Appendix 4: Detailed description of sample preparation and IRMS analyses, at Fera

Sample preparation for HCNS ratio analyses

From each sample an aliquot (50 or 150 g, depending on raw sample available) undergoes freeze-drying, followed by homogenisation with an IKA grinder. The remainder of the raw sample is kept frozen. Using a soxtherm apparatus, 1 g of the freeze-dried material is defatted, as any lipid present in the sample could interfere with the isotope analysis. The treated sample portion is then air dried and homogenised to ensure thorough mixing of the sample prior to analysis.

CNS ratio analyses by IRMS

The tin foil capsules, containing ~ 2mg of freeze-dried defatted sample, are placed in a carousel and then sequentially dropped into an Elementar Pyrocube elemental analyser reaction tube containing tungstic oxide at 1120˚C. The sample is quantitatively converted into carbon dioxide, nitrogen oxides, sulfur oxides and water. The combustion products pass into a second reactor at 850˚C containing copper wires that quantitatively convert the nitrogen oxides to dinitrogen gas and the sulfur oxides to sulfur dioxide. Water is then removed from the carrier stream by a chemical trap containing phosphorus pentoxide and the carbon dioxide and sulfur dioxide are retained on proprietary silica gel traps. After elution of nitrogen these traps are sequentially purged by thermal desorption. The effluent flows into the stable isotope ratio mass spectrometer (Isoprime, UK) via an interface and the ratio of the isotopologues of nitrogen, carbon dioxide and sulfur dioxide are determined by calibration against reference materials of known $^{15}N/^{14}N$, $^{13}C/^{12}C$ and $^{34}S/^{32}S$ ratios. The isotopic data are reported in per mil [‰] on the relevant IAEA δ-scales.

H analyses by IRMS

The isotopic data are reported in per mil [‰] versus V-SMOW (IAEA δ-scale) and acquired on either system 1 and system 2.

System 1

The tin foil capsules, containing ~ 0.8 mg of freeze-dried defatted sample, are placed in a carousel and then sequentially dropped into a high temperature ceramic elemental analyser reaction tube, filled with glassy carbon chips (Elemental Microsanalysis, UK). The ceramic tube is maintained at 1350˚C. Depending on the elemental composition the sample is converted into hydrogen, carbon monoxide, nitrogen, nitrogen monoxide and elemental carbon. The pyrolysis products pass through a packed Molsieve 5Å gas chromatography (GC) column that separates hydrogen and carbon monoxide gas. The GC effluent then

flows into the stable isotope ratio mass spectrometer (Isoprime, UK) via an interface and the ratio of the isotopologues of hydrogen is determined by calibration against reference materials of known $^{2}H/^{1}H$ ratio.

System 2

The tin foil capsules, containing ~ 1 mg of freeze-dried defatted sample, are placed in a carousel and then sequentially dropped into an Elementar Pyrocube elemental analyser reaction tube at 1450˚C. The reaction tube consists of an outer ceramic tube and an inner glassy carbon tube -which allows a constant flow of He between both tubes, the latter is filled with glassy carbon chips and a silver wool plug (Isoprime, UK). Depending on the elemental composition the sample is converted into hydrogen, carbon monoxide, nitrogen, nitrogen monoxide and elemental carbon. Water is then removed from the carrier stream by a chemical trap containing phosphorus pentoxide and sodium hydroxide, whilst the carbon monoxide is retained on a proprietary silica gel trap. After elution of the hydrogen the trap is purged by thermal desorption. The effluent flows into the stable isotope ratio mass spectrometer (Isoprime, UK) via an interface and the ratio of the isotopologues of hydrogen is determined by calibration against reference materials of known $^{2}H/^{1}H$ ratio.