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“Development on the margin”

## Assessing the Sources of Biological Nitrogen Fixation in a Natural, Flooded Rice-field System using a Field $^{15}\text{N}_2$ -labeling Technique

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### Abstract

The importance of biological nitrogen fixation (BNF) to the global nitrogen (N) cycle and plant nutrition is well-known, but common methods for BNF measurement are only qualitative and semi-quantitative. Exposing an intact soil-plant system to a  $^{15}\text{N}_2$ -enriched atmosphere in a natural environment could be the only direct method for quantifying BNF. We introduced a fully enclosed, automated growth chamber to estimate BNF using a  $^{15}\text{N}_2$ -labeling technique in a natural paddy rice field. The controlled growth chamber monitors simultaneously humidity, temperature and carbon dioxide concentration and continuously adjusts these parameters according to the environmental conditions. In this chamber, rice (*Oryza sativa* L.) growing in pots of flooded soil was exposed to a  $^{15}\text{N}_2$ -enriched (approx. 10 atom-%) atmosphere to assess BNF activities associated with rice. A non-enriched,  $^{14}\text{N}_2$ -incubation system was included as control. As limited knowledge is available about the BNF contribution of phototrophic,  $\text{N}_2$ -fixing bacteria, the surface of selected pots was covered by black cloth to manipulate specifically the activity of. After 70 days incubation, phototrophic blue green algae (BGA) from soil surface (0–1 cm) were obtained and subjected to isotope ratio mass spectrometry. Highest  $^{15}\text{N}$ -enrichments were observed in BGA (2.9193 atom-% as opposed to 0.3703 atom-% in the  $^{14}\text{N}_2$ -control chamber). Their significant contribution to N input into flooded rice fields was further confirmed by the higher  $^{15}\text{N}$ -abundance (0.7586 atom-%) found in the soil surface not covered with black cloth as compared to the covered soil surface (0.4387 atom-%). Our results provided evidence that the introduced  $^{15}\text{N}_2$ -labeling technique was suited to assess the sources and amounts of biologically fixed N in a natural, flooded rice-field system. To gain further insights into the ecology and actual contribution of  $\text{N}_2$ -fixing microorganisms in the highly complex soil/rice ecosystem, we will extend our studies by using molecular techniques to study the functional gene encoding nitrogenase (nifH gene), the essential enzyme required for BNF as performed by  $\text{N}_2$ -fixing microorganisms. This gene will be investigated in more detail by  $^{15}\text{N}$ -DNA-based stable isotope probing and quantitative polymerase chain reaction techniques to identify those microorganisms actively contributing to BNF.

**Keywords:**  $^{15}\text{N}_2$ -labelling technique, biological nitrogen fixation, growth chamber