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Hypolithic communities: important nitrogen sources in Antarctic desert soils

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Summary

Hypolithic microbial communities (i.e. cryptic microbial assemblages found on the undersides of translucent rocks) are major contributors of carbon input into the oligotrophic hyper-arid desert mineral soils of the Eastern Antarctic Dry Valleys. Here we demonstrate, for the first time, that hypolithic microbial communities possess both the genetic capacity for nitrogen fixation (i.e. the presence of *nifH* genes) and the ability to catalyse acetylene reduction, an accepted proxy for dinitrogen fixation. An estimate of the total contribution of these communities suggests that hypolithic communities are important contributors to fixed nitrogen budgets in Antarctic desert soils.

Introduction

The soil surface environment in the Dry Valley deserts of Eastern Antarctica is too extreme to support macroscopic microbial communities (Wynn-Williams, 1990), although these desert soils contain a high diversity of microbial phylotypes (Smith *et al.*, 2006; Cary *et al.*, 2010). Hypoliths, prokaryote-dominated biological communities found under translucent stones (see Fig. 1A and B), are 'refuge' habitats which extend the range of life into some of the most extreme desert environments on Earth (Cowan, 2009; Pointing *et al.*, 2009). The overlying quartz or marble allows the transmission of incident light while providing protection from the deleterious effects of extreme desiccation, physical disturbance and high UVa/b fluxes (Cockell and Stokes, 2004).

Hypolithic communities are typically dominated by photoautotrophs including cyanobacteria and green algae (Cockell and Stokes, 2004; Wood *et al.*, 2008), but moss-dominated hypolithons have recently been identified in Antarctic desert soils (Cowan *et al.*, 2010). These communities are now thought to make an important contribution to the carbon input budget in depauperate habitats such as polar deserts (Burkins *et al.*, 2001).

However, little is known of the nitrogen budgets in Antarctic desert soils. Organic nitrogen inputs may be derived from legacy biomass or from lake-derived cyanobacterial biomass distributed within the Dry Valleys by aeolian action (Moorhead *et al.*, 1999). Here we demonstrate, for the first time, that hypolithic communities both possess the genetic capacity for N_2 fixation and exhibit significant nitrogenase activity, making them an important, and hitherto disregarded source of fixed nitrogen in Antarctic deserts.

Results and discussion

Using PCR amplification of metagenomic DNA extracts with *nifH* primers PolF and PolR (widely used universal primers for identification of nitrogenase gene clusters) (Poly *et al.*, 2001), we have demonstrated that all hypolith samples investigated ($n = 6$) contain multiple *nifH* phylotypes, which belong to a wide range of known nitrogen fixers (Fig. 2). All of the cyanobacterial sequences belong to the order *Nostocales*, a group of filamentous cyanobacteria that form specialized cells (heterocysts) where nitrogen fixation is localized. 16S rRNA gene sequences from this order, along with *Oscillatoriales* and *Chroococcales*, can be found in soil environments throughout the Dry Valleys (Wood *et al.*, 2008) and globally in cold environments (Yergeau *et al.*, 2007; Jungblut and Neilan, 2010). However, dry soils in Miers Valley do not show nitrogenase activity (J.A. Sohm and D.G. Capone, unpubl. data). The other sequences were identified as members of the proteobacteria, but it is impossible to predict their capacity to fix nitrogen in hypolithic communities. However, we note that certain proteobacteria (e.g. *Azotobacter vinelandii*) are nitrogen fixing symbionts of plants (Chen *et al.*, 2003).

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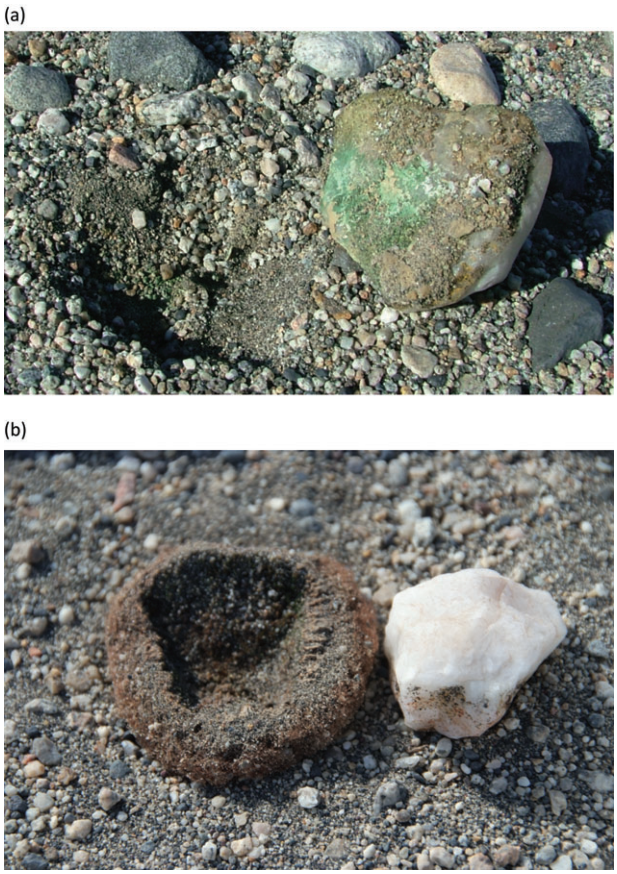

Fig. 1. (A) Cyanobacterial hypolithon; (B) moss-dominated hypolithon.

Nitrogenase activity, determined using the acetylene reduction assay (Capone, 1993), was found in six of the 12 hypolith community samples tested, and ranged over an order of magnitude, 0.02–0.174 nmol N g^{-1} h^{-1} (Fig. 3). Hypoliths selected for this experiment were independent community samples, were not chosen for size or potential stage of development, or for consistency of geochemical factors such as the translucence of the overlying rock. Substantial variability in rates is therefore to be expected.

Acetylene reduction assays were also performed on open soil samples (controls) from 14 different locations in the Miers Valley. No activity was detected in any of these samples.

When acetylene reduction data were recalculated on the basis of total organic carbon content, all values fell within a fourfold range and gave a mean value of 0.73 (SD 0.34) nmol N (mg C)$^{-1}$ h^{-1}. Because these are the first published rates for hypolithic communities, it is difficult to find appropriate examples for comparison. Desert soil crusts are also cyanobacteria-dominated biological communities typical of dry habitats, making them a valid comparative system. Although the range of N fixation rates from the different habitats spans around five orders of magnitude, our surface area normalized acetylene reduction rates (see below) fall within this range (Table 1). Surprisingly, they are also within the range of acetylene reduction found in the wet climate of a Hawaiian rainforest (Matzek and Vitousek, 2003). In comparison, endolithic communities (cyanobacterial- or chlorophyte-dominated communities found within the interstices of granular translucent rocks) both in hot deserts and on the Antarctic continent rarely demonstrate detectable nitrogenase activity (Friedmann and Kibler, 1980).

It was not possible to carry out extensive experiments on the environmental controls on nitrogenase activity in hypoliths because of low sample mass and the desire to minimize environmental disturbances. However, one hypolith was sufficiently large to subdivide into six subsamples in order to assess the effects of light and temperature (sample 5 in Fig. 3). Two samples were incubated under the standard conditions, while the other four were incubated at ambient temperature (ranging from 0°C to +6°C over the course of the 8 h experimental period). Of these four, two were exposed to an ambient sunlight regime, and two were covered in foil to completely exclude light. Nitrogenase activity was not significantly different between any of the treatments ($P > 0.22$), indicating that in the short term, activity is not highly sensitive to the range of temperature or light tested. Assuming that temperature is a major driver of metabolic activity, we note that temperature difference between *in vitro* and *in vivo* incubation conditions was only approximately 10°C, where the maximum difference in activity predicted by Arrhenius behaviour would be twofold.

Over the following austral summer we carried out a second trial on the effects of water on nitrogenase activity. Water availability is known to be very important to nitrogen fixation in cryptic microbial communities (see, for example, Zaady *et al*., 1998; Boison *et al*., 2004). In two experimental samples, nitrogenase activity was not detectable in prior to water addition, but increased to levels equivalent to previous values in samples where 0.5 ml of water was added (0.113 and 0.085 nmol N g^{-1} h^{-1}). While the sample size is small, these results suggest that hypolithic nitrogenase activity is rapidly responsive to water addition. We suggest that this response may be mediated by the state of desiccation of the samples, which might account for the consistently higher acetylene reduction rates in the 2008 experiments and their failure to show a response to water addition.

Using these data on nitrogen fixation in hypoliths together with other data collected as part of an international landscape-scale biocomplexity survey we

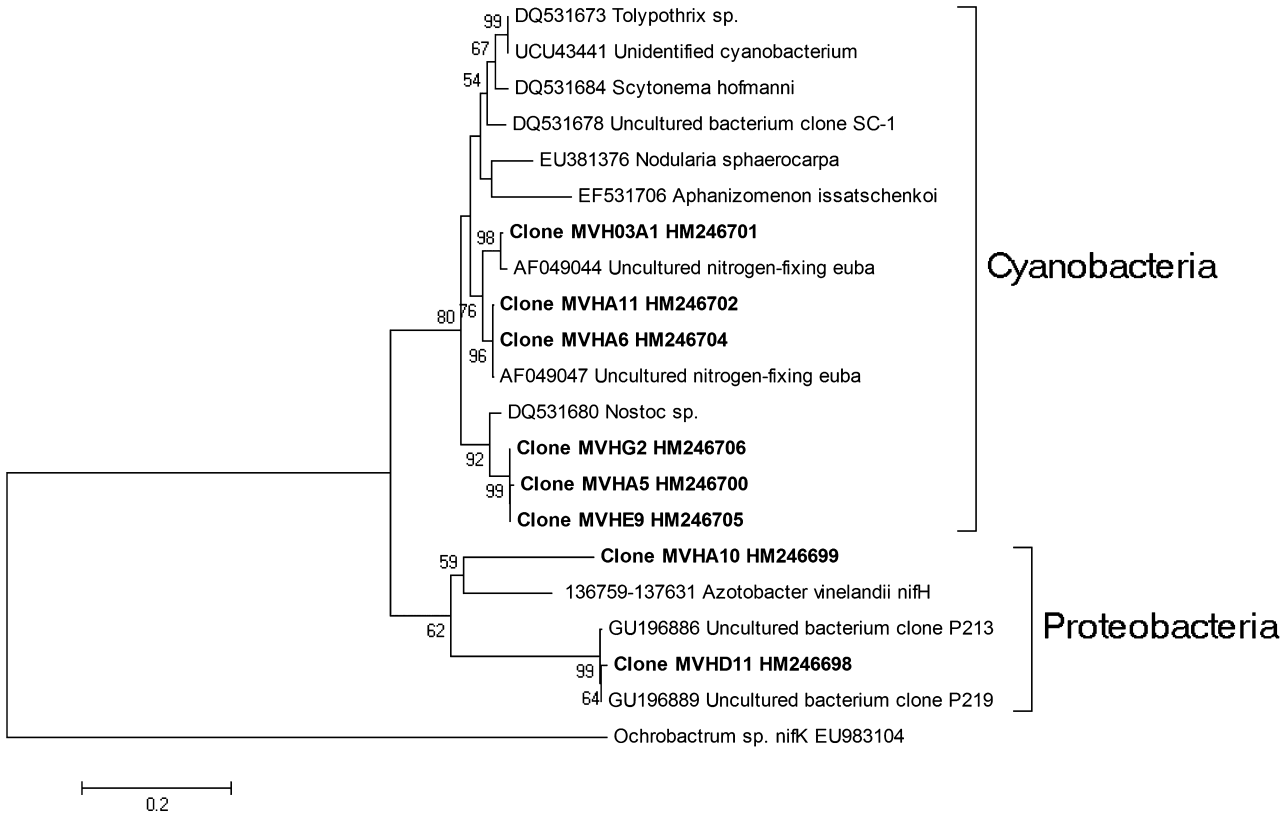

Fig. 2. Phylogenetic tree of *nifH* sequences obtained from hypolith metagenomic DNA. DNA was extracted from six hypolithon samples as described by Miller and colleagues (1999) and used as template for PCR. DGGE analysis of all six samples (data not shown) showed similar 16S rRNA gene amplicon fingerprints, and DNA samples were pooled for further analysis. Primers PolF and PolR (5′-TGCGAY CCS AAR GCB GAC TC-3′ and 5′-ATS GCC ATC ATY TCR CCG GA-3′ respectively) were used to amplify a 360 bp *nifH* fragment. Amplification was carried out as described by Poly and colleagues (2001). PCR products were eluted from agarose gels using a NucleoSpin® Extract II kit. Eluted PCR products were ligated into the pGEM®-T Easy vector (Promega) and transformed into competent *Escherichia coli* Gene Hogs™ (Invitrogen). Plasmids from *nifH* libraries were isolated by use of a Zippy™ Plasmid Miniprep Kit (Zymo Research Cooperation). Sequencing of representative plasmid DNA was carried out by the University of Stellenbosch Sequencing Service using a Hitachi 3730xl DNA Analyser (Applied Biosystems) and the Big Dye Terminator v3.1 system. Chromas® was used for editing sequences. Edited sequences were aligned using Bio-Edit with MEGA 4 (Tamura *et al.*, 2007) being used to construct phylogenetic trees, based on the Maximum Composite Likelihood method and substitution model using Neighbour-Joining. The test of phylogeny was used based on 1000 bootstraps of replication and a pairwise deletion of gaps. DNA sequences were identified by BLAST homology searches against the NCBI non-redundant database. The sequences determined in this study are available at GenBank under Accession No. HM 246698–HM 246706.

attempted to estimate the potential hypolith-derived contributions to the regional nitrogen budget of a 220 km^2 area of three McMurdo Dry Valleys. Using a mean hypolith dry weight value of 29.4 $\pm$ 41.5 g ($n = 31$), we calculate that an 'average' hypolith has the capacity to fix approximately 2.2 $\pm$ 1.6 nmol N h^{-1}. The survey dataset (Fig. 4) showed that 38% of all transects exhibited some colonization by hypolithic communities, and that mean colonization area was 25 cm^2 per transect (i.e. equivalent to an average surface area colonization of 0.024%). Using this value, we calculate that the total hypolith footprint in the three-valley region is approximately 5200 m^2.

Using field measurement data, we estimate the average surface dimensions (i.e. coverage) of a hypolith to be 22.5 $\pm$ 8.4 cm^2 ($n = 43$). Combining the acetylene reduction data with the survey data in order to estimate a value for regional hypolithic N fixation capacity, we obtain an 'instantaneous' value of 5.1 mmol N h^{-1}. The total annual photosynthetically active period for McMurdo Station (some 40 km N-E of the Miers Valley) has been calculated at approximately 2690 h (Frederick and Liao, 2005). We note that this is likely to be a minimum value, since there is evidence that metabolic activity in cold-adapted organisms extends well below freezing point (Bakermans *et al.*, 2003). We

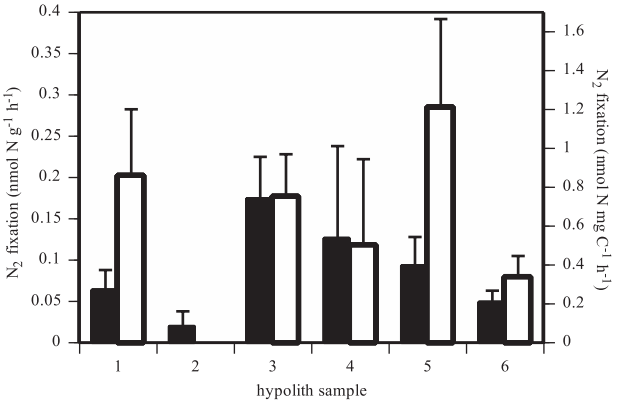

Fig. 3. Specific nitrogen fixation rates calculated from acetylene reduction data. Twelve samples were assayed, with only six showing activity and reported in this figure. No organic carbon measurement was obtained for sample 2, and therefore no normalized carbon rate is reported. Nitrogenase activity was measured using the acetylene (C_2H_2) reduction method, as described by Capone (1993). Multiple hypolithic samples were fragmented and placed into duplicate or triplicate 27 ml serum vials. Vials were sealed immediately, injected with 2 ml of C_2H_2 and incubated at approximately 15°C and 0.4% incident light, in order to mimic *in situ* austral summer conditions. The increase in ethylene (C_2H_4) was monitored in the field over a 24 h period using a gas chromatograph fitted with a flame ionization detector. The rate of increase of C_2H_4 was converted to a N_2 fixation rate by dividing by a C_2H_4:N_2 ratio of 3:1, then multiplying by 2 nitrogen atoms per N_2. Data were expressed as nmol N g^{-1} h^{-1}. Specific rates [nmol N (g C)$^{-1}$ h^{-1}] were calculated by including organic carbon content values. Following measurement of N fixation rates, hypolith samples were retained for organic carbon analysis. Fractions (2–6 g) were ground in a Retsch MM 2000 ball mill to homogenize and reduce the particle size for efficient removal of soil carbonates. An acid digestion method (Midwood and Boutton, 1998) was used to remove soil carbonates. The acid washed soils were dried at 60°C to constant weight, then re-ground in the bead mill. Samples were then weighed into combustible foil sample packages for subsequent analysis. Per cent organic carbon and nitrogen of 0.250 ± 0.002 g samples was determined using a TruSpec Carbon/Nitrogen determinator (LECO Corp., St Joseph, MI, USA) at the Stable Isotope Laboratory, University of Waikato, Hamilton, NZ.

therefore calculate that the minimum annual N input from hypolithic communities to the three valley system investigated is approximately 14 200 mmol N (0.38 kg N).

This result is surprising, given that it has been widely accepted that Dry Valley lake and stream systems are the principal sources of local N input. While the availability of experimental data is very limited, the annual contribution from Lake Hoare in Taylor Valley was estimated to be 0.37 kg N year^{-1} (Moorhead *et al.*, 1999). Since not all of the Antarctic Dry Valleys contain lakes, we suggest that hypolithic communities may be the only significant organic nitrogen contributor to lake-free valleys, and are probably important contributors of fixed nitrogen even in lake-containing valleys.

In conclusion, we have demonstrated that cryptic microbial communities play a much more important role in nutrient cycling in Antarctic desert soils than previously suspected. These results also offer intriguing opportunities for more detailed analysis of the functional behaviour and adaptive strategies of organisms in these highly specialized niches.

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Table 1. Nitrogen fixation rates from other studies of cryptic microbial communities.

Location	Sample type	Acetylene reduction (nmol N cm^{-2} h^{-1})	Study
Miers Valley, Antarctica	Hypolith	0.026–0.23	This study
Gurbantunggut Desert, China	Soil crust	0.028–0.65	Wu *et al.* (2009)
Great Basin Desert, Utah	Soil crust	< 0.033–8.7	Belnap (1996)
Chihuahuan Desert, New Mexico	Soil crust	0.00067	Hartley and Schlesinger (2002)
Negev Desert, Israel	Soil crust	34–61	Zaady *et al.* (1998)
Antarctica (various)	Endolith	Activity in one of 29 samples	Friedmann and Kibler (1980)
Hot deserts (various)	Endolith	No activity	Friedmann and Kibler (1980)
Tropical montane forest, Hawaii	Bryophyte	0.033–0.19	Matzek and Vitousek (2003)
	Lichen	0.10–2.0	Matzek and Vitousek (2003)
	Wood decay	0.054–0.63	Matzek and Vitousek (2003)

Where rates were reported in terms of C_2H_4 production, a conversion factor of 3:1 was used to convert values to N_2 equivalents, multiplied by 2 to yield molar N production.

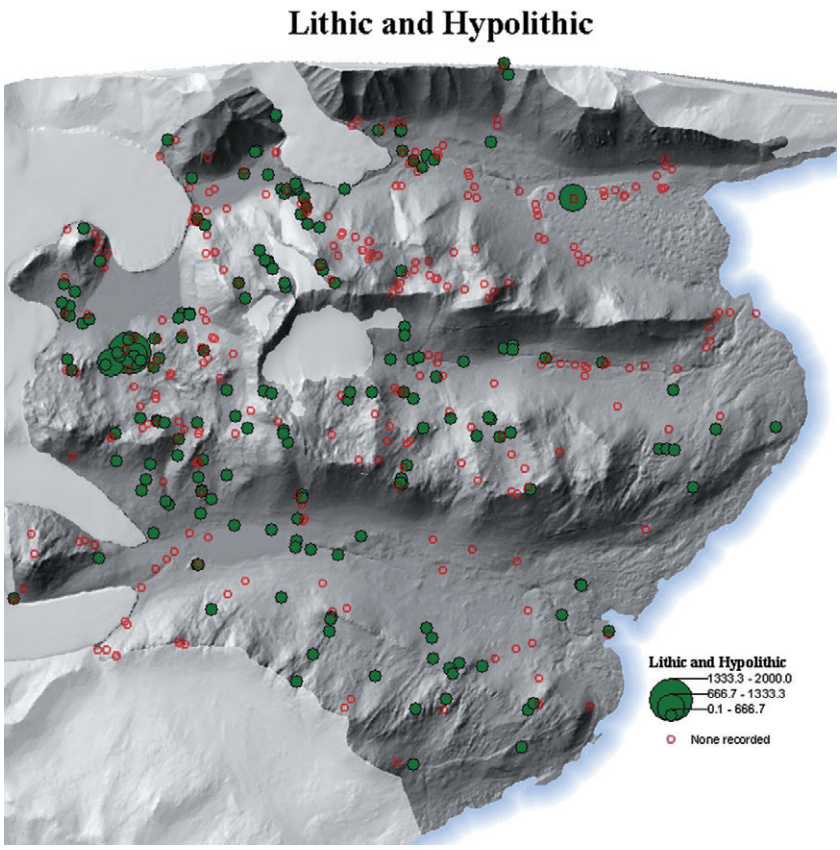

Fig. 4. Survey data of hypolith distribution across the Miers, Marshall and Garwood Valleys, Eastern Antarctica. Hypolith distribution data were recorded as part of a regional survey of surface biology, performed under the auspices of the Waikato University (NZ) FRST project ('Understanding, valuing and protecting Antarctica's unique terrestrial ecosystems: Predicting biocomplexity in Dry Valley ecosystems'). The presence and coverage of hypoliths was recorded in 20 m × 2 m transects at 466 sites, distributed across the Miers, Marshall and Garwood Valleys of the McMurdo Dry Valleys region. Coverage was estimated in units of 10 cm × 10 cm from each transect.

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