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Understory Plants Regulate Soil Respiration through Changes in Soil Enzyme Activity and Microbial C, N, and P Stoichiometry Following Afforestation

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Abstract: Soil respiration (SR) is an important process in the carbon cycle. However, the means by which changes in understory plant community traits affect this ecosystem process is still poorly understood. In this study, plant species surveys were conducted and soil samples were collected from forests dominated by black locust (*Robinia pseudoacacia* L.), with a chronosequence of 15, 25, and 40 years (RP15, RP25, and RP40, respectively), and farmland (FL). Understory plant coverage, evenness, diversity, and richness were determined. We investigated soil microbial biomass carbon (MBC), nitrogen (MBN), phosphorus (MBP), and stoichiometry (MBC:MBN, MBC:MBP, and MBN:MBP). Soil enzyme assays (catalase, saccharase, urease, and alkaline phosphatase), heterotrophic respiration (HR), and autotrophic respiration (AR) were measured. The results showed that plant coverage, plant richness index (R), evenness, and Shannon-Wiener diversity were higher in RP25 and RP40 than in RP15. SR, HR, and AR were significantly higher in the forested sites than in farmland, especially for SR, which was on average 360.7%, 249.6%, and 248.2% higher in RP40, RP25, and RP15, respectively. Meanwhile, catalase, saccharase, urease, and alkaline phosphatase activities and soil microbial C, N, P, and its stoichiometry were also higher after afforestation. Moreover, significant Pearson linear correlations between understory plants (coverage, evenness, diversity, and richness) and SR, HR, and AR were observed, with the strongest correlation observed between plant coverage and SR. This correlation largely depended on soil enzymes (i.e., catalase, saccharase, urease, and alkaline phosphatase), and soil microbial biomass C, N, and P contents and its stoichiometry, particularly urease activity and the MBC:MBP ratio. Therefore, we conclude that plant communities are drivers of soil respiration, and that changes in soil respiration are associated with shifts in soil enzyme activities and nutrient stoichiometry.

Keywords: understory plants; soil enzymes; soil microbial biomass; soil respiration; afforestation ecosystem

1. Introduction

Understory plants play an important role in soil carbon cycling and the future carbon (C) balance of terrestrial ecosystems under climate change [1,2]. The C flux through soil respiration (SR) is a vital component of the global C cycle; it represents approximately 10% of the atmospheric C pool, and is 10 times greater than that from fossil fuel combustion [3]. Consequently, even slight changes in understory plant community composition and traits could affect SR through shifts in productivity [4], changing litter inputs and altering the soil microclimate [5,6]. Many studies have reported that plants can control the balance between plant C inputs and losses [7–10]. However, the means by which changes in plant community traits affect this ecosystem process, especially in afforested ecosystems, is poorly understood. For example, different results are usually reported the effects of plant diversity on SR. Both positive [11] and non-significant relationships [12] between plant diversity and SR have been documented. More importantly, Dias [13] found no significant effect of plant diversity on SR, while Liu [14] observed that plant diversity was the most important driver of SR. Therefore, it is imperative to further investigate plant communities as drivers of SR, and the possible pathways, mechanisms, and significance of SR for global climate change.

Plants affect SR in many ways, one of which is soil enzyme activities [15,16]. Enzyme activity is the most basic driving factor of SR. More than 50% of SR is produced by the enzyme-related decomposition of litter and soil organic matter (SOM) [17,18]. On the other hand, considering the metabolic activities of microorganisms during C deposition into soil, enzymes transform plant residues, decompose plant-derived C, and thus affect SR [19]. These relationships have been revealed through field experiments [15], models [20], and meta-analyses [21,22]. For example, Chen [15] showed a positive correlation between glycosidase activity and SR for most types of vegetation. Ren [9] also documented that enzyme activities, especially oxidative C-degrading enzyme activities, were significantly correlated with SR due to plant litter inputs. However, a lack of clarity regarding afforested ecosystems still remains, because the distribution of both recalcitrant and labile C varies depending on the plant community composition [23]. Furthermore, understanding the effects of the aggradation of afforested ecosystems on belowground C cycles is important for quantifying and predicting the dynamics of terrestrial C, especially under the current scenarios of global climate change. Thus, investigating the role of soil enzyme activities during the aggradation of afforested ecosystems in SR will help to address this knowledge gap.

Plant communities and litter can affect soil respiration rates through nutrient availability [24]. Additionally, ecological stoichiometry is usually regarded as an indicator of microbial nutrient requirements and nutrient availability, especially for C, nitrogen (N), and phosphorus (P) content in microbial biomass [25]. Plant communities also affect soil respiration by plant-trait driven shifts in microbial biomass C, N, and P contents and stoichiometric ratio. Although results from previous studies suggest that ecological stoichiometry, especially the C:N:P ratios of organisms and substrates, could be used as a tool to acquire knowledge to the cycling of these elements [26,27], the question remains as to how plant communities influence SR through changes in the stoichiometry of soil microbial biomass during the aggradation of afforested ecosystems. We raise this question because changes in the plant community composition during aggradation generally produce more litter with higher N content, which is more easily degraded by soil microbes [28,29]. In turn, microorganisms consume nutrients in excess amounts and store them in the form of glycogen or polyphosphates, because afforestation leads to changes in their biomass C:N:P ratio [27,30]. Consequently, understanding how shifts in plant community composition affect SR due to changes in the C:N:P ratio of microbial biomass is key to predicting the dynamics of terrestrial C under future climate change. Thus, to advance our understanding of plant-soil interactions during the aggradation of afforested ecosystems, more information about the regulation of terrestrial C dynamics by plant community through shifts in microbial biomass C, N, and P stoichiometry is needed.

The Loess Plateau, which covers approximately 640,000 km^2 in China, has experienced severe soil erosion and decreased vegetation cover [31]. The abandonment of farmland with slopes >15°

to allow for afforestation is an important management practice to prevent soil erosion and restore ecosystems native to this area [32]. In recent years, numerous studies have been conducted to examine the effects of afforestation on soil physicochemical properties, microbial dynamics, and soil enzyme activities [33–35]. However, information on the relationships among understory plant communities, SR, soil enzymes, and microbial biomass nutrient stoichiometry is scarce. Therefore, we investigated plant community composition, SR, soil enzyme activities, and microbial biomass C, N, and P stoichiometry at three forest stands (aged 15, 25, and 40 years) with *Robinia pseudoacacia* L. (RP) succession after afforestation of former farmland (FL) in the Loess Plateau. We hypothesized that SR changed with plant community traits as aggradation progressed, and that this change in soil respiration was stimulated by the soil C, N, and P stoichiometry of microbial biomass and soil enzymes. The objectives of this study were to (i) evaluate the changes in plant community traits after afforestation, (ii) characterize the changes in soil enzymes and microbial biomass C, N, and P stoichiometry after afforestation, and (iii) demonstrate the relationships between plant community traits, soil enzyme activities, and soil microbial biomass C, N, and P stoichiometry after aggradation of the afforested farmland.

2. Material and Method

2.1. Study Area

The study was conducted at Wuliwan Watershed, Ansai County, Shaanxi Province, northern China (36°46′42″–36°46′28″ N, 109°13′46″–109°16′03″ E) (Figure 1). This area is a fragile, semiarid ecosystem, and has one of the largest global loess areas [35]. The average monthly temperature ranges from −6.2 °C in January to 37.2 °C in July, with a mean annual temperature of 8.8 °C and mean annual precipitation of 505 mm [36]. In this region, the growing season for deciduous species occurs from April to October [37]. The soil is highly erodible, and classified as loessial soil (Calcaric Cambisols, WRB classification, 2014) (Table 1). The dominant tree species in this area is *R. pseudoacacia* L., which was replanted on farmland. The main crop species is *Setaria italica* (L.) P. Beauvois (millet). Water resources for crop growth are dependent entirely on rainfall; irrigation is not practiced during the growing season. The Wuliwan catchment is an experimental site of the Chinese Academy of Science (CAS), and vegetation restoration has been implemented due to serious soil degradation since the 1970s. After 30 years of afforestation, the area of forest has increased significantly from 5% to 40% [38]. Prior to afforestation, all land-use types were essentially farmland, which had been subjected to similar farming practices for more than 20 years with millet and soybean rotations [39]. The understory *Stipa bungeana* Trin. community is the most extensive species in afforested sites. *Stipa grandis* P.A.Smirn. and *Pinus bungeana* Zucc. are the dominant grass species, while *Thymus mongolicus* Ronn. and *Artemisia sacrorum* Ledeb. are the dominant forb species (Table 1).

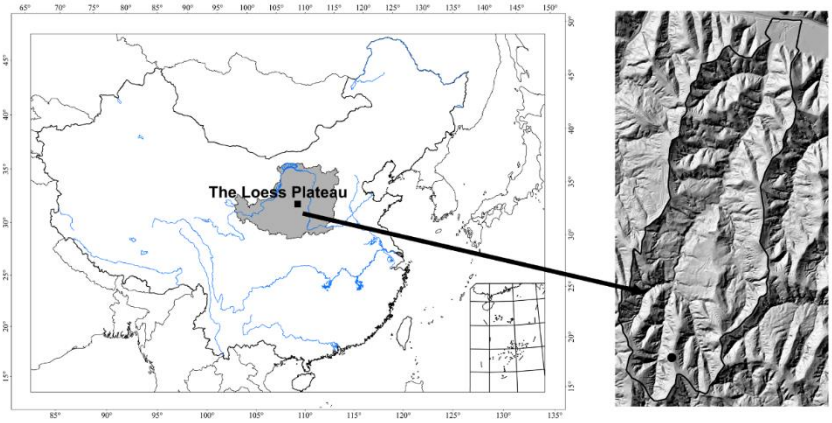

Figure 1. Location of the Loess Plateau and the study site.

Table 1. Geographical information for the four *R. pseudoacacia* L. sites.

Sites	*Farmland*	***Robinia pseudoacacia* Linn. (RP15 year)**	***Robinia pseudoacacia* Linn. (RP25 year)**	***Robinia pseudoacacia* Linn. (RP40 year)**
Elevation (m)	1205	1303	1298	1293
Clay (%)	8.12 ± 0.21 A	8.55 ± 0.14 A	9.54 ± 0.13 A	10.11 ± 0.12 A
Silt (%)	60.45 ± 0.22 A	62.53 ± 0.19 A	60.36 ± 0.19 A	64.35 ± 0.21 A
Sand (%)	31.43 ± 0.10 A	28.92 ± 0.09 A	30.10 ± 0.18 A	25.54 ± 0.11 A
Dominate species		*Artemisia capillaries* Thunb., *Heteropappus altaicus* (Willd.) Novopokr., *Stipa bungeana* Trin., *Oxytropis bicolor* Bunge., *Cleistogenes squarrosa* (Trin.) Keng	*Artemisia capillaries* Thunb., *Heteropappus altaicus* (Willd.) Novopokr., *Stipa bungeana* Trin., *Salsola collina* Pall., *Oxytropis bicolor* Bunge., *Cleistogenes squarrosa* (Trin.) Keng	*Artemisia capillaries* Thunb., *Heteropappus altaicus* (Willd.) Novopokr., *Artemisia sacrorum* Ledeb., *Stipa bungeana* Trin., *Oxytropis bicolor* Bunge., *Cleistogenes squarrosa* (Trin.) Keng

Capital letters indicate significant difference among different land use types ($p < 0.05$); the error bars.

2.2. Experimental Design

Experiments were carried out in June and October, 2014. Based on land use history, three afforested lands, *R. pseudoacacia* L. (RP40), *R. pseudoacacia* L. (RP25), and *R. pseudoacacia* L. (RP15), as well as farmland (FL), were selected. In each different aged stand, three plots with similar slope, gradient, and altitude [38,39] were established. In total, 12 plots (four land use types × three replicate plots) were setup in the study area. In addition, six quadrats (0.5 m × 0.5 m) (three trenched and three untrenched quadrats) were randomly established within the replicate plots, and the trenches (0.5 m wide and 0.8 m deep) were excavated in October 2013. After covering the trenches with a 2 mm thick plastic sheet, we refilled them with soil.

2.3. Soil Respiration Measurement and Soil Sampling

Polyvinyl chloride collars (PVC; 16 cm in diameter × 12 cm in height) were used to measure soil respiration, as described in our previous study [22]. In the experiment sites, six PVC collars were installed to a depth of 10 cm. Three PVC collars in trenched quadrats were used to measure the soil heterotrophic respiration (HR). The other three PVC collars in untrenched quadrats were used to measure SR. In June and August, 2014, AR and HR were measured on a single rain-free day between 9:00 and 11:00 a.m., using the portable soil CO_2 flux system (GXH-3010E1, LI-COR Inc., Lincoln, NE, USA) (μmol CO_2 m^{-2} s^{-1}), and obtained from consecutive 2- or 3-day measurements to represent the average monthly soil respiration. Finally, the three respiration rate observations were averaged to obtain the results for a given plot for both AR and HR.

After removing the litter layer, soil samples were collected at 0–10 cm soil depth using a soil auger (diameter 5 cm) from ten points within an "S" shape in each subplot, and then homogenized to provide one final soil sample per subplot. Overall, 12 samples (four stand age types × three plots) were collected. The samples were sieved through a 2 mm screen, and roots and other debris were removed [39,40]. A fraction of each soil sample was air dried and stored at room temperature prior to analysis of its properties, including water content (SWC) and pH. The other portion of each soil sample was immediately transported to the laboratory (on ice, and then stored at −80 °C) for microbial biomass carbon (MBC), nitrogen (MBN), phosphorus (MBP), and enzymatic assay analyses.

2.4. Analysis of Soil Properties and Enzymes Activities

SWC was determined by oven drying to a constant mass at 105 °C. BD was taken by undisturbed soil and calculated from the gravimetric weight of the cores (using 100 cm^3 cores with a height of 5 cm) before and after oven drying at 105 °C for 24 h from the individual core volume, while soil pH was measured using a pH meter after shaking the soil water (1:5 w/v) suspension for 30 min [22,39]. MBC, MBN, and MBP were estimated from fresh soil samples using a chloroform fumigation-extraction method [39]. Soil catalase, saccharase, urease, and alkaline phosphatase activities were determined as described in our previous study [40].

2.5. Plant Species Identification and Species Diversity Index

Plant species identification was done in situ in June and October of 2014, which was described in our previous study [41]. Five 1 m × 1 m quadrats were established in each replicate plot in June and October, respectively (3 stand age × 3 replicates × 5 quadrats, a total of 45 quadrats at each site in one season). Vegetation surveys of herbaceous plants in the plantation understory were done by tallying stem quantity and plant height for each species. Plant coverage of herb layers was visually estimated using a metal frame of 1 m × 1 m with 100 equally distributed grids above the subplot, and then understory coverage was calculated as the average percentage of ground surface area covered by the shadow of the foliage in each quadrat [41].

Species richness is the number of species in each quadrat [41]. The Richness index (R) was calculated as the total number of species in each community (S), Shannon-Wiener diversity index (H), and Evenness index (E) of the afforested and abandoned land plant communities were calculated using the following equations:

$$\mathrm{H} = \sum_{i=1}^{S} (P_i ln P_i) \quad (1)$$

$$\mathrm{E} = \frac{\mathrm{H}}{ln\mathrm{S}} \quad (2)$$

where S = total number of species in each community, H = Shannon-Wiener diversity index, P_i = density proportion of species "i", ln = natural log.

2.6. Statistical Analyses

All statistical analyses were carried out using SPSS for Windows (version 17.0, SPSS Inc., Chicago, IL, USA). Analysis of variance (ANOVA) and Duncan's Multiple Range Test (DMRT) at 5% level of significance were used to compare the differences in plant community coverage, height, and plant density; R, H, and E indexes; MBC:MBN, MBC:MBP, and MBN:MBP ratios; and soil catalase, saccharase, urease, and alkaline phosphatase activity among different sites. Spearman's rank correlation coefficients were used to investigate the relationships among the plant and soil characteristics at each site. In addition, we used the Beerkan Estimation of Soil Transfer (BEST PRIMER-E, Plymouth, UK) model building procedure, which utilizes all possible combinations of factors to determine which combination of factors (Coverage, Evenness, Diversity, Richness, catalase, alkaline phosphatase, urease, saccharase, MBC:MBN, MBC:MBP, MBN:MBP) account for the greatest proportion of SR (SR, AR and HR). The factors additions were evaluated stepwise and were based on sufficient improvement in the model's R value.

3. Results

3.1. Changes in Soil Properties and Plant Community Traits after Afforestation

We found that after afforestation, soil bulk density (SBD) and Water Holding Capacity (WHC) increased significantly. SBD was higher at RP40 than RP25, RP15, and FL by 3.33%, 5.98%, and 8.77%. WHC was higher at RP40 than RP25, RP15, and FL by 43.79%, 54.48%, and 136.90%. For the increase of humus, pH value was decreased, and the soil was gradually acidic (Table 2). Understory plants showed remarkable variability during aggradation in our study (Figure 2). We found that after afforestation and during aggradation, plant coverage and plant R, E, and H indices increased. These parameters were higher at RP40 than at RP15 both in June and in October. Compared to RP15, plant coverage and plant R, E, and H indices were higher at RP25 by 26.69%, 20.00%, 14.72%, and 8.28% in June and by 27.15%, 89.45%, 12.47%, and 16.72% in October, respectively.

Table 2. Soil properties after afforestation.

Sites	SBD (g·cm^{-3}) [a]	pH	WHC (%) [b]
Farmland	1.14 ± 0.02 A	9.38 ± 0.01 A	10.27 ± 0.74 C
R. pseudoacacia (RP15 year)	1.17 ± 0.01 A	8.67 ± 0.11 A	15.75 ± 0.98 B
R. pseudoacacia (RP25 year)	1.20 ± 0.01 A	8.65 ± 0.01 A	16.92 ± 0.79 B
R. pseudoacacia (RP40 year)	1.24 ± 0.01 A	8.48 ± 0.02 A	24.33 ± 1.21 A

[a] SBD is soil bulk density; [b] WHC is Water Holding Capacity Note: ±SE, Capital letter represents significant difference among sites.

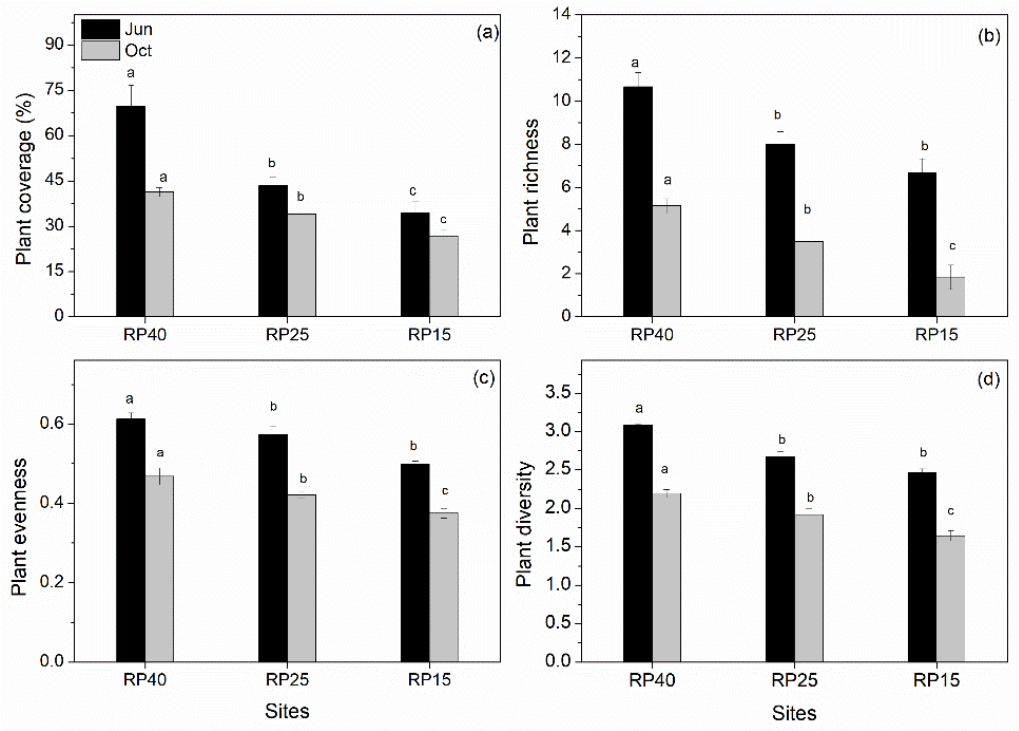

Figure 2. Plant community characteristics ((**a**) plant coverage, (**b**) plant richness, (**c**) plant evenness, (**d**) plant diversity) after afforestation. Different letters denote significant ($p < 0.05$) differences among sites in same month; error bar represents standard error. Note: 40, 25, and 15 years of *Robinia pseudoacacia* L. indicated as RP40, RP25, and RP15.

3.2. Changes in Soil Respiration and Its Components after Afforestation

Significant differences were found in SR, HR, and AR (Figure 3). SR at RP40, RP25, and RP15 was significantly higher than that in FL in June and in October. HR increased as aggradation progressed at afforested sites, with HR at RP40 being 23.68% and 40.06% higher than that at RP25 and RP15 in June, and 14.46% and 90.56% higher in October, respectively. Meanwhile, AR increased following afforestation and yielded average values of 0.54 μmol CO_2 m^{-2} s^{-1} in June and 0.73 μmol CO_2 m^{-2} s^{-1} in October.

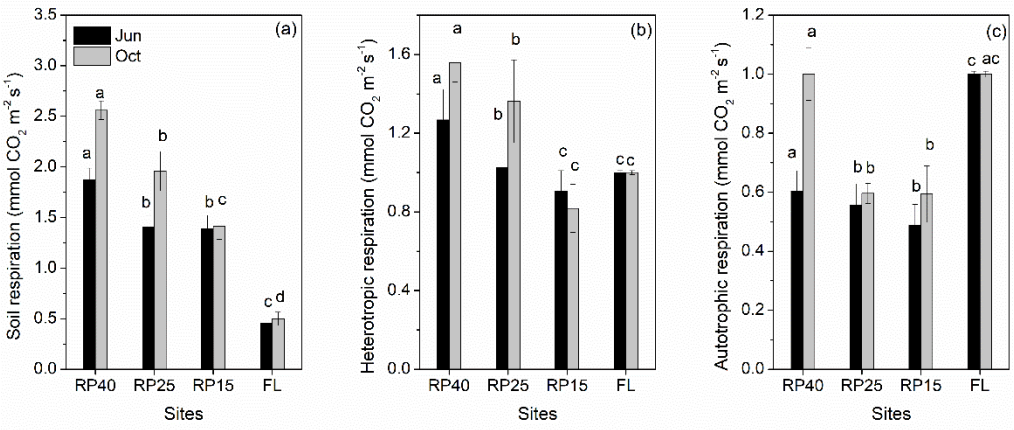

Figure 3. Soil respiration (**a**) and its components ((**b**) soil heterotrophic respiration, (**c**) soil autotrophic respiration) after afforestation. Different letters denote significant ($p < 0.05$) differences among sites in same month; error bar represents standard error. Note: 40, 25, and 15 years of *Robinia pseudoacacia* L. indicated as RP40, RP25, and RP15.

3.3. Changes in Soil Enzyme Activities after Afforestation

Changes in soil enzyme activities are shown in Figure 4. Catalase, saccharase, urease, and alkaline phosphatase contents increased following afforestation. For example, catalase, alkaline phosphatase, urease, and saccharase activities at RP40 were higher than at RP25 in June and October, respectively. Catalase, alkaline phosphatase, urease, and saccharase activities at RP25 were higher than those at RP15 in June and October, respectively. Compared with FL, the average increases of catalase, alkaline phosphatase, urease, and saccharase activities in RP sites were higher in June and October.

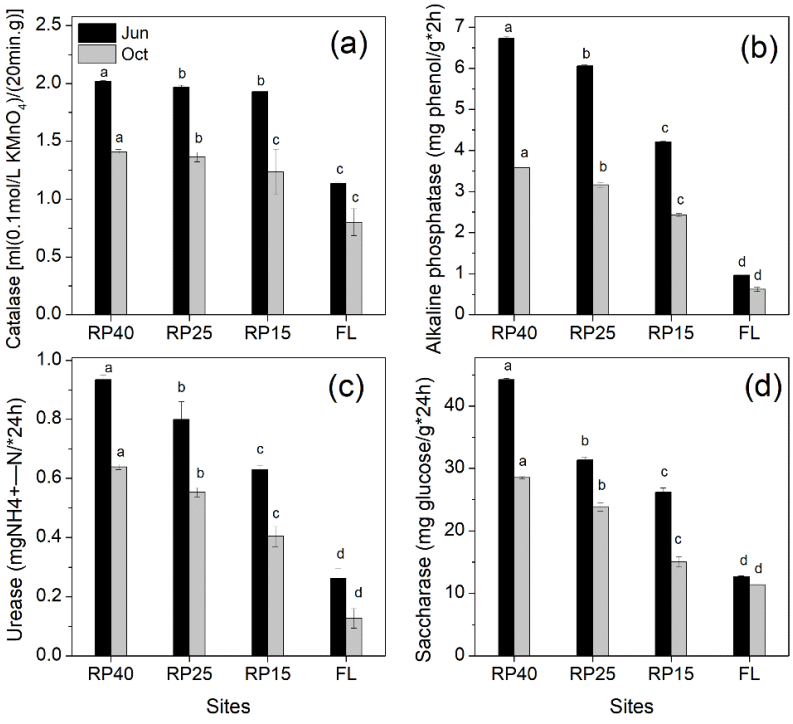

Figure 4. Soil Catalase enzyme activities (**a**), Alkaline phosphatase enzyme activities (**b**), Ureas enzyme activities (**c**) and Saccharase enzyme activities (**d**) after afforestation. Different letters denote significant ($p < 0.05$) differences among sites in same month; error bar represents standard error. Note: 40, 25, and 15 years of *Robinia pseudoacacia* L. indicated as RP40, RP25, and RP15.

3.4. Changes in Soil Microbial Biomass C, N, and P Contents and Its Stoichiometry after Afforestation

Soil microbial biomass C, N, and P contents and its stoichiometry responded differently during aggradation at the afforested sites (Figure 5). The results showed that MBC, MBN, and MBP contents increased significantly at RP40, compared with FL, by 581.7%, 231.5%, and 204.9% in June, and 347.1%, 215.2%, and 113.8% in October, respectively. Further, MBC, MBN, and MBP contents at RP40 were higher than at RP25 and RP15 by approximately 57.62%, 30.90%, and 36.99% in June, and 45.44%, 32.02%, and 18.79% in October, respectively. In addition, MBC:MBN, MBC:MBP, and MBN:MBP ratios were also significantly higher after afforestation. Compared with FL, MBC:MBN, MBC:MBP, and MBN:MBP ratios at RP40 were higher by 105.7%, 127.8%, and 10.69% in June, and 41.96%, 105.4%, and 44.16% in October, respectively. However, among the afforested sites, there were no significant trends observed in these ratios.

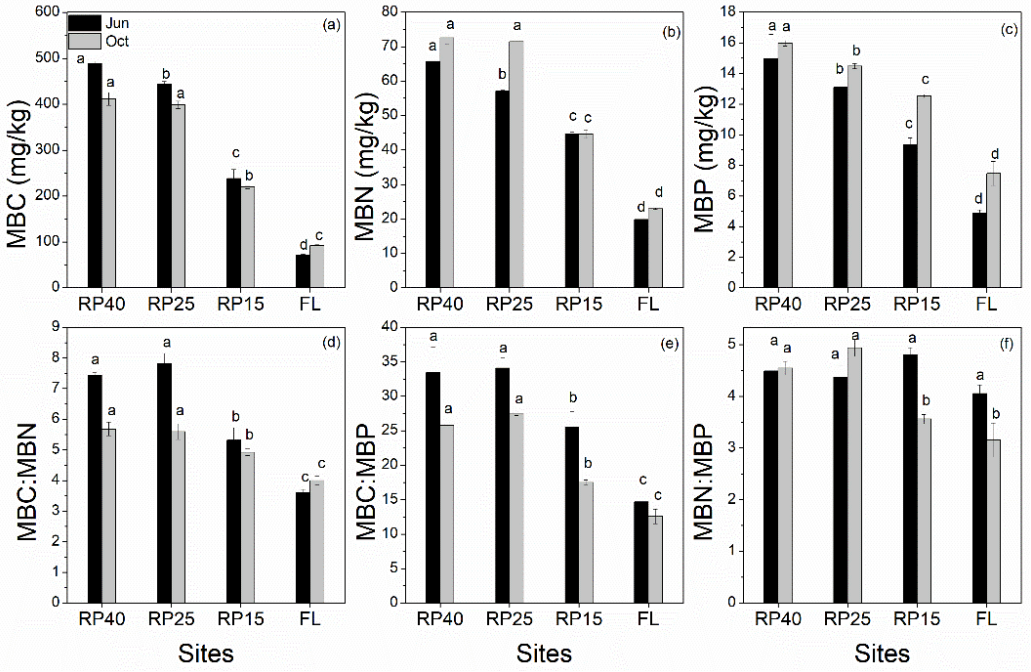

Figure 5. Soil microbial C (**a**), N (**b**), P (**c**) and soil MBC: MBN (**d**), soil MBC: MBP (**e**) and soil MBN: MBP (**f**) after afforestation. Different letters denote significant ($p < 0.05$) differences among sites in same month and error bar represents standard error. Note: 40, 25, and 15 years of *Robinia pseudoacacia* L. indicated as RP40, RP25, and RP15.

3.5. Relationships between Plant and SR Linked to Microbial Biomass C, N, and P Contents and Its Stoichiometry and Soil Enzyme Activities after Afforestation

Spearman's rank correlation coefficients also showed significant relationships among microbial biomasses (C, N, and P), their stoichiometries, soil enzyme activities, and soil respiration components (Table 3). Linear regression results were observed between most of these parameters ($p < 0.05$) (Figure 6), especially for plant coverage (Table 4). The results showed that changes in SR and its components were significantly correlated with catalase, saccharase, urease, alkaline phosphatase, and microbial biomass C, N, and P contents, and MBC:MBN, MBC:MBP, and MBN:MBP ratios ($p < 0.05$). In addition, after performing a "best" model building procedure, we found that urease, MBP, and MBC:MBP ratio were the best predictive factors influencing SR (Table 5).

Table 3. Spearman's rank correlation coefficients between the microbial biomass (C, N, and P) and its stoichiometry (MBC:MBN, MBC:MBP and MBN:MBP) and the soil enzyme actives (catalase, saccharase, urease, and alkaline phosphatase), as well as soil respiration components.

	Soil Enzyme Actives					
	catalase		Alkaline phosphatase		urease	saccharase
SR	−0.653 **		−0.649 **		−0.677 **	−0.757 **
HR	−0.520 *		−0.518 *		−0.410	−0.558 *
AR	−0.595 **		−0.649 **		−0.774 **	−0.717 **
	MBC	MBN	MBP	MBC:MBN	MBC:MBP	MBN:MBP
SR	0.42	0.582 *	0.676 **	0.613 **	−0.695 **	0.447
HR	0.553 *	0.364	0.517 *	0.658 **	−0.699 **	0.481 *
AR	0.24	0.586 *	0.568 *	0.620 **	−0.717 **	0.478 *

Soil respiration (SR); Soil heterotrophic respiration (HR); Soil autotrophic respiration (AR); Microbial biomass carbon (MBC); nitrogen (MBN); phosphorus (MBP); Microbial biomass carbon and nitrogen ratio (MBC:MBN); Microbial biomass carbon and phosphorus ratio (MBC:MBP); Microbial biomass nitrogen and phosphorus ratio (MBN:MBP) ** Correlation is significant at the 0.01 level. * Correlation is significant at the 0.05 level.

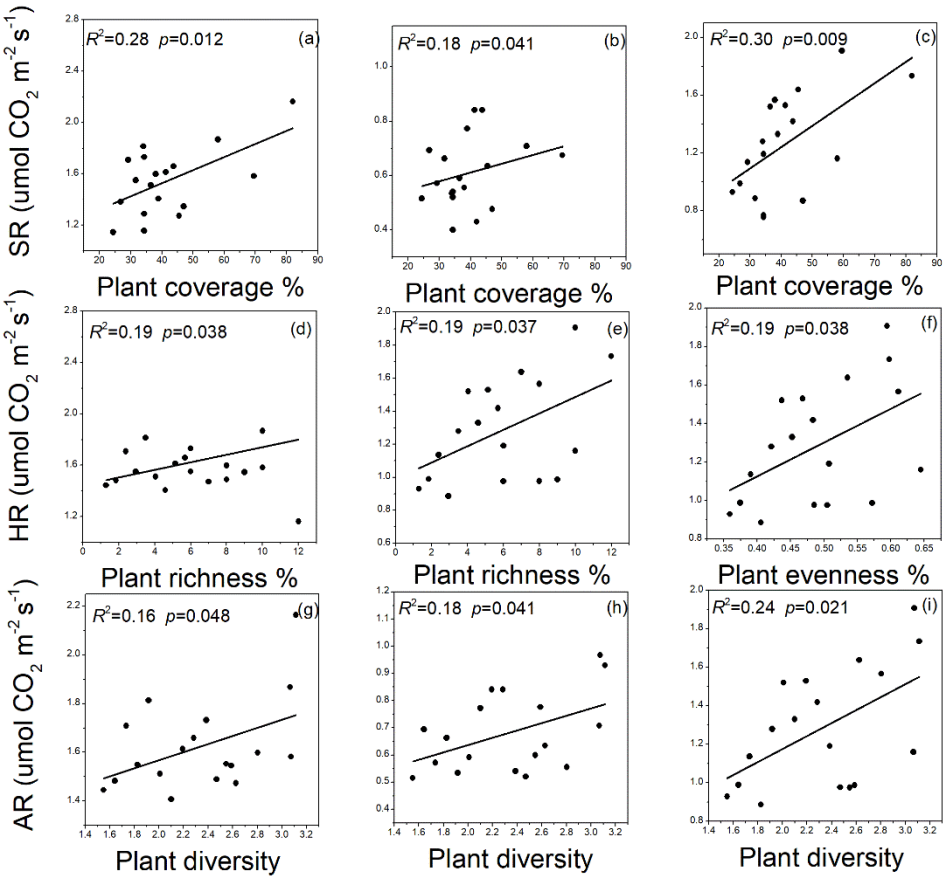

Figure 6. Linear Regression between SR and plant coverage in RP40 (**a**), RP20 (**b**), and in RP15 (**c**); between HR and plant richness in RP40 (**d**), RP20 (**e**), and in RP15 (**f**); between AR and plant diversity in RP40 (**g**), RP20 (**h**), and in RP15 (**i**); Soil respiration (SR); Soil heterotrophic respiration (HR); Soil autotrophic respiration (AR).

Table 4. Results from the BEST model for each number of predictor variables.

Number Variables	*R*	Predictor Variables **
1	0.142	Coverage
2	0.116	Coverage, Evenness
3	0.093	Coverage, Evenness, Diversity
4	0.037	Coverage, Evenness, Diversity, Richness

Number of permutations: 999 (random sample). ** Significance level of sample statistic: 0.1%.

Table 5. Results from the BEST model for each number of predictor variables.

Number Variables	Soil Enzyme Actives		Soil Microbial C, N, P		Soil Microbial Stoichiometry	
	R	Predictor Variables	*R*	Predictor Variables	*R*	Predictor Variables
1	0.131	urease	0.112	MBP	0.136	MBC:MBP
2	0.126	alkaline phosphatase, urease	0.158	MBP	0.193	MBC:MBP, MBN:MBP
3	0.118	catalase, alkaline phos-phatase, urease	0.138	MBP, MBN	0.132	MBC:MBP, MBN:MBP
4	0.036		catalase, alkaline phosphatase, urease, saccharase			

Number of permutations: 999 (random sample). Microbial biomass carbon (MBC); nitrogen (MBN); phosphorus (MBP); Microbial biomass carbon and nitrogen ratio (MBC:MBN); Microbial biomass carbon and phosphorus ratio (MBC:MBP); Microbial biomass nitrogen and phosphorus ratio (MBN:MBP).

4. Discussion

In line with our expectations, plant diversity and coverage significantly influenced SR across our study sites. The most important factors influencing SR were changes in C inputs into soil [7]. These changes in C inputs have been observed by species diversity experiments in grasslands, which documented that more diverse grasslands were more productive [42], and that C inputs further stimulated SR. However, increased plant diversity also showed increased use efficiency [1]. This reduction in N concentration in the soil organic matter may negatively affect SR, including AR and HR [10,13]. On the other hand, understory plant characteristics can have important and diverse effects on soil properties, microclimates, and SR [2]. In particular, the effects of plants on soil temperature and water content are important, because they are drivers of microbial activity and SR [43,44]. For example, a previous study showed that plant canopies often reduce ground level radiation and soil evaporation rates, resulting in lower soil temperature and greater soil moisture content [45]. Therefore, a higher availability of plant-derived resources has a strong and positive affect on SR following afforestation.

The effects of plant diversity on SR may be more pronounced in the presence of soil enzymes [46], which is consistent with our result that species richness mediate soil respiration, partly through changes in soil enzyme activities. Several previous studies also supported our result that soil enzyme activity might significantly affect SR in terrestrial ecosystems [47,48]. For example, Chen [15] reported that changes in microbial enzymatic activities might be the basically drivers of SR, because more than 50% of SR is came from enzyme-related decomposition of litter and SOM. Ren [9] observed that C-degrading extracellular enzyme activities were significantly correlated with SR. A possible explanation was that the litter inputs during afforestation not only provide the building blocks for enzymatic production, because enzymes are basically N-rich molecules, but also increase microbial C demands due to stoichiometry of microbial biomass nutrients [49]. Consequently, increases in microbial C demands were expected to be alleviated by promoting the activities of C-degrading enzymes, influencing SR [50].

More importantly, soil microbial biomass C, N, and P content and its stoichiometry increased significantly with increasing plant diversity in both months of our study. These findings were consistent with previous studies, which showed that the positive effects of plant diversity on soil microbial biomass and functions led to changes in SR [25,51]. In particular, HR came from the microbial

decomposition of root exudates in the rhizosphere. Increased microbial biomass C, N, and P content and its stoichiometry could augment labile soil carbon [52,53], alter microbial communities [9,10], or increase soil moisture [6,54], all of which can impact SR. However, our results contrasted with those of Cong [4] who reported that no significant difference was observed in SR between plant community traits and species richness. This was probably a consequence of the low soil fertility and dry conditions at the time of sampling in their experiment [4]. Therefore, plant species diversity may exceed plant production as a driver of the shifts in soil microbial biomass and stoichiometry in the long term by providing more diverse plant-derived resources, and thus, potentially influence SR. In addition, the species composition of plant communities influences microbial biomass C, N, and P content and stoichiometry, which can lead to changes in microbial bioenergetics and qCO_2. Consequently, changes in these factors influence SR, especially microbial respiration. This conclusion was supported by a previous study that reported the qCO_2 increase with soil and litter carbon to nutrient ratios and underlying stoichiometric controls [55], which may be because microbes in soils with lower microbial C:N ratios have higher growth efficiency and lower release of C through respiration. Conversely, microbes in soils with higher microbial C:N ratios have more C available to be converted into biomass [56–59]. Therefore, changes in plant communities influences the soil microbial C, N, and P stoichiometry, and are important drivers of the trends in microbial bioenergetics and respiration rates of soil microorganisms per unit microbial biomass.

In summary, understory vegetation affects SR through many mechanisms. However, the most important factor is the quantity and quality of organic inputs into the soil from plants. Our results indicate that plants regulate C dynamics through changes in soil enzyme activities and microbial biomass C, N, and P content and stoichiometry. This study has highlighted the role of plants within the plant-soil system, and the fact that plants are necessary for better understanding and simulating the patterns of SR across terrestrial ecosystems.

5. Conclusions

Our study showed that changes in understory plant community traits (coverage, evenness, diversity, and richness), soil respiration and its components, the activities of four enzymes (catalase, saccharase, urease, and alkaline phosphatase), and soil microbial biomass C, N, and P contents and its stoichiometry were significantly driven by afforestation and the aggradation of afforested sites. Plant community traits mediated SR through changes in soil enzyme activities and soil microbial biomass C, N, and P contents and its stoichiometry. Therefore, our results highlight the importance of understory plant community traits in regulating belowground carbon dynamics, and suggested that plant traits, especially plant coverage, altered the components of soil respiration by affecting soil enzyme activities and soil microbial biomass C, N, and P contents and its stoichiometry.

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