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## Effect of fertilization on soil microorganisms in paddy rice systems - A meta-analysis

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### ABSTRACT

Soil microorganisms are considered a sensitive indicator of soil health and quality. In cropping systems, soil microorganisms are strongly affected by crop management, including the application of fertilizers. While studies in natural ecosystems have generally found that increased nitrogen (N) inputs decrease microbial biomass, microorganisms in soils under upland crops often benefit from mineral fertilizer input. Paddy rice soils, being flooded for part of the season, are dominated by different carbon (C) and N cycle processes and microbial communities than soils under upland crops. The objective of this study was to explore the effect of fertilizer on soil microorganisms in paddy rice systems in a meta-analysis of the peer-reviewed literature. Across all studies (n = 55), the addition of mineral fertilizer significantly increased microbial biomass carbon content (MBC) by 26% in paddy rice soils. Mineral fertilizer applications also increased soil organic carbon content (SOC) by 13%. The higher crop productivity with fertilization likely led to higher organic C inputs, which in turn increased SOC and MBC contents. The time of sampling within a season (pre-plant rice, in-season rice, post-harvest rice, or post-harvest rotational crop) did not significantly affect the response of MBC to mineral fertilizer. The positive effect of mineral fertilizer on MBC content did not differ between cropping systems with continuous rice and systems where paddy rice was grown in rotation with other crops. However, compared with upland cropping systems, the increase in the microbial biomass due to mineral fertilizer application is more pronounced in rice cropping systems, even when rice is grown in rotation with an upland crop. Differences in climate and soil oxygen availability likely explain the stronger response of soil microorganisms to mineral fertilizer input in paddy rice systems. Our analysis suggests that fertilization does not consistently select for specific microbial groups (e.g. gram positive or negative bacteria, fungi, actinomycetes) in paddy rice systems; however, it affects microbial community composition through changes in soil properties. How specific groups of microorganisms respond to mineral fertilization likely depends on environmental factors. Overall, our results suggest that in paddy rice systems the application of inorganic fertilizers increases SOC and MBC contents, both of which are important indicators of soil health.

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### 1. Introduction

Soil microorganisms play an important role in soil biochemical processes, such as decomposition of organic material, nutrient

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cycling, and biotransformation of organic pollutants (Schimel, 1995; Thiele-Bruhn et al., 2012). The soil microbial community is therefore an important component of a healthy soil. Even though the soil microbial biomass constitutes only a small portion of soil organic matter, roughly 0.5-6.0%, (Insam, 1990; Shibahara and Inubushi, 1997), it is considered a sensitive indicator of soil health and quality (Doran and Zeiss, 2000). This is due to the fact that the microbial biomass responds more dynamically to management practices than the total soil organic carbon content (SOC) and may show the effect of management on soil health long before such effects can be detected by measuring total SOC (Powlson et al.,



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Abbreviations: CI, confidence interval; CV, coefficient of variation; FYM, farm yard manure; K, potassium; N, nitrogen; NPK, mineral nitrogen, phosphorus and potassium fertilizer; P, phosphorus; PLFA, phospholipid fatty acid; RR, response ratio; MBC, soil microbial biomass carbon; SOC, soil organic carbon.

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In cropping systems, soil microorganisms are strongly affected by crop management, including the application of fertilizers. In a meta-analysis based on more than 100 datasets from long-term trials with annual upland crops from around the world, Geisseler and Scow (2014) found that mineral fertilizer increased microbial biomass carbon contents (MBC) compared with an unfertilized control by an average of 15.1%. This result contrasts to forest and grassland ecosystems where MBC is generally reduced by increased N input (Liu and Greaver, 2010; Geisseler et al., 2016). In upland cropping systems, mineral fertilization also increased SOC content relative to the unfertilized control (Geisseler and Scow, 2014). A close correlation between MBC and SOC suggested that SOC was a major factor contributing to the overall increase in MBC content with mineral fertilization. The analysis did not include data from lowland (paddy) rice systems. However, rice is one of the most important crops globally, being grown on about 160 million ha worldwide, and more than 90% of global rice production is harvested from irrigated or rainfed lowland rice fields (IRRI, 2013).

Compared with natural ecosystems, rice systems share many similarities with upland cropping systems, such as low plant diversity, periods of no plant cover, tillage or crop harvest. However, rice systems differ fundamentally from upland cropping systems in terms of tillage operations and water management. Two unique management practices of lowland rice systems are puddling and flooding. Puddling, which is a very common practice in rice systems in Asia, refers to the repeated tillage of submerged soil before rice is transplanted. By breaking down soil aggregates, reducing macroporosity and dispersing clay particles, puddling greatly restricts water percolation (Chauhan et al., 2012). Flooding creates anoxic conditions in most of the soil. In contrast, aerobic conditions prevail when soils are drained. Thus both puddling and flooding have considerable effects on physical and chemical soil properties, creating a temporally highly variable environment for soil organisms (Banerjee et al., 2006). Furthermore, many paddy rice systems are very intensive with two or even three crops grown each year and nitrogen (N) application rates may in rare cases even be as high as 500 kg ha<sup>-1</sup> per crop (Che et al., 2015). To prevent losses through denitrification, N fertilizer is applied as ammonium or urea. Lack of oxygen prevents nitrification in most of the soil, resulting in prolonged periods of high ammonium concentrations after fertilizer applications under flooded conditions. High N application rates can thus lead to temporarily very high osmotic potentials and potentially toxic concentrations of ammoniacal N (Eno et al., 1955; Omar and Ismail, 1999). Rice field soil thus provides a unique environment for soil microorganisms and their response to fertilizer input may differ between rice systems and upland cropping systems.

To explore the effect of fertilizer on soil microorganisms in paddy rice systems, we carried out a meta-analysis of the peerreviewed literature. The results shall be compared with those from an earlier study in upland crops. Despite the differences between upland and paddy rice systems, we hypothesize that mineral fertilizer in paddy rice systems has a positive effect on the soil microbial biomass as well as on SOC content, as is generally the case in upland systems. Furthermore, we expect that mineral fertilizers lead to changes in the microbial community composition.

### 2. Material and methods

We investigated the effects of fertilization on soil microorganisms in paddy rice systems by meta-analysis. In a first step we searched the online database Web of Science for peer-reviewed papers using the topics 'rice', 'nitrogen', 'microbial' and 'fertili\*'. The articles which met these criteria were then screened for data on microbial biomass. Articles cited in review papers and in the discussion of other articles were also included in our search. The following criteria were applied to select appropriate studies: (i) the data were from field trials with rice cropping systems in paddy soils and (ii) the study reported microbial biomass both from an unfertilized control and a treatment with mineral N addition either alone or in combination with other nutrients. The literature search was concluded in July 2017.

Meta-analysis requires that datasets are independent. To meet this requirement, we only included values from the topsoil when data from several soil layers were reported. When several studies reported data from the same trial, the study with the most complete dataset, including standard deviation and information about other soil properties, such as pH or SOC content, was preferred. When studies measured microbial biomass repeatedly over time or included treatments with different application rates, the datasets were considered dependent and the average response and a composite standard deviation (Borenstein et al., 2009) were calculated and used for the meta-analysis. However, for the analysis of trial duration, time of sampling and N application rate, results from individual sampling times and treatments were included as separate datasets. Meta-analyses were also performed on a subset of studies with both mineral and organic fertilizer treatments and a different subset comparing straw removal with straw retention.

### 2.1. Data analysis

The natural log of the response ratio (RR) was used as effect size in the meta-analysis (Hedges et al., 1999):

$$ln(RR) = ln\left(\frac{X_{+N}}{X_{-N}}\right) \tag{1}$$

where  $X_{-N}$  and  $X_{+N}$  are the means of the target variable in the control and fertilized treatment, respectively. The meta-analysis was performed using the program MetaWin (Rosenberg et al., 2000). MetaWin was also used for meta-regression analyses to investigate the effects of trial duration, N application rate and latitude. Effects of fertilization expressed in percent were calculated as (RR - 1)  $\times$  100%.

Meta-analysis requires a variability estimate for each dataset. Approximately two thirds of the studies reported a measure of variability for MBC content that could be used to calculate the standard deviation, while half the studies reported a measure of variability for SOC content and pH. The missing standard deviations were calculated using the average coefficient of variation (CV) of the datasets where the standard deviation was reported for that soil property.

Effects of fertilization on microbial community composition were assessed using different approaches, namely colony forming units, phospholipid fatty acid (PLFA), direct count, ester-linked fatty acid methyl esters, and selective inhibition. Only a small number of datasets measured the effects of N input on microbial community composition. Due to the small number and the fact that only few datasets included standard deviations, a meaningful meta-analysis was not possible. Instead, we calculated the standard deviation and 95% confidence interval (95% CI) of the RR considering individual datasets as experimental units. This approach results in a smaller error term compared with a meta-analysis and thus is more likely to result in significant differences.

Total PLFA was converted to MBC using a conversion factor of 5.8  $\mu$ g C nmol<sup>-1</sup> PLFA (Joergensen and Emmerling, 2006). When organic matter was reported, we multiplied it by 0.58 (Stevenson and Cole, 1999) to calculate SOC content. These conversions were only relevant for data shown in Fig. 4 and had no effect on the response ratio used for the meta-analysis.

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