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Terrestrial–marine connectivity: Patterns of terrestrial soil carbon deposition in coastal sediments determined by analysis of glomalin related soil protein

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Abstract

Glomalin, an arbuscular mycorrhizal protein component of soil, can be used as an indicator of terrigenous-derived carbon. We measured glomalin in sediments using the terrestrial end-member as a reference in four coastal settings: (1) intertidal seagrass meadows distributed over a rainfall gradient, (2) sediments inshore and offshore from the mouth of a river, (3) coastal coral reefs at various distances from the shore, and (4) intertidal wetlands with varying levels of groundwater influence. Across the rainfall gradient, glomalin in seagrass meadow sediments increased at sites with high mean annual rainfall during the wet season ($r^2 = 0.27$; $F_{1,29} = 5.75$; $p = 0.029$). Glomalin decreased in inshore river sediments (terrestrial) to offshore (marine) sediments ($r^2 = 0.81$; $F_{1,17} = 71.7$; $p \leq 0.0001$). Furthermore, glomalin in reef sediments decreased with distance from the shore. The high intertidal was rich in glomalin where groundwater flowed directly into the wetland compared with those with little groundwater influences. Our data indicate that rivers and groundwater transport terrestrial material, and that mangroves, salt marsh, seagrass meadows, and coral reefs accumulate it, but the connections vary among sites, within sites, and seasonally. Variations in glomalin concentrations are indicative of links between the terrestrial and marine environment that reflect proximity, filtration services, and the level of subsidies that marine ecosystems derive from terrestrial sources. Assessment of glomalin contributes to evaluating terrestrial–marine connectivity, and thus provides knowledge to improve catchment management for the protection of marine ecosystems.

The connectivity of terrestrial and marine ecosystems is important for the productivity of marine and estuarine habitats (Polis et al. 1997). Materials are transported from the terrestrial to the marine environment in dissolved and particulate forms via rivers, runoff, wind, and groundwater. Seaward transport of terrigenous sediment, which contain minerals, organic matter, and pollutants affect the biodiversity and productivity of coastal and estuarine systems (Wooldridge et al. 2006). The connectivity of the terrestrial to the marine environment is crucial in defining many ecological processes.

Conceptual models have been used to explain landscape, sediment, and hydrological connectivity. Hydrological connectivity models in terrestrial landscapes indicate that the overarching factors affecting the passage of water from one part of the landscape to another are the potential for runoff, delivery pathways, lateral buffering capacity, and landscape position, all of which are influenced by climate (Bracken and Croke 2007). Similar models of hydrological connectivity can be extended to the coastal zone in order to provide a framework for investigating the delivery and deposition of terrigenous materials to the marine environment (Fig. 1).

In the coastal zone, pathways of connectivity among landscape components include not only river and groundwater flow, but also tidal exchange. Tidal exchange can transport terrigenous materials delivered into the marine environment by rivers into intertidal habitats (mangroves, salt marsh, and seagrass) where they are accumulated (Wolanski et al. 1992). Additionally, in the coastal zone, important factors that influence delivery of terrestrial materials include distance from land (Wooldridge et al. 2006) and habitat characteristics that determine the potential for deposition (e.g., the friction offered by the habitat and the depth of water). For example, in intertidal habitats, sediment deposition is greater in the lower intertidal, where inundation levels are higher (deeper water) and more frequent compared with the higher intertidal (Adame et al. 2009). Similar to the terrestrial model, climate modifies delivery and deposition in the coastal zone, both through influences on rainfall (runoff potential) and wind and waves that can result in increased sediment availability due to resuspension of fine sediments (Wolanski et al. 1992). In order to assess some of the connections in the coastal model (Fig. 1), we investigated patterns of terrestrial carbon deposition in a range of intertidal and coastal ecosystems. In this study, we analyzed the concentration of a recalcitrant terrestrial glycoprotein called glomalin in marine sediments as an indicator of terrestrial organic matter.

Glomalin is a protein produced by symbiotic arbuscular mycorrhizal (AM) fungi, which colonize the roots of 80% of terrestrial plant taxa (Allen 1991). AM fungi form hyphal networks that absorb nutrients and water from the soil and transfer them to the plant roots, where they are

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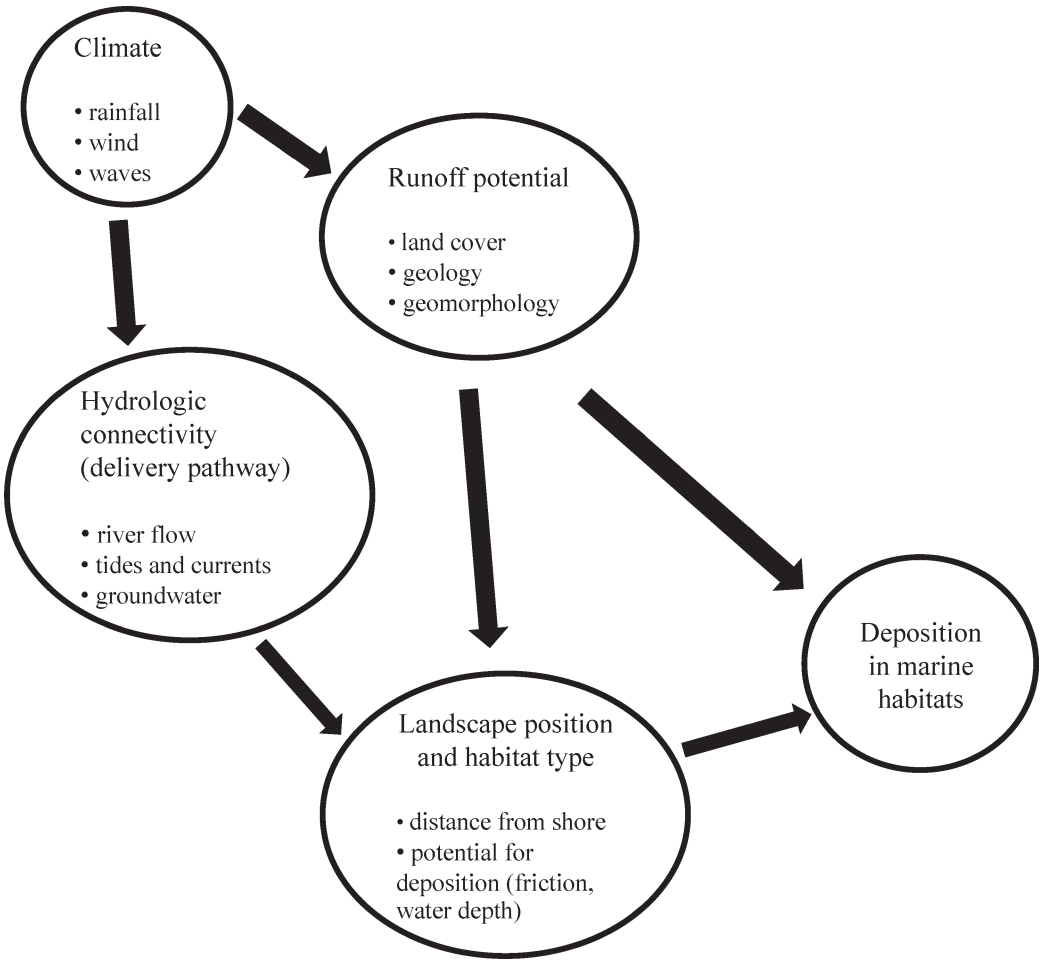

Fig. 1. Conceptual model of factors affecting the connectivity over the terrestrial–marine boundary and the deposition of glomalin within marine habitats.

exchanged with the host plant in structures (arbuscules) within the root cells. The AM fungal symbiosis contributes to the phosphorus nutrition, water uptake, and disease resistance of plants in exchange for photosynthetically fixed carbon (Allen 1991; Wright and Upadhyaya 1996). Glomalin is a constituent of the walls of filaments—hyphae—that constitute the mycelium of the AM symbiosis, which is incorporated into the soil after decomposition (Purin and Rillig 2007). Because glomalin is highly resistant to decomposition, it accumulates in soil and comprises between 1% and 20% of the soil dry weight, reaching concentrations of up to 60 mg cm^{-3} (Rillig et al. 2001). The contribution of glomalin to the total soil carbon can be up to 5%, a contribution that is greater than microbial biomass (Rillig et al. 2001; Lovelock et al. 2004). Glomalin has a turnover time of 7–42 yr (Rillig et al. 2001), which implies a substantial role for glomalin in soil carbon sequestration (Treseder and Allen 2000). It also contains high concentrations of heavy metals, particularly iron (Chern et al. 2007). Glomalin is responsible for soil aggregation; it binds together organic matter and clay particles to form water-stable aggregates (Wright and Upadhyaya 1998). Because of its association with soil particles, glomalin can be leached from eroding soils and washed into streams, where it is transported and deposited in accreting zones and in intertidal ecosystems (Harner et al. 2004; Adame et al. 2009). Glomalin has a known origin (AM fungi), high stability, and is easily measured, which suggests it could be used as a tracer of terrestrial soil components in intertidal and coastal sediments, and thus its abundance in marine sediments may be used as an indicator of terrestrial–marine linkages integrated over time.

Glomalin is normally quantified by measuring several soil protein pools (glomalin-related soil proteins) after extraction at high temperatures (120°C). One pool is operationally defined as Bradford-reactive soil protein (BRSP) and is detected with a nonspecific protein dye that uses Bovine Serum Albumin (BSA) protein as a standard (Wright and Upadhyaya 1996). The other pool is more specific and is detected via an enzyme-linked immunosorbent assay (ELISA) with a monoclonal antibody developed originally against spores of an AM fungus (MAb32B11; Wright and Upadhyaya 1996). The Bradford-based detection shows some cross-reactivity with artificially high leaf-protein additions (Rosier et al. 2006), and with polyphenols (Whiffen et al. 2006). The glomalin-specific antibody MAb32B11 of the ELISA-based detection reacts with a protein band (GiHsp 60) of homologous sequence to fungal

Table 1. Location of study sites and annual rainfall of intertidal seagrass meadow beds in North Queensland, Australia.

Site	Latitude (°)	Longitude (°)	Annual rainfall (mm)
Archer Point	−15.61	145.32	1810
Yule Point	−16.57	145.51	2013
Lugger Bay	−17.96	146.51	2010
Cardwell	−18.28	146.0	2118
Bushland Beach	−19.19	146.68	1102
Pigeon Island	−20.27	148.70	1800
Shoalwater Bay	−22.38	150.82	1019
Gladstone	−23.77	151.30	1020
Urangan	−25.30	152.91	1269

Table 2. Location of study sites and distance from shore of coastal coral reefs in the Whitsundays, Australia.

Site	Latitude (°)	Longitude (°)	Distance from shore (km)
Airlie Harbor	−20.3	148.7	0
Passage Island	−20.1	148.4	0.5
North Edgecombe Bay	−20.1	148.4	3
South Moll	−20.3	148.8	4
Gloucester Island	−20.0	148.4	10
Armit Island	−20.1	148.6	13
False Nara	−20.2	148.9	14
Luncheon Bay	−20.1	148.9	27
Bait Reef	−19.8	149.1	56
Hook Reef	−19.7	149.1	61

heat-shock proteins (Gadkar and Rilling 2006). This more specific fraction of confirmed AM fungal origin is known as immunoreactive soil protein (IRSP; Rosier et al. 2006). As a consequence of the level of confidence with which the different fractions can be attributed to AM fungal production, the IRSP fraction has been recently operationally defined as 'glomalin,' whereas the BRSP fraction has been attributed a less specific protein nature (Purin and Rillig 2007).

In this study, we use glomalin to provide a range of studies in coastal ecosystems to assess the connections suggested in the conceptual model (Fig. 1). We investigated the influence of rainfall on glomalin deposition by assessing glomalin in intertidal seagrass meadow sediments over a coastline that spans a rainfall gradient. We investigated how glomalin can be moved by river flows by measuring glomalin as a function of distance from the mouth of a river. We also investigated deposition of glomalin in coastal coral reefs that extended out to 60 km offshore. We assessed variations in glomalin deposition across intertidal landscapes, where we investigated delivery pathways in tidal waters and in groundwater. Finally, because glomalin is produced by fungi that may occur in some intertidal habitats (Hoefnagels et al. 1993; Sengupta and Chaudhuri 2002), we assessed the occurrence of mycorrhizal fungi within an intertidal wetland.

Methods

Sampling of field sites—Environmental characteristics of the sites used in this study are summarized in Tables 1–3. At each site, surface sediment samples were collected from the top 10 cm and transported in a cool, insulated container to the laboratory.

Sediment samples from intertidal seagrass meadows from the coast of Queensland, Australia (between latitudes 15° and 25°S; Table 1) were collected during two seasons, a dry period from September to October 2005 and a wet period from March to April 2006. All samples were collected within an 8-week period during each of the sampling seasons. On 19 March 2006, the tropical cyclone *Larry* hit the coast near the sampling sites, bringing wind gusts of up to 290 km h^{-1} and intense rain periods for most of March and April.

Sediments from the Brisbane River and Moreton Bay in Southeast Queensland, Australia (27.5°S 153.0°E) were sampled during July 2008 using a Van Veen grab. Samples were taken from the mouth of the Brisbane River and upriver sites (1–15 km inland) and seaward of the river mouth in Moreton Bay (1–20 km seaward). The sediments from the reefs of the Whitsundays Coast in Northeast Queensland, Australia (Table 2) were sampled during July 2007 using a Van Veen grab. Grain size was determined using a combination of wet and dry sieving following methods by Folk (1974), with silt content defined as sediment passing through a 63-μm mesh. Carbonate content was estimated gravimetrically after digestion with 2 mol L^{-1} HCl.

The intertidal soils from Queensland (Tinchi Tamba Wetlands Reserve, Brisbane, described in Adame et al. 2009), Western Australia (Exmouth Gulf, described in Lovelock et al. 2011), and Florida (Indian River Lagoon, mosquito impoundment no. 23, described in Feller et al. 2003; Table 3) were sampled in triplicates along transects from the seaward to the terrestrial side. Samples from intertidal soils were taken over a range of field campaigns between 2004 and 2008. In order to measure groundwater influence on the landward edge of the intertidal wetlands, pore water was drawn at each sampling point. Salinity of the pore water was measured with a handheld refractometer. A decrease in pore-water salinity in the Indian River Lagoon, Florida was found, confirming that fresh groundwater enters the wetlands on the landward edge.

To assess the likelihood of autochthonous production of glomalin in the intertidal, soil roots were assessed for colonization by AM fungi in the Indian River Lagoon, Florida. Roots in the top 10 cm of soil were collected along three transects along the intertidal zone, from the edge of a mangrove forest dominated by *Avicennia germinans* to the terrestrial vegetation. Samples were taken at 10-m intervals. Salinity was simultaneously measured in the pore water at each sampling site. Roots were washed and preserved in 70% ethanol, then cleared and stained (Kormanik and Mcgraw 1982). The point-intercept method was used to assess AM fungi colonization in 10 random 1-cm sections of roots per sample mounted in glycerol and examined at 40 times magnification (Mcgonigle et al. 1990).

Table 3. Location, geomorphological setting, and the influence of groundwater and runoff into coastal wetlands study sites.

Site	Latitude (°)	Longitude (°)	Geomorphological setting	Groundwater influence	Runoff
Tinchi Tamba Wetland Reserve, Australia	−27.5	153.1	Tide- and river-dominated	no	yes
Exmouth Gulf, Western Australia	−22.4	114.3	Tide-dominated	no	yes—during cyclones
Indian River Lagoon, Florida	27.5	−80.3	River-dominated	yes	yes

Analysis of glomalin—Samples were oven-dried at 60°C before analysis. We chose to oven-dry the samples over other methods of storage, because we found that freezing and freeze-drying reduces immunoreactivity in some samples ($p < 0.01$). Freezing samples resulted in a decrease of IRSP of 10–85% of oven-dried samples and freeze-drying resulted in a decrease of 20–70%. Analysis of BRSP showed no significant differences between oven-dried and freeze-dried samples.

Glomalin was measured following the methods of Wright and Updahyaya (1996). (Detailed methods and sources of reagents can be found in: http://www.ars.usda.gov/Research/docs.htm?docid=15971). Soils were extracted multiple times. The first extraction was with 50 mmol L^{-1} of sodium citrate (pH = 8) for 30 min at 120°C, and the second extraction was with 100 mmol L^{-1} of tetrasodium pyrophosphate (pH = 9) for 1 h at 120°C. Samples were centrifuged for 10 min at 3220 × *g* between extractions. Sequential extractions were performed until the supernatant showed none of the red-brown color typical of glomalin. The total volume of the supernatant was recorded and a subsample was centrifuged at 10,000 × *g* for 3 min. Protein concentrations (BRSP) were determined from the second extraction using the Bradford dye-binding assay with protein dye reagent (Bio-Rad Laboratories) and BSA (Sigma-Aldrich) as the standard and were read at 595 nm in a spectrophotometer (Model 680, Bio-Rad Laboratories). BRSP concentrations were extrapolated to mg g^{-1} of aggregated soil particles by correcting for the dry weight and the volume of the extractant. Based on BRSP concentrations, we prepared solutions that contained 0.02 μg of protein per well (Dynex 96 well polyvinyl chloride [PVC] u-bottom plates, Dynex Technologies) for IRSP analysis. Glomalin was analyzed from the first soil extraction with an indirect ELISA with a monoclonal antibody MAb 32B11 against glomalin. The ELISA sequential steps were MAb antibody, ExtrAvidin peroxidase (Sigma-Aldrich), Biotinylated goat antimouse IgM (Jackson Immuno-Research Laboratories), and Horseradish Peroxidase Substrate Kit (Bio-Rad Laboratories). The samples were read at 405 nm in the spectophotometer. Values from ELISA (IRSP) were compared with a standard curve calculated from the highest immunoreactive soil of all of the soils sampled within intertidal sediments in Queensland (Tinchi Tamba Wetlands Reserve, Brisbane). Every assay for IRSP was compared with the dilution curve generated from this soil, which remained constant during the study. Due to the lack of an available commercial standard, IRSP values are shown as unit-less measurements, which provides a comparative mean to test the relative amount of glomalin from different soils tested in a similar way, using the same standard curve.

We conducted three experiments in order to provide verification of the protein assays: (1) We tested the effects of adding different concentrations of BSA to sediment samples in order to understand the capacity of the extraction method to eliminate nonglomalin proteins. We added 0%, 50%, 100%, and 200% of the soil protein content as BSA to three soils and then extracted (*see* above) and analyzed for BRSP and IRSP concentrations. We found no significant difference among different BSA addition treatments and the control samples, which indicates that the extraction procedure is sufficient to denature BSA and render it undetectable by the Bradford assay at the concentrations used in the experiment. However, higher additions of BSA (1000% of protein content) have shown significant increase in BRSP concentrations (Rosier et al. 2006). Thus, BRSP can overestimate glomalin content in samples with very high protein concentrations. (2) We tested how additions of proteins from higher plants, in the form of mangrove leaves, influenced the assay. We included 0.01 g and 0.15 g of leaf addition to 1 g of sediment and then extracted and assayed for IRSP and BRSP. With additions of leaf tissue of 1% of the weight of the soil sample, BRSP and IRSP were unaffected; with additions of 15% of the weight of soil sample, BRSP increased and IRSP concentrations decreased compared with the sediment-only treatment. The extraction of glomalin is therefore sufficient to remove most of the higher plant protein for detection of IRSP, but very high levels of plant protein can affect BRSP and IRSP values obtained. Similar results have been reported by Rosier et al. (2006). (3) To test for cross-reactivity of the BRSP assay with other marine protein sources, we analyzed a range of marine organisms (coral and macroalgal tissues) in conjunction with soil samples. We analyzed 0.5–1 g of freeze-dried ground marine organism tissue. After extraction, BRSP was detected in marine organism samples with values similar to those in sediments ($p > 0.05$); thus, proteins from some marine organisms, similar to observations for leaf protein (above), are able to persist through the extraction process and thus may give rise to higher values of BRSP where they contaminate the soil sample.

In our study sites, BRSP was significantly correlated with IRSP (Fig. 2) probably because only heat-shock proteins persist through the extraction process and the most common heat-shock protein in soil is glomalin. In this study, BRSP was used as a first screening step for estimating glomalin concentrations in soil samples from coastal sedimentary settings. However, due to the potential

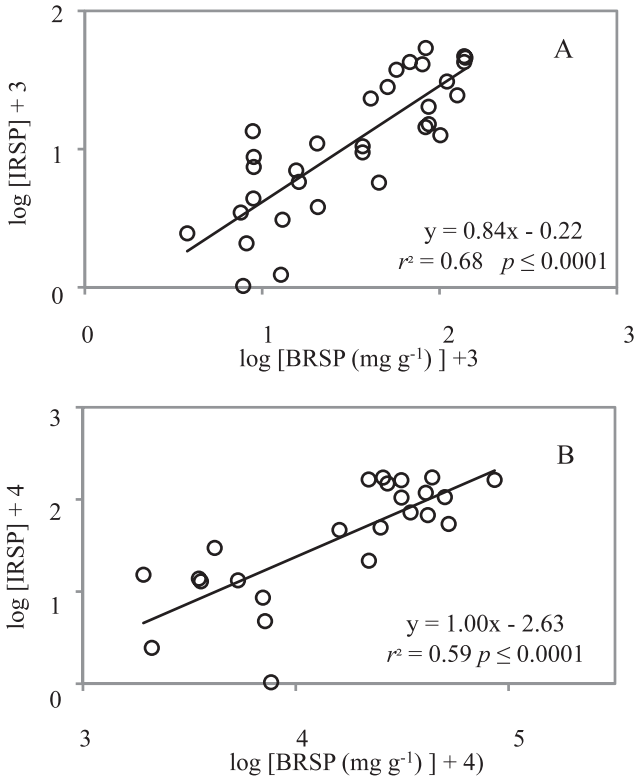

Fig. 2. Correlation between Bradford-reactive soil protein (log BRSP +3) and glomalin as immunoreactive soil protein (log IRSP +4) in two environmental settings with different concentrations of protein: (A) low concentrations in coastal sediments in North Queensland Australia; and (B) high concentrations in mangroves in Moreton Bay, Australia. The regressions are significant: [log (IRSP)] = −0.22 + 0.84 × [log BRSP] (r^2 = 0.68; $F_{1,30}$ = 62.59; $p < 0.001$), and [log (IRSP)] = −2.63 + 1.00 × [log BRSP] (r^2 = 0.59; $F_{1,22}$ = 32.14; $p < 0.001$).

overestimation of glomalin concentrations by using BRSP to estimate glomalin, the use of BRSP as a screening tool for other soil samples and in other environmental settings should be critically assessed. Herein, we refer to BRSP as 'protein' and to IRSP as glomalin.

Finally, in order to determine the proportion of the glomalin in the dissolved and particulate fraction of tidal water during tidal exchange, five replicate tidal-water samples were collected during flood tides from the Indian River Lagoon, Florida. The samples were measured for glomalin twice, before and after being centrifuged at 2000 revolutions min^{-1} for 3 min to remove suspended material.

Statistical analysis—Glomalin and root-colonization data were logarithmically transformed (log_{10}), and in some cases they were converted (+3 or +4) in order to facilitate the visualization of the data (to eliminate negative numbers) and to conform to the requirement for normality and homogenous variances when using linear models. Regression analyses were used to assess the relationship between glomalin and rainfall in seagrass meadow sediments, glomalin and distance from shore, glomalin and silt and carbonate content, and percentage of colonization of roots by AM fungi, and pore-water salinity. Throughout the results, the *F*-value is the ratio of the mean regression sum of squares divided by the mean error sum of squares and represents the significance of the regression. Subscripts next to the *F*-value are the degrees of freedom for each regression. All analyses were performed using Data Desk (version 6.2, Data Description Inc., Ithaca NY).

Results

Deposition of glomalin over a rainfall gradient—Glomalin was observed in sediments of seagrass meadows along the coast of North Queensland (Fig. 3). Mean concentration of BRSP (mean ± SE) was 0.15 mg g^{-1} ± 0.02 mg g^{-1} and for glomalin (IRSP) was 0.099 ± 0.019 (unit-less). A significant relationship between mean annual rainfall, BRSP, and IRSP was found in the wet season (Fig. 3A,C): [log (BRSP) +3] = 1.22 × Rainfall − 1.77, (r^2 = 0.34; $F_{1,29}$ = 8.06; p = 0.012), and [log (IRSP) +3] = 0.86 × Rainfall − 0.89 (r^2 = 0.27; $F_{1,29}$ = 5.75; p = 0.029), but not in the dry season (Fig. 3B,D).

Deposition of glomalin across a riverine landscape—Concentration of glomalin varied in the sediments of the Brisbane River in Moreton Bay, Queensland (Fig. 4). Glomalin concentrations were high in sediments from the terrestrial side of the Brisbane River mouth but rapidly declined in sediments from the mouth of the river into Moreton Bay, with very low concentrations at 25 km from the mouth of the river: [log (BRSP) +3] = −0.023 × Distance from river mouth (km) + 2.33 (r^2 = 0.57; $F_{1,17}$ = 22.4; p = 0.0002), and [log (IRSP) +3] = −0.049 × Distance from river mouth (km) + 1.91 (r^2 = 0.81; $F_{1,17}$ = 71.7; $p \leq 0.0001$). Mean concentrations of BRSP were 0.26 mg g^{-1} ± 0.06 mg g^{-1} and 0.145 ± 0.046 for IRSP.

Deposition of glomalin in coastal coral reefs at increasing distance from shore—Glomalin was measured in sediments from the coast to the adjacent coral reef in the Whitsundays coral reef system. We detected low concentrations of glomalin (0.010–0.050 IRSP) in the sediments over all samples (Fig. 5) compared with values in intertidal seagrass and riverine sediments (cf Figs. 3, 4). Mean concentrations of BRSP were 0.08 mg g^{-1} ± 0.03 mg g^{-1} and for IRSP 0.026 ± 0.004. Maximum glomalin concentrations were measured in sediment from sites close to the mainland (0.054 IRSP in Passage Island 0.5 km off shore and 0.044 IRSP in Arlie Harbor). The lowest glomalin concentrations were measured in sites offshore, at the edge of the coral reefs (0.007 IRSP in Bait Reef 56 km offshore, and 0.01 IRSP in Hook Reef 61 km offshore; Fig. 5A). Glomalin was associated with soil characteristics; glomalin increased with silt content of sediments (%) [log (IRSP) +3] = 0.007 × Silt content + 1.003 (r^2 = 0.78; $F_{1,12}$ = 42.62; $p < 0.01$; Fig. 5B) and decreased with carbonate content (%) (Fig. 5C).

Deposition of glomalin across intertidal landscapes—Concentrations of glomalin in intertidal soils varied among the sites and across the estuaries we sampled (Fig. 6). We distinguished three patterns: the first one was found in Tinchi Tamba Wetlands in Queensland, where we found

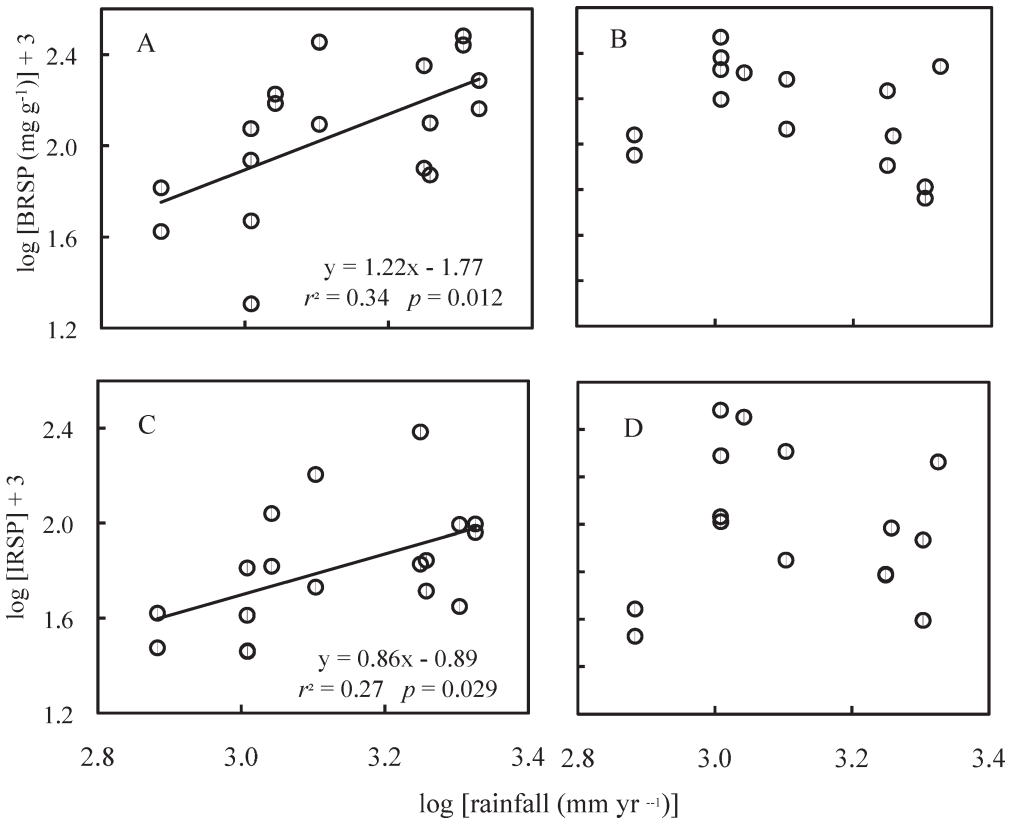

Fig. 3. Variation in Bradford-reactive soil protein (log BRSP +3) and in glomalin as immunoreactive soil protein (log IRSP +3) in intertidal seagrass meadow sediments with variation in mean annual rainfall along the coast of Queensland, Australia. Values were determined in (A, C) the wet season and (B, D) in the dry season. A significant relationship between mean annual rainfall and BRSP and IRSP was found in the wet season: [log (BRSP) +3] = $-1.769 + 1.222 \times$ Rainfall ($r^2 = 0.34$; $F_{1,29} = 8.06$; $p = 0.0118$), [log (IRSP) +3] = $-0.890 + 0.863 \times$ Rainfall, ($r^2 = 0.27$; $F_{1,29} = 5.75$; $p = 0.0291$), but not in the dry season.

higher concentrations of glomalin in the landward intertidal that declined in a seaward direction (Fig. 6B). The second pattern occurred in Exmouth Gulf in the arid zone of Western Australia, where rainfall is very low. At this site, concentrations of glomalin in sediment from the landward edge intertidal were low compared with the seaward fringe, where most of the glomalin was accumulated (Fig. 6D). The third pattern of distribution was found in the Indian River Lagoon, where we found similar concentrations of glomalin in the landward intertidal and the seaward mangrove fringe (Fig. 6F). The Indian River Lagoon site has high levels of groundwater influence on the landward edge of the wetland.

At the Indian River Lagoon, we assessed glomalin in tidal water. We found glomalin at concentrations of 0.084 $L^{-1} \pm 0.007$ L^{-1}. Concentrations were reduced to 0.032 $L^{-1} \pm 0.003$ L^{-1} when water samples were centrifuged, which indicated that most of the glomalin was associated with particulate matter.

To assess whether AM fungi may be contributing to glomalin production in mangroves, we determined root colonization by AM fungi at the Florida site. We found low levels of root colonization in the mangrove *A. germinans* compared with terrestrial tree roots, and we found that the percentage of root colonization with AM fungi declined with increasing salinity (Fig. 7).

Discussion

Glomalin was present in almost all the coastal marine sediments examined. The variations in concentration over gradients in rainfall and among different habitats and environmental settings confirmed many of the pathways proposed for transfer of terrestrial materials to marine habitats.

Deposition of glomalin over a rainfall gradient—Glomalin concentration in intertidal seagrass meadows was significantly correlated with mean annual rainfall (Fig. 3), which suggests that the transport of glomalin to coastal waters is associated with rainfall. Chern et al. (2007) observed low glomalin concentration (IRSP) in river bank sediments in winter compared with summer and suggested that in addition to low production rates in the winter, glomalin was eroded from river banks when water flow rates and sediment transport was higher than in the warmer summer growing season. Thus, glomalin is likely to be moved with sediments as they are flushed to the ocean during high rainfall or flooding episodes.

Variability in the patterns of glomalin deposition in intertidal seagrass meadows are also likely to be affected by factors that influence glomalin concentrations in the terrestrial environment, as well as by the movement of

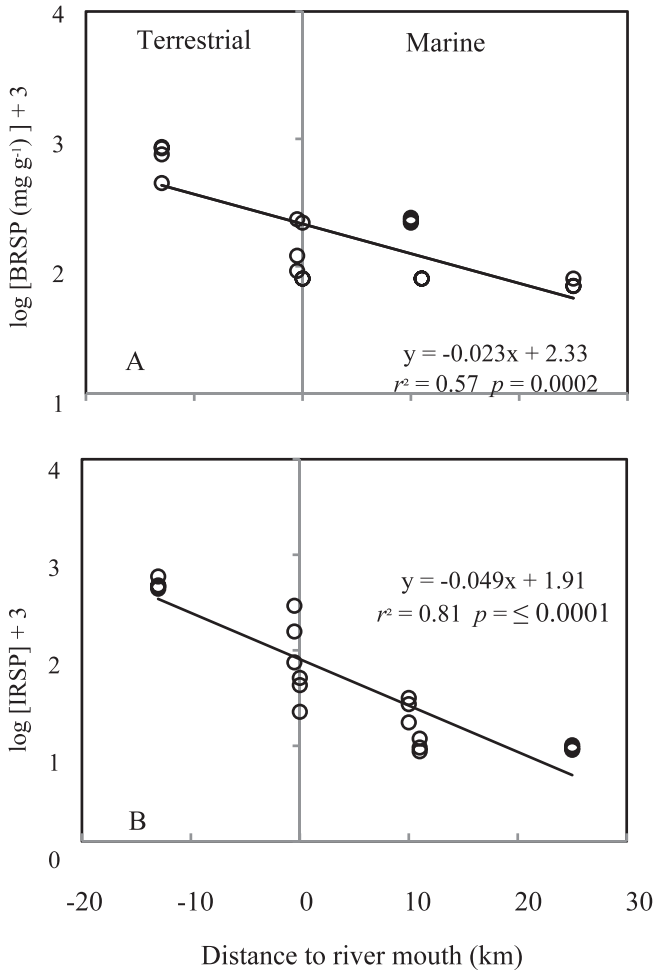

Fig. 4. Variation in (A) Bradford-reactive soil protein (log BRSP +3), and (B) glomalin as immunoreactive soil protein (log IRSP +3) as a function of distance from the mouth of the Brisbane River. On the x-axis, negative values are upriver sites (terrestrial), while positive values are seaward of the river mouth into Moreton Bay (marine). There is an established gradient in water quality over Moreton Bay due to strong flushing from the Pacific Ocean in the Eastern Bay (at the right of both panels).

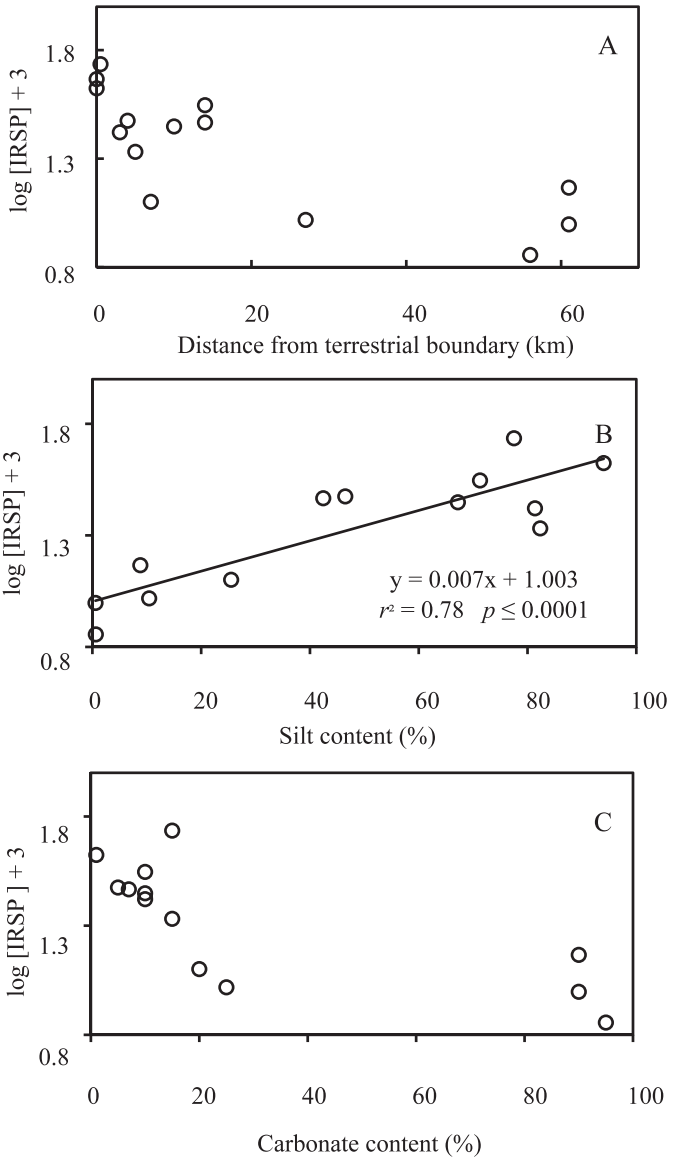

Fig. 5. Glomalin as immunoreactive soil protein (log IRSP +3) and (A) distance from terrestrial boundary, (B) silt content (%) ([log (IRSP) +3] = 0.007 × Silt content + 1.003; $r^2 = 0.78$; $F_{1,12} = 42.62$; $p < 0.01$), and (C) carbonate content (%) from sediment samples from the Whitsundays, Queensland, Australia.

glomalin through runoff, and by the rates of decomposition or remobilization within marine sediments. Autochthonous production of glomalin in seagrass is unlikely because colonization of seagrass roots by AM fungi has not been observed (Nielsen et al. 1999).

In culture experiments in terrestrial sediment, IRSP declined by 46% and BRSP declined by 25% over 150 d, which indicated the turnover rate of glomalin initially after hyphal death (Steinberg and Rillig 2003). Rates of glomalin decomposition in marine sediments are still unknown, although they are likely to vary depending on physical and chemical characteristics of the depositional setting. For example, decomposition of organic molecules is slow in anoxic environments such as mangroves (Lallier-Verges et al. 2008). Other parameters that are likely to influence the decomposition of glomalin could be water depth (Davis et al. 2009), bacterial biomass (Goñi and Hedges 1995), and physical characteristics of the sediment, such as particle size (Rothman and Forney 2007). Finally, glomalin decomposition rates are likely to be influenced by water temperature; from our experiments (*see* Methods), we observed that the immunoreactivity of glomalin decreases at temperatures ≤ 4°C.

Glomalin concentrations vary with soil texture (Rillig and Steinberg 2002); they decline with depth in the soil profile (Rillig et al. 2003), they decrease with greater soil fertility (Lovelock et al. 2004), increase with years after tillage (Wright and Upadhyaya 1999), and accumulate during postfire forest succession (Treseder et al. 2004). Glomalin concentrations also vary with land cover (Rillig et al. 2003) and land management and disturbance (Wright and Upadhyaya 1999). There are no measurements of

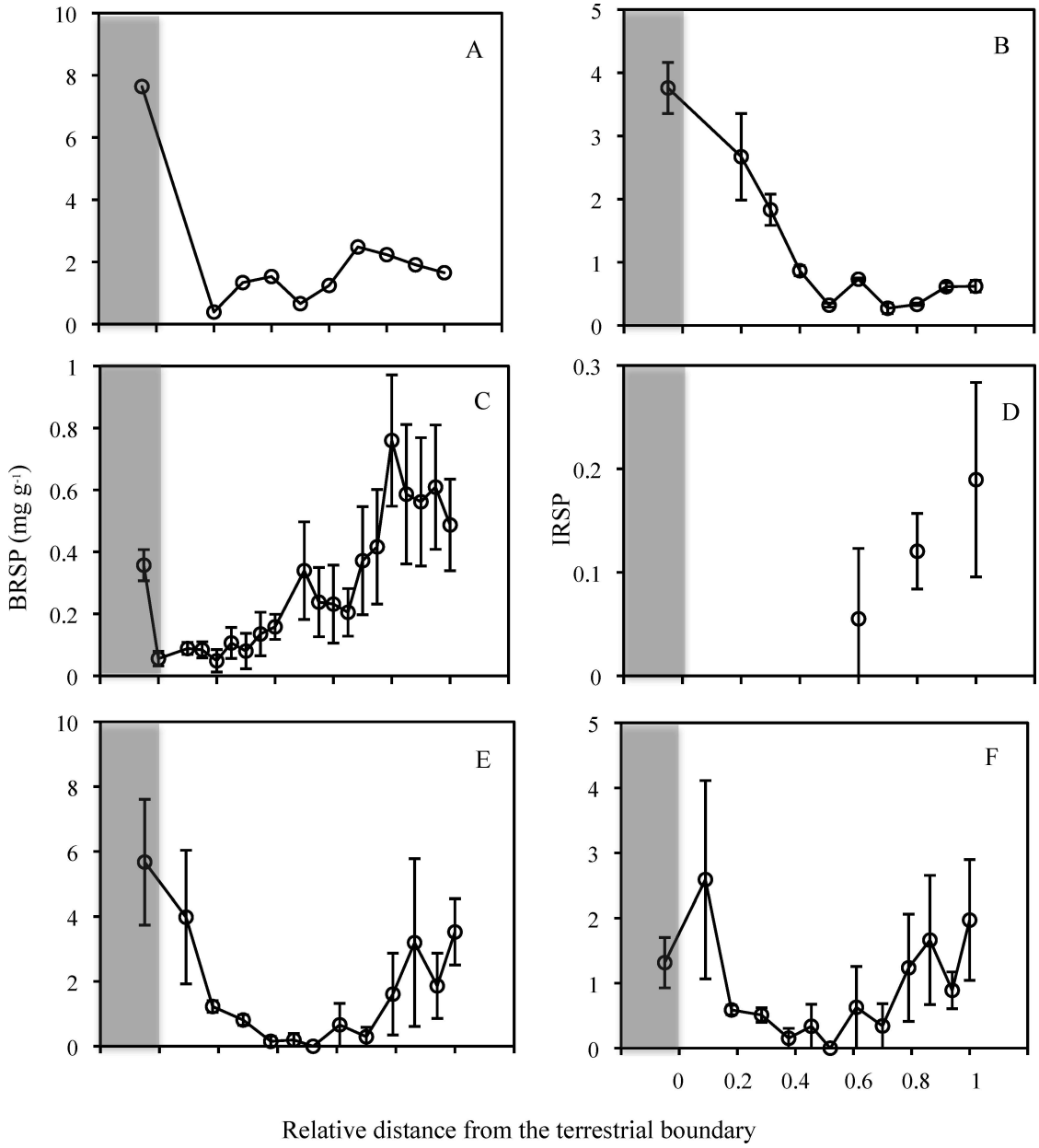

Fig. 6. Variation in Bradford-reactive soil protein (BRSP) and glomalin as immunoreactive soil protein (IRSP) across estuarine landscapes, from the terrestrial boundary to the seaward edge of the mangroves at three locations: (A, B) Tinchi Tamba Wetland Reserve, Queensland; (C, D) Exmouth Gulf, Western Australia; and (E, F) Indian River Lagoon, Florida. Values are means and standard errors of three (Indian River Lagoon) and five (Tinchi Tamba and Exmouth Gulf) samples. Grey sections are values from terrestrial areas. The x-axis is relative distance across the intertidal zone (which varied among sites) where 0 is the terrestrial boundary and 1 is the seaward edge. Note that scales of the y-axes differ among panels.

glomalin concentrations in upland soils of the regions we studied, but future detailed comparisons of glomalin in river catchment and its transport and deposition in the coast could provide insights to improve land management and reduce delivery of sediments into the Great Barrier Reef Lagoon. We would expect high concentrations of glomalin in marine sediments where land-use change (particularly clearing of natural vegetation) is extensive, rainfall is high, and soils are fine-grained and relatively infertile. Glomalin in marine sediments would be expected to decline with increases in natural forest cover, with a high level of intact riparian vegetation (reduced erosion) and in low rainfall areas or periods. Furthermore, assessment of glomalin in sediment cores, if rates of decomposition were known, may provide background levels prior to European colonization to which current rates of glomalin deposition could be compared. Variation in glomalin concentrations in marine sediments may thus provide a sensitive indicator of improvements in watershed management.

Deposition of glomalin associated with river outflow—Sediments sampled throughout the Brisbane River indicat-

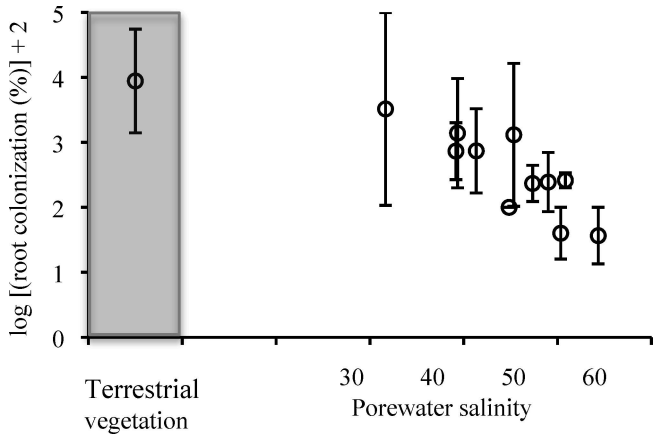

Fig. 7. Root colonization (log (%) +2) by arbuscular mycorrhizal fungi with variation in salinity across the intertidal zone in the Indian River Lagoon, Florida.

ed that large amounts of glomalin are deposited in the river channel and are likely to be transported offshore to Moreton Bay. We measured glomalin 25 km from the river mouth, which indicated the extent of influence of the Brisbane River. The extensive flooding in the region in January 2011 indicated that the flood plume extended to our 25-km sampling station, confirming the extensive terrestrial influence that periodically occurs within the bay (Lybolt et al. 2010).

Deposition of glomalin in coastal coral reefs at increasing distance from shore—Glomalin showed a clear pattern of decreasing concentrations with distance from the shore (Fig. 5A). Furthermore, glomalin was positively correlated with silt content and negatively associated with carbonate content of sediments (Fig. 5B,C). Sediments that are low in carbonate and high in silt are likely to be influenced by material of terrigenous origin (Gagan et al. 1990). The distribution of glomalin with distance from the shore, and the observation that much of the glomalin was associated with particulate matter in tidal water (in the Indian River Lagoon) is consistent with glomalin being attached to particulate material of terrigenous origin that is transported in flood plumes and deposited on coral reefs. Detection of glomalin 60 km offshore is consistent with the magnitude of the Pioneer River flood plume in the region, which has been observed to envelop reefs 50 km from shore (Neil et al. 2002). The magnitude of the terrestrial inputs delivered to the coast has caused negative ecological effects in offshore reefs of the region (van Woesik et al. 1999). The role of glomalin in the sequestration and movement of iron and toxic heavy metals (Chern et al. 2007) may provide important indications of pathways of delivery, deposition, and release of metals in the marine environment, although this is yet to be determined. Moreover, it would be useful to calculate the conservative mixing concentrations of glomalin and compare them to glomalin values measured in the field. This calculation could help estimate rates of glomalin removal during transport, which could be used as a proxy for soil transportation.

Deposition of glomalin across intertidal landscapes—Concentrations of glomalin in intertidal landscapes were variable. Glomalin was differentially deposited in intertidal wetlands, probably as a result of differential connectivity of the terrestrial and marine environment and due to the influence of different vegetation types. In Tinchi Tamba in Queensland, surface flows during flooding may deliver glomalin into the high intertidal, which would result in higher glomalin concentrations in the terrestrial boundary compared with the seaward edge, but additionally, chenopod-dominated salt marsh occurs in the high intertidal. Salt marsh plants, including chenopods, have been observed to have AM fungal symbionts (Hoefnagels et al. 1993) as have mangroves (Sengupta and Chaudhuri 2002), and thus autochthonous production of glomalin in intertidal soils cannot be totally ruled out. But root colonization declines with increasing salinity in mangroves and salt marsh (Juniper and Abbott 1993; Sengupta and Chaudhuri 2002), similar to our observations in the Indian River site.

In contrast, in the Exmouth Gulf, glomalin levels were very low adjacent to the terrestrial boundary (Fig. 6D). This estuary is characterized by extremely low annual rainfall (300 mm), low river inputs, and low groundwater flow. Thus, the intertidal wetland appears to have little direct connection to the terrestrial environment across the terrestrial–marine boundary, except in areas close to creek beds (C. E. Lovelock and S. F. Wright unpubl.). Glomalin, and possibly other terrestrial materials, are delivered to coastal waters during infrequent flood events associated with cyclones in the region (Lovelock et al. 2011). Glomalin is likely to be deposited on the seaward edge of this intertidal zone during tidal exchange, which is consistent with patterns of sedimentation observed in other mangrove wetlands (Adame et al. 2009).

Finally, the Indian River Lagoon site is sandy and has groundwater that flows directly to the wetlands as we can observe from our measurements of interstitial salinity. In this case, the terrestrial soils are rich in glomalin, probably due to low fertility and abundant AM fungi. Thus, conditions of high glomalin production rates combined with porous sands and high levels of groundwater allow glomalin to leach into intertidal mangrove sediments, which results in similar concentrations of glomalin in the terrestrial boundary compared with the seaward edge.

Glomalin as an indicator of terrestrial–marine connectivity—There are many techniques that have been used to trace terrestrial materials (soils and plants) in marine environments. These techniques include (1) biomarkers (n-alkanes, phenolic and hydroxyalkonic compounds, lignin, cutin, loloides, branched and isoprenoid tetraether lipids [Prahl et al. 1994; Bianchi et al. 1997; Hopmans et al. 2004]); (2) carbon isotopes ($\delta^{13}C$ and $\Delta^{14}C$; Raymond and Bauer 2001); (3) chemical parameters, such as nitrogen : carbon ratios and percentage of organic carbon content; (4) physical parameters, including soil color, sediment porosity, grain size; and (5) biological parameters, such as epibenthic fauna, and vascular plant debris (Leithold and Hope 1999). Many of these techniques are extremely powerful, especially when

used with a multiple tracer approach (Prahl et al. 1994; Leithold and Hope 1999; Raymond and Bauer 2001). Despite best efforts, many of the multiple-tracer studies lead to difficulty in interpretation of results, variability of performance of markers within sites, and overlapping of signals.

Glomalin provides an indicator of terrestrial material that can overcome some of the difficulties associated with other indicators. Glomalin's high fidelity to terrestrial soil carbon can overcome the poor specificity of many chemical, physical, and biological tracers (e.g., grain size, sediment color, plant debris, carbon to nitrogen ratios). Glomalin is easily traced, and does not overlap, as isotopic signals sometimes do (Raymond and Bauer 2001), with any marine compound we have tested so far. Furthermore, as far as we know, glomalin values are not confounded by differential degradation rates, such as lignin–phenol ratios and n-alkanes (Bianchi et al. 1997; Hopmans et al. 2004). Finally, glomalin analysis is relatively simple and cheap compared with other effective, but expensive methods, such as biomarkers.

Glomalin can be used as a qualitative tracer to compare relative concentrations among different coastal locations; however, the lack of commercial standards limits broader comparisons among different studies. An improved understanding of the decomposition rates of glomalin in the marine environment and the factors that control glomalin concentrations in soils and its movement across the terrestrial–marine interface would increase confidence in its use as an indicator. Glomalin has the potential to provide an inexpensive and easily interpreted tracer of inputs of terrestrial carbon in the marine environment with which to understand the complexities of terrestrial–marine connectivity.

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