13C-Labelling of lipids to investigate microbial communities in the environment

Richard P Evershed, Zoe M Crossman, Ian D Bull, Hazel Mottram, Jennifer AJ Dungait, Peter J Maxfield and Emma L Brennand

The introduction of 13C-labelled substrates to soils, sediments or cultures followed by 13C analysis of phospholipid fatty acids (PLFAs) provides quantitative and chemotaxonomic information for the groups of microorganisms utilizing a given substrate. Gas chromatography-combustion-isotope ratio mass spectrometry has provided the high precision necessary to measure small isotopic changes (differences in the relative abundances of 13C to 12C expressed as δ13C values) for nanogram amounts of individual compounds, such as microbial PLFAs. This methodology constitutes a powerful new culture-independent method for investigating microbial communities in the environment. The information obtained is highly complementary to that obtained from gene-probe-based methods, and considerable possibilities exist to extend this methodology to include other biochemical components of microorganisms.

Introduction

The complexity of the microbial populations in sedimentary environments is universally acknowledged, with major challenges to their study arising from the unculturable nature of the major proportion of such populations [1]. This places considerable emphasis on the use of indirect approaches (e.g. field- or laboratory-based CO2 flux measurements) and/or culture-independent methods, to investigate the nature and functional ecology of such microbes. Gene probes provide phylogenetic information or identify specific groups of microorganisms (reviewed elsewhere in this issue). Complementary to these approaches are compound-specific stable isotope methods based on microbial lipids. This approach has become possible as a result of the development of gas chromatography-combustion-isotope ratio mass spectrometry (GC/C/IRMS). During the past 15 years very extensive use has been made of δ13C values (relative abundance of 13C to 12C compared to international standard of defined stable carbon isotope composition) of microbial lipid biomarkers (e.g. bacteriohopanes and isoprenoidal lipids) preserved in geologic sediments, to reconstruct ancient environments [2]. An emerging area of application of this methodology is in the investigation of living microbial populations in the environment.

As in the investigation of ancient environments, the use of compound-specific stable isotope approaches to study living microbial populations rests on matching specific compounds, termed biomarkers, to a particular group(s) of organism(s). An essential difference is that if the presence of a given biomarker is taken to indicate the activity of a given microbial group then, on cell death, that biomarker must be rapidly removed from the system. This principle underpins the use of PLFAs to evaluate microbial communities in the environment [3]. Phospholipids are the major components of the cell membranes of living organisms and, crucially, they only remain intact in viable cells [4]; PLFA concentrations and distributions therefore have the capacity to reflect rapid changes in microbial populations. Table 1 summarizes the broad affiliations of PLFAs with known source organisms. Significantly, the specificity of PLFA analysis for assessing the activity of microbial communities in the environment is greatly increased by the use of 13C-labelled substrates in conjunction with GC/C/IRMS. This review highlights recent advances in the use of 13C-labelling of lipids to study microbial communities in the environment. Topics covered include the 13C-labelling of microbes, laboratory and instrumental methods used to determine δ13C values of individual lipids and an overview of recent applications of these approaches.

Labelling approaches

Labelling approaches involve adding a 13C-labelled substrate to a microcosm, sediment, soil or culture medium, then following its fate temporally and/or spatially. Substrates labelled at natural abundance (i.e. those derived from plants having C3 or C4 photosynthetic pathways) provide opportunities for using either whole tissues or biochemical components derived therefrom, as economical and abundant sources of 13C-labelled substrate [5,6]. Likewise, free-air carbon dioxide enrichment
experiments (FACE) provide further useful sources of $^{13}$C-labelled plant tissues (e.g. $\delta^{13}$C = −43%). Despite the high sensitivities of the GC/C/IRMS instrument, the small difference (~15 to 20 %) between such plant tissues and their respective ecosystems imposes practical limits on detecting the fate of label. An alternative approach is to use highly $^{13}$C-labelled substrates produced by raising plants or algae on $^{13}$CO$_2$ or H$^{13}$CO$_3^−$, respectively [8]. Similarly, methanotrophic bacteria can be cultured on $^{13}$CH$_4$ [9–12,13–18]. Another approach is to employ $^{13}$C-labelled substrates produced by organic synthesis. A wide range of $^{13}$C-labelled compounds are available from commercial suppliers. Universally $^{13}$C-labelled synthetic compounds can be expensive, thereby restricting their use in field scale studies, however, the high sensitivities of GC/C/IRMS [19]$^*$ offer considerable potential. Significantly, $^{13}$C-labeling at specific positions in substrates raises opportunities for determining the fate of particular moieties or functional groups during assimilation by microbial communities. A novel approach to $^{13}$C-labeling of below-ground soil microbial communities demonstrated by Ineson and co-workers [20] involves the exposure of photosynthesising plants, either in the field or laboratory, to $^{13}$CO$_2$, resulting in $^{13}$C-labelled root exudates being delivered directly to the rhizosphere.

**Table 1**

<table>
<thead>
<tr>
<th>PLFA</th>
<th>Taxonomic or functional group*</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saturated straight chain</td>
<td>Prokaryotes, eukaryotes</td>
<td>[36]</td>
</tr>
<tr>
<td>&gt;C$_{20}$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Branched chain</td>
<td></td>
<td></td>
</tr>
<tr>
<td>iso/anteiso and others</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10Me</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cyclopropyl</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Monounsaturated 18:1ω8c</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Polysaturated</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
| PLFA listed is indicative of the group of organisms (or is isolated from them). Table adapted from [3].

**Figure 1**

Gas chromatogram of PLFAs extracted from soil from an oak woodland. Peaks are numbered: 1) C$_{19}$ alkane (IS; internal standard), 2) 14:0, 3) 15:0, 4) a15:0, 5) 15:0, 6) 16:0, 7) 16:0, 8) 16:1ω11c, 9) 16:1ω9c, 10) 16:1ω7c, 11) 10Me16:0, 12) 16:1ω5c, 13) ω17:0, 14) a17:0, 15) br17:1 ω 8c, 16) 17:0, 17) 17:1ω 6c, 18) 18:0, 19) 18:1ω9c, 20) 18:1ω7c, 21) 18:1ω5c.
representing the contributions from all the major soil microbial groups. This extract was obtained during an investigation to assess the activities of methanotrophic bacteria. However, on the basis of this profile it is impossible to determine which PLFAs are contributed by methanotrophs and which derive from other classes of microorganism; this can only be revealed by incubating the soil with $^{13}$CH$_4$, then determining PLFA $\delta^{13}$C values by GC/C/IRMS.

**GC/C/IRMS instrumentation**

Isotope ratio monitoring GC/MS was first demonstrated by Matthews and Hayes [21] with Barrie and colleagues [22] coupling a GC via a combustion interface to a dual collector MS to produce the forerunner of today’s GC/C/IRMS instruments (Figure 2). The prerequisite for determination of accurate $\delta^{13}$C values of individual compounds by GC/C/IRMS is good chromatographic separation. Peaks that are not fully resolved can be manually edited to obtain optimum values. In $^{13}$C-labeling studies, closely eluting components may contain widely varying abundances of $^{13}$C owing to utilization of $^{13}$C-labelled substrate by specific members of the microbial community. This is apparent in Figure 3 in the mass/charge ($m/z$) 45/44 ratio trace, where the 18:1ω7c component exhibits a higher abundance of the $m/z$ 45 versus $m/z$ 44 (higher ratio of $^{13}$C/$^{12}$C) than the 18:1ω9c component. An important consideration when analysing highly $^{13}$C-enriched compounds (>10$^3$ ‰) are possible carryover effects between GC analyses [19]. Separate columns and syringes are recommended for determinations of $\delta^{13}$C values at natural abundance.
and high enrichments; likewise septa and injector liners should be changed between the two types of analysis. A further important consideration when analysing highly 13C-enriched PLFAs is the amplification range of the m/z 45 Faraday cup, as high 13C abundances will saturate the detector when set to a range suitable for natural abundance determinations.

Conventional GC/MS instruments operating in the selected ion monitoring mode have been used to follow the fate of 13C-labelled substrates into environmental microbial populations [8,23]. However, complications arise in the selection of ions for monitoring in complex environments where target compounds are unlikely to be fully labelled, resulting in complex ion envelopes and, hence, greatly reduced detection limits compared with GC/C/IRMS.

13C-Labelling of lipids to investigate microbial communities in the environment

Table 2 provides a summary of the range of studies of environmental microbes involving lipids undertaken using 13C-labelling and GC/C/IRMS. Clearly, PLFAs are the major lipids used; an advantage of using 13C-labelling being the additional selectivity achieved compared with PLFA ‘fingerprinting’ alone.

Methanotrophic bacteria

A major area of utility of this methodology is the use of 13CH4 to investigate methanotrophic bacteria in both aerobic and anaerobic environments, including sediments [9,10], soils [11,12,13*,14*,15*,16*], microbial mat [17**] and peat bogs [18**]. A major advantage of using methane arises from its ease of addition to microcosms, providing the opportunity to target an important group of microorganisms. The major finding from studies performed to date are summarized in Table 2.

The ability to detect the effect of a changing environment on a given functional group of microorganisms would be of obvious advantage. Crossman et al. [13*] used 13CH4 incubations in laboratory microcosms to demonstrate variations in methanotrophic bacterial populations with depth through a landfill cover soil (Figure 5); type I methanotrophs were found to be more active in the surface layers, where concentrations of oxygen were highest and methane concentration low, whereas type II methanotrophs dominated in the deepest layers of the cap where methane concentrations were high and oxygen low. This approach was especially effective in investigat-

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13C-Labelling of lipids distributions

The higher δ13C values obtained for several PLFAs from a forest soil confirms incorporation of 13CH4 (Figure 4a). Although this information is useful, a more valuable representation is obtained by calculating the concentration of 13C in the various PLFAs (e.g. nanograms of 13C per gram of soil), which can then be used to provide 13C-PLFA ‘fingerprint’ distributions (Figure 4b) for chemotaxonomic assignments [2,9]. Rearranging the defining equation for δ13C values yields Equation 1:

\[
R_{samp} = \frac{\delta^{13}C_{samp}}{1000} \times R_{std} + R_{std}
\]  

(1)

From \( R_{samp} \) (13C/12C) the fractional abundance (F) of 13C is calculated using Equation 2:

\[
F = \frac{R}{R + 1} = \frac{13C}{13C + 12C}
\]  

(2)

By subtracting the fractional abundance of the unlabelled fatty acid from its fractional abundance following 13C-labelling, the fraction of excess 13C per carbon of the fatty acid is calculated. Multiplying this value by the concentration of carbon present in the target fatty acid (Equation 3) yields the total concentration of 13C-labelled fatty acid.

\[
\text{Amount of } 13C = C(F_{lab} - F_{unlab})
\]  

(3)

Where C is the concentration of carbon in the sample compound in ng (g matrix)−1.

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ing unculturable high-affinity methanotrophs in soils, revealing a novel type II methanotroph producing a br17:0 PLFA [11]. Additional bacterial markers (e.g. hopanoids) can provide complementary chemotaxonomic information to PLFAs [12,14]. Both classes of bacterial biomarker (Figures 6 and 7) indicated that the high-affinity methanotroph population in the forest soil highlighted above was related to type II methanotrophs, with PLFAs indicating a relationship to *Methylocapsa* and *Methylocella* genera of bacteria. In a further study of soil methanotrophs, PLFA and gene probe techniques were adopted by Knief et al. [16]. They reasoned that PCR detection of the *pmoA* gene, although an excellent functional gene marker, does not necessarily indicate the presence of a physiologically active methanotroph community. By contrast, the presence of $^{13}$C-labelled PLFAs, following incubation of soils with $^{13}$CH$_4$, unambiguously confirms the presence of an active population. Significantly, different methanotrophic bacteria were shown to be present and active in different soils.
Table 2

13C-Labelling studies of environmental microbial communities.

<table>
<thead>
<tr>
<th>Environment</th>
<th>13C-labeled substrate</th>
<th>Biomarker</th>
<th>Detection method</th>
<th>Conclusions</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Laboratory microbial cultures</td>
<td>Natural abundance glycerol, glucose, mannose, lactose, complex medium</td>
<td>PLFA</td>
<td>GC/C/IRMS</td>
<td>Complex fractionation patterns varying with substrate and organism</td>
<td>[5]</td>
</tr>
<tr>
<td>Woodland and grassland soils</td>
<td>Universally labelled starch, xylose, vanillin and leaf litter</td>
<td>PLFA</td>
<td>GC/C/IRMS</td>
<td>Similar microbial groups responsible for degrading simple substrates in woodland and grassland soils, but different communities degraded complex substrates</td>
<td>[7*]</td>
</tr>
<tr>
<td>Estuarine sediments</td>
<td>[U-13C]acetate</td>
<td>PLFA</td>
<td>GC/C/IRMS</td>
<td>Acetate consumed by SRB similar to Gram-positive Desulfitomaculum acetoxidans and not by a population of Gram-negative Desulfo bacter spp.</td>
<td>[9]</td>
</tr>
<tr>
<td>Estuarine sediments</td>
<td>H13CO3-</td>
<td>PLFA</td>
<td>GC/C/IRMS</td>
<td>Central role for microphytobenthos in moderating carbon flow in coastal sediments</td>
<td>[33]</td>
</tr>
<tr>
<td>Experimental soils</td>
<td>[1-13C]sodium acetate</td>
<td>PLFA, neutral lipids, glycolipids</td>
<td>GC/MS SIM</td>
<td>Incorporation greatest into PLFA; bacterial growth limited as low pH but occurred at pH 7 and 8</td>
<td>[23]</td>
</tr>
<tr>
<td>Rhizosphere rice paddy soil</td>
<td>13CO2</td>
<td>PLFA</td>
<td>GC/C/IRMS</td>
<td>Microbial populations in rice soil differ in their response to plant photosynthate input</td>
<td>[31]</td>
</tr>
<tr>
<td>Rhizosphere grassland soil</td>
<td>13CO2</td>
<td>PLFA</td>
<td>GC/C/IRMS</td>
<td>13C-labelling showed fundamental differences in the way rhizodeposition was cycled through a microbial community during different stages of plant development</td>
<td>[30*]</td>
</tr>
<tr>
<td>Rhizosphere grassland soil</td>
<td>13CO2</td>
<td>PLFA</td>
<td>GC/C/IRMS</td>
<td>Fungal and Gram-negative bacterial PLFAs showed most 13C-enrichment. Liming did not affect assimilation or turnover rates of 13C-label PLFAs resemble those of PHC-degrading Azotobacter spp.</td>
<td>[32**]</td>
</tr>
<tr>
<td>Sediments from petroleum-contaminated aquifer</td>
<td>[methyl-13C]toluene</td>
<td>PLFA</td>
<td>GC/C/IRMS</td>
<td>Field-scale application of acetate to investigate carbon assimilation and mineralisation</td>
<td>[25]</td>
</tr>
<tr>
<td>Petroleum-contaminated groundwater</td>
<td>[2-13C]acetate</td>
<td>PLFA (FISH)*</td>
<td>GC/C/IRMS</td>
<td>The main SRB degrading acetate in water was Desulfo bacter acetoxidans and Desulfo bacter in sediment</td>
<td>[26]*</td>
</tr>
<tr>
<td>Petroleum-contaminated aquifer water and sediment</td>
<td>[2-13C]acetate</td>
<td>PLFA (FISH)*</td>
<td>GC/C/IRMS</td>
<td>Central role for microphytobenthos in moderating carbon flow in coastal sediments</td>
<td>[33]</td>
</tr>
<tr>
<td>Antarctic soil bacteria</td>
<td>13C-labelled grass</td>
<td>Ergosterol, PLFA, NLFA</td>
<td>GC/MS SIM</td>
<td>Identification of new ambient methane-oxidising methanotroph similar to culturable type II Desulfo bacter acetoxidans and Desulfo bacter in sediment</td>
<td>[11]</td>
</tr>
<tr>
<td>Soil</td>
<td>Ring-labelled [13C]toluene and [U-13C]glucose</td>
<td>PLFA</td>
<td>GC/C/IRMS</td>
<td>Different methanotrophs are present in different soils that oxidise atmospheric methane</td>
<td>[16]</td>
</tr>
<tr>
<td>Batch culture</td>
<td>[U-13C]toluene</td>
<td>PLFA</td>
<td>GC/C/IRMS</td>
<td>Quantified carbon flow along substrate-bacteria-protist food chain</td>
<td>[29*]</td>
</tr>
<tr>
<td>Soil</td>
<td>13CH4</td>
<td>Hopanoids</td>
<td>GC/C/IRMS</td>
<td>Specific bacteriophospholipid labelled</td>
<td>[12]</td>
</tr>
<tr>
<td>Soil</td>
<td>13CH4</td>
<td>PLFA</td>
<td>GC/C/IRMS</td>
<td>Identified new ambient methane-oxidising methanotroph similar to cultivable type II Methylocapsa and Methylocella</td>
<td>[11]</td>
</tr>
<tr>
<td>Upland soils</td>
<td>13CH4</td>
<td>PLFA, DGGE</td>
<td>GC/C/IRMS</td>
<td>Different methanotrophs are present in different soils that oxidise atmospheric methane</td>
<td>[16]</td>
</tr>
<tr>
<td>Landfill cover soils</td>
<td>13CH4</td>
<td>PLFA</td>
<td>GC/C/IRMS</td>
<td>Changes in methanotrophic community from type I to type II with depth</td>
<td>[13*]</td>
</tr>
<tr>
<td>Soil</td>
<td>13CH4</td>
<td>PLFA, hopanoids</td>
<td>GC/C/IRMS</td>
<td>Novel population of methane-oxidising bacteria related to type II methanotrophs, Methylocapsa and Methylocella</td>
<td>[15*]</td>
</tr>
<tr>
<td>Sediment/soil</td>
<td>13CH4</td>
<td>PLFA</td>
<td>GC/C/IRMS</td>
<td>Shift in the composition of the methane-oxidising bacterial community in sediments/soils treated with ammonium</td>
<td>[10,15*]</td>
</tr>
<tr>
<td>Peat bog</td>
<td>13CH4</td>
<td>Hopanoids, sterols (FISH)</td>
<td>GC/C/IRMS</td>
<td>Methanotrophic bacteria associated with Sphagnum mosses provide CO2 for photosynthesis</td>
<td>[18**]</td>
</tr>
<tr>
<td>Anaerobic oxidation of methane</td>
<td>13CH4</td>
<td>PLFA, archaeal lipids</td>
<td>GC/C/IRMS</td>
<td>13C uptake into specific lipids indicates that phylogenetically distinct microbes participate in the anaerobic oxidation of methane</td>
<td>[17**]</td>
</tr>
</tbody>
</table>

PHC, petroleum hydrocarbon contamination; SRB, sulfate-reducing bacteria.
In contrast to soils, wetlands are an important natural source of methane. Indeed, a recent report involving the quantitative carbon isotopic analysis of $^{13}$C-labelled hopanoids and sterols showed that endophytic methanotrophic bacteria provide a significant (10–15%) carbon source for the growth of Sphagnum mosses [18/C15/C15]. The use of fluorescence in situ hybridisation (FISH) analyses in conjunction with scanning electron microscopy (SEM) confirmed the presence of methanotrophic bacteria in stem leaves.

Blumenberg et al. [17]/C15/C15 have extended $^{13}$C-labelling studies of methanotrophs to an anaerobic methane-oxidising microbial mat. GC/C/IRMS analyses were performed on a range of compound classes following in vitro incubation with $^{13}$CH$_4$. Very significant differences were observed in the $\delta^{13}$C values of the various bacterial and archaeal lipids. Largest differences were seen in the $\delta^{13}$C values of bacterial fatty acids, archaeol, a mono-unsaturated archaeol and biphytanes. Incorporation of $^{13}$C-label into a suite of polyunsaturated pentamethylcosanoids indicated that methanotrophic archaea possess a biosynthetic pathway similar to that of methanogenic archaea. Moreover, greater uptake of $^{13}$C into the lipids of sulfate-reducing bacteria (SRB) than those of archaea supports the hypothesis that autotrophic growth of SRB occurs on a methane-derived substrate supplied by the methanotrophs.

**Utilizers of mineralisation products**

Another important use of $^{13}$C-labelling has been for investigations of bacterial communities utilizing the products of organic matter mineralisation. Boschker and co-workers [24] have shown through laboratory incubation of small anoxic/brackish sediment cores that $^{13}$C-acetate and $^{13}$C-propionate were utilized by different members of the microbial community. $^{13}$C from acetate was recovered mainly from PLFAs with even numbers of carbon atoms (16:1$\omega_7c$, 16:0, 18:1$\omega_7c$), whereas fatty acids with odd numbers of carbon atoms (15:0, 15:0, 17:1$\omega_6$, 17:0) were primarily labelled upon incubation with propionate. These findings clearly indicate that the two substrates were predominantly consumed by different specialized groups of SRB. The PLFA labelling pattern for the acetate consumers was similar to Desulfotomaculum acetoxidans and Desulfotigrus spp., two acetate-consuming SRB, while those of the propionate consumers did not resemble any known strain.

Pombo and co-workers [25] have employed $^{13}$C-labelling of PLFAs to trace the assimilation of $^{13}$C-acetate in the mineralisation zone of a petroleum hydrocarbon-contaminated aquifer at the field scale. Some 500L of aquifer water were prepared containing 0.25 mM [2-$^{13}$C]acetate (together with 0.5 mM NO$_3^-$ and 0.5 mM Br$^-$, the latter being added as a conservative tracer) and injected into the aquifer. Samples were removed at 4 h, 23 h and 46 h and PLFA and FISH analyses suggested the presence of active denitrifiers. Label incorporation was seen in PLFAs and dissolved inorganic carbon after 4 h, with a high degree of labelling (>5000%) occurring in certain PLFAs (especially mono-unsaturates) after 46 h. An analogous approach was adopted to investigate acetate-degrading SRB in the same aquifer [26]. These studies emphasized the feasibility of using $^{13}$C-labelled substrates in the field for biogeochemical investigations in a way that was unfeasible with radiocarbon tracers. However, it has been emphasized that laboratory experiments should be undertaken before advancing to field-scale experiments [27].
Toluene-degrading microorganisms

Several reports [27,28,29] have focused on toluene as a microbial substrate, owing to its importance as a pollutant. Incubation of $^{13}$C-ring-labelled toluene with agricultural soil showed $^{13}$C to appear in only 16 of the 59 PLFAs extracted from the soil. A high degree of congruency (85%) was seen between the $^{13}$C-labelled PLFAs and those of the toluene-metabolising microorganisms isolated from the same soil. Interestingly, 91% of the total soil PLFAs were labelled when the same soil was incubated with $^{13}$C-glucose, confirming the power of coupling $^{13}$C tracers with PLFA analysis for investigating substrate metabolism in complex environments. The value of combining $^{13}$C-labeling of PLFAs with gene probe methods to study toluene-degrading microbial populations has also been demonstrated [27]. Additionally, using $^{13}$C-toluene as a substrate, Mauclaire et al. [29] provided important insights into a substrate–bacteria–protist food chain. The stable carbon isotope values, together with biomass and biovolume determinations, were crucial in modelling carbon fluxes through the food chain.

Rhizosphere microbial biomass

A novel means of assessing the activities of rhizosphere microbial communities is to expose photosynthesising plants to $^{13}$CO$_2$ in the laboratory or field and then investigate the incorporation of $^{13}$C into microbial PLFAs obtained from rhizosphere soil. Three recent studies have exploited this approach [30,31,32]. Butler et al. [30] pulse-labelled rye grass by exposure to $^{13}$CO$_2$ in laboratory microcosms, then employed stable carbon isotope analyses of PLFAs from the rhizosphere and bulk soil to investigate carbon dynamics at two stages during plant growth. The analyses showed most extensive labelling of
the fungal PLFA 18:2ω6,9. Lu et al. [31] undertook similar microcosm experiments with rice plants. GC/C/IRMS of PLFAs showed 13C to be incorporated rapidly into rhizosphere microbial PLFAs immediately following 6 h exposure to 13CO2, confirming the tight coupling of rhizodeposition to microbial communities. Field-scale pulsing of 13CO2 in plants in an upland grassland offered the potential to investigate carbon dynamics in situ [20,32**]. By quantifying the amount of 13C in the rhizosphere, and in microbial PLFAs at two different time points, estimates were obtained of turnover rates for each of the PLFAs. The results indicate that liming (a common agricultural practice to increase soil pH and nutrient availability) had little effect on assimilation or turnover rates of the microbial biomass compared with non-limed controls. These investigations clearly show the potential to probe plant–microbe interactions using 13C-labelling approaches.

Conclusions
13C-labelling of microbial PLFAs provides an important new culture-independent approach for the study of environmental microorganisms. The methodology offers several advantages. Firstly, 13C-labelled bacterial PLFAs confirm the presence of an active microbial community, which cannot be confirmed by genetic probes (e.g. PCR). Secondly, 13C-labelling of a specific subset of PLFAs, as a result of incubation with specific substrates, highlights members of the microbial community consuming that substrate. Thirdly, quantified 13C-labelled PLFA profiles can be searched against existing PLFA databases of cultured microorganisms to provide taxonomic information. Lastly, variations in 13C-labelled PLFA profiles between environments following treatment with a substrate indicate population-level differences.

An expansion of the applications of 13C-labelling studies of microbes will be seen over the next few years. The currently available 13C-labelled substrates provide considerable scope for new avenues of enquiry, with the possibility of expanding the range of specialist applications through the custom synthesis of 13C-labelled compounds. Moreover, possibilities exist for enhancing the stable carbon isotopic analysis of PLFAs through the use of other lipid, and indeed non-lipid, biomarkers.

References and recommended reading
Papers of particular interest, published within the annual period of review, have been highlighted as:

* of special interest
** of outstanding interest


15. Chemotaxonomic assignments of methanotrophs based on complementarily analyses of hopanoids and PLFAs.


