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Soil CO_2 flux and photoautotrophic community composition in high-elevation, 'barren' soil

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Summary

Soil-dominated ecosystems, with little or no plant cover (i.e. deserts, polar regions, high-elevation areas and zones of glacial retreat), are often described as 'barren', despite their potential to host photoautotrophic microbial communities. In high-elevation, subnival zone soil (i.e. elevations higher than the zone of continuous vegetation), the structure and function of these photoautotrophic microbial communities remains essentially unknown. We measured soil CO_2 flux at three sites (above 3600 m) and used molecular techniques to determine the composition and distribution of soil photoautotrophs in the Colorado Front Range. Soil CO_2 flux data from 2002 and 2007 indicate that light-driven CO_2 uptake occurred on most dates. A diverse community of *Cyanobacteria*, *Chloroflexi* and eukaryotic algae was present in the top 2 cm of the soil, whereas these clades were nearly absent in deeper soils (2–4 cm). Cyanobacterial communities were composed of lineages most closely related to *Microcoleus vaginatus* and *Phormidium murrayi*, eukaryotic photoautotrophs were dominated by green algae, and three novel clades of *Chloroflexi* were also abundant in the surface soil. During the light hours of the 2007 snow-free measurement period, CO_2 uptake was conservatively estimated to be 23.7 g C m^{-2} $season^{-1}$. Our study reveals that photoautotrophic microbial communities play an important role in the biogeochemical cycling of subnival zone soil.

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Introduction

Microorganisms capable of photoautotrophy are present in a diverse array of habitats including the ocean, freshwater and soil (Callieri *et al*., 2007; Fernández-Valiente *et al*., 2007; Michelou *et al*., 2007). While photoautotrophic microbial communities in soil have been characterized (e.g. Lange, 2001; Fermani *et al*., 2007; Novis *et al*., 2007), soil microbes have most often been studied in environments where plants are abundant. In these environments, soil microbial communities are generally heterotrophic and therefore rely on primarily plant-fixed carbon (C) (Nemergut *et al*., 2005). In contrast, ecosystems of lesser plant cover (e.g. deserts and polar regions) provide open areas of exposed soil that can favour diverse photoautotrophic microbial primary producers that access C from the atmosphere (Garcia-Pichel *et al*., 2001; Casamatta *et al*., 2005; Wood *et al*., 2008). Although the exposed soil of high-elevation mountain ecosystems provides a unique ecosystem in which plant cover is minimal, these areas have received little study (Ley *et al*., 2004; Nemergut *et al*., 2007). Thus, it is unclear how the composition and activity of the photoautotrophic microbial community in this ecosystem may compare to similar soil environments, such as polar and desert regions.

'Barren' areas of mountain ecosystems are often called the 'subnival zone' or 'frost-debris zone' (Troll, 1973) and are found at elevations between the upper limit of the vegetated zone and the lower limit of the permanently snow- and ice-covered zone (cf. King *et al*., 2008). The extent of the subnival zone in high mountains such as the Rockies, Andes and Himalaya is vast and soil exposure in these mountain regions has increased dramatically in recent years due to the retreat of high-elevation glaciers and ice caps (Arendt *et al*., 2002; Barry, 2006; Raper and Braithwaite, 2006). However, it is currently unclear how microbial communities in the subnival zone obtain the C and energy necessary to sustain life in this oligotrophic environment. In many regions, subnival zone soil is snow-covered for more than 9 months of the year, and the short snow-free period provides limited time for the acquisition of C and nutrients (Ley *et al*., 2004). Nutrient fluxes are generally reliant upon snowmelt and precipitation pulses (Williams *et al*., 1996; 1997; Sickman *et al*., 2003), while the sources of available C are less well understood.

Eolian processes are known to transport dust particles over great distances (Psenner, 1999) and have been shown to be an important depositional source of nutrients for some ecosystems (Reynolds *et al*., 2001; 2006). Previous work in the Colorado Front Range suggests that eolian deposition is important to pedogenesis (Litaor, 1987) and it has been proposed that dust and pollen wind-transported from lower elevations provide the primary source of C to subnival zone soil (Ley *et al*., 2004). While eolian deposition is likely an important source of C to subnival zone soil, the contribution of endogenously fixed C to this ecosystem is unknown.

Here we investigate the activity and composition of the microbial photoautotrophic community in subnival zone soil of the Green Lakes Valley, Colorado Front Range. We measured soil CO_2 flux at discrete time points throughout the snow-free period and used cultivation-independent molecular techniques to provide an overview of the photoautotrophic community. We focused our analysis on two primary objectives: (i) to determine the rate and extent of light-driven CO_2 uptake in subnival zone soils, and (ii) to describe the diversity and vertical distribution of the photoautotrophic microbes present. In this study, we show that light-driven CO_2 uptake indeed occurs in subnival zone soil and that a diverse photosynthetic community is found in the top 2 cm of the soil profile.

Results

Field measurements of soil CO_2 flux conducted during the snow-free periods in 2002 and 2007 indicated that subnival zone soil took up CO_2 in the light and mostly respired CO_2 in the dark (Figs 1 and 2). Over the 2002 season, respiration rates across sites ranged from 0.003 to 0.023 g CO_2 m^{-2} h^{-1}, while uptake rates across sites ranged from –0.010 to –0.163 g CO_2 m^{-2} h^{-1}. All dates sampled in 2002 showed a higher rate of CO_2 uptake compared with respiration (Fig. 1A). In the 2007 season, mean respiration rates ranged from 0.018 to 0.036 g CO_2 m^{-2} h^{-1} at the three sites, and across site mean uptake was between –0.026 and –0.046 g CO_2 m^{-2} h^{-1} (Fig. 2A). For the majority of dates sampled in 2007, CO_2 uptake was greater than respiration. In fact, multiple dates in 2007 showed a small amount of CO_2 uptake in the dark (Fig. 2A).

Despite the fact that melt-out occurred almost 1 month earlier in 2002 than in 2007, air temperatures were higher and precipitation was lower during the same summer months (approximately June through August) in 2007 compared with 2002 (Figs 1B and 2B). Specifically, during the overlapping period in which CO_2 measurements were taken in both years, total precipitation in 2007 was 80 mm while total precipitation in 2002 was 106 mm. Likewise, the mean air temperature during this time period was 11.5°C in 2007 and only 10.3°C in 2002. Therefore, the summer of 2007 was warmer and drier than that of 2002, perhaps explaining the difference in CO_2 flux between these years.

Mean CO_2 uptake values for the 2007 season were –0.036 g CO_2 m^{-2} h^{-1} while mean respiration was 0.0016 g CO_2 m^{-2} h^{-1}. Using these data, we conservatively estimate that 23.7 g C m^{-2} $season^{-1}$ was brought into the soil via endogenous carbon fixation during the 2007 measurement period (July through October). We also estimate that during the shorter measurement period in 2002 (June through August), uptake was comparable at 26.0 g C m^{-2} $season^{-1}$.

In order to assess the presence of potentially photoautotrophic microbes in subnival zone soil, soil CO_2 flux was measured on 27 July 2006 (approximately 2 weeks after snowmelt) and the same soils were then used for molecular-phylogenetic analysis. Light and dark measurements of CO_2 flux on this date indicated that both the uptake and respiration of CO_2 were occurring in the soil. CO_2 flux averaged –0.150 g CO_2 m^{-2} h^{-1} uptake and 0.310 g CO_2 m^{-2} h^{-1} respiration across six sites on this date. Bacterial and eukaryotic small-subunit ribosomal RNA (SSU rRNA) gene clone libraries were sequenced from these soils and revealed the presence of a diverse microbial community.

We focused our analysis on known microbial photoautotrophs from four bacterial divisions: *Cyanobacteria*, *Chloroflexi*, *Chlorobi* and *Proteobacteria* (Bryant and Frigaard, 2006). Only a single *Chlorobi* sequence was discovered (from over 1000 bacterial sequences) and comparative analysis of the proteobacterial sequences indicated that none were closely related to known photoautotrophs (data not shown). In contrast, 40 cyanobacterial sequences were found and 152 *Chloroflexi* sequences were also present. In addition, the 42 eukaryotic algal sequences found showed the presence of a diverse community of microscopic green algae in the soil.

Tests of phylogenetic differentiation using the UniFrac metric (Lozupone *et al*., 2006) and the *P*-test (Martin, 2002) suggest that the cyanobacterial communities were significantly different from one another across sites (UniFrac $P = 0.03$; *P*-test $P = 0.03$ for the average pairwise comparison for three sites). In contrast, the algal communities were marginally differentiated across sites (UniFrac $P = 0.09$; *P*-test $P = 0.09$ for the average pairwise comparison across three sites). Finally, assemblages of *Chloroflexi* were not significantly different across sites (UniFrac $P = 0.81$; *P*-test $P = 0.55$ average pairwise comparison across three sites).

Cyanobacterial and algal sequences were almost exclusively in the top 2 cm of the soil profile (Fig. 3); this is expected because light available for photosynthesis diminishes rapidly with depth. While the total number of

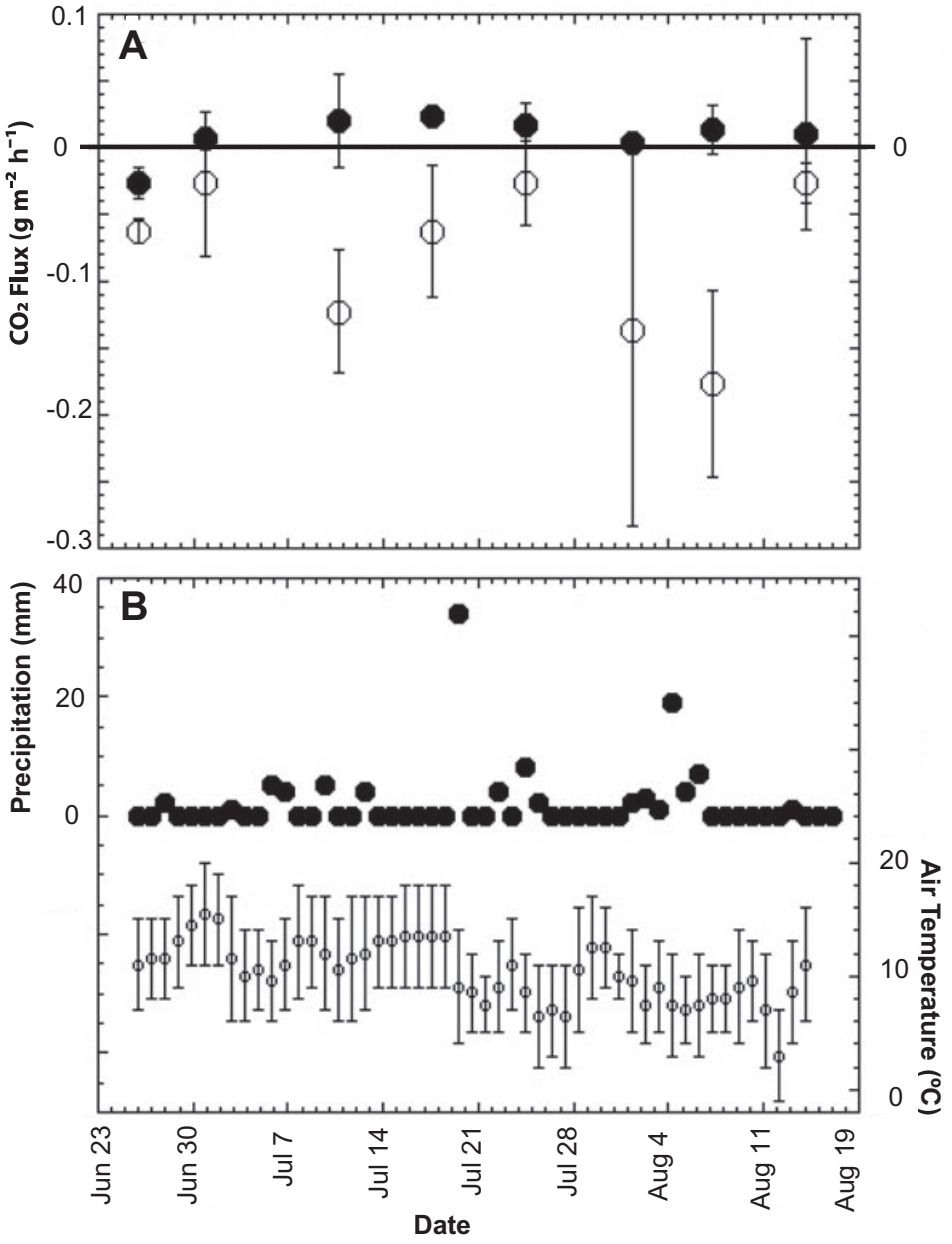

Fig. 1. A. Mean (± standard error of the mean) CO_2 flux values measured between June and August 2002. CO_2 fluxes measured in the light are shown as open circles and measurements of CO_2 in the dark are shown as closed circles.
B. Precipitation and air temperatures during the same period.
Bars indicate daily maxima and minima for air temperatures.

Chloroflexi sequences obtained (152) did not show a similar pattern of predominance in the 0–2 cm soils (data not shown), the assemblages of *Chloroflexi* sequences in the 0–2 cm soil were significantly different from those found in the 2–4 cm soil (UniFrac $P < 0.001$; *P*-test $P < 0.001$). Therefore, the three clades of *Chloroflexi* found almost entirely in the 0–2 cm soil (43 sequences) are the focus of this article, as we can speculate that these are more likely associated with light and therefore potentially photoautotrophic.

Cyanobacterial sequences were present at each of the three sites sampled; however, they were most abundant at the highest elevation site (Site 3) and the only site with some visible signs of dark, crust-like soils. Following the classification of Hugenholtz (2002), many of the cyanobacterial sequences fell within the order *Oscillatoriales*, genus *Tychonema* (Fig. 4). In addition, 13 closely related sequences grouped with the single phylotype previously described as both *Microcoleus glaciei* UTCC 475 (Casamatta *et al*., 2005) and *Phormidium murrayi* (Taton

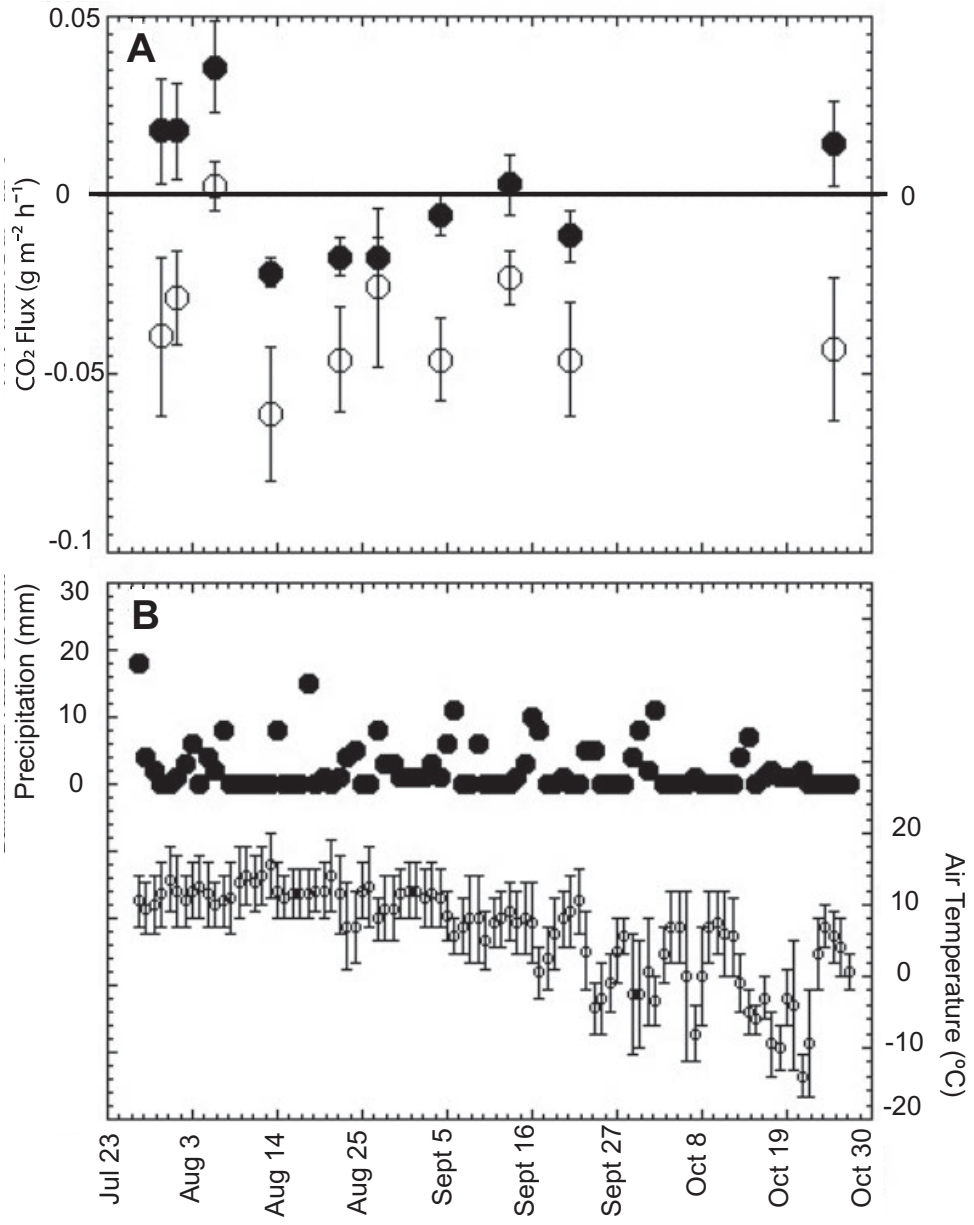

Fig. 2. A. Mean (± standard error of the mean) CO_2 flux values measured between June and October 2007. CO_2 fluxes measured in the light are shown as open circles and measurements of CO_2 in the dark are shown as closed circles.
B. Precipitation and air temperature data during the same period.
Bars indicate daily maxima and minima for air temperatures.

et al., 2006; Comte *et al*., 2007). To our knowledge, this phylotype has been found only in Antarctica (Casamatta *et al*., 2005; Taton *et al*., 2006; Comte *et al*., 2007).

The eukaryotic photoautotrophs in the soil were predominantly green algae with a few sequences from plants (related to the *Bryophyte* family *Mniaceae* and the *Charophyte* families *Klebsormidiophyceae* and *Zygnemataceae*) (Fig. 5). The green algal sequences were predominantly within the class *Chlorophyceae* (also called the *Chlorophyta*), with groups of sequences most closely related to the genera *Chlorococcum*, *Chloromonas* and *Chlamydomonas*. Algal sequences were also found within the class *Trebouxiophyceae* and were most closely related to the genera *Coenocystis* and *Chlorella*.

Analysis of *Chloroflexi*-related sequences revealed the presence of three highly divergent and largely undescribed clades denoted TCh1, TCh2 and TCh3 (Fig. 6). Clade TCh1 was most closely related to the class *Ktedobacteria* and contained two environmental sequences, one isolated from a California grassland soil (GenBank

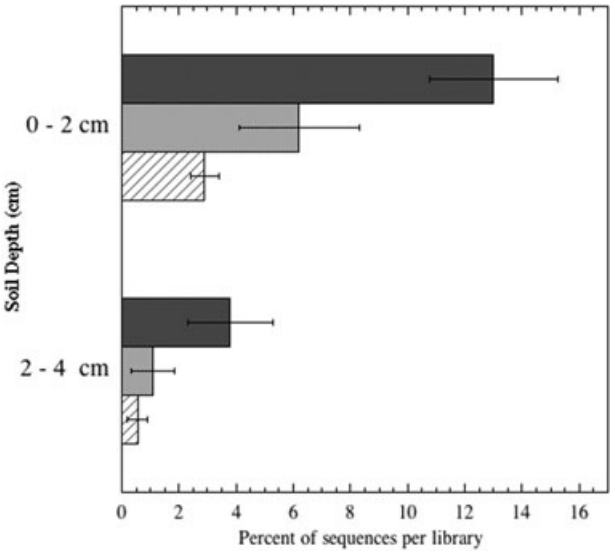

Fig. 3. Mean (± standard error of the mean) percent of *Cyanobacteria* (light grey bars), photosynthetic *Eukarya* (dark grey bars) and novel *Chloroflexi* (hatched bars) sequences in clone libraries ($n = 6$ clone libraries per depth) in the 0–2 cm and 2–4 cm soils. The presence of all groups was significantly higher in the surface soils (0–2 cm).

Accession No. EF516361) and the other from an endolithic sandstone environment in the Rocky Mountains (Walker and Pace, 2007; GenBank Accession No. EF522332). Clade TCh2 was most closely related to all known photoautotrophic *Chloroflexi* and the single heterotrophic genus of this class (*Herpetosiphon*) is deeply basal to this clade. To our knowledge, no other environmental sequences have been identified that fall within this clade. Clade (TCh3) was basal to the known members of the class *Chloroflexi*, distant from, but most closely related to, members of the genus *Herpetosiphon.* To our knowledge there are also no other environmental sequences that fall within the TCh3 clade.

Discussion

Previous work in subnival zone soil of both Colorado and Perú has shown that these high-elevation, unvegetated soil microbial communities are actively cycling C and nitrogen (N) (Ley *et al*., 2004; King *et al*., 2008; Schmidt *et al*., 2008). Furthermore, recent phylogenetic studies have revealed a large diversity and abundance of *Cyanobacteria* in recently deglaciated soil in the high Andes of Perú (Nemergut *et al*., 2007; Schmidt *et al*., 2008). The present study builds upon this previous work and shows that the same Colorado high-elevation sites studied by Ley and colleagues (2004) and King and colleagues (2008) exhibit light-driven CO_2 uptake during the snow-free period and that these soils harbour both known and novel photoautotrophic microorganisms. Moreover, our results contradict the previous suggestion that eolian deposition is the dominant source of C to the subnival zone (Swan, 1992; Ley *et al*., 2004).

Previous work in subnival zone soil of the Colorado Front Range suggested that about 0.5 g C m^{-2} $year^{-1}$ is brought into the soil as a result of eolian deposition (Ley *et al*., 2004). Using our 2007 CO_2 uptake and respiration data, we conservatively estimate that approximately 23.7 g C m^{-2} $season^{-1}$ is added to the soil during the light hours of the short snow-free period. Photosynthetic inputs in 2002 were even higher (an estimated 26.0 g C m^{-2} $season^{-1}$) despite the shorter measurement period (Fig. 1). It should be noted that the summer of 2007 was warmer and drier than that of 2002, perhaps explaining the difference in CO_2 flux between these years. These data counter the previous suggestion that eolian deposition is the dominant input of C to subnival zone soil and show that a significant amount of C is brought into this ecosystem through biological C fixation. The mean rates of C uptake measured in this study are similar to rates estimated for mixed cyanobacterial and algal crusts in polar regions and some desert environments (Garcia-Pichel and Belnap, 1996; Lange, 2001; Novis *et al*., 2007). However, the measured rates are much lower than those for cyanolichen or phycolichen communities (Lange, 2001).

Cyanobacteria and eukaryotic green algae, identified using molecular techniques, may constitute the main groups involved in CO_2 uptake in subnival zone soil. The roles that *Chloroflexi* play in the community remain enigmatic, because this division displays broad physiological diversity and has received little study in soil (Costello and Schmidt, 2006). Nonetheless, all three novel clades of *Chloroflexi* in this study showed the same vertical pattern of distribution in the soil as the *Cyanobacteria* and eukaryotic algae, being more abundant in the top 2 cm of soil compared with the underlying 2 cm, suggesting the potential for phototrophy.

Mixed soil crust communities found in this study are generally comparable to those found in polar and desert regions. The assemblages of *Cyanobacteria* and microscopic eukaryotic algae show similarity in taxonomic grouping despite the fact that these environments may experience very different environmental conditions (e.g. longer summer day lengths in polar regions). Cyanobacterial assemblages at our sites were primarily filamentous, with *Microcoleus* and *Phormidium* phylotypes present, and these organisms are generally considered to be important within a majority of soil crust communities (Garcia-Pichel *et al*., 2001; Belnap, 2003). Interestingly, heterocystous *Cyanobacteria* and phylotypes (e.g. *Leptolyngbya*) that are abundant in many other soil crust communities were not found in this soil. In contrast, a number of unique sequences, most closely related to the

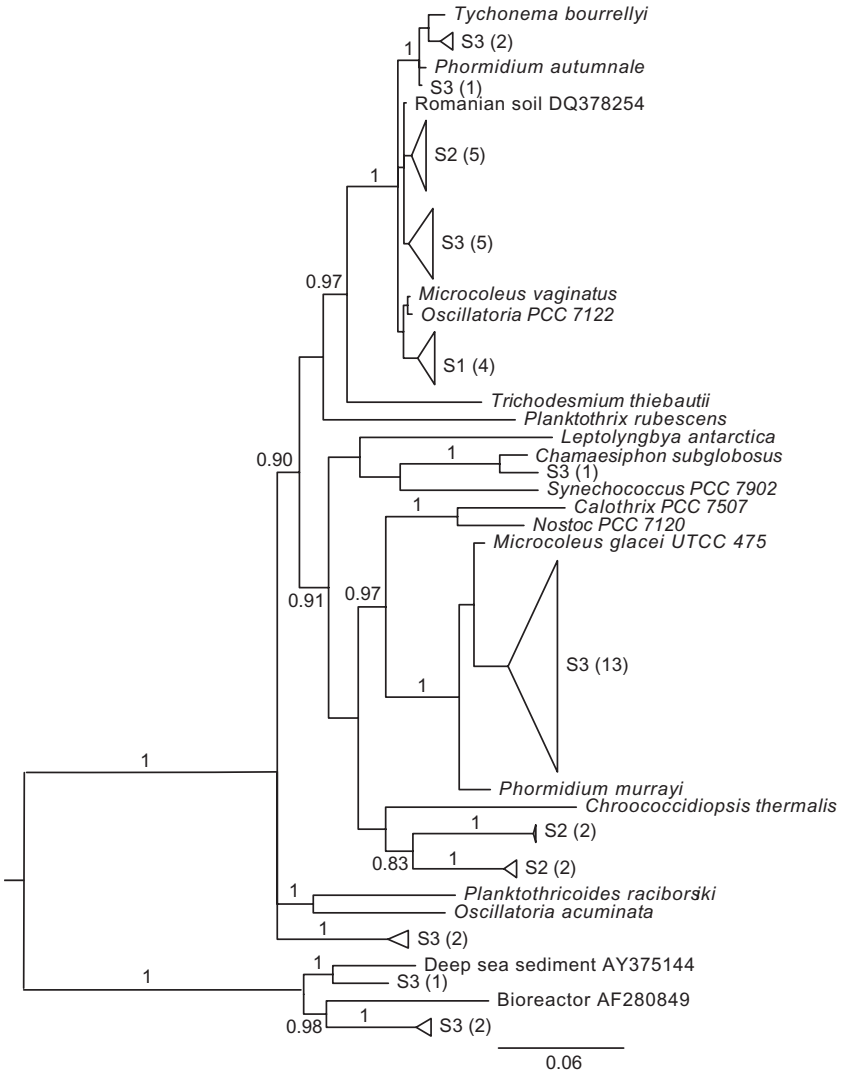

Fig. 4. Bayesian phylogenetic tree of the *Cyanobacteria* found in subnival zone soil. Posterior probabilities are shown for each node. Site 1, Site 2 and Site 3 are indicated as S1, S2 and S3, respectively, with S3 being the highest elevation site (approximately 3798 m). Numbers in parentheses indicate the number of sequences within each wedge. Scale bar represents substitutions per site.

Antarctic phylotype previously described as both *M. glaciei* and *P. murrayi* (Casamatta *et al*., 2005; Taton *et al*., 2006; Comte *et al*., 2007), were found; to our knowledge, this is the first account of this phylotype outside of Antarctica. In addition, the eukaryotic algal sequences in this study were also generally comparable to those found in the few studies of eukaryotic algal communities in polar and desert environments (Broady and Weinstein, 1998; Hu *et al*., 2003; Cardon *et al*., 2008), with a number of *Chlorophyceae* and *Trebouxiophyceae* sequences present. Although they are often abundant in polar and desert soil, the *Desmococcus*-related algae were not found in this study. Furthermore, the novel clades of *Chloroflexi* have been found in very few other environments. To our knowledge, only two closely related sequences have been found in other environments, one of which was a rock endolith of the Colorado Rocky Mountains (Walker and Pace, 2007). Little work to date has examined the role of *Chloroflexi* in soil and to our knowledge research of the diversity and function of *Chloroflexi* in photoautotrophic communities has been limited to the study of hot spring and hypersaline cyanobacterial mats (e.g. Nubel *et al*., 2001; Ley *et al*., 2006). Thus, we can only speculate, based on their vertical distribution, that the *Chloroflexi* groups shown in Fig. 6 are potentially photoautotrophic.

Previous work has shown that *Cyanobacteria* and *Chloroflexi* often cohabitate within hot spring and hypersaline mats (van der Meer *et al*., 2003; 2005; Ley *et al*., 2006). Van der Meer and colleagues (2005) have shown that CO_2 uptake by *Cyanobacteria* and *Chloroflexi* in hot spring mats exhibits a diurnal pattern related to the available wavelengths of light throughout the day. Moreover, the same work suggests that *Cyanobacteria* and *Chloroflexi* utilize different spectra of light for photosynthesis and are photosynthetically active at different times of the day. Previous work has also shown that light penetration through soil is minimal (Woolley and Stoller, 1978; Kasperbauer and Hunt, 1988); however, higher wavelengths, used by

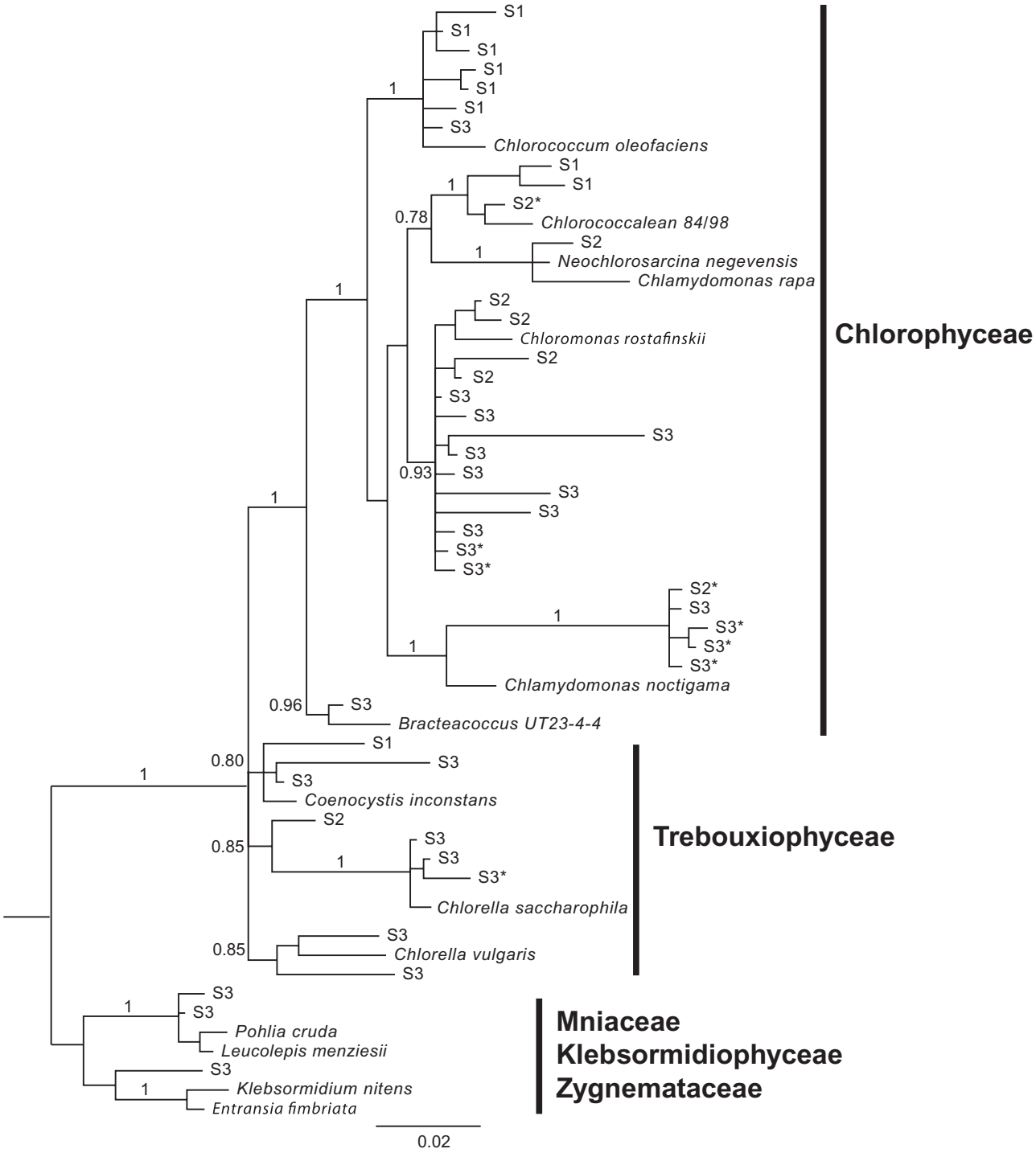

Fig. 5. Bayesian phylogenetic tree of the photosynthetic *Eukarya* found in subnival zone soil. Posterior probabilities are shown for each node. Site 1, Site 2 and Site 3 are indicated as S1, S2 and S3 respectively. An asterisk indicates that the sequence was obtained from the 2–4 cm soils. All other sequences were obtained from the 0–2 cm soils. Scale bar represents substitutions per site.

Chloroflexi (van der Meer *et al*., 2005), have the potential to penetrate much further into the soil (Tester and Morris, 1987). More work is needed to determine the function of the novel clades of *Chloroflexi* and if the *Cyanobacteria*–*Chloroflexi* interactions in soil are similar to those observed in hot spring mats. Furthermore, interactions among *Cyanobacteria*, eukaryotic algae and *Chloroflexi* in soil crusts require additional research.

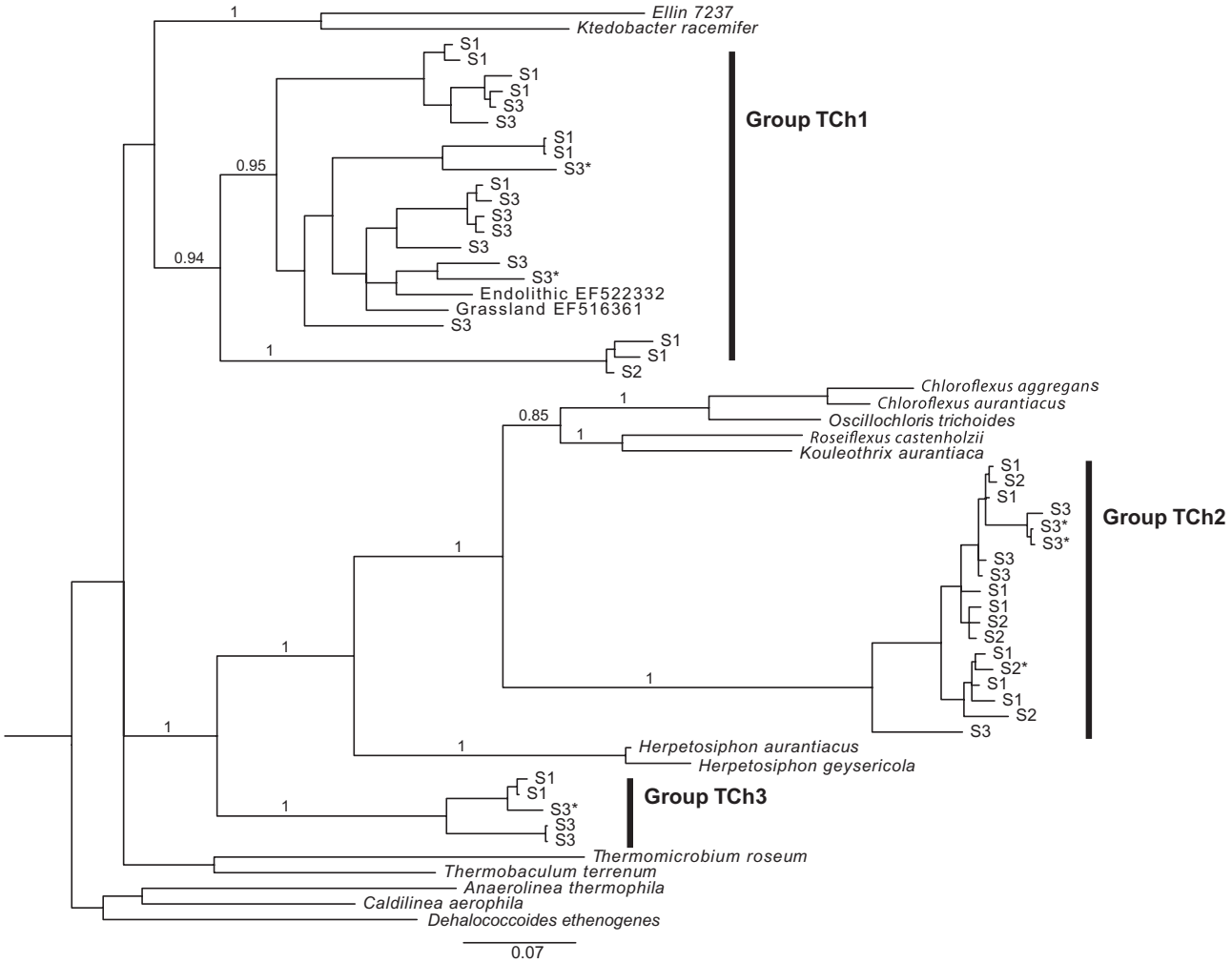

Fig. 6. Bayesian phylogenetic tree of the three novel clades of *Chloroflexi* (Groups TCh1, TCh2 and TCh3) found predominantly in the surface soil (0–2 cm) of the subnival zone. Posterior probabilities are shown for each node. Site 1, Site 2 and Site 3 are indicated as S1, S2 and S3 respectively. An asterisk indicates that the sequence was obtained from the 2–4 cm soils. All other sequences were obtained from the 0–2 cm soils. Scale bar represents substitutions per site.

Our study focused on the identification of photoautotrophic bacteria and eukaryotes in the soil and suggests that these groups contribute to CO_2 uptake. It is important to note that other microorganisms may also contribute to the observed CO_2 uptake. For example, nitrification activity has previously been measured at our sites (Bieber *et al*., 1998) and therefore chemoautotrophic nitrifying bacteria are likely present. While our clone libraries did not suggest an abundance of known chemoautotrophic bacteria in the soil, a few *Nitrospira* sequences were identified and more may be present because rarefaction curves (data not shown) indicated that we have not completely sampled the microbial community (although bacterial rarefaction curves were starting to level off). Moreover, the observed uptake of CO_2 in the dark on some dates in 2002 and 2007 (Figs 1A and 2A) suggests that chemoautotrophy may be an important process contributing to CO_2 fixation. Indeed, previous work has shown that microbial communities can shift in the relative abundance and presence or absence of organisms throughout the season (Lipson *et al*., 2002; Schadt *et al*., 2003; Lipson and Schmidt, 2004; Schmidt and Lipson, 2004). Therefore, it is likely that seasonal shifts in the abundance of photoautotrophic and chemoautotrophic community members may contribute to the variability in light-dependent and light-independent CO_2 fixation throughout the season. A limitation of our study is that we only characterized the potential autotrophs on 1 day. Future work will include multiple sampling dates to help characterize the sea-

sonal fluctuations of photoautotrophs and chemoautotrophs at our study sites.

It has also been suggested that CO_2 flux from barren soils can be abiotically driven. Parsons and colleagues (2004) showed that soils of the Antarctic Dry Valleys can abiotically take up CO_2 as soil temperatures drop and give off CO_2 as soil temperatures rise. This phenomenon is not an explanation for our flux data because we measured CO_2 flux during the morning when air and soil temperatures were rising, yet we almost always observed CO_2 uptake in the light during this period.

The data presented in this study account for CO_2 flux during the light hours of the snow-free season in subnival zone soil; however, the magnitude of night-time and under-snow respiratory losses from the soil are not accounted for. Ample evidence suggests that soil respiration occurs at these and other snow-covered sites during the latter months of the snow-covered period (Brooks *et al*., 1997; Monson *et al*., 2006). Previous work in a subalpine forest has shown that rates of microbial respiration are clearly linked with the depth of the snowpack and therefore the level of insulation provided by the snow (Monson *et al*., 2006). This work suggests that changes in snowpack distribution and extent will likely affect the rates of overwinter microbial respiration. Given that these sites are snow-covered for almost 9 months out of the year, it is possible that all of the C inputs (both eolian and photosynthetic) are respired back to the atmosphere during the snow-covered period. Indeed, previous work suggests that soil organic matter content in subnival zone soil is very low, despite the fact that the soil is quite old (Ley *et al*., 2004; King *et al*., 2008), suggesting that the C balance for these soils may be at steady state. Accordingly, the changes in snow cover distribution and extent, the timing and rate of melt-out, and the increasing temperatures resulting from global change could greatly affect the C balance of these ecosystems, and it is unknown how organisms in subnival zone soil will respond.

The level and extent of environmental change in the subnival zone of the Green Lakes Valley, Colorado has not been quantified; however, studies of mountain ecosystems in this region suggest that change is occurring (e.g. Seastedt *et al*., 2004). We have anecdotally observed an increase in the recruitment of herbaceous plants in some areas of the subnival zone, perhaps indicating that life in this ecosystem may be responding to environmental change. Globally, we have already observed an increase in the area of subnival zone soil as a result of increased glacial retreat (Conen *et al*., 2007; Nemergut *et al*., 2007). Furthermore, it is unclear how changes in snowpack distribution and extent will affect the exposure, activity, and therefore C balance of this ecosystem and future research should investigate the flux and accumulation of carbon in these ecosystems in greater detail. When the vast areas of high-elevation soil present in Asia, South America, Europe and North America are considered, it is clear that the unique microbial communities found in this ecosystem are widespread, but may also be threatened. The subnival zone and the communities present in these environments are deserving of further research.

Conclusions

The data presented here show that both microbially mediated CO_2 uptake and respiration occurred in subnival zone soil, and suggest that the activity of the microbes present varies both seasonally and annually. Temporal variation in C cycling rates is likely linked with some aspect of climate and/or biogeochemical parameters in the soil, as well as with changes in microbial community composition. Furthermore, we have shown that a diverse community of *Cyanobacteria*, *Chloroflexi* and eukaryotic members of the *Chlorophyta*, *Bryophyta* and *Charophyta* are present in the soil. Taken together, our data suggest that microbial photosynthesis provides a significant input of C into subnival zone soil and suggest that eolian deposition, while important, may not be the dominant source of C in the subnival zone.

Experimental procedures

Study site and sample collection

Green Lakes Valley (40°03′N, 105°35′W) is an east-facing headwater catchment in the Colorado Front Range that is approximately 700 ha in area and ranges in elevation from 3250 m to over 4000 m at the Continental Divide. Detailed descriptions of the valley can be found elsewhere (Ley *et al*., 2004) and an aerial photo of the site has been published (top left, fig. 1 in West *et al*., 1999). Bedrock underlying the lower valley is dominated by granites and quartz monzonites of two different intrusions, including Silver Plume monzonite of Precambrian age and Audubon-Albion stock of Miocene age (Caine and Thurman, 1990). The Silver Plume monzonite extends into the upper valley studied here. The subnival zone soils we sampled are Cryic entisols and inceptisols (Williams *et al*., 2001). The catchment is typical of the high-elevation environment of the Colorado Front Range with long, cold winters and a short growing season of 1–3 months (Williams *et al*., 1996).

Unvegetated sites were selected based on previous work in the subnival zone that described microbial biomass, soil moisture and temperature, PAR values and eolian C inputs (Ley *et al*., 2004). Two sites, Sites 1 and 2 (elevation approximately 3657 m), were selected from this previously studied area and an additional higher elevation site, Site 3 (elevation approximately 3798 m), was selected adjacent to the Arikaree glacier within the same watershed. All sampling sites were at least 3 m away from established water-flow paths to avoid sampling of aquatic communities.

CO_2 measurements

Soil CO_2 flux was measured on a weekly or biweekly basis during the snow-free period of 2002 and 2007 and on one date 2 weeks after snowmelt in 2006. CO_2 flux in 2002 was measured using a PP Systems CIRAS-1 (PP Systems, Amesbury, MA, USA) and the 2006 and 2007 CO_2 measurements were taken using the PP Systems EGM-4 Environmental Gas Analyser. Both machines are non-dispersive infrared gas analysers (IRGA) that isolate the infrared band of interest (4.26 μm for CO_2) using an internal filter. The machines include an auto-zero function that corrects for drift in the electronics and dirty optics (PP Systems, Amesbury, MA, USA). Measurements from both machines were taken using a clear 1.18 l chamber in order to allow measurement of both light and dark CO_2 flux. Measurements of CO_2 flux in the light were taken with the clear chamber exposing soil to ambient light conditions, while dark measurements were taken while the chamber was thoroughly covered with an opaque, dark cloth to block out ambient light. The chamber was flushed with ambient CO_2 prior to each soil flux measurement and the auto-zero function was active throughout the measurement process.

Light and dark measurements were taken sequentially from each site. Light measurements integrate both CO_2 uptake and CO_2 respiration, while dark measurements are primarily a measure of respiration. Therefore, light-driven CO_2 uptake rates were calculated by subtracting CO_2 efflux rates measured in the light from those measured in the dark. Data for each site were averaged (mean) across a minimum of three measurement locations at each site and on every date and the standard error was calculated.

Dust versus CO_2

Ley and colleagues (2004) measured dust inputs on the subnival zone slopes in the Green Lakes Valley to be 5 g m^{-2} $year^{-1}$ and showed that C made up 10% of this dust by weight. Therefore we estimated eolian C inputs to be approximately 0.5 g C m^{-2} $year^{-1}$ and used this value to compare photoautotrophic and eolian inputs of C to this ecosystem. A conservative estimate of C inputs from photosynthesis during the 2007 season was calculated from our CO_2 uptake data (averaged over all measurements) by assuming that the period of active photosynthesis lasted for 3 months (late July to late October) and that there were 8 h of usable sunlight per day (actual day length averaged 12.4 h over this period). CO_2 loss from the soil was also determined for the same time period in order to calculate daytime C storage during the 8 h per day photosynthetic period of the 2007 season.

DNA extraction, SSU rRNA gene clone libraries and sequence analysis

Soils were collected on 27 July 2006 at depths of 0–2 cm and 2–4 cm at each of the three sites used for the gas-flux measurements. Samples from each depth were collected from three adjacent areas within each site and were pooled for use in DNA extraction. The soil was sieved to remove rocks greater than 0.5 cm in diameter. The sites are referred to as Site 1, Site 2 and Site 3, with two depths of 0–2 cm and 2–4 cm sampled at each site.

DNA was extracted from the soils using the MO BIO Ultra-Clean Mega Prep Soil DNA kit (MO BIO Laboratories, Carlsbad, CA, USA). Bacterial and eukaryotic community SSU rDNAs were amplified using the *Bacteria*-specific primers 8F (5′-AGAGTTTGATCCTGGCTCAG-3′) and 1391R (5′-GAC GGGCGGTGWGTRCA-3′) and primers 4Fa (5′-TCCGG TTGATCCTGCCRG-3′) and 1492R (5′-GGTTACCTTGTTA CGACTT-3′). Bacterial PCRs were performed with 2.5 mM $MgCl_2$, 0.2 mM each deoxynucleoside triphosphate (dNTP), 0.4 μM each primer, 1 U Taq polymerase (Promega) and buffer supplied with the enzyme using a range of template concentrations. Gradient thermal cycling was carried out for 25 cycles to minimize PCR bias. Amplicons from six different reactions, with different annealing temperatures (± 3°C of optimal), were pooled and gel purified using isolated bands from agarose gels and QIAquick gel purification columns (Qiagen, Valencia, CA, USA). Purified products were ligated into the pCR 2.1 vector (Invitrogen, Carlsbad, CA, USA) and transformed into *Escherichia coli* following the manufacturer's instructions. Transformants were inoculated into a 96-well deep-dish plate containing 1.5 ml of TB Dry nutrient broth (MO BIO Laboratories, Carlsbad, CA, USA). Cultures were shaken at 200 r.p.m. for 16 h at 37°C and then centrifuged. Cell pellets were sent to Functional Biosciences (Madison, WI, USA) for plasmid extraction and were sequenced bidirectionally using sequencing primers T7 and M13R.

Sequences were edited (vector-trimmed and assembled into contigs) using Sequencher 4.1 (Gene Codes, Ann Arbor, MI, USA). Bacterial sequences were aligned using the NAST alignment tool (DeSantis *et al.*, 2006a) and preliminary classifications were obtained using the Greengenes Database (DeSantis *et al.*, 2006b). This alignment was then imported into a 16S rRNA ARB database (Ludwig *et al.*, 2004). Eukaryotic sequences were auto-aligned within ARB and then manually edited. All sequences were chimera checked using Bellerophon (Huber *et al.*, 2004) and Mallard (Ashelford *et al.*, 2006). A mean of 167 non-chimeric bacterial sequences were obtained for each of the three sites at each depth of 0–2 cm and 2–4 cm. The mean number of eukaryotic sequences at each depth was 66 for Sites 1 and 2 and 75 for Site 3.

Phylogenetic analyses were performed using a suite of statistical methods. Phylogenetic trees were inferred using MrBayes (Ronquist and Huelsenbeck, 2003; Altekar *et al.*, 2004) for Bayesian analyses and PAUP 4.0b (Sinauer Associates, Sunderland, MA, USA) for likelihood and parsimony analyses. Bayesian phylogenetic analyses were conducted on the aligned sequences using the GTR + gamma model of evolution with 5 000 000–10 000 000 generations depending on the complexity of the taxa block of interest and the time before convergence. Trees were sampled every 1000 generations. Burnin values were determined using the program Tracer (Rambaut and Drummond, 2007). Consensus trees were created within MrBayes and using PAUP 4.0b. All trees were viewed and modified using FigTree (Rambaut, 2008). In order to assess the significance of phylogenetic differentiation across sites and depths, consensus trees and sequence-

associated environmental data were loaded into the UniFrac website and the UniFrac and *P*-test metrics were calculated (Lozupone *et al*., 2006).

Nucleotide sequence accession numbers

The SSU rRNA sequences generated in this study were submitted to the GenBank database under Accession Nos EU909904–EU910037.

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