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Yields of Buckwheat and Alfalfa in an Intercropping System Inoculated with Dark Septate Endophytes in a Coal Mining Subsidence Dryland Area

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Abstract: Coal mining subsidence areas in north Shaanxi province in north China are commonly low-fertility drylands, and intercropping is a popular method locally of maximizing crop yields. Inoculation with dark septate endophytes (DSE) can increase the yields of conventional crops grown in monoculture, but the effects of DSE on the growth and yield of intercropped plants are unknown. Here, a field experiment was conducted in a coal mining subsidence area on the loess plateau in 2020 and 2021. The two crops were buckwheat and alfalfa, with four treatments for each crop: sole cropping control, sole cropping with DSE inoculation, intercropping control, and intercropping with DSE inoculation. The yields, land equivalent ratios, DSE colonization rates, and yield components of buckwheat and alfalfa were compared in the different treatments. Stepwise regression and path analysis was conducted to identify the factors that determined crop yields. Yields of intercropped buckwheat and alfalfa with DSE inoculation increased in two consecutive years compared with sole cropping control, with yields increasing in 2020 by 117 and 86%, respectively. In 2021, the yield of buckwheat in intercropping with DSE inoculation did not increase significantly, but the yield of alfalfa increased by 120% compared with the sole cropping control. Buckwheat-alfalfa intercropping has the advantage of increasing the yield. DSE inoculation significantly increased the yield of buckwheat-alfalfa intercropping in 2020, but there was no yield advantage in 2021. The yield components of buckwheat and alfalfa showed inconsistent differences among experimental treatments. Stepwise regression and path analysis shows that the DSE colonization rate played an important role in the yield, which was an increased in buckwheat and alfalfa in 2020 and 2021. DSE affected the buckwheat yield indirectly by increasing the grain weight per plant, plant phosphorus uptake, and plant nitrogen content. DSE indirectly affected alfalfa yields by increasing plant nitrogen uptake and plant height. DSE may therefore have some potential to increase yields in buckwheat-alfalfa intercropping systems in coal mining subsidence areas.

Keywords: dark septate endophytes; intercropping; yield; yield components; colonization rate; coal mining subsidence

Citation: Gao, Y.; Bi, Y.; Ma, S.; Zhang, Y.; Guo, Y.; Zhou, Y.; Xu, S.; Christie, P. Yields of Buckwheat and Alfalfa in an Intercropping System Inoculated with Dark Septate Endophytes in a Coal Mining Subsidence Dryland Area. *Agronomy* **2022**, *12*, 2860. https://doi.org/10.3390/agronomy12112860

Academic Editor: Jiaen Zhang

Received: 3 October 2022
Accepted: 14 November 2022
Published: 16 November 2022

1. Introduction

Coal mining remains an important industry in north Shaanxi province, north China, despite its negative effects on climate change. Underground mining can cause land-surface cracking and subsidence, but the filling of the cracks and land leveling treatment can lead to the formation of a raw soil platform or land terrace, which is either abandoned or farmed. There is a degraded area with dry soils of low fertility in the transitional zone between the Loess Plateau and the Mu Us Desert, a typical agro-pastoral transitional zone with strong demand for crops and pasture grasses. It is necessary to introduce more environmentally friendly and efficient methods or technologies to consolidate production and environmental sustainability in order to increase the efficiency of the use of these lands and realize both environmental and industrial economic benefits. Intercropping and application of beneficial microbes are effective means of maximizing productivity and land use efficiency.

Intercropping generally uses combinations of legumes and non-legumes and can be an efficient agronomic measure for increasing crop yields. Intercropping utilizes biological nitrogen fixation of legumes to increase nitrogen retention in the soil. It can ameliorate soil nitrogen and can increase plant yields and nutritional value [1]. Thus, intercropping is an effective means of achieving sustainable development in modern agriculture. There are multiple planting options in intercropping, and cereals-grasses intercropping is an important mode that is popular in maize-producing areas of the midwestern United States, tropical grasslands in Brazil, arid areas in India and South Africa, and slope farmland and the pastoral farming ecotone in China [1–4]. Extensive studies have focused on crop combinations such as maize and alfalfa, wheat and alfalfa, oats and alfalfa, rice and alfalfa, sorghum and alfalfa, maize and palisade grass, and pearl millet and palisade grass [1,2]. The main objectives of these studies have included soil improvement, water, and soil conservation [3], increasing land use efficiency and system productivity, promoting balanced development of farming and husbandry [2,4], and increasing the carrying capacity of degraded grasslands to achieve sustainability of crop-livestock integration (CLI) in tropical agricultural systems [2].

Microorganisms can promote plant growth and have a role in sustainable agricultural practices [5]. They may increase nutrient availability and partially or completely replace synthetic chemical fertilizers in many agricultural systems [5]. Soils contain a vast microbiome that includes arbuscular mycorrhizal fungi (AMF) and dark septate endophytes (DSE) that can colonize host plants and influence host growth and nutrition. However, DSE has a wider host range and can colonize non-mycorrhizal plants that are difficult or impossible to colonize with AMF [6]. DSE can promote the growth of host plants under various stress conditions [6]. Fungal hyphae can extend farther through the soil than roots to take up soil nutrients and water and supply them to the host plants [7]. A meta-analysis of 18 independent studies shows that DSE inoculation increased the nitrogen and phosphorus contents and biomass of a range of plant species by 26–103% [8]. In contrast, another meta-analysis showed that DSE inoculation had negative to neutral effects on plant biomass and nitrogen content [8]. This discrepancy may be due to the use of different species of colonized plants and DSE strains in different studies [8]. However, DSE usually does show positive effects, for example, by promoting the growth and yield of maize [9], tomato [10], purple alfalfa [11], and *Ammopiptanthus mongolicus* [12] significantly. Current studies on DSE focus mainly on their effects on the growth of single plant species.

Microorganisms play an important regulatory role in intercropping systems, and the impact of AMF on intercropping systems has been extensively studied. Although DSE has similar functions to AMF, the effects of DSE on intercropping systems remain unclear. AMF can influence inter- or intraspecies competition and the productivity of plant communities [13]. Common mycorrhizal networks can be formed among the same or different plant species [14], and this can affect the distribution and transfer of carbon, nitrogen, phosphorus, and defense signals [15,16]. However, the role of mycorrhizal networks in plant populations is complex and depends on plant functional characteristics, soil nutrient conditions, and mycorrhizal fungal characteristics [17]. It is unknown whether mycorrhizal networks show different effects on intraspecific and interspecific plant interactions in any system.

Here, we have examined the effects of DSE inoculation combined with grain-grass intercropping on the growth and yield of plants grown on low-fertility soil. The grain is buckwheat, a local multigrain non-mycorrhizal plant, and the grass is alfalfa, a leguminous forage species. This field study on the continuous intercropping of buckwheat and alfalfa was carried out for two years. The aims were to (1) determine if buckwheat-alfalfa intercropping has a yield-increasing effect and the effect of DSE inoculation on yield in a buckwheat-alfalfa intercropping system; (2) determine the effects of DSE inoculation on the yield components of buckwheat and alfalfa in an intercropping system; (3) determine the main factors that influence the yield of buckwheat and alfalfa, and whether any effects of DSE inoculation can be linked to the DSE colonization rate.

2. Materials and Methods

2.1. Site Description

This experiment was conducted from 2020 to 2021 on leveled land (38°59′22′′ N, 110°20′32′′ E; 1185 m altitude) in Zhangjiamao mining subsidence area, Shenmu city, Shaanxi province, north China. The site has a temperate semi-arid continental monsoon climate, with precipitation occurring between June and October. The annual average temperatures during the two years were 9.71 and 9.42 °C, the average annual rainfall was 337 and 318.6 mm, and the average monthly temperature and rainfall during the study period are shown in Figure 1. The average frost-free period was 145 days, the average effective cumulative temperature $\geq$ 10 °C was 3084 °C, and the annual average sunshine duration was 2578 h. The loessial soil at the site had low fertility. The preceding crop was maize. The root stubble was cleared, and the soil was turned over thoroughly after the maize was harvested. The background values of the soil parameters (upper 20 cm) were determined before sowing in 2020, and the results were as follows: soil pH 8.41, electrical conductivity 88.6 µS cm^{-1}, organic matter content 3.28 g kg^{-1}, total nitrogen content 260 mg kg^{-1}, total phosphorus content 462 mg kg^{-1}, total potassium content 9053 mg kg^{-1}, available nitrogen content 22.7 mg kg^{-1}, available phosphorus content 1.57 mg kg^{-1}, and available potassium content 69.1 mg kg^{-1}.

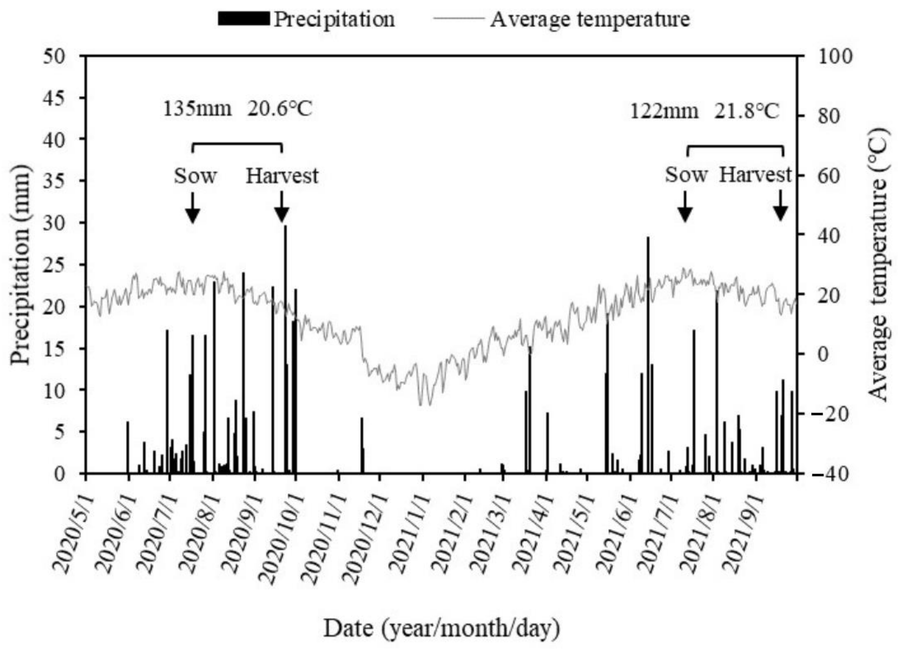

Figure 1. Average temperature and precipitation at the site during the growing seasons in 2020 and 2021. The upper part of the figure shows the average temperature and total precipitation during the growing period from sowing to harvest in both years.

2.2. Experimental Design and Crop Management

A completely randomized block design with two factors was used. The first factor was the three cropping patterns: sole buckwheat, sole alfalfa, and buckwheat-alfalfa intercropping. The second factor was two levels of microbial treatment: CK treatment (control, no active DSE) and DSE treatment. There were three replicate blocks giving a total of 18 plots (Figure 2). The area of each plot was 30 m^2 (5 m $\times$ 6 m), and the spacing between plots was 2 m. The experiment was surrounded by alfalfa. Each plant species had 4 treatments as follows. Buckwheat: (1) sole cropping buckwheat control (B-SCK); (2) sole cropping buckwheat with DSE inoculation (B-SDSE); (3) intercropping buckwheat control (B-ICK); (4) intercropping buckwheat with DSE inoculation (B-IDSE); alfalfa: (1) sole cropping alfalfa control (A-SCK); (2) sole cropping alfalfa with DSE inoculation (A-SDSE); (3) intercropping alfalfa control (A-ICK); (4) intercropping alfalfa with DSE inoculation (A-IDSE). Buckwheat and alfalfa were both sown in drills with sowing depths of 4 and 2 cm, respectively. The row spacing of buckwheat and alfalfa in sole cropping was 0.3 and 0.2 m, respectively. Strip intercropping was used with two rows of buckwheat and two rows of alfalfa, and the row spacing between buckwheat strips and alfalfa strips was 0.25 m. In addition, the row spacing between buckwheat and buckwheat was the same as that of sole-cropped buckwheat, and adjacent rows of alfalfa had the same row spacing as the alfalfa monoculture (Figure 3). Here, each intercropping plot comprised six intercropping strips consisting of twelve rows of buckwheat and twelve rows of alfalfa. In each intercropping plot, buckwheat and alfalfa occupied 11/20 and 9/20 of the area, respectively.

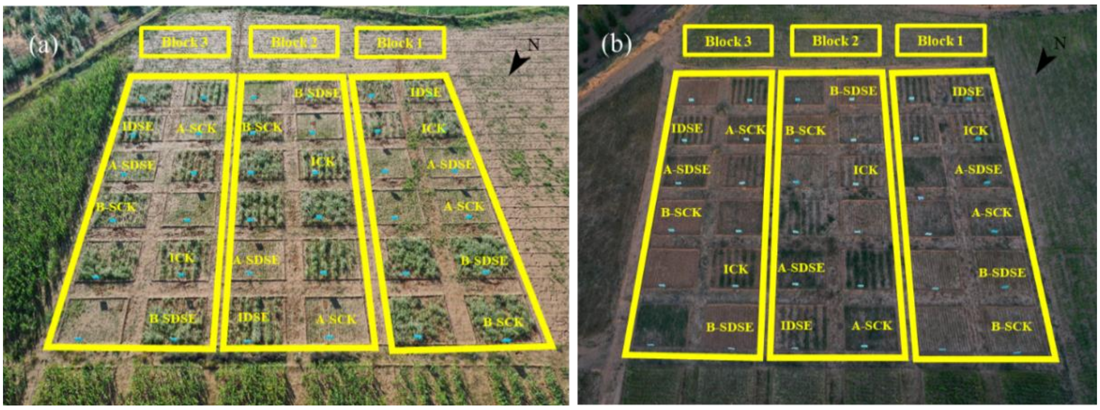

Figure 2. Field test layout in (**a**) 2020 and (**b**) 2021.

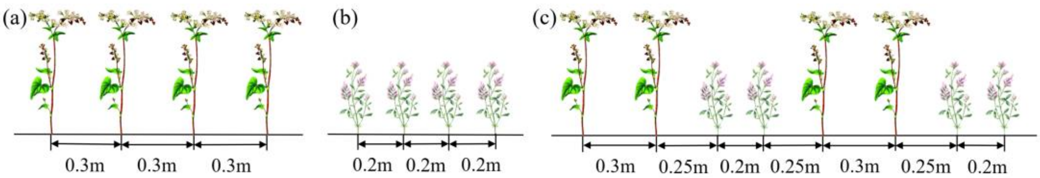

Figure 3. Diagrammatic representation of buckwheat-alfalfa cropping patterns; (**a**) sole buckwheat; (**b**) sole alfalfa; (**c**) buckwheat-alfalfa intercropping.

The test plants were the native crops buckwheat (*Fagopyrum esculentum* Moench.) Yuqiao No. 4 and alfalfa (*Medicago sativa* L.) WL324. The seeds were provided by the Seed Management Station of Shenmu county and were planted for two years. Buckwheat is an annual crop. It was planted by seed drilling for two consecutive years, and the root system of buckwheat was removed after the first-year harvest. Alfalfa is a perennial crop. The aerial part was harvested, and the crown remained for continuing growth the subsequent year.

The DSE used, *Alternaria* sp., was cultured and isolated at the Microbial Reclamation Laboratory of China University of Mining and Technology (Beijing). *Alternaria* sp. was confirmed by molecular identification and deposited in the general microbiology center of the China National Committee (CGMCC, 3, Courtyard 1, Beichen West Road, Chaoyang District, Beijing) with the preservation number CGMCC No. 17463 [9]. Seeds prepared for DSE treatment were immersed in DSE suspension (8×10^5 CFU mL^{-1}), and those intended to be controlled (CK) were immersed in the same volume of DSE suspension, which was previously sterilized by autoclaving at 121 °C for 30 min [9]. Seeds were sown after immersion for 12 h.

Disturbance of the soil microbial community was minimized by avoiding the use of herbicides or insecticides, and artificial weeding was conducted. The crops depended on natural precipitation during the growing season with no irrigation.

2.3. Sample Collection and Measurements

Buckwheat, alfalfa, and their rhizosphere soil were monitored, sampled, and harvested each year when the buckwheat had grown for approximately 70 days. Soil background values were determined, and the plots were sown on 19 July 2020, and the monitoring, sampling, and harvesting date was 25 September 2020. Sowing in the second year was on 15 July 2021, and monitoring, sampling, and harvesting were conducted on 19 September 2021.

Yield-related indicators were the yield and land equivalent ratio. Yield components were divided into agronomic traits and nutrient uptake.

1. DSE colonization rate; DSE colonization rate in roots was determined by acid fuchsin staining [18].
2. Yield and the land equivalent ratio (LER);

Kernels were used to measure the buckwheat yield. The alfalfa yield was measured by taking the stem and leaf parts that were 1–3 cm above the surface of the soil so that the stubble was left for regrowth the following year. The total weight of buckwheat grains in each plot was determined as the yield of buckwheat. The yields per plot were converted to yields per unit area. The land equivalent ratio (LER) of the intercropping system was used to compare the efficiency of sole cropping and intercropping in yield or biomass production and the relative land area required to achieve the same yield of crops grown. This is the sum of the equivalents of the two parts, namely crops X and Y [19], and is calculated as follows:

$$LER = PLER_X + PLER_Y = \frac{B_{iX}}{B_{sX}} + \frac{B_{iY}}{B_{sY}} \quad (1)$$

where, B_{sX} and B_{sY} are the yields of sole X and sole Y, respectively, and B_{iX} and B_{iY} are the yields of intercropped X and intercropped Y, respectively. When LER > 1 the intercropping system has a yield advantage. When LER < 1 the intercropping system has no yield advantage [19].

3. Agronomic traits;

The agronomic trait indicators of buckwheat were plant height, stem diameter, number of main stem nodes, number of main stem branches, number of flower clusters, grain number per plant, grain weight per plant, 100-grain weight, aboveground biomass, and belowground biomass. The agronomic trait indicators of alfalfa were plant height, stem diameter, number of main stem nodes, number of main stem branches, internode length, leaf-to-stem ratio, aboveground biomass, and belowground biomass. When buckwheat was harvested, 10 representative buckwheat plants were randomly selected from each plot to measure plant height, stem diameter, number of main stem nodes, number of main stem branches, and number of flower clusters. After the measurement, the buckwheat plants were collected for the determination of grain number per plant, grain weight per plant, 100-grain weight, aboveground biomass, and belowground biomass. When alfalfa was harvested, 10 representative alfalfa plants were randomly selected from each plot to measure plant height, stem diameter, number of main stem nodes, number of main stem branches, and the internode length. After measurement, the alfalfa plants were collected for the determination of grain number per plant, grain weight per plant, 100-grain weight, aboveground biomass, and belowground biomass. Plant height and internode length were measured using a steel ruler with an accuracy of 1 mm. The stem diameter was measured using a 0.02 mm vernier caliper. The number of main stem nodes, the number of main stem branches, and the number of flower clusters were directly enumerated visually. Grain number per plant, grain weight per plant, and 100-grain weight were enumerated and weighed using an electronic balance with 0.001 g accuracy after threshing. The aboveground and underground parts of buckwheat and alfalfa were collected and oven-dried to constant weight at 75 °C to determine the biomass.

4. Nutrient uptake;

Nutrient uptake indicators were plant nitrogen (N) content, N uptake, phosphorus (P) content, P uptake, soil available N content, and soil available P content. The dried plant samples were used to determine plant N and P contents. Plant N and P uptake were calculated as the elemental concentrations in plant samples × biomass. The rhizosphere soils of 10 plants randomly selected from each plot were mixed thoroughly, air-dried, ground, and sieved to determine the contents of soil available N and soil available P. Plant N content was determined by Kjeldahl digestion [20]. Soil available N content was determined by the alkaline hydrolysis diffusion method [21]. Plant P content and soil available P content were determined by inductively coupled–plasma emission spectroscopy (ICP-OES, Optima 5300DV, Perkin Elmer, Waltham, MA, USA).

2.4. Statistical Analysis

Excel 2016 was used for data processing. SPSS 20.0 (IBM Corp., Armonk, NY, USA) was used for two-way analysis of variance ($p < 0.05$) to test the significance of differences between treatments and the Duncan multiple range tests ($p < 0.05$, $p < 0.01$, denoted by * and **, respectively) to compare mean values. R software (V3.5.2, https://www.r-project.org/, accessed on 21 September 2022) corrplot package was used for Pearson correlation analysis. Regression and path analysis is often used to study the impact of plant yield components on yield [22]. SPSS 20.0 was used for stepwise regression analysis and path analysis to obtain the direct and indirect effects of the DSE colonization rate and yield components on the yields of the two plant species. A correlation coefficient between a yield component and yield is the sum of direct and indirect effects [22].

3. Results

3.1. Effects of Different Treatments on DSE Colonization of Buckwheat and Alfalfa

Typical DSE mycelial structures were observed in the roots of harvested buckwheat and alfalfa (Figure 4a,b), indicating DSE colonization of the roots.

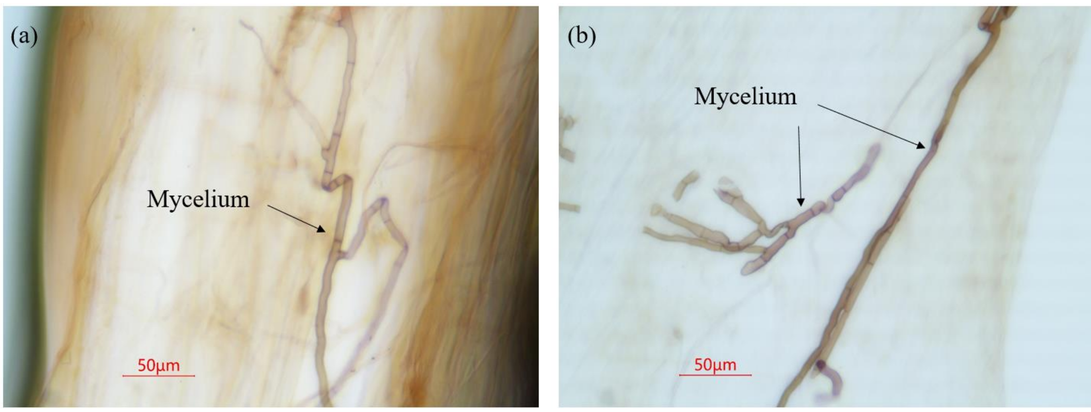

Figure 4. DSE colonization in (**a**) buckwheat and (**b**) alfalfa roots.

DSE colonization rates in B-SCK were 15–25% in 2020 and 2021. DSE inoculation significantly increased DSE colonization rates in B-SCK and B-ICK by 97 and 120.1% in 2020 and by 118.3 and 133.8% in 2021 (Figure 5a). DSE colonization rates in A-SCK were ~15–20% in 2020 and 2021. DSE colonization rates of A-SCK and A-ICK increased significantly by 135 and 170% in 2020 and by 173 and 205% in 2021 following DSE inoculation (Figure 5b). These results indicate that there were indigenous DSE in the soil and the DSE colonization rates of buckwheat and alfalfa roots increased significantly following DSE inoculation. However, intercropping had no significant effect on the DSE colonization rate.

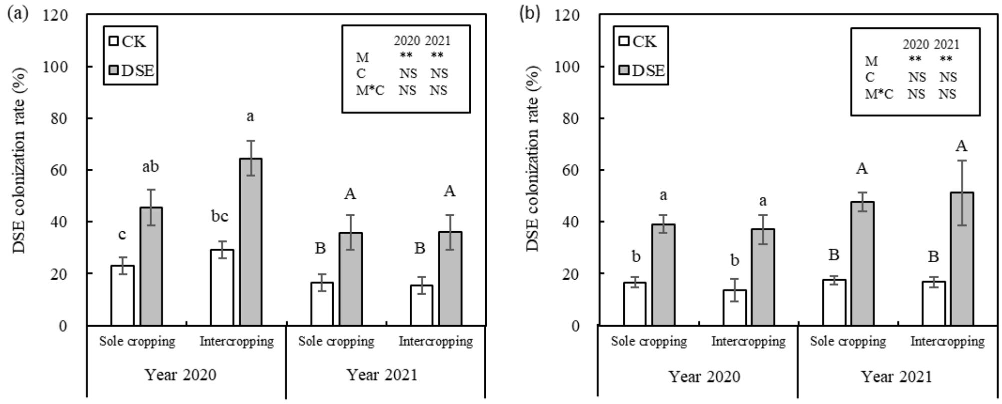

Figure 5. DSE colonization rates of buckwheat and alfalfa under different treatments. (**a**) DSE colonization rate in buckwheat; (**b**) DSE colonization rate in alfalfa. Different lowercase letters indicate a significant difference at the 5% level by LSD between different treatments in 2020. Different capital letters indicate a significant difference at the 5% level by LSD between different treatments in 2021. M, microbial treatment; C, cropping pattern; **, $p < 0.01$; NS, not significant.

3.2. Effects of Different Treatments on Yields of Buckwheat and Alfalfa

The yields of buckwheat in 2020 are shown in Figure 6a. DSE inoculation increased the yields of buckwheat in B-SCK and B-ICK by 31.7 and 85.4%, respectively. Intercropping significantly increased the yields of buckwheat in B-SDSE (by 65.2%) but had no significant effect on the yield of B-SCK. Combined intercropping and DSE inoculation (B-IDSE) increased the yield by 117.5% compared with B-SCK. The sequence of buckwheat yields in different treatments was: B-IDSE > B-SDSE > B-ICK > B-SCK. The yield of buckwheat in B-IDSE increased by 65.2, 85.4 and 117.5% compared with B-SDSE, B-ICK, and B-SCK, respectively. Intercropping combined with DSE inoculation, therefore, produced the maximum yield increase in buckwheat.

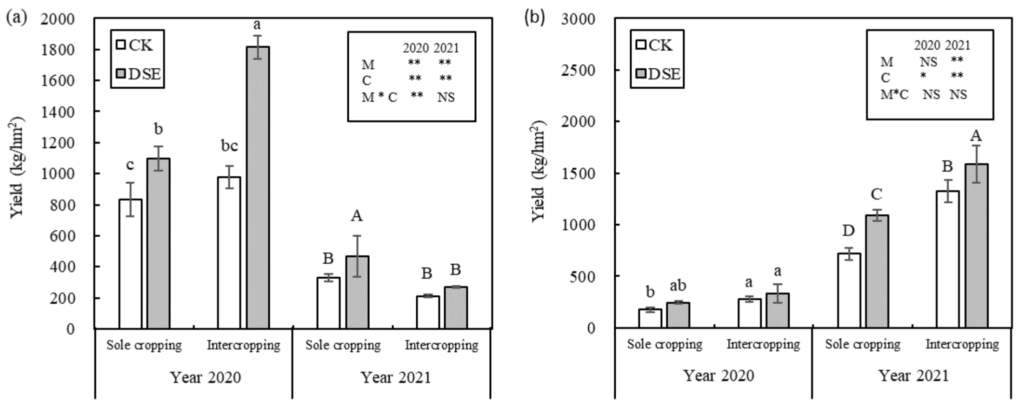

Figure 6. Yields of (**a**) buckwheat and (**b**) alfalfa. Different lowercase letters indicate a significant difference at the 5% level by LSD between different treatments in 2020. Different capital letters indicate a significant difference at the 5% level by LSD between different treatments in 2021. M, microbial treatment; C, cropping pattern; *, $p < 0.05$; **, $p < 0.01$; NS, not significant.

The yields of buckwheat in 2021 are shown in Figure 6a. After the first-year harvest and a second buckwheat crop in the second year in the same plots, the overall yield of buckwheat in 2021 declined by 72.9% compared with 2020. DSE inoculation increased the yield of buckwheat in B-SCK and B-ICK by 41.7 and 27.3%, respectively. Intercropping reduced the yield of B-SCK and B-SDSE by 36.2 and 42.6%. Compared with B-SCK, intercropping with DSE inoculation reduced the yield of buckwheat by 23.2%. The buckwheat yields in different treatments followed the sequence: B-SDSE > B-SCK > B-IDSE > B-ICK. The buckwheat yield in B-SDSE increased by 41.7, 74.4 and 122.3%, respectively, compared with B-IDSE, B-ICK, and B-SCK. Therefore, combining sole cropping with DSE inoculation produced the maximum yield increase in the second year, and this increase was maintained over two consecutive years.

The alfalfa yields in 2020 are shown in Figure 6b. The yields in A-SCK and A-ICK appeared to increase following DSE inoculation, but there was no significant increase. Intercropping increased the alfalfa yield in A-SDSE by 55.8%. Compared with A-SCK, intercropping combined with DSE inoculation increased the yield by 86.2%. The yields of alfalfa in the different treatments followed the order: A-IDSE > A-ICK > A-SDSE > A-SCK, and the yields of alfalfa in A-IDSE were 19.6, 34.3 and 86.2% higher than in A-ICK, A-SDSE, and A-SCK, respectively. Therefore, combining intercropping with DSE inoculation produced the maximum alfalfa yield increase.

The alfalfa yields in 2021 are shown in Figure 6b. After the aboveground parts were harvested in the first year in the same plots, the overall yield of alfalfa in 2021 increased by 356.4% compared with 2020. Compared with the control, DSE inoculation increased the yields of alfalfa in A-SCK and A-ICK by 51.5 and 20%, respectively. Compared with sole cropping, intercropping significantly increased the yields in A-SCK and A-SDSE by 83.7 and 45.5%, respectively. Compared with A-SCK, combining intercropping with DSE inoculation increased the alfalfa yield by 120.5%. The yields in the different treatments followed the sequence: A-IDSE > A-ICK > A-SDSE > A-SCK. The alfalfa yield in A-IDSE increased by 20, 45.5, and 120.5% compared with A-ICK, A-SDSE, and A-SCK, respectively. Therefore, combining intercropping with DSE inoculation produced the maximum alfalfa yield increase in the second year of continuous cropping.

3.3. Effects of the Treatments on the Land Equivalent Ratio (LER) in Intercropping

Statistical analysis was conducted on the $PLER_B$, $PLER_A$, and LER of the buckwheat-alfalfa intercropping system under control and DSE inoculation conditions (Figure 7). In both controls and DSE inoculation, the average LER of the buckwheat-alfalfa intercropping system overall was > 1 in the two years, indicating that intercropping had yield advantages in the field for two consecutive years, with the greatest yield advantage in the first year. From the perspective of the percentages of $PLER_B$ and $PLER_A$, the yield advantage in 2020 depended mainly on overproduction by the buckwheat in the DSE treatment, and the yield advantage in 2021 depended mainly on overproduction by alfalfa. The $PLER_B$ was larger in 2020 than in 2021. DSE inoculation increased the $PLER_B$ in 2020 only. The $PLER_A$ shows that DSE inoculation gave no significant differences in 2020 and 2021. DSE inoculation significantly increased the LER of the buckwheat-alfalfa intercropping system by 11.2% in 2020, mainly due to a significant increase in $PLER_B$. However, DSE inoculation had no significant effect on the LER of the intercropping system in 2021.

3.4. Effects of the Treatments on Agronomic Traits of Both Crop Species

The buckwheat agronomic traits in 2020 are shown in Table 1. Compared with the control, DSE inoculation increased all yield components except 100-grain weight. DSE inoculation significantly increased the stem diameter, the number of main stem branches, and the number of flower clusters in B-SCK by 24.1, 14.6 and 55.3%, respectively. DSE inoculation significantly increased the number of main stem branches, the number of flower clusters, grain number per plant, grain weight per plant, aboveground biomass, and belowground biomass in B-ICK by 22.4, 80.2, 149.9, 161.9, 109.5, and 113.3%, respectively. Compared with sole cropping, intercropping increased all yield components except for the 100-grain weight of B-SCK and B-SDSE. However, the effect of intercropping on B-SCK was not significant. Intercropping increased the number of main stem branches, the number of flower clusters, grain number per plant, grain weight per plant, aboveground biomass, and belowground biomass by 12.7, 42.7, 115.7, 157.8, 100.0, and 105.1%, respectively. Compared with B-SCK, plant height, stem diameter, the number of main stem nodes, the number of main stem branches, the number of flower clusters, grain number per plant, grain weight per plant, aboveground biomass, and belowground biomass in B-IDSE increased by 22.1, 32.8, 18.6, 29.1, 121.7, 190.7, 200.0, 183.9 and 182.8%, respectively.

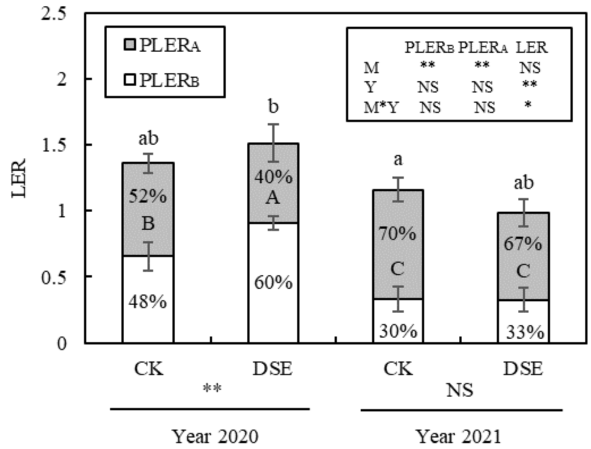

Figure 7. Land equivalent ratio (LER) of the buckwheat-alfalfa intercropping system under different microbial treatments. Different lowercase letters indicate a significant difference at the 5% level by LSD between different treatments in 2020. Different capital letters indicate a significant difference at the 5% level by LSD between different treatments in 2021. M, microbial treatment; Y, year; *, $p < 0.05$; **, $p < 0.01$; NS, not significant.

The agronomic traits of buckwheat in 2021 are shown in Table 1. Compared with the control, DSE inoculation increased all yield components except 100-grain weight. DSE inoculation significantly increased plant height, number of main stem branches, number of flower clusters, and the aboveground biomass of B-SCK by 31.8, 97.4, 104.4, and 143%, respectively. The yield components (except belowground biomass) in B-ICK seemed to increase with DSE inoculation, but there was no significant effect. The yield components in B-ICK and B-IDSE decreased compared to B-SCK, indicating that intercropping reduced the yield components. The significant negative effect of intercropping on B-SCK was reflected in plant height, the number of main stem nodes, and 100-grain weight, which decreased by 36.8, 20.4, and 34.2%, respectively. The significant negative effect of intercropping on B-SDSE was reflected in plant height, number of main stem nodes, number of main stem branches, number of flower clusters, and the aboveground biomass, which decreased by 42.9, 17.0, 56.0, 52.9, and 64.3, respectively. Compared with B-SCK, B-IDSE significantly reduced the 100-grain weight of buckwheat only (by 23.6%), and the apparent decline in each of the other components was not significant.

The agronomic traits of alfalfa in 2021 are shown in Table 2. Compared with the control, yield components (except for stem diameter and internode length) of alfalfa increased following DSE inoculation. DSE inoculation significantly increased plant height and the number of main stem branches of A-SCK by 47.7 and 37.1%, respectively. DSE Inoculation had no significant effect on yield components of A-ICK. Compared with sole cropping, the yield components of A-SCK and A-SDSE indicate that intercropping increased the yield. Intercropping significantly increased plant height, leaf-to-stem ratio, and aboveground biomass of A-SCK by 48.3, 30.7 and 130.4%, respectively. The effect of intercropping on A-SDSE was reflected in the aboveground biomass only, which increased by 61.7%. Compared with A-SCK, plant height, the number of main stem branches, leaf-to-stem ratio, and aboveground biomass of alfalfa in A-IDSE increased significantly by 56.8, 39.3, 31.8 and 169.6%, respectively, indicating that combining DSE inoculation with intercropping significantly increased the yield.

Table 1. Effects of the different treatments on the yield components of buckwheat.

Year	Treatment	Plant Height (cm)	Stem Diameter (mm)	Number of Main Stem Nodes	Number of Main Stem Branches	Number of Flower Clusters	Grain Number per Plant	Grain Weight per Plant (g)	100-Grain Weight (g)	Above-Ground Biomass (g)	Below-Ground Biomass (g)
2020	B-SCK	69.2 ± 0.4 b	5.8 ± 0.3 b	11.8 ± 0.3 b	5.5 ± 0.3 c	65.3 ± 20.2 c	231.4 ± 108.7 b	5.50 ± 2.20 b	2.46 ± 0.25 a	12.40 ± 3.70 b	2.27 ± 0.57 b
	B-SDSE	79.8 ± 5.4 ab	7.2 ± 0.4 a	13.3 ± 0.5 ab	6.4 ± 0.1 b	101.3 ± 5.9 b	311.8 ± 103.3 b	6.40 ± 2.60 b	2.03 ± 0.49 a	17.60 ± 4.70 b	3.13 ± 0.79 b
	B-ICK	78.1 ± 9.1 ab	6.8 ± 0.9 ab	12.7 ± 1.7 ab	5.9 ± 0.5 bc	80.3 ± 20.2 bc	269.2 ± 100.3 b	6.30 ± 2.10 b	2.41 ± 0.34 a	16.80 ± 4.80 b	3.01 ± 0.68 b
	B-IDSE	84.5 ± 6.2 a	7.7 ± 0.3 a	14.0 ± 0.6 a	7.1 ± 0.2 a	144.7 ± 11.7 a	672.6 ± 158.7 a	16.50 ± 2.40 a	2.52 ± 0.28 a	35.20 ± 6.30 a	6.42 ± 1.00 a
2021	B-SCK	44.0 ± 7.3 B	3.8 ± 0.6 AB	10.3 ± 1.0 AB	3.8 ± 1.3 B	36.7 ± 7.6 B	112.0 ± 41.6 AB	2.70 ± 1.24 AB	2.37 ± 0.20 A	5.86 ± 1.27 B	1.51 ± 0.25 A
	B-SDSE	58.0 ± 9.5 A	5.5 ± 1.7 A	11.2 ± 0.6 A	7.5 ± 0.1 A	75.0 ± 27.8 A	134.7 ± 54.5 A	3.14 ± 1.66 A	2.25 ± 0.29 AB	14.24 ± 4.94 A	1.63 ± 0.70 A
	B-ICK	27.8 ± 1.0 C	2.3 ± 0.7 B	8.2 ± 0.8 C	2.5 ± 0.1 B	27.3 ± 4.0 B	43.7 ± 1.5 B	0.68 ± 0.12 B	1.56 ± 0.25 C	3.26 ± 0.63 B	1.02 ± 0.17 A
	B-IDSE	33.1 ± 6.9 BC	3.4 ± 1.0 AB	9.3 ± 0.3 BC	3.3 ± 1.4 B	35.3 ± 2.5 B	66.7 ± 24.2 AB	1.26 ± 0.61 AB	1.81 ± 0.34 BC	5.09 ± 0.60 B	0.66 ± 0.49 A
Significance											
2020	P (M)	*	**	*	***	***	**	**	NS	**	**
	P (C)	NS	*	NS	*	*	*	**	NS	**	**
	P (M × C)	NS	NS	NS	NS	NS	*	**	NS	*	*
2021	P (M)	*	*	*	**	*	NS	NS	NS	**	NS
	P (C)	**	*	**	**	*	*	*	**	**	NS
	P (M × C)	NS	NS	NS	*	NS	NS	NS	NS	NS	NS

Different lowercase letters following mean values in the same column indicate a significant difference at the 5% level by LSD between different treatments in 2020. Different capital letters following mean values in the same column indicate a significant difference at the 5% level by LSD between different treatments in 2020. P (M), difference between groups of microbial treatments; P (C), inter-group differences in cropping patterns; P (M × C), interaction between microbial treatment and cropping pattern; *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; NS, not significant. The agronomic traits of alfalfa in 2020 are shown in Table 2. Compared with the control, DSE inoculation increased the yield components (except 100-grain weight) of alfalfa. DSE inoculation significantly increased the number of main stem nodes and the number of main stem branches of A-SCK by 34.5 and 22.9%, respectively. DSE inoculation had no significant effect on the yield components in A-ICK. Plant height, number of main stem nodes, the number of main stem branches, and leaf-to-stem ratio increased significantly following intercropping with increases of 113.6, 56.7, 48.8, and 27.9%, respectively. The significant effect of intercropping on A-SDSE was reflected in the number of main stem branches and aboveground biomass with rates of increase in 16.9 and 59.1%, respectively. Compared with A-SCK, plant height, the number of main stem nodes, the number of main stem branches, leaf-to-stem ratio, and aboveground biomass of alfalfa in A-IDSE increased significantly with rates of increases of 112.7, 60.0, 51.6, 26.9 and 94.4%, respectively, indicating that combining DSE inoculation with intercropping increased the yield components of alfalfa.

Table 2. Effects of the different treatments on the yield components of alfalfa.

Year	Treatment	Plant Height (cm)	Stem Diameter (mm)	Number of Main Stem Nodes	Number of Main Stem Branches	Internode Length (cm)	Leaf to Stem Ratio	Aboveground Biomass (g)	Belowground Biomass (g)
2020	A-SCK	7.57 ± 1.4 b	1.19 ± 0.23 a	6.17 ± 0.75 b	7.17 ± 0.55 c	1.22 ± 0.08 a	1.04 ± 0.06 b	0.18 ± 0.04 b	0.22 ± 0.04 a
	A-SDSE	10.17 ± 0.93 ab	1.39 ± 0.08 a	8.3 ± 0.3 a	9.3 ± 0.2 b	1.23 ± 0.08 a	1.13 ± 0.03 ab	0.22 ± 0.04 b	0.27 ± 0.03 a
	A-ICK	16.17 ± 3.77 a	1.28 ± 0.03 a	9.67 ± 0.4 a	10.67 ± 0.21 ab	1.66 ± 0.33 a	1.33 ± 0.08 a	0.3 ± 0.06 ab	0.25 ± 0.06 a
	A-IDSE	16.1 ± 6.11 a	1.19 ± 0.27 a	9.87 ± 1.59 a	10.87 ± 1.33 a	1.6 ± 0.4 a	1.32 ± 0.22 a	0.35 ± 0.25 a	0.28 ± 0.19 a
2021	A-SCK	21.17 ± 0.29 B	1.79 ± 0.04 A	3.25 ± 0.25 A	10.7 ± 1.48 B	6.54 ± 0.5 A	0.88 ± 0.04 B	0.69 ± 0.02 C	0.84 ± 0.17 A
	A-SDSE	31.27 ± 1.99 A	1.89 ± 0.1 A	3.75 ± 1.09 A	14.67 ± 2.76 A	8.81 ± 2.47 A	0.97 ± 0.06 AB	1.15 ± 0.14 BC	1.25 ± 0.07 A
	A-ICK	31.4 ± 1.85 A	1.8 ± 0.39 A	3.42 ± 0.14 A	12.8 ± 0.85 AB	9.19 ± 0.26 A	1.15 ± 0.09 A	1.59 ± 0.3 AB	1.19 ± 0.13 A
	A-IDSE	33.2 ± 1.08 A	1.6 ± 0.26 A	4.17 ± 0.52 A	14.9 ± 0.89 A	8.05 ± 1.02 A	1.16 ± 0.24 A	1.86 ± 0.63 A	1.29 ± 0.44 A
Significance									
2020	P (M)	NS	NS	NS	*	NS	NS	NS	NS
	P (C)	**	NS	**	**	*	**	NS	NS
	P (M × C)	NS	NS	NS	NS	NS	NS	NS	NS
2021	P (M)	***	NS	NS	*	NS	NS	NS	NS
	P (C)	***	NS	NS	NS	NS	*	**	NS
	P (M × C)	**	NS	NS	NS	NS	NS	NS	NS

Different lowercase letters following the mean values in the same column indicate a significant difference at the 5% level by LSD between different treatments in 2020. Different capital letters following the mean values in the same column indicate a significant difference at the 5% level by LSD between different treatments in 2020. P (M), difference between groups of microbial treatments; P (C), inter-group differences in cropping pattern; P (M × C), interaction between microbial treatment and cropping pattern; *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; NS, not significant.

3.5. Effects of Different Treatments on Nutrient Uptake by Both Crop Species

Nutrient uptake by buckwheat in 2020 is shown in Table 3. DSE inoculation significantly increased N uptake, plant P content, P uptake, and soil available N content in B-SCK by 64.3, 50.0, 102.3 and 30.5%, respectively. DSE inoculation significantly increased N uptake, P uptake, and soil available N content in B-ICK by 64.9, 79.0 and 34.7%, respectively. Intercropping significantly increased N uptake and P uptake in B-SCK by 44.6 and 50.1%, respectively. Intercropping significantly increased N uptake and P uptake in B-SDSE by 45.1 and 32.8%, respectively, and significantly decreased plant N content and plant P content in B-SDSE by 22.7 and 31.1%, respectively. Combining intercropping with DSE inoculation significantly increased N uptake, P uptake, and soil available N content in buckwheat by 138.4, 32.8 and 23.2%, respectively.

Table 3. Effects of the different treatments on nutrient uptake by buckwheat.

Year	Treatment	Plant N Content (mg g^{-1})	N Uptake (mg)	Plant P Content (mg g^{-1})	P Uptake (mg)	Soil Available N Content (mg kg^{-1})	Soil Available P Content (mg kg^{-1})
2020	B-SCK	22.5 ± 0.8 ab	287.3 ± 12.8 c	3.0 ± 0.3 b	39.3 ± 5.0 d	16.4 ± 1.1 b	4.4 ± 2.1 a
	B-SDSE	26.0 ± 3.5 a	472.1 ± 45.2 b	4.5 ± 0.5 a	79.5 ± 10.4 b	21.4 ± 0.9 a	4.7 ± 1.4 a
	B-ICK	24.3 ± 1.3 ab	415.4 ± 32.0 b	3.5 ± 0.3 b	59.0 ± 2.6 c	15.0 ± 1.6 b	7.5 ± 6.3 a
	B-IDSE	20.1 ± 2.2 b	684.8 ± 42.5 a	3.1 ± 0.3 b	105.6 ± 10.4 a	20.3 ± 1.8 a	3.5 ± 2.4 a
2021	B-SCK	12.4 ± 1.9 A	72.4 ± 18.8 B	4.2 ± 0.6 A	24.3 ± 5.9 AB	17.1 ± 4.3 A	2.0 ± 0.3 B
	B-SDSE	10.4 ± 2.0 A	152.9 ± 78.5 A	3.7 ± 1.0 A	55.3 ± 32.9 A	14.7 ± 1.8 A	2.5 ± 0.4 B
	B-ICK	11.2 ± 0.4 A	36.2 ± 6.1 B	3.2 ± 0.1 A	10.5 ± 2.3 B	19.3 ± 1.4 A	4.1 ± 0.5 A
	B-IDSE	9.5 ± 1.5 A	48.9 ± 12.2 B	3.3 ± 0.6 A	17.2 ± 4.6 B	14.5 ± 1.3 A	2.5 ± 0.1 B
Significance							
2020	P (M)	NS	**	*	**	**	NS
	P (C)	NS	**	*	**	NS	NS
	P (M × C)	*	NS	**	NS	NS	NS
2021	P (M)	NS	NS	NS	NS	*	*
	P (C)	NS	*	NS	*	NS	**
	P (M × C)	NS	NS	NS	NS	NS	**

Different lowercase letters following the mean values in the same column indicate a significant difference at the 5% level by LSD between different treatments in 2020. Different capital letters following the mean values in the same column indicate a significant difference at a 5% level by LSD between different treatments in 2020. P (M), the difference between groups of microbial treatments; P (C), inter-group differences in planting patterns; P (M × C), the interaction between microbial treatment and cropping pattern; *, $p < 0.05$; **, $p < 0.01$; NS, not significant.

Nutrient uptake by buckwheat in 2021 is shown in Table 3. DSE inoculation significantly increased N uptake in B-SCK by 111.2%. DSE inoculation significantly reduced soil available P content in B-ICK by 39%. Compared with sole cropping, intercropping significantly reduced soil available P content in B-SCK by 105%. Intercropping significantly reduced N uptake and P uptake in B-SDSE by 68.0 and 68.9%, respectively. There was no significant difference in the nutrient uptake of buckwheat between B-SCK and B-IDSE, indicating that combining intercropping with DSE inoculation did not affect nutrient uptake.

Nutrient uptake by alfalfa in 2020 is shown in Table 4. Compared with the control, DSE inoculation did not significantly affect nutrient uptake. Compared with sole cropping, intercropping significantly increased plant N content in A-SCK by 7.6%. However, soil available N content declined significantly by 25.0%. Intercropping did not affect nutrient uptake in A-SDSE. Compared with A-SCK, plant N content and N uptake of alfalfa increased significantly by 9.8 and 55.9%, respectively.

Nutrient uptake by alfalfa in 2021 is shown in Table 4. Compared with the control, DSE inoculation reduced the soil available N content in A-SCK by 55.8% and increased the soil available P content by 44.4%. The soil available P content in A-ICK increased significantly by 450% after DSE inoculation. Intercropping significantly increased N uptake and P uptake in A-SCK by 138.5 and 147.4% and significantly decreased soil available N content and soil available P content by 56.9 and 77.8%. Intercropping reduced the soil available P content in A-SDSE by 15.4%. Compared with A-SCK, the N uptake, P uptake, and soil available P content of alfalfa in A-IDSE increased by 171.0, 178.9, and 22.2%, respectively. Soil available N content in A-IDSE declined by 56.9% compared to A-SCK.

Table 4. Effects of the different treatments on nutrient uptake by alfalfa.

Year	Treatment	Plant N Content (mg g^{-1})	N Uptake (mg)	Plant P Content (mg g^{-1})	P Uptake (mg)	Soil Available N Content (mg kg^{-1})	Soil Available P Content (mg kg^{-1})
2020	A-SCK	44.8 ± 2.8 b	7.9 ± 0.8 b	3.00 ± 0.20 a	0.5 ± 0.1 a	23.2 ± 1.7 a	5.9 ± 3.6 a
	A-SDSE	46.7 ± 0.5 ab	10.4 ± 1.1 ab	3.10 ± 0.60 a	0.7 ± 0.2 a	20.6 ± 0.5 ab	5.6 ± 4.3 a
	A-ICK	48.2 ± 0.8 a	14.3 ± 0.4 ab	3.30 ± 0.20 a	1.0 ± 0.1 a	17.5 ± 3.0 b	4.0 ± 3.1 a
	A-IDSE	49.2 ± 0.4 a	17.9 ± 9 a	2.70 ± 0.10 a	1.0 ± 0.6 a	20.4 ± 2.2 ab	6.0 ± 5.5 a
2021	A-SCK	32.2 ± 4.2 A	22.1 ± 2.4 B	2.75 ± 0.14 A	1.9 ± 0.1 B	39.3 ± 2.2 A	1.8 ± 0.1 C
	A-SDSE	36.8 ± 0.7 A	42.1 ± 4.4 AB	2.93 ± 0.02 A	3.4 ± 0.4 AB	17.4 ± 4.5 B	2.6 ± 0.3 A
	A-ICK	32.8 ± 3.0 A	52.7 ± 14.9 A	2.94 ± 0.10 A	4.7 ± 1.1 A	17.1 ± 0.6 B	0.4 ± 0.1 D
	A-IDSE	32.9 ± 3.4 A	59.9 ± 14.7 A	2.84 ± 0.10 A	5.3 ± 1.7 A	16.9 ± 1.6 B	2.2 ± 0.1 B
Significance							
2020	P (M)	NS	NS	NS	NS	NS	NS
	P (C)	**	*	NS	NS	*	NS
	P (M × C)	NS	NS	NS	NS	*	NS
2021	P (M)	NS	NS	NS	NS	**	**
	P (C)	NS	**	NS	**	**	**
	P (M × C)	NS	NS	*	NS	**	**

Different lowercase letters following the mean values in the same column indicate a significant difference at the 5% level by LSD between different treatments in 2020. Different capital letters following the mean values in the same column indicate a significant difference at a 5% level by LSD between different treatments in 2020. P (M), the difference between groups of microbial treatments; P (C), inter-group differences in planting patterns; P (M × C), the interaction between microbial treatment and cropping pattern; *, $p < 0.05$; **, $p < 0.01$; NS, not significant.

3.6. Path Analysis of the Effect of DSE Colonization Rate and Yield Components on the Yields of Both Crop Species

DSE colonization rate and yield components of buckwheat and alfalfa were taken as independent variables, and yield was taken as the dependent variable. Stepwise regression analysis was carried out to identify the factors that had a decisive effect on the yields of buckwheat and alfalfa. DSE colonization rate, plant N content, grain weight per plant, plant P content, P uptake, and soil available N content determined buckwheat yield. Nitrogen uptake, plant height, plant N content, and DSE colonization rate determined alfalfa yield.

Path analysis was carried out on the six factors that played a decisive role in buckwheat yield (Table 5). The absolute value of the coefficient of path analysis indicates the effect of each yield component on buckwheat yield, and this effect was divided into direct and indirect coefficients. Correlation coefficients between the five factors and buckwheat yield were all high and significant ($p < 0.05$). The order of the correlation coefficients of the six determinants was: grain weight per plant (0.947) > P uptake (0.898) > DSE colonization rate (0.730) > plant N content (0.708) > soil available N content (0.418) > plant P content (−0.052). The path coefficient shows that the direct effects of grain weight per plant and plant P content were greater than their indirect effects, and the indirect effects of DSE colonization rate, plant N content, P uptake, and soil available N content on yield were greater than their direct effects. The largest direct effect on yield was exerted by the grain weight per plant (0.510) and the smallest by soil available N content (0.056). P uptake, DSE colonization rate, plant N content, and soil available N content all indirectly increased the yield mainly through a positive response to the grain weight per plant, and their indirect effects were the largest compared to those through other factors. The indirect effect of the DSE colonization rate on yield depended mainly on grain weight per plant (0.332), P uptake (0.141), and plant N content (0.097). The coefficient of determination of the six yield components was 0.993, indicating that these six indicators played a decisive role in the yield of buckwheat. The coefficient of determination of residual factors was 0.084, indicating that other factors and experimental errors had little effect on yield.

Table 5. Path analysis of yield determinants of buckwheat.

Determinant	Correlation Coefficient	Direct Pass Coefficient	Indirect Pass Coefficient						
			Sum	DSE Colonization Rate	Plant N Content	Grain Weight per Plant	Plant P Content	P Uptake	Soil Available N Content
DSE colonization rate	0.730	0.147	0.583		0.097	0.332	0.001	0.141	0.012
Plant N content	0.708	0.285	0.423	0.050		0.267	−0.025	0.114	0.017
Grain weight per plant	0.947	0.510	0.437	0.096	0.149		0.008	0.163	0.020
Plant P content	−0.052	−0.117	0.064	−0.001	0.061	−0.036		0.040	0.000
P uptake	0.898	0.192	0.706	0.108	0.170	0.434	−0.024		0.019
Soil available N content	0.418	0.056	0.362	0.032	0.084	0.182	0.000	0.064	
Coefficient of determination	0.993								
Coefficient of determination of residual factors	0.084								

Path analyses were conducted on the four factors that played a decisive role in alfalfa yield (Table 6), Pearson's correlation coefficient between the determinants and yield was calculated, and the direct and indirect effects of the determinants on yield were obtained. The correlation coefficients between the four components and alfalfa yield were high, and the correlation was significant ($p < 0.05$). The correlation coefficients of the four determinants followed the order: N uptake (0.946) > plant height (0.938) > plant N content (−0.817) > DSE colonization rate (0.373). However, plant N content had an inhibitory effect on alfalfa yield. The path coefficient shows that the indirect effects of the four components on yield were greater than their direct effects. The most direct effect was N uptake (0.454), and the least was the DSE colonization rate (0.373). The indirect effect of N uptake on alfalfa yield (0.493) was greater than its direct effect (0.454), promoting the yield mainly through the positive effect on plant height and plant N content. The indirect effect of plant height on alfalfa yield (0.625) was greater than its direct effect (0.314), promoting the yield mainly through a positive effect on plant N uptake and plant N content. The indirect effect of plant N content on alfalfa yield (−0.555) was greater than its direct effect (−0.262), promoting the yield mainly through negative effects on N uptake and plant height. The indirect effect of the DSE colonization rate on alfalfa yield (0.276) was greater than its direct effect (0.097), and its indirect effect depended mainly on N uptake (0.154), plant height (0.108) and plant N content (0.014). The codetermination coefficient of the four components was 0.974, indicating that they played a decisive role in the yield of alfalfa. The coefficient of determination of residual factors was 0.161, indicating that other factors and experimental errors had little effect on yield.

Table 6. Path analysis of yield determinants of alfalfa.

Determinant	Correlation Coefficient	Direct Pass Coefficient	Indirect Pass Coefficient			
			Sum	N Uptake	Plant Height	Plant N Content
N uptake	0.946	0.454	0.493		0.275	0.185
Plant height	0.938	0.314	0.625	0.398		0.193
Plant N content	−0.817	−0.262	−0.555	−0.319	−0.231	
DSE colonization rate	0.373	0.097	0.276	0.154	0.108	0.014
Coefficient of determination	0.974					
Coefficient of determination of residual factors	0.161					

4. Discussion

4.1. Effects of DSE Inoculation on Plant Colonization Rate, Yield, and Yield Components

DSE is present in a wide range of habitats, especially in stressed environments [6], with little or no host specificity. They can colonize agricultural crops [9] and grassland plants [11], including buckwheat [23] and alfalfa [11], and this concurs with the current results. Indigenous DSE occurs in the barren soils of coal mining subsidence lands. Buckwheat and alfalfa were well colonized by DSE after DSE inoculation (Figure 5).

In the present study, the DSE colonization rate of roots increased significantly after DSE inoculation, and there were significant positive correlations between the DSE colonization rate and the yields of buckwheat and alfalfa. DSE colonization increased the yields and some yield components of buckwheat and alfalfa (Tables 5 and 6). This is consistent with previous studies in which DSE inoculation promoted the growth and increased yields of maize [9], tomato [10], alfalfa [11], *Ammopiptanthus mongolicus* [12], and *Astragalus membranaceus* [24]. Studies had shown significant increases in biomass or yield when the colonization rate of DSE in plant roots reaches 30–40% [9,11,25]. Yield is the result of the coordination of multiple yield components. Each yield component is not independent, and its effect on yield is promoted or restricted by other factors [22]. Here, the formation of alfalfa yield and the accumulation of nutrients were closely related to the morphological development of the plants. Plant height, stem length, leaf area, stem diameter, number of branches, stem-to-leaf ratio, and the accumulation of N and P are important factors that constitute alfalfa yield and are the main indicators of alfalfa yield [26]. Important agronomic traits affecting buckwheat yield capacity included plant height, aboveground biomass, the number of main stem nodes, and the number of flower clusters per plant [27]. The number of seeds in a single inflorescence and the number and weight of seeds per plant was positively correlated with buckwheat yield [27], and this is consistent with the current study. There was a significant positive correlation between some yield components and the yield (Tables 5 and 6). If DSE increased the yield components, this would be an effective means of increasing the yield.

Here, the effect of DSE on plant yield depended mainly on its positive effect on certain plant yield components, thereby indirectly increasing the yield (Tables 5 and 6). Numerous studies have shown that DSE and AMF have similar ecological functions. The DSE hyphae that colonize roots can exchange nutrients with host plants [28]. In addition, hyphae external to the roots increase the volume of soil explored by the root system and hence the amounts of water and nutrients (especially N and P) available for root uptake [28,29]. Both N and P are major elements required for plant growth [30,31]. Previous studies have found that NO_3^- uptake related transporters and NH^{4+} uptake-related transporters, N metabolic enzymes, and glutamate receptors of sweet cherry roots inoculated with DSE were related to the enrichment of nitrate regulation-related genes. These genes may be involved in the regulation of nitrate uptake in plants [32]. Studies have shown that DSE can secrete generous extracellular enzymes [33] and mineralize peptides, amino acids, and insoluble phosphorus in the rhizosphere, making nutrients more easily absorbed by roots [34]. Moreover, metabolites of DSE (mostly amino acids and peptides) also make a contribution to the availability of nutrients to host plants [35]. The current study demonstrates that DSE inoculation significantly increased N and P uptake by buckwheat and alfalfa (Tables 3 and 4). DSE can increase the plant height, aboveground dry weight, and root dry weight of *Astragalus membranacea* [24] and the plant height, stem diameter, shoot and root dry weight, and yield per plant of cucumber [36]. DSE also shows maximum potential in promoting the growth of rice with increased tillering and nitrogen uptake [25]. Here, the DSE colonization rate significantly affected the yield of buckwheat through indirect effects on plant N content, P uptake, and grain weight per plant. The DSE colonization rate significantly affected the yield of alfalfa through indirect effects on N uptake and plant height. This also indicated that the effects of the DSE colonization rate on plant yield were mainly due to indirect effects on yield components (Tables 5 and 6).

4.2. Yield Advantage of Intercropping with DSE Inoculation

Crops have complementary effects on resources in space and time, and this can increase yields and resource utilization efficiency. Intercropping systems have therefore been historically important cropping systems in China [37]. Studies were conducted on the intercropping of buckwheat with soybean [38] and cotton [39]. Intercropping of alfalfa with maize [40,41], triticale [1], oat [1], and sorghum [1] were studied. Here, buckwheat and alfalfa have been used as intercropping species to explore the yield effects. The LER of buckwheat-alfalfa intercropping was >1 in both 2020 and 2021, indicating that buckwheat-alfalfa intercropping gave yield advantages during the two years of the experiment (Figure 5). This is consistent with ancient Chinese records showing that mixtures of alfalfa and buckwheat, a common practice historically, can increase crop yields [42].

Furthermore, the yield advantage of the intercropping system in 2020 depended largely on the increased yield of buckwheat, while the yield advantage in 2021 depended mainly on the increased yield of alfalfa. The LER increase in the intercropping system in 2020 was mainly due to a significant increase in $PLER_B$, and the yield increase in buckwheat was much larger than that of alfalfa. The LER increase in the intercropping system in 2021 depended mainly on a significant increase in $PLER_A$, and the yield increase in alfalfa was much higher than the yield decline of buckwheat (Figure 5), and this is consistent with previous studies. The overyielding performance of intercropping communities is highly dependent on the productivity of the dominant species [4,43]. Studies on intercropping systems dominated by perennial legumes show that perennial legumes are the main competitors and the cereals are weak competitors, e.g., in maize-alfalfa intercropping systems [4]. Studies focused on the complementarity of spatial root distribution, utilization of light and water, nitrogen acquisition, and overyielding [4].

Mycelial networks of DSE have also been found to occur. Numerous studies have observed DSE forming mycelial networks. Initial colonization by DSE usually forms mycelia on the surfaces of plant roots [44]. In heavily colonized plant roots, mycelia often extend deep along the vertical axis of the root to form a mycelial network that is dense in the cells of the root epidermis and cortex or in the intercellular spaces [45]. However, the role of DSE in forming hyphal networks among plants and affecting intercropping plants remains unclear. Microorganisms such as AMF that have similar functions to DSE have been widely used in intercropping systems. Numerous studies show that AMF has positive effects on plant growth in intercropping systems. Mostafa et al. reported that the application of AMF as a biological fertilizer in thyme-soybean intercropping increased the quality and quantity of thyme essential oil compared with sole cropping in semi-arid and arid areas [46]. After AMF inoculation, the highest LER was achieved in the four rows of isabgol and two rows of lentils in an intercropping system [47]. Similarly, our results showed that DSE inoculation in the first year significantly increased the LER of the whole intercropping system. This may be attributed to the formation of a mycorrhizal network that acted as a transmitter between plants under nutrient-poor soil conditions. For example, studies have shown that mycorrhizal networks can benefit maize and broad bean [48]. In soils with low P, mycorrhizal networks can increase the yield in the intercropping system by enhancing aboveground biomass and belowground biomass, nutrient uptake by mycorrhizal-dependent plant species, and root interactions [48]. Similarly, we show that DSE inoculation significantly increased the aboveground biomass and N and P uptake by buckwheat and alfalfa. It is very likely that the mycorrhizal network played an important role in the overyielding of the intercropping system (Tables 1–4). Previous studies have used combined ^{15}N and ^{13}C labeling in a greenhouse experiment to study the mechanics of the contribution of mycorrhizal networks and rhizobia to increase yields and nitrogen uptake in an intercropping system [49]. Higher carbon is imported into the mycorrhizal network in soybean than in maize, and the growth advantage of maize-soybean intercropping was due to the acquisition of nitrogen by maize through the common mycorrhizal network [49]. Here, the significant increase in LER produced by DSE inoculation was due to a significant increase in $PLER_B$ in the first year (Figure 5). Similarly, mycorrhizal networks alleviated competition and produced overproduction by increasing flax growth in flax-sorghum intercropping, while sorghum was unaffected [15]. Mycorrhizal networks can amplify competition and preferentially allocate more water to mycorrhiza-dependent plants. The overyielding is due mainly to the mycorrhizal network-mediated increase in maize biomass compared to faba bean [48]. An upland rice-mung bean intercropping combined mycorrhizal network increased nutrient uptake, nitrogen fixation capacity, and growth of mung bean [50]. However, here, DSE inoculation had no effect on LER, $PLER_B$, and $PLER_A$) in the second year (Figure 5). A possible explanation is that mycorrhizal networks redistributed limited resources among interconnected plants and counteracted interspecific competition through the flow of carbon and mineral nutrients along nutrient gradients [14]. As a result, the effect of the mycorrhizal network on the growth promotion and overyielding of dominant species was lower in the intercropping system than in the sole cropping treatments. The role of DSE among plants and the role of DSE hyphal networks require further exploration and verification.

4.3. Yield Variation Due to Continuous Cropping

The current study found that during a two-year period of buckwheat and alfalfa growth, the yield changes differed between the year of sowing and the second year. The yield of buckwheat in the year of sowing was much higher than in the second year. However, the yield of alfalfa in the second year was much higher than in the first year (Figure 4). Studies show that buckwheat has a high nutrient demand, and continuous cropping will reduce its yield [51]. It is, therefore, necessary to apply appropriate nutrients to meet its demand. Buckwheat is a shallow-rooted plant that relies mainly on the root system to take up nutrients from the shallow plow layer. Thus, in the first year, buckwheat relied on the nutrients in the shallow plow layer for growth. Alfalfa has taproots, and after two years of growth, the roots can take up nutrients from deeper in the soil profile and maintain rapid growth [52]. This is in line with the results of previous studies. For example, alfalfa took up more nutrients for rapid growth with increasing growth stage, elongation of the taproot, and increasing root density [52]. In the case of nutrient deficiency in the shallow soil in the second year, buckwheat could not rely on the root system to penetrate deep into the soil to take up nutrients. It, therefore could not meet its own nutrient requirements for vegetative growth and reproduction, and this is reflected in the decreased soil available P content and plant P uptake (Table 3). Finally, production declined substantially, possibly due to allelopathic effects [53]. The main site of root exudate production is the root tip. Under nutrient imbalance or nutrient deficiency conditions, a large increase in root exudates leads directly to continuous cropping obstacles. Although some studies show that intercropping is also an effective way of alleviating continuous cropping barriers [53], most of these studies have focused on intercropping systems involving annual crops. There is no evidence available from intercropping systems involving perennial grasses. In addition, under natural conditions, except for the biological characteristics of plants, the productivity of vegetation communities will also be affected by climatic factors such as temperature and precipitation [54]. Studies show that changes in environmental conditions have a strong impact on crop nitrogen use, thereby affecting yield changes [55]. Some studies also report that interannual climate change has no significant impact on wheat response to nitrogen fertilizer, except for extreme precipitation and temperature changes, using long-term yield data (1968–2016) at Rothamsted, UK [56]. In some studies, buckwheat was grown under rain-fed conditions for two consecutive years, and similar results were obtained under similar average daily temperatures and similar precipitation, and sufficient fertility [57]. After studying the growth of buckwheat from 2001 to 2017, it was found that the selection of sowing date had a large impact on the grain yield of buckwheat, and irrigation did not greatly affect yield [58]. In semi-arid areas of China, as in our study area here, the yields of wheat and maize in the second year under rainfed conditions did not fluctuate greatly [59]. Yields decreased by 3.4 and 12.8% only when rainfall during the crop growth cycle was similar and decreased by 11.7% [59]. Sorghum and mung bean under the same conditions gave similar results, and similar results were found for wheat and mung bean yields in similar study areas [60]. Here (Figure 1), in 2020, the daily average temperature during the plant growth period was 20.6 °C, and the total precipitation was 135 mm. In 2021 the daily average temperature was 21.8 °C, and the total precipitation was 122 mm. The daily average temperature and the total precipitation were similar in both years. Therefore, the buckwheat and alfalfa under rain-fed conditions in 2021 may have been affected by increasing temperatures and decreasing precipitation, with the yield decreasing. However, previous studies indicate that buckwheat and alfalfa adapted to the local semi-arid climate have drought resistance, and the large changes in their yields in 2021 may not have been due mainly to the interannual changes in temperature and precipitation [26,57].

Here, the buckwheat-alfalfa intercropping system showed different effects on yield in the two years. Compared with sole cropping, the yield of buckwheat in intercropping showed an increasing trend (albeit not significant) in the first year, and the yield declined significantly in the second year. In contrast, yields of alfalfa decreased significantly in the first year and increased significantly in the second year (Figure 6). Previous studies show that crop-legume intercropping can increase crop yields across the entire intercropping system [61], but most intercropping systems are based on annual legumes. Other studies have also shown that intercropping with perennial leguminous forages cannot increase the yield of all crop species in the intercropping system [41], and this is due to numerous factors. First, the length of time that the intercropping system is established is associated with yield variation between different species in the intercropping system [62]. The effect of alfalfa-maize intercropping on crop yield was different in different years [63]. Establishing alfalfa in maize intercropping skipped the low-yielding period of alfalfa in the establishment year [41]. A two-year intercropping experiment indicates that in the sowing year when competition with maize occurred, the yield of alfalfa declined significantly, while the forage yield of intercropped alfalfa in the second year was almost twice that in the year of sowing. These results indicate that the effect of intercropping on alfalfa yield did not exceed the first production year [41]. In intercropping with legumes that have been established for many years, legumes are strong competitors, and cereals are weak competitors, as in, for example, maize corn-alfalfa intercropping [4]. Intercropping maize in the second year significantly reduced root growth by 17–36% and yield by 12%, while continuous cropping of sole alfalfa significantly increased root growth by 26–175% and yield by 137%. Changes in root morphology or distribution in intercropping systems are also an effective means of increasing P uptake and yield [64]. In the current study, alfalfa in the first year was in the low-yield establishment period. Its root system was small, and the aboveground parts were weak. Therefore, the competitive position of alfalfa in intercropping was weak, but this did not affect the normal growth of buckwheat. In the second year, alfalfa root growth was much stronger than in the first year, but the root system of buckwheat was less effective than in the first year. Therefore, the rapid growth of alfalfa under intercropping obtained more underground growth space and nutrients for buckwheat, resulting in a substantial decline in the yield of buckwheat. Buckwheat also failed to compete with alfalfa in the presence of DSE colonization. The negative effect of intercropping on buckwheat was much greater than the beneficial effect of DSE colonization on buckwheat (Tables 1 and 2). Secondly, the planting percentages in intercropping systems are correlated with the yield variation of different plant species in the intercropping systems [65]. In studies of the cultivation technology of alfalfa in Horqin sandy land for stress resistance and yield increase, it was concluded that the final grass yield of the mixed sowing mode of alfalfa and buckwheat with a spacing of 30 cm was significantly lower than that of alfalfa in monoculture. The grass yield of alfalfa decreased with increasing buckwheat sowing and yield, which was inversely proportional to the yield of buckwheat [66]. From the system yield, nitrogen yield, and LER, alfalfa stand density was negatively affected by an increase in maize plant density [40]. The green fodder yields of oats and alfalfa and the hay yields of oats increased significantly under different row ratios. However, in an oat-alfalfa intercropping system with a row ratio of 2:1, the total green feed yield and dry feed yield of oats were maximum [65]. Therefore, the 1:1 row ratio of buckwheat and alfalfa intercropping in this study may have had a greater impact on yield changes, and increasing the number of buckwheat rows in intercropping may have contributed to an increase in buckwheat yield in the second year. Finally, the variation in yield of different plants in the intercropping system was also affected by the tillage conditions brought about by successive years of planting [67]. Compared with no-till alfalfa and wheat, sole cropping and conservation tillage treatments produced significantly higher yields [67]. Even with early planting in northern regions where the length of the growing season is limited, slow growth under no-till conditions may shorten the grain-filling period, thereby limiting the yield potential of full-season hybrids [68]. Therefore, the no-till state of the soil in the second year of buckwheat planting in the current study may have affected buckwheat growth and yield.

5. Conclusions

Both buckwheat and alfalfa in the first year of planting (2020) achieved the maximum yield in the treatment combining intercropping with DSE inoculation to 2.17 times and 1.86 times that of the respective sole cropping controls. In the second year of continuous sole cropping (2021), the highest yield of buckwheat was obtained in sole cropping with DSE inoculation, 41.7% higher than in the sole cropping control. The highest yield of alfalfa was achieved in intercropping combined with DSE inoculation, 120.4% higher than in the sole cropping control. DSE inoculation had a significant effect on the continuing increase in alfalfa yield.

The buckwheat-alfalfa intercropping system had yield advantages in both cropping years (2020 and 2021), but DSE inoculation significantly increased the yield (by 11.2%) in the first year (2020) only.

Grain weight per plant, P uptake of plants, DSE colonization rate, plant N content, soil available N content, and plant P content were the key determinants of buckwheat yield. DSE colonization rate indirectly promoted buckwheat yield by changing the grain weight per plant, P uptake, and plant N content. N uptake, plant height, plant N content, and the DSE colonization rate of alfalfa were key determinants of alfalfa yields. DSE colonization rate promoted alfalfa yield by its positive effects on N uptake and plant height.

Our study conducted an intercropping experiment with buckwheat-alfalfa row ratio of 1:2 for 2 years. The effects of longer time and different intercropping ratios on crop yield and yield compositions under the influence of DSE need to be further studied.

Author Contributions: Conceptualization, Y.B.; methodology, Y.B. and Y.G. (Yakun Gao); formal analysis, Y.G. (Yakun Gao); investigation, Y.G. (Yakun Gao), S.M., Y.Z. (Yang Zhou) and S.X.; data curation, Y.B. and Y.G. (Yakun Gao); writing—original draft preparation, Y.G. (Yakun Gao); writing—review and editing, Y.B., Y.G. (Yakun Gao), S.M., Y.Z. (Yanxu Zhang), Y.G. (Yun Guo) and P.C.; visualization, Y.G. (Yakun Gao); supervision, Y.B.; funding acquisition, Y.B. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded jointly by the National Key Research and Development Program of China (2022YFF1303300), the National Natural Science Foundation of China (51974326), and the Fundamental Research Funds for the Central Universities (2022XJMT01).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Conflicts of Interest: The authors declare no conflict of interest.

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