Patterns of organic acids exuded by pioneering fungi from a glacier forefield are affected by carbohydrate sources

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Abstract

Bare soils in the area of retreating glaciers are ideal environments to study the role of microorganisms in the early soil formation and in processes of mineral weathering. The aim of our study was to investigate whether the source of carbohydrate would influence the patterns of organic acids exuded by fungal species. Three pioneering fungus species, isolated from fine granitic sediments in front of the Damma glacier from the central Swiss Alps, have previously been found to have the capability to exude organic acids and dissolve granite powder. In batch experiments, various carbohydrates, including glucose, cellulose, pectin, pollen, and cell remnants of cyanobacteria, fungi, and algae, were applied as carbohydrate sources and the patterns of exuded organic acids recorded. The results showed that two fungi, the zygomycete fungus Mucor hiemalis and the ascomycete fungus Penicillium chrysogenum, released a significantly higher amount of organic acids in dependence on specific carbohydrate sources. Pollen and algae as carbohydrate sources triggered significantly the exudation of malate in M. hiemalis, and pollen and cellulose that of oxalate in P. chrysogenum. We conclude that the occurrence of complex carbohydrate sources in nutrient-deficient deglaciated soils may positively influence the exudation of organic acids of fungi. In particular, pollen and remnants of other microorganisms can trigger the exudation of organic acids of fungi in order to promote the weathering of minerals and to make nutrients available that would otherwise be trapped in that cryospheric environment.

Keywords: citrate, malate, Mucor, oxalate, Penicillium, retreating glacier, Swiss Alps, Umbelopsis

1. Introduction

Soils of glacier forefields are inhabited by a large variety of microorganisms, such as bacteria, cyanobacteria, archaea, green algae, and fungi (Bardgett et al 2007, Frey et al 2013, Zumsteg et al 2012). Several species of these microorganisms have been shown to exude organic acids, that play an important role in weathering the parent rock material of the glacier forefield (Brunner et al 2011, Frey et al 2010, Lapanje et al 2012). The microorganisms secrete these acids in order to make available the nutrients that would otherwise be trapped in the environment (Gadd 1999, Rosling et al 2007, Rosling 2009). Citrate, malate, and oxalate are three common organic acids, which have been found in the exudates of fungi collected from the glacier forefield (Brunner et al 2011). These acids have the chemical capacity to dissolve granite and increase the concentration of macro- and micronutrients in solution.
mainly Ca, Mg, P, Fe, Mn (Brunner et al. 2011, Hausrath et al. 2009, Neaman et al. 2006).

During the first years after a glacier has retreated, there is no vegetation. Thus, an ecological quandary concerning microorganisms in plant-free, high elevation soils arises regarding how carbon (C) can be obtained for growth and which C can be used (Lynch et al. 2012, Schmidt et al. 2012). Smittenberg et al. (2012) estimated in bare soils without vegetation at the Damma glacier site in Switzerland a C content of around 0.07%, which corresponds to about 50 g C m⁻². It is likely that, besides of the ancient recalcitrant organic matter, deposited C and C from cell remnants of green algae and cyanobacteria, either from supraglacial sources or from forefields, are the major primary C sources in this environment (e.g. Bardgett et al. 2007, Irvine-Fynn et al. 2012, Kastovska et al. 2005, Sattin et al. 2009, Schmidt et al. 2012, Schurig et al. 2013). Deposited C may contain besides of pollen and detritus from wind-blown vegetation also arthropods (Coulson et al. 2003, Edwards 1987, Hawes 2008). Hawes (2008) recorded on a glacier in Spitzbergen, Norway, a maximum deposition of about 0.2 g C m⁻² yr⁻¹, which was, however, composed mainly of plant detritus and only to a minor extent of arthropods. At alpine sites in Colorado, USA, Ley et al. (2004) estimated an aeolian C input of about 0.5 g C m⁻² yr⁻¹, and Schmidt et al. (2012) proposed for the same sites that the deposited C contained large amounts of pine and spruce pollen which were blown from lower elevations to these barren sites during late spring and early summer. Masclaux et al. (2011) estimated maximum inputs from pollen up to 1 g C m⁻² yr⁻¹, from pollen deposited by ‘pollen rains’.

Miltner et al. (2012) postulated a new conceptual model of the C flow in soils, proposing that, although C originally derived from plant organic matter (e.g. pollen), the molecular characteristics of the C are derived from microbial biomass. When microorganisms degrade plant residues, they mainly use easily degradable residues (e.g. proteins, DNA) to build their own biomass. After death, these microorganisms provide the microbial-derived molecular imprint of the soil organic matter (mainly cell wall fragments). The persistent plant parts (e.g. cellulose, lignin) are left ‘relatively’ unaltered, providing the plant-derived molecular imprint (Miltner et al. 2012). Microbial necromass in soils can be up to 40 times higher than the living microbial biomass, and up to 80% of the soil organic C may be derived from microbial biomass (Liang and Balser 2010, Liang et al. 2011). Zumsteg et al. (2013) showed, by applying a ¹³C stable isotope probing method, that bacteria from the glacier forefield do utilize fungal and algal cell material as C source.

In a preliminary study (Brunner et al. 2011) it was shown that glucose as a ‘simple’ and ‘pure’ carbohydrate source can induce the exudation of organic acids. However, it is unlikely that glucose does occur as a carbohydrate source in a glacier forefield. Thus, the question arises whether more ‘complex’ carbohydrate sources than glucose, e.g. cell wall remnants or pollen, would trigger the exudation of organic acids as well. Our hypothesis was that more ‘complex’ carbohydrate sources than glucose would not result in a breakdown of the organic acid exudation. An additional objective of the present study was to find out, which carbohydrate source would trigger the exudation of organic acids the most.

2. Materials and methods

2.1. Fungal strains

Three strains of fungi were used in this study. They were selected from fungi used in a prior dissolution experiment using granite powder, where the production of the organic acids citrate, malate, and oxalate had been observed (Brunner et al. 2011). The chosen strains included two zygomycete strains, Mucor hiemalis (WSL:D30, NCBI:HM002770) and Umbelopsis isabella (WSL:D20, NCBI:HM002767), and one ascomycete strain Penicillium chrysogenum (WSL:D35, NCBI:HM002773). These strains were isolated from granitic sediment material sampled from the Damma glacier forefield in the central Alps of Switzerland (46°38’ N, 08°28’ E) in August 2007 and were identified by sequencing the nuclear small subunit ribosomal DNA (Brunner et al. 2011). Starter material has since been maintained in the fungal collection of the Swiss Federal Institute of Forest, Snow and Landscape Research (WSL). Mother cultures were propagated from this starter material on 2% malt-agar media. For the experiments, mycelial mats were grown by further propagation on malt-agar media topped with cellophane roundels covering the surface of the agar media. The roundels prevented the fungi from adhering to the media, so that once fungal mats had formed on the cellophane surface, requiring about one week, these mats could be lifted off the cellophane surface with a sterilized hook, and transferred to liquid culture Erlemeyer flasks without the cellophane.

2.2. Exudation experiments

A liquid culture system was used for the treatment experiments. One hundred ml Erlenmeyer flasks were filled with 40 ml media, topped with a paper stopper, the paper stopper covered with aluminum foil, and autoclaved. Fungal mats were then transferred into the flasks, which were packed into boxes, covered in aluminum foil to block out light, and put to shake at 98 rpm in a circular motion in a climatic room maintained at 25 °C.

All three fungal strains were used in triplicate for each carbohydrate source, as well as a negative control containing the full media but no fungal inoculation (4 biological treatments = 3 fungal treatments and 1 un-inoculated treatment). Experiments were conducted with seven different carbohydrate source regimes (4 biological treatments × 7 carbohydrate sources × 3 replicates). To the treatment solutions with glucose, cellulose, or pectin as ‘pure’ carbohydrate sources, 0.2 mM CaCl₂ and 0.19 mM NH₄Cl were added to the Millipore water (pH 5.5). The ‘pure’ carbohydrate source was added in concentrations of 20 mg l⁻¹ (glucose) or 17.7 mg l⁻¹ (cellulose, pectin). To the treatment solutions with pollen, fungi, cyanobacteria, or algae as ‘complex’ carbohydrate sources, neither CaCl₂ nor NH₄Cl, was added to the Millipore water because these ‘complex’ carbohydrate sources contain already Ca and N. The ‘complex’ carbohydrate source was added in concentrations of 50 mg l⁻¹. All media ingredients were added before autoclaving, except glucose. Glucose was sterile filtered (0.22 µm, Rotilabo® syringe filter,
Table 1. C- and N-contents and concentrations of the applied carbohydrate sources. All carbohydrate sources were added as dry powders.

| Carbohydrate source | Applied concentration (mg l⁻¹) | C Content (%) | Concentration (mg C l⁻¹) | N Content (%) | Concentration (mg N l⁻¹) |
|---------------------|---------------------------------|---------------|---------------------------|---------------|---------------------------|
| Glucose             | 20.0                           | 40.0          | 8.0                       | 0.00          | 0.0⁹                    |
| Cellulose           | 17.7                           | 42.5          | 7.5                       | <0.03         | 0.0³                    |
| Pectin              | 17.7                           | 35.9          | 6.4                       | 0.11          | 0.0⁹                    |
| Pollen              | 50.0                           | 45.1          | 22.6                      | 2.88          | 1.4                      |
| Cyanobacteria       | 50.0                           | 33.7          | 16.9                      | 3.79          | 1.9                      |
| Fungi               | 50.0                           | 42.8          | 21.4                      | 3.89          | 1.9                      |
| Algae               | 50.0                           | 49.2          | 24.6                      | 6.48          | 3.2                      |

⁹ 2.7 mg N l⁻¹ were added to the solutions in the form of 0.19 mM NH₄Cl.

Carl Roth, Karlsruhe, DE) into the flasks after the flasks were autoclaved and cooled down in order to prevent caramelization of the glucose. The concentrations used are thought to be similar to environmental conditions, and were based on the study of Brunner et al (2011). A detailed description of the media composition across the exudation experiments can be found in Table 1.

The carbohydrate sources used were glucose (Sigma, Switzerland), cellulose (C11, for Chromatography, Whatman, USA), pectin (from apples, No 76282, Fluka, Switzerland), food-grade bee pollen (BIOREX, Ebnat-Kappel, Switzerland), cyanobacteria thalli (Nostoc sp.), fungal mycelia (Penicillium chrysogenum D35), and green algae cells (Chlorella sp.). The cyanobacteria thalli were harvested from the grounds of the WSL Institute, washed with distilled water, dried at 40°C for several days, milled into a fine powder, and suspended in Millipore water for addition into the media. The fungal mycelia was grown on cellophane roundels on malt-agar plates, dried at 40°C, finely milled, and suspended in Millipore water for addition into the media. The fungal mycelia was grown on cellophane roundels on malt-agar plates, dried at 40°C, finely milled, and suspended in Millipore water. The algae, formerly isolated from the Damma glacier forefield, were propagated in BBM media for photobionts (Bischoff and Bold 1963) for six weeks, centrifuged, rinsed, and dried at 40°C before suspension in Millipore water.

2.3. Sampling and processing

After one and two weeks of incubation, 3 ml of the liquid media was removed from the flasks under a sterile flow hood, using a sterile syringe, and sterile filtered (0.22 μm) into small glass ion-chromatography vials which were stored at 4°C until analysis. After two weeks of incubation, the contents of the Erlenmeyer flasks were vacuum filtered through Whatman 0.45 μm filter roundels to collect the fungal materials, which were then dried in a cabinet at 40°C for three days, and then weighed to determine the fungal biomass.

2.4. CN analysis

Total C and N levels of the various carbohydrate sources used in the experiments were measured after combustion and measuring the combustion products CO₂, N₂, and NOₓ with the CN analyzers NC 2500 or NA 1500 (CE Instruments, Wigan, UK) (similarly as described by Horneck and Miller 1998). Both instruments deliver comparable C and N values. The NA 1500 was connected to a mass spectrometer for measuring additionally the stable C and N isotopes, which were used for another study (compare also Zumsteg et al (2013)). Triplicate samples were measured.

2.5. Analysis of glucose

Concentration of glucose in the treatment solutions after one and two weeks of incubation were analyzed by ion chromatography on a Dionex ICS-3000 (Dionex AG, Olten, Switzerland), using a 3 mm × 150 mm CarboPack PA-20 analytical column (similarly as described by Zhu et al (2013)). A 3 mm × 30 mm CarboPack PA-20 column was used as a pre-column to improve run quality. The detection was taken by electrochemical accumulation on an Au-electrode. The detection limit was 0.05 mg l⁻¹.

2.6. Analysis of organic acid

Concentrations of the organic acids citrate, malate, and oxalate in the treatment solutions after one and two weeks of incubation were analyzed by ion chromatography on a Dionex ICS-3000 (Dionex AG, Olten, Switzerland), using a 2 mm × 250 mm IonPac AS19 analytical column (similarly as described by Karthikeyan et al (2007)). A 2 mm × 50 mm IonPac AG19 guard column followed by a 2 mm × 50 mm IonPac AG11-HC guard column were used as pre-columns to improve run quality. Cell temperature of the conductivity detector was set to 35°C and column temperature to 30°C. Separation of the organic acids in the columns was achieved using a NaOH gradient. The detection limit was 0.15 mg l⁻¹ for all organic acids.

Exudation rates of the fungal mycelia were calculated after two weeks of incubation by dividing the concentrations of organic acids in the media by the fungal biomass and the incubation time.

2.7. Statistical analyses

All statistical analyses were performed using STATVIEW 5.0 (SAS Institute Inc., Cary, NC, USA). The effects of the time exposure on the organic acid exudations was analyzed by...
repeated measures ANOVA, and the effects of the various carbohydrate sources and of the various fungus species on the exudation rates of organic acids were analyzed by 'one-way ANOVA' using Fisher's PLSD test. The regression analysis was used to test the relationship between the exudation rates of citrate versus malate, and with using ANOVA to test the significance.

3. Results

3.1. Characterization of carbohydrate sources

All carbohydrate sources had C contents between 33% and 50%, with the cyanobacterial powder having the lowest and the algal powder having the highest value (table 1). Nitrogen was measured in glucose, cellulose, and pectin only in small amounts (<0.2%). In the other carbohydrate sources, N was measured with values between 2.8% and 6.5%, with the highest level in the algae (table 1).

3.2. Growth of fungi

The biomasses of the fungal inoculi after two weeks of growth were, for Mucor hiemalis between 26 and 86 mg, for Umbelopsis isabellina between 38 and 99 mg, and for Penicillium chrysogenum between 30 and 66 mg. The biomasses did not significantly differ based on the carbohydrate source (data not shown).

3.3. Glucose in solutions

Glucose was measured in solutions for all the treatments with a fungal inoculation and for the control. The glucose concentrations with the fungal inoculi were between 0 and 30 µmol l\(^{-1}\), but mainly between 5 and 15 µmol l\(^{-1}\) (figure 1). The two zygomycete fungi, M. hiemalis and U. isabellina, contained in their solutions about double the amount of glucose compared to the ascomycete fungus P. chrysogenum. Glucose decreased significantly between the first and the second week in the cases of M. hiemalis (F = 15.9, P = 0.001) with fungi and algae as carbohydrate sources, and of P. chrysogenum (F = 10.8, P = 0.005) with pectin. In all other cases the amounts of glucose remained about on the same levels.

In the control treatment without fungal inoculation, glucose was present only in the glucose and the pollen treatment (figure 1), but it was not present (or only in traces) in all other carbohydrate-treatments (cellulose, pectin, cyanobacteria, fungi, algae). In the glucose treatment, the glucose concentration was around 160 µmol l\(^{-1}\), which corresponds to about 29 mg l\(^{-1}\) glucose (compared to 20 mg l\(^{-1}\) glucose added). This indicates an overestimation of the glucose of about 45% caused by the method of measurement. The addition of pollen resulted in a glucose concentration of about 50 µmol l\(^{-1}\), which corresponds to about 6.3 mg l\(^{-1}\) glucose (for 50 mg l\(^{-1}\) pollen added).

3.4. Organic acids

The organic acids citrate, malate, and oxalate were detected in all treatment solutions with fungal inoculation. Citrate was most prominent in connection with M. hiemalis (between 40 and 80 µmol l\(^{-1}\)), malate in connection with M. hiemalis (mainly after two weeks of incubation: between 20 and 200 µmol l\(^{-1}\)), and oxalate in connection with M. hiemalis (between 50 and 100 µmol l\(^{-1}\)) and P. chrysogenum (between 100 and 500 µmol l\(^{-1}\); figure 2). In general, there was a slight increase of the organic acids when comparing the concentrations after one week of incubation with that of two weeks. Significant increases were recorded mainly for M. hiemalis and malate (F = 46.8, P < 0.001) and for P. chrysogenum and oxalate (F = 7.91, P = 0.015, figure 2). In the control treatment solutions without fungal inoculation, organic acids were not measured, except in the case with glucose, where a very low amount of citrate (1.4 µmol l\(^{-1}\)) was detected. This value was only slightly above the detection limit of 0.8 µmol l\(^{-1}\) (=0.15 mg l\(^{-1}\)).

Looking at the exudations rates of the organic acids, some of the carbohydrate sources had distinct effects (figure 3).
Overall significant effects were observed for *M. hiemalis* and malate (*F* = 5.38, *P* = 0.005) and for *P. chrysogenum* and oxalate (*F* = 3.87, *P* = 0.020). Pollen and algae as carbohydrate sources triggered the exudation of malate with *M. hiemalis*, and cellulose and pollen that of oxalate with *P. chrysogenum* (figure 3). There were no significant triggering effects on the exudation rates with glucose, pectin, cyanobacteria, and fungi as carbohydrate sources, and no effects for *U. isabellina* in connection with any of the C sources (figure 3). The rates of the organic acids exuded by the three fungal species were only in the case of *P. chrysogenum* for oxalate significantly different (*F* = 24.5, *P* < 0.001), with a manyfold increase of the oxalate compared to citrate and malate (table 2).

The regression analysis showed that the exudation rates of the organic acid citrate were positively related with that of malate (*R*² = 0.57, *P* < 0.001, figure 4). In contrast, the exudation rates of oxalate were not related with that of citrate or that of malate (*R*² < 0.02, *P* > 0.05, data not shown).

### Table 2. Mean exudation rates of organic acid anions (×10^-8 μmol mg^-1 s^-1). One-way ANOVA: Significant *P*-values (*P* < 0.05) are given, and not significant *P*-values are indicated with ‘Ns’. Different letters (a, b) implicate significant differences (*P* < 0.05) between the organic acids.

| Fungus species       | Citrate | Malate | Oxalate | *P*  |
|----------------------|---------|--------|---------|------|
| *Mucor hiemalis*     | 4.16    | 7.16   | 6.51    | Ns   |
| *Umbelopsis isabellina* | 0.17   | 0.46   | 0.12    | Ns   |
| *Penicillium chrysogenum* | 0.22b  | 0.28b  | 18.43a  | <0.001 |

4. Discussion

Pioneering fungi from glacier forefields are able to exude various low-molecular weight organic acids that can promote the weathering of the parent rock material (Brunner et al 2011). Because fungi are heterotrophic organisms, they are dependent on external carbohydrate sources. However, it is...
unclear which carbohydrate sources these fungi can utilize, and how the carbohydrate sources would affect the exudation patterns. In addition, the amount of available organic C in a glacier forefield is extremely scarce (below 0.1%) and the type of C undefined (Bernasconi et al. 2011). It is estimated that the accumulation rate of soil organic C at glacier forefields is between 1 and 36 g m$^{-2}$ yr$^{-1}$ (Düming et al. 2011), and it is likely that the soil organic C is a mixture of ancient recalcitrant C, of recent organic C which was deposited, and of recent C from cell wall remnants of auto- and heterotrophic microorganisms (Bardgett et al. 2007, Miltner et al. 2012). At the Damma glacier forefield the accumulation rate of soil organic C is around 7.1 g m$^{-2}$ yr$^{-1}$ (Düming et al. 2011), and there is evidence, based on radiocarbon age estimates, that ancient C might be the dominant source of soil-respired CO$_2$ (Guelland et al. 2013).

In our batch experiments, the highest rates of organic acid exudation with rates up to $39.7 \times 10^{-8}$ µmol mg$^{-1}$ s$^{-1}$ were observed for oxalate when cellulose or pollen were used as carbohydrate sources in combination with Penicillium chrysogenum. Algal or pollen material as carbohydrate sources were the most predominant triggers for malate in combination with Mucor hiemalis, with maximum exudation rates up to $15.7 \times 10^{-8}$ µmol mg$^{-1}$ s$^{-1}$. In particular, the ‘complex’ carbohydrate sources algae and pollen triggered the exudation of oxalate and malate in the cases described above about three to four times compared to glucose as a carbohydrate source. In contrast, glucose was never the main triggering carbohydrate source for the exudation of organic
acids, and none of the 'complex' carbohydrate sources, e.g. pollen, algal, cyanobacterial, or fungal cell wall remnants, caused a significant breakdown of the organic acid exudation. The patterns of exuded organic acids of *P. chrysogenum* and *M. hiemalis* resembled an earlier study, which used solely glucose as a carbohydrate source (Brunner et al 2011). However, the exudation pattern of *Umbelopsis isabellina* was distinctly different compared to that earlier study, with the concentrations of organic acids being very low. It could be that the powdered granite material, which was not included in the present experimental trials, was the trigger for the exudation of the organic acid in *U. isabellina* in that earlier study.

Oxalate is the most common low-molecular weight organic acid that is produced by fungi (Gadd 1999, Plassard and Fransson 2009, Fransson and Johansson 2010). It plays a crucial role in wood-decay processes, lignocellulose degradation, plant pathogenesis, and weathering (Gadd 1999, Plassard and Fransson 2009). Oxalate is also an unwanted metabolite of the ascomycete *Aspergillus niger* in the industrial production of citrate (Plassard and Fransson 2009). In our experiment with *Penicillium chrysogenum*, a relative of *A. niger*, predominantly oxalate was produced. Citrate and malate exudation rates were rather low, regardless of the carbohydrate sources applied.

It has been shown elsewhere, that species of *Penicillium* exude oxalate and citrate in order to solubilize 'unavailable' forms of P (e.g. Chai et al 2011, Pandey et al 2008). Depending on the P sources, fungal species are also able to adapt production and patterns of the exuded organic acids. For example, the two *Penicillium* species *P. janthinellum* and *P. purpurogenum* can modulate the exudation of citrate and other organic acids in dependence on the P source (Scervino et al 2010). Scervino et al (2011) also showed for *P. purpurogenum* that the type of carbohydrate source modulates the exudation of organic acids and the solubilization of P as well, with glucose and fructose being the best triggers. Reyes et al (1999) reported for *P. rugulosum* that sucrose was the best carbohydrate source for iron phosphate solubilization. In studies using *Aspergillus*, a relative of *Penicillium*, Relwani et al (2008) showed for *A. tubingensis* that glucose and sucrose were the best carbohydrate sources for P solubilization, and Narsian and Patel (2000) showed for *A. aculeatus* that maximum P solubilization was obtained using glucose and arabinose as carbohydrate sources. In the latter two studies it was found that the carbohydrate source lactose was not a good trigger (and with cellulose not being tested in their trials).

In the study of Scervino et al (2011), the nature of the carbohydrate sources, which affected P solubilization by *P. purpurogenum*, were diverse, with glucose being a better trigger that cellulose. Thus, it is surprising that in the present study glucose was never the best trigger for the exudation of the organic acids. Moreover, cellulose and the 'complex' carbohydrate sources algal and pollen materials were in two cases the best triggers, once with *P. chrysogenum* and oxalate, and once with *M. hiemalis* and malate. We assume, that 'complex' carbohydrate sources trigger better the exudation of organic acids, because they contain in addition to the C also the macro- and micronutrients P, Mg, K, Mn, Fe, or Zn. The beneficial effect of cellulose, however, is contradictory: it triggered with *P. chrysogenum* significantly the exudation of oxalate, but, according to our definition, it is not considered as a 'complex' carbohydrate source (is does not contain nutritional elements) but it is a 'pure' carbohydrate source (it is composed of several hundred to over ten thousand linked glucose units).

Several species of *Aspergillus*, which are relatives of *Penicillium*, are known to exude naturally large amounts of organic acids, e.g. *A. niger* exudes citrate and *A. flavus* malate (Papagianni 2007, Battat et al 1991). Papagianni (2007) showed that the rate of citrate production is strongly correlated with amount of available glucose. The excretion of citrate into the medium is thought to be an active process, requiring ATP (Papagianni 2007). Therefore, it would make sense that the 'complex' carbohydrate sources, which contain P, would be more suitable carbohydrate sources than the 'pure' carbohydrate sources. In fungi, the only known and characterized gene, which encodes a carboxylate permease, is most likely the malate transporter Mael (Casal et al 2008). It is thought that the mono-anionic form of the organic acids is transported by a proton-symport mechanism (Sousa et al 1992, Zelle et al 2010). Thus, this could explain why the exudation of citrate showed a positive significant relation with that of malate, because both organic acids would be transported by a similar mechanism.

The easy use of 'complex' carbohydrate sources, e.g. pollen or algae, which contain considerable amounts of cellulose, by the fungi in our study is not surprising. These fungi have a large set of carbohydrate-active enzymes (CAZy). *Penicillium chrysogenum* has more than 40 different enzymes involved in cellulose and pectin degradation as *Aspergillus spp.* (Van den Brink and de Vries 2011). The zygomycete *Rhizopus oryzae*, a relative to *Mucor hiemalis*, however, has only around 27 enzymes to break down cellulose and pectin (Battaglia et al 2011). Based on the CAZy database (www.cazy.org), *P. chrysogenum* has a comparable amount of genes involved in cellulose and pectin degradation as *Aspergillus spp.* (Van den Brink and de Vries 2011). The zygomycete *Rhizopus oryzae*, a relative to *Mucor hiemalis*, however, has only around 27 enzymes to break down cellulose and pectin (Battaglia et al 2011). Consequently, this fungus has difficulties growing on xylan substrates because the genes required for xylan degradation are absent (Van den Brink and de Vries 2011). In our study, cellulose alone was not a trigger for the organic acid exudation neither in *M. hiemalis* nor in *Umbelopsis isabellina*. Mostly,
the use of cellulose resulted in the lowest organic acid exudation rate. The best triggers of malate by *M. hiemalis* were the ‘complex’ carbohydrate sources pollen and algae. However, it is most likely, that *M. hiemalis* utilized from these carbohydrate sources mainly the easy carbohydrates (e.g. glucose and other sugars) and not the cellulose. Consequently, zygomycete fungi have been described as fast growers on easily accessible and digestible substrates (Richardson 2009).

5. Conclusions

In conclusion, we can state that the patterns of exuded organic acid exudation are dependent primarily on the fungal species, secondarily on the available carbohydrate sources, and thirdly on the length of the experiment. It seems that carbohydrate sources, which contain not only C but also nutrient elements, e.g., algal substrates or pollen material, can trigger better the exudation of organic acids compared to pure and simple carbohydrates such as glucose. Thus, the occurrence of complex carbohydrate sources in nutrient-deficient deglaciated soils may positively influence the exudation of organic acids of fungi and, consecutively, the early weathering of minerals as an important process in the formation of soils in these cryospheric environments.

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