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Photosynthetic Carbon Incorporation and Turnover in Antarctic Cryptoendolithic Microbial Communities: Are They the Slowest-Growing Communities on Earth?

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The main forms of terrestrial life in the cold, desolate Ross Desert of Antarctica are lichen-dominated or cyanobacterium-dominated cryptoendolithic (hidden in rock) microbial communities. Though microbial community biomass (as measured by extractable lipid phosphate) was well within the range of values determined for other microbial communities, community lipid carbon turnover times (calculated from community lipid biomass, rates of community photosynthetic carbon incorporation into lipids versus temperature, and the in situ temperature record) were among the longest on Earth (ca. 20,000 years). When the temperature is above freezing and moisture is present, moderate rates of photosynthesis can be measured. Lichen communities had a psychrophilic temperature response (maximal rate of 4.5 ng of C h^{-1} m^{-2} at 10°C) while cyanobacteria communities had maximal rates at 20 to 30°C (3 ng of C h^{-1} m^{-2}). These extraordinarily slowly growing communities were not nutrient limited. No significant changes in photosynthetic metabolism were observed upon additions of 100 nM to 1 mM nitrate, ammonium, phosphate, and manganese. These simple, tenacious microbial communities demonstrate strategies of survival under conditions normally considered too extreme for life.

There are no signs of terrestrial life in the cold, dry Ross Desert (Dry Valleys) of southern Victoria Land, Antarctica. The main life forms are cryptoendolithic (hidden within rock) microbial communities, found in the pore spaces of sandstone, 1 to 5 mm beneath the surface (3). These microbial communities are usually dominated by either lichen or cyanobacteria. Lichen-dominated communities from Linnaeus Terrace (LTL) consist of primary producers (lichen, the occasional green alga, and cyanobacteria) and heterotrophs (fungi, bacteria, and yeasts) (3). Nearby Battleship Promontory is extensively colonized by cyanobacterium-dominated communities (BPC) in which free-living cyanobacteria are the only primary producers. Lichen-dominated communities are also found at Battleship Promontory.

The purpose of this study was to determine annual photosynthetic carbon incorporation, lipid carbon turnover times, and biomass in these frigid microbial communities. The effects of various nutrient concentrations on community photosynthesis were also examined and found to be insignificant. This is the first reported study on the community metabolism and biomass of BPC and extends the literature on the community metabolism of LTL (2, 5, 8, 10, 12, 13, 16–19).

MATERIALS AND METHODS

Sample collection. Linnaeus Terrace in the Upper Wright Valley, Antarctica (77°36′S, 161°05′E), was the source of lichen-dominated rocks (LTL), and Battleship Promontory in Alatna Valley, in the Convoy Range, Antarctica (76°55′S, 161°00′E), was the source of cyanobacterium-dominated rocks (BPC). The biotic zones of colonized rock were excised and ground to a sand consistency in Antarctica (17, 18). This homogenous material was transported and stored frozen in the dark until used in the laboratory.

Photosynthetic carbon incorporation into lipids. [^{14}C]bicarbonate was added to crushed rock samples in the laboratory to determine the effects of temperature on community photosynthetic metabolism. Water was left open to the atmosphere at the experimental temperature to allow CO_2 to equilibrate prior to the incubations. [^{14}C]bicarbonate (1 ml; 5 μCi/ml from a 56-mCi/mmol stock solution) was added to 59 ml of water (equilibrated with CO_2 at the experimental temperature) in 60-ml plastic syringes fitted with Luer Lok three-way valves to avoid CO_2 loss. Then, 6 ml of this solution was added to 3 g of crushed rock (weighed in the dark at 4°C) in 10-ml plastic syringes. Air bubbles were excluded, and the syringes were put on their sides in a Plexiglas chamber thermally regulated with water. Quadruplicate samples of both LTL and BPC were incubated at each temperature (5, 10, 15, 20, and 30°C) under light (~300 μmol of photons m^{-2} s^{-1}) and dark (syringes wrapped in tape) conditions. After 4 h, CO_2, $^{14}CO_2$, and lipid [^{14}C]carbon were measured. CO_2 analysis was as described below, and the lipids were extracted as previously described (18).

Moles of carbon incorporated into lipids were determined by dividing the disintegrations per minute from the lipid extraction by the analytically determined specific activity of CO_2 for each sample. Moles of carbon incorporated per sample were converted to moles of carbon incorporated per square meter by using the ratio of moles of lipid phosphate per gram of colonized rock (in the homogenized samples used for the incubations) to moles of lipid phosphate per square meter (from colonized rock of known surface area).

CO_2 analysis. Subsamples (3 ml) from incubation syringes were injected through 0.4-μm-pore-size Nuclepore filters into 10-ml syringes via three-way valves. The subsamples were then acidified with 1 ml of 0.2 N sulfuric acid to convert dissolved CO_2 to CO_2 gas. Headspace was created with 5 ml of N_2 gas. The syringe was then shaken and turned upside-

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down. The headspace was injected through a septum into a N_2 stream (0.6 ml/s) leading to an infrared gas analyzer (Lira model 3000) for CO_2 analysis. Standard curves were made from 3-ml solutions of sodium bicarbonate in 10-ml syringes and treated and injected as were the samples. Dissolved CO_2 concentrations were 0.1 mM for LTL and 0.4 mM for BPC in these incubations. The high values were due to abundant magnesium and calcium carbonates present in the sandstone (9).

To measure dissolved $^{14}CO_2$, water was filtered through 0.2-μm-pore-size Nuclepore filters and 200 μl was pipetted into 20 ml of Scintiverse BD (Fisher Scientific, Pittsburgh, Pa.). The specific activity of the CO_2 was constant after the first ~10 min of the 4-h incubation (data not shown).

Community biomass, annual photosynthetic carbon incorporation, and lipid carbon turnover time. Community biomass measurements were determined by measuring extractable lipid phosphate as previously described (17, 20). The mass of community lipid carbon was calculated by assuming that a typical phospholipid has 1 phosphate group and 37 carbon atoms.

Annual carbon incorporation into lipids was calculated by multiplying the rates of photosynthetic carbon incorporation into lipids at 5 and 10°C by the time that in situ temperatures inside the sandstone were above 5 and 10°C. Nanoclimate temperature is above 5°C for ~250 h and above 10°C for ~90 h per year in a rock sloped in a northeast orientation which receives maximal sunlight exposure and has the highest in situ temperature (6). Nanoclimate data was obtained from Linnaeus Terrace, so annual carbon incorporation for BPC may not be as reliable as for LTL.

The turnover time of the microbial community lipid carbon pool was calculated by dividing the estimate of annual photosynthetic carbon incorporation into lipids by the mass of lipid carbon in the community.

Nutrient incubations. [^{14}C]bicarbonate incubations of both LTL and BPC were done as described by Vestal (18) with additions of added nutrients (0.1 μM, 1 μM, 10 μM, 0.1 mM, and 1 mM of phosphate, ammonium, nitrate, or manganese) along with water-only controls. The Scheffe multiple-range test ($P = 0.05$, $n = 3$) in the MGLH module of the statistics computer program Systat (Systat Inc.) was used for analysis of variance.

Nutrient analysis. Water-soluble ions were determined from 0.5 g of sandstone samples in 10 ml of distilled, deionized water for 5 h. Samples were filtered through 0.2-μm-pore-size Nuclepore filters and then analyzed for water-soluble nitrate, ammonium, and phosphate by using high-performance liquid chromatography (HPLC). Samples were analyzed for water-soluble phosphorus and manganese by using inductively coupled plasma atomic emission spectrometry (ICP) as described previously (9).

RESULTS AND DISCUSSION

Antarctic cryptoendolithic communities have a unique combination of extremely low rates of photosynthetic carbon incorporation and long carbon turnover times (Table 1). Unproductive ecosystems usually have short carbon turnover times, while long carbon turnover times are usually found in productive ecosystems. Antarctic cryptoendolithic communities (Table 1) are a couple of orders of magnitude less productive than open oceans, the least productive ecosystems discussed by Whittaker (21), which produce 130 g of C m^{-2} $year^{-1}$. The large difference in photosynthetic production between antarctic cryptoendolithic communities and other ecosystems would not be as great if production was calculated just in periods of biological activity and not for the whole year. Antarctic cryptoendolithic communities also have longer carbon turnover times than temperate evergreen forests (carbon turnover time = 27 years), which have the longest carbon turnover time discussed by Whittaker. The extremely long lipid carbon turnover times from this study agree in magnitude with previous estimates (19), with observations that biological and geological processes are occurring at similar rates (7), and with a minimum carbon date calculated at 10^3 years (1). An absolute carbon date cannot be determined since these cryptoendolithic communities are still alive and exchanging CO_2 with the atmosphere (1). The combination of low rates of photosynthetic production and long carbon turnover times in antarctic cryptoendolithic communities may be due to the absence of predators, the lack of darkness (and dark respiration) during warm periods of high metabolic activity, and their ability to rapidly switch metabolism on and off without detrimental effects.

TABLE 1. Biomass (from extractable lipid phosphate), annual photosynthetic carbon incorporation, and lipid carbon turnover times of antarctic cryptoendolithic communities

Community	Lipid P biomass[a]		Lipid C		
	nmol of P g^{-1}	μmol of P m^{-2}	Biomass[b] (mg of C m^{-2})	Incorporation[c] (μg of C m^{-2} yr^{-1})	Turnover[d] (yr)
LTL	88 ± 6	530 ± 70[e]	235	14	17,000
BPC	27 ± 2	170 ± 70[f]	75	4	19,000
BPL[g]	31 ± 2	190 ± 70[h]	84	ND[i]	ND

[a] From triplicate subsamples of the amalgamated colonized zones of several rocks.
[b] Calculated from lipid phosphate biomass assuming the typical phospholipid has 37 carbon atoms.
[c] Calculated from in situ time above 5 and 10°C at Linnaeus Terrace (7) and the photosynthetic response to temperature (Fig. 1).
[d] Lipid C biomass/annual photosynthetic C incorporation into lipids.
[e] From Vestal (17).
[f] From 32 samples of known area excised from 10 rocks.
[g] Lichen-dominated cryptoendolithic community from Battleship Promontory.
[h] From 32 samples of known area excised from 5 rocks.
[i] ND, not determined.

The nanoclimate data used from Friedmann et al. (6) give maximal photosynthetic rates since temperature data from an optimally sun-oriented rock were used. During the two summers discussed by Friedmann et al., the temperature in a horizontal rock was above 5°C for only 10 h and never reached 10°C. Annual photosynthesis in such a rock would be 2 orders of magnitude less than shown in Table 1; such a rock would be by far the least productive photosynthetic community on earth.

Net photosynthetic carbon incorporation was 84 mg of C m^{-2} $year^{-1}$ and the carbon turnover time was 78 years when measurements at 5 and 10°C from Kappen and Friedmann (10) were combined with the nanoclimate temperature record (6). Net photosynthetic carbon uptake is much higher than photosynthetic carbon incorporation into lipids since only a fraction of photosynthetically derived carbon becomes incorporated into lipids. Estimations by Vestal (19) for net photosynthetic carbon uptake (0.1 to 4 mg of C m^{-2} $year^{-1}$) and carbon turnover times (500 to 28,000 years) were closer to measured carbon incorporation into lipids and carbon turnover times in this study (Table 1). Measures of photosynthesis and carbon turnover in the present study are more reliable and precise than previous estimates by Vestal

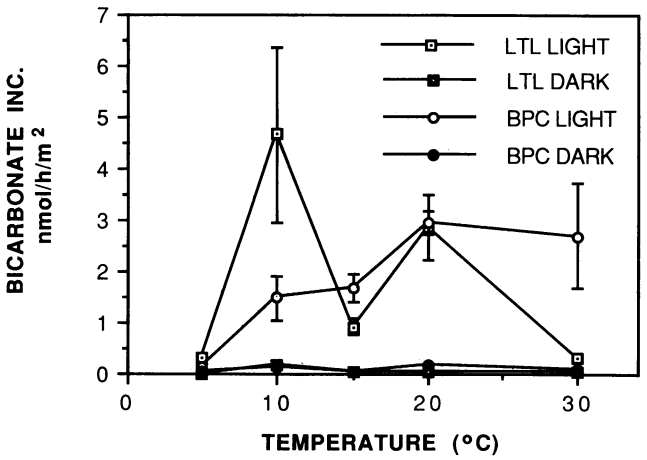

FIG. 1. Photosynthetic response of LTL and BPC to temperature. Rates of photosynthetic carbon incorporation into lipids were measured at various temperatures in light and dark incubations of added [^{14}C]bicarbonate. The amount of carbon incorporated into lipid was calculated from radioactivity in extractable lipids and specific activity of CO_2. Average values (n = 4) plus and minus standard deviations are shown.

(19) since the specific activity of the CO_2 was measured and fewer assumptions were required.

Despite severe conditions, extractable lipid phosphate biomass measurements in antarctic cryptoendolithic communities (Table 1) were not particularly low when compared with similar measurements in other microbial communities (11, 14, 20). Cryptoendolithic biomass values were much greater than those found in sediments from Ace Lake, Antarctica (0.0 to 0.160 nmol g^{-1}), a meromictic lake in the Vestfold Hills (11). Biomass values determined by using ATP extractions from LTL (16) were in good agreement with values determined from extractable lipid phosphate (18).

Biomass values of both lichen-dominated and cyanobacterium-dominated communities at Battleship Promontory were one-third those of lichen-dominated communities at Linnaeus Terrace when independent determinations were made of lipid phosphate per gram and lipid phosphate per square meter (Table 1). The greater biomass of the lichen community at Linnaeus Terrace than of either community (cyanobacteria or lichen) at Battleship Promontory indicate that site differences may be more important than local microenvironments and community type for determining biomass. Annual carbon incorporation into lipids was also much higher in LTL than in BPC, though carbon turnover times were similar.

LTL showed a psychrophilic response to temperature, while photosynthesis in BPC appeared to be mesophilic. Rates of photosynthetic carbon incorporation were highest at 10°C for LTL and were highest above 20°C for BPC (Fig. 1). This indicates that community photosynthetic metabolism in LTL is more specialized for cold temperatures and that BPC may be as well adapted to warmer, less-polar climates. These trends in photosynthetic response to temperature are also seen when compared to the growth response of antarctic lichen phycobionts and cyanobacterium isolates. Growth of phycobionts isolated from LTL tapers off above 15 to 20°C (12). In contrast, a *Chroococcidiopsis* sp., a cyanobacterium isolated from Beacon Valley, Antarctica, does not have a psychrophilic growth response and may belong to the same species as found in hot desert cryptoendolithic communities (12). The bimodal photosynthetic optima in LTL (Fig. 1) may be due to the presence of more than one species of phycobionts (4, 18).

TABLE 2. Average nutrient concentration in 10-ml water extractions of triplicate 0.5-g subsamples from LTL and BPC

Community	Concn (μM)				
	Phosphate[a]	Phosphorus[b]	Ammonium[c]	Nitrate[a]	Manganese[b]
LTL	11 ± 4	4 ± 1	<5	21 ± 3	2.9 ± 0.5
BPC	<4	5 ± 1	<5	116 ± 11	1.5 ± 0.5

[a] Anion analysis by HPLC spectrophotometry.
[b] Elemental analysis by ICP emission spectrometry.
[c] Cation analysis by HPLC spectrophotometry.

Photosynthetic metabolism in these slowly growing, long-lived antarctic cryptoendolithic communities do not show signs of nutrient limitation. There were no significant differences (P = 0.05, n = 3) in photosynthetic incorporation of [^{14}C]bicarbonate into lipids when nutrients (0.1 μM to 1 mM of phosphate, ammonium, nitrate, manganese, or water [control]) were added to either BPC or LTL. In situ nutrient concentrations appear to be sufficient for community metabolism since photosynthetic carbon incorporation in either community did not respond to these large changes in concentration. Ambient water-soluble nutrient concentrations in the biotic zone were similar in both communities, with the exception that nitrate was much higher in BPC than in LTL (Table 2), and are in general agreement with values reported previously for LTL (5).

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