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Crop Water Stress Management in the Tropics and Subtropics



**The Adaptation Mechanism of *Leymus chinensis* to Grazing
and Salt-alkali Stress**

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award of Master of Science degree in Agricultural Sciences

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Abstract

The Songnen grassland, a part of the central Eurasian Steppe ecosystem, is one of the main districts in which Chinese saline-alkali soil concentrates. Overgrazing often enhanced degradation processes and turned out to be an additional major threat for livestock farming. *Leymus chinensis*, a dominant rhizome grass in this semi-arid ecosystem, is considered to be grazing and salt-alkalinity tolerant. However, the adaptation mechanisms of *Leymus chinensis* to grazing and salt-alkalinity stress are unclear. A field experiment was conducted in 2009 and 2010 combining salt-alkali application with clipping to simulate salt-alkali stress and grazing respectively. The aboveground and belowground biomass, plant density and photosynthesis were measured in the field. The proline, oxalic acid and water soluble carbohydrate were measured in the lab.

Our results indicated that salt and clipping had an interactive effect on aboveground biomass (AB) of *L. chinensis*. Salt addition significantly increased AB under no clipping treatment but not under clipping. Clipping significantly decreased AB regardless of salt addition which indicated no compensatory growth. However, relative growth rate and photosynthesis were higher under clipping treatments. In addition, the water soluble carbohydrate contents in rhizomes were reduced under clipping which indicated that sugar was transferred to the aboveground organs for new leaves rebuilding.

The higher productivity of *L. chinensis* under salt stress was mainly due to the integration effect between rhizome and ramets. The ramet density was significantly decreased while the individual ramet biomass was higher under salt stress. The increase of ramet biomass was due to higher leaf area index and net photosynthesis rates therefore they compensated the loss of ramet

number. The reduction of ramet number can not only decrease the material and energy competition, but also can remove salt ions away to lower down the salt stress. However, the root biomass was significantly decreased after two years stress, therefore, the integration function is probably an unsustainable strategy of *L. chinensis* to cope with salt stress, and long-term field studies are needed to understand the combined effects of salt-alkalinity and grazing on growth performance of *Leymus chinensis*.

Key words: *Leymus chinensis*, Salt stress, Grazing, Compensatory growth, Photosynthesis

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List of Abbreviations

AB	Aboveground biomass
S	Salt addition
C	Clipping
EC	Electrical conductivity
RGR	Relative growth rate
R/S	Root to Shoot ratio
Pn	Net photosynthesis rate
Gs	Stomatal conductance
Ci	Intercellular CO ₂ concentration
Tr	Transpiration rate
WUE	Water use efficiency
OA	Oxalic acid
WSC	Water soluble carbohydrate
LAI	Leaf area index
Chl	Chlorophyll

1 Introduction

Soil salinity has been considered a limiting factor to crop production in arid and semi-arid regions of the world (Munns, 2002; Deng et al., 2006). The saline and sodic soils cover about 10% of total arable lands and exist in over 100 countries (Tanji, 1990). Saline and alkaline soils (6.7×10^6 hm²) cover approximately 7% of cultivated lands in China (Deng et al., 2006). Alkalization and salinization exert severe effects on the natural grasslands and farming lands in northeast China. The natural semi-arid steppe ecosystems of Northern China are increasingly affected by overgrazing, hay-production, and inappropriate farming systems. These land-use practices can result in large-scale development of salt-alkaline soil and substantial losses of arable lands (Kato et al., 1998; Zhou et al., 2006; Christensen et al., 2004).

Due to the long evolutionary history of grazing in the central Asian steppe ecosystems, plants have developed mechanisms of grazing tolerance. The grazing tolerance and dominant steppe plant species such as *Leymus Chinensis* (Trin.) Tzvel, is often combined with an adaptation to drought and salt-alkaline stress (Wang et al., 1994). *L. chinensis* is perennial asexual reproductive plant which is composed of the initial “mother” plant and clones growing from the rhizoms called “ramets”. They interact with each other via the rhizome. This integration helps the “plant-network” to cope with different environments by alternate ramet biomass allocation, root to shoot ratio or plant height (Sui et al., 2011).

Although in the Songnen grasslands, grazing and salinity stress often interactively effect on vegetation, not much is known about the underlying mechanisms of stress tolerance due to the lack of studies combining both stress factors in field experiments. A two years field experiment was conducted

in 2009 and 2010 combining mixed salt-alkali solution (NaCl: NaHCO₃: Na₂CO₃ = 1:1:1) addition with clipping (removal 60% of aboveground biomass) to simulate the interactive effect of both stress factors on *L. chinensis*. Several morphological (biomass, ramet density, plant height and leaf area index) and physiological parameters (photosynthesis parameters, proline, oxalic acid and water soluble carbohydrate content) were measured to analyze the adaptation mechanisms of *Leymus chinensis* to defoliation and salt-alkaline stress.

2 Hypotheses & Objectives

The following questions are introduced based on the above consideration: Does *Leymus chinensis* have a compensatory growth capacity in response to clipping? How is compensatory growth affected by salt stress? Which morphological and physiological traits can be linked to compensatory growth?

Hypothesis: (1) ramets subjected to salt stress would benefit from clonal integration. The rhizomes and fine roots of *L. chinensis* may have dilution ability that sacrificing some of their ramets to ensure survival of the whole population under salt stress. (2) The content of water soluble carbohydrate (WSC) in rhizome is determinant factor for grazing and salt tolerance.

3 Literature review

3.1 *Leymus chinensis*

Leymus chinensis (Trin.) Tzvel., a highly palatable grass for livestock, is widely distributed in Russia, Japan, North Korea and northeastern China (Zhu, 2004). It is a perennial species of Gramineae which often grows on plains, hilly, and saline lowlands. In the forest-steppe ecotone and the adjacent dry grassland area of Hulunbeier and Xilin Gol this species is a dominating component of the plant community. Songnen grassland is another main distribution area for *L. chinensis*. The high nutrient content and palatability of *L. chinensis* makes it a very important forage grass for livestock feeding (Wang et al., 2004).

L. chinensis is often found on chernozem and kastanozem soils, alkalized meadow steppe soils, and even on high alkaline soils and they can adapt to extremely cold continental climates as well as, periods of drought during the growing season. They can even grow well in the regions with scarce annual precipitation of about 250 mm (Yang et al., 1995). The preferential soil pH range is pH 6-8 but it can survive in pH 5.5 - 10.3. However, it is sensitive to flooding and water logging (Wang et al., 1994). *L. chinensis* generates long rhizomes in about 10 cm below the soil surface for vegetative (clonal) reproduction (Dong, 1996; Yang et al., 1997). Aboveground organs become senescent at the end of the growing period, individual plants can grow for 10 - 20 years (Zhu, 2004).

3.2 The strategies of grazing tolerance

3.2.1 Morphological adaptation

Grazing is a very common phenomenon in grassland ecosystems. The loss of leaf area reduces the plant's gas exchange capacity and the amount of carbon uptake. Strategies of plants to cope with frequent defoliation by herbivores include morphological avoidance and physiological tolerance (Zhou et al., 2006). Avoidance refers to plants ability of reducing the possibility and extent of consumption by animals which can achieve through changing plant structure and chemical composition (Teng et al., 2004). In order to avoid grazing damage, plants can generate hard cuticle structure, needles, hooks, spines and hair on branches or leaves which make the plants unattractive or unpalatable for grazers (Grant, 1984). Some plants can regulate height, leaf angle; produce secondary metabolites (reduce palatability) or increase asexual reproduction (stolons, adventitious roots) to avoid feeding (Wang et al., 2007; Zhang et al., 2002).

There are some researches on grazing tolerance of *L. chinensis* which indicate that grazing can promote the initiation of dormant buds on the rhizome. High grazing pressure was found to decrease internodes distances of the rhizome which resulted in the production of more aboveground ramets per area (Li and Wang, 1997). Some studies showed that *L. chinensis* changes from the upright growth to prostrate growth or even growth along the soil surface under high grazing pressure to keep aboveground plant organs below the biting height of herbivores (Tang et al., 2010).

3.2.2 Physiological adaption

3.2.2.1 Compensatory growth

Grazing tolerance of plants refers to the ability of offset feeding effect through changing the validity of remaining apical meristem and physiological adjustment to stimulate compensatory growth. Compensatory growth is a positive response to defoliation through increasing plant productivity and fitness, which can be classified into three types: 1) overcompensation occurs when the cumulative total dry weight (including re-moved tissue) of the grazed or clipped plants is greater than the total dry weight of the control plants; 2) equal-compensation occurs when the cumulative dry weight of the treated plants equals that of the controls; and 3) under-compensation occurs when the cumulative dry weight of the treated plants is less than that of the control (Belsky, 1986). It depends on the balance between the promotion and inhibition effects, while this balance is determined by the grazing and environmental conditions, such as grazing intensity, water status, and soil nutrient resources.

3.2.2.2 Herbivore optimization curve

The positive effects of grazing on plant productivity and fitness have also been referred to as herbivore optimization, which shows that the net primary or above-ground productivity of grazed plants increases at low to moderate levels of herbivory until productivity is maximized at an optimal grazing level, beyond which it begins to fall. At high grazing intensities, productivity falls below un-grazed levels (Belsky, 1986).

3.2.2.3 Compensation mechanism

Compensatory growth mechanisms have been discussed by several authors

(Chew 1974; McNaughton 1979, 1983; Hilbert et al. 1981). Briefly stated, these mechanisms are: increase light availability or leaf nitrogen concentration on the remaining leaves (Anten & Ackerly 2001); increase biomass allocation to new leaf production and the using of stored carbohydrates in the stubble and roots to supply energy for photosynthesis (Gao et al. 2009; Liu et al. 2003; McPherson & Williams 1998); removal of older, less photosynthetically active tissue; increased light intensity to underlying tissues; increased tillering or shoot development following removal of apical or dominant meristems; increased water-use efficiency through reduction of transpirational surfaces; and activation of dormant buds.

The contribution of these different mechanisms probably differs between species and their different growing conditions. Some studies found that under moderate grazing intensity *Cleistogenes squarrosa*, a common C4 perennial grass in Northern Chinese grasslands exhibited over-compensation through generating high density tillers, while under heavy grazing intensity it had compensatory growth through consumption stored belowground substance (Wang and Wang, 2001). Rainfall conditions during the growing season also affect the compensatory growth. Under sufficient rainfall condition, plants prone to over-compensate in moderate-intensity grazing (Wang and Wang, 2001). One study showed carbohydrate concentration in the stem bases of *L. chinensis* changed in response to stocking rates, which affected carbohydrate redistribution and thereby promoted the growth of new leaves (Liu 2003). Other studies showed that *L. chinensis* had less compensatory growth under dry compared to wet conditions (Van Staalduinen & Anten 2005; Gao et al. 2008).

3.3 The strategies of salt tolerance

3.3.1 Morphological adaptation

Plants can tolerate salt stress through morphological and physiological adjustment. According to different levels of adaptation they can be divided into the enhalophyte, secretohalophyte and pseudohalophyte (Breckle 1990). Enhalophyte can accumulate salt by special morphology, such as the succulent leaves and stems which absorbed large amounts of water to dilute the salt concentration; while they can also adapt to salt stress by osmotic adjustment and regionalization. Secretohalophyte rely on the morphological structure of salt gland and salt secretion to protect them from salt damage. Pseudohalophyte can survive under salt condition through hardly absorption, even if absorbed the salt ions are stored in the root, stem base rather than stems and leaves (Breckle 1990).

The common secretohalophyte is mainly *Limonium* in Songnen plain. The representative structures are salt glands and epidermal trichome (Lu and Li, 1994). Pseudohalophyte plants include *Puccinellia* and *Artemisia* which almost do not absorb the harmful salt ions from soil. However no halophytes characteristics were found in *L. chinensis*, such as salt glands, leaf carnification or high epidermal stomatal density (Zheng et al. 1995). Instead, some studies show that *L. chinensis* uses asexual reproductive strategy through rhizome to achieve continuous updating of the population. In the optimum environment, the bud on rhizome developed for new plants; while in unfavorable conditions, the bud bank developed for new underground rhizomes or even death (Yang 1995, Wang 1992).

3.3.2 Physiological adaption

3.3.2.1 Salt damage effect on plants

There are two classical views about salt damage to plants: one is physiological drought, the other is ion toxic effect (Flowers et al., 1986; Shi et al., 1998; Guo et al., 2009). The increase of salt concentration in soil solution will increase the osmotic pressure which results in water outflow from cells and then inhibits normal growth of plants. The main cations contained in saline-alkali soil are Na^+ , K^+ , Ca^{2+} , and Mg^{2+} ; anions are Cl^- , HCO_3^- , CO_3^{2-} , SO_4^{2-} and NO_3^- . Ion stress is mainly due to an excess absorption of Na^+ or Cl^- (Guo et al., 2009).

Plant photosynthesis, carbon metabolism and endogenous hormones will have a series of changes under salt stress. The photosynthesis inhibitory is mainly due to the composition and structure damage of thylakoid membrane and light synthase. In addition, physiological drought stress induced abscisic acid (ABA) synthesis in plants which can promote stomatal closure and stop the absorption of CO_2 (Netting, 2000).

3.3.2.2 Salt adaptation mechanisms

Although salt stress caused damage to plants, some of them can tolerate it. The main physiological stress adaptation approaches are: (1) ion compartmentation; (2) cell membrane permeability adjustment; (3) Ca^{2+} regulation; (4) osmotic adjustment.

3.3.2.2.1 Ion compartmentation

In salinity conditions, many plants can resist or reduce salt stress by regulating the absorption of ions and ion compartmentation (Flowers et al., 1986). Some plants transport inorganic ion through the membrane to vacuoles

and isolate them from cytoplasm which not only reduces the cell's osmotic potential, but also protects cytoplasm from ion toxicity. While some others minimize the absorption of harmful ions, and transport them to the old organizations that serve as ions storage which is a special strategy to protect the young tissue at the cost of old organizations (Xu et al., 2000; Zhou et al., 2003; Michelet and Boutry, 1995; Voilmm et al., 1998). Some studies also mentioned that *L. chinensis* can effectively absorb and transport Na⁺ to shoot and then store them in the vacuole under salt stress conditions (Flowers et al., 1986). Some studies also showed that the role of compartmentation is mainly dependent on the proton pump on plasma membrane to achieve transport (Michelet and Boutry, 1995; Voilmm et al., 1998).

3.3.2.2.2 Cell membrane permeability adjustment

Cell membrane is the first barrier between cells and the environment. Under normal growing conditions, the permeability of cell membrane is small. When the plants suffer salt stress, the membrane systems are always damaged due to the ions stress, which expressed as permeability increased, lipid peroxidation accelerated (Michelet and Boutry, 1995). In the neutral salt and alkali salt studies of *L. chinensis*, the cell membrane leakage rate of leaves increased with the increase of Na⁺ concentration, and increased faster under alkali conditions, which indicated that alkali stress caused more serious injury on *L. chinensis* (Shi et al., 1998).

3.3.2.2.3 Ca²⁺ regulation

The level of Ca²⁺ in cytoplasm is an important component of cell regulation and it is a second messenger in the intracellular metabolism (Grant et al., 1985; Cramer et al., 1987). Under normal conditions, Ca²⁺ enters into the cell through the active transport of Ca²⁺-ATPase located on plasma membrane. However, under salt stress, Ca²⁺ uptake was blocked, which probably due to

the damage of membrane and leading to Na^+ replaced Ca^{2+} . If certain Ca^{2+} were added to the external environment, the negative impact of NaCl on plants would be reversed and the extensibility of cell wall can be maintained as well as the plant growth. In addition, the Ca^{2+} channels of vacuole membrane will open under stress or stimulation of some hormonal (Anil et al., 2005). Ca^{2+} is released from the vacuole and banded with calmodulin which can regulate cell metabolism or gene expression, and promote plant to adapt to stress (Grant et al., 1985).

3.3.2.2.4 Osmotic adjustment

Another important adaptation of plants to salt stress was osmotic adjustment i.e. accumulate a certain amount of soluble organic substances in the cytoplasm as osmotic agent to balance the osmotic pressure between the external environment and internal vacuole (Yang et al., 2007). The accumulated osmolytes in plant cells can generally be divided into two categories: inorganic ions and organic solutes. Absorbing and accumulating inorganic ions from the environment requires less energy, but can cause toxicity to plants with excessive accumulation. Therefore, the plants resort to synthesis of small molecules organic solutes in the cell as osmolytes to adapt to stress environment. The soluble organic substances include proline, organic acids and soluble sugar (Wang 2006; Chen et al. 2006; Yang et al. 2007; Yin et al. 2003).

Proline

A large number of plants accumulate proline under osmotic stress, its biological functions are: Firstly, as a cytoplasmic osmolyte to reduce cell water potential, which benefit to absorbing water; secondly, as anti-dehydrating agent. proline is a water soluble neutral un-toxicity substances, high concentrations of proline can reduce water potential of cell, maintain water

content and cell turgor; thirdly, proline can reduce the poisoning of free ammonia generated by proteolysis due to osmotic stress. In addition, proline can service as a nitrogen and carbon skeletons storage which can supply material and energy for recovery growth; fourthly, protect cell membrane and maintain metabolism function (Chen et al., 2006). Proline has a dipole, its hydrophobic side connected with protein, hydrophilic side connected with water which can protect the spatial structure of biological polymers, and prevent protein from denaturation under osmotic stress (Chen et al., 2006; Zhu et al., 1983). Some studies indicated that the concentration of proline in *L. chinensis* was higher in alkali condition than control (Shi et al., 1998; zhou et al., 2003).

Organic acids

Organic acids in plants are mainly from the citric acid cycle, including succinic acid, citric acid, malic acid, lactic acid and oxalic acid (Wang, 2006). Organic acids in plants participate in many important metabolic reactions, such as photosynthesis, respiration and nitrogen metabolism (Yang et al., 2007, 2008, 2009 a b). And they also play an important role in osmotic adjustment and ions balance (Zheng et al., 1995; Zhu, 2003). Many studies indicate that plants can achieve osmotic adjustment through the accumulation and secretion of organic acids under high salt concentration (Yang et al., 2007, 2008). Some studies showed that under alkaline stress the organic acids, citric acid, proline content of *Puccinellia tenuiflora* were increased (Yin et al., 2007); wheatgrass accumulate a large amount of organic acids under alkaline stress (Yang et al., 2007).

Soluble carbohydrate

Carbohydrates are the main products of plant photosynthesis, which are also important energy source for a variety of plant. They can be divided into

structural carbohydrates and non-structural carbohydrates, the former one including lignin, cellulose and semi-cellulose, which are mainly for plant morphogenesis; the latter includes glucose, fructose, starch, sucrose, fructan and mannitol, which are major reactants of plant (Balibera et al., 1997). Soluble carbohydrates help to regulate many developmental and physiological processes, and are also closely related with plant resistance. Some studies have pointed out that the NaCl stress will cause plants to reduce the soluble carbohydrate contents (Bohnert et al. 1995; Ingram and Bartels, 1996; Balibera et al., 1997; Ibrahim, 2004). However, there are differences between different species. In salt-tolerant species, soluble carbohydrate increased in response to salinity, in contrast, the sensitive one showed a decrease in the soluble carbohydrate (Bohnert et al. 1995; Ingram and Bartels, 1996). With the increase of salt concentration, the soluble sugar was reduced in grain sorghum and sweet sorghum (Balibera et al., 1997; Ibrahim, 2004). The study of *Puccinellia distans* under salt stress also show that the total carbohydrate content roots of was decreased (Melis, 1984). Contrary to these reports, Balibera's study showed that with increase of salt concentration, hexanose in stems, roots and leaves of salt-tolerant tomato was decreased, but more sucrose was accumulated (Caldwell et al., 1981).

Belowground organs are important carbohydrate storage of plant to withstand stress. With the increase of salt concentration, soluble sugar content increased in all parts of *L. chinensis*, while, the rhizome had the highest sugar content compared with other parts (Yan et al. 2000). These results show that carbohydrate or their compositions are related to plant salt tolerance, especially in the root part. Interestingly, recent researchers have mentioned that a number of soluble carbohydrates such as glucose, fructose, sucrose and low degree of polymerization fructan may be signaling molecules for plants adapted to the environment (Sheen et al. 1996; Koch 1996; Gibson 2000). Salinization is a serious problem in Songnen plain, there are a lot of studies

about plant salt tolerance, however, the compensatory mechanisms of grazed plants under saline-alkali soil conditions has not been studied.

4 Materials and methods

4.1 Study areas

This study was conducted at the ecological research station of Northeast Normal University, Changling County, Jilin Province China (44°40' to 44°44' N latitude and 123°44' to 123°47' E longitude). The study areas is characterized by a semi-arid, continental climate with a frost-free period of about 140 days; annual mean temperature is 4.9°C and the annual precipitation ranges from 400 to 500 mm (with 67% of the total precipitation between June and August) and annual potential evaporation is about 1600 mm. Main soil type is chestnut (i.e. calcic Kastanozems, which is equivalent to Calcic-orthic aridosol in the US soil taxonomy classification system). The vegetation consists predominantly of grassland plants such as *Leymus chinensis*, *Kochia scoparia*, *Chloris virgata*, *Phragmites communis*, *Puccinellia tenuiflora* and *Artemisia frigida*. *L. chinensis* was dominant species which occupied about 80% in our study plots.

The temperature and precipitation patterns in our study area of 2009 and 2010 were different (Fig 1). In the growing season from April to August, the total rainfall in 2009 and 2010 was 236 mm and 346 mm, respectively. But there was a spring drought in 2009 and summer drought in 2010, i.e. there was almost no rainfall before April in 2009 and June in 2010.

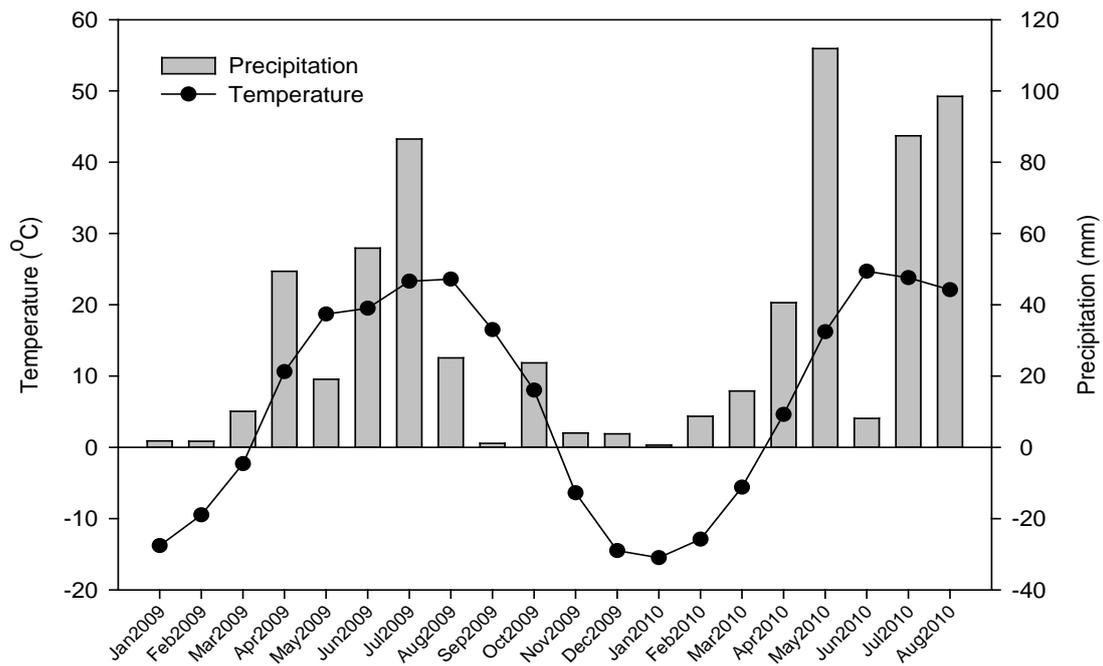


Figure 1 The average monthly temperature and precipitation of Changling research station in 2009 and 2010.

4.2 Field experiment

4.2.1 Experiment designing

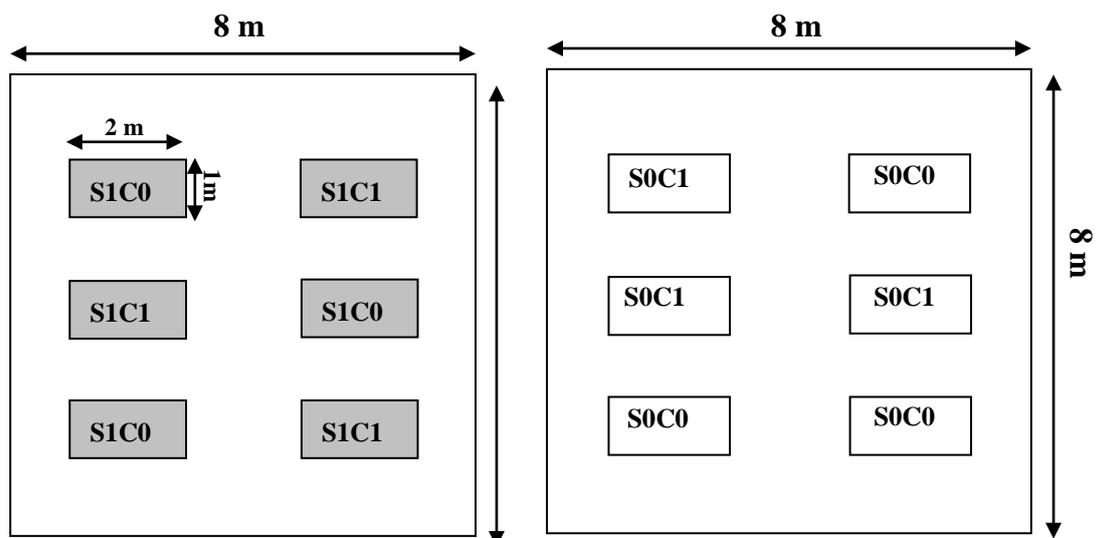


Figure 2 The field experimnet plot design. Gray plots are salt-alkali solution addition plots. S0C0: no salt addition and clipping; S0C1: clipping without salt addition; S1C0: salt addition without clipping; S1C1: clipping with salt.

The field experiment was conducted in 2009 and 2010 including 2 factors: salt-alkali solution addition and clipping with three replicates, i.e. without (S0) and with (S1) salt \times without (C0) and with (C1) clipping treatments \times 3 replicates. In total we installed 12 plots in the size of 1 m \times 2 m.

4.2.2 Salt-alkali solution application

Table 1 The salt-alkali solution application schedule in 2009 and 2010.

Date	[Na ⁺] (mol L ⁻¹)	pH	Salt amount (L m ⁻²)	Salt types (g m ⁻²)			
				NaCl	NaHCO ₃	Na ₂ CO ₃	SUM
15 Jun 2009	0.6	10	4	35.1	50.4	63.6	149.1
23 Jun 2009	0.6	10	2	17.55	25.2	31.8	74.55
11 Jul 2009	0.6	10	2	17.55	25.2	31.8	74.55
21 Jul 2009	1.2	10	2	35.1	50.4	63.6	149.1
07 Aug 2009	1.2	10	4	70.2	100.8	127.2	298.2
13 May 2010	0.6	10	4	35.1	50.4	63.6	149.1
12 Jul 2010	0.6	10	2	17.55	25.2	31.8	74.55
12 Aug 2010	0.6	10	4	35.1	50.4	63.6	149.1
Total				263.25	378	477	1118.25

The salt-alkali solution was applied on each salt treatment plot. It consisted of 3 salt types (NaCl: NaHCO₃: Na₂CO₃ = 1:1:1), the pH was 10 (Table 1). The salt solution addition volume was depending on if the solution can reach to 10 cm soil. The total amount of salt applied in these two years was 1118.25 g m⁻².

4.2.3 Clipping application

The clipping was carried out 11th July, 12th August, 14th October of 2009 and 16th June, 6th August in 2010. Clipping intensity was 60% of the total aboveground biomass (stubble height: 12cm). A prior experiment was carried out to determine the relationship between clipping height and biomass. The regression model is $H = -29.61 \times W + 30.00$, $R^2=0.908$, $P<0.05$ (H, height of the residual; W, dry mass of defoliated shoot).



Pictures 1 a-d. The different performances of *L. chinensis* under salt and clipping treatments. a: S0C0; b: S0C1; c: S1C0; d: S1C1.

4.3 Field sampling

4.3.1 Plant density

Three squares (25 cm × 25 cm) were randomly sited in each plot. Ramet density and plant height of *L. chinensis* in each square was surveyed every month in both years. The relative growth rate (RGR) was calculated by $RGR = (H_2 - H_1) / (t_2 - t_1)$, where H_1 and H_2 are plant height at times t_1 and t_2 .

4.3.2 Soil sampling

The soil samples were taken before salt and clipping applications at the beginning and in the late growing season (14th May and 23th August in 2009; 13th May and 26th August in 2010). A soil auger (length: 1 m; diameter: 3 cm) was used to drill three randomly distributed holes in each plot. The soil cores were subdivided into 4 layers (0-10 cm, 10-20 cm, 20-30 cm, 30-50 cm) in

2009, and 5 layers (0-5 cm, 5-10 cm, 10-20 cm, 20-30 cm, 30-50 cm) in 2010.

4.3.3 Plant sampling

4.3.3.1 Sampling

The plant samples were harvested on 18 August in 2009. The aboveground part was cut out in each 20 cm × 20 cm square subplot and prepared for further determination. The corresponding belowground part was carefully dug out by shovel to a depth of 20 cm. For a more detailed study of the different plant organs we sampled roots with an additional auger (L: 50 cm; D: 10 cm) on 26th August 2010 and subdivided the soil core into different layers of 0-10, 10-20, 20-40 cm. The aboveground biomass in the diameter of the auger was cut out separately and further separated into leaves and stems. The different soil core levels were soaked into water with 80 mesh net bags and divided into stem base, rhizomes and roots. Samples were oven dried at 65 °C until constant weight.

4.3.3.2 Leaf area index (LAI)

Leaves were dried with filter paper. Chose 10 plants with complete leaves from each sample and cut out all of the leaves in each plant. Put the leaves on a white hard paper with a ruler and took pictures with a digital camera and furthermore, calculated leaf areas through SigmaScan 5.0. All of these leaves were dried in the oven (80 °C, 48 h) and then weighted.

4.4 Parameters determination

4.4.1 Soil samples

Electrical conductivity (EC) and pH: The soil samples were sieved by 1mm sieve for soil solution making. The soil solution was composed by soil and

deionized water, and the ration was 1:5. EC was determined by DDS-307 conductivity meter (Leici Shanghai) and pH was measured by PHS-3B pH meter (Leici Shanghai).

4.4.2 Plant samples

4.4.2.1 Photosynthesis

Before harvest (18 June, 12 July) we chose the first fully expanded leaves to determine the net photosynthetic rate (Pn), stomatal conductance (Gs), Intercellular CO₂ concentration (Ci) and transpiration rate (Tr) with LI-6400XT photosynthetic apparatus. The water use efficiency (WUE) was calculated by $WUE = Pn/Tr$. The determination time was from 8 am to 11am, light quantum flux was set to 1000 $\mu\text{mol m}^{-2} \text{s}^{-1}$, and 3 repeats in each each plot were measured.

Photosynthetic diurnal dynamics measurements: the dynamics of plant photosynthesis was measured by a portable photosynthesis system (LI-6400XT) on 10 August 2010 from 8:00 to 18:00, and the measurement was carried out every 2h.

4.4.2.2 Chlorophyll content

The content of Chl a, Chl b and total Chl a + b in the fresh leaves of the plants was determined by UV–vis spectroscopy (Lichtenthaler, 1987). The absorbance of the extracts was measured at 665 and 649 nm wavelengths.

4.4.2.3 Organic acid, praline and total water soluble carbohydrate content

Organic acid: Dry samples (50 mg) were treated with 10 ml deionized water at 100 °C for 20 min, and the extract was used to determine the contents of free inorganic ions. The content of OA⁻ was determined by ion

chromatography.

Dry samples (50 mg) were treated with 10 ml deionized water at 100 °C for 20 min. Proline and total water soluble carbohydrate (WSC) were measured using ninhydrin and anthrone, respectively.

4.5 Statistical analysis

Two-way ANOVA was used to test the effects of salt stress (S), shoot clipping (C) and their interaction (S × C) on all of the parameters mentioned above. With an independent *t*-test the significance of the effect of clipping was tested for the control and salt treatment, in the same way the effect of salt was tested for the control and clipped treatment. Differences were considered significant at $p < 0.05$.

5 Results

5.1 Effects of salt addition on soil EC and pH

EC and pH did not have significant difference in the topsoil (0-10 cm) and deeper soil (10-20 cm) before the study started in 2009 (Table 2). After salt-alkali addition treatment, EC and pH of topsoil (0-10 cm) were significant increased in 2009 and 2010, while not in the other soil layers (Fig 3). EC was more than $1000 \mu\text{s cm}^{-1}$, and pH was about 10 in the topsoil after salt-alkali solution application.

Table 2 The soil electrical conductivity (EC) and pH at the beginning of research.

Soil layers	EC ($\mu\text{s cm}^{-1}$)	pH
0-10 cm		
S0	184.35±16.12ns	8.18±0.15 ns
S1	186.28±14.75ns	8.88±0.34 ns
10-20 cm		
S0	291.20±76.37ns	8.24±0.10 ns
S1	344.17±66.50ns	9.16±0.29 ns

ns: no significant effect

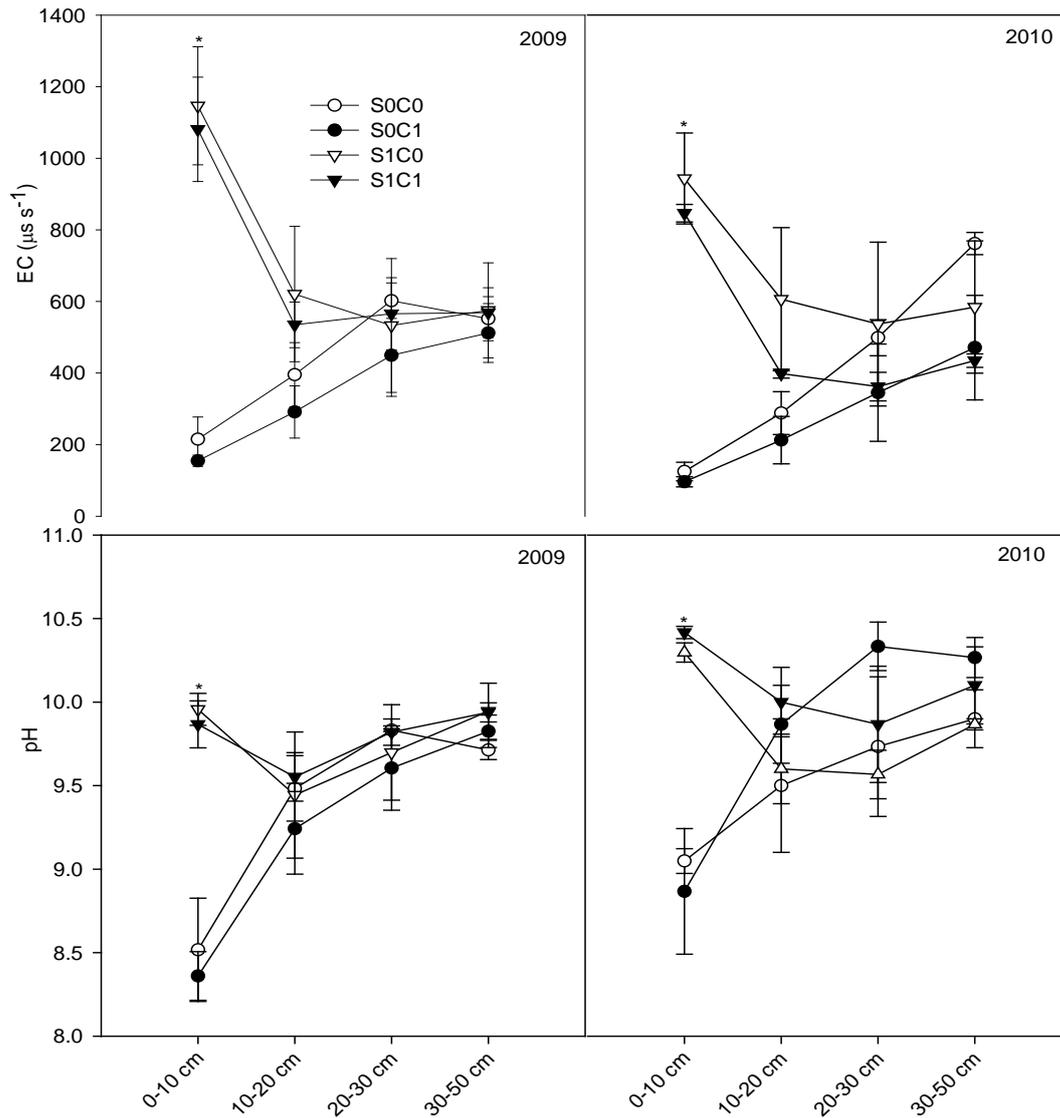


Figure 3 The soil electrical conductivity (EC) and pH in different layers after treatments in 2009 and 2010. S0C0: no salt addition and clipping; S0C1: clipping without salt addition; S1C0: salt addition without clipping; S1C1: clipping with salt. Soil layers: 0-10cm, 10-20cm, 20-30cm, 30-50cm. Values represent means + 1s.e. * means significant difference between salt treatments ($p \leq 0.05$).

5.2 Effects of salt and clipping on plant production

5.2.1 Aboveground biomass

Table 3 Interaction table for the effects of year, salt and clipping on morphological parameters and water soluble carbohydrate (WSC) content of *L. chinensis* in 2009 and 2010.

Sources	df	AB	R/S	Density June	Density July	Density August	WSC stubble	WSC stem base	WSC rhizome	WSC root
Y	1	*	*	NS	*	NS	*	*	NS	*
S	1	*	NS	*	*	*	NS	NS	NS	*
C	1	*	NS	NS	*	NS	*	*	*	NS
S*C	1	*	NS	NS	NS	NS	NS	NS	*	NS
Y*S*C	3	*	NS	NS	*	NS	*	*	*	*

*: significant effect ($P \leq 0.05$); NS: no significant effect

Table 4 Interaction table for the effects of salt and clipping on photosynthesis parameters of *L. chinensis* in June and August of 2010.

Sources	df	LAI	DW ramet	June					July				
				Pn	Cond	Ci	Tr	WUE	Pn	Cond	Ci	Tr	WUE
S	1	*	*	*	*	NS	NS	*	*	*	*	*	*
C	1	*	*	*	*	*	*	NS	*	*	*	*	*
S*C	1	*	*	*	*	*	*	*	*	NS	*	NS	*

*: significant effect ($P \leq 0.05$); NS: no significant effect

The aboveground biomass under clipping conditions was the sum of stubble biomass and the clipped biomass. No clipping or salt effects on aboveground biomass were observed in 2009. There was a significant salt and clipping interaction on aboveground biomass of *L. chinensis* in 2010 ($P=0.009$), but not

in 2009 (Table 3). Aboveground production was increased by salt addition in 2010 under no clipping from 579 g m⁻² at salt free conditions to 899 g m⁻² under salt addition (Fig 4). Clipping significantly reduced aboveground biomass, no matter with or without salt addition in 2010.

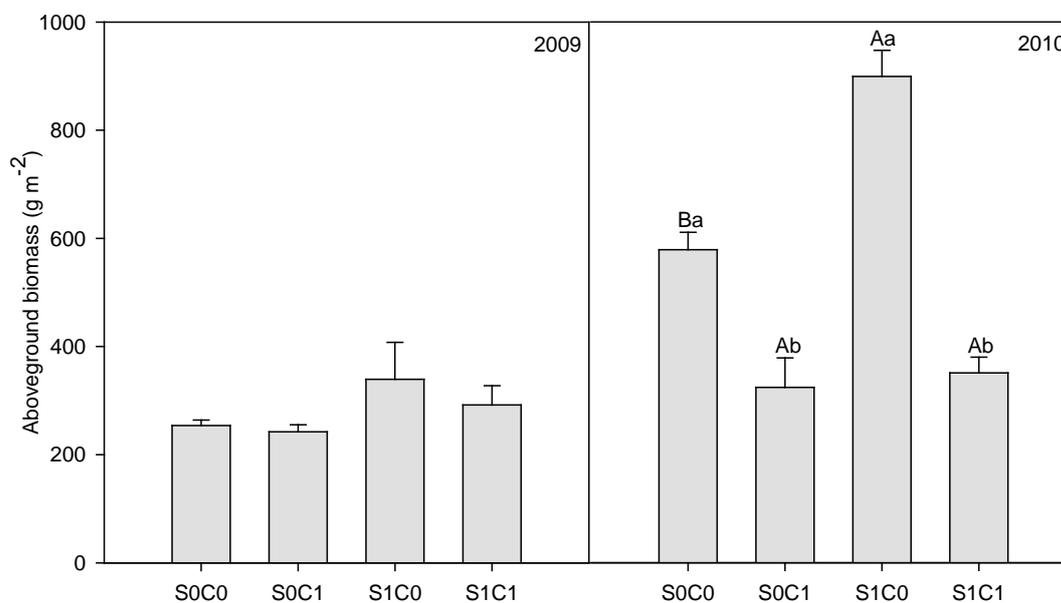


Figure 4 The effects of salt and clipping on aboveground biomass of *L. chinensis* in 2009 and 2010. Values represent means + 1s.e. Different capital letters means significant differences in salt treatments; different small case letters means significant differences in clipping treatments ($P \leq 0.05$).

5.2.2 Ramet density and biomass

There was no significant effect of salt and clipping on ramets density of *L. chinensis* in 2009 (Fig 5). The ramet density was significantly reduced under salt addition in 2010, while the effect of clipping was not significant. The total amount of ramets changed with growing seasons. The ramet density in July was decreased compared with June and August of 2009. After the beginning of growing season *L. chinensis* generated more ramets in June but declined after July in 2010 (Fig 5). Salt and clipping had interaction on individual ramet biomass in 2010 (Table 4). It was significantly increased under salt addition, while, clipping had adverse effect (Fig 6).

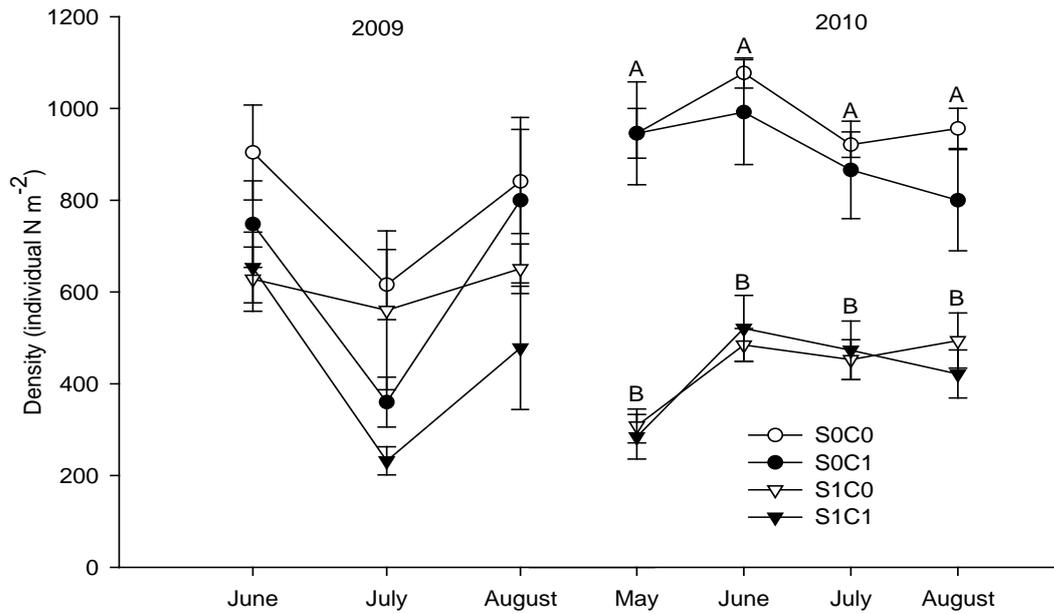


Figure 5 The effects of salt and clipping on ramet density of *L. chinensis* in 2009 and 2010. Values represent means \pm s.e. Different capital letters indicate significant difference between salt treatments ($P \leq 0.05$).

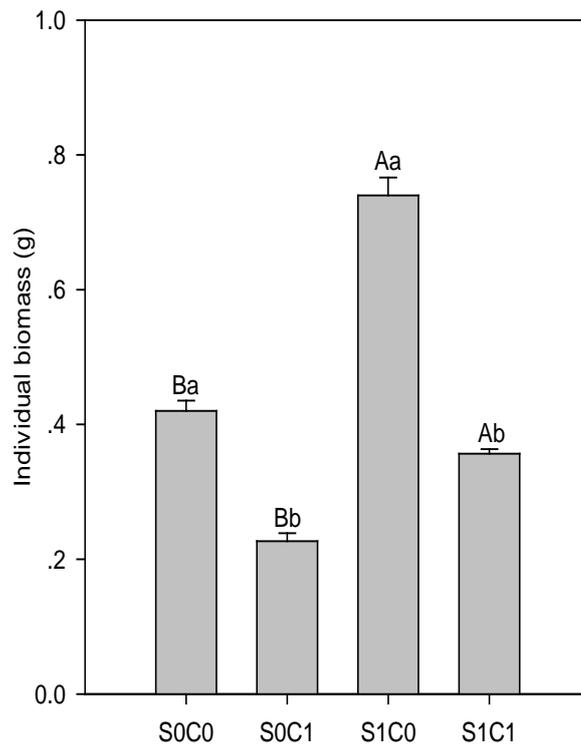


Figure 6 The effects of salt and clipping on individual ramet biomass of *L. chinensis* in 2010. Values represent means + 1s.e. Different capital letters indicate significant difference between salt treatments, small case letters indicate significant difference between clipping treatments ($P \leq 0.05$).

5.2.3 Relative growth rate (RGR)

There was no salt effect on relative growth rate (RGR) of *Leymus chinensis*. But RGR was significantly increased under clipping which means certain level of clipping can stimulate plant elongation growth. In addition, the growth rate in July was higher than August.

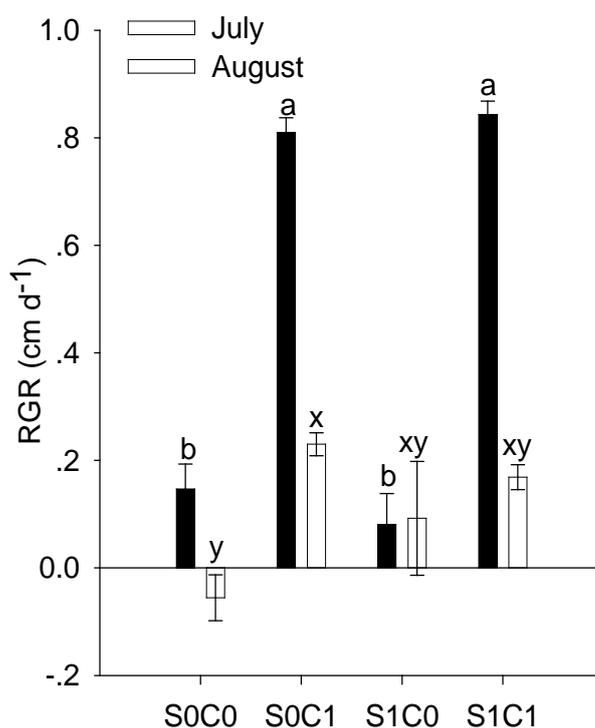


Figure 7 The effects of salt and clipping on RGR of *L. chinensis* in July and August 2010. Different small case letters means significant difference between clipping treatment ($P \leq 0.05$).

5.3 Effects of salt and clipping on belowground growth

5.3.1 R/S ratio

There was no significant interaction between year, salt and clipping treatments on root/shoot ratio (R/S) of *L. chinensis* (Table 3). Salt addition did not affect the R/S without clipping, but under clipping condition salt significantly

decreased the R/S in 2009 (Fig 8). While in 2010 both of the salt, and clipping had no significant effect on R/S (Fig 8). In addition, the R/S ratio was lower in 2010 than 2009.

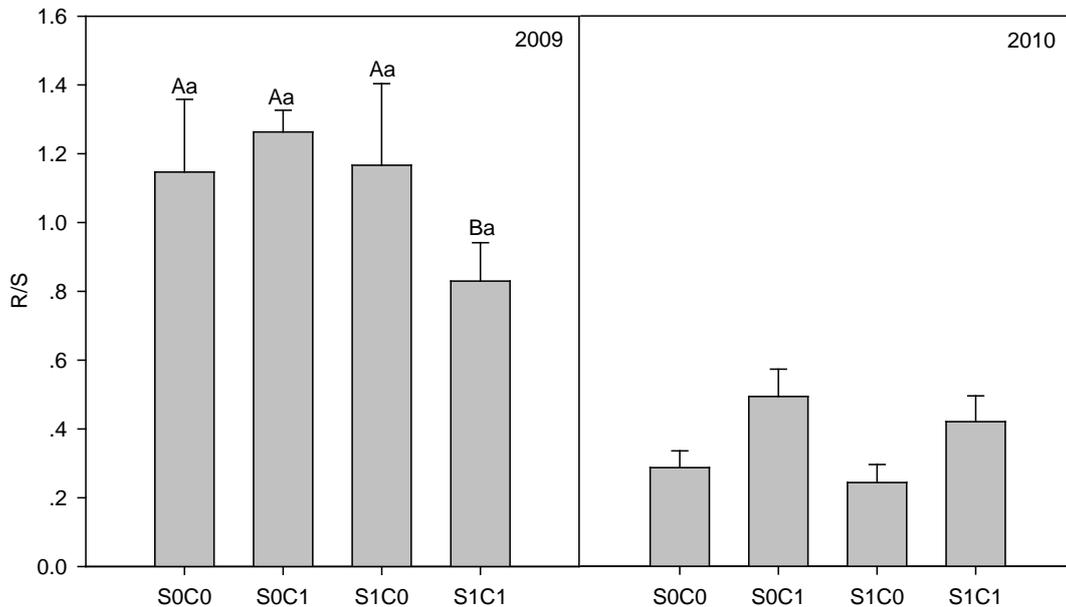


Figure 8 The effects of salt and clipping on R/S ratio of *L. chinensis* in 2009 and 2010. Values represent means + 1s.e. Different capital letters indicate significant difference between salt treatments, small case letters indicate significant difference between clipping treatment ($P \leq 0.05$).

5.3.2 Root biomass distribution

The root biomass in different layers was separately measured. In 0-10 cm soil depth, root biomass was significantly increased under salt addition, while clipping had no significant effect (Fig 9). There was no significant effect of salt and clipping on root biomass in 10-20 cm soil depth. While in the 20-40 cm soil depth, root biomass was significantly reduced under salt addition conditions. Clipping did not have significant effect in all of these soil layers.

The salt stress also affected root distribution in different soil layers. Under the salt free condition, root biomass mainly allocated in the deeper layer

(20-40 cm), and less in the topsoil. Contrary to the salt free condition, salt stress resulted in more root biomass allocated in the topsoil rather than in the deep (Fig 9).

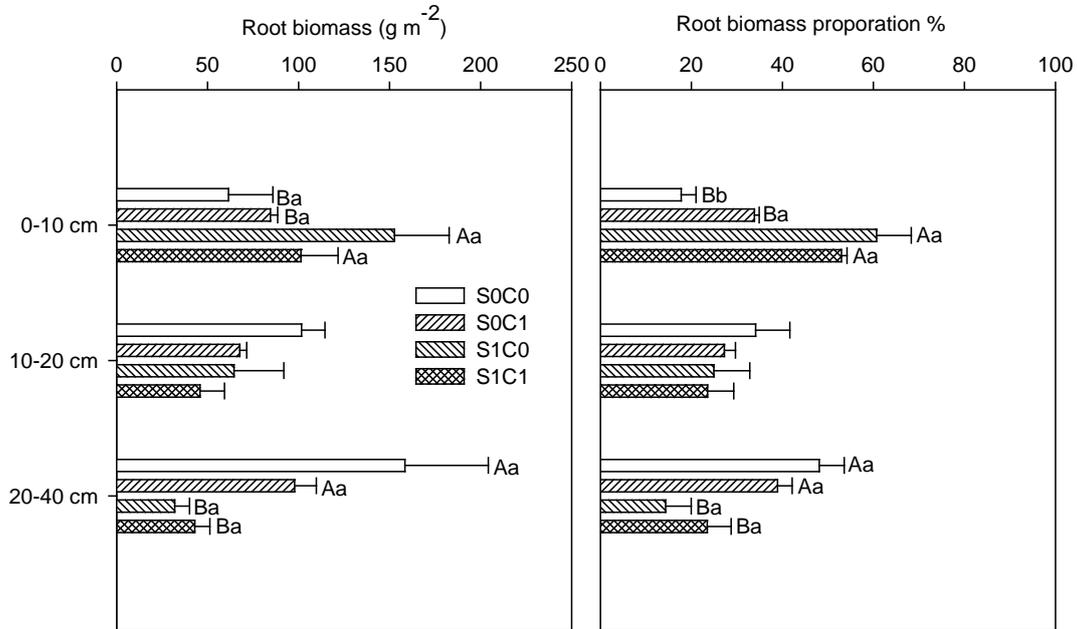


Figure 9 The root biomass distribution of *L. chinensis* in different soil layers in 2010. Values represent means + 1s.e. Different capital letters means significant difference between salt treatments, small case letters means significant difference between clipping treatment ($P \leq 0.05$).

5.3.3 Rhizome biomass

There was no significant interaction between year, salt and clipping treatments on rhizome biomass of *L. chinensis* in 2009 and 2010 (Table 3). Both of the salt and clipping had no significant effect on rhizome biomass in 2009 (Fig 10). Salt addition did not affect the rhizome biomass without clipping, but under clipping condition salt significantly decreased the rhizome biomass in 2010. The rhizome biomass was lower in 2010 than 2009 (Fig 10).

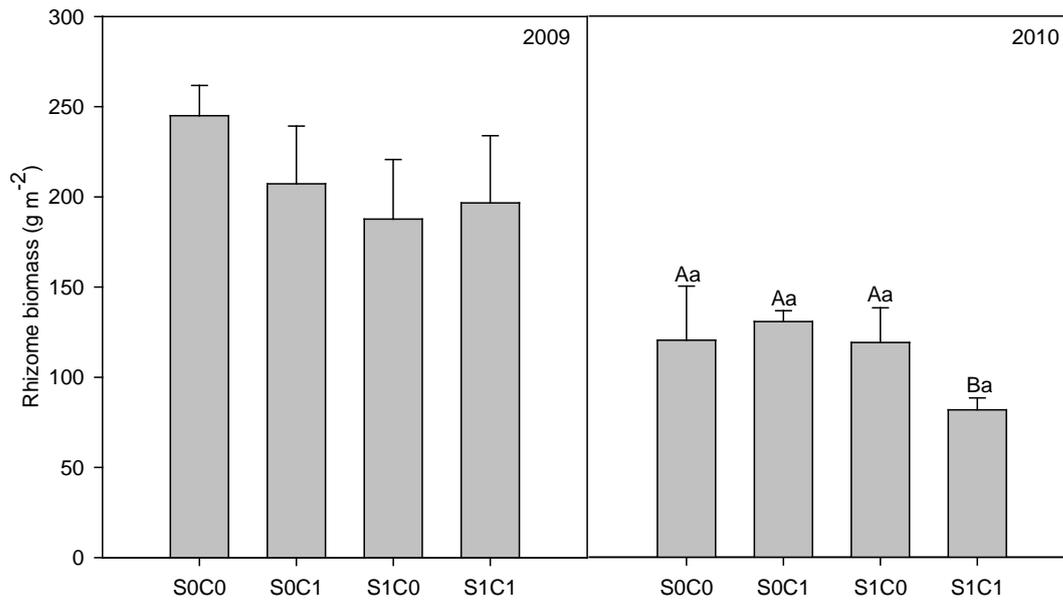


Figure 10 The effects of salt and clipping on rhizome biomass of *L. chinensis* in 0-20 cm soil in 2009 and 2010. Values represent means + 1s.e. Different capital letters indicate significant difference between salt treatments, small case letters indicate significant difference between clipping treatment ($P \leq 0.05$).

5.4 Effect of salt and clipping on photosynthesis

5.4.1 LAI and monthly photosynthesis

Salt addition treatment had significant effect on photosynthesis of *L. chinensis* in all months, except intercellular CO_2 concentration and transpiration rate in June. Clipping also had significant effect on photosynthesis parameters in all months, but not in WUE of June. Interaction between salt and clipping had effect on all parameters of photosynthesis in June, moreover, P_n , C_i , WUE in July (Table 4).

The leaf area index (LAI) was significantly increased by salt addition and decreased by clipping (Fig 11A). Compared with no salt treatment, salt-alkali solution significantly increased net photosynthetic rate of *L. chinensis* in each

month (Fig 11B). The stomatal conductance was significant decline in July under salt treatment (Fig 11C). In addition, intercellular CO₂ concentration, and transpiration rate kept synchronicities changes with stomatal conductance (Fig 11D&E). Water use efficiency (WUE) increased under the salt-alkali stress in June and July (Fig 11F).

Net photosynthetic rate, stomatal conductance, the intercellular CO₂ concentration and transpiration rate were increased under clipping (Fig 11). There was no difference between clipping and no clipping with regard to WUE in June under salt free conditions. However, WUE was increased with clipping treatment in July under no salt condition. Under salt treatment, WUE was significant reduced under clipping in June and July (Fig 11F).

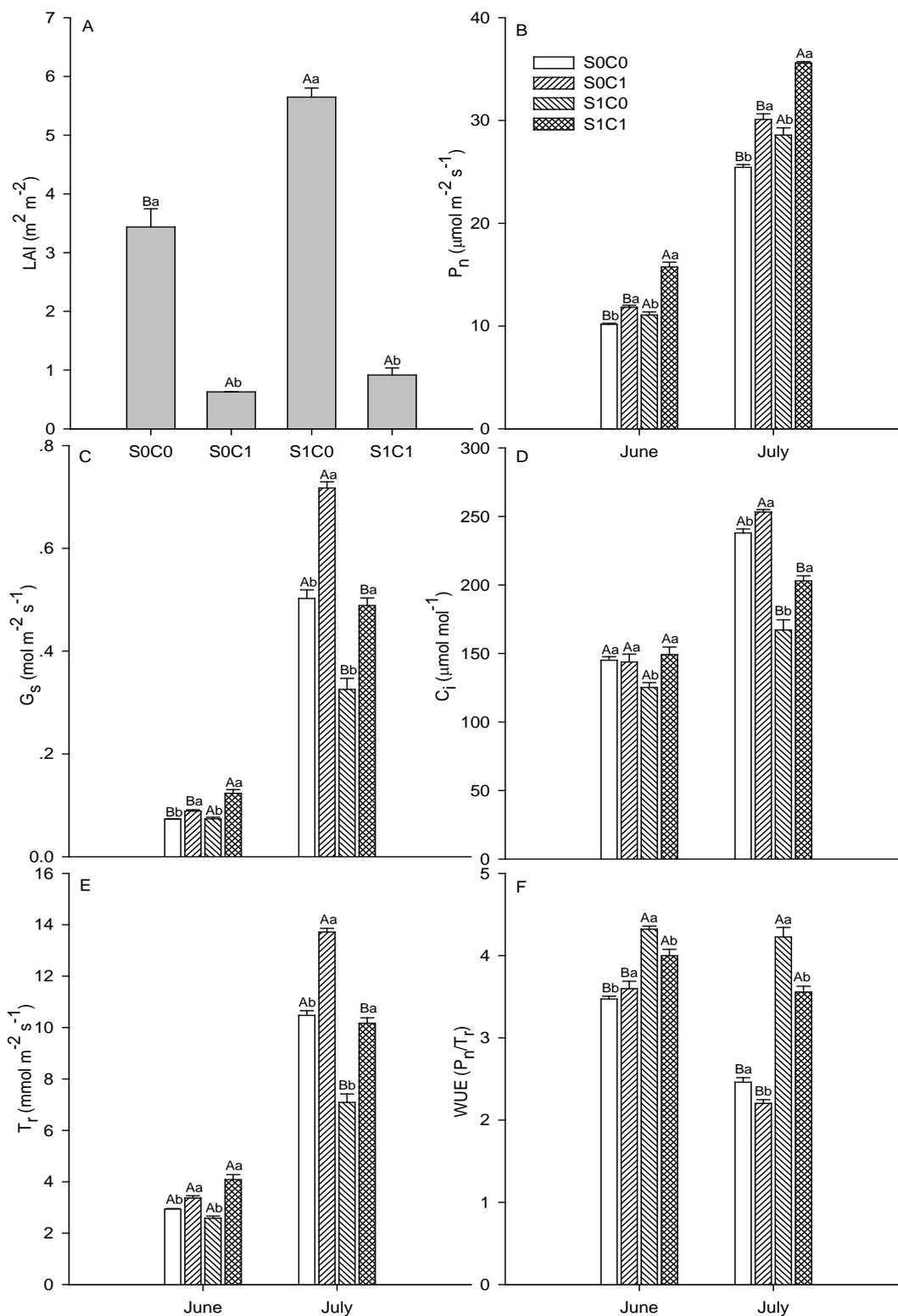


Figure 11 The effects of salt and clipping on LAI and photosynthesis of *L. chinensis* in 2010. Values represent means + 1s.e. Different capital letters indicate significant difference between salt treatments, small case letters indicate significant difference between clipping treatment ($P \leq 0.05$).

5.4.2 Photosynthetic diurnal dynamics

In general, salt stress will have inhibitory effect on photosynthesis of plants which mainly due to the damage of thylakoid membrane and chlorophyll reduction. However, in our experiment the chlorophyll content (chl a, b) was no significant difference under salt treatment compare with control. Clipping also had no significant effect on chl a and chl b.

Two peaks of net photosynthesis rate occurred at about 10 am and 3 pm and a low point from 12 to 2 pm in the salt free conditions. After 5 pm the photosynthesis was continuously decline. However, there was no peek occur under salt stress conditions and the net photosynthetic rate was higher from 7 am to 12 am than under salt free conditions, but after 12 am Pn was lower than control.

In salt stress conditions, the stomatal conductance (Gs), intercellular CO₂ concentration (Ci) and transpiration rate (Tr) showed similar trends. They were lower from 10 am to 2 pm but higher in the other time compared to the salt free conditions. The water use efficiency (WUE) of *L. chinensis* under salt treatment showed higher value before 2 pm compared to salt free conditions. In addition, all parameters were increased under clipping treatments regardless of the salt application.

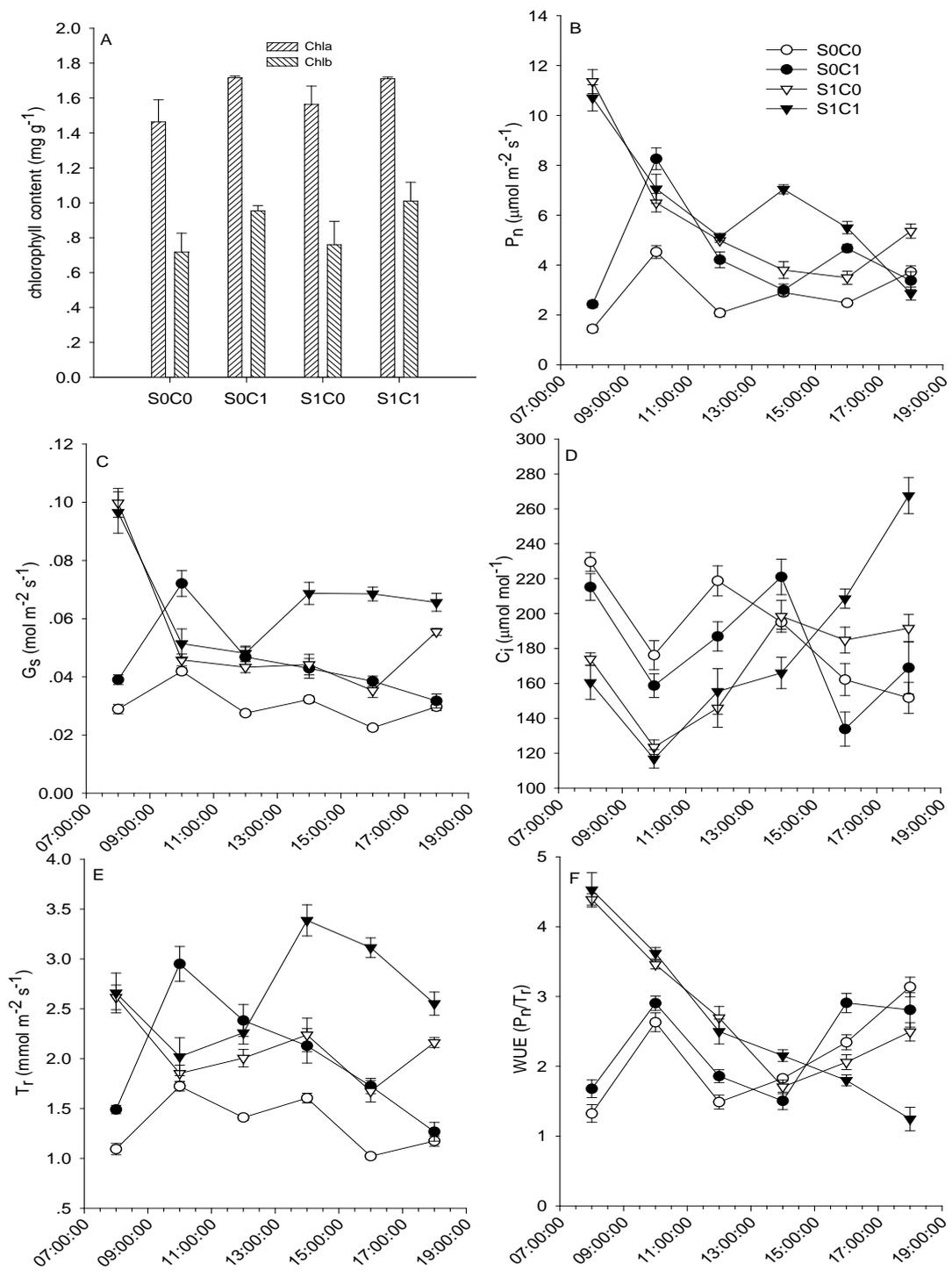


Figure 12 The effects of salt and clipping on Chlorophyll and photosynthesis diurnal dynamics of *L. chinensis* in 2010. A: chlorophyll concentration; B: net photosynthetic rate; C: stomatal conductance; D: intercellular CO₂ concentration; E: transpiration rate; F: Water use efficiency (WUE).

5.5 Effects of salt and clipping on soluble substance content

5.5.1 Proline

Interaction between salt and clipping treatment was significant on proline content in stembase and root of *L. chinensis* in 2010 (Fig 12). The proline content in stembase was significantly reduced under clipping condition and salt stress, but not in salt free conditions. While in the roots, proline content was decreased under clipping in salt free conditions. In addition, leaves had the highest amount of proline.. Under salt stress, the proline content of leaves, stems and roots was significantly reduced, while it was not reduced in rhizomes.

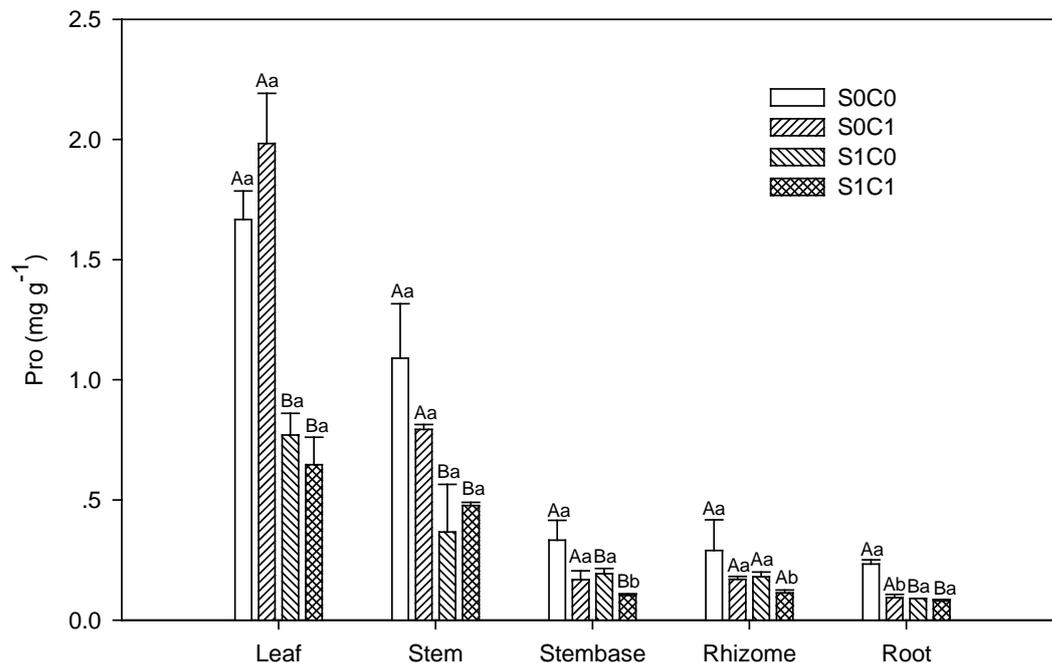


Figure 13 The effects of salt and clipping on Proline (Pro) content in different organs of *L. chinensis* in 2010. Values represent means+1s.e. Different capital letters means significant difference between salt treatments, small case letters means significant difference between clipping treatment ($P \leq 0.05$).

5.5.2 Oxalic acid (OA)

Oxalic acid was the most important organic acid in *L. chinensis*. In our study, there was no significant interaction between salt and clipping treatment on oxalic acid (OA) content in all parts of *L. chinensis* in 2010 (Fig 12). The OA content of stems was significantly increased under salt stress but reduced in all belowground parts.

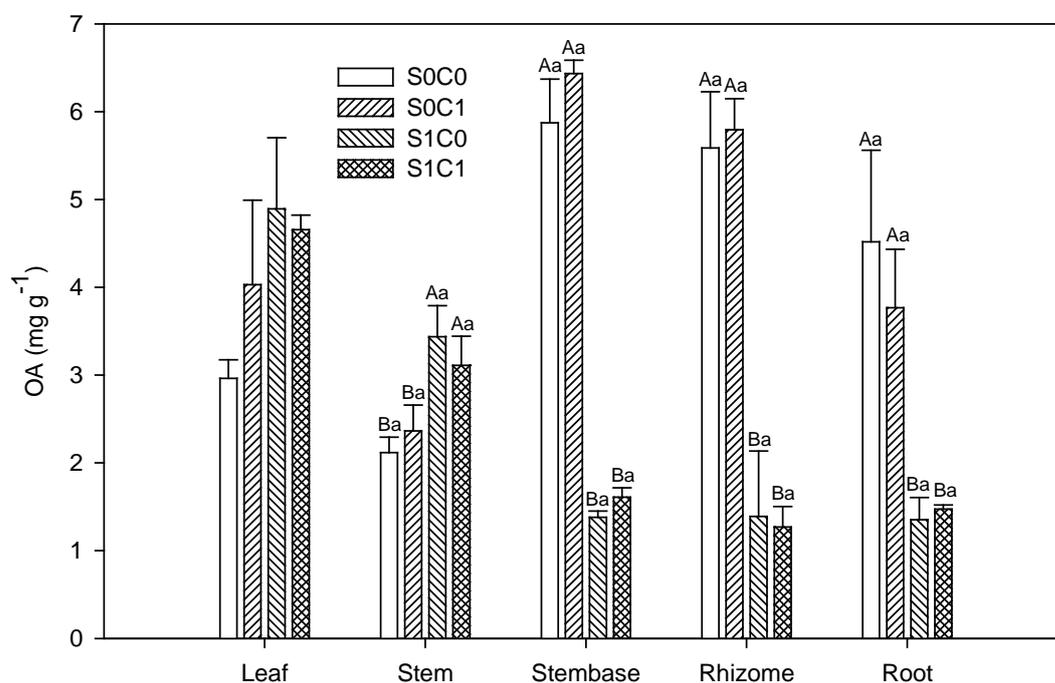


Figure 14 The effects of salt and clipping on Oxalic acid (OA) content in different organs of *L. chinensis* in 2010. Values represent means+1s.e. Different capital letters indicate significant difference between salt treatments, small case letters indicate significant difference between clipping treatment ($P \leq 0.05$).

5.5.3 Water soluble carbohydrate (WSC)

Interaction between salt and clipping treatment was significant on soluble sugar content in stembase and root of *L. chinensis* in 2009 and in rhizome only in 2010 (Table 3). Rhizomes had the highest amount of soluble sugar in both years (Fig 7). Under salt free conditions, clipping had no significant effect on

rhizome sugar content, while it was significant reduced with clipping under salt addition.

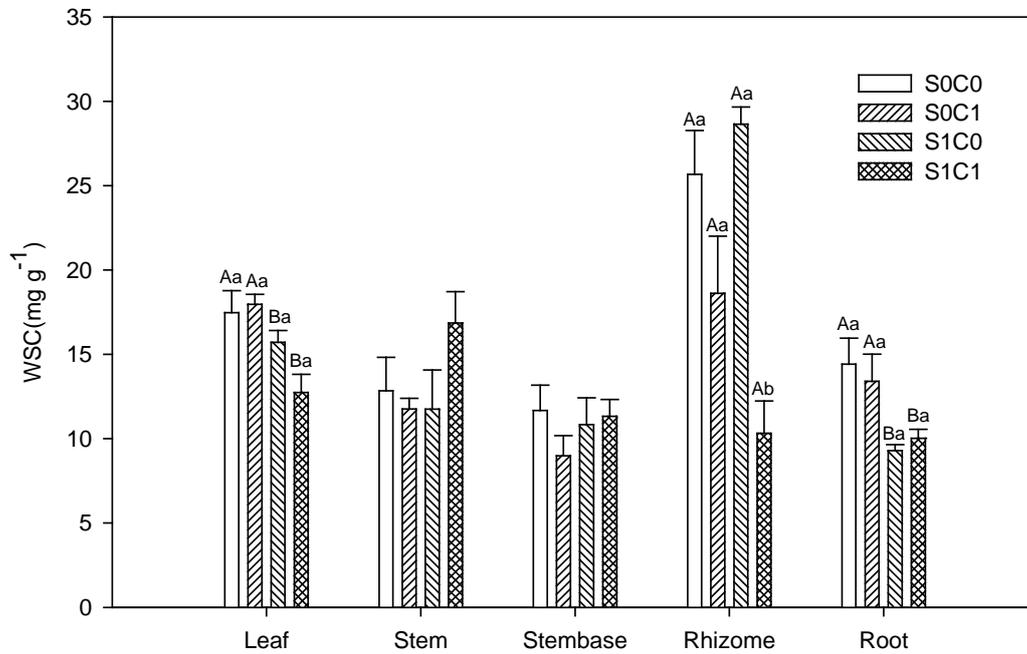


Figure 15 The effects of salt and clipping on water soluble carbohydrate (WSC) content in different organs of *L. chinensis* in 2010. Values represent means+1s.e. Different capital letters indicate significant difference between salt treatments, small case letters indicate significant difference between clipping treatment ($P \leq 0.05$).

6 Discussions

6.1 Effect of clipping and salt stress on compensation growth

Compensatory growth is one of the most important mechanisms for plants tolerant to grazing (Tiffin, 2000). During our studies, *L. chinensis* did not perform equal or over compensate growth, instead clipping significantly reduced the aboveground production (Fig 2). This may be due to the comparable high intensity of clipping in our study (cut off 40% DW from the total shoot). Grazing frequency (interval) and intensity (stubble height) influenced the effect of compensation. Many studies have shown that the higher of stubble height the higher regeneration capacity, but no regrowth occurred if the intensity exceeds a certain extent (Wise and Abrahamson, 2005 and 2007; Zhao et al., 2008). Studies from Inner Mongolia reported that overcompensation of *L. chinensis* could happen under the 20% and 40% clipping levels, and equal compensation was found at the 60% clipping level, but under compensation occurred at 80% clipping level (Zhao et al., 2008). Greenhouse study of *L. chinensis* also showed an over compensation growth under light defoliation in combination with high water and nutrient levels (Gao et al., 2008).

The aboveground biomass was higher under salt-alkali addition in our experiment (Fig 2). This was because the biomass increase per ramet compensated the number of ramets lost under salt conditions (Fig 3 and 4). The buds on rhizomes could develop into new ramets if the environment was suitable, instead dormancy or death would occur under stress conditions (Yang et al., 1995). This integration between rhizomes and ramets in response to salt stress will protect the whole population and secures survival at the expense of the sacrificed ramets. More resources will be available for the remaining ramets which enables a strong vigorous growth under favorable conditions.

Meanwhile, the dead parts can take away some parts of salt ions, thereby reducing the salt stress for the population. Some studies also showed that some plants are to minimize the absorption of harmful ions, and transport them to the old organizations which seem like ions storage. This is a special strategy to protect the young tissue at the cost of old organs (Flowers et al., 1986; Michelet and Boutry, 1995; Voilmm et al., 1998).

The belowground biomass including rhizome and root biomass as well as R/S ratio was not affected by clipping in salt free conditions indicating that there was an equal compensation of belowground biomass. In this case, there was no re-allocation of resources into shoot growth at the cost of root growth. However, other studies showed different response of belowground biomass to clipping (Zhao et al., 2008; Wang, 2004). The study in Inner Mongolia showed significant decrease of belowground biomass with increasing clipping intensity (Zhao et al., 2008). The study carried out in Songnen grassland showed a decreased rhizome biomass but the rhizomes proportion of the total biomass increased considerably with grazing intensity increasing (Wang, 2004). In our study the salt stress resulted in more root biomass allocation to the topsoil rather than to deeper soil horizons. The reason for our contrasting results result may be due to the lower ramet density under salt stress conditions which might have reduced the competition for water among ramets.

6.2 Effect of clipping on the plant photosynthesis

Another important result in our study is that the photosynthesis rate was increased under clipping (Fig 9). In a study of wheatgrass showed that during the first 2 days after clipping the net photosynthetic rate (P_n) was decreased compared to the control, but it increased in the last 8 days and it was even higher than the control (Painter and Detling, 1981). A grazed plant is able to use its reserves to generate new leaves to increase leaf area and

photosynthetic capacity for restoration (Reichman and Smith, 1991). Other studies also proposed that increases in RuDP carboxylase (in C3 plants) or PEP carboxylase (in C4 plants) activity may occur following defoliation due to increased enzyme synthesis (Wareing et al., 1968). However, some studies showed contradicting results. A study of *L. chinensis* in Inner Mongolia indicated that Pn was significantly decreased under heavy grazing compared to ungrazing conditions (Chen et al., 2005); long-term grazing also decreased net photosynthetic rate of *L. chinensis* because of lower photochemical efficiency of PSII and water use efficiency (Zhao et al., 2009). Some researchers reported that these differences depend on the defoliation levels. Defoliation can promote growth by stimulating photosynthesis only under light and moderate defoliation conditions (Wang and Wang, 2001; Zhao et al. 2008). In our study, the increase of photosynthesis rate might result from decreased competition by remaining leaves for mineral nutrients or metabolites supplied by the roots.

Photosynthesis of *L. chinensis* in 2010 was increased by salt addition (Fig 9B). This could mainly be attributed to the density reduction under salt treatments, which increases water availability due to reduced competition. The increased of leaf area might be another reason for increased photosynthetic rate (Fig 9A). In high density plots, the leaf shading may limit the light quantum capture, while, in low density plots, plant can use light more efficient.

6.3 Effect of clipping on the plant carbohydrate

Carbohydrate stored in rhizomes may play an important role in supplying the energy required for rebuilding the leave area. Our results showed that sugar contents in rhizomes were reduced under clipping (Fig 10), suggesting that soluble sugar was transferred to the aboveground plant organs. The study of Cocksfoot indicated that the soluble carbohydrates formed part of a labile pool

which was used for providing substrates for new plant growth (Davidson and Milthorpe, 1966). *L. chinensis* is a rhizomatous clone plant, and rhizomes play an important role as carbohydrate pool or “buffer”. In our case, rhizomes performed like a “source” for carbohydrates. After rebuilding sufficient leaf area, more carbohydrates will be produced by photosynthesis and transported to the rhizomes, now functioning as a carbohydrate sink (Turner et al., 2007). Growth rate models (GRM) also showed that over-compensation may likely occur after low intensity grazing in stress environments, because these adapted plants often have higher matter storage abilities, enabling to initialize increased growth rates respectively over-compensation (Hilbert et al., 1981).

7 Conclusion & Outlook

Our results indicated that salt and clipping had an interactive effect on aboveground biomass (AB) of *L. chinensis*. Salt addition significantly increased AB under no clipping, while the effect was absent under clipping. Clipping decreased AB regardless of salt addition which indicates there was no compensation growth. However, relative growth rates and photosynthesis were higher under clipping indicating increased physiological activity of the defoliated plants, which could not compensate the loss of biomass. In addition, the carbohydrates in rhizomes are likely to play an important role for compensatory growth providing resources to reestablish new leaf area.

The higher productivity of *L. chinensis* under salt stress was mainly due to the integration effect between rhizome and ramets: The individual ramet biomass compensated the loss of ramet number. The increased ramet biomass is due to higher leaf area index and net photosynthesis rates of individual ramets under salt stress. The reduction of ramet number will likely increase the available resources per plant. However, the root biomass was significantly decreased after two years stress, therefore, the integration function is probably an unsustainable strategy of *L. chinensis* to cope with salt stress, and long-term field studies are needed to understand the combined effects of salt-alkalinity and grazing on growth performance of *Leymus chinensis*.

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