Ecological stoichiometry of plant-soil-enzyme interactions drives secondary plant succession in the abandoned grasslands of Loess Plateau, China

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ABSTRACT

Ecological stoichiometry is the study of the interaction and balance of multiple chemical elements in ecological processes. However, how ecological stoichiometric interactions among plants, soils, and enzymes in the rhizosphere affect secondary plant succession is largely unknown. In this study, we collected the dominant and main companion species *Artemisia capillaris*, *A. sacrorum*, and *Stipa bungeana*, along a secondary succession chronosequence (7, 12, 17, 22, and 32-year) following a cropland abandonment in the Loess Plateau, China. We measured the carbon (C), nitrogen (N) and phosphorus (P) concentrations of plant shoots and roots, as well as the concentrations of C, N, P, available N (aN), available P (aP), and the activity of one C-acquiring enzyme (β-glucosidase (BG)), two N-acquiring enzymes (N-acetylglucosaminidase (NAG), leucineaminopeptidase (LAP)), and one P-acquiring enzyme (alkaline phosphatase (AP)) found in the rhizospheric soil to explore the C:N:P stoichiometry that drives secondary plant succession. The C:P and N:P ratios in the shoots of dominant plant species significantly decreased to a minimum value at the 22-year site and then increased with plant secondary succession. Compared with the 7-year site, the rhizosphere soil C:P and N:P ratios, and enzyme N:P ratios (ln (NAG + LAP):ln(AP)) increased 103.6%, 72.0%, and 221.3%, respectively, but enzyme C:N ratios (ln(BG):ln (NAG + LAP)) decreased 48.2% in the dominant plant species at the 32-year site. Principal component analysis indicated that the stoichiometry characteristics of the plant-soil-enzyme continuum differed for each plant species and succession stage. Stoichiometric homeostasis indices for the plant species indicated a relatively strong homeostasis, while redundancy analysis revealed that the variations in soil BG, NAG, and AP activity and enzyme C:N ratio had significant effects on the stoichiometries and nutrient concentrations of the plant tissues. The results indicated that rhizosphere stoichiometry is a powerful tool for evaluating plant-soil interactions in terrestrial ecosystems and that the variation in rhizospheric soil enzyme activity driving the secondary succession of plants.

1. Introduction

Harnessing the restorative processes of the secondary succession of plants is an effective way to improve and restore degraded ecosystems (Lozano et al., 2014; Zhang et al., 2015). Plants can improve soil nutrient availability through interactions with the rhizosphere microbial community that alter soil nutrient status and ultimately facilitate organic substrate decomposition (Bezemer et al., 2010; Eisenhauer et al., 2010). These interactions among plants, mineral soils, and soil enzymes contribute to broad patterns of nutrient cycling and plant succession (Bell et al., 2014; Zechmeister-Boltenstern et al., 2015).

Hence, a holistic grasp of the plant-soil-enzyme continuum will greatly improve our mechanistic understanding of how plant species occurrence and distribution change throughout secondary succession.

Ecological stoichiometry facilitates the understanding of the biogeochemical cycles of multiple elements and is particularly useful for establishing linkages among different ecosystem components such as soils, plant tissues, and microbes (Elser et al., 2000; Zechmeister-Boltenstern et al., 2015; Yang et al., 2018; Li et al., 2019). Soil and plant ecological stoichiometries were found to be tightly coupled in the age sequence of Eucalyptus plantations in a subtropical ecosystem (Fan et al., 2015), the *Caragana korshinskii* plantations on the Loess Plateau

([Zeng et al., 2017](#)), and the chronosequence of *Robinia pseudoacacia* plantations on the Loess Plateau ([Zhang et al., 2019](#)). Although previous studies have improved our understanding of carbon (C) and nutrient cycling, research at the ecosystem level is not sufficient to precisely explain small-scale heterogeneity in ecological stoichiometry ([Zuo et al., 2009; Fan et al., 2015; Xu et al., 2017](#)).

The rhizosphere, composed of tight linkages between the plant and the soil, constitutes a hotbed of nutrient circulation and conversion in the terrestrial ecosystems ([Lambers et al., 2009; Finzi et al., 2015](#)). The dynamics of C and other nutrient cycling between plants and soils are regulated by enzymes that are produced by microbes and plant roots to decompose organic substrates ([Kuzyakov and Razavi, 2019](#)). Because these dynamics are dependent on the soil, microbial community, and plant species, the relationship between soil, enzyme, and plant stoichiometries within the rhizosphere is best evaluated on a plant species-by-species basis ([Hawkes et al., 2005; Diez et al., 2010](#)). [Bell et al. (2014)](#) studied the plant-soil-enzyme stoichiometry in the rhizospheres of different plant species in a semiarid grassland and found close connections between plant and soil nutrient stoichiometries that were independent of microbial stoichiometry and soil enzyme activity. In contrast, [Cui et al. (2018)](#) reported that the ecoenzymatic stoichiometry of the rhizosphere in the Loess Plateau of China was greatly affected by the different restoration plant species and soil properties. Until now, however, there has only been little investigation into the characteristics of the rhizosphere stoichiometry through series of plant secondary succession. Dramatic shifts in species abundance and composition often occur during secondary succession ([Sun et al., 2017](#)). Characterizing the plant, soil, and enzyme stoichiometries in multiple plant species' rhizospheres during plant secondary succession will advance our understanding of the heterogeneity of ecological stoichiometries, both among plant species and over ecological time scales. Furthermore, changes in the ecological stoichiometry may illustrate how biogeochemical cycles contribute to plant species succession after cropland abandonment, which has implications for vegetation restoration.

The Loess Plateau of China is characterized as a fragile ecosystem with serious soil erosion ([Fu et al., 2011; Zhao et al., 2013](#)). Restoration and erosion control are most commonly addressed by cropland abandonment and secondary succession ([Zhang et al., 2016](#)). Variations in plant community, soil nutrients, microbial properties, and enzymatic activity during secondary succession have been well documented ([Sun et al., 2017; Zhang et al., 2016; Wang et al., 2011](#)) and have demonstrated a relationship between the microbial community (microbial diversity, enzyme activity) and the soil and plant characteristics ([Xiao et al., 2013; Zhang et al., 2016; Li et al., 2019](#)). However, information on nutrient concentrations and stoichiometries of soils, plants, and enzymes—especially for individual plant species—is still scarce. Utility of rhizosphere stoichiometry for relating plant species element stoichiometry to below ground C, N and P dynamics would contribute to understand the mechanism of plant secondary succession and to accelerate restoration succession in this semi-arid ecosystem.

In this study, we investigated the relationships among nutrient stoichiometries of plant tissues, soils, and enzymes within the rhizosphere of dominant and main companion species along a secondary succession series on the Loess Plateau. We hypothesized that the C:N:P ratios of plants, rhizospheric soils and enzymes increased with plant secondary succession. Moreover, we hypothesized that the stoichiometry of plant tissues, rhizospheric soils and enzymes are strongly conserved at the plant species level, which is perhaps due to co-evolution ([Blouin, 2018](#)). Finally, due to the relatively high degree of stoichiometric homeostasis of plant species ([Elser et al., 2010; Minden and Kleyer, 2014](#)), we further hypothesized that the variations in rhizospheric soil C, N, and P concentrations and enzyme activity lead to the shifts in plant species abundance and composition during secondary succession.

2. Materials and methods

2.1. Site description

The research was conducted in the Zhifanggou watershed (36°46′N, 109°16′E) located in Ansai county, northern Shaanxi Province, China. The watershed is situated at the hilly-gully region of the Loess Plateau and has a temperate and semiarid climate that is characterized by heavy rainfall during the summer season. The average annual temperature and average annual precipitation are 8.8 °C and 549.1 mm, respectively. The rainfall in wet seasons (July to September) accounted for 70% of the annual precipitation. The soil is classified as a loessial soil that is usually described as Calcaric Cambisols according to the FAO classification system ([Jahn et al., 2006](#)).

In 1980 s, the "Green for Grain" project was implemented by the Chinese government to restore the degraded ecosystems on the Loess Plateau. Much of the sloped cropland had been gradually abandoned for natural secondary succession. After more than 30 years of vegetation restoration, a chronosequence of the vegetation succession series had been developed for the abandoned cropland. It showed that the dominant plant species changed from *Artemisia capillaris* to *A. sacrorum* after a secondary succession recovery of the abandoned cropland ([Xiao et al., 2013](#)).

2.2. Experimental design

Our research focuses on the C:N:P stoichiometry of dominant plant species and main companion species and on the rhizospheric soil nutrients and enzyme activity during the secondary plant succession. Therefore, in May 2016, five sloped croplands that were abandoned for 7, 12, 17, 22, and 32 years were selected to represent a chronosequence of secondary plant succession ([Fig. S1](#)). Within each succession year, three sampling sites measuring 100 m × 100 m at a distance of 300–500 m and having similar slope aspects, gradients, and altitude were selected. We identified dominant and main companion species along the plant succession chronosequences, and three replicate plots (20 m × 20 m) in each site were established for each plant species (dominant and main companion species). Other plant species that did not belong to the species of interest were pulled up by the roots, and PVC collars measuring 10 cm × 20 cm (diameter × height) were installed around individual plant species before conducting the soil sampling to prevent other plant roots from invading the plant rhizospheres under study. To avoid excessive soil drying within the PVC collars during the plant's growth season, a total of 20% of the annual mean precipitation was added to each core twice a month ([Bell et al., 2014; Carrillo et al., 2017](#)). Basic information regarding the sampling sites is presented in [Table 1](#).

2.3. Sampling and chemical determination

We collected rhizospheric soil and plant material within each PVC collars in September 2016. We defined rhizospheric soil as that which remained strongly attached to the roots after the plant was shaken by hand ([Garcia et al., 2005](#)). The rhizospheric soil was collected from the plant roots using a brush immediately after excavation and was subsequently sieved through a 2-mm mesh. One subsample of soil was air-dried and sieved through 1- and 0.25-mm meshes for soil chemical properties analysis. The other soil samples were stored in the refrigerator (4 °C) for two weeks for subsequent soil enzyme activity determination ([Cui et al., 2018](#)).

Plant material was sorted into shoots and roots by clipping the plants at about 30 mm above the soil surface. All tissues were dried to constant weight at 60 °C, and then ground to fine powder using a grinding miller for chemical properties analyses.

C concentrations of the samples were determined using the potassium dichromate oxidation method ([Bao, 2000](#)). Nitrogen (N) concentrations of the samples were measured using the Kjeldahl method after

Table 1
Geographic features and floristic compositions of the sampling sites.

Study sites	Slope aspects	Slope gradient	Altitude	Coordinate	Dominant species	Companion species	Minor species
7-year	W10°N	20°	1303	36°44′47″N, 109°15′12″E	*Artemisia capillaris*	-	*Heteropappus altaicus, Salsola collina*
12-year	E40°N	26°	1276	36°44′02″N, 109°16′31″E	*Artemisia capillaris*	*Artemisia sacrorum*	*Lespedeza davurica, Heteropappus altaicus*
17-year	E25°N	28°	1307	36°44′09″N, 109°16′14″E	*Artemisia sacrorum*	*Stipa bungeana*	*Artemisia capillaris, Heteropappus altaicus, Lespedeza davurica*
22-year	E10°N	30°	1267	36°44′05″N, 109°16′27″E	*Artemisia sacrorum*	*Stipa bungeana*	*Potentilla tanacetifolia, Heteropappus altaicus*
32-year	E16°N	30°	1246	36°44′15″N, 109°15′55″E	*Artemisia sacrorum*	-	*Lespedeza davurica, Potentilla tanacetifolia, Vicia sepium*

digestion by H_2SO_2 and H_2O_2. Soil and plant phosphorus (P) concentrations were measured using the molybdenum antimony reagent colorimetric method. Soil ammonia nitrogen (NH_4^+-N) and nitrate nitrogen (NO_3^--N) were extracted with 1 M KCl and measured with a continuous flow autoanalyzer (FIA Star 5000 analyzer, Foss Tecator, Sweden). We combined the soil NH_4^+-N and NO_3^--N to represent soil available N (aN) pools. Soil available P (aP) was determined through the Olsen method (Bao, 2000).

The activity of soil enzymes, including one C-acquiring enzyme (β-glucosidase, BG), two N-acquiring enzymes (N-acetylglucosaminidase, NAG; leucineaminopeptidase, LAP), and one P-acquiring enzyme (alkaline phosphatase, AP) was measured based on the method described by Saiya-Cork et al. (2002). Briefly, 1.0 g of soil was homogenized with 125 ml buffer (50 mM sodium acetate buffer) to extract the enzymes. Soil slurries (200 μl) and 50 μl of 200 μM fluorometric substrate proxies specific to each enzyme were added to a 96-well microplate. Six analytical replicates and one control (sample without the substrate) were conducted for each sample. The microplates for the determination of AP activity were placed in an incubator at 25 °C for 2 h, others were incubated for 4 h. Finally, the intensity of fluorescence was determined by a microplate reader at 365 nm excitation and 450 nm emission. Calculated soil enzyme activity was expressed as nmol h^{-1} g^{-1} soil. Moreover, the specific enzyme activity expressed as nmol h^{-1} mg SOC^{-1}, was calculated by dividing the absolute soil enzyme activity by the soil organic C concentration (Trasar-Cepeda et al., 2008). The soil enzyme C:N, C:P, and N:P ratios were calculated using ln(BG):ln(NAG + LAP), ln(BG):ln(AP), and ln(NAG + LAP):ln(AP), respectively.

2.4. Statistical methods

We used the two-way analysis of variance to analyze the effects of succession years and plant species, and their interactions on plant-soil-enzyme nutrient concentrations and stoichiometries. Then, mean comparisons were performed using the Duncan's multiple range test at a probability level of 0.05. Cross-correlation analysis was conducted to determine the degree of correlation between plant, soil, and enzyme concentrations and stoichiometries within the plant rhizosphere.

Stoichiometric homeostasis indices (1/H) with corresponding r^2 were calculated using the regression equation ($y = cx^{1/H}$), where y is the plant tissue stoichiometry and x is the rhizosphere stoichiometry, to evaluate the relationship between species-specific plant tissue stoichiometry (shoot and root) and rhizosphere stoichiometry (soil and enzyme). If the regression relationship was not significant, 1/H was set to 0 (as in Makino et al., 2003), and the plant tissue was considered as strictly homeostatic. When the regression relationship was significant, the degree of homeostasis of plant tissues was classified as: strictly homeostatic (1/H ≤ 0), homeostatic (0 < 1/H < 0.25), weakly homeostatic (0.25 < 1/H < 0.5), weakly plastic (0.5 < 1/H < 0.75), and plastic (1/H > 0.75) (Makino et al., 2003; Persson et al., 2010).

In order to explore the variations in the plant-soil-enzyme nutrients and the stoichiometry among different plant species along the secondary plant succession series, the nutrient concentrations and stoichiometries were subjected to principal component analysis (PCA) after standardization to unit variance. A redundancy analysis (RDA) was conducted to evaluate how the rhizospheric soil nutrients, enzyme activities, and their stoichiometries changed relative to the plant tissue concentrations and stoichiometries, and the significance of the soil biochemical factors was tested with Monte Carlo permutations (permutation = 999). The PCA and RDA were implemented by the Vegan and ggplot 2 packages in R (Hadley, 2007; Oksanen et al., 2013; R Core Team, 2017).

3. Results

3.1. Vegetation composition during secondary succession

During secondary succession, *Artemisia capillaris*, which is an annual herb, was the dominant species at the 7- and 12-year sites (Table 1). In the 12-year site, a perennial species, *A. sacrorum*, became an important companion species. In the 17- and 22-year sites, *A. sacrorum* replaced *A. capillaris* as the dominant species, and a number of new species, such as, *Stipa bungeana, Lespedeza davurica, Potentilla tanacetifolia,* and *Heteropappus altaicus* emerged, with *S. bungeana* as the main companion species. In the 32-year site, *A. sacrorum* completely replaced *S. bungeana*.

3.2. Ecological stoichiometry in plants along secondary succession

The C, N, and P concentrations in the shoots, and the P concentration in the roots were significantly different during the secondary succession (Fig. 1A, C, E, F). The C, N, and P concentrations in the dominant plant species first significantly decreased and then increased along the secondary successional gradients, with the lowest value at 17-year site. The C, N, and P concentrations in the shoots of *A. sacrorum* significantly decreased at first, and then increased during the secondary plant succession. Compared with the 7-year site, the P concentration in the roots of *A. capillaris* significantly decreased at the 12-year site. The P concentration in the roots of *A. sacrorum* first decreased and then significantly increased along with increasing successional ages.

The C:P and N:P ratios in the shoots and the C:P ratio in the roots were significantly affected by the plant species, while the C:P ratio in the roots significantly varied for the different succession years (Fig. 2C, D, E). The C:P and N:P ratios in the shoots of dominant plant species first significantly decreased and then increased with plant secondary succession, with the highest value at the 22-year site. Both C:P and N:P ratios in the shoots of *A. capillaris* were significantly lower than that of *A. sacrorum* and *S. bungeana*. In *A. sacrorum*, the C:P ratio in the shoots first significantly increased, and then decreased with successional ages. Compared with the 7-year site, the C:P ratio in the root of *A. capillaris* at the 12-year site was significantly increased. The C:P and N:P ratios in the roots of *A. sacrorum* similarly increased, and then significantly decreased with successional ages.
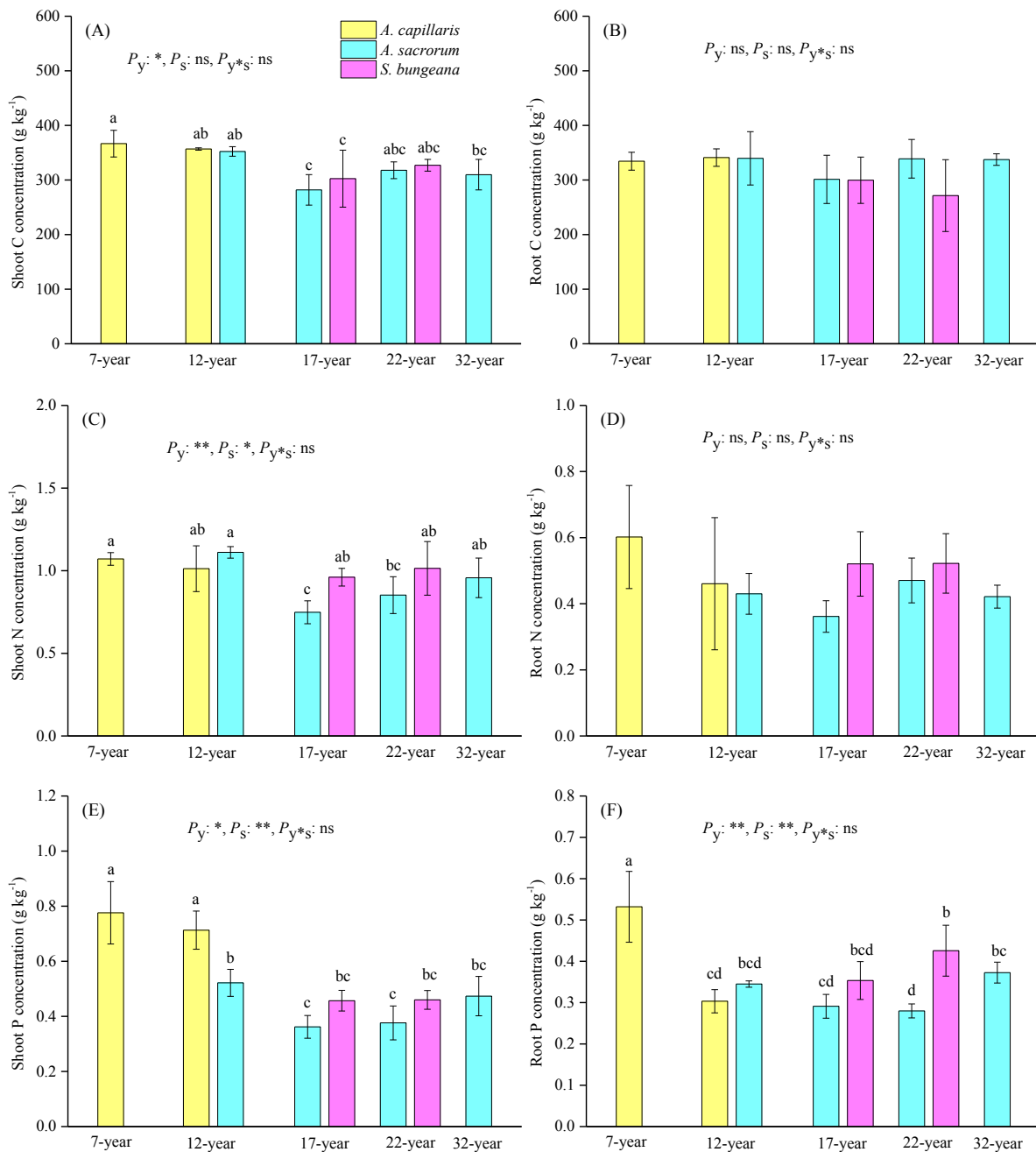
Fig. 1. C, N and P concentrations in shoots (A, C, E) and roots (B, D, F) of dominant species and companion species during secondary plant succession. Note the different scales among the graphs. Letters above the mean ± SD (n = 3) indicate significant differences among plant species at five succession years at $P < 0.05$ using Duncan post hoc tests. P_y, succession years effect; P_s, plant species effect; P_{y*s}, interaction of succession years and plant species effect; *, $P < 0.05$; **, $P < 0.01$; ns, not significant.

3.3. Ecological stoichiometry in rhizosphere soils along secondary succession

The rhizospheric soil C, N, aN, and aP concentrations differed for the successional years ($P < 0.05$ or $P < 0.001$) (Fig. 3A, B, D, E). Compared with the 7-year site, the rhizosphere soil C and N concentrations in the dominant plant species at the 32-year site increased 92.9% and 63.1%, respectively. The C and N concentrations in the rhizosphere of *S. bungeana* were not significantly different between the 17- and 22-year sites, whereas the aN and aP concentrations in the 22-year site were significantly lower than of the 17-year site. The concentration of C and N in the rhizosphere of *A. sacrorum* significantly increased with succession. The aN concentration first significantly increased, and then decreased

with plant succession gradients, while the aP concentration significantly decreased along vegetation succession gradients.

The rhizospheric soil C:P, N:P, and aN:aP were significantly different along the succession gradient (Fig. 4B, C, D). Compared with the 7-year site, the rhizosphere soil C:P and N:P ratios in the dominant plant species at the 32-year site increased 103.6% and 72.0%, respectively. Soil C:P and N:P ratios in the rhizosphere of *A. sacrorum* were significantly increased along the plant succession ages. The aN:aP ratio first significantly increased, and then decreased along the plant succession gradient, with the highest value obtained at the 22-year site.
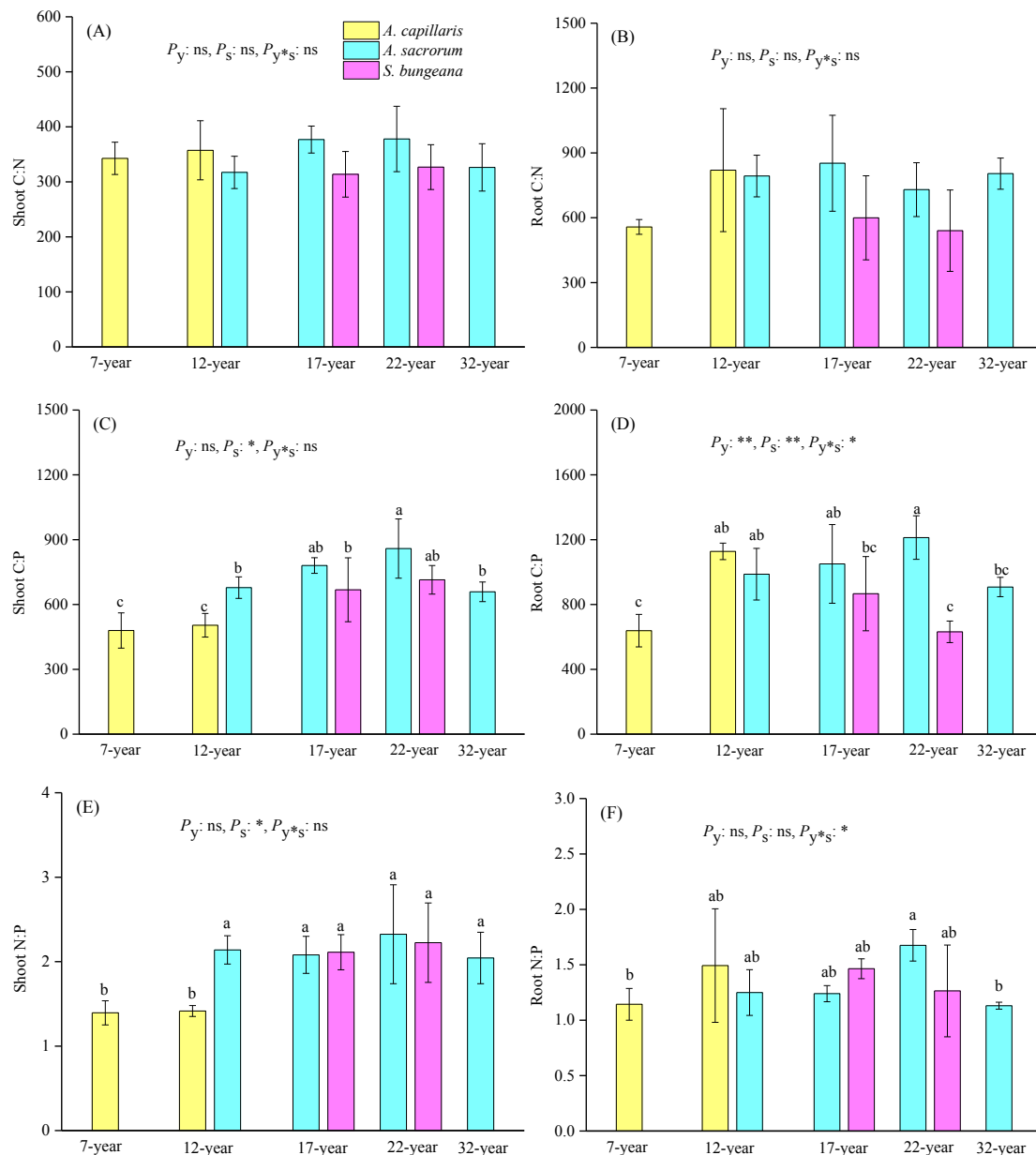
Fig. 2. Ecological stoichiometry ratios for plant tissues during secondary plant succession. Note the different scales among the graphs. Letters above the mean ± SD (n = 3) indicate significant differences among plant species at five succession years at $P < 0.05$ using Duncan post hoc tests. P_y, succession years effect; P_s, plant species effect; P_{y*s}, interaction of succession years and plant species effect; *, $P < 0.05$; **, $P < 0.01$; ns, not significant.

### 3.4. Ecological stoichiometry in rhizosphere soil enzymes

The BG, NAG, LAP, and AP enzyme activity showed significant differences along the vegetation succession ages, and the BG and LAP enzyme activity significantly varied among the different plant species (Fig. 5A, B, C, D). All the enzyme activity decreased over the course of succession, compared with the 7-year site, the rhizosphere soil BG, NAG, LAP, and AP enzyme activity in the dominant plant species at the 32-year site decreased 77.1%, 53.2%, 83.0%, and 49.2%, respectively. In the *A. capillaris* rhizosphere, BG and LAP enzyme activity was lower in the 12-year site than in the 7-year site; in the rhizosphere of *S. bungeana*, the enzyme activity of BG, LAP, and AP in the 22-year site was lower than that in the 17-year site; and in the *A. sacrorum* rhizosphere, BG, NAG, LAP, and AP enzyme activity decreased throughout the vegetation succession gradient.

Enzyme stoichiometries also differed among the different plant species and along the vegetation succession gradient ($P < 0.01$ or $P <$

0.001) (Fig. 6A, B, C). Compared with the 7-year site, the rhizosphere soil enzyme C:N decreased 48.2%, but enzyme N:P ratios increased 221.3% in the dominant plant species at the 32-year site. Enzyme C:N and C:P ratios in the rhizosphere of *A. capillaris* at the 12-year site were significantly lower than that of the 7-year site. The enzyme C:N ratio in the rhizosphere of *A. sacrorum* significantly decreased, while the enzyme C:P and N:P ratios significantly increased along the plant succession gradient.

3.5. Relationships among plant, soil, and enzyme stoichiometry

Principal component analysis showed that ecological stoichiometries vary substantially both among plant species and along the succession gradient (Fig. 7). The first and second components explained 35.73% and 16.43% of the total variance in the ecological stoichiometry, respectively. The plant-enzyme-soil stoichiometries of *A. capillaris* at the 7- and 12-year sites were significantly separated by PC2. The plant-
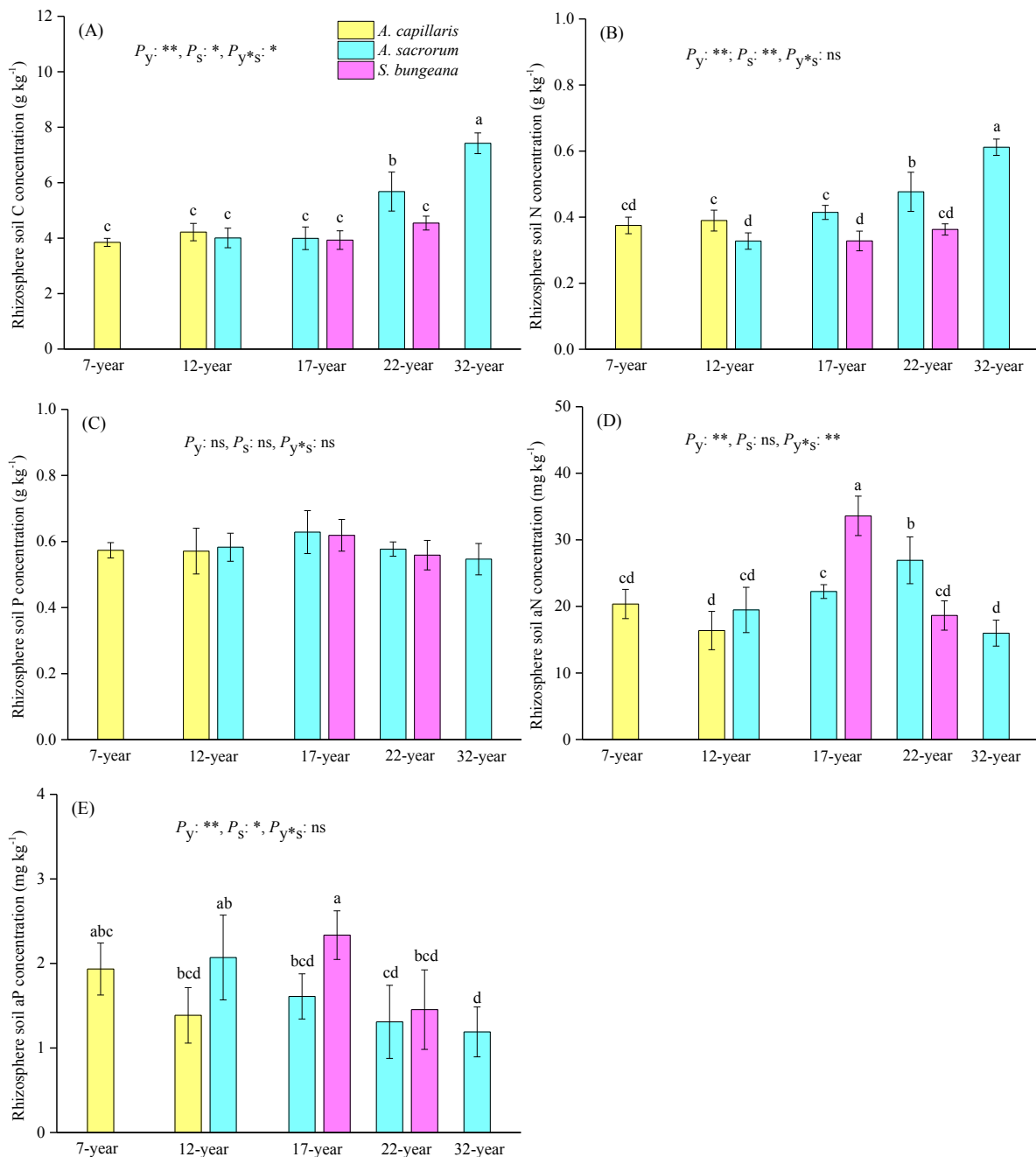
Fig. 3. Soil nutrient concentrations in the rhizosphere of the dominant species and companion species during secondary plant succession. Note the different scales among the graphs. Letters above the mean ± SD (n = 3) indicate significant differences among plant species at five succession years at $P < 0.05$ using Duncan post hoc tests. P_y, succession years effect; P_s, plant species effect; P_{y*s}, interaction of succession years and plant species effect; *, $P < 0.05$; **, $P < 0.01$; ns, not significant.

enzyme-soil stoichiometries of *A. sacrorum* at the 12- and 17-year sites were significantly separated from 22- and 32-year sites along PC1, and the plant-enzyme-soil stoichiometries of *A. sacrorum* at the 22- and 32-year sites were significantly separated by PC2.

In several cases, plant tissues and soil nutrient concentrations, enzyme activity and their stoichiometries were significantly correlated with one another (Fig. 8): shoot N was positively correlated with enzyme AP and negatively correlated with soil aN:aP ratio; shoot P was positively correlated with enzyme AP; root N was positively correlated with enzyme C:N ratio; root P was positively correlated with enzyme BG and enzyme C:N ratio; root C:N and C:P ratios were negatively correlated with enzyme C:N ratio; lastly, root N:P was positively correlated with soil aN:aP ratio. Stoichiometric homeostasis analyses revealed that most

1/H indices between plant tissue and rhizosphere stoichiometry demonstrated a non-significant relationship—indicating strong homeostasis (Table 2). Root C:N in *A. capillaris* demonstrated a strong plastic relationship with the soil C:N ratio, and root N:P ratio in *A. sacrorum* showed a weakly homeostatic relationship with soil aN:aP ratio.

Constrained axes of the RDA explained 80.97% of the variation in plant tissue nutrient concentrations and stoichiometries, with the first and second axes accounting for 42.9% and 19.0%, respectively (Fig. 9). Monte Carlo permutation tests showed that soil BG ($P = 0.035$), NAG ($P = 0.034$) and AP ($P = 0.015$) activity and enzyme C:N ($P = 0.012$) had significant effects on the plant tissue nutrient concentration and stoichiometry during plant secondary succession. Plant C, N, and P concentrations in shoot, and N and P concentrations in root were positively
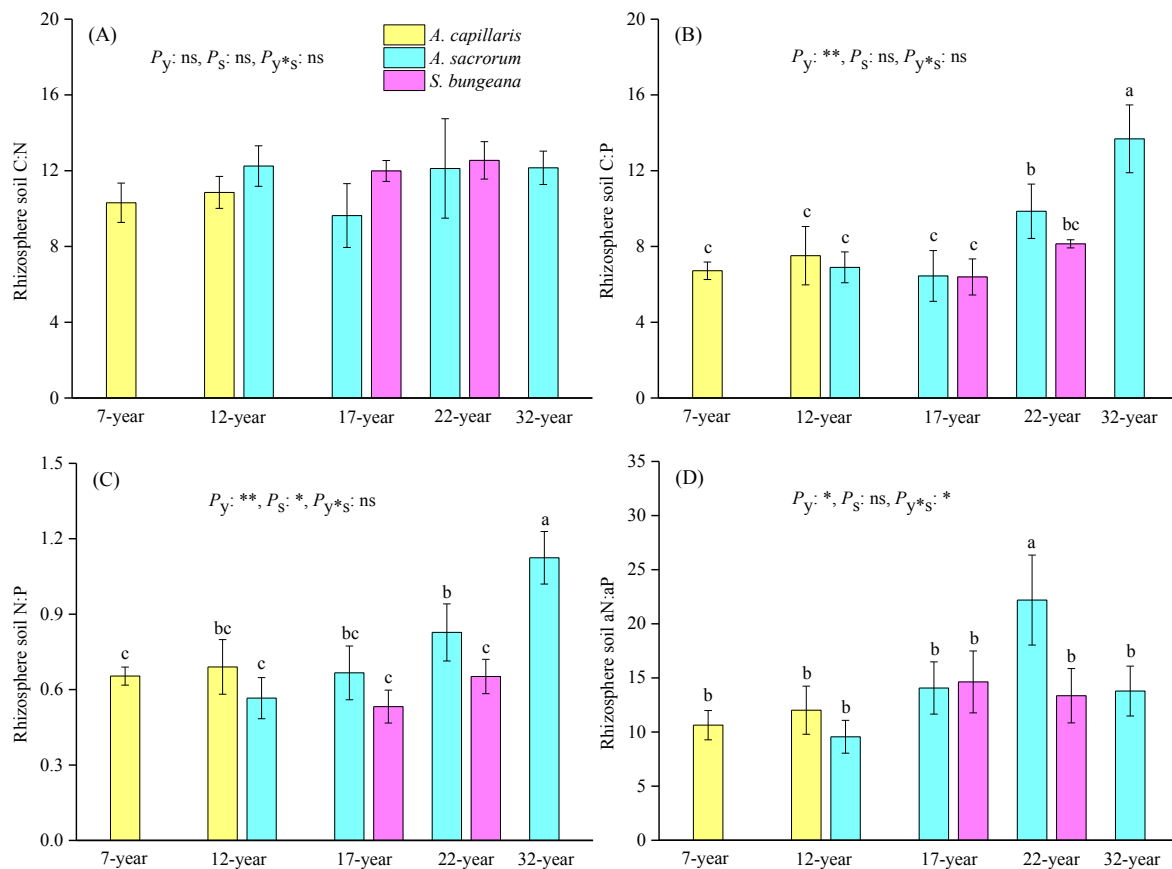
Fig. 4. Soil nutrient stoichiometries in the rhizosphere of the dominant species and companion species during secondary plant succession. Note the different scales among the graphs. Letters above the mean ± SD (n = 3) indicate significant differences among plant species at five succession years at $P < 0.05$ using Duncan post hoc tests. P_y, succession years effect; P_s, plant species effect; P_{y*s}, interaction of succession years and plant species effect; *, $P < 0.05$; **, $P < 0.01$; ns, not significant.

correlated with enzyme BG, NAG, AP activity and enzyme C:N ratio. Enzyme BG, NAG, AP activity and enzyme C:N ratio were strongly negatively correlated with plant tissue stoichiometry.

4. Discussion

4.1. Rhizosphere stoichiometry variation along secondary succession

Plant secondary succession has commonly been shown to alter plant growth, soil properties and soil enzyme activity (Zhang et al., 2016; Cui et al., 2019; Liu et al., 2020a), resulting to differences in the distribution of the C, N, and P concentrations and their stoichiometries. Zeng et al. (2017) and Liu et al. (2020a) reported that vegetation succession greatly affected plant C, N, P concentrations and their stoichiometric ratios. In the present research, we also found that the concentration of C, N, and P in shoot and P in roots (Fig. 1), and the stoichiometries of C:P and N:P in both the shoot and root parts (Fig. 2) were significantly changed along the plant secondary succession gradient. Since the different plant species had distinct plant nutrient-acquisition mechanisms, the changes in plant species composition during secondary succession might contribute to the variation of plant tissue stoichiometry (Han et al., 2005; Mi et al., 2015). Our results also showed that the concentrations of rhizosphere soil organic C and total N increased along secondary succession (Fig. 3). This is consistent with the results of the studies of Jia et al. (2012) and Zhao et al. (2019), suggesting that vegetation secondary succession enhanced the accumulation of C and N in soil by increasing the inputs of organic matter such as plant litter and root exudates. On the other hand, the total P concentration remained stable with site age (Fig. 3). Thus, there was an increase in the soil C:P and N:P ratios with time since cropland abandonment, indicating an increasing P limitation on plant

growth during vegetation succession. These results are in line with some previous studies (Wang et al., 2011; Zhang et al., 2012, 2016; Jiao et al., 2013) and have been further confirmed by the decrease in available soil P concentration. In general, soil enzyme activity plays an important role in C and nutrient cycling and is intimately linked to plant growth in an ecosystem (Jiang et al., 2009). In the present research, we found that the enzyme activity per organic C unit showed a decreasing trend with abandonment age (Fig. 5). Similarly, Raiesi and Salek-Gilani (2018) reported a decreased trend of enzyme activity per microbial biomass C unit with cultivation abandonment. It is possible that the changes in the structure of the soil microbial community and root exudates after abandonment of the cropland modified enzyme production and activity (Zhang et al., 2012; Knelman et al., 2012). Soil enzyme stoichiometry reflects the nutrient requirements of microorganisms (Sinsabaugh et al., 2009). In our research, we found that the enzyme C:N ratio decreased, and the enzyme N:P ratio increased with plant succession (Fig. 6), indicating that the microbial community was more limited by N during secondary succession. This result was confirmed by the study of Li et al. (2020), who reported that the microbial activity in the rhizosphere was limited by nitrogen along the Hailuogou Glacier forefield chronosequence. The variation of plant-soil-enzyme stoichiometries during plant secondary succession reflected that the plant-soil feedback systems can govern the ecological stoichiometries of all ecosystem components involved (Crawford and Knight, 2017; Lekberg et al., 2018). In the present research, the PCA indicated that different plant species—and even the same plant species at different succession stage—could exhibit different stoichiometry characteristics (Fig. 7). Qiu et al. (2013) and Zhang et al. (2018) also demonstrated that plant nutrient stoichiometry varied greatly between plants as well as within the same plant because of the variation of soil nutrient availability and soil microbial community
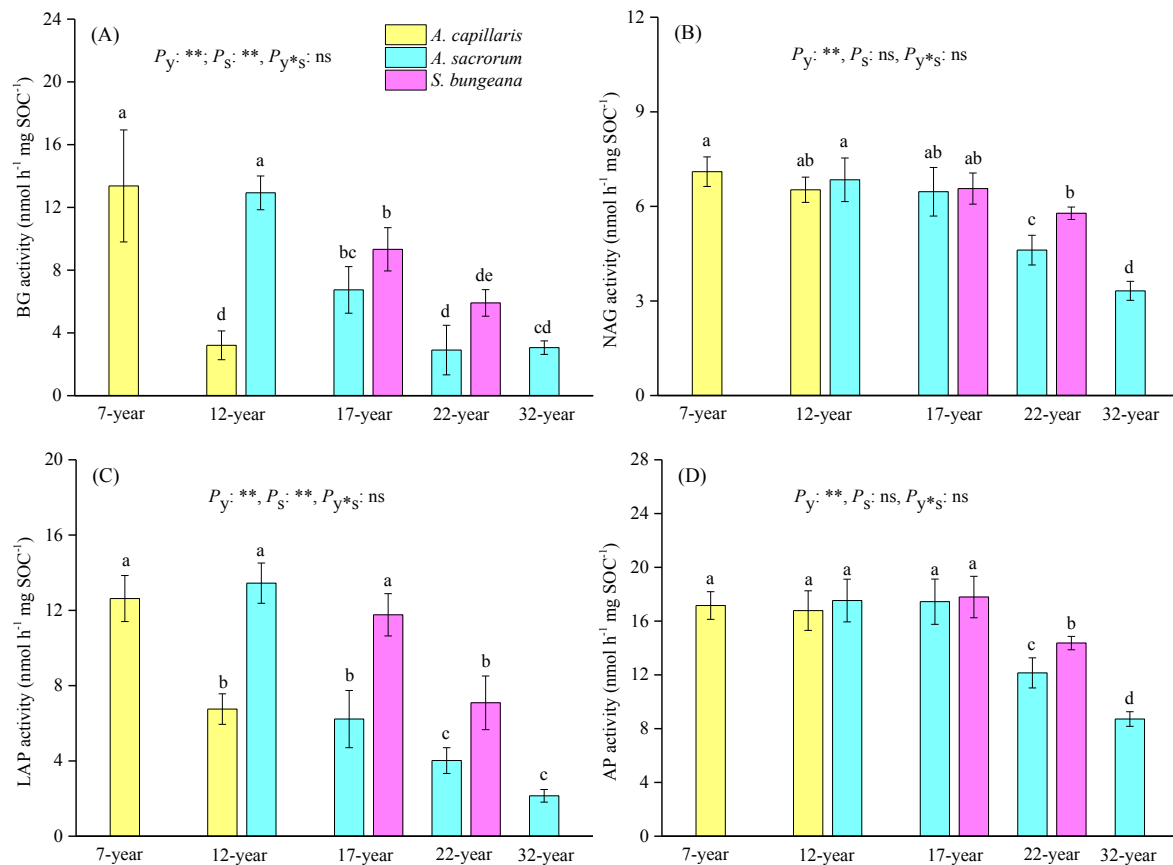
Fig. 5. Soil enzyme activity in the rhizosphere of the dominant species and companion species during secondary plant succession. Note the different scales among the graphs. Letters above the mean ± SD (n = 3) indicate significant differences among plant species at five succession years at $P < 0.05$ using Duncan post hoc tests. P_y, succession years effect; P_s, plant species effect; P_{y*s}, interaction of succession years and plant species effect; *, $P < 0.05$; **, $P < 0.01$; ns, not significant.

composition.

### 4.2. Ecological stoichiometry of plant tissues, rhizosphere soils, and soil enzymes

During secondary plant succession, plant and soil components are tightly linked, and coevolved plant-soil interactions can significantly affect ecological processes (Wardle et al., 2004). In the rhizosphere, nutrient cycling between plants and soils is regulated by soil enzymes produced by rhizosphere soil microbes and plant roots (Luo et al., 2017). Hence, there is a powerful theoretical foundation for forecasting that the stoichiometries of plants, soils, and enzymes should be obviously correlated (Sterner and Elser, 2002; Bell et al., 2014). In the present research, we found that root C:N and C:P ratios were negatively correlated with enzyme C:N ratio, and the root N:P ratio was positively correlated with soil aN:aP ratio (Fig. 8). Those results partly supported our secondary hypothesis, that is, there are strong correlations among plant, soil, and enzyme stoichiometries within the rhizosphere of specific plant species. Indeed, many researchers have found strong correlations between the ecological stoichiometry of the different components of the terrestrial ecosystems (Zeng et al., 2016; Zhang et al., 2019). For example, Yang et al. (2018) reported a strong link among the C:N:P stoichiometry in leaves, root, litter, and soil among different plant communities in a deserted region in Northern China. However, Di Palo and Fornara (2017) reported that soil N:P ratios were not related to plant N:P stoichiometry along natural primary successions distributed across Europe. Our research also showed that some plant, soil, and enzyme stoichiometries were not significantly associated with one other. Those results did not support our secondary hypothesis. The weak correlation between soil and plant stoichiometry that we, as well as other

researchers, have found maybe due to the regulation of high internal elements by plants, such that plant stoichiometry became more of a reflection of the plant's functional identify (Di Palo and Fornara, 2017; Zhao et al., 2014). A conceptual framework of the relationship among soil bacterial diversity, nutrient availability and plant stoichiometry is shown in Fig. 10. Further work is needed to clarify the relative contribution of rhizosphere soil nutrient status and enzyme activities and their stoichiometry to a plant's nutrient stoichiometry during secondary plant succession and to determine under what conditions stoichiometries become coupled or decoupled.

4.3. Factors leading to the change in plant species during secondary succession

Plant and soil nutrient feedbacks within the rhizosphere could significantly influence the competition and coexistence of different plant species (Hawkes et al., 2005; Diez et al., 2010). Previous research suggests that plants exhibit homeostasis and that later successional plant species may show a greater ability to maintain homeostasis as compared to early-stage plant species (Yu et al., 2011; Bai et al., 2019). In the present research, with the exceptions for root C:N ratio in *A. capillaris* and root N:P ratio in *A. sacrorum*, all plant tissues were categorized as strictly homeostasic (Table 2). Yu et al. (2011) evaluated the factors that influence stoichiometric homeostasis in vascular plants and found that species with a lower N:P ratio and higher N and P concentrations were less homeostatic, suggesting that more homeostatic plants tend to conserve nutrients. This suggests that *A. capillaris* maybe less homeostatic than *S. bungeana* and *A. sacrorum*, and this may explain why *A. capillaris* was the first species to colonize the abandoned cropland. However, recent research indicated that plant stoichiometric
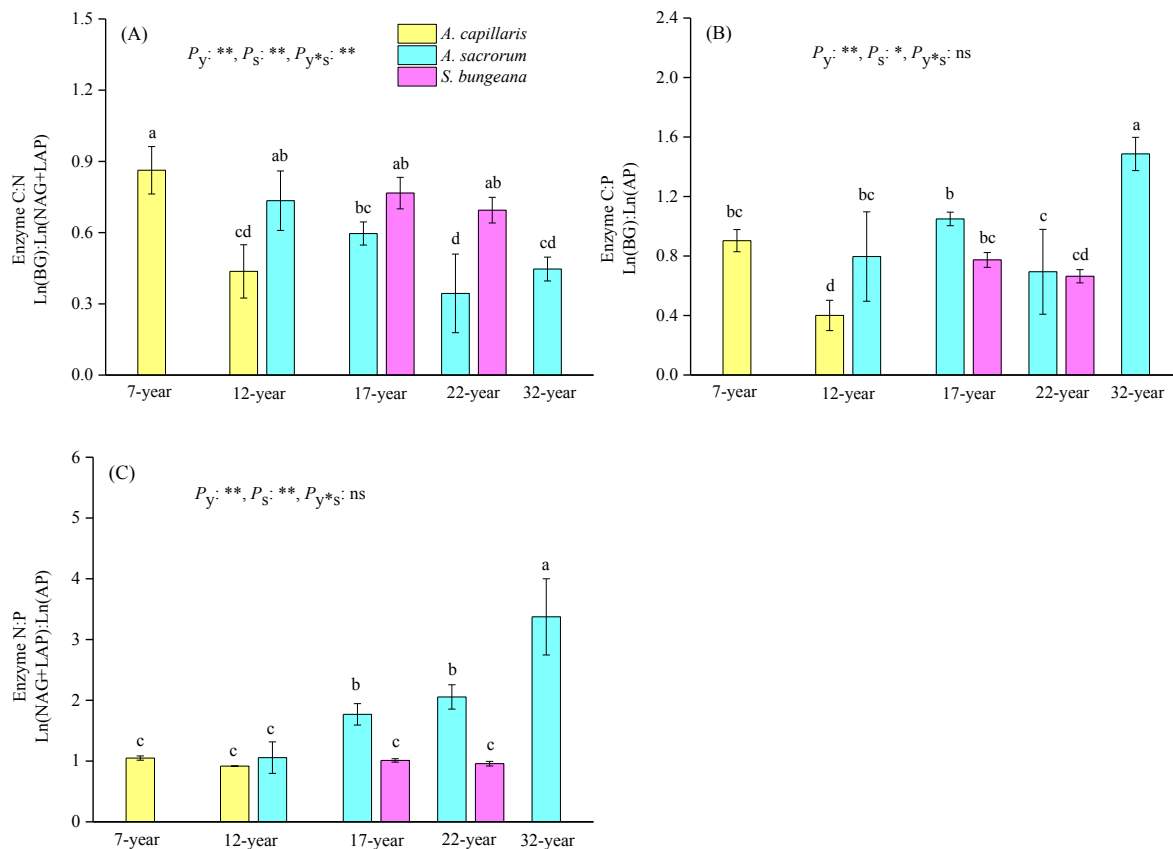
Fig. 6. Soil enzyme stoichiometry in the rhizosphere of the dominant species and companion species during secondary plant succession. Note the different scales among the graphs. Letters above the mean ± SD (n = 3) indicate significant differences among plant species at five succession years at $P < 0.05$ using Duncan post hoc tests. P_y, succession years effect; P_s, plant species effect; P_{y*s}, interaction of succession years and plant species effect; *, $P < 0.05$; **, $P < 0.01$; ns, not significant.
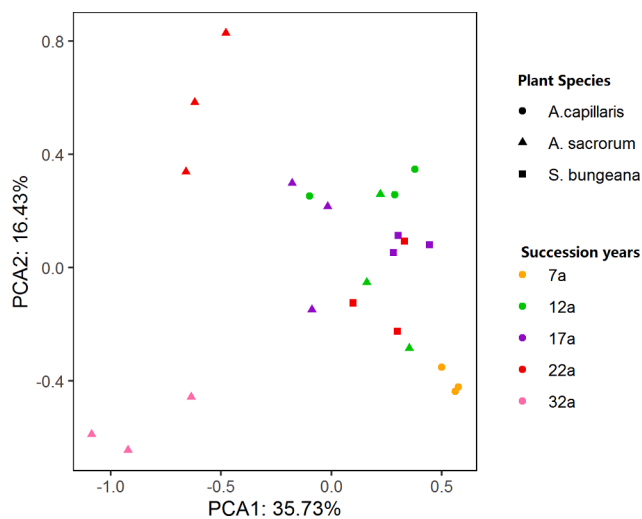
Fig. 7. Principal component analysis (PCA) of plant-soil-enzyme nutrient concentrations and stoichiometries in different plant species along a secondary succession series.

homeostasis is more nuanced (Persson et al., 2010; Yu et al., 2010; Wang et al., 2018). Most plant species exhibit deviations from strict stoichiometric homeostasis and the degree of homeostasis appears to depend on soil nutrient supply, plant species, and plant tissue (Yu et al., 2011; Bai et al., 2019). During plant succession, soil organic carbon and total nitrogen increased while the available nutrients, especially aP, decreased. This decrease may explain the dominance of *A. sacrorum* at various plant

succession stages that showed comparatively lower N and P concentrations. The RDA analysis further indicated that the soil BG, NAG, and AP activity and enzyme C:N ratio had significant effects on the plant tissue nutrient concentration and stoichiometry during secondary plant succession (Fig. 9). Similarly, many studies have reported soil enzyme activity to be a more sensitive indicator for the detection of the influence of restoration processes on terrestrial ecosystems (Raiesi and Salek-Gilani, 2018). The present research indicated that soil enzyme activity was more crucial than soil nutrients in determining plant tissue stoichiometries.

Overall, the present research evaluated the relationships among stoichiometries of plant tissues, soils, and enzymes within the rhizosphere of dominant and main companion species based on only one sampling time point. There is a large body of evidence that soil microbial communities have high temporal dynamics, especially in the rhizosphere (Bell et al., 2015; Nannipieri et al., 2019). Thus contributing to the variation in soil enzyme activity (Rathore et al., 2017). Furthermore, according to the growth-rate hypothesis, the fast growing plant species had relatively high plant P content and lower N:P ratios (Matzek and Vitousek, 2009; Liu et al., 2020b); thus, plant tissue stoichiometry would differ significantly among different growth stages (Gao et al., 2019). It is deemed essential for further studies to perform sampling at different time points to better evaluate the rhizosphere stoichiometries at specific plant species level during secondary plant succession.

5. Conclusions

Here, we explored the ecological stoichiometry of the plant-soil-enzyme continuum in the dominant and main companion species along a secondary succession chronosequence of a grassland on the
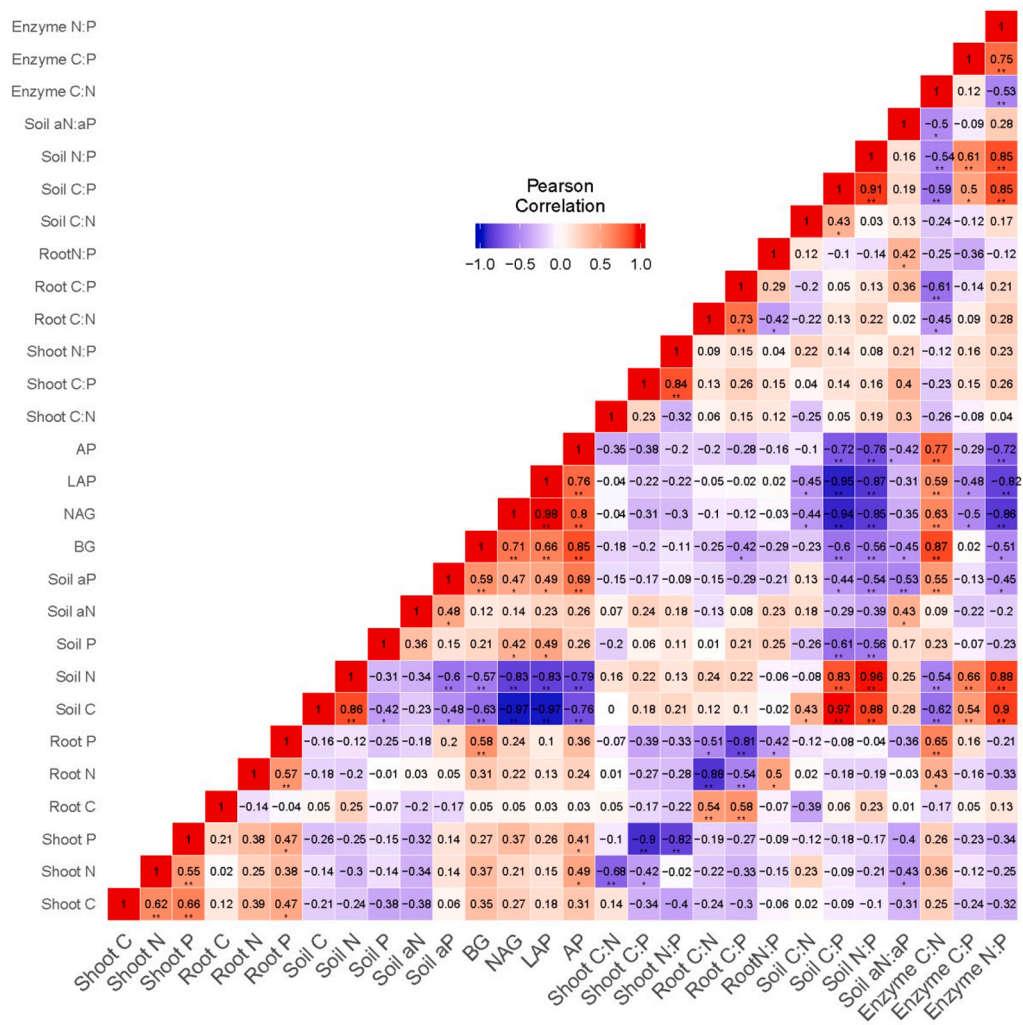
Fig. 8. Correlations between plant, soil, and enzyme concentrations and stoichiometries within the plant rhizosphere.

Table 2
Homeostatic relationships between plant tissue C:N:P and corresponding resources (soil and enzyme) stoichiometric counterparts among three plant species using 1/H indices and associated r^2

| Consumer	Resource	*A. capillaris*		*A. sacrorum*		*S. bungeana*	
		1/H	r^2	1/H	r^2	1/H	r^2
shoot C:N	soil C:N	-0.015	<0.001	-0.329	0.165	1.282	0.444
	enzyme C:N	-0.110	0.191	-0.067	0.043	0.888	0.458
	enzyme C:N	**-0.572**	**0.649**	0.136	0.106	-0.537	0.022
shoot C:P	soil C:P	0.379	0.170	-0.123	0.092	0.389	0.150
	enzyme C:P	-0.083	0.097	-0.095	0.083	0.002	<0.001
root C:P	soil C:P	1.098	0.221	-0.137	0.070	**-1.133**	**0.591**
	enzyme C:P	**-0.613**	**0.827**	-0.159	0.140	0.899	0.141
shoot N:P	soil N:P	-0.034	0.003	-0.049	0.009	0.289	0.084
	soil aN:aP	0.215	0.196	-0.032	0.006	0.380	0.222
	enzyme N:P	-0.302	0.100	-0.430	0.017	0.217	0.004
root N:P	soil N:P	-0.348	0.021	-0.003	<0.001	-0.942	0.426
	soil aN:aP	0.034	<0.001	**0.304**	**0.394**	0.320	0.075
	enzyme N:P	-1.559	0.213	-0.028	0.005	-0.748	0.021

Significant relationships are indicated in bold. Negative H values are by definition homeostatic.

Loess Plateau of China. Plant tissue, rhizosphere soil, and enzyme stoichiometry significantly changed among plant species and succession ages. Rhizosphere soil BG, NAG, and AP activity and enzyme C:N ratio played significant roles in the variation of the plant tissue C, N, P concentrations and stoichiometries. We found that the occurrence and distribution of plant species during secondary succession was significantly associated with the variation of soil enzyme activity and enzyme stoichiometries. This research suggests that assessing the ecological stoichiometry at the plant species' rhizosphere was a useful method for linking plant community to belowground soil nutrient status and enzyme activities, and rhizosphere soil enzyme activity was more crucial than nutrient status in determining plant tissue stoichiometries during
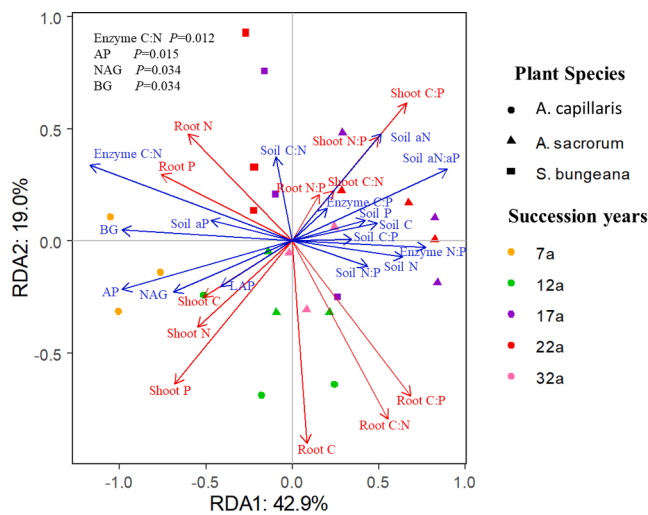
Fig. 9. Redundancy analysis (RDA) conducted to evaluate how the rhizospheric soil nutrients, enzyme activity, and their stoichiometries changed relative to the plant tissue concentrations and stoichiometries. The overall RDA was significant with 999 permutations. The constrained variables explained 80.97% of the total variance.
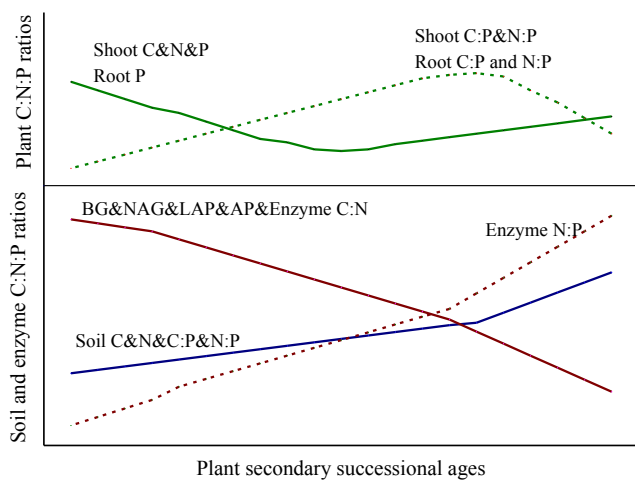
Fig. 10. Conceptual framework exploring the ecological stoichiometric interactions among plants, soils, and enzyme activities in the rhizosphere along a plant succession series. Rhizosphere soil BG, NAG, LAP and AP enzyme activities, and enzyme C:N decreased, but enzyme N:P increased along plant secondary successional gradients, which altered soil organic matter mineralization and nutrient availability. Rhizosphere soil C, N, C:P and N:P ratio increased along the successional gradients. The variation of rhizosphere soil enzyme activity and nutrient status lead to the variation of plant (shoot and root) nutrient concentration and stoichiometry. The C, N, and P concentrations in the shoots, and the P concentration in the roots first decreased and then slightly increased along the plant successional ages. The C:P and N:P ratios in both shoot and root part first significantly increased and then slightly decreased during plant secondary succession.

secondary plant succession on the Loess Plateau.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.catena.2021.105302.

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