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Research Article

Seasonal Dynamics of Rhizosphere Soil Microbial Abundances and Enzyme Activities under Different Vegetation Types in the Coastal Zone, Shandong, China

Microbial community responses to alternative management may be indicative of soil quality change. In this study, soils were collected from research plots over one year. The rhizosphere soils were studied by measuring microbial abundance (bacteria, fungi, actinomycetes, and ammonifiers), enzyme activities (invertase, catalase, urease, and phosphatase), and their relationship. Rhizosphere microbial abundance of different plant species varied greatly across seasons. The rhizosphere environment generated by *Phragmites australis* and *Echinochloa crus-galli* is suitable for microbial growth. In addition, the significant differences in rhizosphere enzyme activities of different plant species across seasons were also observed. There was a significant linear correlation between rhizosphere soil enzyme activities and microbial abundances except for between bacteria and catalase and between fungi and urease, but no such significant relationship was found between all rhizosphere soil microbial abundance and phosphatase except between fungi and acid phosphatase. We concluded that different plant species in coastal areas have different rhizosphere soils, due to the impact of the different root exudates and plant residues of the microbial properties.

1 Introduction

The coastal zone is the area like an interface between the human society and the marine ecological system, and it is one of the most important components in the global system for keeping the sustainable development in the ecology, the economics, and the society. In this region, harsh environments and extreme weather together with inappropriate human activities caused vegetation destruction, which led to serious soil erosion, fertility reduction, and environmental degradation. The spatial and temporal availability of water and nutrients varied extremely in degraded soils together with soil properties changes, which lead to complex interactions between plant and soil, caused by dominant plants [1]. Thus soil degradation is considered as a major threat to the sustainable development of the soil ecosystem in this area. The loss of plant cover is one of the important factors in these soil degradation [2]. The presence of vegetation in this area is important because it provides physical protection and organic matter, which enhances soil fertility levels and water holding capacity [3]. The soil microbial community composition was also affected by these plants [4], which can affect the stability of soil aggregate structure [5, 6].

Soil microorganisms are widely recognized as integrative components of soil quality because of their crucial involvement in many ecosystem processes [7]. Examples of potential biological indicators include microbial biomass [8], microbial abundance [9], and enzyme activities [10, 11]. Physical and chemical properties have been widely used for the assessment of soil quality [12]. However, it will take many years to change significantly for these properties. While microbial populations and enzyme activities of soil properties have been extensively considered to be important factors to reflect any change in soil management and land use because they respond relatively quickly [13, 14]. Additionally, the changes of soil quality can be detected by soil microbial and enzyme activities more sensitively than by physical and chemical properties [15]. So microbial and enzymatic properties are considered to be early and sensitive indicators of future soil changes [16].

Photosynthetic products are provided by plants roots for microbial community, so the rhizosphere can enhance microbial activity [17, 18], further increasing the microbial abundance of rhizosphere [17]. Rhizosphere microbial processes are important factors in determining the survival and sustainable growth of plant [16]. Warembourg et al. [19] and Garcia et al. [20] reported that microbial growth and activity in the rhizosphere is closely related to root exudates, such as low molecular weight organic acids [21, 22] and plant debris, which varied among different plant species.

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Abbreviations: CFU, colony forming unit; **dw**, dry weight

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There is little available information on the rhizosphere soil microbial properties during revegetation in the coastal zone, and most of studies about rhizosphere soil microorganism and enzyme activity were made at a single point in time. So, the objective of the current study was to investigate the rhizosphere soil microbial abundance and enzyme activities of different plant species varied seasonally in response to changes in environmental conditions. The species included two natural grassland (*Phragmites australis* and *Echinochloa crus-galli*), one natural shrubland (*Rosa multiflora*), and one artificial woodland (*Pinus thunbergii*). For this purpose, microbial abundance, enzyme activities, and their relationship were discussed in the rhizosphere soil of four plant species and in a control soil over one year.

2 Materials and methods

2.1 Sampling

The study was carried out at integrated experimental station of coastal zone environment of Yantai Institute of Coastal Zone Research, Chinese Academy of Sciences, where lies in the warm temperate zone, with maritime feature of warm temperate continental monsoon climate. The average temperature and rainfall of the year is 12°C and 620 mm, respectively. Soil samples were taken from the top 20 cm in May, August, November of 2011 and February of 2012. Several randomly located soil cores were collected from the rhizosphere of each species in each plot, hand-shaking method was used to separate the roots and substrates. The soil samples were mixed into a sample and divided into two sub-samples; one of the sub-samples was used to determine the microbial abundances and another was stored at 4°C for enzyme activity analysis.

2.2 Analyses for microbial abundances and enzyme activities

One sub-sample was analyzed for different microbial abundances using tenfold serial dilutions and four different media. Ten grams of the soil samples were diluted in 90 mL sterile distilled water. Bacteria, fungi, actinomycetes, and ammonifiers required different soil solution concentrations for accurate propagule estimates. Bacteria were estimated on full-strength nutrient agar, fungi and actinomycetes were grown on Martin's medium and Gause's No. 1 synthetic medium, respectively. The most probable number method was used to estimate the physiological groups of substrate microbes. Polypetone as the only source of nitrogen in a nutrient solution, which was used to inoculate ammonifiers and incubated for seven days. All microbial abundances were computed on the basis of dried weight of soil. The soil moisture content of each sample was determined by the oven-drying at 105°C for 48 h.

The activities of soil invertase, catalase, urease, and phosphatase were determined according to Lin [23]. Urease activity was measured using 10 g of air-dried soil, 20 mL of citrate solution at pH 6.7 and 10 mL of 10% urea solution. The samples were incubated at 38°C for 3 h and then diluted to 100 mL with distilled water. The suspension was filtered and a 1 mL aliquot was treated with 4 mL of sodium phenol solution and 3 mL of 0.9% sodium hypochlorite solution. The released ammonium was determined colorimetrically with a spectrophotometer (Hitachi, UV2300) at 578 nm. Results were expressed as mg NH_4^+-N g^{-1} soil h^{-1}.

Catalase activity was determined using 5 g of air-dried soil with 40 mL of distilled water and 5 mL of 0.3% H_2O_2, shaken for 30 min (at 120 rpm) and then filtered immediately. The filtrate was titrated with 0.1 mol L^{-1} $KMnO_4$ and the results were expressed as mL $KMnO_4$ g^{-1} soil h^{-1}.

For the assessment of the soil invertase activity, 10 mL of 20% sucrose solution were used as the substrates for the enzyme. Soil portions (10 g of air-dried soil) were incubated at 37°C for 24 h with 1.5 mL of toluene and 10 mL of phosphate buffer at pH 5.5. The filtered soil solution was transferred to a triangular flask with 10 mL of feline solution, 20 mL of distilled water, and heated for 10 min. After the solution reached room temperature, it was treated with 3 mL of 33% KI solution, 4 mL diluted sulfuric acid (1:3) and 0.5 mL of starch indicator solution. The mixture was titrated with 0.1 mol L^{-1} $Na_2S_2O_3$ and the results were expressed as mL $Na_2S_2O_3$ g^{-1} soil h^{-1}.

Acid, alkaline, and neutral phosphatase activities were determined using 10 g of air-dried soil (sieved to <1 mm), then 1.5 mL of toluene, 10 mL of disodium phenyl phosphate solution and 10 mL of 0.05 M borate buffer at pH 5, 7, and 10, respectively, were added to the soil samples. The suspensions were incubated at 37°C for 3 h. Then the suspensions were filtered and the filtrate was colored with 3 mL of 0.5% 4-aminoantipyrine and 3 mL of 2.5% potassium ferrocyanide; the phenol content, released from soil, was determined colorimetrically with a spectrophotometer (Hitachi, UV2300) at 570 nm. Results were expressed as mg phenol g^{-1} soil h^{-1}.

2.3 Statistical analysis

All data were analyzed using SPSS 18.0 for Windows. All measurements were replicated three times and the differences between different soils were compared using one-way analysis of variance (ANOVA) followed by a Student−Newman−Keuls test. The relationships between microbial abundances and enzyme activities were analyzed using correlation analysis, and expressed as Pearson coefficients.

3 Results

3.1 Microbial abundances of different rhizosphere soils

Microbial abundances varied significantly among the rhizosphere soils of different plant species and the different seasons (Fig. 1). Bacterial abundance ranged from 0.1210×10^6 to 10.5150×10^6 colony forming units (CFUs)/g dry weight (dw) in the different rhizosphere substrates across seasons (Fig. 1a). *P. australis* and *E. crus-galli* supported a higher bacterial abundance (varying from 1.9910×10^6 to 10.5150×10^6 and from 1.8083×10^6 CFUs/g dw, respectively) than other species.

Fungal abundance ranged from 0.1483×10^5 to 20.0452×10^5 CFUs/g dw. Rhizosphere substrates harbored higher fungal abundance (varying from 1.5268×10^5 to 20.0452×10^5 CFUs/g dw) in autumn compared to other seasons except *P. australis*, which was higher in summer and winter (Fig. 2a).

Similar with bacterial abundance, actinomycete abundances were higher in *P. australis* (varying from 4.7648×10^5 to 251.3795×10^5 CFUs/g dw) and *E. crus-galli* (53.5798×10^5 to 173.4984×10^5 CFUs/g dw). Actinomycete abundance of *P. australis* was higher than *E. crus-galli* in spring and winter, while lower in summer and autumn (Fig. 1c).
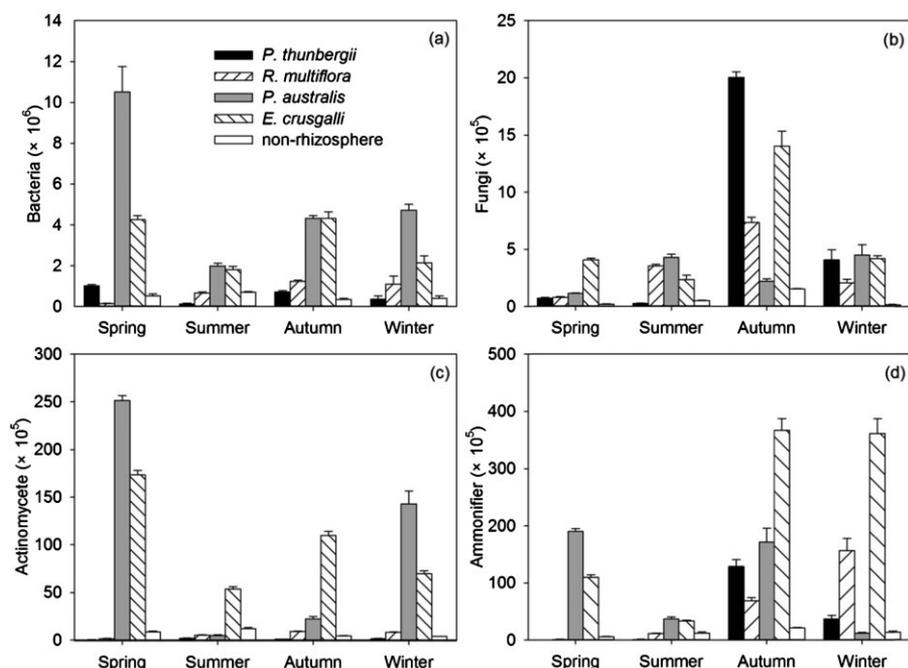
Figure 1. Seasonal changes of soil microbial abundances in rhizosphere substrates among different vegetation types: (a) indicates seasonal dynamics of bacteria from spring to winter; (b) indicates seasonal dynamics of fungi from spring to winter; (c) indicates seasonal dynamics of actinomycete from spring to winter; (d) indicates seasonal dynamics of ammonifer from spring to winter.

A different trend was observed for the ammonifier abundance, which ranged from 0.2706×10^5 to 367.2720×10^5 CFUs/g dw. Ammonifier abundances of different species and non-rhizosphere were higher in autumn or winter than that in spring and autumn (Fig. 1d).

3.2 Enzyme activities in rhizosphere substrates

From Fig. 2a, rhizosphere soil of different plant species across seasons differed significantly for all the enzyme activities tested for. Activities of urease in the rhizosphere of different species ranged from 1.7060 ± 0.5726 to 25.5092 ± 1.1452 mg NH_4-N $g^{-1} h^{-1}$. In general, urease of different species and non-rhizosphere were higher in spring and winter, while the highest value of urease under *P. australis* was found in summer (25.5092 ± 1.1452 mg NH_4-N $g^{-1} h^{-1}$).

The catalase activities in all the rhizosphere soils (varying from 1.3333 ± 0.3055 to 6.5333 ± 0.1155 mL 0.1 mol L^{-1} $KMnO_4 g^{-1} h^{-1}$) was higher than that of the control soil (varying from 0.4667 ± 0.1155 to 1.9333 ± 0.3055 mL 0.1 mol L^{-1} $KMnO_4 g^{-1} h^{-1}$) in different seasons. Soil catalase under *P. thunbergii* and *R. multiflora* were higher in autumn and winter, while other species and non-rhizosphere were higher in spring (Fig. 2b).

Similar with catalase, invertase activities in all the rhizosphere soils of different seasons were higher than that of the non-rhizosphere soil. Invertase activities of different species and non-rhizosphere were higher in autumn and winter (varying from 0.1667 ± 0.0289 to 1.0167 ± 0.0289 mL 0.1 mol L^{-1} $Na_2S_2O_3 g^{-1} h^{-1}$) than that in spring and summer (varying from 0.0833 ± 0.0289 to 0.3333 ± 0.0289 mL 0.1 mol L^{-1} $Na_2S_2O_3 g^{-1} h^{-1}$) (Fig. 2c).

Acid phosphatase levels of different species ranged from 0.0499 ± 0.0086 to 0.4066 ± 0.0086 mg pNP $g^{-1} h^{-1}$, which were higher than that of the non-rhizosphere soil in the corresponding season (Fig. 3a). In general, acid phosphatase activities of different species and non-rhizosphere were lower in spring than in other

seasons. On the contrary, alkaline and neutral phosphatase activities, which varied from 0.0103 ± 0.0149 to 0.3224 ± 0.1362 mg pNP $g^{-1} h^{-1}$ and from 0.0350 ± 0.0172 to 0.4611 ± 0.0944 mg pNP $g^{-1} h^{-1}$, respectively, were significantly higher in spring than in other seasons (Fig. 3b and c). Alkaline phosphatase activities of the control soil were lower than rhizosphere of plant species. While neutral phosphatase activities of non-rhizosphere were higher in the spring and lower in other seasons than all plant species.

3.3 The relationship between microbial abundances and enzyme activities in rhizosphere substrates

The correlation analysis indicated that the relationship between rhizosphere soil microbial abundances and enzyme activities of different plant species across seasons varied in the coastal zone substrates (Table 1). There was a significant linear correlation between rhizosphere soil bacteria abundances and urease, invertase activities, between fungi abundances and catalase, invertase activities, between actinomycete abundance and urease activities. While there has no significant relationship between ammonifier and all enzyme activities. Significant correlations among catalase, urease, invertase, acid phosphatase and alkaline phosphatase were also found, but no such significant relationship was found between neutral phosphatase and microbial abundance and other enzyme activities. In addition, bacteria were correlated significantly with actinomycete and ammonifier. Significant relationships were also found between fungi and ammonifier and between actinomycete and ammonifier.

4 Discussion

Different plant product different root exudates [24] and the vegetation growth [25], by which they support different rhizosphere
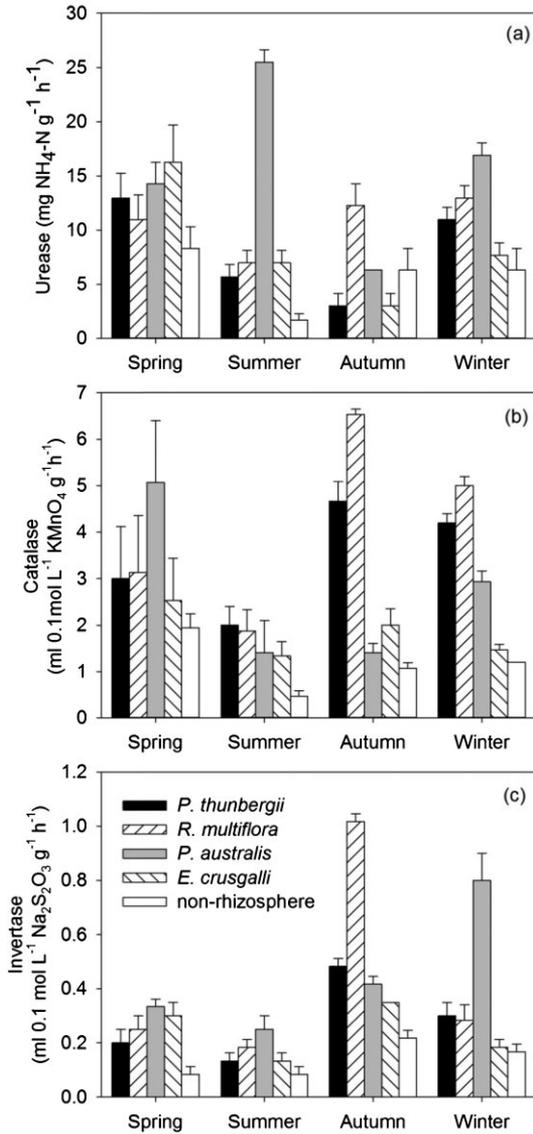
Figure 2. Seasonal changes of soil urease, catalase, and invertase activities in rhizosphere substrates among different vegetation types: (a) indicates seasonal dynamics of urease from spring to winter; (b) indicates seasonal danamics of catalase from spring to winter; (c) indicates seasonal dynamics of invertase from spring to winter.

Figure 3. Seasonal changes of soil phosphatase activities in rhizosphere substrates among different vegetation types: (a) indicates seasonal dynamics of acid phosphatases from spring to winter; (b) indicates seasonal dynamics of alkaline phosphatases from spring to winter; (c) indicates seasonal dynamics of neutral phosphatases from spring to winter.

microbial flora, so this study also showed that rhizosphere microbial abundances varied significantly with different plant species [26, 27]. In the current study, *P. australis* supported a higher bacterial abundance in every season, while fungi abundance peaked in *P. thunbergii*, followed by *E. crus-galli* in autumn. In general, the rhizosphere of *P. australis* and *E. crus-galli* supported higher actinomycete and ammonifier abundance. The above results indicate that the rhizosphere environment generated by *P. australis* and *E. crus-galli* is suitable for microbial growth.

Enzyme activity has been used to assess environmental quality in the past several decades [28]. We determined four rhizosphere enzyme activities relate to nitrogen and phosphorus removal in the present study. We found that the rhizosphere enzyme activities of different plant species varied significantly across seasons. The varied catalase activities of different plant species showed the different
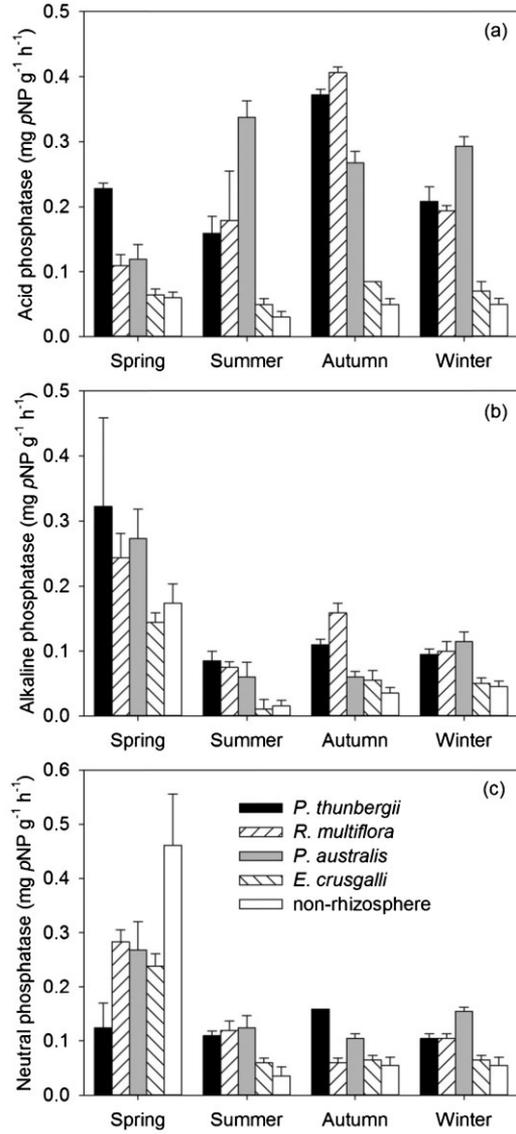
rhizosphere soils oxidative and oxido-reductive potentials of these plants [16]. The higher level of catalase activity was found in the rhizospheres under *P. australis* in spring, but under *R. multiflora* in autumn and winter. Soils under all plant species and non-rhizosphere showed higher values of alkaline phosphatase in spring compared with other seasons, the depletion of available phosphorous or increased adsorption of inorganic phosphorous by plant roots could be responsible for the above results [20]. The season variations in alkaline and neutral phosphatase we observed are consistent with the seasonal dynamics of acid phosphatase activity, which was reported in a study of a beech forest [29]. In addition, Kang and Freeman [30] reported that the acid phosphatase activity was highest in early spring and lowest in autumn and the significant correlationship was found between acid phosphatase with soil temperature or soil water content.

Table 1. The relationship between microbial abundances and enzyme activities in rhizosphere substrates

	Fungi	Actinomycete	Ammonifier	Catalase	Urease	Invertase	Acid phosphatase	Alkaline phosphatase	Neutral phosphatase
Bacteria	0.054	0.909**	0.468**	0.194	0.281*	0.256*	0.000	0.254	0.202
Fungi		0.053	0.450**	0.315*	−0.197	0.427**	0.450**	−0.128	−0.143
Actinomycete			0.427**	0.162	0.269*	0.202	−0.194	0.234	0.243
Ammonifier				0.084	−0.166	0.094	−0.085	−0.131	−0.159
Catalase					0.284*	0.613**	0.548**	0.534**	0.177
Urease						0.268*	0.374**	0.353**	0.235
Invertase							0.705**	0.138	−0.117
Acid phosphatase								0.174	−0.099
Alkaline phosphatase									0.556**

*indicates the significance of $P < 0.5$;
**indicates the significance of $P < 0.05$.

On the other hand, we did not observe seasonal dynamics of some enzyme activities, which was similar with other studies [31]. Additionally, Dick et al. [10] found that the acid phosphatase activity of North American agricultural fields soil did not change significantly with season, Bandick and Dick [11] reported that β-glucosidase activity varied little when soil water content changed in similar systems. Bergstrom et al. [32] also suggested that β-glucosidase activity was small across seasons but large when compared between year and year. Some studies attributed those seasonal variations of soil enzyme activity to pattern of soil temperature and/or water content, but the enzyme activity varied with enzymes, soil properties, and ecosystem types in specific season [31].

In general, hydrolases, such as urease and phosphatase, involved in the N and P cycles and showed higher values in the rhizosphere soils of plant than in the control soils. In the present study, soil enzymes (urease, catalase, invertase, and phosphatase) were higher in the rhizosphere soils of different plant species than in the non-rhizosphere soil except neutral phosphatase in spring. Garcia et al. [20] and Niu et al. [26] also showed that urease activity of non-rhizosphere soil was lower than rhizosphere soil. Speir et al. [33] found that urease activities of planted soils increased, whereas decreases were observed in unplanted soils.

The correlation analysis showed that the relationship between rhizosphere enzyme activities and soil microbial abundances varied in the coastal zone substrates. There was a significant linear correlation between rhizosphere enzyme activities and soil microbial abundances except for between bacteria and catalase and between fungi and urease, but no such significant relationship was found between all rhizosphere soil microbial abundance and phosphatase except between fungi and acid phosphatase. Ge et al. [34] found that microbial abundance and enzyme activity of rhizosphere soil did not consistently increase or decrease for different plant species. The different biological characteristics of plant species, different litter components or the humus decomposition pathway in soil under revegetation plants could be responsible for the above results.

Seasonal dynamics of microbial abundance and enzyme activities depend on plant community composition [35, 36]. The conclusion can be made from above results that soil's chemical, physical, and biological properties, then soil quality of different seasons were influenced by plant species used to establish a plant cover [3, 35, 36]. Microbial abundance and enzyme activity of rhizosphere soils change among different plant species and relate to root exudates and plant growth, which produce differently respond to seasonal change. Because microorganisms play an important role in the nutrient cycle of soil, it is very important to choose suitable plant species, which provide appropriate environment for microbe growth, to reclaim the disturbed coastal sandy land.

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