

Responses to Referee comments (RCs) for “Wet-dry cycles impact DOM retention in subsurface soils” by Yaniv Olshansky et al.

Dear Associate Editor and referees,

We are grateful for the Referees’ and your own comments on our submission; addressing them has certainly improved the paper. We are hereby uploading our numbered responses to referee comments (RCs) as well as our indications of how we implemented the suggested changes.

Please find below our respective response to each RC in *blue*. We have added detail (and made cuts where needed) for improved clarity in this final revision.

Anonymous Referee #1 (R1)

This manuscript describes the effect of wet-dry cycles on DOM retention and composition in two subsurface soils. The manuscript is very well written and the description of the experiment and results are clear and sound. Overall, this manuscript provides valuable insights in the effect of wet-dry cycles on DOM retention and the nm-scale interactions between DOM and mineral soil and is of interest to the scientific community and readers of Biogeosciences in particular.

Specific Comments:

R1C 1: In the methods and results section, it is not really stated clearly that an active microbial community was present while the experiment proceeded. As is discussed line 297-311, page 11, microbial decomposition of DOC was clearly relevant. This should be stated more clearly in the method and result section.

Response 1: We agree with the referee, in this study microbial activity was not suppressed. The revised version includes better clarification on this point. (lines 121-122 and 312-315)

R1C 2: In addition, it would be very helpful to the reader if a carbon mass balance or estimation of the % carbon lost due to mineralization was reported

Response 2: This is a good point. The estimated mass of carbon that was potentially mineralized in the SCM soil systems were, 1370 ± 840 and 1440 ± 680 mg organic carbon (OC) kg^{-1} soil (for wet-dry and continuous-wet treatments respectively). These values represent 11 ± 7 and 11 ± 5 % of the total carbon in the wet-dry and continually-wet systems. These mass balance values were added to the revised version. (lines 202-206)

R1C 3: It is reported that the OC content of SCM was effectively unchanged after treatment (line 195). However, given the large error margin on the solid phase OC content measurement, it is also possible the change in OC content due to DOC retention falls within the margin of error of the measurement. The much larger relative accumulation of DOC in the solid phase of JRB is more easily detected, but judging from figure 1, the OC removal from solution behaves similarly. Unless additional evidence is provided to demonstrate a higher microbial biomass, activity and/or higher carbon mineralization, I am not convinced it can be stated that enhanced mineralization took place in SCM relative to JRB (line 303) based on the data presented here.

Response 3: To address the referee's comment we performed additional parametric statistical analysis for the OC, TN and C to N data. ANOVA and Tukey's HSD were used to test the difference between means. We then compared the mean loss of OC in the reacted soils and the mean amount of OC removed from solution with the student t-test. Results demonstrated a significant mass loss of OC from SCM soil system ($p \leq 0.05$), amounting to 1370 ± 840 and 1440 ± 680 mg OC kg^{-1} soil (for wet-dry and continuous-wet treatments respectively), added at line 204. In the JRB system, the total mass differences were not statistically significant ($p > 0.95$) 150 ± 230 and 260 ± 250 mg OC kg^{-1} soil (for wet-dry and continuous-wet treatments respectively). In addition, C to N ratio of reacted SCM soils significantly decreased compared to unreacted soil, suggesting microbial transformation of the soil organic matter. Therefore,

although this study did not measure microbial biomass or activity directly, our results do support OC mineralization in the SCM soil case. This additional data, analysis and discussion was added to the revised version. (lines 163-166, 202-206 and caption of figure 1)

R1C 4: The effect of mineralization and/or transformation of DOC during the experiment could have a major impact on the results of both quantity and composition of the OC retained in the soil. In addition, the duration of the experiment (4 times 98 hours) is sufficient for microbial growth to take place in response to the DOC addition and treatment. This should be addressed carefully in the discussion, particularly also in relation to the difference between the continually wet and wet-dry treatments.

Response 4: We agree with this RC that microbial growth and respiration contribute to mineralization and transformation of DOC. Our results indicate that OC decomposition is statistically significant in terms of carbon mass balance in the SCM system, whereas it is not clearly detectable above experimental error in the JRB system (see responses 2 and 3 above). Relative increases in peak intensity of O-alkyl from FTIR spectra of the wet-dry reacted SCM soil suggest an increased contribution of microbial derived material. However, spectroscopy (FTIR) and micro-spectroscopy (STXM-NEXAFS) analysis of reacted JRB DOM solutions and soils, suggest that the functional groups removed from reacted JRB DOM solution were similar to the ones accumulated in the reacted JRB soils; therefore, on this basis and the carbon balance, we conclude that the reactions were dominated by sorption and not biotransformation. The revised version contains a more specific discussion of this point. (line 312-315)

R1C 5: It is stated quite strongly in the abstract (line 20) and conclusions (line 354-356) that the spatial fractionation of adsorbed OM is different from what was expected based on previous literature. Though I agree that this is a very interesting observation, a more detailed evaluation of these observations in relation to previous literature is missing and should be added to the discussion.

Response 5: The referee makes an important point that warrants additional information and reference to the literature in the discussion. As has been summarized in the Introduction section (lines 51-65), previous

studies demonstrated the importance of short-range order metal (oxy)hydroxides for the selective adsorption of phenolic and carboxylic moieties, whereas layer silicate clay minerals are shown to preferentially adsorb aliphatic OM (Chorover and Amistadi, 2001). The present study used for the first time STXM-NEXAFS micro-spectroscopy to test if such fractionation occurs on the nano-scale of heterogeneous soil surfaces. Our results demonstrate that although distinct soil surfaces were enriched with iron (i.e. (oxy)hydroxides), the OC associated with these surfaces did not differ detectably from the bulk OC. We emphasized this point with reference to the literature in the discussion section of the revised version. (lines 351-355)

R1C 6: Line 290-296: it could be of interest here to also note the change in ionic strength of the pore-water that occurs due to air drying of the soil and its possible effect on the local Ca^{2+} concentration and thereby potential to form complexes with OM.

Response 6: This is a valid point as it has previously been shown that ionic strength may indeed affect complexation of OM with metal cations and particle surfaces. The revised version will include discussion of the importance of soil solution ionic strength. (lines 307-308)

Anonymous Referee #2 (R2)

General comments: The paper is well written and structured and provides new insights in the retention of dissolved organic matter in soil under varying moisture conditions, which is highly interesting especially for the scientific community dedicated to elucidate organic carbon dynamics along the soil profile. The chosen methodological tool set is innovative and suitable to reach the proposed research aims. I have some comments that should be addressed before the paper is potentially acceptable for publication.

Specific comments:

R2C 1: The entire paper needs to be checked for a proper introduction of the used abbreviation and their constant usage throughout the paper.

Response 1: We thank the referee for addressing this point, the revised version has the proper introductions of all abbreviations.

R2C 2: Lines 15-19: split into two sentences

Response 2: We agree with the referee and changed the sentence accordingly.

R2C 3: Line 24: "considered" not "consider"

Response 3: This was corrected.

R2C 4: Line 27: introduce the abbreviation for carbon and then use this abbreviation constantly throughout the paper

Response 4: The new version includes the correct and consistent abbreviation for carbon throughout.

R2C 5: Lines 68-71: give some explanations why do you expect an increase in surface complexation of carboxyl groups with metal (oxy)hydroxide surfaces or hydroxylated edge surfaces of aluminosilicate clays and promoted associations of hydrophobic fractions with preadsorbed and desiccated DOM components especially in the light of the relatively high pH value of the JRB soil and the statement in lines 348-349 that it is important to note that the studied soils are predominantly composed of silicate and aluminosilicate minerals and are relatively depleted in crystalline and short range order metal oxides.

Response 5: Based on previous work that tested the interactions natural organic matter and reference mineral phases, carboxyl groups demonstrate a higher reactivity toward metal (oxy)hydroxide surfaces or hydroxylated edge surfaces of aluminosilicates than they do for other prevalent mineral surfaces in soils (e.g., charged or uncharged basal plane siloxane sites of clay minerals). The relevant literature is summarized in the introduction section (lines 51-65). In the current study, a combination of STXM-

NEXAFS analysis was used to visualize this phenomenon at the sub-micron scale on natural soil surfaces. The results demonstrate that although distinct soil surfaces were enriched with iron (i.e. (oxy)hydroxides), the OC associated with these surfaces did not differ detectably from the bulk OC. We have clarified the context of this observation in light of prior work in the revised version. (lines 351-355)

R2C 6: Lines 86-89: explain the sampling design in more detail, at the moment the reader is not able to understand if true field replicates, pseudo field replicates, or just laboratory replicates of one composite sample were analysed, this is important in relation to the statistical tests that were performed, which require at least pseudo field replicates from, for example, three different soil pits per site, also I did not find in the text any information about the number of replicates.

Response 6: In this study, soil samples were composited during collection from a single pit excavated in each site. The samples were collected from different locations within each pit and composited to one representative local sample. Replication therefore pertains to the experiment itself, i.e., to test the reproducibility and uncertainty associated with the effects of the wet-dry and continuous-wet treatments for a given soil type. This additional information on sample collection and experimental replication has been added to the revised version. (lines 86-89)

R2C 7: Lines 109-111: the 28 ml that have been filtered, is this the decanted aliquot or did this volume was removed by pipetting? Please explain in more detail.

Response 7: The supernatant was removed by careful pipetting just below the surface to avoid loss of solids. Details have been added to the revised version. (line 111-113)

R2C 8: Lines 133-134: why did you use different preparation techniques for the two soils, and how this affects the comparability of the spectra?

Response 8: Despite the variation mentioned above, it is first most important to point out that transmission FTIR spectroscopy was used in all cases. Also, although not stated in the earlier version of the manuscript, OM samples were subjected to both transmission sample introduction protocols, the KBr pellet and infrared transmission window approach, to provide a direct comparison from which we selected the datasets most informative on the treatment effects within a given soil. Air drying DOM onto IR transmission (Ge or ZnSe) windows is generally considered the approach least prone to artifacts associated with freeze-drying (required for KBr pellets) and potential chemical reaction with the KBr pellet during pressurized preparation (Johnston and Aochi, 1996). Conversely, the potential shortcoming of this method is that spectra may have a lower signal to noise ratio than other sample introduction methods. In the case of the JRB soil, despite high signal to noise for the KBr spectra, we observed alteration of vibrations that suggested potential reaction with the KBr matrix upon pelletization. This type of matrix interaction with DOM is possible and needs to be evaluated on a case-by-case basis (Johnston and Aochi, 1996). Since alteration of spectra in KBr was apparent and transmission window spectra were of high quality, the transmission window data were included as the best available dataset to evaluate the changes with time and treatment for this DOM. Conversely, no alteration of spectra in KBr was observed for SCM soil. Therefore the higher quality KBr pellet spectra are shown for the SCM soil. Most importantly, our FTIR spectral comparisons are focused on characterization of molecular changes that occurred within a given soil by treatment. They were not used to make quantitative comparisons between soils. Hence the sample introduction methods, although different between samples, were consistent across all treatments within a given soil, and so do not alter the inferences made in terms of the effects of treatment and time.

R2C 9: Line 250: for better understanding, please explain “relative”, did you compute ratios or normalized the spectra to the O-alkyl peak?

Response 9: Changes in solution composition of OC were evaluated by the ratio between normalized peak intensity in the FTIR spectra. Clarification has been added to the revised version. (lines 177-178)

R2C 10: Lines 303-305: this statement has to be backed up with references and including a discussion of information from the literature because in this study no direct measurements of microbial parameter were carried out

Response 10: We agree with the RC. The revised version includes additional literature citation and discussion to support this point. (lines 316-320)

R2C 11: Lines 310-311: any recommendation how to address this? How should an experiment be designed to get further information here?

Response 11: Experimental design using isotopically labeled material would provide additional information regarding exchange and decomposition reactions, we have made a note of this (line 326-328).

R2C 12: Line 354-356: this criticism is stressed specifically in the Abstract and in the Conclusions, if the authors wish to make a point here, they should discuss this in more detail including some recommendation for future experimental work.

Response 12: Excellent point. As mentioned in the response to comment 5, additional discussion have been added to the revised version. Future experiments using soils with higher relative proportions of short-range order metal (oxy)hydroxide and lower organic carbon content may provide additional information on nano-scale spatial fractionation of OC, and this has been added to the conclusion. (lines 377-378)

References added:

Chorover, J. and Amistadi, M. K.: Reaction of forest floor organic matter at goethite, birnessite and smectite surfaces, *Geochim. Cosmochim. Acta*, 65(1), 95–109, 2001.

Johnston, C. T., and Y. O. Aochi. 1996. Fourier transform infrared and Raman spectroscopy. In D. L. Sparks (ed.) *Methods of Soil Analysis. Part 3. Chemical Methods-SSSA Book Series no. 5.* Chapter 10, pp. 269-321. Soil Science Society of America, Madison, WI.

Wet-dry cycles impact DOM retention in subsurface soils

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Abstract. Transport and reactivity of carbon in the critical zone are highly controlled by reactions of dissolved organic matter (DOM) with subsurface soils, including adsorption, transformation and exchange. These reactions are dependent on frequent wet-dry cycles common to the unsaturated zone, particularly in semi-arid regions. To test for an effect of wet-dry cycles on DOM interaction and stabilization in subsoils, samples were collected from subsurface (Bw) horizons of an Entisol and an Alfisol from the Catalina-Jemez Critical Zone Observatory and sequentially reacted (four batch steps) with DOM extracted from the corresponding soil litter layers. Between each reaction step, soils either were allowed to air dry (“wet-dry” treatment) before introduction of the following DOM solution or were maintained under constant wetness (“continually-wet” treatment). Microbial degradation was the dominant mechanism of DOM loss from solution for the Entisol subsoil, which had higher initial organic C content, whereas sorptive retention predominated in the lower C Alfisol subsoil. For a given soil, bulk dissolved organic C losses from solution were similar across treatments. However, a combination of Fourier transform infrared (FTIR) and near edge X-ray absorption fine structure (NEXAFS) spectroscopic analyses revealed that wet-dry treatments enhanced the interactions between carboxyl functional groups and soil particle surfaces. Scanning transmission X-ray microscopy (STXM) data suggested that cation bridging by Ca^{2+} was the primary mechanism for carboxyl association with soil surfaces. STXM data also showed that spatial fractionation of adsorbed OM on soil organo-mineral surfaces was diminished relative to what might be inferred from previously published observations pertaining to DOM fractionation on reaction with specimen mineral phases. This study provides direct evidence of the role of wet-dry cycles in affecting sorption reactions of DOM to a complex soil matrix. In the soil environment, where wet-dry cycles occur at different frequencies from site to site and along the soil profile, different interactions between DOM and soil surfaces are expected and needs to be considered for the overall assessment of carbon dynamics.

1 Introduction

Dissolved organic matter (DOM) is the main vehicle of organic carbon (OC) and nutrient transport to the subsoil (Kaiser and Kalbitz, 2012; Kalbitz et al., 2000). There it stimulates key biogeochemical processes including

29 heterotrophic microbial activity (Fontaine et al., 2007), mineral transformation, and organic and inorganic nutrient
30 and contaminant mobilization (Chorover et al., 2007; Polubesova and Chefetz, 2014; Zhao et al., 2011). Interactions
31 with subsoil surfaces act to stabilize DOM against advective transport and microbial degradation (Eusterhues et al.,
32 2014; Kalbitz et al., 2000; Lutzow et al., 2006). Furthermore, prior studies have shown that DOM generated in the
33 surface litter layers can be transported preferentially to clay-enriched subsoils via macropore flow paths that bypass
34 the intervening matrix (Rumpel and Kögel-Knabner, 2010). Particularly in semi-arid vadose zones, these DOM-
35 subsoil interactions occur in a context of frequent wet-dry cycles. Although such cyclic conditions likely impact C
36 dynamics, the nature of their effects on micro- to molecular-scale organo-mineral associations remains poorly
37 known.

38 The principal chemical mechanisms affecting DOM retention at soil particle surfaces – including ligand
39 exchange with surface hydroxyl groups, ion-exchange of organic moieties at charged sites, cation bridging,
40 hydrogen bonding and Van der Waals interactions depend on both DOM molecular composition and mineral
41 surface chemistry (Chorover and Amistadi, 2001; Gu et al., 1994; Kleber et al., 2007, 2014). Interactions of DOM
42 with dissolved polyvalent cations (e.g. Fe^{3+} and Al^{3+}) may also result in its coagulation and co-precipitation with
43 nucleating metal (oxy)hydroxides (Chen et al., 2014a; Eusterhues et al., 2011). Drying of OM-mineral complexes
44 can affect the mode of interaction. These effects may include changing of adsorption mode and product surface
45 chemistry. For example, drying can convert OM adsorbate from outer- to inner-sphere coordination (Kang et al.,
46 2008), promote exposure of hydrophobic functional groups of the adsorbed species, and increased surface catalysed
47 transformation reactions (Olshansky et al., 2014). For systems where cation bridging plays a prominent role in
48 DOM adsorption (e.g., to the siloxane surfaces of 2:1 layer type clay minerals), cation charge and valence effects
49 are important, with increasing exchangeable Ca^{2+} relative to Na^{+} resulting in greater DOM retention (Setia et al.,
50 2013).

51 Due to the heterogeneous nature of both DOM and soil mineral constituents, fractionation of DOM occurs
52 as a result of a gradient of interaction affinities between the DOM components and various soil particle surfaces
53 (Kaiser et al., 1997; Oren and Chefetz, 2012a). DOM fractionation has been studied extensively on single mineral
54 phases (Chorover and Amistadi, 2001; Vazquez-Ortega et al., 2014) and on bulk soils (Guo and Chorover, 2003;
55 Kaiser et al., 1997; Oren and Chefetz, 2012b). Metal (oxy)hydroxides have been suggested as a dominant adsorbent
56 for DOM with the result being preferential retention of high molar mass aromatic and carboxylated moieties
57 (Chorover and Amistadi, 2001; Vazquez-Ortega et al., 2014). Conversely, layered silicates (e.g., smectites,
58 kaolinite) were reported to adsorb mainly low molar mass and aliphatic DOM fractions (Chorover and Amistadi,
59 2001; Polubesova et al., 2008). While the use of specimen mineral phases in adsorption experiments facilitates

60 elucidation of molecular mechanisms of DOM interaction, it does not account for the complexity of competitive
61 interactions associated with heterogeneous assemblies of weathered surfaces as found in natural soils. Conversely,
62 using whole soils in adsorption experiments has traditionally hindered mechanistic interpretations of DOM uptake
63 results. However, increased spatial resolution of spectroscopic methods has helped to overcome these shortcomings
64 by providing micro- and nano-scale information on both soil-mineral phases and associated organic molecules
65 (Chen et al., 2014b).

66 The current study aimed to utilize such methodological advances to elucidate: (i) how wet-dry cycles affect
67 the reactions between DOM and subsoil particle surfaces, and (ii) whether spatial fractionation of DOM is
68 detectable with nanoscale resolution spectroscopic methods. We hypothesized that discontinuous wet-dry cycling
69 during DOM reaction with subsoils would increase complexation of carboxyl groups with metal (oxy)hydroxide
70 surfaces or hydroxylated edge surfaces of aluminosilicate clays and promote association of hydrophobic fractions
71 with pre-adsorbed and desiccated DOM components relative to a continuous-wet condition. Such wetting-drying
72 episodes have been hypothesized to affect OC dynamics in water-limited portions of the critical zone, such as those
73 that occur in the semi-arid southwestern US (Miller et al., 2005; Perdrial et al., 2014), but they have not been
74 previously investigated in controlled laboratory experiments.

75 **2 Materials and Methods**

76 **2.1 Soil samples**

77 Soils were sampled from below mixed conifer forest in the Santa Catalina Mountains (SCM) and Jemez River
78 Basin (JRB) Critical Zone Observatories (CZO) in Arizona and New Mexico, respectively (Chorover et al., 2011).
79 The JRB soil was collected from the south slope of the San Antonio Mountain (35°55'10"N, 106°36'52"W) at an
80 elevation of 2750 m. The SCM soil was collected from the northeast slope of the zero order basin located in the
81 Marshall Gulch experimental site (32°25'44"N, 110°46'14"W) at elevation of 2600 m. The mean annual
82 temperature is 6 and 10.4 °C for the JRB and SCM sites respectively. Both sites are subjected to bimodal annual
83 precipitation patterns with averages of 850 and 940 mm y⁻¹. Parent rock is igneous felsic at both sites; granitic in
84 the SCM and rhyolitic in the JRB. Therefore, the soils used in experiments developed under similar vegetation and
85 climatic condition but in different parent materials. The SCM and JRB soils are classified as Typic Ustorthents and
86 Mixed Psammentic Cryoboralfs, respectively (Soil Survey Staff, 2010, USDA-NRCS., 1999). Soils were collected
87 from the litter layer (0-2 cm) and Bw3 horizon (80-100 cm), from pedons excavated (one in each site) in April
88 2012 and October 2015 for SCM and JRB respectively. The samples were collected from different locations within

89 each pit and composited to one representative local sample. The SCM litter layer was collected in October 2015.
90 Soils were air dried and sieved to obtain the fine earth (< 2 mm) fraction and stored in a closed container. Table 1
91 presents the bulk properties of the studied subsoils as measured using standard methods (Sparks, 1996). The mineral
92 assemblages of both soils were dominated by quartz, feldspars and aluminosilicate clays (Table S1). The SCM soil
93 had higher OM content (1.1 ± 0.5 mg C mg⁻¹) and lower pH (6.1 ± 0.04) than the JRB soil (0.17 ± 0.2 mg C mg⁻¹
94 and 7.05 ± 0.11).

95 2.2 Dissolved organic matter extraction

96 The extraction of DOM was achieved by mixing the air-dried and sieved JRB or SCM litter with ultrapure water
97 (1:5 g/g), and placing the suspension on a reciprocal shaker at 150 rpm for 24 h. Suspensions were centrifuged at
98 15,000 g for 30 min to separate the solids, using polypropylene copolymer (PPCO) centrifuge bottles. Adsorption
99 or contamination of DOM from these bottles was measured to be negligible (Vazquez-Ortega et al., 2014). The
100 supernatant solution was transferred into 50 mL PPCO centrifuge tubes and centrifuged again at 40,000 g for 20
101 min to remove colloidal organic material and the inorganic clay fraction. Supernatant solutions were filtered
102 through pre-combusted and cleaned 0.7 µm glass fiber filters. TOC was measured immediately after extraction
103 (Shimadzu TOC-VCSH, Columbia, MD) and solutions were diluted using ultrapure water to give initial dissolved
104 organic carbon DOC concentrations of 45 mg L⁻¹ (Table 1). DOM solutions were stored at 4°C prior to use.

105 2.3 Sequential batch experiments

106 To model the effect of sequential hydrologic events delivering litter leachate to subsoils in the two CZO sites,
107 subsoils were reacted in a set of four steps with DOM extracted from the litter layer of the corresponding profile.
108 Thirty mL aliquots of DOM ([DOC] = 45 mg L⁻¹) solution were mixed with 3.0 g of soil in 50 mL PPCO centrifuge
109 tubes and agitated (150 rpm, orbital shaker) at room temperature, in the dark. Preliminary kinetic experiments
110 indicated an apparent equilibration time of 98 h, and this was chosen as the equilibration time for each reactor
111 vessel. Suspensions were centrifuged for 30 min at 40,000 g and 28 mL were removed by careful pipetting just
112 below the surface to avoid loss of solids, filtered through precombusted 0.7 µm glass fiber filters and the solutions
113 were stored at 4 °C for a maximum of 24 h prior to analysis, as discussed below. For *continually-wet* treatments, a
114 fresh 28 mL aliquot of DOM solution was added to each tube and suspensions agitated for an additional 98 h (28
115 mL were used because ca. 2 mL remained as entrained solution in the wet soil paste). For *wet-dry* treatments, the
116 soil pastes were air dried for 24 h (drying was accomplished by directing a low-flow circulating dry-air stream to
117 promote desiccation), then an aliquot of 30 mL DOM solution was added to each tube and suspensions were re-

118 agitated for 98 h, for a total of four sequential reaction cycles. Three replicates were prepared for each soil and
119 treatment combination. After the four sequential reaction cycles, soils were freeze-dried and **total organic carbon**
120 **and nitrogen** (TOC and TN) were measured using ECS 4010 CHNSO Analyzer (Costech, MI, Italy). During the
121 experiment samples were maintained under oxic condition by equilibration with oxygenated headspace. **It is**
122 **important to note that microbial activity was not suppressed throughout the reaction steps.**

123 **2.4 Characterization of DOM solutions before and after reaction**

124 Reacted and unreacted DOM solutions were characterized by the following suite of complementary analytical
125 methods: soluble TOC and TN were determined by total elemental analyzer (Shimadzu TOC-L and TNM-L,
126 Columbia, MD), absorbance spectra (190 to 655 nm) were collected using a UV-Vis spectrometer (Shimadzu
127 Scientific Instruments UV-2501PC, Columbia, MD, USA), fluorescence excitation–emission matrices (EEM) were
128 obtained with a FluoroMax-4 equipped with a 150 W Xe-arc lamp source (Horiba Jobin Yvon, Irvine CA, USA),
129 and Fourier transform infrared (FTIR) spectra were collected using a Nicolet NEXUS 670 IR spectrometer
130 (Madison, WI). The EEMs were acquired with excitation (Ex) from 200 to 450 nm and emission (Em) from 250
131 to 650 nm in 5 nm increments. Spectra were collected with Ex and Em slits at 5- and 2-nm band widths,
132 respectively, and an integration time of 100 ms. Ultrapure water blank EEMs were subtracted and fluorescence
133 intensities were normalized to the area under the water Raman peak, collected at excitation 350 nm. Additionally,
134 an inner-filter correction was performed based on the corresponding UV–Vis scans (Murphy et al., 2013).
135 Transmission FTIR spectra were collected with a KBr beam splitter and a deuterated triglycine sulfate (DTGS)
136 detector. Aliquots of two mL of JRB DOM solutions were transferred onto IR transmissive Ge windows, dried
137 under vacuum for 19 h, and spectra collected in transmission mode. For SCM DOM, 2 mL aliquots were freeze
138 dried and mixed with IR-grade KBr, then compressed into pellets. For each sample, 120 scans were collected over
139 the spectral range of 400–4000 cm^{-1} at a resolution of 4 cm^{-1} . Clean Ge windows and KBr pellets were used as
140 background.

141 **2.5 Scanning Transmission X-ray Microscopy and Near Edge X-ray Adsorption Fine Structure (STXM- 142 NEXAFS) analysis of soils**

143 STXM-NEXAFS analyses were conducted on clay-size isolates to avoid particulate organic matter and to overcome
144 possible alteration of C speciation during preparation of thin sections (Chen et al., 2014b). Clay size fractions (<2
145 μm) of the reacted and unreacted JRB soils were separated by sedimentation after dispersion in ultrapure water
146 using a sonication bath. Samples for STXM analysis were prepared by depositing 5 μL of diluted aqueous

147 suspension onto a Si₃N₄ window (75 nm thick) and air-dried. The samples were analyzed by STXM on beamline
148 10ID-1 at the Canadian Light Source (CLS), a 2.9 GeV third-generation synchrotron source. The microscope set
149 up used a 25 nm Fresnel zone plate, which provided a maximum spatial resolution of *ca.* 30 nm. Samples were
150 kept under 1/6 atm of He during measurement.

151 Spatially resolved spectra obtained by collecting stacks of images at energies below and above C 1s, Ca
152 2p, Fe 2p, element edges. The dwell time was set to 1 ms and pixel sizes of 150 nm. Incident energy was calibrated
153 with CO₂ at 290.74 eV.

154 The aXis2000 software package (Hitchcock et al., 2012) was used for STXM image and spectral
155 processing. Stacks were aligned and converted to optical density using a clean area of the Si₃N₄ window for
156 normalization. Regions of interest (ROI) of C, Ca and Fe were extracted from each stack by subtracting below the
157 edge from the optical density (OD) maps. C NEXAFS spectra were extracted by averaging the pixels from the ROI.
158 NEXAFS spectra were normalized and peak deconvolutions were performed using the ATHENA software package
159 (Ravel and Newville, 2005). Peak assignments were based on Cody et al. (1998, 2008), Myneni (2002) and
160 Urquhart et al. (1997).

161 2.6 Data analysis

162 Statistical analyses were performed using *R* software packages (Mangiafico, 2016). Data were checked for
163 normality and equal variance. Means were tested using one-way ANOVA for parametric or Kruskal–Wallis for
164 non-parametric analysis. The differences between means were examined using Tukey’s HSD tests for parametric
165 or Dunn test for non-parametric analysis. Parametric tests used to evaluate the difference of TOC, TN and C to N
166 ratio between treatments, while nonparametric test used to evaluate UV-vis and fluorescence data. The specific UV
167 absorbance (SUVA₂₅₄) was calculated by normalizing absorbance at incident wavelength 254 nm by the cell path
168 length (1 cm) and DOC concentration (M). Fluorescence index (FI, Eq. 1) and humification index (HIX, Eq. 2)
169 values were calculated from the corrected EEMs (McKnight et al., 2001; Ohno, 2002) as follows:

$$170 \quad FI_{Ex370} = \frac{I_{450}}{I_{500}} \quad (1)$$

$$171 \quad HIX_{Ex255} = \frac{\sum(I_{435 \rightarrow 480})}{\sum(I_{300 \rightarrow 345})} \quad (2)$$

172 where Ex is the excitation wavelength (nm) and *I* is the fluorescence intensity at each wavelength.

173 Spectra collected by FTIR were background corrected using KBr pellets or the Ge transmission window
174 as blanks and baseline corrected using the spline function in the OMNIC 8 software program (Thermo Nicolet Co.,
175 Madison, WI). Peak positions were determined using the second-order Savitzky–Golay method. Voigt line shape,
176 (a convolution between mixed Gaussian and Lorentzian line shapes) were fitted to the peaks in the 850-1850 cm⁻¹
177 region using Grams/AI 8.0 spectroscopy software (Thermo Electron Corporation). Changes in DOM molecular
178 composition were evaluated by quantifying peak intensity ratios. Peak assignments were based on Socrates (2004),
179 Mayo et al. (2004), Omoike and Chorover et al. (2004) and Abdulla et al. (2010).
180

181 3. Results

182 3.1 Total OC and Nitrogen

183 The loss of DOC from solution per unit mass of soil was largely independent of reaction step and treatment. The
184 mass loss of DOC upon reaction with SCM soil was 156 ± 5 , 217 ± 3 , 167 ± 17 , and 192 ± 10 mg kg⁻¹ for steps 1-
185 4, respectively, in the wet-dry treatment, and 163 ± 3 , 222 ± 4 , 217 ± 2.5 , and 214 ± 6 mg kg⁻¹ in the continuously-
186 wet treatment. The mass loss of DOC upon reaction with JRB soil was 248 ± 19 , 257 ± 1 , 197 ± 5 , and 200 ± 12
187 mg kg⁻¹ for steps 1-4, respectively, in the wet-dry treatment, and 256 ± 7 , 236 ± 26 , 176 ± 44 , and 208 ± 2 mg kg⁻¹
188 in the continuously-wet treatment. Hence, the mean fraction of OC removed from DOM solution was 58 ± 5 %
189 (SD) after each reaction step with JRB soil and OC uptake values were not significantly different between the
190 continuously-wet and wet-dry treatments. In the SCM soil, the mean fraction of OC removed was $41 \pm 4\%$ of the
191 total after each reaction step in the wet-dry treatment. In contrast to the other three treatments, the continually-wet
192 SCM treatment indicated increasing amounts of OC removed in each step, with $39 \pm 0.8\%$ in the first step, $48 \pm$
193 1% in the second, and $56 \pm 1\%$ in the third and fourth steps (Figure 1). At the end of four reaction steps the TOC
194 of JRB soils increased from $1,700 \pm 74$ mg OC kg⁻¹ for the unreacted soil to $2,750 \pm 87$ mg OC kg⁻¹ and $2,840 \pm$
195 99 mg OC kg⁻¹ for the wet-dry and continuous-wet treatments respectively (Figure 1). For the JRB soil, increases
196 in solid phase OC were not significantly different (student t-test, $p>0.95$) from the cumulative amounts of DOC
197 removed from reacted solutions (902 ± 26 and 876 ± 34 mg OC kg⁻¹ for wet-dry and continuous-wet treatments
198 respectively) and represent a 60% increase in soil TOC. Conversely, for the SCM soil, despite comparable
199 cumulative losses from solution (733 ± 29 and 817 ± 2 mg OC kg⁻¹ for wet-dry and continuous-wet treatments
200 respectively), solid phase analyses indicated that the OC content of the reacted SCM ($11,200 \pm 380$ and $11,200 \pm$
201 290 mg OC kg⁻¹ soil for wet-dry and continuous-wet treatments respectively) soils were effectively unchanged

relative to the unreacted control ($11,800 \pm 180 \text{ mg OC kg}^{-1}$). We then tested for differences between the mean change in OC in the reacted soils and the mean amount of OC removed from solution using the student t-test. Results demonstrate a significant mass loss of OC in the SCM soil ($p \leq 0.05$), amounting to 1370 ± 840 and $1440 \pm 680 \text{ mg OC kg}^{-1}$ soil (for wet-dry and continuous-wet treatments respectively). These values represents 11 ± 7 and $11 \pm 5 \%$ of the total carbon in the wet-dry and continually-wet systems.

Patterns in the removal of total N from the DOM solutions showed similar trends for both soils. In the first two wet-dry steps, a higher proportion of TN was removed from the solution (65 - 70% and 50 - 66% for SCM and JRB soils, respectively) than in the third and fourth steps (31 - 44% for both soils). The measured increase in soil TN by the end of the experiment were 63 and $143 \text{ mg N kg soil}^{-1}$ for SCM and JRB soils respectively. These values are slightly higher than the sum of TN removed from the solution (51 and $88 \text{ mg N kg soil}^{-1}$ for SCM and JRB soils respectively) (Figure 1).

The C:N ratio for all reacted DOM solutions decreased from step 1 to step 4, indicating preferential loss of C from solution, with no significant difference between the continually-wet and wet-dry treatments. However, after the first reaction with the SCM soil, the C:N ratio was 22.0 ± 1.3 , which was higher than the unreacted DOM (14.1 ± 0.8). It is important to note that DOM extracted from unreacted soil had a C:N ratio of 23.7 ± 0.9 , and C:N of DOM decreased during the sequential reaction steps. After the fourth reaction step, ratios of 11.1 ± 0.8 and 9.6 ± 0.8 were observed for the wet-dry and the continually-wet treatments respectively. The C:N of the reacted DOM solution with JRB soil decreased from 10 ± 1.0 after the first reaction step to 4.6 ± 0.5 after the fourth reaction step. The C:N ratio of unreacted DOM solution was 8.4 ± 0.8 . The overall change in soil C:N ratio was evaluated by the differences between unreacted soil and soils reacted four times with DOM solutions (Figure 1). Reacted SCM soils had significantly lower C:N (24.2 ± 1) than unreacted SCM soil (30.5 ± 1.8). However, no change in C:N was detected for reacted versus unreacted JRB soils.

3.2 UV-Vis and Fluorescence Spectroscopy

Reaction with subsoils altered spectroscopic properties of the litter-derived DOM solutions as reflected in UV-Vis (SUVA_{254}) and fluorescence indices (HIX and FI), and there was relatively little variation between continually-wet and wet-dry treatments (Figure 2). For both JRB and SCM the SUVA_{254} values of DOM decreased (relative to unreacted DOM) upon contact with soil (Figure 2), with the exception of the fourth step in wet-dry treatment of SCM soil (Figure 2). This effect of contact with soil on SUVA_{254} was larger for JRB than SCM, although it decreased with progressive reaction steps even for JRB soils from *ca.* 200 ($\text{L mol}^{-1} \text{ cm}^{-1}$) in the first step to *ca.* 50 ($\text{L mol}^{-1} \text{ cm}^{-1}$) by the fourth step. High SUVA_{254} ($905 \pm 35 \text{ L mol}^{-1} \text{ cm}^{-1}$) was measured for DOM extracted from

232 unreacted JRB soil (Table 1). We note that $SUVA_{254}$ values of unreacted DOM also decreased between the first
233 ($393 \text{ L mol}^{-1} \text{ cm}^{-1}$) and subsequent steps ($\sim 350 \text{ L mol}^{-1} \text{ cm}^{-1}$) indicating some alteration of DOM chromophores in
234 the stock DOM solution during the experiment. Although this was a small change relative to soil reaction effects,
235 alteration was also evident in the HIX of unreacted JRB DOM. Therefore, treatment effects (continuous-wet and
236 dry-wet) were evaluated on the basis of differences between reacted and unreacted solutions for the same reaction
237 step. The effect of reaction with soil on $SUVA_{254}$ values were less pronounced for SCM relative to JRB soils. In
238 the wet-dry treatment of SCM soil, $SUVA_{254}$ values of the first three steps were generally consistent at *ca.* $330 \pm$
239 $13 \text{ (L mol}^{-1} \text{ cm}^{-1})$ and in the fourth step the $SUVA_{254}$ increased to $530 \pm 2 \text{ (L mol}^{-1} \text{ cm}^{-1})$. Conversely, $SUVA_{254}$
240 values increased slightly over the course of the experiment from 324 ± 10 to $410 \pm 16 \text{ L mol}^{-1} \text{ cm}^{-1}$ for the
241 continually-wet SCM treatment.

242 Humification index (HIX) values for the reacted DOM were generally higher or similar to the unreacted
243 DOM (Figure 2). As with the $SUVA_{254}$ index, the fourth step of SCM wet-dry treatment was the exception (Figure
244 2), giving a lower HIX for reacted compared to unreacted DOM. The HIX values for DOM reacted with JRB soil
245 were similar for continually-wet and wet-dry treatment. Conversely, with SCM soil, values for the wet-dry
246 treatments were lower than for continually-wet treatments. The relative differences between reacted and unreacted
247 DOM were lower for the JRB system than for the SCM system. For both JRB and SCM soils, higher fluorescence
248 index (FI) values were observed for reacted relative to unreacted DOM (Figure 2) whereas wet-dry versus wet-only
249 treatment effects were negligible. For JRB, FI values increased from 1.31 ± 0.04 (unreacted DOM) to 1.53 ± 0.04
250 whereas corresponding values for SCM were 1.34 ± 0.04 and 1.42 ± 0.02 , respectively. All FI values are in close
251 agreement with the value of DOM associated with predominantly plant material (*ca.* 1.4), as opposed to microbial-
252 derived DOM (*ca.* 1.9) (McKnight et al., 2001).

253 3.3 FTIR

254 Transmission FTIR spectra of reacted and unreacted DOM for the JRB and SCM systems are shown in Figures 3
255 and 4, respectively. The most prevalent peaks in the spectra were associated with amide I and II (1636 and 1560
256 cm^{-1} , respectively), carboxylate (asymmetric and symmetric stretches at 1592 and 1417 cm^{-1} , respectively), alkyl
257 (CH_2 and CH_3 bending vibrations at 1455 and 1380 cm^{-1} , respectively), and aromatic moieties ($\text{C}=\text{C}$ ring vibration
258 at 1500 cm^{-1} , phenol O-H bend 1370 cm^{-1}) and O-alkyl (CO^- stretch at $1030\text{-}1150 \text{ cm}^{-1}$).

259 For JRB soil, the first reaction step in both continually-wet and wet-dry treatments was accompanied by a
260 decrease in peak intensities of carboxylate (1592 cm^{-1} and 1417 cm^{-1}) and amide (1636 and 1560) relative to O-
261 alkyl ($1150\text{-}1030 \text{ cm}^{-1}$). Additionally, primary alcohol (1035 cm^{-1}) peak intensity decreased relative to secondary

262 alcohol (1100 cm^{-1}). This trend persisted in the second step with JRB soil for both treatments, although the pattern
263 was less pronounced and differed by treatment. Specifically, the wet-dry treatment showed a larger decrease in the
264 asymmetric carboxylate stretch (1592 cm^{-1}) whereas the continuous-wet treatment showed a larger decrease in the
265 amide I peak (1636 cm^{-1}). In the third step, the decrease in amide and carboxyl peaks relative to O-alkyl was not as
266 pronounced for the wet-dry as it was in the continually-wet treatment. Finally, in the fourth step of the wet-dry
267 system, a pronounced decrease in amide and carboxyl peaks relative to O-alkyl was again observed, whereas it was
268 not in the continually-wet treatment (Figure 3).

269 Figure 4 shows the spectra of reacted and unreacted DOM in the SCM system. The SCM DOM spectra
270 show similar peaks as the JRB with the addition of carboxyl ($\text{C}=\text{O}$ stretch at 1720 cm^{-1}) and ester ($\text{C}=\text{O}$ stretch
271 1770 cm^{-1} and $\text{C}-\text{O}$ stretch 1265 cm^{-1}). Similar to the JRB system, after reaction with soil, the peaks associated with
272 carboxyl, carboxylate and amide decreased relative to the O-alkyl peaks and this trend was more pronounced in the
273 first step than in the subsequent steps. Similar to the JRB system, in the fourth step of the wet-dry treatment, a
274 pronounced decrease in carboxyl, carboxylate and amide peaks was again observed relative to the O-alkyl peaks.

275 3.4 STXM-NEXAFS

276 Given limitations in beam time, synchrotron analyses were focused on the JRB soil because it showed larger OC
277 accumulation over the course of the experiment. Scanning transmission x-ray microscopy (STXM) images of C,
278 Fe and Ca obtained for the isolated fine fraction of JRB soils reacted four times with DOM in wet-dry and
279 continually-wet treatments are shown in Figures 5 and 6, respectively. The OC signal was observed over all particle
280 surfaces, from continually-wet and wet-dry treatments after four reaction steps. Locations of higher Fe and Ca
281 content were observed for both treatments. Near edge x-ray absorption fine structure (NEXAFS) spectra extracted
282 from C, Ca and Fe-rich regions of interest (ROI) of the STXM maps and C NEXAFS spectra of bulk unreacted soil
283 and DOM are included in Figures 5 and 6. Spectra of the unreacted DOM consist of peaks representing aromatic
284 ($1s \rightarrow \pi^*$ at 285.1 eV), alkyl ($1s \rightarrow 3p/\sigma^*$ at 287.5 eV), amide ($1s \rightarrow \pi^*$ at 288 eV), carboxyl ($1s \rightarrow \pi^*$ at 288.5 and 290
285 eV), O-alkyl ($1s \rightarrow \pi^*$ at 289.5 eV) moieties. The C NEXAFS spectra of unreacted soil show no strong peaks of
286 amide, carboxyl and O-alkyl, similar to the unreacted DOM spectra. However, after four steps of reaction with
287 DOM, soil from both continually-wet and wet-dry treatments exhibited greatly enhanced carboxyl and O-alkyl
288 peaks relative to the unreacted soil. In the wet-dry treatment, the aromatic peak was absent. The O-alkyl peak was
289 more pronounced for the continually-wet than for the wet-dry treatment. Additionally, the amide peak was
290 suppressed in the reacted soil compared to the unreacted DOM, and for the wet-dry treatment this peak was absent
291 and was not included in the fitted spectra (supplementary material). The C NEXAFS spectra of Ca and Fe enriched

ROIs are similar to the average whole image spectra. However in the Ca ROI, the carboxyl peak intensity was enhanced relative to Fe ROI and the averaged whole image spectra. This carboxyl enhancement, which was absent in the unreacted soil, was most pronounced in the wet-dry treatment.

Variations in the C NEXAFS spectra of the reacted soils following each reaction step are displayed in Figure 7. After the first reaction step, intensities of the carboxyl and O-alkyl peaks were relatively increased. For the continually-wet treatment, spectra collected following the second and third steps show an increase in alkyl and O-alkyl peaks, whereas this trend was less evident in the wet-dry treatment.

4. Discussion

Specific surface area (SSA) and OC content are dominant factors controlling sorption of DOM to soil. For comparable mineralogy, higher SSA tends to increase DOM sorption, while higher solid phase OC content suppresses it (Kaiser et al., 1997; Oren and Chefetz, 2012b). In addition, solution chemistry can control DOM-soil interactions. For example, low pH can neutralize weakly acidic OM functionalities, thereby decreasing electrostatic repulsion from negatively-charged surfaces, whereas bivalent cations such as Ca^{2+} can form bridging complexes between negatively-charged surface and DOM sites (e.g., Setia et al., 2013). Further, the presence of polyvalent metal cations in solution can promote precipitation of (meta-)stable OM-metal complexes (Kleber et al., 2014). Gradual drying of pore water changes the ionic strength of the solution, and can potentially promote interactions with metal cations in solution and at organo-mineral surfaces. In the current study, in spite of differences in soil constituents and DOM compositions deriving from the two distinct CZO sites, similar amounts of DOM were removed from solution with both JRB and SCM soils. The fact that OC did not accumulate in the SCM soil solid phase despite significant removal from solution suggests that decomposition and mineralization are dominant factors indicated in the removal of OC from the reacted SCM DOM solutions. Since microbial activity was not suppressed in this study, an active microbial community was presumably present in the soils. Therefore, addition of labile OC in the form of DOM may have resulted in microbial growth and biotransformation of pre-existing soil OC. Indeed, the pronounced decrease in C:N ratio of the reacted soil is consistent with microbial transformation of organic matter (German et al., 2011). Higher HIX for all SCM reacted samples, with the exception of the last step in the wet-dry treatment, further support OM transformation. Enhanced mineralization in the SCM relative to JRB soil may be related to its substantially higher native OC content (Table 1), which would preclude surface stabilizing interactions (Kaiser et al., 1997; Oren and Chefetz, 2012b). Moreover, higher OC content makes the SCM soil more susceptible to the priming effect of the added labile OC as DOM (Blagodatsky et al., 2010). The

321 relatively lower HIX value for the last step of wet-dry treatment coincide with higher SUVA₂₅₄. Since SUVA₂₅₄
322 index is correlated with sample aromaticity (Weishaar et al., 2003), an increase in the aromatic peak in the FTIR
323 spectra was expected. However, FTIR spectra show a relative increase in O-alkyl rather than the aromatic
324 vibrations. It is possible that the decrease observed in 1550 to 1700 cm⁻¹ region is mainly due to a decrease in
325 carboxyl associated peaks rather than increased aromaticity. It is unclear if the removed fraction was exchanged
326 with previously adsorbed OM or preferentially decomposed in the solution. Additional study using isotopically
327 labelled material may provide additional information regarding decomposition and exchange reactions in similar
328 systems.

329 Conversely, significant DOM or soil organic matter decomposition was not observed for the JRB soil
330 experiments, as evidenced from the C mass balance. Therefore, changes in reacted DOM composition can be
331 attributed to preferential adsorption and exchange reactions. The increased FI value of the reacted DOM further
332 suggests preferential adsorption of plant- relative to microbial-derived OM. The slight decrease in SUVA₂₅₄ values
333 is also consistent with this observation, since polyphenols derived from lignin account for most of the aromaticity
334 in DOM.

335 Spectra from C-NEXAFS obtained for the JRB soil fine fraction corroborate the solution data obtained by
336 FTIR. A pronounced increase in the carboxyl peak (288.5 eV) after the first reaction step (Figure 7) is consistent
337 with the decreased intensity of carboxyl in the reacted DOM solutions (Figure 3). NEXAFS spectra collected after
338 the second and third steps of both treatments show additional increases in the O-alkyl (289.5 eV) and alkyl (287.5
339 eV) that corroborate the relative decrease in FTIR peak intensities for these functionalities. The fact that the
340 NEXAFS of the reacted JRB soils clearly shows a relative increase in the carboxyl peak from the third to the fourth
341 step in the wet-dry treatment (Figure 7), suggests that preferential adsorption of the carboxylic component was
342 facilitated by the pre-existing soil-DOM phases of the dried soil. Prior work has shown that soil drying may promote
343 conformational changes in pre-adsorbed DOM that promotes preferential desorption of O-alkyl relative to further
344 inner-sphere coordination of carboxyl components (Kang et al., 2008; Kang and Xing, 2007). Additional support
345 for the formation of inner-sphere carboxyl complexes is from the higher preferential adsorption of carboxyl over
346 amide as observed in FTIR spectra of wet-dry compared to continuous-wet treatments (Figure 3).

347 Due to the heterogeneous composition of soil surfaces and DOM, spatial fractionation of the adsorbed OC
348 moieties was expected. Figures 5 and 6 show that in both wet-dry and continuously-wet treatments, regions
349 containing higher content of Fe and Ca can be distinguished. Interestingly, the C NEXAFS spectra of these distinct
350 locations are generally similar. It is important to note that low Fe spectral signals were detected over all of the
351 particle surfaces images with STXM. This observation contradicts our initial hypothesis, and previous observations

352 (Chorover and Amistadi, 2001; Kaiser et al., 1997; Oren and Chefetz, 2012b; Vazquez-Ortega et al., 2014) that
353 iron (oxy)hydroxides will preferentially adsorb carboxyl containing moieties. These results suggest that weathered
354 particle surfaces, potentially already coated with a thin layer of metal (Fe) oxides and co-associated organic matter,
355 may smear out what might otherwise be observed as a spatial fractionation at this scale (nm).

356 However, close inspection of the C spectra extracted from Fe and Ca enriched zones and whole particle
357 regions reveal that in samples treated with wet-dry steps, the amplitude of the carboxyl peak shows a relative
358 increase preferentially in the Ca enriched regions (Figure 5 and supporting information). This finding suggests that
359 cation bridging interactions are pronounced in stabilizing the carboxyl component in the studied soil. It is important
360 to note that the solution pH was close to 7, and therefore deprotonated carboxylate species were predominant in
361 the suspension. Regions of high Ca are likely associated with charged aluminosilicate surfaces hosting
362 exchangeable cations. The enhancement effect of drying on Ca-carboxylate complex formation can be related to
363 the tendency of the Ca^{2+} hydration shell to become more acidic upon drying (Sposito, 1984). As water molecules
364 are gradually removed during air drying, polarizing forces of the Ca^{2+} cation increases, enhancing the tendency of
365 hydration water to donate protons (Dowding et al., 2005). Therefore, upon drying, protonation of the carboxylate
366 functionality is expected. Protonation of carboxylate decreases the electrostatic repulsion from negatively charged
367 clay surfaces and increases the overall interaction with clays. It is important to note that our studied soils are
368 predominantly composed of silicate and aluminosilicate minerals and are relatively depleted in crystalline and short
369 range order metal oxides.

370 5. Conclusion

371 Results of this study show that wet-dry cycles affect interactions between DOM and subsurface soils, in this case
372 by enhancing the interactions between carboxyl functional group and soil surfaces. Interactions of these
373 functionalities were dominated by Ca^{2+} bridging to soil surfaces. The data also demonstrate that nanoscale spatial
374 fractionation of DOM on soil organo-mineral surfaces was diminished relative to what might be inferred from
375 previous observations pertaining to DOM fractionation on specimen mineral phases. This is likely due to the
376 heterogeneous composition of the weathered soil surfaces and passivation of the underlying mineralogy by metal
377 oxide and OM films. Expanding the experiment to include soils with a higher proportion of short-range order
378 (oxy)hydroxides may result in more pronounced nanoscale spatial fractionation of DOM, but that is unknown at
379 present. Fractionation of DOM in solution was similar under wet-dry conditions for a soil that presented
380 measureable decomposition of the DOM (SCM) as it was for a soil that did not show any detectable decomposition
381 (JRB).

382 This study provides direct evidence of the role of wet-dry cycles in the sorption reactions of DOM to a complex
383 soil matrix. In the soil environment, where wet-dry cycles occur at variable frequencies from site to site and along
384 the soil profile, different interactions between DOM and soil surfaces are expected. This wet-dry effect can partially
385 explain the observation that carbohydrates predominate in subsoil horizons, where soil is less subjected to drying,
386 whereas aromatic and carboxylic compounds are more prevalent in top soils, where wet-dry cycles are more
387 frequent (Kaiser and Kalbitz, 2012). Our findings demonstrate the need to consider the effect of wet-dry cycles in
388 studying the interactions between DOM and soil surfaces.

389

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 515

516 **Table 1. Physico-chemical characteristics of the study soils**

517

| | JRB | SCM |
|--|--------------|--------------|
| Clay (%) | 33.9 | 22.6 |
| Silt (%) | 27.7 | 38.4 |
| Sand (%) | 38.4 | 50.9 |
| SSA (m ² g ⁻¹) _a | 16.6 ± 0.2 | 7.7 ± 0.1 |
| CEC (mmol _c kg ⁻¹) _b | 86.6 ± 4.2 | 61.3 ± 0.8 |
| OC (%) _c | 0.17 ± 0.02 | 1.11 ± 0.5 |
| pH _d | 7.05 ± 0.11 | 6.10 ± 0.04 |
| EC (µS cm ⁻¹) _d | 61.5 ± 26.6 | 36.8 ± 8.8 |
| DOC (mg L ⁻¹) _d | 3.59 ± 0.82 | 13.45 ± 1.30 |
| DOM pH | 6.97 ± 0.06 | 5.91 ± 0.11 |
| DOM EC (µS cm ⁻¹) | 170.7 ± 10.2 | 84.1 ± 12.3 |
| SUVA (L mol ⁻¹ cm ⁻¹) | 905 ± 35 | 539 ± 105 |
| HIX _e | 1.5 ± 0.1 | 4.5 ± 2.3 |
| FI _f | 1.40 ± 0.04 | 1.43 ± 0.03 |

518

519 _a BET-N₂ Specific surface area

520 _b Cation exchange capacity

521 _c Organic carbon

522 _d Obtained in soil aqueous extract (1:10 with 8.2 MΩ, Barnstead water)

523 _e Humification index

524

525 _f Fluorescence index

526

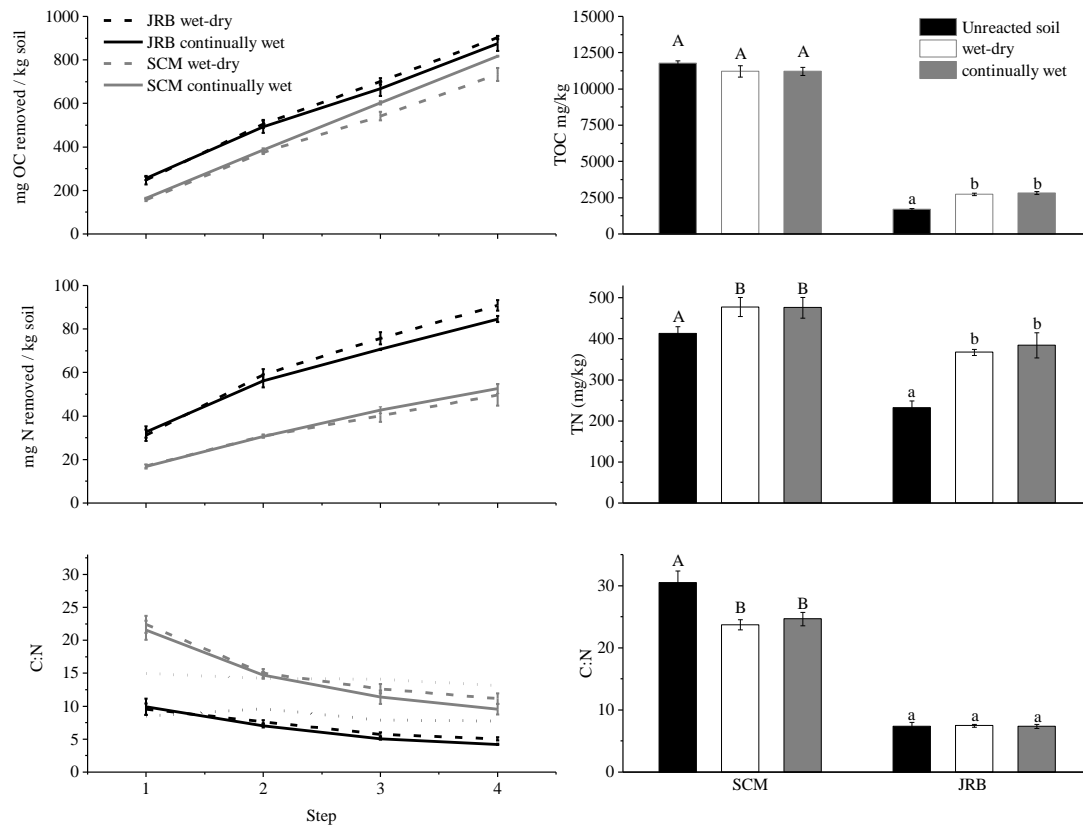
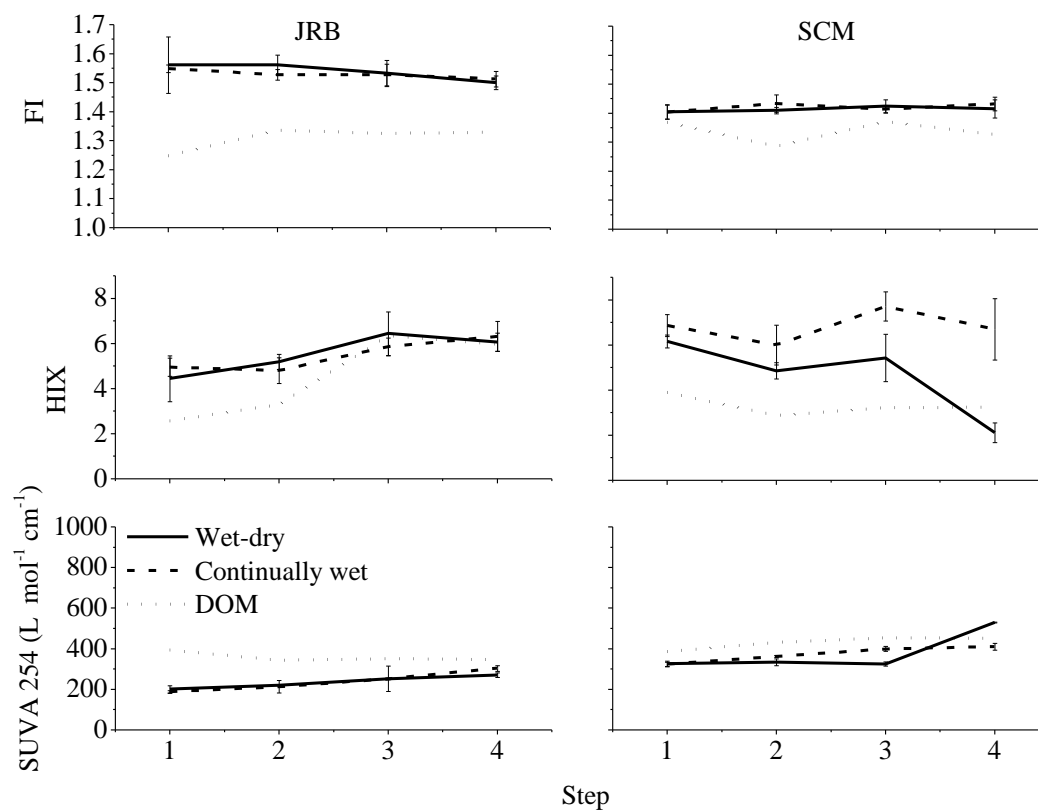


Figure 1: The organic carbon (top), nitrogen (middle) and C:N (bottom), for equilibrated solutions (left) and solid phases after four reaction steps (right). Values for equilibrated solution OC and N represent cumulative removal from solution per soil mass. Dashed lines in OC and N plots show continuous-wet treatments, dotted lines in the C:N plot represent values of unreacted DOM solutions, error bars are the standard deviation, and letters indicate significant difference (ANOVA and Tukey's HSD $p < 0.05$) from unreacted control.



537 **Figure 2. The fluorescence index (FI), humification index (HIX) and specific UV absorbance at 245 nm (SUVA₂₅₄), for equilibrated**
538 **solutions reacted with JRB and SCM soils. The solid lines are wet-dry series, dashed lines are continuous-wet, and dotted lines are**
539 **unreacted DOM; error bars are the standard deviation.**

540

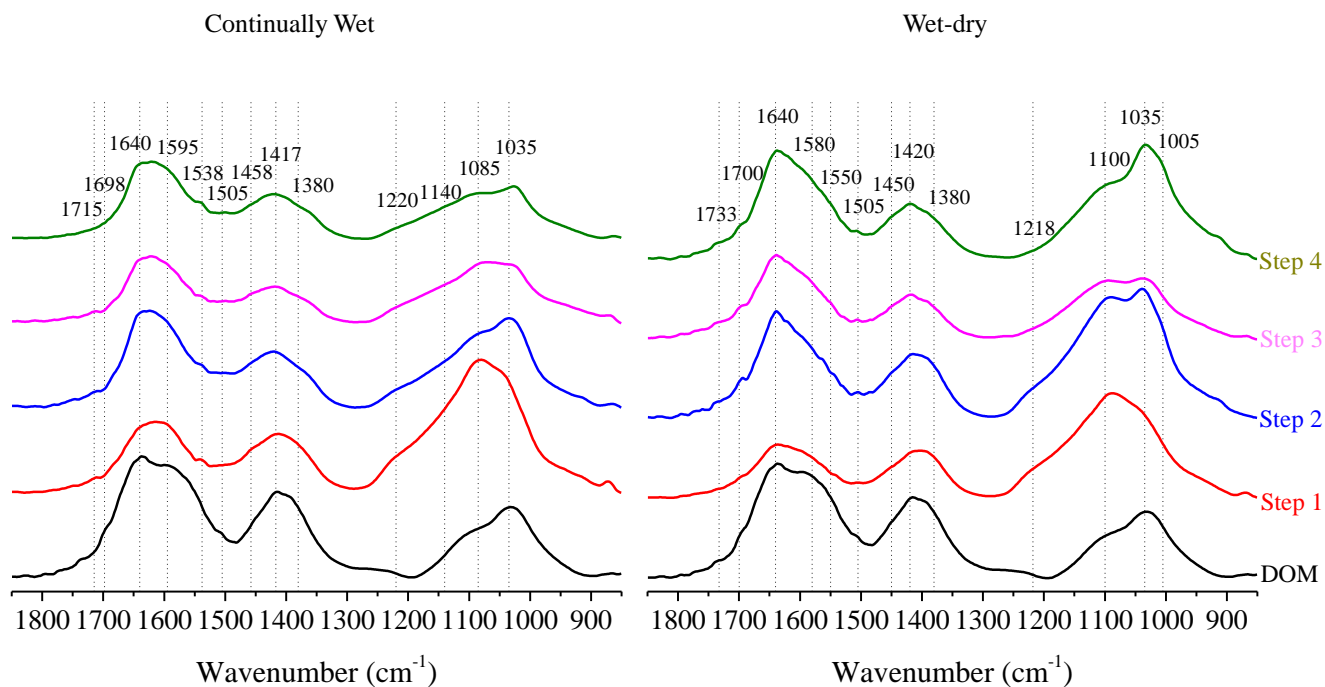
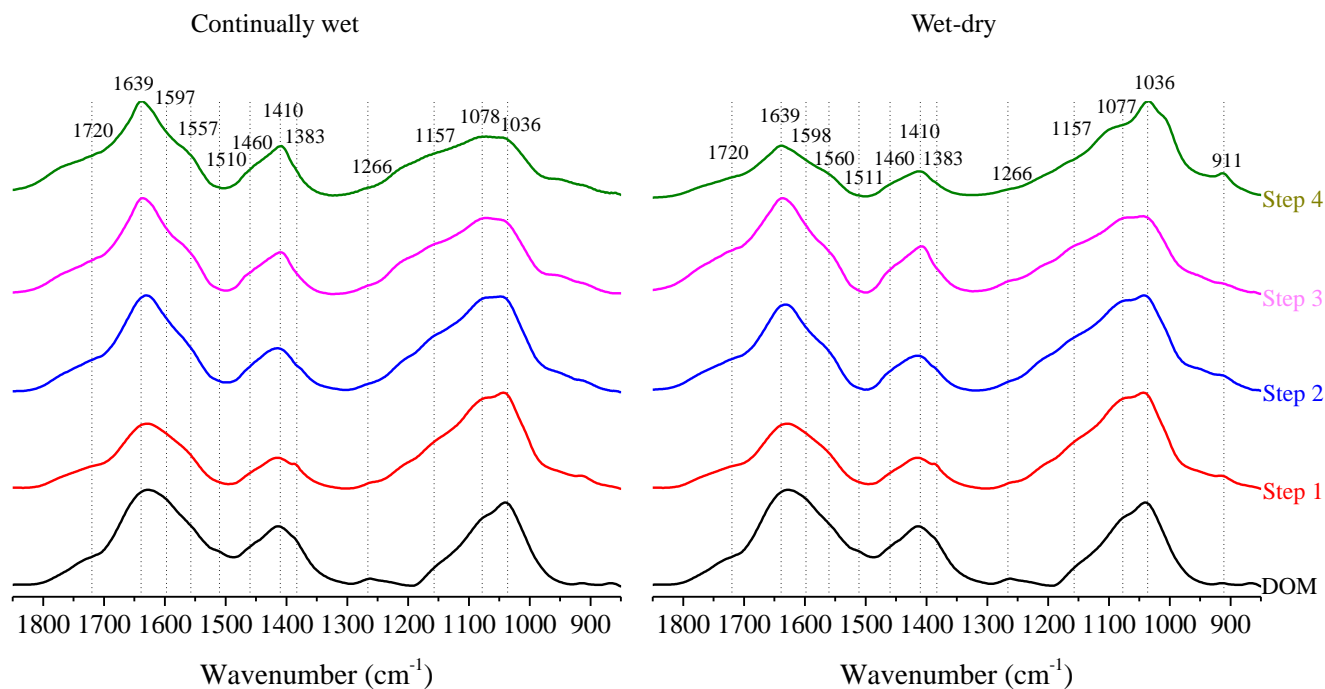


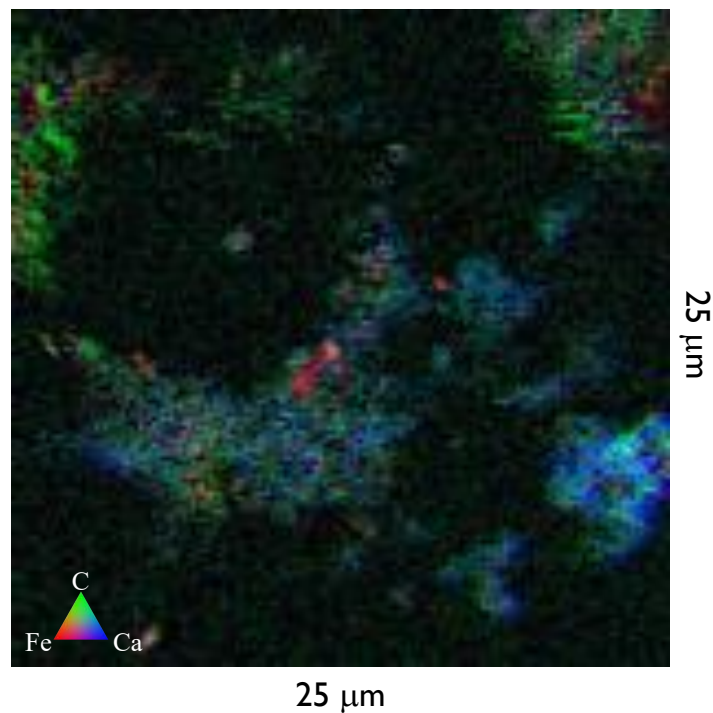
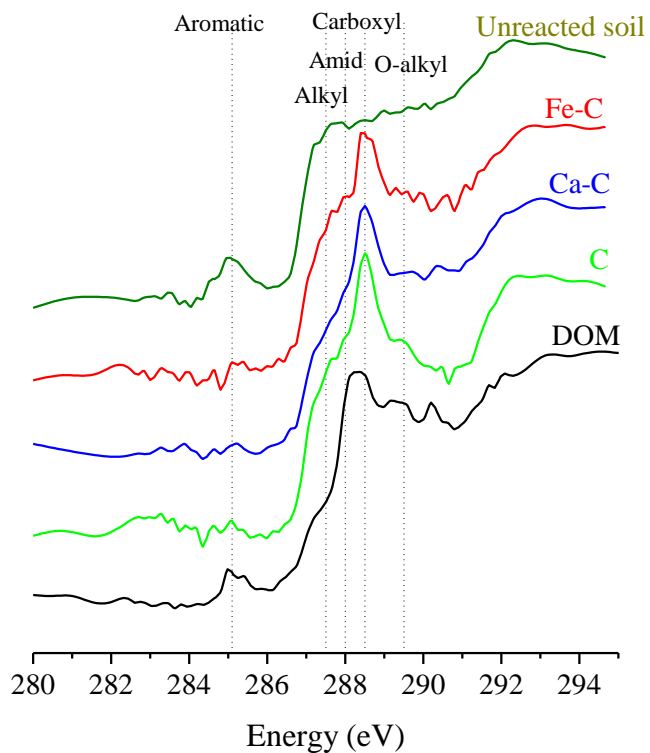
Figure 3. Transmission FTIR spectra of the DOM dried solution reacted with JRB soils from steps 1 to 4 for continuous-wet (left) and wet-dry cycled (right) and the unreacted JRB DOM solution (bottom black line). For color rendering of this image please refer to the online version.



548

549 **Figure 4. Transmission FTIR spectra of the DOM dried solution reacted with SCM soils from steps 1 to 4 for continuous-wet (left)**
 550 **and wet-dry cyclied (right) and the unreacted SCM DOM solution (bottom line). For color rendering of this image please refer to**
 551 **the online version.**

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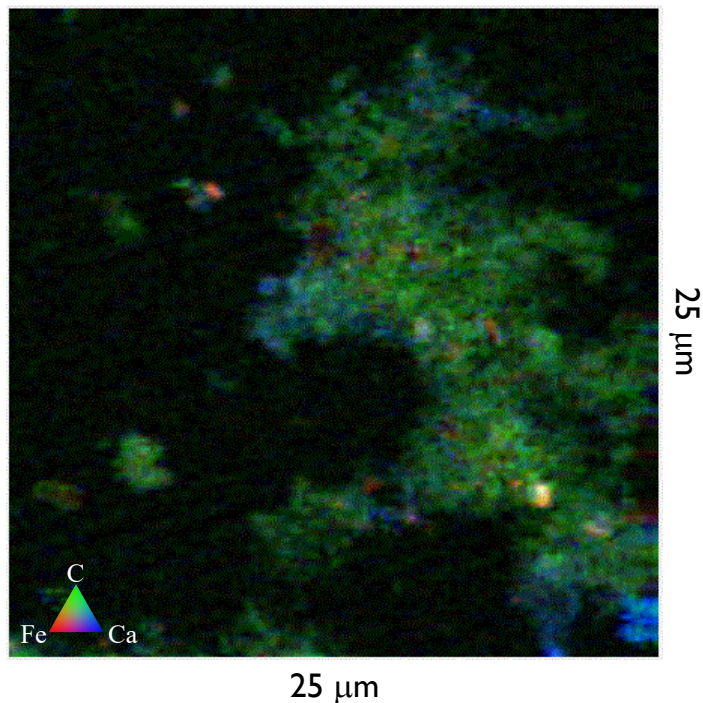
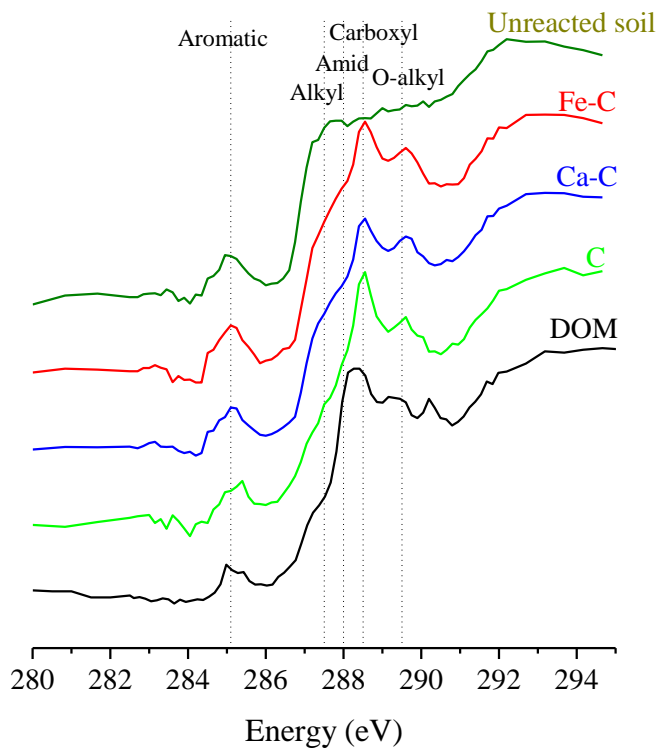


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555 **Figure 5.** JRB soil reacted with DOM under wet-dry cycling. Left, C NEXAFS spectra extracted from C, Ca, and Fe regions of
 556 STXM map. Spectra of unreacted soil (top) and DOM solution (bottom) are presented. Dashed vertical lines point out C species.
 557 Right, tri-colored STXM map of fine fraction from JRB soil reacted four times with DOM under wet-dry cycling; Fe (red), Ca (blue)
 558 and C (green). Image size 25 x 25 μm . For color rendering of this image please refer to the online version.

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560

561 **Figure 6. JRB soil reacted with DOM under continuous-wet conditions. Left, C NEXAFS spectra extracted from C, Ca, and Fe**
 562 **regions of STXM map. Spectra of unreacted soil (top) and DOM solution (bottom) are presented. Dashed vertical lines point out C**
 563 **species. Right, tri-colored STXM map of fine fraction from JRB soil reacted four times with DOM during the continuous-wet**
 564 **treatment. Fe (red), Ca (blue) and C (green). Image size 25 x 25 μm. For color rendering of this image please refer to the online**
 565 **version.**

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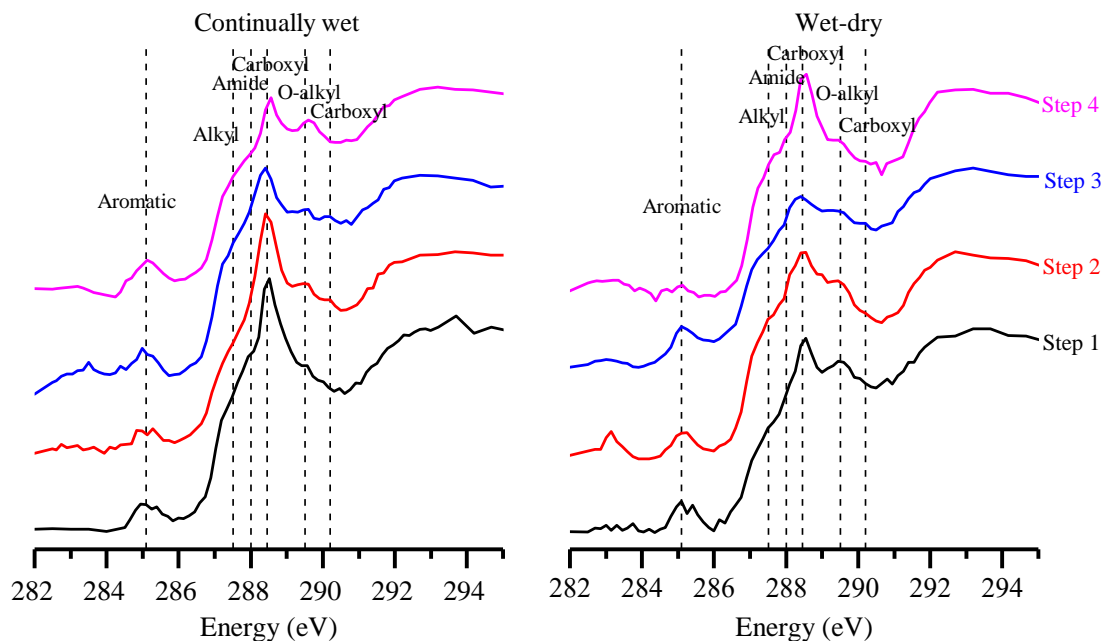


Figure 7. C NEXAFS extracted from C (red in Fig 6) regions of STXM map for the second step of the continuous-wet treatment (left) and from all 4 steps of the wet-dry treatment (right). For color rendering of this image please refer to the online version.