## 4.3 Stable isotopes

### 4.3.1 Bulk C and N

The "bulk" stable isotopic compositions of $^{13}C$ and $^{15}N$ natural abundance (Table 6) show highly variable $\delta^{13}C$ and $\delta^{15}N$ isotopic composition in the terrestrial samples but are very consistent in the mangrove and harbour samples. The flood-plain pasture samples PCR and PPR have more enriched $\delta^{15}N$ values in keeping with deposition of organic matter and animal waste on farm land. The rolling hillside pasture sample PTR has a lower $\delta^{15}N$ value, which would be expected if urea fertiliser were used to promote grass growth. The more enriched $\delta^{13}C$ value in the PTR pasture is indicative of more consistent use of this land for grass and possibly grain (i.e., C4 plants) than the other pasture sites which had stock present.

**Table No. 6**

Soil and sediment composition

| Soil | Nitrogen (%) | $\delta^{15}N$ (‰) | Carbon (%) | $\delta^{13}C$ (‰) |
|---|---|---|---|---|
| PTR | 0.49 | 2.8 | 5.58 | -22.2 |
| PCR | 0.46 | 5.1 | 7.42 | -25.5 |
| PPR | 0.44 | 6.4 | 4.18 | -26.9 |
| NDH | 0.41 | -0.3 | 8.34 | -28.0 |
| NMH | 0.49 | 2.0 | 8.61 | -28.2 |
| NCBR | 0.23 | 1.0 | 6.32 | -25.1 |
| EDH1 | 0.61 | 3.2 | 10.67 | -27.7 |
| EDH2 | 0.33 | 3.7 | 4.39 | -24.1 |
| EMH | 1.10 | 1.0 | 21.10 | -28.7 |
| EMHF | 0.08 | 5.3 | 0.94 | -26.2 |
| M1 | 0.22 | 7.2 | 2.32 | -23.1 |
| M2 | 0.20 | 6.4 | 2.19 | -24.1 |
| M3 | 0.27 | 6.1 | 3.01 | -24.4 |
| H2 0-10 | 0.16 | 5.7 | 1.78 | -25.0 |
| H2 0-20 | 0.13 | 4.6 | 1.57 | -25.1 |
| H2 0-30 | 0.12 | 6.1 | 1.49 | -24.8 |
| H3 0-10 | 0.12 | 5.0 | 1.35 | -25.0 |
| H3 0-20 | 0.11 | 4.7 | 1.30 | -25.0 |
| H3 0-30 | 0.10 | 4.9 | 1.29 | -25.1 |
| H4 0-10 | 0.11 | 4.7 | 1.49 | -25.1 |
| H4 0-20 | 0.11 | 5.1 | 1.30 | -25.0 |
| H4 0-30 | 0.11 | 4.6 | 1.61 | -24.5 |

The native and exotic forest soils had $\delta^{15}N$ and $\delta^{13}C$ values which are consistent with terrestrial non-grass plants (i.e., C3 plants) and low levels of animals. The very depleted $\delta^{15}N$ value for the NDH sample might be explained as an indication of nitrogen fixing plants such as native broom, which was present at that site, or similar plants. The

more enriched $\delta^{15}N$ value for the exotic pine subsoil EMHF probably reflects the effect of decomposition processes as the organic matter was buried and incorporated.

The more enriched $\delta^{15}N$ values in the estuarine sediments are consistent with biological processes including microbial transformations, recycling of organic matter by benthic macrofauna, and faecal pellet production, in these habitats. The consistently higher $\delta^{15}N$ values in the mangrove than in the harbour samples suggests a higher level of decomposition processing in the mangroves. This would be consistent with the ability of mangroves to trap organic debris which, in contrast, would be widely dispersed in the harbour sediments due to the high level of sediment disturbance by wind-waves and tidal resuspension.

The depth-dependent sampling shows an overall pattern of reducing %N and %C with increasing depth and a small difference in the $\delta^{13}C$ values in the 0-30 cm sample relative to the other two depths. This is consistent with biogeochemical processes occurring for a longer time in the deeper sediments than in the sediments down to 20 cm, and may indicate some level of mixing or bioturbation down to 20 cm on the harbour mud flats. The $\delta^{15}N$ values are variable across the increasing depth ranges, as might be expected for a nutrient that can be transformed and biogeochemically recycled within the sediments.

However, as noted in the pilot study results, because it undergoes biogeochemical transformations in the sediment which alter both it's abundance and isotopic signature, $\delta^{15}N$ cannot be used in the mixing model matrix.

### 4.3.2 Compound specific isotopes

The CSI composition of resin and fatty acids in the samples are listed in Table 7A as $\delta^{13}C$ (‰) and the proportion of each is given as the peak area in Table 7B, normalised to a 5.0 g sample extract and a 0.2 µl injection (i.e., 1 mg soil). Quantification of specific compounds is based on the resin and fatty acid analyses (Tables 4 and 5). While the fatty acids identified in the resin acid suite were all present in most samples analysed for CSIs, many of the resin acids were not found in source or harbour samples. Results of CSI analyses from the depth-dependent harbour samples are given in Table 8.

Of interest is the detection of abietic acid in the CSI suite for most samples given the expectation that this compound is easily decomposed in aerobic sediments. Abietic acid and the other resin acids detected in the CSI analyses have been given tentative identification only by the analyst, indicating that these might be interference products with similar peak retention times to the resin acids in the chromatograms. However, as they have also been detected at low levels in the resin acid results (Tables 4 and 5), the CSI results are likely to be real. The additional fatty acids, pentadecanoic and heptadecanoic acid results are given (Tables 7A & B) for future reference because of their broad range of isotopic values. However, as heptadecanoic acid was added as an internal standard to some samples, the affected data cannot be used.

**Table No. 7A**

Compound specific isotope ($\delta^{13}C$ ‰) composition of resin and fatty acids in the matrix of soil samples. Heptadecanoic acid was added to most samples as an internal standard and the affected values have been excluded from the result matrix. (* = tentative identification; missing values less than detection limit)

| Soil Samples | PTR | PCR | PPR | NDH | NMH | NCBR | EDH1 | EDH2 | EMH | EMHF | M1 | M2 | M3 | H2 | H3 | H4 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Resin Acids | | | | | | | | | | | | | | | | |
| Abietic acid* | -27.1 | -31.7 | -30.8 | -32.1 | - | -31.7 | -32.3 | - | -34.5 | -37.7 | -28.7 | -30.0 | -30.7 | -29.5 | | |
| Dehydroabietic acid* | -32.0 | | | | | | | | | | | | | | | |
| Isopimaric acid | | | | | | | | | | | | | | | | |
| Pimaric acid | | | | | | | | | | | | | | | | |
| Sandaracopimaric acid | | | | | | | | | | | | | | | | |
| 7-Oxodehydroabietic acid* | | | | | | | -30.8 | | -32.2 | | | | | | | |
| Fatty Acids | | | | | | | | | | | | | | | | |
| Myristic acid | -27.0 | -28.9 | -29.3 | -32.6 | -33.1 | -28.9 | -37.0 | -33.7 | -40.7 | | -26.0 | -27.3 | -30.2 | -30.2 | -28.2 | -28.9 |
| Palmitic acid | -24.0 | -26.0 | -28.0 | -30.6 | -30.6 | -25.6 | -31.7 | -27.6 | -32.4 | -29.2 | -24.3 | -28.4 | -28.6 | -30.0 | -28.5 | -28.4 |
| Stearic acid | -26.7 | -31.5 | -28.8 | -30.0 | -29.5 | -25.4 | -31.1 | -26.0 | -32.1 | -28.8 | -26.8 | -30.7 | -29.8 | -31.8 | -30.1 | -30.1 |
| Oleic acid | -21.6 | -23.4 | -24.9 | -28.7 | -28.3 | -27.8 | -28.3 | | -29.5 | -25.0 | -21.5 | -26.5 | -27.3 | -22.4 | -22.6 | -23.2 |
| Linolenic acid | -24.1 | -28.5 | -25.5 | -28.3 | -29.0 | -25.6 | -35.0 | -26.5 | -30.8 | -30.8 | -34.3 | -29.5 | | | | |
| Arachidic acid | -24.5 | -30.2 | -27.5 | -32.6 | -34.8 | -29.8 | -32.0 | -25.4 | -33.1 | -27.4 | -27.6 | -29.2 | -30.5 | -30.6 | -28.2 | -28.1 |
| Behenic acid | -30.1 | -34.3 | -27.6 | -33.1 | -34.0 | -31.8 | -30.6 | -30.5 | -33.2 | -29.3 | -27.2 | -27.1 | -30.0 | -29.5 | -19.4 | -24.4 |
| Lignoceric acid | -29.2 | -31.4 | -30.3 | -31.4 | -34.4 | -29.1 | -30.6 | -31.3 | -33.2 | | -25.9 | -30.6 | -27.5 | -29.7 | -26.0 | -28.8 |
| Pentadecanoic acid | -21.3 | -28.7 | -36.0 | -30.3 | -27.7 | -26.9 | -32.3 | -31.2 | -30.0 | | -19.2 | -26.1 | -22.4 | -27.4 | -21.5 | -22.6 |
| Heptadecanoic acid | | -15.5 | | -30.7 | -35.9 | | | -29.3 | | | | | | | -24.2 | -26.9 |

**Table No. 7B**

Compound specific isotope composition of resin and fatty acids in the matrix of soil samples. Heptadecanoic acid was added to most samples as an internal standard and the affected values have been excluded from the result matrix. (Values given in peak area per 1 mg soil, missing values less than detection limit. * = tentative identification)

| Soil Samples | PTR | PCR | PPR | NDH | NMH | NCBR | EDH1 | EDH2 | EMH | EMHF | M1 | M2 | M3 | H2 | H3 | H4 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Resin Acids | | | | | | | | | | | | | | | | |
| Abietic acid* | 3.0 | 2.0 | 0.7 | 1.4 | | 2.3 | 2.8 | | 4.9 | 1.1 | 0.7 | 1.4 | 1.2 | 0.8 | | |
| Dehydroabietic acid* | 1.1 | | | | | | | | | | | | | | | |
| Isopimaric acid | | | | | | | | | | | | | | | | |
| Pimaric acid | | | | | | | | | | | | | | | | |
| Sandaracopimaric acid | | | | | | | | | | | | | | | | |
| 7-Oxodehydroabietic acid* | | | | | | | 2.8 | | 5.6 | | | | | | | |
| Fatty Acids | | | | | | | | | | | | | | | | |
| Myristic acid | 6.0 | 4.0 | 2.1 | 4.3 | 5.4 | 3.8 | 6.9 | 2.4 | 41.2 | | 6.5 | 2.6 | 2.5 | 1.8 | 2.0 | 1.4 |
| Palmitic acid | 43.9 | 34.0 | 17.4 | 31.5 | 44.0 | 64.2 | 25.7 | 17.7 | 121.8 | 4.2 | 30.3 | 13.5 | 11.9 | 9.6 | 12.3 | 10.3 |
| Stearic acid | 11.4 | 10.9 | 4.5 | 7.8 | 7.4 | 9.3 | 9.3 | 6.9 | 27.5 | 1.2 | 3.6 | 2.6 | 2.9 | 2.9 | 3.6 | 2.9 |
| Oleic acid | 31.2 | 13.0 | 5.4 | 22.2 | 12.0 | 37.5 | 16.2 | | 107.6 | 1.4 | 3.9 | 6.4 | 3.0 | 1.9 | 2.7 | 1.4 |
| Linolenic acid | 6.2 | 2.0 | 2.0 | 7.1 | 20.9 | 5.9 | 3.5 | 25.1 | 19.0 | 13.4 | 0.3 | 0.3 | | | | |
| Arachidic acid | 19.4 | 6.7 | 2.3 | 4.9 | 5.1 | 18.6 | 14.4 | 13.4 | 42.6 | 1.1 | 1.1 | 1.5 | 1.5 | 1.6 | 1.2 | 0.7 |
| Behenic acid | 14.5 | 11.2 | 4.6 | 14.3 | 10.6 | 36.3 | 23.0 | 11.0 | 67.3 | 2.3 | 2.0 | 2.9 | 2.3 | 3.2 | 3.4 | 2.8 |
| Lignoceric acid | 15.6 | 26.5 | 4.3 | 7.0 | 16.1 | 26.1 | 20.3 | 12.9 | 33.5 | 2.0 | 2.8 | 4.0 | 2.7 | 3.6 | 2.9 | 1.5 |
| Pentadecanoic acid | 4.0 | 1.2 | 2.0 | 1.4 | 1.6 | 2.5 | 2.8 | 1.0 | 15.4 | | 9.9 | 1.3 | 1.4 | 1.2 | 1.3 | 1.0 |
| Heptadecanoic acid | | 1.9 | | 1.8 | 2.4 | | | 2.4 | | | | | | | 0.6 | 0.7 |

**Table No.8**

Compound specific isotope composition ($\delta^{13}C$ ‰, and peak area per 1 mg soil) of resin and fatty acids in the depth-dependent harbour samples. Heptadecanoic acid was added to most samples as an internal standard and the affected values have been excluded from the result matrix. (missing values less than detection limit).

| Harbour sites | H2 | H2 | H2 | H3 | H3 | H3 | H4 | H4 | H4 |
|---|---|---|---|---|---|---|---|---|---|
| Core depth (cm) | 0-10 | 0-20 | 0-30 | 0-10 | 0-20 | 0-30 | 0-10 | 0-20 | 0-30 |
| CSI (‰) | | | | | | | | | |
| Abietic acid | -30.0 | | | | | | | | |
| Myristic acid | -30.3 | -29.1 | -27.3 | -28.2 | -28.8 | -27.8 | -28.9 | -26.3 | -28.3 |
| Palmitic acid | -30.0 | -29.7 | -29.4 | -28.5 | -28.3 | -28.1 | -28.4 | -28.2 | -29.1 |
| Stearic acid | -31.8 | -31.2 | -30.4 | -30.1 | -30.8 | -32.2 | -30.1 | -30.3 | -28.6 |
| Oleic acid | -22.4 | -27.6 | -22.3 | -22.6 | -23.8 | -22.6 | -23.2 | -23.0 | -22.3 |
| Linolenic acid | | | | | | | | | |
| Arachidic acid | -30.6 | -28.7 | -30.8 | -28.2 | -28.1 | -27.0 | | -33.0 | -29.7 |
| Behenic acid | -29.5 | -28.2 | -23.0 | -19.4 | -25.7 | -31.7 | -24.4 | -42.7 | -36.9 |
| Lignoceric acid | -29.7 | -28.8 | -27.2 | -26.0 | -27.7 | | | -28.8 | -27.4 |
| Pentadecanoic acid | -27.4 | -31.2 | -29.1 | -21.5 | -34.9 | -31.7 | -22.6 | -19.9 | -23.9 |
| Heptadecanoic acid | | -28.1 | | -24.2 | -26.4 | | -26.9 | | -28.2 |
| Peak area per 1 mg soil | | | | | | | | | |
| Abietic acid | 0.8 | | | | | | | | |
| Myristic acid | 1.8 | 1.1 | 1.0 | 2.0 | 1.0 | 0.7 | 1.4 | 0.7 | 1.1 |
| Palmitic acid | 9.6 | 7.4 | 6.4 | 12.3 | 6.0 | 3.3 | 10.3 | 5.1 | 7.3 |
| Stearic acid | 2.9 | 2.6 | 1.9 | 3.6 | 2.3 | 1.0 | 2.9 | 1.5 | 2.7 |
| Oleic acid | 1.9 | 2.9 | 1.0 | 2.7 | 1.3 | 0.6 | 1.4 | 0.8 | 1.4 |
| Linolenic acid | | | | | | | | | |
| Arachidic acid | 1.6 | 1.5 | 0.7 | 1.2 | 1.0 | 0.7 | | 0.7 | 0.8 |
| Behenic acid | 3.2 | 2.7 | 2.6 | 3.4 | 1.8 | 1.4 | 2.8 | 2.8 | 3.0 |
| Lignoceric acid | 3.6 | 2.4 | 2.1 | 2.9 | 1.4 | | | 1.5 | 1.8 |
| Pentadecanoic acid | 1.2 | 0.7 | 0.5 | 1.3 | 0.9 | 0.4 | 1.0 | 0.4 | 0.6 |
| Heptadecanoic acid | | 0.4 | | 0.6 | 0.4 | | 0.7 | | 0.4 |

The depth-dependent harbour sample CSI results (Table 8) are comparable with the resin acid data for these samples (Table 5). However, whereas peak areas show a decline with increasing depth, the isotopic signatures of some compounds show large shifts in $\delta^{13}C$ values between depths which are inconsistent with trends of decreasing mass associated with decomposition and may indicate event-driven deposition of sediment from different sources in the catchment.

## 4.4 Mixtures

Analyses of the five soil mixtures (Tables 9 and 10) were used to examine the reproducibility of the analytical technique as well as provide known mixtures to test the mixing model. The theoretical resin acid content of each mixture was calculated from the analytical results in Table 4 and the known weight proportion of each soil added to the mixture. These results assume that a homogeneous mixture was obtained and that there was no interaction between soils in the mixture. Since the dry soils were mixed and sieved together without loss, the theoretical resin and fatty acid content should be realistic and these have been used to estimate the % recovery (Table 9). A similar calculation was done using the isotopic signatures (Table 10).

The results (Table 9) show that the proportional recoveries of the resin acids from the artificial mixtures of soils were, in almost all instances, higher than expected while the fatty acid recoveries were much closer to the theoretical concentrations, based on the original analyses. As expected, higher "error" levels occurred where the compounds analysed were at low levels (i.e., at a low "signal-to-noise ratio"). However, this was not always the case and some of the higher "error" levels occurred where the compounds were at relatively high concentrations implying a large degree of variability in the analysis method. The source of error was possibly in the extraction efficiency of the routine procedure. This conclusion is drawn from the observation that repeat analyses of resin acids to a non-routine higher level of sensitivity considerably reduced some of the large "error" levels found using the initial results. In general the high senstivity results were about 50% of the original results.

These results raise concerns for the use of resin acids in the final mixing model evaluation and it may be prudent to use them as indicators and use the fatty acids for quantification. Given the mixing model requirement to have each compound present in all samples and the limiting subsoil sample, EMHF, only five of the fatty acids (palmitic acid, stearic acid, arachidic acid, behenic acid, and lignoceric acid) meet these requirements. As these had reasonable recovery % in most mixtures, this may not present a problem.

The exceptionally low recoveries (c. 50% of expected) for the fatty acids in the Mix 5 sample cannot be explained except as an analytical problem with the soil mixture component, EMH (this is the only mixture using that soil). In the Mix 5 test sample either less of each compound was extracted or the original analysis of soil EMH produced high results, or there was a calculation error. It is interesting to note that reducing the resin and fatty acid concentrations in the sample EMH results by a factor of 2 brings the recovery % in line with the other 4 mixtures. When repeat analyses were being done, sample EMH was missed and there was insufficient time to do a further repeat analysis.

This apparent error does not affect the CSI results and reasonably good agreement was obtained between measured and theoretical isotopic values for all mixtures tested. Note, however, that where a source value for a compound is missing, the recovery % for that compound in the mixture has not been calculated.

**Table No.9**

Soil mixture analyses and recovery using resin acids. Columns headed: Mix # are as analysed; Calc values are calculated from results (Table 4) using the known weights added; % are the % recovery based on the observed versus the calculated values. (Apparent differences in % may be due to rounding).

| Soil Mixtures | Mix 1 | Calc | % | Mix 2 | Calc | % | Mix 3 | Calc | % | Mix 4 | Calc | % | Mix 5 | Calc | % |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Abietic acid | 0.58 | 0.79 | 73.0 | 1.33 | 1.99 | 67.0 | 0.64 | 0.82 | 77.9 | 1.68 | 2.01 | 83.5 | 2.18 | 3.94 | 55.3 |
| Dehydroabietic acid | 0.11 | 0.09 | 123.6 | 0.20 | 0.16 | 123.1 | 0.21 | 0.14 | 148.9 | 0.24 | 0.21 | 111.9 | 5.07 | 8.31 | 61.0 |
| Isopimaric acid | 0.02 | 0.01 | 166.7 | 0.04 | 0.03 | 133.3 | 0.03 | 0.01 | 250.0 | 0.05 | 0.03 | 166.7 | 0.96 | 2.11 | 45.5 |
| Pimaric acid | 0.37 | 0.21 | 177.0 | 0.60 | 0.52 | 114.8 | 0.31 | 0.23 | 137.8 | 0.59 | 0.54 | 109.6 | 1.03 | 1.32 | 78.3 |
| Sandaracopimaric acid | 0.86 | 0.59 | 146.3 | 1.41 | 1.32 | 106.8 | 0.69 | 0.55 | 125.9 | 1.40 | 1.28 | 109.4 | 0.85 | 0.98 | 86.9 |
| 7-Oxodehydroabietic acid | 0.23 | 0.12 | 200.0 | 0.29 | 0.23 | 127.5 | 0.17 | 0.11 | 158.9 | 0.27 | 0.22 | 123.0 | 1.13 | 1.65 | 68.7 |
| Myristic acid | 1.3 | 1.1 | 114.0 | 0.9 | 1.1 | 85.7 | 0.7 | 0.7 | 106.1 | 0.6 | 0.6 | 105.3 | 7.2 | 12.2 | 59.0 |
| Palmitic acid | 20.0 | 21.1 | 94.7 | 12.0 | 19.8 | 60.6 | 10.0 | 12.8 | 78.1 | 8.0 | 11.5 | 69.7 | 25.0 | 45.5 | 54.9 |
| Stearic acid | 3.9 | 5.1 | 76.8 | 2.8 | 4.6 | 60.9 | 2.3 | 3.2 | 72.8 | 2.0 | 2.7 | 74.6 | 6.0 | 11.7 | 51.2 |
| Oleic acid | 5.1 | 4.7 | 107.6 | 4.3 | 4.7 | 92.5 | 3.2 | 2.8 | 113.5 | 2.9 | 2.7 | 106.2 | 5.0 | 13.0 | 38.3 |
| Linoleic acid | 1.8 | 1.2 | 147.5 | 1.5 | 1.3 | 120.0 | 1.3 | 0.7 | 175.7 | 0.9 | 0.8 | 116.9 | 5.3 | | |
| Arachidic acid | 8.0 | 7.7 | 104.6 | 6.0 | 7.7 | 77.7 | 5.0 | 4.9 | 102.2 | 4.3 | 5.0 | 86.6 | 7.0 | 13.3 | 52.8 |
| Behenic acid | 21.0 | 18.8 | 111.9 | 16.0 | 21.4 | 74.7 | 12.0 | 13.5 | 89.2 | 14.0 | 16.1 | 86.9 | 17.0 | 29.7 | 57.2 |
| Lignoceric acid | 50.0 | 39.1 | 127.9 | 30.0 | 37.8 | 79.5 | 30.0 | 27.5 | 109.1 | 20.0 | 26.2 | 76.5 | 20.0 | 36.9 | 54.3 |
| Soil mixture by g dry weight | | | | | | | | | | | | | | | |
| PTR | | 80 | | | 50 | | | 40 | | | 10 | | | 10 | |
| NCBR | | 10 | | | 25 | | | 10 | | | 25 | | | 10 | |
| EMH | | 0 | | | 0 | | | 0 | | | 0 | | | 50 | |
| EMHF | | 10 | | | 25 | | | 50 | | | 65 | | | 0 | |
| M3 | | 0 | | | 0 | | | 0 | | | 0 | | | 33 | |

**Table No.10**

Soil mixture compound specific isotopes ($\delta^{13}C$ ‰) analyses and recovery using resin acids. Columns headed: Mix # are as analysed; Calc values are calculated from results (Table 7A) using the known weights added; % are the % recovery based on the observed versus the calculated values. (Apparent differences in % may be due to rounding).

| Soil Mixtures | Mix 1 | Calc | % | Mix 2 | Calc | % | Mix 3 | Calc | % | Mix 4 | Calc | % | Mix 5 | Calc | % |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Abietic acid | -29.6 | -28.6 | 103.5 | -30.9 | -30.9 | 100.1 | -32.9 | -32.8 | 100.3 | -35.4 | -35.1 | 100.7 | -34.3 | -32.3 | 106.0 |
| Dehydroabietic acid | | | | | | | | | | | | | | | |
| Isopimaric acid | | | | | | | | | | | | | | | |
| Pimaric acid | | | | | | | | | | | | | | | |
| Sandaracopimaric acid | | | | | | | | | | | | | | | |
| 7-Oxodehydroabietic acid | | | | | | | | | | | | | -32.4 | -32.2 | 100.6 |
| Myristic acid | -27.7 | | | -27.1 | | | -26.4 | | | -29.6 | | | -38.9 | -34.8 | 111.7 |
| Palmitic acid | -24.3 | -24.7 | 98.4 | -24.7 | -25.7 | 96.1 | -24.2 | -26.8 | 90.2 | -26.0 | -27.8 | 93.6 | -31.1 | -29.7 | 104.8 |
| Stearic acid | -28.5 | -26.8 | 106.5 | -26.6 | -26.9 | 99.1 | -27.9 | -27.6 | 101.1 | -28.0 | -27.7 | 101.0 | -31.1 | -30.2 | 103.1 |
| Oleic acid | -23.1 | -22.5 | 102.7 | -25.1 | -24.0 | 104.6 | -25.0 | -23.9 | 104.5 | -25.8 | -25.4 | 101.4 | -28.1 | -27.9 | 100.8 |
| Linolenic acid | -25.0 | -24.9 | 100.3 | -23.3 | -26.1 | 89.2 | -23.5 | -27.6 | 85.1 | -29.2 | -28.8 | 101.2 | -28.8 | -29.2 | 98.6 |
| Arachidic acid | -25.3 | -25.3 | 100.1 | -26.2 | -26.5 | 98.5 | -26.1 | -26.5 | 98.7 | -27.3 | -27.7 | 98.5 | -31.6 | -31.1 | 101.4 |
| Behenic acid | -28.1 | -30.2 | 93.1 | -29.8 | -30.3 | 98.4 | -30.6 | -29.9 | 102.4 | -25.1 | -30.0 | 83.6 | -30.8 | -31.7 | 97.0 |
| Lignoceric acid | | -29.5 | | -28.6 | -29.8 | 95.9 | -28.7 | -30.5 | 94.1 | -29.5 | -30.9 | 95.4 | -31.2 | -30.6 | 101.9 |
| Bulk $^{13}C$ | -22.3 | -22.9 | 97.4 | -23.3 | -23.9 | 97.4 | -23.4 | -24.5 | 95.5 | -24.9 | -25.5 | 97.6 | -28.0 | -26.3 | 106.3 |
| Soil mixture by g dry weight | | | | | | | | | | | | | | | |
| PTR | | 80 | | | 50 | | | 40 | | | 10 | | | 10 | |
| NCBR | | 10 | | | 25 | | | 10 | | | 25 | | | 10 | |
| EMH | | 0 | | | 0 | | | 0 | | | 0 | | | 50 | |
| EMHF | | 10 | | | 25 | | | 50 | | | 65 | | | 0 | |
| M3 | | 0 | | | 0 | | | 0 | | | 0 | | | 33 | |

## 4.5 Mixing model testing

The Iso-Source mixing model (Phillips & Gregg 2003) has a number of constraints which must be observed:

1 It is a fundamental requirement that any compound used in the mixing model must be present in all source material as well as the mixture (sink sample) being tested.

2 The concentration or isotopic value of each compound used in the mixture must lie within the range of that compound in all sources.

3 Mass must be conserved i.e., a concentration must be used with each CSI value to obtain quantification in the apportionment of sources in the mixture.

4 The sources must be possible.

The following are a number of suggestions which will improve the reliability of the mixing model predictions:

1 The analytical measurements of compounds should all be to the same level of accuracy across all sources and mixtures, using the same analytical method for each compound.

2 Compounds with very low concentrations should be avoided when using the mixing model if there are other compounds with higher concentrations which meet the constraints requirements.

3 The "Tolerance" setting in the mixing model should be set as close to 0.1 as possible to restrict the range of predicted feasible solutions.

Constraints 1-3 effectively restrict the number of compounds that can be used to evaluate a mixture, and either a source or a mixture may be the limiting factor in determining how many compounds can be used. Constraint 4 restricts the number of sources that can be used to evaluate a mixture to those which can influence the mixture.

The 5 prepared mixtures were designed to test the mixing model by comparing the model predictions of soil proportionality with known proportions of specific soils. The results of using 15 different compounds and compound combinations from the 3 or 4 soils used in each mixture are presented in Table 11. The average of all the range of feasible solutions for the proportion of each soil in the mixture is also included.