Correlations between soil microbial and physicochemical variations in a rice paddy: implications for assessing soil health

Ryoichi Doi* and Senaratne Leelananda Ranamukhaarachchi

School of Environment, Resources and Development, Asian Institute of Technology, Klong Luang, Pathumthani 12120, Thailand

**Corresponding author (Email, roird@aeiou.pt)*

This study was conducted to test the hypothesis that spatial variations in soil microbial variables in a Thai rice paddy are accurately described by multivariate profiles of the soil bacterial communities. We found that community-level physiological profiles of soil bacterial communities could better describe the population density of *Rhizoctonia solani* in soil than the physicochemical profiles do. However, soil dehydrogenase levels were closely correlated with soil fertility ($P<0.05$), and these were better described by the physicochemical profiles. Hence, the hypothesis was rejected, and we suspect that soil microbial variables react differently to the same physicochemical changes. The average population density of *R. solani* (35 colony-forming units/g dry soil) was relatively high in the soil we studied, and the soil fertility was found to be among the poorest in Thailand. The soil quality was comparable to the most degraded bare ground soil in an adjacent bioreserve in terms of Shannon diversity index based on the community-level physiological profile as well as values of soil fertility indices. Overall, the soil microbial and physicochemical indicators showed that the paddy soil needs to be supplemented with soil nutrients. Otherwise, *R. solani* may cause a significant reduction in rice production.

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1. Introduction

Soil quality can be assessed by numerous soil variables (Mausbach and Seybold 1998). In addition to physicochemical variables, biotic variables also reflect soil quality (Pankhurst *et al*. 1997). Soil variables often show different patterns of response to the same impact (Jha *et al*. 1992), reflecting the multidimensional quality of soil health (van Straalen 2002). These soil variables are associated with different soil-related variables, including crop yields. Variations in soil-related variables can be empirically predicted from datasets consisting of multiple soil variables. Therefore, analysis of multiple soil biotic variables can provide comprehensive and multidimensional information (Sena *et al*. 2000).

Spatial variations in soil-related variables have different patterns (Covelo *et al.* 2008). These differences are rooted in the complex relationships between each soil-related variable and the aspects of the soil that they describe (Mausbach and Seybold 1998). These different patterns suggest that there may be an ideal dataset to best describe each soil-related variable. Careful observation is necessary to determine which dataset is the most useful in describing each soil-related variable. In addition to physicochemical profiling of soils, various methods of multivariate profiling of soils based on microbial observations have been proposed (Kirk *et al.* 2004). Changes in soil quality can be detected by observing community-level physiological profiles of soil bacterial communities (Garland and Mills 1991). Analysis of the physiological activity of soil bacteria may reveal important information about soil quality which may go undetected by physicochemical analysis, because soil bacterial activity responds differently to impacts than do physicochemical parameters (Doi and Puriyakorn 2007).

Keywords. Bacterial and physicochemical datasets; multivariate analysis; *Rhizoctonia solani*; rice paddy

In this study of soil in a Thai rice paddy, we attempted to determine spatial variations in (1) the population of a plant pathogenic fungus *Rhizoctonia solani,* (2) soil dehydrogenase activity, and (3) soil fertility as defined by soil physicochemical variables. Soil samples were collected from 40 sampling points in a rice paddy in Nakhorn Ratchasima province, Thailand. The soil samples were analysed and profiled. Correlations among these soil-related variables and measures of soil bacterial diversity were analysed to determine differences among the soil-related variables. In this study, soil health was assessed by both physicochemical and bacterial datasets. Community-level physiological profiling was chosen as a suitable method for multivariate bacterial profiling of soils. One goal of the study was to find the most revealing dataset for each soil-related variable, and then determine the best formula for describing soil-related variables. Based on the results, we tried to find correlations between the physicochemical and microbial variations in the soil. Another objective was to make a comprehensive assessment of the quality of the paddy soil as assessed by soil microbial and physicochemical profiles. To compare spatial variations in the physicochemical and microbial profiles of soil, soil samples were gathered from a 40 m × 56 m section of the rice paddy.

2. Materials and methods

2.1 *Site description*

The paddy field is located in Udom Sup Village, Wang Nam Kiao District, Nakhon Ratchasima Province, Thailand (14° 30′ N, 101° 55′ E). Paddy rice cultivation was conducted in the field for seven consecutive years before the sampling in 2005. Cultivation was done as a crop rotation system with another crop. Groundnut (*Arachis hypogaea* L.) was introduced in 2003, planted in February and harvested in May. This rice–groundnut rotation continued until 2005. In late July 2005, the field was plowed and prepared, and rice seedlings were transplanted. The cultivar of rice (*Oryza sativa* L.) was Khao Dok Mali 105. The paddy field was basically rainfed, but supplemental water was available when precipitation was inadequate. In this region, rice grains are harvested around December.

2.2 *Soil sampling*

Soil samples were taken from grids defined by 8 columns and 5 rows. The widths of the columns and rows were 8 m and 10 m, respectively. The soil samples were taken as cubes to a depth of 20 cm, and the other two dimensions were 20 cm; thus the sampled space was 8000 cm^3. In each grid, two soil cubes were taken. The cubes were one meter apart from each other, and the midpoint was the centre of the grid.

After harvesting groundnut in May, the soil was left idle till soils were sampled on 12 July 2005. The sampling took 16 h, during which the area had negligible precipitation (<1 mm). On the day of sampling, the temperature ranged between 24°C and 34°C. The area had 65 mm of precipitation between 1 July and 11 July, the early period of the rainy season (Doi and Ranamukhaarachchi 2009). The soil cubes were immediately placed in a single plastic bag, mixed, passed through a 2 mm sieve, brought to the laboratory at 4°C, and then used for analyses of soil microbial characteristics within 12 h of the sampling. For chemical measurements, the soil samples were air-dried and then sieved through a 2 mm sieve.

2.3 *Physicochemical analyses of soils*

As previously reported by Doi and Sakurai (2004), the following methods were applied to determine the selected physicochemical parameters. Soil moisture content and bulk density were determined by oven drying at 105°C for 48 h. The air-dried and sieved soil was suspended in water at a soil-to-solution ratio of 1:5 and reciprocally shaken at room temperature for 1 h at 120 rpm to determine its pH. Soil organic matter was determined by the loss of ignition method. Exchangeable cations (Ca, K, Mg and Na) were extracted with 1 M ammonium acetate (pH 7.0), and Ca, K and Na contents were determined with an atomic absorption spectrophotometer. The sum of the Ca and Mg contents in the extract was determined by the eriochrome black titration method, and the Ca content was then subtracted from the sum to determine the Mg content. Exchangeable acidity (Al and H) was determined by titration. Available phosphorus was determined by the Bray II method (Bray and Kurtz 1945). Cation exchange capacity was calculated as the sum of the four exchangeable cations (Ca, K, Na and Mg) and the exchangeable acidity.

Values of a soil fertility index (Moran *et al.* 2000) and a soil evaluation factor (Lu *et al.* 2002) were calculated to quantify soil fertility. These indices were known to be indicators of soil fertility in an adjacent bioreserve (Doi and Sakurai 2004). The following equation was used to calculate values of the soil fertility index (Lu *et al.* 2002):

$$\text{Soil fertility index} = \text{pH} + \text{organic matter (\%, dry soil basis)} + \text{available P (mg kg}^{-1}\text{, dry soil)} + \text{exch K (c eq kg}^{-1}\text{ dry soil)} + \text{exch Ca (c eq kg}^{-1}\text{ dry soil)} + \text{exch Mg (c eq kg}^{-1}\text{ dry soil)} - \text{exch Al (c eq kg}^{-1}\text{ dry soil)} \quad (1)$$

Values of soil evaluation factor were calculated using the following equation:

Soil evaluation factor = [exch K (c eq kg^{-1} dry soil) + exch Ca (c eq kg^{-1} dry soil) + exch Mg (c eq kg^{-1} dry soil) – Log (1 + exch Al (c eq kg^{-1} dry soil))] × organic matter (%, dry soil) + 5 (2)

2.4 *Soil microbial analyses*

Soil dehydrogenase activity was determined by colorimetric measurement of the reduction of 2,3,5-triphenyltetrazolium chloride to triphenylformazan according to the method of Casida *et al*. (1964). Five grams of fresh soil was suspended in 4 ml of 250 mM Tris-HCl buffer containing 0.0625% (w/v) glucose. The enzymatic reaction started when 1 ml of 2.5% (w/v) 2,3,5-triphenyltetrazolium chloride solution was added to the soil suspension. The reaction took 16 h in the dark at 37ºC, with occasional mixing of the suspension. The reaction was stopped by adding methanol. The methanol suspension was passed through a cotton plug, filled up to 100 ml with methanol, and formazan was measured colorimetrically at 480 nm.

The population of *Rhizoctonia solani* in the sample was determined according to Ko and Hora (1971). The selective medium was prepared by dissolving final concentrations of 1 g K_2HPO_4, 0.5 g $MgSO_4{\cdot}7H_2O$, 0.5 g KCl, 0.01 g $FeSO_4{\cdot}7H_2O$ and 0.2 g $NaNO_2$ per litre. The pH was then adjusted to 6.5, agar added at 2.5% (w/v) and then autoclaved for 15 min at 121ºC. When the temperature dropped to 50ºC, we added final concentrations of 0.03% (w/v) gallic acid, 0.064 mM streptomycin, 0.116 mM chloramphenicol, 0.227 mM metalaxyl and 0.013 mM prochloraz to the medium. Metalaxyl and prochloraz were prepared from commercial pesticides, Apron® (Syngenta) and *Gerard* (เจอราด ®, Makhteshim), respectively. A volume of 20 ml of this medium was poured into a Petri dish (87 mm in diameter) and solidified at room temperature. Ten g (fresh weight) of the soil sample was suspended in 90 ml of sterilized 0.85% (w/w) NaCl, and reciprocally shaken at room temperature for 30 min at 120 rpm. The suspension was consecutively diluted 10-fold. One ml of the suspension was inoculated and spread onto the selective agar plate, and the number of colonies of *R. solani* was counted after an incubation period of 3 days at 28 ºC in the dark.

The bacterial community in each soil sample was profiled with three Biolog EcoPlates. The above-mentioned soil sample was used within 12 h of completion of the sampling. Ten grams of each soil sample was suspended in 90 ml of sterilized 0.85% (w/v) NaCl and reciprocally shaken at room temperature for 30 min at 120 rpm. The suspension was used as described previously (Doi 2005), and absorbance at 405 nm read using a microplate reader (Perlong DNM-9602G, Nanjing, PR China) at 4–12 h intervals for 7 days. Community-level physiological profiles of the soils were analysed to construct datasets for utilization of the information. The kinetic approach proposed by Lindstrom *et al.* (1998) was applied according to the following formula;

$$y = \text{Absorbance at 405 nm} = K / (1+e^{-R(t-S)}) \quad (3)$$

where K is the asymptote that the well absorbance approaches, R is the exponential rate of change in colour development, t is the time following inoculation of the Biolog plates and S is the time when the mid-point of the exponential portion of the curve (i.e. when $y = K / 2$) is reached. The value of the parameter K was regarded as the asymptote that the value of the average colour development of the well finally reaches. The 0.5 K time point was regarded as the exponential stage (Lindstrom *et al.* 1998) and the 0.95 K time point as the convergence stage. This approach provided 0.5 K and 0.95 K datasets. In addition to these datasets, another dataset called area dataset was constructed (Hackett and Griffiths 1997). For each carbon source, the colour development pattern was plotted and the area under the curve at the 0.95 K time point was regarded as the area for the carbon source. A multivariate profile for the soil sample was then obtained. A ratio-transformation was employed, i.e. each observation was divided by the sum of all the observations for the sample and used for statistical analyses.

Community-level physiological profiling offers bio-diversity indices based on the utilization pattern of the carbon sources by the soil bacterial community (Staddon *et al.* 1997). The Shannon diversity was also calculated using the following equation.

$$\text{Shannon diversity } (H') = -\Sigma pi \ln pi \quad (4)$$

where pi is the proportional absorbance for the ith carbon source over the total absorbance for the soil sample. The Shannon diversity indicates diversity of distribution pattern in the community (Staddon *et al.* 1997). The 0.5 K dataset was used to calculate the Shannon diversity index.

2.5 *Data analyses*

To determine the significance of the column and row effects on soil characteristics, one-way analysis of variance for each of the soil physicochemical characteristics was performed using the statistical software SPSS 10.0.1 (SPSS Inc.). Using the same software, non-linear (quadratic) multiple regression analysis between the population of *R. solani* in soil, values of soil dehydrogenase activity or soil fertility and soil characteristics was performed to obtain the formulae to describe changes in these variables. In the computation, the step-wise method at the default criteria (P=0.05 for inclusion and 0.10 for removal) was chosen. Integrated Land and Water Information System (ILWIS*)* academic version 3.3 (International Institute for Geo-Information Science and Earth Observation) was used to obtain maps

of the distribution of the population of *R. solani* in soil, soil dehydrogenase activity, soil fertility and Shannon diversity based on community-level physiological profiles. The maps were prepared after determining the geostatistics, namely, range, sill and nugget, using the geostatistical software Variowin version 2.2 (Université de Lausanne).

3. Results and discussion

According to one-way analysis of variance, there were significant column effects on most of the soil variables (P<0.05, table 1). Soil moisture, organic matter content, soil fertility index and dehydrogenase activity showed row effects. We found that the *R. solani* population had the greatest coefficient of variation (79%). Soil dehydrogenase activity also had a fairly large coefficient of variation (41%), while variations in the indices of soil fertility and of the Shannon diversity index were relatively small. Table 1 shows the differences among variation patterns of soil fertility. In this table, dehydrogenase activity and community-level physiological profile show significant row and column effects. Most of the values of soil nutrients and soil dehydrogenase activity were poorer than in the adjacent evergreen forest (Doi and Ranamukhaarachchi 2007), which was the original vegetation in the area. The population density of *R. solani* was greater in the rice paddy we studied than in the agricultural fields of peanut (Woodard and Jones 1983), collard (Keinath *et al.* 2003) and cotton (Davis *et al.* 1997), but lower than the *R. solani*-rich soil layer of lawn root mat (10^4/g dry soil, Shim *et al.* 1994). Warm and humid climatic conditions may help to increase the population of *R. solani* in the soil.

Some linear correlations were seen among *R. solani* population, dehydrogenase activity, the indices of soil fertility and the Shannon diversity index (table 2). Dehydrogenase activity, soil fertility index and soil evaluation factor had significant linear relationships with one another (P<0.003), while *R. solani* population and the Shannon diversity index had no linear relationship between each other or with other variables (P>0.379), thus showing their high independence from the others (table 1). Figure 1 shows the distribution

Table 1. Descriptive statistics of soil physicochemical variables, *R. solani* population, soil dehydrogenase activity and diversity of soil bacterial community

Variables	Mean	Minimum	Maximum	Standard deviation	Coefficient of variation (%)	Significance (P value)	
						Column (N=8)	Row (N=5)
Moisture (%)	13.3	9.71	17.0	1.74	13.0	0.956	<u><0.001</u>*
Bulk density (kg l^{-1})	1.52	1.26	1.68	0.09	6.05	<u>0.002</u>	0.890
pH	5.19	4.80	5.96	0.26	4.95	<u><0.001</u>	0.271
Bray II phosphorus (mg kg^{-1} dry soil)	7.22	4.14	15.5	2.73	37.7	<u><0.001</u>	0.834
Exchangeable K (mEq kg^{-1} dry soil)	2.68	1.47	5.44	0.99	37.2	0.389	0.154
Exchangeable Ca (mEq kg^{-1} dry soil)	10.7	5.06	16.7	2.83	26.5	<u><0.001</u>	0.251
Exchangeable Mg (mEq kg^{-1} dry soil)	17.8	6.68	34.1	5.35	30.1	<u>0.002</u>	0.081
Exchangeable Na (mEq kg^{-1} dry soil)	0.85	0.38	1.65	0.37	43.4	<u><0.001</u>	0.846
Exchangeable Al (mEq kg^{-1} dry soil)	5.26	1.21	11.2	3.20	60.9	<u><0.001</u>	0.544
Exchangeable H (mEq kg^{-1} dry soil)	4.49	2.03	7.27	1.10	24.5	<u><0.001</u>	0.057
Organic matter (%)	3.76	3.01	4.58	0.34	9.04	<u>0.002</u>	<u>0.020</u>
Cation exchange capacity (mEq kg^{-1} dry soil)	41.7	30.9	59.0	5.31	12.7	0.069	0.158
Base saturation (%)	75.7	46.9	92.1	12.2	16.2	<u><0.001</u>	0.300
Soil fertility index	14.0	8.96	25.3	3.40	24.3	0.085	<u>0.029</u>
Soil evaluation factor	28.8	15.8	44.4	6.53	22.7	<u><0.001</u>	0.286
Rhizoctonia solani population (colony-forming unit/g dry soil)	35.3	11	205	27.9	79	<u>0.070</u>	0.351
Dehydrogenase activity (μmol formazan g^{-1} dry soil h^{-1})	1.81	0.77	4.24	0.74	40.8	0.088	<u>0.005</u>
Shannon diversity	1.35	1.29	1.43	0.03	2.22	<u>0.022</u>	0.447

*The underlined P values indicate significant column or row effects (P<0.05)

Table 2. Linear correlations (R^2) among soil dehydrogenase activity, *Rhizoctonia solani* population, soil fertility index and soil evaluation factor

	Dehydrogenase	*R. solani*	Soil fertility index	Soil evaluation factor
Rhizoctonia solani population	0.014 (P=0.472)			
Soil fertility index	0.227 (P=0.002)	0.004 (P=0.701)		
Soil evaluation factor	0.219 (P=0.002)	0.014 (P=0.464)	0.447 (P<0.001)	
Shannon diversity	0.015 (P=0.450)	0.001 (P=0.842)	0.024 (P=0.380)	0.014 (P=0.470)

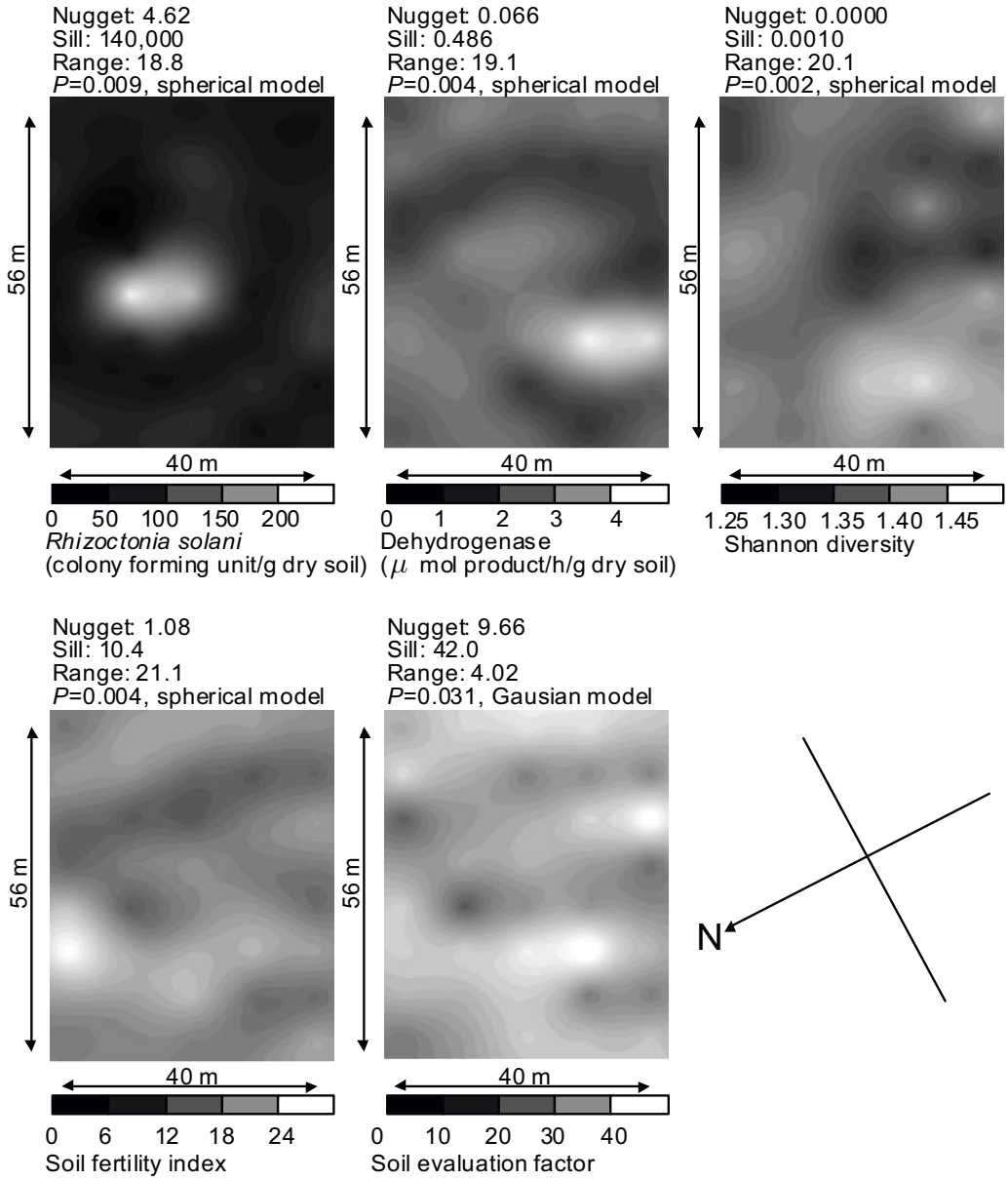

Figure 1. Distribution patterns of *R. solani*, dehydrogenase activity, Shannon diversity and soil fertility in the rice paddy. The values above each map indicate results of the determination of the statistics derived from the semivariogram as a pretreatment of the kriging.

patterns of *R. solani* and soil dehydrogenase activity, and variation patterns of soil fertility index, soil evaluation factor and the Shannon diversity index. As shown in table 2, similar variation patterns of soil dehydrogenase activity, soil fertility index and soil evaluation factor were seen in the rice paddy. However, these variables differed from

R. solani distribution and the Shannon diversity index in their patterns of variation. Because of the correlation between the variation in soil fertility and soil dehydrogenase activity, it appears that these variables can serve as indicators of soil fertility in a relatively compact area such as an agricultural field. In the adjacent area of Sakaerat Bioreserve, where an area of about 20 km^2 was surveyed, dehydrogenase activity was found to serve as a single measure of land degradation and rehabilitation associated with deforestation and reforestation (Doi and Ranamukhaarachchi 2009). Originally, soil fertility index was developed to measure the fertility of soils in cacao fields (Alvim and Cabala-Rosand 1974). Though the development was somewhat intuitive, the variables included in the formula accurately reflect changes in soil fertility (table 1). As indicators of variation in fertility of paddy soil in a relatively compact space (figure 1), soil dehydrogenase activity and soil fertility index were both useful.

The results of a quadratic multiple regression analysis on the above-mentioned differences in the *R. solani* population from the indices of soil fertility and dehydrogenase activity are shown in table 3. Among the dependent variables, soil fertility was best described using regression formulae based on the physicochemical variables (R^2 >0.992), while the bacterial dataset was less useful for describing variations in soil fertility. The variation pattern of soil dehydrogenase activity was also described by a formula based on the physicochemical variables at a high R^2 value (R^2 =0.710). However, this analysis was not useful in finding a way to describe the variation in *R. solani* population based on the physicochemical dataset, though the 0.5 K dataset provided a significant formula. According to this formula, soil samples that resulted in an absorbance value of around 0.45 for phenylethylamine at the 0.5 K time point had the smallest population density of *R. solani*. Because soil dehydrogenase activity, diversity of soil bacterial community and *R. solani* population are soil microbial variables, correlations among these microbial variables were expected. However, variations in Shannon diversity and *R. solani* population were unique while dehydrogenase activity and soil fertility were correlated. A possible explanation is the differences in cause-and-effect relationships among soil variables (Ghini *et al.* 2006). Soil microbial findings are related to the spatial variations in the physicochemical variables and, in turn, they may also alter the soil physicochemical conditions. This assumption is compatible with the area dataset that could describe the variation in soil fertility index well (P<0.001, table 3). Growth of *R. solani* could be suppressed by soil bacteria such as *Pseudomonas* (Pal *et al.* 2000) and *Bacillus* (Mazzola 1999) species. On the other hand, dehydrogenase activity directly reflects soil fertility because the redox consequence represents the total respiratory activity of all soil microbes, not just bacteria. This could explain why dehydrogenase activity was not significantly described by the bacterial dataset (figure 2), and did not correlate with the *R. solani* population.

The fertility and dehydrogenase activity in the rice paddy we studied was very low compared with the forest soil of the Sakaerat Bioreserve, which is close to the paddy (Doi and Ranamukhaarachchi 2009). The soil fertility was comparable with the most degraded soil in the reserve that has a land degradation–rehabilitation gradient in which the bare ground soil has the lowest fertility (Doi and Sakurai 2004). Soil dehydrogenase activity, as an indicator of total soil microbial activity, reflected the fertility of paddy soils (Rautaray 2005). The paddy soil was also poor in Shannon

Table 3. Quadratic multiple regression models for describing variations in *Rhizoctonia solani* population in the paddy field soil, soil dehydrogenase activity, soil fertility index and soil evaluation factor

Dependent variable	Dataset	Model fitness		Formula that describes the variation
		R^2	P	
R. solani	Bacterial 0.5 K	0.344	0.002	(phenylethylamine)x2159+(phenylethylamine)^{2}x2393-(D-xylose)^{2}x961+506
Dehydrogenase	Physicochemical	0.710	<0.001	(pH)^{2}x3.50-(moisture)^{2}x0.0076-pHx36.6–(exch. Al)x0.12+99.1
Soil fertility index	Physicochemical	0.993	<0.001	(base saturation)^{2}x0.00035+(Bray II P)x0.97-(exch. Al)x1.05 + (CEC*)^{2}x0.0011+8.63
	Bacterial 0.95 K	0.133	0.021	-(α-ketobutyric acid)x4.02+16.8
	Bacterial area	0.519	<0.001	-(4-hydroxy benzoic acid)^{2}x7741-(γ-hydroxybutyric acid)x297-(L-threonine)^{2}x10971-(2-hydroxybenzoic acid)^{2}x4889+47.5
Soil evaluation factor	Physicochemical	0.994	<0.001	Mgx0.54+(organic matter)^{2}x0.84+Cax0.55+(CEC)^{2}x0.0026-3.19
	Bacterial 0.5 K	0.377	0.002	(i-erythritol)^{2}x25.2+(glycyl-L-glutamic acid)x39.3-(α-cyclodextrin)^{2}x33.3+(itaconic acid)^{2}x13.0+22.3
	Bacterial area	0.124	0.026	-(pyruvic acid methyl ester)^{2}x6918+40.4

*CEC, cation exchange capacity.

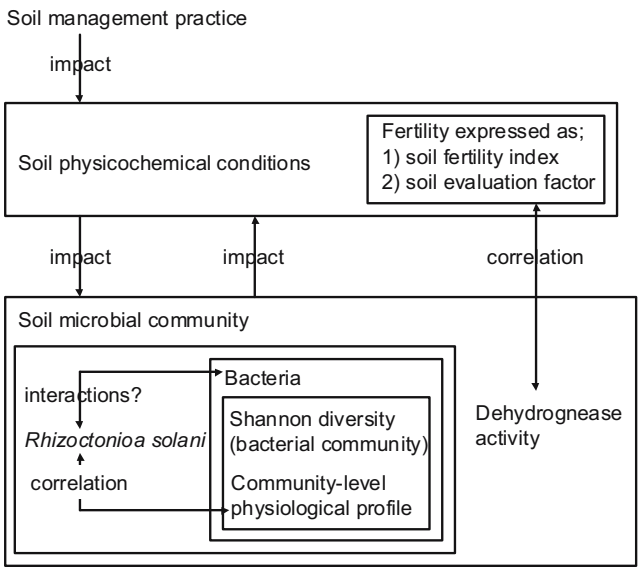

Figure 2. Proposed relationships among soil physicochemical conditions, soil microbial community and soil-related variables

diversity, and the value was comparable with that for the bare ground of Sakaerat (Doi 2005). These findings raise concerns about the possibility of sustainable cultivation in this rice paddy and others like it. In another study, van Elsas *et al.* (2002) suggested the importance of diversity of the soil bacterial community in the suppression of soil-borne diseases. Soil management practices alter the physicochemical properties of soil, and the soil microbial community may respond to these changes in ways that affect the ability of the soil to resist soil-borne diseases. Soil microbial diversity drops when the soil is subjected to intensive exploitation during agricultural production (Matson *et al.* 1997). In the paddy soil we studied, despite the fact that we did not find sheath blight symptoms in the 2005 season, the fertility, soil bacterial community profile and dehydrogenase activity of the soil indicated that the soil had been degraded. The fertility of the field we studied was poorer than many other paddies in Thailand (Prakongkep *et al.* 2008). The severity of sheath blight symptoms in rice is positively correlated with the population of *R. solani* in soil (Belmar *et al.* 1987). Thus, in the near future, due to the high population density of *R. solani* in the soil, the disease may cause a significant reduction in rice production if the soil is not treated in a suitable way. It is important to supply additional nutrients to the soil, and also support antagonistic microbes that decrease the populations of *R. solani*.

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