

1 Co-evolution hints at soil microbial biomass as the entity of soil fertility

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21 **Abstract**

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23 **Nitrogen (N) and Carbon (C) are popular indicators of soil fertility; however,**  
24 **they are not soil fertility itself. In fact, they may be seen as just two aspects of the**

one entity. Soil microbial biomass (SMB) is also one of soil fertility indicators; furthermore, recent study of co-evolution between plants and microorganisms raises an idea that SMB might be the entity of fertility. The correlation between SMB and crop yield has been found in some studies but not in others. Those studies were conducted from the standpoint of N stock balance; therefore, the correlation between soil properties before planting and plant yields were analyzed. Here, we show—in our analysis of harvest-time soil properties and crop yields—that SMB correlates more strongly than inorganic N, total N, or total C with average crop yield under a wide range of cultivation conditions. From the viewpoint of co-evolution, plant biomass is a part of the plant and soil microorganism system; therefore, increasing SMB will balance by increasing plant biomass. In addition, the SMB could increase independently from the plant growth by artificial organic matter input. This concept will break through the yield limitation of conventional farming.

## 41 Introduction

42 Soil fertility is the basic idea of agriculture(Parikh, S. J. & James, B. R., 2012).  
 43 There is a positive correlation between fertilizer use efficiency and soil fertility, and the  
 44 fertility limits the maximum crop yield(Sánchez, 2010; Vanlauwe et al., 2010;  
 45 Musinguzi et al., 2013; Kurwakumire et al., 2014). There are many indicators of soil  
 46 fertility(Andrews & Carroll, 2001). Nitrogen (N) and Carbon (C) are popular indicators  
 47 among then(Fox et al., 1989; Tiessen, Cuevas & Chacon, 1994; Breschini & Hartz,  
 48 2002); however, they are not soil fertility itself. For example, the soil inorganic N is  
 49 mostly used in temperate or cool areas, and soil total C is mostly used in tropical areas.  
 50 The level of soil inorganic N is rich enough to distinguish the difference, but total C is  
 51 too rich in the temperate or cool area. In contrast, inorganic N is too poor but total C is  
 52 poor enough to distinguish the difference in the tropical area(Tiessen, Cuevas & Chacon,  
 53 1994). These are two aspects of the one entity if they are the indicators of fertility.

54 Once, a soil scientist defined soil fertility as follows: “The ability of soil for  
 55 providing water and nutrition which is required as crop growth(Okajima, 1976).” In this  
 56 context, fertile soil provides water and nutrition. Certainly, we imagine the thick  
 57 aggregate when we hear the word “fertile soil”. In addition to the aggregate, however,  
 58 those fertile soils are also fertile in microorganisms(Jastrow, Miller & Lussenhop, 1998;  
 59 Barto et al., 2010). Recent studies revealed a four thousand million-year co-evolution  
 60 between plants and fungi(Redecker, Kodner & Graham, 2000; Humphreys et al., 2010).  
 61 This finding raises an idea that Soil microbial biomass (SMB) might be the entity of  
 62 fertility. Plant biomass and SMB form a system. SMB forms the aggregate and  
 63 improves water-holding capacity. SMB mobilizes the nutrition in soil. This supply of  
 64 water and nutrition is required for crop growth. However, the correlation between SMB

and crop yield has been found in some studies(McGILL et al., 1986; Insam, Mitchell & Dormaar, 1991; Srivastava & Lal, 1994; He et al., 1997) but not in others(Brendecke, Axelson & Pepper, 1993; Entry, Mitchell & Backman, 1996; Holt & Mayer, 1998).

These contradictions are reasonable because each crop yield reflects not only soil fertility but also many other factors such as weather conditions, cultivation conditions, and so on. Therefore, soil fertility must be evaluated as the average productivity across a wide spectrum of conditions(Entry, Mitchell & Backman, 1996). From another point of view, those studies were conducted from the viewpoint of N stock balance(Fox et al., 1989; Breschini & Hartz, 2002; Geisseler et al., 2010). Therefore, researchers analyzed the correlation between soil properties before planting and plant yields. From the viewpoint of co-evolution, plant biomass is a part of both the plant and soil microorganism system; therefore, increasing SMB will balance by increasing plant biomass. Hence, the correlation between soil properties at harvest time and the crop yields should be analyzed. We examined these points in this study and clarified whether SMB is the entity of fertility or not.

## Methods

We chose leafy vegetables for evaluating productivity because (unlike serial crops) their growth directly reflects soil fertility. In addition, leafy vegetables are harvested in the middle of their life stage. We produced SMB gradients from an equal amount of organic matter application under different management and climate conditions. Correlation between soil properties at harvest time and yields was analyzed.

## Study Site

This study was conducted in 2011–2012 at a site in a tropical savanna climate, in a lateritic loamy sand field in Tha Phara village, Khon Kaen Province, Thailand (16° 34' N, 102° 83' E). The Agricultural Production Science Research and Development Center, Khon Kaen, Thailand granted a field experiment permit.

## Plot Design

To generate a local gradient in SMB, 18 plots were established at the field site and treated with 18 treatments (Table 1). The study was consistent with an  $L_{18}$  orthogonal array (Taguchi, 1986), though our aim was to generate a local gradient in soil properties and yield to enable a correlation analysis to determine relationships between SMB N and yield. Our aim was not to test the effects of treatments on soil properties and yield. The  $L_{18}$  orthogonal array is a popular and robust experimental design that can be used under a wide range of conditions. Only the main effects seen in the experiments were presented, and these were considered more robust results than the interactions.

## Cultivation Conditions

Water spinach (season 1, sown August 25, harvested September 26) and lettuce (season 2 to season 4, transplanted and harvested on October 20 and December 7, December 8 and January 17, and January 23 and March 1, respectively) were planted. Season 1 and 2 were during the rainy season, and season 3 and 4 were during the dry season. Total precipitation for seasons 1 to 4 was 248, 7, 0, and 0 mm, respectively. The plot locations were selected randomly in season 1 and fixed the locations in the subsequent seasons. Each plot measured  $3 \times 3$  m. Waste mushroom bed (with a C:N ratio of 40) was applied to each plot in each season at a fresh weight rate of  $1 \text{ kg m}^{-2}$  (equivalent to a C application rate of  $300 \text{ g m}^{-2}$ ). The treatments included watering (1.5

mm twice a day, 3 mm each week, or none), urea incorporation (at a rate of 10, 0.1, or 0 g m<sup>-2</sup>, the 0.1 g m<sup>-2</sup> treatment being applied as a solution in 1 liter of water), waste mushroom bed application method (incorporated, applied to the soil surface, or incorporated after the fungi were killed by packing the material in plastic mulch film and exposing it to sunlight for 1 day), and plant density level (standard, double, or none). No other material was used. The plants were free from diseases and insect pests, so no plant protection procedures were used. The field was kept free of weeds by hand weeding.

### Soil Sampling and Analysis

Crop soil (approximately 14 cm deep) was sampled from each plot just after the crops were harvested. A composite sample, from 10 sampling points, was collected from each plot. Each soil sample was sieved through a 2-mm sieve (through which all of the mushroom waste could pass) while moist, and 500 g of each sample was kept at 2°C until the SMB N content was measured. The SMB N content was measured using the fumigation–extraction method (Amato & Ladd, 1988). The inorganic N concentration in each sample was determined by extracting the sample with 2 M KCl and performing NH<sub>4</sub><sup>+</sup> and NO<sub>3</sub><sup>-</sup> assays on the extract (Keeney & Nelson, 1982). The remaining portion of each sample was air-dried, and the total N and total C contents in the soil were determined using an NC analyzer (SUMIGRAPH NC 200F; Sumitomo Chemical, Tokyo, Japan) using the dry combustion method.

### Correlation Analysis

Simple correlation (Pearson product-moment) analyses between the soil properties (SMB N, total soil C, N, and inorganic N contents) and yields were performed to identify the main effects (as simple averages omitting the non-cropping treatments) of

the treatments in each growing season, in the rainy season, in the dry season, and as an average of those seasons. Soil properties were considered to be robust indicators with regard to the climate conditions when the average of the seasons showed a strong correlation with the yield.

The soil properties at harvest time are shown in Table 2. A wide (32–319  $\mu\text{g g}^{-1}$  soil) range of SMB contents was created from an equal amount (a fresh weight rate of 1 kg  $\text{m}^{-2}$ ) of application of organic matter. This result shows that the difference in management results in ten times increase in SMB.

The top line of Figure 1 shows correlation between soil properties and yields for raw data. No strong correlations were identified between yield and soil properties. We expected this result because each yield is affected by not only soil fertility but also other conditions. On the other hand, summarizing the data for each treatment by simple average, a strong correlation was found between yield and SMB N in season 3 ( $r = 0.880^{**}$ ). Strong correlations were also found in the average values such as rainy season (0.879\*\*), dry season (0.875\*\*), average of the two dry season crop seasons (0.977\*\*\*), and average of all seasons (0.894\*\*; Table 3). They were higher than those of the inorganic N, total N, or total C contents. The exception was inorganic N in the rainy season (0.801\*\*); however, this was caused by the difference in the inorganic N contents in season 1 (11.4–14.2  $\mu\text{g g}^{-1}$ ) and season 2 (7.7–9.6  $\mu\text{g g}^{-1}$ ). The relatively high inorganic N value in season 1 is considered to be a trace of nitrogen flush. However, the soil inorganic N, ranging 11.4–14.2, 7.7–9.6, 7.1–9.8, and 6.3–7.7  $\mu\text{g g}^{-1}$  in season 1 to season 4, respectively, was lower than the lower limit  $\text{NO}_3\text{-N}$  contents of conventional cultivation conditions (20  $\mu\text{g g}^{-1}$ )(Fox et al., 1989; Breschini & Hartz, 2002). Those low inorganic N contents are common under the condition of high C:N

ratio (40) organic matter input(Blair AW & Prince AL, 1928).

## Results

The range of SMB N content in the soils collected in seasons 1 and 2 were significantly different from seasons 3 and 4. The peak of SMB would have been already passed considering they were in seasons 1 and 2. Relationships between SMB N and yield were affected by the range of SMB N contents that were present. For instance, the mean (and range) of SMB N for seasons 1 to 4 were 23 (69–91), 19 (32–50), 190 (235–424), and 98 (222–319)  $\mu\text{g g}^{-1}$ , respectively. The correlation coefficients for the relationships between SMB N contents and yields were 0.198, 0.288, 0.880\*\*, and 0.582 for seasons 1 to 4, respectively.

## Discussion

From the viewpoint of co-evolution, plant biomass is a part of both the plant and the soil microorganism systems. Therefore, plant biomass and SMB will balance each other. On the other hand, from the standpoint of the N stock balance, the SMB N content at harvest time and the yield should be negative(Vitousek & Howarth, 1991). Because the applied waste mushroom bed (giving a concentration of 1715  $\mu\text{g g}^{-1}$  in the top 14 cm of crop soil) was decomposed within each cropping period, no residual particles were observed (Table 2). For soil nitrogen balance, an equal amount of N provided as organic matter was distributed into the crops, soil inorganic N, SMB N, and un-decomposed organic matter. All the organic matter had been decomposed and the soil inorganic N levels were very small, ranging 6.3–14.2  $\mu\text{g g}^{-1}$  through all seasons. The above results suggest the viewpoint of co-evolution, in which plant biomass and SMB are in balance.



In general, SMB can increase independently from plant growth by incorporating organic matter such as plant residue in the soil. In this study, SMB N in the non-cropping treatment was approximately in the middle of the range of all the cropped treatments (Table 2). This means that a breakthrough in yield limit will be possible by increasing soil fertility. On the other hand, the soil fertility is changing, and the change would be more drastic in tropical areas because of the high microbial activity caused by high temperature. Our result shows that the difference of management for organic material results in ten times difference in SMB. Inputting soil organic materials is not enough to improve soil fertility. Adequate soil management for SMB, such as moisture and fertilizing, is necessary to maintain or control soil fertility. The question is: “Will increasing SMB really break through the yield limitation?” There is the case study of this kind of new agriculture(Oda et al., 2014).

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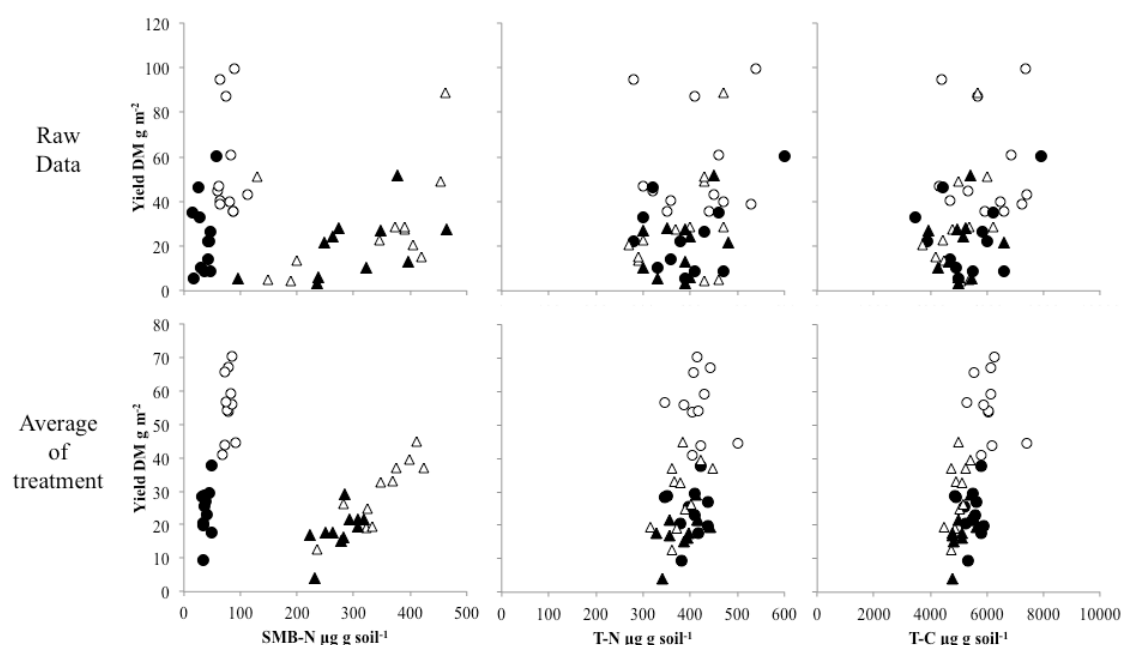
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**Supplementary Information** is linked to the online version of the paper at [www.naturewcom/nature](http://www.naturewcom/nature).

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**Figure 1. Correlation between soil properties and yield**

○Season 1, ●Season 2, △Season 3, ▲Season 4

A local gradient of soil properties was produced by treating plots with an equal amount of organic matter and using different management practices. Relations between soil properties at harvest and the leafy vegetable yields are shown. The top line graphs show the raw data, the bottom line graphs show the average value of each treatment. Seasons 1 and 2 were during the rainy season and seasons 3 and 4 were during the dry season.

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**Table 1. Experimental design using an L<sub>18</sub> orthogonal array**

Plot No.	Watering	Material type and position	Plant density	Nitrogen application
	1: 0 2: Once per week 3: Twice per day	1: Alive, Mixed 2: Surface 3: Dead, Mixed	1: Double 2: Standard 3: None	1: 0 g m <sup>-2</sup> 2: 0.1 g m <sup>-2</sup> 3: 10 g m <sup>-2</sup>
1	1	1	1	1
2	2	2	2	2
3	3	3	3	3
4	1	1	2	2
5	2	2	3	3
6	3	3	1	1
7	1	2	1	3
8	2	3	2	1
9	3	1	3	2
10	1	3	3	2
11	2	1	1	3
12	3	2	2	1
13	1	2	3	1
14	2	3	1	2
15	3	1	2	3
16	1	3	2	3
17	2	1	3	1
18	3	2	1	2

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