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Short communication

Evaluation of sample pre-treatment and potential contamination of soluble carbon in soil extracts during lyophilisation

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ABSTRACT

Lyophilisation of K_2SO_4 soil extracts has been proposed as a sample preparation technique before elemental analysis of carbon or nitrogen. However, previous measurements, based on wet oxidation or catalytic combustion, indicated that C measurements in lyophilised samples not always proved to be accurate. To determine whether the C analysis was affected by the lyophilisation process, an exploratory study was conducted to investigate the potential effects of the sample pre-treatment and of the lyophilisation process itself. This paper puts forward that the use of soil extracts, previously stored at −20 °C, may affect the recovery of salt in the samples and that contamination of the soluble carbon with exogenous C during lyophilisation is feasible. Therefore we recommend to use freshly prepared soil extracts for lyophilisation and always to include an internal standard among the unknown samples to account for a possible contamination.

The use of labelled plant residues (^{13}C, ^{15}N) is most appropriate for tracing the fate of carbon and nitrogen in soil during their decomposition process. However, direct isotopic analysis of soluble carbon and nitrogen in K_2SO_4 soil extracts is generally not feasible with an elemental analyser, coupled to a stable isotope ratio mass spectrometer (EAIMS). Therefore, pre-concentration of the liquid sample is required. Aïta (PhD, 1996) proposed lyophilisation of the soluble extracts before isotopic analysis with an EAIMS. In this context, lyophilisation of liquid samples has been used to determine, e.g. microbial biomass-C and water soluble C or N in soil extracts (Gaillard et al., 1999; Murage and Voroney, 2007; Nicolardot et al., 2007). No anomalies were reported in those studies. However, for extracts low in soluble carbon, Aïta (PhD, 1996) did notice significantly larger total C concentrations in the lyophilised samples, which were measured with an elemental analyser, in comparison to the original liquid samples, which were analysed by means of catalytic combustion. This observation was later confirmed by B. Mary (personal communication, 2004). In addition, a preliminary experiment showed that when K_2SO_4 soil extracts were frozen at −20 °C prior to sampling, the theoretical amount of crystalline K_2SO_4 could not be recovered after lyophilising the aliquots. The objective of the present study was to identify possible bottlenecks during sample preparation and during lyophilisation, which, in the end, could lead to false results.

In a first experiment, the recovery after lyophilisation of the theoretical amount of K_2SO_4 in previously frozen soil extracts (obtained by Coppens et al., 2006; stored at −20 °C) and in freshly prepared soil extracts (both with 0.03 M K_2SO_4) was compared. For each of the 36 replicates provided for both treatments, 7 ml of the potassium sulphate extract was transferred into a small flask covered with nylon material, which theoretically should hold 36.6 mg of K_2SO_4. The 2 sets of 36 flasks were frozen during 24 h at −35 °C before being placed randomly in the lyophiliser (Lyovac GT2). During lyophilisation, the sublimation phase was performed for a period of 48 h at 0.05 MPa and the secondary desiccation for a period of 4 h at 0.008 MPa. After finishing the lyophilisation process, the flasks with potassium sulphate crystals were placed in a desiccator until weighing. The yield of the samples, previously stored at −20 °C, ranged from 10.3 to 36.0 mg K_2SO_4, whereas the amount of salt recovered from the freshly prepared soil extracts

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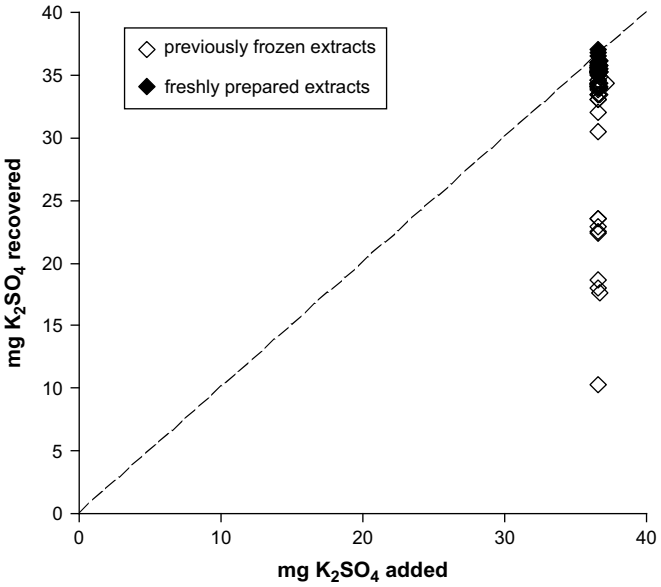

Fig. 1. Amount of potassium sulphate recovered after lyophilising 7 ml of 0.03 M K_2SO_4, obtained from previously frozen soil extracts stored at −20 °C and soil extracts freshly prepared before analysis. The dashed line indicates 100% recovery.

ranged from 35.0 to 37.0 mg K_2SO_4 (Fig. 1). Obviously, freezing and thawing the stored soil extracts affected the solubility of the potassium sulphate. Despite the fact that the defrosted extracts were homogenised before sampling, the result was a variable recovery of K_2SO_4, which was significantly lower than the expected value of 36.6 mg. According to the solid–liquid phase diagram of a salt solution, freezing will tend to concentrate zones of high ionic strength that will be the last to freeze and, when the solubility limit is reached, the salt precipitates out. By thawing the frozen extract, some of the salt precipitate may not turn into solution (e.g. for Ca-rich soils insoluble precipitates of $CaSO_4$ can form) and may result in an inhomogeneous sub-sampling of the solution. In analogy, Fellman et al. (2008) reported that the freezing and thawing of surface water samples significantly decreased dissolved organic matter concentrations, with an absolute decrease in dissolved organic carbon concentrations of 1.5–30%. They found that brown particles precipitated during freezing and that the precipitation was irreversible. These as well as our results demonstrate that soil extracts, which are intended for quantitative measurement of soluble C or N by means of an elemental analyser, should be sampled for lyophilisation immediately after extraction.

A second experiment aimed to evaluate the recovery after lyophilisation of known amounts of C and N in freshly prepared K_2SO_4 solutions and to compare the C measurements of the lyophilised samples by elemental analysis with chemical oxidation of the original liquid samples. Amounts of leucine (54.9% C, 10.7% N) were added to a 0.025 M K_2SO_4 solution in order to obtain 20, 50 and 100 μg C and, respectively, 3.9, 9.7 and 19.4 μg N in 7 ml of this solution, which was transferred into small flasks for lyophilisation. For each of the 3 concentrations, 15 replicates were provided. After lyophilisation (Lyovac GT2), the C and N contents in the K_2SO_4 crystals were determined using an EAIMS. Soluble C in the K_2SO_4 solutions was also performed by wet oxidation using sodium peroxydisulphate. The resulting CO_2 was measured with a non-dispersive infrared analyser (1010, O.I. Analytical). The amount of K_2SO_4 crystals in the flasks after lyophilisation was 30.1 ± 0.5 mg and in good agreement with the theoretical amount to be obtained with 7 ml of 0.025 M K_2SO_4 (30.5 mg). Elemental analysis demonstrated an excellent recovery of leucine-N for the 3 tested

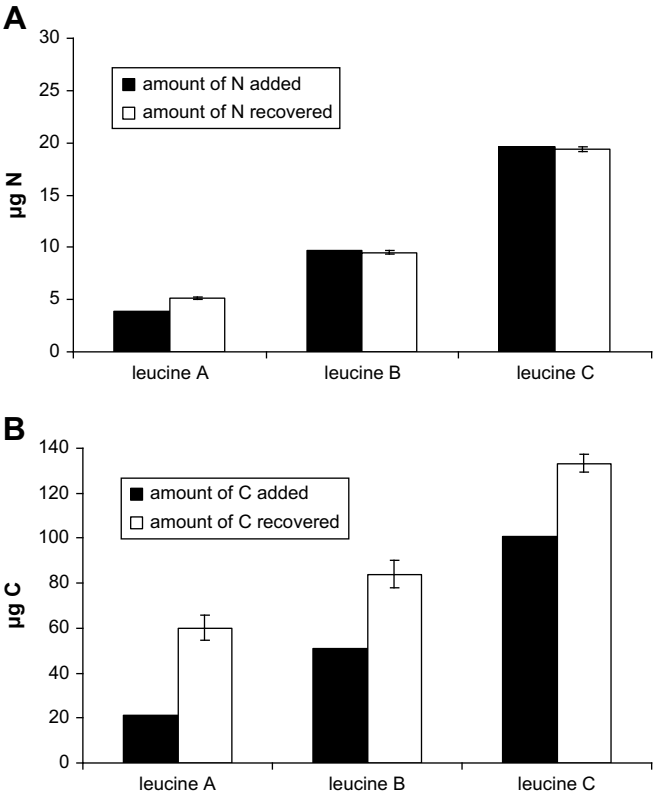

Fig. 2. Recovery of (A) nitrogen and (B) carbon after lyophilisation of 7 ml of 0.025 M K_2SO_4 solutions with increasing amounts of leucine. Black bars represent the theoretical amounts of added nitrogen and carbon. White bars represent the means of 15 measurements, error bars indicate the standard error of the means.

concentrations (Fig. 2A). However, recovery of C was for each concentration significantly larger than the amount of leucine-C added (Fig. 2B), which suggests a contamination of the sample. Analysis of soluble C by wet oxidation of the liquid samples

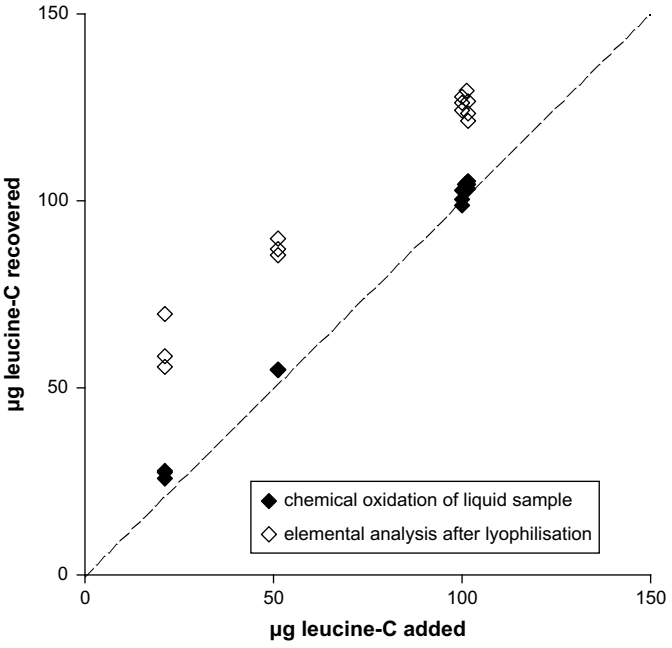

Fig. 3. Comparison of analysis by wet oxidation, and elemental analysis after lyophilisation of the same K_2SO_4 solutions with leucine added. The dashed line indicates 100% recovery.

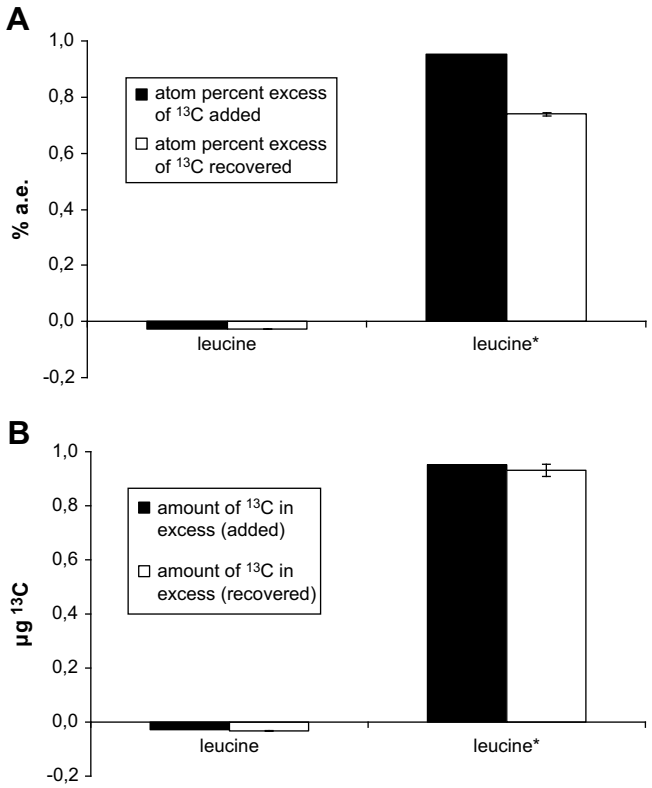

Fig. 4. Recovery of (A) the atom percent excess (% a.e.) for ^{13}C and (B) the total amount of ^{13}C in excess of the natural abundance after lyophilisation of 7 ml of 0.025 M K_2SO_4 solutions with 100 µg leucine-C, and 100 µg leucine-C enriched in ^{13}C (*). Bars represent the means of 15 replicates, error bars indicate the standard error of the means.

matched with the theoretical concentrations (Fig. 3), confirming that contamination occurred during lyophilisation of the samples.

To identify the source of contamination, several additional tests were performed. Firstly, pure K_2SO_4 was analysed for C before and after lyophilisation, which resulted in 0.002% and 0.18% of C, respectively, and excluded initial contamination caused by the used sulphate. Secondly, 7 ml of 0.025 M K_2SO_4 solutions containing (i) 100 µg of leucine-C, and (ii) 100 µg of leucine-C labelled with ^{13}C (atom percent excess of 0.951%), were lyophilised together to check for cross-contamination of the samples. Isotopic analysis of the lyophilised samples showed a significant reduction of the isotopic excess of the labelled samples but showed no enrichment in ^{13}C of the unlabelled samples, thus excluding cross-contamination (Fig. 4A). Despite the reduction in isotopic excess, the recovery of ^{13}C in the labelled samples was 98% (Fig. 4B), which proved that there was no significant loss of C from the samples during the lyophilisation process and that exogenous C was responsible for the contamination of the samples. To examine if CO_2 from the atmosphere was absorbed in the cavities of the lyophilised salt, ^{13}C–CO_2 (atom percent excess 3%) was used to fill the vacuum in the lyophiliser after freeze-drying a 0.025 M K_2SO_4 solution. Isotopic analysis of the formed crystals did not reveal any enrichment in ^{13}C (−0.028% a.e.), thus challenging the hypothesis that samples were contaminated with atmospheric CO_2. As a consequence, samples could only be contaminated during the lyophilisation process itself. We assume that during sublimation, oil vapours from the vacuum pump had entered the sample chamber and were then deposited on the samples. Grounds for this hypothesis are also found in literature (e.g. Jennings, 1999; Niazi, 2004; Oetjen and Haseley, 2004), strongly indicating that C contamination during lyophilisation was not a local problem. Finally, if deposition of oil vapour was the cause of contamination, carbon contents should increase with increasing sample surface. Equal amounts of inert carbon-free SiO_2 were, in triplicate, placed in flasks with diameters of 17 and 66 mm, resulting in potential contact surfaces of 2.3 and 34.2 cm^2, respectively. Carbon analyses after lyophilisation showed 5.6 ± 0.1 µg C g^{-1} SiO_2 for the small and 15.7 ± 0.6 µg C g^{-1} SiO_2 for the large surfaces, corroborating our hypothesis that exogenous C was deposited on the samples during lyophilisation.

In conclusion, our results indicate that freezing soil extracts for storage can induce heterogeneities in the solution once thawed, and they highlight the importance of using freshly prepared soil extracts for analysing soluble C and N. Furthermore, we demonstrated that soil extracts can be contaminated with exogenous carbon during the lyophilisation process, and therefore we strongly recommend an internal standard to be lyophilised among the unknown samples to account for a possible contamination.

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