

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

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10 **Abstract**

11 Nitrogen (N) and Carbon (C) are popular indicators of soil fertility; however, they are not
12 soil fertility itself. In fact, they may be seen as just two aspects of the one entity. Soil
13 microbial biomass (SMB) is also one of soil fertility indicators; furthermore, recent study of
14 co-evolution between plants and microorganisms raises an idea that SMB might be the entity
15 of fertility. The correlation between SMB and crop yield has been found in some studies but
16 not in others. Those studies were conducted from the standpoint of N stock balance; therefore,
17 the correlation between soil properties before planting and plant yields were analyzed. Here,
18 we show—in our analysis of harvest-time soil properties and crop yields—that SMB
19 correlates more strongly than inorganic N, total N, or total C with average crop yield under a
20 wide range of cultivation conditions. From the viewpoint of co-evolution, plant biomass is a
21 part of the plant and soil microorganism system; therefore, increasing SMB will balance by
22 increasing plant biomass. In addition, the SMB could increase independently from the plant
23 growth by artificial organic matter input. This concept will break through the yield limitation
24 of conventional farming.

25

26 **Introduction**

27 Soil fertility is the basic idea of agriculture(Parikh, S. J. & James, B. R., 2012). There is a
28 positive correlation between fertilizer use efficiency and soil fertility, and the fertility limits
29 the maximum crop yield(Sánchez, 2010; Vanlauwe et al., 2010; Musinguzi et al., 2013;
30 Kurwakumire et al., 2014). There are many indicators of soil fertility(Andrews & Carroll,
31 2001). Nitrogen (N) and Carbon (C) are popular indicators among them(Fox et al., 1989;
32 Tiessen, Cuevas & Chacon, 1994; Breschini & Hartz, 2002); however, they are not soil
33 fertility itself. For example, the soil inorganic N is mostly used in temperate or cool areas, and
34 soil total C is mostly used in tropical areas. The level of soil inorganic N is rich enough to
35 distinguish the difference, but total C is too rich in the temperate or cool area. In contrast,
36 inorganic N is too poor but total C is poor enough to distinguish the difference in the tropical
37 area(Tiessen, Cuevas & Chacon, 1994). These are two aspects of the one entity if they are the
38 indicators of fertility.

39 Once, a soil scientist defined soil fertility as follows: “The ability of soil for providing
40 water and nutrition which is required as crop growth(Okajima, 1976).” In this context, fertile
41 soil provides water and nutrition. Certainly, we imagine the thick aggregate when we hear the
42 word “fertile soil”. In addition to the aggregate, however, those fertile soils are also fertile in
43 microorganisms(Jastrow, Miller & Lussenhop, 1998; Barto et al., 2010). Recent studies
44 revealed a four thousand million-year co-evolution between plants and fungi(Redecker,
45 Kodner & Graham, 2000; Humphreys et al., 2010). This finding raises an idea that Soil
46 microbial biomass (SMB) might be the entity of fertility. Plant biomass and SMB form a
47 system. SMB forms the aggregate and improves water-holding capacity. SMB mobilizes the
48 nutrition in soil. This supply of water and nutrition is required for crop growth. However, the
49 correlation between SMB and crop yield has been found in some studies(McGILL et al.,
50 1986; Insam, Mitchell & Dormaar, 1991; Srivastava & Lal, 1994; He et al., 1997) but not in

51 others(Brendecke, Axelson & Pepper, 1993; Entry, Mitchell & Backman, 1996; Holt &
52 Mayer, 1998).

53 These contradictions are reasonable because each crop yield reflects not only soil fertility
54 but also many other factors such as weather conditions, cultivation conditions, and so on.
55 Therefore, soil fertility must be evaluated as the average productivity across a wide spectrum
56 of conditions(Entry, Mitchell & Backman, 1996). From another point of view, those studies
57 were conducted from the viewpoint of N stock balance(Fox et al., 1989; Breschini & Hartz,
58 2002; Geisseler et al., 2010). Therefore, researchers analyzed the correlation between soil
59 properties before planting and plant yields. From the viewpoint of co-evolution, plant biomass
60 is a part of both the plant and soil microorganism system; therefore, increasing SMB will
61 balance by increasing plant biomass. Hence, the correlation between soil properties at harvest
62 time and the crop yields should be analyzed. We examined these points in this study and
63 clarified whether SMB is the entity of fertility or not.

65 **Materials & Methods**

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

72 **Study Site**

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

76 Thailand granted a field experiment permit.

77

78 **Plot Design**

79 To generate a local gradient in SMB, 18 plots were established at the field site and treated
80 with 18 treatments (Table 1). The study was consistent with an L_{18} orthogonal array (Taguchi,
81 1986), though our aim was to generate a local gradient in soil properties and yield to enable a
82 correlation analysis to determine relationships between SMB N and yield. Our aim was not to
83 test the effects of treatments on soil properties and yield. The L_{18} orthogonal array is a
84 popular and robust experimental design that can be used under a wide range of conditions.
85 Only the main effects seen in the experiments were presented, and these were considered
86 more robust results than the interactions.

87

88 **Cultivation Conditions**

89 Water spinach (season 1, sown August 25, harvested September 26) and lettuce (season 2
90 to season 4, transplanted and harvested on October 20 and December 7, December 8 and
91 January 17, and January 23 and March 1, respectively) were planted. Season 1 and 2 were
92 during the rainy season, and season 3 and 4 were during the dry season. Total precipitation for
93 seasons 1 to 4 was 248, 7, 0, and 0 mm, respectively. The plot locations were selected
94 randomly in season 1 and fixed the locations in the subsequent seasons. Each plot measured 3
95 \times 3 m. Waste mushroom bed (with a C:N ratio of 40) was applied to each plot in each season
96 at a fresh weight rate of 1 kg m^{-2} (equivalent to a C application rate of 300 g m^{-2}). The
97 treatments included watering (1.5 mm twice a day, 3 mm each week, or none), urea
98 incorporation (at a rate of 10, 0.1, or 0 g m^{-2} , the 0.1 g m^{-2} treatment being applied as a
99 solution in 1 liter of water), waste mushroom bed application method (incorporated, applied
100 to the soil surface, or incorporated after the fungi were killed by packing the material in

101 plastic mulch film and exposing it to sunlight for 1 day), and plant density level (standard,
102 double, or none). No other material was used. The plants were free from diseases and insect
103 pests, so no plant protection procedures were used. The field was kept free of weeds by hand
104 weeding.

105

106 **Soil Sampling and Analysis**

107 Crop soil (approximately 14 cm deep) was sampled from each plot just after the crops were
108 harvested. A composite sample, from 10 sampling points, was collected from each plot. Each
109 soil sample was sieved through a 2-mm sieve (through which all of the mushroom waste
110 could pass) while moist, and 500 g of each sample was kept at 2°C until the SMB N content
111 was measured. The SMB N content was measured using the fumigation–extraction
112 method (Amato & Ladd, 1988). The inorganic N concentration in each sample was determined
113 by extracting the sample with 2 M KCl and performing NH_4^+ and NO_3^- assays on the
114 extract (Keeney & Nelson, 1982). The remaining portion of each sample was air-dried, and the
115 total N and total C contents in the soil were determined using an NC analyzer (SUMIGRAPH
116 NC 200F; Sumitomo Chemical, Tokyo, Japan) using the dry combustion method.

117

118 **Correlation Analysis**

119 Simple correlation (Pearson product-moment) analyses between the soil properties (SMB N,
120 total soil C, N, and inorganic N contents) and yields were performed to identify the main
121 effects (as simple averages omitting the non-cropping treatments) of the treatments in each
122 growing season, in the rainy season, in the dry season, and as an average of those seasons.
123 Soil properties were considered to be robust indicators with regard to the climate conditions
124 when the average of the seasons showed a strong correlation with the yield.

125

126 **Results**

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

131 The top line of Figure 1 shows correlation between soil properties and yields for raw data.
132 No strong correlations were identified between yield and soil properties. We expected this
133 result because each yield is affected by not only soil fertility but also other conditions. On the
134 other hand, summarizing the data for each treatment by simple average, a strong correlation
135 was found between yield and SMB N in season 3 ($r = 0.880^{**}$). Strong correlations were also
136 found in the average values such as rainy season (0.879^{**}), dry season (0.875^{**}), average of
137 the two dry season crop seasons (0.977^{***}), and average of all seasons (0.894^{**} ; Table 3).
138 They were higher than those of the inorganic N, total N, or total C contents. The exception
139 was inorganic N in the rainy season (0.801^{**}); however, this was caused by the difference in
140 the inorganic N contents in season 1 ($11.4\text{--}14.2 \mu\text{g g}^{-1}$) and season 2 ($7.7\text{--}9.6 \mu\text{g g}^{-1}$). The
141 relatively high inorganic N value in season 1 is considered to be a trace of nitrogen flush.
142 However, the soil inorganic N, ranging $11.4\text{--}14.2$, $7.7\text{--}9.6$, $7.1\text{--}9.8$, and $6.3\text{--}7.7 \mu\text{g g}^{-1}$ in
143 season 1 to season 4, respectively, was lower than the lower limit $\text{NO}_3\text{-N}$ contents of
144 conventional cultivation conditions ($20 \mu\text{g g}^{-1}$) (Fox et al., 1989; Breschini & Hartz, 2002).
145 Those low inorganic N contents are common under the condition of high C:N ratio (40)
146 organic matter input (Blair AW & Prince AL, 1928).

147

148 **Discussion**

149 The range of SMB N content in the soils collected in seasons 1 and 2 were significantly
150 different from seasons 3 and 4. The peak of SMB would have been already passed

151 considering they were in seasons 1 and 2. Relationships between SMB N and yield were
152 affected by the range of SMB N contents that were present. For instance, the mean (and
153 range) of SMB N for seasons 1 to 4 were 23 (69–91), 19 (32–50), 190 (235–424), and 98
154 (222–319) $\mu\text{g g}^{-1}$, respectively. The correlation coefficients for the relationships between
155 SMB N contents and yields were 0.198, 0.288, 0.880**, and 0.582 for seasons 1 to 4,
156 respectively.

157 From the viewpoint of co-evolution, plant biomass is a part of both the plant and the soil
158 microorganism systems. Therefore, plant biomass and SMB will balance each other. On the
159 other hand, from the standpoint of the N stock balance, the SMB N content at harvest time
160 and the yield should be negative (Vitousek & Howarth, 1991). Because the applied waste
161 mushroom bed (giving a concentration of 1715 $\mu\text{g g}^{-1}$ in the top 14 cm of crop soil) was
162 decomposed within each cropping period, no residual particles were observed (Table 2). For
163 soil nitrogen balance, an equal amount of N provided as organic matter was distributed into
164 the crops, soil inorganic N, SMB N, and un-decomposed organic matter. All the organic
165 matter had been decomposed and the soil inorganic N levels were very small, ranging 6.3–
166 14.2 $\mu\text{g g}^{-1}$ through all seasons. The above results suggest the viewpoint of co-evolution, in
167 which plant biomass and SMB are in balance.

168 In general, SMB can increase independently from plant growth by incorporating organic
169 matter such as plant residue in the soil. In this study, SMB N in the non-cropping treatment
170 was approximately in the middle of the range of all the cropped treatments (Table 2). This
171 means that a breakthrough in yield limit will be possible by increasing soil fertility. There is
172 the case study of this kind of new agriculture (Oda et al., 2014). On the other hand, the soil
173 fertility is changing, and the change would be more drastic in tropical areas because of the
174 high microbial activity caused by high temperature.

175

176 **Conclusions**

177 Our result shows that the difference of management for organic material results in ten times
178 difference in SMB. Inputting soil organic materials is not enough to improve soil fertility.
179 Adequate soil management for SMB, such as moisture and fertilizing, is necessary to
180 maintain or control soil fertility. Increasing SMB will break through the yield limitation of
181 conventional farming.

182

183 **Acknowledgments**

184 We sincerely thank S. Thippayarugs for performing the SMB N analyses.

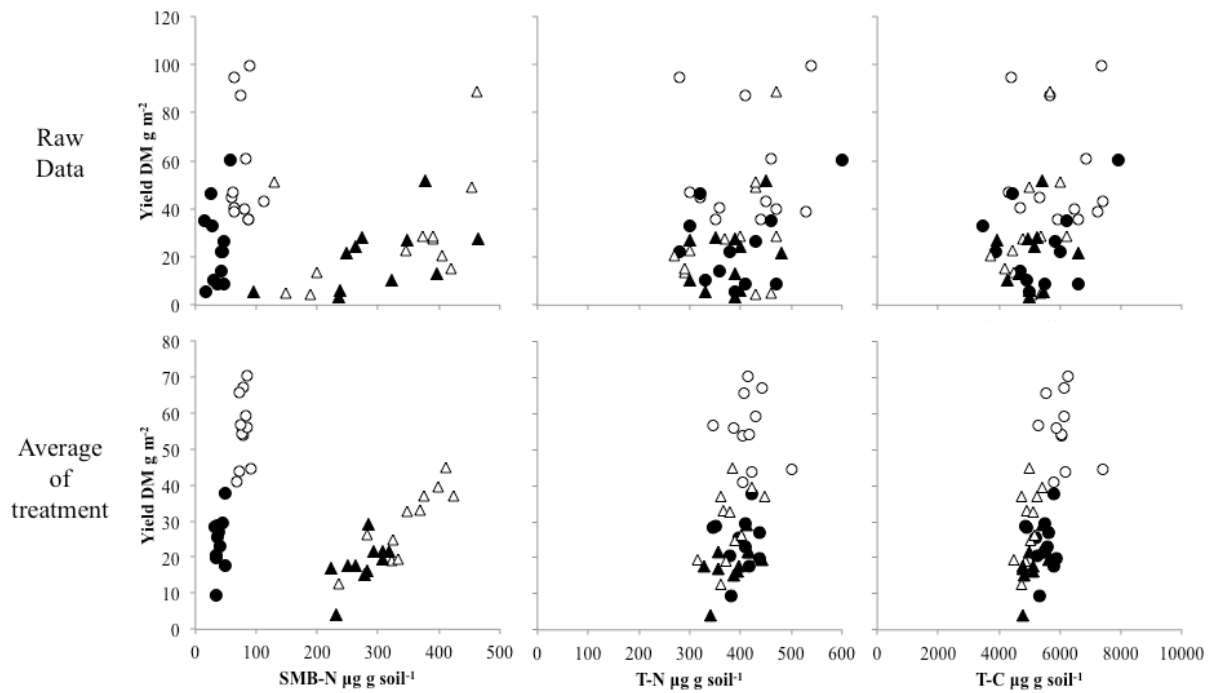
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250
251

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

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

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

259

Table 1. Experimental design using an L₁₈ 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 ⁻² 2: 0.1 g m ⁻² 3: 10 g m ⁻²
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