PROTOCOL: Carbon and Nitrogen Content and Isotope Determination in Dry Vegetation

(i) **Equipment**

- Ball mill for powdering sample
- Sartorius micro balance (0.0001 g)
- Drying Oven (65 °C)
- Carlo Erba Combustion CHN Analyzer
- Isotope Ratio Mass Spectrometer

(ii) **Consumable materials**

- 4 ml polystyrene sample cups with lids
- 7 mm diameter ball bearings
- Styrofoam sample cup storage containers
- Tin foil capsules
- 96 well microplates
- Fine tipped curved tweezers and spatulas

(iii) **Sample preparation**

- Using ground "working sample", fill 4 ml cups until level is between ¼ and ½ full.
- Add 2 ball bearings to each 4 ml cup.
- Cap it tightly and powder the sample in the ball mill for 30 minutes.
- Place sample set in a Styrofoam container in the oven and dry overnight.

(iv) **Measurement procedure**

- Foil Ball Preparation:
  - Calibrate the micro-balance before taking any measurements.
  - Tare the Sartorius scale with a foil capsule to negate its weight.
  - Weigh out 2 mg (± 0.1mg) of powdered sample into a tin foil capsule and fold the opening until no sample can escape using a z-fold technique. This involves folding the opening until it is shaped like a Z and then making 2 horizontal folds, rolling the foil down onto the sample.
  - Tap folded capsule to ensure no sample is leaking.
  - Record your weight and place the capsule in a 96-well microplate, keeping track of each sample's placement in the tray.
- Carbon and Nitrogen Content are determined through combustion analysis on a Costec CHN analyzer.
- A portion of the combustion gas from each sample is routed through a Finnegan Isotope Ratio Mass Spectrometer for determination of δC^{13} and δN^{15} in the sample.

(v) **Data preparation and finalization**

- Calibration standards of peach leaf (NIST standard material) and atropine are used.
- Carbon and Nitrogen concentrations (%) are calculated from the peak heights recorded by the Carlo Erba as referenced to the NIST standards.
- δC^{13} and δN^{15} (‰) are calculated as δR (‰) = $(R_A/R_{standard} - 1)*1000$; where R and R_A are the ratios of the rare and abundant isotopes of the sample and the references collected in the mass spectrometer.

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