

**TRANSPORT OF SOIL COLLOIDS
AND ITS RELATION TO BIOGEOCHEMICAL CYCLING OF ORGANIC CARBON
UNDER DYNAMIC REDOX CONDITIONS**

by

Weila Li

A thesis submitted to the Faculty of the University of Delaware in partial fulfillment
of the requirements for the degree of Master of Applied Sciences

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January, 2019

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ABSTRACT

Release and transport of organic carbon (OC), a large portion of which can complex with mineral colloids, often leads to long-term C sequestration. Despite colloids' potential importance, the role of mobile colloids in carbon cycling is not well understood. In this study, we conducted experiments using syringe columns with objectives to understand 1) the dynamic formation and disassociation processes of mineral-organic associations (MOAs) and transport of OC in relation to soil colloid mobility, 2) the coupled physical and biogeochemical processes of soil OC under dynamic redox conditions.

Regression analyses of chemical properties (pH, Eh, turbidity, ionic strength) for leachate solutions as functions of released water-dispersible colloids were all linear, indicating clear correlations between colloid release with aerobic and anaerobic conditions. Mobilized C and colloids in leachates were quantified by fractionating samples into two particle-size classes (450-1000 nm, 100-450 nm). Under all redox conditions, we found that a larger amount of OC was contained in the 100-450nm size fraction, and a decreased redox potential resulted in an increasing release of this size fraction. In addition, soils in the columns were fractionated into three particle sizes: 450-1000 nm, 100-450 nm, and 2.3-100 nm, and we found that the 2.3-100 nm fraction accumulated the highest C concentration. Moreover, analyses of C and N stocks and their natural abundance of $\delta^{13}\text{C}$, $\delta^{15}\text{N}$ in different sized aggregates indicated that, as redox potential decreased, the most significant change occurred in the 100-450 nm fraction. Overall, expending of oxygen in soil system enhances the release of colloids, which in turn facilitates C turnover in soil. The 100-450 nm colloidal fraction is the most important size fraction as the potential exchangeable or

biogeochemical-reactive fraction due to its high loading capacity of organic carbon and its lability under dynamic redox conditions.

Chapter 1

INTRODUCTION AND BACKGROUND

1.1 Soil Organic Matter (SOM), the Major Carbon Pool on Land

Over the past 150 years, the amount of carbon in the atmosphere, derived from greenhouse gases such as CO₂, CH₄, and N₂O, has increased by 30%. This increased level of particularly CO₂ is extensively associated with global warming. Carbon sequestration refers to a long-term storage of C in oceans, soils, vegetation (especially forests), and geologic formations (Sparks, 2011). Soils contain about 75% of the C (in SOM) pool on land, three times more than stored in the atmosphere, or living plants and animals. Similar to the inorganic components of soil, SOM plays a significant role in affecting the chemistry of the soil. SOM has a strong variability and is usually complexed with clay minerals and metal oxides. Moreover, this major pool of OC is sensitive to changes in climate or local environments. According to Schmidt's perspectives, the persistence of organic matter in soil is largely due to complex interactions between organic matter and its environment, such as the interdependence of compound chemistry, reactive mineral surfaces, climate, water availability, soil acidity, soil redox state and the presence of potential degraders in the immediate microenvironment. Among the different factors, soil microorganisms influence SOM cycling not only via decomposition but also because microbial products like biomass or biofuel are important components of SOM (Kogel-Knabner, 2002).

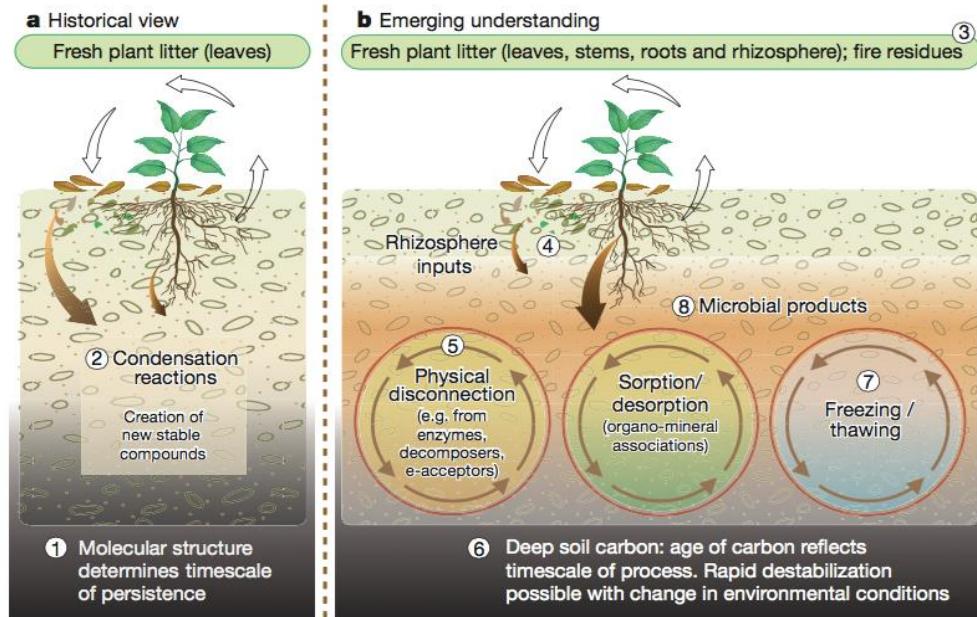


Figure 1.1 A synopsis of the eight insights of soil carbon cycling. (Adapted from Schmidt et al., 2011)

Soil organic matter consists of non-humic and humic substances. Non-humic compounds are mostly derived from undecayed plant and animal tissues and known to be attacked easily by soil microorganisms and persist in the soil only for a brief time while humic substances (HS) are reported to persist in soil for much longer. HS (always in colloid fraction) can be subdivided into humic acid (HA), fulvic acid (FA), and humin, which refer to different stages of SOM decomposition. Sorption of HS to soil clay minerals through adsorption and co-precipitation is a dominant mechanism to stabilize dissolved organic matter and avoid its release to ambient environments, and therefore the stability of mineral-

carbon association determines the quantity and turnover rate of soil organic carbon (Torn et al., 1997).

1.2 Colloids and their Potential Role in Natural Ecosystems

1.2.1 Definition and the Significance of Soil Colloids

Soil colloids, containing the largest amount of SOM, are ~1 nm to 1 μm in size. Because of their small size and large specific surface area, and hence high reactivity and the ability to facilitate the transport of contaminants in subsurface environments, the mobilization, and transport of soil colloids have attracted much research attention (Baalousha et al., 2011; Kretzschmar et al., 1999; Lead and Wilkinson, 2007). In natural ecosystems, colloids are a complex mixture of clay minerals, metal-oxides and oxyhydroxides, and organic substances (Baalousha et al., 2011; Buffle and Leppard, 1995).

1.2.2 The Importance of Colloid Size and the Size Distribution of Natural Colloids

Based on the size-based definition, colloids differ from aqueous solution, bulk soil, and larger particles. Hence, colloidal particles form a critical transition zone between dissolved and particulate phases. The size range and chemical compositions of important colloidal and particulate species are summarized in Figure 1.2 (Docter, D et al, 2015). This size-based physical definition of colloids has been countered with a chemical definition by Gustafsson and Gschwend (1997). They proposed that colloids are constitutes possessing two main properties: (1) they offer molecular milieu into or onto which chemicals can escape from the aqueous solution and (2) its stabilization is dominated by coagulation or aggregation instead of gravitational sedimentation. However, both the physical and

chemical definitions of colloids reveal the important role of the colloidal phase in natural systems due to its special properties compared with the aqueous or particulate phases, for example, the large surface area and variable surface charges.

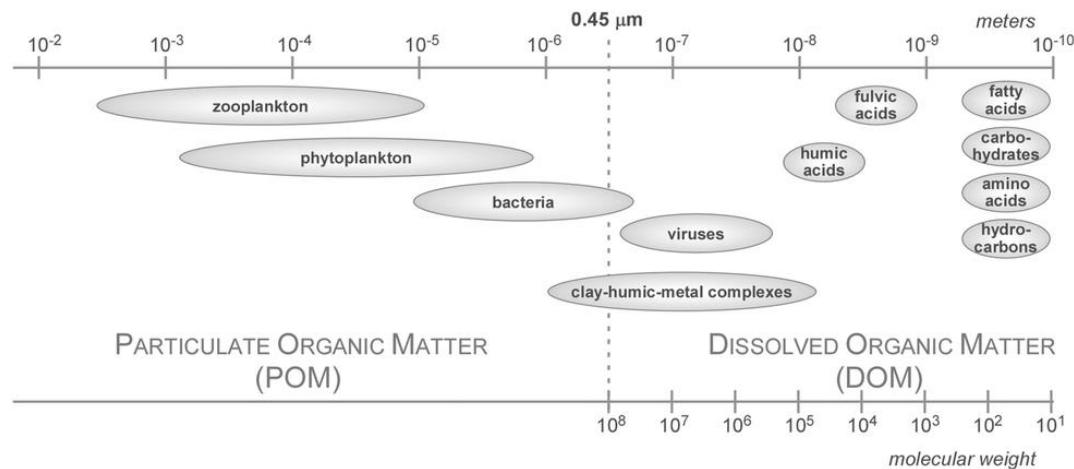


Figure 1.2 The size range of Particulate Organic Matter (POM) and Dissolved Organic Matter (DOM). DOM is defined through filtration. The size limit used to differentiate DOM from particulate organic matter is arbitrarily set to around 0.45 μm . (Adapted from Docter, D et al. 2015)

Natural colloids have been studied for their transport and association with micronutrients, trace metals, and OM in aquatic ecosystems (Buffle and Leppard, 1995). The accumulation and stabilization of natural colloids rely on their source and vary depending on the physical, chemical, and biological processes involved. As a consequence, the colloid size distribution is highly correlated with where colloids are generated, such as in soil pore water, riverine and coastal or marine systems, and how their mobility and stability are being influenced by biogeochemical parameters, such as pH, ionic strength, redox conditions, during their transport. Size separations show great promise in elucidating

the progression of organic matter decomposition and mineral association in soils. Aspects of organic particle and its chemical composition of carbon sources deserve more attention (Wagai and Kitayama, 2009).

1.2.3 Colloid Mobility and its Relation to Cycling of Soil Organic Carbon

Movement of soil organic carbon is induced by soil water flow, during formation and decomposition of mineral-organic matter-association (MOA), thus it plays a significant role in soil forming processes. The transport of soil organic matter facilitates cycling and distribution of soil nutrients, with uptakes by plant roots and soil microorganism species. Along with soil depth profile, the downwards transported soil organic carbon has been reported as a potential source of stabilized carbon occurring in the subsoil. Kaiser and Kalbitz, in 2012, presented a conceptual model of the vertical movement of dissolved organic matter with soil water, assuming the temporal immobilization (sorptive or by co-precipitation), followed by microbial processing, and re-release (by desorption or dissolution) into soil water of altered compounds. The proposed scheme (Figure. 1.3.) explains well the depth-dependent trends in age and composition of dissolved organic matter as well as of solid-phase organic matter in the soil.

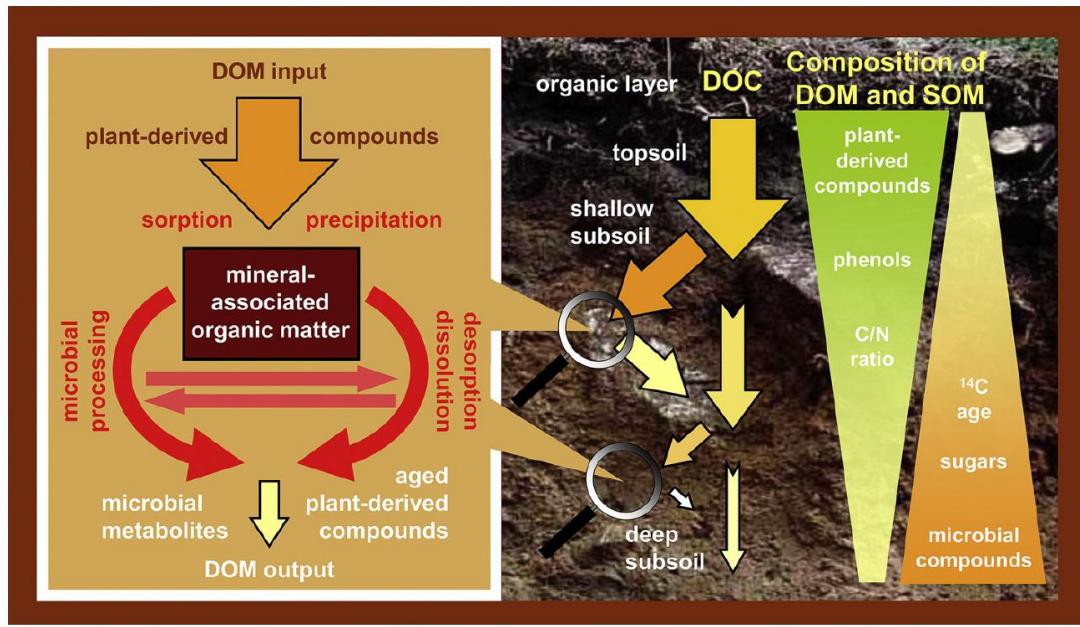


Figure 1.3 Cycling of dissolved organic matter (DOM) in soils. The processes as illustrated in the enlarged outtake on the left-hand side of the figure take place at throughout the soil entire profile. In consequence of continuous sorption and precipitation as well as of microbial processing, desorption and dissolution the proportions of more recent plant-derived compounds decrease with soil depth while those of microbial metabolites and aged/microbially processed plant-derived compounds will increase. Solid soil organic matter (SOM) changes accordingly. Depending on soil properties and hydrological conditions, part of the DOM portion will be transported along preferential flow paths, thus pass through soil without intimate contact with the soil matrix. (Adapted from Kaiser and Kalbitz, 2012)

The OM associated with colloids could be mobilized and potentially contribute to the carbon pool in surface water, freshwater catchments, and soil solutions (Guo and Santschi, 2007) and could be transported with both gravitational and preferential water flow. Subsoils store a vast amount of SOC and global C budgets would be underestimated without considering C storage in deeper soil depths (684–724 Pg C in upper 30 cm vs. 1462–1548 Pg C in upper 100 cm) (Batjes, 1996). The stabilization mechanisms of SOC

in deeper soils have received much attention due to its potential of acting like a C sink (Batjes, 1996; Jobbágy and Jackson, 2000; Chabbi et al., 2009). It is clear that the interactions and association between colloid and DOM demonstrated the role of colloids in carrying or retaining carbon, therefore, it is critical to investigate colloid release and mobilization in order to achieve a better understanding of its successive impact on carbon cycles in natural environments. Furthermore, whether colloidal organic carbon is mobile or immobile, labile or refractory largely determine the residence time and carbon turn-over rate during their transport through soil porous medium and aquatic water column.

1.3 Mobility and Stability of MOAs under Dynamic Redox Conditions.

1.3.1 Dynamic Redox Conditions in Natural Systems

Short-term ponding or long-time flooding conditions have the potential to influence the forming processes of wetland soil matrix through changing chemical conditions, especially redox conditions. It has been observed that the organic matter content in wetlands tends to be significantly higher than that found in surface waters (i.e., rivers, lakes, and marine systems) (Chin et al., 1998). Variations in the redox state of soil occur under low O₂ levels, which are typically important in waterlogged soils. Hence due to the fluctuation of water levels, redox oscillations occur seasonally (Figure. 1.4. Sharifi, 2017). On one hand, soil temperature and water table depth are responsible for seasonal variations in soil CO₂ loss. On the other hand, the vulnerability of wetland soils' carbon storage capacity must be considered as we develop management solutions to adapt to and mitigate the impacts of a changing climate (Steve McNulty, NCSU).

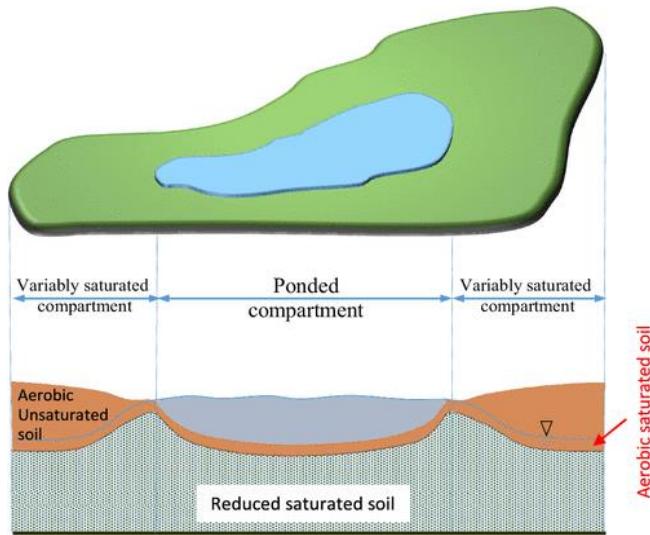


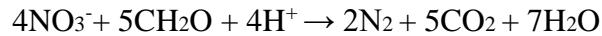
Figure 1.4 Schematics of a hypothetical seasonally flooded wetland. (Adapted from Sharifi, 2017)

When soils are flooded, not enough atmospheric oxygen can diffuse into soil pores, then aerobic microbial respiration will quickly consume the stock of O₂ in the saturated soil and a shift from aerobic to anaerobic respiration occurs (Rowell, 1981). In natural environments, microbes work as drivers of soil redox reaction and mostly mediate the electron transfer from OM, ammonium or other electron donors to electron acceptors. A sequential thermodynamic order of microbially induced reduction reactions (gain of electrons) in soils takes place as a result of O₂ being depleted from minerals or OM. Non-photosynthetic microorganisms in soil are sensitive to the dynamic surroundings and tend to rebuild an equilibrium by decomposing and oxidizing organic compounds back to inorganic forms in energy-yielding reactions. Different from aerobic soils, anaerobic soils provide little or no O₂ as electron acceptors but can provide alternative electron acceptors for microbes to produce energy, either organics leading to fermentation or inorganics

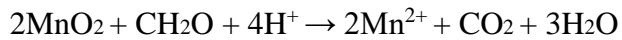
leading to anaerobic respiration (e.g. NO_3^- , manganese or ferric oxides, SO_4^{2-} and CO_2).

The sequential thermodynamic order predicts that after O_2 depletion, NO_3^- is reduced first and the reduction sequence usually then proceeds in the following order: Mn, Fe, S, and organic substrates (Ponnamperuma, 1972; Patrick and Jugsujinda, 1992),

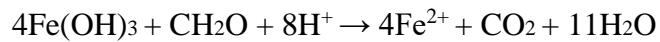
Denitrification:



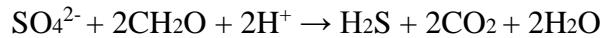
Manganese Reduction:



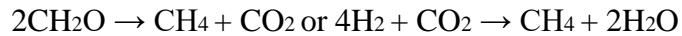
Iron Reduction:



Sulfate Reduction:



Methanogenesis:



The above sequences of microbially induced reduction reactions as a function of saturation time agree with the order of the decreasing change in free energy for the corresponding redox reactions, which are shown in Figure 1.5. The redox and associated chemical and physicochemical changes tend to influence soil structure and SOC storage.

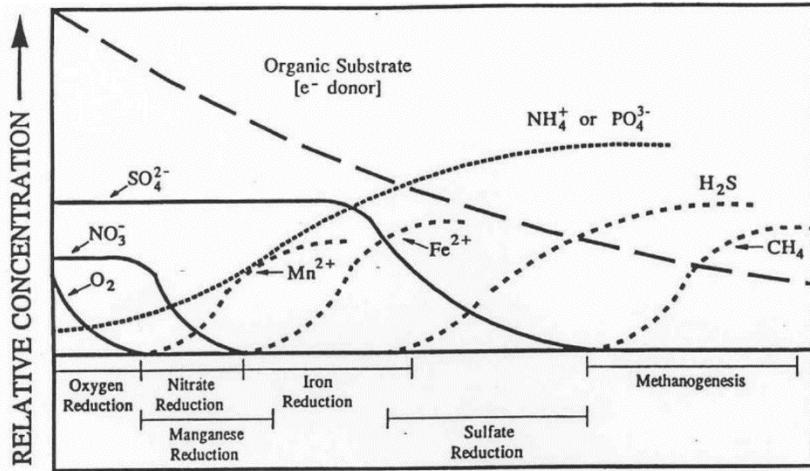


Figure 1.5 A sequence of reductions as time changes (adapted from Lorah et al., 2012)

The soil meta phenotype is also ultimately governed by the highly structured soil environment, resulting in a very heterogeneous availability of electron acceptors and redox chemistry, and both strong spatial and temporal variability (Janet K Jansson. 2018). A strong influence of the interactions between soil microbial species and their environment on the fate of soil C stabilization and sequestration has been emphasized by previous studies, as shown in the mineral-OM-microbes regime (Figure. 1.5). However, the details of these interactions under dynamic redox conditions are still unknown.

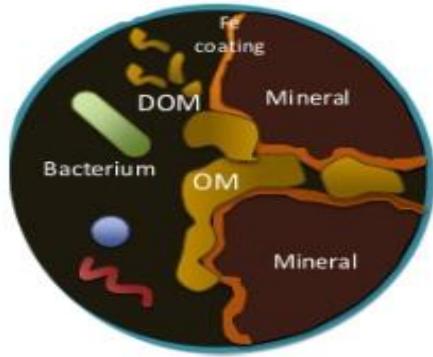


Figure 1.6 Formation of a mineral-OM-microbe regime

1.3.2 Colloid Mobilization under Dynamic Redox Conditions

Soil colloid mobilization and stabilization can be affected by the dynamic oscillation of redox conditions (Thompson et al., 2006). In redox-dynamic soils or sediments, colloid mobility is largely controlled by the processes of dissimilatory Fe^{3+} reduction, shifts in pH and ionic strength, and interactions with organic matter (Yan et al., 2016). Iron reduction is more thermal-dynamically available than the reduction reaction of other oxides. Natural OC forms stable bonds with iron oxides (e.g. ferrihydrite) mainly through ligand exchange (Eusterhues et al., 2014); this association with iron oxides has been well recognized as an important process for long-term C preservation (Kalbitz et al., 2000; Kaiser et al., 2005). Yan et al. (2016) also presented a conceptualization diagram shown in Figure. 1.7. of potential reactions involved in the mobilization and stabilization of colloids in both aerobic and anaerobic environments, which could be a useful guide for further studies. According to previous research results, the complex processes of colloid release tend to be affected by varied redox conditions and DOM concentration. In an

aerobic environment, released colloids can either be stabilized in soil suspension or immobilized by the formation of large aggregates over time, depending on the DOM concentration. At higher DOM concentrations, steric and/or electrostatic repulsion may dominate, whereas at lower concentrations of DOM surface charge neutralization occurs. In terms of an anaerobic environment where O₂ is depleted, dissimilatory microbial iron reduction predominantly leads to the initial release of colloids, due to changes in solution chemistry: the increase in IS and pH values suppress the electric double layer of colloids. On the other hand, released high-molecular-weight DOM induced by iron reduction reaction possibly bridges colloidal particles together through cation bridges and thus leads to a generation of new mineral-OM aggregates where water-dispersed colloids will be stabilized. To date, supporting evidence for all the proposed mechanisms are lacking because of the complex nature of the interactions involved, especially referring to coupled physical and biogeochemical processes.

Soil aggregates have recently been considered to be analogs of evolutionary incubators for soil microbial life. A concept for evolution in soil aggregates was presented as a new insight, suggesting that aggregates worked as a compartmentalized microbial incubator and this would lead to high levels of soil microbial diversity (Rillig et al., 2017). Therefore, it is of great significance to qualify and quantify the colloidal particles with constituents loaded. Through quantifying colloidal pools in natural systems, it was demonstrated that the < 0.45 µm fraction (Colloid_{<0.45}) provided a more accurate assessment in the mobility of colloid-associated-constituent, such as nutrients, contaminants, and trace elements.

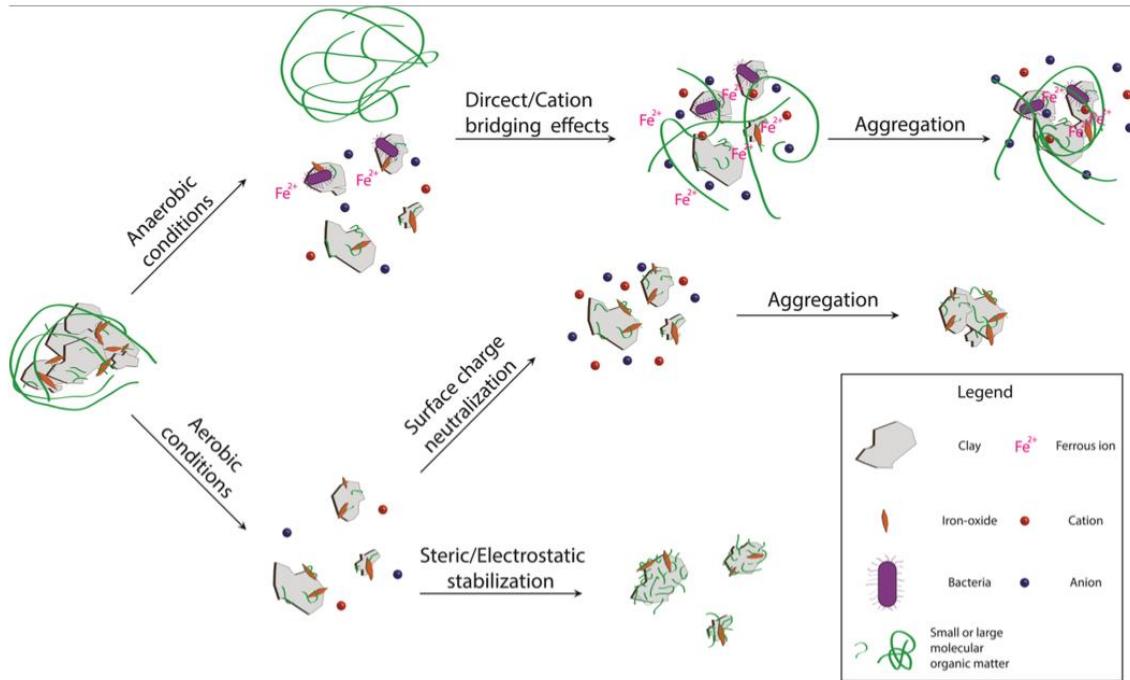


Figure 1.7 Scheme of colloid mobilization and stabilization in the batch system. A more complete conceptualization of potential processes involved in colloid release and stabilization in aerobic and anaerobic soil environments and can serve as a useful guide for additional studies to further elucidate the mechanisms. (adapted from Jing, 2016)

Chapter 2

RESEARCH RATIONALE AND OBJECTIVES

2.1 Research Rationale

Despite the importance of the colloidal phase in binding elements or contaminants in soil and aquatic systems (Baalousha et al., 2011; Kretzschmar et al., 1999; Lead and Wilkinson, 2007; Vold and Vold, 1983), most previous research has underestimated the role of mobile colloids in biogeochemical cycling of soil organic carbon, especially colloids with sizes $< 0.45 \mu\text{m}$. The inability to accurately fractionate colloidal phase from particulate and dissolved phases would hinder accurate assessment of the role of colloid-associated-carbon in biological functions and environmental fate. Therefore, more accurate quantification of soil colloidal pool and understanding its actual contribution to the mobilization and stabilization of colloid-associated-carbon is essential.

Anthropogenic perturbation has made a huge effect on global climate change. Wetland system is taken as a hot-spot ecosystem due to its more sensitive reaction to changing environments among other land types. Seasonally, the water table level of wetland soil varies depending on many environmental parameters such as rainfall and water flow across both surface and subsurface soil, which lead to soil redox oscillations.

The alteration of environmental conditions, especially redox conditions, can greatly impact the mobilization and release of colloids and organic matter into surroundings and

changes the fate and transport of colloid-associated-organic matter. Therefore, identification of the environmental conditions or pathways that could potentially serve as hotspots of soil colloids and colloidal organic carbon (COC) is critical. Recent studies have reported that the release of soil colloids and organic carbon can be induced by microbial-mediated iron reduction (Buettnner et al., 2014; Ryan and Gschwend, 1994a; Thompson et al., 2006), which occurs under redox-dynamic conditions, commonly found in wetland soils, sediments, and natural waters (Kappler and Straub, 2005; Thamdrup, 2000; Weber et al., 2006). The interesting interactions among colloid mobilization, organic carbon release, and varied redox conditions have attracted focused research attention in recent years. However, due to the complicated and heterogeneous nature of the soil system where multiple factors (e.g. mineralogy, topography, hydrology) and processes (e.g., physical and biochemical reactions), the interplays between mobile colloids, organic matter and the potential role that colloids play in carbon cycling under dynamic redox conditions remain poorly understood and thus require systematic investigation.

2.2 Research Objectives

The overall goals of this study are to understand the dynamic processes of mineral-organic associations (MOAs) formation and dissociation, as well as the relations between colloid release and biogeochemical processes of soil organic carbon under dynamic redox conditions. In order to achieve those goals, a set of syringe column experiments were conducted to:

- 1) quantify the release of size-fractionated soil colloids and colloidal organic carbon under stepwise changing redox conditions,

2) quantify colloids size distribution in the soil after drainage and characterize the size-dependent stable carbon and nitrogen isotope signatures in fractionated soils.

We hypothesized that a decrease in soil redox potential would increase the utilization of soil carbon and as well the disassociation of colloidal-OM aggregates. We also hypothesized that the interactions among microorganisms, OM and colloid-OM aggregates regulate the networks of biogeochemical reactions of organic carbon in response to varied redox conditions.

Chapter 3

MATERIALS AND METHODS

3.1 Soil Sample Collection and Preparation

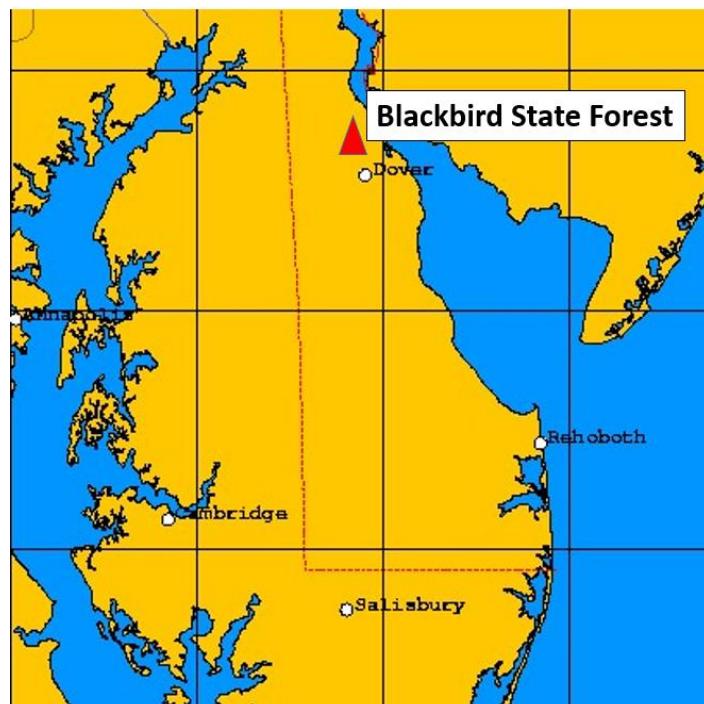


Figure 3.1 Location map of sampling site in this study

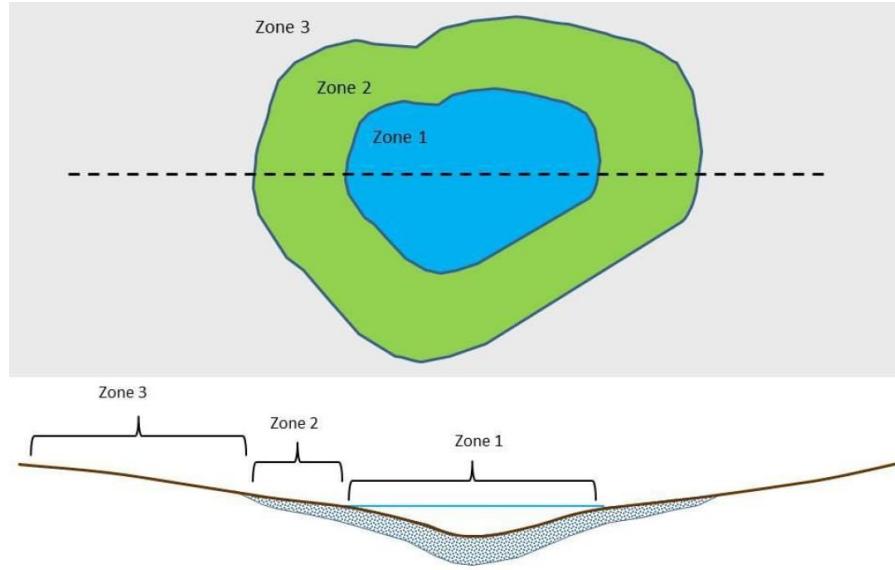


Figure 3.2 A schematic diagram showing the three hydrological zones and a cross section through a typical vernal pool wetland. Zone 1: Ponded zone, ponded water likely to recede below the surface during the summer months; Zone 2: Non-ponded wetland zone, containing hydric soils; Zone 3: Non-wetland(upland) zone.
 (adapted from Rabenhorst et al, 2017, SSSA)

Soil samples were collected from a freshwater wetland, which is located in Blackbird State Forest in New Castle County, DE ($39^{\circ}20' N$, $75^{\circ}40' W$) as part of the Blackbird Creek Watershed that drains into Blackbird Creek and flows into Delaware Bay ultimately; and it is known as a typical vernal pool. This site was selected because it is representative of a natural environment system under dynamic redox conditions, which can directly indicate the perturbation of human activity and climate change. Top-layer soil (0-15cm) was collected from zone 2 (Figure. 3. 2.), a wetland transitional zone marked by saturation, but not significant ponded. Soils were air-dried for 7 days after field sampling, and hand-sieved through 2-mm sieves to make it homogenous. Chemical elements were

measured using CNHS Elementar Cube: C/N ratio was 16.4:1, carbon concentration was 64.7 mg/g soil, nitrogen and sulfur concentration was 3.9 and 0.88 mg/g soil, respectively.

3.2 Syringe Column Experiment under Different Redox Conditions

Syringe column experiments were conducted to quantify soil particle size distribution and colloid transport under dynamic redox potentials, and to characterize the organic carbon in different size fractions under varies redox condition. The experiments consisted of five groups (60 ml Monoject™ Standard Syringe), with two replicates for each group (Shown in Figure 3.3). The syringe columns were packed with 40 g 2-mm sieved dry soil sample. And 40 ml degassed 2 mM-NaCl solutions were synchronously pumped into each column using a multichannel peristaltic pump with 1ml/min flow rate from the bottom until the soil was saturated (pore volume is shown in Table.1). Group A was leached at 0 day, and group B, C, D, E were leached at 9, 16, 22, 27 days respectively (dates selection was based on a preliminary experiment result). Eh value of each group is 333.5mV, 251.5mV, 225.5mV, 161.5mV, 100.5mV. Leaching solutions were measured for chemical properties immediately and stored in 4°C freezer for further characterization; soils were freeze-dried immediately for 2 days and then stored for size fractionation. All procedures were done in a glove bag to avoid being exposed to oxygen. All syringe columns were kept in a box opaque to light to minimize bacterial photosynthesis.

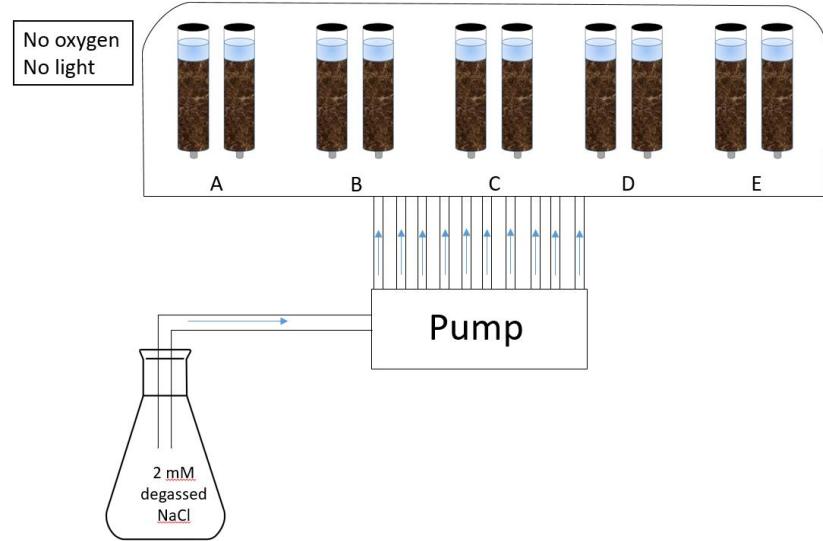


Fig 3.3 Schematic of syringe column experiment setup.

Table 3.1 Calculation of pore volume in syringe columns.

mass (g)	volume (cm ³)	bulk density (g/cm ³)	particle density (g/cm ³)	solid space (g/cm ³)	porosity	pore volume (cm ³)
40	45	0.888889	2.65	0.33543	0.66457	29.90566

3.3 Chemical Analysis of Leaching Solutions

3.3.1 Chemical Characterization

pH, Eh, electrical conductivity (EC) and turbidity of the leaching solution from each column were measured with corresponding probes (Fisher Scientific, Hampton, NH)

in a glove bag. Ionic strength (IS) in leaching solution was calculated from the measured EC values using the equation by Alva et al. and Griffin and Jurinak (Alva et al., 1991; Griffin and Jurinak, 1973; Morrisson et al., 1990):

$$IS = 0.013 \times EC$$

where EC is the solution electrical conductivity (ds/m).

Ferrous iron ($0.22\mu\text{m}$ -filtered samples) concentrations were determined using the ferrozine method (Stookey, 1970). Briefly, solution samples were adjusted the sample pH to less than 2 with concentrated hydrochloric acid (approximately 2 mL per liter) and preserved at room temperature. 1 mL of sample and 1 mL of 1M HCl were transferred to a 15mL tube and 3 mL of buffer reagent solution was added. The buffer reagent solution consisted of 0.1g of ferrozine and 1.192 g of HEPES per 100 mL of solution (Stookey, 1970). The absorbance was measured at 562 nm in 5 cm glass cuvettes by spectrophotometer. The spectrophotometer was calibrated at Fe concentration in the range of 0~0.3mM using ferrous ammonium sulfate standard solutions prepared in acidified water.

3.3.2 Release of Colloids During Leaching Process

The mobility of released colloid was quantified by comparing colloid mass concentrations. Concentration of $< 1\mu\text{m}$ colloids in 1ml solutions (after initial 3-hour settlement by gravity and 30-min centrifugation at 4900 g to separate and remove the supernatant containing $< 0.1\mu\text{m}$ particles, if any, and dissolved ions) was measured gravimetrically. The centrifugation speed, time and the size of the particle settled by the centrifugation were calculated based on the study of Gimbert et al. (2005). The collected

colloids in each microcentrifuge tube were freeze-dried (Labconco, Kansa City, MO) for 24 h until a constant weight was achieved and then quantified with a microbalance. Colloid concentration was reported in a unit of mg/L (dry weight / volume of suspension). Results of colloid concentrations coupled with turbidity curve are shown in the followings. And correlations between solution chemical properties (pH, Eh, Ionic strength (IS) and turbidity) and colloid concentration were analyzed using regression function.

3.3.3 Organic Carbon Quantification and Characterization

To determine and compare the DOC concentration under different redox conditions and in varied size fractions, total organic carbon concentration of <1000nm, <450nm, <100nm leaching solutions were measured using a TOC analyzer (Apollo 900HS) from group A to group E. Additionally to understand the OM degradation status variation in COC (<1000nm), DOC (<450nm) and truly dissolved phase (<100nm) versus the change of redox potential, aromaticity was characterized by UV absorbance. The absorption coefficient at 254nm (a_{254} in m^{-1}) was obtained following the procedure of Green and Blough (1994) and specific UV absorbance (SUVA in $L\ mgC^{-1}m^{-1}$) of OM was calculated as its specific adsorption at 254nm (m^{-1}) divided by the TOC concentration (mgC/L) (Inamdar et al., 2011). Both a_{254} and SUVA could be the index of OM aromaticity measurement.

3.4 Size Fractionation of Soil Samples under Different Redox Potentials

3.4.1 Sequential Size Fractionation of Soil Particles

After leaching, soil samples from the columns were collected and freeze dried for 2 days. Then 35g freeze-dried soil samples were utilized for size fractionation using

sequential physical processes of shaking, sedimentation centrifugation and ultrafiltration(Table 3.2). 350ml DI water was added to 35g soil with a 1:10 ratio of soil/solution, and the soil suspensions were shaken for 24 hours, then followed with 30 min sonication process. After that, the soil suspensions were static settled for 3 hours and then the supernatant was syphoned out for centrifugation process to fractionate soil particles. Centrifugate the supernatant for 1200 rpm(5.36min); 2300rpm(7.2min); 12000rpm(5.36min) to separate soil particles into three fractions: 450~1000nm, 100~450nm, and <100nm (calculation of centrifugation speed and time was based on Gimbert et al). All residuals were re-suspended with 10ml DI water, and dried within oven at 105 °C for 24 hours.

Table 3.2 Method descriptions of particle size fractionation

Fraction	Method	
<1000nm	Sedimentation	3 hours
450-1000nm	Centrifugation	5 min at 1200rpm 7 min at 2300rpm
100-450nm	Centrifugation	5 min at 12000rpm
2.3-100nm	Ultrafiltration	10 kDa UF-Millipore membranes filters

3.4.2 Analysis of C and N in Soil Particle Fractions

Decay process in any ecosystem could be thought as a continuum starting with an input of plant litter or remnants of animal life and leading to the formation of soil organic matter (Melillo et al., 1989). In this study, we tracked the change of C and N chemical dynamics in varied soil size fractions under continuously decreasing of redox potential using standard chemical techniques and stable isotope analyses. Besides, other measurement of H content, S content, C/H ratio and C/N ratio were done to support the C and N dynamic in decay process and the element structure variation during the formation and decomposition processes of mineral-organic matter aggregates.

3.4.2.1 C/H/N/S analysis

After soil size fractionation process, Carbon (%), Nitrogen (%), Sulfur (%), and C/N ratio and C/H ratio of soil samples from each fraction were tested using CHNS Elementar Cube (Shown below).



Figure 3.4 CHNS Elementar Cube with monitor

(<https://www.elementar.de/en/products/organic-elemental-analysis/unicube.html>)

3.4.2.2 C and N Isotopic Analysis

A popular method to assess organic C and N cycling of transformations in soil uses the distribution of ^{13}C and ^{15}N in organic matter pools (Boutton et al., 1998; Liao et al., 2006). Size-fractionated soil samples were analyzed for stable C and N isotopic compositions using EA-IRMS technique (Schema shown below). Each soil particle fraction has six replicates to ensure the accuracy of results. The mass spectrometer analyses are expressed in terms of $\delta(\text{X})$ values, which are parts per thousand differences from a standard:

$$\delta(\text{X}) = [(\text{R}_{\text{sample}}/\text{R}_{\text{standard}}) - 1] * 10^3$$

where X is ^{13}C or ^{15}N and R is the corresponding ratio $^{13}\text{C}/^{12}\text{C}$ or $^{15}\text{N}/^{14}\text{N}$. The δ values are measures of the relative amounts of heavy and light isotopes in a sample. Increases in these values signify relative increases (e.g., more ^{13}C relative to ^{12}C) in the amount of the heavy isotope components.

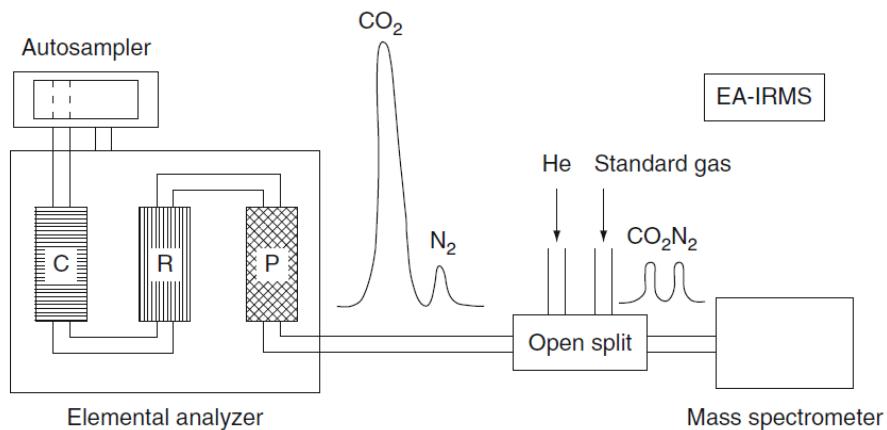


Figure 3.5 Elemental analyzer (EA) coupled to an isotope ratio mass spectrometer (IRMS) (redrawn after Glaser, 2005).

3.5 Data and Statistic Analysis

Sample averages, standard deviations were provided for each of the measured parameters, i.e. solution chemistry, colloid concentration, OC concentration, %OC and isotope values. Statistical analysis was performed by one-way analysis of variance (ANOVA) combined with Turkey-Kramer HSD analysis to test their significance of differences ($p < 0.05$) among different sampling sites as well as different size fractions (JMP®, Version Pro 12. SAS Institute Inc., Cary, NC). Pearson's correlation coefficients

between solution Eh, pH, EC, salinity and OC and colloid concentration in different size fractions were provided ($p < 0.1$, 0.05 and 0.001). To examine the relationships between OC concentration and colloid mass concentration, linear regressions between OC and colloid mass concentration were obtained using the Origin (OriginLab, Northhampton, MA).

Chapter 4

RESULTS AND DISCUSSIONS

4.1 Chemical Characterization of Leaching Solutions

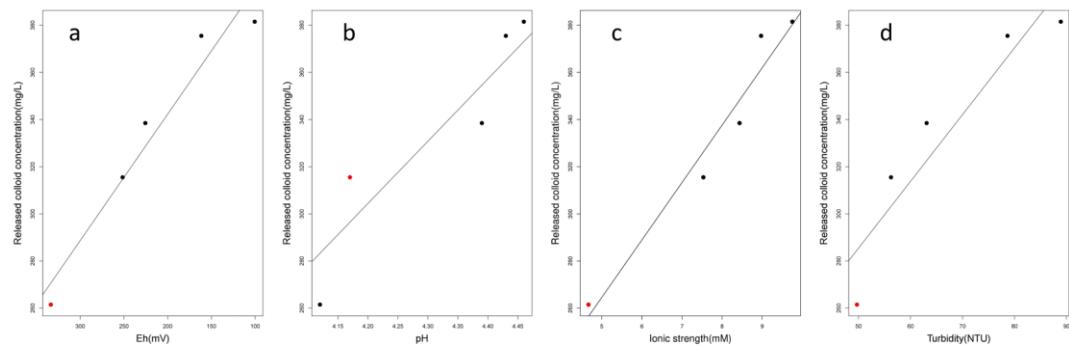


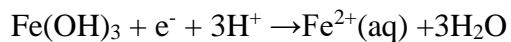
Figure 4.1 Regression analysis of released colloid concentration for leaching solutions as a function of solution chemical properties a) Eh, b) pH, c) Ionic strength (IS), and d) Turbidity.

Batches of syringe column experiment were conducted to better understand the mechanism of soil colloid mobility involving both water-dispersible colloids and stable colloids under various redox conditions. The concentrations of released colloids were taken as an indicator of mobile and water-dispersible colloids, changing as a function of incubation time. The dynamic change of solution acidity and ionic strength influences colloid release and dispersivity by the modification of colloid surface properties. In these study, we additionally tested the dynamic of redox potential and solution turbidity which

were all confirmed to be highly related to colloid stability and organic-clay-aggregates formation in the following discussion. Along with the incubation time, redox condition of leaching samples changed from 333.5mV to 100.5mV as shown in Table.1. Significant variations of the other chemical properties (Table.1.) of leaching samples were also exhibited among the batches.

Relationships of vertical released colloid concentrations versus solution chemical properties are indicated in Figure 4.1, each with a red bias point representing the 0-day leaching sample. Adjusted R-squared values are 0.8459, 0.6796, 0.8717, 0.9197 respectively in terms of pH, Eh, ionic strength and turbidity value versus released colloid. 0-day leaching sample was under an aerobic condition (333.5mV, Table.1.), and as incubation time increased, oxygen remained in soil was constantly consumed due to soil biotic and abiotic chemical reactions, along with a decrease in redox potential. As the redox condition varied from aerobic to anaerobic, soil chemical reactions and microbial reactions inherently varied a lot. When soil oxygen exists, microbial induced soil respiration and abiotic chemical respiration both regulate the mineralization and decomposition of soil organic carbon, during which processes electrons are transferred from soil organic matter to oxygen, and those mechanisms are typically common in fresh topsoil of natural system. Hence there was a trend in decreasing soil acidity and redox potential from the initial 9 days according to the column experiment. As oxygen content reduced and concurrently Eh value dropped, other alternative terminal electron acceptors (TEA) were denoted to play significant roles in soil microbial respiration (Nitrate, manganic manganesees, ferric iron, sulfate, CO₂, or other organic acid, which participated in order as listed). Ferric iron has

been reported to predominantly control the decomposition processes of soil organic matter (reaction shown below):



That process can obviously explain the increasing pattern of pH value after 9 days soil incubation (Table.1.), even though the dissolving of produced CO₂ would have to slightly affect soil acidity.

Soil salinity is another important factor which has effects on mobility and stability of soil colloids, and dissolved ions increases salinity as well as electrical conductivity (EC), as was indicated in the rising value of ionic strength (Figure 4.1.). Fig.1. also exhibited a similar increasing pattern in turbidity of leachate samples among the five groups of column experiment. The sustainable enhancement of downward transported colloids under dynamic redox condition should be a consequence of a combination of complex microbial induced biochemical reactions in soil system, which on the other hand influences the physical and chemical phenomena in soil leaching solutions. To be highlighted, changes in all solution properties between the first two column groups (0-day and 9-day) were confirmed to display a marked variation according to the linear regression analysis (Figure 4.1.). That significant change was assumed to derived from the transformation from aerobic to anaerobic condition in soil regime, during which the dominant electron acceptor varied from oxygen to other elements, especially Fe(III). However, further biochemical examinations are required to learn the inherent mechanism leading to such dramatic variations.

Table 4.1 pH, Eh, IS, turbidity and released colloid concentrations under different leaching solutions. * means index that has p-value less than 5% indicating a statistically significant difference among groups.

Parameters	Unit	Leaching time (day)				
		0	9	16	22	27
Eh*	mV	333.5 ±2.1	251.5 ±7.7	225.5 ±3.5	161.5 ±6.3	100.5 ±4.9
pH	/	4.17 ±0.07	4.12 ±0	4.39 ±0.12	4.43 ±0.04	4.46 ±0.02
Ionic strength*	mM	4.668 ±0.2	7.532 ±0.1	8.435 ±0.05	8.972 ±0.1	9.75 ±0.2
Turbidity*	NTU	49.7 ±1.6	56.25 ±3.7	63.1 ±1.1	78.65 ±4.0	88.9 ±0.84
Released colloid concentration*	mg/L	261.5 ±29.7	315.5 ±8.5	338.5 ±33.9	375.5 ±18.4	381.5 ±7.1

4.2 Release of Colloids During Leaching Process and Associated OC Characterization

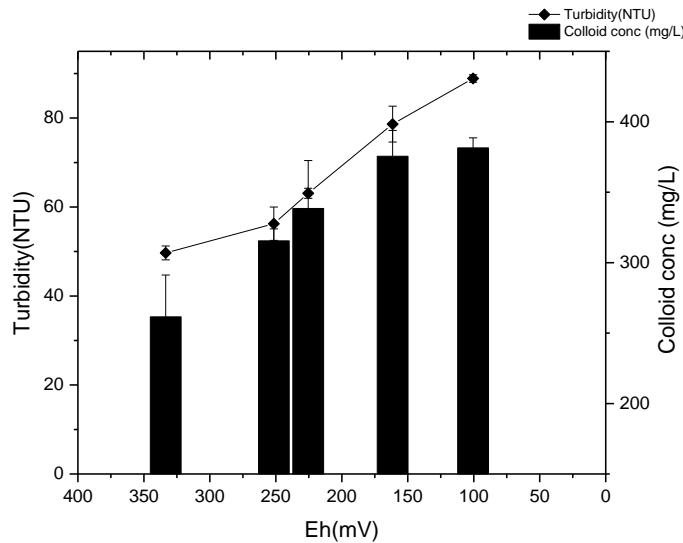


Figure 4.2 Coupled turbidity curve and released colloid concentrations for leaching solutions

Soil colloid mobilization and stabilization can be affected by the dynamic oscillation of redox conditions (Thompson et al., 2006). De-Campos et al. (2009) reported that short term reducing conditions disintegrated soil aggregates and resulted in particle mobilization. A strong positive correlation between soil redox potential (Eh) and soil aggregate stability, the ability of soil aggregates in remaining stable and not being disintegrated, indicated that aggregate stability decreased as Eh decreased (De-Campos et al., 2009). Changes in released colloid concentration as a function of reducing redox potential were compared with turbidity values in leaching solutions in Figure 4.2. Small solid particles dispersed in solution system cause the liquid to appear turbid, indicated in

higher turbidity index. Hence the enrichment of water-dispersible colloids within vertical water flow would increase leachate turbidity, and the declining redox potential facilitated accumulation of downward transported colloidal particles which agree well with previous studies as indicated.

In order to understand how colloidal aggregates behaved under dynamic redox conditions involving association and disassociation of organic-clay complexes in colloidal size, water-dispersible colloids in leachates were fractionated into two sizes (here we define $0.1\text{-}0.45\mu\text{m}$ as $\text{Colloid}_{0.1\text{-}0.45}$ and $0.45\text{-}1\mu\text{m}$ as $\text{Colloid}_{0.45\text{-}1}$). As a function of decreasing redox potential, both $\text{Colloid}_{0.1\text{-}0.45}$ and $\text{Colloid}_{0.45\text{-}1}$ particles were enriched in soil leaching solutions (Figure 4.3.). Furthermore, even if $\text{Colloid}_{0.45\text{-}0.1}$ particles were higher in concentration compared to $\text{Colloid}_{0.1\text{-}0.45}$, the dynamic redox condition preferentially controlled the mobility of $\text{Colloid}_{0.1\text{-}0.45}$. Results were consistent with previous work shown in Figure 2b (supplementary materials): decreasing concentration of $\text{Colloid}_{0.1\text{-}0.45}$ remained in soil level samples but concurrently $\text{Colloid}_{<0.1}$ particles ($<0.1\mu\text{m}$) was accumulated (called as “inherently recalcitrant materials”. Wagai and Kitayama, 2009), which revealed the fact that leaching flow predominantly influenced the mobility and transport of $\text{Colloid}_{0.1\text{-}0.45}$ ($0.1\text{-}0.45\mu\text{m}$) particles while $\text{Colloid}_{<0.1}$ soil particles couldn’t be affected significantly. Assumingly, particle geometrical size and molecular weight play important roles in the control of colloids stabilization and mobilization under dynamic redox conditions. Physical as well as chemical parameters varies among different-sized particles including surface area, sorption capacity, surface charge and etc, all accounting

for the organic-mineral associations formation and dissolution, which could influence the biological utilization of soil organic carbon up to a point.

Dynamic redox reactions also affect the structure and characteristics of OM in soils (Fiedler and Kalbitz, 2003). OM release and retention after mineral dissolution by soil reduction were investigated (Fiedler and Kalbitz, 2003; Grybos et al., 2009; Hagedorn et al., 2000). Hagedorn et al. (2000) found a close correlation between DOC and dissolved iron concentration in forest soils, suggesting that reductive iron dissolution was a major factor for DOC release. Regression analysis in Figure. 2a. (supplementary materials) agreed well with that conclusion. To better understand the interactive correlations between organic carbon storage and colloid mobility, ratios of total organic carbon concentrations and colloids concentrations in different colloid fractions ($0.1\text{-}0.45 \mu\text{m}$, $0.45\text{-}1 \mu\text{m}$) were calculated and compared within the five groups of syringe columns, as an indicator of loading capacity among size-varied colloidal particles (Figure 4.4). Furthermore, the results generally place a focus on the impact of colloids vertical transport in organic carbon persistence and turn-over ability at deep soil horizons. Obviously Colloid_{0.1-0.45} ($0.1\text{-}0.45 \mu\text{m}$) were able to load more organic carbon which was twice that of stocks in Colloid_{0.45-1} ($0.45\text{-}1 \mu\text{m}$). Colloid_{0.1-0.45} had size between truly dissolved fraction and macromolecules, and were dominantly formed by humic aggregates, Fe oxyhydroxides and some proteins (Lead and Wilkinson, 2007). Inherent chemical structures and relatively large surface area could enhance Colloid_{0.1-0.45} fraction's adsorption capacity of organic matter and thus make it as the most dynamic particle fraction participating in biological and chemical soil reactions under dynamic redox conditions.

Across a range of redox potentials (from aerobic and anaerobic), OC loading capacities in both Colloid_{0.1-0.45} and Colloid_{0.45-1} fluctuated in a regular pattern, as was shown in Figure 4.4. Assumingly, a modulation effect adjusted the carbon storage function in organic-mineral associations under a short-term turbulence in redox potential. Once OC uploaded in soil particles reached a maximum content, OM-mineral aggregates would have potential to decompose and release overloaded OC. On the contrary, particles loading less organic carbon tended to stabilize free-OC in the surrounding pore regimes. Those processes could be explained dominantly by the mediation of microbial-induced effects. Under the condition of continuously decline in redox potential, organic matter provide both energy and carbon source for bacteria growth, and electrons are transferred from OC to TEAs. Microbially reduction reaction not only decomposes OC-mineral-microbe aggregates into smaller size fraction, but also influence OC content in aggregates. Nevertheless, if OC content is lower than required, microbes tend to actively hunt for additional OC sources, leading to a sorption of a larger amount of OC on the surface of aggregated particles.

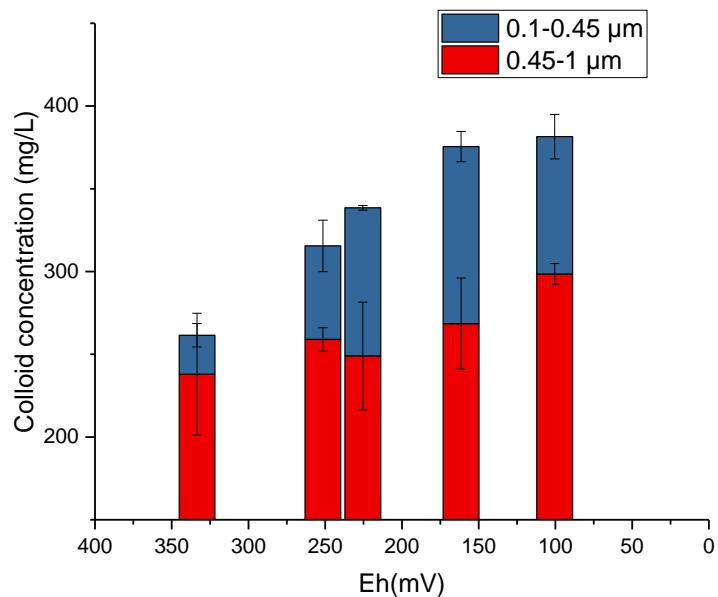


Figure 4.3 Size fractionation of colloids in leaching solutions.

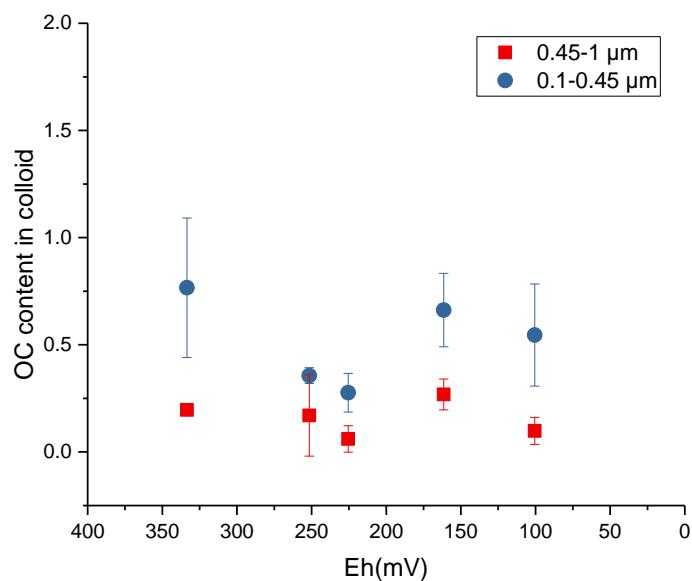


Figure 4.4 The ratio of OC to colloids (%OC) within size fractions of 0.1-0.45, 0.45-1.0 μm.

4.3 Soil Size Fractionation under Different Redox Potentials and Analysis of C and N in Soil Particle Fractions

Colloid_{0.1-0.45} (colloid size of 0.1-0.45µm) was described as the most biologically and redox dynamic fraction among soil colloidal particles with high loading capacity of soil organic carbon in the soil leaching solutions. On the contrary to the phenomena in leaching solutions, there was a decrease in concentration of soil Colloid_{0.1-0.45} from aerobic condition to anaerobic condition, while Colloid_{0.45-1} (colloid size of 0.45-1µm) and Colloid_{<0.1} (colloid size <0.1µm) were gradually accumulated in soils (Figure 4.5.). A number of processes were believed to be responsible for that observations in size distribution of soil colloids. The simplest of these explanations was physical mixing in the soil-water interaction zone where the simultaneous release of Colloid_{0.1-0.45} from the aggregates surfaces to overlying waters and directed diffusion of Colloid_{<0.1} components were supposed to be occurred in the soil-water layer. Moreover, the most obvious and significant observation is that an opposite changing direction took place in the second column group (250mV redox potential) where oxygen was depleted and substituted by nitrate as the dominant role of an electron acceptor. Alteration of the electron acceptors triggered off a series of perturbation in microbial induced reduction reactions, involving formation and release of particles, adhesion of organic carbon on minerals, as well as consumption of organic carbon by microorganisms.

Elemental analyses were performed to explain the role of varied colloid size fractions in redox-dynamic soil systems and how they reacted to the changing environment, which could also be an ideal setting to address the soil reactions facing severe climate change and anthropogenic perturbation. As for all of above three size colloidal fractions,

their C:N ratios were indicated lower than 10 in Figure 4.6(a). According to previous studies, substances with C:N ratios lower than 10 are dominated by microbially processed compounds and/or N-rich compounds, such as peptides, and thus with little inclusion of high C:N plant detritus. Results confirmed that the three colloidal fractions were consisted predominantly of microbially processed organic matter, which was likely some combination of both live and dead microbial biomass and some organo-mineral complexes known as inputs of N-rich materials (the C:N of microbial biomass = 4–7; Paul and Clark 1996). The recalcitrant and complex chemical composition ensured soil colloids or else clay particles under a combination of mineral protection and the recycling of older C within the microbial community (Rasmussen et al, 2018). Moreover, preferential adsorption of N-rich dissolved organic matter on mineral surfaces (Aufdenkampe et al. 2001; Hedges and Oades 1997) might also explain the low C:N ratios of colloidal organic matters. On the other hand, during decomposition, organic C is oxidized by heterotrophic microbes, whereas N is stored in the microbial biomass owing to microbial N demand (their biomass has a much lower C:N ratio than plant biomass) (Wagai and Kitayama, 2009). Based on current results, the C:N ratio declined from larger-size particles to smaller-size particles, and thus Colloid_{<0.1} particles could be definitely treated as the left-over materials of microbial biomass from microbial reduction reactions. In addition, there were wide variations in the C:N ratio and sulfur content of Colloid_{0.1-0.45} fraction (Figure 4.6), especially for group B at 251.5mV. These findings could be interpreted by the significant biological dynamic properties of Colloid_{0.1-0.45} particles. Additionally speaking, microbial reduction reactions and colloid release were most thermodynamically active when a quick

shift from aerobic to anaerobic respiration happened in soil matrix. During those processes, C, N, or S starvation activated the synthesis of amino acid transport systems (Oxender et al., 1980; Wolfenbarger, 1980). Those possibilities were all in line with previous results, including soil leaching solution chemical properties, colloid mobility and stability, as well as the cycling of colloidal organic carbon under dynamic redox conditions, and will also be confirmed in the following isotopic analyses.

Soil organic carbon is composed of a heterogeneous mixture of organic compounds with varied ^{13}C and ^{15}N value due to their biosynthetic pathways (Bianchi and Bauer. 2011). Size-fractionated soil colloid samples were analyzed for stable C and N isotopic compositions using EA-IRMS technique. It is illustrated from Figure 4.7(a) that Colloid_{0.45-1} particles were more depleted in ^{13}C due to the most negative $\delta^{13}\text{C}$ value compared to the other two fractions. And the isotope values became less and less negative from largest to smallest particles, which means ^{13}C enriched materials (for examples, polysaccharides and proteins) dominated the component of smaller-sized colloid particles in soil. Moreover, an obvious result pointed out that $\delta^{13}\text{C}$ value of Colloid_{0.1-0.45} became heavier and heavier along with the continuous decline of redox potential until it eventually kept consistent with that of Colloid_{<0.1} particles. Andrews et al. (2000) suggested that a possible mechanism for changing utilization patterns of soil organic carbon was alteration in microbial community composition, which may lead to changes in whole-community metabolic capacity.

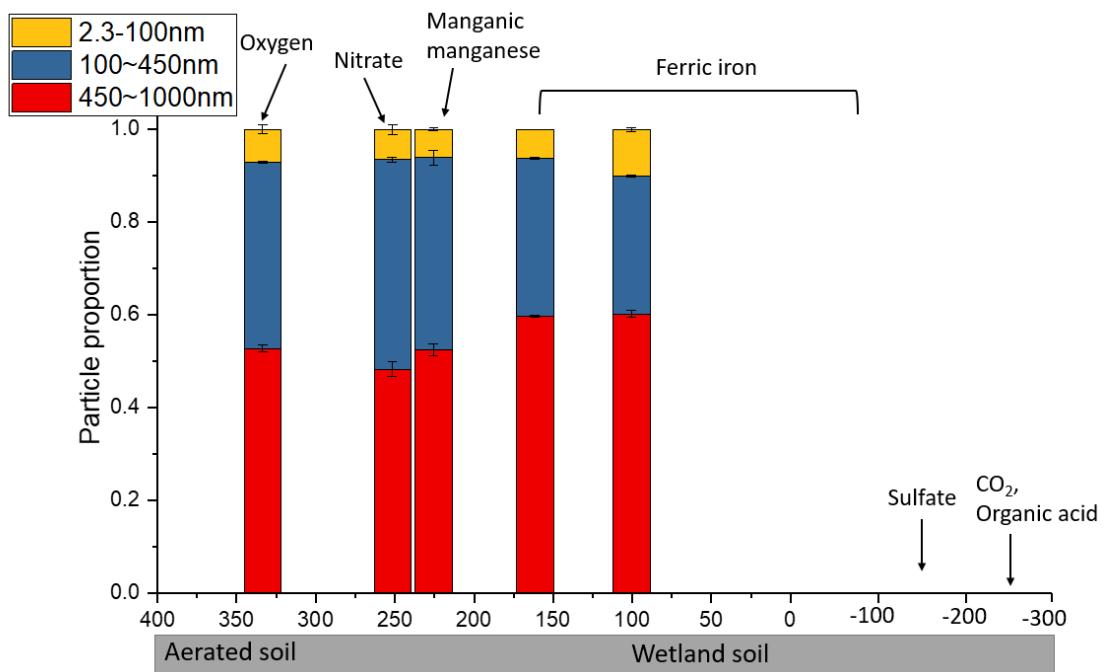
In terms of nitrogen isotopes signatures, the most negative value was exhibited in Colloid_{<0.1} particles, which kept in a stable range from -4 to 0. According to C/N ratio analysis, we concluded that inorganic nitrogen compounds dominantly composited the

Colloid_{<0.1} particles, which was also confirmed in Figure.3b (supplementary material). Isotope ratio of ¹⁵N/ ¹⁴N for Colloid_{0.45-1} fraction is also stable within range of 0 to 1. Significant variation of nitrogen isotope ratio happened in Colloid_{0.1-0.45} fraction, where it decreased from 4 to -1 in the first three redox conditions and after that remained relatively stable, as all indicated in Figure 4.7b.

During the decomposition processes in natural soil systems, soil organic matter is cleaved from large polymers to largely bio-available monomers which are accessible to both plants and microbes (Butterbach-Bahl and Gundersen, 2011). Microbes can further degrade these organic monomers to form ammonium (ammonification or N mineralization). Ammonification is performed by unspecific heterotrophic microorganisms both under aerobic and anaerobic conditions (Jarvis et al., 1996). Both ammonium and nitrate can either be taken up by plants or immobilized by microorganisms, named assimilatory nitrate/ammonium reduction. While dissimilatory nitrate reduction to ammonium (DNRA) is an anaerobic process, catalyzed by fermentative bacteria and reducing NO₃⁻ via nitrite (NO₂⁻) to ammonium (NH₄⁺), during which processes the inorganic N compounds work as N source of microorganisms and as well the terminal electron acceptors. DNRA has the potential to play an important role in ecosystem N retention (Silver et al., 2001) and has been recognized as a significant process mainly in wetland ecosystems. The observed isotope discrimination associated with dissimilatory NO₃⁻ and NO₂⁻ reduction was also shown to vary with redox conditions (Figure 4.7b), namely, with the concentrations of the carbon source and NO₃⁻ or NO₂⁻ (Chien et al., 1977; Mariotti et al., 1981; Bryan et al., 1983; Shearer and Kohl, 1988). In other ways, the ratio of C to electron acceptors seems to control

the partitioning of nitrate to denitrification and DNRA in such a way that DNRA is favored when this ratio is high (Tiedje, 1988), which explains why the highest C/N ratio was observed at 250mV redox condition for Colloid_{0.1-0.45} fraction. In addition, the dissimilatory reduction of nitrate to nitrite or ammonium exhibits significant nitrogen (N) isotope discrimination, where ¹⁴N-bearing nitrate is consumed more rapidly than that containing ¹⁵N, leaving the remaining substrate pool with an elevated 15N:14N ratio (Julie *et al.*, 2008). Therefore, value of $\delta^{15}\text{N}$ for Colloid_{0.1-0.45} fraction decreased in the first three redox states where DNRA process dominated the dissimilatory microbial reduction reactions. Released ammonium ions from DNRA process have stronger trend to bond with colloid-minerals compared with nitrite and as a result Colloid_{0.1-0.45} particles are supposed to absorb a large amount of NH₄⁺ on their surfaces.

The structure of the soil aggregation imparts many of the key properties to soil because it shapes the habitats available to soil microorganisms (Or *et al.*, 2007). Soil aggregates vary in size and are dynamic in terms of their formation and disintegration; these characteristics are partly a function of microbial activities, such as the production of extracellular polymeric materials that help stabilize aggregates (Six *et al.*, 1999; Tisdall and Oades, 1982). For example, microbial N mineralization and immobilization were greatest in intermediate to larger water-stable soil aggregates (Angers *et al.*, 1997; Muruganandam *et al.*, 2010). In conclusion, Colloid_{0.1-0.45} fraction is most actively involved in the sequential microbial reduction reactions when oxygen is depleted in soil system due to its NH₄⁺⁻-bonded structure on surface with a strong hydrogen bonding between molecules.



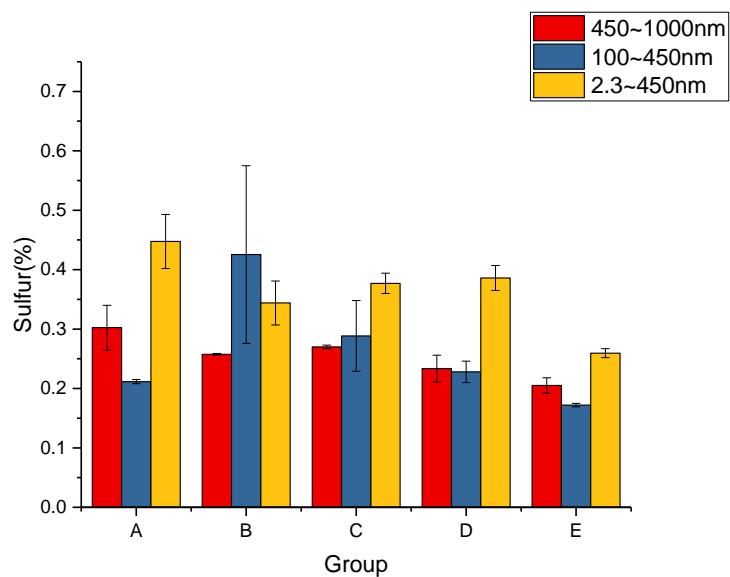
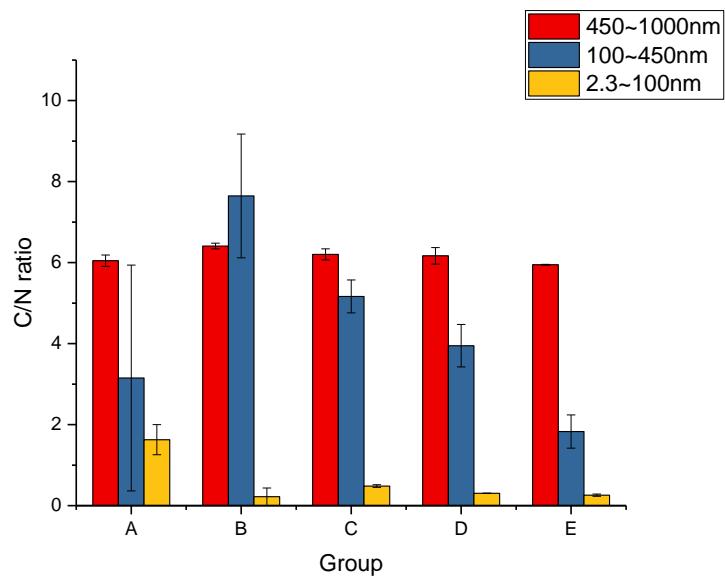


Figure 4.6 C/N ration (a), sulfur content (b), and carbon content (c) of the size-fractionated soil samples versus redox potential in mV.

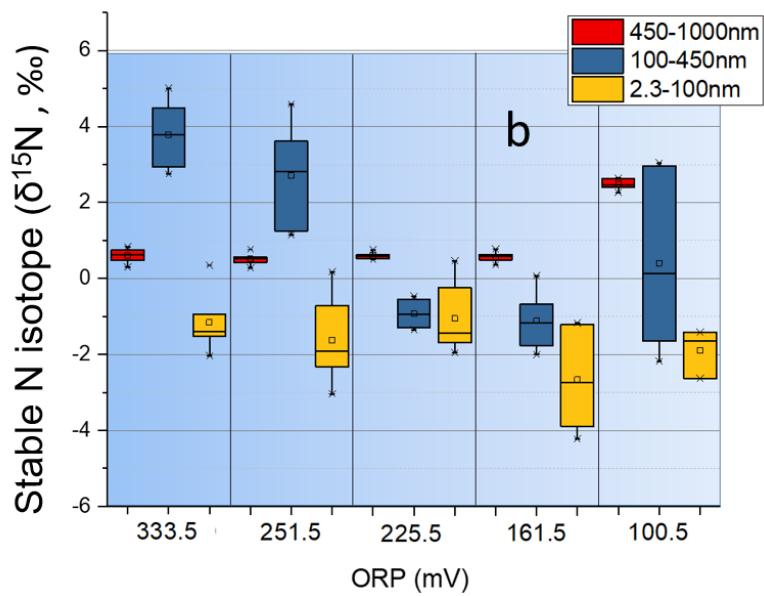
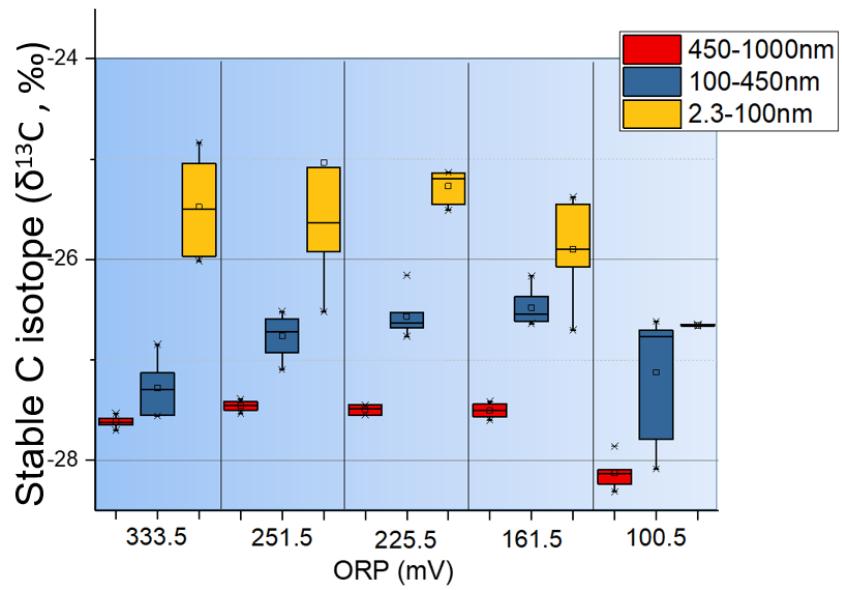


Figure 4.7 The $\delta^{13}\text{C}$, $\delta^{15}\text{N}$ of the size-fractionated soil samples versus redox potential in mV.

Table 4.2 Description and comparison of soil properties for fractionated soil colloids ($\text{Colloid}_{0.1-0.45}$, $\text{Colloid}_{0.45-1}$, $\text{Colloid}_{<0.1}$).

	Size	Morphology	Chemical Composition and Properties	
Colloid_{0.45-1}	0.45-1 μm	A lighter color Loose particles	Higher C:N ratio ^{13}C depleted	Colloid_{0.1-0.45} is the “Potentially exchangeable or reactive soil C pool” (Sanderman. 2008).
Colloid_{0.1-0.45}	0.1-0.45 μm	A darker color Dense soil	Wide range of C:N ratio, $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ More mobile and biochemically dynamic Larger capacity for OC partitioning	
Colloid_{<0.1}	<0.1 μm	A darker color Fluffy soil	Lower C:N ratio ^{13}C enriched More labile	

Chapter 5

CONCLUSION AND FUTURE WORKS

Carbon turnover and sequestration in soil, including its related biogeochemical reactions have been investigated in a large number of studies. As a result, the diversity of biochemical reactions and their differences between aerobic and anaerobic soil conditions are in general well understood. In this study, a more detailed study was carried out to examine the chemical and biological variations under stepwise and specific redox conditions. As is consistent with previous conclusions, an obvious alteration of chemical properties and organic matter features occurs between aerobic and anaerobic conditions. Additionally, the continuous depletion of oxygen in soil system triggers off a series of dissimilatory microbial reduction reactions that leads to facilitated decomposition of soil organic carbon. Those processes coupled with adsorption and disassociation reactions play important roles in the release of colloids and thus guarantee the carbon cycling among small-scale soil particles.

Size distributions in both leaching solution and soil were quantified under each redox condition to further understand soil aggregates formation and disassociation. Results showed that a larger amount of organic C was loaded in Colloid_{0.1-0.45} particles, and the decreased redox potential resulted in an incremental release of those size fraction. Moreover, Colloid_{0.1-0.45} particles were confirmed as the most biochemically-active fraction

due to its large specific surface area and its favoring coating with ammonium (NH_4^+). Sanderman put forward a hypothesis in 2008, telling that a ‘potentially exchangeable or reactive soil C pool’ may exist, and my results help verify the theory by providing the evidence indicating Colloid_{0.1-0.45} fraction was actively involved in both aerobic soil respiration and anaerobic dissimilatory microbial reduction reactions.

Based on the soil biogeochemical reactions, general environmental conditions those which enhance microbial activity will increase decomposition and mineralization of carbon sources. In addition, the biodegradation of dissolved- and colloidal- OM varies with both environmental and ecological factors, like soil fractions, land use and soil contamination (Boyer and Groffman, 1996; Lundquist et al., 1999). In addition, microbial communities and their activities can vary among different sizes of soil aggregates (Mendes et al., 1999; Muruganandam et al., 2009). Knowing about extent and rate of the biodegradation is an approach to best understand the contribution of DOM and COM to C sequestration in soils. What’s more, it is also critical to know how interactions between DOM, COM and the solid soil phase will affect SOM biodegradation. A further controlled lab study is needed to understand how microorganisms change their bio-availability under different conditions in colloidal environments, especially size-based fractions, as well as their contributions to carbon release.

REFERENCES

- Alva, A.K. et al. 1991. Relationship between ionic-strength and electrical conductivity for soil solutions. *Soil Science* 152: 239-242. doi:10.1097/00010694-199110000-00001.
- Aufdenkampe, A.K. et al. 2011. Riverine coupling of biogeochemical cycles between land, oceans, and atmosphere. *Front Ecol Environ* 9(1):53–60.
- Baalousha, M. 2009. Aggregation and disaggregation of iron oxide nanoparticles: Influence of particle concentration, ph and natural organic matter. *Science of the Total Environment* 407: 2093-210. doi:10.1016/j.scitotenv.2008.11.022.
- Baalousha, M. et al. 2008. Aggregation and surface properties of iron oxide nanoparticles: Influence of pH and natural organic matter. *Environmental Toxicology and Chemistry* 27: 1875-1882. doi:10.1897/07-559.1.
- Baalousha, M. et al. 2011. 3.05. natural colloids and manufactured nanoparticles in aquatic and terrestrial systems. In: W. Editor-in-Chief: Peter, editor *Treatise on water science*. Elsevier, Oxford. p. 89-129.
- Batjes, N.H. 1996. Total carbon and nitrogen in the soils of the world. *Eur. J. Soil Sci.* 47 (2), 151 – 163.
- Bauer, J.E. and T.S. Bianchi. 2011. Dissolved organic carbon cycling and transformation. *Treatise on Estuarine and Coastal Science*, Vol 5: Biogeochemistry: 7-67.
- Benner, R. and R.M.W. Amon. 2015. The size-reactivity continuum of major bioelements in the ocean. *Annual Review of Marine Science*, Vol 7 7: 185-205. doi:10.1146/annurev-marine-010213-135126.
- Bianchi, T.S. and Bauer, J.E. 2011. Particulate Organic Carbon Cycling and Transformation. In: Wolanski E and McLusky DS (eds.) *Treatise on Estuarine and Coastal Science*, Vol 5, pp. 69–117
- Boutton, T.W. et al. 1998. d13C values of soil organic carbon and their use in documenting vegetation change in a subtropical savanna ecosystem. *Geoderma* 82, 5–41.

- Boyer, J.N. and Groffman, P.M., 1996. Bioavailability of water extractable organic carbon fractions in forest and agricultural soil profiles. *Soil Biol. Biochem* 28, 783–790.
- Buettner, S.W. et al. 2014. Mobilization of colloidal carbon during iron reduction in basaltic soils. *Geoderma* 221: 139-145. doi:10.1016/j.geoderma.2014.01.012.
- Buffle, J. and G.G. Leppard. 1995. Characterization of aquatic colloids and macromolecules. 1. Structure and behavior of colloidal material. *Environmental Science & Technology* 29: 2169-2175. doi:10.1021/es00009a004.
- Chabbi, A. et al. 2009. Stabilized carbon in subsoil horizons is located in spatially distinct parts of the soil profile. *Soil Biology & Biochemistry* 41, 256-271
- Chen, K.L. et al. 2006. Aggregation kinetics of alginate-coated hematite nanoparticles in monovalent and divalent electrolytes. *Environmental Science and Technology* 40: 1516-1523. 25.
- Chin, Y.P. et al. 1998. Abundance and properties of dissolved organic matter in pore waters of a freshwater wetland. *Limnology and Oceanography* 43: 1287-1296.
- Chin, Y.P., G. Aiken, and E. O'Loughlin. 1994. Molecular weight, polydispersity, and spectroscopic properties of aquatic humic substances. *Environ. Sci. Technol.* 28:1853–1858.
- de Jonge, L.W. et al. 2004a. Colloids and colloid-facilitated transport of contaminants in soils: An introduction. *Vadose Zone Journal* 3: 321-325.
- de Jonge, L.W. et al. 2004b. Particle leaching and particle-facilitated transport of phosphorus at field scale. *Vadose Zone Journal* 3: 462-470.
- De-Campos, A.B. et al. 2009. Short-term reducing conditions decrease soil aggregation. *Soil Science Society of America Journal* 73: 550-559. doi:10.2136/sssaj2007.0425.
- Docter, D. et al. 2015. The nanoparticle biomolecule corona: lessons learned—challenge accepted? *Chem Soc Rev* 44 (17): p. 6094-121.
- Favre, F. et al. 2004. Changes in clay organization due to structural iron reduction in a flooded Vertisol. *Clay Miner* 39:123–134.
- Fiedler, S. and K. Kalbitz. 2003. Concentrations and properties of dissolved organic matter in forest soils as affected by the redox regime. *Soil Science* 168: 793-801. doi:10.1097/01.ss.0000100471.96182.03.

- Gimbert, L.J. et al. 2005. Comparison of centrifugation and filtration techniques for the size fractionation of colloidal material in soil suspensions using sedimentation field-flow fractionation. *Environmental Science & Technology* 39: 1731-1735. doi:10.1021/es049230u.
- Glaser, B. 2005. Compound-specific stable-isotope ($\delta^{13}\text{C}$) analysis in soil science. *Plant Nutr. Soil Sci* 168: 633-648. doi:10.1002/jpln.200521794.
- Green, S.A. and Blough, N.V. 1994. Optical-absorption and fluorescence properties of chromophoric dissolved organic-matter in natural-waters. *Limnology and Oceanography* 39: 1903-1916.
- Griffin, B.A. and Jurinak, J.J. 1973. Estimation of activity coefficients from the electrical conductivity of natural aquatic systems and soil extracts. *Soil Science* 116: 26-30.
- Grybos, M. et al. 2009. Increasing pH drives organic matter solubilization from wetland soils under reducing conditions. *Geoderma* 154: 13-19.
- Guo, L. and Santschi, P.H. 2007. Ultrafiltration and its applications to sampling and characterization of aquatic colloids. In: K. J. Wilkinson and J. R. Lead, editors, *Environmental colloids and particles: Behaviour, separation and characterization*. Wiley, West Sussex, England. p. 159-221.
- Guo, L.D. and Santschi, P.H. 1997. Isotopic and elemental characterization of colloidal organic matter from the Chesapeake bay and Galveston bay. *Marine Chemistry* 59: 1-15. doi:10.1016/s0304-4203(97)00072-8.
- Guo, L.D. et al. 1995. Dynamics of dissolved organic carbon (doc) in oceanic environments. *Limnology and Oceanography* 40: 1392-1403.
- Gustafsson, O. and Gschwend, P.M. 1997. Aquatic colloids: Concepts, definitions, and current challenges. *Limnology and Oceanography* 42: 519-528.
- Hagedorn, F. et al. 2000. Effects of redox conditions and flow processes on the mobility of dissolved organic carbon and nitrogen in a forest soil. *Journal of Environmental Quality* 29: 288-297.
- Hedges, J.I. and Oades, J.M. 1997. Comparative organic geochemistries of soils and marine sediments. *Org Geochem* 27(7-8):319-361
- Inamdar, S. et al. 2011. Fluorescence characteristics and sources of dissolved organic matter for stream water during storm events in a forested mid-atlantic watershed. *Journal of Geophysical Research-Biogeosciences* 116 G03043. doi:10.1029/2011jg001735.

- Jansson, J.K. and Hofmockel, K.S. 2018. The soil microbiome—from metagenomics to metabolomics. *Curr Opin Microbiol* 43:162–168. doi.org/10.1016/j.mib.2018.01.013.
- Jobbágy, E. G. and Jackson, R. B. 2000. The vertical distribution of soil organic carbon and its relation to climate and vegetation. *Ecol Appl* 10:423-436. doi.org/10.1890/10510761(2000)010[0423:TVDO SO]2.0.CO;2
- Kaiser, K. and Kalbitz, K. 2012. Cycling downwards - dissolved organic matter in soils. *Soil Biology and Biochemistry* 52:29-32. doi:10.1016/j.soilbio.2012.04.002.
- Kaiser, M. and Ellerbrock, R.H. 2005. Functional characterization of soil organic matter fractions different in solubility originating from a long-term field experiment. *Geoderma* 127:196-206.
- Kalbitz, K. et al. 2000. Controls on the dynamics of dissolved organic matter in soils: A review. *Soil Science* 165:277-304. doi:10.1097/00010694-200004000-00001.
- Kappler, A. and Straub, K.L. 2005. Geomicrobiological cycling of iron. *Molecular Geomicrobiology* 59:85-108. doi:10.2138/rmg.2005.59.5.
- Karavanova, E.I. 2013. Dissolved organic matter: Fractional composition and sorbability by the soil solid phase (review of literature). *Eurasian Soil Science* 46: 833-844. doi:10.1134/s1064229313080048.
- Kayler, Z.E. et al. 2011. Application of delta c-13 and delta n-15 isotopic signatures of organic matter fractions sequentially separated from adjacent arable and forest soils to identify carbon stabilization mechanisms. *Biogeosciences* 8: 2895-2906.
- Kepkay, P.E. et al. 1997. Colloidal organic carbon and phytoplankton speciation during a coastal bloom. *Journal of Plankton Research* 19:369-389. doi:10.1093/plankt/19.3.369.
- Kirk, G. 2004. The biogeochemistry of submerged soils. John Wiley & Sons, Chichester, UK.
- Kirk, G. et al. 2003. Effects of flooding and redox conditions on solute diffusion in soil. *Eur. J. Soil Sci.* 54:617-624.
- Kögel-Knabner, I. 2002. The macromolecular organic composition of plant and microbial residues as input to soil organic matter. *Soil Biol. Biochem.* 34:139-162.
- Kretzschmar, R. et al. 1995. Influence of natural organic-matter on colloid transport through saprolite. *Water Resources Research* 31:435-445.

- Kretzschmar, R. et al. 1998. Influence of pH and humic acid on coagulation kinetics of kaolinite: A dynamic light scattering study. *Journal of Colloid and Interface Science* 202:95-103. doi:10.1006/jcis.1998.5440.
- Kretzschmar, R. et al. 1999. Mobile subsurface colloids and their role in contaminant transport. *Advances in Agronomy* 66:121-193.
- Stookey, L.L. 1970. Ferrozine---a new spectrophotometric reagent for iron. *Analytical Chemistry* 42 (7):779-781. DOI: 10.1021/ac60289a016.
- Lead, J.R. and Wilkinson, K.J. 2007. Environmental colloids and particles: Current knowledge and future developments. *Environmental colloids and particles*. John Wiley & Sons, Ltd.1-15.
- Liao, D. et al. 2006. GIS approaches for the estimation of residential-level ambient PM concentrations. *Environ Health Perspect* 114:1374-1380. doi:10.1289/ehp.9169.
- Lorah, M.M. et al. 2012. Evaluating nutrient fate and redox controls in groundwater in riparian areas, USGS MD-DE-DC Water Science Center, Baltimore, MD, <http://md.water.usgs.gov/posters/nutrientsRedox/>, Nov. 30
- Lundquist, E.J. et al. 1999. Wet-dry cycles affect dissolved organic carbon in two California agricultural soils. *Soil Biol. Biochem* 31:1031-1038.
- Melillo, J.M. et al. 1989. Carbon and nitrogen dynamics along the decay continuum: plant litter to soil organic matter. In *Ecology of arable land*. Edited by M. Clarholm and L. Bergstrom. Kluwer Academic Publishers, Norwell, MA.
- Morrisson, A.R. et al. 1990. Application of high-performance size exclusion liquid-chromatography to the study of copper speciation in waters extracted from sewage-sludge treated soils. *Analyst* 115:1429-1433. doi:10.1039/an9901501429.
- Newbold, J.D. et al. 1997. Organic matter dynamics in white clay creek, pennsylvania, USA. *Journal of the North American Benthological Society* 16: 46-50. doi:10.2307/1468231.
- Patrick, W.H., and Jugsujinda, A. 1992. Sequential reduction and oxidation of inorganic nitrogen, manganese, and iron in flooded soil. *Soil Sci. Soc. Am. J* 56:1071-1073.
- Patrick, W.H., Grambell, R.P. and Faulkner, S.P. 1996. Redox measurements of soils. 1255-1274. In D.L. Sparks (ed.) *Methods of soil analysis. Part 3. Chemical methods*. SSSA Book Ser. 5. SSSA, Madison, WI.
- Paul, E. A. and Clark, F.E. 1996. *Soil microbiology and biochemistry*. Second edition. Academic Press, San Diego, California, USA.

- Ponnamperuma, F.N. 1972. The chemistry of submerged soils. *Adv. Agron* 24:29-96.
- Ranville, J.F. et al. 2005. Particle-size and element distributions of soil colloids: Implications for colloid transport. *Soil Science Society of America Journal* 69:1173-1184. doi:10.2136/sssaj2004.0081.
- Rasmussen, C. et al. 2018. Controls on Soil Organic Carbon Partitioning and Stabilization in the California Sierra Nevada. *Soil Syst* 2:41.
- Rillig, M.C. et al. 2017. Soil aggregates as massively concurrent evolutionary incubators. *ISME J* 11:1943-1948.
- Wagai, R., Lawrence M.M. and Kitayama, K. 2009. Nature of the “occluded” low-density fraction in soil organic matter studies: A critical review, *Soil Science and Plant Nutrition* 55:1, 13-25. DOI: 10.1111/j.1747-0765.2008.00356.x.
- Rowell, D.L. 1981. Oxidation and reduction. p. 401–461. In D.J. Greenland and M.H.B. Hayes (ed.) *The chemistry of soil processes*. John Wiley & Sons, Chichester.
- Royer, R.A. et al. 2002b. Enhancement of biological reduction of hematite by electron shuttling and fe(ii) complexation. *Environmental Science & Technology* 36:1939-1946. doi:10.1021/es011139s.
- Ryan, J.N. and Gschwend, P.M. 1994a. Effect of solution chemistry on clay colloid release from an iron oxide-coated aquifer sand. *Environmental Science & Technology* 28:1717-1726. doi:10.1021/es00058a025.
- Ryan, J.N. et al. 1999. Bacteriophage prd1 and silica colloid transport and recovery in an iron oxide-coated sand aquifer. *Environmental Science & Technology* 33:63-73. doi:10.1021/es980350+.
- Sharifi, A. et al. 2017. Modeling Nitrogen and Carbon Dynamics in Wetland Soils and Water Using Mechanistic Wetland Model. Rao S. Govindaraju (ed.), *Journal of Hydrologic Engineering*. American Society of Civil Engineers (ASCE), Reston, VA, 22(1):1-18. [https://doi.org/10.1061/\(ASCE\)HE.1943-5584.0001441](https://doi.org/10.1061/(ASCE)HE.1943-5584.0001441).
- Six, J. et al. 2002. Stabilization mechanisms of soil organic matter: Implications for c-saturation of soils. *Plant and Soil* 241:155-176. doi:10.1023/a:1016125726789.
- Thamdrup, B. 2000. Bacterial manganese and iron reduction in aquatic sediments. *Advances in Microbial Ecology* 16:41-84.
- Thomas, G.W. 1996. Soil pH and soil acidity. p. 475-490. In D.L. Sparks (ed.) *Methods of soil analysis. Part 3. Chemical methods*. SSSA Book Ser. 5. SSSA, Madison, WI.

- Thompson, A. et al. 2006. Colloid mobilization during soil iron redox oscillations. *Environmental Science & Technology* 40:5743-5749. doi:10.1021/es061203b.
- Tisdall, J.M., and Oades, J.M. 1982. Organic matter and water-stable aggregates in soils. *J. Soil Sci* 33:141-163.
- Tombacz, E. et al. 1999. Particle aggregation in complex aquatic systems. *Colloids and Surfaces a-Physicochemical and Engineering Aspects* 151:233-244.
- Torn, M. S. et al. 1997. Mineral control of soil organic carbon storage and turnover. *Nature (London)* 389 (6647):170-173.
- Vepraskas, M.J. et al. 2001. Redox chemistry of hydric soils. *Wetland soils: Genesis, hydrology, landscapes, and classification*. Lewis Publishers, Boca Raton, FL. p. 85-106.
- Vold, R.D. and Vold, M.J. 1983. Colloid and interface chemistry. Addison-Wesley, Reading, MA.
- Weber, K.A. et al. 2006. Microorganisms pumping iron: Anaerobic microbial iron oxidation and reduction. *Nature Reviews Microbiology* 4:752-764. doi:10.1038/nrmicro1490.
- Yan, J. et al. 2016. Soil colloid release affected by dissolved organic matter and redox conditions. *Vadose Zone Journal* 15: 10. doi:10.2136/vzj2015.02.0026.

Appendix A

IMAGES OF EXPERIMENT AND SAMPLES

Table A.1 Images of fractionated soil colloids ($\text{Colloid}_{0.1-0.45}$, $\text{Colloid}_{0.45-1}$, $\text{Colloid}_{<0.1}$) under 350mV and 100mV redox conditions.

	$\text{Colloid}_{0.45-1}$	$\text{Colloid}_{0.1-0.45}$	$\text{Colloid}_{<0.1}$
350mV			
100mV			

Figure A.1 Images of column experiment setups





Appendix B

PRELIMINARY COLUMN EXPERIMENT RESULTS

Table B.1 DOC and Fe(II) concentrations in the solutions effluent from the soil column.
And regression statistics between the two parameters.

sample	date	hour	DOC conc. (ppm)	Fe(II) conc. (mM)
A1	10.14	74	316.2857	-0.00254
A2	10.15	98.5	342.9	-9.2E-05
A3	10.16	117	398.1	0.016675
A4	10.19	195	466.35	0.024216
A5	10.25	334.5	1032.6	0.08166
A6	10.27	387	1055.4	0.099838
A7	10.28	408.5	1216.95	0.104497

Regression Statistics	
Multiple R	0.990926
R Square	0.981934
Adjusted R Square	0.97832
Standard Error	57.77901
Observations	7

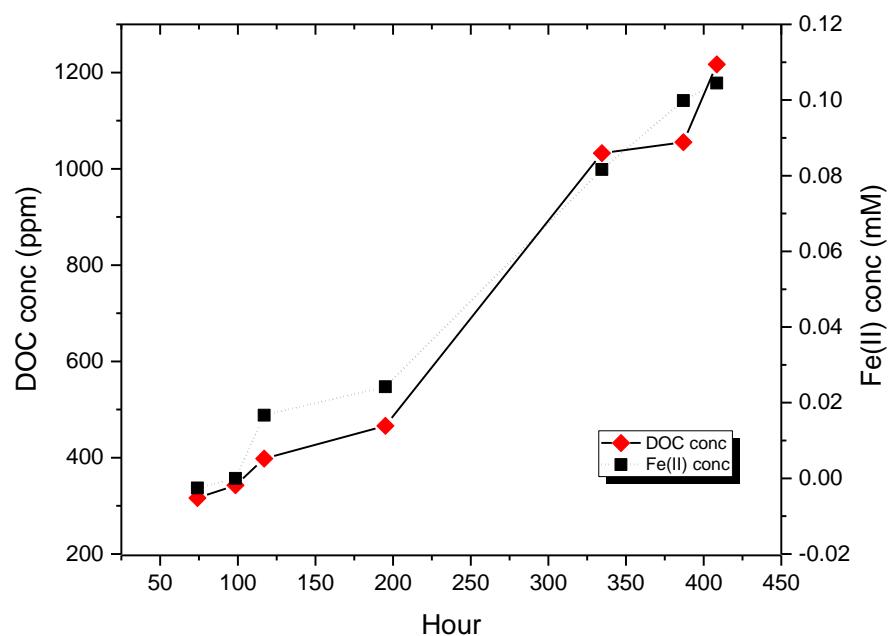


Figure B.1 Concentration change of dissolved organic carbon and Fe(II) in soil solutions. Liquid samples were taken at specific time intervals.

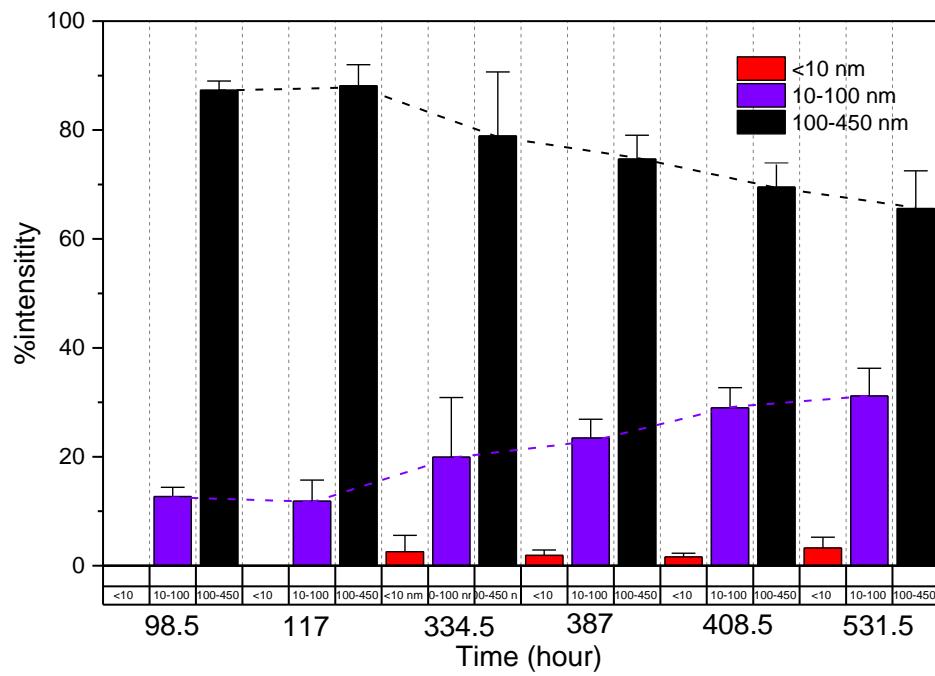


Figure B.2 Size fractionation of particles less than 450nm in soil solutions using DLS (Dynamic Laser Scattering). Liquid samples were taken at specific time intervals.

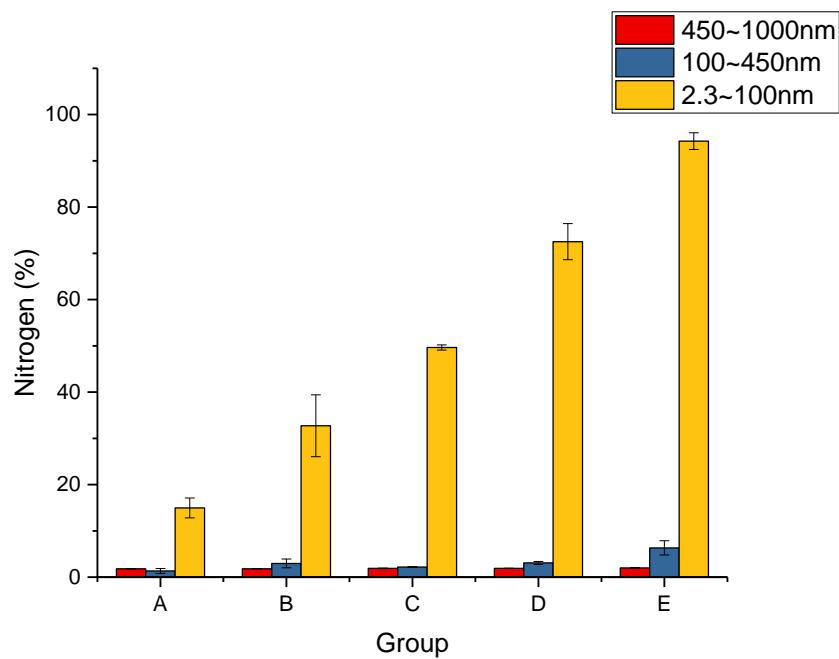


Figure B.3 Nitrogen content in different soil size fractions via the decrease of redox potential.

Appendix C

LIST OF SYMBOLS AND ABBREVIATIONS

Abbreviations

CEC	Cation exchange capacity
COC	Colloidal organic carbon
COM	Colloidal organic matter
DGGE	Denaturing gradient gel electrophoresis
DOC	Dissolved organic carbon
DOCtr	Truly dissolved organic carbon
DOCop	Operationally defined dissolved organic carbon
DOM	Dissolved organic matter
DOMin	Indigenous dissolved organic matter
DIP	Dissolved inorganic phosphorus
DOP	Dissolved organic phosphorus
CEC	Cation exchange capacity
FIFFF	Field flow filtration fractionation
HIX	Humification index
HMW	High molecular weight
ICP-MS	Inductively coupled plasma mass spectrometry

IS	Ionic strength
LIBD	Laser induced break down detection
LMW	Low molecular weight
NOM	Natural organic matter
OM	Organic matter
PCHO	Particulate carbohydrates
PCR	Polymerase chain reaction
PIP	Particulate inorganic phosphorus
POM	Particulate organic matter
POP	Particulate organic phosphorus
PZC	Point of zero charge
SEM-EDs	Scanning electron microscopy with X-ray microanalysis
SOM	Soil organic matter
SSA	Specific surface area
TCHO	Total carbohydrates
TEM	Transmission electron microscopy
TFF	Tangential flow filtration
TOC	Total organic carbon
TP	Total phosphorus
XRD	X-ray diffraction

Appendix D

PERMISSION LETTERS

1/15/2019

University of Delaware Mail - Citation permission of the figure in your published paper



Weila Li <verawei@udel.edu>

Citation permission of the figure in your published paper

2 messages

Weila Li <verawei@udel.edu>
To: michael.schmidt@geo.uzh.ch

Mon, Jan 14, 2019 at 8:21 AM

Hi Dr. Schmidt,

This is Weila, a master student from the University of Delaware, the U.S., department of civil and environmental engineering and I'm working in Dr. Yan Jin's research group. I'm writing the INTRODUCTION part of my master's thesis (The title is 'Transport of soil colloids and its relation to biogeochemical cycling of organic carbon under dynamic redox conditions') and I would like to cite the figure from your published paper in 2011 ('Persistence of soil organic matter as an ecosystem property' / Figure 3 | A synopsis of all eight insights, contrasting historical and emerging views of soil carbon cycling.). I'm writing this email to ask for a permission from you for that citation. I appreciate your kind support for my thesis work!

Thank you!

All the best,

Weila

--
Weila (Vera) Li
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Michael Schmidt <michael.schmidt@geo.uzh.ch>
To: Weila Li <verawei@udel.edu>

Mon, Jan 14, 2019 at 9:11 AM

Dear Weila
just go ahead and use the figure.
as I understand, you are free to use published material for scientific and educational purposes, provided you cite it correctly.
I wish you a successful finish thesis
Cheers, Michael

(sorry for the brief message and potential typos, this message was sent from a mobile device)
[Quoted text hidden]

<https://mail.google.com/mail/u/0/?ik=11b5a4216a&view=pt&search=all&permthid=thread-a%3Ar4425193492656862062&simpl=msg-a%3Ar482652926...> 1/1

Figure D.1 Permission for use of Figure 1.1



Weila Li <verawei@udel.edu>

Citation permission of the figure in your published paper

2 messages

Weila Li <verawei@udel.edu>
 To: docter@uni-mainz.de

Mon, Jan 14, 2019 at 8:29 AM

Hi Dr. Docter,

This is Weila, a master student from the University of Delaware, the U.S. department of civil and environmental engineering and I'm working in Dr. Yan Jin's research group. I'm writing the INTRODUCTION part of my master's thesis ('The title is 'Transport of soil colloids and its relation to biogeochemical cycling of organic carbon under dynamic redox conditions') and I would like to cite the figure from your published paper in 2015 ('The nanoparticle biomolecule corona: lessons learned--challenge accepted? ' Fig. 10. Size range of Particulate Organic Matter (POM) and Dissolved Organic Matter (DOM). DOM is defined through filtration. The size limit used to differentiate DOM from particulate organic matter is arbitrarily set to around 0.45 µm). I'm writing this email to ask for permission from you for that citation. I appreciate your kind support for my thesis work!

Thank you!

All the best,

Weila

--

Weila (Vera) Li
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Docter, Dominic <docter@uni-mainz.de>
 To: Weila Li <verawei@udel.edu>

Tue, Jan 15, 2019 at 3:06 AM

Hi Weila,
 go ahead. Good luck with your thesis
 Kind regards,
 Dominic

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Von: Weila Li [verawei@udel.edu]
 Gesendet: Montag, 14. Januar 2019 16:29
 An: Docter, Dominic
 Betreff: Citation permission of the figure in your published paper
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<https://mail.google.com/mail/u/0/?ik=11b5a4216a&view=pt&search=all&permthid=thread-a%3Ar-155358269573626986&simpl=msg-a%3Ar-640821175...> 1/2

Figure D.2 Permission for use of Figure 1.2



Weila Li <verawei@udel.edu>

Ask for a citation permission of the figure in your published paper
2 messages**Weila Li** <verawei@udel.edu>
To: jyan235@ucmerced.edu

Mon, Jan 14, 2019 at 9:14 AM

Hi Jing,

I'm writing the INTRODUCTION part of my master's thesis (The title is 'Transport of soil colloids and its relation to biogeochemical cycling of organic carbon under dynamic redox conditions') and I would like to cite the figure from your published paper in 2016 ('Soil Colloid Release Affected by Dissolved Organic Matter and Redox Conditions' Fig. 5. Scheme of colloid mobilization and stabilization in the batch system). I'm writing this email to ask for permission from you for that citation. I appreciate your kind support for my thesis work!

Thank you!

All the best,

Weila

--
Weila (Vera) Li
Master Student
Interdisciplinary Science and Engineering Laboratory (ISE Lab)
Department of Civil & Environmental Engineering
University of Delaware
Phone# (302)-333-3097

Jing Yan <jyan235@ucmerced.edu>
To: Weila Li <verawei@udel.edu>

Mon, Jan 14, 2019 at 9:20 AM

Hi Weila,
Yes, no problem. Please use it.
Good luck with writing your thesis.
Bests,
Jing

Get [Outlook for iOS](#)

From: Weila Li <verawei@udel.edu>
Sent: Monday, January 14, 2019 8:14 AM
To: Jing Yan
Subject: Ask for a citation permission of the figure in your published paper

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Figure D.3 Permission for use of Figure 1.7



Weila Li <verawei@udel.edu>

Citation of one of your schematic diagrams

2 messages

Weila Li <verawei@udel.edu>
 To: "Vasilas, Bruce Larue" <bvasilas@udel.edu>
 Cc: Yan Jin <yjin@udel.edu>

Mon, Jul 2, 2018 at 7:46 AM

Hi Dr. Vasilas,

This is Weila, master student from Dr. Jin's group. I'm writing the MATERIALS AND METHODS part of my thesis recently and I want to cite one of your diagrams describing the hydrological zones of a vernal wetland system as attached. This diagram is from your research project (NE 2162: Hydropedology of Vernal Pool Systems). I'm writing this email to ask for a permission from you for that citation. Or if this diagram has been already published then I can cite the certain paper.

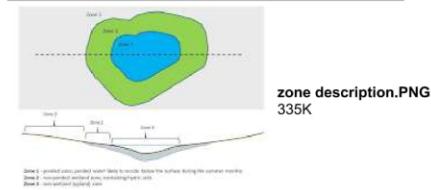
Thank you!

All the best,

Weila

--

Weila (Vera) Li
 Master Student
 Interdisciplinary Science and Engineering Laboratory (ISE Lab)
 Department of Civil & Environmental Engineering
 University of Delaware
 Phone# (302)333-3097



Vasilas, Bruce Larue <bvasilas@udel.edu>
 To: "Li, Weila" <verawei@udel.edu>

Mon, Jul 9, 2018 at 8:00 AM

Weila, You have my permission. It may have been given in a SSSA poster (2017; Rabenhorst et al.). Cite that if it was presented. It should be in the abstract. Rabenhorst also gave a paper on it. Both presentations compared IRIS tubes.

From: Weila Li <verawei@udel.edu>
Sent: Monday, July 2, 2018 10:47 AM
To: Vasilas, Bruce Larue <bvasilas@udel.edu>
Cc: Jin, Yan <yjin@udel.edu>
Subject: Citation of one of your schematic diagrams

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Figure D.4 Permission for use of Figure 3.2