Quantitative DNA assays for detecting Zostera marina DNA in coastal Sediments

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The sequestration of organic carbon (OC) in seagrass meadows has been attracting more attention as global actions to climate change mitigation and adaptation increase. A direct method to detect *Zostera marina* DNA in coastal sediments, which is essential to unravel long-term *Z. marina*-derived OC accumulation, was developed as an environmental DNA (eDNA) detection techniques. Quantitative real-time PCR (qPCR) and droplet digital PCR (ddPCR) were applied to quantify ancient *Z. marina* DNA in coastal sediments, using specifically-designed dual-labeled probes (DLPs) and primers for one nuclear and one chloroplast gene. Suitable pretreatments and methods for extracting *Z. marina* DNA from coastal sediments were examined and their applicability to environmental samples was determined. Surface sediments collected from *Z. marina* meadows contained about 2000 times more DNA than the adjacent unvegetated tidal-flats in the Seto Inland Sea. Moreover, both qPCR and ddPCR successfully detected *Z. marina* DNA in ancient sediments (up to 5000 calibrated years before present (yr cal BP)), evidencing that *Z. marina* DNA can be sequestrated in temperate coastal sediments for several millennia. In addition, qPCR and ddPCR results obtained in the present study were highly correlated, although the later was more accurate than qPCR, particularly at low eDNA concentrations and in PCR inhibitor-rich samples. Thus, the present study sets the basis for clarifying the process of *Z. marina*-derived OC sequestration and demonstrates that seagrass meadows have been present in the Seto Inland Sea for at least 5000 years.

Keywords: Blue carbon, Seagrass, eDNA

