Identification of the key biological nitrification inhibition (BNI) compound from maize roots

Specific plants can suppress nitrification in soil by releasing inhibitory natural products from their roots, a chemical-ecological phenomenon called biological nitrification inhibition (BNI). BNI utilization is a useful strategy for solving environmental problems (e.g., water pollution by NO_3; production of the greenhouse gas N_2O) and improving nitrogen uptake while reducing nitrogen losses from agricultural fields. The crucial property for the isolation of BNI compounds is whether their root exudates, extracts, and compounds are water-insoluble (hydrophobic) or water-soluble (hydrophilic). While hydrophobic compounds with lower mobility are retained in the rhizosphere, hydrophilic compounds can move further away from the roots. In a previous study, two major hydrophobic BNI-contributing compounds from the root surface (zeanone and HDMBOA) were identified in maize, together with two analogs of HDMBOA from inside the roots (Fig. 1). Our objective in this study is to identify the chemical structure and function of a hydrophilic BNI-active compound from maize.

The most BNI-active compound in hydrophilic BNI-activity from maize roots was identified as 6-methoxy-2(3*H*)-benzoxazolone (MBOA). MBOA has been detected in several *Poaceae* species such as maize and wheat. MBOA strongly inhibited the growth of *Nitrosomonas europaea* (Fig. 2). In a soil incubation experiment, NO_3 production was suppressed in the presence of MBOA during incubation for 4 days, and BNI-activity declined in parallel with MBOA biodegradation after incubation for 5 days (Fig. 3). Further experiments suggested that two benzoxazinoids, HDMBOA and HDMBOA-β-glucoside, which are chemically and biologically unstable in soil, respectively, could be converted into the more stable BNI-active MBOA in the soil (Fig. 4). Therefore, MBOA is a key component in the BNI-activity of maize.

MBOA is degraded in soil via microbial reaction, while new MBOA can be constantly produced and released by living maize. Hence, maize can stably exhibit BNI activity. The BNI compounds identified in our study are a promising indicator for evaluating BNI capacity among maize species, and can lead to the development of BNI-enhanced maize.

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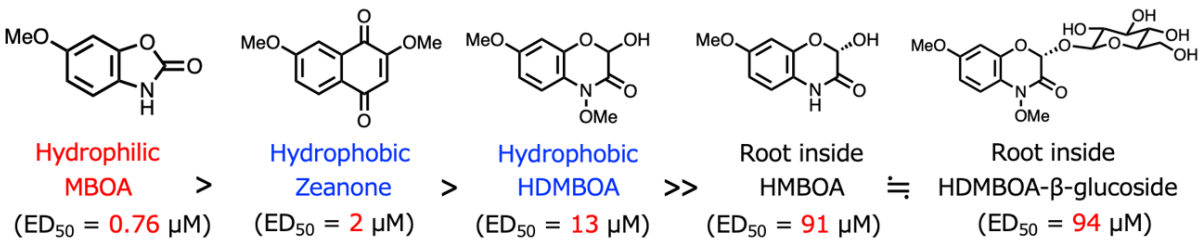

Fig. 1. Structures and BNI activities of identified BNI compounds from maize roots
The values in the parentheses indicate BNI activities. A smaller value means stronger BNI activity.

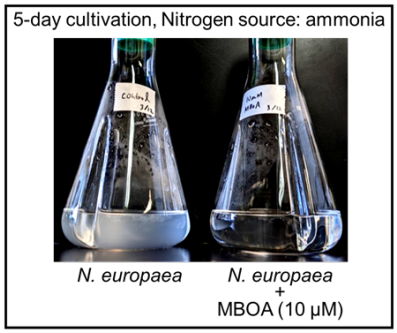

Fig. 2. Effect of MBOA on the growth of *N. europaea*
(Left) *N. europaea* in cloudy medium; (Right) Clear medium caused by suppression of the growth of *N. europaea* in the presence of MBOA.

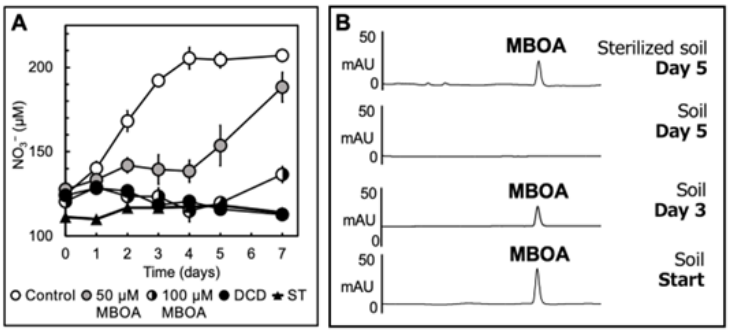

Fig. 3. Effect of MBOA on nitrification and its stability in soil incubation test
Soil was incubated under the presence of MBOA, and the concentrations of NO_3 and MBOA were time-dependently measured. Day 0 (start) means the result 1 hour after application of MBOA. (A) Control: water was used instead of MBOA., DCD: chemical nitrification inhibitor dicyandiamide, ST: sterilized soil. (B) Concentration of MBOA was measured by HPLC.

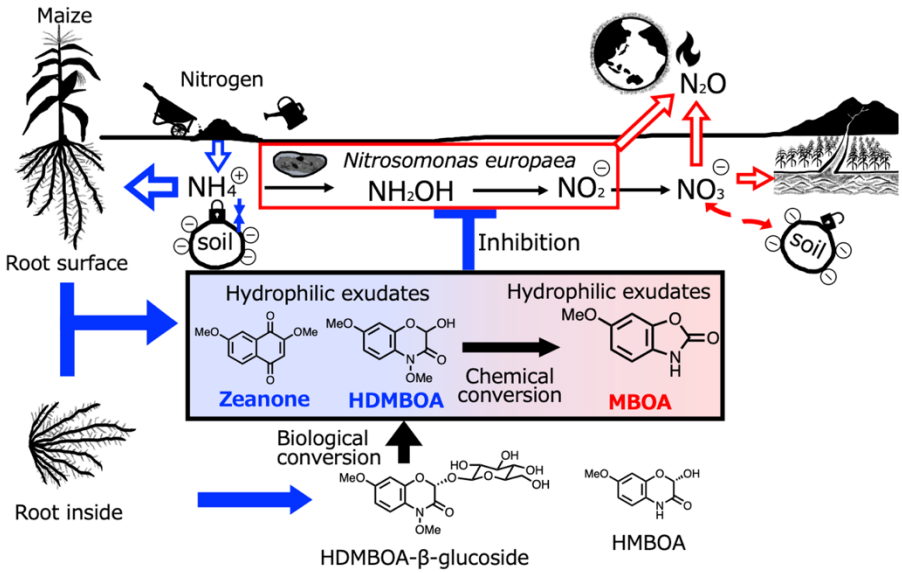

Fig. 4. BNI mechanism of maize
BNI of maize is exhibited by three BNI-active compounds including the key MBOA.

Reference: Otaka, et al. (2023) *Plant Soil* 489: 341-359.