<STUDY_ABSTRACT>
Bacterial community shifts in paddy rice ecosystems under different N fertilizer regimes

<STUDY_DESCRIPTION>

General description. The reduction of fertilizer usage is one of preferable field managements to attain sustainable agriculture. Herein, we applied metagenomic analysis to rice root-associated microbiome to understand microbial community shifts in paddy rice ecosystems under different N fertilizer regimes. Rice was cultivated in paddy fields dressed with low N (LN; 0 kg ha^{-1}), standard N (SN; 30 kg ha^{-1}) and high N (HN; 30o kg ha^{-1}). In particular, LN field has not been subjected to N fertilization for 5 years. Our metagenomic analysis revealed low input of N fertilizer drastically changes rice root-associated microbes relevant to N_2 fixation, plant hormonal effect and methane cycling.

Plant materials and field experimental design. We used rice (*Oryza sativa* L.) cultivar "Nipponbare" whose whole genome sequencing have been finished in 2005 (International Rice Genome Sequencing Project 2005). Nipponbare is improved variety and was bred at Aichi Agricultural Center in Japan in 1963 (Namai et al. 2009). Nipponbare was classified as Japonica based on principal coordinate analysis using 179 restriction fragment length polymorphism data (Kojima et al 2005), and *Temperate Japonica* based on the C.S. Chord distance (Cavalli-Sforza and Edwards 1967) using 169 simple sequence repeats marker data (Garris et al. 2005). Seeds were placed on two layers of filter paper (Advantec-Toyo Ltd., Tokyo, Japan) in a Petri dish (6-cm diameter) containing 4 mL tap water. The Petri dishes were placed in an incubator at 30 °C. After two days (12 April 2009), the germinated seeds were sown in a commercial soil (Mitsui-Toatsu No. 3, Tokyo, Japan) in a 60 × 30 cm cell tray (cell diameter, 1.5 cm; depth, 3 cm) and grown in a greenhouse under natural light conditions for four weeks. Total of 300 seedlings of each cultivar were planted in an experimental field in a square pattern (10 × 10 plants). Hills were spaced 30 cm apart. Rice seedlings were grown in three neighboring fields. These are standard nitrogen (SN), low nitrogen (LN) and high nitrogen (HN). Basal fertilizer (P_2O_5 and K_2O with or without N) was applied to the paddy fields four days before transplanting. In the SN paddy field, N, P_2O_5 and K_2O (Temairazu 666, Co-op Chemical Co.,Ltd., Tokyo, Japan) were fertilized at 30, 30 and 30 kg ha^{-1}, respectively. In the LN field, only P_2O_5 and K_2O (phosphorus-potassium fertilizer with magnesium No.46, Co-op Chemical Co.,Ltd., Tokyo, Japan) were added at 30 kg ha^{-1} each. The LN paddy field has been used for rice cultivation using the same field management procedure employed in the SN field except that no nitrogen has been applied to the LN field since 2004. In the HN paddy field, N, P_2O_5 and K_2O were fertilized at 30, 30 and 30 kg ha^{-1} as basal fertilizer. Additionally, 30 or 60 kg ha^{-1} of ammonium sulfate (Ube Agri-Materials, Ltd., Tokyo, Japan) as additional fertilization fertilized at every two weeks to fertilize total of 270 N kg ha^{-1} in the HN paddy field. 5 L ha^{-1} of chemical herbicide (Kusatory DX Flowable L, Mitsui Chemicals Agro, Inc., Tokyo, Japan) with emulsion formulation was used at May 28, 2009. 5 Kg ha^{-1} of bactericide (Oryzemate, Meiji Seika Kaisha, Ltd., Tokyo, Japan) with granular formulation was used to tolerant to rice blast at 14 June, 2009. 3 L ha^{-1} of insecticide (Nagekomi Trebon, Kumiai Chemical Industry Co., Ltd., Tokyo, Japan) with emulsion formulation was used at 23 June, 2009. 1 L ha^{-1} of insecticide (Starkle mate™, Mitsui Chemicals Agro, Inc., Tokyo, Japan) with liquid formulation was used at 13 August, 2009. Daily Meteorological data during the experiment was obtained from Sendai District Meteorological Observatory (Japan Meteorological Agency, 2009; http://www.jma.go.jp/jma/indexe.html) using Automated Meteorological Data Acquisition System (Fig. 1).

Shoot length, Tiller number and Shoot fresh weight were measured as indication of growth at 90 days after transplanting (Fig. 2). The cut into soil around a rice plant was put in all sides 30 cm with depth about 30 cm from soil surface. The plant was turned up and washed with irrigation. The plant was washed with tap water until the dust disappears from root in laboratory and stored at –80 °C until they were used.

Soil metadata. The paddy field has been continuously managed for wetland rice production since 1940 as an experimental field in Tohoku University. Soil type is classified as Gray Lowland Soil (Classification of cultivated soils in Japan, 3ed approximation) with water table depth 97 cm, which is located on alluvial plain (Sendai plain) as geomorphic position. The characteristics of the field soils in LN used in the present study are pH (H_2O), 5.7; total carbon content, 18.3 %; total nitrogen content, 1.1 %; truog phosphorous content 86.0 mg P_2O_5 kg^{-1}. In similar to LN, that of SN were 5.5, 21.1 %, 1.3 %, 72.5 mg P_2O_5 kg^{-1}, and that of HN were 5.4, 23.6 %, 1.2 %, 66.5 mg P_2O_5 kg^{-1}. A detailed analysis of the soil profile was conducted on December 5, 2003 by Dr. M. Nanzyo (Tohoku University). Soil pedon (Fig. 3) have been sampled from this field and described as follows:

Location: 134-2, Uchinoura, Hironaga, Kashimadai, Osaki, Miyagi, Japan

Latitude: 38-27-39.37'N

Longitude: 141-5-33.33-E

Altitude: 4 m a.s.l.

Classification: Gray lowland soil (classification of cultivated soils in Japan, 3rd approximation)

Geomorphic Position: Alluvial plain

Slope Characteristics: Flat

Precipitation: Udic soil moisture regime

Water Table Depth: 97 cm

Drainage: Poorly drained

Land Use: Paddy rice field

Parent material: Unconsolidated alluvium

Vegetation: Paddy field after harvest

Described by: M. Nanzyo

Date: December 5, 2003

Apg 0 to 10 cm: grayish yellow brown (10YR4/2) moist, clay loam; few faint medium brown (10YR4/6) Fe masses, massive; friable, sticky, plastic; many very fine and common fine roots; clear smooth boundary; positive dipyridyl reaction (++).

Bg 10 to 18 cm: 70% dark olive gray (2.5GY4/1) and 30 % brown (7.5YR4/6) moist, clay; many fine distinct Fe masses; massive; friable, sticky, plastic; many very fine and common fine roots; common very fine tubular pores; abrupt smooth boundary; dipyridyl reaction (±).

Cg 18 to 26 cm: 90% gray yellowish brown (10YR5/2) and 10% brown (7.5YR4/6) moist, clay; common fine to medium Fe masses, massive; friable, sticky, very plastic; common very fine roots; common very fine tubular pores; abrupt smooth boundary; negative dipyridyl reaction.

Ag 26 to 41 cm: 50% gray yellowish brown (10YR4/1) and 30% brownish black (10YR3/1) and 20% brown (7.5YR4/6) moist, clay; massive; friable, sticky, very plastic; few very fine roots; common very fine tubular pores; clear smooth boundary; negative dipyridyl reaction.

Cg2 41 to 65 cm: 90% grayish yellow brown (7.5YR6/2) and 10% yellowish brown (10YR5/6) moist, clay; common medium to coarse prominent Fe masses, massive; friable, sticky, very plastic; few very fine roots; common very fine tubular pores and few medium tubular pores; abrupt; abrupt smooth boundary; negative dipyridyl reaction.

Cg3 65 to 78 cm: greenish gray (7.5GY5/1) moist, sandy loam; few medium prominent dark reddish brown (5YR3/6) Fe masses, massive; friable, slightly sticky, non plastic; no roots; few fine tubular pores; clear smooth boundary; positive dipyridyl reaction (+++).

Cg4 78 to 86 cm: dark olive gray (5GYR4/1) moist, fine loamy sand; massive; friable, slightly sticky, non plastic; no roots; few fine tubular pores; clear smooth boundary; positive dipyridyl reaction (+++).

Cg5 86 to 100+ cm: greenish gray (7.5GR5/1) wet, sandy loamy; massive; friable, slightly sticky, non plastic; no roots; few fine tubular pores; positive dipyridyl reaction (+++).

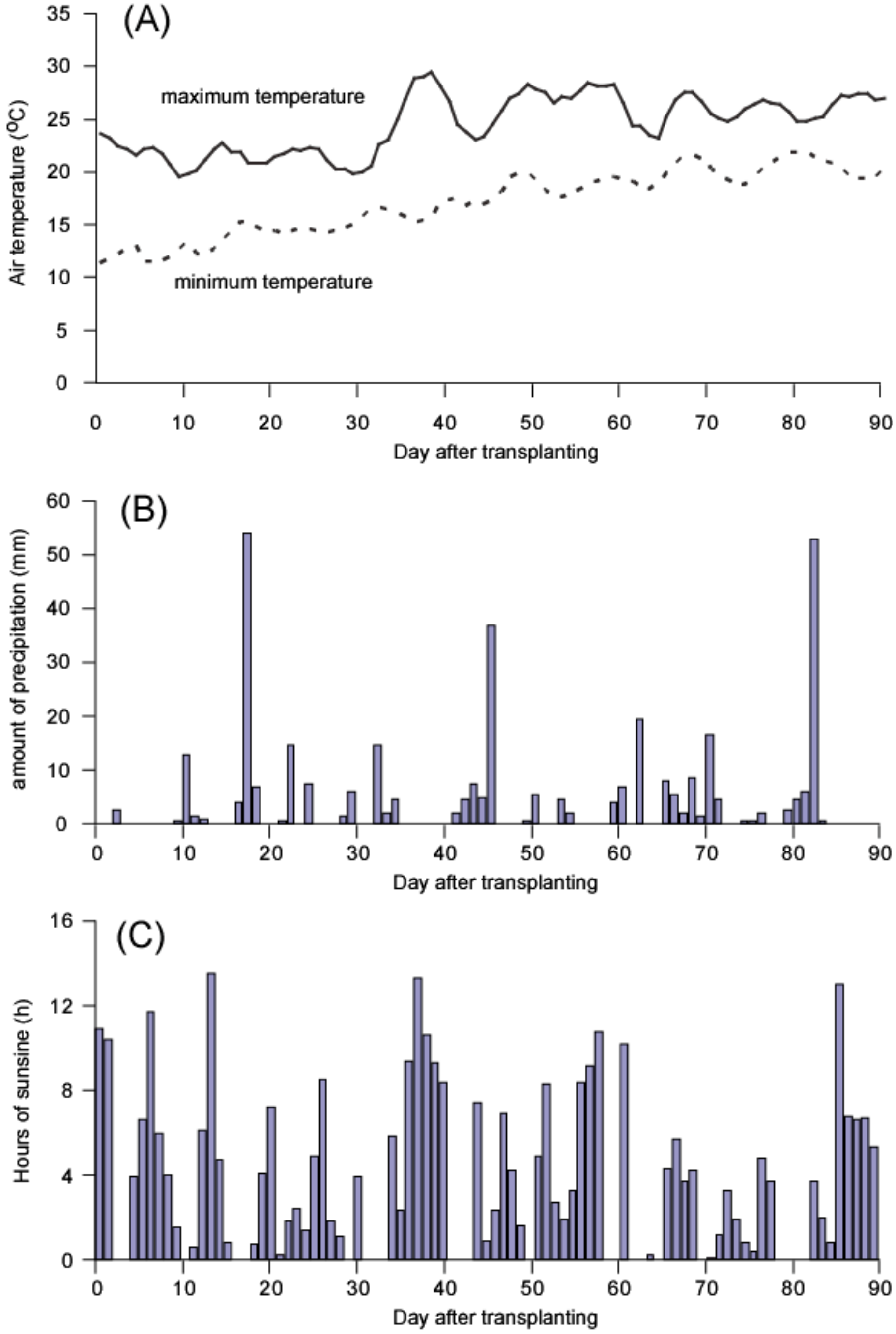

Fig. 1. Meteorological data during cultivation in paddy fields in 2009.
(A) Solid and undulating lines indicate maximum and minimum temperature, respectively (5-day moving average). (B) Total amount of precipitation par a day. (C) Total hours of sunshine par a day.

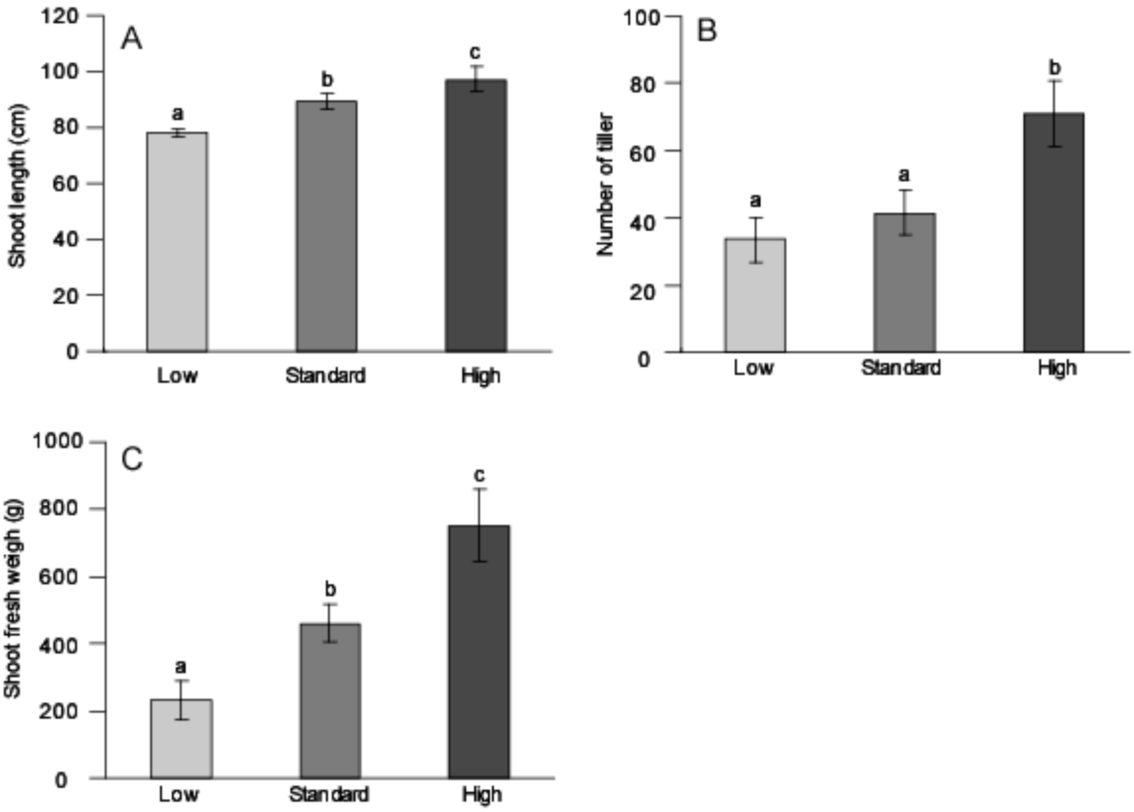

Fig. 2. Growth measurements for (A) shoot length, (B) number of tiller, and (C) fresh weight of shoot of rice under low, standard, and high nitrogen conditions. a common letter (a-c) are not significantly different according to Tukey's test for pair wise mean

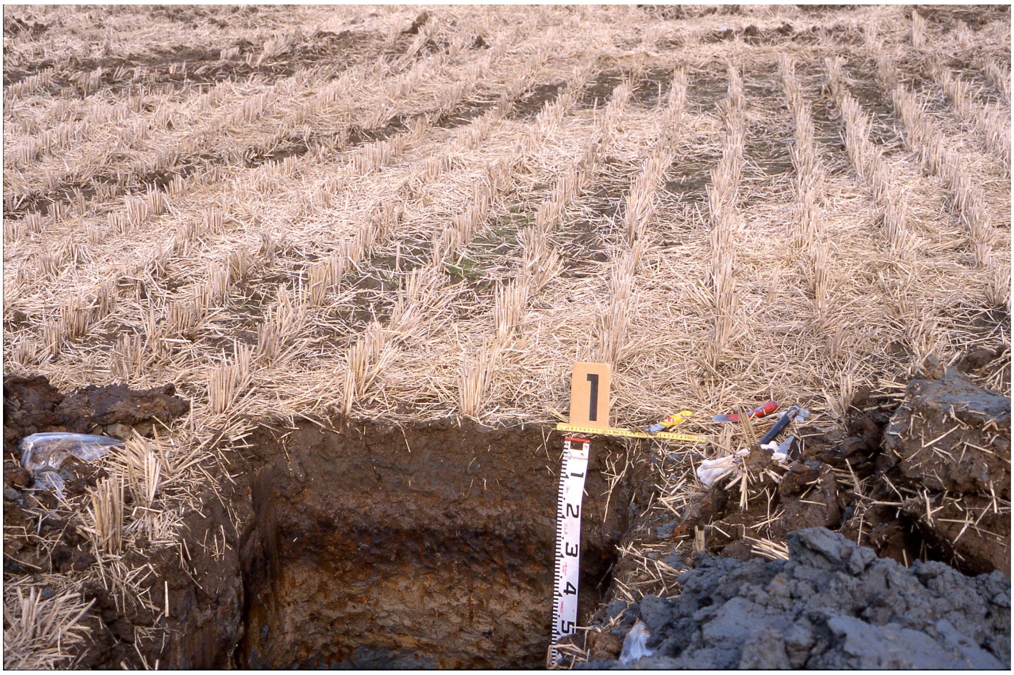

Fig. 3. Soil pedon was sampled on December 5, 2003 by Dr. M. Nanzyo (Tohoku University).

Fig. 3. Continued.

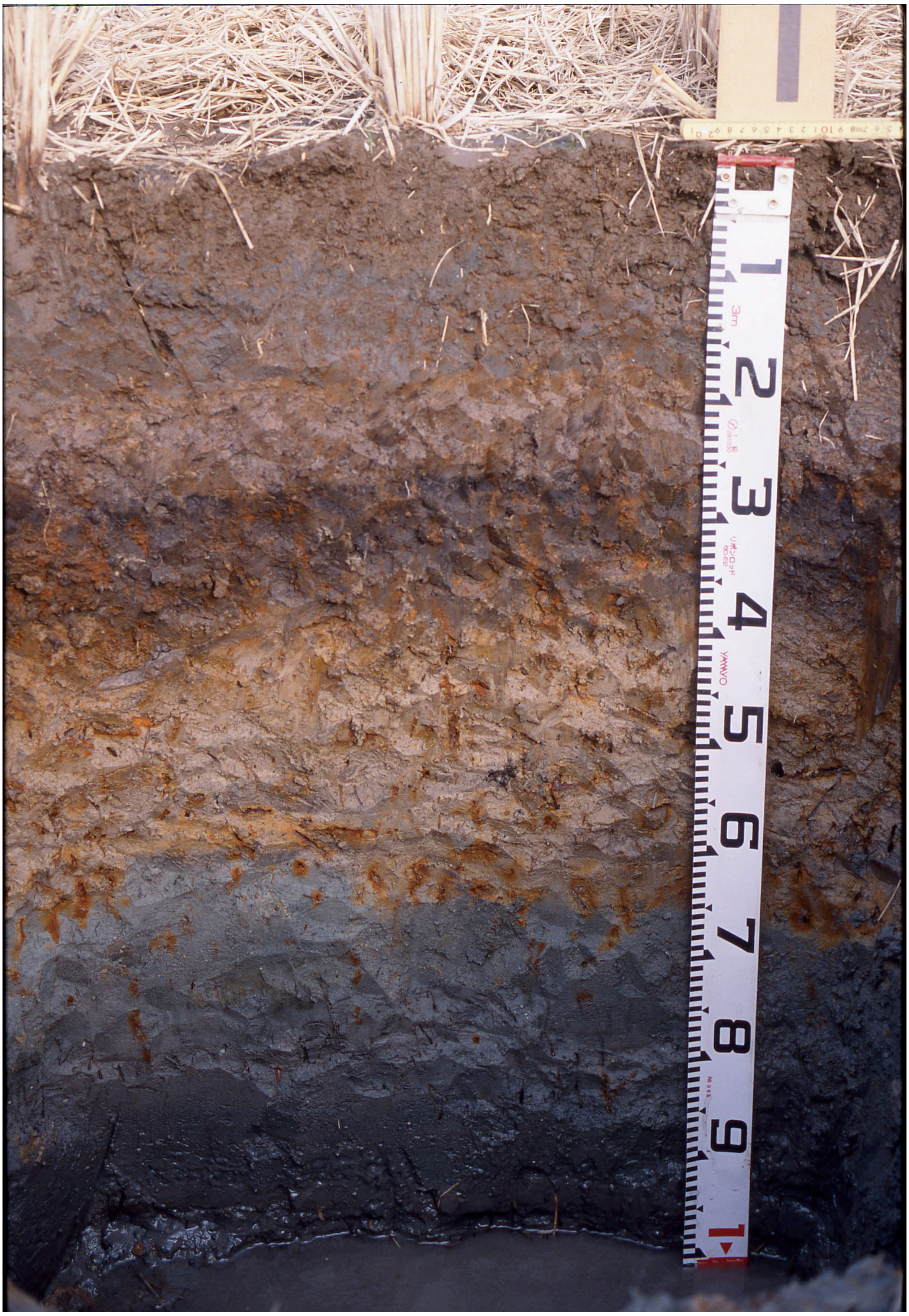

Fig. 3. Continued.

Reference

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