Fall 2023

Biomarker study of Bellingham Bay : identifying how urbanization has affected carbon storage and eelgrass

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BIOMARKER STUDY OF BELLINGHAM BAY: IDENTIFYING HOW URBANIZATION HAS AFFECTED CARBON STORAGE AND EELGRASS

By Jessica L. Shulman

Accepted in Partial Completion

of the Requirements for the Degree

Master of Science

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MASTER'S THESIS

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Jessica L. Shulman

July 16, 2023

BIOMARKER STUDY OF BELLINGHAM BAY: IDENTIFYING HOW URBANIZATION HAS AFFECTED CARBON STORAGE AND EELGRASS

A Thesis

Presented to

The Faculty of

Western Washington University

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Abstract

Understanding sediment sources and fluxes throughout coastal zones is essential to evaluate shoreline stability, ecosystem health, and the potential for carbon storage. In Bellingham Bay, WA, like many developed coastal settings, urban areas have replaced forested cover and altered sediment fluxes, yet little is known of their offshore impacts. I analyzed n-alkanes, found in plant leaf waxes preserved in marine sediments of Bellingham Bay to characterize sediment sources and reconstruct changes in the relative contributions of eelgrass beds to sedimentary organic matter since pre-industrial times using a linear mixing model. Eight 2-meter-long cores were analyzed in order to determine how sediment sources have changed spatially throughout Bellingham Bay. Terrestrial plants contributed the majority of the organic matter to marine sediments in Bellingham Bay. n-Alkane biomarkers show a clear increase in terrestrial sources since pre-industrial times to modern day. The relative contribution of eelgrass to sedimentary organic matter peaked at 28% at a depth of 80 cm, which roughly correlates with the year 1712, and has been in steady decline since. This research has been conducted in collaboration with the U.S. Geological Survey Coastal Habitats in Puget Sound Project, which is examining the impacts of sediment transport on local marine environments. Results from my study show how eelgrass biomarker relative abundances corelate with long-term changes in carbon storage.

Acknowledgements

This manuscript was written because of the hard work from Camilo Ponton. He worked tirelessly to set up collaborative lab group environment, provide thoughtful comments, and share his wealth of knowledge in geochemistry. Although so many things went wrong with world events beyond our control which delayed lab work and prevented in-person meetings he still persisted and went above and beyond as an advisor. Thank you to the committee of Brady Foreman and Eric Grossman for your feedback and knowledge which helped shaped my thesis. Thank you to Ben Paulson who provided technical assistance and allowed us to complete our lab work safely. Thank you to the U.S. Geological Survey Coastal Habitats in Puget Sound Project who allowed us join in with their research. Thank you to graduate students Eston Worthington and Kiana Harris who provided valuable feedback and much needed friendship. Thank you to my partner Ryan Anders who provided me unconditional love and support throughout this entire process.

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1. Introduction

1.1 Importance of Coastal Ecosystems

The Pacific Northwest has experienced a rapid growth in population and urban development in recent decades at the expense of forested cover (Villarreal et al., 2017). This rapid change in land use has altered sediment budget and transport (Grossman et al., 2020) but little is known about sedimentation rates, organic matter sources, and carbon storage in coastal areas (Ward et al., 2003; McLeod et al., 2011). None of these data are readily available for Bellingham Bay, located in northwest Washington State, where urbanization has likely altered sediment transport (Czuba et al., 2011).

Coastal ecosystems are highly sensitive to changes in sediment flux (Kennish 2002). As such, alterations in sediment input to Bellingham Bay are expected to have significant effects on carbon storage, the overall health of the bay, and as a result the economy of Whatcom County. Eelgrass beds are a particularly important component of coastal ecosystems because they are highly productive habitats that stabilize sediment and support complex food webs (Phillips, 1984, Rubin et al., 2018). Eelgrass beds are at an exceptionally high risk because they are sensitive to sediment accumulation; too much sediment can result in burial and abrasion of eelgrass beds (Grossman et al., 2020), while insufficient sediment can result in depleted nutrients (Hauxwell et al., 2003; Czuba et al., 2011). In the last 25 years, eelgrass bed populations have been drastically reduced worldwide and much of this loss has been attributed to changes in sediment flux and resuspension (Dennison et al., 1993; Short and Wyllie-Echeverria, 1996; Hauxwell et al., 2003; Czuba et al., 2011; Rubin et al., 2018).

Bellingham Bay is an essential habitat for wild Chinook and other endangered salmon, as well as other marine animals and waterfowl (Elardo, 2001; Thom et al., 2014). Many of these species depend on sufficient eelgrass beds for feeding, habitat, and/or breeding (Louws, 2015; Sherman and De Bruyckere, 2018). Marine wildlife is also a vital asset for local economies. Whatcom County, which includes Bellingham Bay, was Washington State's second largest commercial fishing port in 2006, with an income of $13.5 million, accounting for 21% of the State fisheries (Louws, 2015).

There has been an increasing interest in assessing the long-term carbon storage potential of coastal ecosystems due to increased carbon dioxide in the atmosphere from anthropogenic sources, resulting in a warming climate (Möller ,1963; Hansen et al., 1997; IPCC, 2022). Carbon stored in coastal ecosystems is often referred to as blue carbon (Nellemann et al., 2009). Coastal ecosystems such as saltmarshes, mangroves, and eelgrass beds account for <2% of seafloor coverage, but account for up to 50% of carbon stored in marine sediments (Duarte at al., 2005). Despite their small coverage, eelgrass beds are very efficient at storing carbon due to their robust root system (Duarte at al., 2005; McLeod et al., 2011; Poppe and Rybczyk 2018; Prentice et al., 2020). Loss of eelgrass beds results in the release of carbon from marine sediments (Piñeiro-Juncal at al., 2021). To estimate how eelgrass bed populations have fluctuated over time in the Bellingham Bay watershed, biomarkers from eelgrass plants preserved in marine sediments were analyzed. Results from this study estimate how eelgrass biomarker relative abundances corelate with long-term changes in carbon storage and urbanization of the area.

1.2 Sedimentary Organic Matter

Sedimentary organic matter is composed of a complex mixture of compounds from various ages and sources (e.g., Diefendorf and Freimuth, 2017). Sediment transport from continental

surfaces to the seafloor results in mixing of organic carbon from different reservoirs (Berner, 2003; Blair et al., 2004; Blair and Aller, 2012). Biomarker analysis can be used to determine the different sources contributing to sedimentary organic matter (Peters et al., 2004; Eglinton and Eglinton, 2008; Diefendorf and Freimuth, 2017; He et al., 2019; He et al., 2020). Active margins, like the Pacific Northwest, are particularly effective at sequestering organic carbon on centennial to millennial time scales because particulate material experiences less sedimentary cycling (Blair and Aller, 2012; Vonk et al., 2019). The sedimentary cycle (storage, transport, deposition, preservation/decay) results in a mixture of organic carbon from various sources and ages preserved in marine sediments. An active margin will store more modern organic matter compared to passive margins which experience more cycling and therefore store more aged organic matter (Figure 1).

Plant leaf waxes are commonly used as biomarkers because they are insoluble in water, resistant to biodegradation and can survive in sediments for millions of years (Freeman and Colarusso, 2001; Peters et al., 2004; Eglinton and Eglinton, 2008). Plant waxes are comprised of straight-chain alkanes (referred to as n-alkanes), fatty acids, and fatty alcohols (Eglinton and Hamilton, 1967, Figure 2). n-Alkanes are long straight chain molecules, lacking a functional group making them especially long lived (Eglinton and Logan 1991). Plants typically produce n-alkanes with one or two dominant carbon chain lengths (Eglinton and Hamilton, 1967). This chain length preference can be used to differentiate terrestrial, aquatic, and primary producer's organic matter (e.g., Wang et al., 2013; Chevalier et al., 2015). Terrestrial plant n-alkane distributions are dominated by compounds of 27 to 31 carbons (C_{27}-C_{31}), aquatic sources of organic matter such as eelgrass mostly produce C_{21}-C_{25}, and primary producer's such as green algae are characterized by the dominance of C_{15} and the absence of n-alkanes longer than C_{20} (Figure 3; Chevalier et al., 2015; Peters et al., 2004). This unique chain length preference by different sources of organic

matter can be used in an end member mixing model (Chevalier et al., 2015). End member mixing analysis consists of selecting components of a mixture that have a well-defined composition ("end members") and using their unique signature to determine their approximate contribution to the mixture (Christophersen and Hooper, 1992). Calculating exact contributions to this mixture can be challenging as there is some overlap in sources. I have created and tested a new linear mixing model based on the Chevalier et al. (2015) model to predict end member relative contributions in Bellingham Bay and that could be applied elsewhere.

1.3 Study Area

Bellingham Bay is a semi-enclosed embayment in Northwestern Washington State. The bay itself is approximately 10 km wide and is relatively shallow with a maximum depth around 30 m (Figure 4). The modern landcover of the Bellingham Bay watershed consists primarily of forested cover (Figure 5). Located directly east of Bellingham Bay is the city of Bellingham. North of Bellingham Bay is a mix of urban developments and agricultural lands (Figure 5). Historical landcover estimates indicate the watershed was dominantly forested cover, with smaller areas of inland wetlands and grasslands before extensive areas were converted to agricultural and urban uses beginning in the mid-1800s.

Bellingham Bay receives drainage from several watersheds. The primary watershed being the Nooksack River Basin, which is approximately 2,050 km^2 (Anderson et al., 2019) between its North, Middle and South forks. The Nooksack River, which has built a large subaqueous delta on the north end of the bay, is the primary source of fresh water, sediment, and terrestrial organic matter to Bellingham Bay with a mean annual sediment load of 1.4 million tons (Czuba et al., 2011).

The Nooksack River originally flowed into both Bellingham and Lummi Bays until 1877 when western settlers removed a mile-long logjam and rerouted the entire river flow to its present course (Collins 1998). The diversion of the Nooksack River into Bellingham Bay altered sediment flux and rerouted a large but unquantified proportion of the river's fine sediment to the bay (Collins 1998). Until recently there were no precise estimates for sedimentation rates in Bellingham Bay. Studies of similar neighboring embayments such as Padilla Bay and Skagit Bay have sedimentation rates ranging from 0.13 to 2 cm/yr (Kairis and Rybczyk, 2010; Grossman et al. 2020). Based on studies using ^{210}Pb sedimentation rates in Bellingham Bay could range from 0.63 to 1.46 cm/yr (Takesue et al., 2023). It is important to accurately measure sedimentation rates because this directly affects marine vegetation, water quality, and carbon storage (Thornton et al., 1980; McLeod et al., 2011).

Bellingham Bay has a history of industry which included several sawmills and shingle mills (Louws, 2015). In the 1950s and 1970s there were manufactures of paper, chemicals, refined oil, aluminum, and processed food (Louws, 2015). Increased urban development and industry transformed land uses and potentially altered sediment discharge, grain size, mineral composition and the character and quality of organic matter to coastal areas, affecting eelgrass beds. Furthermore, increased commercial maritime activity required dredging of shallow embayments (WA State IDD, 1980) enhancing the disturbance of sediments and eelgrass beds.

1.4 Project Goals

Knowledge of ecosystems is limited to relatively short timespans making it difficult to assess the results of human impact on the environment. Recent urban and agriculture developments and subsequent changes in water quality and sedimentation rates are threatening coastal ecosystems in the Pacific Northwest (Czuba et al., 2011; Grossman et al., 2020). The purpose of

my thesis is to use n-alkanes as a biomarker to determine the sources and changes of organic matter flux to the sediments of Bellingham Bay. Furthermore, I evaluate changes in the relative abundance of eelgrass over time and its contributions to sedimentary organic matter, along with carbon storage and relate these variations to ecological shifts and human impact over time. To achieve these goals, biomarkers from multiple 2-meter-long sediment cores from Bellingham Bay were analyzed and a linear mixing model was utilized to interpret the complex mixture of biomarker signatures in marine sediments. In the process, the sensitivity of near mixing models used in similar studies, like Chevalier et al., (2015), was tested to evaluate how accurately these models can track changes in biomarkers from natural environments.

Sediment cores from Bellingham Bay record evidence of a pre-industrial ecosystem. By deconvolving the complex mixture of organic matter in sediment samples using a linear mixing, multiple end member model, I evaluated how increased urbanization and the resulting changes in sediment flux affect eelgrass beds and carbon storage in Bellingham Bay.

2. Methods

2.1 Core Sampling, Sample Processing and Storage

Sediment cores were retrieved through a collaboration between the Geology Department at WWU, the USGS Pacific Coastal and Marine Science Center and Shannon Point Marine Center on February 10-14th 2020. Cored locations along-shore and across-shore from the Nooksack River mouth (Figure 4) were selected to evaluate how sediments and organic matter accumulate across the bay. Using a vibracore drill and 2 m-long aluminum core liners, we were able to retrieve sixteen cores, two at each of the eight locations. The cores retrieved vary in length between 1.36-1.60 m long (Figure 6).

After retrieval, cores were stored in a cold room at (42 ^{o}F). The cores were then processed by first cutting the core liner with aluminum shears, then splitting the core in half (lengthwise). Immediately after opening, cores were described, and one half was photographed. Sediment subsamples were then analyzed for grain size (GS), total organic carbon (TOC), and δ^{13}C values.

Organic biomarker samples were taken in eight cores. Cores VC01-R1, VC02-R1, VC07-R1 and VC08-R1 were sampled every 20 cm. Cores VC03-R2, VC05-R1, VC06-R2, VC09-R1, and VC09-R2 were sampled every 10 cm (Figure 6). All sediment samples were 2 cm thick. Sampled material was stored individually in labeled plastic bags and placed in a freezer until further processing. Gloves were worn at all times during the sampling process, and the spatula was cleaned with deionized (DI) water and ethanol between every sample. Sediment samples were later dried at a low temperature (<40 °C), ground with a mortar pestle, and stored at room temperature in glass vials.

2.2 Vegetation End Member Sampling and Storage

In order to analyze the n-alkane distribution of the pure end members samples of fresh terrestrial leaves, pasture grass, fresh and degraded eel grass, and marine plankton were collected in the Bellingham Bay watershed. Pasture grass, hay, and plant leaves were sampled to represent terrestrial organic matter. Pasture grass and hay were sampled because they account for a significant portion of the landcover and were not present before urbanization of the area. Leaves were sampled from dominant trees such as Red Cedar and Douglas Fir. Pasture grass was sampled from Ben Paulson's property in the northwestern portion of the watershed (western Whatcom County), and tree leaves were sampled from the Sehome Hill Arboretum. All samples were stored at room temperature in brown paper bags, a subsample of each plant was stored in glass vials in a freezer (-4^{o} C).

Plankton samples were collected from Bellingham Bay on October 19th, 2020, by Eston Worthington at 48° 43.307 and 122° 34.53 using a net of mesh towed along the water column. Plankton samples were refrigerated after sampling and then processed in the lab that same day. To separate the plankton, water was decanted from the sample by centrifuge or filtered by gravity inside a pre-combusted glass fiber extraction thimble. Plankton were put in the centrifuge at high (1000 rpm) and low (600 rpm) speeds for 6 minutes each. The supernatant liquid was poured off both samples. All plankton samples were then stored in a freezer (-4^{o} C).

Eelgrass samples were taken at Larrabee State Park on October 4th, 2020, at low tide (11:00AM). During the low tide the beach was able to be walked and live eelgrass beds were sampled. Samples were stored in a brown paper bag and refrigerated until further processing. The following day samples were cleaned to remove any sediment or crustaceans. Samples were then rinsed with DI water and placed on aluminum foil. After cleaning samples were photographed and identified as *Zostera marina*, the native eelgrass species to the area (Poppe and Rybczyk, 2018). Samples were then stored using four different methods; (i) samples were transferred to a glass jar and stored in a refrigerator, (ii) samples were transferred to a glass jar and stored in a freezer, (iii) samples were dehydrated by placing eelgrass in silk paper and applying pressure, (iv) samples were dried overnight in an oven at low temperatures (<40 °C).

2.3 ^{14}C Dating

For the purposes of this study sedimentation rates were calculated using ^{14}C data of organic matter found in the sediment cores. After Samples containing at least 1 mg of carbon were selected from the deepest portions of the cores to estimate the age and sedimentation rate of the cores. Sampled material included shell material from VC05 at 153 cm depth, a mostly intact bivalve shell from VC06 at 116 cm depth and wood material from VC09 at 130 cm depth (Figure 6). Samples

were cleaned, sonicated to remove fine detritus, and submitted to the UC Irvine Keck-CCAMS facility for further analysis. UC Irvine treated the wood sample with an acid-base-acid (1N HCl and 1N NaOH, 75°C) prior to combustion. Carbonate shell samples from VC05 and VC06 were leached with 10% dilute HCl prior to hydrolysis with 85% phosphoric acid. All results were corrected for isotopic fraction (Stuiver and Polach 1977) with $\delta^{13}C$ values measured on a prepared graphite using the AMS spectrometer.

Raw ages were corrected to years BP using the following methodology. The shell material found in VC05 and VC06 was corrected using the average reservoir age (Table 1) corrections from multiple studies (Robinson and Thompson 1981, Hutchison et al., 2004, McNeely et al., 2006). No data for reservoir age of Bellingham Bay existed so averaged data from studies around Puget Sound, British Columbia, and the Oregon coast were used to make reservoir corrections. Data averaged from Robinson and Thompson 1981, Hutchison et al., 2004, and McNeely et al., 2006 estimated the reservoir correction for Bellingham Bay to be -253 ±52 years BP. After adjusting for the reservoir age, calibrated ages were determined using Calib Rev 8.1.0 radiocarbon calibration program (Stuiver and Reimer, 2021).

Table 1. Locations and sources of reservoir age corrections for the ten nearest measurements to Bellingham Bay. Sourced from calib.org

Lon	Lat	Reservoir Age Uncertainty	Reservoir Age	Locality	Location
148	-123	48.6	199	Robinson, S :1981	Orcas Island, WA
151	-123	48.6	344	Robinson, S :1981	Orcas Island, WA
927	-122.5	48	233	McNeely R., :2006	Puget Sound, WA
917	-123.1	48.2	265	McNeely R., :2006	San Juans, WA
920	-123.37	48.43	271	McNeely R., :2006	Victoria, BC
929	-123.37	48.43	236	McNeely R., :2006	Victoria, BC
926	-123.2	49.3	255	McNeely R., :2006	Burrard Inlet, BC
918	-123.2	48.1	256	McNeely R., :2006	Port Crescent, WA
150	-123.43	48.43	146	Robinson, S :1981	Esquimalt, BC
924	-123.7	48.92	257	McNeely R., :2006	Chemainus Bay, BC

2.4 Sedimentation Rates

After making the appropriate corrections for reservoir age and determining the calibrated ages, sedimentation rates were calculated for each core. Calculated calendar ages of each sample were used relative to the sample depth to determine the sedimentation rate for each core. Sedimentation rates were calculated assuming the top of the core as calendar year 2019.

2.5 Loss on Ignition (LOI)

Loss on Ignition (LOI) was used to estimate the amount of water, organic matter, carbonate, and siliciclastic content in sediment core sub-samples. A ceramic crucible was cleaned and put in an oven overnight at 110 ºC to remove any moisture. After drying ceramic crucibles were weighed,

and ~2 g of sediment was added to each crucible and the combined weight was recorded. Sediment samples were dried at 110 ºC for 24 hours, and then a new weight was recorded and subtracted from the initial weight to determine sediment moisture content. The ceramic crucibles were then placed in an oven at 550 ºC for 4 hours. After allowing time for crucibles to cool the combined weight of the crucible and the sample after ignition was recorded. Organic LOI % was calculated by dividing the weight change of the sample after ignition by the fresh sample weight after moisture content was removed. Ceramic crucibles were then placed in a 1000 ºC oven for 2 hours. After allowing time for crucibles to cool the combine weight of the crucible and the sample after ignition was recorded. Carbonate LOI % was calculated by dividing the weight change of the sample during ignition by the sample weight after 550 ºC ignition.

2.6 n-Alkane Analysis

End members sampled included leaves, grasses, eelgrass, and plankton. Eelgrass, grass, and leaves were dried for at least 24 hours. Plankton material was dried and then frozen to prevent decay. Dry leaves, dried eelgrass and frozen plankton were placed individually in a pre-combusted 8ml glass vial with dichloromethane:methanol (9:1, v/v) solution. The beakers with their respective sample were placed in ultrasound cleaner for 1 minute. The total lipid was then blown to dryness at 60 °C over a slow nitrogen flow and stored in the freezer.

Concentrated total lipid extract (TLE) was then loaded into a 2% deactivated silica gel chromatography column and separated into compound classes. The n-alkane fraction was eluted using 10 Ml of hexane and then blown to dryness. For further purification, the n-alkane fraction was loaded onto a $AgNO_3$ coated silica gel chromatography column and eluted with 10 Ml of hexane, blown down fully and stored in the freezer. To test for possible organic contamination in

the total lipid extraction process, a sample of combusted quartz sand was run through the above procedure as a blank.

Sediment samples were dried at ~40º C for 48 hours minimum. Dried samples were ground with a mortar and pestle and stored at room temperature. All tools were cleaned with methanol between samples. Lipids were extracted in a Soxhlet distillation apparatus, a glass condenser commonly used to extract organic matter, for 12 hours in 100Ml of dichloromethane:methanol (9:1, v/v). The Soxhlet, condenser and sediment thimble were all cleaned and rinsed between each run with deionized water as well as dichloromethane and methanol. Total lipid extract (TLE) was fully blown down at 60° C over slow nitrogen flow.

All samples were processed with gas chromatography (GC) analysis. GC analyses were performed using a Varian CP3800 gas chromatograph equipped with aflame ionization detector (FID). A DB-X5 capillary column (30 m × 0.25 mm × 0.25 μm) was used. The GC oven temperature was programmed from 50 to 300 °C at a rate of 3 °C min-1 and held for 20 min. Helium was used as the carrier gas. An auto sampler system injected 5 Ml out of 50-500 Ml of sample according to varying n-alkane concentrations. Hexane blanks were injected before, during, and after each sample sequence. Compound identification was performed by comparison to an external standard injected at the beginning and the end of every sample sequence. The referenced standard was a SUPELCO TraceCERT reference material of n-alkane compounds C7-40. The concentration of the standard was adjusted to match the concentration of the samples in chromatography. The relative abundance was then determined by integrating the area under the peaks from chromatography.

Common parameters used to estimate the composition of sedimentary organic matter from measures of relative abundance include Average Chain Length (ACL), Carbon Preference Index

(CPI), and Terrestrial Aquatic Ratio (TAR). ACL considers all chain lengths present in a mixture and is used to calculate the mean chain length in a sample. A high ACL can indicate that sources are more terrestrial in origin while a low ACL can indicate that sources are more likely from primary producers. The ACL was calculated from the chromatograms using the relative abundance of n-alkanes C_{15} through C_{33}.

$$ACL = (C_{15} * 15 + C_{16} * 16 + \cdots + C_{32} * 32 + C_{33} * 33) / (C_{15} + C_{16} + \cdots + C_{32} + C_{33}) \ (eq. 1)$$

The CPI measures the relative abundance of odd over even chain length n-alkanes (Marzi et al., 1993). A CPI greater than one indicates a sample has more odd n-alkanes, while a CPI less than one indicates a sample has more even n-alkanes. The CPI can be used as an indication of preservation because it has been observed that plants have a preference for producing odd chain length n-alkanes (Eglinton and Hamilton, 1967). Odd chain length n-alkanes will eventually decay into even chain length n-alkanes, therefore CPI's closer to one indicates degradation of organic matter or poor preservation (Cranwell 1981). The CPI was calculated as described in eq. 2 from the chromatograms using the relative abundance of n-alkanes C_{21} through C_{35} using the equation described in Marzi et al., 1993.

$$CPI = \frac{\sum_{odd}(C_{21-33}) + \sum_{odd}(C_{25-35})}{2 * \sum_{even}(C_{22-34})} \ (eq. 2)$$

The Terrestrial to Aquatic ratio (TAR) is an empirical ratio used to estimate the sources present in a mixture of sedimentary organic matter (Bourbonniere and Meyers 1996). The TAR assumes that all C_{27} – C_{31} are terrestrial in origin and that all C_{15} – C_{19} are aquatic in origin. TAR does not consider the n-alkane signature of primary producers or account for the overlap in n-alkane signature for terrestrial and aquatic organic matter.

$$TAR = (C_{27} + C_{29} + C_{31} / C_{15} + C_{17} + C_{19}) \ (eq. 3)$$

2.7 Landcover Maps

Percent landcover was calculated using the 2016 C-CAP High Resolution Land Cover from NOAA and watershed shapefiles for the state of Washington from United States Department of agriculture. Using ARC-GIS landcover data was clipped onto the Bellingham Bay watershed and the percentage landcover was calculated for each of the 24 NOAA landcover types. Landcover data from NOAA only included the United States portion of the watershed. The less than ten percent of the watershed located in Canada was not included in the analyses. The n-alkane distributions of different types of organic matter are the focus of this study, therefore land-cover classifications were grouped based on vegetation cover (i.e. type of plant life). After regrouping, the original set of twenty-four landcover classifications were reduced to the following ten: urban, cultivated crop, grasses (natural and agricultural), forested cover, wetlands (inland and tidal), unconsolidated shore, bare land, and water. Definitions of reach landcover type are as follows:

1. Urban area: This classification contains three levels of urban development defined by NOAA (high, medium, and low). These areas are all described as having significant land covered by concrete or constructed materials.
2. Cultivated crop: Describes areas that are managed for production of annual crops. This includes mostly C3 plants such as wheat, barley, and fruiting plants.
3. Grassland: Includes dominantly C4 terrestrial plants such as grasses and sedges. This category only represents naturally occurring grasslands.
4. Pasture/hay: Includes developed open spaces and pasture hay as defined by NOAA. This category represents man-made areas of grasses.

5. Forested landcover: Includes deciduous forest, evergreen forest, mixed forest, and scrub/shrub land cover classifications. These landcover types all contain dominantly C3 terrestrial plants.
6. Inland wetland: Palustrine wetlands and palustrine aquatic beds.
7. Tidal wetland: Estuarine wetlands, and estuarine aquatic beds.
8. Unconsolidated shore: Defined as areas with silt, sand or gravel that are subject to reworking by water.
9. Bare land: Includes areas barren of vegetation and areas covered by snow/ice as defined by NOAA.
10. Open water: Includes areas of water with less than 25% cover of vegetation or soil.

Data developed by Utah State University in 2004 for the WRIA 1 Watershed Management Project was used to create the historic landcover maps for the Bellingham watershed. This map approximates landcover of the watershed before significant human intervention, around 200 years ago, using interpreted data from soil surveys in Whatcom County (Winkelaar 2004; Gill et al., 2006). The historic landcover data set included ten landcover classifications, these were grouped into the same categories as the modern landcover map. Four landcover classifications from the modern map did not apply or were unmappable in this data set: cultivated crop, pasture/hay, urban cover, and tidal wetlands.

3. Results

3.1 Landcover

Modern and historical landcover maps produced for the Bellingham Bay watershed show the extent and location that land use has changed since modern development (Figure 5, Figure 7). Forested landcover is the dominant landcover class in both the historical and modern watershed,

although it has decreased from 87% landcover to 68% (Figure 8a). The second largest area of landcover in the modern data set was pasture/hay. Pasture was completely absent historically and now represents 11% of the watershed. In the historic watershed in-land wetlands and barren land were tied for the second largest area of landcover, each accounted for 6% of the watershed. Forested cover had the largest decrease of any landcover classification, losing over 400 km^2 of area (Figure 8b). In-land wetlands suffered the second largest decrease in landcover with just under 100 km^2 of area lost (Figure 8b). Grasslands and pasture/hay areas had the largest increase of any landcover classification increasing their cover by over 300 km^2 (Figure 8b). Landcover changes most drastically adjacent to Bellingham Bay, where forested cover and wetlands are replaced by urban and agricultural land (Figure 5, Figure 7). Historical landcover did not include data for Bellingham Bay itself, therefore unconsolidated shore and tidal wetlands were excluded in landcover comparison data.

3.2 Radiocarbon Dating and Sedimentation Rates

Raw carbon ages for the sampled shell material from VC05 and VC06 were over 1,000 years old, but after accounting for the reservoir age (-253 ±52 years) the calculated calibrated age ranged from 1693 to 1820 for the shell material in VC05 and 1496 to 1646 for the shell material in VC06. The wood sample from VC09 did not need to be adjusted for the reservoir age. The calibrated age for the wood sample from VC09 contained three possible ranges due to atmospheric changes in carbon, these ranges are 1697-1723, 1813-1835, and 1880-1910. Sedimentation rates were estimated for each core based on the depth and age range of the sampled material. Sedimentation rates for VC06 were the lowest at 0.22 to 0.31 cm per year. Sedimentation rates for VC05 were 0.47 to 0.76 cm per year. Sedimentation rates for VC09 contained three possible ranges due to the three separate age ranges, 0.40 to 0.43 cm per year, 0.63 to 0.70 cm per year, and 0.83

to 1.18 cm per year (Table 2). Results found based on ^{14}C dating generally agree with sedimentation rates found by Takesue et al. 2023.

Table 2. Summary of results from ^{14}C date where BP is time before 1950, calBP corrections were calculated using CALIB radiocarbon calibration program. Corrected ages are reported to 1 sigma standard deviation.

Sample	Raw Age (yrs.)	Reservoir Age (yrs.)	Calibrated Age (cal BP)	Depth (cm)	Estimated Sedimentation Rate (cm/yr.)
VC05-R1 Shell	1025±15	253 ±52	1693-1820	153	0.47-0.76
VC06-R2 Shell	1175±15	253 ±52	1496-1646	116	0.22-0.31
VC09-R1 Wood	105±15	N/A	1697-1723 1813-1835 1880-1910	130	0.4-0.43 0.63-0.70 0.83-1.18

3.3 Core Description

The cores consisted of dark gray to brown silts and clays, with sulfidic odor, and a gelatinous consistency (Figure 2). Upon opening, cores showed distinct variation in colors of mud with visible layers, but after oxidation all cores displayed a consistent dark gray color. VC08 had a distinct sandy layer at 50 cm depth. Small fragments of shells were found in VC02, VC03, VC05, VC06, VC07, and VC08. Shell fragments found were small, around 2-3 mm in length. No complete, articulated shells were found. Wood fragments and debris were found in cores VC02, VC03, VC05, VC08, and VC09. Wood material and fragments were identified as small fibrous material in sampled mud, ranging from 2-10 mm in length. VC09 contained a distinct layer of

woody material unlike anything found in the other cores (Figure 6). VC09 was located closest to the town of Bellingham, so it is possible that the woody layer was the result of dredging or other anthropogenic activities.

3.4 Sedimentary Organic Matter

Sediment samples when dried weighed approximately 45 g per 45.6 cm^3 of material. LOI of carbonate material for all samples averaged 3.12% with a standard deviation of 0.46% and was stable downcore (Table 3). All samples were primarily composed of siliciclastic material, averaging 90.73% across all sediment samples.

LOI for organic material ranged from 3.6% to 22.5% and averaged 6.15% across all samples. Overall, most cores show a decreasing trend in LOI of organic material downcore. The top 10 cm of all cores was on average 6.32% organic material, while the bottom 10 cm averaged 5.45% organic material. However, cores VC07, VC08, and VC09 did not follow this trend. Cores VC07 and VC08 show almost no change in LOI for organic material downcore, both were relatively uniform around 6% organic matter. VC09 showed or had a large spike of 20% LOI near 130 cm (Figure 9). This depth correlated with a massive woody layer that was unique to VC09.

Table 3: Loss on Ignition percentages for organic matter from various depth in all cores.

Sample	Depth (cm)	% LOI Organic	Sample	Depth (cm)	% LOI Organic
VC01	0	5.34	VC06	0	7.19
	20	3.69		10	5.74
	40	4.13		20	5.60
	60	5.66		30	5.60
	80	4.53		40	5.85
	100	4.51		50	4.42
	120	5.17		61	4.26
	125	4.69		70	4.94
VC02	0	6.15		80	4.22
	40	6.21		90	4.82
	80	5.21		100	4.96
	100	5.90		110	5.26
	140	5.70		120	5.08
	149	6.18		130	4.99
VC03	0	7.26		140	4.66
	10	6.44		148	4.92
	20	5.95	VC07	0	5.65
	30	6.64		80	5.31
	40	6.36		152	6.09

Sample	Depth (cm)	% LOI Organic	Sample	Depth (cm)	% LOI Organic
VC03 continued	50	6.25	VC08	0	6.30
	60	5.44		20	7.25
	70	6.42		60	6.21
	80	5.43		80	6.12
	90	6.22		151	5.85
	100	6.37	VC09	0	5.97
	110	6.36		20	7.03
	120	6.44		40	7.17
	130	5.82		70	7.22
	140	6.15		80	5.99
	147	6.22		100	6.24
VC05	0	6.69		120	6.47
	70	5.25		128	14.85
	150	5.27		130	22.48
	155	5.25		136	8.10
				142	6.11

Organic material preserved in all cores was relatively high (> 5% organic matter). LOI was utilized to quantify the amount of organic matter preserved in sediment samples from Bellingham Bay. When combusting organic material more than just organic carbon is lost on ignition. Other elements such as Hydrogen, Nitrogen, Oxygen, and Sulfur can be lost on ignition. Therefore, the

LOI technique alone can over represent organic carbon in a sample. To estimate total organic carbon (TOC) from LOI results the following formula was utilized (Dean 1974).

$$TOC = LOI / 2.13 \text{ (eq. 7)}$$

TOC ranged from 1.7% to 10.5%. The highest amount of TOC was observed in the bottom of VC09 and correlated with woody material that was unique to that location. Carbon storage in all cores was relatively uniform downcore, except for a significant decrease in the top 20 cm.

3.5 n-Alkane Signatures of Organic Matter and Sediment Cores

N-alkanes relative abundances for all organic matter and sediment samples were measured from C_{15} to C_{33} (Table 4). Terrestrial plants such as native coniferous trees and various pasture grasses peaked at long, odd chain n-alkanes between C_{25} to $C_{31.}$ On average terrestrial organic matter peaked the most at n-alkanes length C_{29} and C_{31} with 22.74% and 24.39% contribution respectively. Eelgrass n-alkane distributions peaked at C_{17} through C_{21}. The strongest peak for eelgrass was at n-alkanes length $C_{19,}$ which accounted for 49.25% of the n-alkanes measured in eelgrass.

Table 4: n-alkane relative abundance (percentage) distributions of all sampled organic matter

	Douglas Fir	Red Alder	Spruce	Pasture Grass 1	Pasture Grass 2	Pasture Grass 3	Eelgrass
C15	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
C16	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
C17	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	**12.55**
C18	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	**0.72**
C19	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	**49.25**
C20	**0.53**	n.d.	n.d.	n.d.	n.d.	n.d.	**1.45**
C21	**0.59**	n.d.	**18.35**	n.d.	n.d.	n.d.	**10.32**
C22	**2.51**	n.d.	**4.77**	n.d.	n.d.	n.d.	**2.06**
C23	**26.04**	**2.50**	**7.54**	n.d.	n.d.	n.d.	**9.85**
C24	**6.03**	**1.26**	**6.99**	n.d.	n.d.	n.d.	**1.97**
C25	**26.50**	**27.77**	**9.20**	n.d.	**7.03**	**1.41**	**4.51**
C26	**2.36**	**4.62**	**8.30**	n.d.	n.d.	n.d.	**2.69**
C27	**8.03**	**51.10**	**9.36**	**1.57**	**3.65**	**3.78**	**1.50**
C28	**2.75**	**1.82**	**3.10**	n.d.	n.d.	n.d.	**1.75**
C29	**18.71**	**10.92**	**11.10**	**37.25**	**6.71**	**51.75**	**0.90**
C30	**1.02**	n.d.	**5.82**	**2.03**	n.d.	n.d.	**0.48**
C31	**4.35**	n.d.	**12.42**	**56.41**	**35.91**	**37.22**	n.d.
C32	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
C33	**0.57**	n.d.	**3.05**	**2.74**	**46.70**	**5.84**	n.d.

Primary producers from Bellingham Bay, such as plankton, were dominated by short, odd chain n-alkanes between C_{15} and C_{19} and peaking in relative abundance at C_{17}. However, these results were unclear when compared to other samples. While other chromatograms of the sampled end members had a flat line with distinct peaks, the plankton sample had a distinct hump caused by a high background frequency from unknown sources (Figure 10). This high background signal is suspected to be related to the plankton being a complex mixture of other organic compounds that are challenging to differentiate from the plankton. Because of this, data from Chevalier's study was used in place to represent primary producers in this case. Chevalier's data for primary producers peaked at C_{15} through C_{17}, with only extremely minor contributions of higher chain length n-alkanes. Each measured source of organic matter to Bellingham Bay had a distinct peak and range of n-alkanes (Figure 11, Table 5).

n-Alkanes extracted from VC01 and VC06 were characterized mainly by odd n-alkanes ranging from C_{17} to C_{33} (Figure 12, Supplementary). The average chain length across all samples was 25.76.

n-Alkane distributions extracted from VC06 have ACLs (eq. 1) ranging from 24.21 to 26.22 and are relatively stable downcore. Standard deviations were less than 1 for all samples (Table 6, Figure 16, Supplementary). ACL decreases from the top of the core until 80 cm depth where it begins to increase again until the bottom of the core.

The CPI (eq. 2) downcore in VC06 ranged from 2.29 to 6.26, peaking at 80 cm depth. The CPI was greater than one for all samples downcore indicating an odd-over-even predominance in n-alkanes from the marine sedimentary organic matter (Table 6). The TAR (eq. 3) is greater than one for all samples down core in VC06. There is a strong increase in TAR at 140 cm in VC06.

4. Discussion and Data Analysis

4.1 Landcover

Landcover in Bellingham has shifted to meet the needs of its growing population. Over 400 km^2 of forested cover and wetlands have been replaced by agricultural and urban areas (Figure 8b). Removal of forested cover can result in increased rates of erosion due to a lack of roots holding top soil together, leading to increased sedimentation rates in Bellingham Bay (McLeod et al., 2011; Ward et al., 2003). Urban watersheds compared to forested watersheds show an increase in suspended sediment load in run off (Corbett et al., 1997). Increased sedimentation rates could lead to burial and death of eelgrass beds, which are vital carbon sinks and ecosystem due to their complex root system which helps to trap carbon in sediment (Piñeiro-Juncal at al., 2021). Thus, changes in landcover to an urban watershed could lead to an overall long-term decrease in carbon sequestration as well as an increase in sedimentary organic matter from terrestrial sources. Landcover data was not available for tidal wetlands which contain significant amounts of eelgrass beds. The linear mixing model will be able to estimate contributions of eelgrass beds and could be used as a proxy to estimate changes in eelgrass beds.

Forested cover areas in the Pacific Northwest contain mostly C_3 plants such as conifer and deciduous trees while agricultural areas contain mostly C_4 plants, such as grasses and hay. This change in landcover has resulted in a distinct shift from very little C_4 plants to over a tenth of the watershed currently being covered in C_4 plants. C_3 and C_4 plants will show a significant difference in their stable carbon isotope signature, with C_4 plants being heavier in ^{13}C than C_3 plants due to how these different plants process carbon during photosynthesis (Vogel, 1993). Further detailed analysis on compound specific isotope data of C_3 vs C_4 would provide further insight.

4.2 Sedimentation Rates

Sedimentation rates were calculated based on carbon dates of shell and wood material found in VC05, VC06, and VC09. Calculated rates based on age and depth suggest a sedimentation rate between 0.22-1.18 cm of sediment per year (Table 2). The relatively high sedimentation rate, presence of a distinct woody layer from 110 cm to 130 cm, and VC09's proximity to several sawmills, shingle mills and paper manufacturers makes it likely that the wood material is anthropogenic in origin and not a reliable tool for an age model. Excluding sedimentation rates from VC09, estimated sedimentation rates in Bellingham Bay ranged from 0.22 to 0.76 cm per year. This range of sedimentation rates are consistent with rates found in nearby embayment's such as Padilla and Skagit bays, which have sedimentation rates ranging from 0.13 to 2 cm/yr (Kairis and Rybczyk, 2010, Grossman et al. 2020). Preliminary results (Takesue et al., 2023) indicate activities of 210Pb that support slightly higher rates of sedimentation than calculated here.

Sedimentation rates were calculated assuming constant accumulation, however it is likely this is not the case. The main source of sediment to Bellingham Bay is the Nooksack River (Czuba et al., 2011). Sediment loads in the Nooksack River are not constant and large discharge events cause more sediment to be deposited (Anderson et al., 2019). Changes in precipitation, which affect the discharge of the river, can cause sedimentation rates to vary over time. Changes in landcover in the watershed from forested cover to agriculture and urban areas can also increase erosion and influence sedimentation rates. It is likely that recent development of urban areas and subsequent decreases in forested cover have increased sedimentation rates up core.

Increases in sedimentation rates can negatively affect the health of eelgrass beds and consequently carbon storage in Bellingham Bay (Kennish 2002; Rubin et al., 2018). Because eelgrass beds are sensitive to sediment accumulation accurate estimates of sedimentation rates in

Bellingham Bay over time are essential in the preservation of eelgrass beds and furthering understanding of carbon storage in Bellingham Bay. Further research should be done to quantify how sedimentation rates have changed in Bellingham Bay as a result of urbanization.

4.3 Mixing Model

The mixing model was developed to incorporate the three main sources of organic matter in Bellingham Bay, terrestrial organic matter, aquatic organic matter, and organic matter from primary producers. n-Alkane distributions for terrestrial plants were measured from trees and pasture grasses found in the watershed. All n-Alkane distributions from terrestrial organic matter were grouped and averaged to create the terrestrial end member. Landcover data for the watershed (Figure 5) suggest that forested cover accounts for 86% of terrestrial organic matter and pasture grasses account for 14% of terrestrial organic matter. The end member for terrestrial input was calculated as a weighted average based on all sampled tree and grass material.

$$Terrestial = 0.86(Tree\ Cover) + 0.14(\ Pasture\ Grass\ )\ eq.4$$

The aquatic end member in the mixing model was based off n-alkane distributions from eelgrass sampled from Bellingham Bay. The primary producer end member in the mixing model was based off n-alkane distributions of algae from Arcachon Bay, France (Chevalier et al., 2015). Primary producers in Bellingham Bay vary throughout the year, with photosynthetic plankton being much more active in the summer (Harris, 2012). Future studies could sample primary producers throughout the year to improve the accuracy of this end member in Bellingham Bay, WA.

The end members from the Bellingham Bay watershed and Arcachon Bay each have unique n-alkane distributions, supporting the validity of an end member mixing equation (Figure 13, Table 5). As suggested by previous studies (Chevalier et al., 2015), primary producers peak at low chain

lengths, aquatic plants peak at mid chain lengths, and terrestrial plants peak at high chain lengths. Because each end member has a unique n-alkane distribution, a mixing model can be used to calculate the approximate percentage that each end member is contributing to a complex mixture of sources, like marine sediments collected from Bellingham Bay.

Table 5: Summary of the relative abundances (%) of n-alkanes in each sampled end member

	Primary		
	Terrestrial	Producers	Aquatic
C_{15}	0	2.28	0
C_{16}	0	0	0
C_{17}	0	56.79	12.55
C_{18}	0	15.18	0.72
C_{19}	0	25.74	49.24
C_{20}	0.15	0	1.45
C_{21}	5.43	0	10.32
C_{22}	2.09	0	2.06
C_{23}	10.34	0	9.85
C_{24}	4.09	0	1.97
C_{25}	18.59	0	4.51
C_{26}	4.38	0	2.69
C_{27}	20.05	0	1.49
C_{28}	2.20	0	1.75
C_{29}	16.14	0	0.90
C_{30}	2.06	0	0.48
C_{31}	10.85	0	0
C_{32}	0	0	0
C_{33}	3.62	0	0

Chevalier et al. (2015) utilized n-alkane distributions in marine sediments from a shallow marine embayment (Arcachon Bay, France), to determine the individual contributions of different carbon sources to sedimentary organic matter. I have utilized the same framework to create a mixing model for Bellingham Bay based on results from end member n-alkane distributions. Compounds with significant overlap between end members were adjusted in the mixing model equations. Note the equations listed below only consider the odd chain lengths as these are the most prominent compounds in plants. Relative contributions for each source were calculated as follows:

Terrestrial contribution: The contribution of terrestrial organic matter to sedimentary organic matter will be estimated assuming that all odd long chain n-alkanes $C_{25\text{-}33}$ are exclusively produced by terrestrial plants. Both aquatic plants (e.g., eelgrass) and terrestrial plants produce C_{23} (Figure 11). To account for this overlap the ratio of C_{23} to odd long chains C_{25}-C_{33} in terrestrial end members was used to estimate C_{23} produced by terrestrial plants in a mixture.

$$\%Terrestrial = \frac{(C_{25-33}) + t_r(C_{25-33})}{(C_{15-33})} * 100 \; (eq. 5)$$

where $t_r = \frac{C_{23}}{(C_{25}-C_{33})}$ in terrestrial plants

Based on results of sampled end members t_r= 0.15

Aquatic plant contribution: The contribution of aquatic plants will be estimated assuming that all $C_{19\text{-}21}$ n-alkanes are produced primarily by eelgrass. Both aquatic plants and primary producers produce C_{17} and the ratio of C_{17} / $C_{19\text{-}21}$ in eelgrass sampled is accounted for by m_r. The term $m_r * C_{19-21}$ accounts for C_{17} that eelgrass is contributing to the

mixture. The term -t_r (C_{25}-C_{33}) accounts for the overlap in C_{23} for eelgrass and terrestrial sources.

$$\%Aquatic = \frac{(m_r * (C_{19-21})) + (C_{19-23}) - (t_r * (C_{25-33}))}{(C_{15-33})} * 100 \quad (eq.6)$$

$$where\ m_r = \frac{(C_{17})}{(C_{19-21})} \text{ in aquatic plants}$$

Based on results of sampled end members m_r=1.5

Primary Productivity contribution: The contribution of primary producers such as plankton and algae will be estimated assuming that $C_{15\text{-}17}$ is primarily from algal origin. The term -m_r*C_{19}-C_{21} accounts for the overlap in C_{17} with aquatic plant sources.

$$\%Algae = \frac{(C_{15-17}) - (m_r * C_{19-21})}{(C_{15-33})} * 100 \quad (eq.7)$$

This model is specific for Bellingham Bay, but the ratios t_r and m_r could be re-calculated and applied for any basin in the world with significant amounts of terrestrial plants, aquatic plants, and primary producers.

In order to test the validity of the mixing model, a series of theoretical mixtures with known, varying amounts of contribution from end members was created and run through the model (Figure 14). For the purpose of this study analyzing changes in eelgrass populations was of the most interest; therefore, the ratio of aquatic plants was adjusted in each scenario. These scenarios

were intended to test the model sensitivity, or how well the model can detect small amounts of changes to an end member. Although each end member has its own unique chain length, there is some overlap so it is not clear how much each source might be contributing to a mixture. This was illustrated by creating graphs of the theoretical mixtures with no division between end members contributions (Figure 14). To test how accurately the model can predict small changes in eelgrass concentrations an iterative loop experiment was created. This model was designed to slightly adjust the percentage of known contributions of aquatic plants in several unique mixing scenarios. Calculated contributions from the model were compared to the known contributions of each source and the difference was graphed for each scenario (Figure 15).

Percent accuracy was calculated based on the difference between percentages the linear mixing model calculated and the actual percentage of each end member in a theoretical mixture. For example, in a 1:1:1 mixture there is a contribution of 33.33% from aquatic plants, primary producers, and terrestrial sources to the total sedimentary organic matter. The model predicted contributions of 34.29 % from aquatic plants, 33.02% from primary producers and 32.68% from terrestrial sources. In this case the model predications deviated by less than 1% for each end member.

Results for more extreme scenarios were less accurate. For example, scenarios were run for sedimentary organic matter mixtures with one tenth the amount of aquatic plant contribution to five times the amount of aquatic plant contribution when compared to terrestrial and primary producers. In these theoretical scenarios when aquatic plant contribution was dominant, mid chain lengths dominated the mixture, and when aquatic plant contributions were minimal the mixture was dominated by short and long chain n-alkanes (Figure 14). In these extreme scenarios with

either very little aquatic plant contribution or aquatic plant dominance, accuracy for predicting end member contributions decreased when compared to the 1:1:1 mixture (Figure 14, Figure 15).

Based on this sensitivity test, even in the extreme scenarios the model was not off by more than 5% (Figure 14, Figure 15). However, it should be noted that the model's accuracy decreases as the end member mixture moves away from the 1:1:1 scenario (Figure 15).

4.4 Composition of Sedimentary Organic Matter

n-Alkane distributions extracted from marine sediments in Bellingham Bay displayed high molecular weights (C_{23}-C_{27}) with a strong odd-over-even predominance (Figure 16) demonstrating that organic matter in Bellingham Bay is predominantly sourced from terrestrial sources (Eglinton and Hamilton, 1967).

When calculating the percentage contribution for all three end members, it was clear that there was no signature for primary producers preserved in marine sediments from Bellingham Bay (No chain lengths below C_{17} were observed). Therefore, only percent contributions for terrestrial and aquatic end members were considered when calculating organic matter contributions downcore. To achieve this the term m_r, which represents the ratio of C_{17} / $C_{19\text{-}21}$ in eelgrass sampled in order to estimate C_{17} produced by eelgrass, was eliminated from equation 3. It was clear that primary producers were not being measured or preserved in the n-alkane content of the marine sedimentary organic matter therefore all n-alkanes C_{17} – C_{23} were produced by aquatic plants.

Table 6: Summary of downcore n-alkane indices and LOI for organic carbon from VC06 in Bellingham Bay.

VC06						
Depth (cm)	Aquatic (%)	Terrestrial (%)	ACL	CPI	TAR	LOI (%)
0	12.48	87.52	26.22	3.15	7.36	7.2
20	15.79	84.21	25.76	2.29	5.41	5.6
40	20.98	79.02	25.23	3.77	10.13	5.6
60	25.80	74.20	25.11	2.52	4.01	4.3
80	28.00	72.00	24.71	6.26	3.85	4.2
100	21.86	78.14	25.27	2.66	5.11	4.9
120	18.40	81.60	25.70	2.57	9.57	5.1
140	11.36	88.64	26.03	5.69	21.68	4.6
148	13.64	86.36	26.04	2.91	7.95	4.9

n-Alkane contributions from terrestrial and aquatic plants were calculated downcore approximately every 20 cm in VC06 and VC01 using the previously discussed linear mixing model. These cores were analyzed to compare organic matter contributions in shallow and deep environments in Bellingham Bay, VC01 is located at approximately 10 m depth near shore while VC06 is located at approximately 25 m depth towards the center of Bellingham Bay.

In VC06 terrestrial plants contributed the majority of the organic matter to marine sediments, 72-87% contribution, while aquatic plant contributions were estimated with the mixing model to range between 11-28% (Table 5). Aquatic plants' contributions decrease towards the surface and bottom of the core. Based on approximate age calculations using carbon dating and assumed sedimentation rates, aquatic plant contributions peaked at 28% contribution around a

depth of 80 cm which roughly correlates with the year 1712. Aquatic plant contributions have been in steady decline since (Figure 17). Chronology was based on ^{14}C dating of mollusk shells found in the core. This chronology is consistent with the most recent report of sedimentation rates in Bellingham Bay based on ^{210}Pb analyses (Nowacki et al., In Press).

In VC01 terrestrial plants also contributed the bulk of organic matter, 86-95% contribution, while aquatic plants contributed only 5-13% of organic matter. Terrestrial contributions increase towards the core top (0 cm) while aquatic contributions decrease. Aquatic organic matter contributions peak at 13% at the bottom of the core (Figure 18). This a similar trend to that observed on core VC06.

ACL decreases from the top of VC06 until 80 cm depth where it begins to increase again until the bottom of the core. The minimum ACL at 80 cm depth correlates with the model's prediction of peak aquatic plant contributions, which have a shorter chain length n-alkanes compared to terrestrial plants. The correspondence between the ACL downcore, and the model's prediction further validate the developed linear mixing model.

The CPI was greater than one for all samples downcore indicating an odd-over-even predominance in n-alkanes from the marine sedimentary organic matter (Table 6). A CPI value greater than one is an indication that organic matter is being preserved mostly intact (Cranwell 1981). The strong odd-over-even predominance present in VC06 suggests consistent preservation of organic matter downcore.

The TAR was greater than one for all samples down core indicating a strong dominance of terrestrial organic matter contributing to sedimentary organic matter in Bellingham Bay. There is a strong increase in TAR at 140 cm which corresponds with the greatest percentage of terrestrial contribution predicted by the linear mixing model. However, TAR does not consider the n-alkane

signature of primary producers or account for the overlap in n-alkane signature for terrestrial and aquatic organic matter.

Results for ACL, CPI, and TAR generally correlate with results predicted by the linear mixing model. The CPI and TAR are useful indicators of changes in sedimentary organic matter composition downcore, however they are based on empirical ratios. Furthermore, TAR does not consider the full spectrum of n-alkanes produced by terrestrial and aquatic sources or the overlap between different sources of organic matter. While these simple equations are useful, they are not as robust as the linear mixing model I used here. The linear mixing model is capable of producing actual percentages of organic matter contributions and accommodates for the observed overlap of n-alkane signatures of different sources of organic matter.

In Bellingham Bay organic carbon is dominated by terrestrial components (produced by land plants). Based on biomarker results, it is apparent that marine plankton contributions to the sedimentary organic matter is minor. This is consistent with a well-oxygenated water column. However, more live plankton collections are needed to better define the n-alkane distributions of plankton communities in Bellingham Bay. Overall, biomarkers are well preserved in marine sediments and show similar patterns in shallow and deep environments, with aquatic plant contributions in decline and an increase in terrestrial organic matter towards the surface. In locations proximal to the river mouth (e.g. VC01) this trend of increased terrestrial carbon towards the surface may be the result of the delta progradation over marine environments. However, this seems less likely at deeper locations (e.g. VC06) where presumably deposition conditions have been more stable.

The linear mixing model shows an increase in terrestrial organic matter contributions at the expense of aquatic plants in the last ~300 years (based on radiocarbon chronology). In a relative

abundance framework, it is not possible to determine if this change is due to an increase in terrestrial plants, a decrease in eelgrass beds, or a combination of the two since the results are based on percentages. Nonetheless, complementary data sets support an increase of terrestrial plant contribution to the sediments. The LOI data shows an increase in organic material in the top 40 cm of the core (Figure 9). This is not consistent with a decrease in eelgrass beds, or at least implies the increase in terrestrial plant contributions was larger than the decrease in eelgrass. Furthermore, there has been a significant change in landcover since preindustrial times in the Bellingham Bay watershed. Landcover maps show a significant decrease in forested cover and inland wetlands which are replaced by urban and agricultural areas (Figure 5, Figure 7, Figure 8). It is likely that the decrease in forested cover in the watershed has led to an increase in terrestrial organic matter input to Bellingham Bay due to increased erosion from exposed soils. The rerouting of the Nooksack River in 1877 would also result in a significant increase in terrestrial organic matter (Collins 1998). The resulting increase in sediment flux could bury eelgrass beds, resulting in their decline. The results of downcore biomarkers derived from the linear mixing model are consistent with the reconstructed change in landcover.

In order to produce more accurate results and to better understand human impact on organic matter sources, compound specific isotopic analysis should be measured. Before industrialization of the area, terrestrial land cover mainly consisted of C3 plants, while many crops brought to the northern part of the watershed are C4 plants. Understanding the isotopic signature of organic matter in cores could better indicate how urbanization has affected organic matter sources.

4.5 Carbon Storage

TOC ranged from 1.7% to 10.5%. The highest amount of TOC was observed in the bottom of VC09 and correlated with woody material that was unique to that location. This increased

amount of TOC storage at the bottom of VC09 was unprecedented, and significantly higher than all other samples. VC09 was sampled near an industrial area directly off the coast of Bellingham (Figure 4). This area has been dredged and included several sawmills, shingle mills, and paper manufactures (Louws, 2015), so it is likely that this woody material is anthropogenic in origin. For this reason, VC09 was not included when calculating average TOC. Excluding VC09, TOC averaged 2.64 ± 0.38%. Carbon storage in all cores was relatively uniform downcore, except for a significant decrease in the top 20 cm which likely represents the sediment re-working zone. Based on observed LOI and TOC, approximately the top 20 cm of the core could be subject to re-working; anything below that threshold represents long term, stable storage of organic carbon.

Sediment samples when dried weighed approximately 45 grams per 45.6 cm^3 of material. Estimated sedimentation rates for Bellingham Bay, based on estimates from VC05 and VC06, were on average 0.49 cm/yr. The mass accumulation rate (MAR) is calculated as the product of the sedimentation rate and the dry weight density of sediment sampled. The estimated MAR for sediments in Bellingham Bay is 0.49 g cm^{-2} per year. Across all cored locations, excluding VC09, sediments on average store 2.64 ± 0.38% organic carbon by weight. With a sediment MAR of 0.49 cm/yr, the flux of organic carbon in Bellingham Bay is 1.1 - 1.4 x 10^8 g C km^{-2} per year. Using Google Earth, the entire surface area of Bellingham Bay was estimated to be approximately 68 km^2 (Supplementary Figure 1S). Using this surface area, Bellingham Bay has the potential to store 7.5 - 9.5 x 10^9 g C per year, or 7500 to 9500 tons of carbon per year.

Eelgrass beds cover an estimated 0.19 ± 0.02 km^2 (Gaeckle 2009) in Bellingham Bay and have the capability to store approximately 1.0 x 10^8 g C km^{-2} per year (McLeod et al., 2011). If eelgrass beds alone can store 1.0 x 10^8 g C km^{-2} per year and sediments in Bellingham Bay store 1.1 x 10^8 to 1.4 x 10^8 g C km^{-2} per year, then areas with eelgrass beds could potentially store double

the amount of carbon than just sediment alone. Future studies should quantify carbon storage within areas with significant eelgrass bed coverage and compare results to areas without eelgrass bed coverage to see if the root systems store a significant amount of organic carbon. More research quantifying carbon sinks such as eelgrass beds and further understanding into how to maximize their storage potential is required to effectively offset atmospheric carbon emissions.

Currently eelgrass beds are in decline (Rubin et al., 2018;), causing their root systems to degrade and release trapped carbon (Duarte at al., 2005; Poppe and Rybczyk 2018). There is also opposing research which claims that eelgrass beds are a source of carbon to the atmosphere due to biological activity from eelgrass that produces more carbon than is stored in eelgrass biomass, however this study does not consider carbon trapped by eelgrass root systems or sedimentary organic matter in general (Dam at al., 2021).

Sedimentary organic matter stored in marine environments provides significant long term carbon storage that should be considered as a part of the solution to offset carbon released into the atmosphere (Nellemann et al., 2009; McLeod at al., 2011). Eelgrass beds can potentially help to increase sedimentary organic matter stored in marine environments (McLeod et al., 2011; Poppe and Rybczyk 2018) and therefore could be a solution to help mitigate carbon released into the atmosphere. Future research in this area, specifically looking at carbon stored in the root system of eelgrass could help to determine their effectiveness as carbon sinks. Future sampling should include coring locations with significant eelgrass bed coverage to more accurately estimate their ability to trap carbon within their root system and comparing carbon stored to other areas within Bellingham Bay that have no eelgrass bed coverage.

5. Conclusion

Understanding sediment sources and fluxes of carbon throughout coastal zones is essential to evaluating ecosystems' health and the potential for carbon storage. Using n-alkanes biomarkers to understand changes in sediment provenance can lead to a better understanding of how urbanization can affect sediment sources in coastal environments by comparing changes in sediment composition from pre-industrial times to current sediment sources.

In this study sixteen marine sediment cores were analyzed from eight locations in order to determine organic matter sources in Bellingham Bay. Plant wax biomarkers (n-alkanes) were extracted from two cores at separate locations, one near shore (VC01) and one near the deepest part of Bellingham Bay (VC06) to examine environmental changes. Biomarker distributions from all extracted sediment samples exhibited good preservation state (CPI > 1) and were dominated by long chain n-alkanes ($>C_{25}$) which indicate a dominantly terrestrial plant source. A linear mixing model was developed to convert the n-alkane compound distributions into percentage contributions of difference sources to the organic matter in marine sediments. Results from the linear mixing model estimate that aquatic plant (eelgrass) contributions to sedimentary organic matter peaked at 28% at 80 cm depth, which roughly correlates with the year 1712, and have been in steady decline since. Terrestrial plants contributed the bulk of the organic matter to all marine sediment sampled in Bellingham Bay. n-Alkane biomarkers show a clear increase in terrestrial sources since pre-industrial times. The increase in terrestrial carbon content in the marine sediments is consistent with landcover change that shows a decrease in forested cover in the watershed since pre-industrial times. The conversion of forested cover to urban areas is suspected to have led to increased erosion and therefore an increase in terrestrial sediments and organic

matter delivered to Bellingham Bay. Although this linear mixing model was created for Bellingham Bay, the methods used in this study could be applied to other coastal ecosystems.

Carbon storage at all cored locations was relatively stable downcore, except for the inferred re-working zone. On average, sediments in the studied cores store 2.64% organic carbon by weight. If carbon storage in the entire bay is consistent with the sites examined, then Bellingham Bay has the potential to store 7500 to 9500 tons of carbon per year. It was estimated that areas with eelgrass beds could potentially store double the amount of carbon than just sediment alone. Although there was no clear correlation between carbon storage and relative abundances of organic matter sources downcore, further research should be done sampling sediment in areas with eelgrass beds to assess how their root system hold carbon compared to areas sampled here with no eelgrass beds.

The high CPI suggests that organic matter is well preserved in sampled marine sediments, and that future research could be done on these cores. Continued research into organic matter sources in developed coastal ecosystems will help to inform how urbanization has affected the ecosystems and carbon sequestration rates in the watershed. The methods used here such as the linear mixing model could be applied to additional coastal ecosystem. Future research should be focused in areas with higher eelgrass bed densities to further determine how eelgrass bed densities and long-term carbon storage have been affected by urbanization.

Figures

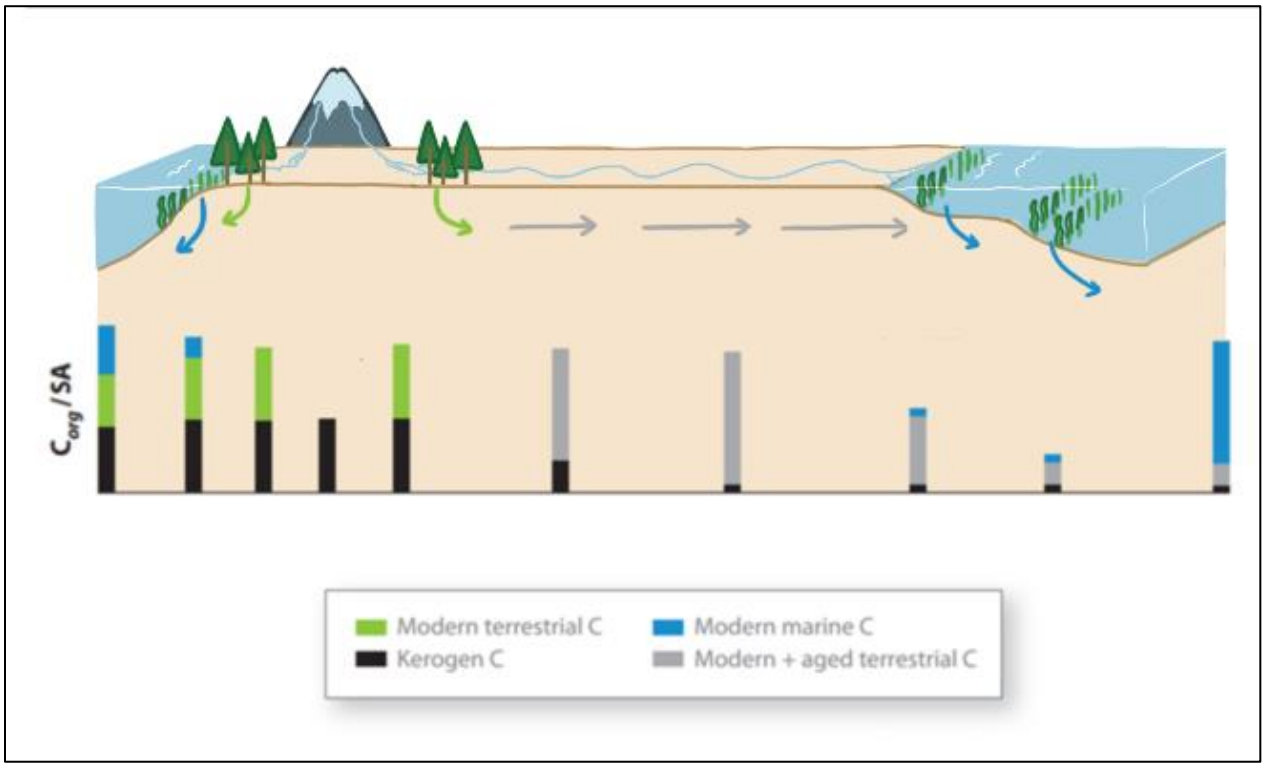

Figure 1: Modified from Blair and Aller, 2012. The contrasting active- and passive-margins and their respective preservation of modern terrestrial and marine organic matter. In passive margins (right) modern terrestrial organic matter travels much further and is mixed with more aged terrestrial material form bedrock before being deposited in marine sediments. In active margins (right) modern terrestrial organic matter is preserved on a much faster scale, preserving more modern terrestrial organic matter and less aged material.

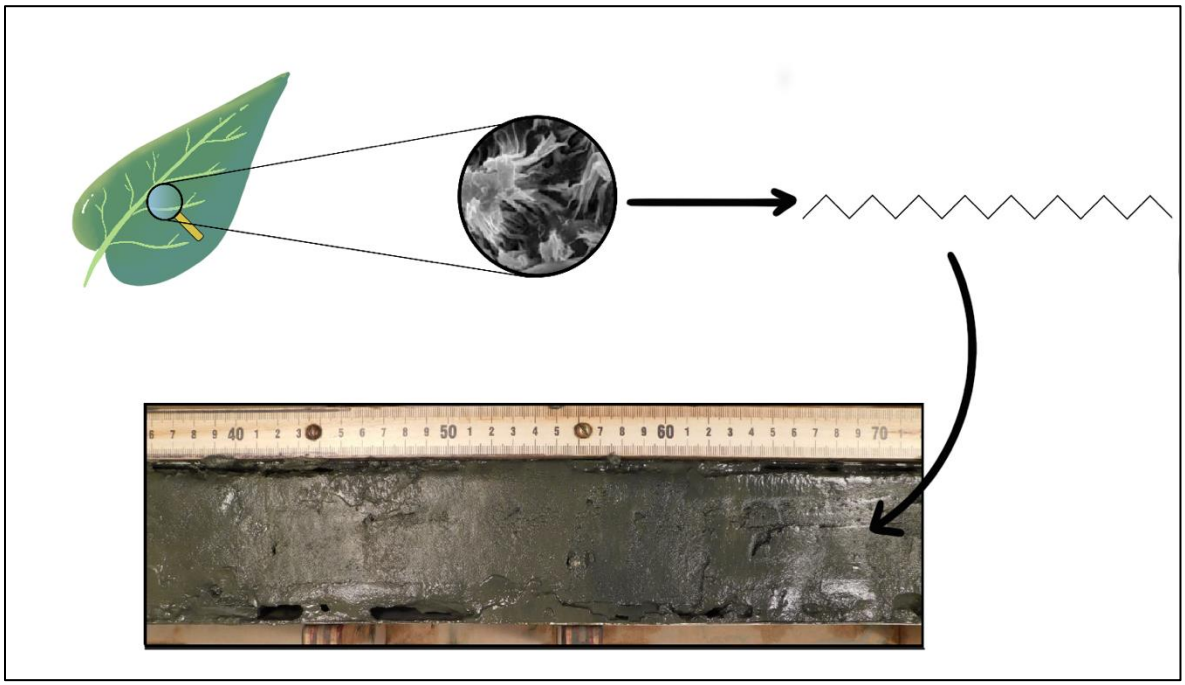

Figure 2 – Image depicting plant leaf waxes and their compostion and preservation in marine sediments. The top middle panel is a scanning electron microscope image of a plant leaf, showing an SEM image of the epicuticular wax coating (adapted from Szafranek et al., 2008). An important component of these plant waxes are single chain hydrocarbons (n-alkanes) of various lengths which are resistant to environmental degradation and preserve well in sediments. The sediment core from Bellingham Bay shown above, exhibits black to dark-gray silty clays.

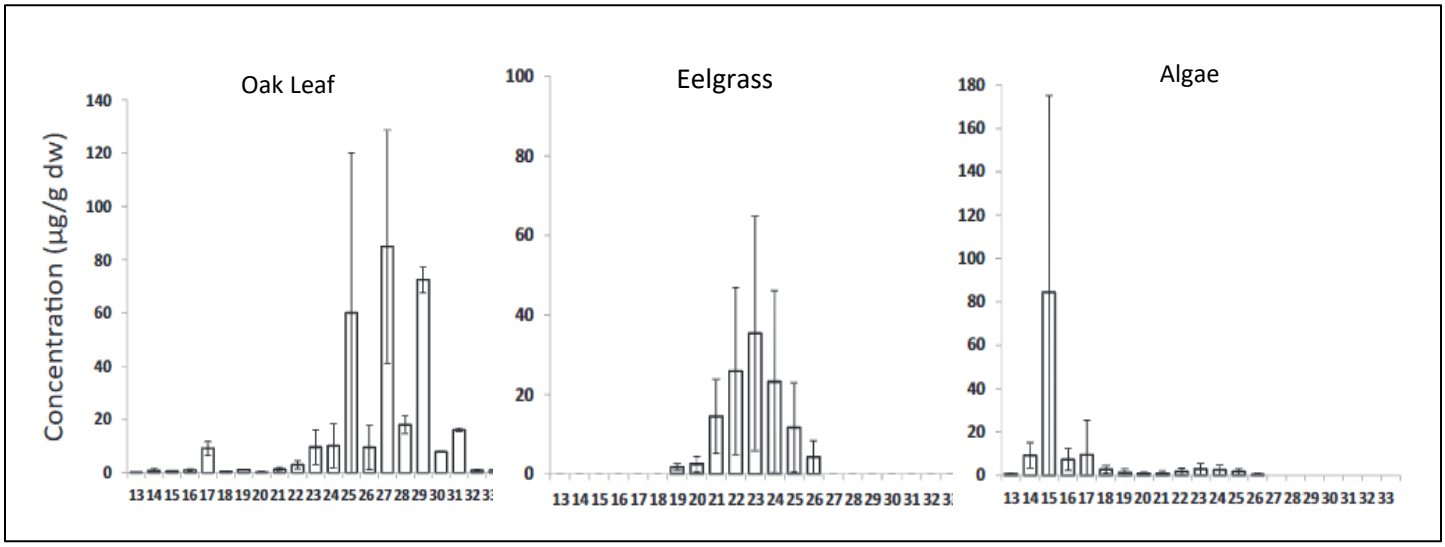

Figure 3 - Concentration of n-alkanes (C_{13}-C_{33}) in different sources of sedimentary organic matter. Note how eelgrass has a distinctive n-alkane distribution characterized by the dominance of mid-carbon chains (C_{21}-C_{25}) and the absence of long-carbon chains (C_{27}-C_{33}) [Modified from: Chevalier et al., 2015].

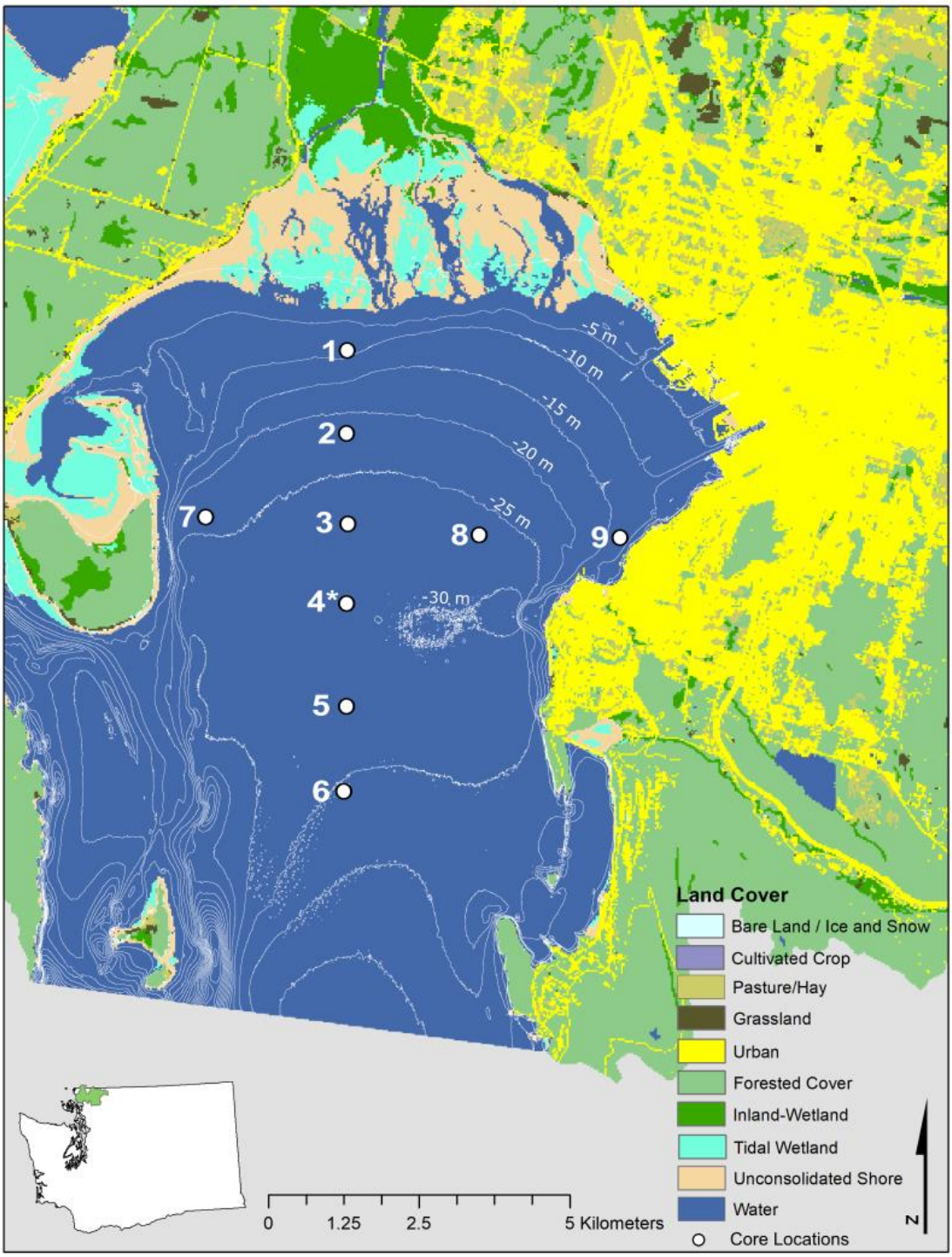

Figure 4- Map of Bellingham Bay with land cover (NOAA), bathymetry data (USGS), and successfully cored locations. Eelgrass beds can be found in tidal wetlands and unconsolidated shore areas. Cores are located parallel and perpendicular to the Nooksack Delta to achieve maximum bay coverage around the main sediment source. Core location 4 was too close to a disposal site and was therefore not retrieved.

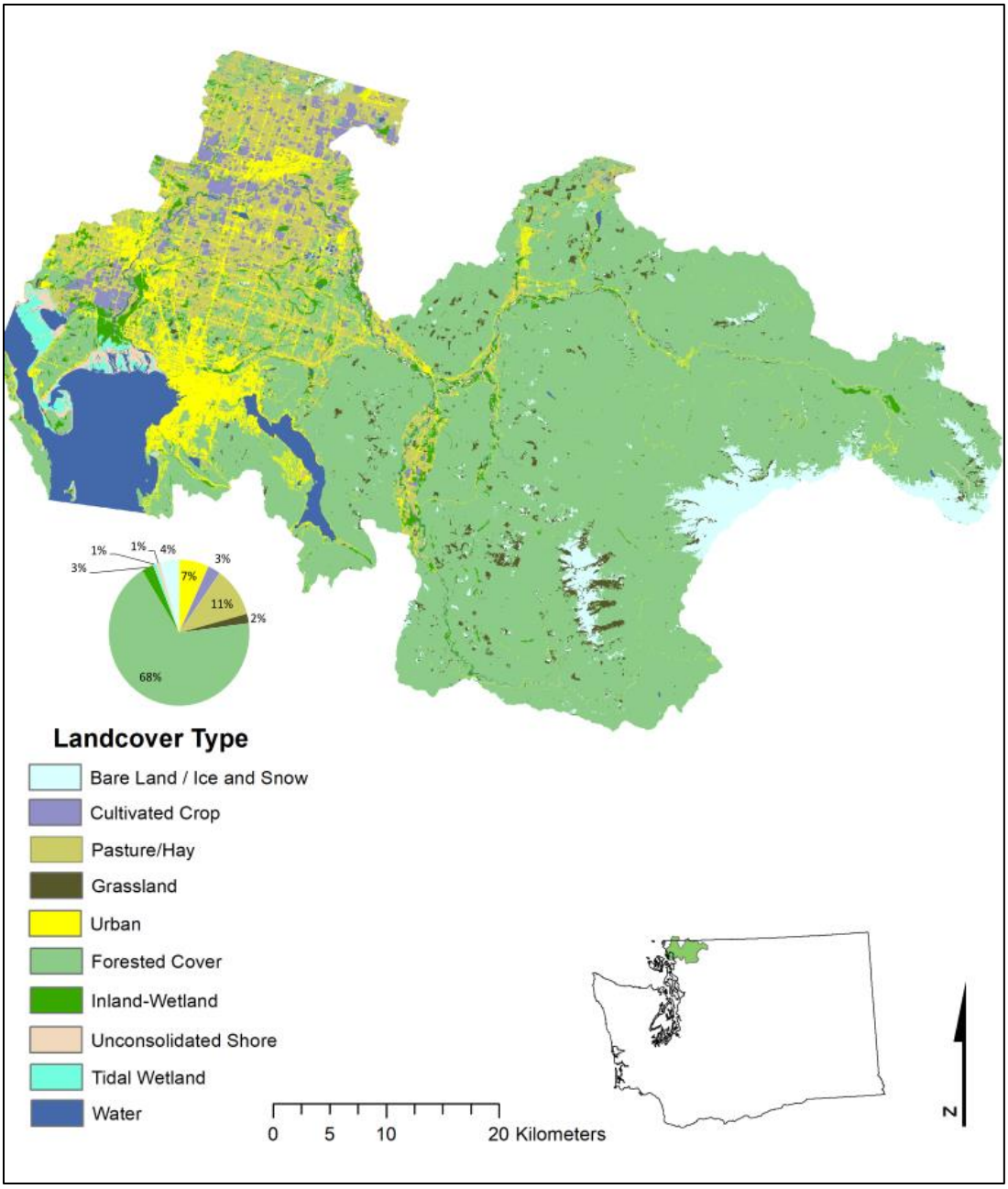

Figure 5: Current landcover for the Bellingham Bay watershed. Data used from 2016 C-CAP High Resolution Land Cover from NOAA. The watershed shapefile was provided by the United States Department of agriculture. Pie chart representing modern landcover in the Bellingham Bay watershed. Most of the watershed is forested cover. Manmade environments (urban, pasture/hay, and cultivated crop account for almost a quarter of the watershed.

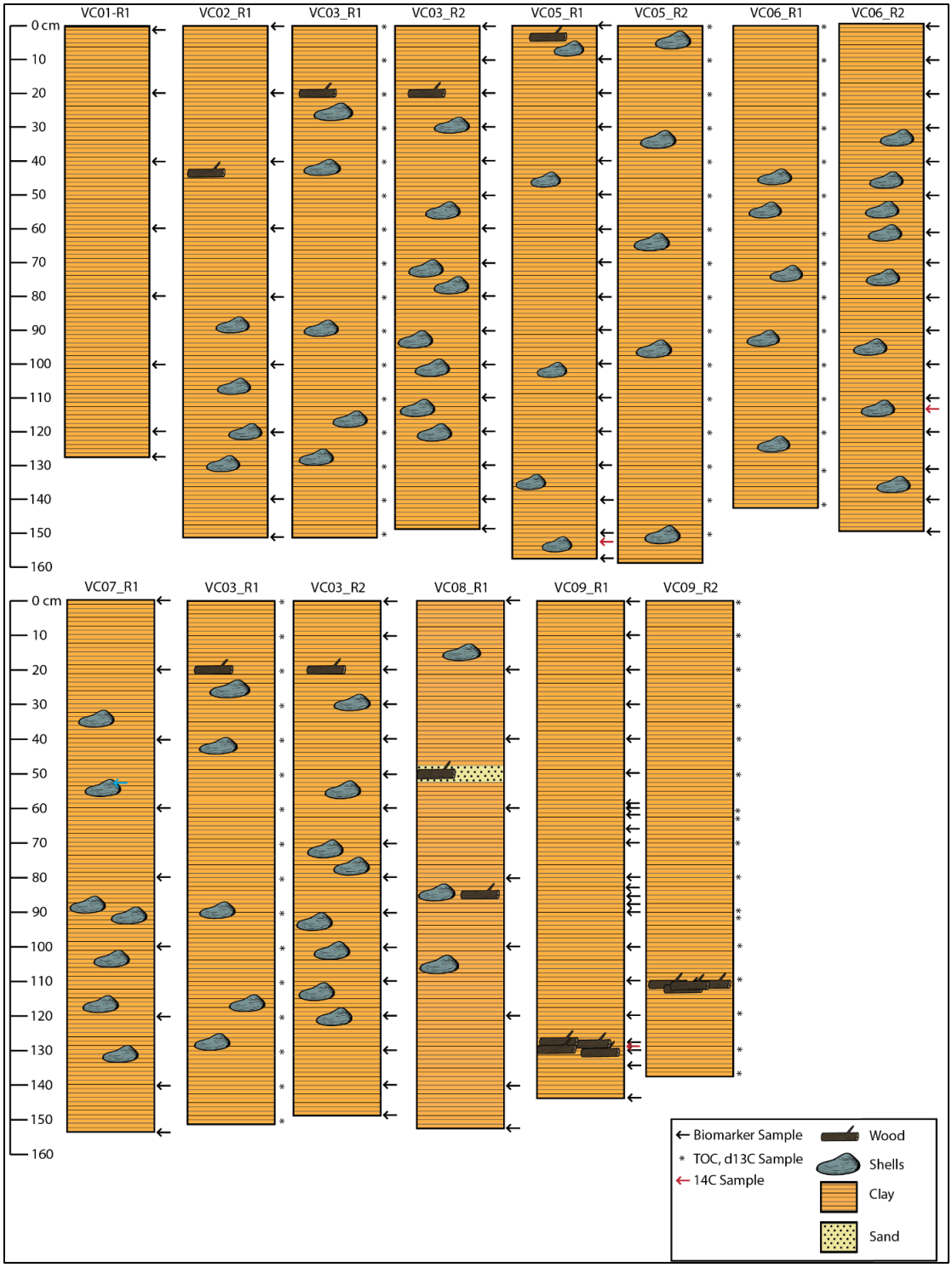

Figure 6: Core logs and sampling interval for all cores that were sampled. The top row shows the N-S cores and bottom E-W cores. Organic biomarker samples were taken in eight cores. Cores VC01-R1, VC02-R1, VC07-R1 and VC08-R1 were sampled every 20 cm. Cores VC03-R2, VC05-R1, and VC06-R2 were sampled every 10 cm, VC09-R1 was sampled at every 10 cm as well as corelating samples VC09-R2 to account for the 10 cm difference in the major wood layer.

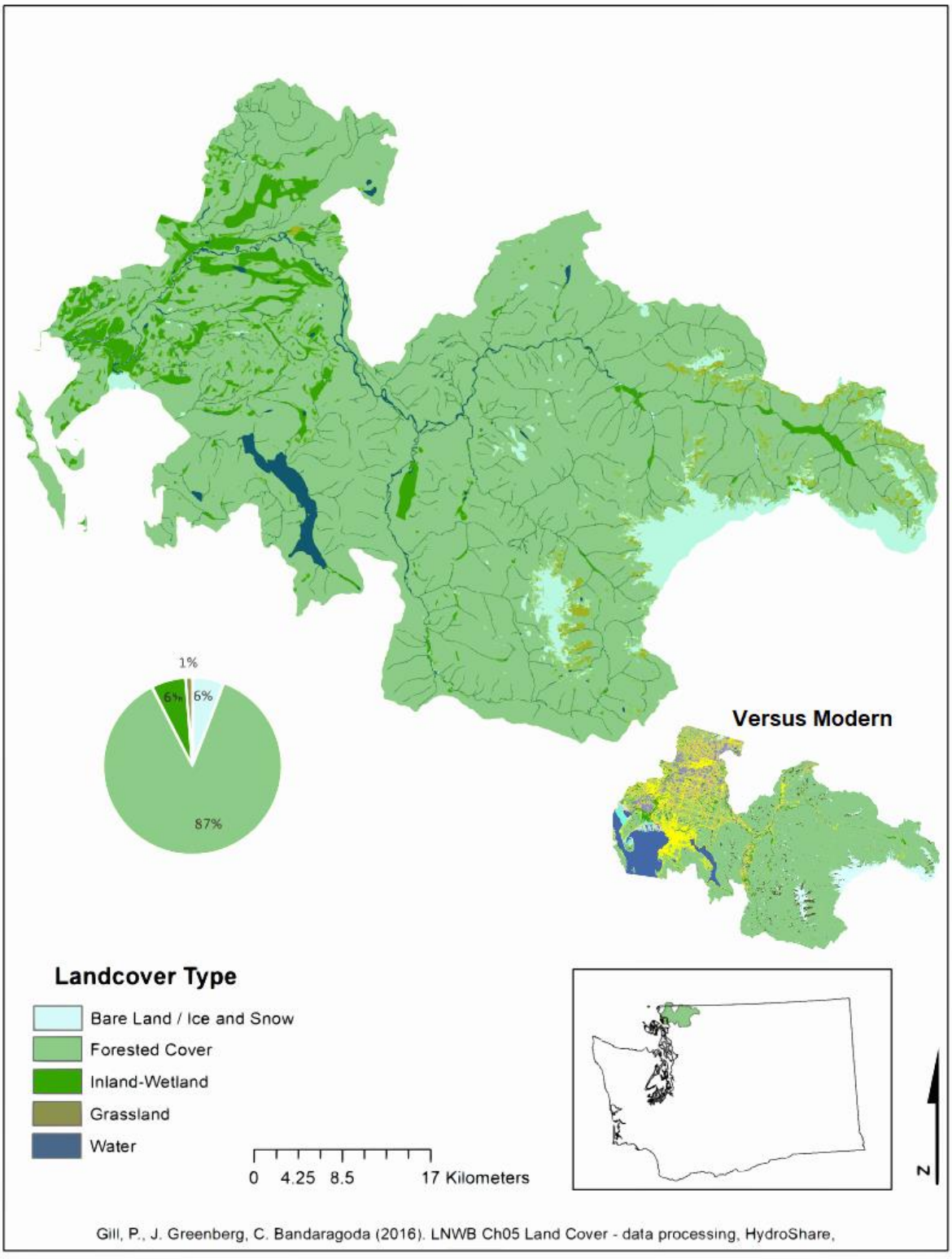

Figure 7: Map of historic landcover for Bellingham Bay watershed and pie chart and modern map for comparison. Developed using soil coverage of Whatcom County to infer past environmental conditions. Historic land cover mapping was developed by Utah State University (Winkelaar, 2004) for the WRIA 1 watershed. Watershed shapefile was provided by the United States Department of agriculture.

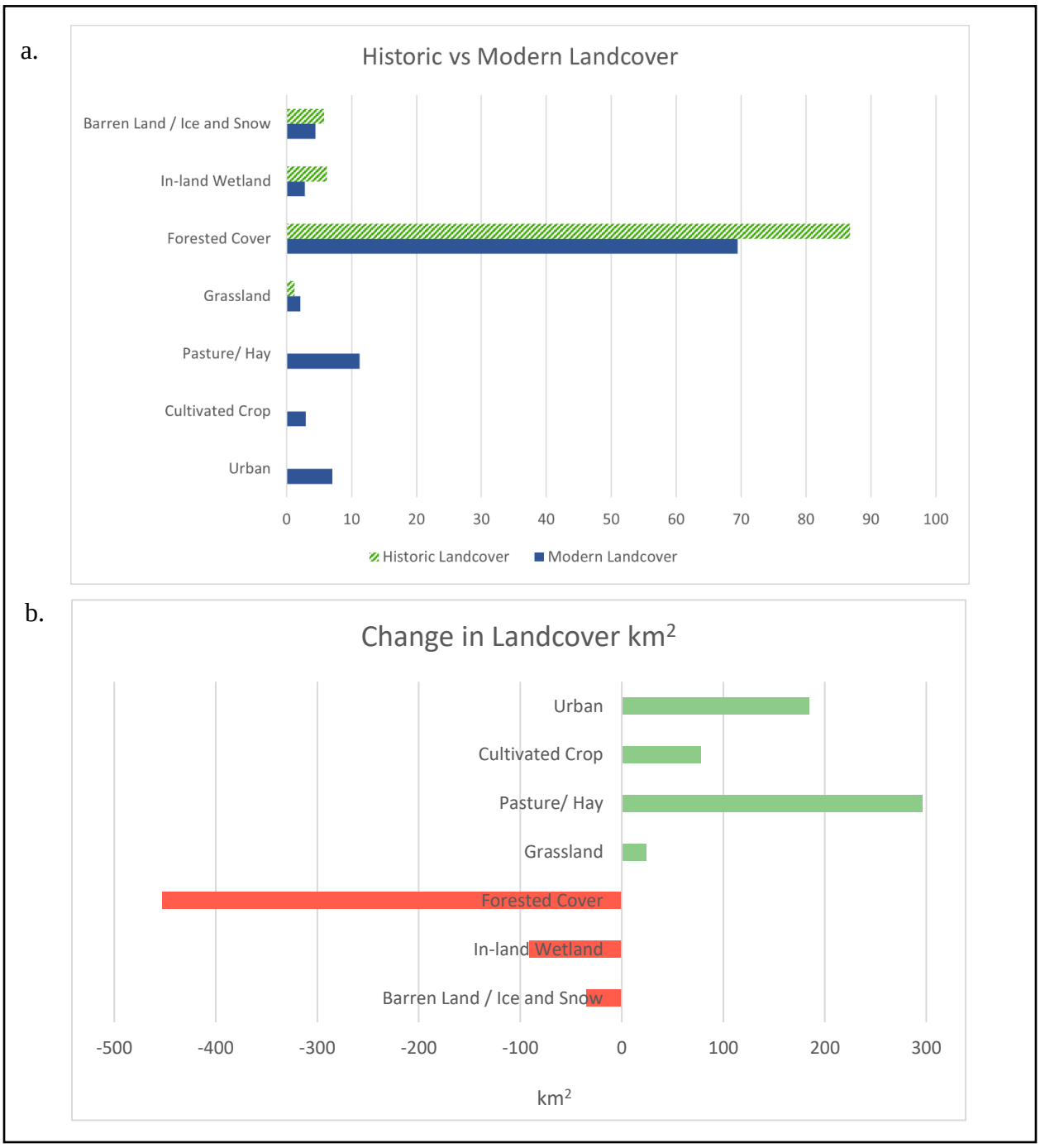

Figure 8a. Percentage of landcover types in the Bellingham Bay watershed for modern (2016 NOAA C-CAP landcover) and historic landcover. Upper bar (green) represents historic, the lower bar (blue) represents modern. Historical data was produced by Utah State University to represent landcover for ~200 years ago using soil data to reconstruct past environments (Winkelaar 2004). In both cases forested cover is the majority of the watershed. **Figure 8b.** Change in landcover by km^2. Red bars on left represent a percent decrease, green bars on the right represent a percentage increase. Notice the large decrease in forested cover, and the large increase in agricultural and urban areas.

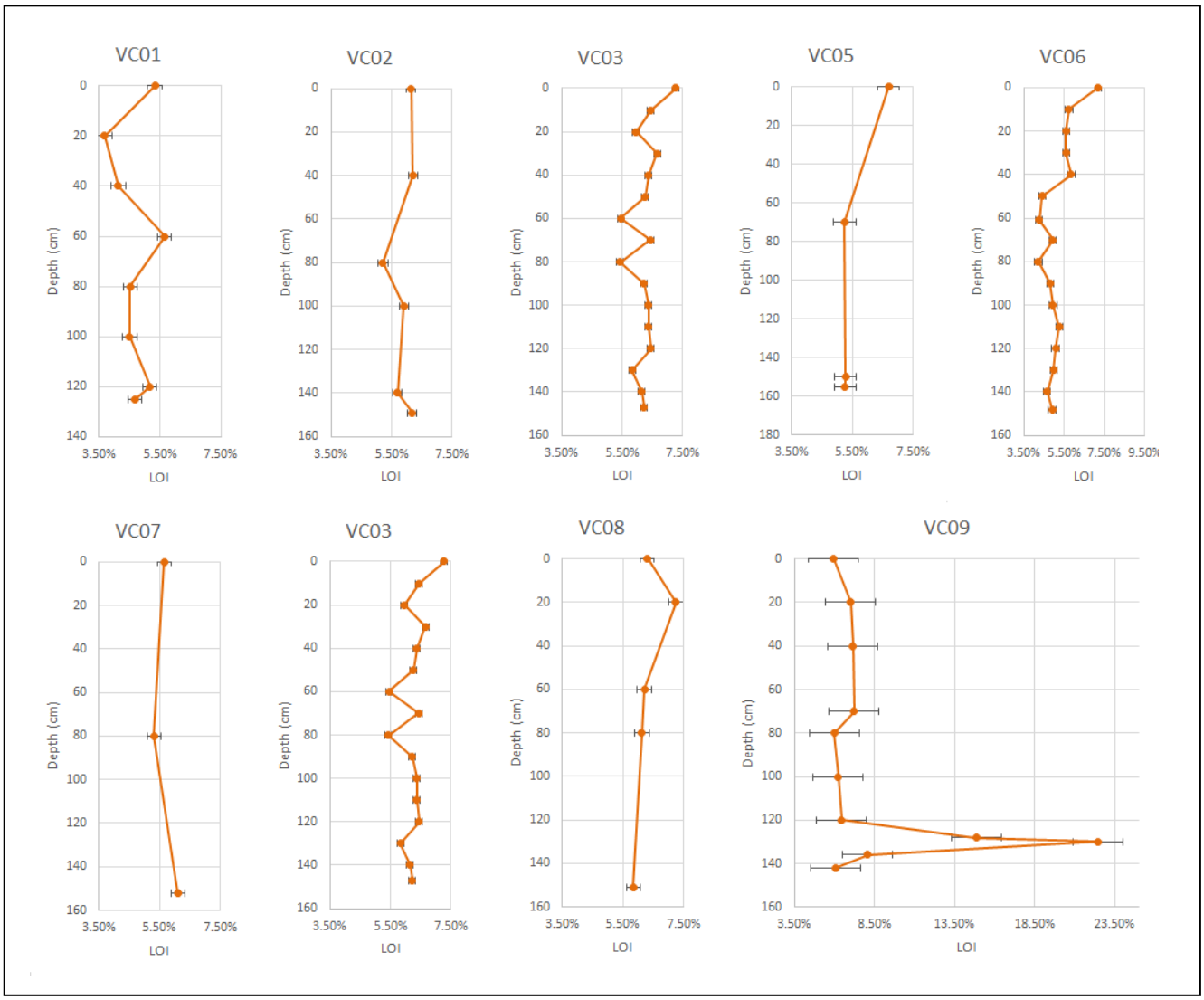

Figure 9: Loss on Ignition (LOI) of organic material down core. Top row represents the North-South transect and the bottom row represents the East-West transect of cores. Y-axis represents depth in core and X-axis is LOI % increasing from left to right. Organic matter typically ranges from 3-7% except for the spike in organic matter in VC09 at 130 cm depth which peaks at 22.5% organic matter. In general, there is a sharp decrease in organic matter from the top 0-20cm, this threshold likely represents the reworking area. Further downcore organic matter is stable showing that organic matter is preserved in Bellingham Bay.

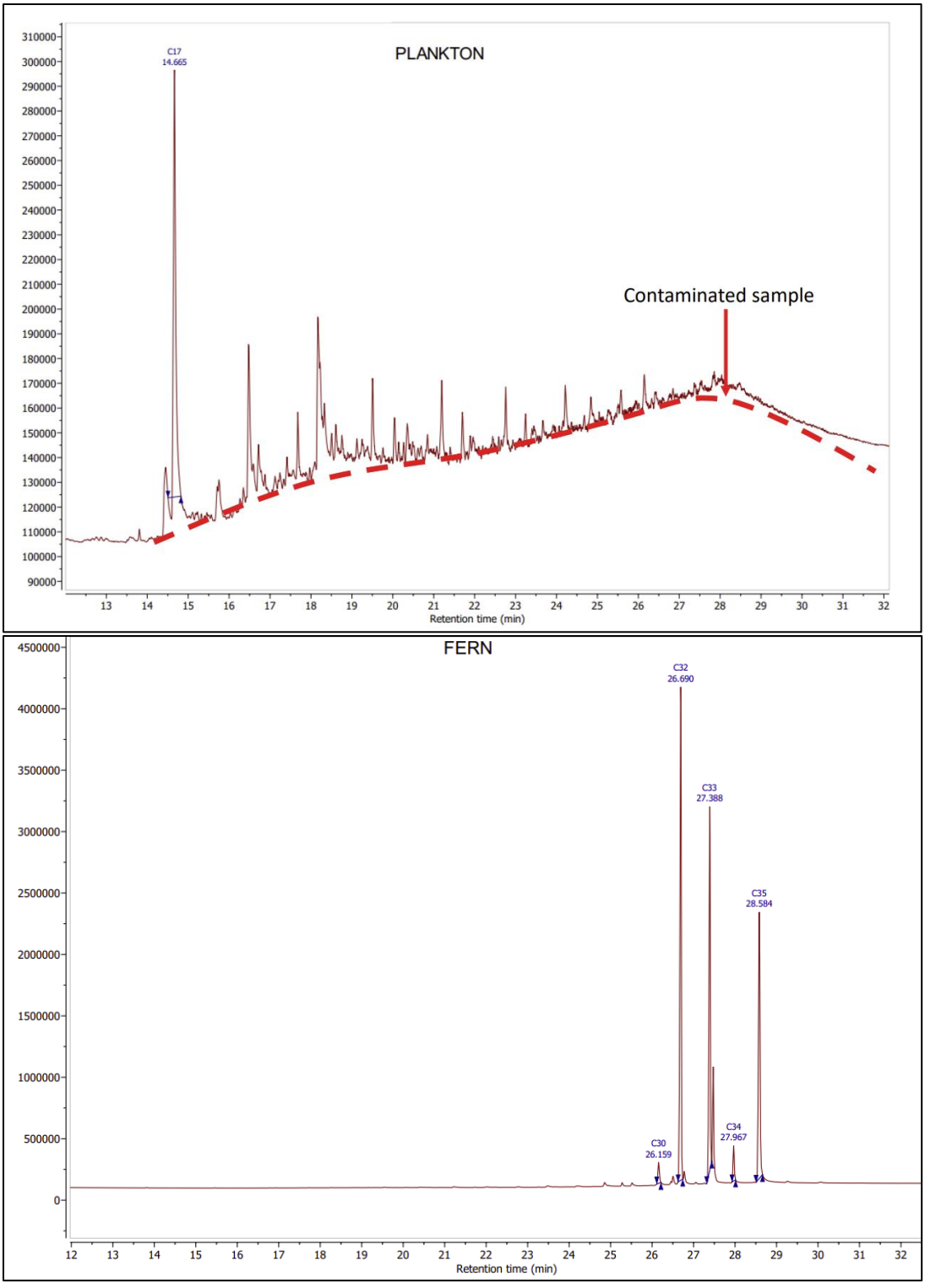

Figure 10: Raw gas chromatography results for plankton collected from Bellingham Bay. The plankton sample (top) from Bellingham Bay showed characteristic signs of possible contamination highlighted by the dashed line. The fern sample shows no sign of contamination and is flat, minus the peaks at select chain lengths of n-alkanes.

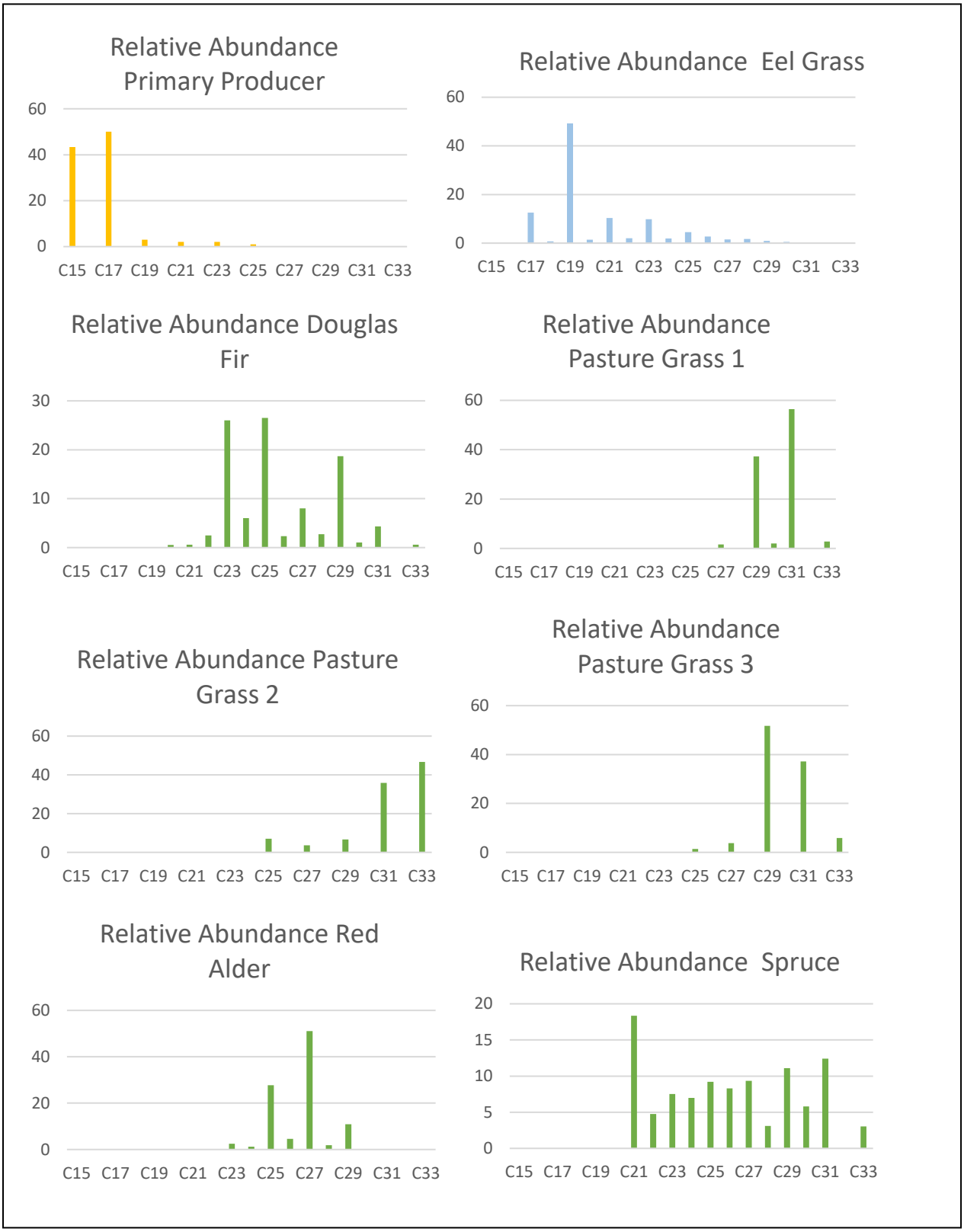

Figure 11: Relative abundances of all sampled plant material. Notice that primary producers like Plankton peak at much lower carbon chain lengths than terrestrial plants like the Red Alder or grasses. Y axis represents relative abundance and X axis represents n-alkane chain lengths.

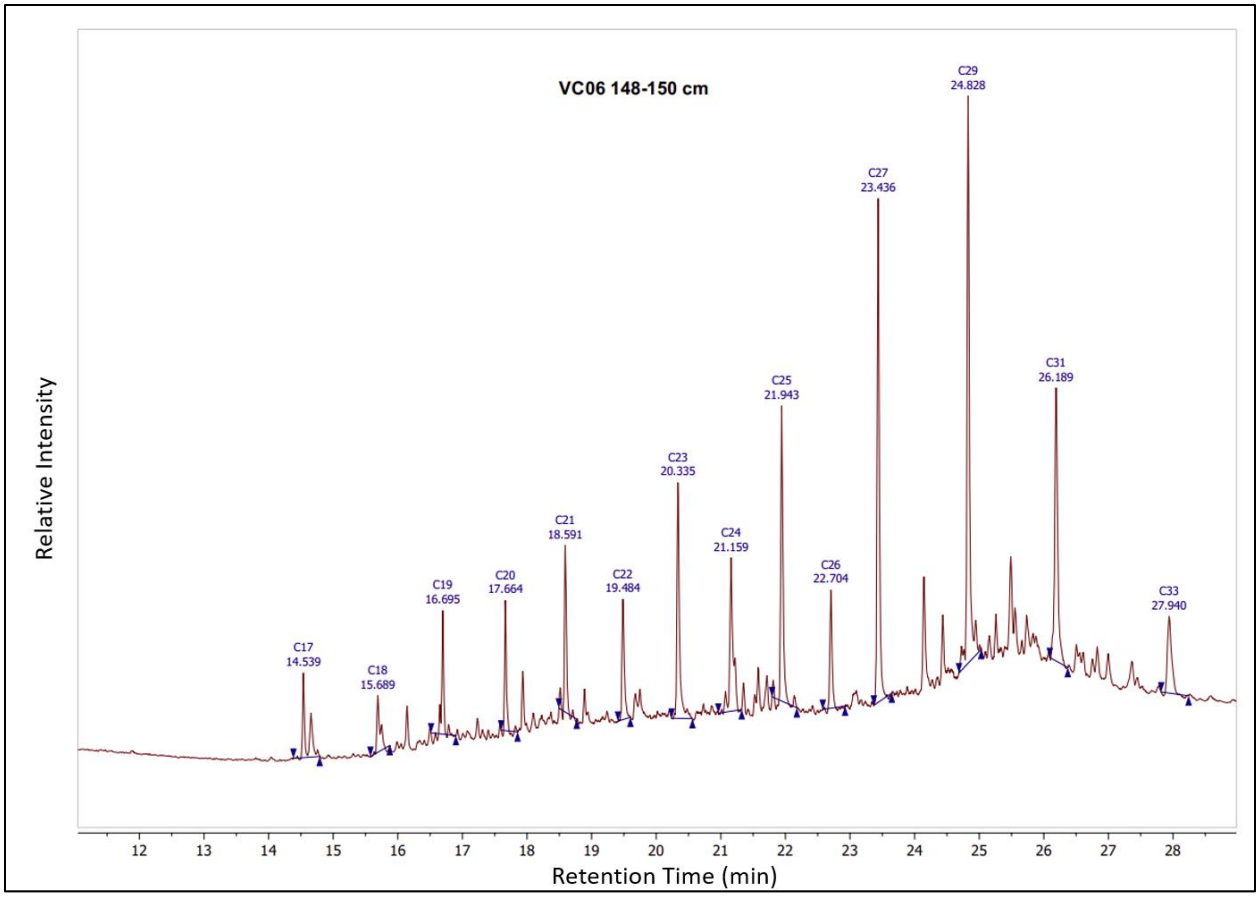

Figure 12: Chromatogram of n-alkane fractions from VC06 at 148 cm depth. Peaks are labeled with retention time in minutes and the corresponding n-alkane chain length. The y-axis represents the intensity, or how abundant that particular chain length is, and the x-axis represents the retention time in minutes.

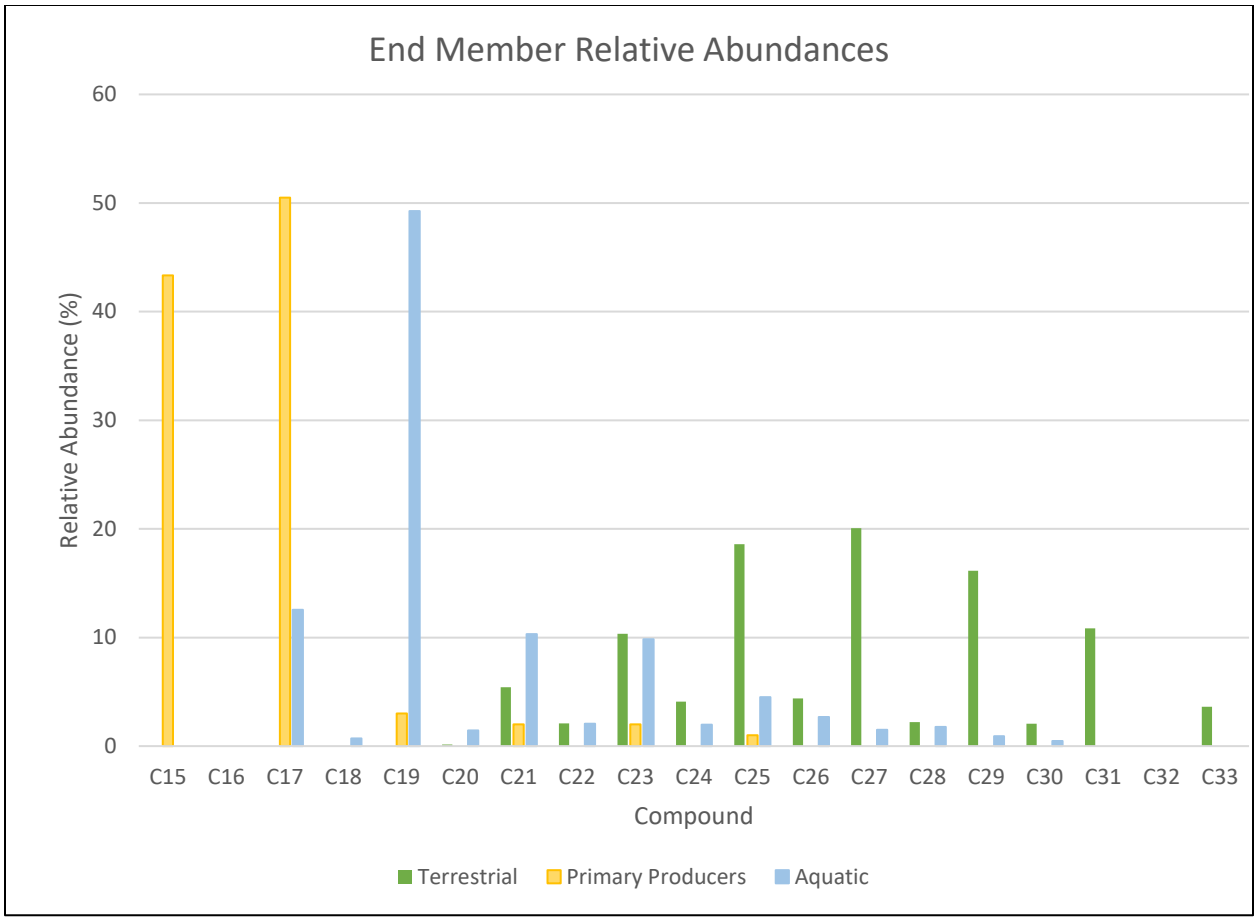

Figure 13: Relative abundances of each end member. X axis represents compound C_{15}-C_{33} and the Y axis represents relative abundances. Primary producers shown in yellow, peak at C_{15} and C_{17} while terrestrial end members, shown in green, peak much higher chain lengths between C_{25} and C_{33} and aquatic end members peak at midchain lengths, between C_{19} and $C_{21.}$ Notice that each end member has a unique distribution.

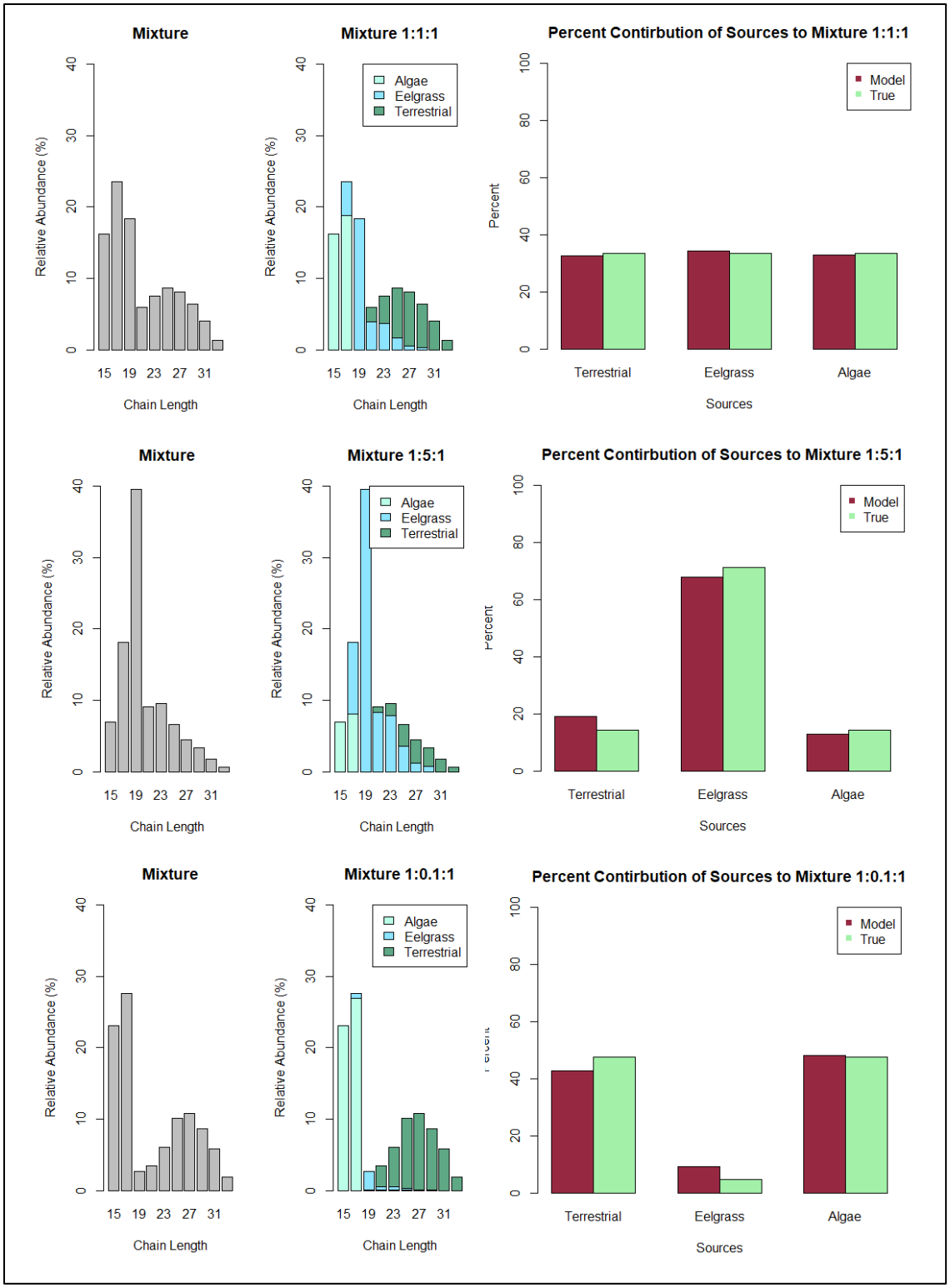

Figure 14: On the left, bar plots visualizing hypothetical mixing scenarios. The all-gray figure shows what mixing results will look like in a mixture with no distinction between end members. The colored bar plots show how end members contribute to the mixture. On the right, results of model productions of end member amounts in three mixing scenarios (1:1:1, 1:5:1, and 1:0.01:1) mixing versus the hypothetical true results. Results were most accurate for large amounts and less accurate at predicting when there is almost none of an end member.

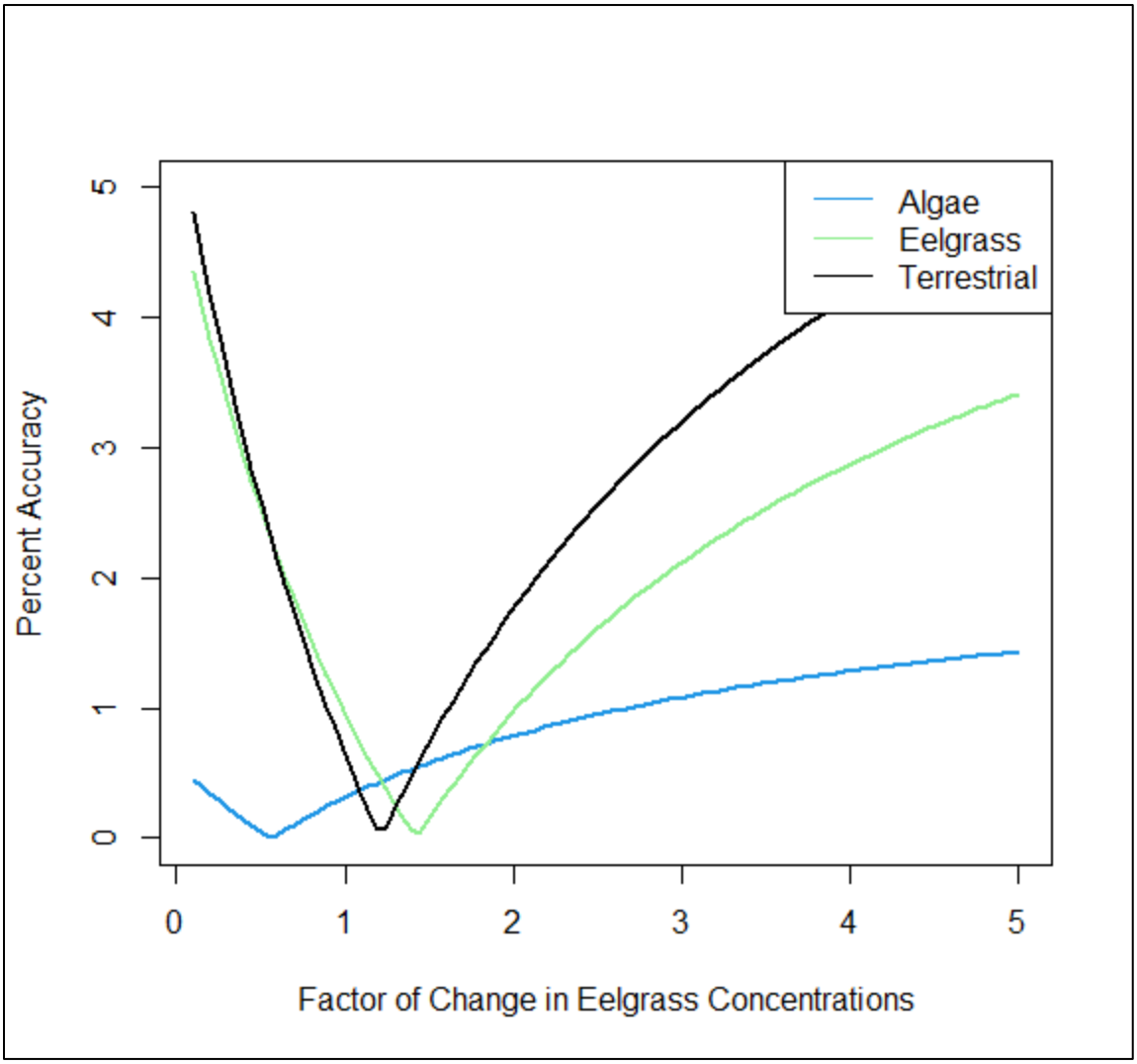

Figure 15: Graph depicting the accuracy of the mixing model. Eelgrass concentrations were changed by a factor of 0 to 5 in order to assess how the model adjust calculations for each end member. A percentage difference of 0 indicates when the model was most accurate. Around 1, (indicating a 1:1:1 mixture) is when the model is the most accurate for all sources. As the factor of change in eelgrass away from 1 the accuracy in the model decreases.

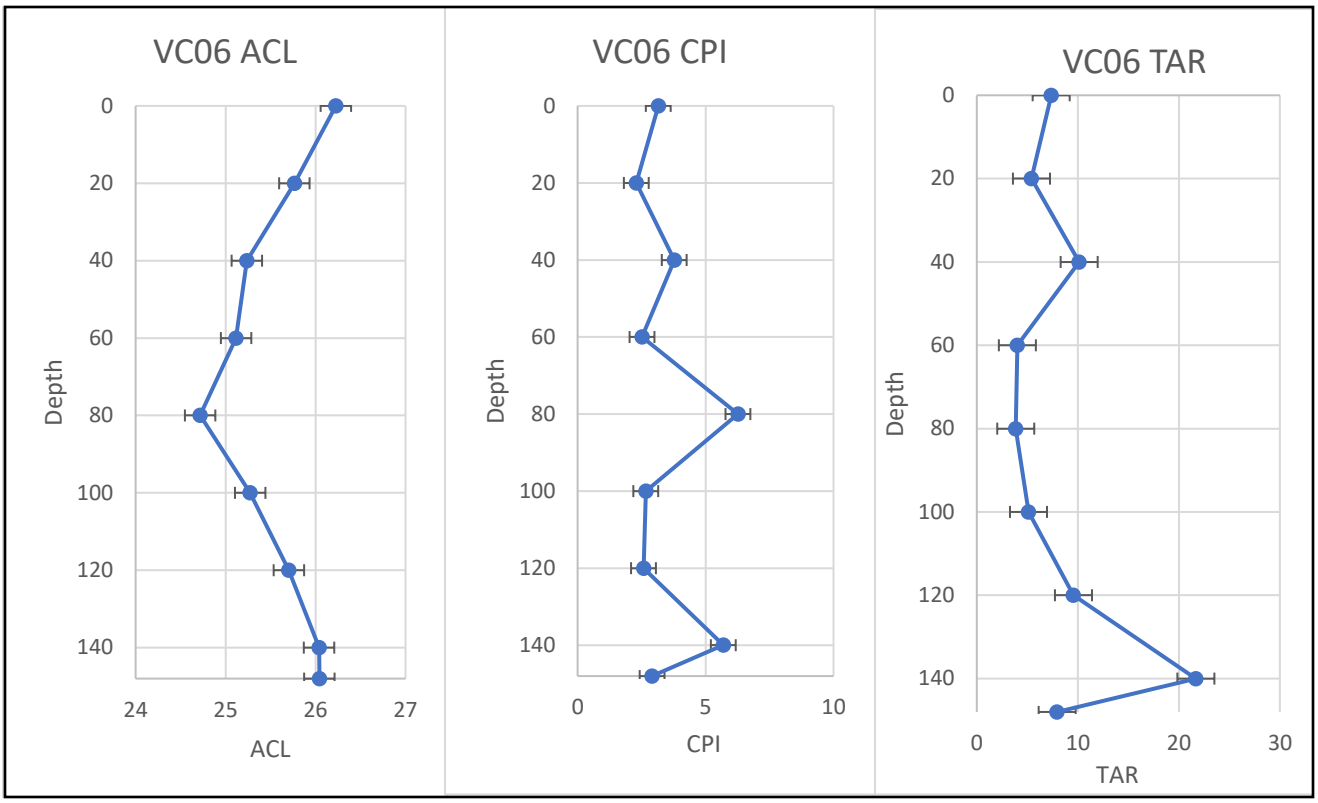

Figure 16: Downcore results for Average Chain Length (ACL), Carbon Preference Index (CPI), and Terrestrial Aquatic Ratio (TAR) for VC06. The ACL downcore was between C_{24} and C_{27}, indicating that VC06 is dominated by terrestrial organic matter. The CPI downcore remained above 1, indicating that odd chain lengths dominated sedimentary organic matter. The TAR was significantly above 1 at all depths, indicating a strong dominance of terrestrial organic matter compared to aquatic organic matter.

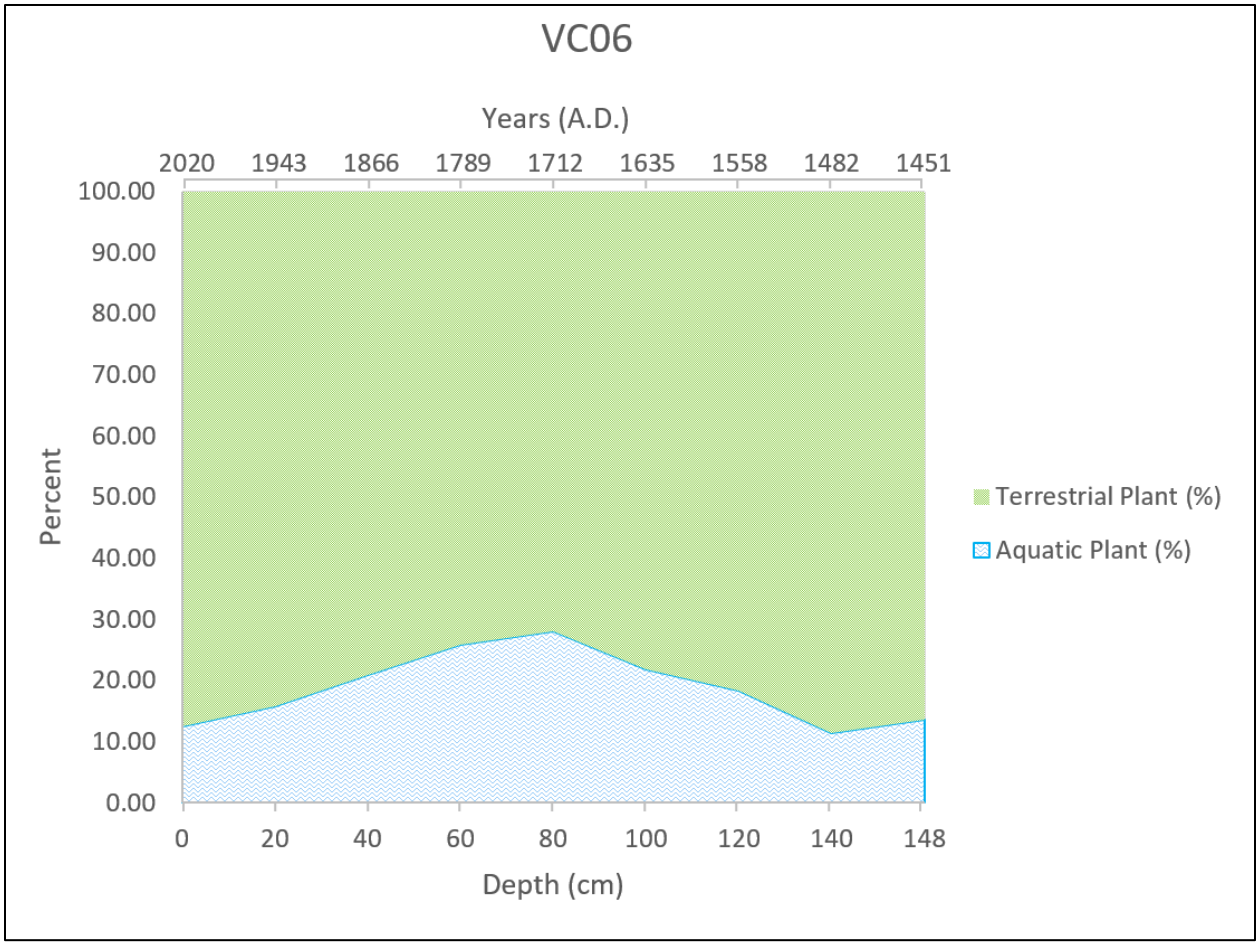

Figure 17: N-alkane contributions downcore for VC06. Solid green represents terrestrial contributions while chevron blue represents eelgrass contributions. The axis represents depth and time. Notice that eelgrass contributions peak at 80 cm and decrease towards the top and bottom of the core. Based on age calculations of shells found downcore 80 cm downcore roughly correlates to the year 1712. Note, chronology was calculated assuming an average sedimentation rate of 0.26 cm/yr. The margin of error on the chronology calculations is ± 50 years.

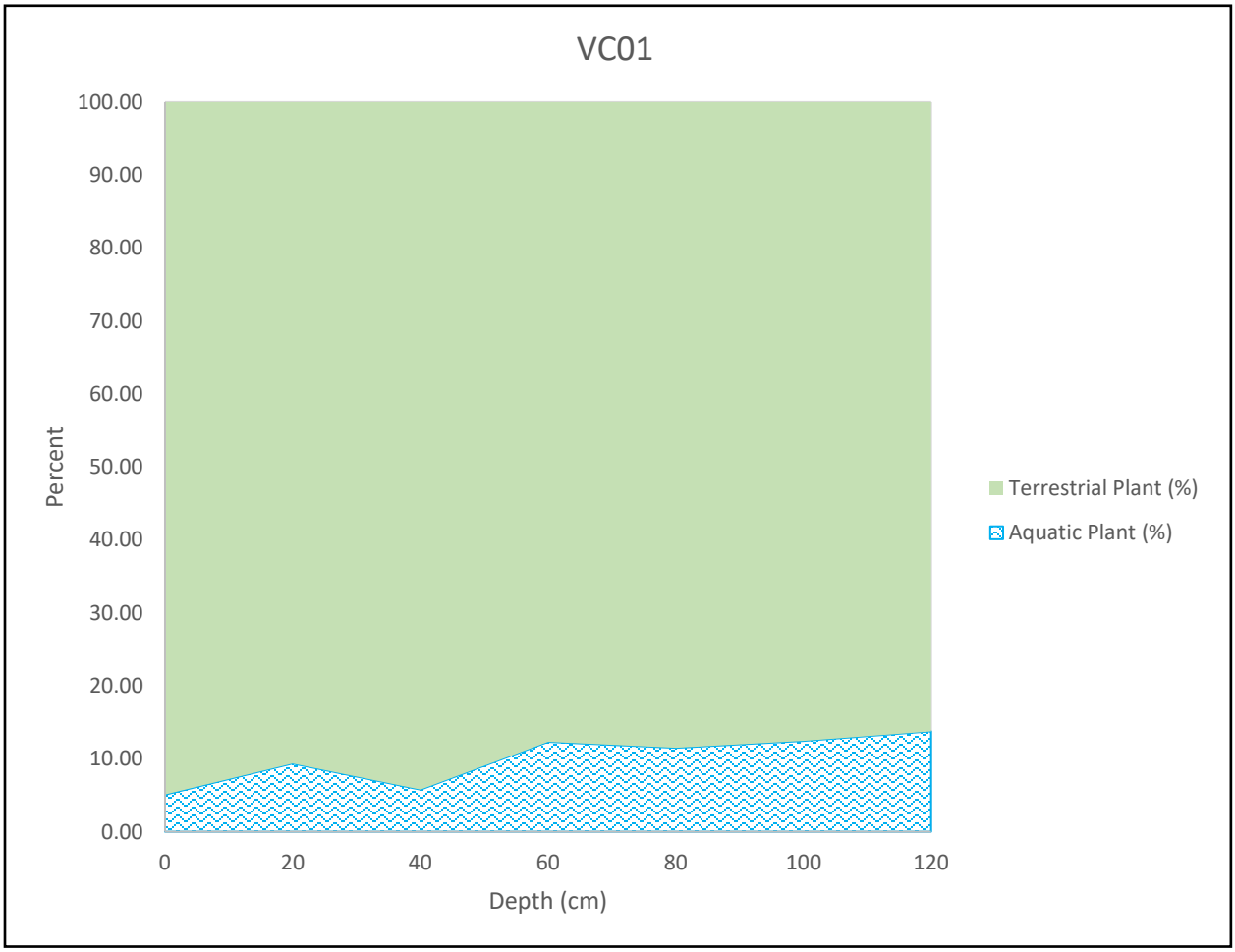

Figure 18: N-alkane contributions downcore for VC01. Solid green represents terrestrial contributions while chevron blue represents eelgrass contributions. The x axis represents depth. Notice that aquatic plant contributions decrease towards the surface (0 cm).

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