DIWPA: DIVERSITAS in the Western Pacific and Asia

DIWPA News Letter

Office: Center for Ecological Research, Kyoto University, Otsu, Shiga, Japan

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Message from the chairperson

Shin-ichi Nakano

We are delighted to present our 50th newsletter edition to you. Your participation in our journey is deeply appreciated. Additionally, DIWPA celebrated its 30th anniversary last year.

Our institution, CER, has obtained certification as a Joint Usage/Research Center (JURC) from the Ministry of Education, Culture, Sports, Science, and Technology (MEXT) of Japan. Consequently, we actively facilitate research in ecology and related sciences by providing open access to our research facilities and resources for both domestic and international researchers.

http://www.ecology.kyoto-u.ac.jp/en/joint.html

In the fiscal year 2023 (until the end of March 2024), MEXT is set to evaluate each of the approximately 70 JURC institutions and research centers in Japan. The primary objectives of this evaluation are: 1) to identify outstanding JURC institutions and research centers, 2) to certify them as International JURC (I-JURC) from FY 2024 to 2026, and 3) to provide financial support. Many JURC institutions and research centers in Japan have diligently prepared for I-JURC in the upcoming period, including our own application.

If we CER successfully get the position, the DIWPA Secretariat will expand its functions and strengthen its organization to better support hosting international workshops and symposia. We, at CER, aspire to demonstrate leadership in international collaborative research in Asia, initially serving as a core/hub for international ecology in East Asia. In the long run, our goal is to establish ourselves as the foremost core/hub for ecological research across all of Asia, bolstering our international presence. Additionally, we aim to attract collaborative researchers worldwide to CER, combining cutting-edge research information and technology from well-developed countries such as Europe and the United States. This approach will enable us to explore original and innovative research themes related to the rich ecosystems and biodiversity of the Asia-Pacific region, renowned for its 'Mega biodiversity. We earnestly hope to secure the I-JURC position.

Message from the Secretary General

Atsushi Ishida

Currently, Japan is facing the 10th wave of the novel coronavirus (COVID-19). However, social restrictions within the country have eased, and the number of visitors from foreign countries has fully recovered compared to the pre-pandemic period. Therefore, we anticipate the successful organization of the DIWPA International Field Biology Course (IFBC) in 2024. In that year, we plan to focus the IFBC on forest ecosystems and will hold it in the Ogasawara Islands, a UNESCO World Natural Heritage site in Japan. More detailed information about the DIWPA IFBC will be provided in the next issue or on our homepage.

In July 2021, Amami-Oshima Island, Tokuno-shima Island, the northern part of Okinawa Island, and Iriomote Island were designated as the fifth UNESCO World Natural Heritage site in Japan, following the recognition of Shirakami, Yakushima Island, Shiretoko, and the Ogasawara Islands. In this volume of the DIWPA Newsletter, we feature a report on the ecophysiology of mangrove plants conducted at Iriomote Island, a UNESCO World Natural Heritage site. We look forward to receiving reports from DIWPA members in future newsletters. Alongside the DIWPA newsletter, we hope to continue enhancing its function as a platform for the exchange of information on biodiversity conservation, as well as related ecosystems and societies.

Photos at Ogasawara

Report

Research on nitrogen uptake mechanisms in mangrove trees

Tomomi Inoue

National Institute for Environmental Studies (Japan)

Mangroves—the mysterious and rich forests

When you make your way through a mangrove forest, you may be surprised by how many things live there. For my field work in mangrove forests, I prefer ebb tide, when the forest floor is drained, facilitating access (Fig. 1a). Cute mudskippers skip along the mud (Fig. 1b), giant brackish-water snails gnaw fallen leaves (Fig. 1c), colorful crabs dance as they wave their claws in synchrony, and waterfowl peck at them. When you look up into the branches of the mangrove trees, you may spot the brilliant blue of kingfishers, and monkeys may be watching you back (Fig. 1d). Large mammals, such as deer and tigers, also live in mangrove forests. But although ebb tide facilitates field work, high tide brings other rewards, even though you'll get wet. At high tide, the sea invades the forest, and the landscape changes dramatically. A variety of fish swim swiftly through the intertwining tree roots and between your legs as you watch (Fig. 2). I was excited to watch a fever of large stingrays swim calmly into the forest. All of these inhabitants draw their energy from the mangrove trees' production of organic matter. Being adapted to intertidal environments that are physically and chemically harsh for plants, mangrove trees have developed various features that allow them to grow under highly saline hypoxic soils and tidal turbulence. The one that particularly catches my interest is their nitrogen uptake mechanisms.

Fig.1. (a) Field work is easier to conduct at ebb tide. (b-d) Living things in mangrove forests: (b) mud skipper in Vietnam; (c) brackish-water snails in Japan; (d) proboscis monkeys in Malaysia.

Fig.2. Fish swim swiftly through intertwining tree roots.

How do they grow so large on low-nitrogen tidal flats?

Tidal flats, where mangrove trees grow, tend to have low soil nitrogen content owing to repeated tidal export of nitrogen-containing litter to the ocean (Alongi *et al.*, 1992; Feller *et al.*, 2003a, 2003b; Lovelock *et al.*, 2004; Reef *et al.*, 2010). Yet mangrove trees actively produce leaves and can grow large (Fig. 3), which suggests mechanisms that support nitrogen uptake, but the process has been long debated. While taking a walk in a mangrove forest, I came up with an experimental design to test the relation between mangrove trees and nitrogen fixers—diazotrophs, which convert atmospheric nitrogen to ammonia. If diazotrophs live near or in mangrove tree roots, the trees can acquire nitrogen, as long as the diazotrophs have contact with the atmosphere. I decided to measure soil nitrogen fixing activity in isolated mangrove trees of various sizes each day for a month on Iriomote Island, Japan. The results showed high levels of nitrogen fixation around the roots (Fig. 4; Inoue *et al.*, 2020). Around the roots of smaller and therefore likely

Fig. 3. Giant *Sonneratia alba* tree on Pohnpei Island, Federated States of Micronesia.

younger trees, the diazotrophic community most closely resembles that found in unvegetated mudflats, but as trees grow and develop higher root biomass, it evolves to recruit plant-growth-promoting bacteria (Inoue *et al.*, 2020). The results reveal mutualistic relationships between mangrove trees and diazotrophs. However, mysteries remain, as at high tide, the nitrogen supply through the water to diazotrophs can be limited, and the partial pressure of N_2 is significantly lower in submerged soil than in the atmosphere (Inoue *et al.*, 2024).

Gifted roots

Mangrove trees have distinctive aerial roots (Tomlinson, 1986) that can provide support for the trees on the unstable muddy flats. Different tree species have specific

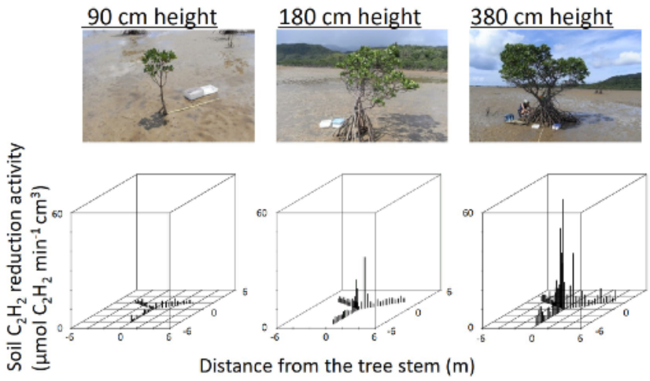

Fig. 4. Soil nitrogen fixing activity (measured as C_2H_2, reduction activity) around roots of *Rhizophora stylosa* trees.
Nitrogen fixation increased with tree size.

root types, resembling octopus arms (stilt roots, Fig. 5a), bamboo shoots (pneumatophores, Fig. 5b), or bent knees (knee roots, Fig. 5c). In all types of aerial roots, many small pores called lenticels on the root surface provide a route of entry for air via inner spongy tissues, called columnar lacunae, that provide an air path down to the buried roots (Scholander *et al.*, 1955; Curran *et al.*, 1986; Ish-Shalom-Gordon & Dubinsky, 1992). Evolution of these features allows aerobic respiration in the root cells of mangrove trees (Scholander *et al.*, 1955; Curran *et al.*, 1986; Skelton & Allaway, 1996; Allaway *et al.*, 2001). But that may be not all: as about 80% of air is nitrogen, the path may carry nitrogen to the diazotrophs in the mangrove roots.

Isotope tracer techniques are useful to track the movement of elements. I used $^{15}N_2$ tracer gas to track the movement of nitrogen gas through the mangrove root system in the field (Fig. 6). The $^{15}N_2$ tracer gas that passed through the lenticels into the aerial roots was detected incorporated into organic forms of nitrogen in the belowground parts of the roots, in which rates of diazotrophic nitrogen fixation were high (Inoue *et al.*, 2019; Inoue *et al.*, 2024). These facts confirm that N_2 is supplied to diazotrophs through the internal air path of mangrove aerial roots, which we observed in two mangrove species in different families, namely the Acanthaceae and the Rhizophoraceae.

Fig. 5. Aerial roots of mangrove trees: (a) stilt roots; (b) pneumatophores; (c) knee roots.

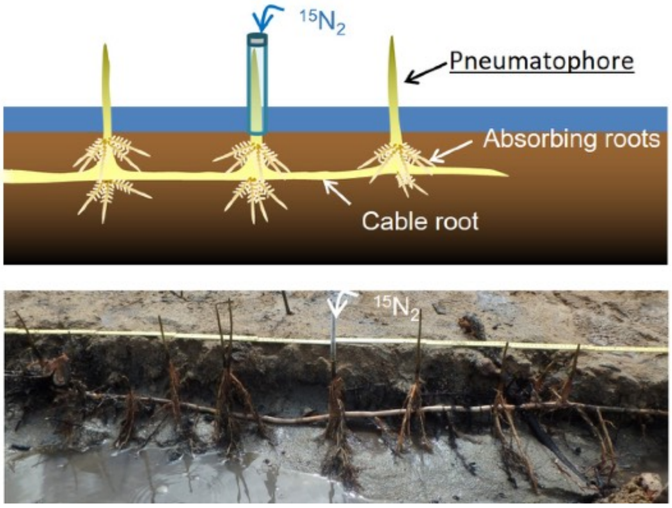

Fig. 6. Field experiments using $^{15}N_2$ tracer gas to track the movement of nitrogen gas through the mangrove root system. $^{15}N_2$ gas was injected into a chamber attached to an aerial root, whole roots were later sampled, and $\delta^{15}N$ of root samples was analyzed.

Adaptation to low-nitrogen tidal flats?

Mangrove trees evolved around 55 million years ago from terrestrial plants. According to the results of a genomic study, multiple taxa have independently invaded tidal flats, and mangroves of the Acanthaceae and the Rhizophoraceae, as mentioned above, originated from different invasions (He *et al.*, 2022). In addition, symbiosis between plants and diazotrophs have also been observed in seagrasses, which invaded seacoasts around 100 million years ago (Welsh, 2000; Mohr *et al.*, 2021). Establishing an association with diazotrophs may be a key to adapting to low-nitrogen coastal ecosystems, and the distinctive structure of mangrove aerial roots could be an adaptation not only to anoxic soils but also to nitrogen-limited tidal flats.

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From DIWPA Office

We are very happy to share our **50th** ✨ newsletter edition with you! Celebrate with us! We ask for your continued support for our next phase.
Please see the back issues on
https://diwpa.ecology.kyoto-u.ac.jp/category/diwpa-news-letter/

2024 DIWPA International Field Biology Course will be held in the Ogasawara Islands. The details will be announced on our website. Don't miss it!

DIWPA New Letter is published in PDF format only on our website to promote paperless. Thank you very much for your understanding.

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