Spatial distributions of core and intact glycerol dialkyl glycerol tetraethers (GDGTs) in the Columbia River basin and Willapa Bay, Washington: Insights into origin and implications for the BIT index

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ABSTRACT

Branched and isoprenoid glycerol dialkyl glycerol tetraethers (BrGDGTs and IsoGDGTs, respectively) are used to reconstruct carbon flow from terrestrial landscapes to the ocean in a proxy called the branched vs isoprenoid tetraether index, or BIT Index. The index is based on analysis of core GDGTs (C-GDGT), that are produced from intact GDGTs (I-GDGT) upon cell death. GDGTs are a component of the cell membranes of bacteria living in soils (Br) and archaea (Iso) living primarily in the marine environment. However, uncertainty in the identity of the organisms that biosynthesize BrGDGTs, variation in the extent to which GDGTs represent endmember carbon pools, and suspected production of BrGDGT and IsoGDGTs in both marine and terrestrial environments hinder interpretation of the BIT Index. Since BrGDGTs remain our only tool for measuring the presence of unknown BrGDGT producing organisms, it is particularly important to use the intact form of BrGDGTs present in living cells (rather than core BrGDGT in relict biomass) to infer distributions of the likely source organisms. In situ production within riverine, lacustrine, and marine environments is currently thought to be possible, yet few measures of I-BrGDGTs are available in these environments to support these claims. Here we assess the spatial distribution of core and intact GDGTs in the Columbia River basin and nearby Willapa Bay (Washington and Oregon) in order to elucidate source environments and behavior of I-GDGTs relative to C-GDGTs. The presence of I-BrGDGTs throughout the studied soils, rivers and estuaries suggests *in situ* production across the continuum from soil to marine environments. Likewise, intact crenarchaeol, the marine end member IsoGDGT used in the BIT index, was present in all samples. Widespread production of each GDGT class along terrestrial carbon transport paths likely alters the BIT Index along this continuum. Core to intact ratios and the weak correlation between I-GDGT derived BIT values and carbon isotope signatures suggest

48 a mixture of allochthonous and autochthonous sources of GDGTs in riverine and marine
49 environments. Our findings highlight the need for further work into the provenance and behavior
50 of GDGTs to improve interpretations of the BIT index and other environmental proxies that rely
51 on these compounds.

52

1. Introduction

Estimates suggest that 2.7 Pg of carbon enter inland aquatic systems each year, with approximately 0.45 Pg/y of that total carbon pool being exported to the ocean in the form of dissolved and particulate organic carbon (Battin et al., 2009; Cole et al., 2007). A fraction of this organic carbon (OC) is deposited in marine sediments and is then preserved. The branched vs isoprenoid tetraether index, or BIT Index, based on branched glycerol dialkyl glycerol tetraether (BrGDGT) membrane lipids of bacteria and isoprenoidal GDGTs (IsoGDGTs) of archaea (Fig. 1), is one of the tools used to reconstruct past input of this terrestrial OC into coastal marine sediments (e.g. Hopmans et al., 2004; Walsh et al., 2008; Schouten et al., 2013).

As the terrestrial end member, BrGDGTs are considered to have a terrestrial bacterial origin, but the specific source organisms remain largely unknown (e.g. Sinninghe Damsté et al., 2000; Weijers et al., 2005; 2009a; Sinninghe Damsté et al., 2011) and recent evidence also suggests that BrGDGT origins extend beyond soils and into freshwater and marine environments (e.g. Peterse et al., 2009; Sinninghe Damsté et al., 2009; Tierney and Russel, 2009; Bechtel et al., 2010; Sun et al., 2011; Zhu et al., 2011b; Fietz et al., 2012; Kim et al., 2012; Zhang et al., 2012; Yang et al., 2013; Zell et al., 2013a, 2013b, 2014, 2015; De Jonge et al., 2014, 2015; Weijers et al., 2014; Weber et al., 2015). While source organisms remain largely unknown for BrGDGTs (Grossi et al., 2015), a significant correlation between BrGDGT abundance and the presence of acidobacteria in soils implicates these bacteria as producers of BrGDGTs (Weijers et al., 2006). However, in a study of 17 cultures of acidobacteria, only one of nine BrGDGTs was detected in extracts from two of the cultured species (Sinninghe Damsté et al., 2011).

The marine endmember, crenarchaeol, is an isoprenoidal GDGT (IsoGDGT) currently thought to be produced only by a group of archaea formerly assigned to the marine group I *Crenarchaeota*, now called *Thaumarchaea* (Sinninghe Damsté et al., 2002; Brochier-Armanet et al., 2008; de la Torre et al., 2008). Although recent work has implicated *Euryarchaea* in the production of crenarchaeol (Lincoln et al., 2014), this result is not yet undisputed (Schouten et al., 2014). Studies have also shown that crenarchaeol is present in an array of environments, spanning soils and marine sediments (e.g. Weijers et al., 2006; Fietz et al., 2011, 2012; Smith et al., 2012).

A major shortcoming in our understanding of the processes that control GDGT-based proxy record production stems from a reliance on measurements of core GDGTs (C-GDGTs) in studies attempting to trace the origin of GDGT producing organisms. C-GDGTs represent the pool of membrane lipids that have lost their polar head groups following cell death, as opposed to intact GDGTs (I-GDGTs) that represent the polar intact form of GDGTs that are present in living cells (e.g. Harvey et al., 1986; Lipp and Hinrichs, 2009; Huguet et al., 2010; Tierney et al., 2012). While core lipid measurements are appropriate for reconstructing past conditions from sediment cores, this approach is not an appropriate means to study the distribution of living organisms or the processes affecting proxy emplacement (Schouten et al., 2010). Further, the BIT Index is a ratio of lipids, leading to a disregard for the importance of knowing the concentration of these trace components in sediment relative to the much larger pools of OC for which they are a proxy (e.g. Smith et al., 2012).

Comparisons between the BIT index and more traditional indicators of terrigenous OC such as $\delta^{13}\text{C}$, C:N ratios, and higher plant derived lignin-phenols have helped validate the BIT index as a measure of the relative inputs of terrestrial and marine carbon (e.g. Walsh et al., 2008;

Weijers et al., 2009b; Smith et al., 2010). In general, $\delta^{13}\text{C}$ compares more favorably to the BIT Index than the abundance of the higher plant derived lignin-phenols, indicating differences in the production, transport and burial between OC originating from higher plant and soil sources (e.g. Walsh et al., 2008; Smith et al., 2010). Comparison of the BIT Index with other soil microbial proxies such as the Rsoil index based on bacteriohopanepolyols (BHPs) may provide better insights into soil bacterial origins than comparison with plant derived lignin phenols (Zhu et al., 2011a; Selver et al., 2012, 2015). Other work suggests that autochthonous production of GDGTs can significantly bias the BIT Index, hindering its use as a tracer of soil organic matter in certain areas (Zink et al., 2010; Fietz et al., 2011). Several studies also suggest that variation in autochthonous crenarchaeol production may influence the BIT index when inputs of BrGDGTs remain unchanged (e.g. Fietz et al., 2011; Smith et al., 2012).

Studies that incorporate quantitative analysis of both C- and I-GDGTs hold some promise for constraining environments in which the source organisms live and can help differentiate autochthonous from allochthonous production (e.g. Lipp and Hinrichs, 2009; Ingalls et al., 2012; Kim et al., 2012; Zhang et al., 2012). Comparison of the relative abundance of various GDGTs among sample locations can also aid in determining the origins of C-GDGTs in a given environment (e.g. Sinninghe Damsté et al., 2009; Kim et al., 2012; Zell et al., 2013a, 2013b; De Jonge et al., 2014). Some work has suggested that intact polar lipids can be preserved in sediments (e.g. Bauersachs et al., 2010), with selective degradation of lipids with certain polar head groups occurring in oxidized sediments (e.g. Lengger et al., 2013, 2014). While debate continues regarding the exact lifespan of I-GDGTs in various environments following cell death or polar head group loss, I-GDGTs are the only tool we have to trace unidentified populations of

119 BrGDGT producing cells (Harvey et al., 1986; Lipp and Hinrichs, 2009; Schouten et al., 2010;
120 Xie et al., 2013).

121 Here we focus on the BIT Index and attempt to identify the source environments of
122 newly produced GDGTs by quantitatively relating I- and C-GDGTs to each other and to the total
123 OC pool. We investigated 43 sample sites across a soil and sediment transect from the Columbia
124 River basin and nearby riverine and marine environments. Sample sites in the basin and
125 surrounding areas were chosen to represent an array of environments, ranging from soils to
126 marine sediments and encompassing small and large rivers and substrates from various water
127 depths and salinities. Through an analysis of the spatial distribution and composition of C- and I-
128 GDGTs as well as $\delta^{13}\text{C}$ and BIT index values, we attempt to target locations that may host
129 GDGT-producing organisms and characterize their concentration relative to the OC pool that
130 they are intended to trace.

131 **2. Methods**

132 *2.1. Study area*

133 The Columbia River basin covers more than 670,000 square kilometers, and its stream
134 course spans 2,000 km in portions of seven U.S. states as well as southeastern British Columbia.
135 Due to its size, the watershed contains a diverse range of topographic elements, lithologies, and
136 climates. The dry, eastern portions of the basin receive less than 500 mm of precipitation per
137 year, with mean annual air temperatures (MAAT) ranging from -15 to 35°C (Ferguson et al.,
138 1999). The Western portions of the Columbia River basin are predominantly moderated by a
139 moist, maritime climate and receive upwards of 175 cm of precipitation each year with MAAT

140 ranging from 5-20°C (Western Regional Climate Center, 2009). Soil types are variable, with
141 thick deposits of loess as well as undeveloped sand and gravel from eroding basaltic rock
142 (Busacca, 1991). Predominant soil orders are inceptisols originating from volcanic ash and wind-
143 blown mollisols, which are moist in the winter and dry in summer (Oregon State University,
144 1993). The sedimentation regime of the Columbia is influenced by seasonal variability in river
145 discharge, which reflects climatic patterns of snowmelt, rainfall, and dry summers (Whetten et
146 al., 1969). However, much of the natural variation in coastal delivery of sediments has been
147 reduced by large scale damming along the main stem of the river. Mean annual discharge of the
148 Columbia River at its mouth is 7,500 cubic meters per second.

149 Sample locations throughout the Columbia River basin and Willapa Bay were chosen to
150 represent the array of environmental conditions present in the watershed and depositional
151 environments of both a large river as well as tributaries and small rivers (Figure 2). Terrestrial
152 sites ranged from semi-arid shrub-steppe soils near Twisp, Washington (N48°19'14.5200"
153 W120°17'49.5600", 500 m) to subalpine Mt. Hood meadows (N45°19'31.9794"
154 W121°39'39.8874", 1600 m). Tributary sediments from the Deep River in Washington and the
155 John Day and Hood Rivers in Oregon as well as marine sites at Astoria Canyon near the
156 Columbia River estuary span a range of water depths and salinities (Table 1). Willapa Bay is a
157 tidally influenced embayment on the Washington coast and is partially enclosed by the Long
158 Beach Peninsula, a coastal formation maintained by accretion of Columbia River sediments.
159 Willapa Bay sediments are comprised of inputs spanning a riverine to marine continuum, with
160 salinity ranging from 15-32 psu. Sample sites within two of the bay's major tributaries, the Palix
161 and Willapa Rivers, were also included in this study.

2.2. Sample Collection

Soils (horizon A or AB transition zone) and river shore sediments (0-2 cm depth) from the John Day River, OR; Beaver Army Dock (BAD), OR; Young River, OR; and Twisp, WA were collected with a clean trowel between May and September in 2008 and 2011. Soils were air dried at room temperature in the dark and sieved prior to storage. Willapa Bay sediments were sampled in August of 2009 and Columbia River sediments were collected in August of 2010 using a shipboard multicore or handheld clam gun. Marine sediments from stations CR-7 – CR-15, and CR-40 were collected by a shipboard multicore during an April 2008 Coastal Margin Observation and Prediction (CMOP) cruise aboard the R.V. Wecoma. Riverine and estuarine sample collection was conducted during the dry period in this region. All samples were stored frozen until lipid extraction.

2.3. GDGT analysis

GDGTs were soxhlet extracted for 72 h at 45°C with a solvent mixture of 9:1 (vol:vol) dichloromethane (DCM): methanol (MeOH) (Huguet et al., 2010). Solvent extracts were evaporated in a TurboVap (Caliper LifeSciences) and total lipid extracts (TLE) dried over anhydrous sodium sulfate with 1:1 (vol:vol) DCM:MeOH. TLEs were concentrated by evaporating under ultra-pure N₂(g). To achieve separation of core and intact lipids, samples were eluted sequentially with ethyl acetate (C-GDGTs) and MeOH (I-GDGTs) through a glass column filled with 1.5 g silica gel according to Liu et al. (2011) and the resulting samples stored at -20°C. Polarfractions containing I-GDGTs were hydrolyzed in 5% HCl:MeOH for 4 h at 70°C. Hydrolyzed samples were extracted in DCM (4x) and rinsed with MilliQ water (6x) to remove

any remaining acid. C-GDGT containing fractions were analyzed without additional pretreatment.

It has been reported previously that total separation of C- and I-GDGTs may not be achieved by sequential elution over silica gel (Pitcher et al., 2009). Thus, to assess the effectiveness of our separation protocol, in a subset of 15 samples we evaluated carryover. To do this, we analyzed C-GDGTs in half of the I-GDGT fraction without prior hydrolysis and half of the C-GDGT fraction was re-analyzed for C-GDGTs following hydrolysis. I-GDGTs eluting in the core fraction were calculated by subtracting non-hydrolyzed C-GDGT concentrations from hydrolyzed C-GDGT concentrations. Carryover was considered negligible, with an average of 1.4% for core and 2% for intact and did not influence BIT index values. All fractions were syringe-filtered (0.45 μ m pore size PTFE) into HPLC vials with 300 μ L 99:1 (vol:vol) hexane:isopropanol. C₄₆ GDGT (500 ng) was added to each sample as an internal standard to enable C-GDGT quantification (Huguet et al., 2006).

Samples were analyzed according to Hopmans et al. (2000) and Schouten et al. (2007) using an Agilent 1100 Series liquid chromatograph coupled to an Agilent ion trap (XCT) mass spectrometer (LC-MS) equipped with an autoinjector and ChemStation chromatography manager software. Separation was achieved on a Prevail Cyano column (2.1 by 150 mm and 3 μ m; Alltech, Deerfield, IL) fitted with a Prevail Cyano guard cartridge (7.5 by 4.6 mm and 5 μ m) maintained at 30°C. GDGTs were eluted isocratically first with 99:1 (vol:vol) hexane:isopropanol for 5 min and then by using a linear gradient to 1.8% isopropanol over 65 min and a flow rate of 0.2 ml/min. The column was back flushed after each analysis with 90:10 (vol:vol) hexane:isopropanol for 8 min at 0.2 mL/min and then equilibrated back to initial conditions for 10 min. Detection was achieved by atmospheric pressure chemical ionization

(APCI) of the eluent with the following conditions: nebulizer pressure of 60 lb/in², vaporizer temperature of 400°C, N₂ drying gas flow of 6 liters/min at 200°C, capillary voltage of -3 kV, and a corona at 5 µA (~3.2 kV). The scanning range was 730 to 2,000 *m/z*, and the target mass was 1,300 *m/z*. Absolute abundances of GDGTs in samples were determined by comparing GDGT peak areas to those of the C₄₆ internal standard for each sample (Huguet et al., 2006). Analytical error was determined by comparison of triplicate injections for isoprenoid and BrGDGTs ranging in concentration from 3 - 150 µg gOC⁻¹. Triplicate injections were compared for 8 samples and the analytical error was determined to be 4 percent for C-GDGTs and 7 percent for I-GDGTs.

2.3. Elemental analysis

Triplicate samples of 40 to 100 mg (dry weight) were weighed into silver capsules and acidified with HCl vapor in a desiccator for 24 hours (Komada et al., 2008). The percent carbon and nitrogen as well as δ¹³C and δ¹⁵N isotopic compositions were determined using elemental analysis interfaced with isotope ratio mass spectrometry (EA-IRMS). Samples were analyzed using a Costech ECS 4010 EA coupled with a Finnigan MAT 253 IRMS in the IsoLab at the University of Washington's department of Earth and Space Sciences. Additional analyses were completed using a Carlo Erba NC2500 EA in P. Quay's stable isotope lab in the School of Oceanography at the University of Washington, Seattle and at Skidaway Institute of Oceanography in Savannah, Georgia using a ThermoFinnigan Flash EA Series 1112 coupled with a ThermoFinnigan Delta V IRMS. Errors are ±0.1% for elemental analyses and ±0.6‰ for stable isotope analyses.

231

232 2.4. Principal Component Analysis

233 A principal component analysis (PCA) was applied to core and intact fractional GDGT
234 abundances, BIT indices, inverse $\delta^{13}\text{C}$ values, $\delta^{15}\text{N}$, % TOC, % TON, and salinity for all sample
235 sites ($n = 43$; 16 species evaluated) to determine statistical relationships between sampled
236 environments. GDGT fractional abundances represent the concentration of each compound
237 relative to the total GDGT pool for that sample site and enable comparison of GDGT
238 distributions across large ranges in concentration (e.g. Sun et al., 2011; Kim et al., 2012; De
239 Jonge et al., 2014; Ding et al., 2015). All sample data were normalized using a log
240 transformation prior to PCA. PCA was performed using the R v3.0.2 platform and results were
241 plotted in RStudio v0.98.501 software.

242

243 3. Results

244 3.1. GDGT concentrations and the BIT index

245 Isoprenoid and branched GDGTs were present in all sites ($n = 43$). Total GDGT
246 concentrations relative to OC were highly variable throughout the study area with values
247 spanning from 2 – 690 $\mu\text{g gOC}^{-1}$ (Figs. 3 & 4; Appendix Tables 1a and 1b). Mass normalized
248 GDGT concentrations (relative to sediment analyzed) are presented in Appendix Tables 2a and
249 2b. Both C- and I-BrGDGT concentrations relative to OC were low in soils except at Young
250 River A, and highest in riverine sites, with higher concentrations in the tributaries to the
251 Columbia, Palix and Willapa Rivers, and in the upstream sample sites within the Columbia

mainstem (Figs. 3A& 4A). Deep River contained the highest concentrations of both C-BrGDGTs (229 $\mu\text{g gOC}^{-1}$) and I-BrGDGTs (69 $\mu\text{g gOC}^{-1}$, Fig. 3A). Young River A contained the highest mass normalized concentrations of both C-BrGDGTs (12 $\mu\text{g g sed}^{-1}$) and I-BrGDGTs (3 $\mu\text{g g sed}^{-1}$, Appendix Tables 2a and 2b). Both C- and I-BrGDGT concentrations were lowest in the marine sites. C-Crenarchaeol concentrations were highest in marine sample sites, with concentrations ranging from 272-647 $\mu\text{g gOC}^{-1}$ (Fig. 3B). Intact crenarchaeol was more variable, with the highest OC normalized concentration at the riverine station CR St. 5 (33 $\mu\text{g gOC}^{-1}$, Fig. 3B) and the highest mass normalized concentration at marine station CR-40 (Appendix Table 2b). The sample locations near North Channel had high concentrations of both core and intact crenarchaeol (Fig. 3B). Total crenarchaeol concentrations were lowest in soil sites (n=5), ranging from 0-5 $\mu\text{g gOC}^{-1}$ with an average concentration of 1 $\mu\text{g gOC}^{-1}$ (Fig. 3B).

BIT index values were highest in soils (n=5, average core BIT = 0.91 ± 0.12) and upland riverine sediments in the Deep and John Day Rivers (n=5, BIT = 0.96 ± 0.27 ; Fig. 3C) despite some of these soils containing extremely small concentrations of BrGDGTs (2 – 8 $\mu\text{g gOC}^{-1}$ total BrGDGT). Young River soils were the only soil sites with total BrGDGT concentrations greater than 8 $\mu\text{g gOC}^{-1}$. With the exception of the North Channel and Beaver Army Dock (BAD), corelipid derived BIT index values for riverine sites within the Columbia River generally decreased with distance downstream (Fig. 2 & 3C). Core BIT values did not show consistent trends in and near the Columbia River estuary. Marine sample sites had the lowest BIT values, with average core BIT = 0.02 ± 0.02 and intact BIT = 0.06 ± 0.03 (Fig. 3C). C-GDGT derived BIT values were generally higher than I-GDGT values (Figs 3C & 4C), with the exception of the marine sites, CR St. 11 and 12, and the Young River soils (Fig 3C). Average I-GDGT derived BIT values (0.42 ± 0.32) were more variable ($p < 0.001$) than those derived from C-GDGTs

(0.65 ± 0.27). Soils and upland riverine sites generally had the highest intact BIT values. Three stations near the Columbia River estuary (CR. St. 9.1, 9.3, & 13) had higher intact BIT values than nearby sites.

Core and I-BrGDGT and crenarchaeol were present throughout the Palix and Willapa Rivers (Fig. 4). With the exception of WB St. 6, all sites in the Palix and Willapa Rivers had higher amounts of C-GDGTs when compared to the intact pool (Fig. 4). Willapa Bay Station 10 (WB St. 10) had the highest concentration of C-BrGDGTs (Fig. 4A). Intact Br-GDGT concentrations were highest at WB St. 3, which also had the highest concentration of both core and intact crenarchaeol (Fig 4B). Within Willapa River, the core BIT values generally increase with distance upstream, with the highest BIT values at WB St. 6 (Fig. 4C). However, the intact BIT values do not show the same trend.

Relative proportions of C and I-GDGTs varied in both the Columbia River and Willapa Bay sites (Figs. 5 & 6). Sample sites with the highest proportions of I-BrGDGTs include CR-7, and CR. St. 10, 9.3, and 13 (Fig. 5A). All of these stations had very low concentrations of BrGDGTs (Fig. 4A). An average of 11 % of BrGDGTs were intact in upland Columbia River tributaries (Deep and John Day Rivers), where BrGDGT concentrations were highest (Fig. 5A). For nearly all sample sites, a higher proportion of crenarchaeol was intact when compared to the BrGDGTs (Figs. 5 & 6). Those sites with the highest proportion of intact crenarchaeol include CR St. 8 and 14 (Fig. 5B), both of which had very low overall (core and intact) concentrations of crenarchaeol (Fig. 3B). An average of 40 % of crenarchaeol was intact for riverine sites within the Columbia mainstem (Fig. 5B). The proportion of intact crenarchaeol was generally lowest in marine sediments except for a few samples in Beaver Army Dock, Deep River, and Young River, CR St.11 where concentrations were also lower (Fig. 5B). Willapa Bay sites had an

average of 15 % I-BrGDGTs and 35 % intact crenarchaeol (Fig. 6). WB St. 6 had the highest intact proportions of both BrGDGTs and crenarchaeol (Fig. 6). There was a significant correlation ($R^2 = 0.69$, p -value < 0.01) between I-BrGDGTs and the total BrGDGT pool. Intact and total crenarchaeol concentrations were more weakly correlated ($R^2 = 0.26$, p -value < 0.01).

3.2. BIT Index values and $\delta^{13}C$

With the exception of offshore marine sites, Columbia River sample sites generally had more depleted $\delta^{13}C$ signatures, with $\delta^{13}C$ values ranging from -24.3 to -27.8 ‰ and a mean of -26.0 ± 0.9 ‰ (Fig. 7). The overall signature within Willapa Bay is more enriched, with $\delta^{13}C$ values ranging from -22.6 to -25.2 ‰ and a mean of -24.3 ± 0.9 ‰ (Fig. 7). Upland riverine sediments in the Columbia River watershed had the most depleted $\delta^{13}C$ signatures, with an average $\delta^{13}C$ value of -27.2 ± 0.9 ‰ (Appendix Table 1c). Marine sediments had the most enriched $\delta^{13}C$ signatures with an average $\delta^{13}C$ value of -21.9 ± 0.6 ‰. $\delta^{13}C$ values were more strongly correlated with C-GDGT derived BIT values than intact BIT indices in both the Willapa Bay and the Columbia River (Fig. 7). Willapa Bay had a stronger correlation ($R^2 = 0.60$, $p < 0.01$) between $\delta^{13}C$ and C-GDGT derived BIT values than Columbia River sample sites ($R^2 = 0.44$, p -value < 0.01 ; Fig. 7). No significant correlation was observed between intact BIT indices and $\delta^{13}C$ signatures.

3.3. GDGT composition and PCA analysis

The composition of I and C-isoGDGTs differ from each other and differ among environmental types (Fig. 8). GDGT-1-3 showed a decreasing trend along the soil to marine

continuum and from I-isoGDGTs to C-isoGDGTs (Fig. 8). Core BrGDGT compositions were very uniform with a slight deviation in composition of soils relative to other environments. Intact BrGDGTs displayed more variability (Fig. 9).

The first two principal components of the PCA analysis explain 53.8 percent of total variance for C-GDGTs and 48.2 percent of total variance for the I-GDGTs (Fig. 10). Distribution of principal component scores resolve several subgroups within the data (Figs. 10A & 10B). Principal component scores for the various environmental types are distinctly clustered for C-GDGTs (Fig. 10A). Also, marine samples form a distinct group along PC1 where salinity is a major distinguishing variable. Within the I-GDGT dataset, this grouping is less pronounced (Fig. 10B). PC1 scores for estuarine, riverine, and soil sample sites also follow a general trend with higher positive scores on the marine end-member and lower negative scores on the terrestrial end-member (Fig. 10A). BrGDGTs showed clear trends of enrichment in terrestrial samples that had lower $\delta^{13}\text{C}$ values and higher BIT index values (Fig. 10A). Conversely, crenarchaeol was enriched in samples with greater salinities and higher $\delta^{15}\text{N}$ values (Fig. 10A & B). Environmental types were better separated in C-BrGDGTs than I-BrGDGTs (Fig. 10B). Within the I-GDGT data, distributions of principal component scores for soil sites and marine sites are distinctly clustered, whereas scores for riverine and estuarine environments are more variable (Fig. 10B).

4. Discussion

4.1. GDGT origins

BrGDGTs are the soil endmember in the BIT Index. Because the organisms that make BrGDGTs are unknown, production of BrGDGTs in soil and non-soil environments is now commonly inferred from distributions of C-BrGDGTs (e.g. Fietz et al., 2012; Schouten et al., 2013 and references therein; Weijers et al., 2014). For example, marine production was inferred from an increase in C-BrGDGT concentrations toward the Lost City hydrothermal field and in anoxic portions of the marine water column, suggesting origins from distinct marine environments (e.g. Lincoln et al., 2013; Liu et al. 2014; Xie et al., 2014). Other work has examined variations in C-BrGDGT distributions between terrestrial inputs and marine sediments to suggest possible marine production of BrGDGTs (e.g. Peterse et al., 2009; Zhu et al., 2011b; Weijers et al., 2014; Zell et al., 2014, 2015). Since C-GDGTs have not been shown to be present in living cells (Huguet et al., 2006; Schouten et al., 2013; Elling et al., 2014), they cannot indicate the presence, present or past, of living biomass in a particular location. In our study site, significant amounts of I-BrGDGTs, indicators of living or recently living cells, were detected in the Columbia, Palix, and Willapa Rivers and their estuaries. The highest concentration of I-BrGDGTs was found in Deep River, a small tributary to the Columbia mainstem (Fig. 3). While surrounding soils are certainly a likely source of BrGDGTs to many riverine and marine settings, our findings support other works that suggests production of BrGDGTs not only extends into freshwater and likely estuarine environments (e.g. Tierney and Russel, 2009; Bechtel et al., 2010; Tierney et al., 2010; Sun et al., 2011; Kim et al., 2012; Zhu et al., 2011b, Zhang et al., 2012; Yang et al., 2013; Zell et al., 2013a, 2013b, 2014; De Jonge et al., 2014, 2015), but that these sources may exceed soil sources in some cases (e.g. Peterse et al., 2009; Zell et al., 2014, 2015).

With the exception of Young River A, soils sampled in this study contained only small amounts ($2 - 8 \mu\text{g gOC}^{-1}$) of I- or C-BrGDGTs relative to river and estuary sites, suggesting that these soil endmembers may not be the primary source of BrGDGTs to the rivers studied. However, our soil sample size ($n = 5$) is small and may not be representative of the entire study area. Future work is needed to examine soil sources of BrGDGTs in the Columbia and Willapa basins. Despite our small sample size, total BrGDGT concentrations in our soil samples ($2 - 119 \mu\text{g gOC}^{-1}$) are within the range of those reported in previous studies ($3 - 84 \mu\text{g gOC}^{-1}$) when peat soils are excluded (e.g. Weijers et al. 2006, 2010; Tierney and Russel, 2009). This suggests that our data are representative of other soil environments. Further examination of bulk geochemical data for our sample sites suggests that percent OC is not a strong predictor of OC normalized I-BrGDGTs (Appendix Table 1c). Excepting Young River A (12% OC), which has some of the highest Br-GDGT concentrations relative to OC in our study area (Fig. 3A), the average wt%OC of soil samples (2.65%) is similar to riverine (1.6%) and estuarine (2.1%) sites. This finding is in conflict with the intended interpretation of BIT Index values as tracers of OC carbon pools. We detected I-BrGDGTs in all but one sample site (CR St. 11), which provides indirect evidence for possible soil, riverine, estuarine, and marine origins for these compounds, although marine stations contained only small ($1 - 2 \mu\text{g gOC}^{-1}$) quantities of I-BrGDGTs, suggesting that *in situ* populations of BrGDGT producing organisms are minor or that small amounts of I-BrGDGTs can be redistributed after cell death, but prior to head group cleavage.

Based on our data, it remains unclear whether C-BrGDGTs sampled at downstream locations originated within the main river channel, in tributary channels, or in soils. It is also unclear how tidal reworking of sediment may influence distributions of these compounds in the estuarine environment. BrGDGTs remain orphan biomarkers, with the suite of bacteria that

produce them largely remaining unknown (Sinninghe Damsté et al., 2011). Thus, as in our work, other evidence for non-soil production of BrGDGTs is largely based on indirect evidence, such as variation in BrGDGT composition, reconstructed air temperature, sea surface temperature, pH values, or shifts in proportions of I-BrGDGTs relative to the total branched pool among sample areas (e.g. Zhu et al., 2011b; Kim et al., 2012; Zhang et al., 2012; Yang et al., 2013; Zell et al., 2013a, 2013b, 2014; De Jonge et al., 2014, 2015). Based on I-BrGDGT concentrations throughout our study area, the environmental conditions in which these compounds originate are diverse, spanning a range of salinities and distances from shore (Fig. 2 & Table 1). Temporal variability in runoff and river discharge between various portions of the watershed may result in a diverse array of origins and ages for GDGTs in estuarine or marine end members (e.g. Zell et al., 2013a).

The relatively low proportion of I-BrGDGTs relative to total BrGDGTs in many sample locations suggests a small population of BrGDGT-producing organisms around the time of sampling (Figs. 5A & 6A). Additionally, the correlation between OC normalized concentrations of core and intact BrGDGTs differs among environments with the highest correlation in soils ($R^2=0.99$, p -value < 0.01 , $n=5$) and progressively weaker correlations in riverine ($R^2=0.59$, p -value < 0.01 , $n=20$), estuarine ($R^2=0.35$, p -value = 0.02 , $n=15$) and marine ($R^2=0.34$, p -value = 0.6 , $n=3$; negative correlation) sites. Scatter in these data along the soil-marine continuum suggests that patchy production, sediment movement, tidal influence, hydrodynamic sorting of particles, and variable degradation rates are all potentially important controls on observed C- and I-BrGDGT distributions. Other studies have shown that 80-90 % of BrGDGTs in soils can be core lipids (e.g. Peterse et al., 2010; Weijers et al., 2011) and turnover times for C-BrGDGTs are

thought to be ~20 years in mid-latitude, arable soils (Weijers et al., 2010), which could lead to an accumulation of core lipids in soils where BrGDGT production is taking place.

Several sample stations (CR-7, CR St. 10) had higher proportions of I-BrGDGTs relative to the total BrGDGT pool, but the total BrGDGT pool at these sites is low ($< 4 \mu\text{g gOC}^{-1}$). The low concentrations mean that even trace quantities of I-BrGDGTs in these samples could translate to high proportions of intact lipids relative to the total pool. Two sites in the Columbia River had higher I-BrGDGT concentrations and high proportions of I-GDGTs and thus appeared to have significant *in-situ* production of branched lipids. CR St. 13 contained $55 \mu\text{g BrGDGT gOC}^{-1}$, 45 percent of which was intact (Fig. 5A). CR St. 9.3 contained $63 \mu\text{g BrGDGT gOC}^{-1}$, 44 percent of which was intact (Fig. 5A). These values strongly suggest production within riverine sediments, and are exceptional sites to further study the biological origins of these molecules.

4.2. Crenarchaeol distributions

Ammonia-oxidizing archaea (AOA) belonging to the *Thaumarchaeota* are the only confirmed biological source of crenarchaeol (e.g. Sinninghe Damsté et al., 2002; de la Torre et al., 2008; Pitcher et al., 2011; Kim et al., 2012). Although a recent field study has suggested the possibility that members of the Euryarchaea may also produce this lipid (Lincoln et al., 2014), confirmation from cultured cells is still absent (Schouten et al., 2014). C-Crenarchaeol represents an enriched component of the total OC pool in marine compared to terrestrial environments (Figs. 3B, 4B and 10). However the presence of I-crenarchaeol at all sample sites throughout the Columbia River basin and Willapa Bay indicates that crenarchaeol is produced throughout our sample area (Figs. 3B & 4B). Two sites in the Palix River had higher concentrations of I-

430 crenarchaeol than marine sediments (Fig. 3B & 4B), suggesting *in situ* production within the
 431 riverine sedimentary environment. The presence of I-crenarchaeol in terrestrial soil and riverine
 432 samples is also consistent with the near ubiquitous presence of AOA in soils (e.g. Leininger et
 433 al., 2006; Pitcher et al., 2011) and other findings pointing to a combination of marine and
 434 terrestrial, riverine, or estuarine inputs of crenarchaeol to marine sedimentary environments (e.g.
 435 Fietz et al., 2011, 2012; Smith et al., 2012). The low proportion of I-crenarchaeol and the high C-
 436 crenarchaeol concentrations in marine sediments (Fig. 5B) compared with other sample areas in
 437 our study suggests primarily non-sedimentary sources, reflecting deposition from the overlying
 438 water column where AOA are abundant members of the planktonic community and only reach
 439 the sediment after cell death and degradation during sinking (Karner et al., 2001). The correlation
 440 between C- and I-crenarchaeol is lowest in soils ($r^2=0.02$, $n=4$), increases in riverine ($r^2=0.71$,
 441 $n=20$) and estuarine ($r^2=0.90$, $n=15$) sites and declines slightly in the marine environment
 442 ($r^2=0.83$, $n=3$). This trend suggests that production and preservation of crenarchaeol are closely
 443 coupled in riverine, estuarine and marine environments.

444 Large ranges in the organic carbon normalized concentrations of both C- and I-
 445 crenarchaeol suggest that, like C-BrGDGTs in our study, C-crenarchaeol does not represent total
 446 carbon equally among all sources (Figs. 3B and 4B). The highest concentrations of I-
 447 crenarchaeol normalized to OC were in the Palix River and the Columbia River mainstem, a
 448 terrestrial environment. This suggests that crenarchaeol coming from production within these
 449 rivers may result in delivery of crenarchaeol to coastal systems beyond what would be expected
 450 from soils alone. Our data indicate that the composition of I-isoGDGTs differs from C-
 451 isoGDGTs extracted from the same samples (Fig. 8). This may be further evidence for mixed
 452 sources and varying lability or residence times of C and I-GDGTs in sediments from across our

site. Other work has shown two to six fold differences in preservation efficiencies for various I-isoGDGTs (Lengger et al., 2013). The resilience of I-GDGTs is related to the polar head group structure, as headgroups from glycosidic polar lipids are lost more slowly than those from phospholipids (Harvey et al., 1986; Lipp and Hinrichs, 2009; Schouten et al. 2010; Xie et al., 2013). *Thaumarchaeae* produce isoGDGTs with both phospholipid and glycolipid headgroups (Pitcher et al., 2011), thus it is possible that differences in composition between core and intact pools in different environments may also be related to relative rates of polar head group loss during diagenesis.

4.3. The BIT Index

The BIT index was originally used to track soil organic carbon input into the marine environment (e.g. Hopmans et al., 2004; Walsh et al., 2008). Our data suggest that crenarchaeol and BrGDGTs employed in the BIT index reflect spatially, temporally, and biologically complex OC pools. In order to develop meaningful interpretations of BIT index values, several assumptions are implied. For example, that OC normalized concentrations of BrGDGTs and crenarchaeol are consistent throughout the environment or system (e.g. terrestrial to marine continuum), production must be restricted to appropriate endmember environments, transport of endmembers must be quantitative and decomposition must be uniform relative to source carbon pools across all GDGTs (e.g. Fietz et al., 2012; Smith et al., 2012). Our results and other recent research into GDGT sources and sinks have challenged these assumptions.

Our data indicate a large degree of variability in the organic carbon normalized concentrations of both crenarchaeol and BrGDGTs among the environments sampled (Figs. 3 &

475 4). When crenarchaeol concentrations vary greatly, the BIT index may exaggerate or
476 underestimate the amount and variability of terrestrial OM exported from the surrounding
477 watershed due to the fact that the BIT index is a ratio influenced by both crenarchaeol and
478 BrGDGTs. Other work has shown that changing crenarchaeol concentrations can exert a
479 significant control on BIT Index values due to the smaller concentration ranges of BrGDGTs
480 (Castañeda et al., 2010; Schmidt et al., 2010; Fietz et al. 2012; Kim et al., 2012; Sinninghe
481 Damsté et al., 2012; Smith et al., 2012; Zell et al., 2013a). This scenario has been validated in
482 other portions of the Washington coastline (Walsh et al., 2008). However, it might also be that
483 autochthonous production of BrGDGTs in riverine or estuarine environments may have the
484 opposite effect on BIT index values if crenarchaeol production is low (e.g. Fietz et al., 2011; Zell
485 et al., 2013a). This may explain the high I-GDGT derived BIT index values of the upland
486 riverine sites within the Columbia basin, where BrGDGT concentrations were much higher than
487 crenarchaeol (Fig. 3).

488 Our data also agree with other studies that have shown that production of crenarchaeol
489 and BrGDGTs are not restricted to the marine and terrestrial environments, respectively (e.g.
490 Castañeda et al., 2010; Schmidt et al., 2010; Fietz et al., 2012, Kim et al., 2012; Smith et al.,
491 2012). Weijers et al. (2006) examined core BrGDGTs in globally distributed soils and found
492 concentrations ranging from 0.2 to 4.5 $\mu\text{g gOC}^{-1}$ and detected significant concentrations of the
493 marine endmember, crenarchaeol, in nearly all terrestrial sites. Recent studies have also
494 provided evidence for production of BrGDGTs in marine, riverine, estuarine, and lacustrine
495 environments (e.g. Sinninghe Damsté et al., 2009; Tierney and Russel, 2009; Zhu et al., 2011b;
496 Kim et al., 2012; Zhang et al., 2012; Zell et al., 2013a, 2013b, 2014; De Jonge et al., 2014, 2015;
497 Weber et al., 2015), thus distorting sedimentary BIT index values during transport of terrestrial

signals. These trends in production would also alter other BrGDGT based proxies such as the MBT/CBT indices, in which variations have been used to infer in situ production of BrGDGTs (e.g. Zhang et al, 2012; Zell et al., 2013, 2014, 2015). Our data indicate that C-GDGT derived BIT index values are generally higher than I-GDGT derived BIT index values (Figs. 3C & 4C), implying that in-situ production of crenarchaeol relative to BrGDGTs is an important process affecting this measure of terrestrial soil carbon exports (e.g. Fietz et al., 2011; Smith et al., 2012).

The observed differences in C- and I-BIT values might also be due to differences in preservation of Br versus IsoGDGTs. Matrix effects within soils and sedimentary particles can protect organic compounds from degradation (e.g. Hedges and Keil, 1995). Differential matrix effects experienced by soil and marine organic matter can result in differential degradation of BrGDGTs and IsoGDGTs. Huguet et al. (2008, 2009) showed that crenarchaeol degrades at a faster rate than branched GDGTs in oxygenated environments, and in some cases preservation of soil-derived BrGDGTs was an order of magnitude greater than crenarchaeol over long time scales. Thus, the lower I-GDGT derived BIT values in our data could indicate that portions of C- crenarchaeol are degraded in the riverine environment prior to being buried in riverine sediments while C-BrGDGTs may be accumulating in sediment end members. It is also possible that BrGDGT production in the riverine setting is low relative to the C-BrGDGT pool derived from a mixture of freshwater and terrestrial sources (e.g. Peterse et al., 2015). Although C-BIT index values in the Columbia and Willapa Rivers follow an increasing trend with distance upstream, I-GDGT derived BIT index values are more variable, possibly reflecting in situ GDGT production. In soils and the upland riverine sites, C- and I-GDGT derived BIT values are more similar (Fig.

3C), indicating that C-GDGT pools in these areas are largely comprised of *insitu* production and may also reflect preservation of all GDGTs within the soil matrix.

Our PCA suggests that using C-GDGT values results in a greater separation of marine and soil endmember environments along PC1 (Fig. 10). The closer grouping of samples along PC1 in the analysis of I-GDGTs suggests again that production is patchy and that *in situ* production is likely imposed on top of a background of core lipids that are integrated over a much longer history.

Because $\delta^{13}\text{C}$ signatures can reflect the relative contributions of terrestrial and marine OM to sediments, comparing the BIT index with $\delta^{13}\text{C}$ values of total organic carbon (TOC) can put important constraints on how well BIT values are tracking the relative proportion of terrestrial carbon (e.g. Huguet et al., 2007; Walsh et al., 2008; Weijers et al., 2009b, 2010). The stronger correlation between $\delta^{13}\text{C}$ signatures and core BIT index values relative to intact BIT index values in our data (Fig. 7) may illustrate the effect of *in situ* GDGT production on the BIT index. Other work suggests that stronger correlations between $\delta^{13}\text{C}$ values and core BIT index values indicate that a higher proportion of riverine OM was soil derived (e.g. Weijers et al., 2009b; Smith et al., 2010) while weaker correlations may be indicative of a greater contribution of plant-derived OM (that of terrestrial vegetation) relative to soil OM (that of soil microbial communities) (Walsh et al., 2008). Our data suggest that the carbon signature of the I-BrGDGTs used in the BIT index may be different than that of the core lipids, providing further indirect support for multiple environmental sources of BrGDGTs. Recent work examining the carbon isotope composition of purified BrGDGTs from soils and those from a nearby lake provides strong evidence for lacustrine BrGDGT production (Weber et al., 2015). Similar work is needed to expand this evidence to the riverine setting.

543

544 **5. Conclusions**

545 Intact branched and isoprenoid GDGTs were present throughout the Columbia River
546 basin and nearby estuarine environments in Willapa Bay. Taking this into account along with the
547 diversity of our sample sites, it is reasonable to conclude that BrGDGTs are biosynthesized in an
548 array of environments, not simply soil environments as originally suggested. The spatial
549 distribution of intact BrGDGTs suggests a mix of autochthonous and allochthonous inputs to
550 riverine/estuarine sediments. Our findings are consistent with other earlier studies that suggest
551 riverine production of BrGDGTs and some terrestrial production of crenarchaeol (e.g. Kim et al.,
552 2012; Zell et al., 2013a, 2013b, 2014; De Jonge et al., 2014). Further work examining the
553 provenance of these compounds is needed to employ the BIT index as a proxy for soil organic
554 matter inputs to marine sediments, as originally intended (Hopmans et al., 2004). The mixing of
555 autochthonous and allochthonous inputs of GDGTs to the endmembers studied resulted in
556 variability between core and intact derived BIT index values. GDGT distributions differed
557 between soils, tributaries, and the Columbia River mainstem. Thus, the BIT index may
558 inadvertently be a measure of both terrestrial and aquatic organic matter inputs, via a balance
559 between production and degradation, to sediments. $\delta^{13}\text{C}$ values showed a stronger relationship
560 with C-GDGT derived BIT index values compared with I-GDGT BIT index values and may be
561 used concurrently with the BIT index to verify the provenance of the organic matter being
562 studied. We suggest that future work utilizing the BIT index include quantification of both core
563 and intact pools of GDGTs due to the uncertain origins of core branched GDGTs in riverine and
564 marine environments in which this index has been applied.

565

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Figure Captions

Figure 1. The BIT index and GDGT structures and (m/z):Glycerol trialkyl glycerol tetraether (GTGT-0, 1304), GDGT-0 (1302), GDGT-1 (1300), GDGT-2 (1298), GDGT-3 (1296), Crenarchaeol (1292) and its regioisomer, BrGDGT-I (1050), BrGDGT-II (1036), and BrGDGT-III (1022) (modified from Yang et al., 2013; Pitcher et al., 2011).

Figure 2. Sample site locations distributed across Columbia River basin and Willapa Bay. Inset Map A shows distribution of Columbia River mainstem and estuary sites (note rotated map orientation). Inset Map B shows distribution of Willapa Bay sites. Symbols represent environmental types for sample locations: riverine (triangle), estuarine (diamond), marine (star), and soils (pentagon).

Figure 3. Branched GDGT abundances (A) and Crenarchaeol abundances (B) as proportions of organic carbon; BIT Indices for Columbia River sites (C). Note that core GDGT derived Crenarchaeol abundances for CR-7, -15, and -40 exceed the scale of the chart (see Appendices 1a & 1b for values). Core GDGT derived branched abundances exceed the chart scale for Deep River Mud. Sample sites listed in order of increasing distance from marine environment.

Figure 4. Branched GDGT abundances (A) and Crenarchaeol abundances (B) as proportions of organic carbon; BIT Indices for Willapa Bay sites (C). Note that Crenarchaeol abundances

exceed the chart scale for WB. St. 1 and 3 (see Appendices 1a & 1b for values). Sample sites listed in order of increasing distance from marine environment for Palix and Willapa Rivers.

Figure 5. Intact branched GDGTs as percentage of total branched GDGT pool (A) and intact Crenarchaeol as percentage of total Crenarchaeol pool (B) listed in order of increasing distance from marine environment.

Figure 6. Intact branched GDGTs as percentage of total branched GDGT pool (A) and intact Crenarchaeol as percentage of total Crenarchaeol pool (B) listed in order of increasing distance from marine environment for Palix and Willapa River estuaries.

Figure 7. Scatter plots, regression equations, and statistical values for comparison of $\delta^{13}\text{C}$ (‰) and BIT indices for Columbia River (left) and Willapa Bay (right) sample sites. Core BIT (upper plots) values show a stronger correlation with $\delta^{13}\text{C}$ values when compared with Intact BIT (lower plots)

Figure 8. Mean compositions of core (A) and intact (B) isoprenoid GDGTs as proportions of the total isoprenoidal GDGT pool for various study areas. Compounds arranged by mass to charge ratio (m/z).

Figure 9. Mean compositions of core (A) and intact (B) branched GDGTs as proportions of the total branched GDGT pool for various study areas. Compounds arranged by mass to charge ratio (m/z).

Figure 10. PCA Biplots for PC1 & PC2 scores and loadings for core (A) and intact (B) GDGTs. GTGT 0, GDGTs 0 – 3 and BrGDGT I – III are displayed in figure 1. Additional vector arrow labels correspond to variables analyzed and are abbreviated as follows: ‘ ^{13}C ’ = $\delta^{13}\text{C}$, ‘%C’ = % Carbon, ‘%N’ = % Nitrogen, ‘ ^{15}N ’ = $\delta^{15}\text{N}$.

901 **Figures**

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903 Figure 1.

Figure 2.

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925 Figure 8.

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929 Figure 9.

Figure 10.

932 Table 1. Sample site characteristics. Columbia River (CR), Willapa Bay (WB), Beaver Army
 933 Dock (BAD).

<u>Type</u>	<u>Name</u>	<u>Latitude</u>	<u>Longitude</u>	<u>Depth/Horizon</u>	<u>Salinity</u>	<u>Date</u>
Estuarine	CR St.8	N 46° 12' 41.2992"	W 123° 54' 49.4994"	0-1 cm	11	8/13/2010
	CR St.9.1	N 46° 15' 59.1006"	W 124° 0' 20.196"	0-3 cm	11	8/13/2010
	CR St.9.2	N 46° 15' 59.1006"	W 124° 0' 20.196"	0-3 cm	11	8/13/2010
	CR St.9.3	N 46° 15' 59.1006"	W 124° 0' 20.196"	0-2 cm	11	8/13/2010
	CR St.10	N 46° 16' 9.0012"	W 124° 0' 36.399"	0-1 cm	11	8/13/2010
	WB St.1	N 46° 35' 51.8994"	W 123° 54' 1.4754"	0-2 cm	15	8/12/2009
	WB St.2	N 46° 36' 15.1992"	W 123° 54' 48.1998"	0-2 cm	20	8/12/2009
	WB St.3	N 46° 36' 52.4988"	W 123° 55' 1.7004"	0-2 cm	22	8/12/2009
	WB St.4	N 46° 38' 4.4982"	W 123° 56' 42.6006"	0-2 cm	30	8/12/2009
	WB St.6	N 46° 41' 32.0346"	W 123° 43' 58.2414"	0-2 cm	20	8/12/2009
	WB St.8	N 46° 42' 0.2010"	W 123° 53' 11.2986"	0-2 cm	30	8/13/2009
	WB St.9	N 46° 42' 27.1008"	W 123° 51' 6.7998"	0-2 cm	-	8/13/2009
	WB St.10	N 46° 40' 1.4016"	W 123° 48' 26.7984"	0-2 cm	24	8/13/2009
Marine	WB St.11	N 46° 40' 31.5978"	W 123° 47' 14.1000"	0-2 cm	24	8/13/2009
	WB St.12	N 46° 42' 24.9984"	W 123° 51' 3.4986"	0-2 cm	28	8/13/2009
	CR-15	N 46° 9' 59.3994"	W 124° 20' 1.2114"	0-1 cm	32	4/14/2008
Riverine	CR-40	N 46° 9' 59.3994"	W 124° 54' 36.6114"	0-1 cm	32	4/14/2008
	CR-7	N 46° 9' 56.4114"	W 124° 9' 34.8120"	0-1 cm	32	4/14/2008
	BAD	N 46° 11' 1.2114"	W 123° 11' 13.8114"	0-1 cm	-	5/7/2008
	BAD Mud Bank	N 46° 10' 10.0914"	W 123° 8' 57.0480"	0-1 cm	-	5/7/2008
	CR St.1	N 46° 13' 37.8012"	W 123° 46' 42.3978"	0-1 cm	1	8/12/2010
	CR St.2	N 46° 13' 43.8996"	W 123° 46' 31.6986"	0-1 cm	1	8/12/2010
	CR St.3	N 46° 14' 52.0002"	W 123° 43' 15.0990"	0-1 cm	0	8/12/2010
	CR St.4	N 46° 14' 57.9978"	W 123° 37' 58.1874"	0-1 cm	0	8/12/2010
	CR St.5	N 46° 14' 1.9998"	W 123° 42' 11.4006"	0-1 cm	1	8/12/2010
	CR St.6	N 46° 12' 58.6008"	W 123° 44' 32.7012"	0-1 cm	1	8/12/2010
	CR St.7	N 46° 11' 18.1026"	W 123° 51' 16.9884"	0-1 cm	5	8/13/2010
	CR St.11	N 46° 11' 12.9012"	W 123° 54' 57.9996"	0-4 cm	-	8/13/2010
	CR St.12	N 46° 10' 48.6984"	W 123° 53' 25.9008"	0-4 cm	4	8/13/2010
	CR St.13	N 46° 10' 45.4002"	W 123° 52' 43.4994"	0-4 cm	5	8/13/2010
	CR St.14	N 46° 12' 45.2988"	W 123° 38' 35.7720"	0-1 cm	1	8/9/2010
	CR St.15	N 46° 10' 42.8016"	W 123° 43' 3.5004"	0-1 cm	1	8/29/2010
	Deep River Bank	N 46° 20' 4.9914"	W 123° 41' 57.0114"	0-5 cm	-	5/7/2008
	Deep River Mud	N 46° 20' 4.9914"	W 123° 41' 57.0114"	0-5 cm	-	5/7/2008
	John Day 2"	N 44° 45' 2.6100"	W 120° 14' 35.0880"	0-2 cm	0	8/6/2011
	John Day 2ft	N 44° 45' 2.6100"	W 120° 14' 35.0880"	0-2 cm	0	8/6/2011
	John Day Bank	N 44° 45' 2.6100"	W 120° 14' 35.0880"	0-2 cm	0	8/6/2011
	North Channel 2cm	N 46° 14' 4.9986"	W 123° 52' 45.9840"	0-2 cm	-	5/7/2008
Soil	Hood River A	N 45° 42' 12.888"	W 121° 30' 8.7120"	A	-	5/7/2008
	Hood River AB	N 45° 42' 12.888"	W 121° 30' 8.7120"	A-B	-	5/7/2008
	Mt Hood A	N 45° 19' 31.9794"	W 121° 39' 39.8874"	A	-	5/7/2008
	Twisp Surface	N 48° 19' 14.5200"	W 120° 17' 49.5600"	A	-	9/7/2008
	Young River A	N 46° 4' 5.6274"	W 123° 47' 15.7914"	A	-	5/7/2008

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Figure 1

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