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Emergency control of *Spartina alterniflora* re-invasion with a chemical method in Chongming Dongtan, China

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Abstract

The exotic species *Spartina alterniflora* (*S. alterniflora*) seriously threatens the stability and functioning of saltmarsh ecosystems in the Yangtze Estuary. Ambitious efforts have been undertaken to control this species, but subsequent re-invasion is frequent, presenting a significant barrier to restoration. The complexity and high cost of integrated physical control programs has necessitated a shift in focus, leading to considerable attention being paid to the potential of herbicides to control *S. alterniflora*. To find a strategy for emergency control of small and scattered patches of re-invading *S. alterniflora*, an in situ field experiment using Gallant (Haloxyfop-R-methyl) herbicide was conducted. The growth parameters of plant density and height were used to evaluate the control efficiency of different treatment dosages and times and sediment samples were taken for environmental toxicity analysis. The results show the following: (1) the control efficacy of the maximum proposed application dose (2.70 g/m^2) was 92% for continuous swards and 100% for small patches, while those of other dosages (0.45 g/m^2, 0.90 g/m^2, and 1.35 g/m^2) were lower than 40%; (2) the appropriate implementation time was July to August with 100% mortality resulting from a single application, while *S. alterniflora* was shown to be capable of recovering rapidly after treatment in May; and (3) there were no significant differences in the community structure of meiofauna among the herbicide treatments and the control, and no herbicide residues were detected in sediment samples collected from treatment areas. This chemical control method was implemented in the Shanghai Chongming Dongtan National Bird Nature Reserve (CDNR). The results of this study indicate that Gallant is an environmentally friendly herbicide with high efficiency, which can be adopted for emergency control of re-invading *S. alterniflora*.

Keywords: Spartina alterniflora; Re-invasion; Chemical control method; Gallant; Yangtze Estuary

1. Introduction

The invasion of exotic species is one of the most significant threats posed by global environmental change (Theoharides and Dukes, 2007). Invasive species threaten the integrity of natural systems throughout the world by disrupting interactions between species and changing patterns of resource availability, resulting in losses of ecosystem function and spiraling economic costs (Cook-Patton and Agrawal, 2014; Strong and Ayres, 2016). Ambitious efforts have been implemented to control or eradicate invasive species (Yuan et al., 2011; Strong and Ayres, 2016). However, treated areas often face the daunting challenge of re-invasion by the same or other unwanted exotic species (e.g., Galatowitsch and Richardson, 2005; Richardson and Kluge, 2008). The re-invading species might be taking advantage of newly available resources and habitat disturbance resulting from treatments (Kettenring and Adams, 2011; Cutting and Hough-Goldstein, 2013). Legacy

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effects on environmental properties that persist after control programs can also allow re-invaders to make quick adaptation and spread rapidly before native species are able to recover, which may present a significant barrier to the restoration of natural areas threatened by invasive plants (Cuddington, 2011; Gabler and Siemann, 2012; Pearson et al., 2016).

Spartina alterniflora (*S. alterniflora*, smooth cordgrass), a halophyte native deeply rooted in the Atlantic and Gulf coasts of North America, is an invasive species of coasts at the global scale (An et al., 2007; Zheng et al., 2016). For the purposes of reducing tidal wave energy, mitigating erosion, and trapping sediments, *S. alterniflora* was introduced to the Shanghai Chongming Dongtan National Bird Nature Reserve (CDNR) in 1995 (Yuan et al., 2011). Since then, *S. alterniflora* has expanded rapidly to form monocultures dominating the intertidal zone in the CDNR over the past twenty years, with a distribution area exceeding 18 km^2 (Huang et al., 2008; Yuan et al., 2014). The area dominated by *S. alterniflora* is characterized by tall (higher than 1 m) and dense vegetation, reduced biodiversity, and deteriorated habitat conditions, significantly affecting suitable stopover sites for migratory water birds and disrupting the delicate ecological balance in these areas (Zou et al., 2016; Liao et al., 2018). In 2013, an ecological engineering project covering an area of 24 km^2 aimed at *S. alterniflora* removal and bird habitat restoration was conducted in the CDNR (Hu et al., 2015). While the practice of control method successfully relieved the severity of the infestation, there were still large areas of the *S. alterniflora* community left intact outside the CDNR. Over the last two years, unpredictable and sporadic re-invasions of the controlled areas have been spotted. Before these re-invasion events seriously deter the effects of ecological restoration in these areas, it is necessary to find useful methods to control the small and scattered patches of re-invading *S. alterniflora* and restore the natural ecosystem in the CDNR.

Considering the complexity and high cost of physical or mechanical control methods (e.g., cutting plus waterlogging), as well as the sometimes unpredictable effects of biological methods (Yuan et al., 2011), using an environmentally friendly herbicide with high efficiency should be a high priority for use in eliminating the small and scattered patches of re-invading *S. alterniflora*. At present, many herbicides, such as Gallant, Glyphosate, Glufosinate ammonium, and Imazapyr have been used to control *S. alterniflora* in North America, Europe, New Zealand, and China (Miller and Croyhers, 2004; Mateos-Naranjo et al., 2012; Knott et al., 2013; Sheng et al., 2014). Of the existing trials with different herbicides, the Gallant used in New Zealand was the most successful herbicide, with up to 95% mortality (Miller and Croyhers, 2004), thus providing an appealing option. However, evidence also showed that the control efficacy of the same herbicides varies greatly in different areas with different habitat characteristics and plant morphology (Knott et al., 2013; Patten et al., 2017). The geographical variability of the estuarine environment will influence the control efficiency (Patten, 2003; Major et al., 2003). For example, the tidal range in the Yangtze Estuary is approximately 1.9 m for neaps and 4.6 m for springs, while in Kaipara Harbor (the largest natural estuary in New Zealand) it is approximately 1.9 m for neaps and approximately 2.8 m for springs (Hu et al., 2019; Mark and Malcolm, 2017). The greater tidal range in the Yangtze Estuary increases the submerged time of *S. alterniflora*, which shortens the absorption time of Gallant herbicide and decreases the control efficiency. In addition, *S. alterniflora* in the Yangtze Estuary grows denser and higher, and has wider genetic exchange than that in New Zealand (Hayward et al., 2008; Xiao et al., 2009; Yuan et al., 2011; Liu et al., 2016). These phenotypic and genetic variations will result in different responses and sensitivity of *S. alterniflora* to Gallant herbicide (Knott et al., 2013). Therefore, to implement Gallant herbicide to control *S. alterniflora* in China's coastal areas, appropriate dose concentration, spraying time, and environmental safety parameters need to be determined under specific local environmental conditions.

In this study, a field experiment was undertaken to use Gallant herbicide to control re-invading *S. alterniflora* patches in the CDNR from 2016 to 2017. The objectives of the study were the following: (1) to identify the suitable dose and time for using Gallant to control *S. alterniflora* patches, (2) to analyze the possible environmental impacts of using this herbicide, and (3) to provide useful emergency control strategies to eliminate the small and scattered patches of *S. alterniflora* re-invading the CDNR. Overall, our study will shed a new light on an emergency control strategy for the small and scattered *S. alterniflora* re-invading patches, which is relevant to our understanding of *S. alterniflora* management in China's coastal regions more generally.

2. Materials and methods

2.1. Study area

The CDNR (31°25′N to 31°38′N, and 121°50′E to 122°05′E) (Fig. 1), one of the most important wetland ecosystems in eastern China, is located between the North Branch and the South Branch's North Channel of the Yangtze Estuary (Li et al., 2014). This region is characterized by irregular semidiurnal tides, with maximum and average tide heights of

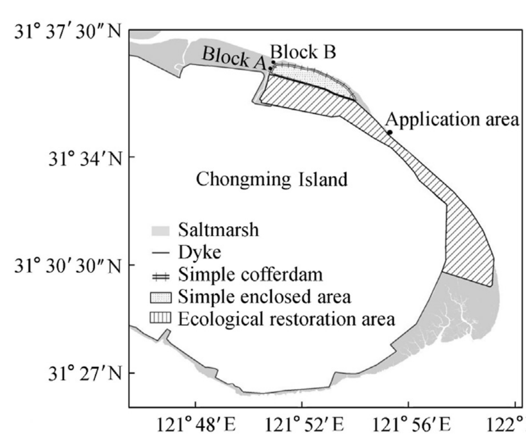


Fig. 1. Location of CDNR and study area.

4.62–5.95 m and 1.96–3.08 m, respectively (Hu et al., 2019). It covers 326.10 km^2 and serves as an important stopover habitat for shorebirds migrating between Australia and Siberia (Yuan et al., 2011). The three dominant salt-marsh species in the CDNR are *S. alterniflora*, *Scirpus mariqueter*, and *Phragmites australis* (Yuan et al., 2011). The latter two are native species.

2.2. Experimental materials

The herbicide used in this study was Gallant, also called Haloxyfop-R-methyl ($C_{16}H_{13}ClF_3NO_4$, 108 g of active ingredient per liter). It belongs to the aromatic aryloxyphenoxy propionic acid herbicides group and is suitable for treating both stems and leaves (Wu et al., 2013). This herbicide inhibits acetyl coenzyme A carboxylase in plants, resulting in blocked fatty acid synthesis and mortality of target plants (Wu et al., 2013). It can be used to eradicate grassy weeds with a long efficacy period, but is ineffective against Cyperaceae plants (Miller and Croyhers, 2004).

2.3. Experimental design

The field experimental site was located in a floodplain area outside the simple cofferdam north of the Chongming Dongtan Ecological Restoration Project area (Fig. 1). The elevation of this site is around 3.50 m (the local Wusong bathymetric benchmark), and the site is periodically flooded. According to the distribution of *S. alterniflora*, the experimental site was divided into two blocks: (A) continuous swards with average coverage of 80%–95%, and (B) scattered small patches with diameters of 0.1–1 m (Figs. 1 and 2). Block A was used to identify the control efficacy (defined in section 2.4) of Gallant herbicide with different treatment dosages, while block B was used to identify the control efficacy of Gallant herbicide with different treatment times during the growing season.

In August 2016, fifteen permanent plots (5 m × 5 m) with different treatment dosages were established randomly in Block A (Fig. 2(a)). There was an interval of at least 5 m between each plot. *S. alterniflora* plants on the peripheries of each plot were regularly removed through manual harvesting from the bottom of the stem to avoid diffusion. Five dosages of Gallant herbicide (0 g/m^2, 0.45 g/m^2, 0.90 g/m^2, 1.35 g/m^2, and 2.70 g/m^2) were used and denoted as G0, G1, G2, G3, and G4, respectively (Table 1 and Fig. 2(a)). Each treatment dose had three replicates. On sunny and windless days, at least six hours (an empirical value according to the local tidal rhythm and the instructions of Gallant herbicide) before the next rising tide, herbicide was sprayed on the canopy of *S. alterniflora* in each of the plots according to the dosages from low to high levels. After each treatment, the spraying equipment was rinsed three times with pure water to ensure the accuracy of the dose in the next treatment.

In April 2017, four transects were established perpendicularly to the dyke towards the sea (Fig. 2(b)). Within each transect, six quadrats (2 m × 2 m, about 40 m apart) were established that had similar coverage (29.3% ± 3.8%) of

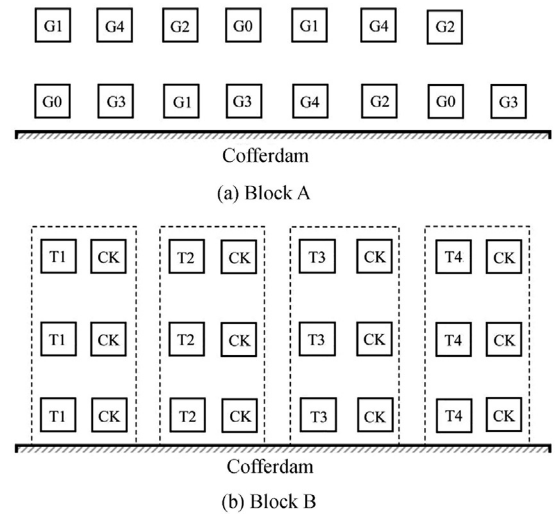


Fig. 2. Experimental design and layout of treatment plots in experimental blocks (the solid line boxes represent the treated quadrats, and the dashed line boxes represent the experimental transects; G0, G1, G2, G3, and G4 represent five dosages of Gallant herbicide of 0 g/m^2, 0.45 g/m^2, 0.90 g/m^2, 1.35 g/m^2, and 2.70 g/m^2, respectively; T1 indicates dosing in May, T2 indicates dosing in June, T3 indicates dosing in July, and T4 indicates dosing in August; and CK means blank control).

S. alterniflora patches (Fig. 2(b)). From May to August 2017, Gallant herbicide was sprayed onto the three longitudinally arranged quadrats along one transect on a monthly basis, and the adjacent quadrat was used as the blank control, thus giving three replicates and three blank controls for each time treatment and a total of four types of time treatments (i.e., May, June, July, and August, denoted as T1, T2, T3, and T4, respectively) (Table 1 and Fig. 2(b)). The dosages of the herbicide for each time treatment were determined by the experiment in 2016, and the implementation method we used here was consistent with that of the experiment in 2016.

To verify whether the control strategies drawn from the two experiments described above can be used as useful emergency control strategies for eliminating the small and scattered patches of re-invading *S. alterniflora*, one block (100 m × 200 m) was established in July 2018 in an area where re-invasion of *S.*

Table 1
Summary of locations and treatments for experiment on use of Gallant herbicide to control *S. alterniflora*.

Variable	Treatment dose (g/m^2)	Abbreviation	Treatment date	Plot size (m^2)	Replicates
Dosage	0	G0	Aug. 11, 2016	25	3
	0.45	G1	Aug. 11, 2016	25	3
	0.90	G2	Aug. 11, 2016	25	3
	1.35	G3	Aug. 11, 2016	25	3
	2.70	G4	Aug. 11, 2016	25	3
Time	2.70	T1	May 10, 2017	4	3
	2.70	T2	Jun. 10, 2017	4	3
	2.70	T3	Jul. 10, 2017	4	3
	2.70	T4	Aug. 10, 2017	4	3

alterniflora had occurred in the CDNR (Fig. 1). Then, the emergent control method proposed in our study was implemented in this block. For two months, the control efficacy was monitored and evaluated.

2.4. Sampling and measurement

At 1 d before treatment, and 1 d, 3 d, 7 d, 14 d, 21 d, and 30 d after treatment, the status of *S. alterniflora* in each plot was visually assessed as alive or dead. Then, the density and height of surviving *S. alterniflora* plants were surveyed. The control efficacy (%), defined as the proportion of an infestation that is killed as a result of treatment, was calculated using Eq. (1):

$$E_c = \frac{N-n}{N} \times 100\% \tag{1}$$

where E_c is the control efficacy, n is the number of surviving ramets after treatment, and N is the number of ramets before treatment (Hedge et al., 2003).

Samples from the upper 2 cm of sediment (approximately 4 mL) were randomly collected from each plot using a syringe (Xu et al., 2018) 1 d before, and 1 d, 3 d, 7 d, 14 d, 21 d, and 30 d after treatment. At each sampling point, each sample contained three surface sediments, giving three pooled sample replicates per plot. After mixing with glutaraldehyde (2% final concentration, i.e., 20 g/L), meiofauna (e.g., threadworms and copepods) were extracted from and stained in all 270 samples according to Xu et al. (2010). Enumeration and identification were conducted under microscopes (Olympus DP80) at 100 times to 200 times magnifications.

For herbicide residue analysis, another sample of the upper 2 cm of sediment (25 cm × 25 cm) was randomly collected from each plot 1 d, 3 d, 7 d, 14 d, 21 d, and 30 d after treatment. All samples were stored at −20°C in a portable refrigerator and sent to a professional testing institute (Weipu Analysis & Testing Center, Shanghai, China). After pretreatment (i.e., ultrasound extraction (Zhang et al., 2016)), herbicide residues in the sediments were detected through liquid chromatography-mass spectrometry (LC-MS), with detection limit and reporting limit of 5.75×10^{-5} mg/kg and 2×10^{-4} mg/kg, respectively. Values lower than the reporting limit indicate that no active ingredient of the herbicide has been detected.

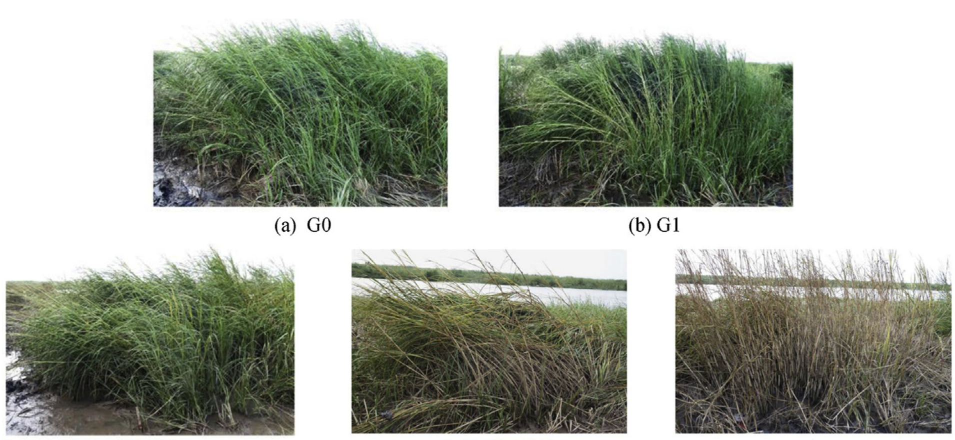
2.5. Data analysis

The plant data collected from field measurements were analyzed using one-way analysis of variance (ANOVA) to test for significant differences among the control and treatments. The least significant difference (LSD) method was used to make multiple comparisons. The level of statistical significance (P) was set as 0.05. Non-metric multidimensional scaling (nMDS) analysis was used to visualize the variation in the species composition of meiofauna among different treatments on each sampling date. We also tested for the statistically significant differences in community structure of meiofauna overall among treatments and among sampling dates using an analysis of similarities (ANOSIM), which is a non-parametric test based on the Mantel test and a standardized rank correlation between two distance matrices. If the resulting statistic (R) is close to 1, it indicates that the most similar samples are in the same groups, while if R is close to 0, it indicates that the similarities do not have any relationship with the groups. nMDS and ANOSIM were computed using PRIMER v7.0.13 (Clarke et al., 2014). One-way ANOVAs were implemented using SPSS23.0.

3. Results

3.1. Control efficacy of Gallant herbicide with different dosages

Photographs illustrating the efficacy of the different herbicide dosages on the control of *S. alterniflora* 30 d after treatment in 2016 are shown in Fig. 3. The G4 treatment was able to control *S. alterniflora* effectively, as shown by the



(a) G0 (b) G1 (c) G2 (d) G3 (e) G4

Fig. 3. Control efficacy after application of Gallant herbicide for one month with different dosages.

brown and withered ramets, and blackened and rotting roots. The G3 treatment had some effect, with a small number of ramets turning yellow. The G1 and G2 treatments had no significant effect on controlling *S. alterniflora*, as the plants grew normally with green terminal buds.

Changes in *S. alterniflora* density and height under different treatments during the experiment in 2016 are shown in Fig. 4(a) and (b). After 30 d, the ramet densities under the G3 and G4 treatments were both significantly lower than those under the G0, G1, and G2 treatments ($P < 0.05$). The ramet density under the G4 treatment was significantly lower than that under the G3 treatment ($P < 0.05$), but there were no significant differences in density among the G0, G1, and G2 treatments ($P > 0.05$). Thirty days after treatment, compared with the initial density before treatment, the mean densities of the G0 and G1 treatments increased by (35 ± 13) ind./m^2 and (18 ± 2) ind./m^2, respectively, while the reductions of mean density were 5.70% ± 5.15%, 32.36% ± 6.04%, and 92.02% ± 2.06% for the G2, G3, and G4 treatments, respectively (Fig. 4(a)). In addition, the height of surviving ramets under the G4 treatment was significantly lower than those under other types of treatments 30 d after treatment ($P < 0.05$), and there were no significant differences among the other treatments ($P > 0.05$) (Fig. 4(b)).

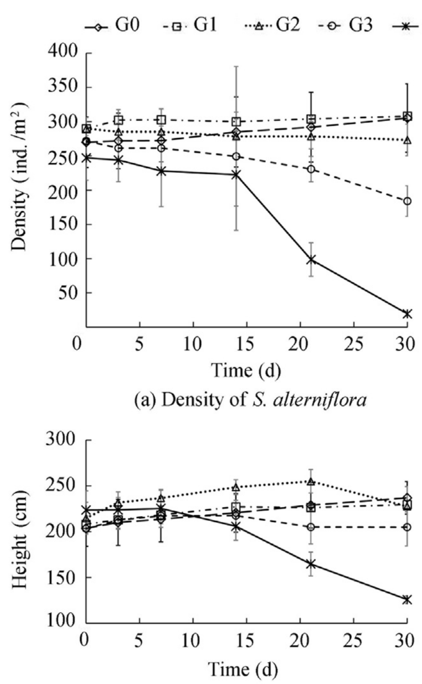


Fig. 4. Effects of different treatment dosages of Gallant herbicide.

3.2. Control efficacy of Gallant herbicide with different treatment times

The efficacy of using the herbicide at different times during the growing season on the control of *S. alterniflora* during 2017 is shown in Fig. 5. Spraying herbicide with the same dosage at different times was shown to have different efficacies. Spraying Gallant in July and August resulted in a high degree of control of *S. alterniflora*. The density of surviving ramets and control efficacy were 0 and 100%, respectively, under both the T3 and T4 treatments one month after treatment. Within three months of treatment, no emergence of new ramets had been observed (Table 2). When herbicide was sprayed in June, the control efficacy one month after treatment was 57.31% ± 15.68%, while all the ramets were dead by two months after treatment (Table 2). When sprayed in May, the density of *S. alterniflora* initially decreased (66.79% ± 24.83% one month after treatment and 90.85% ± 13.61% two months after treatment), but new ramets appeared in the quadrat three months after treatment (Table 2).

3.3. Evaluation of environmental impacts of herbicide treatment

Of all the meiofauna identified in the sediment samples, threadworms accounted for the largest proportion, followed by polychaetes, copepods, and cladocerans, while rotifers, ostracods, and oligochaetes accounted for lower proportions. The nMDS analyses of meiofauna community structure (i.e., species composition and abundance) under different herbicide dosages at different sampling dates are presented in Fig. 6. From each sampling date, the community of meiofauna collected from the blank control and different herbicide dosages are grouped together (Fig. 6). Based on the results of ANOSIM analysis, the difference in community structure was only significant among different sampling dates ($R = 0.368$, $P < 0.01$) but not significant among different herbicide treatments ($R = 0.02$, $P > 0.1$). This indicates that spraying the proposed dosages (i.e., G1 to G4) of Gallant to control *S. alterniflora* does not seem to be harmful to benthos when applied over the one-month timescale used in this study.

(a) May (b) June (c) July (d) August

Fig. 5. Control efficacy after application of Gallant herbicide for one month at different times.

Table 2
Control efficacy of Gallant herbicide with different treatment times during growing season (mean ± standard deviation).

Treatment time	Before treatment		One month after treatment		Two months after treatment		Three months after treatment	
	Density (ind./m^2)	Control efficacy (%)	Density (ind./m^2)	Control efficacy (%)	Density (ind./m^2)	Control efficacy (%)	Density (ind./m^2)	Control efficacy (%)
May (T1)	37 ± 2	0.00 ± 0.00	13 ± 10	66.79 ± 24.83	4 ± 5	90.85 ± 13.61	5 ± 8	86.83 ± 20.20
June (T2)	35 ± 12	0.00 ± 0.00	14 ± 1	57.31 ± 15.68	0 ± 0	100.00 ± 0.00	0 ± 0	100.00 ± 0.00
July (T3)	32 ± 9	0.00 ± 0.00	0 ± 0	100.00 ± 0.00	0 ± 0	100.00 ± 0.00	0 ± 0	100.00 ± 0.00
August (T4)	38 ± 10	0.00 ± 0.00	0 ± 0	100 0.00 ± 0.00	0 ± 0	100.00 ± 0.00	0 ± 0	100.00 ± 0.00

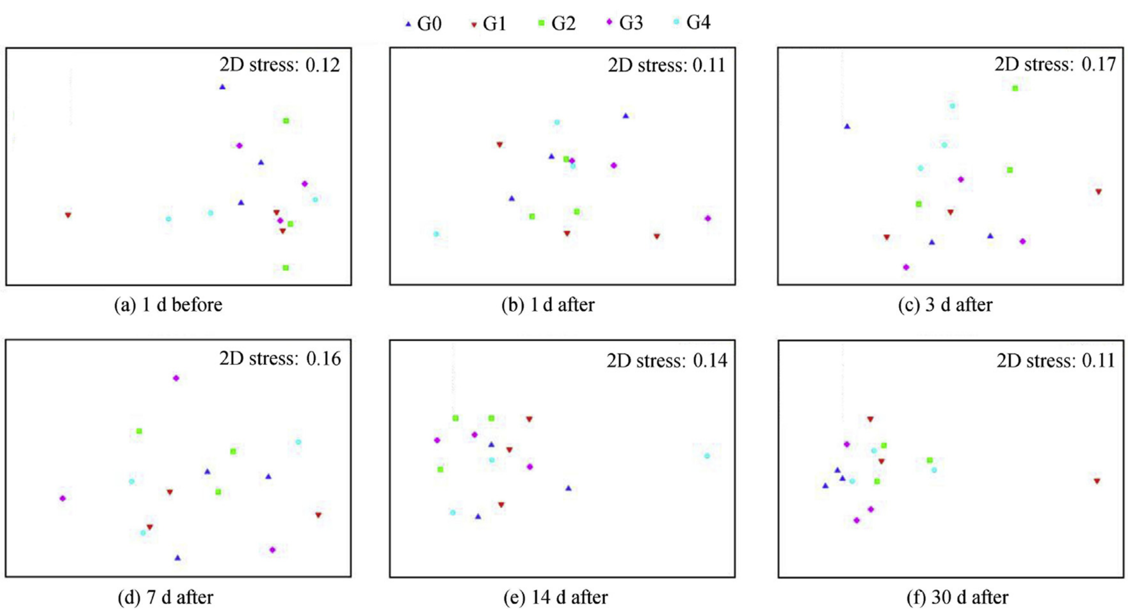


Fig. 6. Non-metric multidimensional scaling analyses of different Gallant herbicide application dosages on community structure of meiofauna.

The results of herbicide residues provided by a professional testing institution showed that herbicide residues of all samples were not detected (less than 0.000 2 mg/kg, the reporting limit of LC-MS), and no significant differences were found between treatments and the control. This indicates that the Gallant sprayed on the canopy of the *S. alterniflora* community was almost fully absorbed by the *S. alterniflora* plants, and the little herbicide that had been scattered on the mudflat could also be degraded rapidly.

3.4. Implementation of emergency control strategies for re-invading S. alterniflora

The implementation effects of emergency control strategies for controlling *S. alterniflora* re-invasion are shown in Fig. 7. Two months after application, the re-invaders were brown and withered with a 100% mortality and no new ramets had appeared. The distribution of the native species *S. mariqueter* (Cyperaceae) was not reduced by Gallant and expanded because Gallant is a specific herbicide aimed at Gramineae plants. This indicates that Gallant herbicide, when used in appropriate dosage and time, is a promising candidate for

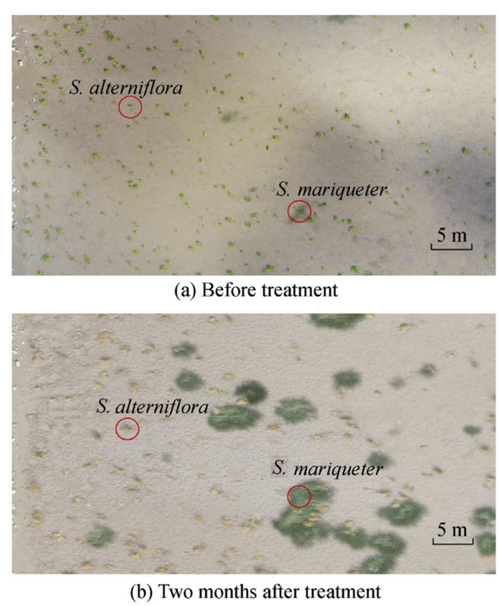


Fig. 7. Application effects of emergency control strategies for managing *S. alterniflora* re-invasion.

effectively eliminating small and scattered patches of re-invading *S. alterniflora* in coastal areas, potentially over the long term.

4. Discussion

4.1. Control efficacy of Gallant herbicide

Herbicide is widely used to control weeds in cropland management, and some herbicides work by restraining growth of weeds by inhibiting the physiological and metabolic activities of plants or by destroying fibers and cell structures (Wu et al., 2013; Knott et al., 2013). In coastal areas, the limited practicality of physical control programs has resulted in considerable attention and hopes being focused on the potential of herbicides to exterminate invasive species (Shimeta et al., 2016; Patten et al., 2017). To date, various herbicides have been tested in a series of trials to control *S. alterniflora*. Glyphosate and Imazapyr have been widely used in most European countries and the United States for a number of years (Simenstad et al., 1996; Hedge et al., 2003; Shimeta et al., 2016). However, evidence has been presented that these herbicides are not able to satisfactorily control established *S. alterniflora* communities, with control efficacies usually less than 65% and repeated spraying often necessary (Patten, 2004; Mateos-Naranjo et al., 2012; Knott et al., 2013). Compared with these results, the Gallant herbicide used in this study is characterized by high efficiency, with 100% control efficacy.

The dosage of an herbicide is a critical factor affecting control effectiveness (Patten, 2003; Major et al., 2003). As shown in this study, the control efficacy of Gallant herbicide changes greatly with different dosages. The appropriate dose in this study (i.e., 2.70 g/m^2) could control *S. alterniflora* effectively, while a lower dose (i.e., 0.45 g/m^2) had no control effect at all, and new ramets emerged later. The different control efficacies (92% in 2016 and 100% in 2017) of Gallant using the same dose (2.70 g/m^2) can be attributed to mutual occlusion by dense stems and leaves or uneven spraying. When applied to small patches in 2017, it was easy to spray evenly and almost every ramet was exposed to herbicide. For the continuous swards in 2016, it was common that the higher ramets were preferentially controlled with some covered shorter ramets, which were able to survive due to the lesser amount of herbicide absorbed.

The efficacy of herbicides is also affected by application time or the life-history stage of target plants (Hedge et al., 2003; Knott et al., 2013). In previous studies, the control efficacy of Glyphosate on *S. alterniflora* in May was significantly lower than in June, July, and August (Hedge et al., 2003; Major et al., 2003), and the control efficacy of Glyphosate at the same dose was 93% on *S. alterniflora* seedlings but less than 25% on mature plants (Knott et al., 2013). In this study, spraying Gallant herbicide during the rapid germinating stage (i.e., May) could suppress the growth of *S. alterniflora* but not control it. When Gallant herbicide was sprayed on the flowering phase (July and August), the control efficacy was significantly increased with a 100% mortality within one month. This can be explained by the fact that most of the energy produced by photosynthesis during the flowering phase is devoted to sexual reproduction, with less energy reserved in underground parts. As the number of asexual ramets remains constant, it becomes almost impossible to re-generate after herbicide disturbance (Yuan et al., 2011; Liu et al., 2017). Therefore, this could represent the best stage to control *S. alterniflora* by spraying herbicide at a suitable dosage.

4.2. Ecological and economic consequences of Gallant herbicide treatment

Chemical methods are usually considered to have negative ecological impacts, due to the possibility of residual toxicity at spraying sites that could poison non-target plants and animals, and negatively affect the local soil and water ecosystems (Hedge et al., 2003; Major et al., 2003). In this study, short-term evidence of the toxic effects of Gallant herbicide treatments on the local ecological environment was not detected when an appropriate spraying method was used. Meiofauna are extremely sensitive to environmental changes and are often used as indicators of environmental safety (Xu et al., 2018). In this study there were no significant changes in the community structure of meiofauna after spraying with Gallant herbicide in the treatment year. Similar harmless effects of herbicide spraying on benthos have been reported by many studies (Patten, 2003; Back et al., 2012; Shimeta et al., 2016). Furthermore, our results showed that no herbicide residues were detected in the sediment samples in the treatment year. These results indicated that spraying an appropriate dose of Gallant with appropriate methods can effectively avoid environment residual and poisoning of the surrounding environment, primarily due to the following three factors: (1) Gallant is a highly absorptive herbicide that can be quickly absorbed by stems and leaves, and has a short exposure time in the environment; (2) *S. alterniflora* communities or patches were characterized by dense coverage and, therefore, very little herbicide could penetrate the canopy of the plants and reach the sediment; and (3) inherent tidal processes could have washed away the possible dripping herbicide and hence reduce local environmental residue. However, it should also be noted that if an overdose of herbicide or non-tidal-disturbance time after application is not sufficient, the unabsorbed herbicide drops will enter the water circulation or sedimentary environments, which may lead to long-term environmental impacts. Therefore, choosing appropriate dosing concentrations, implementation periods, and methods is essential and critical to minimizing the influence of herbicide on environmental safety, and further on-site studies are required to identify and assess other potential environmental impacts (e.g., on water quality) that the Gallant herbicide treatment may have on a larger space-time scale. In addition, specificity is a salient feature of evaluating the viability of herbicide option (Patten, 2003; Major et al., 2003), especially when invasive *S. alterniflora* and the native species *S. mariqueter* share almostoverlapping niches in the Yangtze Estuary (Huang et al., 2008). Inappropriate herbicides may also decrease the growth of native species while eliminating exotic species. However, Gallant herbicide was shown to be a species-specific herbicide, which could selectively kill *S. alterniflora* without harming *S. mariqueter.*

Compared with expensive and demanding physical control measurements, programs adopting herbicide treatments to control invasive plants are attractive due to relatively low costs and easy application (Shimeta et al., 2016). According to our estimates, the cost of spraying Gallant herbicide to eradicate *S. alterniflora* was 13 000 dollars per km^2 in the CDNR (including 1 000 dollars for herbicide, 2 000 dollars for spraying tools, and 10 000 dollars for labor), which is economically superior to other already-used chemical methods. For instance, the cost for spraying Rodeo to control *S. alterniflora* seedlings was around 170 000 dollars per km^2, and the cost for spraying Imazapyr to control *S. alterniflora* was around 60 000 dollars per km^2 (Hedge et al., 2003). It is worth noting that most of the spraying work was carried out by human labor in this study. The cost could decrease if improved tools and methods are used. Similar proposals were made in another study, in which an all-wheel-drive amphibious vehicle (Argo), a helicopter, and an unmanned aerial vehicle were suggested as efficient and sustainable options for spraying (Miller and Croyhers, 2004).

4.3. Emergency control of *S. alterniflora* re-invasion

After removal of invasive plants, ecosystems generally exhibit vacant niches, weak competition, and high resource availability (Panetta and Sparkes, 2001; Buckley et al., 2007; Kettenring and Adams, 2011), which can enable *S. alterniflora* to re-adapt quickly and rapidly re-form small and scattered patches. Adopting emergency control strategies against re-invasion at an early stage could prevent a much larger effort later (Grevstad, 2005). Small-scale control methods such as physical tillage, breaking of rhizomes, or mowing are labor-intensive, and the most cost-effective control is considered to be herbicide treatment (Li and Zhang, 2008). As we have shown here, adopting Gallant herbicide as an emergency control strategy to eliminate the small and scattered patches of re-invading *S. alterniflora* not only resulted in high control efficacy but also had no short-term detrimental impact on the environment in the treatment year. However, long-term observations are required to continue future work to monitor the regrowth of *S. alterniflora* and multi-year persistence of control efficiency, as well as to assess more detailed environmental impact (e.g., water quality). Therefore, when this herbicide method is used to control small re-invading patches in an estuary area, the following guidelines need to be followed: (1) the appropriate dose is 2.70 g/m^2 to guarantee high control efficacy and low impacts on the environment; (2) the appropriate implementation time is July to August, when there is no rain or tide predicted within six hours of application; and (3) herbicides need to be sprayed evenly on the canopy of target patches, with no spraying of exposed mudflats. Furthermore, *S. alterniflora* can not only re-invade mudflats but also re-invade the habitats of native vegetation (e.g., *S. mariqueter* and *P. australis*). Therefore, when spraying herbicide to control *S. alterniflora* in certain areas, attention should be paid to the protection of non-target Graminaceae species.

In addition, like in many coastal plants, propagules (seeds or vegetative fragments) of *S. alterniflora* can be transferred over long distances by tidal currents and establish successfully at a settlement site (Xiao et al., 2009; Zhu et al., 2014). These floating propagules may lead to unpredictable and sporadic re-invasion events. Thus, it is essential to actively monitor treated areas and keep searching for incoming re-invaders. Where such infestations are discovered, the appropriate control action (e.g., the Gallant herbicide treatment mentioned in our study) needs to be taken at the appropriate time. Also, restoration of native species in post-control areas can also function as a barrier against re-invasion by *S. alterniflora*. Finally, the risk of the propagules from possible source areas immigrating to controlled regions should be regulated. For example, removing all flowering heads prior to seed ripening (Grevstad, 2005) or spraying herbicides to suppress the growth of *S. alterniflora* before flowering (Anderson, 2007) could be effective methods for lowering re-invasion success. In summary, control of infection sources and protection of susceptible habitats (eradication of newly recruited invasive individuals and rejuvenation of native species) should be combined to achieve the goals of controlling re-invasion by *S. alterniflora* and restoring natural coastal ecosystems.

5. Conclusions

With a background of frequent re-invasion of *S. alterniflora* in ecological restoration areas, this study investigated the appropriate dose concentration, spray time, and possible environmental impacts of use of Gallant herbicide to control small and scattered patches of re-invading *S. alterniflora* in the CDNR. The results indicated that spraying Gallant herbicide in small *S. alterniflora* patches at the dose of 2.70 g/m^2 in July and August can result in a 100% mortality within a month. Moreover, there was no detrimental short-term impact on the meiofauna community structure and no herbicide residues in sediment. To ensure efficient control of *S. alterniflora* re-invading patches and minimize possible long-term environmental impacts, the guidelines for using Gallant as an emergency control strategy are provided. The results of this study provide a novel emergency control strategy for the small and scattered *S. alterniflora* patches that could be applied to other similar coastal areas.

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References

An, S.Q., Gu, B.H., Zhou, C.F., Wang, Z.S., Deng, Z.F., Zhi, Y.B., Li, H.L., Chen, L., Yu, D.H., Liu, Y.H., 2007. *Spartina* invasion in China: Implications for invasive species management and future research. Weed Res. 47(3), 183–191. https://doi.org/10.1111/j.1365-3180.2007.00559.x.

Anderson, L.W.J., 2007. Potential for sediment-applied acetic acid for control of invasive *Spartina alterniflora*. J. Aquat. Plant Manag. 45(2), 100–105. https://doi.org/10.1093/icesjms/fsm095.

Back, C.L., Holomuzki, J.R., Klarer, D.M., Whyte, R.S., 2012. Herbiciding invasive reed: Indirect effects on habitat conditions and snail-algal assemblages one year post-application. Wetl. Ecol. Manag. 20(5), 419–431. https://doi.org/10.1007/s11273-013-9296-4.

Buckley, Y.M., Bolker, B.M., Rees, M., 2007. Disturbance, invasion and re-invasion: Managing the weed-shaped hole in disturbed ecosystems. Ecol. Lett. 10(9), 809–817. https://doi.org/10.1111/j.1461-0248.2007.01067.x.

Clarke, K.R., Gorley, R.N., Somerfield, P.J., Warwick, R.M., 2014. Change in Marine Communities: An Approach to Statistical Analysis and Interpretation, 3rd ed. Primer-E, Plymouth.

Cook-Patton, S.C., Agrawal, A.A., 2014. Exotic plants contribute positively to biodiversity functions but reduce native seed production and arthropod richness. Ecology 95(6), 1642–1650. https://doi.org/10.1890/13-0782.1.

Cuddington, K., 2011. Legacy effects: The persistent impact of ecological interactions. Biol. Theory 6(3), 203–210. https://doi.org/10.1007/s13752-012-0027-5.

Cutting, K.J., Hough-Goldstein, J., 2013. Integration of biological control and native seeding to restore invaded plant communities. Restor. Ecol. 21(5), 648–655. https://doi.org/10.1111/j.1526-100X.2012.00936.x.

Gabler, C.A., Siemann, E., 2012. Environmental variability and ontogenetic niche shifts in exotic plants may govern re-invasion pressure in restorations of invaded ecosystems. Restor. Ecol. 20(5), 545–550. https://doi.org/10.1111/j.1526-100X.2012.00901.x.

Galatowitsch, S., Richardson, D.M., 2005. Riparian scrub recovery after clearing of invasive alien trees in headwater streams of the Western Cape, South Africa. Biol. Conserv. 122(4), 509–521. https://doi.org/10.1016/j.biocon.2004.09.008.

Grevstad, F.S., 2005. Simulating control strategies for a spatially structured weed invasion: *Spartina alterniflora* (Loisel) in Pacific Coast estuaries. Biol. Invasions 7(4), 665–677. https://doi.org/10.1007/s10530-004-5855-1.

Hayward, B.W., Grenfell, H.R., Sabaa, A.T., Morley, M.S., 2008. Ecological impact of the introduction to New Zealand of Asian date mussels and cordgrass: The foraminiferal, ostracod and molluscan record. Estuar. Coast 31(5), 941–959. https://doi.org/10.1007/s12237-008-9070-7.

Hedge, P., Kriwoken, L.K., Patten, K., 2003. A review of *Spartina* management in Washington State, US. J. Aquat. Plant Manag. 41(2), 82–90.

Hu, M.Y., Ge, Z.M., Li, Y.L., Li, S.H., Tan, L.S., Xie, L.N., Hu, Z.J., Zhang, T.Y., Li, X.Z., 2019. Do short-term increases in river and sediment discharge determine the dynamics of coastal mudflat and vegetation in the Yangtze Estuary? Estuarine. Coast Shelf Sci. 220, 176–184. https://doi.org/10.1016/j.ecss.2019.03.004.

Hu, Z.J., Ge, Z.M., Ma, Q., Zhang, Z.T., Tang, C.D., Cao, H.B., Zhang, T.Y., Li, B., Zhang, L.Q., 2015. Revegetation of a native species in a newly formed tidal marsh under varying hydrological conditions and planting densities in the Yangtze Estuary. Ecol. Eng. 83, 354–363. https://doi.org/10.1016/j.ecoleng.2015.07.005.

Huang, H.M., Zhang, L.Q., Guan, Y.J., Wang, D.H., 2008. A cellular automata model for population expansion of *Spartina alterniflora*, at Jiuduansha Shoals, Shanghai, China. Estuar. Coast Shelf Sci. 77(1), 47–55. https://doi.org/10.1016/j.ecss.2007.09.003.

Kettenring, K.M., Adams, C.R., 2011. Lessons learned from invasive plant control experiments: A systematic review and meta-analysis. J. Appl. Ecol. 48(4), 970–979. https://doi.org/10.1111/j.1365-2664.2011.01979.x.

Knott, C.A., Webster, E.P., Nabukalu, P., 2013. Control of smooth cordgrass (*Spartina alterniflora*) seedlings with four herbicides. J. Aquat. Plant Manag. 51, 132–135.

Li, H.P., Zhang, L.Q., 2008. An experimental study on physical controls of an exotic plant *Spartina alterniflora* in Shanghai, China. Ecol. Eng. 32(1), 11–21. https://doi.org/10.1016/j.ecoleng.2007.08.005.

Li, X., Zhou, Y.X., Zhang, L.P., Kuang, R.Y., 2014. Shoreline change of Chongming Dongtan and response to river sediment load: A remote sensing assessment. J. Hydrol. 511, 432–442. https://doi.org/10.1016/j.jhydrol.2014.02.013.

Liao, Y.B., Shou, L., Tang, Y.B., Gao, A.G., Chen, Q.Z., Yan, X.J., Chen, J.F., 2018. Influence of two non-indigenous plants on intertidal macrobenthic communities in Ximen Island special marine protected area, China. Ecol. Eng. 112, 96–104. https://doi.org/10.1016/j.ecoleng.2017.12.023.

Liu, H.Y., Lin, Z.S., Zhang, M.Y., Qi, X.Z., 2017. Relative importance of sexual and asexual reproduction for range expansion of *Spartina alterniflora* in different tidal zones on Chinese coast. Estuar. Coast Shelf Sci. 185, 22–30. https://doi.org/10.1016/j.ecss.2016.11.024.

Liu, W.W., Maung, D.K., Strong, D.R., Pennings, S.C., Zhang, Y., 2016. Geographical variation in vegetative growth and sexual reproduction of the invasive *Spartina alterniflora* in China. J. Ecol. 104(1), 173–181. https://doi.org/10.1111/1365-2745.12487.

Major, W.W.I., Grue, C.E., Grassley, J.M., Conquest, L.L., 2003. Mechanical and chemical control of smooth cordgrass in Willapa Bay, Washington. J. Aquat. Plant Manag. 41(1), 6–12. https://doi.org/10.1023/A:1022946700883.

Mark, P., Malcolm, G., 2017. Trapping and episodic flushing of suspended sediment from a tidal river. Continent. Shelf Res. 143, 286–294. https://doi.org/10.1016/j.csr.2016.07.007.

Mateos-Naranjo, E., Cambrolle, J., Garcia De Lomas, J., Parra, R., Redondo-Gomez, S., 2012. Mechanical and chemical control of the invasive cordgrass *Spartina densiflora* and native plant community responses in an estuarine salt marsh. J. Aquat. Plant Manag. 50, 106–111.

Miller, G., Croyhers, K., 2004. Controlling invasive *Spartina*: The New Zealand success story. In: Proceedings of the Third International Conference on Invasive *Spartina*, pp. 247–248. San Francisco.

Panetta, F.D., Sparkes, E.C., 2001. Re-invasion of a riparian forest community by an animal-dispersed tree weed following control measures. Biol. Invasions 3(1), 75–88. https://doi.org/10.1023/A:1011408703336.

Patten, K., 2003. Persistence and non-target impact of imazapyr associated with smooth cordgrass control in an estuary. J. Aquat. Plant Manag. 41, 1–6.

Patten, K., 2004. Comparison of chemical and mechanical control efforts for invasive *Spartina* in Willapa Bay, Washington. In: Proceedings of the Third International Conference on Invasive *Spartina*, San Francisco, pp. 249–254.

Patten, K., O'Casey, C., Metzger, C., 2017. Large-scale chemical control of smooth cordgrass (*Spartina alterniflora*) in Willapa Bay, WA: Towards eradication and ecological restoration. Invasive Plant Sci. Manag. 10(3), 284–292. https://doi.org/10.1017/inp.2017.25.

Pearson, D.E., Ortega, Y.K., Runyon, J.B., Butler, J.L., 2016. Secondary invasion: The bane of weed management. Biol. Conserv. 197, 8–17. https://doi.org/10.1016/j.biocon.2016.02.029.

Richardson, D.M., Kluge, R.L., 2008. Seed banks of invasive Australian Acacia species in South Africa: Role in invasiveness and options for management. Perspect. Plant Ecol. Evol. Systemat. 10(3), 161–177. https://doi.org/10.1016/j.ppees.2008.03.001.

Sheng, Q., Huang, M.Y., Tang, C.D., Niu, D.L., Ma, Q., Wu, J.H., 2014. Effects of different eradication measures for controlling *Spartina alterniflora* on plants and macrobenthic invertebrates. Acta Hydrobiol. Sin. 38(2), 279–290 (in Chinese). https://doi.org/10.7541/2014.41.

Shimeta, J., Saint, L., Verspaandonk, E.R., Nugegoda, D., Howe, S., 2016. Long-term ecological consequences of herbicide treatment to control the invasive grass, *Spartina anglica*, in an Australian saltmarsh. Estuar. Coast Shelf Sci. 176, 58–66. https://doi.org/10.1016/j.ecss.2016.04.010.

Simenstad, C.A., Cordell, J.R., Lucinda, T., Weitkamp, L.A., Paveglio, F.L., Kilbride, K.M., Fresh, K.L., Grue, C.E., 1996. Use of rodeo® and x-77®

spreader to control smooth cordgrass (*Spartina alterniflora*) in a southwestern Washington Estuary: 2. Effects on benthic microflora and invertebrates. Environ. Toxicol. Chem. 15(6), 969–978. https://doi.org/10.1002/etc.5620150620.

Strong, D.R., Ayres, D.A., 2016. Control and consequences of *Spartina* spp. invasions with focus upon san Francisco Bay. Biol. Invasions 18(8), 2237–2246. https://doi.org/10.1007/s10530-015-0980-6.

Theoharides, K.A., Dukes, J.S., 2007. Plant invasion across space and time: Factors affecting nonindigenous species success during four stages of invasion. New Phytol. 176(2), 256–273. https://doi.org/10.1111/j.1469-8137.2007.02207.x.

Wu, X.T., Zhao, J., Wu, J.L., Wang, D.L., Deng, Q.H., Huang, Y., 2013. Determination of Haloxyfop-R-methy in andrographis herba and soil by gas chromatography with electron capture detector. J. Anal. Sci. 29(2), 227–230 (in Chinese).

Xiao, D.R., Zhang, L.Q., Zhu, Z.C., 2009. A study on seed characteristics and seed bank of *Spartina alterniflora* at saltmarshes in the Yangtze Estuary, China. Estuarine. Coast Shelf Sci. 83(1), 105–110. https://doi.org/10.1016/j.ecss.2009.03.024.

Xu, K.D., Du, Y.F., Lei, Y.L., Dai, R.H., 2010. A practical method of ludox density gradient centrifugation combined with protargol staining for extracting and estimating ciliates in marine sediments. Eur. J. Protistol. 46(4), 263–270. https://doi.org/10.1016/j.ejop.2010.04.005.

Xu, Y., Stoeck, T., Forster, D., Ma, Z.H., Zhang, L.Q., Fan, X.P., 2018. Environmental status assessment using biological traits analyses and functional diversity indices of benthic ciliate communities. Mar. Pollut. Bull. 131, 646–654. https://doi.org/10.1016/j.marpolbul.2018.04.064.

Yuan, L., Zhang, L.Q., Xiao, D.R., Huang, H.M., 2011. The application of cutting plus waterlogging to control *Spartina alterniflora* on saltmarshes in the Yangtze Estuary, China. Estuarine. Coast Shelf Sci. 92(1), 103–110. https://doi.org/10.1016/j.ecss.2010.12.019.

Yuan, L., Ge, Z.M., Fan, X.Z., Zhang, L.Q., 2014. Ecosystem-based coastal zone management: A comprehensive assessment of coastal ecosystems in the Yangtze Estuary coastal zone. Ocean Coast Manag. 95, 63–71. https://doi.org/10.1016/j.ocecoaman.2014.04.005.

Zhang, C.H., Yang, Z.C., Wang, J.J., Yang, F.Z., Fan, X.Z., 2016. Simultaneous determination of acetochlor and clomazone residues in soil by HPLC with ultrasonic extraction. Agrochemicals 55(6), 434–436 (in Chinese).

Zheng, S.Y., Shao, D.D., Asaeda, T., Sun, T., Luo, S.X., Cheng, M., 2016. Modeling the growth dynamics of *Spartina alterniflora* and the effects of its control measures. Ecol. Eng. 97, 144–156. https://doi.org/10.1016/j.ecoleng.2016.09.006.

Zhu, Z.C., Bouma, T.J., Ysebaert, T., Zhang, L.Q., Herman, P.M.J., 2014. Seed arrival and persistence at the tidal mudflat: Identifying key processes for pioneer seedling establishment in salt marshes. Mar. Ecol. Prog. 513, 97–109. https://doi.org/10.3354/meps10920.

Zou, Y.A., Tang, C.D., Niu, J.Y., Wang, T.H., Xie, Y.H., Guo, H., 2016. Migratory waterbirds response to coastal habitat changes: Conservation implications from long-term detection in the Chongming Dongtan wetlands, China. Estuar. Coast 39(1), 273–286. https://doi.org/10.1007/s12237-015-9991-x.