

RESEARCH ARTICLE

Linking ecology and systematics of acidobacteria: Distinct habitat preferences of the *Acidobacteriia* and *Blastocatellia* in tundra soils

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Abstract

The *Acidobacteria* is one of the major bacterial phyla in soils and peatlands. The currently explored diversity within this phylum is assigned to 15 class-level units, five of which contain described members. The ecologically relevant traits of acidobacteria from different classes remain poorly understood. Here, we compared the patterns of acidobacterial diversity in sandy soils of tundra, along a gradient of increasing vegetation—unfixed aeolian sand, semi-fixed surfaces with mosses and lichens, and mature soil under fully developed plant cover. The *Acidobacteria*-affiliated 16S rRNA gene sequences retrieved from these soils comprised 11 to 33% of total bacterial reads and belonged mostly to members of the classes *Acidobacteriia* and *Blastocatellia*, which displayed opposite habitat preferences. The relative abundance of the *Blastocatellia* was maximal in unfixed sands and declined in soils of vegetated plots, showing positive correlation with soil pH and negative correlation with carbon and nitrogen availability. An opposite tendency was characteristic for the *Acidobacteriia*. Most *Blastocatellia*-affiliated reads belonged to as-yet-undescribed members of the family *Arenimicrobiaceae*, which appears to be characteristic for dry, depleted in organic matter soil habitats. The pool of *Acidobacteriia*-affiliated sequences, apart from *Acidobacteriaceae*- and *Bryobacteraceae*-related reads, had a large proportion of sequences from as-yet-undescribed families, which seem to specialize in degrading plant-derived organic matter. This analysis reveals sandy soils of tundra as a source of novel acidobacterial diversity and provides an insight into the ecological preferences of different taxonomic groups within this phylum.

Introduction

The *Acidobacteria* is one of the most abundant and highly diverse bacterial phyla in soils and peatlands [1–6]. The proportion of *Acidobacteria*-affiliated 16S rRNA gene reads in sequence

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pools retrieved from various soil habitats ranges between 5 and 50% of the total bacterial community [3,7–10]. Our knowledge of the roles of acidobacteria in soils includes decomposition of various biopolymers and participation in the global cycling of carbon, iron and hydrogen, but this list of functional capabilities remains far from being complete and is attributed to several sub-groups of this phylum only.

The currently explored diversity within the *Acidobacteria* is commonly addressed as corresponding to 26 major 16S rRNA gene sequence clades or subdivisions (SD) [11]. Recently, these 26 subdivisions were assigned to 15 class-level units, five of which contain described members [12]. These include three earlier established classes *Acidobacteriia*, *Blastocatellia* and *Holophagae* [13–15] as well as two recently proposed classes *Vicinamibacteria* and *Thermoanaerobaculia* [12]. The phylogenetic range of the class *Acidobacteriia* accommodates 16S rRNA gene sequences from several SDs including 1, 2, 3, 5, 11, 12, 13, 14, 15, and 24. The classes *Blastocatellia*, *Vicinamibacteria* and *Thermoanaerobaculia* correspond to one subdivision each, i.e. SDs 4, 6, and 23, while the class *Holophagae* includes SDs 8 and 22. In soil, acidobacterial SDs 1, 2, 3, 4 and 6 are the most abundant ones [8]. This is true for a wide range of ecosystem types, including boreal and tropical forests, grasslands and pastures, as well as arid landscapes [3–5, 8–10]. The abundances of subdivisions 1, 2 and 3 show negative correlation with soil pH, while the opposite tendency is characteristic for subdivisions 4 and 6 [8]. Peatlands are also among the preferred habitats of *Acidobacteria*. Acidic *Sphagnum*-dominated boreal peatlands are colonized mainly by members of SDs 1 and 3 [16–18]. Subarctic peatlands, in addition to SDs 1 and 3, may contain a relatively high proportion of SD2 *Acidobacteria* [19,20].

Despite their wide distribution in various soils, acidobacteria remain strongly underrepresented in culture collections due to difficulties in their cultivation and laboratory maintenance [6]. Much of the currently described diversity (ca. 15 genera) belong to the class *Acidobacteriia*, which includes the orders *Acidobacteriales* and *Bryobacteriales* (SDs 1 and 3), and accommodates acidophilic or acidotolerant, mesophilic and psychrotolerant, chemoheterotrophic bacteria that utilize various sugars and polysaccharides and possess a number of hydrolytic capabilities [12]. The class *Blastocatellia* contains 7 genera of aerobic, mesophilic or thermophilic, chemo-heterotrophic bacteria that specialize on degradation of complex proteinaceous compounds and one genus of microaerophilic thermophilic anoxygenic photoheterotrophs, *Chloracidobacterium* [21]. Other classes of *Acidobacteria* contain only a limited number of characterized representatives and, therefore, their ecologically relevant traits remain poorly understood.

In the course of our recent study of prokaryotic community succession following sand fixation and soil formation in the tundra zone, *Acidobacteria* were identified as one of the most abundant bacterial groups, which was present along a whole gradient of increasing vegetation, from unfixed aeolian sands to mature soils under fully developed plant cover [22]. Since the phenotype of bacteria that colonize dry and nutrient-poor sands is clearly different from that of bacteria in organic-rich soils under plant cover, the acidobacterial populations in these sites with contrasting characteristics should have been quite distinct with regard to their lifestyles and environmental adaptations. The analysis presented here was undertaken in order to verify this hypothesis and to examine diversity patterns of the *Acidobacteria* along a gradient of increasing vegetation in tundra.

Materials and methods

Sampling sites

The 16S rRNA gene sequence dataset retrieved by Zhelezova et al. [22] and deposited with GenBank under the Bioproject accession number PRJNA497067 was used for the analysis.

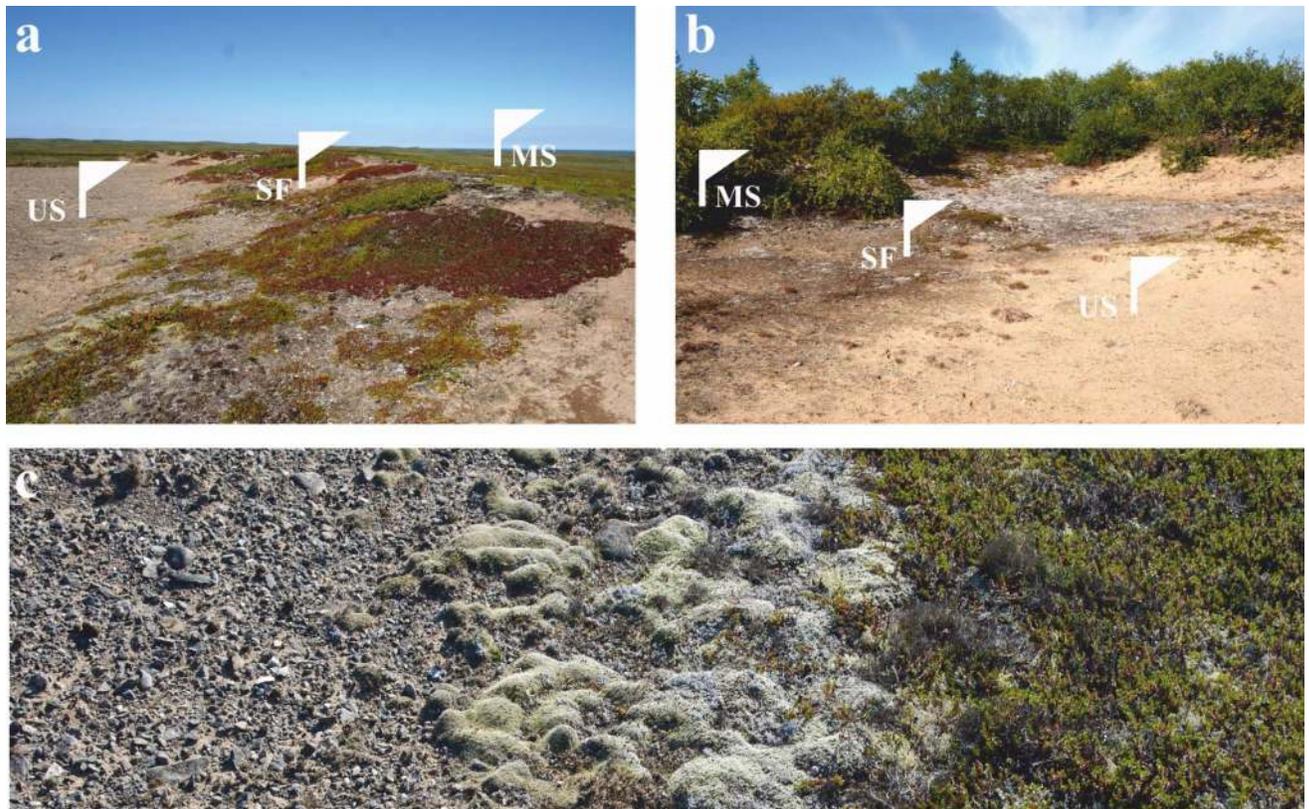


Fig 1. Two sampling sites examined in the study. (a) flat sand hills near Nelmin Nos (site I) and (b) small sand dunes near Naryan-Mar (site II). White flags indicate different types of plots along a gradient of increasing vegetation: US—unfixed sand, SF—semi-fixed surface, MS—mature soil. (c) Example of vegetation gradient from sand with gravel to cover of lichens and mosses (left to right) in site I near Nelmin Nos.

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Two chronosequences of soil formation on aeolian sands with similar initial stages and different mature vegetation (typical tundra and wooded tundra) corresponded to the two sampling sites located on the shores of the Pechora River (Northwestern Russia, Nenetsia region). The two sites differed in the plant cover that developed on mature soil, i.e. typical tundra vegetation with subshrubs (Site I) and wooded tundra with rare trees and subshrubs (Site II). These sampling sites are shown in Fig 1 and the characteristics of soil samples used for the analysis are given in Table 1. Detailed description of the sampling sites are reported elsewhere [22]. No specific permits were required for sampling at these tundra sites. These locations are not privately-owned or protected in any way and are also not parts of national parks or reserves. Our sampling did not involve endangered or protected species.

Table 1. Locations of sampling sites and characteristics of sampled substrates (values are shown as means ($n = 2$ for TOC and TN, $n = 5$ for pH) \pm standard deviations).

Sampling sites	Surface type	Moisture, %	TOC, %	TN, %	pH
I—Nelmin Nos, 67°58'34.3"N, 52°55'19.9"E	US	3.10	0.06 \pm 0.00	0.03 \pm 0.00	6.27 \pm 0.27
	SF	2.61	0.15 \pm 0.02	0.04 \pm 0.00	5.60 \pm 0.29
	MS	27.86	1.71 \pm 0.06	0.12 \pm 0.01	5.37 \pm 0.22
II—Naryan-Mar, 67°36'23.2"N, 53°08'12.2"E	US	0.39	below detection limit	0.02 \pm 0.00	6.12 \pm 0.10
	SF	0.82	0.18 \pm 0.00	0.04 \pm 0.00	5.66 \pm 0.41
	MS	5.72	1.67 \pm 0.26	0.09 \pm 0.01	4.77 \pm 0.25

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For both sites, sampling was performed on three types of surfaces: 1- unfixated aeolian sand (US), 2- semi-fixed surface with mosses and lichens (SF), and 3—mature soil under developed plant cover (MS) (Fig 1, Table 1). Sampling plots of different types were located on a transect with 3–5 m between each plot. For every surface type on each site, five soil samples (~50 g of soil free of plants, mosses and lichens) were taken from depths of 1–5 cm. For molecular analyses, samples were stored at -70 °C. The total organic carbon (TOC) and total nitrogen (TN) contents were determined for the pooled sample of the five replicates taken for the molecular analysis from each plot using a Vario MACRO Cube CN-analyzer (Elementar Analysensysteme GmbH, Germany) [22]. pH was measured in soil suspension in distilled water in the ratio of 1:2.5.

Analysis of SSU rRNA gene sequences

The sequences retrieved by Zhelezova et al. [22] were represented by the fragments of V3–V4 regions of bacterial and archaeal 16S rRNA genes. This pool of sequences was reanalyzed with QIIME 2 v.2018.8 (<https://qiime2.org>) [23]. DADA2 plugin was used for sequence quality control, merging of paired-end reads and chimera filtering [24]. Since the number of reads obtained from two replicates of MS plot in site I was too low (below 5,000 sequences), the comparative analysis of all three plots in site I was performed based on three replicates.

Operational Taxonomic Units (OTUs) were clustered applying VSEARCH plugin [25] with open-reference function using db Silva132 [26,27] with 97% identity. Taxonomy assignment was performed using BLAST against db Silva132 with 97% identity. 16S rRNA sequences affiliated with the *Acidobacteria* were extracted and used for further detailed analyses. The alpha-diversity indices were calculated using the core-metrics-phylogenetic method implemented in QIIME 2 v.2018.8. Phylogenetic analysis was carried out using the ARB program package [28] (version 6.0.3) and the sequence alignment based on dbSilva132. Short sequences were added to the existing tree using the quick ARB parsimony insertion tool. The network diagram was constructed using Gephi [29].

Statistical analyses

Statistical evaluations were made with GraphPad Prism (v. 7.0) applying multiple t-tests. Corrections for multiple comparisons were made using Holm-Sidak method. The significance level alpha was set at 0.05. Pearson correlation test was performed to check correlations between soil chemical properties and sequence abundances of different acidobacterial groups.

Results

Characteristics of sampled substrates along a gradient of increasing vegetation

The sampling plots corresponding to the three successional stages of soil formation in tundra—unfixated aeolian sand, semi-fixed surfaces with mosses and lichens, and mature soil under fully developed plant cover—differed from each other with regard to various physico-chemical parameters (Table 1). Most pronounced differences were observed with regard to moisture and total organic carbon contents. Unfixated and semi-fixed sands were extremely dry, while soils under plant cover contained more water. The content of organic carbon was nearly non-measurable in unfixated sands and increased gradually with formation of vegetation cover.

Community composition of the *Acidobacteria*

A total of 1,019,619 partial 16S rRNA gene sequences (mean amplicon length 260 bp) were retrieved from the examined soil samples (Table 2). Of these, 463,507 sequences were retained after merging of paired-end reads, quality filtering, removing chimeras and singletons. The pool of *Acidobacteria*-affiliated reads included 74037 sequences, which accounted for 9–31% of all bacterial reads in different samples. Overall, the acidobacterial alpha-diversity in unfixed aeolian sands was slightly lower than that in soils of vegetated plots (see mean values of Shannon indexes in Table 2) but this difference was not statistically significant.

As revealed by principle coordinate analyses, the acidobacterial communities corresponding to the three successional stages of soil formation in tundra were also clearly distinct (Fig 2).

The *Acidobacteria*-affiliated sequences retrieved from the two tundra sites belonged to members of the classes *Acidobacteriia* (53–100% of all acidobacterial reads in different samples), *Blastocatellia* (0–34%), and *Holophagae* (0–13%) (Fig 3). The proportion of sequences that could not be assigned to these classes was in the range of 0–6% in unfixed sands and 0–1% in mature soils under plant cover. The pool of sequences from the *Acidobacteriia* was composed of reads from representatives of the orders *Acidobacteriales* (SD1) and *Bryobacterales* (SD3) as well as SD2 acidobacteria, which comprise an as-yet-undescribed order of this class. Notably, a large proportion of *Acidobacteriales*-affiliated sequences (6–37% of all

Table 2. Sequencing statistics and various alpha-diversity metrics.

Sampling site	Sample ID	Raw reads	Filtered reads*	<i>Acidobacteria</i> reads	<i>Acidobacteria</i> /Filtered reads (%)	Diversity indices		
						Shannon	Observed OTUs	Pielou's evenness
SI (Nelming Nos)	US	26888	11429	2392	21	4.12 ±0.09	24	0.88
		48969	21871	4509	21		29	0.85
		39219	19133	3658	19		31	0.84
	SF	43054	21171	3302	16	4.81 ±0.16	40	0.93
		34967	16873	3026	18		37	0.89
		36727	17525	4368	25		44	0.89
	MS	40752	17570	3991	23	4.57 ±0.03	40	0.92
		28471	11381	1910	17		24	0.92
		30914	12816	3168	25		37	0.89
SII (Naryan-Mar)	US	35900	17014	1585	9	4.08 ±0.26	26	0.87
		43734	22605	2278	10		31	0.85
		83916	36714	3559	10		30	0.89
		37253	16094	1599	10		19	0.86
		42662	15079	1615	11		26	0.86
	SF	51797	22265	2963	13	4.34 ±0.07	24	0.95
		52038	23126	3344	14		30	0.86
		51165	23019	3379	15		32	0.86
		59837	26503	3848	15		34	0.86
		66002	29843	3221	11		29	0.91
	MS	45105	24362	3721	15	4.44 ±0.65	40	0.92
		36054	16322	3039	19		23	0.87
		22707	11672	3606	31		59	0.91
		32450	14527	3218	22		23	0.88
		29109	14593	2738	19		24	0.88

*Filtered reads: number of merged paired-end sequences excluding low quality reads, singletons and chimeras.

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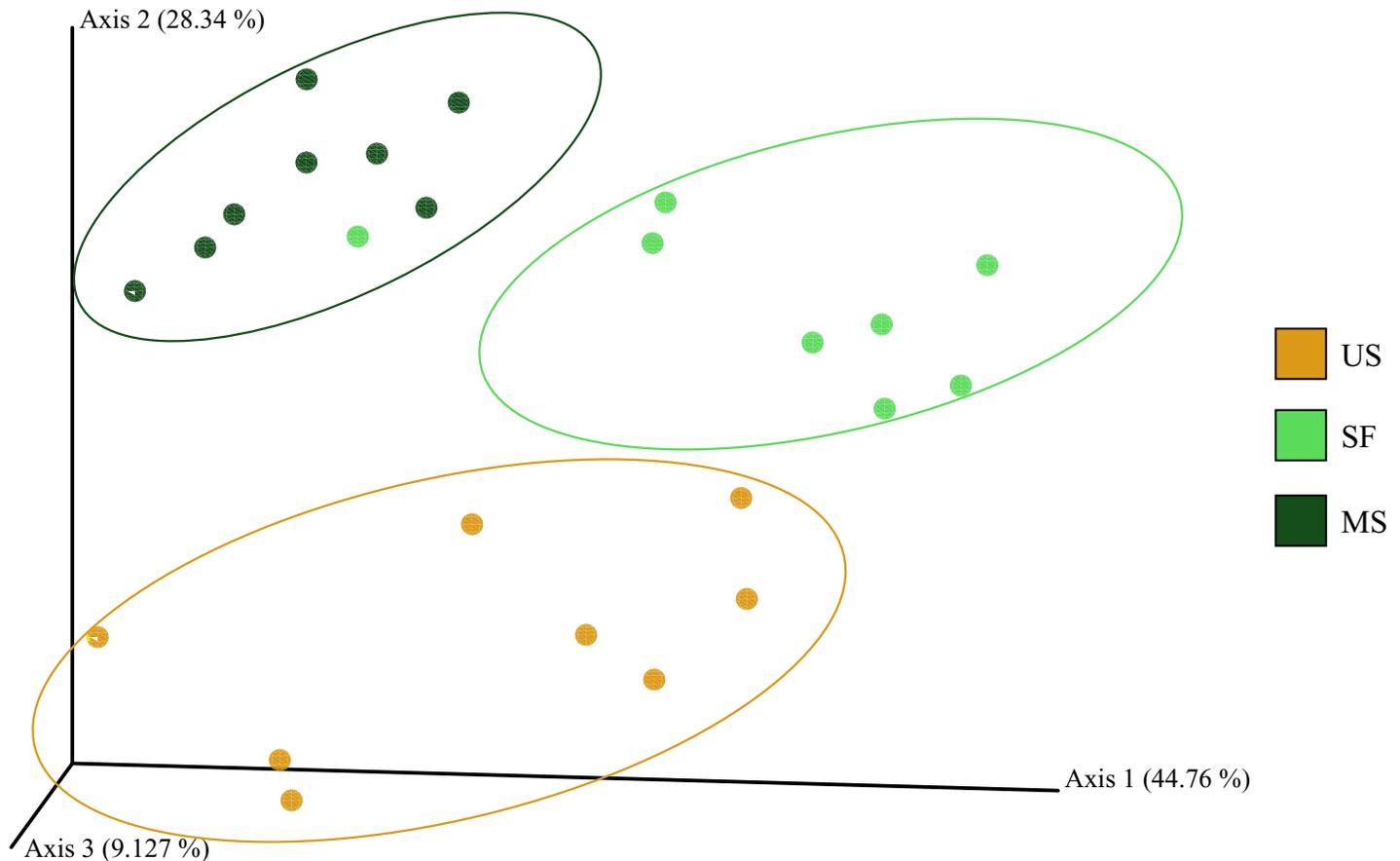


Fig 2. Comparison of the *Acidobacteria* community composition in samples examined in this study by principle coordinate analyses (PCoA). PCoA plot is based on the weighted UniFrac distance of the sequencing dataset.

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acidobacterial reads in different samples) could not be assigned to the only currently described family of this order, i.e. *Acidobacteriaceae*. This group of sequences is further addressed as ‘uncultured *Acidobacteriales*’ in accordance with the taxonomic classification used in db Silva132 and indicated by black hatching in Fig 3.

Taxonomy-based analysis revealed a clear shift in the *Acidobacteria* community composition along a gradient of increasing vegetation in both study sites (Fig 3). The most characteristic feature of acidobacterial communities in unfixed sands was a prominent presence of the *Blastocatellia* (SD4), which comprised $31\pm 2\%$ and $14\pm 5\%$ of all *Acidobacteria*-affiliated reads obtained from sites I and II, respectively (S1 Table). The relative abundance of these bacteria declined dramatically in vegetated soils of both sites, down to $7\pm 4\%$ of reads in mature soils from the site I and a complete disappearance in mature soils from site II. The same trend was observed for members of the *Holophagae*, which accounted for $12\pm 1\%$ and $7\pm 3\%$ of all acidobacterial reads in unfixed sands from sites I and II, respectively, but were not detected in mature soils (Fig 3). The opposite trend was detected for members of the *Acidobacteriaceae* and SD2, which were either absent (*Acidobacteriaceae* in site I) or present in a low abundance in unfixed sands ($13\pm 5\%$ for *Acidobacteriaceae* in site I and 4–7% for SD2 in both sites) but became major community members (with relative abundances of approximately 20–30%) in vegetated mature soils (S1 Table). The *Bryobacteriales*-affiliated acidobacteria (SD3) displayed highest relative abundances in semi-fixed soils, accounting for $36\pm 9\%$ and $50\pm 4\%$ of all

acidobacterial reads in sites I and II, respectively. No clear trend could be observed for the uncultured group within the order *Acidobacteriales* (Fig 3).

Most abundant OTUs of *Acidobacteria* and their distribution patterns

Using 97% sequence identity, a total of 232 acidobacterial OTUs were identified in all studied samples. Of these, 157 OTUs were detected in the site I and 159 OTUs were identified in the site II (Table 2); 80 OTUs were shared between sites I and II (S1 Fig). The numbers of OTUs shared between the two sites within each of the succession stages were 23, 24 and 42 for US, SF and MS plots, respectively (S1 Fig).

Our further, detailed analysis was focused on five major taxonomic groups within the *Acidobacteria*. Two of these groups, *Blastocatellia* (SD4) and *Acidobacteriaceae* (SD1), were chosen because they displayed two clearly opposite trends along a gradient of increasing vegetation in tundra soils. These groups of *Acidobacteria* contain a number of cultured and characterized representatives, thus offering a possibility of analyzing specific reasons behind their environmental distribution. Two other groups, SD2 and uncultured *Acidobacteriales*, do not contain described representatives and were chosen, therefore, in order to find out their eco-niche preferences. One additional group that was included in the analysis, *Holophagae* (SD8), contains only few cultured representatives; the physiology and functional capabilities of these acidobacteria remain poorly understood. The OTUs of these bacteria comprising $\geq 1\%$ of all *Acidobacteria*-affiliated reads in at least one of the examined sites are listed in Table 3 and are displayed in S2 Fig.

Although the same taxonomic representation of *Acidobacteria* at the order and family levels was observed for both sites, diversity of OTUs identified in the sites I and II was clearly different (S2 Fig). For example, *Blastocatellia*-affiliated OTUs 1–3, which were among the most abundant OTUs in site I, were absent from site II. Vice versa, the most abundant *Blastocatellia*-affiliated OTU in site II, OTU 7, was not detected in site I. Other groups of *Acidobacteria* in the two sites were also represented by different OTUs, which may be due to some differences between the sites I and II, such as the plant cover composition and the moisture level (Table 1).

The most abundant OTUs of the *Blastocatellia* (OTUs 1–3 and 7–9) belonged to the as-yet-uncultivated clade RB41 within the family *Pyrinomonadaceae* and occurred mainly in unfixed sands in both tundra sites. Only three OTUs were classified at the genus level as representing the genera *Blastocatella* (OTUs 11 and 12) and *Stenotrophobacter* (OTU 5).

By contrast, nearly all of the *Acidobacteriaceae*-affiliated OTUs could be classified at the genus level and represented the genera *Granulicella* (OTUs 24–28, 30, 33, 34), *Acidipila* (OTUs 23, 32) and *Bryocella* (OTU 29). The most abundant OTU from the *Acidobacteriaceae* (No 31), however, could not be assigned to any of the described genera and displayed 96.0–96.5% similarity with 16S rRNA gene sequences from '*Acidisarcina*'/*Acidipila* group.

The most abundant SD2-affiliated OTUs (No 13–15) displayed high similarity with environmental 16S rRNA gene sequences retrieved from soils or sediments of various northern locations (GenBank numbers FJ004757, KM200371, FJ625349). These were detected mainly in mature soils, although OTU 13 was also highly abundant in semi-fixed sand from site I.

Finally, OTUs comprising the group of uncultured *Acidobacteriales* were present in all examined samples but varied with regard to their identity and relative abundance. Thus, OTUs 38 and 48 were abundant in unfixed sands but were not detected in semi-fixed sands and soils under plant cover. By contrast, OTU 39 was highly representative in semi-fixed sands but was also present in other samples. Environmental 16S rRNA gene sequences representing this group of acidobacteria were retrieved mainly from various grasslands and forest soils but

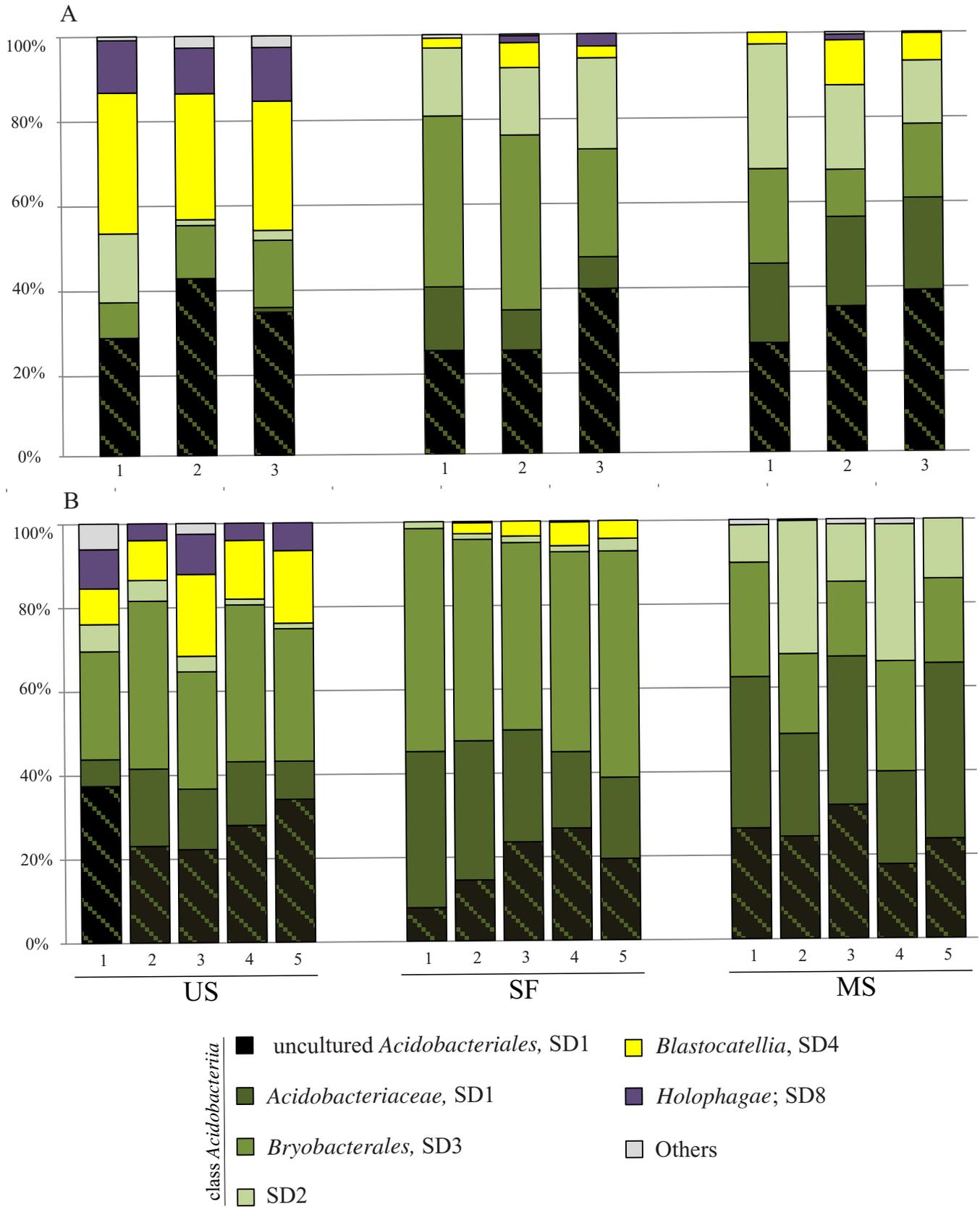


Fig 3. Community composition of the *Acidobacteria* along a gradient of increasing vegetation—unfixed aeolian sand, semi-fixed surfaces with mosses and lichens, and mature soil under fully developed plant cover—based on 16S rRNA gene sequence analysis. A—site I, B—site II. The taxonomic analysis was performed according to dbSilva 132. The values of the relative abundance of different acidobacterial groups in individual samples are given in [S1 Table](#). Significant differences in relative abundances of particular acidobacterial groups between unfixed sand and different stages of soil formation were revealed for the *Acidobacteriaceae* (P -value < 0.01), *Bryobacteriales* (P -value < 0.001) and *Blastocatellia* (P -value < 0.001) ([S2 Table](#)).

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also from microbial mats of lava tube walls, orthoquartzite caves, uranium and potassium mines ([Table 3](#)).

Major trends of changes in the *Acidobacteria* community composition in sandy soils of tundra along the gradient of increasing vegetation are seen in the network diagram illustrating the most abundant OTU distribution between the three examined types of plots ([Fig 4](#)). An apparent shift from the community composed of the *Blastocatellia* and uncultured *Acidobacteriales* in aeolian sands to the community dominated by various subgroups of the *Acidobacteriia* in mature soils is observed in this diagram.

Specific phylogenetic sub-groups of *Acidobacteria* revealed in tundra soils

A large group of OTUs affiliated with the class *Blastocatellia*, which were identified mostly in unfixed sands (OTUs 1–4, 6–10), were classified as belonging to the as-yet-uncultivated clade RB41 within the family *Pyrinomonadaceae* ([Table 3](#)). This group was named after the environmental clone sequence RB41 (GenBank accession No Z95722), which was retrieved from the Roggenstein field site soil near Munich (Germany) in the seminal work of Ludwig et al. [1]. Detailed phylogenetic analysis of these sequences revealed that they form a distinct phylogenetic group, which clusters separately from the other described families of the order *Blastocatellales*, i.e. *Blastocatellaceae*, *Arenimicrobiaceae* and *Pyrinomonadaceae* ([Fig 5](#)). These sequences displayed 91–92% similarity to 16S rRNA gene sequences from members of the genera *Brevitalea* and *Arenimicrobium*, while their sequence similarity to other described members of the *Blastocatellia* was in the range of 86–89%.

Another large group of numerically abundant OTUs identified in tundra soils affiliated with the order *Acidobacteriales* but did not belong to the only currently described family *Acidobacteriaceae* ([Fig 6](#)). In db Silva 132, this group is addressed as ‘uncultured *Acidobacteriales*’, which is not fully correct since it comprises a number of isolates named ‘Ellin’ (Ellin7137, 7522, 6547, 6528, 6527, 5106, 323 and others). These isolates were obtained by Janssen and co-workers [30–33]; none of them, however, was characterized, so that no information about these bacteria is currently available. 16S rRNA gene sequence similarity of these bacteria to described members of the family *Acidobacteriaceae* is below 91%.

Correlations between chemical soil properties and the abundances of different acidobacterial groups

To reveal the influence of several chemical soil properties (pH, C and N contents) on the relative abundances of different acidobacterial groups, Pearson correlation test was performed. The positive correlation was revealed between pH and the relative abundances of the *Holophagae* (SD8) ($R = 0.82$, $p = 0.05$) and *Blastocatellia* (SD4) ($R = 0.80$, $p = 0.06$). The highest relative abundance of these groups was detected in the acidobacterial communities of unfixed sands in both sites. The negative correlation was revealed between pH and the relative abundances of the *Acidobacteriaceae* (SD1) ($R = -0.83$, $p = 0.04$) and SD2 ($R = -0.73$, $p = 0.1$), which tended to dominate in mature soils. These correlations could be explained by the gradual decrease of pH during the process of soil formation. No significant correlations, however, were revealed between C and N contents and the abundance of particular acidobacterial groups ([Table 4](#)).

Table 3. The most abundant operational taxonomic units (OTUs) of *Acidobacteria* detected in tundra soils.

No OTU	Silvadb match	Taxonomy	Reported habitat	Similarity (%)
1	EU132342	SD4, <i>Pyrinomonadaceae</i> RB41	soil from an undisturbed mixed grass prairie preserve USA	97.7
2	EF019176	SD4, <i>Pyrinomonadaceae</i> RB41	trembling aspen rhizosphere USA	98.0
3	HQ645212	SD4, <i>Pyrinomonadaceae</i> RB41	soil samples from meadow in the Tibet Plateau China	98.4
4	Z95722	SD4, <i>Pyrinomonadaceae</i> RB41	soil sample Germany	99.2
5	EU132039	SD4, <i>Stenotrophobacter</i>	soil from an undisturbed mixed grass prairie preserve USA	96.9
6	EF494321	SD4, <i>Pyrinomonadaceae</i> RB41	River granitic landscape Australia	97.3
7	EU150230	SD4, <i>Pyrinomonadaceae</i> RB41	dry meadow soil USA	97.3
8	JN615840	SD4, <i>Pyrinomonadaceae</i> RB41	yellow microbial mat from lava cave wall Portugal	96.1
9	AB294343	SD4, <i>Pyrinomonadaceae</i> RB41	stream Japan	97.7
10	EU132394	SD4, <i>Pyrinomonadaceae</i> RB41	soil from an undisturbed mixed grass prairie preserve USA	99.2
11	JN020220	SD4, <i>Blastocatella</i>	Chernobyl concrete microbial biofilm Ukraine	100.0
12	LC026845	SD4, <i>Blastocatella</i>	dust particles China	96.1
13	FJ004757	SD2, uncultured	bulk soil Netherlands	99.2
14	KM200371	SD2, uncultured	Tobacco rhizospheric soil China	98.8
15	FJ625349	SD2, uncultured	boreal pine forest soil Finland	96.5
16	EF516082	SD2, uncultured	grassland soil USA	98.4
17	EU150221	SD2, uncultured	Soil from spruce fir forest USA	98.0
18	DQ450697	SD2, uncultured	saturated alpine tundra wet meadow soil USA	99.2
19	KJ623626	SD2, uncultured	volcanic ice cave sediments Antarctica	99.6
20	EF019283	SD2, uncultured	trembling aspen rhizosphere USA	98.0
21	AB821147	SD2, uncultured	forest soil South Korea	98.4
22	Y11632	SD1, uncultured	zinc-polluted soil Belgium	99.6
23	HQ598413	SD1, <i>Acidipila</i>	woodland soil Germany	98.8
24	JN023390	SD1, <i>Granulicella</i>	temperate highland grassland Mexico	95.3
25	JN023710	SD1, <i>Granulicella</i>	temperate highland grassland Mexico	98.0
26	FR667798	SD1, <i>Granulicella</i>	iron snow from acidic coal mining-associated Lake Germany	100.0
27	JN023799	SD1, <i>Granulicella</i>	temperate highland grassland Mexico	97.6
28	JN023530	SD1, <i>Granulicella</i>	temperate highland grassland Mexico	99.6
29	JN023102	SD1, <i>Bryocella</i>	temperate highland grassland Mexico	98.0
30	GU731314	SD1, <i>Granulicella</i>	soil sample with arsenic Germany	97.6
31	HQ674949	SD1, ' <i>Acidisarcina</i> '	weathered feldspar mineral China	98.8
32	FJ625317	SD1, <i>Acidipila</i>	boreal pine forest soil Finland	99.6
33	FPLS01053045	SD1, <i>Granulicella</i>	unknown	97.2
34	JN023174	SD1, <i>Granulicella</i>	temperate highland grassland	96.9
35	FPLL01007473	SD1, uncultured	peat soil Japan	100.0
36	JN023389	SD1, uncultured	temperate highland grassland Mexico	96.1
37	AB364756	SD1, uncultured	peat soil Japan	97.6
38	EF018888	<i>Acidobacteriales</i> , uncultured	trembling aspen rhizosphere USA	96.9
39	HM445289	<i>Acidobacteriales</i> , uncultured	microbial mat from lava tube walls Portugal	98.4
40	HQ598756	<i>Acidobacteriales</i> , uncultured	woodland soil Germany	97.2
41	AB364808	<i>Acidobacteriales</i> , uncultured	peat soil Japan	97.6
42	FJ004744	<i>Acidobacteriales</i> , uncultured	bulk soil Netherlands	99.6
43	AJ536862	<i>Acidobacteriales</i> , uncultured	uranium mining waste pile Germany	98.8
44	HM445280	<i>Acidobacteriales</i> , uncultured	microbial mat from lava tube walls Portugal	97.2
45	AY963371	<i>Acidobacteriales</i> , uncultured	soil China	98.8
46	EF516179	<i>Acidobacteriales</i> , uncultured	grassland soil USA	98.4
47	EF516150	<i>Acidobacteriales</i> , uncultured	grassland soil USA	97.7

(Continued)

Table 3. (Continued)

No OTU	Silvadb match	Taxonomy	Reported habitat	Similarity (%)
48	JF833567	<i>Acidobacteriales</i> , uncultured	potassium mine soil China	98.4
49	HM062461	<i>Acidobacteriales</i> , uncultured	soil USA	97.2
50	EF516275	<i>Acidobacteriales</i> , uncultured	grassland soil USA	95.7
51	HQ598546	<i>Acidobacteriales</i> , uncultured	woodland soil Germany	94.5
52	EF018794	<i>Acidobacteriales</i> , uncultured	trembling aspen rhizosphere USA	99.2
53	GU205282	<i>Acidobacteriales</i> , uncultured	sediment from orthoquartzite cave Venezuela	95.3
54	HQ598572	<i>Acidobacteriales</i> , uncultured	woodland soil Germany	97.6
55	HQ118387	<i>Acidobacteriales</i> , uncultured	loamy soil of Eucalyptus forest USA	98.0
56	FJ004707	<i>Acidobacteriales</i> , uncultured	rhizosphere <i>Lotus corniculatus</i> Netherlands	99.6
57	HQ598769	<i>Acidobacteriales</i> , uncultured	woodland soil Germany	97.6
58	JN023645	<i>Acidobacteriales</i> , uncultured	temperate highland grassland Mexico	96.9
59	KJ410541	<i>Acidobacteriales</i> , uncultured	<i>Pinus massoniana</i> soil China	99.2
60	FJ624925	<i>Acidobacteriales</i> , uncultured	boreal pine forest soil Finland	99.2
61	EU132294	<i>Holophagae</i> , uncultured	prairie grass soil USA	96.5
62	JX114379	<i>Holophagae</i> , uncultured	rhizosphere soil Spain	95.3
63	EF018757	<i>Holophagae</i> , uncultured	trembling aspen rhizosphere USA	95.7
64	EF516932	<i>Holophagae</i> , uncultured	grassland soil USA	94.9
65	EF018864	<i>Holophagae</i> , uncultured	trembling aspen rhizosphere USA	98.0
66	KJ081622	<i>Holophagae</i> , uncultured	copper contaminated soil China	95.7
67	JF428950	<i>Holophagae</i> , uncultured	rhizosphere soil China	96.5
68	EU132431	<i>Holophagae</i> , uncultured	prairie grass soil USA	96.5

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Discussion

All previously available knowledge of *Acidobacteria* diversity in the tundra zone refers to the ecosystems with developed plant communities, i.e. tundra heaths with a mosaic vegetation of dwarf shrubs and alpine grasses [19,34], wetlands with mixed cover of lichens and mosses [20] or forested tundra with lichen cover [35]. All of these studies report SDs 1, 2 and 3 (belonging to the class *Acidobacteriia*) as the major groups of tundra-inhabiting *Acidobacteria*. The results obtained in our study for vegetated plots (Fig 3) are in agreement with the previous reports. The search for information on *Acidobacteria* diversity in aeolian sand dunes and the corresponding chronosequence of soil formation on sands in the tundra zone, however, yielded no results. As revealed in our study, sandy soils of tundra are characterized by a prominent presence of the *Blastocatellia* (SD4), which may comprise up to one third of the acidobacterial community. This finding was somewhat unexpected because none of the currently described members of the *Blastocatellia* were obtained from cold environments. Thermophilic representatives of this class, *Pyrinomonas methylaliphatogenes* (family *Pyrinomonadaceae*) and *Chloracidobacterium thermophilum* (family '*Chloracidobacteriaceae*'), were isolated from a hot spring and a thermal soil, respectively [36,37]. All other described representatives, i.e. members of the genera *Blastocatellia*, *Aridibacter*, *Tellurimicrobium*, *Stenotrophobacter* (family *Blastocatellaceae*) and the genera *Arenimicrobium*, *Brevitalea* (family *Arenimicrobiaceae*), were obtained from Namibian semiarid savanna soils [14,38,39]. These bacteria were characterized as possessing wide growth temperature ranges (from 8–11°C to 45–52°C) and, yet, their growth optima were recorded at 28–45°C. The 16S rRNA gene sequences retrieved from aeolian sands of tundra affiliated only with the families *Arenimicrobiaceae* and *Blastocatellaceae*. Most of these sequences displayed low similarity (below 91–92%) to those from described members of these families, suggesting that the phenotypes of these bacteria are also different from those of

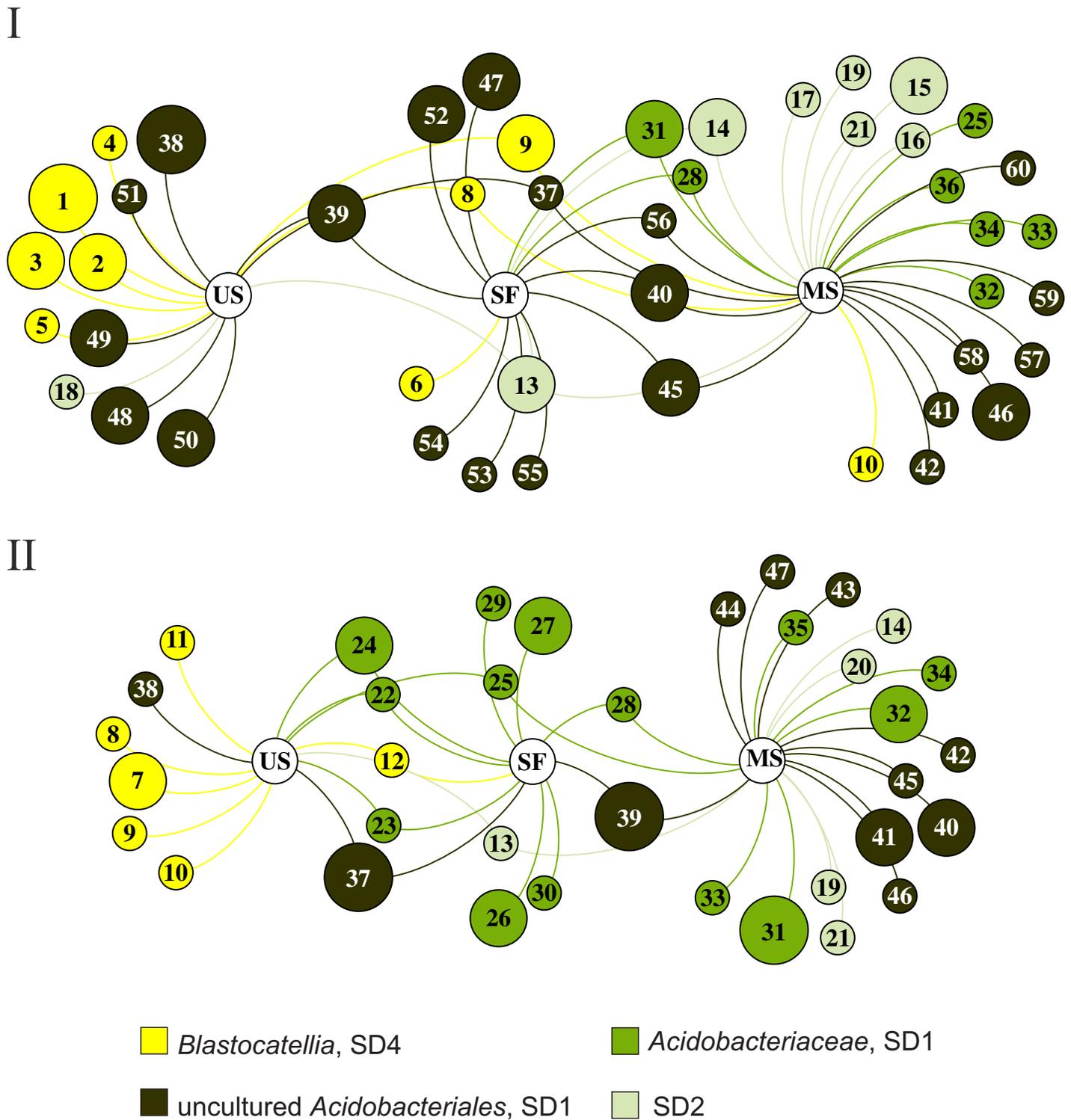


Fig 4. Network diagram illustrating the most abundant OTU distribution between unfixed aeolian sand, semi-fixed surfaces with mosses and lichens, and mature soil under fully developed plant cover. The size of the OTU nodes is weighted according to the relative abundance of the particular OTU. The diagram was constructed using *gephi* [29].

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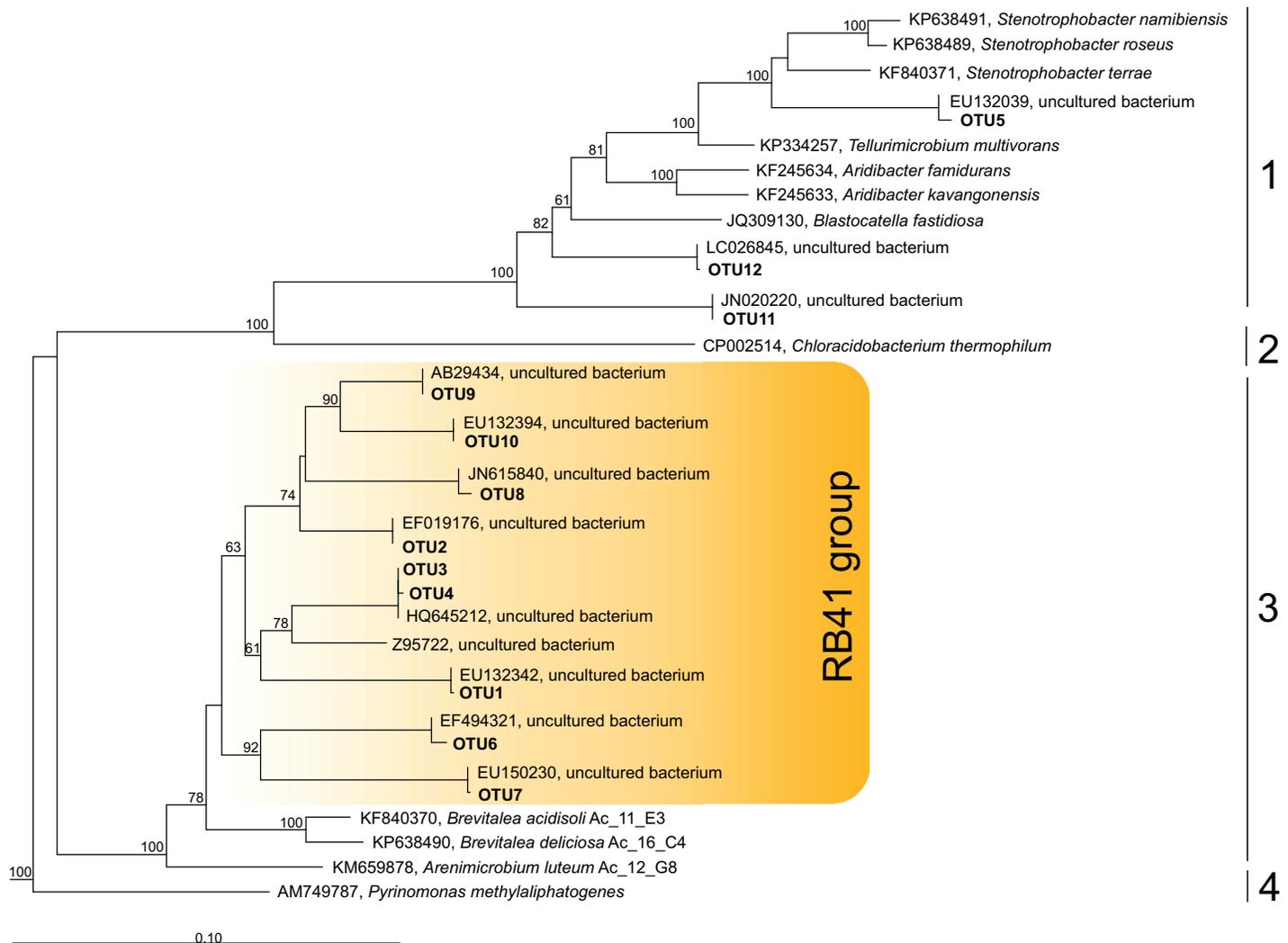


Fig 5. Maximum parsimony tree showing the phylogenetic position of the most abundant acidobacterial OTUs from the *Blastocatellia* in relation to closest relatives of described species and/or environmental 16S rRNA gene sequences. Various families within the *Blastocatellia* are indicated as follows: 1—*Blastocatellaceae*, 2—*Chloracidobacteriaceae*, 3—*Arenimicrobiaceae*, 4—*Pyrinomonadaceae*. Bootstrap values are derived from 1000 pseudoreplicates. An outgroup was composed of three 16S rRNA gene sequence from members of the *Holophagae*, *Holophaga foetida* (X77215) and two related environmental sequences (FQ658676 and FQ659446). The scale bar indicates 10% estimated sequence divergence.

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acidobacteria from Namibian savanna soils. One particular group of sequences characteristic for sands of tundra, which were classified by using dbSilva 132 as ‘uncultured *Pyrinomonadaceae* RB41’ (Table 3), formed a common clade with 16S rRNA gene sequences from the *Arenimicrobium* and *Brevitalea* (Fig 5), suggesting that these sequences should be addressed as affiliating with the family *Arenimicrobiaceae*. Final conclusions about the taxonomic position of these bacteria, however, should await their isolation and characterization. Future efforts in culturing these bacteria could possibly benefit from the evidence for their ability to survive drought, nutrient limitation and low temperatures (as suggested by Wüst et al. [40] and our study).

The trend observed for members of the family *Acidobacteriaceae* (Table 4) agrees well with our current knowledge of their preference for low pH and high availability of plant-derived organic matter [4,6,8,10]. Notably, a large group of OTUs in semi-fixed sands or mature soils

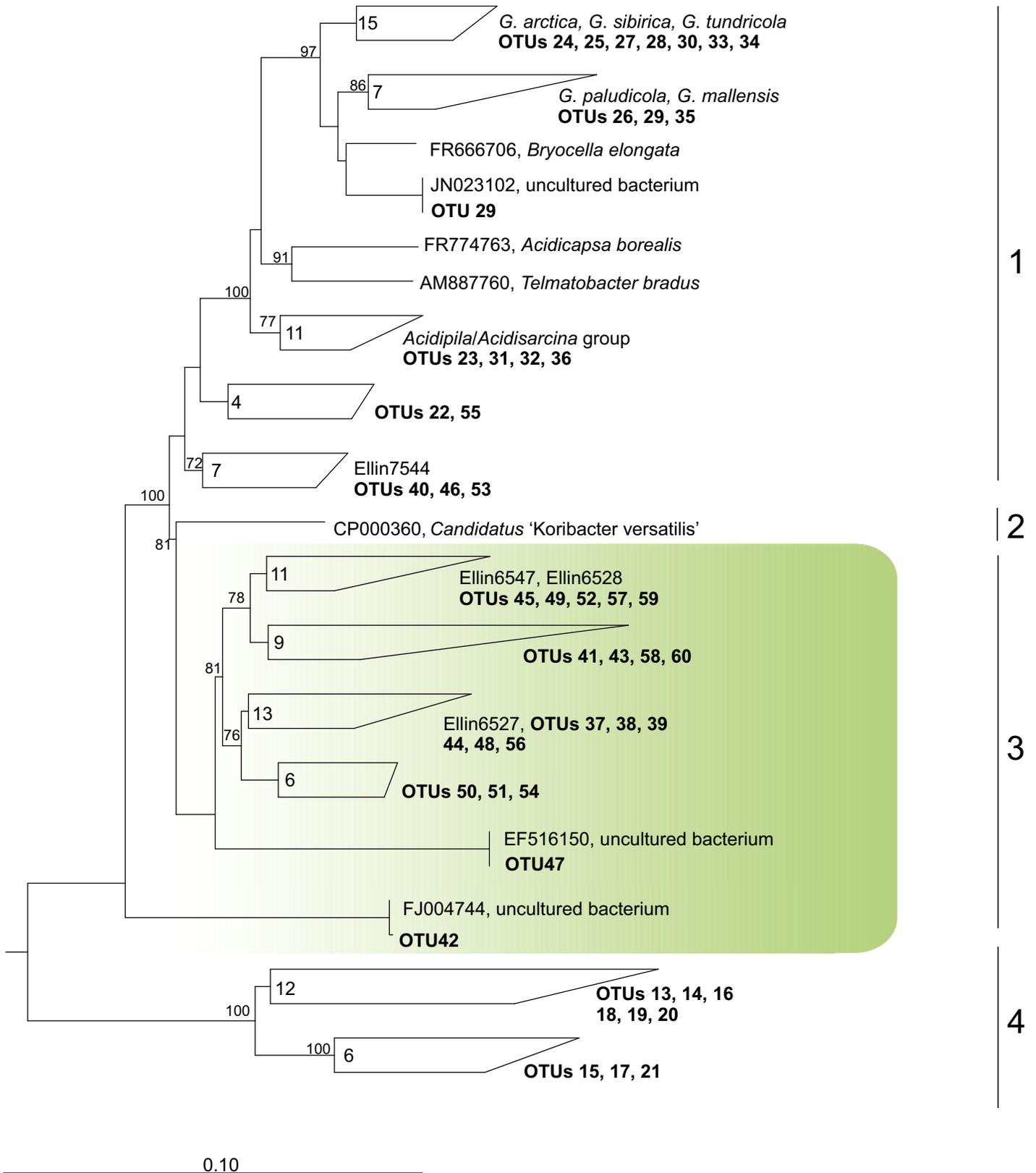


Fig 6. Maximum parsimony tree showing the phylogenetic position of the most abundant acidobacterial sequences from the *Acidobacteriia* in relation to closest relatives of described species and/or environmental 16S rRNA gene sequences. Various families and groups within the *Acidobacteriia* are indicated as follows: 1—*Acidobacteriaceae*, 2—‘*Koribacteraceae*’, 3—uncultured *Acidobacteriales*, 4—SD2. Bootstrap values are derived from 1000 pseudoreplicates. An outgroup was composed of three 16S rRNA gene sequence from members of the *Holophagae*, *Holophaga foetida* (X77215) and two related environmental sequences (FQ658676 and FQ659446). The scale bar indicates 10% estimated sequence divergence.

<https://doi.org/10.1371/journal.pone.0230157.g006>

were represented by *Granulicella* species (OTUs 24–28, 30, 33, 34). These bacteria were isolated from tundra soils or northern boreal wetlands [40–42] and are commonly associated with mosses and lichens. Good tolerance of low temperatures, wide repertoires of carbohydrate-active enzymes encoded in their genomes and pronounced hydrolytic capabilities explain wide distribution of *Granulicella* species in tundra habitats [42–45]. The OTU31, which was shared between SF and MS plots in site I and was highly abundant in MS plots of site II, is represented by ‘*Acidisarcina*’-like bacteria, which were also isolated from lichen-dominated tundra soils and displayed chitinolytic and xylanolytic capabilities [35].

A notable group of OTUs in unfixed sands was represented by *Holophagae*-affiliated 16S rRNA gene sequences (OTUs 61–68). This finding was somewhat unexpected because these acidobacteria are commonly not abundant in soils [9]. At present, the class *Holophagae* contains only four described representatives [12]. Three of these acidobacteria are strict anaerobes and none of them was obtained from soils. 16S rRNA gene sequences retrieved in our study from upper, aerobic layers of sandy tundra soils were only distantly related (83–86% sequence similarity) to those of described *Holophagae* members. Most likely, these sequences belong to aerobic, psychrotolerant bacteria with as-yet-unknown functional potential.

An insight into community changes of the *Acidobacteriia* may also provide valuable information regarding some as-yet-uncultivated sub-groups within this class. One example is SD2 *Acidobacteria* which, by now, does not contain characterized representatives. Similar to members of the *Acidobacteriaceae*, the relative abundance of SD2 *Acidobacteria* was negatively correlated with pH and positively correlated with organic carbon content (Table 4). However, their correlation with nitrogen availability was more strongly pronounced than that of *Acidobacteriaceae*, thus suggesting the need for increasing nitrogen content in cultivation media. Another numerically abundant group of *Acidobacteriia*-affiliated 16S rRNA gene sequences, which were retrieved from all examined samples and addressed as ‘uncultured *Acidobacteriales*’, was classified as belonging to an as-yet-undescribed family (Fig 6). Phylogenetic diversity within this group is relatively wide and no clear correlation was observed with any of the examined parameters (Table 4). As noticed above, several representatives of this group (isolates named Ellin7137, 7522, 6547, 6528, 6527, 5106, 323) were earlier obtained and reported as being represented by extremely slow-growing bacteria that formed mini-colonies (with

Table 4. Correlations between pH, organic carbon, nitrogen and the number of sequences from different groups of *Acidobacteria*.

Acidobacterial group	pH	C	N
<i>Acidobacteriaceae</i> (SD1)	-0.83*	0.63	0.56
<i>Acidobacteriales</i> uncultured (SD1)	0.37	0.05	0.11
<i>Bryobacterales</i> (SD3)	0.02	-0.45	-0.42
SD2	-0.73	0.79	0.80
<i>Blastocatellia</i> (SD4)	0.80	-0.47	-0.45
SD8	0.82	-0.56	-0.59
Other	0.64	-0.39	-0.49

*Statistically significant values (P-value confidence level < 0.05) are indicated by bold.

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diameters of 25–200 μm) after long (> 12 weeks) periods of incubation [33]. It is hardly surprising that none of these isolates were characterized and taxonomically described. The occurrence of these slow-growing and presumably oligotrophic bacteria in aeolian sands of tundra appears to be logical based on their phenotype. Cultivation and characterization of these microorganisms represents a challenge for further taxonomic studies on *Acidobacteria*.

Conclusions

In summary, our analysis revealed two clearly distinct profiles of ‘ecological fitness’ of the *Acidobacteriia* and *Blastocatellia*. The latter, in particular members of the family *Arenimicrobiaceae*, appear to be characteristic for dry, depleted in organic matter sandy soils. An opposite habitat preference was demonstrated by the *Acidobacteriia*, which seem to specialize in degrading plant-derived organic matter and, therefore, become a major group of acidobacteria in soils under fully developed plant cover. This linkage between the taxonomic affiliation and the potential functional capabilities could help interpreting the results of molecular diversity surveys and navigate our efforts in obtaining representatives of as-yet-uncultivated groups of soil *Acidobacteria*.

Supporting information

S1 Table. Relative abundance of different acidobacterial groups.

(PDF)

S2 Table. Statistical values calculated by multiple t-test comparisons for acidobacterial groups from Sites 1 and 2.

(PDF)

S1 Fig. Venn diagrams showing the number of shared and unique OTUs in Sites 1 and 2, and in three types of successional stages examined in this study.

(PDF)

S2 Fig. Heat map showing the relative abundances of well-represented acidobacterial OTUs.

(PDF)

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References

1. Ludwig W, Bauer SH, Bauer M, Held I, Kirchhof G, Schulze R, et al. Detection and *in situ* identification of representatives of a widely distributed new bacterial phylum. *FEMS Microbiol Lett.* 1997; 153: 181–190. <https://doi.org/10.1111/j.1574-6968.1997.tb10480.x> PMID: 9252585
2. Hugenholtz P, Pitulle C, Hershberger KL, Pace NR. Novel division level bacterial diversity in a Yellowstone hot spring. *J Bacteriol.* 1998; 180: 366–76. Available: <http://www.ncbi.nlm.nih.gov/pubmed/9440526> PMID: 9440526
3. Janssen PH. Identifying the dominant soil bacterial taxa in libraries of 16S rRNA and 16S rRNA genes. *Applied and Environmental Microbiology.* 2006. pp. 1719–1728. <https://doi.org/10.1128/AEM.72.3.1719-1728.2006> PMID: 16517615
4. Kielak AM, Barreto CC, Kowalchuk GA, van Veen JA, Kuramae EE. The ecology of *Acidobacteria*: moving beyond genes and genomes. *Frontiers in Microbiology.* 2016. p. 744. <https://doi.org/10.3389/fmicb.2016.00744> PMID: 27303369
5. Eichorst SA, Trojan D, Roux S, Herbold C, Rattei T, Woebken D. Genomic insights into the *Acidobacteria* reveal strategies for their success in terrestrial environments. *Environ Microbiol.* 2018; 20: 1041–1063. <https://doi.org/10.1111/1462-2920.14043> PMID: 29327410
6. Dedysh SN, Sinninghe Damsté JS. *Acidobacteria*. eLS. Chichester, UK: John Wiley & Sons, Ltd; 2018. pp. 1–10. <https://doi.org/10.1002/9780470015902.a0027685>
7. Lee SH, Ka JO, Cho JC. Members of the phylum *Acidobacteria* are dominant and metabolically active in rhizosphere soil. *FEMS Microbiol Lett.* 2008; 285: 263–269. <https://doi.org/10.1111/j.1574-6968.2008.01232.x> PMID: 18557943
8. Jones RT, Robeson MS, Lauber CL, Hamady M, Knight R, Fierer N. A comprehensive survey of soil acidobacterial diversity using pyrosequencing and clone library analyses. *ISME J.* 2009; 3: 442–453. <https://doi.org/10.1038/ismej.2008.127> PMID: 19129864
9. Lauber CL, Hamady M, Knight R, Fierer N. Pyrosequencing-based assessment of soil pH as a predictor of soil bacterial community structure at the continental scale. *Appl Environ Microbiol.* 2009; 75: 5111–5120. <https://doi.org/10.1128/AEM.00335-09> PMID: 19502440
10. Foessel BU, Nägele V, Naether A, Wüst PK, Weinert J, Bonkowski M, et al. Determinants of *Acidobacteria* activity inferred from the relative abundances of 16S rRNA transcripts in German grassland and forest soils. *Environ Microbiol.* 2014; 16: 658–675. <https://doi.org/10.1111/1462-2920.12162> PMID: 23802854
11. Barns SM, Cain EC, Sommerville L, Kuske CR. Acidobacteria phylum sequences in uranium-contaminated subsurface sediments greatly expand the known diversity within the phylum. *Appl Environ Microbiol.* 2007; 73: 3113–6. <https://doi.org/10.1128/AEM.02012-06> PMID: 17337544
12. Dedysh SN, Yilmaz P. Refining the taxonomic structure of the phylum Acidobacteria. *Int J Syst Evol Microbiol.* 2018; 3796–3806. <https://doi.org/10.1099/ijsem.0.003062> PMID: 30325293
13. Thrash J, Coates J. Class I. Acidobacteria Cavalier-Smith 2002, 12VP. In: Krieg NR, Staley JT, Brown DR, Hedlund BPPNW, editor. *Bergey's Manual of Systematic Bacteriology*, 2nd edn, vol 4. Springer, New York; 2011. p. 727.
14. Pascual J, Wüst PK, Geppert A, Foessel BU, Huber KJ, Overmann J. Novel isolates double the number of chemotrophic species and allow the first description of higher taxa in Acidobacteria subdivision 4. *Syst Appl Microbiol.* 2015; 38: 534–544. <https://doi.org/10.1016/j.syapm.2015.08.001> PMID: 26460220
15. Thrash J, Coates J. Class II. Holophagae classis nov. *Bergey's Manual of Systematic Bacteriology*, 2nd edn, vol 4. 2011. p. 731.
16. Dedysh SN. Cultivating uncultured bacteria from northern wetlands: knowledge gained and remaining gaps. *Front Microbiol.* 2011; 2: 184. <https://doi.org/10.3389/fmicb.2011.00184> PMID: 21954394
17. Pankratov TA, Ivanova AO, Dedysh SN, Liesack W. Bacterial populations and environmental factors controlling cellulose degradation in an acidic Sphagnum peat. *Environ Microbiol.* 2011; 13: 1800–1814. <https://doi.org/10.1111/j.1462-2920.2011.02491.x> PMID: 21564458
18. Serkebaeva YM, Kim Y, Liesack W, Dedysh SN. Pyrosequencing-based assessment of the bacteria diversity in surface and subsurface peat layers of a northern wetland, with focus on poorly studied phyla and candidate divisions. *PLoS One.* 2013; 8: e63994. <https://doi.org/10.1371/journal.pone.0063994> PMID: 23700443
19. Männistö MK, Kurhela E, Tirola M, Häggblom MM. Acidobacteria dominate the active bacterial communities of Arctic tundra with widely divergent winter-time snow accumulation and soil temperatures. *FEMS Microbiol Ecol.* 2013; 84: 47–59. <https://doi.org/10.1111/1574-6941.12035> PMID: 23106413

20. Danilova O V., Belova SE, Gagarinova I V., Dedysh SN. Microbial community composition and methanotroph diversity of a subarctic wetland in Russia. *Microbiology*. 2016; 85: 583–591. <https://doi.org/10.1134/S0026261716050039>
21. Huber KJ, Pascual J, Foesel BU, Overmann J. *Blastocatellia*. *Bergey's Manual of Systematics of Archaea and Bacteria*. 2017. <https://doi.org/10.1002/9781118960608.cbm00060>
22. Zhelezova A, Chernov T, Tkachkova A, Xenofontova N, Semenov M, Kutovaya O. Prokaryotic community shifts during soil formation on sands in the tundra zone. Esposito A, editor. *PLoS One*. Public Library of Science; 2019; 14: e0206777. <https://doi.org/10.1371/journal.pone.0206777> PMID: [30939175](https://pubmed.ncbi.nlm.nih.gov/30939175/)
23. Caporaso J, Kuczynski J, Stombaugh J. QIIME allows analysis of high-throughput community sequencing data. *Nat Methods*. Nature Publishing Group; 2010; 7: 335–336. <https://doi.org/10.1038/nmeth0510-335>
24. Callahan BJ, McMurdie PJ, Rosen MJ, Han AW, Johnson AJA, Holmes SP. DADA2: High-resolution sample inference from Illumina amplicon data. *Nat Methods*. 2016; 13: 581–583. <https://doi.org/10.1038/nmeth.3869> PMID: [27214047](https://pubmed.ncbi.nlm.nih.gov/27214047/)
25. Rognes T, Flouri T, Nichols B, Quince C, Mahé F. VSEARCH: a versatile open source tool for metagenomics. *PeerJ*. 2016; 4: e2584. <https://doi.org/10.7717/peerj.2584> PMID: [27781170](https://pubmed.ncbi.nlm.nih.gov/27781170/)
26. Quast C, Pruesse E, Yilmaz P, Gerken J, Schweer T, Yarza P, et al. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. *Nucleic Acids Res*. 2013; 41: D590–D596. <https://doi.org/10.1093/nar/gks1219> PMID: [23193283](https://pubmed.ncbi.nlm.nih.gov/23193283/)
27. Glöckner FO, Yilmaz P, Quast C, Gerken J, Beccati A, Ciuprina A, et al. 25 years of serving the community with ribosomal RNA gene reference databases and tools. *J Biotechnol*. 2017; 261: 169–176. <https://doi.org/10.1016/j.jbiotec.2017.06.1198> PMID: [28648396](https://pubmed.ncbi.nlm.nih.gov/28648396/)
28. Ludwig W, Strunk O, Westram R, Richter L, Meier H, Yadhukumar A, et al. ARB: a software environment for sequence data. *Nucleic Acids Res*. 2004; 32: 1363–1371. <https://doi.org/10.1093/nar/gkh293> PMID: [14985472](https://pubmed.ncbi.nlm.nih.gov/14985472/)
29. Bastian M, Heymann S, Jacomy M. Gephi: an open source software for exploring and manipulating networks. *Third International AAAI Conference on Weblogs and Social Media*. 2009. pp. 361–362. <https://doi.org/10.1136/qshc.2004.010033>
30. Sait M, Hugenholtz P, Janssen PH. Cultivation of globally distributed soil bacteria from phylogenetic lineages previously only detected in cultivation-independent surveys. *Environ Microbiol*. 2002; 4: 654–666. <https://doi.org/10.1046/j.1462-2920.2002.00352.x> PMID: [12460273](https://pubmed.ncbi.nlm.nih.gov/12460273/)
31. Joseph SJ, Hugenholtz P, Sangwan P, Osborne CA, Janssen PH. Laboratory cultivation of widespread and previously uncultured soil bacteria. *Appl Environ Microbiol*. 2003; 69: 7210–7215. <https://doi.org/10.1128/AEM.69.12.7210-7215.2003> PMID: [14660368](https://pubmed.ncbi.nlm.nih.gov/14660368/)
32. Davis KER, Joseph SJ, Janssen PH. Effects of growth medium, inoculum size, and incubation time on culturability and isolation of soil bacteria. *Appl Environ Microbiol*. 2005; 71: 826–834. <https://doi.org/10.1128/AEM.71.2.826-834.2005> PMID: [15691937](https://pubmed.ncbi.nlm.nih.gov/15691937/)
33. Davis KER, Sangwan P, Janssen PH. Acidobacteria, Rubrobacteridae and Chloroflexi are abundant among very slow-growing and mini-colony-forming soil bacteria. *Environ Microbiol*. 2011; 13: 798–805. <https://doi.org/10.1111/j.1462-2920.2010.02384.x> PMID: [21108723](https://pubmed.ncbi.nlm.nih.gov/21108723/)
34. Männistö MK, Tirola M, Häggblom MM. Bacterial communities in Arctic fields of Finnish Lapland are stable but highly pH-dependent. *FEMS Microbiology Ecology*. 2007. pp. 452–465. <https://doi.org/10.1111/j.1574-6941.2006.00232.x> PMID: [17328122](https://pubmed.ncbi.nlm.nih.gov/17328122/)
35. Belova SE, Ravin N V., Pankratov TA, Rakitin AL, Ivanova AA, Beletsky A V., et al. Hydrolytic capabilities as a key to environmental success: chitinolytic and cellulolytic Acidobacteria from acidic sub-arctic soils and boreal peatlands. *Front Microbiol*. 2018; 9: 1–14. <https://doi.org/10.3389/fmicb.2018.00001>
36. Crowe MA, Power JF, Morgan XC, Dunfield PF, Lagutin K, Rijpstra WIC, et al. *Pyrinomonas methylaliphatogenes* gen. nov., sp. nov., a novel group 4 thermophilic member of the phylum Acidobacteria from geothermal soils. *Int J Syst Evol Microbiol*. 2014; 64: 220–227. <https://doi.org/10.1099/ijs.0.055079-0> PMID: [24048862](https://pubmed.ncbi.nlm.nih.gov/24048862/)
37. Tank M, Bryant DA. *Chloracidobacterium thermophilum* gen. nov., sp. nov.: an anoxygenic microaerophilic chlorophotoheterotrophic acidobacterium. *Int J Syst Evol Microbiol*. 2015; 65: 1426–1430. <https://doi.org/10.1099/ijs.0.000113> PMID: [25667398](https://pubmed.ncbi.nlm.nih.gov/25667398/)
38. Foesel BU, Rohde M, Overmann J. *Blastocatella fastidiosa* gen. nov., sp. nov., isolated from semiarid savanna soil—the first described species of Acidobacteria Subdivision 4. *Syst Appl Microbiol*. 2013; 36: 82–89. <https://doi.org/10.1016/j.syapm.2012.11.002> PMID: [23266188](https://pubmed.ncbi.nlm.nih.gov/23266188/)
39. Wüst PK, Foesel BU, Geppert A, Huber KJ, Luckner M, Wanner G, et al. *Brevitalea aridisoli*, *B. deliciosa* and *Arenimicrobium luteum*, three novel species of Acidobacteria subdivision 4 (class Blastocatellia)

- isolated from savanna soil and description of the novel family Pyrinomonadaceae. *Int J Syst Evol Microbiol.* 2016; 66: 3355–3366. <https://doi.org/10.1099/ijsem.0.001199> PMID: 27255677
40. Pankratov TA, Dedysh SN. *Granulicella paludicola* gen. nov., sp. nov., *Granulicella pectinivorans* sp. nov., *Granulicella aggregans* sp. nov. and *Granulicella rosea* sp. nov., acidophilic, polymer-degrading acidobacteria from Sphagnum peat bogs. *Int J Syst Evol Microbiol.* 2010; 60: 2951–9. <https://doi.org/10.1099/ijse.0.021824-0> PMID: 20118293
 41. Männistö MK, Rawat S, Starovoytov V, Häggblom MM. *Granulicella arctica* sp. nov., *granulicella mallensis* sp. nov., *granulicella tundricola* sp. nov. and *granulicella sapmiensis* sp. nov., novel acidobacteria from tundra soil. *Int J Syst Evol Microbiol.* 2012; 62: 2097–2106. <https://doi.org/10.1099/ijse.0.031864-0> PMID: 22058325
 42. Oshkin IY, Kulichevskaya IS, Rijkstra WIC, Sinninghe Damsté JS, Rakitin AL, Ravin N V., et al. *Granulicella sibirica* sp. nov., a psychrotolerant acidobacterium isolated from an organic soil layer in forested tundra, West Siberia. *Int J Syst Evol Microbiol. Microbiology Society;* 2019; 69: 1195–1201. <https://doi.org/10.1099/ijsem.0.003290> PMID: 30775959
 43. Rawat SR, Männistö MK, Bromberg Y, Häggblom MM. Comparative genomic and physiological analysis provides insights into the role of *Acidobacteria* in organic carbon utilization in Arctic tundra soils. *FEMS Microbiol Ecol.* 2012; 82: 341–355. <https://doi.org/10.1111/j.1574-6941.2012.01381.x> PMID: 22486608
 44. Rawat SR, Männistö MK, Starovoytov V, Goodwin L, Nolan M, Hauser LJ, et al. Complete genome sequence of *Granulicella mallensis* type strain MP5ACTX8T, an acidobacterium from tundra soil. *Stand Genomic Sci.* 2013; 9: 71–82. <https://doi.org/10.4056/sigs.4328031> PMID: 24501646
 45. Rawat SR, Männistö MK, Starovoytov V, Goodwin L, Nolan M, Hauser L, et al. Complete genome sequence of *Granulicella tundricola* type strain MP5ACTX9T, an Acidobacteria from tundra soil. *Stand Genomic Sci.* 2014; 9: 449–461. <https://doi.org/10.4056/sigs.4648353> PMID: 25197431