**A Statistics and Analyses System of Microbial Community Level Physiological Profiles (CLPP) Based on Excel**

Lin Li 1,2, Yanjie Zhu 2, Suyu Liang 2,3, Songyan Tian 2,3, Qian Du 2,3, Shenkui Liu 2

1 Alkali Soil Natural Environmental Science Center (ASNESC), Northeast Forestry University, Harbin 150040, PR China
2 Key Laboratory of Forest Ecology and Forestry Ecological Engineering in Heilongjiang Province, Heilongjiang Forestry Engineering and Environment Institute, Harbin 150081, PR China
3 Mudanjiang Forest Ecosystem Research Station, Mudanjiang, Heilongjiang, 157500, PR China
4 State Key Laboratory of Subtropical Silviculture, Zhejiang A&amp;F University, Lin’an, Hangzhou, 311300, PR China

Corresponding author email: shenkui8u@nefu.edu.cn

Molecular Soil Biology, 2019, Vol.10, No.1 doi: 10.5376/msb.2019.10.0001

**Abstract** Microbial community level physiological profiles (CLPP) assessed using Biolog MicroPlates has effectively demonstrated the metabolic activities of multiple microbes. Due to a large volume of data, and complicated calculation process, the conventional low efficiency calculations delay the study. To empower researchers to quickly tabulate, compute, analyze and present data, we introduced A Biolog EcoPlate data statistics and analyses system (A-BEP-DSAS). It is implemented with Biolog EcoPlate data tested by an ELISA microplate reader (Molecular Devices Versamax). The data input-output process and visual calculation flow are performed using Excel table linked with built-in VBA programming language. We tested A-BEP-DSAS by data from a soil sample, and rapidly and accurately obtained the average well color development (AWCD) data, carbon source utilization, six common diversity indices and other calculation results. A-BEP-DSAS is an automated program based on Microsoft Excel VBA, with the advantages of easy operation, rapid analysis, process visualization, and also allows the user to choose the data source and optimum time for analysis.

**Keywords** Biolog EcoPlate data analyses, Excel VBA, Microbial community level physiological profiles, Microbial diversity index, Carbon source utilization

**1 Introduction**

Soil ecosystem is one of the most important places for material cycle and energy conversion (Gupta, 1998). Soil microorganisms play a key role in the transformation of soil organic matter, and are also significant factors that affect plant nutrition activity (Powlson et al., 2001; Maestre et al., 2011; Jin et al., 2017). Thus, soil microbial diversity and activity are the foundations of a stable and healthy soil ecosystem. Among the microbial indicators, community level physiological profiles, assessed by the method of Biolog EcoPlate™, has effectively demonstrated the metabolic activities of multiple microbes, which reflect the condition of soil ecological health.

Microbial community level physiological profiling analysis using Biolog MicroPlates was originally described by J. Garland and A. Mills (Garland and Mills, 1991). The Biolog EcoPlate, specifically adapted from GN and GP plates to characterize environmental communities, contains three sets of 31 useful carbon sources (http://www.biolog.com/pdf/eco_microplate_sell_sheet.pdf), including carbohydrates, amino acids, carboxylic acids, polymers, amines and phenolic compounds, and a blank for soil community analysis (Insam, 1997). The community reaction patterns were typically analyzed at defined time intervals over 2–5 days, which is the logarithmic growth period of microorganisms (Hackett and Griffiths, 1997). Other researchers measured for 10 consecutive days, and analyzed over 6–7 days, which is the stable growth period of microorganisms (Girvan et al., 2003; Liu et al., 2015; Kumar et al., 2017). The reaction patterns were most effectively analyzed with a microplate reader, which provided optical density (OD590, OD750) values. The Principle Components Analysis (PCA), indicating the correlation between different sample points, and the Constrained Ordination (redundancy analysis or canonical correspondence analysis), indicating the correlation between sample points and quantitative environmental factors, via the average well color development (AWCD) data, calculated from original optical
density values, were the most popular methods of data analysis (Rutgers et al., 2016; Zhang et al., 2018). The changes observed in the fingerprint pattern provide key data on the microbial population changes over time (Daou et al., 2017; Jiang et al., 2017).

There is no commercial software available for the data processing of Biolog EcoPlate. Due to the large volume of data, and the complicated calculation process, Excel is an optimal data platform for analysis. Biolog Eco data analysis using Excel data table is suitable for the data continuity, and the data can be automatically imported in combination with Excel built-in VBA programming language. The researchers can select corresponding data parameters according to their specific requirements, which makes the data processing more intuitive, simple and flexible.

In this study, an auto data input and output process with visual calculation flow was carried out using Excel table linked with built-in VBA programming language. This user-friendly software provides a useful resource for researchers by reducing monotonous repetitive work in Biolog EcoPlate data statistics and analyses.

2 Materials and Methods
2.1 Availability and requirements
Project name: A-BEP-DSAS
Operating system(s): Windows
Programming language: Excel VBA
Other requirements: Biolog EcoPlate data tested by ELISA microplate reader for ten days in a row of soil samples (4-6h.xls, 24h.xls, to 240h.xls). A-BEP-DSAS.xls and Summary Statement.xls were stored in the same file folder.
Availability of data and materials: The dataset analyzed during the current study is available from http://www.hljifee.org.cn/.

2.2 Data statistics and analyses methods
The average well color development (AWCD) for all carbon sources was calculated to assess soil microbial metabolic activity (Garland and Mills, 1991).

\[ AWCD = \sum \frac{C_i - R}{n} \]

Where \( C_i \) is the optical density (OD590-OD750) value of the reaction wells, \( R \) is the OD value of the control well, \( n \) is the number of 31 different carbon sources. The AWCD reflects the overall situation of soil microbes utilizing different carbon sources (Choi and Dobbs, 1999).

The Richness index (\( S, \) absorbance \( \geq 0.2 \)), Shannon-Weaver index (\( H \)), Pielou index (\( EH \)), Shannon evenness index (\( E \)), Simpson dominance index (\( D \)) and McIntosh index (\( U \)), which were derived from absorbance obtained from Biolog EcoPlates, demonstrated the functional diversity of soil microbial communities. The formulae are as follows:

Richness index (\( S, \) absorbance \( \geq 0.2 \))

\[ S(\text{absorbance} \geq 0.2) = N(n_i \geq 0.2) \]

Shannon-Weaver index (\( H \))

\[ H = -\sum P_i \ln P_i \]

Pielou index (\( EH \))

\[ EH = H / \ln S \]

McIntosh index (\( U \))

\[ U = \left( \sum n_i \right)^{1/\sum n_i} \]

Shannon evenness index (\( E \))

\[ E = \left( \sum n_i - U \right) / \left( \sum n_i - \sum n_i / S^{1/2} \right) \]

Simpson dominance index (\( D \))

\[ D = 1 - \sum P_i^2 \]

Where, \( P_i = (C_i - R) / \sum (C_i - R) \); \( N \) is the number of wells with value \( (C_i - R) \geq 0.2 \); \( n_i = C_i - R \) (Zak et al., 1994; Fang et al., 2009).

3 Results
The Biolog EcoPlate data tested by ELISA microplate reader for ten consecutive days was stored in the same file folder with A-BEP-DSAS.xls, the main site of data analysis. The Summary Statement.xls is an Excel table that held the exported data. All these excel tables can be viewed at http://www.hljifee.org.cn/.
In the “A-BEP-DSAS.xls”, users open the dialog box (Fig. 1) by clicking the “Analysis” button. This dialog box is the main interface of the specific conditions based on the user's request to enter the relevant information and selection. Biolog EcoPlate initial data tested by ELISA microplate reader are added to the “A-BEP-DSAS.xls” by clicking “DataInput” button, followed by choosing whether or not to eliminate the background values and time for analysis. Then, after clicking the “Analysis” button, the calculated data including AWCD curve, diversity indexes (Richness Index, Shannon-Winner Index, Pielou Index, Simpson Index, McIntosh Index, and McIntosh Evenness Index), microbial consumption of six major groups of carbon sources, and data used for the analysis of the PCA or RDA are added in the corresponding sheets of “A-BEP-DSAS.xls”. “Data Output” button can export the data to the Excel table named “Summary Statement.xls”.

![Initialization dialog box of A-BEP-DSAS](image-url)

**Figure 1 Initialization dialog box of A-BEP-DSAS**

### 3.1 Data input

Biolog EcoPlate data tested by ELISA microplate reader (Molecular Devices Versamax) at 590 nm and 750 nm wavelengths during 10 days were auto-input into A-BEP-DSAS.xls, sheet “Original Data”, cell C4 to AA91.

### 3.2 Data analyses

The AWCD in each tested time node is shown in the sheet of “AWCD Curves”. Based on the specific research needs, the researchers selected the time nodes to be analyzed from the drop-down list box of “Select time”. “Eliminate BV” option button indicated whether the background values were eliminated, if you chose “Yes”, the absorbance values <750 nm were subtracted, and only the absorbance values <590 nm were used for the subsequent calculations.

After making personalized choices, the results of diversity index were shown in the sheet of “Diversity Index”, and the results of carbon sources utilized were shown in the sheet of “Carbon Source Classification”. The cell location corresponding to each data result are shown in Tables 1 and 2.

### 3.3 Data output

The results obtained from the A-BEP-DSAS were summarized in the table of “Summary Statement.xls”, one row per sample.

**Table 1 The cell location of diversity index**

| Diversity index | Richness (S) | Shannon-Winner (H) | Pielou (EH) | Simpson (D) | McIntosh (U) | McIntosh Evenness (E) |
|-----------------|--------------|--------------------|-------------|-------------|---------------|-----------------------|
| Cell of results | C6           | B18                | C18         | B25         | B33           | C33                   |

**Table 2 The cell location of carbon sources utilized**

| Carbon Source Classification | Carbohydrates | Amino Acids | Carboxylic acids | Amines | Polymers | Aromatics |
|-----------------------------|---------------|-------------|------------------|--------|----------|-----------|
| Cell of results             | B20           | C20         | D20              | E20    | F20      | G20       |
4 Discussion
A-BEP-DSAS provides a visual calculation flow to the large community of users with Biolog EcoPlate data processing requirements. This application can be extended, modified and adapted by transforming the Excel formulae, functions and VBA codes based on the user’s needs, ensuring that Biolog EcoPlate data can be effectively used in user-specific analyses.

Authors' contributions
LL conceived and designed the research, performed the program, data analyses and wrote the manuscript, and contributed the experimental materials. YZ played an important role in maintaining the operation of download data website. SL, ST and QD revised the manuscript, tested data, and helped perform the analysis with constructive discussions. SL designed the research and approved the final version.

Acknowledgments
This work was supported by the National Key Research and Development Program of China (2016YFC0501202-03), Applied research of Heilongjiang forest industry general administration (sggjY2016017), and the Natural Science Foundation of China (31400388, 41475123). The authors thank Peng Li at Oxford Nanopore Technologies for guidance and support.

References
Choi K.H., and Dobbs F.C., 1999, Comparison of two kinds of Biolog microplates (GN and ECO) in their ability to distinguish among aquatic microbial communities, J. Microbiological Methods, 36(3): 203-213. https://doi.org/10.1016/S0167-7012(99)00034-2
Daou L., Luglia M., Périssol C., et al., 2017, Sporulation and physiological profiles of bacterial communities of three Mediterranean soils affected by drying-rewetting or freezing-thawing cycles, Soil Biology & Biochemistry, 113: 116-121. https://doi.org/10.1016/j.soilbio.2017.06.008
Fang H., Yu Y.L., Chu X.Q., Wang X.G., Yang X., and Yu J.Q., 2009, Degradation of chlorpyrifos in laboratory soil and its impact on soil microbial functional diversity, J. Environ. Sci., 21(3): 380-386. https://doi.org/10.1016/S1001-8571(08)62280-9
Garland J.L., and Mills A.L., 1991, Classification and characterization of heterotrophic microbial communities on the basis of patterns of community level sole-carbon-source utilization, Applied and Environmental Microbiology, 57(8): 2351-2359
Girvan M.S., Bullimore J., Pretty J.N., et al., 2003, Soil type is the primary determinant of the composition of the total and active bacterial communities in arable soils, Applied & Environmental Microbiology, 69(3): 1800-1809. https://doi.org/10.1128/AEM.69.3.1800-1809.2003
Gupta V., 1998, The living soil: Soil microorganisms and their role in soil processes, Australian Cotton Growers Research Association, 43-48
Hackett C.A., and Griffiths B.S., 1997, Statistical analysis of the time-course of biology substrate utilization, Journal of Microbiological Methods, 30(1): 63-69. https://doi.org/10.1016/S0167-7012(97)00045-6
Insam H., 1997, A new set of substrates proposed for community characterization in environmental samples, Microbial Communities, 163(6): 259-260. https://doi.org/10.1007/978-3-642-40694-6_25
Jiang L.L., Han G.M., Yu L., et al., 2017, Corn cob biochar increases soil culturable bacterial abundance without enhancing their capacities in utilizing carbon sources in Bi-oLog Eco-plates, Journal of Integrative Agriculture, 16(3): 713-724. https://doi.org/10.1007/S10295-0119(16)61338-2
Jin W.H., Yang J.S., Yao R.J., et al., 2017, Effects of rice-wheat rotation and afforestation on microbial biomass carbon in coastal salt-affected soils of eastern China, Pedosphere, 27(5): 938-948. https://doi.org/10.1016/S1002-0160(17)60397-7
Kumar U., Shahid M., Tripathi R., et al., 2017, Variation of functional diversity of soil microbial community in sub-humid tropical rice-rice cropping system under long-term organic and inorganic fertilization, Ecological Indicators, 73: 536-543. https://doi.org/10.1016/j.ecolind.2016.10.014
Liu B., Li Y., Zhang X., et al., 2015, Effects of chlortetracycline on soil microbial communities: comparisons of enzyme activities to the functional diversity via Biolog EcoPlates™, European Journal of Soil Biology, 68: 69-76. https://doi.org/10.1016/j.ejsobi.2015.01.002
Maestre F.T., Bowker M.A., Cantón Y., et al., 2011, Ecology and functional roles of biological soil crusts in semi-arid ecosystems of Spain, Journal of Arid Environments, 75(12): 1282-1291. https://doi.org/10.1016/j.jaridenv.2010.12.008
Powison D.S., Hirsch P.R., and Brookes P.C., 2001, The role of soil microorganisms in soil organic matter conservation in the tropics, Nutrient Cycling in Agroecosystems, 61(1-2): 41-51. https://doi.org/10.1023/A:1013338028454
Rutgers M., Wouterse M., Drost S.M., et al., 2016, Monitoring soil bacteria with community-level physiological profiles using Biolog™ ECO-plates in the Netherlands and Europe, Applied Soil Ecology, 97: 23-35
Zak J.C., Willig M.R., Moorhead D.L., and Wildman H.G., 1994, Functional diversity of microbial communities: A quantitative approach, Soil Biol. Biochem., 26(9): 1101-1108
Zhang L, Lyu T, Zhang Y , et al., 2018, Impacts of design configuration and plants on the functionality of the microbial community of mesocosm-scale constructed wetlands treating ibuprofen, Water Research, 131: 228-238