Biology and Fertility of Soils (2021) 57:179–192
https://doi.org/10.1007/s00374-020-01515-3

ORIGINAL PAPER

Correlation of the abundance of bacteria catalyzing phosphorus and nitrogen turnover in biological soil crusts of temperate forests of Germany

Julia Katharina Kurth[1,2] · Martin Albrecht[3] · Ulf Karsten[3] · Karin Glaser[3] · Michael Schloter[1,2] · Stefanie Schulz[2]

Received: 30 March 2020 / Revised: 25 September 2020 / Accepted: 30 September 2020 / Published online: 15 October 2020

Abstract

Soil P pools are strongly driven by microbial activities, and vice versa, P pools shape bacterial communities and their functional potential. Biological soil crusts (biocrusts) represent a microbial hotspot for nutrient turnover. We compared biocrusts and bulk soil samples from different temperate beech (*Fagus sylvatica* L.) forests representing a gradient in soil texture, nutrient concentrations, and pH values at biocrust peak biomass. We measured the total and plant-available P and N concentrations and assessed the bacterial potential to mineralize (*phoD*, *phnX*), solubilize (*gcd*), and take up P (*pstS* and *pitA*) and mineralize (*chiA*, *apr*) and fix N (*nifH*) by quantifying the respective marker genes (qPCR). We found an increase of absolute and relative bacterial abundance involved in P turnover in biocrusts, but the strategy to acquire P differed between the regions as bacteria harboring the starvation-induced *pstS* gene were most abundant where labile P was lowest. In contrast, the region with lowest total P concentrations has a higher potential to utilize more stable phosphonates. N mineralization was strongly correlated to P turnover at regions with increased labile N and P concentrations. Interestingly, the potential to fix N was highest in the bulk soil where total P concentrations were highest. Even though the correlation of N and P turnover is strongest if their ratio is low, the acquisition strategy strongly depends on soil properties.

Keywords Biological soil crust · Microbial N turnover · Microbial P turnover · Temperate forest · qPCR · Biodiversity Exploratories

Introduction

Many terrestrial habitats are limited in the major nutrients P and N (Elser et al. 2007). The P concentration of the parent rock material, the turnover of the internally bound organic P, and the sorption of P onto soil particles most importantly determine the P availability in forest soils, as fertilizer hardly plays a role (Walker and Syers 1976). Changes in P pools are strongly driven by microbial activities and dependent on microbial community composition and its activity pattern (Richardson and Simpson 2011; Rodríguez et al. 2006). Vice versa, the composition of P pools in soil shapes microbial communities and determines their functional potential (Bergkemper et al. 2016a). However, not only P pools and P availabilities drive P transformation processes, but also the overall nutrient stoichiometry strongly influences microbial P turnover, as microorganisms keep a stable ratio of macronutrients in their biomass (Cleveland and Liptzin 2007). For example, Sorkau et al. (2018) described a positive correlation of microbial P and N in soils of different temperate forest regions. Moreover, a positive correlation of bioavailable P fractions in soil and microbes potentially able to fix N was found (Bergkemper et al. 2016a). Vice versa, inorganic P (P_i) limitation can repress N assimilation as the respective genes

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s00374-020-01515-3) contains supplementary material, which is available to authorized users.

✉ Stefanie Schulz
stefanie.schulz@helmholtz-muenchen.de

[1] Department of Ecology and Ecosystem Management, TUM School of Life Science Weihenstephan, Chair of Soil Science, Technical University of Munich, Emil-Ramann-Straße 2, 85354 Freising, Germany

[2] Research Unit Comparative Microbiome Analyses, Helmholtz Zentrum München, Ingolstädter Landstraße 1, 85764 Neuherberg, Germany

[3] Department of Applied Ecology & Phycology, Institute of Biological Sciences, University of Rostock, Albert-Einstein-Strasse 3, 18057 Rostock, Germany

are under the control of the *Pho* regulon, which contains several genes controlled by a two-component system, which detects P_i concentrations in the environment (Santos-Beneit 2015). Highest turnover rates for plant-available nutrients like N, P, or C have been described for biological hotspots like rhizosphere or drilosphere in soil (Hoang et al. 2016; Kuzyakov and Blagodatskaya 2015; Lipiec et al. 2016; Reinhold-Hurek et al. 2015; Schulz et al. 2013; Uksa et al. 2015). Biological soil crusts (biocrusts) can be considered as such hotspots. Biocrusts are mostly dominated by organisms like phototrophic cyanobacteria, microalgae, lichens, or mosses, which drive the input of nutrients into the system. Together with associated heterotrophic microorganisms such as archaea, bacteria, or fungi, they create stable microhabitats, for example, by the excretion of polysaccharides (Cania et al. 2020; Mugnai et al. 2018, Vuko et al. 2020). Biocrusts have been mostly studied in arid and nutrient-poor habitats (Belnap et al. 2001), where they are the predominant vegetation form and their growth is limited by water and nutrient availability. Biocrusts from those regions contribute about half of the terrestrial N_2 fixation (Elbert et al. 2012). Moreover, it was demonstrated by Beraldi-Campesi et al. (2009) that biocrusts are not only enriched in N but that this also comes along with higher total P concentrations underlining the importance of balanced nutrient concentrations as proposed by Cleveland and Liptzin (2007). Less is known and only a few studies exist, which describe biocrusts as hotspots for nutrient turnover in temperate regions (Baumann et al. 2019; Brankatschk et al. 2013; Corbin and Thiet 2020; Gypser et al. 2016; Schaub et al. 2019; Schulz et al. 2016; Szyja et al. 2018), especially within well-developed ecosystems like forests (Baumann et al. 2017; Glaser et al. 2018; Williams et al. 2016). Atmospheric P inputs by dry and wet deposition in forests contribute as well since forests are dust traps also for particles from irrigated, highly fertilized agricultural soils (Aciego et al. 2017; Berthold et al. 2019) and those might be entrapped in the polymeric matrix of biocrusts. However, the relative contribution to P pools strongly depends on the bulk soil P concentration (Aciego et al. 2017). The role of biocrusts in P transformation in temperate forests has been described by Baumann et al. (2017), who found that, similar to biocrusts from arid regions, the P concentrations in biocrusts are enriched compared with adjacent bulks soils. Additionally, they demonstrated that especially the concentration of P-containing minerals decreased and of organic P concentrations increased in biocrusts compared with bulk soil. Thus, we hypothesize that (i) microorganisms colonizing biocrusts are involved in solubilization of mineral P and its transformation to biomass and thus abundances of those microorganisms are higher in biocrusts compared with bulk soil. (ii) As it was supposed that P and N turnover are closely linked, we further hypothesize that similar to P mineralization, also the potential for N mineralization is more pronounced in biocrusts, because of the higher microbial abundance, while the energy demanding fixation of N is less important in biocrusts from nutrient-rich forest soils. (iii) The strength of correlations between N and P turnover strongly depends on the N/P ratio of the bulk soil.

To test these hypotheses, we compared biocrust and bulk soil samples from temperate beech (*Fagus sylvatica* L.) forests, along a gradient in soil texture, nutrient concentrations, and pH values. Samples were taken once when biomass of biocrusts was highest. As the lifetime of biocrusts in temperate forests is short (they typically occur only at the end of the winter period before trees develop leaves) and the size of biocrusts is small as they newly develop every season, which excludes repeated sampling of the same biocrust, we abstained from repeated samplings but instead increased the number of analyzed replicates. Bacterial genes coding for proteins catalyzing P mineralization (alkaline phosphatase—*phoD*; phosphonoacetaldehyde hydrolase—*phnX*), solubilization (quinoprotein glucose dehydrogenase—*gcd*), and uptake (substrate binding protein of the phosphate ABC transporter—*pstS*; low-affinity Pi transporter—*pitA*) as well as N mineralization (bacterial chitinase group A—*chiA*; alkaline metalloprotease—*apr*) and N_2 fixation (dinitrogenase reductase subunit of the nitrogenase—*nifH*) were used as proxies for the abundance of the respective functional bacterial groups. We used qPCR to measure the abundance of bacterial genes involved in P and N turnover and correlated the data with the stable and labile P and N pools.

Material and methods

Sampling regions and procedure

All sampled forest sites are part of the Biodiversity Exploratories, a platform for interdisciplinary biodiversity research in Germany, with sites located in the southwest (Schwäbische Alb (ALB)), central (Hainich (HAI)), and northeast (Schorfheide-Chorin (SCH)) part of Germany (Fischer et al. 2010, www.biodiversity-exploratories.de). The three experimental regions differ gradually from south to north in altitude (ALB: 758 m, HAI: 415 m, SCH: 68 m), mean annual precipitation (year 2018, ALB: 806 mm, HAI: 357 mm, SCH: 539 mm), soil texture (silty clay in ALB and HAI, loamy sand in SCH), pH (ALB: pH 5.7 ± 0.8, HAI: pH 5.8 ± 1.2, SCH: pH 4.9 ± 1.5), and nutrient status (Alt et al. 2011; Grüneberg et al. 2010). The size of the regions ranges from 420 to 1300 km^2 and each exploratory includes 50 grassland and 50 forest plots with differing land use intensity.

Samples were taken in spring 2018 before bud break from 3 to 4 plots per region focusing on managed beech (*Fagus sylvatica* L.) forest plots with an even-aged tree population. At each plot, biocrust and bulk soil samples were collected from

skid trails at one to three spots, depending on their prevalence. Overall, 27 biocrust samples, visually recognized as green cover on the ground, were collected (see Fig. 1 and Supplement Table 1 for original plot numbers and number of biocrusts sampled). Biocrust samples (approx. 10 cm in diameter and 5 mm in thickness) were taken with a sterile Petri dish and transported on ice to the lab. As control, we sampled biocrust-free bulk soil (0–5 mm depth) close to the biocrust (max. distance of approx. 1 m), resulting in 54 samples in total. Samples for nutrient analyses were frozen at − 20 °C and samples for microbial analysis at − 80 °C until further analysis.

Abiotic soil properties

The pH was measured using 2 g of fresh sample material after 15 min shaking in 20 ml of a 0.01 M $CaCl_2$ solution. The water content was determined gravimetrically by the loss of weight after drying (60 °C, 24–48 h). The dried material was further used for phosphate and N_{total} analyses.

N_{total} was measured using approx. 30 mg of dried sample material in a CNS-Analyzer (vario EL, Elementar, Germany). The more alkaline soil samples of ALB were treated with 10% HCl and subsequently dried at 60 °C to remove carbonates prior measurement.

For the measurement of inorganic N compounds (exchangeable NH_4^+–N and NO_3^--N) as well as total dissolved nitrogen (TDN), 0.5 g material was extracted using 20 ml of a 0.01 mol l^{-1} $CaCl_2$ solution. The extracts were then filtered and stored frozen until analysis. The two inorganic fractions were directly analyzed using a constant flow analyzer (Flowsys, Alliance Instruments, Austria). For TDN, an aliquot of 10-ml extraction filtrate was digested according to Berthold et al. (2015) with peroxydisulfate for 24 h at 90 °C. The samples were neutralized after digestion and measured for NO_3^--N concentration like described above. The values for dissolved organic nitrogen (DON) were calculated by the subtraction of summed inorganic N fractions from the total dissolved N values.

All measurements of inorganic phosphate (after extraction or digestion) were carried out using the molybdenum blue photometric method (Hansen and Koroleff 1999). The absorbance was measured at 885 nm in a 5-cm optical glass cuvette using a photometer (UV1200, Shimadzu, Japan). Two easily available inorganic phosphate fractions of the Hedley fractionation (Hedley et al. 1982) were sequentially analyzed for H_2O-soluble phosphate (P_{H2O}) and $NaHCO_3$-fraction (P_{NaHCO3}) using approx. 1.5 g dried material. For total P (P_{total}) analysis, approx. 100 mg dried material was digested according to Berthold et al. (2015) for 24 h at 90 °C using an acid peroxydisulfate solution and neutralized before measurement.

DNA extraction and quantification

Phenol chloroform extraction of nucleic acids was performed with approx. 0.5 g of sample material according to Töwe et al. (2011) or without added material for extraction blanks, which served as extraction control (four processed controls). For homogenization, Lysing Matrix Tubes E (MP Biomedicals, USA) and the Precellys24 Instrument (Bertin Technologies, France) were used. The purity of the extract was checked by measuring the ratios of adsorption of 260 nm/280 nm and 260 nm/230 nm as given by NanoDrop® ND-1000 spectrophotometer (Thermo Fisher Scientific, USA). Yields of extracted DNA were quantified using Quant-IT™ Picogreen® dsDNA Assay Kit (Thermo Fisher Scientific, USA). The DNA concentration of the four extraction controls was below detection limit, and thus, DNA contamination during the extraction could be excluded.

Real-time quantitative PCR

A SYBR Green® based approach was applied by using a 7300 real-time qPCR machine (Applied Biosystems, Germany) to quantify gene abundances according to the manufacture's protocol. The reaction mix contained 12.5 µl of SYBR Green® (Thermo Fisher Scientific, USA), forward (F) and reverse (R) primers (Metabion, Germany), 0.5 µl BSA (3%, Sigma, Germany), and DEPC-treated water and

Fig. 1 Biological soil crusts in skid trails of region. **a** ALB, **b** HAI, **c** SCH

was set to 25 µl. Reaction conditions, primers, calibration standards, and full names of the chosen marker genes are summarized in Table 1. To exclude inhibitory effects, dilution tests were performed prior running qPCRs. Standard series ($r^2 > 0.99$), no template controls and samples diluted to 1/64 were included in each run. To evaluate the quality of the qPCR, melting curve analyses were performed and randomly chosen samples were checked by electrophoresis on a 1.5% agarose gel. The qPCR efficiency was calculated for each primer pair with the formula $Eff_{slope} = 10\text{^}(-1/slope) - 1$ and was in all measurements between 74 and 90%. Values below detection limit of 10 copies (according to manufacturer's protocol) per reaction were set NA.

Statistical analysis

Data analysis was performed in R version 3.6.1 (R Core Team 2019). Linear mixed effect models (function lme in R package nlme (Bates and Pinheiro 1998)), which are particularly well suited for unbalanced sample designs, were used to investigate variances caused by the region (ALB, HAI, SCH) and sample type (biocrust, bulk soil). Plot number was set as random factor to consider multiple sampling within one plot. Models were fit by maximizing the restricted log-likelihood. To validate the linear mixed effects models, residual vs. fitted plots and plots showing sample quantiles vs. theoretical quantiles based on the model were tested for homogenous variance and normal distribution of residuals. If either conditions of normality were not met or homogeneity of variance had to be improved, data was log transformed, except DON and P_{H2O} that were square root transformed. To test for significant differences ($p < 0.05$) between the investigated factors, pairwise comparisons were conducted by a Tukey post hoc test (R package lsmeans) (Lenth 2016). The dot and crossbar plots were created using the ggplot2 package (Wickham 2016); for Pearson correlation plots, the qgraph package (Epskamp et al. 2012) was chosen.

To see how abiotic properties and gene abundances differ on average between biocrust and bulk soil samples, we defined the ratio r, which is based on the mean of biocrust and bulk soil for each variable. In case the values of biocrust were higher than bulk soil values, this was calculated as r = biocrust/bulk soil, else as $r = -$bulk soil/biocrust.

Results

Soil chemical properties

Soil chemical properties are shown in Fig. 2 and results of statistical analysis are summarized in Tables 2 and 3. The differences between the values of each pair of biocrust and bulk soil are shown in Supplement Fig. 1. N_{total}, P_{total}, and NO_3^--N concentrations and pH increased significantly ($p \leq 0.001$) in biocrusts and bulk soil to the same extent from SCH (N_{total}

Table 1 qPCR reaction conditions and calibration standards for qPCR of functional genes

Protein	Target gene	Reaction conditions				Calibration standard source
		F- and R-primer (pmol μl^{-1})	Thermal profile[a]	Primer	Reference	
Dinitrogenase reductase subunit of the nitrogenase	*nifH*	0.5	45 s/95 °C, 45 s/55 °C, 45 s/72 °C	nifH-f and -r	Rösch et al. (2002)	*Sinorhizobium meliloti* 30136
Alkaline metalloprotease	*apr*	1	45 s/95 °C, 45 s/53 °C, 45 s/72 °C	apr-f, apr-r	Bach et al. (2001)	*Pseudomonas aeruginosa* 5071
Bacterial chitinase group A	*chiA*	1	30 s/95 °C, 30 s/60 °C, 60 s/72 °C	chiA-f, chiA-r	Xiao et al. (2005)	*Streptomyces griseus*
16S ribosomal RNA	16S rRNA gene	0.5	45 s/94 °C, 45 s/58 °C, 45 s/72 °C	FP and RP 16S	Bach et al. (2002)	*Pseudomonas putida*
Alkaline phosphatase	*phoD*	0.8	20 s/95 °C, 60 s/60 °C, 30 s/72 °C, 60 s/81 °C	phoD-F, phoD-R	Bergkemper et al. (2016b)	*Bradyrhizobium japonicum*
Phosphonoacetaldehyde hydrolase	*phnX*			phnX-F, phnX-R		*Salmonella enterica* DSM 17058 (DSMZ)
Quinoprotein glucose dehydrogenase	*gcd*			gcd-F, gcd-R		*Salmonella enterica* DSM 17058 (DSMZ)
Phosphate inorganic transporter	*pitA*			pitA-F, pitA-R		*Pseudomonas fluorescens*
Phosphate-specific transporter (periplasmic phosphate-binding protein)	*pstS*			pstS-F, pstS-R		*Bradyrhizobium japonicum*

[a] Performed in 40 cycles

2289 ± 919 mg kg^{-1} dw; P_{total} 149 ± 47 mg kg^{-1} dw; NO_3^--N 7.26 ± 5.5 mg kg^{-1} dw) over HAI (N_{total} 3782 ± 768 mg kg^{-1} dw; P_{total} 327 ± 114 mg kg^{-1} dw; NO_3^--N 12.88 ± 5.8 mg kg^{-1} dw) to ALB (N_{total} 4588 ± 1402 mg kg^{-1} dw; P_{total} 624 ± 263 mg kg^{-1} dw; NO_3^--N 18.09 ± 7.7 mg kg^{-1} dw)). The same trend was observed for TDN, which spanned a range from ALB to SCH from 37.70 ± 8.74 mg kg^{-1} dw to 25.74 ± 9.15 mg kg^{-1} dw. In contrast, P_{NaHCO3} values were lower in HAI (9.79 ± 2.33 mg kg^{-1} dw) and SCH (12.48 ± 3.42 mg kg^{-1} dw) than in ALB (31.96 ± 21.14 mg kg^{-1} dw). Interestingly, P_{H2O} (11.2 ± 8.0 mg kg^{-1} dw) was detected at highest concentrations in SCH and DON was on the same level for all regions even though N_{total} and P_{total} were lowest.

Differences between biocrusts and bulk soil for N concentration were most obvious in SCH, where NO_3^--N concentration and exchangeable NH_4^+-N and TDN concentrations were twice as high in biocrusts, and in ALB, where the concentrations of TDN and DON decreased in biocrusts, even though none of them was significant (see Fig. 2 and Table 2). In SCH also, the amount of P_{H2O} was higher in biocrusts than in bulk soil. In contrast, P_{NaHCO3} was detected in lower concentrations in biocrusts of SCH and ALB.

Gene abundances

Absolute gene abundances were calculated as gene copy numbers g^{-1} dw and are displayed in Fig. 3, and the statistical results are shown in Tables 2 and 3. The differences between the values of each pair of biocrust and bulk soil are shown in Supplement Fig. 2.

Table 2 *p* values of linear mixed effect models. Significant values ($p < 0.05$) are shown in italics

	Variable	$p_{Exploratory}$	$p_{Sample\ type}$
Abiotic soil properties	N_{total}	*0.024*	0.283
	TDN	*0.011*	0.951
	NH_4-N	0.744	0.184
	NO_3-N	*0.042*	0.875
	DON	0.766	0.924
	P_{total}	*0.005*	0.707
	P_{NaHCO3}	0.081	0.143
	P_{H2O}	0.125	0.521
	N/P	0.284	0.265
	pH	*> 0.001*	0.322
Bacteria	16S rRNA gene	0.732	0.487
N cycle	*nifH*	*> 0.001*	0.344
	chiA	*> 0.001*	0.647
	apr	0.486	0.745
P cycle	*phoD*	*0.002*	*0.021*
	phnX	0.989	*0.007*
	gcd	0.298	*0.003*
	pstS	0.061	0.310
	pitA	0.894	*0.010*

Abundances were 1.1 (*pstS*, HAI) and up to 2.37 times (*pitA*, SCH) higher in biocrust samples for most of the genes. Regarding solubilization of P, the abundance of *gcd* gradually decreased from ALB to SCH from 2.3 × 10^7 ± 1.4 × 10^7 to 1.0 × 10^7 ± 8.3 × 10^6 mean gene copies g^{-1} dw. The abundance of *gcd* in SCH was the single significant difference between biocrust and bulk soil in P turnover, where *gcd* showed higher values in biocrusts. The abundances of the genes for the mineralization of P (*phoD*, *phnX*) were generally lowest. Especially, *phoD* was six to five times lower in SCH compared with ALB and HAI, respectively, and only reached 1.3 × 10^6 ± 6.1 × 10^5 copies g^{-1} dw in SCH. The gene abundance of *phnX* did not differ between regions. However, the *phnX* abundance in biocrusts of HAI was two times as high as in bulk soil of HAI. In SCH, the abundance of *phnX* was more than six times higher in biocrusts compared with the respective bulk soil. Regarding the abundance of marker genes for P uptake, *pstS* was higher compared with *pitA*, especially in ALB and HAI. The abundance of *pitA* in bulk soil and biocrusts was stable across all regions with mean gene abundances of 8.7 × 10^6 ± 5.1 × 10^6 and 1.5 × 10^7 ± 9.9 × 10^6 copies g^{-1} dw, respectively. In contrast, *pstS* abundance was lowest in SCH where at the same time the difference between biocrust and bulk soil ($r = 1.43$) was highest.

The gene abundances of microbes catalyzing N turnover were higher compared with genes triggering P turnover in all regions, except for *nifH* in SCH, which was in the same range as genes linked to P transformation processes. *nifH* was one order of magnitude higher abundant ($p < 0.015$) in ALB (2.1 × 10^8 ± 1.6 × 10^8 copies g^{-1} dw) and HAI (1.4 × 10^8 ± 9.2 × 10^7 copies g^{-1} dw) compared with SCH (1.2 × 10^7 ± 9.4 × 10^6 copies g^{-1} dw). Moreover, the *nifH* gene abundance was nearly two times lower ($p = 0.029$) in the biocrust of the ALB compared with the bulk soil. The opposite trend was observed in SCH where in the biocrust, the *nifH* gene abundance was two times higher. The same pattern as for *nifH* was observed for *chiA* abundance with lowest values in SCH (4.1 × 10^7 ± 1.8 × 10^7 copies g^{-1} dw) and highest in ALB (2.1 × 10^8 ± 1.2 × 10^8 copies g^{-1} dw). Even though biocrusts and bulk soil did not differ significantly in *chiA* abundance, gene abundances in biocrusts of HAI and SCH were 1.3 and 1.5 times higher, respectively. The *apr* gene abundance did not differ between regions and sample type and ranged from 5.7 × 10^7 to 1.2 × 10^8 copies g^{-1} dw. Still, in SCH, 1.7 times more *apr* could be detected in the biocrust compared with the bulk soil. The abundance of bacterial 16S rRNA genes (see Fig. 3c) did not differ significantly between the regions or sample type (Table 2). Detected values varied from 3.4 × 10^{10} (bulk soil—SCH) to 5.96 × 10^{10} gene copies g^{-1} dw (biocrust—HAI).

Table 3 Significant *p* values of Tukey post hoc test of linear mixed effect models. Only variables being significantly affected by region or sample type are shown

	Variable	Contrast	$p_{\text{Post Hoc}}$
Abiotic soil properties	pH	ALB, bulk soil - SCH, bulk soil	> 0.001
		HAI, bulk soil - SCH, bulk soil	0.003
		ALB, biocrust - SCH, biocrust	> 0.001
		HAI, biocrust - SCH, biocrust	0.001
	N_{total}	ALB, bulk soil - SCH, bulk soil	0.039
	TDN	ALB, bulk soil - SCH, bulk soil	0.013
	P_{total}	ALB, bulk soil - SCH, bulk soil	0.016
		ALB, biocrust - SCH, biocrust	0.016
N cycle	*nifH*	ALB, bulk soil - SCH, bulk soil	> 0.001
		ALB, bulk soil - ALB, biocrust	0.029
		HAI, bulk soil - SCH, bulk soil	0.003
		ALB, biocrust - SCH, biocrust	0.011
		HAI, biocrust - SCH, biocrust	0.015
	chiA	ALB, bulk soil - SCH, bulk soil	0.002
		HAI, bulk soil - SCH, bulk soil	0.020
		ALB, biocrust - SCH, biocrust	0.022
		HAI, biocrust - SCH, biocrust	0.033
P cycle	*phoD*	ALB, bulk soil - SCH, bulk soil	0.005
		HAI, bulk soil - SCH, bulk soil	0.042
	gcd	SCH, bulk soil - SCH, biocrust	0.013

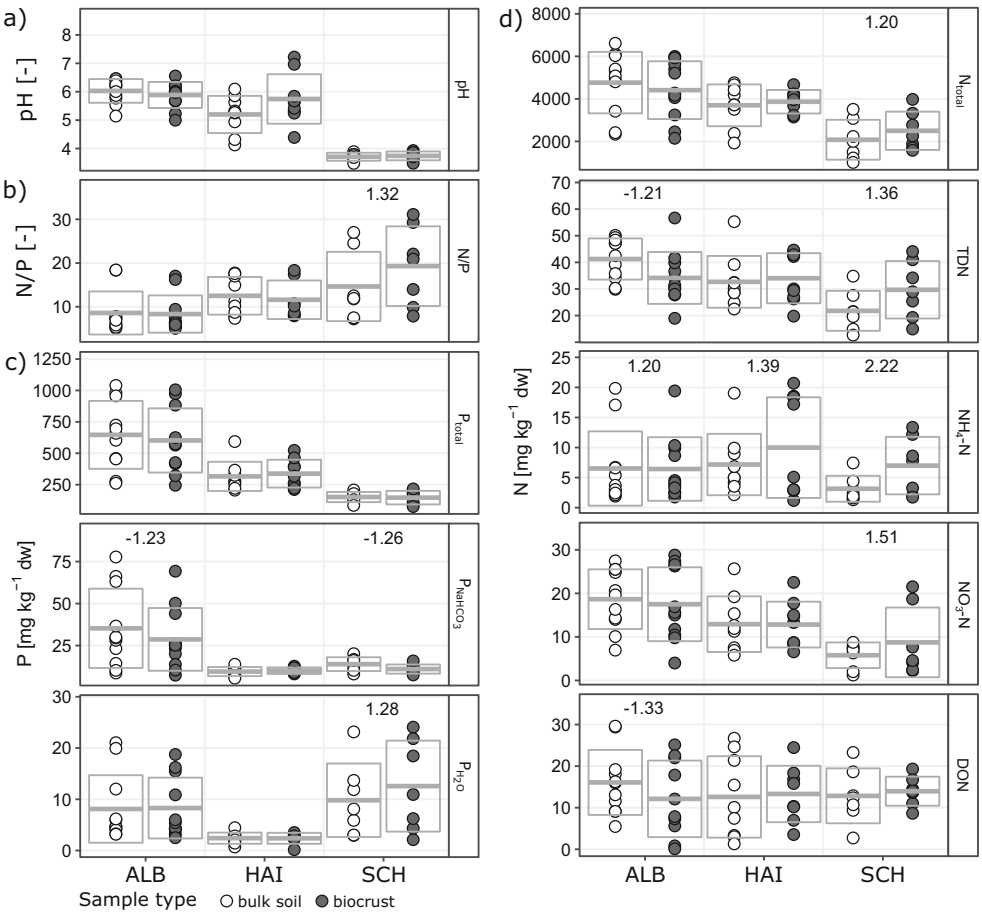

Fig. 2 Abiotic soil properties as pH (**a**), the N/P ratio (based on mg/kg) (**b**), the P (**c**), and N pools (**d**) are shown as dot plots and crossbars. Crossbars are marked by the mean values and standard deviation. The P pools include total P (P_{total}) and labile P (P_{H2O} and P_{NaHCO3}) and the N pools include total N (N_{total}), total dissolved N (TDN), exchangeable NH_4^+-N, NO_3^--N, and dissolved organic N (DON). The values are grouped by region and sample type. Above each group, the ratio *r* is displayed to see how biocrust and bulk soil differ on average. Values with $-1.1 \geq r \leq 1.1$ are not shown

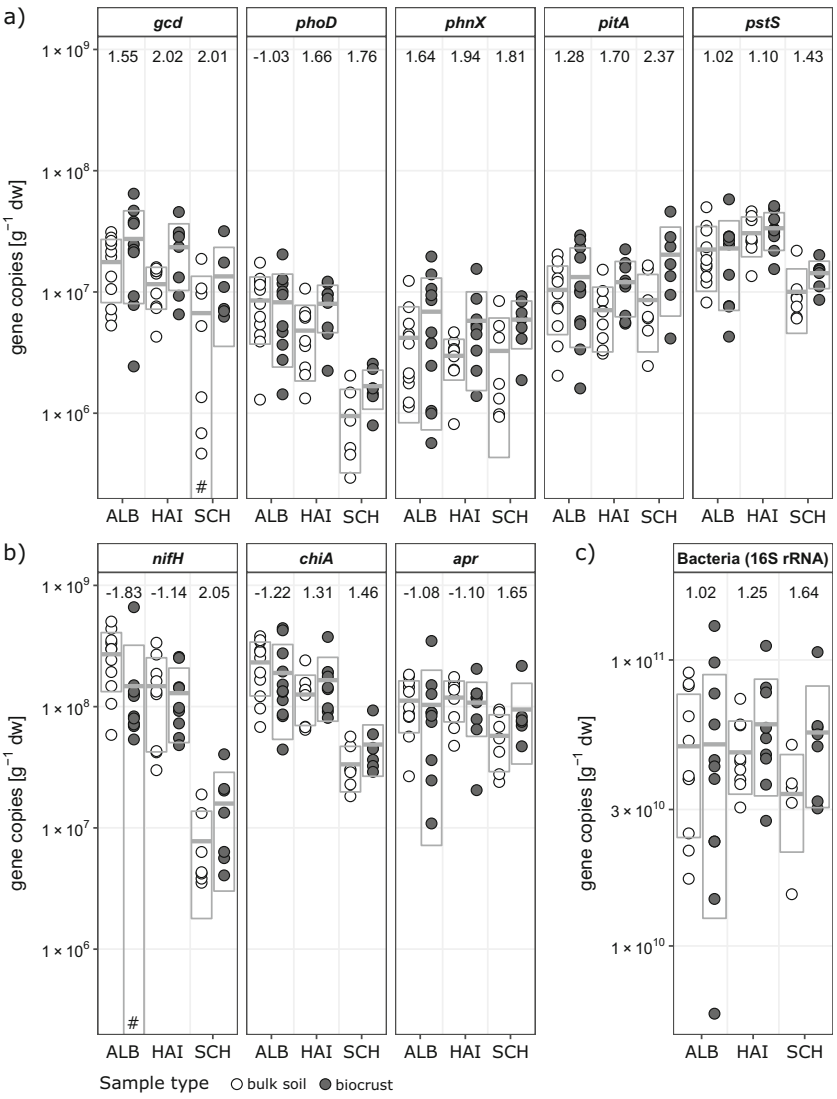

Fig. 3 Functional gene copy numbers per gram dry weight are shown as dot plots and crossbars. Crossbars are marked by the mean values and standard deviation and dots show the distribution of the single values. **a** P turnover (*gcd, phoD*, *phnX*, *pitA*, *pstS*), **b** N turnover (*nifH*, *chiA*, *apr*), and **c** the abundance of the 16S rRNA gene as marker for total bacterial abundance. "#" indicates that standard deviation is higher than the mean value. The values are grouped by region and sample type. Above each group, the ratio *r* is displayed to see how biocrust and bulk soil differ on average

To also account for minor changes in bacterial biomass between bulk soil and biocrust, relative gene abundances were calculated as copy numbers per 16S rRNA gene abundance. The results are shown in Supplement Fig. 3 and statistical results are shown in Supplement Table 2 and 3. Regarding N cycle genes, tendencies of relative gene abundances were similar to absolute gene abundances, and only for *nifH* gene abundances, differences between bulk soil and biocrust in ALB were less pronounced. Also, for P cycle genes, the pattern of relative gene abundances was mostly comparable with those of the absolute gene abundances with more pronounced differences between exploratories for *phoD* relative gene abundance and between bulk soil and biocrust in HAI for *pitA* relative gene abundance. Only, the relative gene abundance of *gcd* was higher in the bulk soil of SCH, while it was higher in the biocrust for the absolute gene abundances.

Correlation of soil chemical properties and gene abundances

In total, 18 variables were included in the correlation analyses. The significant Pearson correlations ($r^2 > 0.6$, $r^2 < -0.6$, $p < 0.05$) are shown in Fig. 4. From Table 4 and Fig. 4, it can be concluded that the total number of significant correlations is comparable between sample types in each region but the type of connections can be quite different. In general, the total number of correlations decreased from ALB and SCH to HAI, while the number of negative correlation was lowest in ALB and highest in SCH.

In particular, in ALB, the difference in numbers of correlations was highest between biocrust and bulk soil and resulted in 30 and 42 positive correlations, respectively. Here, we found strong correlations of genes driving N and P turnover with each other. The P pools revealed a strong intra-correlation in bulk soil and biocrusts in ALB. In bulk soil,

Table 4 Number of significant Pearson correlations as shown in Fig. 4

	ALB		HAI		SCH	
	Bulk soil	Biocrust	Bulk soil	Biocrust	Bulk soil	Biocrust
$r^2 > 0.6$	42	30	29	26	27	29
$r^2 < -0.6$	1	5	7	4	9	12
Total sum per sample type	43	35	30	36	36	41

TDN and NO_3^--N concentrations were correlated with all abundances of genes, except *phnX* to TDN. In contrast, TDN concentration was only correlated to *phoD* abundance and NO_3^--N concentrations to *chiA* abundance in biocrusts while the other abundances of genes (*pstS*, *phnX*, *nifH*, and *apr*) were correlated to exchangeable NH_4^+-N concentration. Interestingly, no correlations for *nifH* abundances were found in bulk soil, even though it was the highest abundant gene in bulk soil of ALB compared with all other samples. However, we detected a strong correlation between *nifH* abundance and exchangeable NH_4^+-N concentrations in biocrusts.

In HAI, we observed fewest correlations compared with the other regions ($n_{total} = 66$). In bulk soil, a strong correlation of *phoD*, *pitA*, and *chiA* abundances could be observed, while *pstS* abundance was not correlated even though it was highly abundant. pH was strongly correlated in biocrust and bulk soil, in biocrusts especially to abundances of P turnover genes. In biocrusts, a similar correlation pattern between gene abundances was found as described for ALB.

The highest number of negative correlations was detected in SCH ($n_{total} = 77$, $n_r{}^2_{<0} = 21$) which were equally distributed between biocrust ($n = 29$, $n_r{}^2_{<0} = 12$) and bulk soil ($n = 27$, $n_r{}^2_{<0} = 9$). In bulk soil, *chiA* and *nifH* abundances revealed the highest number of correlations, mostly with abundances of genes involved in P turnover. We found a strong correlation of *pitA* and *nifH* abundances, and N_{total}, which was also obvious in bulk soil of HAI, but weaker. Further, P_{NaHCO3} concentration was negatively correlated to pH, *phoD*, *chiA*, and *apr* abundances in bulk soil and to TDN and exchangeable NH_4^+-N concentrations in biocrusts of SCH samples. No correlations for *pstS* abundance in biocrusts were found, even though it was much higher abundant compared with bulk soil.

Discussion

Parent material determines P acquisition strategy in biocrusts

The aim of this study was to investigate the correlation of the bacterial driven P and N turnover in biocrusts from forests, which differ in nutrient stocks, soil texture, and pH. To be able to correlate nutrient concentrations and abundance of major functional microbial groups, we did one extensive sampling campaign during peak biomass of the biocrusts. In nutrient-poor habitats, biocrusts are important first colonizers and described as "mantels of fertility" by Garcia-Pichel et al. (2003) as they are known to increase nutrient concentrations due to activities of C and N_2 fixing phototrophic organisms and thus contain higher abundances of microorganisms compared with bulk soils in arid environments (Castillo-Monroy et al. 2011; Maier et al. 2018). In the forest biocrusts, we neither detected significantly higher nutrient concentrations nor significantly increased bacterial abundance (16S rRNA gene) in most of the biocrusts (Table 2), even though well-developed biocrusts were present at all investigated plots (see Fig. 1) which was further corroborated by the conspicuous presence of algae (Glaser et al. 2018). The well-developed forest soils are generally rich in nutrients and organic substances, which allow for already high microbial abundances in bulk soil. For example, the sample with the lowest bacterial abundance reached 3.4×10^{10} gene copies g^{-1} dw (bulk soil—SCH), which can be hardly reached in biocrusts from arid environments but is definitely several orders of magnitude higher compared with the respective bulk soils (e.g., Couradeau et al. 2016; Maier et al. 2018; Nagy et al. 2005). Moreover, in temperate forests, biocrusts are mostly only temporally abundant during spring, when soil temperature is above freezing point and light is available in sufficient photon flux rates before the vegetation period. Further, strong disturbances of biocrusts, e.g., by management, are likely. Both factors result in high dynamics of biocrust development in temperate forests and yearly formations are typical for such environments. Only at the early vegetation period, the additional input of C by the phototrophic organisms may favor the establishment of a biocrust community. The combination of high nutrient concentrations in bulk soil and short periods of biocrust formation and persistence explain the less pronounced difference of bacterial abundance and nutrient concentrations between biocrusts and bulk soil.

In ALB, the P pools and gene abundances of bacteria involved in P turnover did not differ significantly between bulk soil and biocrust (Table 3). In contrast in HAI and SCH, the absolute and relative abundance of bacteria specifically involved in P turnover increased much more in the biocrust, while P_{total} did not differ in any region. P_{NaHCO3} on the other hand was slightly decreased in biocrusts compared with bulk

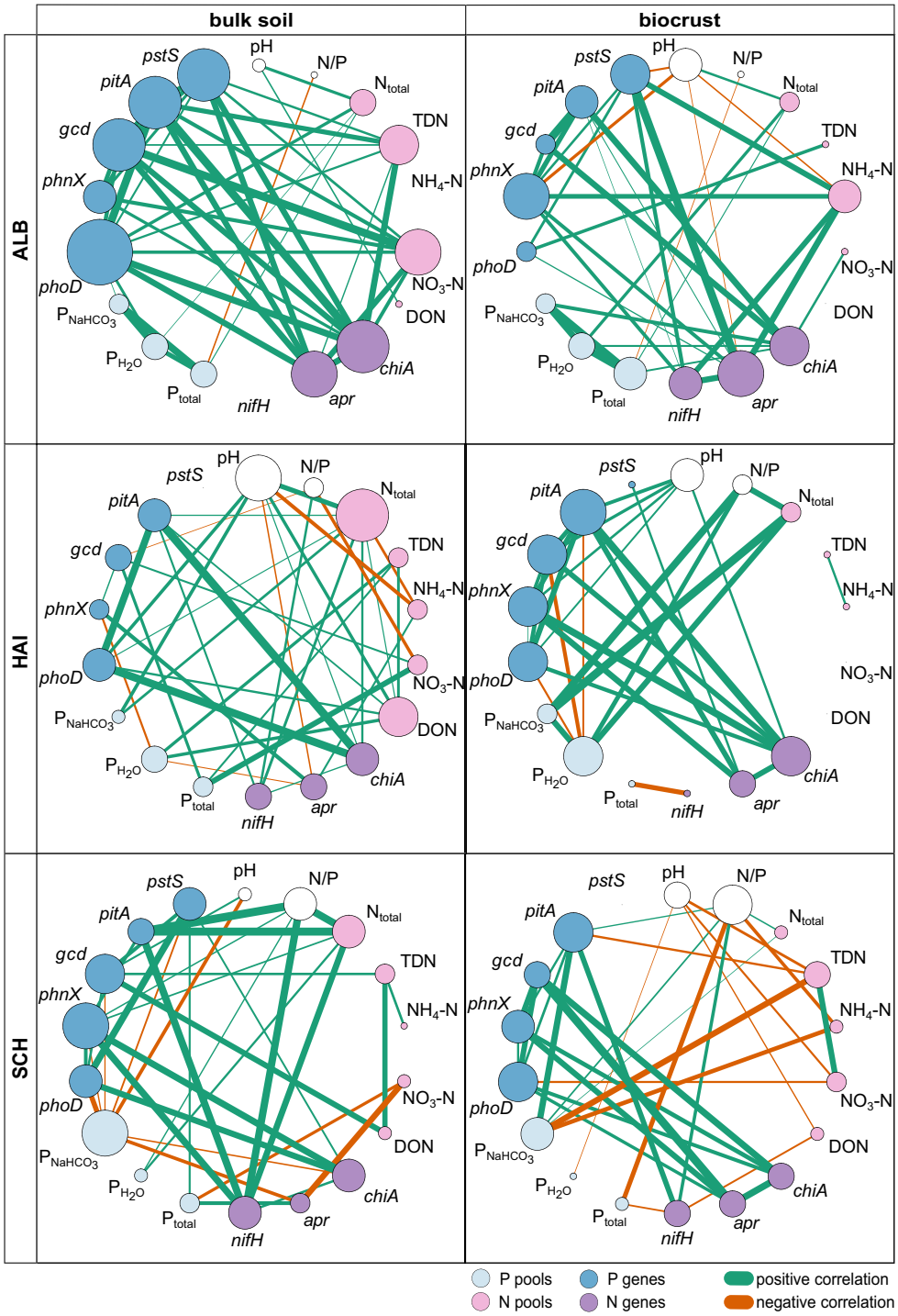

Fig. 4 Correlations within biocrusts and bulk soil are shown as Pearson correlation plot for each region. The analysis included pH, N/P ratio (based on mg/kg), N pools (N_{total}, TDN, NH_4^+-N, NO_3^--N, DON), P pools (P_{total}, P_{H2O}, P_{NaHCO3}), as well as the gene abundance for N (*nifH*, *chiA*, *apr*) and P cycle genes (*phoD*, *phnX*, *gcd*, *pitA*, *pstS*); those categories are indicated by circle colors. The size of each circle indicates the degree of correlations while the thickness of each line indicates the strength of correlation, ranging between $r^2 = 0.6$ and $r^2 = 0.97$ with $p < 0.05$

soil in ALB and SCH. Moreover, the ALB had highest *gcd* gene abundances, indicating the highest potential for P solubilization. Solubilization was also identified as key process in other P-rich forest regions (Bergkemper et al. 2016a) as well as in agricultural fields with sufficient P supply (Grafe et al. 2018). The additionally high P_{NaHCO3} concentration might point to an efficient release of P from iron and aluminum oxides, which have a good solubility in the pH range of the ALB (Lindsay 1979).

In comparison with ALB, the P_{total} concentrations of HAI and SCH were significantly lower. Interestingly, the bacterial potentials to acquire P in the biocrusts differed between those two sites with respect to P uptake and mineralization of organic P. In HAI, similar to ALB, the mineralization of P in biocrusts was dominated by bacteria carrying the *phoD* gene, which are able to hydrolyze phosphomonoesters and –diesters under favorable conditions. Furthermore, the labile P pools were very low in HAI, which coincides with the highest

abundance of the phosphate-inducible transporter gene *pstS*. In biocrusts and bulk soil from SCH, bacteria carrying the *phnX* gene were much more abundant than *phoD* and increased in biocrusts. However, as our analysis was done on DNA level, we cannot conclude that this is also true for the expression of those genes. For P-poor soils, it was demonstrated that the pool of easily degradable phosphomono- and diesters is depleted quickly, and that instead more recalcitrant phosphonates remain in the soil as organic P source (Bergkemper et al. 2016a; Condron et al. 2005; Lang et al. 2017). For SCH, this could indicate that the supply with mono- or diesters is not high enough, so that the P demand is fed by the additional degradation of phosphonates. In this regard, Baumann et al. (2017) concluded that P solubilized from the minerals is directly transferred and immobilized by the microbiome of the biocrusts, because the combination of low pH and sandy texture reduces the sorption capacity of soils in SCH, as low concentrations of iron and aluminum minerals can be assumed (Lang et al. 2017). This is further underlined by the high P_{H2O} and low P_{NaHCO3} concentrations. Thus, immobilization by sorption to minerals is unlikely and corroborated by the significantly reduced relative abundance of *gcd* in biocrusts of SCH, even though absolute *gcd* numbers slightly increased in biocrusts their relative abundance compared with total 16S rRNA gene abundance dropped. Additionally, an increase of *pstS* was observed in the biocrusts of SCH. *pstS* is part of the *Pho* regulon and important for P uptake under P limitation (Santos-Beneit 2015). Consequently, during biocrust development, P might get limited and uptake via the *pstS* pathway displays an advantage. Surprisingly, also the abundance of *pitA* was higher in biocrusts from SCH compared with the other sites. The *pitA* gene codes for the unspecific P_i transporter (Wanner 1993; Willsky and Malamy 1980). Thus, bacteria from these biocrusts are well equipped for the uptake of P under fluctuating conditions.

Labile pools determine the correlation of bacterial P and N turnover

For forests, Cleveland and Liptzin (2007) postulated a well-constrained molar N/P ratio of 9:1 for microbial biomass and of 15:1 for forest soils. Regarding the different sites of our study, a similar ratio between N and P in the soil were measured for the ALB (N/P ratio of 8.5 ± 4.6), while the difference between N_{total} and P_{total} concentrations increased from HAI to SCH. Thus, we assumed that in ALB processes to acquire N and P are closely interlinked, while in HAI and SCH, the microbes are facing P limitation which is in line with higher potentials for P acquisition.

Regarding ALB, our data underlines a strong link between N and P turnover; both abundances of N mineralization genes (*apr*, *chiA*) measured are significantly correlated to abundances of all P cycle genes. However, it has to be taken into account that there are much more enzymes able to mineralize organic N sources, like neutral proteases or subtilisin, than the ones which we analyzed in this study. Nonetheless, our chosen marker genes cover chitinolytic and proteolytic proteins. Moreover, our observation is based on statistical analyses, with a lack of additional experimental testing, for example, by fertilization, which is a classical drawback of field studies, but compensated by a high number of replicates in this study (up to 11 replicates). The strong correlation between N and P cycle genes might indicate that the mineralization of organic sources is used to deliver both N and P to the same extent. A similar observation was described by Heuck and Spohn (2016), who demonstrated in an incubation experiment of organic horizons from temperate forests that net N and P mineralization are strongly correlated and increased with decreasing N/P ratio. Against this backdrop, it is not surprising that the abundance of bacteria harboring the *nifH* gene was generally highest in the ALB and contrary to our hypothesis, significantly increased in the bulk soil compared with the biocrust ($p = 0.029$). Taken into account that the fixation of one molecule N_2 needs 16 molecules of ATP (Zehr et al. 2003), a relatively better supply with P and also C could potentially increase N_2 fixation rates (Reed et al. 2007). Thus, in the ALB, this displays an additional source to fuel the labile N pool. Even though the *nifH* gene abundance was significantly lower in the biocrusts, the nutrient pools did not differ between bulk soil and biocrusts in the ALB, which is not compensated by higher N mineralization gene abundance. However, we also have to consider that *nifH* is phylogenetically widely distributed (Gaby and Buckley 2012) and that the primers used only cover sequences of *nifH* cluster I, which comprises aerobic N fixers like *Proteobacteria*, *Frankia*, *Paenibacillus*, and some *Cyanobacteria* like *Anabaena*, *Nostoc*, and *Plectonema* but excludes others, which might be associated with biocrusts (Rösch et al. 2002). Within this study, we also determined the nitrification potentials as revealed by the quantification of the *amoA* marker gene coding for a subunit of the ammonia monooxygenase of bacteria and archaea. And indeed, when *nifH* was increased in bulk soils, also ammonia-oxidizing bacteria or archaea were increased in over 70% of the replicates (data not shown). The ammonium produced during N_2 fixation might be used as substrate for the nitrification process, which in turn might have caused a reduction of exchangeable NH_4^+-N. This hypothesis is supported by a strong positive correlation of *nifH* abundance to NH_4^+-N in the biocrusts, which is a plausible hint that the NH_4^+-N is not immediately oxidized to NO_3^--N in the biocrusts. Moreover, Sorkau et al. (2018) demonstrated that 86% of microbial P variation in the forest soils of the same regions can be explained by microbial N while also organic C, soil moisture, and soil type were main drivers for microbial P. A limitation of P is unlikely in ALB, neither in biocrusts nor in

bulk soil, as we could detect high P_{total}, quite high P_{NaHCO3} concentrations, and high potentials for solubilization (*gcd*).

Only in SCH, the abundance of the microbes which drive N and P turnover was always higher in the biocrust compared with the bulk soil samples. Moreover, their abundances were always positively correlated with each other. Probably, the sandy soil and the generally low nutrient stocks in SCH rather reflect a nutrient-poor habitat, where biocrusts are described as nutrient and microbial hotspots (Brankatschk et al. 2013; Hernandez and Knudsen 2012; Kidron et al. 2015; Schulz et al. 2013; Xiao and Veste 2017). While total nutrient stocks were significantly lowest in SCH, the labile nutrient pools were comparable with the other regions or even higher in the biocrusts for P_{H2O}. One explanation might be that the P and N mineralization and subsequent accumulation were driven by the microbial need for C. This phenomenon was already described in other forest ecosystems (Spohn and Kuzyakov 2013) but also in glacier forefields where especially at well-developed sites, microbes were limited by C rather than N or P (Göransson et al. 2011). Consequently, N and P remain in the soil and built up a higher labile nutrient pool. Moreover, the transcription of the ammonium transporter gene *amtB* is under the control of the *Pho* regulon (Santos-Beneit 2015). Thus, under P starvation, also NH_4^+-N uptake is reduced.

In contrast to SCH and ALB, lowest number of correlations were found in HAI as well as lowest labile P concentrations, while exchangeable NH_4^+-N was highest in HAI and even enriched in the biocrusts but less than the ones from SCH. On the one hand, this could point towards different uptake strategies for N and P or on the other hand different mineralization activities. However, as this study is based on DNA analyses, which only reflect the bacterial potential and nutrient pools in the soil, it is impossible to disentangle these strategies. Biocrusts are small micro-habitats, which do not allow to sample large amounts, which is for example needed for additional microbial biomass analyses or RNA sampling, as therefore much more sub-samples per biocrusts need to be analyzed to account for daily fluctuations of gene transcription pattern. However, what was remarkable in bulk soil samples from HAI was the prominent correlation of *pitA*, *chiA*, and *phoD* abundance. This would rather point towards a specific mineralization of either N- or P-containing sources in HAI bulk soil samples, as chitin for example does not contain any P moieties. What was conspicuous in HAI was the high variability of the pH, which ranged from pH 4.13 to 7.22. This might mask a lot of effects, as pH differences are frequently described as main driver for differences in other soil properties, microbial community composition, and functionality (Fierer and Jackson 2006; Rousk et al. 2010; Stempfhuber et al. 2015, 2017). In the frame of the Biodiversity Exploratories, Stempfhuber et al. (2017) demonstrated that pH has a strong impact on the nitrifying community as the ratio of ammonium to ammonia is changed and causes changes in potential activities and the community composition. The lower the pH, the more ammonia-oxidizing archaea prevail, which have a lower turnover rate (Stempfhuber et al. 2017). Thus, especially the extremely low pH values measured in the HAI biocrusts might have caused an accumulation of exchangeable NH_4^+-N.

Conclusion

In conclusion, our data demonstrate that the potential to biogeochemically turnover P is an essential microbial function in biocrusts, as such microbes were more abundant in the biocrusts compared with bulk soil at all investigated regions on the level of absolute and relative abundance. In contrast to our initial hypothesis that low nutrient concentrations cause a strong correlation of N and P turnover, we found most positive correlations in the region with the highest nutrient concentrations but the lowest N/P ratio. Especially, the potential for additional N input by fixation seems to be of importance at sites with low N/P ratios. Potential N mineralization was strongly correlated to P turnover at sites where labile N and P pools were increased. Even though the correlation of N and P turnover is strongest if their availability is comparable, the potential acquisition strategy obviously strongly depends on soil properties. Future studies should confirm the abundance pattern of certain functional microbial groups in subsequent years. However, as biocrusts in temperate regions newly develop every year at distinct places, a direct comparison is often difficult, as (micro)environmental conditions may differ even if the same site is analyzed. Additionally, despite the short life time of biocrusts mainly in temperate forests, also repeated samplings during the succession would be of interest to understand tipping points of different developmental stages. This would need possibilities for a miniaturization of analytical tools to ensure the availability of material for all needed analysis as a result of the small sizes of biocrusts. However, especially, in regions with inconclusive results as in HAI, in-depth microbial community analyses including non-targeted metagenomic approaches might help to disentangle region-specific and biocrust-specific effects. As our data based on measurements on DNA level future work should consider combining microbial activity measurements and actual turnover rates to identify under which conditions the detected potential is accessed.

Acknowledgments We thank the managers of the three Exploratories, Kirsten Reichel-Jung, Iris Steitz, Sandra Weithmann, Florian Straub, Juliane Vogt, and Miriam Teuscher and all former managers for their work in maintaining the plot and project infrastructure; Christiane Fischer for giving support through the central office, Andreas Ostrowski for managing the central data base, and Markus Fischer, Eduard Linsenmair, Dominik Hessenmöller, Daniel Prati, Ingo Schöning, François Buscot, Ernst-Detlef Schulze, Wolfgang W. Weisser, and the late Elisabeth Kalko for their role in setting up the Biodiversity Exploratories project. The work has been funded by the DFG Priority Program 1374 "Infrastructure-Biodiversity-Exploratories"

(KA 899/28-2; SCHL 446/31-2). Field work permits were issued by the responsible state environmental offices of Baden-Württemberg, Thüringen, and Brandenburg. We are very grateful to the Biological Station Zingst (University of Rostock), Laura Fuchs, Juliane Müller, Akane Chiba, Jasmin Danzberger for their help during sampling and in the lab.

Authors' contributions All authors contributed to the study conception and design. Material preparation, data collection, and analysis were performed by Julia Katharina Kurth and Martin Albrecht. The first draft of the manuscript was written by Julia Katharina Kurth and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Funding Open Access funding enabled and organized by Projekt DEAL. The work has been funded by the DFG Priority Program 1374 "Infrastructure-Biodiversity-Exploratories" (KA 899/28-2; SCHL 446/31-2).

Data availability All data generated or analyzed during this study are included in this published article and its supplementary information files. Additionally, datasets are available in the BExIS database of the Biodiversity Exploratories project: ID 26166 (https://www.bexis.uni-jena.de/PublicData/PublicDataSet.aspx?DatasetId=26166) and ID 26206 (https://www.bexis.uni-jena.de/PublicData/PublicDataSet.aspx?DatasetId=26206).

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethics approval Not applicable.

Consent to participate The authors declare that they consent to participate.

Consent for publication The authors declare that they consent for publication.

References

Aciego SM, Riebe CS, Hart SC, Blakowski MA, Carey CJ, Aarons SM, Dove NC, Botthoff JK, Sims KWW, Aronson EL (2017) Dust outpaces bedrock in nutrient supply to montane forest ecosystems. Nat Commun 8:1–10. https://doi.org/10.1038/ncomms14800

Alt F, Oelmann Y, Herold N, Schrumpf M, Wilcke W (2011) Phosphorus partitioning in grassland and forest soils of Germany as related to land-use type, management intensity, and land use-related pH. J Plant Nutr Soil Sci 174:195–209. https://doi.org/10.1002/jpln.201000142

Bach H-J, Hartmann A, Schloter M, Munch JC (2001) PCR primers and functional probes for amplification and detection of bacterial genes for extracellular peptidases in single strains and in soil. J Microbiol Methods 44:173–182. https://doi.org/10.1016/S0167-7012(00)00239-6

Bach H-J, Tomanova J, Schloter M, Munch J (2002) Enumeration of total bacteria and bacteria with genes for proteolytic activity in pure cultures and in environmental samples by quantitative PCR mediated amplification. J Microbiol Methods 49:235–245. https://doi.org/10.1016/S0167-7012(01)00370-0

Bates DM, Pinheiro JC (1998) Linear and nonlinear mixed-effects models. Conf Appl Stat Agric. https://doi.org/10.4148/2475-7772.1273

Baumann K, Glaser K, Mutz JE, Karsten U, MacLennan A, Hu Y, Michalik D, Kruse J, Eckhardt KU, Schall P, Leinweber P (2017) Biological soil crusts of temperate forests: their role in P cycling. Soil Biol Biochem 109:156–166. https://doi.org/10.1016/j.soilbio.2017.02.011

Baumann K, Siebers M, Kruse J, Eckhardt K-U, Hu Y, Michalik D, Siebers N, Kar G, Karsten U, Leinweber P (2019) Biological soil crusts as key player in biogeochemical P cycling during pedogenesis of sandy substrate. Geoderma 338:145–158. https://doi.org/10.1016/J.Geoderma.2018.11.034

Belnap J, Kaltenecker JH, Rosentreter R, Williams J, Leonard S, Eldridge D (2001) Biological soil crusts: ecology and management. United States Department of the Interior, Denver, USA

Beraldi-Campesi H, Hartnett HE, Anbar A, Gordon GW, Garcia-Pichel F (2009) Effect of biological soil crusts on soil elemental concentrations: implications for biogeochemistry and as traceable biosignatures of ancient life on land. Geobiology 7:348–359. https://doi.org/10.1111/j.1472-4669.2009.00204.x

Bergkemper F, Schöler A, Engel M, Lang F, Krüger J, Schloter M, Schulz S (2016a) Phosphorus depletion in forest soils shapes bacterial communities towards phosphorus recycling systems. Environ Microbiol 18:1988–2000. https://doi.org/10.1111/1462-2920.13188

Bergkemper F, Kublik S, Lang F, Krüger J, Vestergaard G, Schloter M, Schulz S (2016b) Novel oligonucleotide primers reveal a high diversity of microbes which drive phosphorous turnover in soil. J Microbiol Methods 125:91–97. https://doi.org/10.1016/J.MIMET.2016.04.011

Berthold M, Zimmer D, Schumann R (2015) A simplified method for total phosphorus digestion with potassium persulphate at subboiling temperatures in different environmental samples. Rostock Meeresbiologische Beitr 25:7–25

Berthold M, Wulff R, Reiff V, Karsten U, Nausch G, Schumann R (2019) Magnitude and influence of atmospheric phosphorus deposition on the southern Baltic Sea coast over 23 years: implications for coastal waters. Environ Sci Eur 31:27. https://doi.org/10.1186/s12302-019-0208-y

Brankatschk R, Fischer T, Veste M, Zeyer J (2013) Succession of N cycling processes in biological soil crusts on a Central European inland dune. FEMS Microbiol Ecol 83:149–160

Cania B, Vestergaard G, Kublik S, Köhne JM, Fischer T, Albert A, Winkler B, Schloter M, Schulz S (2020) Biological soil crusts from different soil substrates harbor distinct bacterial groups with the potential to produce exopolysaccharides and lipopolysaccharides. Microb Ecol 79:326–341. https://doi.org/10.1007/s00248-019-01415-6

Castillo-Monroy AP, Bowker MA, Maestre FT, Rodriguez-Echeverria S, Martinez I, Barraza-Zepeda CE, Escolar C (2011) Relationships between biological soil crusts, bacterial diversity and abundance, and ecosystem functioning: insights from a semi-arid Mediterranean environment. J Veg Sci 22:165–174

Cleveland CC, Liptzin D (2007) C:N:P stoichiometry in soil: is there a "Redfield ratio" for the microbial biomass? Biogeochemistry 85: 235–252. https://doi.org/10.1007/s10533-007-9132-0

Condron LM, Turner BL, Cade-Menun BJ, Sims JT, Sharpley AN (2005) Chemistry and dynamics of soil organic phosphorus. In: Sims T, Sharpley AN, Westermann DT (eds) Phosphorus: agriculture and the environment, vol 46. American Society of Agronomy, Inc. Crop Science Society of America, Inc. Soil Science Society of America, Inc., Madison, pp 87–121

Corbin JD, Thiet RK (2020) Temperate biocrusts: mesic counterparts to their better-known dryland cousins. Front Ecol Environ. https://doi.org/10.1002/fee.2234

Couradeau E, Karaoz U, Lim HC, Nunes da Rocha U, Northen T, Brodie E, Garcia-Pichel F (2016) Bacteria increase arid-land soil surface temperature through the production of sunscreens. Nat Commun 7: 10373

Elbert W, Weber B, Burrows S, Steinkamp J, Büdel B, Andreae MO, Pöschl U (2012) Contribution of cryptogamic covers to the global cycles of carbon and nitrogen. Nat Geosci 5:459–462. https://doi.org/10.1038/ngeo1486

Elser JJ, Bracken MES, Cleland EE, Gruner DS, Harpole WS, Hillebrand H, Ngai JT, Seabloom EW, Shurin JB, Smith JE (2007) Global analysis of nitrogen and phosphorus limitation of primary producers in freshwater, marine and terrestrial ecosystems. Ecol Lett 10:1135–1142. https://doi.org/10.1111/j.1461-0248.2007.01113.x

Epskamp S, Cramer AOJ, Waldorp LJ, Schmittmann VD, Borsboom D (2012) Qgraph: network visualizations of relationships in psychometric data. J Stat Softw 48:1–18

Fierer N, Jackson RB (2006) The diversity and biogeography of soil bacterial communities. Proc Natl Acad Sci U S A 95:6578–6583. https://doi.org/10.1073/pnas.95.12.6578

Fischer M, Bossdorf O, Gockel S, Hänsel F, Hemp A, Hessenmöller D, Korte G, Nieschulze J, Pfeiffer S, Prati D, Renner S, Schöning I, Schumacher U, Wells K, Buscot F, Kalko EKV, Linsenmair KE, Schulze E-D, Weisser WW (2010) Implementing large-scale and long-term functional biodiversity research: the Biodiversity Exploratories. Basic Appl Ecol 11:473–485. https://doi.org/10.1016/J.BAAE.2010.07.009

Gaby JC, Buckley DH (2012) A comprehensive evaluation of PCR primers to amplify the *nifH* gene of Nitrogenase. PLoS One 7: e42149. https://doi.org/10.1371/journal.pone.0042149

Garcia-Pichel F, Johnson SL, Youngkin D, Belnap J (2003) Small-scale vertical distribution of bacterial biomass and diversity in biological soil crusts from arid lands in the Colorado Plateau. Microb Ecol 46: 312–321

Glaser K, Baumann K, Leinweber P, Mikhailyuk T, Karsten U (2018) Algal richness in BSCs in forests under different management intensity with some implications for P cycling. Biogeosciences 15: 4181–4192. https://doi.org/10.5194/bg-15-4181-2018

Göransson H, Olde Venterink H, Bååth E (2011) Soil bacterial growth and nutrient limitation along a chronosequence from a glacier forefield. Soil Biol Biochem 43:1333–1340. https://doi.org/10.1016/J.SOILBIO.2011.03.006

Grafe M, Goers M, von Tucher S, Baum C, Zimmer D, Leinweber P, Vestergaard G, Kublik S, Schloter M, Schulz S (2018) Bacterial potentials for uptake, solubilization and mineralization of extracellular phosphorus in agricultural soils are highly stable under different fertilization regimes. Environ Microbiol Rep 10:320–327. https://doi.org/10.1111/1758-2229.12651

Grüneberg E, Schöning I, Kalko EKV, Weisser WW (2010) Regional organic carbon stock variability: a comparison between depth increments and soil horizons. Geoderma 155:426–433. https://doi.org/10.1016/j.geoderma.2010.01.002

Gypser S, Herppich WB, Fischer T, Lange P, Veste M (2016) Photosynthetic characteristics and their spatial variance on biological soil crusts covering initial soils of post-mining sites in lower Lusatia, NE Germany. Flora Morphol Distrib Funct Ecol Plants 220:103–116. https://doi.org/10.1016/j.flora.2016.02.012

Hansen HP, Koroleff F (1999) Determination of nutrients. In: Grasshoff K, Kremling K, Ehrhardt M (eds) Methods of seawater analysis. Wiley-VCH Verlag GmbH, Weinheim, pp 159–228

Hedley MJ, Stewart JWB, Chauhan BS (1982) Changes in inorganic and organic soil phosphorus fractions induced by cultivation practices and by laboratory incubations1. Soil Sci Soc Am J 46:970–976. https://doi.org/10.2136/sssaj1982.03615995004600050017x

Hernandez RR, Knudsen K (2012) Late-successional biological soil crusts in a biodiversity hotspot: an example of congruency in species richness. Biodivers Conserv 21:1015–1031. https://doi.org/10.1007/s10531-012-0236-z

Heuck C, Spohn M (2016) Carbon, nitrogen and phosphorus net mineralization in organic horizons of temperate forests: stoichiometry and relations to organic matter quality. Biogeochemistry 131:229–242. https://doi.org/10.1007/s10533-016-0276-7

Hoang DTT, Pausch J, Razavi BS, Kuzyakova I, Banfield CC, Kuzyakov Y (2016) Hotspots of microbial activity induced by earthworm burrows, old root channels, and their combination in subsoil. Biol Fertil Soils 52:1105–1119. https://doi.org/10.1007/s00374-016-1148-y

Kidron GJ, Posmanik R, Brunner T, Nejidat A (2015) Spatial abundance of microbial nitrogen-transforming genes and inorganic nitrogen in biocrusts along a transect of an arid sand dune in the Negev Desert. Soil Biol Biochem 83:150–159. https://doi.org/10.1016/J.SOILBIO.2015.01.024

Kuzyakov Y, Blagodatskaya E (2015) Microbial hotspots and hot moments in soil: concept review. Soil Biol Biochem 83:184–199. https://doi.org/10.1016/J.SOILBIO.2015.01.025

Lang F, Krüger J, Amelung W, Willbold S, Frossard E, Bünemann EK, Bauhus J, Nitschke R, Kandeler E, Marhan S, Schulz S, Bergkemper F, Schloter M, Luster J, Guggisberg F, Kaiser K, Mikutta R, Guggenberger G, Polle A, Pena R, Prietzel J, Rodionov A, Talkner U, Meesenburg H, von Wilpert K, Hölscher A, Dietrich HP, Chmara I (2017) Soil phosphorus supply controls P nutrition strategies of beech forest ecosystems in Central Europe. Biogeochemistry 136:5–29. https://doi.org/10.1007/s10533-017-0375-0

Lenth RV (2016) Least-squares means: the R package lsmeans. J Stat Softw. https://doi.org/10.18637/jss.v069.i01

Lindsay WL (1979) Chemical equilibria in soils. Wiley, New York

Lipiec J, Frąc M, Brzezińska M, Turski M, Oszust K (2016) Linking microbial enzymatic activities and functional diversity of soil around earthworm burrows and casts. Front Microbiol 7:1361. https://doi.org/10.3389/fmicb.2016.01361

Maier S, Tamm A, Wu D, Caesar J, Grube M, Weber B (2018) Photoautotrophic organisms control microbial abundance, diversity, and physiology in different types of biological soil crusts. ISME J 12:1032–1046

Mugnai G, Rossi F, Felde VJMNL, Colesie C, Büdel B, Peth S, Kaplan A, De Philippis R (2018) Development of the polysaccharidic matrix in biocrusts induced by a cyanobacterium inoculated in sand microcosms. Biol Fert Soils 54:27–40

Nagy ML, Pérez A, Garcia-Pichel F (2005) The prokaryotic diversity of biological soil crusts in the Sonoran Desert (Organ Pipe Cactus National Monument, AZ). FEMS Microbiol Ecol 54:233–245

R Core Team (2019) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna

Reed SC, Seastedt TR, Mann CM, Suding KN, Townsend AR, Cherwin KL (2007) Phosphorus fertilization stimulates nitrogen fixation and increases inorganic nitrogen concentrations in a restored prairie. Appl Soil Ecol 36:238–242. https://doi.org/10.1016/J.APSOIL.2007.02.002

Reinhold-Hurek B, Bünger W, Burbano CS, Sabale M, Hurek T (2015) Roots shaping their microbiome: global hotspots for microbial

activity. Annu Rev Phytopathol 53:403–424. https://doi.org/10.1146/annurev-phyto-082712-102342

Richardson AE, Simpson RJ (2011) Soil microorganisms mediating phosphorus availability update on microbial phosphorus. Plant Physiol 156:989–996. https://doi.org/10.1104/pp.111.175448

Rodríguez H, Fraga R, Gonzalez T, Bashan Y (2006) Genetics of phosphate solubilization and its potential applications for improving plant growth-promoting bacteria. Plant Soil 287:15–21. https://doi.org/10.1007/s11104-006-9056-9

Rösch C, Mergel A, Bothe H (2002) Biodiversity of denitrifying and dinitrogen-fixing bacteria in an acid forest soil. Appl Environ Microbiol 68:3818–3829. https://doi.org/10.1128/aem.68.8.3818-3829.2002

Rousk J, Bååth E, Brookes PC, Lauber CL, Lozupone C, Caporaso JG, Knight R, Fierer N (2010) Soil bacterial and fungal communities across a pH gradient in an arable soil. ISME J 4:1340–1351. https://doi.org/10.1038/ismej.2010.58

Santos-Beneit F (2015) The pho regulon: a huge regulatory network in bacteria. Front Microbiol 6:402. https://doi.org/10.3389/fmicb.2015.00402

Schaub I, Baum C, Schumann R, Karsten U (2019) Effects of an early successional biological soil crust from a temperate coastal sand dune (NE Germany) on soil elemental stoichiometry and phosphatase activity. Microb Ecol 77:217–229. https://doi.org/10.1007/s00248-018-1220-2

Schulz S, Brankatschk R, Dümig A, Kögel-Knabner I, Schloter M, Zeyer J (2013) The role of microorganisms at different stages of ecosystem development for soil formation. Biogeosciences 10:3983–3996

Schulz K, Mikhailyuk T, Dreßler M, Leinweber P, Karsten U (2016) Biological soil crusts from coastal dunes at the Baltic Sea: cyanobacterial and algal biodiversity and related soil properties. Microb Ecol 71:178–193. https://doi.org/10.1007/s00248-015-0691-7

Sorkau E, Boch S, Boeddinghaus RS, Bonkowski M, Fischer M, Kandeler E, Klaus VH, Kleinebecker T, Marhan S, Müller J, Prati D, Schöning I, Schrumpf M, Weinert J, Oelmann Y (2018) The role of soil chemical properties, land use and plant diversity for microbial phosphorus in forest and grassland soils. J Plant Nutr Soil Sci 181:185–197. https://doi.org/10.1002/jpln.201700082

Spohn M, Kuzyakov Y (2013) Phosphorus mineralization can be driven by microbial need for carbon. Soil Biol Biochem 61:69–75. https://doi.org/10.1016/J.SOILBIO.2013.02.013

Stempfhuber B, Engel M, Fischer D, Neskovic-Prit G, Wubet T, Schöning I, Gubry-Rangin C, Kublik S, Schloter-Hai B, Rattei T, Welzl G, Nicol GW, Schrumpf M, Buscot F, Prosser JI, Schloter M (2015) pH as a driver for ammonia-oxidizing archaea in forest soils. Microb Ecol 69:879–883. https://doi.org/10.1007/s00248-014-0548-5

Stempfhuber B, Richter-Heitmann T, Bienek L, Schöning I, Schrumpf M, Friedrich M, Schulz S, Schloter M (2017) Soil pH and plant diversity drive co-occurrence patterns of ammonia and nitrite oxidizer in soils from forest ecosystems. Biol Fertil Soils 53:691–700. https://doi.org/10.1007/s00374-017-1215-z

Szyja M, Büdel B, Colesie C (2018) Ecophysiological characterization of early successional biological soil crusts in heavily human-impacted areas. Biogeosciences 15:1919–1931. https://doi.org/10.5194/bg-15-1919-2018

Töwe S, Wallisch S, Bannert A, Fischer D, Hai B, Haesler F, Kleineidam K, Schloter M (2011) Improved protocol for the simultaneous extraction and column-based separation of DNA and RNA from different soils. J Microbiol Methods 84:406–412. https://doi.org/10.1016/J.MIMET.2010.12.028

Uksa M, Schloter M, Endesfelder D, Kublik S, Engel M, Kautz T, Köpke U, Fischer D (2015) Prokaryotes in subsoil—evidence for a strong spatial separation of different phyla by analysing co-occurrence networks. Front Microbiol 6:1269. https://doi.org/10.3389/fmicb.2015.01269

Vuko M, Cania B, Vogel C, Kublik S, Schloter M, Schulz S (2020) Shifts in reclamation management strategies shape the role of exopolysaccharide and lipopolysaccharide-producing bacteria during soil formation. Microb Biotechnol 13:584–598. https://doi.org/10.1111/1751-7915.13532

Walker TW, Syers JK (1976) The fate of phosphorus during pedogenesis. Geoderma 15:1–19. https://doi.org/10.1016/0016-7061(76)90066-5

Wanner BL (1993) Gene regulation by phosphate in enteric bacteria. J Cell Biochem 51:47–54. https://doi.org/10.1002/jcb.240510110

Wickham H (2016) ggplot2 elegant graphics for data analysis. Springer, New York

Williams L, Loewen-Schneider K, Maier S, Büdel B (2016) Cyanobacterial diversity of western European biological soil crusts along a latitudinal gradient. FEMS Microbiol Ecol 92:fiw157. https://doi.org/10.1093/femsec/fiw157

Willsky GR, Malamy MH (1980) Characterization of two genetically separable inorganic phosphate transport systems in Escherichia coli. J Bacteriol 144:356–365

Xiao B, Veste M (2017) Moss-dominated biocrusts increase soil microbial abundance and community diversity and improve soil fertility in semi-arid climates on the Loess Plateau of China. Appl Soil Ecol 117-118:165–177. https://doi.org/10.1016/j.apsoil.2017.05.005

Xiao X, Yin X, Lin J, Sun L, You Z, Wang P, Wang F (2005) Chitinase genes in lake sediments of Ardley Island, Antarctica. Appl Environ Microbiol 71:7904–7909. https://doi.org/10.1128/AEM.71.12.7904-7909.2005

Zehr JP, Jenkins BD, Short SM, Steward GF (2003) Nitrogenase gene diversity and microbial community structure: a cross-system comparison. Environ Microbiol 5:539–554. https://doi.org/10.1046/j.1462-2920.2003.00451.x