Contribution of iron in clay minerals to redox cycle in paddy soils; enhancement of nitrogen-fixing iron-reducing bacteria in paddy soils

*Yuki Shimizu[1], Yoshio Takahashi[1], Yoko Masuda[2], Sakura Sato[2], Michinari Sunamura[1,3], Toshihiro Kogure[1], Keishi Senoo[2,3]

1. Department of Earth and Planetary Science, Graduate School of Science, The University of Tokyo, 2. Graduate School of Agricultural and Life Sciences / Faculty of Agriculture, The University of Tokyo, 3. Collaborative Research Institute for Innovative Microbiology, The University of Tokyo

Iron (Fe) is the most abundant element on Earth, and it circulates in the Earth's surface layers interacting with various chemical components and biota. In particular, the redox reactions of Fe(II) and Fe(III) are deeply related to the evolution of the Earth's atmosphere and microbial metabolism, and are very important from a geochemical point of view. In this study, we focused on the oxidation state and chemical species of Fe.

The redox state of Fe has a great influence on the behavior of various elements (such as arsenic and potassium; Takahashi et al., 2004; Khaled and Stucki, 1991) in the surface soil environment. However, until now, only the redox state of clay minerals (Stucki, 2011) and ferrihydrite in soils have been focused on individually. In this study, we analyzed the contribution of Fe-bearing clay minerals in soil to the redox reactions in soil.

Among the interactions between microorganisms and Fe, this study focused on microbial Fe reduction and nitrogen fixation in paddy soils. In this study, in addition to hematite and ferrihydrite (Masuda et al., 2021), we investigated whether Fe(III) in the octahedral structure of clay minerals (smectite) can be utilized by iron-reducing bacteria. In addition, we investigated whether Fe(III) in clay minerals enhances their nitrogen fixation ability.

In this study, we used the μ-XRF (X-ray Fluorescence)-XAFS method, STXM (Scanning Transmission X-ray Microscopy), and Mössbauer spectroscopy. In addition, we conducted incubation (reduction) and re-oxidation experiments using paddy soil and clay minerals. In these experiments, we mainly used the 1,10-phenanthroline method and XANES analysis to analyze Fe concentrations and species in the liquid and solid phases. In addition, culture experiments were carried out in a pure culture system and the nitrogen-fixing activity at that time was measured using the acetylene reduction activity (ARA) assay.

A new method was developed to determine the Fe(II)/Fe(III) ratio in clay minerals using Fe(II)-smectite and Fe(III)-smectite as the end components of linear combination fitting of XAFS spectra. This method enables us to investigate not only the simple Fe(II)/Fe(III) ratio but also the ratio of Fe(II) and Fe(III) chemical species in μ-XRF-XAFS analysis. From the soil incubation and re-oxidation experiments, it was found that about 30% of Fe(III)-smectite in the paddy soil was reduced. This is about 15% of total Fe, indicating the importance of Fe(III)-smectite in soil as an oxidizing Fe species. It was also shown that Fe(II) in the reduced clay minerals is re-oxidized by the atmosphere. Furthermore, it was found that most of the dissolved Fe(II) in the soil is probably present in the form of adsorbed smectite. Pure culture experiments revealed that *Geomonas terrae* utilizes Fe(III)-smectite as an electron acceptor and that Fe(III)-smectite enhances its nitrogen-fixing activity.

Unlike ferrihydrite and hematite, the Fe(III)-smectite investigated in this study has the advantage that

Fe(II) is not easily dissolved when reduced. Therefore, if the clay minerals remain in the paddy soil, a cycle can be formed in which Fe(II) in the clay minerals is oxidized to Fe(III) during the water fall period and reduced to Fe(II) by microorganisms during the waterlogged period. If the application of Fe(III)-smectite can enhance the nitrogen-fixing activitiy in actual paddy soil, it is expected to contribute to the reduction of artificial nitrogen fertilizer, which is one of the causes of the increase in CO_2 concentration.

Keywords: paddy soil, redox reaction, smectite, iron-reducing bacteria, nitrogen fixation, XAFS

