



# Arctic willow (*Salix polaris*) exudation as a driver of microbial activity and soil formation in the high arctic tundra

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**Abstract** Colonization by pioneer plants, among which the arctic willow (*Salix polaris*) is one of the most important, accelerates soil development after deglaciation. This is achieved through the increased input of organic matter from plant biomass and the exudation of low molecular mass organic compounds (LMMOA), predominantly organic acids, which facilitate mineral dissolution and nutrient release. These exudates support microbial activity and contribute to the formation of soil organic matter. While there is quite a lot of data on the exudation and acceleration of microbial activity in the rhizosphere of various

plants, similar data concerning arctic plants, including willow, are scarce. Furthermore, there is a lack of data on the effect of C, N, P root stoichiometry on nutrient content in exudates and the rhizosphere microbiome during soil succession after deglaciation. In this study, we analysed various habitats of high-arctic tundra in Petuniabukta (Billefjorden, Svalbard), representing different stages of vegetation development. Our objectives were (i) to assess soil and rhizosphere carbon and nutrient content and availability, as well as microbial biomass CNP; (ii) to evaluate the rhizosphere effect on nutrient availability and the microbiome of arctic willow; and (iii) to measure root and exudation CNP and quality, primarily LMMOA, in arctic willow from the studied habitats. The exudates released to deionised water were analysed for LMMOA and inorganic anions (ion chromatography)

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as well as the total content of C and N. The plants roots were analysed for CNP content. Soil chemical properties (e.g. pH, organic C, total and exchangeable content of elements, water extractable  $\text{PO}_4^{3-}$ ) and microbial parameters (microbial biomass and quantity of bacteria and fungi) were assessed in both rhizosphere and bulk soils, with the rhizosphere effect calculated accordingly. The most abundant LMMOA species in willow exudates were lactate, acetate, formate, malate and citrate, followed by pyruvate, quinate and oxalate, collectively representing approximately 2% of the total exuded C. The rhizosphere effect of willows on nutrient availability and microbial parameters was the most significant at sites with early soil development and diminished with increasing vegetation cover. A link was observed between nitrogen and phosphorus exudation and plant root stoichiometry. These trends underscored the essential role of root exudation in overcoming microbial nutrient limitations during early soil development, particularly in sites with lower nitrogen availability by reducing the soil C/N ratio.

**Keywords** Svalbard · Low molecular mass organic acids · Elements stoichiometry · Rhizosphere effect · Roots · High arctic · Soil chemistry

## Introduction

Deglaciation of high arctic glaciers (Ludwigsen et al. 2020) leads to newly formed soil from sediments (Bradley et al. 2014; Kabala and Zapart 2012). The pedogenetic process is highly influenced by the colonization of primary producers—cyanobacteria, algae, and moss, which enhance rock weathering by their other respiration and organic substances exudation (Borin et al. 2010). These organic substances are resources for the growth of heterotrophic communities (Bardgett et al. 2007), which play a key role in nutrient cycling.

Later on, as soil development progresses, the role of initial colonisation is taken over by vascular plants (Jones and Henry 2003). The influence of plants on the soil environment is first evident in the roots rhizosphere. Easily consumable exudates (organic acids, sugars) and dead root biomass support rhizosphere microbiome which is more active in rhizosphere soil than in surrounding bulk soil (Orgiazzi et al. 2018).

These oases of life with more easily available nutrients significantly accelerate further biological colonization (Miniaci et al. 2007).

Shrub growth is generally considered as important indicator of Arctic zone evolution because of its high sensitivity to climate changes (Myers-Smith et al. 2015). Willow shrubs emerge as one of the first vascular plants after deglaciation in the High Arctic (Nakatsubo et al. 2010; Prach and Rachlewicz 2012). With reported lifespan of up to 120 years (Le Moullec et al. 2019), willows serve as valuable indicators for reconstructing long-term ecosystem dynamics throughout the entire Arctic region. The willow shrubs expansion is positively correlated with increasing temperatures (Clemmensen and Michelsen 2006; Forbes et al. 2010) and negatively associated with extensive snow cover in early spring (Boulanger-Lapointe et al. 2014; Schmidt et al. 2006). Conversely, a denser shrub layer can result in increased snow depth during the winter, providing better insulation for the soil, leading to higher soil temperatures and increased microbial activity (Sturm et al. 2005). Fast colonization of deglaciated or colder areas by willow species has been documented (Boulanger-Lapointe et al. 2014) and their increasing impact on soil formation and C sequestration in the soil can be expected. This effect is particularly profound in the High Arctic, expected to be a highly sensitive ecosystem to these changes (Post et al. 2009).

Root exudation represents important flux of easily available organic C to the soil environment. It can generally account for 5–25% of plant net C assimilation (Farrar et al. 2003; Jones and Darrah 1994; Jones et al. 2004). Exudation is a strategy of plants for increasing nutrient availability and for the detoxification of potentially risk elements (e.g. Al, Cd) (Jones and Darrah 1994; Neumann and Römheld 2007). Exudates also play an important role as carbon and energy sources for microorganisms (Wang et al. 2023), and so they influence microbial community structure and enhance their activity in the close surrounding of roots (Esperschütz et al. 2009; Shi et al. 2011). The input of easily available organic compounds accelerates decomposition of old soil organic matter (Blagodatskaya and Kuzyakov 2008; Kaštovská et al. 2022; Wild et al. 2014; Yang et al. 2016) and nutrient release from decomposing organic matter and thus stimulate soil development and nutrient cycling in poor soils or primary substrate (e.g.

Fontaine et al. 2011; Phillips et al. 2011). However, the development of the rhizosphere microbiome and nutrient release to soil depend not only on the exudation of available C but also on N and P availability, that the microorganisms can obtain either from soil organic matter decomposition or directly from exudates. While there is quite a lot of data on exudation of C and nutrients, and on acceleration of microbial activity in rhizosphere of various plant (Drake et al. 2013; Kaštovská et al. 2015; Prescott et al. 2020), similar data concerning arctic plants including willow are scarce. Furthermore, there is a lack of data on the effect of CNP root stoichiometry on the content of nutrients in exudates and on rhizosphere microbiome during soil succession after deglaciation.

Within root exudation, low molecular mass organic acids (LMMOA) can make high proportion of exudation. For example LMMOA exudation by lupin roots under P deficiency can make up to 25% of plant photosynthetically fixed C (Jones 1998). They play a crucial role as C and energy resources, chemoattractant signals to microbes, chelators, etc., but also as soil acidifiers (Haichar et al. 2014). Therefore LMMOA are fundamental for the plant–microbial interactions and soil formation (Shi et al. 2011) and their importance is increasing with global warming (Bertin et al. 2003; Lynch et al. 2018). Many authors (Drake et al. 2015; Wild et al. 2014; Yang et al. 2016) highlighted the need for a better understanding of the origin and behaviour of LMMOA, particularly in arctic soils, where the role of shrub exudation was found to be important in mitigating soil carbon losses due to enhanced CO<sub>2</sub> fixation and increased soil C storage/stabilisation/sequestration (Lynch et al. 2018).

Shrubs play important role in enhancement of total amount and availability of nutrients and this effect is most pronounced in the close surrounding of roots due to input of organic compounds via exudation and enhancement of microbial activity. Rising temperatures are accelerating shrubs development, with Arctic willow (*Salix polaris*) as a pioneer species (Ślupianek et al. 2019). We postulate that the exudation of LMMOA will accelerate nutrient availability and soil formation. In addition, it will depend on root and exudate stoichiometry to shape the rhizosphere microbiome. The effect will be more pronounced in the early stages after deglaciation as compared with later stages, which will be indicated by the larger rhizosphere effect, as defined

by the ratio of rhizosphere-to-bulk soil, on nutrient availability and microbial biomass. We run a detailed study of bulk soil, arctic willow rhizosphere and exudates in the High Arctic habitats with different plant cover and in different stages of soil formation to assess the influence of willow on the development of the High Arctic soils. The aims of these contributions are (1) To measure soil and rhizosphere C and nutrient content and availability as well as microbial biomass CNP across the different soil habitats. (2) To evaluate the rhizosphere effect on available nutrients and microbiome of arctic willow in different habitats; (3) To measure in microcosm experiment root and exudates CNP, and quality LMMOA, in the arctic willow from the studied habitats. Soil chemistry was characterized by the total contents of elements, CEC, base saturation and the content of exchangeable and water-extractable carbon and nutrients. Microbial biomass and quantity of bacteria and fungi were used as the biological parameters. Rhizosphere effect (REs) was assessed from the rhizosphere-to-bulk soil ratios. Root exudation was measured in a microcosm experiment.

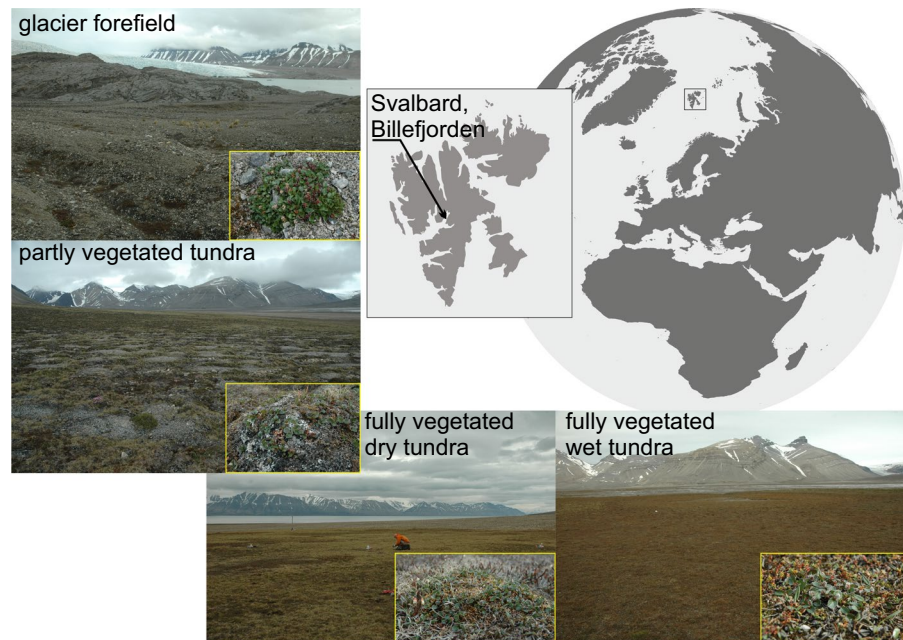
## Material and methods

### Locality description

Sampling area was situated in a High Arctic site Petuniabukta fjord (Billefjorden, Svalbard, Fig. 1). Four different habitats were selected according to time since deglaciation and vegetation covers: (i) glacier forefield (25–79 years after deglaciation, for age determination see Luláková et al. (2023), (ii) partly vegetated (iii) fully vegetated dry and (iv) fully vegetated wet tundra. The last three sites have been deglaciated since the end of the last ice age—more than 10,000 years ago (Karczewski and Rygielski 1989). Sampling plots at the glacier forefield of Nordenskiöldbreen are covered with biological soil crusts (35–55%); the sparse vascular vegetation (cover of 7–15%) is dominated by *Saxifraga oppositifolia* and *Dryas octopetala*, followed by *Salix polaris*.

Partly vegetated tundra developed on gentle slope (1°) is represented by lichen-dominated biological soil crusts (70%) and patches and strips of vascular vegetation of low shrubs (15%; *D. octopetala*, *S. polaris*, *S. oppositifolia*).

**Fig. 1** Map of Svalbard and example of sampling habitats



Old tundra (fully vegetated dry tundra) vegetation developed on an elevated sea terrace with dense vegetation of low shrubs (53%; *S. oppositifolia*, *S. polaris*, *Cassiope tetragona*) and *Carex* species (27%), followed by a herb *P. viviparum* (11%).

Fully vegetated wet tundra developed on fine sediment of an alluvial fan (slope, 1°). Dense vegetation cover was co-dominated by hummock-forming low shrubs (55%; *S. oppositifolia*, *S. polaris*, *D. octopetala*) and mosses (20%) with significant cover of *Polygonum viviparum* (20%). The soil varied in elemental composition indicating a different parent rock (Suppl. Table 1).

#### Willow age determination

The age of willows in each habitat (Table 1) was determined by a standard dendrological procedure from high-quality thin cross-sections (Schneider and Gärtner 2013; Schweingruber et al. 2007, 2013). Those were  $\times 100$  magnified and photographed for further visual analysis and age determination. We used rather a conservative approach to age estimation—only counting the complete and wedging rings. Such count gives us the minimal plant age rather than ultimate dating of establishment. For a detailed description of all methods used for age determination, please see the supplementary.

**Table 1** Basic statistics of *Salix polaris* ring count (minimal age estimation) at studied habitats (5 replicates per habitat)

| Habitat                    | Average amount of ring $\pm$ SD | Maximal amount of rings |
|----------------------------|---------------------------------|-------------------------|
| Glacier forefield          | 17.2 $\pm$ 4.1                  | 23                      |
| Partly vegetated tundra    | 42 $\pm$ 8.8                    | 51                      |
| Fully vegetated dry tundra | 38.6 $\pm$ 5.7                  | 44                      |
| Fully vegetated wet tundra | 38.8 $\pm$ 6.3                  | 48                      |

All habitats but the glacier forefield show similar values of minimal age (Table 1), indicating that shrubs sampled in the glacier forefield are the delegates of the first generation with respect to the deglaciation period (25–79 years ago) as well as the usual life span of *S. polaris* in the fjord which is approximately 40 to 50 years (Buchwal et al. 2013).

#### Soil sampling and preparation

Five independent samples of rhizosphere (RH) soil of the arctic willow and surrounding bulk (BS) soils were collected from each habitat in July 2015. Each independent sample consisted of four subsamples taken from random points. The uppermost (litter layer or soil crust) was removed (if present and soil crust was in partly vegetated tundra habitat) before

sampling of BS soil, and then a 5 cm layer of mineral soil was taken. For sampling RH soil, willow plants were carefully removed from the soil, bulk soil was separated by hand and then adjacent RH soil was gently shaken from roots into the plastic bag. Samples were then homogenized and sieved (2 mm mesh size) and a part for chemical analyses was quickly air-dried in a thin layer, and a part for microbial analyses was kept in a cold room (at 4 °C) until further analyses. Part of the soil was immediately frozen for DNA analyses.

#### Quantification of exudation and LMMA

Five willow plants of comparable size with intact roots and surrounding soil were collected on each habitat and transported in plastic bags to the laboratory and acclimated for 3–5 days in plant growth chamber (Conviron E8 with CMP6050 control, Canada; under relevant environmental conditions: day/night (12 h/12 h) photosynthetic active radiation flux of 800/100  $\mu\text{mol m}^{-2} \text{s}^{-1}$ , temperature 10/6 °C, and 80% relative air humidity).

After the acclimation, plants were carefully removed from the soil and roots were gently washed with deionised water and regenerated in 1 mM  $\text{CaCl}_2$  for 0.5 h. After this step the regeneration solution was thoroughly rinsed away, and roots were immersed in known volume of deionised water (conductivity  $<0.055 \mu\text{S cm}^{-1}$ ) for 2 h over the light period. During the regeneration, protoplast of mechanically damaged cells is washed out into the solution and cell membranes are stabilized by  $\text{Ca}^{2+}$  (Neumann and Römhild 2007). Therefore, the exudation rates in following step are not likely affected by the manipulation, nor by changes in membrane permeability. Additionally, the use of water as a trap solution was reported to give similar results as nutrient solutions or KCl (Shepherd and Davies 1994). Collected water with exudates was immediately frozen and kept at  $-22^\circ\text{C}$  until analysis. Amount of exuded C and N were recalculated per plant dry mass ( $\text{nmol g}^{-1} \text{plant dm h}^{-1}$ ). Each LMMA were recalculated as a C exudation ( $\text{nmol C g}^{-1} \text{plant dm h}^{-1}$ ).

#### Soils, roots and exudates chemical analyses

Total content of Si, Al, Ca, K, Fe, Mg and P (in %) in soil was determined by X-ray fluorescence (XRF)

(Delta Premium XPD 6000, OLYMPUS INNOV-X, USA) under standard lab conditions (Tejnecký et al. 2015). Exchangeable Ca, Mg, K, Na, Al, Fe and Mn ( $\text{Ca}_{\text{ex}}$ ,  $\text{Mg}_{\text{ex}}$ ,  $\text{K}_{\text{ex}}$ ,  $\text{Na}_{\text{ex}}$ ,  $\text{Al}_{\text{ex}}$ ,  $\text{Fe}_{\text{ex}}$  and  $\text{Mn}_{\text{ex}}$  in  $\text{mg kg}^{-1}$ ) in soil were extracted in 0.1 M  $\text{BaCl}_2$  (2.5:30, w/v) according to UNECE (2006), effective cation exchange capacity (CEC in  $\text{meq kg}^{-1}$ ) was calculated as a sum of exchangeable cations ( $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ ,  $\text{K}^+$ ,  $\text{Na}^+$ ,  $\text{Al}^{3+}$ ,  $\text{Fe}^{3+}$  and  $\text{Mn}^{2+}$  in  $\text{meq kg}^{-1}$ ) and potentiometrically determined  $\text{H}^+$ .

Soil carbonate content ( $\text{C}_{\text{inorg}}$ ) was determined using the volumetric calcimeter method (Loeppert and Suarez 1996).

Total organic carbon ( $\text{C}_{\text{org}}$ ) and total nitrogen ( $\text{N}_t$ ) contents in soils, roots and exudates were measured using an elemental analyser (Vario MICRO cube, Elementar Analysensysteme GmbH, Germany).

Certain soil samples contained a significant amount of carbonates; therefore, prior to conducting  $\text{C}_{\text{org}}$  and  $\text{N}_t$  analyses, carbonates were removed from all soil samples through acid fumigation using HCl vapours (Harris et al. 2001). Results for  $\text{C}_{\text{inorg}}$ ,  $\text{C}_{\text{org}}$ , and  $\text{N}_t$  were reported in %. The total P content in roots was determined by means of ICP-OES (iCAP 7000, Thermo Scientific, USA) after  $\text{HNO}_3$  digestion in Teflon vessels (Vondráčková et al. 2015).

Water extractable elements and anions ( $\text{Ca}$ ,  $\text{K}$ ,  $\text{Fe}$ ,  $\text{Al}$ ,  $\text{Cl}^-$ ,  $\text{SO}_4^{2-}$  in  $\text{mg kg}^{-1}$ ) in soil were extracted by cold water (1:10, w/v) and the pH of the solution (active soil pH) was measured potentiometrically (pH meter inoLab pH Level 1 WTW, Germany). The concentration of extracted elements was determined by means of ICP-OES (iCAP 7000, Thermo Scientific, USA). Soil dissolved organic carbon (DOC) was extracted with cold water (sample: water ratio of 1:5) by shaking at 20 °C for 1 h and subsequently analysed after transport for total organic carbon on a LiquiTOC II (Elementar, Germany). Water extractable phosphorus ( $\text{P-PO}_4^{3-}$ ) was determined after sampling as soluble reactive phosphorus according to Murphy and Riley (1962) using a field portable spectrophotometer (Hach Lange DR 2800).

The ion chromatograph ICS 1600 (Dionex, USA) equipped with IonPac AS11-HC (Dionex, USA) guard and analytical columns was used to analyse  $\text{PO}_4^{3-}$  (as a  $\text{P-PO}_4^{3-}$ ) and LMMA of exudates. The eluent composition was 1.00–43.22 mM KOH with a gradient of 1–80 min; the flow rate was set to 1  $\text{mL min}^{-1}$ . An ASRS 300—4-mm suppressor

(Dionex, USA) and Carbonate Removal Device 200 (Dionex, USA) were used to suppress eluent conductivity. Chromatograms were processed and evaluated using the software Chromeleon 6.80 (Dionex, USA). Standards were prepared from 1 g L<sup>-1</sup> each LMMA and PO<sub>4</sub><sup>3-</sup> concentrates (Analytika, CZ and Inorganic Ventures, USA) and deionised water (conductivity < 0.055 µS cm<sup>-1</sup>; Millipore, USA) in the concentration 0.1, 1, 5, 10, 40 mg L<sup>-1</sup>. For each analyte, a separate calibration quadratic curve in Chromeleon 6.80 (Dionex, USA) software was calculated from the area (µS \* min) and analyte concentration (mg L<sup>-1</sup>). Detection limits were calculated from a 3:1 signal-to-noise ratio (Shabir 2003). For determined organic acids, the limits of detection were: 0.012 µmol C L<sup>-1</sup> for quinate, 0.011 µmol C L<sup>-1</sup> for lactate, 0.014 µmol C L<sup>-1</sup> for acetate, 0.019 µmol C L<sup>-1</sup> for propionate, 0.008 µmol C L<sup>-1</sup> for formate, 0.018 µmol C L<sup>-1</sup> for pyruvate, 0.014 µmol C L<sup>-1</sup> for malate, 0.011 µmol C L<sup>-1</sup> for oxalate and 0.022 µmol C L<sup>-1</sup> for citrate (Mercl et al. 2017) (Table S3).

Dissolved organic C (DOC) and total nitrogen (TN) in exudates were analysed by Shimadzu TOC-LCPH with TNM-L module, catalytic combustion at 720 °C, chemiluminescence detection for TN and NIDR detection for DOC.

#### Microbial analyses

Carbon, nitrogen and phosphorus in microbial biomass (C<sub>mic</sub>, N<sub>mic</sub> and P<sub>mic</sub> in mg kg<sup>-1</sup>) were estimated using the chloroform-fumigation extraction method (Brookes et al. 1985, 1982; Vance et al. 1987). To calculate C<sub>mic</sub> and N<sub>mic</sub>, extracts were measured for C (DOC) and N (DN) on the TOC/TN analyzer LiquiTOC II (Elementar, Germany). Concentration of reactive P in extracts to calculate P<sub>mic</sub> were analysed spectrophotometrically according to Brookes et al. (1982). The nutrient concentrations in microbial biomass were calculated as the difference between concentrations in fumigated and non-fumigated soil extracts. Results were corrected for incomplete extraction of all microbial C (K<sub>ec</sub>=0.45), all microbial N (K<sub>en</sub>=0.54) and microbial P (K<sub>ep</sub>=0.4).

The amount of 0.25 g of soil was used for DNA extraction using PowerSoil DNA isolation kit (MOBIO

Laboratories, USA). The DNA was quantified fluorometrically using Qubit (ThermoFisher Scientific, UK) following the standard protocol and kit for dsDNA quantification. Quantification of bacterial and fungal SSU rRNA genes was performed using the FastStart SybrGREEN Roche® Supermix and Step One system (Life Technologies, USA). All microbial methods (microbial biomass, DNA extraction and bacteria and fungi quantification) are described in more detail in Luláková et al. (2023).

#### Statistic and calculations

Basic statistical analysis, such as the correlation between soil properties and two-way ANOVA with habitat and rhizosphere effect as factors with four levels (ANOVA) and their interaction, were performed (Statgraphics XVI.I Centurion). The F-tests identified the significant factors and multiple range tests were used for significant differences between the means at the 95.0% confidence level ( $p < 0.05$ ) or in some cases confidence level was higher than 95% but not higher than 90.0%. Homogeneous Groups (H.G.) were used for bulk soils to show significant differences between habitats. The method used to discriminate among the means is Fisher's least significant difference (LSD) test. Correlations between soil organic C and microbial biomass and fungi across all sites were visualized in R version 4.0.2 (R Core Team 2020) with R package *ggplot2*. Tukey multiple comparisons were used to calculate a significant effect of the rhizosphere effect within habitats. If any determined value was lower than the detection limit of the used analytical methods (e.g., IC or ICP-OES), then it was replaced (for statistical tests) by one-half of the detection limit. Rhizosphere effect was expressed as a relative difference between rhizosphere and bulk soil ( $REs = RH/BS$ ). Rhizosphere effect is referred as negative when  $REs < 1$  and as positive when  $REs > 1$ . Variability of REs was calculated using error propagation for division. Correlations and habitat associations between the total content of chosen elements determined by XRF and water extractable compounds were visualized using principal component analyses (PCA) in CANOCO for Windows 5.0 (ter Braak and Šmilauer 2012).

## Results

### Chemistry and biology of the bulk soils

The multivariate principal component analysis (PCA) was used to visualize correlations and habitat associations of total element contents measured by XRF analysis and content of water extractable nutrients (Fig. 2). The bulk soils of individual sites differ in the total element contents while three out of four sites exerted similarity in content of water extractable nutrients (Fig. 2a, b). The bulk soil of the fully vegetated dry tundra site represented environment with the lowest content of  $K_t$  and  $Fe_t$  ( $0.44 \pm 0.03\%$  and  $1.35 \pm 0.03\%$  respectively) among all sites, while the glacier forefield had the highest content of  $K_t$  and  $Fe_t$  ( $1.93 \pm 0.31\%$  and  $2.46 \pm 0.25\%$  respectively) (Table S1). The highest content of  $Ca_t$  ( $6.78 \pm 1.09\%$ ) was determined in the bulk soils of the partly vegetated tundra. Magnesium ( $Mg_t$ ) was detected only in the partly vegetated ( $2.31 \pm 0.78\%$ ) and fully vegetated dry tundra ( $0.91 \pm 0.04\%$ ).  $P_t$  was detected only in the soils of glacier forefield habitat ( $0.025 \pm 0.01\%$ ) (Table S1).

Concentrations of water extractable elements in the bulk soil of the fully vegetated dry tundra were markedly higher ( $p < 0.05$ ) than in the bulk soils of the other sites. Content of  $Ca_{H_2O}$  increased in the sequence: glacier forefield habitat < partly vegetated < fully wet < fully vegetated dry tundra ( $p < 0.001$ ) (Table 2). DOC was more than four times higher in the soil of the fully vegetated dry tundra than on the other sites ( $p < 0.001$ ). Bioavailable  $P_{H_2O}$  was generally very low and it was in the soil of the partly vegetated tundra three times higher than that in all the soils ( $p < 0.001$ ). Similarly to  $P_{H_2O}$ ,  $N_{min}$  content was low, ranging from  $0.79 \text{ mg kg}^{-1}$  (fully vegetated wet tundra) to  $2.82 \text{ mg kg}^{-1}$  (partly vegetated tundra). Content of  $C_{inorg}$ , which was highest in the partly vegetated tundra, ranged from 0.39 to 6.80% (Fig. 3, Table S1).

The bulk soils were alkaline (active pH 7.24–9.19) with high base saturation (average  $98.0 \pm 1.79\%$ ). Cation exchange capacity (CEC), was the lowest in the bulk soils of the glacial forefield and increased in the same sequence as  $Ca_{H_2O}$ : forefield < partly vegetated < fully vegetated wet < fully vegetated dry tundra.  $Ca_{ex}$  was found to be a main exchangeable cation

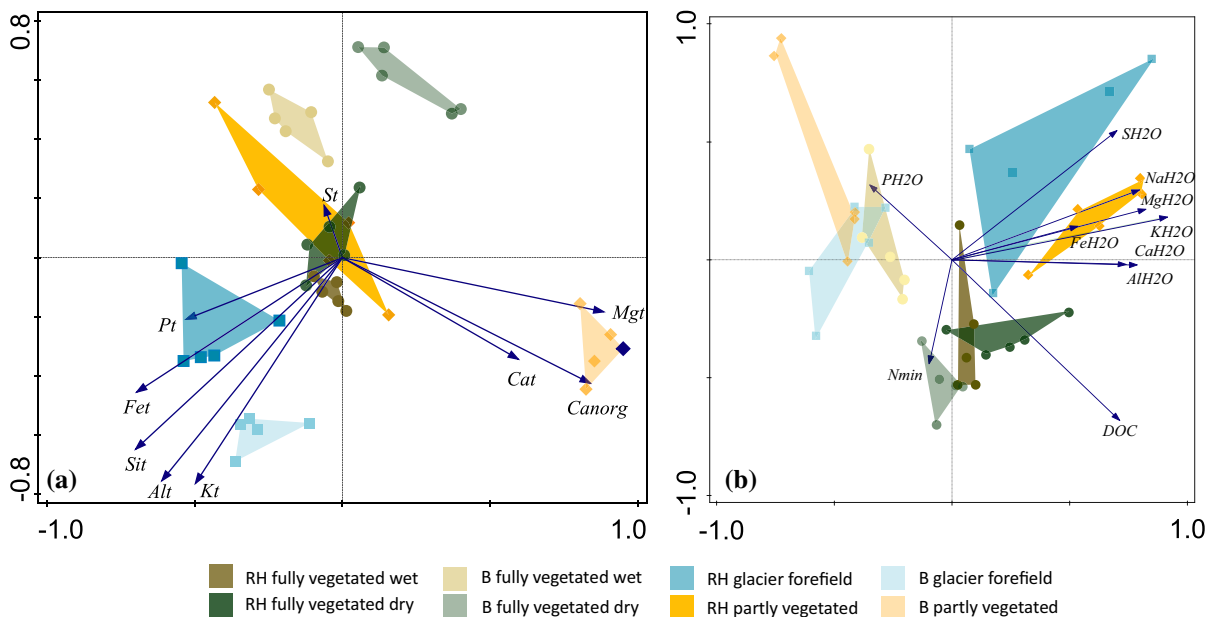


**Fig. 2** Principal component analysis (PCA) of **a** total element content measured by XRF and **b** water extractable elements and anions in the bulk (B) and rhizosphere (RH) soil, respectively. Correlations of the variables with the principal compo-

nents (PC1 and PC2) are indicated by length and direction of the arrows. The affiliation of the soil sample to the habitat and to the type of soil (bulk × rhizosphere soil sample) is shown by the colour of polygon

**Table 2** pH and content of water extractable K, Ca, Mg, Fe, Al,  $\text{SO}_4^{2-}$  (in  $\text{mg kg}^{-1}$ ) in the bulk soil and rhizosphere effect (RE=RH soil /BS) (mean and standard deviation, n = 5)

| Content in the bulk soil   | pH   | K (mg kg <sup>-1</sup> ) |     |      | Ca (mg kg <sup>-1</sup> ) |     |      | Mg (mg kg <sup>-1</sup> ) |     |      | Fe (mg kg <sup>-1</sup> ) |     |      | Al (mg kg <sup>-1</sup> ) |     |      | SO <sub>4</sub> <sup>2-</sup> (mg kg <sup>-1</sup> ) |     |      |      |     |
|----------------------------|------|--------------------------|-----|------|---------------------------|-----|------|---------------------------|-----|------|---------------------------|-----|------|---------------------------|-----|------|------------------------------------------------------|-----|------|------|-----|
|                            |      | Mean                     | SD  | H.G  | Mean                      | SD  | H.G  | Mean                      | SD  | H.G  | Mean                      | SD  | H.G  | Mean                      | SD  | H.G  | Mean                                                 | SD  | H.G  |      |     |
| Glacier forefield          | 9.05 | 0.15                     | b   | 9.41 | 2.99                      | b   | 78.6 | 11.4                      | a   | 7.89 | 2.11                      | a   | 3.31 | 1.12                      | a   | 1.92 | 0.43                                                 | a   | 38.9 | 30.8 | a   |
| Partly vegetated tundra    | 8.27 | 0.26                     | a   | 5.52 | 0.22                      | a   | 128  | 3.66                      | b   | 22.0 | 3.54                      | c   | 3.35 | 0.52                      | a   | 1.62 | 0.38                                                 | a   | 73.7 | 18.5 | b   |
| Fully vegetated dry tundra | 8.14 | 0.08                     | a   | 13.8 | 1.75                      | c   | 193  | 15.3                      | d   | 26.9 | 1.44                      | d   | 4.37 | 0.65                      | ab  | 4.78 | 2.35                                                 | b   | 15.9 | 4.92 | a   |
| Fully vegetated wet tundra | 8.17 | 0.17                     | a   | 15.5 | 1.79                      | c   | 150  | 8.31                      | c   | 12.2 | 1.73                      | b   | 4.95 | 0.80                      | b   | 3.69 | 0.82                                                 | b   | 22.1 | 8.61 | a   |
| Rhizosphere effect         | pH   | K                        |     |      | Ca                        |     |      | Mg                        |     |      | Fe                        |     |      | Al                        |     |      | SO <sub>4</sub> <sup>2-</sup>                        |     |      |      |     |
|                            | Mean | SD                       | H.G | Mean | SD                        | H.G | Mean | SD                        | H.G | Mean | SD                        | H.G | Mean | SD                        | H.G | Mean | SD                                                   | H.G | Mean | SD   | H.G |
| Glacier forefield          | 0.86 | 0.05                     | a   | 25.1 | 26.1                      | b   | 3.78 | 2.82                      | a   | 6.60 | 5.68                      | b   | 2.58 | 1.22                      | b   | 4.66 | 3.66                                                 | bc  | 33.0 | 46.8 | b   |
| Partly vegetated tundra    | 0.90 | 0.00                     | a   | 15.6 | 12.8                      | ab  | 70.0 | 72.0                      | b   | 4.44 | 1.19                      | ab  | 1.64 | 0.49                      | ab  | 5.16 | 2.33                                                 | c   | 7.24 | 3.01 | ab  |
| Fully vegetated dry tundra | 1.00 | 0.07                     | b   | 4.38 | 3.02                      | a   | 2.42 | 0.98                      | a   | 1.40 | 0.26                      | a   | 1.24 | 0.44                      | a   | 1.76 | 0.87                                                 | ab  | 1.56 | 0.17 | a   |
| Fully vegetated wet tundra | 1.00 | 0.00                     | b   | 2.18 | 0.64                      | a   | 3.40 | 1.00                      | a   | 1.26 | 0.24                      | a   | 1.22 | 0.45                      | a   | 0.80 | 0.37                                                 | a   | 2.58 | 1.11 | ab  |

The small letter indicates the difference between habitats

H.G. homogeneous groups; SD standard deviation

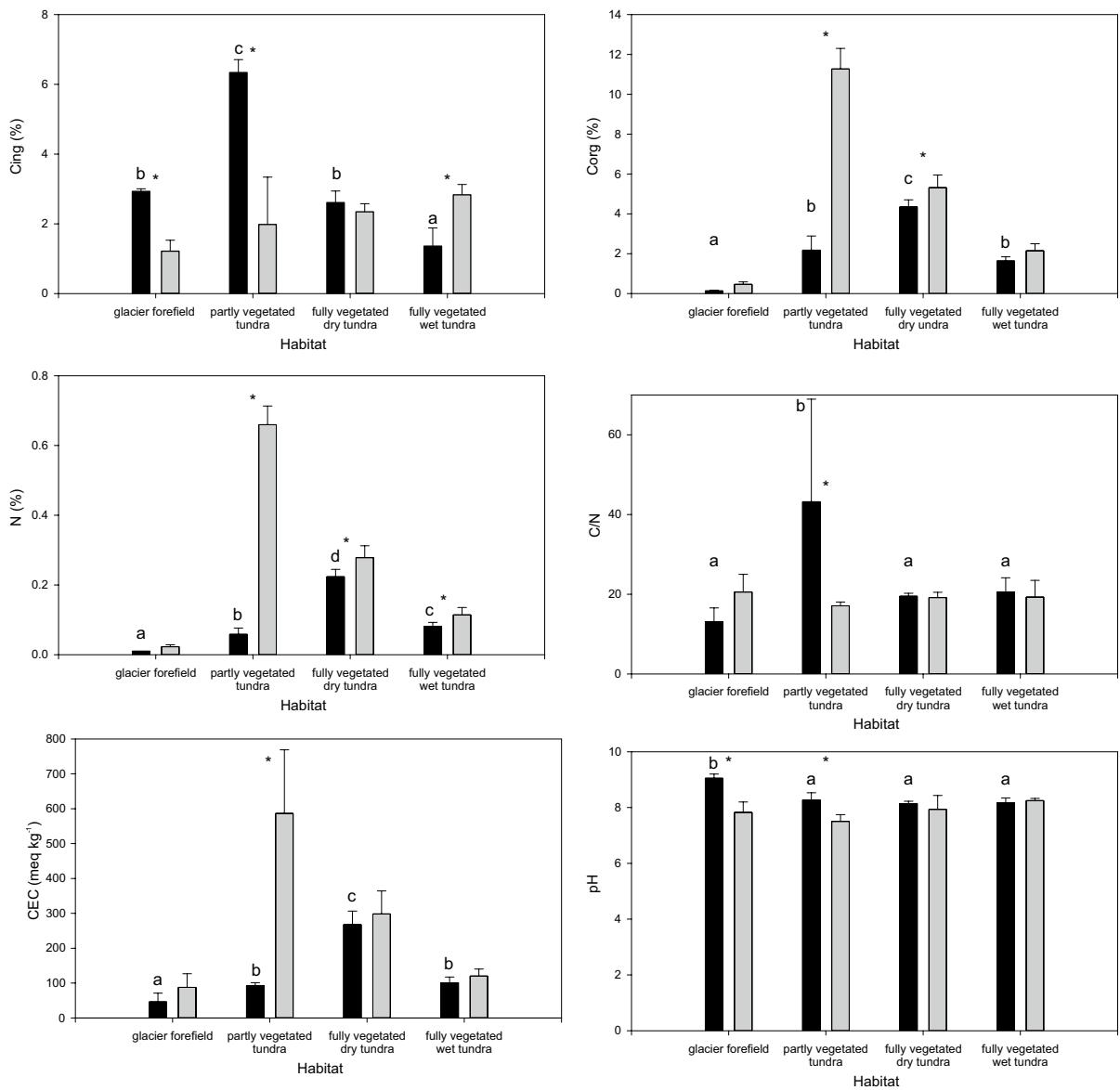


**Fig. 3** Total inorganic and organic C contents, CEC, N contents, C/N and  $\text{pH}_{\text{H}_2\text{O}}$  in the bulk (black column) and rhizosphere (grey column) soil (mean and standard error,  $n=5$ , small letter indicates difference between habitats and \* between bulk and rhizosphere soil)

in all soils (Fig. 3, Table S2).  $\text{Ca}_{\text{ex}}$  and  $\text{Mg}_{\text{ex}}$  were the highest in the bulk soils of the fully vegetated dry tundra ( $4814 \pm 741$ ,  $285 \pm 21.7 \text{ mg kg}^{-1}$  respectively) and the lowest in the bulk soils of the glacier forefield habitat ( $741 \pm 459$ ,  $40.4 \pm 10.1 \text{ mg kg}^{-1}$  respectively, Table S2). Neither the concentration of exchangeable elements nor the concentration of water extractable elements mirrored the total element contents of the

bulk soil. The strong correlations between exchange elements ( $\text{Ca}_{\text{ex}}$ ,  $\text{Mg}_{\text{ex}}$ ), CEC and water extractable elements ( $\text{Ca}_{\text{H}_2\text{O}}$ ,  $\text{Mg}_{\text{H}_2\text{O}}$ ) were observed (Table S4).

Organic carbon content ( $\text{C}_{\text{org}}$ ) ranged from 0.13 to 4.34% and  $\text{C}_{\text{org}}$  correlated with CEC (Fig. 3; Table S4). CNP in microbial biomass, microbial DNA and bacterial and fungal genes copies followed the trend of DOC being higher in the bulk soil of the

bulk soil. The strong correlations between exchange elements ( $\text{Ca}_{\text{ex}}$ ,  $\text{Mg}_{\text{ex}}$ ), CEC and water extractable elements ( $\text{Ca}_{\text{H}_2\text{O}}$ ,  $\text{Mg}_{\text{H}_2\text{O}}$ ) were observed (Table S4).

fully vegetated dry tundra sites comparing the other sites (Fig. 4). Fungi to bacteria ratio was the highest in the bulk soil of the partly vegetated tundra dominated by lichens because of high abundance of fungi and low abundance of bacteria (Fig. 4). Microbial C and fungal genes copies were positively correlated to  $C_{org}$  (Fig. 5), but their increase with  $C_{org}$  was small. These correlations were driven mainly by site differences and the inter-site trends were not significant. No dependence on  $C_{org}$  was found for bacterial gene copies (Fig. S2).

### Chemistry and biology of rhizosphere

From the geochemistry perspective, the rhizosphere soil differed from the bulk soil by amounts of total, extractable and water-soluble elements and pH (Tables S1 and S2, 2, Fig. 3). The effect of the rhizosphere on the total element contents was not significant. However, the multivariate PCA analysis revealed interaction between sites and rhizosphere with convergence in the total element contents except for the glacial forefield (Fig. 2a). Simultaneously, a significant increase in the levels of water extractable nutrients was observed in the rhizosphere across all sites (Fig. 2b). Microbial C and fungal genes copies linearly increased with increasing  $C_{org}$  in the rhizosphere soil similarly to the bulk soil and, in contrast to the bulk soil, in the rhizosphere soil the increase of microbial C and fungal gene copies were faster (Fig. 5). Fungi-to-bacteria ratio was higher in the rhizosphere than in bulk soil at all sites except for the partly vegetated tundra.

### Rhizosphere effect

The rhizosphere effects (REs) on the total cation content, measured by the ratio of rhizosphere to bulk soil, exhibited modest variations, ranging from 0.7 to 1.8. In the fully vegetated dry and fully vegetated wet tundra, the REs consistently showed a positive trend ( $REs > 1$ ), indicating a larger impact of rhizosphere on total cation contents compared to the less developed glacial forefield and partly vegetated tundra ( $REs \leq 1$ , Table S1).

REs on exchangeable and water extractable cations, as well as cation exchange capacity (CEC), exhibited a positive trend and surpassed those observed for total cations (REs from 0.16 to 6.78).

Notably, this positive impact was more pronounced on the less developed sites compared to the fully vegetated dry and fully vegetated wet tundra (Tables S2 and 2).

In both the partly vegetated tundra and glacial forefield, unlike the fully vegetated dry and fully vegetated wet tundra, the rhizosphere exerted a negative influence on  $N_{min}$ , with REs 0.3 and 0.9, respectively. Intriguingly, in the rhizosphere of the fully vegetated wet tundra,  $N_{min}$  experienced a remarkable nearly tenfold increase ( $REs = 5.30$ ), but still, its content remained comparable with other sites (Table S2).

Available  $P_{H2O}$  displayed only small REs at all the sites except for the partly vegetated tundra, where  $P_{H2O}$  was less available in the rhizosphere than in the bulk soil ( $REs = 0.28$ ) (Table 3).

Rhizosphere consistently exhibited positive effect on  $C_{org}$ ,  $N_t$ , microbial CNP, and DNA (Table 3). The most substantial impact on  $C_{org}$ ,  $N_t$ , microbial CNP and DNA was observed in the partly vegetated tundra and glacial forefield ( $REs = \text{up to } 72$  for Cmic). The rhizosphere effects on fungal and, particularly, bacterial gene copies were most pronounced in the soil of the glacial forefield ( $REs = 79.6$  and  $170$ , respectively, Table 3).

### Exudation of willow roots

Carbon in the roots varied within a narrow range from 42.0 to 56.2%, while N and P differences were wider ( $0.92\text{--}2.05\%$  N and  $106\text{--}702$  mg P kg<sup>-1</sup>) bringing significant differences in C/P and C/N ratios between the roots from different habitats (Table 4). The C/P ratio decreased in order the fully vegetated dry > partly vegetated > fully vegetated wet > glacial forefield and C/N ratio in similar order the fully vegetated dry > partly vegetated > fully vegetated wet = glacial forefield.

DOC exudation was similar between habitats but roots of willows from the fully and partly vegetated tundra released significantly higher amount of DN ( $p = 0.019$ ) and lower amount of P (Table 5). Consequently, molar C/N and N/P ratios in exudates showed significant differences between willows from different habitats ( $p < 0.001$ ). C/N was roughly twice as high in exudates from willows taken at the glacier forefield as compared to other habitats and N/P was roughly one order in magnitude higher in exudates of willows from partly and fully vegetated dry tundra

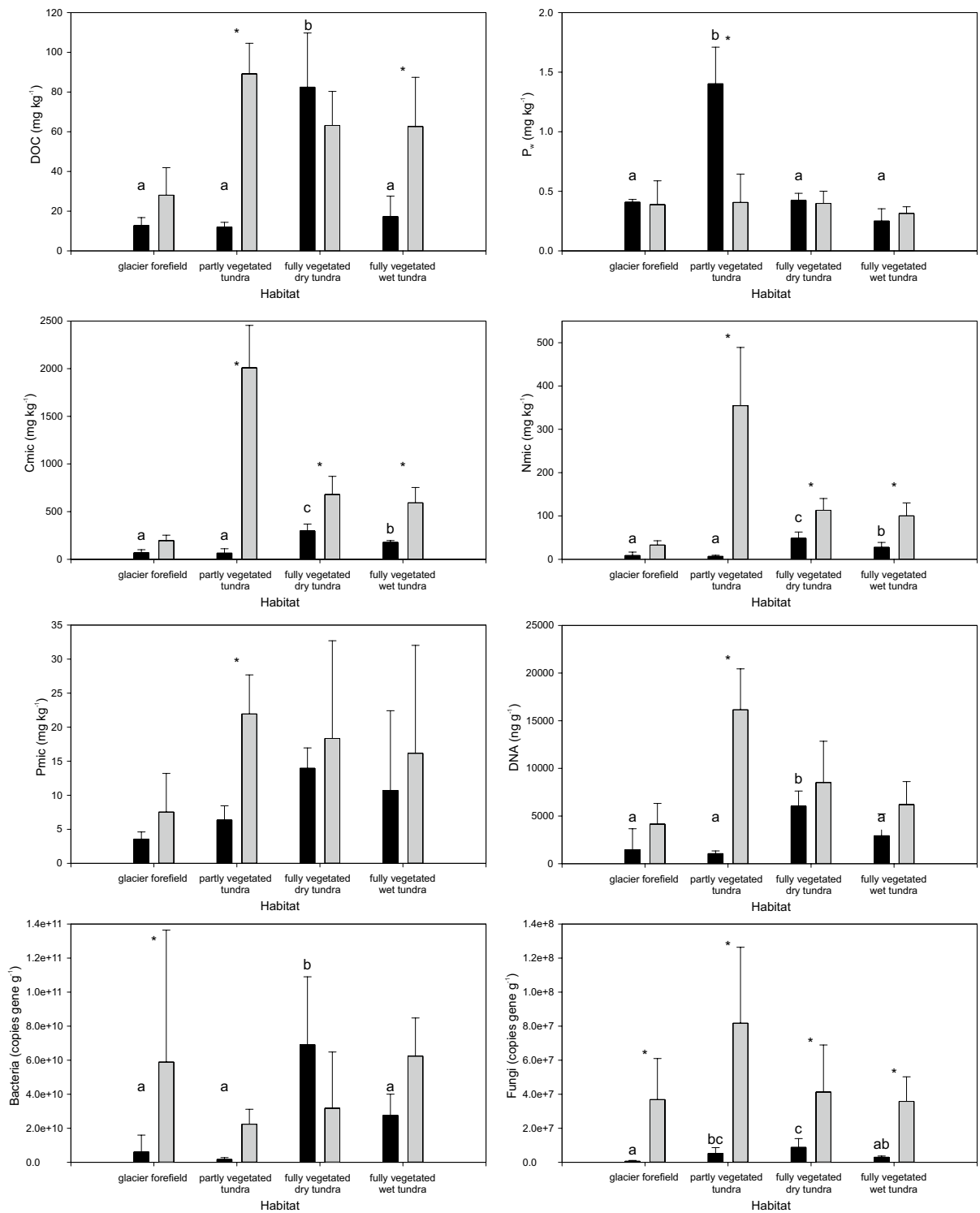


**Fig. 4** Dissolved organic carbon and phosphorus, microbial CNP, DNA, bacterial and fungal gene copies in the bulk (black column) and rhizosphere (grey column) soil (mean and 95%

confidence intervals, n=5). The small letters indicate the difference between habitats and \* between bulk and rhizosphere soil)

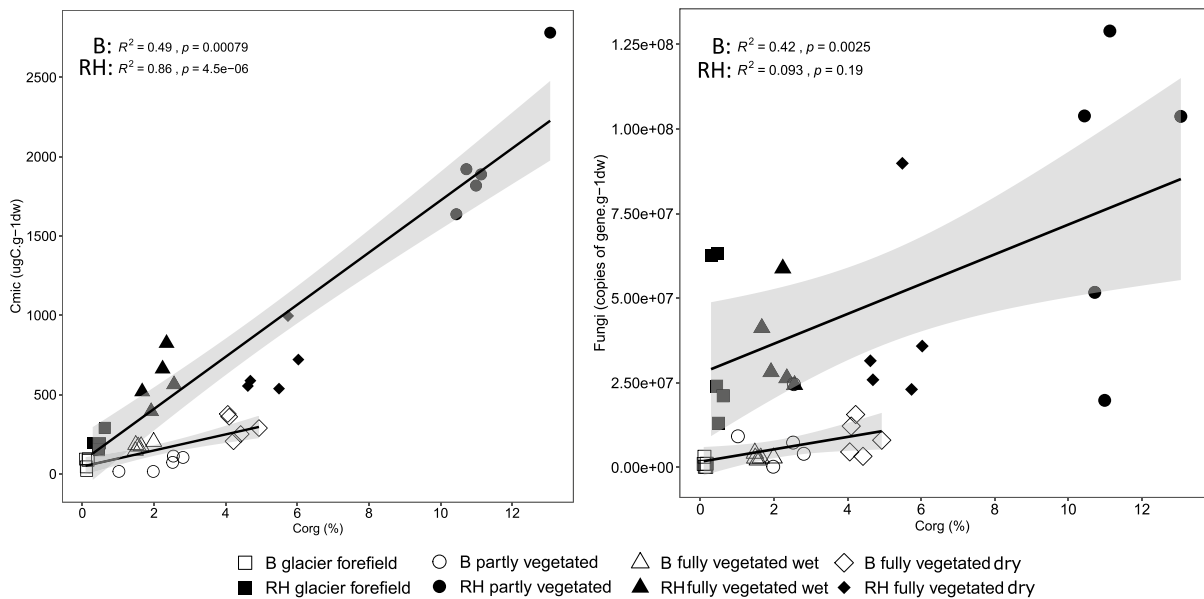


**Fig. 5** Correlations between soil organic C and microbial biomass and fungi, respectively, counted across all sites. Open symbols indicate the bulk soil (B), and closed symbols indicate rhizosphere soils (RH)

**Table 3** Rhizosphere effect (RE = RH soil / BS) of Corg, Ntot, Cmic, Nmic, Pmic,  $P_{H_2O}$ , DOC, DNA, bacteria and fungi (mean and standard deviation,  $n = 5$ )

| Rhizosphere effect         | Corg       |      |     | Ntot  |      |     | Cmic  |       |     | Nmic     |        |     | Pmic  |      |     |
|----------------------------|------------|------|-----|-------|------|-----|-------|-------|-----|----------|--------|-----|-------|------|-----|
|                            | Mean       | SD   | H.G | Mean  | SD   | H.G | Mean  | SD    | H.G | Mean     | SD     | H.G | Mean  | SD   | H.G |
| Glacier forefield          | 3.56       | 0.62 | a   | 2.30  | 0.53 | a   | 3.78  | 2.82  | a   | 14.0     | 19.6   | a   | 3.55  | 2.33 | ab  |
| Partly vegetated tundra    | 6.14       | 3.77 | b   | 12.4  | 4.72 | b   | 70.0  | 72.0  | b   | 56.5     | 16.2   | b   | 3.88  | 1.90 | b   |
| Fully vegetated dry tundra | 1.22       | 0.23 | a   | 1.26  | 0.22 | a   | 2.42  | 0.98  | a   | 2.50     | 0.94   | a   | 1.44  | 1.18 | a   |
| Fully vegetated wet tundra | 1.32       | 0.26 | a   | 1.44  | 0.39 | a   | 3.40  | 1.00  | a   | 4.30     | 2.12   | a   | 2.50  | 1.66 | ab  |
| Rhizosphere effect         | $P_{H_2O}$ |      |     | DOC   |      |     | DNA   |       |     | Bacteria |        |     | Fungi |      |     |
|                            | Mean       | SD   | H.G | Mean  | SD   | H.G | Mean  | SD    | H.G | Mean     | SD     | H.G | Mean  | SD   | H.G |
| Glacier forefield          | 0.96       | 0.48 | b   | 11.26 | 10.6 | b   | 11.26 | 10.59 | b   | 169.94   | 266.68 |     | 79.6  | 46.6 | b   |
| Partly vegetated tundra    | 0.28       | 0.16 | a   | 15.50 | 2.14 | b   | 15.50 | 2.14  | b   | 20.52    | 18.15  |     | 34.6  | 39.1 | a   |
| Fully vegetated dry tundra | 0.94       | 0.25 | b   | 1.42  | 0.60 | a   | 1.42  | 0.60  | a   | 0.70     | 0.79   |     | 5.48  | 2.68 | a   |
| Fully vegetated wet tundra | 1.44       | 0.58 | b   | 2.12  | 0.53 | a   | 2.12  | 0.53  | a   | 2.46     | 0.62   | n.s | 12.4  | 5.0  | a   |

The small letter indicates the difference between habitats

H.G. homogeneous groups; SD standard deviation; n.s. not significant difference

than that from the glacial forefield (Table 5). P exudation was negatively correlated with N/P ratio of roots ( $r = -0.560$ ,  $p < 0.0157$ ,  $n = 19$  and Fig. S3), and positively to C/N in exudates ( $r = 0.621$ ,  $p < 0.0285$ ,  $n = 19$  and Fig. S3). On the contrary, N exudation was positively correlated to N/P ratio in roots ( $r = 0.400$ ,

$p < 0.086$ ,  $n = 19$  and Fig. S3) and negatively with the C/N of exudates ( $r = -0.681$ ,  $p < 0.0001$ ,  $n = 20$  and Fig. S3).

Sum of C released from roots in form of LMMOA expressed per plant dry mass and per hour was affected by habitat only slightly ( $p = 0.072$ ) and

**Table 4** Total C, N and P contents, C/N, C/P and N/P molar ratios in willow roots (*Salix polaris*) from microcosm experiment (mean and standard deviation, n=5)

| Habitat                    | C (%) |      |     | N (%) |      |     | P (mg kg <sup>-1</sup> ) |      |     | C/N  |      |     | C/P  |      |     | N/P  |      |     |
|----------------------------|-------|------|-----|-------|------|-----|--------------------------|------|-----|------|------|-----|------|------|-----|------|------|-----|
|                            | Mean  | SD   | H.G | Mean  | SD   | H.G | Mean                     | SD   | H.G | Mean | SD   | H.G | Mean | SD   | H.G | Mean | SD   | H.G |
| Glacier forefield          | 44.5  | 2.05 | a   | 1.47  | 0.35 | b   | 355                      | 127  | bc  | 37.3 | 10.5 | a   | 3683 | 1633 | a   | 104  | 47.0 |     |
| Partly vegetated tundra    | 47.3  | 0.69 | ab  | 1.28  | 0.22 | ab  | 202                      | 57.6 | ab  | 44.1 | 7.64 | ab  | 6544 | 2252 | bc  | 153  | 67.1 |     |
| Fully vegetated dry tundra | 48.7  | 5.18 | b   | 1.01  | 0.08 | a   | 148                      | 34.0 | a   | 57.0 | 10.1 | b   | 8260 | 2389 | c   | 143  | 15.4 |     |
| Fully vegetated wet tundra | 45.3  | 1.55 | ab  | 1.58  | 0.40 | b   | 375                      | 196  | c   | 35.5 | 10.2 | a   | 3819 | 1938 | ab  | 105  | 32.2 | n.s |

The small letter indicates the difference between habitats

H.G. homogeneous groups; SD standard deviation; n.s. not significant difference

decreased in a sequence: glacier forefield  $\geq$  fully vegetated dry  $\geq$  fully vegetated wet = partly vegetated tundra. Proportion of LMMOA-C from DOC was similar for all tundra habitats (in average 2%).

Determined speciation of LMMOA in *S. polaris* exudates were highly variable (Table S3). In general, lactate, acetate, formate, malate and citrate were more abundant LMMOA species than oxalate, pyruvate and quinate. Amounts of butyrate, valerate, tartrate, malate, isocitrate, cis and trans-aconitate were mostly below the detection limit. An example of a chromatogram of LMMOA released from the willow roots of the glacier forefield sample is presented in Fig. S1.

The exudation rate of LMMOA and their speciation were affected by the habitat from which the willow originated (Table 5, Fig. 6). Total flux of C in the form of LMMOA was in a range from 179 to 340 nmol C g<sup>-1</sup> plant dm h<sup>-1</sup> (mean 254 nmol g<sup>-1</sup> plant dm h<sup>-1</sup>) being higher in the roots from the glacier forefield (294 nmol C g<sup>-1</sup> plant dw h<sup>-1</sup>, sum of C as a LMMOA, mean) and fully vegetated dry tundra (340 nmol C g<sup>-1</sup> plant dw h<sup>-1</sup>, sum of C as a LMMOA, mean) was higher than those from other habitats.

Exudation of measurable LMMOA species expressed as nmol C of individual LMMOA (Fig. 6) varied depending on the willow origin. Willows from glacial forefield and fully vegetated dry tundra released more acetate, malate and citrate, but the differences were not significant. Citrate exudation rate was 14.0 nmol C g<sup>-1</sup> plant dm h<sup>-1</sup> for glacier forefield and 7.46 nmol C g<sup>-1</sup> plant dm h<sup>-1</sup> for fully vegetated dry tundra comparing the mean value of 7.55 nmol C g<sup>-1</sup> plant dm h<sup>-1</sup> for all habitats. The only significant difference in LMMOA species was the higher oxalate exudation rate at the glacial forefield ( $p=0.034$ ).

## Discussion

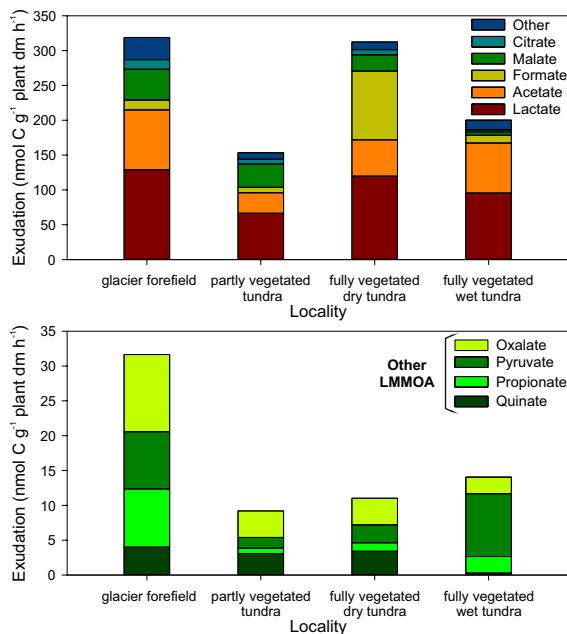
Differences in geochemistry detected in the bulk soil of partly and fully vegetated dry and wet tundra were not present in their willow rhizospheres and all the RH soils did not significantly differ (Fig. 2a). The geochemistry distinction compared to the RH soils of these three sites persisted only in the rhizosphere of the glacial forefield, coinciding with previously published results that substrate geochemistry is a predominant factor for the growth of microbial communities

**Table 5** Exudation rate and total carbon, nitrogen, LMMOA-C, and exudate C/N and N/P stoichiometry as measured in the microcosm experiment with willow plants taken from different sites (mean and standard deviation, n = 5)

| Habitat                                                                           | DOC ( $\mu\text{mol g}^{-1}$ plant dm h <sup>-1</sup> ) |      |     | DN ( $\mu\text{mol g}^{-1}$ plant dm h <sup>-1</sup> ) |      |     | C/N  |      |     | N/P             |      |     | Sum of LMMOA C ( $\text{nmol C g}^{-1}$ plant dm h <sup>-1</sup> ) |      |     |
|-----------------------------------------------------------------------------------|---------------------------------------------------------|------|-----|--------------------------------------------------------|------|-----|------|------|-----|-----------------|------|-----|--------------------------------------------------------------------|------|-----|
|                                                                                   | Mean                                                    | SD   | H.G | Mean                                                   | SD   | H.G | Mean | SD   | H.G | Mean            | SD   | H.G | Mean                                                               | SD   | H.G |
| Glacier forefield                                                                 | 16.3                                                    | 2.56 |     | 0.09                                                   | 0.62 | a   | 22.7 | 3.43 | c   | 57.4            | 31.9 |     | 294                                                                | 107  |     |
| Partly vegetated tundra                                                           | 12.1                                                    | 3.66 |     | 1.22                                                   | 0.49 | ab  | 9.33 | 4.41 | ab  | 657             | 833  |     | 179                                                                | 84.6 |     |
| Fully vegetated dry tundra                                                        | 16.5                                                    | 7.47 |     | 1.66                                                   | 0.66 | b   | 8.90 | 3.20 | a   | 501             | 322  |     | 340                                                                | 196  |     |
| Fully vegetated wet tundra                                                        | 14.1                                                    | 2.77 | n.s | 0.92                                                   | 0.28 | a   | 13.7 | 2.91 | b   | 149             | 36   | n.s | 213                                                                | 44.8 | n.s |
|                                                                                   |                                                         |      |     |                                                        |      |     |      |      |     |                 |      |     |                                                                    |      |     |
| P-PO <sub>4</sub> <sup>3-</sup> ( $\text{nmol g}^{-1}$ plant dm h <sup>-1</sup> ) |                                                         |      |     |                                                        |      |     |      |      |     |                 |      |     |                                                                    |      |     |
|                                                                                   | Mean                                                    |      |     | SD                                                     |      |     | H.G  |      |     | LMMOA C/DOC (%) |      |     | Mean                                                               |      |     |
|                                                                                   |                                                         |      |     |                                                        |      |     |      |      |     |                 |      |     | Mean                                                               |      |     |
| Glacier forefield                                                                 | 14.4                                                    |      |     | 10.5                                                   |      |     | b    |      |     | 1.79            |      |     | 0.46                                                               |      |     |
| Partly vegetated tundra                                                           | 3.76                                                    |      |     | 2.29                                                   |      |     | a    |      |     | 1.50            |      |     | 0.53                                                               |      |     |
| Fully vegetated dry tundra                                                        | 4.59                                                    |      |     | 2.77                                                   |      |     | a    |      |     | 2.44            |      |     | 1.80                                                               |      |     |
| Fully vegetated wet tundra                                                        | 6.31                                                    |      |     | 1.73                                                   |      |     | a    |      |     | 1.54            |      |     | 0.36                                                               |      | n.s |

The small letter indicates the difference between habitats

H.G. homogeneous groups; SD standard deviation; n.s. not significant difference

**Fig. 6** Mean exudation rates of main (a) and less (b) abundant LMMOA species in willow (*Salix polaris*) from different habitats (n = 5;  $\text{nmol C g}^{-1} \text{ plant dm h}^{-1}$ ) used in a microcosm experiment

in the early stages of soil development on bare surfaces before vascular plants begin to develop (Bryan 1977; Greinwald et al. 2023; Luláková et al. 2023). The mostly positive rhizosphere effect of willows on nutrient contents and microbial communities was greater at the glacier forefield and partly vegetated tundra with soils in the very early stage of development compared to the vegetated dry and wet tundra. At the latter two sites with developed vegetation cover, the rhizosphere effect on nutrient content was reduced by influence of higher content of available organic matter originating from other plant species roots, rhizodeposition and/or lichens in the bulk soil (e.g. *Carex* species and *Dryas octopetala*), more complex plant–microorganism interactions (Richardson et al. 2009; Schulz et al. 2013) and by more weathering resistant substrate (Sauer 2015). These observations align with findings from other studies that highlight a more pronounced plant effect on sites characterized by harsh conditions, as opposed to those with more developed and mature soils (Orgiazzi et al. 2018).

#### Soil nutrients and rhizosphere effect

Higher amount of available nutrients, higher CEC and lower pH in the rhizosphere in comparison to the

bulk soils are linked to high input of organic material, fast decomposition (Rasse et al. 2005) and mineral weathering, which are usually enhanced in the rhizosphere by the activity of roots and associated microorganisms (Borin et al. 2010; Collignon et al. 2011). In general, an increase in soil organic matter content leads to a decrease in soil pH, and soil organic matter serves as the dominant sorbent, providing CEC (Gruba and Mulder 2015). This corresponds to a strong correlation between  $C_{org}$  and CEC found in BS and RH soils ( $r=0.938$ ,  $p<0.001$ ,  $n=40$ ). Weathering is promoted by increased acidity associated mainly with respiration, organic acid and  $H^+$  release from roots and microflora (Banfield et al. 1999; Frey et al. 2010). Moreover, weathering itself promotes acidity by releasing anions and acid cations into the soil. All these mechanisms are reported to contribute to increased acidity in the rhizosphere by one to two units (Hinsinger et al. 2009). In more developed soils of the vegetated tundra sites, acidity increased by about half a unit, likely due to organic matter accumulation. In the very young soils of the partly vegetated tundra and glacial forefield with scarce vegetation cover, however, acidity increased more by about one unit. The pronounced increase of sulphates and available  $Al_{H_2O}$  in the rhizosphere of the glacial forefield and partly vegetated tundra indicate that weathering, alongside soil organic matter and biological activity, significantly affected rhizosphere acidity at these sites with high levels of sulphates and carbonates in the bulk soil (Table 2 and Fig. 3). Dissolution of gypsum and carbonate induced by RH acidification is reported by Borin et al. (2010), and more intense weathering connected with acidification, in turn, contributed to distinctly positive REs on exchangeable and water extractable nutrients and CEC at the partly vegetated tundra and glacial forefield.

Easily available (water extractable) elements, such as  $Ca_{H_2O}$  and  $K_{H_2O}$ , the bulk and also rhizosphere soils had their primary source in soil sorption complex and in easily dissolved minerals (Table S4), which is documented by correlations between  $Ca_{ex}$  and  $Ca_{H_2O}$  ( $r=0.873$  by  $p<0.001$ ,  $n=40$ ) and between  $K_{ex}$  and  $K_{H_2O}$  ( $r=0.972$  by  $p<0.001$ ,  $n=40$ ). These correlations might suggest, together with literature (Doner and Lynn 1989; Singh et al. 2012) that available  $K_{H_2O}$  could be also released from highly soluble K minerals such as sylvite (KCl) or from soluble K, Na, Mg and  $SO_4^{2-}$  salts (see correlations between

$K_{H_2O}$  and  $K_t$   $r=0.409$  by  $p=0.009$ ,  $K_{H_2O}$  and  $Cl_{H_2O}^-$   $r=0.738$  by  $p<0.001$ ,  $K_{H_2O}$  and  $SO_4^{2-}$   $r=0.709$  by  $p<0.001$ ; Table S4). However, this statement has not been supported by mineralogical analysis. Exchangeable Ca, K and Mg were the main cations comprising CEC and positive REs on these cations were reflected in increased CEC higher content of water extractable nutrients in the rhizosphere as determined mainly on the partly vegetated tundra and glacial forefield.

Bioavailable  $P_{H_2O}$  was very low at all sites, at the lower limit of the values reported for Svalbard by Kylander et al. (2013). Nevertheless, bulk soil of the partly vegetated tundra had higher availability of P, which could be explained by a higher content of sulphates and carbonates than on other sites and also by the high abundance of lichen-dominated crusts. Lichens are hot spots of biological activity in the young habitats (Agnelli et al. 2021), and biocrust-forming lichens have been shown as important modulators of P release and cycling (Concostrina-Zubiri et al. 2022; Farrar 1976).

#### Exudation rate and exudate composition

To obtain more detailed information on root exudation, especially LMMA release, we arrange a microcosm experiment. We cultivated willow from different habitats in the growth chamber under the same conditions in sterile deionised water to eliminate microbial activity. At the same time, care was taken to minimize root injury and to eliminate its possible influence on organic acid release by pre-incubating the roots before the measurements. Thus, the measured exudation rate does not directly correspond to natural conditions but is ecologically relevant (Williams et al. 2021) and can be compared with some caution to the results obtained from rhizosphere soil.

Root exudation rate that comprises available C and nutrients depends on soil environmental conditions, root–soil concentration gradient, plant genotype, plant developmental stage and spatial location of solutes in the roots. Exudation rates are greater at root tips than more mature regions of roots (Badri and Vivanco 2009; Jones et al. 2009). Willows used in our microcosm experiment were comparable in age; they were cultivated in the same conditions, and the exudates from the entire root system were collected. The main factors that could affect the exudation rate

calculated per plant were the growth history of the plants, the physiology of the roots, and the architecture. The measured rate of C exudation of willows from different habitats was not significantly different. Based on this and spatial constraints of the rhizosphere, we suggest in accordance with Sokol and Bradford (2019) that soil enrichment by C depends on the proportion of rhizosphere soil in the soil, and willow abundance on the site will be the important controlling factor of the increase of organic matter in the soil.

Exudation of nitrogen and phosphorus from willow roots and the C/N ratio of exudates was affected by root stoichiometry, which is consistent with the literature data dealing with other plant species (Callahan et al. 2022; Cardenas et al. 2021; Cunha et al. 2022; Prescott et al. 2020). The nitrogen exudation rate of willow from the individual sites positively correlated to N/P of roots and negatively to C/N in exudates. Contrariwise, the phosphorus exudation rate negatively correlated to N/P roots and positively to C/N in exudates. This shows that N and P exudation depends on root N/P stoichiometry. Roots poor in N relative to P (low root N/P ratio; P in excess) exudate low amount of N and high amount of P and exudates have high C/N ratio and vice versa roots rich in N relative to P (high N/P ratio, N in excess) exudate more N, which results in their low C/N.

The high amount of N in exudates with a low C/N ratio was released from roots of the fully vegetated dry tundra and partly vegetated tundra, corresponding to a high N/P ratio and low P content of roots. We suggest that these plants could experience more P than N deficiency there. In contrast, the lowest N exudation with high C/N was detected in the glacial forefield that had the lowest root N/P and high P content, which may indicate plant exposition to low N availability relative to P.

The speciation of LMMOA in root exudates determined in our experiment was comparable with previously published results for *Salix* plants (e.g. *S. viminalis*; Magdziak et al. 2013). Input of LMMOA into soils increases microbial activity in the rhizosphere and subsequently accelerates the SOM decomposition (Haichar et al. 2014; Cheng et al. 2014; Wild et al. 2014) and mobilizes nutrients from inorganic sources by acidifying the environment. The later processes are particularly important for P acquisition at all sites where P can be bound in insoluble inorganic

complexes (Richardson et al. 2009). Oxalic, malic and citric acids were found to be the major LMMOAs exuded from roots to P deficient soils (Jones 1998) accelerating mobilization and solubilisation of adsorbed and bonded P via chelation of  $\text{Fe}^{3+}$ ,  $\text{Al}^{3+}$  in acid soils and  $\text{Ca}^{2+}$  in alkaline soils (Richardson et al. 2009; Vance et al. 2003). In our experiment, acetate, malate and citrate were always the most abundant LMMOA species indicating willow adaptation on alkaline soils with low P accessibility.

Of monocarboxylic acids, lactate was one of the most abundant LMMOA in exudates in willow roots from all habitats. As plants can rely on lactate fermentation and secretion when deprived of anoxia (Rivoal and Hanson 1994) we suggest that willows could experience hypoxia during our experiment. Acetate has been referred to improve drought tolerance and alleviate oxidative stress (Ellsworth et al. 2022), which is important in unstable and harsh conditions of the High Arctic environment.

#### Rhizosphere effect on microbiome and C, N and P availability

It is beyond doubt that the rhizosphere positively affects C and nutrient availability and related development of the soil microbiome and its metabolic activities and that accelerates organic material transformation and nutrient mining via priming effect (Wild et al. 2016). Accordingly, we measured significant increase in organic C and N in the rhizosphere and, consequently, in parameters of microbial biomass, fungi and bacteria. Microbial biomass ( $C_{\text{mic}}$ ) increased with  $C_{\text{org}}$  faster in the rhizosphere than in the bulk soil (Fig. 5) showing higher availability of  $C_{\text{org}}$  in the rhizosphere than in the bulk soil. The highest DOC content in the rhizosphere of the partly vegetated tundra coincided with the highest microbial biomass ( $C_{\text{mic}}$  and  $N_{\text{mic}}$ ), and vice versa, the lowest DOC in the rhizosphere of the glacial forefield was followed by the lowest microbial biomass.

Similarly to  $C_{\text{mic}}$ , fungal gene copies increased with the increase of  $C_{\text{org}}$  in contrast to bacteria gene copies, which decreased (Fig. S2). This might imply that the microbial biomass increase was mainly caused by fungal expansion (Figs. 4. and 5), which is in agreement with observed trends in other studies (Balasooriya et al. 2014; Carreiro et al. 2000; Eserschütz et al. 2009).

Fungal ability to produce enzymes effectively to decompose more complex SOM (Rousk et al. 2015) is together with fungal mycelium network capable to explore surrounding soil essential for further soil aggregation (Guggenberger et al. 1999; Miller and Jastrow 1990), and C sequestration (Lynch et al. 2018). Roots and root-associated symbiotic fungi improve aggregate formation, improving the physical protection of organic matter (Feng et al. 2022). In accordance with this, Wang et al. (2022) have shown that vegetation development and subsequent increase of dissolved organic C and N brought about an increase of organic C in all soil fractions. This trend was observed by Wang et al. (2022) already from the early stages of soil development on the same Nordenskiöldbreen glacial forefield. In addition to fungal activities, DOC exudation, microbial-derived DOC, and necromass formation are essential and efficient ways of stabilising organic matter (Sokol and Bradford 2019). They form organo-mineral associations which are not easily decomposed by microbes and have a long resistance time in soil (Lavalée et al. 2020). In the present study, we detected much higher REs on the abundance of fungi and bacteria on young sites of the glacial forefield and partly vegetated tundra as compared with the fully vegetated sites (see Fig. 4 and Table 3). Also, the most pronounced rhizosphere effect on C forms was detected on these habitats, underlining essential importance of root exudation for soil formation and organic matter stock.

Root input of labile C provides microbes with energy to synthesize a variety of enzymes (Blagodatskaya and Kuzyakov 2008; Fontaine et al. 2011), which accelerates microbial decomposition of SOM (Drake et al. 2013; Kuzyakov 2002) and leads to priming of soil organic matter decomposition and nutrient mining (Esperschütz et al. 2009; Chen et al. 2014). However, enzyme production, which is needed for organic matter decay and overall microbial growth, is not only C and energy but also N-demanding processes that can be strengthened by N sufficiency. In the partly vegetated tundra, high  $C_{mic}$  in the rhizosphere and also high rhizosphere effect on microbial parameters were accompanied by high C and N availability and low C/N; C content in the rhizosphere was six times higher ( $REs=6.14$ ), and N content was even 12 times higher ( $REs=12.4$ ) than in the bulk soil. Different increases of C and N availability in the rhizosphere caused the sharp decline of

C/N (from 18.5 to 21.3) compared with the bulk soil ( $50.4 \pm 0.33$ ). Such a sharp decline may be explained by the dominant effect of exudates released by P-limited plants (see above) on the soil poor in organic C and N. The high N exudation of roots in the partly vegetated tundra supplied microbes with N, which was limiting in the bulk soil and could promote the production of N-rich phosphatases. This alleviated P limitation and accelerated microbial growth. In addition, it could also help to overcome stress caused by high amounts of carbonates and sulphates originating from gypsum in the bedrock at this site. These findings allow us to speculate that plants that were P-limited and consequently released more N in exudates reduced not only N but also, at least in part, P limitation of microbial growth, resulting in a pronounced rhizosphere effect on microbial parameters.

Carbon content in the rhizosphere of the glacial forefield was, by one order and magnitude, lower than at other sites, and concurrently, the C/N ratio was high. It matched the high C/N ratio of exudates and the low N exudation rate of N-limited plants. Since the bulk soil at this habitat was extremely poor in organic matter and input of available organics was low, priming of soil organic matter transformation could not sufficiently eliminate microbial C and N colimitation. An important mechanism contributing to increased C accessibility during the very early stage of soil formation at this site could be the abiotic liberating of C. Exudates such as oxalate may promote C transformation, directly liberating organic compounds from organo-mineral complexes and improving the accessibility of previously protected C (Keiluweit et al. 2015). Willow roots from the glacial forefield exuded three times more oxalate than those from other habitats, and similar trends (not significant differences) were observed also for malate or citrate.

We did not find correspondence in the C/N ratio of exudates and rhizosphere soil in the fully vegetated habitats, which could be connected with the more complex effect of developed bulk soil with higher matter content and more interspecific interactions.

The content and availability of soil phosphorus is often a (co-)limiting factor for plant growth in arctic soils (Giesler et al. 2012). Available P is released either from primary and secondary minerals by chemical and biological weathering through the action of acids (P solubilization) or from organic material by hydrolytic enzymes (Zhou et al. 2013), which are

secreted mainly by soil microorganisms and, to a lesser extent, also by plant roots. Microbes need to be supplied by C, energy, and N to produce hydrolytic enzymes and maintain overall metabolic activity, as mentioned above. They can accumulate P in their cells and gradually release it both during their short lifetime and after death into the environment, keeping P relatively accessible to plants. In our study, P<sub>mic</sub> was more than one order of magnitude higher than the amount of P<sub>H<sub>2</sub>O</sub> at all studied sites and was a more important source of P than P<sub>H<sub>2</sub>O</sub> in the bulk soil as well as in the rhizosphere. It coincides with the findings that P<sub>mic</sub> is the largest source of available P in tundra (Weintraub 2011). P<sub>mic</sub> in the rhizosphere at the glacial forefield was more than two times lower as compared with the rhizosphere of the partly vegetated tundra even though amount of P<sub>H<sub>2</sub>O</sub> was similar at both sites and roots from the glacial forefield exuded three times more P than roots from the partly vegetated tundra. The low ability of the glacial forefield rhizosphere microbiome to use available P could be caused by C and N colimitation, as mentioned above. At the partly vegetated tundra, however, plants appeared to be P-limited and released exudates rich in N, with low C/N but poor in P. It was mirrored by higher C and N and C/N in the rhizosphere. In such conditions, microbes could mine P from soil organic matter and accumulate it in biomass and/or successfully compete with plants for P.

## Conclusions

Our results confirm that exudation by pioneer arctic plants such as dwarf willows enables faster release of nutrients after deglaciation, promotes microbial biomass in the rhizosphere and accelerates soil development. The rhizosphere effect, as defined by an increase of nutrients and microbial parameters in the rhizosphere as compared with the bulk soil on nutrient availability and microbial biomass, was greater at the glacial forefield and partly vegetated tundra with early soil development and decreased with increasing vegetation cover on the fully vegetated dry and wet tundra. The measured rate of C exudation by roots was comparable at all habitats, but the N and P exudation was found to be affected by plant root stoichiometry. High N exudation with a low C/N ratio was observed in the roots from the partly and fully

vegetated dry tundra with the presence of willow roots with a high N/P ratio. Conversely, the lowest N exudation with a high C/N ratio was found in the roots from the glacier forefield with the low root N/P. Similarly, willows with low N/P of roots excreted more P. These trends allowed us to deduce the effect of root stoichiometry on nutrient release and availability to regulate microbial activity through them.

A detailed study of the LMMOA exudation by *S. polaris* showed that LMMOAs make up 2% of total exuded C. Lactate, acetate, formate, malate, and citrate are dominant LMMOA species. It confirms the important role of exudates in nutrient release via chelation, solubilisation, acidification and acceleration of mineral weathering in the alkaline soil environment. Our study sheds light in detail on some of the plant–microbial interactions which are crucial during the initial soil development in order to face the harsh condition after deglaciation.

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**Data Availability** The datasets are available as a supplement.

## Declarations

**Conflict of interests** All authors declare they have no financial interests.

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