Complete factorial design:

Two treatment levels, four replicates

No CPOM or CPOM

Ambient N &P or N & P addition

**Study Site:**

-Include CPOM data only describe how we got data

-include initial nutrient data (ambient) only describe how we got data

Procedure for sediment collection:

1. Collect replicate Ekman samples from LPP littoral (1600 ml sediment needed)
2. Sieve sediments through 250 um mesh and retain in buckets\*
3. 100 ml of sediment in BOD= ~3.5 cm
4. Add ~200 ml of pond water, gently so as not to disturb the sediments (3200 ml water needed)

\*By definition, a “macroinvertebrate” is any invertebrate that is 250 um or larger. By using a 250 um mesh net, we effectively eliminated macroinvertebrates from our samples.

CPOM density for LPP

1. Run sediment (littoral) from Ekman through 250 um sieve
2. Whatever was retained by the net was put into 1L bottles
3. Back at the lab, the contents of the 1 L bottles were run through 1 mm sieve
4. The CPOM that remained in the sieve was placed in a pre-weighed paper bag
5. CPOM was dried at 50 degrees C, weighed, and then ashed at 550 degrees C to determining AFDM

\*CPOM calculation was used to determine how much CPOM to add to the BOD bottles

Nutrient analysis

1. Dr. Dina Leech filtered 50 ml of the collected pond water (29 May 2014) through GFF
2. N was tested using Hach Test Kit N1- 12 (cat. # 14081-00) using the provided instructions
3. Nitrate- read as below detection (bd)- no color developed (<8.8 mg/L)
4. Nitrite- read as bd- no color developed (<0.066mg/L)
5. Ammonia- read as bd- color was yellow (<0.2mg/L)
6. Orthophosphate- read as bd- no color developed (<0.2 mg/L)

Final BOD Samples Collected (July 3, 2014)

All- C:N ratio sediment and Sediment ergosterol

CPOM- C:N ratio of leaves and Leaf ergosterol

CPOM Flux Sampling Protocol:

1. Remove 15 ml with glass syringe and place in 10 ml vial
2. Seal vial and fix for T-O
3. Remove 15 ml with glass syringe place in 10 ml vial
4. Incubate vial in dark for 5 hr
5. Remove 3ml and add to bacterial vial and preserve
6. Remove 30 ml and filter through GFF into 50 ml Falcon tube
7. Remove 5ml and filter through GFF for absorbence into 15 ml Falcontube
8. Add 90 ml water back to BOD and seal
9. Incubate BOD for 5 hr in dark
10. Remove 15ml from BOD and place in 10 ml vial\*
11. Fix incubation and T-1 vial

\*BOD bottles were incubated with ~15 ml of water removed (285ml total) on rocker-shakers (Speed 8, Tilt 8) in the dark

Leaf OM content determination:

1. 5 leaves were randomly selected from the tulip poplar leaves collected from fall 2013
2. Each leaf was gently submersed in DI water until it was soft enough to core (About 5 min)
3. A single leaf disk (10 mm; #5 Cork Borer) was cut from the leaf bade avoiding the midrib
4. The disk was placed into a pre-weighed crucible and dried at 50 degrees Celcius, then ashed at 550 degrees C

Find File Sediment Dry Mass and AFDM

Find File Sediment BOD jar setup