



# Effects of watershed land-cover on the biogeochemical properties of estuarine tidal flat sediments: A test in a densely-populated subtropical island



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## ABSTRACT

The effects of watershed land cover on the biogeochemical properties of estuarine tidal flat sediment were examined in estuarine tidal flats of 16 watersheds in a densely populated, subtropical island of Japan. Despite the small sizes of the watersheds (<16.5 km<sup>2</sup>), a redundancy analysis showed that river water quality explained 62% of the cross-estuary variation in the biogeochemical properties of estuarine tidal flat sediment by the first two ordination axes. We also found that the dissolved nutrient concentrations of river water and pheophytin *a* content of tidal flat sediment were positively related to agricultural and urbanized land cover in the watersheds. These results indicate that human nutrient inputs significantly increase algae-derived deposits in estuaries with relatively more developed watersheds. The  $\delta^{13}\text{C}$  of particulate organic matter (POM) was negatively related to watershed forest cover. This suggests that terrestrially derived-origin POM deposits are substantial in the estuaries connected to watersheds with relatively high forest cover. However, the chemical properties of tidal flat sediment were not related to chemical indicators of POM in the base flow. We hypothesize that substantial terrestrially derived POM is discharged to estuaries of high-forest-cover watersheds during high flow, and this partially controls the chemical properties of estuarine sediments. Our results demonstrate that the chemical properties of estuarine tidal flats are associated with watershed land cover, and that the dominant processes controlling estuarine sediment properties differ among watersheds depending on land cover composition.

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## 1. Introduction

Estuarine tidal flats are important habitats for various organisms and contribute to nutrient cycling in coastal marine systems (Levin et al., 2001). In particular, the biogeochemical properties of sediment, such as the quantity and quality of organic matter, affect oxygen consumption, nutrient cycling, and food acquisition by benthic organisms in tidal flats (Mortimer et al., 1999; Pratt et al., 2014; Sutula et al., 2014). In many watersheds, land cover is altered by various types of human activity (e.g., forest logging, urbanization, and agricultural land development), and the quantity,

quality, and origin of materials transported through rivers to estuaries reflect those human activities on land (Ahearn et al., 2005; Sakamaki and Richardson, 2011; Young et al., 1999). For instance, nutrient concentrations in river water tend to be high in areas with high urban and agricultural land use (Correll et al., 1992), and are generally lower in watersheds with relatively high pristine forest cover and/or plains (Basnyat et al., 1999; Ngoye and Machiwa, 2004). The quantity and quality of riverine particulate organic matter (POM) also vary according to riparian and watershed environments (e.g., coverage and type of vegetation, land use type) (Frost et al., 2009; Sakamaki and Richardson, 2011). Therefore, examining the relationships between the biogeochemical properties of estuarine tidal flat sediments and watershed land cover may provide a good measure of human impacts on estuarine tidal flats. Materials transported from watershed are expected to be a major component of those depositing in estuarine tidal flats (Howarth

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et al., 1991). However, there have been no studied statistically testing the relationships between watershed land use, chemical properties of river water and tidal flat sediment properties.

Carbon and nitrogen stable isotope ratios ( $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ ) and the C/N ratio are useful chemical indicators to identify the origins of POM (e.g., Cloern et al., 2002; Meyers, 1994). In particular, human-derived nitrogen (e.g., manure, sewage treated water) tends to have relatively higher  $\delta^{15}\text{N}$  compared with naturally derived nitrogen (Umezawa et al., 2002; Viana and Bode, 2013). These tracers are widely used to measure the relative contributions of organic matter sources with different origins in biogeochemical and ecological studies of estuaries (e.g., Volkman et al., 2008; Zhou et al., 2006). However, the biogeochemical properties of estuarine sediments are affected not only by material inputs from adjacent systems (e.g., watershed and offshore ocean systems), but also by various other factors, such as hydrodynamic conditions, geomorphology, and biological processes of benthic organisms (e.g., Sakamaki and Nishimura, 2007; Widdows and Brinsley, 2002). The complexity of the processes controlling estuarine environments may obscure watershed effects in estuaries, and poses a challenge in our general understanding of the linkage between watershed land cover and biogeochemical properties of estuarine sediments.

In general, sediment properties are spatially heterogeneous, both across and within estuaries (e.g., Sakamaki et al., 2010). Thus, to find general patterns of the effect of watershed land cover on estuarine tidal flat sediment, proper statistical designs for data sampling and analyses are needed. In particular, variation in chemical properties of tidal flat sediments across estuaries needs to be measured, taking into account variation within estuaries, and examined with respect to watershed conditions. In fact, Sakamaki et al. (2010) reported a significant relationship between the content of watershed-derived organic matter in estuarine tidal flat sediment and watershed size using a field sampling design that accounted for variation in sediment properties both across and within estuaries in twenty watersheds of the Pacific Northwest Coast. This example suggests the validity of the combined application of chemical markers, such as stable isotopes, and statistical analyses with a proper field sampling design. However, no study has tested whether or how watershed land cover affects the biogeochemical properties of sediments in estuarine tidal flats.

In Okinawa Island, located in the subtropical, southwestern part of Japan, we investigated 16 estuarine tidal flats that belong to independent watersheds with different land cover compositions of forest, urban, and agricultural areas. Specifically, we aimed to answer the following questions. 1) Can we detect significant effects of watershed land cover on the biogeochemical properties of sediment in estuarine tidal flats? 2) What are typical biogeochemical characteristics of tidal flat sediment specific to watersheds predominated by forested, urbanized, or agricultural area? To answer these questions, we used multiple chemical markers, such as the carbon: nitrogen ratio and their stable isotope ratios, to analyze our field samples, and tested the statistical relationships between watershed land cover composition, river water chemistry, and sediment properties of estuarine tidal flats.

## 2. Methods and materials

### 2.1. Study sites and field sampling

A total of 16 estuarine tidal flats and rivers entering those estuaries in Okinawa Island of southwestern Japan were studied ( $26^{\circ}52' - 26^{\circ}04'\text{N}$ ,  $127^{\circ}38' - 128^{\circ}19'\text{E}$ ) (Fig. 1 & Fig. S1). The watershed areas ranged from  $0.4 \text{ km}^2$  to  $16.5 \text{ km}^2$ . From all the study sites, tidal flat sediments and river water were sampled in September 2010. Three tidal flat sediment samples were collected

from each of the low (L), upper (U), and middle (M) parts of the intertidal area along a main river channel in each estuary at low tide during spring tides. Tidal flat sediments were collected using a core sampler with a depth of 7 cm and a diameter of 12 cm, and the 0–1-cm depth layer was used for the chemical analysis. River water was also sampled around the most downstream freshwater reach of each river at base flow. All sediment and water samples were stored in an icebox and carried to the laboratory.

### 2.2. Sample analyses

In the laboratory, the collected sediment and water samples were stored in a refrigerator and processed for analyses in a couple of days. To measure chlorophyll *a* (Chl.*a*) and pheophytin *a* (Pheo.*a*), subsamples of tidal flat sediment of ca.  $1 \text{ cm}^3$  in volume were collected from the 0–1-cm depth layer of each of the three core sediments obtained within each part (i.e. U, M or L) of estuary. Three subsamples were mixed to composite a representative sample of each part of estuary in the laboratory. The composited samples were soaked in 90% acetone for 24 h in a refrigerator to extract algal phytopigments. After the extraction, samples were manually shaken and centrifuged. Chl.*a* and Pheo.*a* were estimated as the amounts of pigment per dry weight of sediment using a spectrophotometer (V-630, JASCO Corporation, Tokyo, Japan) following Lorenzen's method (Lorenzen, 1967).

For analyses of fine fraction of tidal flats sediment, subsamples were taken from each of the three samples collected within each part of estuary and mixed to composite a representative sample of the part of estuary. To determine the mud content of tidal flat sediments, the dry masses of the composited samples from each part of estuaries were measured before and after samples sifted using a  $63\text{-}\mu\text{m}$  sieve to remove mud. We further used the sediment samples of  $<63 \mu\text{m}$  for chemical analysis, since we aimed to analyze biogeochemical properties of organic particles and finer particles generally contain more organic matter compared with coarser particles (Sakamaki and Nishimura, 2006). The sediment samples of  $<63 \mu\text{m}$  were acidified with 10% HCl to remove carbonate in plastic tubes for at least 24 h. The sediment samples were centrifuged and rinsed with deionized water. This process was repeated three times to remove HCl, and the sediment samples were dried in an oven at  $60 \text{ }^{\circ}\text{C}$ . Suspended POM in river water was collected on precombusted glass fiber filters (Whatman GF/F) by filtration. To remove carbonate, the filter samples were acidified with 10% HCl and then rinsed with deionized water. Those samples were dried in an oven at  $60 \text{ }^{\circ}\text{C}$ , and preserved in a desiccator until the following elemental analyses. Carbon and nitrogen contents and the stable isotope ratios ( $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$ ) of the riverine POM and  $<63 \mu\text{m}$  tidal flat sediment were measured using an element analyzer (Flash EA 1112, Thermo Electron, Waltham, MA, USA) connected to a mass spectrometer (Delta Plus Advantage, Thermo Electron).  $\text{L-Alanine}$  ( $\delta^{13}\text{C}$ :  $-19.6\text{‰} \pm 0.2\text{‰}$ ,  $\delta^{15}\text{N}$ :  $8.7\text{‰} \pm 0.2\text{‰}$ ) was used as standard reference material for above analysis.

The river water samples were filtered through membrane filters with pore sizes of  $0.45 \mu\text{m}$ . The filtered river water was analyzed for nutrient constituents by applying the following conventional methods (Rice et al., 2012). The analyses for nitrate ( $\text{NO}_3\text{-N}$ ), nitrite ( $\text{NO}_2\text{-N}$ ), ammonium ( $\text{NH}_4\text{-N}$ ) and phosphate-phosphorus ( $\text{PO}_4\text{-P}$ ) were conducted by using a continuous flow autoanalyzer (QuAAtro 2HR, BL TEC K. K., Osaka, Japan).  $\text{NO}_3\text{-N}$  and  $\text{NO}_2\text{-N}$  were determined by naphthyl ethylenediamine absorptiometry.  $\text{NH}_4\text{-N}$  and  $\text{PO}_4\text{-P}$  were analyzed by the indophenol blue method and molybdenum blue method, respectively. Dissolved total phosphorus and dissolved total nitrogen were analyzed using an auto analyzer (TRACCS800, BL TEC K. K., Osaka, Japan) after digestion with sodium hydroxide and potassium peroxydiphosphate. All of

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