

## Effects of *Phragmites* Invasion on Microbial Diversity and Enzyme Activities in a Brackish Marsh

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**Abstract.** Invasion and expansion of *Phragmites communi Trin.* are a widespread phenomenon in many regions. This study assessed effects of invasion of *P. communi* on microbial diversity and enzyme activities in a wetland, which play a key role soil nutrient cycle. Soil samples from three locations (native, mixed and invaded) were collected and microbial diversity along with a suite of enzyme activities were determined in a brackish marsh of Korea. No significant differences in enzyme activities and bacterial diversity were found among sites. However, significantly higher fungal diversity and N-acetylglucosaminidase activity were found in the mixed site. This result suggests that plant diversity may induce higher diversity of fungal community resulting in faster mineralization of organic nitrogen.

**Keywords:** Invasive plant, Soil microorganism, Organic matter decomposition, T-RFLP

### 1. Introduction

Invasive species have been noted as the main threatening factor for biodiversity from a global perspective [1]. Expansion of invasive species is mainly mediated by human activities such as land use change, nutrient enrichment and physical introduction, along with additive effects of global climate change. Previous studies have focused on the mechanism of invasion and influences of invasive plants on overall biodiversity of various ecosystems. However, much less studies have been conducted in relation to soil microorganisms, which play a key role in soil nutrient cycling.

Wetlands are a transient zone located between aquatic and terrestrial ecosystems, which exhibit diverse importance [2]. For example, brackish wetlands are one of the most productive ecosystems providing habitats for various vegetation and waterfowls. They also play a key role in organic matter decomposition and often represent ‘biogeochemical hotspot’ at a landscape scale. Such an important ecosystem, however, is vulnerable to invasive plants, of which impacts have drawn much attention. For example, rapid invasion and expansion of *Phragmites* sp. to native species in brackish wetlands all over the world has widely been observed. Similarly, brackish marshes used be dominated by a native species, *Scirpus planiculmis*, have rapidly been invaded and replaced by *Phragmites communi Trin.* in East Asian countries including Korea. Previous studies have mainly concerned with invasion mechanism and overall effects on plant community, while much less attention was given to soil microorganism. Bacterial and fungal communities in soils play a key role in decomposition and other biogeochemical processes in soils. For example, they mediate rate-limiting steps in organic matter decomposition and mineralization by producing and releasing various extracellular enzymes. Microorganisms in soils are believed to be extremely diverse, but due to limitation in methodology, relatively less is known about them. Recent development employing molecular approaches such as fingerprinting methods, qPCR or sequencing have allowed researchers to obtain information about microbial diversity without depending on culture-based methods.

The aim of this study was to assess influences of an invasive plant on microbial diversity and enzyme activities in a wetland ecosystem. For this, we collected soil samples from 3 locations in a brackish marsh in Korea, and bacterial/fungal diversities and activities of a suite of enzymes were determined.

## 2. Materials and Methods

### 2.1. Site Description and Soil Characteristics

The study site was a tidal brackish marsh located on the estuary of Han River in Korea (37°46'N / 126°40'E), which has rare history of disturbance by human activities. Five replicate samples were collected from each of three sites, namely a native site (dominated by *S. planiculmis*), a mixed site (co-dominated by *P. communi Tri.* and *S. planiculmis*) and an invaded site (dominated by *P. communi Tri.*) over one growing season on a monthly basis (Total 6 samplings). Soil samples were collected from the surface to 10 cm depth and transferred to the lab on ice. Soils of all sites are classified as silt loam (Sand 1 %, silt 73 %, and clay 26%) and no physical differences were discernible. No significant differences in water content, organic matter content, extractable ammonium and pH were found among three sites (Table 1), indicating that chemical properties were also similar.

Table 1: Chemical properties of soil samples collected from three sites. All values are means with standard error of mean.

Sites	Water content (%)	Organic matter (%)	Ext. ammonium (ng g <sup>-1</sup> )	pH
Native	0.31 ±0.01	0.06 ±0.00	2.00 ±0.52	7.38 ±0.06
Mixed	0.30 ±0.01	0.05 ±0.00	2.01 ±0.52	7.51 ±0.05
Invaded	0.29 ±0.01	0.05 ±0.00	1.90 ±0.46	7.46 ±0.16

### 2.2. Microbial Diversity

Bacterial and fungal diversities were assessed by T-RFLP analysis. Soil DNA was extracted and fragments of 16s-RNA and ITS genes were amplified by PCR using the FAM (6carboxyfluorescein) labelled specific primers for bacteria and fungi, respectively. Purified PCR products were digested with *HhaI* and then terminal fragments were analysed by an automated sequencer. Microbial diversity was presented by Shannon's index, evenness and richness of T-RF patterns.

### 2.3. Enzyme Activities

Extracellular enzyme activities were determined using fluorogenic model substrates, methylumbelliferyl (MUF) compounds.  $\beta$ -glucosidase, N-acetylglucosaminidase, phosphatase and arylsulfatase were determined to assess C, N, P and S mineralization rates, respectively.

## 3. Results and Discussion

### 3.1. Microbial Diversity

No significant differences were found for diversity, evenness and richness for bacterial community among three sites (Table 2). However, fungal diversity presented as Shannon's Index was the highest in the mixed site followed by native site and invaded site (Table 2). Since no differences in chemical or physical properties of soils, the differences in fungal diversity among three sites appear to be mainly caused by differences in vegetation. It is also interesting to note that fungal diversity was the highest in the mixed site. This may be related to colonization of mycorrhizal fungi which often exhibit plant-specific patterns as noted in other salt marshes [3]. In mixed site in my study, two types of vegetation could provide more diverse habitats for fungi resulting in higher fungal diversity.

### 3.2. Enzyme Activities

No significant differences were found in enzyme activities among sites except for N-acetylglucosaminidase (Table 3). Particularly, N-acetylglucosaminidase activity was the highest in mixed site. This result is in accordance with fungal diversity and suggests that higher diversity of fungal community is associated with nitrogen mineralizing enzyme. It has been reported that fungal community composition is related to nitrogen mineralization or N-acetylglucosaminidase activity in forest floor [4]. In the present study, mixed growth of two types of vegetation induced higher diversity of fungal community which then may

induces higher N-acetylglucosaminidase activity. This further suggests that partial invasion of *P. communi* *Trin.* could accelerate nitrogen mineralization in an ecosystem.

Table 2: Diversity, evenness and richness of bacteria and fungi in soils from 3 sites. Significantly different values among sites are labelled with different letters (One-way ANOVA at  $P < 0.05$ ).

Microbes	Sites	Diversity	Evenness	Richness
Bacteria	Invaded	2.29 ± 0.26	0.79 ± 0.05	28.7 ± 3.5
	Mixed	2.83 ± 0.05	0.82 ± 0.05	21.2 ± 6.4
	Native	2.55 ± 0.14	0.83 ± 0.05	29.5 ± 5.1
Fungi	Invaded	1.50 ± 0.16 <sup>a</sup>	0.68 ± 0.12	18.2 ± 2.6
	Mixed	2.43 ± 0.17 <sup>b</sup>	0.64 ± 0.07	50.5 ± 13.4
	Native	1.78 ± 0.18 <sup>a</sup>	0.67 ± 0.10	30.2 ± 6.5

Table 3: Extracellular enzyme activities in soils from 3 sites. Significantly different values among sites are labelled with different letters (One-way ANOVA at  $P < 0.05$ ).

Sites	β-glucosidase	N-acetylglucosaminidase	Phosphatase	Arylsulfatase
Invaded	2.29 ± 0.26	2.29 ± 0.26 <sup>a</sup>	0.79 ± 0.05	28.7 ± 3.5
Mixed	2.83 ± 0.05	2.83 ± 0.05 <sup>b</sup>	0.82 ± 0.05	21.2 ± 6.4
Native	2.55 ± 0.14	2.55 ± 0.14 <sup>ab</sup>	0.83 ± 0.05	29.5 ± 5.1

Overall results of this study indicate that entire replacement of *S. planiculmis*-dominated brackish marsh to *P. communi* would affect bacterial and fungal community minimally. Likewise influences on microbial activity such as extracellular enzymes are also negligible. However, mixed growth of two vegetation during expansion of *P. communi* can result in higher fungal diversity and faster turnover of organic nitrogen.

#### 4. Acknowledgements

This study was supported by NRF (2012-0008675; 2012-0009247).

#### 5. References

- [1] O.E. Sala, F.S.III Chapin, J.J. Armesto, E. Berlow, J. Bloomfield, R. Dirzo, E. Huber-Sanwald, L.F. Huenneke, R.B. Jackson, A. Kinzig, R. Leemans, D.M. Lodge, H.A. Mooney, M. Oesterheld, N.L. Poff, M.T. Sykes, B.H. Walker, M. Walker, D.H. Wall. Global biodiversity scenarios for the year 2100. *Science* 2000, **287**: 1770-1774.
- [2] W.J. Mitsch, and J.G. Gosselink. *Wetlands*. John Wiley & Sons, 2000.
- [3] D.J. Burke, E.P. Hamerlynck, and D. Hahn. Interactions among plant species and microorganisms in salt marsh sediments. *Appl. Environ. Microbiol.* 2002, **68** (3): 1157-1164.
- [4] H. Chung, D.R. Zak, and E.A. Lilleskov. Fungal community composition and metabolism under elevated CO<sub>2</sub> and O<sub>3</sub>. *Oecologia* 2006, **147**: 143-154.