**Experimental setup for labelled litter incubation**

Background: Soils subjected to higher long-term soil moisture (which also have higher exchangeable Ca content) have lower mineralization per unit C (and higher SOC content). Prelim incubations show that adding CaCl2 to initially low- and high-Ca soils reduces CO2 emission further. But when soils are leached of excess CaCl2 before incubation CO2 emission is only reduced in the initially high-Ca soils. Since the EC w/o leaching is very high, we only focus on this treatment (preincubation with CaCl2 and leaching before incubation on HIGH soils).

Objective: Investigate the effects of and (interactions between) Ca addition and incubation water content, on C dynamics in soils with high exchangeable Ca/high long-term water content.

Methods: Soils from high-Ca/high-moisture quintile were combined to obtain a composite HIGH sample.

1. **Pre incubation** –HIGH soil (~100 g) were placed in mason jars and either deionized water (DW) or 200 meq/L CaCl2 were added at 0.55 g/g water content and “incubated” for 10 days. Lid was opened daily for 1 week, to allow added Ca to exchange other cations (although exchange is an order of minutes reaction). Mason jars were then opened and air-dry soil was transferred from each mason jar to a Buchner funnel with a Whatman 1 and leached with DW until the EC of the leachate was <100 uS/cm.

**2**. **Incubation** **and CO2 measurement** – Leached HIGH soils were air dried and weighed into Qorpaks (~9 g). 180 mg of air dried and ground 13C15N-labeled willow leaves (or only soils as control) were added to the soils (2% w/w OM) and wetted with DW to obtain a water content of 40% or 60% WFPS (3.6 and 5.5 mL, respectively). The Qorpak vials were place inside XXX mL sealed mason jars along with scintillation vials containing alkaline traps made of 15 mL of 0.18 M KOH (made with CO2-free water). 5 mL of CO2-free water was added to the bottom of the mason jar to maintain moist conditions. A calibration curve was constructed by regressing the EC of the alkaline trap 24 hours after injection of a known amount of CO2 (99.999%). The CO2 emitted from each jar was measured on days x,y,z. To remove the effect of the small amount of CO2 present in the air in the jar at the time of setup, measurements from “blank” jars with no soil additions were used. To determine total C mineralized by the sample, the average (n = 3) blank EC value for the corresponding sampling day was subtracted from each jar’s EC measurement. This delta EC value was then converted into total CO2 released by the sample, using the standard curve. After each measurement, the alkaline traps were replaced with fresh solutions in new vials and new DW was added to the bottom of the jar.

**3. delta13C-CO2 measurement** –On days x, y, z of the incubation, the EC of the KOH traps is measured and then KOH is transferred to 50 mL conical vials containing 5 mL of 0.3 M BaCl2. The BaCl2 mixed with the KOH trap results in the precipitation of absorbed CO2 as BaCO3. Precipitated solutions were centrifuged at 2500 rpm for 5 min. Supernatant solution was decanted, leaving the precipitate. The remaining precipitate was rinsed with 10 mL DIW, centrifuged again, and solution decanted, for a total of three rinses. The remaining precipitate was dried at 70 °C. BaCO3 samples were acidified using H3PO4 and the released CO2 was analyzed for δ13C on a Thermo Scientific DELTA V isotope ratio mass spectrometer interfaced with a Gasbench II (Thermo Scientific, West Palm Beach, FL).

**4. Additional analyses–** On day 4 and at the end of incubation, 6 jars from each treatment level were destructively sampled to determine how soil biogeochemical properties change during the incubation. On day 4 – the 4.18.2020 – the Qorpaks were capped and put in a -20C freezer to await analysis. On day 112 (5.8.2020) 60% WFPS were put in the freezer. On day 132 (25.8.2020) 40% WFPS were put in the freeze

* Microbial biomass C and DOC were measured using the chloroform fumigation and 0.05 M K2SO4 extraction method. Extracts of fumigated and non-fumigated soils were filtered thru 0.45um paper into 50 mL conical vials**.** Aliquots (8 mL) were analyzed for NPOC and TN with a Shimadzu TOC. The remaining extracts were freeze dried and the solids were analyzed for delta13C and delta 15N of the fumigated and un-fumigated samples. The CN concentrations and isotopes signature were used in the mixing model. teaching
* A subsample of the bulk soil was taken, air-dried and ball-milled.
* Soils were fractionated to obtain a free light fraction (fPOM), an occluded light fraction (oPOM) and a heavy mineral fraction (silt+clay, or silt and clay separately?). 1. To isolate the fPOM, 5 g of soil was shaken with SPT 1.75 g/cm3 for 30 min, allowed to settle, centrifuged (2000 g 30 min) and separated on glass filter paper, washed with DI to remove SPT, and then the filter was washed into a pre-tared container, dried in the oven and weighed. To isolate the oPOM, the pellet was re-dispersed using a vortex mixer and sonicated at 350 J/mL. The dispersion was left to settle overnight, centrifuged (2500 g/30 min), and separated on glass filter paper (GF/A or GF/D), washed with DI to remove SPT, and then the filter was washed into a pre-tared container, dried in the oven and weighed. To isolate the silt+clay fraction, the residue is washed with DI and centrifuged (2-3 times) to remove SPT until density of supernatant ~1. Sodium hexametaphosphate is added and shaken for 16 hours to re-disperse the soil, which is then passed through a 53 um sieve to remove sand. Bulk soil, fPOM, oPOM and silt+clay fractions are weighed, ground or ball-milled and sent to COIL for CN isotope analysis. Clay can be further separated by centrifugation or sedimentation.
* Other C tests pre and post incubation - pyrophosphate extractable DOC and Na2SO4 extractable DOC
* Soil aggregate stability – no differences in slaking value was seen between DW and Ca soil (before incubation).
* DOM adsorption to soils

**5. Mass of soil needed for analyses**

CO2 and delta13CO2 measurements – no soil needed

Microbial biomass C and DOC – 10 g per sample

1. 0.2 mg/g soil 60-80 g per treatment if COIL

Soil fractionation – 5 g per sample = 15 g per treatment

Fractionation + MBC= 85-90 g per treatment. 8 treatment per soil 800 g per soil for 1 measurement.

Exchangeable cations (before incubation) – 60 g per soil

~~Aggregate stability – 30 g per soil (5 per wetting rate\*2wetting rate\*3 replicates)~~

DOM adsorption isotherm – 45 g per soil (3 g per concentration\*5 concentration\*triplicates)

**6. Mixing model equations**

A standard isotope mixing model was used to determine 13C (and 15N) values of all C pools:

For example, the concentration of C in a POM fraction would equal

Clitter-derived-POM= Ctotal-POM × (delta13Cafter-POM − delta13Ccontrol-POM)/(delta13Clitter − delta13Ccontrol-POM)

where Ctotal-POM is the total amount of C in the POM pool, delta13Cafter-POM is the delta13 value of the C in the POM pool at the end of the experiment, delta13Ccontrol-POM is the delta value of C in the POM pool in the unlabeled controls and delta13Clitter is the delta13 value of the labelled litter.