

# Impacts of space mutation and endophyte on the drought and salt stress tolerance of perennial ryegrass

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## Research Article

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# Abstract

**Aims** This study revealed whether endophyte or space mutation have effects on the resistance of perennial ryegrass to drought and salt stress and whether space mutation have impacts on endophyte function.

**Methods** Growth performance, phytohormone contents, stoichiometry of C, N and P, and Na<sup>+</sup> and K<sup>+</sup> ion transport of endophyte-infected (E+) and endophyte-free (E-) plants, including space mutation and wild type plants, were evaluated under four different treatments (control, drought, salt, and drought combined with salt stress).

**Results** The results showed that stress treatments significantly inhibited ( $P<0.05$ ) the growth of perennial ryegrass and significantly changed ( $P<0.05$ ) SA contents, significantly increased ( $P<0.05$ ) ion transport from underground to aboveground and changed stoichiometry of C, N and P. Endophytes only significantly improved ( $P<0.05$ ) host growth in wild type plants, and had different effects on CTK, GA and SA contents. Endophytes had little effects on regulating the rational distribution of ions and stoichiometry of C, N and P in both mutant and wild type plants, and its role was inconsistent. Space mutation significantly improved ( $P<0.05$ ) host growth under some stress, significantly reduced ( $P<0.05$ ) IAA, ABA, GA and SA contents, Na<sup>+</sup>/K<sup>+</sup> had different effects on stoichiometry of C, N and P which depends on treatments.

**Conclusions** Plants regulate stoichiometry of C, N and P and ion transport in response to environmental changes. Either endophyte and space mutation had beneficial effects on the stress tolerance of plant only under some treatments. Space mutation did not affect endophyte function.

# Introduction

Perennial ryegrass (*Lolium perenne*) is a globally important forage and turf grass species owing to its desirable agronomic performance in temperate climates. Extensive studies have confirmed that the *Epichloë festucae* var. *lolii* endophyte is an essential component of perennial ryegrass cultivation systems owing to its capacity to enhance agronomic performance (Latch et al. 1984; Johnson et al. 2013). *E. festucae* var. *lolii* mutualistically interact with perennial ryegrass because it provides major fitness enhancements and protection against both biotic and abiotic stresses (Johnson et al. 2013; Young et al. 2013; Ross 2016). Especially about stress tolerance, there were many reports about endophytes have increased host tolerance to drought and salt stress (Wang et al., 2020; Cheplick et al., 2000; Malinowski et al., 2000). Furthermore, these results indicated that the complicated interaction between endophyte and host were also influenced by genotype, environment and some other factors.

Most of the commercial perennial ryegrass cultivars widely used in China are imported from overseas. Several approaches to breed new perennial ryegrass varieties with domestic adaptive traits and promote seed production have been implemented. Space mutation has been applied to plant breeding in the past 30 years in China and a number of new cultivars or selections of rice, wheat, maize, green pepper, and watermelon were developed using this method (Liu et al. 2007; Paran et al. 2007). To overcome some of the limitations of traditional introgression and selective breeding, some endophyte-infected (E+) and endophyte-free (E-) perennial ryegrass seeds were treated with space mutation. Mutant germplasm resources were established, some growth parameters of individual plants were measured, and some plants were selected for further evaluation and utilisation. In order to further select individuals with strong stress tolerance, it is necessary to further evaluate their performance and physiology response under different stress conditions. As these mutant germplasms included endophyte associations, the effects of space mutation on function of endophyte in perennial ryegrass and their interactions were also need clarify. Hopefully, this clarification will help us to understand the effects of these two different breeding methods including endophyte and space mutation and make clear how to utilize them.

Thus, in the present study, the further evaluation of the stress resistance of the E+ and E- plants was conducted. The objectives of the present study were to 1) reveal whether endophyte or space mutation have effects on host stress resistance, 2) identify whether space mutations have impacts on endophyte function, and 3) understand the physiological factors of the underlying stress tolerance of perennial ryegrass for the breeding of stress-tolerant cultivars.

## Materials And Methods

### Plants materials

Seeds of perennial ryegrass were supplied by Lanzhou University and were screened for infection in 2014 and 2015 at the Yuzhong Experimental Station of Lanzhou University (104°39' E, 35°89' N, Altitude 1653 m), Gansu Province, China (Chen et al. 2020 a,b). The seeds from endophyte-infected (E+) and endophyte-free (E-) sub-populations were marked and stored at 4°C. Two hundred seeds per E+ and E- population were carried using “Shenzhou11” spaceship in October, 2016. These seeds were marked as space mutation (SPE+ and SPE-) and stored at 4°C. In April 16, 2017, these space mutation seeds and wild type seeds without space mutation (UE+ and UE-) were planted in experimental field blocks at the Yuzhong Experimental Station of Lanzhou University. The growth and morphology of all the plants were evaluated, and the seeds were harvested and stored at 4°C.

### Experimental design

In May 2019, the well filled, healthy-looking E+ and E- seeds harvested from SPE+, SPE-, UE+ and UE- were planted in plastic trays (30 cm × 25 cm × 8 cm) filled with 1.5 kg soil (commercial fine sandy soil, Lanzhou) that had been sterilized in an oven at 130 °C for 30 min. Five rows with 10 seeds were planted per tray at a depth of 1 cm. Four trays per seed lot were prepared and placed in a temperature-controlled greenhouse (18°C - 24°C) with 10 h of illumination per day on the Yuzhong campus of Lanzhou University. After the plants produced four tillers, the endophyte viability in each seedling was determined using microscopic examination of the host leaf sheath pieces after they had been stained with aniline blue (Nan 1996). The seedlings germinated from E+ seeds with longitudinally-orientated hyphae of the endophyte were marked as E+ and the seedlings germinated from E- seeds without hyphae were marked as E-. The marked E+ or E- seedlings were transplanted into round pots (upper diameter - 13 cm × lower diameter - 10 cm × height - 11 cm) containing the same amount of media (sterilised commercial vermiculite and black soil with a w/w ratio 1:3). Each pot had only one seedling and an equal initial water treatment. After one month of stabilisation with same irrigation, four different treatments were established. The pots were weighed and watered to maintain the appropriate relatively soil moisture content (RSMC), and they were irrigated with the same volume of 250 mmol NaCl before treatment to keep the conditions as following: CK (45% RSMC), drought stress treatment (D, 15% RSMC), salt stress treatment (S, 45% RSMC with 250 mmol NaCl), and drought combined with salt treatment (DS, 15% RSMC with 250 mmol NaCl). Each treatment had eight replicates for SPE+, SPE-, UE+, and UE-, and they were randomly placed in a greenhouse that is maintained at a constant condition (temperature: 25 ± 2 °C, humidity: 42 ± 5%). After another month of growth, the plants were destructively harvested for evaluation.

### Experimental evaluations

After 28 days of growth, 2 g of fresh leaves were collected from each plant for gibberellin (GA<sub>3</sub>), indole-3-acetic acid (IAA), cytokinins (CTK), salicylic acid (SA), and abscisic acid (ABA) contents test using enzyme-linked immunosorbent assay (Danshi biology, Shanghai, China).

After 28 days of growth, plant height and tiller number of each plant were recorded. The whole plants were then carefully removed from the pots, washed with distilled water, and dried on filter paper. All harvested plants were separated into roots and shoots, and their fresh weights were recorded. Dry weight was obtained after oven-drying the

tissue at 60°C until a constant weight was reached. After weighing, the plant materials were ground twice using a mixer mill (Retch 400MM, German) to analyse for ion contents and nutrient elements.

Na<sup>+</sup>, K<sup>+</sup>, and Ca<sup>2+</sup> ion contents were analysed using atomic absorption spectrometry (M6AA system, Thermo, USA) after mineralisation in a mixture of acids (Hanway and Heidel 1952). Based on these results, the ratios of Na<sup>+</sup>/ K<sup>+</sup> and S<sub>K,Na</sub> were calculated as follows:  $S_{K,Na} = (\text{aboveground K}^+ / \text{Na}^+) / (\text{underground K}^+ / \text{Na}^+)$  (Flowers and Yeo 1988).

Carbon (TC) content was determined using the K<sub>2</sub>CrO<sub>7</sub> oxidation method (Tanveer et al. 2014). Total nitrogen (TN) and total phosphorus (TP) contents were determined following digestion with sulfuric acid (H<sub>2</sub>SO<sub>4</sub>) at a temperature of 420°C, and the concentrations in the digested solutions were determined using flow injection analysis using a FIAstar 5000 Analyser (FOSS Analytical, Denmark; Xia et al. 2018).

## Statistical analyses

Statistical data analysis was performed using SPSS, Inc. (Released 2009. PASW Statistics for Windows, version 25.0. Chicago: SPSS Inc). Means were reported with their standard errors. Univariate analysis of general linear models was employed to estimate the effects of single factors and their interactions on indices of perennial ryegrass in the present study (Supplementary Table S1). Based on these effects, significant differences between single factors (stress treatment, endophyte and mutation) were assessed using the least significant difference (LSD) test at  $P < 0.05$ , generated from one-way analysis of variance (ANOVA) based on separated dataset. Statistical significance was set at 95% confidence level.

## Results

### Plant growth

Stress treatments significantly inhibited ( $P < 0.05$ ) the plant heights of SPE-, UE+, and UE- (Fig. 1A). Endophyte only had significant positive effects ( $P < 0.05$ ) on the height of wild type plants under control. Mutation treatment had no effect on plant height.

Stress treatments had significant inhibitory effects ( $P < 0.05$ ) on tiller numbers (Fig. 1B). For mutant plants, the tiller numbers of E+ plants were significantly higher ( $P < 0.05$ ) than those of E- plants under drought stress; for wild type plants, the tiller numbers of E+ plants were significantly higher ( $P < 0.05$ ) than those of E- plants under these four treatments. Mutation treatment had significant effects on tiller numbers; mutant plants had significantly more tiller numbers ( $P < 0.05$ ) than wild type plants under drought and salt stresses.

Stress treatments had significant inhibitory effects ( $P < 0.05$ ) on plant biomass (Fig. 2). For mutant plants, the aboveground biomass of E+ plants was significantly lower ( $P < 0.05$ ) than that of E- plants under salt stress; underground biomass of E+ plants was significantly lower ( $P < 0.05$ ) than that of E- plants under control. For wild type plants, the aboveground biomass of E+ plants was significantly higher ( $P < 0.05$ ) than that of E- plants under salt stress and drought with salt stress. Mutation treatment had significant effects on underground biomass; mutant plants had significantly more underground biomass ( $P < 0.05$ ) than wild type plants under drought and salt stresses.

### Phytohormones

Both stress treatments and endophytes had no significant effect on IAA and ABA contents (Table 1 and Table 2). Mutation treatment had significant effects on IAA and ABA contents. Mutant plants had significantly lower IAA content ( $P < 0.05$ ) than wild type plants under drought stress and drought with salt stress. However, mutant plants had significantly lower ABA contents ( $P < 0.05$ ) than wild type plants under control and salt stress.

Table 1  
IAA contents of different plants under different treatments ( $\mu\text{mol/g}$ )

	CK	D	S	DS
<b>SPE+</b>	0.1070 $\pm$ 0.0035a	0.1023 $\pm$ 0.0125a	0.1088 $\pm$ 0.0184a	0.1215 $\pm$ 0.0182a
<b>SPE-</b>	0.1076 $\pm$ 0.0070a	0.1110 $\pm$ 0.0179a	0.1025 $\pm$ 0.0078a	0.1076 $\pm$ 0.0156a
<b>UE+</b>	0.1328 $\pm$ 0.0138a	0.1296 $\pm$ 0.0184a	0.1300 $\pm$ 0.0095a	0.1340 $\pm$ 0.0205a
<b>UE-</b>	0.1216 $\pm$ 0.0189a	0.1379 $\pm$ 0.0091a	0.1301 $\pm$ 0.0131a	0.1206 $\pm$ 0.0102a
		*		*

Note: SPE+ is endophyte-infected (E+) space mutant plants; SPE- is endophyte-free (E-) space mutant plants; UE+ is wild type E+ plants; UE- is wild type E- plants. CK, control treatment; D, drought stress treatment; S, salt stress treatment; DS, drought with salt stress treatment. Value are mean $\pm$ standard errors (n=8). Lower case letters compare the same plant under different treatments; asterisk (\*) on the corresponding right column indicates significant ( $P < 0.05$ ) difference between SPE+ and SPE- plants or UE+ and UE- under the same treatment; asterisk (\*) at the corresponding bottom line indicates significant ( $P < 0.05$ ) difference between space mutant plants and wild type plants under the same treatment. Similarly, for the following tables.

Table 2  
ABA contents of different plants under different treatments (ng/g)

	CK	D	S	DS
<b>SPE+</b>	250.9573 $\pm$ 9.7602a	255.7750 $\pm$ 10.9165a	180.0618 $\pm$ 10.4126a	244.0913 $\pm$ 6.9820a
<b>SPE-</b>	220.9874 $\pm$ 10.1011a	234.8816 $\pm$ 9.6489a	253.9608 $\pm$ 10.5770a	236.0461 $\pm$ 7.5880a
<b>UE+</b>	382.1603 $\pm$ 9.6322a	303.3523 $\pm$ 11.3902a	320.4423 $\pm$ 10.7538a	262.3333 $\pm$ 8.0732a
<b>UE-</b>	321.5470 $\pm$ 12.5662a	355.4404 $\pm$ 12.5107a	299.7063 $\pm$ 8.9676a	290.6521 $\pm$ 11.9967a
	*		*	

Stress treatments and mutations had no significant effects on CTK content; however, the CTK content in UE- plants under salt stress was significantly higher ( $P < 0.05$ ) than that in the other three treatments (Table 3). For wild type plants, the CTK content in E+ plants was significantly lower ( $P < 0.05$ ) than that in E- plants under salt stress; however, that in E+ plants was significantly higher ( $P < 0.05$ ) than that in E- plants under drought with salt stress.

Table 3  
CTK contents of different plants under different treatments (ng/g)

	CK	D	S	DS
<b>SPE+</b>	212.9478 $\pm$ 8.4511a	209.9735 $\pm$ 8.7595a	237.3190 $\pm$ 7.8143a	226.1775 $\pm$ 9.6290a
<b>SPE-</b>	208.6571 $\pm$ 7.0949a	167.5465 $\pm$ 8.2164a	205.7379 $\pm$ 9.8508a	217.4881 $\pm$ 9.2511a
<b>UE+</b>	208.1583 $\pm$ 4.0061a	218.4908 $\pm$ 3.3992a	207.2505 $\pm$ 3.8942a	* 226.9398 $\pm$ 5.8704a *
<b>UE-</b>	203.6920 $\pm$ 5.5422b	202.5411 $\pm$ 4.3526b	229.5993 $\pm$ 3.5950a	188.8349 $\pm$ 4.9269b

Stress treatments had different effects on GA content (Table 4). Salt stress and drought with salt stress treatments significantly increased ( $P<0.05$ ) the GA content in SPE-, and drought stress and drought with salt stress treatments significantly reduced ( $P<0.05$ ) the GA contents in UE+. However, the three stress treatments significantly increased ( $P<0.05$ ) the GA content in UE-. For mutant plants, the GA contents in E+ plants was significantly higher ( $P<0.05$ ) than that in E- plants under control. For wild type plants, the GA content in E+ plants was significantly higher ( $P<0.05$ ) than that in E- plants under control; however, that in E+ plants was significantly lower ( $P<0.05$ ) than that in E- plants under drought stress and drought with salt stress. Mutation treatment had significant effects on GA content; mutant plants had significantly lower GA contents ( $P<0.05$ ) than wild type plants under control, drought stress, and salt stress.

Table 4  
GA contents of different plants under different treatments (ng/g)

	CK		D		S		DS
<b>SPE+</b>	169.4732±4.6129a	*	173.3297±6.4820a		150.0513±4.0086a		170.4556±5.8386a
<b>SPE-</b>	135.1193±2.1167b		141.7357±4.5575b		185.0565±6.5959a		187.5610±4.3187a
<b>UE+</b>	256.8241±6.8590a	*	175.7738±4.5292b	*	234.1401±2.9267a		151.0450±4.4011b
<b>UE-</b>	185.4488±4.0993b		213.8393±3.8096a		219.5878±4.1464a		219.3913±3.2128a
	*		*		*		

Stress treatments had different effects on SA content (Table 5). Salt stress and drought with salt stress significantly reduced ( $P<0.05$ ) the SA content in SPE-, and drought and drought with salt stress significantly reduced ( $P<0.05$ ) the SA contents in UE+. However, drought stress significantly improved ( $P<0.05$ ) the SA content in UE-. For mutant plants, the SA content in E+ plants was significantly higher ( $P<0.05$ ) than that in E- plants under salt and drought stress. For wild type plants, the SA content in E+ plants was significantly higher ( $P<0.05$ ) than that in E- plants under control and salt stress conditions. However, that in E+ plants was significantly lower ( $P<0.05$ ) than that in E- plants under drought stress. Mutation treatment had significant effects on SA content, mutant plants had significantly lower SA contents ( $P<0.05$ ) than wild type plants under control, drought, and drought with salt stress.

Table 5  
SA contents of different plants under different treatments (µg/g)

	CK		D		S		DS
<b>SPE+</b>	44.3238±2.9377a		44.1920±1.5626a	*	47.8488±6.5260a	*	42.9746±9.2800a
<b>SPE-</b>	42.9753±1.3518a		41.8161±1.3181a		38.0270±1.4630b		37.0010±4.5899b
<b>UE+</b>	53.4005±7.0468a	*	41.0515±5.2912c	*	50.5940±1.4162ab	*	46.3788±4.2903bc
<b>UE-</b>	46.3510±3.4617b		53.9558±2.3493a		41.4178±8.8112b		46.2239±4.5437b
	*		*				*

## Ion contents

Stress treatments significantly promoted ( $P<0.05$ ) aboveground Na<sup>+</sup> content (Fig. 3). For mutant plants, the aboveground Na<sup>+</sup> content in E+ plants was significantly lower ( $P<0.05$ ) than that in E- plants under control. For wild type

plants, the aboveground  $\text{Na}^+$  content in E+ plants was significantly lower ( $P<0.05$ ) than that in E- plants under salt stress, however, aboveground  $\text{Na}^+$  content in E+ plants was significantly higher ( $P<0.05$ ) than that in E- plants under drought with salt stress. The underground  $\text{Na}^+$  content in E+ plants was significantly lower ( $P<0.05$ ) than that in E- plants under control, whereas the aboveground  $\text{Na}^+$  content in E+ plants was significantly higher ( $P<0.05$ ) than that in E- plants under drought with salt stress (Fig. 3B). Mutation treatment only had significant effects on aboveground  $\text{Na}^+$  contents. The aboveground  $\text{Na}^+$  content of mutant plants was significantly lower ( $P<0.05$ ) than that in wild type plants under control and drought stress, however, that of mutant plants was significantly higher ( $P<0.05$ ) than that in wild type plants under salt stress.

Stress treatments had different effects on aboveground and underground  $\text{K}^+$  contents (Fig. 4). Stress treatments had significant inhibitory effects ( $P<0.05$ ) on the aboveground  $\text{K}^+$  contents in UE+ and UE- (Fig. 4A) and significant inhibition effects ( $P<0.05$ ) on the underground  $\text{K}^+$  content in SPE+, SPE-, and UE- (Fig. 4B). For mutant plants, the underground  $\text{K}^+$  content in E+ plants was significantly higher ( $P<0.05$ ) than that in E- plants under drought stress. For wild type plants, the aboveground  $\text{K}^+$  content in E+ plants was significantly higher ( $P<0.05$ ) than that in E- plants under drought stress and drought with salt stress. Mutation treatment had significant effects on  $\text{K}^+$  contents. The aboveground  $\text{K}^+$  content in mutant plants was significantly higher ( $P<0.05$ ) than that in wild type plants under control and salt stress. The underground  $\text{K}^+$  content in mutant plants was significantly higher ( $P<0.05$ ) than that in wild type plants under control.

Stress treatments had different effects on aboveground and underground  $\text{Ca}^{2+}$  contents (Fig. 5). Stress treatments significantly increased ( $P<0.05$ ) the aboveground  $\text{Ca}^{2+}$  contents in SPE+ and SPE-, but had (Fig. 5A) significant inhibitory effects ( $P<0.05$ ) on the aboveground  $\text{Ca}^{2+}$  content in UE+. Drought stress treatment significantly increased ( $P<0.05$ ) the underground  $\text{Ca}^{2+}$  content in UE- (Fig. 5B). For mutant plants, the aboveground  $\text{Ca}^{2+}$  content of E+ plants was significantly lower ( $P<0.05$ ) than that in E- plants under control and salt stress conditions. The underground  $\text{Ca}^{2+}$  content in E+ plants was significantly higher ( $P<0.05$ ) than that in E- plants under the three stress treatments. For wild type plants, both the aboveground and underground  $\text{Ca}^{2+}$  contents in E+ plants were significantly higher ( $P<0.05$ ) than those in E- plants under control. Mutation treatment had significant effects on  $\text{Ca}^{2+}$  content. The aboveground  $\text{Ca}^{2+}$  content in mutant plants was significantly lower ( $P<0.05$ ) than that in wild type plants under control; however, that in mutant plants was significantly higher ( $P<0.05$ ) than that in wild type plants under salt stress. The underground  $\text{Ca}^{2+}$  content in mutant plants was significantly higher ( $P<0.05$ ) than that in wild type plants under control and drought with salt stress.

Salt and drought with salt stress treatments significantly improved ( $P<0.05$ ) the aboveground ratio of  $\text{Na}^+/\text{K}^+$  (Fig. 6A). Only salt stress significantly improved ( $P<0.05$ ) the underground ratio of  $\text{Na}^+/\text{K}^+$  in SPE+ (Fig. 6B). For mutant plants, the aboveground ratio of  $\text{Na}^+/\text{K}^+$  in E+ plants was significantly lower ( $P<0.05$ ) than that in E- plants under control. For mutant plants, the aboveground ratio of  $\text{Na}^+/\text{K}^+$  in E+ plants was significantly lower ( $P<0.05$ ) than that in E- plants under control. For wild type plants, the aboveground ratio of  $\text{Na}^+/\text{K}^+$  in E+ plants was significantly lower ( $P<0.05$ ) than that in E- plants under salt stress, and the underground ratio of  $\text{Na}^+/\text{K}^+$  in E+ plants was significantly lower ( $P<0.05$ ) than that in E- plants under control. Mutation treatment had significant effects on both aboveground and underground ratio of  $\text{Na}^+/\text{K}^+$  because the aboveground and underground ratio of  $\text{Na}^+/\text{K}^+$  in mutant plants were significantly lower ( $P<0.05$ ) than those in wild type plants under control and drought stress.

Stress treatments significantly improved ( $P<0.05$ )  $S_{\text{K,Na}}$  (Fig. 7). For wild type plants,  $S_{\text{K,Na}}$  in E+ plants was significantly higher ( $P<0.05$ ) than that in E- plants under control. Mutation treatment had no significant effect on  $S_{\text{K,Na}}$ .

## Elements

Stress treatments had significant inhibitory effects ( $P<0.05$ ) on aboveground C content (Fig. 8A). Stress treatments significantly reduced ( $P<0.05$ ) the underground C content in SPE+ and UE-. Drought stress treatments significantly reduced ( $P<0.05$ ) the underground C content in SPE-, whereas salt stress improved that in SPE- (Fig. 8B). For mutant plants, the aboveground C content in E+ plants was significantly lower ( $P<0.05$ ) than that in E- plants under drought with salt stress. The underground C content in E+ plants was significantly higher ( $P<0.05$ ) than that in E- plants under control, drought, and drought with salt stress, whereas that in E+ plants was significantly lower ( $P<0.05$ ) than that in E- plants under salt stress. For wild type plants, the aboveground C content in E+ plants was significantly lower ( $P<0.05$ ) than that in E- plants under control, drought stress, and drought with salt stress. The underground C content in E+ plants was significantly higher ( $P<0.05$ ) than that in E- plants under drought stress. Mutation treatment had significant effects on underground C contents. Underground C content in mutant plants was significantly higher ( $P<0.05$ ) than that in wild type plants under control, salt stress, and drought with salt stress.

Stress treatments significantly increased ( $P<0.05$ ) the aboveground N content in SPE-, UE+, and UE- (Fig. 9A). Stress treatments significantly improved ( $P<0.05$ ) the underground N content in SPE+ and SPE-, whereas salt stress and drought with salt stress significantly reduced ( $P<0.05$ ) the underground N content in UE+ (Fig. 9B). For mutant plants, E+ plants had significantly lower ( $P<0.05$ ) underground N content than E- plants under control. For wild type plants, E+ plants had significantly higher ( $P<0.05$ ) aboveground N content than E- plants under drought stress and significantly higher ( $P<0.05$ ) underground N content than E- plants under control. However, E+ plants had significantly lower ( $P<0.05$ ) underground N contents than E- plants under salt stress. Mutation treatment had significant effects on aboveground and underground N content. The aboveground N content in mutant plants was significantly higher ( $P<0.05$ ) than that in wild type plants under salt and drought with salt stress conditions. The underground N contents in mutant plants was significantly lower ( $P<0.05$ ) than that in wild type plants under control and drought stress; however, that in mutant plants was significantly higher ( $P<0.05$ ) than that in wild type plants under salt and drought with salt stress.

Stress treatments significantly inhibited ( $P<0.05$ ) on aboveground P content (Fig. 10A). Stress treatments significantly improved ( $P<0.05$ ) the underground P content in SPE+ and UE- (Fig. 10B). For mutant plants, E+ plants had significantly higher ( $P<0.05$ ) aboveground and underground P contents than E- plants under salt stress. However, E+ plants had significantly lower ( $P<0.05$ ) underground P content than E- plants under drought stress. For wild type plants, E+ plants had significantly lower ( $P<0.05$ ) aboveground P content under drought with salt stress and significantly lower ( $P<0.05$ ) underground P content under drought stress. Mutation treatment had significant effects on the aboveground and underground P content. Mutant plants had significantly lower ( $P<0.05$ ) aboveground P content than wild type plants under salt and drought stress; however, they had significantly higher ( $P<0.05$ ) underground P content than wild type plants under salt stress.

Stress treatments significantly reduced ( $P<0.05$ ) aboveground C/N (Fig. 11A) and underground C/N in SPE+ and SPE-, respectively, but improved the underground C/N in UE+ (Fig. 11B). For mutant plants, the underground C/N in E+ plants was significantly higher ( $P<0.05$ ) than that in E- plants under control and drought stress whereas that of E+ plants was significantly lower ( $P<0.05$ ) than that of E- plants under salt stress. For wild type plants, E+ plants had significantly lower ( $P<0.05$ ) aboveground C/N under drought stress but significantly higher ( $P<0.05$ ) underground C/N under drought with salt stress. Mutation treatment had significant effects on aboveground C/N because the aboveground C/N in mutant plants was significantly lower ( $P<0.05$ ) than that in wild type plants under salt stress and drought with salt stress. Underground C/N in mutant plants was significantly higher ( $P<0.05$ ) than those in wild type plants under control and drought stress. The aboveground C/N in mutant plants was significantly lower ( $P<0.05$ ) than that in wild type plants under drought with salt stress.

Stress treatments significantly increased ( $P<0.05$ ) aboveground C/P (Fig. 12A) and reduced underground C/P in SPE+ and UE- (Fig. 12B). For mutant plants, underground C/P in E+ plants was significantly higher ( $P<0.05$ ) than that in E-

plants under control and drought stress, whereas that in E+ plants was significantly lower ( $P<0.05$ ) than that in E- plants under salt stress. For wild type plants, E+ plants had significantly higher ( $P<0.05$ ) underground C/P under drought stress than E- plants. Mutation treatment had significant effects on aboveground C/P because the aboveground C/P in mutant plants was significantly lower ( $P<0.05$ ) than that in wild type plants under drought stress; however, it was significantly higher ( $P<0.05$ ) than that in wild type plants under drought with salt stress.

Stress treatments significantly improved ( $P<0.05$ ) the aboveground N/P (Fig. 13A) and underground N/P in SPE- but reduced the underground N/P in UE+ (Fig. 13B). Endophytes had no significant effects on either aboveground or underground N/P. Mutation treatment had significant effects on N/P because the aboveground N/P in mutant plants was significantly higher ( $P<0.05$ ) than that in wild type plants under drought with salt stress. Underground N/P in mutant plants was significantly lower ( $P<0.05$ ) than that in wild type plants under control and drought stress; however, it was significantly higher ( $P<0.05$ ) than that in wild type plants under drought with salt stress.

## Discussion

Previous studies revealed that endophytes improve host tolerance to stress through a variety of morphological and physiological adaptations and adjustments by promoting host growth and photosynthesis, increasing the levels of beneficial metabolites, activating antioxidant systems to scavenge ROS, modulating plant growth phytohormones, improving nutrient uptake, and maintaining ionic homeostasis (Malinowski et al. 2000, 2019; Song et al. 2015; Hume et al. 2016; Nagabhyru et al. 2013). In the present study, we evaluated the plant growth, contents of phytohormones, stoichiometry of C, N and P, and  $\text{Na}^+$  and  $\text{K}^+$  ion transport in perennial ryegrass under different treatments. The results showed that endophyte only regulated phytohormones, the rational distribution of ion content and nutrient elements in host plants of both mutation and wild types under some treatments; however, its role was inconsistent depend on treatments. Endophytes had either beneficial or harmful effects on plant stress tolerance under certain treatments.

## The effects of stress and endophyte on the growth of perennial ryegrass

In the present study, stress treatments inhibited the growth of perennial ryegrass. While, *E. festucae* var. *lolii* only had beneficial effects on host growth for wild type plants, but had no effects on mutant plants which suggested the mutation treatment may have effects on endophyte function. Some previous studies have indicated that the effects of endophytes on the growth of perennial ryegrass may be either beneficial or harmful, depending on a combination of biotic and abiotic factors, as well as the interaction between the host and endophyte genotypes (Marks et al. 1991; Cheplick 1997, 1998).

## The effects of stress and endophyte on hormones contents of perennial ryegrass

In the present study, both stress treatments and endophytes had no significant effect on IAA and ABA contents, however, had significant effects on GA and SA contents. These changes in endogenous hormones in plants under different conditions confirmed that plants utilise hormones in response to stress. Phytohormones are important signalling molecules that are related to plant growth and physiological and developmental processes (Aaron et al. 2009). IAA is the most common auxin produced by plants, and its concentration is key in the regulation of plant growth and development (Müller 2003). ABA is known to induce stomatal closure as a water-conserving response, in which plants benefit in the short term by reducing water loss via transpiration (Lemichez et al. 2001). CTK plays a key role in

improving grain yield by affecting the source/sink transition (Peleg et al. 2011). GA is a vital plant growth regulator, which plays an important role in seed germination growth of floral organs, and in lateral shoot formation, which is also observed to encourage plant growth and improvement under numerous abiotic stress conditions (Olszewski et al. 2002; Tuna et al. 2008; Ahmad et al. 2010). SA is a well-known signalling molecule that affects plant growth and development and is involved in plant immune system responses to pathogens and insect herbivores (Hayat et al. 2010; Bastías et al. 2018).

In the present study, *Epichloë* endophyte different effects on CTK, GA and SA contents; this is consistent with previous studies, which revealed that *Epichloë* endophytes change hormones to improve host stress tolerance (De Battista et al. 1990; Saikkonen et al. 2004, 2013; Xia et al. 2018). Bunyard and McInnis (1990) reported that E+ tall fescue plants produced significantly more ABA in response to drought stress than E- plants. Some glasshouse-based studies have indicated a similar endophyte-enhancement of ABA concentration in tall fescue leaf tissue in response to drought (Joost 1995). Similar results were also observed in some Chinese native grasses, such as *Achnatherum inebrians* and *Festuca sinensis*. E+ Chinese wildrye (*Leymus chinensis*) plants have a higher SA content than E- plants, especially when they are exposed to *B. sorokiniana* and *C. lunata* (Wang et al. 2016). Some endophytes have been reported to produce IAA and related indole compounds in cultures (De Battista et al. 1990, Yue et al. 2000). Phytohormone production *in planta* may also induce defense-related secondary metabolism in plants. However, very little is known about the role of hormones in symbiosis and their direct effects on host fitness traits.

## The effects of stress and endophyte on the Na<sup>+</sup>/K<sup>+</sup> transport of perennial ryegrass

In the present study, compared with the control, Na<sup>+</sup> ion content significantly increased ( $P < 0.05$ ), whereas K<sup>+</sup> ion content significantly decreased ( $P < 0.05$ ) under stress treatments. Na<sup>+</sup>/K<sup>+</sup> and  $S_{K,Na}$  also significantly increased ( $P < 0.05$ ) under stress treatments. Ca<sup>2+</sup> levels did not change in most plants. Accumulation of inorganic ions, such as Na<sup>+</sup> and K<sup>+</sup>, is one of the mechanisms for osmotic adjustment in plants during stress response (Shabala et al. 2011). This is very important for alleviating host damage because Na<sup>+</sup> accumulation in plant cells causes extreme damages by inhibiting enzymes, disrupting K<sup>+</sup> acquisition, inhibiting K<sup>+</sup>-dependent metabolic processes, and causing oxidative stress (Pan et al. 2016; Zhu 2001). Maintaining constant intracellular K<sup>+</sup> and Na<sup>+</sup> balance is essential for metabolic processes in cells and is crucial for plant adaptation in response to saline environments (Zhu 2003). Previous studies have reported that endophyte infection could adjust Na<sup>+</sup> and K<sup>+</sup> concentrations in host plants under stress. For example, Bayat et al. (2009) found that *Epichloë* endophytes increased K<sup>+</sup> and Ca<sup>2+</sup> contents in *F. arundinacea* under drought stress. Reza and Mirlohi (2010) showed that *E. coenophiala* and *E. uncinata* endophyte infection reduced Na<sup>+</sup> and Cl<sup>-</sup> concentrations in tall fescue and meadow fescue roots, but increased K<sup>+</sup> concentrations in the shoots under salt stress. Both Song et al. (2015) and Chen et al. (2018) reported that *E. bromicola* infection reduced Na<sup>+</sup> content, Na<sup>+</sup>/K<sup>+</sup> ratio, and shoot Ca<sup>2+</sup> content, but increased K<sup>+</sup> content and root Ca<sup>2+</sup> content in *Hordeum brevisubulatum* under salt and alkali stresses. The Na<sup>+</sup> content decline in E+ plants led to better plant growth under stresses compared to that in E- plants. Restriction of Na<sup>+</sup> transportation and increase in K<sup>+</sup> concentration to ensure a high cytosolic K<sup>+</sup>/Na<sup>+</sup> ratio are very important for plants to tolerate high salt levels (Berthomieu et al. 2003; Cuin et al. 2003). These changes could decrease the levels of toxic ions and osmotic influence on plants under stress treatments. Ca<sup>2+</sup> is essential for selective ion transport mechanisms and maintenance of K<sup>+</sup> influx and Na<sup>+</sup>/K<sup>+</sup> selectivity. In this study, *Epichloë* endophyte infection did not show significant effects on these indices in most plants.

# The effects of stress and mutation on stoichiometry of C, N and P of perennial ryegrass

In the present study, C and P contents decreased under stress, whereas N content increased. C, N, and P are essential elements, and the tissue elemental stoichiometry has a mechanistic linkage with the growth rate of the organism. The growth rate hypothesis proposes that higher growth rates are associated with lower C/N, C/P, and N/P ratios (Hessen et al. 2007). N availability can stimulate phosphatase activity in the roots (Fujita et al. 2010), which could potentially promote P uptake. P is required to meet the protein synthesis demands for increased growth rates (Hessen et al. 2007). *Epichloë* endophytes also adjusted C, N, and P contents and C/N and C/P ratios to increase host growth (Song et al. 2015; Song et al. 2016; Chen et al. 2018; Xia et al. 2018). For example, Vázquez-de-Aldana et al. (2013) noted that *E. festucae* alters the nutrient content of *F. rubra* regardless of water availability. Song et al. (2015) showed that E+ *H. brevisubulatum* plants had higher contents of N, P and lower ratios of C/N and C/P under salt stress, corroborating the report of Chen et al. (2018). For salt and alkali stresses, Xia et al. (2018) reported that E+ *A. inebrians* plants had higher N and P contents under soil water deficit. However, endophytes alleviate these changes only in very few cases.

Endophyte-derived alkaloid production is one of the key traits considered by pasture breeders when selecting endophyte strains for pasture grass breeding program. Alkaloids may play some roles in helping endophytes confer protection to plants against abiotic and biotic stresses (Nagabhyru et al. 2013; Schardl et al. 2013). For example, alkaloid levels of E+ *A. inebrians* increases as NaCl concentration increases, and decreases as water content increases in the soil (Zhang et al. 2011). Nagabhyru et al. (2013) showed that loline alkaloid levels increased in response to drought stress in E+ tall fescue. The chemical ecology mediated by endophytes in grasses has been revealed to be far more complex (Saikkonen et al. 2013). The content variations of N and P contents under stress in our study may also have impacts on alkaloid concentrations, because N is an important component for alkaloid biosynthesis and P availability influences ergot alkaloid production in endophyte-infected grasses (Belesky et al. 1987; Malinowski et al. 1998; Faeth and Fagan 2002). Alkaloid production in these individual plants will be evaluated as soon as possible in the near future to provide a more comprehensive understanding for selection and breeding of these materials.

## The effects of space mutation on perennial ryegrass

Space mutations result in abundant and non-directional mutations, which create genetic variability to improve various complicated traits in plants. Space-induced mutation breeding is an effective way to breed new varieties and enhance genetic diversity (Liu et al. 2008). In the present study, space mutation had effects on plant performance as space mutation increased tiller number and underground biomass, plant  $K^+$  and  $Ca^{2+}$  contents, underground C content, and underground ratio of C/N; however, it reduced plant phytohormone contents, aboveground  $Na^+$  content,  $Na^+/K^+$ , and aboveground C/N ratio under some treatments. The mutant individuals provided new methods and resources for perennial ryegrass breeding with strong stress tolerance. Using space-induced radiation, a number of advantageous mutations, which were used to make a breakthrough in most desired crop yield, were also achieved. China has produced 41 varieties developed through space-induced mutation breeding of various crop species such as rice, wheat, cotton, sesame, pepper, tomato, and alfalfa (Liu et al. 2008). However, space-mutation approach results in abundant, non-directional mutations (He et al. 2006). This breeding methodology needs to be followed by many studies, such as material selection, molecular screening of mutants, and earlier generation identification of quality characters to successfully breed new varieties. We should continue to select excellent individual plants from the second generation of these perennial ryegrass germplasms with space mutations and conduct characterisation and genetic analysis in combination with molecular techniques. There are no reports on the effects of space mutations on *Epichloë* endophytes. In the present study, space mutations did not affect endophyte function. *Epichloë* endophyte, which lived

inside E+ seeds, went through space-induced radiation, possibly having radiation mutations in the genome. Studies on endophyte isolation from E+ mutation plants and its characteristics are in progress to reveal the mutation site and mechanism.

## Conclusion

We concluded that plants went through significant variations in physiology and regulate stoichiometry of C, N and P and Na<sup>+</sup>/K<sup>+</sup> transport in response to environmental changes. Both endophyte and space mutations improved perennial ryegrass growth. However, either endophyte and space mutations had beneficial or harmful effects on regulating the rational distribution of ions and stoichiometry of C, N and P and the adjustment varied with treatments. Space mutations did not affect endophyte function. These results suggest that the selection of mutant germplasm for cultivation may only depend on individual plants with excellent performance.

## Declarations

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## Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Author contributions

PT designed the experiments, BHM and YL did the experiment and analysis, ZJC, CJL and ZBN provided seeds, BHM, YL, PT wrote the manuscript. All authors contributed to the article and approved the submitted version.

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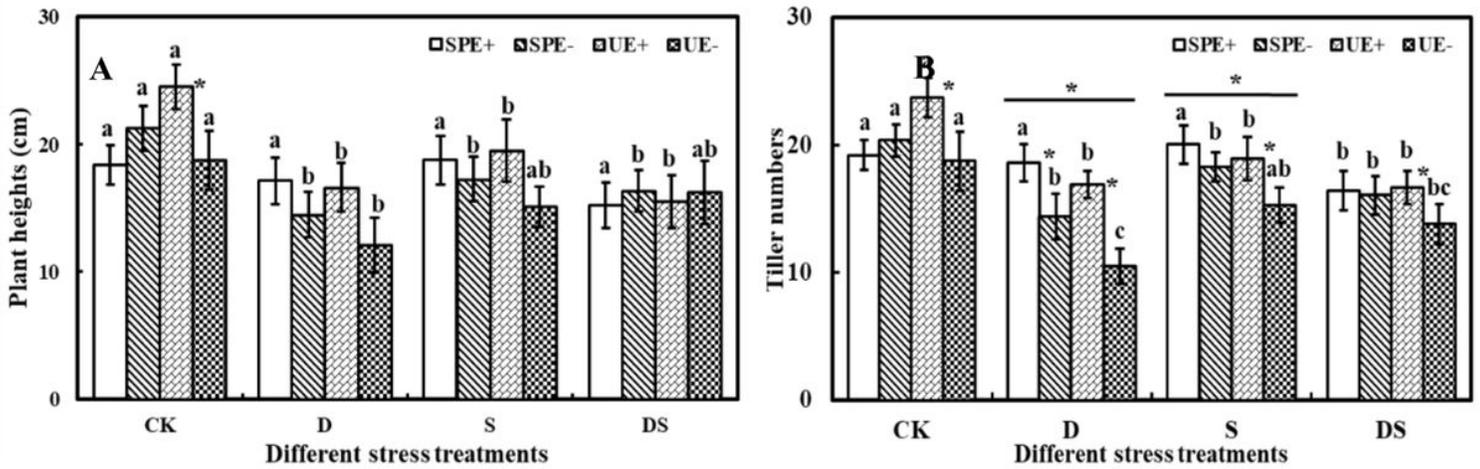
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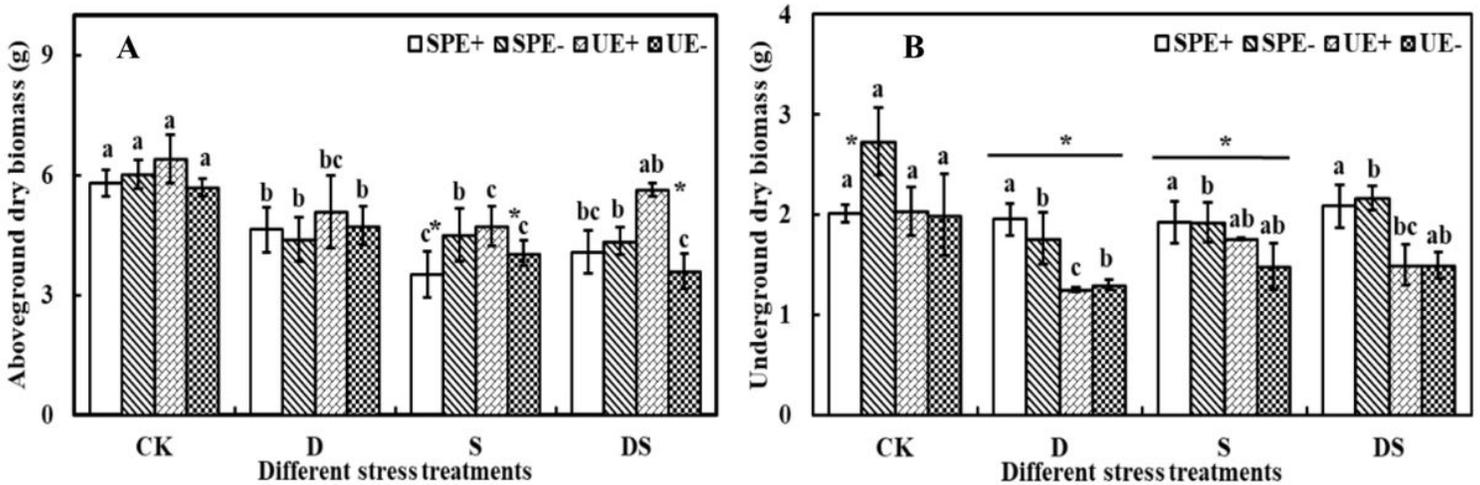
## Figures



**Figure 1**

Plant height (A) and tiller number (B) of different plants under different treatments.

Note: SPE+ is endophyte-infected (E+) space mutant plants; SPE- is endophyte-free (E-) space mutant plants; UE+ is wild type E+ plants; UE- is wild type E- plants. CK, control treatment; D, drought stress treatment; S, salt stress treatment; DS, drought with salt stress treatment. Capped lines represent the standard errors of the mean. Lower case letters compare the same plant under different treatments; asterisk (\*) above bars indicates significant ( $P < 0.05$ ) difference between SPE+ and SPE- plants or UE+ and UE- under the same treatment; asterisk (\*) above the corresponding line indicates significant ( $P < 0.05$ ) difference between space mutant plants and wild type plants under the same treatment. Similarly, for the following figures.



**Figure 2**

Aboveground (A) and underground (B) dry biomass of different plants under different treatments.

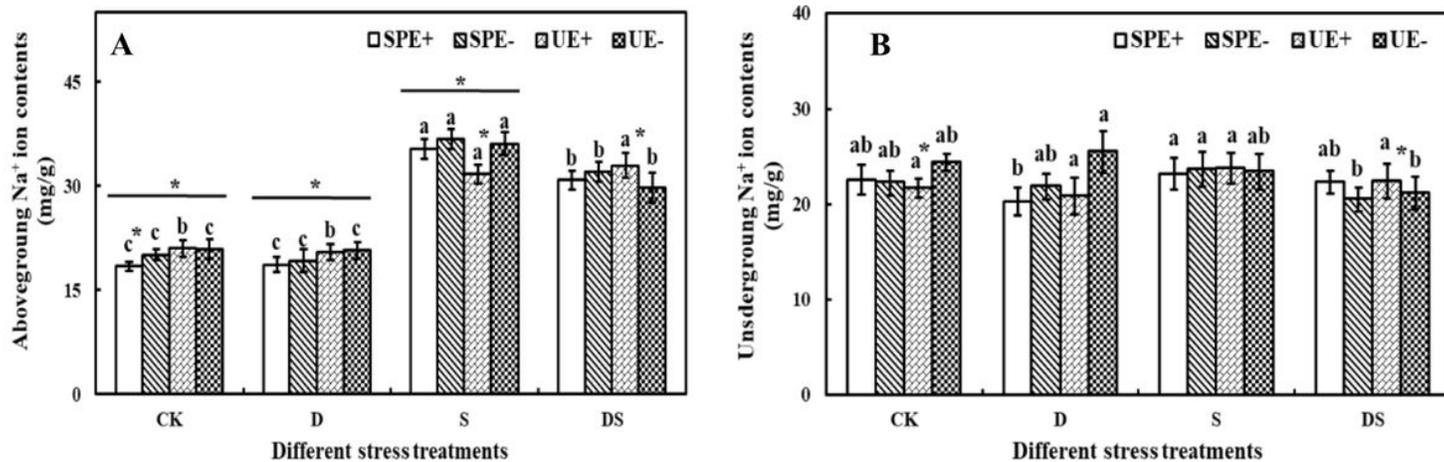


Figure 3

Aboveground (A) and underground (B) Na<sup>+</sup> ion contents of different plants under different treatments.

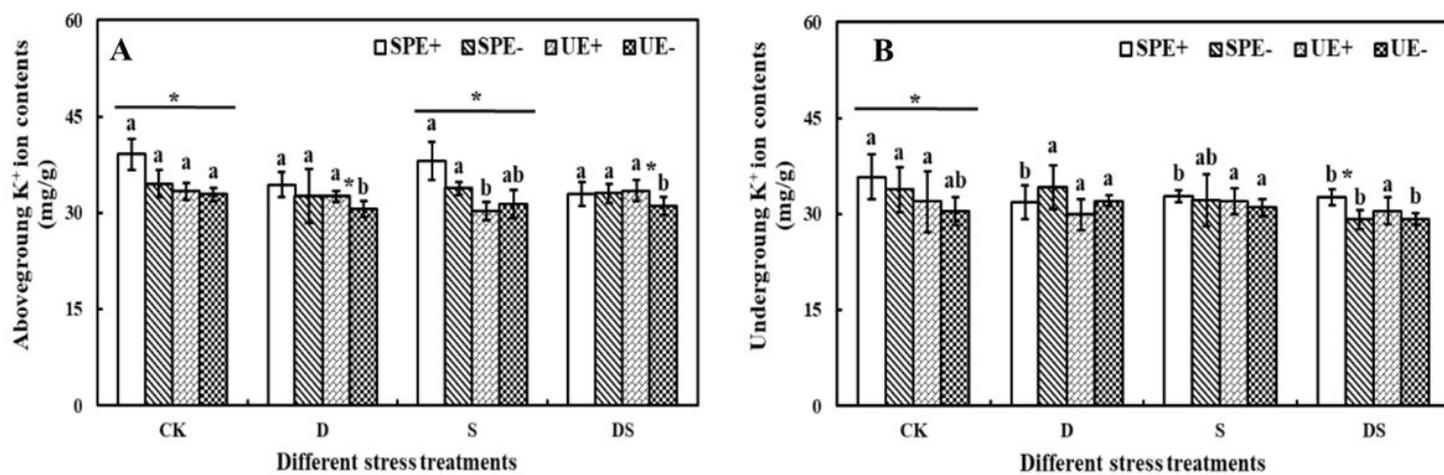


Figure 4

Aboveground (A) and underground (B) K<sup>+</sup> ion contents of different plants under different treatments.

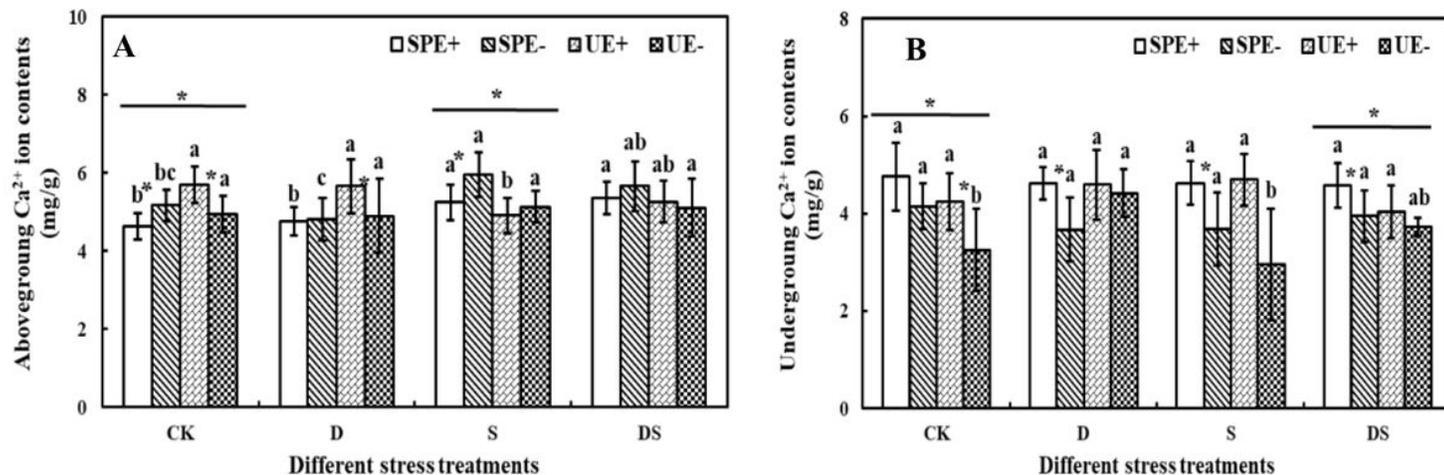


Figure 5

Aboveground (A) and underground (B)  $\text{Ca}^{2+}$  ion contents of different plants under different treatments.

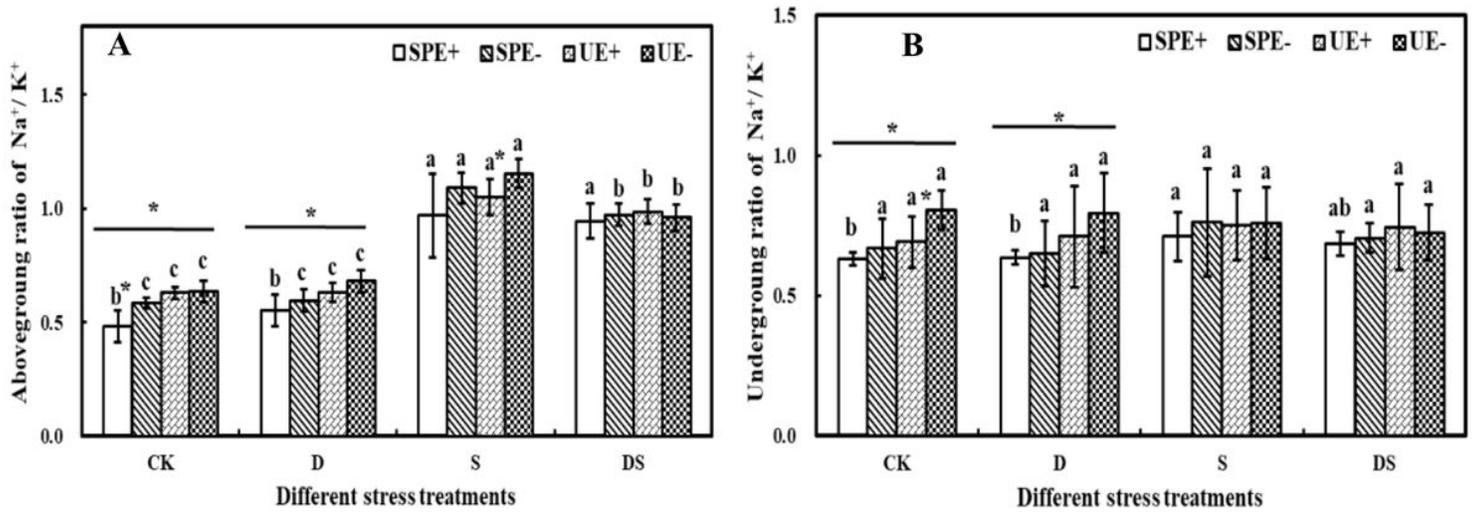


Figure 6

Aboveground (A) and underground (B) ratio of  $\text{Na}^+/\text{K}^+$  of different plants under different treatments.

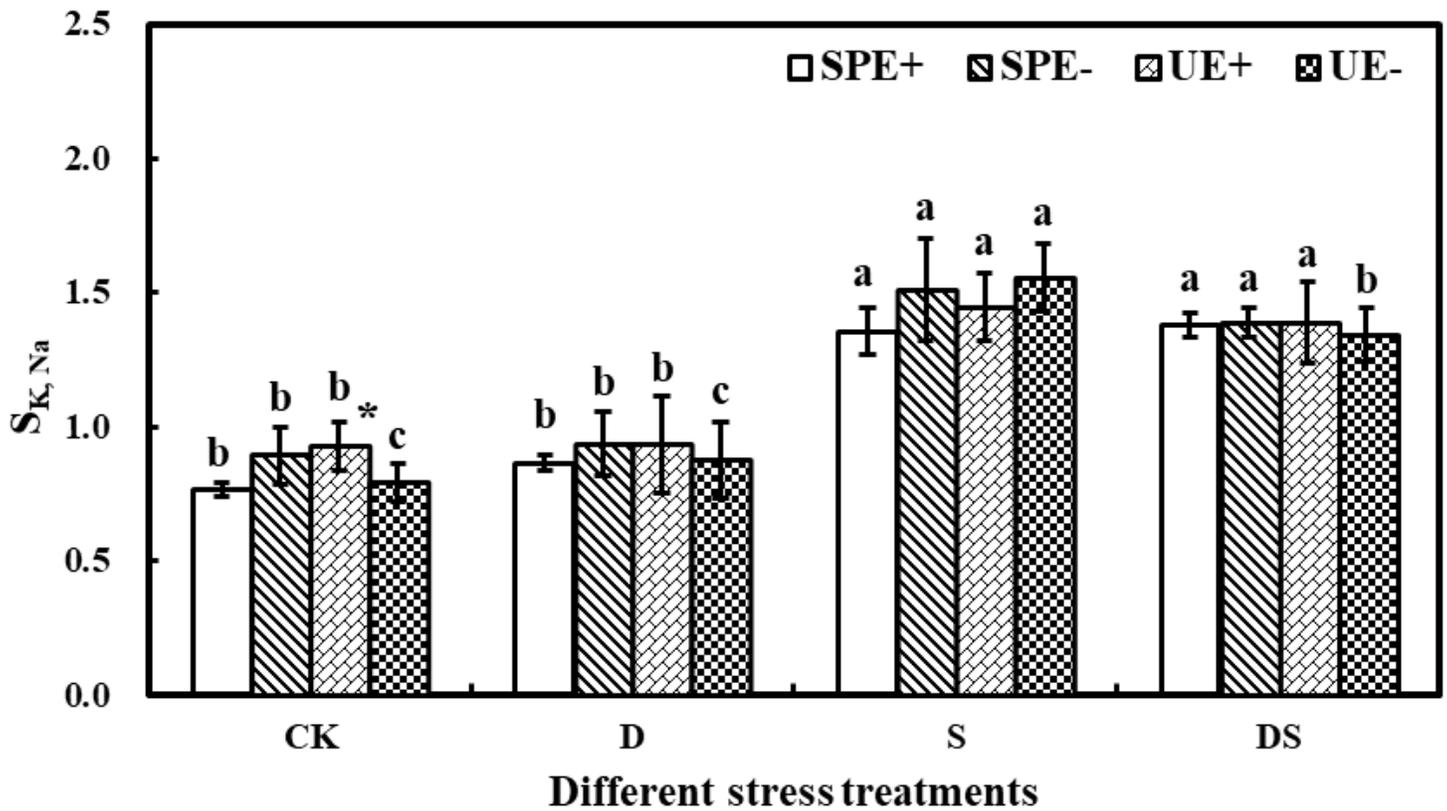
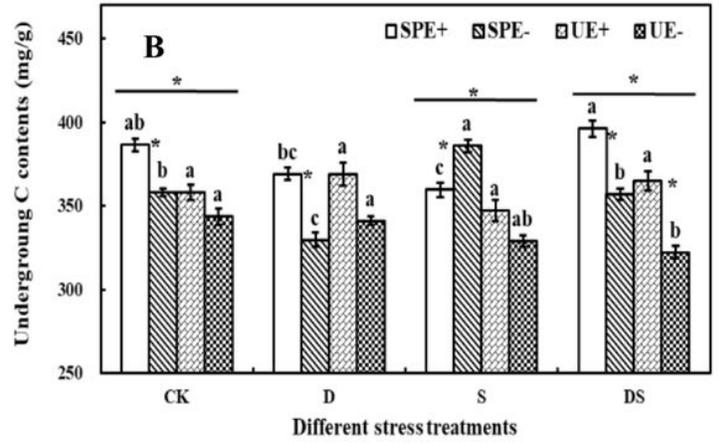
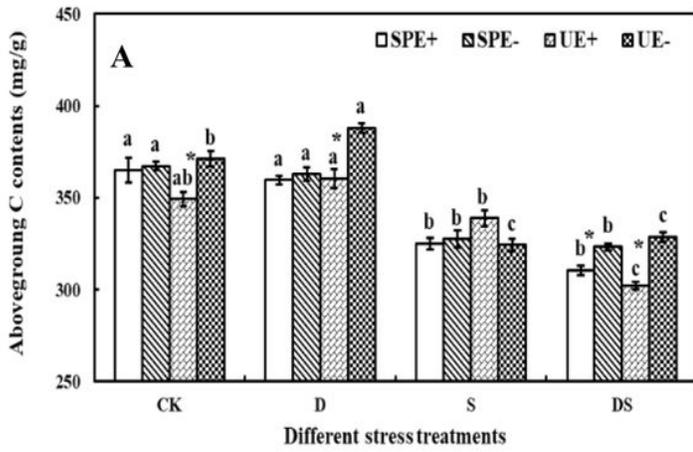


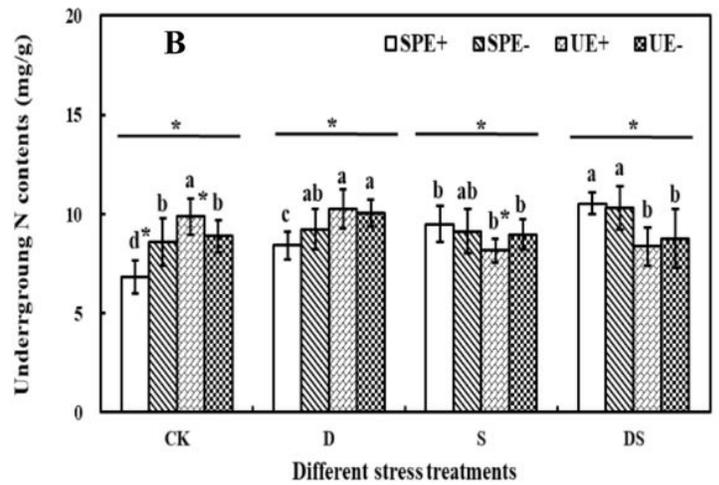
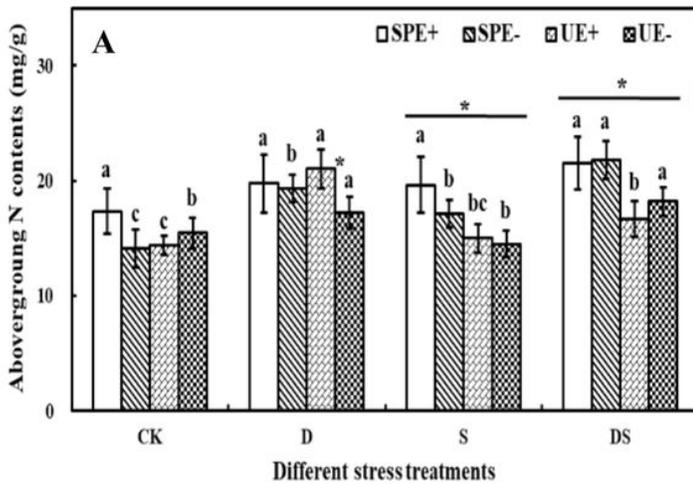
Figure 7

$S_{K,Na}$  of different plants under different treatments.



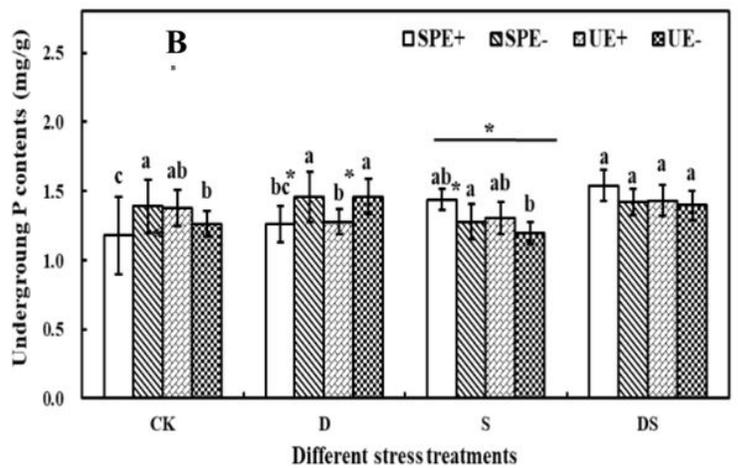
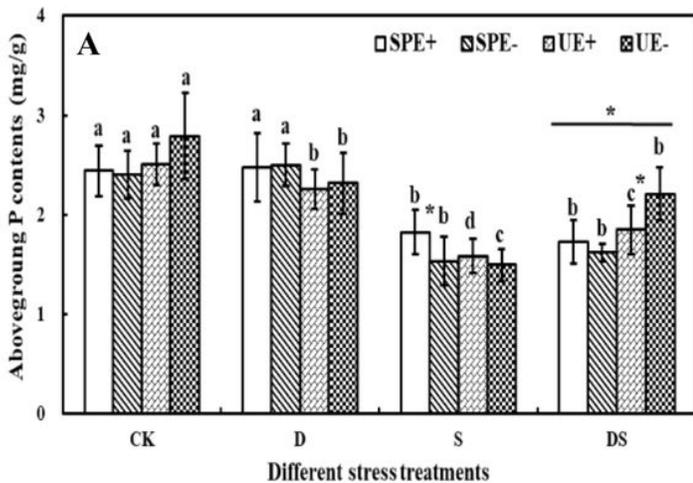
**Figure 8**

Aboveground (A) and underground (B) C contents of different plants under different treatments.



**Figure 9**

Aboveground (A) and underground (B) N contents of different plants under different treatments.



**Figure 10**

Aboveground (A) and underground (B) P contents of different plants under different treatments.

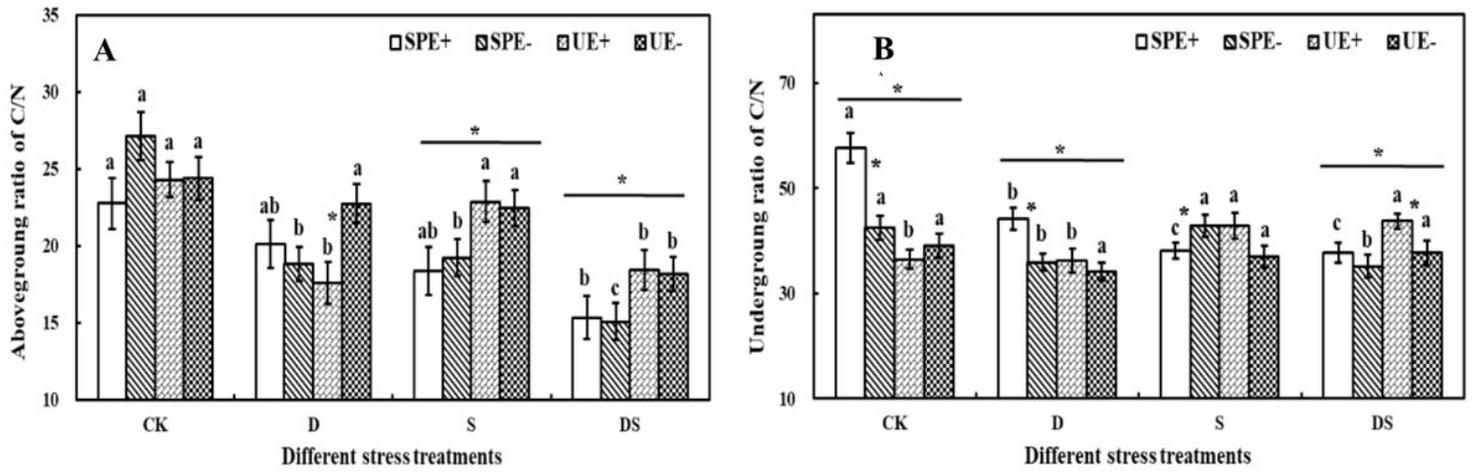


Figure 11

Aboveground (A) and underground (B) ratio of C/P of different plants under different treatments.

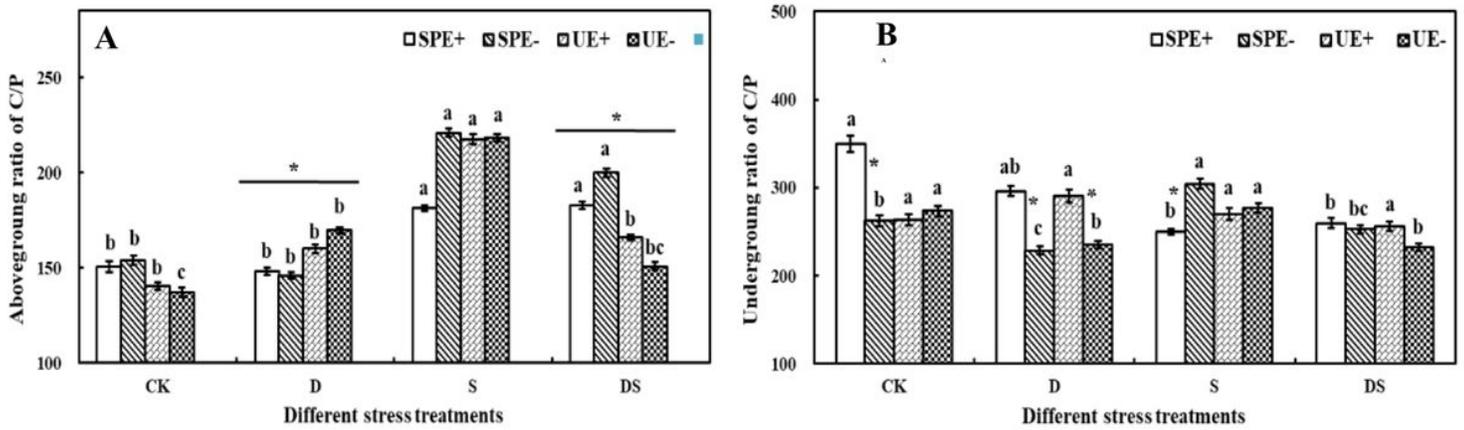


Figure 12

Aboveground (A) and underground (B) ratio of C/P of different plants under different treatments.

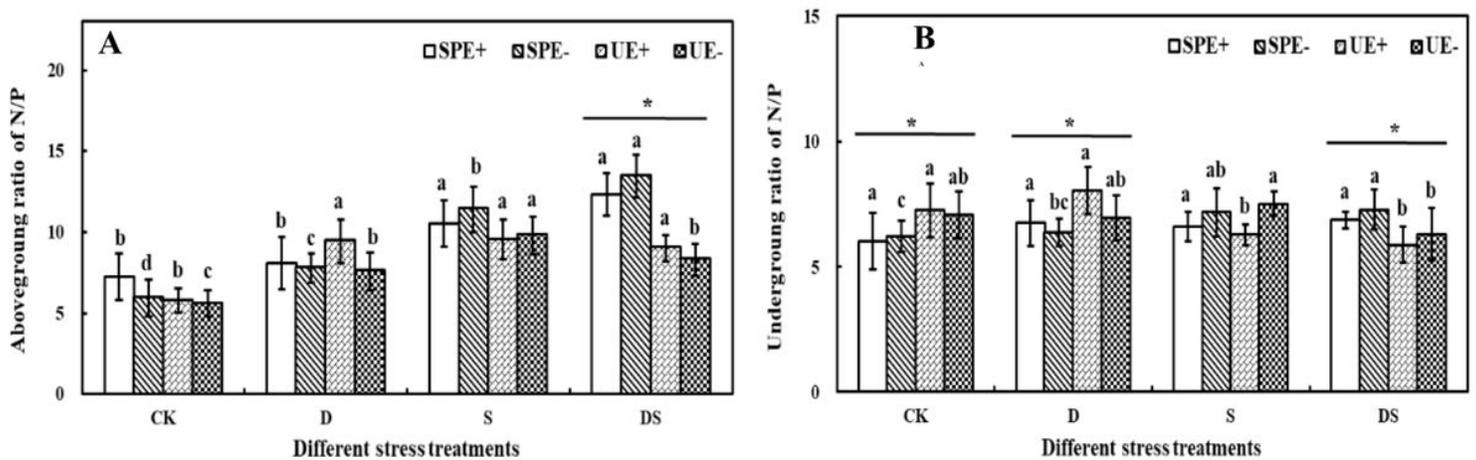


Figure 13

Aboveground (A) and underground (B) ratio of N/P of different plants under different treatments.

## Supplementary Files

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- [TableS1.xlsx](#)