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Community ecology

Photoautotrophic microorganisms as a carbon source for temperate soil invertebrates

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We tested experimentally if photoautotrophic microorganisms are a carbon source for invertebrates in temperate soils. We exposed forest or arable soils to a $^{13}CO_2$-enriched atmosphere and quantified ^{13}C assimilation by three common animal groups: earthworms (Oligochaeta), springtails (Hexapoda) and slugs (Gastropoda). Endogeic earthworms (*Allolobophora chlorotica*) and hemiedaphic springtails (*Ceratophysella denticulata*) were highly ^{13}C enriched when incubated under light, deriving up to 3.0 and 17.0%, respectively, of their body carbon from the microbial source in 7 days. Earthworms assimilated more ^{13}C in undisturbed soil than when the microbial material was mixed into the soil, presumably reflecting selective surface grazing. By contrast, neither adult nor newly hatched terrestrial slugs (*Deroceras reticulatum*) grazed on algal mats. Non-photosynthetic $^{13}CO_2$ fixation in the dark was negligible. We conclude from these preliminary laboratory experiments that, in addition to litter and root-derived carbon from vascular plants, photoautotrophic soil surface microorganisms (cyanobacteria, algae) may be an ecologically important carbon input route for temperate soil animals that are traditionally assigned to the decomposer channel in soil food web models and carbon cycling studies.

1. Introduction

Biological soil crusts formed by algae and other photoautotrophic microorganisms are known to be important carbon (C) sources in extreme climatic regions [1,2]. By contrast, few studies have considered such C inputs into soil food webs in temperate regions, focusing on soil organic matter [3] and microorganisms [4]. For soil invertebrate animals, traditional models assumed that vascular plants are the principal C sources, either as living plants (for herbivores such as molluscs) or as plant litter (for detritivores such as earthworms) [5]. However, recent research has shown that C inputs from living roots also need to be considered [6,7]. A third route of C input into temperate soil food webs not yet quantified is via photoautotrophic soil microorganisms. Grazing on and ingestion of soil algae, for example, have been observed in a number of soil animal taxa [8–10], but the functional significance of this C route for animal ecology and soil C dynamics is not known. Here, we report preliminary experiments under controlled laboratory conditions in which we quantified assimilated (rather than ingested) ^{13}C tracer in soil invertebrates derived from atmospheric $^{13}CO_2$ in the absence of vascular plants.

2. Material and methods

(a) Experimental set-up

Mineral soil (top 5 cm) was collected from a Sitka spruce (*Picea sitchensis* (Bong.) Carr.) stand in Portlaois, Ireland, and from a cultivated cereal plot at Thornfield, Belfield campus of University College Dublin, Ireland. The forest soil was a clay with 3.8% C, 0.34% N, and the arable soil was a loam with 2.52% C, 0.27% N.

Six large (2.7 l), square glass jars with rubber-seal cliptops (Kilner™, Rayware Group, Liverpool, UK), lying on the side, were filled with a 2 cm layer of laboratory sand (0.1–0.3 mm particle size), onto which a 2 cm layer of sieved (2 mm) forest or arable soil was placed (see the electronic supplementary material, figure S1). The sand drainage layer ensured moist conditions during daytime (high temperatures, high evaporation) and prevented flooding of the soil surface during night-time (lower temperatures and condensation). Soil was kept near water capacity, but never water-logged. To let photoautotrophic microbial surface communities establish, open jars were initially incubated in a plant growth chamber at 20°C for 21 days; light was provided by fluorescent and tungsten lamps at an irradiance of 300 µE in a 12 L/12 D cycle.

Isotopically labelled $^{13}CO_2$ was produced in 60 ml syringes attached to a three-way valve by releasing 0.15 M HCl onto $NaH^{13}CO_3$ (99 atom% ^{13}C) and injected into the jars through a rubber septum (see the electronic supplementary material, figure S1). Initial nominal ^{13}C enrichment of the CO_2 in the jars' atmosphere was 74 atom% ^{13}C with a concentration of 1300 ppm CO_2. Three $^{13}CO_2$ pulses were administered, after 1, 4 and 8 days. Closed jars (two each with forest and arable soil) were incubated in the same growth chamber at 20°C in the light for 12 days in total. To estimate non-photosynthetic C fixation from $^{13}CO_2$ in the dark, two additional jars with forest soil were wrapped in aluminium foil and incubated along with the other jars. Replication of jars for the ^{13}C labelling step was kept low ($n = 2$ per treatment) because variation between jars in ^{13}C uptake efficiency was not of interest and false negative results due to a labelling failure were unlikely with repeated $^{13}CO_2$ injections.

After 12 days, intact soil cores (8.5 cm diameter) were excised and transferred from the Kilner jars into individual round polypropylene containers (250 ml). The forest soil with the microbial crust was either left intact or mixed manually to simulate soil disturbance that distributes superficial organic matter; the arable soil was left intact. Then, representatives of three important soil animal groups were introduced: earthworms (forest soil) and springtails and slugs (both arable soil), and incubated in the dark at 15°C for 7 days (slugs 3 days).

(b) Soil invertebrates

Adult *Allolobophora chlorotica* (Savigny 1826) (Annelida: Lumbricidae), a common, endogeic, geophagous earthworm species [11], were collected manually from a vegetable garden in County Dublin. Five adults (mean live weight 268 mg, range 137–406 mg) were introduced per container. There were four containers for light-incubated forest soil (two each with soil left intact and soil mixed) and two containers with dark-incubated, intact forest soil.

Ceratophysella denticulata (Bagnall 1941) (Hexapoda: Collembola) is a common, surface-dwelling (hemiedaphic life form) springtail species that is expected to feed on soil algae [12]. Adults were obtained from own laboratory cultures and 20–25 individuals were introduced into each container ($n = 3$) with light-incubated, arable soil.

Adult grey field slugs, *Deroceras reticulatum* (O. F. Müller 1774) (Mollusca: Agriolimacidae) [13], were collected manually from the same cultivated cereal plot at Thornfield. Adults were kept in laboratory culture, and eggs that were laid were incubated until hatching. Four newly hatched *D. reticulatum* (mean live weight 2 mg) and two freshly collected adult *D. reticulatum* (mean live weight 294 mg, range 115–601 mg) were introduced into each container ($n = 2$) with light-incubated, arable soil.

(c) Microbial, isotopic and statistical analysis

Microbial crusts and soil were sampled on the day when invertebrates were introduced. Small samples containing microbial mats were scraped off the soil surface and dispersed in water. Photoautotrophic microorganisms (cyanobacteria, algae) were classified roughly according to Archibald [14] under a light microscope (100× and 400× magnification).

Soil was sampled by scraping the surface layer (about 1 mm) or in bulk after mixing, dried at 50°C and powdered in a ball mill. Invertebrates were starved on moist filter paper for 24 h at 15°C (to allow gut clearance), frozen and freeze-dried for 24 h. Earthworms and adult slugs were ground individually with mortar and pestle before weighing of subsamples (about 1 mg dry weight each) into tin capsules. To achieve sample masses required for mass spectrometry, between 20 and 25 *C. denticulata* were taken from each container and pooled as one sample per container (0.2–0.3 mg dry weight).

Samples were analysed by continuous flow isotope ratio mass spectrometry (CF-IRMS) as described previously [11]. Isotopic ratios are expressed in conventional delta (‰) notation. Control animals of each species from unlabelled jars or the field (earthworms, large slugs) were analysed and compared statistically to animals that had access to labelled mats by non-parametric Mann–Whitney *U*-tests (StatView v. 5). Data are shown as scatterplots and medians, as advocated by Weissgerber *et al*. [15] for such small sample size studies. Data points for the large-bodied species (earthworms and slugs) are shown for individual animals (considered here as replicates) because the main interest was in the variation in ^{13}C assimilation between individuals from the same algal mats (with a known, measured ^{13}C enrichment).

3. Results

In the dark (figure 1*a*), non-photosynthetic fixation of $^{13}CO_2$ in forest soil was negligible and no ^{13}C was assimilated by earthworms (*U*-test $p > 0.10$). In the light, significant amounts of ^{13}C were fixed photosynthetically by microorganisms, as evidenced by high ^{13}C values of the bulk soil organic matter (figure 1*b*) and very high enrichments ($\delta^{13}C + 3014‰ \pm 1045$, mean ± s.d., $n = 4$) of the surface mat samples. This ^{13}C was also assimilated by earthworms, *A. chlorotica*, especially where the soil had been left intact, but also from mixed soil (figure 1*b*). All earthworm $\delta^{13}C$ comparisons between control, mixed, unmixed treatments were significantly different (*U*-test $p < 0.001$, $n = 9$ or 10).

Springtails initially had a median $\delta^{13}C$ value of −22.5‰ ($n = 3$) and were very highly labelled (median $\delta^{13}C + 380‰$, $n = 3$, *U*-test $p < 0.05$) (figure 2*a*) after 7 days' feeding on soil microbial mats ($\delta^{13}C + 2360‰ \pm 231$, mean ± s.d., $n = 3$), on arable soil. Remarkably, the measured C content of the soil surface crust including microbial mats was only 6.1% C ± 0.6, just over double that of bulk soil (2.5% C ± 0.1).

Neither adult nor newly hatched slugs, *D. reticulatum*, assimilated any labelled C derived from CO_2 (figure 2*b*). Start and end whole-body $\delta^{13}C$ values were not significantly different (medians −24.2‰ versus −23.8‰ for adults, −25.9‰ versus −25.0‰ for newly hatched, all $n = 4$ or 5) (*U*-test all $p > 0.10$).

Types of photoautotrophic microorganisms in the soil surface samples included (in order of frequency): Cyanobacteria;

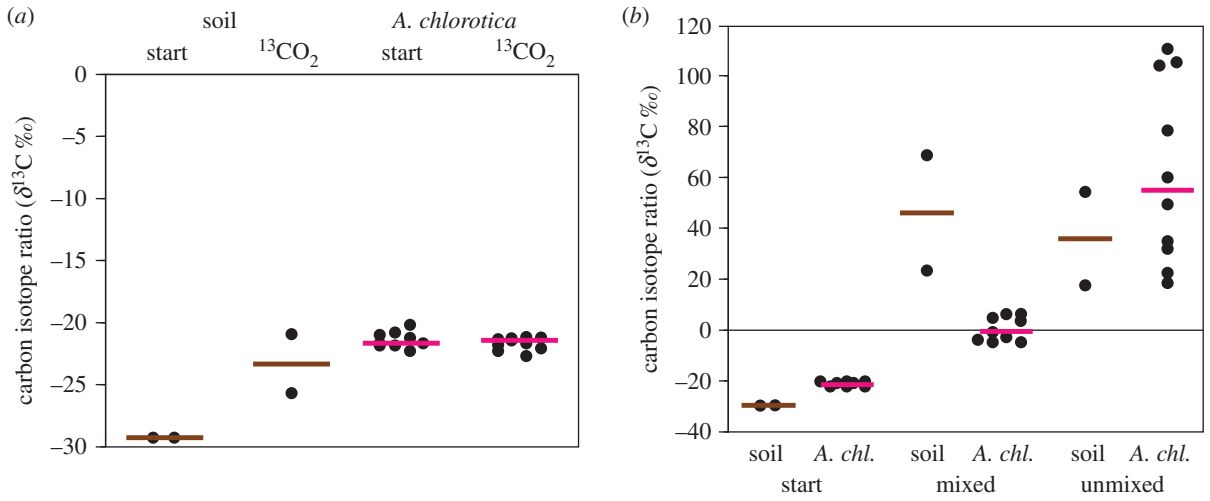

Figure 1. Scatterplots showing (*a*) ^{13}C fixation in soil in the dark (note narrow range of *Y*-axis scale) and (*b*) detection of ^{13}C in soil incubated under labelled $^{13}CO_2$ atmosphere with light, and its assimilation by earthworms (*A. chl.*, *Allolobophora chlorotica*) from mixed or unmixed forest soil. Coloured bars are medians. (Online version in colour.)

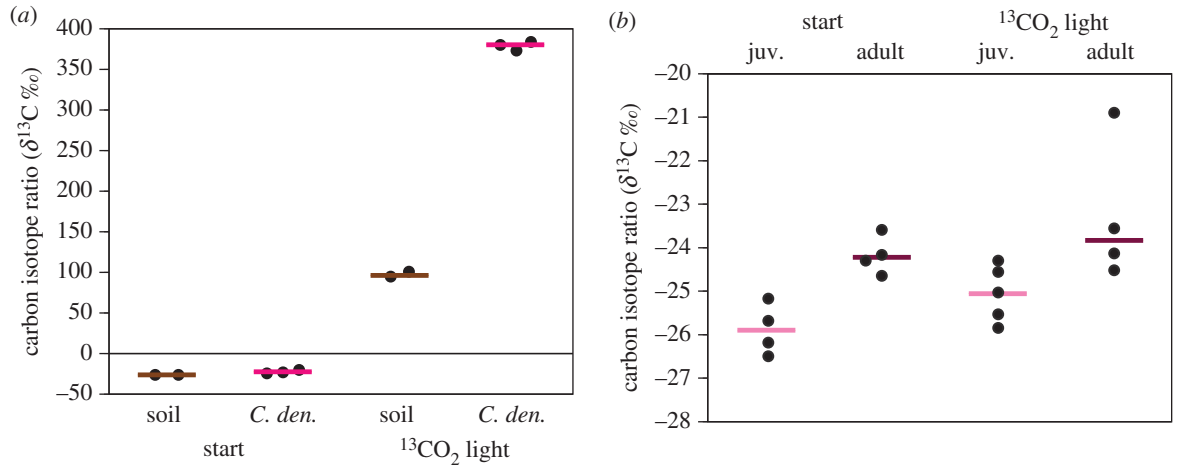

Figure 2. (*a*) Detection of microbially fixed ^{13}C in arable soil and collembolans (*C. den.*, *Ceratophysella denticulata*) and (*b*) no evidence for assimilation of microbially fixed ^{13}C by newly hatched (juv.) or adult slugs, *Deroceras reticulatum*. Coloured bars are medians. (Online version in colour.)

unidentified unicellular, coccal cells; Bacillariophyta; Chlorophyta; Euglenophyta.

4. Discussion

C fixed from CO_2 by photoautotrophic soil microorganisms was used as a C source by important soil decomposer animals. These results show that a third route of C inputs into temperate soil food webs exists, independently of vascular plants that contribute litter and root-derived inputs [5]. Previous studies have surmised that such C sources must exist, for instance [16] concluded that 20–40% of microbial C sources in a temperate grassland were not derived from vascular plants. Recently, C and nitrogen assimilation by soil microfauna (stylet-bearing nematodes and tardigrades) from cyanobacteria of desert soil crusts was demonstrated [17].

Simple isotope mixing equations suggest that, in just 7 days, earthworms derived 3.0% and 0.7% of their total body C from the autotrophic microbial C source in undisturbed (see the electronic supplementary material, figure S2) and mixed forest soil, respectively, while the proportion of new C on the bulk soil C was 1.8 and 2.1% and just 0.2% in the lower soil layer (table 1). Springtails, which are smaller and have faster tissue turnover than earthworms, derived 17.0% of their body C from the microbial source in unmixed arable soil. These estimates suggest that the microbial C input route could be of ecological importance, representing a small [3,18] but very productive and reactive [1,19] C pool that is grazed selectively by soil animals. As in a study on desert soil crusts [17], the present data cannot distinguish between direct assimilation of photoautotrophs and prior C flow through heterotrophic microorganisms, but the short incubation times tend to support the direct route. Further research is required to establish the significance of such microbial C inputs in natural soils, so that

Table 1. C (% of total) in forest soil and *A. chlorotica* derived from photoautotrophic microbial sources (mean ± s.d.). n.a., not applicable.

compartment	undisturbed soil	mixed soil
total soil	1.8 (0.7)	2.1 (0.9)
lower soil layer	0.2 (0.2)	n.a.
earthworms	3.0 (0.8)	0.7 (0.2)

soil food web models [5] and C cycling studies [2] account for this route. Grazing by animals, in turn, probably controls soil photoautotrophic microbial communities [10].

Having dwelled on highly enriched microbial mats, in the absence of vascular plants, slugs did not assimilate CO_2-derived C. Newly hatched *D. reticulatum* in particular could be expected to graze on algal mats, but the present findings confirm both life stages as herbivores of vascular plants [13].

Ethics. Animals used in experiments are not endangered, nor subject to animal research ethics regulations in the country where the work was conducted.

Data accessibility. Data are available as the electronic supplementary material, dataset S1.

Authors' contributions. O.S. and J.D. conceived and designed the experiment; J.D. carried out the experiment; S.S. handled Collembola and identified algae; O.S. coordinated the study and drafted the manuscript. All authors gave final approval for publication. All authors agree to be held accountable for the work performed.

Competing interests. We declare we have no competing interests.

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References

1. Belnap J, Lange OL (eds). 2003 *Biological soil crusts: structure, function, and management*. Berlin, Germany: Springer.
2. Elbert W, Weber B, Burrows S, Steinkamp J, Budel B, Andreae MO, Poschl U. 2012 Contribution of cryptogamic covers to the global cycles of carbon and nitrogen. *Nat. Geosci.* **5**, 459–462. (doi:10.1038/ngeo1486)
3. Shimmel SM, Darley WM. 1985 Productivity and density of soil algae in an agricultural system. *Ecology* **66**, 1439–1447. (doi:10.2307/1938006)
4. Ge TD *et al.* 2013 Microbial phototrophic fixation of atmospheric CO_2 in China subtropical upland and paddy soils. *Geochim. Cosmochim. Acta* **113**, 70–78. (doi:10.1016/j.gca.2013.03.020)
5. DeRuiter PC, Neutel AM, Moore JC. 1995 Energetics, patterns of interaction strengths, and stability in real ecosystems. *Science* **269**, 1257–1260. (doi:10.1126/science.269.5228.1257)
6. Pollierer MM, Langel R, Korner C, Maraun M, Scheu S. 2007 The underestimated importance of belowground carbon input for forest soil animal food webs. *Ecol. Lett.* **10**, 729–736. (doi:10.1111/j.1461-0248.2007.01064.x)
7. Scheunemann N, Scheu S, Butenschoen O. 2010 Incorporation of decade old soil carbon into the soil animal food web of an arable system. *Appl. Soil Ecol.* **46**, 59–63. (doi:10.1016/j.apsoil.2010.06.014)
8. Curry JP, Schmidt O. 2006 The feeding ecology of earthworms: a review. *Pedobiologia* **50**, 463–477. (doi:10.1016/j.pedobi.2006.09.001)
9. Shtina EA, Kozlovskaya LS, Nekrasova KA. 1981 Relations of soil oligochaetes and algae. *Soviet J. Ecol.* **12**, 44–48.
10. Jahnke J, Wehren T, Priefer UB. 2007 *In vitro* studies of the impact of the naked soil amoeba *Thecamoeba similis* Greef, feeding on phototrophic soil biofilms. *Eur. J. Soil Biol.* **43**, 14–22. (doi:10.1016/j.ejsobi.2006.11.002)
11. Melody C, Schmidt O. 2012 Northward range extension of an endemic soil decomposer with a distinct trophic position. *Biol. Lett.* **8**, 956–959. (doi:10.1098/rsbl.2012.0537)
12. Buse T, Filser J. 2014 Mucilaginous seeds and algal diets attract soil Collembola in preference tests. *Eur. J. Soil Biol.* **65**, 1–6. (doi:10.1016/j.ejsobi.2014.08.005)
13. Pallant D. 1972 Food of grey field slug, *Agriolimax reticulatus* (Müller), on grassland. *J. Anim. Ecol.* **41**, 761–769. (doi:10.2307/3208)
14. Archibald PA. 1990 Soil algae. In *Soil biology guide* (ed. DL Dindal), pp. 69–96. New York, NY: John Wiley & Sons.
15. Weissgerber TL, Milic NM, Winham SJ, Garovic VD. 2015 Beyond bar and line graphs: time for a new data presentation paradigm. *PLoS Biol.* **13**, e1002128. (doi:10.1371/journal.pbio.1002128)
16. Kramer C, Gleixner G. 2006 Variable use of plant- and soil-derived carbon by microorganisms in agricultural soils. *Soil Biol. Biochem.* **38**, 3267–3278. (doi:10.1016/j.soilbio.2006.04.006)
17. Darby BJ, Neher DA. 2012 Stable isotope composition of microfauna supports the occurrence of biologically fixed nitrogen from cyanobacteria in desert soil food webs. *J. Arid Environ.* **85**, 76–78. (doi:10.1016/j.jaridenv.2012.06.006)
18. Jeffery S, Harris JA, Rickson RJ, Ritz K. 2009 The spectral quality of light influences the temporal development of the microbial phenotype at the arable soil surface. *Soil Biol. Biochem.* **41**, 553–560. (doi:10.1016/j.soilbio.2008.12.014)
19. Abed RMM, Polerecky L, Al-Habsi A, Oetjen J, Strous M, de Beer D. 2014 Rapid recovery of cyanobacterial pigments in desiccated biological soil crusts following addition of water. *PLoS ONE* **9**, e112372. (doi:10.1371/journal.pone.0112372)