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Biological $^{12}C-^{13}C$ fractionation increases with increasing community-complexity in soil microcosms

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ABSTRACT

Isotope fractionation is a ubiquitous phenomenon in natural ecosystems. When chemical elements move through food chains, natural isotope ratios change because biological processes tend to discriminate against heavier isotopes. This effect can be used to trace flows of matter, estimate process-rates and determine the trophic level of organisms in biological systems. While it is widely accepted that 15N-accumulates in natural food-chains, it is disputed to which extent this is the case for ^{13}C. We constructed sand-microcosms inoculated with a dilution series of soil organisms and amended with glucose as the source of organic carbon. We demonstrated that the proportion of ^{13}C in respiratory CO_2 correlated inversely with community complexity. Our results therefore suggest that increasing community complexity, with increasing synergy, competition and predation, facilitates increasing $^{12}C-^{13}C$ isotopic fractionation.

1. Introduction

In most chemical and biochemical reactions different isotopes of the same element will essentially be functionally equivalent. Some biological processes, however, discriminate slightly against heavier isotopes (Peterson and Fry, 1987). This causes small differences in the relative occurrence of natural isotopes between different ecosystem components, which has provided essential information about mass flow pathways in ecosystems (Dittert et al., 1998; Bengtson and Bengtsson, 2007; Cabral et al., 2010). Within plants ^{13}C discrimination during photosynthesis leads to significantly different isotopic signatures in similar components of C_3 and C_4 plants (Hobbie and Werner, 2004). This has been utilized to trace carbon sources in many ecological studies, for example in relation to vegetation change from C_3 to C_4 plants or by amendment of C_4 plant material to C_3 soils in laboratory experiments (Werth and Kuzyakov, 2010). Biological fractionation of ^{15}N and ^{14}N is high compared to the fractionation of ^{13}C and ^{12}C (Peterson and Fry, 1987). This is the basis for using ^{15}N enrichment through trophic transfer as a method to study trophic structure. This method has been applied on single species (Scheu, 2002; Illig et al., 2005; Oelbermann and Scheu, 2010), on whole communities (Layman et al., 2007) and recently also on soil microfauna (Darby and Neher, 2012).

There is an ongoing debate among ecologists about the extent of carbon isotope discrimination in soil food webs. Ehleringer et al. (2000) asserted that progressive $\delta^{13}C$ increase of soil organic matter (SOM) was related to a gradual shift in the relative contributions of microbial vs. plant components in the residual SOM, rather than to microbial discrimination during decomposition. Along this line, some researchers claim that ^{13}C discrimination is negligible during microbial respiration (DeNiro and Epstein, 1978; Lin and Ehleringer, 1997). Others have suggested that there may be a considerable $^{13}C-^{12}C$ fractionation during microbial decomposition and respiration (Blair et al., 1985; Mary et al., 1992; Andresen et al., 2011). In a comprehensive literature review, Werth and Kuzyakov (2010) reported that the difference between $\delta^{13}C$-values of the respired CO_2 and the microbial biomass varied between +4.3‰ (^{13}C enrichment)

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to −3.2‰ (^{13}C depletion) for C_3 soils whereas the respired CO_2 was more depleted, up to −5.7‰ for C_4 soils. Hence, the extent of microbial discrimination during decomposition in soils is not clear, but Lerch et al. (2011) stress that discrimination during microbial processing, even if small, may be responsible for significant shifts in the stable isotope composition of the soil organic matter over long time periods.

Experimental investigations of isotope discrimination in soil pose special challenges. Detection of small changes in isotope composition of CO_2 released from material added to soil is difficult. This is because CO_2 derived from decomposition of the huge pool of native soil carbon will overshadow the detection of small changes in the isotopic composition of CO_2 derived from a freshly amended resource. Thus, experimental conditions in which the resource is decomposed in an essentially carbon free soil matrix are warranted.

In this study, we used a simple microcosm approach in systems with very low levels of native soil carbon. We investigated how the $\delta^{13}C$ ratio in respired CO_2 derived from glucose with a known $\delta^{13}C$-value varied with different microbial food web complexity. We constructed microcosms with a series of decreasing species-richness, and thus biological complexity, by inoculation of sterilized sand with a serially diluted soil suspension. The rationale behind this approach is firstly that microcosms inoculated with increasingly more dilute soil-suspensions will contain increasingly fewer microbial species ending with zero in the highest dilutions. Franklin et al. (2001) and Wertz et al. (2006) used similar dilution approaches that demonstrated that colony morphology and DGGE-based species-richness decreased with dilution. Secondly, we reckon that in more taxon-rich systems there will be more, and increasingly complex, interactions between organisms. Hence carbon will be cycled more times and therefore sequentially more depleted in ^{12}C due to respiratory discrimination. Thus, if organisms preferentially respire ^{12}C (and thus preferentially retain ^{13}C in the produced biomass), then more complex communities with several trophic levels will retain more ^{13}C. This will take place when protozoan predators consume bacteria, and when larger protozoa consume primary grazers, but also through interactions between bacteria with different trophic demands. Thus, we hypothesize that in systems with higher complexity proportionally less ^{13}C will be produced through respiration due to trophic recycling, and hence repeated retention of ^{13}C, of carbon from the lower levels of the food chain.

2. Material and methods

We established a gradient of soil microbial complexity using serial dilutions of a soil slurry, which we obtained by suspending 100 g unsterile pasture soil (Löddeköpinge, Sweden) in 1 l sterile water. The suspension was shaken for 2 h, and allowed subsequently to settle for 1 h. From the supernatant we then prepared a 14-step repeated 10-fold dilution-series. Each of the 14 serial dilutions was then transferred to a sterilized soil–sand mixture (one part Löddeköpinge soil, two parts sand) ending up with 10^{-1} to 10^{-14} g of non-sterile soil per gram sterile soil–sand mixture. We incubated these soil–sand mixtures under sterile conditions in darkness for 8 months, at 15 °C. We used soil from these 14 incubations to inoculate the present experiment. The long pre-incubation period (8 months) ensured that the microbial biomass in the soil had stabilized and hence there were not any systematic differences in the amount of organisms added to our treatments with different species-richness and complexity.

From each of the 14 stock incubations with different species-richness, we prepared five microcosms, i.e. we set up a total of 70 microcosms. Each microcosm consisted of a 117 ml serum bottle containing 10.0 g dry quartz sand (sand, 0.3–1 mm, treated to remove organic carbon in a muffle furnace (550 °C for 4 h)). The bottles were autoclaved (120 °C, 20 min) to eliminate any indigenous organisms. We then supplied each microcosm with 1.5 ml sterilized liquid that contained a nutrient solution (1.5 mg glucose, ($C_6H_{12}O_6 \times H_2O$), 6.8 mg KH_2PO_4, 24.7 mg $MgSO_4$, 2.9 mg NaCl, 3.93 mg $CuSO_4 \times 5H_2O$, 0.75 mg MoO_3, 0.14 mg H_3BO_3, 0.077 mg $MnSO_4 \times H_2O$, 0.66 mg Fe–Na–EDTA, 25 mg KNO_3, 59 mg $Ca(NO_3)_2 \times 4H_2O$ in 1.0 l of distilled water). The result of this procedure was that we added 1.5 mg glucose, corresponding to 0.6 mg carbon, to each microcosm resulting in a concentration of 0.06 mg C g^{-1} sand.

We then prepared suspensions of the stock soil samples prepared from serial dilutions as described above. From each of the 14 dilution treatments, we prepared a suspension by mixing 2.0 g of soil–sand mixture with 18 ml sterile phosphate buffer (Neff's Modified Amoeba Saline, Page, 1988). From each of these 14 suspensions, we then supplied five replicate sterile serum bottles with 0.5 ml of; i.e. we prepared a total of 70 microcosms. We then sealed the microcosms with sterile Teflon-coated rubber stoppers and incubated them in the dark, at 15 °C, for 68 days. During this incubation, the microcosms were regularly opened to avoid anaerobic conditions in the sand. Whenever the microcosms were opened they were flushed with CO_2-free air to eliminate the contamination from atmospheric CO_2, then immediately sealed again. This procedure was carried out in a laminar flow bench to minimize the risk of contamination.

After the 68-day incubation, we started the proper experiment by adding more glucose to the microcosms. We transferred 50 μl of a glucose solution (60 g l^{-1}) to each microcosm resulting in a concentration of 0.12 mg C g^{-1} of sand. The rationale behind this procedure, involving a two-step incubation, is that we minimize the importance of any original soil carbon added with the soil suspensions. Most of the easily degradable soil carbon added with the soil suspension at the beginning of the experiment will already be respired. Based on the respiratory activity in the 14 soil–sand mixtures used for inoculation, we calculated that the amount of degradable carbon transferred to our soil–sand microcosms during inoculation made up less than 1.1% of the glucose amendment. Respiration activity based on soil-C is therefore insignificant compared to respiration on glucose-C and by far most of the microbial biomass in the system will be formed by growth on the added glucose.

The proper experiment lasted for 43 days with destructive sampling on the last day. During the experimental period, respiration rate was calculated from regular measurements of CO_2. Headspace in the microcosms (0.5 ml) was analysed on a gas chromatograph with a thermal conductive detector and 1.8 m × 3 mm Poropak Q column operated at 35 °C. At three occasions, on day 3, 11, and 43, we transferred 5.0 ml headspace to 5.9 ml Exetainer vials for $^{13}C/^{12}C$ ratio analysis on Gas Chromatography Isotope Ratio Mass Spectrometer (GC–MS, Agilent GHG GC 7890A, Agilent Technologies, Hørsholm, Denmark) equipped with an ML Gas Auto-sampler (v. 3.0, Mikrolab Aarhus, Højbjerg, Denmark). At these three occasions, the microcosms were opened after sampling and flushed with CO_2-free air to ensure that the accumulated CO_2 in the samples represented the CO_2 that was evolved in the three periods (day 0–3, day 3–11, and day 11–45).

At the destructive sampling, at day 43, we quantified various organism-related parameters in the soil. Protozoa were enumerated in microtitre plates (Costar, No. 3598; Biotech Line ag., Slangerup, Denmark) by a modified version of the "most probable number method" using dilute Tryptic Soy Broth (0.1 g l^{-1}, Difco, BD, Brøndby, Denmark) as medium (Rønn et al., 1995). Microtitre plates were incubated at 15 °C in darkness, and individual wells were inspected for the presence or absence of protozoa after 1 and 3