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Indirect effects of non-native *Spartina alterniflora* and its fungal pathogen (*Fusarium palustre*) on native saltmarsh plants in China

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Summary

1. Pathogens can affect their hosts and change community composition and structure. Pathogens may be key determinants of biological invasions. However, few empirical studies exist examining how non-native plants drive their invasions through indirect effects involved with pathogens. Here, indirect effects refer to how one species alters the effect that another species has on a third.

2. *Fusarium palustre* was associated with the dieback of *Spartina alterniflora* in its native North American saltmarshes. Native plant *Phragmites australis* was also found to die back in the Dongtan wetland of the Chinese Yangtze River estuary invaded by non-native *Spartina alterniflora*. This phenomenon suggests that *Spartina* might not escape from its pathogen when being introduced from its native North America and has indirectly caused the dieback of *Phragmites* in China.

3. To investigate the indirect effect of *Spartina* involving *Fusarium*, we sampled plants and soils in dieback patches to isolate the pathogen. Next, we used an artificial inoculation study to determine the virulence of *Fusarium* to both *Phragmites* and *Spartina*. Finally, the spatial distribution of *Fusarium* was studied through examining its incidence in saltmarshes along the east coast of China.

4. The endophytic fungus *F. palustre* was found to be closely associated with *Phragmites* dieback in the Dongtan wetland and it is likely that it was transported by non-native *Spartina* from its native North American saltmarshes to the Chinese saltmarshes. The spillover of *F. palustre* from non-native *Spartina* to native *Phragmites* might subsequently facilitate *Spartina* invasion.

5. *Synthesis*. Invasive plants do not only directly compete with native plants, but also indirectly cause pathogen infection on the latter, by acting as vectors and reservoirs for pathogens shared with native plants. Our findings highlight the significance of indirect effects involving pathogens in biological invasions. It is necessary to consider these pathogen-mediated indirect effects of non-native plant species in multi-host-pathogen systems for management and restoration purposes.

Key-words: dieback, *Fusarium*, indirect effects, invasion ecology, pathogen spillover, *Spartina*, Yangtze River estuary

Introduction

Biological invasions create considerable damage to natural and managed ecosystems (Wilcove *et al.* 1998; Mack *et al.* 2000) as well as economic losses and human health impacts (Pimentel *et al.* 2001; Keesing *et al.* 2010). So far, multiple hypotheses (e.g. enemy release, invasional meltdown and enemy-of-my-enemy) have been proposed to explain how invading species succeed in colonizing new areas, which focus mainly on traits of the invading species and/or properties of the invaded ecosystems (Catford, Jansson & Nilsson 2009). Recently, a growing literature demonstrates that pathogens may act as drivers for biological invasions and affect invasion processes (Strauss, White & Boots 2012; Flory & Clay 2013).

Pathogens play important roles in shaping community structure and affecting ecosystem processes and functioning

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(Thomas, Renaud & Guegan 2005; Hatcher, Dick & Dunn 2006; Flory & Clay 2013). Pathogens themselves are often invasive species; they spread via increased global trade and travel and may attack new hosts in introduced habitats (Keesing *et al.* 2010; Hatcher, Dick & Dunn 2012a,b). For example, plant pathogen *Cryphonectria parasitica* causes chestnut blight and *Ophiostoma ulmi* causes Dutch elm disease resulting in a loss of native plants (Anagnostakis 1987; Potter *et al.* 2011). Pathogens may also hitchhike on other invaders to the new habitats (Liebhold *et al.* 1995; Husson *et al.* 2011; Vilcinskas *et al.* 2013). Two cases of hitchhiking non-natives include *Batrachochytrium dendrobatidis* causing chytrid disease that is transmitted by the invasive American bullfrog and *Aphanomyces astaci* which results in crayfish plague carried by the invasive signal crayfish (Hatcher, Dick & Dunn 2012a). Sometimes, soil-borne plant pathogens can be accumulated by non-native plants, which in turn threaten native plants (Eppinga *et al.* 2006; Mangla, Inderjit & Callaway 2008). In the latter two cases, pathogens are involved in the competitive interactions between the non-native and native species and may indirectly facilitate one of the two competitors (White, Wilson & Clarke 2006; Dunn *et al.* 2012). These indirect effects demonstrate 'how one species alters the effect that another species has on a third' (Strauss 1991) and are considered to be important in influencing the success of species and structuring ecological systems (Holt 1977; Wootton 1994). Despite their potentially vital impacts, the indirect effects involved with pathogens in the context of biological invasions have not been widely studied (White, Wilson & Clarke 2006; Dunn *et al.* 2012). Additionally, study cases that have examined both the mechanism and impact of an indirect effect have been scarce (but see Lenz & Taylor 2001; Adam, Pearl & Bury 2003; White, Wilson & Clarke 2006). In this study, we report on a study concerning the pathogen-mediating indirect effect in an invaded saltmarsh ecosystem on the world's largest alluvial island, Chongming Island, in the Yangtze River estuary of China.

The saltmarshes on Chongming Island were historically dominated by native sedges (e.g. *Scirpus* spp. and *Carex* spp.) and reed (*Phragmites australis* (Cav.) Trin. ex Steud., hereafter referred as to *Phragmites*) (Xu & Zhao 2005). In 2001, for promoting sediment accretion in tidal flats, the ramets of smooth cordgrass (*Spartina alterniflora* Loisel, hereafter referred as to *Spartina*) were intentionally introduced from Yancheng in Jiangsu Province where the seeds and ramets of *Spartina* were initially introduced from the southeast of North America (i.e. Morehead City, NC, Altamaha Estuary and Sapelo, GA and Tampa Bay, FL) in 1979 (An *et al.* 2007; Li *et al.* 2009). As then, *Spartina* has caused serious ecological disruptions on Chongming Island, such as displacing local plant species, driving *Scirpus mariqueter* Wang et Tang to local extinction (Chen *et al.* 2004), reducing native biodiversity (Li *et al.* 2009) and altering nutrient cycling (Liao *et al.* 2008). In 2008, it was observed that native *Phragmites* was experiencing dieback, but only when growing in patches containing or surrounded by non-native *Spartina*. The dieback has subsequently continued to advance to new areas as *Spartina* spreads (personal observations). Our recent field observations have revealed that the dieback can reduce the competitive ability of *Phragmites* relative to *Spartina*, consequently favouring *Spartina* colonization in these marshes (Li *et al.* 2013). However, the specifics of the mechanism of *Phragmites* dieback are still to be confirmed.

Various hypotheses such as herbivory by crabs (Holdredge, Bertness & Altieri 2009; Coverdale, Altieri & Bertness 2012), snails (Silliman *et al.* 2005) and stem borers (Gaeta & Kornis 2011) and altered soil physicochemistry (Mendelssohn & McKee 1988; Armstrong & Armstrong 2001; Brown, Pezeshki & DeLaune 2006) have been proposed to explain vegetation dieback in wetlands. Yet, these factors do not seem to be responsible for *Phragmites* dieback on Chongming Island (Li *et al.* 2013; Li 2014). Chen (2004) has first reported that 2 years after its introduction, non-native *Spartina* on Chongming Island also experienced local dieback for a period of time, which seemed to be the result of an unidentified pathogenic *Fusarium* fungus. Recently, the dieback of *Spartina* in its native coastal saltmarshes of North America has been found to be associated with the endophytic fungus *Fusarium palustre* (Elmer & Marra 2011; Elmer *et al.* 2013). Accordingly, we hypothesized that an indirect effect of invasive *Spartina* involved with a *Fusarium* fungus might be related to Chinese *Phragmites* dieback. To test this hypothesis, we addressed the following questions: (i) Does a particular *Fusarium* fungus that is associated with non-native *Spartina* exist on Chongming Island? We sampled plants and soils on Chongming Island to screen out and identify the fungus, (ii) Is the fungus responsible for the dieback of native *Phragmites*? We used an artificial inoculation study in the glasshouse to determine the virulence of the fungus to *Phragmites* compared with *Spartina*, and (iii) Is there any evidence that the fungus is spreading to *Phragmites* populations in other Chinese saltmarshes? We sampled plants and soils in *Phragmites* patches without *Spartina* invasion along the east coast of China to determine the spatial distribution of the fungus. Addressing these questions will be helpful to understand both the mechanisms of *Phragmites* dieback and the pathogen-mediating indirect effect in the *Spartina* invasion of coastal China.

Materials and methods

FIELD SAMPLING AND IDENTIFICATION OF THE PATHOGENIC *FUSARIUM*

Field sampling was conducted in the Dongtan wetland on Chongming Island in the Yangtze estuary, China (31°25′–31°38′N, 121°50′–122°05′E). The Dongtan wetland is recognized as a 'Wetland of International Importance' and was set aside as a National Nature Reserve for migratory birds. This area has a northern subtropical monsoon climate with the mean annual temperature of 15.3 °C and average annual precipitation of 1022 mm (Xu & Zhao 2005). The wetland covers a total area of 32 600 ha, consisting of shallow water, muddy flats and saltmarshes that are dominated by native sedges (e.g. *Scirpus* spp. and *Carex* spp.) and *Phragmites*, and non-native *Spartina*. *Spartina* has driven *Scirpus mariqueter* locally extinct in their co-occurring marshlands (Chen 2004) and

also invades *Phragmites* and sedge communities where they form two-species mixtures or mosaics of their pure patches in the saltmarshes (Wang 2007).

To identify the *Fusarium* fungus associated with *Phragmites* dieback, four study sites with the straight line distance of about 1 km between the nearest sites were randomly selected as spatial replicates in March, 2012. These sites were based on our field observations for monitoring the dynamics of *Phragmites* dieback from 2008 to 2011. Each study site contained four types of plant communities (1.5 m × 1.5 m per plot) including *Phragmites* monoculture, *Spartina* monoculture and their mixtures that were healthy or exhibiting dieback. No significant differences of environmental variables (e.g. oxidation reduction potential, electric conductivity, pH and water content) were observed among the four communities monitored (Li *et al.* 2013). Plants and rhizosphere soils were sampled once a month from early April to mid-September in 2012, given that *Phragmites* and *Spartina* begin to produce ramets in April and *Phragmites* dieback occurs from late July to August. At each sampling, three individual *Phragmites* and/or *Spartina* plants with intact above-ground parts and 20-cm-depth below-ground parts (i.e. rhizomes) were collected from each of the 16 plots. Rhizosphere soil samples were also collected using a 5 cm (diameter) × 25 cm (height) earth boring auger. Standing litter and the rhizosphere soils of *Phragmites* and *Spartina* were collected, and these samples were transported to the laboratory in sealed plastic sampling bags, stored at 4 °C and assayed within 24 h.

Fusarium was screened out from plant and rhizosphere soil samples according to Booth (1971). Isolates were subcultured and tested for the pathogenicity to *Phragmites* and *Spartina* using a detached leaf assay (Yu *et al.* 1991), which was repeated twice to ensure the stable pathogenicity of the fungi. The infected leaf was re-sampled to isolate the tested fungus. The most virulent *Fusarium* was picked out to be identified according to morphological traits (Booth 1971; http://www.ctu.edu.vn/colleges/agri/gtrinh/fuskey.pdf) and phylogenetic analysis based on the nuclear ribosomal internal transcribed spacer (ITS) region (White *et al.* 1990) and the beta-tubulin (β-tu*b*) gene region (O'Donnell & Cigelnik 1997). The amplification of the two gene regions was performed on a Bio-Rad C1000 Touch™ Thermal Cycler (Bio-Rad, Hercules, California, USA) in 25 μL volumes with 1 μL DNA template, 12.5 μL 2× Taq MasterMix (CWBIO, Beijing, China), 1 μL volume per primer and 11.5 μL RNase-free water. Amplification of each primer-isolation system was repeated three times to ensure consistency. Sterile double-distilled H_2O instead of DNA template was added as a negative control. Amplification products were detected by 1.2% agarose gel electrophoresis and visualized under UV light. PCR products were purified and sequenced in both directions with the corresponding primers (BGI, Shanghai, China). Sequences were edited and assembled with CHROMAS 2.22 computer program (Techelysium, Tewantin, Australia) and SeqMan™ II software (DNASTAR, Inc, Madison, Wisconsin, USA). The sequences of known *Fusarium* species were downloaded from GenBank data base (Fig. 1), and sequence alignment was performed with the BLAST program in NCBI. Phylogenetic analysis was performed in ClustalX (Thompson *et al.* 1997) and MEGA 5.05 (Tamura *et al.* 2011).

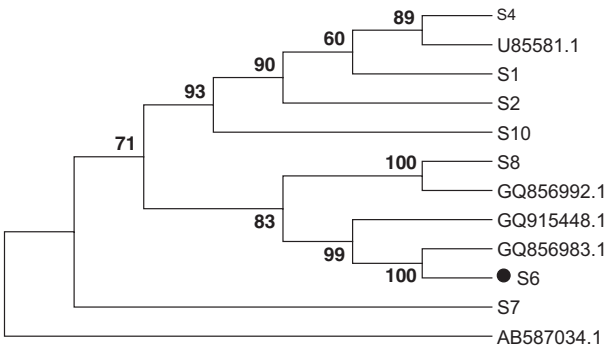

Fig. 1. Phylogenetic relationships among *Fusarium* isolates collected from the Dongtan wetland. The phylogenetic tree was built using a neighbour-joining method based on the partial gene sequences of β-tubulin. Numbers above the branches represent the bootstrap values based on bootstrap test (1000 replicates). The reference strains downloaded from GenBank data base are *Fusarium* cf. *incarnatum* (GQ856992.1), *Fusarium kyushuense* (U85581.1), *Fusarium palustre* (GQ856983.1) and *Fusarium sporotrichioides* (GQ915448.1), and the outgroup strain is *Microdochium nivale* (AB587034.1). S1, S2, S4, S6, S7, S8 and S10 are the seven strains into which all 72 isolates from the Dongtan wetland were grouped.

VIRULENCE OF THE PATHOGENIC *FUSARIUM*

We conducted an artificial inoculation study in the glasshouse to determine the virulence of the fungus to both *Phragmites* and *Spartina*. Seeds of *Phragmites* and *Spartina* collected from healthy patches in the Dongtan wetland were surface-sterilized by dipping them in 75% ethanol for 5 min and then rinsing them with sterile deionized water. Sterilized seeds were allowed to germinate in autoclaved soil collected from the Dongtan wetland. After germination, two seedlings were placed in a plastic pot (13 cm × 10 cm × 15 cm) with sterilized soil and fertilized with slow release fertilizer bimonthly. Three plant combinations with eight replicates each were established in the glasshouse including *Phragmites* monoculture (two seedlings per pot), *Spartina* monoculture (two seedlings per pot) and their mixture (one seedling of each plant species).

Stem inoculations were performed 6 months later. The inoculation site was sterilized with 75% ethanol cotton and stuck with an agar plug (1 cm × 0.5 cm) with mycelia and then wrapped in moist sterilized cotton and sealed with parafilm. The inoculation site was sprayed with tap water to maintain humidity. Inoculation of the sterile agar plug served as a control, and the second inoculation was performed on the first inoculated position after 1 week. Symptoms of infection (i.e. red or brown spots or stripes on stem; dark brown spots on sheath) were observed every 3 days for 1 month after inoculation. According to Li *et al.* (2011), disease incidence (Di) was calculated by $\mathrm{Di} = \frac{n_1}{N} \times 100\%$, where n_1 is the number of diseased plants and N is the total number of all investigated plants. Disease index (DI) was calculated by $\mathrm{DI} = \frac{\sum g \times n_2}{N \times 5}$, where g is the grade of disease severity, n_2 is the number of plants at that grade. Infection severity was scored on a five-point scale based on the fraction of leaf infected, where 0 = no disease; 1 ≤ 25%; 2 = 25%–50%; 3 = 50%–75%; 4 ≥ 75%. One month after inoculation, plant height and leaf number were measured. The averages of these data from each plant species in each pot were recorded for statistical analysis. Plants were sampled again to reisolate the target fungus and fulfil Koch's postulates.

SPATIAL DISTRIBUTION OF THE PATHOGENIC *FUSARIUM* IN CHINESE SALTMARSHES

To study the spatial distribution of *Fusarium* in the Dongtan wetland, we sampled plants and soils in the above-mentioned field plots once a month from early April to mid-September, 2012. Three individual *Phragmites* and/or *Spartina* plants with intact above-ground parts and

20-cm-depth below-ground parts (i.e. rhizomes and rhizosphere soils) were collected. We also extended our sampling to determine whether *Fusarium* occurs in marshland *Phragmites* populations along the east coast of China. We collected above- and below-ground samples of *Phragmites* in the patches where no *Spartina* exists, including Tanghai (39°11.462′N, 118°20.283′E, Hebei Province), Dongying (38°01.176′N, 118°58.085′E, Shandong Province), Yancheng (33°34.205′N, 120°32.29 3′E, Jiangsu Province), Shanghai (31°28.863′N, 121°56.582′E), Songmen (28°23.000′N, 121°38.027′E, Fujian Province) and Zhuhai (22°23.814′N, 113°36.789′E, Guangdong Province). Three to five *Phragmites* individuals and their 20-cm-depth rhizosphere soils were sampled at each site (a total of 48 individual *Phragmites* and 48 portions of soil samples for the six sites). The targeted *Fusarium* was screened out and its incidence in plants was expressed as the percent colonization, which was considered as the number of isolate/total number of tissue pieces plated from each *Phragmites* and *Spartina* plant. The abundance of *Fusarium* in soils was expressed as the number of spores in 1 g of soil sample.

STATISTICAL ANALYSIS

Statistical analyses were performed with software R (version 2.12.2, R Development Core Team 2011). The effects of plant community type (i.e. *Phragmites* monoculture, *Spartina* monoculture and their mixture), plant species (i.e. *Phragmites* in monoculture and mixture, *Spartina* in monoculture and mixture) and their interactions on disease incidence were analysed using binomial logistic regression. Considering that the number of infected plants followed a binomial distribution and the prior probability distribution of disease incidence was a uniform distribution between 0 and 1, disease incidence was estimated by Bayesian analysis, which was performed using WinBUGS (version 1.4.3, http://www.mrc-bsu.cam.ac.uk/bugs/). Then, the significance of the difference between plant species was tested by comparing the 95% confidence intervals. If the intervals did not overlap, the differences among plant types were considered to be significant. The data of disease index did not meet the assumptions of analysis of variance (ANOVA); so, permutation test (Venables & Ripley 2002) was used to analyse the effects of plant community type, plant species and their interactions on disease index. Kruskal–Wallis test was used to further analyse the differences of disease index between plant species.

Multivariate analysis of variance (MANOVA) and a *post hoc* Tukey's HSD test were used to analyse the effects of plant community type, plant species, fungal treatment and their interactions on plant height. The effects of plant species and fungal treatment on plant height were further analysed using *t*-test, respectively. Kruskal–Wallis test was used to analyse the differences of leaf number of *Phragmites* and *Spartina* after being inoculated with *Fusarium*.

In the field study, only the targeted *Fusarium* was screened out from plant samples in the Dongtan wetland but not from the samples collected along the east coast of China; so, we just analysed the data of the Dongtan wetland to determine its spatial distribution. A randomized block design with plant community type as fixed factor and site as a block was used in the Dongtan wetland. To explore the relationships of *Fusarium* with site and plant community type, the data of the incidence of *Fusarium* from the same plant community were combined and analysed by using Kruskal–Wallis test, because the data did not meet the assumptions of ANOVA. To explore the relationship between *Fusarium* and plant species, the differences of the incidence of *Fusarium* in each plant species alone or in combinations (i.e. *Phragmites* in monoculture, healthy mixture and dieback mixture, *Spartina* in monoculture, healthy mixture and dieback mixture) were analysed using binomial logistic regression. The significance of difference among plant species was tested by comparing the 95% confidence intervals.

Results

IDENTIFICATION OF THE PATHOGENIC *FUSARIUM* IN THE DONGTAN WETLAND

A total of 72 *Fusarium* strains were isolated from samples in the Dongtan wetland and classified into five known species (*Fusarium acuminatum*, *F. kyushuense*, *F. incarnatum* (synonym *F. semitectum*), *F. palustre* and *F. poae*). More than 90% of the *Fusarium* strains were collected from aboveground plant tissues. Only one isolated strain was picked out for its virulence on *Phragmites* (*ca.* 1 cm lesion) in the detached leaf assay. Its ITS sequence (495 bp) was more than 98% identical to *Fusarium* species in GenBank, and β-tu*b* gene sequences (1168 bp) evidently confirmed that the strain clustered with the known *F. palustre* (Fig. 1), which is an endophytic fungus that was initially isolated from *Spartina* in dieback sites in its native North American saltmarshes (Elmer & Marra 2011). We hereafter refer to the identified fungus as *F. palustre*.

VIRULENCE OF THE PATHOGENIC *FUSARIUM*

Artificial inoculation in the glasshouse demonstrated that both native *Phragmites* and non-native *Spartina* were susceptible to the infection by *F. palustre*. The disease symptoms on *Phragmites* were similar to those on dieback *Phragmites* observed in the field. Moreover, the fungus was reisolated from the infected plants. Plant community type did not affect the disease incidence of all plants ($\chi^2 = 1.89$, d.f. = 1, $P = 0.17$). In the monoculture, the disease incidence of *Phragmites* was significantly higher than that of *Spartina* (Fig. 2a; $P < 0.05$), but there was no significant difference of disease incidence between the two plants in their mixture (Fig. 2a; $P > 0.05$). Disease index was only affected by plant species (permutation test, d.f. = 1, $P = 0.02$), but not by plant community type (permutation test, d.f. = 1, $P = 0.15$). The disease indices of *Phragmites* were significantly higher in both monoculture and mixture, compared with those of *Spartina* (Fig. 2b; $\chi^2 = 12.66$, d.f. = 3, $P = 0.01$).

Plant height differed between *Phragmites* and *Spartina* ($F_{1,60} = 8.65$, $P < 0.01$) and between the fungal treatment and control treatment ($F_{1,60} = 7.79$, $P = 0.01$). For *Phragmites*, there was no significant difference of plant height between the treatment (42.6 ± 2.1 cm) and control groups (46.5 ± 3.1 cm) ($t = 1.27$, d.f. = 25, $P = 0.21$). However, *Spartina* height decreased significantly from 52.9 ± 2.7 to 46.1 ± 2.9 cm after inoculation ($t = 2.62$, d.f. = 30, $P = 0.01$). Leaf number differed between *Phragmites* and *Spartina* (permutation test, d.f. = 1, $P = 0.01$) exposed to the fungal treatment. After inoculating the fungus, leaf numbers of the two plant species decreased significantly, from 6.0 ± 0.4 to 4.7 ± 0.4 for

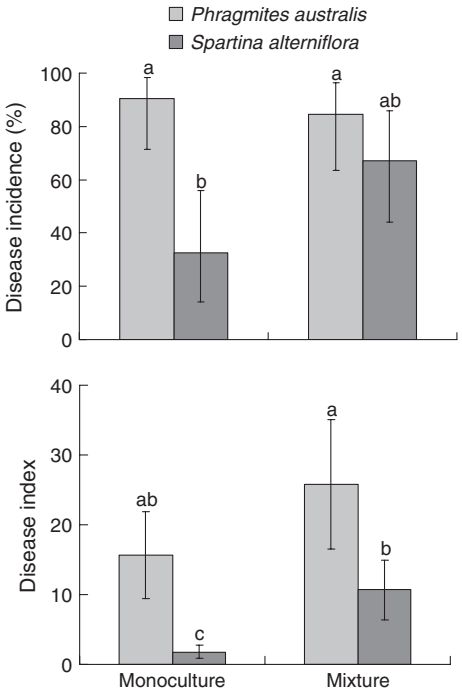

Fig. 2. Disease incidence (a) and disease index (b) in the glasshouse experiment. Error bars in A represent the 95% confidence intervals. Error bars in (b) represent the standard errors (n = 8). Different letters indicate significant differences ($P < 0.05$).

Phragmites ($\chi^2 = 6.20$, d.f. = 1, $P = 0.01$) and from 4.6 ± 0.7 to 3.9 ± 0.2 for *Spartina* ($\chi^2 = 4.33$, d.f. = 1, $P = 0.04$).

SPATIAL DISTRIBUTION OF THE PATHOGENIC *FUSARIUM* IN CHINESE SALTMARSHES

Fusarium palustre was detected in *Spartina* monoculture and in the *Phragmites–Spartina* mixtures in the Dongtan wetland. However, the fungus was not found in pure *Phragmites* stands along the east coast of China. The distribution of *F. palustre* on *Phragmites* and *Spartina* varied temporally and spatially and it was not isolated from rhizosphere soil samples during the growing season. *Fusarium palustre* existed in rhizomes of both *Phragmites* and *Spartina* plants in April and May with the incidence of 5.56 ± 3.40%, but the fungus was found more frequently in the above-ground tissues including leaves and stems. In June, the incidence of *F. palustre* in the Dongtan wetland showed no variability among the four sites ($\chi^2 = 2.66$, d.f. = 3, $P = 0.45$), but it was significantly higher in dieback mixture (18.8 ± 4.0%) than in *Spartina* monoculture (4.2 ± 4.2%) or in healthy mixture (8.3 ± 5.9%) ($\chi^2 = 8.48$, d.f. = 3, $P = 0.04$). Moreover, *F. palustre* tended to accumulate on *Phragmites* in dieback mixture (25.0 ± 9.0%) more profusely than on *Spartina* in dieback mixture (12.5 ± 2.8%), on *Phragmites* (8.3 ± 1.3%) and *Spartina* (8.3 ± 1.3%) in healthy mixture (Fig. 3; $\chi^2 = 10.93$, d.f. = 5, $P = 0.05$). In July, its incidence declined in all communities but still tended to be higher in dieback mixture (7.5 ± 4.8%) than in healthy mixture (2.8 ± 2.8%) ($\chi^2 = 4.18$, d.f. = 3, $P = 0.24$).

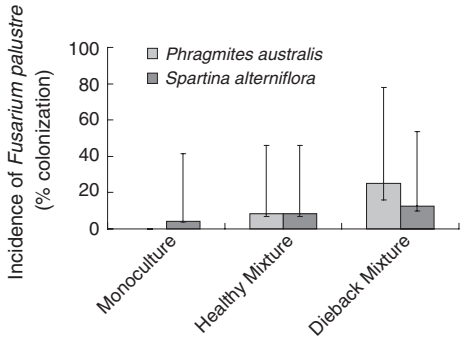

Fig. 3. Incidence of *Fusarium palustre* at the individual plant species level in the Dongtan wetland in June, 2012. Error bars represent the 95% confidence intervals.

Discussion

In this study, we explored the indirect effects of non-native *Spartina* involving a pathogen associated with dieback on native *Phragmites* in Chinese saltmarshes. *Fusarium palustre* was confirmed as the endophytic fungus in the Dongtan wetland as being the same pathogen driving *Spartina alterniflora* dieback in North American saltmarshes according to morphological traits and phylogenetic relationships (Elmer, LaMondia & Caruso 2011; Elmer & Marra 2011).

In the field, prior to the dieback outbreak, *F. palustre* tended to accumulate more on *Phragmites* compared with *Spartina* and its incidence on *Phragmites* in dieback mixture was greater than three times as much as that in healthy mixture. Our glasshouse study showed that *F. palustre* expressed greater virulence on *Phragmites* than *Spartina*. However, *F. palustre* alone did not kill *Phragmites*. The outcome in the glasshouse experiment may not reflect the patterns observed in nature (Schulz & Boyle 2005), suggesting that more field studies are still needed to determine whether *F. palustre* is directly responsible for *Phragmites* dieback in the Dongtan wetland.

The patterns revealed in this study indicate that the distribution of *F. palustre* in the Dongtan wetland is strongly dependent on non-native *Spartina*. Interestingly, *F. palustre* was only isolated from *Spartina* monoculture and its mixtures with *Phragmites*, but not in pure *Phragmites* stands along the east coast of China. In south-eastern North America, *Spartina* experienced massive dieback several times during 1975–2001 and *Fusarium* infection is considered to be one of the causes of this event (Alber *et al.* 2008). In 1979, seeds and ramets of *Spartina* were introduced to China in Jiangsu Province, from which its ramets were then transplanted to the Dongtan wetland in 2001 (Li *et al.* 2009). Although no report exists of whether there was *Spartina* dieback in Jiangsu Province, these sources of *Spartina* may have served as the vectors for *F. palustre* from North America to China. This potential scenario is supported based on spatial pattern and phylogenetic relationships of this pathogen. From the literature on biological invasions, we know that invasions may be facilitated if non-natives can escape from enemies such as pathogens upon

entry into novel habitats according to the enemy release hypothesis (Keane & Crawley 2002; Colautti *et al.* 2004; Torchin & Mitchell 2004). However, *Spartina*'s transition from North America to China is interesting because it seems at odds with this scenario as it was not associated with a loss of the fungal pathogen. Given that *F. palustre* could not be isolated from pure *Phragmites* stands and does not appear to be a soil-borne pathogen, *Spartina* might be the source of *F. palustre* in the Dongtan wetland.

There are two main avenues for how host-shifting indirect effects can occur when pathogens interact with non-natives and natives (D' Antonio & Kark 2002; Strauss, White & Boots 2012; Flory & Clay 2013). In particular, the non-native species can act as reservoir hosts for pathogens to cause 'spillover effect', whereby disease levels for a native species (non-reservoir host) are driven by transmission from a reservoir host sustaining a relatively high pathogen population density (Power & Mitchell 2004). Alternatively, they can act as new hosts for native pathogens to cause 'spillback effect', whereby the non-native species is a competent host for a native pathogen with the presence of the additional hosts increasing disease impacts in native species (Holt 1977). Both spillover and spillback effects can potentially threaten native species and affect community composition and structure, while these effects have been reported more in crop communities rather in natural plant communities (Power & Mitchell 2004; Kelly *et al.* 2009). In this study system, the non-native *Spartina* can be regarded as a reservoir host for the pathogen because *F. palustre* was not found in pure *Phragmites* stands. Consequently, *Spartina* played dual roles acting both as the source of the pathogen and as the reservoir for maintaining the pathogen population. Owing to its higher susceptibility to the pathogen and greater levels of infection, it is most likely that *Phragmites* acted as a non-reservoir.

Combined with our previous study (Li *et al.* 2013), it appears that non-native *Spartina* benefited from its spillover effect. *Spartina*'s invasion in *Phragmites* communities is possibly a consequence of pathogen spillover resulting in apparent competition between the shared hosts. This is expected to enhance the negative impacts on the non-reservoir host mediated indirectly by the shared pathogen (Holt 1977). Alternatively, competition with *Spartina* may weaken *Phragmites* rendering it more susceptible to *F. palustre* and subsequent dieback. This may explain why no *F. palustre* was isolated from *Phragmites* growing in monoculture. The fact that *F. palustre* did exist, but no dieback was observed in healthy mixture may also be explained by this hypothesis. The findings that *Spartina* ramet density and *F. palustre* loads on *Spartina* in dieback mixture were higher than those in healthy mixture (Li *et al.* 2013), suggest that there may be a threshold for the occurrence of *Phragmites* dieback. Clearly, given that spillover and spillback processes take time to occur, monitoring populations of reservoir hosts is crucial to manage the multi-host-pathogen communities.

Whether indeed spillover is operating in Chinese saltmarshes raises the question of how this is occurring. In plants, the reservoir may either be reproductive or vegetative tissue. In the case of *Pyrenophora semeniperda*, the pathogen was transmitted to native plants via the invasive cheatgrass (*Bromus tectorum*) seed bank (Beckstead *et al.* 2010). Although many *Fusarium* species are wind- and rain-splashed disseminated (Paul *et al.* 2004), whether *F. palustre* spills over through seeds or other tissues (e.g. leaves, stems and rhizomes) requires further study. However, the long-term outcome in the *Spartina–Fusarium–Phragmites* system will be complex, depending on the cascading impacts of altered ecosystem properties and the potential of the evolution of host defence and pathogen virulence (Clay & Kover 1996; Parker & Gilbert 2004; Seabloom *et al.* 2009).

Overall, the results of this study suggest that non-native *Spartina* acting as a vector and reservoir for a pathogen shared with native *Phragmites*, poses double threats to Chinese saltmarsh native plant via direct competition with *Spartina* and pathogen infection. Non-native plants alter the native community composition and provide a source of potential hosts for pathogens to be transmitted. Additionally, the pathogens can mediate the interactions among hosts resulting in apparent competition to feed back to the community composition. Ultimately, understanding the indirect effects involved with pathogens becomes critical as biological invasions occur at a greater frequency than ever before due to increased globalization. Elucidating the impacts of pathogen-mediated indirect effects of invasive plant species on native plant species in multi-host-pathogen nature systems will hopefully aid in the management and restoration of natural ecosystems.

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