Verrucomicrobial elevational distribution was strongly influenced by soil pH and carbon/nitrogen ratio

Congcong Shen^{1,2,3} · Yuan Ge² · Teng Yang^{1,3} · Haiyan Chu¹

Received: 30 September 2016 / Accepted: 10 February 2017 / Published online: 6 March 2017
© Springer-Verlag Berlin Heidelberg 2017

Abstract

Purpose The bacterial phylum Verrucomicrobia plays important roles in biogeochemical cycling processes, while the ecology of this phylum is still unclear. Previous elevational studies mainly focused on whole bacterial communities, while no study exclusively picked out Verrucomicrobia. Our objectives were to investigate the abundance, diversity and community composition of soil Verrucomicrobia across an elevation gradient on Changbai Mountain.

Materials and methods In total, 24 soil samples representing six elevation gradients were collected. Primer set 515F/806R was used for PCR amplifications and sequencing was conducted on an Illumina HiSeq2000 platform. Data sets comprising of Verrucomicrobial phylum were culled from all quality sequences for the further analyses of Verrucomicrobial diversity and community composition.

Results and discussion The relative abundance of Verrucomicrobia accounted for ~20% of the total bacterial communities, and Spartobacteria and DA101 were the most

dominant class and genus, respectively. Verrucomicrobia community composition differed significantly among elevations, while the Verrucomicrobia diversity showed no apparent trend along elevation although the richness of some classes or genera significantly changed with elevation. The Verrucomicrobial community composition, diversity, and relative abundance of specific classes or genera were significantly correlated with soil pH and carbon/nitrogen ratio (C:N ratio).

Conclusions These results indicated that Verrucomicrobia were abundant in Changbai Mountain soils, and Verrucomicrobial elevational distribution was strongly influenced by soil pH and C:N ratio. Our results also provide potential evidence that the dominant genus DA101 occupies different ecological niches and performs oligotrophic life history strategy in soil environment.

Keywords C:N ratio · Elevational distribution · Illumina HiSeq sequencing · pH · Soil Verrucomicrobia

Responsible editor: Jianming Xu

Electronic supplementary material The online version of this article (doi:10.1007/s11368-017-1680-x) contains supplementary material, which is available to authorized users.

✉ Haiyan Chu
hychu@issas.ac.cn

¹ State Key Laboratory of Soil and Sustainable Agriculture, Institute of Soil Science, Chinese Academy of Sciences, East Beijing Road 71, Nanjing 210008, China

² State Key Laboratory of Urban and Regional Ecology, Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, Beijing 100085, China

³ University of the Chinese Academy of Sciences, Beijing 100049, China

1 Introduction

The bacterial phylum Verrucomicrobia has been known to be present in a wide variety of terrestrial and aquatic ecosystems (Buckley and Schmidt 2001; Fierer et al. 2013; Sharp et al. 2014; Bergen et al. 2014). Despite they are ubiquitous, we know little about their physiologies because species are difficult to obtain in pure culture and isolation (Janssen et al. 2002). Data showed that only 31 isolates has been obtained from soil samples (Ribosomal Database Project (RDP) II) (Hedlund et al. 1997; Hedlund 2011). Based on pure culture method, researchers gradually found that members of this group participated in methane oxidation (Dunfield et al. 2007; Pol et al. 2007), nitrogen fixation (Khadem et al. 2010), and polysaccharide degradation (Martinez-Garcia et al. 2012), suggesting that they play

an important ecological role. However, their abundance, diversity, and distribution attributes across environmental gradient are virtually unknown. This may derive from traditional knowledge that Verrucomicrobia are present at low frequency. But a recent study pointed out that Verrucomicrobial relative abundances in soils have been underestimated (Bergmann et al. 2011). Given that this group may drive key biogeochemical cycling processes, it is urgent to fill our knowledge gaps about the ecological significance of the Verrucomicrobia.

The distribution of an organism in relation to biotic and abiotic characteristics of its environment can provide important clues to understand the basis of the organism physiology and its function within an ecosystem. Recently, studies about the ecology of specific groups are receiving increasing attention. For example, the diversity of Acidobacterial phylum has been revealed to be comparable to that of Proteobacterial, and researchers found that different subgroups respond differently to pH or other particular environmental factors (Barns et al. 2007; Naether et al. 2012; Zhang et al. 2014; Liu et al. 2016). One study found a weak positive correlation between the abundance of Verrucomicrobial ribosomal ribonucleic acid (rRNA) and soil moisture content, and another study found strong correlation between the abundance of Verrucomicrobia (by quantitative PCR) and soil carbon content (Buckley and Schmidt 2001; Ranjan et al. 2015). Elevational gradient, with many changing environmental variables, provides natural platform for studying biodiversity patterns (Bryant et al. 2008). Previous elevational studies mainly focused on whole bacterial communities, while no study exclusively picked out Verrucomicrobia (Fierer et al. 2011; Shen et al. 2013; Singh et al. 2014; Yuan et al. 2014). Here, our objectives were to investigate the abundance, diversity, and community composition of soil Verrucomicrobia across an elevation gradient on Changbai Mountain. Primer set 515F/806R was used for PCR amplifications, because previous work has demonstrated that this primer set was unbiased against Verrucomicrobia phylum (Bergmann et al. 2011).

2 Materials and methods

2.1 Soil sampling and physicochemical analyses

The detailed background of Changbai Mountain, sampling scheme, and physicochemical analyses were described in Shen et al. (2013). We presented all these information in brief. We collected 24 soil samples from the northern slope of Changbai Mountain (126° 55' E–129° 00' E, 41° 23' N–42° 36' N) on 18 June 2009. Six elevational gradients (530, 760, 1250, 1680, 1950, 2200 m), representing six typical vegetation types, were chosen. At each elevation, soil samples were collected from four plots (10 × 10 m) as four independent replicates. The main characteristics along the elevational gradient were shown in Table S1 (Electronic Supplementary

Material). These samples were divided into two parts; one was stored at 4 °C for physical and chemical analyses, and the other was stored at –20 °C prior to DNA extraction. We measured six main physicochemical characteristics in laboratory (Table S2, Electronic Supplementary Material), including pH, moisture, total carbon (TC), total nitrogen (TN), microbial biomass C (MBC), and biomass N (MBN).

2.2 Biological analyses

Sample processing, sequencing, and core amplicon data analysis were performed by the Earth Microbiome Project (www.earthmicrobiome.org). DNA was extracted from soils using the MoBio PowerSoil DNA Isolation Kit (MO BIO, Carlsbad, CA, USA). PCR amplifications were conducted in triplicate reactions for each sample using the primer set 515F (5'-GTGCCAGCMGCCGCGGTAA-3') and 806R (5'-GGAC TACVSGGGTATCTAAT-3') that amplifies the V4 region of the 16S rRNA gene. This primer set was selected as it exhibits few biases against different bacterial taxa. DNA was amplified in triplicate PCR reactions following the protocol described in Caporaso et al. (2010). Sequencing was conducted on an Illumina HiSeq2000 platform following the 2 × 100-bp paired-end protocol (Caporaso et al. 2012). Quality filtering and processing of reads were conducted as described in Caporaso et al. (2010). Reads were assigned to operational taxonomic units (OTUs) at the 97% sequence similarity level using the open-reference OTU picking protocol in QIIME (Caporaso et al. 2010). Taxonomy was then assigned to all representative sequences using the RDP classifier. Data sets comprising of Verrucomicrobial phylum were culled from all quality sequences for the further analyses of Verrucomicrobial communities. Relative Verrucomicrobial abundances were calculated as the percentage of bacterial sequences in each sample assigned to the Verrucomicrobial phylum. Sequences have been deposited to the MG-RAST metagenomic analysis server (<http://metagenomics.anl.gov/>) for public access (accession numbers from 4,555,016.3 to 4,555,037.3).

2.3 Statistical analyses

QIIME was used to estimate both taxonomic and phylogenetic matrices of the pairwise distances between communities (Bray–Curtis and unweighted Unifrac distances, respectively). Alpha diversity was determined using both taxonomic metrics (numbers of OTUs and Shannon index, H') and a phylogenetic metric (Faith's phylogenetic diversity). Pearson's correlation analyses were used to identify the significant correlations ($P < 0.05$) between biological variables (Verrucomicrobial diversity and relative abundances of specific class or genus) and elevation or soil characteristics using SPSS 20.0 for Windows. Non-metric multidimensional scaling (NMDS) based on Bray–Curtis distance was used to

analyze the differences in Verrucomicrobial community composition among elevations. The *ordisurf* function in R software was employed to fit a smooth surface of the elevation gradient onto the NMDS ordination (v.3.3.0; R Development Core Team 2010). In addition, we tested for significant differences in community composition among elevations using analysis of similarities (ANOSIM) with R statistical software. A Mantel test was used to determine the environmental factors that significantly correlate with community composition (abundance of OTUs), and significant factors were used to construct the soil property matrix for canonical correspondence analysis (CCA) in the vegan package of R. All above analyses were performed based on matrices of the rarefied data (5000 Verrucomicrobial sequences per sample). Two samples were abandoned because of the pollution in the transportation.

3 Results

3.1 Verrucomicrobial community composition

Across all soil samples, we obtained 1,534,018 bacterial high-quality sequences, with 40 phyla identified in total (Table S3, Electronic Supplementary Material). Proteobacteria, including four classes of alpha-, beta-, delta-, and gamma-proteobacteria, was the most abundant phylum. The second and third most abundant phyla were Acidobacteria and Verrucomicrobia. The three dominant phyla accounted for more than 80% of the bacterial sequences (Fig. 1).

Sequences assigned to the phylum Verrucomicrobia made up 302,671 (19.73%) of the total sequences. Within the phylum, class Spartobacteria was predominant, with 85.98% of Verrucomicrobial sequences assigned to this group (Fig. 2 and

Table S5, Electronic Supplementary Material). Class Pedosphaerae was the second dominant group, constituting 9.6% of sequences. Other sequences were assigned to the following three classes: Methylocidiphilae, Opitutae, and Verrucomicrobiae, accounting for 2.6, 1.47, and 0.32%, respectively. We further calculated the relative abundance of genera within Spartobacteria class (Fig. 2). DA101 has the highest relative abundance (84.38%), and Candidatus Xiphinematobacter was the second dominant genus (11.63%). Other genera like Chthoniobacter, Ellin506, heteroC45_4W, and OR-59 were identified at relatively low abundances.

3.2 Verrucomicrobial diversity

In terms of number of OTUs, Shannon index, and Faith's PD (phylogenetic diversity), we calculated Verrucomicrobial diversity at 5000-sequence standardization (5000 Verrucomicrobial sequences per sample). The Verrucomicrobial diversity did not significantly correlate with elevation, neither with other soil characteristics. We further calculated number of OTUs at the class and genus levels within Verrucomicrobial phylum. The OTU richness of Pedosphaerae, Methylocidiphilae, and Opitutae class have significant and positive correlations with elevation. These significant relationships were also found in Chthoniobacter and Ellin506 genus (Table 1). Soil pH and C:N ratio have close relationships with OTU richness of these classes and genera, showing positive or negative correlations (Table 1).

3.3 Factors influencing Verrucomicrobial community composition

The NMDS plot showed that the Verrucomicrobial communities clustered together within the same elevation and changed

Fig. 1 Percentages of sequences assigned to each phylum within total bacterial sequences (top 22 phyla were listed). Primer set 515F/806R was used for PCR amplifications

Fig. 2 Relative abundances of five classes belonging to Verrucomicrobial phylum (a) and relative abundances of six genera belonging to Spartobacteria class (b) in all soil samples and each of six sampling location along elevation

along the elevation gradient (Fig. 3). Furthermore, community composition differed significantly among elevations according to the ANOSIM test (Table S8, Electronic Supplementary Material). Mantel test showed that Verrucomicrobial community composition significantly correlated with soil pH, elevation, C:N ratio, TN, moisture, and TOC. Of all of the environmental variables examined, pH showed the highest correlation

with the community composition ($r = 0.72$, $P < 0.001$; Table 2). Specifically, the relative abundance of four classes (Spartobacteria, Pedosphaerae, Methylocidiphilae, and Opitutae) and two genera (DA101, Chthoniobacter) showed significant correlations with elevation, pH, and C:N ratio (Figs. 4 and 5 and Table S9, Electronic Supplementary Material). TC, TN, and MBC also showed significant

Table 1 Pearson correlations (R) between OTUs richness and soil and site characteristics at the phylum, class, and genus levels

r	Elevation	SM	pH	TC	TN	C:N ratio	MBC	MBN
p__Verrucomicrobia	0.312	0.204	0.112	−0.18	−0.263	0.266	0.053	0.154
c__Methylocidiphilae	0.898	0.165	−0.778	−0.311	−0.631	0.582	0.085	0.028
c__Pedosphaerae	0.659	0.322	−0.535	−0.09	−0.376	0.508	0.139	0.089
c__Spartobacteria	−0.184	−0.074	0.712	−0.082	0.142	−0.261	−0.165	−0.055
c__Opitutae	0.862	0.093	−0.653	−0.366	−0.7	0.628	−0.14	−0.124
c__Verrucomicrobiae	−0.251	0.311	0.622	0.377	0.402	−0.161	0.1	0.273
g__Candidatus Xiphinematobacter	0.17	0.4	−0.248	0.3	0.006	0.382	0.448	0.341
g__Chthoniobacter	0.524	0.173	−0.49	−0.127	−0.405	0.48	0.061	0.001
g__DA101	−0.338	−0.288	0.762	−0.187	0.179	−0.449	−0.327	−0.2
g__Ellin506	0.686	−0.239	−0.158	0.346	−0.326	0.712	−0.321	−0.277
g__heteroC45_4W	0.016	0.488	0.261	0.365	0.333	−0.061	0.292	0.398

The Verrucomicrobial communities were randomly sampled at the 5000 sequences level. Values in italic indicate significant correlations ($P < 0.05$)

TC total carbon, TN total nitrogen, SM soil moisture, MBC soil microbial biomass C, MBN soil microbial biomass N

Fig. 3 Verrucomicrobial community compositional structure in soils indicated by non-metric multidimensional scaling (NMDS) using Bray–Curtis dissimilarity. The elevational gradients were treated as the smooth surfaces to fit on the NMDS ordination

correlations with the relative abundance of some classes or genus (Table S9, Electronic Supplementary Material).

4 Discussion

4.1 Verrucomicrobial community composition

Our study here showed that Verrucomicrobia accounted for about 20% percentage of whole bacterial sequences, indicating a relatively higher abundance of Verrucomicrobia in soils. While this result is inconsistent with our previous investigation, which obtained only 0.28% Verrucomicrobial sequences (Shen et al. 2013). Primer difference might be a primary cause (515F/907R vs. 515F/806R), as Bergmann et al. (2011)

Table 2 The biogeochemical factors that significantly correlated with Verrucomicrobial communities were listed below

Variables	<i>r</i>	<i>P</i>
pH	0.72	0.001
Elevation	0.662	0.001
C:N ratio	0.5	0.001
TN	0.31	0.014
Moisture	0.248	0.006
TC	0.213	0.034
MBC	0.203	0.051
MBN	0.114	0.152

The correlations (*r*) and significance (*P*) were determined by Mantel tests between the community structure and environmental variables

TC total carbon, *TN* total nitrogen, *MBC* soil microbial biomass C, *MBN* soil microbial biomass N

proposed that primer set 515F/806R was unbiased against Verrucomicrobia phylum. Previous work using oligonucleotide probe to target the 16S rRNA genes of Verrucomicrobia revealed that members of Verrucomicrobia are very difficult to target (Sangwan et al. 2005). Janssen (2006) identified the dominant bacterial phyla in libraries of 16S rRNA and 16S rRNA genes and found that members of the phylum Verrucomicrobia make up an average of 7% (range 0 to 21%) of soil bacterial communities. Using primer set 515F/806R and high-throughput sequencing (454 or Illumina), percentages of Verrucomicrobia in soil bacterial communities have reached above 20% (Bergmann et al. 2011; Fierer et al. 2013). Some studies designed specific primer and used clone library sequencing to investigate Verrucomicrobial diversity, but the pattern seemed not clear enough due to limit sequences (Parveen et al. 2013; Ranjan et al. 2015). Our study provides evidence that the abundance of soil Verrucomicrobia was underestimated and suggests that primer set 515F/806R combining with high-throughput Illumina sequencing might be a better choice for studying the Verrucomicrobial diversity patterns.

Lineages of Verrucomicrobia have been informally classified into five subdivisions or seven subdivisions numbered 1 to 7 (Hugenholtz et al. 1998; Schlesner et al. 2006). Commonly, classes Verrucomicrobiae, Spartobacteria, and Opitutae were recognized as subdivisions 1, 2, and 4, and subdivision 3 mainly included bacteria Ellin515, Ellin518, Ellin5102, etc. (Sangwan et al. 2005). Our results found that Spartobacteria and Pedosphaerae (mainly Ellin515) were the first and second dominant classes, which are identical with previous findings that the majority of the sequences (90%) belonged to subdivisions 2 and 3 (Bergmann et al. 2011; Ranjan et al. 2015). However, the dominance of Spartobacteria class observed in most terrestrial environments was generally lost in the ocean and marine sediments (Freitas et al. 2012). Within Spartobacteria class of our result, DA101 genus was predominant in both forest and tundra soils. DA101 was firstly described in 1998 as a particularly abundant 16S rRNA sequence recovered from a grassland soil in the Netherlands (Felske and Akkermans 1998). It is likely that this genus is more abundant in grassland than other soil ecosystems. According to metadata statistics, a recent study found that DA101 was averagely six times more abundant in grassland than in forest soils (Brewer et al. 2016).

4.2 Verrucomicrobial elevational diversity pattern

Elevational pattern is a fundamental pattern in ecology. Here, we found that Verrucomicrobial diversity exhibited no evident pattern with elevation (Table 1). This is in line with many previous studies, which also found non-significant bacterial elevational patterns (Fierer et al. 2011; Shen et al. 2013; Yuan et al. 2014). But at the class or genus level, OTU richness for Pedosphaerae, Methylococcoides, Opitutae, Chthoniobacter, and Ellin506 significantly increased with elevation. We infer

Fig. 4 The relationships between relative abundances of five classes and five genera and soil pH illustrated by linear regressions

that this pattern was largely driven by pH and C:N ratio, because OTU richness for such class and genus are significantly and negatively correlated with pH, whereas positively with C:N ratio. Apparently, the Verrucomicrobial elevational patterns are determined by different trends in the subdivision elevational patterns. But these classes which showed significant increasing richness pattern are at low relative abundance and exhibit inverse trends with dominant Spartobacteria (non-significant richness pattern with elevation), which may result in a non-significant Verrucomicrobial elevational diversity pattern.

4.3 Factors influencing Verrucomicrobial community composition

Factors influencing patterns in the distribution of microbial taxa have been well studied (Martiny et al. 2006;

Hanson et al. 2012). Particularly, it is recognized that pH strongly controls bacterial communities. Expectedly in this study, pH showed the highest correlation with the Verrucomicrobial community composition ($r = 0.72$, $P < 0.001$; Table 2). Besides pH, other soil features like C:N ratio, soil moisture, TC, and TN also significantly affected the Verrucomicrobial community composition. Though some previous studies provided evidences of the association of soil moisture and carbon content with changes in Verrucomicrobial community structure (Buckley and Schmidt 2001; Ranjan et al. 2015), they did not investigate the correlation at the class and genus levels. By contrast, we used linear regression analysis to study the relationship between the relative abundance of specific class or genus and soil characteristics and found that the effects of pH and C:N ratio are still powerful at

Fig. 5 The relationships between relative abundances of five classes and five genera and soil C:N ratio illustrated by linear regressions

the class and even genus level. Specifically, our result found that the relative abundance of class Methylacidiphilae showed highly negative correlation with pH. Members of Methylacidiphilae are nitrogen-fixing acidophiles capable of methane oxidation, and many studies have discovered this group in extremely acidic environments (Pol et al. 2007; Dunfield et al. 2007; Islam et al. 2008). We also found that genus *Candidatus Xiphinematobacter* abundance have significant correlations with pH and C:N ratio. *Xiphinematobacter* species live as endosymbionts in the ovarian and intestinal epithelium of nematodes in the *Xiphinema americanum* group (Schlesner et al. 2006). It has been documented that nematode abundances were significantly influenced by pH and C:N ratio (Wu et al. 2011; Shen et al. 2014). Thus, *Candidatus Xiphinematobacter* should adapt to similar conditions with nematodes. We all know that carbon resource is important for microbial growth. Notably, we found that the relative abundance of genus DA101 was significantly and negatively correlated with C:N ratio. This means that members of genus DA101 are likely to prefer low C:N ratio. In other words, members of genus DA101 are more competitive in low carbon resource condition. A previous study suggested that colony development by members of the class Spartobacteria was inhibited by other culturable bacteria, given that Spartobacteria groups were only found on plates receiving a more dilute inoculum (Sangwan et al. 2005). Recently, Brewer et al. (2016) assembled a draft genome named *Candidatus Udaeobacter copiosus*, which is a representative of DA101 clade, and speculated that *Ca. U. copiosus* is an oligotrophic soil bacterium that reduces its requirement for soil organic carbon by acquiring costly amino acids and vitamins from the environment. Together, these studies provide potential evidence that dominant genus DA101 occupies different ecological niches and performs oligotrophic life history strategy in soil environment. Yet, further researches are needed to identify this physiological property by cultural means (Janssen et al. 2002; Senechkin et al. 2010).

5 Conclusions

We first characterized changes in Verrucomicrobial diversity and community composition along elevation at the class or genus level. These results indicated that Verrucomicrobia were abundant in Changbai Mountain soils, and Verrucomicrobial elevational distribution was strongly influenced by soil pH and C:N ratio. Our results also provide potential evidence that the dominant genus DA101 occupies different ecological niches and performs oligotrophic life history strategy in soil environment.

Acknowledgements We thank Wenju Liang and Xinyu Li for their assistance with soil sampling and Yu Shi and Yingying Ni for their assistance in lab analysis. We also thank Earth Microbiome Project (EMP) for the sample processing, sequencing, and core amplicon data analysis. This work was supported by the National Natural Science Foundation of China (41371254), the National Program on Key Basic Research Project (2014CB954002), and the Strategic Priority Research Program (XDB15010101, XDB15010302) of Chinese Academy of Sciences.

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

References

- Barns SM, Cain EC, Somerville L, Kuske CR (2007) Acidobacteria phylum sequences in uranium-contaminated subsurface sediments greatly expand the known diversity within the phylum. *Appl Environ Microbiol* 73:3113–3116
- Bergen B, Herlemann DPR, Labrenz M, Jürgens K (2014) Distribution of the verrucomicrobial clade Spartobacteria along a salinity gradient in the Baltic Sea. *Environ Microbiol Rep* 6:625–630
- Bergmann GT, Bates ST, Eilers KG, Lauber CL, Caporaso JG, Walters WA, Knight R, Fierer N (2011) The under-recognized dominance of verrucomicrobia in soil bacterial communities. *Soil Biol Biochem* 43:1450–1455
- Brewer T, Handley K, Carini P, Gibert J, Fierer N (2016) Genome reduction in an abundant and ubiquitous soil bacterial lineage. *bioRxiv* 2016:053942
- Bryant JA, Lamanna C, Morlon H, Kerkhoff AJ, Enquist BJ, Green JL (2008) Colloquium paper: microbes on mountainsides: contrasting elevational patterns of bacterial and plant diversity. *Proc Natl Acad Sci U S A* 115:11505–11511
- Buckley DH, Schmidt TM (2001) Environmental factors influencing the distribution of rRNA from verrucomicrobia in soil. *FEMS Microbiol Ecol* 35:105–112
- Caporaso JG, Kuczynski J, Stombaugh J, Bittinger K, Bushman FD, Costello EK, Fierer N, Pena AG, Goodrich JK, Gordon JI, Huttley GA, Kelley ST, Knights D, Koenig JE, Ley RE, Lozupone CA, McDonald D, Muegge BD, Pirrung M, Reeder J, Sevinsky JR, Tumbaugh PJ, Walters WA, Widmann J, Yatsunenko T, Zaneveld J, Knight R (2010) QIIME allows integration and analysis of high-throughput community sequencing data. *Nat Methods* 7:335–336
- Caporaso JG, Lauber CL, Walters WA, Berg-Lyons D, Huntley J, Fierer N, Owens SM, Betley J, Fraser L, Bauer M, Gormley N, Gilbert JA, Smith G, Knight R (2012) Ultra-high-throughput microbial community analysis on the Illumina HiSeq and MiSeq platforms. *ISME J* 6:1621–1624
- Dunfield PF, Yuryev A, Senin P, Smirnova AV, Stott MB, Hou S, Ly B, Saw JH, Zhou Z, Ren Y, Wang J, Mountain BW, Crowe MA, Weatherby TM, Bodelier PL, Liesack W, Feng L, Wang L, Alam M (2007) Methane oxidation by an extremely acidophilic bacterium of the phylum verrucomicrobia. *Nature* 450:879–882
- Felske A, Akkermans ADL (1998) Prominent occurrence of ribosomes from an uncultured bacterium of the Verrucomicrobiales cluster in grassland soils. *Lett Appl Microbiol* 26:219–223
- Fierer N, Ladau J, Clemente JC, Leff JW, Owens SM, Pollard KS, Knight R, Gilbert JA, McCulley RL (2013) Reconstructing the microbial diversity and function of pre-agricultural tallgrass prairie soils in the United States. *Science* 342:621–624
- Fierer N, McCain CM, Meir P, Zimmermann M, Rapp JM, Silman MR, Knight R (2011) Microbes do not follow the elevational diversity patterns of plants and animals. *Ecology* 92:797–804
- Freitas S, Hatosy S, Fuhrman JA, Huse SM, Welch DB, Sogin ML, Martiny AC (2012) Global distribution and diversity of marine verrucomicrobia. *ISME J* 6:1499–1505

- Hanson CA, Fuhrman JA, Horner-Devine MC, Martiny JB (2012) Beyond biogeographic patterns: processes shaping the microbial landscape. *Nat Rev Microbiol* 10:497–506
- Hedlund BP, Gosink JJ, Staley JT (1997) Verrucomicrobia div. nov., a new division of the bacteria containing three new species of Prostheco bacter. *Antonie Van Leeuwenhoek* 72:29–38
- Hedlund BP (2011) Phylum XXIII. Verrucomicrobia phyl. nov. In: Krieg NR, Ludwig W, Whitman WR, Hedlund BP, Paster BJ, Staley JT et al (eds) *Bergey's manual of systematic bacteriology*. Williams & Wilkins, New York, pp 795–841
- Hugenholtz P, Goebel BM, Pace NR (1998) Impact of culture-independent studies on the emerging phylogenetic view of bacterial diversity. *J Bacteriol* 180:4765–4774
- Islam T, Jensen S, Reigstad LJ, Larsen O, Birkeland NK (2008) Methane oxidation at 55 °C and pH 2 by a thermoacidophilic bacterium belonging to the verrucomicrobia phylum. *Proc Natl Acad Sci U S A* 105:300–304
- Janssen PH (2006) Identifying the dominant soil bacterial taxa in libraries of 16S rRNA and 16S rRNA genes. *Appl Environ Microbiol* 72:1719–1728
- Janssen PH, Yates PS, Grinton BE, Taylor PM, Sait M (2002) Improved culturability of soil bacteria and isolation in pure culture of novel members of the divisions Acidobacteria, Actinobacteria, Proteobacteria, and Verrucomicrobia. *Appl Environ Microbiol* 68:2391–2396
- Khadem AF, Pol A, Jetten MS, Op den Camp HJ (2010) Nitrogen fixation by the verrucomicrobial methanotroph “*Methyloacidiphilum fumarolicum*” SolV. *Microbiology* 156:1052–1059
- Liu J, Sui Y, Yu Z, Yao Q, Shi Y, Chu H, Jin J, Liu X, Wang G (2016) Diversity and distribution patterns of acidobacterial communities in the black soil zone of northeast China. *Soil Biol Biochem* 95:212–222
- Martinez-Garcia M, Brazel DM, Swan BK, Arnosti C, Chain PS, Reitenga KG, Xie G, Poulton NJ, Lluersma Gomez M, Masland DE, Thompson B, Bellows WK, Ziervogel K, Lo CC, Ahmed S, Gleasner CD, Detter CJ, Stepanauskas R (2012) Capturing single cell genomes of active polysaccharide degraders: an unexpected contribution of verrucomicrobia. *PLoS One* 7:e35314
- Martiny JB, Bohannan BJ, Brown JH, Colwell RK, Fuhrman JA, Green JL, Horner-Devine MC, Kane M, Krumins JA, Kuske CR, Morin PJ, Naeem S, Ovrees L, Reysenbach AL, Smith VH, Staley JT (2006) Microbial biogeography: putting microorganisms on the map. *Nat Rev Microbiol* 4:102–112
- Naether A, Foesel BU, Naegele V, Wust PK, Weinert J, Bonkowski M, Alt F, Oelmann Y, Polle A, Lohaus G, Gockel S, Hemp A, Kalko EK, Linsenmair KE, Pfeiffer S, Renner S, Schoning I, Weisser WW, Wells K, Fischer M, Overmann J, Friedrich MW (2012) Environmental factors affect acidobacterial communities below the subgroup level in grassland and forest soils. *Appl Environ Microbiol* 78:7398–7406
- Parveen B, Mary I, Vellet A, Ravet V, Debroas D (2013) Temporal dynamics and phylogenetic diversity of free-living and particle-associated verrucomicrobia communities in relation to environmental variables in a mesotrophic lake. *FEMS Microbiol Ecol* 83:189–201
- Pol A, Heijmans K, Harhangi HR, Tedesco D, Jetten MS, Op den Camp HJ (2007) Methanotrophy below pH 1 by a new verrucomicrobia species. *Nature* 450:874–878
- R Development Core Team (2010) R: a language and environment for statistical computing. R Foundation for statistical computing, Vienna
- Ranjan K, Paula FS, Mueller RC, Jesus Eda C, Cenciani K, Bohannan BJ, Nusslein K, Rodrigues JL (2015) Forest-to-pasture conversion increases the diversity of the phylum verrucomicrobia in Amazon rainforest soils. *Front Microbiol* 6:779
- Sangwan P, Kovac S, Davis KE, Sait M, Janssen PH (2005) Detection and cultivation of soil verrucomicrobia. *Appl Environ Microbiol* 71:8402–8410
- Schlesner H, Jenkins C, Staley JT (2006) The phylum verrucomicrobia: a phylogenetically heterogeneous bacterial group. *Prokaryotes* 7:881–896
- Senechkin IV, Speksnijder AG, Semenov AM, van Bruggen AH, van Overbeek LS (2010) Isolation and partial characterization of bacterial strains on low organic carbon medium from soils fertilized with different organic amendments. *Microb Ecol* 60:829–839
- Sharp CE, Smimova AV, Graham JM, Stott MB, Khadka R, Moore TR, Grasby SE, Strack M, Dunfield PF (2014) Distribution and diversity of verrucomicrobia methanotrophs in geothermal and acidic environments. *Environ Microbiol* 16:1867–1878
- Shen C, Liang W, Shi Y, Lin X, Zhang H, Wu X, Xie G, Chain PSG, Grogan P, Chu H (2014) Contrasting elevational diversity patterns between eukaryotic soil microbes and plants. *Ecology* 95:3190–3202
- Shen C, Xiong J, Zhang H, Feng Y, Lin X, Li X, Liang W, Chu H (2013) Soil pH drives the spatial distribution of bacterial communities along elevation on Changbai Mountain. *Soil Biol Biochem* 57:204–211
- Singh D, Lee-Cruz L, Kim W-S, Kerfahi D, Chun J-H, Adams JM (2014) Strong elevational trends in soil bacterial community composition on Mt. Halla, South Korea. *Soil Biol Biochem* 68:140–149
- Wu T, Ayres E, Bardgett RD, Wall DH, Garey JR (2011) Molecular study of worldwide distribution and diversity of soil animals. *Proc Natl Acad Sci U S A* 108:17720–17725
- Yuan Y, Si G, Wang J, Luo T, Zhang G (2014) Bacterial community in alpine grasslands along an altitudinal gradient on the Tibetan Plateau. *FEMS Microbiol Ecol* 87:121–132
- Zhang Y, Cong J, Lu H, Li G, Qu Y, Su X, Zhou J, Li D (2014) Community structure and elevational diversity patterns of soil Acidobacteria. *J Environ Sci (China)* 26:1717–1724