Effects of vegetation restoration and environmental factors on understory vascular plants in a typical karst ecosystem in southern China

Zhiyang Ou*, Shilong Pang, Qinfei He, Yuhua Peng, Xiaorong Huang & Wenhui Shen

Understory vegetation is an important component in most forest ecosystems. It is very important for soil and water conservation in karst region, study on understory will provide valuable information for understanding the interaction mechanism between understory flora and karst environment. Thirty-two plots were sampled in three vegetation types along with a restoration gradient (shrubland, forest–shrub transition, and mixed-species broadleaf forest) in typical karst mountains in Southwest Guangxi, China. Overstory trees, understory vascular plants, soil nutrients, and topographic factors were recorded in each 400-m² plot. Multivariate statistics were used, including the multi-response permutation procedure (MRPP), indicator species analysis, and canonical correlation analysis (CCA). MRPP showed understory species composition significantly differed among the three vegetation types, with the greatest difference between the shrubland and the mixed forest. Twenty-one understory species were identified as significant indicator species, with 13 species being identified as indicators of the shrubland, two of forest–shrub transition, and six of the mixed forest. Light-demanding herbaceous seed plants were common in shrubland, while shade-tolerant calcicole assembled under the mixed forest. Forward selection of CCA ordination revealed that understory plant distribution was most strongly influenced by elevation, followed by soil pH, the concentration of total potassium and exchangeable calcium, slope aspect, slope degree, and the concentration of available potassium. The result reveals that vegetation types affect understory species composition by modifying understory environments. Elevation affects the spatial distribution of vegetation and soil factors, and then the understory plants. Meanwhile, soil Ca content also plays a key role in the understory species distribution. Understory diversity increased with increasing canopy structure complexity from shrubland to mixed-species forest. Thus, it is necessary to take measures to promote natural vegetation restoration and to protect the mixed forests in degraded karst areas.

Understory flora represents the important component of biodiversity and plays a key role in maintaining structure and ecosystem functions in forests1-3. Although understory vegetation contributes relatively little to the total forest vegetation biomass, it contributes a great proportion of floristic diversity4,5. In some areas of the Hyrcanian zone, herbaceous plants constitute over 90% of the total plants with diverse life forms6. Understory flora also influences soil enzyme activity and soil fertility and improves material cycling and energy flow in forest ecosystems8,9. Understory flora provides a nursery, facilitates the colonization of target plants, improves the survival and growth of tree seedlings, changes the understory microhabitat, and affects forest hydrology7-10. Study on understory flora could help improve understanding community dynamics, plant responses to disturbance, and forest regeneration and helps guide conservation and management11-17.

The forest canopy is a major determinant in the forest ecosystem. It strongly influences the understory flora through heterogeneous microhabitat caused by species composition and structure18-20. Thus, understory species
composition differs markedly among forests\textsuperscript{21,22}. Previous research found forest canopy controls understory flora by modifying understory environments and resource availability by competing with understory plants for resources both above and below ground\textsuperscript{23–25}. Giesbrecht et al. stated that overstory structure drives fine-scale spatial structure in the understory plant community by light transmission\textsuperscript{26}. Studies have demonstrated a significant relationship between understory flora and soil–chemical gradient modified by canopy cover\textsuperscript{27,28}. For instance, Bartels and Chen\textsuperscript{29} asserted that increasing overstory broad-leaved tree abundance increased the richness of herbaceous-layer plants via its impact on soil nutrients. In addition, topography partially influences microenvironment by controlling the spatial redistribution of light, temperature, water availability, and soil nutrients and, thereby, affects understory flora. In hilly regions, slope aspect exerts an especially strong influence on understory flora, with northeast-facing plots exhibiting higher values of species richness and Hill’s diversity than southwest-facing plots\textsuperscript{30,31}. The spatial patterns of understory flora are also closely associated with slope aspect\textsuperscript{12,23}. In comparison, other studies have shown that the richness and diversity of understory species vary with elevation, not with slope aspect\textsuperscript{14,32}.

The karst landforms of China are mainly distributed in the southwestern region, where the ecological environment is fragile and exhibits a low resistance to interference. Intensive human activity, combined with the distinct geological background, is leading to the continuous degradation of forests to shrubland and grassland. In some karst areas, rocky desertification or seasonal rocky desertification has appeared, with large areas of bare bedrock or block accumulation. The natural vegetation is sparse in karst rocky desertification area, and the soil and water loss is quite serious. Therefore, the ecological security of this area has been severely affected, so ecosystem restoration and reconstruction of the vegetation is urgent. Many studies have focused on the spatial patterns of forest communities, and the vegetation dynamics and restoration in this region\textsuperscript{33–38}. The current study was carried out in Pingguo County, southwest Guangxi, China. There is a large area of typical karst landforms, with an appropriate area of rocky desertification. As a result of many years of ecological conservation, there has been a noticeable increase in forest coverage. During this period of ecological conservation, the economic value of ecological restoration and soil quality under different vegetation restoration models were studied\textsuperscript{39,40}. Some studies demonstrated that the spatial patterns and diversity of woody plant species were significantly affected by soil and topographic factors\textsuperscript{41,42}. Understory is very important for soil and water conservation in karst region, but few studies have addressed the understory in this area. Whether the factors affecting arbor layer also impact the understory, and how the environmental factors systematically interpret the species diversity variation of understory? Knowledge about it in this area is still lacking. Therefore, we examined understory vascular plant communities under three types of overstory canopy (shrubland, forest–shrub transition, and mixed-species broadleaf forest) in a typical karst ecosystem and attempted to elucidate the effects of vegetation types and environmental factors (soil nutrients and topography) on the understory. Our specific objectives were to: (1) detect whether and how vegetation type controls the composition and distribution of understory flora and (2) assess the relative importance of soil properties and topographic features in regulating the understory flora. We expect that our findings will provide valuable information for understanding the interaction mechanism between understory flora and karst environment in the karst areas.

Materials and methods

Study area. The study was carried out in the karst mountains of Pingguo County (23° 12′–23° 51′ N, 107° 21′–107° 57′ E), located in southwestern Guangxi, China. The landforms of Pingguo are mainly composed of karst, where the vegetation very easily degrades, leading to rocky desertification. In 2014, areas without obvious rocky desertification in Pingguo County only accounted for 18.47% of the total karst area\textsuperscript{43}. The climate of the area is of the south subtropical monsoon. Annual average temperature ranges from 19 to 21.5°C, and annual mean precipitation ranges from 1,200 to 1,500 mm mainly occurs from May to September\textsuperscript{41}. The soil has been identified as brown and black limestone soil. Due to a long history of human interference, the local vegetation has deteriorated across large areas. The prevailing vegetation types are shrub–grassland, shrubland, open forest, and secondary forest. The shrubland is dominated by Vitex negundo, Cipadessa cinerascens, and Alchornea trewioides. The remaining secondary forests are patchily distributed near villages, and the tree layer is mostly dominated by Excentrodendron hsiennum, with common species including Sapium rotundifolium, Ficus concinna, Choerospondias axillaris, and Walsura robusta.

Sampling. In 2012, we established 32 plots of 20 × 20 m, of which 9 plots were established in shrubland, 11 plots were in forest–shrub transition forest (forest–shrub), and 12 plots were in mixed-species broadleaf secondary forest (mixed-forest). Field investigations were carried out in May and June 2012. Woody plants with DBH (diameter at breast height at 1.3 m, measured to 0.1 cm accuracy) ≥1.0 cm and height ≥1.5 m were defined as stand tree. In each plot, the DBHs and heights of stand trees were recorded. The details of canopy characteristics are found in Table 1. Understory plants with heights < 1.5 m were censused inside five 2 × 2 m quadrats, with one in the centre and four at the corners of the plot. In each quadrat, data were collected regarding the number of vascular plants, height, and coverage by species. Data from each quadrat were pooled for the plot-level analyses. Topographic factors were recorded, including the ratio of rock bareness (Rock), elevation (Elv), slope degree (Degree), slope aspect (Aspect), and slope position (Position).

Because the soil is shallow in the karst mountains, soil samples were only collected at a depth of 0–15 cm at eight to ten random locations along an S-shaped transect in each plot. Samples were mixed to obtain approximately 1 kg per 20 × 20 m plot for subsequent chemical analysis. The soil samples were air-dried in the laboratory and sieved through a 0.25-mm mesh screen. The soil was tested at the Soil and Research Division of the Guangxi Forestry Research Institute, China, following the methods prescribed in Bao\textsuperscript{44}. Soil pH value was measured with a acidity meter; soil organic matter (SOM) was measured by using potassium dichromate oxidation-outer
heating; total nitrogen (TN) and available nitrogen (AvN) were measured by using distillation method, total phosphorus (TP) was analysed by using NaOH melting-molybdate antimony anti color development-UV Spectrophotometry method; available phosphorus (AvP) was measured by using 0.5 mol L⁻¹ NaHCO₃ extraction-molybdate antimony anti color development-UV Spectrophotometry method; total potassium (TK) was measured by using NaOH melting-flame photometry method; available potassium (AvK) was analysed by using NH₄OAc extraction-flame photometry method, and exchangeable calcium (Ca) was determined by using atomic absorption spectrophotometry method.

**Data analysis.** Slope aspect was divided into four slope directions: (1) shaded slope (0°–45° and 315°–360°); (2) half-shaded slope (45°–90° and 270°–315°); (3) half-sunny slope (90°–135° and 225°–270°); and (4) sunny slope (135°–225°). Defined values were assigned to the qualitative variables, aspect and position. The sunny slope was assigned 0.3, the half-sunny slope was assigned 0.5, the half-shaded slope was assigned 0.8, and the shady slope was assigned 1.0. The top of the slope was assigned 0.4, the mid-slope was assigned 1.0, and the downslope was assigned 0.8.

The number of individual understory plants per plot and the Shannon–Wiener diversity (α-diversity) and its components (i.e., species richness (S), Shannon–Wiener index (H'), and evenness) were used to describe vegetation biodiversity. These indices were calculated with PC-ORD version 5.0⁹, using the following equations:

\[ N = \sum N_i; \]  
\[ H' = -\sum_{i=1}^{S} \ln p_i \]  
\[ E = H' / \ln s \]

where \( N_i \) is the number of individual understory plants per plot, \( S \) is the species richness, and \( p_i \) is the relative abundance of species \( i \), calculated as the number of plants of species \( i \) as a proportion of the total number of plants of all species in the \( j \)th plot.

Duncan's multiple comparison tests were used to test for differences in overstory and understory characteristics among the three vegetation types. Pearson correlation analysis was used to detect relationships between environmental factors and overstory and understory flora. Differences in understory flora species composition were tested with the means of the pair-wise multi-response permutation procedure (MRPP), using a presence/absence species data matrix with rank transformed Sorensen (Bray–Curtis) distance.
|                      | O-density | O-S | O-E | O-H | O-height | O-DBH | U-individual | U-S | U-E | U-H |
|----------------------|-----------|-----|-----|-----|----------|-------|--------------|-----|-----|-----|
| Soil pH              | −0.29     | 0.20| 0.31| 0.34| 0.42*    | 0.44* | 0.11         | 0.41*| −0.16| 0.08|
| Soil organic matter  | −0.20     | −0.18| −0.15| −0.20| 0.29     | 0.39* | −0.41*       | −0.30| 0.04 | −0.12|
| Total nitrogen       | −0.26     | −0.10| −0.08| −0.11| 0.38*    | 0.48**| −0.39*       | −0.21| −0.02| −0.12|
| Total phosphorus     | −0.14     | 0.04| −0.17| −0.05| 0.08     | 0.22  | −0.09        | 0.02 | 0.10 | 0.11|
| Total potassium      | −0.42*    | 0.16| 0.06| −0.03| 0.44*    | 0.49**| −0.05        | 0.07 | 0.09 | 0.10|
| Available nitrogen   | −0.32     | 0.03| 0.07| 0.02 | 0.54**   | 0.60**| −0.36*       | 0.06 | 0.10| 0.04|
| Available phosphorus | −0.38*    | −0.27| −0.09| −0.21| 0.36*    | 0.42* | −0.38*       | −0.23| 0.23 | 0.04|
| Available potassium  | −0.41*    | 0.11| 0.03| 0.02 | 0.44*    | 0.45**| −0.20        | 0.10 | 0.15 | 0.16|
| Exchangeable calcium | −0.03     | −0.33| −0.35*| −0.40*| 0.31     | 0.39* | −0.19        | −0.29| 0.03 | −0.13|
| Ratio of rock barreness| −0.42* | 0.13| 0.29| 0.25 | 0.51**   | 0.57**| −0.12        | 0.16 | 0.00 | 0.07|
| Slope degree         | 0.39*     | 0.24| 0.33| 0.34 | 0.31     | 0.26  | 0.08         | 0.38*| 0.55**| 0.49**|
| Slope position       | 0.20      | 0.04| −0.28| −0.11| −0.18    | −0.09 | 0.25         | 0.21 | 0.09 | 0.19|
| Elevation            | 0.26      | −0.13| −0.26| −0.26| −0.47**  | −0.45**| −0.34        | −0.50**| 0.14 | −0.15|
| U-individual         | 0.19      | 0.12| 0.08| 0.16 | −0.31    | −0.21 |             |      |      |      |
| U-S                  | −0.03     | 0.54**| 0.34| 0.55**| 0.29     | 0.24  |             |      |      |      |
| U-E                  | −0.05     | 0.18| 0.03| 0.10 | 0.31     | 0.15  |             |      |      |      |
| U-H                  | 0.04      | 0.48**| 0.17| 0.39* | 0.32     | 0.18  |             |      |      |      |

Table 2. Correlation coefficients between environmental factors and characteristics of overstory and understory. O-density means overstory tree density; O-S means overstory species richness; O-E means overstory evenness; O-H means overstory Shannon–Wiener index; O-height means overstory tree height; O-DBH means diameter at breast height of overstory; U-individual means number of individuals in understory; U-S means understory species richness; U-E means understory evenness; U-H means understory Shannon–Wiener index. *P < 0.05; **P < 0.01.

Results

Characteristics of understory flora, soil, and topographic properties in the three vegetation types. A total of 186 understory plants, belonging to 147 genera and 76 families, were recorded. Among these plants, 91, 85, and 138 species were recorded in shrubland, forest–shrub, and mixed forest, respectively. The forest–shrub had the fewest species but the highest value for species diversity (i.e., α-diversity index; Table 1). The mixed forest contained the most species, while the shrubland had the lowest value for the α-diversity index.

Soil pH and TK were significantly higher in the mixed forest than the other two vegetation types. SOM, TN, and AvN were significantly lower in the shrubland than the other two vegetation types. Soil AvP and Ca were significantly higher in the mixed forest than in the shrubland, and soil AvK was significantly higher in the shrubland and the mixed forest than in the forest–shrub. However, soil TP did not significantly differ among the three vegetation types. The mixed forest showed the highest value for Rock, and the forest–shrub exhibited the highest value for Degree. Of note, the observed forest–shrub was distributed at higher elevation.

Correlations of environmental factors with canopy characteristics and understory flora. Overstory tree density (O-density) was significantly negatively correlated with TK, AvP, AvK, and ratio of rock barreness. Exchangeable calcium was significantly negatively correlated with overstory evenness (O-E) and Shannon diversity (O-H) (Table 2). Except for SOM, TP, and exchangeable calcium, significantly positive correlations of environmental variables with overstory mean height were observed, and significantly positive correlations between overstory mean DBH and soil properties (except for SOM and TP) were also found. Overstory species richness was significantly positively related to understory species richness and Shannon diversity, and the relationships of overstory Shannon diversity with understory species richness and Shannon diversity were also significantly positive. Soil parameters, i.e., SOM, TN, AvN, and AvP, were significantly negatively correlated with individual numbers of understory (U-individual), but the correlation between understory species richness (U-S) and soil pH was significantly positive (Table 2). In addition, the slope aspect was significantly positively related.
to understory species richness (U-S), evenness (U-E), and Shannon diversity (U-H), but a significantly negative correlation between elevation and understory species richness (U-S) was found (Table 2).

**Effect of vegetation type on understory flora.** MRPP showed that the three vegetation types were significantly different \( (P < 0.0001) \) from one another with respect to understory species composition, with the greatest difference being detected between the shrubland and the mixed forest (Table 3). Overall, the foreset–shrub was more similar than the shrubland in species composition to the mixed forest (Table 3).

Of the 186 understory species, ISA identified 21 species as significant indicators. The three communities had different indicator species. Thirteen species were identified as indicators of the shrubland, two of the forest–shrub, and six of the mixed forest (Table 4). Indicators of the shrubland were mainly herbaceous seed plants, while those of the mixed forest were mainly ferns.

**Controls of soil and topographic factors on understory flora.** For the CCA ordination, a Monte Carlo permutation test showed that the eigenvalues for the first and those for all canonical axes clearly achieved statistical significance \( (P < 0.01) \), indicating the soil and topography had significant comprehensive effects on the distribution of understory plants. The eigenvalues of the four axes were 0.982, 0.857, 0.764, and 0.698. The coefficients describing the species-environment correlations were 0.999, 0.981, 0.988, and 0.970. The four axes in the CCA ordination explained 50.7% of the variance in the relationship between understory flora and the environmental variables. The first axis was strongly and significantly correlated to Elv, followed by soil pH, TK, Ca, AvK, slope degree, slope aspect, rock bar, and AvP. The second axis was strongly related to AvN, followed by TN, TP, and SOM (Table 5, Fig. 1). Because the eigenvalues of the first two axes were greater than those of the others, we considered factors that were highly correlated with the first two axes to have greater effects on the understory flora.

---

**Table 3.** Summary of multi-response permutation procedure tests on similarity in understory species composition among contrasting forest patch types. \(^a\) Separation among groups by Sorensen (Brey-Curtis) distance; separation strengthens with more negative values. \(^b\) Within-group similarity; the value ranges from 0 to 1, 1 indicating identical items within the group. \(^c\) Significance level of the corresponding \( T \)-statistic.

| Forest patch types pair-wise comparison | \( T \) statistic\(^a\) | \( A \) value\(^b\) | \( P \) value\(^c\) |
|----------------------------------------|--------------------------|----------------|----------------|
| Shrubland vs. forest-shrub transition  | − 6.42                   | 0.071          | < 0.0001       |
| Shrubland vs. mixed-species broadleaf forest | − 7.24                  | 0.072          | < 0.0001       |
| Forest-shrub transition vs. mixed-species broadleaf forest | − 5.01                  | 0.041          | < 0.0001       |

**Table 4.** Indicator values (IV) of major species in the understory per vegetation type.

| Forest patch type                      | Species                  | Value (IV) | \( P \) value |
|----------------------------------------|--------------------------|------------|--------------|
| Shrubland                              | Apluda mutica            | 42.3       | 0.013        |
|                                         | Barleria cristata        | 33.3       | 0.015        |
|                                         | Bauhinia corymbosa       | 44.4       | 0.001        |
|                                         | Caryopteris incana      | 33.3       | 0.018        |
|                                         | Chromolaena odorata      | 63.0       | 0.000        |
|                                         | Cratoxylum cochinichene  | 55.6       | 0.000        |
|                                         | Cudrania tricuspidata    | 44.4       | 0.002        |
|                                         | Mallotus philipensis     | 33.3       | 0.016        |
|                                         | Microstegium nodosum     | 62.3       | 0.000        |
|                                         | Miscantus floridulus     | 33.3       | 0.015        |
|                                         | Phyllanthus emblica      | 48.3       | 0.003        |
|                                         | Senecio scandens         | 37.4       | 0.015        |
|                                         | Viburnum proinquum       | 39.9       | 0.036        |
| Forest-shrub transition                 | Lepionurus sylvestris    | 50.4       | 0.002        |
|                                         | Ulmus parvifolia         | 47.3       | 0.003        |
| Mixed-species broadleaf forest          | Asplenium sumpsoni       | 34.2       | 0.021        |
|                                         | Asplenium saxicola       | 33.3       | 0.021        |
|                                         | Boehmeria nivea          | 63.1       | 0.000        |
|                                         | Laportea violacea        | 51.2       | 0.003        |
|                                         | Nephrolepis auriculata   | 51.2       | 0.003        |
|                                         | Streblus tonkinensis     | 33.3       | 0.025        |
Forward selection of the variables showed that Elv had the greatest explanatory power, followed by soil pH, TK, Ca, Aspect, Degree, and AvK, showing that the distribution of understory species was primarily affected by topography (Table 6).

The pure and overlapping effects of soil and topographic variables were shown in Table 7. The soil and topographic variables jointly explained 52.53% of the variation in understory plant distribution, of which 30.23%

### Table 5. Correlation coefficients of soil and topographic factors for the first four species axes. Eigenvalues and cumulative percentages of variance were explained by canonical correspondence analysis ordinations. *P < 0.05; **P < 0.001.

|                  | Axis 1     | Axis 2     | Axis 3     | Axis 4     |
|------------------|------------|------------|------------|------------|
| Soil pH          | 0.933**    | 0.093      | 0.003      | 0.069      |
| Soil organic matter | −0.294    | 0.520**    | −0.168     | 0.276      |
| Total nitrogen   | 0.039      | 0.623**    | −0.178     | 0.267      |
| Total phosphorus | −0.169     | 0.566**    | −0.251     | 0.002      |
| Total potassium  | 0.849**    | 0.054      | −0.119     | −0.027     |
| Available nitrogen | 0.135     | 0.630**    | 0.085      | 0.213      |
| Available phosphorus | −0.628**  | 0.016      | 0.066      | −0.136     |
| Available potassium | 0.758**   | −0.136     | −0.123     | −0.013     |
| Exchangeable calcium | 0.812**   | 0.201      | 0.086      | 0.164      |
| Ratio of rock bareness | 0.652**  | 0.059      | −0.138     | 0.081      |
| Slope degree     | 0.675**    | 0.445**    | −0.158     | 0.259      |
| Slope aspect     | 0.667**    | 0.429*     | 0.354*     | −0.221     |
| Slope position   | 0.410*     | 0.330      | −0.402*    | −0.154     |
| Elevation        | −0.968**   | 0.132      | −0.073     | 0.094      |
| Eigenvalues      | 0.982      | 0.857      | 0.764      | 0.698      |
| Species-environment correlations | 0.999 | 0.981 | 0.988 | 0.970 |
| Cumulative percentage variance |
| Of species data | 7.9 | 14.8 | 21 | 26.6 |
| Of species-environment relation | 15.1 | 28.2 | 40 | 50.7 |

**Figure 1.** The canonical correspondence analysis (CCA) ordination of 32 plots and environmental factors. Arrows indicate the environmental variables [soil pH, soil organic matter (SOM), total nitrogen (TN), available nitrogen (AvN), total phosphorus (TP), available phosphorus (AvP), total potassium (TK), available potassium (AvK), exchangeable calcium (Ca), the ratio of rock bareness (Rock), elevation (Elv), slope degree (Degree), slope aspect (Aspect), and slope position (Position)]. Plots are identified by vegetation types as shrubland (circle), forest-shrub transition (square), and mixed forest (triangle).
and 17.73% were explained by pure soil factors and pure topographic variables, respectively. Overlapping effect between soil factors and topographic variables was 4.57%. The residual fraction was 47.47%.

### Discussion

The canopy of a plant community determines the understory flora over a large part of the area. Many studies have found that stand types have significant effects on floristic composition, species diversity, and cover of understory vegetation. The three vegetation types investigated significantly differed in overstory tree composition, canopy structure, and selected soil variables except for TP (Table 1). Meanwhile, the canopy plant diversity was significantly correlated with that of the understory (Table 2). The shrubland had not yet closed for the lowest height and DBH. Light-demanding Gramineous and Compositae plants, i.e. *Apluda mutica*, *Microstegium nodosum*, *Miscanthus floridulus*, and *Chromolaena odoratum*, were common and dominant in the shrubland (Table 4). The crown density of forest–shrub transition community was larger than that of shrubland with higher height and DBH. Shade-tolerant shrub, *Lepionurus sylvestris*, appeared in the understory, companying with light-demanding species, *Ulmus parvifolia* (Table 4). The canopy of mixed forest was dense with the highest height and DBH (Table 1). Shade-tolerant ferns, i.e. *Asplenium sampsoni* and *Asplenium saxicola* etc., were common in the understory and were detected as the indicators of this community. The result reveals the contrasting canopy types may control the understory species composition by light regimes.

In this study, the species richness and α-diversity of the overstory usually increased from the shrubland to the forest (Table 1). Community structure became more complex and provided more diverse habitats for understory plants occupying different niches; thus, the understory species richness very markedly increased with increasing overstory species richness and α-diversity, with the mixed forest having the most understory species. The understory α-diversity also significantly increased, with the greatest α-diversity index being detected in the forest–shrub (Table 1, Table 2). In a karst ecosystem in China, the species diversity rose steadily with community succession, where the vine-shrub community had the most abundant species, and the highest α-diversity index was detected in secondary forests, and then the diversity decreased in old-growth evergreen broad-leaved forest. Plant diversity generally increases with succession and tends to be exceptionally low in oldfield ecosystems when there is strong species dominance. Our results demonstrate that plant diversity tends to increase with direct community succession from the shrubland to the forest, but the communities have not yet reached steady stages. The mixed forest supported the most species in the overstory and understory; it plays a key role in maintaining plant diversity in the study area, so it is very necessary to take measures to protect the mixed forests in the degraded karst areas.

The forest canopies also support different understory flora by modifying the environment. Studies concluded that forest types controlled the understory flora by influencing soil nutrient availability. In addition, topographies also are important factors affecting plant distribution. Several studies explored the effect of topographical
variables on plant spatial patterns in the karst areas of China, but only few focus on the coupling effect of soil and topographies on understory flora. Our study detected that canopy structure influenced on understory flora through affecting on soil pH, SOM, TN, AvN, and AvP, and elevation and slope aspect were also significantly related to understory structure and understory species diversity. CCA revealed that elevation was the most important factor correlated with the variation in understory species distribution, followed by soil pH, TK, and Ca (Table 6), it also significantly negatively correlated with soil pH, TK, and Ca (Table 5). The shrubland was mainly distributed at higher altitude region, and light-demanding herbaceous seed plants were common in this community. Karst calcicolous trees, such as E. hisenmu, S. rotundifolium, and Lysidice rhodostegia, assembled at lower altitude with soil rich Ca content to a dense mixed-species broadleaf forest. Light-demanding understory species disappeared from this community for weak light condition, but shade-tolerant calcicole, i.e. Asplenium saxicola, Asplenium sampsoni and Streblus tonkinensis assembled under the canopy. Our results reveals that elevation controls the spatial distribution of vegetation and soil factors, and then the understory plants, and soil Ca content is another important factor affecting the distribution of understory plants. The soil Ca content of karst ecosystem is higher than that of non-calcareous area based materially on carbonate rock. The adaptation of calcicole to high Ca content limestone soil needs to be studied to detect the formation of plant habitat diversity. However, CCA ordination can only explain 50.7% of the variance in the understory flora, indicating that other factors also affect the understory flora and need to be further studied.

Conclusion
Overall, the three vegetation types were significantly different (P < 0.0001) in understory species composition. Twenty-one understory species were identified as indicator species. Indicators of the shrub were mainly light-demanding herbaceous seed plants, while those of the mixed forest were mainly shade-tolerant calcicoles. Vegetation types controlled understory species composition by modifying understory environments. Elevation controlled the spatial distribution of vegetation and soil properties, and then the understory plants. Meanwhile, soil Ca content was also important for the understory species distribution. In view of the importance of understory for soil, water and biodiversity conservation in karst region, and the investigated communities have not yet reached a steady state and easily degenerate, it is critical to protect the mixed forest and take measures to promote the vegetation restoration in the study area.

Data availability
The datasets generated and/or analysed in the current study are available from the corresponding author on reasonable request.

Received: 23 June 2019; Accepted: 15 June 2020
Published online: 21 July 2020

References
1. Gilliam, F. S. The ecological significance of the herbaceous layer in temperate forest ecosystems. *Bioscience* **57**, 845–858 (2007).
2. Cervellini, M. et al. Relationships between understory specialist species and local management practices in coppiced forests—evidence from the Italian Apennines. *For. Ecol. Manag.* **385**, 35–45 (2017).
3. Hamelin, C., Cagnon, D. & Truax, B. Exotic invasive shrub glossy buckthorn reduces restoration potential for native forest herbs. *Sustainability* **9**, 1–13 (2017).
4. Lü, Y. L., Wang, S. L. & Yan, S. K. Short-term effects of understory vegetation removal on nutrient cycling in litter layer of Chinese fir plantation. *Chin. J. Appl. Ecol.* **22**, 2560–2566 (2011) (in Chinese with English abstract).
5. Yazdanshenas, H., Kalagar, M. & Toularoud, M. M. Understory plant species diversity of Asalem's forests, northern Iran. *For. Res. Eng. Int. J.* **3**, 56–62 (2019).
6. Li, Y. L., Wang, S. L. & Yan, S. K. Short-term effects of understory vegetation removal on nutrient cycling in litter layer of Chinese fir plantation. *Chin. J. Appl. Ecol.* **22**, 2560–2566 (2011) (in Chinese with English abstract).
7. Yang, Y. et al. Mechanism of litter and understory vegetation effects on soil carbon and nitrogen hydrolase activities in Chinese fir forests. *Acta Ecol. Sin.* **36**, 8102–8110 (2016) (in Chinese with English abstract).
8. Berkes, F. A.R., Canham, C. D. & Kelly, V. R. Competition vs. facilitation of tree seedling growth and survival in early successional communities. *Ecology* **76**, 1156–1168 (1995).
9. Padilla, F. M. & Pugnaire, F. I. The role of nurse plants in the restoration of degraded environments. *Front. Ecol. Environ.* **4**, 196–202 (2006).
10. Feng, Q. H. et al. Effects of density adjustment on ground cover and soil hydrological function of *Picea asperata* plantation in the subalpine region of western Sichuan Province, China. *J. Nanjing For. Univ. For. Sci. Ed.* **42**, 98–104 (2018) (in Chinese with English abstract).
11. Rasingam, L. & Parthasarathy, N. Diversity of understory plants in undisturbed and disturbed tropical lowland forests of Little Andaman Island, India. *Biodiv. Conserv.* **18**, 1045–1065 (2009).
12. Boonstra, R., Krebs, C. J. & Cowxill, K. Responses of key understory plants in the boreal forests of western North America to natural versus anthropogenic nitrogen levels. *For. Ecol. Manag.* **401**, 45–54 (2017).
13. Ou, Z. Y., Su, Z. Y., Ye, Y. C., Zhu, J. Y. & Liu, S. S. Ground vegetation as indicators of topsoil chemical properties in Dongguan, South China. *Acta Ecol. Sin.* **29**, 984–992 (2009) (in Chinese with English abstract).
14. Su, Z. Y., Ke, X. D. & Zhang, S. J. Vascular plants as indicators of organic carbon gradient in subtropical forested soil. *Pol. J. Environ. Stud.* **21**, 1393–1398 (2012).
15. Dolan, B. & Kilgore, J. Forest regeneration following emerald ash borer (*Agrilus planipennis* Fairenna) enhances mesophication in eastern hardwood forests. *Forests* **9**, 353–366 (2018).
16. Zhang, J. W., Young, D. H., Oliver, W. W. & Fiddler, G. Effect of overstory trees on understory vegetation in California (USA) ponderosa pine plantations. *Forest. Int. J. Forest Res.* **89**, 91–99 (2016).
17. Curzon, M., Baker, S., Kern, C., Palik, B. J. & D’Amato, A. W. Influence of mature overstory trees on adjacent 12-year regeneration and the woody understory: Aggregated retention versus intact forest. *Forests* **8**, 31, https://doi.org/10.3390/f8020031 (2017).
18. Adám, R., Ódor, P. & Bölöni, I. The effects of stand characteristics on the understory vegetation in *Quercus petraea* and *Q. cerris* dominated forests. *Commun. Ecol.* **14**, 101–109 (2013).
19. Navrroud, B. R., Vajari, K. A., Piliehrav, B. & Kooch, Y. Interactions between tree and herb layers vegetation along a gradient of tree composition in Hyrcanian forests. Russ. J. Ecol. 46, 483–486 (2015).

20. Mestre, L. et al. The influence of canopy-layer composition on understory plant diversity in southern temperate forests. For. Ecosyst. 4, 6. https://doi.org/10.1186/s40663-017-0993-z (2017).

21. Yu, M. & Sun, O. J. Effects of forest patch type and site on herb-layer vegetation in a temperate forest ecosystem. Forest Ecology and Management 1300, 14–20 (2013).

22. Hsu, H., Peng, Q. & Su, Y. H. The influence of canopy species and topographic variables on understory species diversity and composition in coniferous forests. Sci. World J. https://doi.org/10.1155/2014/252489 (2014).

23. Hicks, D. J. & Taylor, M. S. Effects of Aesculus glabra canopy on understory community structure and environment in a temperate deciduous forest. Castanea 80, 8–19 (2015).

24. Riegel, G. M., Miller, R. F. & Krueger, W. C. Competition for resources between understory vegetation and overstory vegetation in northeastern Oregon. Ecol. Appl. 2, 71–85 (1992).

25. Barbier, S., Gosselin, F. & Balandier, P. Influence of tree species on understory vegetation diversity and mechanisms involved—a critical review for temperate and boreal forests. For. Ecol. Manag. 254, 1–15 (2008).

26. Giesbrecht, I. J. W., Saunders, S. C., MacKinnon, A. & Lertzman, K. P. Overstory structure drives fine-scale coupling of understory light and vegetation in two temperate rainforest floodplains. Can. J. For. Res. 47, 1244–1256 (2017).

27. Mataji, A. et al. Understory vegetation as environmental factors indicator in forest ecosystems. Int. J. Environ. Sci. Tech. 7, 629–638 (2010).

28. Warren, R. J. Mechanisms driving understory evergreen herb distributions across slope aspects: As derived from landscape position. Plant Ecol. 198, 297–308 (2008).

29. Song, T. Q. et al. Spatial pattern of forest communities and environmental interpretation in Mulun National Nature Reserve, karst cluster-peak depression region. chin. J. Plant Ecol. 34, 298–308 (2010) (in Chinese with English abstract).

30. Liu, Y. G., Liu, C. C., Wei, Y. F., Liu, Y. G. & Guo, K. Species composition and community structure at different vegetation successional stages in Puding, Guizhou Province, China. chin. J. Plant Ecol. 35, 1009–1018 (2011) (in Chinese with English abstract).

31. Ou, Z. Y., He, B. Y. & Kou, J. F. Landsat thermal remote sensing to investigate the present situation and variation characteristics of karst rocky desertification in Pingguo County of Guangxi, Southwest China. Univ. For. Technol. 30, 60–66 (2016) (in Chinese with English abstract).

32. Ou, Z. Y. et al. Effect of soil fertility and topographic factors on woody plant communities in the karst mountains of Southwest Guangxi, China. Acta Ecol. Sin. 34, 3672–3681 (2014) (in Chinese with English abstract).

33. Ou, Z. Y., Zhu, J. Y., Peng, Y. H., He, Q. F. & Pang, S. L. Relationship between plant diversity and environmental factors of Excentrodendron hsiennu community in karst mountainous areas of Guangxi. chin. J. Cent. South Univ. For. Technol. 36, 60–66 (2016) (in Chinese with English abstract).

34. Liu, Y., He, B. Y. & Kou, J. F. Landsat thermal remote sensing to investigate the present situation and variation characteristics of karst rocky desertification in Pingguo County of Guangxi, Southwest China. Sci. Soil Water Conserv. 15, 125–131 (2017) (in Chinese with English abstract).

35. Bao, S. D. The Agro-Chemical Analysis of Soil (China Agriculture Press, Beijing, 2000) (in Chinese).

36. McCune, B. & Meford, M. J. PC-ORD. Multivariate Analysis of Ecological Data (Version 5) (MjM Software Design, Oregon, 2006).

37. Ister, S. I. & Golubukal, F. Effect of stand types on understory vegetation. J. Environ. Biol. 30, 595–600 (2009).

38. Légare, S., Bergeron, Y. & Paré, D. Influence of forest composition on understory cover in boreal mixedwood forests of western Quebec. Silva Fenn 36, 353–366 (2002).

39. Hameed, M. A. et al. Influence of planting type on ground flora composition and diversity in Gatwa artificial forest plantation. Pak. J. Bot. 43, 1867–1872 (2011).

40. Sagar, R., Singh, A. & Singh, J. S. Differential effect of woody plant canopies on species composition and diversity of ground vegetation: A case study. Trop. Ecol. 49, 189–197 (2008).

41. Shi, B., Yao, X. H. & Ben, H. D. Species composition and diversity in the process of natural succession of Karst vegetation in Central Guizhou: Case study of Puding Country in Guizhou. For. Res. 21, 669–674 (2008) (in Chinese with English abstract).

42. Bazzaz, F. A. Plant species diversity in old-field successional ecosystems in southern Illinois. Ecology 56, 485–488 (1975).

43. Xia, D. G. & Burdman, M. A. Influences of edaphic factors on the distribution and abundance of a rare palm (Cyrtoistachys renda) in a peat swamp forest in eastern Sumatra, Indonesia. Austral. Ecol. 31, 964–974 (2006).

44. Zhang, Z. H., Hu, G., Zhu, J. D. & Ni, J. Spatial heterogeneity of soil nutrients and its impact on tree species distribution in a karst forest of Southwest China. chin. J. Plant Ecol. 35, 1038–1049 (2011) (in Chinese with English abstract).

45. Song, T. Q. et al. Community composition and biodiversity characteristics of forests in Karst cluster-peak-depression region. Biodivers. Sci. 18, 355–364 (2010) (in Chinese with English abstract).

46. Ou, Z. Y. et al. Coupling relationships between woody plants in Excentrodendron hsiennu community and related edaphic and topographic factors. chin. J. Ecol. 32, 3182–3189 (2013) (in Chinese with English abstract).

47. Tan, Y. B. et al. Effect of environmental factors on understory species diversity in Southwest Guangxi Excentrodendron tonkinense forests. Biodivers. Sci. 27, 970–983 (2019) (in Chinese with English abstract).

Acknowledgements

This project was supported by the Guangxi Science and Technology Plan Project (AB16380300) and the Fundamental Research Funds of the Guangxi Forestry Research Institute (2018131).
Author contributions
Z.O. designed the research and wrote the paper; S.P., Q.H., Y.P., X.H., and W.S. conducted the field survey and analysed the data.

Competing interests
The author declares no competing interests.

Additional information
Correspondence and requests for materials should be addressed to Z.O.

Reprints and permissions information is available at www.nature.com/reprints.

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons license, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons license and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this license, visit http://creativecommons.org/licenses/by/4.0/.

© The Author(s) 2020