Spatial gradients in soil-carbon character of a coastal forested floodplain are associated with abiotic features, but not microbial communities

Aditi Sengupta¹, Julia Indivero², Cailene Gunn², Malak M. Tfaily³,⁴, Rosalie K. Chu⁴, Jason Toyoda⁴, Vanessa L. Bailey¹, Nicholas D. Ward³,⁵, James C. Stegen¹

1. Biological Sciences Division, Pacific Northwest National Laboratory, PO Box 999
   MSIN: J4-18, Richland, WA 99352
2. Marine Sciences Laboratory, Pacific Northwest National Laboratory, 1529 West Sequim Bay Road, Sequim, WA 98382
3. Department of Soil, Water, and Environmental Sciences, University of Arizona, Tucson, AZ 85719
4. Environmental Molecular Sciences Laboratory, Pacific Northwest National Laboratory, Richland, WA 99352
5. School of Oceanography, University of Washington, Seattle, WA 98195

Correspondence to: aditi.sengupta@pnnl.gov
Abstract

Coastal terrestrial-aquatic interfaces (TAIs) are dynamic zones of biogeochemical cycling influenced by salinity gradients. However, there is significant heterogeneity in salinity influences on TAI soil biogeochemical function. This heterogeneity is perhaps related to unrecognized mechanisms associated with carbon (C) chemistry and microbial communities. To investigate this potential, we evaluated hypotheses associated with salinity-associated shifts in organic C thermodynamics, biochemical transformations, and heteroatom content in a first-order coastal watershed in the Olympic Peninsula of Washington state, USA. In contrast to our hypotheses, thermodynamic favorability of water soluble organic compounds in shallow soils decreased with increasing salinity, as did the number of inferred biochemical transformations and total heteroatom content. These patterns indicate lower microbial activity at higher salinity that is potentially constrained by accumulation of less favorable organic C. Furthermore, organic compounds appeared to be primarily marine/algal-derived in forested floodplain soils with more lipid-like and protein-like compounds, relative to upland soils that had more lignin-, tannin-, and carbohydrate-like compounds. Based on a recent simulation-based study, we further hypothesized a relationship between microbial community assembly processes and C chemistry. Null modelling revealed strong influences of dispersal limitation over microbial composition, which may be due to limited hydrologic connectivity within the clay-rich soils. Dispersal limitation indicated stochastically assembled communities, which was further reflected in the lack of an association between community assembly processes and C chemistry. This suggests a disconnect between microbial community composition and C biogeochemistry, thereby indicating that the salinity-associated gradient in C chemistry was driven by a combination of spatially-structured inputs and salinity-associated metabolic responses of microbial communities that were independent of community composition. We propose that impacts of salinity on coastal
soil biogeochemistry need to be understood in the context of C chemistry, hydrologic/depositional dynamics, and microbial physiology, while microbial composition may have less influence.

1. Introduction

The interface between terrestrial and aquatic ecosystems represent a dynamic and poorly understood component of the global carbon (C) cycle, particularly along the tidally-influenced reaches of coastal watersheds where terrestrial and marine biospheres intersect (Krauss et al., 2018; Neubauer et al., 2013; Tank et al., 2018; Ward et al., 2017b). Moreover, the nutrient cycles occurring at these terrestrial-aquatic interfaces (TAIs) influence locally important ecosystem services like contaminant fate and transport and water quality (Conrads and Darby, 2017; Vidon et al., 2010). While coastal soil C stocks are being increasingly quantified (Hinson et al., 2017; Holmquist et al., 2018; Krauss et al., 2018), the impact of tidally-driven salinity gradients on molecular level features of the soil-C pool and the processes driving soil organic matter (OM) cycling are poorly studied (Barry et al., 2018; Hoitink et al., 2009; Sawakuchi et al., 2017; Ward et al., 2017b), particularly in settings with low freshwater inputs that allows significant seawater intrusion compared to large river systems (Hoitink and Jay, 2016). Moreover, there is some indication that microbial diversity and composition impact soil C storage and mineralization (Mau et al., 2015; Trivedi et al., 2016). This points to the intriguing possibility that processes governing microbial community assembly may be associated with OM chemistry, but evaluations of such associations are lacking. This lack of mechanistic knowledge combined with significant ecosystem heterogeneity in biogeochemical function across salinity gradients (more below), highlights a need to understand how molecular-level processes vary with seawater exposure along coastal TAIs. Doing so will help enhance predictive models of TAI.
biogeochemistry that can be potentially included in ecosystem models to more accurately represent the role of TAIs in the broader Earth system (U.S. DOE., 2017).

Modeling of coastal TAIs is currently impeded by poor knowledge of the mechanisms underlying salinity-driven variation in biogeochemical function of associated soils. Previous studies have evaluated function primarily as carbon dioxide (CO$_2$) and methane (CH$_4$) flux measurements from soil, and/or soil OM concentrations measured as bulk soil C, percent OM and porewater dissolved organic C (DOC) concentrations in large scale coastal plain river systems. Results from field-based natural salinity gradient studies, long-term field manipulations of salinity exposure, and lab-based incubation studies subjecting soils to varying levels of salinity broadly show the following trends: increases in CO$_2$ and decreases in CH$_4$ emissions in freshwater soils exposed to increasing salinity (Chambers et al., 2011, 2013, 2014; Liu et al., 2017; Marton et al., 2012; Neubauer et al., 2013; Steinmuller and Chambers, 2018; Weston et al., 2006, 2011), and decreases in CO$_2$ and CH$_4$ emissions from soils with a natural history of being exposed to saline environment when exposed to elevated salinity (Chambers et al., 2013; Herbert et al., 2018; Neubauer et al., 2005, 2013; Weston et al., 2014) (also see Table S1). Two exceptions have been observed where CO$_2$ emissions decreased in historically freshwater coastal wetland soils exposed to seawater (Ardón et al., 2018; Herbert et al., 2018). These observations suggest that microbial activity usually increases with salinity in soils that were not previously exposed to saline conditions, while simultaneously indicating reduced microbial activity with increasing salinity in soils that have a historical exposure to elevated salinity. In contrast to relatively consistent responses of gas fluxes to changes in salinity, there are strong inconsistencies in DOC responses, including no change (Weston et al., 2006, 2011, 2014),
increased DOC (Chambers et al., 2014; Tzortziou et al., 2011), and decreased DOC (Ardón et al., 2016, 2018; Liu et al., 2017; Yang et al., 2018) with increasing salinity.

Relatively consistent gas flux responses combined with inconsistent DOC responses of soils exposed to elevated salinity suggest at least a partial decoupling between microbially driven biogeochemical rates and the concentration of DOC. This apparent decoupling between the size of the C pool and microbial activity suggests that C biogeochemistry is influenced by salinity-exposure history, which in turn influences nutrient resources available to soil microbial communities. Specifically, any systematic shifts in soil organic carbon (SOC) chemistry profiles that occur along natural salinity gradients (Bischoff et al., 2018; Neubauer et al., 2013), which cannot be observed with bulk C measurements may result in unpredictable carbon fluxes. Moreover, bulk C content can show no change across gradients of salinity (Neubauer et al., 2013) and may fail to capture an integrated view of microbial-activity driven C cycling dynamics at TAIIs. In contrast, detailed molecular-level evaluation of SOC composition can provide a more mechanistic view of OC transformations, relative to bulk measures of C content or gas flux measurements. Analyses of specific chemical biomarkers such as lignin phenols, amino acids, and lipids have been used in soils, sediments, and water to quantitatively evaluate the provenance of terrestrial-derived OM (Hedges et al., 1997), the reactivity of OM as it travels through a soil column (Shen et al., 2015), and microbial community composition (Langer and Rinklebe, 2009), respectively. While biomarkers provide quantitative details on OC cycling, they generally represent a small fraction of the total OM pool, thus, non-targeted approaches such as analysis of thousands of peaks via Fourier Transform Ion Cyclotron Resonance Mass Spectrometry (FTICR-MS) have become increasingly widespread for determining molecular-level organic compound signatures (Rivas-Ubach et al., 2018) across a variety of terrestrial (Bailey et al., 2017; Simon et
Despite its potential importance, a detailed understanding of the characteristics of soil organic compounds (Zark and Dittmar, 2018) and their association with microbial communities in coastal TAIs is currently not available. However, starting with an assumption of increases in microbial activity with increasing salinity (Nyman and Delaune, 1991; Smith et al., 1983; Tzortziou et al., 2011) provides a series of expectations. First, it is generally expected that microbes preferentially degrade compounds with higher nominal oxidation states (NOSC) or lower Gibbs Free Energy ($\Delta G_{\text{ox}}^{0}$) due to greater thermodynamic favorability (Boye et al., 2017; Ward et al., 2017a), although factors such as redox state, mineral associations, and microbial community composition can alter this generality (Schmidt et al., 2011). The basic assumption that OM reactivity follows NOSC leads to the expectation that the average $\Delta G_{\text{ox}}^{0}$ of the compounds in the resource environment will increase with increasing salinity as organic compounds with greater thermodynamic favorability are preferentially depleted (LaRowe and Van Cappellen, 2011). Second, actively growing microbial communities are known to enhance biochemical transformations and generate heteroatom containing organic molecules [sulfur (S), nitrogen (N) and phosphorus (P)] (Guillemette et al., 2018; Koch et al., 2014; Ksionzek et al., 2016); therefore greater heteroatom content and more biochemical transformations are expected with increasing salinity. Third, studies have also shown that microorganisms adapt to saline conditions through the production or sequestration of osmolytes (Gouffi et al., 1999; Roberts, 2005; Sleator and Hill, 2002), a strategy that requires organic N mining. This suggests a potential increase in N-containing biochemical transformation with increasing salinity. Fourth, soil OM in saturated environments like floodplains are expected to be less oxygenated and can
also receive deposition of suspended sediments during flooding, both of which may result in a greater abundance of marine/algal derived OM exhibiting low oxygen to carbon (O/C) and high hydrogen to carbon (H/C) ratio as compared to upland soils (Seidel et al., 2016; Tfaily et al., 2014; Ward et al., 2019b). We therefore expect a greater relative abundance of lipids and proteins and less lignin and tannin compounds in the floodplain soils, relative to upland (i.e., drained) soil.

While we expect systematic shifts in C chemistry across landscape scale salinity gradients, an open question is the degree to which C chemistry is associated with ecological assembly processes governing microbial communities. Soil microorganisms transform soil C, but there is limited evidence of direct links between microbial community assembly processes and molecular-level soil C chemistry (Kubartová et al., 2015; Rocca et al., 2015; Trivedi et al., 2016; van der Wal et al., 2015). Assembly processes, broadly divided into deterministic (selective) and stochastic (random) factors, function over space and time to structure microbial communities, which in turn mediate biogeochemical cycles (Graham et al., 2016, 2017b; Nemergut et al., 2013a; Stegen et al., 2015). These processes can be inferred from phylogenetic distances among microbial taxa using ecological null models, which have been widely employed to understand community assembly processes in subsurface microbial ecology (Caruso et al., 2011; Dini-Andreote et al., 2015; Graham et al., 2017a, 2018; Stegen et al., 2012). Furthermore, a recent study used ecological simulation modeling to show that environments experiencing increasing rates of dispersal processes are linked to reduced biogeochemical functioning (Graham and Fine, 2008). This leads to the hypothesis that the influence of deterministic selection will progressively increase with salinity-driven increases in microbial activity.
The objective of the current study was to test the following hypotheses in a coastal forested floodplain and adjacent upland forest: (i) the overall Gibbs Free Energy of organic compounds will increase with increasing salinity; (ii) biochemical transformations, heteroatom content, and N-containing biochemical transformation will increase with increasing salinity; (iii) the lipid and protein compound classes will be prevalent in the floodplain soils compared to upland soils in which lignin- and tannin-type molecules will dominate; and (iv) microbial community assembly processes will be increasingly deterministic as salinity increases. The chemical forms of C in these soils were characterized using FTICR-MS. We also employed ecological null model analysis to evaluate the relationship between C chemistry and the influences of assembly processes on microbial communities. Based on our results, we propose a conceptual model of organic C processing in a coastal forested floodplain in which landscape-scale gradients in C chemistry are driven by a combination of spatially-structured inputs and salinity-associated metabolic responses of microbial communities that are independent of community composition.

2. Materials and Methods:

2.1 Site Information and Soil Sampling

Soils along a coastal watershed draining a small first order stream, Beaver Creek, in the Washington coast were selected for this study. Beaver Creek is a tributary of Johns River and experiences a high tidal range of up to 2.5 m that extends midway up the first-order stream’s channel and inundates the landscape in its floodplains. The confluence of Beaver Creek and Johns River is roughly 2.5 km upstream of the Grays Harbor estuary and 14.5 km from the Pacific Ocean, and experiences variable exposure to saline waters at high tide (Fig. 1). Surface water salinity near Beaver Creek’s confluence ranges from 0 psu at low tide to 30 psu at high tide during dry periods (Ward, unpublished). Tidal exchange to Beaver Creek was restored after
2014 when a culvert near the creek’s confluence with Johns River was removed (Washington Department of Fish and Wildlife, 2019). Due to the minimal past tidal exchange, the floodplain is dominated by gymnosperm trees (*Picea sitchensis*) that are rapidly dying since the culvert removal (Ward et al., 2019a). The headwaters (before the river channel forms) is a sparsely forested, perennially inundated freshwater wetland with tidal exchange blocked by a beaver dam, followed downstream by a densely forested setting along the river channel. Towards Beaver Creek’s confluence salt tolerant grasses such as *Agrostis stolinifera* become the most dominant land cover as forest cover becomes more sparse. The watershed’s hillslope/uplands is dominated by *Tsuga heterophylla* trees, but *Picea sitchensis* are also present.

Two sampling transects perpendicular to the river along the up/downstream salinity gradient were established and represent a high salt exposure site close to the culvert breach location and a moderate salt exposure site upstream of the high salt exposure site. These transects represent a coastal forested wetland with brackish (semi-salty) groundwater and consisted of three terrestrial sampling points at each transect extending from the riparian zone to the beginning of the steep upslope. An additional soil sampling point ~20m uphill from the moderate salt exposure site transect served as a purely terrestrial upland endmember. The floodplain transects represented hydric soils classified as Ocosta silty clay loam while the upland site was a well-drained Mopang silt loam. The transects experience periodic inundation episodes which result in surface pooling of tidal water.

Soil samples were collected in triplicate at each of the seven locations (Fig. 1) [BC2, BC3, and BC4 at the high-salt exposure transect, locations BC12, BC13, BC14 at the moderate salt exposure transect, and BC15 as upland site]. The high-salt exposure transect was 230 m from the...
moderately saline transect (0.6 km from the confluence of Beaver Creek with Johns River), and each site at the transect was ~25 m apart from the next. For data comparison’s sake, we classify BC2, BC3, BC12, and BC13 as floodplain sites while BC4 and BC14 are further inland and ~75 m away from the creek at the base of the densely wooded hillslope. Soil samples for molecular characterization studies were collected at two depths—shallow (10 cm) and deep (19-30 cm). Samples were collected from the face of soil pits using custom mini-corers, placed into sterile amber glass vials, purged with N₂ to maintain anaerobic conditions, frozen in the field within an hour at -20 °C, and stored at -80 °C on return to the lab. Bulk samples were collected for soil physicochemical characterization including texture classification with hydrometer method after organic matter removal, dry combustion with direct measure of total C, nitrogen (N) and sulfur (S) by Elementar Macro Cube, plant-available N as ammonium-nitrogen (NH₄-N) and nitrate-nitrogen (NO₃-N) with 2M KCl quantified on Lachat as colorimetric reaction, pH, specific conductivity, gravimetric water content, bulk density, and porosity. Molecular characterization included ultra-high resolution C characterization using FTICR-MS and microbial community assembly analyses using amplicon-based 16S rRNA gene sequencing.

2.2 FTICR-MS solvent extraction and data acquisition

Soil organic compounds were extracted using a sequential extraction protocol with polar {water (H₂O)} and non-polar {chloroform (CHCl₃) and methanol (CH₃OH)} solvents per standardized protocols (Graham et al., 2017a; Tfaily et al., 2015, 2017). Briefly, extracts were prepared by adding 5 ml of MilliQ H₂O to 5 g of each of the replicate samples in sterile polypropylene centrifuge tubes (Genesee Scientific, San Diego, USA) suitable for organic solvent extractions and shaking for 2 h on a Thermo Scientific LP Vortex Mixer. Samples were removed from the shaker and centrifuged for 5 minutes at 6000 rpm, and the supernatant was removed into a fresh
centrifuge tube. This step was repeated two more times, with the 15 ml supernatant pooled for each sample and stored at -80 °C until further processing. Next, Folch extraction with CHCl₃ and CH₃OH was performed for each soil pellet left over from the water extraction. Folch extraction entailed adding 2 ml CH₃OH, vortexing for 5 seconds, adding 4 ml CHCl₃, vortexing for 5 seconds, followed by of 0.25 ml of MilliQ H₂O. The samples were shaken for 1 hr and another 1.25 ml MilliQ H₂O was added and left overnight at 4 °C to obtain bi-layer separation of upper (polar) layer and the lower (non-polar) layer. The extracts were stored in glass vials at -20 °C until ready to be used. The water soluble organic carbon (WSOC) fraction was further purified using a sequential phase extraction protocol to remove salts as per Dittmar et al., 2008. For the purpose of this study, purified WSOC and CHCl₃ fractions were used. The extracts were injected into a 12 Tesla Bruker SolariX FTICR-MS located at Environmental Molecular Sciences Laboratory (EMSL) in Richland, WA, USA. Detailed methods for instrument calibration, experimental conditions, and data acquisition are provided in Graham et al., 2017a and Tfaily et al., 2017.

### 2.3 FTICR-MS Data Processing

One hundred forty-four individual scans were averaged for each sample and internally calibrated using an organic matter homologous series separated by 14 Da (–CH₂ groups). The mass measurement accuracy was less than 1 ppm for singly charged ions across a broad m/z range (100 - 900 m/z). Data Analysis software (Bruker Daltonik version 4.2) was used to convert raw spectra to a list of m/z values applying FTMS peak picker module with a signal-to-noise ratio (S/N) threshold set to 7 and absolute intensity threshold to the default value of 100. Chemical formulae were then assigned using in-house software following the Compound Identification Algorithm, proposed by Kujawinski and Behn (2006), modified by Minor et al. (2012), and
described in Tolić et al. (2017). Peaks below 200 and above 900 were dropped to select only for calibrated and assigned peaks. Chemical formulae were assigned based on the following criteria: S/N >7, and mass measurement error < 0.5 ppm, taking into consideration the presence of C, H, O, N, S, P, and excluding other elements. Detected peaks and associated molecular formula were uploaded to the in-house pipeline FTICR R Exploratory Data Analysis (FREDA) to obtain: (i) NOSC values from elemental composition of the organic compounds (Koch and Dittmar, 2006, 2016), (ii) thermodynamic favorability of the compounds calculated as Gibbs Free Energy for the oxidation half reactions of the organic compounds ($\Delta G_{\text{ox}}^{0}$) based on the equation $\Delta G_{\text{ox}}^{0} = 60.3 - 28.5 \times \text{NOSC}$ (LaRowe and Van Cappellen, 2011), where a higher $\Delta G_{\text{ox}}^{0}$ indicates a less thermodynamically favorable species than a lower value (LaRowe and Van Cappellen, 2011), (iii) abundance of compounds grouped into elemental groups (CHO, CHOS, CHOP, CHNOS, CHNO, CHNOP, CHOSP, and CHNOSP), and (iv) abundance of compound classes (carbohydrate-, lipid-, protein-, amino sugar-, lignin-, tannin-, condensed hydrocarbon-, and unsaturated hydrocarbon-like) based on molar H:C and O:C ratios of the compounds (Bailey et al., 2017).

Biochemical transformations potentially occurring in each sample were inferred from the FTICR-MS data by comparing mass differences in peaks within each sample to precise mass differences for commonly observed biochemical transformations (Breitling et al., 2006; Stegen et al., 2018b). The ultra-high mass accuracy of FTICR-MS allows precise mass differences to be counted for the number of times each transformation was observed within each sample. Briefly, the mass difference between m/z peaks extracted from each spectrum were compared to commonly observed mass differences associated with 92 common biochemical transformations.
provided in previous publications (Graham et al., 2017a; Stegen et al., 2018c). All possible pairwise mass differences were calculated within each extraction type for each sample. For example, a mass difference of 97.05 corresponds to a gain or loss of the amino acid proline, while a difference of 43.98 corresponds to the gain or loss of a carboxylate molecule.

### 2.4 Ecological Modeling

Null modeling was used to estimate influences of ecological processes on microbial community composition from rarefied (10000) 16S rRNA amplicon-dependent microbial community composition and phylogenetic relatedness. The extraction, purification, and sequencing of soil microbial DNA were performed according to published protocol (Bottos et al., 2018). Sequence pre-processing, operational taxonomic unit (OTU) table construction and phylogenetic tree building were performed using an in-house pipeline, HUNDO (Brown et al., 2018). Null modeling was performed as described previously (Stegen et al., 2013, 2015) with a total of 35 samples to estimate relative influences of deterministic and stochastic selection processes. Briefly, samples that passed quality control and rarefaction threshold were evaluated for pairwise phylogenetic turnover between communities, calculated as the difference between the mean-nearest-taxon-distance ($\beta$MNTD) metric and mean of the null distribution in units of standard deviation. The difference was significant if the $\beta$-nearest taxon index ($\beta$NTI) > 2 or <-2 signifying variable or homogenous selection, respectively. Comparisons within the null distribution (2> $\beta$NTI>-2) represent stochastic processes including homogenizing dispersal and dispersal limitation or undominated processes. These processes were evaluated using the Raup-Crick metric extended to account for species relative abundances (RC\textit{bray})(Stegen et al., 2013, 2015). Homogenizing dispersal was inferred if deviations were 2>$\beta$NTI>-2 and RC\textit{bray}<0.95, while deviations 2>$\beta$NTI>-2 and RC\textit{bray}>0.95 suggested dispersal
limitation. Undominated processes were represented by comparison within the null distribution of both metrics ($2 > \beta_{NTI} > -2$ and $0.95 > RC_{Bray} > -0.95$). Raw sequences are archived at NCBI (BioProject PRJNA541992) with reviewer link (https://dataview.ncbi.nlm.nih.gov/object/PRJNA541992?reviewer=b55qu29emsinvk3udb2rmuffqh).

2.5 Statistical Methods

Samples were separately analyzed for WSOC and CHCl$_3$ fractions. Within each solvent fraction, samples were grouped into shallow or deep depths. FTICR-MS dependent metrics including $\Delta G^\circ_{\text{Cox}}$, and relative abundance of compound classes, total transformations, nitrogen-containing transformations, and organic nitrogen containing compounds were regressed against specific conductivity. Regressions were considered significant if $R^2 \geq 0.50$ and $p \leq 0.05$. The transformation profiles were also regressed with the community assembly processes to determine the relationship between deterministic/stochastic processes and organic compound transformations. Mantel tests were used to evaluate similarity between BNTI matrix and Sorensen matrix of peak presence/absence. The Sorensen distance matrices of WSOC and CHCl$_3$ peaks were regressed against measured variables (soil physicochemical properties) and community assembly process-variables to determine correlations. Finally, a redundancy analysis –based stepwise model building with forward model choice was performed to determine variation in the Hellinger-transformed water-fraction peaks and CHCl$_3$ fraction peaks as explained by explanatory variables (which included measured soil variables, modeled community assembly variables, and categorical variables depth and location). All statistical analyses were performed in the statistical computing language R version 3.5.3 (R Development Core Team, 2019).
3. Results

3.1 Soil characterization. The percent of total soil C (%C) in the shallow soils (26.3 ± 8.3%) was higher than the deeper soils (4.0 ± 1.3%) for the lowland soils (i.e. “floodplain” and “inland” sites), while the upland site had an average %C of 7.4 ± 0.27% at 10 cm and 2.13 ± 0.06% at 30 cm (Table S2). No significant relation was observed between %C in the shallow inland and floodplain soils along the salinity gradient. The percent of total soil N (%N) of the shallow soils were higher (1.5 ± 0.40%) than the deeper soils (0.4 ± 0.08%) for the lowland soils and co-varied with %C (r²=0.95). The pH of all soils were acidic (5.64 ± 0.70). The concentrations of NH₄-N and NO₃-N showed a consistent trend where NH₄-N was 1-2 orders of magnitude higher than NO₃-N in all samples. The specific conductivity (used as a measurement of salinity in this study) of the shallow soils ranged from 206-866 (±12) µS cm⁻¹ in the lowland soils to 43+5 µS cm⁻¹ in the terrestrial end-member site. The deep soils exhibited specific conductivity ranging from to 148-524 (±11) µS cm⁻¹ in the lowland soils to 29.2±8 µS cm⁻¹ in the terrestrial end-member site. Texture analysis revealed a broad range of sand (4.1 – 40 %), silt (21.4 – 57.9%), and clay (28.6 – 64.8%) fractions.

3.2 Thermodynamics, compound classes, and elemental composition. The calculated ΔG₀Cox WSOC (Table S3)in shallow soils was consistent with our hypothesis of decreasing thermodynamic favorability with increasing conductivity. Average ΔG₀Cox ranged from 53-71 kJ mol C⁻¹ (R²= 0.78, p <0.00001), while no significant relationship between ΔG₀Cox and specific conductivity was observed for WSOC fraction in the deeper soils (averaging 51-54 kJ mol C⁻¹) for the floodplain and inland samples (Fig. 2). The upland site had significantly higher average ΔG₀Cox (67-70 kJ mol C⁻¹) than the soils near the lowland. The ΔG₀Cox (CHCl₃) at both depths
(Table S4) was higher than the water fractions (ranging between 96-105 kJ mol C\(^{-1}\)) but did not show significant relationship with respect to specific conductivity.

Peak profiles for each solvent extraction showed distinct compound classes in the van Krevelen space, with peaks assigned to specific compound classes according to rules outlined in Kim et al., 2003 and modified by Bailey et al., 2017. The WSOC fraction was dominated by compounds classified as protein-, amino sugar-, lignin-, condensed hydrocarbon-, carbohydrate-, and tannin-like compounds (Table 1), while the CHCl\(_3\) fraction had relative high abundances (75\% and higher) of lipid-like compounds (data not shown). A modest percentage of peaks (11-17\%) did not have classes assigned. Unique and common peaks extracted in the WSOC fraction in samples grouped according to their landscape position and depth [four sites in the floodplain (BC2, BC3, BC12, and BC13), two sites inland (BC4 and BC14), and one upland site (BC15)] are represented as H:C to O:C ratio of the compounds (p = 0.05) in Fig. S1.

The shallow WSOC in the floodplain had greater relative abundance of unique lipid (28\%)- and protein (25\%)-like compounds with relatively high H:C and low O:C ratios as compared to the upland site (BC15), which had an 31\%, 30\%, and 19\% unique peaks representing lignin-, tannin-, and carbohydrate-like compounds respectively. About 23\% of peaks were common in both groups, including lignin- and condensed hydrocarbon-like compounds (Fig. S1a). Between the floodplain and the inland samples, high H:C and low O:C ratios representing 47\% lipid-, 38\% protein-, and 22\% amino sugar-like peaks were uniquely present in the floodplain samples (Fig. S1b). The inland shallow soils had 19\% unique higher H:C peaks representing condensed hydrocarbon-like compounds compared to 1.2\% in the upland soil, though most of the compound classes were observed at both locations (Fig. S1c). Linear regression with specific conductivity profiles showed significant positive correlation with amino sugar-, protein-, lipid-,
and unsaturated hydrocarbon-like compounds, while condensed hydrocarbon-like compounds were significantly negatively correlated (Table S5).

For the deep soils, the upland site had 32% unique peaks with relatively high H:C ratios and low O:C ratios commonly associated with unsaturated hydrocarbon-like compounds, as compared to the 0.7% in the floodplain which had higher prevalence of unique peaks representing condensed hydrocarbon (36%), and tannin-like (35%) compounds (Table 1, Fig. S1d). The floodplain vs inland samples had thrice as many unique peaks with high H:C and low O:C ratios representing lipid-like compounds in the floodplain samples. Comparisons between inland and upland end-member samples revealed 43% and 37% unique peaks representing low H:C and high O:C ratio hydrocarbon- and tannin-like compounds respectively in inland samples, while 32%, 14% 9%, and 12% of unique peaks were matched to unsaturated hydrocarbon-, lipid-, protein-, and amino sugar-like compounds respectively in the latter (Table 1, Fig. S1e, f). No significant relationship was observed with specific conductivity (Table S5). For the CHCl₃ fraction, peaks of lipid-like and unsaturated hydrocarbon-like compounds were observed to be common in all samples (data not shown) and regressions against specific conductivity were not significant for the compound classes.

Compositional differences of the organic compounds showed variable heteroatom abundances, with cumulative heteroatom abundance decreasing with increasing salinity ($R^2=0.43$, $p = 0.009$) for shallow fraction of the WSOC. For the WSOC fraction, heteroatom abundance of CHOP ($R^2 = 0.61$) and CHNOP ($R^2 = 0.50$) containing compounds was consistent with our hypothesis and significantly ($p < 0.01$) increased, while CHNOS ($R^2 = 0.66$), and CHNOSP ($R^2 = 0.62$) abundances were inconsistent with our hypothesis and significantly decreased with increasing...
specific conductivity. The elemental composition of the WSOC compounds for deep soils did not show any significant trend with respect to conductivity. For the CHCl₃ fraction, relative abundance of CHNOP in the shallow soils significantly decreased with specific conductivity ($R^2 = 0.57$, $p < 0.01$).

3.3 Transformation profiles. In contrast to our expectations, the number of transformations decreased with increasing salinity in the water fraction of shallow soils ($R^2 = 0.60$, $p < 0.01$) (Fig. 3a, Table S3). We also evaluated N-containing transformations and the abundance of N-containing compounds in the system. Total nitrogen-containing transformations also decreased significantly with conductivity but the correlation was not as strong ($R^2 = 0.40$, $p < 0.01$). Total N-containing compounds (Fig. 3b, Table S3) as well as their relative abundance decreased significantly ($R^2 = 0.74$, $p < 0.01$), with increasing conductivity in the shallow soils for water fraction.

3.4 Ecological processes impacting community composition

Null modeling revealed that community assembly processes were influenced by variable selection ($\beta_{NTI} > 2$), homogenous selection ($\beta_{NTI} < -2$), dispersal limitation ($2 > \beta_{NTI} > -2$ and $RC_{bray} > 0.95$), homogenizing dispersal ($2 > \beta_{NTI} > -2$ and $RC_{bray} < -0.95$), and undominated processes ($2 > \beta_{NTI} > -2$ and $0.95 > RC_{bray} > -0.95$) (Fig. 4). Dispersal limitation had the greatest influence, responsible for 54% of the variation in community composition. The lowest signal was for homogenizing dispersal (1%), and the signal for homogenous selection (23%) was higher than variable selection (9%). Together, deterministic processes (variable selection plus homogeneous selection) were responsible for 32% of the variation in community composition, with 55% contributed by stochastic processes (dispersal limitation plus homogenizing dispersal).
Variation not accounted by dispersal or selection (i.e., influenced by a mixture of processes) accounted for the remaining signal (23%). Consistent with influences from both stochastic and deterministic processes, $\beta$NTI relationships with environmental variables were significant ($p < 0.05$ by Mantel test), but relatively weak ($r=0.46$ for pH and $r=0.31$ for specific conductivity) (Fig. S2).

To evaluate associations between microbial community assembly processes and chemistry, process estimates were regressed against features of the organic C profiles. Deterministic processes decreased (Fig S3a) while community assembly processes influenced by non-deterministic processes increased with increasing number of transformations of organic compounds (Fig. S3b), although no strong relationships were observed between assembly processes and transformations ($p = 0.027$, $R^2 = 0.11$ for deterministic/non-deterministic processes, $p = 0.475$, $R^2 = 0.015$ for variable selection, $p = 0.054$, $R^2 = 0.10$ for homogenous selection, $p = 0.514$, $R^2 = 0.007$ for homogenizing dispersal). No significant relationships were observed between assembly processes and the number of N-containing transformations. Sorensen dissimilarity values based on the detected FTICR peaks for the water fraction were correlated with distance matrices of measured environmental variables and estimates of community assembly processes. Weak positive correlations were observed with $\text{NH}_4$-N ($r = 0.28$), pH ($r = 0.27$), specific conductivity ($r = 0.41$), $\text{NO}_3$-N, silt, and clay ($r = 0.30$) while for the CHCl$_3$ fraction, weak positive correlations were observed with specific conductivity and $\text{NO}_3$-N ($r = 0.26$) (Fig. S4). A Mantel test of FTICR Sorensen dissimilarity vs $\beta$NTI values yielded a significant relationship ($r = 0.213$, $p = 0.003$) for water fraction but not for CHCl$_3$ fraction ($r=0.076$, $p = 0.152$). The stepwise model building yielded a combination of five variables that were weakly associated with the
composition of water fraction peaks (p=0.026, adj. $R^2 = 0.217$), including sand, dispersal limitation, $NH_4^-$-N concentration, specific conductivity, and location. The model explaining variation in the composition of CHCl$_3$ fraction peaks was non-significant (p = 0.1, adj. $R^2 = 0.05$).

4. DISCUSSION

Sea level rise is increasing the inland extent of tides and exacerbating storm surge, resulting in greater salinity intrusion and altered ecosystem behavior across coastal TAI s (Conrads and Darby, 2017; Ensign and Noe, 2018; Langston et al., 2017; McCarthy et al., 2018; Neubauer et al., 2013). Site-driven variations in the responses of bulk soil biogeochemical processes (i.e., gas flux and DOC release) to elevated salinity suggests potentially important influences of underlying features such as C chemistry and microbial communities. To provide a foundation for understanding the role of C chemistry and microbial communities on biogeochemical cycling in coastal soils, we evaluated associations among a landscape-scale soil salinity gradient, molecular-level soil carbon chemistry, and microbial community assembly processes in order to ultimately inform future improvements for predictive models. In soils associated with a coastal first-order drainage basin, we observed salinity-associated gradients in soil organic carbon character that were not associated with microbial community assembly processes. Our results are consistent with C chemistry being driven by a combination of spatially-structured inputs and salinity-associated metabolic responses of microbial communities that are independent of microbial community composition.

4.1 Molecular characterization reveals chemical gradients not seen in the bulk C pool
The systematic shifts observed in the molecular signatures compared to non-significant changes in bulk C chemistry shows that molecular-level investigations are particularly relevant to process-based resolution of C biogeochemistry. The absence of bulk C signals mimicking molecular C signals parallel studies indicating rapid change in molecular constituents of the soil C pool with no change in gross C content (Graham et al., 2018; Reynolds et al., 2018). A faster turnover time of C has been observed in microbial biomass as compared to bulk soil organic matter (Kramer and Gleixner, 2008), which is likely to impact microbe-mediated biochemical C transformations and lead to chemically complex heterogeneous C signatures likely to be missed in bulk analysis (Tfaily et al., 2015). The systematic C character shifts exhibited by samples at the shallow depth suggests that organic C compound pools in shallower soil depths are sensitive to salinity gradients while deeper depth signatures do not vary systematically across the landscape. The landscape gradient observed in the shallow soils is likely influenced by a combination of reduced litterfall from the recently suffering trees, changing understory vegetation, and algae-rich particulate OM deposition during inundation events that presumably initiated after the recent culvert removal. In contrast, the deeper soil depths were more similar to older organo-mineral complexed C in terrestrial soils across various ecosystems and land uses (Conant et al., 2011; Dungait et al., 2012; Jobbágy and Jackson, 2000; Kramer and Gleixner, 2006, 2008). The lack of any systematic gradients in the mineral-associated soil C provides further evidence in support of these interpretations, in addition to previous studies showing mineral-associated soil C to be less responsive to environmental forcings, relative to water soluble C (Reynolds et al., 2018).

4.2 Decreases in organic C thermodynamic favorability may restrict microbial activity
Consistent with our first hypothesis, systematic changes in soil organic C character were observed with thermodynamically less favorable C present at high salinity in shallow soils. This gradient was expected to emerge from increased microbial activity at higher salinity leaving behind less favorable organic C. However, decreases in the number of inferred biochemical transformations and heteroatom abundances with increasing salinity suggests that microbial activity decreased with increasing salinity. While difficult to infer direction of causality, these patterns suggest that less favorable C at higher salinities may constrain microbial activity, leading to fewer biochemical transformations of the organic C. Thermodynamic limitation of organic C transformation is likely due to anaerobic conditions (LaRowe and Van Cappellen, 2011), which are indicated by high-moisture content of soils, high NH₄-N, and low NO₃-N. Anaerobic conditions restrict oxidation of C compounds based on thermodynamic properties (i.e., NOSC and ΔG°ₐₓ) (Boye et al., 2017), and our data suggest that this has the potential to lead to lower microbial activity in conditions with less favorable organic C.

4.3 Compound class landscape gradients suggest influences of spatially structured inputs

Similar to patterns in C thermodynamic favorability, C compound classes showed significant heterogeneity in shallow soils but had conserved characteristics in deeper soils. The lipid-like peaks observed in the shallow floodplain samples suggest marine-associated algal-derived lipid organic matter similar to results observed by Ward et al., 2019 in a coastal wetland setting. In contrast, lignin-like signatures in the upland site suggest terrestrially derived OM, as has been observed in other environments where terrestrially-derived organic molecules have a high abundance of vascular-plant derived material such as lignin (Hedges and Oades, 1997; Ward et al., 2013). These characteristics also align with reports of saturated soil environments (e.g., floodplains) exhibiting greater abundance of less-oxygenated organic matter than aerobic environments (e.g., upland soils) as reported by Tfaily et al., 2014 in organic matter.
transformation of a peat column. Our observed landscape gradients in compound class composition indicate spatially structured inputs of organic C such as particulate OM deposition (Langley et al., 2007). Combining this outcome with gradients observed in the total number of biochemical transformations and the contribution of heteroatoms suggests that sources of C (marine vs terrestrial) and in situ processing combine to influence landscape-scale gradients molecular-level organic C chemistry.

4.4 Ecological assembly processes are weakly associated with organic C

Our results show that microbial community assembly is driven by a combination of dispersal limitation (a stochastic process) and deterministic selection most likely associated with pH, as is often observed in soils (Fierer, 2017; Fierer and Jackson, 2006; Garbeva et al., 2004). In contrast, variation in organic C character was associated primarily with specific conductivity. This suggests that the composition of microbial communities is not mechanistically related to C chemistry. Consistent with this inference, we found a very weak association between βNTI and organic C characteristics. Furthermore, and contrary to our hypothesis, we observed a weak negative association between the influence of deterministic processes and the number of organic C transformations.

Relatively fast dynamics of organic C compounds compared to relatively slow changes in microbial composition may underlie the lack of association between assembly processes and C chemistry (Bramucci et al., 2013). Supporting this interpretation, a recent study evaluating microbial community composition and C biogeochemistry of soils in a mesohaline marsh following saltwater intrusion reported immediate changes in C mineralization rates with delayed shifts in microbial community composition (Dang et al., 2019). Similarly, a 17-year dryland soil
transplant experiment showed large shifts in microbial activity with no change in community composition (Bond-Lamberty et al., 2016). Furthermore, studies across diverse systems show disconnect in function and composition. For example, C chemistry and not microbial community structure or gene expression was found to significantly influence freshwater hyporheic zone organic matter processing (Graham et al., 2018); environmental conditions influenced the distribution of functional groups, but not taxonomic composition of marine bacterial and archaeal communities (Lima-Mendez et al., 2015; Louca et al., 2016); and dynamic community shifts did not impact functional stability of a methanogenic reactor (Fernández et al., 1999).

Combining our study with these previous investigations provides evidence that soil microbial community composition does not strongly influence biogeochemical function.

In our system, lack of an association between microbial composition and organic C chemistry is also likely due to a strong influence of stochastic community assembly. Our null modeling indicated that dispersal limitation was responsible for 54% of variation in community composition. Dispersal limitation influences composition by restricting the movement of organisms through space. Restricted movement enhances the influences of stochastic ecological drift, which arises through birth and death events that are randomly distributed across taxa (Green et al., 2004, 2008; Hubbell, 2001; Martiny et al., 2006; McClain et al., 2012; Stegen et al., 2015). This can result in functionally redundant taxa across the landscape (Loreau, 2004).

Moreover, one can argue as per Louca et al., 2018 that in an open system with regular exposure to external inputs (e.g., via tides), functional redundancy is expected to occur and lead to a decoupling of microbial structure and function (Burke et al., 2011; Liebold and Chase, 2017; Nemergut et al., 2013b).
Conclusions

Our results have revealed landscape scale gradients in soil C chemistry in a coastal forested floodplain, but also show that such gradients are different across soil depths and OC fractions—occurring only in the shallow, water soluble C pool. In addition, we found little evidence of an association between C chemistry and microbial community assembly processes, likely due to a dominant influence of stochastic community assembly (as indicated by a strong influence of dispersal limitation). We propose that the disconnect between C chemistry and microbial communities is enhanced by differences in the time scales for which C chemistry and microbial community composition shift.

Our findings suggest that cross-system heterogeneity observed in coastal soil biogeochemical responses to salinity are likely associated with molecular-level C chemistry and microbial physiological responses that are contingent on historical conditions (Goldman et al., 2017; Hawkes and Keitt, 2015; Hawkes et al., 2017; Stegen et al., 2018a). We further suggest that microbial community composition may not strongly influence biogeochemical function in coastal soils. Processes associated with molecular-level C chemistry dynamics are therefore likely to be a critical component of ecosystem responses to changing salinity dynamics in coastal TAI s. A full elucidation of these processes will lay a foundation for the development of mechanistic models of coastal TAI biogeochemical dynamics, providing an opportunity for better representation of these ecosystems in local, regional, and Earth system models.

Code and data availability

Raw sequence data has been uploaded to the National Center for Biotechnology Information’s (NCBI) Sequence Read Archive (SRA) under BioProject PRJNA541992. All other data files and
codes will be uploaded to the Department of Energy’s (DOE) Environmental Systems Science Data Infrastructure for a Virtual Ecosystem (ESS-DIVE) upon manuscript acceptance. Original codes for community assembly metric calculation are available at Stegen_etal_ISME 2013 github repository https://github.com/stegen/Stegen_etal_ISME_2013.

Author contribution

AS designed the study, performed the experiments, conducted data analyses and interpretation, and wrote the original draft. JI and CG collected the samples and created site maps. MTF, RKC, and JT provided input on FTICR methodology, conducted the FTICR-MS instrument run, and handled quality filtering and pre-processing of FTICR scans. VLB and NDW contributed to funding acquisition, site selection, study design conceptualization, interpretation of results and editing. JCS contributed to funding acquisition, study design conceptualization, interpretation of results, reviewing and editing. All authors provided feedback on the manuscript.

Competing interests

The authors declare no conflict of interest.

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Table 1. Relative peak abundances (%) of compound classes in the water extracted organic carbon fraction averaged across replicates per site. Samples are ordered according to their depth profile (shallow and deep) and their relative position in the landscape: floodplain (Fp), inland (I), and upland (U). Abbreviations: Con HC (condensed hydrocarbon), UnsatHC (unsaturated hydrocarbon), Other (no classification assigned)

<table>
<thead>
<tr>
<th>Site/Depth</th>
<th>Landscape position</th>
<th>Protein (%)</th>
<th>Amino Sugar (%)</th>
<th>Lipid (%)</th>
<th>Lignin (%)</th>
<th>Con HC (%)</th>
<th>Tannin (%)</th>
<th>Other (%)</th>
<th>Carb (%)</th>
<th>Unsat HC (%)</th>
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<tbody>
<tr>
<td>BC2_Shallow</td>
<td>Fp</td>
<td>17.2</td>
<td>3.3</td>
<td>9.4</td>
<td>31.0</td>
<td>22.3</td>
<td>13.2</td>
<td>0.5</td>
<td>1.8</td>
<td>1.3</td>
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<tr>
<td>BC3_Shallow</td>
<td>Fp</td>
<td>21.6</td>
<td>3.8</td>
<td>11.5</td>
<td>27.3</td>
<td>23.0</td>
<td>9.8</td>
<td>0.4</td>
<td>1.5</td>
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<tr>
<td>BC4_Shallow</td>
<td>I</td>
<td>1.6</td>
<td>0.6</td>
<td>0.3</td>
<td>45.3</td>
<td>32.2</td>
<td>18.9</td>
<td>0.04</td>
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<tr>
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<td>1.8</td>
<td>4.0</td>
<td>38.1</td>
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<td>1.5</td>
<td>1.3</td>
<td>51.8</td>
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<td>1.5</td>
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<td>0.3</td>
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Figure 1. Study site Beaver Creek in the Olympic Peninsula in western Washington. The creek is a first order stream with tidal exchange restored in 2014. Top panel shows site location in western Washington with inset panel zoomed in to show site close to Johns River. Bottom panel shows soil sampling locations at the high salt exposure (BC2, BC3, BC4) transect, moderate salt exposure (BC12, BC13, BC14) transect, and terrestrial upland (BC15) site. The transects with six sampling sites experience periodic inundation episodes which result in surface pooling of tidal water. Map was created using ArcGIS 10.5 software (ESRI, 2017). Coordinate System: GCS WGS 1984.
Figure 2. Average Gibbs Free Energy (GFE) of samples in the water fraction of shallow soils increased with increasing specific conductivity (a) while no change was observed in the deeper soils (b).
Figure 3. The total number of inferred transformations (a) and total abundance of N-containing compounds (b) in the water fraction of shallow soils show significant negative correlations with increasing specific conductivity. No significant relationships were observed for water fraction of deeper soils or for the CHCl₃ fraction in shallow or deeper soils.
Figure 4. Histograms representing the observed distribution of comparisons based on (a) Beta-near taxon index ($\beta$NTI) and (b) Raup Crick metric ($RC_{Bray}$). Red lines represent the significance thresholds, whereby values outside their bounds are significantly different from the null distribution.