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Microbial Activity and Diversity Vary with Plant Diversity and Biomass in Wetland Ecosystems

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Abstract
Wetland ecosystems have low plant diversity, dominated only by one or two types of vegetation. Species with high biomass can easily invade wetlands and the effect of plant invasion on ecosystem process rates has drawn considerable attention within the field of microbial ecology. However, the effects of high biomass plants on microbes, which play a central role in wetland biogeochemistry, are notably understudied. This study investigates the relationship between plant species and their enzyme activities with microbial diversity in wetland ecosystems. Samples were collected from both natural and constructed wetlands to test whether high and low biomass species of submerged aquatic plants would have significant effects on microbial activity and diversity. This study found that plant species with high biomass increased activities of hydrolase activities such as β-glucosidase and N-acetyl-glucosaminidase. Microbial diversity was higher in rhizosphere with two high biomass plant species present compared to one plant species, due to niche competition, as indicated by a higher Shannon–Weaver index value.

Keywords Microbial activity · Microbial abundance · Plant diversity · Wetlands

Introduction

Wetlands provide many important ecosystem services such as biomass production and carbon sink (Mitsch and Gosselink 2000). Soil enzyme activity can affect the microbial diversity, by releasing exudates and oxygen into the rhizosphere (Singh and Kumar 2008). Enzyme activities also mediate by regulation of above-ground and below-ground litter production (Kang et al. 2005) due to predominating plant species of greater biomass. However, previous studies have shown conflicting evidence for the effects of wetland plant species on microbial activities and diversity. For example, it has been reported that high plant species richness increases concentration of microbial C and dehydrogenases (Zhang et al. 2011a, 2011b). Additionally, Calheiros et al. (2009) used denaturing gradient gel electrophoresis (DGGE) to demonstrate that high plant richness could increase bacterial abundance and community structure profiles in wetlands. Conversely, Kantawanichkul et al. (2009) reported that non-invasive wetland plants could alter the abundance of ammonia-oxidizing bacteria (*Nitrosomonas* and *Nitrobacter*) and denitrifying bacteria. Ahn et al. (2007) found addition of plants increased microbial community size but had no effect on structure, compared to un-vegetated plots. Similarly, DeJournett et al. (2007) reported no effect of plant species types on size and structure of methanotrophic bacterial communities in wetlands.

Microbial diversity and composition are known to be affected by plant diversity in various ecosystems. The relationship between plant and microbial diversity has been particularly well-studied in terrestrial ecosystems, with many studies reporting positive (Tilman et al. 1997; Naeem and Li 1997; Griffiths et al. 2000; Muller et al. 2002), negative (Rodriguez and Gomez-sal 1994; Pfisterer and Schmid 2002; Dukes 2001) and neutral (Engelhardt and Kadlec 2001; Griffiths et al. 2001) due to variation in physiological characteristics between plant species. According to the "complementarity theory," net primary productivity (NPP) increases with plant species richness in terrestrial ecosystem (Tilman et al. 2001). High plant species richness can induce high heterogeneity of organic resource

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environments and consequently lead to the development of diverse microbial groups (Sugiyama et al. 2008; Zak et al. 2003). However, studies that have focused on the relationship between biodiversity and microbial function have primarily been conducted in grassland ecosystems (Tilman et al. 2001; Roscher et al. 2004).

Relevant data for the relationship between plant and microbial diversity for wetlands are lacking. Most wetland microbial function experimentation has focus on wastewater treatment wetlands, which often contain extremely high amounts of nitrogen. Additionally, salt marsh have very low plant diversity and dominated by one or two types of vegetation that differ considerably in their primary productivity rates. High biomass plants can easily invade wetland ecosystems due to their greater nutrient use efficiency, early maturation, and allelopathy (Ruiz and Carlton 2003; Callaway et al. 2004). Many invasive species are also tolerant of or pre-adapted to environmental disturbances, which can further accelerate their expansion (Mitchell and Gopal 1991). As a result, they can often displace native species, resulting in a loss of plant diversity (Mack et al. 2000). The current study was conducted in natural marshes (brackish), constructed wetlands, and mesocosms with either inorganic or organic soils in order to investigate the pattern by which microbes are affected by high biomass plant species.

The objectives of this study were to evaluate the effects of high plant biomass species on microbial activity (b-glucosidase and N-acetylglucosaminase) and microbial diversity in wetlands. We hypothesized that (1) greater biomass plants would increase microbial activity due to higher C input to soil and (2) plant diversity would have an effect on microbial diversity and composition. We used 5 plant species in total, with richness levels of 1 and 2 entailing high and low biomass, respectively. We collected sediment samples of *Phragmites australis* and *Scripus planiculmis* from natural wetlands. We also constructed wetlands and mesocosms with *Phragmites australis*, *Thypha angustata*, and *Sphagnum palustre* in all possible combinations. We observed and analyzed microbial activities, as well as identifying bacterial communities and fungi under 1- and 2-plant species conditions using T-RF (Table 2).

Materials and Methods

Study Sites and Sampling

We assessed microbial activity and diversity within three different types of wetland sediments: natural, constructed, and mesocosm wetlands that were exposed to different plant species. We collected samples from total eight different wetlands: natural marshes (marshK, marshS), constructed wetlands (ConA, ConB), mesocosm wetlands with organic soils (OrgA, OrgB), and mesocosm wetlands with inorganic soils (InoA, InoB) (Table 1).

Natural marshes are Kirkpatrick marsh (Marsh K) and Seongdong wetlands (Marsh S). Marsh K is located on the Rhode River, a sub-estuary of Chesapeake Bay, in Maryland, USA (lat. 38° 53′N, long. 76°33′W). Mean salinity at this site was 10 ppt and mean tidal range was 44 cm. The high-marsh platform was 40–60 cm above the mean low water level and soils were predominantly organic (> 80%) to 5-m depth. Mean daily air temperatures ranged from − 4 to 31 °C and mean annual precipitation was 108 cm. Dominant plant species were dominant C4 grass, *Spartina patens*, which covered 30,105 m^2. The plant with the highest biomass in Marsh K was *Phragmties australis*, which covered 15,665 m^2 of the site. Samples were collected from triplicate quadrats (0.5 × 0.5 m) during period of peak high biomass in summer. Three replicate samples, comprising a total of 9 pooled soil cores (3 sites per 3 replicates), were taken from *Scripus*-only-*Phragmites*-only and mixed communities, respectively.

Marsh S is a natural brackish marsh, a typical wetland type in Korea, located northeast of Seoul (37°46′N, 126°40′E). Salinity ranged between 2.8 and 8.2, with tidal range of 3–6.8 (Park 2004; Baek and Yim 2011). Dominant plant species were sedge, *Schoenoplectus*, and *Phragmties australis*. Rhizosphere (10–30 cm below soil surface) samples were collected from triplicate quadrats (0.5 × 0.5 m) during period of peak high biomass in summer. Three replicate samples, comprising a total of 9 pooled soil cores (3 sites per 3 replicates), were taken from *Scripus*-only–*Phragmites*-only and mixed communities, respectively.

Constructed wetlands (ConsA, ConsB) were created at Mangsan park, Seongnam, Kyunggi-do, Korea (37°40′N, 127°14′E). The experiment was conducted during the main growing season (April–November). We created 2 plots and planted 2 plant species per plot (2 m × 2 m × 40 cm deep), including typical water-tolerant species found within subtropical monsoonal climate region (Table 1). For constructed wetlands, plants were collected from natural wetland sites in Ansan, Kyunggi-do, Korea (37°17′10.68″N, 137°55′25.96″E; 6,353 m^2) and cultivated in natural conditions with high moisture. The Ansan wetlands have warm, humid conditions during the summer and cold, dry conditions during the winter. The main water source was surface water and mean precipitation at the study site was 1235.2 mm per year. The physicochemical properties of the water were as follows: pH = 5.4–5.7, EC = 75–79 μs cm^{-1} and DO = 19.6–7.5 mg l^{-1}. Rhizosphere soils were collected using pooled soil cores (10 cm deep, 5 cm diameter) from each constructed wetland during peak plant biomass in summer.

Mesocosms were created at Yonsei University campus (outside), Seoul, Korea, with 2 soil types (organic

Table 1 Species composition for each sampling sites

Wetland types	Location	Species name	Site name	Soil texture
	High biomass	*Phragmites australis*		
Marsh K	Mixed		Tidal marsh	Clay
	Low biomass	*Spartina patens*		
	High biomass	*Phragmites australis*		
Marsh S	Mixed		Marsh	Silt loam
	Low biomass	*Schoenoplectus americanus*		
Constructed	High biomass	*Typha angustata*	ConA	
	Mixed			
	Low biomass	*Zizania latifolia*		Silt loam
	High biomass	*Typha angustata*	ConB	
	Mixed			
	Low biomass	*Phragmites australis*		
Mesocosm	High biomass	*Typha angustata*	OrgA	
Organic	Mixed			
	Low biomass	*Sphagnum palustre*		Sandy
	High biomass	*Phragmites australis*	OrgB	Clay
	Mixed			Loam
	Low biomass	*Sphagnum palustre*		
Mesocosm	High biomass	*Typha angustata*	InoA	
Inorganic	Mixed			
	Low biomass	*Sphagnum palustre*		
	High biomass	*Phragmites australis*	InoB	Sandy loam
	Mixed			
	Low biomass	*Sphagnum palustre*		

and inorganic soil) (Table 1). A total of 24 mesocosm (4 types of site × 3 plant types × 2 replicate) systems of 65L (L 745 mm, W 530 mm, D 415 mm) were set up following a randomized block design in early March. The experiment was conducted during the main growing season (April–November). The systems were established with 2 types of soil (organic and inorganic): organic mixed peat moss and agricultural soil; mixed sand and agricultural soil. Mesocosm wetlands were exposed to precipitation and amended with groundwater, when necessary, to maintain a water level of 10 cm above the soil surface. We collected plant from same place (Ansan natural wetlands) as constructed wetlands. We used *sphagnum*–module with medium for low biomass plant. We planted *Phragmites,* and *Typha* (height, 20–25 cm) in each of the mesocosms for high biomass plant. Rhizosphere soils were collected using pooled soil cores from each mesocosms during peak plant biomass in summer ($n = 10$). All plant composition and sampling sites are shown in Table 1.

Plant samples were collected from triplicate quadrats (0.5 × 0.5 m) in each site at the same time as soil sampling during period of peak high biomass in summer (August). Dry mass of plant was determined by drying a subsample at 75 °C for 3 days.

Measurement of Physicochemical Factors

For each quadrat, we measured pH, water content, soil organic matter content (SOM), dissolved organic carbon (DOC) content, and phenolic content. Soil pH was determined by adding soil to water at a ratio of 1:5 (w/v) and detected using an electrode. Soil water content was determined via measurement of weight loss after drying samples overnight at 105 °C. To estimate SOM weight, soil samples were incubated and dried in a furnace at 600 °C for 24 h, after which ash-free dry weight was measured and the difference between initial and final weights were calculated. DOC was determined by adding soil to a slurry of distilled water at a ratio of 1:10 (w/ v) for 1 h and filtered through a 0.45-mm filter paper. A TOC-V CPH (Shimadzu, Model TOC-5000, Japan) was used to analyze the DOC concentration, using potassium hydrogen phthalate for a standard calibration curve. A phenolic compound content assay was conducted using Folin–Ciocalteau phenol reagent (Box 1983). One milliliter of each sample was mixed with 1.5 ml of Na_2CO_3 (50 g L^{-1}) and 0.5 ml of Folin–Ciocalteu reagent and was incubated for 2 h at 20 °C in darkness. A standard calibration curve was created with phenolic content against absorption (750 nm), measured by spectrophotometer (FLUO-star OPTIMA, BMG LABTECH).

Analysis of Enzyme Activity

As an index of microbial activity, we measured the activities of two hydrolases, β-glucosidase and N-acetylglucosaminidase, using the MUF-substrate method (Kang et al. 1998). Those activities are important in carbon (C) and nitrogen (N) cycling in soil decomposition because it participates in the processes whereby chitin is converted to amino sugars, which are major sources of mineralizable C and N in soils (Ekenler and Tabatabai 2004). The concentrations of each substrate solution were 400 µM Sigma, St. Louis, MO, USA; MUF-β-glucoside; and MUF-N-acetylglucosamine. The mixture of sample suspension and substrate solution was incubated for 60 min at 25 °C and centrifuged at 5000×*g* for 5 min in order to halt enzyme activity. A fixed volume (300 ml) of supernatant was then transferred to a 96-well black plate and absorbance was measured (conditions: emission, 460 nm; excitation, 355 nm; FLUO-star OPTIMA, BMG LABTECH). An MUF-free substrate was used as a negative control and hydrolytic enzyme activity was expressed as nMol/MUF g dry soil^{-1} min^{-1}. These enzymes are ubiquitous in bacteria, fungi, plants, and animals and are indicative of overall microbial activity.

Analysis of Microbial and Fungal Diversity

Microbial DNA was extracted from 0.5 g of soil using Power Soil DNA isolation kit (MoBio, USA). To screen for sample contamination, a parallel DNA extraction procedure was performed with an unused filter, considered as "blank" for further analysis. To compare microbial community composition of each site, we conducted Terminal Restriction Fragment Length Polymorphism (T-RFLP) analysis of soil DNA. DNA samples were amplified by PCR using the fluorescently labeled forward primer 27F (5′[6FAM]-AGAGTTTGATCC TGGCTCAG-3′) and unlabeled reverse primer 927R (5′-CCG TCAATTCCTTTRAGTTT-3′), which targeted bacterial 16S rRNA genes (Lane 1991) (Table 2). For fungal communities, PCR was performed using the fluorescently labeled forward primer ITS1F (5′[Hex]-CTTGGTCATTTAGAGGAAGTAA-3′) and unlabeled reverse primer ITS4 (5′-TCCTCCGCTTAT TGATATGC-3′), which targeted the fungal internal transcribed spacer (ITS) region of the rRNA gene (White et al. 1990; Heid et al. 1996) (Table 2). ~300 ng of purified PCR product was added to a reaction mixture (final vol. 25 µl) containing 10U of restriction endonuclease HhaI (Promega, Madison, WI) and was then incubated at 37 °C for 4 h. Digests were desalted and 1 µl aliquot was used for subsequent T-RFLP analysis. Terminal fragment-size analysis was performed using ABI 377 DNA Analyzers (Applied Biosystems) in conjunction with the GeneScan software (Applied Biosystems). Shannon indices (Shannon 1948) were calculated based on Complete Linkage Clustering data and T-RFLP.

Statistical Analysis

A two-way ANOVA was conducted with soil enzyme activity and microbial quantity data, in order to determine significant differences between sites/group, using SPSS 21.0 software. Standard deviations are displayed as numerals in tables and as error bars in graphical figures. A One-way ANOVA with Tukey's analysis was conducted in order to compare differences in physicochemical variables and extracellular enzyme activity between plant types. Microbial community structure and diversity were determined and quantified using TR-F profiles. Bacterial and fungal diversity were quantified by Shannon's diversity index (*H*) based on abundance of fragments at each site (Shannon 1948).

Results

Soil Biochemical Change

Soil characteristics were not significantly different (Table 3) between sites with different levels of plant species and richness except soil organic carbon contents. These results suggested that any changes in microbial activity or community composition were not attributable to differences in soil characteristics (excl. soil organic C contents). Soil organic C contents were 0.7 to 2.2 higher at high plant biomass sites compared to low biomass sites ($p < 0.001$) in mesocosms. Although organic contents were higher in natural wetlands than in mesocosms, organic contents were not significantly different with different levels of plant species in Marsh K.

Table 2 Primer for T-RF used in this study

Target genes	PCR primer	Primer sequence (5′ – 3′)	Product size (bp)	Reference
Bacteria	27F	CCTACGGGAGGCAGCAG	900	(Lane 1991)
	927R	CCGTCAATTCCTTT(A/G)AGTTT		
Fungi	ITS	CTTGGTCATTTAGAGGAAGTAA		(White et al. 1990)
		TCCTCCGCTTATTGATATGC		

Table 3 Changes in soil physicochemical factors of the wetland types with mono- and bi-culture (means ± standard deviation)

Wetland types	Location	pH	WC(%)	OM((%)	DOC (mg g^{-1})	Phenolic (mg g^{-1})
	High	7.2 ± 0.4	69.5 ± 9.8	49.7 ± 5.3	2.8 ± 0.8	0.001 ± 0.0001
Marsh K	Mix	7.0 ± 0.1	87.0 ± 2.5	59.8. ± 5.3	3.5 ± 0.3	0.001 ± 0.0001
(*n* = 9)	Low	7.0 ± 0.3	87.8 ± 1.5	53.8 ± 0.4	2.8 ± 0.4	0.001 ± 0.0001
Marsh S	High	7.4 ± 0.1	31.0 ± 0.8	6.1 ± 0.2[b]	0.5 ± 0.1	0.001 ± 0.0001
(*n* = 9)	Mix	7.5 ± 0.1	30.0 ± 1.0	4.7 ± 0.2[a]	0.4 ± 0.1	0.01 ± 0.001
	Low	7.5 ± 0.2	39.0 ± 0.7	4.6 ± 0.4[a]	0.3 ± 0.05	0.01 ± 0.02
	High	4.9 ± 0.3	63.0 ± 0.3	2.9 ± 0.4[a]	0.01 ± 0.001	0.001 ± 0.002
Con A	Mix	6.0 ± 0.2	39.4 ± 11	3.5 ± 0.3[a]	0.01 ± 0.003	0.004 ± 0.001
(*n* = 9)	Low	5.8 ± 0.4	44 ± 13	3.1 ± 0.7[a]	0.03 ± 0.01	0.1 ± 0.01
	High	4.9 ± 0.3	63.0 ± 0.3	2.9 ± 0.4[a]	0.01 ± 0.001	0.001 ± 0.002
Con B	Mix	6.0 ± 0.3	47.3 ± 7.0	3.5 ± 0.3[a]	0.01 ± 0.004	0.002 ± 0.001
(*n* = 9)	Low	5.8 ± 0.4	43.9 ± 13	3.1 ± 0.7[a]	0.03 ± 0.01	0.001 ± 0.005
	High	4.9 ± 0.1	86 ± 0.03	38 ± 1.2[b]	0.01 ± 0.004	0.004 ± 0.003
Org A	Mix	4.9 ± 0.1	76.5 ± 3.3	23 ± 5.3[a]	0.01 ± 0.003	0.01 ± 0.001
(*n* = 10)	Low	4.9 ± 0.1	83 ± 0.03	33 ± 0.7[a]	0.01	0.01
	High	4.8 ± 0.2	82.2 ± 7.5	35.4 ± 1[b]	0.01 ± 0.005	0.005 ± 0.002
Org B	Mix	4.8 ± 0.1	74 ± 12.2	40 ± 2.5[b]	0.01 ± 0.005	0.005 ± 0.008
(*n* = 10)	Low	4.9 ± 0.1	83 ± 0.03	32 ± 0.7 [a]	0.01	0.01
	High	7.7 ± 0.07	23.2 ± 0.5	3.2 ± 0.2[b]	0.03 ± 0.001	0.03 ± 0.001
Ino A	Mix	7.6 ± 0.05	27.7 ± 1.1	1.2 ± 0.2[a]	0.03 ± 0.02	0.02 ± 0.005
(*n* = 10)	Low	7.3 ± 0.08	22.5 ± 1.0	1.4 ± 0.5[a]	0.03	0.004
	High	8.0 ± 0.02	29.2 ± 2	1.9 ± 0.5[a]	0.03 ± 0.007	0.02 ± 0.01
Ino B	Mix	7.4 ± 0.1	25.6 ± 0.3	1 ± 0.1 [a]	0.03 ± 0.001	0.01
(*n* = 10)	Low	7.3 ± 0.08	22.5 ± 1.0	1.4 ± 0.5[a]	0.03	0.004
Two-way ANOVA site	(*p*-value)	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
Plant		–	–	0.017	–	–
Site × plant						

Values are means ± SD. Values in plant species followed by the same letter do not differ significantly from one another (as determined by ANOVA/Tukey analysis)

Microbial Enzyme Activity

Both β-glucosidase and N-acetylglucosaminidase were similar base levels across all sites (Fig. 1). However, both enzymes exhibited a significant increase in activity at sites with high plant biomass. The extracellular activity both of ß-D-glucosidase and N-acetyl-glucosaminidase positively correlated with soil water and organic matter contents.

Bacterial and Fungal Diversity Index

The microbial diversity of soils with two plant species present was significantly higher than that of samples with a single species present. Microbial diversity index (*H′*) among plant species was significantly different for both fungi and bacteria (Fig. 2). Moreover, bacterial and fungal diversity was higher in soils from the natural marsh than mesocosm soils.

Discussion

In wetland ecosystems, invasion of high biomass plant species leads to more efficient uptake of nutrients and greater productivity, although the exact relationship between plant species diversity and microbial diversity remains unclear (Ruiz and Carlton 2003; Callaway et al. 2004). The current study presents two contrasting mechanisms that influence microbial community activity and composition in wetland ecosystems. Primary productivity of plant species induced variation in microbial community activity, as indicated by β-glucosidase and N-acetylglucosaminidase activity changes (Fig. 2). However, changes in soil bacterial and fungal composition were dictated by plant species richness (Fig. 3).

Our results indicate that microbial enzyme activities increase with high plant biomass (*Phragmites* and *Typha*) rather than low plant biomass (*sphagnum* or *Spartina*), due to higher organic carbon levels in high plant biomass wetlands (Table 4). These results might be related

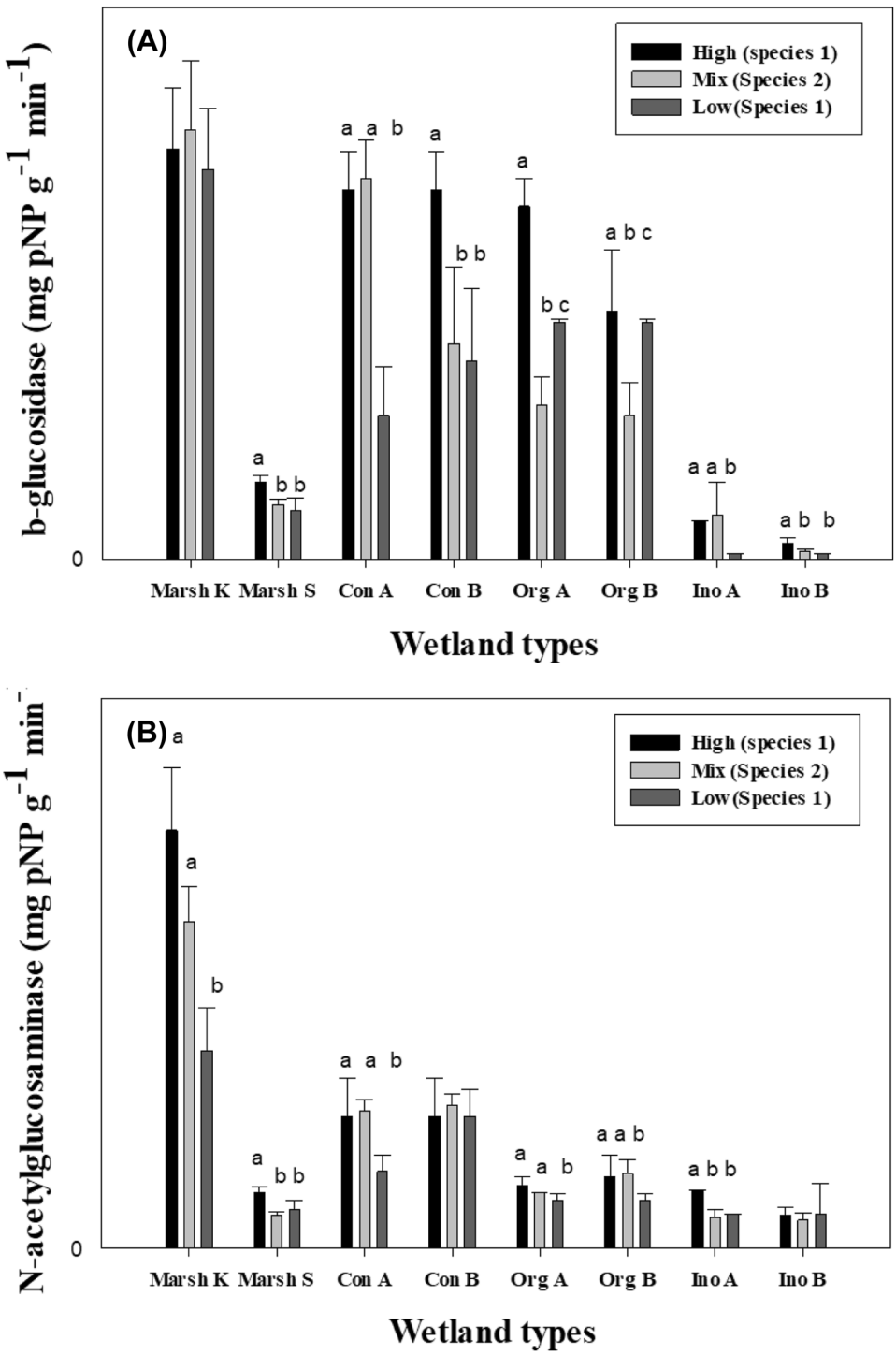

Fig. 1 Enzyme activities: **A** β-glucosidase and **B** N-acetylglucosaminidase across the sites. Different letters denote variation plant species at $p < 0.05$ level according to Tukey's test

to introduction of deep-rooting species (e.g., *P. australis*) into wetlands causes structural changes in root depth distribution that can lead to an increase in soil organic matter mineralization (Mozdzer et al. 2016). Plant species with high rooting depth and root productivity positively affect soil carbon contents (Calheiros et al. 2009; Kyambadde et al. 2004). Therefore, relative magnitude of soil carbon inputs from both above- and below-ground may depend upon plant species. High biomass plant such as *P. australis* stimulate extracellular enzyme activity (Ravit et al. 2003; Song et al. 2014), both of which are key in the degradation of recalcitrant organic compounds (Sinsabaugh 2010). Kim et al. (2018) also reported that higher primary production plant increased enzyme activity and microbial abundance due to increase organic carbon in tidal marsh.

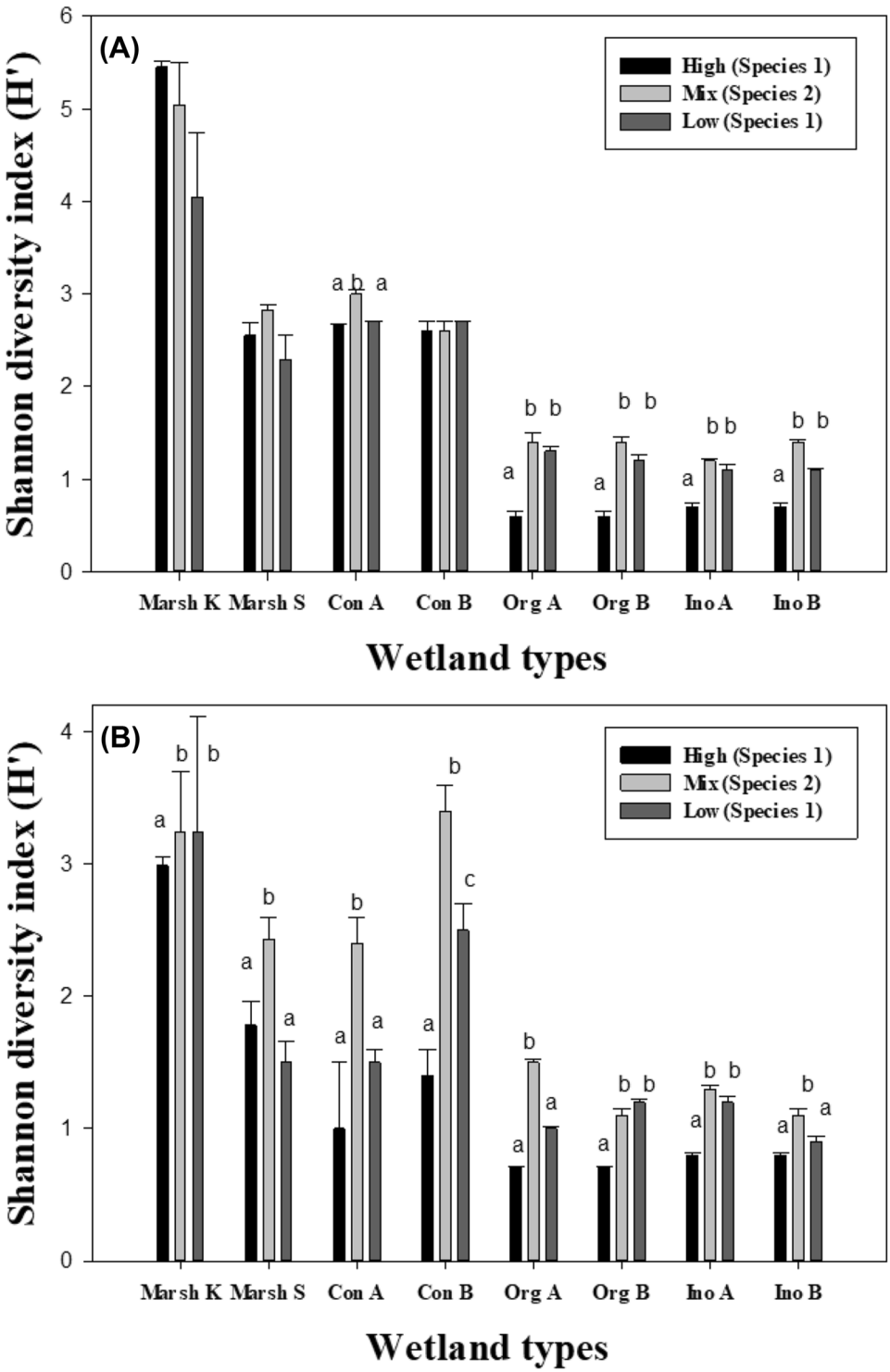

Fig. 2 Shannon's diversity index calculated from the numbers and areas of T-RFs (terminal restriction fragments) for bacteria (**A**) and fungi (**B**) in rhizosphere soils from all plants. Different letters denote variation plant species at $p < 0.05$ level according to Tukey's test

Rhizodeposition denotes a continuous flow of carbon containing compounds from roots to soil. Plant litter is a primary source of soil carbon; however, root exudates are also important, accounting for 5–33% of daily photo-assimilate, especially in actively growing plants (Bardegett et al. 2005). A potential explanation for the observed differences in enzyme activities across plant biomass levels may be that high biomass plant species contribute to soil carbon pools via input of large amounts of new carbon, whereas low biomass plant species provide low-quality plant material. Furthermore, we found that microbial activity increased alongside high organic carbon levels with high plant biomass species. Increased belowground accumulation of C stimulates the abundance

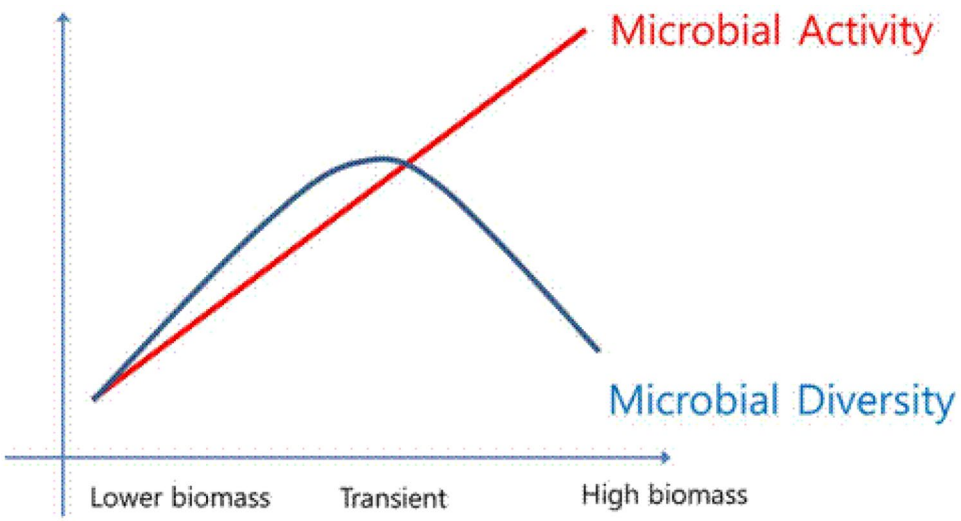

Fig. 3 A conceptual model for mechanism of plant diversity in relation to microbial activities and diversity in soil systems

and activity of microorganisms, resulting in further processing of C substrates and soil organic matter (Angers and Caron 1998). In addition to biomass and necromass inputs, microorganisms facilitate decomposition of plant detritus and subsequent cycling of soil organic matter through production of extracellular enzymes (Freeman et al. 2001; Sinsabaugh 2010; Fenner and Freeman 2011). Our results are supported by previous mesocosm and field studies: Wang et al. (2009) suggested that high plant biomass can contribute to microbial biomass and soil C. Factors that potentially affect root production or plant biomass allocation can be important to C storage in marshes because belowground biomass composes a large fraction of the organic matter that is preserved in wetlands. This indicates that belowground production is a crucial factor influencing C storage rates in wetland ecosystems (Chen and Twilley 1999; Middleton and Mckee 2001). Terrestrial ecosystem behaves in a similar pattern: Lange et al (2015) found that higher plant diversity increased rhizosphere inputs into microbial communities, resulting in both increased microbial activity and carbon storage. Evidence to explain these changes include that deep root vegetation in highly diverse plant communities reduces evaporation from topsoil, which in turn promotes higher soil microbial activity and growth, soil carbon storage is linked to root inputs, including root exudation, which are known to influence shifts in microbial activity and composition (Bais et al. 2006; Kramer et al. 2010; Rasse et al. 2005).

Therefore, we predict that the increased enzyme activity observed at sites with high biomass plant species is due to increased levels of available C.

Table 4 Total plant biomass (g) at the wetland types with mono-culture and bi-culture (means ± standard deviation)

Wetland types	Location	Biomass, dry weight (g)
Marsh K	High	410.4 ± 55.8
	Mix	380.5 ± 29.9
	Low	217.4 ± 6.9
Marsh S	High	305.4 ± 26.6
	Mix	290.8 ± 38.2
	Low	210.5 ± 19.5
Con A	High	57.2 ± 8.6
	Mix	40.3 ± 12.0
	Low	18.9 ± 2.8
Con B	High	55.0 ± 2.8
	Mix	30.5 ± 2.8
	Low	5.1 ± 0.14
Org A	High	10.8 ± 2.3
	Mix	10.5 ± 2.5
	Low	1.2 ± 0.2
Org B	High	3.8 ± 7.5
	Mix	5.4 ± 6.3
	Low	1.0 ± 0.5
Ino A	High	5.9 ± 2.6
	Mix	7.6 ± 2.1
	Low	1.5 ± 0.8
Ino B	High	7.2 ± 2.4
	Mix	6.2 ± 2.3
	Low	1.6 ± 0.5
Two-way ANOVA site		< 0.001
Plant		< 0.001
Site × plant	(*p*-value)	< 0.001

Change in Microbial Diversity

We found that microbial diversity of soils exposed to two species was higher than that of soils with a single species present. Interestingly, both bacterial and fungal diversities

were higher when two plant species were present (coefficient value $x = 1.3$, $R^2 = 0.2$). This finding suggested that higher plant diversity supports microbial diversity. Those finding (Fig. 3) suggests proportional relationship between plant diversity and microbial diversity. Greater plant species richness can create a more heterogeneous environment and consequently induce microbial diversity by reducing niche competition and overlap (Eisenhauer et al. 2013; Zak et al. 2003). These findings clearly show that structurally and functionally distinct microbial communities develop under different plant species in wetlands, suggesting that it is important to consider the diversity of plant species, along with the abiotic factors, when investigating the abundance and coexistence of different microbial species. Higher biomass plant species could have more intense competition with other plant species, which may increase their secondary compound contents, such as lignin (Welker et al. 2015). Moreover, microbial diversity of natural wetlands was higher than that of constructed wetlands. This finding may also be related to the increased competition of plants under natural conditions and which may induce high heterogeneity of carbon resources to microbes (Palacios et al. 2012). The diversity and composition of rhizosphere communities seemed to variate more dramatically under natural conditions. Natural wetlands with high potential productivity and competitiveness could provide better quality carbon resources for microbes. Our results imply that management practices that maintain diversity of aquatic macrophytes in wetlands may increase the efficacy of conservation efforts by further promoting the ecosystem services of wetlands.

Ecological Implications

The relationship between microbial diversity and activity is highly important within many ecosystems, potentially relating to plant biomass and diversity, and biogeochemical cycling processes. In this study, organic carbon and enzyme activities remarkably increased with high plant biomass in mesocosms. However, the nature of this relationship remains uncertain; an accurate definition necessitates experimental methods that match the complexity and changeability of microbial composition and activity. Further research is needed to better understand the high biomass plant effects on microbes, and more research should be long-term period conducted in natural wetlands.

High biomass plant invasion of wetlands would increase abundance, quality, and heterogeneity of soil organic C, due to their high primary productivity and improved nutrient retention ability. However, it is uncertain what effect high microbial activity has on carbon stabilization within these ecosystems and the wider-scale consequences.

Conclusions

(1) High plant biomass species increases microbial enzyme activity, likely due to increased levels of soil organic C.
(2) High plant richness induces greater bacterial and fungal diversity, suggesting the key factors that affect microbial activity and diversity in wetlands are similar to that of terrestrial ecosystems. Therefore, it could be feasible to extrapolate data from terrestrial studies to those of wetlands, which collectively could help to improve our understanding of the relationship between plant and microbial diversity. Future studies should focus on the microbial quantity and quality analysis of invasive plant with high biomass in wetland.

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