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Molecular and cultural assessment of copiotrophic bacteria in the forefield of a receding glacier

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Abstract Glacier forefield soils often exhibit a gradient of carbon content, the copiotrophic bacteria diversity of which may provide insights into bacterial population development. Glacier forefield soils were assessed for total carbon, copiotroph counts, and restriction fragment length polymorphism (RFLP) phylotyping of copiotroph isolates to determine trends in diversity. Total carbon and bacteria counts, copiotroph counts, and copiotroph diversity were found to increase with soil age. We conclude that copiotroph diversity dynamics can be useful to characterize bacteria population development in the forefield environment.

Keywords RFLP, soil, alpine, Shannon-Wiener index, Simpson's index

INTRODUCTION

The forefield of a receding glacier presents a unique opportunity for ecological research as it provides a real-time view of several decades of organism succession that can be studied in the space of a few hundred meters. Although much of our current knowledge of glacial forefield ecosystems has evolved from numerous diversity studies of forefield plant communities (FRENOT *et al.* 1998, JUMPPONEN *et al.* 1999), little is known about the diversity of bacteria and how bacterial populations evolve along a glacier forefield. The development of bacterial populations in forefield soils is an important component of the glacial environment, as bacteria are most likely the primary pioneers of these sites (SMITH 1991). Forefield bacteria populations must evolve and adapt to changing conditions including nutrient concentrations, especially carbon. Carbon is often undetectable in the youngest forefield soils, but may approach a few percent in older soils several hundred meters from the glacier terminus (MATTHEWS 1992). With the exception of photoautotrophs and chemoautotrophs, a prerequisite for microbial community development is a sufficient supply of organic carbon to support growth and maintenance of biomass (ANDERSON & DOMSCH 1985). Copi-

otrophic bacteria require elevated amounts of carbon for growth and cellular maintenance (OLSEN & BAKKEN 1987) and may be capable of providing ecological information about the development of bacterial populations along a glacier forefield. We feel that the development of copiotroph populations in a gradient of carbon availability such as that provided by the glacier forefield may provide ecological insights into the role of diversity in the development of bacteria population.

Throughout the previous decade, the use of molecular methods has become commonplace for studying the microbial communities present in environmental samples (OVREAS & TORSVIK 1998, TIEDJE *et al.* 1999). Specifically, restriction fragment length polymorphism (RFLP) analysis has been useful in assessing the diversity of bacteria in the soil environment (KIRCHHOF *et al.* 1997, WIDMER *et al.* 1998), however no study to date has implemented molecular tools to assess diversity in a forefield environment. Although much emphasis has been placed on use of molecular methods in community assessment studies, exclusive reliance on such methods can place unwarranted emphasis on minor, inactive members of the microbial community, while some truly active members may go undetected. Thus, we maintain that valuable information can be gained from the judicious combination of both molecular tools and traditional culturing methods.

This research was conducted in order to characterize the population diversity of culturable copiotrophic bacteria harvested from glacier forefield soils of different ages. The strong gradient of carbon concentration present may help reveal ecological patterns of copiotroph diversity that define the forefield environment and lead to a better understanding of microbial population succession. Additionally, copiotrophic bacteria may provide a biological discrimination between the differing stages of forefield soil succession.

MATERIALS AND METHODS

Field site, soil sampling, and soil characteristics

Soils were harvested from the forefield of the Dammaglacier in the Canton of Uri, Switzerland. A 500 m sampling transect was established, beginning from the glacier terminus (2053 m a.m.s.l.) running parallel to the forefield. Soils were collected from five sites (Tab. 1) along the transect by pooling at least six sub-samples harvested from a roughly 10m diameter circle surrounding the given sampling point. The soils were sieved on site (2 mm) and processed immediately upon return to the laboratory. The time since deglaciation assigned to each soil was inferred from data compiled by the

Tab. 1: Site and soil characteristics of the Dammaglacier forefield.

Site	Distance from glacier (m)	Time since deglaciation (y)	Elevation (m.a.m.s.l.)	Soil type	% total C	pH
1	0	0-1	2053	Coarse sand	0.017 ± 0.001	6.09
2	60	10	2052	Sand	0.047 ± 0.002	5.37
3	100	46	2058	Fine sand	0.375 ± 0.009	5.20
4	350	70	1985	Loamy sand	0.457 ± 0.001	4.63
5	500	> 70	1979	Sand	0.558 ± 0.041	4.58

Laboratory of Hydraulics, Hydrology and Glaciology (Swiss Federal Institute of Technology, Zürich). Distances from the glacier terminus and altitudes were determined with Global Positioning with an accuracy of approximately ± 3 m.

- Site 1. Site 1 was 0 m from the glacier terminus and was coarse sand with a large proportion of the particles greater than 3 mm in size, although some finer sands were present. There were no plants growing at this site. Since it was in immediate contact with the glacier terminus, annual melt-off created continuous wet conditions throughout the summer months. However, the coarse nature of the soil allowed for considerable drainage.
- Site 2. Site 2 was 60 m from the glacier terminus and was well-drained sand with the majority of particles smaller than 2 mm with some larger particles (3-4 mm) present. Sporadic grass cover was evident at site 2, mostly consisting of *Poa* spp. Lichens were also present. Site 2 was situated directly in front of a large terminal moraine, which marked the extent of the last progression of the Dammaglacier that ended in 1992.
- Site 3. Site 3 was 100 m from the glacier terminus and was fine sand with a brown coloring, indicative of the accumulation of organic matter. Diverse vegetation was present, including both perennial and annual grasses and small shrubs.
- Site 4. Site 4 was 350 m from the glacier terminus and exhibited extensive vegetation and a vast root zone. Soils were mostly fine sands, which were well-drained, and exhibited distinct "O"-horizon development.
- Site 5. Site 5 was 500 m from the glacier terminus and was very similar to site 4 but with a more extensive root zone and coarse texture.

Approximately 5 g of fresh soil was suspended in 15 ml of 0.1 M CaCl₂, stirred for 20 min, and then measured for pH. Carbon analysis was performed with a LECO 932 CHNS analyzer according to the manufacturer's instructions (LECO, Krefeld, Germany).

Culturing soil bacteria

Soils were assessed for copiotroph and oligotroph (organisms capable of growth on low-carbon medium) plate counts according to the method of HU & VAN BRUGGEN (1997). Briefly, 5 g of field moist soil was vigorously shaken in 10ml sterile water for 1 min followed by serial dilution of the soil suspension. Fifty ml of each suspension was plated in triplicate onto copiotroph and oligotroph medium. After 1 week (copiotrophs) or two weeks (oligotrophs) of incubation at room temperature in the dark, plates containing between 30 and 300 colonies were counted to assess cell numbers per gram of soil.

To collect individual isolates for genetic analysis, 32 colonies were picked from each soil (160 total) at random from the triplicate plates used for bacteria count assessment. Picked colonies were transferred to fresh medium, and re-cultured as described above.

Phylotype analysis of soil isolates

Each of the 160 randomly picked copiotroph colonies chosen for phylotype analysis were subjected to DNA extraction, PCR amplification, and restriction fragment length polymorphism (RFLP) analysis. For DNA extraction, an individual colony of each isolate was scraped from the agar plate, resuspended in a 500 µl centrifuge tube containing 100 µl sterile water, and heated to 100°C for 10 min. Tubes were then centrifuged for 1 min at 10,000 x g to pellet cell debris. Three µl of the DNA-containing supernatant were used in the subsequent 75-µl volume PCR reactions. PCR was performed on each DNA sample as described previously, using primers Eub 338-f and UniB-r, which amplify approximately 1100 bp of the 16S rRNA gene of most bacteria (AMANN *et al.* 1995). Following PCR, DNA templates were cleaned using chloroform, precipitated with isopropanol, and resuspended in 30µl of a mixture containing 0.2 µl *Taq* I, 0.2 µl *Hae* III, 3 µl 10x multi-core buffer (Catalys Inc., Wallisellen, Switzerland), and 26.6 µl sterile, nuclease-free water. The restriction digest reactions were incubated overnight at 37°C then separated by running 5 µl on a 8% polyacrylamide gel (200 V, 2 h). Gels were stained with SYBR-green nucleic acid stain as described by the manufacturer (Molecular Probes, PoortGebouw, The Netherlands), visualized with UV transillumination and photographed.

Copiotroph diversity assessment

RFLP patterns were compared to determine the number of unique patterns representing unique copiotroph phylotypes. The number and distribution of each phylotype was assessed for each soil and the copiotroph diversity determined according to the Shannon-Wiener diversity index (H'),

$$H' = \sum_{i=1}^S (n_i / N) \ln(n_i / N)$$

and Simpson's diversity index (D),

$$D = \sum_{i=1}^S n_i^2, \text{ MAGURRAN (1988)}$$

Where n_i is the proportional abundance of each phylotype in the soil sample and S is the total number of phylotype patterns in each soil. For clarity, we have expressed Simpson's index as $1/D$.

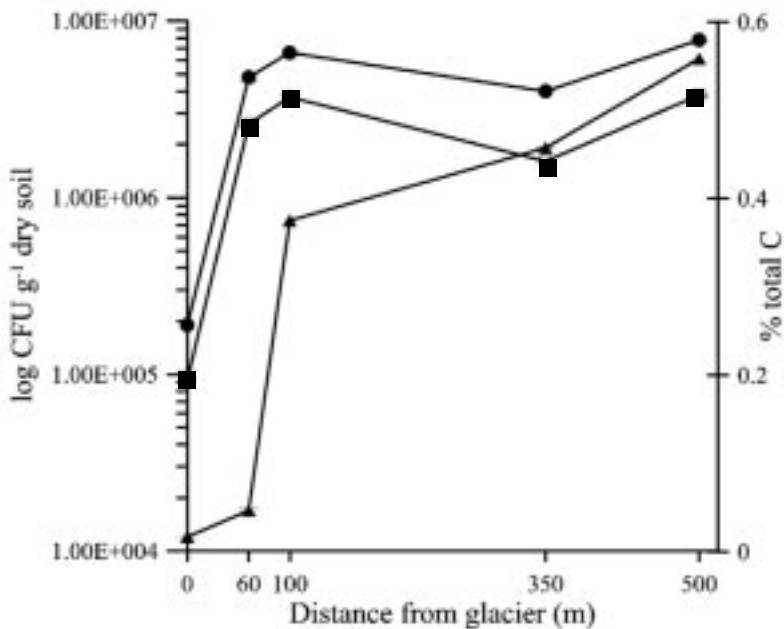


Fig. 1: Forefield soil bacteria numbers and total carbon percentage at selected distances from the Dammaglacier. Legend: ●, total bacteria; ■, copiotrophic bacteria; ▲, % total carbon. Error bars represent the standard errors of triplicate measurements.

RESULTS

Culturable bacteria numbers

As shown in figure 1, the number of total bacteria as assessed through the addition of oligotroph and copiotroph counts, increased rapidly over the first 60m of terrain from 1.9×10^5 c.f.u. g^{-1} dry soil to 6.6×10^6 c.f.u. Total bacteria counts decreased at the 350 m site to 4.0×10^6 c.f.u., then increased again to a maximum of 7.8×10^6 c.f.u. at 500 m. The numbers of copiotrophs followed the same trend as the total bacteria counts, but were detected at approximately one-half the number of total bacteria.

Isolate phylotype analysis

Following DNA extraction, PCR, and RFLP analysis, each of the 32 randomly picked copiotroph isolates from each forefield soil was grouped according to RFLP-phylotype (Fig. 2). The number of detected phylotypes increased from 14 from site 1 to 18 from site 5, indicating an increase in phylotype diversity associated with increasing soil age and distance from the glacier (Tab. 2). Interestingly, site 2 was characterized by a noticeable decrease in copiotroph diversity, which was attributable to one-third of the picked isolates belonging to the same phylotype.

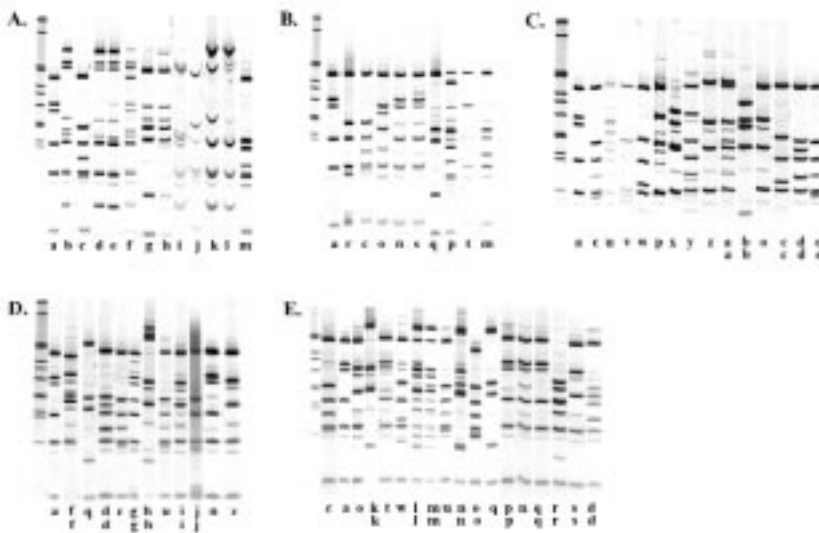


Fig. 2: Composite images of RFLP patterns generated from copiotroph isolates harvested from: A, site 1; B, site 2; C, site 3; D, site 4; E, site 5 of the Dammaglacier forefield. The letter below the pattern indicates the RFLP type.

Tab. 2: Copiotroph RFLP types and abundance detected in each site of the Dammaglacier forefield.

Site	RFLP type*	RFLP type abundance	Site	RFLP type	RFLP type abundance
1	a	7	4	a	10
	b	5		ff	5
	c	3		q	4
	d	2		dd	3
	e	1		c	1
	f	1		gg	1
	g	1		hh	1
	h	1		u	1
	i	1		ii	1
	j	1		jj	1
	k	1		n	1
	l	1		z	1
	m	1			
			5	c	4
2	a	20	a	3	
	r	2	o	3	
	c	2	kk	3	
	o	1	w	3	
	n	1	ll	2	
	s	1	mm	2	
	q	1	u	1	
	p	1	nn	1	
	t	1	oo	1	
	m	1	q	1	
			pp	1	
3	a	8	n	1	
	c	6	qq	1	
	u	2	rr	1	
	v	2	ss	1	
	w	1	dd	1	
	p	1			
	x	1			
	y	1			
	z	1			
	aa	1			
	bb	1			
	o	1			
	cc	1			
	dd	1			
	ee	1			

*Letters in bold denote phlotypes found at more than one site

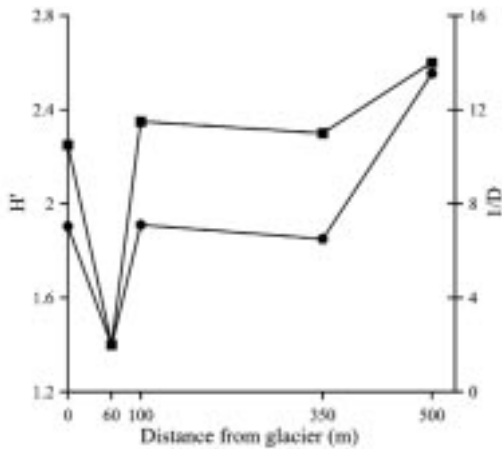


Fig. 3: Forefield soil copiotroph H' and $1/D$ at Dammaglacier forefield sites investigated in this study. Legend: ■ = H' ; ● = $1/D$.

The number and distribution of each phylotype were used to generate Shannon-Wiener diversity index (H') and Simpson's index ($1/D$) values in order to compare the copiotroph diversity of each soil. As predicted by the number of phylotypes detected, H' and $1/D$ both indicated an overall increase in copiotroph diversity as soil age and distance from the glacier increased. H' increased from 2.25 at site 1 to 2.6 at site 5, and $1/D$ increased from 7.04 to 13.54 (Fig. 3). The diversity index values for site 2 also exhibited a large decrease as expected following the assessment of the number of phylotypes isolated from the site.

DISCUSSION

This study was initiated to assess the diversity of copiotrophic bacteria in the soils of a glacier forefield. The forefields of receding glaciers can be viewed as physical timelines where several decades of microbial population development can be studied in the space of a few hundred meters. By focusing on the copiotrophic bacteria, which require elevated concentrations of carbon for growth and maintenance, we have used the gradient of nutrient status inherent to the glacier forefield soils to develop basic ecological principles that define the forefield environment.

Of particular importance to glacial forefield soil ecology is the concept of a forefield being viewed as a physical timeline with the youngest soils (or soil precursors) found at the glacier terminus and the oldest ones found distant from the glacier. In the current study, soil bacterial biomass was

observed to follow a similar trend previously shown in the same forefield (SIGLER & ZEYER, in press) in addition to other investigated forefields (INSAM & HASELWANDTER 1989, OHTONEN *et al.* 1999). However, in the previous studies, copiotrophs as a sub-group were not accounted for. We attribute the increase in both total and copiotrophic bacteria numbers to site parameters such as the increase in the amount of carbon detected in each soil, which increased in a similar pattern as the bacteria numbers (Fig. 1). It is assumed that the increased carbon content was directed by an increase in vegetation coverage in older soils. However, it is also plausible that plant growth promoting bacteria could also create conditions favoring increased vegetation coverage (NAUTIYAL 2000). Of course, it is a widely accepted fact that only 1% of all environmental bacteria are culturable (OLSEN & BAKKEN 1987), thus the numbers generated in this study are intended to be used as a guideline to describe ecological properties, and not as absolute biomass estimates. Parameters such as soil biomass and metabolic efficiency have been previously investigated in other forefields (INSAM & HASELWANDTER 1989, OHTONEN *et al.* 1999), but we investigated the perspective of molecular diversity of the forefield bacterial community. In general, H' and $1/D$ suggested that copiotroph diversity increased along the glacier forefield. This trend in diversity is probably driven by an increased number of carbon sources, for instance, plant root exudates. The diversity of plants has been shown to increase in the first several years of forefield succession (FRENOT *et al.* 1998, JUMPPONEN *et al.* 1999) and could be playing a major role in copiotroph diversity on our sites. Copiotrophic bacteria require moderate to elevated amounts of carbon for their metabolism. A shift in population from low diversity in younger soils to higher diversity in older soils may be associated with an overall shift from populations that inefficiently metabolize what little carbon is available to them, to populations that carry out metabolism with greater efficiency (PICKETT 1976). However, given the dramatic changes in site characteristics from site 1 to site 5, especially in total soil carbon concentration, a larger overall change in H' was expected. Regardless, the discovery of diverse microbial communities was not unexpected. The isolation of diverse assemblages of bacteria from a mixture of rock and glacial ice has been previously reported (DANCER *et al.* 1997) and, combined with our observations, reinforces our characterization of glacial forefields as microbially diverse environments. It is interesting that the soil nearest the glacier (site 1) exhibited relatively high H' and $1/D$ values. We hypothesize that low cell numbers allow for a diverse bacterial assemblage, free from spatial competition, to inhabit this site even though carbon was seemingly limiting. Furthermore, wide niche availability is a feature of early suc-

cession environments (SANTEGOEDS *et al.* 1998) and may play a key role in the high bacterial diversity in early forefield succession investigated at the Dammaglacier.

Site 2 exhibited a dramatic decrease in copiotroph diversity as compared to the soils from the other sites (Fig. 3). This decrease was caused by the isolation of a large proportion of similar phylotypes (approximately two-thirds of the total isolates from site 2). The ecological explanation for the high abundance of a common phylotype at this site is unknown. It does, however, indicate that even under oligotrophic environmental conditions such as those found at site 2, the copiotroph population can exhibit dominance of certain community members.

Phylotype analysis was able to illustrate the succession character of the glacier forefield environment. Several phylotypes were found in adjacent sites, indicating a generalized adaptation behavior, while others were subjected to phylotype replacement and were only detected in one sample (Tab. 2). Such ecological patterns are common to succession environments where changing conditions coupled with diverse organisms lead to the replacement of some organisms but also persistence of others (MC CORMICK *et al.* 1991).

In conclusion, copiotroph number and diversity were found to increase along the forefield succession as soil age increased. The trend in cell number was coupled with an increase in total carbon, however it is unknown which mechanisms were responsible for the increase in copiotroph diversity. In general, late succession organisms focus their energy toward maintenance and not reproduction (GADGIL & SOLBRIG 1972). Thus, it is likely that increased soil carbon content and number of carbon sources are factors in the copiotroph diversity increase, since specialist organisms in late succession can more effectively take advantage of diverse forms of carbon, provided by an increased diversity of plants. Despite this, other factors such as niche development and predator-prey dynamics cannot be ruled out, warranting further investigation into the mechanisms of forefield diversity.

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