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Effect of rice variety and fertilizer type on the active microbial community structure in tropical paddy fields in Sri Lanka

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ABSTRACT

Tropical rice fields are a special class of wetlands, which are vital for world food production. Microbial communities that drive biogeochemical processes and nutrient availability for rice growth rely on rhizodeposited C as a source of energy. The current study revealed information on the structure of the active microbial community in tropical paddy fields in Sri Lanka by employing in situ Phospholipid Fatty Acids based Stable Isotope Probing (PLFA-SIP) with $^{13}CO_2$ pulse labeling. The study was conducted in selected rice fields cultivated long-term with two rice varieties (traditional, KH vs. improved, BG) in combination with two types of fertilizers (inorganic, ING vs. organic, ORG).

Multivariate analysis on relative ^{13}C concentration ($^{13}CC_r$) of the 0–10 cm soil layer revealed that the active microbial community structure varied significantly between rice varieties and fertilizers, whereas the effect of variety × fertilizer was significant as well ($p < 0.05$). Microbial community at the lower soil depth (10–20 cm) was less abundant compared with the surface layer and indicated negligible reliance on rhizodeposit ^{13}C.

Factorial ANOVA on $^{13}CC_r$ revealed significant effects of variety, type of fertilizer and variety × fertilizer type on active C uptake by actinomycetes. Rice variety significantly influenced the active fungal and Gram-negative bacterial groups, while none of the factors affected Gram-positive bacteria. Active Gram-negative bacteria were higher with BG variety, whereas fungi and actinomycetes were higher with KH variety in both inorganic and organic fertilizer applied fields. Gram-negative and Gram-positive bacteria were the most active functional groups in the paddy soil microbial community, whereas actinomycetes only relied to a minor extent on rhizodeposit C to meet their energy requirement. It is concluded that rice microbial communities differ significantly as a function of cultivation practices, potentially showing a large impact on ecosystem processes such as greenhouse gas emissions and plant nutrient availability.

1. Introduction

Lowland paddy fields are identified as a special class of manmade wetlands for food production. Almost one fifth of the global wetland area is covered by rice fields, which are mainly distributed in the tropical belt (Kirk, 2004). A 35% increase of rice production is expected to meet the demand by the growing global population in the next two decades (Nguyen and Ferrero, 2006), which therefore requires intensification of cultivation.

Lowland rice soils are characterized by anaerobic conditions during the period of early crop growth, where at least 4–5 cm of standing water level is maintained. An oxic soil environment prevails between the mature crop and the crop establishment in the next season, with soil drainage that is generally situated after the grain filling stage. Fluctuations in the water table drive an interchange between reduction and oxidation status of these soils governing anaerobic and aerobic processes, which are mostly functioned by microorganisms (Conrad, 2002; Kumaraswamy et al., 2000). On the other hand microbial diversity is influenced vice versa varying the latter spatio-temporally. In addition to water management, agricultural inputs such as fertilizers directly add substrates for microbial growth and impact upon the microbial diversity in these soil ecosystems. Plant factors influencing the spatio-temporal variations in microbial diversity and functioning may include rice variety and growth stage as root exudates are a major microbial energy source. Although specific microbial processes in rice soils have gained much research interest, those frequently do not combine information on the active microbial community (Gutknecht et al., 2006).

The assessment of the active soil microbial community employs various biochemical techniques, including nucleic acids (DNA and RNA) and cell membrane phospholipid fatty acids (PLFA) as biomarkers.

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Among the latter, PLFA analyses in combination with stable isotope probing (PLFA-SIP) are a very sensitive technique to characterize metabolically active microorganisms at functional group level (Boschker et al., 1998), in comparison with DNA/RNA-SIP. Latter methods advance PLFA-SIP, with respect to taxonomic resolution, despite less liability of DNA and difficulty in extractability of RNA in soil.

PLFA-SIP has been successfully used to label active microbial communities during rhizodeposit carbon uptake in rice soils, however, based on controlled microcosms (Lu et al., 2007, 2004). A dynamic environmental gradient that exists in the rice fields, which cannot be matched by controlled laboratory conditions, require in situ studies to reveal the active soil microbial community in rhizodeposit C metabolism. In the current study in lowland rice fields in Sri Lanka, we used the in situ PLFA-SIP methodology developed by authors for temperate grasslands (Denef et al., 2007) and wetlands (Balasooriya et al., 2008) using $^{13}CO_2$ pulse labeling. We hypothesized that the active microbial community vary with environmental gradients in rice fields including rice variety and type of fertilizer input. The study aimed at assessing the effect of rice variety and type of fertilizer input on the active soil microbial communities in rice fields that have two different plant varieties in combination with two types of fertilizer input.

2. Materials and methods

2.1. Site description

The study was conducted in irrigated rice fields located in Doluwa farming area, on the Mahaveli river banks in Kandy district (7° 17′ N, 80° 31′ E) of Sri Lanka. The soil is characterized with a nearly flat landscape and classified locally as low humic gley an equivalent to soil great group Fluvaquents which are primarily stratified wet soils on flood plains (Mapa et al., 1999). The site is located in the mid country (ca. 500 m a. s. l) wet zone, which receives an annual rainfall of more than 2500 mm and the mean annual temperature is 26 °C (Panabokke, 1996).

Four rice fields cultivated with two different rice varieties: Bg94-1 (BG, improved rice variety) and *Kalu Heenati* (KH, traditional rice variety) and two types of fertilizer: organic fertilizer (ORG) and mineral fertilizer (ING) were selected for the pulse labeling study. The selected fields were continuously cropped with each variety × fertilizer type combination (BG variety with inorganic fertilizer (BI) or organic fertilizer (BO), KH variety with inorganic fertilizer (KI) or organic fertilizer (KO) for at least 4 cropping seasons (2 years).

The BG variety is popular as a high yielding semi-dwarf short duration (105 days) rice variety possessing better plant development with high number of tillers (Suriyagoda et al., 2011), whereas the KH variety is grown for the medicinal qualities of its grain, however with longer duration for maturity (125 days), taller plants with low number of tillers and lower yield (Suriyagoda et al., 2011). Mineral fertilizer application consisted of N, P and K according to the recommendations by the Department of Agriculture, Sri Lanka (Urea 135 kg/ha, triple super phosphate [$Ca(H_2PO_4)_2$] 62 kg/ha and muriate of potash (KCl) 62 kg/ha). Basal dressing at planting consisted of N, P and K, whereas first top dressing (N) applied at the seedling stage and second top dressing applied at the flower initiation (N, K). The organic fertilizer (4 t/ha) consisted of a mixture of green manure (*Gliricidia sepium*, *Tithonia diversifolia*, *Erythrina indica* and *Leucaena leucocephala*), rice straw and compost made of cow manure and green manure (Wijewardhana, 2008). The fields were submerged with approximately 4–5 cm of standing water level throughout the growing season. Seedlings were transplanted at about 20 × 15 cm inter- and intra-row spacing (2 seedlings per hill) to obtain a uniform density of plants. Weeds were managed regularly by manual weeding.

2.2. In situ PLFA-SIP in rice fields

Pulse labeling with $^{13}CO_2$ was conducted using Plexiglas chambers (30 × 30 × 80 cm, n = 3)in each field during late flowering stage of the rice plants. A total of 500 ml of 13C labeled (99 atom %) CO_2 was supplied to photosynthesizing plants within each chamber distributed over a period of 6 daylight hours. The three replicate chambers were placed in line with 2 m distance in between and on top of stainless-steel collars firmly inserted in the soil to a depth of ca. 10 cm. First injection of 100 ml of $^{13}CO_2$ in each chamber was done 15 min after placing the chamber, in order to get the CO2 level in the chamber to fell below atmospheric levels (Balasooriya et al., 2008). The injections were repeated four times more, with 1 h intervals. After the last $^{13}CO_2$ injection, Plexiglas chambers were sealed during the night to allow maximum incorporation of 13C in plant and soil microbial biomass.

2.3. Soil and plant sampling and analysis of ^{13}C

After 24 h from pulse labeling, chambers were removed and soil (0–10 cm and 10–20 cm depth) and plant samples were taken (n = 3) (Balasooriya et al., 2008). In addition, composite samples of soil and shoots from each paddy field were taken prior to labeling as control. All samples were immediately stored on ice and transported to the laboratory where soil samples were frozen (−20 °C). Frozen samples from Sri Lanka were air transported on dry ice to the Ghent University (Belgium) for laboratory analysis.

Soil samples were wet sieved (250 μm) to separate soil from roots, followed by centrifuging and freeze drying of the pellet in order to obtain a homogeneous dry soil sample free of roots and soil fauna (Balasooriya et al., 2008). Root samples were collected during wet sieving for further analysis. Shoot and root samples were oven dried (24 h at 60 °C) and then ground using an ultra-centrifugal mill (ZM200, Retsch, Germany). A subsample of ground shoot, root and soil samples was analyzed for $\delta^{13}C$ (‰) using an Elemental Analyzer (EA) (ANCA–SL, SerCon, UK) coupled to an Isotope Ratio Mass Spectrometer (IRMS) (20–20, SerCon, UK). The net ^{13}C enrichment ($\Delta\delta^{13}C$, expressed in ‰) of shoot, root and soil samples were calculated by subtracting pre-labeling natural abundance $\delta^{13}C$ values of control samples from the post-labeling $\delta^{13}C$ values.

2.4. Soil analysis for selected properties

During the drained period of the rice fields, after harvesting, soil samples were taken from 0 to 10 cm and 10–20 cm depth for determination of physico-chemical soil characteristics. At each experimental field, three replicate samples were taken using a gauge auger and mixed to obtain a composite sample. Air dried soil samples were analyzed for particle size distribution, pH, C and N. Soil texture analysis was performed using the pipette method following the procedure described by Gee and Bauder (1986). The organic C content was determined by the Walkley and Black method described by Nelson and Sommers (1996). Soil pH (1:2.5 KCl) was measured with a pH meter. Total Nitrogen was determined by Kjeldahl distillation method as described by Keeny and Nelson (1982).

2.5. PLFA extraction and GC–C–IRMS

The extraction, quantification and compound specific $\delta^{13}C$ analysis of PLFAs were performed following the method described by Denef et al. (2007). In brief, total lipids were extracted from 6 g of freeze-dried soil using phosphate buffer/chloroform/methanol (Bossio and Scow, 1998). Phospholipids were isolated from other less polar lipids by solid phase extraction and subsequently trans-esterified by mild methanolysis to form volatilizable fatty acid methyl esters (FAMEs).

FAMEs were analyzed by capillary gas chromatography combustion-isotope ratio mass spectrometry (GC–c–IRMS) (GC–C/Delta PLUS XP,