Root-Driven Weathering Impacts on Mineral-Organic Associations Over Pedogenic Time Scales

Mariela Garcia Arredondo
University of Massachusetts Amherst

Follow this and additional works at: https://scholarworks.umass.edu/masters_theses_2

Part of the Biogeochemistry Commons, Geochemistry Commons, and the Soil Science Commons

Recommended Citation
https://doi.org/10.7275/13338943 https://scholarworks.umass.edu/masters_theses_2/739

This Open Access Thesis is brought to you for free and open access by the Dissertations and Theses at ScholarWorks@UMass Amherst. It has been accepted for inclusion in Masters Theses by an authorized administrator of ScholarWorks@UMass Amherst. For more information, please contact scholarworks@library.umass.edu.
ROOT-DRIVEN WEATHERING IMPACTS ON MINERAL-ORGANIC ASSOCIATIONS OVER PEDOGENIC TIME SCALES

A Thesis Presented

By

MARIELA GARCIA ARREDONDO

Submitted to the Graduate School of the University of Massachusetts Amherst in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

February 2019

Environmental Conservation
ROOT-DRIVEN WEATHERING IMPACTS ON MINERAL-ORGANIC ASSOCIATIONS OVER PEDOGENIC TIME SCALES

A Thesis Presented

By

MARIELA GARCIA ARREDONDO

Approved as to style and content by:

___________________________________________
Marco Keiluweit, Chair

___________________________________________
Baoshan Xing, Member

___________________________________________
Isaac Larsen, Member

___________________________________________
Curt Griffin, Department Head
Environmental Conservation
ACKNOWLEDGEMENTS

I would like to acknowledge my committee Marco Keiluweit, Isaac Larsen, and Baoshan Xing for taking the time to work with me. I would also like to recognize all those involved in this work: Corey Lawrence, Marjorie Schulz, Malak M. Tfaily, Ravi Kukkadapu, Morris E. Jones, Kristin Boye, and especially Marco Keiluweit. Your expertise, patience, and assistance are what really helped to make this project come to fruition. I have learned a lot in the past two years which would not have been possible without your unwavering support. Thank you to K. Campbell and T. Kane of the USGS for their help in collecting the X-ray diffraction data. I would also like to thank Jay Dynes and Jian Wang for their help at the SM beamline at the Canadian Light Source. I also thank A. Chica and H. Mashayekhi for their laboratory assistance.

This work was supported by the National Science Foundation Graduate Research Fellowship Program, the SLAC SFA research program (SLAC FWP 10094), which is funded by the U.S. Department of Energy (DOE) Subsurface Biogeochemical Research (SBR) program within the Office of Biological and Environmental Research. The Canadian Light Source is supported by the Canada Foundation for Innovation, Natural Sciences and Engineering Research Council of Canada, the University of Saskatchewan, the Government of Saskatchewan, Western Economic Diversification Canada, the National Research Council Canada, and the Canadian Institutes of Health Research. A portion of this research was performed using EMSL, an Office of Science User Facility sponsored by the Office of Biological and Environmental Research.

I would also like to thank my partner, siblings, and friends for their continual belief in me, their understanding and encouragement. Y al último, si bien no menos importante, también quisiera agradecerles a mis padres, Lourdes y Salome, siempre
puedo contar con ustedes por su apoyo aunque sé que mucho de lo que hago todavía se sienta misterioso y como que nunca terminara. Ustedes me criaron conociendo el amor intenso por la tierra, algo que siempre mantendré cerca al corazón e integral a la fuente de mi pasión. Espero que con mi trabajo les pueda mostrar que sus esfuerzos y sacrificios si valieron la pena y son la razón por la que pude seguir por adelante. Los quiero mucho.
ABSTRACT

ROOT-DRIVEN WEATHERING IMPACTS ON MINERAL-ORGANIC ASSOCIATIONS OVER PEDOGENIC TIME SCALES

FEBRUARY 2019

MARIELA GARCIA ARREDONDO
B.S., CORNELL UNIVERSITY, ITHACA, NY
M.S., UNIVERSITY OF MASSACHUSETTS, AMHERST, MA

Directed by: Marco Keiluweit

Plant roots are critical weathering agents in deep soils, yet the impact of resulting mineral transformations on the vast deep soil carbon (C) reservoir are largely unknown. Root-driven weathering of primary minerals may cause the formation of reactive secondary minerals, which protect mineral-organic associations (MOAs) for centuries or millennia. Conversely, root-driven weathering may also transform secondary minerals, potentially enhancing the bioavailability of C previously protected in MOAs. Here we examined the impact of root-driven weathering on MOAs and their capacity to store C over pedogenic time scales. I compared soil that experienced root-driven weathering, resulting in the formation of discrete rhizosphere zones in deep soil horizons (100-160 cm) of the Santa Cruz Marine Terrace chronosequence (65 ka-226 ka), with adjacent soil that experienced no root growth. Using a combination of radiocarbon, mass spectrometry, Mössbauer spectroscopy, and X-ray spectromicroscopy approaches, we characterized MOA transformations in relation to changes in C content, turnover and chemistry across four soils ranging in age (65 ka-226 ka). We found that the onset of root-driven weathering (65-
90 ka) increased the amount of C associated with poorly crystalline iron (Fe) and aluminum (Al) phases, particularly highly-disordered nano-goethite. The increase in C coincided with greater overall C concentrations, longer C residence times, and a greater abundance of microbially-derived C. Continued root-driven weathering (137-226 ka) did not significantly change the amount of C associated with crystalline Fe and Al phases, but resulted in a decline in the amount of C associated with poorly crystalline metal phases. This decline in C associated to poorly crystalline phases coincided with a decrease in C concentrations and potential turnover rates, and a shift toward plant-derived C. In contrast, soil not affected by root-driven weathering showed low amounts of C bound to poorly crystalline Fe and Al phases regardless of soil age and, correspondingly, lower C concentrations and estimated residence times. My results demonstrate that root-driven formation and disruption of poorly crystalline Fe and Al phases directly controls both C accrual and loss in deep soil. Hence root impacts on soil C storage are dependent on soil weathering stage, a consideration critical for predictions of the vulnerability of deep soil C to global change.
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>CHAPTER</th>
<th>PAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACKNOWLEDGMENTS</td>
<td>iii</td>
</tr>
<tr>
<td>ABSTRACT</td>
<td>v</td>
</tr>
<tr>
<td>LIST OF TABLES</td>
<td>ix</td>
</tr>
<tr>
<td>LIST OF FIGURES</td>
<td>x</td>
</tr>
<tr>
<td>CHAPTER</td>
<td></td>
</tr>
<tr>
<td>1. INTRODUCTION</td>
<td>1</td>
</tr>
<tr>
<td>2. METHODS</td>
<td>6</td>
</tr>
<tr>
<td>2.1. Study Site</td>
<td>6</td>
</tr>
<tr>
<td>2.2. Soil Sampling</td>
<td>7</td>
</tr>
<tr>
<td>2.3. Total Carbon</td>
<td>8</td>
</tr>
<tr>
<td>2.4. Radiocarbon Analysis</td>
<td>8</td>
</tr>
<tr>
<td>2.5. Quantitative X-Ray Diffraction</td>
<td>9</td>
</tr>
<tr>
<td>2.6. Sequential Extractions</td>
<td>9</td>
</tr>
<tr>
<td>2.7. Fourier-Transform Ion Cyclotron Resonance Mass Spectrometry (FT-ICR-MS) Analysis</td>
<td>10</td>
</tr>
<tr>
<td>2.8. $^{57}$Fe-Mossbauer Spectroscopy</td>
<td>12</td>
</tr>
<tr>
<td>2.9. Scanning X-ray Transmission Microscopy (STXM) Imaging</td>
<td>13</td>
</tr>
<tr>
<td>2.10. Statistical Analysis</td>
<td>14</td>
</tr>
<tr>
<td>3. RESULTS</td>
<td>15</td>
</tr>
<tr>
<td>3.1. Elemental and Radiocarbon Analyses</td>
<td>15</td>
</tr>
<tr>
<td>3.2. Mineralogical Composition</td>
<td>15</td>
</tr>
<tr>
<td>3.2.1. X-ray diffraction</td>
<td>15</td>
</tr>
<tr>
<td>3.2.2. Sequential extractions</td>
<td>16</td>
</tr>
<tr>
<td>3.2.3. Mössbauer spectroscopy</td>
<td>17</td>
</tr>
<tr>
<td>3.3. Organic Matter Composition</td>
<td>18</td>
</tr>
<tr>
<td>3.3.1. Mass spectrometry</td>
<td>18</td>
</tr>
<tr>
<td>3.4. Micron-scale Visualization of Mineral-Organic Associations</td>
<td>20</td>
</tr>
<tr>
<td>3.4.1. Spatial distribution of C, Fe and Al</td>
<td>20</td>
</tr>
<tr>
<td>3.4.2. Quantitative image analysis</td>
<td>20</td>
</tr>
<tr>
<td>4. DISCUSSION</td>
<td>22</td>
</tr>
<tr>
<td>4.1. Rhizogenic weathering effects on quantity, age, and composition of soil carbon</td>
<td>22</td>
</tr>
<tr>
<td>4.2. Rhizogenic weathering effects on mineralogy</td>
<td>24</td>
</tr>
</tbody>
</table>
4.3. Evolution of mineral-organic associations in rhizosphere soil ..................26
4.5. Rhizogenic weathering generates and disrupts protective mineral-organic associations .................................................................29
4.6. Predicting the vulnerability of deep soil carbon to rhizogenic weathering impacts ..................................................................................31

5. CONCLUSION ..................................................................................................................33

APPENDIX: TABLES & FIGURES ..................................................................................35
BIBLIOGRAPHY ..............................................................................................................49
# LIST OF TABLES

<table>
<thead>
<tr>
<th>Tables</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Changes in total extractable organic C and metals, and clay mineral composition as a function of soil age in rhizosphere and non-rhizosphere soil</td>
<td>35</td>
</tr>
<tr>
<td>2. Modeled Mössbauer spectral parameters of iron oxides and clays as a function of soil age in rhizosphere and non-rhizosphere soil</td>
<td>36</td>
</tr>
<tr>
<td>3. Mineralogical changes as a function of soil age in rhizosphere (R) and non-rhizosphere (NR) soil based on fitting of X-ray diffractograms</td>
<td>38</td>
</tr>
</tbody>
</table>
### LIST OF FIGURES

<table>
<thead>
<tr>
<th>Figures</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Study site is Santa Cruz Terrace Chonosequence, photo credits to Marjorie Schulz (Schulz et al., 2016). There are five terraces ages 65kyr – 226kyr with distinct root weathering profiles.</td>
<td>39</td>
</tr>
<tr>
<td>2. Changes in C concentration and $\Delta^{14}$C values in rhizosphere and non-rhizosphere soil across the chronosequence. Bars represent standard error of the mean of three replicate samples.</td>
<td>40</td>
</tr>
<tr>
<td>3. Changes in extractable Fe and Al for (a) poorly crystalline and (b) crystalline phases, as well as C associated with each phase, in non-rhizosphere and rhizosphere soil across the chronosequence. Poorly crystalline phases are presented as the sum of metals and C extracted by water, pyrophosphate and hydroxylamine, crystalline phases are the sum of metals and C released by dithionite. Bars represent standard error of the mean of three replicate samples.</td>
<td>41</td>
</tr>
<tr>
<td>4. Changes in abundance of Fe oxides of varying degree of disorder in (a) non-rhizosphere and (b) rhizosphere soil across the chronosequence as determined by Mössbauer spectroscopy. Note np = nano-particle.</td>
<td>42</td>
</tr>
<tr>
<td>5. Changes in abundance of different organic compound classes in (a) non-rhizosphere and (b) rhizosphere soil across the chronosequence as determined by FT-ICR-MS. Classes given in darker colors (tannins, lignin, and carbohydrates) are attributed to plant-derived compounds, those given in lighter colors compounds (amino sugars, proteins, lipids, and unsaturated hydrocarbons) are classified as microbially-derived.</td>
<td>43</td>
</tr>
<tr>
<td>6. Changes in the micro-scale distribution of C in relation to Fe and Al phases in rhizosphere and non-rhizosphere soil across the chronosequence. To visualize the spatial distribution of C, Fe and Al, overlay maps (Red=Fe, Green=Al, Blue=C) for non-rhizosphere (b-d) and rhizosphere soil (e-g) at each terrace are presented. Scale bars are 5 µm except for non-rhizosphere terrace 2 which is 2 µm. Terrace 1 (a) has no rhizosphere differentiation, so only a map for bulk soil is shown. To quantify spatial relationships between Fe and C, we calculated Spearman’s rank correlation coefficient for three replicate maps per sample. Changes in the correlation coefficient describing the association of C and Fe are shown in panel (h). To further assess the degree of association between C and Fe, a co-localization analysis was performed (see methods for details) on three replicate images. Changes in the percentage of C associated with Fe across the chronosequence are shown in panel (i). For each analysis, bars represent standard error of the mean of three replicate images.</td>
<td>44</td>
</tr>
<tr>
<td>7. Conceptual diagram denoting that the formation of poorly crystalline phases and therefore commonly noted patterns in pedogenesis are due to root weathering. Root weathering of parent material leads to mineral-organic association formation and the increase in total organic carbon found in soils.</td>
<td>46</td>
</tr>
</tbody>
</table>
8. Mössbauer spectra as a function of soil age in rhizosphere and non-rhizosphere soil. Variable temperature Mossbauer spectra of terrace 2, non-rhizosphere (a-d) and rhizosphere samples (e-h) ........................................................................................................47

9. Differences in the presence and absence of Al-goethite nano-particles (np) in Mössbauer spectra at terrace 2 as a function of rhizosphere and non-rhizosphere soil. (a-c) 77 K Mossbauer spectra of non-rhizosphere samples from depths of 100, 110, and 140cm respectively, showing precipitation of bulk soil Fe as np-goethite’s of varying particle-sizes/Al-contents (particle sizes: type 1>type 2>type 3; Al-contents type 1<type 2<type 3) and minor amounts of Fe as Fe (III) in clay lattices. (d-g) 8 K Mossbauer spectra of terrace 1 bulk soil and rhizosphere soil samples from depths of 100, 110, and 140cm respectively showing somewhat equal distribution of soil Fe between clay minerals and np-goethite: types 2, 3, and 4 (type 4 is unique to rhizosphere soil samples)........................................................................................................48
CHAPTER 1
INTRODUCTION

Soils play a critical role in the global carbon (C) cycle because they contain 3300 Pg of C, which is more than twice the amount stored in the atmosphere (Jobbagy & Jackson, 2000; Tarnocai et al., 2009). More than half of total soil C stocks reside in deep (>1m) soils (Harper and Tibbett, 2013), where C can be stored for millennia (Ewing et al., 2006). However, several studies have demonstrated a dramatic response of deep soil C to environmental change (Bernal et al., 2016; Richter et al., 1999; Fontaine et al., 2007). To model and predict how deep soil C responds to disturbance, a better understanding of the mechanisms controlling soil C storage at depth is needed.

Due to the greater abundance of reactive metal phases, mineral-organic associations (MOAs) are assumed to be the quantitatively most important C protection mechanism in deeper soils (Rumpel and Kögel-Knabner, 2011). Poorly crystalline iron (Fe), manganese (Mn) and aluminum (Al) (hydr)oxides, amorphous metal-organic complexes, and aluminosilicates have a high affinity for organic C due to large surface areas and multiple bonding sites (Mikutta et al., 2007). The resulting MOAs often dramatically reduce microbial and enzymatic access to organic C (Kleber et al., 2015), thereby protecting C from loss processes for millennia (Torn et al., 1997). However, MOAs are subject to weathering during soil development (Chadwick & Chorover, 2001; Chorover et al., 2004), altering the crystallinity of mineral phases (Thompson et al., 2006) and their ability to protect C (Torn et al., 1997; Mikutta et al., 2009). While significant progress has been made in elucidating the impact of weathering on MOAs over pedogenic time scales, the influence of root-driven weathering reactions has been largely ignored.

Roots release 40-60% of photosynthetically fixed C into the soil as sloughed-off root cells and tissues, mucilage and exudates (Heinemeyer et al., 2007), which contribute up to 50-70% of
total soil C stocks (Clemmensen et al., 2013). The release of reactive C compounds induces an intense weathering regime in the near-root soil environment (often termed rhizosphere) (Richter et al., 2007), thereby altering geochemical and microbial processes (Fimmen et al., 2008; Schulz et al., 2016). For example, exudates such as organic acids, reductants, and metal chelators can directly dissolve minerals (Hinsinger and Courchesne, 2008; Richter et al., 2007). Further, proton release and CO₂ production acidify the soil and increase the solubility of many MOA-forming minerals (Tschiersch et al., 2012). Roots may also drive mineral weathering indirectly. Oxygen consumption by roots and microbes is often strong enough to create reducing conditions in the rhizosphere (Husson, 2013), which promotes reductive dissolution of minerals. Yet, to date, studies focusing on the development and C storage potential of MOAs (Chorover et al., 2004; Mikutta et al., 2009; Torn et al., 1997) have largely overlooked the potential impacts of such root-driven weathering reactions.

This is particularly true of deep soils, where weathering is generally perceived as driven by chemical processes (Lawrence et al., 2015; Moore et al., 2010), even though root systems extend to depths far beyond 1 m (Richter et al., 2007; Neung-Hwan et al., 2005). Roots and associated microbes are increasingly recognized as principal weathering agents in soils and bedrock (Leake et al., 2008), promoting the transformation of primary into secondary minerals (Brantley et al. 2011). Rhizosphere soil can thus be enriched in secondary phases, such as poorly crystalline Fe and Al hydroxides (April & Keller, 1990; Seguin et al., 2005). Schulz et al. (2016) compared deep soil that had experienced rhizogenic weathering with deep soil of the same age but directly unaffected by roots. Root-driven weathering had created poorly crystalline Fe and Al phases, which are known to have a strong effect on C storage in the same soils (Masiello et al.,
2004; Lawrence et al., 2015). In fact, recent studies suggest root exudate-mediated weathering of primary minerals produces crystalline Fe and Al phases that act as templates for the formation of MOAs (Yu et al., 2017), suggesting that root-driven weathering is essential for the protection of C in MOAs.

Root-driven weathering also transforms and dissolves secondary mineral phases. Seasonal variations in root activity have been shown to transform secondary minerals by dissolving Fe and Al hydroxides (Collignon et al., 2012) while hydroxylating clay mineral surfaces and interlayers (Augusto et al., 2001; Turpault et al., 2007). Observations of root-driven weathering over longer time scales by Fimmen et al. (2008) found that roots promote solubilization and re-precipitation of Fe away from the root, causing the relative enrichment of Al hydroxides in the near-root environment (i.e., rhizosphere soil). Dissolution and/or transformations of secondary minerals may directly affect reactive surface sites and their capacity to bind organic compounds (Chorover et al., 2004; Thompson et al., 2006). In fact, a recent study by Keiluweit et al. (2015) demonstrates that selected root exudates can disrupt MOAs, solubilizing previously mineral-bound organic C and making it more susceptible to microbial conversion to CO₂. The notion that root exudates can disrupt MOAs is further supported by the significant loss of old C protected in deep soils (Bernal et al., 2016; Fontaine et al., 2007) and associated with poorly crystalline Fe and Al phases (Rasmussen et al., 2007; Finley et al., 2018) in response to the addition of root exudates. Combined, these studies suggest that root-driven weathering may not only promote the formation, but also the disruption of MOAs, with unclear consequences for soil C storage.

The main objective of this study was to determine the impact of rhizogenic weathering on MOAs and soil C over pedogenic time scales. To accomplish our objective, we examined the MOA
transformations in relation to soil C dynamics in deep soil horizons of the Santa Cruz Marine Terraces chronosequence (White et al., 2008; Schulz et al., 2016). Deep soil horizons at this site are particularly suited for this study because they feature discrete zones created by root-driven weathering (here termed “rhizosphere soil”) (Schulz et al., 2016). The formation of this rhizosphere soil is an effect of repeated growth of deep roots into pores or fractures that have previously been colonized by roots (White & Kirkegaard, 2010). Preferential growth of new roots and root decay into the same soil spaces created a weathering regime that – over pedogenic timescales – caused the formation of mineralogically distinct rhizosphere soil (Schulz et al., 2016). In contrast, adjacent soil zones did not experience root growth and were not subjected to direct root-driven weathering. In the absence of roots, this “non-rhizosphere soil” experienced a different weathering regime and, thus, developed mineralogical characteristics distinct from those of rhizosphere soil. While this non-rhizosphere soil may have been influenced by solute exchange with the rhizosphere soil, the direct weathering impact of roots can be considered negligible (Schulz et al. 2016).

To follow the impact of root-driven weathering over time, we analyzed samples from four soil profiles ranging in age from 65 ka to 226 ka across the chronosequence, and directly compared rhizosphere to non-rhizosphere soil in each profile. Sample characterization was guided by three objectives: to determine the effects of rhizogenic weathering on (i) C concentration, turnover time and chemistry, (ii) mineralogy, particularly the changes in poorly crystalline Fe and Al phases, and (iii) the degree of association between C and Fe and Al phases at the micron-scale. To this end, we used a suite of methods for solid-phase analysis of C, mineralogy and MOAs, including radiocarbon measurements, high-resolution Fourier-transform ion cyclotron mass spectrometry
(FT-ICR-MS), quantitative sequential extractions, Mössbauer spectroscopy, and scanning X-ray transmission microscopy (STXM). Our results indicate a strong coupling between root-driven MOAs transformations and soil C accrual and loss over pedogenic time scales.
CHAPTER 2
METHODS

2.1 Study Site

The Marine Terrace soil chronosequence is located in coastal California just north of Santa Cruz, CA, USA, and consist of five spatially distinct coastal terraces ranging in age from 65 ka to 226 ka (Fig. 1) (White et al., 2008). The terraces increase in age (and elevation) with distance from the modern coastline (Fig. 1) (Schulz et al., 2018; Perg et al., 2001). The soils developed on the Marine terraces are generally classified as Mollisols and have developed from shallow marine sediment deposits (Schulz et al., 2018). Terrace deposits are predominantly composed of granodiorite to quartz diorite, with some amounts of gabbro, sandstone and mudstone (White et al., 2008). The mudstone contains little clay and ranges from a siliceous, organic-rich mudstone to a porcelanite (Clark, 1981; El-Sabbagh & Garrison, 1990). The marine terraces near Santa Cruz have been dated using $^{10}$Be and $^{26}$Al (Perg et al., 2001). The age determination has facilitated quantitative testing and development of reactive transport models for weathering and soil-profile development (Maher et al., 2009; Lawrence et al., 2014). The predominant vegetation is open grassland (combination of native and European grasses) interspersed with California oak woodlands. The current climate is Mediterranean, with cool wet winters and warm dry summers with mean annual precipitation and temperature of ~730 mm and 13.4 ºC, respectively (Schulz et al., 2016). The youngest soil (65 ka, terrace 1) has an A horizon but no Bt horizon and, while indirect root influences cannot be excluded, no evidence of rhizosphere development at depths greater than 1 m is currently observed. In the 65 ka soil, orange-hued Fe oxides have formed a relatively uniform coating on primary mineral grains throughout the sediment deposit. Soils on
older surfaces of the Santa Cruz chronosequence (90–226 ka, terraces 2–5) display Bt horizons at depths greater than 1 m. These Bt horizons feature gray, low-chroma weathering zones subjected to root activity (rhizosphere soil) and orange, high-chroma zones that did not experience root growth and are similar to the Fe oxide rich subsoils in terrace 1 (non-rhizosphere soil) (Fig. 1) (Schulz et al., 2016). These distinct color (and mineralogical) differences between rhizosphere and non-rhizosphere soil are comparable to those observed by Fimmen et al. (2008) in humid forested ecosystems.

2.2. Soil Sampling

Soil samples from terraces 1-3 and 5 were collected using a hand-auger from depths showing the most pronounced rhizosphere differentiation (depths = 120-170 cm). Triplicate cores were taken on each terrace at random locations. In the lab, rhizosphere soil was carefully separated from non-rhizosphere soil for terraces 2, 3 and 5, based on the color differences described above, and dried in the dark in an anaerobic chamber. All soil from terrace 1 was considered to be non-rhizosphere soil due to the absence of visible rhizogenic weathering zones. Terrace 4 was not sampled because it is known to have been subjected to recent anthropogenic disturbance where the top 30 cm had been removed (White et al., 2008). Analyses were generally performed on all replicates except for those with very low throughput and high analytical cost (radiocarbon, Mössbauer, FT-ICR-MS, and X-ray spectromicroscopy). For the low through but high-cost analyses, equal amounts (on a mass basis) of three replicates were pooled and one composite sample was analyzed.
2.3. Total Carbon

To determine total C concentration, samples were analyzed via an ECS 4010 CHNS-O elemental analyzer (Costech Analytical Technologies, Inc., Valencia, CA, USA), with acetanilide as a standard. These C analyses were performed on all samples (triplicate cores from each terrace separated into rhizosphere and non-rhizosphere soil). The soils contained very little to no inorganic C, and C concentrations are thus considered to reflect organic C concentrations.

2.4. Radiocarbon Analysis

Composite samples from each zone and terrace were submitted to the University of California, Irvine W.M. KECK Carbon Cycle Accelerator Mass Spectrometry Laboratory (UCI-KECK AMS), where sample carbon was converted to CO$_2$ through combustion with CuO at 900°C. The CO$_2$ was then cryogenically extracted and purified on a vacuum line and yield was measured using pressure change in a known volume (Xu et al., 2007). Approximately 1 mg of CO$_2$ was partitioned into a glass tube containing Zi + TiH$_2$ catalyst filament and sealed with a torch prior to graphitization in a furnace for 500-550°C for 7 hours. Any remaining CO$_2$ was retained for $\delta^{13}$C measurement by IRMS. After graphitization, graphite was packed into a 1 mm diameter aluminum target and loaded on the UCI-KECK AMS for $^{14}$C measurement. The $^{14}$C abundance was normalized to a constant $\delta^{13}$C using AMS measurements for both values, allowing for correction of natural isotope fractionation as well as any fractionation that occurs within the AMS system (Xu et al., 2007).
2.5. Quantitative X-ray Diffraction

Composite samples were prepared for XRD using a modified method based on Eberl (2003). One gram of homogenized sample was mixed with 20% corundum and ground in a McCrone micronizing mill with 4mL ethanol for 5 minutes, generating particle sizes on the order of 10-30μm. After drying at 60°C, the mixture was transferred to a plastic scintillation vial with three delrin balls (~1cm in diameter) along with 200-800μL Vertrel® solution (Dupont) and shaken for 10 minutes. The powder was passed through a 250-μm sieve to break up larger aggregates and loaded onto an XRD sample holder. Samples were analyzed using a Siemens D500 X-ray diffractometer from 5 to 65 degrees two theta using Cu Kα X-ray radiation, with a step size of 0.02 degrees and a dwell time of 2 seconds per step. Quantitative mineralogy was calculated using the USGS software, RockJock (Eberl, 2003), which fits XRD intensities of individual mineral standards to the measured diffraction pattern.

2.6. Sequential Extractions

To determine the amount of poorly crystalline and crystalline material present in these soil samples, sequential wet-chemical extractions were performed on samples from terrace 1, as well as rhizosphere and non-rhizosphere soil from terraces 2, 3 and 5. Triplicate samples were hand-ground with mortar and pestle and 300 mg of each sample was weighed into 15 ml centrifuge tubes. Our sequential extraction followed the procedure developed by Heckman et al. (2018), which avoids C-based extractants to allow for the quantification of metal phases as well as the organic C compounds associated with them. The sequential extraction consisted of ultrapure H2O to extract dissolved metals, pyrophosphate to solubilize metal-organic complexes (Ross & Wang,
1993), hydroxylamine to extract easily reducible phases (Courchesne & Turmel, 2008), and dithionite to quantify total reducible metal phases (Wagai and Mayer, 2007). In each step, tubes received 10 ml of initial extractant and the soil was suspended by briefly placing the mixture on a vortexer (30 s). The tubes were then placed on shaker for 16 h (at 300 rpm), subsequently centrifuged (4000 x g) for 1 hr for pyrophosphate and 20 min for all other extractants. Between each extraction step, the remaining soil pellet was washed with 7 ml of ultrapure H₂O to remove excess extractant. Samples re-suspended in H₂O were placed on a shaker for 2 hours and centrifuged at 4000 x g for 20 min. The supernatant was then combined with the previously recovered extract. The extract was filtered through 0.2-µm syringe filters and stored in the dark for further analysis. Aliquots were diluted, acidified with 2% nitric acid, and analyzed by ICP-MS (Shimadzu ICPMS-2030, Shimadzu Scientific Inst., Columbia, MD, USA) to determine Fe, Al and Si concentrations and separate aliquots were diluted and analyzed by TOC (Shimadzu TOC-L CPH with an ASI-L, Shimadzu Scientific Inst., Columbia, MD, USA) to determine dissolved organic C concentrations in the extracts.

2.7. Fourier-Transform Ion Cyclotron Resonance Mass Spectrometry (FT-ICR-MS) Analysis

A 12 Tesla Bruker SolariX FTICR spectrometer located at Environmental Molecular Science Lab a DOE-BER national user facility located in Richland, WA, was used to obtain molecular information on the organic compounds in composites of water extracts. Other attempts to characterize the composition of the bulk and mineral-associated organic matter based on X-ray absorption spectroscopy and nuclear magnetic resonance spectroscopy failed due to the extremely low C concentrations in our samples. The FT-ICR-MS analyses were focused on water extracts
because water has been shown to extract the most diverse pool of organic compounds (Tfaily et al., 2015). Equal aliquots from triplicate water extracts (see sequential extraction above) were combined into a composite sample for analysis. Samples were then introduced into a standard Bruker ESI source to generate negatively charged molecular ions. The instrument was externally calibrated weekly to a mass accuracy of < 0.1 ppm using a tuning solution from Agilent, which contains the following compounds: C$_2$F$_3$O$_2$, C$_6$HF$_9$N$_3$O, C$_{12}$HF$_{21}$N$_3$O, C$_{20}$H$_{18}$F$_{27}$N$_3$O$_8$P$_3$, and C$_{26}$H$_{18}$F$_{39}$N$_3$O$_8$P$_3$ with m/z ranging between 112 to 1333. The instrument settings were optimized by tuning on a Suwannee River Fulvic Acid (SRFA) standard. Blanks (HPLC grade MeOH) were also run at the beginning and the end of the day to monitor potential carry over from one sample to another. The instrument was flushed between samples using a mixture of water and methanol. The ion accumulation time (IAT) was varied between 0.1 and 0.3 s to account for differences in C concentration between samples. Ninety-six individual scans were averaged for each sample and internally calibrated using OM homologous series separated by 14 Da (–CH2 groups). The mass measurement error was less than 1 ppm for singly charged ions across a broad m/z range (i.e. 200 < m/z < 1200). To further reduce cumulative errors, all sample peak lists for the entire dataset were aligned to each other prior to formula assignment to eliminate possible mass shifts that would impact formula assignment. Putative chemical formula were assigned using Formularity software made using Visual Studio (Microsoft Corporation) (Tolić et al., 2017). Chemical formula were assigned based on the following criteria: S/N > 7, and mass measurement error < 1 ppm, taking into consideration the presence of C, H, O, N, S and P and excluding other elements. Peaks with large mass ratios (m/z values > 500 Da) often have multiple possible candidate formulas. These peaks were assigned formula through propagation of CH$_2$, O, and H$_2$ homologous series. If
multiple formula candidates were found, we consistently picked the formula with the lowest error and lowest number of heteroatoms, and the assignment of one phosphorus atom required the presence of at least four oxygen atoms. Peaks that were present in the blanks were subtracted from the sample data sets. Additionally, peaks that were present in only one sample were removed and are not included in the downstream analysis. On average, 4427 ± 428 formula were identified in each sample. To emphasize differences between samples, we further eliminated those formula compounds that are common among all samples. The remaining “unique” formula amounted to an average 1523 ± 622 per sample. To elucidate changes in the relative abundance of different compound classes, unique formulas were plotted on a van Krevelen diagram corresponding to their H/C (hydrogen to carbon) vs. O/C (oxygen to carbon) ratios (Kim et al., 2003). Using the H/C and O/C thresholds defined in Tfaily et al. (2015), compounds were broadly categorized as lipid-, protein-, lignin-, tannin-, carbohydrate-, amino sugar-, and unsaturated hydrocarbon-like compounds.

2.8. $^{57}$Fe-Mössbauer Spectroscopy

Variable-temperature Mössbauer spectra were collected using a Web Research Company (St. Paul, MN) instrument that included a closed-cycle cryostat SHI-850 obtained from Janis Research Company, Inc (Wilmington, MA), a Sumitomo CKW-21 He compressor unit, and a Ritverc (St. Petersburg, Russia) NaI detection system. $^{57}$Co/Rh source (75-mCi, initial strength) was used as the gamma energy source. The raw data was folded to 512 channels to provide a flat background and a zero-velocity position corresponding to the center shift (CS) of a metal Fe foil at room temperature (RT). Calibration spectra were obtained with a 7-μm-thick Fe foil
(Amersham, England) placed in the same position as the samples to minimize any geometry errors. The Mössbauer data were modeled with Recoil software (University of Ottawa, Canada) using a Voigt-based structural fitting routine (Rancourt and Ping, 1991). Sample preparation and sample holder were identical to the procedures reported in Peretyazhko et al. (2013).

2.9. Scanning X-ray Transmission Microscopy (STXM) Imaging

Scanning transmission X-ray microscopy (STXM) analyses were conducted at beamline SM 10ID-1 of the Canadian Lights Source (CLS) in Saskatoon, Canada. Composite soil samples were suspended in ultrapure H$_2$O (1:10 soil:solution ratio) and an aliquot of 1 µl was drop deposited on Si$_3$N$_4$ windows (Silson) and dried under vacuum. The sample chamber for STXM image collection was He-filled to minimize attenuation of the soft X-rays. Images were collected from three replicate regions of interest (25 x 25 µm) containing representative collections of particles for all samples. Images were collected at energies above and below the C K-edge (280 eV and 288.5 eV), Fe L-edge (700 eV and 709.5 eV), and Al K-edge (1560 eV and 1568 eV), respectively. Images were recorded with dwell times of 1 (C and Fe) or 3 ms (Al) and a pixel size of 40 x 40 nm. Elemental maps for C, Fe and Al were generated by subtracting the aligned pre-edge image from the post-edge image using aXis2000 (McMaster University, Hamilton, Ontario, Canada). The spatial relationship between C, Fe and Al in each region of interest were further analyzed in the open-source image processing software Fiji (Schindelin et al., 2012; Rueden et al., 2017). To determine the spatial correlation of C with either Fe or Al, the Coloc2 plugin (Abramoff et al., 2004) was used to determine the Spearman’s rank correlation coefficient. To quantify the degree of association between C and Fe or Al, a co-localization analysis was performed using the
Colocalization Threshold plugin (Abramoff et al., 2004). The underlying algorithm calculates the number of pixels in which significant absorption of both C and Fe (or Al) is measured, relative to the total number of pixels in which significant absorption of only C is measured. The resulting values (expressed in percent) is a measure of the proportion of the total C in each image that is co-localized with Fe or Al. Correlation and co-localization analyses were performed for both C-Fe and C-Al pairs, but only the C-Fe pair is reported here. Because the Al K-edge is at significantly higher energy than the C K-edge and Fe L-edge, the spatial resolution and sensitivity for Al was insufficient for our quantitative imaging analysis.

2.10. Statistical Analyses

One-way Analyses of Variance (ANOVA) were conducted using OriginPro2018 by Origin Lab followed by Fisher’s LSD pairwise comparison tests with a p value of less than 0.05 indicating statistical significance. For synchrotron imaging Spearman’s Rank Correlation analyses and Colocalization analyses were conducted in Fiji and the results were further compared using Fisher’s LSD in OriginPro2018 with a p value of less than 0.05 indicating statistical significance.
CHAPTER 3
RESULTS

3.1. Elemental and radiocarbon analyses

Elemental and radiocarbon analysis revealed consistently greater C concentrations (Fig. 2), and less depleted (i.e., more negative) $\Delta^{14}$C values (Fig. 2) in rhizosphere than non-rhizosphere soil across the chronosequence. Relative to bulk soil from terrace 1, C concentrations in terrace 2 decreased significantly by approx. 50% ($p < 0.05$, Fisher’s LSD) in non-rhizosphere soil. However, in rhizosphere soil in terrace 2, C concentrations were significantly increased by more than 30% ($p < 0.05$, Fisher’s LSD). Between terraces 2 and 5, C significantly decreased by about 43% in rhizosphere soil ($p < 0.01$, Fisher’s LSD). Contrastingly, C steadily decreased by over 67% in the non-rhizosphere soil from terrace 1 to 5 ($p < 0.01$, Fisher’s LSD). Terrace 2 showed substantially more depleted $\Delta^{14}$C values in both rhizosphere and non-rhizosphere soil than in the other terraces.

3.2. Mineralogical Composition

3.2.1. X-ray diffraction

The impact of rhizogenic weathering on the relative abundance of secondary minerals, particularly the formation and presence of phyllosilicate clays, was evaluated using XRD. Fitting of diffractograms showed that phyllosilicate clays in non-rhizosphere soils constituted approximately 30% of all XRD-detectable phases, and only increased at terrace 5 (Table 3). However, for rhizosphere soils the phyllosilicate clay portion more than doubled between terraces 1 and 2 to exceed 60%, and further increased to more than 70% in terraces 3 and 5. Conversely, the contributions from primary minerals diminished with soil age in rhizosphere soil. These
observed trends indicate that phyllosilicate clays formed during the initial stages of rhizogenic weathering.

3.2.2. Sequential extractions

Sequential extractions were used to determine the impact of rhizogenic weathering on the relative abundance of poorly crystalline and crystalline Fe and Al phases, as well as the amount of organic C released upon extraction (Fig. 3). The amount of water, pyrophosphate, and hydroxylamine extractable Fe and Al (here defined as poorly crystalline phases) in non-rhizosphere soil remained relatively stable and was significantly lower than in rhizosphere soil across the chronosequence (p < 0.05, Fisher’s LSD) (Fig. 3a). In contrast, the abundance of poorly crystalline Fe and Al phases increased significantly between terraces 1 and 2 in rhizosphere soil, and declined thereafter (p < 0.01, Fisher’s LSD). The extraction of greater amounts of poorly crystalline Fe and Al phases in rhizosphere soil coincided with a significantly larger mobilization of C when compared to non-rhizosphere soil (p < 0.01, Fisher’s LSD) (Fig. 3a).

The amount of dithionite-extractable Fe and Al (here defined as crystalline phases) was significantly lower in non-rhizosphere soil compared to rhizosphere soil (p < 0.01, Fisher’s LSD) (Fig. 3b). However, the trends for crystalline Fe and Al phases differ from those observed for poorly crystalline phases. The amount of crystalline Fe and Al phases in non-rhizosphere soil, as well as the amount of associated C, did not change significantly with soil age (p < 0.05, Fisher’s LSD). In contrast, crystalline Fe and Al phases in rhizosphere soil initially increased 4-fold from terrace 1 to 2 and plateaued thereafter. This increase in crystalline phases is mirrored by our XRD data which shows that the abundance of crystalline phyllosilicate clays in rhizosphere soils at
terrace 2 increased more than in non-rhizosphere soils. The amount of C associated with these crystalline phases in rhizosphere soil followed the same pattern – an initial increase by 75% in terrace 2, and unchanged values in terrace 3 and 5. The observed dynamics in rhizosphere soil indicate that initial rhizogenic weathering created poorly crystalline Fe and Al phases. However, more crystalline Fe and Al phases and phyllosilicate clays began to dominate the rhizosphere with continued weathering.

3.2.3. Mössbauer spectroscopy

Mössbauer spectra were obtained at room temperature (RT) and below RT to gain insights into type and nature of Fe oxides in terms of their particle size, extent of metal or C coatings (Peretyazhko et al., 2013; Chen et al., 2017) (Figs. 8 and 9). Mössbauer spectra showed that neither rhizosphere nor non-rhizosphere soils contained ferrihydrite-like phases (Kaplan et al., 2016) or Fe(III)-organic matter complexes (Jung et al., 2012). In non-rhizosphere soil, Mössbauer spectra showed precipitation of ~90% of the total Fe as nano-particulate goethite (np-goethite, < 10 nm particle size, Table 2) (Cook et al., 1999) with the remaining Fe (~10%) found in clay minerals. Furthermore, modeling suggested that np-goethite phases consist of three different general pools (or broad classes) labeled as type 1, type 2, and type 3. These differences were attributed to variations in particle size, which is a function of metal (Al) substitution (goethite with Al/(Al + Fe) ratio 0.16 display doublet at RT) (Fysh and Clark, 1982), with disorder increasing in the following order: type 1 > type 2 > type 3. The relative distribution (and, thus, the degree of disorder) of np-goethite phases in the rhizosphere soil are distinctly different from non-rhizosphere
soils. For example, type 3 is absent, and a significant pool of a highly disordered np-goethite - type 4, with the lowest particle size and highest degree of metal substitution – is evident.

Rhizosphere and non-rhizosphere soil showed divergent trends in the np-goethite contributions with increasing soil age (Fig. 4). The Fe oxide composition in terrace 1 was dominated by relatively ordered np-goethite phases (types 1 and 2), and minor contributions of lepidocrocite and np/Al-hematite. In terrace 2, however, distinct differences in Fe oxide mineralogy emerged between non-rhizosphere and rhizosphere soil. A relatively more disordered np-goethite (type 3) emerged in non-rhizosphere soil (Fig. 4a). The np-goethite (type 3), as well as the relatively ordered np-goethite (types 1 and 2), dominated Fe oxide composition in non-rhizosphere soil; their relative contributions (~34-38% and 62-66%, respectively) remain constant from terrace 2 to 5. However, in terrace 2 rhizosphere soil, type 3 oxide is absent. In this sample a highly disordered np-goethite (type 4) emerged (Fig. 4b and Fig. 9). Np-goethite (type 4) represented 58% of the Fe oxides detected in terrace 2, and its contribution steadily declined to ~50% between terrace 3 and 5. Our Mössbauer spectroscopy results indicate that these initial stages of rhizogenic weathering (terrace 2) promoted the formation of highly disordered np-goethite phases (type 4), the contribution of which declined with continued weathering (terrace 3-5).

3.3. Organic Matter Composition

3.3.1. Mass spectrometry

To determine the impact of root weathering on the composition of organic matter, we analyzed water extracts by high-resolution FT-ICR-MS. In terrace 1, over 80% of the unique
masses can be categorized as tannin-, lignin-, and carbohydrate-like compounds, while the remaining 20% of these unique masses were categorized as lipid-, protein-, amino sugar-, and condensed hydrocarbon-like compounds (Fig. 5). With increasing soil age, non-rhizosphere soil showed a steady decline in tannin-, lignin-, and carbohydrate-like compound contribution relative to terrace 1. This decline was concurrent with proportional increases in the abundance of protein, lipid and amino sugar-like, which were the most dominant compound classes (> 60% of total) in terrace 5 (Fig. 5a). On the other hand, with the initial formation of the rhizosphere (terrace 2, Fig. 5b), the contribution of lignin-, tannin-, and carbohydrate-like decreased by almost 50%, while that of lipid-, protein- and amino sugar-like compounds increased 3-fold. As rhizosphere soil aged further (from terrace 2 to 5), lipid-, protein-, and amino sugar-like compounds decreased in their contribution. In terrace 5, the contribution of tannin-, lignin-, and carbohydrate-like compounds was dominating rhizosphere soil, making up to ~60% of all mass peaks. In sum, non-rhizosphere and rhizosphere soil showed notably different patterns for the relative abundance of compound classes that can be attributed to plant material (e.g., lignin, tannins, and carbohydrates) and those that may be microbially derived (e.g., lipids, proteins, and amino sugars).

Due to the low C concentrations of our samples, other spectroscopic methods that would provide additional information on relative abundance of specific C functional groups and degree of oxidation could not be used for this analysis (i.e. Carbon 1s Near-edge X-ray Absorption Fine Structure (NEXAFS) Spectroscopy).
3.4. Micron-scale Visualization of Mineral-Organic Associations

3.4.1. Spatial Distribution of C, Fe and Al.

Elemental maps recorded by STXM revealed differences in the micron-scale distribution of C, Fe, and Al between non-rhizosphere and rhizosphere soil (Fig. 6). Fig. 6a-g show normalized false-color overlays of C, Fe, and Al maps of clay-sized particles. In terrace 1 (Fig. 6a), we observed larger (< 5µm), discrete Al-rich particles as well as smaller (< 1µm), Fe- and C-containing particles. In non-rhizosphere soil, discrete Fe-rich particles dominated, the abundance of which gradually increased from terrace 2 to 5 (Fig. 6b-d). When present, C-rich spots were only found in association with these discrete Fe particles (Fig. 6b-d). Concurrently, the abundance of Al-rich particles declined. Images of rhizosphere soil contained visibly more C than non-rhizosphere soil (Fig. 6e-g). Most notably in terrace 2, which contained the most C in a patch-like distribution. These patches contained notable amounts of small (< 1 µm), dispersed Fe-rich particles (Fig. 6e). Diffuse C-rich patches were not visible in terraces 3 and 5 (Fig. 6f and g). Here, C-rich spots appeared more concentrated and increasingly co-localized with larger (> 1 µm) Fe- and Al-rich particles. The small and dispersed Fe-rich particles were much less prominent in terraces 3 and 5 (Fig. 6f and g).

3.4.2. Quantitative Image analysis.

In addition to the qualitative image analysis presented above, we quantified how rhizogenic weathering altered the degree to which C is associated with Fe by subjecting our Fe and C maps (n = 3) to correlation (Fig. 6h) and colocalization analyses (Fig. 6i). We focused our imaging analysis on Fe (as opposed to Al) due to the higher spatial resolution images that can be achieved.
at the Fe L-edge (see methods section for detailed explanation). From terrace 1 to terrace 2, the Fe and C distributions became significantly less correlated in non-rhizosphere soil, as evidenced by declining r values from ~0.63 to ~0.30 (Fig. 6h). The r value remained low and did not change significantly thereafter. In contrast, the distribution of Fe and C in rhizosphere soil became more strongly correlated with the onset of weathering. Between terrace 1 and 2, the r value increased from 0.63 to ~0.83, a value significantly greater than that in non-rhizosphere soil (p < 0.05, Fisher’s LSD). With continued weathering (terrace 2 to 5), r values declined gradually and significantly, approaching those in non-rhizosphere soils (r = ~0.34).

Our co-localization analysis showed analogous trends for the fraction of C that is co-localized with Fe (Fig. 6i). The amount of C associated with Fe in non-rhizosphere soil did not change significantly across the age gradient and remained between 40-50%. In contrast, the proportion of C co-located with Fe in rhizosphere soil increased to 80% in terrace 2, a value significantly greater than that found in the non-rhizosphere soil (p < 0.05, Fisher LSD). Between terrace 2 and 5, the amount of C co-localization with Fe gradually declined towards values observed in non-rhizosphere soils (~45%).

Taken together, our elemental mapping revealed that the initial rhizosphere differentiation (terrace 2) coincided with the formation of Fe-C associations, where small Fe oxide particles appeared to be embedded within a matrix of organic matter (Fig. 6e). As weathering in rhizosphere soil continued, larger Fe oxide particles increasingly dominated and the degree of association with C declined (Fig. 6e-f). In contrast, non-rhizosphere soils displayed consistently low amounts of C associated with Fe across the age gradient (Fig. 6b-d).
CHAPTER 4
DISCUSSION

More than 50% of soil C is found in soils deeper than 1 m and protected within MOAs (Rumpel & Kögel-Knabner, 2012). Although the effects of root activity on deep soil C dynamics have received increased attention (Yu et al., 2017; Poirier et al., 2018), the impact of root-driven weathering on MOAs and C storage over pedogenic time scales have yet to be understood. Our comparison of rhizosphere and non-rhizosphere soil across the Santa Cruz chronosequence highlight the significant impact of root activity on the evolution of mineralogy, organic matter chemistry and, consequently, the nature of MOAs during soil development.

4.1 Rhizogenic weathering effects on quantity, age, and composition of soil carbon

Continuous root C inputs such as dead root tissue, border cells, mucilage, or exudates promote rhizogenic weathering over pedogenic time scales (Fimmen et al., 2008; Schulz et al., 2016). Our results show that root C significantly changed the concentration (Fig. 2), $\Delta^{14}$C values (Fig. 2), and chemistry of organic matter (Fig. 4b) in soil directly impacted by rhizogenic weathering. Across the age gradient, rhizosphere soil contained more C than non-rhizosphere soil (Fig. 2), which is consistent with previous observations of root-promoted C accumulation in deep soil (Fimmen et al., 2008; Richter et al., 1999). C accumulation became particularly significant in terrace 2, where direct rhizogenic weathering effects were first observable, with C concentrations in non-rhizosphere soil only half of those found in rhizosphere soil (Fig. 2). C accumulation at this weathering stage coincided with a steep decline in $\Delta^{14}$C values (Fig. 2), suggesting longer C residence times, and a significant increase in the relative abundance of organic compounds that
can be attributed to microbial sources (e.g., lipids, amino sugars, and proteins) (Fig. 5b). With continuous rhizogenic weathering (terraces 3 and 5) C concentrations, Δ¹⁴C values (Fig. 2), and the abundance of microbially-derived compounds (relative to that of plant-derived compounds such as lignins, tannins, and carbohydrates) in rhizosphere soil declined (Fig. 5b).

The dynamics of C content and radiocarbon content reflect those observed for bulk soil in comparable chronosequences, which generally show an increase in C concentration and potential residence time during initial weathering stages and a decline thereafter (Masiello et al., 2004; Torn et al., 1997; Mikutta et al., 2009; Lawrence et al., 2015; Chorover et al., 2004). The increase in microbially-derived products during initial weathering stages and their subsequent decline in late stages follows concentrations of bacterial biomass C in bulk soils at our site (Moore et al., 2010). Fimmen et al (2008) also noted increased microbial biomass in rhizogenically weathered soil, suggesting that this increase in microbially-derived C may stem from root-associated microbes. Our results thus indicate that root activity during initial root-driven weathering promoted C accumulation by triggering microbial biomass growth in the rhizosphere.

The C dynamics in non-rhizosphere soil are in sharp contrast to that in rhizosphere soil, and those observed in the aforementioned chronosequences (Masiello et al., 2004; Torn et al., 1997; Mikutta et al., 2009; Chorover et al., 2004; Lawrence et al., 2015). C concentrations gradually declined with soil age (Fig. 2), paralleled by a gradual shift from predominantly plant-derived to microbially-derived C compounds (Fig. 5a). In the absence of direct root C inputs, the relatively slow shift towards microbially derived compounds could be a result of long-term changes in the amount and composition of surface DOC leached into deeper soil horizons (Kramer
et al. 2012) or the result of continuous microbial cycling of the C available in the non-rhizosphere soil over time (Rumpel and Kögel-Knabner, 2012).

4.2 Rhizogenic weathering effects on mineralogy

Roots and associated microbes create an intensive weathering regime in the rhizosphere through the introduction of organic reductants, chelators, acids or protons (e.g., Hinsinger et al., 2005), which dissolve primary minerals (Brantley et al. 2011; Violante & Caporale, 2015; Yu et al., 2017) and transform secondary minerals (Collignon et al., 2012; Fimmen et al., 2008; April & Keller, 1990; Turpault et al., 2007). Our results show that these root-driven weathering reactions significantly altered the mineralogy across the chronosequence, with the effect on the formation and transformation of poorly crystalline minerals being particularly notable.

The initial development of the rhizosphere coincided with a rapid increase in poorly crystalline phases (3-fold) (Fig. 3b), which was mirrored by the appearance of highly disordered np-goethite (Fig. 4b). At terrace 2, the most disordered np-goethite phases (type 4) represented more than 50% of all Fe oxides present. Disorder arises from substitution with Al and C (Chen et al., 2017), forming smaller and more disordered np-goethite (Cornell & Schwertmann, 2003). The greater abundance of highly disordered Fe and Al phases during initial weathering stages in rhizosphere soils is consistent with the accumulation of Al and Fe hydroxides (e.g., ferrihydrite, imogolite, and allophane) observed in younger soils of comparable chronosequences (Torn et al., 1997; Chorover et al., 2004; Mikutta et al., 2009; Masiello et al., 2004). However, we did not find this trend in increasing Al and Fe hydroxides in bulk soil across the chronosequence (Lawrence et
al. in prep). This discrepancy strongly suggests that the dynamics of poorly crystalline phases are directly related to rhizogenic weathering.

With continued weathering the abundance of these poorly crystalline minerals decreased from terraces 2 to 5. During these later weathering stages, the contribution of crystalline Fe and Al phases increased (Fig. 4b) and shifted from highly disordered (type 4) to more ordered np-goethite (types 1-3) (Fig. 4b). This transformation from small, poorly crystalline to larger, thermodynamically stable crystalline minerals through continued weathering in rhizosphere soils is consistent with observations in more weathered soils of other chronosequences (Masiello et al., 2004; Chorover et al., 2004; Chadwick & Chorover et al., 2001; Torn et al., 1997). In our case, transformation (or “aging”) of highly disordered np-goethite into more ordered and thermodynamically stable phases may be due to repeated redox cycles (Thompson et al., 2006), as induced by root activity (Fimmen et al., 2008). Hence, our results indicate that with recurring root colonization of rhizosphere soil over pedogenic time scales, more ordered and, thus, stable Fe phases have been created.

Mineral composition and transformations in non-rhizosphere soil were distinctly different from that in rhizosphere soil across the chronosequence. Non-rhizosphere soil showed significantly lower concentrations of poorly crystalline Fe and Al phases than rhizosphere soil, with no significant changes in concentration along the soil age gradient (Fig. 3a). However, sequential extractions indicated an increase in the amount of crystalline Fe and Al with time (Fig. 3b), which is consistent with the dominance of the most ordered np-goethite phases (types 1-2) in late weathering stages (Fig. 4a). The relatively low abundance of poorly crystalline materials and
slow transformation of crystalline Fe and Al phases indicate lower weathering rates, which we attribute to the lack of root C inputs and associated microbial activity.

4.3 Evolution of mineral-organic associations in rhizosphere soil

Root impacts on organic matter composition and mineralogy during initial and late weathering stages resulted in dramatic transformations of MOAs across the chronosequence. Initial rhizogenic weathering (terrace 2) coincided with a sharp increase in the amount of C associated with poorly crystalline Fe and Al phases (Fig. 3a) and was accompanied by significant increases in C accumulation and potential C residence times (Fig. 2), highlighting the importance of such MOAs for C storage in these soil systems (Masiello et al., 2004; Lawrence et al., 2015).

The importance of the interaction of organic compounds with poorly crystalline Fe and Al phases in controlling C storage during initial rhizogenic weathering stages was further supported by STXM images, which showed the strongest spatial associations between Fe and C in terrace 2 (Fig. 6h and i). Fe-C associations at this weathering stage show a unique morphology, with sub-micron sized Fe minerals embedded within a matrix of diffuse C (Fig. 6e). The observation of small Fe particles closely associated with C (Fig. 6e) is consistent with our Mössbauer results, which revealed the formation of highly disordered np-goethite phases substituted with Al and/or C (Fig. 4b) at this stage. These diffuse C patches embedded within agglomerations of Fe particles are like those of amorphous organic matter documented using high-resolution imaging elsewhere (Keiluweit et al., 2015; Thompson et al., 2006), suggesting a degree of microbial processing. Our mass spectrometry results (Fig. 5b) further support this notion, showing a sharp increase in the contribution of potentially microbially-derived organic compounds, such as amino sugars, proteins.
and lipids (from 20 to 80%), with the onset of rhizogenic weathering. Strong affinities between poorly crystalline minerals and microbially-derived compounds are in good agreement with reports by Mikutta et al. (2009), who showed that the presence of aliphatic and amide C, which are indicative of microbial residues, is highly correlated with the abundance of poorly crystalline Fe phases.

Continued rhizogenic weathering (terrace 3-5) resulted in the gradual loss of associations between poorly crystalline phases and microbially-derived C. STXM imaging revealed that the degree of micron-scale association of C and Fe steadily decreased during late weathering stages (Fig. 6a, e-g). This decrease in the amount of C associated with poorly crystalline Fe and Al phases (Fig. 3a) was paralleled by a decline in the contribution of highly disordered, potentially C-substituted, np-goethite phases (Fig. 4b) and microbially-derived compounds (Fig. 5b). We attribute these observations of root-induced disruption of MOAs, possibly via ligand-promoted or reductive dissolution (Keiluweit et al., 2015; Clarholm et al., 2015) as well as changes in pH or Eₜ (Fimmen et al., 2007). Past studies have shown that root exudates can mobilize previously Fe- and Al-associated C, enhancing its bioavailability and so causing C loss through microbial mineralization (Keiluweit et al., 2015). Similarly, Rasmussen and co-workers (Rasmussen et al., 2007; Finley et al., 2018) found that C loss in response to the addition of root exudates was most pronounced in soils with the greatest contents of poorly crystalline phases, suggesting that MOAs consisting of poorly crystalline Fe and Al are the most susceptible to root impacts. The fact that root-induced disruption of poorly crystalline Fe and Al phases in later weathering stages (terrace 3-5) coincided with a decline in C concentrations and increase in Δ¹⁴C (Fig. 2) suggests that older,
microbially-derived C previously-protected in such MOAs (terrace 2) was lost to microbial decomposition and/or leaching.

Although the amount of C stored in association with poorly crystalline phases diminished in late weathering stages, the fraction of C associated with crystalline Al and Fe phases remained relatively constant (Fig. 3b). At the same time, highly disordered np-goethite was gradually replaced by more ordered phases (Fig. 4b). Because the increasing order in np-goethite phases suggests a lower degree of substitution, less C may be protected within these structures. This notion is confirmed by our STXM imaging results, which show larger Al- and Fe-rich particles with only trace amounts of C (Fig. 6f-g). These findings demonstrate that MOAs dominating the later weathering stages consist of discrete, crystalline Fe and Al particles with surface coatings of organic compounds, similar to those reported by Chorover and Chadwick (2001) in highly weathered soils. Combined, our results suggest that as continued weathering disrupts highly protective MOAs between C and poorly crystalline Fe and Al phases, less protective MOAs between C and more stable, crystalline phases are left behind.

4.4. Evolution of mineral-organic associations in non-rhizosphere soil

In contrast to the dramatic change in nature of MOAs in rhizosphere soil, MOAs in non-rhizosphere soil showed no or only gradual transformations and, consequently, no C accumulation. In fact, the amount of C associated with poorly crystalline phases, which was significantly lower than that in rhizosphere soil, steadily decreased with soil age (Fig. 3a). The decrease in C associated with poorly crystalline phases was accompanied by a gradual decline in C concentrations (Fig. 2). These findings suggest that root activity and associated weathering is
necessary to form poorly crystalline minerals and supply C compounds for the formation of MOAs, which could provide a mechanism for C accumulation in deep soils. In the absence of roots, as observed in the non-rhizosphere soil here, weathering driven by surface DOC alone (e.g., Kramer et al., 2012) does not seem to generate significant amounts of protective MOAs and, thus, fails to accumulate C at the same rate.

Compared to rhizosphere soil, a significantly larger fraction of C in the non-rhizosphere was associated with crystalline Fe and Al phases (Fig. 2). Nevertheless, the total amount of C associated with these phases is low, consistent with the low degree of micron-scale association between Fe and C at the sub-micron scale as evidenced by STXM (Fig. 6h-i). Carbon, if present, was found in association with large discrete Fe and Al particles across the chronosequence (Fig. 6e-g). These findings imply that Fe and Al phases with greater crystallinity might have lower C loadings and, consequently, have a lower capacity to protect MOA from microbial decomposition. Overall, our results suggest that non-rhizosphere soils store less C than rhizosphere soils due to the lack of poorly crystalline phases and the relative dominance of highly crystalline Fe and Al phases with low C loadings.

4.5. Rhizogenic weathering generates and disrupts protective mineral-organic associations

Our comparison of rhizosphere and non-rhizosphere soil demonstrate that roots are important agents of formation, transformation, and disruption of MOAs over pedogenic time scales. The evolution of MOAs under the influence of rhizogenic weathering at our site is conceptualized in Fig. 7 and summarized as follows: Stage I represents the initial weathering stage prior to root colonization of deep soils. In the absence of root weathering of primary minerals,
accumulation of C in association with poorly crystalline Fe and Al phases is slow. In Stage II, root colonization triggers intense rhizogenic weathering of primary minerals (feldspar and quartz), either directly through root exudates (Hinsinger and Courchesne, 2008; Richter et al., 2007; Bonneville et al., 2011) or indirectly through changes in the geochemical regime (e.g., pH or Eh) (Fimmen et al., 2008; Schulz et al., 2016). Rhizogenic weathering accelerates formation of MOAs between poorly crystalline Fe and Al phases with microbially-derived organic matter, thereby driving C accumulation in the newly formed rhizosphere. In Stage III and IV, continual rhizogenic weathering disrupts associations between organic matter and poorly crystalline phases, leaving behind chemically more stable, yet less protective MOAs consisting of more crystalline phases. This change in the nature and reactivity of MOAs is paralleled by C loss during later weathering stages. The dynamics in adjacent soils not directly affected by rhizogenic weathering (non-rhizosphere soil) were distinctly different, showing no significant generation of MOAs consisting of poorly crystalline phases. Instead, the amount of C associated with crystalline mineral phases gradually declined, corresponding to a loss of C over time.

The formation of MOAs between poorly crystalline Fe and Al phases in early weathering stages and disruption of such MOAs during later weathering stages in rhizosphere soil is consistent with observations in other chronosequence studies (Torn et al., 1997; Mikutta et al., 2009; Chorover et al., 2004). In these studies, the abundance of poorly crystalline Fe and Al phases across soils of different weathering stages was directly related to the amount of mineral-associated C, overall C stocks and turnover rate. However, because these studies focused on relatively shallow and/or well-homogenized bulk soils, the effect of rhizogenic weathering could not be isolated. Since the general temporal pattern of initial MOA formation followed by disruption with
increasing soil weathering observed in other chronosequences match those observed in the rhizosphere soil examined here, but not that of our non-rhizosphere soil, we suggest that root activity is a principal, yet largely overlooked driver of MOA dynamics during soil formation.

4.6. Predicting the vulnerability of deep soil carbon to rhizogenic weathering impacts

Anthropogenic and environmental drivers such as increasing atmospheric CO$_2$ levels (Kuzyakov 2011), land use change (Richter et al. 1999), nutrient depletion (Husson, 2013), or drought (Schenk, 2008) are thought to increase root growth into deeper soil horizons. But whether enhanced root activity in deep soil will enhance C storage at depth or deplete old C stored in MOAs remains a topic of debate (Richter et al., 1999; Fontaine et al., 2007). Our results suggest that the impact of root growth in deep soils will depend on the developmental stage and mineralogical composition of the soil. Here we find that enhanced root activity in relatively young soils may lead to C accrual through formation of new MOAs. Conversely, increased root colonization of older, more weathered soil may disrupt existing MOAs and cause the loss of C protected therein. These insights may therefore be useful in distinguishing soils that lend themselves to root-promoted C storage at depth and those that would be vulnerable to root-induced soil C loss.

Although roots are now widely recognized as the predominant source of soil C in many ecosystems particularly important in deep soils (Rumpel and Kögel-Knabner, 2011), and their physical traits are increasingly relied upon to predict soil C storage (Poirier et al., 2018), the effect of root-induced weathering has largely been overlooked. Current models reflect roots as passive conduits of C and nutrients (Dwivedi et al., 2017). While models have begun incorporating root effects on microbial activity (e.g. priming effects) (Sulman et al., 2014; Dwivedi et al., 2017), the
direct impacts of rhizogenic weathering reactions on MOAs formation (Yu et al., 2017) and disruption (Keiluweit et al., 2015; Clarholm et al., 2015) are not currently considered. Our results show that the ability of roots to create new mineral phases that protect organic matter and increase soil C storage capacity, as well as disrupt C currently protected in MOAs leading to C loss, may warrant model incorporation. Recent approaches using reactive transport models that begin to prioritize the incorporation of rhizogenic weathering reactions hold great promise (Lawrence et al., 2014; Li et al., 2017).
CHAPTER 5
CONCLUSION

Here we investigated root-driven transformations of MOAs and their impact on soil C storage over pedogenic time scales across the Santa Cruz soil chronosequence. By comparing changes in rhizosphere and non-rhizosphere soils over time, we found that periods of C accrual and loss are directly linked to root-driven weathering formation and disruption of MOAs. Initial root-driven weathering (65-90ka) caused a notable increase in the amount of C associated with poorly crystalline Fe and Al phases, which coincided with the accumulation of C, particularly microbially-derived compounds. The strong relationship between C accumulation and the formation of poorly crystalline Fe and Al phases is likely due to the concurrent formation of highly disordered, Al- and C- substituted nano-goethite. However, continued rhizogenic weathering (137-226ka) led to a decline in both the abundance of poorly crystalline phases and the amount of associated C. The decrease in abundance of poorly crystalline phases, such as highly disordered nano-goethite, was accompanied by a gradual loss of C and increasing contributions of plant-derived compounds. The observed dynamics of MOAs in rhizosphere soil with increasing soil age reflects those observed in other chronosequence studies conducted in bulk soil, where the formation of poorly crystalline Fe and Al phases during initial or intermediate weathering stages coincided with greater C accumulation and residence times. Since we did not observe these dynamics in soil unaffected by roots, which showed significantly and consistently lower amounts of poorly crystalline phases, we conclude that root-driven formation and disruption of MOAs are a primary control on soil C accrual and loss over pedogenic time scales. Our results indicate that the effects of increasing root growth - resulting from climate or land use change - on the net soil C balance in deep soils may depend on soil weathering stage. The ability of roots to create new
reactive mineral phases and increase the capacity of soil C storage in MOAs may be greater in young soils, while in old, highly weathered soils, root activity may destabilize C currently protected in MOAs. Recognizing that roots are important agents of both formation and disruption of MOAs is an important next step towards more complete conceptual and numerical models for predicting the vulnerability of deep soil C to environmental change.
APPENDIX
TABLES & FIGURES

TABLE 1: Changes in total extractable organic C and metals, and clay mineral composition as a function of soil age in rhizosphere and non-rhizosphere soil.

<table>
<thead>
<tr>
<th>Terrace</th>
<th>Age (kyr)</th>
<th>Zone</th>
<th>Org. C_{tot} &amp;</th>
<th>Fe_{tot} &amp;</th>
<th>Al_{tot} &amp;</th>
<th>Si_{tot} &amp;</th>
<th>Clay %</th>
<th>Clay Mineral Composition</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>µmol g^{-1}</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>65</td>
<td>Bulk</td>
<td>59 (5)</td>
<td>9.3 (0.4)</td>
<td>26 (1)</td>
<td>19 (1)</td>
<td>29</td>
<td>k, b, sa, i</td>
</tr>
<tr>
<td>2</td>
<td>90</td>
<td>Rhizosphere</td>
<td>94 (9)</td>
<td>12.1 (0.7)</td>
<td>101 (6)</td>
<td>57 (5)</td>
<td>63</td>
<td>H, k, s, b, c, m</td>
</tr>
<tr>
<td>Non-rhizosphere</td>
<td>44 (6)</td>
<td>10.2 (0.9)</td>
<td>23 (1)</td>
<td>25 (4)</td>
<td>34</td>
<td>H, k, s, b, c, m</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>137</td>
<td>Rhizosphere</td>
<td>72 (11)</td>
<td>10.6 (1.1)</td>
<td>110 (10)</td>
<td>51 (8)</td>
<td>76</td>
<td>H, k, s, b, c, m</td>
</tr>
<tr>
<td>Non-rhizosphere</td>
<td>38 (7)</td>
<td>14.9 (1.0)</td>
<td>25 (1)</td>
<td>21 (2)</td>
<td>23</td>
<td>h, k, s, b, c, m</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>226</td>
<td>Rhizosphere</td>
<td>66 (12)</td>
<td>9.1 (1.4)</td>
<td>97 (10)</td>
<td>37 (7)</td>
<td>74</td>
<td>H, k, s</td>
</tr>
<tr>
<td>Non-rhizosphere</td>
<td>28 (7)</td>
<td>12.1 (1.3)</td>
<td>33 (2)</td>
<td>28 (2)</td>
<td>73</td>
<td>H, k, s, b, c, m</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* H: Halloysite, K: Kaolinite (disordered), S: Smectite (Ca-Kinney montmorillonite), B: biotite (1M), C: chlorite (Fe-rich;Tusc), M: muscovite(2M1) alternative, Sa: saponite, I: illite (1Md+dioctmica & smectite). Capital letters > 15% abundance, lowercase letters indicate < 15% abundance.

& Sum of H_{2}O, pyrophosphate, hydroxylamine, and dithionite extractable organic C and metals. Numbers in parentheses indicate the standard error of the mean of three replicate samples.
TABLE 2: Modeled Mössbauer spectral parameters of iron oxides and clays as a function of soil age in rhizosphere and non-rhizosphere soil

<p>| Sample*+ | Split | Phase† | $&lt;CS&gt;$ | $&lt;\Delta&gt;$ | $\sigma^4$ | $&lt;\varepsilon&gt;$ | $&lt;|H|&gt;$ | $\sigma_{HF}$ | phase% | $\chi^{2,9}$ |
|----------|-------|--------|--------|---------|---------|---------|--------|---------|--------|-------------|
| Terrace 1+ | Bulk | Fe(III)-clay | 0.5 | 0.66 | 0.27 | — | — | 54 | 1 | 27 | 1.5 |
| | | Fe(II)-clay | 1.3 | 2.9 | 0.5 | — | — | 42.2 | 0.8 | 7 | — |
| | | np/Al-hematite | — | — | 0.37 | -0.2 | 49.2 | 1.5 | 28 | — |
| | | Lepidocrocite | — | — | 0.49 | -0.12 | 49.2 | 1.5 | 28 | — |
| | | Fe(III) | — | — | 0.4 | -0.1 | 6.7 | 5 | 29 | — |
| Terrace 2 | Non-rhizosphere* | Fe(III)-clay | 0.47 | 0.69 | 0.37 | na | — | 48.7 | 0.7 | 36 | — |
| | | np/Al-goethite-1 | 0.49 | — | — | -0.12 | 46.9 | 1.3 | 34 | — |
| | | np/Al-goethite-2 | 0.5 | — | — | -0.14 | 46.9 | 1.3 | 34 | — |
| | | np/Al-goethite-3 | 0.46 | — | — | -0.16 | 43.2 | 3.8 | 18 | — |
| Rhizosphere+ | | Fe(III)-clay | 0.5 | 0.77 | 0.53 | — | — | 54.3 | 1 | 6 | — |
| | | np/Al-hematite | 0.45 | — | — | -0.1 | 49.1 | 1.85 | 16 | — |
| | | np/Al-goethite-1&amp;2 | 0.45 | — | — | -0.12 | 49.1 | 1.85 | 16 | — |
| | | np/Al-goethite-4 | 0.4 | — | — | -0.1 | 21.2 | 16 | 30 | — |
| Terrace 3 | Non-rhizosphere* | Fe(III)-clay | 0.47 | 0.78 | 0.38 | — | — | 48.3 | 0.7 | 28 | — |
| | | np/Al-goethite-1 | 0.49 | — | — | -0.12 | 46.4 | 1.5 | 31 | — |
| | | np/Al-goethite-2 | 0.5 | — | — | -0.13 | 46.4 | 1.5 | 31 | — |
| | | np/Al-goethite-3 | 0.47 | — | — | -0.15 | 44 | 4.5 | 34 | — |
| Rhizosphere+ | | Fe(III)-clay | 0.49 | 0.6 | 0.3 | — | — | 55.2 | 1 | 9 | — |
| | | Fe(II)-clay | 1.2 | 3 | 0.3 | — | — | 49.3 | 1.5 | 20 | — |
| | | np/Al-hematite | 0.47 | — | — | -0.03 | 55.2 | 1 | 9 | — |
| | | np/Al-goethite-1&amp;2 | 0.5 | — | — | -0.11 | 49.3 | 1.5 | 20 | — |
| | | np/Al-goethite-4 | 0.4 | — | — | -0.12 | 23.6 | 17.8 | 30 | — |</p>
<table>
<thead>
<tr>
<th>Terrace 5</th>
<th>Non-rhizosphere*</th>
<th>Fe(III)-clay</th>
<th>0.46</th>
<th>0.77</th>
<th>0.54</th>
<th>__</th>
<th>__</th>
<th>__</th>
<th>11</th>
<th>3.4</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>np/Al-goethite-1</td>
<td></td>
<td>0.45</td>
<td>___</td>
<td>___</td>
<td>-0.08</td>
<td>48.2</td>
<td>1.3</td>
<td>29</td>
<td>___</td>
</tr>
<tr>
<td></td>
<td>np/Al-goethite-2</td>
<td></td>
<td>0.56</td>
<td>___</td>
<td>___</td>
<td>-0.2</td>
<td>47.6</td>
<td>2.11</td>
<td>31</td>
<td>___</td>
</tr>
<tr>
<td></td>
<td>np/Al-goethite-3</td>
<td></td>
<td>0.42</td>
<td>___</td>
<td>___</td>
<td>-0.1</td>
<td>45.2</td>
<td>4.5</td>
<td>29</td>
<td>___</td>
</tr>
<tr>
<td>Rhizosphere+</td>
<td>Fe(III)-clay</td>
<td></td>
<td>0.5</td>
<td>0.54</td>
<td>0.27</td>
<td>__</td>
<td>__</td>
<td>__</td>
<td>45</td>
<td>2.1</td>
</tr>
<tr>
<td></td>
<td>Fe(II)-clay</td>
<td></td>
<td>1.2</td>
<td>3</td>
<td>0.3</td>
<td>__</td>
<td>__</td>
<td>__</td>
<td>2.5</td>
<td>___</td>
</tr>
<tr>
<td></td>
<td>np/Al-hematite</td>
<td></td>
<td>0.4</td>
<td>___</td>
<td>___</td>
<td>-0.02</td>
<td>55.5</td>
<td>1</td>
<td>5</td>
<td>___</td>
</tr>
<tr>
<td></td>
<td>np/Al-goethite-1&amp;2</td>
<td></td>
<td>0.49</td>
<td>___</td>
<td>___</td>
<td>-0.13</td>
<td>49.4</td>
<td>1.6</td>
<td>22</td>
<td>___</td>
</tr>
<tr>
<td></td>
<td>np/Al-goethite-4</td>
<td></td>
<td>0.4</td>
<td>___</td>
<td>___</td>
<td>-0.12</td>
<td>16.8</td>
<td>12.7</td>
<td>26</td>
<td>___</td>
</tr>
</tbody>
</table>

Spectra obtained at 77K; spectra generated at 8 K; Spectral component, 2center shift, 3quadrupole splitting, 4std. dev. of Δ, 5quadrupole shift parameter, 6absolute average HF, 7std. deviation of hyperfine field distribution (HFD), 8relative percent of Fe in different phases, 9goodness of fit, Lorentzian half widths at half maximum (HWHM) of all elemental doublets is fixed at 0.14 mm/sec; No coupling was allowed between CS and HFD; the A+/A- areas of the doublets is fixed at 1; No coupling is allowed between HFD and HF, and A1/A3 and A2/A3 areas are fixed at 3 and 2, respectively; 10not applicable; Modeling: Voight-based structural fitting routine (Rancourt and Ping, 1991); 11np = nano-particle
TABLE 3: Mineralogical changes as a function of soil age in rhizosphere (R) and non-rhizosphere (NR) soil based on fitting of X-ray diffractograms

<table>
<thead>
<tr>
<th>Terrace</th>
<th>Age</th>
<th>Zone</th>
<th>Mineral</th>
<th>Bulk</th>
<th>NR</th>
<th>R</th>
<th>NR</th>
<th>R</th>
<th>NR</th>
<th>R</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>65</td>
<td>2</td>
<td>Quartz</td>
<td>35</td>
<td>48</td>
<td>26</td>
<td>57</td>
<td>19</td>
<td>22</td>
<td>21</td>
</tr>
<tr>
<td></td>
<td>90</td>
<td>3</td>
<td>Kspar</td>
<td>6</td>
<td>58</td>
<td>32</td>
<td>64</td>
<td>22</td>
<td>25</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td>137</td>
<td>5</td>
<td>Plagioclase</td>
<td>31</td>
<td>5</td>
<td>3</td>
<td>6</td>
<td>2</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>226</td>
<td>2</td>
<td>Goethite</td>
<td>0</td>
<td>3</td>
<td>1</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Ferrihydrite</td>
<td>n.d.</td>
<td>0</td>
<td>1</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Total other (non-phyllosilicates)</td>
<td>71</td>
<td>66</td>
<td>37</td>
<td>77</td>
<td>24</td>
<td>26</td>
<td>26</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Halloysite</td>
<td>n.d.</td>
<td>18</td>
<td>41</td>
<td>15</td>
<td>60</td>
<td>52</td>
<td>55</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Kaolinite (disordered)</td>
<td>7</td>
<td>1</td>
<td>4</td>
<td>0</td>
<td>2</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Smectite (Ca-Kinney montmorillonite)</td>
<td>n.d.</td>
<td>12</td>
<td>12</td>
<td>5</td>
<td>12</td>
<td>11</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Biotite (1M)</td>
<td>3</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Chlorite (Fe-rich; Tusc)</td>
<td>n.d.</td>
<td>2</td>
<td>5</td>
<td>3</td>
<td>2</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Muscovite (2M1) alternative</td>
<td>n.d.</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Total phyllosilicates</td>
<td>29</td>
<td>34</td>
<td>63</td>
<td>23</td>
<td>76</td>
<td>73</td>
<td>74</td>
</tr>
</tbody>
</table>

* Weight percent of mineral phases determined using RockJock (Eberl, 2003)
NR = non-rhizosphere
R = rhizosphere
FIGURE 1: Study site is Santa Cruz Terrace Chronosequence, photo credits to Marjorie Schulz (Schulz et al., 2016). There are five terraces ages 65kyr – 226kyr with distinct root weathering profiles.
FIGURE 2: Changes in C concentration and $\Delta^{14}$C values in rhizosphere and non-rhizosphere soil across the chronosequence. Bars represent standard error of the mean of three replicate samples.
FIGURE 3: Changes in extractable Fe and Al for (a) poorly crystalline and (b) crystalline phases, as well as C associated with each phase, in non-rhizosphere and rhizosphere soil across the chronosequence. Poorly crystalline phases are presented as the sum of metals and C extracted by water, pyrophosphate and hydroxylamine, crystalline phases are the sum of metals and C released by dithionite. Bars represent standard error of the mean of three replicate samples.
FIGURE 4: Changes in abundance of Fe oxides of varying degree of disorder in (a) non-rhizosphere and (b) rhizosphere soil across the chronosequence as determined by Mössbauer spectroscopy. Note np = nano-particle
FIGURE 5: Changes in abundance of different organic compound classes in (a) non-rhizosphere and (b) rhizosphere soil across the chronosequence as determined by FT-ICR-MS. Classes given in darker colors (tannins, lignin, and carbohydrates) are attributed to plant-derived compounds, those given in lighter colors compounds (amino sugars, proteins, lipids, and unsaturated hydrocarbons) are assigned to microbially-derived.
FIGURE 6: Changes in the micro-scale distribution of C in relation to Fe and Al phases in rhizosphere and non-rhizosphere soil across the chronosequence. To visualize the spatial distribution of C, Fe and Al, overlay maps (Red=Fe, Green=Al, Blue=C) for non-rhizosphere (b-d) and rhizosphere soil (e-g) at each terrace are presented. Scale bars are 5 µm except for non-rhizosphere terrace 2 which is 2 µm. Terrace 1 (a) has no rhizosphere differentiation, so only a map for bulk soil is shown. To quantify spatial relationships between Fe and C, we calculated Spearman’s rank correlation coefficient for three replicate maps per sample. Changes in the correlation coefficient describing the association of C and Fe are shown in panel (h). To further assess the degree of association between C and Fe, a co-localization analysis was performed (see methods for details) on three replicate images. Changes in the percentage of C associated with Fe across the chronosequence are shown in panel (i). For each analysis, bars represent standard error of the mean of three replicate images.
FIGURE 7: Conceptual diagram denoting that the formation of poorly crystalline phases and therefore commonly noted patterns in pedogenesis are due to root weathering. Root weathering of parent material leads to mineral-organic association formation and the increase in total organic carbon found in soils.

a) Rhizogenic weathering

b) Mineral-organic associations
FIGURE 8: Mössbauer spectra as a function of soil age in rhizosphere and non-rhizosphere soil. Variable temperature Mossbauer spectra of terrace 2, non-rhizosphere (a-d) and rhizosphere samples (e-h).
FIGURE 9: Differences in the presence and absence of Al-goethite nano-particles (np) in Mössbauer spectra at terrace 2 as a function of rhizosphere and non-rhizosphere soil. (a-c) 77 K Mossbauer spectra of non-rhizosphere samples from depths of 100, 110, and 140 cm respectively, showing precipitation of bulk soil Fe as np-goethite’s of varying particle-sizes/Al-contents (particle sizes: type 1 > type 2 > type 3; Al-contents type 1 < type 2 < type 3) and minor amounts of Fe as Fe (III) in clay lattices. (d-g) 8 K Mossbauer spectra of terrace 1 bulk soil and rhizosphere soil samples from depths of 100, 110, and 140 cm respectively showing somewhat equal distribution of soil Fe between clay minerals and np-goethite’s: types 2, 3, and 4 (type 4 is unique to rhizosphere soil samples).
BIBLIOGRAPHY


55