

## Article

# Sex-Related Ecophysiological Responses of *Hippophae rhamnoides* Saplings to Simulate Sand Burial Treatment in Desertification Areas

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**Abstract:** (1) Background: In recent years, *Hippophae rhamnoides* has been used extensively to prevent desertification in China due to its nitrogen (N) fixation and sand stabilization abilities. However, as a dioecious species, few studies have focused on the sexual dimorphism of *H. rhamnoides* in response to sand burial, which frequently presents in desertification areas. (2) Methods: In this paper, we explored the ecophysiological responses of female and male saplings of *H. rhamnoides* under unburied treatment (control) and different sand burial depths (denoted as T33, T67, T90 and T133, corresponding to sand burial depths of 33, 67, 90 and 133 percent of the mean initial height of the saplings, respectively). (3) Results: Compared with unburied controls, the T33 treatment significantly promoted biomass accumulation and photosynthetic capacity, whereas T67 and T90 treatments inhibited biomass and physiological parameters of the two sexes. Deeper sand burial treatments, i.e., T90 and T133, significantly decreased the survival rates of the two sexes. Furthermore, the sex-specific responses of the two sexes of *H. rhamnoides* were affected by different depths of sand burial. Males had higher levels of stem starch and root sucrose and exhibited a larger increase in root nodule biomass under the T33 treatment, indicating better carbohydrate utilization and N fixation, whereas females showed lower total biomass and fewer root nodules, as well as more inhibition of photosynthetic and chlorophyll fluorescence parameters, water potential and root carbohydrates, indicating more negative effects on females than males under the T67 treatment. (4) Conclusions: We conclude that sex-related response and adaptation to sand burial depths may potentially affect the colonization, sex ratio and ecological function of the two sexes of *H. rhamnoides* in desertification areas.

**Keywords:** sexual dimorphism; sand burial; photosynthetic capacity; carbohydrates; chlorophyll fluorescence



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## 1. Introduction

Currently, many countries are encountering serious land desertification, i.e., continuous land degradation caused by climate change and excess human activities in arid and semi-arid regimes [1–3]. Sand burial is a common disturbance for plants in desertification areas. The soil microenvironment, including moisture, temperature, aeration, etc., changes with different depths of sand burial of plants [4,5]. Plants have various characteristics that allow them to adapt to sand burial, including by shifting biomass allocation, elongating their stems and increasing photosynthetic capacity, water-use efficiency and the chlorophyll content of unburied leaves [6–9]. Sand burial may be a driver that regulates the distribution and composition of vegetation in desertification areas [8,10].

Sea buckthorn (*Hippophae rhamnoides* L.), an important dioecious tree, plays important roles in fixing N, preventing soil erosion, regulating the microclimate and retaining ecological stabilization [11,12]. A trend of aeolian desertification has been developing in the Zoige Basin, eastern Tibetan Plateau, due to overgrazing, wind erosion and global climate change [13]. A number of studies have focused on vegetation and soil restoration of desertification areas in the field of restoration ecology [3,8,14]. In recent years, many *H. rhamnoides*

saplings have been planted in such areas to prevent desertification, owing to its functions of N fixation and sand stabilization. Therefore, it is essential to understand the responses and adaptations of *H. rhamnoides* saplings to sand burial for successful afforestation and restoration in local ecosystems.

Different reproductive investment in two genders can trigger asymmetric growth and functional strategies with sex-specific responses to photosynthetic assimilation, resource allocation and life history in dioecious species, especially in an adverse environment, even before they reach reproductive maturity [15–20]. Moreover, sexual dimorphism appears to vary among species. Most studies indicate that males of some species, such as *Populus cathayana*, *Ilex aquifolium*, *Corema album*, etc., exhibit better performance than females in stressful environments [21,22]. It has been reported that male plants have higher photosynthetic rates, long-term water-use efficiency, osmotic regulation and antioxidant activity than female plants under conditions of drought, salt and waterlogging stress [20,23,24]. However, an opposite trend has been found in some herb species [25,26]. Additionally, some studies indicate no significant sexual differences under some conditions [27,28]. It was reported that *H. rhamnoides* presented with sexual dimorphisms in response to water stress and altitudinal gradient. Sexual differences in dry matter accumulation, root/shoot ratio, net photosynthesis, transpiration, instantaneous water-use efficiency (WUEi) and carbon isotope composition ( $\delta^{13}\text{C}$ ) were observed under water-deficiency treatment. Moreover, male individuals had a conservative water-use strategy in response to drought stress [29,30]. In addition, the male: female ratio of *H. rhamnoides* was biased toward males along the altitudinal gradient, indicating that stressful environments had a more negative impact on females [30]. Sex-specific responses to sand burial in dioecious plants employed to prevent desertification have not been explored to date. Based on previous literature, we hypothesized that male saplings of *H. rhamnoides* may have better survival rates, growth and physiological responses in a sand burial habitat due to their lower reproductive investment and conservative resource use strategy, resulting in a stronger influence on the local desertification ecosystem. The photosynthetic and chlorophyll fluorescence parameters, as well as water potential and tissue carbohydrates, are useful indices to evaluate plant adaptation to environmental stress [17,20]. We want to answer the following questions: (1) Do *H. rhamnoides* saplings exhibit sex-specific responses in survivorship, growth and ecophysiological parameters when buried in sand?; (2) Do sand burial depths correlate with negative or positive effects on ecophysiological parameters of *H. rhamnoides* saplings?; and (3) Do different sand depths enhance or diminish sexual differences between the two sexes?

## 2. Materials and Methods

### 2.1. Plant Material and Experimental Design

Female and male cuttings of *H. rhamnoides* collected from 10 adult trees (5 females and 5 males) with similar genetic and age were selected for sapling formation in a plantation in Fuxin County, Liaolin Province, China. Both female and male saplings of *H. rhamnoides* came from the cultivation plantation. The experiment was completely randomized and included the following factors: two sexes and five levels of sand burial. Twenty replicates per treatment were included in the experiment. On 10 May 2016, 200 healthy saplings (100 males and 100 females) with approximately the same crown size and equal height were chosen and replanted in 30 L sand-soil-filled plastic pots (45 cm diameter, 50 cm height). After growing for four weeks, the mean seedling height ( $30.02 \pm 0.73$  cm) was obtained by measuring the height of each sapling in every pot. The saplings were buried in sand to different depths for 45 days. The depths of sand burial were determined according to the methods described by Shi et al. (2004) with some modifications according to observation in the desertification area. An unburied sapling was used as a control (denoted as control), and sand burial treatments were conducted at different depths (denoted as T33, T67, T90 and T133, corresponding to sand burial depths of 33, 67, 90 and 133 percent of the mean initial height of the saplings). Sand was taken from the moving sandy land in the desertification area of the Zoige Basin, Sichuan Province, China. Sandy soil was added to pots at different

burial depths, and each sapling was kept vertical while being buried. The pots were placed randomly in an experimental greenhouse at Mianyang Teacher's College located at N 31°19' and E104°78', Mianyang City, Sichuan Province, China. The temperature and humidity mimicked realistic scenarios in a desertification area with a day temperature range of 15–22 °C, a night temperature range of 8–12 °C and a relative air humidity range of 50%–70%. All pots were watered with 1 L water every three days, and no additional fertilizer was added to the pots to ensure close to natural soil conditions.

## 2.2. Determination of Growth Parameters

All saplings were harvested after 45 days of sand burial treatments, and the survival rate was determined. The surviving saplings were evaluated by fresh phloem, which was found to occur in both stems and roots, as well as in green tissue on leaves. Five randomly selected surviving saplings were dug out in each treatment, and roots were picked up as intact as possible from sandy soils. First, sampled saplings were cleaned with tap water, and the number of branches was counted. Then, they were divided into leaves, stems, roots and root nodules, which were carefully collected from roots. Finally, these parts were oven-dried at 80 °C for 72 h and weighed individually. The total biomass was calculated as the sum of dry mass of leaves, stems and roots, and the root/shoot ratio (R/S ratio) was calculated as root biomass/(leaf biomass + stem biomass).

## 2.3. Determination of Gas Exchange Parameters

The gas exchange parameters were measured two days before the end of the experiment. The fourth or fifth intact leaf from five randomly chosen saplings in each treatment was used to measure gas exchange parameters between 08:00 and 11:30 using a portable photosynthesis system (LI-6400; Li-Cor, Inc., Lincoln, NE, USA). Saturated photosynthetic photon flux density (PPFD) was determined by preliminary experiments. Prior to measurements, samples were illuminated with saturated PPFD provided by an LED light source for 10–30 min to achieve full photosynthetic induction. The net photosynthetic rate ( $P_n$ ), transpiration rate ( $E$ ), stomatal conductance ( $G_s$ ) and leaf internal  $CO_2$  concentration ( $C_i$ ) were measured under the following conditions: leaf temperature, 25 °C; leaf-to-air vapor pressure deficit,  $1.5 \pm 0.5$  kPa; PPFD,  $1500 \mu\text{mol m}^{-2} \text{s}^{-1}$ ; relative air humidity, 50%; and ambient  $CO_2$  concentration,  $400 \pm 5 \mu\text{mol mol}^{-1}$ . Photosynthetic water-use efficiency (WUE) was calculated as the ratio of  $P_n$  to  $E$ .

## 2.4. Determination of Chlorophyll Fluorescence

The fourth or fifth fully expanded leaf from five saplings randomly sampled from each treatment was used to measure chlorophyll fluorescence. Chlorophyll fluorescence kinetics parameters ( $F_v/F_m$ , dark-adapted quantum yield of PSII;  $\Phi$ , effective quantum yield of PSII;  $q_N$ , non-photochemical quenching coefficient;  $q_P$ , photochemical quenching coefficient) were measured and calculated according to van Kooten and Snel (1990) [31] with a PAM chlorophyll fluorometer (PAM 2100, Walz, Effeltrich, Germany). First, leaf samples were placed in the dark for 30 min using an aluminum foil cover. The minimal fluorescence yield ( $F_0$ ) and the maximal fluorescence yield ( $F_m$ ) were measured. Then, the leaves were illuminated with actinic light at an intensity of  $250 \mu\text{mol m}^{-2} \text{s}^{-1}$ , which was the light intensity at the time of measurements. The actinic light was removed, and the minimal fluorescence ( $F_{0'}$ ) was measured by illuminating the leaves with 3 s of far-red light. A saturating white light pulse of  $8000 \mu\text{mol m}^{-2} \text{s}^{-1}$  was applied for 0.8 s when  $F_m$  and maximal fluorescence ( $F_{m'}$ ) were measured.

## 2.5. Determination of Leaf Water Potential

At the end of experiment, the third fully expanded and intact leaf from five randomly chosen saplings in each treatment was cut and sealed immediately in a small plastic bag containing moist paper towels, and the predawn leaf water potential was determined with a WP4 dew-point potentiometer (Decagon Devices, Inc., Pullman, WA, USA) before sunrise.

### 2.6. Determination of Leaf Chlorophyll Content

Fresh leaves (0.2 g) from five saplings randomly sampled from each treatment were placed in a centrifuge tube, extracted in 8 mL of 80% chilled acetone (*v/v*), sealed and placed in the dark to avoid light degradation. After 72 h extraction, the tubes were centrifuged at  $5000 \times g$  for 10 min. Then, the absorbance of extracts was measured using a Unicam UV-330 spectrophotometer (Unicam, Cambridge, UK) at 645 and 663 nm. The total chlorophyll content (Tchl<sub>a+b</sub>) was determined as the sum of chlorophyll a (Chl<sub>a</sub>) and chlorophyll b (Chl<sub>b</sub>).

### 2.7. Determination of Starch and Sucrose

The powdered dry leaf, stem and root materials (0.2 g) sampled from five saplings of each treatment were transferred separately into a centrifuge tube, incubated in 8 mL of 80% (*v/v*) ethanol at 80 °C for 30 min and centrifuged at  $5000 \times g$  for 10 min. The ethanol extract was used to determine sucrose content. Then, 1 mL of ethanol extract was transferred into a test tube. After adding 0.1 mL of 2 mol L<sup>-1</sup> NaOH solution, the test tube was placed into a boiling water bath for 10 min. Then, the test tube was cooled and combined with a 1 mL of 0.1% resorcinol solution (*w/v*) and 3 mL of 10 mol L<sup>-1</sup> HCl solution. The reaction mixture was placed into a 80 °C water bath for 30 min and cooled; then, the absorbance was measured at 480 nm (Unicam, Cambridge, UK). The residue after ethanol extraction was used to determine starch content in glucose equivalents. After the addition of 2 mL of water, the tubes were placed in a boiling water bath for 15 min. After cooling, 2 mL of 9.2 M perchloric acid was added and centrifuged. Supernatants were collected and re-extracted twice with 2 mL of 4.6 M perchloric acid. After centrifugation, supernatants were combined and brought to a final volume of 10 mL with water. Then, the starch content was determined by an anthrone reagent according to Yemm and Willis (1954) [32].

### 2.8. Data Analysis

Analyses of variance (ANOVA) and a regression model analysis were performed using the SPSS 19.0 software package. Before statistical analysis, normality and homogeneity of variances were checked and log-transformed to correct deviations from these assumptions when needed. A two-way ANOVA was conducted to evaluate the effects of sex, sand burial and their interaction on each variable. Non-linear regression analysis was used to analyze the correlation between total biomass and various parameters and to explore the effect of ecophysiological parameters on biomass accumulation of *H. rhamnoides*. A principal component analysis (PCA) with all variables was further undertaken to gain insight into trait-variable loadings and their dependence on the interactive effect of sex and sand burial depth, as well as to distinguish the differences in adaptation abilities between the sexes under different sand burial depths.

## 3. Results

### 3.1. Sex-Specific Responses of Survival and Growth Traits to Sand Burial Depths

The results show that sand burial depths significantly affected the survival of saplings. The survival rate of saplings was 100% in the unburied control and the T33 and T67 treatments, whereas the survival rates of female and male saplings were 30% and 35%, respectively, under the T90 treatment. None of saplings of either sex survived under the T133 burial treatment. There were significant differences in total biomass and its component organs in the experiment (Table 1). The total biomass of the T33 treatment was the highest, whereas that of T90 treatment was the lowest. Both sexes showed higher leaf, root, root nodule, total biomass and number of stem branches under the T33 treatment compared to controls and other sand burial treatments. Moreover, males had more root nodules, higher total biomass and more stem branches than females under the T67 treatment. Sex significantly affected all growth parameters, except for leaf biomass and the R/S ratio, whereas sand burial had a significant effect on all determined growth traits. An interactive

effect between sex and sand burial was observed on total biomass, stem branch number and root nodule biomass.

**Table 1.** Growth parameters of female and male saplings of *Hippophae rhamnoides* under different sand burial depth treatments.

Treatment	Leaf Biomass (g DW)	Stem Biomass (g DW)	Root Biomass (g DW)	Total Biomass (g DW)	R/S Ratio	Branch Number	Root Nodule Biomass (g DW)
Female control	8.54 ± 0.34 bc	8.62 ± 0.37 b	2.13 ± 0.15 cd	19.30 ± 0.45 b	0.13 ± 0.01 a	8.6 ± 0.4 cd	0.20 ± 0.01 bc
T33	10.59 ± 0.33 a	10.05 ± 0.35 ab	2.97 ± 0.16 ab	23.61 ± 0.47 a	0.14 ± 0.01 a	11.2 ± 0.58 ab	0.23 ± 0.02 ab
T67	6.56 ± 0.34 d	6.53 ± 0.31 bc	1.66 ± 0.23 de	14.74 ± 0.36 c	0.13 ± 0.02 a	6.4 ± 0.5 de	0.13 ± 0.01 d
T90	2.89 ± 0.20 e	5.24 ± 0.26 c	1.1 ± 0.07 e	9.24 ± 0.37 d	0.14 ± 0.01 a	4 ± 0.32 e	0.10 ± 0.01 d
Male control	7.05 ± 0.33 cd	8.77 ± 0.64 b	2.53 ± 0.19 bc	18.35 ± 0.39 b	0.16 ± 0.01 a	9.6 ± 0.4 bc	0.21 ± 0.01 bc
T33	9.93 ± 0.41 ab	11.50 ± 0.38 a	3.59 ± 0.13 a	25.03 ± 0.78 a	0.17 ± 0.02 a	13 ± 0.45 a	0.28 ± 0.02 a
T67	6.72 ± 0.52 d	9.07 ± 0.66 b	2.24 ± 0.08 cd	18.03 ± 1.17 b	0.14 ± 0.02 a	12.2 ± 0.92 a	0.26 ± 0.02 ab
T90	3.02 ± 0.24 e	6.39 ± 0.35 c	1.08 ± 0.05 e	10.48 ± 0.40 d	0.12 ± 0.01 a	5.2 ± 0.37 e	0.16 ± 0.01 cd
Sex effect	NS	***	***	**	NS	***	***
Sand burial effect	***	***	***	***	*	***	***
Sex × sand burial effect	NS	NS	NS	*	NS	***	***

Notes: Each value represents the mean ± SE of five replicates. Different lowercase letters in the same column indicate significant differences between females and males of *H. rhamnoides* under different sand burial depths according to multiple comparisons followed by Bonferroni's test ( $p < 0.05$ ). The effects of sex, sand burial and their interaction on the growth parameters were determined according to a two-way ANOVA. Control, unburied treatment; T33, T67, T90 and T133 correspond to sand burial depths of 33, 67 and 90 percent of the initial height of the saplings at the beginning of the experiment. \*  $0.01 < p < 0.05$ ; \*\*  $0.001 < p \leq 0.01$ ; \*\*\*  $p \leq 0.001$ ; NS, not significant.

### 3.2. Sex-Specific Response of Photosynthetic Traits to Sand Burial Depths

Both sexes showed the highest  $P_n$  and  $G_s$  values under the T33 treatment, whereas the lowest  $E$  and  $G_s$  values were observed under the T90 treatment (Figure 1a–c). Moreover, females and males differed significantly in their responses to sand burial depths. Female saplings showed more inhibited  $P_n$  under T67 and lower WUE values than males under the T67 and T90 treatments (Figure 1a,d). However, male saplings had lower  $G_s$  values than females under the T90 treatment (Figure 1b). Sex, sand burial depth and their interaction significantly affected  $P_n$ ,  $E$ ,  $G_s$  and WUE (Table 2).

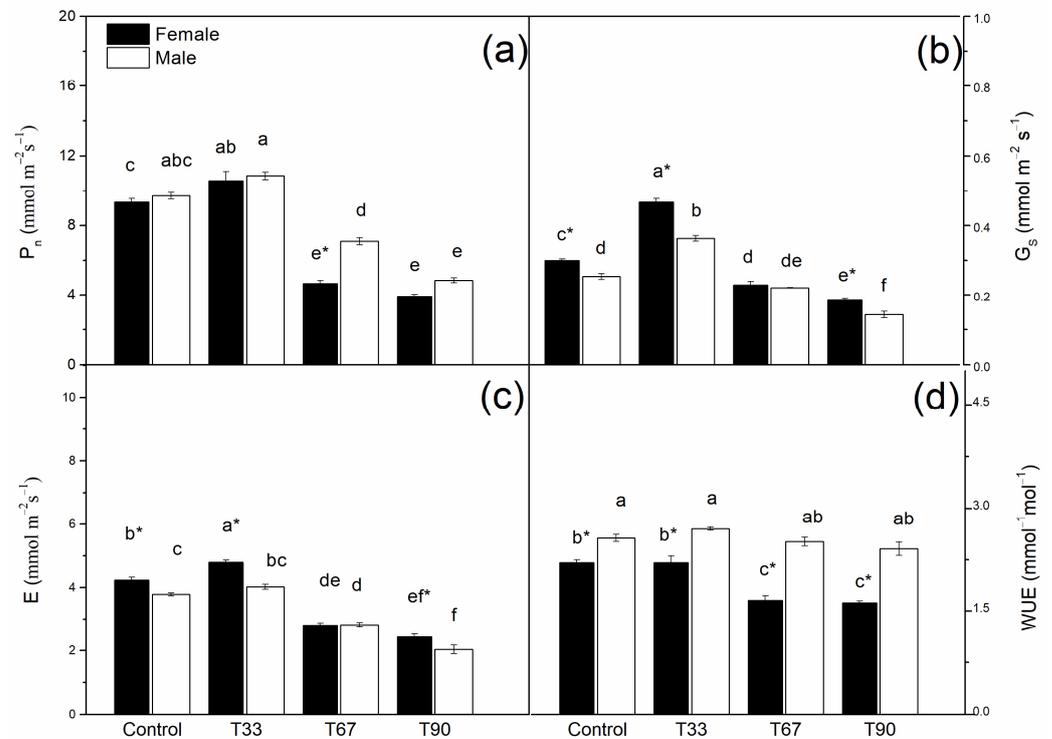
### 3.3. Sex-Specific Response of Leaf Chlorophyll Fluorescence to Sand Burial Depths

Female saplings showed a greater inhibition effect on  $F_v/F_m$  under the T67 treatment (Figure 2a), which was decreased by 7.59% compared to the controls. However, there were no significant differences between the sexes under other sand burial treatments. The  $\Phi$  of females significantly decreased by 7.20% and 10.52% under the T67 and T90 treatments, respectively. Female saplings showed the highest  $qP$  under the T33 treatment, whereas both sexes had the lowest  $qP$  values under the T90 treatment (Figure 2c). Female saplings had the lowest  $qN$  values under the T90 treatment, whereas no significant difference in  $qN$  was observed between the two sexes under the T33 and T67 treatments (Figure 2d). The sand burial depth had a significant effect on all chlorophyll fluorescence parameters. Sex alone and the interaction between sex and sand burial significantly affected  $F_v/F_m$  and  $qN$  (Table 2).

### 3.4. Sex-Specific Responses of Leaf Chlorophyll Pigment and Water Potential to Sand Burial Depths

In females, the T67 and T90 sand burial treatments significantly decreased chlorophyll<sub>a</sub> (Chl<sub>a</sub>) and chlorophyll<sub>a+b</sub> (Tchl<sub>a+b</sub>) contents, whereas the T33 treatment showed no significant difference compared with controls. In males, the Chl<sub>a</sub> and Tchl<sub>a+b</sub> contents significantly increased by 30.91% and 29.60%, respectively, under the T33 treatment, whereas only the T90 treatment decreased Chl<sub>a</sub> and Tchl<sub>a+b</sub> (Figure 3a–c). The T90 treatment significantly decreased the water potential of both sexes, whereas the water potential of females de-

creased by 31.87% under the T67 treatment, indicating a more negative effect on females (Figure 3d). Sex independently affected water potential. Sand burial had a significant effect on chlorophyll pigment and water potential. The interaction between sex and sand burial affected  $Chl_a$ ,  $Tchl_{a+b}$  and water potential (Table 2).



**Figure 1.** Mean values ( $\pm$ SE,  $n = 5$ ) of photosynthetic parameters in the leaves of females and males of *H. rhamnooides* under different sand burial depths: (a)  $P_n$ , net photosynthetic rate; (b)  $G_s$ , stomatal conductance; (c)  $E$ , transpiration rate; (d) WUE, water-use efficiency. Different lowercase letters indicate significant differences between sexes and sand burial treatments according to Bonferroni's tests ( $p < 0.05$ ). \* indicates significant differences between female and male saplings at the same treatment depth ( $p < 0.05$ ). Control, unburied treatment; T33, T67 and T90 correspond to sand burial depths of 33, 67 and 90 percent of the original plant height, respectively.

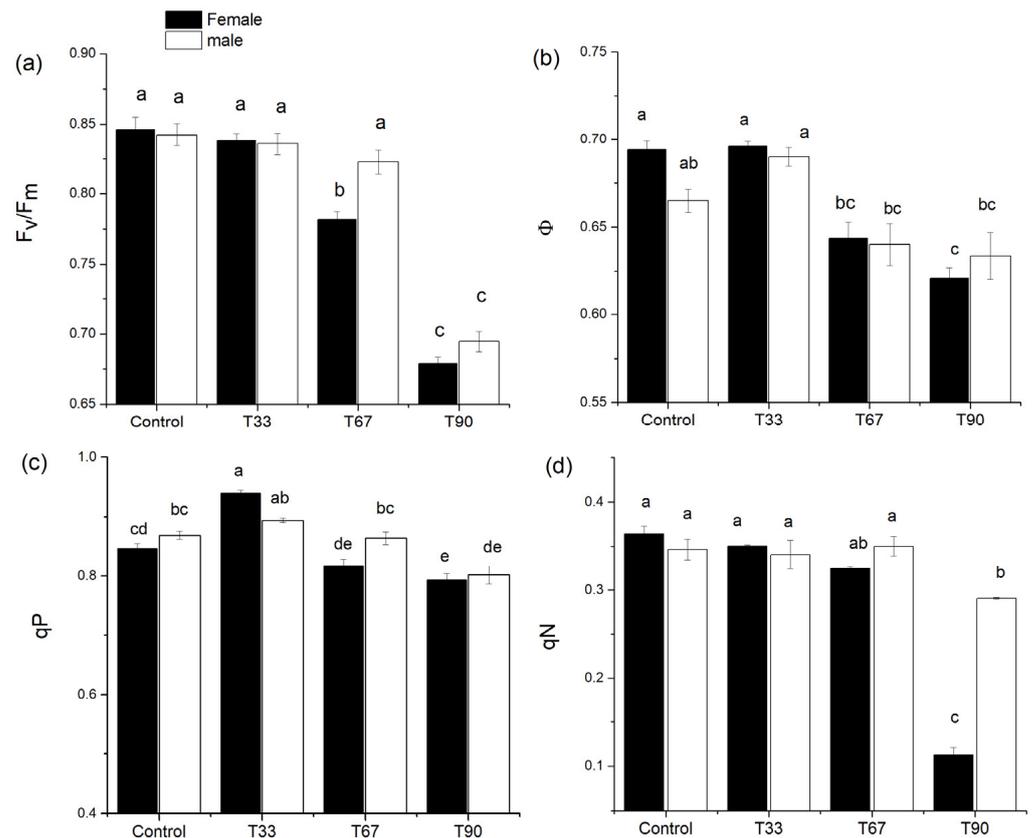
**Table 2.** Statistical significance of single and interactive effects of sex and sand burial depth on physiological parameters of *H. rhamnooides* saplings based on two-way ANOVA.

Variable	Sex		Sand Burial		Sex $\times$ Sand Burial	
	F	<i>p</i>	F	<i>p</i>	F	<i>p</i>
$P_n$	31.776	***	281.891	***	7.874	***
$G_s$	79.091	***	367.463	***	12.994	***
$E$	47.44	***	302.007	***	7.766	***
WUE	182.406	***	21.982	***	6.423	**
Water potential	8.589	**	24.588	***	3.727	*
$Chl_a$	1.728	NS	51.297	***	5.696	**
$Chl_b$	0.422	NS	9.289	***	1.239	NS
$Tchl_{a+b}$	0.542	NS	57.401	***	6.26	**
$F_v/F_m$	6.083	*	205	***	4.123	*
$\Phi$	1.231	NS	28.151	***	2.187	NS
qP	1.367	NS	52.836	***	8.135	***
qN	45.297	***	123.922	***	48.920	***
leaf starch	0.155	NS	32.656	***	0.458	NS
root starch	175.023	***	83.528	***	12.244	***

Table 2. Cont.

Variable	Sex		Sand Burial		Sex × Sand Burial	
	F	p	F	p	F	p
stem starch	24.998	***	54.232	***	7.488	***
leaf sucrose	45.424	***	510.238	***	12.663	***
root sucrose	56.552	***	154.046	***	34.931	***
stem sucrose	8.828	**	14.049	***	2.49	NS

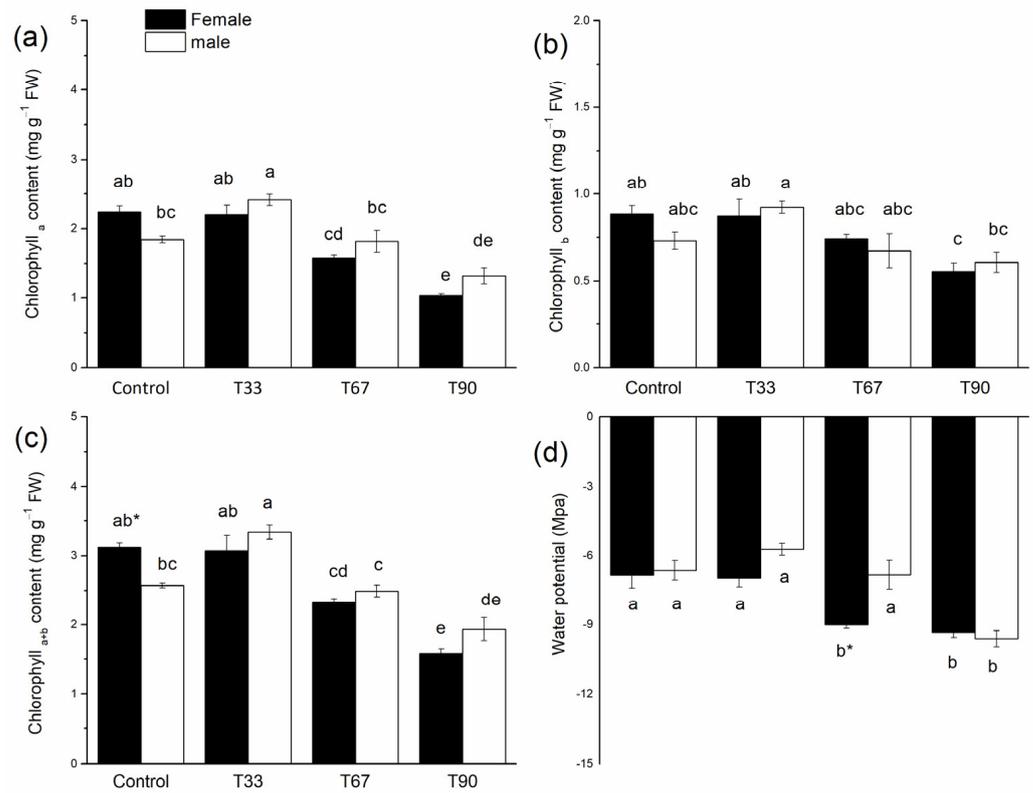
Note: \*  $0.01 < p < 0.05$ ; \*\*  $0.001 < p \leq 0.01$ ; \*\*\*  $p \leq 0.001$ ; NS, not significant.



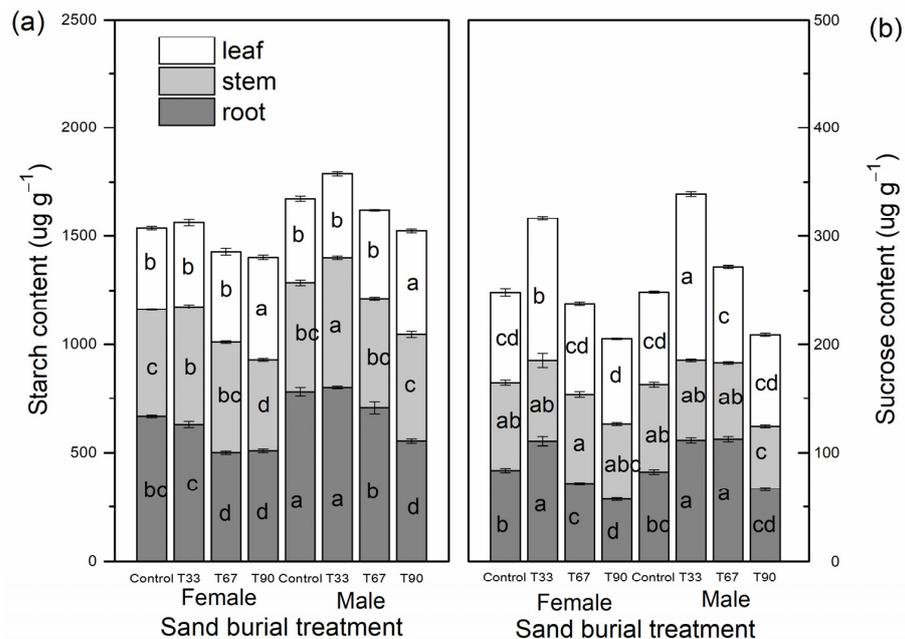
**Figure 2.** Mean values ( $\pm$ SE,  $n = 5$ ) of chlorophyll fluorescence parameters of female and male saplings of *H. rhannoides* under different sand burial treatments: (a)  $F_v/F_m$ , dark-adapted quantum yield; (b)  $\Phi$ , effective quantum yield of PSII; (c)  $qP$ , photochemical quenching coefficient; (d)  $qN$ , non-photochemical quenching coefficient. Different lowercase letters indicate significant differences between sand burial treatments according to Bonferroni's test ( $p < 0.05$ ).

### 3.5. Sex-Specific Response of Carbohydrate Content to Sand Burial Depths

Under the T90 treatment, root starch and sucrose decreased and leaf starch increased in both sexes; moreover, females showed lower stem starch than males. Compared with controls, the T67 treatment decreased root starch in both sexes (9.88%~24.72%) and sucrose in females (14.30%) and increased root sucrose in males (36.39%). Moreover, males showed higher root starch and sucrose contents than females under the T67 treatment. Compared with controls, stem starch and leaf and root sucrose contents increased in both sexes under the T33 treatment. Moreover, males showed higher stem and root starch and leaf sucrose contents than females under the T33 treatment (Figure 4a,b). Sex independently affected carbohydrate contents, except for leaf starch. Sand burial had a significant effect on carbohydrate contents. The interaction between sex and sand burial significantly affected carbohydrate contents, with the exceptions of stem sucrose and leaf starch contents (Table 2).



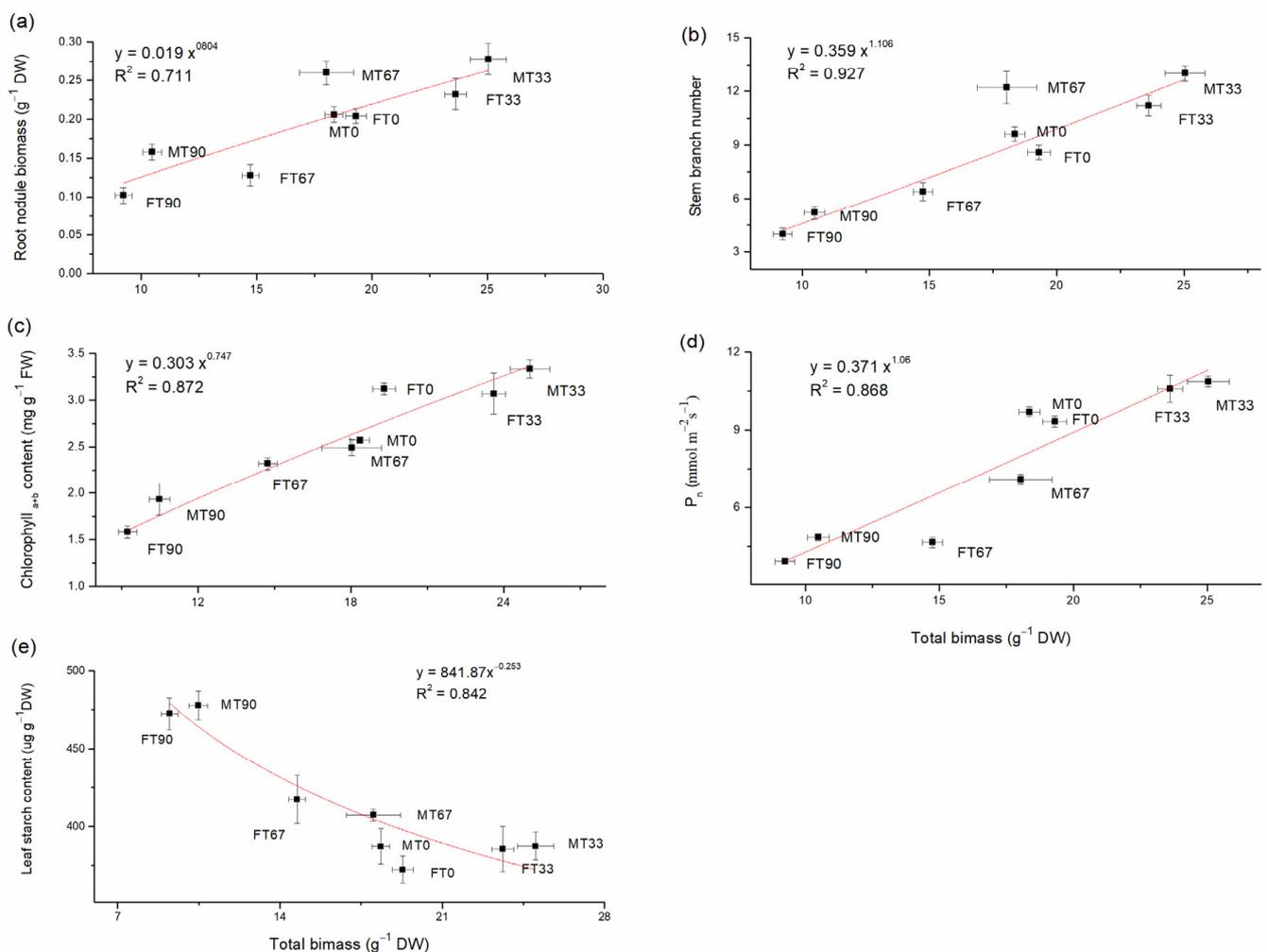
**Figure 3.** Mean values (±SE, n = 5) of chlorophyll content and water potential of female and male saplings of *H. rhamnoides* under different sand burial treatments: (a) chlorophyll<sub>a</sub> content; (b) chlorophyll<sub>b</sub> content; (c) total chlorophyll<sub>a+b</sub> content; (d) water potential. Different lowercase letters indicate significant differences between sand burial treatments according to Bonferroni's test (p < 0.05). \* indicates significant differences between female and male individuals at the same burial depth (p < 0.05).



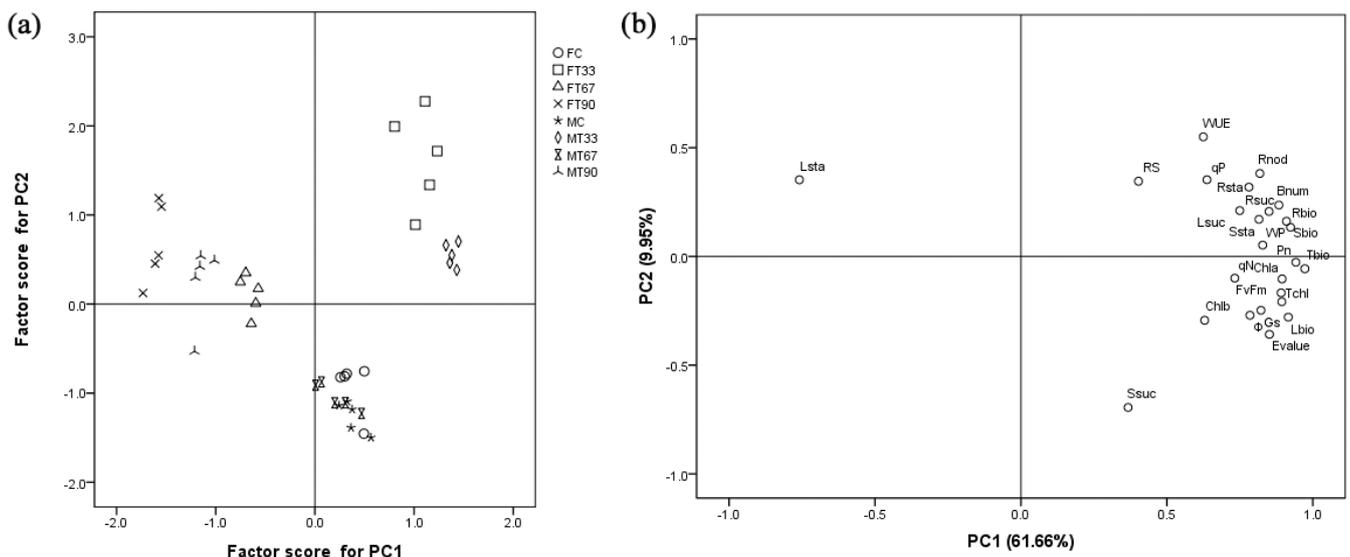
**Figure 4.** Mean values (±SE, n = 5) of carbohydrates of female and male saplings of *H. rhamnoides* under different sand burial depths. (a) Starch content. (b) Sucrose content. Different lowercase letters indicate significant differences between sand burial treatments according to Bonferroni's test (p < 0.05).

### 3.6. Results of Correlation and Principal Component Analysis

Across sexes and sand burial depth treatments, total dry biomass had a significant positive relationship with root nodule biomass (Figure 5a,  $y = 0.019x^{0.804}$ ,  $R^2 = 0.711$ ), stem branch number (Figure 5b,  $y = 0.359x^{1.106}$ ,  $R^2 = 0.927$ ), chlorophyll<sub>a+b</sub> content (Figure 5c,  $y = 0.303x^{0.747}$ ,  $R^2 = 0.872$ ) and  $P_n$  (Figure 5d,  $y = 0.371x^{1.06}$ ;  $R^2 = 0.868$ ). However, total biomass had a significant negative relationship to leaf starch content (Figure 5e,  $y = 841.87x^{0.253}$ ,  $R^2 = 0.842$ ). PCA showed a clear demarcation between the two sexes under different sand burial treatments (Figure 6a). The PCA model with two components explained 71.61% of total variances under different treatments. The first component (61.66%) was strongly influenced by leaf, stem, root and total biomass; branch number; root nodules;  $F_v/F_m$ ;  $\Phi$ ;  $qP$ ;  $qN$ ;  $P_n$ ;  $G_s$ ;  $E$ ; WUE; starch contents of leaves, stem and roots; sucrose content of leaves and roots;  $Chl_b$ ;  $Tchl$ ; and water potential, which were negatively correlated with leaf starch. The second component (9.95%) was strongly influenced by WUE and negatively correlated with stem sucrose content (Figure 6b).



**Figure 5.** Correlation analysis and non-linear model regression between total biomass (mean  $\pm$  SE,  $n = 5$ ) and (a) root nodule biomass, (b) stem branch number, (c) chlorophyll<sub>a+b</sub> content (d) net photosynthetic rate ( $P_n$ ) and (e) leaf starch content of two sexes of *H. rhamnooides* under different sand burial treatments. FT0 and MT0, female and male unburied control treatment, respectively; FT33 and MT33, females and males, respectively, under 33% sand burial treatment; FT67 and MT67, females and males, respectively, under 67% sand burial treatment; FT90 and MT90, females and males, respectively, under 90% sand burial treatment.



**Figure 6.** Results of the principal component analysis (PCA) showing (a) distributions of females and males of *H. rhamnoides* under different sand burial treatments (b) correlations of factor loadings for all ecophysiological parameters in the interactive treatments of sex and sand burial. The variable acronyms are as follow:  $P_n$ , light photosynthetic rate;  $E$ , transpiration rate,  $G_s$ , stomatal conductance;  $F_v/F_m$ , dark-adapted quantum yield of PSII;  $\Phi$ , effective quantum yield of PSII;  $qN$ , non-photochemical quenching coefficient;  $qP$ , photochemical quenching coefficient;  $Lsta$ , leaf starch content;  $Ssta$ , stem starch content,  $Rsta$ , root starch content;  $Lsuc$ , leaf sucrose content;  $Ssuc$ , stem sucrose content;  $Rsuc$ , root sucrose content;  $Chl_a$ ,  $Chl_b$  and  $Tchl$ , content of chlorophyll  $a+b$  and total chlorophyll content, respectively;  $Lbio$ , leaf biomass;  $Sbio$ , stem biomass;  $Rbio$ , root biomass;  $RS$ , root-to-shoot ratio;  $WP$ , water potential.

#### 4. Discussion

Plants develop various adaptive characteristics to acclimate to sand burial and show different survival and growth performance under desertification conditions [5,33–36]. Our study demonstrated that sand burial depths differently affected the ecophysiological parameters of two sexes of *H. rhamnoides*. Growth traits of both sexes were greater under the T33 treatment compared to controls, as reflected higher leaf, root and total biomass, indicating a positive effect caused by a shallow sandy burial treatment. It was reported that shallow burial depth increased relative growth rates of *Salix gordejewii*, *Artemisia wudanica* and *Artemisia halodendron*, which is similar to our results [37,38]. A suitable sand burial depth can reduce soil temperature and maintain adequate moisture for the roots, both of which are beneficial to the survival and resource capturing of saplings [39,40]. The survival rate of both sexes decreased sharply under the T90 treatment, and all saplings died under the T133, possibly due to photosynthesis inhibition, respiratory failure and energy and resource exhaustion [7,33,34,41]. It was reported that the mortality of male *Silene otites* was significantly higher than that of females under drought stress [25], whereas females of *Baccharis dracunculifolia* showed a significant increase in mortality relative to males [42]. However, our results revealed no significant sexual difference in survival rates or determined parameters under the T90 treatment, indicating that excess sand burial depths may diminish sexual dimorphism of *H. rhamnoides*.

Dioecious plants exhibit sexual dimorphism in response to stressful environments. Previous studies indicated that males show a higher photosynthetic rate, a lower resource demand and higher resource utilization efficiency in stressful environments [14,30]. Sex-specific growth and physiological responses of *H. rhamnoides* to water stress have been reported [29]. Our results reveal sex-specific responses of *H. rhamnoides* saplings exposed to different sand burial depths. Males had more stem branches; greater stem branches, root nodule and total biomass; and better photosynthetic and carbon balance capacities than

females under the moderate T67 treatment, which indicates that males of *H. rhamnoides* may have better resource capture and utilization capacities than females to adapt to sand burial. Different sand burial treatments have striking effects not only on biomass allocation but also on physiological processes [33,41]. Plants are expected to maximize their photosynthetic efficiency and to enhance the probability of survival under sand burial conditions [36,40]. In both sexes of *H. rhamnoides* significantly increased leaf and root biomass was closely correlated with the highest  $P_n$  and  $G_s$  under the T33 treatment. Both sexes had significantly decreased  $P_n$ ,  $G_s$  and  $\Phi$  values under the T67 and T90 sand burial treatments, resulting in further growth and biomass inhibition. Chlorophyll fluorescence reflects PSII function and light-harvesting efficiency. Both sexes showed significant decreases in  $F_v/F_m$ ,  $\Phi$ ,  $qP$  and  $qN$  under deeper sand burial treatments (Figure 2), indicating a decrease in C metabolism and a photoinhibitory effect on PSII [8]. These changes may affect membrane lipid peroxidation, which has been reported to cause oxidative death of cells and plant mortality under sand burial conditions [35,42]. An inhibition effect of deep sand burial (T67 or T90) on chlorophyll content was observed in both sexes, with a more negative effect on females. Under the T90 treatment, both sexes had decreased root starch and sucrose contents and increased leaf starch, causing photosynthetic depression. Males showed higher root starch and sucrose contents under the T67 treatment and higher stem and root starch and leaf sucrose content than females under the T33 treatment, indicating a significant sexual difference in carbohydrates and C balance strategies under sand burial conditions. The higher stem starch and root sucrose in males implies better transportation and utilization of carbohydrates from resource to sink organs under sand burial treatments, which reflects a balance between carbohydrate production during photosynthesis and use during plant growth [43].

Considering both sexes and depths of sand burial treatments, total biomass had a significant positive relationship with dry mass of root nodules, stem branch number, total chlorophyll<sub>a+b</sub> content and  $P_n$  and a significantly negative relationship with leaf starch content (Figure 5). Functional traits showed different contributions to adaptation of males and females to sand burial treatments, as detected by PCA (Figure 6b). These intrinsic functional traits of *H. rhamnoides* related to N absorption, light capture and photosynthesis and C metabolism may be critical for successful colonization and growth in a sand burial environment. The adaptation of the two sexes differed significantly, varying with sand burial depths. As shown in Figure 6a, both sexes had higher factor scores under T33 than other treatments, indicating a more obvious promoting effect of shallow sand burial on both sexes. Both sexes under T90 and females under T67 treatment were closely distributed and could be regarded as a group, whereas both sexes under control conditions and males under the T67 treatment had a similar factor score, indicating better ecophysiological parameters in males under the T67 treatment than those of females. Sand deposition may change the ratio between aerobic and anaerobic microbes and reduce the amount of mycorrhizal fungi [44], ultimately affecting soil N and C cycle in the ecosystem. Adaptation differences between the two sexes to sand burial may play an important role in a given soil ecosystem. It was reported that males of *Populus cathayana* promoted the amount of soil acid phosphatase and the colonization rate and biomass of arbuscular mycorrhizal, indicating better phosphorus absorption under conditions of phosphorus deficiency [45]. It is known that Hippophae can form N-fixing root nodules when infected with *Actinomyces Frankia* [46–48]. In our study, higher root nodule biomass was observed in males than females under T33 and T67 treatments, which may be related to more nodule bacteria and a higher N-fixation capacity. Previous studies reported that nitrate strongly inhibited nodule biomass and nitrogenase activity in Hippophae and Casuarina [49,50]. In addition, phosphorus (P), N and their interaction affected N fixation and partitioning in *H. rhamnoides* [51]. Therefore, the dynamics of root nodules of *H. rhamnoides* during sand burial treatments may imply potential changes in N and P content in soils. From the perspective of longer-term dynamics, possible changes in C-based biochemical components and N fixation under the sand burial condition may influence C circulation and storage in a given desertification ecosystem [52].

## 5. Conclusions

In this study, female and male saplings of *H. rhamnoides* exhibited significantly different responses to sand burial depths. The shallow sand burial treatment (T33) promoted the growth of both sexes, whereas T67 and T90 treatments inhibited biomasses and physiological parameters of both sexes. Different depths of sand burial affected sex-specific responses. Males had higher stem starch and root sucrose contents, exhibited a greater increase in root nodule biomass under T33 treatment and had a stronger ability to withstand sand burial than female saplings under the moderate sand burial treatment (T67). Although deeper sand burial treatments (T90 and T133) diminished sex-specific responses, the sexual dimorphism in the responses of *H. rhamnoides* to sand burial may affect the coexistence of the two sexes and the sex ratio, as well as soil N fixation, in desertification areas in the future.

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