A role of a herbivorous crab, *Neosarmatium smithi*, in dissolved iron elution from mangrove ecosystems

Yasuhiro Nakanishi1*, Tatsuma Matsutani1, Ko Hinokidani1, Takashi Nagai2 and Mami Irie1

1 Graduate School of Agriculture, Tokyo University of Agriculture, 1-1-1 Sakuragaoka, Setagaya-ku, Tokyo 156-8502, Japan
2 Environment Science Department, Incorporated Foundation Okinawa Prefecture Environment Science Center, 720 Kyozuka, Urasoe, Okinawa 901-2111, Japan
* Corresponding author: ynaka@nodai.ac.jp

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ABSTRACT

Iron solubilization in mangrove soils associated with polyphenols leached out from leaf-litter can improve iron bioavailability. In this context, the leaf-removing process by mangrove crabs would increase reacting frequency of the polyphenols in mangrove leaves with iron in the soils. In this study, we investigated ecological roles of a leaf-removing crab, *Neosarmatium smithi*, on the iron solubilization process. After the fallen leaves carried by the crabs to their burrows and eaten by them, polyphenols may be remained in their feces. If so, contact of polyphenols in the feces with mangrove soils could promote elution of dissolved iron from the soils. In order to demonstrate this hypothesis, we firstly surveyed the appearance ratio of the black part in crab burrows and measured total phenolic content in feces of *N. smithi* as well as in the black part soil. Then, we examined influences of the crab feces on dissolved iron elution from mangrove soils. As the results, the appearance ratio of the black part in the burrow was 67% and the phenolic content in the feces, the black part, and the yellow part in crab burrows were 9.93, 0.49, and 0.12 mg g⁻¹, respectively. Dissolved iron content in the solution (soil + water extract from feces) was 0.65 μg g⁻¹ and this content was 4.5 times higher than the control (soil + distilled water). We suggest that the polyphenols remained in the feces affect to solubilize insoluble forms of iron by iron reduction and chelating properties.

Key words: mangrove, dissolved iron, herbivorous crab, polyphenols, tannins, feces

INTRODUCTION

In coastal marine ecosystems, riverine inputs are the dominant sources of nutrients, which contribute to the high bio-productivity. Among the various nutrients, iron is a micronutrient essential for phytoplankton growth, and it is indicated that the iron supplying system from land contributes significantly to primary production in marine ecosystem, especially of coastal area (Martin and Fitzwater 1988; Martin 1992; Matsunaga et al. 1998; Takeda 1998). Attention has been paid to a hypothesis that forest is a major source of dissolved iron to seawaters. For example, it was reported that organic complex iron (e.g. fulvic acid-iron compound) originated in humus layer in upland forest soils was a major source for the growth of marine phytoplankton (Matsunaga et al. 1998; Kuma et al. 1996).

It is known that mangrove species which grow in the intertidal zone of the tropics and subtropics contain high amounts of tannins which is classified as polyphenols, in their bark, leaves, and roots. For instance, it was reported that tannins content in leaves of nine mangrove species were 14.56–40.11% in dry-weight (Basak et al. 1999), and their ecological effects on nutrients cycle also have been investigated (Kraus et al. 2003). Those polyphenols such as tannins leached from leaf-litter can form organic complexes with insoluble forms of manganese and iron in soils, making them water-soluble forms (i.e. organic metal complexes; Levanidov 1957; Arakawa et al. 1993; Matsutani et al. 2013a).

We have focused on this chemical property and simply demonstrated that the role of the polyphenolic components in mangrove leaf-litter on the iron solubilization processes in mangrove forest soils by a laboratory experiment, and found the amounts of dissolved form of iron were significantly increased by mixing the water-extracts, which was extracted from mangrove leaves by distilled water, with soils compared with controls (soil + distilled water). Furthermore, the significant positive correlation was shown between amounts of iron eluted from soil mixed with the water extract and polyphenolic contents in the water-extract which was added to the soil (Matsutani et al. 2013a). This result indicated that water-soluble polyphenolic components
contained in mangrove leaves functioned to solubilize the insoluble iron in mangrove soils.

However, the chemical reaction above mentioned is not simple in the field, but it should be more complicated. In the intertidal zone and river mouth where mangroves grow, most of mangrove leaf-litter can be exported by tidal and river activities, which will reduce the reaction frequency of polyphenols in the fallen leaves with the forest soil. On the other hands, the presence of benthic animals that directly consume mangrove leaf-litter could increase the reaction between polyphenolic components leached from leaf-litter and mangrove floor soils by their feeding activities. For example, removal of fallen leaves by crabs into their burrows are generally known to be an important trophic pathway in mangrove forests, and this process can prevent the tidal export of detritus from mangrove ecosystems to adjacent ecosystems (Micheli 1993; Robertson and Daniel 1989; Ashton 2002; Kristensen 2008; Chen and Ye 2008).

According to our previous study conducted in a subtropical mangrove forest in Okinawa, southern Japan, it was found that a leaf-removing crab, *Neosarmatium smithi*, removed 71, 70 and 37 % of the total annual litter fall of *Bruguiera gymnorrhiza*, *Rhizophora stylosa* (mangrove species), and *Derris trifoliata* (non-mangrove species), respectively (Matsutani et al. 2013b). From the results, it has become clear that leaf-removing crabs carry most of the leaf-litter into their burrows and the removing process would mean an increased reacting frequency between polyphenols in mangrove leaves and iron in the soil. In fact, when we have dug up the burrows, many undegraded (or under fed) mangrove leaves were observed in the inner wall of their burrows (Fig. 1) as well as black colored part (Fig. 2). To identify the black part, we have observed behavior of *N. smithi* in captivity in laboratory. Then, it was found that the crab evacuates feces as it was rubbed against the wall of the experimental box (Fig. 3), and this behavior explains that the black parts were stemmed from the feces.

After the fallen leaves carried by the crabs to their burrows and eaten by them, polyphenolic components may be remained in their feces. If so, contact of polyphenols in the feces with the burrow soil could generate elution of dissolved iron from the soil. In order to demonstrate this hypothesis, we firstly surveyed the appearance ratio of the black part in crab burrows, and measured total phenolic content in feces of *N. smithi* as well as in the black part collected from the crab burrows. Finally, we examined influences of the crab feces on dissolved iron elution from mangrove soils.
METHODS

Study area

The study area selected was Shimajiri estuary (24°52′ N, 125°17′E) on Miyako Island, Okinawa as the area is conserved by the local municipality to maintain natural conditions and located close to our research station (Miyako Subtropical Farm, Tokyo University of Agriculture), being only about 15 km away. The climate of this area is subtropical, and the average annual rainfall and air temperature are 2,034 mm and 23.6 °C, respectively. The difference between the annual highest and lowest tide levels in the estuary is 2.3 m.

Vegetation in the estuary is a mixed forest containing five species; Rhizophora stylosa, Bruguiera gymnorrhiza, Derris trifoliata, Avicennia marina, and Kandelia obovata in order of stem densities. Except for D. trifoliata, all of the species are classified as mangroves (Spalding et al. 2010). The width from the creek edge to the bank edge in the forest is about 2 to 20 m, and the length of forest from the creek mouth to the upper end is about 500 m. Detritivorous crabs inhabiting the estuary were Neosarmatium smithi, Helice leachi, and Parasesarma bidens in order of population density. A detailed description of the study site can be found in Matsutani et al. (2013b).

Appearance survey of black part in crab burrows

Field surveys on appearance ratio of the black part on the wall of crab burrows were carried out using a study plot (10 m × 10 m) in an upstream area of Shimajiri estuary. N. smithi was the dominant detritivorous crab species in this site (Matsutani et al. 2013b). Firstly, the number of burrow (diameter; <25 mm) was counted in each small quadrat (1 m × 1 m; n = 8) which randomly put on the floor of study plot. Secondly, the burrow was dug up (to depth of 40 cm) to observe whether the black part was there on the inner wall. The colour characteristic of the inner wall soil was identified by the standard soil colour chart, and the black and the yellow parts were found to be black 2.5 Y 2/1 and dull yellow 2.5 Y 6/4, respectively.

Phenolic content in feces of N. smithi and the inner wall soils

Because the polyphenols are the organic compound synthesized by plants, phenolic substances found in environment (e.g. soils) should be stemmed from plants. Regarding the black part observed on the inner wall of crab burrows, if the black part contains much polyphenols, it would demonstrate that the black part is faecal material deposited by the crabs.

For this reason, we examined the total phenolic content in the inner wall soils of their burrow and in feces of N. smithi.

Faecal material: We collected 16 individuals of N. smithi crabs (male: n = 8; female: n = 8), with carapace width from 25 to 40 mm, from the study site on April and November in 2012. Each crab was kept individually in a plastic box (Polypropylene; L400 × W250 × H200 mm; n = 16) with 5 mm depth of brackish water (salinity: 1.5 %) and starved for 2 days in captivity (room temp.; 25 ± 5 °C). After the starvation, faecal samples (n = 16) which were adhered on the plastic wall were gathered from each box.

Inner wall soil: In the upstream area of Shimajiri estuary on November 11 in 2012, we collected two kinds of soil samples from the inside wall surface of the crab burrows; soils black in colour (the black part: n = 16) and soils yellow in colour (the yellow part: n = 16). Each part was collected carefully using a spatula. As the reference, we also collected upland soils (dark red soil: n = 6) from the neighbouring sugarcane fields, which were assumed to be the original source of mangrove floor soil in the estuary. The feces and soil samples were dried for 24 hours in a forced-air drier and ground into powder in a mortar.

Measurement of total phenolics: Total phenolic content in the samples were measured by the following method. Each 100 mg sample was mixed with 10 mL of an acetone-and-water solution (7: 3 v/v) and shaken for 1 hour. The solution was filtered through a glass fibre filter with a pore size of 1.7 μm (GF/A, Whatman). This extraction procedure was repeated three times for each sample. The total phenolic content in the filtrate was determined using the Folin-Ciocalteu method (Julkunen-Tiitto, 1985). In a 10 mL measuring flask, 1 mL of 1 N Folin-Ciocalteu reagent was added to 1 mL of the filtrate and the flask was vigorously shaken. After 3 minutes, 1 mL of 10 % sodium carbonate solution was added to the flask, and it was shaken thoroughly again. After 1 hour at room temperature (25 ± 5 °C), the absorbptivity of the solution was read at 700 nm using a spectrophotometer. In this analysis, tannic acid (Chinese gallotannin, with a molecular weight of 1701.2) solution was used as the standard reagent.

The aqueous acetone solution is generally used as efficient solvent for extraction of polyphenols, especially tannins (e.g. Hernes et al. 2001). Since tannins are the one of major target compounds in present study, the aqueous
acetone solution was selected as solvents in this experiment and tannic acid was used as a standard compound for determination of total phenolic content.

**Elution of dissolved iron from soil with extract solution from crab feces**

When the crab feces contain much polyphenolic substances, dissolved iron can be generated by meeting the polyphenols with iron in soil. To experimentally demonstrate this chemical reaction (i.e. complexation), we have conducted the simple experiment in which mangrove soils were mixed with water-extracts from crab feces in order to verify whether crab feces promote generation of dissolved iron in mangrove soil. In this study, dissolved iron was defined as under 0.2μm size following Gledhill and Buck (2012). The dissolved iron includes soluble forms (<0.02μm) and colloidal forms (0.02 to 0.2μm). The former includes inorganic ions (Fe$^{2+}$ and Fe$^{3+}$) and organically complexed iron, and the latter includes inorganic and organic colloidal iron (Gledhill and Buck 2012; Lough et al. 2019).

**Faecal materials and soils:** In order to collect faecal materials, we separately kept 10 individuals of *N. smithi* in plastic boxes and fed leaves of *B. gymnorrhiza* and *R. stylosa* by turns for 6 months. Faecal samples were collected every day, dried for 24 hours with a forced-air drier, and ground into powder in a mortar (the sum was about 50 g in dry weight). Mangrove soil samples were taken from surface layer (depth from 0 to 10 cm) of mangrove forest around *N. smithi* habitats in the study site where dominant mangrove species were *R. stylosa* and *B. gymnorrhiza*.

**Measurement of dissolved iron:** Extract of the feces was obtained from shaking the powder sample with distilled water (1:10 w/v) for 1 hour and filtering the resultant suspension through a glass filter with a pore size of 1.7μm (GF/A, Whatman). The soil sample was mixed with the extract or distilled water (as a control) at a ratio of 1:10 (w/v). After shaking the resultant suspension for 1 hour, it was filtered through a membrane filter with a pore size of 0.2μm (Millipore). The particulate iron (>0.2μm) was removed by this filtration processes, but the fine colloids and the soluble species passed the filter. The filtrate collected in a 50 mL Erlenmeyer flask was buffered at pH 3.2 with a 10 M formic acid 2.4 M ammonium formate buffer solution (0.25 M / 50 mL; Nishioka and Takeda, 2000). The amounts of dissolved iron eluted from the soil samples were measured by atomic absorption spectrometry.

As a reference, dissolved iron content in the supernatant obtained the feces was mixed only with distilled water was also measured in the same manner.

For a reference, concentration of phenolic compound in the extract solution from the feces was determined by Folin-Ciocalteu method (see described above section 2.3). It was found that the extract contained 0.03 mM phenolic compound (tannic acid equivalent).

**Statistical analyses**

Triplicate samples were used to calculate the mean and standard error values. One-way analysis of variance (ANOVA) was used to compare phenolic content and dissolved iron content among four types of materials (feces of *N. smithi*, black and yellow parts of the inner wall soil, and upland soil). To clarify the difference of phenolic content and dissolved iron content among the materials, the Kruskal-Wallis test was used to identify. When a significant difference was shown in the phenolic content, Steel-Dwass multiple comparison test was performed to compare the content among the groups. All analyses were performed using Excel Toukei 2010 (SSRI).

**RESULTS**

**Appearance survey of black part in crab burrow**

The average of density of crab burrow (diameter; <25 mm) was 20 ± 2 m⁻². Among them, the appearance ratio (AR) of the black part in the burrow was 67 ± 7% (mean ± S.E.) and the range of AR was 39 to 100% (Table 1).

**Phenolic content in feces of *N. smithi* and the burrow soils**

Phenolic content (mean ± S.E.) in feces of *N. smithi*, the black part, the yellow part, and dark red soil were 9.93 ± 3.14, 0.49 ± 0.07, 0.12 ± 0.02, and 0.11 ± 0.02 mg g⁻¹, respectively (Table 2). Thus, phenolic content in the feces was significantly higher than those in the other samples. Phenolic content in the black part was significantly higher (about 4 times higher) than those in the yellow part and dark red soil which were assumed to be the original source of mangrove soils in Shimajiri estuary.
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Dissolved iron elution from soil with extracted solution from crab feces

Fig. 4 shows influence of feces of *Neosarmatium smithi* on dissolved iron elution from mangrove soil. According to this result, dissolved iron content in the supernatant solution obtained after the mangrove soil was mixed with water extracts from feces of the crab was 0.65 ± 0.015 μg g⁻¹ (mean ± S.E.) and this content was 4.5 times higher than the control (soil + distilled water; 0.15 ± 0.015 μg g⁻¹). Dissolved iron content in the supernatant obtained after the feces was mixed only with distilled water remained very low (0.08 ± 0.003 μg g⁻¹).

**DISCUSSION**

What is the black part in crab burrows?

When we selected burrows of *Neosarmatium smithi*, as judged from the diameter of the burrow mouth, black parts were observed adhering to the inner soil wall of many burrows, and the appearance ratio was very high (over 60% in average). We have thought that the black part in burrow is originated in faecal materials of crabs, therefore we measured phenolic content in the black part and the neighbouring yellow part as well as in the feces of the crabs. As the results, we found that the feces contained almost 1% (w/w) of phenolics and the black parts (black 2.5 Y 2/1) also contained phenolics at approximately 4.5 times higher than those in the yellow parts (dull yellow 2.5 Y 6/4). These results suggest that polyphenols stemmed from mangrove leaves remained not only in the feces also in the black part on the inner soil wall of the burrows.

On the other hand, since the phenolic content in original crab feces collected in captivity was 20 times higher than that in the black part collected in the fields, it may need to discuss the other origins of the black parts rather than feces themselves. When iron in soil is under reductive condition, the colour of iron presents black (iron sulfides) or grey (reduced iron). However, as the inner surfaces of the crab burrows are exposed to air at low tide,
which induces the surface to be aerobic condition, iron in the surface sediments should be oxide. Despite of the aerobic condition, black parts were often observed in the inner wall, which is assumed to be 1) complexed iron produced by a reaction of phenolic substances with feces of the crab, and 2) attributed to accumulation of organic matter in the feces.

Influences of leaf-removing crabs on iron solubilization

Biomass and litter production in mangrove forests are huge (Komiyama et al. 2008), and although the mangroves supply significant amount of the leaf-litter into forest floor, the amount of leaf removal by crabs is also significant levels (Schories et al. 2003). For example, N. smithi is known to be a major leaf-removing crab species in mangrove forest and it is indicated that the sweeping behaviour by the crab are an important trophic pathway in mangrove ecosystems. In previous mangrove studies, there are some researches which focused on roles of the crab in macro nutrient cycling (e.g. litter decomposition; Robertson 1986; Lee 1997), but few studies have focused on the changes of chemical character in soil (or sediment) by intervention of the macrobenthos.

Mangroves highly contain polyphenols (e.g. tannin) in their leaf, and as the compounds have reductive properties for iron, they can make organic metal complex with iron (Kennedy and Powell 1985). Tannins can be divided into two groups (hydrolysable tannins and condensed tannin) and reported mostly water-soluble (Kuiters 1990; Hättenschwiler and Vitousek 2000, Kraus et al. 2003). Therefore, the polyphenols such as tannins easily leached out from the leaf tissue can significantly affect to the soil chemical character, especially iron dynamics.

The surface inner wall of crab burrows exposes to oxidative conditions by ventilation caused by burrowing activity by crabs (Mokhtari et al., 2016). For this reason, it was thought that iron oxides (e.g. crystalline Fe and hydroxide) were the dominant forms of iron in the surface soil. The above iron species are insoluble in water and not bioavailable. However, when polyphenols (from leaf-litter, or feces of N. smithi) are supplied to inner wall soil in the crab burrows, the iron oxides can be reduced by the reduction property of the polyphenols. And then, Fe^{2+} or Fe^{3+} produced by the reduction process can be complexed with catechol groups or carboxyl groups of polyphenols (the former is a catecholate, the latter is a carboxylate). The stability constants of organically complexed iron with polyphenols are relatively high (Perron and Brumaghim, 2009). According to the results of present study, feces of leaf-removing crab, N. smithi, contained phenolic compounds, and dissolved iron elution was promoted when the water extract obtained from crab feces was mixed with mangrove soil. On this iron solubilization in soil by the feces, we suggest that the phenolic compounds have affected to insoluble forms of iron (e.g. iron hydroxides) by iron reduction and chelating properties. However, as it cannot exclude possibility that other natural iron chelators (e.g. organic acids) than phenolic compounds help iron solubilize, further studies on this issue are in progress.

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