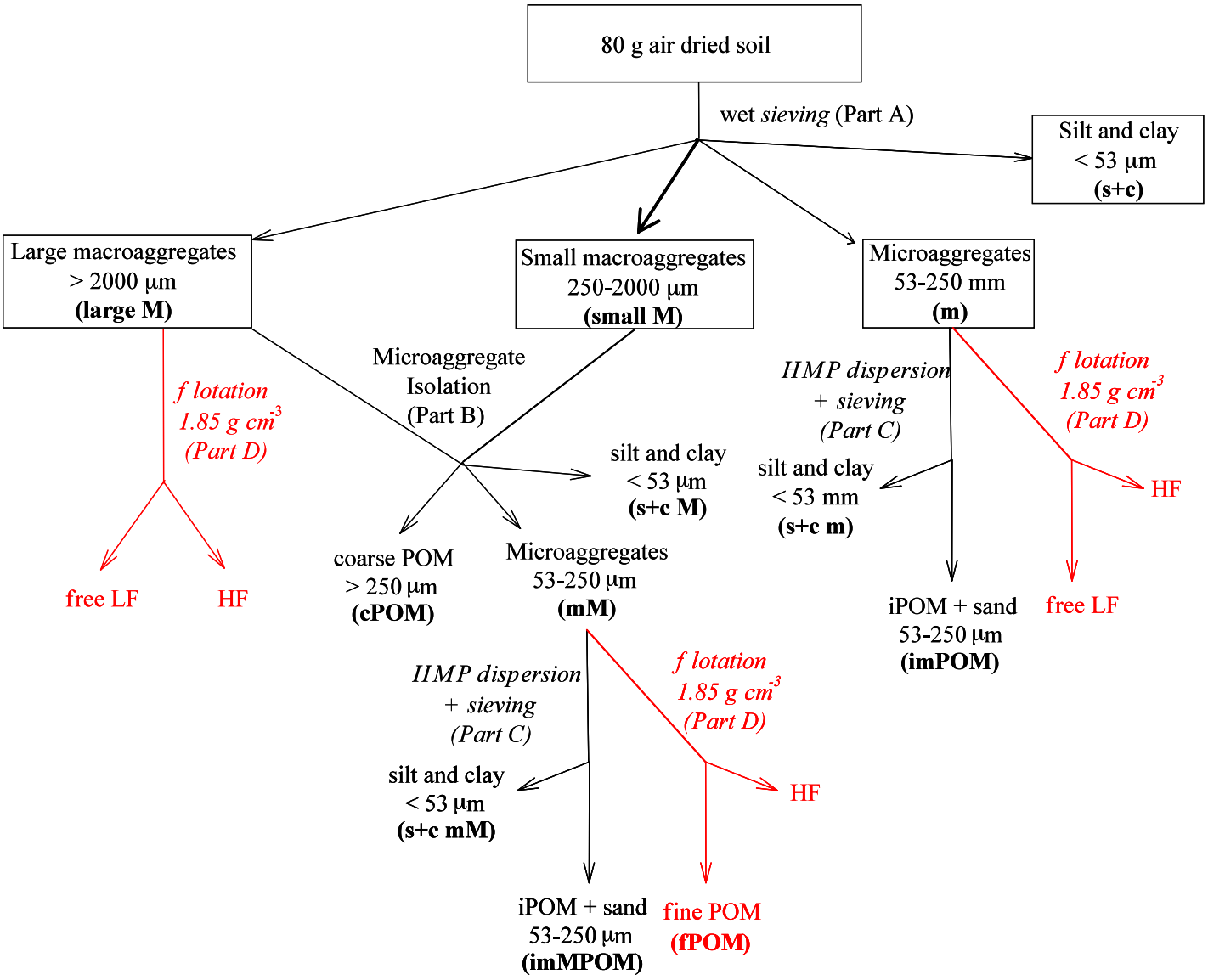

80 g air dried soil
wet *sieving* (Part A)
Silt and clay
< 53 µm
**(s+c)**
Large macroaggregates
> 2000 µm
**(large M)**
Small macroaggregates
250-2000 µm
**(small M)**
Microaggregates
53-250 mm
**(m)**
*f lotation*
*1.85 g cm-3*
*(Part D)*
free LF
HF
Microaggregate
Isolation
(Part B)
silt and clay
< 53 µm
**(s+c M)**
coarse POM
> 250 µm
**(cPOM)**
Microaggregates
53-250 µm
**(mM)**
*HMP dispersion*
*+ sieving*
*(Part C)*
silt and clay
< 53 mm
**(s+c m)**
*f lotation*
*1.85 g cm-3*
*(Part D)*
HF
iPOM + sand
53-250 µm
**(imPOM)**
free LF
*HMP dispersion*
*+ sieving*
*(Part C)*
silt and clay
< 53 µm
**(s+c mM)**
*f lotation*
*1.85 g cm-3*
*(Part D)*
HF
iPOM + sand
53-250 µm
**(imMPOM)**
fine POM
**(fPOM)**

*Protocol: Part A: Wet-sieving of whole soil.*

1. Take a 80 g subsample from air-dried whole soil (weigh on digital balance and record weight; two significant numbers).
2. Fill up white basin (30 cm diameter; 8 cm deep) with water until water level is approximately 1 cm above 2000 μm (2 mm) sieve-mesh.
3. Sprinkle soil evenly out on sieve and wait for 5 minutes.
4. After the 5 minutes, sieve soil for two minutes by moving sieve 50 times up and down (approx. 3 cm amplitude) with a slight angle to ensure that water and small particles go through the mesh.
5. Take sieve out of the water and rinse of the sides plus bottom of the sieve with water in order to have all particles in suspension.
6. Backwash > 2000 μm aggregates (i.e. large macroaggregates) into preweighed small drying pan with sufficient water.
7. Decant off the floating litter into the waste bucket.
8. Put drying pan with large macroaggregates in the 60°C forced air oven (overnight).
9. Pour water and particles that went through the 2000 μm sieve remaining in white basin onto a 250 μm, which you hold above the second white basin, and repeat sieving procedure (in 2 minutes move sieve 50 times up and down (approx. 3 cm amplitude) with a slight angle to ensure that water and small particles go through the mesh).
10. Take sieve out of the water and rinse of the sides plus bottom of the sieve with water in order to have all particles in suspension.
11. Backwash 250-2000 μm aggregates (i.e. small macroaggregates) into preweighed small drying pan.
12. Put drying pan with small macroaggregates in the 60°C forced air oven (overnight).
13. Pour water and particles that went through the 250 μm sieve remaining in white basin onto a 53 μm, which you hold above the second white basin, and repeat sieving procedure (in 2 minutes move sieve 50 times up and down (approx. 3 cm amplitude) with a slight angle to ensure that water and small particles go through the mesh).
14. Take sieve out of the water and rinse of the sides plus bottom of the sieve with water in order to have all particles in suspension.
15. Backwash 53-250 μm aggregates (i.e. microaggregates) into preweighed small drying pan.
16. Put small drying pan with microaggregates in the 60°C forced air oven (overnight).
17. Pour water + < 53 μm particles (i.e. silt + clay) remaining in white basin into a preweighed large drying pan.
18. Put large drying pan with silt + clay particles in the 60°C forced air oven (overnight).
19. Next day, weigh all fractions, grind subsample (1-2 g) of the large and small macroaggregates and microaggregates and put all grounded fractions, 5-10g non grounded large and small macroaggregates and microaggregates, and 2g of silt and clay fraction in vials to be used for C and N analysis

*Protocol: Part B: Isolating micro's from large and small macroaggregates*

1. KENYA/EMBU SOIL: DAY BEFORE: Take a subsample (5 g) from oven-dried macroaggregates (weigh on digital balance and record weight; two significant numbers) and put them in a beaker with approximately 50 ml of water; put them in cooler 4C overnight). OTHER SOILS: Take a subsample (15 g) from oven-dried macroaggregates (weigh on digital balance and record weight; two significant numbers) and put them in a beaker with approximately 50 ml of water (let them stand for 20 minutes until you are ready with the device).
2. Put device (i.e. microaggregate isolator) on reciprocal shaker, put 50 glass beads on 250 μm mesh, connect small tubing to device and faucet, and connect large tubing to device plus put it through clamp attached to stand. Clamp should be positioned at a height that the water level in the device is 1.5 cm above the mesh (not above the plexiglass border, but above the mesh). Fill up the device with water until water is running out of the outlet into the white basin and regulate the water flow in order to have a slow, but stable water flow through the device. Collect all the water needed to accomplish this in a white basin (30 cm diameter, 8 cm deep).
3. Once water flow is stable, close of the water with the red valve, move the outlet from the device to a 53 μm sieve in a white basin, add the soil to the glass beads on the 250 μm mesh, and put the lid on the device. SHAKING PROCEDURE FOR ALL SOILS EXCEPT EMBU: start the reciprocal shaker at low (= 150 rpm) speed and shake (5 minutes max.) the device until water flowing out of the device onto the 53 μm sieve is clear and all aggregates on top of the 250 μm screen are broken up. SHAKING PROCEDURE FOR EMBU: start the reciprocal shaker at high (=250 rpm) speed and shake for 3 minutes, stop water flow by closing red valve, take off lid, rinse with water until aggregates are clearly visible on mesh, break up the larger visible aggregates by putting some pressure with spatula directly on aggregate. Put lid back on, start water flow by opening red valve and shake for another minute. After that minute, stop shaking, turn off the water by closing red valve, take off lid, rinse with water until aggregates are clearly visible on mesh, break up aggregates by putting some pressure with spatula directly on aggregate. Put lid back on, start water flow by opening red valve and shake for another minute (all aggregates should be broken up).
4. Close the red valve, rinse off the lid of the device and sides of the Plexiglas tube. See (with spatula) if all the macroaggregates are broken up, if not put lid back on device, open the red valve, and start shaker at low (or high) speed. If all aggregates are broken up, close red valve, drain device and tubing by removing tubing from clamp and lowering the white basin with 53 μm sieve (but do this slowly because otherwise the water will spill over). Rinse tubing and make sure the outlet is completely drained.
5. **If two people are available, then Step 5 to 8 is done by one person while the other conducts step 9.** Sieve the material left on the 53 μm sieve by moving the sieve 50 times up and down (approx. 3 cm) with a slight angle to ensure that water and small particles go through the mesh.
6. Take the sieve out of the water, rinse the sides and bottom to ensure that all <53 μm particles are in the suspension.
7. Backwash > 53 μm (i.e. microaggregates-within-macroaggregate) into a preweighed small drying pan and put it in the 105°C forced air oven (overnight).

8. Transfer water and particles that went through the 53 μm sieve remaining in white basin to a preweighed large drying pan and put it in the 105°C forced air oven (overnight).
9. **Other person:** Take device from shaker, put it onto a 2000 μm and 53 μm sieve (the 2000 μm is to collect the beads and the 53 μm sieve to collect organic matter), and rinse water through the bottom of the device to collect beads, sand and organic matter on the sieves (make sure that all organic matter is rinsed off the sieve! Once the beads are collected on the 2000 μm and all the organic matter is collected on the 53 μm sieve, transfer material that is on 53 μm sieve in a preweighed small drying pan and put it in 105°C convection oven.
10. Next day weigh all fractions, grind subsample (2 g) of the 53-250 μm fraction (i.e. mM), grind the > 250 μm fractions and put grounded fractions, 2-5g of non-grounded 53-250 μm (i.e. mM) and silt and clay fraction in vials to be sent to Dr. Johan Six, UCDavis.

*Protocol: Part C: Isolation of intra-aggregate POM associated with microaggregate fractions (free m and mM).*

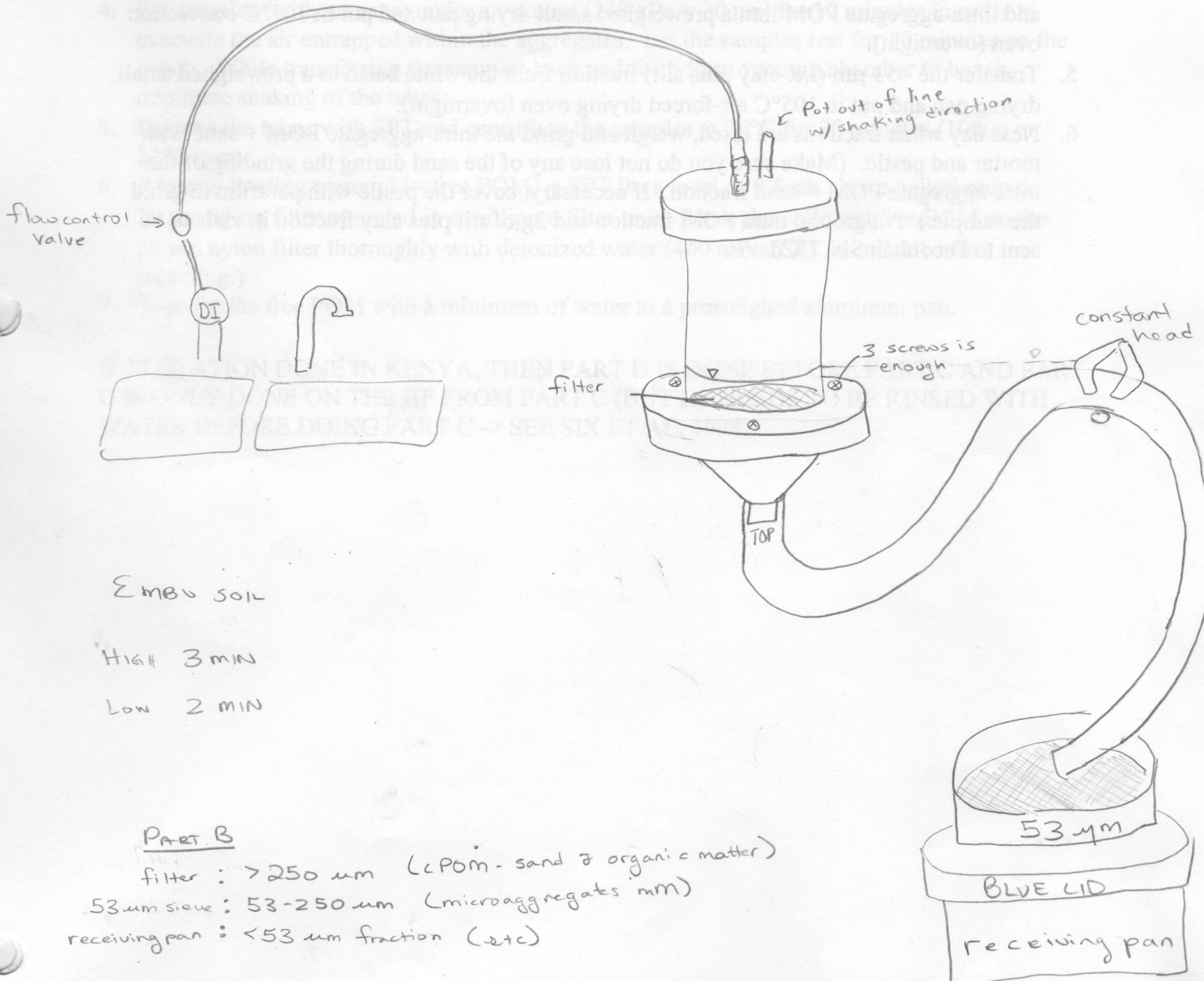
1. Sample preparation the evening before: place ~5g subsamples (if not enough material, use less material, but not less than 2.5 g) of the aggregate fractions in preweighed weigh cups and dry overnight in the 105°C convection oven.
2. Next morning: take the fractions out of the oven and let them cool off in the desiccator and weigh the sample on 4-place balance and transfer to a centrifuge tube.
3. Add 15 ml 0.5% (5 g/L) NaHMP to the soil sample in the centrifuge tube. Shake on a reciprocal for 18 hours (overnight). NOTE: if necessary add 10 glass beads to the centrifuge to accomplish full dispersion).
4. Next day: pour the dispersed sample over a 53 μm sieve in white basin (30 cm diameter; 8 cm deep), rinse thoroughly until water coming through the sieve is clean, backwash the sand and intra-aggregate POM into a preweighed small drying pan and put in 105°C convection oven (overnight).
5. Transfer the <53 μm (i.e. clay plus silt) fraction from the white basin to a preweighed small drying pan and put in 105°C air-forced drying oven (overnight).
6. Next day when fractions are dried, weigh and grind the intra-aggregate POM + sand with mortar and pestle. (Make sure you do not lose any of the sand during the grinding of the intra-aggregate POM + sand fraction. If necessary, cover the pestle with parafilm to grind the sample.) Put ground intra POM fraction and 2g of silt plus clay fraction in vials to be sent to Dr. Johan Six, UCdavis.

*Protocol: Part D: Flotation of free POM associated with aggregate fractions (lM, sM, mM, and m)*. WILL BE DONE IN DAVIS.

1. Sample preparation the evening before: place up to eight ~5g subsamples (if not enough material, use less material, but not less than 2.5 g) of the aggregate fractions in weigh pans and dry overnight in the 105°C convection oven.
2. Next morning: take the fractions out of the oven and let them cool off in the desiccator. Fill 8 centrifuge tubes (50ml) with 30 ml of 1.85 g $cm^{-3}$ sodium polytungstate (SPT).
3. Place each centrifuge tube with SPT in a beaker on 4-place balance and tare. Add a dried subsample and record the exact weight of added soil. Cap the centrifuge tube. Mix subsample with SPT by shaking gently by hand (10 strokes). Rinse cap and sides of centrifuge tube with SPT to 40-ml line.
4. Put samples (without caps) under a vacuum (138 kPa = 20 psi) for 10 minutes in order to evacuate the air entrapped within the aggregates. Let the samples rest for 10 minutes on the bench. While transferring the samples back and forth from vacuum chamber to bench, minimize shaking of the tubes.
5. Balance the tubes with SPT and centrifuge the samples at 20°C for 30 minutes (1000g or 2200 rpm).
6. Aspirate floating material (= free POM) + SPT (to a level of 0.5 cm above pellet) onto a 20 μm nylon filter mounted in a millipore filter unit. Rinse the collected free POM on the 20 μm nylon filter thoroughly with deionized water (400 ml). (SPT is collected for recycling.)
7. Transfer the free POM with a minimum of water to a preweighed aluminum pan.

IF FLOTATION DONE IN KENYA, THEN PART D IS DONE BEFORE PART C AND PART D IS ONLY DONE ON THE HF FROM PART C (BUT HF NEEDS TO BE RINSED WITH WATER BEFORE DOING PART C -> SEE SIX ET AL. 1998).

8. Transfer water and particles that went through the 53 µm sieve remaining in white basin to a preweighed large drying pan and put it in the 105°C forced air oven (overnight).
9. **Other person:** Take device from shaker, put it onto a 2000 µm and 53 µm sieve (the 2000 µm is to collect the beads and the 53 µm sieve to collect organic matter), and rinse water through the bottom of the device to collect beads, sand and organic matter on the sieves (make sure that all organic matter is rinsed off the sieve! Once the beads are collected on the 2000 µm and all the organic matter is collected on the 53 µm sieve, transfer material that is on 53 µm sieve in a preweighed small drying pan and put it in 105°C convection oven.
10. Next day weigh all fractions, grind subsample (2 g) of the 53-250 µm fraction (i.e. mM), grind the > 250 µm fractions and put grounded fractions, 2-5g of non-grounded 53-250 µm (i.e. mM) and silt and clay fraction in vials to be sent to Dr. Johan Six, UCDavis.




EMBU SOIL

HIGH 3 MIN

LOW 2 MIN

PART B

filter : > 250 µm (cPOM - sand & organic matter)

53 µm sieve : 53-250 µm (microaggregates mM)

receiving pan : < 53 µm fraction (silt etc)