affect soil nutrients, and soil also provides feedback on the effectiveness of plant nutrients (Mcgroddy et al., 2004). Previous studies have found a strong relation between CNP in plants and soils (Yang and Luo, 2011; Liao et al., 2014; Yu et al., 2014), and our results also show that since the decomposition of SOC is the main source of soil N (Rustad et al., 2001), SOC was positively correlated with soil N. However, few studies had shown details on relations between concentrations of elements in soils and roots (Jobbágy and Jackson, 2001; Qiang et al., 2010). In addition, many studies in subtropical and tropical areas have shown that plant nutrients correlate with soil (Hedin, 2004; Fan et al., 2015), and in this way we also found consistent results showing a positive correlation between soil P and plant nutrients. Generally speaking, the soil C:N is negatively related to soil N mineralization rate (Elser et al., 2000; Yong et al., 2012). We found a correlation among fine root and soil C, N, and P stoichiometry, a negative correlation between fine root and soil C:N and C:P, which indicates that with the increase of vegetation C:N and C:P, soil N utilization rate and circulation increase, because N has a positive impact on soil N mineralization rate (Mooshammer et al., 2014). Furthermore, fine root N:P was significantly related to soil N:P, the re-translocation of nutrients between soil and fine root confirms these relations. Previous studies found that the re-translocation patterns of N and P contents in leaves of different plants were similar (Fife et al., 2008). However, the stoichiometric characteristics of plants and soil in karst areas need to be further studied in microorganism and litters.

5. Conclusion

In this study, we provide the scientific basis for the degraded karst ecosystem in terms of underground stoichiometric characteristics and found some interesting details. The fine root and soil C, N, and P and their stoichiometry produced a great response with a succession of vegetation. The fine root N, P, C:N and C:P are significantly affected by vegetation succession, and the secondary forest is different from the other three types. Meanwhile, plants fine root limited by phosphorus in each restoration stages. In terms of soils, SOC and soil N are relatively stable in karst forests at four restoration stages. Soil C:P was significantly affected by vegetation type, and were larger in primary and secondary forests than in shrubs and grasses in surface soil layer. Moreover, soil N accumulated giving a comparatively stable condition in primary forest. In addition, we found that SOC was positively correlated with soil N, and a negative correlation between the fine root and soil in C:N and C:P, indicate that the increase of vegetation C:N and C:P increase with soil N utilization rate and circulation.

Acknowledgements

This work was supported by the National Key Research and Development Program of China (2016YFC0502405), the Guangxi Key Research and Development Program (AB17129009, AB16380255), the National Natural Science Foundation of China (31400412, 31870712, 41701293), and the Guangxi Provincial Program of Distinguished Expert in China supported this research project financially, the Major Project of the Ministry of China Agriculture and Ministry of China Finance (No. 2015–38); the Special Fund for Local Scientific and Technological Development under the Leadership of the Central Government (No. 2017CT5010); National Key Research and Development Programs in China (No. 2017YFD0200803); and the National Natural Science Foundation of China (No. 414013w58).

References

Benjamin, Z.H., Ying-Ping, W., Peter M, V., Christopher B, F., 2008. A unifying framework for dinitrogen fixation in the terrestrial biosphere. Nature 454, 327.

Berhongaray, G., Janssens, I.A., King, J.S., Ceulemans, R., 2013. Fine root biomass and turnover of two fast-growing poplar genotypes in a short-rotation coppice culture. Plant Soil 373, 269–283.

Chen, H., Zhang, W., Wang, K., Hou, Y., 2012. 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. 92, 1086–1093.

Chen, H., Nie, Y., Wang, K., 2013. Spatio-temporal heterogeneity of water and plant adaptation mechanisms in karst regions: a review. Acta Ecol. Sin. 33, 317–326.

Cheng, B., Zhao, Y., Zhang, W., Shuqing, A.N., 2010. The research advances and prospect of ecological stoichiometry. Acta Ecol. Sin. 7, 195–196.

Copley, J., 2000. Ecology goes underground. Nature 406 (6795), 452–454.

W, David.A., W, Lawrence.R., B, Richard.D., 2004. Ecosystem properties and forest decline in contrasting long-term chronosequences. Science 305, 509–513.

Davidson, E.A., Trumbore, S.E., Amundson, R., 2000. Biogeochemistry: soil warming and organic carbon content. Nature 408 (6814), 789.

Dj, T.J.R., 2003. Use of nitrogen to phosphorus ratios in plant tissue as an indicator of nutrient limitation and nitrogen saturation. J. Appl. Ecol. 40, 523–534.

Du, H., Song, T., Zeng, F., Wang, K., Peng, W., Fu, W., Li, S., et al., 2015. Carbon storage and its controlling factors under different vegetation types in depressions between karst hills,southwest China. Acta Ecol. Sin. 35 (14), 4658–4667.

Du, H., Hu, F., Zeng, F.P., Wang, K.L., Peng, W.X., Zhang, H., et al., 2017. Spatial distribution of tree species in evergreen-deciduous broadleaf karst forests in southwest China. Sci. Rep. 7, 15664.

Du, H., Liu, L., Su, L., et al., 2019. Seasonal changes and vertical distribution of fine root biomass during vegetation restoration in a karst area, southwest China. Front. Plant Sci. 9, 2001.

Elser, J.J., Fagan, W.F., Denno, R.F., Dobberfuhl, D.R., Folarin, A., Huberty, A., Interlandi, S., Kilham, S.S., Mccauley, E., Schulz, K.L., 2000. Nutritional constraints in terrestrial and freshwater food webs. Nature 408, 578–580.

Eshel, A., Beeckman, T., 2013. Plant Roots: the Hidden Half. CRC press.

Fan, H., Wu, J., Liu, W., Yuan, Y., Hu, L., Cai, Q., 2015. Linkages of plant and soil C:N:P stoichiometry and their relationships to forest growth in subtropical plantations. Plant Soil 392, 127–138.

Fife, D.N., Nambiar, E.K.S., Saur, E., 2008. Retranslocation of foliar nutrients in evergreen tree species planted in a Mediterranean environment. Tree Physiol. 28, 187–196.

Gallaher, R.N., Weldon, C.O., Boswell, F.C., 1976. A semiautomated procedure for total nitrogen in plant and soil Samples 1. Soil Sci. Soc. Am. J. 40 (6), 887–889.

Gamboa, A.M., Galicia, L., 2011. Differential influence of land use/cover change on topsoil carbon and microbial activity in low-latitude temperate forests. Agric. Ecosyst. Environ. 142, 280–290.

Gray, J.T., 1983. Nutrient use by evergreen and deciduous shrubs in southern California: I. Community nutrient cycling and nutrient-use efficiency. J. Ecol. 71, 21–41.

Gregory, P.J., 2014. Plant Roots: Growth, Activity and Interaction with Soils.

Han, W., Fang, J., Guo, D., Zhang, Y., 2010. Leaf nitrogen and phosphorus stoichiometry across 753 terrestrial plant species in China. New Phytol. 168, 377–385.

Hedin, L.O., 2004. Global organization of terrestrial plant-nutrient interactions. Proc. Natl. Acad. Sci. 101 (30), 10849–10850.

Hessen, D.O., Ågren, G.I., Anderson, T.R., et al., 2004. Carbon sequestration in ecosystems: the role of stoichiometry. Ecology 85 (5), 1179–1192.

Heyburn, J., Mckenzie, P., Crawley, M.J., Fornara, D.A., Heyburn, J., Mckenzie, P., Crawley, M.J., Fornara, D.A., 2017. Effects of grassland management on plant C:N:P stoichiometry: implications for soil element cycling and storage. Ecosphere 8 (10), 1963.

Hobbie, S.E., Vitousek, P.M., 2000. Nutrient limitation of decomposition in Hawaiian forests. Ecology 81, 1867–1877.

Jackson, R.B., Mooney, H.A., Schulze, E.D., 1997. A global budget for fine root biomass, surface area, and nutrient contents. Proc. Natl. Acad. Sci. 94 (14), 7362–7366.

Jl, D.F., Burton, A.J., Allen, M.F., Ruess, R.W., Hendrick, R.L., 2002. Fine root architecture of nine North American trees. Ecol. Monogr. 72, 293–309.

Jobbágy, E.G., Jackson, R.B., 2001. The distribution of soil nutrients with depth: global patterns and the imprint of plants. Biogeochemistry 53, 51–77.

Li, D., Wen, L., Yang, L., Luo, P., Xiao, K., Chen, H., Zhang, W., He, X., Chen, H., Wang, K., 2017. Dynamics of soil organic carbon and nitrogen following agricultural abandonment in a karst region. J. Geophys. Res. Biogeosci. 122, 230–242.

Liao, Y., Mccormack, M.L., Fan, H., Wang, H., Wu, J., Jie, T., Liu, W., Guo, D., 2014. Relation of fine root distribution to soil C in a Cunninghamia lanceolata plantation in subtropical China. Plant Soil 381, 225–234.

Lili, C., Qiang, D., Zhiyou, Y., et al., 2018. Age-related C:N:P stoichiometry in two plantation forests in the Loess Plateau of China. Ecol. Eng. 120, 14–22.

Liu, X.Z., Zhou, G.Y., Zhang, D.Q., Liu, S.Z., Chu, G.W., 2010. N and P stoichiometry of plant and soil in lower subtropical forest successional series in southern China. Chin. J. Plant Ecol. 34, 64–71.

Liu, X., Ma, J., Ma, Z.-W., Li, L.-H., 2017. Soil nutrient contents and stoichiometry as affected by land-use in an agro-pastoral region of northwest China. Catena 150, 146–153.

Liu, L., Ni, J., Zhong, Q., Hu, G., Zhang, Z., 2018. High mortality and low net change in live woody biomass of karst evergreen and deciduous broad-leaved mixed forest in southwestern China. Forests 9 (5), 263.

López, B., Sabaté, S., Gracia, C.A., 2001. Annual and seasonal changes in fine root biomass of a Quercus ilex L. forest. Plant Soil 230, 125–134.

Makkonen, K., Helmisaari, H.S., 1999. Assessing fine-root biomass and production in a Scots pine stand–comparison of soil core and root ingrowth core methods. Plant Soil 210, 43–50.

Mcgroddy, M.E., Daufresne, T., Hedin, L.O., 2004. Scaling of C:N:P stoichiometry in forests worldwide: implications of terrestrial redfield-type ratios. Ecology 85, 2390–2401.

Michaels, A.F., 2003. The ratios of life. Science 300, 906–907.

Mooshammer, M., Wanek, W., Hämmerle, I., Fuchslueger, L., Hofhansl, F., Knoltsch, A., Schnecker, J., Takriti, M., Watzka, M., Wild, B., 2014. Adjustment of microbial nitrogen use efficiency to carbon:nitrogen imbalances regulates soil nitrogen cycling. Nat. Commun. 5, 3694.

Nelson, D.W., Sommers, L.E., Sparks, D.L., Page, A.L., Helmke, P.A., Loeppert, R.H., Soltanpour, P.N., Tabatabai, M.A., Johnston, C.T., Sumner, M.E., 1996. Total carbon, organic carbon, and organic matter. Methods Soil Anal. 9, 961–1010.

Niu, L.A., Hao, J.M., Zhang, B.Z., et al., 2011. Influences of long-term fertilizer and tillage management on soil fertility of the north China plain. Pedosphere 21 (6), 813–820.

Pan, F.J., Zhang, W., Liu, S.J., Li, D.J., Wang, K.L., 2015. Leaf N:P stoichiometry across plant functional groups in the karst region of southwestern China. Trees (Berl.) 29 (3), 883–892.

Paul, K.I., Polglase, P.J., Richards, G.P., 2003. Predicted change in soil carbon following afforestation or reforestation, and analysis of controlling factors by linking a C accounting model (CAMFor) to models of forest growth (3PG), litter decomposition (GENDEC) and soil C turnover (RothC). For. Ecol. Manag. 177, 485–501.

Pei, S., Fu, H., Wan, C., 2008. Changes in soil properties and vegetation following exclosure and grazing in degraded Alxa desert steppe of Inner Mongolia, China. Agric. Ecosyst. Environ. 124, 33–39.

Peterson, B.G., Carl, P., Boudt, K., et al., 2014. Performance analytics: econometric tools for performance and risk analysis. R Package Version 1 (3541), 107.

Powers, J.S., 2004. Changes in soil carbon and nitrogen after contrasting land-use transitions in northeastern Costa Rica. Ecosystems 7, 134–146.

Pregitzer, K.S., 2002. Fine roots of trees – a new perspective. New Phytol. 154, 267–270.

Pregitzer, K.S., Kubiske, M.E., Yu, C.K., Hendrick, R.L., 1997. Relationships among root branch order, carbon, and nitrogen in four temperate species. Oecologia 111, 302–308.

Qiang, Y., Quansheng, C., Elser, J.J., Nianpeng, H., Honghui, W., Guangming, Z., Jianguo, W., Yongfei, B., Xingguo, H., 2010. Linking stoichiometric homoeostasis with ecosystem structure, functioning and stability. Ecol. Lett. 13, 1390–1399.

R Core Team, 2018. R: A Language and Environment for Statisticalcomputing. R Foundation for Statistical Computing, Vienna, Austria. Available at: https://www.R-project.org/.

Reich, P.B., 2010. Global biogeography of plant chemistry: filling in the blanks. New Phytol. 168, 263–266.

Reich, P.B., Tjoelker, M.G., Machado, J.L., Oleksyn, J., 2006. Universal scaling of respiratory metabolism, size and nitrogen in plants. Nature 439, 457–461.

Ru-kun, L., 1999. Soil Argrochemistry Analysis Protocoes. China Agriculture Science Press.

Rustad, L., Campbell, J., Marion, G., et al., 2001. A meta-analysis of the response of soil respiration, net nitrogen mineralization, and aboveground plant growth to experimental ecosystem warming. Oecologia 126 (4), 543–562.

Ruttenberg, K.C., 2013. The global phosphorus cycle. Treatise on Geochem. 8, 585–643.

Sardans, J., Rivas-Ubach, A., Peñuelas, J., 2012. The elemental stoichiometry of aquatic and terrestrial ecosystems and its relationships with organismic lifestyle and ecosystem structure and function: a review and perspectives. Biogeochemistry 111, 1–39.

Sistla, S.A., Schimel, J.P., 2012. Stoichiometric flexibility as a regulator of carbon and nutrient cycling in terrestrial ecosystems under change. New Phytol. 196, 68–78.

Song, Z., Xie, Z., Zhang, J., 2012. Soil organic carbon distribution in relation to land use and its storage in a small watershed of the Loess Plateau, China. Catena 88, 6–13.

U, N., U, T., 2005. Species differences in timing of leaf fall and foliage chemistry modify nutrient resorption efficiency in deciduous temperate forest stands. Tree Physiol. 25, 1001.

Vitousek, P., 1982. Nutrient cycling and nutrient use efficiency. Am. Nat. 119, 553–572.

Vitousek, P.M., Olander, L., Allison, S., 2002. Nitrogen and nature. S. Auml, ttenschwiler Ambio 31, 97.

S Q, W., Guirui, Y., 2008. Ecological stoichiometry characteristics of ecosystem carbon, nitrogen and phosphorus elements. Acta Ecol. Sin. 28 (8), 3937–3947.

Wang, M.M., Chen, H.S., Zhang, W., Wang, K.L., 2018. Soil nutrients and stoichiometric ratios as affected by land use and lithology at county scale in a karst area, southwest China. Sci. Total Environ. 619, 1299–1307.

Yang, Y., Luo, Y., 2011. Carbon: nitrogen stoichiometry in forest ecosystems during stand development. Glob. Ecol. Biogeogr. 20, 354–361.

Yang, Y., Liu, B.R., An, S.S., 2018. Ecological stoichiometry in leaves, roots, litters and soil among different plant communities in a decertified region of Northern China. Catena 166, 328–338.

Yong, L., Wu, J., Liu, S., Shen, J., Huang, D., Su, Y., Wei, W., Keith, S.J., 2012. Is the C:N:P stoichiometry in soil and soil microbial biomass related to the landscape and land use in southern subtropical China? Glob. Biogeochem. Cycles 26, GB4002.

Yu, Y.F., Peng, W.X., Song, T.Q., Zeng, F.P., Wang, K.L., Wen, L., Fan, F.J., 2014. Stoichiometric characteristics of plant and soil C, N and P in different forest types in depressions between karst hills, southwest China. Yingyong Shengtai Xuebao 25 (4), 947–954.

Zeng, Z.X., Wang, K.L., Liu, X.L., et al., 2016. Stoichiometric characteristics of live fresh leaves and leaf litter of typical plant communities in a karst region of northwestern Guangxi. Acta Ecol. Sin. 36 (7), 1907–1914.

Zeng, X., Xiao, Z., Zhang, G., et al., 2018. Speciation and bioavailability of heavy metals in pyrolytic biochar of swine and goat manures. J. Anal. Appl. Pyrolysis 132, 82–93.

Zhou, Y., Boutton, T.W., Wu, X.B., 2018. Soil C:N:P stoichiometry responds to vegetation change from grassland to woodland. Biogeochemistry 140, 341–357.