**ABSTRACT**

In this inquiry activity, community-level physiological profiling (CLPP) with EcoPlates is described to quantitatively depict the community of microorganisms situated in a natural environment. Students can develop their own hypotheses and carry out experimentation about the makeup of microbial communities using EcoPlates. There are many independent variables that can be assessed or changed to determine how environmental differences can play important roles in the types of microorganisms that are found in an environment.

**Key Words:** Diversity; metabolism; community metabolic diversity; carbon-source utilization; engagement; EcoPlates; microbial diversity; natural environment; soil.

**Introduction**

There are many ways in which microbiology laboratory classes strive to engage students and teach microbial diversity and metabolism. For example, one customary set of labs is the “microbiological unknown” activities in which students either isolate or are given an unknown microorganism and perform a series of assays and growth tests to determine the bacterial species (e.g., Wagner & Stewart, 2000; Deutch, 2001). These labs have often been changed to encourage more engagement (e.g., Martinez-Vaz et al., 2015), to utilize polymerase chain reaction and sequencing techniques (Boomer et al., 2002), and to add additional bioinformatics skills (Gibbens et al., 2015). An alternative method to studying individual microorganisms or isolates is to analyze the community of microbes present in an environment, such as deep-sequencing an entire microbial community to identify individual members present, a costly and labor-intensive approach.

Microbial community physiological profiling, a method of determining microbial diversity by metabolic means, is another option for studying all microorganisms present in a sample. Previously, we described using community-level physiology profiling (CLPP) with Biolog EcoPlates to study soil microbe communities as a quick and powerful method to introduce students to microbiology (Marshall & Sweat, 2008). Biolog EcoPlates are 96-well plates that are supplied with 31 different carbon sources and one water control, seeded in triplicate with dehydrated media and a tetrazolium dye. Different microbial communities will have markedly different abilities to utilize these carbon sources, depending on the individual bacterial species present, and carbon-source use is indicated by the reduction of the dye and formation of a purple color. These communities can be described by carbon-source utilization (CSU) for each individual carbon source and the total community metabolic diversity (CMD). CSU is the average utilization by the community of each individual carbon source (the more utilization, the more purple the well becomes and the higher the CSU). CMD is the total number of different carbon sources utilized by the community, which can range from zero (no utilization) to 31 (community is able to utilize all carbon sources) (Marshall & Sweat, 2008).

We have been performing Biolog EcoPlate analysis as a student-driven inquiry laboratory in a summer undergraduate program focusing on experimental design. Students receive limited instruction in microbiology and community-level profiling and then are challenged to design their own hypotheses and experimental design, utilizing the EcoPlates. Students analyze soil from several locations around campus and compare the microbial communities. They are encouraged to assess not only location but other soil variables such as moisture, pH, and nutrient levels (using soil kits such as Rapitest).
Hypotheses that students have tested in relation to microbial communities include soil location, soil type, species of plants growing in the sample, soil chemical composition, and invertebrate species count. Student can also vary the temperature at which they incubate their plates and the amount of incubation time.

**Materials**
- Biolog EcoPlates (http://www.biolog.com; catalog no. 1506, $10.72 each, quantity discounts available)
- Soil Rapitest (Luster Leaf, available at hardware stores; approximate price is $10 for 10 individual tests each for pH, nitrogen, phosphorus, and potassium)
- Soil moisture probe (available at hardware stores for ~$10)

**Procedure**
1. Students develop hypotheses about microbial communities and variables within and among those communities.
2. Students sample soil from the environment, taking care to wear gloves to minimize contamination from skin. They use a clean weigh dish as a shovel and place the soil into a 50 mL conical vial. They note the location(s) of samples. For a more robust analysis, students could construct transects and sample those sites.
3. Students use the moisture probe to sample the soil location and record the data.
4. Students weigh 0.5 g of soil and add it to 50 mL of sterile water in a tube. The tube is then capped, shaken vigorously, and the particulate matter is allowed to settle or can be centrifuged out. The remaining soil is saved for future analyses.
5. Students pipette 100 µL of the supernatant (from step 4) into each well of the EcoPlate. A multichannel pipettor works better for this, but a regular pipettor will work as well.
6. The plate is allowed to incubate. We usually read the plates after 4 days. Generally 25°C is used, but we have also changed the temperatures and then CSU and CMD will change due to differential temperature growth of different microbes. Increasing temperature could speed up color development but also would change the CSU and CMD, as noted above.
7. Plates are read. Students use a microtiter plate reader set at A590. The positive growth is denoted by a purple well, and students could simply analyze, by eye, each well for purple color to generate a CMD. However, the CSU for each carbon source could not be generated without a plate reader.
8. Students then analyze data for CSU, CMD, and correlation analysis (Marshall & Sweat, 2008). CSU = absorbance mean carbon-source well – absorbance mean water. CMD is achieved by summing the number of positive carbon sources. A sample correlation table is show in Table 1, and a graph is shown in Figure 1.
9. While the plates are incubating, the soil is analyzed for additional variables. Students can use the Rapitest Soil Test kit, which has individual tests for pH, nitrogen, phosphorus,

### Table 1. Community-level physiology profiling (CLPP) data table with added soil analysis. Soil was taken from indicated locations, and moisture was analyzed by a handheld probe. Community metabolic diversity (CMD) was determined with Biolog EcoPlates (see text) using a four-day incubation period at 25°C. Soil analysis was performed using Rapitest kits according to manufacturer’s instructions.

<table>
<thead>
<tr>
<th>Location</th>
<th>CMD</th>
<th>Moisture</th>
<th>pH</th>
<th>Phosphorus</th>
<th>Nitrogen</th>
<th>Potassium</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zen garden</td>
<td>28</td>
<td>Wet</td>
<td>7.5</td>
<td>Surplus</td>
<td>Depleted</td>
<td>Adequate</td>
</tr>
<tr>
<td>Left side tree</td>
<td>30</td>
<td>Moist</td>
<td>7.5</td>
<td>Sufficient</td>
<td>Depleted</td>
<td>Adequate</td>
</tr>
<tr>
<td>Right side tree</td>
<td>31</td>
<td>Wet</td>
<td>7.5</td>
<td>Sufficient</td>
<td>Deficient</td>
<td>Adequate</td>
</tr>
<tr>
<td>Fletcher bush</td>
<td>30</td>
<td>Moist</td>
<td>7</td>
<td>Deficient</td>
<td>Depleted</td>
<td>Sufficient</td>
</tr>
<tr>
<td>Flowerpot</td>
<td>31</td>
<td>Moist</td>
<td>7.5</td>
<td>Surplus</td>
<td>Deficient</td>
<td>Adequate</td>
</tr>
<tr>
<td>Bush sands</td>
<td>29</td>
<td>Wet</td>
<td>7.5</td>
<td>Deficient</td>
<td>Depleted</td>
<td>Surplus</td>
</tr>
<tr>
<td>Bush UCB</td>
<td>26</td>
<td>Moist</td>
<td>7.5</td>
<td>Surplus</td>
<td>Depleted</td>
<td>Adequate</td>
</tr>
<tr>
<td>Grass</td>
<td>30</td>
<td>Moist</td>
<td>7</td>
<td>Sufficient</td>
<td>Depleted</td>
<td>Sufficient</td>
</tr>
<tr>
<td>Sdfc</td>
<td>29</td>
<td>Moist</td>
<td>6.5</td>
<td>Deficient</td>
<td>Depleted</td>
<td>Depleted</td>
</tr>
<tr>
<td>Verde</td>
<td>29</td>
<td>Moist</td>
<td>7</td>
<td>Deficient</td>
<td>Deficient</td>
<td>Deficient</td>
</tr>
<tr>
<td>Hysa</td>
<td>24</td>
<td>Dry</td>
<td>7</td>
<td>Depleted</td>
<td>Depleted</td>
<td>Adequate</td>
</tr>
<tr>
<td>Fab</td>
<td>28</td>
<td>Moist</td>
<td>7.5</td>
<td>Deficient</td>
<td>Depleted</td>
<td>Depleted</td>
</tr>
<tr>
<td>North parking</td>
<td>31</td>
<td>Wet</td>
<td>7.5</td>
<td>Deficient</td>
<td>Depleted</td>
<td>Depleted</td>
</tr>
<tr>
<td>Bush parking</td>
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<td>Dry</td>
<td>7.5</td>
<td>Adequate</td>
<td>Sufficient</td>
<td>Deficient</td>
</tr>
<tr>
<td>Tree desert</td>
<td>18</td>
<td>Dry</td>
<td>7.5</td>
<td>Sufficient</td>
<td>Depleted</td>
<td>Deficient</td>
</tr>
</tbody>
</table>
and potassium. The soil can be tested the same day or in a later class period.

(10) After students have completed the soil analysis, they may revise their hypotheses on the basis of the new information. For example, a student might hypothesize that a soil very low in nitrogen or “Depleted for Nitrogen” in the test would have a low CMD since nitrogen is required for amino acid synthesis.

(11) Students can analyze their data for additional parameters, such as:

- average well-color development over time (Choi & Dobbs, 1999);
- Shannon-Weaver diversities or diversity of substrate utilization (DSU; Muñiz et al., 2014), comparable to CMD;
- analysis of which substrates are utilized, calculated by Shannon-Weaver evenness value (Tam et al., 2001); and/or
- correlation or principal component analysis of grouped substrate types (polymers, carbohydrates, carboxylic acids, amino acids, amines, or phenolic compounds; Choi & Dobbs, 1999).

Table 1 and Figure 1 give an example of how students could present and analyze their data. Indeed, each student or team will have different data, as their hypotheses and site or sites will differ.

**Extensions**

There are myriad extensions that can be done with CLPP. Soils can be spiked with inorganic, organic nontoxic, or toxic elements and/or compounds to probe differences in CMD and CSU. Sampling locations can vary widely; waterways can be sampled; and CLPP assayed at different times of year can be tracked. Time courses of plate incubation can be included as well (Choi & Dobbs, 1999). There are many additional variables that can be studied, such as soils under different types of plants (e.g., native and nonnative, monocotyledons and dicotyledons, evergreen and deciduous, with and without nitrogen-fixing root nodules). Students themselves should be generating these variables and hypotheses, perhaps after a short research project. Moreover, more extensive analysis can occur, such as statistical analysis (Marshall & Sweat, 2008) and much more complex modeling (Muñiz et al., 2014). Furthermore, for programs studying microbial biochemistry, further analysis of the carbon sources used can inform conclusions drawn about the community (Choi & Dobbs, 1999).

This activity can be adapted and performed at many levels, including high school, community college, and undergraduate courses of different levels, from first-year biology to upper-level microbial ecology classes. Even for institutions that do not have a microtiter plate reader, CMDs can be generated, because analysis can be performed even in the absence of the plate reader, by comparing qualitatively the purple color that develops in the well. For younger students, analyzing one or two variables in the context of the CMD would be an effective method of data analysis; in upper-level classes, more sophisticated analysis can occur (Muñiz et al., 2014). We use this activity in an experimental-design unit and assess it accordingly, using the TIPS II test (Burns et al., 1985). Additional assessment can include the Experimental Design Ability Test or EDAT (Sirum & Humburg, 2011) and the Biological Experimental Design Concept Inventory or BEDCI (Deane et al., 2014).

**Conclusion**

EcoPlates are an economical method for analyzing the community of microorganisms in a given setting and allow one to capture the metabolic activities of a microbial community (Garland, 1997). Giving students opportunities to develop their own hypotheses and experimental design and to analyze authentic data helps them understand that science is a way of knowing, and not a set of facts to be memorized. EcoPlate analysis is an effective method to study microorganism communities, and these plates can be utilized in an inquiry setting in which student generate and test their own hypotheses. Data generated from EcoPlates is quantitative and easily analyzed — statistically, graphically, and mathematically. Community-level physiological profiling using EcoPlates is amenable to many levels of students and different types of analysis and can be used in a wide variety of courses to help students develop experimental-design and data-analysis skills.

**References**


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