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Tidal Marsh Plant Community Development within Four Restored Lowland Estuaries in the Western Peninsulas of Washington State, USA

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Abstract

Vegetation and soil within estuarine ecosystems play an integral role in ecological processes within pocket estuaries. However, physical barriers, caused by culverts diminish hydrological inputs, sediment exchange, and habitat connectivity. The restoration of estuaries by bridge replacement reconnects the aquatic corridor, however, the recovery of plant communities and soil substrate is not well understood. This observational study monitored four estuary restoration sites of variable ages (0 - 12 years) in Western Washington, USA. Plant community composition, soil organic carbon, organic matter, and soil nutrients were assessed. Percent soil carbon was different among the pre-restoration and youngest (3-year) post-restoration site ($P = 0.03$), suggesting an initial decrease in carbon and organic matter during restoration. Both N and P were deficient at the newest, lower restoration site, presumably linked to the lack of organic matter required for adequate cation exchange capacity and nutrient/plant exchange ($P < 0.05$). Plant species diversity was higher at the intermediate (9-year) and oldest post-restoration sites (12-year; $P = 0.02$). Vegetation composition was primarily native species with few invasive plants present. The results of this study illustrate that tidal marsh plant communities are influenced by the development of salinity and vertical gradients with older sites having an increase in species diversity. Future surveys are ongoing to better understand how these sites recover organic matter and tidal marsh communities to form adequate estuarine habitat over time.

Keywords

Estuary Restoration, Habitat Recovery, Culvert Removal, Bridge Replacement

1. Introduction

Estuarine ecosystems are the link between freshwater and marine environments and are considered one of the most productive systems on the planet [1]. Hydrogeomorphology, vegetation, and soil are all functionally interrelated and facilitate energy production, biogeochemical cycling, and gene flow within habitats [2] [3] [4]. Within estuarine wetlands, sediment and vegetation play integral roles in denitrification, carbon sequestration, and physical stability through sediment stabilization and organic matter production [5] [6] [7]. These estuarine processes support valuable ecosystem services including flood mitigation, storm abatement, water quality and quantity improvements, and wetland and aquifer recharge [8]. The ecological complexity of estuary vegetation and sediment accretion also forms intricate food webs, thereby creating essential habitat for many aquatic and terrestrial wildlife species. This is of particular importance for the conservation and restoration of habitat required to support many marine, freshwater, and terrestrial wildlife species.

Despite their importance, 74% of estuary habitat in the Pacific Northwest has been lost to maritime industries, private landownership, and commercial development [9]. In Washington and Oregon alone, up to 5000 streams with over 196,000 kilometers of roadways are affected by culverts that have severely altered hydrological regimes. Loss of connectivity through the implementation of physical barriers deeply channelizes streams, diminishes flood plains, and interrupts gene flow for many species [10]. Declining shellfish, salmon (*Oncorhynchus* spp.), and resident Orca (*Orcinus orca*) populations have catalyzed an emphasis to restore estuary habitat by culvert removal and bridge replacement. It is hypothesized that the restoration of estuaries will promote energy movement and the biophysical processes required for sediment exchange, the creation of flood plains, tidal marsh succession, and improved stream morphology [11] [12] [13]. Therefore, successful estuary restoration will improve migration routes and tidal marsh habitat required to restore nursery grounds and food webs for many iconic wildlife species [14].

Due to the wide variety of restoration outcomes, effective monitoring becomes an essential component to assessing ecosystem recovery [15]. Because native tidal marsh habitat is an aspect of estuary restoration, the early detection and eradication of invasive plant species are required to encourage resilient plant communities that resemble native reference sites. Secondary disturbances during culvert restoration results in newly disturbed estuarine sediments that may lack the abiotic and biotic complexity required for native plant establishment [16]. These disturbed substrates tend to promote fast-growing exotic plant species that could impact processes such as marsh plant succession, soil development, and water relations [17] [18]. Some sites may become so invaded and degraded that they are transformed into novel systems incapable of obtaining the level of tidal marsh functioning required for estuary recovery [19]. Therefore, restoration should emphasize early detection and management of exotic species before they get well-established in restored estuary projects [15].

The objective of this current project is to investigate the development of soil carbon, soil organic matter (SOM), plant nutrients, and plant communities within various urban estuary restoration sites in Western Washington, USA. This observational study monitored four estuary restoration sites of variable ages (0 - 12 years) in Western Washington, USA: 1) pre-restored culverted site, 2) three-year-old restored estuary, 3) nine-year-old restored estuary, and 4) 12-year-old restored estuary. In doing so, this study will contribute information regarding soil development in various stages of estuary recovery. Additionally, this study will provide early exotic plant species detection and baseline data for long-term study of restored tidal marsh plant communities within the Kitsap Peninsula of Washington State.

2. Material and Methods

2.1. Study Sites

The four study sites are located on the Kitsap Peninsula in Washington State (**Figure 1**). The four sites selected included: The first site is the pre-site, Harper Creek Estuary (47˚30'00.9"N 122˚30'58.4"W) in Port Orchard, Washington. This pre-restoration site within this study contained two working culverts (60 cm in diameter). Soil directly adjacent to this site is composed of Tacoma silt loam and Harstine gravelly ashy sandy loam [20]. The second site, Carpenter Creek Estuary (47˚47'42.471"N 122˚30'26.7114"W), located in Kingston, Washington, was restored in 2013 after removal of a 3 m × 3 m box culvert on South Kingston Road and installation of a 27.4 m bridge that spans the entire channel width of Carpenter Creek and can withstand natural tidal inundation from Appletree Cove. The majority of soil adjacent to this site is classified as an alluvial beach deposit with minor components of Poulsbo gravelly sandy loam [20]. The third site sampled was the nine-year-old site at the Beaver Creek Estuary (47˚34'12.1938"N 122˚33'7.2468"W), located in Manchester, Washington, at the head of Clam Bay and was restored in 2007. Restoration of Beaver Creek with the removal of the lower culvert and installation of a 6.7 m bottomless arch bridge at the head of Clam Bay were completed in 2007. Soil in this region is composed of Kapowsin gravelly ashy loam [20]. The forth site was the 12-year-old site located at the Dogfish Creek Estuary (47˚44'48.3144"N 122˚39'7.3368"W), located in Poulsbo, Washington at the head of Liberty Bay. This site was restored in 2004 with the removal of a 1.5 m culvert, which was replaced with a 26.8 m bridge. Soil composition adjacent to Dogfish Creek includes urban land-Alderwood complex and Kitsap silt loam [20].

2.2. Vegetation Community Survey

Transects were placed parallel to the water along the lower bank edge where the first perennial vegetation was located nearest the edge of water, as described in the PacFish InFish Biological Opinion Monitoring Program [21]. Three, 50-m

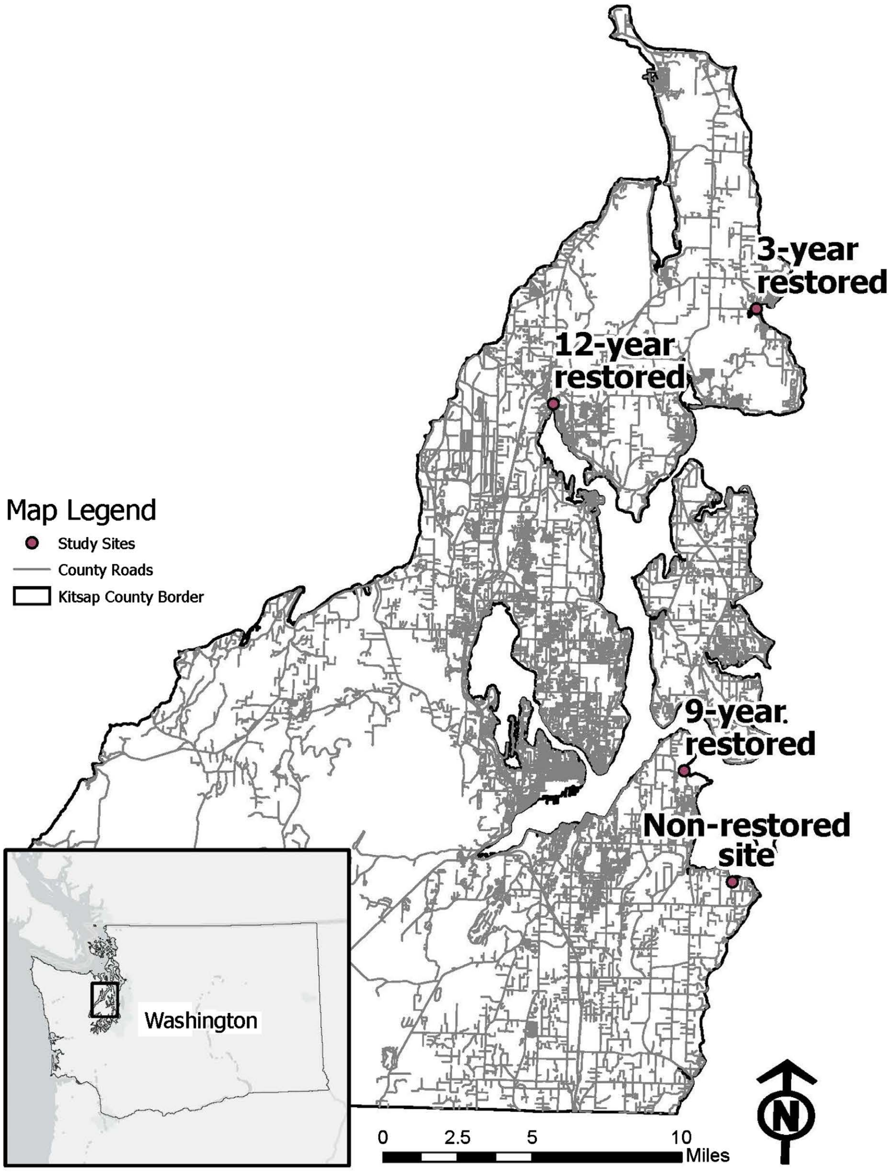

Figure 1. Map of study sites in Kitsap County, Washington State. Located on the Olympic Peninsula, sites within this study include Harper Creek (pre-restoration), Carpenter Creek (3-Yr), Beaver Creek (9-Yr), and Dogfish Creek (12-Yr). Map provided by Andrew McCay.

transects were place above the headwater of the restoration location on the freshwater influence side and three 50-m transects were placed below the tailwater on the marine influence side. The initial transect began at the bridge and subsequent transects followed the remaining estuarine vegetation, along both sides of the stream channel. Line-point intercept data was recorded along each meter of each transect wherein the species the nearest stem, leaf, or plant base intercepted was recorded using a four-letter code based on the first two letters of the genus and species. A total of 48 transects were completed in this study (4 sites × 2 visits × 6 transects). Along each transect, three 1 m × 1 m quadrats were randomly placed (4 sites × 6 transects × 3 quadrats). Within each quadrat, soil was collected and vegetation height (cm) from the center and each corner, along with visual percent cover of each species present was recorded. A 25 cm × 25 cm sample of biomass, including all plant matter one cm above the soil, was collected from a fourth randomly chosen quadrat (4 sites × 6 biomass samples). Hitchcock and Cronquist [22] and Pojar and MacKinnon [23] were used to identify plant species.

2.3. Sample Preparation and Analysis

Soil was dried at 45˚C for one week, sieved (screen 1 cm × 1 cm) to remove rocks greater than 1 cm in diameter. From each sieved soil sample (n = 72), approximately 60 g was taken from each sample to make a homogenized composite sample representing each transect, totaling 24 composite samples. From each composite sample, a 30 g sub-sample was ground into a fine powder using a SPEX Mixer Mill in the Geology Department at WWU, which uses a hardened stainless-steel mortar and two stainless steel ball bearings. The ground composite sub-samples were used to test for P and CEC at Spectrum Analytical (Washington Court House, OH). Soil organic matter from the composited soil samples was determined using an optimized weight loss-on-ignition (WLOI) methodology specific to estuarine sediment [24]. Soil was dried overnight at 110˚C to remove any adsorbed water. Approximately two to three grams of soil was added to a ceramic crucible and the combined weight of the sample and crucible was recorded. The samples were then placed in a muffle furnace at 550˚C for four hours. Crucibles were removed and cooled for 10 minutes before being weighed. Carbon content was determined by percent WLOI and was calculated by determining the difference of final soil weight after samples were heated for four hours, from the initial soil weight.

2.4. Carbon-to-Nitrogen Elemental Analysis

Dried, homogenized soil and vegetation samples were analyzed for total carbon and nitrogen using a Thermo Electron NC Soil Analyzer Flash EA 1112 Series (Thermo Electron Corporation, Milan, Italy). A mass of approximately 100 mg was placed in tin capsules and compressed to remove any air prior to carbon-to-nitrogen analysis. A calibration curve for nitrogen was established using an analytical standard of atropine, which contained 48.4 $g \cdot kg^{-1}$ nitrogen. The calibration curve for nitrogen had an R^2 value of 0.99 and the mass of atropine ranged from 0.05 mg to 0.96 mg. The calibration curve for carbon also had an R^2 value of 0.99 and the mass of atropine ranged from 0.77 mg to 13.67 mg.

2.5. Statistical Analyses

A multiple analysis of variance (MANOVA) was used followed by a two-way fixed-effects analysis of variance (ANOVA) to compare percent carbon, soil organic matter, macro and micronutrients, species richness, species abundance using the Shannon-Wiener (H'), and number of invasive species occurrences at each site. The fixed effects were site (pre, 3-Yr, 9-Yr, and 12-Yr) and location (above and below) with an interaction term. Levene's test and Shapiro-Wilk's was used to assess how well data fit the assumption of homogeneity of variances and normally distributed populations, respectively. A log transformation was applied, when needed, prior to the ANOVA. A post-hoc pairwise comparison was used for sites and locations with significant ANOVA results (P-value < 0.05) using the "holm" P-adjustment method. When data could not be transformed to meet the assumptions of ANOVA, a Kruskal-Wallis non-parametric test was used.

The Chao and bootstrap population estimations were used to assess sampling efficiency. Principal components analysis (PCA) was used to determine which combination of species explained the most variance in the plant communities [25]. Because several species comprised nearly 20 percent of the entire vegetation population across all sites, a row-centered and scaled PCA was used to create homoscedasticity in the dataset, and thus scale species with large counts. Using the prcomp function in R, based on a singular value decomposition of the data matrix, identification of which components accounted for the most variation and separation in each principal component was determined. Analysis of similarity (ANOSIM) was used to calculate dissimilarity by using a ranked dissimilarity matrix. Based on these assumptions, a two-way ANOSIM was calculated between site and location, with 1000 permutations. All data was analyzed by R Version 3.3.0; [26].

3. Results

3.1. Percent Soil Carbon, Organic Matter, and Nutrients

Cation exchange capacity (CEC) differed per site with higher levels associated with the oldest restoration sites and the and pre-site and lowest levels recorded in sediments associated with the 3-year-old sites, below the bridge (P = 0.002). The newest restoration site (3-Yr) below the point of culvert restoration was significantly lower in soil carbon when compared across the other sites. Pair-wise post-hoc tests indicate similarities between the pre-restoration site and the oldest post-restoration site (12-Yr) with intermediate values observed from the 9-Yr plots. There were also significant differences in percent soil organic matter between sites (P = 0.003). Similar with soil carbon, the newest site (3-Yr) below the location of culvert restoration was significantly lower in soil organic matter when compared to the other sites (**Table 1**). There was a significant interaction between site and location (above and below) when concentrations of N and P were compared among the sites driven by a significant N and P nutrient decreases below the bridge at the 3rd year, newest site (P < 0.05; **Table 1**).

3.2. Vegetation

No differences existed when plant height and aboveground biomass was compared among sites and locations. A significant difference between site was noted

Table 1. Soil cation exchange capacity (CEC), percent soil carbon (%C), soil organic matter (%SOM), total percent nitrogen (%N), phosphorus (P), and plant species richness and plant species diversity from four estuary restoration sites in Kitsap County, Washington. Samples were pooled for analysis from two distinct locations (above and below; n = 23) at each site: pre-restoration (pre), three (3-Yr), nine (9-Yr), and 12 years (12-Yr) post-restoration. Means connected by the same letter do not differ according to Tukey's HSD ($\alpha = 0.05$).

	CEC (cmol/kg)	%C	%SOM	%N	P (mg/g)	Plant Sp. Diversity	Plant Sp. Richness
Pre-site							
Above	12.5 ± 0.4^{ab}	3.5 ± 1.0^{a}	10.9 ± 2.5^{ab}	0.2 ± 0.1^{a}	60.7 ± 2.3^{ab}	1.2 ± 0.07^{a}	5 ± 0.3^{a}
Below	15.3 ± 3.4^{a}	7.5 ± 2.9^{a}	17.2 ± 6.2^{ab}	0.5 ± 0.2^{a}	127.0 ± 28.6^{a}	1.5 ± 0.2^{b}	7.3 ± 1.5^{b}
3-yr site							
Above	10.2 ± 0.5^{ab}	2.4 ± 0.4^{ab}	6.8 ± 1.2^{ab}	0.2 ± 0.1^{a}	222.3 ± 65.3^{a}	1.1 ± 0.1^{a}	5.5 ± 0^{ab}
Below	2.1 ± 1.1b	0.9 ± 0.5^{c}	2.8 ± 1.0^{c}	0.03 ± 0.01^{b}	35.7 ± 8.7^{b}	1.4 ± 0.07^{ab}	6.0 ± 0.3^{ab}
9-yr site							
Above	9.2 ± 0.8^{ab}	2.0 ± 0.3^{bc}	5.8 ± 0.7^{ab}	0.1 ± 0.01^{a}	36.7 ± 3.5^{b}	1.6 ± 0.04^{b}	10.7 ± 0.8^{c}
Below	8.5 ± 3.4^{ab}	2.2 ± 0.7^{b}	6.8 ± 2.2^{ab}	0.1 ± 0.1^{a}	56.0 ± 15.5^{ab}	1.5 ± 0.01^{b}	7.7 ± 0.4^{b}
12yr site							
Above	16.6 ± 2.3^{a}	2.6 ± 0.9^{ab}	11.9 ± 2.6^{ab}	0.2 ± 0.1^{a}	92.7 ± 6.9^{a}	2.0 ± 0.06^{c}	10.2 ± 0.3^{c}
Below	14.4 ± 3.0^{a}	4.6 ± 1.1^{ab}	7.1 ± 1.7^{ab}	0.3 ± 0.1^{a}	129.7 ± 52.8^{a}	2.0 ± 0.07^{c}	10.2 ± 1.2^{c}

Superscript letters indicate homogenous subsets, as determined by Tukey's HSD.

for plant species diversity by site ($F_{(3,16)} = 23.58$, $P < 0.001$; Table 1). The pre-restoration site was the only site with significant differences in plant species diversity between above vs. below location ($P < 0.005$). Additionally, the Shannon-Wiener (H') index of species diversity was significantly highest at the oldest post-restoration site (1.98 ± 0.04) than any other site. A trend toward a more diverse plant community assemblage can be seen over time. A similar trend was noted for plant species richness between site and location (Table 1).

Summer vegetation surveys documented a total of 64 plant species (Table 2). The Chao estimated 73 (±9) species followed by the bootstrap method, which estimated a population size of 66 (±3). The most abundant native plant species throughout all the sites included: pickleweed (*Salicornia virginica*; 19.2%), orache (*Atriplex patula*; 9.5%), gumweed (*Grindelia squarrosa*; 8.0%), saltgrass (*Distichlis spicata*; 6.4%), meadow grass (*Hordeum brachyantherum*; 4.9%), and dune grass (*Elymus mollis*; 4.2%). ANOSIM indicated significant dissimilarities between location (above and below) at all sites (All $P < 0.05$; Table 3).

The principal components ordination was used to illustrate the vegetation community sampled during this study. Principal components I-II accounted for 32.3% of the total variance and illustrates salinity and vertical gradients based on species variable loadings ($\chi^2 = 23.4$; df = 6; $P < 0.0001$; Figure 2). Principal component I describes the species distribution by salt-tolerance, whereas salt tolerant species (illustrated by green ellipse) cluster in the upper left of the ordination and differentiate into freshwater marsh species (illustrated by blue ellipse)

Table 2. Complete species list of 64 species recorded along transects (n = 24), including scientific name, common name, location (above and below), native status, and relative species abundance (%) for all transect measurements located at four estuary restoration sites in Kitsap County, Washington including: one pre-restoration (Pre) site and three post restoration sites aged: three (3-yr), nine (9-Yr) and 12 years (12-Yr). Species are listed by their functional group and native status (N = Native and naturalized; NX = Noxious).

Species name	Abbrev	Common Name	Site (Pre, 3, 9, and 12)		Native Status	Relative Abundance (%)
			Above	Below		
Forbs and vines		-	-	-	-	-
Achillea millefolium	*AcMi*	Yarrow	N/A	Pre	N	<0.01
Argentina egedii	*ArEg*	Pacific Silverweed	12-Yr	9-Yr	N	0.25
Cakile edentula	*CaEd*	American Sea Rocket	3-Yr	3-Yr	N	0.71
Atriplex patula	*ChAl*	Orache	All Sites	All Sites	N	9.46
Cirsium arvense	*CiAr*	Canadian Thistle	9-Yr	N/A	NX	0.13
Convolvulus arvensis	*CoAr*	Bindweed	N/A	12-Yr	NX	<0.01
Cuscuta pacifica	*CuPa*	Dodder	3-Yr	N/A	N	<0.01
Cytisus scoparius	*CySc*	Scotch Broom	9-Yr	3-Yr, 12-Yr	NX	0.46
Daucus carota	*DaCa*	Queen Anne's Lace	N/A	12-Yr	NX	<0.01
Equisetum hymale	*EqHy*	Scouring Rush	9-Yr	N/A	N	0.04
Galium aparine	*GaAp*	Sticky Weed	N/A	3-Yr	N	<0.01
Grindelia squarrosa	*GrSq*	Gumweed	All Sites	All Sites	N	7.96
Honkenya peploides	*HoPe*	Seabeach Sandwort	N/A	3-Yr	N	0.29
Hypochaeris radicata	*HyRa*	Hairy Cat's Ear	3-, 9-, 12-Yr	9-Yr, 12-Yr	NX	0.13
Jaumea carnosa	*JaCa*	Fleshy Jaumea	Pre	Pre, 12-Yr	N	1.63
Lathyrus odoratus	*LaOd*	Sweet Pea	N/A	Pre, 12-Yr	N	0.75
Leucanthemum vulgare	*LeVu*	Ox-Eye Daisy	N/A	9-Yr	NX	0.08
Lotus corniculatus	*LoCo*	Bird's-foot Trefoil	N/A	9-Yr	N	1.50
Montia linearis	*MoLi*	Montia	Pre, 12-Yr	N/A	N	0.33
Plantago lanceolata	*PlLa*	English Plantain	9-Yr	9-Yr, 12-Yr	N	0.25
Plantago major	*PlMa*	Round Leaf Plantain	9-Yr	9-Yr	N	0.08
Plantago maritima	*PlMa*.1	Sea Plantain	3-, 12-Yr	Pre, 12-Yr	N	1.83
Polygonum aviculare	*PoAv*	Knotgrass	12-Yr	12-Yr	N	0.04
Polystichum munitum	*PoMu*	Sword Fern	9-Yr	N/A	N	0.04
Prunella vulgaris	*PrVu*	Self-Heal	N/A	9-Yr	N	<0.01
Ranunculus repens	*RaRe*	Creeping Buttercup	9-Yr	N/A	N	0.04
Rumex acetosa	*RuAc*	Sorrel	N/A	9-Yr	N	0.04
Rumex crispus	*RuCr*	Curly Dock	N/A	9-Yr	N	0.04
Sagina maxima	*SaMa*	Coastal Pearlwort	3-, 12-Yr	N/A	N	1.54
Salicornia virginica	*SaVi*	Pickleweed	All Sites	All Sites	N	19.21
Spergularia canadensis	*SpCa*	Sand Spurry	12-Yr	Pre, 3-Yr	N	2.38
Symphyotrichum subspicatum	*SySu*	Douglas Aster	12-Yr	N/A	N	0.17

Continued

Tanacetum vulgare	*TaVu*	Tansy	9-Yr	N/A	NX	<0.01
Trifolium wormskioldii	*TrWo*	Red Clover	9-Yr	12-Yr	N	0.38
Triglochin maritima	*TrMa*	Seaside Arrowgrass	3-, 12-Yr	Pre, 3-Yr	N	0.75
Graminoids		-	-		-	-
Agrostis capillaris	*AgCa*	Colonial Bentgrass	All Sites	All Sites	N	11.79
Agrostis exarata	*AgEx*	Spike Bent Grass	12-Yr	N/A	N	0.04
Ammophila arenaria	*AmAr*	European Beachgrass	N/A	Pre	NX	<0.01
Calamagrostis canadensis	*CaCa*	Blue Joint Grass	12-Yr	N/A	N	0.25
Deschampsia cespitosa	*DeCe*	Tufted Hair Grass	Pre	N/A	N	<0.01
Distichlis spicata	*DiSp*	Saltgrass	Pre, 3-, 12-Yr	Pre, 12-Yr	N	6.38
Elymus glaucus	*ElGl*	Blue Wild Rye	N/A	Pre, 12-Yr	N	0.29
Elymus mollis	*ElMo*	Dune grass	N/A	3-Yr	N	4.17
Elymus repens	*ElRe*	Quack Grass	All Sites	All Sites	N	1.25
Holcus lanatus	*HoLa*	Velvet Grass	9-, 12-Yr	9-Yr	N	0.21
Hordeum brachyantherum	*HoBr*	Meadow Barley	3-, 12-Yr	Pre, 3-Yr	N	4.92
Phalaris arundinacea	*PhAr*	Reed Canary Grass	9-, 12-Yr	9-, 12-Yr	NX	0.46
Sedges and Rushes		-	-		-	-
Carex lyngbyei	*CaLy*	Lyngby Sedge	Pre, 3-, 12-Yr	N/A	N	0.79
Eleocharis palustris	*ElPa*	Spike Rush	9-Yr	N/A	N	0.13
Juncus effusus	*JuEf*	Common Rush	9-Yr	9-, 12-Yr	N	0.33
Juncus gerardii	*JuGe*	Saltmeadow Rush	Pre	Pre, 12-Yr	N	1.25
Woody plants		-	-		-	-
Acer macrophyllum	*AcMa*	Big Leaf Maple	9-Yr	Pre, 9-Yr	N	0.58
Alnus rubra	*AlRu*	Red Alder	9-Yr	9-Yr	N	0.58
Oemleria cerasiformis	*OeCe*	Oso Berry	N/A	Pre	N	0.67
Pinus contorta	*PiCo*	Shore Pine	N/A	12-Yr	N	0.63
Pseudotsuga menziesii	*PsMe*	Douglas Fir	N/A	Pre	N	0.21
Robinia pseudoacacia	*RoPs*	Black Locust	N/A	9-Yr	N	0.42
Rosa nutkana	*RoNu*	Nootka Rose	Pre	Pre, 9-Yr	N	0.63
Rubus armeniacus	*RuAr*	Himalayan Blackberry	Pre, 9-, 12-Yr	Pre, 9-, 12-Yr	NX	1.25
Rubus ursinus	*RuUr*	Trailing Blackberry	N/A	9-Yr	N	0.42
Salix sitchensis	*SaSi*	Sitka Willow	9-Yr	N/A	N	0.17
Salix hookeriana	*SaHo*	Hooker Willow	N/A	9-Yr	N	1.08
Symphoricarpos albus	*SyAl*	Snowberry	N/A	Pre, 9, 12-Yr	N	0.08
Tsuga heterophylla	*TsHe*	Western Hemlock	N/A	9-Yr	N	0.04
Other						
Bare Ground	*BaGr*	-	All Sites	All Sites	-	9.00
Large Woody Debris	*LWD*	-	All Sites	All Sites	-	1.17

Table 3. Analysis of similarity between locations (above and below) at each site: one pre-restoration (Pre) site and three post restoration sites aged: three (3-Yr), nine (9-Yr) and 12 years (12-Yr), within this study. The number of permutations equaled 999. High ANOSIM R-values indicate larger dissimilarity in vegetation composition between locations.

ANOSIM	Pre		3-Yr		9-Yr		12-Yr	
R-Value	0.43		0.81		0.56		0.35	
P-Value	0.007**		0.005**		0.002**		0.008**	
SIMPER	Species	P	Species	P	Species	P	Species	P
	Pickleweed	0.01	Pickleweed	0.02	Orache	0.003	Saltgrass	0.003
	Fleshy Jaumea	0.03	Dune grass	0.003	Colonial Bentgrass	0.009	Coastal Pearlwort	0.05
	Shore Pine	0.03	Gumweed	0.00	Gumweed	0.01	Gumweed	0.02
					Common Rush	0.02	Pacific Silverweed	0.04

*Significant at $P < 0.05$; **Significant at $P < 0.01$

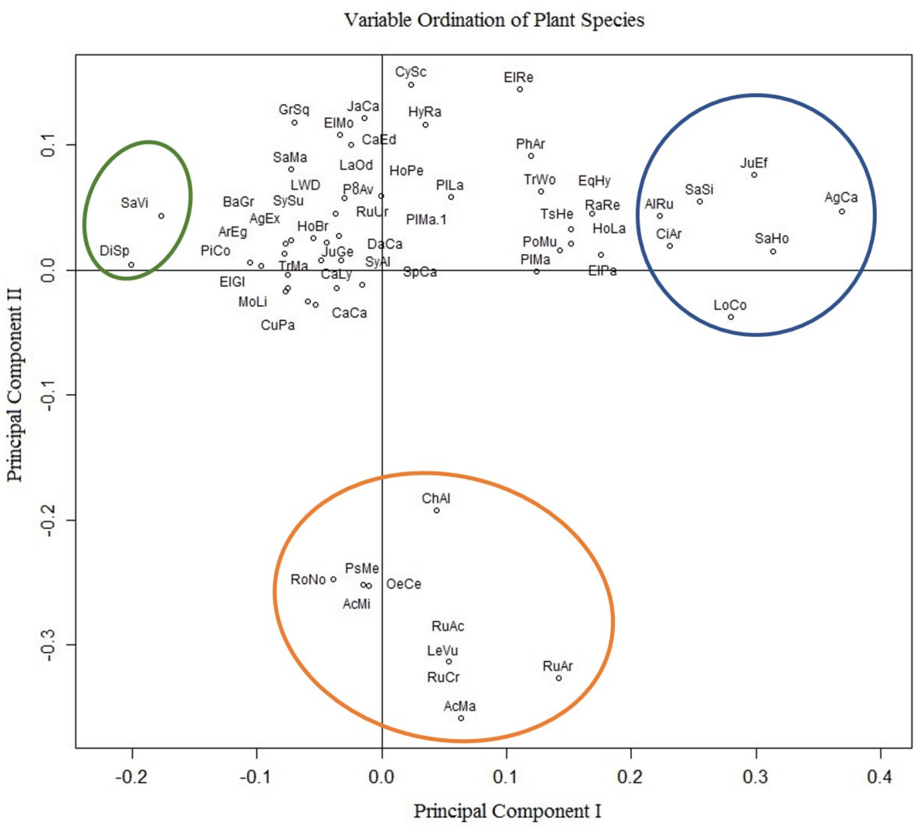

Figure 2. Variable ordination of principal component I and II (PCA) of plant species from four estuary restoration sites in Western Washington, USA. The four sites include one pre-restoration site (pre) and three post-restoration sites aged: three (3-Yr), nine (9-Yr), and 12 years (12-Yr). PCA by plant species using principal components I-IV from two distinct locations (above and below; n = 24). On PC I, the green ellipse indicated pickleweed (SaVi) and saltgrass (DiSp), two halophytic species. Those encircled by the blue ellipse, indicate species adapted to freshwater ecology. PC II is mainly separated by a vertical gradient, where species within the yellow ellipse form higher up on estuarine banks. All species abbreviations are defined in Table 2.

into the right quadrate of the graph. Principal component II likely describes a vertical gradient, wherein older, woody native and non-native upland species (orange ellipse) differentiated from herbaceous marsh perennials and annuals (**Figure 2**).

Invasive species were relatively uncommon at each site and marginal evidence of a difference was observed in the interaction of site and location of the number of invasive occurrences in point-intercept data ($F_{(3,40)} = 2.52$, $P = 0.07$; **Table 2**). Nine invasive plant species were documented: Himalayan blackberry (*Rubus armeniacus*; 1.3%), Scotchbroom (*Cytisus scoparius*; 0.5%), reed canary grass (*Phalaris* arundinacea; 0.5%), hairy cat's ear (*Hypochaeris* radicata; 0.1%), Canada thistle (*Cirsium* arvense; 0.1%), ox-eye daisy (*Leucanthemum vulgare*; <0.1%), European beachgrass (*Ammophila arenaria*; <0.01%), common tansy (*Tanacetum vulgare*; < 0.1%), field bindweed (*Convolvulus arvensis*; <0.1%), and Queen Anne's lace (*Daucus carota*; <0.1%).

4. Discussion

Differences in CEC, N, P, soil carbon and SOM existed among sites, with the lowest levels sampled from the newest post-restoration site. These results are consistent with other post-restoration sites [27], where large carbon deficits occur in initially restored systems [28] [29]. This reduction in soil carbon and organic matter could be due to several mechanisms. For one, heavy machinery and plant removal during bridge construction were responsible for displacing fine sediment and organic matter. Secondly, the reconnection of the estuary after culvert removal promoted movement by riverine outflows, daily tides, and weather events causing the displacement of lightweight, fine sediment particles into the estuary, leaving coarse sediment deposition at the tailwater of the new bridge. Estuaries are characterized by the dynamic deposition and resuspension of fine sediment particles; sediment accretion naturally occurs in the low-sloped areas when water velocity is reduced. Because the sites within this study are shallow-water estuaries with flat topography, velocity of silt and fine sediment slows and allows for the accretion of coarse sediment along estuarine edges near vegetation, which reduced the amount of fine sediment and SOM [30].

None of the sites displayed carbon or SOM concentrations representative of undisturbed, temperate coastal marshes on the eastern coasts of the U.S., which estimate normal soil carbon ranging between 12% - 20% and organic matter ranging between 22% - 35% [31]. Further, it is suggested that 18% - 26% of estuarine soil (mineral or organic) is composed of organic matter [28], with 21% - 25.6% of that organic matter being comprised of carbon [32]. Further, both N and P were deficient at the newest, lower restoration site. This is presumably linked to the lack of organic matter, which is required for adequate cation exchange capacity and nutrient/plant exchange [33]. This is especially true for nitrogen, which has been reported as deficient after 30 years post-restoration [29]. Authors note that climate and dominant plant community will differ for the west coast and influence soil carbon and organic matter differently; therefore, future studies that document Pacific Northwest projects over time will help better understand how restoration is improving ecosystem functioning.

Plant species diversity and richness increased over time, with the oldest post-restoration site (12-Yr) showing the highest species diversity of all study sites. Plants surveyed totaled 64 species between all sites and fell within the range of our statistical estimates. Forbs were the most common functional group sampled between all sites and comprised over half of species. Grasses, sedges, and rushes comprised just under one quarter of all species followed by woody species, which comprised 20% of all species sampled. Common estuarine species native to the Pacific Northwest were encountered in relative proportion to functional group composition typical of a temperate pocket estuary, including non-native and non-tidal marsh plant species associated with culvert disturbance [34] [35].

Based on plants surveyed, pickleweed, saltgrass, orache, gumweed, and dunegrass are the most common at recent post-restoration sites, with gumweed, and dunegrass especially successful colonizing rocky, disturbed sites. The oldest post restoration site (12-Yr) had the highest species diversity and is relatively similar to other restored, temperate estuaries in terms of overall diversity based on Shannon-Weiner diversity index [36] [37]. Authors note that this site also had greater SOM, which may play roles in water relations and nutrient availability. This site harbored plants such as monita, sea plantain, coastal pearlwort, sand spurry, seaside arrowgrass and Pacific silverweed and may represent mid-successional species. Based on the community analyses, vegetation communities separated into two distinct groups: halophytic (salt tolerant) and glycophytic (salt intolerant), due to the formation of both a salinity and vertical gradient within the recovering floodplain and marshes. The salinity from the marine input is effective in deterring invasive species recruitment [38]. However, their presence was a result of a vertical gradient, which disconnected the plant community from the tidal flood plain, harbored non-native species. The second, glycophytic, group was composed of salt intolerant grasses, rushes, and early successional woody species. These were survey from areas of either strong freshwater influence, or also perched above the tidal flood plain. It is noted that species that appeared on the restoration planting lists were not sampled in this survey.

In summary, soil carbon and organic matter are initially lost during restoration with no sites having ranges comparable to natural estuaries. Borja *et al.* [27] suggest recovery from sediment modification and habitat creation is estimated to take at least two decades. Plant communities do not appear to homogenize between locations (above and below), due to the development of a salinity gradient, which is a common component of a natural estuary. However, vertical gradients at the shoreline resulted in perched vegetation communities that are removed from tidal influence, and conducive to woody riparian and non-native plant species establishment. Authors acknowledge these are results from an unreplicated, observational study that seeks to capture current state of local estuary soil and vegetation, which can be followed over time. This information hopes to inform future projects where direct seeding of pickleweed, saltgrass, seaside plantain, and Lyngby's sedge, can be initiated upon restoration. After the development of the salinity gradient, woody plantings (e.g. Nootka Rose, Oso Berry, Hooker's and Sitka Willow, Shore Pine) can be incorporated along the vertical gradient where inundation is infrequent. Time and additional sampling are required to determine the recovery of these estuaries over time, and if recovered floodplains to adequately create marsh habitat in lowland pocket estuaries required for the migration of anadromous fish species of Western Washington.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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