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Plant rhizopsphere is a hotspot for greenhouse gas emissions

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Nitrous oxide (N_2O) is a greenhouse gas that also degrades stratosphere ozone. Marked N_2O emission were detected from soybean root systems with degraded nodules during late growth stage in field-grown soybeans. A model system developed to produce N_2O emissions from soybean fields. Soybean plants inoculated with *nosZ* mutant of *Bradyrhizobium japonicum* USDA110 (lacking N_2O reductase) were grown in aseptic jars. After 30 days, shoot decapitation (D, to promote nodule degradation), soil addition (S, to supply soil microbes), or both (DS) were applied. N_2O was emitted only in the DS treatment. Thus, both soil microbes and nodule degradation are required for the emission of N_2O from the soybean rhizosphere. The N_2O flux peaked at 15 days after DS treatment. A ^{15}N tracer experiment indicated that the N_2O was derived from N fixed in the nodules. As for nitrification, the addition of nitrification inhibitors significantly reduced N_2O flux. Both AOA and AOB were detected by PCR analysis with N_2O emission profile in soybean rhizosphere. The N_2O flux from the *nirKnosZ* mutant rhizosphere was significantly lower than that from *nosZ* mutant, but was still 30% to 60% of that of *nosZ* mutant, suggesting that N_2O emission is due to both *B. japonicum* and other soil microorganisms. Only *B. japonicum nosZ+* strains could take up N_2O. In particular, *Fusarium* spp., a soil fungus may contributed to N_2O emission in soybean rhizoshere. From these results, the organic-N inside of the nodules was mineralized to NH_4^+, and N_2O producing processes (nitrification and denitrification) simultaneously occur in the soybean rhizopsphere. We continue to examine which microbes really mediated N_2O methabolism using isotopic techniques including ^{15}N site preference of N_2O molecules. N_2O emissions from soybeans ecosystems can be mitigated by inoculting *B. japonicum* mutants with increased N_2O reductase activity (Nos++ strains). The mutation of *nasS* gene is responsible for the Nos++ phenotype. We propose that *nasS* mutation might be an effective strategy to induce higher Nos activities in N_2O-reducing rhizobia, such as indigenous isolates from local soybean fields or even from other important leguminous crops such as alfalfa, and thus to mitigate N_2O emission.

Plants have mutualistic symbiotic relationships with rhizobia and fungi by the common symbiosis pathway, in which Ca_2^+/calmodulin-dependent protein kinase (encoded by *CCaMK*) is a central component. Although *OsCCaMK* is required for fungal accommodation in rice roots, little is known about the role of *OsCCaMK* in rice symbiosis with bacteria. Here, we report the effect of a *tos17*-induced *OsCCaMK* mutant (NE1115) on CH_4 flux in low-nitrogen (LN) and standard-nitrogen (SN) paddy fields as compared with wild-type (WT) Nipponbare. Growth of NE1115 was significantly decreased compared with that of WT, especially in the LN field. The CH_4 flux of NE1115 in the LN field was significantly higher (156?407% in 2011 and 170?816% in 2012) than that of WT, although no difference was observed in the SN field. The copy number of pmoA was significantly higher in the roots and rhizosphere soil of WT than those of NE1115. However, mcrA copy number did not differ between WT and NE1115. These results were supported by a ^{13}C-labeled CH_4-feeding experiment. In addition, the natural abundance of ^{15}N in WT shoots (3.05 permile) was significantly lower than in NE1115 shoots (3.45 permile), suggesting higher N_2 fixation in WT due to dilution with atmospheric N_2 (0.00 permile). Thus, CH_4 oxidation and N_2 fixation were simultaneously activated in the root zone of WT rice in the LN field, and both processes are likely controlled by *OsCCaMK*.

Keywords: methane, nitrous oxide, rhizosphere, Bradyrhiozbia, bacteria, stable isotope