https://doi.org/10.17221/9/2024-PSE

The influence of timing and planting proportion on the intraspecific competitiveness ability of drunken horse grass (*Achnatherum inebrians* (Hance) Keng) by fungal endophyte infection

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Citation: Cao J.X., Yao X., Wei X.K., Zhang R.C., Liu J., Li C.J. (2024): The influence of timing and planting proportion on the intraspecific competitiveness ability of drunken horse grass (*Achnatherum inebrians* (Hance) Keng) by fungal endophyte infection. Plant Soil Environ., 70: 418–429.

Abstract: Drunken horse grass (*Achnatherum inebrians* (Hance) Keng) is a toxic perennial bunchgrass native to Northwestern China. *Epichloë* endophytic fungi infection could enhance the stress tolerance of drunken horse grass. However, there is a scarcity of literature regarding the effects of intraspecific competition. As a result, we anticipated that the intraspecific competitive dynamics between endophyte-infected (EI) and endophyte-free (EF) plants would become more transparent for four years by planted as the proportions of 2:1, 1:1, and 1:2. The results showed the EI plants exhibited more biomass, seed yields, and survival rates than EF plants. Endophyte infection also facilitated a competitive advantage by enhancing photosynthesis and soil nutrition. Our findings constituted the inaugural investigation into the influence of the intraspecific competitive ability of grass infected with *Epichloë* endophyte fungi. EI plants caused them to become stronger and stronger, while EF became weaker and weaker by timing and planting proportion increasing, and EF drunken horse grass could be replaced by EI. These conclusions were instrumental in elucidating why the endophytic fungal infection rate of drunken horse grass is 100% observed in natural wilderness. *Epichloë* endophyte could reduce plant diversity and enhance the dominance of EI plants in intraspecific competition; drunken horse grass may be threatening the persistence of native plant species.

Keywords: endure; environmental stresses; asymmetric competition; grass-fungal symbioses; endophytic fungi

Drunken horse grass (*Achnatherum inebrians* (Hance) Keng) is a toxic perennial bunchgrass species native to several provinces in Northwest China (Zhang et al. 2015a). Due to its exceptional tolerance to various environmental stresses including drought (Cui et al. 2022), grazing (Yao et al. 2015), cold temperatures (Chen et al. 2016), sandy environments (Wang et al. 2021), pests (He et al. 2021), pathogenic fungi (Kou et al. 2021), and heavy metals (Jin et al. 2022), drunken horse grass is capable of enduring these challenges.

Supported by the National Basic Research Program of China, Project No. 2014CB138702.

https://doi.org/10.17221/9/2024-PSE

Drunken horse grass is also recognised for infecting *Epichloë* endophytes at a rate of 100%, which frequently colonises its seeds without manifesting any symptoms (Zhang et al. 2012). Many grasses form symbiotic associations with *Epichloë* endophytes, which grow between plant cells, producing bioactive alkaloids potentially advantageous to the host. Ergot alkaloids, including ergonovine and lysergic acid amide (Zhang et al. 2011a). However, the endophytes have also been documented to enhance the tolerance of grass to drought, disease, and herbivory (Li et al. 2007). The interaction between drunken horse grass and its *Epichloë* endophyte is complex, whereas alkaloids may prevent overgrazing but present hazards to livestock when consumed in large quantities for an extended duration (Xia et al. 2015). The identification of its correlation with *Epichloë* endophytes that produce ergot alkaloids offers prospects for enhancing comprehension of grass-fungal symbioses and the ramifications they have on the administration of grasslands (Li et al. 2007, Xia et al. 2015).

Tilman (2004) developed intraspecific competition, which occurs when members of the same species compete with one another. This competition is ubiquitous among plants and has a profound effect on the structure of communities and populations. Increased asymmetric competition results in a disproportionate distribution of limited resources among specific individuals, which hinders the growth, reproduction, and survival of those who are competitively inferior (Moora and Zobel 1996).

The extent of asymmetric competition is frequently determined by the proportion of plants as measured by relative yield total (RYT) (Weigelt and Jolliffe 2003). Competition is symmetric at low proportions, as indicated by the RYT value approaching 1. However, as proportion increases, the RYT value decreases, suggesting an increase in asymmetric competition. Dense populations result in larger plants attaining disproportionate benefits while smaller individuals suffer the consequences (Damgaard 1999, Zhang et al. 2020).

Many grass species form symbioses with *Epichloë* endophytes, which can impart a competitive advantage by enhancing species' tolerance to environmental stresses (Torres et al. 2012). Endophytes have been demonstrated to increase host growth rate, size, seed production, and survival of their hosts (Chen et al. 2018). However, endophyte effects on the dynamics of intraspecific competition over time have been the subject of few investigations. Further, an extensive symbiotic association exists between grasses and *Epichloë* endophytes. Nearly one hundred percent of infection rates were detected in inebriated horse grass in the Northwest Chinese provinces of Gansu, Xinjiang, Qinghai, and Ningxia (Zhang et al. 2015b). Endophytic fungi, which develop intercellularly within aboveground tissues, have the ability to enhance the viability of the host grass by bolstering its tolerance to both biotic and abiotic stresses. Furthermore, endophytes have the ability to promote growth through the enhancement of photosynthetic rate (Clay and Schardl 2002) and nitrogen uptake (Christian et al. 2019, Bayat et al. 2023).

At present, despite the systematic and detailed research on tall fescue (Triplett et al. 2022) and ryegrass (Jastrzbska et al. 2015), there are few studies on the effects of grass endophytic fungi symbionts on the structure, succession direction and diversity of vegetation community. Although the impact of endophytes on individual host grasses has been well-documented, there is a scarcity of research examining the potential influence of endophytes on competitive dynamics between infected and uninfected individuals in relation to the planting proportion. Competition experiments that investigate the impact of planting proportions and years on the intraspecific competitive ability of drunken horse grass would yield valuable insights into the ecological benefits that symbiosis imparts. Furthermore, by analysing competitive interactions and fitness traits across consecutive years, the effects of endophyte symbiosis on the dynamics of the host population could be better understood.

MATERIAL AND METHODS

Materials. Drunken horse grass seeds of endophyte-infected (EI) and endophyte-free (EF) were collected in September 2012 originating from a single EI and EF plant to reduce variability at the Yuzhong campus (103°51'E, 35°36'N, altitude 1 874 m a.s.l.) of Lanzhou University, China. Nearly 100% of populations are infected with *Epichloë* in nature, which was planted in 2011. Before planting, a test was carried out to verify whether the endophyte-infected, with 75% alcohol disinfected for 5 min and 10 min with 1% sodium hypochlorite disinfection, with sterile water, rinsed clean, with germination on double-layer filter paper. After 7 days, seedlings were planted in the field; plants were examined microscopically using the aniline blue-staining method to visualise

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mycelium within culms and leaves after growing for 10 weeks to inform the rate of endophytes infection; we tested 12 plants in every plot from 2013 to 2016, the results displayed that the rates of EI plants infecting endophytes were more than 95%. The rates of EI plants are less than 5%.

Preprocessing and study design. Before planting, tests were carried out to verify whether the endophyte-infected seeds (He et al. 2021). This study adopted the De (1996) alternative experimental design. In September 2012, the collected seeds were sown in the experimental field of Yuzhong campus, Lanzhou University as the following proportions: (1) 2 rows EI *vs.* 1-row EF; (2) 1 row EI *vs.* 1-row EF; (3) 1 row EI *vs.* 2 rows EF, compared to EI or EF seeds sown by monoculture (mono). The plant spacing was 15 cm, and each hole had 6 seeds at 3–4 cm depth. Seed selection was carried out before sowing to insure that the germination rate of drunken horse grass seeds was no less than 95% (Yue et al. 2021). After calculating the emergence rate in the field in March 2013, the plant's endophytic fungi carrying rate was determined by microscopic examination with aniline blue to visualise mycelium within culms and leaves (Zhang et al. 2009, 2011b). Each hole was established and maintained with one seedling. Each plot area was 2.5 m × 2.5 m, and each treatment had 3 replicates.

Measurement of growth indices. Survival and photosynthesis of drunken horse grass. From 2013 to 2016, twelve plants were randomly selected from each replicate and measured in September. The plant's tiller, seed yield, above and underground biomass dry weight, and plant height were all assessed. The tillers counted 3 plants from one row; these plants were measured plant height with a tape, then were cut down to weight biomass; 12 plants of every treatment were harvested and separated into roots and shoots, then washed with distilled water; these plants were oven-dried at 80 °C until a constant weight to determine the dry weight. Seed yield was determined by weighing seeds per plant after the seeds matured. The survival rate was determined for all living plants in the experimental field every March (revival period). Plants were watered once established, and no fertiliser was added to the fields during the experimental period. The data on weather conditions we gained from the Yuzhong County Meteorological Bureau, as well as the mean air temperature and precipitation during this period, are shown in Figure 1 and Figure 2. The count of surviving plants in each plot was documented for the computation of the survival rate in March of each year between 2013 and 2016. LI-6400 portable photosynthetic apparatus (Lincoln, USA) was utilised to quantify photosynthesis parameters such as intercellular CO_2 concentration, net photosynthetic rate, and stomatal conductance. The parameters were all computed in accordance with the procedures outlined by Caemmerer and Farquhar (1981).

Measurement of soil physicochemical property under drunken horse grass community. Soil samples were collected near the root of drunken horse grass. After removing debris, stones and other impurities from the soil's surface, the profiles were dug at 0–15 cm, with soil samples of 1 kg for each treatment. Each bag of soil samples was coordinated and marked. After returning to the laboratory, the soil samples were oven-dried and sundries removed. Then, the samples were suspended, sieved, mixed, and bottled for further examination. The physical

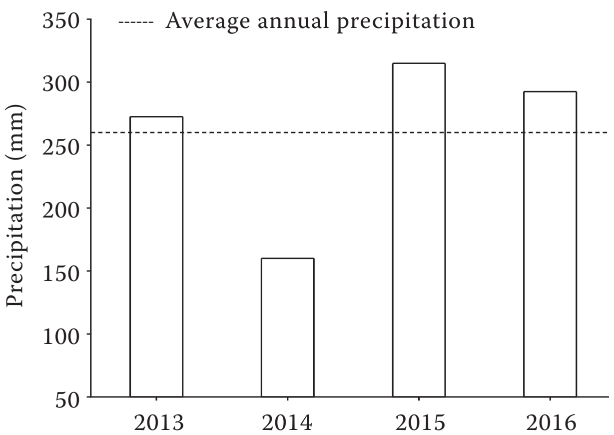

Figure 1. Precipitation in Yuzhong County from 2013 to 2016

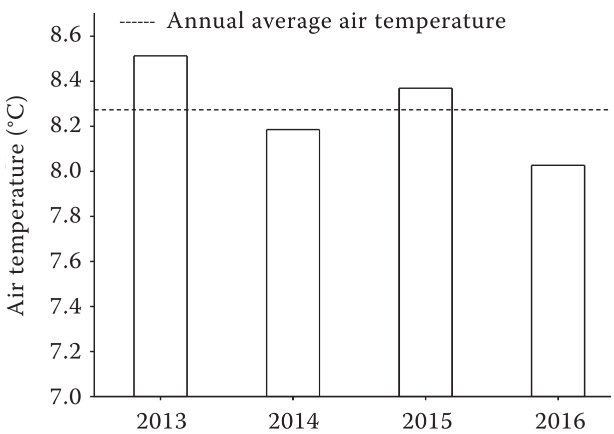

Figure 2. Air temperature of Yuzhong County from 2013 to 2016

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Table 1. Results of three-way ANOVA for the effects of timing (T), planting proportion (P), and *Epichloë* (E) on plant height, tiller number, biomass, seed yield, and survival rate of drunken horse grass

Treatment	*df*	Plant height		Tiller number		Biomass		Seed yield		Survival rate	
		F	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*
T	3	2 710.61	< 0.001	10 431.85	< 0.001	17 732.51	< 0.001	130 716.41	< 0.001	5 076.07	< 0.001
P	3	1 936.83	< 0.001	1 051.64	< 0.001	8 270.69	< 0.001	9 757.81	< 0.001	4 368.48	< 0.001
E	1	13 470.32	< 0.001	411.78	< 0.001	2 831.37	< 0.001	5 853.64	< 0.001	2 725.91	< 0.001
T × P	9	48.47	< 0.001	109.11	< 0.001	639.52	< 0.001	2 068.36	< 0.001	769.02	< 0.001
T × E	3	636.54	< 0.001	78.60	< 0.001	320.66	< 0.001	1 379.97	< 0.001	250.74	< 0.001
P × E	3	458.95	< 0.001	183.95	< 0.001	812.37	< 0.001	3 289.99	< 0.001	816.35	< 0.001
T × P × E	9	112.45	< 0.001	47.97	< 0.001	91.27	< 0.001	766.31	< 0.001	151.33	< 0.001

df – degrees of freedom

and chemical properties of the soils at various points were then obtained. The soil pH was determined with a potentiometer (Boston, USA). The soil organic carbon (SOC) and total nitrogen (TN) contents were determined using the Walkley-Black and the Kjeldahl digestion methods, respectively (Marks and Clay 2010, Iqbal et al. 2012). The total phosphorus (TP) content was quantitatively determined by $HClO_4$-HNO_3-H_2SO_4 digestion used in conjunction with a colourimetric method (Jin et al. 2019).

Data analysis. Intraspecific competition can be quantified by De Wit's relative yield total. RYT is calculated as follows:

$$RYT = (RY_a + RY_b)/2 \quad (1)$$

where: RY_a – $Y_{a\ (mix)}$ divided by $Y_{a\ (mono)}$; RY_b – $Y_{b\ (mix)}$ divided by $Y_{b\ (mono)}$. *a* and *b* – EI and EF, respectively. RYT values near 1 indicate symmetric competition where individuals are equally affected. Values less than 1 indicate asymmetric competition where one individual experience a greater reduction in performance than the other individual.

All statistical analyses were performed with SPSS software (version 20.0, Inc., Chicago, USA). A three-way analysis of variance (ANOVA) was used to analyse the effects of timing, planting proportion, and *Epichloë*, and the differences between the means for different factors were compared using Duncan's multiple-range tests at $P < 0.05$. All figures were drawn using Origin 2021 software (Hampton, USA).

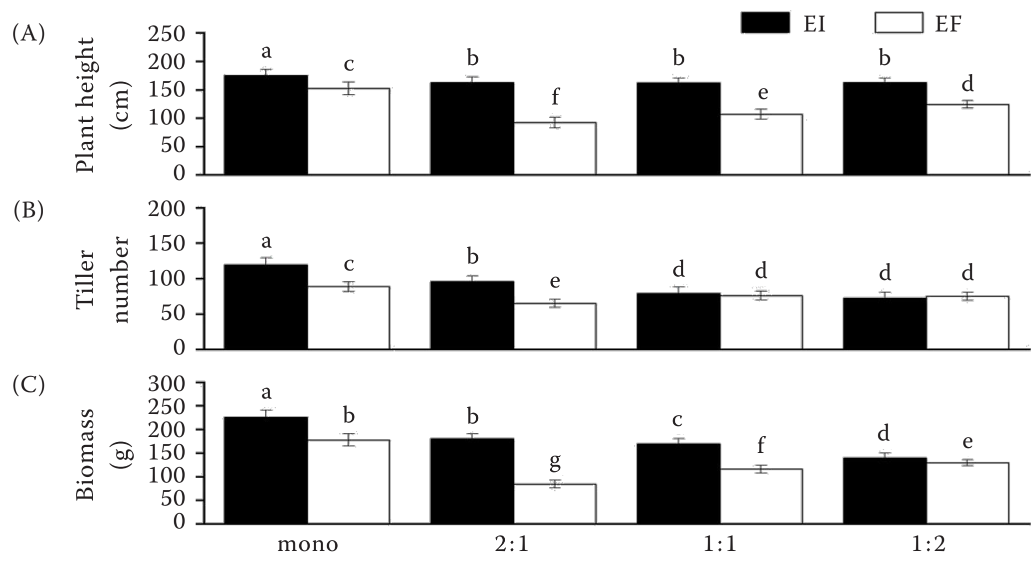

Figure 3. (A) Plant height; (B) tiller number, and (C) biomass were measured in September 2016. Twelve plants were randomly selected for measurement in each replication (EI – endophyte-infected; EF – endophyte-free). Values are means with standard error bars ($n = 3$). Different letters indicate the means are significantly different ($P < 0.05$)

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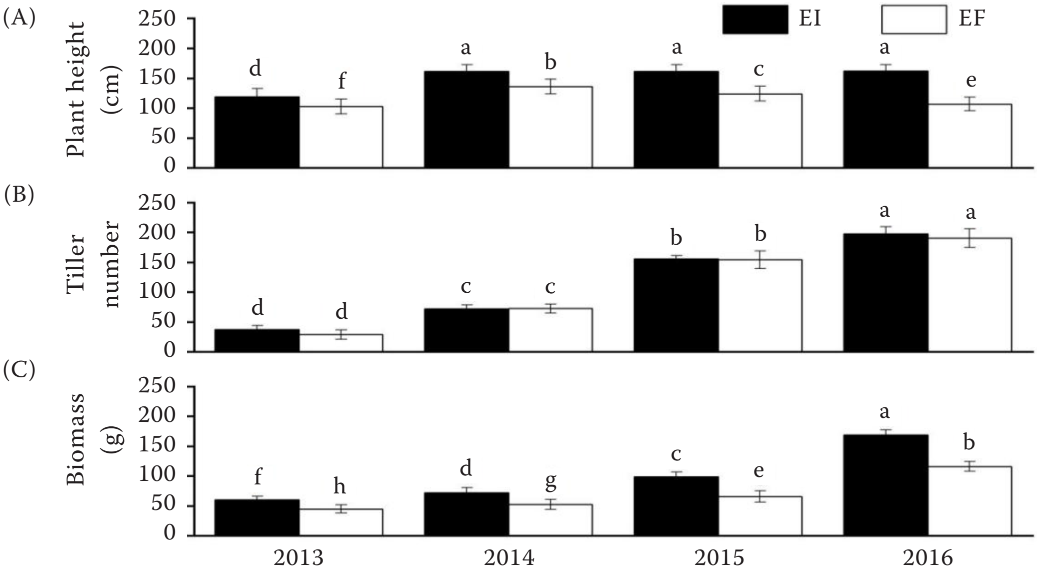

Figure 4. Plant growth parameters at different proportions. (A) Plant height; (B) tiller number, and (C) biomass were measured in September from 2013 to 2016. Twelve plants were randomly selected for measurement in each replication (EI – endophyte-infected; EF – endophyte-free). Values are means with standard error bars ($n = 3$). Different letters indicate the means are significantly different ($P < 0.05$)

RESULTS

Plant growth parameters. The plant height, tiller number, and biomass were significantly affected by timing (T), planting proportion (P), and *Epichloë* (E) (Table 1), and T × P × E significant ($P < 0.001$). Diverse proportions of the plant height of EI remained relatively stable in 2016. Figure 3B demonstrates that EI produced more tillers than EF in the 2:1 proportion, whereas no significant difference ($P > 0.05$) was observed in the 1:1 and 1:2 proportions. There were substantial variations in biomass between EI and EF across all three proportions. In particular, at 2:1, 1:1, and 1:2 proportions, EI had a biomass that was 213.24, 145.70, and 107.86% greater than EF plants, respectively (Figure 3C).

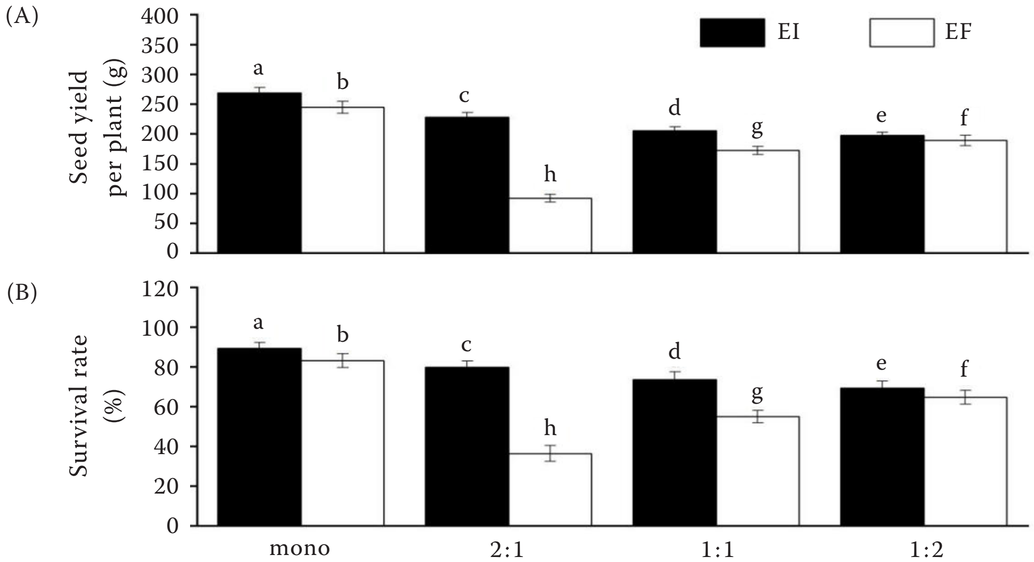

Figure 5. Seed yield and plant survival in the same year. (A) Seed yield per plant and (B) survival rate of different proportions were measured in September 2016. Twelve plants were randomly selected for measurement in each replication (EI – endophyte-infected; EF – endophyte-free). Values are means with standard error bars ($n = 3$). Different letters indicate the means are significantly different ($P < 0.05$)

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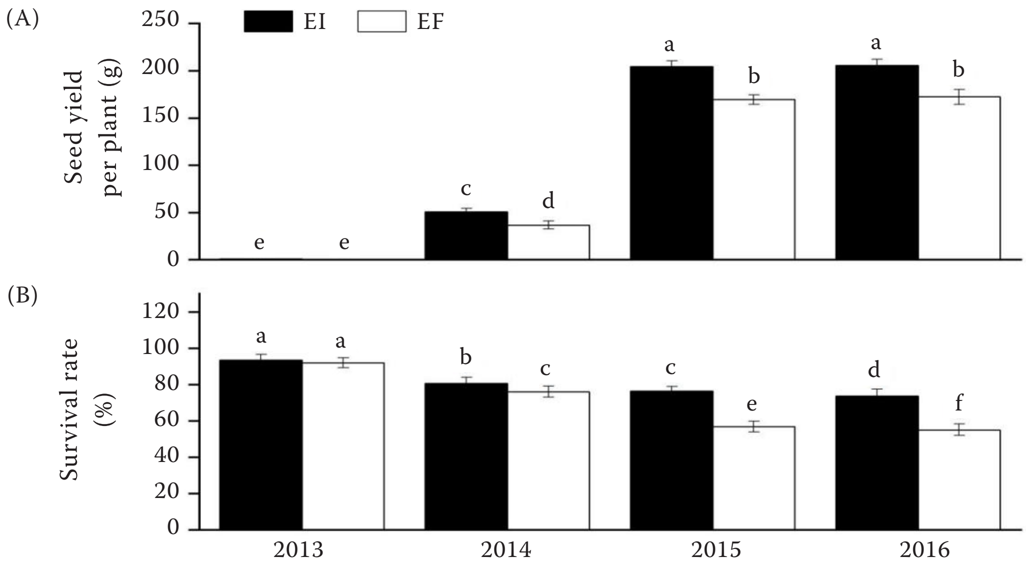

Figure 6. Seed yield and plant survival at different proportions. (A) Seed yield per plant and (B) survival rate were measured in September from 2013 to 2016. Twelve plants were randomly selected for measurement in each replication (EI – endophyte-infected; EF – endophyte-free). Values are means with standard error bars (n = 3). Different letters indicate the means are significantly different (P < 0.05)

The height of EI plants remained stable from 2014 to 2016 (Figure 4A). The tiller number of both EI and EF plants significantly increased by years (P < 0.05), while no differences were found between EI and EF in each year (Figure 4B). Biomass of both EI and EF plants increased by years with significant differences between each other in each year (Figure 4C, P < 0.05).

Seed yield and plant survival. Seed yield and plant survival were significantly affected by timing, planting proportion, and *Epichloë* (Table 1), and T × P × E significant (P < 0.001). In 2016, EI plants exhibited seed yields of 247.72, 119.45, and 104.70% and survival rates of 219.74, 133.95, and 107.36% compared to the EF counterparts at 2:1, 1:1, and 1:2 proportions (Figure 5), but the plant survival rate declined over the years (Figure 6)

Photosynthetic indicators. Net photosynthetic rate and transpiration rate were significantly affected by planting proportion, and *Epichloë* (Table 2), and P × E significantly (P < 0.001). Intercellular CO_2 concentration and stomatal conductivity were significantly affected by planting proportion, and *Epichloë*, T × P and P × E were significant (P < 0.001). The net photosynthetic rate, transpiration rate, intercellular CO_2 concentration and stomatal conductivity (Figure 7A–D) of different proportions were mea-

Table 2. Results of three-way ANOVA for the effects of timing (T), planting proportion (P), and *Epichloë* (E) on net photosynthetic rate, transpiration rate, intercellular CO_2 concentration, and stomatal conductivity of drunken horse grass

Treatment	*df*	Net photosynthetic rate		Transpiration rate		Intercellular CO_2 concentration		Stomatal conductivity	
		F	*P*	*F*	*P*	*F*	*P*	*F*	*P*
T	3	0.64	0.59	0.08	0.97	1 384.12	< 0.001	1 067.45	< 0.001
P	3	151.37	< 0.001	614.57	< 0.001	4 034.06	< 0.001	2 058.18	< 0.001
E	1	171.03	< 0.001	593.82	< 0.001	2 320.04	< 0.001	3 432.12	< 0.001
T × P	9	0.39	0.94	0.00	1.00	64.06	< 0.001	17.37	< 0.001
T × E	3	0.59	0.62	0.00	1.00	0.00	1.00	0.00	1.00
P × E	3	47.89	< 0.001	215.99	< 0.001	2 527.10	< 0.001	833.23	< 0.001
T × P × E	9	0.20	0.99	0.00	1.00	0.00	1.00	0.00	1.00

df – degrees of freedom

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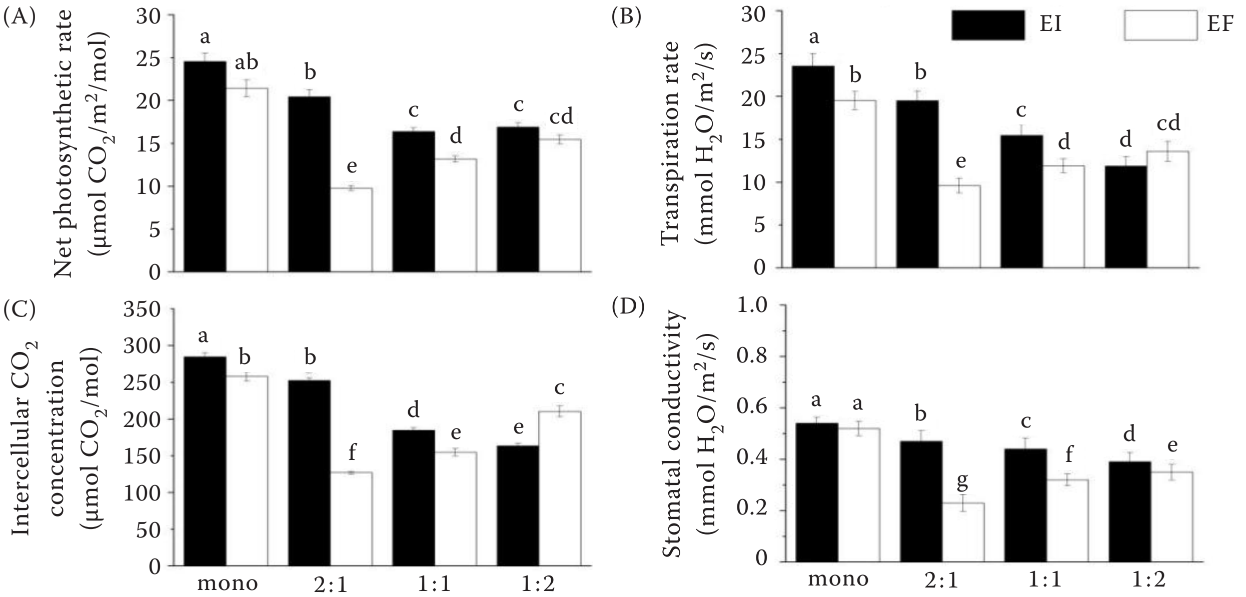

Figure 7. Photosynthetic indicators in the same year. (A) Net photosynthetic rate; (B) transpiration rate; (C) intercellular CO_2 concentration, and (D) stomatal conductivity of different proportions were measured in September 2016. Twelve plants were randomly selected for measurement in each replication (EI – endophyte-infected; EF – endophyte-free). Values are means with standard error bars (n = 3). Different letters indicate the means are significantly different (P < 0.05)

sured in September 2016. These four photosynthetic indicators increased with an increasing planting proportion for EI and EF plants, respectively (Figure 7, P < 0.05). Further analysis showed that these four photosynthetic indicators had an increasing trend by years in the proportion of 1:1 (Figure 8).

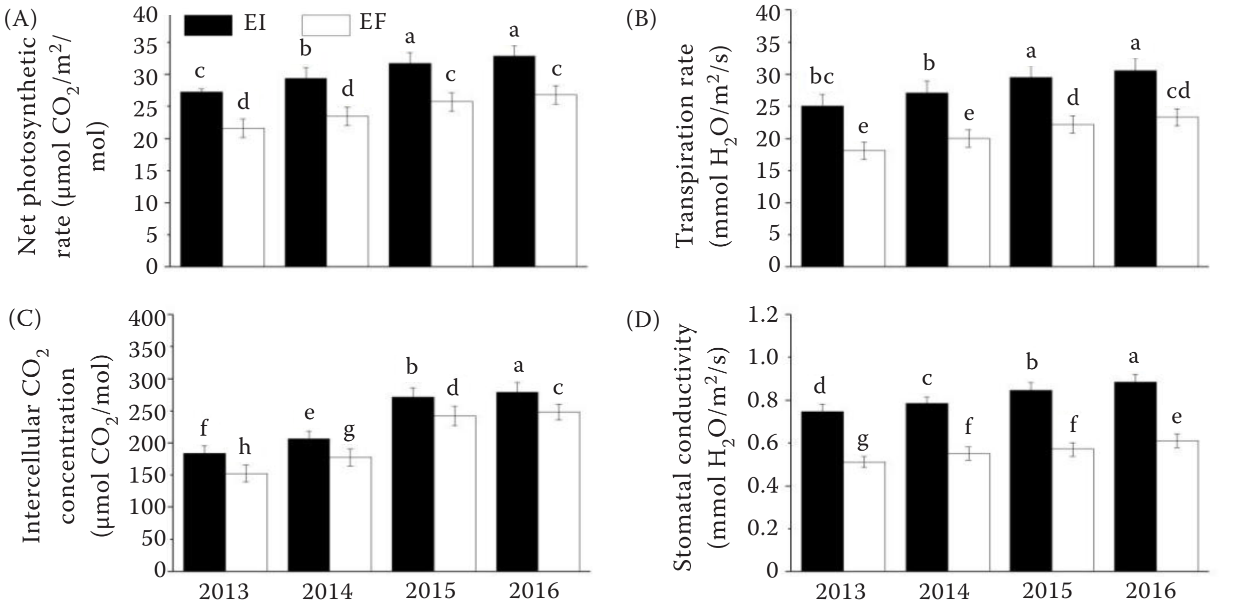

Figure 8. Photosynthetic indicators in different years at the 1:1 proportion treatment. (A) Net photosynthetic rate; (B) transpiration rate; (C) intercellular CO_2 concentration, and (D) stomatal conductivity were measured from September 2013 to 2016. Twelve plants were randomly selected for measurement in each replication (EI – endophyte-infected; EF – endophyte-free). Values are means with standard error bars (n = 3). Different letters indicate that the means differ significantly (P < 0.05)

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Table 3. Results of three-way ANOVA for the effects of timing (T), planting proportion (P), and *Epichloë* (E) on pH value, soil organic matter, total N and total P content of soil of drunken horse grass

Treatment	*df*	pH value		Soil organic matter		Total N content of soil		Total P content of soil	
		F	*P*	*F*	*P*	*F*	*P*	*F*	*P*
T	3	4.61	< 0.001	151.07	< 0.001	74.61	< 0.001	331.27	< 0.001
P	3	15.19	< 0.001	6 850.99	< 0.001	189.89	< 0.001	334.40	< 0.001
E	1	1.10	0.30	35 035.93	< 0.001	490.98	< 0.001	1 028.36	< 0.001
T × P	9	0.08	1.00	2.14	0.03	1.46	0.17	6.76	< 0.001
T × E	3	0.00	1.00	0.00	1.00	0.00	1.00	0.00	1.00
P × E	3	5.38	< 0.001	3 926.72	< 0.001	121.30	< 0.001	187.92	< 0.001
T × P × E	9	0.00	1.00	0.00	1.00	0.00	1.00	0.00	1.00

df – degrees of freedom

pH value and soil nutrient. pH value was significantly affected by timing and planting proportion (Table 3), and P × E was significant ($P < 0.001$). Soil organic matter and total N were significantly affected by timing, planting proportion, and *Epichloë* (Table 3), P × E significant ($P < 0.001$). The effects of timing, planting proportion, and *Epichloë* on total P content of soil were all significant, T × P and P × E significant (Table 3, $P < 0.001$). There was no significant difference in the pH value among different proportion treatments (Figure 9A, $P > 0.05$). Soil organic matter increased with the proportion increased for the EI and EF plants, respectively (Figure 9B, $P < 0.05$). The total nitrogen content of the soil of the EI plants remained stable in the three proportions, while the total nitrogen content of the EF plants rose as the planting proportions increased (Figure 9C). The total phosphorus content of the soil of the EI plants showed no difference in the 2:1 and 1:1 proportion treatment. In contrast, EF showed no difference in the 1:1 and 1:2 treatments (Figure 7D, $P > 0.05$).

Regarding EI and EF plants in the proportion of 1:1, pH value (Figure 10A) and soil nutrients (Figure 10B–D) were found to increase from 2013 to 2016, and there were significant differences ($P < 0.05$)

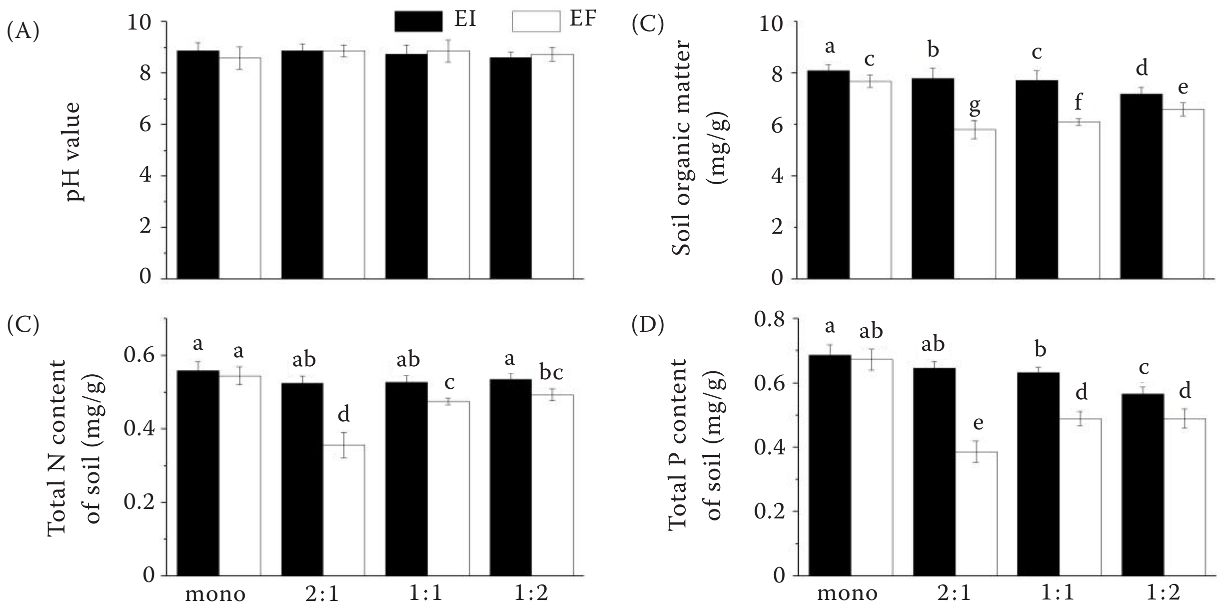

Figure 9. pH value and soil nutrient in the same year. (A) pH value; (B) soil organic matter; (C) total nitrogen (N) content of soil, and (D) total phosphorus (P) content of soil of different proportions were measured in September 2016. Twelve plants were randomly selected for measurement in each replication (EI – endophyte-infected; EF – endophyte-free). Values are means with standard error bars ($n = 3$). Different letters indicate that the means differ significantly ($P < 0.05$)

https://doi.org/10.17221/9/2024-PSE

Table 4. The relative yield total of drunken horse grass in different proportions at the same time (September 2016) and the same proportion (1:1)

Group	Treatment	RYT values
Relative yield total of drunken horse grass in different proportions at the same time (September 2016)	EI:EF = 2:1	0.64 ± 0.03^{c}
	EI:EF = 1:1	0.70 ± 0.08^{a}
	EI:EF = 1:2	0.67 ± 0.11^{b}
Relative yield total of drunken horse grass at the same proportion (1:1)	2013yr	0.87 ± 0.16^{a}
	2014yr	0.75 ± 0.08^{b}
	2015yr	0.70 ± 0.06^{c}
	2016yr	0.70 ± 0.14^{c}

Relative yield total (RYT) values were shown as mean ± standard error. Different lowercase letters indicate significant differences in the same group (P < 0.05). EI – endophyte-infected; EF – endophyte-free

Relative yield total. The relative yield total of drunken horse grass was calculated in different proportions at the same time (September 2016) and the same proportion (1:1) (Table 4). All the RYT values were less than 1, and the average RYT values were 0.7 in 2015 and 2016. The average RYT values were 0.64 and 0.67 in the proportions of 2:1 and 1:2, respectively 2016. The RYT value decreased as the EI planting proportion increased, and the minimum RYT value was found in the 2:1 proportion treatment (Table 4, P < 0.05).

DISCUSSION

Biomass can directly reflect the competitiveness of plants, and the higher the plant biomass accumulation, the stronger the competitiveness of plants (Kolb et al. 2002). Our results demonstrated that *Epichloë* endophyte infection enhanced the intraspecific competitive ability of drunken horse grass through various mechanisms. Specifically, when EI plants were cultivated alongside EF in 2:1, 1:1, and 1:2 proportions after four years, the EI plants exhibited more biomass values (Figure 3C), seed yields (Figure 5A),

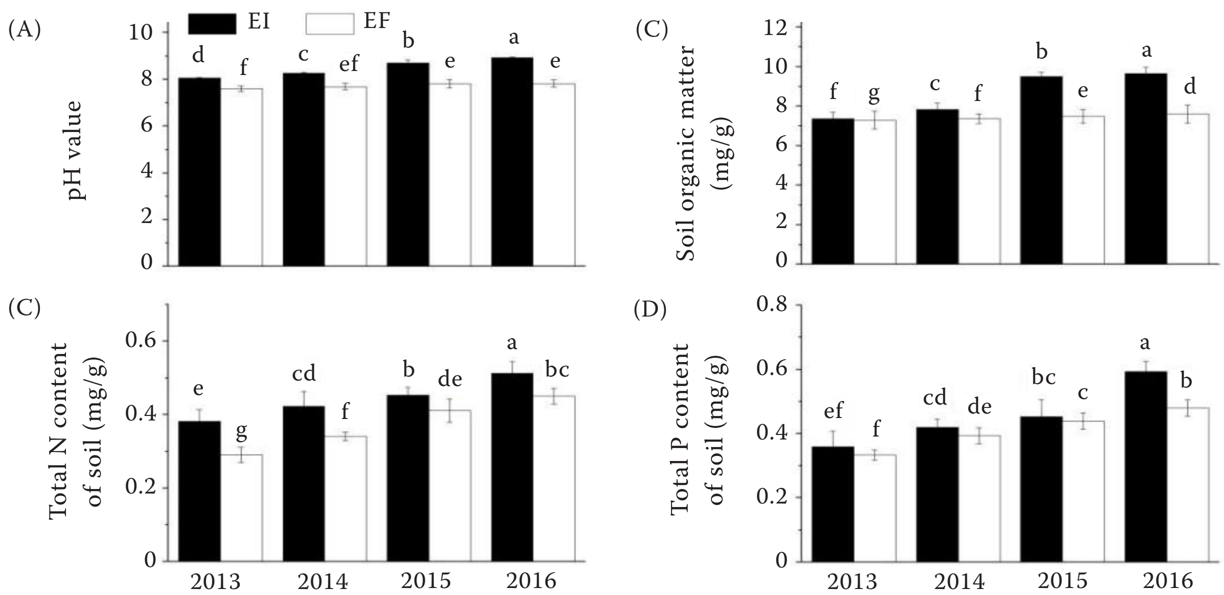

Figure 10. pH value and soil nutrient in different years at the 1:1 proportion treatment. (A) pH value; (B) soil organic matter; (C) total nitrogen (N) content of soil, and (D) total phosphorus (P) content of soil were measured in September from 2013 to 2016. Twelve plants were randomly selected for measurement in each replication (EI – endophyte-infected; EF – endophyte-free). Values are means with standard error bars (n = 3). Different letters indicate that the means differ significantly (P < 0.05)

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and survival rates (Figure 5B) than EF. Yang et al. (2021) and Zhang et al. (2018) studied the mixed seeding of EI and EF under low nitrogen stress conditions in the greenhouse and under soil water deficit conditions. They showed that *Epichloë* endophyte had a gain effect on the plant height, tillering and biomass of the drunken horse grass. These previous research results are consistent with ours.

This study showed that the photosynthetic capacity of EI or EF plants increased along with increasing planting proportion and timing. Furthermore, endophyte-infected plants demonstrated enhanced resource capture ability in every proportion treatment compared to endophyte-free counterparts (Figure 10). Previous research on antioxidant enzyme activities and photosynthesis of *Elymus dahuricus* (Zhang et al. 2018) and tall fescue (Nagabhyru et al. 2013, Hosseini et al. 2015) under water-deficit stress indicated that infection with *Epichloë* endophyte had an advantage to enhance the host's photosynthetic capacity. The results of intraspecific competition on orchard grass (*Dactylis glomerata*) (Rozpadek et al. 2015) showed the fungal endophyte *Epichloë typhina* improved the photosynthesis efficiency of its host, and this research confirmed our results.

The findings of this study indicated that endophyte-infected drunken horse grass could compete more effectively with endophyte-free conspecifics for soil nutrients, irrespective of proportion (Figure 10). The soil organic carbon content of endophyte-infected tall fescue plots was greater than that of endophyte-free plants (Franzluebbers and Stuedemann 2002, 2005). Under high nitrogen concentration conditions, *Epichloë* endophyte significantly increased soil C content in mixed seeding ratios (Jin et al. 2022). While regarding the single sowing and 1:1 mixed sowing of drunken horse grass, it was found that *Epichloë* endophyte significantly increased the N content in the upper part of the single sowing and the aboveground P content in the mixed sowing (Xia et al. 2018) and *Epichloë* infected plants also had higher C, N and P contents under salt and alkali stresses (Chen et al. 2018). We found that *Epichloë* endophyte significantly increased the N and P content in the soil because of the enrichment of soil nutrients. The average RYT values were less than 1 with a decreasing trend from 2013 to 2016 (Table 4). This indicated the intraspecific competitiveness of drunken horse grass was greater than its interspecific capacity. In 2016, the RYT value was 0.70 in the 1:1 proportion, while it was 0.64 and 0.67 in the 2:1 and 1:2 proportions, respectively (Table 4), which showed a significant difference. These results indicated the asymmetric competition intensified along with an increasing proportion of the endophyte-infected plants. The results of other field experiments showed that when the mixed planting proportion was 1:1, the RYT value of drunken horse grass EI and EF was less than 1 (Xia et al. 2018). Clay and Holah (1999) discovered that fungal endophyte symbiosis reduces plant diversity and enhances fescue dominance in successional fields and predicted tall fescue may threaten the persistence of native plant species, affect successional dynamics and modify food webs in plant communities. In this study, it was found that in the 1:1 ratio, the RYT values of drunken horse grass EI and EF were less than 1 and decreasing by years, and this indicated that EF drunken horse grass could be replaced by EI plants as time increased, *Epichloë* endophyte enhanced dominance of EI plants.

The intraspecific competition of drunken horse grass led to disproportionate gains for the endophyte-infected plants at the expense of endophyte-free individuals, such as inhibiting plant height, tiller and biomass (Figures 3–4), reducing seed yield and survival rate (Figures 5–6) and reducing photosynthesis and soil nutrient content (Figures 7–10). EF drunken horse grass could be replaced by EI as planting time increasing. These diverse mechanisms synergistically improved the fitness and competitive abilities of drunken horse grass when infected by *Epichloë* endophyte.

To the best of our knowledge, this work represented the first study on the influence of the intraspecific competitive ability of grass infected with *Epichloë* endophyte. It was found that both the proportion and timing could intensify the intraspecific competition of drunken horse grass. The endophyte-infected plants outperformed the endophyte-free individuals by affecting photosynthesis and soil nutrient content, leading to the slow growing and a low seed yield of the endophyte-free counterparts and resulting in a low survival rate. These findings provided crucial evidence for comprehending the plant intraspecific competition between endophyte-infected and endophyte-free plants. The effects on the drunken horse grass community's long-term dynamics further elucidated the 100% rate of *Epichloë* endophyte infection within the natural wilderness; *Epichloë* endophyte reduces plant diversity and enhance EI plants dominance in the intraspecific competition; drunken horse grass may threatening the persistence of native plant species.

Acknowledgement. We wish to thank Drs. Yubing Fan and Toba Olasehinde for editing and providing helpful comments on the manuscript, and the anonymous reviewers for reviewing it.

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Received: January 4, 2024
Accepted: May 6, 2024
Published online: May 23, 2024