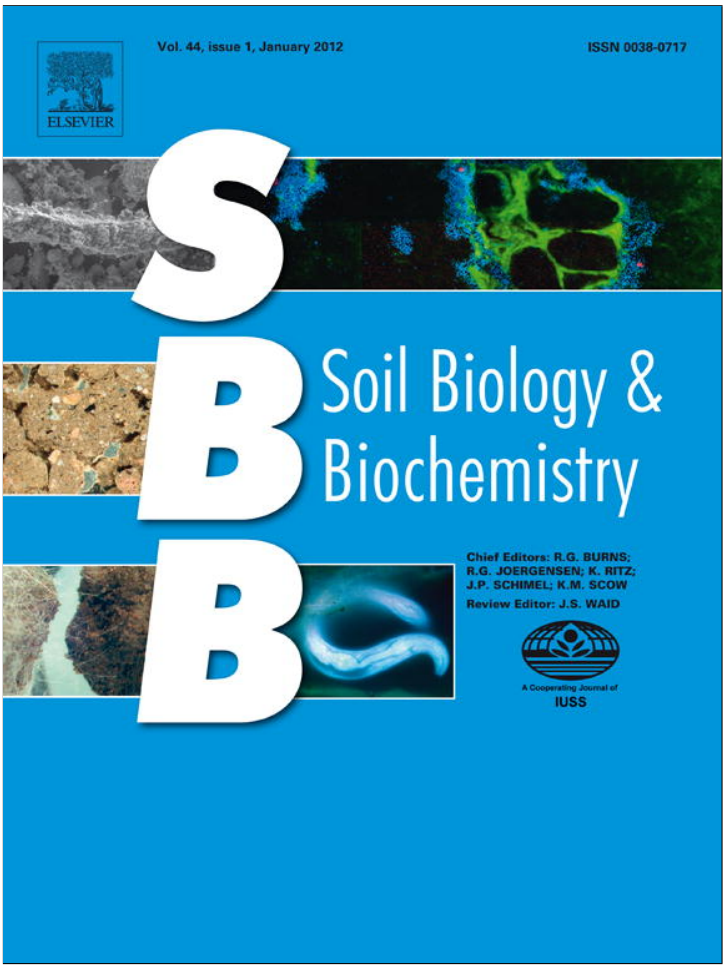


(This is a sample cover image for this issue. The actual cover is not yet available at this time.)

Soil Biology & Biochemistry 50 (2012) 58–65

Contents lists available at SciVerse ScienceDirect

Soil Biology & Biochemistry

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

# Digging deeper to find unique microbial communities: The strong effect of depth on the structure of bacterial and archaeal communities in soil

Kathryn G. Eilers [a], Spencer Debenport [b], Suzanne Anderson [c], Noah Fierer [a,d,*]

[a] Department of Ecology and Evolutionary Biology, University of Colorado, Boulder, CO 80309, USA
[b] Department of Plant Pathology, The Ohio State University – OARDC, Wooster, OH 44691, USA
[c] Department of Geography, University of Colorado, Boulder, CO 80309, USA
[d] Cooperative Institute for Research in the Environmental Sciences, University of Colorado, Boulder, CO 80309, USA

ARTICLE INFO

*Article history:*
Received 28 September 2011
Received in revised form
3 January 2012
Accepted 12 March 2012
Available online 27 March 2012

*Keywords:*
Soil depth
Pyrosequencing
16S rRNA gene
Soil bacteria
Soil archaea
Microbial diversity

ABSTRACT

Microorganisms exist throughout the soil profile and those microorganisms living in sub-surface horizons likely play key roles in nutrient cycling and soil formation. However, the distributions of microbes through the soil profile remain poorly understood, as most studies focus only on those communities found in near-surface horizons. Here we examined how microbial community structure changes within soil profiles, whether these changes are similar across soils from different landscape positions, and how the community-level variation within individual soil depth profiles compares to the variation across surface soils from a wide range of biomes. We characterized changes in bacterial and archaeal community composition and diversity with depth through nine soil profiles located in a forested montane watershed in Colorado, USA. Microbial community composition was determined by barcoded pyrosequencing of the 16S rRNA gene employing a primer set that captures both bacteria and archaea. Relative microbial biomass and soil carbon concentrations decreased exponentially with depth while soil pH increased in nearly all of the profiles examined. Bacterial diversity was typically highest in the top 10 cm of the profile; diversity typically dropped by 20–40% from the surface horizons to the deepest horizons sampled. Community composition was significantly affected by soil depth in all profiles, driven primarily by a decline in the relative abundance of *Bacteroidetes* with depth and the peak in the relative abundance of *Verrucomicrobia* between 10 and 50 cm. Microbial community composition across the nine pits was most variable in the surface horizons; communities at deeper soil depths were relatively similar regardless of landscape position. When compared to the microbial communities from 54 previously-analyzed surface soils collected across a wide range of biome types, we found that there was as much variation within individual soil pits as across surface soils from different biomes, emphasizing the importance of soil depth as an environmental gradient structuring soil microbial communities.



## 1. Introduction

Although soil microbes influence biogeochemical processes throughout the soil profile, our understanding of the structure and diversity of soil microbial communities is predominantly limited to surface horizons, with the vast majority of studies focusing solely on the top 15 cm of the soil column. As a result, microbial communities that exist at deeper soil depths have not been well characterized and the spatial variability exhibited by these communities remains poorly understood.

While microbial biomass often exhibits exponential decreases with depth (Blume et al., 2002; Fierer et al., 2003; Hartmann et al., 2009), there are still active cells in deeper soil horizons and in saprolite (Buss et al., 2005; Richter and Markewitz, 1995). On a depth-weighted basis, a significant portion of the microbial biomass contained in soil (35% and 50%, Fierer et al., 2003 and Schutz et al., 2010; respectively) is located in sub-surface horizons (defined as below 25 cm in Fierer et al. and below 40 cm in Schutz et al.). On a per cell basis, these deeper microbes may, arguably, have a greater influence on soil formation processes than their counterparts at the surface due to their proximity to parent material (Buss et al., 2005). Likewise, sub-surface microbes likely

* Corresponding author. Department of Ecology and Evolutionary Biology, University of Colorado, CIRES Bldg. Rm. 318, UCB 216, Boulder, CO 80309-0216, USA. Tel.: +1 303 492 5615; fax: +1 303 492 1149.
*E-mail address:* Noah.Fierer@colorado.edu (N. Fierer).

0038-0717/$ – see front matter © 2012 Elsevier Ltd. All rights reserved.
doi:10.1016/j.soilbio.2012.03.011

have a critical influence on longer-term soil carbon sequestration given that sub-surface horizons can harbor substantial quantities of organic C with long turnover times (Fierer et al., 2005; Rumpel and Kogel-Knabner, 2011; Trumbore, 2000).

A wide range of edaphic factors can shape the composition of soil microbial communities, including: pH, nutrient levels, the quantity and quality of organic C, moisture availability, and $O_2$ levels. Since nearly all of these edaphic factors typically change with soil depth, we would expect strong, corresponding shifts in the structure of microbial communities through soil profiles. Indeed, previous work has demonstrated significant changes in microbial communities with soil depth. The diversity of microorganisms typically decreases with depth, whether diversity is measured by DNA fingerprinting (Agnelli et al., 2004; Goberna et al., 2005; LaMontagne et al., 2003), phospholipid fatty acid profiles (Fierer et al., 2003), or 16S rRNA gene sequences (Will et al., 2010). We also know that the composition of microbial communities typically changes with soil depth (Ekelund et al., 2001; Fritze et al., 2000; Hansel et al., 2008; Hartmann et al., 2009; Will et al., 2010; Zvyagintsev, 1994). However, with a few notable exceptions (e.g. Hansel et al., 2008; Hartmann et al., 2009; Will et al., 2010), much of this previous work has lacked the phylogenetic and/or taxonomic resolution to document which specific microbial taxa changed in abundance through the soil profiles examined. We still have only a limited understanding of how depth gradients in microbial community structure vary across distinct soil profiles and how landscape position influences the communities found in deeper soil horizons. Likewise, there is little information available on whether certain bacterial or archaeal taxa are restricted to specific soil depths and how the magnitude of variation in microbial communities within a given soil profile compares to the known variation in surface soil communities across biomes.

Here, we used high-throughput, barcoded pyrosequencing to examine shifts in soil bacterial and archaeal communities with depth across nine soil pits located within a forested, montane watershed. Our objective was to document changes in overall community structure and the relative abundances of individual taxa within and across soil profiles at a detailed level of spatial, taxonomic, and phylogenetic resolution. By sub-sampling thick horizons and sampling as deeply as possible, we were able to gain a relatively detailed picture of how community composition changes with depth. We hypothesized that community composition within each pit would vary depending on landscape position since soil edaphic factors are heterogeneous across the landscape. We also hypothesized that the pronounced changes in environmental conditions with soil depth generate differences between surface and sub-surface microbial communities within individual profiles that are similar in magnitude to those differences observed between surface communities from very different soil types.

## 2. Methods

### 2.1. Site Description

Sampling was conducted within two regions of the Gordon Gulch watershed (105.47 W, 40.01 N), part of the Boulder Creek Critical Zone observatory east of Boulder, Colorado, USA (Supplementary Fig. 1). Gordon Gulch is an upper montane forest (average elevation = 2627 m) with clear north/south-facing slopes, a central meadow in the upper portion of the watershed, and a forested riparian area in the lower portion. Ponderosa pines (*Pinus ponderosa*) dominate south-facing slopes and lodgepole pines (*Pinus contorta*) dominate north-facing slopes. Soils are derived from paleoproterozoic biotite schist and biotite gneiss bedrock (Cole and Braddock, 2009). The nine sampled pits were located on south-facing slopes (Pits 1, 4, 5, and 8) and on north-facing slopes (Pites 3, 7, and 9). Pit 2 is located in a forested riparian area and Pit 6 is located in a meadow in the upper portion of the watershed (Supplementary Fig. 1).

### 2.2. Sample collection

Samples were collected from the nine soil pits over a five-day period in July, 2009 and there were no measurable precipitation events during the sampling period. Pits were excavated as deeply as possible (20–180 cm in depth), with all of the pits excavated to the soil/saprolite boundary except for Pit 6, which was excavated to the water table without encountering bedrock. Soil pits were sampled by horizon via horizontal coring of cleaned pit faces, with multiple samples collected from thicker horizons for a total of 69 samples (3–12 samples per pit depending on soil depth, see Supplementary Table 1). Samples for microbial analyses were collected last (deepest into the face) to minimize cross-contamination. Soil samples were sieved to 2 mm, stored at 4 °C and split for analysis within a 2 d of collection. Samples used for soil microbial community analysis were frozen at −80 °C until DNA was extracted.

### 2.3. Soil characteristics

Relative changes in microbial biomass with depth were determined using the substrate-induced respiration (SIR) method as described previously (Fierer et al., 2003). Briefly, 10 mL of yeast extract solution (3 g in 250 mL of de-ionized water) was added to 5 g of field-moist soil and shaken horizontally for 4 h at 20 °C. $CO_2$ production was measured at 1.5, 3, and 4.5 h with an infrared gas analyzer (IRGA) (CA-10a, Sable Systems, Inc., Las Vegas, NV, USA). Soil pH was assessed by mixing soil and water in a 1:2 ratio (by volume) and then measured with a pH probe (Accumet AB15 basic pH meter) after 1 h of incubation. Moisture content was calculated by oven drying soil at 60 °C for 48 h. About l g of the dry soil was then ground in a ceramic mortar and pestle and concentrations of soil carbon and nitrogen were measured on a Costech, ECS 4010 CHN Analyzer. Extractable $NH_4^+$ and $NO_3^-$ concentrations were assessed by shaking 5 g of field-moist soil with 40 mL 0.5 M $K_2SO_4$ for 1 h. The soil slurries were then filtered through 0.2 μm filters and frozen at −20 °C until $NH_4^+$ and $NO_3^-$ levels were measured on a Lachat QuikChem 8500 spectrophotometric flow injection analyzer (Hach, Loveland, CO, USA).

### 2.4. Microbial community analysis

DNA was extracted from each of the 69 collected soils using a MoBio PowerSoil Kit (MoBio Laboratories, Carlsbad, CA, USA) following the manufacturer's instructions. DNA was PCR-amplified in triplicate using barcoded 16S rRNA gene prokaryotic primers following the protocol described in Bates et al. (2011). This primer set (515f, 806r) was designed to amplify the hypervariable V4 region of the 16S rRNA gene from nearly all bacteria and archaea (Bates et al., 2011; Bergmann et al., 2011), with the amplified region well-suited for accurate phylogenetic and taxonomic placement of sequences (Liu et al., 2007). Amplicons from each sample were pooled in equimolar concentrations into a single aliquot that was shipped on dry ice to the Environmental Genomics Core Facility at the University of South Carolina for pyrosequencing on a 454 Life Sciences Genome Sequencer FLX (Roche) machine running the Titanium chemistry. Pyrosequencing yielded 213,088 quality sequences in total with read lengths equal to the full lengths of the amplicons.

Raw sequence data was processed using QIIME (Caporaso et al., 2010). Sequences shorter than 250 bp or longer than 500 bp and

with a quality score lower than 25 were eliminated. Sequences were assigned to samples by the 12-bp barcodes and then grouped into phylotypes using Cd-hit (Li and Godzik, 2006) with a phylotype threshold of ≥97% sequence similarity. Representative sequences were classified using nucleotide BLAST against the Greengenes core set (DeSantis et al., 2006). If there were conflicting classifications the sequence was discarded and only sequences classified as either bacterial or archaeal were included in downstream analyses.

Shifts in community structure were visualized using a principle coordinates analysis of the pairwise weighted UniFrac distances (Lozupone and Knight, 2005, 2008). The Unifrac metric was used to assess beta diversity patterns, the differences in community composition between pairs of samples. In particular, Unifrac assesses the phylogenetic distance between pairs of communities by quantifying the relative proportion of shared lineages with the weighted Unifrac algorithm taking the abundances of individual phylotypes into account. Alpha diversity metrics, including both the number of observed phylotypes per sample and Faith's phylogenetic diversity (Faith, 1992) were calculated using QIIME by rarifying all samples to the same sequencing depth (826 sequences per sample).

To assess changes in community relatedness with depth, we averaged the pairwise weighted UniFrac distances for soil depth intervals for the six pits that were deeper than 80 cm. We then compared the average pairwise UniFrac distance between samples within each depth interval. To determine if differences in community composition were significant, we used ANOSIM analyses and Mantel tests, as implemented in PRIMER (Clarke and Gorley, 2006), relating pairwise Unifrac distances to depth within individual profiles.

### 2.5. Comparison with global soils

We directly compared the microbial communities found in the collected pit soils to a previously-analyzed set of 54 surface soils (top 5 cm) collected from a wide range of biomes (forest, desert, and grassland soils from humid and dry tropical, temperate, and polar biomes). The microbial communities in these soils were analyzed in the same manner described above; details on these samples are provided in Bates et al. (2011) and in Supplementary Table 3. All sequence data (the 213,088 sequences from the pit soils plus 69,714 quality sequences from the cross-biome survey of surface soils) were analyzed together in QIIME using the method described above with minor modifications. Briefly, new phylotypes were picked from the combined sequence files using the BLAST method in QIIME against the GreenGenes core set (DeSantis et al., 2006) with a phylotype threshold of ≥90% sequence similarity to decrease the complexity of the data set. The resulting phylotype table was then rarified to 800 sequences per sample and the weighted Unifrac metric was used to calculate pairwise distances between all 123 communities. We used ANOSIM analyses to determine if those communities found within individual profiles are significantly different from the range of communities found across the 54 surface soils.

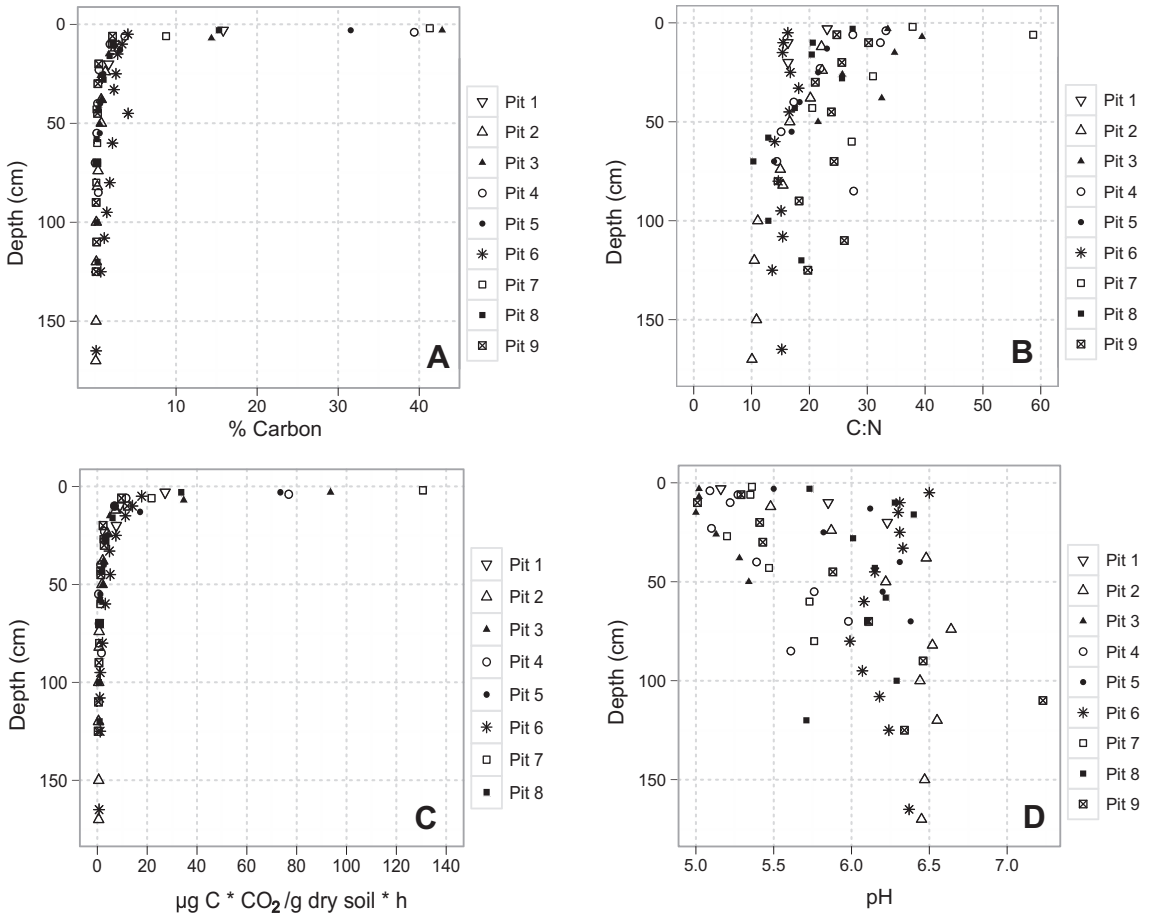


**Fig. 1.** Key soil profile characteristics in the nine pits. Plots show depth profiles (symbols in legend) of (A) percent soil carbon plotted on log scale, (B) C:N ratio, (C) relative microbial biomass as measured by substrate-induced respiration, plotted on log scale, and (D) soil pH. Complete soil characteristics are available in Supplementary Table 1.

## 3. Results

### 3.1. Soil characteristics

Soil characteristics varied with depth and between soil pits (Fig. 1 and Supplementary Table 1). Briefly, soil carbon and nitrogen concentrations decreased exponentially with depth. The C:N ratio decreased with depth (from 16 to 37 at the surface to 10 to 15 in sub-surface horizons; Fig. 1b) while pH tended to increase with depth (ranging from 5.0 to 7.2 across all samples, Fig. 1d). Soil moisture content at the time of sampling was variable throughout the soil profiles with the deepest sample from Pit 6 having a very high moisture content as it was taken near the water table (Supplementary Table 1). Relative microbial biomass decreased exponentially with depth, decreasing by nearly two orders of magnitude from the near-surface horizons to the deepest depths sampled (Fig. 1c); however, even the deepest soils had microbial biomass levels that were measurable and well above our detection limits.

### 3.2. Alpha diversity patterns

Diversity levels typically decreased with soil depth, whether diversity was measured using phylotype richness (number of phylotypes per sample, Fig. 2a) or phylogenetic diversity (Faith's PD, Fig. 2b). We divided all samples into two categories: shallow communities (0–10 cm) and deeper communities (those below 11 cm) and found significant differences in diversity regardless of the diversity metric employed (*T*-test *P* values $< 0.01$ in both cases). Within individual pits, the relationships between soil depth and either phylotype richness or phylogenetic diversity were significant in 7 of the 9 pits (Fig. 2). Likewise, there were significant relationships between both alpha diversity metrics and two of the measured soil variables indicated in Supplementary Table 1 (%C and pH) in the same 7 pits (Spearman's $r > 0.5$, $P < 0.01$ in all 7 cases).

### 3.3. Beta diversity patterns

There were pronounced changes in microbial community composition with depth and, across all samples, the shallow horizons (0–10 cm) harbored significantly different communities from the deeper horizons (>11 cm in depth)(ANOSIM $P < 0.001$). This pattern held in nearly every pit examined; the surface communities were distinct from the sub-surface communities with the transition from shallow to sub-surface communities generally occurring between 10 and 25 cm in depth (Fig. 3). Across the 9 pits, the communities in the near-surface horizons and in the deepest horizons were most variable and those communities between 20 and 60 cm in depth were relatively similar regardless of the pit sampled or the landscape position of the pit (Fig. 4). The beta diversity levels within each pit (i.e. the weighted Unifrac values) were only correlated with one of the measured soil variables listed in Supplementary Table 1 (%C) as determined by Mantel tests (Spearman's $r > 0.4$, $P < 0.02$ in all nine pits). However because %C is strongly correlated with soil depth, we do not know if the changes in %C are indeed the sole factor driving the observed beta diversity patterns.

### 3.4. Shifts in taxon abundances with depth

The microbial communities in the soil profiles were dominated by the following bacterial phyla: *Verrucomicrobia*, *Proteobacteria*, *Acidobacteria, Actinobacteria, Bacteroidetes*, *Gemmatimonadetes*, and *Planctomycetes* (average relative abundances of 28, 18, 16, 13, 5, and 3% across all 69 pit samples analyzed). The relative abundances of

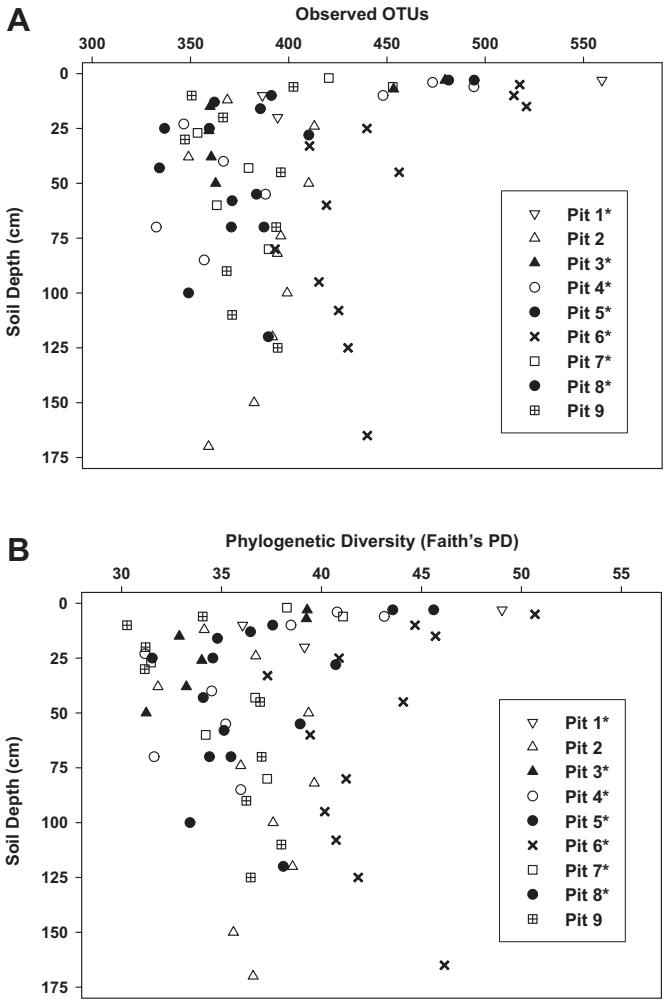


**Fig. 2.** Changes in relative alpha diversity levels with depth. Panel A shows average observed number of phylotypes (OTUs) in each sample while panel B shows differences in phylogenetic diversity (Faith's PD). In both cases, the diversity metrics were calculated using rarefied data (826 total sequences per sample). Asterisks after the pit names in the legend indicate those pits which exhibited a statistically significant correlation between depth and diversity levels ($P < 0.01$).

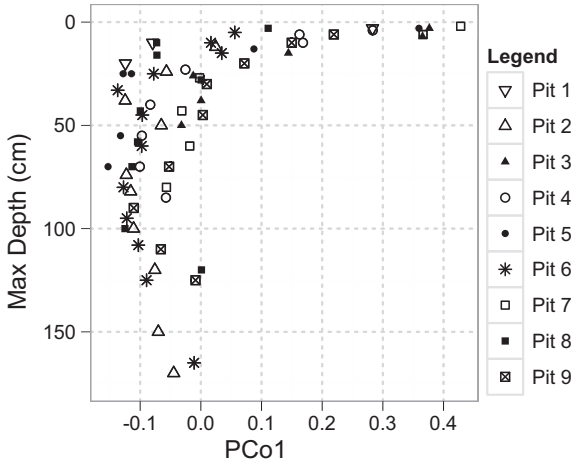


**Fig. 3.** Change in microbial community composition with depth (beta diversity) as measured by the score on the first principle coordinate axis of a principle coordinates analysis of the weighted UniFrac distance matrix.

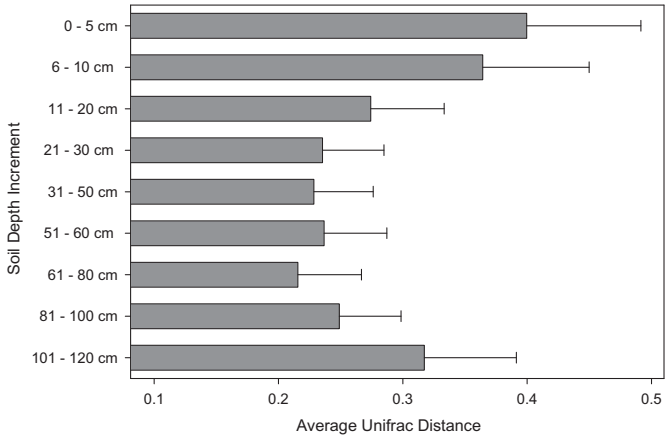


**Fig. 4.** Variations in community structure with depth as measured by the average weighted UniFrac distance within each depth interval (*y*-axis). Error bars are the standard deviation of the Unifrac distances between samples within each depth interval.

individual bacterial taxa were highly variable across the collected samples (see Supplementary Table 2 for full taxon abundance data) with a subset of major taxonomic groups largely driving the depth-related beta diversity patterns evident from Fig. 3. Relative abundances of the phylum *Bacteroidetes* declined exponentially with depth while *Actinobacteria* were most abundant at the surface and in the deepest regions of the pits (Fig. 5), but this pattern was not uniform across all of the sampled pits. Likewise, *α-Proteobacteria*, decreased in relative abundance with depth (Fig. 5), particularly the Caulobacterales, the dominant α-proteobacterial group (1–20% of all bacterial sequences), which decreased with depth in seven pits from an average of approximately 15% in the top 10 cm to <5% in the deeper horizons (see Supplementary Table 2). Members of the *β-Proteobacteria* and *γ-Proteobacteria* sub-phyla also decreased in relative abundance with depth (Fig. 5). The phylum *Verrucomicrobia* peaked in relative abundance between 20 and 40 cm where they comprised up to 52% of the community in some samples. The relative abundances of *Acidobacteria*, *Gemmatimonadetes*, and *Planctomycetes* did not exhibit any clear shifts in relative abundances with depth across the pits (Fig. 5, Supplementary Table 2).

Archaea, represented by only 1% of all sequences analyzed, had relative abundances in individual samples that ranged from 0 (no archaeal sequences detected) to 9% (Fig. 5). Although archaeal sequences were identified from all pits, they were most commonly found in four pits, including the two deepest pits (Pits 2 and 6) that were located by a stream and in a meadow (respectively). The relative abundance of archaea increased with depth in some of the pits (Fig. 5), although they remained only a small proportion of the community in all pits. The archaeal community was dominated by five phylotypes, which comprised 76% of the archaeal sequences, with nearly all of these sequences (95%) classified as members of crenarchaeotal group I.1b (also known as the Thaumarchaeota, Pester et al., 2011).

### 3.5. Community changes with depth versus changes across a range of surface soils

To put the overall changes in microbial community composition with soil depth into context, we compared all soils sampled for this study to 54 surface soils (0–5 cm) collected from locations throughout North and South America that were analyzed in the same manner as the soil profile samples from Gordon Gulch. We found that the differences in community structure with depth in Gordon Gulch soils were comparable to differences in community structure across the collection of surface soils collected from many different types of ecosystems (Fig. 6). In other words, the magnitude of the community changes with depth in the Gordon Gulch soil profiles (average pairwise Unifrac distances across samples collected from individual pits ranged from 0.32 to 0.45) was equivalent to the magnitude of the community changes observed across surface samples collected from a wide range of soil types, biomes, and climates (where the average Unifrac distance was 0.39). Although each of the 123 soils included in the Fig. 6 meta-analysis harbored distinct bacterial communities, the soil microbial communities from the deeper soil horizons analyzed in this study were most similar to the surface soils collected from a variety of temperate and tropical forest sites (see Supplementary Table 3). This pattern is confirmed by the ANOSIM analyses, when we compared those communities found in individual pits to those communities found across the 54 surface soils, none of the individual pits harbored communities distinct from those found across the range of surface soils sampled from other sites ($P > 0.1$ in

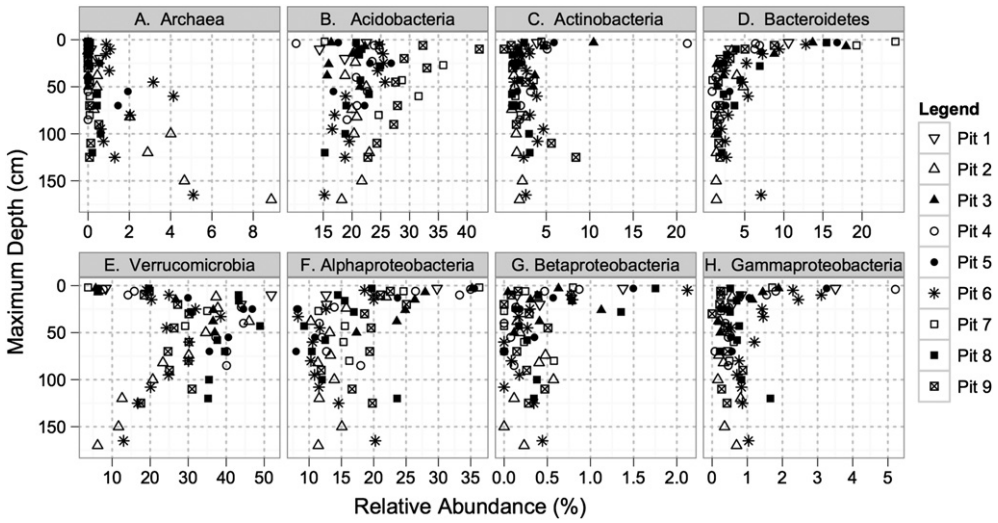


**Fig. 5.** Change in the relative abundance of archaea and selected bacterial taxa with depth in the nine soil pits. For full details on the relative abundances of archaeal and bacterial taxa, see Supplementary Table 2.

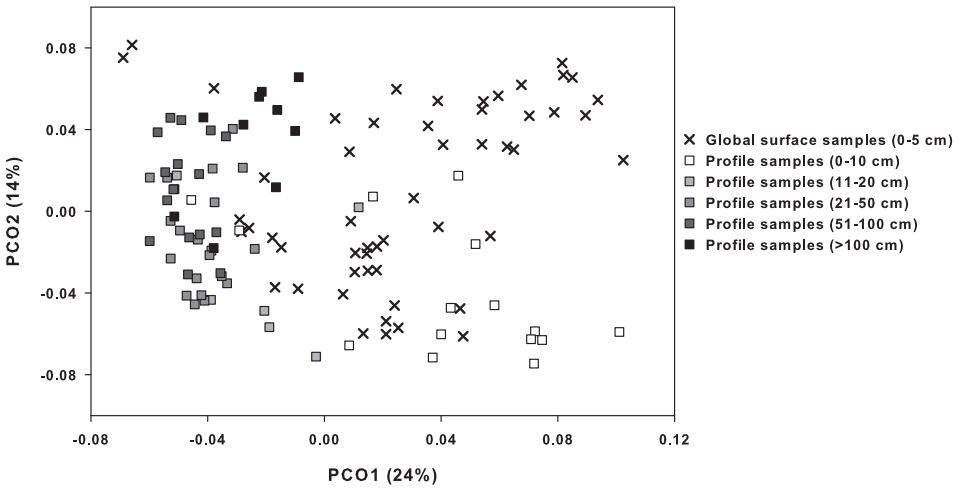


**Fig. 6.** Comparison of the Gordon Gulch pit samples to previously-analyzed global surface soils based on principle coordinates analysis of pairwise weighted UniFrac distances. For further details on the soils included in this analysis, see Supplementary Table 3.

all nine cases). What this means is that the depth-related gradients in community composition were as strong (or stronger) than the gradients in community composition found across the wide range of biomes sampled.

## 4. Discussion

The studied soil profiles represent strong environmental gradients, with multiple edaphic factors changing with depth (Fig. 1 Supplementary Table 1). One of the most pronounced changes through the profiles was the nearly exponential decrease in microbial biomass with depth, a pattern that parallels the decrease in carbon quantity (Fig. 1) and a presumed decrease in carbon quality with depth (Rumpel and Kogel-Knabner, 2011). Although the substrate-induced respiration method we used to estimate changes in microbial biomass probably overestimates microbial biomass at shallow depths and underestimates microbial biomass at deeper depths (Fierer et al., 2003; Wardle and Ghani, 1995), the observed changes in microbial biomass through the soil profiles are consistent with other studies (Ekelund et al., 2001; Fierer et al., 2003; Fritze et al., 2000; Hartmann et al., 2009). We also observed decreases in microbial diversity in nearly all of the pits (Fig. 2), a pattern similar to those found in other fingerprinting-based studies (Agnelli et al., 2004; Fierer et al., 2003; Goberna et al., 2005; LaMontagne et al., 2003; Will et al., 2010). Together these results suggest that changes in environmental conditions with soil depth represent a strong ecological filter, and that many surface-dwelling microorganisms are less likely to thrive in the environments of the deeper soil horizons. This hypothesis is supported by the results presented in Fig. 3; the overall structure of the microbial communities changed markedly with depth in all profiles, with the most pronounced changes occurring within the top 25 cm.

The magnitude of the depth-related changes in microbial community composition are clearly evident from Fig. 6, where we directly compared the soil profile communities to communities from surface soils collected from across the globe (which included soils from deserts, tropical rainforests, prairies, boreal forests, and temperate forests). Microbial communities within the same profile, even communities separated by as little as 10–20 cm in depth, can be as distinct from one another as soil communities from completely different biomes separated by thousands of kilometers (Fig. 6, Supplementary Table 3), highlighting the strength of the environmental gradient present within the soil profiles. Interestingly, the communities from the deeper soil horizons are most similar to those surface soils from forested sites in both semi-arid and more humid regions (Supplementary Table 3), even though such soils do not have particularly low organic carbon concentrations. This pattern suggests that it may be the type of carbon, not necessarily the amount of carbon, that is driving the apparent similarity between the deeper soils in the forested watershed studied here and the surface soils from other forested sites.

When we compared the microbial communities from the different pits to one another, we found that the communities in the near-surface horizons were most variable across the nine pits. Communities from intermediate depths (approximately 20–60 cm) were relatively uniform in composition across all pits, regardless of landscape position or vegetation cover. The greater variability in the near-surface soil communities across the nine pits is likely a product of the greater variability in edaphic factors (including pH and organic C concentrations, Fig. 1) and environmental conditions (particularly differences in temperature and moisture regimes between the north and south-facing slopes). In contrast, the intermediate soil depths are more uniform in edaphic characteristics and temperature or moisture differences between pits are likely to be less pronounced Furthermore, the influences of differences in vegetation types on microbial community structure may be more apparent in surface horizons where root densities and litter inputs are highest. Interestingly, the communities at the deepest soil depths are, like the surface communities, highly variable across the different pits. This may, in part, reflect differences in parent material across the watershed, the degree of weathering of the parent material, or differences in hydrologic conditions in the deeper soil depths. Alternatively, the high variability in community composition across the deeper soil horizons may be a product of stochastic processes; alpha diversity levels are lower in the deeper soil depths (Fig. 2) and, just by chance, the taxa that get dispersed into the deeper soil horizons vary across the pits sampled.

From Fig. 5 it is apparent which specific microbial taxa were responsible for the overall community shifts summarized in Figs. 3 and 6. Changes in the relative abundance of *Bacteroidetes* with depth were particularly striking, as members of this bacterial phylum were far more abundant in the near-surface horizons than in deeper depths (Fig. 5), a pattern also observed by Will et al. (2010). Although *Bacteroidetes* is a diverse phylum, there is some evidence to suggest that soil *Bacteroidetes* are typically copiotrophic

and are most abundant in soils that have relatively large amounts of labile organic carbon, including rhizosphere soils (Fierer et al., 2007). Thus, the higher abundances of *Bacteroidetes* in the surface horizons may, in part, reflect increased organic C availability. This hypothesis may also explain decreases in the abundances of the proteobacterial taxa with depth that were observed here (Fig. 5) and in other studies (Hansel et al., 2008; Will et al., 2010), as a variety of soil proteobacterial taxa are also considered to be copiotrophic (Eilers et al., 2010; Goldfarb et al., 2011; Nemergut et al., 2010).

In contrast, the Verrucomicrobia exhibited a very different pattern with soil depth. There was a clear, mid-profile peak in the relative abundances of Verrucomicrobia that was fairly consistent across all of the profiles with Verrucomicrobia representing >40% of the sequences from individual samples at these depths (Fig. 5). We do not know why this mid-profile peak in verrucomicrobial abundances exists, but it has also been observed in other profiles (Hansel et al., 2008). Although *Verrucomicrobia* are likely to be far more abundant in soil than previous studies would suggest (as most of the commonly-used 'universal' bacterial primers are biased against Verrucomicrobia, (Bergmann et al., 2011)) the ecological niches inhabited by soil Verrucomicrobia remain largely undetermined and there are few cultured isolates representing those verrucomicrobial taxa most commonly found in soil, (Bergmann et al., 2011). However, it has been suggested that many soil Verrucomicrobia are oligotrophic and able to grow under conditions of low C availability, which may, in part, explain their distributions through the profiles examined here. Clearly more work is needed to understand the ecology of this bacterial phylum which appears to be dominant in the sub-surface soil horizons examined here.

Previously published studies have shown that other bacterial phyla including *Actinobacteria* and *Acidobacteria* change in relative abundance with soil depth (Hansel et al., 2008; Hartmann et al., 2009; Will et al., 2010), yet these taxa did not exhibit consistent shifts in relative abundance with depth through the profiles examined here. This inconsistency suggests that changes in bacterial community composition with depth are, to some degree, site specific and dependent on specific characteristics of the profiles being studied.

Although relatively rare in nearly all profile samples, members of the archaeal domain did appear to be most abundant in the deepest soil depths (Fig. 5). Most of these archaea were classified as members of crenarchaeotal group I.1b (Thaumarchaeota), the dominant archaeal taxon in soils (Bates et al., 2011). Previous work has also shown that members of 'group 1' Crenarchaeota can be found in deeper soil depths (Hansel et al., 2008; Hartmann et al., 2009) and that ratios of archaeal:bacterial ratios increase with soil depth (Kemnitz et al., 2007). Although the ecology of this taxon remains unclear, there is widespread speculation that they are ammonia oxidizers and may be driving autotrophic nitrification in the deeper soil depths (Leininger et al., 2006), but this cannot be confirmed with this study.

## 5. Conclusions

We found pronounced shifts in microbial community structure with depth through the nine soil profiles with the observed changes in microbial communities within individual profiles exceeding the changes in surface soil communities observed across biomes. Finding soils with very distinct microbial communities may be as simple as digging down half a meter instead of traveling thousands of kilometers to sample across distinct ecosystem types. These strong depth-related gradients should be carefully considered when designing surveys of soil microbial diversity as even small differences in sampling depth across sites could make it difficult to resolve cross-site differences in microbial diversity or composition. Landscape position had the largest effect on the composition of microbial communities in the near-surface horizons and in the deepest horizons; those communities found at intermediate depths were relatively similar across the pits suggesting that edaphic factors are more homogeneous at these depths. Although we could clearly identify a number of microbial taxa that exhibited pronounced changes in relative abundance with depth, it was difficult to identify the specific factors driving these taxon distributions because multiple environmental factors change with soil depth (including carbon quantity, quality, nutrient availability, and the moisture regime) and the basic ecology of many of these taxa remains largely undetermined. As those microbes living throughout the soil profile, not just those living in near-surface horizons, are likely to have important effects on carbon sequestration, nutrient cycling, and weathering processes, a more integrated and comprehensive understanding of the microbial ecology of the soil profile is clearly needed.

## Acknowledgments

We would like to thank Rachel Gabor, Jeana Lee, Rory Cowie, Gaddy Bergmann, and the many undergraduates who helped dig the pits used in this study. We would also like to thank Donna Lyons for assistance with pyrosequencing as well as Antonio Gonzales and Greg Caparoso for assistance with QIIME. We thank Christine Seibold and Wendy Roth for their assistance with the soil characterization. We would also like to thank Scott Bates, Robert Bowers, Chris Lauber, and Kelly Ramirez for their helpful comments on the manuscript. This study was funded by the Boulder Creek Critical Zone Observatory, (NSF-0724960).

## Appendix. Supplementary material

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.soilbio.2012.03.011.

## References

Agnelli, A., Ascher, J., Corti, G., Ceccherini, M.T., Nannipieri, P., Pietramellara, G., 2004. Distribution of microbial communities in a forest soil profile investigated by microbial biomass, soil respiration and DGGE of total and extracellular DNA. Soil Biology & Biochemistry 36, 859–868.

Bates, S., Caporaso, J.G., Walters, W.A., Knight, R., Fierer, N., 2011. A global-scale survey of archaeal abundance and diversity in soils. ISME Journal 5, 908–917.

Bergmann, G., Bates, S., Eilers, K., Lauber, C., Caporaso, J., Walters, W., Knight, R., Fierer, N., 2011. The under-recognized dominance of *Verrucomicrobia* in soil bacterial communities. Soil Biology & Biochemistry 43, 1450–1455.

Blume, E., Bischoff, M., Reichert, J., Moorman, T., Konopka, A., Turco, R., 2002. Surface and subsurface microbial biomass, community structure and metabolic activity as a function of soil depth and season. Applied Soil Ecology 592, 1–11.

Buss, H.L., Bruns, M.A., Schultz, M.J., Moore, J., Mathur, C.F., Brantley, S.L., 2005. The coupling of biological iron cycling and mineral weathering during saprolite formation, Luquillo Mountains, Puerto Rico. Geobiology 3, 247–260.

Caporaso, J.G., et al., 2010. QIIME allows analysis of high-throughput community sequencing data. Nature Methods 7, 335–336.

Clarke, K., Gorley, R., 2006. PRIMER, sixth ed. PRIMER-E Ltd., Plymouth, UK.

Cole, J., Braddock, W., 2009. Geologic Map of the Estes Park 30′ × 60′ Quadrangle, North-central Colorado. U.S. Geological Survey Scientific Investigations Map 3039.

DeSantis, T.Z., Hugenholtz, P., Larsen, N., Rojas, M., Brodie, E.L., Keller, K., Huber, T., Dalevi, D., Hu, P., Andersen, G.L., 2006. Greengenes, a chimera-checked 16S rRNA gene database and workbench compatible with ARB. Applied and Environmental Microbiology 72, 5069–5072.

Eilers, K.G., Lauber, C.L., Knight, R., Fierer, N., 2010. Shifts in bacterial community structure associated with inputs of low molecular weight carbon compounds to soil. Soil Biology & Biochemistry 42, 896–903.

Ekelund, F., Ronn, R., Christensen, S., 2001. Distribution with depth of protozoa, bacteria and fungi in soil profiles from three Danish forest sites. Soil Biology & Biochemistry 33, 475–481.

Faith, D.P., 1992. Conservation evaluation and phylogenetic diversity. Biological Conservation 61, 1–10.

Fierer, N., Bradford, M., Jackson, R., 2007. Toward an ecological classification of soil bacteria. Ecology 88, 1354–1364.
Fierer, N., Chadwick, O.A., Trumbore, S.E., 2005. Production of $CO_2$ in soil profiles of a California annual grassland. Ecosystems 8, 412–429.
Fierer, N., Schimel, J., Holden, P., 2003. Variations in microbial community composition through two soil depth profiles. Soil Biology & Biochemistry 35, 167–176.
Fritze, H., Pietikainen, J., Pennanen, T., 2000. Distribution of microbial biomass and phospholipid fatty acids in Podzol profiles under coniferous forest. European Journal of Soil Science 51, 565–573.
Goberna, M., Insam, H., Klammer, S., Pascual, J.A., Sanchez, J., 2005. Microbial community structure at different depths in disturbed and undisturbed semiarid Mediterranean forest soils. Microbial Ecology 50, 315–326.
Goldfarb, K.C., Karaoz, U., Hanson, C.A., Santee, C.A., Bradford, M.A., Treseder, K.K., Wallenstein, M.D., Brodie, E.L., 2011. Differential growth responses of soil bacterial taxa to carbon substrates of varying chemical recalcitrance. Frontiers in Microbiology 2.
Hansel, C.M., Fendorf, S., Jardine, P.M., Francis, C.A., 2008. Changes in bacterial and archaeal community structure and functional diversity along a geochemically variable soil profile. Applied and Environmental Microbiology 74, 1620–1633.
Hartmann, M., Lee, S., Hallam, S.J., Mohn, W.W., 2009. Bacterial, archaeal and eukaryal community structures throughout soil horizons of harvested and naturally disturbed forest stands. Environmental Microbiology 11, 3045–3062.
Kemnitz, D., Kolb, S., Conrad, R., 2007. High abundance of Crenarchaeota in a temperate acidic forest soil. Fems Microbiology Ecology 60, 442–448.
LaMontagne, M.G., Schimel, J.P., Holden, P.A., 2003. Comparison of subsurface and surface soil bacterial communities in california grassland as assessed by terminal restriction fragment length polymorphisms of PCR-amplified 16S rRNA genes. Microbial Ecology 46, 216–227.
Leininger, S., Urich, T., Schloter, M., Schwark, L., Qi, J., Nicol, G., Prosser, J., Schuster, S., Schleper, C., 2006. Archaea predominate among ammonia-oxidizing prokaryotes in soils. Nature 442, 806–809.
Li, W., Godzik, A., 2006. Cd-hit: a fast program for clustering and comparing large sets of protein or nucleotide sequences. Bioinformatics 22, 1658–1659.
Liu, Z., Lozupone, C., Hamady, M., Bushman, F., Knight, R., 2007. Short pyrosequencing reads suffice for accurate microbial community analysis. Nucleic Acids Research 35, e120.
Lozupone, C., Knight, R., 2005. UniFrac: a new phylogenetic method for comparing microbial communities. Applied and Environmental Microbiology 71, 8228–8235.
Lozupone, C., Knight, R., 2008. Species divergence and the measurement of microbial diversity. FEMS Microbiology Reviews 32, 557–578.
Nemergut, D.R., Cleveland, C.C., Wieder, W.R., Washenberger, C.L., Townsend, A.R., 2010. Plot-scale manipulations of organic matter inputs to soils correlate with shifts in microbial community composition in a lowland tropical rain forest. Soil Biology & Biochemistry 42, 2153–2160.
Pester, M., Schleper, C., Wagner, M., 2011. The Thaumarchaeota: an emerging view of their phylogeny and ecophysiology. Current Opinion in Microbiology 14, 300–306.
Richter, D., Markewitz, D., 1995. How deep is soil? Bioscience 45, 600–609.
Rumpel, C., Kogel-Knabner, I., 2011. Deep soil organic matter-a key but poorly understood component of terrestrial C cycle. Plant and Soil 338, 143–158.
Schutz, K., Kandeler, E., Nagel, P., Scheu, S., Ruess, L., 2010. Functional microbial community response to nutrient pulses by artificial groundwater recharge practice in surface soils and subsoils. FEMS Microbiology Ecology 72, 445–455.
Trumbore, S., 2000. Age of soil organic matter and soil respiration: radiocarbon constraints on belowground C dynamics. Ecological Applications 10, 399–411.
Wardle, D., Ghani, A., 1995. Why is the strength of relationships between pairs of methods for estimating soil microbial biomass often so variable? Soil Biology & Biochemistry 27, 821–828.
Will, C., Thurmer, A., Wollherr, A., Nacke, H., Herold, N., Schrumpf, M., Gutknecht, J., Wubet, T., Buscot, F., Daniel, R., 2010. Horizon-specific bacterial community composition of german grassland soils, as revealed by pyrosequencing-based analysis of 16S rRNA genes. Applied and Environmental Microbiology 76, 6751–6759.
Zvyagintsev, D., 1994. Vertical distribution of microbial communities in soils. In: Ritz, K., Dighton, J., Giller, K. (Eds.), Beyond the Biomass. John Wiley and Sons, West Sussex, U.K, pp. 29–37.