

Article

Plant–Soil Feedback of Companion Species during Grassland Community Succession

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Abstract: The responses of dominant species to plant–soil feedback (PSF) are well established; however, the changes in the PSF of companion species remain unclear. This study aims to assess the direction and intensity of PSF, determine the main factors influencing it, and interpret the ecological significance of PSF in companion species within the context of grassland community succession. Three typical companion species, namely *Artemisia sacrorum*, *Artemisia capillaris*, and *Artemisia giraldii*, were planted in soils at three grassland community succession stages (early, middle, and late) on the Loess Plateau. Our results indicate that during both plant growth periods, the shoot biomass of *A. sacrorum*, *A. capillaris*, and *A. giraldii* in early- and late-stage soils was higher than that in the middle-stage soil, suggesting consistent growth of the three companion species during the two growth periods. However, plant growth simultaneously led to a reduction in soil nutrient content and microbial biomass, resulting in an overall decrease in the biomass of the three species, indicating a negative PSF effect in companion species. In conclusion, the negative PSF observed in all three associated species explains the temporary dominance of companion species during succession. This study enhances our understanding of the mechanisms driving PSF in community succession.

Keywords: community succession; companion species; plant–soil feedback; soil microbial activity; soil nutrients; plant traits



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1. Introduction

For a scientific understanding of community succession, it is crucial to comprehend the changes in plant community structure and composition [1]. The forms and functions of terrestrial ecosystems are continually changing due to human activities and climate change, particularly in the Loess Plateau region of China [2,3]. Since the 1950s, factors such as reclamation, deforestation, and extreme weather events (e.g., droughts, heavy rain, hailstorms, and strong winds) have severely damaged the vegetation on the Loess Plateau, leading to significant soil degradation and erosion [4]. However, after the implementation of China's 'Grain-for-Green' Program in 1999, there was a notable improvement in plant growth, coverage, and plant community diversity [5]. Returning farmland to grassland has proven to be an effective measure in enhancing soil fertility and controlling erosion in this region [6]. Nevertheless, due to the unique environmental conditions of the Loess Plateau, such as drought and nutrient-poor soil resulting from severe erosion [7], vegetation restoration in this area requires an extended period. Therefore, understanding the dynamics of plant communities during restoration is vital for comprehending community succession.

While significant research has been conducted on changes in soil nutrients, microbial activity, and plant community structure during plant succession [8], more researchers are now focusing on changes in plant–soil feedback (PSF) during succession. PSF refers to the changes in soil biotic and abiotic properties caused by plant growth, which, in turn, influence the growth of the plants themselves or other plants [9,10]. Increasing evidence suggests that PSF plays a crucial role in driving community succession [11,12]. By studying PSF, we can gain a better understanding the changes in plant community composition and structure, as well as the role of PSF in species replacement.

PSF varies significantly among species at different successional stages. Recent studies have shown that the PSF of early-successional grass species tends to be negative, which may be attributed to the greater influence of pathogenic fungi compared to mycorrhizal fungi [13,14]. Conversely, the PSF of late-successional grass species exhibits a positive feedback effect, indicating that the role of mycorrhizal fungi is more significant during late succession than that of pathogenic fungi [15]. Dominant and companion species display notable differences in photosynthesis and resource utilization due to their distinct ecological niches [16]. Additionally, research has confirmed that the growth of dominant species can produce allelopathy, inhibiting the seed germination and growth of associated species. This process eventually leads to the gradual disappearance of the community of associated species, driving the plant community to change in a specific direction and ultimately develop into a top-level dominant community [2]. Scholars have found that dominant and companion species have different PSF effects, which contribute to species replacement within the community when studying PSF at different successional stages [15,17]. For instance, during the invasion of exotic species, dominant species demonstrate a significant advantage in the PSF process, whereas companion species gradually disappear [16]. This discrepancy is primarily due to companion species typically having a relatively low importance value in the community and facing disadvantages in photosynthetic competition and soil nutrient utilization [18]. These findings underscore the importance of PSF in companion species for community succession [15]. However, current research on PSF is primarily focused on dominant species, with a lack of study on the PSF of common companion species during community succession. Thus, a comprehensive analysis is urgently needed to evaluate the PSF of common companion species in the grassland succession sequence of the Loess Plateau. This analysis will aid in a better understanding of changes in plant community composition and structure, as well as the role of PSF in species replacement.

In this study, we have identified three typical companion species (*Artemisia sacrorum*, *Artemisia capillaris*, and *Artemisia giraldii*) in the soils of three grassland community succession stages (early, middle, and late) on the Loess Plateau. Through a two-year PSF experiment, the aims of this study were to (1) assess the direction and intensity of PSF in the three companion species, (2) determine the main factors influencing the PSF of these species, and (3) provide an ecological interpretation of the impact of companion species' PSF on grassland community succession. The results of this study are expected to provide a scientific basis for vegetation restoration and stability on the Loess Plateau.

2. Materials and Methods

2.1. Experimental Site and Design

2.1.1. Plant, Seed, and Soil Collection

The dominant species of secondary succession in semiarid grasslands, *Setaria viridis* (abandoned 1–2 years ago), *Stipa bungeana* (abandoned 10–20 years ago), and *Bothriochloa ischaemum* (abandoned >30 years ago), were selected from the Loess Plateau in China as representative plants of early-, middle-, and late-successional species, respectively [2,3]. Rhizosphere soil samples were collected from the three dominant species, representing early-, middle-, and late-stage species. The soil samples were collected at a depth of 5–20 cm from soil adhering to the plant roots and were obtained from the Ansai Research Station of the Chinese Academy of Sciences (36°51' N, 109°19' E). Subsequently, the collected soil samples were passed through a 5 mm sieve and stored at 4 °C.

As experimental plants, we selected three companion species: *A. capillaris*, *A. sacrorum*, and *A. giraldii*. These three companion species are common in the grass succession process of the Loess Plateau. Specifically, *A. capillaris* serves as the companion species in the early stage of succession, while *A. sacrorum* and *A. giraldii* act as companion species in the middle and late stages of succession, respectively. The seeds of these three companion species were obtained from mature plants.

2.1.2. Plant–Soil Feedback (PSF) Experiment

To investigate the PSF effects of the three companion species in soils of three different succession stages, PSF experiments were carried out at the State Key Laboratory of Soil Erosion and Dryland Farming in Loess Plateau, Institute of Soil and Water Conservation, Chinese Academy of Sciences, and the Ministry of Water Resources (34°12' N, 108°07' E, 530 m a.s.l.). For this, *A. sacrorum*, *A. capillaris*, and *A. giraldii* were planted in the soils at three different succession stages, resulting in 9 treatments (12 pots/replicates: 6 replicates per year) planted in each treatment, totaling 108 pots (Figure S1).

The planting method involved placing crushed stones with a diameter of approximately 1 cm at the bottom of each pot, serving as a watering channel with a diameter of about 1.5 cm. Subsequently, 2.8 kg of the soil sample (based on dry weight) was added. On 4 May 2018, seeds were sown by scattering them in each pot, and sufficient water was supplied to enhance seed germination. After two weeks of growth, thinning was conducted to control the final number of plants per pot at four. Throughout the plant growth period, the positions of the pots were randomly changed once a week, and water was controlled twice a week to maintain 80% of the field water capacity. To accurately simulate field conditions, no fertilizers were added during the entire experiment. Plant height was measured four times a year during the growth period (20 May, 20 June, 20 July, and 20 August). After four months of plant growth (the first year or first plant growth period), five pots (6 pots/replicates) were chosen at random to obtain plant and soil samples. The aboveground and belowground parts of the remaining pots were removed (drop out), and the soil was retained for the second-year experiment (the second year or second plant growth period). The next year, we replanted the plants (using plant seeds) with the same planting and management methods as in the first year.

2.1.3. Plant–Soil Sampling

After removing the dust from the plant leaves, the shoots were cut along the soil surface using scissors, and the roots were obtained using the whole-plant harvesting method. The plant roots were rinsed with distilled water. Then, both the shoots and roots were stored in archive bags and dried to a constant weight at 65 °C, which is the root biomass (RTB) and shoot biomass (STB). The dried plant sample was pulverized with a spheroidal graphite apparatus and sieved through a 1 mm sieve for determination of plant carbon (C), nitrogen (N), and phosphorus (P), including STB carbon (STBC), nitrogen (STBN), phosphorus (STBP), RTB carbon (RTBC), nitrogen (RTBN), and phosphorus (RTBP).

After the root of the plants was obtained, the remaining soil was sufficiently mixed, sieved through a 2 mm sieve, and the soil samples were divided into two parts. One part was air-dried and used for determination of the soil mineral nutrient content (soil organic carbon (SOC), total nitrogen (TN), total phosphorus (TP), available nitrogen (SAN), and available phosphorus (SAP)), whereas the other part was stored at 4 °C for determinations of the soil microbial biomass (microbial carbon (MBC) and microbial nitrogen (MBN)) and soil enzyme activity (β -1,4-glucosidase (BG), cellobiose hydrolase (CBH), β -1,4-acetylglucosamine glycosidase (NAG), leucine aminopeptidase (LAP), and phosphatase (AP)).

2.2. Library Analysis

The H₂SO₄-K₂Cr₂O₇ oxidation heating method was used for the determination of the C content in the plant and soil, the Kjeldahl method was used to determine the plant and soil N content, and the P content in the plant and soil was determined by the molybdenum

antimony anti-colorimetric method [19–21]. The contents of nitrate nitrogen (NO_3^- -N) and ammonium nitrogen (NH_4^+ -N) in the soil were determined by ultraviolet spectrophotometry and flow analysis, respectively. The molybdenum antimony anti-colorimetric method was used to determine the SAP content in the soil [22]. Soil microbial biomass was determined by the chloroform fumigation method [23], and soil enzyme activity was determined using the microplate fluorescence method [24]. Before the pot experiment, the soil mineral nutrient content, microbial biomass, and enzyme activities of the three succession stages soil were measured, and the results are shown in Tables S1 and S2.

2.3. Statistical Analysis

The PSF index is utilized to evaluate the direction and strength of the feedback effect of various plant species during different growth periods (year). The formula for calculating the PSF index is as follows [25]:

$$\text{PSF index} = \frac{(\text{biomass}_2 - \text{biomass}_1)}{\text{biomass}_1}$$

where biomass_1 and biomass_2 are the biomass of the first and second growth periods (referring to the first and second years of the experiment), respectively.

The data presented in both figures and tables are shown as the mean \pm standard error. SAN is the sum of NO_3^- -N and NH_4^+ -N. A three-way ANOVA was used in comparing the effects of the plant growth period, species, and soil type on the content of shoot biomass, root biomass, SOC, TN, TP, SAP, SAN, MBC, MBN, BG, CBH, NAG, LAP, AP, STBC, STBN, STBP, RTBC, RTBN, and RTBP. Tukey post-hoc tests were used for multiple comparisons ($p < 0.05$). Before ANOVA analyses, the normality and homogeneity of variance were tested. Differences in plant characteristics (biomass, plant height, and plant C, N, and P) between the first and second growth periods were assessed using t-tests. Relationships between plant and soil characteristics were assessed using correlation matrices, and data were analyzed and visualized using R 4.0.2 (corrplot package).

3. Results

3.1. Plant Biomass, PSF Index, and Plant Height

The shoot and root biomass of *A. sacrorum*, *A. capillaris*, and *A. giraldii* are significantly influenced by the soil type and plant growth period (Figure 1a–f; Table S3). In both the first and second growth periods (referring to the first and second years of the experiment), the shoot and root biomass of the three species grown in the early- and late-stage soils were higher than those grown in the middle-stage soil. Furthermore, the shoot biomass of *A. sacrorum* and *A. capillaris* grown in early- and late-stage soil, and the shoot biomass of *A. giraldii* grown in the soil of all three stages were significantly higher in the first growth period than that in the second growth period.

During the first growth period, the plant height of *A. sacrorum*, *A. capillaris*, and *A. giraldii* grown in late-stage soil (30 d (day), 60 d, 90 d, and 120 d) was higher overall than that of plants grown in early- and middle-stage soils (Figure 2a–c). In the second growth period, the heights of *A. sacrorum* (60 d, 90 d, and 120 d) and *A. giraldii* (120 d) grown in late-stage soil were overall higher than those of plants grown in early- and middle-stage soils (Figure 2d–f). Additionally, the plant height of *A. sacrorum* in middle- and late-stage soil, of *A. capillaris* (90 d and 120 d) in the three stages of soil, and *A. giraldii* in early-stage soil (90 d and 120 d) were significantly greater in the second growth period than in the first (Table S4).

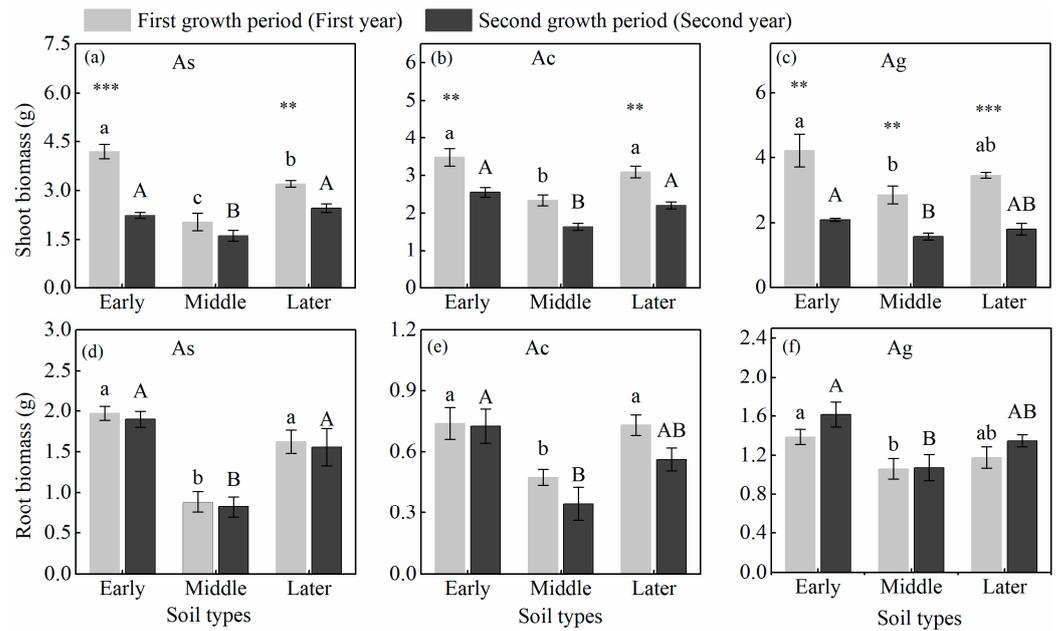


Figure 1. Shoot and root biomass for *Artemisia sacrorum* (As, **a,d**), *Artemisia capillaris* (Ac, **b,e**), and *Artemisia girdalii* (Ag, **c,f**) over the two growth periods (refers to the first and second years of the experiment hereafter). Different letters indicate significant differences in the mean biomass among the three soil types based on a Tukey post-hoc test ($p < 0.05$), and asterisks indicate significant differences between growth periods ($*** p < 0.001$, $** p < 0.01$).

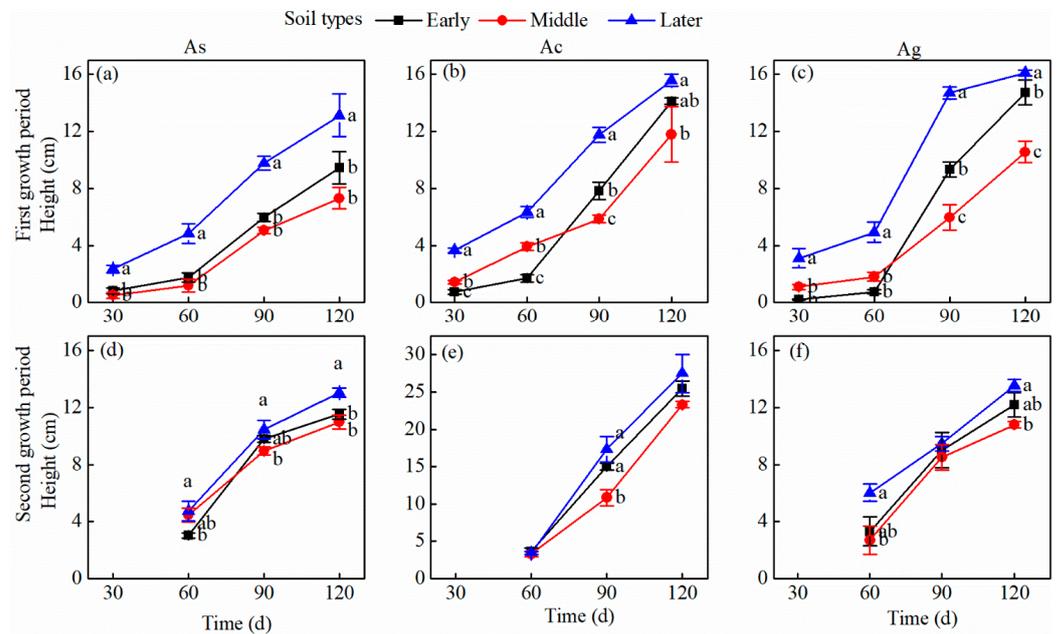


Figure 2. Plant height for *Artemisia sacrorum* (As, **a,d**), *Artemisia capillaris* (Ac, **b,e**), and *Artemisia girdalii* (Ag, **c,f**) over the two growth periods. Different letters indicate significant differences in mean plant height among the three soil types based on a Tukey post-hoc test ($p < 0.05$).

The PSF indexes of *A. sacrorum*, *A. capillaris*, and *A. girdalii* were all negative, with indexes ranging from -0.16 to -0.46 , -0.26 to -0.29 , and -0.42 to -0.48 , respectively (Figure 3a–c).

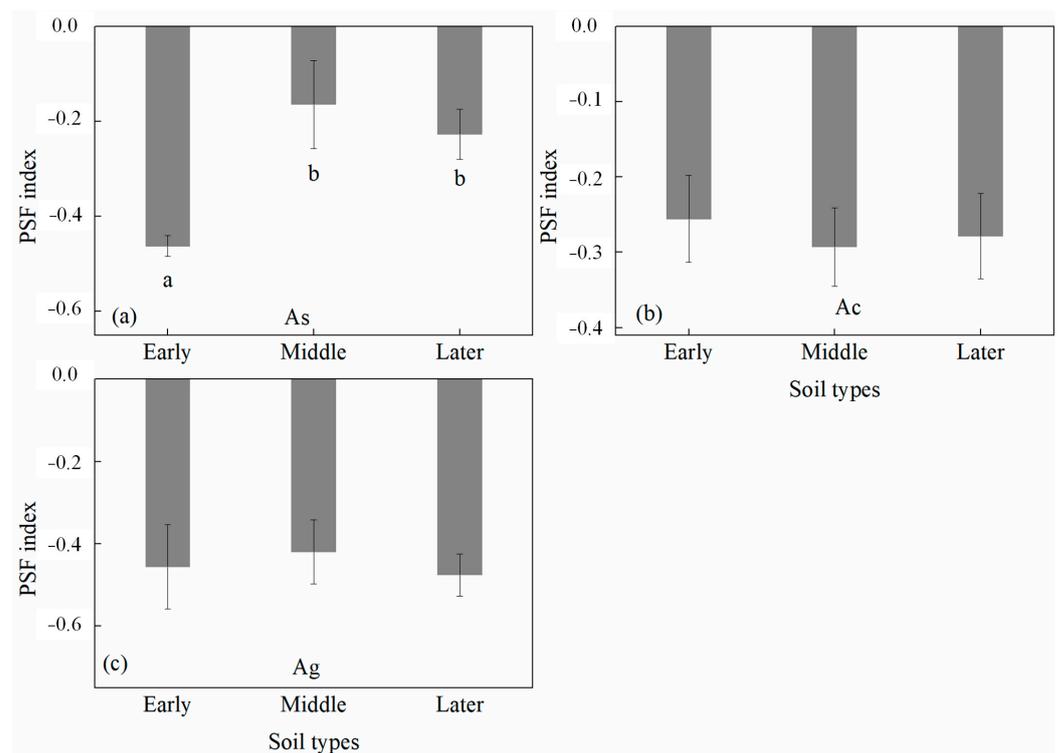


Figure 3. Plant–soil feedback (PSF) index of *Artemisia sacrorum* (As, **a**), *Artemisia capillaris* (Ac, **b**), and *Artemisia giraldii* (Ag, **c**). Different letters indicate significant differences in PSF index among the three soil types based on a Tukey post-hoc test ($p < 0.05$).

3.2. Carbon, Nitrogen, and Phosphorus Characteristics of Plants

In the first growth period, *A. sacrorum* and *A. capillaris* grown in early- and late-stage soils generally had higher STBC, STBN, STBP, RTBC, RTBN, and RTBP contents than those grown in middle-stage soil. Similarly, *A. giraldii* grown in early- and late-stage soil had higher STBC, STBN, RTBC, and RTBN contents than those grown in middle-stage soil. In the second growth period, the STBC, STBN, STBP, RTBN, and RTBP contents of the three species grown in early- and late-stage soil were higher than those grown in middle-stage soil. Additionally, the RTBN and RTBP contents of *A. giraldii* grown in early-stage soil were significantly higher than those of plants grown in middle- and late-stage soils (Tables 1 and 2).

Table 1. Mean (\pm SE) shoot biomass C, N, and P contents of *Artemisia sacrorum*, *Artemisia capillaris*, and *Artemisia giraldii*.

Growth Period	Species	Soil Types	STBC (g kg ⁻¹)	STBN (g kg ⁻¹)	STBP (g kg ⁻¹)
First	<i>A. sacrorum</i>	Early	434.85 \pm 13.02 a ***	9.26 \pm 0.35 a **	2.14 \pm 0.07 b ***
		Middle	363.60 \pm 5.90 b **	7.51 \pm 0.44 b	1.81 \pm 0.05 c ***
		Late	428.20 \pm 3.91 a ***	9.54 \pm 0.26 a **	2.45 \pm 0.05 a ***
	<i>A. capillaris</i>	Early	438.20 \pm 7.65 a ***	9.80 \pm 0.76 b **	1.74 \pm 0.04 a ***
		Middle	323.46 \pm 5.55 c	7.11 \pm 0.07 c *	0.66 \pm 0.22 b
		Late	391.98 \pm 6.10 b ***	11.72 \pm 0.28 a **	1.67 \pm 0.04 a ***
	<i>A. giraldii</i>	Early	433.92 \pm 3.67 a **	9.80 \pm 0.31 ab **	1.12 \pm 0.09 b
		Middle	403.79 \pm 7.15 c *	8.34 \pm 0.40 b *	0.49 \pm 0.04 c **
		Late	452.30 \pm 3.01 a ***	10.15 \pm 0.48 a	1.81 \pm 0.15 a **

Table 1. Cont.

Growth Period	Species	Soil Types	STBC (g kg ⁻¹)	STBN (g kg ⁻¹)	STBP (g kg ⁻¹)
Second	<i>A. sacrorum</i>	Early	407.65 ± 4.46 A	8.70 ± 0.25 A	1.10 ± 0.1 A
		Middle	325.11 ± 8.87 C	7.01 ± 0.49 B	0.76 ± 0.06 B
		Late	381.38 ± 4.53 B	8.34 ± 0.10 A	1.24 ± 0.05 A
	<i>A. capillaris</i>	Early	377.59 ± 3.80 A	8.02 ± 0.23 B	1.08 ± 0.04 A
		Middle	305.00 ± 6.97 C	6.57 ± 0.21 C	0.67 ± 0.02 C
		Late	329.32 ± 4.56 B	9.86 ± 0.23 A	0.94 ± 0.03 B
	<i>A. giraldii</i>	Early	416.95 ± 2.53 A	8.52 ± 0.13 B	1.11 ± 0.02 A
		Middle	376.67 ± 7.16 B	7.34 ± 0.12 C	0.72 ± 0.01 B
		Late	411.87 ± 6.53 A	9.21 ± 0.08 A	1.05 ± 0.04 A

Different letters indicate significant differences in mean C, N, and P among the three soil types based on a Tukey post-hoc test. Asterisks indicate significant differences between growth periods (** $p < 0.01$, *** $p < 0.001$, * $p < 0.05$). STBC, shoot biomass carbon; STBN, shoot biomass nitrogen; STBP, shoot biomass phosphorus.

Table 2. Mean (± SE) root biomass C, N, and P contents of *Artemisia sacrorum*, *Artemisia capillaris*, and *Artemisia giraldii*.

Growth Period	Species	Soil Types	RTBC (g kg ⁻¹)	RTBN (g kg ⁻¹)	RTBP (g kg ⁻¹)
First	<i>A. sacrorum</i>	Early	367.99 ± 1.08 b ***	8.78 ± 0.25 a ***	0.57 ± 0.04 a ***
		Middle	323.81 ± 5.24 c ***	5.54 ± 0.46 b ***	0.31 ± 0.05 b ***
		Late	401.89 ± 9.25 a ***	7.79 ± 0.26 a ***	0.58 ± 0.00 a ***
	<i>A. capillaris</i>	Early	370.16 ± 1.66 a ***	7.80 ± 0.06 a ***	0.55 ± 0.00 a ***
		Middle	345.42 ± 7.07 b ***	5.94 ± 0.4 b ***	0.45 ± 0.00 b ***
		Late	376.50 ± 8.64 a ***	7.96 ± 0.2 a ***	0.59 ± 0.01 a ***
	<i>A. giraldii</i>	Early	366.62 ± 7.18 a ***	9.02 ± 0.77 a ***	0.61 ± 0.08 ***
		Middle	318.49 ± 0.94 b ***	6.05 ± 0.08 b ***	0.43 ± 0.01 ***
		Late	362.53 ± 10.43 a ***	8.06 ± 0.35 ab ***	0.51 ± 0.02 ***
Second	<i>A. sacrorum</i>	Early	348.67 ± 3.39 A	7.10 ± 0.25 A	0.46 ± 0.03
		Middle	312.33 ± 4.58 B	5.38 ± 0.15 B	0.42 ± 0.03
		Late	334.42 ± 5.01 A	6.63 ± 0.17 A	0.44 ± 0.01
	<i>A. capillaris</i>	Early	354.78 ± 7.94 A	6.74 ± 0.34 A	1.07 ± 0.02 A
		Middle	327.39 ± 2.95 B	5.31 ± 0.13 B	0.69 ± 0.01 C
		Late	351.72 ± 4.34 A	6.69 ± 0.12 A	0.89 ± 0.04 B
	<i>A. giraldii</i>	Early	334.69 ± 4.02 A	8.01 ± 0.15 A	1.12 ± 0.03 A
		Middle	307.35 ± 7.27 B	5.62 ± 0.17 C	0.86 ± 0.01 B
		Late	338.28 ± 2.36 A	6.95 ± 0.17 B	0.86 ± 0.02 B

Different letters indicate significant differences in mean C, N, and P among the three soil types based on a Tukey post-hoc test. Asterisks indicate significant differences between growth periods (** $p < 0.01$, *** $p < 0.001$). RTBC, root biomass carbon; RTBN, root biomass nitrogen; RTBP, root biomass phosphorus.

Furthermore, in the soil of all three stages, the STBC, STBP, RTBC, RTBN, and RTBP contents of *A. sacrorum* were significantly lower in the second growth period than the first. Similarly, the STBN, RTBC, RTBN, and RTBP contents of *A. capillaris* were significantly lower in the second growth period than the first. The STBC, STBN, RTBC, RTBN, and RTBP contents of *A. giraldii* were also significantly lower in the second growth period than the first (Tables 1 and 2).

3.3. Soil Carbon, Nitrogen, and Phosphorus, Enzyme Activity, and Microbial Biomass

The SOC and TN contents were significantly higher in the early- and late-stage soils than in the middle-stage soil (Figure 4a–o). Additionally, the SOC, TP, and SAP contents in the early-, middle-, and late-stage soil in the second growth period were significantly lower than those in the first growth period (Table S5).

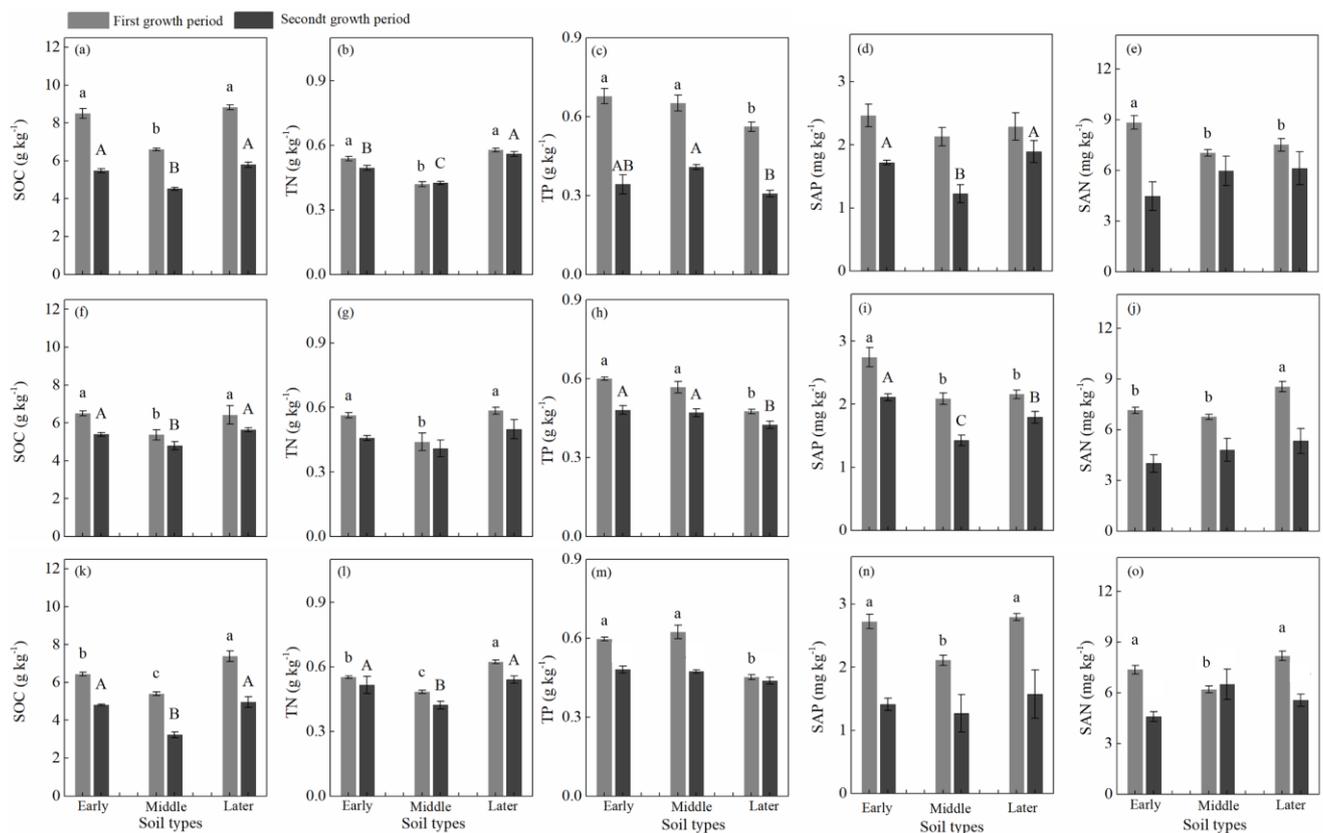


Figure 4. Soil chemistry of *Artemisia sacrorum* (a–e), *Artemisia capillaris* (f–j), and *Artemisia giralddii* (k–o). Different letters indicate significant differences in soil chemicals among the three soil types based on a Tukey post-hoc test ($p < 0.05$). SOC, soil organic carbon; TN, soil total nitrogen; TP, soil total phosphorus; SAP, soil available phosphorus; SAN, soil available nitrogen.

The enzyme activities of BG, CBH, NAG, LAP, and AP were higher in the early- and late-stage soils than in the middle-stage soil (Figure 5a–o). Additionally, in *A. sacrorum*, the BG, CBH, NAG, LAP, and AP activities were significantly higher in the second growth period than in the first growth period in the three soil stages. In *A. capillaris*, the activities of BG and LAP in the three soil stages and the activity of NAG in the early- and late-stage soils were significantly higher in the second growth period than in the first growth period. In *A. giralddii*, the activities of AP and LAP in the three soil stages and CBH in the middle- and late-stage soil were significantly higher in the second growth period than those in the first growth period (Table S6).

In the soils of the three companion plant species, MBC and MBN contents were generally higher in the early- and late-stage soils than in the middle-stage soil (Figure 6a–f). In the early soil stage, the MBC and MBN contents of the three companion plant species were significantly lower in the second growth period than those in the first growth period (Table S7).

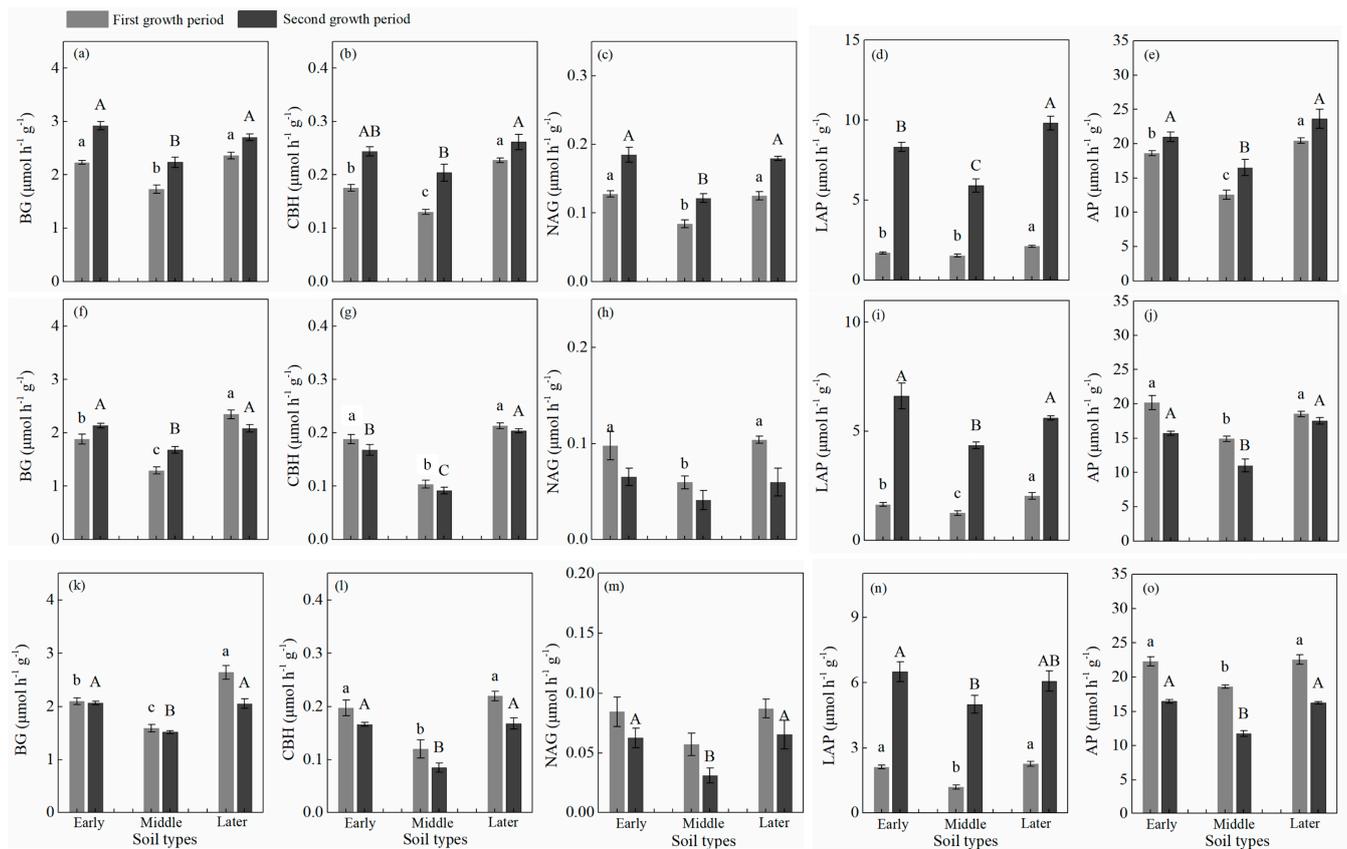


Figure 5. Soil enzyme activities of *Artemisia sacrorum* (a–e), *Artemisia capillaris* (f–j), and *Artemisia giraldii* (k–o). Different letters indicate significant differences in enzyme activity among the three soil types based on a Tukey post-hoc test ($p < 0.05$). BG, β -1,4-glucosidase; CBH, cellobiose hydrolase; NAG, β -1,4-acetyl-glucosamine glycosidase; LAP, leucine aminopeptidase; AP, phosphatase.

3.4. Correlation Analysis between Plant–Soil–Enzyme Activities and Plant Biomass

The results for *A. sacrorum* showed significant positive correlations between STB and the following factors: STBC, STBN, STBP, RTBC, TN, SAP, SAN, MBC, MBN, BG, CBH, NAG, LAP, and AP ($p < 0.05$). Moreover, plant height showed a significant and positive correlation with the following factors: RTBN, RTBP, TN, SAP, SAN, and CBH activities ($p < 0.05$). For *A. capillaris*, STB exhibited significant positive correlations ($p < 0.05$) with STBC, STBN, STBP, RTBC, TN, SAP, SAN, MBC, MBN, BG, CBH, NAG, LAP, and AP activity ($p < 0.05$). Additionally, plant height showed significant and positive correlations with RTBN, RTBP, and SOC ($p < 0.05$). Regarding *A. giraldii*, there were significant positive correlations between STB and STBC, STBN, STBC, TN, SAN, MBC, MBN, BG, CBH, NAG, LAP, and AP activity ($p < 0.05$). In addition, plant height was significantly and positively correlated with STBC, STBN, TN, MBC, MBN, BG, CBH, NAG, LAP, and AP activity ($p < 0.05$) (Figure 7).

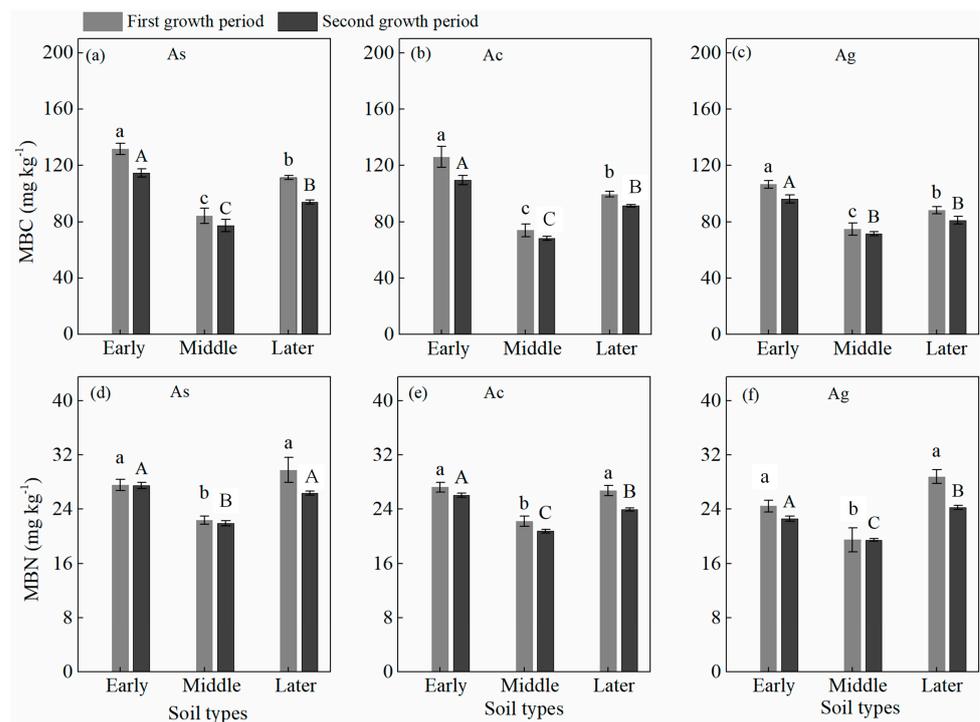


Figure 6. Soil microbial biomass of *Artemisia sacrorum* (a,d) *Artemisia capillaris* (b,e), and *Artemisia giraldii* (c,f). Different letters indicate significant differences in soil microbial biomass among the three soil types based on a Tukey post-hoc test ($p < 0.05$). MBC, microbial biomass carbon; MBN, microbial biomass nitrogen.

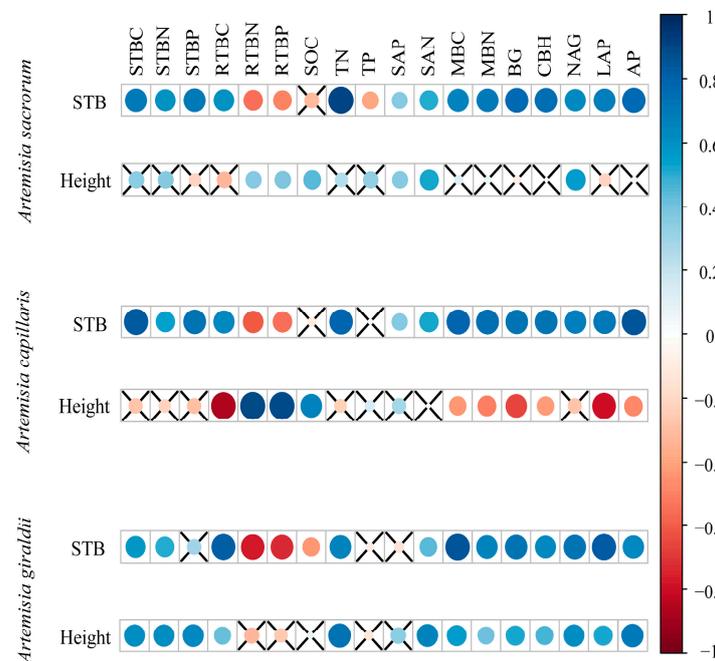


Figure 7. Correlation network analysis of plant and soil characteristics of *Artemisia sacrorum*, *Artemisia capillaris*, and *Artemisia giraldii*. ×, not significant. STB, shoot biomass; STBC, shoot biomass carbon; STBN, shoot biomass nitrogen; STBP, shoot biomass phosphorus; RTBC, root biomass carbon; RTBN, root biomass nitrogen; RTBP, root biomass phosphorus; SOC, soil organic carbon; TN, soil total nitrogen; TP, soil total phosphorus; SAP, soil available phosphorus; SAN, soil available nitrogen; MBC, microbial biomass carbon; MBN, microbial biomass nitrogen; BG, β -1,4-glucosidase; CBH, cellobiose hydrolase; NAG, β -1,4-acetyl-glucosamine glycosidase; LAP, leucine aminopeptidase; AP, phosphatase.

4. Discussion

Observations of *A. sacrorum*, *A. capillaris*, and *A. giraldii* growth during the two growth periods revealed consistent responses in the soils of different successional stages. Specifically, plants grown in early- and late-stage soils exhibited a higher shoot biomass and plant height compared to those in middle-stage soil. This variation was primarily attributed to the higher soil nutrient content, microbial biomass, and enzyme activities in early- and late-stage soils than that in middle-stage soil [26]. Furthermore, the correlation analysis results indicated a significant positive relationship among shoot biomass, soil nutrient content, and enzyme activity for all three species. Moreover, plants grown in early- and late-stage soils demonstrated higher plant C and N contents, important indicators of their ability to cope with environmental stress. Generally, these indicators are strongly correlated with plant biomass [27,28], as confirmed in this study. Overall, a positive correlation was found between shoot biomass and plant C and N contents in all three species (Figure 7). Consequently, these three companion species grown in the early- and late-stage soils exhibited a strong growth advantage in this study.

Investigating PSF with companion species helps us to better understand changes in community composition and structure during plant community succession, as well as its driving role in community succession [15]. In this study, we observed negative PSF effects for all three species in the time series, indicating a strong inhibitory effect of the plant growth period on their companion species' growth, which gradually increased over time. Plant growth influences the biotic and abiotic environments of the soil by altering soil structure and root secretions [29,30]. In other words, plant growth inevitably leaves residual effects on the soil, significantly altering its physical, chemical, and biological properties [31,32]. These residual soil effects can either promote or inhibit the growth of other plants [33,34]. Prior studies have reported that negative PSF effects are mainly attributed to soil resource depletion [32], a finding supported by other research [35,36]. Thus, the negative PSF effects observed in this study on *A. sacrorum*, *A. capillaris*, and *A. giraldii* could be attributed to an overall decrease in soil nutrient content and soil microbial biomass during the plant growth period. Furthermore, soil nutrient depletion negatively impacts soil microbe growth, as soil nutrients provide essential nutrition for microbes' development [8]. Studies have shown that the interaction between soil nutrients and soil microbes can affect plant growth [33,37]. Hence, soil nutrient depletion may indirectly influence the negative PSF effects on plant species by reducing soil microbial activity. Additionally, soil microbial activity significantly affects soil nutrient cycling. Soil microorganisms, as essential decomposers in the ecosystem, facilitate the decomposition of dead roots and the cycling of soil nutrients [38]. Simultaneously, they decompose challenging-to-degrade substances into substances that plants can directly absorb and utilize [39]. However, soil microorganisms themselves are an active part of the soil organic matter [40], and their level of activity directly affects the soil's nutrient content [30]. Consequently, significant reductions in soil microbial load and enzyme activity weaken soil nutrient cycling and restrict plant growth. Moreover, the correlation matrix results showed that the overall STB of the plants was positively correlated with soil nutrient content and microbial biomass (Figure 7), further supporting our hypothesis.

Plant C and N serve as structural and nutritional elements, respectively, and play important roles in plant growth [41,42]. This study found a significant positive correlation between STB and plant height and plant C and N content, with higher C and N contents in the first growth period. This suggests that a reduction in C and N storage capacities might limit plant growth to a certain extent, leading to a negative PSF. Previous studies have shown that dominant species have clear advantages over companion species in response to invasive species [16], mainly due to the inferiority of companion species during resource competition [16,18]. Conversely, dominant species occupy more ecological niches in the community [16] and thus exhibit clear advantages in resource competition over invasive species. Moreover, our previous studies have confirmed that PSF is negative, neutral, and positive for early-, middle-, and late-stage species, respectively, in the successional

sequence [26,43]. This difference in the direction of feedback is mainly attributed to the effects of plant growth on soil nutrient content and microbial activity. Our findings indicate that companion species are at a disadvantage compared to dominant species, especially in later soil stages. This is mainly because companion species usually have a relatively low importance in the community and are at a disadvantage in terms of photosynthetic competition and soil nutrient use [18].

5. Conclusions

In this study, we systematically analyzed the PSF effects of the companion species of *A. capillaris*, *A. sacrorum*, and *A. giraldii*. Our results emphasize that the negative PSF of companion species explains the temporary advantage of species in the plant community and their eventual replacement by dominant species in successional sequences. The results of this study enhance our understanding of the mechanisms underlying PSF stability in community succession.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/f14081634/s1>, Figure S1: Plant–soil feedback experiment design of companion species *Artemisia sacrorum* (As), *Artemisia capillaris* (Ac), and *Artemisia giraldii* (Ag); Table S1: Soil chemical of field soils that support early-, middle-, and late-successional species; Table S2: Soil microbial biomass and enzyme activity of field soils that support early-, middle-, and late-successional species; Table S3: F and p values of independent factors (plant growth period (P), species (S), and soil types (ST)) and their interactions to various parameters studied by a three-way ANOVA. Table S4: ANOVA analysis parameters of plant height during the plant growth period of *Artemisia sacrorum*, *Artemisia capillaris*, and *Artemisia giraldii*; Table S5: ANOVA analysis parameters of soil chemicals during growth of *Artemisia sacrorum*, *Artemisia capillaris*, and *Artemisia giraldii*; Table S6: ANOVA analysis parameters of soil enzyme activity during the plant growth of *Artemisia sacrorum*, *Artemisia capillaris*, and *Artemisia giraldii*; Table S7: ANOVA analysis parameters of soil microbial biomass during the plant growth of *Artemisia sacrorum*, *Artemisia capillaris*, and *Artemisia giraldii*.

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