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Links between plant diversity, carbon stocks and environmental factors along a successional gradient in a subalpine coniferous forest in Southwest China

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ABSTRACT

In all, 48 sites of subalpine coniferous forest that had undergone natural regeneration for 5–310 years were selected as study locations in the Southwest China. We compared species richness (S), plant diversity (Shannon–Wiener index, H'; Margalef index, R), and above- and below-ground ecosystem carbon (C) pools of six plant communities along a chronosequence of vegetation restoration, and we also examined evidence for a functional relationship between plant diversity and C storage. Our results showed that above-ground C increased significantly (over 52-fold), mainly due to the increase of C in aboveground living plants and surface litter. Soil organic carbon (SOC) content increased from the herb community type (dominated by *Deyeuxia scabrescens*, P1) to mixed forest type (dominated by *Betula* spp. and *Abies faxoniana*, P4), which constituted the main C pool of the system (63–89%), but decreased thereafter (communities P5–P6). The mean C stock in the whole ecosystem – trees, litter layer and mineral soil – ranged from 105 to 730 Mg C ha^{-1} and was especially high in the spruce forest community type (dominated by *Picea purpurea*, P6). On the other hand, the relationships between C stocks (soil, aboveground) and mean annual temperature or altitude were generally weak ($P > 0.05$). Moreover, we did not detect a relationship between S and aboveground C storage, while we found a significant negative relationship between H', R and aboveground C storage. In addition, our experiment demonstrated that total root biomass and litter C/N ratio were significant functional traits influencing SOC, while S, R, and H' had little effect. Path analysis also revealed that litter C/N ratio predominantly regulated SOC through changes in the quantity of microorganisms and soil invertase enzyme activity.

1. Introduction

Conservation of biodiversity and mitigation of global warming are two major environmental challenges nowadays. Forest biomass and soils are considered to have a large potential for temporary and long-term carbon (C) storage (Houghton, 2005; Peichl and Arain, 2007). Enhancing C sequestration by increasing forested land area has been suggested as an effective measure to mitigate elevated atmospheric CO_2 concentrations and hence to contribute towards the prevention of global warming (Watson, 2000). However, there has been some disagreement about whether an increase in soil C may be achieved through forests (Peichl and Arain, 2007). Environmentalists have also become interested in potential functional relationships between plant diversity and C storage. There is evidence that plant assemblages with a high species diversity may promote a more efficient use of resources and greater net primary production (Vandermeer, 1989) and, consequently, a higher rate of C sequestration (Catovsky et al., 2002; Kirby and Potvin, 2007) compared with sites with a lower species diversity. So far, only few investigations have indicated a positive impact of plant diversity on C sequestration in soils (Fornara and Tilman, 2008).

It has been argued, however, that the results achieved in experimental studies may have little predictive value in natural ecosystems. Therefore, Loreau (2000) has suggested that the scope of studies on biodiversity and ecosystem functioning should be expanded to natural ecosystems. On the other hand, such studies are equivocal, mainly because in natural ecosystems differences in diversity are often confounded with land use history or abiotic parameters. C stocks are sensitive to a range of factors, including climate, topography, soil and vegetation management, and other anthropogenic conditions. Meanwhile, changes in the C storage and diversity may be related to different stages of succession. Several studies have estimated differences in soil C stocks in relation to vegetation and topography, land use and climate (Lemenih and Itanna, 2004; Li et al., 2010), but only few studies have evaluated how these environmental factors affect C store at different temporal scales. The best way to examine the relationship between diversity and C stock could be to use succession data, since succession (rather than normal community fluctuation) usually covers broad ranges of

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diversity, biomass and other community variables. Nevertheless, data collected for both diversity and C storage that simultaneously span an entire successional sequence are particularly rare.

Subalpine coniferous forest ecosystems play an important role in the terrestrial ecosystem of China. At the same time, the subalpine zone of Southwest China has been considered a hotspot of biodiversity. However, increased grazing, cutting and harvesting of medicinal plants have all been implicated as growing threats to biodiversity in this region (Wang, 2004). Although the vegetation of this region has been profoundly modified by human activity, records indicate no disturbance history, such as windfall, insect break and fire. In efforts to revive and restore the forest landscape, China conducted afforestation projects in the 1950s, followed by the Natural Forest Protection Program (NFPP) launched in 2000. Consequently, natural regeneration communities are at different secondary succession stages with little human activities. In this study, we measured above- and belowground C stocks, plant diversity and environmental factors of six plant communities during different stages of succession in the subalpine zone of Southwest China. The objectives were (1) to elucidate the distribution of plant diversity and C stocks along successional gradients in a subalpine coniferous forest, (2) to examine the influence of environmental factors on C stocks, and (3) to quantify the relationships between C stocks and plant diversity.

2. Materials and methods

2.1. Study site

Our experiment was conducted in the Wanglang Nature Reserve (32°49′–33°02′N, 103°55′–104°10′E, 2300–4980 m above sea level), Southwest China. The mean annual temperature ranges from 1.5 to 2.9 °C. The annual cumulative temperature ($\geqslant$10 °C) is 1056.5 °C, and the absolute maximum and minimum temperatures are 26.2 °C and −17.8 °C, respectively. Annual precipitation ranges from 801 to 825 mm depending on the elevation, with most rain falling between May and August. In winter, soil stays frozen longer than 150 days, and the depth of the frozen soil is more than 40 cm. The vertical distribution of vegetation types in this region includes mixed forest of conifers and broadleaf trees, and broadleaf deciduous forest (2300–2600 m), fir forest (dominated by *Abies faxoniana*) and spruce-cypress forest (dominated by *Picea purpurea* and *Sabina saltuaria*, 2600–3500 m), subalpine shrubs and meadow (3500–4400 m), and sparse vegetation (4400–4900 m).

2.2. Sampling methods

We selected communities with herbs (dominated by *Deyeuxia scabrescens*, P1), shrubs (dominated by *Salix paraqplesia*, P2), broadleaf deciduous forest (dominated by *Betula platyphylla*, P3), mixed forest (dominated by *Betula* spp. and *A. faxoniana*, P4), fir forest (dominated by *A. faxoniana*, P5) and climax phase (dominated by *P. purpurea*, P6) as the restoration series in the Wanglang Nature Reserve, and studied C stocks and their allocation, and association types in these six communities using a field survey method with a replacement of space to time. The sites were selected from 2620 to 2840 m in elevation. The number of years was determined using tree rings and through interviews with local supervisors. All sampling sites were located within the nature reserve with little disturbance. The total studied area of 3000 ha has not undergone a comprehensive plan for forest management. Site information and specific characteristics of each community were recorded (Table 1). In all, 8 square sites (plots) of 20 m × 20 m of each stage of succession were selected for data collection (Mallik and Robertson, 1998). The tree stratum (48, 80, 143 and 310 years old) was sampled within four 10 m × 10 m subplots in each plot. The shrub stratum was sampled from four 5 m × 5 m quadrats nested within each 10 m × 10 m subplot. The herb stratum was investigated in four 1 m × 1 m quadrats nested within each 10 m × 10 m subplot. A total of 48 plots, 192 subplots, and 768 shrub and herb quadrats were established. For every subplot and quadrat, a floristic inventory was performed for three strata: (1) herb stratum (species <0.5 m in height), (2) shrub stratum (0.5–5 m) and (3) tree stratum (>5 m). The species were identified according to the International Code of Botanical Nomenclature (Greuter et al., 2000) and the Flora of China. In each quadrat, the abundance, height and cover of every species, the number of individuals, and all trees and shrubs with >2.0 cm in diameter at breast height (DBH) were recorded in each plot. The cover of every species was visually estimated as percentage of canopy cover.

2.3. C content in biomass and soil

Five dominant trees, representing the stand-specific DBH range, were selected and destructively sampled in each chronosequence stand. The biomass was estimated directly from height, DBH and age (Fang et al., 2006; Peichl and Arain, 2007). Two types of equations for each tree component as well as for the total tree biomass were developed: the first equation with tree diameter as the single input variable, Eq. $y_i = c(x_1)^a$ (1), and the second equation with tree diameter as the first variable in combination with an additional explaining variable, Eq. $y_i = c(x_1)^a (x_2)^b$ (2), where y is the dry biomass (kg) of the tree component (e.g. foliage, live branches, dead branches, stem wood, total aboveground, roots, and total tree), c is the constant, a and b are equation parameters, x_1 is tree diameter at DBH (cm); x_2 is tree height (m) or age (years). Biomasses of understory shrubs and herbs were destructively harvested and oven-dried (60 °C) to constant weight and weighed. For all vegetation compartments, a carbon concentration of 50% (on dry matter basis) was assumed (Matthews, 2003). The litter was collected using a 25 cm × 25 cm frame. All coarse woody debris was re-

Table 1
Stand characteristics of the study plots.

No.	Community type (dominant species)	Altitude (m)	Aspect (°)	Slope (°)	Soil type	AverageDBH (cm)	Averageage (years)	Woody plant density (trees hm^{-2})	Averageheight (m)	Plant coverage
P1	Gramineous	2730	SE10	42	Brown soil	–	3	–	0.8	0.90
P2	Willow shrub	2680	SE10	30	Brown soil	–	5	57500	1.5	0.80
P3	Birch forest	2600	SE30	40	Brown soil	12.4	48	1975	13.5	0.60
P4	Mixed forest	2700	EN35	38	Dark brown soil	22.5	80	740	27.0	0.75
P5	Fir forest	2620	E	4	Dark brown soil	28.3	143	210	35.0	0.70
P6	Spruce forest	2840	EN15	25	Dark brown soil	35.8	310	206	43.8	0.65

DBH, diameter at breast height.

moved from the samples prior to analysis, and dry weights of litter samples were recorded in the laboratory. For the litter layer, C contents were calculated by multiplying the concentrations of C by the average weight of the layer (i.e. Balboa et al., 2006). The roots of the sampled trees were excavated and sub-samples were taken in order to determine the total root biomass. Sequential soil coring method was used to investigate fine root (diameter <2 mm) biomass. Fine root samples were oven-dried at 60 °C and then weighed. For the measurement of above-ground litter decomposition, litter from all plant types was air-dried and placed in litterbags. Litterbags (10 cm × 10 cm) were made using a 1-mm nylon mesh on the top surface and a 0.1-mm mesh on the bottom to prevent loss of material. The total of 48 litterbags were placed about 30–50 cm apart in the same location. For each forest stand type, 8 bags (one bag per plot) were collected after 2 years (700 days). Litter was oven-dried (60 °C) and weighed to determine mass loss. Remaining mass was expressed on ash-free basis for each sampling date.

Soil samples (100 cm^3 per sample) were collected at 0–10, 10–20, and 20–30 cm with a core sampler. From three to five sets of samples were collected from each plot. The soil samples were weighed immediately and transported to the laboratory, where they were oven-dried at 105 °C for 48 h and again weighed. The bulk density (g of dry soil per cm^3 of soil) and volumetric soil water content were calculated afterwards. SOC stock was calculated according to soil carbon concentration, and bulk density of the fine earth and stoniness (the volumetric percentage of fragments of >2 mm) were determined as described by Grimma et al. (2008). The total carbon content of the 30-cm deep layer was finally estimated by summing all layers. Total C was analyzed in litter and mineral soil samples with an Elemental Analyzer.

2.4. Soil environment parameters

The analysis of soil nutrients, including nitrogen (N) and available phosphorus (P), was conducted by micro-kjeldahl and molybdenum blue methods, respectively (Jackson, 1973). The determination of biological properties (the number of living microorganisms) was estimated by viable count on serial spread plates. A series of 10-fold dilutions was prepared for each sample starting with 90 ml of sterilized phosphate buffered saline added to 10.0 g of soil sample. The flask was then closed and the contents were stirred for 30 min, and 1.0 ml suspension was added to 9.0 ml of sterilized phosphate buffered saline. The dilutions were repeated to produce six continuous dilutions. Finally, 0.1 ml from each serial dilution of the sample suspension was spread over an agar plate with Martin's medium for fungi, a plate with beef extract peptone medium for bacteria and a plate with Gauze's No. 1 synthetic medium for actinomycetes. The plates were incubated at 28 °C until colonies appeared (2 days for bacteria, and 3 days for fungi and actinomycetes), and colony forming units (CFU) were then counted.

Soil pH was measured in 1:2.5 (w/v) ratio of soil to distilled water suspension. Soil enzyme activities were assayed in triplicate air-dried samples as described by Guan (1986). Briefly, the urease activity was determined using urea as a substrate: the soil mixture was first incubated at 37 °C for 24 h, the produced NH_3–N was determined by a colorimetric method, and the urease activity was expressed as μg NH_4–N/g/h. The invertase activity was determined using sucrose as a substrate, incubating the soil mixture at 37 °C for 24 h and measuring the produced glucose with the colorimetric method. The invertase activity was expressed as μg glucose g^{-1} h^{-1}. The catalase activity was measured using H_2O_2 as a substrate and shaking the mixture for 20 min, after which the filtrate was titrated with 0.1 mol l^{-1} $KMnO_4$. The catalase activity was expressed as ml 0.1 mol l^{-1} $KMnO_4$ g^{-1} h^{-1}. Three repeats were made for each type of a test.

2.5. Species diversity analyses

The species importance values (IV) for each quadrat were calculated using the following formulae (Zhang et al., 2005):

$$IV_{tree} = (RD + RF + RC)/3 \quad (1)$$

$$IV_{shrub\ and\ herb} = (RC + RH)/2 \quad (2)$$

where RD was the relative density (defined as the percentage of individuals of a tree species to the total individuals of all tree species per plot), RF was the relative frequency (defined as the percentage of frequency of a tree species to the total frequency of all tree species), RC was the relative coverage (defined as the percentage of the cover of a species to the total cover of all species, and RH was the relative height (defined as the percentage of the total height of a species to the total height of all species). The IV value was used to calculate species diversity in each plot.

$$\text{Margalef index}(R) R = (S-1)/\ln(N) \quad (3)$$

$$\text{Shannon-Wiener index}(H') : H' = -\sum P_i \ln(P_i) \quad (4)$$

where S was the number of species in a quadrat, N was the sum of IV of all species in the same quadrat, P_i was the proportional IV of the ith species in a quadrat and $P_i = N_i/N$, N_i was the IV of the ith species in a quadrat.

2.6. Statistical analyses

To assess differences among the six plant communities, the results of species richness, plant diversity (Shannon–Wiener index, Margalef index), and soil and above-ground carbon stocks were analyzed using one-way ANOVAs. Multiple comparisons of species richness and plant diversity means among plant communities were performed using Duncan's multi-range test at $P < 0.05$. Data that were not normally distributed were transformed prior to analysis, using square-root or log transformations. Analyses were performed using SPSS 13.0 for Windows. Redundancy analysis (RDA) was applied to quantify and test effects of altitude, soil properties and vegetation traits on the plant diversity variation. The whole process was based on computations made with Canoco for Windows 4.5. The relationships of C stocks with species richness, plant diversity and environmental parameters were examined with Pearson correlation analyses. In addition, a multivariate analysis was carried out to test for causal models linking changes in the main litter quality, soil microorganisms, soil enzyme, vegetation traits and soil carbon storages following Shipley's d-sep method (Shipley, 2004). This method tests the statistical constraints imposed by the model, which are in the form of conditional independence relationships. For example, if we state that litter quality (L) affects soil carbon storage (C) through soil microorganisms (M) only, L and C should be independent when M is held constant statistically. In other words, L and C are independent conditional to M. We measured independence using Pearson (partial) correlations, where no significant (partial) correlation corroborated the stated independence relationship. For each model, the implied separate tests of independence (pi) were combined using the Fisher's C statistic to provide a test for the entire model:

$$C = -2\sum_{i=1}^{n} \ln(pi)$$

where n is the number of tests of independence and C is distributed as a χ^2 variable with $df = 2n$. Significance was fixed at the 0.05 level throughout the study.

3. Results

3.1. Variation of soil properties and vegetation characteristics

All investigated properties exhibited great spatial variation across the sampling sites. The coefficients of variation (CV) ranged as follows: plant biomass (103.7%) > total root biomass (92.3%) > tree height (80.9%) > invertase activity (52.6%) > fine root biomass (50.0%) > urease activity (44.3%) > litter N (41.5%) > mean annual temperature (41.1%) > tree diameter at breast height (40.0%) > soil moisture (39.8%) > catalase activity (38.2%) > soil P (36.4%) > litter C/N ratio (36.3%) > litter production (31.2%) > soil N/P ratio (29.8%) > species richness (22.8%) > soil bulk density (22.2%) > soil N (18.8%) > Margalef index (17.9%) > Shannon–Wiener index (17.7%) > soil pH (13.4%) > plant coverage (13.3%) > the quantity of microorganisms (6.2%) (Table 2).

3.2. Carbon stocks and aboveground litter decomposition

The total C pool in the ecosystems ranged from 105 to 730 Mg C ha^{-1}. When all above-ground components were considered together, above-ground C increased from P1 to P6 ($P < 0.001$; Fig. 1). C storage in tree layer represented 24%, 26%, 57% and 61% of the C pool system in the P3, P4, P5, P6 forest stands, respectively. Compared with the amount of C stored in the tree layer, C storage in understory small trees was very little, representing less than 3% of the C pool system in the P3–P6 forest stands. The contribution of litter to the C pool system was less than 4% in all cases. Meanwhile, C storage in total roots represented less than 10% of the C pool system in the P1–P6 forest stands. In the six plant communities, SOC varied between 94 and 222 Mg C ha^{-1} in the top 30 cm. SOC constituted the main C pool of the P1–P4 stands (62–89%). On average, 80–90% of SOC was located in the soil down to 20 cm, while only 10–20% was stored in 20–30 cm. The total SOC increased significantly ($P < 0.001$) in P1 from 94 Mg C ha^{-1} to 222 Mg C ha^{-1} in P4, then decreased to 171 Mg C ha^{-1} in P6 (Fig. 2). In addition, fine root biomass was lower while the litter production was higher in P5 and P6 forest stands than in P4 forest type (Fig. 3a and b). Conifer litter (P5 and P6 forest stands) decomposed more slowly than other forest type litters. After 2 years, the mass of remaining conifer litter was 78–82%, whereas only 40–55% of other forest type litter mass remained (Fig. 3c). Moreover, conifer litter showed a higher C/N ratio compared with that of other forest type litters (Fig. 3d).

Table 2
Descriptive statistics (means, standard deviations (SD) and coefficients of variation (CV)) of plant diversity, soil variables and vegetation characteristics of the 48 investigated sites.

Variable	Mean	SD	CV (%)
Soil moisture (%)	30.9	12.3	39.8
Mean annual temperature (°C)	1.7	0.7	41.1
Soil bulk density (g cm^{-3})	0.45	0.1	22.2
Soil pH	6.5	0.9	13.4
Soil N (mg g^{-1})	6.0	1.1	18.8
Soil P (mg g^{-1})	3.7	1.4	36.4
Soil N/P ratio	1.7	0.5	29.8
Litter production (Mg ha^{-1})	3.2	1.0	31.2
Litter C (mg g^{-1})	244.6	15.2	6.2
Litter N (mg g^{-1})	3.3	1.4	41.5
Litter C/N ratio	68.6	25.0	36.5
Quantity of microorganisms ($\times 10^4$ CFU g^{-1} dry soil)	344.3	21.5	6.2
Urease (NH_4–N mg g^{-1} dry soil, 24 h, 37 °C)	1.7	0.8	44.3
Invertase (glucose mg g^{-1} dry soil, 24 h, 37 °C)	2.7	1.4	52.6
Catalase (0.1 mol/L $KMnO_4$ g^{-1} dry soil, 24 h, 37 °C)	2.4	0.9	38.2
Species richness	51.0	11.6	22.8
Shannon–Wiener index	2.4	0.4	17.7
Margalef index	5.7	1.0	17.9
Plant biomass (Mg ha^{-1})	344.5	357.3	103.7
Total root biomass (Mg ha^{-1})	61.1	56.4	92.3
Fine root biomass (Mg ha^{-1})	3.4	1.7	50.0
Average tree diameter at breast height (cm)	24.8	9.9	40.0
Plant coverage	0.8	0.1	13.3
Tree height (m)	20.3	16.4	80.9

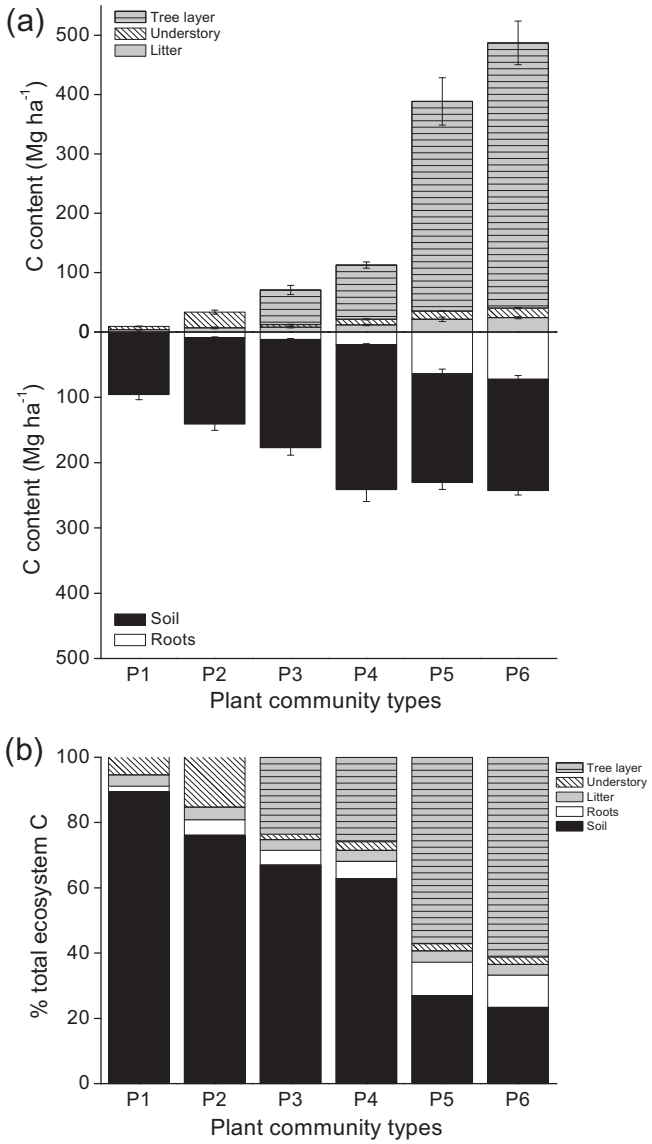

Fig. 1. Total carbon stock and its allocation in six plant community types during different stages of succession in a subalpine coniferous forest in Southwest China. Horizontal bars indicate standard errors of means ($n = 8$). Herb community dominated by *D. scabrescens* (P1), shrub community dominated by *S. paraqplesia* (P2), broadleaf deciduous forest dominated by *B. platyphylla* (P3), mixed forest dominated by *Betula* spp. and *A. faxoniana* (P4), fir forest dominated by *A. faxoniana* (P5), and spruce forest dominated by *P. purpurea* (P6).

3.3. Species richness and plant diversity

The species richness of the six plant communities showed a humped pattern of variation along the succession (Fig. 4a). From P1 to P3, species richness showed an increasing trend, but after P3 it sharply decreased. The Shannon–Wiener index displayed a similar trend as species richness during succession. The maximum species richness and Shannon–Wiener index occurred in P3 with a total of 69 for species richness and 2.93 ± 0.06 for Shannon–Wiener index (Fig. 4b and c). The Margalef index increased from 5.74 in P1 to 7.38 in P2 and decreased thereafter.

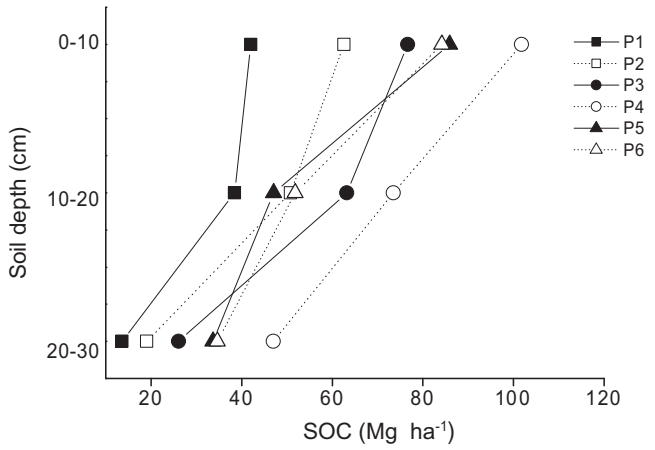

Fig. 2. Soil organic carbon (SOC) content across soil depths in six plant community types during different stages of succession in a subalpine coniferous forest in Southwest China. Horizontal bars indicate standard errors of means ($n = 8$). Herb community dominated by *D. scabrescens* (P1), shrub community dominated by *S. paraqplesia* (P2), broadleaf deciduous forest dominated by *B. platyphylla* (P3), mixed forest dominated by *Betula* spp. and *A. faxoniana* (P4), fir forest dominated by *A. faxoniana* (P5), and spruce forest dominated by *P. purpurea* (P6).

3.4. Contribution of environmental factors to species richness and plant diversity

The RDA diagram (Fig. 5) shows the relationships between the species richness, plant diversity and environmental variables. Each plant diversity/environmental variable is represented by an arrow. The longer the arrow the higher is the importance of the diversity/environmental variable for the distribution of the data. The position of plant diversity relative to an environmental variable indicates how strongly the plant diversity is associated with the particular variable. Eigen values for the first, second, third and fourth axes were 0.597, 0.272, 0.081 and 0.048, respectively. Axis 1 explained most of the diversity variation, and the first two RDA axes accounted for 86.9% of the variation in plant diversity and associated environmental variation. The environmental variables best correlated with the first ordination axis included soil pH, litter production, litter C, litter N, litter C/N ratio, urease and catalase activity. The second ordination axis strongly correlated with soil moisture. The variables explaining the largest amount of variation were litter production ($P < 0.001$), litter N ($P < 0.001$) and catalase activity ($P < 0.001$). By contrast, the mean annual temperature and altitude generally had weak impacts on the species richness and plant diversity.

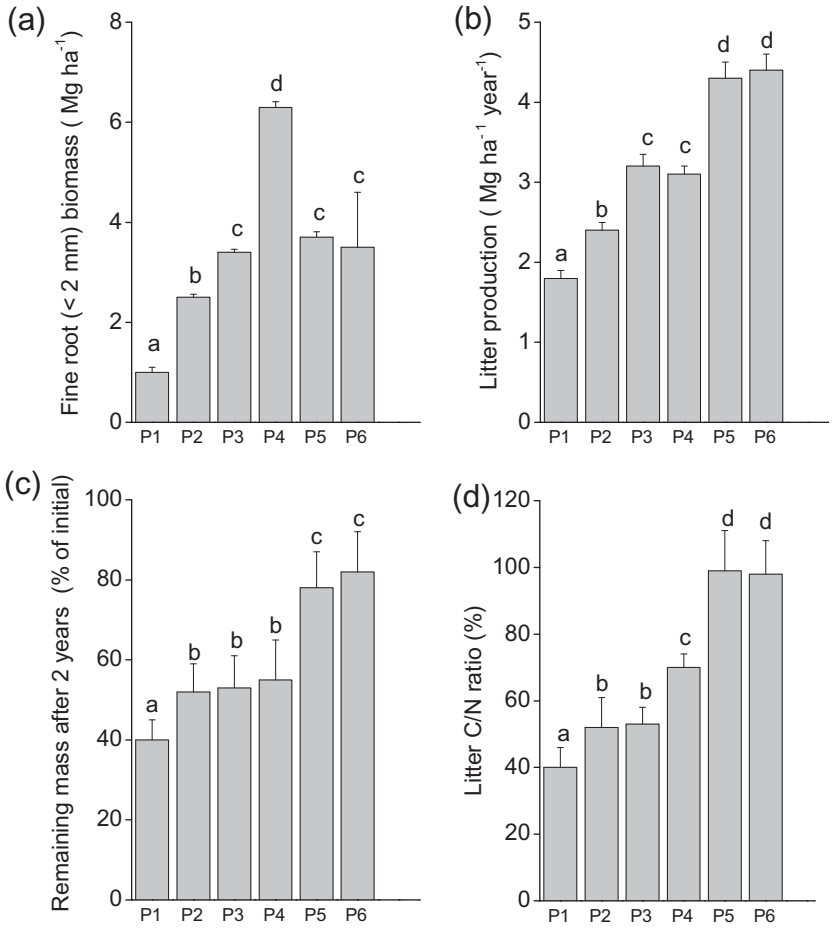

Fig. 3. Fine root biomass, litter production, remaining mass and litter C/N ratio in six plant community types during different stages of succession in a subalpine coniferous forest in Southwest China. Horizontal bars indicate standard errors of means ($n = 8$). Herb community dominated by *D. scabrescens* (P1), shrub community dominated by *S. paraqplesia* (P2), broadleaf deciduous forest dominated by *B. platyphylla* (P3), mixed forest dominated by *Betula* spp. and *A. faxoniana* (P4), fir forest dominated by *A. faxoniana* (P5), and spruce forest dominated by *P. purpurea* (P6). The values not sharing the same letters are significantly different ($P < 0.05$) according to Duncan's multi-range test.

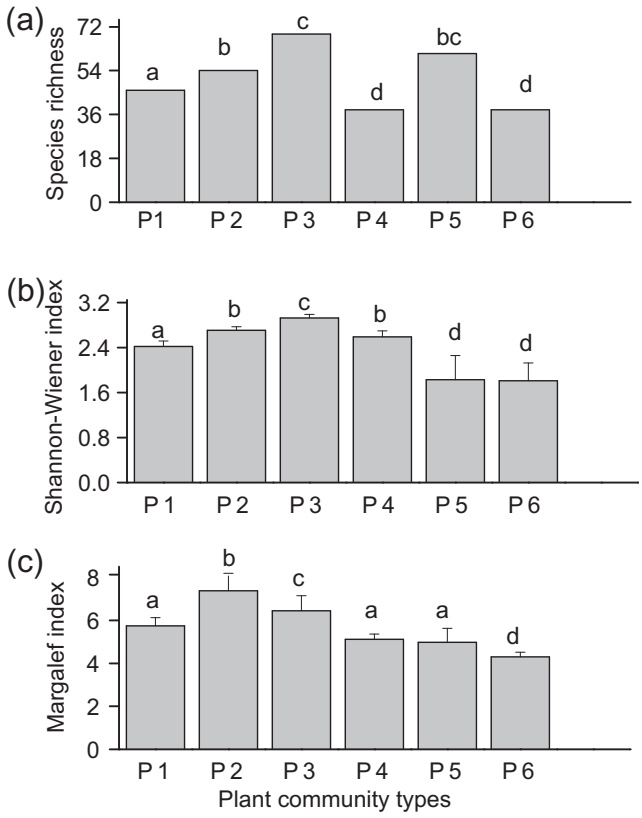

Fig. 4. Species richness, Shannon–Wiener index and Margalef index in six plant community types during different stages of succession in a subalpine coniferous forest in Southwest China. Horizontal bars indicate standard errors of means (n = 8). Herb community dominated by *D. scabrescens* (P1), shrub community dominated by *S. paraqplesia* (P2), broadleaf deciduous forest dominated by *B. platyphylla* (P3), mixed forest dominated by *Betula* spp. and *A. faxoniana* (P4), fir forest dominated by *A. faxoniana* (P5), and spruce forest dominated by *P. purpurea* (P6). The values not sharing the same letters are significantly different ($P < 0.05$) according to Duncan's multi-range test.

3.5. The relationship between C stocks and environmental parameters

Correlation analysis was used to examine the relationships between soil and above-ground C stocks and various environmental variables as listed in Table 3, ranging from mean annual temperature and altitude to aboveground vegetation properties and soil properties. However, the relationships between C stocks (soil, above-ground C), and the mean annual temperature and altitude were generally weak ($P > 0.05$). Significant relationships with above-ground C stocks were shown by soil moisture and invertase activity (positive) as well as by plant diversity (negative) (Table 3, Fig. 6). Among measured environmental attributes, the quantity of microorganisms and litter C/N ratio showed the strongest (positive) relationship with SOC, followed by total root biomass and fine root biomass, and, to a lesser extent, invertase activity (Table 3). On the other hand, the species richness and plant diversity did not emerge as significant predictor variables for SOC (Fig. 6).

3.6. Structural equation modeling (path analysis)

The results of the multivariate analyses (d-sep test) of causal models linking litter quality (litter C/N ratio, L), soil microorganisms (the quantity of microorganisms, M), soil enzyme activity (soil invertase activity, E), vegetation traits (total root biomass, V) and soil carbon storages (C) are given in Fig. 7. The results showed that the probability that the causal relationships hypothesized by models a, b, c, d, e, g and h actually generated the observed data was lower than 0.01. Therefore, these models were rejected. By contrast, there was a high probability (0.421) of the causal processes hypothesized by model f, which indicated that litter C/N ratio affects SOC through changes in the quantity of microorganisms and soil invertase activity (Fig. 7). Soil microorganisms act as a control over soil enzyme activity.

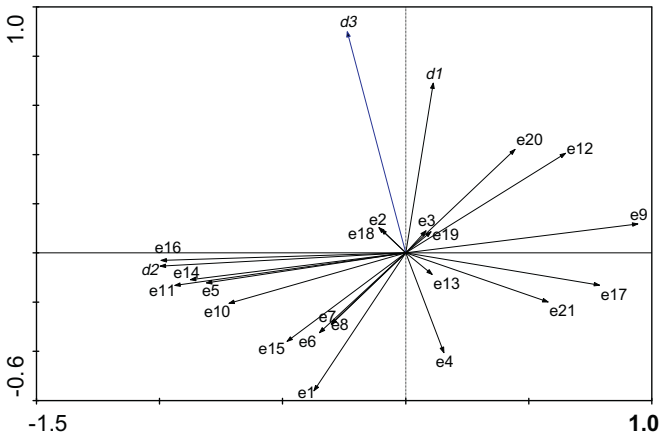

Fig. 5. Bioplot diagram of the redundancy analysis (RDA) on species richness and plant diversity (Shannon–Wiener index, Margalef index) by environmental parameters: soil moisture (e1), mean annual temperature (e2), altitude (e3), soil bulk density (e4), soil pH (e5), soil N (e6), soil P (e7), soil N/P ratio (e8), litter production (e9), litter C (e10), litter N (e11), litter C/N ratio (e12), the quantity of microorganisms (e13), urease activity (e14), invertase activity (e15), catalase activity (e16), plant biomass (e17), total root biomass (e18), average tree diameter at breast height (e19), plant coverage (e20), tree height (e21), species richness ($d1$), Shannon–Wiener index ($d2$) and Margalef index ($d3$). Axis 1 (59.7%, $P < 0.001$) and axis 2 (27.2%, $P < 0.001$).

Table 3
Correlation coefficients among variables. Significances of correlations are indicated as:$^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

Variable	Soil organic C	Above-ground C
Soil moisture	0.197, $P = 0.180$	0.429, $P = 0.002$ **
Mean annual temperature	0.193, $P = 0.187$	−0.576, $P = 0.231$
Altitude	0.133, $P = 0.370$	0.682, $P = 0.136$
Soil bulk density	0.086, $P = 0.560$	−0.132, $P = 0.371$
Soil pH	0.116, $P = 0.433$	−0.019, $P = 0.898$
Soil N	−0.165 $P = 0.263$	−0.238, $P = 0.104$
Soil P	−0.142, $P = 0.336$	−0.169, $P = 0.250$
Soil N/P ratio	−0.142, $P = 0.336$	−0.168, $P = 0.251$
Litter production	−0.076, $P = 0.607$	−0.393, $P = 0.006$
Litter C	−0.205, $P = 0.163$	−0.061, $P = 0.679$
Litter N	0.094, $P = 0.525$	0.038, $P = 0.798$
Litter C/N ratio	0.470, $P = 0.001$**	0.034, $P = 0.820$
Quantity of microorganisms	0.994, $P = 0.000$***	0.084, $P = 0.572$
Urease	0.123, $P = 0.406$	0.030, $P = 0.842$
Invertase	0.292, $P = 0.044$*	0.320, $P = 0.027$*
Catalase	0.110, $P = 0.456$	0.016, $P = 0.914$
Plant biomass	0.040, $P = 0.789$	0.159, $P = 0.282$
Total root biomass	0.291, $P = 0.045$*	0.009, $P = 0.950$
Fine root biomass	0.279, $P = 0.047$*	−0.061, $P = 0.679$
Remaining mass	0.209, $P = 0.166$	0.289, $P = 0.050$
Woody plant density	0.037, $P = 0.801$	0.010, $P = 0.948$
Plant coverage	0.007, $P = 0.964$	0.210, $P = 0.153$
Tree height	0.092, $P = 0.533$	0.269, $P = 0.064$

4. Discussion

We compared the species diversity, and above- and below-ground C stocks of six plant communities along a chronosequence of vegetation restoration. Our study is one of only a few investigations that have assessed the function of diversity in a forest ecosystem undergone the complex successional dynamics that

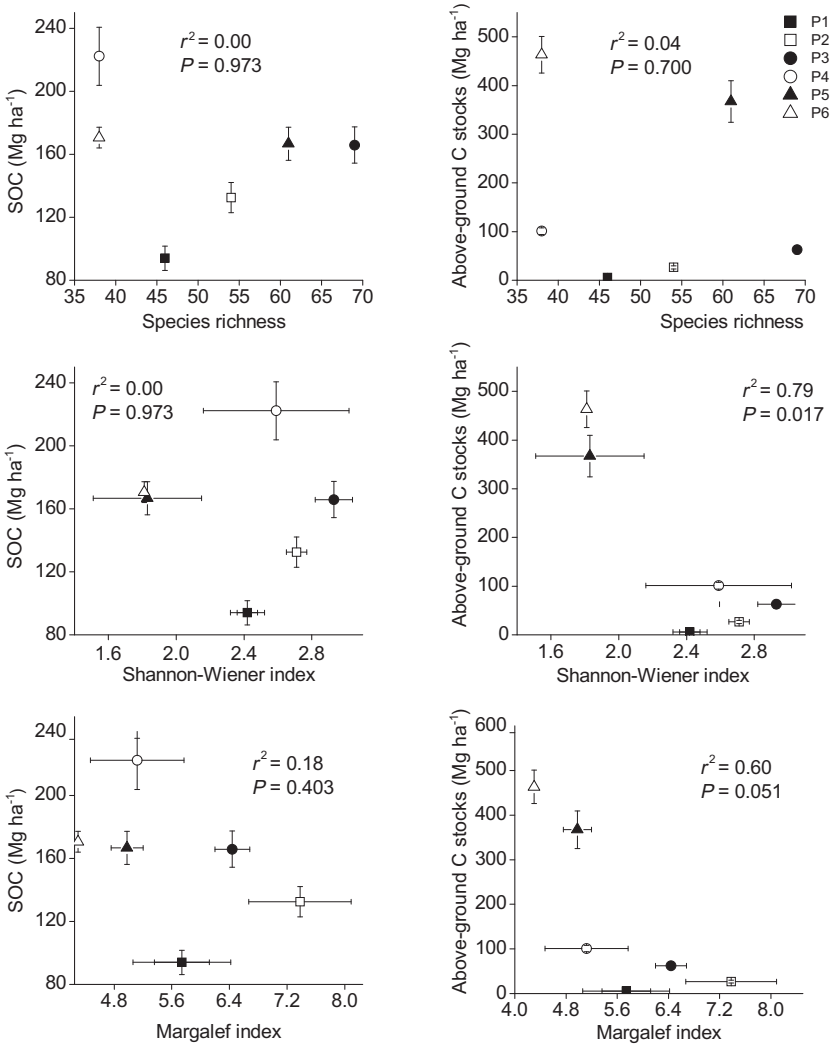

Fig. 6. Relationships between soil organic carbon (SOC) and aboveground C stocks and species richness, Shannon–Wiener index and Margalef index at the level of association type in a subalpine coniferous forest in Southwest China. Vertical and horizontal bars indicate standard errors of means ($n = 8$). Herb community dominated by *D. scabrescens* (P1), shrub community dominated by *S. paraqplesia* (P2), broadleaf deciduous forest dominated by *B. platyphylla* (P3), mixed forest dominated by *Betula* spp. and *A. faxoniana* (P4), fir forest dominated by *A. faxoniana* (P5), and spruce forest dominated by *P. purpurea* (P6).

characterizes natural environments (Troumbis and Memtsas, 2000; Firn et al., 2007; Vila et al., 2007), as opposed to an artificially derived system with species selected and then assembled. These results clearly demonstrated the tremendous range in C storage within subalpine coniferous forest ecosystems in Southwest China. This variation appears unrelated to regional differences in annual temperature and altitude and, therefore, is most likely biological or microclimate related. All sampling sites were located within a nature reserve with little disturbance and management practice. Thus, the design of the study did not allow the detection of changes in plant diversity and soil C storage that were attributable to forest management. RDA indicated that species richness and plant diversity were strongly related to soil moisture, litter mass, litter N and soil catalase activity, but less related to mean annual temperature and altitude. In other words, soil moisture, litter production, litter N and soil catalase activity play an important role in regulating plant diversity in the subalpine coniferous forest in Southwest China. This result was underscored by the community of a highly productive, low-diversity gymnosperm-dominated (*A. faxoniana* and *P. purpurea*) stands, which were found to be associated with lower soil moisture and soil enzyme activity, and higher litter production compared with less productive stands.

Worldwide, SOC stocks up to 1 m average 12.2 kg m^{-2} (122 Mg ha^{-1}) in temperate forests (Prentice, 2001; Lal, 2005), and 11.3 kg m^{-2} (113 Mg ha^{-1}) among all forests (Sombroek et al., 1993). In our study, the soil C stores at 0–30 cm depth ranged from 94 Mg C ha^{-1} to 222 Mg C ha^{-1}, indicating high levels and high spatial variability. About 80% of SOC was allocated in the top 20 cm soil layers. Secondary succession is therefore expected to affect predominantly the SOC contents of top layers. Nevertheless, SOC increased from P1 to P4, providing a major C reservoir and remaining as an important component of the overall forest ecosystem C budget prior to the restoration time of 80 years. However, we observed that SOC declined between restoration times of 80 and 310 years, suggesting that *A. faxoniana* sites may accumulate carbon only until the age of 80 years. We suggest the following explanations for the declined net C sequestration in P5 and P6 forest stands: (a) the lower belowground carbon input. The decreased SOC in the latter succession stage could be attributed to the decreased fine root input (Fig. 3a). Fine roots may enhance C accumulation in soils by several SOC protection mechanisms other than high fine root C inputs (Balesdent and Balabane, 1996; Jastrow et al., 1998; Wang et al., 2010); (b) the more limited C incorporation into mineral soil in coniferous stands (P5 and P6) than in other

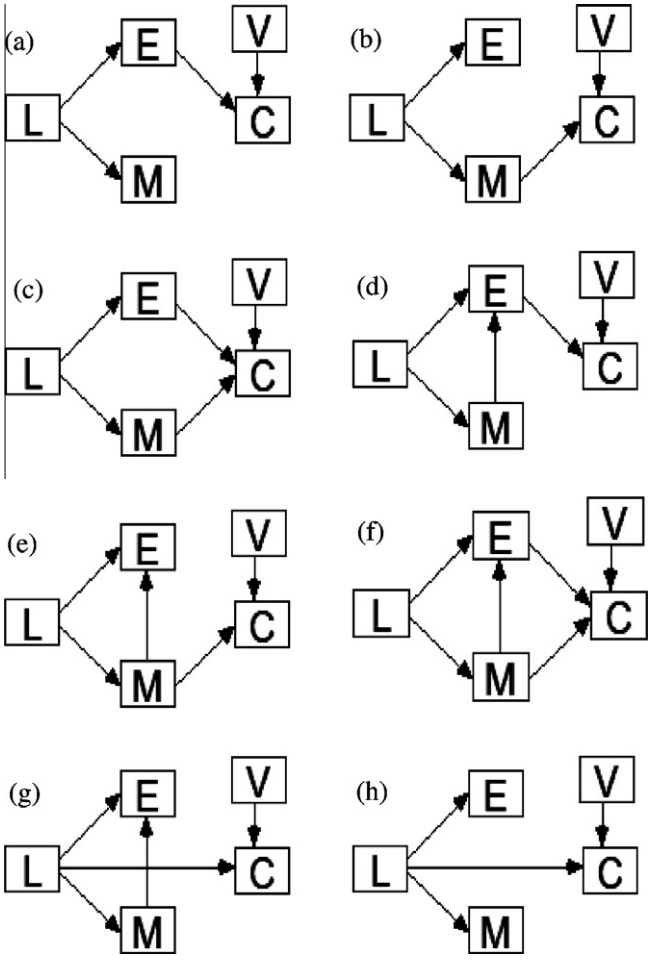

Fig. 7. Path models describing relationships between litter C/N ratio (L), the quantity of living microorganisms (M), invertase activity (E), total root biomass (V) and soil organic carbon (C). Model a, B = {(L,V)|{Ø}, (L,C)|{V,E}, (V,E)|{L}, (V,M)|{L}, (E,M)|{L},(M,C)|{L,V,E}}, 40.83 (<0.001); Model b, B = {(L,V)|{Ø}, (L,C)|{V,s3}, (V,E)|{L}, (V,M)|{L}, (E,C)|{L,V,M}}, 28.33 (0.002); Model c, B = {(L,V)|{Ø}, (L,C)|{V,E,M}, (V,E)|{L}, (V,M)|{L}, (E,M)|{L}}, 24.28 (<0.001); Model d, B = {(L,V)|{Ø}, (L,C)|{V,E}, (V,E)|{L, M}, (V,M)|{L}, (M,C)|{L,V, E}}, 55.69 (0.001); Model e, B = {(L,V)|{Ø}, (L,C)|{V,M}, (V,E)|{L,M}, (V,M)|{L}, (E,C)|{L,V,M}}, 36.31 (0.003); Model f, B = {(L,V)|{Ø}, (L,C)|{V,E,M}, (V,E)|{L,M}, (V,M)|{L}}, 2.53 (0.421); Model g, B = {(L,V)|{Ø}, (V,E)|{L,M}, (V,M)|{L}, (E,C)|{L,V,M}, (M,C)|{L,V}}, 32.84 (<0.001); Model h, B = {(L,V)|{Ø}, (V,E)|{L}, (V,M)|{L}, (E,M)|{L}, (E,C)|{L,V}, (M,E)|{L,V}}, 6.23 (0.002). The notation (*x*, *y*)|{*z*}means the Pearson correlation between variables *x* and *y*, conditional on variable *z*. In other words, *x* and *y* are independent but conditional to *z*. Ø represents the null (empty) set. The overall model is tested with Fisher's *C* statistic $P < 0.05$. Values are results from Fisher's *C* tests: the χ^2 value and its probability in parentheses. Models that are accepted at the 5% level are shown in bold.

stands. The slower decay of conifer litter compared to the litter of deciduous trees will lead to more remaining organic matter, eventually this will contributes partly to the lower accumulation of C in the mineral soil under coniferous than deciduous trees (Wang et al., 2010). The slow decomposition of conifer litter may be explained by its chemical composition. Its C/N ratio is higher than that of other stands litter (Fig. 3d). The substrate with a high C/N ratio represents a low quality and lower transfer rate of C from substrate to mineral soil than that with a lower C/N ratio. Nevertheless, we cannot rule out that changes in microclimatic conditions (soil humidity and temperature) may have modified the rate of litter decomposition; (c) the higher litter production. The increased litter production in P5 and P6 forest stands may exert a priming effect on soil respiration, resulting in decreased surface soil C storage (Schaefer et al., 2009). According to our experimental results, the first two explanations are likely the main processes that lead to decreases in C sequestration in P5 and P6 forest stands, as indicted by the fact that the fine root biomass and litter C/N ratio showed a significant (positive) relationship with SOC (Table 3).

The mean C stocks in the whole ecosystem – trees, litter layer and mineral soil – ranged from 105 to 730 Mg C ha^{-1}. The above-ground C pool increased significantly, mainly due to the increase of C in above-ground living plants and surface litter. This finding is consistent with the idea that the C stock in tree biomass increases with the stand age and with the number of larger trees (Vanninen et al., 1996). The result indicates that large trees play a more important role in the carbon storage of subalpine forest in this area. Understory and litter contributed only little to the total ecosystem biomass and C pool. Below-ground ecosystem C (which is the sum of C in mineral soil and tree roots) was considerably higher in the P6 plant community due to a more pronounced contribution of the tree root biomass. The net difference in the total ecosystem C between P1 and the P6 stand was 625 Mg C ha^{-1}, which demonstrated the considerable C sequestration potential of the pine plantation forests growing in the subalpine coniferous forest in Southwest China. Thus, protecting natural regeneration poses a low cost but has a high natural value as a restoration practice, especially in the mountainous regions where resources and labor available for restoration are limited (Zhang et al., 2010). Although P5 and P6 forest stands were ranked as the highest in carbon store, lower plant diversity (Fig. 4), substantial acidification, depletion of nutrients and disruption of biogeochemical cycles are common soil changes associated with these two forest stand types (Wang, 2004). Our findings highlight the need that the future strategy of tree species selection to prevent C impoverishment of these forests in subalpine China should consider the potential effects of tree species on the chemical composition in addition to the quantity of C stocks. Protective measures undertaken in later succession stages should enhance biodiversity and the role of both soil and tree components as long-term C sinks. In addition, mixed forest (dominated by *Betula* spp. and *A. faxoniana*, P4) with a higher plant diversity, species richness, SOC, ecosystem stability and soil fertility (Wang, 2004) are advantageous for the restoration of soil health in subalpine china. The present results will enhance our ability to evaluate the role of forests in regional C cycles and have important implications for conservation planning. As mentioned earlier, the age-sequence of our stands was not replicated and it does not represent growth patterns of a single stand over time. Therefore, the dependence of ecosystem C pools on stand age should be viewed in accordance with these limitations.

Traditionally, the C cycle has been addressed independently from the species involved, but recently several studies have shown that both plant species diversity and composition can have important impacts on C dynamics (Fornara and Tilman, 2008; Steinbeiss et al., 2008; Dias et al., 2010). We tested whether plant diversity in the observed communities had an effect on C stocks but found that species richness possessed no significant relationship with above-ground C, while a strong negative association occurred between plant diversity (measured as Simpson's biodiversity index) and above-ground C stocks. The negative relationship could indicate that above-ground C stock is mainly driven by the presence of a particular species, i.e. a selection effect (Creed et al., 2009). The dominant effects of two species, *A. faxoniana* and *P. purpurea,* indicate that ecosystem functions, such as C stocks, are not determined solely by the number of species but are more likely to be determined by the characteristics of the species present. This result is analogous to results obtained in several experimental biodiversity studies, where species composition or functional traits of specific species were better predictors for ecosystem functioning than species richness (Kahmen et al., 2005).

A number of mechanisms have been proposed by which plant diversity could influence the storage of C in soils (Gleixner et al., 2005). However, there was no significant effect of plant diversity at any of the three levels tested in the present study, which suggests that the relationship between biodiversity and ecosystem function is not straightforward and cannot be generalized across forest ecosystems. Although simple measures of plant diversity were insignificant predictors of SOC, litter C/N ratio, the quantity of microorganisms, invertase activity and fine root biomass had a significant effect on SOC. Accordingly, we suggest that the differences in the quality of gymnosperm and angiosperm litter, and the quantity of the organic matter input to soil through fine-root turnover from belowground, rather than tree diversity, accounted for the observed differences in SOC. In order to explain the empirical patterns of direct and indirect covariation between variables, we further used a multivariate analysis to better understand how litter quality controls the soil processes and followed the approach suggested by Shipley (2004). We designed models to test whether litter C/N ratio affects SOC through changes in invertase activity only (model a), the quantity of microorganisms only (model b) or both (model c). As soil microorganisms have been suggested to be related to soil enzymes, we included an effect of soil microorganisms on soil enzymes (models d, e and f). Finally, we tested whether litter C/N ratio had a direct effect on SOC (model g) or not (model h). The vegetation parameter that had the strongest relationship with SOC, i.e. total root biomass, was included in the models. The rejection of models a, b, d, e, g and h confirmed that litter C/N ratio affects SOC both through changes in soil microorganism and soil enzyme activities. The proposed accepted model f is similar to model c, but soil microorganisms act as a control over soil enzyme activities. The multiple controls by which the litter quality can affect SOC suggest that a multi-factor approach may be necessary to obtain a better mechanistic understanding of how changes in litter can affect this ecosystem process.

In conclusion, we inferred that protecting forests from a conversion to herb plantations would have the greatest positive impact on C stocks in the context of mitigating global climate change. On the other hand, we did not detect a relationship between S and above-ground C storage, while we found a significant negative relationship between H', R and above-ground C storage. In addition, our experiment demonstrated that litter C/N ratio and root biomass are significant functional traits influencing SOC, while S, R and H' have little effect. The path analysis revealed that litter C/N ratio predominantly regulates SOC through changes in the quantity of microorganisms and soil invertase enzyme activity.

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