Brief Report

The ubiquitous soil verrucomicrobial clade 'Candidatus Udaeobacter' shows preferences for acidic pH

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Abstract

Members of the verrucomicrobial clade 'Candidatus Udaeobacter' rank among the most dominant bacterial phylotypes in soil. Nevertheless, despite this global prevalence, in-depth analyses with respect to pH preferences of 'Ca. Udaeobacter' representatives are still lacking. Here, we utilized a recently designed primer pair, specifically targeting 'Ca. Udaeobacter', to investigate links between soil pH and the abundance as well as phylotype composition of this largely unexplored verrucomicrobial clade. Based on 150 forest and 150 grassland soils, comprising a broad pH range, we determined the highest total abundance of 'Ca. Udaeobacter' in strongly acidic soil (pH, ~5.1) and, noteworthy, in ultra-acidic soil (pH < 3.5) and at a pH ≥ 7, its abundance drastically declined. When we analysed the six most dominant amplicon sequence variants affiliated with 'Ca. Udaeobacter' separately, their abundances peaked within a pH range of approximately 4.7–5.2, and only in one case at slightly acidic soil pH (pH, 6.1). Our study benefits from a combination of quantitative real-time PCR and high-throughput amplicon sequencing, enabling for the first time a highly specific abundance analysis of representatives affiliated with 'Ca. Udaeobacter', which revealed that this globally abundant verrucomicrobial clade shows preferences for acidic soil.

Introduction

Soil pH is regarded as a key driver of bacterial community composition in terrestrial ecosystems worldwide (Lauber *et al*., 2009; Griffiths *et al*., 2011; Kaiser *et al*., 2016; Delgado-Baquerizo *et al*., 2018). Bacterial taxa adapted to a broad soil pH range, but also those depending on a more specific soil pH have previously been reported (Lehtovirta-Morley *et al*., 2011; Huber *et al*., 2014; Kaiser *et al*., 2016). Nevertheless, although numerous surveys analysed pH effects on soil-resident bacteria, the pH preferences of a globally abundant verrucomicrobial clade, designated 'Candidatus Udaeobacter' (Brewer *et al*., 2016), have so far not been comprehensively investigated. This can be explained by the fact that '*Ca*. Udaeobacter' evades cultivation and many commonly used PCR primers are largely biased against verrucomicrobial 16S rRNA genes (Bergmann *et al*., 2011; Guo *et al*., 2016).

Currently, only two studies focusing on the ecology, genomic properties and potential lifestyle features of '*Ca*. Udaeobacter' are available (Brewer *et al*., 2016; Willms *et al*., 2020). These studies indicate vitamin and multiple amino acid autotrophies based on two metagenome-assembled genomes (MAGs). Notably, both reported MAGs, affiliated with '*Ca*. Udaeobacter', also encode several components allowing complementation of auxotrophies such as serine/threonine exchangers and amino acid permeases (Brewer *et al*., 2016; Willms *et al*., 2020). In addition, compared to available genomes of other ubiquitous soil bacteria, the MAGs are relatively small with estimated sizes of ~2.81 Mbp (Brewer *et al*., 2016) and ~3.67 Mbp (Willms *et al*., 2020). It is possible that a small genome size and reducing the metabolic expense for synthesizing, e.g., costly amino acids, which might be efficiently acquired from, e.g., lysed cells present in the environment (Willms *et al*., 2020), confer a selective advantage in soil ecosystems. So far, it is unknown whether other uncultivated abundant soil taxa also contain reduced genomes and thereby sacrifice metabolic versatility. Therefore, in order to extend the knowledge on dominant soil bacteria exhibiting this characteristic, analyses of the distribution of '*Ca*. Udaeobacter' as well

as enriching or isolating representatives of this verrucomicrobial clade are clearly important next steps.

Recently, we detected arginine-dependent acid resistance and cadaverine biosynthesis genes, providing protection against acidic conditions, in our MAG assigned to '*Ca*. Udaeobacter' (Willms *et al*., 2020). Furthermore, Brewer *et al*. (2016) detected no significant correlation between the abundance of an abundant phylotype affiliated with '*Ca*. Udaeobacter' and other prokaryotic or eukaryotic taxa, indicating that '*Ca*. Udaeobacter' is most probably not involved in a symbiotic or host–pathogen interaction. Thus, it can be assumed that in contrast to verrucomicrobial endosymbionts belonging to '*Ca*. Xiphinematobacter' (Vandekerckhove *et al*., 2000; Brown *et al*., 2015), '*Ca*. Udaeobacter' representatives are not exposed to pH levels occurring, e.g., in nematodes, but have to cope with pH conditions in diverse soil ecosystems.

Our recently designed and evaluated 16S rRNA gene-based primer pair UDBAC_F/UDBAC_R (Willms *et al*., 2020) opened the door to comprehensive exploration of pH preferences with respect to '*Ca*. Udaeobacter', as it allows targeted detection of soil prokaryotes affiliated to this poorly characterized verrucomicrobial clade. Taking into account that 16S rRNA gene relative abundances derived from amplicon sequencing can lead to misinterpretations of microbial community structure (the increase of one taxon can lead to the concurrent decrease of other taxa in compositional data), Jian *et al*. (2020) suggested to integrate quantitative PCR (qPCR)-based microbiome profiling into standard marker gene sequencing-based microbiome analysis. Therefore, we used the UDBAC primer pair with respect to both, qPCR and amplicon production for next-generation sequencing, which allowed us to extensively study the relationship between soil pH and '*Ca*. Udaeobacter' abundance. Our study is based on a total of 150 forest and 150 grassland soils, covering a broad pH range (Supporting Information Table S1). These soils were derived from three geographic regions in Germany (Hainich-Dün, Schorfheide-Chorin and Schwäbische Alb) (Supporting Information Fig. S1), located up to 700 km apart, which enabled statistically robust analysis (see Supporting Information for details on experimental procedures).

Our discovery of genes providing protection against acidic conditions in a MAG affiliated with '*Ca*. Udaeobacter' (Willms *et al*., 2020) set the stage for hypothesis testing. In this context, we speculated that different '*Ca*. Udaeobacter' representatives show high abundances in soils exhibiting low or very low pH values.

Results and discussion

The primer pair UDBAC (Willms *et al*., 2020) revealed numerous amplicon sequence variants (ASVs) and thus diverse '*Ca*. Udaeobacter' phylotypes in forest as well as grassland soil (Fig. 1A; Supporting Information Tables S2 and S3). We normalized relative abundances of ASVs using qPCR data (Supporting Information Table S4 and S5) to estimate '*Ca*. Udaeobacter' phylotype absolute abundance (here designated artificial absolute abundance) (Fig. 1B and C) as described by Jian *et al*. (2020). This type of analysis has so far not been performed with respect to '*Ca*. Udaeobacter' and confirmed that ASV2 represents the most abundant phylotype across all analysed soils (Supporting Information Fig. S2).

The pH with respect to the 300 analysed samples ranged from 3.3 to 7.6 and was more acidic in forest (pH range, 3.3–7.2) than in grassland soil (pH range, 4.7–7.6) (Supporting Information Table S1 and Fig. 1C). Strikingly, within a pH range of approximately 4.5–5.5, we determined the highest '*Ca*. Udaeobacter' artificial absolute abundance (peak at pH ~5.1) (Fig. 1B and Supporting Information Table S6). According to the classification of the United States Department of Agriculture Natural Resources Conservation Service, soil pH ranges of 4.5–5.0 and 5.0–5.5 are considered very strongly acidic and strongly acidic, respectively (Soil Science Division Staff, 2017). It can be assumed that genes providing protection against acidic conditions, recently detected in a '*Ca*. Udaeobacter' MAG (Willms *et al*., 2020) from Schwäbische Alb sample AEW3 (pH, ~5.5), contribute to the successful colonization of acidic soils. To verify whether the preference for acidic pH can be detected in forest as well as grassland soil with respect to each study region, we performed separate analyses. Indeed, the preference for acidic pH was statistically significant with respect to forest as well as grassland soil of Schwäbische Alb, Hainich-Dün and Schorfheide-Chorin region (Supporting Information Table S6).

A large fraction of the Schwäbische Alb forest as well as grassland soils and the forest soils derived from Hainich-Dün exhibited a (very) strongly acidic pH (Fig. 1C and Supporting Information Table S1). In contrast, many of the soils from Hainich-Dün grassland, Schorfheide-Chorin grassland and Schorfheide-Chorin forest showed pH values ≥ 7 or ultra-acidic pH values, which were accompanied by comparably lower '*Ca*. Udaeobacter' artificial absolute abundances (Fig. 1C and Supporting Information Table S1). Accordingly, in a recent study, including five soils collected in Slovakia, '*Ca*. Udaeobacter' relative abundance was approximately fourfold higher in very strongly acidic soil (pH 4.98) than in soils with a pH of 7.36–7.81 (Böhmer *et al*., 2020).

In order to gain a deeper understanding with respect to pH preferences of '*Ca*. Udaeobacter', the most dominant ASVs were analysed separately (Fig. 2). This analysis revealed a peak of ASV1, ASV2 (most abundant ASV
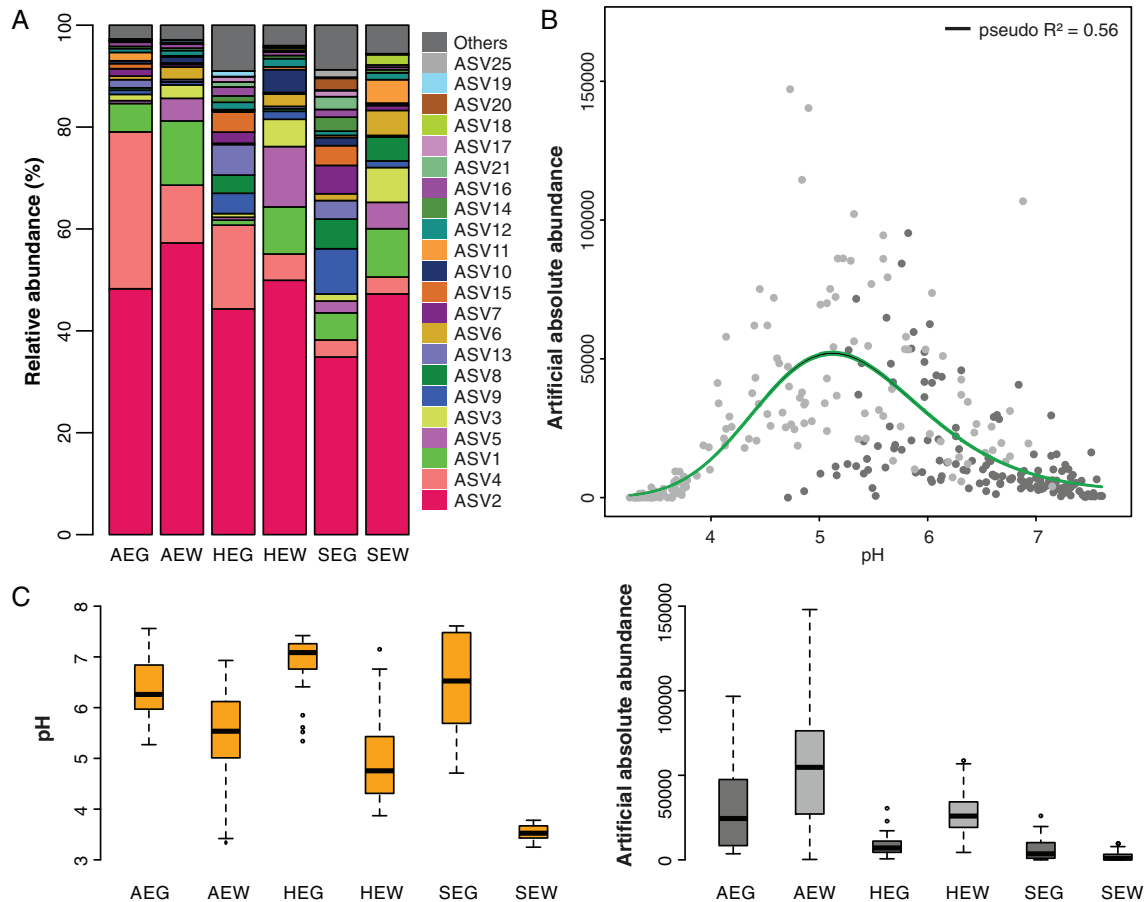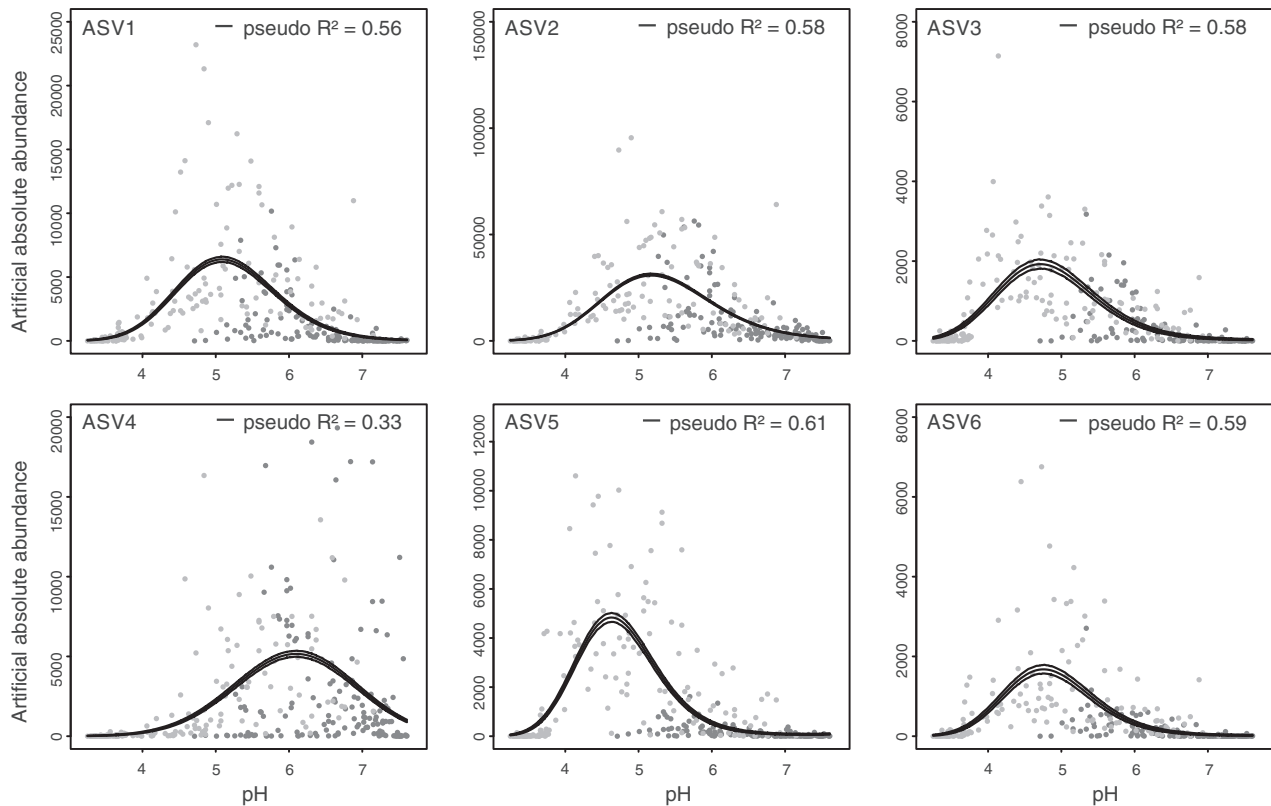
Fig. 1. Abundances of ASVs in forest and grassland soils covering a broad pH range (pH 3.3–7.6).A. Relative abundances of ASVs detected with the UDBAC primer pair in forest and grassland soils. The relative abundances were calculated based on high-throughput amplicon sequencing data. AEG, HEG and SEG represent grassland soil samples derived from Schwäbische Alb, Hainich-Dün and Schorfheide-Chorin, respectively. AEW, HEW and SEW represent forest soil samples derived from Schwäbische Alb, Hainich-Dün and Schorfheide-Chorin, respectively. ASV13, ASV15 and ASV21 were classified as uncultured *Verrucomicrobiaceae*. All other ASVs were classified as '*Candidatus* Udaeobacter' representatives. Others, ASVs showing less than 1% relative abundance.B. Relationship between soil pH and artificial absolute abundance of all ASVs classified as '*Ca*. Udaeobacter'. Artificial absolute abundances per ng DNA were calculated based on a combination of high-throughput amplicon sequencing and quantitative real-time PCR data (in both cases the UDBAC primer pair was considered). Light grey and dark grey circles represent forest and grassland soils, respectively. The solid line represents the predicted regression line and the green dashed line indicates the 99% prediction interval. The pseudo R^2 of the regression is shown in the top right corner.C. Boxplots showing pH values and artificial absolute abundance of all ASVs classified as '*Ca*. Udaeobacter' with respect to forest as well as grassland soils derived from the three considered geographic regions. The middle line in the box represents the median framed by the first and third quartile. The whiskers visualize 1.5 times the interquartile range.

detected in this study), ASV3, ASV5 and ASV6 artificial absolute abundance within a pH range of approximately 4.7–5.2 (Fig. 2 and Supporting Information Table S6). Only in case of ASV4, a preference for slightly acidic soil (abundance peak at pH 6.1) was determined (Fig. 2). This illustrates that analyses at high phylogenetic resolution are necessary to gain a more holistic understanding on links between pH and bacterial community composition.

Within the past two decades, several studies reported that pH represents a major driver of soil bacterial communities (Fierer and Jackson, 2006; Rousk *et al.*, 2010; Ren *et al.*, 2018; O'Brien *et al.*, 2019). Nevertheless, as many

of the diverse soil bacteria are differentially affected by changes in pH (Bartram *et al.* 2014; Kaiser *et al.*, 2016), more data on single bacterial phylotypes are required. Here, we found that one of the most abundant soil bacterial clades on Earth, '*Ca*. Udaeobacter', thrives under acidic pH conditions. Taking into account that many standard laboratory media used to enrich soil bacteria typically exhibit a (near-)neutral pH, this finding should be considered within future attempts tailored to the retrieval of '*Ca*. Udaeobacter' cultivates. These cultivates will pave the way for extensive physiological studies, enabling an improved understanding of lifestyle features with respect to '*Ca*. Udaeobacter'. In this study, the

Fig. 2. Relationships between soil pH and abundances of dominant ASVs in forest and grassland soils. Artificial absolute abundances per ng DNA were calculated based on a combination of high-throughput amplicon sequencing and quantitative real-time PCR data (in both cases the UDBAC primer pair was considered). Light grey and dark grey circles represent forest and grassland soils, respectively. The solid line represents the predicted regression line and the dashed line indicates the 99% prediction interval. The pseudo R^2 of the regression is shown in the top right corner.

apparent pH preferences of '*Ca*. Udaeobacter' were detected based on temperate forest and grassland soil samples derived from three geographic regions in Germany and our findings may or may not extrapolate to other sites globally depending on site-specific variables.

Data availability statement

The 16S rRNA gene-based amplicon sequencing data generated in this study were deposited in the Sequence Read Archive (SRA) of the NCBI under the accession number SRP273389 (bioproject accession: PRJNA647619). With respect to samples AEG2, AEG8, AEG16, AEG21, AEW2, AEW7, HEG4, HEG7, HEG21, HEW3 and HEW5, amplicon sequencing data were deposited in the Sequence Read Archive (SRA) under the accession number SRP226057 (SRX7071889 to SRX7071895 and SRX7071874, SRX7071875, SRX7071876 and SRX7071886).

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Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Appendix 1. Experimental procedures.

Fig. S1. Location of the three geographic regions considered in this study and distribution of sampling plots. The sampling plots are located within the German Biodiversity Exploratories Schorfheide-Chorin (a.), Hainich-Dün (b.) and Schwäbische Alb (c.) (modified from Fischer *et al*., 2010b). Grassland plots are indicated by circles and forest plots are indicated by triangles. The Biodiversity Exploratories serve as research platform for the entire German biodiversity research community. Each of the three Exploratories comprises 50 experimental plots (EPs) located in forest and 50 EPs located in grassland. From the 100 experimental plots per Exploratory, a subset of very intensive plots (VIPs) is utilized by the German biodiversity research community for studying biodiversity or ecological processes in extreme detail.

Fig. S2. Artificial absolute abundances of dominant ASVs in forest and grassland soils derived from three geographic regions. Artificial absolute abundances per ng DNA were calculated based on a combination of high-throughput amplicon sequencing and quantitative real-time PCR data (in both cases the UDBAC primer pair was considered).

Table S1. Characteristics of forest and grassland soils considered in this study as well as the number of generated high-quality amplicon sequences per sample.

Table S2. ASV table resulting from high-throughput amplicon sequencing data processing.

Table S3. Sequences of ASVs classified as 'Candidatus Udaeobacter' and showing ≥1% relative abundance.

Table S4. Quantitative real-time PCR data used for normalization of ASVs in the ASV table resulting from high-throughput amplicon sequencing data processing.

Table S5. ASV table after performing normalization with quantitative real-time PCR data.

Table S6. Characteristics of regression models to analyse the effect of pH on the abundance of 'Candidatus Udaeobacter' and associated ASVs. df stands for degrees of freedom. [1] = Grassland, [2] = Forest, [3] = Grassland Schorfheide-Chorin, [4] = Grassland Schwäbische Alb, [5] = Grassland Hainich-Dün, [6] = Forest Schorfheide-Chorin, [7] = Forest Schwäbische Alb, [8] = Forest Hainich-Dün.