NGEE-Tropics BNL AC_i and AQ protocols for red blue light source

This protocol is for measuring A-C_i and AQ curves with the LiCOR 6400XT and the 2x3cm Red Blue light source, software version 6.3.2, adapted from previous NGEE-Arctic and NGEE-Tropic protocols by Kim Ely and Alistair Rogers, February 2017.

Instruments & Equipment

Gas Exchange

- LI-COR 6400XT gas exchange system with a R/B light source
- Sufficient batteries and chargers for the 6400 to allow measurements to be made without interruptions for charging.
- Gaskets: 2x3 white (2 day^{-1} $machine^{-1}$) and black (1 day^{-1} $machine^{-1}$), 3-hole (1 $per\ campaign^{-1}$ $machine^{-1}$)
- CO_2 cartridges (2 day^{-1} $machine^{-1}$)
- Chemicals: soda lime, Drierite
- Zero gas (UHP nitrogen), regulator, tubing
- Spare parts (see list)
- Bar codes (sample number stickers)
- Sample envelopes
- Medium ziplock bags (4 x 6 inch) for holding barcoded envelop on machine
- Laptop or other alternative for downloading data

Advance Preparation

1. Ensure instrument is recently calibrated (<2 years)
2. Ensure most recent software is loaded (6.3.3)
3. Load NGEE Tropics config files
4. Ensure chemicals and CO_2 cartridges are in place at field site
5. Ensure any safety training is complete e.g. working at height
6. Instruments zero with zero gas at start of campaign

Sampling Plan

Branches will be collected predawn / very early in the day using the canopy crane, from top of canopy (sunlit leaves). Branches to be cut while submerged in water. Using a transfer cup the branches will be transferred to buckets and kept in the shade for Aci measurements.

GAS EXCHANGE

Start of Day

1. Turn on (CO_2 on Scrub, Desiccant on Bypass)
2. Open config NGEE_TropicsA_RB_ACi_age or TEST_NGEE_Tropics_RB_AQ_2017
3. Follow warm up procedures
4. Open logfile and name using the following format
    a. date: YYYYMMDD
    b. site: PA-SLZ
    c. measurement type ACi or AQ
    d. instrument ID: i.e. Derek, Mariano, Andy, Jorge, Bernie, Johnny

    e.g. 20160217_SLZ_ACi_Bernie

Warm up procedures

Immediately

1. CO_2 cylinder in ***replace after 6h - set a timer***
2. Temperature (block, air, leaf) all within 2°C
3. PARout - responds to light
4. PARin - light comes on and is stable
5. Pressure - OK (c. 100 kPa at sea level)
6. Leaf fan working
7. Flowmax > 750µmol s-1
8. Flow restrictions - drops <15 µmol s-1 on full scrub
9. Set Flowzero (pump off, leaf fan off)

After >10 minutes (full scrub desiccant and soda lime)

10. Check CO2 zero (± 5 µmol mol-1 of zero)
11. Check H2O zero (0.2 mmol mol-1 and falling after 2 minutes)
12. Tleaf - responds
13. Tleaf zero (block and leaf within 0.1°C)
14. Leak test - (CO2R to 400 mol mol-1, Flow to 200 µmol s-1) blow through tube around all gaskets and seals - CO2S should change by <1 µmol mol-1
15. Match

Logging and sample tracking

1. The NGEE Tropics config will prompt you to enter information in the following fields
    a. barcode: e.g. BNL10854 (all BNL bar codes have human readable five digit numbers, proceed this number with the BNL prefix)

b. species: there are pull down menus for species IDs e.g. CASTEL
c. location: e.g. San Lorenzo
d. machine name: e.g. Bernie
e. serial number: e.g. PSC-3613

2. Keep ziplock bag with barcode on machine during measurement
3. After each measurement label the leaf with the barcode number using surgical tape. The leaf will be collected for spectral measurement and tissue sampling later.
4. Record any notes or comments in log file using "Add Remark" function on LI-COR.

Instrument stability

1. Set chamber conditions: flow 500 µmol s^{-1}, just saturating PAR (2000 µmol m^{-2} s^{-1}), CO_2R = 400 ppm, T_{block} at T_{air} +1 or 2°C (dew point buffer).
2. Wait for instrument stability
3. Match

Leaf acclimation

1. Insert leaf and close chamber.
2. Leak test with a straw (<1 µml departure from set point in CO2S)
3. If necessary fix leaks by reclamping, tightening, removing tension on leaf or using Molycote 111 on gaskets
4. If leaf does not fill chamber adjust Area with an estimate.
5. Monitor A and g_s for a ***minimum of 20 minutes*** and then ensure stability (flat lines for 5 minutes) - some species took >45 minutes to stabilize.
   a. Check dew points - T_{block} should be 1-2°C above the dew points for sample and reference (see graph F)
   b. Check VPD < 1.5 kPa
   c. Check **ΔCO2 > 10 µmol s^{-1}** (adjust flow if necessary 500-200 µmol s^{-1})
   d. Match after any adjustments

Autoprogram for ACi

1. Ensure you make some at least one log during stabilization to load up the metadata that will be added to the data from the auto program
2. Launch autoprogram "TEST_Arctic_Aci_2015" Note on Johnny launch "A-Ci Curve2"
3. Configure autoprogram settings
   a. CO_2 values = 400, 325, 250, 175, 100, 66, 33, 400, 400, 400, 475, 575, 675, 800, 1000, 1400, 1800, 400
   b. Min wait = 60 s
   c. Max wait = 120 s
   d. Match before log = "always"

    e. Stats = yes, Means log file = no, control changes = no, Echo to Com = no
    f. Stability: CO_2R SD <0.75 over 20 s, A SD < 0.25 over 20 s.
    g. Note b,c and f may need to be adjusted depending on species.
4. Start curve and monitor progress of A-c_i curve.
5. After program has finished mark up leaf if area correction is required then release from chamber. Flag leaf for collection for spectra measurement.

Autoprogram for AQ

1. Ensure you make some at least one log during stabilization to load up the metadata that will be added to the data from the auto program
2. Launch autoprogram “TEST_Arctic_AQ_2015” Note on Johnny launch “Light Curve2”
3. Configure autoprogram settings
    a. PAR values = 2000, 2000, 1500, 1250, 1000, 750, 500, 250, 200, 150, 100, 75, 50, 20, 0, 1500
    b. Min wait = 30 s
    c. Max wait = 200 s
    d. Match before log = “never”
    e. Stats = yes, Means log file = no, control changes = no, Echo to Com = no
    f. Stability: A SD < 0.2 over 20 s, PARin SD < 1 over 20 s, Tleaf SD < 0.2 over 20s.
    g. Note b,c and f may need to be adjusted depending on species.
4. Start curve and monitor progress of A-Q curve.
5. After program has finished mark up leaf if area correction is required then release from chamber. Flag leaf for collection for spectra measurement.

Expected stable values (based on Feb 2016 diurnal measurements)

Code	Species	Photosynthesis	Conductance
CASTEL	Castilla elastica	12	0.1
LUEHSE	Luehea seemannii	22	0.3

ANACEX	Anacardium excelsum	10	0.08
CORDAL	Cordia alliodora	10	0.1
CALYCA	Calycophyllum candidissimum	10	0.1
FICUIN	Ficus insipida	18	0.2
ALIBED	Pseudosamanea guachapele	20	0.25
ANTITR	Pittoniotis trichantha	16	0.25
PSE1SE	Pseudobombax septenatum		
TERMAM	Terminalia amazonia	18	0.17
TOCOPI	Tocoyena pittieri	9	0.1
CARAGU	Carapa guianensis	12	0.15
TACHVE	Tachigali versicolor	15	0.5
VOCHFE	Vochysia ferruginea	20	0.45
VIROSP	Virola multiflora	7	0.05
MICOBO	Miconia borealis	15	0.25
APEIME	Apeiba membranacea	20	0.5
GUATDU	Guatteria dumetorum	15	0.6