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Plant Community and Nutrient Development within Four Estuary Restoration Sites in Kitsap County, Washington

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PLANT COMMUNITY AND NUTRIENT DEVELOPMENT
WITHIN FOUR ESTUARY RESTORATION SITES
IN KITSAP COUNTY, WASHINGTON

By
Shannon Marie Call

Accepted in Partial Completion
Of the Requirements for the Degree
Master of Science

Kathleen L. Kitto, Dean of the Graduate School

ADVISORY COMMITTEE

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MASTER’S THESIS

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Shannon Marie Call
June 23, 2017
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A Thesis
Presented to
The Faculty of
Western Washington University

In Partial Completion
Of the Requirements for the Degree
Master of Science

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Shannon Marie Call
June 2017
Abstract
Coastal wetland ecosystems are some of the most productive ecosystems on the planet and link freshwater and marine environments. Coastal wetlands provide invaluable ecosystem services such as carbon sequestration, storm abatement, biogeochemical cycling, and water filtration. However, estuaries affected by physical barriers, such as culverts, experience reduced hydrological inputs and reduced connectivity above and below the site of impact. Loss of connectivity results in loss of ecosystem function such as carbon and nitrogen cycling. We investigated soil nutrients and vegetation composition of estuarine communities in four estuary restoration locations in Kitsap County, Washington and the following questions were addressed: 1) is there a linear trajectory in recovery of soil carbon and organic matter due to length of time since ecological restoration (i.e. culvert removal), 2) is there a recovery of soil nutrients optimal for plant growth, 3) does plant species diversity increase over time, 4) will plant communities homogenize between restoration location (i.e., above or below the culvert) over time, and 5) does time since restoration affect invasibility?

Differences in percent soil carbon and organic matter existed among sites. The carbon-to-nitrogen ratio was highest below the culvert restoration location at the newest post-restoration site, indicating nitrogen deficiency. Percent soil carbon and organic matter initially dropped in newly restored sites, and was highest at the pre-restoration site (pre). Soil nutrients were analyzed and nitrogen, potassium, magnesium, sulfur, boron, copper, and manganese were positively correlated with dried plant biomass. Potassium, magnesium, boron, iron, and manganese were all below common soil ranges. A total of 65 plant species were surveyed, with a significant increase in species richness and diversity (H’) at the oldest restoration site, with decreasing differences in diversity as age since restoration increased.

Community composition was dominated by pickleweed (Salicornia virginica), colonial
bentgrass (*Agrostis capillaris*), and fat hen (*Chenopodium album*) among all sites. Nine invasive species were surveyed, but were not significantly different within and among sites. The pre-restoration site (pre) showed the lowest species richness above the culvert and the intermediate site had the highest, with a trend of increasing species richness over time. The oldest post-restoration site had the highest diversity using the Shannon-Wiener (H’) diversity index. Locations (above or below) were significantly different from one another determined by principal component analysis (PCA), analysis of similarity (ANOSIM), and similarity percentages (SIMPER). The results indicate salinity is the largest environmental driver of vegetative assemblages, and homogenization of plant communities between locations (above vs. below) has not occurred at any site.
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1.0 Introduction

1.1 Ecosystem Connectivity

The application of restoration landscape ecology within this project is a synthesis of spatial connectivity and temporal dynamics of species assemblages between habitat types (Burgman, Lindenmayer, and Elith 2005). Coastal wetland and estuarine ecosystems spatially link freshwater and marine environments (Mitsch and Gosselink 2015), which are supported by functional and structural connectivity between freshwater riverine and marine ecosystems (Downing 2005). Functional connectivity sustains biotic components of an ecosystem, like populations of plants and animals, allowing species to move between marine and freshwater ecosystems (Cloern 2007). Structural connectivity promotes the movement of abiotic factors, such as sediment, into estuarine systems and creates habitat that supports biodiversity (Spooner and Vaughn 2009). Because biogeochemical reactions and carbon sequestration occur within estuarine sediment, connectivity to adjacent habitat is crucial for sediment accretion and vegetation succession (Craft 2007). A high degree of ecological connectivity between freshwater riparian and marine ecosystems facilitates energy production, biogeochemical cycling, and gene flow within habitats (Borja et al. 2009; Fulford, Russell, and Rogers 2016; Horskins, Mather, and Wilson 2006; McRae et al. 2008).

Estuarine ecosystems are highly dynamic, interrelated systems that facilitate the movement of materials between two otherwise distinct ecosystems (Thorpe et al. 2010). Functional ecological connectivity between highly heterogeneous estuarine and riverine habitat facilitates many processes that support ecosystem services such as storm abatement from sediment accretion, aquifer recharge as water filters through estuarine sediment, and resource output through habitat particularly crucial for native and endangered anadromous
fish species (Hall, Jordaan, and Frisk 2012; Helfield and Naiman 2002). Additionally, estuaries are highly dynamic regions; influenced by a constant influx of sediment that mitigates the force of tidal action and provides protection to coastal property (Koch et al. 2009). Estuaries and coastal wetlands provide ecological processes such as primary productivity, sequestering and storing carbon, and creating habitat structure (Reid et al. 2005).

1.1.1 Ecosystem Services

Ecosystem services are functions performed by ecosystems, deemed valuable from the benefits provided to humans. Coastal wetlands perform services such as storm abatement, attenuating waves and severe weather, and water filtration (Barbier et al. 2011; McRae et al. 2008; Sheaves 2009). Hydrogeomorphology, vegetation, and soil are all functionally interrelated and influence net primary productivity, carbon sequestration rates, bioaccumulation of minerals and metals, and the creation of wildlife habitat (Beaumont et al. 2007). Within an estuarine ecosystem, vegetation provides bank stabilization, bioaccumulates pollutants, and is a source of organic matter input into sediment (Lytle and Lytle 2001; Negrin et al. 2016), while estuarine soils reduce nitrogen leaching, provide physical stability through sediment and organic matter accretion, and act as a sink for carbon (Burdige and Zheng 1998; Craft 2007; Craft, Broome, and Seneca 2016; Marks, Chambers, and White 2016; Mueller, Jensen, and Megonigal 2016). This interaction between soil, vegetation, and organic matter are strongly dependent on an influx of sediment from both freshwater and marine sources. As a result of this dynamic relationship, a constant influx of marine and freshwater derived nutrients supports biodiversity and sediment accretion (Grange et al. 2000). Functional connectivity allows for the migration and movement of biotic
communities, while structural connectivity then facilitates the movement of material through intertidal estuaries (Moreno-Mateos et al. 2012).

1.1.2 Carbon in Estuaries

As a result of this movement of materials, up to 87 teragrams of carbon are stored each year in salt marshes (McLeod et al. 2011). Carbon in estuarine systems originates from two sources: within the ecosystem (autochthonous) and from outside the ecosystem (allochthonous). Atmospheric carbon dioxide (CO\textsubscript{2}) is utilized by plants and algae during photosynthesis and incorporated into chlorophyll, carbohydrates, and cellulose (Evert and Eichhorn 2013; Pregitzer 2003). In coastal estuarine systems, carbon sequestration occurs when soil organic matter, plant detritus, and particulate organic matter are input into the soil and stored in the sediment, referred to as blue carbon. About half of annual blue carbon sequestration occurs in ecologically connected shallow-water salt marshes (Kirwan and Mudd 2012), where it accumulates as algal cells in water bodies, organic matter within sediment, and in above- and belowground vegetative biomass (Drake et al. 2015; McLeod et al. 2011; Mueller, Jensen, and Megonigal 2016; Negrin et al. 2016). Estuaries experience such high blue carbon sequestration rates (Kirwan et al. 2013; Zhang et al. 2016) because flat topography (Noe and Hupp 2009) and frequent flooding deposits vegetative detritus (organic matter) and particulate organic matter (Bertilsson and Jones 2002) from riparian and marine inputs respectively (Davidson and Janssens 2006).

Natural factors influence carbon sequestration efficacy, such as hydrologic regimes, topography, microbial and plant community composition, ambient temperature, and salinity (Wang et al. 2011). Once organic matter enters the sediment, particles suspended in water during tidally induced flooding, get trapped in the matrix formed by the organic detritus
(Brady 1974). This matrix allows for the continued accumulation of organic matter in estuarine sediment (Doane and Horwáth 2004; Mudd, D’Alpaos, and Morris 2010; Zhang et al. 2016). Increased vegetative detrital inputs and sedimentation facilitates carbon sequestration rates as anaerobic conditions are created by inundation and aerobic decomposition efficiency is reduced. Duration of tidal inundation (Voss, Christian, and Morris 2013), sediment accretion (Redfield 1972), tidal influence (Miller, Neubauer, and Anderson 2001), the microbial and vegetation communities that promote the rate at which carbon is cycled or sequestered in sediment.

Approximately 1-4% of organic matter is comprised of nitrogen (Cabaniss et al. 2005; Craft, Seneca, and Broome 1991), and because the nitrogen cycle depends on recovered microbial communities and input from plant material, when little carbon (i.e. plant material) is in the system, even less reactive nitrogen will be available for plant uptake (Taylor and Townsend 2010). Nitrogen is highly mobile within a wetland system and is highly water soluble (Brady 1974; Evert and Eichhorn 2013). As a result, microbial communities have adapted to denitrify or fix nitrogen as part of the nitrogen cycle (Mitsch and Gosselink 2015). Because coarse sediment has lower water holding capacity, nitrogen retention is reduced. Consequently, coarse sediment negatively affects microbial communities as nitrogen is leached from the system rather than being adsorbed onto clay particles and within organic matter (Liao et al. 2008; Tobias et al. 2001). When sediment begins accreting, nitrogen cations more readily bind to clay particles (Howarth and Cole 1985; Manzoni et al. 2010). Estuaries are significant drivers of nitrogen loss through denitrification, the process by which facultative anaerobic bacteria reduce oxidized nitrate to N₂ (Bernhard et al. 2015; Day et al. 2013; Mitsch and Gosselink 2015). When nitrogen is
leached from the system, microbial communities are unable to metabolize and facilitate the transfer of inert atmospheric N\textsubscript{2} captured by plants into reactive nitrogen (Bernhard et al. 2015; Vitousek et al. 1997). Nitrogen may be retained within the sediment in a positive feedback loop between increased organic matter input and sediment accretion. Organic matter input has important consequences for microbial populations, thus the reduction in organic matter increases nitrogen leaching, reduces nutrient transformation, and reduces carbon sequestration (Parker 2005).

1.1.3 Vegetation in Estuaries

Estuarine plant species in an intertidal zone are restricted by two gradients: salinity and flooding (Bockelmann and Neuhaus 1999). Salinity gradients occur on the landscape, where a pattern of plant distribution can be seen based on salinity influence (Crain et al. 2004). Flooding gradients vary as the depth and duration of inundation is altered and as tidal influence interacts with riverine flow (Odum 1988). Plant species richness decreases as salinity or flooding influence increases (Engels and Jensen 2009), while freshwater influence recruits more diverse species assemblages. Additionally, plants can be classified by the function and resources they perform and use in an ecosystem (Elliott and Quintino 2007; McLaren and Turkington 2010). When functional groups (e.g. forbs, graminoids, sedges/rushes, and woody) of estuarine vegetative communities are diverse, ecosystem health is maintained through resource use partitioning (Cardinale, Palmer, and Collins 2002). The presence of plant material may reside in soil for up to a century (Whalen et al. 2014), making vegetation an important factor when considering carbon sequestration and ecosystem services. Estuarine vegetation sequesters a major portion of atmospheric carbon, as well as sustains microbial communities. Diverse species assemblages strongly influence carbon
storage because higher plant diversity supports increased microbial activity, which in turn increases the carbon storage ability. This ability of a soil system to store carbon is a function of the integration of new carbon into the soil (Lange et al. 2015). When many species are present, and partitioning of resources occurs, larger volumes of plant matter are input into the system. This suggests that newly restored sites with low vegetative diversity will consequently store less carbon. With continual input from organic matter into the sediment (Hobbie 2015), diverse microbial communities are supported (Buchan et al. 2003; Hoorens, Aerts, and Stroetenga 2003; Kirwan et al. 2013; Mulholland 2002).

Plant species colonization is strongly influence by salinity gradients, wherein this gradient influences the relative abundance of individual species (La Peyre et al. 2001). When a community is driven by a saline influence, one or two halophytic species tend to dominate the estuarine system and their abundance out-competes other halophytic species. In salt marsh ecosystems, vegetation is generally separated by a salinity gradient ranging from 0.5 parts-per-thousand to 30 parts-per-thousand (Greenberg, Maldonado, and Droge 2006; La Peyre et al. 2001; Odum 1988; Sharpe and Baldwin 2009).

1.1.4 Microbes and Nutrients in Estuaries

Microbes are key facilitators in the decomposition and transformation of vascular plant detritus in aquatic systems, like salt marshes (Benner, Moran, and Hodson 1986). Microbial assemblages (including phytoplankton) within estuaries are also driven by salinity gradients and flooding regimes. Microbes directly influence nutrient cycling in estuarine soil (Ryther and Dunstan 1971a) as they decompose and mineralize vegetation into its elemental components; replacing nutrients and maintaining ecosystem productivity (Hoorens, Aerts, and Stroetenga 2003). This occurs when plant litter and carbon are broken down by microbial
communities in soil (Duarte and Cebrián 1996; Knops and Tilman 2000). It is this nutrient cycling and transformation that allows macro- and micronutrients to be absorbed by plants (Mcguirk et al. 2008). The hydroperiod influences nutrient cycling in wetland systems. The amount of oxidation or reduction of chemicals in a wetland system is directed by hydrologic conditions; this is known as the redox potential. When anaerobic conditions persist as a result of flooding, bacterial respiration is decreased (Cao, Green, and Holden 2008; McLeod et al. 2011; Morris et al. 2002), and thus the reduction and oxidation potential of nutrients are altered. Flooding influences the type of nutrient processing. This cycle between organic matter input, microbial decomposition, and vegetation uptake creates a perpetuating feedback loop between primary productivity and nutrient cycling (Apple and del Giorgio 2007; Bruesewitz et al. 2013), wherein ecosystem productivity and services are maintained.

Water movement within and between marine and freshwater ecosystems is highly dynamic and facilitates the influx of nutrients. Of necessary nutrients, estuarine plants, phytoplankton, and microalgae utilize carbon, nitrogen, and phosphorus the most, yet in proportional quantities. These specific proportions are described as the Redfield Ratio, where the atomic ratio of carbon, nitrogen, and phosphorus are present in organic matter as carbon-to-nitrogen-to-phosphorus = 106:16:1 (Neill 2005; Redfield 1958). Of these three macronutrients, the two most commonly deficient in an estuarine system are nitrogen and phosphorus (Lui and Chen 2011). Nitrogen is often the most limiting nutrient from the marine ecosystem and may be the limiting factor of organic matter production in estuaries (Ryther and Dunstan 1971b). Alternatively, phosphorus is often the most limiting nutrient in freshwater system (Fisher et al. 1992). This relationship between marine and freshwater systems facilitates the input and mixing of nutrients within an estuarine system, supporting
prodigious primary productivity rates (Cloern 2007; Morris et al. 2002; Piehler and Smyth 2011). While nitrogen and phosphorus may be limiting in a natural estuary, over two thirds of estuaries in the contiguous United States are negatively influenced by excess nitrogen and phosphorus input, including the Puget Sound (Williams and Kimball 2013). Because of their location between land and sea, estuaries are particularly vulnerable to large influxes of urban, agricultural, and industrial effluents (Dolbeth et al. 2007; Valiela and Bowen 2002). When this influx of nutrients, particularly nitrogen and phosphorus, exceed that which can be utilized by vascular plants, phytoplankton populations increase rapidly and cause harmful algal blooms leading to estuary degradation (Woodland et al. 2015).

Nutrient transformation is supported by sediment accretion, spatial complexity, and organic matter transported into an estuarine system (Biggs and Howell 1984; Perez et al. 2011). This nutrient availability is directly translatable to the amount of sequestered carbon stored as organic matter (Beach et al. 2012). While the movement of material from adjacent habitat facilitates the movement of nutrients into an estuary, the ability of nutrient adsorption by biotic communities is salinity dependent (Duinker 1980; Seitzinger, Gardner, and Spratt 1991). Additionally, soils with high clay content tend to hold negative cations more readily than soils with relatively smaller clay composition (Brady 1974). Seasonal temperatures, sediment composition, and hydrologic connectivity will ultimately influence the rate at which an estuary acts as a sink or source for nutrients (Boyle, Collier, and Dengler 1974). Because of the dynamic mixing of water in estuaries, adequate nutrient influxes in the Pacific Northwest can be seen (Davis et al. 2014). This influx occurs because upwelling ocean-derived nutrients strongly influence increased estuarine nutrients (Day Jr. et al. 1989).

However, a wide variability exists among estuaries as a result of hydrologic mixing, spatial
and temporal distribution of nutrients, and size, making it difficult to assess a common range of nutrients (Li and Li 2011; Lui and Chen 2011; Renjith et al. 2016; Wilson and Morris 2012).

Estuarine plants have developed efficient nutrient usage systems (Enoksson 1993; Mitsch and Gosselink 2015). Many halophytes (salt-tolerant) utilize alternative photosynthetic pathways (C₄ and CAM) which allows increased water conservation by reducing photorespiration (Bromham and Bennett 2014; Flowers and Colmer 2015; Richards, Pennings, and Donovan 2005). This photosynthetic efficiency supports carbon gain and nutrient retention in a harsh environment (Richards et al. 2010). However, a lack of organic matter may result in a loss of soil nutrients. This occurs because organic matter retains nutrients and this provides a source of nutrients for microbial and bacterial populations (Lehmann and Kleber 2015).

1.2 Degradation of Estuaries

Estuaries comprise 4% of total landmass globally, yet nearly 50% are estimated to be heavily impaired from anthropogenic sources, such as coastal development and urbanization. (Fetscher et al. 2010; Lemly, Kingsford, and Thompson 2000). Anthropogenic activity such as agricultural waste runoff, industrial pollution, and mechanical disturbance harm functional integrity of estuaries worldwide (Lemly, Kingsford, and Thompson 2000; Lotze et al. 2006; Nichols et al. 1986). In addition to degradation from excess nutrients, loss of connectivity from impediment by a physical barrier reduces structural and functional connectivity. This disconnection has a negative impact on gene flow, seed dispersal, and marsh habitat in which migration routes are interrupted (Bartz et al. 2006; Beaumont et al. 2007). Additionally, a restriction of sedimentation rates, biogeochemical cycling, and hydrological mixing can be
seen in sites that are structurally disconnected (Moreno-Mateos et al. 2012; Ward, Malard, and Tockner 2002). Many estuaries have been affected by loss of connectivity through physical alterations in landscape, such as culverts, levees, and dated drainage infrastructure (Puget Sound Estuaries Implementation Strategy Narrative 2015).

In Washington and Oregon alone, up to 5,000 streams with over 196,000 kilometers of road are affected by culverts (United States General Accounting Office 2001). Culverts have altered hydrological regimes and caused the endangerment of Pacific Northwest aquatic salmonid species by restricting their migration through the development of disconnected habitat, impassable culverts, and scour pools (Escarameia and May 1999). Specifically, estuaries in the Pacific Northwest face increased anthropogenic pressure as a result of resource use, private development, and commercial estuarine use (Huppert et al. 2003). Within the Puget Sound of Washington State, 74% of estuary habitat has been lost and now comprises only 14,640 acres (Puget Sound Estuaries Implementation Strategy Narrative 2015).

As a result of culvert blockage, restricted tidal flow and sediment accretion creates additional stressors on already highly stressed estuarine communities by reducing flocculation ability of fine-grained sediment (Leussen and Cornelisse 1993), biogeochemical cycling, hydrologic mixing, and reduced carbon sequestration (Eberhardt, Burdick, and Dionne 2011; Maynard, Dahlgren, and O’Geen 2011; Petrone et al. 2011; Wohl 2014; Valiela and Bowen 2002). Culverts cause increased sediment impoundment at the headwater, where water enters the culvert (Poplar-Jeffers et al. 2009). Because of reduced particle entrainment downstream and increased velocity where water exits the culvert, scour pools may develop below the tailwater (Giannico and Souder 2004). This displacement of sediment
may create a perched culvert, which elevates the tailwater of the culvert above the river or estuary, structurally disconnecting it from the stream channel. During low tide and low flows, the scour pools trap aquatic species, and fully disconnect the freshwater ecosystem from marine habitat. Alteration of sediment input in estuaries results in reduced resilience from rising sea levels, increased salinity in the tidal freshwater zone, and reduced nutrient transport into the system (Beaumont et al. 2007; Callaway, Delaune, and Patrick Jr. 1997). Permanent alteration in hydrology increases flood duration and drying periods in estuaries (Eberhardt, Burdick, and Dionne 2011), which in turn negatively influences functional connectivity within biotic communities (Janousek 2004). Consequently, culvert placement alters the pathways of energy and material flows by reducing and/or altering sediment organic matter accretion rates (Cardinale et al. 2012).

Soil organic matter input is restricted by the structural disconnectivity of a culvert, which reduces sediment and organic matter accretion in estuarine ecosystems. Sediment and organic matter input is then decreased below the culvert, reducing biogeochemical cycling. Physically separated populations may then experience loss of genetic diversity, increase in interbreeding, and reduction of resilience because of isolation or habitat fragmentation (Horskins, Mather, and Wilson 2006; Balkenhol et al. 2016). Not only does altering connectivity influence genetic dispersal, extended periods of flooding and drying create longer periods of aerobic conditions. This extended drying period allows facultative bacterial communities to metabolize organic matter at increased rates, where dinitrogen (N₂), methane (CH₄), and carbon dioxide (CO₂) are released into the atmosphere (Flynn 2008; Mitsch and Gosselink 2015; Vincent, Burdick, and Dionne 2013). Fragmented plant assemblages experience reduced net primary productivity, creating temporal instability and loss of
ecosystem services. The alteration in functional connectivity then leads to a reduction of hydrological mixing that decreases carbon sequestration rates, separates populations, and restricts migration (Fellman, Petrone, and Grierson 2011; Søndergaard, Stedmon, and Borch 2003; Srivastava and Vellend 2005).

1.3 Remediation of Estuaries

Process-based restoration is currently the focus in restoration ecology because supporting services, such as nutrient cycling, primary productivity, and carbon sequestration are the foundational elements that drive all other ecosystem services (Apostol and Sinclair 2006). Restoration of estuaries seeks to improve the abiotic conditions by culvert removal and bridge replacement (described in methods), thereby restoring the aquatic corridor and floodplain. However, many processes should be considered in a holistic approach to reconnect ecosystems, including connectivity throughout the intertidal zone to promote sediment/soil stability and the recovery of native estuarine vegetation. This in turn would enhance energy movement, population resilience, migration routes, and biophysical processes between abiotic factors and biotic communities (D’Agostini, Gherardi, and Pezzi 2015; Ward, Malard, and Tockner 2002). Early succession sediments and soils within restoration sites lack soil organic matter and depend on pioneer species to influence abiotic and biotic conditions that facilitate the arrival of other estuarine species (Mason, French, and Jolley 2013). However, path dependence within plant recovery suggests that natural processes lead to varied outcomes (Desjardins 2015), thereby, the recovery pathway can be influenced by the order and timing of plant colonization. Thus, newly restored corridors may be susceptible to invasion by exotic plant species that may alter native plant recovery and the ecosystem processes such as soil accretion rates, organic inputs, and water cycling by
transpiration (Levine et al. 2003; Hobbs et al. 2006). Restoration efforts may be altered by less predictable weather caused by climate change (Harris and van Diggelen 2006), increased nutrient loading from anthropogenic sources (Vitousek et al. 1997), or length of spatial alteration (Smith et al. 2009).

When considering vegetation restoration of estuaries, there is a fundamental gap in the trajectory regarding the succession of native floral species. Though vegetation is projected to recover in one to two decades (Byers and Chmura 2007; Borja et al. 2010), long-lasting effects of sediment impoundment and structural disconnectivity on microbial communities can still be seen several decades after restoration (Bernhard, Marshall, and Yiannos 2012). In recent restoration efforts, a large variation in sediment dynamics within the first five years were noted in a Pacific Northwest estuary restoration site, but estimations for sediment accretion and soil carbon recovery range between 75-150 years (Desjardins 2015; Thom, Zeigler, and Borde 2002). Vegetation recovery data is sparse in the Pacific Northwest, though pickleweed (Salicornia spp) appears to be one of the initial pioneer species in tidal salt marshes (Lonard, Judd, and Stalter 2012). This pioneer species creates positive input that supports accretion rates by trapping fine particles in stems and roots and adding organic material to the sediment (Sánchez, SanLeon, and Izco 2001). Sedimentation and soil development is the first stage of estuary recovery, which then promotes microbial communities and the succession of other plant species (Sacco, Seneca, and Wentworth 1994). It appears nutrient levels are typically lower in post-restoration sites than in a recovered system, especially nitrogen (Strange et al. 2002), but a lack of consistency when identifying and prioritizing restoration projects has created a gap in knowledge.
Due to the wide variety of potential restoration outcomes, implementation and effectiveness monitoring become essential components in assessing ecosystem recovery, especially when determining which systems drive recovery (Apostol and Sinclair 2006; Society for Ecosystem Restoration 2004). Because the end goal of restoration is to encourage a self-replicating natural system that will resemble native reference sites, early monitoring and identifying alternative pathways in estuary restoration becomes an important management strategy. However, the lack of post-restoration effectiveness monitoring of soil carbon and vegetation communities continues increasing the fundamental gap of knowledge in the recovery of estuarine systems (Suding, Gross, and Houseman 2004). The objective of this thesis project is to monitor the development of soil carbon, organic matter, nutrients, and plant communities within four estuary restoration sites in Kitsap County, WA. Several factors are considered within the scope of this project to determine the role of time in restoration including: 1) soil carbon, organic matter, and nutrient development within sediment (Brady 1974), 2) estuary vegetation succession, and 3) invasive species recruitment, (Davis, Grime, and Thompson 2000; Mason, French, and Jolley 2013).
2.0 Research Objectives and Statistical Hypotheses

The objective of this project is to assess the development and timing of soil carbon, organic matter, nutrients, and plant communities as a function of estuary restoration. Within the scope of this project, recovery is defined as a return to normal levels of soil carbon, organic matter, nutrients, native plant assemblages, and low invasive species recruitment. To inform this objectives, the following four questions were posed: 1) is there a linear relationship in recovery of soil carbon, organic matter, and nutrients due to length of time since ecological restoration (i.e. culvert removal), 2) does plant species diversity increase over time, 3) will plant communities homogenize between restoration location (i.e., above or below the culvert) over time, and 4) does time since restoration affect invasibility? Because of the gap in knowledge regarding, development of soil carbon, organic matter, soil nutrients, homogenization of estuarine vegetation communities, and the invasibility of these sites, suggested direction in current research, the following questions, and statistical hypotheses, were established to address this gap:

Question 1. Is there a linear relationship in the recovery of soil carbon, organic matter, and nutrients due to length of time since ecological restoration (i.e. culvert removal)?

\[ H_0 = \text{There will be no difference in soil carbon, organic matter, and soil nutrients as length of time since restoration has increased.} \]

\[ H_A = \text{There will be an increase in soil carbon, organic matter, and soil nutrients as length of time since restoration has increased, because vegetation communities will have diversified over time and will provide increased sediment input and facilitate accelerated accretion rates. Additionally, there will be a recovery of carbon, nitrogen, phosphorus, potassium, magnesium, calcium, sulfur, boron, copper, iron, manganese,} \]

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and zinc because time since restoration has increased sediment accretion and movement of materials, thus facilitating biogeochemical cycling.

Question 2. Does species diversity increase over time?

\( H_0 \) = There will be no difference in plants species diversity as time since restoration has increased because estuarine plant communities in the Pacific Northwest are ubiquitous.

\( H_A \) = There will be an increase in plant species diversity between sites as time since restoration has increased. Specifically, recently restored sites will still show lower species diversity because the culvert historically restricted sediment, nutrient, and genetic movement.

Question 3. Will plant communities homogenize between restoration location (above the headwater or below the tailwater) over time?

\( H_0 \) = Vegetation communities will not homogenize between restoration location over time because estuarine plant communities in the Pacific Northwest are ubiquitous.

\( H_A \) = Vegetation communities will homogenize between restoration location (i.e, above and below the restoration location). Specifically, the oldest post-restoration site will show the most similarity between upstream and downstream communities as the reconnected aquatic corridor will facilitate dispersal of genetic material, sediment, and nutrients.
Question 4. Do we see greater invasive species recruitment in older post-restoration sites?

\[ H_0 = \text{There will be a smaller recruitment of invasive species as time since restoration has increased because native estuarine colonize and native plant species outcompete invasive species.} \]

\[ H_A = \text{There will be an increase in invasive species as time since restoration has increased because sites are located within heavily urbanized areas and the chance for initial invasive species recruitment is greater, resulting in an alternative stable state.} \]
3.0 Methods

3.1 Sample Sites

The four salt marsh study sites are located on the Olympic Peninsula in Kitsap County, Washington State (Figure 3-1). Kitsap County contains over 400 km of saltwater shoreline (Puget Sound Partnership 2010). Pollution and degradation of estuarine habitat in Kitsap County has increased as a result of increased urbanization and industrialization (Landahl et al. 1997). Various types of industry and culverts have affected each site, including undersized pipe culverts less than 3 m wide and fish ladders less than 2 m wide (Washington State Recreation and Conservation Office 2016). Chemical cycling is heavily influenced by hydrological connectivity, soil organic matter, and microorganism communities, as such, novel ecosystems often recover a hybrid of characteristics from pre-restoration states (Hobbs, Higgs, and Harris 2009), so a pre-restoration site was included within the project for reference. Sites, ordered in ascending restoration age, are 1) pre-restoration (Harper Creek), 2) 3-year-old post-restoration (Carpenter Creek), 3) 9-year-old post-restoration (Beaver Creek), and 4) 12-year-old post-restoration (Dogfish Creek). Sampling took place in July and September of 2016.
Figure 3-1. 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).
3.1.1 Harper Creek

Harper Creek Estuary (47°30′00.9″N 122°30′58.4″W) is located in Port Orchard, Washington. Ecological connectivity, hydrology, and sediment movement has been impacted by a culvert. The dumping of brick waste and road fill used for the roads Olympiad Drive and Southworth Drive (Dunagan 2014) have impacted this site for nearly a century. Additionally, the Harper Brick Factory affected this site and was closed in 1932 and later demolished in the 1940s (Heytvelt 2013). Upon demolition, bricks and industrial debris were dumped into the estuary and remnants of brick can still be seen today. Harper Creek is the pre-restoration site within this study and contained two working culverts at the time of sampling. Since sampling, the culvert has been removed and replaced with a large-span bridge (Small and Cook 2016a). Soil directly adjacent to this site is composed of Tacoma silt loam and Harstine gravelly ashy sandy loam (USDA 2017). Bankfull width is 4 m with an annual water discharge for this site is 1.1 cubic feet per second (Watershed Health Monitoring Program 2014). Annual mean discharge for this site is unknown.

Restoration at this site is to occur in two phases. Since sampling, phase one of two has occurred, with phase two nearly complete. In November 2016 the upper 0.6 m-diameter culvert under Southworth Drive was replaced with a 4.9 m wide box culvert (Small and Cook 2016b). The lower culvert, located under Olympiad Drive SE, is a 60.9 cm in diameter and approximately 30.5 m long (Figure 3-2) and will be replaced with a 36 m single span bridge (Small 2015). No planting revegetation plan has been provided.
Figure 3-2. Harper Creek and estuary located in Yukon Bay, Port Orchard, Washington. The culvert located under Olympiad Drive SE, contained a 60.9 cm culvert and was replaced with a 36 m bridge in January of 2017.
3.1.2 Carpenter Creek

Because salmon and steelhead were listed as endangered species, habitat restoration to improve fish habitat has been coordinated among many organizations to remove culverts. Due to culvert influence, high velocity tidal flow created large scour holes at the headwater and tailwater, trapping juvenile salmonids at low tide, where they became easy targets for birds of prey, such as Great Blue Heron and Bald Eagles (Maasberg 2011). 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 x 3 m box culvert on South Kingston Road and installation of a 27.4 m bridge (Figure 3-3). The final bridge spans the entire channel width of Carpenter Creek and can withstand natural tidal inundation from Appletree Cove (Maasberg 2011). Upon bridge installation, approximately 30 acres of coastal wetland habitat were reestablished (Washington State Recreation and Conservation Office 2016a). Not only do plant communities’ migration movement and genetic dispersal ability benefit from habitat reconnection, but Carpenter Creek Estuary also serves as a vital migration stopover for juvenile salmonids. Reconnection of this site provides salmonids greater access to resources throughout entire estuary. The majority of soil adjacent to this site is classified as an alluvial beach deposit with minor components of Poulsbo gravelly sandy loam (USDA 2017). Work to replace a 1.5 m culvert on West Kingston Road with a 45.7 m bridge is planned for 2017.

Revegetation notes at this site listed the following number of native woody species to be planted along the estuary bank that receives infrequent tidal inundation: shore pine (10; East bank only), red-osier dogwood (33), Western crabapple (15), red-flowering currant (33), red elderberry (15), and Hooker willow (150; along lower edge). The following kilograms of species were planted along the bottom estuary bank that is inundated daily: seawatch
angelica (0.45 kg), Douglas aster (0.45 kg), marsh clover (0.91 kg), Oregon bentgrass (2.27 kg), Pacific reedgrass (2.27 kg), and tufted hairgrass (2.27 kg). No additional regions (i.e. riparian and upper shoreline) were mentioned in the planting plant.
Figure 3-3. Carpenter Creek is located in Kingston, Washington, this site was restored in 2013 after removal of a 3 m x 3 m box culvert (left) on South Kingston Road followed by the installation of a 27.4 m bridge (right). The bridge spans the entire channel width of Carpenter Creek and is the youngest post-restoration site (3-Yr) within this study.
3.1.3 *Beaver Creek*

Beaver Creek Estuary (47°34′12.1938″N 122°33′7.2468″W), located in Manchester, Washington, at the head of Clam Bay, was restored in 2007. Beaver Creek was filled by the United States Navy in the 1940s and used as a firefighter training facility on the Manchester Fuel Depot Base. The original route of Beaver Creek was diverted and two concrete fish ladders were installed approximately 75 years ago (Figure 3-4; left), blocking the natural function of the estuary and migration routes for salmonids (GeoEngineers 2007). The upper fish ladder was removed in 2003 with the help of Mid Sound Fisheries Enhancement Group, GeoEngineers, and the Manchester Fuel Depot (GeoEngineers 2007). Restoration of Beaver Creek stream meanders, along with the removal of the lower culvert and installation of a 6.7 m bottomless arch culvert (Figure 3-4; right) at the head of Clam Bay were completed in 2007. The restoration provided 4.5 acres of riparian habitat, 0.4 km of stream accessibility, and re-establishment of the historical floodplains and estuary habitat. Soil in this region is composed of Kapowsin gravelly ashy loam (USDA 2017). In addition to stream morphology restoration, this restoration project was more extensive and included barriers further inland than all other sites. Native plants were planted in all regions including: estuarine, riparian, and upper shoreline. Annual mean discharge at this site is between 0.59 and 0.66 cubic feet per second (USGS 2012).

Plants provided by Fleet and Industrial Supply Center Puget Sound Manchester Fuel Depot included 7,500 Douglas-fir, 3,000 grand fir, 500 shore pine, and 1,000 Western red cedar in the between February and March of 2010. Plants were planted in the following zones: floodplain, riparian slope, middle bench, upper bench 1, upper bench 2, and marine
shoreline. Those species in the marine shoreline zone included two bitter cherry, six oceanspray, and 798 dunegrass plugs.
Figure 3-4. Beaver Creek restoration was completed with the removal of a 2 m fish ladder (left) and installation of a 6.7 m bottomless arch culvert (right) in Manchester, Washington. The restoration provided 4.5 acres of riparian habitat, 0.4 km of stream accessibility, and re-establishment of the historical floodplains (GeoEngineers 2006).
3.1.4  *Dogfish Creek*

Dogfish Creek Estuary (47°44′48.3144″N 122°39′7.3368″ W), located in Poulsbo, Washington at the head of Liberty Bay, was restored in 2004 with the removal of a 1.5 m culvert, which was replaced with a 26.8 m bridge (Figure 3-5). The restoration reconnected 1.6 hectares of estuarine habitat and 9.7 kilometers of stream. Reconnections made the upper reaches of Dogfish Creek accessible to chinook (*Oncorhynchus tshawytscha*), coho (*O. kisutch*), and chum (*O. keta*) salmon. Additionally, steelhead (*O. mykiss*) and cutthroat trout (*O. clarkia*) benefited from the restoration. Approximately 5.4 hectares of upland habitat and 365.8 m of estuarine shoreline were restored with the completion of the restoration. Currently, this site is a designated passive open space, for the use of sitting, relaxing and leisure walking. Soil composition adjacent to Dogfish Creek includes urban land-Alderwood complex and Kitsap silt loam (USDA 2017). Annual mean flow at this site is between 6.3 and 18.3 cubic feet per second (Department of Natural Resources and Parks Water and Land Resources Division 2013).

The planting plan provided for this site included: six Pacific wax myrtle (*Myrica californica*), three strawberry tree (*Arbutus unedo*), three burning bush (*Euonymus alatus*), and three pampas grass (*Cortaderia selloana*). Of these, only Pacific wax myrtle is native to the Pacific Northwest.
Figure 3-5. Dogfish Creek Estuary is located in Poulsbo, Washington, was restored in 2004 with the removal of a 1.5 m culvert (left) and replaced with a 26.8 m bridge (right). The restoration reconnected 1.6 hectares of estuarine habitat and 9.7 kilometers of stream was made accessible to anadromous fish populations.
3.2 Vegetation Community Survey

To assess the objective of development and timing of plant communities as a function of estuary restoration, transects were used to assess plant populations. 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 (Archer et al. 2016). Three, 50-m 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 (Herrick et al. 2005). A total of 48 transects were completed in this study (4 sites x 2 visits x 6 transects). Quadrat data will be used in a future publication, and was not analyzed for this project.

Along each transect, three 1 m x 1 m quadrats were randomly placed (4 sites x 6 transects x 3 quadrats). Within each quadrat, vegetation height (cm) from the center and each corner, along with visual percent cover of each species present. A 25 cm x 25 cm sample of biomass, including all plant matter one cm above the soil, was collected from a fourth randomly chosen quadrat (4 sites x 6 biomass samples). At each randomly placed quadrat, five soil plugs were collected at a depth of 18 cm, totaling 72 soil samples (4 sites x 18 soil samples per site). Hitchcock and Cronquist (1973) and MacKinnon and Pojar (1994) were used to identify plant species.
The Chao (1987) and bootstrap population (1984) estimations were used and assume the plant community is composed of a fixed number of species. These methods estimate the standard error of the estimates and assume there is no variance in the observed plant species richness. The Chao method assumes equal sampling of plant species is not possible, and thus estimates the number of unseen species and adds them to the measured species richness. The Chao method does correct for bias of large species occurrences, but it does not completely remove all bias. The population estimation was repeated using the bootstrap method, which has been found to be the most robust population estimator (Otis et al. 1978). The bootstrap method effectively reduces bias, yet may underestimate the actual number of species if many rare species are sampled. Because the robust line-point intercept produced a large data set, the bootstrap is the preferential estimation method (Smith and van Belle 1984).

3.3 Sample Preparation and Analysis

3.3.1 Soil Processing and Homogenization

Soil was dried at 45°C for one week at Olympic College in Poulsbo, Washington. Dried soil samples were manually broken up using a plastic tub and rubber mallet. Soil samples were sieved using a plastic sieve with 1 cm x 1 cm holes 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 tested for carbon-to-nitrogen at WWU. The remaining composite sample was sent to Spectrum Analytical (Washington Court House, OH) for
macro- and micronutrient analysis including: phosphorus, potassium, magnesium, calcium, sulfur, boron, copper, iron, manganese, and zinc.

3.3.2 Vegetation Processing and Homogenization

All vegetative biomass was dried at 45°C for one week at Olympic College in Poulsbo, Washington. A homogenized sample of dried vegetation was coarsely ground using a coffee grinder. The coarsely ground biomass was then ground in acid washed polystyrene vials and Plexiglass® pestles with a Wig-L-Bug Electric Mill (Ridgewood, New Jersey, USA). The dried vegetation was further processed by mechanical grinding using a marble mortar and pestle. Ground samples were tested for carbon and nitrogen at WWU to determine if there were differences among sites. These data will be combined with heavy metal analysis performed by another student to be used in a future publication.

All laboratory work was completed at Western Washington University. Carbon-to-nitrogen analysis was carried out through the Department of Environmental Sciences and weight loss-on-ignition (WLOI) was carried out through the Department of Geology.

3.3.3 Weight Loss on Ignition

Soil organic matter from the compositied soil samples was determined using an optimized weight loss-on-ignition (WLOI) methodology specific to estuarine sediment (Wang, Li, and Wang 2011). Soil was dried overnight at 110°C to remove any absorbed 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 (Eq. 1).

\[
\% \text{ WLOI} = 100 \times \left( \frac{\left[ (W_{\text{dry soil wt}} - \text{Crucible wt}) - (W_{550 \text{ g}} - \text{Crucible wt}) \right]}{W_{\text{dry soil wt}} - \text{Crucible wt}} \right)
\]

Eq. 1: Percent Weight Loss-on-Ignition

3.3.4 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 kg\(^{-1}\) nitrogen (Homann 2016). 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.

3.3.5 Calculating Differences

The difference (\(\Delta\)) between transects (n=3) of the following variables were calculated: soil carbon, soil organic matter, the carbon and nitrogen ratio, species diversity (\(H'\)). This was done by subtracting values from transects located above the culvert restoration location (i.e. headwater) from transects located below the culvert restoration location (i.e. tailwater). Then, values were standardized by adding the same number to make all values positive within each variable. This allowed visualization of the pattern between sites.
3.3.6 *Quality Control*

Accuracy was determined by the use of standardized reference materials, duplicate samples, and sample blanks. Two Standard Reference Materials (SRM) were used for soil quality control: 1) Terreno SRM (Lot 414A) contained 16.97 g kg\(^{-1}\) carbon and 1.86 g kg\(^{-1}\) nitrogen and 2) Corvallis Long-Term Ecosystem Productivity research network which contained 81.5 g kg\(^{-1}\) carbon and 3.51 g kg\(^{-1}\) nitrogen (BIS Lot A; 200-270 MESH). Samples from SRM Terreno Lot 414A ranged from 1.76 mg carbon to 1.77 mg carbon and 0.198 mg nitrogen to 0.199 mg N nitrogen Corvallis SRM ranged from 8.27 mg carbon to 8.73 mg carbon and 0.360 mg nitrogen to 0.376 mg nitrogen. One SRM was used for vegetation quality control: “Apple Leaves” 1515 (National Institute of Standards and Technology, Gaithersburg, MD) and contained 22.5 g kg\(^{-1}\) nitrogen. Samples of SRM “Apple Leaves” ranged from 95.0 g kg\(^{-1}\) nitrogen to 475 g kg\(^{-1}\) nitrogen. Relative percent difference for carbon was <1% for all soil samples. Relative percent difference for nitrogen was <2%. Field samples were interspersed with SRM samples throughout analysis. Initial “Apple Leaves” 1515 relative difference was >3%, but were consistent throughout analysis. As a result, initial sample volume was decreased to obtain relative percent differences less than 2 percent.
4.0 Statistical Analyses

All data management was completed using Microsoft Excel (Version 2016). Statistical analysis and plotting were conducted using R (Version 3.3.0; R Core Team 2016) in the RStudio environment. The ‘stats’ core package (R Core Team 2016) was used for ANOVA, PCA using the ‘prcomp’ function, pairwise-t-test, Kendall Tau correlation test using the ‘cor.test’ function, and the Kruskal-Wallis test. The ‘vegan’ package (Oksanen et al. 2016) was used for the Shannon-Wiener diversity, ANOSIM, and SIMPER. All plotting was completed with the ‘boxplot’ function in the core ‘graphics’ package of R (R Core Team 2016) and the ‘RColorBrewer’ package was used for all color in the boxplots (Neuwirth 2014).

4.1 Parametric Analyses

A two-way fixed-effects analysis of variance (ANOVA) was used to compare percent carbon, carbon and nitrogen and among ratio, percent soil organic matter, species richness, species abundance using the Shannon-Wiener (H’), and number of invasive species occurrences at each site. In the analysis, site (pre, 3-Yr, 9-Yr, and 12-Yr), location (above and below), and the interaction effects between them were compared. Both factors were considered fixed, as sites and locations were not randomly selected. Levene’s test was used to assess how well data fit the assumption of homogeneity of variances among sites and locations. If Levene’s P-value was greater than 0.05, a log transformation was applied to reduced heterogeneity prior to ANOVA because of outliers. Shapiro-Wilk’s normality test was used to test the assumption that samples came from normally distributed populations. 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 (Holm 1979) which
sequentially rejects hypotheses until no further rejections can be performed and reduces false positive error rates. When data could not be transformed to meet the assumptions of ANOVA, a Kruskal-Wallis non-parametric test was used.

Plant species richness fails to incorporate relative abundance of each species. Therefore, the Shannon-Wiener Diversity Index ($H'$) was used to quantify species richness and abundance. This method is widely used, despite the inability to compare across communities when uneven samples are collected. As such, the number of line-point intercept between site and location were equal across all sites (Barrantes and Sandoval 2009).

$$H' = - \sum_{i=1}^{S} \left[ \left( \frac{n_i}{n} \right) \times \ln \left( \frac{n_i}{n} \right) \right]$$

\(n_i\) = Number of individuals belonging to the \(i\)th of \(S\) species

\(n\) = Total number of individuals

Eq. 2. Shannon-Wiener Diversity Index ($H'$)

4.2 Non-Parametric Analyses

4.2.1 Soil and Plant Biomass Correlation

Twelve Kendall rank correlation coefficients were used to measure the association of plant height and soil macro- and micronutrient data at \(\alpha=0.05\). Because one transect below the culvert at the pre-restoration site contained high levels of soil nitrogen, carbon, organic matter, magnesium, sulfur, and boron, it was removed from the correlation analysis.

4.2.2 Plant Community Composition Analysis

Relative species abundance, an aspect of biodiversity, was calculated by dividing plant species relative abundance by its rank abundance. Hierarchical clustering, principal
component analysis (PCA), and analysis of similarity (ANOSIM) to create a low-dimensional representation of the variation of species assemblages and identify potential patterns in plant species distribution. Association analysis, using chi-square goodness of fit, was used to determine if there was a statistical relationship between site and location of hierarchical clusters. Cluster analysis was completed using squared Euclidean distance and Ward’s minimum variance method.

PCA was used to determine which combination of species explained the most variance in the plant communities (Gotelli and Ellison 2004). Several key assumptions are that all principal components (PC) are independent, orthogonal, and homoscedastic. Data were independent, orthogonal, and heteroscedastic, meaning variability across the species counts was unequal, with several species occurring in frequencies several orders of magnitude larger. The occurrence of species with large counts can obscure species with low occurrences. 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. Because PCA requires at least one observation per species in order to determine which species influenced community composition, species which were absent in transects, but were present in the quadrat survey, were removed from transect PC analysis. Using the prcomp function in R, based on a singular value decomposition of the data matrix, identification of which species accounted for the most variation and separation in each principal component was determined.

To determine which features produced a stable PCA cluster, the procedure from Ben-Hur and Guyon (2003) was modified by repeatedly using less principal components until a stable cluster was formed. Principal components one through eight (PC I-VIII) were initially
used as variables in the agglomerative, hierarchical clustering approach. This procedure was repeated with consecutive removal of each principal component, until the minimum number of components was identified with the fewest misclassifications (PC I-IV). Clustering stability was achieved using the first four principal components by the process of eliminating principal components sequentially. Plant species variable ordination was used to visualize species distribution patterns.

Hierarchical clustering on principal components was repeated with transects without any occurrence of pickleweed (*Salicornia virginica*). Since pickleweed had such frequent species occurrence, it dominated the analysis. The goal of removing pickleweed was to reveal possible underlying biological factors that may be driving plant species separation.

Analysis of similarity (ANOSIM) does not depend on multivariate normality and calculates dissimilarity by using a ranked dissimilarity matrix. As such, ANOSIM is appropriate for a one-way and two-way crossed and nested ANOVA-type design, where the main difference resides in how distances are converted into ranks prior to calculation (Gotelli and Ellison 2004; Somerfield, Clarke, and Olsgard 2002). Based on these assumptions, a two-way ANOSIM was calculated between site and location, with 1,000 permutations.

4.3 Statistical Errors

The ANOVA and Kendall’s Tau correlation tests were performed using an unadjusted test-wise error rate of $\alpha=0.05$. For the pairwise-t-tests, a posterior comparison controlled for simultaneous comparisons, and thus the $P$-value is adjusted downward, which is why the “holm” correction method was used. When performing a posteriori contrasts, the probability of making a Type I error increases with multiple tests, which is why it is not
recommended to use the Bonferroni method, as results are overly conservative. Adjusting the $\alpha$-value penalizes researchers for conducting multiple tests.

When using an uninformed approach, such as with hierarchical clustering or PCA, the constraints of \textit{a posteriori} are removed. This allows the user freedom to explore clusters in the dataset without preconceived notions. Care should be taken with biological interpretation, and a fundamental understanding of the system being studied is crucial.

The ANOSIM analysis is used in many multivariate abundance and community data sets, yet may be calculated using the wrong distance metric based upon the community structure, which confounds the differences between groups. Additionally, the \textit{post hoc} SIMPER analysis fails to differentiate taxa with strong between-group effects, but also taxa with large within-group variance. This may be misleading as strong between-group effects may be given little weight when they had small variance (Warton, Wright, and Wang 2012). Because of this shortcoming, hierarchical clustering and PCA were used to support ANOSIM and SIMPER analysis.
5.0 Results

5.1 Soil

5.1.1 Percent Soil Carbon

There was a significant interaction between site and location when soil carbon percentages were compared among all sites ($F_{(3,16)} = 3.68, P = 0.03$). The newest restoration site (3-Yr) below the point of culvert restoration was significantly lower in soil carbon (0.92% ± 0.54) when compared across the other sites (2.04% - 7.53%). An initial decrease in soil carbon after disturbance, with gradual increase over time is illustrated (Figure 5-1). This is evident by the pair-wise post-hoc test that shows similarities between the pre-restoration site and the oldest post-restoration site (12-Yr) with intermediate values observed from the 9-Yr plots.
Figure 5-1. Boxplots illustrating percent soil carbon values at four estuary restoration sites in Kitsap County, Washington. Sites included one pre-restoration (Pre) site and three sites at varying post-restoration ages: three (3-Yr), nine (9-Yr), and 12 years (12-Yr). Two distinct locations were sampled at each site, above and below the restored culvert (above and below; n=24). There was significantly lower soil carbon below the location of restoration at the newest post-restoration site (3-Yr) when compared to all other sites. A gradual increase in soil carbon is illustrated over time, with similar values at the pre-restoration site and the site aged 12 years. Different letters indicate statistical differences as determined by post-hoc pairwise comparisons (α=0.05).
5.1.2 Soil Carbon Differences between Site and Location

There were no significant differences among sites in relative soil carbon differences, calculated as the differences between soil carbon percentages above and below the culvert or bridge at each site ($F_{(3,8)} = 1.57, P= 0.27$). The pre-restoration site (Pre) shows a wider range of soil carbon change compared to all other sites (0.60 % - 13.47%). Though not statistically different, a visual trend illustrates that the largest difference in percent soil carbon between the above and below restoration location was observed at the newest post-restoration site (3-Yr), with the percent difference decreasing between the headwater and tailwater as time of recovery progresses (Figure 5-2).
Figure 5-2. Percent difference (Δ) of soil carbon between the headwater and tailwater transects from four estuary restoration sites in Kitsap County, Washington. Sites included one pre-restoration (pre) site and three sites at varying post-restoration ages: three (3-Yr), nine (9-Yr), and 12 years (12-Yr). A one-way analysis of variance was used to test differences in soil carbon between sites (n=3). Boxplots illustrate the difference (Δ) between percent soil carbon from above and below the restoration location each site.
5.1.3 Percent Soil Organic Matter

There were significant differences in percent soil organic matter between sites ($F_{(3,16)} = 6.92$, $P= 0.003$), with a marginal interaction with location ($F_{(3,16)} = 2.71$, $P= 0.08$). The newest site (3-Yr) below the location of culvert restoration was significantly lower in soil organic matter (2.80% ± 1.20) when compared to the pre-site above (10.88% ± 2.51) and below (17.20% ± 6.22) and the location above the 12-year-old site (9.51% ± 1.74). After restoration, it appears locations below the site of restoration are most affected, shown by an initial loss of soil organic matter and a return to pre-restoration values only within the above locations by year 12 (Figure 5-3).
Figure 5-3. Percent soil organic matter boxplots from four estuary restoration sites in Kitsap County, Washington. Sites were included: pre-restoration (pre) site, three (3-Yr), nine (9-Yr), and 12 years (12-Yr). Two distinct locations were sampled at each site, above and below the restored culvert (above and below; n=24). There was significantly lower soil organic matter below the location of restoration at the newest post-restoration site (3-Yr) when compared to all other sites. A gradual increase in soil organic matter is illustrated over time, with similar values at both the pre-restoration and the oldest (12-Yr) site. Different letters indicate statistical differences determined by post-hoc pairwise comparisons (α=0.05).
5.1.4 Soil Organic Matter Differences between Site and Location

There were no significant differences among sites in above vs. below difference in percent soil organic matter change ($F_{(3,8)} = 1.44, P= 0.30$). The pre-restoration site shows the largest range of soil organic matter change (0.97% - 27.88%), when compared to all other sites (14.36% - 27.89%). No clear trend is apparent (Figure 5-4).
Figure 5-4. Percent difference (Δ) in soil organic matter from four estuarine restoration sites of varying ages in Kitsap County, Washington were compared, including one pre-restoration (Pre) and three post restoration sites aged: three (3-Yr), nine (9-Yr), and 12 years (12-Yr). Two distinct locations were sampled at each site, above and below the restored culvert (above and below; n=24). Boxplots illustrate the difference (Δ) between percent soil organic matter from above and below each site. There were no significant differences in soil organic matter between locations at each site.
5.1.5 Soil Carbon-to-Nitrogen Ratio between Site and Location

There was a significant interaction between site and location in percent soil carbon-to-nitrogen ratios among all sites \( F_{(3,16)} = 4.35, P= 0.03 \). The percent soil carbon-to-nitrogen ratio at the newest post-restoration site (3-Yr) below the point of culvert restoration was significantly higher (24.56% ± 0.89) than all other sites (13.58% - 19.18%). The nine-year-old site showed intermediate soil carbon to nitrogen ratios. A sharp increase in percent soil carbon-to-nitrogen ratio is evident in substrate below the most recent culvert restoration (Figure 5-5).
Figure 5-5. Carbon-to-nitrogen ratio boxplots illustrating four estuary restoration sites in Kitsap County, Washington. Sites include one pre-restoration (pre) and three post-restoration sites: three, (3-Yr), nine (9-Yr), and 12 years (12-Yr). Within each site, two locations (above and below; n=24) the site of culvert restoration were sampled. A significant difference in the soil carbon-to-nitrogen ratio was noted between location. A significant interaction of soil carbon-to-nitrogen between site and location was observed among all sites. Letters indicate statistical differences in sites and locations determined by post-hoc pairwise comparisons ($\alpha=0.05$).
5.1.6 Carbon-to-Nitrogen Differences between Site and Location

There were no significant differences observed in differences (Δ) among sites in above vs. below difference in percent soil carbon-to-nitrogen ratios among sites ($F_{(3,20)} = 1.62, P = 0.20$). A visual trend suggests that carbon-to-nitrogen ratios may decrease over time since restoration (Figure 5-6).
Figure 5-6. Difference ($\Delta$) in % soil carbon-to-nitrogen ratio illustrating the one-way analysis of variance used to test differences in soil carbon-to-nitrogen ratios between four estuary restoration sites in Kitsap County, Washington. Boxplots illustrate the difference ($\Delta$) between percent carbon-to-nitrogen ratio from above and below the restoration location each site. Sites include one pre-restoration (pre) site and three sites at varying post-restoration ages: three (3-Yr), nine (9-Yr), and 12 years (12-Yr). While no significant difference was observed between sites ($n=3$), an initial increase in soil carbon-to-nitrogen ratio can be seen post-restoration, followed by a decrease in the difference of soil carbon-to-nitrogen ratios over time.
5.1.7 Non-Metric Multidimensional Scaling of Soil Nutrients

The result of the NMDS showed that soil nutrients have significantly homogenized with regard to location within site at the oldest two post-restoration sites, indicated by the blue and green ellipses overlap ($P<0.005$; Figure 5-7). Ordination exploration indicated that homogenization of soil nutrients between locations has not occurred at the pre-restoration and newest restoration location. The newest post-restoration site showed a nutrient deficiency below the restoration-location. Two dimensions were reported, with a stress value converging on 0.10, indicating a reliable ordination.
Figure 5-7. Non-metric multidimensional scaling of soil nutrients between four restoration locations in Kitsap County, Washington. Ellipses that are closer together indicate similar soil nutrient content, while ellipses further apart indicate larger differences. Samples were analyzed 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.
5.1.8 Correlation of Soil Nutrients and Plant Biomass

No difference between plant height and soil nutrient correlations was observed among sites, so data were pooled. Of the 10 soil nutrients analyzed (n=23), nitrogen, potassium, magnesium, sulfur, boron, copper, and manganese were positively correlated with plant dry weight (all $P<0.05$; Table 5-1). Potassium, magnesium, boron, iron, and manganese were all considered deficient (Table 5-2). Nitrogen, potassium, and sulfur are all utilized by plants in relatively high quantities and support physical growth of plant bodies. Magnesium, boron, copper, and manganese are also incorporated into the structural development of plants and support photosynthetic elements needed for carbon assimilation.
Table 5-1. Soil nutrient correlations 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. Dried plant weight was positively correlated with the following soil nutrients: nitrogen, potassium, magnesium, sulfur, boron, copper, and manganese (P< 0.05; α=0.05). Common ranges of soil macro and micronutrients are reported, along with sediment nutrient ranges across all sites.

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>τ</th>
<th>P-value</th>
<th>Site Range ppm ± SE</th>
<th>Common Range ppm&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total N</td>
<td>0.36</td>
<td>0.02*</td>
<td>1,763 ±233</td>
<td>200– 5,000</td>
</tr>
<tr>
<td>P</td>
<td>0.27</td>
<td>0.07</td>
<td>92 ±16</td>
<td>100– 2,000</td>
</tr>
<tr>
<td>K&lt;sup&gt;+&lt;/sup&gt;</td>
<td>0.43</td>
<td>0.004**</td>
<td>238 ±19</td>
<td>1,700– 33,000</td>
</tr>
<tr>
<td>Mg&lt;sup&gt;+&lt;/sup&gt;</td>
<td>0.36</td>
<td>0.02*</td>
<td>652 ±69</td>
<td>1,200– 15,000</td>
</tr>
<tr>
<td>Ca</td>
<td>0.10</td>
<td>0.53</td>
<td>747 ±100</td>
<td>700– 36,000</td>
</tr>
<tr>
<td>S</td>
<td>0.31</td>
<td>0.04*</td>
<td>284 ±41</td>
<td>100– 2,000</td>
</tr>
<tr>
<td>B&lt;sup&gt;+&lt;/sup&gt;</td>
<td>0.33</td>
<td>0.03*</td>
<td>2.98 ±0.44</td>
<td>1– 150</td>
</tr>
<tr>
<td>Cu</td>
<td>0.31</td>
<td>0.04*</td>
<td>2.09 ±0.34</td>
<td>1– 150</td>
</tr>
<tr>
<td>Fe&lt;sup&gt;+&lt;/sup&gt;</td>
<td>-0.14</td>
<td>0.37</td>
<td>253.38 ±17.20</td>
<td>5,000– 50,000</td>
</tr>
<tr>
<td>Mn&lt;sup&gt;+&lt;/sup&gt;</td>
<td>0.36</td>
<td>0.02*</td>
<td>62.91 ±9.29</td>
<td>200– 10,000</td>
</tr>
<tr>
<td>Zn</td>
<td>0.26</td>
<td>0.08</td>
<td>11.17 ±3.34</td>
<td>10– 250</td>
</tr>
</tbody>
</table>

<sup>a</sup>Standard soil macro- and micronutrient ranges were drawn from Brady (1974), Day et al. (Day Jr et al. 1989), and Evert and Eichhorn (Evert and Eichhorn 2013).

* indicates significance at P<0.05; ** indicates significance at P<0.01

<sup>+</sup> indicates deficiency based on common ranges.
Table 5-2. Soil nutrient deficiencies among four estuary restoration sites in Kitsap County, Washington. Potassium, magnesium, boron, iron, and manganese were deficient in soil at all sites (pre, 3-Yr, 9-Yr, and 12-Yr) and location of culvert restoration (above and below).

<table>
<thead>
<tr>
<th>Site</th>
<th>Nutrient</th>
<th>Site Range (ppm)</th>
<th>Normal Range (ppm(^a))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre</td>
<td>Potassium (K)(^A)</td>
<td>204 – 461</td>
<td>100 – 2,000</td>
</tr>
<tr>
<td></td>
<td>Magnesium (Mg)(^A)</td>
<td>600 – 1713</td>
<td>1,200 – 15,000</td>
</tr>
<tr>
<td></td>
<td>Boron (B)(^A)</td>
<td>1.6 – 10.9</td>
<td>5 – 150</td>
</tr>
<tr>
<td></td>
<td>Iron (Fe)(^AB)</td>
<td>104.9 – 375.9</td>
<td>5,000 – 50,000</td>
</tr>
<tr>
<td></td>
<td>Manganese (Mn)(^A)</td>
<td>35 – 189</td>
<td>200 – 10,000</td>
</tr>
<tr>
<td>3-Yr</td>
<td>Potassium (K)(^B)</td>
<td>65 – 305</td>
<td>100 – 2,000</td>
</tr>
<tr>
<td></td>
<td>Magnesium (Mg)(^A)</td>
<td>69 – 914</td>
<td>1,200 – 15,000</td>
</tr>
<tr>
<td></td>
<td>Boron (B)(^A)</td>
<td>0.2 – 7.8</td>
<td>5 – 150</td>
</tr>
<tr>
<td></td>
<td>Iron (Fe)(^B)</td>
<td>110.6 – 212.7</td>
<td>5,000 – 50,000</td>
</tr>
<tr>
<td></td>
<td>Manganese (Mn)(^B)</td>
<td>8 – 62</td>
<td>200 – 10,000</td>
</tr>
<tr>
<td>9-Yr</td>
<td>Potassium (K)(^B)</td>
<td>209 – 237</td>
<td>100 – 2,000</td>
</tr>
<tr>
<td></td>
<td>Magnesium (Mg)(^A)</td>
<td>205 – 969</td>
<td>1,200 – 15,000</td>
</tr>
<tr>
<td></td>
<td>Boron (B)(^A)</td>
<td>0.8 – 2.4</td>
<td>5 – 150</td>
</tr>
<tr>
<td></td>
<td>Iron (Fe)(^A)</td>
<td>298.7 – 363.2</td>
<td>5,000 – 50,000</td>
</tr>
<tr>
<td></td>
<td>Manganese (Mn)(^B)</td>
<td>17 – 87</td>
<td>200 – 10,000</td>
</tr>
<tr>
<td>12-Yr</td>
<td>Potassium (K)(^AB)</td>
<td>240 – 362</td>
<td>100 – 2,000</td>
</tr>
<tr>
<td></td>
<td>Magnesium (Mg)(^A)</td>
<td>441 – 1,180</td>
<td>1,200 – 15,000</td>
</tr>
<tr>
<td></td>
<td>Boron (B)(^A)</td>
<td>0.9 – 6.0</td>
<td>5 – 150</td>
</tr>
<tr>
<td></td>
<td>Iron (Fe)(^AB)</td>
<td>211.4 – 324.6</td>
<td>5,000 – 50,000</td>
</tr>
<tr>
<td></td>
<td>Manganese (Mn)(^AB)</td>
<td>39 – 126</td>
<td>200 – 10,000</td>
</tr>
</tbody>
</table>

\(^a\) Standard soil macro- and micronutrient ranges were drawn from Brady (1974); Superscript letters indicate homogenous subsets, as determined by Tukey’s HSD.
5.2 Vegetation

5.2.1 Plant Species List as a Table

Surveys in July and September 2016 included 65 total plant species (Table 5-3). Using the Chao population estimation method, 73 (±9) species were estimated to make up the entire population size. This was repeated using the bootstrap method, which estimated a population size of 66 (±3). The proportion of each most abundant species was calculated based on how many individuals were observed out of the total occurrences from line-point intercept data. The most abundant native plant species include pickleweed (*Salicornia virginica*; 19.2%), fat hen (*Chenopodium album*; 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%).

Nine invasive plant species were documented along with the percentage they comprised from all point-line intercept data. All invasives encountered are considered noxious by the Washington State Noxious Weed Control Board 2017) including 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%), common tansy (*Tanacetum vulgare*; <0.1%), field bindweed (*Convolvulus arvensis*; <0.1%), and Queen Anne’s lace (*Daucus carota*; <0.1%).
Table 5-3. Complete species list of 65 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).

<table>
<thead>
<tr>
<th>Species name</th>
<th>Abbrev</th>
<th>Common Name</th>
<th>Site (Pre, 3, 9, and 12)</th>
<th>Native Status</th>
<th>Relative Abundance (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Above</td>
<td>Below</td>
<td></td>
</tr>
<tr>
<td><strong>Forbs and vines</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Achillea millefolium</td>
<td>AcMi</td>
<td>Yarrow</td>
<td>N/A</td>
<td>Pre</td>
<td>N</td>
</tr>
<tr>
<td>Argentina edgewii</td>
<td>ArEd</td>
<td>Pacific Silverweed</td>
<td>12-Yr</td>
<td>9-Yr</td>
<td>N</td>
</tr>
<tr>
<td>Cakile edentula</td>
<td>CaEd</td>
<td>American Sea Rocket</td>
<td>3-Yr</td>
<td>3-Yr</td>
<td>N</td>
</tr>
<tr>
<td>Chenopodium album</td>
<td>ChAl</td>
<td>Fat Hen</td>
<td>All Sites</td>
<td>All Sites</td>
<td>N</td>
</tr>
<tr>
<td>Cirsium arvense</td>
<td>CiAr</td>
<td>Canadian Thistle</td>
<td>9-Yr</td>
<td>N/A</td>
<td>NX</td>
</tr>
<tr>
<td>Convolvulus arvensis</td>
<td>CoAr</td>
<td>Bindweed</td>
<td>N/A</td>
<td>12-Yr</td>
<td>NX</td>
</tr>
<tr>
<td>Cuscuta pacifica</td>
<td>CuPa</td>
<td>Dodder</td>
<td>3-Yr</td>
<td>N/A</td>
<td>N</td>
</tr>
<tr>
<td>Cytisus scoparius</td>
<td>CySc</td>
<td>Scotch Broom</td>
<td>9-Yr</td>
<td>3-Yr, 12-Yr</td>
<td>NX</td>
</tr>
<tr>
<td>Daucus carota</td>
<td>DaCa</td>
<td>Queen Anne's Lace</td>
<td>N/A</td>
<td>12-Yr</td>
<td>NX</td>
</tr>
<tr>
<td>Equisetum hynale</td>
<td>EqHy</td>
<td>Scouring Rush</td>
<td>9-Yr</td>
<td>N/A</td>
<td>N</td>
</tr>
<tr>
<td>Galium aparine</td>
<td>GaAp</td>
<td>Sticky Weed</td>
<td>N/A</td>
<td>3-Yr</td>
<td>N</td>
</tr>
<tr>
<td>Grindelia squarrosa</td>
<td>GrSq</td>
<td>Gumweed</td>
<td>All Sites</td>
<td>All Sites</td>
<td>N</td>
</tr>
<tr>
<td>Honkenya peploides</td>
<td>HoPe</td>
<td>Seabeach Sandwort</td>
<td>N/A</td>
<td>3-Yr</td>
<td>N</td>
</tr>
<tr>
<td>Hypochaeris radicata</td>
<td>HyRa</td>
<td>Hairy Cat's Ear</td>
<td>3-, 9-, 12-Yr</td>
<td>9-Yr, 12-Yr</td>
<td>NX</td>
</tr>
<tr>
<td>Jaumea carnosa</td>
<td>JaCa</td>
<td>Fleshy Jaumea</td>
<td>Pre</td>
<td>Pre, 12-Yr</td>
<td>N</td>
</tr>
<tr>
<td>Lathyrus odoratus</td>
<td>LaOd</td>
<td>Sweet Pe</td>
<td>N/A</td>
<td>Pre, 12-Yr</td>
<td>N</td>
</tr>
<tr>
<td>Leucanthemum vulgare</td>
<td>LeVu</td>
<td>Ox-Eye Daisy</td>
<td>N/A</td>
<td>9-Yr</td>
<td>NX</td>
</tr>
<tr>
<td>Lotus corniculatus</td>
<td>LoCo</td>
<td>Bird's-foot Trefoil</td>
<td>N/A</td>
<td>9-Yr</td>
<td>N</td>
</tr>
<tr>
<td>Montia linearis</td>
<td>MoLi</td>
<td>Montia</td>
<td>Pre, 12-Yr</td>
<td>N/A</td>
<td>N</td>
</tr>
<tr>
<td>Plantago lanceolata</td>
<td>PlLa</td>
<td>English Plantain</td>
<td>9-Yr</td>
<td>9-Yr, 12-Yr</td>
<td>N</td>
</tr>
<tr>
<td>Plantago major</td>
<td>PlMa</td>
<td>Round Leaf Plantain</td>
<td>9-Yr</td>
<td>9-Yr</td>
<td>N</td>
</tr>
<tr>
<td>Plantago maritima</td>
<td>PlMa.1</td>
<td>Sea Plantain</td>
<td>3-, 12-Yr</td>
<td>Pre, 12-Yr</td>
<td>N</td>
</tr>
<tr>
<td>Polygonum aviculare</td>
<td>PoAv</td>
<td>Knotgrass</td>
<td>12-Yr</td>
<td>12-Yr</td>
<td>N</td>
</tr>
<tr>
<td>Polystichum munitum</td>
<td>PoMu</td>
<td>Sword Fern</td>
<td>9-Yr</td>
<td>N/A</td>
<td>N</td>
</tr>
<tr>
<td>Prunella vulgaris</td>
<td>PrVu</td>
<td>Self-Heal</td>
<td>N/A</td>
<td>9-Yr</td>
<td>N</td>
</tr>
<tr>
<td>Ranunculus repens</td>
<td>RaRe</td>
<td>Creeping Buttercup</td>
<td>9-Yr</td>
<td>N/A</td>
<td>N</td>
</tr>
<tr>
<td>Rumex acetosa</td>
<td>RuAc</td>
<td>Sorrel</td>
<td>N/A</td>
<td>9-Yr</td>
<td>N</td>
</tr>
<tr>
<td>Rumex crispus</td>
<td>RuCr</td>
<td>Curly Dock</td>
<td>N/A</td>
<td>9-Yr</td>
<td>N</td>
</tr>
<tr>
<td>Sagina maxima</td>
<td>SaMa</td>
<td>Coastal Pearlwort</td>
<td>3-, 12-Yr</td>
<td>N/A</td>
<td>N</td>
</tr>
<tr>
<td>Salicornia virginica</td>
<td>SaVi</td>
<td>Pickleweed</td>
<td>All Sites</td>
<td>All Sites</td>
<td>N</td>
</tr>
<tr>
<td>Species</td>
<td>Abbr.</td>
<td>Common Name</td>
<td>Duration</td>
<td>Exclusions</td>
<td>Notes</td>
</tr>
<tr>
<td>--------------------------------------------</td>
<td>-------</td>
<td>-----------------------</td>
<td>----------</td>
<td>------------</td>
<td>--------</td>
</tr>
<tr>
<td>Spergularia canadensis</td>
<td>SpCa</td>
<td>Sand Spurry</td>
<td>12-Yr</td>
<td>Pre, 3-Yr</td>
<td>N</td>
</tr>
<tr>
<td>Symphyotrichum subspicatum</td>
<td>SySu</td>
<td>Douglas Aster</td>
<td>12-Yr</td>
<td>N/A</td>
<td>N</td>
</tr>
<tr>
<td>Tanacetum vulgare</td>
<td>TaVu</td>
<td>Tansy</td>
<td>9-Yr</td>
<td>N/A</td>
<td>NX</td>
</tr>
<tr>
<td>Trifolium wormskiiolii</td>
<td>TrWo</td>
<td>Red Clover</td>
<td>9-Yr</td>
<td>12-Yr</td>
<td>N</td>
</tr>
<tr>
<td>Triglochin maritima</td>
<td>TrMa</td>
<td>Seaside Arrowgrass</td>
<td>3-, 12-Yr</td>
<td>Pre, 3-Yr</td>
<td>N</td>
</tr>
<tr>
<td><strong>Graminoids</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Agrostis capillaris</td>
<td>AgCa</td>
<td>Colonial Bentgrass</td>
<td>All Sites</td>
<td>All Sites</td>
<td>N</td>
</tr>
<tr>
<td>Agrostis exarata</td>
<td>AgEx</td>
<td>Spike Bent Grass</td>
<td>12-Yr</td>
<td>N/A</td>
<td>N</td>
</tr>
<tr>
<td>Calamagrostis canadensis</td>
<td>CaCa</td>
<td>Blue Joint Grass</td>
<td>12-Yr</td>
<td>N/A</td>
<td>N</td>
</tr>
<tr>
<td>Deschampsia cespitosa</td>
<td>DeCe</td>
<td>Tufted Hair Grass</td>
<td>Pre</td>
<td>N/A</td>
<td>N</td>
</tr>
<tr>
<td>Distichlis spicata</td>
<td>DiSp</td>
<td>Saltgrass</td>
<td>Pre, 3-, 12-Yr</td>
<td>Pre, 12-Yr</td>
<td>N</td>
</tr>
<tr>
<td>Elymus glaucus</td>
<td>ElGl</td>
<td>Blue Wild Rye</td>
<td>N/A</td>
<td>Pre, 12-Yr</td>
<td>N</td>
</tr>
<tr>
<td>Elymus mollis</td>
<td>ElMo</td>
<td>Dunegrass</td>
<td>N/A</td>
<td>3-Yr</td>
<td>N</td>
</tr>
<tr>
<td>Elymus repens</td>
<td>ElRe</td>
<td>Quack Grass</td>
<td>All Sites</td>
<td>All Sites</td>
<td>N</td>
</tr>
<tr>
<td>Holcus lanatus</td>
<td>HoLa</td>
<td>Velvet Grass</td>
<td>9-, 12-Yr</td>
<td>9-Yr</td>
<td>N</td>
</tr>
<tr>
<td>Hordeum brachyantherum</td>
<td>HoBr</td>
<td>Meadow Barley</td>
<td>3-, 12-Yr</td>
<td>Pre, 3-Yr</td>
<td>N</td>
</tr>
<tr>
<td>Phalaris arundinacea</td>
<td>PhAr</td>
<td>Reed Canary Grass</td>
<td>9-, 12-Yr</td>
<td>9-, 12-Yr</td>
<td>NX</td>
</tr>
<tr>
<td><strong>Sedges and Rushes</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carex Lyngbyei</td>
<td>CaLy</td>
<td>Lyngby’s Sedge</td>
<td>Pre, 3-, 12-Yr</td>
<td>N/A</td>
<td>N</td>
</tr>
<tr>
<td>Eleocharis palustris</td>
<td>ElPa</td>
<td>Common Spike Rush</td>
<td>9-Yr</td>
<td>N/A</td>
<td>N</td>
</tr>
<tr>
<td>Juncus effusus</td>
<td>JuEf</td>
<td>Common Rush</td>
<td>9-Yr</td>
<td>9-, 12-Yr</td>
<td>N</td>
</tr>
<tr>
<td>Juncus gerardii</td>
<td>JuGe</td>
<td>Saltmeadow Rush</td>
<td>Pre</td>
<td>Pre, 12-Yr</td>
<td>N</td>
</tr>
<tr>
<td><strong>Woody plants</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acer macrophyllum</td>
<td>AcMa</td>
<td>Big Leaf Maple</td>
<td>9-Yr</td>
<td>Pre, 9-Yr</td>
<td>N</td>
</tr>
<tr>
<td>Alnus rubra</td>
<td>AlRu</td>
<td>Red Alder</td>
<td>9-Yr</td>
<td>9-Yr</td>
<td>N</td>
</tr>
<tr>
<td>Oemleria cerasiformis</td>
<td>OeCe</td>
<td>Oso Berry</td>
<td>N/A</td>
<td>Pre</td>
<td>N</td>
</tr>
<tr>
<td>Pinus contorta</td>
<td>PiCo</td>
<td>Shore Pine</td>
<td>N/A</td>
<td>12-Yr</td>
<td>N</td>
</tr>
<tr>
<td>Pseudotsuga menziesii</td>
<td>PsMe</td>
<td>Douglas Fir</td>
<td>N/A</td>
<td>Pre</td>
<td>N</td>
</tr>
<tr>
<td>Robinia pseudoacacia</td>
<td>RoPs</td>
<td>Black Locust</td>
<td>N/A</td>
<td>9-Yr</td>
<td>N</td>
</tr>
<tr>
<td>Rosa nutkana</td>
<td>RoNu</td>
<td>Nootka Rose</td>
<td>Pre</td>
<td>Pre, 9-Yr</td>
<td>N</td>
</tr>
<tr>
<td>Rubus armeniacus</td>
<td>RuAr</td>
<td>Himalayan Blackberry</td>
<td>Pre, 9-, 12-Yr</td>
<td>Pre, 9-, 12-Yr</td>
<td>NX</td>
</tr>
<tr>
<td>Rubus ursinus</td>
<td>RuUr</td>
<td>Trailing Blackberry</td>
<td>N/A</td>
<td>9-Yr</td>
<td>N</td>
</tr>
<tr>
<td>Species</td>
<td>Code</td>
<td>Common Name</td>
<td>Age</td>
<td>Lifeform</td>
<td>Other Site</td>
</tr>
<tr>
<td>-------------------------</td>
<td>------</td>
<td>------------------</td>
<td>------</td>
<td>----------</td>
<td>------------</td>
</tr>
<tr>
<td><em>Salix sitchensis</em></td>
<td>SaSi</td>
<td>Sitka Willow</td>
<td>9-Yr</td>
<td>N/A</td>
<td>N</td>
</tr>
<tr>
<td><em>Salix hookeriana</em></td>
<td>SaHo</td>
<td>Hooker Willow</td>
<td>N/A</td>
<td>9-Yr</td>
<td>N</td>
</tr>
<tr>
<td><em>Symphoricarpos albus</em></td>
<td>SyAl</td>
<td>Snowberry</td>
<td>N/A</td>
<td>Pre, 9, 12-Yr</td>
<td>N</td>
</tr>
<tr>
<td><em>Tsuga heterophylla</em></td>
<td>TsHe</td>
<td>Western Hemlock</td>
<td>N/A</td>
<td>9-Yr</td>
<td>N</td>
</tr>
</tbody>
</table>

**Other**

<table>
<thead>
<tr>
<th>Category</th>
<th>Code</th>
<th>Sites</th>
<th>Other Sites</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bare Ground</td>
<td>BaGr</td>
<td>All Sites</td>
<td>All Sites</td>
<td>-</td>
</tr>
<tr>
<td>Large Woody Debris</td>
<td>LWD</td>
<td>All Sites</td>
<td>All Sites</td>
<td>-</td>
</tr>
</tbody>
</table>
5.2.2 Plant Height and Biomass

There were no significant differences in plant height and dry weight between site and location or the interaction between the two effects. No trend showing a difference of plant height between location and above or below the restoration location was observed. No significant difference in plant dry biomass weight between locations (above and below) for sites was observed (Table 5-4).

Table 5-4. Plant height, biomass, and invasive species recruitment between four estuarine restoration locations in Kitsap County, Washington. No significant differences were observed between site, location, and the interaction.

<table>
<thead>
<tr>
<th></th>
<th>Df</th>
<th>Sum Sq</th>
<th>Mean Sq</th>
<th>F-value</th>
<th>P-value</th>
<th>η²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plant Height</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Site</td>
<td>3</td>
<td>423.80</td>
<td>141.28</td>
<td>0.69</td>
<td>0.57</td>
<td>0.11</td>
</tr>
<tr>
<td>Location</td>
<td>1</td>
<td>719.40</td>
<td>719.42</td>
<td>3.51</td>
<td>0.08</td>
<td>0.18</td>
</tr>
<tr>
<td>Site:Location</td>
<td>3</td>
<td>1775.20</td>
<td>591.75</td>
<td>2.89</td>
<td>0.07</td>
<td>0.35</td>
</tr>
<tr>
<td>Residuals</td>
<td>16</td>
<td>3276.10</td>
<td>204.75</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plant Biomass</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Site</td>
<td>3</td>
<td>3280.8</td>
<td>1093.6</td>
<td>0.58</td>
<td>0.64</td>
<td>0.10</td>
</tr>
<tr>
<td>Location</td>
<td>1</td>
<td>6600.8</td>
<td>6600.8</td>
<td>3.50</td>
<td>0.08</td>
<td>0.18</td>
</tr>
<tr>
<td>Site:Location</td>
<td>3</td>
<td>4601.0</td>
<td>1533.7</td>
<td>0.81</td>
<td>0.51</td>
<td>0.13</td>
</tr>
<tr>
<td>Residuals</td>
<td>16</td>
<td>30205</td>
<td>1888</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
5.2.3 *Invasive Plant Species*

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 B-10). The largest abundant invasive species include Scotch broom, reed canary grass, and Himalayan blackberry each comprising less than one percent of all species recorded (Table B-11). The interaction effect accounts for 16% of the variability in the dataset. Older sites appear to have more invasive species below the restoration location (Table B-12).
5.2.4  *Plant Species Diversity*

A significant difference between site was noted for plant species diversity by site ($F_{(3,16)} = 23.58, \ P < 0.001$). The pre-restoration site was the only site with significant difference 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 ($1.98 \pm 0.04$) than any other site. A trend toward a more diverse plant community assemblage can be seen over time (Figure 5-8). A similar trend was noted for plant species richness between site and location ($F_{(3,16)} = 4.15, \ P = 0.02$; Figure B-1, Appendix B).
Figure 5-8. Plant species diversity from four estuary restoration sites in Kitsap County, Washington. 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). Two distinct locations at each site were measured (above and below culvert restoration; n=24). The 12-Yr site was significantly more diverse than all other sites. An increase in species diversity can be seen over time. Differences in letters indicate statistical differences determined by post-hoc pairwise comparisons (α=0.05).
5.2.5  *Difference in Plant Diversity by Site and Location*

A marginally significant difference (Δ) calculated as the differences between plant diversity above and below the culvert or bridge at each site using the Shannon-Wiener Index (H’) at each site \( F_{(3,20)} = 2.88, P= 0.06 \) was observed. No trend was observed in species richness. The difference in plant diversity between site restoration location (above and below) at each site is decreasing over time. This shows a trend toward homogenization of plant diversity over time (Figure 5-9).
Figure 5-9. Difference (Δ) in plant diversity by site. A marginal difference was observed at four estuary restoration sites in Kitsap County, Washington. The difference in diversity between location (above and below) shows sites homogenize over time. Sites include one pre-restoration (pre) site and three post-restoration sites: three (3-Yr), nine (9-Yr), and 12 years (12-Yr) since culvert removal. Differences in plant diversity were taken from two distinct locations within each site (above and below culvert restoration; n=24).
5.2.6 Hierarchical Clustering of Vegetative Communities

Three distinct clusters were formed, the first contained only transects from the pre-restoration (pre) and newest post-restoration (3-Yr) sites, the second contained only transects from the nine-year-old site (9-Yr), and the third contained all other transects (Figure 5-10). To test the significance of group clusters, association analysis between groups was determined using the chi-square goodness of fit test ($\chi^2 = 58.34; \text{df} = 6; P<0.0001$). Transects from both the nine and 12 year post-restoration sites clustered into groups with 100% accuracy, while transects at the three and pre restoration sites clustered into the third cluster with 58% accuracy. The newest post-restoration site (3-Yr) below the culvert removal may be more similar to the oldest post restoration site, but no real trends are clear using data not centered or scaled (Figure 5-10). Additionally, the first cluster contains high occurrences of salinity, the second cluster shows low salinity, and the remaining transects cluster together in the third group (Figure 5-11).
Figure 5-10. Dendrogram of hierarchical clusters by site from four estuary restoration sites in Kitsap County, Washington. Sites include one pre-restoration site (pre) and three post-restoration sites: three (3 yr), nine (9 yr), and 12 years (12 yr). Two distinct locations at each site were measured (above and below; n=24). Sites significantly clustered into three distinct groups, the nine-year-old site in a unique cluster, the Pre, 3 yr and 12 yr sites cluster into a unique group. The remaining 3 yr and pre sites clustered into a third cluster with 58% accuracy.
Figure 5-11. Dendrogram of hierarchical clusters by salinity from four estuary restoration sites in Kitsap County, Washington. Sites include one pre-restoration site (pre) and three post-restoration sites: three (3-Yr), nine (9-Yr), and 12 years (12-Yr). Two distinct locations at each site were measured (above and below; n=24). Sites significantly clustered into four distinct groups by low and high salinity influence.
5.2.7 *Principal Component Analysis using Hierarchical Clustering*

The first four principal components (PC I-IV) were used based on the stable clusters produced when separating transects by location. While principal components I-IV only accounted for 32.3% of the total variance, a salinity gradient is illustrated based on species variable loadings ($\chi^2 = 23.4; \text{df} = 6; P<0.0001; \text{Figure 5-12}$). Principal component I describes the species distribution by salt-tolerance. Principal component II likely describes a successional gradient, wherein older, woody, species differentiate from herbaceous perennials and annuals. Principal components III and IV describe a refined salinity gradient, where brackish-tolerating species differentiate from salinity-intolerant species. The following species accounted for 10 percent of the correlation within PC-I and are listed with their variable loading scores: pickleweed (-0.17) and saltgrass (-0.20), while colonial bentgrass (0.36), Hooker’s willow (0.31), common rush (0.30), Sitka willow (0.25), red alder (0.22), Canada thistle (0.23), and birdsfoot trefoil (0.28) showed greatest influence on positive variable scores. Species with higher variable scores, whether negative or positive, are the most important species in identifying the biological explanation (Table B-19; Appendix B).
Figure 5-12. Variable ordination of principal component I and II of plant species from four estuary restoration sites in Kitsap County, Washington. 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). Variable ordination by plant species using principal component analysis and principal components I-IV from two distinct locations at each site were measured (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.
A distinction between location (above and below) was seen, with nearly all transects above the restoration location clustering together and nearly all transects below the restoration location clustering in separate groups (Figure 5-13). Group one contained all above transects from the intermediate site (9-Yr). Group two contained only below transects from the youngest post-restoration (3-Yr) and oldest post-restoration site (12-Yr). Group three contained mostly above transects, while group four contains mostly below transects. Group five contained two outlier transects, which do not match any other sites. Additionally, within group three, the oldest post-restoration site clustered separately from the others in the same group, indicating additional differences.
Figure 5-13. Site clusters using PCA by location (above and below) and principal components 1-4 from four estuary restoration sites in Kitsap County, Washington represented with a cluster dendrogram. 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). Two distinct locations at each site were measured (n=24).
5.2.8 *Analysis of Similarity and Similarity Percentages*

Analysis of similarity (ANOSIM) indicates that there are significant dissimilarities between location (above and below) at all sites (Table 5-5). The newest post-restoration site showed the biggest difference between locations (ANOSIM R = 0.81, *P*=0.005). The intermediately aged post-restoration site showed the second biggest difference between locations (ANOSIM R = 0.56, *P*=0.002). The pre-restoration location had the next largest difference (ANOSIM R = 0.43, *P*=0.007) and the oldest post-restoration site showed the smallest difference, but locations were still significantly dissimilar (ANOSIM R = 0.35, *P*=0.008).

Using similarity percentage (SIMPER), the dissimilarity between locations at the pre-restoration site was primarily characterized by pickleweed and fleshy Jaumea below the culvert. The dissimilarity at the newest post-restoration site was primarily characterized by pickleweed, dune grass, and gumweed primarily below the restoration location, while dissimilarity at the intermediate post-restoration site were primarily characterized by fat hen, colonial bentgrass, gumweed, and common rush above the culvert restoration location. The dissimilarity at the oldest post-restoration was primarily characterized by saltgrass, coastal pearlwort, gumweed, and Pacific silverweed above the restoration location.
Table 5-5. 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.

<table>
<thead>
<tr>
<th>ANOSIM</th>
<th>Pre</th>
<th>3-Yr</th>
<th>9-Yr</th>
<th>12-Yr</th>
</tr>
</thead>
<tbody>
<tr>
<td>R-Value</td>
<td>0.43</td>
<td>0.81</td>
<td>0.56</td>
<td>0.35</td>
</tr>
<tr>
<td>P-Value</td>
<td>0.007**</td>
<td>0.005**</td>
<td>0.002**</td>
<td>0.008**</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>SIMPER</th>
<th>Species</th>
<th>P</th>
<th>Species</th>
<th>P</th>
<th>Species</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pickleweed</td>
<td>0.01</td>
<td>Pickleweed</td>
<td>0.02</td>
<td>Fat Hen</td>
<td>0.003</td>
</tr>
<tr>
<td></td>
<td>Fleshy Jaumea</td>
<td>0.03</td>
<td>Dune grass</td>
<td>0.003</td>
<td>Colonial Bentgrass</td>
<td>0.009</td>
</tr>
<tr>
<td></td>
<td>Shore Pine</td>
<td>0.03</td>
<td>Gumweed</td>
<td>0.00</td>
<td>Gumweed</td>
<td>0.01</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Common Rush</td>
<td>0.02</td>
<td>Pacific Silverweed</td>
<td>0.04</td>
</tr>
</tbody>
</table>

* Significant at P < 0.05; ** Significant at P < 0.01
6.0 Discussion

6.1 Soil

6.1.1 Soil Carbon and Organic Matter

Carbon is sequestered in coastal estuarine ecosystems by the accumulation of soil organic matter in sediment by aquatic algae, above- and belowground plant biomass, and aquatic phytoplankton (McLeod et al. 2011; Mueller, Jensen, and Megonigal 2016; Negrin et al. 2016). Current research indicated newly restored sites experience a decrease in soil carbon and organic matter, whereas sites which have been restored for several decades show a near return to values seen in pristine estuaries (Borja et al. 2010). In our study, differences in percent soil carbon and organic matter existed among sites, with the lowest levels sampled from the newest post-restoration site (3-Yr).

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 (Kitsap County Dept. of Public Works 2009), thus reducing the percent soil carbon. Because carbon comprises a large proportion of organic matter, when fine particulates and organic matter are not held in the system, a loss of carbon also occurs. Secondly, when the aquatic corridor was restricted with a culvert, tidal inundation was interrupted, restricting the flow of sediment movement. Because of impoundment, sediment accretion was restricted, and accumulated at the headwater of the culvert. When the aquatic corridor was reconnected, riverine outflows, daily tides, and weather events caused the displacement of lightweight, fine sediment particles, leaving only coarse sediment deposition at the tailwater of the culvert. This displacement of fine particles was demonstrated by Cooper, Palmer, and Nevins (2015). Coarse sediment generally
contains little organic matter, silt, and clay. These components of sediment facilitate biogeochemical cycling (Fellman, Petrone, and Grierson 2011), adsorption of cations (Brady 1974), sequestration of carbon (Mitsch and Gosselink 2015), and organic matter (Mueller, Jensen, and Meganigal 2016). Lastly, because 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 sediment along estuarine edges near vegetation. At the time of sampling, vegetation recruitment had not yet occurred below the newest bridge, which reduced the amount of fine sediment and organic accretion (Fagherazzi et al. 2006).

Current research indicates at least five years are needed for biotic components of estuarine ecosystems to recover (Borja et al. 2010; Craft et al. 1988), like macroinvertebrates and vegetation, yet at least a decade is needed to recover ecosystem services, like carbon sequestration and primary productivity (Harwell, Cropper, and Ragsdale 1977; Jones and Schmitz 2009). In our study, carbon sequestration and soil organic matter recruitment have not yet been recovered in the newest post-restoration site. Though not statistically significant, the intermediatively aged (9-Yr) and oldest restoration site (12-Yr) supports a trend of increasing soil carbon and organic matter, important for decreasing pore space and increasing microbial activity. However, neither have returned to normal ranges for an undisturbed, temperate coastal marsh (Vincent, Burdick, and Dionne 2013). Estimates for normal soil carbon ranges between 12 – 20% and organic matter ranges between 22 – 35% for an undisturbed, temperate coastal marsh (Mitsch and Gosselink 2015; Vincent, Burdick, and Dionne 2013). Research suggest between 18 – 26% of estuarine soil (mineral or organic) is
composed of organic matter (Craft et al. 1988), with 21 – 25.6% of that organic matter being comprised of carbon (Walsh, Ingalls, and Keil 2008). Borja et al. (2010) suggest recovery from sediment modification and habitat creation is estimated to take at least two decades. Our sites appear to follow this trend. As such, supporting ecosystem services like carbon sequestration, have not yet returned to undisturbed estuarine levels (Craft 2007; Vincent, Burdick, and Dionne 2013; Walsh, Ingalls, and Keil 2008).

Soil carbon and organic matter at the oldest post-restoration site were similar to pre-restoration levels in 12 years. Because the pre-restoration site has been affected by a culvert for at least a century, sediment accretion and movement of organic matter was likely restricted. Based on our sites, once the culvert is removed, soil carbon and organic matter initially decrease and return to pre-restoration levels after a decade. This timeline suggests that once habitats are structurally reconnected, approximately 5% of soil carbon and organic matter are recovered within the first decade. This trajectory indicates recovery may take nearly half a century to recover soil carbon levels. We also see a similar recovery timeline of 40 years in salt marshes located on the West Coast (Moreno-Mateos et al. 2012). Because vegetation recruitment, flocculation, and microbial communities are drivers of soil carbon and organic matter, their recruitment rates may increase in an exponential trajectory, which could shorten the time until ecosystem processes are recovered.

When comparing differences in soil carbon from above and below the restoration location, it appears sites are on a linear trend toward decreased differences between locations. Soil carbon has not homogenized between locations at the newest post-restoration site, evident by the lack of carbon. These results are consistent with soil carbon recruitment in other post-restoration sites over time (Borja et al. 2010), where large carbon deficits occur in
initially restored systems (Craft et al. 1988; Moreno-Mateos et al. 2012). The coarse sediment with large pore spaces increases percolation rates of water, decreasing fine sediment and flocculation ability, thereby delaying the succession of microbial communities. However, while restoration efforts initially degrade habitat, the end goal of recovering ecosystem services may be seen with time (Harwell, Cropper, and Ragsdale 1977). Hydrological regimes may return to historic reconnection levels rather quickly, but recovery of ecosystem services may be slow, taking up to four decades after perturbation removal (Jones and Schmitz 2009). Among our sites, it appears 12 years after restoration soil carbon has recovered what the pre-restoration location developed within a century.

Moreover, when considering soil carbon and organic matter change differences among sites, there is a trend of decreased differences, though not statistically supported. However, this trend may be the result of a return of primary productivity, restored sediment deposition, and a return to normal tidal inundation. These factors are noted in a natural, functioning estuary (Callaway et al. 2012). Primary productivity facilitates the entrapment of tidal sediment, which then facilitates the addition of organic matter into the sediment (Hussein, Rabenhorst, and Tucker 2004). It is this process that reduces erosion; thus fulfilling a positive feedback-loop wherein estuarine plant species may continue colonizing the low tidal ecosystem. We note a probable trajectory of the homogenization between location (i.e. above and below) in our post-restoration sites.

6.1.2 Soil Carbon-to-Nitrogen Ratio

The 3 year post-restoration site (3-Yr) had the highest carbon-to-nitrogen ratio below the point of culvert restoration, indicating lower relative nitrogen levels within this site (Brady 1974; Evert and Eichhorn 2013). This is presumably connected to soil organic matter,
which plays a major role in nitrogen cycling as most nitrogen enters the system as a component of organic matter (Day et al. 2013). Because of the effects of heavy machinery, restored hydrology, and displacement of fine material, biogeochemical cycling and nitrogen retention may be reduced. Because soil health is directly influenced by aquatic and terrestrial connectivity, soil at this site was greatly affected by the disruption of fines, which increased the pore space. Pore space in soil is the proportion occupied by air and water, which is directly determined by sediment size. Sediment with low porosity, that is, reduced air and water, tends to experience greater gas and nutrient loss. The sediment below the restoration location at our newest restoration site contains large sandy particles and we saw a reduction in nitrogen at this location. Upon the return of fine particles, porosity will increase, a retention of organic matter will occur, and the site may then begin accreting sediment to keep pace with relative se-level rise. The carbon-to-nitrogen ratios at the remaining sites and locations within our study appear to be within normal ranges in the Pacific Northwest, between 15:1 and 19:1 (Littke et al. 2011).

Among all sites, the difference in carbon-to-nitrogen between location (above and below) was not significantly different. Research has suggested sites begin functioning normally after a decade with regard to many biotic functions, yet nitrogen deficiency may still be apparent up to 30 years post-restoration (Moreno-Mateos et al. 2012). The pre-restoration and oldest post-restoration site carbon-to-nitrogen ratios are below 20:1, suggesting nitrogen is not limiting in the sites not recently disturbed, whether from culvert installation or removal (Craft 2001). The return of carbon-to-nitrogen to pre-restoration levels around year nine supports the hypothesis that time since restoration may partially recover regulating ecosystem services, like nitrogen cycling.
6.1.3 Correlation of Soil Nutrients and Plant Biomass

Of the twelve essential macro- and micronutrients that were measured, nitrogen, potassium, magnesium, sulfur, boron, copper, and manganese were positively correlated with plant dry biomass. Based on rough estimates, potassium, magnesium, boron, iron, and manganese were in the lower range expected within all study sites (Brady 1974; Day Jr. et al. 1989; Evert and Eichhorn 2013). However, when compared among the sites, we do note a linear trajectory in nutrient availability with time. Because estuaries are dynamic, and pockets of deposition and deficiency may be observed within a site, it is possible for an estuary to be considered nutrient deficient in one area, and considered nutrient rich in an adjacent region (Fisher et al. 1999; Renjith et al. 2016). This is especially prevalent in our sites where low salinity, turbidity of flowing water, and small freshwater outflow are present (Howarth 1993). We saw a homogenization in soil nutrients between locations in our site restored 12 years ago. Consequently, nutrients significantly correlated with plant height are all utilized in structural and photosynthetic processes within plants, indicating that as nutrients are incorporated, plants are utilizing them efficiently to grow.

Estuarine homeostasis is maintained through nutrient transformation in the soil, which plants are then able to absorb (Berner 2003). Through increased primary productivity, and the subsequent input of dissolved organic matter, nutrient transformation is supported. The relationship between nutrient cycling and primary productivity will likely be altered as sediment input is altered, (e.g. through culverts, or dams), with the support of dynamic plant assemblages within estuarine systems may mitigate additional nutrient inputs (Kirwan and Mudd 2012; Mudd, Howell, and Morris 2009). The biodiversity-ecosystem hypothesis states conserving biodiversity is necessary for maintaining ecosystem services (Srivastava and
Vellend 2005), like nutrient cycling, that vegetation assemblages are a critical component to foundational ecosystem services.

6.2 Vegetation

6.2.1 Plant Species Diversity

Species diversity of estuarine vegetative communities increased over time, with the oldest post-restoration site (12-Yr) showing the highest species diversity of all study sites. Plant species richness followed the same trend. Plants surveyed totaled 65 species between all sites and fell within the range of our statistical estimates 66 (±3) based on the bootstrap method, which has shown to be a more dependable population estimation method (Efron 1979). The forbs functional group was the most common group sampled between all sites and comprised over half of species sampled. Grasses, sedges, and rushes comprised just under one quarter of all species. Woody species comprised 20% of all species sampled. In estuaries of the Pacific Northwest, it is common to sample fat hen, saltgrass, pickleweed, fleshy Jaumea, seaside arrowgrass, seaside plantain, and dune grass. All common estuarine species native in the Pacific Northwest were encountered in relative proportion to functional group composition typical of a temperate estuary (Gabler et al. 2017), with the exception of the intermediately aged (9-Yr) post-restoration site.

The pre-restoration site (pre) had the lowest species richness and diversity above the existing culvert. The species composition at the pre-restoration site included halophytic succulent forbs, graminoids, and rushes including fleshy Jaumea, pickleweed, salt-meadow rush, and saltgrass. Pickleweed was located both above and below the culvert, while and fleshy Jaumea was mainly located below the culvert, suggesting greater tidal inundation above the culvert. Additionally, two culverts were in place at the time of study, which caused
an obstruction to water flow, both upstream and in the marine confluence creating a large holding area that remains inundated for longer periods. Reduced channel width, increased water flow velocity, and high directional flow caused a localized scour above the culvert (Escarameia and May 1999), creating steep banks, primarily saline inundation, and restricting vertical colonization of estuarine vegetation gradients. Ecosystem disconnectivity structurally and functionally disconnects ecosystems, reducing plant species diversity and ecosystem services (Brudvig 2011; Hooper et al. 2005; Worm et al. 2006). The pre-restoration site was the only site within this study to show a significant difference between location (above vs. below).

The newest post-restoration site (3-Yr) below the bridge was also the lowest with regard to species diversity. The majority of species composition at this site included halophytic forbs, graminoids, and rushes. Pickleweed and saltgrass were the main two species colonized above the restoration point. Estuary banks were mostly comprised of pickleweed and gumweed. Based on the SIMPER analysis, the largest separation between plant communities occurred with the presence of dune grass, which only occurred below the restoration location, while pickleweed was the most frequently sampled above the restoration site. Gumweed was ubiquitous throughout. The revegetation plan included three functional groups: woody, forbs, and graminoids. These included, but were not sampled in our study, shore pine (*Pinus contorta*), red-osier dogwood (*Cornus stolinifers*), Western crabapple (*Malus fusca*), red-flowering currant (*Ribes sanguineum*), red elderberry (*Sambucus racemose*), and Hooker willow (*Salix hookeriana*). In the forb functional group seawatch angelica (*Angelica lucida*), Douglas aster (*Aster subspicatus*), and marsh clover (*Trifolium wormskjoldii*) were planted in the cleared areas, but did not persist in the three years post-
restoration. The graminoid functional group contained Oregon bentgrass (*Agrostis oregonensis*), Pacific reedgrass (*Calamagrostis nutkaensis*), and tufted hairgrass (*Deschampsia cespitosa*), of which, only tufted hairgrass (<0.01%) was actually sampled in this study.

The intermediately aged post-restoration site (9-Yr) had the second highest diversity when compared between all sites. The majority of species at this site included: halophytic forbs, graminoids, and rushes, with few salt-tolerant woody species. Fat hen, pickleweed, and gumweed were most common below the restoration location. Creeping buttercup, bird’s-foot trefoil, and colonial bentgrass were most prevalent above the restoration site. Estuary banks were mostly comprised of pickleweed, colonial bentgrass, and bird’s-foot trefoil. The SIMPER analysis supports this separation, as colonial bentgrass and common rush were generally located above the restoration location, whereas gumweed and fat hen were located only below the restoration site. Additionally, the PC analysis supports the development of a vertical gradient, as Himalayan blackberry and red alders were located above the restoration location, above areas with tidal influence. The revegetation plan included three woody species for the revegetation of the marine shoreline including bitter cherry (*Prunus emarginata*), oceanspray (*Holodiscus discolor*), and dunegrass. These species were not surveyed. No graminoid species were included in the planting plan, though two graminoid species were surveyed. These species included velvet grass and reed canary grass, which were recruited naturally. Most species included in the planting plan tolerate salt spray and occasional inundation, with the exception of dune grass. Because of the daily inundation, these species were inappropriate for the marine shoreline. None of the plants surveyed were actually planted during the restoration of this site. However, increased species diversity was
affected by the location of the culvert, which was further into the freshwater riparian habitat, where tidal inundation is minimal (Thom, Zeigler, and Borde 2002).

The oldest post restoration site (12-Yr) had the highest species diversity, likely due to the time since restoration. The most numerous species above the restoration location included pickleweed, saltgrass, montia, sea plantain, coastal pearlwort, and sand spurry. Species encountered most often below the restoration location include gumweed, fat hen, pickleweed, English plantain, sea plantain. Saltgrass and Pacific silverweed were encountered above the restoration location. These occurrences are supported by the SIMPER and PC analysis, both show a separation between species that tolerate full tidal inundation. The planting plan provided for this site included six Pacific wax myrtle (*Myrica californica*), three strawberry tree (*Arbutus unedo*), three burning bush (*Euonymus alatus*), and three pampas grass (*Cortaderia selloana*). Of these, only Pacific wax myrtle is native to the Pacific Northwest. None of the species indicated on the planting plan were sampled. This site had the highest diversity within the study and is relatively similar to other restored, temperate estuaries in terms of overall diversity (Craft et al. 1999; Morgan and Short 2002). Craft et al. (1999) showed natural and constructed salt-marshes have a Shannon’s Index (H’) between 1.92 and 1.96, which is comparable to this site. Morgan and Short (2002) estimated newly restored sites have a low H’ between 0.0-0.2, which was shown to increase by year 15 to 0.5, which is lower than our observed diversity. This illustrates that species diversity indices alone should not be used to assess the recovery of an ecosystem (McCann 2000).

Among all sites, the difference in plant species diversity between location (above and below) was calculated. When comparing those differences of diversity from above the restoration location to the diversity below the restoration location, it appears sites are on a
linear trend toward decreased differences between locations, with higher species colonization below the restoration location. Species diversity is likely increasing because of time since restoration and the accretion of organic matter and sediment over time. This may be attributed to the recent culvert restoration. However, a trend toward decreasing mean difference between locations within sites was observed, yet plant communities have not homogenized.

It has been noted that plant species diversity affects ecosystem processes such as nutrient cycling, primary productivity and biogeochemical cycling (Cardinale et al. 2007; Tilman et al. 1997; Tilman 1999). As such, we do see an upward trend of plant diversity with increased soil carbon and thus, a return of carbon sequestration among all our sites. Diversity is a passive response to ecosystem regulating mechanisms (McCann 2000) and should be considered as one aspect in a holistic approach (Zedler 2017). Once fine sediment returns to the site, species diversity is expected to increase (Buchan et al. 2003; Hobbie 2015; Partyka and Peterson 2008).

6.2.2 Salinity Gradient

Based on several community analyses including principal component analysis, non-metric multidimensional scaling, and analysis of similarity, vegetation communities separated into two distinct groups: halophytic and glycophytic (salt intolerant), presumably due to a salinity gradient. The first group was comprised of halophytic species: pickleweed, saltgrass, and fleshy Jaumea. The second, glycophytic, group was composed of colonial bentgrass, Hooker’s willow, common rush, Sitka willow, red alder, Canada thistle, and bird’s-foot trefoil. This distinct grouping suggests salinity is driving species composition. Additionally, SIMPER and PC analysis show plant communities at sites are separating by
location of transect (i.e. above or below) among sites. Halophytic species that group together also tend to occur more frequently below the restoration location.

Plant species colonization is strongly influenced by salinity gradients, which influences the relative abundance of individual species. As such, species diversity at the oldest post-restoration site is likely the highest due to time since restoration, yet vegetation recruitment is still influenced by a salinity gradient. Halophytic plants are particularly critical in estuarine habitat as coastal areas experience rising sea levels (Flowers and Colmer 2008). Recent research has shown that estuarine plant species support ecosystem services such as carbon sequestration, sediment accretion, and shore stability (Flowers and Muscolo 2015) by colonizing harsh environmental conditions where salt concentrations are around 30 parts per thousand. Although we did not sample salinity, the separation of community composition supports the presence of a salinity gradient driving vegetation recruitment in estuarine ecosystems based on the known ecology of species sampled (Alaback et al. 1994, Crain et al. 2004; Guo et al. 2014; Sharpe and Baldwin 2009; Pennings, Grant, and Bertness 2005).

Invasive species appear to follow the freshwater gradient, where a reduction of salinity influence allowed for the colonization of invasive species, such as reed canary grass. Nine invasive species were noted at older sites, all of which are considered Class B or C Noxious Weeds by the State of Washington (WA State Noxious Weed Control Board 2017). An important component of ecosystem function is maintenance of native species assemblages (Boumans, Burdick, and Dionne 2002) and it appears invasibility within our study sites is reduced due to the presence of a salinity gradient. While there were no statistical differences of invasive species presence between our study sites or locations, post-restoration monitoring will be critical in controlling the spread of invasive plants species.
No invasive species were surveyed within transects at the pre-restoration location, yet they were observed at the site. The newest post restoration site contained Scotch broom, and hairy cat’s ear below the tailwater of the restoration location. At the intermediately aged site Canada thistle, Scotch broom, hairy cat’s ear, reed canary grass, Himalayan blackberry, common tansy, and ox-eye daisy were present. The higher recruitment of invasive species occurred above the headwater of the restoration location. At the oldest post-restoration site scotch broom, Queen Anne’s lace, reed canary grass, Himalayan blackberry, bindweed, and hairy cat’s ear were present throughout the site. Based on our results, it is the presence of a salinity gradient that effectively influences invasive species recruitment (La Peyre et al. 2001).

Within our sites, it appears the plant species initially planted in the intertidal zone during restoration may have been inappropriately selected due to the severe salinity influence of our sites. When comparing the actual plant assemblage surveyed to the planting plans provided for the newest and intermediate restoration sites, only two individuals of tufted hairgrass had persisted (Fleet and Industrial Supply Center Puget Sound Manchester Fuel Depot 2010; Kitsap County Dept. of Public Works 2009; Makers architecture and urban design 2004). Recruitment of all other plants surveyed occurred through unassisted succession. This recruitment shows applying costly seed mixes and native woody plants has reduced efficacy for repopulating the area. Therefore, reseeding post-restoration sites with a mix of the plant species surveyed in this study would increase vegetation recolonization may facilitate a quicker return of ecosystem function.
6.2.3 Ecological Succession

Based on plants surveyed, pickleweed, saltgrass, fat hen, gumweed, and dunegrass are the most common at recent post-restoration sites, with gumweed, and dunegrass especially successful colonizing rocky, disturbed sites. Plants that tend to recruit later in time include monita, sea plantain, coastal pearlwort, sand spurry, seaside arrowgrass and Pacific silverweed. Plant succession is critical in restoration and when implementing effectiveness monitoring criteria, the species listed above thrive in disturb areas and should be considered in planting plans. Restoration plans should include those species listed above that are the first to colonize post-disturbance habitat. Additionally, planting the species listed above may speed up the recovery of ecosystem function, like a quicker return of ecosystem processes, like carbon sequestration and biogeochemical cycling.

Once plant colonization occurs, sediment is further stabilized because of roots. This begins to reduce the amount of sediment that is washed into open marine water during high tide. Plants also slow the velocity of water, which carries suspended fine sediment particles, and caused the sediment to drop from the water column. This cycle of reducing velocity of water encourages sediment accretion. In addition, plant roots created a matrix smaller pore spaces in the sediment and facilitates proper aeration of roots as well as holding and transporting nutrients. Appropriate pore space, combined with sediment accretion, then encourages flocculation, whereupon small particles conglomerate together. This creates an even greater area for microbes and biogeochemical cycling. This cycle of primary succession, sediment accretion, and flocculation is all dependent on connectivity between habitats.
Restored ecosystems may never return to pre-disturbance conditions, as such, management plans should focus on returning function to ecosystem processes rather than focus on a return to a reference state. Future estuarine restoration will depend on site-specific plans based on soil characteristics, geographic extent, and species present. Nutrients appeared within normal ranges, with the exception of the newest post-restoration site below the installed bridge. A trend of increasing soil and organic matter was observed after post-restoration. Future research should include vertical transects to capture the gradation of vegetation distribution and salinity measurements, especially in larger estuarine systems. While species richness and diversity are consistent with other Pacific Northwest estuarine communities, invasive species may be an issue. Facilitating restoration catered to individual sites based on salinity gradients and pioneer species will be central to the continued restoration of supporting ecosystem services. Maintenance and effectiveness monitoring will also play a key role in ensuring native plant species are not outcompeted by invasive plant species.
6.2.4 *Implications for Restoration*

- Soil organic matter development is central to vegetation recruitment. During restoration, incorporating woody debris and organic matter from onsite could enhance sediment with material that may speed up organic matter accretion and support microbial communities.

- In coastal estuarine systems where hydrology has been historically altered and then reconnected, the development of a salinity gradient should be considered when re-vegetating the intertidal zone with the utilization of early successional species such as pickleweed, saltgrass, and Lyngby’s sedge in place of woody species.

- It appears the timeline for recovering soil organic matter to natural levels will take approximately 30 - 40 years, while plant assemblages appear to resemble a natural estuary by year 12.
7.0 Conclusion

7.1 Evaluation of Objectives

In this study carbon, nutrients, and organic matter were measured at four estuary restoration locations in Kitsap County. Plant vegetative communities were assessed for homogenization between locations (above and below) the restoration location and drivers of community assemblages were explored. Below are the originally stated statistical hypotheses and their evaluation.

Question 1. Is there a linear trajectory in recovery of soil carbon, organic matter, and soil nutrients due to length of time since ecological restoration (i.e. culvert removal)?

$H_0 =$ There will be no difference in soil carbon, organic matter, and soil nutrients as length of time since restoration has increased.

$H_A =$ There will be an increase in soil carbon, organic matter, and soil nutrients as length of time since restoration has increased, because vegetation communities will have diversified over time, sediment accretion will return, and vegetation will provide increased organic matter into the sediment.

Neither hypotheses was fully accepted. Soil carbon and organic matter may increase as length of time since restoration increases, yet there was not a definitive trend in our data. Specifically, there was more carbon and soil organic matter in the pre-restoration and oldest-post restoration locations. An initial decrease in carbon and organic matter can be seen at the newest-post restoration site. Based on restoration literature, habitat degradation occurs initially in post-restoration sites. Additionally, the same amount of soil carbon and organic
matter in the pre-restoration site was found in the oldest post-restoration site, indicating the negative impact the culvert had on sediment accretion.

We accepted the alternative hypothesis that soil nutrients would increase with time since restoration. This is demonstrated by a deficiency in potassium, magnesium, boron, iron, and manganese below the restoration location at the newest post-restoration site. Correlation analysis showed a positive relationship between plant height and nitrogen, potassium, magnesium, sulfur, boron, copper, and manganese. Based on known morphology of the plant species encountered within this study, the hypothesis that plants are acquiring nutrients needed for growth was supported (Table 5-1). Phosphorus, calcium, iron, and zinc were not correlated with plant biomass, thus did not support the hypothesis. Although potassium, magnesium, boron, iron, and manganese were considered deficient based on literature estimates, plants did not show any deficiencies (Table 5-2). While there were no outward signs of deficiency, nutrient dynamics are unique among sites, so it may be difficult to assess the magnitude of deficiency.

Question 2. Does species richness and diversity of estuarine vegetative communities increase over time?

H₀= There will be no difference in plants species richness and diversity as time since restoration has increased because estuarine plant communities in the Pacific Northwest are ubiquitous.

Hₐ= There will be an increase in plant species richness and diversity between sites as time since restoration has increased. Specifically, recently restored sites will still
show lower species richness because of the culvert historically restricting sediment, nutrient, and genetic movement.

There was a significant difference in plant species richness and diversity as time since restoration has increased (Table B-16; Appendix B). There was also a significant difference in plant diversity between sites (Table B-13; Appendix B). Plant richness and diversity both show an increase over time, likely due to soil nutrient accumulation. This is the result of increased organic matter input. This supports the positive feedback loop seen in other restoration sites, where a positive increase in organic matter drives positive response in a return to ecosystem services.

Question 3. Will plant communities of estuarine vegetative communities homogenize between restoration location (above or below) over time?

\( H_0 = \) Vegetation communities will not homogenize between restoration location over time because estuarine plant communities in the Pacific Northwest are ubiquitous.

\( H_A = \) Vegetation communities will homogenize between restoration location over time as the reconnected aquatic corridor will facilitate dispersal of genetic material, sediment, and nutrients.

Plant species assemblages did not homogenize over time between restoration locations. Vegetation communities were significantly different between locations, as determined by hierarchical clustering on principal components (Figure 5-13), and analysis of similarity (Table 5-5). While it was determined that estuarine communities are relatively ubiquitous in the Pacific Northwest, homogenization did not occur between locations even 12 years post-restoration. The lack of homogenization is likely a result of a salinity gradient,
which is driving vegetative community assemblage more than any other environmental factor. Further, the location of the bridge within the ecotone between freshwater and marine systems may vary; illustrating that homogenization between communities is an inappropriate assessment of system recovery. Salinity should be used to determine the location of the bridge within the broad context of the gradient. Additionally, a flooding (i.e. vertical) gradient should be incorporated into plant assemblages. Estuaries naturally stratify based on the influence of marine waters and measuring both vertical gradation and salinity will provide a complete vegetation assemblage and location within the ecotone.

Question 4. Does time since restoration affect invasive species recruitment?

\( H_0 \): There will be no difference in invasive species as time since restoration has increased because estuarine sites show relatively low species diversity due to saline influence.

\( H_A \): There will be an increase in invasive species as time since restoration has increased because sites are located within heavily urbanized areas, and the chance for invasive species recruitment increases with time.

There were more invasive species noted at older sites, yet this invasive species occurrence was not significantly different between sites, thus the hypothesis was not supported. It should be considered that a vertical gradient of vegetation was not measured, resulting in a lower number of invasive species encountered. Because a salinity gradient is ultimately driving vegetation community structure, and there are relatively few invasive species in the intertidal zone that studied sites. As such, the true invasibility was not captured.
8.0 References


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Appendices

Appendix A. Soil Tables and Figures

Table A-1. Two-way ANOVA of percent soil carbon using log transformed data to fit a normal distribution. Sites and the interaction effect are significantly different from four estuary restoration sites in Kitsap County, Washington.

<table>
<thead>
<tr>
<th></th>
<th>df</th>
<th>Sum Sq</th>
<th>Mean Sq</th>
<th>F-value</th>
<th>P-value</th>
<th>η²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Site</td>
<td>3</td>
<td>5.26</td>
<td>1.84</td>
<td>5.87</td>
<td>0.007**</td>
<td>0.52</td>
</tr>
<tr>
<td>Location</td>
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<td>0.01</td>
<td>0.01</td>
<td>0.03</td>
<td>0.86</td>
<td>0.001</td>
</tr>
<tr>
<td>Site:Location</td>
<td>3</td>
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<td>1.15</td>
<td>3.68</td>
<td>0.03*</td>
<td>0.41</td>
</tr>
<tr>
<td>Residuals</td>
<td>16</td>
<td>5.021</td>
<td>0.31</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* significant at $P < 0.05$; ** significant at $P < 0.01$
Table A-2. One-way ANOVA of differences in percent soil carbon between locations at each site. No significant differences were observed between four estuary restoration sites in Kitsap County, Washington.

<table>
<thead>
<tr>
<th></th>
<th>df</th>
<th>Sum Sq</th>
<th>Mean Sq</th>
<th>F-value</th>
<th>P-value</th>
<th>η²</th>
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<tr>
<td>Site</td>
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<td>50.30</td>
<td>16.77</td>
<td>1.57</td>
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<td>0.37</td>
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<tr>
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<td>85.64</td>
<td>10.70</td>
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<td></td>
</tr>
</tbody>
</table>

* significant at $P < 0.05$; ** significant at $P < 0.01$
Table A-3. Post-hoc pairwise comparisons of percent soil carbon using t-test with pooled standard deviation site and location using the “holm” p-adjustment method. Samples were taken from four estuary restoration locations in Kitsap County Washington.

<table>
<thead>
<tr>
<th></th>
<th>Pre^A</th>
<th>Pre^B</th>
<th>3-Yr^A</th>
<th>3-Yr^B</th>
<th>9-Yr^A</th>
<th>9-Yr^B</th>
<th>12-Yr^A</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre^B</td>
<td>1.00</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>3-Yr^A</td>
<td>1.00</td>
<td>0.73</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>3-Yr^B</td>
<td>0.11</td>
<td>&lt;0.005**</td>
<td>0.48</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>9-Yr^A</td>
<td>1.00</td>
<td>0.48</td>
<td>1.00</td>
<td>0.73</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>9-Yr^B</td>
<td>1.00</td>
<td>0.48</td>
<td>1.00</td>
<td>0.73</td>
<td>1.00</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>12-Yr^A</td>
<td>1.00</td>
<td>0.73</td>
<td>1.00</td>
<td>0.48</td>
<td>1.00</td>
<td>1.00</td>
<td>-</td>
</tr>
<tr>
<td>12-Yr^B</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td>&lt;0.05*</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
</tr>
</tbody>
</table>

^A = Above; ^B = Below; * significant at $P < 0.05$; ** significant at $P < 0.01$

Pre = pre-restoration, 3-Yr = Newest Post-Restoration, 9-Yr = Intermediate Post-Restoration, 12-Yr = Oldest Post-Restoration
Table A-4. Two-way ANOVA of percent soil carbon-to-nitrogen ratios. Location and the interaction effect show significance. Soil samples were taken from four estuary restoration locations in Kitsap County Washington.

<table>
<thead>
<tr>
<th>Df</th>
<th>Sum Sq</th>
<th>Mean Sq</th>
<th>F-value</th>
<th>P-value</th>
<th>η²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Site</td>
<td>3</td>
<td>0.23</td>
<td>0.08</td>
<td>2.75</td>
<td>0.08</td>
</tr>
<tr>
<td>Location</td>
<td>1</td>
<td>0.13</td>
<td>0.13</td>
<td>4.65</td>
<td>&lt;0.05*</td>
</tr>
<tr>
<td>Site: Location</td>
<td>3</td>
<td>0.36</td>
<td>0.12</td>
<td>4.35</td>
<td>0.02*</td>
</tr>
<tr>
<td>Residuals</td>
<td>16</td>
<td>0.44</td>
<td>0.03</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* significant at $P <0.05$;
Table A-5. One-way ANOVA of soil carbon-to-nitrogen ratios. Sites were not significantly different from four estuary restoration locations in Kitsap County, Washington.

<table>
<thead>
<tr>
<th></th>
<th>Df</th>
<th>Sum Sq</th>
<th>Mean Sq</th>
<th>F-value</th>
<th>P-value</th>
<th>$\eta^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Site</td>
<td>3</td>
<td>84.8</td>
<td>28.26</td>
<td>1.62</td>
<td>0.22</td>
<td>0.20</td>
</tr>
<tr>
<td>Residuals</td>
<td>20</td>
<td>348.0</td>
<td>17.40</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table A-6. Post-hoc pairwise comparisons of soil carbon to nitrogen ratios using t-test with pooled standard deviation between site and location at four estuary restoration sites in Kitsap County, Washington.

<table>
<thead>
<tr>
<th></th>
<th>Pre&lt;sup&gt;A&lt;/sup&gt;</th>
<th>Pre&lt;sup&gt;B&lt;/sup&gt;</th>
<th>3-Yr&lt;sup&gt;A&lt;/sup&gt;</th>
<th>3-Yr&lt;sup&gt;B&lt;/sup&gt;</th>
<th>9-Yr&lt;sup&gt;A&lt;/sup&gt;</th>
<th>9-Yr&lt;sup&gt;B&lt;/sup&gt;</th>
<th>12-Yr&lt;sup&gt;A&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre&lt;sup&gt;B&lt;/sup&gt;</td>
<td>1.00</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>3-Yr&lt;sup&gt;A&lt;/sup&gt;</td>
<td>1.00</td>
<td>1.00</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>3-Yr&lt;sup&gt;B&lt;/sup&gt;</td>
<td>0.05*</td>
<td>0.05*</td>
<td>0.02*</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>9-Yr&lt;sup&gt;A&lt;/sup&gt;</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>9-Yr&lt;sup&gt;B&lt;/sup&gt;</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td>0.77</td>
<td>1.00</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>12-Yr&lt;sup&gt;A&lt;/sup&gt;</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td>0.07</td>
<td>1.00</td>
<td>1.00</td>
<td>-</td>
</tr>
<tr>
<td>12-Yr&lt;sup&gt;B&lt;/sup&gt;</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td>0.13</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
</tr>
</tbody>
</table>

<sup>A</sup> = Above; <sup>B</sup> = Below; * significant at $P < 0.05$;
Pre = pre-restoration, 3-Yr = Newest Post-Restoration, 9-Yr = Intermediate Post-Restoration, 12-Yr = Oldest Post-Restoration
Table A-7. Two-way ANOVA of soil organic matter above and below the bridge and/or culvert at all fours sites in Kitsap County, Washington.

<table>
<thead>
<tr>
<th></th>
<th>Df</th>
<th>Sum Sq</th>
<th>Mean Sq</th>
<th>F-value</th>
<th>P-value</th>
<th>η²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Site</td>
<td>3</td>
<td>4.20</td>
<td>1.40</td>
<td>6.92</td>
<td>0.003**</td>
<td>0.56</td>
</tr>
<tr>
<td>Location</td>
<td>1</td>
<td>0.41</td>
<td>0.41</td>
<td>2.03</td>
<td>0.17</td>
<td>0.11</td>
</tr>
<tr>
<td>Site:Location</td>
<td>3</td>
<td>1.65</td>
<td>0.55</td>
<td>2.71</td>
<td>0.08</td>
<td>0.34</td>
</tr>
<tr>
<td>Residuals</td>
<td>16</td>
<td>3.34</td>
<td>0.20</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

** significant at $P < 0.005$
Table A-8. One-way ANOVA of the difference in soil organic matter above and below the bridge and/or culvert between all sites.

<table>
<thead>
<tr>
<th></th>
<th>Df</th>
<th>Sum Sq</th>
<th>Mean Sq</th>
<th>F-value</th>
<th>P-value</th>
<th>η²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Site</td>
<td>3</td>
<td>236.20</td>
<td>78.74</td>
<td>1.44</td>
<td>0.30</td>
<td>0.35</td>
</tr>
<tr>
<td>Residuals</td>
<td>8</td>
<td>438.10</td>
<td>54.76</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* significant at $P < 0.05$
Table A-9. Post-hoc pairwise comparison of soil organic matter by site and location using t-tests with pooled SD and p-adjustment method “holm”. Four estuary restoration sites were surveyed, all located in Kitsap County, Washington.

<table>
<thead>
<tr>
<th></th>
<th>Pre (^B)</th>
<th>Pre (^A)</th>
<th>3-Yr (^B)</th>
<th>3-Yr (^A)</th>
<th>9-Yr (^B)</th>
<th>9-Yr (^A)</th>
<th>12-Yr (^B)</th>
<th>12-Yr (^A)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre (^B)</td>
<td>1.00</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>3-Yr (^A)</td>
<td>1.00</td>
<td>0.74</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>3-Yr (^B)</td>
<td>0.04*</td>
<td>0.004**</td>
<td>0.41</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>9-Yr (^A)</td>
<td>1.00</td>
<td>0.42</td>
<td>1.00</td>
<td>0.74</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>9-Yr (^B)</td>
<td>1.00</td>
<td>0.57</td>
<td>1.00</td>
<td>0.57</td>
<td>1.00</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>12-Yr (^A)</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td>0.02*</td>
<td>1.00</td>
<td>1.00</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>12-Yr (^B)</td>
<td>1.00</td>
<td>0.74</td>
<td>1.00</td>
<td>0.41</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
</tr>
</tbody>
</table>

\(^A\) = Above; \(^B\) = Below; * significant at \(P < 0.05\); ** significant at \(P < 0.01\)

Pre = pre-restoration, 3-Yr = Newest Post-Restoration, 10 Yr = Intermediate Post-Restoration, 13-Yr = Oldest Post-Restoration
Appendix B. Vegetation Tables and Figures

Table B-10. Two-way ANOVA of invasive plant species from four estuary restoration locations in Kitsap County, Washington. A marginal difference was observed.

<table>
<thead>
<tr>
<th></th>
<th>df</th>
<th>Sum Sq</th>
<th>Mean Sq</th>
<th>F-value</th>
<th>P-value</th>
<th>$\eta^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Site</td>
<td>3</td>
<td>8.23</td>
<td>2.74</td>
<td>1.33</td>
<td>0.28</td>
<td>0.09</td>
</tr>
<tr>
<td>Location</td>
<td>1</td>
<td>4.69</td>
<td>4.69</td>
<td>2.27</td>
<td>0.14</td>
<td>0.05</td>
</tr>
<tr>
<td>Site:Location</td>
<td>3</td>
<td>15.56</td>
<td>5.19</td>
<td>2.52</td>
<td>0.07</td>
<td>0.16</td>
</tr>
<tr>
<td>Residuals</td>
<td>40</td>
<td>82.50</td>
<td>2.06</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* significant at $P < 0.05$
Table B-11. Noxious and invasive species recorded at transects (n=24), including scientific name, abbreviation, common name, location (above and below), 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).

<table>
<thead>
<tr>
<th>Species name</th>
<th>Abbrev</th>
<th>Common Name</th>
<th>Site (Pre, 3-, 9-, and 12- yr)</th>
<th>Native Status</th>
<th>Relative Abundance (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cytisus scoparius</td>
<td>CySc</td>
<td>Scotch Broom</td>
<td>9-Yr; 3-Yr; 12-Yr</td>
<td>NX</td>
<td>0.46</td>
</tr>
<tr>
<td>Phalaris arundinacea</td>
<td>PhAr</td>
<td>Reed Canary Grass</td>
<td>9-Yr; 12-Yr</td>
<td>NX</td>
<td>0.46</td>
</tr>
<tr>
<td>Rubus armeniacus</td>
<td>RuAr</td>
<td>Himalayan Blackberry</td>
<td>9-Yr; 12-Yr; Pre; 9-Yr; 12-Yr</td>
<td>NX</td>
<td>0.46</td>
</tr>
<tr>
<td>Cirsium arvense</td>
<td>CiAr</td>
<td>Canadian Thistle</td>
<td>9-Yr</td>
<td>NX</td>
<td>0.13</td>
</tr>
<tr>
<td>Hypochaeris radicata</td>
<td>HyRa</td>
<td>Hairy Cat's Ear</td>
<td>3-Yr; 9-Yr; 9-Yr; 12-Yr</td>
<td>NX</td>
<td>0.13</td>
</tr>
<tr>
<td>Leucanthemum vulgare</td>
<td>LeVu</td>
<td>Ox-Eye Daisy</td>
<td>-</td>
<td>NX</td>
<td>0.08</td>
</tr>
<tr>
<td>Daucus carota</td>
<td>DaCa</td>
<td>Queen Anne's Lace</td>
<td>-</td>
<td>NX</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Convolvulus arvensis</td>
<td>CoAr</td>
<td>Bindweed</td>
<td>-</td>
<td>NX</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Tanacetum vulgare</td>
<td>TaVu</td>
<td>Tansy</td>
<td>9-Yr</td>
<td>NX</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>
Table B-12. Post-hoc pairwise comparison of invasive plant species at four estuary restoration sites in Kitsap County, Washington. The p-value was corrected using the “holm” method with $\alpha < 0.05$.

<table>
<thead>
<tr>
<th></th>
<th>Pre A</th>
<th>Pre B</th>
<th>3-Yr A</th>
<th>3-Yr B</th>
<th>9-Yr A</th>
<th>9-Yr B</th>
<th>12-Yr A</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre B</td>
<td>1.00</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>3-Yr A</td>
<td>1.00</td>
<td>1.00</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>3-Yr B</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>9-Yr A</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>9-Yr B</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>12-Yr A</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td>-</td>
</tr>
<tr>
<td>12-Yr B</td>
<td>0.35</td>
<td>0.35</td>
<td>0.35</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td>0.35</td>
</tr>
</tbody>
</table>

A = Above; B = Below; * significant at $P < 0.05$; ** significant at $P < 0.001$

Pre = pre-restoration, 3-Yr = Newest Post-Restoration, 9-Yr = Intermediate Post-Restoration, 12-Yr = Oldest Post-Restoration
Table B-13. Two-way ANOVA of plant species diversity from four estuary restoration sites in Kitsap County, Washington. Two location at each site were measured, above and below the bridge and/or culvert.

<table>
<thead>
<tr>
<th></th>
<th>Df</th>
<th>Sum Sq</th>
<th>Mean Sq</th>
<th>F-value</th>
<th>P-value</th>
<th>η²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Site</td>
<td>3</td>
<td>2.12</td>
<td>0.71</td>
<td>23.58</td>
<td>&lt;0.001***</td>
<td>0.82</td>
</tr>
<tr>
<td>Location</td>
<td>1</td>
<td>0.08</td>
<td>0.08</td>
<td>2.53</td>
<td>0.13</td>
<td>0.14</td>
</tr>
<tr>
<td>Site:Location</td>
<td>3</td>
<td>0.22</td>
<td>0.07</td>
<td>2.39</td>
<td>0.11</td>
<td>0.31</td>
</tr>
<tr>
<td>Residuals</td>
<td>16</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*** significant at P < 0.001
Table B-14. One-way ANOVA of plant species diversity above and below the bridge and/or culvert at four estuary restoration sites in Kitsap County, Washington.

<table>
<thead>
<tr>
<th></th>
<th>Df</th>
<th>Sum Sq</th>
<th>Mean Sq</th>
<th>F-value</th>
<th>P-value</th>
<th>η²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Site</td>
<td>3</td>
<td>2.12</td>
<td>0.71</td>
<td>18.35</td>
<td>&lt;0.001***</td>
<td>0.73</td>
</tr>
<tr>
<td>Residuals</td>
<td>20</td>
<td>0.77</td>
<td>0.04</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*** significant at $P < 0.001$
Table B-15. Post-hoc pairwise comparison of plant species diversity at four estuary restoration sites in Kitsap County, Washington. The p-value was corrected using the “holm” method with $\alpha < 0.05$.

<table>
<thead>
<tr>
<th></th>
<th>Pre^A</th>
<th>Pre^B</th>
<th>3-Yr^A</th>
<th>3-Yr^B</th>
<th>9-Yr^A</th>
<th>9-Yr^B</th>
<th>12-Yr^A</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre^B</td>
<td>0.56</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>3-Yr^A</td>
<td>1.00</td>
<td>0.23</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>3-Yr^B</td>
<td>1.00</td>
<td>1.00</td>
<td>0.56</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>9-Yr^A</td>
<td>0.09</td>
<td>1.00</td>
<td>0.03*</td>
<td>0.80</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>9-Yr^B</td>
<td>0.47</td>
<td>1.00</td>
<td>0.18</td>
<td>1.00</td>
<td>1.00</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>12-Yr^A</td>
<td>&lt;0.001***</td>
<td>0.04*</td>
<td>&lt;0.001***</td>
<td>0.01*</td>
<td>0.27</td>
<td>0.05*</td>
<td></td>
</tr>
<tr>
<td>12-Yr^B</td>
<td>&lt;0.001***</td>
<td>0.04*</td>
<td>&lt;0.001***</td>
<td>0.01*</td>
<td>0.27</td>
<td>0.05*</td>
<td>1.00</td>
</tr>
</tbody>
</table>

^A = Above; ^B = Below; * significant at $P < 0.05$; *** significant at $P < 0.001$

Pre = pre-restoration, 3-Yr = Newest Post-Restoration, 9-Yr = Intermediate Post-Restoration, 12-Yr = Oldest Post-Restoration
Table B-16. Two-way ANOVA of plant species richness at two distinct locations (above and below) four estuary restoration sites in Kitsap County, Washington.

<table>
<thead>
<tr>
<th></th>
<th>Df</th>
<th>Sum Sq</th>
<th>Mean Sq</th>
<th>F-value</th>
<th>P-value</th>
<th>η²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Site</td>
<td>3</td>
<td>86.03</td>
<td>28.68</td>
<td>16.19</td>
<td>&lt;0.001***</td>
<td>0.75</td>
</tr>
<tr>
<td>Location</td>
<td>1</td>
<td>0.01</td>
<td>0.01</td>
<td>0.006</td>
<td>0.94</td>
<td>0.00</td>
</tr>
<tr>
<td>Site:Location</td>
<td>3</td>
<td>22.03</td>
<td>7.34</td>
<td>4.15</td>
<td>0.02*</td>
<td>0.44</td>
</tr>
<tr>
<td>Residuals</td>
<td>16</td>
<td>28.33</td>
<td>1.77</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*indicates significance at $P<0.05$; *** indicates significance at $P<0.001$
Table B-17. One-way ANOVA of plant species richness between four estuary restoration sites in Kitsap County, Washington.

<table>
<thead>
<tr>
<th></th>
<th>Df</th>
<th>Sum Sq</th>
<th>Mean Sq</th>
<th>F-value</th>
<th>P-value</th>
<th>η²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Site</td>
<td>3</td>
<td>86.03</td>
<td>28.68</td>
<td>11.39</td>
<td>&lt;0.001***</td>
<td>0.63</td>
</tr>
<tr>
<td>Residuals</td>
<td>20</td>
<td>50.38</td>
<td>2.52</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*** significant at $P < 0.001$
Table B-18. Post-hoc pairwise comparison of plant species richness at four estuary restoration sites in Kitsap County, Washington using the “holm” $P$-value correction method and $\alpha=0.05$.

<table>
<thead>
<tr>
<th></th>
<th>Pre $^A$</th>
<th>Pre $^B$</th>
<th>3-Yr $^A$</th>
<th>3-Yr $^B$</th>
<th>9-Yr $^A$</th>
<th>9-Yr $^B$</th>
<th>12-Yr $^A$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre $^B$</td>
<td>0.57</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>3-Yr $^A$</td>
<td>1.00</td>
<td>1.00</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>3-Yr $^B$</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>9-Yr $^A$</td>
<td>0.002**</td>
<td>0.14</td>
<td>0.006</td>
<td>0.01</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>9-Yr $^B$</td>
<td>0.39</td>
<td>1.00</td>
<td>0.70</td>
<td>1.00</td>
<td>0.25</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>12-Yr $^A$</td>
<td>0.006**</td>
<td>0.32</td>
<td>0.01*</td>
<td>0.03*</td>
<td>1.00</td>
<td>0.49</td>
<td>-</td>
</tr>
<tr>
<td>12-Yr $^B$</td>
<td>0.006**</td>
<td>0.32</td>
<td>0.01*</td>
<td>0.03*</td>
<td>1.00</td>
<td>0.49</td>
<td>1.00</td>
</tr>
</tbody>
</table>

$^A$ = Above; $^B$ = Below; * significant at $P < 0.05$; ** significant at $P < 0.01$

Pre = pre-restoration, 3-Yr = Newest Post-Restoration, 9-Yr = Intermediate Post-Restoration, 12-Yr = Oldest Post-Restoration
Figure B-1. Plant species richness of four estuary restoration sites in Kitsap County, Washington represented using boxplots. 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). Two distinct locations at each site were measured (above and below; n=24). There were significantly more plant species at older, post-restoration sites. An increase in species richness can be seen over time. Differences in letters indicate statistical differences determined by post-hoc pairwise comparisons (α=0.05).
Figure B-2. Difference (Δ) of plant species richness representing the difference between location of restoration (above and below). A one-way analysis of variance was used to test differences between above and below the restoration location among four estuary restoration sites in Kitsap County, Washington. Sites include one pre-restoration (pre) site and three sites at varying post-restoration ages: three years (3-Yr), nine years (9-Yr), and 12 years (12-Yr). No significant difference was observed between sites over time.
Figure B-3. Site clusters using PCA by location (above and below) and principal components 1-2 and 1-3 from four estuary restoration sites in Kitsap County, Washington represented with a cluster dendrogram. 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). Two distinct locations at each site were measured (n=24).
Table B-19. Principal components (I-IV) variable loading scores for species from four estuary restoration sites in Kitsap County, Washington. Proportion of principal component variance is indicated under principal component.

<table>
<thead>
<tr>
<th>Principal Component</th>
<th>Species</th>
<th>Variable Loading Score</th>
<th>Principal Component</th>
<th>Species</th>
<th>Variable Loading Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>PC 1 (10%)</td>
<td>Saltgrass</td>
<td>0.187</td>
<td>PC 3 (7%)</td>
<td>Nootka Rose</td>
<td>0.322</td>
</tr>
<tr>
<td></td>
<td>Pickleweed</td>
<td>0.174</td>
<td></td>
<td>Hooker Willow</td>
<td>0.251</td>
</tr>
<tr>
<td></td>
<td>Canada Thistle</td>
<td>-0.228</td>
<td></td>
<td>Colonial Bentgrass</td>
<td>0.250</td>
</tr>
<tr>
<td></td>
<td>Common Rush</td>
<td>-0.297</td>
<td></td>
<td>Ox Eye Daisy</td>
<td>-0.281</td>
</tr>
<tr>
<td></td>
<td>Hooker Willow</td>
<td>-0.321</td>
<td></td>
<td>Curly Dock</td>
<td>-0.281</td>
</tr>
<tr>
<td></td>
<td>Colonial Bentgrass</td>
<td>-0.377</td>
<td></td>
<td>Sorrel</td>
<td>-0.281</td>
</tr>
<tr>
<td>PC 2 (9%)</td>
<td>Big Leaf Maple</td>
<td>0.330</td>
<td>PC 4 (7%)</td>
<td>Scotch Broom</td>
<td>0.31</td>
</tr>
<tr>
<td></td>
<td>Curly Dock</td>
<td>0.330</td>
<td></td>
<td>Dunegrass</td>
<td>0.28</td>
</tr>
<tr>
<td></td>
<td>Ox Eye Daisy</td>
<td>0.330</td>
<td></td>
<td>Am. Searocket</td>
<td>0.26</td>
</tr>
<tr>
<td></td>
<td>Him. Blackberry</td>
<td>0.322</td>
<td></td>
<td>Quack Grass</td>
<td>0.24</td>
</tr>
<tr>
<td></td>
<td>Jaumea Carnosa</td>
<td>-0.10</td>
<td></td>
<td>Seaside Arrowgrass</td>
<td>-0.16</td>
</tr>
<tr>
<td></td>
<td>Scotch Broom</td>
<td>-0.11</td>
<td></td>
<td>Pacific Silverweed</td>
<td>-0.19</td>
</tr>
<tr>
<td></td>
<td>Quack grass</td>
<td>-0.13</td>
<td></td>
<td>Saltgrass</td>
<td>-0.26</td>
</tr>
</tbody>
</table>