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Use of ^{13}C-labelled plant materials and ergosterol, PLFA and NLFA analyses to investigate organic matter decomposition in Antarctic soil

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Abstract

The relationship between organic matter decomposition and changes in microbial community structure were investigated in Antarctic soils using ^{13}C-labelled plant materials. Soils with and without labelled *Deschampsia antarctica* (a native Antarctic grass) were incubated for 42 days and sampled at 0, 7, 14, 21, 28 and 42 days. Changes in microbial community structure were assessed using phospholipid fatty acid analysis (PLFA) and an analysis of the fatty acids associated with the neutral lipid fraction (NLFA). These studies showed that there were no significant changes in PLFA or NLFA profiles over time suggesting no change in microbial community structure during residue decomposition. There was a marked increase however, in ergosterol levels in these soils indicative of growth of the fungal biomass. Analysis of this ergosterol using gas chromatography-mass spectrometry confirmed the transformation of the plant residue by showing the incorporation of ^{13}C-plant C into the ergosterol. This incorporation of ^{13}C into the ergosterol increased over the incubation period. Importantly, these changes associated with fungal growth were not evident in the analysis of either the PLFA or NLFA fractions thus questioning the reliability of such approaches for studying changes in microbial communities associated with the decomposition of plant residues.

Keywords: Maritime Antarctic; Phospholipid fatty acids; Neutral lipid fatty acids; ^{13}C-labelled ergosterol; Decomposition; Microbial communities

1. Introduction

The beginning of the 21st century has been marked by an increased awareness of the impacts of human activity on the sustainability of the biosphere. As the major drivers of the biogeochemical cycles that promote primary productivity and therefore sustain life, microorganisms are the focus of considerable attention. However, as yet we know relatively little about the complexity and structure of natural microbial assemblages (Davison et al., 1999; Derry et al., 1999) or of how essential this complexity is to function (Kennedy and Gewin, 1997; McGrady-Steed and Morin, 2000; Lipson et al., 2002). Until relatively recently, attempts to understand these complexity: function relationships have been hampered by a lack of suitable methods (Kennedy and Gewin, 1997; O'Donnell and Goerres, 1999) but with the availability of novel molecular and biochemical techniques significant advances are a realistic possibility (O'Donnell and Goerres, 1999). The fact that specific chemical components can be used to indicate changes in microbial communities (Tunlid and White, 1992; Frostegård et al., 1993) and that stable isotopes can be used to follow the transfer of nutrients through the microbial compartments in the soil (Arao, 1999; Radajewski et al., 2000; Zhang, 2002) has made it possible to open the 'microbial black-box' and to examine in detail the relationship between specific groups of microorganisms and nutrient cycling.

One of the difficulties in working with soils is the marked heterogeneity of the environment and the impact this has on trophic interactions and ecological complexity. In degraded ecosystems and in extreme environments this complexity is often reduced making it potentially easier to investigate the relationship between microbial diversity and ecosystem function. For plants and animals the number of taxa decreases with increasing latitude (Kanda and Komárková, 1997); this is also true for arthropods (Block, 1985), algae (Broady, 1996) and protozoa (Smith, 1996). This gradient of

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decreasing biodiversity may reflect the fact that as latitude increases the environment becomes more extreme leading to a short growing season, low temperatures and limited productivity.

Antarctic soils are extreme and inhospitable to many organisms and as such diversity and growth rates are usually very low (Kennedy, 1993; Bölter, 1995; Wynn-Williams, 1996). These low growth rates and low diversity make Antarctic ecosystems relatively simple and potentially vulnerable to disruption (Kanda and Komárková, 1997). However, as simple systems with comparatively simple trophic structures, they provide a useful paradigm for studying the relationship between microbial communities and function in soils (Wynn-Williams, 1996; Kanda and Komárková, 1997).

The work reported here started with the assumption that the absence of higher organism interactions and the simpler food webs typical of Antarctic soils (Kanda and Komárková, 1997) would make it easier to investigate the relative importance of different components of the microbial community in the decomposition of plant residues. The aims of the study were to assess the roles of bacteria and fungi in the decomposition processes in Antarctic soils and to investigate, using labelled plant residues, the extent to which the transfer of plant-C to fungal biomass can be directly assessed.

2. Materials and methods

2.1. Soil and plant materials

The study site (Fossil Bluff) is on the eastern coast of Alexander Island (71°19′S, 68°17′W), Antarctica and is one of several, small, ice-free areas along the eastern margin of Alexander Island near the George VI Sound (Salvatore, 2001). Fossil Bluff is characterized by a mineral, rocky soil without extensive areas of vegetation (Convey and Smith, 1997). Surface soil samples (2–5 cm) were taken from beneath a lichen-moss tundra and from pockets within coarse scree in January 1997 and bulked to give one composite sample. Soils were transported frozen (−20 °C) to the UK and kept frozen (−80 °C) until used. Soils contained 1.6% total C, 38 μg g^{-1} total N, $pH_{(water)}$ 7.6, and had a moisture content at collection of 0.42 g H_2O g^{-1} soil (dw).

Deschampsia antarctica Desv. (Poaceae) is one of the two flowering plants colonising the Antarctic islands and can be found in most of Maritime Antarctica down to approximately 68°S. It does not extend to continental Antarctica (Greene and Holtom, 1971; Grobe et al., 1997). Plant material was collected at the same time as the soil and propagated in a controlled environment cabinet (Hopkins et al., 1997). Plants were grown at 10–12 °C in open glass vials on vermiculite supplied with mineral nutrient solution in which the N source was 99 at.% ^{15}N. To label plant-C, ^{13}C-CO_2 was injected daily to the chamber during the 12 h light period. This resulted in a 4.1 at.% ^{13}C enrichment (Hopkins et al., 1997). The green leaves were then harvested and shredded (approx. size 2–3 mm) prior to use in the microcosms.

2.2. Microcosms

Soils were thawed at 4 °C, sieved to 2 mm, and immediately used in the microcosms. The microcosms (36×100 ml^2 Erlenmeyer flasks) were set up with 6 g (fresh weight) of soil in each flask. Control soils (18 flasks) were left unamended (no labelled grass material) whilst the amended soils were mixed with 0.01% (0.6 mg) of shredded ^{13}C labelled *D. antarctica*. Microcosms were then sealed with rubber Subaseal® stoppers. To standardise gravimetric water content, water was added to 50% by weight (w/w) and maintained at 50% throughout the incubation period. Microcosms were incubated in the dark at 12–15 °C (Gallenkamp Cooled Incubator) for 42 days and sampled destructively (three control and three amended microcosms) at 0, 7, 14, 21, 28 and 42 days. Soil samples from each microcosm were divided and 2 g used for PLFA and NLFA analysis and 2 g for ergosterol analysis.

2.3. Soil respiration

Soil respiration was monitored daily as described by Hopkins et al. (1997) by removing 10 ml of headspace gas from the microcosms using an air-tight syringe (SGE International Ltd). Gas samples were transferred to evacuated gas sample tubes (IsoChem) and kept at room temperature prior to analysis.

CO_2 samples were analysed isothermally at room temperature by gas chromatography (GC) on a Varian 90-P gas chromatograph fitted with a 1.32 m long, 3 mm internal diameter stainless steel column packed with Poropak Q. Helium was used as the carrier gas and peaks were detected using a thermal conductivity detector. Peaks were measured manually and quantified with an external 1% (v/v) CO_2 standard in nitrogen. Curves were fitted in Excel using a linear function ($R^2 = 0.83$ and 0.95 for the control and amended soils respectively) and the rates of CO_2 evolution (as mol CO_2 g^{-1} soil d^{-1}) calculated using the method described by Heilmann and Beese (1992) where the difference between the initial and final CO_2 concentrations in the microcosms is divided by the number of incubation days (42 days).

2.4. Fatty acids methyl ester extraction and GC analysis

FAMEs were extracted from 2 g of soil using minor modifications to the method described by Bligh and Dyer (1959). The procedure involved the addition of 25 ml of chloroform: methanol: citrate buffer—0.15 molar trisodium citrate dihydrate, 0.15 molar citric acid,

pH 4—(1:2:0.8, v/v/v) followed by mixing for 2 h after which time another 6.6 ml of chloroform and 6.6 ml of water were added to give a final ratio of chloroform: methanol: citrate buffer of 1:1:0.9 (v/v/v). Tubes were then agitated vigorously and allowed to settle overnight. The upper aqueous layer was then removed and the chloroform filtered through a Whatman No. 2 filter paper that had been previously washed with chloroform: methanol (2:1, v/v). The chloroform phase was next transferred to a screw capped test tube and dried under nitrogen at 37 °C then redissolved in 0.5 ml of chloroform. These samples were then fractionated on silicic acid columns (0.5 g of 200–400 mesh size, Sigma) using 10 ml of chloroform (neutral lipids), 10 ml of acetone (glycolipids) and 10 ml of methanol (phospholipids) (Frostegård et al., 1991). Both phospholipid (methanol) and neutral lipid (chloroform) fractions were analysed. Fractions (both methanol and chloroform) were dried at 37 °C under a stream of nitrogen and saponified using 1 ml of methanol and 1 ml of aqueous KOH 30% (w/v) at 75 °C overnight. After cooling, samples were acidified to pH 1.0 with 10% hydrochloric acid: water (v/v) and extracted with 1 ml diethyl ether. The upper layer was removed and the procedure repeated twice. The combined fractions were evaporated to dryness at 37 °C under a stream of nitrogen. Fatty acids were then esterified using 1 ml of dichloromethane, 1 ml of a phase transfer catalysis solution [0.1 molar tetrabutylammonium hydrogen sulphate in 0.2 molar NaOH] and 25 μl of iodomethane. Tubes were agitated for 30 min at room temperature and allowed to settle for 10 min after which time the upper phase was discarded and the lower phase was evaporated to dryness. Nonadecanoate (19:0, Sigma) was added as internal standard and the purified fatty acid methyl esters (FAME) were dissolved in 100 μl of hexane and analysed by gas chromatography (GC) on a 30 m fused silica, OV-1 (methyl silicone) capillary column. Helium was used as the carrier gas and peaks were detected by flame ionisation. Peaks were quantified automatically using a computing integrator and identified by reference to the Supelco™ 37 Component FAME Mix and their BAME (Bacterial Acid Methyl Esters) mix. Fatty acids were identified by co-chromatography using the nomenclature described by Tunlid and White (1992).

Selected fatty acid data were organised in an Excel spreadsheet and exported to the Multi-Variate Statistical Package (MVSP version 3.01, Kovach Computing Services, http://www.kovcomp.co.uk/mvsp/) for correspondence analysis (CA).

Prior to CA the data matrix was analysed to assess whether the data were normally distributed (Tabachnick and Fidell, 2001) using the Normality Test option of the Basic Statistics procedure of Minitab for Windows release 11 (Minitab Inc. http://www.minitab.com). Where the data were not found to be normally distributed (PLFA) the individual peak measurements (nmol g^{-1} soil) were $\log_{10}$ transformed prior to CA. Where the analysis showed the data to be normally distributed (NLFA) no transformation was carried out.

2.5. Ergosterol extraction and HPLC analysis

Ergosterol was extracted according to Seitz et al. (1977) with only minor modifications. A 2 g sample of soil was mixed with 10 ml of absolute ethanol and agitated for 2 h. Samples were then transferred to 50 ml tubes and centrifuged at $12,000 \times g$ for 10 min at 10 °C. An aliquot (5 ml) of the supernatant was then transferred to a 15 ml screw cap test tube and 1 ml KOH 60% (w/v) added. Test tubes were sealed tightly and heated at 90 °C for 30 min in a water bath. After cooling, samples were partitioned by adding 1 ml distilled water and 3 ml hexane to the tubes, agitating and allowing the two phases to separate. The hexane phase was removed to a fresh tube and the extraction repeated. The second hexane phase was added to the first and the tubes were evaporated to dryness under a flow of nitrogen. Ergosterol extracts were dissolved in 1.5 ml of HPLC grade methanol and stored in the dark at 4 °C until analysed.

Ergosterol was quantified using high performance liquid chromatography (HPLC) by injecting 10 μl of sample onto a Spherisorb column (S5ODS2, 25 cm, Phase Separations Ltd). HPLC grade ethanol was used as the mobile phase at a flow rate of 1 ml min^{-1}. Ergosterol was detected at 282 nm (Seitz et al., 1977; Newell, 1992) and the retention time was approximately 4 min.

A standard curve derived from an external ergosterol standard diluted in methanol was used to quantify (μg ergosterol g^{-1} soil) the amount of ergosterol in each sample.

2.6. Ergosterol derivatisation and GCMS analyses of ^{13}C

Samples used for HPLC analysis were subsequently derivatised to their trimethylsilyl (TMS) ethers to facilitate their analysis by gas chromatography and to reduce decomposition during injection and analysis (Axelsson et al., 1995; Nielsen and Madsen, 2000). Derivatisation was achieved by dissolving the extracts in 200 μl of dichloromethane (DCM) and adding 50 μl of 10% trimethyl-chlorosilane (TMCS) in N,O-bis(-trimethylsilyl)-trifluoro-acetamide (BSTFA), and heating at 60 °C for 1 h. The excess reagents were evaporated and the TMS ether derivatives dissolved in 100 μl of DCM.

Gas-chromatography-mass spectrometry (GCMS) analysis of ergosterol derivatives was done using a Hewlett-Packard 5890 II gas chromatograph fitted with a split/splitless injector (280 °C) and linked to a Hewlett-Packard 5972 mass selective detector (electron voltage 70 eV, filament current 220 μA, source temperature 180 °C, multiplier voltage 2000 V, interface temperature 300 °C). Spectra acquisition was controlled by a HP Vectra 486 PC chemstation computer, either in full scan mode

(50–550 amu s^{-1}) or in selective ion monitoring (20 ions 0.7 cps 35 ms dwell) for greater sensitivity. When set for selective ion monitoring (SIM) the ions targeted were *m/z* 337–340, 363–366 and 468–471, respectively. Each sample (1 μl) in DCM was injected using an HP7673 autosampler in the splitless mode and then vented after 1 min. After the solvent peak had passed the GC, temperature programme and data acquisition commenced. Separation was performed on a fused silica capillary column (30 m × 0.25 mm^2 i.d.) coated with 0.25 μm 5% phenyl methyl silicone (HP-5). The GC was held isothermally at 80 °C for 5 min, programmed to 300 °C at 10 °C min^{-1} then held at 300 °C for 18 min. Helium was used as the carrier gas (flow 1 ml min^{-1}, pressure of 70 kPa, split at 30 ml min^{-1}). The acquired data was stored on DAT tape for later data processing, integration and printing. Peak identification and area integration were performed automatically on a computing integrator.

3. Results

3.1. Soil respiration

Fig. 1 shows the increase in CO_2 evolution from the amended and control soils. Over the 42-day incubation period, respiration in the amended soils was significantly higher than that in the control soils. Microbial activity was calculated as the difference in CO_2 evolution between amended and control treatments (Anderson and Ingram, 1993). The rate of CO_2 production (Heilmann and Beese, 1992) in the control soil was 0.7×10^{-6} mol CO_2 g^{-1} soil d^{-1} and in the amended soil was 2.1×10^{-6} mol CO_2 g^{-1} soil d^{-1}. The data show that the respiration rate resulting from plant-C amendment is three times higher than that in the unamended soils. This represents the mineralization of 9 times the amount of C added as plant-C, and is indicative of a significant priming effect.

3.2. Fatty acid methyl ester analysis

Fatty acid methyl esters (FAMEs) were used to study changes in both the phospholipid and neutral lipid fractions. Total phospholipid was calculated from the sum of quantities of individual fatty acids and shown to range (average values, $n = 3$) from 2.6 to 10.3 nmol g^{-1} soil in the amended soil and from 4.7 to 10 nmol g^{-1} soil in the control soil. Comparison of the total phospholipid data using ANOVA showed no significant difference ($P > 0.05$) between amended and unamended soils. The most abundant fatty acids in both treatments were 18:2ω6, a peak co-chromatographing with 18:2t, 16:0 and 18:0. Of these, 18:2ω6 (Fig. 4) has been used to indicate fungal biomass (Frostegård and Bååth, 1996) and accounted for approximately 24% of the total PLFA in the amended soil and 21% in the control soil. Of the PLFA normally associated with bacterial communities, 13Me 14:0, 12Me 14:0, 3OH 14:0, 15:0, 15Me 16:0 and 17:0 were found in both treatments. PLFA fingerprints contained 24 fatty acids and 19 were selected on the basis that they could be measured reliably and reproducibly in all profiles. These data were $\log_{10}$ transformed and subjected to CA. Fig. 2 shows that the first two axes accounted for 50% of the between sample variance (32 and 18%, respectively). The control samples form two small clusters in different parts of the plot and are separated from the amended samples. No systematic differences in community structure were found in either set of samples related to sampling time suggesting that there was no change in community structure throughout the incubation period.

Total neutral lipid was calculated in the same way as the phospholipid and the average ($n = 3$) shown to range from 14 to 22.6 nmol g^{-1} soil in the amended soil and from 20.7

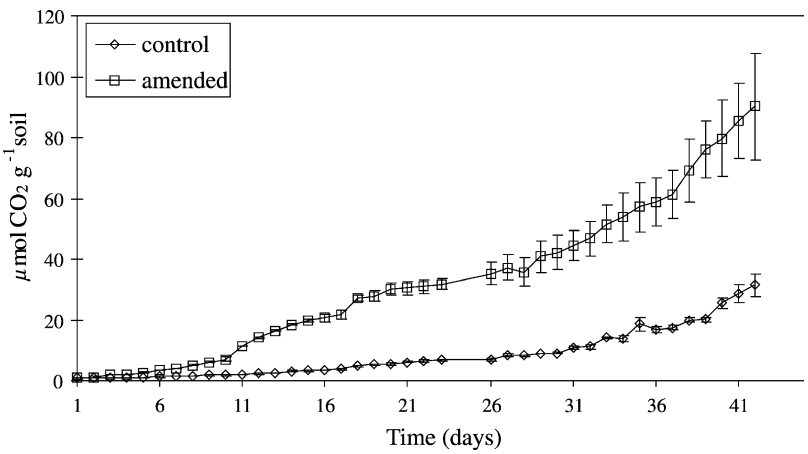

Fig. 1. Changes in CO_2 evolution in Antarctic soils amended with *Deschampsia antarctica* (□, 0.01% w/w) and in unamended control soils (◇). Although polyphasic, both curves could be described using a linear function ($R^2 = 0.95$ and $R^2 = 0.83$ for amended and unamended, respectively). Respiration rates for the amended soil were calculated (Heilmann and Beese, 1992) as 2.1×10^{-6} mol CO_2 g^{-1} soil d^{-1} and for the unamended soil as 0.7×10^{-6} mol CO_2 g^{-1} soil d^{-1}. Data are means ± SE ($n = 3$).

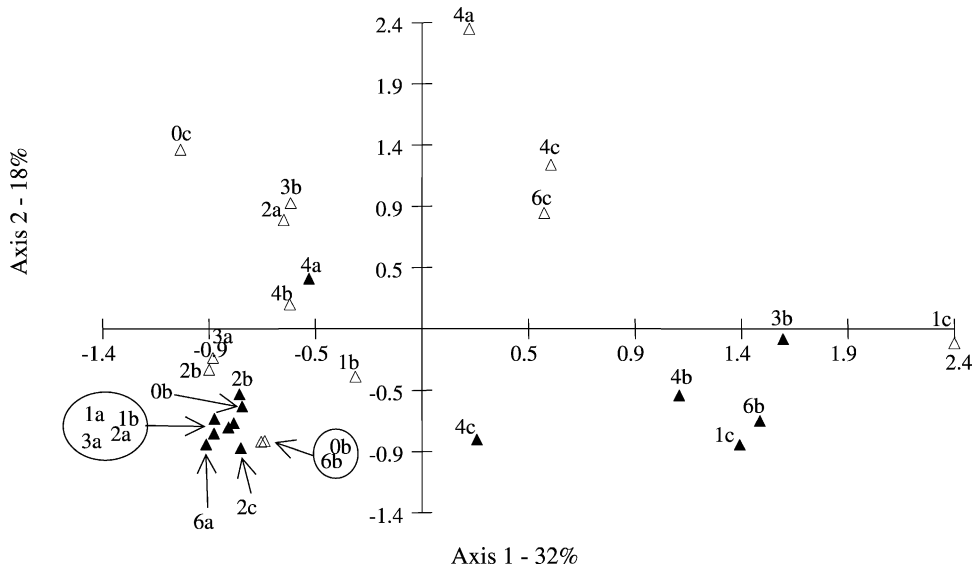

Fig. 2. Correspondence analysis of PLFA profiles ($\log_{10}$ of nmol FA g^{-1} soil) of Fossil Bluff soils incubated over 6 weeks with and without plant residues. The plot explains 50% of the variance in the data. Hollow symbols indicate amended soil and solid symbols the control soil. Numbers indicate the week of microcosm sampling and the letters represent the microcosm replicates.

to 30.8 nmol g^{-1} soil in the control soil. Comparison of the total neutral lipid data (NLFA) using ANOVA showed significant differences ($P = 0.02$) between amended and unamended soils. The most abundant fatty acids in both treatments were 22:2, 2OH 14:0, 3OH 14:0 and 18:2ω6, the latter accounting, on average, for 10% of the total NLFA in the amended soil and 6% in the control soil. Forty different fatty acids were identified in this analysis and were present in both treatments. As with the PLFA data 19 selected fatty acids from amended and control microcosms were analysed using CA and the result of plotting the first and second CA axes are shown in Fig. 3. These accounted for 39 and 18% of the total variance, respectively but, as with the PLFA data, no systematic difference in the microbial communities was found either between treatments or sampling time.

3.3. Changes in fungal biomass and the incorporation of ^{13}C into ergosterol

Ergosterol extracts were analysed by HPLC using the characteristic absorbance of the conjugated double bond at 282 nm (Seitz et al., 1977), and used to investigate changes in fungal biomass. The chromatogram contained a peak with a retention time of 4 min that co-chromatographed with an

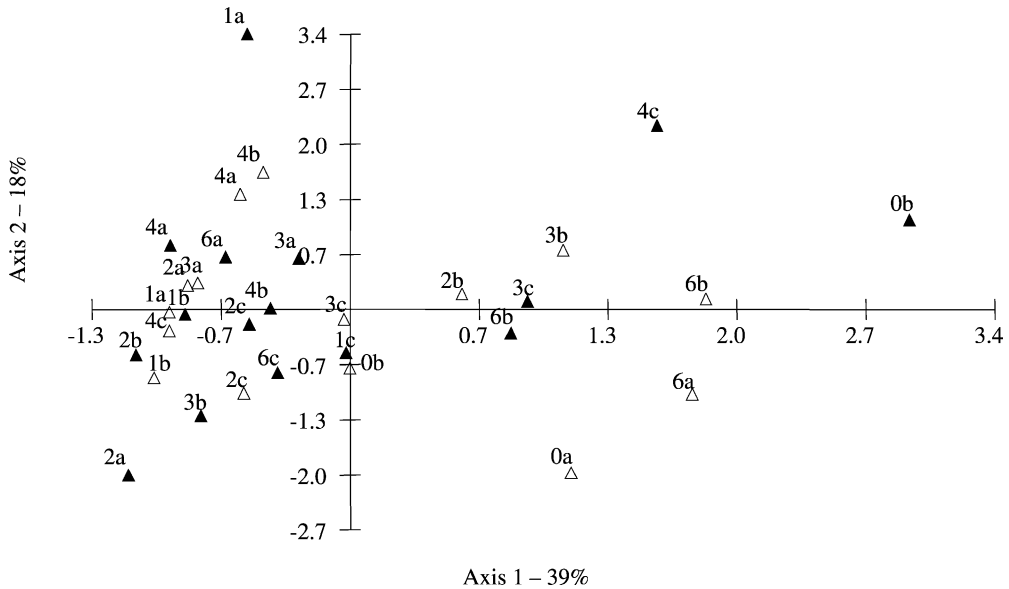

Fig. 3. Correspondence analysis of NLFA profiles (nmol FA g^{-1} soil) of Fossil Bluff soils incubated over 6 weeks with and without plant residues. The plot explains 57% of the variance in the data. Hollow symbols indicate amended soil and solid symbols the control soil. Numbers indicate the week of microcosm sampling and the letters represent the microcosm replicates.

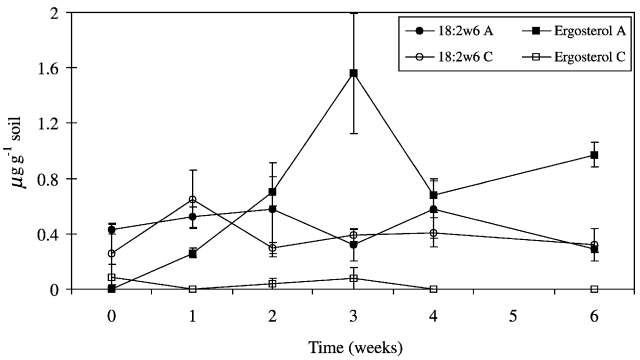

Fig. 4. Changes in ergosterol and 18:2ω6 concentration in soil of Fossil Bluff, Alexander Island, over 6 weeks of incubation in microcosms. Amended microcosms had 0.01% (w/w) of ^{13}C-labelled *D. antarctica* residues added to the soil. Data are means ± SE ($n = 3$). A and C indicate amended and control microcosms, respectively.

authentic ergosterol standard (Sigma). The mean (three separate microcosms) amounts of ergosterol at each sampling date are given in Fig. 4. Ergosterol contents in amended and unamended soils differed significantly (ANOVA, $P < 0.05$) and reached a maximum in the amended soil after 3 weeks incubation. There were no significant changes in the ergosterol in the unamended soils (ANOVA, $P > 0.05$).

Fig. 5(a) shows the gas chromatogram of a pure ergosterol standard following derivatisation with TMS. The ergosterol peak (30.3 min) was analysed by SIM and the spectrum compared with that of published spectra (Axelsson et al., 1995; Nielsen and Madsen, 2000). These analyses revealed a fragmentation pattern characteristic of ergosterol with a molecular ion at *m/z* 468 and abundant ions at *m/z* 337 and *m/z* 363 (Fig. 5b). These ions were due to the loss of the TMS group and the C_1–C_3 fragment and the TMS group and one methyl group, respectively. Analysis of the soil extracts (Fig. 6) following TMS derivatisation and GCMS showed that over the incubation period the relative atomic mass of these peaks increases by 1, 2 and 3 amu indicating replacement of ^{12}C-carbon with ^{13}C-carbon derived from the plant residue. The control soil (no ^{13}C amendment) showed no enrichment for the heavier carbon isotope (Fig. 7).

4. Discussion

The work done here shows that in these Antarctic soils, biological activity (low basal CO_2, low fungal biomass) is low. However, on addition of plant material, CO_2 evolution increased from 1×10^{-6} to over 9×10^{-5} mol CO_2 accumulated over the 42 days (Fig. 1). This increase in CO_2 evolved from the amended soils exceeded the amount of C added as plant residue and demonstrates that addition of plant residues has a marked priming effect (Kuzyakov et al., 1997, 1999; Kuzyakov and Cheng, 2001; Leifeld et al.,

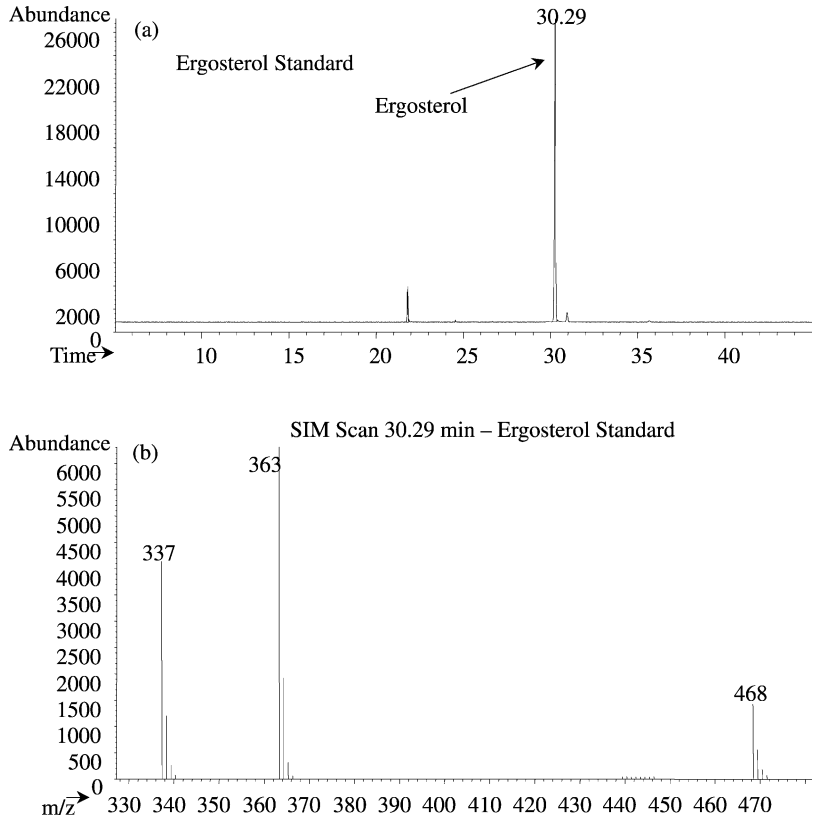

Fig. 5. (a) Chromatogram showing ergosterol retention time and (b) SIM spectrum of trimethylsilyl derivative of pure ergosterol showing fragmentation pattern of the ergosterol molecule.

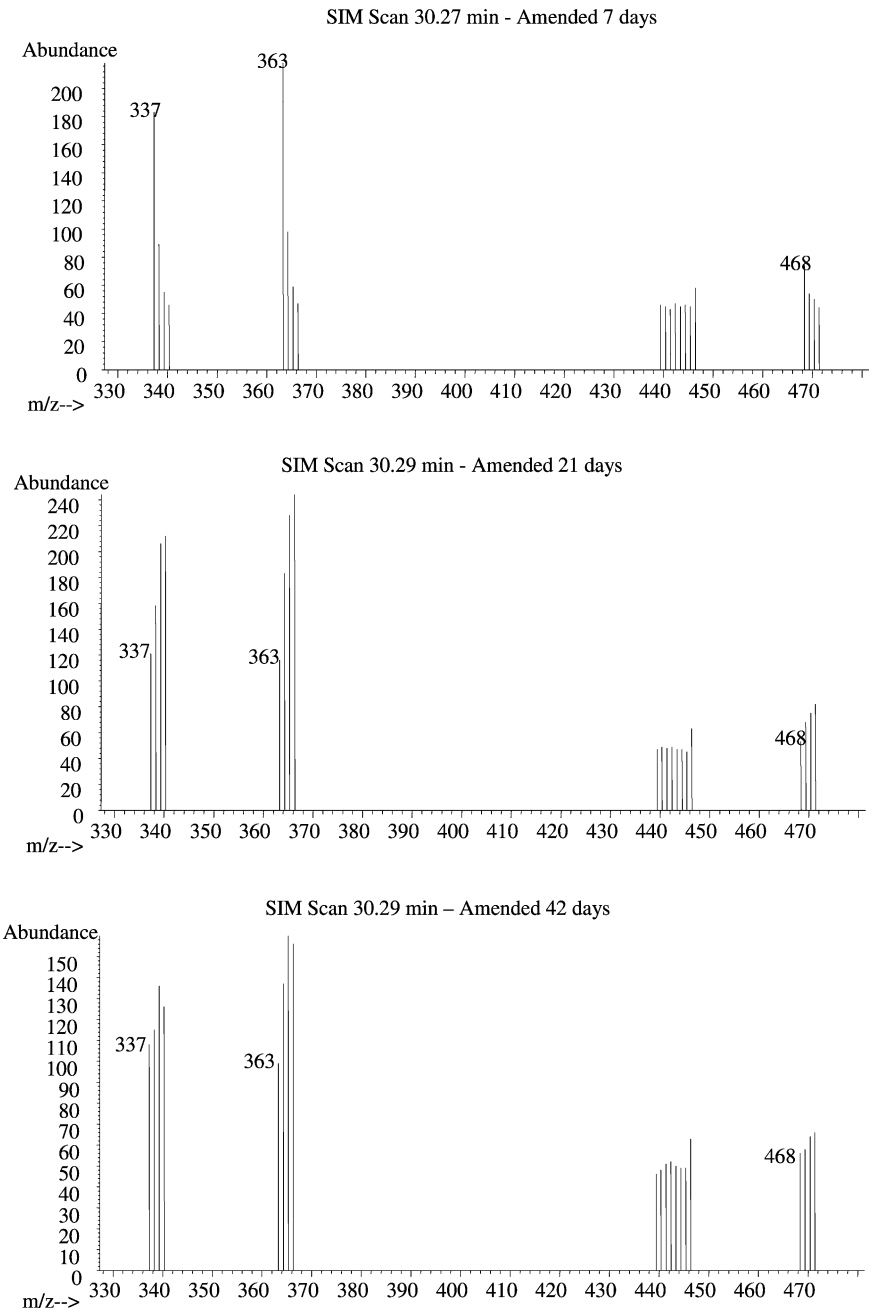

Fig. 6. SIM spectra of trimethylsilyl derivatives of ergosterol form Antarctic soil samples incubated with ^{13}C labelled plant material at 7, 21 and 42 days of incubation showing ^{13}C enrichment of the ergosterol molecule over time.

2002; Luna-Guido et al., 2003) resulting in enhanced mineralization of the native soil organic matter. The amount of ^{13}C-CO_2 (data not shown) was, on average, 1.2 and 7% of the total CO_2 evolved from the control and amended soils, respectively, during the first 4 weeks of incubation. The additional ^{13}C in the CO_2 evolved from the amended soil suggests complete mineralization of at least some components of the labelled plant material. Although one needs to exercise caution in interpreting the extent of the priming effect from the difference between amended and unamended soils, any errors are expected to be small because of the low C content of these soils. Tate (1987) concluded that priming effects in soils are probably limited to special environmental conditions or to certain types of laboratory studies. Whilst microcosm effects cannot be entirely ruled out here, it is possible that such priming effects are characteristic of soils from extreme environments where restrictions imposed by nutrient limitation or low diversity and biomass of decomposer microorganisms are alleviated by the addition of plant residues. Thus, on addition of plant-C to these soils, there is a rapid increase in respiration (CO_2 levels increase to 90 times those of the control after 1 day) suggesting that the decomposer community is adapted to periodic inputs of organic C and (probably) to water that is largely unavailable

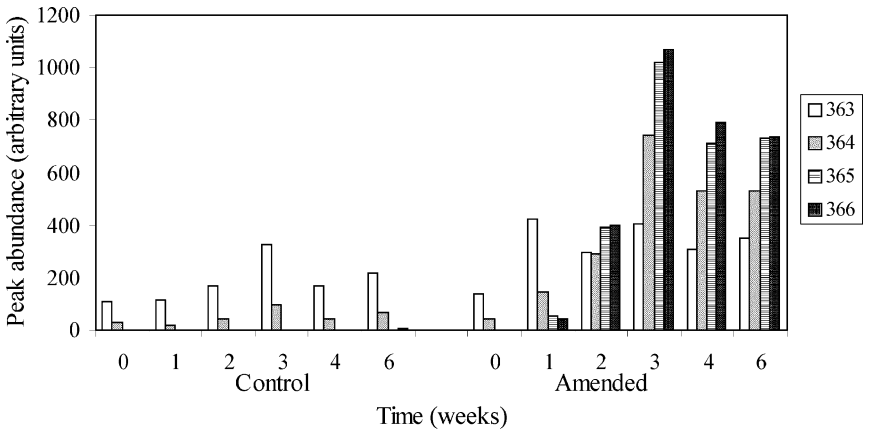

Fig. 7. Changes in the average quantity ($n = 3$) of ^{13}C incorporated to the main fragmentation ion of the ergosterol molecule (m/z 363) in the control and plant amended microcosms over 6 weeks of incubation of Fossil Bluff soil.

most of the year in much of Antarctica (Kennedy, 1993; McRae and Seppelt, 1999). De Nobili et al. (2001) have proposed that at least some microorganisms respond to sporadic substrate additions by reacting rapidly to small quantities of 'trigger' molecules. Low carbon soils receiving limited inputs, such as these Antarctic soils provide an ideal opportunity to test this hypothesis under extreme conditions. Since the experiment was destructively sampled it was not possible to extend the sampling beyond the planned 42 day period. However, as seen in Fig. 1 the respiration rate in the amended soil at 42 days is accelerating and any subsequent studies should be designed to investigate the decomposition process and associated microbial community dynamics over an extended period.

Phospholipid fatty acids (PLFA) are used to indicate the microbial biomass since they are present in all microbial cells and, as discussed by Tunlid and White (1992), are rapidly turned over. Since the demand for phosphorus is high in most soils, as soon as the cells die the phosphate group in phospholipids is mineralised, probably by phosphatase enzymes (Olander and Vitousek, 2000). It is expected that while the phospholipid fraction of the total lipids would give an estimate of the live (active) biomass, the neutral lipid fraction, produced by the substitution of the phosphate group of the glycerol backbone for a hydroxyl, would indicate the recently dead biomass. Thus, a comparison of these two fatty acid fractions provides information on changes in microbial structure and the turnover of the microbial biomass. However, like all biomarker approaches, the results need to be interpreted cautiously since NLFA can occur as energy storage compounds in mycorrhizal fungi (Olsson and Wilhelmsson, 2000). To determine whether mycorrhizae were important in these systems we checked for the mycorrhizal marker 16:1ω5 (Olsson et al., 1998; Madan et al., 2002) in both the PLFA and NLFA profiles. Significant amounts of 16:1ω5 were absent from both fractions suggesting, quite reasonably, that mycorrhizal fungi are not significant components of the microbial community in this soil. This, together with the absence of plant and animal tissues where NLFA are also found as storage polymers (Christie, 1982) and the finding that NLFA levels are generally low in bacteria (Niedhardt et al., 1990) support our contention that the NLFA patterns reflect recently turned over biomass and that like the PLFA this shows no systematic change over the 42-day incubation period.

The amounts of total NLFA (14–22.6 nmol g^{-1} soil) were significantly higher than those of PLFA (2.6–10.3 nmol g^{-1} soil), indicating a high demand for P and rapid turnover of phospholipid-P (Tunlid and White, 1992). Comparing the values of total PLFA found in the Fossil Bluff soil used in this experiment with those found by other researchers in temperate and in tropical soils showed the Antarctic values for total PLFA to be considerably lower. Zelles et al. (1994) reported that the PLFA content of a German agricultural soil ranged between 42 and 163 nmol g^{-1} and Zeller et al. (2001) have reported a lower value (23.3 nmol g^{-1}) for European grassland soil. These values for temperate soils are markedly lower than those recorded for a tropical soil (rice paddy) where total PLFA contents of 192 nmol g^{-1}soil were found (Zelles, 1999). The low values found in the Antarctic soil are consistent with a low prokaryote biomass.

In this study we did not rely solely on the FAME data to assess changes in community structure. Ergosterol was also used to estimate fungal biomass (Gessner and Chauvet, 1993; Frostegård and Bååth, 1996; Newell et al., 2000) since it is generally regarded as providing a good index of fungal growth (Seitz et al., 1977; Nylund and Wallander, 1992) and has been shown to correlate well with the amount of 18:2ω6 (Frostegård and Bååth, 1996). The increase in ergosterol concentration in the amended soils shows that the fungal community responds promptly to the incorporation of plant residues with a marked increase in ergosterol content after only 7 days (from undetectable to 0.26 μg g^{-1} soil) increasing to a maximum 3 weeks after amendment (1.56 μg g^{-1} soil). The GCMS studies and the enrichment in ^{13}C-carbon over time show clearly that this biomass

increase is a result of residue decomposition. Since an increase in ergosterol indicates fungal growth (fungal mycelium was visible in the amended soil) one might expect to see a corresponding increase in the relative amounts of fungal fatty acid markers such as 18:2ω6. However, this was not the case and there was neither a systematic change in the PLFA patterns over time (Fig. 2) nor any measurable increase in the amounts of the fungal fatty acid marker 18:2ω6. These results provide direct evidence of the danger of relying on only one marker such as PLFA to monitor changes in microbial communities. Other workers have also expressed concerns over the use of 18:2ω6 in this way based on the fact that different fungal taxa contain different amounts of 18:2ω6 (Stahl and Klug, 1996; Drijber et al., 2000). Similar results to those reported here are provided by Olsson et al. (1998) who investigated the use of specific fatty acids to monitor mycorrhizal growth in calcareous sand dunes and also by Zeller et al. (2001) in studies of European grasslands where PLFA failed to detect management effects even though they were evident from changes in ergosterol and microbial biomass C. This lack of congruence between the different biomarkers may indicate differences in community structure and differences in 18:2ω6 and ergosterol content between species. It may also reflect the fact that under certain environmental conditions fungi may not synthesise 18:2ω6.

This study shows the direct incorporation of ^{13}C into ergosterol from labelled plant material showing that the plant is being degraded with the products of decomposition immobilised in the fungal biomass. This and studies with other biomarkers (Arao, 1999) should enable more detailed analysis of C turnover in soils and in particular the role and succession of different microbial components (e.g. bacteria, fungi, actinomycetes) in decomposition processes in soils. This could help refine models of C turnover and enhance our understanding of microbial community structure and nutrient flux in soil. Sun (2000) has recently shown the advantages of this approach and has used fatty acids to investigate the transfer of ^{13}C from labelled microalgae to the microbial community of marine sediments. Zhang (2002) also sees the incorporation of ^{13}C-labelled substrates into microbial biomarkers as providing exciting new insights into biogeochemical cycles and to the role of specific microbial components in the movement and transformation of substrates. To achieve the potential offered by these approaches, however, requires more studies and the investigation of other biomarkers and labels.

5. Conclusions

This paper shows that biomarkers can be used to investigate the incorporation of ^{13}C-labeled plant material into the microbial biomass. Respiration measurements showed that the addition of these plant residues has a major impact on CO_2 evolution and that the amount of CO_2 evolved exceeds that added in the plant indicating that the biomass response to residues has a pronounced priming effect in these Antarctic soils. Changes in PLFA profiles and in particular the increase in the 18:2ω6 did not correlate with increases in ergosterol and failed to detect any change in community structure during decomposition despite a marked increase in ergosterol. This shows the need to exercise caution when interpreting lipid biomarker data and also emphasises the need for a polyphasic approach to microbial community analysis where no single technique should be relied upon without corroborating information from other methods.

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