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APPLICATION OF COMPOUND-SPECIFIC ^{13}C-IRMS TO TRACE THE FATE OF PLANT-DERIVED C IN SOIL MICROBIAL COMMUNITIES

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INTRODUCTION: Phospholipid fatty acids (PLFAs) of microbial membranes can be used to quantify soil microbial abundance and community diversity. PLFAs form a relatively constant proportion of cellular biomass, are rapidly degraded on cell-death and are characteristic of specific microbial groups. In combination with isotopic labelling, ^{13}C-enrichment of individual PLFAs (determined by GC-C-IRMS) allows C-fluxes through components of the soil microbial biomass to be determined. Often in previous studies investigating the fate of substrate additions to soil, the amount of labelled substrate added was itself sufficient to cause change in the soil microbial community. In this study we followed the fate of soil additions and assessed the disturbance to the pre-existing soil microbial community structure.

MATERIALS AND METHODS

A mix of *Agrostis capillaris* and *Festuca rubra* (ratio 2:1) was grown in soil within pots containing two isolated plant-free soil cores (Figure 1). Mesh windows in the wall of the cores allowed access of either a) both roots and mycorrhizal hyphae b) the mycorrhizal hyphae alone or c) neither roots nor mycorrhizal hyphae.

Three differing carbon substrates were added to soil cores, all were derived from the shoot material of *Lolium perenne* plants ^{13}C-labelled to approximately 60 atom %. They comprised of either the whole tissue (freeze dried and milled) or the soluble and insoluble fractions of a hot water extract of the tissue. In each case an equivalent of just 5.25 mg C was added to each soil core.

Seven days after the ^{13}C-additions the soil cores were harvested. For treatments which allowed root access into the core, rhizosphere soil (adhering tightly to roots) was separated from the bulk soil. PLFAs were extracted from the soil and converted to methyl esters prior to analysis. Total concentrations of the PLFAs were determined by GC using an Agilent Technologies 6890N Network GC system. The ^{13}C/^{12}C isotope ratios of the individual PLFAs was determined by GC-C-MS using a Trace GC Ultra coupled via a GC Combustion Interface III to a Finnigan DELTA^{plus} XP.

Figure 1: A mix of Agrostis capillaris and Festuca rubra growing within a pot containing two plant free soil cores. To the right of the pot is a core illustrating the mesh windows.

Principal component analysis (PCA) of PLFA relative concentration and ^{13}C-distribution within the PLFA fractions were determined to assess treatment impact upon microbial community structure and fate of substrate-derived C.

RESULTS

The PCA plot of the relative PLFA concentration indicated that rhizosphere soil had a different microbial community compared with bulk soil. However, the data spread indicates for both rhizosphere and bulk soil the substrate additions had no effect on the microbial community present in the cores at harvest (Figure 2). In contrast the PCA plot of the relative concentration of the ^{13}C-labelled PLFAs separated into clusters based on the type of carbon substrate added (Figure 3), indicating that distinct groups within the total microbial community utilised each substrate type.

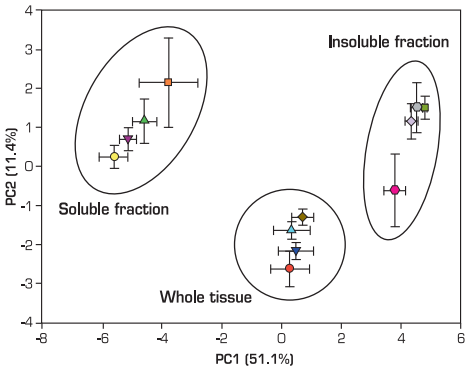

Figure 2: Principal component analysis of the relative concentration (mol% abundance) of the PLFA compounds in the rhizosphere and bulk soils (determined by GC) seven days after substrate addition. The principal component scores are plotted for individual replicates.

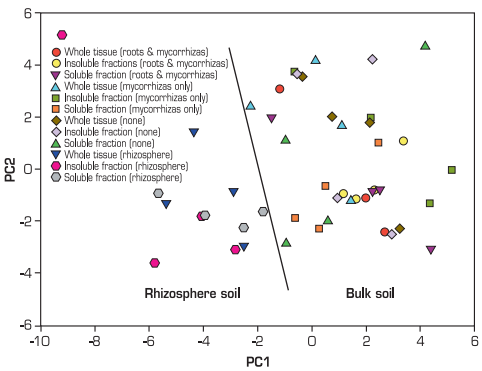

Figure 3: Principal component analysis of the relative concentration of ^{13}C within the PLFA profile (determined by GC-C-MS) seven days after substrate addition. Results are the mean of four replicates ± S.E.M. Symbols are as in Figure 2.

CONCLUSIONS: With tracer additions of ^{13}C-enriched plant fractions, GC-C-IRMS of soil PLFAs allowed identification of distinct microbial communities responsible for assimilation of contrasting substrate types. In contrast to previous studies, the tracer rates of addition did not significantly affect microbial community structure. Therefore, the results are representative of native C-transfers in soil, as opposed to also reflecting microbial growth in response to unrealistically high substrate additions.

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