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이학박사학위논문

**Effects of elevation and herbivores
on the distribution of halophytes
in a Korean salt marsh**

한국 염습지에서 고도와 초식동물이
염생식물 분포에 미치는 영향

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서울대학교 대학원

생명과학부

방 정 환

**Effects of elevation and herbivores
on the distribution of halophytes
in a Korean salt marsh**

Jeong Hwan Bang

Advisor: Professor Eun Ju Lee, Ph.D.

**A Thesis Submitted for the
Degree of Doctor of Philosophy**

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School of Biological Sciences

Seoul National University

Effects of elevation and herbivores on the distribution of halophytes in a Korean salt marsh

지도교수 이 은 주

이 논문을 이학박사 학위논문으로 제출함

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방정환의 이학박사 학위논문을 인준함

2018년 6월

위 원 장 박 종 욱 (인)

부위원장 이 은 주 (인)

위 원 김 재 근 (인)

위 원 박 상 규 (인)

위 원 이 일 하 (인)

Abstract

Jeong Hwan Bang

School of Biological Sciences

The Graduate School

Seoul National University

The distribution of halophytes in salt marshes is generally determined by environmental gradients, and it is important to identify the principal factors involved. This thesis addresses the influence of main factors on halophyte distribution and growth. First study recorded how marsh plants were distributed along elevational gradients, and investigated the environmental factors affecting their distribution on the Siheung Tidal Flat, which has one of the large tidal ranges in the world. I found that each halophyte species, which have received limited attention, had a distinct zonation according to elevation. Also, soil salinity in the high-elevation plots varied over a wide range. Cluster analysis classified plots into four clusters reflecting plant community composition (Cluster 1 at high elevation, defined by *Suaeda glauca*, *Zoysia sinica*, and *Phragmites australis*; Cluster 2 at mid-high elevation, defined by *Phacelurus latifolius*; Cluster 3 at low elevation, defined by *Suaeda japonica*; Cluster 4 at mid-high elevation, defined by *Carex scabrifolia*). Non-metric multidimensional scaling indicated that the distribution of *S. japonica* was strongly influenced by elevation and flood frequency, whereas that of *P. latifolius* was negatively influenced by soil salinity and soil cations (Na^+ ,

Mg²⁺, K⁺, Ca²⁺). Understanding the relationship between halophyte distribution and environmental factors along elevational gradients in a natural salt marsh provides important ecological information that may contribute to salt marsh restoration.

In second study, I identified halophytic plant species consumed by crabs and determined the relative contributions of halophytes and other food sources to the diet. Crabs live at high densities in intertidal zones with various halophytes in salt marshes. However, the ecological relationships between crabs and halophytes as well as the impacts of herbivorous crabs on the plant distribution are not fully understood. I found that the dominant crab species *Helice tientsinensis* consumed *Suaeda japonica*, even though several halophytes inhabited the study site. These results indicated that *H. tientsinensis* is a selective feeder. Based on a stable isotope analysis, I observed a dietary shift during crab development. Immature individuals mainly ate soil organic matter, whereas mature individuals showed more diverse food sources. I observed greater plant consumption by middle-sized crabs than by crabs of other sizes. These results for the feeding relationship extend our understanding of the interaction between crabs and halophytes in salt marshes.

Lastly, I investigated the effects of seed dispersion distance and the combination of burrowing crabs and marsh plants on soil environment factors and plant growth. Crabs and halophytes are important indicators of soil composition and fertility in salt marsh ecosystems. Many previous studies have examined the effects of crab excavation on soil properties, but little is known about the combined effects of crabs and halophytes on salt marsh soils. I found that plant biomass and

height of individual plants tended to be higher at distances > 1 m from stands of parent plants. Crabs preferred habitats with high vegetation cover. Low densities of burrowing crabs and marsh plants also caused considerable changes in soil properties. The combination of crabs and halophytes increased the spatial variability of physicochemical parameters in these salt marsh soils. These changes can also affect growth of halophyte. Crabs and halophytes often occur at high densities in salt marshes, and in combination may have a significant impact on salt marsh ecosystems.

Keyword: Environmental gradient, Halophytes, Korea, Restoration, Salt marshes, Siheung Tidal Flat, Zonation

Student Number: 2012-20312

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CHAPTER 1.

General introduction

The distribution of halophytes is generally determined by environmental gradients and is characterized by a distinct zonation according to shore elevation (Chapman, 1974). Salt marsh halophytes typically show a band-like pattern of zonation with respect to elevation. Previous researchers have suggested that this zonation pattern is related to plant succession (Chapman, 1976), but De Leeuw et al. (1993) showed that factors other than succession can affect the distribution of halophytes. Subsequent studies have shown that plant distribution in salt marshes is associated with numerous abiotic and biotic factors (Rogel et al., 2001; Costa et al., 2003; Lenssen et al., 2004). Among these factors, tidal inundation and salinity according to elevation are the main drivers of halophyte distribution (Bertness, 1991; Pennings et al., 2005; Rasser et al., 2013). However, these environmental gradients can vary depending on the natural conditions in salt marshes and can directly or indirectly affect community dynamics and halophyte distribution. Plant density is a biotic factor, and in the case of annual plants, their distribution or density is significantly related to distance from parent plants (Ellison, 1987). Recent studies show that crab bioturbation and herbivory can also regulate the distribution of halophytes (He et al., 2015; Martinetto et al., 2016). This thesis therefore addresses the influence of four main factors on plant distribution: the abiotic factor of shore elevation and three biotic factors, namely, crab herbivory, crab bioturbation, and plant density.

The surface elevation of salt marshes creates a gradient of abiotic factors that can affect the distribution of halophytes (Bertness, 1991; Adam, 1993; Rasser et al., 2013). Salt marshes experience twice-daily flood and ebb tides, with daily variation in tidal height. The period of seawater immersion and flood frequency therefore depends on the surface elevation and tidal pattern (Adam, 1993). Varty and Zedler (2008) showed that microtopographic variations could increase immersion stress in perennial plants. In addition, plants in deeply-inundated regions can easily be damaged by pressure (Gordon et al., 1985). Soil salinity in salt marshes tends to increase with elevation during most of the year (Callaway et al., 1990). Salinity variations can limit the geographic distribution of halophytes (Weber and D'Antonio 1999). Allakhverdiev et al. (2000) emphasized that salinity is an important environmental factor limiting plant growth and productivity. The elevation (inundation) gradient can also directly affect soil moisture and can change soil drying conditions in salt marshes. Therefore, elevation, inundation and salinity are important factors regulating the distribution of halophytes.

The feeding behavior of herbivores can affect halophyte distributions (Alberti et al., 2012; He et al., 2015). Previously, it was assumed that herbivores have no significant impacts on halophyte communities, but there is now growing evidence of their importance (Silliman et al., 2005). Previous studies have shown that herbivores can affect plant distribution (Lubchenco, 1980), primary productivity (Bortolus and Iribarne, 1999), and ecosystem function (Jefferies et al., 2006). Recent studies now show that small herbivorous animals such as crabs can exert top-down control in salt marshes and can limit wetland boundaries (Alberti et al., 2010, 2012). Crabs are one of the dominant herbivore groups in salt marshes and can feed on a wide variety of plants (Qin et al., 2010; He et al., 2015). The influence of herbivores on plant communities may depend on herbivore habitat and food preference (Farrell, 1991). In addition, food sources may vary depending on the size and sex of the crab (Divine et al., 2015, Hübner et al., 2015). Despite this ecological importance, no research has been conducted into the use of halophytes as a food source for crabs in the study area.

Bioturbation refers to the biological reworking of soils and sediments, and the consequent biogeochemical changes (Meysman et al., 2006). Burrowing activity by crabs is a form of bioturbation that can control the distribution of halophytes in salt marshes by changing the physicochemical properties of soil (Martinetto et al., 2016). Recent studies have shown that crab bioturbation involves complex interactions with both positive and negative consequences for soil nutrient cycling (Wang et al., 2010; Martinetto et al., 2016). The burrowing activity of crabs can affect sediment oxygen content and aerobic decomposition rates (Hemminga et al., 1991; Fanjul et al., 2008; Fanjul et al., 2011) Daleo et al. (2008) demonstrated that increased sediment oxygen content can affect plant biomass in association with arbuscular mycorrhizal fungi. The density of the halophyte can be affected by the distance from the parent plants. Annual plants that scatter their seeds can vary in initial density depending on seed dispersal distance from parent plants (Ellison, 1987). The initial density of a plant can cause intraspecific competition for limited resources and can have a significant impact on plant growth (Weiner and Thomas, 1986). The emergence of halophytes in salt marshes can affect physicochemical properties of the soil. The changes in soil factors may be dependent on the distribution of plants and crabs, and it remains unclear how these combinations affect plant communities in macrotidal salt marshes.

In this thesis, I investigate the major factors affecting the distribution and growth of halophytes in salt marsh ecosystem with different plant density. This study was conducted from 2014 to 2016 as part of a long-term monitoring programme on the Siheung Tidal Flat, a Coastal Wetland Protected Area. The area has one of the large tidal ranges in the world and is one of the typical salt marshes on the west coast of Korea (Bang et al., 2018). Tidal flats and halophytes are widely-distributed along deep tidal channels. The site is a suitable location to study the determinants of plant species distribution because there is a distinct pattern of halophyte zonation along the elevation gradient (Adam, 1990). Halophytes are a potential food source for the dominant crab species (*Helice tientsinensis*). Crabs use the halophytes as a habitat and may control plant density through feeding behavior. Given the high density of *H. tientsinensis* in the study area, it was expected to affect halophyte distribution and growth. The mud-flat crab (*Helice tientsinensis*) occurs mainly in the upper intertidal zone on the west coast of Korea (Marine Arthropod Depository Bank of Korea, 2009). It is the dominant species in the study area, found mostly among *Suaeda japonica* communities (patchy distribution). It was expected that crab burrow density will vary depending on the growth condition of the plant. Different habitat types were defined by the combination of crabs and plants in the study area, and these combinations were expected to have different effects on salt marsh soil properties and halophyte communities.

I hypothesized that the main drivers that determine the distribution of halophytes are different based on the plant densities. In this study, I investigated

the following three aspects of a saltmarsh ecology: (1) the effect of elevation on the distribution of halophytes at a high plant density, (2) the role of halophyte species as a food source for crabs and their importance relative to other food sources, and (3) the effects of burrowing crabs and the distance from the parent plants on the distribution of halophytes at a low plant density.

CHAPTER 2.

Plant distribution along an elevational gradient in a macrotidal salt marsh on the west coast of Korea

* This chapter was accepted in journal 'Aquatic botany' in June 2018.

2.1. Introduction

The distribution of salt marsh plants is generally determined by environmental gradients, including those caused by physicochemical and biotic factors such as tidal inundation, variations in salinity, and interactions with other species (Ungar, 1998). Surface elevation and tidal inundation are among the main drivers of halophyte distribution (Bertness, 1991; Adam, 1993; Rasser et al., 2013). In salt marshes, topographic features formed by the tides, such as channels, levees, and ponds, are irregularly distributed (Stribling et al., 2007). The resulting topographic heterogeneity results in elevational variations in surface microtopography. In salt marshes, small changes in elevation can affect plant occurrence and distribution by affecting the level of waterlogging in soil (Varty and Zedler, 2008). Changes in elevation have a direct influence on flood frequency and inundation time, and can indirectly affect other environmental factors (Castillo et al., 2000; Davy et al., 2011). At lower elevation, flood frequency and inundation time increase, low oxygen content generates a reducing state in the soil, and halophytes must be able to tolerate anaerobic conditions (Colmer and Flowers, 2008). Although the tide provides water and nutrients to salt marsh plants (Steever et al., 1976), it can affect their distribution by flooding and by increasing salinity stress (Detling and Klikoff, 1973; Flowers and Colmer, 2015).

Soil salinity in salt marshes is often influenced by elevation (Pennings et al., 2005), but also by other environmental factors. Flood frequency varies with elevation, with consequent effects on exposure time, temperature, and solar

radiation, all of which affect soil water evaporation and salinity (Pennings et al., 2005). Soil salinity can also vary depending on fresh water inputs (Wang et al., 2017) and the presence of halophytes (Pennings and Bertness, 1999), which may influence soil nutrient concentrations. Salt marsh plants in high-salinity regions make limited use of water absorption via osmotic pressure, and their growth and biomass are limited by salt stress, which affects photosynthesis, protein synthesis, and energy metabolism (Debez et al., 2004; Parida and Das, 2005). Salt marsh plant species can survive in soils with widely different salinities, as they have different salt tolerance ranges and adaptation strategies. Therefore, understanding how environmental factors and marsh plant distribution varies with elevation can reveal how each species responds to physico-chemical environmental factors and provide important information on key factors determining plant distribution and productivity.

The distribution of halophytes in salt marshes can also be affected by interspecific competition, commensalism and other interspecific interaction along the development of ecological succession (Figueroa et al., 2003). Generally, the lower limit of a species distribution may be determined by tide-related abiotic factors, whereas interspecific competition may be an important biological factor affecting plant distribution in the upper intertidal zone (Crain et al., 2004). However, Pennings and Callaway (1992) showed that interspecific competition may not be the sole factor determining halophyte distribution in the upper intertidal zone. Distribution and abundance of salt marsh plants can be measured using a variety of indices such as biomass, cover, and frequency. Plant cover is easy to

record, but the error range between individual samples can be large depending on the sampling technique and the number of samples (Hanley, 1978). By contrast, plant biomass can be measured by anyone using an electronic balance, and small individual samples can be measured accurately. Statistical analysis of biomass data can clarify the relationship between vegetation distribution and environmental factors and how these vary with elevation.

Recently, the total area covered by tidal flats in South Korea has decreased rapidly due to human activities, such as the construction of sea walls and harbors and reclamation projects converting salt marshes to agricultural and industrial land (Hong et al., 2010). The total area of tidal flats has been reduced from an estimated 3900 to 2400 km² over 30 years (Park et al., 2015). This habitat loss and the resulting disturbance has impacted plant communities, migratory birds, and other mudflat organisms. Several factors have led to a recent growth of interest in salt marsh restoration. Salt marshes provide a protective buffer zone against tsunami waves, storms (hurricanes), and sea level rise (Moeller et al., 1996; Wolters et al., 2005). Tidal flat ecosystems are highly productive, and restoration may promote coexistence between humans and the natural environment (Wolters et al., 2005). Despite the increased interest in salt marsh restoration, most studies have focused on vegetation establishment and development (Warren et al., 2002). For successful restoration, it is also important to monitor the structure and function of the original ecosystem. The ultimate goal of wetland restoration is to create a sustainable and naturally functioning ecosystem. However, the success of salt marsh restoration is still controversial. One reason is the limited monitoring information on natural

ecosystems (Zhao et al., 2016). Monitoring natural salt marshes is essential to provide reference data for restoration. Understanding the relationship between halophyte distributions and physical environmental factors, especially elevation, is an important key to salt marsh restoration (Williams and Faber, 2001) and may also provide important data on the potential effects of sea level rise.

The distribution of salt marsh plants is expected to vary depending on the region and natural conditions. The Siheung Tidal Flat has one of the large tidal ranges (from 4 to 9 m) in the world and is one of the typical salt marshes of the west coast of Korea (Wells et al., 1990). Despite its ecological importance, there are no data on marsh plant distributions in relation to elevation in this part of Korea. This study therefore aimed to address the question of how environmental factors affect halophyte distributions on the Siheung Tidal Flat. I tested two hypotheses: (1) each salt marsh plant species has a different distribution range with respect to elevation; (2) elevation, flood frequency, salinity, available phosphorus and soil cations (Na^+ , Mg^{2+} , K^+ , Ca^{2+}) significantly influence salt marsh plant distribution and biomass. To test the first hypothesis, I recorded the distribution range of seven salt marsh plant species in the intertidal zone of the Siheung Tidal Flat using quadrats and precise measurements of elevation. To test the second hypothesis, I calculated the flood frequency based on the elevations measured at each plot on the Siheung Tidal Flat, and conducted a quantitative field survey to sample vegetation and soil environmental factors.

2.2. Material and methods

2.2.1. Study site

The field study was conducted at Siheung Tidal Flat (37° 23' 40" N, 126° 46' 05" E), which is located in the back bay at Gyeonggi Bay, facing the Yellow Sea in South Korea. Siheung Tidal Flat (~0.71 km²) was designated a Coastal Wetland Protected Area in 2012 and consists of salt marsh, mudflats, and tidal channels (Fig. 2-1). The tidal channels, a major feature of this wetland, are formed as a result of the high tidal range (from 4 to 9 m), and facilitate the movement of seawater (Wells et al., 1990). The study area is located in the temperate climatic zone. Mean annual temperature is 12.1°C, and annual precipitation is 1234 mm. Most rainfall occurs from June to September, this period accounting for 70% of the total annual precipitation (Korea Meteorological Administration, <http://www.kma.go.kr/>).

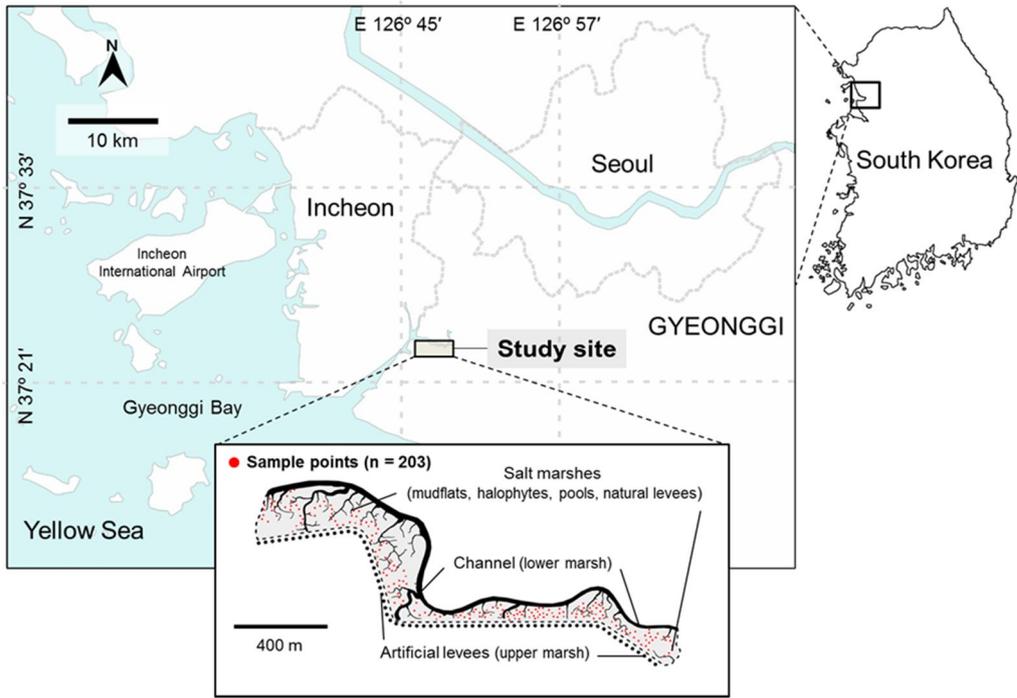


Figure 2-1. Map showing the location of the study site and sampling plots (n = 203), Siheung Tidal Flat, on the west coast of South Korea.

Suaeda japonica Makino (Chenopodiaceae) forms the dominant vegetation at the study site. This annual plant is widely distributed throughout the west coast of Korea and can tolerate wide ranges of waterlogging and salinity (Hayakawa and Agarie, 2010). Another dominant plant is the perennial *Phacelurus latifolius* Ohwi (Gramineae). *Phragmites australis* Trin ex Steud (Gramineae), *Suaeda glauca* Bunge (Chenopodiaceae), *Carex scabrifolia* Steudel (Cyperaceae), *Artemisia fukudo* Makino (Asteraceae), and *Zoysia sinica* Hance (Poaceae) are less common at the study site than *S. japonica* and *P. latifolius*.

2.2.2. Field survey

The field survey was conducted in September 2015. Two hundred and three sampling plots (1 × 1 m) were randomly selected across the site, including the seven dominant salt marsh plants and bare ground at various elevations (Fig. 2-1). Each plot was installed over 10 m away from any adjacent plots to avoid spatial autocorrelation (Schlesinger et al., 1996). All samples were collected from 203 plots in the intertidal zone where halophytic plants grow in the study area (Fig. 2-2). At each sampling point, the relative elevations of the plots in the intertidal zone were measured with a height accuracy of <2.0 mm using a digital level instrument (DL-200, South, China). Then, the elevations reached by high tide in the study area were obtained from real-time tide observations (absolute elevation value; m) provided by the Korea Ocean Observing and Forecasting System (KOOFS), which relate to the Korea original bench mark. After installing PVC pipes perpendicular to the ground at the points of high tides, the relative elevations of the points of high

tide in the study area were also measured using a digital level instrument (DL-200, South, China). All relative elevation values were then converted to absolute elevation values using the information provided by KOOFS for the study area. Flood frequency was calculated using the following method: the high tide elevation value during tidal period > the elevation value at each plot = 1 (flood frequency). The flood frequency at each sampling plot was calculated using the database (1 year of data) of the Korea Hydrographic and Oceanographic Agency, which provided Automated Real-Time Tidal Elevation information (absolute elevation value; m) for the study area.

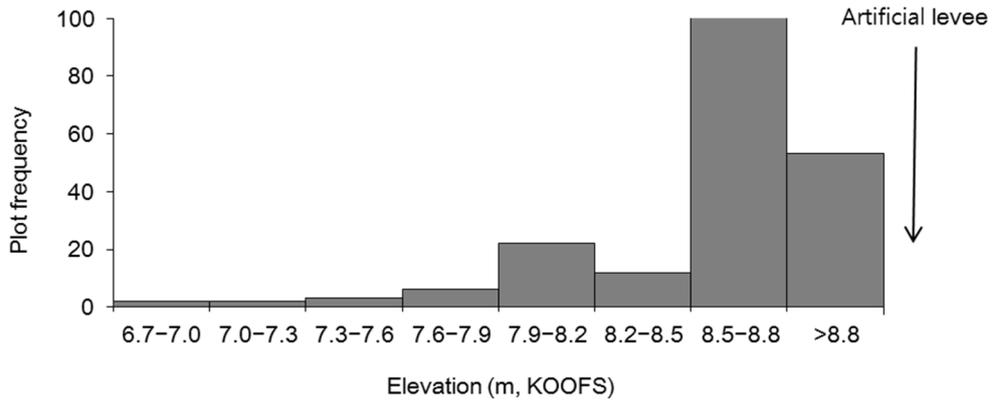


Figure 2-2. Frequency, according to elevation, of the 203 sampling plots (1 × 1 m) on the Siheung Tidal Flat in 2015. Elevation data from the Korea Ocean Observing and Forecasting System (KOOFS).

The vegetation survey carried out in each plot measured above-ground fresh biomass, frequency of occurrence, and percentage cover of each plant species. The percentage cover of all plant species was recorded to the nearest 5%, except for rare species (nearest 1%). The percentage cover of bare ground was also recorded. Above-ground fresh biomass was measured on September 24th, 2015, and soil samples were taken on September 23rd, 2015, at the lowest tide periods in September to eliminate the influence of tide and precipitation. Soil samples were taken from five random subsamples at 5 cm depth in each plot. The five subsamples representing each plot were mixed completely (~500 ml volume per sample) and then stored in a sealed plastic bag at 4°C until soil analysis was conducted.

2.2.3. *Soil analysis*

In each of the 203 soil samples, water content was calculated by measuring the difference in sample weight (10 g) before and after heating in an oven at 105°C for 48 h. Organic matter content was determined by the loss-on-ignition method (John, 2004). To measure soil pH and soil salinity, air-dried soil samples were passed through 2 mm sieves and mixed with distilled water (1:5) for 1 h. Soil pH and electrical conductivity were then measured using a multiparameter bench meter (PC2700, Eutech, Singapore). The electrical conductivity was converted into salinity using the formula, $\text{Salinity (ppt)} = 0.064 \times \text{EC} \times (\% \text{ water in soil}/100) \times 10$, where EC = Electrical conductivity (m mho cm^{-1}) (Joshi and Ghose, 2003). The cations Na^+ , Mg^{2+} , K^+ , and Ca^{2+} were determined by the Mehlich-3 extraction

method (Ziadi and Tran, 2007) and measured using an Inductively Coupled Plasma Emission Spectrometer (ICP-730ES, Varian, Australia). Soil available phosphorus was extracted by Mehlich-3 solution and measured using colorimetry (Ziadi and Tran, 2007).

2.2.4. Data analysis

Three analysis steps were conducted in R software (R Core Team, 2016) in order to relate variation in salt marsh plant communities to environmental factors. Nonparametric statistics were used because of non-normality of the data. Firstly, to identify patterns of similarity in plant community structure, a cluster analysis (CA) based on species biomass data was computed. Cluster analysis was performed using the UPGMA linkage method and the Bray-Curtis dissimilarity matrix. Multi-response permutation procedures were applied to check for significant differences between the clusters. Secondly, Kruskal-Wallis tests were conducted to evaluate variation in environmental factors and plant community structure in the different clusters identified by CA. Multiple comparison tests were then performed using the *kruskalmc* function in the ‘pgirmess’ package if there were significant differences in the Kruskal-Wallis test ($P < 0.05$). Thirdly, non-metric multidimensional scaling (NMS) was used based on Bray-Curtis distances between sampling sites. The ‘vegan’ package in R was applied for NMS computation. In this package, the function ‘*metaMDS*’ was used to find the best solution with the lowest stress value (Bae et al., 2014), and the function ‘*envfit*’ was then used to evaluate the relationships between the salt marsh plant community and environmental factors.

2.3. Results

2.3.1. Environmental factors

Flood frequencies in the lowest (+6.67 m) and highest (+9.13 m) elevation plots in the study area were 583 and 19 inundations per year, respectively, an almost 30-fold difference (Table 2-1). Soil water content decreased significantly with increasing elevation, ranging from 12.5% to 79.0% ($r = -0.488$, $n = 203$, $P < 0.001$).

Table 2-1. Environmental factors measured at each of the 203 study plots at SiheungTidal Flat in Korea. Elevation data from the Korea Ocean Observing and Forecasting System (KOOFS).

Variables	Mean	Standard error	Range
Elevation (m)	8.59	0.03	6.76 – 9.13
Flood frequency (no. of days year ⁻¹)	109	7	583 – 19
Soil water content (%)	30.8	0.7	12.5 – 79.0
Soil organic matter (%)	4.9	0.1	2.8 – 8.7
Soil pH	7.08	0.03	6.24 – 8.24
Soil salinity (ppt)	8.64	0.22	3.31 – 16.99
Soil Na ⁺ (mg g ⁻¹)	11.79	0.32	4.59 – 26.40
Soil Mg ²⁺ (mg g ⁻¹)	2.41	0.05	1.21 – 4.34
Soil K ⁺ (mg g ⁻¹)	1.11	0.01	0.70 – 1.74
Soil Ca ²⁺ (mg g ⁻¹)	0.79	0.01	0.42 – 1.51
Soil available phosphorus (mg g ⁻¹)	0.06	0.002	0.02 – 0.14

Although soil salinity did increase with elevation, there was substantial variation, particularly at high elevations (+8.5 to +9.0 m) (Fig. 2-3a). Soil available phosphorus was negatively correlated with elevation ($r = -0.675$, $n = 203$, $P < 0.001$). Available phosphorus values were high at low-elevation plots, and decreased strikingly with increases in elevation (Fig. 2-3b). Na^+ and Mg^{2+} were both positively correlated with elevation ($r = 0.194$, $n = 203$, $P < 0.010$ and $r = 0.083$, $n = 203$, $P = 0.239$ respectively), whereas K^+ and Ca^{2+} were negatively correlated with elevation ($r = -0.164$, $n = 203$, $P < 0.050$ and $r = -0.242$, $n = 203$, $P < 0.001$, respectively).

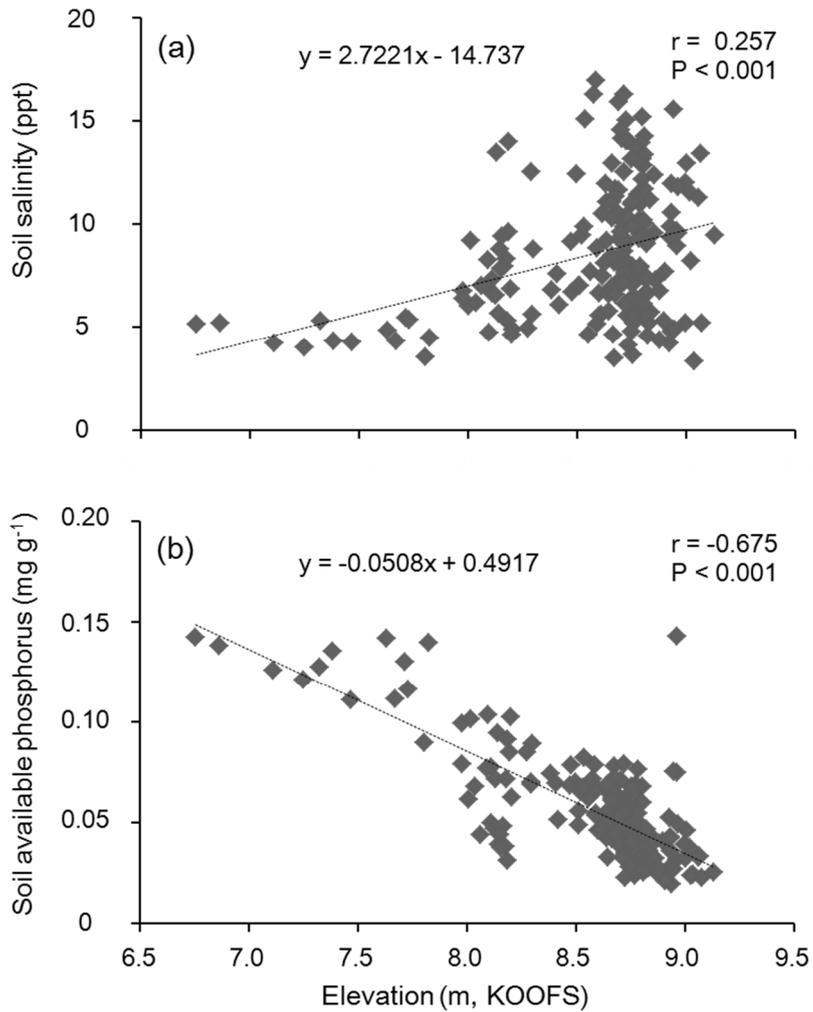


Figure 2-3. Relationships between elevation and (a) soil salinity ($r=0.257$, $P < 0.001$) and (b) soil available phosphorus ($r=-0.675$, $P < 0.001$) in the 203 sampling plots at Siheung Tidal Flat. Correlation between variables was assessed by Spearman's rank correlation (r). Elevation data from the Korea Ocean Observing and Forecasting System (KOOFS).

2.3.2. Plant distribution

Suaeda japonica and *P. latifolius* were the most common plant species, accounting for 40% of the total cover at the study site. There was also much bare ground (Table 2-2). The distribution of bare ground and the seven plant species differed according to elevation and soil salinity (Fig. 2-4). Spatial extent of bare ground was 100% at the lowest elevation, declining with increasing elevation as plants appeared (Fig. 2-4o). Despite some differences in distribution, all plant species occurred at elevations above +7.0 m. *Suaeda japonica* and *C. scabrifolia* showed unimodal distributions, with their peak biomass at +8.2 to +8.5 m and +8.5 to +8.8 m, respectively (Fig. 2-4c and m). *Zoysia sinica* and *P. latifolius* (Fig. 2-4a and k) became gradually more abundant with increasing elevation, reaching maximum biomass in the highest elevation plots (above +8.8 m). *Phragmites australis* and *S. glauca* were absent in plots at elevations below +8.5 m, and had peak abundance at +8.5 to +8.8 m (Fig. 2-4e and i).

Table 2-2. Frequency of occurrence (%) and mean (standard error) coverage (%) of bare ground and salt marsh plants occurring at Siheung Tidal Flat (n = 203 plots).

	Frequency (%)	Coverage (SE) (%)
Bare ground	98	51.8 (3.9)
<i>Suaeda japonica</i>	41	22.4 (3.9)
<i>Phacelurus latifolius</i>	32	18.2 (3.8)
<i>Carex scabrifolia</i>	10	2.0 (0.9)
<i>Suaeda glauca</i>	5	2.6 (1.7)
<i>Zoysia sinica</i>	2	1.8 (1.5)
<i>Phragmites australis</i>	2	1.0 (1.0)
<i>Artemisia fukudo</i>	1	0.2 (0.2)

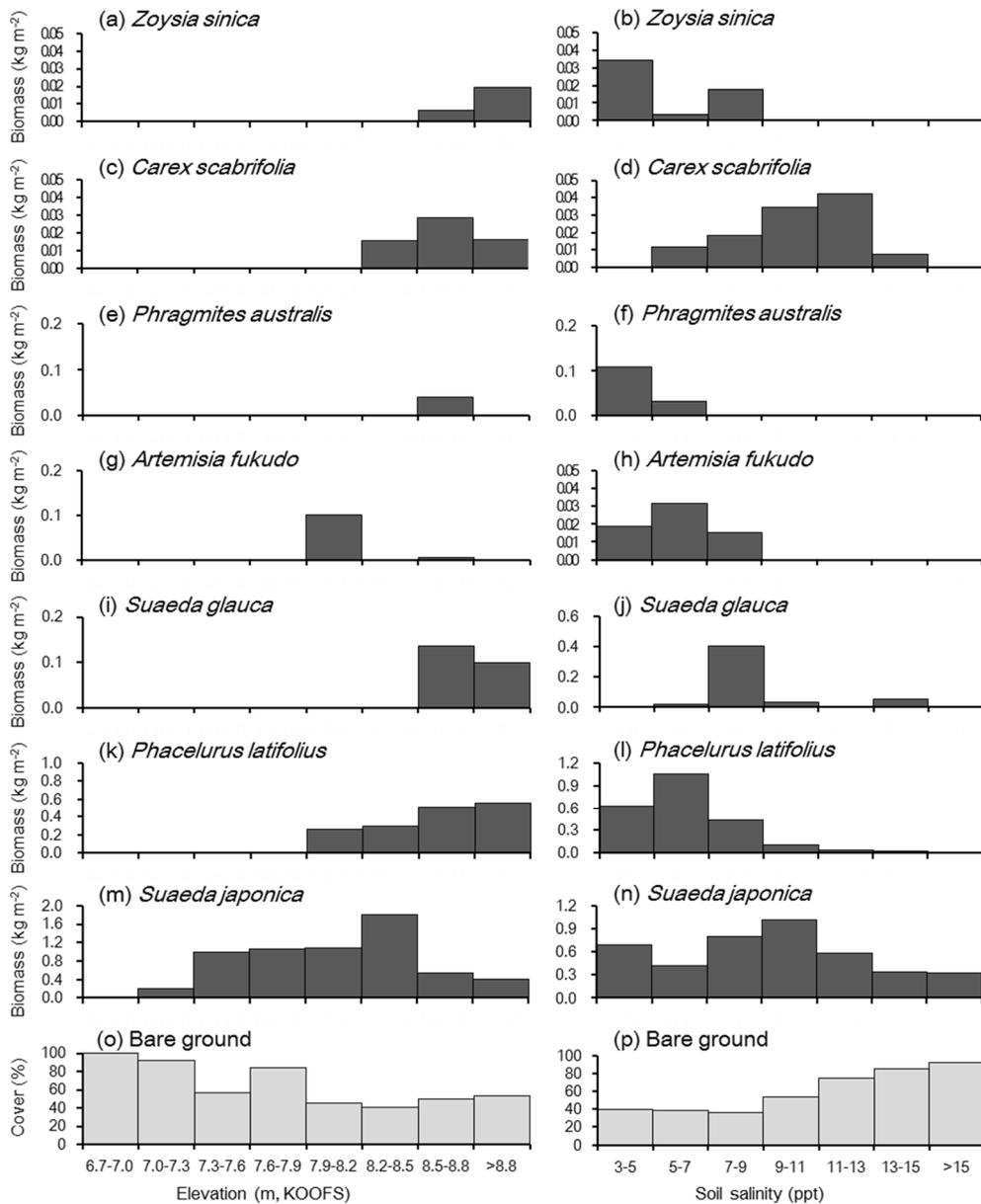


Figure 2-4. Distribution of salt marsh plants (above-ground fresh biomass) and bare ground (cover) in relation to elevation and soil salinity in the 203 sampling plots at Siheung Tidal Flat. (a, b) *Zoysia sinica*, (c, d) *Carex scabrifolia*, (e, f) *Phragmites australis*, (g, h) *Artemisia fukudo*, (i, j) *Suaeda glauca*, (k, l) *Phacelurus latifolius*, (m, n) *Suaeda japonica*, (o, p) bare ground. Black rectangles (■), salt marsh plants; grey rectangles (□), bare ground data.

Plant distribution patterns relative to soil salinity were the inverse of those seen with respect to elevation. The percentage of bare ground was relatively low in low-salinity plots, and tended to increase progressively with increasing salinity (Fig. 2-4p). With the exception of *C. scabrifolia* and *S. japonica*, the maximum biomass of plant species coincided with low soil salinity (cf. *Z. sinica* and *P. australis* 3–5 ppt; *A. fukudo* and *P. latifolius* 5–7 ppt; *S. glauca* 7–9 ppt) (Fig. 2-4b, f, h, l and j). *Suaeda japonica*, the most common plant, is well-adapted to a high-salinity waterlogged environment, and occurred at a wide range of salinities (Fig. 2-4n). Biomass of *P. latifolius* tended to be high in low-salinity plots, and declined steeply with increasing salinity, especially at >7 ppt (Fig. 2-4l). Biomass of *Z. sinica* and *P. australis* peaked at low salinity (Fig. 2-4b and f), and that of *C. scabrifolia* peaked at high salinity (Fig. 2-4d). *Suaeda glauca* peaked at low-mid salinity, whereas *A. fukudo* was not found at salinities >9 ppt (Fig. 2-4h and j).

Cluster analysis of plots based on plant community composition (Fig. S2-1) identified four clusters (1–4) related to variation in environmental characteristics (Table 2-3). *Suaeda japonica* was dominant in Cluster 3, associated with the lowest elevation and highest flood frequency ($P < 0.050$). Cluster 2 was characterized by *P. latifolius* and showed the lowest values for salinity, soil cations (Na^+ , Mg^{2+} , K^+ , Ca^{2+}), and available phosphorus ($P < 0.050$). *Carex scabrifolia* was the dominant species in Cluster 4, associated with the highest values for salinity and soil cations ($P < 0.050$). Cluster 1 was defined by high abundances of *S. glauca*, *Z. sinica*, *P. australis*, and *A. fukudo*, and by relatively low salinity compared with Cluster 4

($P < 0.050$). Differences in plant community composition were also reflected in NMS (stress value = 3.4) (Fig. 2-6 and Table 2-4). Regarding plant species, each cluster is clearly separated. Plots in Cluster 2 were mostly located on the left of the ordination. They were low salinity, soil cations (Na^+ , Mg^{2+} , K^+ , Ca^{2+}), and available phosphorus. Also, species with a higher demand for flood frequency and salinity are more common in Cluster 3 on the upper part of the ordination.

Table 2-3. Mean values of environmental factors and salt marsh plant biomass across the four clusters. The values in parenthesis show the standard error. Different letters indicate significant differences in the factor between clusters based on the multiple comparison tests following the Kruskal-Wallis test ($P < 0.050$).

Variables	1	2	3	4
Elevation (m)	8.7 (0.03) ^{ab}	8.7 (0.03) ^a	8.5 (0.04) ^b	8.8 (0.04) ^a
Flood frequency (no. of days year ⁻¹)	70 (5) ^{ab}	74 (5) ^b	135 (11) ^a	67 (6) ^b
Soil water content (%)	29.8 (1.36)	29.3 (0.58)	32.4 (1.19)	28.6 (0.76)
Soil organic matter (%)	5.2 (0.36)	4.9 (0.11)	4.9 (0.12)	4.3 (0.18)
Soil pH	6.9 (0.11) ^b	6.8 (0.04) ^b	7.2 (0.04) ^a	7.4 (0.10) ^a
Soil salinity (ppt)	7.2 (0.72) ^{bc}	6.7 (0.26) ^c	8.9 (0.30) ^{ab}	10 (0.46) ^a
Soil Na ⁺ (mg g ⁻¹)	9.8 (1.07) ^{bc}	8.8 (0.36) ^c	12.2 (0.4) ^{ab}	14.1 (1.03) ^a
Soil Mg ²⁺ (mg g ⁻¹)	2.1 (0.16) ^b	2.0 (0.05) ^b	2.6 (0.07) ^a	2.7 (0.15) ^a
Soil K ⁺ (mg g ⁻¹)	1.1 (0.05) ^{ab}	1.0 (0.02) ^b	1.1 (0.02) ^a	1.2 (0.05) ^a
Soil Ca ²⁺ (mg g ⁻¹)	0.7 (0.06) ^{ab}	0.7 (0.02) ^b	0.8 (0.02) ^a	0.9 (0.06) ^a
Soil available phosphorus (mg g ⁻¹)	0.06 (0.007) ^a	0.04 (0.002) ^b	0.06 (0.003) ^a	0.05 (0.004) ^a
<i>S. japonica</i> (kg m ⁻²)	0.19 (1.10) ^b	0 (0) ^b	1.62 (0.13) ^a	0 (0) ^b
<i>P. latifolius</i> (kg m ⁻²)	0.08 (0.05) ^b	1.49 (0.09) ^a	0.01 (0.01) ^b	0 (0) ^b
<i>C. scabrifolia</i> (kg m ⁻²)	0.01 (0.01) ^b	0 (0) ^b	0 (0) ^b	0.20 (0.02) ^a
<i>S. glauca</i> (kg m ⁻²)	1.27 (0.45) ^a	0 (0) ^b	0 (0) ^b	0 (0) ^b
<i>Z. sinica</i> (kg m ⁻²)	0.10 (0.05)	0 (0)	0 (0)	0 (0)
<i>P. australis</i> (kg m ⁻²)	0.24 (0.15)	0.01 (0.01)	0 (0)	0 (0)
<i>A. fukudo</i> (kg m ⁻²)	0.03 (0.03)	0 (0)	0.03 (0.02)	0 (0)

Table 2-4. Relationship between environmental factors and NMS ordination of the salt marsh plant community based on *envfit* (1000 permutations).

Factors	NMS1	NMS2	r ²	P
Elevation (m)	-0.39021	-0.92073	0.1698	0.001
Flood frequency (no. of days year ⁻¹)	0.38205	0.92414	0.1600	0.001
Soil water content (%)	0.18920	0.98194	0.0505	0.010
Soil organic matter (%)	-0.25882	0.96593	0.0141	0.312
Soil pH	0.98920	0.14657	0.1735	0.001
Soil salinity (ppt)	0.99990	0.01446	0.2128	0.001
Soil Na ⁺ (mg g ⁻¹)	0.99971	0.02393	0.2343	0.001
Soil Mg ²⁺ (mg g ⁻¹)	0.98934	0.14565	0.2398	0.001
Soil K ⁺ (mg g ⁻¹)	0.99552	0.09458	0.2100	0.001
Soil Ca ²⁺ (mg g ⁻¹)	0.99979	-0.02030	0.1550	0.001
Soil available phosphorus (mg g ⁻¹)	0.96545	0.26058	0.0945	0.001

Plant cover and biomass were positively correlated, but the linear relationship varied depending on plant species (Fig. 2-5). The positive correlation between plant cover and biomass was higher for *C. scabrifolia* ($r = 0.937$, $n = 83$, $P < 0.001$) than for *P. latifolius* ($r = 0.735$, $n = 64$, $P < 0.001$) or *S. japonica* ($r = 0.743$, $n = 83$, $P < 0.001$). *Suaeda japonica* showed a weak correlation with biomass at $>60\%$ plant cover compared with *P. latifolius*.

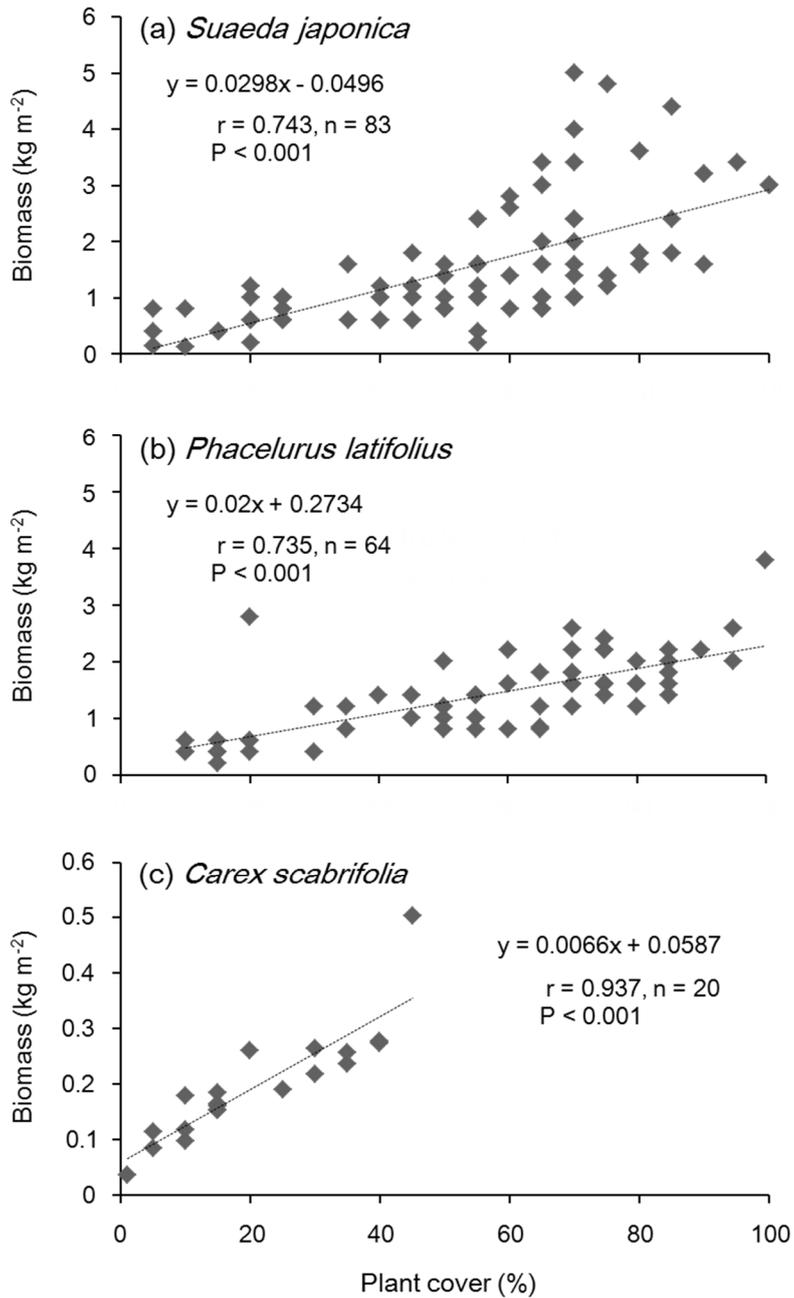


Figure 2-5. The relationship between plant cover and biomass in (a) *Suaeda japonica*, (b) *Phacelurus latifolius*, and (c) *Carex scabrifolia*.

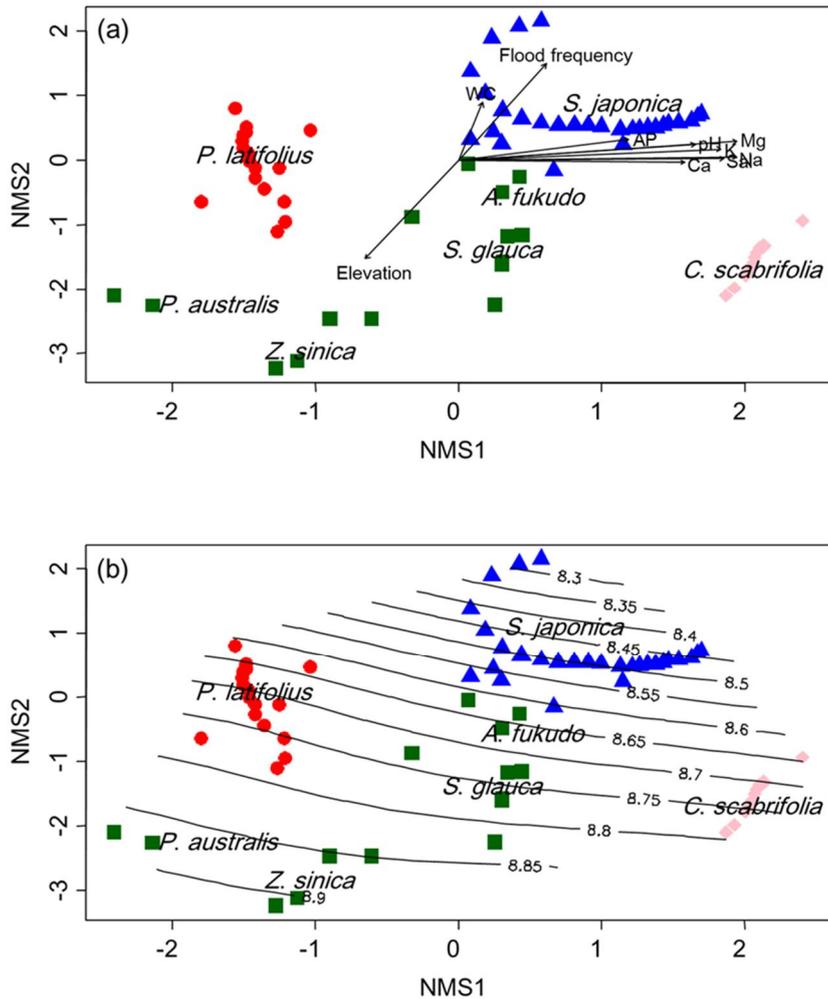


Figure 2-6. NMS ordination based on the above-ground fresh biomass of salt marsh plant species at Siheung Tidal Flat. (a) Ordination with fitted vectors of environmental variables, (b) NMS ordination fitted by elevation. Seven salt marsh plants showed significant correlations with environmental factors ($P < 0.050$). Each symbol shape and color represents one of the four clusters (green: *A. fukudo*, *S. glauca*, *P. australis*, *Z. sinica*; red: *P. latifolius*; blue: *S. japonica*; pink: *C. scabrifolia*).

2.4. Discussion

In salt marshes, halophyte zonation patterns are generally determined by environmental gradients (Ungar, 1998). At the Korean study site, each of seven salt marsh plant species had a distinct distribution range along gradients of elevation and salinity (Fig. 2-4). *Suaeda japonica* was the most dominant species and was found at a wide range of elevations; *P. latifolius* and *Z. sinica* were distributed further landward than other species; *C. scabrifolia* and *P. australis* were confined to higher-elevation plots; *A. fukudo* was mainly found at mid-range elevation plots. Thus, each salt marsh plant species had a different distribution range depending on the elevation and was likely influenced by different flood frequency ranges (Fig. 2-7). Bare ground coverage gradually increased with decreasing elevation because plants in low-elevation plots may not survive long periods of inundation (Dunton et al., 2001). The tidal pattern according to elevation is one of the important factors determining the distribution of marsh plants (Adams, 1963; Congdon, 1981; Isacch et al., 2006; Hladik and Alber, 2014). Tidal flooding leads to decreased soil oxygen and results in anaerobic conditions (Ponnamperuma, 1984). The oxygen concentration in soil depends on the inundation time and flood frequency (Setter and Waters, 2003). When plants are completely submerged, decreased light, O₂, and CO₂ levels restrict photosynthesis (Colmer and Flowers, 2008). Plant growth is also restricted by characteristics of anoxic soil such as ion toxicity, ion transport interruption, and deficiencies in available minerals (Alhdad et al., 2015). Therefore, the elevation-related zonation pattern seems to reflect differences in the ability of each plant species to withstand flooding stress. In addition, most of the salt marsh

plants are distributed at high elevation, which may indicate a realized niche reflecting potential interspecific competition in the upper intertidal zone.

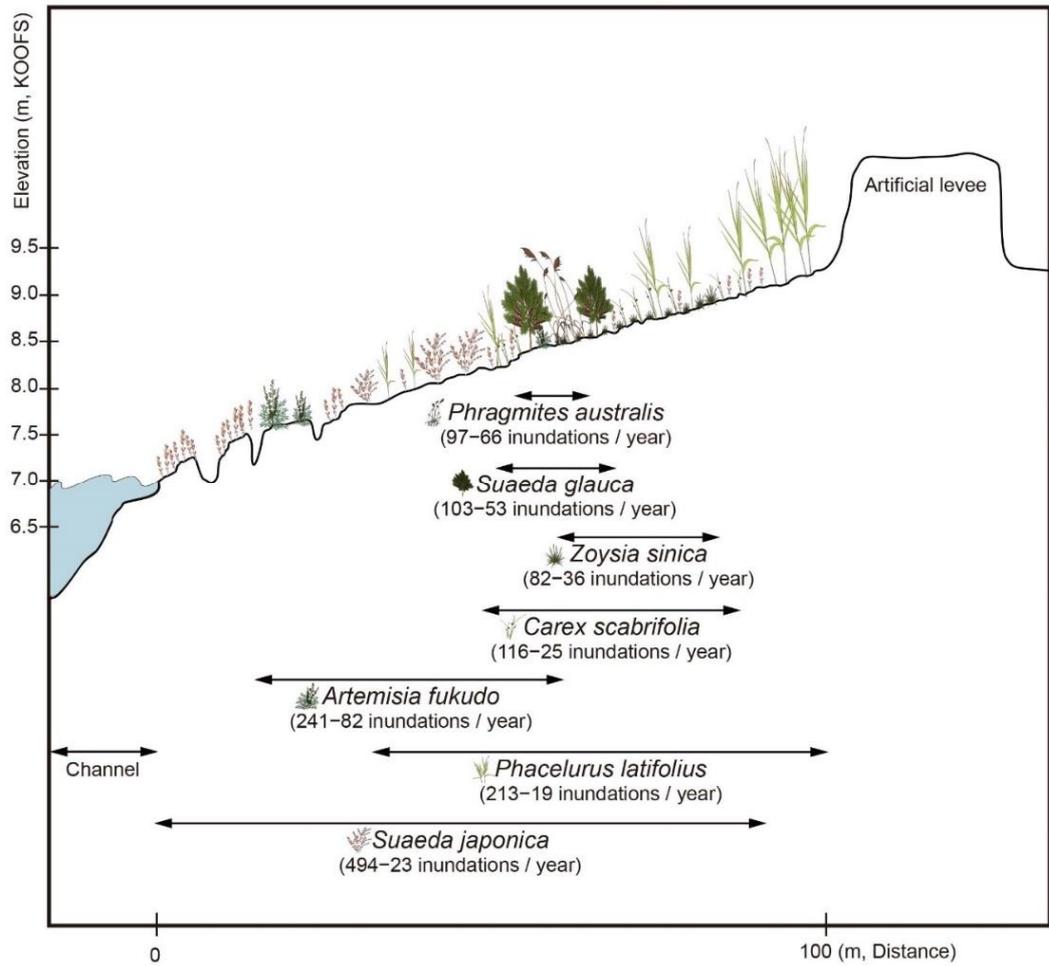


Figure 2-7. Plant distribution and flood frequency in relation to elevation at Siheung Tidal Flat. Elevation data from the Korea Ocean Observing and Forecasting System (KOOFS).

Halophyte distribution is also related to salinity (Pennings et al., 2005). Although halophytes can tolerate some salt stress, individual species differ in their tolerance range (Flowers and Colmer, 2008). In contrast to its relationship with elevation, bare ground coverage tended to increase with increasing salinity. *Suaeda japonica* was common across the salinity range; *Z. sinica*, *P. australis*, *A. fukudo*, and *P. latifolius* were found at low salinities, whereas *S. glauca* and *C. scabrifolia* were found at mid-range salinities. Salt marsh plants can adapt to salt stress by regulating ion concentrations for water potential, regulating ion exchange, and accumulating ions. Their differential abilities may influence species distributions along the salinity gradient (Flowers and Colmer, 2008).

Salt marshes experience twice-daily flood and ebb tides, with daily variation in tidal height. The period of seawater immersion therefore depends on elevation. As elevation increases, the aerial exposure and drying times increase, leading to higher soil salinity (Pennings et al., 2005). At this study site, soil salinity did tend to increase with elevation, but with a wide range of variation in high-elevation plots (Fig. 2-3a). Most halophytes were found in high-elevation plots, and the extent of plant cover can influence soil water evaporation and hence soil salinity (Pennings and Bertness, 1999). In addition, irregular topography in the upper intertidal zone may affect soil salinity by determining the level of soil water saturation (De Rijk, 1995). Freshwater inflows from precipitation and rivers can also contribute to differences in salinity between bare grounds and plant-covered habitats (Horton and Murray, 2007). Soil available phosphorus tended to decrease as elevation increased (Fig. 2-3b). Higher flood frequencies at lower elevation may

increase the supply of available phosphorus to plants through mineralization, whereas at higher elevation (lower flood frequency) increased desiccation can inhibit nutrient diffusion and mass flow (He and Dijkstra, 2014). Plant cover had a smaller effect on soil available phosphorus than on soil salinity (Fig. 2-3a and b), indicating that phosphorus concentration may be determined by sediment water content rather than by biological processes (Shao et al., 2013). Available phosphorus concentration can therefore be significantly affected by elevation.

These results showed a high correlation between plant cover and biomass but with some differences among plant species (Fig. 2-5). In particular, the most abundant plant, *S. japonica*, showed considerable variability in the relationship between percentage cover and biomass. This variation may be driven by a combination of individual growth form, vegetation structure, habitat type, and environmental gradients (Jiang et al., 2017). At this study site, plant cover was recorded to the nearest 5% at each plot. A previous study showed that the sampling technique can increase the error for individual species (Hanley, 1978). However, plant biomass can also be quantitatively measured for small individual species using an electronic balance, which can provide ecologically important information about plant distribution and productivity (Jiang et al., 2017). Therefore, the biomass data collected in this study are a more objective index, and allowed a more rigorous statistical analysis because the error range between samples is smaller than for plant cover data.

The relationship between species distribution and environmental factors in

this salt marsh is shown clearly by the results of CA and NMS analysis (Fig. 2-6 and Table 2-3). In particular, the distribution of seven salt marsh plants showed significant correlations with environmental factors ($P < 0.05$). *Suaeda japonica*, defining Cluster 3, was the dominant species at the lowest elevation and at the highest flood frequency and occurred across the range of soil salinities. These results are consistent with those of previous studies. *Suaeda japonica*, an annual plant that grows rapidly in its early stages, is widely distributed throughout salt marshes on the west coast of Korea (Lee and Ihm, 2004). The species copes with salt stress by means of increased levels of betaine and accumulation of glycinebetaine (Yokoishi and Tanimoto, 1994; Hayakawa and Agarie, 2010). These characteristics allow it to dominate in areas with wide ranges of immersion and salinity. Therefore, *Suaeda japonica*, the most dominant species in the study site, is also well-adapted to a high-salinity waterlogged environment. *Phacelurus latifolius*, the principal species in Cluster 2, dominated at mid-high elevations where soil salinity, cations (Na^+ , Mg^{2+} , K^+ , Ca^{2+}), and available phosphorus negatively affected distribution and productivity. Min (2015) showed that *P. latifolius* is a high-elevation marsh species. This plant is dominant in freshwater or brackish wetlands, where it is confined to the upper intertidal zone (Shim et al., 2009). Available phosphorus may also be reduced by the more prolonged drying of the soil at high elevation (He and Dijkstra, 2014). Elevation, salinity, cations, and available phosphorus may therefore determine the distribution of *P. latifolius*. *Carex scabrifolia*, in Cluster 4, was distributed at mid-high elevation and at the highest salinity and cation values in the study area. This species commonly occurs

together with *P. australis* at high elevations in salt marshes (Chen et al., 2007; Zhou et al., 2007). In Japan, *C. scabrifolia* is typically found on sandbars in lagoons and estuaries (Hodoki et al., 2014), which may experience high salinities. In general, lagoons and the upper reaches of salt marshes may have some high-salinity microhabitats, and *C. scabrifolia* may prefer these areas because of reduced competition with other plant species. *Suaeda glauca*, *Z. sinica*, and *P. australis* defined Cluster 1, which was distributed at high elevation, whereas *A. fukudo* (Cluster 1) was found at mid-elevation areas in the study site. Salinity in Cluster 1 plots was relatively low compared with that in other cluster plots. *Phragmites australis* is widely distributed throughout freshwater, brackish, and salt marsh habitats, tolerating a wide range of salinities, but usually predominates in brackish wetlands. Its distribution is limited to salt marches with a high elevation (Chambers et al., 1999). At this study site, *Zoysia sinica*, a pioneer species on salt marsh mudflats (Kim et al., 1986), occurred in areas with high elevation and relatively low salinity. This is consistent with previous studies on this halophyte showing that it occurs in salt marshes on the convex surfaces of highly elevated regions (Min and Je, 2002), a pattern suggesting that it is vulnerable to flooding.

CHAPTER 3.

Diet of the mud-flat crab *Helice tientsinensis* in a Korean salt marsh

* This chapter will be submitted in journal 'Wetlands' in August 2018.

3.1. Introduction

Crabs are abundant in salt marshes (Jones, 1984); they are dominant species in these ecosystems with a high biomass (Golley et al., 1962). The feeding behavior of crabs can control the abundance and distribution of prey in salt marshes (Hoffman et al., 1984). Crabs also affect nutrient dynamics (Alberti et al., 2015; Martinetto et al., 2016) and can directly or indirectly affect community dynamics and food web interactions in salt marshes (Trussell et al., 2002).

The shore crab *Helice tientsinensis* lives in the upper part of the mud flat of the southwestern coast of Korea with salt marsh plants, and it is a very economically and commercially useful species for fishermen and local residents (Baek, 2014). The crabs use halophytes as food sources and can affect their distribution (He et al., 2015). Extensive studies of mangroves have evaluated the effects of herbivorous crabs on plant communities. For example, crabs generally feed directly on mangrove leaves or store the leaves in burrows for later ingestion (Martinetto et al., 2016). Feeding by these crabs can result in the removal of 30–90% of annual litter in a mangrove habitat (Robertson, 1986; Micheli, 1993; Slim et al., 1997). Recent studies report that small herbivorous animals, such as crabs, can limit the marsh seaward boundaries (Alberti et al., 2010; He et al., 2015). In addition, crabs play important roles in salt marsh ecosystems, e.g., as herbivores, predators, and sediment consumers, and utilize diverse food types, depending on the species (Bertness, 1999; Hsieh et al., 2002; Qin et al., 2010). Differences in food sources are also related to the size and sex of crabs (Divine et al., 2015;

Hübner et al., 2015). However, little is known about the feeding relationships between the crab *H. tientsinensis* and Korean native marsh plant species. Understanding the food sources of dominant crabs may provide insights into their roles in the food web and their impacts on the salt marsh plant community.

Stable isotope analyses are commonly used in feeding ecology to identify predator diet compositions and trophic levels (Peterson and Fry, 1987; Post, 2002; Layman et al., 2007). The isotopic signatures of consumers are often similar to those of plants, and food resources of consumers can be characterized by comparisons between the isotopic signatures of consumers and food resources (Fry et al., 1978; DeNiro and Epstein, 1981). However, it is difficult to distinguish plant species from the particular diet of a consumer based on isotope analyses alone. Recently, increasing ecological studies have included DNA analyses to evaluate feeding habits (Valentini et al., 2009). A DNA analysis of food residues in the stomach can provide substantial and accurate prey information. DNA analyses of stomach contents and stable isotope analyses can provide complementary information about food sources (Peterson, 1999).

In this study, I used a stable isotope analysis and a DNA analysis of stomach contents to identify the main food sources of the dominant crab *Helice tientsinensis* in the Siheung Tidal Flat, which is a Coastal Wetland Protected Area with a high tidal range (from 4 to 9 m) in Korea (Wells et al., 1990). *H. tientsinensis* and salt marsh plants are widely distributed in Korean salt marshes, but their feeding relationships have not been studied. I determined the types of halophytes that are

eaten by crabs and their importance relative to other food sources. I also used a stable isotope mixing model (SIAR) to evaluate the impact of the herbivorous crabs on salt marsh plant communities in the Siheung Tidal Flat. I hypothesized that (1) *H. tientsinensis* feeds on a variety of salt marsh plants at the study site and (2) the main food sources vary depending on the sex and carapace width (CW) of the crab.

3.2. Material and methods

3.2.1. Study site

Field surveys were conducted in the Siheung Tidal Flat (37°23'52"N, 126°45'33"E), which is one of the macrotidal salt marshes in South Korea. Halophytes and tidal flats are widely distributed among the deep tidal channels formed by the high tidal range from 4 to 9 m (Wells et al., 1990), which has been designated a Coastal Wetland Protected Area (ca. 0.71 km²) since 2012 owing to its ecological importance. The study area is geographically located in the mid-latitude temperate climatic zone characterized by a mean annual temperature of 12.1°C, and a mean annual precipitation of 1234 mm, with 70% of annual precipitation falling in the rainy season from June to September (Korea Meteorological Administration, <http://www.kma.go.kr/>).

The dominant salt marsh plants in this area are *Suaeda japonica* Makino and *Phacelurus latifolius* Ohwi; other species include *Carex scabrifolia* Steudel, *Artemisia fukudo* Makino, *Phragmites australis* Trin ex Steud and *Zoysia sinica* Hance, and are potential food sources for crabs. *Helice tientsinensis* Rathbun is the most abundant crab species at this study site, with estimated densities of 27–47 individuals per m².

3.2.2. Sampling and sample processing

All samples were collected in August 2016 at three sites; these locations were dominated by *S. japonica* and *P. latifolius* in the Siheung Tidal Flat. Sampling sites

were separated by at least 50 m (Fig. 3-1). Crab samples collected at the site using standard commercial crab traps (33 × 33 × 58 cm [width × height × length], 1-cm mesh) were immediately placed in an ice box to minimize digestion. After collection, the samples were transferred to the laboratory and the sex and CW, as determined using calipers, were recorded. Samples were then frozen at -20°C until analysis. The potential food sources included halophytes, polychaetes, soil organic matter (SOM), and benthic microalgae (BMI), based on the literature and observations (Qin et al., 2010; Han et al., 2012; Mao et al., 2016). The leaves of marsh plants (*S. japonica*, *P. latifolius*, *C. scabrifolia*, *A. fukudo*, *P. australis* and *Z. sinica*) were collected by hand. Polychaetes were collected in the soil. Samples for SOM analyses were collected from depths of 0–1 cm in the surface soil and then stored in an ice box. Reference samples for BMI were collected in the same season (summer) near the study site (Kang, 2016). To estimate the crab density in the study area, the number of burrows at each sampling point was counted in August 2016.

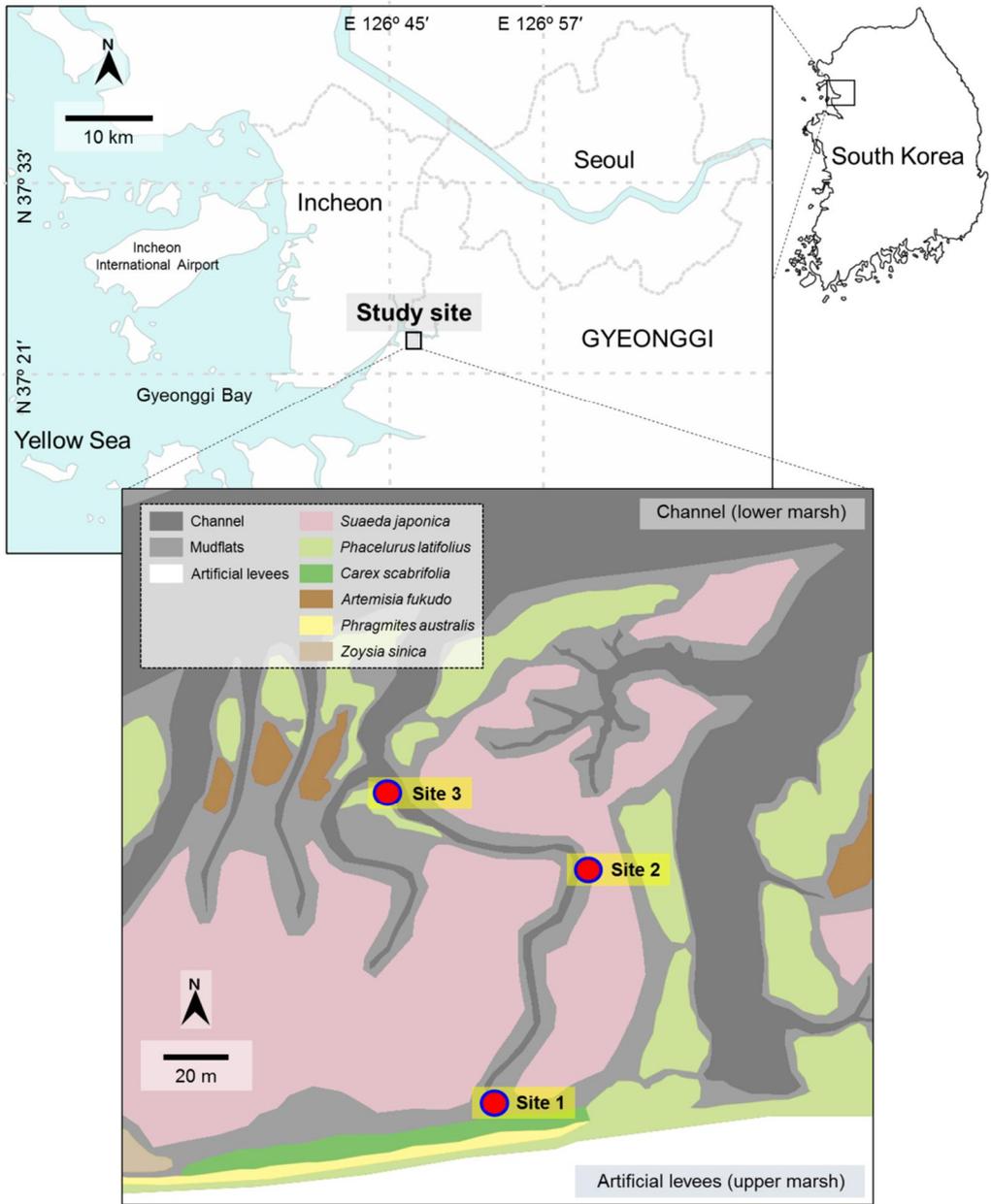


Figure 3-1. Map showing the location of the sampling sites (n = 3), Siheung Tidal Flat.

For the DNA analysis of stomach contents, the stomachs of 18 crabs were incised and stored in 70% ethanol. Muscle tissue of the leg of the crab was dissected for the stable isotope analysis because these tissues in decapods have less variation in ^{13}C and ^{15}N than that in other tissue types (Yokoyama et al., 2005). In addition, debris from the exoskeleton or other inorganic carbonates was not included in the samples and muscle samples were not oxidized. In total, 30 crabs were used for the stable isotope analysis. All crab samples were classified into the following categories according to the CW or sex: G1 (2.0– 2.5 cm, n = 10), G2 (2.5– 3.0 cm, n = 10), and G3 (3.0– 3.5 cm, n = 10); Female (n = 15) and Male (n = 15).

Four groups of potential food sources (n = 5) were selected for the stable isotope analysis as follows: plant (*Suaeda japonica*), animal (polychaetes), SOM, and BMI. All samples were freeze-dried (Kim, 2016) to remove moisture and then milled to a uniform particle size using a ball mill (Retsch MM301; Dusseldorf, Germany). The pulverized samples were added to a tin disc (Perkin Elmer, Waltham, MA, USA), made into compact spheres, and stored in plates, and then a stable isotope analysis was performed at the Stable Isotope Biogeochemistry Center at UC Berkeley.

3.2.3. *Stable isotope analyses*

The carbon and nitrogen isotope ratios were analyzed using a CHNOS Elemental Analyzer (vario ISOTOPE cube; Elementar, Hanau, Germany) coupled

with a mass spectrometer (IsoPrime 100; Isoprime Ltd., Manchester, UK). The stable isotope ratio expressed as δX (‰) was determined according to the following formula:

$$\delta X (\text{‰}) = [(R_{\text{sample}}/R_{\text{standard}}) - 1] \times 1000,$$

where X is ^{15}N or ^{13}C and R is the ratio of $^{15}\text{N}/^{14}\text{N}$ or $^{13}\text{C}/^{12}\text{C}$. R standards were the Vienna Pee Dee Belemnite (VPDB) standard for carbon and atmospheric N_2 for nitrogen. The analytical precision of these measurements was 0.20‰ for $\delta^{13}\text{C}$ and 0.30‰ for $\delta^{15}\text{N}$.

The relative contribution of each food source to the consumers (three groups of crabs) was assessed by the isotope mixing model using the ‘SIAR’ package in R. Potential food sources were classified as plant (*S. japonica*), animal (polychaetes), SOM, and BMI, based on analyses of stomach content DNA and references. The following fractionation factors used for decapods in previous studies (McCraith et al., 2003; Vanderklift and Ponsard, 2003) were applied to this model: $0.4 \pm 1.2\%$ for $\delta^{13}\text{C}$ and $2.2 \pm 1.6\%$ for $\delta^{15}\text{N}$.

3.2.4. DNA analysis of stomach contents

DNA was extracted from the stomach content samples using the QIAamp DNA Stool Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol, except for the lysis step. One or two 5-mm stainless steel beads (Qiagen) were added for sufficient homogenization in the lysis step and the samples were

mixed by shaking on a Mixer Mill (Retsch) at 20 Hz for 1 min. The extracted DNA was finally eluted in 200 μ l of AE buffer and stored at -20°C until further analysis.

The ITS2 region of the ribosomal RNA (rRNA) gene was amplified using a universal ITS primer set, i.e., ITS2_S2F (5'-ATGCGATACTTGGTGTGAAT-3') and ITS2_S2R (5'-GACGCTTCTCCAGACTACAAT-3') (Chen et al., 2010). For PCR amplification, 2 μ l of extracted DNA was added to amplification mixture, Intron FastMix/Frenche™ Premix (iNtRON Biotechnology, Seoul, South Korea), and forward and reverse primers (5 pmol) in a final reaction volume of 20 μ l. PCR conditions were as follows: an initial denaturation at 94°C for 5 min, 40 cycles of denaturation at 94°C for 30 s, annealing at 56°C for 30 s, and elongation at 72°C for 45s, and a final extension step at 72°C for 10 min. All PCR products were purified using the MEGAquick-spin™ Total Fragment DNA Purification Kit (iNtRON Biotechnology). Purified PCR products were ligated into the pGEM-T Easy Vector according to the manufacturer's protocols (Promega, Madison, WI, USA) and transformed into DH5a chemically competent cells. Cells were plated in Luria–Bertani agar + ampicillin medium with 40 μ l of X-gal solution (2% w/v) for antibiotic selection and blue-white screening. After the cloning step, white colonies were selected and used for colony PCR with M13F and M13R primers. PCR conditions were as follows: an initial denaturation at 95°C for 10 min, 35 cycles of denaturation at 94°C for 30 s, annealing at 55°C for 30 s, and elongation at 72°C for 1 min, and a final extension step at 72°C for 7 min. After amplification, PCR products were purified and delivered to a commercial sequencing service (Genotech, Daejeon, South Korea). All DNA sequences were identified by

BLASTN searches against the GenBank database and aligned using MEGA6 (Tamura et al., 2013). In addition, all BLASTN results were confirmed by comparisons with sequences of salt marsh plants collected in the field.

3.2.5. Data analyses

Statistical analyses were performed using R (R Core Team, 2017). Before the analysis, all data were checked for normality and homogeneity of variances. The correlations between the $\delta^{13}\text{C}$ or $\delta^{15}\text{N}$ values and the CW of crabs were evaluated using Pearson correlation coefficients. Two-way ANOVA was used to explain variation in the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ signatures of consumer groups using the CW and sex of crabs as the main fixed factors. Tukey's multiple comparison tests were used only if significant differences were detected by ANOVA ($P < 0.05$).

3.3. Results

3.3.1. Stable isotope analysis

The $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values for consumer crabs (*H. tientsinensis*) differed according to the CW and sex of crabs (Table S3-1). There were no significant effects of Sex or the CW \times Sex interaction, whereas the carbon and nitrogen isotope values were significantly different according to the CW of crabs (ANOVA, $P < 0.001$) (Table 3-1). As the CW of the crab increased, $\delta^{13}\text{C}$ increased ($G1 < G2 < G3$). The $\delta^{15}\text{N}$ values were higher in G2 than in G1 and G3. In particular, the difference in $\delta^{15}\text{N}$ between G2 and G3 was approximately 2–3‰.

Table 3-1. Summary of two-way ANOVA results for consumer crabs in the Siheung Tidal Flat. (df: degrees of freedom; Sum Sq: sum of squares; Mean Sq: mean square; TSS: total sum of squares; CW: carapace width (CW) class).

Factor	df	Sum Sq	Mean Sq	<i>F</i> -value	Pr(> <i>F</i>)	Variance explained (%)
$\delta^{13}\text{C}$						
CW	2	23.29	11.65	11.86	<0.001	46.5
Sex	1	2.04	2.04	2.08	0.1620	4.1
CW \times Sex	2	1.15	0.58	0.59	0.5633	2.3
Residuals	24	23.56	0.98			
TSS		50.05				
$\delta^{15}\text{N}$						
CW	2	32.6	16.3	15.1	<0.001	52.5
Sex	1	0.55	0.55	0.51	0.4814	0.9
CW \times Sex	2	3.04	1.52	1.41	0.2642	4.9
Residuals	24	25.91	1.08			
TSS		62.1				

The $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values differed among the potential food sources collected from the Siheung Tidal Flat (Table S3-2). *Suaeda japonica* was the most ^{13}C -depleted food source in the Siheung Tidal Flat ($-27.99 \pm 0.67\text{‰}$), while *P. latifolius* was the most ^{13}C -enriched ($-14.52 \pm 0.26\text{‰}$). The $\delta^{13}\text{C}$ values for SOM ($-24.54 \pm 0.35\text{‰}$) and polychaetes ($-20.88 \pm 0.31\text{‰}$) were intermediate between those for *S. japonica* and *P. latifolius*. The $\delta^{15}\text{N}$ values for potential food sources ranged from *P. latifolius* ($2.36 \pm 1.35\text{‰}$) to *C. scabrifolia* ($7.15 \pm 1.95 \text{‰}$).

Potential food sources and consumer crabs were well-differentiated with respect to carbon and nitrogen stable isotopes (Fig. 3-2). The $\delta^{13}\text{C}$ values for all crabs were distributed within the range of $\delta^{13}\text{C}$ values for potential food sources.

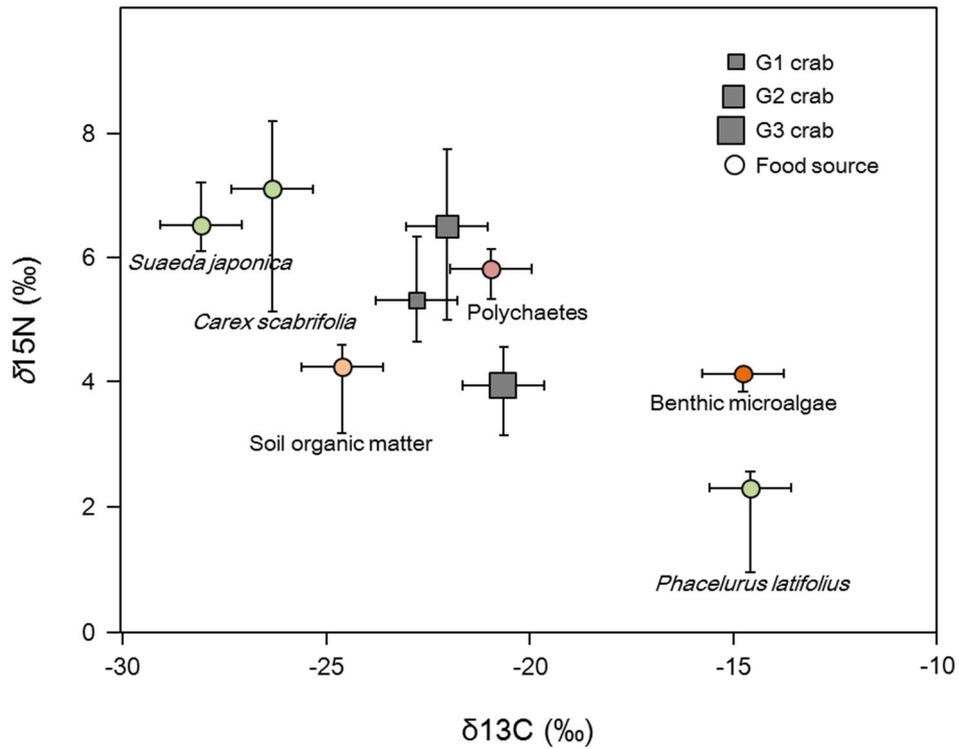


Figure 3-2. Stable isotope signatures ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, mean \pm SD, $n = 5$) for crabs (gray symbols) and food sources (other colors) collected in the Siheung Tidal Flat. G1: CW (2.0–2.5 cm); G2: CW (2.5–3.0 cm); G3: CW (3.0–3.5 cm); CW: carapace width.

3.3.2. DNA analysis of stomach contents

DNA was extracted from 23 samples, including six salt marsh plants and the stomach contents of 18 crab individuals (*H. tientsinensis*) in the Siheung Tidal Flat. Thirty unique sequences were detected from genes amplified from the stomach content samples and only *S. japonica* was detected in all samples (Table 3-2). The amplification success rate was 57.5% (Table S3-3). Additionally, the amplification success rate differed depending on the CW class and sex of crabs and was higher in G3 (77.5%) than in G2 (37.5%) and in Females (77.5%) than in Males (37.5%).

Table 3-2. Plant food sources detected in the stomach contents of crabs.

No.	ID	Sex	Class	Pairwise alignment with collected plants in study site	% identity
1	Sh_1	F	G2	<i>Suaeda japonica</i>	100
2				<i>Suaeda japonica</i>	99.71
3	Sh_2	F	G2	<i>Suaeda japonica</i>	100
4	Sh_3	F	G2	<i>Suaeda japonica</i>	98.73
5	Sh_4	F	G3	<i>Suaeda japonica</i>	100
6				<i>Suaeda japonica</i>	100
7				<i>Suaeda japonica</i>	99.71
8				<i>Suaeda japonica</i>	100
9				<i>Suaeda japonica</i>	100
10	Sh_5	F	G3	<i>Suaeda japonica</i>	99.71
11				<i>Suaeda japonica</i>	97.56
12				<i>Suaeda japonica</i>	100
13	Sh_6	F	G3	<i>Suaeda japonica</i>	99.71
14				<i>Suaeda japonica</i>	100
15				<i>Suaeda japonica</i>	100
16	Sh_7	F	G3	<i>Suaeda japonica</i>	99.72
17				<i>Suaeda japonica</i>	99.71
18				<i>Suaeda japonica</i>	100
19				<i>Suaeda japonica</i>	100
20	Sh_8	M	G3	<i>Suaeda japonica</i>	100
21				<i>Suaeda japonica</i>	100
22				<i>Suaeda japonica</i>	99.71
23	Sh_9	M	G3	<i>Suaeda japonica</i>	100
24				<i>Suaeda japonica</i>	100
25				<i>Suaeda japonica</i>	99.71
26	Sh_10	M	G3	<i>Suaeda japonica</i>	100
27				<i>Suaeda japonica</i>	100
28				<i>Suaeda japonica</i>	100
29				<i>Suaeda japonica</i>	100
30				<i>Suaeda japonica</i>	100

3.3.3. Stable isotope mixing model

A stable isotope mixing model (SIAR) was used to estimate the relative contributions of the four main categories of food sources to crabs assigned to the three CW classes (Fig. 3-3). The ratios of SOM, BMI, animal, and plant materials varied according to the CW class. SOM was the most substantial food source in G1 (57.2%) but contributed less to the food compositions of G2 (37.6%) and G3 (32.0%). BMI showed the inverse trend to that observed for SOM: G1 (15.0%) < G2 (22.4%) < G3 (33.5%). The contribution of *S. japonica* was lower in G1 and G3 (14.0%) than in G2 (20.0%). In the case of animal food sources, the ratio was higher in G2 (20.0%) and G3 (20.5%) than in G1 (13.9%).

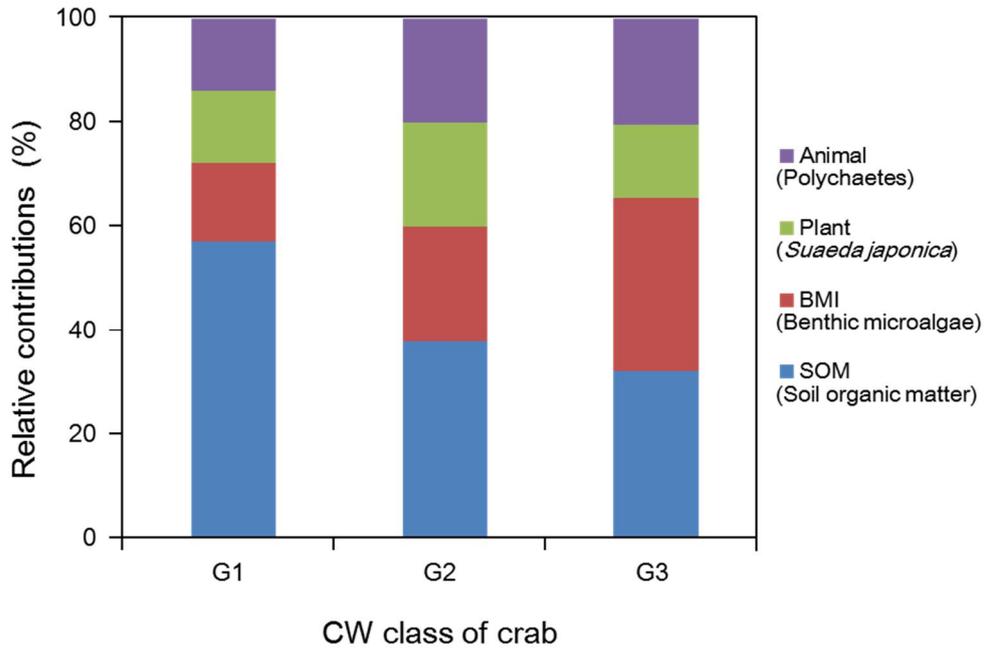


Figure 3-3. SIAR model estimates of the relative contributions of food sources (expressed as percentages) for each CW class of crabs collected in the Siheung Tidal Flat. G1: CW 2.0–2.5 cm; G2: CW 2.5–3.0 cm; G3: CW 3.0–3.5 cm; CW: carapace width of crabs.

3.4. Discussion

Information on the main food sources of herbivorous crabs can improve our understanding of the interaction between crabs and plants in salt marshes. DNA analyses of stomach contents can provide direct evidence for preferred food sources based on comparisons between sequences in the GenBank database and sequences of salt marsh plants collected in the field (Joo et al., 2014). Generally, this analysis shows recently consumed food. A stable isotope analysis of muscle tissues reflects the mean composition of the assimilated diet and provides relatively long-term information (Divine et al., 2015). These two methods are complementary and suitable for analyses of decapod food sources.

In a previous study, *H. tientsinensis* consumed plants as its main food source in a salt marsh, particularly the leaves of *Phragmites australis* and *Spartina alterniflora* (Qin et al., 2010). At this study site, only *S. japonica* DNA was detected in the stomach contents of crabs, regardless of the CW and sex, even though several halophytes inhabited the study site (Table 3-2). These results indicated that the dominant crabs (*H. tientsinensis*) are selective feeders in the Siheung Tidal Flat. *Helice tientsinensis* may have used *S. japonica* as its main food source for several reasons. First, *S. japonica* is the dominant plant in the Siheung Tidal Flat and is thus easily accessible. Second, *S. japonica* is shorter than other salt marsh plants, and energy expenditure may be lower for *S. japonica* consumption than for the consumption of other plants. Third, leaves and plant tissues are soft in *S. japonica*, and these properties may be preferable for *H.*

tientsinensis. To improve our understanding of the prey preferences of crabs, food preference experiments or comparisons of food sources at various sites with different vegetation structures should be performed.

The range of $\delta^{13}\text{C}$ values for potential food sources in the Siheung Tidal Flat ranged from -27.99‰ (*S. japonica*) to -14.52‰ (*P. latifolius*) (Fig. 3-2 and Table S3-2). A wide range of $\delta^{13}\text{C}$ values for the food sources can allow high source discrimination in mixed models (Mao et al., 2016). In addition, the $\delta^{13}\text{C}$ values for the consumers were distributed within the $\delta^{13}\text{C}$ range for potential food sources, indicating that the carbon sources are potential food sources for consumers. The $\delta^{13}\text{C}$ values for SOM and polychaetes were intermediate, suggesting that they are major food sources, and were similar to the $\delta^{13}\text{C}$ values for consumers. Additionally, the $\delta^{13}\text{C}$ values for crabs differed significantly according to the CW (Table 3-1). These results show that diet of crabs may vary with respect to the CW. The range of $\delta^{15}\text{N}$ values of potential food sources was 2.36‰ (*P. latifolius*) to 7.15‰ (*C. scabrifolia*) and *C. scabrifolia* showed the most highly $\delta^{15}\text{N}$ -enriched signatures (Fig. 3-2 and Table S3-2). The $\delta^{15}\text{N}$ values for the crabs also varied considerably depending on the CW category (Table 3-1). Among the consumers, the most highly $\delta^{15}\text{N}$ -enriched signatures were those of the G2 group. G2 group crabs probably consumed mostly *S. japonica* or *C. scabrifolia*. In contrast, G3 crabs showed the most $\delta^{15}\text{N}$ -depleted signatures. Based on the similar $\delta^{15}\text{N}$ values, G3 crabs probably consumed substantial BMI, unlike the G2 crabs. A sharp decrease in the $\delta^{15}\text{N}$ value for G3 crabs may be related to the molting period after achieving sexual maturation (Bodin et al., 2007). There was no significant

difference in the isotopic results between sexes, unlike the results for CW (Table 3-1). However, unlike the other groups, there was a difference between $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ isotopes in G2. This may be due to differences in the activity area in the G2 crabs, which may be the most vigorous group (Squires and Dawe, 2003). There may also be a difference due to the timing of pregnancy. Therefore, understanding the life history of crabs can improve the interpretation of ontogenic dietary changes in food source analyses.

These results provided evidence for a mixed diet consisting of polychaetes, SOM, *S. japonica*, and BMI in crabs. Using SIAR, I evaluated the relative contributions of the major food resources (Fig. 3-3). I found that SOM was the most highly represented food source in G1 crabs. This result indicated that relatively immature, small crabs may not eat a wide variety of food and SOM may be readily available and digested (Han et al., 2012). As the CW class increased, the proportion of SOM in the diet gradually decreased and the proportions of other food sources increased. G2 crabs tended to consume a variety of foods. The food sources for G3 were expected to be dominated by BMI. Similar trends were observed in a study of the food sources of an estuarine crab (*Sesarma dehaani*) in Korea, indicating that immature individuals mainly consumed detrital sediments, whereas mature individuals used various carbon sources (Han et al., 2012). The stomach contents and stable isotope analysis showed that the primary salt marsh plant consumed by the crab was *S. japonica*, with a relative contribution of 10–15%. Considering the high density of crabs, grazing may regulate the distribution of *S. japonica* in Siheung Tidal Flat. Previous studies have shown that crabs have a

potential role in determining the plant distribution in wetlands (Costa et al., 2003). An herbivorous crab consumed *S. alterniflora* leaves and damaged up to 75% of the leaves (Alberti et al., 2007), and damaged leaves can also cause biomass loss due to infection by fungi (Daleo and Iribarne, 2009; Freitas et al., 2015). He et al. (2015) recently reported that grazing crabs can limit the distribution of halophytes, together with abiotic stress or competition. These findings suggest that consumer pressures need to be considered in studies of the halophyte distribution.

The PCR amplification success rates for the stomach contents differed according to CW class and sex (Table S3-3). The amplification success rate for G3 samples (CW: 3.0–3.5 cm) was greater than that for G2 samples (CW: 3.0–3.5 cm). These differences may be explained by differences in the food source composition and in the amount of sample. For example, a small individual crab contains a relatively small amount of food compared with that in a large crab. Small sample volumes may be associated with lower DNA contents and lower amplification success rates. Squires and Dawe (2003) showed that female crabs consume highly accessible food sources, and male and female crabs consume different foods.

The ITS gene is an appropriate marker for food source analyses of herbivorous crabs. It contains considerable variation compared to that in other conserved regions (Baldwin et al., 1995). In previous studies, the ITS2 region was used to classify medicinal plants, with an identification success rate of 92.7% at the species level. In this study, the amplification success rate for whole stomach contents was 57.5%. To increase the amplification success rate, it is necessary to

obtain sufficient sample volumes for large individual crabs. In addition, the use of specific blocking primers will selectively prevent DNA amplification from fungal species and increase the accuracy of food source analyses (Lewis et al., 1994; Vestheim and Jarman, 2008).

CHAPTER 4.

Interacting effects of crab burrowing and halophyte growth on soil properties in a Korean salt marsh

* This chapter is under review in journal 'Ecological indicators' in June 2018.

4.1. Introduction

Burrowing animals play important roles in soil ecosystems as consumers, degraders, and habitat disturbers (Wang et al., 2010), exerting a significant influence on organic matter and nutrient dynamics (Hättenschwiler and Gasser, 2005). Crabs are one of the important constituents of salt marsh ecosystems and can have a significant impact on the salt marsh sedimentary environment (Smith et al., 1991). Their burrowing activity is a form of bioturbation (Meysman et al., 2006), which changes the structure of anoxic wetland soils, transports particulate matter and accelerates ecosystem nutrient cycling (Bertness, 1985; Warren and Underwood, 1986). Changes in the physicochemical composition of the soil as a result of crab burrowing can directly or indirectly affect the salt marsh plant community (Iribarne et al., 1997; Botto et al., 2006).

Plants can also have a significant impact on soil chemical processes by changing physical factors in the soil (Vitousek and Walker, 1989; Wedin and Tilman, 1990; Vinton and Burke, 1995). Individual plant species play an important role in determining soil fertility and have a direct positive feedback impact on soil nutrient cycling (Pastor et al., 1984; Berendse et al., 1987; Hobbie, 1992). Litter decomposition rate, which is affected by microenvironmental conditions such as pH, temperature, and other physical factors, drives changes in soil nutrient concentrations (Hector et al., 2000). The plant rhizosphere can also affect soil organic matter decomposition and nutrient dynamics (Hättenschwiler and Gasser, 2005). Also, plant density is affected by levels of disturbance and spatial

heterogeneity in soils (Grace, 1999). For example, annual plants which scatter their seeds may show variation in the dispersal distance from individual parent plants. Variations in seed density according to distance from the parent plants may determine the degree of intraspecific competition for limited resources, and may have a considerable influence on plant growth (Weiner and Thomas, 1986).

In salt marshes, crabs are the dominant macroinvertebrates in terms of abundance (Jones, 1984) and biomass (Golley et al., 1962). Crab burrowing disturbs anoxic soil, affecting its physical structure (Fanjul et al., 2007; Fanjul et al., 2008), nutrient cycling and energy flow (Montague, 1981; Botto et al., 2006), and the structure and productivity of mangrove forests (Smith III, 1988; Smith et al., 1991). To date, most studies of these processes have been carried out in mangrove wetlands. The salt marsh ecosystem is one of the important carbon storage pools (Livesley and Andrusiak, 2012). Halophytes are highly productive and play an important role in the biogeochemistry of salt marsh soils. Although there has been some researches into the structure and ecological characteristics of salt marshes (Ungar, 1998; Varty and Zedler, 2008; Davy et al., 2011), little is known of the interactions between plant communities and burrowing crabs, despite their potential importance to the functioning of salt marsh ecosystems.

The Siheung Tidal Flat is one of the large salt marshes in Korea and has a wide range of halophyte species distributed in relation to the tidal range (Wells et al., 1990). The burrowing crab *Helice tientsinensis* is common in the *Suaeda japonica* community, the dominant halophyte of the Siheung Tidal Flat. Nothing is

known of the role of crabs and halophytes in sedimentary characteristics in Korean salt marshes. Therefore, this study aimed to identify the distribution of the dominant crabs and halophytes on the Siheung Tidal Flat and determine effects of the combination of crabs and halophytes on the physicochemical properties of soils. The specific questions were: (1) What determines the distribution of plant populations and community characteristics? (2) How does the presence of crab and plant affect soil characteristics?

4.2. Material and methods

4.2.1. Study site

The Siheung Tidal Flat, a macrotidal salt marsh, is one of the large tidal flats in Korea. Deep tidal channels are created by the high tidal range (from 4 to 9 m), and salt marsh plants are widely distributed in the intertidal zone (Wells et al., 1990). The government has recognized the ecological importance of this area, and designated as a Coastal Wetland Protected Area since 2012 (area ~0.71 km²). This study was conducted in the upper intertidal zone of the Siheung Tidal Flat (37° 23' 40.0" N, 126° 46' 13.6" E), which has a relatively consistent elevation (8.7–8.8 m) and occupies a large proportion of the study area. The burrowing crab *Helice tientsinensis* Rathbun is mainly distributed in the upper intertidal zone of tidal flats on the west coast of Korea (Marine Arthropod Depository Bank of Korea, 2009). *Suaeda japonica* Makino is the dominant halophyte at the study site (Bang et al., 2018). This annual plant is patchily-

distributed across the study area, with the patches separated by unvegetated mudflats (Fig. 4-1). Flowers of *S. japonica* bloom from July to September, with seeds forming from October to November. Seeds dispersed from the parent plant each year begin to germinate in the spring of the following year. *Suaeda japonica* communities support dense populations of *Helice tientsinensis*, the most abundant burrowing crab species in this salt marsh. Densities are estimated as 8–48 individuals/m². The crab burrows are easily identified in the *S. japonica* community and also occur in areas with little or no vegetation cover. The fiddler crab *Uca arcuata* De Haan is mainly observed in the unvegetated low intertidal zone, which was excluded from the study area.

The study area is located in the mid-latitude temperate climatic zone. The annual precipitation in 2014 was 788.1 mm, with > 56% of the rain falling between July and September. The mean annual temperature in 2014 was 12.8°C (Korea Meteorological Administration, <http://www.kma.go.kr/>). The mean height above sea level is 8.8 m and the area is irregularly submerged (1–12 times per month).

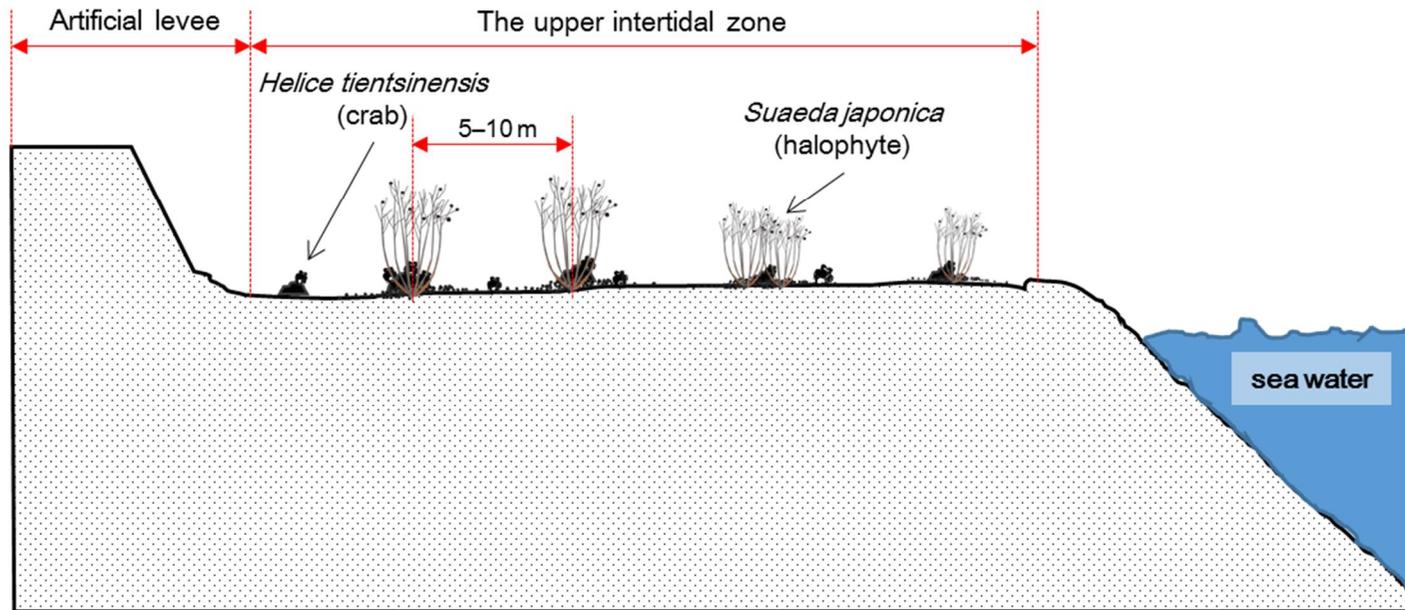


Figure 4-1. Cross-section of study site showing the distribution of the dominant crabs and halophytes.

4.2.2. Field survey

The first field survey investigated the growth of *S. japonica* and soil properties according to the distance of seed dispersal from the parent plants, which are dead plants from the previous year. These dead plants occur in patches with a diameter of ~50 cm. In salt marsh plants of the Family Chenopodiaceae, most seeds disperse < 1 m from the parent plants, but tidal movement can scatter seeds at low density over distances > 1 m (Ellison, 1987). Based on these patterns, three zones were defined in relation to distance from parent plants of *S. japonica*: a Center zone (0 m) at the location of the parent plants; a Near zone, within 1 m radius of them; and a Remote zone covering the area > 1 m (range 2 – 5 m) distant from the parent plants. Ten replicate permanent plots (0.5 × 0.5 m) were randomly established in each zone (Center, Near, Remote). The vegetation survey was conducted every 2 months from April to October 2014. In each plot, the number of stems and plant height were measured and above-ground dry weight biomass was measured in October 2014. Soil samples were taken during the lowest tidal periods of October 2014 to eliminate the effects of tides and precipitation on each plot. Soil samples were taken from three random subsamples at 5 cm depth in each plot. The three subsamples representing each plot were mixed thoroughly (~300 ml volume per sample) and stored at 4°C in a sealed plastic bag until soil analysis was performed. The second field survey investigated the differences in soil properties according to the habitat types distinguished by the combination of burrowing crabs and salt marsh plants.

To investigate the second field survey, I identified the distribution pattern of the dominant crabs and halophytes in the study area. Recent studies have shown that burrowing crabs preferred well-vegetated habitats to reduce hazards from drying and predators (He and Cui, 2015; Chen et al., 2016). At the study site, the burrow density was higher in the vegetated habitats than in bare ground. Also, the burrow density was higher at high plant coverage than at low plant coverage (Fig S4-1). To measure their relative effects, four habitat types were defined in the study area according to the densities of burrowing crabs (BC) and plants (P) (Table 4-1): the ‘Control’ habitat consisted of a mudflat lacking both crab burrows and plants; the ‘Low BC’ habitat lacked vegetation and had soil disturbed by a low density of burrowing crabs; the ‘Low BC+P’ habitat had a low density of burrowing crabs (degree of soil disturbance was similar to Low BC) and a sparse cover of *S. japonica* (1–2 individuals); the ‘High BC+P’ habitat had higher densities of both crabs and *S. japonica*. Each habitat type was randomly distributed throughout the salt marsh. Ten replicate plots (0.5 × 0.5 m) were randomly established in each habitat (Control, Low BC, Low BC+P, High BC+P). To avoid spatial autocorrelation (Schlesinger et al., 1996), each plot was installed > 5 m away from any neighboring plots. A vegetation survey and soil sampling was conducted in each plot in October 2014. The vegetation survey measured plant density, height, and coverage. Percentage cover was recorded to the nearest 5%. Soil samples were collected simultaneously in the same manner as in the first field survey. To estimate burrowing crab density in the study area, numbers of burrows in each treatment plot were counted in October 2014.

Table 4-1. Habitat types defined according to crab and plant abundance on Siheung Tidal Flat.

Habitat type	No. burrows (no. 0.25 m ⁻²)	No. plants (no. 0.25 m ⁻²)	Plant height (cm)	Plant coverage (%)	Material composition
Control					Mudflat
Low BC	2–3				Mudflat, crab
Low BC+P	2–4	1–2	52–59	15–35	crab, plant
High BC+P	8–12	4–9	59–73	70–90	crabs, plants

† Low BC: low density of crab burrows; Low BC+P: low density of both crab burrows (Low BC) and plants (P); High BC+P: higher densities of both crab burrows (High BC) and plants (P).

4.2.3. Soil analysis

Soil water content (WC) was measured by weight loss after drying at 105°C for 48 h. Soil organic matter (OM) content was determined by loss-on-ignition (combustion at 550°C for 4 h) (John, 2004). To measure soil pH and electrical conductivity (EC), the soil samples were dried naturally in the shade for 2 weeks and then the soil was passed through a 2 mm sieve; next, the soil was mixed in distilled water (1:5) for 1 h, and pH and EC were measured using a multiparameter bench meter (PC2700, Eutech, Singapore). Total soil carbon (C) and total soil nitrogen (N) content was measured using an elemental analyzer (Flash EA 1112, Thermo Electron Corporation, USA). Available phosphorus (AP) and cations (Na^+ , Mg^{2+} , K^+ , Ca^{2+} , Fe^{2+}) were extracted by the Mehlich-3 extraction method (Ziadi and Tran, 2007). The ferric ion in the soil is one of the variables indicating the redox potential state (Takai and Kamura, 1966). This ion can exist in the form of oxidized iron in a marshy soil layer with reduced oxygen (Kristensen, 2000). AP was measured by colorimetry (Ziadi and Tran, 2007) and cations measured using an Inductively Coupled Plasma Emission Spectrometer (ICP-730ES, Varian, Australia).

4.2.4. Data analysis

All data were analyzed using R software version 3.3.3. (R Core Team, 2017). Nonparametric statistics were used because the variables were not normally-distributed and the number of samples was small. Kruskal-Wallis tests were used to

compare plant growth or soil properties in relation to distance from the parent plants. Kruskal-Wallis tests were also used to compare soil properties in the different habitat types defined by crab burrow density and vegetation cover. Multiple comparison tests were performed using the *kruskalmc* function in the ‘pgirmess’ package within the R program if there were significant differences in the Kruskal-Wallis test ($P < 0.05$). For Cluster Analysis using Ward’s method, the sampling plots defined in relation to distance from parent plants were classified into two groups, and the sampling plots in different habitat types were classified into four groups using the *hclust* function within the R program. Principal Component Analysis was used to investigate the relationship between soil properties and sampling plots (seed dispersal distances, habitat types). PCA was performed using a correlation matrix of 12 soil variables, with the variables standardized before analysis to compensate for different scales.

4.3. Results

4.3.1. Vegetation survey according to distance from parent plants

The number of *S. japonica* stems decreased over time, with the pattern of change varying with distance from the parent plants (Fig. 4-2a). Stem density decreased most rapidly in the Center zone from April to June. After June, the rate of decline was very similar in the Center and Near zones. At each time point, the Remote zone had the lowest number of *S. japonica* stems. The mean density of stems was significantly different in October ($\chi^2 = 25.39$, d.f. = 2, $P < 0.001$), with

final values of 34.0 ± 1.05 stems 0.25 m^{-2} (Near zone), 24.0 ± 1.34 stems 0.25 m^{-2} (Center zone), and 5.4 ± 0.62 stems 0.25 m^{-2} (Remote zone).

The height of the *S. japonica* plants was inversely related to distance from the parent plants, with highest values in the Remote zone (Fig. 4-2b). The effect of distance on plant height was greatest in October ($\chi^2 = 19.36$, d.f. = 2, $P < 0.001$). Mean height was very similar in the Near (51.06 ± 1.85 cm) and Center zones (52.08 ± 0.86 cm), but was significantly greater ($P < 0.05$) in the Remote zone (64.38 ± 1.33 cm).

Above-ground biomass also differed significantly ($\chi^2 = 19.73$, d.f. = 2, $P < 0.001$) with distance from parent plants (Fig. 4-2c). Biomass was nearly three times greater ($P < 0.05$) in the Remote zone (219.27 ± 14.99 g 0.25 m^{-2}) than in the Near (69.43 ± 6.88 g 0.25 m^{-2}) and Center zones (72.86 ± 4.05 g 0.25 m^{-2}).

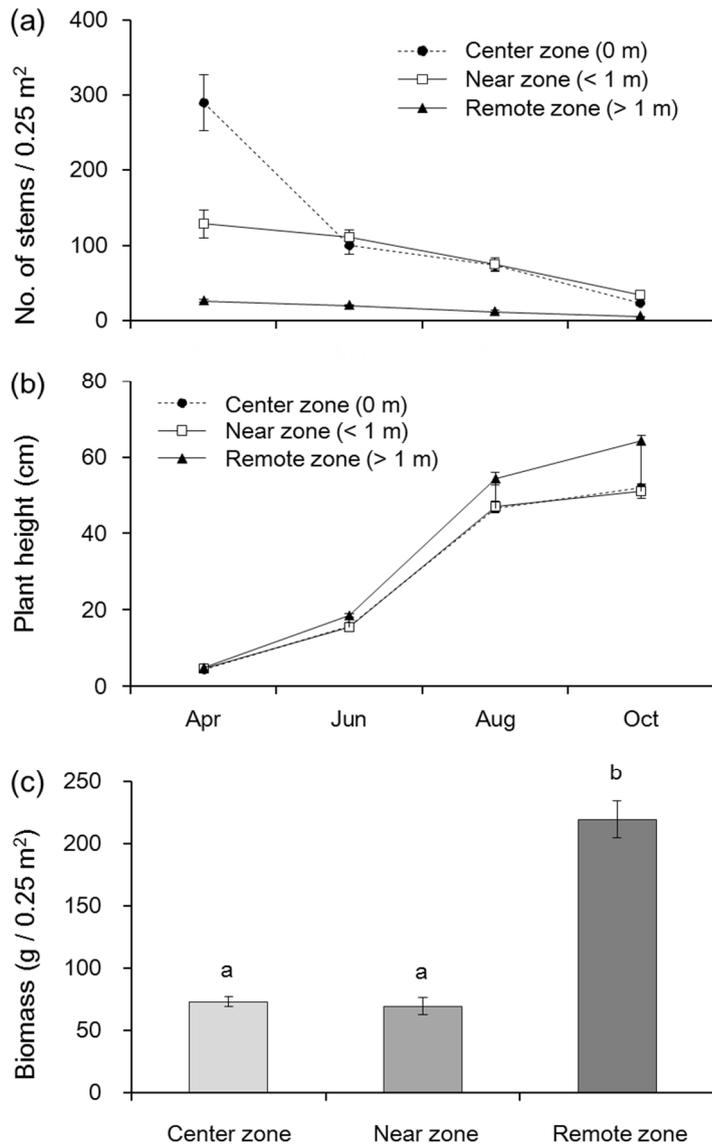


Figure 4-2. (a) Number of stems, (b) plant height, and (c) above-ground biomass of *Suaeda japonica* in three zones defined with respect to distance from parent plants. Different lower-case letters above the bars in (c) indicate significant ($P < 0.05$) differences between zones in multiple comparisons following the Kruskal-Wallis test. Each error bar represents the standard error ($n = 10$).

4.3.2. Soil properties according to distance from parent plants

Physicochemical properties of the soil varied with respect to distance from the parent plants. There was little difference between the Center and Near zones, but several soil parameters in Remote zone were significantly different: WC ($\chi^2 = 15.35$, d.f. = 2, $P < 0.001$), OM ($\chi^2 = 15.58$, d.f. = 2, $P < 0.001$), C ($\chi^2 = 14.00$, d.f. = 2, $P < 0.001$), AP ($\chi^2 = 15.80$, d.f. = 2, $P < 0.001$), and K^+ ($\chi^2 = 9.01$, d.f. = 2, $P < 0.05$) (Table 4-2). Specifically, the Remote zone showed significantly higher values for WC, OM, C and K^+ and significantly lower AP than the Center and Near zones ($P < 0.05$).

Two groups of plots were identified by CA according to distance from parent plants (Fig. S4-2). Group A contained plots from the Center and Near zones, whereas Group B consisted mainly of Remote zone plots plus two plots from the Center zone. Principal Component Analysis indicated two groups of soil properties according to the distance from the parent plants (Fig. 4-3). On the left side of the graph, the plots of the Center zone and the Near zone overlap; most of the plots in the Remote zone are towards the right side of the graph. The first component (PC1) explained 50.66% of the data variance (Table 4-3). PC1 loaded positively from OM, pH, C, N, K^+ , and Ca, and negatively from AP. The second component (PC2) explained 21.53% of the data variance. PC2 loaded negatively from EC, Na^+ , and Mg^{2+} , and positively from WC, pH, C, and N.

Table 4-2. Mean (\pm SE) values for soil properties in three zones defined with respect to distance from parent plants of *Suaeda japonica*.

Soil properties	Center zone (Mean \pm SE)	Near zone (Mean \pm SE)	Remote zone (Mean \pm SE)
Water Content (%)	30.9 \pm 0.38 ^a	31.6 \pm 0.41 ^a	33.8 \pm 0.52 ^b
Organic Matter (%)	3.32 \pm 0.07 ^a	3.41 \pm 0.10 ^a	3.97 \pm 0.12 ^b
pH	5.96 \pm 0.01 ^a	6.03 \pm 0.02 ^{ab}	6.09 \pm 0.03 ^b
Electric Conductivity (mS cm ⁻¹)	10.38 \pm 0.51	10.96 \pm 0.53	10.73 \pm 0.44
Carbon (%)	29.21 \pm 2.80 ^a	25.03 \pm 1.34 ^a	38.08 \pm 3.07 ^b
Nitrogen (%)	9.82 \pm 0.18	9.83 \pm 0.17	10.92 \pm 0.42
Available Phosphorus (mg kg ⁻¹)	20.71 \pm 0.91 ^b	19.44 \pm 1.23 ^b	11.42 \pm 1.40 ^a
K ⁺ (mg g ⁻¹)	0.65 \pm 0.03 ^a	0.65 \pm 0.02 ^a	0.79 \pm 0.04 ^b
Ca ²⁺ (mg g ⁻¹)	0.43 \pm 0.03 ^{ab}	0.39 \pm 0.01 ^a	0.50 \pm 0.02 ^b
Na ⁺ (mg g ⁻¹)	8.13 \pm 0.54	8.48 \pm 0.46	8.03 \pm 0.37
Mg ²⁺ (mg g ⁻¹)	1.85 \pm 0.12	2.08 \pm 0.11	2.12 \pm 0.12
Fe ²⁺ (mg g ⁻¹)	0.24 \pm 0.01 ^b	0.20 \pm 0.01 ^a	0.25 \pm 0.03 ^{ab}

† Different superscript letters indicate significant differences between the means based on multiple comparisons following the Kruskal-Wallis test ($P < 0.05$).

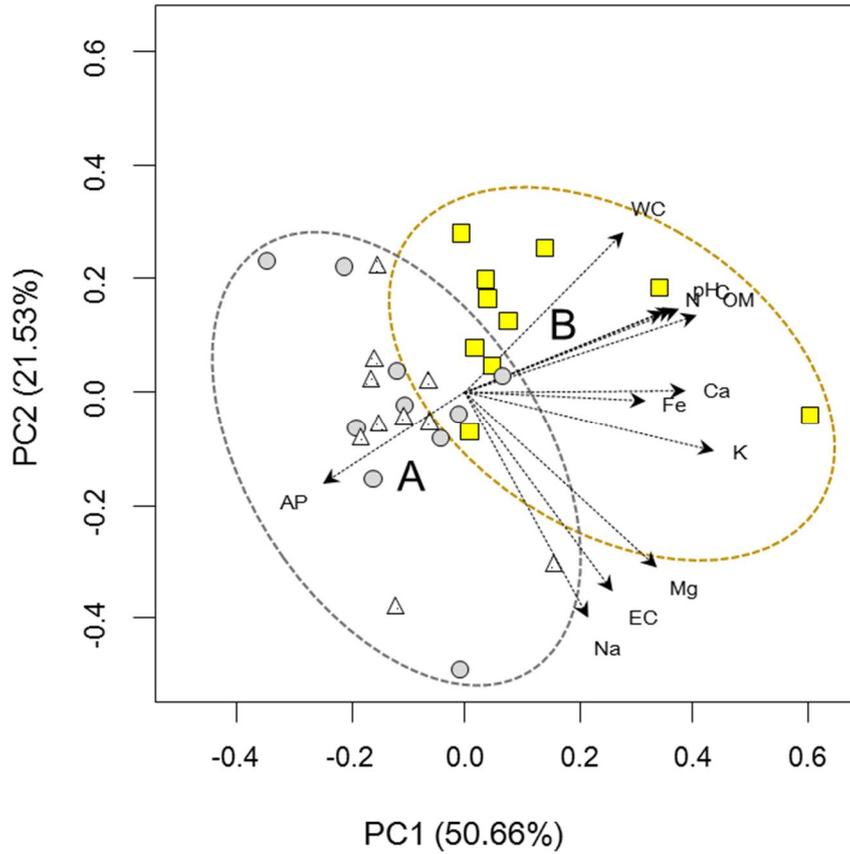


Figure 4-3. Principal Component Analysis of soil properties for seed dispersion distances in the sampling plots on Siheung Tidal Flat. Seed dispersion distances: A, Center (●) and Near (△), B, Remote (■); WC, water content; OM, organic matter; EC, electrical conductivity; AP, available phosphorus.

Table 4-3. PCA loadings and explained variance for soil properties between seed dispersion distances on Siheung Tidal Flat.

Soil factors	PC1	PC2	PC3	PC4	PC5	PC6
Water Content (%)	0.238	0.370	0.310	-0.191	0.361	-0.304
Organic Matter (%)	0.348	0.178	0.052	-0.200	0.149	0.271
pH	0.311	0.191	0.297	0.143	0.406	-0.153
Electric Conductivity (mS cm ⁻¹)	0.221	-0.465	0.142	-0.145	0.150	0.069
Carbon (%)	0.322	0.190	-0.489	0.106	-0.078	-0.105
Nitrogen (%)	0.299	0.185	-0.428	-0.395	0.038	0.375
Available Phosphorus (mg kg ⁻¹)	-0.214	-0.215	-0.509	0.195	0.741	-0.066
K ⁺ (mg g ⁻¹)	0.183	-0.524	0.004	-0.161	-0.016	-0.095
Ca ²⁺ (mg g ⁻¹)	0.287	-0.409	0.146	-0.077	0.075	-0.103
Na ⁺ (mg g ⁻¹)	0.372	-0.139	-0.104	-0.045	-0.162	0.117
Mg ²⁺ (mg g ⁻¹)	0.331	0.005	-0.245	0.277	-0.265	-0.627
Fe ²⁺ (mg g ⁻¹)	0.272	-0.023	0.139	0.752	0.014	0.473
% of Variance	50.660	21.530	6.578	6.252	5.442	3.293
Cumulative %	50.660	72.190	78.766	85.018	90.460	93.753

4.3.3. Soil properties by habitat type

Physicochemical parameters of the soil also varied in relation to crab burrow density and the structure of the *S. japonica* community (plant density, height, and coverage), as shown by significant differences among habitat types (d.f. = 3, $P < 0.001$) (Table 4-4). The presence of burrowing crabs (Low BC) was associated with significantly higher values for OM, EC, Ca^{2+} , Na^+ and Mg^{2+} , and significantly lower pH and Fe^{2+} compared to the Control habitat ($P < 0.05$). Soil WC, pH, C and N tended to be higher in the presence of *S. japonica* (Low BC+P, High BC+P), whereas EC, AP, K^+ , Ca^{2+} , Na^+ , and Mg^{2+} tended to decrease with increasing plant density compared to the Low BC habitat. Values of WC, OM, C and N were significantly higher, and AP and Fe^{2+} concentrations significantly lower, in the High BC+P habitat compared to the Control habitat ($P < 0.05$).

The Cluster Analysis by habitat identified four groups of plots (Fig. S4-3). Group A represented the mudflat with no crab burrows or vegetation (Control habitat). Group B consisted mostly of plots from the unvegetated burrowing crab habitat (Low BC) but also included some plots from the Low BC+P habitat. Group C contained most of the Low BC+P habitat plots (low crab burrow density, low density vegetation). Group D represented the High BC+P habitat, defined by the highest densities of crab burrows and vegetation. In addition, PCA revealed a clear separation between the soil properties of the four habitats (Fig. 4-4). The first component (PC1) explained 43.06% of the data variance (Table 4-5). Groups B and D were clearly separated along the PC1 axis, but there was some overlap between

sampling plots in Groups B and C. PC1 loaded negatively from EC, AP, Na⁺, Mg²⁺, and K⁺, and positively from WC, pH, C, and N. The second component (PC2) explained 33.28% of the data variance. The Control habitat (Group A) was clearly separated from the other three groups by PC2. PC2 loaded negatively from WC, OM, C, N, and K⁺, and positively from Fe²⁺ and AP.

Table 4-4. Mean (\pm SE) values for soil properties in four defined habitat types on Siheung Tidal Flat.

Soil Properties	Control (Mean \pm SE)	Low BC (Mean \pm SE)	Low BC+P (Mean \pm SE)	High BC+P (Mean \pm SE)
Water Content (%)	27.58 \pm 0.35 ^a	30.38 \pm 0.56 ^{ab}	31.59 \pm 0.63 ^{bc}	33.8 \pm 0.52 ^c
Organic Matter (%)	3.03 \pm 0.08 ^a	3.81 \pm 0.09 ^b	4.06 \pm 0.12 ^b	3.97 \pm 0.12 ^b
pH	6.04 \pm 0.01 ^{bc}	5.94 \pm 0.02 ^a	5.97 \pm 0.02 ^{ab}	6.09 \pm 0.03 ^c
Electric Conductivity (mS cm ⁻¹)	12.5 \pm 0.24 ^{ab}	17.3 \pm 0.40 ^c	15.0 \pm 0.59 ^{bc}	10.7 \pm 0.44 ^a
Carbon (%)	14.06 \pm 0.64 ^a	22.34 \pm 0.49 ^{ab}	28.93 \pm 1.26 ^{bc}	38.08 \pm 3.07 ^c
Nitrogen (%)	8.83 \pm 0.11 ^a	9.22 \pm 0.11 ^{ab}	10.1 \pm 0.18 ^{bc}	10.92 \pm 0.42 ^c
Available Phosphorus (mg kg ⁻¹)	28.1 \pm 1.82 ^{bc}	32.9 \pm 1.73 ^c	19.9 \pm 2.42 ^{ab}	11.4 \pm 1.40 ^a
K ⁺ (mg g ⁻¹)	0.85 \pm 0.02 ^{ab}	1.14 \pm 0.03 ^{bc}	1.03 \pm 0.04 ^b	0.79 \pm 0.04 ^a
Ca ²⁺ (mg g ⁻¹)	0.32 \pm 0.03 ^a	0.71 \pm 0.02 ^c	0.68 \pm 0.02 ^{bc}	0.50 \pm 0.02 ^{ab}
Na ⁺ (mg g ⁻¹)	9.54 \pm 0.38 ^{ab}	12.69 \pm 0.32 ^c	10.81 \pm 0.64 ^{bc}	8.03 \pm 0.37 ^a
Mg ²⁺ (mg g ⁻¹)	2.58 \pm 0.10 ^{ab}	3.74 \pm 0.15 ^c	3.07 \pm 0.20 ^{bc}	2.12 \pm 0.12 ^a
Fe ²⁺ (mg g ⁻¹)	0.54 \pm 0.05 ^b	0.33 \pm 0.01 ^a	0.32 \pm 0.02 ^a	0.25 \pm 0.03 ^a

† Different superscript letters indicate significant differences between the means based on multiple comparisons following the Kruskal-Wallis test ($P < 0.05$).

†† Low BC: low density of crab burrows; Low BC+P: low density of both crab burrows (Low BC) and plants (P); High BC+P: higher densities of both crab burrows (High BC) and plants (P).

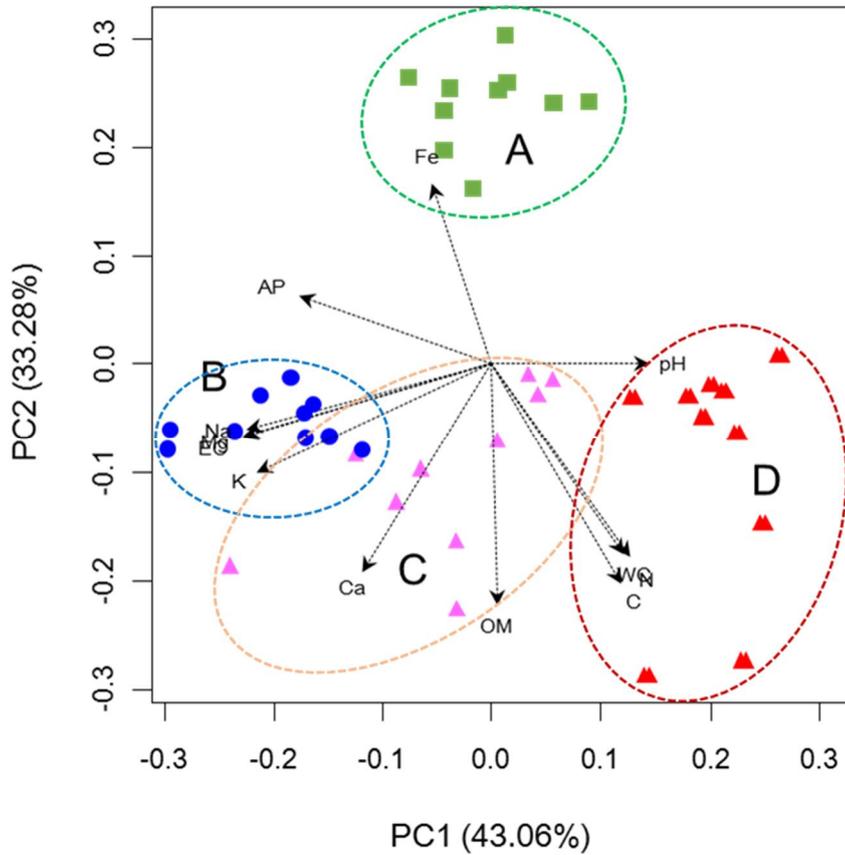


Figure 4-4. Principal Component Analysis of soil properties for habitat types in the sampling plots on Siheung Tidal Flat. Habitat types: A, Control (■); B, Low BC (●); C, Low BC+P (▲); D, High BC+P (▲). WC, water content; OM, organic matter; EC, electrical conductivity; AP, available phosphorus.

Table 4-5. PCA loadings and explained variance for soil properties between habitat types on Siheung Tidal Flat.

Soil factors	PC1	PC2	PC3	PC4	PC5	PC6
Water Content (%)	0.213	-0.357	-0.222	0.190	-0.372	0.501
Organic Matter (%)	0.008	-0.450	0.069	0.105	-0.278	-0.518
pH	0.256	0.000	0.706	-0.140	0.231	0.442
Electric Conductivity (mS cm ⁻¹)	-0.410	-0.138	0.039	-0.135	-0.103	0.102
Carbon (%)	0.209	-0.408	0.200	0.093	0.084	-0.040
Nitrogen (%)	0.225	-0.360	0.215	0.287	0.300	-0.269
Available Phosphorus (mg kg ⁻¹)	-0.316	0.129	-0.057	0.808	0.343	0.161
K ⁺ (mg g ⁻¹)	-0.403	-0.123	0.104	-0.191	0.232	-0.007
Ca ²⁺ (mg g ⁻¹)	-0.406	-0.133	0.175	-0.171	-0.047	0.051
Na ⁺ (mg g ⁻¹)	-0.382	-0.203	0.205	-0.024	0.069	-0.095
Mg ²⁺ (mg g ⁻¹)	-0.212	-0.387	-0.070	0.066	-0.200	0.389
Fe ²⁺ (mg g ⁻¹)	-0.097	0.338	0.517	0.317	-0.636	-0.108
% of Variance	43.060	33.280	9.148	4.529	2.725	2.586
Cumulative %	43.060	76.340	85.489	90.017	92.743	95.329

4.4. Discussion

This study provides new insights into how the dominant halophyte and crab species affect the biogeochemical properties of soil in a Korean macrotidal salt marsh. In contrast to previous studies (Botto et al., 2005; Escapa et al., 2008), I was able to compare the relative effects of plants and crabs on soil characteristics. Data on these biological factors are essential for understanding nutrient cycling and energy flow in salt marsh ecosystems.

Soil nutrient concentrations in salt marshes is influenced by various biological factors (Vernberg, 1993), including the growth of wetland plants. The growth characteristics (height and biomass) of *S. japonica* in the study area were significantly affected by the stem density (Fig. 4-2). The stem density of the early *S. japonica* increased with decreasing distance from the parent plants. These results are consistent with those of Ellison (1987), and suggest quite limited seed dispersal in this species, with most seeds spread within 1 m from the parent plants. Tidal movements can transport seeds of *S. japonica* further from the parent plants (Huiskies et al., 1995; Min, 2005), but most seeds settle within 1 m (our Center and Near zones), and only a small number would have reached our Remote zone by tidal movements. Seeds of *S. japonica* may also be moved by rain. The extent of seed transport by these mechanisms will probably determine the seedling density of *S. japonica* throughout the salt marsh. In this study area, over time, the density of *S. japonica* stems decreased sharply in the Center and Near zones, so that at the end of the growing season stem density and height were similar in the two zones. This

is probably due to increased intraspecific competition for limited resources and adjustment of stem densities by self-thinning (Yu et al., 2014). On the other hand, growth (biomass and height) of *S. japonica* was considerably higher in the Remote zone where density was relatively low. These individual plants experienced lower levels of intraspecific competition, and seem to have used more soil nutrients for growth than those in the Center and Near zones (Ellison, 1987; Cabaço et al., 2013).

In addition to significant differences in plant height and biomass with the change in stem density of *S. japonica*, the plant density was also associated with significant changes in soil properties (Table 4-2). In the CA and PCA results, plots from the Center and the Near zones (within 1 m from parent plants) formed a group showing similar soil properties. Remote zone plots were mostly separate from this first group but with some overlap (Fig. 4-3 and Fig. S4-2). Halophytes in salt marshes play an important role in soil nutrient cycling (Sollie and Verhoeven, 2008). They can increase the concentrations of carbon and nitrogen in salt marsh soil (Sousa et al., 2010). Halophytes also play a role in maintaining and accumulating P in biomass (Sekiranda and Kiwanuka, 1997). In this study area, soil properties in the Remote zone were significantly different from those in the Center and Near zones. The higher WC in Remote zone plots may be a function of higher plant biomass, which reduces soil water evaporation (Pennings and Bertness, 1999). The highest contents of OM, C, N, K⁺, Ca²⁺, and Mg²⁺ were found in the high-biomass Remote zone, whereas AP content was lowest in this zone. This pattern suggests that differences in the growth of the halophyte depending on distance from the parent plants can affect soil organic content and therefore the C,

N, P, and cation concentrations of the soil.

Suaeda japonica and the burrowing crab *Helice tientsinensis* are the dominant species in the study area and occupy similar habitats. In this salt marsh, the relationship between the habitat type and soil characteristics is shown clearly by the results of CA and PCA (Fig. 4-4 and Fig. S4-3). In particular, there were significant differences in soil characteristics depending on habitat type (Table 4-4). The Control habitat without vegetation or burrowing crabs (Group A, Control) was clearly differentiated from the other habitats. Tidal flat soils are strongly influenced by oxygen supply and the rate of OM decomposition, with a clear vertical zonation according to redox conditions. A near-surface 'oxic' zone is determined by the depth of oxygen penetration into the sediment; below this, a 'suboxic' zone contains nitrate, oxidized manganese and iron; the deepest 'reduced' zone is dominated by sulfate (Kristensen, 2000). In the study area, the Control habitat, consisting of bare ground without bioturbation, showed relatively low values of WC, OM, C, and N, while values for Fe^{2+} were relatively high compared to other habitats.

The habitat with soil disturbed by burrowing crabs (Group B, Low BC) was clearly separated from other habitats. Gutiérrez et al. (2006) reported that soil disturbed by crabs accounts for 23% to 58% of salt marsh surface area. This pattern results from the mixing of surface and deep soil by the burrowing crabs (McCraith et al., 2003), an activity that changes the microtopography of the soil surface by forming mounds (Warren and Underwood, 1986). By allowing oxygen to penetrate

anoxic soils and increasing the transport of particulate matter, crab burrowing modifies the physicochemical properties and redox potential of soil (Fanjul et al., 2007). In this study area, WC, OM, C, and N were higher in the Low BC habitat compared to the Control habitat. This contrast may be a result of burrowing by the crabs. Crab burrowing activity can increase the surface area and create more interstitial spaces in which moisture is retained (Koo et al., 2007). The feeding activity of crabs can increase organic matter, C, and N contents (Botto et al., 2006; Gutiérrez et al., 2006). Burrowing activity accelerates the mineralization of decomposing OM (Otani et al., 2010) and increases release of inorganic nutrients in forms available as nutrients for plants (Takeda and Kurihara, 1987; Mayer et al., 1995; Fanjul et al., 2007). In this study area, AP, K⁺, Ca²⁺, Na⁺, and Mg²⁺ tended to be higher in areas excavated by crabs, but values of Fe²⁺ were lower than in the Control habitat. This may be the result of the excavation of the crabs changing the vertically compartmentalized anaerobic soil layer to aerobic soil (Kristensen, 2000).

Plots in Group C (Low BC+P) were similar to Group B (Low BC) in degree of crab disturbance, but differed in supporting a low density (1–2 individuals) of plants. These two groups therefore allowed us to measure the effects of low density vegetation on soil characteristics in crab-disturbed soil. In Principal Component Analysis, Groups B and C were separated, but with some overlap. In particular, the Low BC+P habitat showed higher values for WC, OM, C, and N content than the Low BC habitat. These results suggest that the emergence of plants in crab-disturbed soil may promote water retention by increasing the surface area of salt marsh soil (Botto and Iribarne, 2000; Li et al., 2017). Shading by plants may affect

evapotranspiration in strong sunlight and the relatively high water content would then reduce the EC value (Pennings and Bertness, 1999). In addition, increased exudates from leaves and roots may have contributed to increased OM, C, and N content (Davis and Van der Valk, 1983; Neira et al., 2006). In this study area, cations (Na^+ , Mg^{2+} , K^+ , and Ca^{2+}) and AP concentrations were highest in soil disturbed by crabs (Low BC), but decreased with the emergence of plants. This pattern suggests that these nutrients may be used for plant growth.

Group D (High BC+P) is a habitat with a high density of both burrowing crabs and plants. In this study area, crab burrows tended to be more numerous where vegetation was denser than in the Low BC+P habitat (Table 4-1). These results indicate that crabs may prefer a relatively well-vegetated habitat. Recent studies have shown that salt marsh vegetation may be a favorable habitat for crabs because it provides shelter from both dessication and from predators, and the plants themselves can be used as a food source (He and Cui, 2015; Chen et al., 2016). Principal Component Analysis showed that Group D was clearly distinguished from Group A (without soil disturbance) and also from Groups B and C (with low soil disturbance by crab or crab + plant). These results indicate that the combination of crab burrowing and the growth pattern of *S. japonica* can have a significant impact on soil properties in the study area. In particular, Groups C and D showed similar patterns, but the highest values for WC, C, and N content were found in Group D (High BC+P). High densities of crab burrows and plants increased salt marsh soil disturbance. Consequently, water content would have increased due to plant shading and increased soil surface area (Botto and Iribarne,

2000; Li et al., 2017). In addition, high plant productivity (Montemayor et al., 2014), burrowing activity, and feeding behavior (Martinetto et al., 2016) may have contributed to the high C and N contents. On the other hand, cations and AP concentrations were lowest in the High BC+P habitat. This is probably due to the uptake of nutrients by the high density of growing vegetation.

The plant density is determined by the distance from the parent plants. The plant density is controlled by self-thinning in the center and near zones. Plant growth was affected by the plant density, and the biomass was low in these zones. In the remote zone, the plant density was relatively low, and the biomass was high. In addition, crab bioturbation is influenced by plant coverage. Plant coverage was relatively high in the center, near and remote zones (similar coverage). Crabs affect the soil nutrients through active bioturbation at high plant coverages and thereby facilitate plant growth. The intensity of bioturbation was relatively low in areas with a low plant coverage (Low BC+P) or no vegetation (Control, Low BC).

CHAPTER 5.

General discussion and conclusions

This thesis showed that halophytes, crabs and the soil are involved in complex interactions that have both positive and negative effects (Fig. 5-1). In the relationship between halophytes and crabs, halophytes can be a favorable habitat for crabs because the risk of predation is low in a well-vegetated habitat, and the halophytes can be used as a food source. However, crabs can affect the growth of halophytes negatively by their predation on plant leaves and can regulate plant density. Bioturbation by crabs can affect soil aeration and provide available nutrients for halophytes in the disturbed soil.

In the relationship between halophytes and the soil, the halophytes can create aerobic conditions around the rhizosphere soil and can change the status of the soil nutrients. The soil can be a habitat for halophytes, and it provides water and nutrients. Each halophyte species has positive or negative effects on their distribution according to flooding and salinity stress.

In the relationship between crabs and the soil, the burrowing activity of the crabs can affect soil aeration and can change the status of the soil nutrients. However, the soil can be an unfavorable habitat for crabs because this habitat can be desiccated easily, and the risk of predation is high. In addition, the soil can be used as a food source, e.g., soil organic matter.

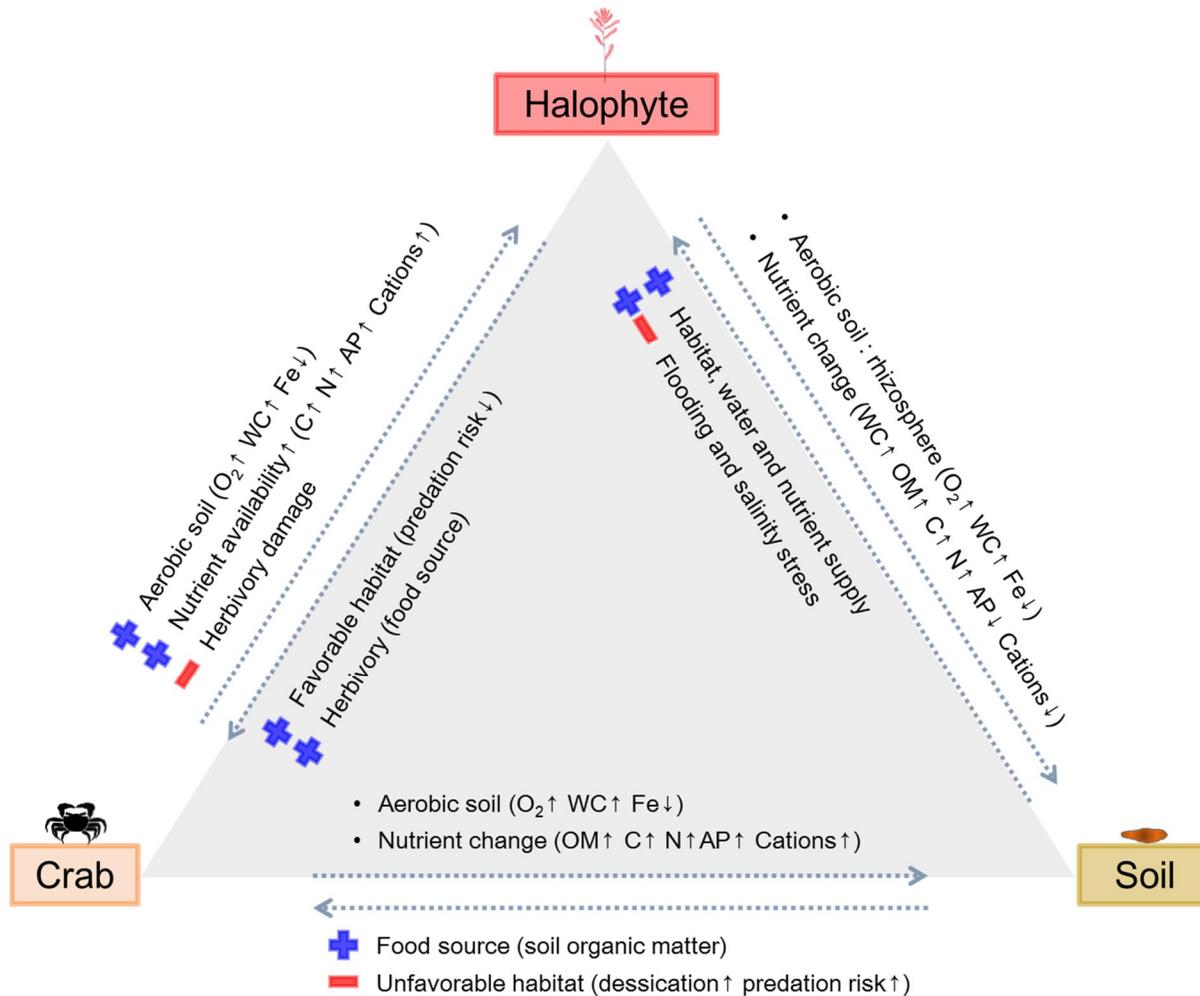


Figure 5-1. Interactions among plant-animal-soil.

In conclusion, surface elevation is the main driver of halophyte distribution at a high plant density. First, I found that each halophyte species showed a distinct distribution range in relation to elevation gradients (Chapter 2). *Suaeda japonica* was characteristic of regions with low elevation that experienced frequent flooding. *Carex scabrifolia* was distributed at mid-high elevation. The limited ability of *P. latifolius* and *Zoysia sinica* to endure inundation resulted in its landward distribution. The study site may be used as a reference wetland for restoration of macrotidal salt marshes. The quantitative field data provide valuable basic information for species selection and planting locations, which may promote plant establishment and improve survival rate in restored salt marshes.

Secondly, the dominant crab species (*Helice tientsinensis*) can be a selective feeder (Chapter 3). The crab fed on *S. japonica* among the halophytes of the study area, and the diet changed according to the size of the individual. Considering the high densities of crabs in salt marshes, I suggest that crab herbivory can affect the distribution of *S. japonica* in these environments.

Thirdly, burrowing crabs and plant density are the main drivers of halophyte distribution at a low plant density (Chapter 4). Plant density or distribution was dependent on the distance from the parent plants. Plant growth was affected by the plant density. Crabs preferred a habitat with high plant cover. The combined effects of plants and crabs caused considerable changes in soil properties. I suggest that the combination of crabs and plants and the plant density can affect the distribution of halophytes.

Future studies

For future studies, I suggest that understanding the mechanisms underlying the distribution of halophytes requires intensive field sampling, supplemented by transplant experiments of species along elevational gradients. Controlled experiments could improve our understanding of halophyte environmental tolerances and interspecific competition. In addition, a long-term monitoring study of halophyte population dynamics would add to our knowledge of temporal and spatial changes in their distribution.

Studying the primary productivity of the plant, the secondary productivity of the crab, and the relationship with consumers at higher trophic levels would increase understanding of the food web in macrotidal salt marshes. This study makes it possible to understand energy flow through the salt marsh food web.

Data on crab distribution, excavation, and feeding behavior illustrate the interaction between plants and crabs. The relationship between hibernation and plant germination is still unclear.

Experiments on crab bioturbation and soil microbial processes could also increase our understanding of nutrient cycling and biogeochemistry of salt marshes.

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APPENDIX

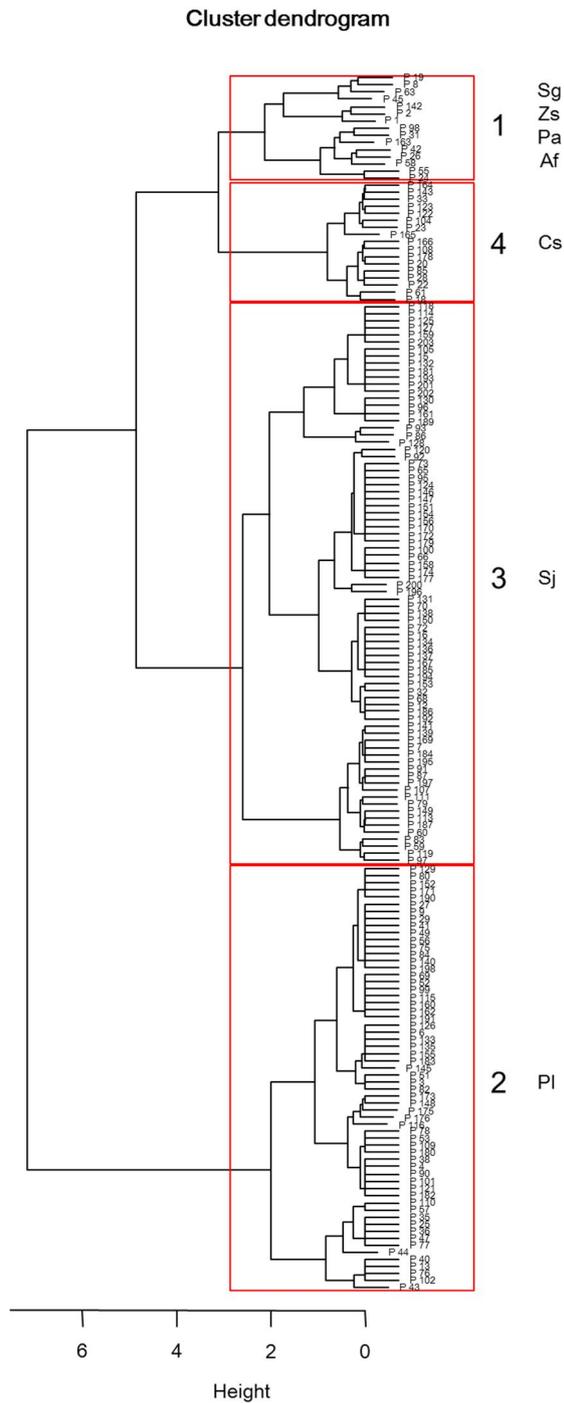


Figure S2-1. Dendrogram resulting from cluster analysis of the distribution of salt marsh plants at Siheung Tidal Flat (Pl: *P. latifolius*, Sj: *S. japonica*, Cs: *C. scabrifolia*, Sg: *S. glauca*, Zs: *Z. sinica*, Pa: *P. australis*, Af: *A. fukudo*).

Table S3-1. Size class and stable isotope signatures ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, mean \pm SD, n = 5) for consumer crabs in the Siheung Tidal Flat.

Class	CW range (cm)	Sex	$\delta^{13}\text{C}$ (‰)	$\delta^{15}\text{N}$ (‰)
G1	2.0– 2.5	F	-22.43 ± 0.80	5.54 ± 0.79
G2	2.5– 3.0	F	-21.46 ± 1.23	5.98 ± 1.94
G3	3.0– 3.5	F	-20.55 ± 0.80	3.99 ± 0.66
G1	2.0– 2.5	M	-22.97 ± 1.22	5.20 ± 0.50
G2	2.5– 3.0	M	-22.45 ± 1.16	7.12 ± 0.62
G3	3.0– 3.5	M	-20.59 ± 0.51	4.00 ± 1.01

Table S3-2. Stable isotope signatures ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, mean \pm SD, n = 5) for food sources in the Siheung Tidal Flat.

Food sources	$\delta^{13}\text{C}$ (‰)	$\delta^{15}\text{N}$ (‰)
Polychaetes (Animal)	-20.88 ± 0.31	5.87 ± 0.48
Soil organic matter (SOM)	-24.54 ± 0.35	4.03 ± 1.08
<i>Suaeda japonica</i> (Plant)	-27.99 ± 0.67	6.58 ± 0.43
<i>Phacelurus latifolius</i> (Plant)	-14.52 ± 0.26	2.36 ± 1.35
<i>Carex scabrifolia</i> (Plant)	-26.24 ± 1.08	7.15 ± 1.95
Benthic microalgae (BMI)	-14.70 ± 0.10	4.20 ± 0.30

Table S3-3. PCR ampification success rates for the stomach contents of crabs.

Sex	Class	CW range (cm)	No. of samples	No. of successes	Amplification success (%)		
					Class	Sex	Total
F	G2	2.5–3.0	4	3	75.0	77.5	57.5
	G3	3.0–3.5	5	4	80.0		
M	G2	2.5–3.0	5	0	0.0	37.5	
	G3	3.0–3.5	4	3	75.0		
Total			18	10			

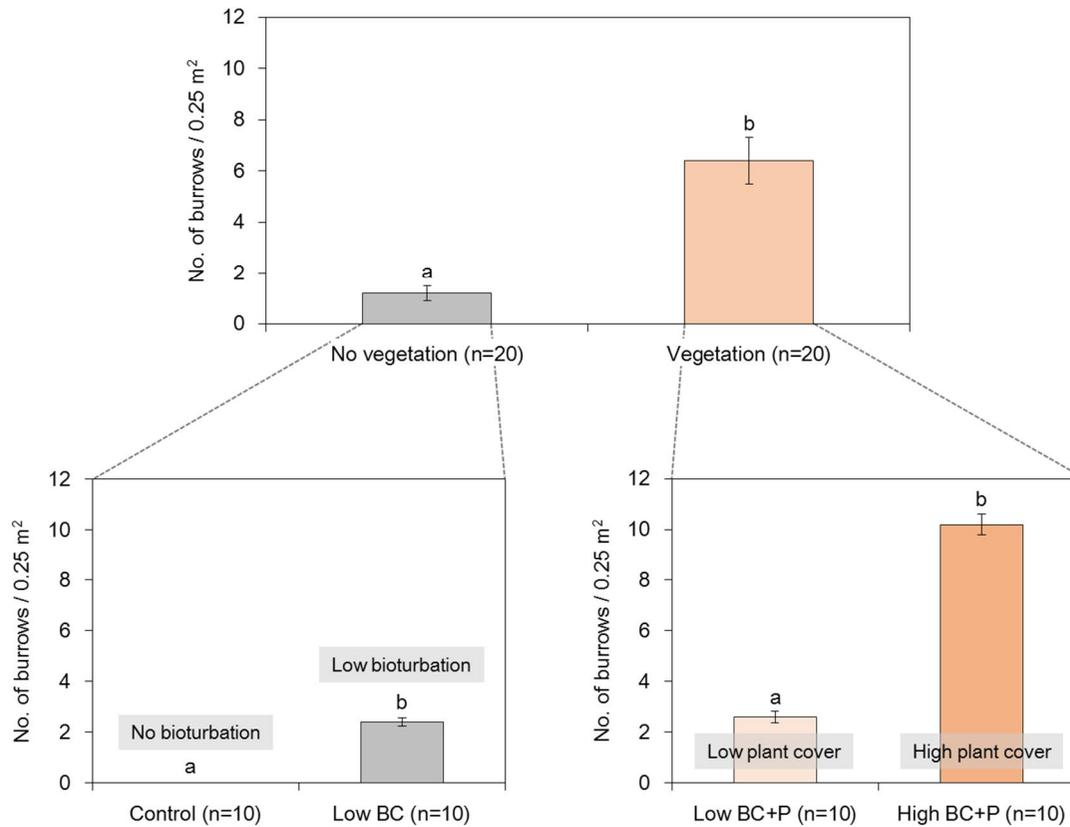


Figure S4-1. The number of burrows by the different habitat types. Different letters represent significant differences between three marsh zones based on multiple comparison tests after the Kruskal-Wallis test ($p < 0.05$).

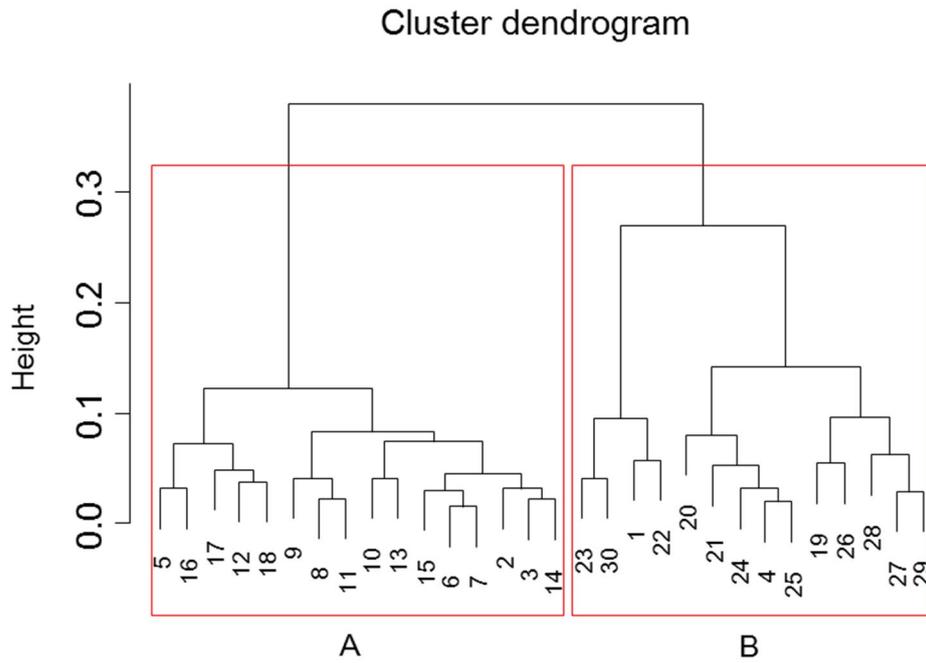


Figure S4-2. Dendrogram obtained by Cluster Analysis of the sampling plots for seed dispersion distances on Siheung Tidal Flat. Seed dispersion distances: A, Center (1–10) and Near (11–20); B, Remote (21–30).

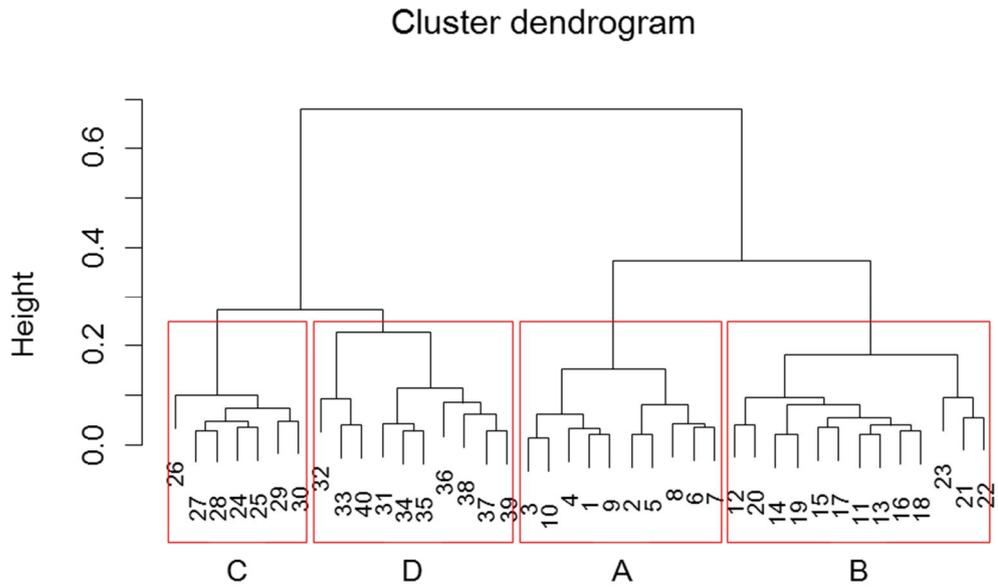


Figure S4-3. Dendrogram obtained by Cluster Analysis of the sampling plots for habitat types on Siheung Tidal Flat. Habitat types: A, Control (1–10); B, Low BC (11–20); C, Low BC+P (21–30); D, High BC+P (31–40).

국문초록

염습지에서 염생식물(halophytes) 분포는 일반적으로 환경구배에 의해 결정되며 관련된 주요 요인을 확인하는 것이 중요하다. 본 학위논문은 염생식물 분포와 생장에 영향을 주는 주요 요인을 다룬다. 첫 번째 연구는 염생식물이 고도 기울기에 따라 어떻게 분포하는지를 기록하고 분포에 영향을 미치는 환경 요인을 조사했다. 조사지역은 세계에서 가장 큰 조수간만의 차를 보이는 곳 중 하나인 시흥 갯벌이다. 나는 관심이 집중되지 않은 각 염생식물 종이 고도에 따라 분명한 대상분포를 하고 있다는 것을 발견했다. 또한, 고도가 높은 곳의 토양 염분은 매우 다양했다. 식물 군집 구성을 반영한 클러스터분석(Cluster analysis)을 통해 4 개의 그룹으로 분류했다 (나문재, 갯잔디, 갈대에 의해 정의된 높은 고도 그룹 1; 모새달에 의해 정의된 중간-높은 고도 그룹 2; 칠면초에 의해 정의된 낮은 고도 그룹 3; 천일사초에 의해 정의된 중간-높은 고도 그룹 4). NMS 분석은 칠면초(*S. japonica*)의 분포가 고도와 침수 빈도에 긍정적인 영향을 받는 반면에 모새달(*P. latifolius*)는 토양 염분과 토양 양이온에 의해 부정적으로 영향을 받는다는 것을 보여 주었다. 자연적인 염습지에서 고도 구배에 따른 환경요인과 염생식물 분포 사이의 관계를 이해하는 것은 염 습지 복원에 기여할 수 있는 중요한 생태 정보를 제공할 수 있다.

두 번째 연구에서는 게가 섭식한 염생식물의 종류를 확인하고 염생식물과 다른 먹이원의 상대적인 기여도를 결정했다. 게는 조간대에서 고밀도로 서식하며 염습지의 다양한 염생식물과 함께 서식한다. 그러나 게와 염생식물 사이의 생태학적 관계뿐만 아니라 초식 게가 염생식물 분포에 미치는 영향은 명확하게 알려지지 않았다. 연구 대상지에는 다른 염생식물이 서식함에도 불구하고 우점하는 갈게(*Helice tientsinensis*)는 칠면초만 소비한다는 것을 발견했다. 이 결과는 갈게가 선택적 섭식자라는 것을 나타낸다. 안정동위원소 분석을 통해, 게 발달과정 동안 먹이 변화를 확인했다. 미성숙한 개체는 주로 토양 유기물을 섭식했지만, 성숙한 개체는 더 다양한 먹이원을 섭식했다. 특히, 다른 크기의 게보다 중간 크기의 게가 더 많은 식물을 소비했다. 염습지에서 먹이 관계에 대한 연구 결과는 게와 염생식물 간의 상호작용에 대한 우리의 이해를 넓혀준다.

마지막으로, 식물의 밀도와 염생식물과 게 조합이 토양환경요인과 식물 생장에 미치는 영향을 조사했다. 게와 염생식물은 염습지 생태계의 토양 구성과 비옥도를 나타내는 중요한 지표이다. 많은 선행 연구에서 게 굴착이 토양 특성에 미치는 영향을 조사했지만 게와 염생식물이 염습지 토양에 미치는 결합 영향에 대해서는 거의 알려지지 않았다. 식물의 생물량과 개체의 높이는 모식물체(parent plants)에서 1m 이상 떨어진 지점에서 더 높다는 것을 확인했다. 게는 높은 식생 피도가 있는 서식지를 선호했다. 저밀도 게와 식물은 토양 특성에 상당한 변화를

일으켰다. 그리고 계와 염생식물의 조합은 염습지 토양의 물리화학적 매개변수의 공간적 다양성을 증가시켰다. 또한 이들의 변화는 염생식물의 생장에 영향을 줄 수 있다. 계와 염생식물은 염습지에서 고밀도로 출현하며, 이 조합은 염습지 생태계에 상당한 영향을 줄 수 있다.

주요어 : 굴착 계, 환경구배, 염생식물, 갈게, 염습지, 시흥 갯골, 칠면초

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