

# Effects of mechanical and chemical control on invasive *Spartina alterniflora* in the Yellow River Delta, China

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**Abstract:** *Spartina alterniflora* is one of the most noxious invasive plants in China and many other regions. Exploring environmentally friendly, economic and effective techniques for controlling *S. alterniflora* is of great significance for the management of coastal wetlands. In the present study, different approaches, including mowing and waterlogging, mowing and tilling and herbicide application, were used to control *S. alterniflora*. The results suggest that the integrated approach of mowing and waterlogging could eradicate *S. alterniflora*, the herbicide haloxyfop-r-methyl could kill almost all the *S. alterniflora*, and the integrated approach of mowing and tilling at the end of the growing season was a perfect way to inhibit the germination of *S. alterniflora* in the following year. However, no matter which control approach is adopted, secondary invasion of *S. alterniflora* must be avoided. Otherwise, all the efforts will be wasted in a few years.

**Keywords:** invasion; mowing; waterlogging; tilling; herbicide; haloxyfop-r-methyl

## Introduction

*Spartina alterniflora* Loisel (smooth cordgrass) is a perennial C<sub>4</sub> grass native to the eastern and gulf coasts of the United States and has important ecological functions in its native ecosystems (Mobberley 1956). Due to intentional or unintentional introduction, *S. alterniflora* is now distributed in coastal marshes almost all around the

world. Because of its vigorous vitality, strong salt tolerance, waterlogging tolerance, strong asexual reproduction and rapid expansion, *S. alterniflora* poses a serious threat to the biodiversity and ecological security of many coastal tidal wetlands (Li et al. 2009; Strong and Ayres 2013). It is now a notorious invader of coastal ecosystems in many regions of the world, including estuaries in New Zealand, China, Africa and the Pacific coast of the USA (Adams et al. 2016; An et al. 2007; Buhle et al. 2012; Knott et al. 2013).

*Spartina alterniflora* was artificially introduced to China in 1979 (An et al. 2007) and can now be found in all coastal provinces of the country (Liu et al. 2018). Since it was artificially introduced to the Yellow River Delta (YRD) in 1990, *S. alterniflora* has become widely distributed in the intertidal zone of the YRD, with a total area of 3278 ha in 2015 (Yang et al. 2017). In the invaded area in the YRD, zooplankton biomass and diversity have decreased, benthic species abundance has decreased, economic shellfish have disappeared, and bird foraging and habitat have also become threatened (Shen et al. 2009; Tian et al. 2009; Tian et al. 2008). *Spartina alterniflora* has also significantly altered the soil physicochemical characteristics and microbial communities in the YRD (Zhang et al. 2018).

In order to minimize its negative ecological effects, the control of *S. alterniflora* has become an important issue in coastal wetland management. *Spartina alterniflora* is a perennial herb, and its reproductive modes include sexual reproduction via seeds and asexual reproduction via rhizomes or plant fragments (Strong and Ayres 2016). The objectives of the various approaches used to control *S. alterniflora* are to solely or simultaneously inhibit its growth, sexual reproduction and asexual reproduction. Managers and scientists have attempted to develop techniques for controlling this

species, including mechanical, chemical and biological approaches (Knott et al. 2013; Gao et al. 2014; Xie et al. 2018). Some mechanical approaches, such as mowing and flooding, mowing and shading, can achieve good weeding effect and have little impact on the environment (Yuan et al. 2011; Smith and Lee 2015). Chemical control methods usually use herbicides, which are easy to implement and have achieved good control effect in some areas. However, herbicides may cause environmental pollution and damage the health of animals and plants (Patten et al. 2017; Qiao et al. 2019). Biological control methods need to be improved, and there is also a risk of ecological invasion of new alien species (Xie et al. 2018).

Although some of the previous approaches can achieve good control results, there are still many aspects to be improved. 1) The control efficacy of a approach may vary greatly in different regions (Patten 2004). 2) The cost of control needs to be further reduced. (Riddin et al. 2016; Yuan et al. 2011). 3) It takes too long to eliminate or eradicate *S. alterniflora*, ranging from a few years to more than a decade (Kerr et al. 2016; Patten 2004; Patten et al. 2017; Riddin et al. 2016). The drawbacks of the control approaches may be due to the various growth periods of *S. alterniflora* or environmental conditions, such as climate, topography, and elevation.

A series of in situ field experiments which included mechanical and chemical control methods were performed in the YRD of China during 2016 - 2018. The aim of this study is to explore or improve the control technology of *S. alterniflora* so as to reduce its cost, improve its efficiency and widen its application.

## Materials and methods

### *Site description*

The YRD (118°20'E~119°20'E, 37°16'N~38°16'N), one of the most active regions of land-ocean interaction in the world, is a fan-shaped area located on the southern bank of the Bohai Sea and the western part of Laizhou Bay in China. The YRD has a warm-temperate and semi-humid continental monsoon climate with distinctive seasons and a rainy summer. The annual average temperature is 12.9 °C, with minimum and maximum mean daily temperatures of -2.8 °C in January and 26.7 °C in July, respectively. The average annual precipitation is 560 mm, nearly 70% of which occurs from July to September (Han et al. 2018). The in situ field experiment site in this study is located on the south side of the Yellow River estuary (37°43'46.36"N, 119°15'13.29"E). The area has frequent tides, and the highest tide level exceeds 2 m. *Zostera japonica*, *Spartina alterniflora*, *Suaeda salsa*, *Phragmites communis* and *Tamarix chinensis* are sequentially distributed from sea to land. The niche of *S. alterniflora* overlaps with that of *Zostera japonica* and *Suaeda salsa* on the low-tidal and mid-tidal beaches, respectively. Figure 1 shows the experimental site of this study and the distribution of *S. alterniflora* in YRD.

### **Figure 1 Distribution of *S. alterniflora* in the Yellow River Delta of China**

### *Experimental design*

Mowing and waterlogging to control *S. alterniflora*

*Control of the clonal ramets of S. alterniflora.* The reproductive modes of *S. alterniflora* include sexual reproduction via seeds and asexual reproduction via rhizomes. Accordingly, the sprouts of *S. alterniflora* include seedlings and clonal ramets. To ex-

plore the optimal combination scheme of mowing and waterlogging for the control of clonal ramets, experiments involving the interaction between mowing and waterlogging were designed.

Two mowing treatments, which were mowing in early June and early August 2017 (hereafter referred to as Mow\_6 and Mow\_8), were established, each having 6 replicates. The height of the stubble was less than 2 cm. There were 5 waterlogging treatments in each mowing plot, and the waterlogging levels were 0, 10, 20, 30 and 40 cm, respectively. Waterlogging lasted from early June 2017 to November 2018. The 0 cm waterlogging treatment is considered the control treatment (CK) and was neither mowed nor waterlogged. The area of each mowing plot was approximately 10 m<sup>2</sup>, in which PVC pipes with an inner diameter of 31 cm were buried to 40 cm underground. The heights of the PVC pipes above the ground were 0, 10, 20, 30 and 40 cm, respectively (Fig. 2). Tidal water was trapped in the pipes and maintained at the corresponding water level.

**Figure 2 Schematic diagram of the integrated control experiment involving mowing and waterlogging.** Mow\_6 and Mow\_8 indicate that the time of mowing was early June or early August, respectively. 0, 10, 20, 30 and 40 indicate the depth of waterlogging (cm).

*Effects of different stubble heights on clonal ramet control efficacy.* Under a given waterlogging level, a lower stubble height results in a better control effect. When a mechanical equipment is used to mow *S. alterniflora*, the height of the stubble is unlikely to be 0 cm. However, the height of the stubble can be easily limited to less than 10 cm or even 5 cm. An experiment testing the interaction between stubble height and waterlogging level was established in early June 2018. The height of stubble was 5 or 10 cm. The depth of waterlogging was 0, 30 or 40 cm. According to the experimental

results from 2017, the treatment of 5 cm stubble did not include 40 cm of waterlogging.

*Control of the seedlings of S. alterniflora.* The seedlings are slender and grow slowly in the early stages of growth. Although previous studies have shown that the integration of mowing and waterlogging can kill seedlings, this mortality may be caused by trampling during mowing. To determine the effect of waterlogging on the growth of seedlings, eight waterlogging plots were constructed in late May 2017 using PVC pipes with an inner diameter of 31 cm. Two waterlogging depths were established: 10 and 20 cm, and each treatment included four replicate plots. At the beginning of the experiment, the seedlings had 3 leaves, and the plant height was 5-7 cm. The growth of seedlings in the plot was regularly assessed.

#### Mowing and tilling to control *S. alterniflora*

Five replicate plots were established in 2016 to carry out an experiment involving mowing together with tilling (hereafter referred to as MT) to control *S. alterniflora*. Each plot had an area of approximately 10 m<sup>2</sup>, and the distance between the plots was more than 10 m. A control plot without MT was established near each MT plot. One fixed subplot with an area of 1 m<sup>2</sup> in each plot was set up for a later survey. At the end of the growing season (mid-October 2016), the aboveground plants of *S. alterniflora* were mowed and removed, and then the soil was tilled with a shovel. The tillage depth was approximately 20 cm, and the roots of *S. alterniflora* remained in the soil.

#### Spraying herbicides to control *S. alterniflora*

In early June 2018, haloxyfop-r-methyl and glyphosate were sprayed onto the *S.*

*alterniflora* canopy, the height of which was approximately 50 cm. Different doses of the herbicides were sprayed, each over an area of 100 m<sup>2</sup>. The doses of haloxyfop-r-methyl were 0.15, 0.3 and 0.45 kg ha<sup>-1</sup> (hereafter referred to as H1, H2 and H3). The doses of glyphosate were 4.0 and 8.0 kg ha<sup>-1</sup> (hereafter referred to as G1 and G2). Three square plots with an area of 1 m<sup>2</sup> were randomly established for each vegetation survey. In the herbicide treatments, when the vast majority of *S. alterniflora* died, the survey area was expanded to 4 m<sup>2</sup>.

#### *Field sampling and survey*

The seasonal variation in the plant density and canopy height of *S. alterniflora* in the different plots were regularly investigated. Spike parameters, such as density and length, were investigated at the end of the growing season.

#### *Statistical analysis*

One-way ANOVA was used to identify significant differences in the parameters of *S. alterniflora* among the various treatments. The parameters included plant density, canopy height, panicle density and so on. After testing for the homogeneity of variance (Levene's test), the least significant difference (LSD) method was used to carry out multiple comparison analysis. Significance for all statistical analyses was accepted at  $p = 0.05$  level.

### **Results**

#### *Control of S. alterniflora by mowing and waterlogging*

Control of the asexual propagation of *S. alterniflora* by mowing and waterlogging

*Effects of mowing and waterlogging on the density of S. alterniflora.* All mowing and

waterlogging combinations significantly inhibited the germination of *S. alterniflora* (Fig. 3). The waterlogging at 30 and 40 cm depth after mowing in June or August completely inhibited the germination of *S. alterniflora*, with no *S. alterniflora* germinating from August 2017 to November 2018. The density of *S. alterniflora* in the 10 and 20 cm waterlogging treatments was less than 3.9% that in the CK treatment in 2017. The different mowing times also had an important effect on the germination of *S. alterniflora*. Under the same waterlogging level, Mow\_6 resulted in better control than Mow\_8. In the Mow\_6 treatment, only one new ramet of *S. alterniflora* was found in the 12 plots at a 10 or 20 cm waterlogging depth. In the Mow\_8 treatment, new ramets were found in 2 plots with a 10 cm waterlogging depth and 3 plots with a 20 cm waterlogging depth.

**Figure 3 Density of *S. alterniflora* under different combinations of mowing and waterlogging.** 10, 20, 30 and 40 cm indicate the various waterlogging levels. CK indicates the treatment with neither mowing nor waterlogging.

*Effects of mowing and waterlogging on the canopy height of S. alterniflora.* Clonal ramets sprouted from the rhizomes of *S. alterniflora* almost throughout the entire growing season; thus, we investigated the canopy height of *S. alterniflora*. The canopy height increased almost linearly in the control treatment and reached a maximum ( $93.1 \pm 4.8$  cm, mean  $\pm$  standard error) in early November 2017. The height of the clonal ramets that regenerated after mowing was significantly affected by the waterlogging level. The regenerated clonal ramets in Mow\_6 were taller than those in Mow\_8 because of the longer growth time. At the end of the growing season in 2017, the height of the regenerated clonal ramets in the 10 and 20 cm waterlogging treatments in Mow\_6 was 96% ( $p > 0.1$ ) and 68% ( $p < 0.01$ ), respectively, of the height in



the CK treatment (Fig. 4A). The height of the regenerated clonal ramets in the 10 and 20 cm waterlogging treatments in Mow\_8 was 27% ( $p<0.01$ ) and 30% ( $p<0.01$ ), respectively, of the height in CK (Fig. 4B). In 2018, the height in the 10 and 20 cm waterlogging treatments was close to that in CK at the end of the growing season. In addition, the height of *S. alterniflora* in 2018 was much higher than that in 2017 ( $p<0.01$ ).

**Figure 4 Canopy height of *S. alterniflora* under different combinations of mowing and waterlogging.** 10, 20, 30 and 40 cm indicate the various waterlogging levels. CK indicates the treatment without any mowing or waterlogging.

*Effects of different stubble heights on asexual reproduction control efficacy.* *Spartina alterniflora* could be completely controlled if the stubble was waterlogged at a suitable water level after mowing. A single mowing could not effectively control *S. alterniflora*. The density of newly cloned ramets was approximately half that before mowing (Fig. 5A). Under the condition of long-term waterlogging at a level of 30 or 40 cm after mowing, no ramets germinated regardless of whether the stubble height was 5 cm or 10 cm (Fig. 5A). The height of the stubble affected the growth of the new clonal ramets. Within 46 days after germination, the heights of the ramets showed no significant difference between the 10 and 5 cm stubble height treatments. However, 77 days after germination, the height of the ramets in the former treatment was 61% higher than that in the latter treatment ( $p<0.01$ , Fig. 5B), and this difference was maintained until the end of the growing season.

**Figure 5 Density (A) and canopy height (B) of *S. alterniflora* under different stubble height and waterlogging level treatments.** S5 W0 indicates that the height of the stubble (S) is 5 cm and the level of waterlogging (W) is 0 cm, and the others are similar.

## Control of the sexual propagation of *S. alterniflora* by mowing and waterlogging

*Effects of mowing and waterlogging on heading of S. alterniflora.* Because there were few regenerated clonal ramets after mowing and waterlogging, the spike density of *S. alterniflora* was far lower following those treatments than that in the control treatment at the end of the growing season in 2017. Among the 24 waterlogging plots under the Mow\_6 treatment, spikes were found only in one 10 cm waterlogging plot and one 20 cm waterlogging plot. In the Mow\_8 treatment, spikes were found only in one 10 cm waterlogging plot. The average spike density in the 10 cm and 20 cm plots was no more than 3.1% of that in the control treatment (Fig. 6A). The spike density of *S. alterniflora* in 2018 was similar to that in 2017 in all cases except the 10- and 20-cm waterlogging treatments in Mow\_8 (Fig. 6D). The spike data indicate that the integrated approach could significantly inhibit the sexual propagation of *S. alterniflora* via seeds for two years.

The spike rate refers to the ratio of the number of plants with spikes to the number of all plants. The spike rate of *S. alterniflora* was  $45\% \pm 6\%$  in the control treatment in 2017. Waterlogging and mowing significantly decreased the spike rate (Fig. 6B). The spike rate in the 20 cm waterlogging plots in Mow\_6 was similar to that in the control treatment. However, this value was not representative because there were only 2 plants in one of the 12 plots. In 2018, the spike rate was slightly higher than that in 2017. Moreover, the difference between the mowing and waterlogging treatments and the control treatment was smaller than that in the last year (Fig. 6E).

The length of the spike was not affected by 10 cm of waterlogging. A waterlogging level of 20 cm seemed to inhibit strike length; however, there was only one spike

in this treatment, and its value might not be representative of all spikes (Fig. 6C and 6F).

**Figure 6** Spike parameters of *S. alterniflora* under different combinations of mowing and waterlogging in 2017 (A-C) and 2018 (D-F).

*Effects of waterlogging on the growth of S. alterniflora seedlings.* At the beginning of the waterlogging experiment in late May, the seedling densities in the two treatments were very similar, at  $159 \pm 35$  and  $152 \pm 34$  stems  $\text{m}^{-2}$  in the 10 cm and 20 cm waterlogging treatments, respectively (Fig. 7). After 15 days, many leaves turned yellow, and a few seedlings died, and the densities were  $139 \pm 28$  and  $146 \pm 31$  stems  $\text{m}^{-2}$ , respectively. All of the seedlings in the 8 plots died 43 days after waterlogging.

**Figure 7** Density dynamics of the seedlings of *S. alterniflora* under different waterlogging treatments. 10 cm and 20 cm indicate the water level of waterlogging.

#### *Control of S. alterniflora by mowing and tilling*

##### Effects of mowing and tilling on *S. alterniflora* density

The sprouts of *S. alterniflora* include seedlings germinating from seeds and clonal ramets arising from rhizomes. In the early growing season, the seedlings from seeds were slender and grew very slowly, while the cloned ramets were robust and grew fast; thus, it was easy to distinguish them morphologically before July.

Mowing and tillage at the end of the growing season in 2016 almost completely inhibited the asexual reproduction of *S. alterniflora* in 2017 ( $p < 0.001$ ). The density of cloned ramets in the MT treatment in early May 2017 was 2.4 plants  $\text{m}^{-2}$ , which was only 0.6% of that in the control treatment. One month later, the density of cloned ramets remained almost unchanged (2.6 plants  $\text{m}^{-2}$ , Fig. 8A). Although all the seeds of *S. alterniflora* were removed, the seeds of nearby *S. alterniflora* could enter the open

MT plots via tidal or wind transportation. Thus, there were still some seedlings germinating from seeds in the MT plots. From May to June 2017, the seedling density in the MT treatment was lower than that in the CK treatment by 28% - 31% (Fig. 8B,  $p > 0.05$ ). After July, it was impossible to distinguish seedlings from cloned ramets in terms of morphology. During the reproductive growing period (July to November 2017), the density of *S. alterniflora* in the MT treatment was 5% - 31% of that in the CK treatment ( $p < 0.001$ , Fig. 8C). *Spartina alterniflora* was completely restored in 2018. There was no significant difference in the density of *S. alterniflora* between the MT and CK treatments during the reproductive growing period in 2018 (Fig. 8D-F).

**Figure 8** Dynamics of the density of *S. alterniflora* after mowing and tilling. The white bars indicate mowing and tilling. The grey bars indicate the control treatment with neither mowing nor tillage.

Effects of mowing and tilling on the canopy height of *S. alterniflora*

Mowing and tillage effectively inhibited both the germination and growth of *S. alterniflora* the following year (Fig. 9). The clonal plant height in the MT and CK treatments increased continuously, but the growth rate of clonal ramets was significantly inhibited by MT, and the canopy height in the MT treatment was always 25%-45% of that in the CK treatment ( $p < 0.001$ , Fig. 9A). From germination to early June in 2017, the seedlings grew very slowly, plant height remained below 7 cm, and there was no significant difference in seedling height between the MT and CK treatments (Fig. 9B). During the reproductive growth period, the canopy height of *S. alterniflora* in the MT treatment remained much lower than that in the CK treatment. At the end of the growing season in early November, the canopy height in the CK treatment was 105.7 cm, which was higher than that in the MT treatment by 53.4%

( $p < 0.001$ ). Therefore, mowing and tilling inhibited both the germination and growth of *S. alterniflora* in the following year. However, the growth rate of *S. alterniflora* was well restored in 2018, and the canopy height in the MT treatment was very close to that in the CK treatment, especially during the reproductive period (Fig. 9D-F).

**Figure 9** Dynamics of the canopy height of *S. alterniflora* after mowing and tilling. The white bars indicate mowing and tilling. The grey bars indicate the control treatment with neither mowing nor tillage.

#### Effects of mowing and tilling on the heading of *S. alterniflora*

Mowing and tilling at the end of the growing season significantly inhibited the spike density, spike rate and spike length of *S. alterniflora* in the following year (Fig. 10). The spike density of *S. alterniflora* in the MT treatment was only 21.9% ( $p < 0.01$ ) of that in the CK treatment. Tillage also inhibited the growth of *S. alterniflora*, resulting in a lower spike rate, which was the ratio of the number of spikes to the number of *S. alterniflora* stems. The growth of spikes was also inhibited, and the spike length in the MT treatment was shorter than that in the CK treatment by 13% ( $p < 0.05$ ). The spike data indicated that the seed yield in the following year was highly inhibited by mowing and tilling. As a result, the sexual propagation of *S. alterniflora* will be continuously inhibited.

Due to the restoration of asexual reproductive capacity and the secondary invasion of seeds, *S. alterniflora* in the MT treatment grew very well in 2018, and its panicle growth features were even better than those in the CK treatment (Fig. 10).

**Figure 10** Effects of mowing and tilling on the heading of *S. alterniflora*. The white bars indicate mowing and tilling. The grey bars indicate the control treatment with neither mowing nor tillage.

## *Control of S. alterniflora by herbicides*

### Effects of herbicides on the growth of *S. alterniflora*

The 0.15-0.45 kg ha<sup>-1</sup> dose of haloxyfop-r-methyl had a strong weed control effect. Haloxyfop-r-methyl was sprayed with a backpack sprayer in early June 2018. One and a half months later, the vast majority of *S. alterniflora* were dead (Fig. 11A). Although all three doses of haloxyfop-r-methyl had good herbicidal effects, there were significant differences among the different doses. At the end of the growing season, in comparison to the control treatment, the density of *S. alterniflora* in the H1, H2 and H3 treatments decreased by 58.6% ( $p<0.01$ ), 98.3% ( $p<0.01$ ) and 99.5% ( $p<0.01$ ), respectively. The application of 0.3 and 0.45 kg ha<sup>-1</sup> haloxyfop-r-methyl achieved a perfect weed control effect. Although a small number of *S. alterniflora* survived in haloxyfop-r-methyl plots, their growth was significantly inhibited. The plant height of *S. alterniflora* in haloxyfop-r-methyl plots was approximately half that in the CK treatment at the end of the growing season ( $p<0.05$ , Fig. 11B).

The control effect of glyphosate on *S. alterniflora* was inferior to that of haloxyfop-r-methyl. At the end of the growing season, the density of *S. alterniflora* in the G1 and G2 treatments decreased by 16.1% ( $p>0.1$ ) and 23.4% ( $p>0.05$ ), respectively, and the canopy height in the G1 and G2 treatments was shorter than that in the CK treatment by 36.8% ( $p<0.01$ ) and 44.6% ( $p<0.01$ ), respectively.

**Figure 11** Effects of herbicides on the growth of *S. alterniflora*. CK indicates the control treatment without herbicide. H1, H2 and H3 indicate the treatments with 0.15, 0.30 and 0.45 kg ha<sup>-1</sup> haloxyfop-r-methyl, respectively. G1 and G2 indicate the treatments with 4.0 and 8.0 kg ha<sup>-1</sup> glyphosate, respectively.

## Effects of herbicides on the heading of *S. alterniflora*

In the herbicide treatments, the surviving *S. alterniflora* grew slowly, and their spikes were also poisoned by herbicides. The spike density of *S. alterniflora* in the haloxyfop-r-methyl treatments was only 0.6%-16.4% of that in the CK treatment ( $p<0.01$ , Fig. 12A). However, there were no significant differences among the different doses. The spike length of *S. alterniflora* in the H1, H2 and H3 treatments was 88.3% ( $p<0.1$ ), 65.4% ( $p<0.01$ ) and 36.1% ( $p<0.01$ ) of that in the CK treatment, respectively (Fig. 12B). However, there were no significant differences among the different doses. Panicle development was severely inhibited in the H2 and H3 treatments, and there were no mature seeds. Therefore, the sexual reproduction of *S. alterniflora* was completely inhibited by the two treatments.

Glyphosate significantly inhibited the spike density of *S. alterniflora*, and the spike density in the G1 and G2 treatments was 41.0% and 12.7% of that in the CK treatment ( $p<0.01$ , Fig. 12A). However, the growth of spikes in the G1 and G2 treatments was good. The spike length was almost the same as that in the CK treatments, and many mature seeds were produced, which indicated that glyphosate application could not satisfactorily inhibit the sexual reproduction of *S. alterniflora* in the following year.

**Figure 12** Effects of haloxyfop-r-methyl on the spikes of *S. alterniflora*. CK indicates the control treatment without herbicide. H1, H2 and H3 indicate the treatments with 0.15, 0.30 and 0.45 kg ha<sup>-1</sup> haloxyfop-r-methyl, respectively. G1 and G2 indicate the treatments with 4.0 and 8.0 kg ha<sup>-1</sup> glyphosate, respectively.

## Cost of controlling *S. alterniflora*

On the basis of our experimental study, 200 m<sup>2</sup> of *S. alterniflora* were treated

with each control approach to preliminarily estimate the control cost. The control costs of mowing + 30 cm waterlogging, mowing + tillage and spraying 0.3 kg ha<sup>-1</sup> of haloxyfop-r-methyl were 4104, 3284 and 1067 dollars per hectare, respectively. Because these approaches were nearly 100% effective in eliminating *S. alterniflora*, we assumed that 10% of the cost would be used for maintenance or supplementary control in the second year.

## Discussion

### *Control efficacy of mowing and waterlogging*

Mowing can prevent the photosynthesis of *S. alterniflora*, and waterlogging after mowing may lead to the gradual death of roots due to hypoxia (Xie et al. 2018). Many studies have shown that mowing in addition to waterlogging can eradicate *S. alterniflora* (Table 1). This study aimed to improve control effectiveness of this integrated approach and reduce its cost.

The control efficacy of mowing is closely related to mowing timing. Improper timing of mowing, especially during the later growing season, may promote the regeneration of *S. alterniflora* (Tan et al. 2010). The sprouts of *S. alterniflora* come from seed germination and rhizome cloning. The clonal reproduction of rhizomes occurs almost throughout the entire growing season. From the germination stage, the density of *S. alterniflora* increased gradually. Due to the death of the seedlings germinating from seeds, the plant density began to decline in May and reached a minimum in early June. After that, there were no newly germinated seeds, and the density of *S. alterniflora* increased continuously due to the enhancement of the clonal reproductive ability of rhizomes (CK in Fig. 3). The seasonal variation in plant density indicates



that the clonal reproductive capacity of the *S. alterniflora* community may be weakest in early June. Therefore, early June, that is, the end of the vegetative growth period, may be the best time to control the asexual reproduction of *S. alterniflora*. Our study confirmed that mowing in early June is more effective than mowing in early August under the same waterlogging conditions (Fig. 3). However, differences in climate or topography may lead to differences in optimal mowing times. In the Yangtze Estuary, Tang et al. (2009) found that mowing during the flowering stage in early July had a better control effect than mowing during other periods. Yuan et al. (2011) also found that mowing along with waterlogging during the flowering stage in early July can eradicate *S. alterniflora* in the Yangtze Estuary. In summary, the optimal time for mowing is from the end of vegetative growth to the flowering stage.

**Table 1 A summary of the control efficacy of mowing and waterlogging**

Although *S. alterniflora* has strong resistance to flooding, continuous waterlogging stress will inhibit its growth. The control effect of waterlogging is closely related to the phenological phase and water level. The effect of continuous waterlogging on seedlings is greater than that on ramets. Our results showed that 10-20 cm waterlogging killed all of the seedlings, which were 5-7 cm high in 43 days. Chen et al. (2011) also found that continuous waterlogging at a depth of 20 cm could lead to the death of seedlings (height 7-10 cm) within 3 months. This is likely due to the poor resistance of seedlings to waterlogging. We observed that the seedlings of *S. alterniflora* were very slim and grew slowly. Two months after germination, the height of seedlings was still less than 7 cm, and the number of leaves was no more than 3.

The water level of waterlogging has a great influence on the control efficacy. The

ramets of *S. alterniflora* grow fast and have strong adaptability to waterlogging stress. When the level of waterlogging is lower than the height of plants, the chlorophyll content in the leaves increases significantly, and the growth of *S. alterniflora* is promoted. When water levels are higher than the plants, waterlogging leads to a significant decline in chlorophyll content and photosynthetic rate and eventually significantly inhibits the growth and reproduction of *S. alterniflora* (Mateos-Naranjo et al. 2007; Yuan and Zhang 2010). Even if the water level is as high as 100 cm, it is very difficult to completely kill *S. alterniflora* by waterlogging alone (Yuan and Zhang 2010). The integrated method of mowing and waterlogging can achieve better control efficacy. Studies in the Yangtze Estuary of China have found that *S. alterniflora* can be eradicated by waterlogging at 30-70 cm after mowing (Sheng et al. 2014; Yuan et al. 2011). Our study in the Yellow River Estuary of China found that the waterlogging level after mowing could be reduced to 20 cm if *S. alterniflora* was controlled at the end of the vegetative growth period. The control efficacy of *S. alterniflora* could be as high as 90% even if the waterlogging level was only 10 cm after mowing. Our previous studies showed that the rhizomes of *S. alterniflora* were dead after mowing and waterlogging at a level of 20 cm for 4.5 months (Xie et al. 2018).

In summary, the integration of mowing and waterlogging can eradicate *S. alterniflora*, and the timing of mowing from the late vegetative growth stage to the flowering stage of *S. alterniflora* is suitable, but the depth of waterlogging may vary in different regions. A lower waterlogging level implies easier control and lower cost. Therefore, it is better to conduct experimental research before the large-scale control of *S. alterniflora* is implemented.

### *Control efficacy of mowing and tilling*

There are few studies on controlling *S. alterniflora* by mowing and tilling (Table 2). Mowing is the pretreatment of tilling, and tilling plays the main role in control. *S. alterniflora* enters dormancy in the cold winter. Tillage can destroy the rhizome and make it vulnerable to cold and the tide and therefore easily affects the asexual reproductive capacity of the rhizome. Therefore, the asexual reproduction of *S. alterniflora* was almost completely inhibited the following spring (Fig. 8A). However, if the secondary invasion of seeds is unavoidable, the control area will be reoccupied by *S. alterniflora* after a certain period of time (Fig. 8D-F).

The choice of tillage time may have a great influence on control efficacy. This study suggested that mowing and tilling at the end of the growing season reached a satisfactory control efficacy. As described in Section 4.1, the asexual reproductive capacity of *S. alterniflora* may be weakest at the end of the vegetative growth stage, so tillage during this period may also achieve very good control efficacy. In the coastal zone of Fujian Province of China, mowing and tillage in early July could eradicate *S. alterniflora*, which did not reappear in the second and third years (Tan 2008). Some studies have reported that the control efficiency of winter tillage is only 73% (Patten 2004). If the rhizome of *S. alterniflora* is further broken after tillage, the control efficiency may be improved (Mateos-Naranjo et al. 2012).

**Table 2 A summary of the control efficacy of mowing and tilling**

### *Control efficacy of herbicides*

Chemical control is usually carried out by applying herbicides to eradicate *S. alterniflora*. Many herbicides, such as haloxyfop-r-methyl, glyphosate, glufosinate am-

monium, and imazapyr, have been used to control *S. alterniflora*. The control efficacy of different herbicides varies greatly, ranging from 12% to 100% (Table 3). The U. S. Environmental Protection Agency only allows glyphosate and imazapyr to be used in estuarine environments (Knott et al. 2013). Glyphosate is a widely used herbicide, but its efficacy for control of *S. alterniflora* is unsatisfactory, the vast majority of which is less than 62% (Knott et al. 2013; Mateos-Naranjo et al. 2012; Patten 2004). Although some studies have shown that the control efficacy of haloxyfop-r-methyl against *S. alterniflora* is very poor, the present study found that the control efficacy of haloxyfop-r-methyl is close to 100% at an appropriate dose. In addition to the dosage of herbicides, the growth period of *S. alterniflora* also affects the control efficacy of herbicides. For example, the same dosage of glyphosate showed 93% control efficacy against *S. alterniflora* seedlings but only 16% - 25% control efficacy against mature plants (Knott et al. 2013). Glufosinate and imazapyr have better mortality effects on the seedlings of *S. alterniflora*, but their control effect against mature *S. alterniflora* is also poor, usually less than 33% (Knott et al. 2013; Patten 2004). The control efficacy of herbicides is also influenced by wind, tidal cycles and sediment cover on stems and leaves (Hedge et al. 2003).

**Table 3 A summary of the control efficacy of sprayed herbicides**

It must be noted that it is almost impossible to achieve 100% control efficacy by spraying herbicides due to the mutual occlusion of dense stems and leaves or uneven spraying. In this study, 0.30 kg ha<sup>-1</sup> haloxyfop-r-methyl could kill 98% of *S. alterniflora*. when increasing the dosage of from 0.30 to 0.45 kg ha<sup>-1</sup>, there were still a few surviving plants in some areas (1-5 stems m<sup>-2</sup> on average). Other studies have found similar results. The application of glyphosate in the same location for several consec-

utive years did not improve the overall control significantly compared with the application of glyphosate for only one year, and there were still several *S. alterniflora* plants per square meter (Patten 2004). This sporadic survival of *S. alterniflora* may lead to large-scale secondary invasion over the next few years. It is not enough to spray herbicides only once. The surviving *S. alterniflora* should be sprayed with herbicides for a second or even a third time.

Chemical methods for controlling *S. alterniflora* are likely to have negative effects. On the one hand, chemical agents usually cause some residual toxicity; on the other hand, they often cause harm to other plants and animals, thereby destroying the local soil and water ecosystems (Kilbride and Paveglio 2001; Paveglio et al. 1996). Qiao et al. (2019) found that the crab density was significantly lower than that in the control treatment after spraying haloxyfop-r-methyl or glyphosate for 4 months, but the crab population recovered 11 months after spraying herbicides. However, many studies have found that herbicides are not harmful organisms on beaches or in estuaries (Patten 2003; Shimeta et al. 2016). Species richness and diversity value of native plants were not affected by glyphosate (Mateos-Naranjo et al. 2012). Our study found that haloxyfop-r-methyl had no effect on *Suaeda salsa*, a native vegetation. This harmlessness may be due to low-dose exposure to herbicides because herbicides are mainly taken up by plant stems and leaves or washed away by tidal water, and only a small amount reaches sediments (Shimeta et al. 2016). The influence of herbicides on the environment is closely related to the amount and time of herbicide application. In the future, the optimal time and minimum dosage of herbicides should be evaluated to minimize their negative effects on the environment.

### *Cost of controlling S. alterniflora*

The cost of controlling *Spartina alterniflora* is very high. The mean annual cost to manage Willapa *S. alterniflora* during five years was ~\$3,500 km<sup>-1</sup> of shoreline per year (Patten et al. 2017). The cost of chemical control in this study was 1067 dollars per hectare. By comparison, the average annual cost for the chemical control of *S. alterniflora* was 2414 dollars per hectare in South Africa (Riddin et al. 2016). The control costs of mowing and waterlogging in this study was 4104 dollars per hectare. Yuan et al. (2011) reported that the control cost of mowing and waterlogging was only 500 dollars per hectare. Because of the higher waterlogging level than that in this study and the need for pumping, the low cost reported by Yuan is almost impossible.

### **Conclusion**

This study covers a variety of approaches to control *S. alterniflora*, and all of the approaches can achieve very high control effectiveness. This study also provides a possibility to reduce the cost of controlling *S. alterniflora*.

The integrated approach of mowing and waterlogging can completely inhibit the sexual and asexual reproduction of *S. alterniflora*, thus achieving the goal of eradicating *S. alterniflora*. It is recommended that this method be used in areas with frequent tidal flooding. The technical details of this approach include (1) mowing *S. alterniflora* at the end of the vegetative growth stage, with the height of the stubble being less than 10 cm, and (2) continuous waterlogging after mowing until the end of the year, with the water level being 20-30 cm.

The integrated approach of mowing and tilling at the end of the growing season can almost completely inhibit the asexual reproduction of *S. alterniflora*, and the re-

moval of *S. alterniflora* can effectively inhibit its sexual reproduction by removing seeds from the system. Therefore, this integrated approach is a good way to control *S. alterniflora*. This method may be more suitable for places with cold winters. In addition, if we want to extend this approach, special machinery suitable for muddy tidal flats is needed.

The application of haloxyfop-r-methyl at a dose of 0.3-0.45 kg ha<sup>-1</sup> can almost eradicate *S. alterniflora*, and its control efficacy was more than 98%. The control efficacy of glyphosate at a dose of 4.0-8.0 kg ha<sup>-1</sup> was less than 23%. Therefore, haloxyfop-r-methyl can be used to control *S. alterniflora*. To minimize the potential environmental pollution, herbicides are recommended for new invasive patches of *S. alterniflora*.

Finally, it is difficult to eradicate *S. alterniflora* only once. Later investigation and re-control for the remaining *S. alterniflora* are probably necessary. Large-scale control is also needed. Otherwise, the controlled areas are likely to be re-invaded by the seeds of *S. alterniflora* from seawater in a few years.

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