<u>Soil respiration measurement using Micro-Oxymax Respirometer</u>

<u>Basal respiration</u>
I.   Soil preparation
     a. 50g (dry weight) soil in 250ml flask[1] (with GL 45 fitting)
     b. Setup the (water) bottles and tubing to Extension Interfaces
     c. Weight wet soil based on moisture content measurement into numbered (according to channel number) Duran bottles.
     d. Assemble as bottles are weighed except a tubing to Extension Interface to equilibrate with atmosphere
     e. After all bottles are weighed, finish setup making sure O-rings and metal plates connects are intact.
     f. Pre-incubate for 12-24 hours at incubation temperature (RT)[2]
II.   Setting up the Micro Oxymax Respirometer
     a. Calibration (`Tools-Calibration`)
         i. Offsetting gases other than O_2 (bottle 1 – lime soda column)
        ii. Offsetting O_2 (bottle 2 – calibration gas cylinder[3])
       iii. Gaining O_2 (bottle 1)
       iv. Gaining gases other than O_2 (bottle 2[4])
     b. Leakage check (`Tools-Utilities`)
         i. Click on <u>L</u>eakage button for automatic testing (pp. 76 for source of leakage)
     c. Setup the experiment (`Experiment-Setup`)
         i. Can be done while leakage test is on
        ii. `Setup` tab
           1. **Channels** – End channel – # channels to use
           2. Mark 'Auto volume Measurement', 'O2 Consumption Positive'
           3. **Timing** – Sample Interval(Hours) – 2.5hours
           4. **Data Units** – Normalization Unit – g
       iii. `Chamber Setup` tab
           1. Type 'Normalization Units' and 'Channel Label'
           2. 'Volume' and 'Leakage' will be determined by test
     e. Start the incubation by clicking `Run`

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[1] If available soil samples are not enough, try with as much as possible but be consistent with all samples within an experiment.
[2] Pre-incubation is needed for restoration of metabolic equilibrium of the population after storing at 4C (12hr) and re-wetting (24hr).
[3] Open the cylinder first, open regulator knob until hearing hissing sound, then adjust pressure to 5psi.
[4] Current composition (H_2S 179.8ppm, H_2 1801ppm, CO_2 0.906%, CO 0.9015%, CH_4 0.9054%) – balanced by N_2 (cylinder # SG9147282, reference # 83-124147696-1). It should be modified in System Properties at Sensors tab (pp. 70)

III.   Incubation condition & measurement
   a.   23C (or RT), 2 ½ hour interval[5] for 120 hours[6]
   b.   Humidifying bottles (50ml with GL32 cap) for each sample to maintain moisture level in gas after going through drier (Figure 1)
   c.   Collect final results (accumulation) and graph in both rate ($\mu l\ O_2\ g^{-1}$ soil h) and accumulation ($\mu l\ O_2\ g^{-1}$ soil) for further inference
IV.   After incubation
   a.   Disconnect Duran bottles, empty soil, wash, oven-dry and cap with aluminum foil for long term storage; otherwise, re-use them for next incubation.
   b.   Leave water bottles connected for immediate next incubation; otherwise cover with the original orange caps

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[5] Or shortest intervals possible. (# channel + 1) * 6 min
[6] 72 hours by Chuck, 150 hours by Hollender *et al* 2003