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Highlights

► Protists where enriched by 6.8 atom% for ^{13}C and 16.4 atom% for ^{15}N. ► Assimilation rates were calculated to yield a net production efficiency of 36.8%. ► Protist respiration equated to an increase of 0.15 atom% excess $^{13}CO_2$ respired min^{-1}. ► Protist respiration amounted to an accumulation of 0.7 fg CO_2–C $protist^{-1}$ min^{-1}. ► First time amount of CO_2 respired was measured for soil protists to this accuracy.

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Short communication

Measuring soil protist respiration and ingestion rates using stable isotopes

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ABSTRACT

Protists have a direct effect on soil nutrient cycling due to their abundance, diversity and assimilation rates of bacterial biomass. Here for the first time stable isotopes have been utilised to quantify respiration and ingestion rates of soil protists. We show that microcosms can generate values for these variables within the instrument detection range. Through consumption of enriched bacteria, indigenous agricultural grassland soil protists obtained an enrichment of 6.8 atom% (±1.67) for ^{13}C and 16.4 atom% (±4.34) for ^{15}N. Bacteria were consumed at a rate of 41 (±0.04) bacteria h^{-1} protist^{-1} during the initial 24 h period of incubation. Protist respiration monitored over time equated to an increase of 0.15 atom% excess (±0.036) in $^{13}CO_2$ respired per minute and an accumulation of 0.7 fg (±0.36) CO_2–C protist^{-1} min^{-1}. These results provide numbers quantifying the assimilation efficiencies of soil protists, their effect on labile biomass turnover and the flow of C and N through the soil food web.

Microbial communities are the foundation of soil food webs, with nearly all biogeochemical transformations directly resulting from microbial activity (Konhauser, 2007). Soil protists are highly diverse and abundant (Foissner, 1999), with typical active abundances of 10^5–10^7 cells g^{-1} (Adl and Coleman, 2005). Protists have a direct effect on nutrient cycling as a large proportion of ingested food is excreted back into the soil environment (Fenchel, 1982). Protist grazing activity stimulates rates of C and N cycling in soil by releasing the nutrients immobilised in bacterial biomass, as part of the soil microbial loop, thus promoting plant growth (Bonkowski, 2004). Earlier data from field studies in a variety of cropping systems (Paustian et al., 1990) demonstrated that protist respiration is the largest, single component of total soil respiration by consumers and protists consume an amount equivalent to that of nematodes. Other data from grazing studies have indicated a strong influence of protist grazing on bacterial community structure through selective grazing (Adl, 2003; Rønn et al., 2002).

Studies using stable isotopes to assess protist grazing are common in aquatic environments (Frias-Lopez et al., 2009; Gonzalez, 1999; Moodley et al., 2000). The application of stable isotopes in soil zoology provides a glimpse *in situ* of biological interactions (Tiunov, 2007), and was recently reviewed by Crotty et al. (in press) for protist ecology. To date, investigations of the soil microbial food web have been unable to ascertain bacterivory by protists or their impact on labile biomass turnover (Lueders et al., 2006). Here, for the first time measurements were taken of soil protist respiration and grazing rates utilising stable isotopes to improve the accuracy of the data.

Soil was collected from a permanent grassland site (N 50′46′54.55019 W 3′55′1.03173) in South West England. The soil was of the Hallsworth series (Harrod and Hogan, 2008) and had undergone the same management treatments for the last 20 years (see Crotty et al., 2012). Protists were cultured by taking 1.5 g of soil added to 70 ml deionised water, and 15 ml lettuce leaf solution (Sonneborn, 1970) and incubated at 18 °C for three days. To produce a concentrated protist culture, the solution was centrifuged at 1600 *g* for 2 min and the supernatant discarded, leaving ~10 ml concentrated solution. To enrich the protist culture with stable isotopes, the protists were fed a 0.5 ml enriched *Pseudomonas lurida* (Behrendt et al., 2007) culture, prepared according to Murray et al. (2009). The bacterial inoculum had been grown with labelled glucose and ammonium chloride as the sole carbon (C) and nitrogen (N) sources, 2.5 g $^{13}C_6$ – glucose; 1 g ^{15}N–NH_4Cl (both 99 atom%, SerCon, Crewe, UK); to become 99 atom% enriched. *P. lurida* is resistant to the antibiotics ampicillin and rifampicin, and therefore retrievable after experimental introductions when grown on selective media. A sub-sample of the concentrated protist culture was used as a control.

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Protist numbers were enumerated at the beginning and end of the enrichment period using a haemocytometer (Adl et al., 2007; Adl and Coleman, 2005). To measure bacterial consumption over time, bacterial plate counts were taken at intervals of 24, 30 and 54 h, using the method of Clegg et al. (1995). At each time point, 10-fold serial dilutions were made in sterile ¼ Ringer's solution, before spread-plating onto King B agar containing ampicillin and rifampicin (50 μg ml^{-1}) and replicated in triplicate. Agar plates were incubated at 27 °C for three days before counting colonies. The level of protist enrichment was adapted from Crotty et al. (2011). The sample was washed via centrifugation before re-suspension in 1 ml deionised water. Sub-samples of 100 μl were analysed using a stable isotope mass spectrometer (20/20, PDZ-Europa, Crewe, UK). To evaluate the amount of $^{13}CO_2$ respired by the protists over time, 0.45 ml of the concentrated protist suspension was incubated in a 1.5 ml septum vial with a 0.05 ml addition of *P. lurida* at natural isotope abundance to prevent encystment of the concentrated protists. Respiration samples (1 ml) were taken at varying time intervals from the resulting headspace atmosphere using a gas syringe, collected and stored in evacuated 12 ml butyl septum-capped vials (Exetainer®, Labco Limited, High Wycombe, UK) (Knohl et al., 2004; Midwood et al., 2006) and overfilled with Helium, before analysis by mass spectrometry. After all respiration samples were taken, each Exetainer was analysed using a trace gas analyser (ANCA TGII, PDZ-Europa, Crewe, UK) linked to a stable isotope analyser mass spectrometer (20/20, PDZ-Europa, Crewe, UK) with a Gilson model 221 auto-sampler.

Protist numbers increased by 2.5 times over the 54 h incubation, from 5×10^5 ml^{-1} to 1.25×10^6 ml^{-1}. Protist consumption of bacteria averaged 41 (±0.04) bacteria h^{-1} $protist^{-1}$ during the initial 24 h period of incubation, providing an initial rate of consumption of 0.186% ^{13}C min^{-1}; this reduced to 1.2 (±0.01) bacteria h^{-1} $protist^{-1}$ after 30 h and 0.2 (±0.0003) bacteria h^{-1} $protist^{-1}$ after 54 h, as bacterial abundance decreased. After 54 h, the bacteria were reduced to 0.004% of the initial inoculum and the protist culture had started to encyst. The remaining bacteria at the end of the incubation contribute a negligible amount to the calculations especially since they were kept in the stationary phase of growth. The protists were found to have a mean enrichment (±standard error, $n = 5$) of 6.8 atom% (±1.67) for ^{13}C and 16.4 atom% (±4.34) for ^{15}N. This level of enrichment is significantly greater than natural abundance ($P = 0.019$ (^{13}C) and $P = 0.014$ (^{15}N)), and approximates the assimilation efficiency, using initial rates of reaction and food vacuole processing times (Berger and Pollock, 1981). This yields a net production efficiency of 36.8%. There was an increase in atom% excess within the headspace atmosphere as there was a continued accumulation of protist $^{13}CO_2$ respiration over time (Fig. 1 – bar), with an average 0.15 atom% excess (±0.036) increase in $^{13}CO_2$ respired min^{-1}. The μg CO_2–C accumulated in the headspace also increased over the time period (Fig. 1 – dot symbol), equating to an average of 2.4 μg (±1.15) CO_2–C g^{-1} dry weight of protists min^{-1}, or 0.7 fg (±0.36) CO_2–C $protist^{-1}$ min^{-1}. At the initial rate of consumption 5.2×10^{-3} g ^{13}C accumulated in the protists and 3.56×10^{-6} g C min^{-1} was respired. This is the first time the amount of CO_2 respired has been measured for soil protists with this level of accuracy. Loss of assimilated C through excretion could not be measured, limiting our ability to carry out further calculations on energy efficiency.

This is the first study to quantify the consumption efficiencies of a mixed culture of soil protists, and indicate their rate of soil C turnover. In this simplification of the soil food web, we have measured a high level of consumption of bacteria and the loss of ^{13}C label through protist respiration. These results provide empirical evidence for the movement of C through trophic levels, that could be used to provide more accurate models (e.g. Holtkamp et al. (2011) or Moore et al. (2005) where there is a ~4% difference in production efficiency). Employing this novel approach to quantify consumption by soil fauna will help unravel the complexity of interactions within this food web, thus contributing to our understanding of how each trophic level functions as an energy converter within the soil ecosystem. Further method development to understand whether respiration and ingestion rates are similar *in situ* could involve the utilisation of soil columns, implementing methods used in Adl (2007). We have tested successfully an approach using undisturbed soil cores inoculated with protists enriched with stabled isotopes to act as part of the food chain (Crotty et al., 2012). Future work should concentrate on building additional steps into the food chain and quantifying the effect different species (bacterial and protist) have on the assimilation efficiencies, supplemented by *in-situ* studies.

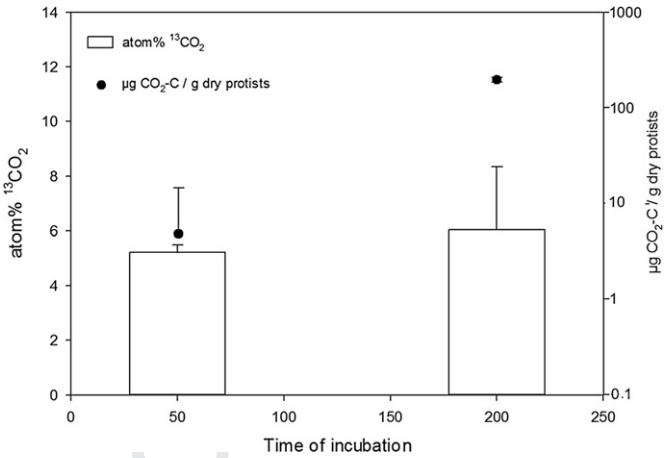

Fig. 1. Bar and dot graph showing difference in atom% excess and μg CO_2–C/g for protist respiration over time (min) ($n = 3$).

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