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Decreases of carbon and nitrogen in the soils of a 20-year chronosequence of restored wetlands, Washington State, USA

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Abstract

Freshwater wetland restoration is intended to replace both area and function as part of compensatory mitigation, including restoration of biogeochemical soil processes. This study examined the amount of carbon (C) and nitrogen (N) found in the soils of twenty-two wetland restoration sites in the Puget Sound region of Washington State, USA, and assessed whether vegetative strata, hydrologic regime, hydrogeomorphic class, and soil type influenced C and N accumulation rates. The wetland restoration sites were constructed between 1993 and 2013, representing a 20-year chronosequence with multiple sites constructed each year. Soil samples and data regarding vegetative strata, hydrologic regimes, and soil characteristics were collected in July and August 2015, and additional soil samples and hydrologic data was collected in December 2015. Overall, total C decreased over time, with an estimated rate of -0.70 Mg C ha^{-1} yr^{-1}, while total N increased slightly over time (0.004 Mg N ha^{-1} yr^{-1}). Soil characteristics such as color and texture were used to divide soils into upper and lower soil layers. The soil layer distinction was a significant variable in assessing trends of C, N, and bulk density (g cm^{-3}). Bulk density (gr cm^{-3}) was found to generally increase with increasing time. There were no statistically significant relationships between the amount of C or N in the soils and hydrogeomorphic class, hydrologic regime, or soil type. Nevertheless, C:N ratio had a negative relationship with increased age, suggesting that C loses were greater than N losses through time. This study reports the surprising result that rather than increasing through time (as is common in natural ecosystems), mitigation processes can lead to an initial decrease through time in average soil C and N. The results suggest that C and N are not specifically influenced by distinct restoration site design components (i.e., vegetation, hydrology, soil type), but that soil amendments added to wetland restoration sites may influence soil biogeochemical processes in unexpected ways.

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Introduction

Wetlands are one of the most ecologically important natural resources in the world and provide key ecosystem services. They provide food, retain floodwaters, improve water quality and prevent soil erosion (Mitsch & Gosselink, 2007). Concerns about global climate change have placed even greater importance on wetlands for their ability to retain carbon (C). As a result, C sequestration in wetlands has recently become the subject of much research (Li et al., 2004; Badiou et al., 2011; Olander et al., 2012; Marton et al., 2014). It is estimated that wetlands store as much as 30 percent of the earth's total soil C even though they comprise only 4 to 6 percent of its land area (Mitra et al., 2005; Mitsch & Gosselink, 2007).

The quantity and rate of C accumulation depends on the type of wetland. Peat wetlands, which have been studied extensively, have been found to contain the largest soil C pool of all wetlands types (Bridgham et al., 2006). However, their C accumulation rate is very slow, reported to range between 20 to 30 g C m^{-2} yr^{-1} (Gorham, 1991; Roulet, 2000). Conversely, estuarine wetlands have smaller C soil pools but C accumulation rates are exceptionally high, up to ten times the sequestration rate of other wetland types (Bridgham et al., 2006), and have been most recently estimated at 244.7 g C m^{-2} yr^{-1} in the Northern and Southern hemispheres and approximately 10.2 Tg C yr^{-1} globally (Ouyang & Lee, 2014). The extent of study on northern peatlands and tidal wetlands is understandable because of their C pools and accumulation rates. However, in today's world, some of the most often-impacted wetlands in the developed world are inland, freshwater (palustrine) systems in temperate climates. It is important to understand the role these systems play in worldwide C sequestration, as their loss has been both incremental and staggering over the past century.

In addition to C accumulation, understanding bulk density, nitrogen (N) availability and C to N (C:N) ratios are important because they determine soil health and C processing by microbial communities. Bulk density in wetland soils, a measurement of the dry mass of soil per unit volume, can vary widely depending on organic soil content and soil type, and has tremendous impact on C, N, C:N ratio, and microbial dynamics in soils. The bulk density of mineral wetland soils range between 1.0 to 2.0

g cm^{-3}, whereas organic soils range between 0.04 and 0.30 g cm^{-3}, the lower end of the spectrum of which is indicative of *Sphagnum* peatlands (Mitsch & Gosselink, 2007). While densities higher than 1.5 g cm^{-3} begin to impact plant robustness (i.e., the ability of plants to resist disease and other environmental stressors), densities higher than 2.0 g cm^{-3} can contribute to failed plant establishment, limited nutrient availability, decreased porosity, and reduced water availability at wetland restoration sites.

Carbon storage in soils is stoichiometrically dependent on availability of other elements like N. The largest pool of N in wetlands is found in soils, followed by plants (Bowden, 1987). Vegetation characteristics, hydrologic regimes, human sources of nutrient loading (e.g., fertilizers), and precipitation and temperature can affect N pools in wetlands. While N deposition may be relatively constant within an ecosystem, N fixation can also result from actinorhizal relationships with plants (esp. in families Fabaceae and Betulaceae), and recycling N from plants to soils is dependent on plant decomposition rates. The balance of C versus N in soils is fundamentally important because of C and N limitations to microbes, which govern N release and immobilization in soils (Kaye & Hart, 1997). Microbes regulate soil C release through decomposition of dead organic material (e.g., plant material), transforming it into humic compounds and respiring CO_2 into the soil and eventually the atmosphere.

The amount of C that remains in the soil is affected by microbial activity and N availability. Temperature, moisture, and other soil minerals further temper the amount of C that accumulates in the soil because of their influence on organic matter decomposition. Carbon inputs to soils are also highly dependent on N availability, because N regulates plant growth in many temperate ecosystems. In wetland systems, the N content of soils can vary tremendously. Bowden (1987) compiled a number of studies of total N pools in the upper 30 cm of a variety of wetland habitats and the numbers ranged from ~90 g m^{-1} for a papyrus swamp to 1700 g m^{-1} in a Wisconsin reed marsh. In concert with C accumulation, the amount of N in soils is critical to plant survival and overall wetland health, and its bioavailability to plants is linked to soil C, which is best expressed by the C:N ratio.

Soil C:N ratios are important indicators of soil quality. In general, high C:N ratios (i.e., wide ranges) typically exist in wetland soils with fresh detrital plant inputs whereas lower C:N ratios (narrower ranges) are typical of sites with lower detrital decomposition rates and therefore sufficient N for plant uptake (Bowden, 1987). A high soil C:N ratio indicates that either plant matter in the soil is decomposing at a slower rate, creating high C concentrations, or that organic matter C is locked into recalcitrant compounds in the soil. High C:N ratios can also occur in response to large additional of C-rich plant material (like woody debris) to soils, or large N losses from soils due to rapid plant uptake of N or denitrification). Under high C:N ratio conditions, soil microbes will immobilize the existing N from the soil to complete decomposition processes and use the soil C for energy, resulting in low nutrient availability for plant survival (Bowden, 1987; USEPA, 2008).

Different wetland types, especially those with high organic content in their soils, can have different C:N ratios. A recent literature summary (Ballantine et al., 2012) indicated that the C:N ratio for restored wetlands ranges between 6.7 and 28.2. Hunt et al. (2014) found relatively higher C:N ratios in natural wetlands (13.8 to 19.7) and lower ratios in the restored and converted systems (9.1 to 9.3 and 7.5 to 9.3, respectively). The large variation is likely due to the different wetland types, locations, vegetative structure, and nutrient inputs into the systems from various sources. Regardless of variability, soil development is critical to overall wetland health. As such, soil health becomes an important variable to consider when evaluating whether soils are functionally replaced during wetland restoration.

Wetland loss from human impact has reduced the amount of wetland habitat available to sequester C. It is estimated that over half of the world's wetlands, approximately 12.8 million km^2, have been lost to human development (Zedler & Kercher, 2005), almost 1 million km^2 of which have been lost in the United States after 1492 CE (Dahl, 1990). To combat their overall loss, the United States requires project proponents to restore or replace wetlands that are damaged or filled by development. This restoration is largely regulated via enforcement of the 1977 Clean Water Act. However, restored wetlands are often monitored only for vegetative performance standards, hydrology, and coarse-soil indicators (Hossler et al., 2011). Regulatory agencies rarely require testing of C content, nutrient dynamics, or soil quality (e.g., soil C:N ratios) as part of mitigation for wetland impacts. Even on the limited occasions that soil organic matter or C sequestration is analyzed in restored wetlands, there are not enough data to know how site design can affect C accumulation rates or overall soil health.

Many recent studies have been undertaken to understand C sequestration in restored wetlands (Badiou et al., 2011; Ballantine et al., 2012; Burden et al., 2013; Crooks et al., 2014; Larkin et al., 2014). Estuarine wetlands have received the bulk of attention for C sequestration relative to restoration because of their high C sequestration rates. Studies conducted on freshwater emergent wetlands have estimates it can take anywhere from 12 over 100 years for restoration of freshwater emergent systems to result in soil organic C levels equivalent to those found in natural wetlands (Li et al., 2004; Ballantine et al., 2012; Besasie & Buckley, 2012; Osland et al., 2012; Song et al., 2012; Burden et al., 2013). These wetland types have a slower accumulation rate than estuaries. Studies. Accumulation rate studies are often conducted across a chronosequence, ranging anywhere from 0 to 50 years old. Sites are then compared to natural wetlands to determine sequestration rates relative to natural systems. Most of the reviewed studies have low replication for same-aged sites (a common issue with chronosequence studies (Walker et al., 2010), or have been limited to studying emergent systems. This lack of replication in same-aged sites and variety of vegetative cover is a gap in the ability to identify methods for storing C in restored wetlands.

The compensatory mitigation program of the Washington State Department of Transportation (WSDOT) in Washington State, USA, has constructed over 200 wetland restoration sites statewide in order to restore or replace wetlands lost as a result of highway construction. Wetland restoration sites are permitted under Sections 401 and/or 404 of the 1977 Clean Water Act and must meet specific criteria in order to be considered successful replacements for lost wetlands. The WSDOT monitors wetland restoration sites for up to 15 years, during which biologists assess hydrology, vegetative growth and strata types, and soil structure (WSDOT, 2008, 2014). With anywhere from five to 15 new sites constructed each year for the past 30 years, the WSDOT data set provides a unique, well-documented chronosequence for research on mitigated wetland development through time.

The objectives of my study were to quantify total organic C stored in the surface soils of restored freshwater wetlands, estimate the surface C accumulation rate for restored wetlands, and assess whether the rate is affected by variables such as soil N concentration, vegetative strata, hydrology, hydrologic regime, or soil type. Through this study, I hoped to answer the following research questions:

1) What is the estimated C accumulation rate for restored freshwater wetlands; and
2) Do vegetative strata, hydrology, hydrologic regime, or soil type influence C or N accumulation, or bulk density in the soils of these systems?

The ultimate goal of my research is to provide information about the effectiveness of different vegetative strata and hydrologic regimes for C accumulation in restoration site design. It is my hope that the data can help planners and wetland scientists design wetland restoration sites that maximize C storage while still meeting critical biodiversity needs, potentially helping in the challenge of C sequestration.

Methods

Study area

The study area encompasses areas of the Puget Sound lowland region of western Washington State, USA, between latitudes and 46^{o} 43' N and 48^{o} 59' N, and longitudes 122^{o} 44' W and 122^{o} 57' W (Figure 1). Puget Sound is a linear, fjord-like water body that runs north-south and connects to the Pacific Ocean via the Strait of Juan de Fuca. Located west of the Cascade mountain range, Puget Sound and many of the existing water bodies in the region were formed as a result of glacial retreat approximately 14,000 years ago (Kruckeberg, 1991; Shipman, 2004). The melting of the glaciers carved many depressions out of the landscape and left deep layers of glacial alluvial material that became the basis of the soil series observed today.

Soils in the Puget Sound region range from mineral and mixed mineral-organic to organic. More common soil series in the region are mineral soils such as sandy loams and silt loams with clay; rich silt loams are often found in alluvial areas (Kruckeberg, 1991). The area's soils have been disturbed through a long history of land reclamation, infrastructure development, and agriculture practices. Expansive tracts have been drained in order to lower the water level and to use the fertile alluvial soils for growing crops. Deep organic soils and peat soils are rare in the Puget Sound

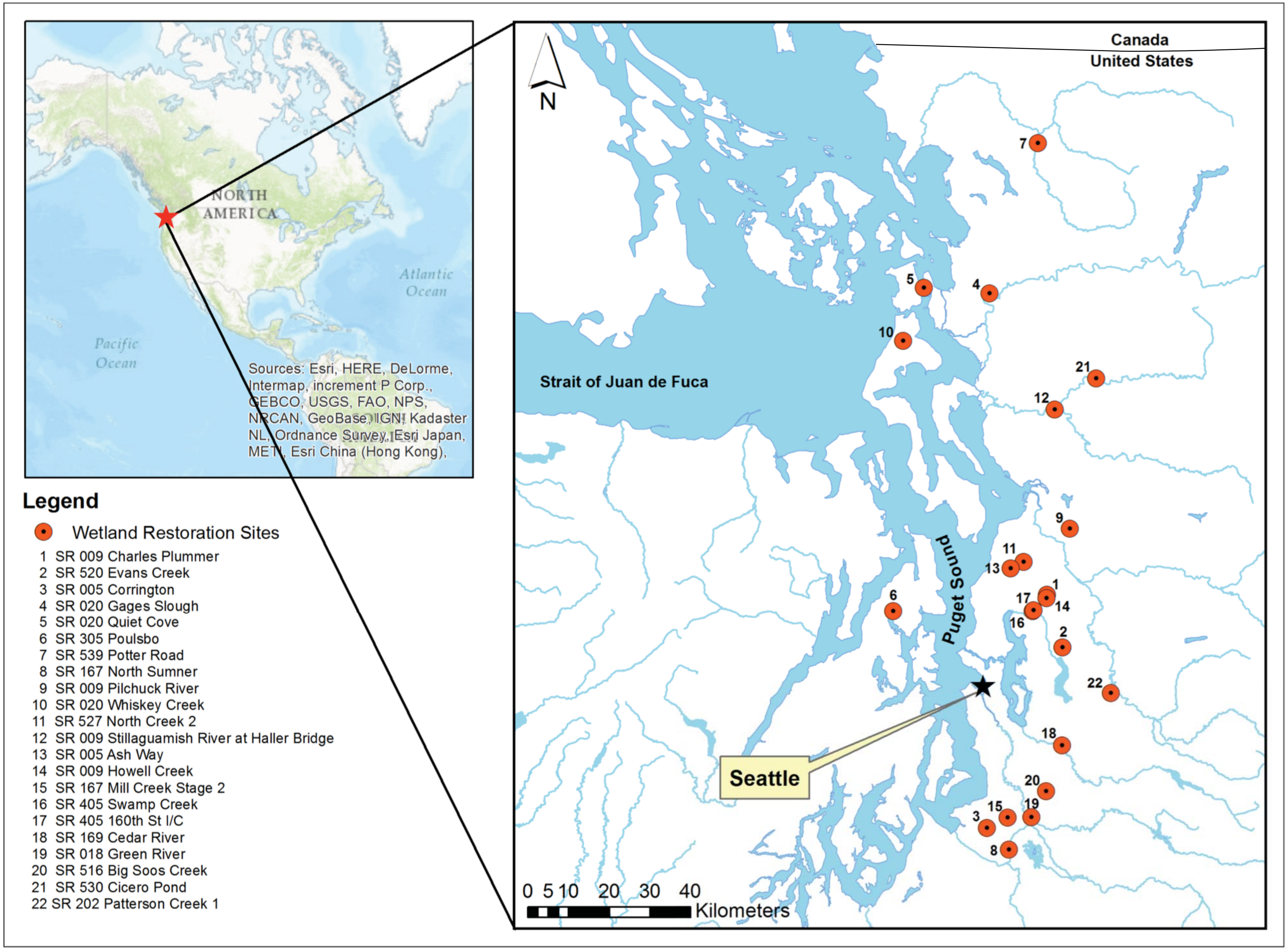

Figure 1. Study area, located in lowland Puget Sound, Washington, USA (orange dots = wetland restoration sites included in study).

lowlands given past anthropogenic disturbances. Moderate organic horizons can be found in soils that have been minimally disturbed or farmed and then abandoned for a number of years.

Site selection

Twenty-two sites were selected from the comprehensive list of freshwater wetland restoration sites constructed and monitored by WSDOT as part of their compensatory wetland mitigation program (Table 1). The chosen sites were depressional closed, flow-through, or outflow, wetlands that were either constructed from upland, or were historically wetland but had been converted to non-wetland use prior to restoration. Only sites mapped and recorded as contemporary mineral soils were selected in order to avoid C data skewing from naturally present organic soils (USDA-NRCS, 2013). Ideal sites had three vegetative strata present based on the Cowardin wetland classification system: palustrine (freshwater) forested (PFO), palustrine scrub-shrub (PSS) and palustrine emergent (PEM) (Cowardin et al., 1979), although in some cases sites with fewer strata were chosen. As-built grading and planting plans for each site were reviewed to verify the created strata, which were then field verified. Site ages ranged from 2 to 22 years old; year zero was defined as the year that construction and planting was completed. Where possible, three to five sites were selected from each year, but when this was not possible, sites were selected from years slightly earlier or later than the targeted year.

Data collection and analysis

Vegetation, hydrology, and soil data were collected in July and August 2015, and additional soil samples and hydrologic data were collected in December 2015. A variety of data were collected for each of the three wetland attributes (Table 2). Up to four, 1 m^2 quadrats (sampling units) were randomly established within each vegetative stratum (sample) using a PVC-constructed grid at each wetland restoration site (Figure 2; Appendix A). The plot locations were recorded using the application “GPS Averaging” on a GPS- and GLONASS-enabled iPhone 5s (Apple, Cupertino, CA). A total of 179 quadrats were established as part of the study, and up to 12 quadrats were established per wetland restoration site (n=46; Appendix B). The number of quadrats varied from site to site due to fewer than three strata present and unexpected site conditions such as access, inundation, or lack of wetland characteristics.

Table 1. Characteristics of the 22 wetland restoration sites included in the study. (WGS = World Geodetic System; SR = State Route, PFO = palustrine forested, PSS = palustrine scrub-shrub, PEM = palustrine emergent; HGM = hydrogeomorphic class; C = depressional closed, O = depressional outflow, FT = depressional flow-through).

Nr.[1]	Site name	Latitude (WGS84)	Longitude (WGS84)	Age	Vegetation class[2] PFO	PSS	PEM	Mapped soil series	HGM
1	SR 009 Charles Plummer	47.793391000	-122.143739000	2	x	x	x	Norma loam	O
2	SR 520 Evans Creek	47.678050000	-122.089520000	2	x	x		Puget silty clay loam	FT
3	SR 005 Corrington	47.275289000	-122.322018000	3	x	x	x	Bellingham silt loam; Shalcar muck	O
4	SR 020 Gages Slough	48.454153617	-122.354017730	6	x	x	x	Sumas silt loam; Field silt loam, protected	O
5	SR 020 Quiet Cove	48.463160909	-122.571937834	6		x	x	Hydraquents, tidal	C
6	SR 305 Poulsbo	47.749071811	-122.644957273	6	x	x	x	Norma fine sandy loam	O
7	SR 539 Potter Road	48.787693910	-122.205489857	6	x	x		Briscot silt loam, drained, 0 to 2 percent slopes	FT
8	SR 167 North Sumner	47.228479260	-122.249184797	9		x	x	Semiahmoo muck; Puget silty clay loam	O
9	SR 009 Pilchuck River	47.939968418	-122.072663461	11	x	x	x	Puyallup fine sandy loam; Puget silty clay loam	O
10	SR 020 Whiskey Creek	48.345244472	-122.635824444	11	x	x	x	Swantown gravelly sandy loam, 0 to 5 percent slopes	O
11	SR 527 North Creek 2	47.864733176	-122.222201985	13	x	x	x	Norma loam; Alderwood gravelly sandy loam, 2 to 8 percent slopes	O
12	SR 009 Stillaguamish River at Haller Bridge	48.201818943	-122.130080993	16	x		x	Puget silty clay loam; Puyallup fine sandy loam	O
13	SR 005 Ash Way	47.849679170	-122.264216200	17	*	x		Norma loam; Alderwood urban land complex, 2-8 percent slopes	O
14	SR 009 Howell Creek	47.785383620	-122.144523100	17	*	x	*	Norma loam	O
15	SR 167 Mill Creek Stage 2	47.299405760	-122.255281600	17		x		Norma sandy loam	O
16	SR 405 Swamp Creek	47.756557030	-122.188440500	17		x	x	Snohomish silt loam	O
17	SR 405 160th Street I/C	47.758182820	-122.185387600	19	x	x	x	Snohomish silt loam	FT
18	SR 169 Cedar River	47.461059400	-122.084505700	20	*	x	*	Alderwood and Kitsap soils, very steep	FT
19	SR 018 Green River	47.300885110	-122.178755600	21	x			Urban land	O
20	SR 516 Big Soos Creek	47.359125190	-122.132148000	21	x		x	Everett gravelly sandy loam, 5 to 15 percent slopes	O
21	SR 530 Cicero Pond	48.272020080	-121.995458700	21	x	x		Pilchuck loamy sand	O
22	SR 202 Patterson Creek 1	47.577904860	-121.927724000	22	x	*		Everett gravelly sandy loam, 0 to 5 percent slopes	C

[1]Site number corresponds to mapped sites on Figure 1. [2]Vegetative classes represented by an asterisk (*) were initially included in site design but were eliminated from the study due to either change in vegetative design or safety issues.

Table 2. Data parameters used to characterize wetland attributes of studied restoration sites.

Wetland Attribute	Data Collected
Vegetation	Absolute aerial cover (USACE, 1987) Cowardin classification (Cowardin et al., 1979) - Palustrine emergent (PEM) - Palustrine scrub-shrub (PSS) - Palustrine forested (PFO) Wetland plant indicator status (Lichvar et al., 2014) - Facultative (FAC) - Facultative wetland (FACW) - Obligate wetland (OBL)
Hydrology	Hydrogeomorphic class (Brinson, 1993) - Depressional closed - Depressional outflow - Depressional flow-through Hydroperiod (Hruby, 2014) - Saturated - Occasionally flooded or inundated - Seasonally flooded or inundated - Occasionally flooded or inundated Soil hydrology (USACE, 2010) - Hydrologic indicators - Saturated soils - High groundwater table/inundated soils - Surface water
Soils	Soil taxonomy (USDA-NRCS, 2006, 2010; Schoeneberger et al., 2012) - Color - Texture - Redoximorphic features Soil type (USDA-NRCS, 2013) - Mineral - Mixed organic / mineral Nutrient level and density - Carbon (percent [mg C g^{-1}]; Mg C ha^{-1}) - Nitrogen (percent [mg N g^{-1}]; Mg C ha^{-1}) - C:N ratio - Bulk density (g cm^{-3})

Vegetation

The vegetation rooted within each quadrat was identified to species and its wetland indicator status was recorded (Lichvar et al., 2014). The absolute cover of each species was visually estimated using methods from the United States Army Corps of Engineers (USACE, 1987). Vegetation that overhung but was not rooted within each plot was not included in aerial cover calculations; a general note was made of its presence. The intended stratum of each plot was verified based on the percent aerial cover of the tallest dominant species. For example, a plot with over 20 percent aerial cover by red alder trees (*Alnus rubra*) was considered to be a

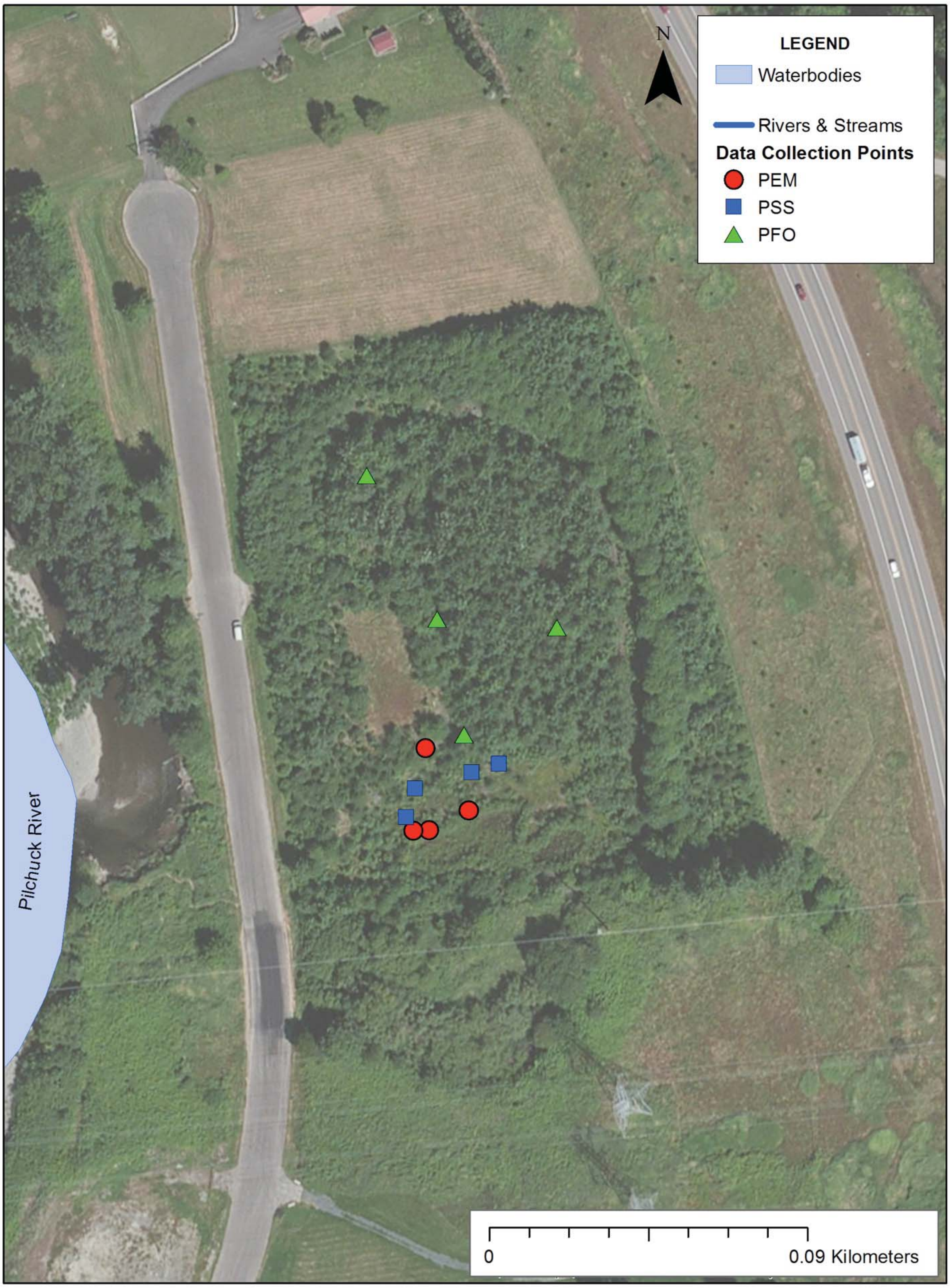

Figure 2. Established 1 m^2 quadrats at the SR 009 Pilchuck River restoration site, Site 9 (PEM = palustrine emergent, PSS = palustrine scrub-shrub, PFO = palustrine forested). Quadrats were established in the same way on all other study sites and all quadrat GPS positions are given in Appendix A.

palustrine forested plot, regardless of whether shrub or emergent species had a greater aerial cover percentage. The vegetative stratum and species composition of each plot was verified against the as-built planting plan. At each restoration site, effort was made to replicate the plant community from plot to plot within each stratum to increase reproducibility and minimize variation between sample units. Sampling was limited to vegetated areas that were planted as part of the restoration work; vegetated wetland areas that existed prior to restoration site construction were not sampled.

Hydrology

Hydrology at each sample site was assessed in two ways. First, the hydroperiod was assessed using the intended hydroperiod documented in the site's restoration plan and verified in the field. Each quadrat was then assigned one of the following hydroperiods identified in Hruby (2014), from 'driest' to 'wettest': saturated, occasionally flooded or inundated, seasonally flooded or inundated, or permanently flooded or inundated.

Next, the soil hydrology was assessed in each of the formal soil pit excavated in each vegetative stratum at the sites. The depth to saturation, depth to standing water from the soil surface, and/or depth of surface water, were recorded after ten minutes. Where no water was present, indicators of wetland hydrology as established by USACE (2010) were recorded if there were present. Rather than use the actual water depths, the soil hydrology for each plot was converted to one of the five above-mentioned categorical levels using the 'wettest' condition present. For example, if soils were saturated to the surface but also had standing water in the excavated pit within 8 cm of the surface, then the condition was categorized as 'high water table.' Soil hydrology was recorded twice at each quadrat: once in July/August at the height of the growing season and once in December, outside of the growing season.

Soils

Three different steps were taken to assess soils: soil characterization based on field conditions at a single formal soil pit for site, soil collection for C and N analysis, and soil collection for bulk density analysis. Soils were collected for analysis in each quadrat established within the vegetative strata at the sites. Within each stratum, a formal soil pit was dug at the first quadrat, which included assessment of soil texture, color, and presence of hydric indicators. The pit was excavated with a sharpshooter shovel to a maximum depth of 40 cm.

Soils were characterized by dividing the soil from the formal soil pit into upper and lower layers based on color differentiation, and the depth of each layer was recorded in cm. The soil texture, color, morphological features, and presence of redoximorphic features indicating hydric soil conditions were recorded for each layer using identification methods outlined in USDA-NRCS (2010) and Schoeneberger et al. (2012). Soil colors were assigned using Munsell® Soil Color charts. Informal soil pits were excavated at the subsequent plots within each stratum to verify that soil conditions were similar to the formal sample pit for the stratum. Any substantial differences in soil color or layer depth was noted. The layer depths for each layer were averaged across a stratum for each site to provide a constant layer depth for the stratum in statistical analysis.

Soil samples for bulk density and nutrient analysis were collected from every quadrat an 18-inch (46 cm), 0.75-inch diameter (1.91 cm), stainless steel hand soil sampler (JMC, Newton, IA). The sampler was pounded into the soil to a maximum 40 cm depth with a rubber mallet. Two soil cores were collected from each quadrat. To collect soils for bulk density analysis, the first core was left intact, its depth (cm) measured, and then placed in a paper bag labeled with “BD”, the site name, vegetative stratum type, and collection date. The second core was collected for C and N analysis and was divided into upper and lower layers based on a distinct break in color/texture differences. The depth (cm) of each layer was recorded and each was placed in a separate bag labeled with “upper” and “lower,” the site name, vegetative stratum type, and collection date. Cores were taken in the same manner from the subsequently established quadrats and combined with the other samples to provide a more homogenous sampling of soils. A maximum of four cores for bulk density and four cores for C:N ratio analysis were taken per stratum at each site.

All sites were sampled in the same manner, although in a few cases site conditions prevented collection of four samples within each stratum. The top mulch or detrital layer was removed to the extent possible before samples were measured, separated, and bagged. A total of 358 soil samples were taken for nutrient analysis and bulk density. However, at each site the soil cores were pooled (physically averaged) into three sample bags for each stratum: Bag A contained soil from the upper layers of up to four soil cores; Bag B contained the soils from the lower soil layers the four soil cores; and Bag C contained up to four complete soil cores. This manual pooling of soil cores allowed for a more even distribution of soil types for a site, resulting in a mean effect of soil characteristics. As a result, the number of collected soil samples was reduced to 138: 46 for bulk density analysis and 92 split soil samples for nutrient analysis (46 upper layer samples and 46 lower layer samples).

Soil analysis for percent C and N was conducted in concert with bulk density analysis. All soil samples were dried for 72 hours at 70^{o} C in a laboratory oven (Thelco GCA/Precision Scientific, Winchester, VA), but were processed differently thereafter depending on the test parameter. Each collected bulk density sample was sifted through a 2 mm metal sieve and separated into two paper coin envelopes per sample, one with the 2 mm sample and one with the coarse (>2 mm) material. The 2 mm sample and coarse sample were then weighed separately using a portable toploading scale (M-power, Sartorius, Bohemia, NY). Bulk density (g cm^{-3}) for each sample was calculated by:

$$BD = Sm/(\pi r^2 \text{ x } D)$$

where *BD* is the bulk density (g cm^{-3}), *Sm* is the mass of the 2 mm sieved soil sample, *r* is the radius of the hand soil sampler (0.953 cm) and *D* is the depth (expressed in cm) of the collected layer.

Percent C and N (mg g^{-1} dry weight) were analyzed by running prewashed aluminum capsules packed with 1 to 3 mg of the 0.5 mm-sieved soil samples through a PerkinElmer CNHO 2400 CHN elemental analyzer (PerkinElmer, Waltham, MA). Every fifth soil sample was replicated to provide quality control and minimize sampling error. Carbon mass was calculated as:

$$Cm = (P \text{ x } BD)/100$$

where *Cm* is the C mass (expressed as g C cm^{-3}), *P* is the percent C, and *BD* is bulk density (expressed as g cm^{-3}). Total C density was then calculated as:

$$Cd = (Cm \text{ x } D) \text{ x } 100$$

where *Cd* is the total C (expressed as Mg C ha^{-1}), *Cm* is C dry mass (expressed as g C cm^{-3}), and *D* is the depth of the collected layer in cm (Appendix B). Total N density was calculated in the same manner as total C density. The C:N ratio was calculated by dividing percent C by percent N. The annual accumulation rates of C and N were calculated as the difference between the average C or N density (Mg C ha^{-1} or Mg N ha^{-1}) of the 20 to 22 year-old sites and the average C or N density of the 2 to 3 year-old sites, and dividing by the maximum number of years since site restoration (i.e., 20 years). Rates were calculated for all vegetative strata and both soil layers together, and then for each individual stratum and both soil layers, and finally for each individual stratum and each soil layer.

Statistical analysis

Statistical analysis and modeling was completed using the statistical software packages R version 3.2.3 (R-Core-Team, 2015) and RStudio version 0.99.879 (RStudio-Team, 2015), along with the packages stats and ggplot2 (Wickham, 2009). The data were transformed prior to analysis when necessary to meet normality assumptions. Specifically, C, N, and C:N values were transformed to $\sqrt{C}$, $\sqrt{N}$, and ln C:N to reduce skewness and provide for more symmetric, normal distributions. Both the Shapiro-Wilks test and quantile-quantile plots conducted on transformed data indicated that data were normally distributed.

An analysis of covariance (ANCOVA) was used to test the data for each response variable as both continuous and categorical data were used as predictor variables. Percent C, percent N, and C density were used as individual response variables and were numeric values. Multiple predictor variables and interactions were used to fit the model to test their significance to the model ($p \leq 0.05$; Table 3). Of the predictor variables, vegetative stratum, soil layer, soil type, hydroperiod, and hydrogeomorphic class were treated as categorical data. For duplicate samples, the percent C, N, and C:N ratio were averaged with the other sample result for the individual sample point rather than included as a separate data point in statistical analysis.

In order to validate each model, the model residuals were compared to the fitted values to determine whether a model was a proper fit ($p \leq 0.05$). Models were fitted individually with the entire data set to test for significance. Additional statistical analysis included plotting vegetative strata against percent C and N, C:N ratio, bulk density, and C density to detect any significant relationships between the predictor variables and nutrient levels or bulk density.

Table 3. Variables and interactions of data examined through statistical analysis. All response variables are continuous and site age was also treated as continuous; remaining predictor variables are categorical.

Response variables (y)	Predictor variables (x)	Interaction(s)
• √C • √N • ln C:N • Bulk density (gr cm^{-3}) • Total C (Mg C ha^{-1}) • Total N (Mg N ha^{-1})	• Site age (yrs) • Vegetative stratum - palustrine emergent - palustrine scrub-shrub - palustrine forested • Soil layer - upper - lower • Soil type - mineral - mixed organic / mineral • Hydroperiod - indicators - saturated - occasionally flooded or inundated - seasonally flooded or inundated - occasionally flooded or inundated • Hydrogeomorphic class - depressional closed - depressional outflow - depressional flow-through	• Site age : vegetative stratum • Site age : hydroperiod • Site age : hydrogeomorphic class • Site age : soil type • Soil type : vegetative stratum • Hydroperiod : vegetative stratum • Hydroperiod : hydrogeomorphic class • Hydrogeomorphic class : vegetative stratum

Results

Structural and functional site characteristics

Of the 22 sites studied, the most represented vegetative stratum was scrub-shrub (n=18), followed by forested (n=15) and emergent (n=13). Multiple same-aged sites were included in the study, but not for every year that was included in the chronosequence (Table 1). Four sites were 6-years old and four were 17-years old; three sites were 21 years old; two sites were 11-years old; and two other sites were 2-years old. The remaining seven sites in the chronosequence accounted for one site per year. Eight restoration sites had three created vegetative strata present, eight had two strata, and six had one stratum. The sites mean and median ages of the examined restoration sites were 12.9 and 14.5, respectively; the modal ages were of 6 and 17 (n=4). The most common hydroperiod for the sites was seasonally flooded (n=10) while the most common hydrogeomorphic classification was depressional outflow (n=15). These analyses were completed for the full dataset, but were also completed by dividing the dataset into two sub-datasets relative to their soil layer (upper and lower).

Carbon and nitrogen pools

Overall, upper layer soils tended to have a higher percentage of C than lower layer soils (Table 4; Figures 3a and 3b). Across all vegetative strata, the mean concentration of C in the upper layer was measured at 8.88 percent, with a range of 2.64 to 21.27 percent. The lower layer soils had a mean concentration C of 3.91 percent and a range of 0.40 to 12.73 percent. When all strata were considered together, the percent C decreased with site age relative to both soil layers, but was statistically significant for the upper layer ($p = 0.040$, $r^2 = 0.18$; Figures 3c and 3d). Percent C in the samples also generally decreased in relationship to increasing age for all strata when strata were considered individually, but none of these relationships were significant (Figures 4a and 4b). Mean total C across all strata was measured at 82.00 Mg C ha^{-1} for the upper layer and 42.65 Mg C ha^{-1} for the lower layer (Table 4).

Total C ranged from 10.31 to 327.56 Mg C ha^{-1} for the upper layer and 7.09 to 190.14 Mg C ha^{-1} for the lower layer. No significant relationships were found between total C and age regardless of whether strata were considered individually (Figures 4c and 4d) or together (Figures 5a and 5b). There was a general trend of decreasing total C over time in the upper soil layer. However, when all strata were considered together, total C in the lower layer increased slightly over time. Lower layer soils associated with forested and scrub-shrub sites had small increases in total C with increasing age (forested: $p = 0.378$, $r^2 = 0.06$; scrub-shrub: $p = 0.746$, $r^2 =0.01$), while total C in soils associated with emergent sites decreased over time ($p = 0.461$, $r^2 = 0.05$; Figure 4d), as well as when considering all strata together ($p = 0.622$, $r^2 = 0.01$; Figure 5b). None of these were significant relationships ($p \leq 0.05$). There was larger variation in total C in upper layer soils than lower layer soils when considering strata individually, and there were more outliers associated with total C relative to lower layer soils than upper soils (Figures 5c and 5d).

Accumulation rates were calculated for a multitude of data combinations (Table 5). This approach was taken to ascertain if accumulation rates varied depending on

Table 4. Mean ± standard error (SE) of dependent variables relative to vegetative stratum and soil layer (PEM n = 13, PSS n = 18, PFO n = 15).

	Upper soil layer $\bar{x}$ ± SE	Lower soil layer $\bar{x}$ ± SE
Palustrine emergent (PEM)		
% C	9.04 ± 1.52	4.07 ± 0.98
% N	0.53 ± 0.08	0.23 ± 0.04
C:N	16.76 ± 0.98	16.38 ± 1.44
BD (g cm^{-3})	0.73 ± 0.06	0.74 ± 007
TC (Mg C ha^{-1})	71.94 ± 10.82	39.22 ± 9.93
TN (Mg N ha^{-1})	4.36 ± 0.68	2.44 ± 0.56
Palustrine scrub-shrub (PSS)		
% C	8.19 ± 0.81	4.29 ± 0.50
% N	0.48 ± 0.05	0.27 ± 0.03
C:N	17.53 ± 1.10	18.66 ± 2.09
BD (g cm^{-3})	0.80 ± 0.06	0.78 ± 0.06
TC (Mg C ha^{-1})	85.27 ± 12.08	47.85 ± 10.84
TN (Mg N ha^{-1})	4.96 ± 0.77	3.21 ± 0.69
Palustrine forested (PFO)		
% C	9.56 ± 1.26	3.38 ± 0.59
% N	0.59 ± 0.09	0.22 ± 0.04
C:N	17.02 ± 1.11	18.49 ± 4.05
BD (g cm^{-3})	0.70 ± 0.06	0.72 ± 0.06
TC (Mg C ha^{-1})	86.8 ± 19.49	39.91 ± 10.20
TN (Mg N ha^{-1})	5.66 ± 1.51	2.53 ± 0.72
All strata combined (PEM, PSS, PFO)		
% C	8.88 ± 0.66	3.91 ± 0.38
% N	0.53 ± 0.04	0.24 ± 0.02
C:N	17.14 ± 0.62	17.96 ± 1.63
BD (g cm^{-3})	0.75 ± 0.04	0.75 ± 0.03
TC (Mg C ha^{-1})	82.00 ± 8.36	42.65 ± 6.04
TN (Mg N ha^{-1})	5.02 ± 0.60	2.77 ± 0.39

whether certain strata or certain soil layers were included. The carbon accumulation rate varied based on strata and soil layer(s) that were considered in the estimate. Considering all strata and both soil layers, total C decreased rather than accumulated in the soils at the examined sites over the 20-year chronosequence, estimated at -0.70 Mg C ha^{-1} yr^{-1}. When calculated separately for the upper and lower soil layers, C accumulation rate in the upper soil layer was estimated to be -1.18 Mg C ha^{-1} yr^{-1}, and -0.12 Mg C ha^{-1} yr^{-1} in the lower layer. It is important to note, however, that declines in C for individual strata relative to soil layer should be interpreted relative to the reduced sample sizes used for these calculations (Table 5).

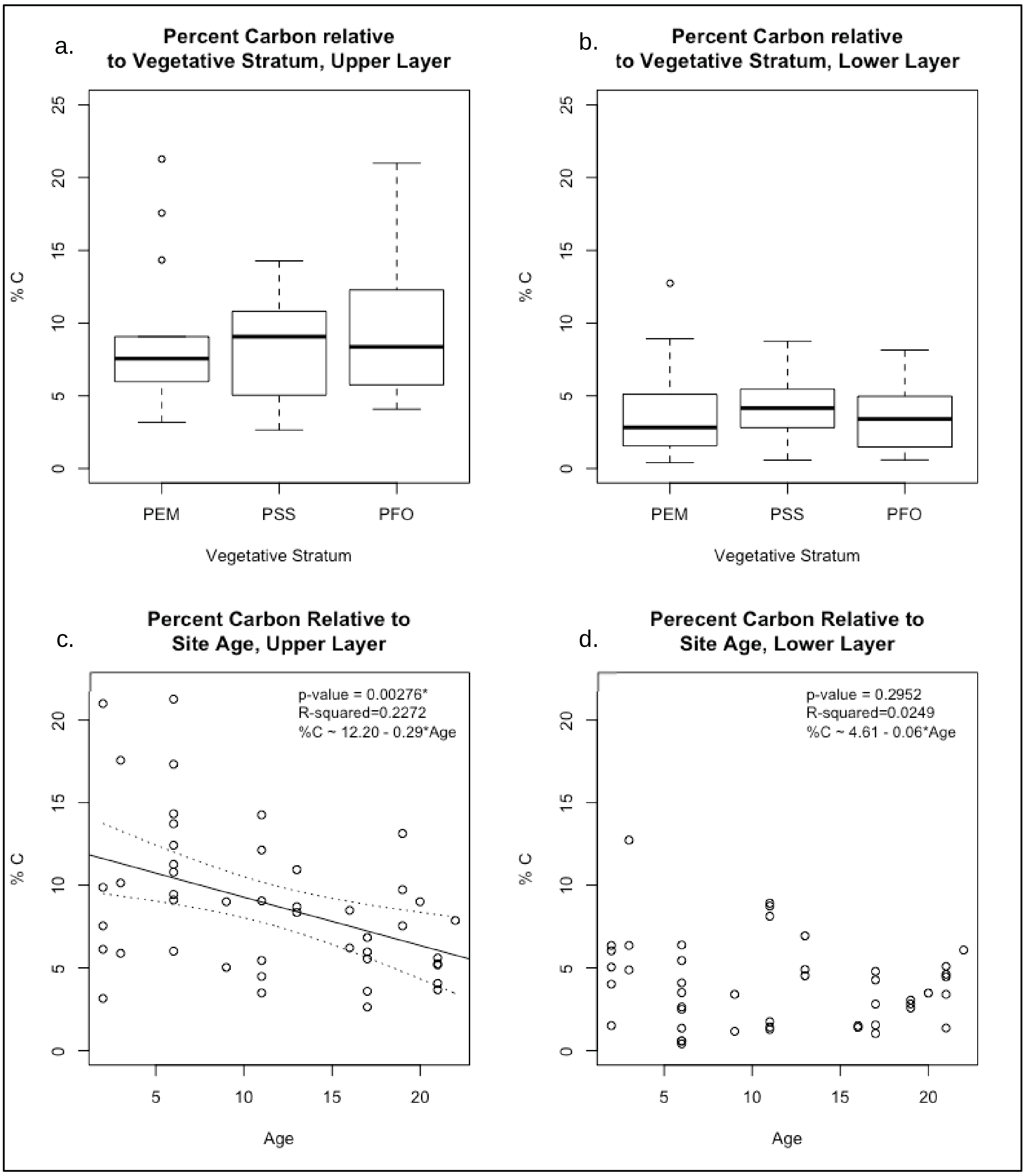

Figure 3. Box whisker plots of percent C relative to vegetative strata for the upper (a.) and lower (b.) soil layers. The upper whisker represents the 75th percentile, the box represents the range of sites within the 27th to 25th percentile, and the lower whisker represents the sites within the 25th percentile of the variable for each stratum. Dark line represents the mean variable measurement, and open circles represent outliers (PEM n = 13, PSS n = 18, PFO n = 15). Percent C relative to age for the upper (c.) and lower (d.) soil layers, with combined vegetative strata (n = 46; CI = 95%). '*' denotes significant p-values (≤ 0.05); solid lines = regression lines fitted to model with significant p-values; dotted lines = confidence intervals.

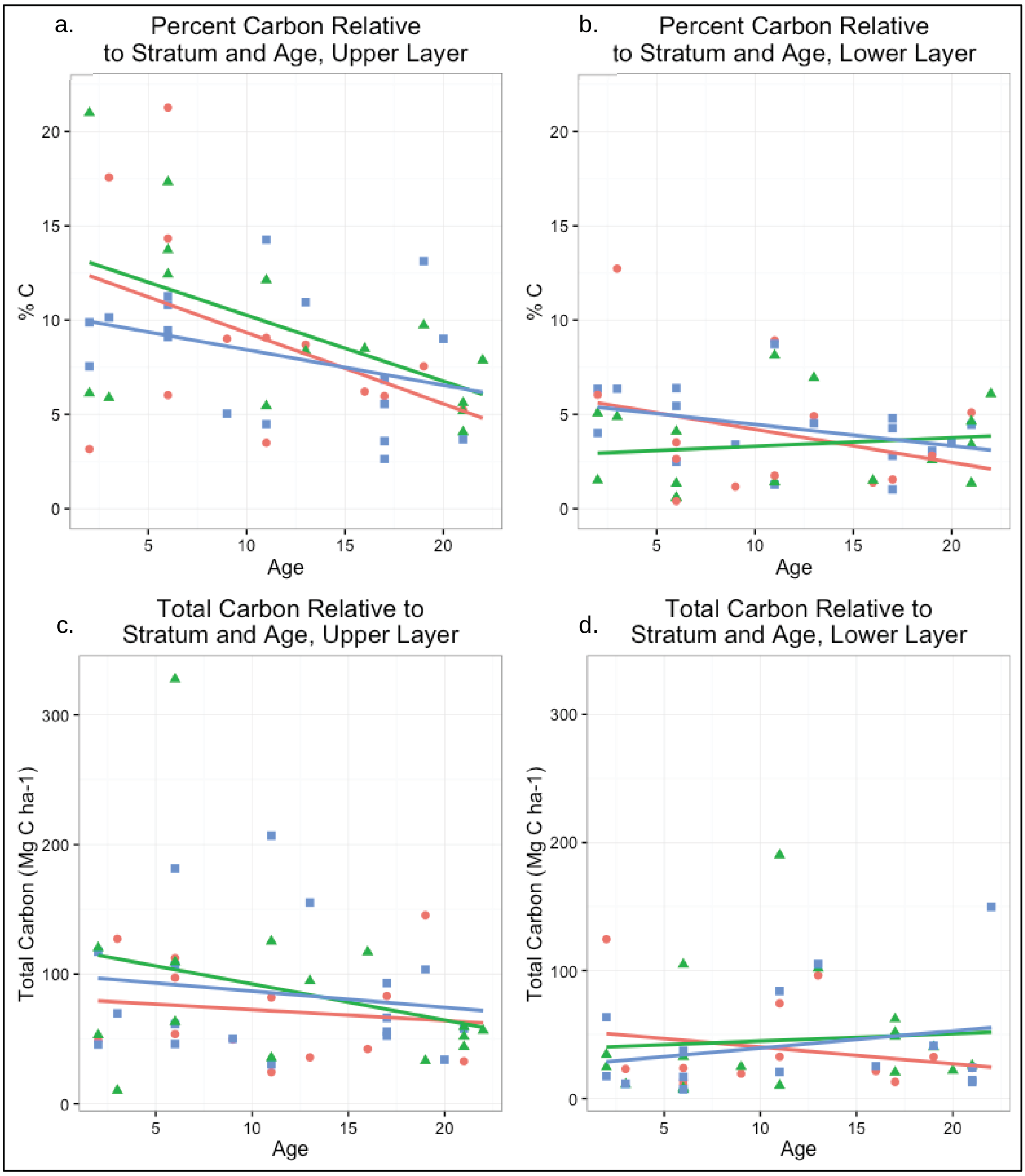

Figure 4. Percent C in upper (a.) and lower (b.) soil layers relative to age and vegetative strata; and total C in upper (c.) and lower (d.) soil layers relative to age and vegetative strata. Red circles and red fitted line = emergent sites (n = 13); blue squares and blue fitted lines = scrub-shrub sites (n = 18); green triangles and green fitted line = forested sites (n = 15).

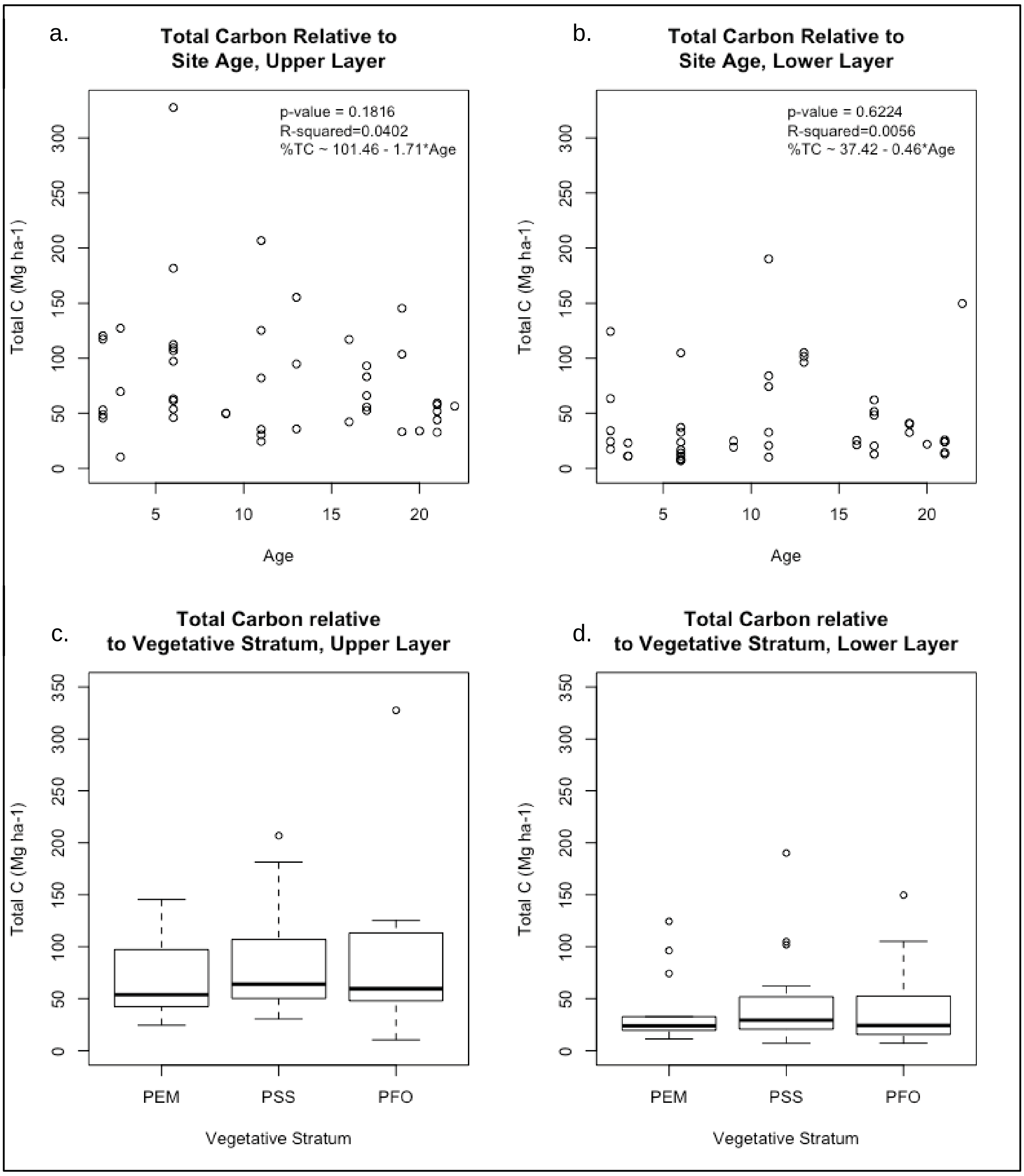

Figure 5. Total C relative to age in the upper (a.) and lower (b.) soil layers, with combined vegetative strata (n = 46; CI = 95%). Box whisker plots of total C relative to vegetative strata for the upper (c.) and lower (d.) soil layers. The upper whisker represents the 75th percentile, the box represents the range of sites within the 27th to 25th percentile, and the lower whisker represents the sites within the 25th percentile of the variable for each stratum. Dark line represents the mean variable measurement, and open circles represent outliers (PEM n = 13, PSS n = 18, PFO n = 15).

Table 5. Carbon and nitrogen accumulation rates calculated for total C and total N. The 'X' designates the variables included in the calculation for total C and total N accumulations. Sample size indicates the number of sample units used in calculating accumulation rates (i.e., number of 20-22 year old sites, and number of 2 to 3 year old sites).

Vegetative stratum			Soil layer		Accumulation rate	Accumulation rate	Sample size (n)
PEM	PSS	PFO	Upper	Lower	(Mg C ha^{-1} yr^{-1})	(Mg N ha^{-1} yr^{-1})	(20-22 y.o. sites, 2-3 y.o. sites)
x	x	x	x	x	-0.70	0.004	14, 15
x	x	x	x		-1.18	-0.01	7, 8
x	x	x		x	-0.12	0.03	7, 7
x			x	x	-2.39	-0.10	2, 4
	x		x	x	-0.70	0.003	4, 6
		x	x	x	-0.64	-0.31	7, 5
x			x		-2.51	-0.10	1, 2
x				x	-2.26	-0.13	1, 2
	x		x		-1.44	-0.02	2, 3
	x			x	0.03	0.05	2, 3
		x	x		-1.53	-0.05	4, 2
		x		x	0.58	0.07	4, 2

Nitrogen concentrations were greater in the upper soil layer than the lower soil layer (Figures 6a and 6b). The mean percent N in the upper soil layer was 0.53, with a range of 0.17 to 1.35 percent (Table 4). In the lower layer, the mean percent N was 0.24 and had a range of 0.02 to 0.55 percent. Neither vegetative strata type nor age had a statistically significant relationship with percent N present relative to either soil layer. Soil N generally decreased with age in both the upper and lower soil layers when considering all strata together. As with C, there was greater variance in N concentrations relative to upper layer soils than lower layer soils when considering strata individually (Figures 6c and 6d). When percent N was evaluated by stratum, concentrations tended to decrease with increasing age in both the upper and lower soil layers, with the exception of lower layer soils associated with forested sites (Figures 7a and 7b). In this case, N increased slightly over the 20-year period. However, none of these relationships with N as a response variable were statistically significant. Total N increased over time in upper layer soils at emergent sites, but decreased at scrub-shrub and forested sites when strata were considered individually (Figures 7c and 7d). The inverse relationship was found in lower layer soils: total N decreased at emergent sites and increased at scrub-shrub and forested sites.

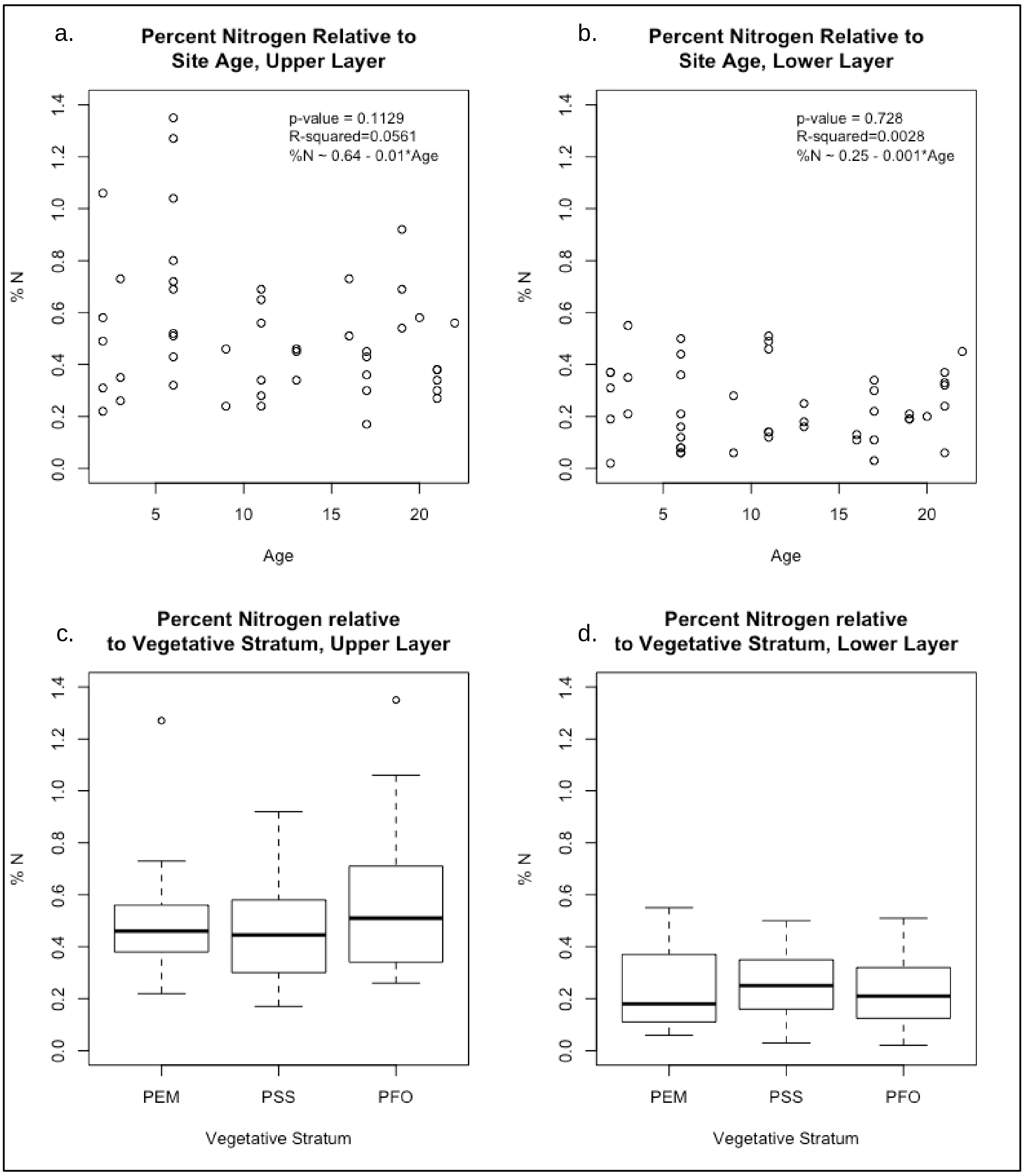

Figure 6. Percent N relative to age in the upper (a.) and lower (b.) soil layers, with combined vegetative strata (n = 46; CI = 95%). Box whisker plots of percent N relative to vegetative strata for the upper (c.) and lower (d.) soil layers. The upper whisker represents the 75th percentile, the box represents the range of sites within the 27th to 25th percentile, and the lower whisker represents the sites within the 25th percentile of the variable for each stratum. Dark line represents the mean variable measurement, and open circles represent outliers (PEM n = 13, PSS n = 18, PFO n = 15).

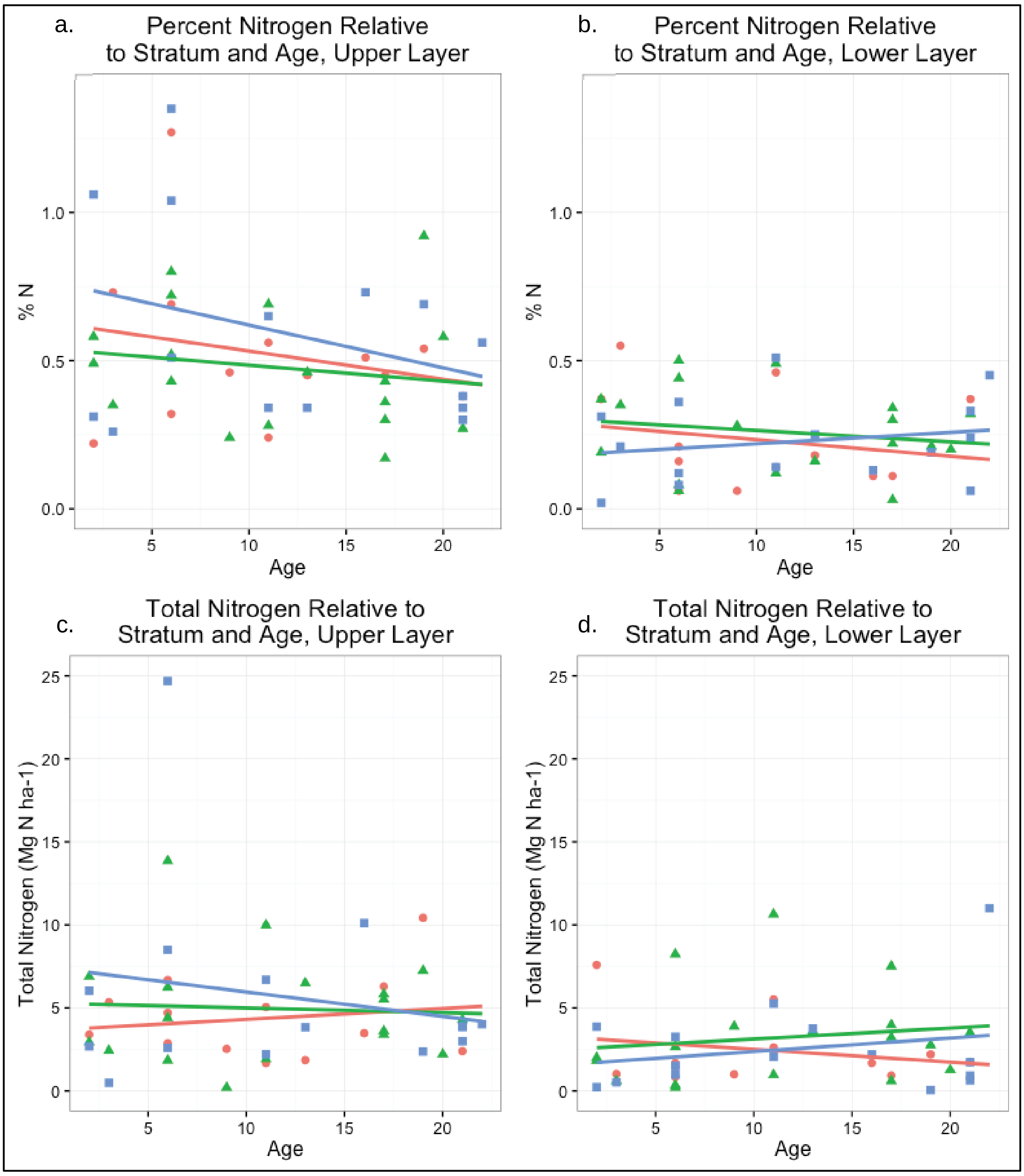

Figure 7. Percent N in upper (a.) and lower (b.) soil layers relative to age and vegetative stratum; and total N in upper (c.) and lower (d.) soil layers relative to age and vegetative strata. Red circles and red fitted line = emergent sites (n = 13); blue squares and blue fitted lines = scrub-shrub sites (n = 18); green triangles and green fitted line = forested sites (n = 15).

As with percent N, there was greater variation in total N concentrations relative to upper layer soils than lower layer soils when considering strata individually (Figures 8a and 8b). Emergent sites associated with lower layer soils exhibited the least variation in total N concentrations. Overall, total N concentrations were higher in the upper layer than in the lower layer, but exhibited a negative relationship with age in the upper layer soils and a slightly positive relationship with age in the lower layer soils (Figures 8c and 8d).

The N accumulation rate for all strata considering both upper and lower soils was estimated at 0.004 Mg N ha^{-1} yr^{-1}. As with C, the N accumulation rate varied by strata and soil layer, but overall saw several more positive accumulation rates than with C. These increases in N were all associated with the lower layer soils or a combination of both soils (Table 5). This pattern is mimicked in the upper and lower soil layers when all strata are combined: upper level soils have a negative accumulation rate while lower level soils have a slightly positive accumulation rate. Though provided, the other N accumulation rates are based on sample sizes too small to support statistically viable results (Table 5).

The C:N ratios generally decreased over the 20-year chronosequence at the study sites. This was true for C:N ratios relative to both the upper and lower soil layers, regardless of whether vegetative strata were combined or considered individually (Figures 9a and 9b; 10a and 10b). Considering all strata, the range for C:N ratios in lower layer soils was 6.67 to 75.50 ($\bar{x}$ = 17.95); while in the upper layer soils, the range was limited to between 11.66 and 26.38 ($\bar{x}$ = 17.14). The lowest mean C:N ratio was associated with lower layer soils at emergent sites while the highest was associated with lower layer soils at scrub-shrub sites (Table 4).

Significant relationships were found in several models with C:N ratio as the response variable:

- With age relative to upper layer soils when combining all strata ($p = 0.001$, $r^2 = 0.23$; Figure 10a);
- With age when considering both soil layers together and combining all strata ($p = 0.007$, $r^2 = 0.07$; Figure 10c); and
- With age relative to upper layer soils at emergent sites ($p = 0.021$, $r^2 = 0.40$; Figure 10d).

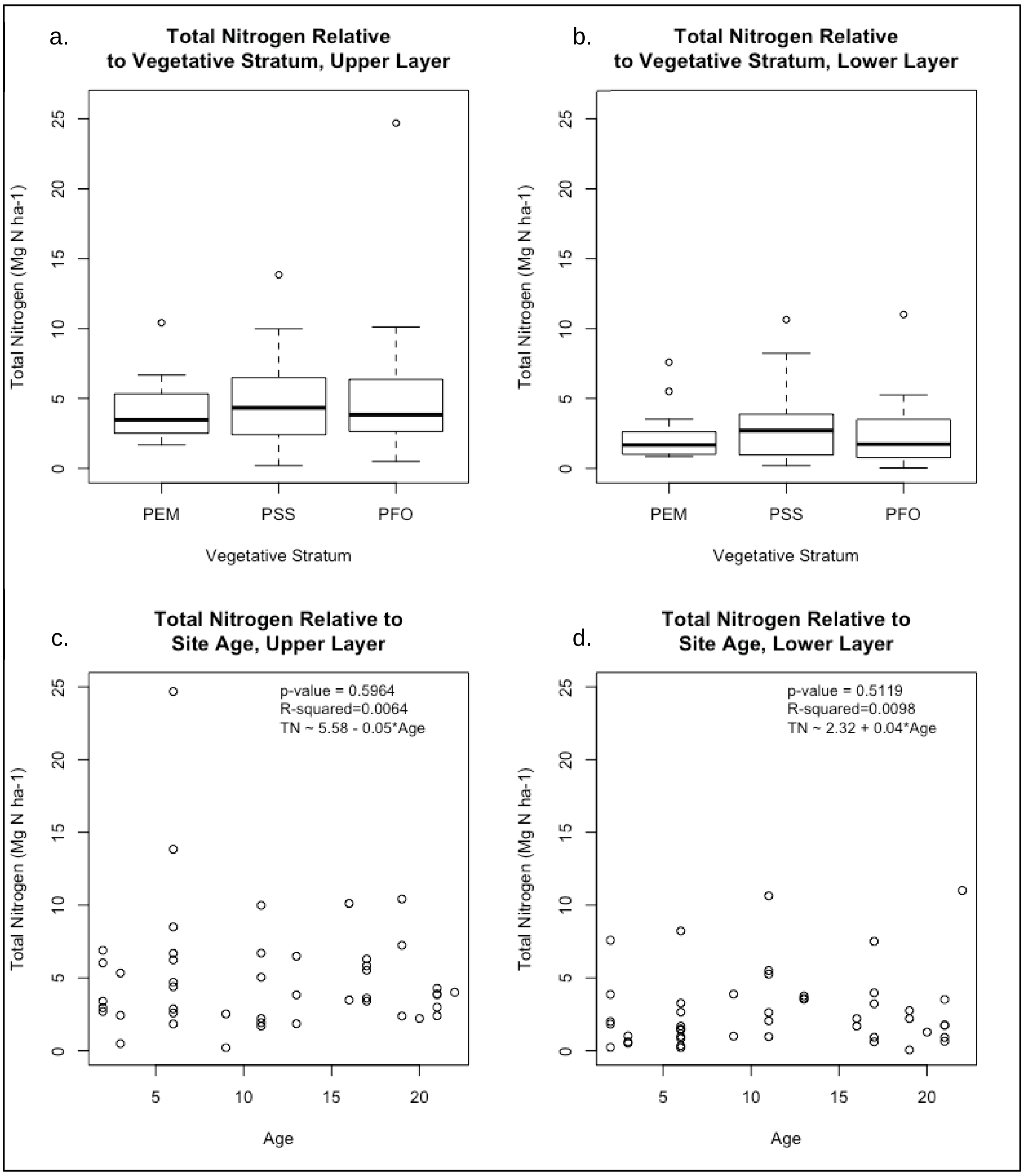

Figure 8. Box whisker plots of total N relative to vegetative strata for the upper (a.) and lower (b.) soil layers. The upper whisker represents the 75th percentile, the box represents the range of sites within the 27th to 25th percentile, and the lower whisker represents the sites within the 25th percentile of the variable for each stratum. Dark line represents the mean variable measurement, and open circles represent outliers (PEM n = 13, PSS n = 18, PFO n = 15). Total N relative to age for the upper (c.) and lower (d.) soil layers, with combined vegetative strata (n = 46; CI = 95%). No significant p-values (≤ 0.05) were found.

While other models tested with C:N as the response variable did not exhibit significant relationships, they did exhibit the same negative relationship between C:N ratio and increased age for each vegetative stratum when delineated by soil layer (Figures 10a and 10b).

There was greater variance in the C:N ratios in lower layer soils relative to stratum than in the upper layer soils (Figures 11a and 11b).

Bulk density

Overall, bulk density ranged from 0.12 to 1.45 g cm^{-3} across all vegetative strata and ages and generally increased with increasing age in both upper and lower layer soils when all strata were considered together (Figures 12a and 12b). There was larger variation of bulk density per stratum relative to the upper layer soils than to the lower layer soils; the lower layer soils exhibited smaller ranges of variation relative to stratum (Figures 12c and 12d). When data were separated by stratum, bulk density decreased over time at emergent sites and increased over time at forested and scrub-shrub sites (Figures 13a and 13b). The relationship was only significant between bulk density and age for the forested stratum in the upper soil layer ($p = 0.029$, $r^2 = 0.34$; Figure 14).

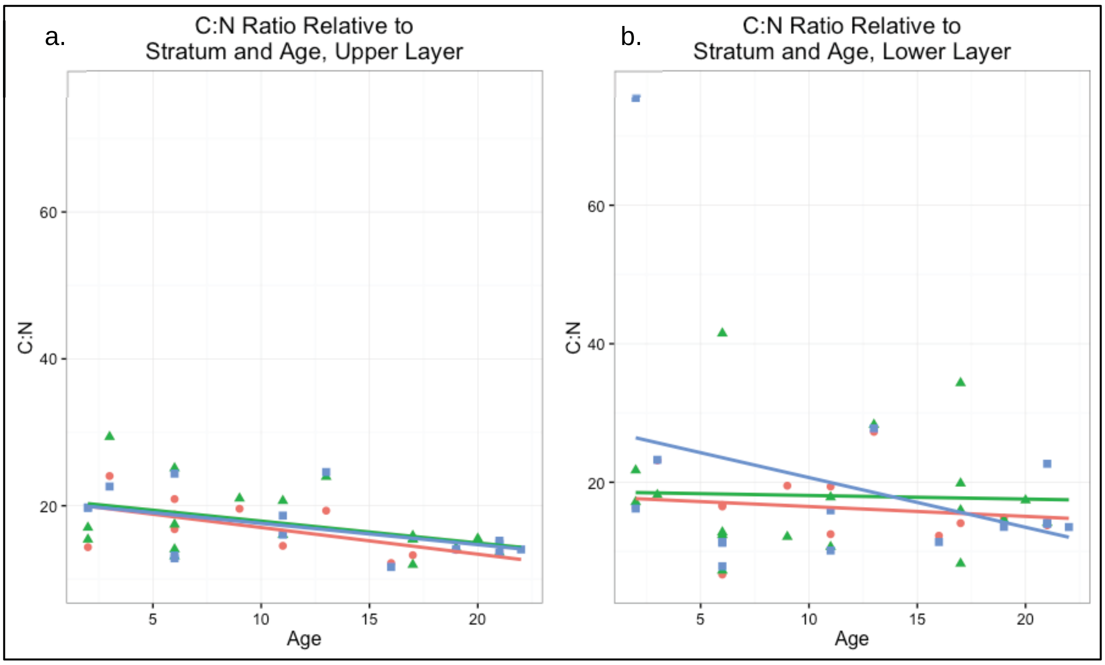

Figure 9. C:N ratio of upper (a.) and lower (b.) soil layers relative to age and vegetative strata. Red circles and red fitted line = emergent sites (n = 13); blue squares and blue fitted lines = scrub-shrub sites (n = 18); green triangles and green fitted line = forested sites (n = 15).

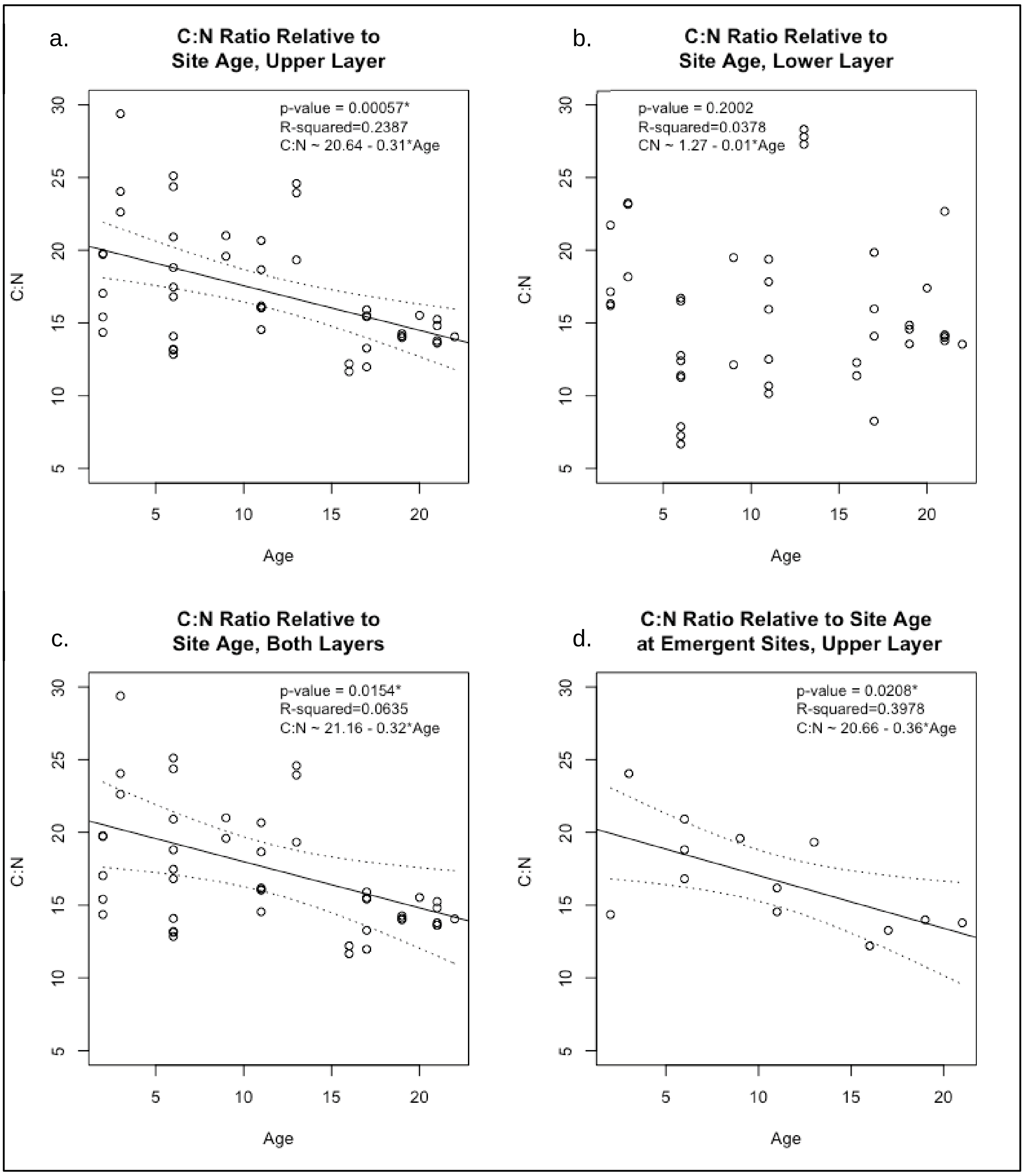

Figure 10. C:N ratio relative to age in the upper (a.) and lower (b.) soil layers, with combined vegetative strata (n = 46; CI = 95%). Significant relationships between C:N ratio and age relative to age across both soil layers (c.), and relative to age at emergent sites in the upper soil layer (d.). '*' denotes significant p-values (≤ 0.05); solid lines = regression lines fitted to model with significant p-values; dotted lines = confidence intervals.

Forested plots also had the lowest mean bulk density of the three strata relative to both upper and lower soil layers (Table 4). No significant relationships were found for bulk density relative to the emergent or scrub-shrub stratum, regardless of whether soils layers were combined or considered separately.

Influences of wetland attributes on carbon and nitrogen pools

The results from the ANOVA tables for each of the tested models indicate there were no statistically significant relationships between soil type, hydrologic regime, or hydrogeomorphic class and any of the tested response variables. The type of vegetative stratum was also not a statistically significant predictor for any of the response variables ($p > 0.05$; Appendix C). However, the soil layer was statistically significant in most of the full models, and became a critical distinction in the data analysis (Appendix C). As a result of splitting data into two different data sets, one for upper and one for lower soil layers, five models were determined to best fit the data (Table 6).

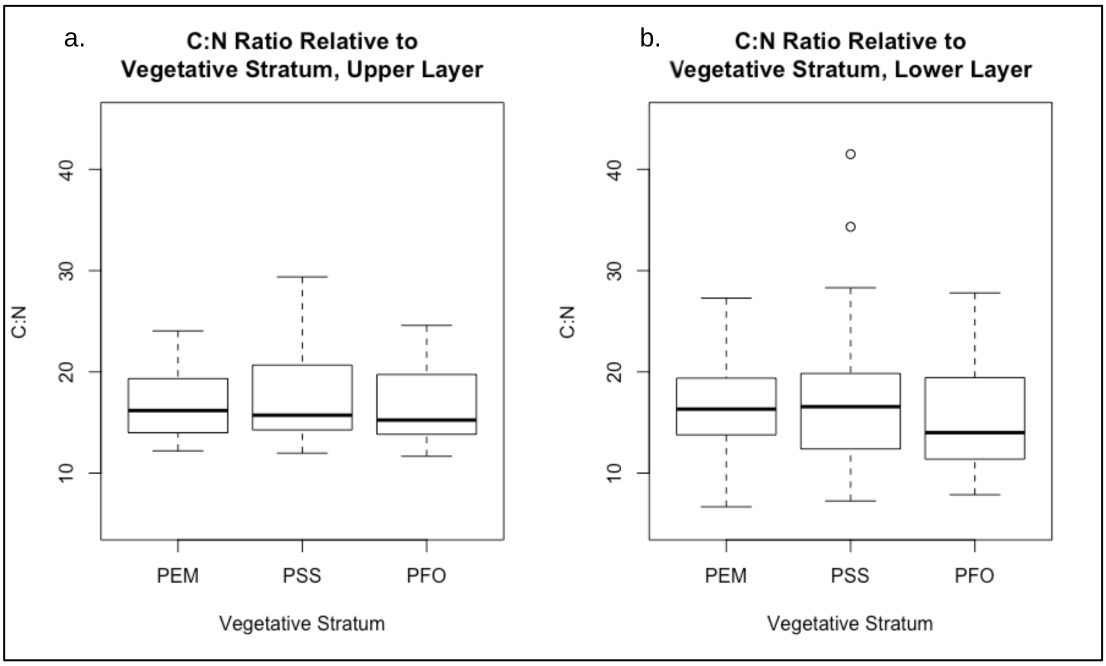

Figure 11. Box whisker plots of bulk density relative to vegetative strata in the upper (a.) and lower (b.) soil layers. The upper whisker represents the 75th percentile, the box represents the range of sites within the 27th to 25th percentile, and the lower whisker represents the sites within the 25th percentile of the variable for each stratum. Dark line represents the mean variable measurement, and open circles represent outliers (PEM n = 13, PSS n = 18, PFO n = 15).

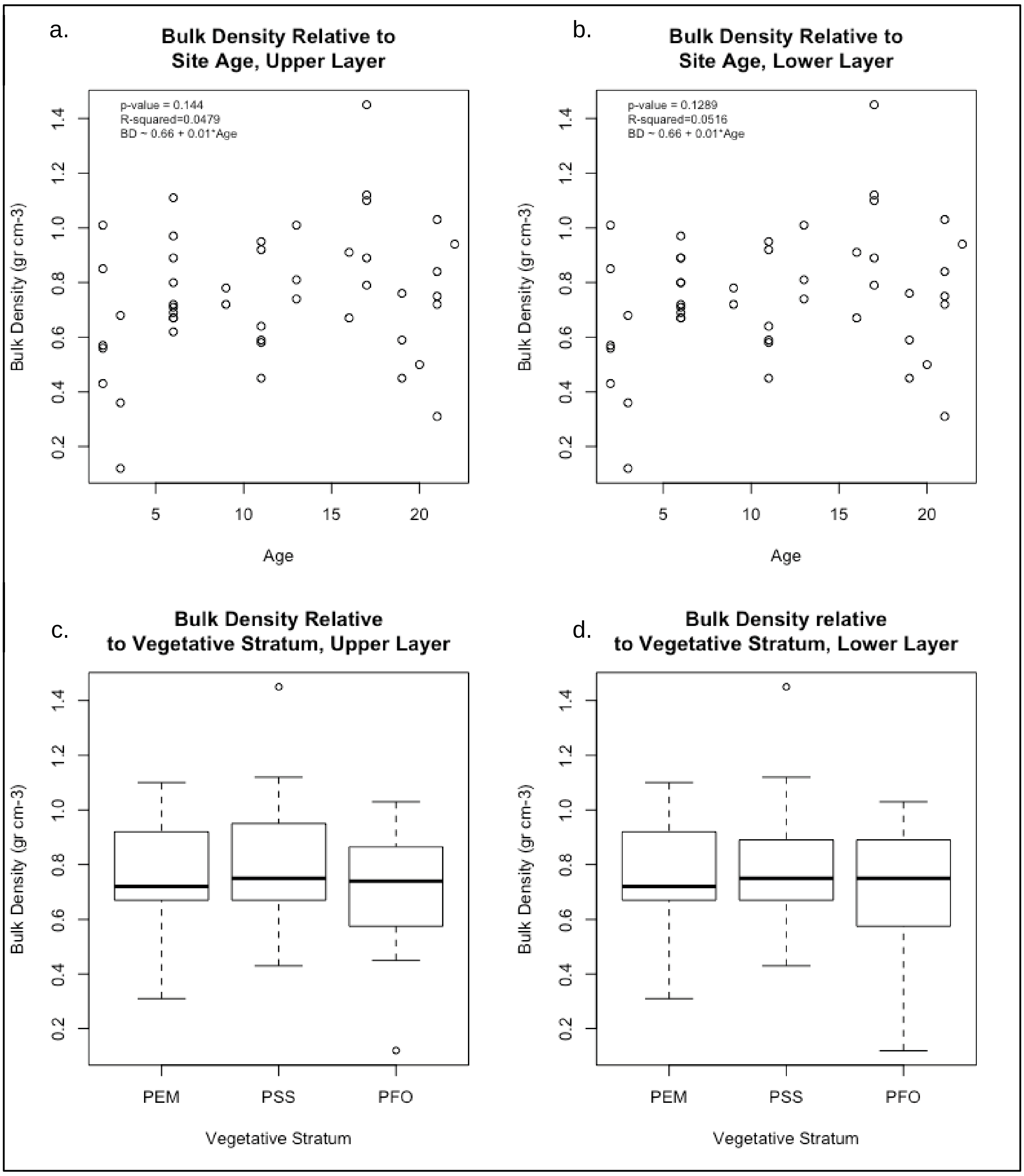

Figure 12. Bulk density in the upper (a.) and lower (b.) soil layers relative to age, with combined vegetative strata (n = 46; CI = 95%). No significant p-values (≤ 0.05) were found. Box whisker plots of bulk density relative to vegetative strata in the upper (c.) and lower (d.) soil layers. The upper whisker represents the 75th percentile, the box represents the range of sites within the 27th to 25th percentile, and the lower whisker represents the sites within the 25th percentile of the variable for each stratum. Dark line represents the mean variable measurement, and open circles represent outliers (PEM n = 13, PSS n = 18, PFO n = 15).

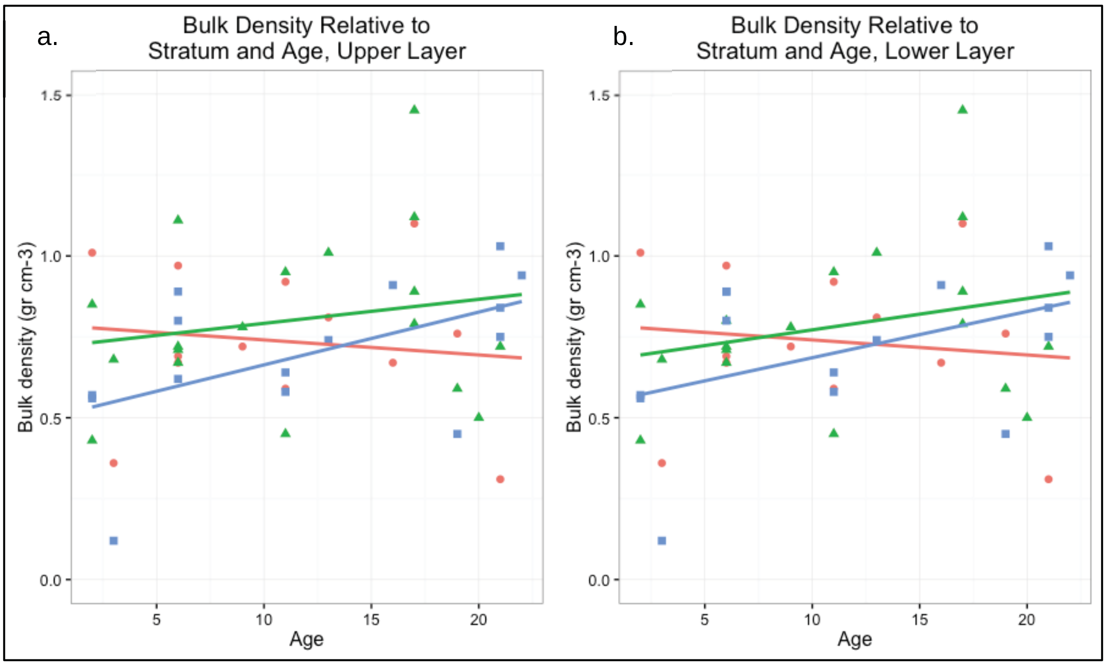

Figure 13. Bulk density of upper (a.) and lower (b.) soil layers relative to age and vegetative strata. Red circles and red fitted line = emergent sites (n = 13); blue squares and blue fitted lines = scrub-shrub sites (n = 18); green triangles and green fitted line = forested sites (n = 15).

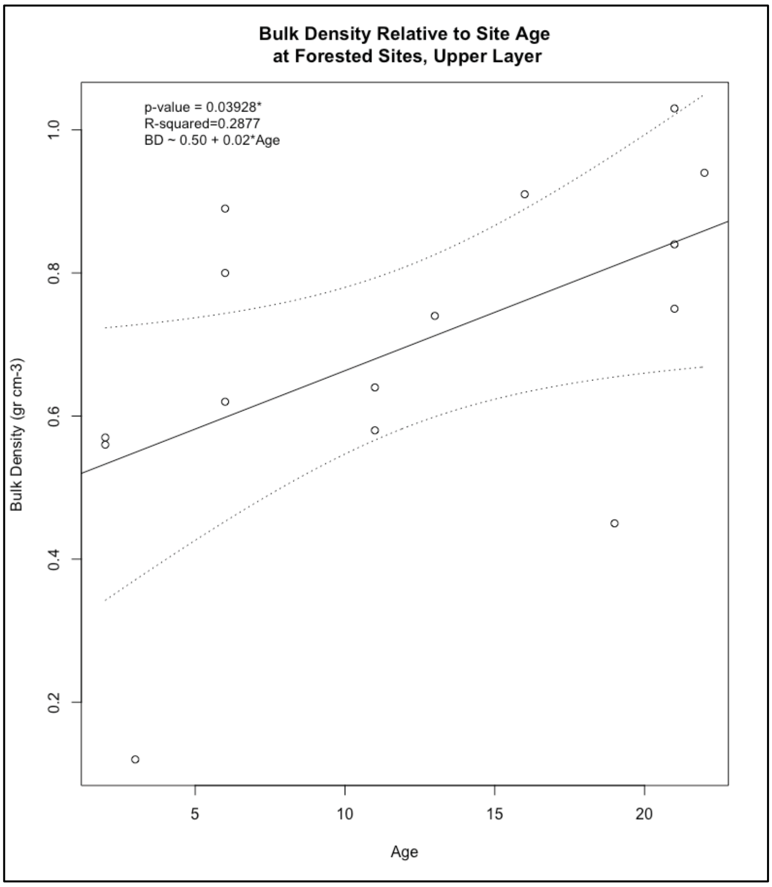

Figure 14. Significant relationship between bulk density of upper layer soils relative to age at forested sites ($p \leq 0.05$; CI = 95%, n = 15). Solid line = regression lines fitted to model with significant p-value; dotted lines = confidence interval.

Table 6. Fitted models of statistically significant relationships between analyzed response variables and predictor variables (SE = residual standard error; DF = degrees of freedom).

Fitted model	p-value	r^2	SE	F-statistic	DF
%C ~ 12.20 – 0.29Age *(all strata, upper soil layer)*	0.0028	0.186	4.104	10.06	44
C:N ~ 21.16 – 0.32Age *(all strata, both soil layers)*	0.0154	0.063	8.107	6.101	90
C:N ~ 20.64 – 0.31Age *(all strata, upper soil layer)*	0.0006	0.239	3.685	13.79	44
C:N ~ 20.66 – 0.36Age *(emergent stratum, upper soil layer)*	0.0208	0.399	2.863	7.266	11
BD ~ 0.46 + 0.02Age *(forested stratum, upper soil layer)*	0.0393	0.288	0.201	5.250	13

Fitting models with separate data sets for upper and lower soil layers indicated two significant relationships between vegetative strata and a response variable, one relative to C:N ratio and one relative to bulk density. In the first relationship, C:N ratios decreased in upper soil second relationship, bulk density increased over time in upper layer soils at forested sites (Figure 14). None of the proposed interactions were found to be statistically significant, regardless of whether the data was split by soil layer (Appendix C). All significant models had relatively low r^2 values, and so were not considered to be a strong indicator of prediction.

Discussion

Carbon and nitrogen pools

The ranges for percent C and N, total C, C:N ratios, and bulk density that were calculated as part of this study are consistent with ranges of these variables presented in the literature for wetland soils in both natural and restored wetlands (Bowden, 1987; Bridgham et al., 2006; Mitsch & Gosselink, 2007; Ballantine et al., 2012). layers over time at emergent sites (Figure 10d). Ballantine et al. (2012) studied vegetative development relative to soil properties, and reviewed multiple studies of chronosequences of restored wetlands. As such, the Ballantine et al. study provides a useful tool for comparing the study results to peer-reviewed research (Table 7).

Table 7. Literature comparison for attributes of created and natural wetlands, reproduced from Ballantine et al. (2012). Brackish and salt marsh sites were removed from the table (Amendments = soil amendments, No. WL = number of wetlands included in study. C:N ratio calculated by C. Kroe based on organic C and Total N.

Study	Wetland type	Location	Age	Amendments	No. WL	Depth (m)	Organic C (g kg^{-1})	Total N (g kg^{-1})	Bulk density (g cm^{-3})	C:N ratio
Ahn and Peralta (2009)	palustrine shrub/scrub	Virginia	1,5,8	0.2 m topsoil	3	0.1	15.8	1.4	1.01	11.29
Ballantine and Schneider (2009)	palustrine emergent	New York	3–5	no			30.3		1.10	
Bishel-Machung et al. (1996)	slope, riverine, depression, fringe	Pennsylvania	1–8	sometimes w/0.1–0.3 m topsoil	44	0.05	31.0	1.1	1.15	28.18
Bruland and Richardson (2006)	headwater riverine, mainstem riverine, non-riverine mineral soil flat, non-riverine organic soil flat	North Carolina	3–9	not specified	11		59.0			
Bruland et al. (2009)	non-tidal forested	Virginia	5	pre-amendment	1	0–0.2	11.4	0.8	1.25	14.25
Campbell et al. (2002)	palustrine emergent	Pennsylvania	2–10	not specified	12	0.05	24.0		1.20	
Card et al. (2010)	prairie potholes	Saskatchewan, Alberta	1–3	no	28	0.06	47.60			
Fennessy et al. (2008)	created, emergent marshes	Ohio	1–9	not specified	10	0.1	24.0	2.6	1.75	9.23
Fennessy et al. (2008)	comparable natural wetlands	Ohio	natural	natural	9	0.1	75.5	11.3	0.72	6.68
Galatowitsch and vanderValk (1996)	emergent wet meadow Minnesota	Iowa	3	not specified	10		38.3		0.90	
Hogan et al. (2004)	palustrine emergent	Maryland	5,5,12	not specified	3	0.13	12.0		1.10	
Hogan et al. (2004)	palustrine forest	Maryland	natural	natural	3	0.13	57.0		0.90	
Hossler and Bouchard (2010)	palustrine emergent	Ohio	3–8	1 w/wetland soil	5	0–0.05	0.2–0.45			
Hossler and Bouchard (2010)	palustrine emergent	Ohio	natural	natural	4	0–0.05	0.5–2.4			
Mitsch and Gosselink (2000)	organic wetland soil		natural	natural			120–200		0.2–0.3	
Nair et al. (2001)	phosphate-reclaimed wetlands	Florida	1–16	not specified	5	0.1	0.56	0.04	0.70	14.00
Nair et al. (2001)	adjacent native wetlands	Florida	natural	natural	5	0.1	1.93	0.09	0.40	21.44
Shaffer and Ernst (1999)	freshwater palustrine	Oregon	5	not specified	50	0.05	29.2		1.1–1.6	
Stauffer and Brooks (1997)	not specified, mitigation site	Pennsylvania	1	unamended control plots	1	0.15	4.5	0.5		9.00
Stauffer and Brooks (1997)	not specified, mitigation site	Pennsylvania	1	salvaged marsh surface soil	1	0.15	27.5	2		13.75
Stolt et al. (2000)	palustrine forested shrub/scrub	Virginia	4–7	not specified	3	0.05–0.15	0.093			
Taylor and Middleton (2004)	coal slurry pond	Illinois	5	no	1	0.05–0.15	6.0			
Ballantine et al. (2012)	palustrine emergent	New York	3	control, straw, topsoil, mix, biochar	4	0.1	23.02	1.2	1.42	19.18

Note: Adapted from Ballantine et al. 2012. "Soil properties and vegetative development in four restored freshwater depressional wetlands." Soil Science Society of America Journal 76(4): 1482-1495.

Carbon

The overall trend with the present study results was for small reductions in both percent carbon and total carbon over time (Figures 3c and 3d; 5a and 5b). These small reductions did not result in wetland soils that were outside the range of expected values for soil C and N (Table 7), but the trends are interesting and unexpected. The overall decreases in C accumulation found as part of my study are in contrast to my original hypothesis, that C accumulation rates would be discernible and related to one or more predictor variables. They are also largely contrast with published studies, which typically have shown increases in C accumulation in restored, freshwater wetlands over time (Anderson & Mitsch, 2006; Badiou et al., 2011; Larkin et al., 2014).

Despite the contrast between my study and other studies regarding C accumulation rates, the C concentration values in my study (2.64 to 21.27 mg C gr-1) are consistent with those in Ballantine et al. (2012; Table 7). Excluding salt marshes and sites with organic soils, these studies had a range from 0.09 to 75.5 g C kg-1. The range of C recorded as part of my study, however, was notably narrower than the range presented in Ballantine et al. (2012) even with the exclusion of studies that focused on salt marshes and organic soils (Table 7).

Why are constructed wetlands losing C in surface soils over time in the present study? To answer, we must interpret the decline in soil C over time in a statistical context. The sole statistically significant model with percent C as the response variable has a low r-squared (0.186). This indicates that only 19% of the variability of the response variables (% C) was explained by age. The dynamics of ecosystem C loss over time following disturbance may explain some of these results. Huang et al. (2013) found that there might be a short-term loss of C concentration (as well as N) associated with freshwater marshes before C begins to accumulate again due to natural processes. This is a classic pattern in soil C dynamics in managed forests referred to as the "Covington Curve" (Covington, 1981; Yanai et al., 2003). If this were the case with the current study, I may have expected to observe a curvilinear regression in relation to C accumulation over time. If this dynamic is present, there was no clear indication of a curvilinear pattern in the soil C data.

Nitrogen

I anticipated that percent N and total N would increase over time, but this pattern was not uniformly present. Percent nitrogen and total nitrogen were not significantly correlated to any predictor variables. Overall, the examined sites showed an increase in percent N (mg g^{-1}) and of total N (Mg N ha^{-1}) when all vegetative strata and both soil layers were considered. Total nitrogen appeared to increase in the lower soil layers relative to both scrub-shrub and forested communities, but decreased relative to emergent communities (Table 5; Figure 7d). The increased biomass production of older forested and scrub-shrub communities may support the storage of N in the soil. However, there were no statistically significant relationships between N and any of the examined independent variables.

The range of soil N included in Ballantine et al. (2012) was 0.04 to 11.3 g N kg^{-1}, which was substantially broader than the range of N found in my study (0.02 to 0.55 mg N gr^{-1}). Excluding the 'natural' wetlands, the highest N soil level recorded in Ballantine et al. (2012) is 2.6 g N kg^{-1} (Table 7), which is still almost five times greater than the highest soil N value recorded at my study sites. Though the sites included in my study clearly have a narrower range of soil N, it is unclear why this may be the case. Nitrogen found in wetland soils is naturally several magnitudes lower when compared to C, and the nutrient is a primary limiting factor in the biogeochemistry of wetland soils, and its level tends to increase in the soils of restored wetlands in concert with C (Mitsch & Gosselink, 2007). Organic soils often have less N available to plants, as it is in organic rather than inorganic form. However, N has multiple paths both into and out of the wetland soils via denitrification (loss) and mineralization (gain), and can also be affected by hydrology, temperature, and hydrologic regime, creating a complicated web of N sinks and sources (Bowden, 1987; Sleutel et al., 2008; Noe et al., 2013).

I did not observe any positive, significant relationships between N and any predictor variables as part of my study. Sutton-Grier et al. (2009) found that increased soil organic matter results in increased availability of N in wetland soils, and determined it to be a short-term affect. They found decreases in N and soil organic matter over the long-term, indicating that soil amendments added as part of their study only contributed to nutrient increases for a few years. One potentially critical difference between my study and the Sutton-Grier et al. study is hydrogeomorphic class: I focused depressional systems whereas their study focused on a single riparian (riverine) wetland. This may indicate that the overall hydrogeomorphic class (e.g., depressional, riverine, slope) plays a larger role in N accumulation. Conversely, the overall trend in my study was for total N (Mg N ha^{-1}) to increase slightly over time (Table 5). However, given the lack of statistical correlation between soil N and the independent variables I examined, it is difficult to determine not so much *how*, but *why* the amount of N in the system changes as it does over time.

C:N ratios

Of the five statistically significant relationships resulting from my study, three of them were related to C:N ratio (Table 6). Sites included in my study include a broad range of C:N ratios (6.67 and 41.5), indicating there is a mix of both immobilization and mineralization occurring at the sites. In looking at individual C:N ratios for sample sites, the widest C:N ratios were recorded in the lower soil layer (Appendix B). There were also several outliers for C:N ratios in the lower layer, and it is possible that these have inadvertently skewed the lower layer data. The removal of these outliers from the dataset did not substantially improve any of the models tested with C:N ratio as the response variable; it only reduced the mean a small degree. Wang et al. (2016) also found higher C:N ratios in the lower soil layers of wetlands with an approximate depth of 30 to 40 cm; higher N amounts were found in the upper 10 cm. However, Wang et al. focused on coastal wetlands, which are subjected to different nutrient influxes due to their landscape position. Interestingly, though, Ma et al. (2012) also found that the C:N ratio in freshwater reed wetlands decreased with soil depth. This indicated that freshwater influx in the deeper soils causes decomposition rates to slow because of reduced microbial activity. These two studies could provide explanations for the overall higher C:N ratios and downward trends associated with lower layer soils in my study. This, however, does not explain the reduced C:N ratios over time.

Microbes in wetland soils strive to maintain a biomass C:N ratio of biomass of approximately 10 as a steady state through the constant processes of ammonification (conversion of organic N to ammonium) and immobilization (conversion of inorganic N to organic N) (Reddy & DeLaune, 2008). This ‘balance’ is affected by multiple conditions, including influxes of C in the form of plant detritus, detritus, and other external inputs such as hydrology. Most of the sample sites had a C:N ratio less than

25 (n = 83), which is a critical threshold for C:N ratios. In aerobic conditions, microbes will assimilate N through immobilization if the C:N ratio is greater than 25. If the ratio is less than 25, they will release inorganic N via ammonification (i.e., detrital decomposition) (Reddy & DeLaune, 2008). Considering that most of the sample sites in my study had a C:N ratio less than 25, mineralization appears to be occurring more often at the sites included in my study.

The trends of C:N ratios over time found in this study correspond with the expected trend for C:N in wetland soils: a decrease in C:N ratio over time, toward the ideal ratio of 10. Recent studies reflect only a slightly narrower range of C:N ratios and age than the present study (Table 7) (Inglett & Inglett, 2013). While there was a general negative relationship between C:N ratio and age across all strata and both soil layers, only one stratum had a statistically significant relationship to C:N ratio: emergent sites associated with upper layer soils ($p = 0.020$, $r^2 = 0.39$; Figure 10d). Despite somewhat consistent trends in this study for these two significant relationships, these analyses should be considered with caution given the small sample size on which it was based (n = 13).

Bulk density

As with C and N studies, the overall temporal trends of bulk density in my study were contrary to previously published studies of both restored and natural wetlands. Previous studies indicate that bulk density largely decreases as sites age, indicating an increased input of organic material into the wetland system, which helps to increase soil porosity, water retention, and therefore plant survival (Mitsch & Gosselink, 2007). Both the forested and scrub-shrub communities included in my study showed a general trend in increased, rather than decreased, bulk density over time (Figures 13a and 13b). Only one positive, significant relationship was noted, between bulk density and age at forested sites relative to upper layer soils (Figure 14; Table 6). While it is possible that this is due to increased input of organic material from forested canopies, I did not measure biomass input at the sites and so any correlation between bulk density and biomass on the examined sites is unknown.

The bulk density results from my study were on average slightly lower (range 0.12 to 1.45 g cm^{-3} across all strata and soil layers) than the range of the studies summarized by Ballantine et al. (2012) (0.7 to 1.75 g cm^{-3}). However, another study found lower ranges in wetland restoration sites, between 0.46 and 0.82 g cm^{-3} in the upper soil layer (Inglett & Inglett, 2013). Soils with a lower bulk density (~ 0.2 to 0.3 g cm^{-3}) are typical of well-decomposed organic soils, while mineral soils have a bulk density that ranges between 1.0 and 2.0 g cm^{-3} (Mitsch & Gosselink, 2007). Of the sites included in my study, only one sample site, the forested community at the SR 005 Corrington site, had a bulk density less than 0.30 g cm^{-3} (0.12 g cm^{-3}). This may be explained by the fact the site is located in an area mapped with both mineral and organic soil series (Table 1). Although the percent C and total C amounts for the SR 005 Corrington site's forested community aren't unusually high relative to the other sites in my study (% C = 4.88; total C = 11.41 Mg C ha^{-1}), the bulk density could either be because the site was successfully constructed to mimic natural bulk density levels, or that historic soil conditions have influenced bulk density. It is also a very young site, and it is possible that too much soil amendment was collected, misrepresenting the soils on the site.

Influences of wetland attributes on carbon and nitrogen pools

Although several statistically significant relationships were found, vegetative strata, hydrology, hydrologic regime, and soil type were not effective in explaining C or N accumulation rates. With regard to both N and C, nutrient levels found in the soils are only small portions of the overall budgets for each constituent. Both nutrients enter and leave wetlands through multiple aquatic and atmospheric processes, and are subjected to multiple biochemical transformations. Plants are also critical factor in the C and N cycling in wetlands, and their influence on the amount of these nutrients found in the soil cannot be discounted in the overall analysis of soil pools. The scope of my study was limited to C and N levels in the soils, and did not consider input and outputs, which would better reflect the overall cycling of nutrients in the system.

My study showed a general increase of bulk density with time. While this condition is not necessarily consistent with natural wetlands, it is a trend that has been found to be consistent for disturbed wetlands and wetlands with overall loss of C and organic matter (Ballantine et al., 2012). Wetland restoration often involves construction with heavy equipment as well as the removal of the upper soil layers during the grading process. Heavy equipment can compact soils and create higher bulk density; if soils are not properly scarified at the end of site grading the compaction will continue through site development and bulk density can remain unusually high for many years post construction. When the soils are graded during construction and the upper layers removed, the soils with the highest proportions of organics are also removed, further exacerbating and potentially increasing bulk density (Sutton-Grier et al., 2009). While not a trend found exclusively with the sites I studied ranging from 20 to 22 years of age, construction-related impact to bulk density is a possible condition present on several older examined sites, specifically at SR 405 Swamp Creek (PSS bulk density [BD] = 1.45; PEM BD = 1.10), SR 167 Mill Creek (PSS BD = 1.12), and SR 516 Big Soos Creek (PFO BD = 1.03; Appendix B). These sites range in age from 17 to 21 years. The older sites included in the study were constructed in the late 1990s, before the advent of many of today's best management practices, including improved soil amendments and construction techniques that limit soil compaction. As such, it seems that the presence of substantial soil amendments coupled with improved construction practices could potentially account for lower bulk density levels in the younger sites. However, his cannot be proven, as I did not study site construction methods and site-specific soil amendment data was not readily available.

Soil moisture (hydroperiod) did not appear to be a reliable predictor of any of the response variables (Appendix C). I also did not find that hydrogeomorphic class was statistically correlated to the response variables. These findings contrast with other studies that indicate soil moisture content can influence, and at times drive, nutrient dynamics in wetlands (Takatert et al., 1999; Mitsch & Gosselink, 2007; Sleutel et al., 2008; Maynard et al., 2011; Noe et al., 2013). The role of flow-through systems in transporting of nutrients is obvious, but perhaps not as easy to measure with categorical data. Rather than simply characterize the hydroperiod with categorical levels as I did, these other studies have measured the depth and frequency of flooding and groundwater in wetlands, sediment accumulation depths, etc. It may be that further parsing of my data into quantitative depths of water/inundated soils would reveal a statistical correlation between hydroperiod and one or more of the response variables. Transforming hydrogeomorphic class into a meaningful statistical predictor seems unlikely, though, without setting up additional experiments to study flow patterns and sediment accretion.

As with hydrologic variables, soil type did not appear to be a robust enough predictor of C, N, and bulk density. While I recorded soil texture at each sample site, I parsed the data by whether the sample site was mapped in a mineral soil series or a mixed mineral-organic soils series. Soil texture, specifically percent clay, has been shown to influence net nitrification rates and annual net ammonification rates in floodplain wetlands, and has been found to have a positive correlation with N mineralization rates (Sleutel et al., 2008; Noe et al., 2013). I did not analyze percent sand, silt, and clay as part of my study. This data may have provided more useful data regarding correlations between soil type and nutrient levels. Further research could be done on the studied restoration sites to test for correlations between soil texture and response variables.

It was surprising that there was not a more consistent correlation between vegetative stratum and the response variables over time. This lack of a pattern may be due to the processes involved in restoring or creating a wetland. Typically, WSDOT uses small (i.e., < 2 m in height) woody plants for planting tree and shrub species, rather than taller, more established plants. This method is more cost effective and makes planting a site with thousands of saplings much easier and less invasive (i.e., can be done by hand). As a result, 'forested' communities are functionally scrub-shrub communities for a number of years, until they provide a dense enough canopy and have a large enough footprint to effectually function as a tree species. With regard to emergent plantings, plugs or seedlings are often use and planted to promote rapid colonization. Still, emergent plantings are often more widely spaced during than what is found in natural wetlands, and so their aboveground biomass contributions are limited until they have established a robust root system. As a result, younger sites are limited in their ability to mimic the influences vegetative strata have on natural sites, possibly making the influence of one vegetative stratum over another indiscernible at a young age. This could especially be applicable to young forested and scrub-shrub sites, where only differences in planting palettes and not necessarily site structure, would be evident.

There are two other possible explanations for the disparity between my results and other published studies regarding C and N concentrations. First of all, fertilizer, mulch, and highly organic topsoil have been used in the construction of the study sites. The addition of these materials is intended to prevent weed growth and promote successful growth of the planted trees, shrubs and emergent vegetation. Anderson and

Mitsch (2006) noted that both N and C should increase in created riparian wetlands over time as a result of organic matter accumulation and denitrification. However, Oren et al. (2001) proposed that soil fertilization can decrease C sequestration in upland forests, and it has also been shown that different treatments, composts, or mulch types can affect C concentrations in wetland soils (Ballantine et al., 2012). Ballantine et al. (2012) also studied soil amendments added to wetland restoration sites and theorized that they can be used to decrease bulk density and increase soil health. However, the input of such highly organic substrates onto a site could falsely elevate the soil organic C amount in the system. Especially with restoration sites constructed by WSDOT, topsoil and mulch is often scarified or mixed into the upper layer of soils, which made it difficult to exclude this material from collection and analysis as a part of the upper soil layer. Both the upper and lower soil layers were examined as part of this study, and decreases in C and N were recorded in both soil layers. This indicates that soil amendments could also influence lower soil layers via downward migration through the soil profile. The influx of C into the system from high amounts of soil amendments is similar to the effect of forest clear-cutting studied in Covington (1981) and more recently reassessed by Yanai et al. (2003). Both studies detailed a substantial decrease in organic mass in temperate hardwood forests during the first 20 years following a clear-cut, after which point C began to steadily increase. This trend is known in forestry science as the ‘Covington Curve.’ The decreases in soil C had been attributed to potentially elevated levels of decomposition in the upper soil layers, as well as a loss of C from erosion and increased leaching of dissolved organic carbon. Certainly, there are substantial differences between upland forests and wetlands in terms of soil biogeochemistry, but these studies regarding clear-cuts give pause and present an interesting question about the overall impact of high levels of soil amendments on soil C in restored wetlands.

Secondly, N-fixing species are often common on restoration sites, either as volunteers that revegetated sites on their own or has intentionally included in the plantings. A common species is red alder (*Alnus rubra*), especially on a number of the older sites included in the study when it was included as part of the planting plan. Red alder is a deciduous tree species that is known as a highly productive N fixer. Stands of red alder have been shown fix N at a range of 100 to 200 kg ha^{-1} yr^{-1}, storing N in their roots until they decay and die (Binkley et al., 1994). Modern-day site restoration does not often include red alder in planting design in the Puget Sound region due to its weedy nature and high propensity for establishing itself as a volunteer species. The inclusion of high percentage of red alder on older sites could account for the higher amount of N on younger sites and lower amounts on older sites: N on older sites is stored in the alder until its eventual decay, limiting its availability. I did not, however, study the effect of individual species on soil C and N pools as part of this study.

Conclusion

The lack of a relationship between C and N and vegetative strata, soil type, or hydrologic characteristics suggests that wetlands are complex ecological systems with multiple variables that affect soil biochemistry. While few significant relationships were found in this study, the trends of decreasing C and N and increasing bulk density countered those trends found in other studies of both natural and restored wetlands. These findings were also in contrast to my original hypothesis that C and N would increase over time. They also did not provide any clear answers regarding a path for how to maximize C accumulation through wetland restoration site design. However, my results did indicate that upper and lower soil layers exhibit important differences in C and N content, and may provide insight on how soil processes differ in the two soil layers of restored wetlands.

In the United States, vegetative cover is often one of the principal drivers of restoration site design because of regulatory requirements. Adding high amounts of carbon-rich soil amendments may expedite plant establishment, allowing the site to meet performance standards quickly and be deemed successful mitigation. However, it may also have unintended consequences for soil biochemistry in the short-term, creating nitrogen-limited systems that initially deplete C and N soil pools.

Further research of freshwater wetland restoration should focus on their role in the overall carbon and nitrogen cycles. I also recommend that future studies consider pre-treatment C concentrations in soils of proposed sites, as well as C concentrations in soil amendments added to the sites. Incorporating both of these data into a study design would help establish a better baseline for studying changes in C concentrations. While wetland restoration sites may mimic natural sites in the long-term, the question as to whether soil functions and biogeochemical processes are being replaced in the short term – and how to best do it – remains unanswered.

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Appendix A: Study site maps

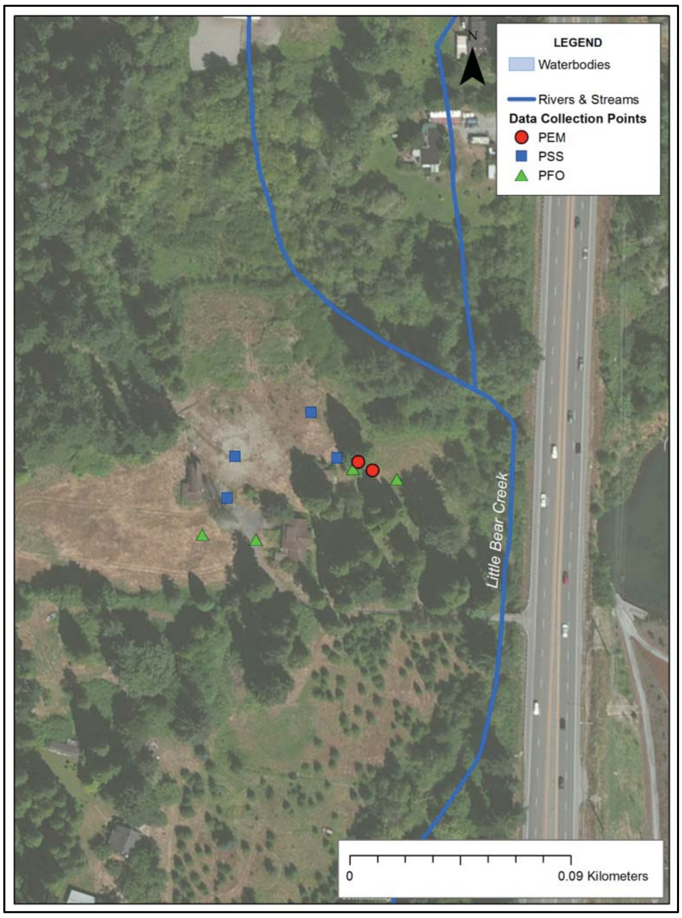

Figure A1. SR 009 Charles Plummer wetland restoration site (site nr. 1).

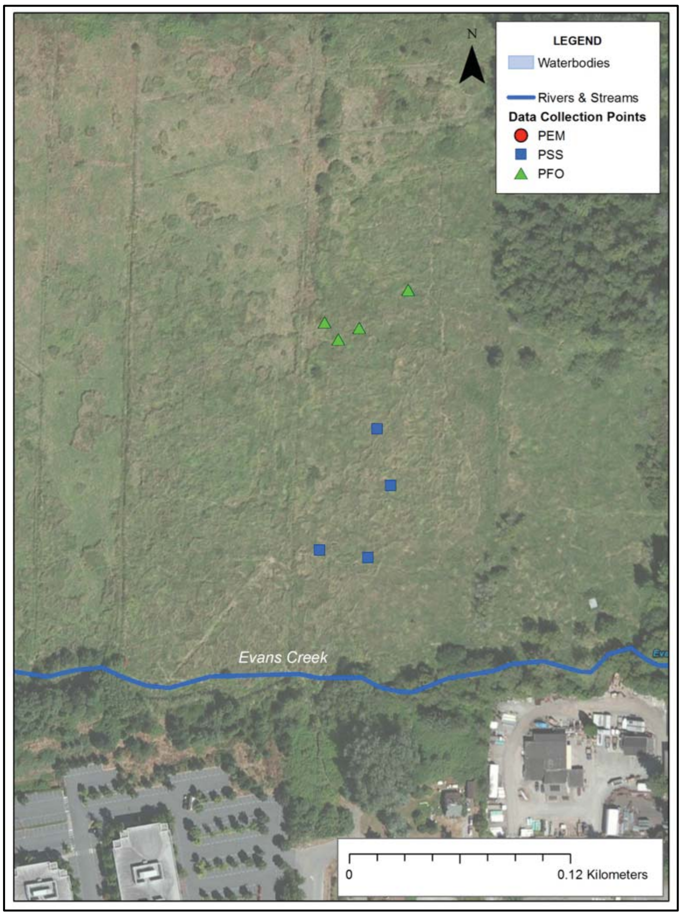

Figure A2. SR 520 Evans Creek wetland restoration site (site nr. 2).

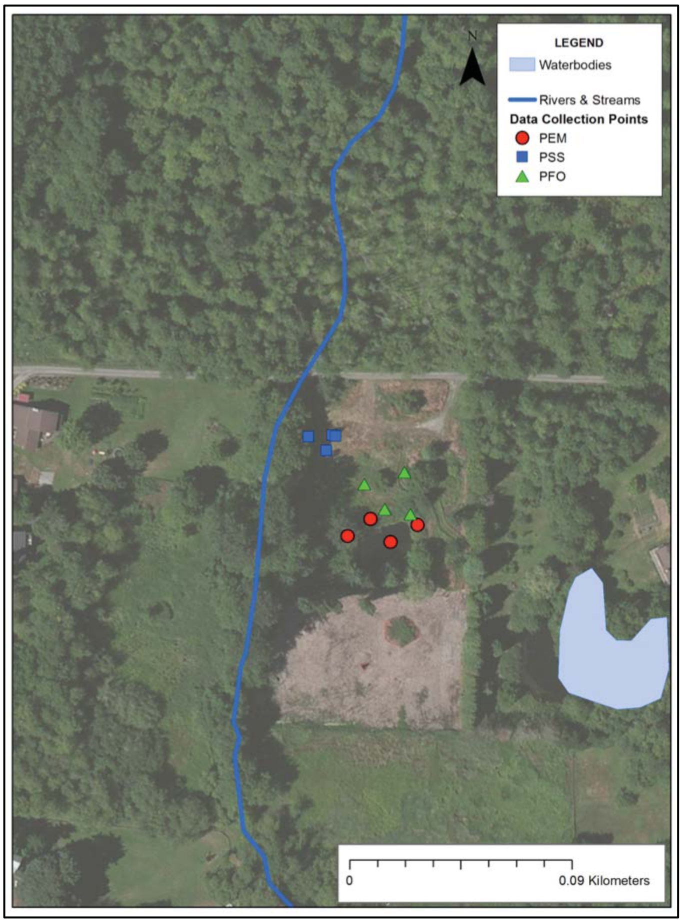

Figure A3. SR 005 Corrington wetland restoration site (site nr. 3).

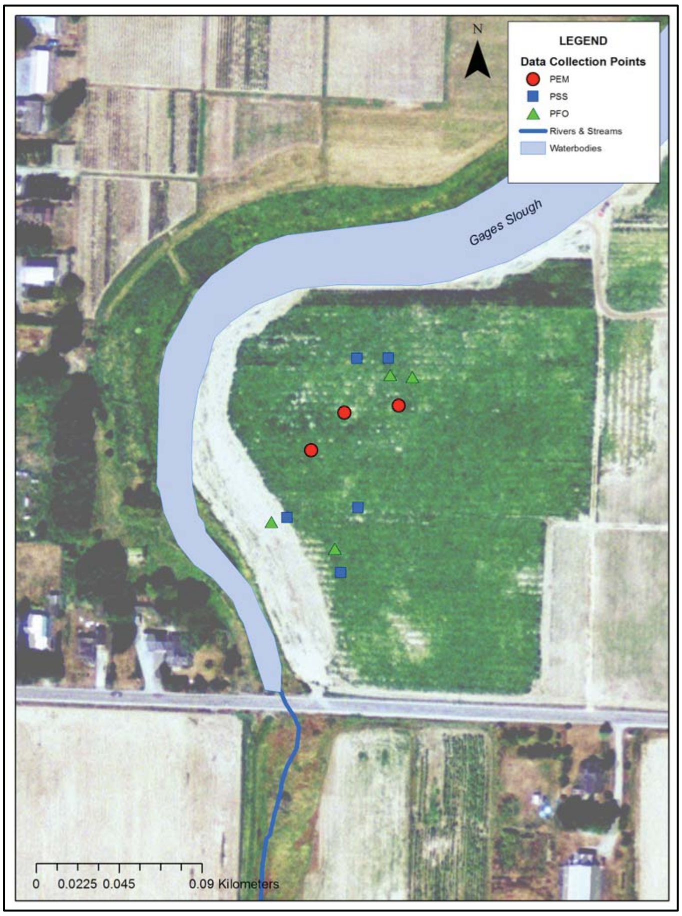

Figure A4. SR 020 Gages Slough wetland restoration site (site nr. 4).

Figure A5. SR 020 Quiet Cove wetland restoration site (site nr. 5).

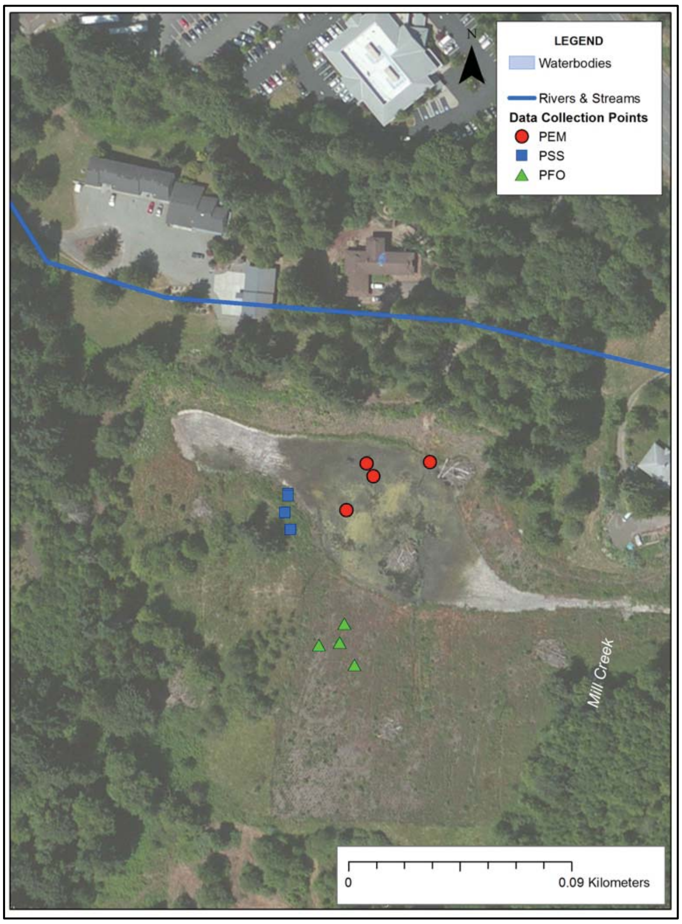

Figure A6. SR 305 Poulsbo wetland restoration site (site nr. 6).

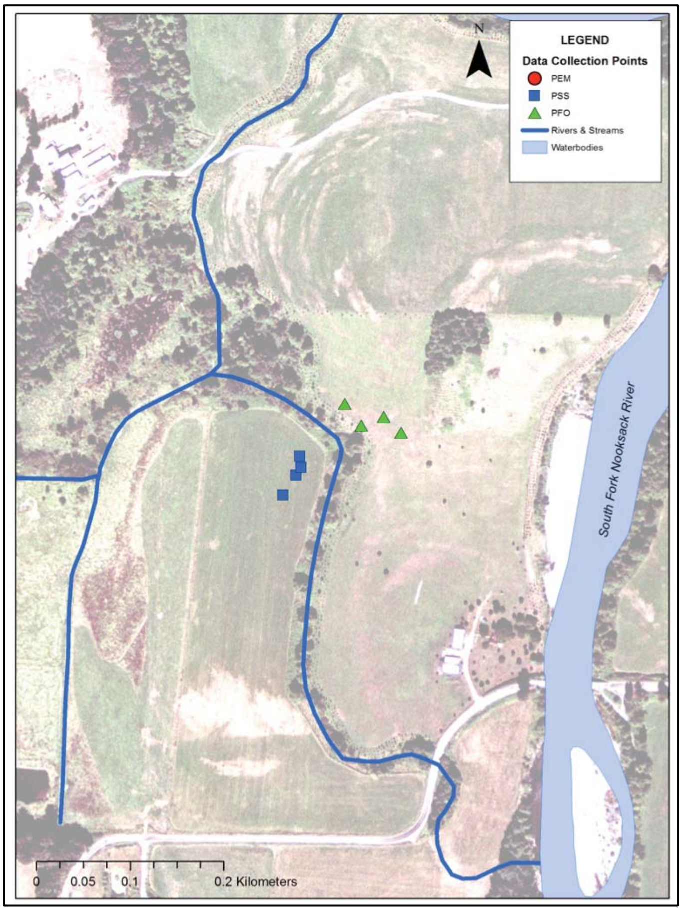

Figure A7. SR 539 Potter Road wetland restoration site (site nr. 7).

Figure A8. SR 167 North Sumner wetland restoration site (site nr. 8).

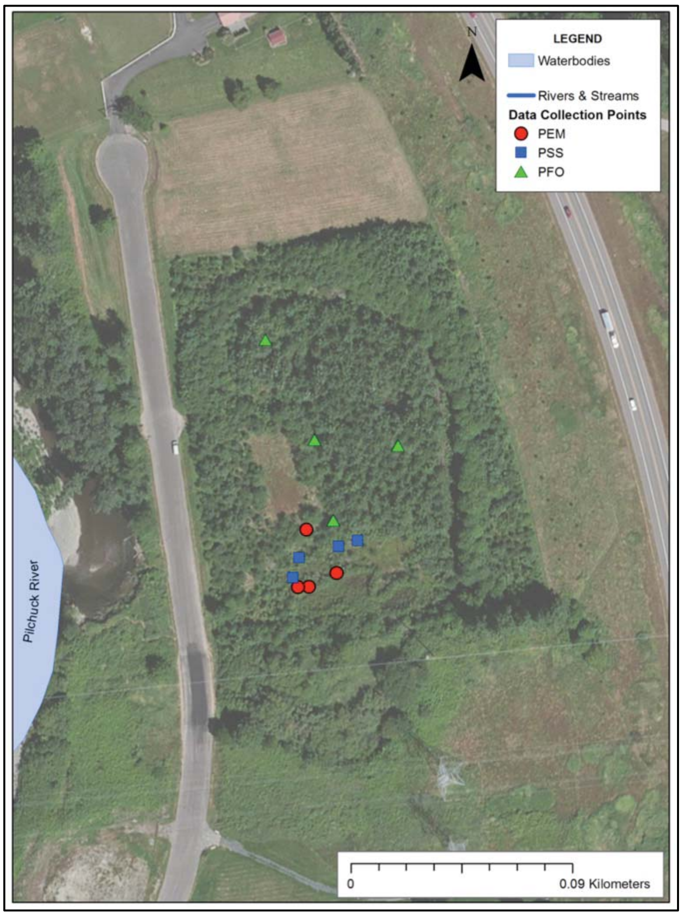

Figure A9. SR 009 Pilchuck River wetland restoration site (site nr. 9).

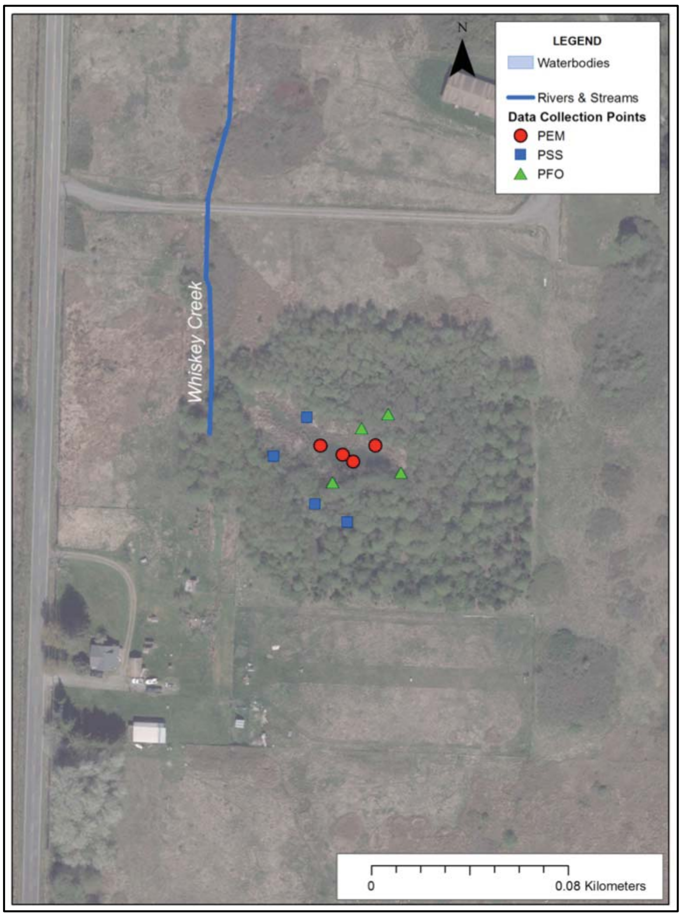

Figure A10. SR 020 Whiskey Creek wetland restoration site (site nr. 10).

Figure A11. SR 527 North Creek 2 wetland restoration site (site nr. 11).

Figure A12. SR 009 Stillaguamish River at Haller Bridge wetland restoration site (site nr. 12).

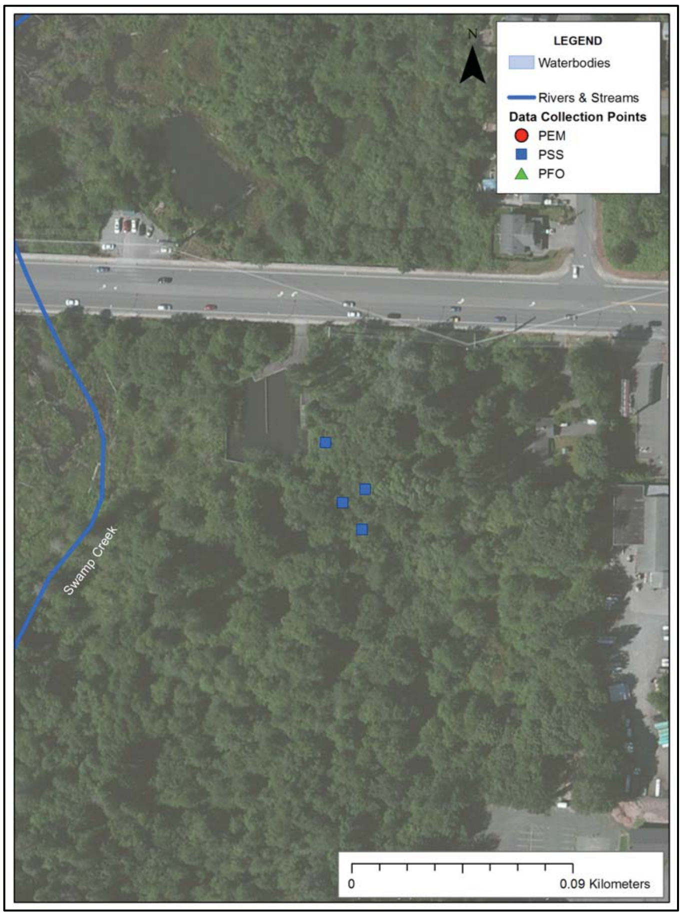

Figure A13. SR 005 Ash Way wetland restoration site (site nr. 13).

Figure A14. SR 009 Howell Creek wetland restoration site (site nr. 14).

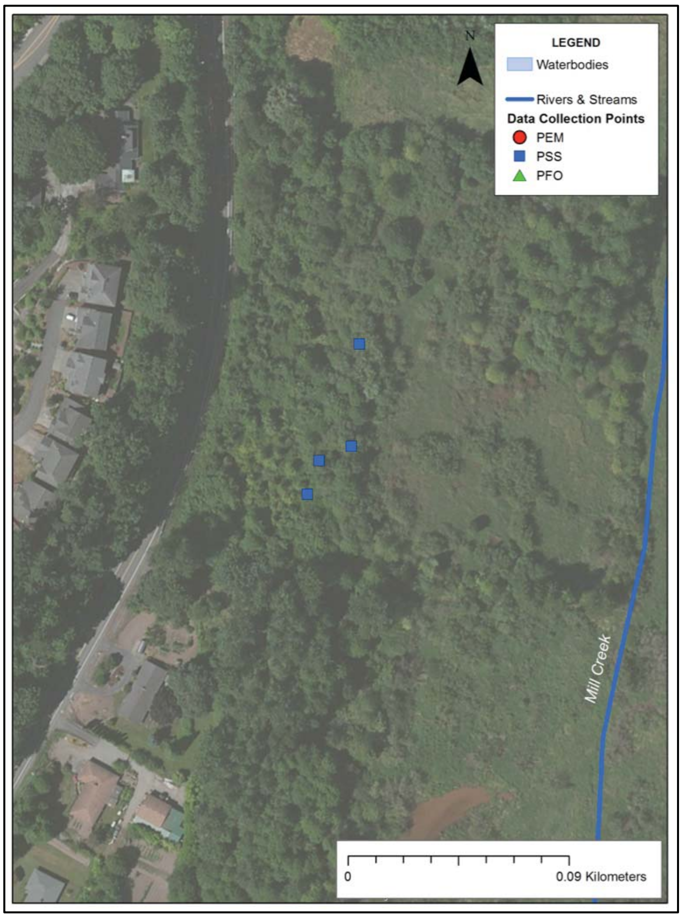

Figure A15. SR 167 Mill Creek Stage 2 wetland restoration site (site nr. 15).

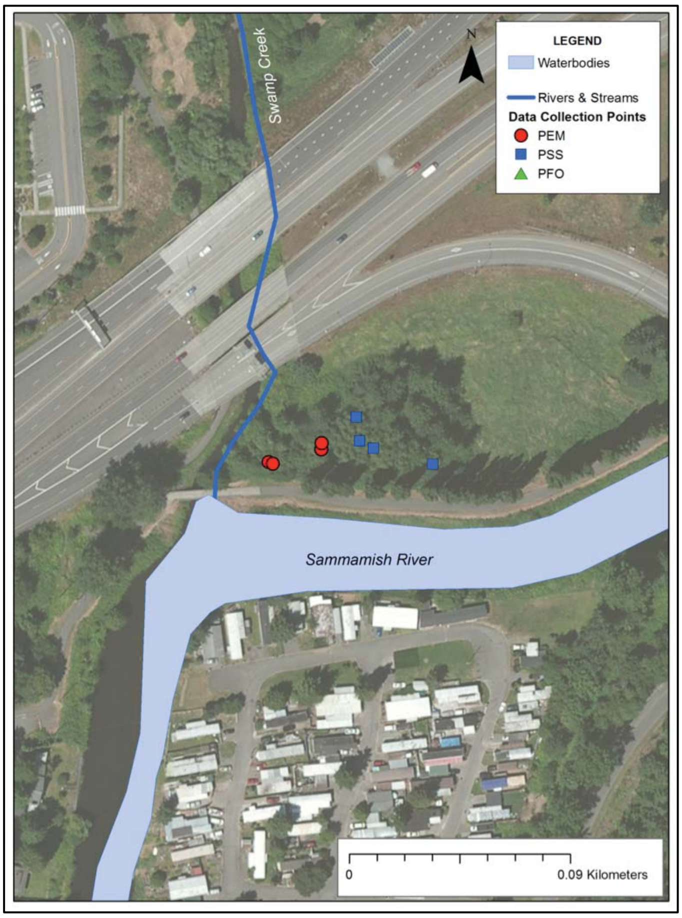

Figure A16. SR 405 Swamp Creek wetland restoration site (site nr. 16).

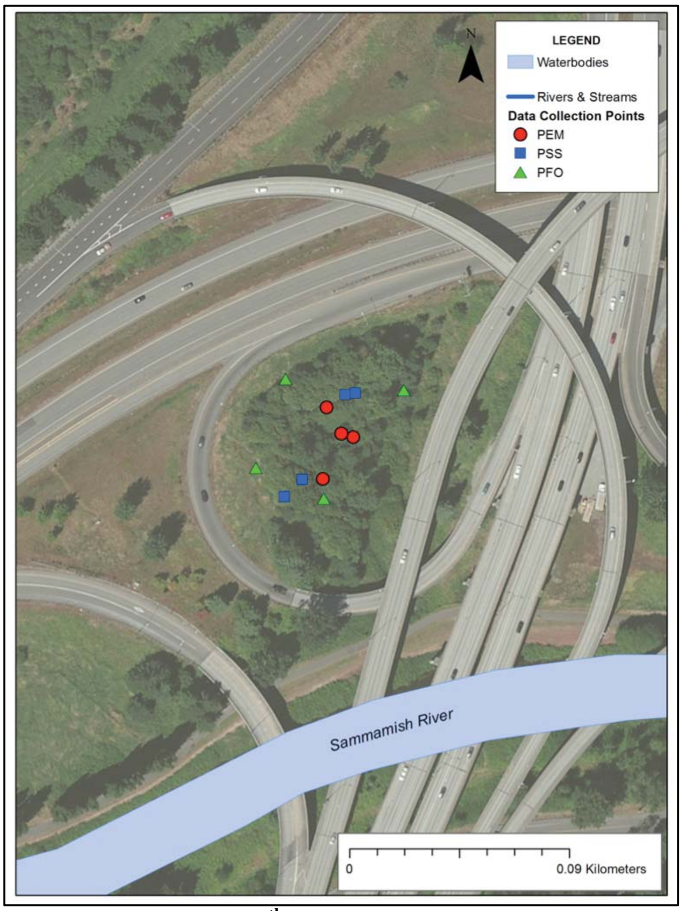

Figure A17. SR 405 160th St I/C wetland restoration site (site nr. 17).

Figure A18. SR 169 Cedar River wetland restoration site (site nr. 18).

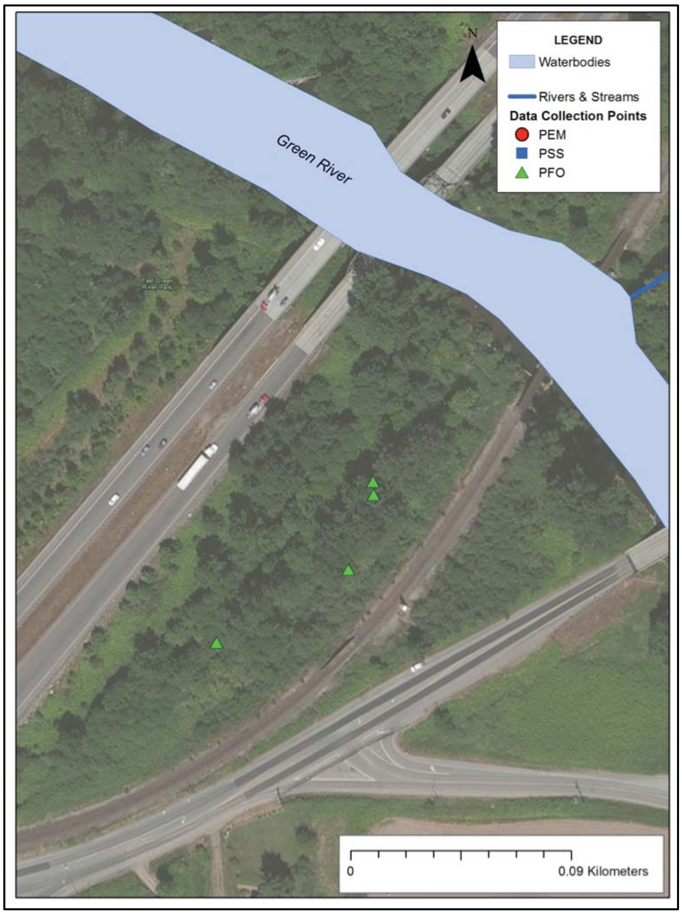

Figure A19. SR 018 Green River restoration site (site nr. 19).

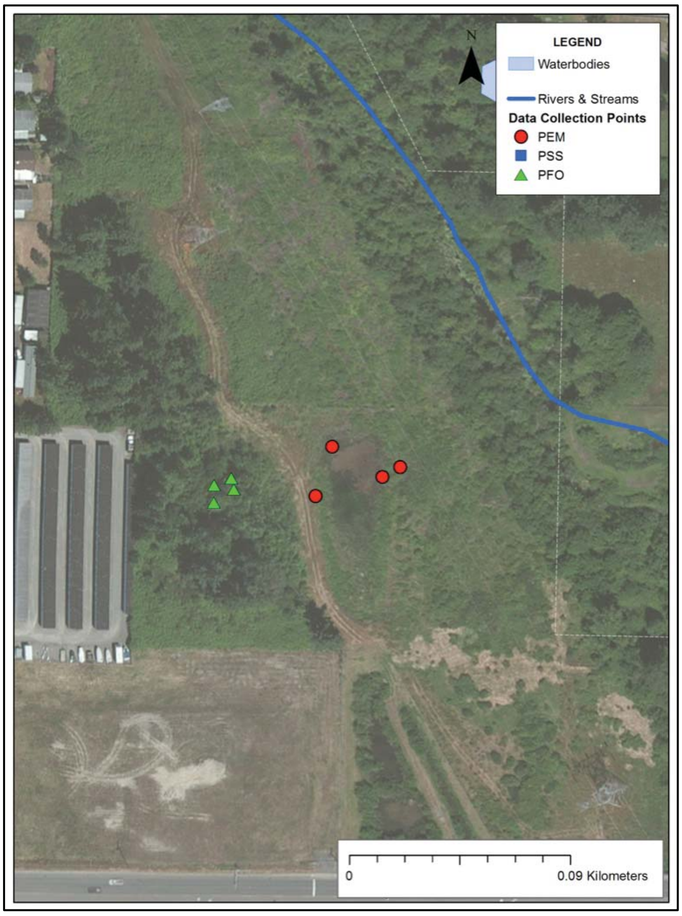

Figure A20. SR 516 Big Soos Creek wetland restoration site (site nr. 20).

Figure A21. SR 530 Cicero Pond wetland restoration site (site nr. 21).

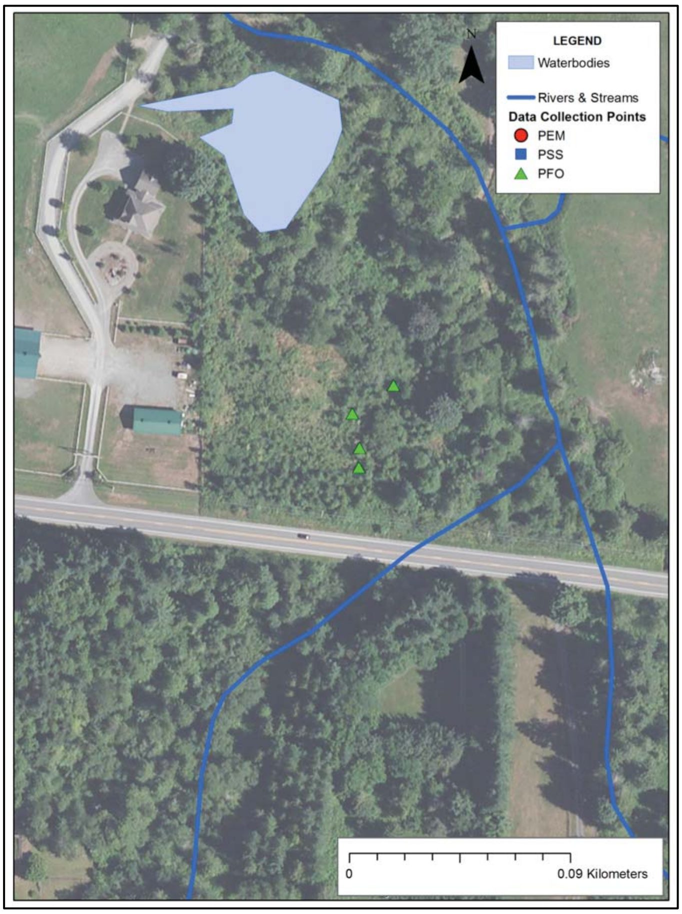

Figure A22. SR 202 Patterson Creek 1 wetland restoration site (site nr. 22).

Appendix B: Sample site data and calculations

Site Nr.	Site Name	Sample Name	Site Age (yrs)	Avg. Sample Depth (cm)	Vegetation Type	Hydrologic Regime	Avg. Hydroperiod July 2015	Avg. Hydroperiod Dec 2015	Hydrogeomorphic Class
1	**SR 009 Charles Plummer**	**PEM.009PLU.150724**	2	15.24	palustrine emergent	permanently flooded	surface water	surface water	depressional outflow
1	**SR 009 Charles Plummer**	**PEM.009PLU.150724**	2	20.32	palustrine emergent	permanently flooded	surface water	surface water	depressional outflow
1	**SR 009 Charles Plummer**	**PSS.009PLU.150724**	2	13.97	palustrine scrub-shrub	seasonally flooded	saturated	saturated	depressional outflow
1	**SR 009 Charles Plummer**	**PSS.009PLU.150724**	2	6.35	palustrine scrub-shrub	seasonally flooded	high water table	saturated	depressional outflow
1	**SR 009 Charles Plummer**	**PFO.009PLU.150721**	2	10.16	palustrine forested	saturated	saturated	saturated	depressional outflow
1	**SR 009 Charles Plummer**	**PFO.009PLU.150721**	2	22.23	palustrine forested	saturated	saturated	saturated	depressional outflow
2	**SR 520 Evans Creek**	**PSS.520EVA.150728**	2	13.97	palustrine scrub-shrub	semi-permanently flooded	indicators	high water table	depressional flowthrough
2	**SR 520 Evans Creek**	**PSS.520EVA.150728**	2	22.23	palustrine scrub-shrub	semi-permanently flooded	indicators	high water table	depressional flowthrough
2	**SR 520 Evans Creek**	**PFO.520EVA.150728**	2	15.24	palustrine forested	saturated	indicators	saturated	depressional flowthrough
2	**SR 520 Evans Creek**	**PFO.520EVA.150728**	2	20.32	palustrine forested	saturated	indicators	saturated	depressional flowthrough
3	**SR 005 Corrington**	**PEM.005COR.150804**	3	20.32	palustrine emergent	seasonally flooded	indicators	high water table	depressional outflow
3	**SR 005 Corrington**	**PEM.005COR.150804**	3	5.08	palustrine emergent	seasonally flooded	indicators	high water table	depressional outflow
3	**SR 005 Corrington**	**PSS.005COR.150804**	3	10.16	palustrine scrub-shrub	saturated	indicators	saturated	depressional outflow
3	**SR 005 Corrington**	**PSS.005COR.150804**	3	2.54	palustrine scrub-shrub	saturated	indicators	saturated	depressional outflow
3	**SR 005 Corrington**	**PFO.005COR.150804**	3	15.24	palustrine forested	saturated	saturated	saturated	depressional outflow
3	**SR 005 Corrington**	**PFO.005COR.150804**	3	20.32	palustrine forested	saturated	saturated	saturated	depressional outflow
4	**SR 020 Gages Slough**	**PEM.020GAG.150708**	6	9.21	palustrine emergent	semi-permanently flooded	indicators	high water table	depressional outflow
4	**SR 020 Gages Slough**	**PEM.020GAG.150708**	6	28.89	palustrine emergent	semi-permanently flooded	indicators	surface water	depressional outflow
4	**SR 020 Gages Slough**	**PSS.020GAG.150708**	6	10.80	palustrine scrub-shrub	saturated	indicators	saturated	depressional outflow
4	**SR 020 Gages Slough**	**PFO.020GAG.150708**	6	6.35	palustrine forested	saturated	indicators	saturated	depressional outflow
4	**SR 020 Gages Slough**	**PFO.020GAG.150708**	6	15.24	palustrine forested	saturated	indicators	saturated	depressional outflow
4	**SR 020 Gages Slough**	**PSS.020GAG.150708**	6	5.24	palustrine forested	saturated	indicators	saturated	depressional outflow
5	**SR 020 Quiet Cove**	**PEM.020QUE.150709**	6	10.16	palustrine emergent	seasonally flooded	indicators	high water table	depressional closed
5	**SR 020 Quiet Cove**	**PEM.020QUE.150709**	6	10.16	palustrine emergent	seasonally flooded	indicators	high water table	depressional closed
5	**SR 020 Quiet Cove**	**PSS.020QUE.150709**	6	6.35	palustrine scrub-shrub	saturated	indicators	saturated	depressional closed
5	**SR 020 Quiet Cove**	**PSS.020QUE.150709**	6	5.08	palustrine scrub-shrub	saturated	indicators	saturated	depressional closed
6	**SR 305 Poulsbo**	**PEM.305POU.150807**	6	7.62	palustrine emergent	semi-permanently flooded	indicators	surface water	depressional outflow
6	**SR 305 Poulsbo**	**PEM.305POU.150807**	6	7.62	palustrine emergent	semi-permanently flooded	indicators	surface water	depressional outflow
6	**SR 305 Poulsbo**	**PSS.305POU.150807**	6	7.62	palustrine scrub-shrub	saturated	indicators	saturated	depressional outflow
6	**SR 305 Poulsbo**	**PSS.305POU.150807**	6	22.86	palustrine scrub-shrub	saturated	indicators	saturated	depressional outflow
6	**SR 305 Poulsbo**	**PFO.305POU.150807**	6	10.16	palustrine forested	saturated	indicators	saturated	depressional outflow
6	**SR 305 Poulsbo**	**PFO.305POU.150807**	6	10.16	palustrine forested	saturated	indicators	saturated	depressional outflow
7	**SR 539 Potter Road**	**PSS.539POT.150706**	6	27.09	palustrine scrub-shrub	semi-permanently flooded	high water table	saturated	depressional flowthrough
7	**SR 539 Potter Road**	**PSS.539POT.150706**	6	8.47	palustrine scrub-shrub	semi-permanently flooded	high water table	saturated	depressional flowthrough
7	**SR 539 Potter Road**	**PFO.539POT.150706**	6	26.67	palustrine forested	seasonally flooded	indicators	high water table	depressional flowthrough
7	**SR 539 Potter Road**	**PFO.539POT.150706**	6	13.97	palustrine forested	seasonally flooded	indicators	high water table	depressional flowthrough
8	**SR 167 North Sumner**	**PEM.167SUM.150730**	9	7.62	palustrine emergent	semi-permanently flooded	high water table	surface water	depressional outflow
8	**SR 167 North Sumner**	**PEM.167SUM.150730**	9	22.86	palustrine emergent	semi-permanently flooded	high water table	surface water	depressional outflow
8	**SR 167 North Sumner**	**PSS.167SUM.150730**	9	1.06	palustrine scrub-shrub	seasonally flooded	high water table	high water table	depressional outflow
8	**SR 167 North Sumner**	**PSS.167SUM.150730**	9	17.78	palustrine scrub-shrub	seasonally flooded	high water table	high water table	depressional outflow
9	**SR 009 Pilchuck River**	**PEM.009PIL.150803**	11	7.62	palustrine emergent	seasonally flooded	saturated	surface water	depressional outflow

Site Nr.	Site Name	Sample Name	Site Age (yrs)	Avg. Sample Depth (cm)	Vegetation Type	Hydrologic Regime	Avg. Hydroperiod July 2015	Avg. Hydroperiod Dec 2015	Hydrogeomorphic Class
9	**SR 009 Pilchuck River**	**PEM.009PIL.150803**	11	20.32	palustrine emergent	seasonally flooded	saturated	surface water	depressional outflow
9	**SR 009 Pilchuck River**	**PSS.009PIL.150803**	11	15.24	palustrine scrub-shrub	seasonally flooded	saturated	surface water	depressional outflow
9	**SR 009 Pilchuck River**	**PSS.009PIL.150803**	11	17.78	palustrine scrub-shrub	seasonally flooded	saturated	surface water	depressional outflow
9	**SR 009 Pilchuck River**	**PFO.009PIL.150803**	11	10.16	palustrine forested	seasonally flooded	indicators	surface water	depressional outflow
9	**SR 009 Pilchuck River**	**PFO.009PIL.150803**	11	22.86	palustrine forested	seasonally flooded	indicators	surface water	depressional outflow
10	**SR 020 Whiskey Creek**	**PEM.020WHI.150713**	11	15.24	palustrine emergent	semi-permanently flooded	saturated	surface water	depressional outflow
10	**SR 020 Whiskey Creek**	**PEM.020WHI.150713**	11	20.32	palustrine emergent	semi-permanently flooded	saturated	surface water	depressional outflow
10	**SR 020 Whiskey Creek**	**PSS.020WHI.150713**	11	15.24	palustrine scrub-shrub	seasonally flooded	indicators	surface water	depressional outflow
10	**SR 020 Whiskey Creek**	**PSS.020WHI.150713**	11	22.86	palustrine scrub-shrub	seasonally flooded	indicators	surface water	depressional outflow
10	**SR 020 Whiskey Creek**	**PFO.020WHI.150713**	11	17.78	palustrine forested	seasonally flooded	indicators	high water table	depressional outflow
10	**SR 020 Whiskey Creek**	**PFO.020WHI.150713**	11	17.78	palustrine forested	seasonally flooded	indicators	high water table	depressional outflow
11	**SR 527 North Creek 2**	**PEM.527NOR.150716**	13	5.08	palustrine emergent	seasonally flooded	saturated	surface water	depressional outflow
11	**SR 527 North Creek 2**	**PEM.527NOR.150716**	13	24.13	palustrine emergent	seasonally flooded	saturated	surface water	depressional outflow
11	**SR 527 North Creek 2**	**PSS.527NOR.150716**	13	13.97	palustrine scrub-shrub	semi-permanently flooded	indicators	not recorded	depressional outflow
11	**SR 527 North Creek 2**	**PSS.527NOR.150716**	13	22.23	palustrine scrub-shrub	semi-permanently flooded	indicators	not recorded	depressional outflow
11	**SR 527 North Creek 2**	**PFO.527NOR.150716**	13	15.24	palustrine forested	saturated	indicators	not recorded	depressional outflow
11	**SR 527 North Creek 2**	**PFO.527NOR.150716**	13	20.32	palustrine forested	saturated	indicators	not recorded	depressional outflow
12	**SR 009 Stillaguamish River at Haller Bridge**	**PEM.009STI.150721**	16	10.16	palustrine emergent	seasonally flooded	high water table	surface water	depressional closed
12	**SR 009 Stillaguamish River at Haller Bridge**	**PEM.009STI.150721**	16	22.86	palustrine emergent	seasonally flooded	high water table	surface water	depressional closed
12	**SR 009 Stillaguamish River at Haller Bridge**	**PFO.009STI.150720**	16	15.24	palustrine forested	seasonally flooded	high water table	high water table	depressional closed
12	**SR 009 Stillaguamish River at Haller Bridge**	**PFO.009STI.150720**	16	18.63	palustrine forested	seasonally flooded	high water table	high water table	depressional closed
13	**SR 005 Ash Way**	**PSS.005ASH.150717**	17	15.24	palustrine scrub-shrub	saturated	indicators	saturated	depressional outflow
13	**SR 005 Ash Way**	**PSS.005ASH.150717**	17	12.07	palustrine scrub-shrub	saturated	indicators	saturated	depressional outflow
14	**SR 009 Howell Creek**	**PSS.009HOW.150724**	17	12.70	palustrine scrub-shrub	seasonally flooded	indicators	surface water	depressional outflow
14	**SR 009 Howell Creek**	**PSS.009HOW.150724**	17	22.86	palustrine scrub-shrub	seasonally flooded	indicators	surface water	depressional outflow
15	**SR 167 Mill Creek Stage 2**	**PSS.167MIL.150731**	17	17.78	palustrine scrub-shrub	seasonally flooded	saturated	high water table	depressional outflow
15	**SR 167 Mill Creek Stage 2**	**PSS.167MIL.150731**	17	17.78	palustrine scrub-shrub	seasonally flooded	saturated	high water table	depressional outflow
16	**SR 405 Swamp Creek**	**PEM.405SWA.150717**	17	12.70	palustrine emergent	seasonally flooded	indicators	surface water	depressional outflow
16	**SR 405 Swamp Creek**	**PEM.405SWA.150717**	17	7.62	palustrine emergent	seasonally flooded	indicators	surface water	depressional outflow
16	**SR 405 Swamp Creek**	**PSS.405SWA.150717**	17	12.70	palustrine scrub-shrub	saturated	indicators	saturated	depressional outflow
16	**SR 405 Swamp Creek**	**PSS.405SWA.150717**	17	15.24	palustrine scrub-shrub	saturated	indicators	saturated	depressional outflow
17	**SR 405 160th St I/C**	**PEM.405160.150727**	19	25.40	palustrine emergent	seasonally flooded	saturated	surface water	depressional flow-through
17	**SR 405 160th St I/C**	**PEM.405160.150727**	19	15.24	palustrine emergent	seasonally flooded	saturated	surface water	depressional flow-through
17	**SR 405 160th St I/C**	**PSS.405160.150727**	19	13.34	palustrine scrub-shrub	saturated	indicators	saturated	depressional flow-through
17	**SR 405 160th St I/C**	**PSS.405160.150727**	19	22.23	palustrine scrub-shrub	saturated	indicators	saturated	depressional flow-through
17	**SR 405 160th St I/C**	**PFO.405160.150727**	19	7.62	palustrine forested	saturated	indicators	saturated	depressional flow-through
17	**SR 405 160th St I/C**	**PFO.405160.150727**	19	0.46	palustrine forested	saturated	indicators	saturated	depressional flow-through

Site Nr.	Site Name	Sample Name	Site Age (yrs)	Avg. Sample Depth (cm)	Vegetation Type	Hydrologic Regime	Avg. Hydroperiod July 2015	Avg. Hydroperiod Dec 2015	Hydrogeomorphic Class
18	**SR 169 Cedar River**	**PSS.169CED.150803**	20	7.62	palustrine scrub-shrub	seasonally flooded	indicators	high water table	depressional flow-through
18	**SR 169 Cedar River**	**PSS.169CED.150803**	20	12.70	palustrine scrub-shrub	seasonally flooded	indicators	high water table	depressional flow-through
19	**SR 018 Green River**	**PFO.018GRE.150731**	21	15.24	palustrine forested	semi-permanently flooded	saturated	surface water	depressional outflow
19	**SR 018 Green River**	**PFO.018GRE.150731**	21	12.70	palustrine forested	semi-permanently flooded	saturated	surface water	depressional outflow
20	**SR 516 Big Soos Creek**	**PEM.516BIG.150731**	21	20.32	palustrine emergent	semi-permanently flooded	saturated	high water table	depressional outflow
20	**SR 516 Big Soos Creek**	**PEM.516BIG.150731**	21	15.24	palustrine emergent	semi-permanently flooded	saturated	high water table	depressional outflow
20	**SR 516 Big Soos Creek**	**PFO.516BIG.150731**	21	7.62	palustrine forested	temporarily flooded	indicators	high water table	depressional outflow
20	**SR 516 Big Soos Creek**	**PFO.516BIG.150731**	21	5.08	palustrine forested	temporarily flooded	indicators	high water table	depressional outflow
21	**SR 530 Cicero Pond**	**PSS.530CIS.150721**	21	22.01	palustrine scrub-shrub	saturated	saturated	surface water	depressional outflow
21	**SR 530 Cicero Pond**	**PSS.530CIS.150721**	21	15.24	palustrine scrub-shrub	saturated	saturated	surface water	depressional outflow
21	**SR 530 Cicero Pond**	**PFO.530CIS.150721**	21	15.24	palustrine forested	saturated	indicators	saturated	depressional outflow
21	**SR 530 Cicero Pond**	**PFO.530CIS.150721**	21	5.08	palustrine forested	saturated	indicators	saturated	depressional outflow
22	**SR 202 Patterson Creek 1**	**PFO.202PAT.150728**	22	7.62	palustrine forested	temporarily flooded	indicators	high water table	depressional closed
22	**SR 202 Patterson Creek 1**	**PFO.202PAT.150728**	22	26.04	palustrine forested	temporarily flooded	indicators	high water table	depressional closed
18	**SR 169 Cedar River**	**PSS.169CED.150803**	20	7.62	palustrine scrub-shrub	seasonally flooded	indicators	high water table	depressional flow-through
18	**SR 169 Cedar River**	**PSS.169CED.150803**	20	12.70	palustrine scrub-shrub	seasonally flooded	indicators	high water table	depressional flow-through
19	**SR 018 Green River**	**PFO.018GRE.150731**	21	15.24	palustrine forested	semi-permanently flooded	saturated	surface water	depressional outflow
19	**SR 018 Green River**	**PFO.018GRE.150731**	21	12.70	palustrine forested	semi-permanently flooded	saturated	surface water	depressional outflow
20	**SR 516 Big Soos Creek**	**PEM.516BIG.150731**	21	20.32	palustrine emergent	semi-permanently flooded	saturated	high water table	depressional outflow
20	**SR 516 Big Soos Creek**	**PEM.516BIG.150731**	21	15.24	palustrine emergent	semi-permanently flooded	saturated	high water table	depressional outflow
20	**SR 516 Big Soos Creek**	**PFO.516BIG.150731**	21	7.62	palustrine forested	temporarily flooded	indicators	high water table	depressional outflow
20	**SR 516 Big Soos Creek**	**PFO.516BIG.150731**	21	5.08	palustrine forested	temporarily flooded	indicators	high water table	depressional outflow
21	**SR 530 Cicero Pond**	**PSS.530CIS.150721**	21	22.01	palustrine scrub-shrub	saturated	saturated	surface water	depressional outflow
21	**SR 530 Cicero Pond**	**PSS.530CIS.150721**	21	15.24	palustrine scrub-shrub	saturated	saturated	surface water	depressional outflow
21	**SR 530 Cicero Pond**	**PFO.530CIS.150721**	21	15.24	palustrine forested	saturated	indicators	saturated	depressional outflow
21	**SR 530 Cicero Pond**	**PFO.530CIS.150721**	21	5.08	palustrine forested	saturated	indicators	saturated	depressional outflow
22	**SR 202 Patterson Creek 1**	**PFO.202PAT.150728**	22	7.62	palustrine forested	temporarily flooded	indicators	high water table	depressional closed
22	**SR 202 Patterson Creek 1**	**PFO.202PAT.150728**	22	26.04	palustrine forested	temporarily flooded	indicators	high water table	depressional closed

Site Nr.	Site Name	Sample Name	Soil Layer	Mapped Soil Type	%C	%N	C:N	√ %C	√ %N	ln C:N	Bulk Density (gr cm^{-3})	C Mass (gr cm^{-3})	Total C (Mg ha^{-1})	N Mass (gr cm^{-3})	Total N (Mg ha^{-1})
1	**SR 009 Charles Plummer**	**PEM.009PLU.150724**	upper	mineral	3.16	0.22	14.36	1.778	0.469	1.157	1.01	0.032	48.81	48.64	3.39
1	**SR 009 Charles Plummer**	**PEM.009PLU.150724**	lower	mineral	6.04	0.37	16.32	2.458	0.608	1.213	1.01	0.061	124.40	123.96	7.59
1	**SR 009 Charles Plummer**	**PSS.009PLU.150724**	upper	mineral	9.88	0.58	17.03	3.143	0.762	1.231	0.85	0.084	117.39	117.32	6.89
1	**SR 009 Charles Plummer**	**PSS.009PLU.150724**	lower	mineral	6.36	0.37	17.15	2.522	0.609	1.234	0.85	0.067	34.35	34.33	2.00
1	**SR 009 Charles Plummer**	**PFO.009PLU.150721**	upper	mineral	20.99	1.06	19.71	4.582	1.032	1.295	0.56	0.070	120.28	119.42	6.03
1	**SR 009 Charles Plummer**	**PFO.009PLU.150721**	lower	mineral	5.06	0.31	16.20	2.250	0.559	1.209	0.56	0.036	63.47	62.99	3.86
2	**SR 520 Evans Creek**	**PSS.520EVA.150728**	upper	mineral	7.55	0.49	15.41	2.748	0.700	1.188	0.43	0.033	45.68	45.35	2.94
2	**SR 520 Evans Creek**	**PSS.520EVA.150728**	lower	mineral	4.02	0.19	21.73	1.590	0.278	1.530	0.43	0.011	24.45	38.43	1.82
2	**SR 520 Evans Creek**	**PFO.520EVA.150728**	upper	mineral	6.13	0.31	19.77	2.476	0.557	1.296	0.57	0.035	53.11	53.25	2.69
2	**SR 520 Evans Creek**	**PFO.520EVA.150728**	lower	mineral	1.51	0.02	75.50	1.229	0.141	1.878	0.57	0.009	17.44	17.49	0.23
3	**SR 005 Corrington**	**PEM.005COR.150804**	upper	mineral & organic	17.56	0.73	24.05	4.190	0.854	1.381	0.36	0.063	127.22	128.45	5.34
3	**SR 005 Corrington**	**PEM.005COR.150804**	lower	mineral & organic	12.73	0.55	23.15	3.568	0.742	1.365	0.36	0.045	23.06	23.28	1.01
3	**SR 005 Corrington**	**PSS.005COR.150804**	upper	mineral & organic	10.14	0.35	29.38	3.184	0.587	1.468	0.68	0.069	69.71	70.06	2.42
3	**SR 005 Corrington**	**PSS.005COR.150804**	lower	mineral & organic	6.36	0.35	18.17	2.522	0.592	1.259	0.68	0.043	10.94	10.98	0.60
3	**SR 005 Corrington**	**PFO.005COR.150804**	upper	mineral & organic	5.88	0.26	22.62	2.425	0.510	1.354	0.12	0.007	10.31	10.75	0.48
3	**SR 005 Corrington**	**PFO.005COR.150804**	lower	mineral & organic	4.88	0.21	23.24	2.209	0.458	1.366	0.12	0.006	11.41	11.90	0.51
4	**SR 020 Gages Slough**	**PEM.020GAG.150708**	upper	mineral	6.02	0.32	18.81	2.454	0.566	1.274	0.97	0.058	53.77	53.78	2.86
4	**SR 020 Gages Slough**	**PEM.020GAG.150708**	lower	mineral	0.40	0.06	6.67	0.632	0.245	0.824	0.97	0.004	11.21	11.21	1.68
4	**SR 020 Gages Slough**	**PSS.020GAG.150708**	upper	mineral	9.11	0.52	17.46	3.019	0.723	1.244	1.11	0.102	106.84	109.16	6.23
4	**SR 020 Gages Slough**	**PFO.020GAG.150708**	upper	mineral	12.43	0.51	24.37	3.526	0.714	1.387	0.80	0.100	63.27	63.14	2.59
4	**SR 020 Gages Slough**	**PFO.020GAG.150708**	lower	mineral	0.59	0.08	7.87	0.766	0.274	0.893	0.80	0.005	7.21	7.19	0.98
4	**SR 020 Gages Slough**	**PSS.020GAG.150708**	lower	mineral	0.58	0.08	7.25	0.762	0.283	0.860	0.80	0.005	7.09	2.43	0.34
5	**SR 020 Quiet Cove**	**PEM.020QUE.150709**	upper	mineral	14.33	0.69	20.91	3.785	0.828	1.320	0.67	0.096	97.20	97.55	4.70
5	**SR 020 Quiet Cove**	**PEM.020QUE.150709**	lower	mineral	3.51	0.21	16.71	1.873	0.458	1.223	0.67	0.023	23.77	23.89	1.43
5	**SR 020 Quiet Cove**	**PSS.020QUE.150709**	upper	mineral	10.80	0.43	25.12	3.286	0.656	1.400	0.67	0.073	46.09	45.95	1.83
5	**SR 020 Quiet Cove**	**PSS.020QUE.150709**	lower	mineral	2.49	0.06	41.50	1.578	0.245	1.618	0.67	0.017	8.50	8.47	0.20
6	**SR 305 Poulsbo**	**PEM.305POU.150807**	upper	mineral	21.27	1.27	16.81	4.612	1.125	1.226	0.69	0.148	112.43	111.83	6.68
6	**SR 305 Poulsbo**	**PEM.305POU.150807**	lower	mineral	2.64	0.16	16.50	1.625	0.400	1.217	0.69	0.018	13.95	13.88	0.84
6	**SR 305 Poulsbo**	**PSS.305POU.150807**	upper	mineral	11.26	0.80	14.08	3.356	0.894	1.149	0.72	0.081	61.55	61.78	4.39
6	**SR 305 Poulsbo**	**PSS.305POU.150807**	lower	mineral	6.39	0.50	12.78	2.528	0.707	1.107	0.72	0.046	104.79	105.17	8.23
6	**SR 305 Poulsbo**	**PFO.305POU.150807**	upper	mineral	17.33	1.35	12.84	4.163	1.162	1.109	0.62	0.108	109.40	109.17	8.50
6	**SR 305 Poulsbo**	**PFO.305POU.150807**	lower	mineral	4.10	0.36	11.39	2.025	0.600	1.057	0.89	0.037	37.28	37.07	3.26
7	**SR 539 Potter Road**	**PSS.539POT.150706**	upper	mineral	9.45	0.72	13.13	3.074	0.849	1.118	0.71	0.067	181.58	181.76	13.85
7	**SR 539 Potter Road**	**PSS.539POT.150706**	lower	mineral	5.45	0.44	12.39	2.335	0.663	1.093	0.71	0.039	32.73	32.77	2.65
7	**SR 539 Potter Road**	**PFO.539POT.150706**	upper	mineral	13.72	1.04	13.20	3.705	1.020	1.121	0.89	0.123	327.56	325.66	24.69
7	**SR 539 Potter Road**	**PFO.539POT.150706**	lower	mineral	1.35	0.12	11.25	1.162	0.346	1.051	0.89	0.012	16.88	16.78	1.49
8	**SR 167 North Sumner**	**PEM.167SUM.150730**	upper	mineral & organic	9.01	0.46	19.59	3.002	0.678	1.292	0.72	0.065	49.71	49.43	2.52
8	**SR 167 North Sumner**	**PEM.167SUM.150730**	lower	mineral & organic	1.17	0.06	19.50	1.082	0.245	1.290	0.72	0.008	19.37	19.26	0.99
8	**SR 167 North Sumner**	**PSS.167SUM.150730**	upper	mineral & organic	5.04	0.24	21.00	2.245	0.490	1.322	0.78	0.040	50.20	4.17	0.20
8	**SR 167 North Sumner**	**PSS.167SUM.150730**	lower	mineral & organic	3.40	0.28	12.13	1.336	0.272	1.385	0.78	0.014	24.89	47.15	3.88
9	**SR 009 Pilchuck River**	**PEM.009PIL.150803**	upper	mineral	3.49	0.24	14.54	1.868	0.490	1.163	0.92	0.032	24.47	24.47	1.68
9	**SR 009 Pilchuck River**	**PEM.009PIL.150803**	lower	mineral	1.75	0.14	12.50	1.323	0.374	1.097	0.92	0.016	32.72	32.72	2.62

Site Nr.	Site Name	Sample Name	Soil Layer	Mapped Soil Type	%C	%N	C:N	√ %C	√ %N	ln C:N	Bulk Density (gr cm^{-3})	C Mass (gr cm^{-3})	Total C (Mg ha^{-1})	N Mass (gr cm^{-3})	Total N (Mg ha^{-1})
9	**SR 009 Pilchuck River**	**PSS.009PIL.150803**	upper	mineral	4.49	0.28	16.04	2.119	0.529	1.205	0.45	0.020	30.46	30.79	1.92
9	**SR 009 Pilchuck River**	**PSS.009PIL.150803**	lower	mineral	1.28	0.12	10.67	1.131	0.346	1.028	0.45	0.006	10.13	10.24	0.96
9	**SR 009 Pilchuck River**	**PFO.009PIL.150803**	upper	mineral	5.45	0.34	16.07	2.334	0.583	1.206	0.64	0.035	35.54	35.44	2.21
9	**SR 009 Pilchuck River**	**PFO.009PIL.150803**	lower	mineral	1.42	0.14	10.14	1.192	0.374	1.006	0.64	0.009	20.83	20.78	2.05
10	**SR 020 Whiskey Creek**	**PEM.020WHI.150713**	upper	mineral	9.06	0.56	16.18	3.010	0.748	1.209	0.59	0.054	82.05	81.46	5.04
10	**SR 020 Whiskey Creek**	**PEM.020WHI.150713**	lower	mineral	8.92	0.46	19.38	2.479	0.599	1.235	0.59	0.037	74.27	106.94	5.51
10	**SR 020 Whiskey Creek**	**PSS.020WHI.150713**	upper	mineral	14.26	0.69	20.67	3.776	0.831	1.315	0.95	0.136	206.82	206.46	9.99
10	**SR 020 Whiskey Creek**	**PSS.020WHI.150713**	lower	mineral	8.74	0.49	17.84	2.956	0.700	1.251	0.95	0.083	190.14	189.81	10.64
10	**SR 020 Whiskey Creek**	**PFO.020WHI.150713**	upper	mineral	12.13	0.65	18.66	3.483	0.806	1.271	0.58	0.070	125.32	125.09	6.70
10	**SR 020 Whiskey Creek**	**PFO.020WHI.150713**	lower	mineral	8.13	0.51	15.94	2.851	0.714	1.202	0.58	0.047	83.99	83.84	5.26
11	**SR 527 North Creek 2**	**PEM.527NOR.150716**	upper	mineral	8.70	0.45	19.33	2.950	0.671	1.286	0.81	0.071	35.89	35.80	1.85
11	**SR 527 North Creek 2**	**PEM.527NOR.150716**	lower	mineral	4.91	0.18	27.28	2.216	0.424	1.436	0.81	0.040	96.21	95.97	3.52
11	**SR 527 North Creek 2**	**PSS.527NOR.150716**	upper	mineral	10.95	0.46	23.95	3.308	0.676	1.380	1.01	0.111	155.31	154.50	6.49
11	**SR 527 North Creek 2**	**PSS.527NOR.150716**	lower	mineral	4.53	0.16	28.31	2.128	0.400	1.452	1.01	0.046	101.80	101.71	3.59
11	**SR 527 North Creek 2**	**PFO.527NOR.150716**	upper	mineral	8.36	0.34	24.59	2.891	0.583	1.391	0.74	0.062	94.85	94.28	3.83
11	**SR 527 North Creek 2**	**PFO.527NOR.150716**	lower	mineral	6.95	0.25	27.80	2.636	0.500	1.444	0.74	0.052	105.13	104.51	3.76
12	**SR 009 Stillaguamish River at Haller Bridge**	**PEM.009STI.150721**	upper	mineral	6.21	0.51	12.20	2.493	0.714	1.086	0.67	0.065	42.33	42.27	3.47
12	**SR 009 Stillaguamish River at Haller Bridge**	**PEM.009STI.150721**	lower	mineral	1.40	0.11	12.28	1.184	0.338	1.089	0.67	0.008	21.47	21.44	1.68
12	**SR 009 Stillaguamish River at Haller Bridge**	**PFO.009STI.150720**	upper	mineral	8.49	0.73	11.66	2.914	0.853	1.067	0.91	0.084	117.10	117.74	10.12
12	**SR 009 Stillaguamish River at Haller Bridge**	**PFO.009STI.150720**	lower	mineral	1.50	0.13	11.37	1.225	0.363	1.056	0.91	0.003	25.30	25.43	2.20
13	**SR 005 Ash Way**	**PSS.005ASH.150717**	upper	mineral	6.84	0.43	15.91	2.615	0.656	1.202	0.89	0.061	93.13	92.78	5.83
13	**SR 005 Ash Way**	**PSS.005ASH.150717**	lower	mineral	4.79	0.30	15.97	2.189	0.548	1.203	0.89	0.043	51.63	51.46	3.22
14	**SR 009 Howell Creek**	**PSS.009HOW.150724**	upper	mineral	5.55	0.36	15.42	2.356	0.600	1.188	0.79	0.044	55.72	55.68	3.61
14	**SR 009 Howell Creek**	**PSS.009HOW.150724**	lower	mineral	4.27	0.22	19.84	1.635	0.429	1.162	0.79	0.021	48.43	77.11	3.97
15	**SR 167 Mill Creek Stage 2**	**PSS.167MIL.150731**	upper	mineral	2.64	0.17	15.50	1.623	0.412	1.191	1.12	0.030	52.50	52.57	3.39
15	**SR 167 Mill Creek Stage 2**	**PSS.167MIL.150731**	lower	mineral	1.03	0.03	34.33	1.015	0.173	1.536	1.12	0.012	20.52	20.51	0.60
16	**SR 405 Swamp Creek**	**PEM.405SWA.150717**	upper	mineral	5.97	0.45	13.27	2.443	0.671	1.123	1.10	0.065	83.14	83.40	6.29
16	**SR 405 Swamp Creek**	**PEM.405SWA.150717**	lower	mineral	1.55	0.11	14.09	1.245	0.332	1.149	1.10	0.017	12.95	12.99	0.92
16	**SR 405 Swamp Creek**	**PSS.405SWA.150717**	upper	mineral	3.59	0.30	11.97	1.895	0.548	1.078	1.45	0.052	66.17	66.11	5.52
16	**SR 405 Swamp Creek**	**PSS.405SWA.150717**	lower	mineral	2.81	0.34	8.26	1.676	0.583	0.917	1.45	0.041	62.15	62.10	7.51
17	**SR 405 160th St I/C**	**PEM.405160.150727**	upper	mineral	7.55	0.54	13.99	2.748	0.735	1.146	0.76	0.057	145.40	145.75	10.42
17	**SR 405 160th St I/C**	**PEM.405160.150727**	lower	mineral	2.82	0.19	14.84	1.679	0.436	1.171	0.76	0.021	32.56	32.66	2.20
17	**SR 405 160th St I/C**	**PSS.405160.150727**	upper	mineral	13.13	0.92	14.27	3.624	0.959	1.154	0.59	0.078	103.63	103.34	7.24
17	**SR 405 160th St I/C**	**PSS.405160.150727**	lower	mineral	3.06	0.21	14.57	1.749	0.458	1.163	0.59	0.018	40.25	40.13	2.75
17	**SR 405 160th St I/C**	**PFO.405160.150727**	upper	mineral	9.73	0.69	14.10	3.119	0.831	1.149	0.45	0.044	33.23	33.36	2.37
17	**SR 405 160th St I/C**	**PFO.405160.150727**	lower	mineral	2.58	0.19	13.55	1.814	0.520	1.086	0.45	0.015	41.26	0.53	0.04
18	**SR 169 Cedar River**	**PSS.169CED.150803**	upper	mineral	9.01	0.58	15.53	3.002	0.762	1.191	0.50	0.045	34.03	34.33	2.21
18	**SR 169 Cedar River**	**PSS.169CED.150803**	lower	mineral	3.48	0.20	17.40	1.865	0.447	1.241	0.50	0.017	21.91	22.10	1.27
19	**SR 018 Green River**	**PFO.018GRE.150731**	upper	mineral	4.07	0.30	13.62	2.018	0.547	1.134	0.84	0.027	51.94	52.10	3.84
19	**SR 018 Green River**	**PFO.018GRE.150731**	lower	mineral	1.36	0.06	22.67	1.166	0.245	1.355	0.84	0.011	14.45	14.51	0.64

Site Nr.	Site Name	Sample Name	Soil Layer	Mapped Soil Type	%C	%N	C:N	√ %C	√ %N	ln C:N	Bulk Density (gr cm^{-3})	C Mass (gr cm^{-3})	Total C (Mg ha^{-1})	N Mass (gr cm^{-3})	Total N (Mg ha^{-1})
20	**SR 516 Big Soos Creek**	**PEM.516BIG.150731**	upper	mineral	5.24	0.38	13.79	2.289	0.616	1.140	0.31	0.016	32.78	33.01	2.39
20	**SR 516 Big Soos Creek**	**PEM.516BIG.150731**	lower	mineral	5.10	0.37	13.78	2.258	0.608	1.139	0.31	0.016	23.93	24.09	1.75
20	**SR 516 Big Soos Creek**	**PFO.516BIG.150731**	upper	mineral	5.61	0.38	14.81	2.369	0.616	1.171	1.03	0.058	44.05	44.03	2.98
20	**SR 516 Big Soos Creek**	**PFO.516BIG.150731**	lower	mineral	4.62	0.33	14.00	2.149	0.574	1.146	1.03	0.048	24.14	24.17	1.73
21	**SR 530 Cicero Pond**	**PSS.530CIS.150721**	upper	mineral	3.68	0.27	13.63	1.918	0.520	1.134	0.72	0.026	57.92	58.32	4.28
21	**SR 530 Cicero Pond**	**PSS.530CIS.150721**	lower	mineral	4.46	0.32	14.14	1.541	0.463	1.044	0.72	0.017	25.88	48.94	3.51
21	**SR 530 Cicero Pond**	**PFO.530CIS.150721**	upper	mineral	5.18	0.34	15.24	2.276	0.583	1.183	0.75	0.039	59.43	59.21	3.89
21	**SR 530 Cicero Pond**	**PFO.530CIS.150721**	lower	mineral	3.40	0.24	14.17	1.844	0.490	1.151	0.75	0.026	13.00	12.95	0.91
22	**SR 202 Patterson Creek 1**	**PFO.202PAT.150728**	upper	mineral	7.87	0.56	14.05	2.805	0.748	1.148	0.94	0.074	56.58	56.37	4.01
22	**SR 202 Patterson Creek 1**	**PFO.202PAT.150728**	lower	mineral	6.09	0.45	13.53	2.468	0.671	1.131	0.94	0.057	149.60	149.07	11.01

Appendix C: ANOVA tables for full models

Table C1. ANOVA table for full model of predictor variables and interactions, with percent nitrogen as response variable. Model run using RStudio version 0.99.879 (Df = degrees of freedom; Sum Sq = sum of squares; Mean Sq = mean square; Pr(>F) = significance probability associated with F value).

Response variable: % Carbon					
Predictor variable	Df	Sum Sq	Mean Sq	F value	Pr(>F)
vegetative stratum	2	3.97	1.99	0.1579	0.854287
site age	1	125.46	125.46	9.9722	0.002488 **
hydrologic regime	5	50.05	10.01	0.7957	0.557092
soil type	1	0.52	0.52	0.0415	0.839318
hydrogeomorphic class	2	0.81	0.41	0.0323	0.968250
soil layer	1	559.99	559.99	44.5094	9.063e-09 ***
site age:hydrogeomorphic class	2	94.04	47.02	3.7374	0.029533 *
site age:hydrologic regime	3	50.27	16.76	1.3318	0.272474
site age:soil type	1	23.99	23.99	1.9065	0.172477
vegetative stratum:hydrogeomorphic class	4	13.35	3.34	0.2653	0.899106
vegetative stratum:hydrologic regime	3	17.55	5.85	0.4650	0.707779
vegetative stratum:soil type	2	66.01	33.00	2.6231	0.080884 .
vegetative stratum:site age	2	16.83	8.41	0.6687	0.516168
hydrologic regime:hydrogeomorphic class	2	3.23	1.62	0.1285	0.879618
Residuals	60	754.89	12.58		

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Table C2. ANOVA table for full model of predictor variables and interactions, with percent nitrogen as response variable. Model run using RStudio version 0.99.879 (Df = degrees of freedom; Sum Sq = sum of squares; Mean Sq = mean square; Pr(>F) = significance probability associated with F value).

Response variable: % Nitrogen					
Predictor variable	Df	Sum Sq	Mean Sq	F value	Pr(>F)
vegetative stratum	2	0.02884	0.01442	0.2971	0.74407
site age	1	0.12206	0.12206	2.5143	0.11808
hydrologic regime	5	0.07509	0.01502	0.3094	0.90546
soil type	1	0.07520	0.07520	1.5491	0.21811
hydrogeomorphic class	2	0.03740	0.01870	0.3852	0.68200
soil layer	1	1.91862	1.91862	39.5221	4.06e-08 ***
site age:hydrogeomorphic class	2	0.32948	0.16474	3.3935	0.04016 *
site age:hydrologic regime	3	0.21627	0.07209	1.4850	0.22771
site age:soil type	1	0.00567	0.00567	0.1168	0.73372
vegetative stratum:hydrogeomorphic class	4	0.08206	0.02051	0.4226	0.79172
vegetative stratum:hydrologic regime	3	0.06607	0.02202	0.4536	0.71568
vegetative stratum:soil type	2	0.07808	0.03904	0.8042	0.45221
vegetative stratum:site age	2	0.04960	0.02480	0.5109	0.60256
hydrologic regime:hydrogeomorphic class	2	0.10318	0.05159	1.0627	0.35194
Residuals	60	2.91273	0.04855		

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Table C3. ANOVA table for full model of predictor variables and interactions, with total carbon (Mg C ha^{-1}) as response variable. Model run using RStudio version 0.99.879 (Df = degrees of freedom; Sum Sq = sum of squares; Mean Sq = mean square; Pr(>F) = significance probability associated with F value).

Response variable: Total carbon (Mg C ha^{-1})					
Predictor variable	Df	Sum Sq	Mean Sq	F value	Pr(>F)
vegetative stratum	2	1664	832	0.3217	0.7261414
site age	1	1737	1737	0.6718	0.4156571
hydrologic regime	5	11231	2246	0.8686	0.5075805
soil type	1	7485	7485	2.8945	0.0940572.
hydrogeomorphic class	2	326	163	0.0631	0.9389008
soil layer	1	34727	34727	13.4293	0.0005264 ***
site age:hydrogeomorphic class	2	8788	4394	1.6992	0.1915074
site age:hydrologic regime	3	12367	4122	1.5942	0.2001999
site age:soil type	1	1	1	0.0004	0.9845020
vegetative stratum:hydrogeomorphic class	4	8205	2051	0.7932	0.5342483
vegetative stratum:hydrologic regime	3	5709	1903	0.7359	0.5347324
vegetative stratum:soil type	2	1504	752	0.2909	0.7486470
vegetative stratum:site age	2	2084	1042	0.4030	0.6700803
hydrologic regime:hydrogeomorphic class	2	4735	2368	0.9156	0.4058014
Residuals	60	155155	2586		

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Table C4. ANOVA table for full model of predictor variables and interactions, with total nitrogen (Mg N ha^{-1}) as response variable. Model run using RStudio version 0.99.879 (Df = degrees of freedom; Sum Sq = sum of squares; Mean Sq = mean square; Pr(>F) = significance probability associated with F value).

Response variable: Total nitrogen (Mg N ha^{-1})					
Predictor variable	Df	Sum Sq	Mean Sq	F value	Pr(>F)
vegetative stratum	2	8.87	4.435	0.3783	0.686618
site age	1	0.24	0.242	0.0207	0.886185
hydrologic regime	5	44.07	8.813	0.7519	0.587996
soil type	1	47.49	47.489	4.0513	0.048630*
hydrogeomorphic class	2	9.56	4.778	0.4076	0.667086
soil layer	1	115.43	115.432	9.8476	0.002636**
site age:hydrogeomorphic class	2	52.93	26.464	2.2576	0.113411
site age:hydrologic regime	3	49.61	16.535	1.4106	0.248493
site age:soil type	1	0.54	0.541	0.0462	0.830618
vegetative stratum:hydrogeomorphic class	4	45.75	11.439	0.9758	0.427563
vegetative stratum:hydrologic regime	3	33.17	11.057	0.9433	0.425503
vegetative stratum:soil type	2	4.18	2.088	0.1782	0.837254
vegetative stratum:site age	2	15.61	7.803	0.6657	0.517666
hydrologic regime:hydrogeomorphic class	2	39.30	19.648	1.6762	0.195727
Residuals	60	703.31	11.722		

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Table C5. ANOVA table for full model of predictor variables and interactions, with C:N ratio as response variable. Model run using RStudio version 0.99.879 (Df = degrees of freedom; Sum Sq = sum of squares; Mean Sq = mean square; Pr(>F) = significance probability associated with F value).

Response variable: C:N ratio					
Predictor variable	Df	Sum Sq	Mean Sq	F value	Pr(>F)
vegetative stratum	2	36.91	18.46	0.3589	0.699937
site age	1	419.67	419.67	8.1606	0.005874 **
hydrologic regime	5	160.77	32.15	0.6252	0.681094
soil type	1	30.05	30.05	0.5844	0.447587
hydrogeomorphic class	2	167.05	83.53	1.6242	0.205620
soil layer	1	21.76	21.76	0.4231	0.517867
site age:hydrogeomorphic class	2	386.83	193.41	3.7609	0.028920 *
site age:hydrologic regime	3	485.03	161.68	3.1438	0.031620 *
site age:soil type	1	17.40	17.40	0.3383	0.562963
vegetative stratum:hydrogeomorphic class	4	233.73	58.43	1.1362	0.348158
vegetative stratum:hydrologic regime	3	241.04	80.35	1.5623	0.207866
vegetative stratum:soil type	2	96.83	48.42	0.9415	0.395746
vegetative stratum:site age	2	277.24	138.62	2.6955	0.075686.
hydrologic regime:hydrogeomorphic class	2	656.71	328.36	6.3850	0.003062 **
Residuals	60	3085.60	51.43		

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Table C6. ANOVA table for full model of predictor variables and interactions, with bulk density (g cm^{-3}) as response variable. Model run using RStudio version 0.99.879 (Df = degrees of freedom; Sum Sq = sum of squares; Mean Sq = mean square; Pr(>F) = significance probability associated with F value).

Response variable: Bulk density (g cm^{-3})					
Predictor variable	Df	Sum Sq	Mean Sq	F value	Pr(>F)
vegetative stratum	2	0.12127	0.06063	2.0704	0.1350547
site age	1	0.26118	0.26118	8.9181	0.0040835 **
hydrologic regime	5	0.49690	0.09938	3.3935	0.0091379 **
soil type	1	0.33515	0.33515	11.4442	0.0012679 **
hydrogeomorphic class	2	0.50235	0.25118	8.5766	0.0005295 ***
soil layer	1	0.00000	0.00000	0.0000	0.9955097
site age:hydrogeomorphic class	2	0.10624	0.05312	1.8139	0.1718454
site age:hydrologic regime	3	0.08875	0.02958	1.0101	0.3946137
site age:soil type	1	0.23663	0.23663	8.0800	0.0061082 **
vegetative stratum:hydrogeomorphic class	4	0.35976	0.08994	3.0711	0.0228260 *
vegetative stratum:hydrologic regime	3	0.58521	0.19507	6.6609	0.0005883 ***
vegetative stratum:soil type	2	0.04477	0.02239	0.7644	0.4700874
vegetative stratum:site age	2	0.07452	0.03726	1.2724	0.2876210
hydrologic regime:hydrogeomorphic class	2	0.17502	0.08751	2.9882	0.0579293 .
Residuals	60	1.75716	0.02929		

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

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