

Surface gas-exchange processes of snow algae

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The red-colored chlorophyte *Chlamydomonas nivalis* is commonly found in summer snowfields. We used a modified Li-Cor gas-exchange system to investigate surface gas-exchange characteristics of snow colonized by this alga, finding rates of CO₂ uptake up to 0.3 μmol m⁻²s⁻¹ in dense algal blooms. Experiments varying the irradiance resulted in light curves that resembled those of the leaves of higher plants. Red light was more effective than white and much more effective than green or blue, because of the red astaxanthin that surrounds and masks the algal chloroplasts. Integrating daily course measurements of gas exchange showed CO₂ uptake around 2,300 μmol·m⁻²·day⁻¹ in heavily colonized patches, indicating that summer snowfields can be surprisingly productive.

In high-altitude and high-latitude regions where winter snow persists into the local summer, one can almost always find patches of snow colored in various ways, but particularly red or pink. In the U.S., such snow is often called “watermelon snow” because of its pink color and also because it sometimes exudes a faint odor of ripe watermelon when disturbed. The organism responsible for the pink color (and presumably the odor) is *Chlamydomonas nivalis* (Bauer) Wille, a unicellular alga in the Volvocales. *C. nivalis* is apparently global, as it has been reported from New Zealand (1), Australia (2), and Europe (3–6). In North America, it occurs in Alaska (5), the Cascade Mountains of the Pacific Northwest (7, 8), the Sierra Nevada of California (7, 9), and the Rocky Mountains of the western United States and Canada (7, 10, 11).

The life-cycle stage most abundant in snow comprises spherical cells 10–50 μm in diameter (2, 9, 12, 13) that appear bright red under the microscope, a color that comes from the carotenoid astaxanthin (14, 15). These spherical cells are called “aplanospores” and are designated “resting cells” in the algal literature, although this does not mean that they are metabolically inactive. The cells are not motile and drift passively with the meltwater as it flows along and through the snow (16), although this movement may be somewhat restricted by a mucilaginous coat that causes them to stick slightly to snow crystals (4).[§] Snowfields typically show a very patchy distribution of pink color, with more intense pink often concentrated along meltwater courses.

The environment in which *C. nivalis* lives is a harsh one for photosynthetic organisms. At the high altitudes where they are typically found (above 2,500 m), UV-radiation levels can be quite high (17), and clear skies coupled with reflection within the snow can result in spherically integrated photosynthetically active radiation (PAR) fluence rates that often reach 4,500 μmol·m⁻²·s⁻¹ and occasionally reach 6,000 μmol·m⁻²·s⁻¹ (18), about triple the maximum light load on a horizontal leaf at the top of a canopy. Moreover, the algae live in snow that is never much warmer than 0°C, and cold temperatures exacerbate photoinhibition (19, 20). Nonetheless, the cells are clearly photosynthetic. Thomas (9) measured photosynthesis by using ¹⁴C near Tioga Pass, California, finding rates from 6 to 34 μg C mm⁻³·hr⁻¹ (≈6.5 × 10⁻¹¹ μmol CO₂·s⁻¹ per cell). Thomas also reported that subsurface samples, which received less light, photosynthesized more than samples from the surface, suggesting that photoinhibition might have occurred in the surface layers. Mosser *et al.* (10), however, reported no photoinhibition

up to 8.5 × 10⁴ lux (≈1,600 μmol·m⁻²·s⁻¹). Mosser *et al.* (10) also studied the effects of temperature on ¹⁴CO₂ uptake, finding that most populations had temperature optima between 10°C and 20°C. (They reported their CO₂-uptake rates in raw cpm.)

Although ¹⁴CO₂ methods can provide information useful to the study of the alga itself, it is also important to know how much CO₂ exchange takes place between the snow and the air. Most investigators who have studied carbon exchange between snow and air have made their measurements in winter, when they generally find that CO₂ flows out of the snow because of soil respiration under it (21–23); photosynthesis in snow algae could absorb CO₂ from the air, or the algae could simply tap into this efflux, reducing CO₂ emissions from the snow. Gas-exchange from snow-algae-covered areas might represent a small but significant global carbon sink not previously noticed, because summer snowfields cover significant areas of the Earth. Regardless of the source of CO₂, algal photosynthesis could play an important role in carbon and nutrient cycling in the local ecosystem of the snowfields and their surroundings. Therefore, we decided to examine the gas-exchange characteristics of pink snow by using a modified gas-exchange apparatus that is normally used to measure leaf-level photosynthesis.

Materials and Methods

We used a Li-Cor LI-6200 photosynthesis system (Li-Cor, Lincoln, NE) as the basis of our snow-surface gas-exchange system. Because no commercially available chamber met our purposes, we constructed a shallow square Plexiglas chamber, ≈1 m square by 8 cm high, and attached the LI-6200 sensor head (containing humidity and temperature sensors and hose connections to the rest of the LI-6200 system). We installed two brushless motor fans (F62LM-012GK, Micronel, Vista, CA) to promote good gas mixing within the chamber. Recently, Hooper *et al.* (24) pointed out that using the LI-6200 with large chambers can lead to calculation errors caused by (i) changes in chamber water-vapor pressure and (ii) mixing in a preexisting boundary layer that might have a very different [CO₂] from that of the bulk air. Because the snow is so cold, evaporation from the surface was extremely low (vapor pressure in the chamber typically changed <0.001 mbar/min), obviating the first problem, and our site was quite windy, obviating the second.

To allow us to resample the same area of snow several times, we constructed an aluminum frame that sealed onto the Plexiglas box and extended down into the snow ≈5 cm. We pressed the frame down into the snow at the beginning of a series of measurements and left it in place, clamping the box onto the frame for a measurement (which lasted ≈3 min; Fig. 1) and propping it up on one edge between measurements. The box was sealed to the frame with a closed-cell foam gasket.

We used natural sunlight as our sole light source, reducing the incident irradiance with multiple layers of wire and fiberglass window-screening material when we made light curves. For the

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Abbreviation: PAR, photosynthetically active radiation.

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[§]Hoham, R. W., Sixtieth Annual Western Snow Conference, April 14–16, 1992, Jackson, WY, pp. 78–83.

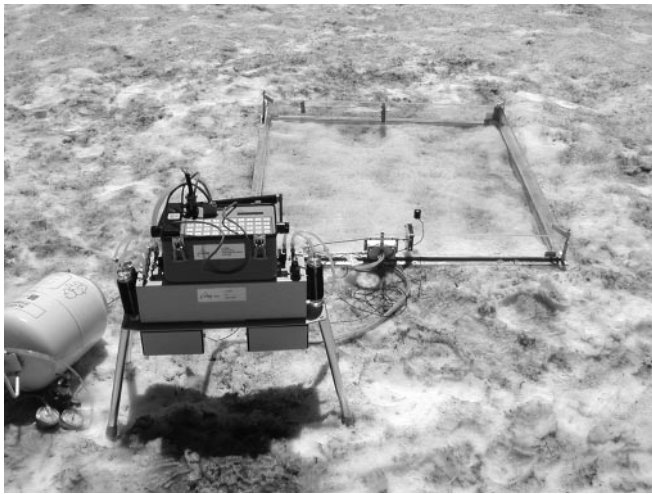


Fig. 1. The gas-exchange apparatus in place on the snow. Patches that appear dark in this black-and-white photograph are pink watermelon snow colonized by *C. nivalis*.

colored-light experiments, we used Roscolene filters 863 medium blue, 874 medium green, and 823 medium red (Roscoe Laboratories, Stamford, CT). Fig. 2 shows the spectra of these filters and also of the Plexiglas box obtained with a Hewlett-Packard Diode-Array Spectrophotometer (model 8452A, Hewlett-Packard, Waldbronn, Germany). We measured light with a quantum sensor (LI-190SA, Li-Cor) at snow level under the chamber for light curves and color experiments or on top of the chamber (adjusting light value for the Plexiglas) for daily courses.

Our field site was in the Snowy Range of the Rocky Mountains, in a persistent snowfield $\approx 41^{\circ} 21' N$ latitude, $106^{\circ} 16' W$ longitude, at an altitude of $\approx 3,250$ m (10,700 feet). Like most of the snowfields in this area, this field showed both white areas and deep pink areas, indicating the absence and presence, respectively, of snow-algal communities. Snow depths ranged from

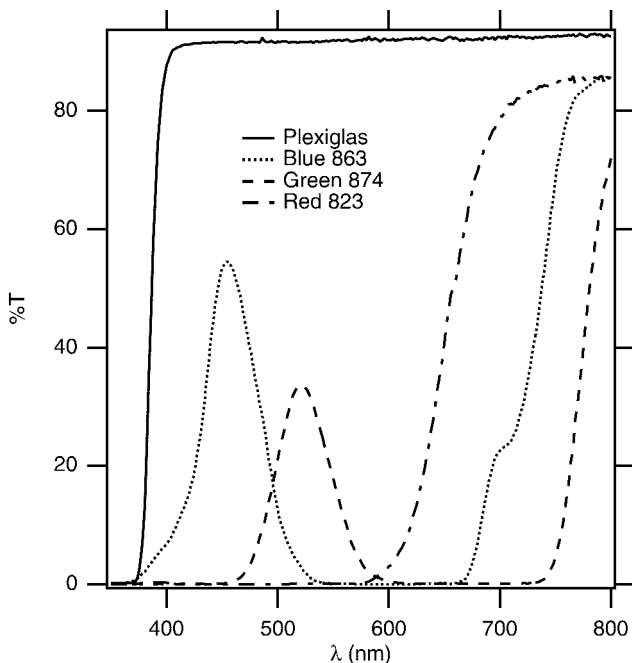


Fig. 2. Transmittance spectra of the Roscolene filters and the Plexiglas box.

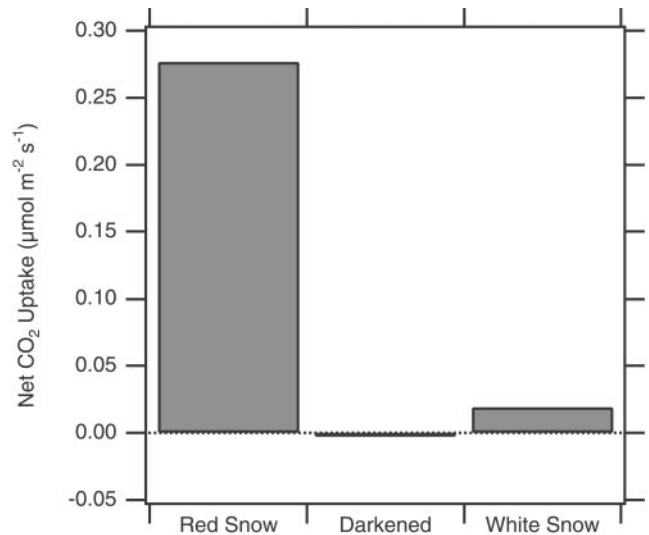


Fig. 3. Comparison of pink and white snow, and the pink snow patch darkened with a black velvet curtain.

>2 m to as little as 13 cm. We made measurements in the summers of 1999, 2000, and 2001, usually in early July.

To determine the concentration of *C. nivalis* cells contributing to CO₂ exchange, we collected the snow under the gas-exchange chamber at the end of the day to a depth where we no longer saw any pink color, usually ≈ 10 cm (although there was considerable variability in the maximum depth to which we found algae). We melted the sample completely, measured its volume, filtered it through a coarse screen to remove debris such as pine and fir leaves, and took several 50-ml samples after thorough mixing. Each 50-ml sample was centrifuged at 1,300 rpm for 1.5 min, after which we carefully decanted the supernatant, leaving a volume of 5.0 ml. We resuspended the cells in this volume and counted them by using a hemacytometer, thus getting an estimate of 10 times the concentration of cells in the original snow sample.

Results

To refine our setup and determine initially whether we could observe any net CO₂ exchange at all, we picked a brilliant sunny day, placed the box directly on the snow, and compared a snow patch that was obviously red with one that had no apparent red coloring (Fig. 3). The pink snow had a net CO₂ uptake rate of $\approx 0.3 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, whereas a nearby patch of snow with no pink color showed an order of magnitude less. The pink patch had a measurable rate of CO₂ efflux ($0.016 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) when we placed a black cloth over the box. These results demonstrated that the apparatus had sufficient sensitivity to detect snow-surface gas exchange and that the CO₂ uptake was coming from algal photosynthesis. Because we had not yet constructed the sealing frame, the specific numbers may not be accurate, and indeed they are substantially higher than any other rates we measured.

To develop an idea of how much carbon exchange occurs in algae-colonized patches of snow over the long term, we placed the frame in the snow and measured gas-exchange rates roughly every hour, over several dawn-to-dusk periods (Figs. 4 and 5; other experiments showed similar patterns). We recorded light (Figs. 4B and 5B) and snow temperature (Figs. 4C and 5C) by using the PAR sensor and leaf thermocouple supplied with the gas-exchange system at the same time we measured gas exchange. CO₂-exchange rates always peaked near mid-day and were lowest at dawn and sunset, and the difference between

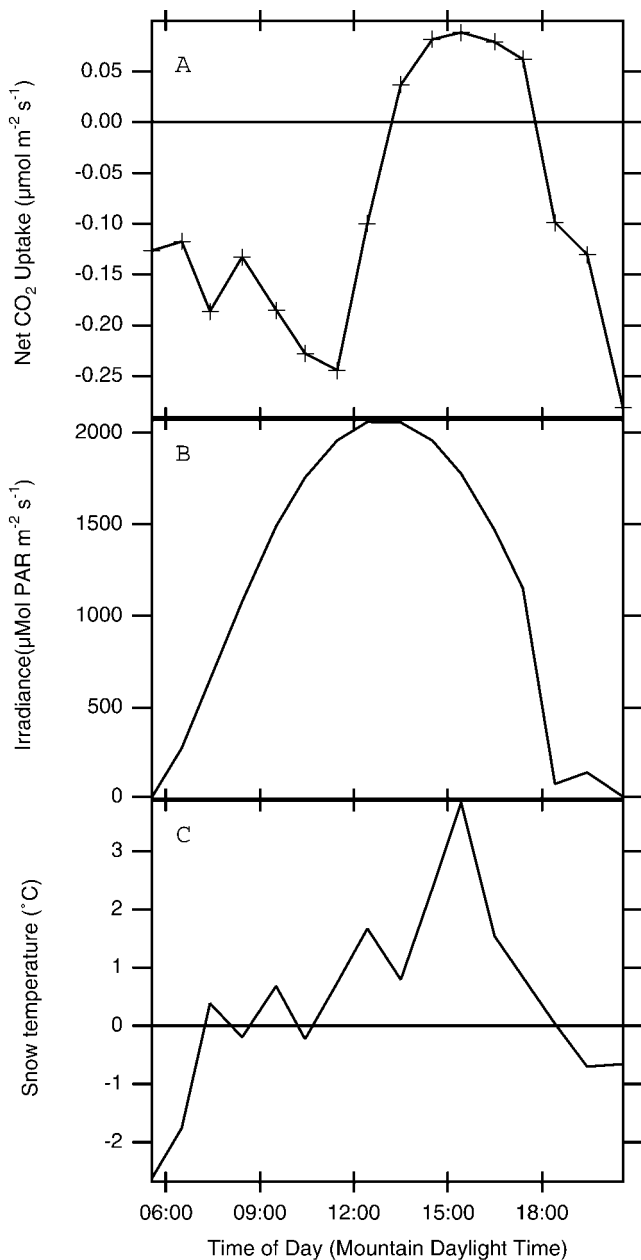


Fig. 4. Daily course of gas exchange over a single patch of algae-colonized snow obtained on June 21, 2000. (A) Area-specific rates of photosynthesis. (B) Photosynthetically active radiation outside the chamber. (C) Snow temperature.

dark-measured rates and peak rates was usually on the order of $0.1\text{--}0.3 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, or $\approx 1 \times 10^{-9} \mu\text{mol per cell}\cdot\text{s}^{-1}$. However, the actual values of net CO_2 uptake were quite variable, often starting from negative values, sometimes as low as $-0.5 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ and only barely reaching positive values at mid-day (compare Fig. 4 with Fig. 5).

To investigate photosynthetic light response under field conditions, we generated a light curve beginning with full sun and reducing the light by successively adding layers of window screen. The resulting light curve (Fig. 6) seems to be quite similar to that typically found in higher plants, with a linear increase at low light, saturation at high light, and no indication of photoinhibition even when PAR was at its maximum for the day, $1,872 \mu\text{mol PAR}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. The maximum rate of photosynthesis we found was $\approx 0.1 \mu\text{mol CO}_2\cdot\text{m}^{-2}\cdot\text{s}^{-1}$.

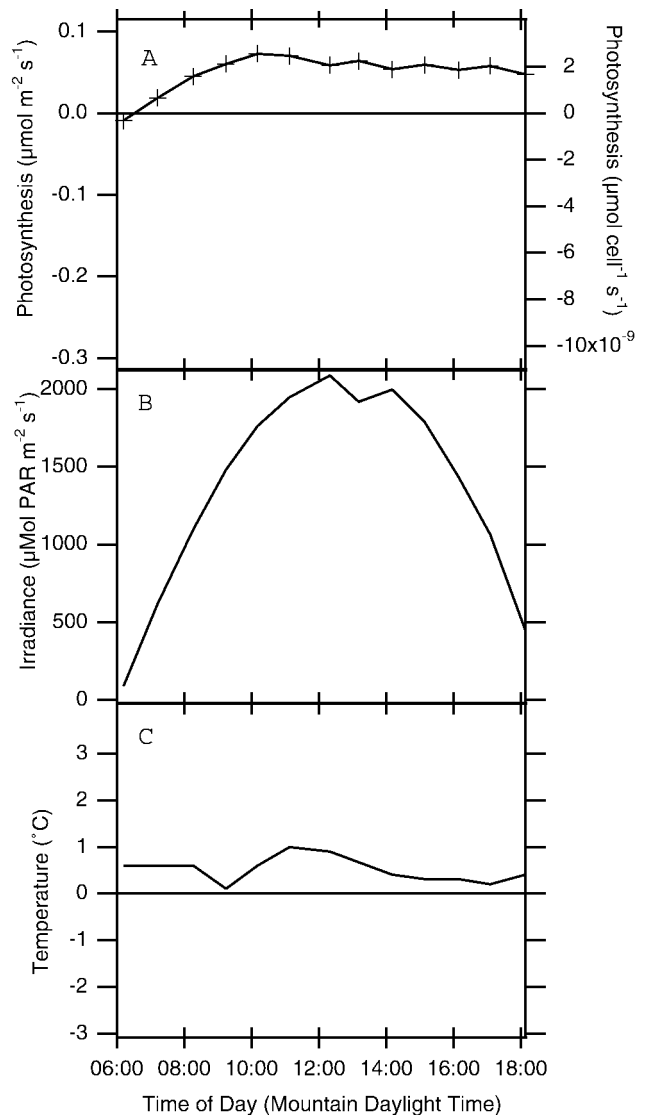


Fig. 5. Similar to Fig. 4, but taken on July 19, 2000.

We measured CO_2 exchange in different colors of light in the field to see how gas-exchange measurements correlated with the spectral properties of individual cells we reported previously, where cells were found to screen out light of wavelengths $<550 \text{ nm}$ (18). We used broad-band filters to determine the photosynthetic response to red, blue, and green light by using window screen to adjust the total PAR inside the cuvette so it was approximately the same for each color. We superimposed these results on a light curve for white light obtained the same day (Fig. 6). Red light clearly drove more photosynthesis than the equivalent irradiance of white, and blue and green both drove significantly less, because astaxanthin prevented the short-wavelength light from reaching the chloroplast. The blue filter we used had some transmittance in the red (Fig. 2), and we expect that a purer blue would have shown even less photosynthesis because astaxanthin blocks almost all of the blue light (19).

Discussion

C. nivalis clearly takes up CO_2 from the air, and some particularly rich patches of snow have area-specific rates $\approx 10\%$ of the rates of many green plants. We found large patch-to-patch variations in gas-exchange rates, usually correlated with algal densities but

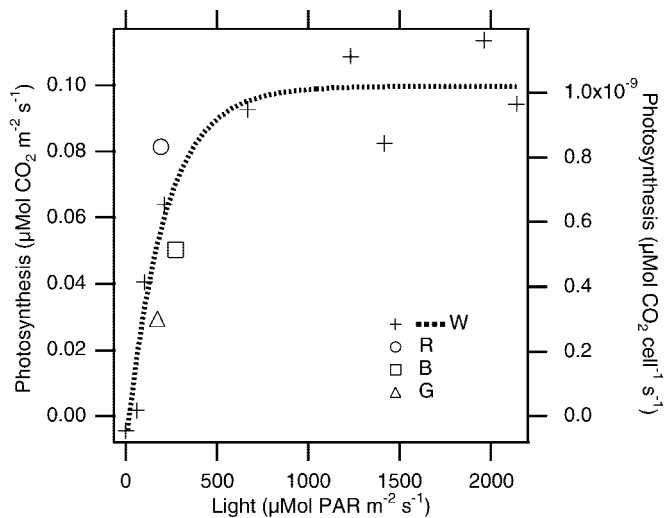


Fig. 6. White-light curve with superimposed points taken with red, blue, and green filters.

not always (data not shown). We did the light curve (Fig. 6) in the year 2001, the black/white experiment (Fig. 3) in 1999, and daily course measurements in 2000 and 2001; 2000 was a very low snow year for the Snowy Range, and there was clearly much less snow algae present than in the year before, even in those few snowfields that persisted over the summer. The year 2001 was intermediate between 1999 and 2000. Rates at light saturation varied from 0 (with dark rates strongly negative) to $0.3 \mu\text{mol CO}_2\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, and algal densities only seemed to account for part of this variability. In particular, even very red patches (when they were possible to find) from the low-snow year of 2000 seemed to have much lower rates than moderately pink patches from the year before. Algal densities averaged $\approx 2 \times 10^7$ cells m^{-2} in pink patches in all years sampled, so variation in cell concentration apparently cannot account for all of the variation we saw in photosynthetic rates.

It is likely that *C. nivalis* gets much of its CO_2 from respiration within or beneath the snow, as indicated by some of our daily courses in which practically all values of photosynthesis were negative; i.e., the patches of snow were giving off CO_2 rather than taking it up (Fig. 4). Presumably, curves such as these occur because the algae's photosynthetic activity acts against a background of constant CO_2 efflux from the snow surface. Such efflux certainly does occur: in winter and spring, CO_2 effluxes from the snow ranging from 0.2 to $0.5 \mu\text{mol CO}_2\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ have been calculated from a location quite close to our field site (21, 22). As a rule, our more negative gas-exchange rates came from algal patches growing in relatively thin snow, which is consistent with these studies. Thus, some of the patch-to-patch variation we observed is probably caused by localized variations in under-snow respiration rates and is, perhaps, caused by different substrates (i.e., rock slabs vs. soil) and different snow depths. Perhaps stable-isotope studies could illuminate what proportion of the algae's carbon comes from the atmosphere and what comes from soil respiration.

We saw no indication of photoinhibition in the field (Fig. 6) despite the near-freezing temperatures, high incident light levels, and reflection from the underlying snow. The algae's high content of astaxanthin, located around the chloroplast where it can absorb light that might otherwise strike the photosynthetic apparatus, probably protects the cells from excessive light. In addition, the pigments in any single cell will absorb some of the light that might otherwise strike neighboring cells, reducing light

levels below what might be found in algae-free snow. Astaxanthin in *C. nivalis* absorbs blue light strongly, as shown by spectrophotometric measurements of monolayers of cells and of individual cells (18). The astaxanthin can also confer substantial UV protection, and the algae also contain phenolics that may help protect against UV damage; the concentration of phenolics is regulated in part by UV exposure (25). It may be that the algae close to the surface were being photoinhibited while increased contributions from the lower layers of algae, which were receiving less light, compensated for this. It is also possible that our experimental conditions were simply not severe enough to cause photoinhibition. The Plexiglas cuvette transmitted $\approx 92\%$ of the visible light but virtually no UV light, so algae under the box were exposed to somewhat less severe radiation stress than they would be likely to experience outside the apparatus.

With the data presented here, it is possible to estimate what contribution photosynthesis in the snow might make to large-scale CO_2 exchange. If we integrate the data from Fig. 5, we get a daily rate of $2,256 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{day}^{-1}$, or $7.78 \times 10^{-5} \mu\text{mol}$ per cell per day. If we knew the total quantity of algae in a given area of snow, we could multiply it by such an integrated rate to obtain daily CO_2 flux. Painter *et al.* (26) in the Sierra Nevada Mountains of California have recently developed the use of remote sensing to estimate snow-algae densities: a trial scan of their field site found a mean concentration of $1,300$ cells ml^{-1} over an area of 0.495 km^2 of snow. Recent reanalysis of their data suggests that the number should be closer to 300 cells ml^{-1} (T. H. Painter, personal communication). Their assumption that all of the cells are in the top 10 cm of snow would give an average areal density of 3×10^7 cells m^{-2} . Multiplying by the integrated photosynthesis rate of $7.78 \times 10^{-5} \mu\text{mol}$ per cell per day gives a mean daily CO_2 -fixation rate of $2.3 \times 10^3 \mu\text{mol}\cdot\text{m}^{-2}$ per day, or $2.8 \times 10^4 \mu\text{g C m}^{-2}$ per day. Assuming that half of the algal biomass is carbon (27) and that the snow algae are active for 90 days per year, we get an overall annual net primary production (NPP) of $\approx 5 \text{ g m}^{-2}$. This result is small but significant, $\approx 2\%$ of the area-specific productivity of arctic heathlands.[†]

This approach, combining gas-exchange measurements with remote sensing of algal concentrations, is promising, but obviously more work needs to be done before we can develop large-scale estimates of snow-algae productivity with confidence. For example, the estimate of average algal concentration over a large area by Painter *et al.* (26) is similar to our measurements of algal concentration in patches consciously chosen for their conspicuous red color. Possibly the area of the Sierra where they worked is much richer in snow algae than the area of the Rockies where we worked, or perhaps there are differences in our methods of measurement or other calculations that could lead to this discrepancy. Also, the area covered by snow algae and the concentration within that area vary greatly during the season: the algae multiply at the same time the snow-cover decreases because of melting. Thus, periodically taking a census of the algal population would be required.

It would be illuminating to know more about the overall ecology of snow algae. We have observed that the algae tend to concentrate on the surface in areas where liquid water runs down into the snow or over the snow. This pattern could be caused by simple passive distribution with the flowing water, but the alga's mucilaginous sheath might also allow it to stay preferentially in areas where liquid water was more available. We also observed that algae seem to concentrate in areas where soil run-off flows onto the surface of the snow (rather than under the snow as happens more frequently). It could be that the nutrients that

[†]Shaver, G. R. (2001) *NPP Tundra: Toolik Lake, Alaska, 1982* (Oak Ridge National Laboratory Distributed Active Archive Center), Vol. 2002, www.daac.ornl.gov.

accompany soil run-off stimulate algal blooms, or perhaps the algal propagules come from the soil. The highest concentrations of snow algae of which we are aware occur on Svalbard (6, 15), below cliffs that support extensive bird-nesting areas, suggesting that nutrient availability can exert a powerful effect on algal concentrations.

Clearly, snow cannot be assumed to be a net CO₂ producer, particularly in the summer months. In fact, under some circumstances it can be a significant CO₂ sink.

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