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ORIGINAL PAPER

Primary succession of soil enzyme activity and heterotrophic microbial communities along the chronosequence of Tianshan Mountains No. 1 Glacier, China

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Abstract We investigated the primary successions of soil enzyme activity and heterotrophic microbial communities at the forefields of the Tianshan Mountains No. 1 Glacier by investigating soil microbial processes (microbial biomass and nitrogen mineralization), enzyme activity and community-level physiological profiling. Soils deglaciated between 1959 and 2008 (0, 5, 17, 31 and 44 years) were collected. Soils >1,500 years in age were used as a reference (alpine meadow soils). Soil enzyme activity and carbon-source utilization ability significantly increased with successional time. Amino-acid utilization rates were relatively higher in early, unvegetated soils (0 and 5 years), but carbohydrate utilization was higher in later stages (from 31 years to the reference soil). Discriminant analysis, including data on microbial processes and soil enzyme activities, revealed that newly exposed soils (0–5 years) and older soils (17–44 years) were well-separated from each other and obviously different from the reference soil. Correlation analysis revealed that soil organic carbon, was the primary factor influencing soil enzyme activity and heterotrophic microbial community succession. Redundancy analysis suggested that soil pH and available P were also affect microbial activity to a considerable degree. Our results indicated that glacier foreland soils have continued to develop over 44 years and soils were significantly affected by the geographic location of the glacier and the local topography. Soil enzyme activities and heterotrophic microbial communities were also significantly influenced by these variables.

Keywords Tianshan Mountain No. 1 Glacier · Primary succession · Heterotrophic microbial community · Soil enzyme activity · Biolog ecoplates

Jun Zeng and Xiao-xia Wang have contributed equally to this work.

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Abbreviations

MBC	Microbial biomass carbon
MBN	Microbial biomass nitrogen
C_t	Total carbon
N_t	Total nitrogen
SOC	Soil organic carbon
S_t	Total sulfur
P_t	Total phosphorus
P_{al}	Available phosphrous
K_{al}	Available potassium
N_{al}	Available nitrogen
S_{at}	Total salt
Ref	Reference soil
RDA	Redundancy analysis
AWCD	The average well colour development
CLPP	Community-level physiological profiling

Introduction

The forefields exposed after a glacier has retreated are widely considered to be ideal for the study of soil formation and biogeochemical cycling (Hopkins et al. 2007). As a glacier retreats, the barren land experiences a succession of soil processes, including transformation of organic material, nitrogen deposition and nutrient cycling (Brankatschk et al. 2011; Duc et al. 2009; Nemergut et al. 2007; Schmidt et al. 2008). Soil microorganisms (i.e., lichen, fungi, and bacteria) are an indispensable part of these processes and are considered to form pioneering communities. Their establishment influences the physicochemical properties of soil, indirectly facilitates pedogenesis and paves the way for later plant propagation (Hahn and Quideau 2013; Kaštovská et al. 2005; Nemergut et al. 2007; Philippot et al. 2011; Schüette et al. 2009; Sigler and Zeyer 2002; Wu et al. 2012; Zumsteg et al. 2012). For example, soil microorganisms contribute to (1) the decomposition of organic matter and the release of nutrients for plant growth; (2) the formation of soil humic acid and the development of soil colloid; (3) the fixation of nitrogen to improve soil nitrogen content; and (4) the enrichment, movement and utilization of dissolved soil minerals (Bardgett and Walker 2004; Duc et al. 2009; Tscherko et al. 2003). These processes, however, are still poorly understood (Schüette et al. 2009).

To date, a majority of the studies on microbially-mediated soil processes in glacier forelands have focused on shifts in soil enzyme activity, microbial biomass (microbial carbon and nitrogen, or C and N, respectively), and N cycling (nitrogen fixation, ammonia oxidation, and denitrification) during soil development (Brankatschk et al. 2011; Sigler and Zeyer 2002; Tscherko et al. 2003). For example, soil enzyme activity [involved in C, N, phosphorus (P), and sulfur (S) cycling] usually showed an increasing trend during succession, whereas some conflicting studies found that enzyme activity was in a slower or temporary steady state in older soils from two alpine glacier forefields (Brankatschk et al. 2011; Schmidt et al. 2008; Tscherko et al. 2003). Microbial biomass C (MBC) and N (MBN) and the MBC/MBN ratio changed irregularly. Some of these parameters increased dramatically along the chronosequence, while others showed a decreasing trend, suggesting shifts in the microbial community (Göransson et al. 2011; Tscherko et al. 2003). N was almost completely derived from photosynthetic and nitrogen-fixing bacteria, while, in developed soils, nitrification and denitrification rates were significantly increased in recently de-glaciated soils from a high-elevation glacier (Schmidt et al. 2008). However, those important processes were all limited by the amount of available organic matter in the soil. Increasing concentrations of organic matter along chronosequences increase microbial activity (respiration, soil-enzyme activity, and mineralization), microbial biomass, ammonium oxidation, and nitrification and denitrification rates (Wardle et al. 2004). Therefore soil organic matter was considered to be the primary driving force for the primary succession of soil microbial organisms in barren glacier forelands (Göransson et al. 2011). Owing to the lack of available organic matter, it is generally believed that autotrophic communities would be the first colonizers in newly exposed soils (Walker and Moral 2003). In contrast, a wide variety of heterotrophic microorganisms are also active in newly exposed glacial substrates, decompose organic matter derived from atmospheric deposition and assimilate ancient carbon, secondary microbial material and debris (Duemig et al. 2011; Göransson et al. 2011; Nemergut et al. 2007). Therefore, heterotrophic microorganisms play an important role in labile-C accumulation in newly exposed soils (Nemergut et al. 2007; Tscherko et al. 2004, 2003). Primary successions of heterotrophic microorganisms in the forelands of retreated glaciers from European, South

American, and high altitudes arctic regions have been well documented. Most of these studies have used molecular biology methods, and the results presented a relatively clear picture of microbial composition and structural shift along chronosequences (Hahn and Quideau 2013; Kaštovská et al. 2005; Nemergut et al. 2007; Philippot et al. 2011; Schüette et al. 2009, 2010; Sigler and Zeyer 2002; Wu et al. 2012; Zumsteg et al. 2012). Although those studies were informative, 16S rRNA gene sequences did not reflect microbial community-level physiological profiling. Therefore, the shift in ecological function and heterotrophic carbon metabolism following spatial and temporal changes are still largely unknown (Wu et al. 2012). Furthermore, glacial geomorphology, glaciations, soil formation and evolution, and mass balance of glaciers from the arid and semi-arid regions of central Asia, like the Tianshan Mountains No. 1 glacier, have been well-documented since the 1950s, but research on microbial succession has been rare (Li et al. 2013; Wang et al. 2005).

In this study, we examined the shift in soil enzyme activity and microbial processes along the chronosequence to reflect the succession of a functional community. Further, we used Biolog EcoPlates to detect changes in heterotrophic microbial communities and characterize carbon-source utilization in different successional stages. We hypothesized that (1) soil organic carbon was the main influencing factor than total nitrogen to the primary succession of heterotrophic microbial communities and activities; (2) heterotrophic microbial community in different successional stages could project some clear successional traits.

Materials and methods

Site description and soil sampling

Tianshan Mountain No. 1 glacier is a small cirque-valley glacier, located at the headwaters of the Urumqi River, western Tianshan Mountains, Xinjiang, China (43°06′N, 86°49′E). It is about 3,840 m above sea level and is surrounded by deserts and Gobi (Fig. 1a). The observation of Glacier No. 1 started in 1959 and the over 50 years' data showed that the air temperature has been continuously rising since 1985, and the tendency of rise has accelerated since 1995. From 1997 up to the present, the average temperature has increased by 1 °C, hence the glacier has split into two separate parts since the end of 1993 (Fig. 1b) (Li et al. 2013). From 1959 to 1993, the glacier retreated at an average rate of 4.5 m year^{-1}, and then the retreating rate was slower in the east branch (3.5 m year^{-1}) but faster in the west branch (5.8 m year^{-1}) from 1993 to 2004 (Xia et al. 2012; Xu et al. 2011).

In this study we selected only the well documented east branch for investigation. On 17 August 2008 we set up five transects that ran parallely along the direction of the glacial retreat. Sampling sites were located at 0, 18, 60, 120 and 180 m from the glacier terminus, representing the successional ages 0, 5, 17 (a glacier mean retreating rate of 3.5 m year^{-1}), 31 and 44 years (4.5 m year^{-1}), respectively (Fig. 1c, d). The deglaciation time of each soil site was inferred from datasets compiled by the Tianshan Glaciological station of Chinese Academy of Science. Samples of the top 5 cm of soil were sampled (three replicates for each site) with a small spade at an interval of 15–20 m along each transect. In addition, an alpine meadow soil about 500 m away from the glacier terminus was selected as the reference soil (Ref). Soil samples were stored in ice box and immediately transported to laboratory within 2 h and divided into two aliquots: one was stored at −80 °C, and the other was stored at 4 °C for biogeochemical analyses.

Soil chemical properties

Soil pH was determined after diluting 3 g of air-dried soil in 7.5 ml of 0.01 M $CaCl_2$ solution and measured by a glass electrode using a Mettler DL-25 pH meter (Shanghai, China). The N_t and S_t contents were determined by dry combustion at 1,200 °C on an elemental analyzer (LECO CNS 2000) with infrared and thermal conductivity detectors, respectively. The SOC content was measured using the potassium dichromate oxidation titrimetric method. Available nitrogen was detected by the alkaline hydrolysis. Available phosphorous was determined by the sodium hydroxide-molybdenum stibium anti-color method. Available potassium was evaluated by the ammonium acetate-flame photometer method. Total phosphorus was measured by means of perchloric acid-Sulphate, and total salt was determined by hot roasting.

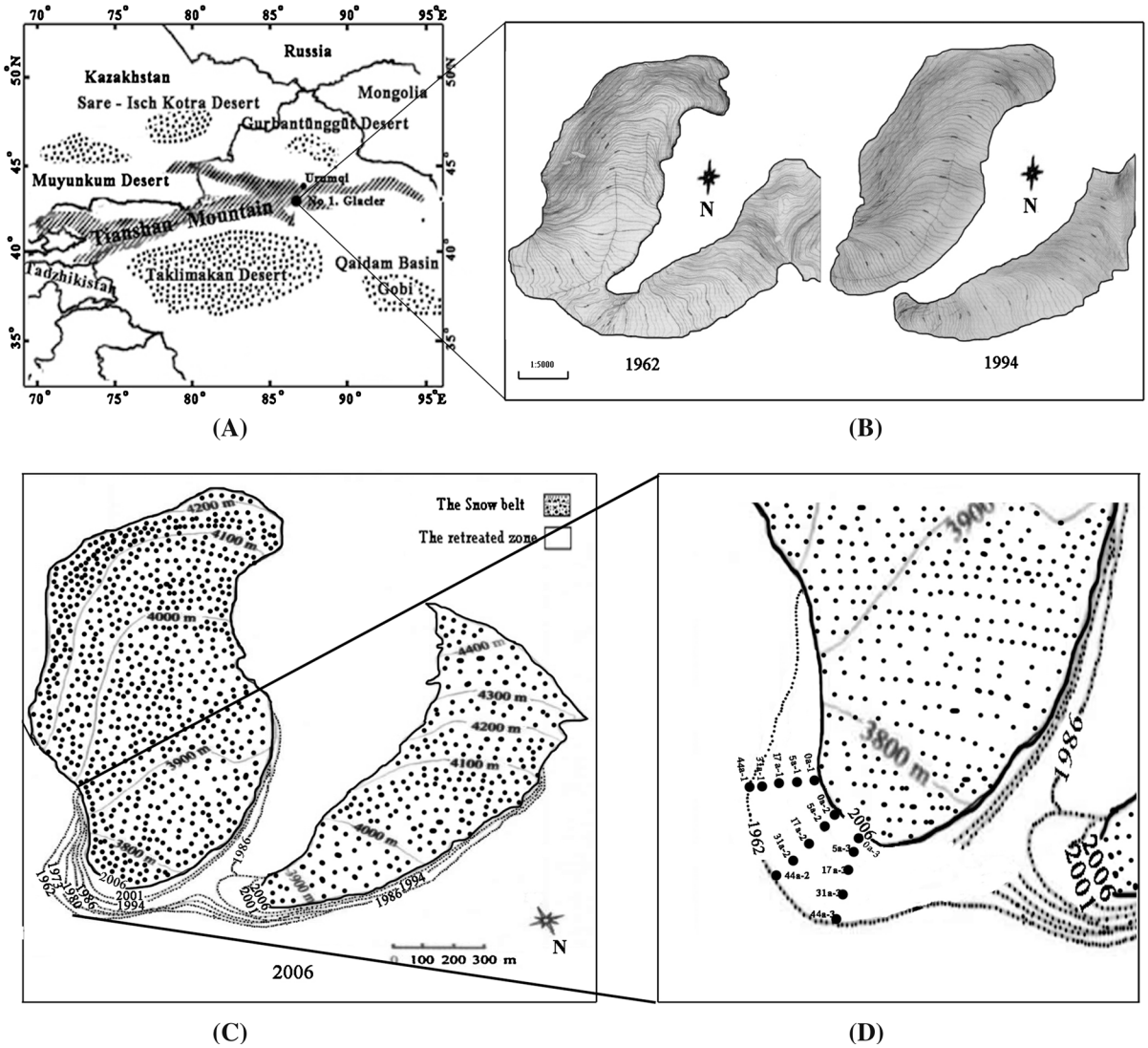

Fig. 1 Map of the sampling sites on the east branch of the Tianshan Mountains glacier No. 1. **a** Location of the Glacier No. 1. **b** Changes in Glacier No. 1 during the period of 1962–1994. **c** The boundaries of glacier No. 1 in different periods (*dashed lines* represent the glacial boundaries from 1962 to 2001, and the *solid line* represents the glacial boundary in 2006). **d** Five transect sampling sites are denoted by the *dotted rectangle*

Microbial processes

Mineralization of nitrogen was measured under waterlogged conditions, and the soils were incubated at 40 °C for 7 days, and the produced NH_4^+–N was measured at a wavelength of 631 nm (Kandeler and Gerber 1988). Microbial biomass carbon was determined by the chloroform-fumigation-extraction (CFE) protocol (Sparling and West 1988). After fumigation with chloroform for 24 h at room temperature in the dark, microbial C was extracted with 0.5 M K_2SO_4, and the extracted solutions were measured on a TOC-V_{CPH} analyzer (Shimadzu, Japan). Microbial biomass nitrogen was determined as described by Brookes et al. (1985).

Assays of soil enzyme activity

We determined the urease activity according to Kandeler and Gerber (1988), using urea as the

substrate. The protease activity was measured as described by Ladd and Butler (1972), and soil samples were incubated with sodium butyric at 50 °C for 2 h, and the produced NH_2 was measured photometrically at 578 nm. The arylsulphatase activity was assayed following Tabatabai and Bremner (1970), using *p*-nitrophenylsulphate solution as the substrate. After incubation at 37 °C for 1 h, the reaction product *p*-nitrophenol was measured colorimetrically at 400 nm. To estimate the sucrase activity, sucrose was used as the substrate, followed by colorometric determination of the reducing sucrose (Guan et al. 1986). The acid phosphatase activity was assayed in disodium phenylphosphate solution (37 °C, 1 h), and the produced phenol was estimated colorimetrically at 400 nm. The arginine deaminase activity was measured by deaminase of arginine solution (2 h, 37 °C) and the produced NH_4^+ was determined colorimetrically at 630 nm (Alef and Kleiner 1986).

Biolog assays

Biolog Eco Plates (Biolog Inc., Hayward, CA, USA) were used to assess the community-level physiological profiling to reflect the shift of microbial community in the six soil samples. Briefly, 10.0 g of dry soil was mixed with 100 ml sterile saline 0.85 % NaCl solution buffer and shaken for 10 min, followed by centrifugation at 800 rpm for 10 min. Aliquots from 10^{-2} dilution (inoculation density around 10^5 cells mL^{-1}) were pipetted into Biolog Eco Plates™ with 31 different carbon sources and a negative control (water) using an 8-channel micropipetter set to 150 µl. Plates were incubated in the dark at 10 °C for 10 days without agitation, and the absorbance was determined at 24 h intervals starting at 0 h using a Microplate E-Max Reader model 3550 (Bio-Rad, Richmond, Calif., USA) at a wavelength of 590 nm (Weber and Legge 2010).

Statistical analysis

For the Biolog assay, the average well color development (AWCD) value was calculated *as per* the formula: AWCD $= \sum$(C−R)/31, where *C* is the color production within each well; R is the absorbance value of the control well. The shifts of soil properties, enzyme activities, and AWCD were evaluated by one-way ANOVA to statistically test for differences along the chronosequences, using LSD post hoc analyses. Discriminant analysis was applied to examine differences in microbial activity pattern between sites, to group similar sites to one successional stage, and to identify the discriminatory importance of each microbial variable (N mineralization, deaminase, protease, urease, arginine deaminase, sucrase, acid phosphate, arylsuphatase and AWCD) (Tscherko et al. 2003). The groups were defined according to the site age. Wilkes's criterion was used for the stepwise selection of the variables. The shift of microbial community was analyzed by principal component analysis (PCA). All data were analyzed by using the SPSS software package (SPSS, Version 19.0).

The relationships between soil chemical properties (SOC, C_t, N_t, P_t, P_{al}, K_{al}, N_{al} and S_{at}), and soil enzyme activities (urease, protease, arylsulphatase, acid phosphate, sucrase), microbial processes (nitrogen mineralization and arginine deaminase), and CLPP were evaluated by multivariate statistics using Canoco 4.5. Multivariate statistical analyzes were conducted on centered and standardized data matrices. Detrended correspondence analysis (DCA) indicated a short length of the gradient (0.621, and 0.759 for enzyme activities and CLPP, respectively), which justified employment of redundancy analysis (RDA). Statistical significance of the relationship between microbial processes, soil enzyme activity, AWCD and environmental variables was evaluated using the Monte Carlo permutation test (unrestricted permutation, reduced model, 999 permutations). The relationship between SOC, N_t, N_{al}, K_{al} and microbial biomass, enzyme activities and AWCD was tested by bivariate correlation analysis.

Results

Chemical properties of soils

Selected soil parameters are listed in Table 1. SOC content showed a slight increase from 8.03 to 8.47 g kg^{-1} ($P > 0.05$) prior to the 17-year time point, but significantly increased from 13.48 to 75.59 g kg^{-1} ($P < 0.05$) in 44 years and Ref site, respectively. Other soil parameters, like P_{al} and P_t, also followed a similar pattern. However, the S_t and K_{al} content followed the opposite pattern and

Table 1 Selected soil properties of the successional sites across the Urumqi Glacier No. 1

Soil properties	Successional age					
	0a	5a	17a	31a	44a	Ref
pH	7.57 ± 0.18a	7.68 ± 0.12a	7.58 ± 0.15a	7.73 ± 0.09a	7.88 ± 0.09a	7.30 ± 0.08b
SOC (g kg^{-1})	8.03 ± 0.19c	8.33 ± 0.18c	8.47 ± 0.09c	4.75 ± 0.06d	13.48 ± 1.15b	75.59 ± 1.69a
N_t (mg kg^{-1})	172.11 ± 0.047b	313.38 ± 0.080b	292.50 ± 0.040b	143.68 ± 0.041b	209.08 ± 0.016b	1,114.76 ± 0.40a
S_t (g kg^{-1})	0.30 ± 0.052a	0.24 ± 0.038a	0.25 ± 0.062a	0.17 ± 0.039b	0.18 ± 0.057b	0.12 ± 0.011b
P_t (g kg^{-1})	0.67 ± 0.10b	0.92 ± 0.12a	0.73 ± 0.04b	0.73 ± 0.08b	1.08 ± 0.10a	0.99 ± 0.09a
P_{al} (mg kg^{-1})	4.30 ± 0.26d	4.80 ± 0.11d	3.90 ± 0.12d	13.04 ± 1.15b	10.21 ± 1.00c	16.63 ± 0.85a
K_{al} (mg kg^{-1})	56.92 ± 2.24c	78.24 ± 12.66b	56.90 ± 1.48c	39.03 ± 4.11d	31.90 ± 1.03d	324.01 ± 15.00a
N_{al} (mg kg^{-1})	23.32 ± 0.62c	25.60 ± 0.72b	14.05 ± 0.28d	23.34 ± 0.57c	23.30 ± 1.05c	77.02 ± 1.22a
S_{at} (g kg^{-1})	2.05 ± 0.17a	0.34 ± 0.04f	1.62 ± 0.08c	1.83 ± 0.06b	1.31 ± 0.06d	1.06 ± 0.09e
P_{al}/P_t (%)	0.65 ± 0.059c	0.52 ± 0.041c	0.544 ± 0.029c	1.86 ± 0.15a	0.97 ± 0.039b	1.71 ± 0.11a
N_{al}/N_t	0.14 ± 0.0036b	0.082 ± 0.0021d	0.048 ± 0.0016f	0.16 ± 0.0041a	0.11 ± 0.0040c	0.069 ± 0.00082e
SOC/N_t	46.65 ± 1.1b	26.57 ± 0.58d	28.84 ± 0.48d	32.76 ± 0.9c	66.00 ± 3.46a	68.26 ± 0.91a

SOC Soil organic carbon; *N* total nitrogen; S_t total sulfur; P_t total phosphorus; P_{al} available phosphorous; K_{al} available potassium; N_{al} available nitrogen; S_{at} total salt

remained relatively stable before the 17-year time point, while significantly declining in later stages. The N_t content significantly increased before the 17-year time point ($P < 0.05$) and then presented a declining trend from 17 to 44 years of succession. Soil pH values showed a slight, but not significant increase during 44 years of succession. The P_{al}/P_t ratio also followed that no obvious changes were detected before 17 years, but this parameter significantly increased from 31 years onward relative to the reference soil. The SOC/N_t and N_{al}/N_t ratios significantly declined before 17 years but both increased from 31 years on.

Microbial processes and soil enzyme activity

The MBC content showed a slight decrease from 17.19 to 3.42 mg kg^{-1} over the course of 31 years, and the lowest value was recorded at 31 years of succession. However, this parameter significantly increased to 25.33 mg kg^{-1} at the 44-year time point. The MBN content did not change significantly over the course of 44 years (except for 31 years), but was much lower than in reference soil. N_{min} showed a clear increase before 5 years, but remained relatively unchanged from 5 to 44 years (Table 2).

Generally, levels of soil enzymes involved in N, C, P and S cycling (i.e., protease, urease, arginine deaminase, sucrase, acid phosphate and arylsuphatase) increased along the successional time gradient (Fig. 2). However, sucrase, protease, arginine deaminase and acid phosphatase activity increased significantly ($P < 0.05$) by the 44-year time point. Arylsuphatase activity was essentially not detectable before 31 years, but clearly increased after the 44-year time point. Urease activity in recently uncovered soils (0–5 years) was much lower relative to the reference.

Bivariate correlation coefficient analysis showed most soil parameters were significantly correlated with MBC, MBN and enzyme activities ($P < 0.01$). Partial analysis (set SOC and N_t as control variable) showed none of parameters were correlated with microbial processes but pH, P_{al} and K_{al} were significantly correlated with soil enzyme activities (Supplementary Table s1). Discriminant analysis, including data on microbial processes (N mineralization, and arginine deaminase) and soil enzyme activities (protease, urease, sucrase, acid phosphatase and arylsuphatase), revealed that newly exposed soils on young moraines (0–5 years) were grouped together, and older soils (17–44 years) were well-separated from each other and obviously different from reference soil. Discriminant function 1 (DF1) explained 97.6 % of the total variance, and arylsulphatase was the most important variable for discriminating soils in different successional stages (Fig. 3).

Shifts in microbial community composition and carbon source utilization by CLPP

Principal component analysis illustrated the shift of microbial functional structure based on their carbon substrate utilization patterns (Supplementary Fig. 1). The first and the second principal component (PC1 and PC2) explained 39.2 and 23.1 % of data variance, and their eigenvalues were larger than the pooled average of all eigenvalues, suggesting microbial community shifted significantly. Soil samples could separate into two parts that younger soils (0, 5 and 17 years) were in the first quadrant, the older (31 and 43 years) and Ref

Table 2 Microbial biomass carbon and nitrogen, N mineralization, C_{mic}/N_{mic} ratio of microbial biomass of soils along the chronosequence

Successional age years	C_{mic} (mg kg^{-1})	N_{mic} (mg kg^{-1})	C_{mic}/N_{mic}	N_{min} (μg NH_4-N^{-1} $g^{-1}d$)
0a	17.19 ± 3.55c	1.79 ± 0.16b	9.54 ± 1.25a	0.15 ± 0.02c
5a	14.05 ± 1.51c	2.47 ± 0.57b	6.78 ± 0.72a	0.42 ± 0.05b
17a	12.80 ± 1.55c	2.26 ± 1.05b	6.35 ± 2.29a	0.34 ± 0.06b
31a	3.42 ± 0.41d	0.64 ± 0.01c	5.59 ± 0.56a	0.34 ± 0.04b
44a	25.33 ± 5.98b	2.29 ± 1.66b	7.35 ± 0.43a	0.44 ± 0.08b
Ref	111.26 ± 15.35a	11.59 ± 2.39a	9.75 ± 1.30a	0.87 ± 0.17a

C_{mic} microbial biomass carbon; N_{mic} microbial biomass nitrogen; N_{min} mineralization of nitrogen

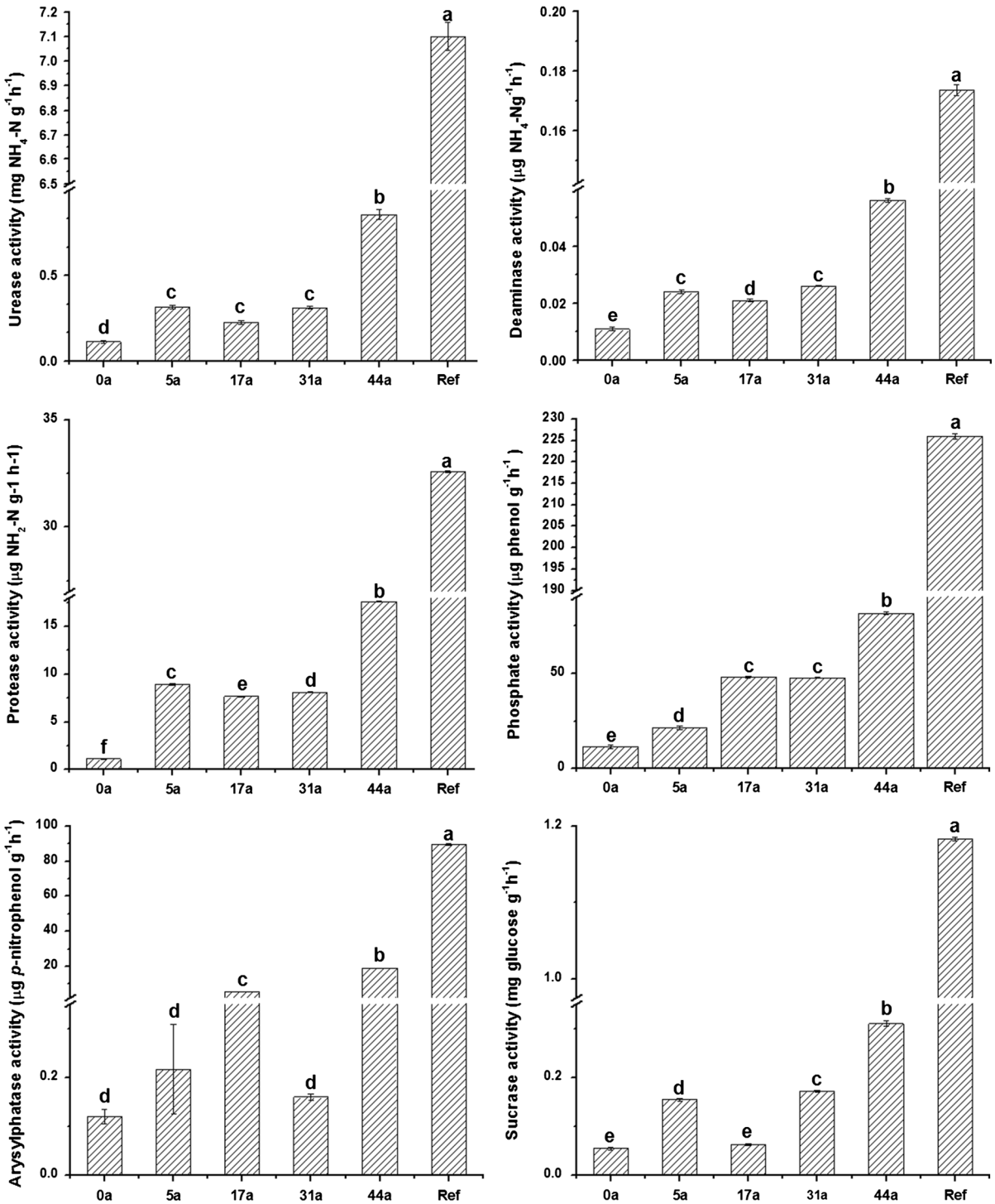

Fig. 2 Microbial enzyme activity (urease, deaminase, protease, phosphate arylsuphatase and sucrase) along the chronosequence

soils were in the fourth quadrant. Of them, 0 and 5 years samples were clustered together; and 31 years sample was more similar to 44 years samples, suggesting microbial communities within the two groups were very similar. The rotated component matrix showed a trend that the number of carbon

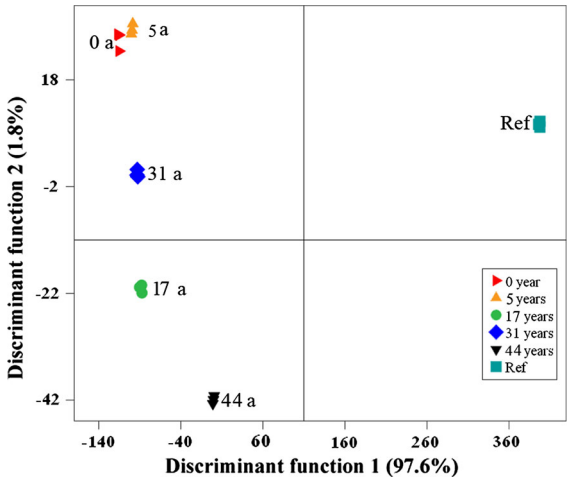

Fig. 3 A scatter diagram of discriminant functions 1 and 2 for the Tianshan Mountains No. 1 Glacier chronosequence. Groups are defined according to the age of the soil

sources with high loading (>0.7) for PC1 and PC2 belonging to amino acids, carbohydrates and polymers (Fig. 4a and supplementary Table s2). Further analyses showed that the utilization of several carbon sources presented a clear successional trend. For example, the utilization of D-glucosaminic acid was high in 0- and 5-year soils; D-galactonic acid γ-lactone was dominant in 17-year soil; N-acetyl-D-glucosamine was dominant in 31 years. D-galacturonic acid was relatively higher after 31 years (Fig. 4b). Amino acid utilization followed the same trend. For example, glycyl-L-glutamic acid was one of the three most utilizable carbon sources in 0-year samples, but utilization gradually decreased to an almost undetectable level in other samples. To the contrary, L-serine utilization ability was increased along the time sequences (Fig. 4c).

Discriminant analysis based on the AWCD values of the 31 carbon sources after 240 h of incubation revealed that newly exposed soils on young moraines were distinctly different from soils on older moraines (5- to 44-year samples and the reference). Discriminant function 1 (DF1) explained 95.5 % and DF2 explained 3.9 % of the total variance. i-Erythritol (carbohydrate), 2-hydroxybenzoic acid (phenolic acid) and putrescine (amine) were the best carbon sources for discriminating the soils according to the successional stage (Fig. 4d).

The influence of soil properties on microbial processes, enzyme activity, and CLPP

We analyzed soil microbial processes, enzyme activity, and CLPP in selected soil samples and their relationships with environmental factors (e.g., soil pH, P_t, P_{al}, and S_{at}) (Fig. 5). Of these factors, N_t, SOC, K_{al} and N_{al} were rejected in the RDA analysis because their inflation factors were larger than 20, implying that these variables were redundant with other variables in the datasets. Figure 5 shows that RDA explained over 83.1 % of dataset variability. Soil pH and available P were the dominant factors of microbial processes, soil enzyme activity and CLPP. The microbial processes, enzyme activity, and CLPP of young soils (0 and 5 years) were clearly separated from older soils (from 17 years to the reference soil). Monte Carlo significance tests revealed that the axes explained a significant amount of the variation in all of the data ($P < 0.001$).

Discussion

Anthropogenic activities and geographic location were more important than soil age for changes in soil properties

In this study, we adopted the concept of distance as a substitute for successional time to study microbial and functional communities in soils during primary succession along the forefields of Glacier No. 1 at the headwaters of Urumqi River in the Tianshan Mountains. Generally, soil properties and nutrient content were not correlated with soil age within a relatively short time frame (soil age <50 years). This observation was in agreement with previous studies (Tscherko et al. 2003, 2004; Göransson et al. 2011). For instance, the SOC, N_{al} and N_t content were only strongly correlated with soil age between the 0-year time point and the reference soil ($R^2 > 0.6$), but this correlation was not obvious at the 44-year time point ($R^2 < 0.2$) (data not shown), suggesting that soils after 44 years of glacier retreat are still under development.

To note, SOC content was significantly higher (four- to eight-fold) than in other unvegetated, recently deglaciated glacier forelands in alpine and arctic

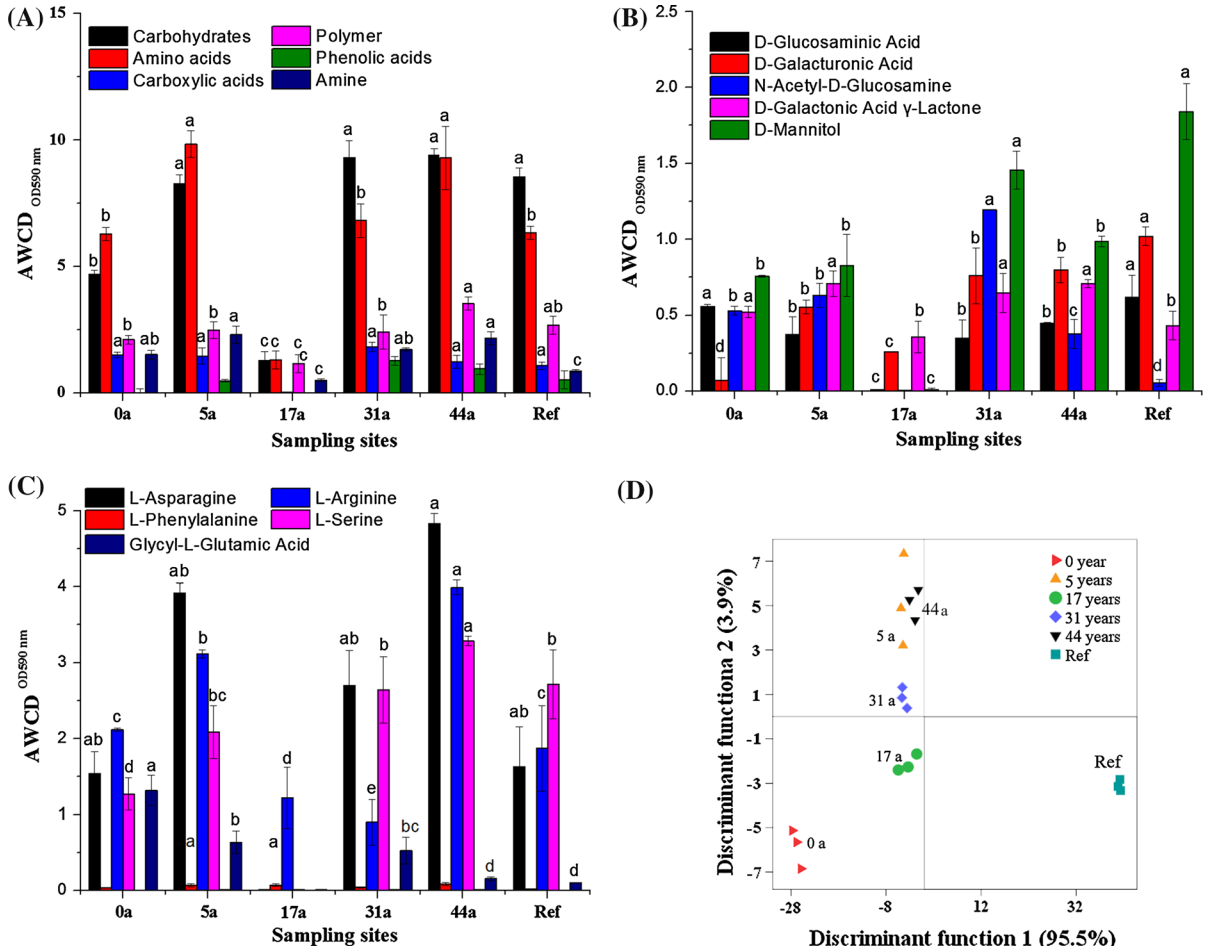

Fig. 4 The utilization efficiency of different carbon sources by the soil microbial community at different successional times. Values are the means of three independent readings. Standard deviations (±SD) are represented by *bars*. **a** Categorized carbon substrate utilization patterns of the six soil samples. **b** Utilization efficiency of carbohydrates at different successional times. **c** Utilization efficiency of amino acids at different successional times. **d** Discriminant analysis of the AWCD for 31 carbon sources from soils at different successional ages

regions. For example, SOC content in glacier foreland soils from Alaska ranged from 0.5 to 1.8 g kg^{-1} (from 0 to 4 years), and SOC content varied from 0.9 to 3.6 g kg^{-1} in Ödenwinkel and Rotmoos glacier soils (from 0 to 50 years) (Tscherko et al. 2003). This was likely due to the long-term anthropogenic activities, such as industrial and agricultural production and domestic livestock production, and regional properties, such as dust transportation, geographical location, topography and local climate conditions (Zhang et al. 2011; You and Dong 2011; Li et al. 2011; Wang et al. 2012). Glacier No. 1 is situated in the central Tianshan Mountains and surrounded by the Gurbantünggüt desert in the north and by the Taklamakan and Gobi deserts in the south and west. It is also bordered by industrial cities, including Urumqi (China, about 40 km away), Almaty (Kazakhstan), Bishkek (Kyrgyzstan) and Tashkent (Tajikistan) (Fig. 1a). As this glacier is characterized as a combination of high mountain and desert basin and thus is frontogenetic, dust particles, or individual fly ash particles, and domestic waste from the north (Urumqi) and west (Almaty, Bishkek and Tashkent) are blocked and precipitated with rain and snow (Li et al. 2000, 2011; Wang et al. 2012; You and Dong 2011; Zhang et al. 2011). For example, Li et al. (2000) found that during the past 40 years the mean organic acid content (methanoic acid, acetate, oxalate and pyruvate) in ice

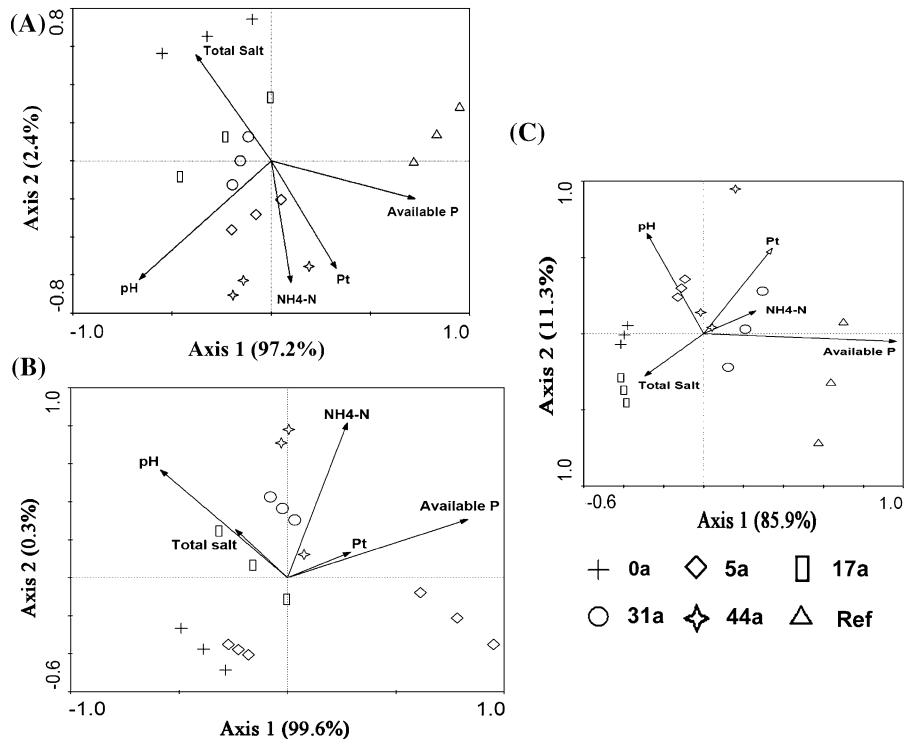

Fig. 5 Redundancy analysis plots of microbial processes (**a**), enzyme activity (**b**) and CLPP (**c**)

cores was higher than levels found in glaciers in the Arctic and Greenland. In particular, the acetate concentration (392.3 ng g^{-1}) was significantly higher than the concentration of methanoic acid (147 ng g^{-1}), and the pyruvate content was also found to be higher, suggesting that the major pollutants were from industrial production. Wang et al. (2012) studied the carbonaceous aerosol concentration of snow and ice from the glacier and found that the concentration of total carbon was as high as 1,943 ng g^{-1} soil. Additionally, Zhang et al. (2011) reported that more than 90 % of fly ash particles from the four Asian industrial cities near the glacier contained C (mainly from metallurgical industries and coal-fired power plants).

SOC was the most limiting factor for microbial process and activity in early, unvegetated soils

Despite the fact that some soil parameters did not change in a statistically significant manner after 44 years of succession, functional soil communities did change significantly. Because microorganisms are near the bottom of the food chain, microbial community changes are often a precursor to changes in environmental parameters as a whole. Therefore, they are typically the first organisms to react to chemical and physical changes in the environment. Discriminant analysis based on microbial biomass (MBC and MBN), processes (N_{min} and arginine deaminase) and enzyme activity revealed that soil samples were clustered according to soil age. Soils exposed from 0 to 5 years were grouped together, and the reference sample was significantly different from the other samples. DF1 explained 97.6 % of the total variance, and arylsulphatase was the most important variable in discriminating the soils, suggesting that functional soil communities changed regularly with soil development. These results were consistent with the result derived from foreland soils in alpine areas reported by Tscherko et al. (2003). Göransson et al. (2011) believed that SOC and N were the most limiting resources that influenced soil development and microbial activity in early soils. Furthermore, Wardle et al. (2004) showed that the SOC was the most limiting factor for microbial growth and activity in early, unvegetated, barren soils, and the N-limitation effect occurred with carbon accumulation. Our results were accord with them evidenced by the bivariate and partial correlation coefficient analysis and the ratio of

SOC/N_t. In addition, other soil parameters could also affect microbial activity to a considerable degree. For example, RDA and partial correlation analysis showed that soil microbial process and enzyme activity were significantly correlated with the pH P_{al} and K_{al}. Göransson et al. (2011) found that a small increase in pH could dramatically increase the availability of dissolved organic matter (DOM) in soil and thus significantly affect microbial activity. Our results showed that pH increased slightly during 44 years, which is consistent with their results.

SOC availability maybe the main driving force to the shift of soil heterotrophic microbial communities along the chronosequences

Discriminant analysis based on the utilization of 31 carbon sources revealed that the microbial communities of the 0-year and reference samples were significantly distinct from the other samples. This may suggest that soil organic carbon availability maybe contribute to the shift of heterotrophic microbial communities. As soil developed, the availability of organic matter decomposable by the microbial community declined. Therefore, the community may change from faster growing (R-strategy) in younger soils to K-strategists in late succession due to the higher availability of organic matter in earlier soils (Sigler et al. 2002). Such as, in this study, we noticed that amino acid-utilization rates were relatively high in early, unvegetated soils (0 and 5 years), but carbohydrate-, polymer- and phenolic acid-utilization activity was higher at later stages (31 years to the reference). Those results were in agreement with the above mentioned shift in the functional communities observed in this study and with the hypothesis that the combination of C and N addition may dramatically stimulate heterotrophic microbial activity in the youngest soil (Göransson et al., 2011).

Topography affects soil enzyme activity and microbial processes

The topography of the glacier also influenced soil nutrient distribution, and large stones and glacial streams could exert considerable influence on the microclimate of soil and increase heterogeneity (Hodkinson et al. 2003). Glacier No. 1 is a small cirque-valley glacier, and the terrain sloped upward from the glacier terminus. The terrain at 0–18 m (0–5 years) away from the glacier terminus formed a steep descent with an almost 30° angle to the horizontal and was covered with a large number of rocks. The terrain then became more even and flat from 60 to 180 m (17–44 years), and soils were poorly exposed to the sunlight. The topography of the early stages (0–5 years) formed a "safe site" that allowed protection from wind and temperature extremes, further influencing nutrient distribution (Sigler and Zeyer, 2002). However, at sites >17 years, UV radiation may have had a large impact on nutrient release. For example, the ratio of P_{al}/P_t was approximately 0.6 % in 0- to 17-year samples, but this parameter increased significantly by 31 years to the reference. Values >1 % further supported the hypothesis that older soils (31 years to the reference) were directly exposed to sunlight, which may enhance P weathering and nutrient availability in bulk soil relative to earlier soils (0–17 years) covered with large rocks. These results agree well with ratios reported for soils of the alpine glaciers (Tscherko et al. 2003, 2004).

Conclusions

In conclusion, most examined soil properties from glacier No. 1 experienced a clear succession over time in the long term, and significant changes generally occurred in newly exposed soils. Compared with the same period for other foreland soils, the rate of soil development was relatively higher. The most likely causes for these observations were the glacier-specific geographical location in arid and semi-arid central Asia, the adjacent deserts and industrial cities and the topography. Specifically, glacier No. 1 is a valley glacier that is bare in the middle and top with large rocks at the bottom. Along with soil development, the heterotrophic microbial communities and soil enzyme activity showed a clear shift along the chronosequence, and each successional stage had unique carbon-source metabolic traits. Soil C and N accumulation, nutrient availability and the geographical location of the glacier were the main impetus and factors that influenced microbial succession.

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References

Alef K, Kleiner D (1986) Arginine ammonification, a simple method to estimate microbial activity potentials in soils. Soil Biol Biochem 18:233–235

Bardgett RD, Walker LR (2004) Impact of coloniser plant species on the development of decomposer microbial communities following deglaciation. Soil Biol Biochem 36:555–559

Brankatschk R, Towe S, Kleineidam K, Schloter M, Zeyer J (2011) Abundances and potential activities of nitrogen cycling microbial communities along a chronosequence of a glacier forefield. ISME J 5:1025–1037

Brookes PC, Landman A, Pruden G, Jenkinson D (1985) Chloroform fumigation and the release of soil nitrogen: a rapid direct extraction method to measure microbial biomass nitrogen in soil. Soil Biol Biochem 17:837–842

Duc L, Noll M, Meier BE, Buergmann H, Zeyer J (2009) High diversity of diazotrophs in the forefield of a receding Alpine glacier. Microb Ecol 57:179–190

Duemig A, Smittenberg R, Koegel-Knabner I (2011) Concurrent evolution of organic and mineral components during initial soil development after retreat of the Damma glacier, Switzerland. Geoderma 163:83–94

Göransson H, Olde Venterink H, Bååth E (2011) Soil bacterial growth and nutrient limitation along a chronosequence from a glacier forefield. Soil Biol Biochem 43:1333–1340

Guan S, Zhang DS, Zhang ZM (1986) Soil enzymes and its methodology. Agric Press, Beijing Chinese

Hahn AS, Quideau SA (2013) Shifts in soil microbial community biomass and resource utilization along a Canadian glacier chronosequence. Can J Soil Sci 93:305–318

Hodkinson ID, Coulson SJ, Webb NR (2003) Community assembly along proglacial chronosequences in the high Arctic: vegetation and soil development in north-west Svalbard. J Ecol 91:651–663

Hopkins DW, Badalucco L, English LC, Meli SM, Chudek JA, Ioppolo A (2007) Plant litter decomposition and microbial characteristics in volcanic soils (Mt Etna, Sicily) at different stages of development. Biol Fertil Soils 43:461–469

Kandeler E, Gerber H (1988) Short-term assay of soil urease activity using colorimetric determination of ammonium. Biol Fertil Soils 6:68–72

Kaštovská K, Elster J, Stibal M, Šantrůcková H (2005) Microbial assemblages in soil microbial succession after glacial retreat in Svalbard (high Arctic). Microb Ecol 50:396–407

Ladd J, Butler J (1972) Short-term assays of soil proteolytic enzyme activities using proteins and dipeptide derivatives as substrates. Soil Biol Biochem 4:19–30

Li XQ, Qin DH, Zhou H (2000) Organic acids: differences in ice core records between Glac- bier 1, Tianshan, China and the polar areas. Chin Sci Bull 45:1419–1423

Li Z, Zhao S, Edwards R, Wang W, Zhou P (2011) Characteristics of individual aerosol particles over Urumqi Glacier No. 1 in eastern Tianshan, central Asia, China. Atmos Res 99:57–66

Li SS, Zhang MJ, Li ZQ, Li HL, Luo SF (2013) Variation of glacier terminuses in the Tianshan Mountains, China, during the Period of 1960–2009. Arid Zone Res 30: 378–384

Nemergut DR, Anderson SP, Cleveland CC, Martin AP, Miller AE, Seimon A, Schmidt SK (2007) Microbial community succession in an unvegetated, recently deglaciated soil. Microb Ecol 53:110–122

Philippot L, Tscherko D, Bru D, Kandeler E (2011) Distribution of high bacterial taxa across the chronosequence of two alpine glacier forelands. Microb Ecol 61:303–312

Schmidt SK et al (2008) The earliest stages of ecosystem succession in high-elevation (5000 metres above sea level), recently deglaciated soils. Proc R Soc B 275:2793–2802

Schüette UME, Abdo Z, Bent SJ, Williams CJ, Schneider GM, Solheim B, Forney LJ (2009) Bacterial succession in a glacier foreland of the high Arctic. ISME J 3:1258–1268

Schüette UME, Abdo Z, Foster J, Ravel J, Bunge J, Solheim B, Forney LJ (2010) Bacterial diversity in a glacier foreland of the high Arctic. Mol Ecol 19:54–66

Sigler WV, Zeyer J (2002) Microbial diversity and activity along the forefields of two receding glaciers. Microb Ecol 43:397–407

Sigler W, Crivii S, Zeyer J (2002) Bacterial succession in glacial forefield soils characterized by community structure, activity and opportunistic growth dynamics. Microb Ecol 44:306–316

Sparling G, West AW (1988) A direct extraction method to estimate soil microbial C: calibration in situ using microbial respiration and ^{14}C labelled cells. Soil Biol Biochem 20:337–343

Tabatabai M, Bremner J (1970) Arylsulfatase activity of soils. Soil Sci Soc Am J 34:225–229

Tscherko D, Rustemeier J, Richter A, Wanek W, Kandeler E (2003) Functional diversity of the soil microflora in primary succession across two glacier forelands in the Central Alps. Eur J Soil Sci 54:685–696

Tscherko D, Hammesfahr U, Marx MC, Kandeler E (2004) Shifts in rhizosphere microbial communities and enzyme activity of Poa alpina across an alpine chronosequence. Soil Biol Biochem 36:1685–1698

Walker LR, Del Moral R (2003) Primary succession and ecosystem rehabilitation. Cambridge University Press, Cambridge

Wang GY, Shen YP, Mao WY (2005) Climate warming at headwater of Urumqi River, Xinjiang in past 44 years and its impact on glacier shrinking. J Glaciol Geocryol 27: 813–819

Wang SJ, Zhang MJ, Wang FT, Li ZQ (2012) Characteristics of carbonaceous aerosol concentration in snow and ice of glaciers in Tianshan Mountains. Huan jing ke xue 33: 679–686

Wardle DA, Walker LR, Bardgett RD (2004) Ecosystem properties and forest decline in contrasting long-term chronosequences. Science 305:509–513

Weber KP, Legge RL (2010) Community-level physiological profiling. In: Bioremediation, Springer, pp 263–281

Wu X et al (2012) Bacterial diversity in the foreland of the Tianshan No. 1 glacier, China. Environ Res Lett 7:014038

Xia MY, Li ZQ, Wang WB (2012) Research on characteristics variation of east and west branchs of Glacier No. 1 at the headwaters of Urumqi River. J Arid Land Res Environ 26:40–44

Xu X, Pan B, Hu E, Li Y, Liang Y (2011) Responses of two branches of Glacier No. 1 to climate change from 1993 to 2005, Tianshan, China. Quatern Int 236:143–150

You X, Dong Z (2011) Deposition process of dust microparticles from aerosol to snow-firn pack on Glacier No. 1 in Eastern Tianshan Mountains, China. J Earth Sci 22: 460–469

Zhang X, Wu G, Yao T, Zhang C, Yue Y (2011) Characterization of individual fly ash particles in surface snow at Urumqi Glacier No. 1, Eastern Tianshan Chinese. Sci Bull 56:3464–3473

Zumsteg A et al (2012) Bacterial, archaeal and fungal succession in the forefield of a receding glacier. Microb Ecol 63:552–564