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Carbon routes from decomposing plant residues and living roots into soil food webs assessed with ^{13}C labelling

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ABSTRACT

This field experiment investigated how C from fresh organic amendments and from a growing leek crop was allocated into different soil microbial and faunal groups in an arable field. A ^{13}C-enriched red clover green manure was incorporated in one treatment, while the growing leek crop was pulse labelled with $^{13}CO_2$ in another. Incorporation of ^{13}C into microbial fatty acids, micro- and macroarthropods, enchytraeids and earthworms was determined on several occasions during the growing season in order to determine whether different groups or species of microorganisms and fauna were specialised on either the decomposing green manure material or root-derived C. Compound-specific stable isotope ratio analysis showed fatty acid markers of actinomycetes and Gram-positive bacteria to be more strongly linked to C originating from the decomposing green manure material, whereas the marker for arbuscular mycorrhizal fungi was more linked to C from the growing leek crop. In contrast, several markers for Gram-negative bacteria were the most ^{13}C-enriched and had incorporated more ^{13}C than the other phospholipid fatty acids in both treatments, indicating a general dominance irrespective of C source. Most soil fauna seemed to derive their C directly or indirectly from the decomposing plant material, while C from the growing crop appeared to be of secondary importance in this agroecosystem.

1. Introduction

Primary plant production is the basis for belowground food webs and supplies soil organisms with energy and nutrients. The rate of transfer may not only be dependent on the plant community, as there are feedback mechanisms between the above- and belowground communities that also determine plant community development (Bever et al., 1997; Wardle, 2002). Thus, decomposer soil organisms that feed on detritus affect plant communities through nutrient supply, whereas herbivores, pathogens and symbionts affect plant communities directly through their interaction with living plant roots (Wardle et al., 2004). However, the multitude of ways by which soil organism communities affect plant growth and C allocation belowground are poorly understood. These two different ways for soil organisms to directly or indirectly link to plant carbon (C) are crucial in understanding the feedback between above- and belowground organism communities.

Plant community composition greatly influences the community composition of organisms in the rhizosphere, which is characterised by close, mutualistic interactions between plants and organisms in the root zone and a high degree of species specificity, whereas organisms in decomposer communities are considered to be food generalists to a higher degree (Scheu and Setälä, 2002). However, plants interact only with a subset of the large species pool in soils. Bacterial community composition and symbiotic fungi have been found to be strongly influenced by plants, whereas saprophytic fungi are not influenced by growing plants but by dead organic matter (Klamer and Hedlund, 2004; Singh et al., 2006).

In organic crop production, leguminous green manure crops are commonly used as nitrogen (N) and C sources, especially on farms without access to farmyard manure, and also function as a base for improving soil structure and increasing the biodiversity of food webs. Thus, green manuring can increase populations of soil microbes (Manici et al., 2004), microarthropods (Aagard-Axelsson and Thorup-Kristensen, 2000) and earthworms (Schmidt et al., 2003). Both the green manure and the growing crop can also have a selective influence on soil organism community composition (Schutter and Dick, 2001; Kowalchuk et al., 2002; Viketoft et al., 2005; Wardle et al., 2006).

Studies of C allocation from different organic sources into specific organisms are now possible through the combination of molecular methods and biomarkers for identification of soil

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organisms with radioactive or stable isotope techniques. Addition of a substrate with a distinct isotopic signature can be traced as an isotopic fingerprint in newly synthesised microbial and faunal compounds. This approach has recently been utilised in studies on microbial communities in soil ecosystems, examining incorporation of labelled substrates of varying complexity (Phillips et al., 2002; Waldrop and Firestone, 2004; McMahon et al., 2005; Williams et al., 2006, 2007) or root-derived C (Gavito and Olsson, 2003; Butler et al., 2003; Treonis et al., 2004) into microbial fatty acids. Trophic structures of soil faunal communities have been identified based on differences in natural abundance of ^{15}N (Schneider et al., 2004; Chahartaghi et al., 2005; Albers et al., 2006) and C flow in soil animal food webs has been traced and quantified using ^{13}C (Albers et al., 2006; Ostle et al., 2007; Pollierer et al., 2007).

The objective of this study was to explore the utilisation of C from fresh red clover used as green manure and from root-derived C from a growing leek crop (rhizodeposition, root grazing and arbuscular mycorrhizal (AM) fungal symbiosis) by different soil microbial and faunal groups in an arable field. Our hypothesis was that the green manure and leek crop support different organism communities, i.e. that different groups and species of microorganisms and fauna can, to various degrees, be linked to either the green manure material (decomposer food chain) or the growing leek crop (grazer food chain). In particular, we wanted to determine whether different microbial taxonomic groups, determined by phospholipid, PLFA, and neutral lipid, NLFA, fatty acids, and different soil fauna groups (microarthropods, enchytraeids and earthworms) used different sources of C.

2. Materials and methods

2.1. Field experiment: site description and layout

The field experiment was established in an agricultural field at the Krusenberg estate outside Uppsala, Sweden (59°N, 17°E, 5 m above sea level) in 2004. The soil at the site was classified as a silty clay loam consisting of 34% clay and 66% silt (Cambisol according to the FAO soil classification). The organic matter content was 2.1% and the pH (H_2O) 6.1 at the start of the experiment. The soil had a total C content of 14.1 g kg^{-1} soil, total N content of 1.3 g kg^{-1} soil and total S content of 220 mg kg^{-1} soil. The plant-available P content was 20 mg kg^{-1} soil (extracted with an ammonium lactate/acetic acid solution as described by Egnér et al., 1960) and the HCl-extractable P content 560 mg kg^{-1} soil. Barley (*Hordeum vulgare* L. cv Cecilia) was grown the year before the experiment. The monthly mean air temperature ranged from 11 °C in May to 17 °C in August and the cumulative precipitation during the cropping season was 229 mm. The experiment was irrigated at 25 mm deficit and the total irrigation during the cropping season was 85 mm.

All treatments were planted with leek and amended with green manure. Since legumes and leek have similar C isotopic signatures, a tracer was needed to be able to differentiate between the two C sources. This was done by incorporation of ^{13}C-enriched red clover green manure and by pulse labelling of the growing leek crop with $^{13}CO_2$ in two separate treatments. The treatments were:

(A) ^{13}C-labelled red clover as green manure and unlabelled leek;
(B) unlabelled green manure and ^{13}C-labelled leek; and
(C) control with unlabelled green manure and leek.

The treatments were randomly replicated in four blocks. The treatment plots measured 84 cm × 84 cm and were placed at 1 m distance from each other.

The red clover green manure was incorporated into the soil by spade on 3 June. In each plot, 1.9 kg fresh weight (FW) shoots, 0.5 kg FW stubble and 1.8 kg FW roots (with adhering soil) were incorporated, corresponding to in total 0.5 kg dry weight (DW) green manure material. The plots were cultivated with a rotary cultivator on 13 June, to mix the clover with the soil more homogeneously. The experiment was planted with leek (*Allium porrum* L. cv. Hilari), on 21 June. In each plot, 24 plants were planted in three rows with an inter-row spacing of 25 cm and an intra-row spacing of 10 cm. The experiment was kept free of weeds by hand-weeding.

2.2. ^{13}C labelling of green manure

To prepare green manure, red clover (*Trifolium pratense* L. cv. Vivi) was sown in large pots (84 cm × 84 cm) of soil in a greenhouse on 9 February 2004 and harvested before flowering at a plant age of 4 months. The red clover was fertilised with a nutrient solution (Wallco, Cederroth International AB, Sweden), containing 51 mg N, 10 mg P and 43 mg K per litre plus micronutrients, starting 1 month after sowing. Of 14 pots sown, six were labelled with ^{13}C. The labelling was initiated 2 months after sowing. During labelling, an acrylic chamber with the same base dimensions as the pots was placed on the pots and sealed in place with duct tape. An electric fan mixed the air in the chamber to keep the mixture of $^{12}C/^{13}C$ homogeneous and the CO_2 concentration in the chamber was monitored using an infrared gas analyser (IRGA). For the first 3 weeks, the labelling was conducted twice a week, each labelling period consisting of four CO_2 pulses lasting for in total 2–3 h. To avoid high temperatures inside the chamber, the labelling procedure was changed after 3 weeks so that each pot was labelled for a shorter period, two pulses per labelling period, each pulse lasting approximately 30–40 min, but four times a week. At each pulse, 80 ml of CO_2 enriched to 99% with ^{13}C (Larodan, Sweden) were injected with a syringe through a rubber septum when the CO_2 concentration had declined to approximately 100–150 ppm in the chamber. The 80-ml injection raised the CO_2 concentration in the chamber up to approximately 700 ppm. The labelling procedure was continued for 2 months before the red clover was harvested. Four days after the last ^{13}C pulse, the shoots were cut approximately 5 cm above the soil surface. Thereafter the stubble was cut as close to the soil surface as possible. The roots were removed from the pots, and shaken to remove most of the adhering soil. The three fractions shoots, stubble and roots were weighed and mixed separately and thereafter stored in plastic bags at +2 °C until incorporation into the field 1 day later. Duplicate samples of all three fractions were taken for isotopic analysis. The samples were freeze-dried and ball-milled prior to isotopic analysis.

2.3. ^{13}C labelling and sampling of leek

During the growing season, the leek was pulse labelled for 5 days in a row 4 (13–17 July), 7 (3–7 August) and 10 (25–29 August) weeks after planting. The labelling procedure was similar to that in the greenhouse. A 20 cm high metal frame, with the same base dimensions as the acrylic chamber, was pressed into the soil. The acrylic chamber was placed on top and sealed to the metal frame with duct tape. The CO_2 concentration inside the chamber was monitored with a photoacoustic gas analyser (INNOVA AirTech Instruments, Denmark). When the initial CO_2 concentration declined to 100–150 ppm in the chamber, 80 ml of $^{13}CO_2$ were injected. This procedure was repeated twice each day during the first 2 weeks and three times a day during the third week. Leek crop samples were taken after each labelling period (approximately 48 h after the last pulse), i.e. on 19 July, 9 August, 31 August, plus before harvest on 29 September. In each plot, one old leaf (the oldest leaf that had not withered) from a total of three plants and one young leaf (the next youngest) from another three plants were sampled. Close to these six plants, soil cores were taken to a depth of 15 cm for