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TRACKING THE FATE AND RECYCLING OF ^{13}C-LABELED GLUCOSE IN SOIL

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A short-term incubation of soil amended with ^{13}C-glucose was conducted to determine the extent of labeled C recycling that might occur within the microbial community. Changes in the production and isotopic composition of CO_2 and biomass suggest that two phases of microbial activity occurred after the glucose addition. The initial phase due directly to the metabolism of the added glucose was characterized by an increase in biomass and a high growth efficiency. A second phase appeared to be driven by less available substrates (e.g., cell wall structures, soil organic matter) and characterized by insignificant changes in biomass but significant generation of CO_2 suggestive of low growth efficiency. Glucose-C supported 12 to 73% of the CO_2-C evolved and 17 to 21% of biomass-C, suggesting glucose was the principle energy rather than a C source during the 15- to 48-hour phase of the incubation. Variation in δ^{13}C composition of individual phospholipid fatty acids (PLFA) during the incubation indicated that different components of the microbial community played different roles in the cycling of the added glucose. The most enriched δ^{13}C values were initially those PLFA associated with Gram-positive bacteria, suggesting they were responsible for much of the initial incorporation. By contrast, at the end of the 48-hour incubation, 4 of 24 PLFA biomarkers were not labeled with ^{13}C. Actinomycetes, however, probably played a larger role in the use of recycled glucose-derived C, as suggested by the enrichment in ^{13}C of 10-methyl 18:0 PLFA after the exhaustion of glucose. Results from this study show that the element of time needs to be considered carefully in the interpretation of any stable isotope labeling and biomarker study. (Soil Science 2005;170:767–778)

Key words: Phospholipid fatty acids, stable isotopes, isotope biomarkers, ^{13}C-labeling, stable isotope probing.

RECENT advances in molecular techniques have revealed substantial variation in microbial community structure in many environments including soil (Griffiths et al., 2000; Roose-Amsaleg et al., 2001). Little, however, is understood regarding the ecological significance of such differences in community structure. Direct links between components of the microbial community and biogeochemical processes have traditionally relied on culture-dependent techniques. Alternatively, the use of stable isotopes in combination with microbial biomarkers can provide a powerful approach to elucidate the links between microbial structure and its function (Boschker and Middelburg, 2002; Manefield et al., 2004). Phospholipid fatty acids (PLFA), among other lipids, have been used as biomarkers, compounds with biological specificity, in geochemical and ecological studies (Calderon et al., 2001; Canuel and Martens, 1993; White and Ringelberg, 1998). With recent development of gas chromatography-combustion-isotope ratio mass spectrometry (GC-C-IRMS), it has become possible to analyze biomarkers for

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their stable isotopic composition. Recent studies suggest, for example, that $\delta^{13}C_{PLFA}$ can be used as indicators of the mean $\delta^{13}C$ of microbial substrate in aerobic soils (Burke et al., 2003; Cifuentes and Salata, 2001).

In addition to natural abundance studies, the use of labeled substrates offers opportunity to study processes carried out by specific groups of microorganisms. The idea behind this approach is that a portion of the labeled compound or tracer is incorporated into the biomass of the metabolically active populations, which can be detected in a variety of biomarkers. Stable isotope-labeled compounds do not have the legal or health restrictions of their radioactive counterparts and can be used directly in the field. Recent studies have used ^{13}C-labeled substrates in combination with the isotopic composition of biomarkers to quantify and identify the degradation rates and pathways of the substrate and the organisms involved (Bull et al., 2000; Hanson et al., 1999; Portais et al., 1999).

The addition of stable isotope-labeled compounds can also provide information regarding the impact of global climate or environmental change on soil microbial ecology. Potential consequences of global climate change on the soil environment could affect biogeochemical cycling by stimulating or inhibiting specific groups of microbes (Bardgett et al., 1996; Ronn et al., 2002). Increased utilization of cellobiose by the fungal component of the microbial community, for example, occurred under elevated CO_2 in forest soil (Phillips et al., 2002). After the addition of ^{13}C-labeled toluene in soil, ^{13}C-labeling occurred in PLFA primarily associated with genera within the order *Actinomycetales*, demonstrating that degradation was carried out by a small and defined component of the microbial community (Hanson et al., 1999). Even shifts in the activity of major groups, such as bacteria versus fungi, can greatly affect the transformation and long-term storage of soil carbon (C) or cycling of nutrients (Bailey et al., 2002).

The use of labeled substrates and subsequent measurement of biomarker compounds such as PLFA have been instructive in a number of studies including those of anoxic metabolism, methanotrophy, autotrophy, and food web dynamics (Boschker and Middelburg, 2002). Most studies using stable isotope labels, however, have relied on a single measurement of the isotopic composition of biomarker PLFA after a given incubation time. Little is understood about the rate and amount of ^{13}C label that is recycled within the microbial community over time. If substantial recycling of the added label occurs during the experiment erroneous interpretation of the data could result.

The purpose of this study was to determine the extent of recycling that might occur after the addition of a labeled substrate. We conducted a short-term incubation of soil amended with ^{13}C-labeled glucose with frequent sampling to determine the change in PLFA biomarkers labeled over time. The addition of glucose was used to simulate short-term change in soil organic C common in tracer addition studies. In the soil environment, short-term changes in soil organic C, although not always resulting in labile forms such as glucose, may include crop residue, animal and human wastes, contaminants, root exudates, drying/wetting regimes, or mechanical treatment of soil (Kruzyakov et al., 2000).

MATERIALS AND METHODS

Experimental Setup

Captina silt loam (fine-silty, siliceous, mesic Typic Fragiudults) was collected from the University of Arkansas Agricultural Experimental Farm in Fayetteville, Arkansas, from depth of 0 to 15 cm, sieved through a 2-mm sieve, and stored at 6 °C until use. A subsample was sent to the University of Arkansas Diagnostics Laboratory for chemical analysis. The soil contained 12, 74, and 14% sand, silt, and clay, respectively, and had a pH of 6.6. The soil contained 920 and 9210 mg kg^{-1} total N and C, respectively. Twenty-five gdw equivalent soil was placed in 42 individual 473 mL mason jars and brought to 10% H_2O by weight with distilled water. The jars were covered with plastic wrap and preincubated at 25 °C for 2 weeks before experiments were initiated.

Nine jars containing unamended soil and nine jars containing soil amended with 1000 mg unlabeled glucose (D(+)-glucose anhydrous, Fluka, MicroSelect grade; >99.5%; $\delta^{13}C = -10.73 \pm 0.04‰$) and 114 mg NH_4NO_3/kg soil, equivalent to 400 and 40 mg C and N/kg soil, respectively, or an amendment C:N ratio of 10. One amended and one unamended sample were analyzed for glucose remaining at 0, 2.5, 5, 7.5, 10, 12.5, 15, 24, and 48 hours.

Twelve jars, representing two replicates to be sampled at 0, 5, 10, 15, 24, and 48 hours, were amended with 1000 mg 95‰ ^{13}C-glucose and 114 mg NH_4NO_3/kg soil. The NH_4NO_3

was added to prevent N-limitation and ensure complete utilization of the added glucose. The ^{13}C-labeled jars were sampled for the stable isotopic composition of CO_2 and soil PLFA used to follow the fate of the glucose C during the incubation. In addition to the glucose-amended samples, a set of 12 jars with no glucose or NH_4NO_3 were sampled at 0, 5, 10, 15, 24, and 48 hours and used to determine the $\delta^{13}C_{PLFA}$ in the absence of the ^{13}C-glucose. This unlabeled set of incubation jars provided a means to determine the isotopic composition of the PLFA when the microorganisms were only supported by SOM. The ^{13}C-glucose used was made from a mixture of uniformly labeled D-glucose ($^{13}C_6$, 99 atom% ^{13}C, CDN Isotopes) and unlabeled glucose dissolved in distilled water, lyophilized, ground with a mortar and pestle, and divided into 10 subsamples for isotopic analysis where average value was determined as 95.00 ± 0.32‰. Solutions containing glucose and NH_4NO_3, or distilled water, were applied to appropriate jars, increasing the gravimetric H_2O content to 18%, or −0.033 Mpa and soil in all jars were thoroughly mixed.

Test tubes containing 5 mL of 1 M KOH and 10 mg/L tropaeolin O indicator (Sigma-Aldrich Company, Milwaukee, WI) were placed inside each mason jar to collect evolved CO_2. Jars were sealed with commercially available lids and incubated at 25 ± 2 °C. The tubes containing the KOH were removed, $BaCl_2$ was added to precipitate the collected carbonate, and the remaining KOH was titrated with HCl to determine CO_2 evolved (Wolf et al., 1994). Glucose was extracted from soil by using distilled water added to give a 1:1 soil:water ratio, jars shaken for 15 minutes, and a 2.0 mL aliquot of the aqueous slurry was transferred to a 2.5-mL centrifuge tube and centrifuged for 2 minutes at 15,000*g*. The percent glucose remaining was measured by using Sigma Diagnostics Glucose Assay, Procedure No. 510 (Sigma-Aldrich Chemical Company, St. Louis, MO). As glucose oxidation was still proceeding in the extract, the time interval between glucose extraction and the deproteinization reaction stopping step was monitored and kept constant at each sample time. One milliliter of the supernatant fraction was placed in a test tube, 1 mL 5% $ZnSO_4$*$7H_2O$ added, followed by 1 mL 0.3 N $BaOH_2$, and the mixture vortexed and centrifuged again for 2 minutes at 15,000*g*. One milliliter of the final supernatant was reacted with 4 mL of the PGO enzyme/o-Dianisidine dihydrochloride solution for 30 minutes, and color development was measured as adsorption at 450 nm (Bausch and Lomb Spectronic 21). Absorbance values were compared with a standard curve made by using a glucose solution subjected to the same deproteinization and colorization procedure to obtain percent glucose remaining in soil solution at each time interval. The percent glucose remaining values are reported as micrograms of glucose C gdw soil^{-1}.

Two replicates of the unamended soil samples and those samples containing ^{13}C-labeled glucose were analyzed at time = 0, 5, 10, 15, 24, and 48 hours for the stable isotopic composition of soil PLFA and the evolved CO_2. The soil samples were immediately placed in a freezer (−20 °C) upon collection. Frozen samples were lyophilized and extracted within 1 week of collection using the modified Bligh-Dyer method which included phosphate buffer (Pinkart et al., 2002; White and Ringelberg, 1998). Samples were fractionated into neutral lipids, glycolipids, and phospholipids and retained for analysis following (Agren et al., 1992). Fractionation was carried out by using solid-phase extraction with silicic acid phase on a vacuum manifold system to facilitate elution (Dobbs and Findlay, 1993; White and Ringelberg, 1998). After saponification, fatty acids in the phospholipid fraction were converted to their corresponding fatty acid methyl esters (FAME), using BF_3 in methanol (Dobbs and Findlay, 1993). The concentrations of FAME were determined by gas chromatography–flame ionization detection (GC-FID), using a Hewlett Packard 5890 series II plus GC equipped with a 50 m SGE BPX-70 (70% cyanopropyl polysilphenylene-siloxane) capillary column. Samples were also analyzed by gas chromatography–mass spectrometry, using a Hewlett Packard GC (HP 5890 series II plus) interfaced with a mass selective detector (HP5970B) to identify each PLFA. Both retention time and mass spectra of known standards both individual (12-methyl-tetradecanoate, 13-methyl-tetradecanoate, methyl linoleate; Sigma Aldrich Chemical Company, St. Louis, MO) and mixtures (Bacterial Fatty Acid Methyl Esters and 37 Component FAME Standards; Supelco Co., Rockford, IL) were used to identify PLFA in each sample. For those FAME where standards were not available, the mass spectra data were compared with an available spectral database (NBS). The same chromatographic conditions including column type, length, and phase were used in both the

GC-FID and GCMS analyses. Weight percent recovery was 80 to 92% ($n = 5$) for the Captina silt-loam used in the experiment as determined from phospholipid recovery standards used (phosphatidylcholine dedodecanoyl, phosphatidylcholine deheptadecanoyl, and phosphatidylcholine nonadecanoyl; Sigma-Aldrich Chemical Company, St. Louis, MO).

Analyses

The C isotopic composition of PLFA was determined by GC-IRMS. Isotopic composition of each individual FAME was determined using a GC (HP6890) coupled to a stable isotope ratio mass spectrometer (Finnegan Delta$^+$) via a combustion interface (Finnegan GC/CIII). A correction for the addition of the methyl C from BF_3/methanol derivatization was calculated for each fatty acid by mass balance from the analysis of free and methylated internal standard nonadecanoic acid (Abrajano et al., 1994). The same capillary column, analysis conditions and standards used for the GCMS and GCFID were used for the GC-IRMS. Stable isotopic ratios were measured relative to high purity, calibrated, and reference gas standards expressed relative to international standard PDB (Pee Dee Belemnite).

Assignment of general microbial groups to the PLFA identified and analyzed isotopically was carried out according to Ringelberg et al., (1989), Zelles et al., (1992), and White et al. (1996). The position of the double bond is defined by the symbol ω followed by the number of carbons from the methyl end of the molecule. Location of methyl groups (Me) are designated by a number proceeding the chain length and refer to the number of C atoms from the carboxylic C.

Microbial biomass was estimated from total PLFA content (μg PLFA) of each soil sample, using the conversion factor described by Zelles et al., (1992) where 280 μg PLFA is equivalent to 1 mg C of microbial biomass.

The isotopic composition of the respired CO_2 was determined from the carbonate collected as $BaCO_3$. The $BaCO_3$ was collected, washed, and dried on glass fiber filters. Subsamples of each were weighed into iconel boats that were dropped into an evacuated vessel of chromic-H_3PO_4. Each acidification reaction was allowed to proceed, with stirring, for 1 hour at 90 °C, after which the CO_2 evolved was cryogenically purified on a vacuum line and collected in a sealed glass tube. Samples were analyzed for the C isotopic composition using a multiport tube cracker on a Finnegan Delta+ isotope-ratio mass spectrometer. Procedures used were those designed for $CaCO_3$ (Hassan, 1982) (Swart et al., 1991); therefore, we tested this approach for $BaCO_3$ by generating a number ($n = 10$) of $BaCO_3$ samples in the same way as samples but from standard solutions of $NaCO_3$ with a known stable isotopic composition. Stable isotopic ratios were measured relative to high purity, calibrated, reference gas standards expressed relative to international standard PDB. Values obtained from the $BaCO_3$ generated were within 0.7‰ of the original $NaCO_3$ and exhibited a standard deviation of 0.5‰. Standards (House Faux PDB; Calcite) were prepared in between each sample to verify the completeness of the reaction, the cryogenic preparation, and IRMS analysis.

Statistical Analyses

Replicate measures of CO_2 evolved, biomass, individual PLFA content, $\delta^{13}C$ of CO_2, and $\delta^{13}C$ of each individual PLFA were used in the statistical analyses. The design was a completely randomized two-factor factorial (glucose and time) with two replications for each glucose by time treatment combination. Data were subjected to an analysis of variance and means separated by using an least significant difference, with $P < 0.05$ level of significance using SAS software, version 8.2 (SAS Institute, Inc., Cary, NC).

RESULTS AND DISCUSSION

Two Major Phases of Microbial Activity Suggests Recycling of Added Substrate

The glucose added to the soil was rapidly metabolized and below detection limits by the 15-hour sampling (Fig. 1a). First-order kinetics were used to evaluate the glucose C degradation. The first-order rate constant (κ) for glucose C degradation for the 0- to 10-hour time period was 0.062 h^{-1} ($r^2 = 0.88$). Ladd and Paul (1973) reported that <4% of the added glucose remained after 36 hours of incubation in a fine sandy loam soil, but 65% of the glucose C remained in the soil. Similar results were reported by Tateno (1985) in a study in which a decline in microbial activity occurred after the depletion of glucose after 2 days.

The production of CO_2 and changes in biomass exhibited a significant time by treatment interaction and suggested that two major phases of microbial activity occurred in the glucose amended treatment (Fig. 1a). Significant increase

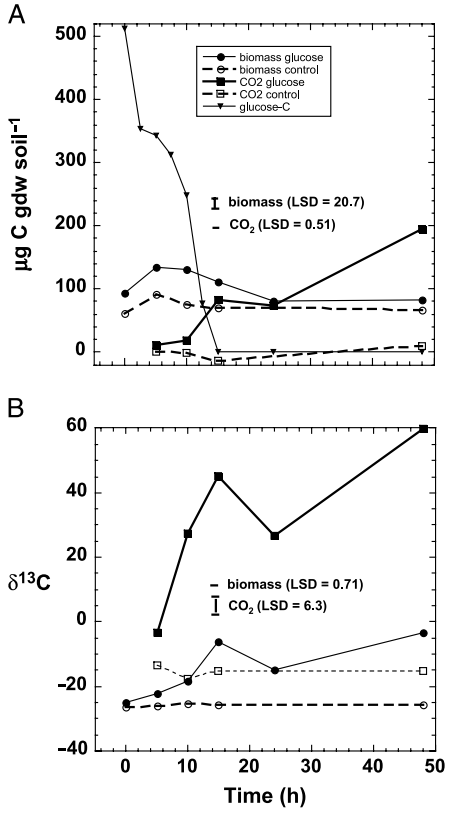

Fig. 1. The μg C gdw soil^{-1} (A) and the stable isotopic composition ($\delta^{13}C$; B) of the microbial biomass and respired CO_2 from the control treatments (dashed lines) where no glucose was added and from the treatments where ^{13}C-labeled glucose was added (solid lines). These values are plotted versus incubation time, and least significant difference (LSD; $P < 0.05$), determined from the analysis of variance, is given as a bar to scale.

in production of CO_2 occurred between each time point until the glucose supply was depleted at 15 hours. The significant change in CO_2 evolved, primarily between 10 and 15 hours, was followed by a period of more rapid and significant CO_2 production from 24 to 48 hours (Fig. 1a). This pattern suggests two phases of growth, one (0 to 15 hours) directly fueled by the added glucose and the other (24 to 48 hours) fueled by less available substrates possibly generated from the original glucose. It is likely that once the glucose was depleted there was no longer enough energy to support the larger biomass generated by the original addition of the glucose (Wagner, 1975). Less available substrates probably were generated through the breakdown of the unsupported biomass, which likely supported some of the microbial activity that occurred after 15 hours (Chahal and Wagner, 1965; Wagner, 1975).

Within the glucose-amended soil, biomass was significantly higher than the unamended soil at time zero suggesting microbial growth was stimulated in the glucose amended soil before freezing the initial samples (Fig. 1a). Biomass significantly increased between 0 and 5 hours in both the amended and control treatments. This initial increase was followed by a significant decline in biomass to levels similar to the starting biomass within the unamended control suggesting that the initial increase in biomass was due to physical mixing before the incubation of the soil (Fig. 1a). Within the unamended soil, no significant changes in biomass were detected other than the increase at 5 hours. After the initial, significant increase at 5 hours, biomass remained constant until 10 hours, significantly decreased at 15 and 24 hours, and remained constant at 48 hours in the glucose amended soil. Estimates of growth efficiency within the glucose amended soil were based on the change in biomass, calculated from total PLFA, and CO_2 evolved. Microbial growth efficiency during the initial stages of the incubation was estimated to be 79 ± 17%. This extremely high value is similar to the theoretical maximum for aerobically grown microorganisms (70%; Wagner, 1975) as well as the initial stages (0 to 8 hours) of microbial utilization of glucose in the same soil (83%; Gilmour and Gilmour, 1985) but higher than most estimates of soil microbial growth efficiencies for carbohydrates in general (40 to 60%, Paul and Clark 1996). The change in biomass was insignificant but CO_2 production during the final 24 hours of incubation was significant suggesting growth efficiency was low. Substrate possibly made available through recycling of microbial biomass, would be more complex therefore primarily oxidized to CO_2 rather than incorporated into biomass during the final stages of the incubation. The addition of NH_4NO_3 with the glucose and the low initial C:N ratio (~9) suggests that the lower efficiency had more to do with the composition and lower lability of the produced biomass used in the later part of the experiment and less to do with nitrogen availability.

Tracking the ^{13}C-label into Respired CO_2 and Biomass Revealed the Recycling of the Label Incorporated During the Early Stages of Glucose Degradation

The CO_2 and biomass served as independent measures of the isotopic composition of the

microbial community during the course of the experiment. Tracking the ^{13}C-label of the original glucose in the CO_2 and biomass formed suggested that two phases of microbial activity were supported by the added glucose. The isotopic composition of both the respired CO_2 and the biomass followed a similar pattern to the changes observed in biomass and CO_2 produced over the course of the incubation (Fig. 1b). The isotopic composition of both the respired CO_2 and biomass within the ^{13}C-glucose amended soil were significantly (analysis of variance; $P < 0.05$) enriched in ^{13}C relative to the control and became progressively enriched in ^{13}C up to 15 hours at which point both pools became significantly more deplete in ^{13}C (Fig. 1b). The depleted $\delta^{13}C$ values coincided with the time when glucose levels were exhausted and suggests that microbial respiration became dependent on another source of C rather than the C derived from the added glucose. The subsequent and significant increase in ^{13}C of both biomass and CO_2 suggests that between 24 and 48 hours the microbes began to use recycled forms of C originally derived from the added glucose. The change in $\delta^{13}C$ of the biomass and CO_2 between 0 and 15 hours occurred at a greater rate than that between 24 and 48 hours suggesting the bioavailability of substrate used was quite different between these two incubation periods. The trends in isotopic composition further support the different growth efficiencies for these periods which also suggest differences in substrate bioavailability.

An estimated mass balance of measured C suggests a substantial part of the glucose-derived C may have been rapidly converted to microbial storage components not accounted for in our estimate of biomass. The unaccounted C, as calculated from the total of all three pools measured (CO_2, biomass, glucose) at t = 0 hours minus the total at each time point, appeared to have increased until the added glucose was exhausted at t = 15 hours. After 15 hours, a smaller increase in the unaccounted C was calculated at 24 hours and a decrease at 48 hours (Table 1). One conversion factor was used to calculate biomass from PLFA content and was applied to samples from all stages of the experiment. It is possible that a greater microbial biomass C was associated with lower levels of PLFA, caused by an increase in cell size or storage compounds, during the initial stages of the incubation (Tunlid and White, 1992). This may have caused an underestimate of biomass at the start of the experiment, creating an overestimate in the unaccountable C. The biomass, however, was within range of levels determined for this same soil during an earlier study using different methods (Gilmour and Gilmour, 1985).

The $\delta^{13}C$ of the respiration, biomass, and original substrate suggests that a portion of the C that supported the later, slower period of activity was fueled by the added glucose. The mean $\delta^{13}C$ value for the unaccounted C ($\delta^{13}C_{ua}$) was calculated as $\delta^{13}C_{ua} = M_0(\delta^{13}C_0) - M_T(\delta^{13}C_T)]/(M_0 - M_T)$, where M_0 is the mass of the total C measured at t = 0 hours, $\delta^{13}C_0$ is the total weighed average $\delta^{13}C$ at t = 0 hours, M_T is the mass of the total C at the respective time point, and $\delta^{13}C_T$ is the total weighed average $\delta^{13}C$ of the measured pools of C at the respective time point. The mean $\delta^{13}C_{ua}$ was 109‰ and did not vary much throughout the incubation, suggesting the unaccounted C calculated was probably derived from the added glucose (95‰). The fact that these values exceed the isotopic composition of the added glucose (95‰) may be attributed to the underestimation of microbial biomass. A portion of the C that remained unaccounted for during the initial stages of the incubation appears to have been subsequently used at a much slower rate later in the experiment. The increase in the amount of C accounted for between 24 and 48 hours was primarily in the form of respiration (Table 1). The $\delta^{13}C$ of CO_2 suggest that much of the respiration was fueled by the added glucose. Calculated values for the $\delta^{13}C$ of the unaccounted C suggest that decaying biomass originally derived from the added glucose was likely the form of C fueling the later increase in respiration.

The amount of C in CO_2 or biomass that was derived from the added glucose versus the SOM was estimated using a simple mixing model by assuming that SOM and the added glucose were the only potential sources of C supporting the microorganisms. The estimates of the amount of C derived from glucose and SOM used both the mass of each C pool (biomass and CO_2) and the $\delta^{13}C$ of the added glucose (95‰) and an assumed $\delta^{13}C$ of −25.8‰ for the SOM (Table 1). The average $\delta^{13}C_{PLFA}$ (−25.8‰) of microbial biomass in the incubations without added glucose was used to approximate the $\delta^{13}C$ of SOM since average $\delta^{13}C_{PLFA}$ has been found to closely approximate the $\delta^{13}C$ of substrate in aerobic soil (Burke et al., 2003; Cifuentes and Salata, 2001). In terms of absolute quantities, glucose-derived C appeared in the biomass relative to CO_2 at the

TABLE 1
Size of each carbon pool measured or calculated within ^{13}C-glucose amended incubations at each time point

Time (h)	CO_2-C			Biomass-C†					
	Glucose-derived*	SOM-derived*	Total	Glucose-derived*	SOM-derived	Total	Glucose-C	Total C	Unaccounted for C‡
	μg C gdw soil^{-1}			μg C gdw soil^{-1}			μg C gdw soil^{-1}		
0	–	–	0	–	93	93 (10)	512	604	–
5	2	10	12 (7)	22	110	132 (15)	344	488	116
10	15	5	20 (8)	23	109	132 (8)	248	400	204
15	35	49	84 (0.5)	23	89	112 (10)	0	196	408
24	25	47	72 (28)	13	67	80 (4)	0	152	452
48	65	131	196 (0.9)	18	66	84 (4)	0	280	324

The total C refers to the sum of all pools measured. Calculated values include quantity of total carbon in the CO_2 and biomass that was derived from the added glucose (glucose derived) or soil organic matter (SOM derived), and the amount of C not accounted for (Unacc. C) in the three pools measured (CO_2, biomass, glucose) at each given time point is provided. Standard error of each value provide in parentheses.

*Values are calculated from the mean weighed δ^{13}C value of the CO_2 and biomass pools, the δ^{13}C of the added glucose (95.0 ‰), and assuming δ^{13}C of SOM is –25.8 ‰. The δ^{13}C of SOM is based upon the average δ^{13}C of the microbial biomass in the control incubations determined from the $\delta^{13}C_{PLFA}$.

†Microbial biomass was estimated from the total PLFA content (μg PLFA gdw soil^{-1}) divided by the conversion factor (280 μg PLFA mg C biomass^{-1}) from Zelles et al. (1992).

‡Unaccounted for C defined as all carbon originally (t = 0 h) measured in CO_2, biomass and glucose not measured in those pools following the given period of incubation, and calculated as the difference between total C measured at t = 0 h and total C measured at a given time point.

start of the experiment (0 to 5 hours; Table 1). Proportionally, however, glucose C appeared to only make up 12% of CO_2-C and 17% of biomass-C suggesting that SOM or stored microbial C supported the energy and C for the initial microbial growth (Table 1). The greatest proportion of glucose-derived C in microbial biomass appeared at 15 and 48 hours. Glucose-derived C never dominated either CO_2 or biomass C throughout the experiment except at 10 hours, when it represented ~75% of CO_2-C (Table 1).

In terms of quantity, the amount of glucose-derived C found in the CO_2 was greatest at 15 and 48 hours and exceeded the amount found in biomass during this period. Throughout the experiment, however, the amount of glucose-derived C in biomass was stable and remained between 13 and 23 μg C gdw soil^{-1} (Table 1). Tracking the ^{13}C-label from the added glucose demonstrated that a portion of the glucose C went into microbial biomass early in the experiment. The added glucose, however, was never the major source of C for microbial biomass production throughout the experiment. The δ^{13}C of CO_2 suggests that the added glucose became an important energy source early on in the incubation and again after the depletion of the original glucose, suggesting that substrate derived from added glucose fueled a "second" period of activity after 15 hours. The new forms of glucose C, whether it was reworked into new, more resistant compounds or used to make microbial storage compounds, was used primarily to fuel microbial activity and not to build biomass.

Tracking Use and Recycling of Labile Substrate Through the Soil Microbial Community Using $\delta^{13}C_{PLFA}$

Within all soil samples analyzed, 24 individual PLFA were detected and quantified with 12 resolved isotopically at most time points in both the glucose-amended and unamended treatments. Not all PLFA detected could be analyzed isotopically due to coelution or concentrations below the detection limit (0.4 μg gdw soil^{-1}). The initial (t = 0 samples) $\delta^{13}C_{PLFA}$ composition of the soil used in the experiment ranged from −27.9 to −24.0‰, indicating a potential for small differences in substrate utilization by different components of the microbial community. The 3‰ range in the isotopic composition of PLFA in the soil used in this study was within the range for aerobic agricultural soil reported in other studies (Burke et al., 2003; Cifuentes and Salata, 2001).

Statistical analysis of the PLFA content (nmol gdw soil^{-1}) and $\delta^{13}C_{PLFA}$ showed that there was a significant ($P < 0.05$) time by glucose

treatment interaction for the seven PLFA reported in Fig. 2. During the incubation with ^{13}C-labeled glucose, the range in $\delta^{13}C_{PLFA}$ varied greatly both among the individual PLFA and over time. The most enriched δ^{13}C values occurred at 15 and 48 hours (19.6 and 24.9‰, respectively) within ^{13}C-labeled glucose soil and were for a15:0, one biomarker indicative of Gram-positive bacteria (Ringelberg et al., 1989; Zelles et al., 1992). The results show that some Gram-positive bacteria were responsible for much of the initial incorporation of glucose C. Significant increases in δ^{13}C values of a15:0, i15:0, i16:0, a16:0, and i17:0, all PLFA indicative of Gram-positive bacteria, were detected between 0 and 15 hours (Figs. 2 and 3). The δ^{13}C of 10Me18:0, indicative of actinomycetes in soil (Zelles, 1999), suggests actinomycetes were not as involved in the initial incorporation of the glucose added as other Gram-positive bacteria but potentially important in some recycling of the added glucose (Fig. 3).

The quantity and δ^{13}C composition of each individual PLFA varied throughout the course of the incubation, indicating that different components of the microbial community played different roles in the utilization and cycling of the added glucose. Those PLFA that represented

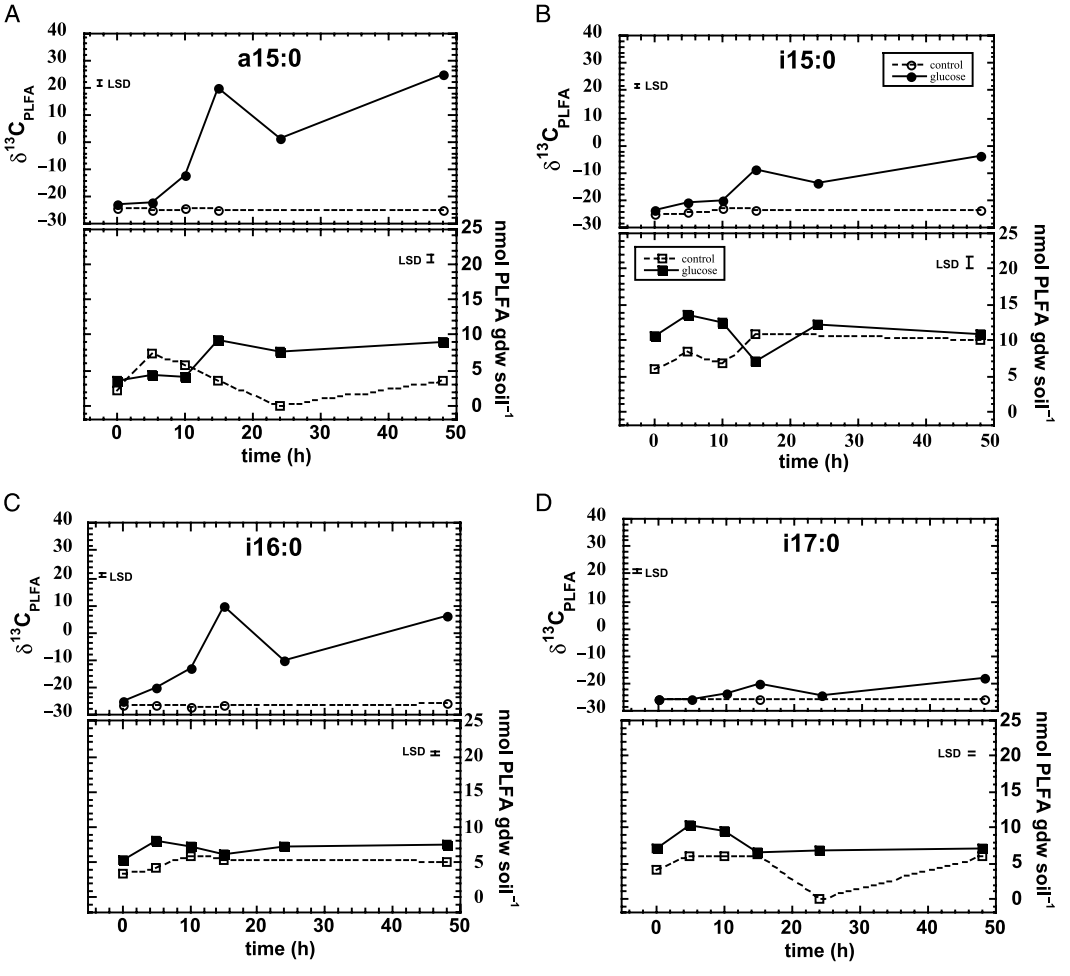

Fig. 2. Quantity (nmol PLFA gdw soil^{-1}; squares) and stable carbon isotopic composition ($\delta^{13}C_{PLFA}$; circles) of (A) 12-methyl-tetradecanoate (a15:0), (B) 13-methyl-tetradecanoate (i15:0), (C) 14-methyl-hexadecanoate (i16:0), (D) 15-methyl-hexadecanoate (i17:0), (E) hexadecenoate (16:1ω7), (F) hexadecanoate (16:0), and (G) octadecanoate (18:0) from the unamended control (dashed lines and open symbols) and ^{13}C-gluose–labeled treatment (solid lines and symbols). Values are plotted versus incubation time, and least significant difference (LSD; $P < 0.05$), determined from the analysis of variance, is provide as a bar to scale.

the largest fraction of the PLFA in the soil, i15:0, 16:0, and i17:0, had a similar δ^{13}C pattern over the course of the experiment (Fig. 2). Each of these biomarkers exhibited a significant decrease in abundance at 15 hours when their respective C isotopic composition was relatively enriched in ^{13}C. This decrease may be associated with ''a crash'' of some components of the microbial community after depletion of glucose. The 16:0 marker is a general marker integrating the signature of many microbial groups. The significant reduction in 16:0 probably represents the loss of some microorganisms and the simultaneous ^{13}C enrichment of 16:0 suggests the use of labeled substrates resulting from microbial biomass originally supported by the added glucose (Fig. 2F).

The quantity and isotopic composition of a15:0 and 16:1ω7 followed a similar pattern, indicating that the use of glucose-derived C fueled growth of some Gram-positive and Gram-negative bacteria (Figs. 2, A and E). Both of these PLFA were relatively small components of the initial soil microbial community (3.0 to 3.2 nmol gdw soil^{-1}) and became important components after the addition of glucose (≥10 nmol gdw soil^{-1}). This suggests that the microbial response to added glucose was more specific to particular groups and not a general response by most components of the soil microbial community. Significant peaks in the abundance of a15:0 and 16:1ω7 occurred at 15 hours, when the glucose was exhausted, followed by a significant decrease at 24 hours (Fig. 2, A and E). The larger increase in both quantity and δ^{13}C of a15:0 at 48 hours suggests that Gram-positive bacteria incorporated recycled C derived from the added glucose.

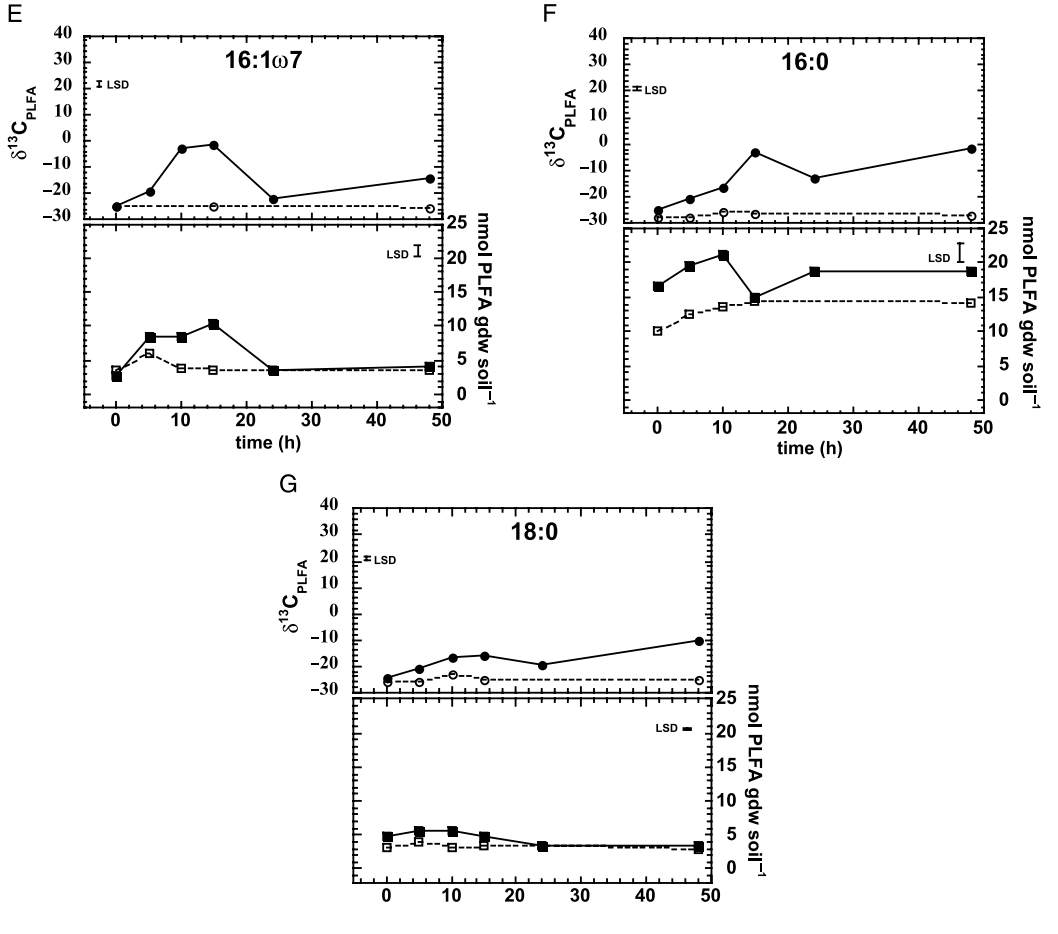

Fig. 2. (Continued)

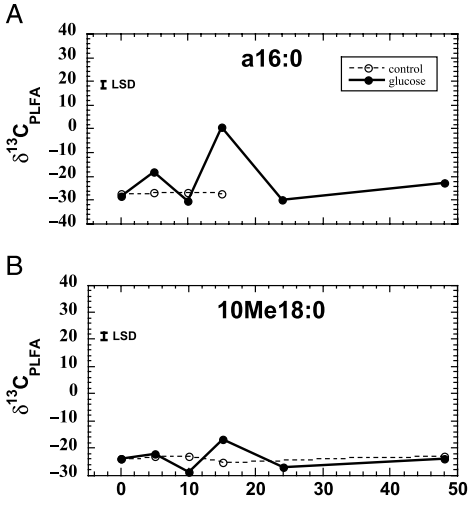

Fig. 3. Stable carbon isotopic composition ($\delta^{13}C_{PLFA}$) of (A) 13-methyl-hexadecanoate (a16:0) and (B) 10-methyl-octadecanoate (10Me18:0). Values are from the unamended control (dashed lines and open symbols) and ^{13}C-gluose–labeled treatments (solid lines and symbols). Values are plotted versus incubation time, and least significant difference (LSD; $P < 0.05$), determined from the analysis of variance, is provided as a bar to scale.

Potential fungal biomarkers were not abundant in this soil during the experiment, and, consequently, we could not resolve them isotopically. Although inconclusive, the data suggest the fungal community had a less important role in the use and recycling of the added glucose in this soil during the 48-hour incubation (Fig. 4).

The quantity of 10Me18:0, often associated with actinomycetes (Zelles, 1999), exhibited significant time and treatment main effects. It was a minor component in the initial samples, significantly increased at 5 hours and was significantly higher in the glucose amended relative to the unamended soil (data not shown). The isotopic composition of 10Me18:0 had a significant time by treatment interaction and contained more glucose-derived C late in the incubation as indicated by significant increases in $\delta^{13}C$ (Fig. 3B). This PLFA only became enriched in ^{13}C at 15 hours when the added glucose was depleted. Overall, the pattern of $\delta^{13}C$ exhibited by 10Me18:0 suggests that the actinomycetes played a minor role in the initial uptake of the glucose and may have relied upon the glucose-derived products of the Gram-positive bacteria in the soil. This shift in the role of components of the microbial community involved in the cycling of the added glucose C is congruent with observations of initial dominance of opportunistic microbial populations after glucose additions (Shen and Bartha, 1996).

The 16:0 and 18:0 are ubiquitous PLFA and are therefore likely to represent the integration of the most dominant groups in the soil studied. These common PLFA may have been largely derived from both the Gram-positive bacteria and actinomycetes, given the dominance of terminally methylated PLFA. In the glucose-amended soil, both of these PLFA exhibited a significant increase in quantity initially (0 to 5 hours), decreased in quantity between 10 and 15 hours, and no significant change between 24 and 48 hours (Figs. 2, F and G). The change in the quantity of 16:0 and 18:0 followed that of the total biomass, indicating that they indeed reflect a dominant component of the microbial community (Table 1). The $\delta^{13}C$ of the 16:0 and 18:0 exhibited a peak in $\delta^{13}C$ at 15 hours and subsequently significantly enriched again at 48 hours, similar to a15:0, a Gram-positive PLFA. The $\delta^{13}C$ of these general PLFA, however, did not change as dramatically as most of the more specific Gram-positive PLFA and instead exhibited a more gradual enrichment over the course of the incubation. This may due to other components of the microbial community, such as actinomycetes, which were not involved in the utilization of the labeled glucose but appeared to have become more important after the depletion of the added glucose.

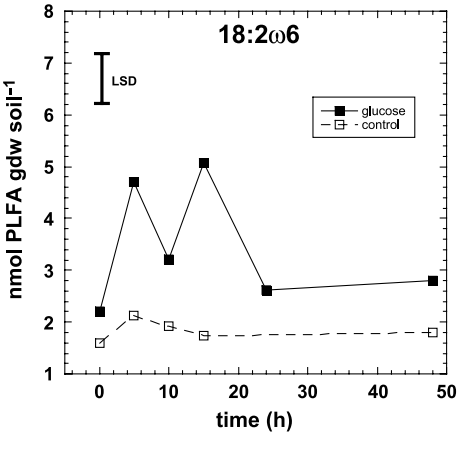

Fig. 4. Quantity (nmol PLFA gdw soil^{-1}) of methyl linoleate (18:2ω6) from the unamended control (dashed line) and ^{13}C-gluose–labeled treatment (solid line). Values are plotted versus incubation time, and least significant difference (LSD; $P < 0.05$), determined from the analysis of variance, is provided as a bar to scale.

CONCLUSIONS

Tracking the ^{13}C label through respiration and microbial biomass is instructive for understanding the fate of organic substrates introduced into the soil environment. The use of $\delta^{13}C_{PLFA}$ can be useful in identifying what components of the soil microbial community respond to specific substrate additions. Results from this study suggest that formation of microbial byproducts may occur rapidly during the early stages in the degradation of bioavailable forms of C. Formation of these byproducts from glucose appeared to support more respiration than biomass production. The contribution of these byproducts to biomass, however, was great enough to significantly increase the δ^{13}C of PLFA after exhaustion the added glucose suggesting that recycling of added ^{13}C label can occur rapidly in soil.

Few studies have followed stable isotopically labeled elements over time (Middelburg et al., 2000), and little is understood about how they are recycled. Supporting previous work, the response of the soil microbial community to the addition of glucose in this study suggests that even a labile substrate does not illicit a general response by the entire microbial community. Instead, specific groups of the community appear to be more involved in the entire degradation process than others. Meanwhile, other groups are only involved in specific aspects of substrate degradation. Gram-positive bacteria probably dominated the use of glucose C throughout the experiment. Actinomycetes, however, were not as important in the initial utilization of the glucose but may have played a more important role in the use of microbial products derived from the original glucose. If this experiment were conducted with only one 48-hour time point, for example, the $\delta^{13}C_{PLFA}$ data could have erroneously indicated that actinomycetes played a role in the initial uptake of the added glucose. Results from this study show that the element of time needs to be carefully considered in the interpretation of any stable isotope-labeling and biomarker study.

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