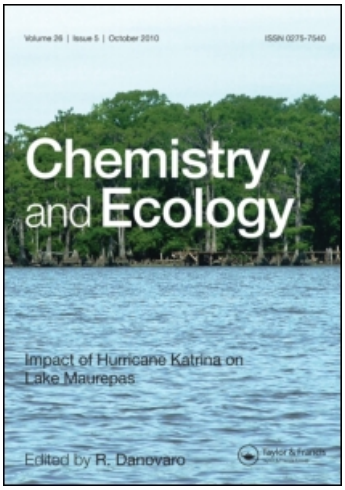


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# Positive relationships between phenol oxidase activity and extractable phenolics in estuarine soils

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Decomposition of recalcitrant materials such as phenolics is known to play a pivotal role in organic matter decomposition and nutrient cycling in estuaries. The specific goals of this study were to determine temporal and spatial variations of phenol oxidase and phenolics in estuarine soils, and to elucidate controlling factors for phenol oxidase activity. To achieve these goals, phenol oxidase activity and phenolic content were measured in soils developed along the side of an estuary in the Han River, Korea. Soil samples were collected in three locations with different vegetation: mud flats, *Zizania*-dominated soils, and *Salix*-dominated soils. Monthly measurements were also made in a *Zizania*-dominated site over a year period. Phenol oxidase activity varied between 0.00 and 0.28 diqc $min^{-1}$ $g^{-1}$, whilst phenolic content ranged from 0.0–10.5 $\mu g\, g^{-1}$. A correlation analysis revealed that phenol oxidase activity exhibited positive correlations with phenolic content in both seasonal and spatial data. The same relationship was found when the data were analysed separately for each site. Unlike peatlands or upland forest soils where negative correlations were often found between phenol oxidase activity and phenolics, substrate induction appears to account for the positive correlation in the present study.

**Keywords:** phenol oxidase; soil enzyme; wetland; phenolic compound, estuary

## 1. Introduction

Estuaries are a transient zone located between freshwater and seawater ecosystems. Estuarine ecosystems are highly dynamic in their chemical, physical and biological characteristics due to influences from tidal fluxes and runoff from upstream watershed [1]. In particular, shallow sediment and vegetation developed along the sides of estuaries are of great ecological importance, because of their high primary productivity as well as active decomposition mediated by soil microorganisms. Microorganisms produce various enzymes by which organic matter is decomposed, and hence information about microbial enzymes is essential for the understanding of biogeochemical processes. However, enzymatic analysis of organic matter decomposition in the surface soils of estuaries from an ecological perspective has not been well documented, whilst decomposition of organic matter in water bodies or underwater sediments has been widely studied [2,3].

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Organic matter decomposition in various ecosystems is often limited by degradation of the recalcitrant proportion of organic matter [4]. Of the various soluble products of decomposition, phenolics have attracted particular interest for their inhibitory effects on extracellular enzymes [5]. Further, phenolics are highly recalcitrant, which often determines the overall degradation rates of organic matter. Phenol oxidase is one of the few enzymes involved in phenolic degradation, and hence its ecological roles and regulating factors have drawn much attention. For example, Freeman et al. [6] have proposed an 'enzyme latch' hypothesis which indicates critical roles of phenol oxidase and phenolics in the carbon accumulation of peatlands. Phenol oxidase in soils has also been assessed in forest ecosystems in relation to inhibitory effects of excessive nitrogen on litter decomposition [7]. It was reported that increased N input through atmospheric deposition may result in the accumulation of phenolics by inhibitory effects of inorganic nitrogen on phenol oxidase.

Considering the ecological importance of estuarine sediment in organic matter decomposition and nitrogen enrichment by non-point pollutants from upland areas [8], information about phenol oxidase and phenolics in estuaries would be highly valuable. To date, however, there have been few reports on phenol oxidase activity and its ecological roles in estuarine ecosystems. To fill the gap in this knowledge, we conducted a field survey of phenol oxidase and other chemical properties of estuarine sediment in the Han River of Korea. The specific aims of this study were to determine temporal and spatial variations of phenol oxidase and phenolics in estuarine soils, and to elucidate controlling factors for phenol oxidase activity.



## 2. Materials and methods

### 2.1. *Study site*

The sampling site was Jang-Hang estuary, which is located in the lower reach of the Han River (N 37° 38′; E 126° 44′). The annual mean temperature of the site is 15°C and the annual mean precipitation is around 1,500 mm. The areas are flooded several times during summer due to the influence of the summer monsoon, but they are rarely flooded during the other seasons. The site has discernible zones of different vegetation perpendicular to the water flow. Areas adjacent to the water are mud flats without any surface vegetation, whilst herbaceous species (*Zizania latifolia*-dominated areas) and then woody plants (*Salix koreensis*-dominated areas) are sequentially developed towards the upland fringe. The area has been well protected from any human interference because a civilian's approach is strictly prohibited by military fences.

### 2.2. *Soil sampling*

Soil samples were collected from three locations with the distinctively different vegetation areas described above. At each location, three soil cores were collected at a 5 cm depth from the soil surface. To compare the three locations, soil samples were collected in June, August, October, and December 2006. Soil samples were also collected in the *Zizania*-dominated area from July 2006 to June 2007 on a monthly basis to assess temporal variations.

### 2.3. *Soil chemical and physical properties*

Organic matter content, total nitrogen, water content, soil texture, soil pH, extractable nitrate, extractable ammonium, and extractable DOC were determined by standard methods [9].

### 2.4. *Phenol oxidase and phenolics*

Phenol oxidase activity was determined by using L-DOPA (L-3,4-dihydroxyphenylamine) as a model substrate [10]. In short, 1 g of soil was added with 10 ml of L-DOPA solution (10 μM) and incubated for 15 minutes. The incubation was terminated by centrifuging samples at 2,200 *g* for 5 minutes. Absorbance of the supernatant was measured at 450 nm by a micro-plate reader (Thermo Labsystems, Multiskan Ascent). The activity was expressed in the unit of μMol 2,3-dihydroindole-5,6-quinone-2-carboxylate (diqc) per minute per g of dry soil. Internal controls (e.g. samples reacted with deionised water only, and absorbance of L-DOPA itself) were employed to remove any interference from the soil matrix or substrate itself. Phenolic content was determined by Folin–Ciocalteu solution, after extracting soil with deionised water [11].

### 2.5. *Statistical analysis*

Site-specific and monthly differences in phenol oxidase and phenolic contents were assessed by a one-way ANOVA test. Statistical significance was tested at $p < 0.05$. Correlation analysis between two variables was also conducted. SPSS 12.0 was used for all statistical analyses.



## 3. Results and discussion

No significant differences were found in soil organic matter content in the three locations, whilst total N was the lowest at the mud flat (Table 1). All soils were classified as silt loam, but substantial differences were observed for extractable nitrate and ammonium contents. Extractable nitrate was highest at the *Salix*-dominated site and decreased substantially towards the river. In contrast, extractable ammonium was highest at the mud flat, and lowest at the *Salix*-dominated area (Table 1). Such differences could be explained by two mechanisms. First, the mud flat is flooded often and hence anaerobic conditions can be induced more frequently than at the *Zizania*- or *Salix*-dominated areas. Under anaerobic conditions, ammonium oxidation is strongly inhibited, which can result in the accumulation of ammonium in flooded areas [12]. Secondly, denitrification, which can remove nitrate from a soil under anaerobic conditions, could more actively occur at the mud flat compared with the *Salix*-dominated area.

Figure 1 exhibits monthly variations of phenolics and phenol oxidase activity at the *Zizania*-dominated area. Phenolic contents varied between 0.0 and 10.5 $\mu g\, g^{-1}$, with the maximum value in October and the minimum value in March. Two peaks are discernible as a seasonal variation; one in autumn and the other one in late winter (Figure 1(A)). For phenol oxidase, much higher activities were observed in September and October, and variations were minimal in the other months (Figure 1(B)). Bonnett et al. [13] have reported a similar seasonal trend in northern

Table 1. Physico-chemical properties of soils. Mean ± SE. Values labelled with different letters are significantly different at $p < 0.05$ (one-way ANOVA).

| | Organic matter content | Total N | Water content | Soil texture | pH | Ext. $NO_3^-$ | Ext. $NH_4^+$ | Ext. DOC |
|---|---|---|---|---|---|---|---|---|
| Mud flat | 4.10 ± 2.76 | 0.042 | 30.91 ± 3.72 | Silt loam | 7.80 ± 0.03 | 4.52 ± 3.34a | 98.20 ± 8.58a | 35.35 ± 7.66 |
| *Zizania* site | 4.49 ± 3.31 | 0.062 | 18.50 ± 2.90 | Silt loam | 7.52 ± 0.14 | 7.36 ± 3.59a | 5.70 ± 1.16b | 25.23 ± 3.35 |
| *Salix* site | 3.90 ± 0.59 | 0.063 | 28.11 ± 1.87 | Silt loam | 7.26 ± 0.01 | 40.64 ± 41.81b | 1.18 ± 1.55b | 36.20 ± 12.96 |

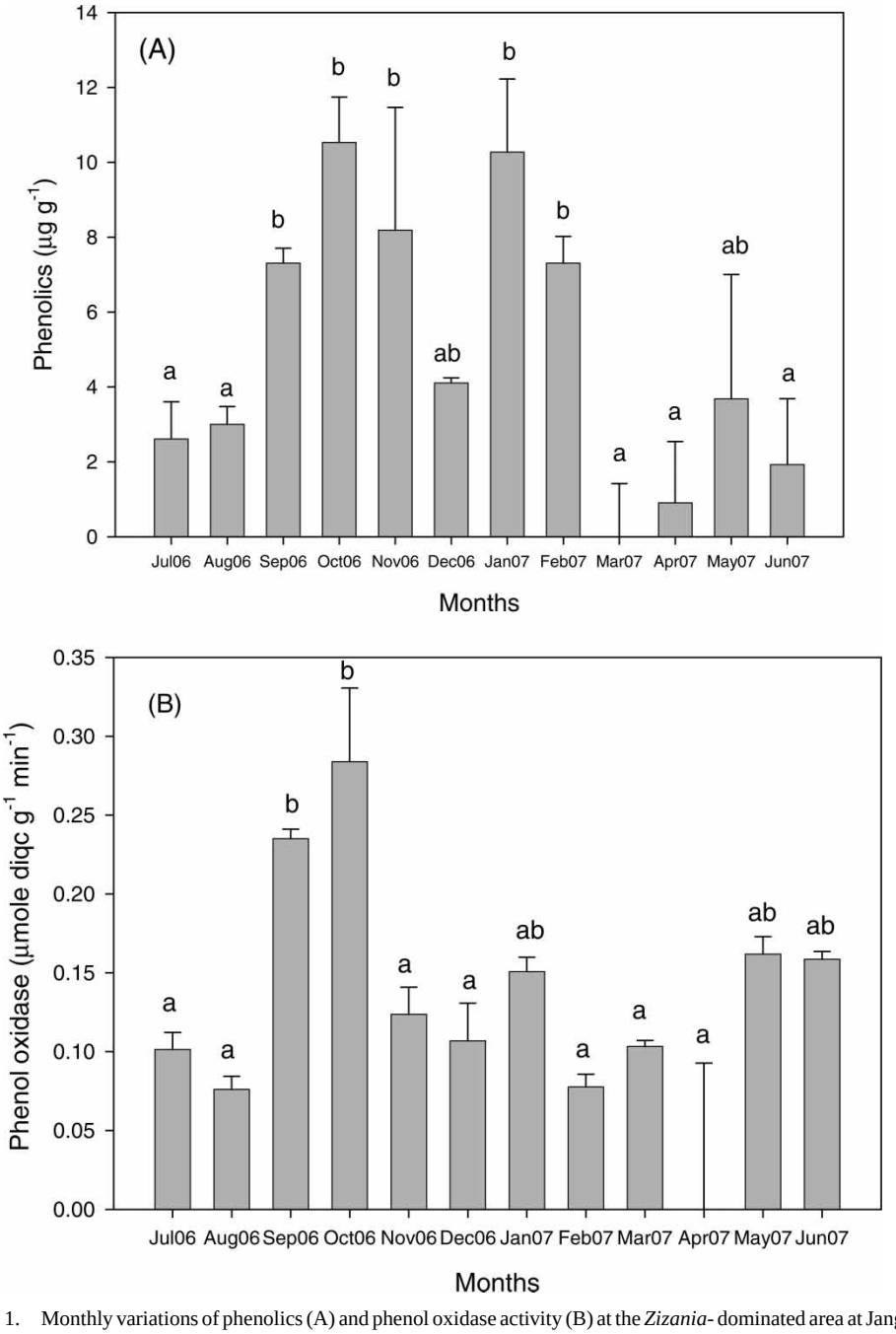


Figure 1. Monthly variations of phenolics (A) and phenol oxidase activity (B) at the *Zizania*- dominated area at Janghang estuary, Korea. Data labelled with different letters are significantly different to each other at $p < 0.05$ (one-way ANOVA).

peatlands, where phenol oxidase activity exhibited a peak over late autumn, which appeared to be related to substrate quality.

Over the four sampling occasions, phenolic content was highest at the mud flat with a range of $8.6 \pm 3.8$ to $15.0 \pm 2.3\,\mu g\,g^{-1}$. Likewise, phenol oxidase activity was higher at the mud flat than the other two areas in August and October (Figure 2).

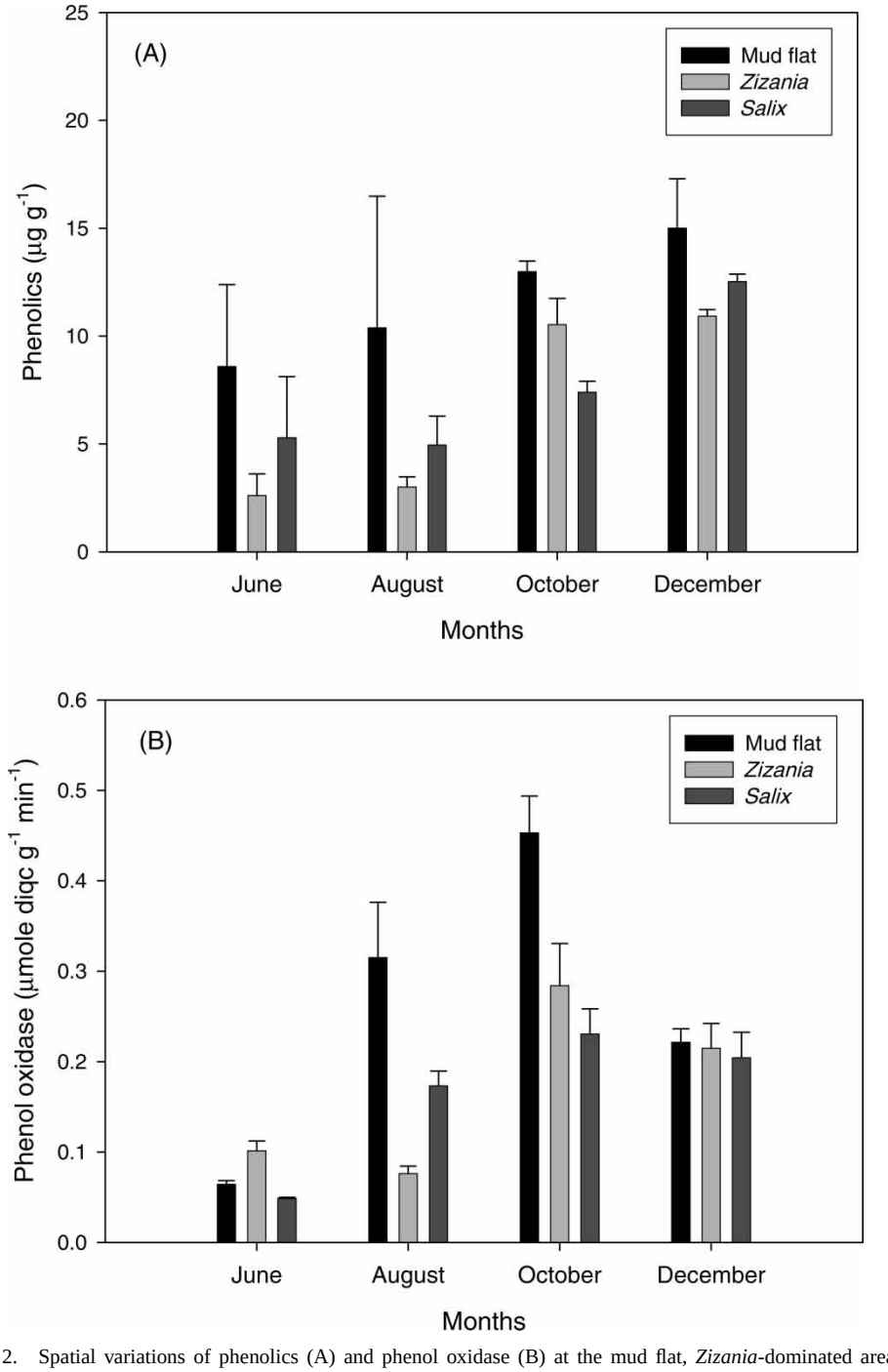


Figure 2. Spatial variations of phenolics (A) and phenol oxidase (B) at the mud flat, *Zizania*-dominated area and *Salix*-dominated area at Janghang estuary, Korea in June, August, October, and December in 2006.

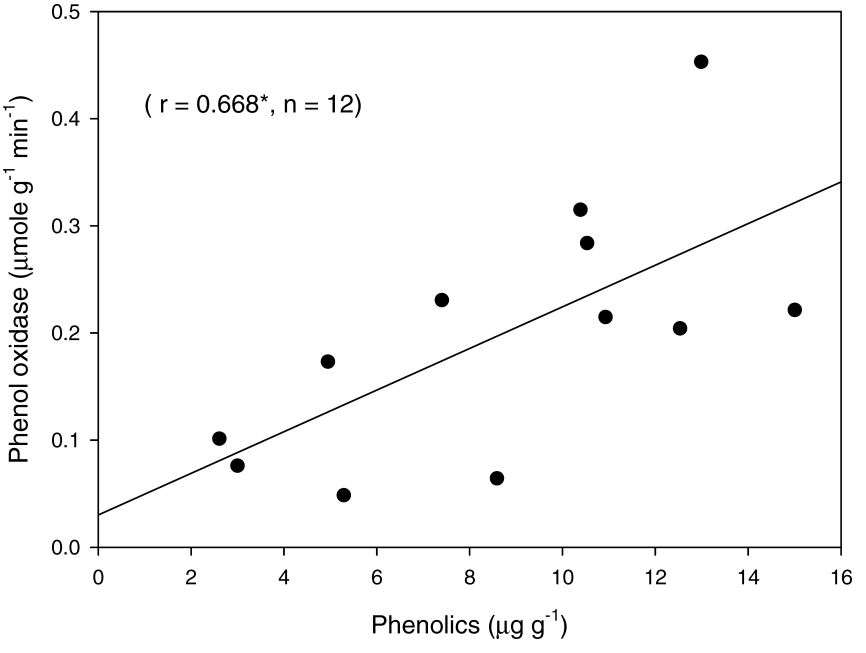


Figure 3. Relationship between phenol oxidase activity and phenolics in three sites over four sampling occasions. Each data point is a mean of three replicate samples.



A correlation analysis revealed a positive correlation between phenol oxidase activity and phenolics when the data from the three areas were combined (Figure 3). The positive correlation could be caused by two factors, namely spatial differences among three areas, or temporal variations among four sampling occasions. The former is supported by the fact that both phenolics and phenol oxidase activity were higher at the mud flat than those of vegetated areas (Figure 2). In addition, temporal variations in each site also contributed to a positive correlation between phenol oxidase activity and phenolics. This is supported by the fact that a similar pattern of positive relationships between phenolics and phenol oxidase activity were observed when data in each area were analysed separately (Figure 4).

These overall patterns of positive correlation between phenolics and phenol oxidase activity are contradictory to previous reports where negative correlations were often reported between the two in peatlands [13]. Such negative correlations were also reported during N addition experiments in temperate forests [7,14], where N addition decreased phenol oxidase activity whilst phenolic contents increased. Those findings suggested that lower phenol oxidase activity may result in the accumulation of phenolics in soil ecosystems. However, Fenner et al. [15] have reported positive correlations between phenol oxidase and phenolics during all four seasons in peatlands under the water logged conditions. They proposed that activation of phenol oxidase may result in mobilisation of phenolics from the organic matrix of peat. The system studied is fairly dry compared to their sites, but probably similar mechanisms may function, which result in positive correlations.

The finding of contradicting reports from peatlands or upland forest soils could probably result from differences in chemical properties. The estuarine sediment we assessed may differ from other systems in terms of chemical properties of the soil. For example, phenol oxidase can be activated by the addition of $Fe^{2+}$ [16], which we did not measure in the present study. In addition, the main carbon input to soil in our system is different from that in peatlands or forest soils. Peat soils are often enriched in high concentrations of phenolics which originated from *Sphagnum*.

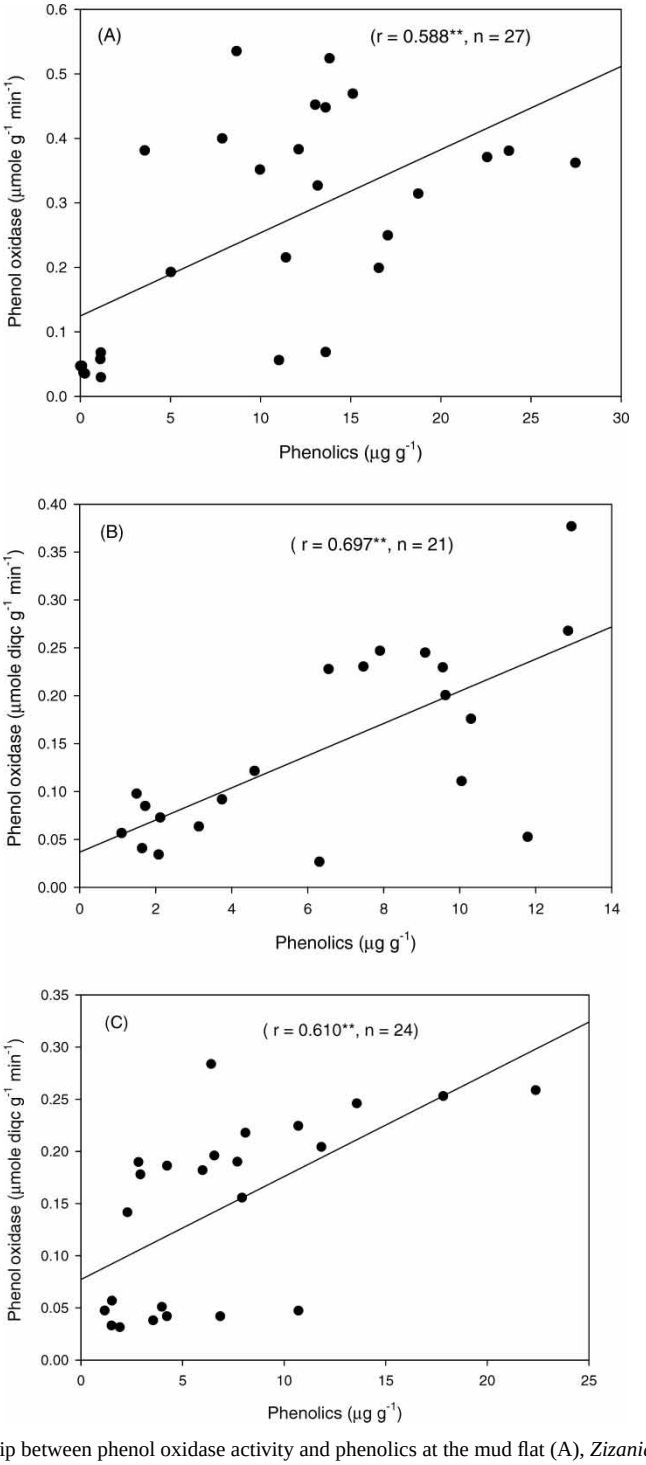


Figure 4. Relationship between phenol oxidase activity and phenolics at the mud flat (A), *Zizania*-dominated area (B) and *Salix*-dominated area (C) at Janghang estuary, Korea in 2007.

In such systems, phenolics would accumulate if phenol oxidase decreased. In contrast, the low concentration of phenolics in our system could induce enzyme production by the mechanism of substrate induction [17], which can result in a positive correlation between phenolics and phenol oxidase. Another possibility is that the phenolic levels determined in this study are just a product generated by phenol oxidase, as noted by Fenner et al. [15].

In this study, our results demonstrated that phenol oxidase would be positively correlated with phenolics in estuarine sediment at various spatio-temporal scales. Various types of recalcitrant organic matter can be transported from upland areas and deposited in estuary ecosystems. Therefore, the results of this study suggest that such systems could oxidize and then remove recalcitrant organic matter effectively up to a certain range.

Because we did not determine microorganisms directly which may produce phenol oxidase, further study should focus on diversity and biomass of phenol oxidase-producing microorganisms under different concentrations of phenolics.

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