Environmental Microbiology (2007) **9**(2), 482–491 doi:10.1111/j.1462-2920.2006.01165.x

Impact of carbon metabolism on ^{13}C signatures of cyanobacteria and green non-sulfur-like bacteria inhabiting a microbial mat from an alkaline siliceous hot spring in Yellowstone National Park (USA)

Marcel T. J. van der Meer,[1] Stefan Schouten,[1]* Jaap S. Sinninghe Damsté[1] and David M. Ward[2]
[1]*Royal Netherlands Institute for Sea Research (NIOZ), Department of Marine Biogeochemistry and Toxicology, PO Box 59, 1790 AB Den Burg, Texel, the Netherlands.*
[2]*Montana State University, Department of Land Resources and Environmental Sciences, PO Box 173120, Bozeman, MT 59717, USA.*

Summary

Alkaline siliceous hot spring microbial mats in Yellowstone National Park are composed of two dominant phototropic groups, cyanobacteria and green non-sulfur-like bacteria (GNSLB). While cyanobacteria are thought to cross-feed low-molecular-weight organic compounds to support photoheterotrophic metabolism in GNSLB, it is unclear how this could lead to the heavier stable carbon isotopic signatures in GNSLB lipids compared with cyanobacterial lipids found in previous studies. The two groups of phototrophs were separated using percoll density gradient centrifugation and subsequent lipid and stable carbon isotopic analysis revealed that we obtained fractions with a ~60-fold enrichment in cyanobacterial and an approximately twofold enrichment in GNSLB biomass, respectively, compared with the mat itself. This technique was used to study the diel cycling and ^{13}C content of the glucose pools in and the uptake of ^{13}C-bicarbonate by the cyanobacteria and GNSLB, as well as the transfer of incorporated ^{13}C from cyanobacteria to GNSLB. The results show that cyanobacteria have the highest bicarbonate uptake rates and accumulate glucose during the afternoon in full light conditions. In contrast, GNSLB have relatively higher bicarbonate uptake rates compared with cyanobacteria in the morning at low light levels. During the night GNSLB take up carbon that is likely derived through fermentation of cyanobacterial glucose enriched in ^{13}C. The assimilation of ^{13}C-enriched cyanobacterial carbon may thus lead to enriched ^{13}C-contents of GNSLB cell components.

Received 24 July, 2006; accepted 11 September, 2006. *For correspondence. E-mail schouten@nioz.nl; Tel. (+31) 222 369565; Fax (+31) 222 319674.

Introduction

Cyanobacterial mats in alkaline siliceous hot springs have been studied as models of microbial community composition, structure and function (e.g. Ward *et al*., 1998; 2002; 2006), as well as modern analogues of stromatolites (Ward *et al*., 1992). These mats contain unicellular cyanobacteria, *Synechococcus* spp., that live together with filamentous anoxygenic phototrophs, which may benefit directly or indirectly from cyanobacterial photosynthesis. In such mats cyanobacteria are thought to be the main primary producers (Ward *et al*., 1987; Nold and Ward, 1996). Green non-sulfur-like bacteria (GNSLB), particularly phylogenetic relatives of *Chloroflexus aurantiacus* and *Roseiflexus* species, are the major filamentous anoxygenic phototrophs (e.g. Bauld and Brock, 1973; Weller *et al*., 1991; Ruff-Roberts *et al*., 1994; Ferris and Ward, 1997; Ward *et al*., 1998; Nübel *et al*., 2002). As *Chloroflexus* and *Roseiflexus* grow better photoheterotrophically (Pierson and Castenholz, 1974; Hanada *et al*., 2002; Madigan *et al*., 2005), and low-molecular-weight organic compounds are photoassimilated into mat filaments (Sandbeck and Ward, 1981; Anderson *et al*., 1987; Ward *et al*., 1987), it has been thought that GNSLB grow mainly photoheterotrophically *in situ*, deriving organic compounds directly from cyanobacteria (Bateson and Ward, 1988; Nold and Ward, 1996). While similar isotopic compositions would be expected in case of cross-feeding, lipid biomarkers characteristic of GNSLB (wax esters ranging from C_{30} to C_{37}) are enriched in ^{13}C by ~10–15‰ relative to a cyanobacterial lipid biomarker (the C_{17} n-alkane) in these mats (Summons *et al*., 1996; van der Meer *et al*., 2000).

The most obvious explanation for this is photoautotrophy by GNSLB. *C. aurantiacus* grows photoautotrophically using the 3-hydroxypropionate pathway for CO_2 fixation (Strauss and Fuchs, 1993) and *Roseiflexus* has recently been found to contain genes of this pathway (C.G. Klatt, D.A. Bryant and D.M. Ward, unpubl. results). The 3-hydroxypropionate pathway imparts a smaller

isotopic fractionation (~13–14‰; Holo and Sirevåg, 1986; van der Meer *et al*., 2001) than the Calvin Cycle (~20–25‰; Madigan *et al*., 1989; Sakata *et al*., 1997; Popp *et al*., 1998) and thus yields ^{13}C-enriched fixed carbon. Recent ^{13}C labelling studies confirmed GNSLB autotrophy especially during low light conditions (van der Meer *et al*., 2005).

Alternatively, ^{13}C-enriched carbon from cyanobacteria may be taken up by GNSLB. Nold and Ward (1996) demonstrated that up to 85% of the CO_2 fixed in the mat during daytime photosynthesis can be accounted for as polyglucose. This polyglucose appears to be fermented during the night with the release of short-chain fatty acids, primarily acetate. Recent *in situ* gene expression studies also documented a shift from daytime photosynthesis to night-time fermentation in mat *Synechococcus* populations (Steunou *et al*., 2006). Acetate and other fermentation products are photoincorporated into filaments (Sandbeck and Ward, 1981; Anderson *et al*., 1987), suggesting a mechanism of cross-feeding from cyanobacteria to GNSLB, which van der Meer and colleagues (2005) experimentally demonstrated. Interestingly, sugars have been shown to be strongly enriched in ^{13}C compared with lipid biomarkers in cyanobacteria and other Calvin Cycle photoautotrophs (Deines, 1980; van Dongen *et al*., 2002). It is thus possible that part of the heavy isotopic signatures of GNSLB cell components results from the photoheterotrophic uptake by GNSLB of fermentation products derived from isotopically heavy polyglucose produced by cyanobacteria. Indeed, the dominant mat sugar, glucose, was shown to be strongly enriched in ^{13}C (van der Meer *et al*., 2003). However, as both cyanobacteria and GNSLB are known to produce polyglucose storage materials (Holo and Grace, 1987; Konopka, 1992) it is unclear whether cyanobacteria within the mat produce ^{13}C-enriched polyglucose and how this pool varies over time.

In order to improve the association of cell components and stable isotope signatures with their different sources, we physically separated cyanobacterial biomass from GNSLB biomass by density centrifugation. Analysis of guild-specific glucose ^{13}C signatures, diel cycling of glucose pools and ^{13}C-labelling experiments suggest that both photoautotrophy and photoheterotrophic uptake of cyanobacterial fermentation products can lead to a heavier isotopic signature in GNSLB.

Results and discussion

Density separation of Synechococcus *and GNSLB fractions*

Centrifugation of a gently homogenized microbial mat sample using a percoll density gradient resulted in the formation of two distinct fractions, a green fraction at the bottom and a floating brown-red fraction at the top (Fig. 1). The positioning of the green fraction at the bottom of the density gradient (average density of 1.078 g ml^{-1}) is in agreement with the cyanobacterial cell density of approximately 1.09 g ml^{-1} reported by Sitz and Schmidt (1973). Microscopic inspection revealed that the green fraction was mainly composed of unicellular cyanobacteria, though filaments likely representing GNSLB were also observed; the brown fraction was predominantly comprised of filamentous bacteria but some cyanobacterial cells were also observed. Due to the strong clumping of filamentous bacteria it was impossible to accurately determine the relative percentages of the two cell types.

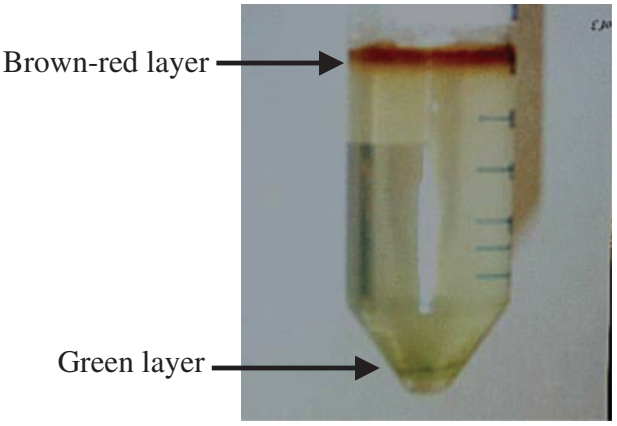

Fig. 1. Photograph of the percoll density gradient separation of GNSLB (brown-red layer) from the cyanobacteria (green layer) present in a hot spring microbial mat sample from Mushroom Spring.

To further evaluate the composition of the fractions, lipid and isotope analysis was performed on the green and brown fractions and compared with the whole mat. The lipid extracts of the whole mat were dominated by C_{16}–C_{18} fatty acids, which are derived from mixed sources [including both *Synechococcus* (Fork *et al*., 1979) and GNSLB (Knudsen *et al*., 1982; van der Meer *et al*., 2001; 2002)], C_{32}–C_{36} wax esters derived from GNSLB (Knudsen *et al*., 1982; van der Meer *et al*., 2001; 2002) and lower amounts of the cyanobacterial C_{17} n-alkane (Gelpi *et al*., 1970; M.T.J. van der Meer, S. Schouten and D.M. Ward, unpubl. results) similar to what has previously been reported (Dobson *et al*., 1988; Shiea *et al*., 1991; Summons *et al*., 1996; van der Meer *et al*., 2000). Differences in lipid concentrations in the mats sampled in the morning and afternoon were only minor and within analytical error (Fig. 2A). The lipid composition of the density separated green and brown fractions were markedly different compared with each other and to the whole mat. The green fraction contained predominantly the C_{16} and C_{18} fatty acid and the C_{17} n-alkane in relatively high amounts (Fig. 2B) and only

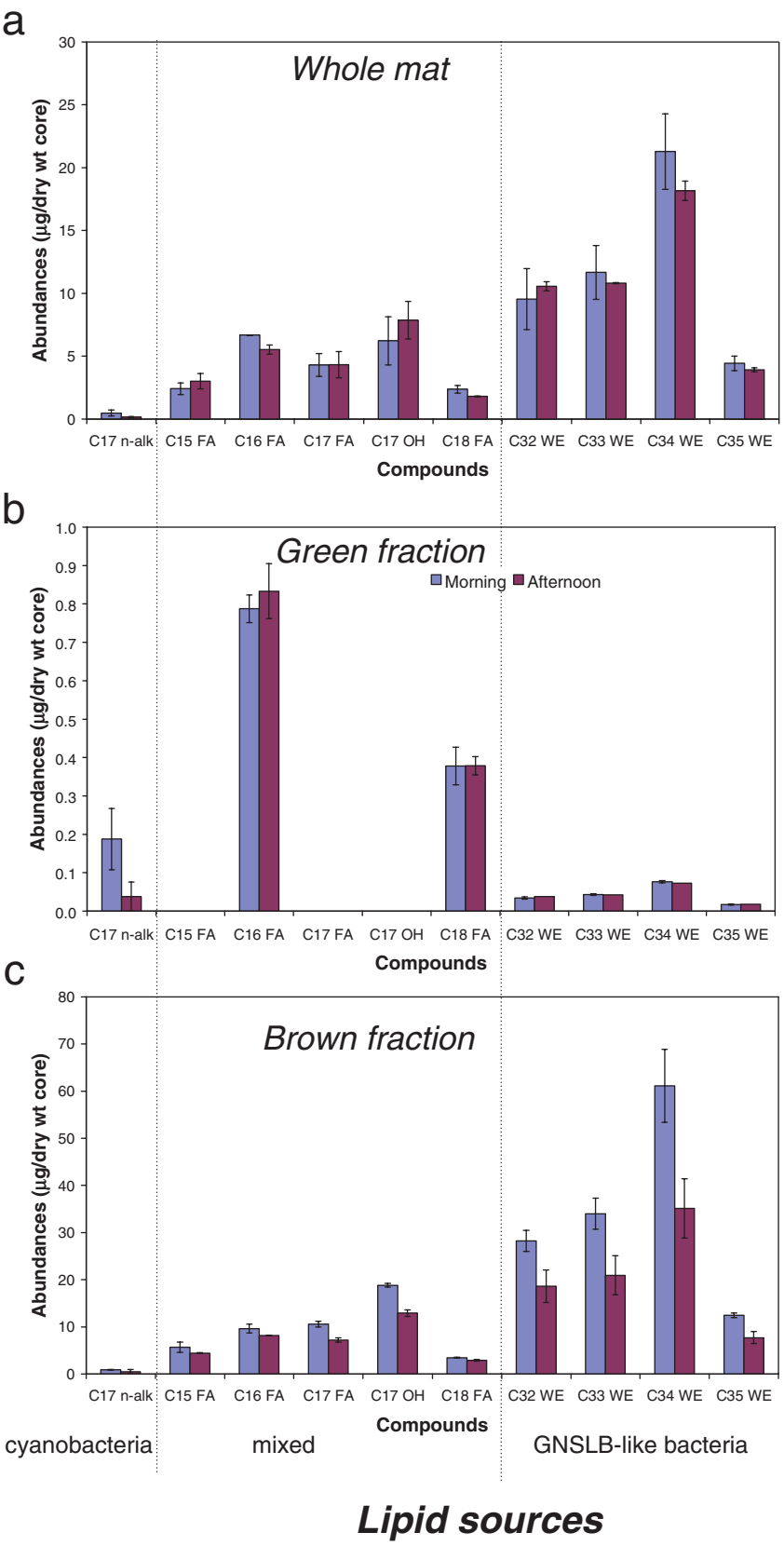

Fig. 2. Concentrations of lipids in (A) whole mat, (B) density separated green fraction, and (C) density separated brown fraction during the morning (blue bars) and afternoon (purple bars). Error bars are standard deviations from replicate core analysis.

Table 1. Average stable carbon isotopic composition (in ‰ versus Vienna Pee Dee Belemnite) of bulk carbon, glucose and lipids of the whole mat and of density separated green and brown fractions.

	Morning			Afternoon		
	Whole mat	Green fraction	Brown fraction	Whole mat	Green fraction	Brown fraction
Bulk	–16.9	–19.4	–15.0	–16.6	–18.3	–16.4
Glucose	–11.0	–10.0	–9.9	–11.4	–15.4	–10.3
C_{17} n-alkane	–27.6	–27.9	n.d.	n.d.	–27.9	n.d.
C_{15} FA	–21.5	n.d.	–20.1	–20.2	n.d.	–21.1
C_{16} FA	–24.7	–28.1	–21.1	–22.9	–27.6	–22.1
C_{17} FA	–21.0	n.d.	–19.8	–19.9	n.d.	–20.2
C_{17} OH	–20.2	n.d.	–19.0	–18.9	n.d.	–19.4
C_{18} FA	–25.1	–27.0	–21.1	–24.1	–26.9	–22.2
C_{32} WE	–20.2	n.d.	–19.6	–19.5	n.d.	–19.9
C_{33} WE	–19.8	n.d.	–19.4	–19.5	n.d.	–19.9
C_{34} WE	–20.0	n.d.	–19.6	–19.5	n.d.	–19.2
C_{35} WE	–19.9	n.d.	–19.9	–19.0	n.d.	–19.4

n.d., not determined due to low abundances. Standard error of replicate analysis was < 1‰.

low abundance of the C_{32}–C_{36} wax esters. In contrast, the brown fraction was dominated by C_{15}–C_{18} fatty acids and C_{32}–C_{36} wax esters with the C_{17} n-alkane in relatively lower abundance (Fig. 2C). These results suggest that the green fraction was indeed predominantly comprised of cyanobacterial cells but not exclusively as evidenced by the presence of wax esters. The brown fraction consisted predominantly of GNSLB though the presence of C_{17} n-alkane indicated that some cyanobacteria cells were still present. Based on changes in the relative ratio of the C_{17} n-alkane and wax esters we estimate that the green fraction was enriched ~60-fold in *Synechococcus* and the brown fraction approximately twofold in GNSLB compared with the mat itself.

Isotopic composition of lipids and glucose in Synechococcus *and GNSLB fractions*

The separation of the two dominant phototrophs allowed us to investigate their ^{13}C-signatures in more detail. The $\delta^{13}C$ of the whole mat was *c.* –17‰ both in the morning and afternoon (Table 1, Fig. 3A and B), similar to what has been reported previously (van der Meer *et al.*, 2000; 2003). In comparison, the biomass contained in the brown fraction was slightly enriched in ^{13}C compared with the whole mat while the biomass of the green fraction was significantly depleted in ^{13}C (Table 1, Fig. 3A and B). This observation was extended by ^{13}C analysis of the lipids specific for cyanobacteria and GNSLB. The C_{32}–C_{36} wax esters were enriched by *c.* 7‰ relative to the cyanobacterial C_{17} n-alkane both in the whole mat and in the brown and green fractions respectively (Table 1). Intriguingly, the C_{16} and C_{18} fatty acids which are abundant in both the brown and green fractions are isotopically distinct, i.e. they are enriched by 5–7‰ in the brown fraction compared with the green fraction. The $\delta^{13}C$ values of these acids in the whole mat have intermediate values suggesting that these compounds are derived from both cyanobacteria and GNSLB. Finally, glucose was isotopically much heavier than lipid biomarkers, showing enrichment in ^{13}C of 12–17‰ relative to the C_{17} alkane in the green fraction and of ~10‰ relative to wax esters in the brown fraction. Glucose in the green fraction was more depleted in ^{13}C in the afternoon samples and this value may be more representative of the ^{13}C signature of cyanobacterial polyglucose, which would have accumulated to near maximum levels by that time (see below). This ^{13}C signature of –15.4‰ is heavier than that of GNSLB lipids (*c.* –19‰ to –21‰), suggesting that organic compounds derived from fermentation of cyanobacterial polyglucose, if incorporated by GNSLB, could lead to a ^{13}C-enrichment of GNSLB biomarkers.

Spatio-temporal distribution of glucose in the mat and Synechococcus *and GNSLB fractions*

Association of much of the glucose in the mat with phototrophs was indicated by the observation that most of the glucose was found in the upper ~1 mm of the mat (Fig. 4A), where light is sufficient to support photosynthesis (Ramsing *et al.*, 2000; Ward *et al.*, 2006). The abundance of total glucose was determined over a diel cycle both in the whole mat and in the density separated green and brown fractions. The results showed that both cyanobacteria and GNSLB contribute to the glucose pool (Fig. 4B). Although it appears that the GNSLB contribution is larger, this may have been an artefact of sampling to a depth of ~5 mm, because cyanobacteria are restricted to the uppermost 1 mm interval at this temperature, while GNSLB are also found deeper in the mat (Ramsing *et al.*, 2000).

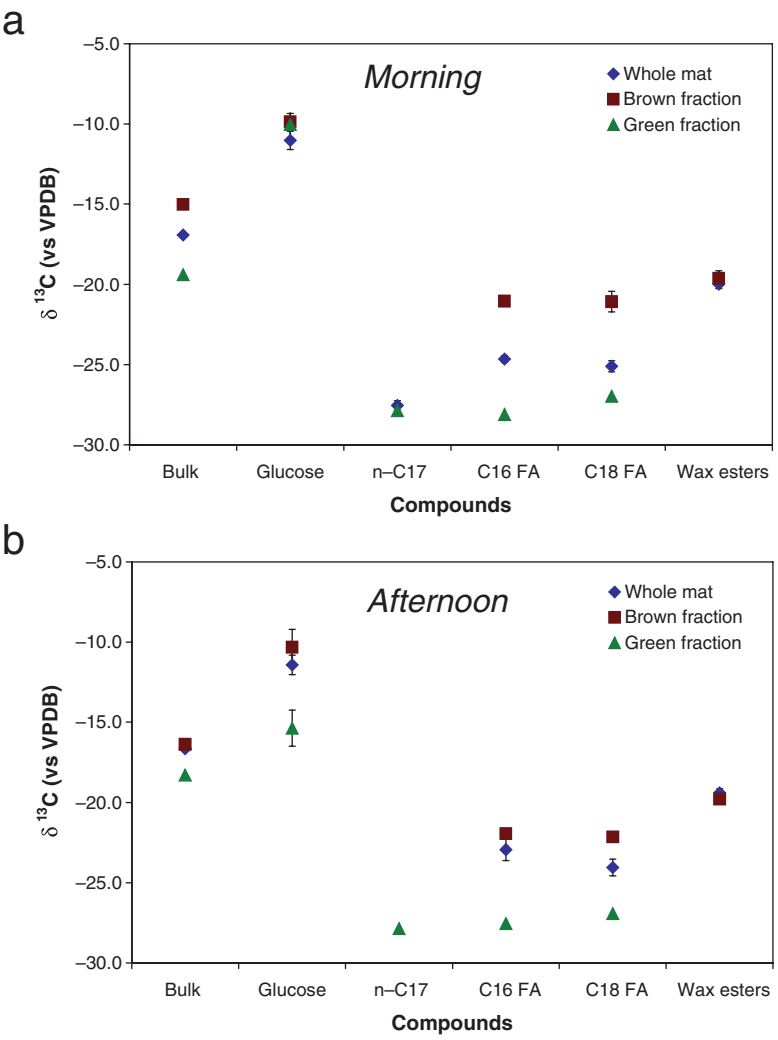

Fig. 3. Stable carbon isotopic composition of lipids in the whole mat (blue diamonds) and density separated green (green triangles) and brown (brown squares) fractions (A) during the morning and (B) during the afternoon. Error bars are standard deviations from replicate isotope analysis.

Interestingly, there is a diel pattern in the abundance of glucose which is particularly evident in the whole mat and in the green fraction containing predominantly cyanobacterial biomass (Fig. 4B). In fact, this pattern can also be visualized by the position of the green fraction in the percoll gradient (Fig. 4C). Beginning in the morning of the first day of sampling (09:22 in Fig. 4C), the green fraction descended to the bottom of the gradient through the afternoon, then migrated upwards throughout the evening and reached a maximum by early morning (08:50) before descending again to the bottom throughout the next afternoon. This was probably due to changes in *Synechococcus* cell density upon the synthesis during the day and fermentation during the night of polyglucose (Konopka, 1992; Nold and Ward, 1996). A diel cycle was less obvious for the glucose concentration in the brown fraction containing GNSLB.

^{13}C-bicarbonate uptake by Synechococcus *and GNSLB fractions*

To further investigate carbon metabolism in these mat phototrophs we performed ^{13}C-bicarbonate labelling experiments to estimate bicarbonate uptake rates (see van der Meer *et al.*, 2005) during the morning, when glucose concentrations are low, and during the afternoon when glucose concentrations are high. This showed that the uptake of bicarbonate in the whole mat was significantly lower in the morning than in the afternoon (Table 2, Fig. 5A). This is not surprising as light levels were still low in the morning, thereby limiting photosynthesis. Interestingly, during the morning the highest uptake rate of the bicarbonate was in the brown fraction, i.e. the GNSLB with 1.1 ± 0.1 mg C g^{-1} dry weight h^{-1}, compared with the green fraction mostly comprised of cyanobacteria with 0.8 ± 0.5 mg C g^{-1} dry weight h^{-1} (Table 1, Fig. 5A). These

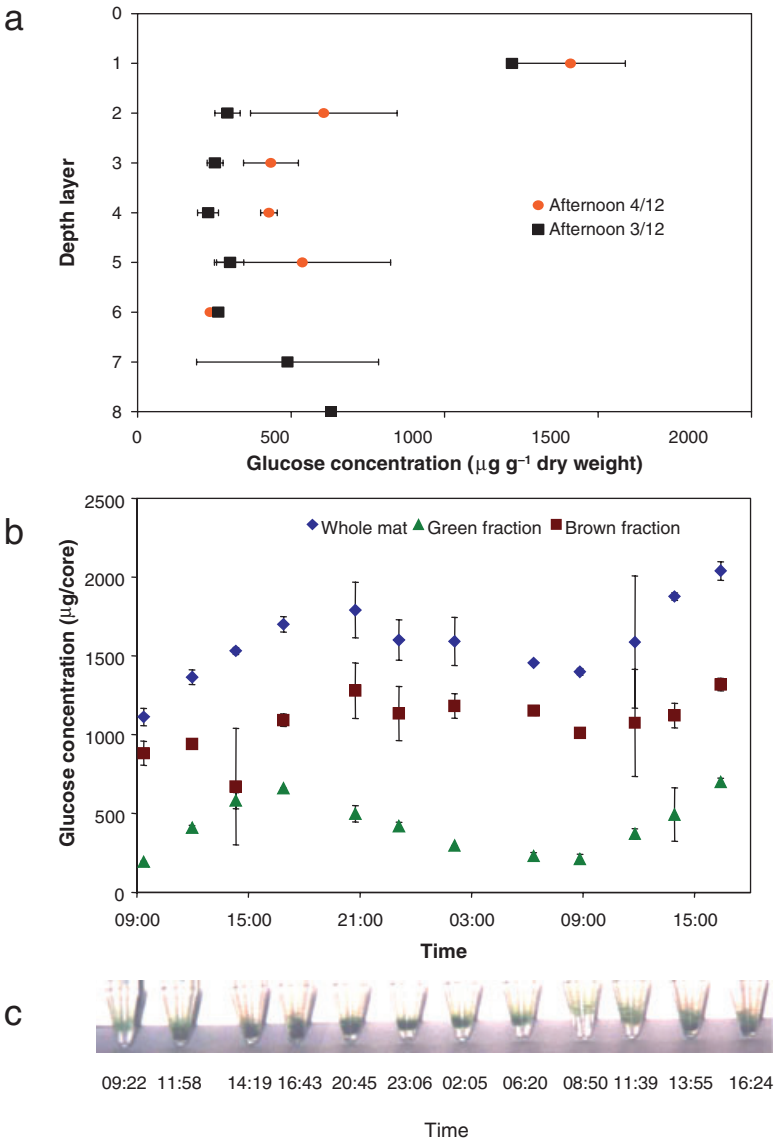

Fig. 4. Glucose distribution in Mushroom Spring mat and percoll fractions.
A. Glucose concentration with depth in the mat.
B. Diel variation in the total glucose content of the whole mat and density separated green and brown fractions.
C. Photo of the percoll density gradient separation of the green fraction from the mat samples collected over a diel cycle.

Table 2. Uptake and uptake rates of ^{13}C label into biomass and density separated green and brown fractions during the ^{13}C-bicarbonate labelling experiment and ^{13}C redistribution following afternoon pulse-labelling and subsequent overnight incubation.

Time	Biomass	Bicarbonate uptake (mg C g^{-1} dry weight)	Bicarbonate uptake rate (mg C g^{-1} dry weight h^{-1})
Morning	Whole mat	1.7 ± 0.7	0.9 ± 0.2
	Green fraction	0.8 ± 0.2	0.4 ± 0.2
	Brown fraction	1.1 ± 0.1	0.6 ± 0.1
Afternoon	Whole mat	10.4 ± 4.8	10.4 ± 3.0
	Green fraction	4.5 ± 0.6	4.5 ± 0.6
	Brown fraction	3.0 ± 0.9	3.0 ± 0.9
Next morning	Whole mat	6.4 ± 0.9	n.r.
	Green fraction	3.5 ± 0.7	n.r.
	Brown fraction	4.1 ± 1.3	n.r.

n.r., not relevant.

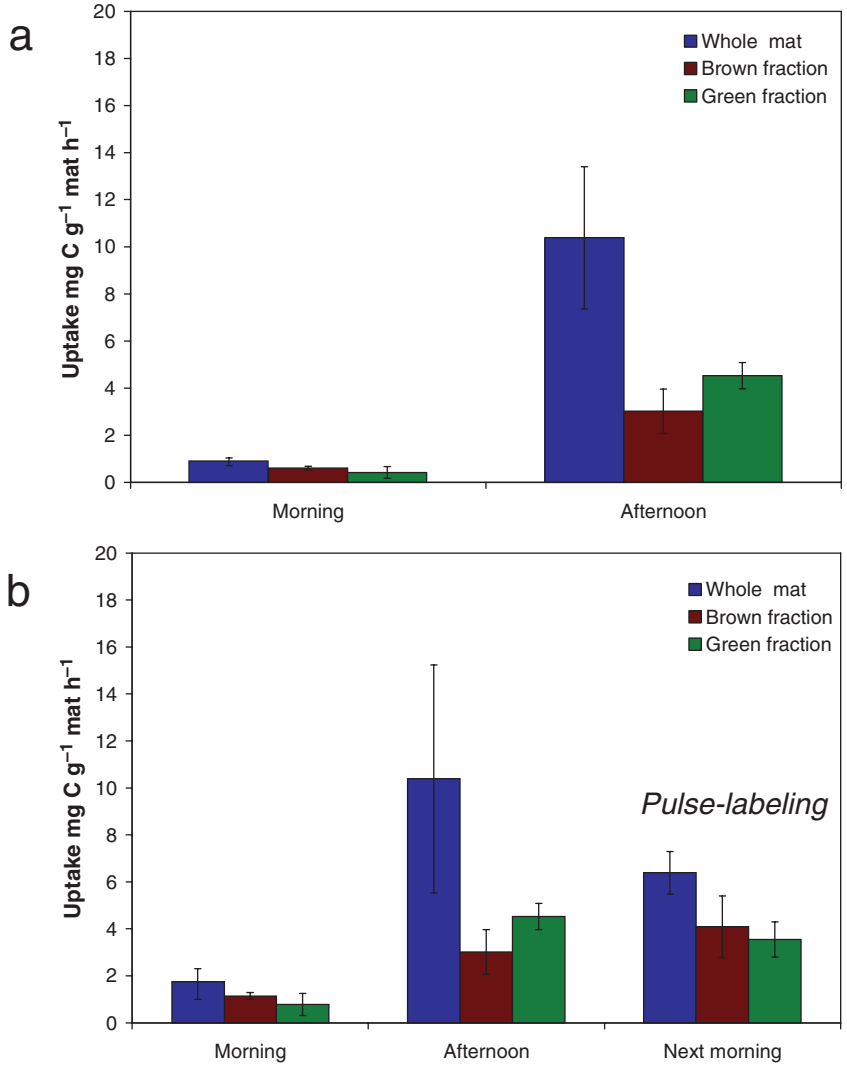

Fig. 5. Results of ^{13}C-bicarbonate labelling experiments.
A. Morning and afternoon uptake rates of ^{13}C-bicarbonate of microbial mat from Mushroom Spring and density separated green and brown fractions derived from this mat.
B. Amount of ^{13}C-bicarbonate taken up in the whole mat and density separated green and brown fractions during incubation with ^{13}C-bicarbonate in the morning and afternoon and amount of ^{13}C-bicarbonate still present the next morning in the whole mat and density separated green and brown fractions after removal of the ^{13}C-bicarbonate.

numbers compare very well with previous estimates based on ^{13}C label incorporation in specific biomarkers in a 1999 ^{13}C-bicarbonate labelling experiment, i.e. 0.8 and 0.5 mg C g^{-1} dry weight h^{-1} for GNSLB and cyanobacteria respectively (van der Meer *et al.*, 2005). This suggests that the two methods used, i.e. one based on incorporation of ^{13}C in specific compounds and one based on incorporation of ^{13}C in density separated cell material, resulted in very similar estimates of carbon uptake rates.

The bicarbonate uptake rate in the afternoon was an order of magnitude higher in the green fraction compared with the morning and most of the bicarbonate incorporation was present in this fraction. The brown fraction had a lower rate of bicarbonate uptake in the afternoon compared with the green fraction. However, as with the green fraction, the rate in the brown fraction was also significantly higher in the afternoon than in the morning. This may not necessarily be associated with mid-day autotrophy by GNSLB, because the brown fraction contains some cyanobacterial cells (see above) and rapid cross-feeding of cyanobacterial photosynthate to GNSLB occurs (Bateson and Ward, 1988). The extent of GNSLB ^{13}C incorporation is, however, rather high during the afternoon, raising the interesting question of whether such anoxygenic photoautotrophy occurs, and if so, how photoautotrophy by this anoxygenic phototroph is shielded from the superoxic conditions of the photic zone at that time of day (Ramsing *et al.*, 2000; Ward *et al.*, 2006). Our results are in agreement with the hypothesis that GNSLB are the main autotrophs during low light levels, while at full light levels cyanobacteria are the dominant autotrophs (van der Meer *et al.*, 2005).

Carbon transfer between Synechococcus *and GNSLB fractions*

In order to examine carbon transfer between cyanobacteria and GNSLB, we performed a pulse-labelling

experiment during the natural day/night cycle by incubating mat samples with ^{13}C-bicarbonate in vials during the afternoon, then, following incubation, removing the overlying water containing unincorporated ^{13}C-bicarbonate before continuing incubation through the rest of the afternoon, evening and night. After pulse labelling in the afternoon more than half of the ^{13}C label which was incorporated into the mat was present in the biomass of the green fraction (Table 2, Fig. 5B). By early next morning the amount of ^{13}C label in the green fraction had somewhat decreased while the amount of ^{13}C label in the brown fraction had slightly increased, suggesting transfer of carbon from cyanobacteria to GNSLB. However, the statistical evidence to support this conclusion is not strong ($P < 0.08$ and 0.17 respectively; one tail, unequal variance *t*-test). This may have been due to our experimental procedure, as we purposefully terminated the overnight incubation before light shone on the mat in order to exclude possible interference from photoautotrophic fixation of $^{13}CO_2$ produced during the night from cyanobacterial fixed carbon. However, this may have prevented the photoheterotrophic uptake of fermentation products. In previous pulse labelling studies (van der Meer *et al*., 2005) we noted dramatically greater ^{13}C incorporation into GNSLB upon continued morning incubation, suggesting that light is important for the incorporation of fermentation products in GNSLB. Furthermore, addition of a mixture of acetate and glycolate prevented this incorporation, suggesting that acetate was a likely intermediate. Anderson and colleagues (1987) demonstrated an increase in fermentation products, notably acetate, in morning mat samples. Thus, it is likely that acetate and other fermentation products derived from *Synechococcus* fermentation accumulate during the night, consistent with the nighttime expression of fermentation genes in *Synechococcus* (Steunou *et al*., 2006) and are available for uptake once light is available. Indeed, during daytime there is a build-up of cyanobacterial glucose while it disappears during the night (Fig. 4B).

Conclusions

Our data show that percoll gradients successfully separate cyanobacteria and filamentous GNSLB from a hot spring microbial mat to a large degree. Application of this technique to ^{13}C labelling experiments showed that GNSLB are photoautotrophic during low light levels in the morning and that cyanobacteria are the dominant photoautotrophs at full light levels. Isotopically heavy polyglucose biosynthesized by cyanobacteria during the day is subsequently fermented and the fermentation products consumed heterotrophically by GNSLB during the night and early morning. Thus, photoautotrophy and cross-feeding of polyglucose-derived fermentation products represent two mechanisms that likely both contribute to the heavy isotopic content of GNSLB cell components.

Experimental procedures

Sampling

Samples were taken from an ~60°C site in the microbial mat of Mushroom Spring located in Yellowstone National Park, Wyoming, USA. Duplicate cores (*c*. 0.5 cm depth) were taken using a No. 4 cork borer at 09:22 h in the morning and at 16:43 h in the afternoon on 3rd November 2001 to obtain microbial mats for density separation and vertical profiling; the latter were subsampled by cutting parallel to the mat surface with a razor blade at ~1 mm intervals. To determine the diel variation in glucose duplicate mat samples were taken at 2–3 h intervals over a 36 h period during 3rd to 4th November 2001. Mat samples were kept frozen until they were separated by density separation and/or analysed for glucose content.

^{13}C labelling experiments

The ^{13}C labelling experiments were performed using ^{13}C-labelled sodium bicarbonate (Cambridge Isotope Laboratories, 99% ^{13}C, CLM-441) on cores taken just before incubation. The core samples were placed in 4 ml spring water in 7 ml glass screw cap vials, which were capped with Teflon/silica septa and sealed with tape to avoid contamination of the mat with ^{13}C-labelled material. The vials were incubated horizontally with the cores in the upright position in the effluent channel at approximately the same temperature as at the sampling site. A 60 mM stock solution of labelled sodium bicarbonate was made so that 100 μl of stock solution added to 4 ml of *in situ* spring water would result in 10% of the inorganic carbon being labelled; the total concentration of inorganic carbon also increased by 10%. A 1 h dark pre-incubation period was included in the morning to allow the labelled substrate to diffuse further into the cores (van der Meer *et al*., 2005). A dark pre-incubation was not done in the afternoon as this would have created an unnatural light regime. Samples were incubated in the morning from 7:00 to 8:30 and in the afternoon from 13:30 to 14:30 on 3rd November 2001.

In order to evaluate the carbon transfer from cyanobacteria to GNSLB during the night (van der Meer *et al*., 2005) a pulse-labelling experiment was performed by incubating mat samples in vials during the afternoon, as described above, except that the water containing the unincorporated ^{13}C-bicarbonate was removed after the incubation period. The cores were then rinsed with spring water, resealed and incubated overnight in fresh spring water. The incubations were stopped the next morning at 6:45 before light was strong enough for oxygenic photosynthesis to make the mat oxic.

Labelling experiments were done on triplicate cores and cores were sampled in triplicate for bulk isotope measurements. All cores were sampled once for density separation.

Density separation of mat fractions

Samples of the microbial mat collected in the morning and afternoon and from the labelling experiments were homogenized in 10 ml spring water using a Dounce tissue homogenizer. The homogenized samples were separated by percoll density gradient centrifugation (see Pertoft, 2000 and references cited therein). A percoll gradient of *c.* 1.078 g ml^{-1} average density was obtained by mixing the 10 ml of hot spring water, that contained the homogenized cell material, with 15 ml percoll (1.13 g ml^{-1}, ICN Biochemicals). The percoll mixture was centrifuged for 1.5 h in an Eppendorf 5804 centrifuge (5000 rpm/4500G, swing-out rotor with 16.1 cm arm).

Sugar and isotopic analysis

Whole mat samples and density separated fractions were analysed for total sugar content according to the method of Herbert and colleagues (1971).

Stable carbon isotopic composition of bulk cell material was determined by elemental analysis on decalcified (by reaction with 1 N HCl for 18 h) samples using a Carlo Erba Flash elemental analyser coupled to a Thermofinnigan Delta^{PLUS} irmMS system. All samples were analysed at least in duplicate. Lipids were extracted and analysed by GC-irMS as described previously by van der Meer and colleagues (2005). The isotopic composition of glucose was analysed following the procedure of van Dongen and colleagues (2001).

Acknowledgements

We thank M.M. Bateson and B. Lindstrom for help with field work and W.I.C. Rijpstra for analytical assistance. This study was supported by the US National Aeronautics and Space Administration (NASA) Grants NAG5-8824 and 13468 and NASA's support of the Montana State University Thermal Biology Institute (NAG5-8807). The US National Park Service is thanked for permission to conduct research in Yellowstone National Park.

References

Anderson, K.L., Tayne, T.A., and Ward, D.M. (1987) Formation and fate of fermentation products in hot spring cyanobacterial mats. *Appl Environ Microbiol* **53:** 2343–2352.

Bateson, M.M., and Ward, D.M. (1988) Photoexcretion and consumption of glycolate in a hot spring cyanobacterial mat. *Appl Environ Microbiol* **54:** 1738–1743.

Bauld, J., and Brock, T.D. (1973) Ecological studies of *Chloroflexis*, a gliding photosynthetic bacterium. *Arch Microbiol* **92:** 267–284.

Deines, P. (1980) The isotopic composition of reduced organic carbon. In *Handbook of Environmental Isotope Geochemistry.* Fritz, P., and Fontes, J.Ch. (eds). Amsterdam, the Netherlands: Elsevier Scientific Publishing, pp. 329–406.

Dobson, G., Ward, D.M., Robinson, N.R., and Eglinton, G. (1988) Biogeochemistry of hot spring environments: free lipids of a cyanobacterial mat. *Chem Geol* **68:** 155–179.

van Dongen, B.E., Schouten, S., and Sinninghe Damsté, J.S. (2001) Gas chromatography/combustion/isotope-ratio-monitoring mass spectrometric analysis of methylboronic derivatives of monosaccharides: a new method for determining natural ^{13}C abundances of carbohydrates. *Rapid Commun Mass Spectrom* **15:** 496–500.

van Dongen, B.E., Schouten, S., and Sinninghe Damsté, J.S. (2002) Carbon isotopic variability in algal and terrestrial carbohydrates. *Mar Ecol Prog Ser* **232:** 83–92.

Ferris, M.J., and Ward, D.M. (1997) Seasonal distributions of dominant 16S rRNA-defined populations in a hot spring microbial mat examined by denaturing gradient gel electrophoresis. *Appl Environ Microbiol* **63:** 1375–1381.

Fork, D.C., Murata, N., and Sato, N. (1979) Effect of growth temperature on the lipid and fatty acid composition and the dependence on temperature of light-induced redox reactions of cytochrome f and of light energy redistribution in the thermophilic blue-green alga *Synechococcus lividus*. *Plant Physiol* **63:** 524–530.

Gelpi, E., Schneider, H., Mann, J., and Oró, J. (1970) Hydrocarbons of geochemical significance in microscopic algae. *Phytochem* **9:** 603–612.

Hanada, S., Takaichi, S., Matsuura, K., and Nakamura, K. (2002) *Roseiflexus castenholzii* gen. nov., sp. nov., a thermophilic, filamentous, photosynthetic bacterium that lacks chlorosomes. *Int J Syst Bacteriol* **52:** 187–193.

Herbert, D., Phipps, P.J., and Strange, R.E. (1971) Chemical analysis of microbial cells. In *Methods in Microbiology*, Vol. 5B. Norris, J.R., and Ribbons, D.W. (eds). London, UK: Academic Press, pp. 209–344.

Holo, H., and Grace, D. (1987) Polyglucose synthesis in *Chloroflexus aurantiacus* studied by ^{13}C-NMR. *Arch Microbiol* **148:** 292–297.

Holo, H., and Sirevåg, R. (1986) Autotrophic growth and CO_2 fixation of *Chloroflexus aurantiacus*. *Arch Microbiol* **145:** 173–180.

Knudsen, E., Jantzen, E., Bryn, K., Ormerod, J.G., and Sirevåg, R. (1982) Quantitative and structural characteristics of lipids in *Chlorobium* and *Chloroflexus*. *Arch Microbiol* **132:** 149–154.

Konopka, A. (1992) Accumulation and utilization of polysaccharide by hot-spring phototrophs during a light-dark transition. *FEMS Microb Ecol* **102:** 27–32.

Madigan, M.T., Takigiku, R., Lee, R.G., Gest, H., and Hayes, J.M. (1989) Carbon isotope fractionation by thermophilic phototrophic sulfur bacteria: evidence for autotrophic growth in natural populations. *Appl Environ Microbiol* **55:** 639–644.

Madigan, M.T., Jung, D.O., Karr, E.A., Sattley, W.M., Achenbach, L.A., and van der Meer, M.T.J. (2005) Diversity of anoxygenic phototrophs in contrasting extreme environments. In *Geothermal Biology and Geochemistry in Yellowstone National Park.* Inskeep, W.P., and McDermott, T.R. (eds). Bozeman, MT, USA: Thermal Biology Institute, Montana State University, pp. 203–219.

van der Meer, M.T.J., Schouten, S., de Leeuw, J.W., and Ward, D.M. (2000) Autotrophy of green non-sulphur bacteria in hot spring microbial mats: biological explanations

for isotopically heavy organic carbon in the geological record. *Environ Microbiol* **2:** 428–435.

van der Meer, M.T.J., Schouten, S., van Dongen, B.E., Rijpstra, W.I.C., Fuchs, G., Sinninghe Damsté, J.S., *et al.* (2001) Biosynthetic controls on the ^{13}C-contents of organic components in the photoautotrophic bacterium *Chloroflexus aurantiacus*. *J Biol Chem* **276:** 10971–10976.

van der Meer, M.T.J., Schouten, S., Hanada, S., Hopmans, E.C., Sinninghe Damsté, J.S., and Ward, D.M. (2002) Alkane-1,2-diol-based glycosides and fatty glycosides and wax esters in *Roseiflexus castenholzii* and hot spring microbial mats. *Arch Microbiol* **178:** 229–237.

van der Meer, M.T.J., Schouten, S., Sinninghe Damsté, J.S., de Leeuw, J.W., and Ward, D.M. (2003) Compound specific isotopic fractionation patterns suggest different carbon metabolisms among *Chloroflexus*-like bacteria in hot spring microbial mats. *Appl Environ Microbiol* **69:** 6000–6006.

van der Meer, M.T.J., Schouten, S., Bateson, M.M., Ulrich Nübel, U., Wieland, A., Kühl, M., *et al.* (2005) Diel variations in carbon metabolisms of green nonsulfur-like bacteria in alkaline silicious hot spring microbial mats from Yellowstone National Park, USA. *Appl Environ Microbiol* **71:** 3978–3986.

Nold, S.C., and Ward, D.M. (1996) Photosynthate partitioning and fermentation in hot spring microbial mat communities. *Appl Environ Microbiol* **62:** 4598–4607.

Nübel, U., Bateson, M.M., Vandieken, V., Kühl, M., and Ward, D.M. (2002) Microscopic examination of distribution and phenotypic properties of phylogenetically diverse Chloroflexaceae-related bacteria in hot spring microbial mats. *Appl Environ Microbiol* **68:** 4593–4603.

Pertoft, H. (2000) Fractionation of cells and subcellular particles with percoll. *J Biochem Biophys Methods* **44:** 1–30.

Pierson, B.K., and Castenholz, R.W. (1974) Studies of pigments and growth in *Chloroflexus aurantiacus*, a phototrophic filamentous bacterium. *Arch Mikrobiol* **100:** 283–305.

Popp, B.N., Laws, E.A., Bridigare, R.R., Dore, J.E., Hanson, K.L., and Wakeham, S.G. (1998) Effect of phytoplankton cell geometry on carbon isotopic fractionation. *Geochim Cosmochim Acta* **62:** 69–77.

Ramsing, N.B., Ferris, M.J., and Ward, D.M. (2000) Highly ordered vertical structure of *Synechococcus* populations within the one-millimeter-thick photic zone of a hot spring cyanobacterial mat. *Appl Environ Microbiol* **66:** 1038–1049.

Ruff-Roberts, A.L., Kuenen, J.G., and Ward, D.M. (1994) Distribution of cultivated and uncultivated cyanobacteria and *Chloroflexus*-like bacteria in hot spring microbial mats. *Appl Environ Microbiol* **60:** 697–704.

Sakata, S., Hayes, J.M., McTaggart, A.R., Evans, R.A., Leckrone, K.J., and Togasaki, R.K. (1997) Carbon isotopic fractionation associated with lipid biosynthesis by a cyanobacterium: relevance for interpretation of biomarker records. *Geochim Cosmochim Acta* **61:** 5379–5389.

Sandbeck, K.A., and Ward, D.M. (1981) Fate of immediate methane precursors in low sulfate hot spring algal-bacterial mats. *Appl Environ Microbiol* **41:** 775–782.

Shiea, J., Brassell, S.C., and Ward, D.M. (1991) Comparative analysis of extractable lipids in hot spring microbial mats and their component photosynthetic bacteria. *Org Geochem* **17:** 309–319.

Sitz, T.O., and Schmidt, R.R. (1973) Purification of *Synechococcus lividus* by equilibrium centrifugation and its synchronization by differential centrifugation. *J Bacteriol* **115:** 43–46.

Steunou, A.-S., Bhaya, D., Bateson, M.M., Melendrez, M.C., Ward, D.M., Brecht, E., *et al.* (2006) In situ analysis of nitrogen fixation and metabolic switching in unicellular thermophilic cyanobacteria inhabiting hot spring microbial mats. *Proc Natl Acad Sci USA* **103:** 2398–2403.

Strauss, G., and Fuchs, G. (1993) Enzymes of a novel autotrophic CO_2 fixation pathways in the phototrophic bacterium *Chloroflexus aurantiacus*, the 3-hydroxypropionate cycle. *Eur J Biochem* **215:** 633–643.

Summons, R.E., Jahnke, L.L., and Simoneit, B.R.T. (1996) Lipid biomarkers for bacterial ecosystems: studies of cultured organisms, hydrothermal environments and ancient sediments. In *Evolution of Hydrothermal Ecosystems on Earth (and Mars?).* Block, G.R., and Goode, J.A. (eds). Wiley, Chichester, UK: Ciba Foundation Symp. 202, pp. 174–194.

Ward, D.M., Tayne, T.A., Anderson, K.L., and Bateson, M.M. (1987) Community structure, and interactions among community members in hot spring cyanobacterial mats. *Symp Soc Gen Microbiol* **41:** 179–210.

Ward, D.M., Bauld, J., Castenholz, R.W., and Pierson, B.K. (1992) Modern phototrophic microbial mats: anoxygenic, intermittently oxygenic/anoxygenic, thermal, eucaryotic and terrestrial. In *The Proterozoic Biosphere: A Multidisciplinary Study.* Schopf, J.W., and Klein, C. (eds). Cambridge, UK: Cambridge University Press, 309–324.

Ward, D.M., Bateson, M.M., Ferris, M.J., and Nold, S.C. (1998) A natural view of microbial biodiversity within hot spring cyanobacterial mat communities. *Microbiol Mol Biol Rev* **62:** 1353–1370.

Ward, D.M., Papke, R.T., Nübel, U., and McKitrick, M.C. (2002) Natural history of microorganisms inhabiting hot spring microbial mat communities: clues to the origin of microbial diversity and implications for micro- and macrobiology. In *Biodiversity of Microbial Life: Foundation of Earth's Biosphere.* Staley, J.T., and Reysenbach, A.-L. (eds). New York, NY, USA: John Wiley and Sons, pp. 25–48.

Ward, D.M., Bateson, M.M., Ferris, M.J., Kühl, M., Wieland, A., Koeppel, A., and Cohan, F.M. (2006) Cyanobacterial ecotypes in the microbial mat community of Mushroom Spring (Yellowstone National Park, Wyoming) as species-like units linking microbial community composition, structure and function. In *Species and Speciation in Micro-Organisms.* Spratt, B., Staley, J.T., and Fisher, M. (eds). *Phil Trans Royal Soc Ser B* (in press).

Weller, R., Weller, F.W., and Ward, D.M. (1991) 16S rRNA sequences retrieved as randomly primed cDNA from a hot spring cyanobacterial mat community. *Appl Environ Microbiol* **57:** 1146–1151.

