**Protocol for Rpf soil Microcosm Experiment**

**Description**: Measuring microbial functionality in soil with Rpf addition to optimize concentration and application times.

**Materials Required**:

* Microcosm vials
* Live soil from Griffy Teaching Preserves
* Rpf – Recombinant protein from rpf gene KBS0714 expressed in E. coli and captured on NiNTA column and filter sterilized (0.22um) and concentration measured (1.4 mg/uL)
* Rpf IgG – Antibodies against Rpf activity from rabbit serum concentration measured (\_\_\_\_\_ug/uL)
* Sterile dH20

**Controls**: (4 reps each)

1. Media – 2mL R2B
2. Rpf – 1.95mL R2B + 50uL Rpf
3. Baseline growth – 2mL R2B + 10uL KBS0714
4. Rpf Positive control – 1.95mL R2B + 50uL Rpf + 10uL 5X washed late stationary KBS0714
5. Rpf negative control – 1.95mL R2B + 50uL Rpf + 25uL IgG + 10uL KBS0714
6. Soil negative control – 100uL H2O in 3X autoclave soil (4 reps)
7. Soil positive control – 75 uL Rpf + 450 uL H2O in 3X autoclave soil (4reps)

**Treatments**: (4 reps each) live soil

1. 12.5uL Rpf + 87.5uL H20 x 4 = 50uL Rpf + 350uL H2O stock
2. 25uL Rpf + 75uL H20 x 4 = 100uL Rpf + 300uL H2O stock
3. 50uL Rpf + 50uL H20 x 4 = 200uL Rpf + 200uL H20 stock
4. 75uL Rpf + 25uL H2O x 8 = 600uL Rpf+200uL H2O stock
5. 500uL H20

**Methods**:

1. Prepare Treatment mixes before starting experiment and keep chilled on ice
2. Pull out soil from 4°C fridge on first floor
3. Homogenize soil by shaking thoroughly in bag
4. Measure out 5g of live soil into each vial and then cap tightly
5. Lay on side and allow to incubate for 24 hours in environmental room
6. Take CO2 reading using instrument in the environmental room of Lennon Lab following Nathan’s instruction
7. Aliquot appropriate amounts of treatment into the vials using sterile pipette tips to distribute solution evenly in soil matrix as discussed
8. Cap tightly and lay on side
9. Take 0, 24, 48, and 72 hour time point readings of CO2
10. The treatments will be reapplied to the same vials to measure if resuscitation consistently increases microbial activity with Rpf application or if microbial activity declines. Take another 0, 24, 48, and 72 hour time point reading of CO2.
11. Be sure to allow some O2 into vials after each reading by unscrewing caps under the bench hood
12. Record data by hand (keep note of what time point) and also export file into flash drive/ somewhere safe so that data can be exported into excel file