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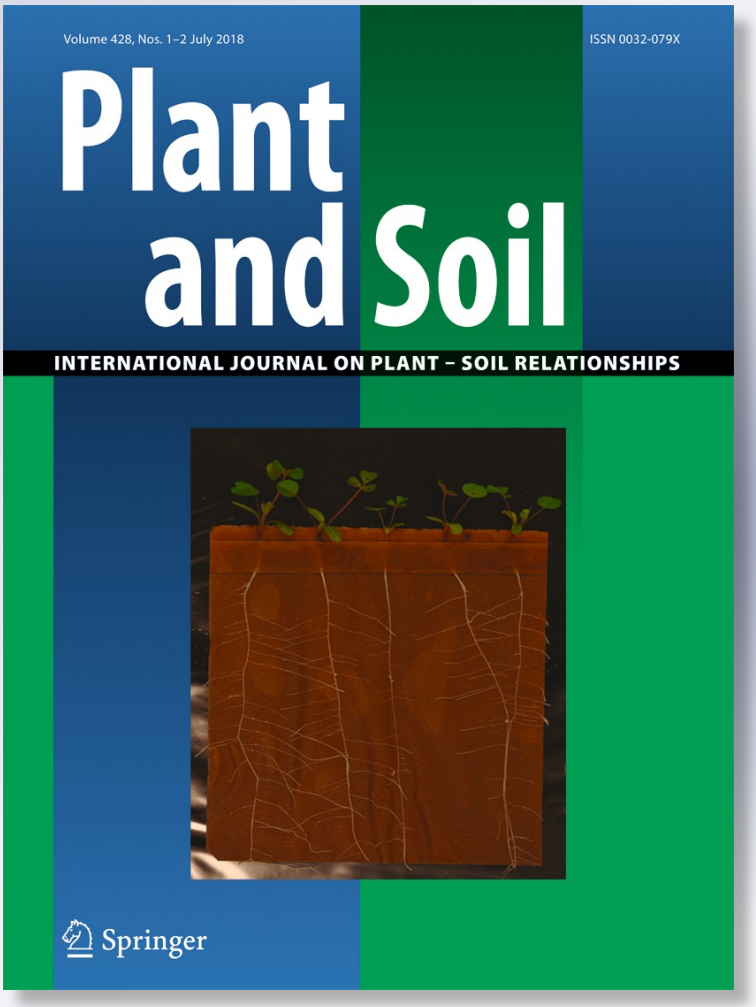

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REGULAR ARTICLE

Infection by the fungal endophyte *Epichloë bromicola* enhances the tolerance of wild barley (*Hordeum brevisubulatum*) to salt and alkali stresses

Taixiang Chen · Richard Johnson · Shuihong Chen · Hui Lv · Jingle Zhou · Chunjie Li

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Abstract

Background and aims Salinization is considered as a major environmental threat to agricultural systems. Infection with *Epichloë* fungal endophytes has been shown to increase tolerance to NaCl stress for several host grass species, but limited information is available regarding the effects of these endophytes under mixed salt (NaCl and Na_2SO_4) and mixed alkali ($NaHCO_3$ and Na_2CO_3) stresses. Since these four compounds are considered very harmful to many inland areas in China, we conducted a study to determine the impact of *Epichloë* fungal endophyte infection on wild barley (*Hordeum brevisubulatum*) under both salt stress (SS) and alkali stress (AS).

Methods Wild barley with (E+) and without (E-) *Epichloë* endophyte was subjected to mixed salt (molar ratio of $NaCl:Na_2SO_4 = 1:1$) and mixed alkali (molar ratio of $NaHCO_3:Na_2CO_3 = 1:1$) treatments (0, 100, 200, 300 and 400 mM). Photosynthetic parameters and chlorophyll content were measured after 21 days exposure to stress, and growth parameters, physiological indexes, sodium (Na^+), potassium (K^+), calcium (Ca^{2+}), nitrogen (N), phosphorus (P) and carbon (C) contents were determined after 22 days exposure to stress.

Results The harmful effect of alkali stress on the growth of wild barley was stronger than those of salt stress, irrespective of endophyte infection. Alkali stress had a greater impact on photosynthesis and chlorophyll content compared to salt stress and also accumulated more of the osmoprotectant glycine betaine. However, salt stress appeared to increase total antioxidant capacity as well as increasing K^+ content (resulting in a relative low Na^+/K^+ ratio). Under alkali stress, Ca^{2+} content sharply increased in roots as opposed to a decrease under salt stress. In roots, the C, N, P contents and the C:N ratio was higher under salt stress compared to alkali stress whereas the C:P and N:P ratios were lower. In shoots, the N and P contents were higher under salt stress compared to alkali stress whereas the C content and the C:P and C:N ratios were lower. Interestingly, the presence of *Epichloë* endophyte infection on wild barley under both salt stress and alkali stress led to significant amelioration of both stresses. *Epichloë* infection significantly increased photosynthesis, chlorophyll content, total antioxidant capacity and glycine betaine content, whilst lowering leaf malondialdehyde content. Furthermore, *Epichloë* infection reduced Na^+ content, the Na^+/K^+ ratio and shoot Ca^{2+} content but increased K^+ content and the root Ca^{2+} content. *Epichloë* infected plants also had higher C, N and P contents but lower ratios of C:N, C:P and N:P than uninfected plants.

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Conclusions The presence of the *Epichloë* endophyte suppresses the negative effect of salt stress and alkali stress on wild barley seedling growth. The possible mechanisms by which the presence of *Epichloë* endophyte enhances growth of plants exposed to those two stresses include improved photosynthetic ability, increased antioxidant potential, increased nutrient absorption, and osmotic and ionic adjustment. The study also found that alkali stress is more harmful to wild barley than salt stress.

Keywords *Epichloë* endophyte · *Hordeum brevisubulatum* · Salt stress · Alkali stress

Abbreviations

E +	endophyte-infected plant
E-	endophyte-free plant
SS	salt stress
AS	alkali stress
Na^+	sodium
K^+	potassium
Ca^{2+}	calcium
C	total organic carbon
N	total nitrogen
P	total phosphorus
ANOVA	analysis of variance
ROS	reactive oxygen species

Introduction

Soil salinity is a major constraint to crop production (Tavakkoli et al. 2011), and often occurs alongside alkali stress (Wang et al. 2011). Salinity affects approximately 20% of arable land and 50% of irrigated land worldwide (FAO 2005). Although soil salinity is more prevalent in arid and semi-arid regions, it is variable both spatially and temporally (Flowers and Yeo 1995). Soil remediation and specific agricultural practices can reduce the damaging effects of saline soil, but the cultivation of plant varieties resistant to saline soils is the best approach for agriculture in saline areas (Flowers and Yeo 1995).

Salt imposes several stresses upon plants. First, it changes the osmotic potential of the soil solution, which reduces the amount of water available to plants. Second, it induces osmotic stress and ion injury by disrupting the ion balance in plant cells (Munns 2002). Alkali stress imposes the same adverse stresses, but to a greater degree because of the high pH (Munns 2002; Yang et al. 2009). High pH can also destroy root membrane structures and strongly affects the intracellular ion balance in plant cells (Guo et al. 2009). Thus, plants growing in alkaline soil must cope with physiological drought and ion toxicity, maintain their intracellular ion balance, and regulate the pH outside the roots (Yang et al. 2008). Despite this, far fewer studies have focused on alkali stress than on salt stress.

Epichloë endophytes [Clavicipitaceae, Hypocreales, Ascomycota] are biotrophic fungi that form symbioses with temperate grasses in the Pooideae subfamily (Bayat et al. 2009; Saikkonen et al. 2013). Asexual *Epichloë* species live asymptomatically and intercellularly within the above-ground parts of the host, including the developing inflorescence and seeds (Christensen et al. 2008; Saikkonen et al. 2013). Asexual *Epichloë* symbionts are only vertically transmitted via the mother plant lineage (Saikkonen et al. 2002). *Epichloë* endophytes either produce a range of alkaloids and other secondary metabolites, leading to enhanced resistance to biotic and abiotic stresses including drought, waterlogging, salt, cold, heat, heavy metals, insects, nematodes and diseases (Schardl et al. 2004; Song et al. 2015a). *Epichloë* endophyte infection can also affects the growth of the host plant, as well as its N assimilation, resource allocation, and mineral uptake abilities (Hamilton et al. 2012; Malinowski and Belesky 2000; Pan and Clay 2002). Some studies have found that *Epichloë* endophytes play a key role in salt tolerance of host grasses (Reza and Mirlohi 2010; Rodriguez et al. 2008). The results of field studies have indicated that salinity and alkalinity affect the frequency of asexual *Epichloë* endophyte infection (Wang et al. 2005). However, there is little direct experimental evidence that *Epichloë* endophytes affect the tolerance of the host grass to alkali soils. Taking previous survey data and reports about the effect of *Epichloë* endophytes on host grasses exposed to salt stress into account, we hypothesized that: (1) infection by the *Epichloë* endophyte would enhance the tolerance of plants to salt and alkali stresses; and (2) the harmful effects of salt and alkali stresses on the growth of plants would differ in intensity. In this work, therefore, our objectives were three fold: (1) to confirm that the presence of the *Epichloë* endophyte in wild barley has beneficial effects when plants are exposed to salt and alkali stresses. (2) To understand the physiological mechanisms of *Epichloë*-mediated tolerance to salt and alkali stresses. (3) To determine

differences between the salt and the alkali stress responses in the host.

Wild barley (*Hordeum brevisubulatum*) is an important forage crop that is widely adaptable to a variety of stress conditions, including salt, alkali and water stresses. For this reason, wild barley is often used as a model plant in studies on salinity (Wang et al. 2016). A strictly asexual *Epichloë* endophyte commonly infect wild barley with infection frequencies ranging from 80% to 90% in the saline–alkali area of Linze County, Gansu Province, China (Song et al. 2015a). Phylogenetic analyses indicate that the *H. brevisubulatum* endophyte is *Epichloë bromicola* (Song et al. 2016).

In this study, two salt ($NaCl:Na_2SO_4$) and alkali ($NaHCO_3:Na_2CO_3$) combinations were used to mimic the salt components and characteristics of alkaline soil in northern China, where wild barley is widely distributed. Endophyte-infected (E+) and endophyte-free (E-) wild barley seedlings were treated with these salt and alkali stresses for 21 days, and then plant growth characteristics, photosynthetic and physiological parameters, and nutrient stoichiometry were analyzed. These parameters were also used to determine the differences between the effects of salt stress and alkali stress on wild barley.

Materials and methods

Plant material

Wild *H. brevisubulatum* plants with mature reproductive tillers were collected from the Linze Experimental Station of Lanzhou University, Gansu Province, China, in 2014. The endophyte infection status (E+ or E-) was monitored by staining and microscopic examination and by PCR using the following *Epichloë*-specific PCR primer pair: (tub2-exon 1d-1: GAGAAAATGCGTGA GATTGT, tub2-exon 4u-2: GTTTCGTCCGAGTT CTCGAC). Seeds from one E+ mature inflorescence were divided into two groups and one group was treated for 1.5 h with a 100-times dilution of thiophanate-methyl (Jiangsu Rotam Chemistry Co. Ltd., Jiangsu, China) to kill the *Epichloë* endophyte, and the other group was treated with water. The seeds were carefully rinsed with water to remove the fungicide. Treated and untreated seeds were planted separately in a greenhouse. Although the fungicide treatment killed some of the seeds, those that survived had outgrown the negative effects of the fungicide after about 3 weeks. In order to obtain sufficient seeds for experiments, plants were clipped to 5 cm above the soil surface to acquire enough tillers and irrigated with water and modified ½-strength Hoagland's nutrient solution as required. Seeds were collected from these E+ and E- tillers in 2015, and the endophyte infection status was monitored again by PCR using *Epichloë*-specific PCR primers. In this way, we acquired two populations of *H. brevisubulatum* seeds that differed only in the presence (E+) or absence (E-) of the *Epichloë* endophyte. During the experiments, plants were microscopically examined several times to confirm the presence or absence of the asexual *Epichloë* endophyte. All seeds were stored at 4 °C to maintain endophyte viability.

Experimental design

Seeds harvested from E+ and E- plants were sown in two seed-raising trays (1 tray for E+ and 1 tray for E-) filled with vermiculite that had been sterilized at 150 °C for 24 h. The seedlings were supplied with modified ½-strength Hoagland's nutrient solution and irrigated with water as required. After 14 days, seedlings were transplanted into sterilized vermiculite in pots (21 cm diameter × 16 cm high; 3 seedlings per pot). Each pot was placed in a plastic tray (28 cm diameter × 8 cm high), and all trays were supplied with the same volume of modified ½-strength Hoagland's nutrient solution. The nutrient solution was replaced every 7 days to maintain nutrient concentration.

After 35 days, seedlings were treated with ½-strength Hoagland's solution with or without salt (SS: molar ratio of $NaCl:Na_2SO_4 = 1:1$) or alkali (AS: molar ratio of $NaHCO_3:Na_2CO_3 = 1:1$). The E+ and E- seedlings were subjected to the following salt and alkali treatments: 0 mM (pH of SS: 7.02 ± 0.014; pH of AS: 6.98 ± 0.064), 100 mM (pH of SS: 7.56 ± 0.014; pH of AS: 10.03 ± 0.011), 200 mM (pH of SS: 7.48 ± 0.024; pH of AS: 9.97 ± 0.006), 300 mM (pH of SS:7.36 ± 0.068; pH of AS: 9.94 ± 0.010) and 400 mM (pH of SS: 7.28 ± 0.084; pH of AS: 9.90 ± 0.006). The experiment had a randomized complete block design with five replicates (pots) of E+ and E- seedlings. The seedlings receiving these salt and alkali treatments were treated with 50 mM salt or alkali on the first and second days to avoid salt and alkali shock. The final concentrations of salt and alkali in each treatment were applied from day 3 onwards. Every 3 days, the treatment solution was replaced to maintain consistent stress conditions, and the position of each pot was changed randomly. The growth

conditions in the greenhouse were as follows: 28 °C/25 °C (day/night), 16-h light/8-h dark photoperiod, with light supplied at about 800 μmol m^{-2} s^{-1} and 65% relative humidity.

Plant biomass and physiological indexes measurements

Photosynthetic parameters and chlorophyll content were measured before harvest (after 21 days of treatment). The photosynthetic parameters measured were transpiration rate, photosynthesis rate, stomatal conductance and intercellular carbon dioxide concentration. These parameters were measured using a Li-6400 portable photosynthesis system (Li-Cor Inc., Lincoln, NE, USA). The chamber was equipped with a red/blue LED light source (Li6400-02B) with photosynthetically active radiation set at 1500 μmol m^{-2} s^{-1}, air carbon dioxide concentration of 400 ± 10 μmol CO_2 mol^{-1}, and temperature at 28 ± 1 °C. Measurements were conducted between 9:00 and 11:00 AM. Chlorophyll content was measured with a chlorophyll meter (SPAD-502 Plus, Konica Minolta Sensing, Inc., Japan), as the average of five measurements on each leaf, taken near the site of photosynthesis measurements. The relative value for the chlorophyll concentration is the SPAD value.

All plants were harvested on day 22 after the start of the treatments to measure biomass and other parameters. All the shoots and roots were rinsed with water to remove vermiculite then thoroughly washed with distilled water to remove the salt and alkali. After harvest, shoot height and shoot tiller numbers were manually measured. Some of the leaf material was frozen in liquid nitrogen to measure total antioxidant capacity and malondialdehyde content. To determine dry weight, shoots and roots samples were oven-dried at 80 °C to a constant weight. Dried tissues were weighed and ground to obtain homogenous samples for cation and elemental analyses.

To determine the osmotic adjustment, glycine betaine, which always functions as an osmoprotectant (Ashraf and Foolad 2007) was measured using a kit method. Briefly, leaves from plants in each treatment were dried at 80 °C for 3 days and ground to pass through a 40-mesh sieve. Each finely ground dried sample (0.2 g) was shaken with 2 ml 80% methanol (*v*/v) at 60 °C for 30 min. The mixture was centrifuged at 10.000×g at 25 °C for 15 min, the glycine betaine concentration was then measured with a Reinecke Salt Kit (Comin Biotechnology, Co. Ltd., Suzhou, China) following the manufacturer's instructions. Finally, the absorbance was measured at 525 nm using a SP-723-type visible spectrophotometer (Spectrum Instruments, Co. Ltd., Shanghai, China). The glycine betaine concentration was calculated by comparison with a standard sample in the kit.

The total antioxidant capacity, which is of importance for detoxifying reactive oxygen species (ROS) produced under salinity stress (Boaretto et al. 2014; Gratão et al. 2005), was measured with a Fe^{3+}-TPTZ Kit (Comin Biotechnology, Co. Ltd., Suzhou, China) following the manufacturer's instructions. Briefly, 0.1 g fresh leaf tissue stored in liquid nitrogen was homogenized with 1 ml extraction buffer on ice. The mixture was centrifuged at 10,000×g at 4 °C for 10 min. A 30 μl aliquot of the supernatant was mixed with 900 μl of the kit solution and 90 μl double-distilled water, and then incubated at 37 °C for 10 min. Finally, the absorbance was measured at 593 nm using an SP-723-type visible spectrophotometer. The total antioxidant capacity was calculated in comparison with the blank control.

To determine the degree of oxidative stress, malondialdehyde, which is an indicator of membrane damage, was extracted and measured using the thiobarbituric acid method (Han et al. 2017; Wang et al. 2006). Briefly, 0.5 g fresh leaf tissue stored in liquid nitrogen was homogenized with 5 ml 5% trichloroacetic acid with a mortar and pestle. The ground material was centrifuged at 3000 rpm for 10 min. A 2 ml aliquot of the supernatant was mixed with 2 ml 0.67% thiobarbituric acid, heated at 100 °C for 30 min, cooled on ice, and then centrifuged at 3000 rpm for 10 min. The absorbance of the supernatant was measured at 450, 532 and 600 nm, and the malondialdehyde concentration was calculated using the following formula: $C = 6.45(A_{532} - A_{600}) - 0.56A_{450}$.

To quantify cations (Na^+, K^+ and Ca^{2+}) in plant tissues, dried shoots and roots were extracted in 100 mM acetic acid at 90 °C for 3 h. The extractant was filtered through filter paper, and then made up to 40 ml before determining the cation concentrations using a Sherwood 410 Flame Spectrophotometer (Keer Instrument Co. Ltd., Beijing, China). The cation concentrations were calculated by comparison with standard samples. The Na^+ and K^+ concentrations in the samples were then used to calculate the Na^+:K^+ ratio in the shoots and roots (Pan et al. 2016).

Total C content was determined using the oil bath–K_2CrO_7 titration method (oxidization with dichromate in the presence of H_2SO_4 by heating at 180 °C for 5 min,

followed by titration with $FeSO_4$) (Olsen 1954). To estimate the total N and total P contents in shoots and roots, dried samples were digested with H_2SO_4, K_2SO_4 and $CuSO_4$ in a heated block (Hanon Instruments, Co., Ltd., Jinan, China) at 420 °C for 35 min, and then the N and P concentrations were determined with a flow injection analyzer (FIAstar 5000 Analyzer, Foss, Denmark). The stoichiometric ratios of C, N and P in shoots and roots (C:N, C:P and N:P) were then calculated (Nanjing Agriculture University 1996).

Statistical analyses

Data were analyzed using SPSS statistical software (Ver. 19.0, SPSS, Inc., Chicago, IL, USA). Three-way analysis of variance (ANOVA) was used to determine the effects of endophyte (E), stress type (S) and stress level (L) on growth parameters, photosynthetic measurements, physiological indexes and stoichiometric ratios. Significant differences between E+ and E- plants under the same stress (SS or AS) were determined by independent *t*-test. Significant differences between salt stress (SS) and alkali stress (AS) were also determined by independent *t*-test. Statistical significance was defined at the 95% confidence level. Data shown in figures are mean ± standard error.

Results

Growth parameters

Comparisons of E+ and E- plants under salt stress and alkali stress demonstrated that the presence of the endophyte significantly positively influenced plant height, tiller numbers, shoot dry matter and root dry matter under stress treatment but not under the control (Fig. 1, Table 1). Both the salt stress and alkali stress negatively affected the growth of the plants, and to a greater extent under alkali stress than under salt stress, the tiller numbers, shoot dry matter and root dry matter were higher under salt stress than under alkali stress (Fig. 1, Table 1).

Photosynthesis

Both salt stress and alkali stress negatively affected the transpiration rate, photosynthesis rate, stomatal conductance and intercellular carbon dioxide concentration of the *H. brevisubulatum* plants (Fig. 2, Tables 1 and 2). The presence of the endophyte significantly increased the photosynthesis ability of *H. brevisubulatum* seedlings under both salt and alkali treatments compared with plants without this fungus (Fig. 2, Tables 1 and 2). The transpiration rate, photosynthesis rate, stomatal conductance and intercellular carbon dioxide concentration was higher in the E+ plants than in the E- plants in the 100, 200, 300 and 400 mM salt and alkali stress treatments, while the E+ and E- plants showed no significant differences under the control conditions (Fig. 2). Irrespective of endophyte infection, plants under salt stress had significant higher transpiration rate, photosynthesis rate and stomatal conductance than plants under alkali stress, however, the intercellular carbon dioxide concentration was significantly higher under alkali stress (Fig. 2).

Physiological indexes

Chlorophyll content was significantly higher in E+ than in E- plants in the 200, 300, 400 mM salt stress treatments and 300, 400 mM alkali conditions (Fig. 3a). Within the measured range of salt stress and alkali stress, E+ plants had higher total antioxidant capacity than E- plants. The total antioxidant capacity of E- plants was 36.3% lower in the 400 mM salt treatment than in the 300 mM salt treatment, whereas the total antioxidant capacity of E+ plants increased with increasing stress levels. The total antioxidant capacity of E+ plants were 2.04-fold and 1.53-fold higher than that of E- plants in the 400 mM salt stress treatment and 400 mM alkali stress treatment, respectively. There were significant differences in total antioxidant capacity between the E+ and E- plants in the 200, 300 and 400 mM salt treatments, and in the 100 and 400 mM alkali treatments (Fig. 3b). The presence of the endophyte significantly increased the glycine betaine content in the 200 mM alkali treatment, and in the 300 and 400 mM salt and alkali treatments (Fig. 3c). Endophyte infection reduced the malondialdehyde concentration under various stress conditions, the malondialdehyde content was significantly higher in E- than in E+ plants in the 300, 400 mM salt treatments and 400 mM alkali treatment (Fig. 3d). Comparing the plants under salt treatment and alkali treatment, the chlorophyll content and total antioxidant capacity of plants under salt stress were significantly higher than that of plants under alkali treatment (Fig. 3a, b), but glycine betaine content and malondialdehyde content showed a different pattern, being significantly higher under alkali treatment than under salt stress (Fig. 3c, d).

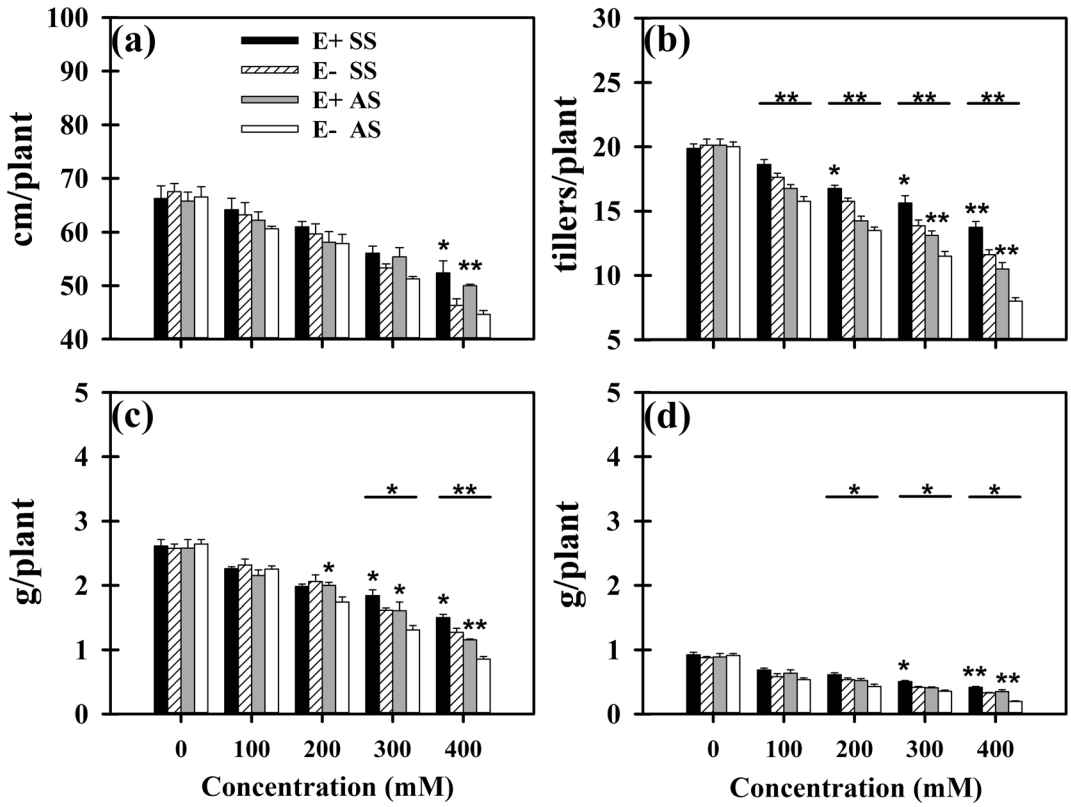

Fig. 1 Morphological characteristics of *Hordeum brevisubulatum* with (E+) and without (E-) endophyte under salt stress (SS) and alkali stress (AS). (**a**) Plant height, (**b**) tiller numbers, (**c**) shoot biomass, and (**d**) root biomass. Values are mean ± standard error (SE). * and ** on top of bars indicate significant difference at $P < 0.05$ and $P < 0.01$, respectively (independent *t*-test), between E+ and E- plants under the same stress type (SS or AS). * and ** above line means significant difference at $P < 0.05$ and $P < 0.01$, respectively (independent *t*-test), between plants under salt stress (SS) and alkali stress (AS)

Na^+, K^+, Ca^{2+} content and Na^+:K^+ ratio

The results showed that in the shoots and roots of *H. brevisubulatum* seedlings Na^+ content increased while K^+ content declined as the stress concentrations increased. However, the presence of the endophyte alleviated this change, especially under the high stress conditions (Fig. 4). In all salt and alkali stress treatments, E+ plants had significantly lower Na^+ content in shoots than E- plants (Fig. 4a). For roots, the Na^+

Table 1 Three-way ANOVA for the effects of endophyte (E), stress type (S) and stress level (L) on plant height, tiller numbers, shoot dry weight, root dry weight, transpiration rate and photosynthesis rate of *Hordeum brevisubulatum*

Variable	Plant height	Tiller numbers	Shoot dry weight	Root dry weight	Transpiration rate	Photosynthesis rate
E	7.85 **	45.35 ***	9.70 **	34.42 ***	87.89 ***	62.03 ***
S	5.80 *	135.92 ***	26.11***	23.51 ***	128.89 ***	171.98 ***
L	76.70 ***	321.33 ***	207.44 ***	218.84 ***	100.49 ***	165.55 ***
E × S	0.01 ns	0.05 ns	1.01 ns	0.03 ns	1.00 ns	4.45 *
E × L	2.53 *	5.38 ***	4.27 **	1.94 ns	4.29 **	6.27 ***
S × L	0.17 ns	11.19 ***	4.14 **	1.96 ns	9.94 ***	15.70 ***
E × S × L	0.10 ns	0.14 ns	1.22 ns	0.71 ns	0.36 ns	1.69 ns

The numeric data in the table is F-value

*,**,*** and ns represent significant at $p \leq 5\%$, 1%, 0.1% levels and not significant, respectively

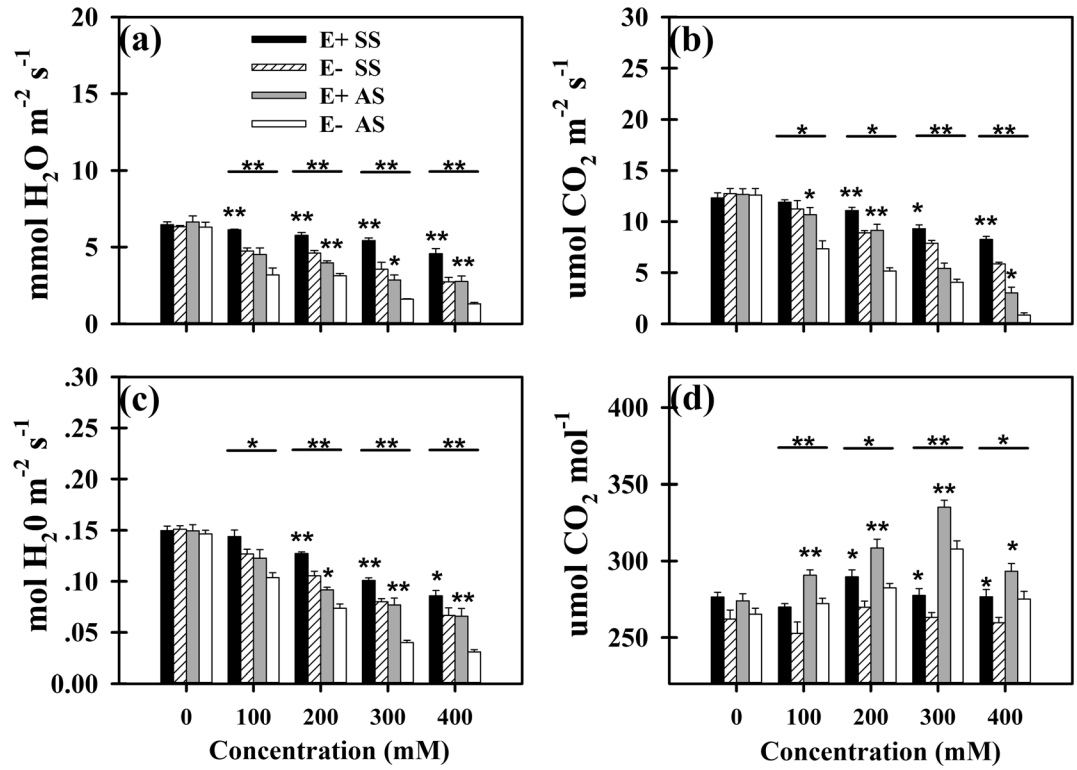

Fig. 2 Photosynthetic characteristics of *Hordeum brevisubulatum* with (E+) and without (E-) endophyte under salt stress (SS) and alkali stress (AS). (**a**) Transpiration rate, (**b**) photosynthesis rate, (**c**) stomatal conductance, and (**d**) intercellular carbon dioxide concentration. Values are mean ± standard error (SE). * and ** on top of bars indicate significant difference at $P < 0.05$ and $P < 0.01$, respectively (independent *t*-test), between E+ and E- plants under the same stress type (SS or AS). * and ** above line means significant difference at $P < 0.05$ and $P < 0.01$, respectively (independent *t*-test), between plants under salt stress (SS) and alkali stress (AS)

content of E+ plants was significantly lower than of E- plants at the higher stress levels (Fig. 4b). The E+ plants had significantly higher K^+ content in shoots and roots in the 300 and 400 mM salt and alkali treatments (Fig. 4c, d). Decrease of Ca^{2+} content was observed in the shoots as the stress concentrations increased. However, the presence of the endophyte strengthened this change, especially under higher

Table 2 Three-way ANOVA for the effects of endophyte (E), stress type (S) and stress level (L) on stomatal conductance, intercellular carbon dioxide concentration, chlorophyll content, total antioxidant capacity, glycine betaine content and malondialdehyde (MDA) content of *Hordeum brevisubulatum*

Variable	Stomatal conductance	Intercellular CO_2 concentration	Chlorophyll	Total antioxidant capacity	Glycine betaine	Malondialdehyde
E	73.29 ***	80.37 ***	30.64 ***	74.38 ***	45.29 ***	21.29 ***
S	113.62 ***	104.34 ***	59.15 ***	137.47 ***	99.66 ***	71.96 ***
L	206.62 ***	24.88 ***	108.31 ***	35.24 ***	719.58 ***	131.30 ***
E × S	2.40 ns	0.64 ns	0.58 ns	10.68 **	0.67 ns	0.06 ns
E × L	5.01 **	0.90 ns	1.58 ns	13.40 ***	4.50 **	3.69 **
S × L	6.58 ***	16.90 ***	5.56 ***	11.42 ***	13.02 ***	4.43 **
E × S × L	0.80 ns	0.59 ns	0.27 ns	2.83 *	0.25 ns	0.20 ns

The numeric data in the table is F-value

*,**,*** and ns represent significant at $p \leq 5\%$, 1%, 0.1% levels and not significant, respectively

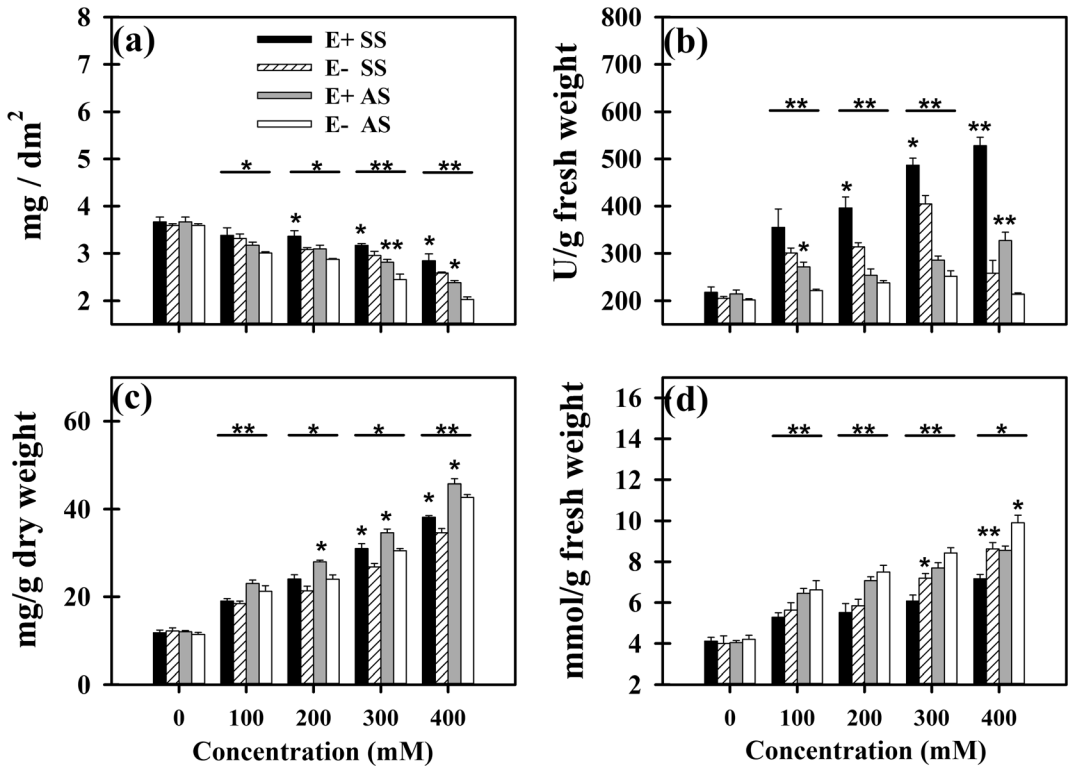

Fig. 3 Physiological indexes of *Hordeum brevisubulatum* with (E+) and without (E-) endophyte under salt stress (SS) and alkali stress (AS). (**a**) Chlorophyll content, (**b**) total antioxidant capacity, (**c**) glycine betaine content, and (**d**) malondialdehyde content. Values are mean ± standard error (SE). * and ** on top of bars indicate significant difference at $P < 0.05$ and $P < 0.01$, respectively (independent *t*-test), between E+ and E- plants under the same stress type (SS or AS). * and ** above line means significant difference at $P < 0.05$ and $P < 0.01$, respectively (independent *t*-test), between plants under salt stress (SS) and alkali stress (AS). Chlorophyll content is expressed as SPAD units

stress conditions. The E+ plants had significantly lower Ca^{2+} content in shoots than E- plants in the 200, 300 and 400 mM treatments (Fig. 4e). The Ca^{2+} content of roots under alkali stress showed a different pattern to under salt stress being significantly increased in the 200 (E+) and 300 mM (E+ and E-) alkali stress treatments, however, it was decreased as the salt stress concentration increased. Endophyte infection alleviated the decrease under salt stress but strengthened the increase under alkali stress, except in the control and 100 mM salt and alkali stress treatments where the E+ plants had significantly higher root Ca^{2+} content than E- plants (Fig. 4f). An increase in the Na^+:K^+ ratio was observed in the shoots and roots as the stress concentrations increased. The E+ plants had a significantly lower Na^+:K^+ ratio than E- plants when exposed to salt and alkali stress treatments (Fig. 5). Irrespective of endophyte infection, the plants under salt stress had significantly higher K^+ content and significantly lower Na^+ content, Ca^{2+} content and Na^+:K^+ ratio in shoots and roots than plants under alkali treatment (Figs. 4 and 5).

Carbon, nitrogen and phosphorus contents

The C content in shoots and roots was higher in E+ than in E- plants (Fig. 6a, b), but salt or alkali conditions did not have a significant main effect on C content in shoots ($P = 0.625$) (Table 3). The N and P contents under stress were higher in E+ than in E- plants both in shoots and roots, but there was no difference between E+ and E- plants in the control. In addition, the N and P contents were higher in shoots than in roots (Fig. 6c-f). Comparing the plants under salt treatment and alkali treatment, the C content in shoots was significant higher in plants under alkali treatment in the 300 and 400 mM treatments, but C content in roots was higher under salt stress. The N and P contents were higher in plants under salt stress

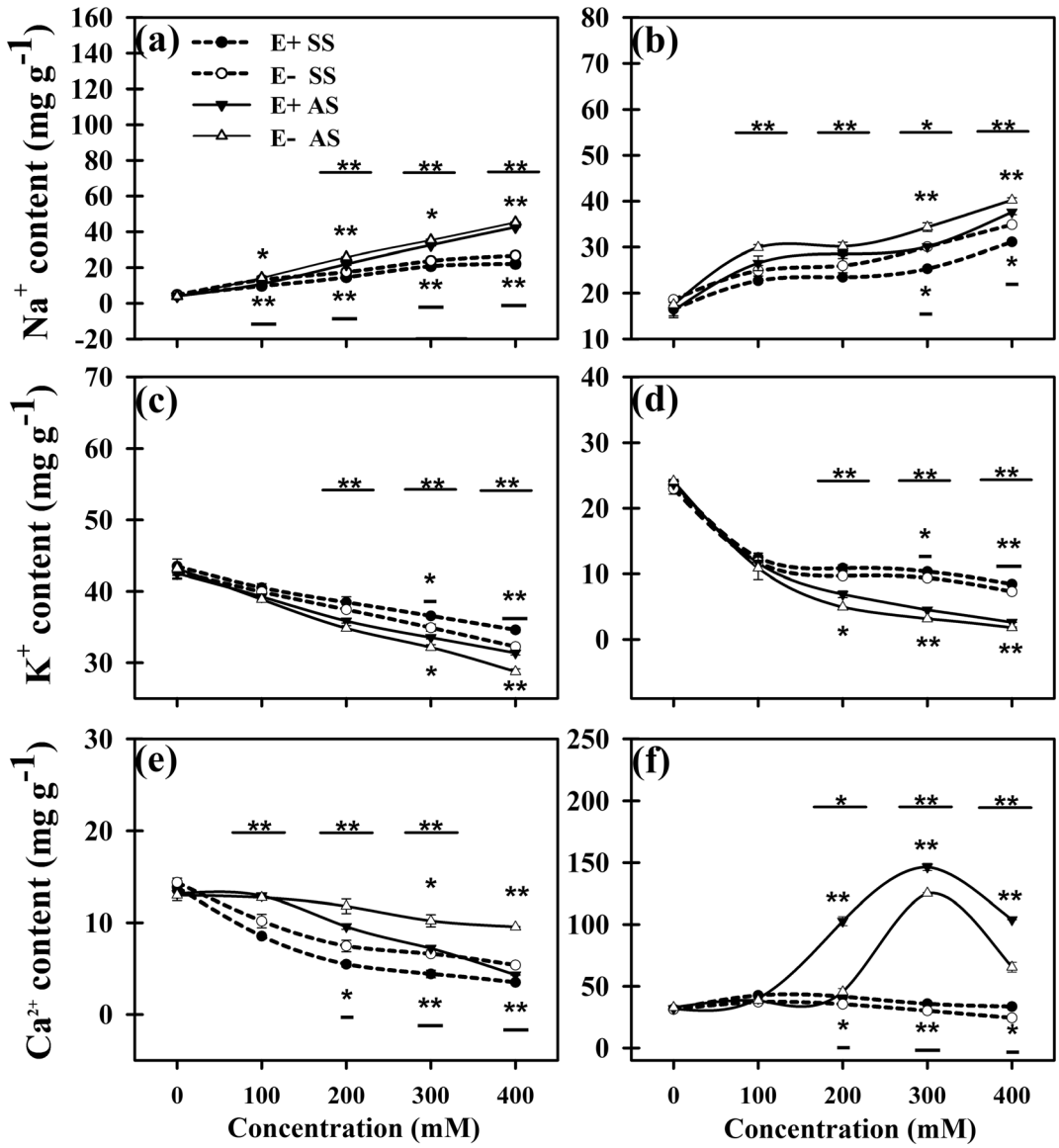

Fig. 4 Na^+, K^+, Ca^{2+} content in shoots and roots of *Hordeum brevisubulatum* with (E+) and without (E-) endophyte under salt stress (SS) and alkali stress (AS). (**a**) Shoot Na^+ content, (**b**) root Na^+ content, (**c**) shoot K^+ content, (**d**) root K^+ content, (e) shoot Ca^{2+} content, (f) root Ca^{2+} content. Values are mean ± standard error (SE). Asterisks above relative short line (**, *) indicate significant difference ($P < 0.01$ and $P < 0.05$, respectively; independent *t*-test) between E+ and E- plants under salt stress (SS). Asterisks without underline (**,*) indicate significant difference ($P < 0.01$ and $P < 0.05$, respectively; independent *t*-test) between E+ and E- plants under alkali stress (AS). * and ** above relative long line means significant difference at $P < 0.05$ and $P < 0.01$, respectively (independent *t*-test), between plants under salt stress (SS) and alkali stress (AS)

than in plants under alkali stress both in shoots and roots (Fig. 6).

C:N:P stoichiometry

Endophyte infection did not have a significant main effect on the C:N ratio in shoots ($P = 0.114$) (Table 4), yet, under higher salt and alkali stress conditions, lower C:N ratio were found in E+ than in E- plants and significant difference were present at 400 mM for shoots and roots, and 200 mM under alkali stress for roots and 300 mM under salt stress for roots. The C:P ratio were lower in E+ than in E- plants under salt and alkali stresses, and the difference between the E+ and E-

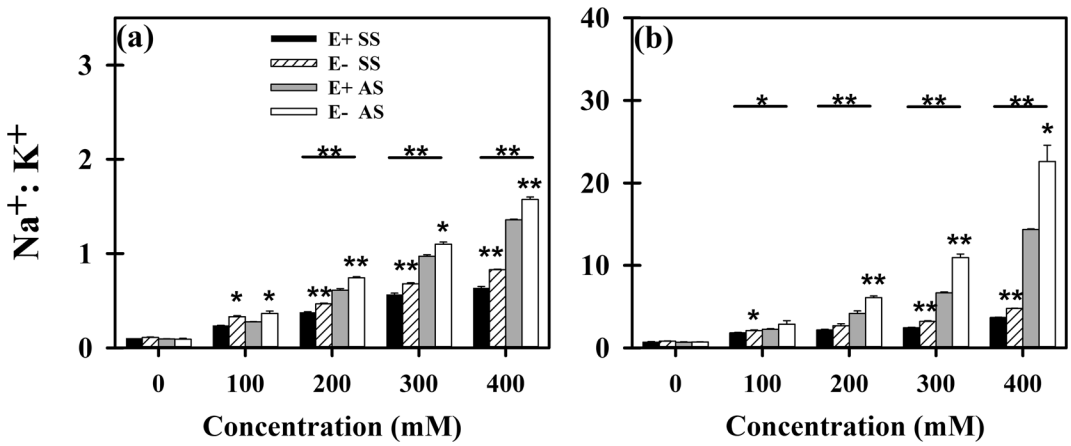

Fig. 5 $Na^+:K^+$ ratio in shoots and roots of *Hordeum brevisubulatum* with (E+) and without (E-) endophyte under salt stress (SS) and alkali stress (AS). (**a**) Shoot $Na^+:K^+$ ratio and (**b**) root $Na^+:K^+$ ratio. Values are mean ± standard error (SE). * and ** indicate significant difference at $P < 0.05$ and $P < 0.01$, respectively (independent *t*-test), between E+ and E- plants under the same stress type (SS or AS). * and ** above line means significant difference at $P < 0.05$ and $P < 0.01$, respectively (independent *t*-test), between plants under salt stress (SS) and alkali stress (AS)

plants, in both shoots and roots, became significant as the stress concentrations increased (Fig. 7c, d). The N:P ratio in the shoots showed a similar pattern to the C:P ratio under salt and alkali stress, which was significantly higher in E- plants than in E+ plants in salt and alkali treatments (Fig. 7e). In the roots, the differences in the N:P ratio was not significantly different between E+ and E- plants in the various treatments (Fig. 7f). Irrespective of endophyte infection, the plants under alkali stress had significant higher C:N ratio than plants under salt treatment for shoots. In roots, the plants under salt stress had significantly higher C:N ratio than under alkali stress in the 100 and 400 mM treatments. The C:P ratio in the shoots and roots showed a similar pattern with N:P ratio in the roots, which were significantly higher under alkali stress than under salt stress. In terms of N:P ratio in roots, the difference between plants under salt and alkali stress was only found in 400 mM treatment (Fig. 7).

Discussion

This study has contributed significant new data on the interaction between *Epichloë* endophytes and their host plants under abiotic stress. The key findings were that alkali stress is more harmful than salt stress to the growth and photosynthesis of wild barley (*H. brevisubulatum*) and that *Epichloë* infection can ameliorate both of these.

Effects of *Epichloë* endophyte infection on resistance of *Hordeum brevisubulatum* to salt and alkali stresses

The impact of *Epichloë* infection to increase host resistance to biotic and abiotic stresses has been demonstrated in many studies (Saikkonen et al. 2004; Song et al. 2015b; Xia et al. 2015). In the present study we demonstrated that *Epichloë* infected *H. brevisubulatum* consistently outperformed uninfected plants only under stress, which is consistent with other studies demonstrating that *Epichloë* positively affects plants under biotic and abiotic stress conditions (Saikkonen et al. 2004; Saikkonen et al. 2006; Song et al. 2015b; Xia et al. 2015; Zhang et al. 2010). However, some studies have shown that *Epichloë* can confer benefits generally to their host, even under non-stress conditions (Rudgers et al. 2005). The impact of *Epichloë* infection to increase resistance to NaCl stress has been demonstrated in many studies (Reza and Mirlohi 2010; Rodriguez et al. 2008; Song et al. 2015b). However, this paper is the first study demonstrating the effect of *Epichloë* infection to increase resistance to alkali stress. In addition, most studies have focused on a single physiological stress (i.e., NaCl) and very little is known about how *Epichloë* infection impacts multiple salt (NaCl and Na_2SO_4) or alkali ($NaHCO_3$ and Na_2CO_3) treatments. Since NaCl, Na_2SO_4, $NaHCO_3$ and Na_2CO_3 are the main harmful salts in many inland areas of China (Kawanabe and Zhu 1991), our results have both theoretical and practical significance.

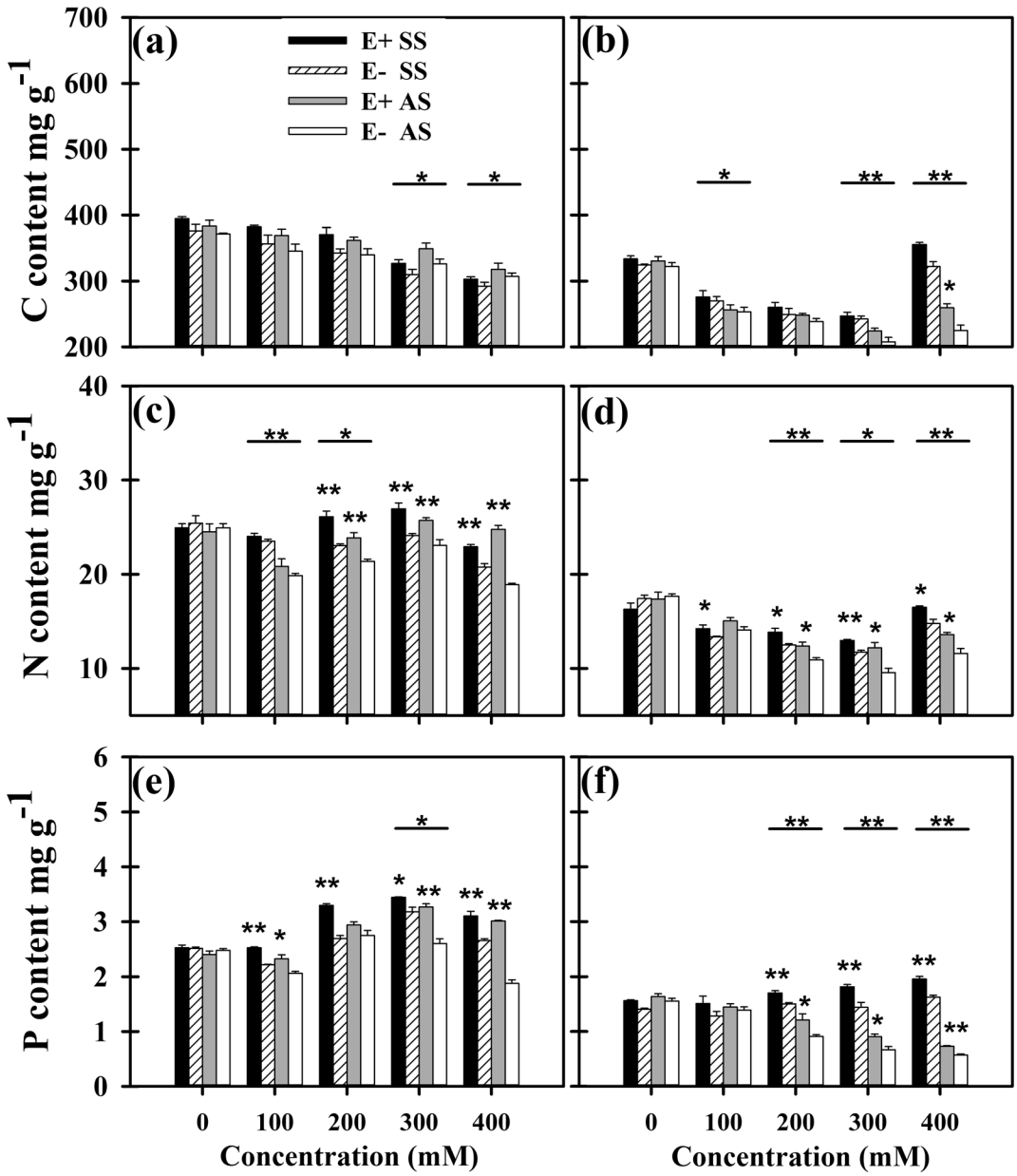

Fig. 6 Carbon, nitrogen and phosphorus contents in shoots and roots of *Hordeum brevisubulatum* with (E+) and without (E-) endophyte under salt stress (SS) and alkali stress (AS). (**a**) Shoot C content, (**b**) root C content, (**c**) shoot N content, (**d**) root N content, (**e**) shoot P content, (**f**) root P content. Values are mean ± standard error (SE), with bars indicating SE. Asterisks (**,*) on top of bars indicate significant difference ($P < 0.01$ and $P < 0.05$, respectively; independent *t*-test) between E+ and E- plants under the same stress type (SS or AS). * and ** above line means significant difference at $P < 0.05$ and $P < 0.01$, respectively (independent *t*-test), between plants under salt stress (SS) and alkali stress (AS)

Several mechanisms have been proposed to explain the finding that *Epichloë* infection can ameliorate the effect of salt and alkali on plants. One relates to photosynthesis, which is an important indicator of physiological sensitivity to abiotic stress (Chaves et al. 2009), that is significantly decreased under stress, leading to decreased carbon assimilation by plants and ultimately lower crop yield (Liu et al. 2015) and biomass (Xia et al. 2015). It has been reported that *Epichloë* endophyte-infected plants have increased net photosynthetic rates, higher intercellular carbon dioxide concentration, higher stomatal conductance and greater transpiration rates than

Table 3 Three-way ANOVA for the effects of endophyte (E), stress type (S) and stress level (L) on Na^+, K^+, Ca^{2+} content, ratio of Na^+:K^+ and C content in shoots and roots of *Hordeum brevisubulatum*

Variable	Na^+ content		K^+ content		Ca^{2+} content		Na^+: K^+		C content	
	shoot	root	shoot	root	shoot	root	shoot	root	shoot	root
E	326.54 ***	56.10 ***	20.77 ***	14.28 ***	93.58 ***	319.95 ***	314.40 ***	71.13 ***	29.68 ***	23.30 ***
S	2870.56 ***	97.78 ***	72.64 ***	181.59 ***	169.63 ***	2332.60 ***	2324.05 ***	491.46 ***	0.24 ns	124.00 ***
L	5316.88 ***	256.37 ***	263.69 ***	756.86 ***	223.13 ***	503.54 ***	3699.62 ***	302.74 ***	59.36 ***	144.41 ***
E × S	1.12 ns	0.31 ns	0.45 ns	0.01 ns	0.98 ns	127.69 ***	1.56 ns	33.57 ***	0.06 ns	0.09 ns
E × L	20.56 ***	1.63 ns	3.05 *	1.02 ns	10.57 ***	55.73 ***	29.94 ***	15.45 ***	0.62 ns	3.33 *
S × L	621.16 ***	9.08 ***	5.63 ***	30.35 ***	23.50 ***	568.21 ***	533.94 ***	151.07 ***	3.35 *	35.33 ***
E × S × L	2.58 ns	0.31 ns	0.35 ns	0.43 ns	5.24 **	39.78 ***	0.59 ns	9.79 ***	0.11 ns	0.24 ns

The numeric data in the table is F-value

*,**,*** and ns represent significant at $p \leq 5\%$, 1%, 0.1% levels and not significant, respectively

endophyte-free plants under biotic or abiotic stress (Amalric et al. 1999; Rozpadek et al. 2015; Xia et al. 2015). Results from the present study (Fig. 2, Tables 1 and 2) support these findings. *Epichloë* infected *H. brevisubulatum* plants had increased stomatal conductance and transpiration rates resulting in increased gas exchange capacity and increased intercellular carbon dioxide concentration, both of which lead to improved net photosynthesis. An alternative or additional explanation is that the respiration of the infecting *Epichloë* fungus could also be increasing under high stress, increasing the amount of CO_2 available for photosynthesis. This interesting finding warrants further study.

Reactive Oxygen Species (ROS) have also been proposed as a mechanism to enhance salt stress and alkali stress tolerance of *Epichloë* infected *H. brevisubulatum* through ROS scavenging systems and osmotic adjustment. Osmotic stress and excessive Na^+ affect functions of salinity-stressed plants, leading to the generation of ROS such as O_2^- (Kar 2011; Guo et al. 2015). ROS produced under salinity stress may act as a signal to trigger defense responses via transduction pathways (Miller et al. 2010) but at higher concentrations can damage plant cells. Consequently, ROS-scavenging systems involving non-enzymatic and enzymatic antioxidants work together to detoxify ROS (Boaretto et al. 2014; Gratão et al. 2005). Non-enzymatic antioxidants include flavonoids, alkaloids, phenolic compounds, tocopherol and carotenoids, which donate electrons to the glutathione-ascorbate cycle in which oxidized glutathione is regenerated into reduced glutathione (Gratão et al. 2005). Enzymatic antioxidants include superoxide dismutase, ascorbate peroxidase, catalase and peroxidase (Gallego et al. 2012; Gratão et al. 2005). Studies on tall

Table 4 Three-way ANOVA for the effects of endophyte (E), stress type (S) and stress level (L) on N and P contents, and ratios of C:N, C:P and N:P in shoots and roots of *Hordeum brevisubulatum*

Variable	N content		P content		C:N		C:P		N:P	
	shoot	root	shoot	root	shoot	root	shoot	root	shoot	root
E	84.37 ***	36.54 ***	236.20 ***	61.44 ***	2.19 ns	15.87 ***	34.72 ***	45.12 ***	55.04 ***	10.38 **
S	42.38 ***	26.63 ***	96.39 ***	313.57 ***	31.37 ***	5.35 *	47.86 ***	324.86 ***	1.91 ns	277.43 ***
L	39.86 ***	116.43 ***	146.05 ***	20.83 ***	29.00 ***	10.23 ***	126.76 ***	66.21 ***	47.14 ***	37.63 ***
E × S	2.87 ns	2.29 ns	4.87 *	2.83 ns	1.05 ns	0.28 ns	5.59 *	3.70 ns	0.12 ns	0.36 ns
E × L	14.03 ***	7.48 ***	28.96 ***	1.73 ns	4.85 **	3.79 **	14.44 ***	3.83 **	6.02 ***	0.43 ns
S × L	8.06 ***	16.73 ***	7.49 ***	80.39 ***	2.75 *	7.32 ***	8.79 ***	98.87 ***	8.09 ***	63.36 ***
E × S × L	3.20 *	0.46 ns	15.24 ***	0.88 ns	0.49 ns	0.28 ns	8.19 ***	2.08 ns	1.19 ns	0.79 ns

The numeric data in the table is F-value

*,**,*** and ns represent significant at $p \leq 5\%$, 1%, 0.1% levels and not significant, respectively

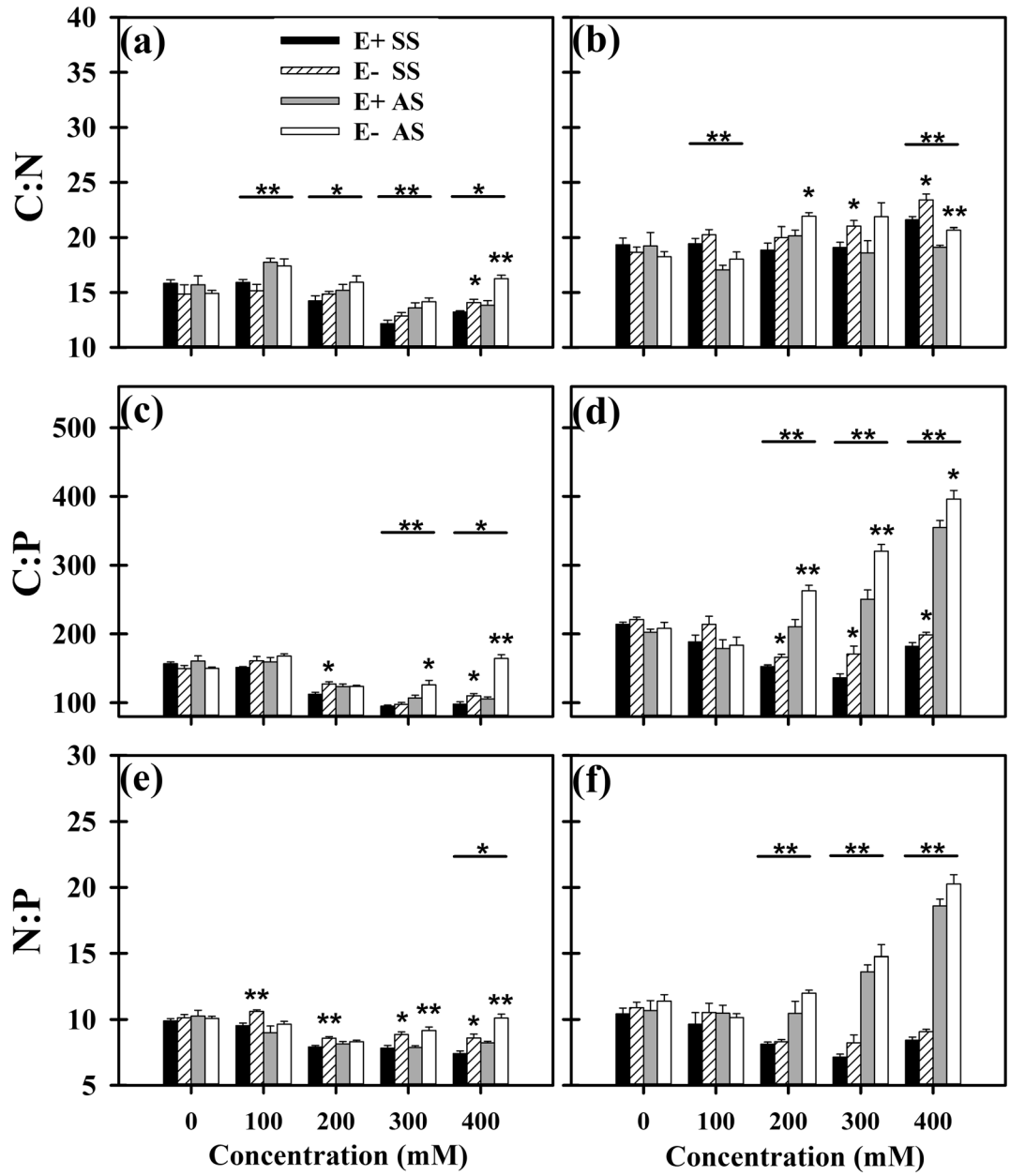

Fig. 7 Stoichiometric ratios of carbon, nitrogen, and phosphorus in *Hordeum brevisubulatum* with (E+) and without (E-) endophyte under salt stress (SS) and alkali stress (AS). (**a**) Shoot C:N, (**b**) root C:N, (**c**) shoot C:P, (**d**) root C:P, (**e**) shoot N:P, (**f**) root N:P. Values are mean ± standard error (SE), with bars indicating SE. Asterisks (**,*) on top of bars indicate significant difference ($P < 0.01$ and $P < 0.05$, respectively; independent *t*-test) between E+ and E- plants under the same stress type (SS or AS). * and ** above line means significant difference at $P < 0.05$ and $P < 0.01$, respectively (independent *t*-test), between plants under salt stress (SS) and alkali stress (AS)

fescue, fine fescue, drunken horse grass and perennial ryegrass suggested that *Epichloë* endophyte infection increased the production of enzymatic and non-enzymatic antioxidants including flavonoids and other phenolic compounds (Hamilton et al. 2012; Saikkonen et al. 2013; Torres et al. 2009; Zhang et al. 2010). We have also shown increased production of the total antioxidant capacity in *Epichloë* infected *H. brevisubulatum* plants and as such benefits their host plants under stress. Salinity leads to hyperosmotic stress by increasing

intracellular osmolality, and plants can counteract this through the uptake or synthesis of organic osmolytes (Shabala and Shabala 2011) such as glycine betaine (Yang et al. 2003). In this study, glycine betaine content was higher in *Epichloë* infected plants than in uninfected plants and likely mitigates the damaging effects of oxidative stress by activating or stabilizing ROS-scavenging enzymes and/or by repressing ROS production (Chen and Murata 2008). The higher malondialdehyde content, a product of membrane lipid peroxidation (Yazici et al. 2007) and a physiological indicator of stress tolerance in plants (Luna et al. 2000), of uninfected *H. brevisubulatum* plants under salt and alkali stresses, compared to infected plants in this study, suggests a positive role of the endophyte in the host plant response to salt and alkali stresses.

Accumulation of inorganic ions such as Na^+, K^+ and Cl^- is another mechanism for osmotic adjustment in plants (Shabala and Shabala 2011). Our results demonstrated that *Epichloë* endophyte infection significantly affected Na^+, K^+ and Ca^{2+} absorption and the Na^+:K^+ ratio of *H. brevisubulatum* under salt stress and alkali stress. This has significant implications for the host since excess Na^+ in the cytosol damages plant cells by inhibiting enzymes, disrupting K^+ acquisition, inhibiting K^+-dependent metabolic processes, and causing oxidative stress (Flowers et al. 2015; Pan et al. 2016; Zhu 2001). The accumulation of K^+ in plant cells under stress is also important for stomatal conductance (Bayat et al. 2009), and decreased K^+ concentrations may hamper the normal plant activities (Wyn et al. 1979). Maintaining constant intracellular K^+ and Na^+ balance is essential for metabolic processes in cells and is crucial for plant adaptation to saline environments (Zhu 2003). Our results support earlier work in which *Epichloë* endophyte infection was shown to decrease Na^+ and increase K^+ concentrations of plants under NaCl stress (Reza and Mirlohi 2010; Song et al. 2015b). In higher plants, Ca^{2+} is a key signaling component in the SOS (salt overly sensitive) system under salinity stress (Zhu 2003) and is essential for selective ion transport mechanisms and for the maintenance of K^+ influx and Na^+/K^+ selectivity (Epstein 1961; Hare and Cress 1997). Bayat et al. (2009) found that *Epichloë* endophyte increased the K^+ and Ca^{2+} contents in *F. arundinacea* under drought stress. In our study, *Epichloë* endophyte infection increased Ca^{2+} uptake but decreased Ca^{2+} transport from roots to shoots under stress. The change in Ca^{2+} concentration in response to endophyte infection under stress may be related to mechanisms controlling signal transduction and ion balance. However, to fully understand the influence of Ca^{2+} on stress tolerance, further detailed studies are needed.

C, N and P are essential elements (Chen et al. 2010; Vrede et al. 2004), and their dynamics are important in aquatic and terrestrial ecosystems. Several studies have suggested a mechanistic linkage between tissue elemental stoichiometry and the growth rate of the organism (Kyle et al. 2003). The growth rate hypothesis (GRH) proposes that higher growth rates are associated with lower C:N, C:P and N:P ratios (Hessen et al. 2007). Our study showed that *Epichloë* endophyte infected plants had lower C:N and C:P ratios which could partly explain the higher biomass of E+ plants under salt stress and alkali stress. The N:P ratio in plants is an important index to identify limiting nutrients (Koerselman and Meuleman 1996). The results of the present study indicated that the growth of E- plants under salt stress and alkali stress was more limited by P, which is required to meet the protein synthesis demands under higher growth rates (Hessen et al. 2007). However, it is possible that the lower N:P ratio in E+ plants was because of increased synthesis of N-containing antioxidants.

Taken together, we can conclude that the effect of *Epichloë* on *H. brevisubulatum* under salt and alkali stresses may be multifaceted, involving photosynthesis, ion balance, nutrient stoichiometry and physiological processes. The presence of the endophyte in shoots can increase nutrient acquisition, and possibly increase photosynthate allocation to the roots. Our results partly explained the enhanced growth of endophyte infected *H. brevisubulatum* under salt stress and alkali stress.

Comparative effects of salt-stress and alkali-stress of *Hordeum brevisubulatum*

In addition to lower water potential and ion toxicity, the high pH under alkali stress would lead to a lack of protons and the destruction or inhibition of transmembrane electrochemical potential gradients in cells (Guo et al. 2015). These changes would ultimately damage root structure and functions such as ion absorption (Fig. 4), the contents of C, N and P (Figs. 6 and 7), and the membrane system (Fig. 3d), leading to severe reductions in photosynthesis (Fig. 2). Furthermore, higher amounts of energy and nutrients must be consumed under alkali stress, which could explain the greater growth inhibition under alkali stress than under salt stress. Thus it is not

surprising that the plants growing in the presence of elevated levels of Na^+ and in high pH (alkali) will perform more poorly than plants growing in the same levels of Na^+ but in neutral pH conditions (salt).

The intercellular carbon dioxide concentration was only slightly affected by salt stress, and increased with increasing stress intensity under alkali stress (Fig. 2d), similar to the responses of *H. vulgare* to alkali and salt stresses reported previously by Yang et al. (2009). However, the results of studies on the effects of alkali stress and salt stress on photosynthesis have often been inconclusive. Guo et al. (2015) reported that *Triticum aestivum* showed higher transpiration rate, photosynthesis rate, stomatal conductance and intercellular carbon dioxide concentration under salt stress than under alkali stress. The stomatal conductance is correlated with changes in water potential, and reductions in stomatal conductance and transpiration rate might be a response to decreased water potential (Koyro 2006), the leaf water potential decreased with increasing stress levels under both salt stress and alkali stress (Shabala and Shabala 2011). The stomatal conductance and transpiration rate of wild barley decreased to a greater extent under alkali stress than under salt stress, therefore, we conclude the high pH under alkali stress decreased water absorption capacity of roots, affected stomatal movement and gas exchange and also resulted in a reduction of stomatal conductance and transpiration rate in *H. brevisubulatum* leaves. A reduction in photosynthesis rate under stress results from either a decrease in intercellular carbon dioxide concentration induced by stomatal closure, or from non-stomatal factors such as changes in biochemical constituents of the leaf, the cumulative effects of changes in leaf water and osmotic potential, and changes in photosynthetic pigment content (Koyro 2006). Our results showed that intercellular carbon dioxide concentration was significantly higher under alkali stress treatments, which indicated that the reduced photosynthesis rate under salt stress and alkali stress may have not resulted from a decrease in intercellular carbon dioxide concentration, but might have resulted from photosynthetic pigment damage, decreased stomatal conductance, and ion toxicity in the cytosol. Therefore, the depression of photosynthesis in wild barley may be due to the combined effects of decreased stomatal conductance and decreased transpiration rate. Such reductions in photosynthetic parameters have been shown to reduce salt loading into the leaves, which extends their longevity by maintaining salts at subtoxic levels for longer than would occur if the transpiration rate and stomatal conductance were not decreased (Koyro 2006). We suggest this may represent an adaptive mechanism to cope with excessive salt stress and alkali stress, rather than being an indicator of stress. Glycine betaine has been shown to accumulate in response to dehydration and salinity stress in many crop plants (Tsutsumi et al. 2015; Yang et al. 2003), and it was able to protect the photosynthetic machinery against salinity (Hassine et al. 2008). The relationship between glycine betaine accumulation and stress tolerance is species- or even genotype-specific (Jones et al. 1984; Varshney et al. 1988; Tsutsumi et al. 2015; Yang et al. 2003). Our results showed that the glycine betaine content increased to a greater extent under alkali stress than under salt stress (Fig. 3c). An alternative explanation is that the increased glycine betaine in the cytoplasm of *H. brevisubulatum* cells may participate in osmotic adjustment under higher salt stress and alkali stress to balance osmotic pressure from the vacuoles, more glycine betaine accumulated under alkali stress than under salt stress implied that the high pH under alkali stress enhanced glycine betaine synthesis to maintain the intracellular ion balance, prevent dehydration and protect biomacromolecules.

Na^+/K^+ ratio is an important feature of salt-tolerant plants (Flowers and Colmer 2008; Shabala and Cuin 2008). Our results support earlier work conducted by Yang et al. (2009) on *H. vulgare* in which that the Na^+/K^+ ratio increased slowly under salt stress, but increased sharply under alkali stress. N and P contents in *H. brevisubulatum* were significantly higher under salt stress than under alkali stress (Fig. 6), supporting the growth rate hypothesis of Sterner and Elser (2002), which proposes that plants with rapid growth rates absorb more nutrients. The differences in P concentration among organisms are driven by differences in allocation to P-rich ribosomal RNA (rRNA) to meet the protein synthesis demands of increased growth rates (Hessen et al. 2007). N is an essential component of antioxidants (Khan et al. 2014). In *H. brevisubulatum*, more ROS-scavenging antioxidants were produced under salt stress than under alkali stress (Fig. 3b). It is possible that the high pH under alkali stress may have interfered with the control of phosphatase secretion and the P-uptake capacity of roots. On the other hand, stomatal closure was greater under alkali stress than under salt stress (Fig. 2c), decreasing transpiration and so decreasing CO_2 assimilation (Flexas et al. 2004). Additionally, the high pH under alkali stress might have severely damaged the structure and functions of the roots including their nutrient absorption capacity. The growth rate hypothesis

proposes that higher growth rates are coupled to lower C:N, C:P and N:P ratios. In this study, compared with seedlings in the alkali stress treatment, those in the salt stress treatment had lower shoot C:N, shoot C:P, root C:P and root N:P ratios (Fig. 7), consistent with the growth rate hypothesis. There was no difference in shoot N:P ratio between salt stress and alkali stress, but the seedlings in the salt stress treatment had higher root C:N ratio (Fig. 7b), contrary to expectations that these ratios would be lower in plants with a higher growth rate (Elser et al. 2000). However, it is possible that the lower root C:N ratio (Fig. 7b) under alkali stress was due to increased synthesis of N-containing metabolites which may be an adaptive strategy to alkali environment.

Conclusions

The presence of the asexual *Epichloë* endophyte suppresses the negative effect of salt stress and alkali stress on wild barley seedling growth. The possible mechanisms by which the presence of *Epichloë* endophyte enhances growth of plants exposed to those two stresses include improved photosynthetic ability, increased antioxidant potential, increased nutrient absorption, and osmotic and ionic adjustment. The findings of this study also confirmed that the effect of salt stress and alkali stress are different in intensity and the growth factors and physiological indicators were more adversely affected in the plants exposed to the alkali stress than those exposed to the salt stress. The results have enhanced knowledge of the application of endophytes that will enable new cultivar breeding for stress tolerance and ecological conservation under conditions of salt and alkali.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflicts of interest.

Human and animal studies The article does not contain any studies with human participants or animals performed by any of the authors.

Informed consent This study did not involve human participants, and so informed consent was not required.

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