Short Communication

How does sample preparation affect the $\delta^{15}N$ values of terrestrial ecological materials?

Francis Q. Brearley[1]*

[1] Department of Environmental and Geographical Sciences, Manchester Metropolitan University, Chester Street, Manchester, M1 5GD, UK

Key words: carbonates / mass spectrometry / nitrogen-isotope ratios / sample acidification / stable isotopes / temperature

Accepted April 1, 2009

1 Introduction

The use of stable isotopes in terrestrial ecosystem ecology is increasing: their great benefit is that they can provide integrated measures of ecological processes over time. Nitrogen (N) isotopes are used to examine the N cycle and plant–soil relationships, where $\delta^{15}N$ values of ecological materials result from many different and interacting biogeochemical and physiological processes (*Högberg*, 1997; *Robinson*, 2001; *Dawson* et al., 2002).

Prior to analysis on an isotope-ratio mass spectrometer (IRMS), samples must be dried and ground. However, there is no standard procedure for this and researchers often dry materials at different temperatures. The most common temperature appears to be 60°C (*Erskine* et al., 1998; *Fry* et al., 2000; *BassiriRad* et al., 2003) but some researchers have used the relatively high temperature of 105°C (*Koba* et al., 2003) whereas others dry samples at the considerably lower temperature of 40°C–50°C (*Hawke*, 2001), and some researchers do not quote the temperature they used (*Ostle* et al., 1999). With dual isotope analysis, ^{15}N and ^{13}C can be measured simultaneously and often carbonates are removed by acid treatment prior to analysis. *Mateo* et al. (2008) have reviewed the effects of sample preparation, and specifically acidification, on marine samples but there is far less information on preparation effects on soils or terrestrial material (but see *Taylor* et al., 1997; *Harris* et al., 2001). In some cases, samples have also been ground in liquid N before analysis (*Brearley* et al., 2005) and it is questioned if this may affect ^{15}N values if N is taken up during grinding. In this paper, I therefore examine the effects of drying temperature, acid fumigation, and grinding in liquid N on the N-isotope values of soils from two contrasting sites and leaves from one tree species.

2 Materials and methods

Soils and leaves were chosen in an attempt to span the range of $\delta^{15}N$ values found in ecological materials. They were (1) serpentine soil from Croagh Patrick, Co. Mayo, Ireland (53°46′ N, 9°39′ W), (2) soil from Robinson Ridge in the Windmill Islands region of Antarctica (66°16′ S, 110°33′ E), and (3) leaves from *Cyrilla racemiflora* L. (Cyrillaceae) from the Blue Mountains in Jamaica (18°05′ N, 76°39′ W). Soils from Ireland were air-dried shortly after collection and ground in a pestle and mortar to pass a 0.5 mm mesh. Subsamples which were not further treated were considered controls. Other subsamples were lightly reground in liquid N. Further subsamples were dried in a drying oven at 40°C, 60°C, or 105°C for 24 h. These were then weighed accurately into tin capsules for analysis on an IRMS (see below). Subsamples for acid fumigation were weighed into silver capsules, and carbonates were removed using the method of *Harris* et al. (2001) by moistening them to field capacity, exposing them to 12 M HCl vapour for 24 h, and then air-drying them. There is no evidence that capsule material affects the isotope ratio (*Bosley* and *Wainwright*, 1999). Antarctic soils were sieved to 2 mm, frozen at –20° C for 7 y, and then air-dried. Subsamples ground to pass a 0.5 mm mesh were considered controls. Other subsamples were treated as the controls, then lightly reground in liquid N or dried at 40°C, 60°C, or 105°C for 24 h before being weighed into tin capsules. Leaves from Jamaica were taken from the canopies of three trees and dried at 50°C for 1 week. They were then cut into small pieces (approx. 1 cm^2) and mixed thoroughly. Controls were subsamples which were ground in a Fritsch Pulverisette ball mill and weighed directly into tin capsules. Other subsamples were ground in liquid N or ground in a ball mill and dried at 60°C or 105°C for 24 h before being weighed into tin capsules. All samples were analyzed using a ThermoFinnegan Delta^{plus} IRMS interfaced with a CE Instruments 1112 Flash elemental analyzer *via* a Conflo III. Nitrogen-isotope ratios are expressed as delta (δ) notation which is the per mille deviation from atmospheric N: $\delta^{15}N$ (‰) = $[(R_{sample} / R_{standard}) - 1] \times 1000$ where R_{sample} is the ^{15}N : ^{14}N ratio of the sample and $R_{standard}$ is the ^{15}N : ^{14}N ratio of the atmospheric-N standard. L-alanine was run as an internal standard; precision (one standard deviation) of repeated analysis of this standard was 0.12 ‰. One-way ANOVAs followed by *Dunnett's* (1955) test were used to analyze differences between treatments and controls. There were three replicates of each treatment in every case.

* Correspondence: Dr. F. Q. Brearley;
e-mail: f.q.brearley@mmu.ac.uk

3 Results

The overall mean $\delta^{15}N$ value for the soils from Ireland was 4.0‰. All treatments led to a reduction in the $\delta^{15}N$ values when compared with the control. This was statistically significant for grinding in liquid N and heating at 60°C and 105°C where the reductions were approx. 1 ‰ ($F_{5,12} = 7.31$, $p = 0.002$; Fig. 1a). The Antarctic soils had high overall mean $\delta^{15}N$ values (21.4‰) as they have been historically enriched in ^{15}N through penguin excrement (*Wasley* et al., 2006). Heating at 60°C (but not at 105°C) led to a significant reduction in the $\delta^{15}N$ value by approx. 0.6 ‰ ($F_{4,10} = 4.48$, $p = 0.025$) despite the large variation following this treatment. Grinding in liquid N led to a small (nonsignificant) increase in $\delta^{15}N$ (Fig. 1b). The leaves from Jamaica were depleted in ^{15}N (overall mean = –4.3‰). Heating at 60°C and 105°C led to a reduction in $\delta^{15}N$ values by approx. 0.75 ‰ ($F_{3,8} = 7.42$, $p = 0.011$). Grinding in liquid N led to a (nonsignificant) reduction in $\delta^{15}N$ values (Fig. 1c).

4 Discussion

These results show that a number of pretreatments of ecological materials for $\delta^{15}N$ analysis affect the ^{15}N : ^{14}N ratio. The greatest effects were seen for drying at 60°C and 105°C and grinding in liquid N. However, these effects were not always consistent among samples as only heating at 60°C had an effect on all three. The change of 0.6‰ to 1.0‰ following pretreatments is considerable given that most ecological materials fall within a range of –5‰ to +5‰. Furthermore, a change in $\delta^{15}N$ of 0.04‰ can represent a 10% change between reactants and products (*Peterson* and *Fry*, 1987), therefore these changes are likely to be ecologically meaningful.

The most consistent effect was seen for heating at temperatures >60°C which led to reductions in $\delta^{15}N$ values. This is most likely due to selective removal of ^{15}N-rich compounds such as proteins or amino acids (*Ostle* et al., 1999). *DeNiro* et al. (1985) found heating had a large effect on bone N-isotope ratios (depletion of up to 4‰ after heating at 200°C); in contrast, *Taylor* et al. (1997) did not find drying temperature (40°C–105°C) to affect the $\delta^{15}N$ values of fruiting bodies of four species of fungi, again showing that this reduction in $\delta^{15}N$ is not always consistent. There was less effect of heating on the Antarctic soils, perhaps because they had been stored frozen and may already have lost ^{15}N-enriched compounds. Whilst the most common temperature for drying materials appears to be 60°C, I show here that drying at this temperature or above is likely to have an effect on the $\delta^{15}N$ values of plant and soil materials.

Grinding in liquid N led to a reduction in the $\delta^{15}N$ value of the soils from Ireland. This might have resulted from uptake of N from the liquid N if it was depleted in ^{15}N (although no data are available); however, this is probably not likely as grinding in liquid N led to a (nonsignificant) increase in the Antarctic soils. *Feuchtmayr* and *Grey* (2003) showed that freezing had an effect on $\delta^{15}N$ although, in their case, there was an enrichment in ^{15}N (liquid N did not come into direct contact with their samples). Physical disruption of plant or microbial cells may lead to a loss of nitrogenous compounds *via* leaching when thawed. My results therefore call into question the validity of grinding material in liquid N as has been done in some studies or, perhaps, storage in this medium if not protected, *e.g.*, by plastic vials.

In some cases, it may be helpful to remove carbonates by acid fumigation for reliable $\delta^{15}N$ analysis in low-N, but carbonate-rich, soils because during analysis of such soils there is often a shortage of oxidation in the IRMS combustion tube producing CO which has the same mass as N_2 and therefore interferes with the N peak (R. Goodhue, pers. comm.). Only the soils from Ireland were acid fumigated and showed a (nonsignificant) decrease in $\delta^{15}N$—perhaps due to an improvement in the N_2 peak following fumigation. This broadly agrees with the results of *Kennedy* et al. (2005) who found that acidification sometimes decreased the $\delta^{15}N$ value of marine sediments, but this was dependent upon the acid used and its molarity. In contrast, *Harris* et al. (2001) found that acid fumigation increased the $\delta^{15}N$ value of soils, but only significantly in one of the four soils they studied; samples with greater carbonate content also showed a greater $\delta^{15}N$ increase following acidification in the study of *Jacob* et al. (2005).

In conclusion, I show that samples for $\delta^{15}N$ analysis should not be dried at >60°C or ground in liquid N as these can affect

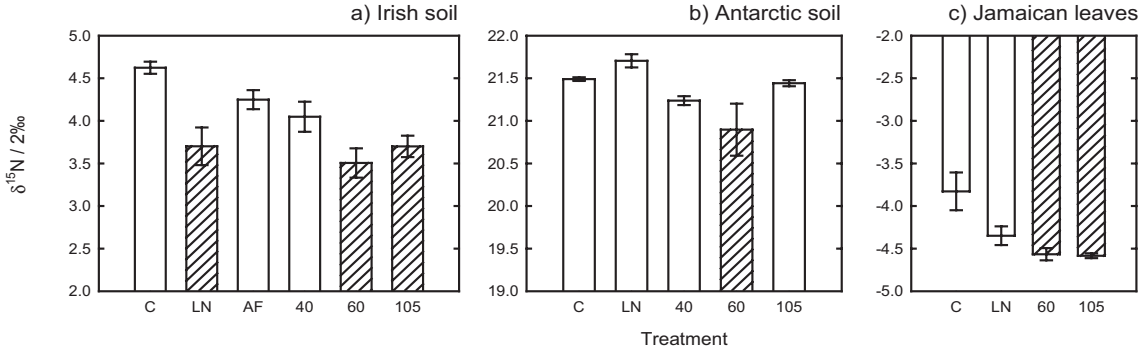

Figure 1: Nitrogen-isotope values ($\delta^{15}N$) for three sample types after grinding in liquid nitrogen (LN), acid fumigation (AF), and heating at 40°C (40), 60°C (60), or 105°C (105) for 24 h, relative to a control (C). Hatched bars are significantly different from the control according to Dunnett's test with $p < 0.05$. $n = 3$ for all treatments.

the $\delta^{15}N$ signature to a significant degree. The effect of sample acidification is less clear but its use is not recommended unless samples are required for carbon-isotope analysis as some samples show a change in $\delta^{15}N$ values.

Acknowledgments

Many thanks to *Robbie Goodhue* (Trinity College, Dublin) for sample analysis and helpful contributions and *Jane Wasley* and *Sharon Robinson* (University of Wollongong) for providing the soil samples from Antarctica.

References

BassiriRad, H., Constable, J. V. H., Lussenhop, J., Kimball, B. A., Norby, R. J., Oechel, W. C., Reich, P. B., Schlesinger, W. H., Zitzer, S., Sehtiya, H. L., Silim, S. (2003): Widespread foliar $\delta^{15}N$ depletion under elevated CO_2: inferences for the nitrogen cycle. *Glob. Change Biol.* 9, 1582–1590.

Bosley, K. L., Wainwright, S. C. (1999): Effects of preservatives and acidification on the stable isotope ratios (^{15}N : ^{14}N, ^{13}C : ^{12}C) of two species of marine animals. *Can. J. Fish. Aquat. Sci.* 56, 2181–2185.

Brearley, F. Q., Scholes, J. D., Lee, S. S. (2005): Nitrogen nutrition and isotopic discrimination in tropical ectomycorrhizal fungi. *Res. Microbiol.* 156, 184–190.

Dawson, T. E., Mambelli, S., Plamboeck, A. H., Templer, P. H., Tu, K. (2002): Stable isotopes in plant ecology. *Annu. Rev. Ecol. Syst.* 33, 507–559.

DeNiro, M. J., Schoeninger, M. J., Hastorf, C. A. (1985): Effect of heating on the stable carbon and nitrogen isotope ratios of bone collagen. *J. Archaeol. Sci.* 12, 1–7.

Dunnett, C. W. (1955): A multiple comparison procedure for comparing for comparing several treatments with a control. *J. Am. Stat. Assoc.* 50, 1096–1121.

Erskine, P. D., Bergstrom, D. M., Schmidt, S., Stewart, G. R., Tweedie, C. E., Shaw, J. D. (1998): Subantarctic Macquarie Island – a model ecosystem for studying animal-derived nitrogen sources using ^{15}N natural abundance. *Oecologia* 117, 187–193.

Feuchtmayr, H., Grey, J. (2003): Effects of preparation and preservation procedures on carbon and nitrogen stable isotope determinations from zooplankton. *Rapid Comm. Mass Spectrom.* 17, 2605–2610.

Fry, B., Bern, A. L., Ross, M. S., Meeder, J. F. (2000): $\delta^{15}N$ studies of nitrogen use by the red mangrove, *Rhizophora mangle* L. in south Florida. *Estuar. Coast. Shelf Sci.* 50, 291–296.

Harris, D., Horwáth, W. R., van Kessel, C. (2001): Acid fumigation of soils to remove carbonates prior to total organic carbon or carbon-13 isotopic analysis. *Soil Sci. Soc. Am. J.* 65, 1853–1856.

Hawke, D. J. (2001): Variability of $\delta^{15}N$ in soil and plants at a New Zealand hill country site: correlations with soil chemistry and nutrient inputs. *Aust. J. Soil Res.* 39, 373–383.

Högberg, P. (1997): ^{15}N natural abundance in soil-plant systems. Tansley Review No. 95. *New Phytol.* 137, 179–203.

Jacob, U., Mintenbeck, K., Brey, T., Knust, R., Beyer, K. (2005): Stable isotope food web studies: a case for standardized sample treatment. *Mar. Ecol Prog. Ser.* 287, 251–253.

Kennedy, D. P., Kennedy, H., Papadimitriou, S. (2005): The effect of acidification on the determination of organic carbon, total nitrogen and their stable isotopic composition in algae and marine sediment. *Rapid Comm. Mass Spectrom.* 19, 1063–1068.

Koba, K., Hirobe, M., Koyama, L., Kohzu, A., Tokuchi, N., Nadelhoffer, K. J., Wada, E., Takeda, H. (2003): Natural ^{15}N abundance of plants and soil N in a temperate coniferous forest. *Ecosystems* 6, 457–469.

Mateo, M. A., Serrano, O., Serrano, L., Michener, R. H. (2008): Effects of sample preparation on stable isotope ratios of carbon and nitrogen in marine invertebrates: implications for food web studies using stable isotopes. *Oecologia* 145, 105–115.

Ostle, N. J., Bol, R., Petzke, K. J., Jarvis, S. C. (1999): Compound specific $\delta^{15}N‰$ values: amino acids in grassland and arable soils. *Soil Biol. Biochem.* 31, 1751–1755.

Peterson, B. J., Fry, B. (1987): Stable isotopes in ecosystem studies. *Ann. Rev. Ecol. Syst.* 18, 293–320.

Robinson, D. (2001): $\delta^{15}N$ as an integrator of the nitrogen cycle. *Trends Ecol. Evol.* 16, 153–162.

Taylor, A. F. S., Högbom, L., Högberg, M. N., Lyon, A. J. E., Näsholm, T., Högberg, P. (1997): Natural ^{15}N abundance in fruit bodies of ectomycorrhizal fungi from boreal forests. *New Phytol.* 136, 713–720.

Wasley, J., Robinson, S. A., Lovelock, C. E., Popp, M. (2006): Climate change manipulations show Antarctic flora is more strongly affected by elevated nutrients than water. *Glob. Change Biol.* 12, 1800–1912.

