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Epichloë endophyte enhances intraspecific competition of Achnatherum inebrians under nitrogen stress

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Research Article

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Abstract

Background and aims Drunken horse grass (DHG, *Achnatherum inebrians*) is a perennial grass widely distributed in the grasslands of northwestern China, forming a symbiosis with the *Epichloë* endophyte, and has a strong growth advantage in the community. However, the mechanism of its competitive success remains unclear.

Methods Endophyte-infected (EI) and endophyte-free (EF) DHG were employed in greenhouse experiments. EI plants served as competitive species, while EF plants were the target species. Aggressivity was employed to quantify the competitiveness between EI and EF plants. The effects of endophyte, plant density, and three different nitrogen levels in pot on the competitiveness of DHG plants were investigated.

Results The presence of *Epichloë* endophytes significantly improved growth performance, photosynthetic capacity, and nitrogen uptake efficiency of the host grasses, especially under low nitrogen conditions. the competitive abilities of DHG. Importantly, the gain effect of the endophyte on the competitiveness of DHG gradually weakened with the decrease in the number of EI plants.

*Conclusion*The *Epichloë* endophyte enhances DHG competitiveness under nitrogen limitation but diminishes as soil nitrogen content increases. These results provide insights into the strong competitive advantage of EI DHG and the high infection rate of DHG in natural grasslands with harsh habitats.

Introduction

Competition among plant individuals is a prevalent phenomenon in the nature world (Wang et al. 2022). This phenomenon extends beyond interactions between diverse plant species (interspecies competition) to interactions among distinct individuals of the same plant species (intraspecies competition) (Zhang et al. 2022). Empirical investigations have underscored that competition is the predominant factor shaping community structure, thereby serving as the primary catalyst for species exclusion within these ecosystems (Liu et al. 2021). Plant competition is influenced by an array of factors, encompassing both external and internal elements that shape plant competitiveness. At the core of plant competition lies the intricate struggle among plants for vital resources such as light, water, and nutrients (Craine and Dybzinski 2013). Light stands as a fundamental prerequisite for photosynthesis, enabling plants to manufacture the organic resources crucial for their growth (Loudari et al. 2022). Remarkably, the diminution of available light emerges as the paramount dimension in the competitive interplay among plants, shaping their struggle for growth and survival (Craine and Dybzinski 2013). In contrast to the above-ground contest for light, below the surface, a distinct arena of competition unfolds as water and nutrients amass within the soil (Klinerova and Dostal 2020; Weemstra and Valverde-Barrantes 2022), instigating a fervent struggle among plant roots. Among the spectrum of essential nutrients, nitrogen emerges as a particularly scarce mineral resource, thereby assuming a pivotal role in constraining and dictating plant growth. Notably, these external dynamics, along with other environmental factors, exert a significant impact on the competitive prowess of plants (Aschehoug et al. 2016). Studies on plant

competition can help understand the survival strategies of plants, the role of intra- and interspecific relationships in community development and succession, and the ecological niche differentiation of populations (Aschehoug et al. 2016; Wang et al. 2015).

Among various internal plant factors, fungal endophytes play a crucial role (Hume et al. 2016). Endophytic fungi, residing within plant tissues without causing apparent harm, are integral components of plant biology (Hou et al. 2021). Among these, the *Epichloë* genus plays a significant role in the health and stress response of its grass hosts (Christensen et al. 2008). The associations between them are generally considered to be mutualistic unless under extreme stresses (Bastias et al. 2021). Host plants provide photosynthesis for endophytes, and the presence of endophytes improves the survival of host grass in abiotic (Becker et al. 2016) and biotic stresses (Matsukura et al. 2014) increasing the survival ability and adaptive capacity of host grasses under various harsh conditions, further enhancing competitiveness. To date, some studies indicate that *Epichloë* endophytes positively affect the competitive ability of their hosts such as *Lolium arundinaceum* and *Festuca pratensis*, while other research indicates negative or neutral effects of endophytes on the competitive ability of their host grasses (Mersch and Cahoon 2012). The notable disparities in competitive mechanisms may be attributed to species-specific variations or the intricate interplay of ecological interactions and environmental conditions. Consequently, there is a need to investigate these competitive mechanisms across a broader range of species within diverse environmental conditions (Xiong et al. 2015).

Achnatherum inebrians (drunken horse grass, DHG), a perennial native to semi-arid and arid grasslands of northern and northwestern China, provides a unique model for studying these interactions (Yao et al. 2016). In natural condition, DHG plants are symbiotic with either *Epichloë gansuensis* or *E. inebrians* (Chen et al. 2015; Li et al. 2004), rendering DHG toxic to grazing livestock due to the production of abundant alkaloids (Zhang et al. 2011). The study found that 19 populations were 100% infected with *E. gansuensis* in low rainfall areas (Nan and Li 2000). Over the past decades, the endophytic fungal symbiosis of DHG has been systematically investigated. The presence of *Epichloë* endophyte provides DHG with a robust ability to enhance host resistance and tolerance to various abiotic stresses including drought (Li et al. 2007; Xia et al. 2018), salt (Chen et al. 2018), cold (Liu et al. 2007), heavy mental (Zhang et al. 2014) and low nutritions (Wang et al. 2018). Additionally, it enhances resistance to biotic stresses such as insects (Guo et al. 2016), diseases (Liu et al. 2015) and grazing by livestock (LI et al. 2018). Undoubtedly, avoiding herbivores' ingestion help DHG gain greater competitiveness than any other edible plants in natural grassland. It has been observed that the *Epichloë* endophyte confers a strong competitive ability ,ensuring that endophyte-infected (EI) DHG, rather than endophyte-free (EF) DHG, occupies the dominant place in their drought and infertility habitat.

The purpose of this study is to investigate how the endophyte can enhance the competitiveness of the host DHG. We hypothesize that endophytes have the potential to enhance the capacity of their host DHG for acquiring nutrients and water, so that EI DHG could occupy the dominant place in the natural habitat. To test this hypothesis, we established different gradients of soil nitrogen content in a pot-based experiment, simulating the infertile soil conditions typical of DHG habitats. We compared the abilities of

photosynthesis, water and nutrients acquisition and utilization between EI and EF under different seedling densities to determine the ways in which *Epichloë* endophyte enhances the competitiveness of EI DHG grass.

Materials and Methods

Plant growth conditions and nitrogen treatments

Prior to planting, EI and EF seeds were tested for infection rates. Each pot was filled with vermiculite, containing two monocultures (EI and EF, respectively) and three mixtures (EI: EF = 1:1, EI: EF: 3:1: EI: EF: 1:3). 150 mL pour of Hoagland nutrient solution was added to each pot. After 30 days, three nutrient solutions of 0.1 mM, 1 mM, and 7.5 mM were applied to establish a gradient treatment with varying nitrogen concentrations. This testing phase spanned 110 days, during which pots were randomly placed in the greenhouse and were relocated every other week.

A. inebrians plants harvested from the greenhouse were gently shaken to remove soil. The aboveground and underground parts were separated, freshly weighed, and then brought to the laboratory for drying in an oven at 105°C to obtain the dry weight. The average value was calculated to represent the biomass of each *A. inebrians* plant.

Determination of photosynthetic parameters and chlorophyll content

On September 1st, photosynthetic parameters were measured. For each treatment, 5 top leaves of *A. inebrians* plants, randomly selected from both EI and EF, were used. The measurements were conducted using a GFS-3000 device (WALZ, Germany), following the method outlined by Dahal et al. (2017). The chamber was set with an air carbon dioxide concentration of 440 ± 10 μmol·mol-1, and the testing conditions maintained a temperature range of 25–28℃. Additionally, a chlorophyll meter, SPAD-502Plus (Konica Minolta Sensing, Inc., Japan), was employed to measure chlorophyll in the top leaf of each plant in every treatment. The relative SPAD values were translated into concentration values (mg/dm^2).

Determination of growth parameter

Following the determination of photosynthesis parameters and chlorophyll content, all samples were dried in an oven at 80°C until reaching a constant weight. Subsequently, each dried leaf portion from every treatment was weighed to determine the total dry matter of each plant. The data on dry matter weight were then used to calculate the aggressivity (A) using the following formula:

$A = Y_{ab}/Y_{aa}\text{-}Y_{ba}/Y_{bb}$

Where Y_{ab} and Y_{ba} represent the total dry weight of EI or EF plants, respectively, when EI and EF were mixed during sowing. Y_{aa} and Y_{bb} denote the dry weight of EI or EF plants, respectively, when EI or EF were monocultured.

Determination of nitrogen content and other relative indicators

Total nitrogen contents in the plant samples were assayed using flow injection analysis (FIAstar 5000 Analyzer, FOSS, Sweden). Data on nitrogen content from the samples were then used to calculate total nitrogen accumulation (TNA), nitrogen utilization efficiency (NUtE), and nitrogen uptake efficiency (NUpE), according to the following formulas :

TNA (mg) = Σ N concentration × total plant dry weight

NUtE (g^2/mg) = total plant dry weight/N concentration

NUpE (mg/g) = TNA/root dry weight

Statistical analysis

The data were statistically integrated using Microsoft Excel 2010 and analyzed and processed with SPSS 21.0. A two-way ANOVA was employed to compare the effects of endophytic fungi and plant competition on the growth status and nutrient utilization of DHG. Additionally, a three-way ANOVA was used to analyze the effects of low nitrogen stress, endophytic fungi and plant competition on photosynthesis, nutrient use efficiency, etc., of DHG. Statistical significance was set as 95% confidence level. Means and their standard errors are reported.

The relationships between the variables-endophyte, seedling density, nitrogen concentration, plant height, photosynthetic rate, chlorophyll content, and biomass-were also assessed. Initially, bivariate correlations between the variables were examined, to ensure the linear models made sense. Subsequently, using structural equation modeling (SEM), possible causal relationships between the various components and biomass were identified. The R package "piecewiseSEM" was employed to conduct the SEM steps (Nakagawa et al. 2017).

Results

Plant growth indicator and photosynthetic indicators

Significant effects of endophyte ($p < 0.001$), nitrogen concentration ($p < 0.001$) and their interactions ($p = 0.047$) were detected on plant height (Table 1). Under the 0.1 mM Nitrogen concentration treatment, the plant height of EI at a 1:3 sowing rate condition was significantly lower than EI plants at the other 3:1 sowing rate condition ($p < 0.05$). Conversely, the plant height of EF at 1:1 sowing rate condition was significantly lower than in the monoculture condition ($p < 0.05$). However, under higher nitrogen concentrations (1.0 mM and 7.5 mM), sowing rate did not significantly affect plant height ($p > 0.05$). When comparing EI and EF effect, EI plants consistently exhibited greater height under low (0.1 mM) and moderate (1.0 mM) nitrogen concentrations across all sowing rates (monoculture, 1:1, and 3:1) ($p < 0.05$).

However, under the highest nitrogen concentration (7.5 mM), endophyte effect were not significantly influenced by sowing rate ($p > 0.05$) (Fig. 1).

The significant effects of endophyte ($p < 0.001$), nitrogen concentration ($p < 0.05$) and sowing rate ($p < 0.05$) on aboveground and underground biomass were detected (Table 2). Under 0.1 mM and 1.0 mM concentration treatments, the aboveground biomass of EI was significantly greater than that of EF plants ($p < 0.05$). Conversely, the aboveground biomass of EI at a 1:1 sowing rate condition was significantly lower than EF under 7.5 mM nitrogen concentrations(Fig. 2A). Under 1.0 mM and 7.5 mM concentration treatments, the underground biomass of EI plants at the monoculture sowing rate condition was significantly lower than that of EF ($p < 0.05$). The EI plant had a significantly higher underground biomass than EF only at 1:3 sowing rate condition ($p < 0.05$) (Fig. 2B).

The significant effects of endophyte ($p < 0.001$) and nitrogen concentration on chlorophyll content ($p = 0.013$) were detected (Table 1). Under the 0.1 mM Nitrogen concentration treatment, the chlorophyll content of EF plants at the 1:1 sowing rate condition was significantly lower than at 1:3 sowing rate condition ($p < 0.05$). Meanwhile, the chlorophyll content of EI plants was not siginificantly affected by the sowing rate ($p > 0.05$). In monoculture and 1:1 condition, EI plants had significantly higher chlorophyll content than EF ($p < 0.05$). Under 1.0 mM Nitrogen concentration treatment, the chlorophyll content of EI plants at the 3:1 sowing rate condition was significantly higher than at the 1:3 sowing rate condition ($p < 0.05$). Conversely, the chlorophyll content of EF plants at the monoculture condition was significantly higher than at the 1:3 sowing rate condition ($p < 0.05$), In monoculture, 1:1 and 3:1 conditions, the chlorophyll content of EI plants was significantly higher than EF ($p < 0.05$). Under the 7.5 mM Nitrogen concentration treatment, the chlorophyll content of both EI and EF plants was not significantly affected by the sowing rate ($p > 0.05$). EI plant had a significantly higher chlorophyll content than EF only at the 1:1 sowing rate condition ($p < 0.05$) (Fig. 3).

Only endophyte (p = 0.006) significantly increased the photosynthetic rate of *A. inebrians* under various treatments (Table 1). Under the 7.5 mM Nitrogen concentration treatment, the photosynthetic rate of EF plants at the 1:1 sowing rate condition was significantly lower than at the 3:1 and 1:3 sowing rate condition ($p < 0.05$). Additionally, EI plants had a significantly higher photosynthetic rate than EF under this Nitrogen concentration treatment ($p < 0.05$). Under the 1.0 mM Nitrogen concentration treatment, EI plants had a significantly higher photosynthetic rate than EF at the 3:1 sowing rate condition ($p < 0.05$). Under the 0.1 mM Nitrogen concentration treatment, EI plants had a significantly higher photosynthetic rate than EF in monoculture, 1:1 and 1:3 sowing rate conditions ($p < 0.05$) (Fig. 4).

Carbon, nitrogen and phosphorus concentration

There was no marked difference in endophyte ($p > 0.05$) between the aboveground carbon contents of EI and EF. Under the 1 mM Nitrogen concentration treatment, the carbon content of EF plants at the 1:1 sowing rate condition was significantly lower than at the 3:1 and 1:3 sowing rate conditions ($p < 0.05$). Under other sowing conditions and nitrogen concentration treatments, the carbon content was not

significantly affected by the sowing rate ($p > 0.05$). Under the 0.1 mM Nitrogen concentration treatment, the carbon content of EI plants at the 1:1 sowing rate condition was significantly lower than at the 3:1 and 1:3 sowing rate conditions ($p < 0.05$). Under the 7.5 mM Nitrogen concentration treatment, the carbon content of EF plants at the 3:1 sowing rate condition was significantly higher than at the 1:1 sowing rate condition ($p < 0.05$) (Fig. 5).

With the increase in soil nitrogen content, the nitrogen content of both EI and EF plants increased both above and underground. Under the 0.1 mM Nitrogen concentration treatment, the endophyte significantly increased the nitrogen content of EI and EF plants under monoculture conditions ($p < 0.05$) The nitrogen content of EF plants at a 1:3 sowing rate condition was significantly higher than at 3:1 and 1:1 sowing rate conditions ($p < 0.05$). Under the 1 mM Nitrogen concentration treatment, the endophyte significantly increased the aboveground nitrogen content of both EI and EF plants under monoculture conditions, and the nitrogen content of EI plants at a 1:1 sowing rate condition was significantly higher than at a 1:3 sowing rate condition ($p < 0.05$). Under the 7.5 mM Nitrogen concentration treatment, the nitrogen contents of EI plants under monoculture conditions was significantly higher than under the other sowing conditions ($p < 0.05$) (Fig. 6).

The absorption of phosphorus content decreased with an increasing soil nitrogen content. Under the 0.1 mM Nitrogen concentration, the phosphorus content of EI was considerably higher than that of EF plants at 1:1 and 3:1 sowing rate conditions ($p < 0.05$), and the phosphorus content of EI plants at a 1:3 sowing rate condition was significantly higher than at 3:1 and 1:1 sowing rate conditions ($p < 0.05$). Under the 1 mM Nitrogen concentration treatment, the endophyte significantly increased the underground phosphorus content of *A. inebrians* at a 1:3 sowing rate condition ($p < 0.05$). Under the 7.5 mM Nitrogen concentration treatment, differences in endophyte between aboveground and underground P content of EI and EF were not significant ($p > 0.05$) (Fig. 7).

Nitrogen accumulation, utilization and uptake efficiency

The Total Nitrogen Accumulation (TNA) of *A. inebrians* was predominantly influenced by nitrogen concentration ($p < 0.001$) (Table 3). At a low nitrogen concentration (0.1 mM), the TNA in EI plants in monoculture was significantly greater than those in the 1:1 and 1:3 sowing rates ($p < 0.05$). TNA of EF plants at monoculture condition was significantly higher than those at the 1:1 sowing rate condition ($p < 0.05$). Additionally, EI plants had a higher TNA than EF at the 1:1 sowing rate condition ($p < 0.05$). Under the 1.0 mM Nitrogen concentration treatment, EI plants had a higher TNA than EF at the 1:1 sowing rate condition ($p < 0.05$) (Fig. 8A).

Nitrogen Utilization Efficiency (NUtE) was significantly influenced by both nitrogen concentration ($p = 0.004$) and sowing rate ($p < 0.001$) (Table 3). Under 0.1 mM Nitrogen concentration treatment, EI plant had a higher NUtE than EF at the 3:1 sowing rate condition ($p < 0.05$). Under the 1.0 mM Nitrogen concentration treatment, NUtE of EI plant at the monoculture condition was significantly higher than 3:1 sowing rate condition ($p < 0.05$). EI plants had a higher TNA than EF at monoculture and 1:1 sowing rate

condition ($p < 0.05$). Under the 7.5 mM Nitrogen concentration treatment, NUtE of EI plants at the 1:3 sowing rate condition was significantly higher than those at the other 3 sowing rate conditions ($p < 0.05$) (Fig. 8B).

The Nitrogen Uptake Efficiency (NUpE) showed significant effects of endophyte ($p < 0.001$), nitrogen concentration ($p < 0.001$), and sowing rate (p = 0.024). Additionally, significant interactions were observed between nitrogen concentration and sowing rate ($p < 0.001$), as well as among endophyte, nitrogen concentration, and sowing rate ($p = 0.025$) (Table 3). Under the 0.1 mM Nitrogen concentration treatment, EI plant had a higher NUpE than EF at monoculture, 1:1 and 3:1 sowing rate condition ($p < 0.05$). Under the 1.0 mM Nitrogen concentration treatment, NUpE of EI plants at the 1:3 sowing rate condition was significantly higher than those at the other 3 sowing rate conditions ($p < 0.05$). Additionally, EI plants had a higher NUpE than EF at monoculture, 1:1 and 1:3 sowing rate condition ($p < 0.05$). Under the 7.5 mM Nitrogen concentration treatment, NUpE of EI plants at the 1:3 sowing rate condition was significantly lower than those at the other 3 sowing rate conditions ($p < 0.05$). NUpE of EF plants at the 1:3 sowing rate condition was significantly lower than those at monoculture and 3:1 sowing rate conditions ($p < 0.05$) (Fig. 8C).

Competitive ability and SEM for the Interactive Effects on DHG Biomass

The effects of nitrogen concentration, sowing rate, and their interactions on aggressivity were not significant ($p > 0.05$) (Table 4). Under 0.1 mM and 1.0 mM Nitrogen concentration treatment, all aggressivity values were greater than 0, indicating that the competition of EI was stronger than EF plants, regardless of whatever the sowing rate was 1:1 or 1:3. However, at a seeding ratio of 3:1, the competitive advantage over EF plants is reduced with the increase in EI plants. Conversely, under the 7.5 mM Nitrogen concentration treatment, the aggressivity values were less than 0, indicating that EF plants had stronger competition than EI (Fig. 9).

Plant biomass was found to be influenced by the interaction of endophyte, seedling density, and N concentration through the pathways of plant height, photosynthetic indicators, and nitrogen content, as demonstrated by SEM analysis. The best-fitting SEM adequately explained the data with $\chi^2 = 5.992$, $p = 0.115$, and GFI = 0.994, successfully capturing the variance in plant biomass ($R^2 = 97\%$). In the final model, seedling density ($p < 0.01$) and N concentration ($p < 0.01$) had a direct positive impact on biomass, while endophyte ($p < 0.001$) had a negative direct impact. The final model accounted for 94%, 25%, and 94% of the variation in nitrogen, chlorophyll, and plant height, respectively. Furthermore, through the nitrogen content, endophyte, seedling density, and N concentration also had favorable indirect influences on plant biomass ($p < 0.05$) (Fig. 10).

Discussion

This study elucidates that the presence of *Epichloë* endophytes significantly enhances the competitive abilities of DHG, especially under low nitrogen conditions. This enhancement is manifested in the improved growth performance, photosynthetic capacity, and nitrogen uptake efficiency of the host grasses. Notably, the beneficial effects of endophytes intensify as soil nitrogen content decreases, suggesting that in nutrient-poor soils, EI DHG could gain a competitive edge and become more dominant. Concurrently, the impact of endophytes on DHG competitiveness diminishes with the decreasing number of EI plants, providing insight into the prevalence of EI *Achnatherum inebrians* in challenging grassland environments.

Previous research has consistently shown that endophyte infestation has a beneficial impact on the growth of various host grasses, increasing plant height, the number of reproductive branches, and overall biomass (Wang et al. 2020; Xia et al. 2018). Competition, a crucial factor in determining community composition and dynamics, is often reflected in enhanced biomass—an essential indicator of a plant's competitive ability. Biomass, in turn, is influenced by processes such as respiration, photosynthesis, and assimilation (Collalti et al. 2019; Keddy et al. 2010). Photosynthesis, a fundamental source of energy for plants, converts absorbed light energy into chemical energy for storage, supporting the organism's growth and development (Shi et al. 2022). While some studies have suggested that plants with *Epichloë* endophytes accumulate more biomass due to enhanced photosynthetic activity (Belesky et al. 1989), our study diverges from this trend. Contrary to previous findings, we observed a negative impact of *Epichloë* endophyte on host biomass. This discrepancy may be attributed to the metabolic activity of *Epichloë* hyphae within the host, resulting in energy loss for the host plant. The net photosynthetic rate of plants is closely tied to their biomass accumulation (Rozpądek et al. 2015) and significantly influenced by plant height. Taller plants typically access more light, potentially enhancing their photosynthetic capacity (Chen et al. 2022). In our study, under low nitrogen stress conditions, the height of EI DHG surpassed that of EF plants. Consequently, the photosynthetic capacity of EI DHG was higher than that of EF plants across various mixed seeding treatments.

It is well-established that the process of photosynthesis involves the assimilation of carbon (C) elements by plants, and the efficiency of this process is intricately linked to the concentration of available CO_2 within the cell. This concentration, in turn, is often influenced by the stomatal conductance of plant leaves (Arena et al. 2016). Reduced stomatal conductance directly results in decreased CO_2 assimilation in chloroplasts, leading to lower photosynthetic rates and, consequently, inhibiting leaf and overall plant growth (Woodruff et al. 2015). Therefore, in instances of competition between two species, the dominant plant can enhance its competitive advantage by regulating the opening of leaf stomata (Rozpądek et al. 2015) This study reveals that the endophyte had a positive effect on the stomatal conductance of DHG at 0.1 mM and 1 mM Nitrogen concentrations. This indicates that the photosynthetic parameter was higher for EI plants than EF plants. The efficiency observed suggests that EI plants possess a superior ability to regulate stomatal closure compared to EF plants.

Root systems play a crucial role in determining the competitive advantage of plants, especially in nutrient and water uptake (Schiefelbein and Benfey 1991; Wilson 1988). The presence of endophytes significantly

influences nutrient absorption, transportation, and utilization within host plants (Malinowski et al. 1999). Nitrogen (N) and Phosphorus (P), essential nutrients for plant growth and metabolic processes (Gaude et al. 2007; Shaukat et al. 2012; Verzeaux et al. 2017), are particularly impacted by endophyte symbiosis. Alkaloids, paramount secondary metabolites of *Epichloë* endophytes and grass symbionts, contain nitrogen as a crucial component (Irmer et al. 2015). Previous research has demonstrated that endophytes can enhance nitrogen uptake in ryegrass and promote plant root growth, particularly under low nitrogen conditions (Ravel et al. 1997). The current study validates that endophytes significantly increase aboveground nitrogen content in monocultures under 0.1 mM and 1 mM Nitrogen concentrations. Under low nitrogen conditions, endophytes promote plant root growth, indicating an enhanced ability to absorb and supply nitrogen to the shoot apical region and leaves, even when the endophyte mycelium is not present in the roots (Hou et al. 2021). Considering the effect of alkaloids on plant competition, research by Malinowski et al. (1999) explored how endophyte infection altered the root morphology of tall fescue in a greenhouse experiment. The changes observed improved the absorption of essential nutrients, increased the synthesis and release of alkaloids, thereby boosting the plant's competitive advantage. In natural grazing grasslands, the toxicity of alkaloids ensures that livestock and herbivorous insects naturally avoid consuming plants containing them during growth. This avoidance mechanism contributes to plant survival, fostering resilience and a level of resistance. Moreover, symbionts produce secondary metabolites, especially alkaloids, and secrete antimicrobial substances to enhance their resistance to various diseases caused by fungi and bacteria, further improving their competitive ability (Schardl et al. 2004).

Phosphorus is an important element for plant growth (Bouain et al. 2022). Previous study has showed that endophytes can mitigate growth limitations by influencing the accumulation of phosphorus in host plants (Cui et al. 2022). The current research suggests that the aboveground phosphorus content of EI plants surpasses that of EF plants under monosowing conditions. Specifically, the endophyte significantly increases the aboveground phosphorus content under 0.1 mM Nitrogen concentration treatments in the 1:1 and 3:1 sowing ratios. This observed phenomenon could potentially be attributed to the greater diversity of phosphate-solubilizing fungal (PSE) species in the inter-rhizosphere soils of EI plants. The elevated diversity of PSE is thought to enhance plant assimilation of phosphorus (Zaidi and Khan 2007). As phosphorus and nitrogen are integral components of ribosomal RNA, essential for protein synthesis and overall plant growth, their enhanced levels in EI plants could explain the improved resistance to both biotic and abiotic stresses (Elser et al. 2001; Hessen et al. 2007; Smith et al. 2011). In the current study, grasses respond to high nitrogen levels by enhancing the uptake of other nutrients to alleviate nitrogen limitation. This response leads to rapid growth, increased aboveground biomass, and an intensified asymmetry in competition for belowground nutrient resources. Consequently, this asymmetry affects competition for aboveground light resources (Zhai et al. 2017). Therefore, the concurrent increase in phosphorus and nitrogen levels in plants may contribute to the ways in which endophytes enhance the competitive ability of host grasses.

The composition of grass mixes has the potential to influence grassland ecosystems by fostering ecological niche complementation (Zolotarev et al. 2021). Changes in nutrient levels can alter the

competitive interaction environment of target species by regulating the behavior of current mutualistic species (Calama et al. 2019).. Nitrogen application and seeding density are essential factors affecting crop yields (Ma et al. 2010). The presence of *Epichloë* endophyte facilitates nutrient uptake and decreases competition. Under low nitrogen conditions, competitive were found to adversely affect the growth of DHG,, resulting in a greater capacity for growth in monocultures than in mixtures. When the sowing ratio was 1:1 and 1:3, EI plants exhibited a significant competitive advantage over EF. However, when the mixing ratio was 3:1, the competitiveness of EI against EF was less than the competitiveness of EI itself. The competitive advantage did not increase with an increase in EI plant density, which may be attributed to the fact that when there were too many EI plants, the nutrients required by themselves increased, and the intraspecific competition of the EI plants increased, thus this may be due to the fact that when there are too many EI plants, their nutrient requirements increase, leading to increased intraspecific competition among EI plants. This phenomenon could result in a reduction in competitive ability against EF plants (Xu et al. 2022).Mixed communities often exhibit a 'compromising' effect. When one plant is at a competitive disadvantage for light energy, another plant may actively reduce its height to improve the fair energy use of the entire mixed system (Wilson 1995). Intraspecific competitive interactions had a beneficial impact on the growth of DHG, resulting in a more significant growth capacity when sown in mixtures compared to monocultures (Dai et al. 2014). In this study, a suitable increase in sowing density enhanced plant biomass under the same nitrogen application conditions. A higher planting density leads to a larger population size, resulting in increased plant community biomass. This increased biomass facilitates the upward transfer of nutrients stored in stems and leaves, leading to higher yields (Ye et al. 2023). However, it has been observed that sowing density has a negative impact on the chlorophyll content of *Achnatherum inebrians*. Under low-density conditions, plants may not compete fiercely for nutrients. Conversely, under high-density conditions, plants engage in fierce competition for resources, leading to a limited supply that affects photosynthesis. Consequently, lower chlorophyll content and reduced harvest index are observed (Ma et al. 2010). Therefore, the response of forage morphological traits to interspecific relationships varies at different mixing ratios (Kong et al. 2014).

Conclusion

The present study indicates that the *Epichloë* endophyte enhances the competitiveness of DHG by promoting plant growth, facilitating photosynthesis, and aiding nutrient absorption in the host plant. The findings suggest that the competitive advantage of EI plants over EF plants weakens as the number of EI plants increases. This information provides insight into the strong competitive advantage and high infection rate of *Achnatherum inebrians* in natural grasslands with harsh habitats, as well as the higher dispersal rate of EI plants in the field. However, due to the limitations of the pot trials, future field experiments are needed to conduct further research and fully explain the effect of *Epichloë* endophyte on intraspecific competition in DHG.

Declarations

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Conflict of interests The authors declare that they have no competing interests

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Figures

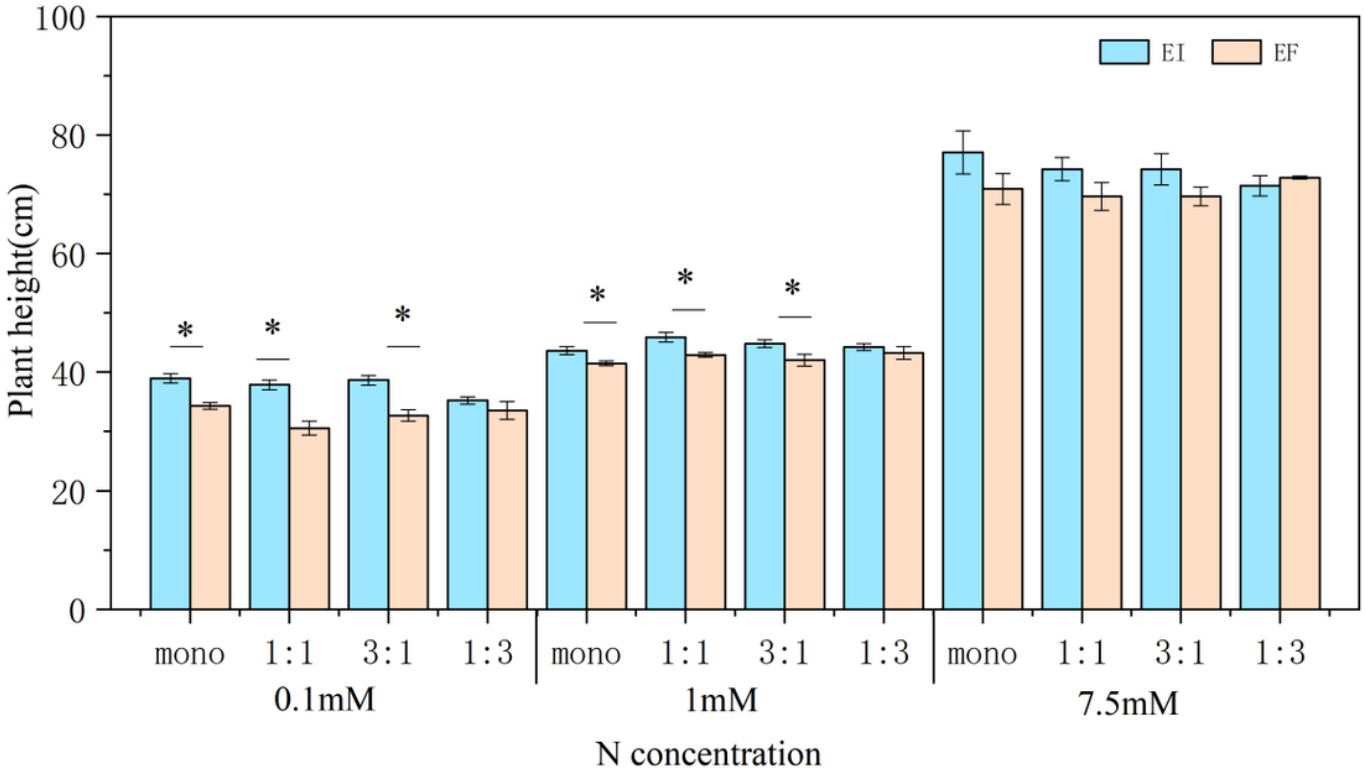


Figure 1

Plant height of *A. inebrians* plants under different nitrogen concentrations and different sowing conditions

Note: The marked asterisk (*) indicates the significant difference between plant height of EI / EF plants under the same seeding conditions.(p <0.05).The same below.

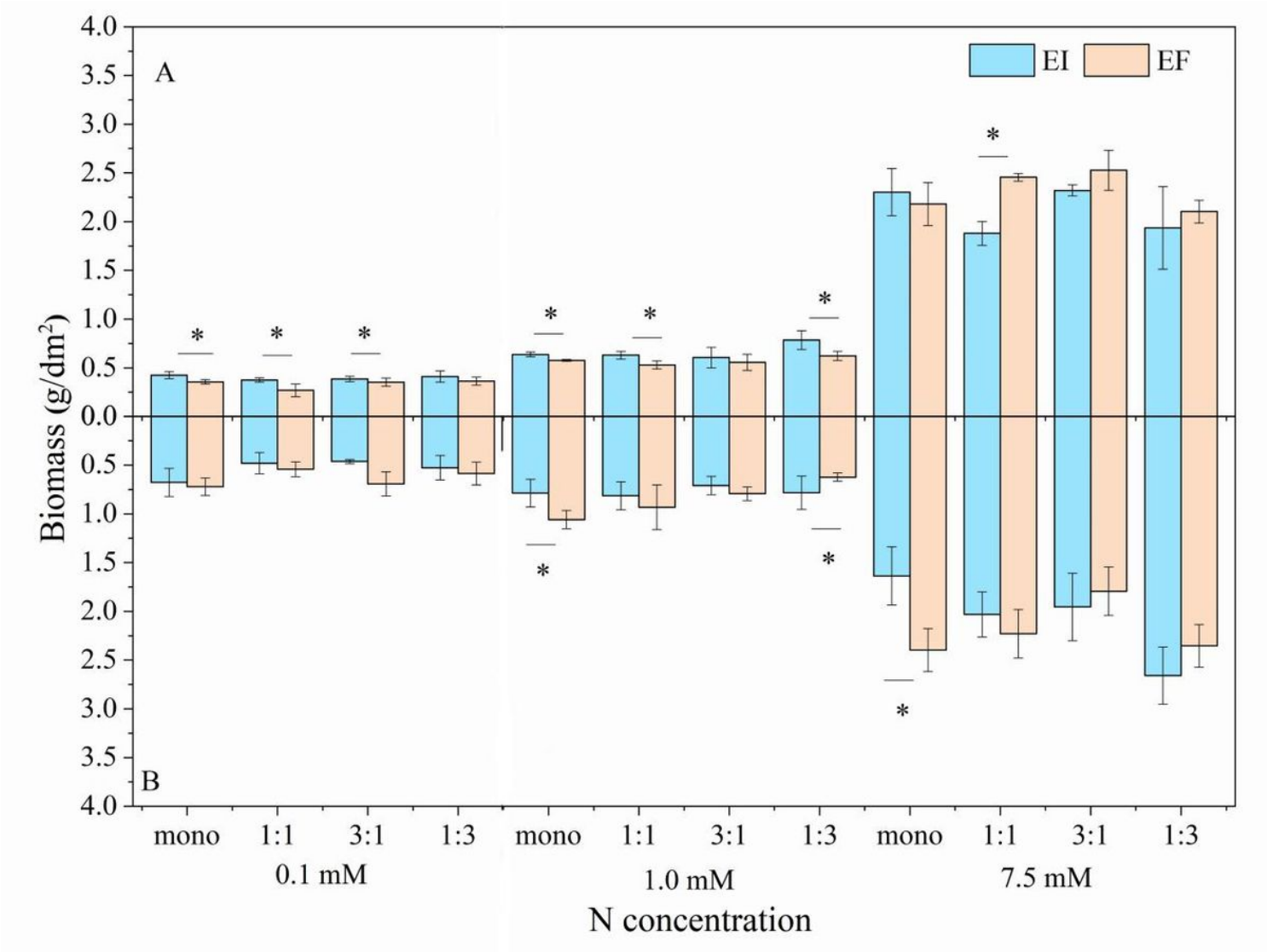


Figure 2

Biomass of the plants under different nitrogen concentrations and sowing conditions (A) aboveground biomass (g / dm2), (B) underground biomass (g / dm^2)

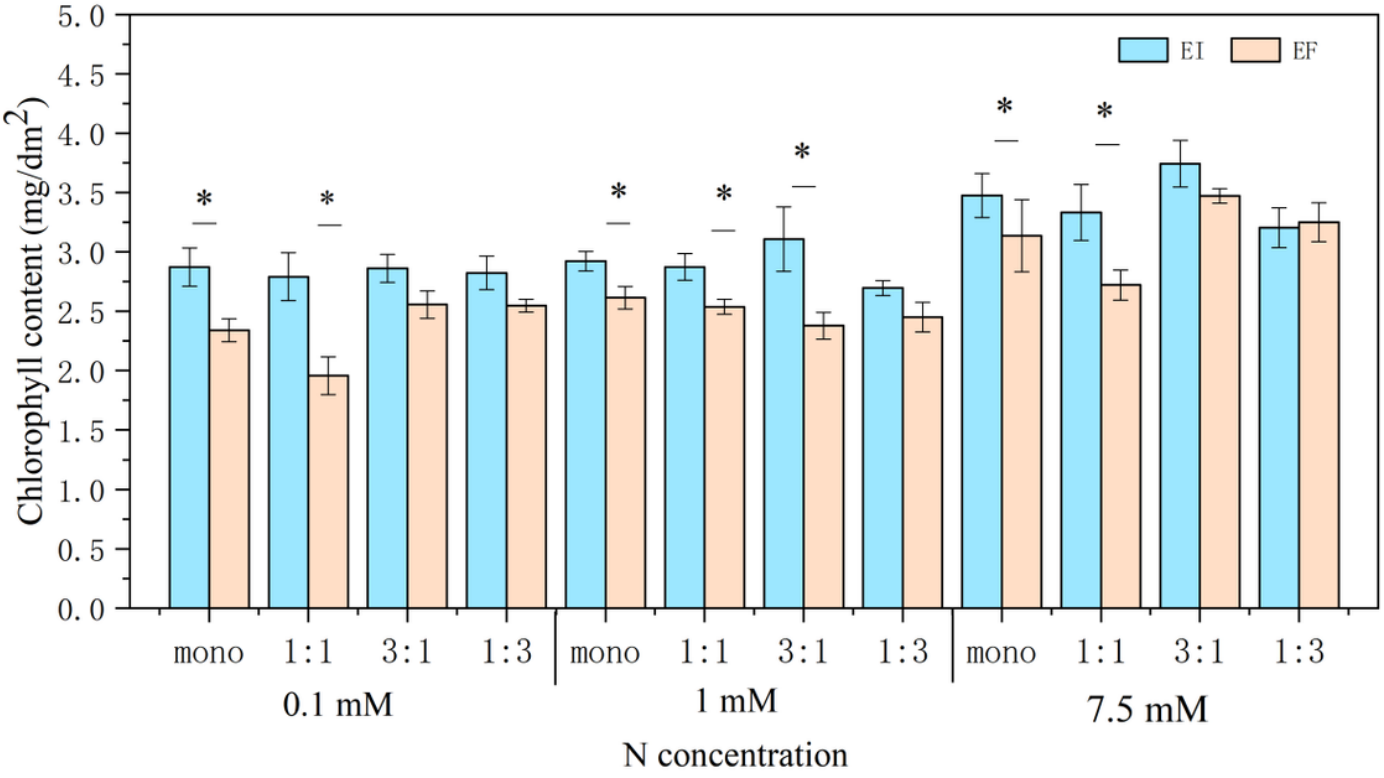


Figure 3

Chlorophyll content (mg/dm^2) under different nitrogen concentrations and different seeding treatments

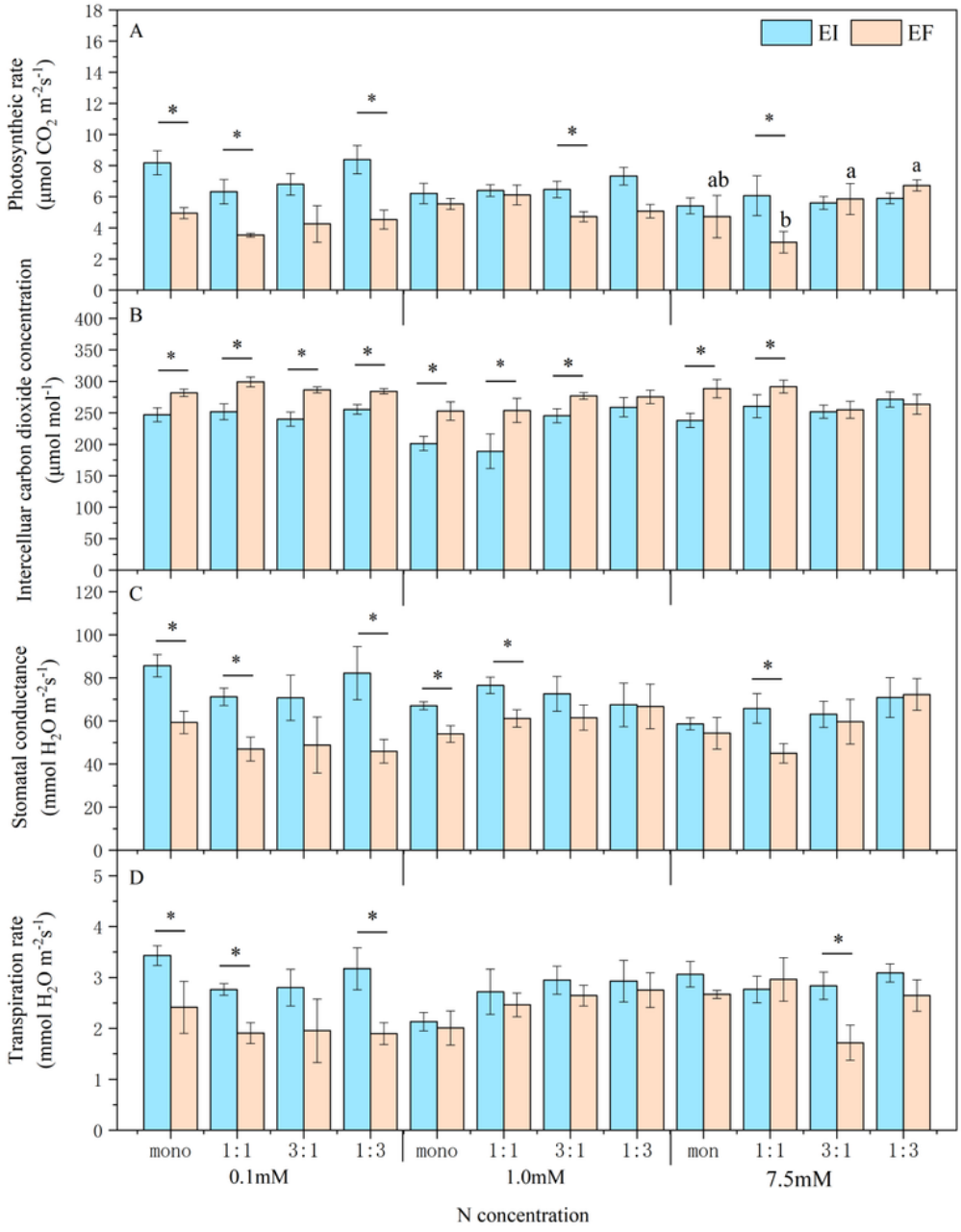


Figure 4

Leaf photosynthetic index under different nitrogen concentrations and different seeding treatments (A) Photosynthetic rate (μmol CO_2 $m^{-2}s^{-1}$), (B) Intercellular carbon dioxide concentration (μmol CO2mol^{-1}), (C) Stomatal conductance (mg/dm^2), (D) Transpiration rate (mmol H_2O $m^{-2}s^{-1}$)

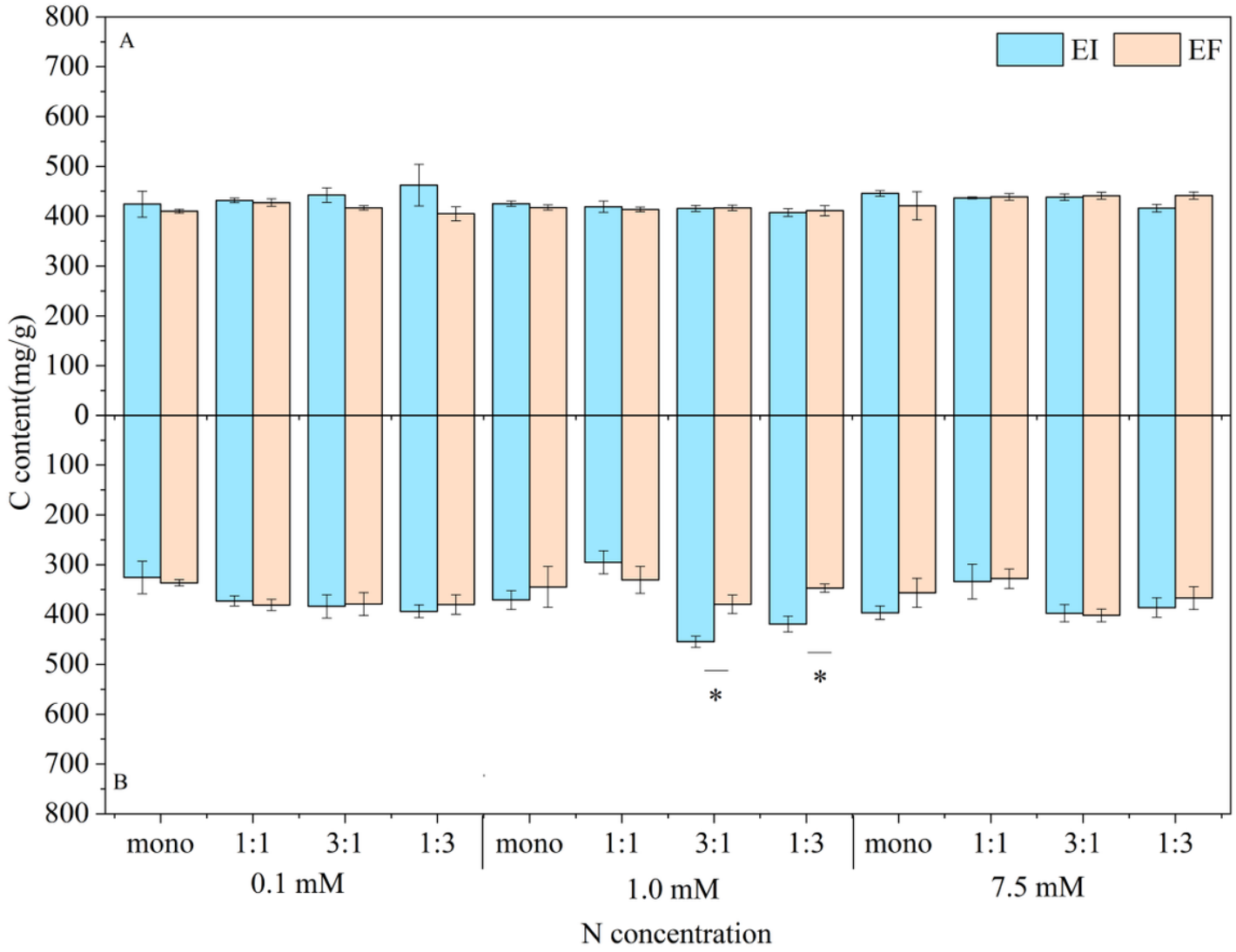


Figure 5

C content of *A. inebrians* plants (A) aboveground and (B) underground carbon content (mg / g) under different nitrogen concentrations and different sowing conditions

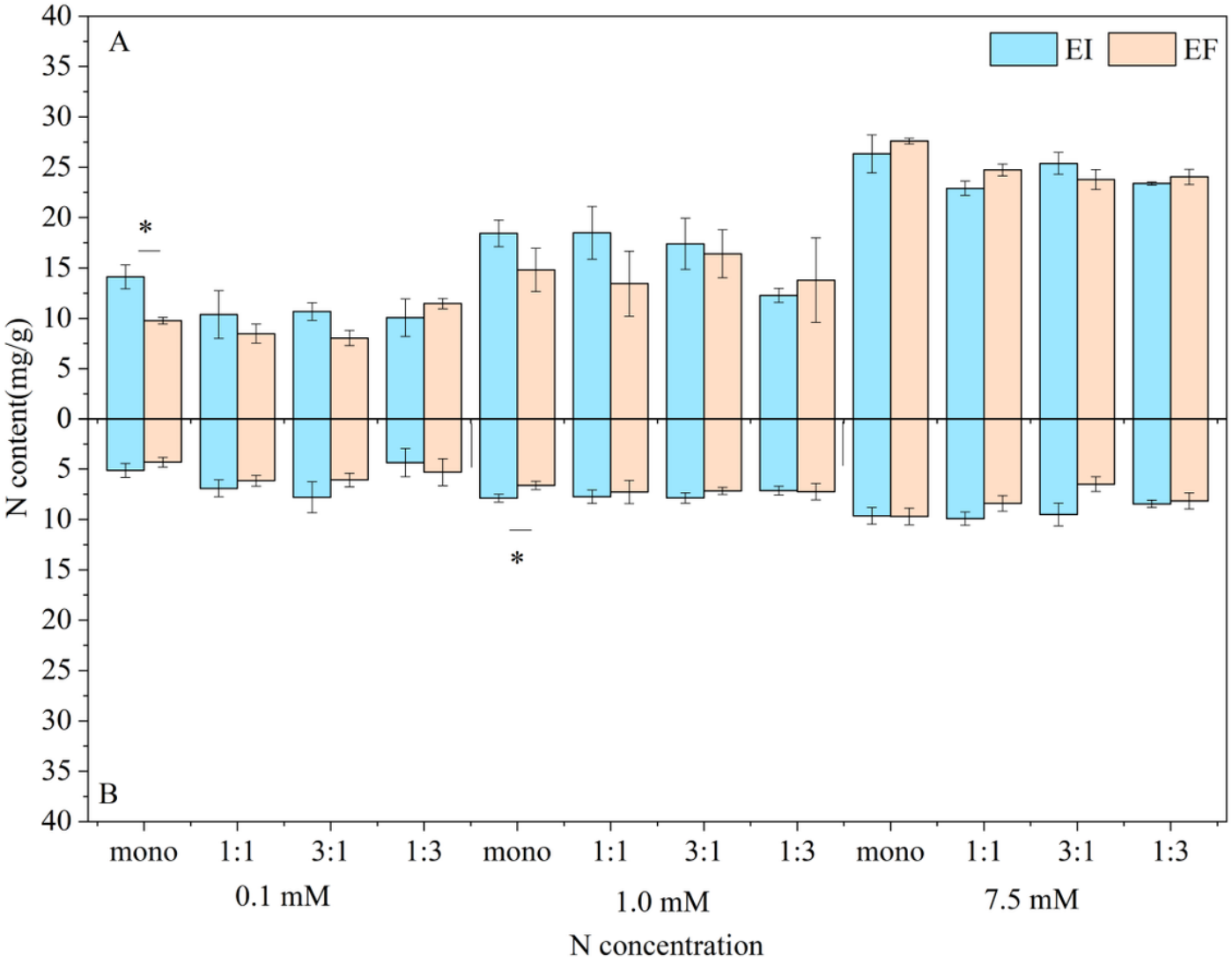


Figure 6

Nitrogen content of *A. inebrians* plants (A) aboveground and (B) under ground nitrogen content (mg / g) under different nitrogen concentrations and different sowing conditions

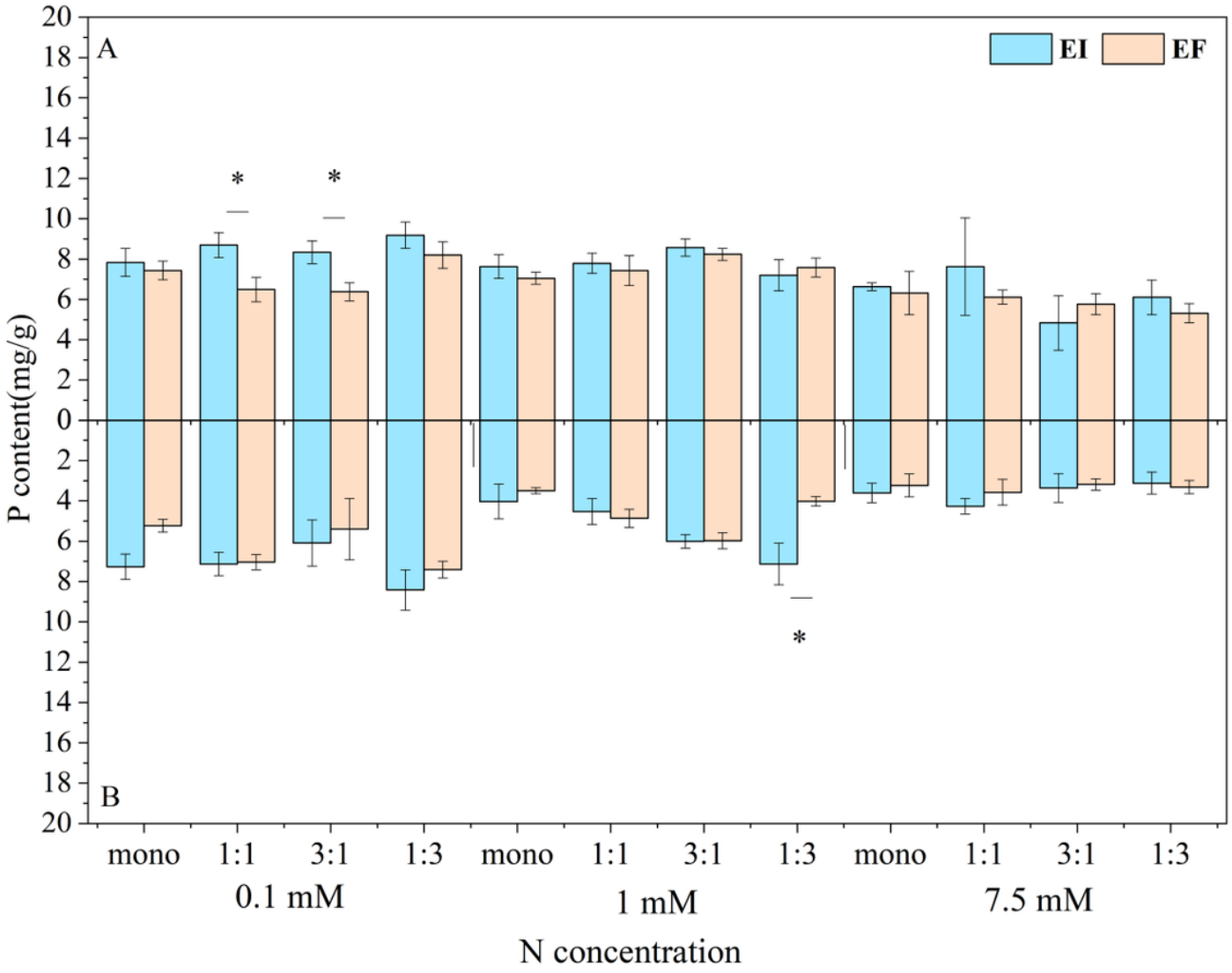


Figure 7

P content of *A. inebrians* plants (A) aboveground and (B) underground phosphorus content (mg / g) under different nitrogen concentrationas and different sowing conditions

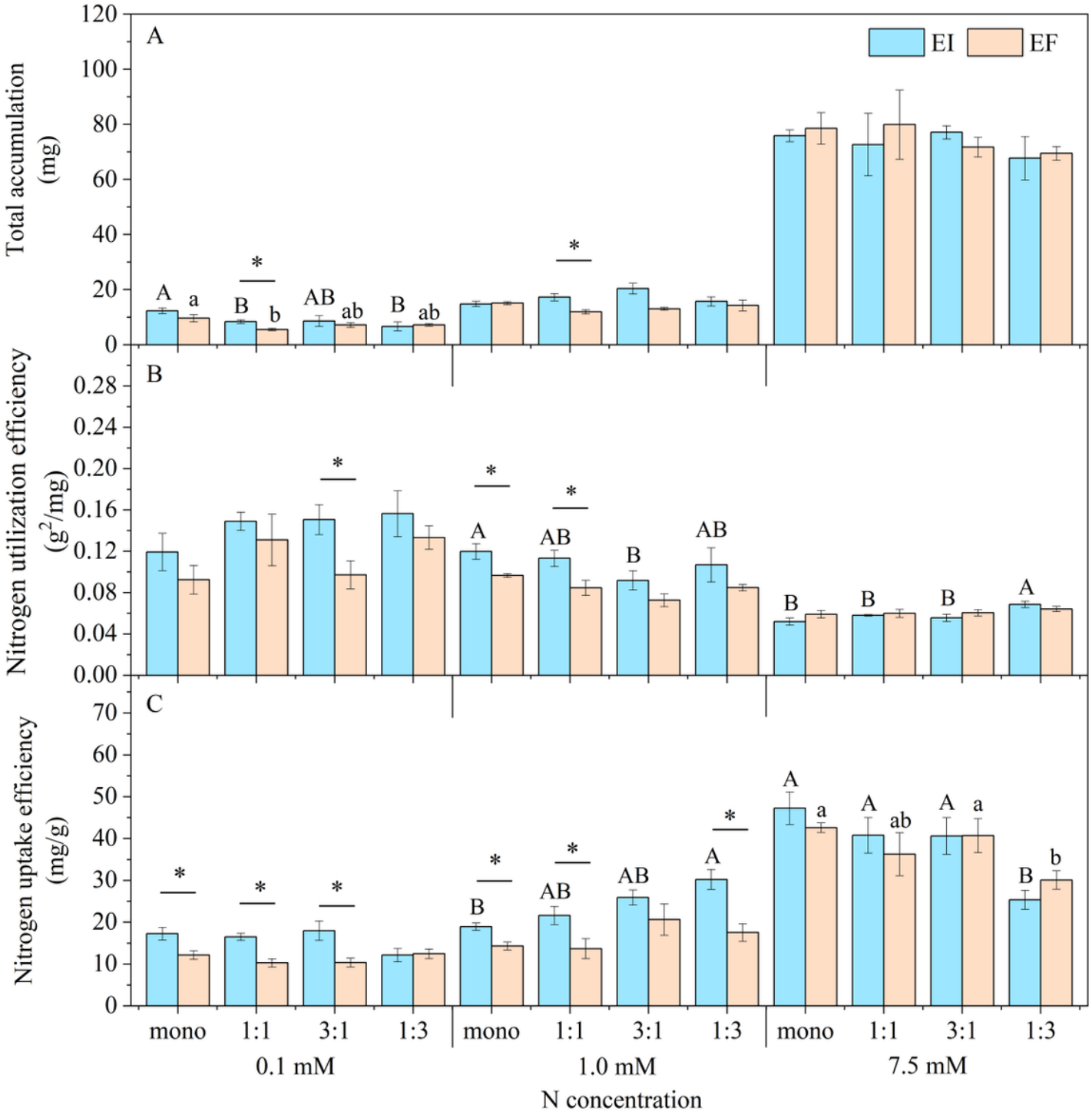


Figure 8

Nutrient use efficiency of the plants under different nitrogen concentrations and sowing conditions (A) Total Nitrogen accumulation (mg), (B) Nitrogen utilization efficiency (g^2 / mg), (C) Nitrogen uptake efficiency (mg / g)

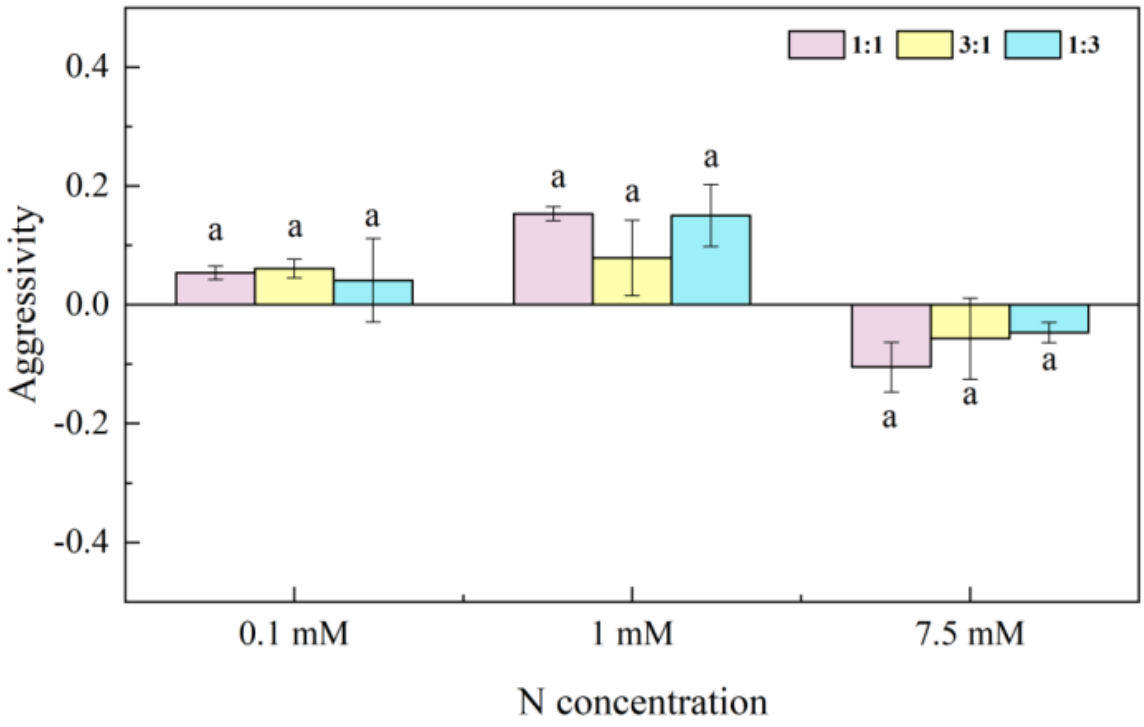


Figure 9

Aggressivity of EI plants at different nitrogen concentrations and different mixing conditions

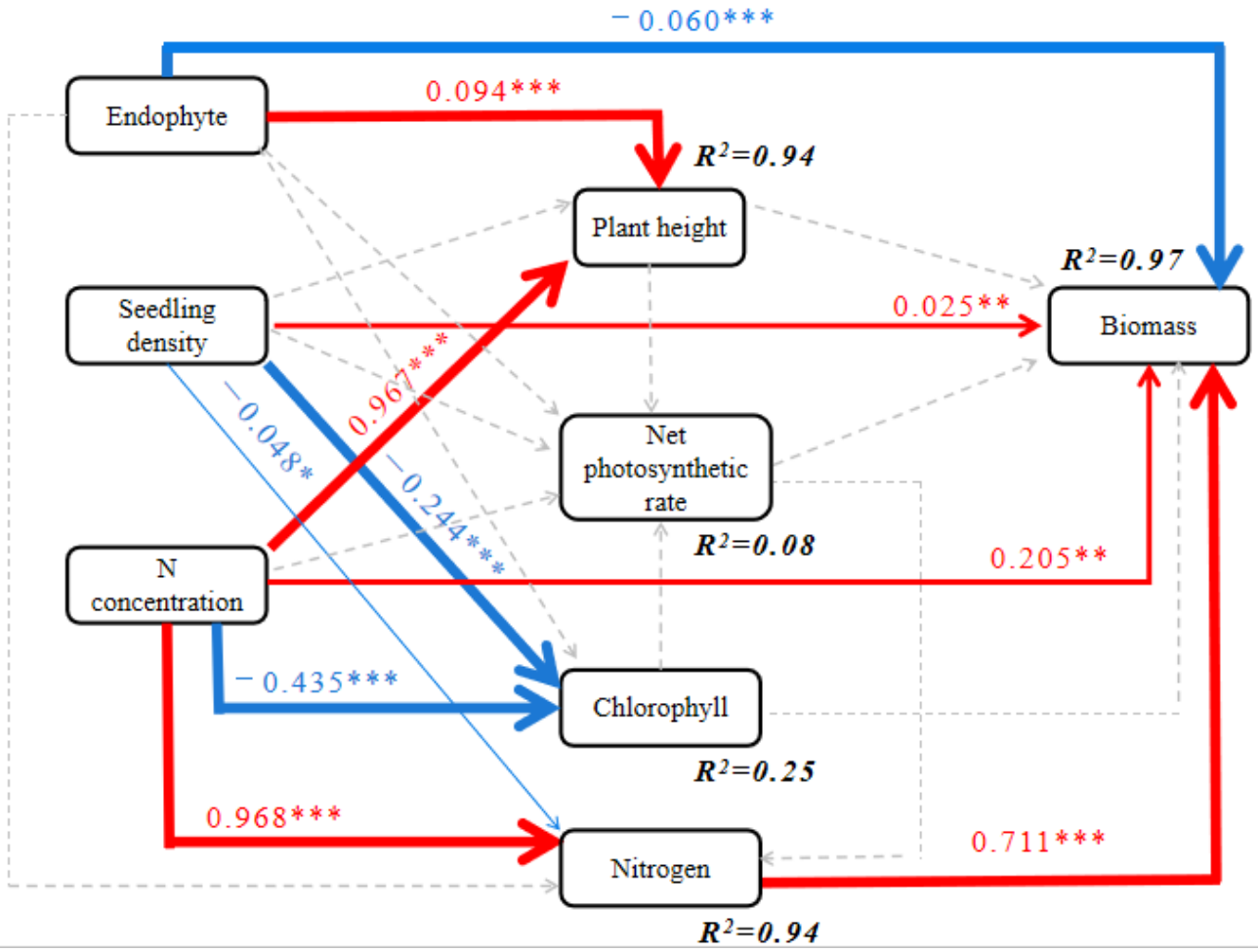


Figure 10

Endophyte, sowing rate, and N concentration on biomass of *A. inebrians* seedlings as determined by structural equation modeling (SEM) analysis. Dotted arrows, insufficient statistical evidence for path coefficients (p >0.05). Red arrows indicate relationships that are positive, and blue arrows indicate relationships that are negative. Next to each response variable in the model is the proportion of variance explained (R^2). Asterisks denote statistical significance (***, p <0.001; **, p <0.01; *, p <0.05); the width of the arrows indicates the intensity of the causal association; and the values next to the arrows are standardized path coefficients, which reflect the extent of the relationship. The finished model suited the data well enough: GFI =0.994, p =0.115, and $\chi 2$ =5.992

Supplementary Files

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