Aberystwyth University

Measuring soil protist respiration and ingestion rates using stable isotopes

Crotty, Felicity V.; Adl, Sina M.; Blackshaw, Rod P.; Murray, Philip J.

Published in:

Soil Biology and Biochemistry

DOI:

[10.1016/j.soilbio.2012.10.039](https://doi.org/10.1016/j.soilbio.2012.10.039)

Publication date:

2013

Citation for published version (APA):

Crotty, F. V., Adl, S. M., Blackshaw, R. P., & Murray, P. J. (2013). Measuring soil protist respiration and ingestion rates using stable isotopes. *Soil Biology and Biochemistry*, 57, 919-921.
<https://doi.org/10.1016/j.soilbio.2012.10.039>

General rights

Copyright and moral rights for the publications made accessible in the Aberystwyth Research Portal (the Institutional Repository) are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from the Aberystwyth Research Portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain
- You may freely distribute the URL identifying the publication in the Aberystwyth Research Portal

Take down policy

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

tel: +44 1970 62 2400
email: is@aber.ac.uk

AUTHOR QUERY FORM

ELSEVIER	Journal: SBB Article Number: 5342	Please e-mail or fax your responses and any corrections to: E-mail: corrections.esch@elsevier.tnq.co.in Fax: +91 44 24426088
--	--	--

Dear Author,

Please check your proof carefully and mark all corrections at the appropriate place in the proof (e.g., by using on-screen annotation in the PDF file) or compile them in a separate list. Note: if you opt to annotate the file with software other than Adobe Reader then please also highlight the appropriate place in the PDF file. To ensure fast publication of your paper please return your corrections within 48 hours.

For correction or revision of any artwork, please consult <http://www.elsevier.com/artworkinstructions>.

Any queries or remarks that have arisen during the processing of your manuscript are listed below and highlighted by flags in the proof.

Location in article	Query / Remark: Click on the Q link to find the query's location in text Please insert your reply or correction at the corresponding line in the proof
Q1	Please update reference "Crotty et al., in press".
Q2	Please confirm that given names and surnames have been identified correctly. <div style="border: 1px solid black; padding: 10px;"> Please check this box if you have no corrections to make to the PDF file <input data-bbox="794 1181 877 1266" type="checkbox"/> </div>

Thank you for your assistance.

Contents lists available at [SciVerse ScienceDirect](#)

Soil Biology & Biochemistry

journal homepage: www.elsevier.com/locate/soilbio

Highlights

► Protists were 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 $^{13}\text{CO}_2$ respired min^{-1} . ► Protist respiration amounted to an accumulation of 0.7 fg $\text{CO}_2\text{-C protist}^{-1} \text{ min}^{-1}$. ► First time amount of CO_2 respired was measured for soil protists to this accuracy.

Contents lists available at SciVerse ScienceDirect

Soil Biology & Biochemistry

journal homepage: www.elsevier.com/locate/soilbio

Short communication

Measuring soil protist respiration and ingestion rates using stable isotopes

Q2 **Felicity V. Crotty^{a,b,c,*}, Sina M. Adl^a, Rod P. Blackshaw^b, Philip J. Murray^c**^a Department of Soil Science, University of Saskatchewan, 51 Campus Drive, Saskatoon S7N 5A8, Canada^b Centre for Agricultural and Rural Sustainability, Plymouth University, Drake Circus, Plymouth PL4 8AA, UK^c Sustainable Soil and Grassland Systems, Rothamsted Research, North Wyke, Okehampton, Devon EX20 2SB, UK

ARTICLE INFO

Article history:

Received 28 August 2012

Received in revised form

29 October 2012

Accepted 30 October 2012

Available online xxx

Keywords:

Assimilation efficiencies

Microbial ecology

Protist

Soil respiration

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 $^{13}\text{CO}_2$ respired per minute and an accumulation of 0.7 fg (± 0.36) $\text{CO}_2\text{-}^{13}\text{C}$ 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.

© 2012 Elsevier Ltd. All rights reserved.

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 $10^5\text{--}10^7$ 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 $^{13}\text{C}_6\text{--glucose}$; 1 g $^{15}\text{N--NH}_4\text{Cl}$ (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.

* Corresponding author. Current address: Animal and Microbial Sciences, Institute of Biological, Environmental and Rural Sciences, Aberystwyth University, Gogerddan, Aberystwyth SY23 3EB, UK. Tel.: +44 (0) 1970 823061; fax: +44 (0) 1970 823245.

E-mail address: fec3@aber.ac.uk (F.V. Crotty).

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 ¹³CO₂ 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% ¹³C 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 ¹³C and 16.4 atom% (±4.34) for ¹⁵N. This level of enrichment is significantly greater than natural abundance ($P = 0.019$ (¹³C) and $P = 0.014$ (¹⁵N)), 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 ¹³CO₂ respiration over time (Fig. 1 – bar), with an average 0.15 atom% excess (±0.036) increase in ¹³CO₂ respired min⁻¹. The µg CO₂-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) CO₂-C g⁻¹ dry weight of protists min⁻¹, or 0.7 fg (±0.36) CO₂-C protist⁻¹ min⁻¹. At the initial rate of consumption 5.2×10^{-3} g ¹³C accumulated in the protists and 3.56×10^{-6} g C min⁻¹ was respired. This is the first time the amount of CO₂ 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 ¹³C 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.

Fig. 1. Bar and dot graph showing difference in atom% excess and µg CO₂-C/g dry protists for protist respiration over time (min) ($n = 3$).

(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 stable 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.

References

- Adl, S.M., 2003. The Ecology of Soil Decomposition. CABI Publishing, Wallingford, 335 pp.
- Adl, S.M., 2007. Motility and migration rate of protozoa in soil columns. *Soil Biology & Biochemistry* 39, 700–703.
- Adl, S.M., Coleman, D.C., 2005. Dynamics of soil protozoa using a direct count method. *Biology and Fertility of Soils* 42, 168–171.
- Adl, S.M., Acosta-Mercado, D., Anderson, R.T., Lynn, H.D., 2007. Protozoa. In: Carter, M.R., Gregorich, E.G. (Eds.), *Soil Sampling and Methods of Analysis*, second ed. CRC Press, Boca Raton, p. 1264.
- Behrendt, U., Ulrich, A., Schumann, P., Meyer, J.-M., Spröer, C., 2007. *Pseudomonas lurida* sp. nov., a fluorescent species associated with the phyllosphere of grasses. *International Journal of Systematic and Evolutionary Microbiology* 57, 979–985.
- Berger, J.D., Pollock, C., 1981. Kinetics of food vacuole accumulation and loss in *Paramecium tetraurelia*. *Transactions of the American Microscopical Society* 100, 120–133.
- Bonkowski, M., 2004. Protozoa and plant growth: the microbial loop in soil revisited. *New Phytologist* 162, 617–631.
- Clegg, C.D., Anderson, J.M., Lappin, H.M., Vanelsland, J.D., Jolly, J.M., 1995. Interaction of a genetically-modified *Pseudomonas fluorescens* with the soil-feeding earthworm *Octolasion Cyaneum* (Lumbricidae). *Soil Biology & Biochemistry* 27, 1423–1429.
- Crotty, F.V., Adl, S.M., Blackshaw, R.P., Murray, P.J. Review: using stable isotopes to differentiate trophic feeding channels within soil food webs. *Journal of Eukaryotic Microbiology*, in press.
- Crotty, F.V., Adl, S.M., Blackshaw, R.P., Murray, P.J., 2012. Protozoan pulses unveil their pivotal position within the soil food web. *Microbial Ecology* 63, 905–918.
- Crotty, F.V., Blackshaw, R.P., Murray, P.J., 2011. Tracking the flow of bacterially derived ¹³C and ¹⁵N through soil faunal feeding channels. *Rapid Communications in Mass Spectrometry* 25, 1503–1513.
- Fenchel, T., 1982. Ecology of heterotrophic microflagellates 2. Bioenergetics and growth. *Marine Ecology-progress Series* 8, 225–231.
- Foissner, W., 1999. Protist diversity: estimates of the near-imponderable. *Protist* 150, 363–368.
- Frias-Lopez, J., Thompson, A., Waldbauer, J., Chisholm, S.W., 2009. Use of stable isotope-labelled cells to identify active grazers of picocyanobacteria in ocean surface waters. *Environmental Microbiology* 11, 512–525.

- Gonzalez, J.M., 1999. Bacterivory rate estimates and fraction of active bacterivores in natural protist assemblages from aquatic systems. *Applied and Environmental Microbiology* 65, 1463–1469.
- Harrod, T.R., Hogan, D.V., 2008. The Soils of North Wyke and Rowden, Soil Survey of England and Wales. Rothamsted Research, Okehampton.
- Holtkamp, R., van der Wal, A., Kardol, P., van der Putten, W.H., de Ruiter, P.C., Dekker, S.C., 2011. Modelling C and N mineralisation in soil food webs during secondary succession on ex-arable land. *Soil Biology & Biochemistry* 43, 251–260.
- Knohl, A., Werner, R.A., Geilmann, H., Brand, W.A., 2004. Kel-F (TM) discs improve storage time of canopy air samples in 10-mL vials for CO₂-delta C-13 analysis. *Rapid Communications in Mass Spectrometry* 18, 1663–1665.
- Konhauser, K.O., 2007. Introduction to Geomicrobiology. Wiley-Blackwell, Hoboken, 440 pp.
- Lueders, T., Kindler, R., Miltner, A., Friedrich, M.W., Kaestner, M., 2006. Identification of bacterial micropredators distinctively active in a soil microbial food web. *Applied and Environmental Microbiology* 72, 5342–5348.
- Midwood, A.J., Gebbing, T., Wendler, R., Sommerkorn, M., Hunt, J.E., Millard, P., 2006. Collection and storage of CO₂ for C-13 analysis: an application to separate soil CO₂ efflux into root- and soil-derived components. *Rapid Communications in Mass Spectrometry* 20, 3379–3384.
- Moodley, L., Boschker, H.T.S., Middelburg, J.J., Pel, R., Herman, P.M.J., de Deckere, E., Heip, C.H.R., 2000. Ecological significance of benthic foraminifera: (13)C labeling experiments. *Marine Ecology-progress Series* 202, 289–295.
- Moore, J.C., McCann, K., de Ruiter, P.C., 2005. Modeling trophic pathways, nutrient cycling, and dynamic stability in soils. *Pedobiologia* 49, 499–510.
- Murray, P.J., Clegg, C.D., Crotty, F.V., de la Fuente Martinez, N., Williams, J.K., Blackshaw, R.P., 2009. Dissipation of bacterially derived C and N through the meso- and macrofauna of a grassland soil. *Soil Biology & Biochemistry* 41, 1146–1150.
- Paustian, K., Andren, O., Clarholm, M., Hansson, A.C., Johansson, G., Lagerlof, J., Lindberg, T., Pettersson, R., Sohlenius, B., 1990. Carbon and nitrogen budgets of 4 agroecosystems with annual and perennial crops, with and without N-fertilization. *Journal of Applied Ecology* 27, 60–84.
- Rønn, R., McCaig, A.E., Griffiths, B.S., Prosser, J.I., 2002. Impact of protozoan grazing on bacterial community structure in soil microcosms. *Applied and Environmental Microbiology* 68, 6094–6105.
- Sonneborn, T.M., 1970. Methods in paramecium research. In: Prescott, D.M. (Ed.), *Methods of Cell Physiology*. Academic Press, New York and London, pp. 241–339.
- Tiunov, A.V., 2007. Stable isotopes of carbon and nitrogen in soil ecological studies. *Biology Bulletin* 34, 395–407.