Soil Biology & Biochemistry 43 (2011) 1450–1455

Contents lists available at ScienceDirect

Soil Biology & Biochemistry

journal homepage: www.elsevier.com/locate/soilbio

The under-recognized dominance of *Verrucomicrobia* in soil bacterial communities

Gaddy T. Bergmann [a,b], Scott T. Bates [a,b], Kathryn G. Eilers [a,b], Christian L. Lauber [a,b], J. Gregory Caporaso [c], William A. Walters [d], Rob Knight [c,e], Noah Fierer [a,b,*]

[a] Cooperative Institute for Research in Environmental Sciences, University of Colorado at Boulder, Campus Box 216, Boulder, CO 80309-0216, USA
[b] Department of Ecology and Evolutionary Biology, University of Colorado at Boulder, Campus Box 334, Boulder, CO 80309-0334, USA
[c] Department of Chemistry and Biochemistry, University of Colorado at Boulder, Campus Box 215, Boulder, CO 80309-0215, USA
[d] Department of Molecular, Cellular, and Developmental Biology, University of Colorado at Boulder, Campus Box 347, Boulder, CO 80309-0347, USA
[e] Howard Hughes Medical Institute, 4000 Jones Bridge Road, Chevy Chase, MD 20815-6789, USA

ARTICLE INFO

Article history:
Received 17 November 2010
Received in revised form
18 January 2011
Accepted 10 March 2011
Available online 5 April 2011

Keywords:
Verrucomicrobia
Spartobacteria
Bacteria
Relative abundance
Ecological dominance
Primer bias
Barcoded pyrosequencing
16S rRNA genes
Oligotroph
Subsurface horizon

ABSTRACT

Verrucomicrobia are ubiquitous in soil, but members of this bacterial phylum are thought to be present at low frequency in soil, with few studies focusing specifically on verrucomicrobial abundance, diversity, and distribution. Here we used barcoded pyrosequencing to analyze verrucomicrobial communities in surface soils collected across a range of biomes in Antarctica, Europe, and the Americas (112 samples), as well as soils collected from pits dug in a montane coniferous forest (69 samples). Data collected from surface horizons indicate that *Verrucomicrobia* average 23% of bacterial sequences, making them far more abundant than had been estimated. We show that this underestimation is likely due to primer bias, as many of the commonly used PCR primers appear to exclude verrucomicrobial 16S rRNA genes during amplification. *Verrucomicrobia* were detected in 180 out of 181 soils examined, with members of the class *Spartobacteria* dominating verrucomicrobial communities in nearly all biomes and soil depths. The relative abundance of *Verrucomicrobia* was highest in grasslands and in subsurface soil horizons, where they were often the dominant bacterial phylum. Although their ecology remains poorly understood, *Verrucomicrobia* appear to be dominant in many soil bacterial communities across the globe, making additional research on their ecology clearly necessary.

1. Introduction

Bacteria belonging to the phylum *Verrucomicrobia* are nearly ubiquitous in soil (Zhang and Xu, 2008), but are usually considered to be one of the less frequent bacterial phyla. A recent meta-analysis of published clone library results indicated that, on average, only about 7% of soil bacterial sequences were verrucomicrobial (Janssen, 2006). *Verrucomicrobia* are also typically difficult to cultivate (Janssen et al., 1997, 2002; Janssen, 1998; Joseph et al., 2003; Davis et al., 2005). As a result, this phylum is currently represented by <100 isolates in public databases, such as Greengenes (http://greengenes.lbl.gov/) and the Ribosomal Database Project (http://rdp.cme.msu.edu/). To date, nearly all *Verrucomicrobia* cultured from soil have been free-living (Schlesner et al., 2006; Ward et al., 2006; Griffiths and Gupta, 2007) and most are mesophilic (Sangwan et al., 2004), facultatively or obligately anaerobic (Chin et al., 2001), saccharolytic (Janssen, 1998), and oligotrophic (da Rocha et al., 2009; Senechkin et al., 2010). Several studies have documented the distribution of *Verrucomicrobia* across specific soil gradients (O'Farrell and Janssen, 1999; Buckley and Schmidt, 2001; Sangwan et al., 2005), but relatively little information is available on the composition, diversity, and overall distribution of verrucomicrobial communities across a range of soil types.

In this study, we set out to obtain improved estimates of the relative abundance and distribution of *Verrucomicrobia* in soil, and identify the dominant taxa in this phylum across a range of soil types. In order to meet these objectives, we analyzed 181 soils from Antarctica, Europe, and the Americas, including samples from subsurface soil horizons in a montane coniferous forest. All soils were analyzed with a recently developed primer set that amplifies a phylogenetically informative region of the 16S rRNA gene with few selective biases

* Corresponding author. University of Colorado at Boulder, Department of Ecology and Evolutionary Biology, CIRES, Box 216 UCB, Boulder, CO 80309-0216, USA. Tel.: +1 303 492 5615 (Office), +1 303 492 2099 (Lab); fax: +1 303 492 1149.
E-mail address: Noah.Fierer@Colorado.edu (N. Fierer).

0038-0717/$ – see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.soilbio.2011.03.012

against *Verrucomicrobia* or any other known bacterial taxa. A barcoded pyrosequencing protocol similar to that described previously (Fierer et al., 2008; Hamady et al., 2008) was used to sequence all PCR products. Estimates of verrucomicrobial abundance were then compared to those from previously published datasets. Patterns of diversity and distribution were examined in order to provide some insight into the ecology of soil *Verrucomicrobia*.

2. Methods

2.1. Sample collection

We collected 112 mineral soil samples from relatively undisturbed sites across North America, South America, Europe, and Antarctica. These represent a wide range of soil types and span a range of edaphic and environmental characteristics (e.g. latitudes between 68°N and 78°S, pH between 3.4 and 9.9, and total organic carbon concentrations between 0.12 and 24.94 mg C kg soil^{-1}). Sampling procedures followed the protocol outlined by Fierer and Jackson (2006). Briefly, samples were taken near the time of the peak growing season for vascular plants (except those from Antarctica where plants are absent). At each site, five to ten randomly selected samples were collected (to a depth of ~5 cm) within an area of about 100 m^2. These were combined into a single composite sample for each site, and then shipped at 4 °C to the University of Colorado at Boulder. Upon receipt, samples were homogenized, sieved to 4 mm, and stored at −20 °C until DNA extraction. To assess how verrucomicrobial abundance varied across soil depth profiles (from the surface down to 170 cm in depth), we also included an additional 69 samples from nine soil pits excavated in a montane coniferous forest in Colorado, USA (Pit 6 was in a meadow). These samples were processed following the procedure described above. Sampling locations and characteristics for all 181 soils included in this study are provided in Supplementary Table S1.

2.2. DNA extraction, PCR amplification and sequence analysis

DNA was extracted from soils using the MO BIO PowerSoil® DNA Isolation Kit, and following the method described in Lauber et al. (2008). Amplicons for barcoded pyrosequencing were generated using the methods described in Lauber et al. (2009), except that triplicate PCR reactions were performed for each sample using the primer set 515F (5′-GTGCCAGCMGCCGCGGTAA-3′) and 806R (5′-GGACTACVSGGGTATCTAAT-3′). The 515F primer included the Roche 454-B pyrosequencing adapter and a GT linker, while 806R included the Roche 454-A sequencing adapter, a 12-bp barcode (unique to each sample), and a GG linker. The region amplified by this primer set is well suited for accurate phylogenetic placement of bacterial sequences (Liu et al., 2007) and, as explained below, the primer set should amplify nearly all bacterial taxa with few biases. Amplicons from the triplicate reactions were combined, cleaned and quantified to determine the volume needed to produce a single composite sample with equal representation of each individual sample. The composite sample was sent to Engencore at the University of South Carolina for sequencing on a Roche 454 automated sequencer.

Pyrosequencing data were processed using the QIIME pipeline (Caporaso et al., 2010). Briefly, sequences were assigned to specific samples based on their unique barcodes and binned into phylotypes at 97% sequence similarity. A representative sequence of each phylotype was chosen and classified using the RDPII taxonomy (Cole et al., 2006). Relative verrucomicrobial abundances were calculated as the percentage of bacterial sequences in each sample assigned to the verrucomicrobial phylum.

Primer coverage predictions were based on the results from the Primer Prospector analysis pipeline (http://pprospector.sourceforge.net/). The Silva dataset (http://www.arb-silva.de/) was used in the *in silico* primer coverage prediction, and filtered by length and percent identity. Length was based on domain of life, with 1450 bp as a minimum sequence length for Archaea and Bacteria, and 1800 bp for Eukarya. Sequences were also filtered to 97% identity using Uclust (Edgar, 2010). Primers were scored by finding the best local alignment to Silva dataset sequences, and assessing a weighted score based on 3′ and non-3′ mismatches. Primer pair coverage (Fig. S1) was generated by using the lowest primer score of the pair.

2.3. Meta-analysis

We reviewed studies that used “universal” bacterial primers for sequence-based surveys (clone library/Sanger sequencing or pyrosequencing) of 16S rRNA genes from soil samples in order to compare our results to previous estimates of relative verrucomicrobial abundances. Only those studies with at least 50 sequences per sample were used for meta-analysis. The ten studies included in the meta-analysis, and the primer sets used in each study are described in Tables S2 and S3, respectively.

3. Results

From the 181 soils included in this study, we obtained a total of 207,015 quality bacterial sequences, with an average read length of

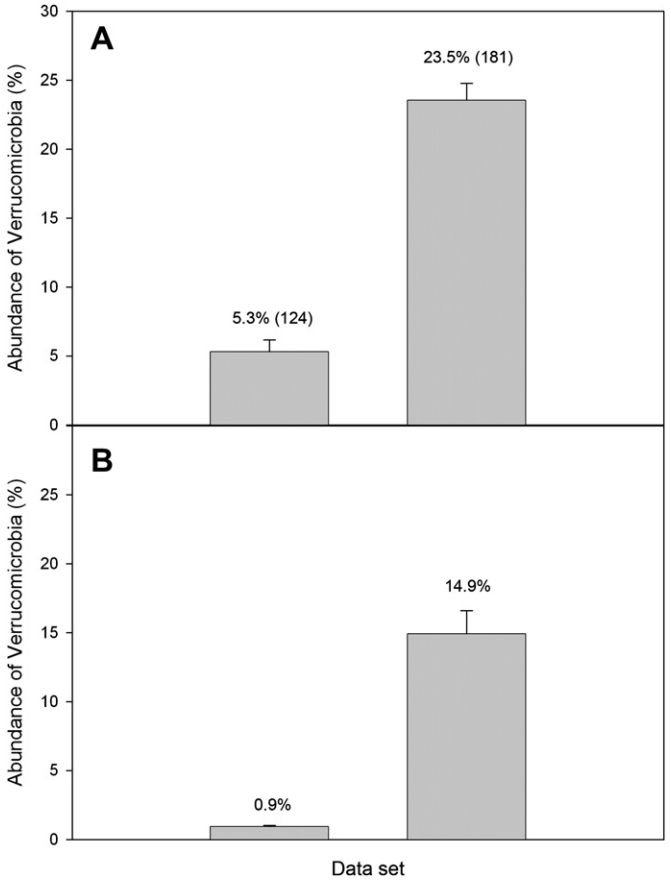

Fig. 1. Relative abundance of *Verrucomicrobia* in soil. A) Percent abundance of *Verrucomicrobia* in previously published datasets (124 soils; left column) and this study (181 soils in all, including both surface and subsurface samples; right column). B) Percent abundance of *Verrucomicrobia* for a subset of 55 soils, analyzed using the 27F/338R primer set (Lauber et al., 2009; left column), then reanalyzed in the present study using the 515F/806R primer set (right column). Error bars represent standard error of the mean.

Table 1
Relative abundance of individual verrucomicrobial phylotypes across surface soils from different biome types. The biome categories are AT (Arctic tundra; 134 total sequences), ASAG (arid/semi-arid grassland; 398 total sequences), ASAS (arid/semi-arid shrubland; 123 total sequences), BF (boreal forest; 508 total sequences), AF (agricultural field; 20 total sequences), CF (coniferous forest; 318 total sequences), DF (deciduous forest; 1298 total sequences), GP (grassland/prairie; 13,460 total sequences), PD (polar desert; 418 total sequences), SACF (semi-arid coniferous forest; 1979 total sequences), and TF (tropical forest; 1327 total sequences). The 15 most abundant phylotypes (ID numbers given to the left, indicated by "PT") are depicted, and the table to the right gives the percent that each phylotype (by row) represents of all verrucomicrobial sequences (across all samples; $n = 112$), the frequency of a phylotype's occurrence (across all samples), and the closest matching cultured isolate. All phylotypes are known to be within the class *Spartobacteria*, except one (PT02535) for which the family-level classification could not be unequivocally determined. The origin of each sample is reported by biome in Supplementary Table S1.

Phylotype	% of sequences	Frequency	Closest matching isolate	GenBank #	% Identity	AT	ASAG	ASAS	BF	AF	CF	DF	GP	PD	SACF	TF
PT00001	30.01	89	*Chthoniobacter flavus*	AY388649	91	4	22	5	24	10	31	27	45	1	23	23
PT00002	19.18	77	*Chthoniobacter flavus*	AY388649	91	0	7	0	0	5	23	7	29	0	4	45
PT06005	6.69	73	*Chthoniobacter flavus*	AY388649	90	0	6	2	9	5	5	5	9	0	10	6
PT03824	6.08	81	*Chthoniobacter flavus*	AY388649	90	31	40	46	11	15	4	5	3	0	31	3
PT00683	4.50	89	*Chthoniobacter flavus*	AY388649	92	9	10	2	15	5	3	5	4	24	11	4
PT02737	2.36	72	*Chthoniobacter flavus*	AY388649	91	26	2	3	10	5	8	13	1	0	3	9
PT13121	1.96	40	*"Candidatus Xiphinematobacter brevicolli"*	AF217462	89	5	0	0	3	25	12	21	1	0	0	1
PT04198	1.76	101	*Chthoniobacter flavus*	AY388649	99	3	5	26	4	10	3	3	1	9	5	1
PT05991	1.69	62	*Chthoniobacter flavus*	AY388649	91	1	1	6	0	0	1	0	0	65	3	2
PT05512	1.35	55	*Chthoniobacter flavus*	AY388649	90	1	0	0	1	0	2	1	2	0	0	3
PT06508	1.02	59	*Chthoniobacter flavus*	AY388649	93	1	4	9	1	0	0	0	1	1	6	0
PT02553	0.98	52	*Chthoniobacter flavus*	AY388649	92	0	1	0	0	0	1	4	1	0	1	1
PT07240	0.97	47	*"Candidatus Xiphinematobacter brevicolli"*	AF217462	89	4	0	0	15	0	3	3	0	0	1	0
PT05715	0.93	40	*Chthoniobacter flavus*	AY388649	90	0	1	1	2	0	0	1	1	0	1	1
PT02535	0.77	58	*"Candidatus Xiphinematobacter brevicolli"*	AF217462	81	16	0	0	4	20	5	3	0	0	1	2

approximately 260 bp. Sequences assigned to the phylum *Verrucomicrobia* made up 48,802 of these sequences. *In silico* tests indicate that most of the commonly used primer sets considered to be "universal" for bacteria are biased against *Verrucomicrobia*, while the primer set used here (515F/806R) is relatively unbiased against this and other bacterial phyla (Tables S4 and S5). This primer bias has a clear effect on estimates of verrucomicrobial abundance, as the average relative abundance of verrucomicrobial sequences was 5.3% (0.0–20.0%) in our meta-analysis of 124 soils from previously published studies using other primer sets, but 23.5% (0.0–72.6%) in our direct analysis of 181 soils (Fig. 1a). In addition, for a subset of 55 soils that were analyzed using both 27F/338R (another commonly used "universal" primer set) and 515F/806R, verrucomicrobial abundances were only 0.9% with 27F/338R (Lauber et al., 2009), but they were 14.9% for these same soils analyzed using 515F/806R (Fig. 1b).

The mean number of verrucomicrobial phylotypes per soil for all 181 soils was 49. Within the phylum, class *Spartobacteria* was dominant, with 92% of all verrucomicrobial sequences (44,847 sequences in total) assigned to this group (Table 1). Subdivision 3

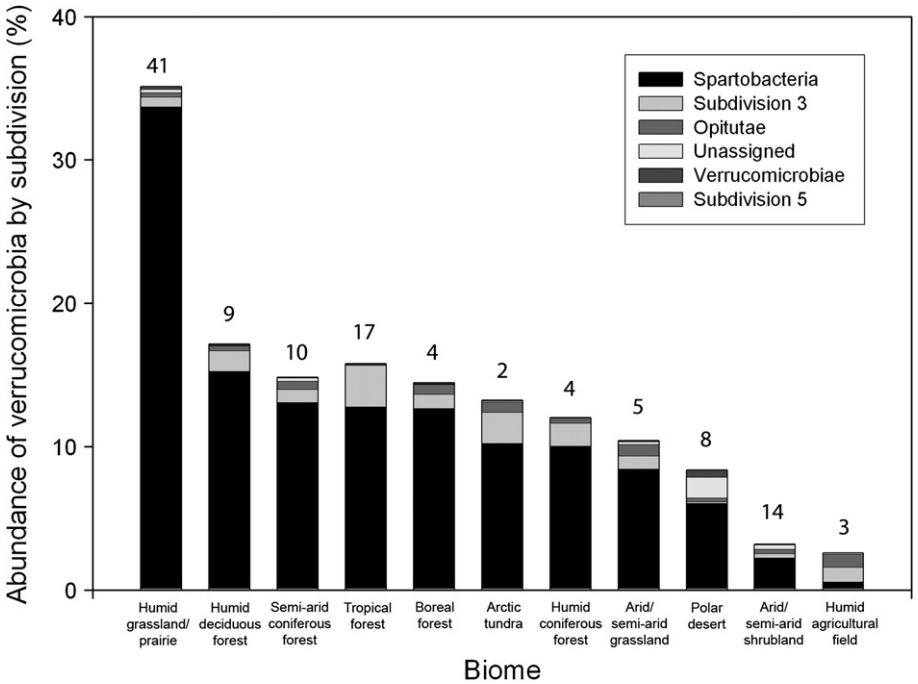

Fig. 2. Abundance of *Verrucomicrobia* by biome. Numbers over each bar represent the number of soil samples for that biome. Total height of each bar represents the mean abundance of *Verrucomicrobia* (relative to all bacteria) in each biome.

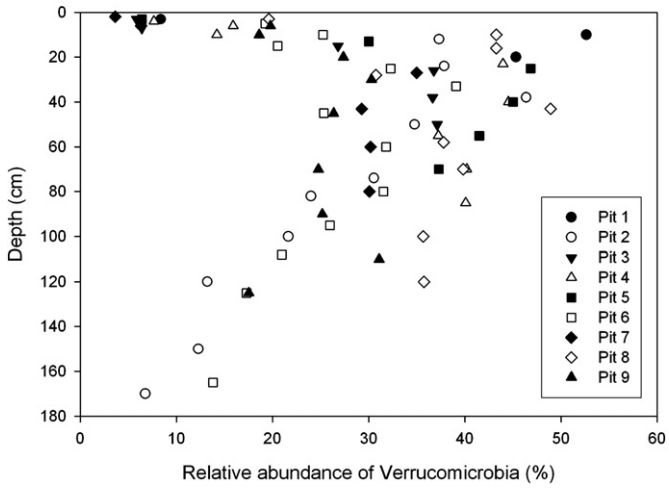

Fig. 3. Relative abundance of *Verrucomicrobia* relative to other bacterial phyla in nine soil pits, ranging in maximum depth from 20 to 170 cm.

and *Opitutae* were the second and third most abundant classes, constituting 5% and 1% of sequences, respectively. A few phylotypes were most closely related to "*Candidatus Xiphinematobacter brevicolli*," an endosymbiont of the nematode *Xiphinema*, which inhabits the rhizosphere as a plant pathogen (Vandekerckhove et al., 2000, 2002). However, the majority of dominant phylotypes were most closely related to *Chthoniobacter flavus*, a free-living soil heterotroph (Sangwan et al., 2004) (Table 1).

Although sampling was unequal across biomes, soil from grasslands and prairies had the highest average relative abundances of *Verrucomicrobia*, averaging 35% of all bacterial sequences (Fig. 2) in individual soils from these biomes. *Spartobacteria* were the most abundant class, but they dropped to only 36% in agricultural fields, while Subdivision 3 and *Opitutae* rose to 44% and 19%, respectively (Fig. 3). We also examined verrucomicrobial distribution in nine pits from a montane coniferous forest, two of which were excavated below 160 cm. The relative abundances of *Verrucomicrobia* ranged from 2 to 20% near the surface (0–6 cm), but were as high as 52% at intermediate depths (approximately 10–50 cm). Class *Spartobacteria* was dominant in all horizons (Fig. 4).

4. Discussion

It is likely that verrucomicrobial abundances have been underestimated in many studies, as a number of commonly used PCR primers targeting 16S rRNA are biased against members of the *Verrucomicrobia* (see Fig. S1, and Tables S4 and S5; see also: Andersson et al., 2008; Hamady and Knight, 2009; Wang and Qian, 2009). Although the discrepancy in estimated verrucomicrobial abundances between the 515F/806R primer set used here and other primer sets (Fig. 1a) could be due, in part, to differences among soils, or the taxonomic schemes used in the different studies, analyses conducted on the same 55 soils with identical methods, but different primers, indicate that verrucomicrobial abundances are likely many times higher than previously estimated (Fig. 1b). Together with *in silico* tests (Tables S4 and S5), these results show that 515F/806R achieves good coverage with no significant bias against the bacterial phyla evaluated, including *Verrucomicrobia*, making it an effective "universal" primer set for bacterial analyses. *Verrucomicrobia* appear to be far more common in soil than previously thought, their average abundance in soil (23.5%) being comparable to that of other phyla considered to be dominant in soil, namely *Proteobacteria* (39%), *Acidobacteria* (20%), and *Actinobacteria* (13%) (Janssen, 2006).

We found that the dominant verrucomicrobial class in nearly all soils was *Spartobacteria* (Table 1), only one representative of which has been cultivated to date (Schlesner et al., 2006). However, other molecular studies have shown that *Spartobacteria* is the most common verrucomicrobial class in soil (Sangwan et al., 2004; Janssen, 2006).

The relative abundance of *Verrucomicrobia* was highly variable across soils, as confirmed in other studies (Buckley and Schmidt,

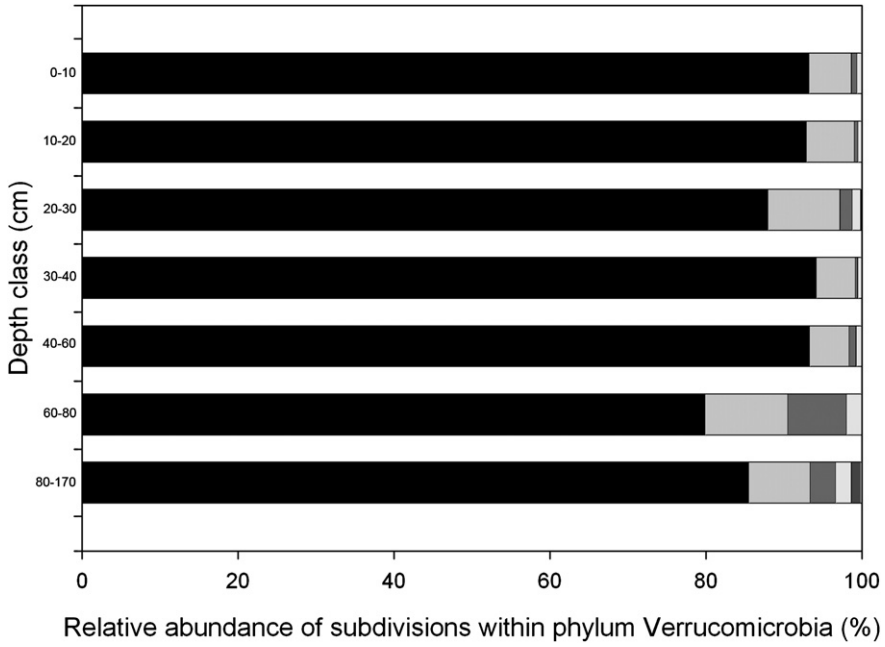

Fig. 4. Abundance of classes within the phylum *Verrucomicrobia* in nine soil pits, ranging in maximum depth from 20 to 170 cm. Classes depicted on bar from left to right: *Spartobacteria*, Subdivision 3, *Opitutae*, Unassigned, and *Verrucomicrobia*.

2001, 2003; Janssen et al., 2002; Janssen, 2006), with members of this phylum apparently most abundant in soils from grasslands and prairies (Fig. 2). With so few soil *Verrucomicrobia* having been cultured, the ecological relevance of this finding remains to be determined. However, it is possible that *Verrucomicrobia* are more abundant in grasslands due to an association with eukaryotic hosts. The most abundant verrucomicrobial class, *Spartobacteria*, contains free-living taxa, as well as a number of endosymbionts associated with nematode worms in the genus *Xiphinema* (Vandekerckhove et al., 2000, 2002; Wagner and Horn, 2006). Because temperate grasslands typically harbor relatively high levels of nematode biomass (Fierer et al., 2009), this may, in part, explain why *Verrucomicrobia* appear to be so abundant in this biome (Janssen, 2006). However, further research is required to test this hypothesis.

In the soil profiles examined from a single, forested watershed, there was a striking increase in verrucomicrobial abundances at intermediate depths (Fig. 3), with *Verrucomicrobia* dominating some of the bacterial communities in horizons 10–50 cm below the surface. Additional research is required to determine if this pattern is observed in profiles from other ecosystem types. Because C availability decreases as one moves down a soil profile (Fierer et al., 2003), *Verrucomicrobia* may be relatively abundant in subsurface horizons due to their oligotrophic life history strategy (da Rocha et al., 2009; Senechkin et al., 2010). Bacteria that are difficult to culture may be specialized for oligotrophy (Garland et al., 2001; Sigler et al., 2002), so the slow growth rate of *Verrucomicrobia* (Janssen et al., 1997, 2002; Janssen, 1998) may allow them to exploit sparse resources in subsurface soils. Alternatively, if there are methanotrophic members of this phylum in soil, as there are in hot springs (Semrau et al., 2008; den Camp et al., 2009; Lee et al., 2009), then the subsurface peak in verrucomicrobial abundance may correspond to the peak in methane oxidation that occurs at similar depths in other forested soil profiles (Mohanty et al., 2006, 2007). *Verrucomicrobia* are likely to occupy very different ecological niches in both surface and subsurface horizons, but the environmental factors regulating their diversity and abundance within and across soil types remains to be determined.

Acknowledgments

We want to thank Spencer Debenport, Rebecca McCulley, and the large number of other collaborators who helped us collect soils from all of the sites included here. We also want to thank Donna Berg-Lyons for help with the laboratory work and Joe Jones from Engencore at the University of South Carolina who conducted the pyrosequencing run. This research was funded by grants to R.K. and N.F. from the Bill and Melinda Gates Foundation, Crohn's and Colitis Foundation of America, National Institutes of Health, National Science Foundation, and the U.S. Department of Agriculture.

Appendix. Supplemental materials

Supplemental materials related to this article can be found online at doi:10.1016/j.soilbio.2011.03.012.

References

Andersson, A.F., Lindberg, M., Jakobsson, H., Baeckhed, F., Nyrén, P., Engstrand, L., 2008. Comparative analysis of human gut microbiota by barcoded pyrosequencing. PLoS ONE 3, e2836. doi:10.1371/journal.pone.0002836.

Buckley, D.H., Schmidt, T.M., 2001. Environmental factors influencing the distribution of rRNA from *Verrucomicrobia* in soil. FEMS Microbiology Ecology 35, 105–112.

Buckley, D.H., Schmidt, T.M., 2003. Diversity and dynamics of microbial communities in soils from agro-ecosystems. Environmental Microbiology 5 (6), 441–452.

Caporaso, J.G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F.D., Costello, E.K., Fierer, N., Pena, A.G., Goodrich, J.K., Gordon, J.I., Huttley, G.A., Kelley, S.T., Knights, D., Koenig, J.E., Ley, R.E., Lozupone, C.A., McDonald, D., Muegge, B.D., Pirrung, M., Reeder, J., Sevinsky, J.R., Turnbaugh, P.J., Walters, W.A., Widmann, J., Yatsunenko, T., Zaneveld, J., Knight, R., 2010. QIIME allows analysis of high-throughput community sequencing data. Nature Methods. doi:10.1038/nmeth.f.303.

Chin, K.J., Liesack, W., Janssen, P.H., 2001. *Opitutus terrae* gen. nov., sp. nov., to accommodate novel strains of the division "*Verrucomicrobia*" isolated from rice paddy soil. International Journal of Systematic and Evolutionary Microbiology 51, 1965–1968.

Cole, J.R., Chai, B., Farris, R.J., Wang, Q., Kulam-Syed-Mohideen, A.S., McGarrell, D.M., Bandela, A.M., Cardenas, E., Garrity, G.M., Tiedje, J.M., 2006. The ribosomal database project (RDP-II): introducing myRDP space and quality controlled public data. Nucleic Acids Research 35 (Database issue), D169–D172. doi:10.1093/nar/gkl889.

da Rocha, U.N., Andreote, F.D., de Azevedo, J.L., van Elsas, J.D., van Overbeek, L.S., 2009. Cultivation of hitherto-uncultured bacteria belonging to the *Verrucomicrobia* subdivision 1 from the potato (*Solanum tuberosum* L.) rhizosphere. Journal of Soils and Sediments 10, 326–339.

Davis, K.E.R., Joseph, S.J., Janssen, P.H., 2005. Effects of growth medium, inoculum size, and incubation time on culturability and isolation of soil bacteria. Applied and Environmental Microbiology 71, 826–834.

den Camp, H.J.M.O., Islam, T., Stott, M.B., Harhangi, H.R., Hynes, A., Schouten, S., Jetten, M.S.M., Birkeland, N.K., Pol, A., Dunfield, P.F., 2009. Environmental, genomic and taxonomic perspectives on methanotrophic *Verrucomicrobia*. Environmental Microbiology Reports 1, 293–306.

Edgar, R.C., 2010. Search and clustering orders of magnitude faster than BLAST. Bioinformatics. doi:10.1093/bioinformatics/btq461.

Fierer, N., Allen, A.S., Schimel, J.P., Holden, P.A., 2003. Controls on microbial CO_2 production: a comparison of surface and subsurface soil horizons. Global Change Biology 9, 1322–1332.

Fierer, N., Jackson, R.B., 2006. The diversity and biogeography of soil bacterial communities. Proceedings of the National Academy of Sciences of the United States of America 103, 626–631.

Fierer, N., Hamady, M., Lauber, C.L., Knight, R., 2008. The influence of sex, handedness, and washing on the diversity of hand surface bacteria. Proceedings of the National Academy of Sciences of the United States of America 105, 17994–17999.

Fierer, N., Strickland, M.S., Liptzin, D., Bradford, M.A., Cleveland, C.C., 2009. Global patterns in belowground communities. Ecology Letters 12, 1238–1249.

Garland, J.L., Cook, K.L., Adams, J.L., Kerkhof, L., 2001. Culturability as an indicator of succession in microbial communities. Microbial Ecology 42, 150–158.

Griffiths, E., Gupta, R.S., 2007. Phylogeny and shared conserved inserts in proteins provide evidence that *Verrucomicrobia* are the closest known free-living relatives of *Chlamydiae*. Microbiology 153, 2648–2654.

Hamady, M., Knight, R., 2009. Microbial community profiling for human microbiome projects: tools, techniques, and challenges. Genome Research 19, 1141–1152.

Hamady, M., Walker, J.J., Harris, J.K., Gold, N.J., Knight, R., 2008. Error-correcting barcoded primers for pyrosequencing hundreds of samples in multiplex. Nature Methods 5, 235–237.

Janssen, P.H., 1998. Pathway of Glucose catabolism by strain VeGlc2, an anaerobe belonging to the *Verrucomicrobiales* lineage of bacterial descent. Applied and Environmental Microbiology 64, 4830–4833.

Janssen, P.H., 2006. Identifying the dominant soil bacterial taxa in libraries of 16S rRNA and 16S rRNA genes. Applied and Environmental Microbiology 72, 1719–1728.

Janssen, P.H., Schuhmann, A., Moerschel, E., Rainey, F.A., 1997. Novel anaerobic ultramicrobacteria belonging to the *Verrucomicrobiales* lineage of bacterial descent isolated by dilution culture from anoxic rice paddy soil. Applied and Environmental Microbiology 63, 1382–1388.

Janssen, P.H., Yates, P.S., Grinton, B.E., Taylor, P.M., Sait, M., 2002. Improved culturability of soil bacteria and isolation in pure culture of novel members of the divisions *Acidobacteria, Actinobacteria, Proteobacteria*, and *Verrucomicrobia*. Applied and Environmental Microbiology 68, 2391–2396.

Joseph, S.J., Hugenholtz, P., Sangwan, P., Osborne, C.A., Janssen, P.H., 2003. Laboratory cultivation of widespread and previously uncultured soil bacteria. Applied and Environmental Microbiology 69, 7210–7215.

Lauber, C.L., Strickland, M.S., Bradford, M.A., Fierer, N., 2008. The influence of soil properties on the structure of bacterial and fungal communities across land-use types. Soil Biology and Biochemistry 40, 2407–2415.

Lauber, C.L., Knight, R., Hamady, M., Fierer, N., 2009. Pyrosequencing-based assessment of soil pH as a predictor of soil bacterial community structure at the continental scale. Applied and Environmental Microbiology 75, 5111–5120.

Lee, K.C., Webb, R.I., Janssen, P.H., Sangwan, P., Romeo, T., Staley, J.T., Fuerst, J.A., 2009. Phylum *Verrucomicrobia* representatives share a compartmentalized cell plan with members of bacterial phylum *Planctomycetes*. BMC Microbiology 9, 5.

Liu, Z., Lozupone, C., Hamady, M., Bushman, F.D., Knight, R., 2007. Short pyrosequencing reads suffice for accurate microbial community analysis. Nucleic Acids Research 35, e120.

Mohanty, Santosh R., Bodelier, Paul L.E., Floris, Virgilio, Conrad, Ralf, 2006. Differential effects of nitrogenous fertilizers on methane-consuming microbes in rice field and forest soils. Applied Environmental and Microbiology 72, 1346–1354. doi:10.1128/AEM.72.2.1346-1354.2006.

Mohanty, Santosh R., Paul Bodelier, L.E., Conrad, Ralf, 2007. Effect of temperature on composition of the methanotrophic community in rice field and forest soil. FEMS Microbiology Ecology 62, 24–31. doi:10.1111/j.1574-6941.2007.00370.x.

O'Farrell, K.A., Janssen, P.H., 1999. Detection of *Verrucomicrobia* in a pasture soil by PCR-mediated amplification of 16S rRNA genes. Applied and Environmental Microbiology 65, 4280–4284.

Sangwan, P., Chen, X., Hugenholtz, P., Janssen, P.H., 2004. *Chthoniobacter flavus* gen. nov., sp. nov., the first pure-culture representative of subdivision two, *Spartobacteria* classis nov., of the phylum *Verrucomicrobia*. Applied and Environmental Microbiology 70, 5875–5881.

Sangwan, P., Kovac, S., Davis, K.E.R., Sait, M., Janssen, P.H., 2005. Detection and cultivation of soil *Verrucomicrobia*. Applied and Environmental Microbiology 71, 8402–8410.

Schlesner, H., Jenkins, C., Staley, J.T., 2006. The phylum *Verrucomicrobia*: a phylogenetically heterogeneous bacterial group. In: Dworkin, M., Falkow, S., Rosenberg, E., Schleifer, K.H., Stackebrandt, E. (Eds.), The Prokaryotes. Springer, New York, pp. 881–896.

Semrau, J.D., DiSpirito, A.A., Murrell, J.C., 2008. Life in the extreme: thermoacidophilic methanotrophy. Trends in Microbiology 16, 190–193.

Senechkin, I.V., Speksnijder, A.G.C.L., Semenov, A.M., van Bruggen, A.H.C., van Overbeek, L.S., 2010. Isolation and partial characterization of bacterial strains on low organic carbon medium from soils fertilized with different organic amendments. Microbial Ecology. doi:10.1007/s00248-010-9670-1.

Sigler, W.V., Crivii, S., Zeyer, J., 2002. Bacterial succession in glacial forefield soils characterized by community structure, activity and opportunistic growth dynamics. Microbial Ecology 44, 306–316.

Vandekerckhove, T.T.M., Willems, A., Gillis, M., Coomans, A., 2000. Occurrence of novel verrucomicrobial species, endosymbiotic and associated with parthenogenesis in *Xiphinema americanum* group species (*Nematoda*, *Longidoridae*). International Journal of Systematic and Evolutionary Microbiology 50, 2197–2205.

Vandekerckhove, T.T.M., Coomans, A., Cornelis, K., Baert, P., Gillis, M., 2002. Use of the *Verrucomicrobia*-specific probe EUB338-III and fluorescent in situ hybridization for detection of "*Candidatus Xiphinematobacter*" cells in nematode hosts. Applied and Environmental Microbiology 68, 3121–3125.

Wagner, M., Horn, M., 2006. The *Planctomycetes, Verrucomicrobia, Chlamydiae* and sister phyla comprise a superphylum with biotechnological and medical relevance. Current Opinion in Biotechnology 17, 241–249.

Wang, Y., Qian, P.Y., 2009. Conservative fragments in bacterial 16S rRNA genes and primer design for 16S ribosomal DNA amplicons in metagenomic studies. PLoS ONE 4, e7401. doi:10.1371/journal.pone.0007401.

Ward, N., Staley, J.T., Fuerst, J.A., Giovannoni, S., Schlesner, H., Stackebrandt, E., 2006. The order Planctomycetales, including the genera *Planctomycetes*, *Pirellula*, *Gemmata*, *Isosphaera* and the Candidatus genera *Brocadia*, *Kuenenia* and *Scalindua*. In: Dworkin, M., Falkow, S., Schleifer, K.H., Stackebrandt, E. (Eds.), The Prokaryotes. Springer, New York, pp. 757–793.

Zhang, L., Xu, Z., 2008. Assessing bacterial diversity in soil. Journal of Soils and Sediments 8, 379–388.