Soil Microbial Dynamics Modeling in Fluctuating Ecological Situations by Using Subtractive Clustering and Fuzzy Rule-Based Inference Systems

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Abstract: Microbial population and enzyme activities are the significant indicators of soil strength. Soil microbial dynamics characterize microbial population and enzyme activities. The present study explores the development of efficient predictive modeling systems for the estimation of specific soil microbial dynamics, like rock phosphate solubilization, bacterial population, and ACC-deaminase activity. More specifically, optimized subtractive clustering (SC) and Wang and Mendel's (WM) fuzzy inference systems (FIS) have been implemented with the objective to achieve the best estimation accuracy of microbial dynamics. Experimental measurements were performed using controlled pot experiment using minimal salt media with rock phosphate as sole carbon source inoculated with phosphate solubilizing microorganism in order to estimate rock phosphate solubilization potential of selected strains. Three experimental parameters, including temperature, pH, and incubation period have been used as inputs SC-FIS and WM-FIS. The better performance of the SC-FIS has been observed as compared to the WM-FIS in the estimation of phosphate solubilization and bacterial population with the maximum value of the coefficient of determination ($R^2 = 0.9988$) in the estimation of previous microbial dynamics.

Keywords: Phosphate solubilizing bacteria, bacterial population, ACC-deaminase activity, subtractive clustering, fuzzy rule-based prediction system.

1 Introduction

The soil is the lively part of the terrestrial environment that supports all forms of life. Soil condition is the result of continuous conservation and degradation processes and represents its continued capacity to function as vital living ecosystems [Carter, Gregorich, Anderson et al. (1997); Doran, Jones, Arshad et al. (1999); Doran and Zeiss (2000); Ghosh, Palsaniya and Kumar (2017)]. A unique balance of chemical, physical and biological (including microbial) components contribute to maintaining soil strength [Or,

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Smets, Wraith et al. (2007); Schoenholtz, Van Miegroet and Burger (2000); Doran (2002); Nielsen, Winding, Binnerup et al. (2002)]. Consequently, the assessment of soil strength necessitates the estimations of its components. Microorganisms possess the ability to contribute an integrated measure of soil condition that cannot be obtained with physical/chemical measures [Nielsen, Winding, Binnerup et al. (2002); Winding, Hund-Rinke and Rutgers (2005); Fine, Van Es and Schindelbeck (2017)]. Microorganisms respond quickly to changes, hence they rapidly adapt to environmental conditions. The microorganisms that are best adapted will be the ones that flourish [Singh, Pandey and Singh (2011); Ali, Hayat, Begum et al. (2017)]. This adaptation potentially allows microbial analyses to be discriminating in soil fitness assessment, and changes in microbial populations and activities, therefore, function as an excellent indicator of change in soil condition [Schloter, Dilly and Munch (2003); Gil-Sotres, Trasar-Cepeda, Leirós et al. (2005); Van Bruggen and Semenov (2000); Hermans, Buckley, Case et al. (2016)]. Microbial indicators of soil condition cover a diverse set of microbial capacities due to the multifunctional properties of microbial communities in the soil ecosystem that support to (i) control plant diseases as well as insect and weed pests; (ii) form beneficial symbiotic associations with plant roots (e.g. nitrogen-fixing bacteria and mycorrhizal fungi); (iii) recycle plant nutrients; (iv) improve soil structure with positive repercussions for its water- and nutrient-holding capacity; and (v) increase crop production [Ros, Goberna, Moreno et al. (2006); Alkorta, Aizpurua, Riga et al. (2003); Havlicek, (2012); Garbach, Milder, DeClerck et al. (2017); Tamez-Hidalgo, Christensen, Lever et al. (2016)]. One of the most important objectives in assessing the condition of a soil is the establishment of indicators for evaluating its current status [Doran, Jones, Arshad et al. (1999); Doran (2002); Schipper and Sparling (2000)]. Microbial population and enzyme activity are significant soil microbial condition indicators. These factors can be modeled using statistical and artificial intelligence techniques with significantly less engineering effort [Barberán, Bates, Casamayor et al. (2012); Hughes, Hellmann and Ricketts et al. (2001); Haider, Pakshirajan and Singh et al. (2008); Liang, Das and McClendon (2003); Tajik, Ayoubi and Nourbakhsh (2012); Kim, Yoo and Ki et al. (2011); Ebrahimi, Sinegani and Sarikhani et al. (2017); Ludwig, Vormstein and Niebuhr et al. (2017); Mukhlisin, El-Shafie and Taha (2012); Taghavifar and Mardani (2014)]; meanwhile, soil microbial, enzyme activity prediction by mathematical models is a tough task. In recent years, the trends towards modeling of machining processes using artificial intelligence methods have been increased due to their advanced computing capability. Researchers have used various intelligent techniques, including artificial neural network (ANN), fuzzy logic, neuro-fuzzy, adaptive neuro-fuzzy inference system (ANFIS) etc., for the prediction of machining parameters and to enhance manufacturing automation [Sen, Mandal and Mondal (2017); Yu, Yu, Wang et al. (2016); Hanafy, Zaini, Shoush et al. (2014)]. ANN and fuzzy logic are two important methods of artificial intelligence in modeling nonlinear problems. For example, ANN model has been implemented in the prediction of biosurfactant production under variable environmental conditions [Ahmad, Crowley, Marina et al. (2016)]. A neural network can learn from the data and feedback, however understanding the knowledge or the pattern is difficult. On the other hand, fuzzy logic models are easy to comprehend because they use linguistic terms in the form of *if-then* rules. A neural network with their learning capabilities can be also used to

learn the fuzzy decision rules, to create a hybrid intelligent system. A powerful subtractive clustering (SC) and Wang and Mendel's (WM) rule-based fuzzy inference systems (FIS) have been implemented in various application domains [Eftekhari and Katebi (2008); Lohani, Goel and Bhatia (2014); Wang (2003); Yang, Yuan, Yuan et al. (2010)]. Both methods use the advantages of fuzzy systems in a different way in efficient predicting and modeling. Though, we hardly noticed the implementation of FIS methods in soil microbial dynamics prediction in published literature. The FIS method in modeling and optimization problems is an effective way for the number of trials, and saving time and materials as well as offering a complete evaluation of the experimental process through creating a regression relation between dependent and independent variables. With this motivation, in the present research, evaluation and comparison of the prediction and simulation efficiency of SC-FIS and WM-FIS methods have been accomplished for estimation of soil microbial dynamics under fluctuating environmental situations.

2 Fuzzy rule based systems

Fuzzy logic is based on degrees of truth than completely true or false, which is similar to the functioning of the human brain. Both gather data about an incident, create a number of partial truths, and finally compose them into a higher truth. In data mapping, fuzzy logic assigns partial membership to each data point rather than the complete membership. After that, partial memberships are composed using certain *if-then* rules to find out the complete membership [Klir and Yuan (1995)]. Fuzzy inference is the process of constructing the map from a given input to an output using the fuzzy logic approach. Mainly, clustering methods, including the c-means clustering, fuzzy c-means clustering, mountain clustering, and subtractive clustering are used for generating the fuzzy rules in inference system. In the present study, most widely used subtractive clustering method is used in the implementation of fuzzy inference system and their performance is compared with the general fuzzy inference system based on Wang and Mendel's rule. A short description of both methods is as follows.

2.1 Wang and Mendel's fuzzy rule based system

Wang and Mendel's (WM) fuzzy rule based system (FRBS) is the simplest type of inference system. It is implemented in the present study by using the *frbs* package in R [Riza, Bergmeir, Henera et al. (2015)]. For a particular data set $\mathbf{D} = (x_1^1, x_2^1, y^1)(x_1^2, x_2^2, y^2)...(x_1^n, x_2^n, y^n))$, where y^n represent the output of input pair x^n , basic steps of method are as follows [Wang and Mendel (1982)]: (a) partitioning of input and output spaces into fuzzy regions by dividing the domain interval of each input and output variable into 2N+1 regions, where N may be different for each of the variables with either equal or unequal length, and assigning membership function to each partitioning from the previous step by deciding the degrees of each input-output pairs to different regions, assigning pairs to a maximum degree and obtaining one if-then rule for one pair; (c) assigning a degree to each rule, this is done to avoid the inconsistent rules of similar *if* part and different then part for

different data pairs; (d) creating a combined rule base, and (e) mapping based on a combined fuzzy rule. The details of the method can be seen in Ref. [Wang and Mendel (1982)].

2.2 Subtractive clustering fuzzy rule based system

The Subtractive clustering (SC) finds fuzzy clusters by assigning each data point a potential p_i for the likelihood of it according to a Gaussian measure $p_i = \sum e^{-\alpha ||x_i - x_j||^2}$,

where $\alpha = 4/r_a^2$ and r_a represents the cluster radius. The Euclidean norm $||\cdot||$ signifies the vector distance between data points (x_i, x_j) . The data point with maximum potential is selected as the first cluster center and all the data points within distance r_a of cluster center are linked to the first cluster. The second cluster center is determined in a similar way after excluding the data points associated with the first cluster. The process is continued until all data points lie within r_a of a cluster center [Chiu (1994); Chiu (1997)]. In this way, the subtractive clustering method produces a number of clusters in the data set for generating fuzzy rules in following steps: (a) degree of fulfillment of i^{th} fuzzy rule from i^{th} cluster center is calculated as $\mu_i = e^{-\alpha ||Y-X_i||^2}$; (b) defining the membership function for each j^{th} input to i^{th} fuzzy rule as; $A_{ij}(Y_j) = e^{-\alpha (Y_j - X_i)^2}$ (c) estimation of output variable by using the fuzzy i_f – then rule. The details of fuzzy rule extraction are described in [Chiu (1994); Chiu (1997)]. The method is implemented using the frbs package in R [Riza, Bergmeir, Herrera et al. (2015)].

3 Materials and methods

3.1 Soil sampling

The rhizospheric soil was collected from the wheat field. For this purpose, wheat plants were uprooted at tillering stage and stored in polythene bags. The non-rhizospheric soil was removed by agitating the roots strongly and the soil strictly adhering to the roots was used for the desired soil sample. Seven different wheat rhizospheric samples were collected and pooled up to make a composite sample and designated wheat root rhizospheric samples (WRS). The measured input experimental parameters using the Taguchi design are summarized in Table 1. Measured values of the bacterial population, rock phosphate solubilization, and ACC deaminase activity is given in Table 2.

Independent variables	Units	Valu	e		
Temperature	°C	25	30	35	40
pН	Adim	6	7	8	9
Incubation period	Days	3	6	9	12

Table 1: Values of different input variables.

Incubation period	Temperature	рН	Rock phosphate solubilization	Bacterial population	ACC- deaminase activity
3	25	6	10.520	2×10^5	1240.05
3	25	7	10.520	1×10^{7}	657.230
3	25	8	123.24	4×10^7	1311.63
3	25	9	169.29	3×10^{5}	430.010
3	30	6	146.27	3×10^{5}	821.970
3	30	7	151.53	6×10^{3}	1399.11
3	30	8	6.8800	1×10^{2}	3.81000
3	30	9	170.39	4×10^{6}	848.100
3	35	6	142.10	2×10^{5}	1063.96
3	35	7	79.820	1×10 ⁵	252.780
3	35	8	120.83	3×10^{4}	269.820
3	35	9	74.560	3×10 ⁶	859.460
3	40	6	3.7700	1×10^{2}	2.34000
3	40	7	124.56	4×10^{5}	393.660
3	40	8	0.1300	1×10^{2}	7.02000
3	40	9	5.1300	1×10^{2}	3.37000
6	25	6	131.79	5×10^5	94.8600
6	25	7	103.72	1×10 ⁶	422.060
6	25	8	113.59	2×10^{6}	440.240
6	25	9	71.710	2×10^{6}	531.120
6	30	6	119.51	1×10^{4}	305.040
6	30	7	9.2900	1×10^{2}	1.15000
6	30	8	119.95	7×10^4	366.390
6	30	9	133.11	1×10^{5}	401.610
6	35	6	122.58	1×10^{5}	61.9100

Table 2: Measured average value of rock phosphate solubilization, bacterial population, and ACC deaminase activity.

6	35	7	117.10	1×10^{4}	138.030
6	35	8	8.6800	1×10^{2}	8.60000
6	35	9	91.660	2×10^4	295.950
6	40	6	138.59	7×10^4	170.980
6	40	7	0.7400	1×10^{2}	0.46000
6	40	8	12500	3×10 ⁵	433.420
6	40	9	111.40	1×10^{5}	336.850
9	25	6	50.870	1×10^{7}	587.930
9	25	7	123.90	5×10^{5}	309.580
9	25	8	124.78	1×10^4	199.380
9	25	9	122.36	2×10^4	255.050
9	30	6	128.28	9×10^{3}	677.680
9	30	7	270.39	9×10^{3}	551.570
9	30	8	116.66	2×10^4	328.900
9	30	9	100.00	1×10^{5}	906.040
9	35	6	102.85	1×10^{5}	215.290
9	35	7	111.62	2×10^{5}	308.450
9	35	8	95.610	2×10^5	408.420
9	35	9	105.92	3×10 ⁵	1118.49
9	40	6	9.8600	1×10^{2}	6.61000
9	40	7	117.54	7×10^3	516.350
9	40	8	96.920	1×10^{5}	128.940
9	40	9	138.37	1×10^{5}	257.320
12	25	6	132.67	2×10^4	1155.98
12	25	7	0.2400	1×10^{2}	0.53000
12	25	8	4.6400	1×10^{2}	2.88340
12	25	9	0.4200	1×10^{2}	0.99890
12	30	6	113.59	1×10^4	311.861

12	30	7	122.58	1×10^{5}	267.553	
12	30	8	119.73	9×10^{4}	472.052	
12	30	9	0.5200	1×10^{2}	1.83640	
12	35	6	112.71	2×10^{6}	351.625	
12	35	7	117.10	3×10^{4}	431.152	
12	35	8	125.21	4×10^5	343.672	
12	35	9	137.50	1×10^{6}	168.712	
12	40	6	1600.0	16×10 ⁵	20.6100	
12	40	7	3700.0	37×10^{5}	8.77000	
12	40	8	9500.0	95×10 ⁵	13.8100	
12	40	9	3400.0	34×10 ⁵	13.8100	

3.2 Analytical measurements

3.2.1 Phosphate solubilization activity measurements

The phosphorus solubilizing activity of bacterial isolates present in soil sample was determined on the basis of the extent of solubilization of rock phosphate in NBRIP broth media [Nautiyal (1999)]. Briefly, 0.1 g rhizospheric soil sample was dissolved in 100 mL Tryptic soy broth in a conical flask and placed in shaking incubator at 100 rpm at 28°C. After 18 h incubation, the bacterial growth was observed by turbidity. For rock phosphate solubilization, 20 μ L of prepared inoculums was added to 50 mL, modified NBRIP broth (Glucose 10 g, Rock phosphate 5 g, MgCl₂.6H₂O 5 g, MgSO₄.7H₂O 0.25 g, KCl 0.2 g, (NH₄)₂SO₄ 0.1 g/L). Respective control was run without inoculums from rhizospheric soil samples. After 72 h of incubation, in shaking incubator, broth inoculated media was filtered and available P contents were measured at 410 nm [Olsen and Sommers (1982)]. The experiment was performed in replicate.

3.2.2 Bacterial population measurement

Cultivable attached rhizospheric bacteria were counted by the spread plate method on Marine Agar (Difco 2216) sterilized by autoclaving (121°C, 1 atm for 20 min). Dilutions were performed in 34 g/L sterile sodium chloride solution. Plates were set up in duplicate for each dilution. Incubation times were, according to the experimental at 25°C. Bacterial concentrations were expressed as CFU per ml for cultivable bacteria [Leonard, Blancheton and Guiraud (2000)].

3.2.3 Quantification of ACC deaminase activity measurement

ACC deaminase activity was assayed according to the method described by Penrose and Glick [Penrose and Glick (2003)], which measures the amount of α -ketobutyrate

produced after the enzyme ACC deaminase, cleaves ACC. The quantity of α -ketobutyrate (Sigma-Aldrich Co., U.S.A.) produced by this reaction was determined by comparing the absorbance of a sample to a standard curve of α -ketobutyrate ranging between 0.1-1.0 nmol at 540 nm. A stock solution of α -ketobutyrate was prepared in 0.1 M Tris-HCl (pH 8.5) and stored at 4°C. In order to measure the specific activity of the cultures, protein estimation was carried out according to the procedure detailed in [Lowly, Rosebrough, Farr et al. (1951)]. The data were subjected to analysis of variance using Statix software and means were compared by Duncan's multiple range tests at 5% probability [Steel and Torrie (1980)].

4 Modeling outcomes of FRBS

The predicted values of phosphate solubilization (PS), bacterial population (BP), and ACC deaminase activity (ACCA) by WM-FIS and SC-FIS methods are exhibited in Figure 1-3 respectively. The accuracy of both FIS methods has been evaluated in terms of the Pearson correlation coefficient (ρ), root mean square error (RMSE), and coefficient of determination (R²) are computed according to Eqs. 1-3 [Steel and Torrie (1980)], respectively and have been summarized in Table 3.



where A_i and P_i represents the actual and FIS method predicted values of experimental measurements, respectively.

5 Discussion

WM-FIS and SC-FIS methods have been implemented in the estimation of phosphate solubilization, bacterial population and ACC deaminase activity and their performance are compared in terms of the Pearson correlation coefficient, root mean square error and coefficient of determination. The Pearson correlation coefficient indicates the strength of the relationship between the actual values of phosphate solubilization, bacterial population and ACC deaminase activity and their estimated values by WM-FIS and SC-FIS methods, but the coefficient of determination measures the definite strength.



Measurement number

Figure 1: Phosphate solubilization estimation results using WM-FIS and SC-FIS methods.



Measurement number

Figure 2: Bacterial population estimation results using WM-FIS and SC-FIS methods.



Figure 3: Bacterial population estimation results using WM-FIS and SC-FIS methods.

FIS methods	Soil microbial dynamics types	Accuracy measures			
		Pearson correlation coefficient (ρ)	Root mean square error (RMSE)	Coefficient of determination (R ²)	
	1. PS	0.7762	1020.00	0.6025	
WM-FIS	2. BP	0.4992	4660000	0.2492	
	3. ACCA	0.5410	363.000	0.2926	
	1. PS	0.9994	45.2800	0.9988	
SC-FIS	2. BP	0.9673	1350000	0.9356	
	3. ACCA	0.7179	271.000	0.5153	

Table 3: Values of different input variables.

The correlation coefficient close to one indicates an approximately linear relationship between the actual and model predicted values of a dependent variable as well as better performance of model used for estimation. The RMSE is a significant measure to explain the precision of the model used for prediction, though; it is sensitive to large errors. The coefficient of determination is a significant measure to check the performance of models used in the estimation as it helps to understand the inconsistency of dependent variables. Figure 1 presents the actual phosphate solubilization vs. WM-FIS and SC-FIS model predicted phosphate solubilization on a log scale. It is obvious that the SC-FIS model predicted values of phosphate solubilization are comparable to the experimental values of phosphate solubilization (except for measurement number 40). Though, the WM-FIS model predicted values of phosphate solubilization exhibit larger deviation to the experimental values. This fact is further confirmed by the minimum value of *RMSE* = 45.28 and maximum values of the correlation coefficient $\rho = 0.9994$ and the coefficient of determination $R^2 = 0.9998$ (Table 3) for the SC-FIS model than the WM-FIS model predicted values of phosphate solubilization. Also, SC-FIS model has a better estimation efficiency for the phosphate solubilization than bacterial population and ACC deaminase activity in terms of *RMSE*, ρ and R^2 . The prediction results of the bacterial population using SC-FIS and WM-FIS methods have been shown in Figure 2. Again, SC-FIS method exhibits better estimation efficiency than the WM-FIS method (except measurement number 2). This is also established with the lesser value of *RMSE*=1350000 and higher values of the correlation coefficient ρ =0.9673 and the coefficient of determination $R^2 = 0.9356$ for the SC-FIS model than the WM-FIS model in the prediction of bacterial population (Table 3). The ACC deaminase activity estimation results are shown in Figure 3. In most of the measurements, the SC-FIS model estimated values of ACC deaminase activity are closer to their real measured values. This confirms the better performance of the SC-FIS model than the WM-FIS model. This fact is further approved in terms of a lesser value of *RMSE* and higher values of ρ and R^2 for the SC-FIS model than the WM-FIS model (Table 3). During the analysis, it is observed that there is some combination of input variables that results in the SC-FIS and WM-FIS model

predicted values of phosphate solubilization, bacterial population and ACC deaminase activity close to their actual values. Table 4 summarizes three best combinations of such variables. In case of phosphate solubilization, SC-FIS method for the combination of input parameters: INC = 6, Temp = 25, and pH = 8 results in almost 100% accuracy in prediction (actual value of phosphate solubilization 113.59 and SC-FIS method predicted value of phosphate solubilization 113.56). A similar situation is observed for two combinations of input parameters (INC = 9, Temp = 25, pH = 7, and INC = 12, Temp = 25, pH = 6) in the estimation of bacterial population using the SC-FIS method (Table 4). For ACC deaminase activity estimation using the SC-FIS method three combinations of input (INC = 3 , Temp = 35 , pH = 8 , INC = 6 , Temp = 25 , pH = 7 , parameters and INC = 12, Temp = 25, pH = 9) results in almost 100% prediction accuracy. The best combinations of input parameters summarized in Table 4 can be used in searching the optimal environmental conditions that result in the best estimation of microbial dynamics. Fuzzy methods are accurate in the modeling of data while controlling the imprecision. Due to this reason, WM-FIS method has been implemented in several applications, like the prediction of dissolved oxygen in river water [Shaghaghian (2010)], operator performance using electroencephalographic (EEG) variables [Zhang, Xia and Garibaldi et al. (2017)], and energy forecasting [Jozi, Pinto and Praça et al. (2016)]. Also, the WM-FIS method has reliable prediction performance than ANN and support vector machine (SVM) methods in the latter application. Though, the application of WM-FIS method in microbial dynamics estimation is not noticed in published literature. Also, the performance of WM-FIS method has been enhanced in some recent studies, like using an evolutionary algorithm in controlling fuzzy sets [Kato, Morandin, Sgavioli et al. (2009)], and inducing cooperation for fuzzy rules [Casillas, Cordón and Herrera (2000)], etc. Another option is to evaluate the performance of WM-FIS method with some other FIS method like SC-FIS which has better efficiency in several applications like road header performance prediction [Yazdani-Chamzini, Razani, Yakhchali et al. (2013)], fault detection [Chudasama, Shah and Shah (2016)], modeling demand response of smart grid [Pereira, Fagundes, Melicio et al. (2014)], and soil cation exchange capacity [Keshavarzi, Sarmadian, Rahmani et al. (2012)], etc. The better performance of the SC-FIS method is noticed than the WM-FIS method in the present analysis. Since the SC method recognizes similarities in the data set and creates an FIS to model the data behavior using a minimum number of efficient fuzzy rules. The prediction of ACC deaminase is significant as it is an important factor to promote the growth of a plant.

6 Conclusion

The study presents the estimation of microbial dynamics, including phosphate solubilization, bacterial population, and ACC-deaminase activity by using SC-FIS and WM-FIS methods and their performance is compared in terms of correlation coefficient, root mean square error and coefficient of determination. The temperature, pH, and incubation period show variation during the measurement and affects microbial dynamics, therefore used as input of SC-FIS and WM-FIS methods. The SC-FIS method has abetter estimation efficiency than the WM-FIS method of estimation of microbial

dynamics. Also, the best estimation efficacy is observed for the phosphate solubilization by using the SC-FIS method. Estimation of ACC-deaminase activity by using WM-FIS method results in the least accuracy.

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