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# Environmental factors influencing the distribution of rRNA from Verrucomicrobia in soil

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**Abstract**

The Verrucomicrobia constitute a newly discovered division of the Bacteria identified as a numerically abundant component of soil microbial communities in numerous sites around the world. The relative abundance of rRNA from Verrucomicrobia was investigated in the soil to examine the influence of specific environmental factors on the distribution of Verrucomicrobia and to better understand the distribution of this group in terrestrial ecosystems. The abundance of the verrucomicrobial rRNA was determined by using a novel oligonucleotide probe that is specific for verrucomicrobial 16S rRNA. The abundance of verrucomicrobial 16S rRNA in soil microbial communities was determined in relation to plant community composition and soil management history over a period of 2 years. Additional samples were analyzed to determine if verrucomicrobial rRNA relative abundance changes in relation to either soil depth or soil moisture content. The Verrucomicrobia composed $1.9 \pm 0.2\%$ of the microbial community rRNA present in the 85 soil samples examined. The distribution of verrucomicrobial rRNA in the soil reveals that Verrucomicrobia are significantly affected by environmental characteristics that change in relation to time, soil history, and soil depth, and reveals that a statistically significant amount of the variation in verrucomicrobial rRNA abundance can be explained by changes in soil moisture content. 

*Keywords:* 16S rRNA; 16S rRNA probe; Soil moisture; Seasonal variability; Microbial distribution; Effect of cultivation; Verrucomicrobia

## 1. Introduction

Molecular phylogenetic studies continue to redefine our definitions of microbial diversity as microorganisms representing novel lines of bacterial descent are recovered almost routinely from environmental samples. For each newly discovered phylotype, questions remain about their distribution and abundance in the environment as well as about the roles these organisms play in the ecosystem. The Verrucomicrobia constitute a newly identified bacterial division that appears to be widely distributed in both aquatic and terrestrial systems, but for which only a handful of organisms have been cultivated in isolation [1]. Verrucomicrobia are present in 91% of the 16S rDNA clone libraries generated from soil microbial communities and on average represent $11 \pm 4\%$ (S.E.M.) of the 16S rDNA clones in these libraries as calculated from published reports [2–10]. In addition, Verrucomicrobia are a numerically abundant component of certain soil microbial communities as determined by RT-PCR analysis of rRNA [11] and by quantitative PCR analysis of rDNA [6]. Despite their widespread distribution in the environment and evidence that they are a numerically abundant component of soil microbial communities nothing is known 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 understanding the basis of the organisms physiology and its function within an ecosystem. Thus careful observation of the environmental distribution of an organism or group of organisms that have not yet been studied in cultivation can help us to improve our understanding of the nature of these organisms and can provide insights about the conditions required for their enrichment and isolation. We determined the abundance of the Verrucomicrobia in a series of replicated fields located at the Kellogg Biological Station Long Term Ecological Research site (KBS-LTER, Hickory Corners, MI, USA) over a period of 2 years. An oligonucleotide probe was designed and tested that is specific for 16S rRNA

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from the Verrucomicrobia. This probe was used to determine the abundance of verrucomicrobial rRNA in soil RNA extracts. Soil sampling at the KBS-LTER site allowed us to describe the influence that management history, time, soil depth, and soil moisture content have on the abundance of verrucomicrobial rRNA in the soil.

## 2. Methods

### 2.1. Site description and soil sampling

Soil samples were taken from the LTER site located at the Michigan State University KBS in Hickory Corners, MI, USA. The KBS-LTER site, established in 1989 to study ecological processes in agroecosystems, includes a large-scale replicated field experiment located on 48 hectare of land that had been uniformly farmed for over 50 years prior to its establishment, and also includes nearby successional fields that have never been cultivated [12]. Soils are Typic Hapludalfs, sandy to silty clay loam and are of moderate fertility [12]. The site has a mean annual air temperature of 9.4°C and a mean annual rainfall of 860 mm [13]. A total of 30 fields were sampled representing four sets of fields from the main experiment site, and two sets of successional fields that had never been cultivated but were located near the experimental site. The fields sampled from the main experimental site consisted of conventionally tilled (CT) agricultural fields managed under a corn/soybean/wheat rotation (in corn during 1996), fields planted with poplar trees (PL), historically cultivated successional (HCS) fields that had been abandoned from cultivation in 1989, and subplots of the HCS fields that received annual tillage (HCST). The other fields sampled consisted of never cultivated successional (NCS) fields located several hundred meters from the main experimental site, and late successional (LS) fields that were located approximately 1 km from the main experimental site. Plant communities in the NCS and LS fields were dominated by herbaceous forbs and closely resembled the plant communities in HCS fields. Fields were sampled by taking a single soil core (2.5 cm diameter, 10 cm depth) from each of five permanent sampling stations distributed across each field replicate. The soil cores from each field replicate were pooled, sieved (4 mm mesh), frozen in liquid nitrogen (generally within 10 min of sampling), and stored at −80°C.

Soil samples were obtained at four times over a period of 2 years to ensure that any observed treatment effects are not artifacts of temporal variability. Though this sampling scheme was meant to control for temporal variability in treatment effects it also allows for a limited analysis of the nature of temporal variability in verrucomicrobial abundance in soils. On October 3, 1996 and May 23, 1997 soil was sampled from three field replicates of the CT, HCS, and NCS treatments. On June 6, 1998 soil was sampled from five field replicates of treatments CT, HCS, HCST, and NCS. June 1998 sampling included both 5-cm deep soil cores and 10-cm deep soil cores to assess potential differences in microbial community structure due to soil depth. On July 28, 1998 five field replicates were sampled from all of the described treatments (CT, PL, HCS, HCST, NCS, LS). Subsets of the July 1998 samples were used to determine the gravimetric soil moisture content.

### 2.2. Probe design

To determine the abundance of Verrucomicrobia in the soil an oligonucleotide probe that targets verrucomicrobial 16S rRNA (ver47, Fig. 1) was designed with the help of the ARB software package [14]. The specificity of the probe was empirically tested against RNA extracted from *Verrucomicrobium spinosum* ATCC 43997 and from closely related non-target organisms consisting of *Ketogulonigenium vulgarem* DSM4025, *Nitrosomonas europaea* ATCC 25978, *Planctomycetes limnophilus* ATCC43296, and *Acidobacterium capsulatum* ATCC 51196. RNA was extracted from bacterial cultures and 100 ng, 80 ng, 60 ng, 40 ng, and 20 ng of RNA were immobilized on nylon membranes for use in hybridization experiments. The wash temperature providing the appropriate probe specificity was determined empirically. Hybridization experiments were carried out as described below.

### 2.3. Nucleic acid extraction and hybridization

RNA for use in hybridization experiments was extracted from soil as previously described [15,16]. In brief, a 10-g portion of each frozen soil sample was suspended in a buffer suitable for sample homogenization and RNA stabilization. Microbial cells were lysed using beadmill homogenization with 10 g of 0.1 mm silica/zirconia beads in a 32-ml chamber for a duration of 2 min (Beadbeater, Biospec Products, Inc.). RNA from homogenized samples was concentrated by precipitation with polyethylene glycol and then purified using both hydroxyapatite and Sephadex G-75 columns. RNA samples were finally precipitated, resuspended in 200 μl of RNAse-free dd$H_2O$, and stored at −20°C.

Quantitative filter hybridization was performed as previously described [15,17]. Nucleic acids from soil samples and standards were denatured with 0.5% glutaraldehyde–50 mM $Na_2HPO_4$, serially diluted to provide a range of sample concentrations, blotted onto nylon membranes using a 96-well dot blot manifold, and immobilized by UV crosslinking. RNA isolated from the pure cultures mentioned above was included on all membranes as standards to control for differences in the specific activity of labeled probes and to account for the possibility of non-specific probe binding. All membranes used for hybridization were prepared in duplicate for hybridization with either the probe Ver47, specific for Verrucomicrobia 16S rRNA, or

the probe Univ1390, specific for 16S rRNA from the Bacteria, Archaea, and Eukarya. Hybridization protocols for $^{32}$P-5′-labeled oligonucleotide probes were previously described in detail [17]. Hybridization between radio-labeled probes and RNA immobilized on filters proceeded at 45°C for at least 12 h. Following probe hybridization, filters were washed twice for 30 min at 45°C. The specifically bound probe that remained on the membrane was visualized using a phosphorimaging system (Storm 860, Molecular Dynamics), signal intensity was quantified using Image Quant software v 5.0 (Molecular Dynamics).

### 2.4. Data analysis

Within a soil sample, the relative abundance of rRNA derived from a specific group was measured as the ratio of the signal derived from a group-specific probe to the signal derived from the universal probe. This approach for determining microbial rRNA abundance has been used previously to describe aspects of microbial community structure [17]. Relating specific probe binding to universal probe binding controls for variability in the total amount of RNA recovered from each soil sample. This approach also controls for the presence of hybridization inhibitors that may co-purify with RNA from soil. Positive controls were included on each membrane to correct for variations in the labeling efficiency of different oligonucleotide probes while negative controls were used to correct for the possibility of non-specific probe binding. In these experiments RNA obtained from *V. spinosum* was used as the positive control, while RNA from *K. vulgarem*, *P. limnophilus*, *N. europaea*, *A. capsulatum*, *Cytophaga johnsonae* ATCC 17061, *Arthrobacter globiformis* ATCC 8010, and *Saccharomyces cerevisiae* American Ale Yeast 1056 (Wyeast Labs, INCS.) were all used as negative controls. Every RNA sample was represented by five aliquots in a dilution series to examine potential differences in signal intensity due to inhibition or membrane saturation. The ratio of signal intensities obtained for specific and universal probe binding to an RNA sample was defined as $R = \sum_{i=1}^{n} [G_i(U_i)^{-1}]n^{-1}$, where $G_i$ and $U_i$ represent, respectively, the corresponding signal intensities obtained for group-specific and universal probe binding to each aliquot representing the sample, and $n$ equals the total number of aliquots representing the RNA sample. The value $R$ was calculated for each soil RNA sample ($R_s$), and a mean value of $R$ was determined for all positive ($R_p$) and negative ($R_n$) controls present on each membrane. The relative abundance (expressed as a percentage) of rRNA from a specific microbial group was then defined as $(R_s - R_n)(R_p - R_n)^{-1} \times 100$.

Percent rRNA abundance data were arcsine-transformed prior to statistical analyses to control for statistical artifacts that may result when the variance is proportional to the mean in a dataset, as is common for percentage data. Repeated measures ANOVA was used to examine the main effects of treatment and sampling time, as well as the interaction of these effects on verrucomicrobial rRNA abundance in treatments CT, HCS, and NCS over all four sampling dates. Repeated measures ANOVA was also used to examine the main effects of soil depth and treatment on verrucomicrobial rRNA abundance in samples from June 1998. ANCOVA was used to examine the effects that field treatment and soil moisture content have on verrucomicrobial rRNA relative abundance in samples from July 1998. Significant ANOVA results were investigated using Fisher's Protected Least Significant Difference test to perform all pairwise comparisons. Statistical tests were performed using StatView v 5.0 (SAS Institute, Inc.).

## 3. Results

### 3.1. Development of an oligonucleotide probe for the Verrucomicrobia

The probe Ver47 (Fig. 1) is complementary to 100% of the Verrucomicrobia 16S rRNA genes that have been sequenced in the probe target region and are present in public databases (29 sequences total). In addition, the probe has two or more base pair mismatches with 99.9% of the 16S rRNA sequences in the Ribosomal Database Project Release 8.0 (>22 000 sequences, [18]). In total there are only 24 gene sequences that have a single base pair mismatch to the probe. The specificity of the probe was determined empirically by hybridization against RNA from Verrucomicrobia and against RNA from microorganisms in closely related phylogenetic groups (Fig. 2). A final wash temperature of 45°C was found to be sufficient to achieve probe specificity under the hybridization conditions described.

### 3.2. Verrucomicrobial rRNA abundance

In the 30 fields examined over a period of 2 years, verrucomicrobial rRNA accounted for between 0 and 9.8% of the rRNA present in the soil. The mean abundance of

| Ver47 Sequence | 5' GAC | TTG | CAT | GTC | TTA | WC | 3' |
|---|---|---|---|---|---|---|---|
| a) *V. spinosum* | 3' CUG | AAC | GUA | CAG | AAU | AG | 5' |
| Controls | | | | | | | |
| b) *K. vulgarum* | 3' • • • | • • • | • • • | • • C | • • • | CC | 5' |
| c) *N. europaea* | 3' • • • | • • • | • • • | • • C | • • • | • C | 5' |
| d) *P. limnophilus* | 3' • • • | • • • | • • • | • G • | • U • | • • | 5' |
| e) *A. capsulatum* | 3' • • • | • • • | • • • | • • C | • • • | CC | 5' |

Fig. 1. Sequence of the Ver47 probe specific for rRNA from Verrucomicrobia. The Ver47 probe sequence is depicted along with the complementary 16S rRNA sequence from the verrucomicrobial positive control and homologous sequences from species used as negative controls. The letter W indicates that either of the bases A or T can be present at this position.

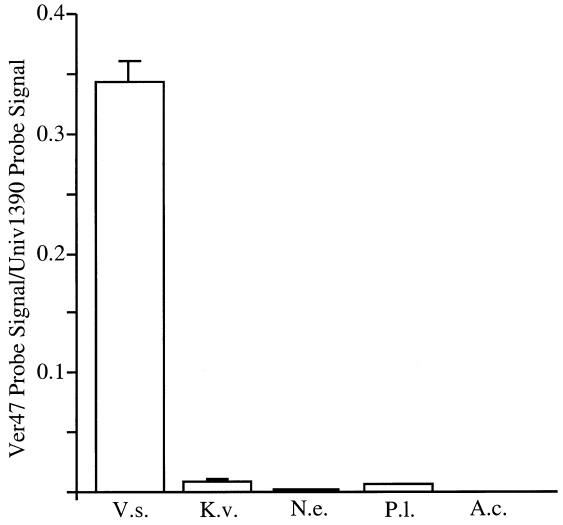


Fig. 2. A summary of hybridization results with probe Ver47. Each bar represents the signal intensity for probe Ver47 bound to sample RNA divided by the signal intensity for probe Univ1390 bound to RNA from the same sample, thereby controlling for differences in the amount of 16S rRNA present in each sample. Values are expected to be less than unity as a result of differences in the labeling and hybridization efficiency of the two probes. Each bar represents the mean and standard error from six separate experiments each performed at five different RNA concentrations. The RNA samples used are *V. spinosum* (V.s.), *K. vulgarum* (K.v.), *N. europaea* (N.e.), *P. limnophilus* (P.l.), and *A. capsulatum* (A.c.).

verrucomicrobial rRNA in KBS-LTER soil microbial communities was 1.9 ± 0.2% (standard error (S.E.M.), $n = 85$). Analysis of CT, HCS, and NCS fields at four times indicated slight differences in verrucomicrobial rRNA abundance between the treatments but these differences were not found to be significant by ANOVA (Fig. 3A). In contrast, ANOVA revealed that sampling time has a significant impact on the abundance of verrucomicrobial rRNA in the soil ($F_{3, 18} = 9.913$, $P = 0.004$). The highest abundance of verrucomicrobial rRNA occurred in May 1997 (2.66% ± 0.37% (S.E.M.)) with significantly lower values occurring in June 1998 (1.4% ± 0.24% (S.E.M.)), July 1998 (1.24% ± 0.39% (S.E.M.), and October 1996 (0.72% ± 0.35% (S.E.M.) (Fig. 3B). The interaction between the main effects of treatment and sampling time was not significant.

### 3.3. Verrucomicrobial rRNA relative abundance and soil depth

In June 1998 the effect that soil depth has on verrucomicrobial rRNA relative abundance was investigated by sampling four sets of fields (CT, HCST, HCS, NCS) by using either 5-cm deep soil cores or 10-cm deep soil cores. Consistent with previous analyses, differences in verrucomicrobial rRNA relative abundance between the different treatments were not significant. Verrucomicrobial rRNA abundance, however, did vary significantly with depth ($F_{1, 15} = 23.159$, $P = 0.0002$) (Fig. 4). Verrucomicrobial rRNA was more than twice as abundant in the 5-cm deep soil cores (3.87% ± 0.53%) than in the 10-cm deep soil cores (1.59% ± 0.17%). The interaction of the main effects of treatment and depth was not significant indicating that treatment effects do not influence differences due to depth.

### 3.4. Verrucomicrobial rRNA relative abundance and soil moisture

For the July 1998 samples both gravimetric soil moisture content and verrucomicrobial rRNA abundance were determined. Verrucomicrobial rRNA abundance demonstrated a slight positive correlation with soil moisture content (correlation coefficient = 0.510, $P = 0.0041$) (Fig. 5A). ANCOVA was used to determine if a significant amount of the variability in the abundance of the verrucomicrobial rRNA is explained by differences in soil moisture content.

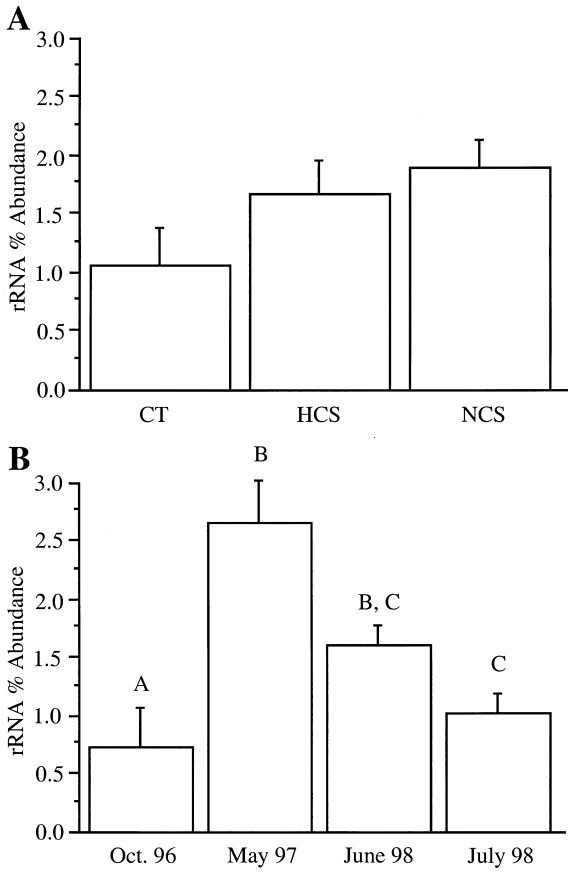


Fig. 3. Abundance of verrucomicrobial rRNA in fields from the KBS-LTER site at different times. Average abundance of verrucomicrobial rRNA in fields that have either been cultivated (CT), abandoned from cultivation (HCS), or never cultivated (NCS) for samples from October 1996, May 1997, June 1998, and July 1998 (A). Abundance of verrucomicrobial rRNA in October 1996, May 1997, June 1998, and July 1998 averaged over all fields examined (B). Effects detected to be significant by ANOVA are indicated by the placement of letters above each bar so bars with different letters are significantly different from each other (Fishers PLSD, $P < 0.05$). Bars and whiskers represent the mean and standard error respectively.

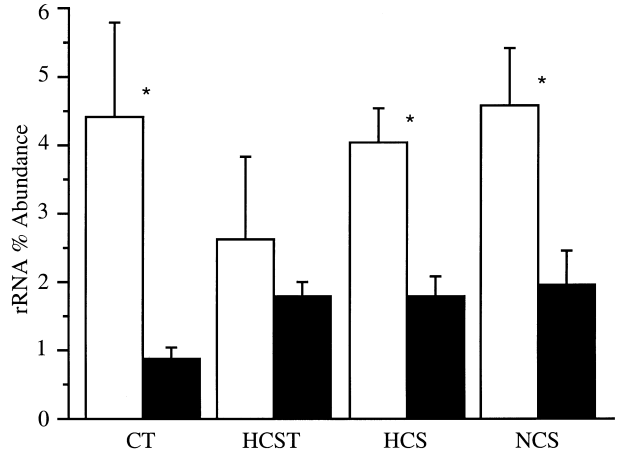


Fig. 4. The effect of depth on verrucomicrobial rRNA abundance. Abundance of verrucomicrobial rRNA in either 5-cm deep soil cores (open bars) or 10-cm deep soil cores (black bars) in fields that have either been cultivated (CT), abandoned from cultivation (HCS), abandoned from cultivation but tilled annually (HCST), or never cultivated (NCS). Asterisks indicate significant differences between 5-cm and 10-cm cores that were detected using unpaired *T*-tests ($P < 0.01$). Bars and whiskers represent the mean and standard error respectively.

A test of the homogeneity of slopes revealed that the effect of moisture on verrucomicrobial rRNA relative abundance does not differ significantly between the treatments examined and so interaction effects were excluded from subsequent analyses. ANCOVA provides evidence that there are significant differences in verrucomicrobial rRNA abundance among the treatments examined in July 1998 ($F_{5, 22} = 4.500$, $P = 0.0056$). Subsequent tests revealed that verrucomicrobial rRNA abundance tended to be significantly lower in fields with a history of cultivation (CT, PL, HCST, HCS) than in fields that had never been cultivated (LS, NCS) (Fig. 5B). This observation is consistent with the data obtained from other sampling times as verrucomicrobial rRNA abundance tended to be lower in historically cultivated fields than in fields that had never been cultivated (Fig. 3A). In addition, ANCOVA provides evidence that there is a statistically significant relationship between soil moisture content and the abundance of verrucomicrobial rRNA ($F_{1, 22} = 8.182$, $P = 0.0091$) indicating that soil moisture may be a useful variable for predicting differences in verrucomicrobial rRNA relative abundance in soil.

## 4. Discussion

While analyses of rRNA genes (rDNA) with PCR-based techniques are useful for identifying the organisms present within microbial communities, measurement of the relative proportions of the rRNA molecules themselves can be used to determine patterns of microbial abundance [19]. It is important to note, however, that there is a positive correlation between intracellular concentrations of rRNA and the growth rate of microorganisms [20–22]. Thus, equal measures of rRNA relative abundance may be obtained for microbial groups that are either numerically abundant and growing slowly, or less abundant and growing rapidly. In this way measurement of rRNA abundance may differ from measurements of rDNA, though Felske et al. (1998) have observed similar patterns of community structure when observing either rRNA or rDNA from soil microbial communities [23]. It is also interesting to note that similar measurements of microbial relative abundance have been obtained for soil microbial communities both from studies that have used fluorescent in situ hybridization and from those that have used rRNA hybridization [24].

The oligonucleotide probe Ver47 proved useful for determining the relative abundance of verrucomicrobial rRNA in soil microbial communities. With this new probe we provide the first measurements of verrucomicrobial rRNA relative abundance in the soil by a technique that

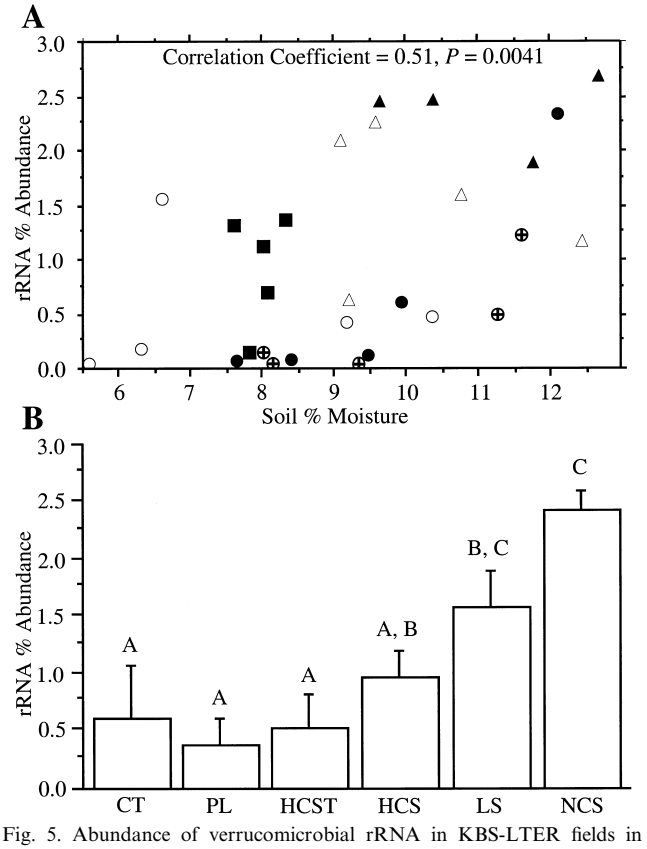


Fig. 5. Abundance of verrucomicrobial rRNA in KBS-LTER fields in July 1998 with respect to soil moisture and management. Verrucomicrobial rRNA abundance in soil samples ($n = 29$) from July 1998 are plotted against soil moisture content (A). Soil samples are from fields that have either been cultivated (CT, ●), planted with poplar trees (PL, ⊕), abandoned from cultivation but tilled annually (HCST, ○), abandoned from cultivation (HCS, ■), or never cultivated (LS, △; NCS, ▲). Differences in verrucomicrobial rRNA abundance are also shown in relation to field management (B). Bars and whiskers represent the mean and standard error of rRNA abundance in fields from each of the treatments described above. Different letters are used to indicate bars found to be significantly different (Fishers PLSD, $P < 0.05$).

does not require PCR amplification of nucleic acids from the microbial community. The mean verrucomicrobial rRNA abundance found at four different times in fields from the KBS-LTER site was 1.9% ± 0.2% (S.E.M.). Sampling at the KBS-LTER site was designed to address the relative importance of plant community composition, soil management history, soil depth and soil moisture on the abundance of verrucomicrobial rRNA in the soil. In addition, samples were taken at different times to allow for an assessment of the temporal variability in verrucomicrobial rRNA relative abundance.

The HCS and NCS fields both support diverse plant communities that are similar to one another but very different from the nearly monoclonal plant community present on the CT fields. In contrast, the HCS fields prior to their abandonment in 1989 were treated identically to the CT fields resulting in total soil carbon and nitrogen concentrations that are similar to those found in CT fields and are significantly lower than those found in the NCS fields [13,25]. If plant community composition was a major influence on the abundance of Verrucomicrobia in soil then verrucomicrobial rRNA relative abundance should be similar in the HCS and NCS fields. Alternatively, if soil characteristics relating to cultivation history are a major influence on the abundance of Verrucomicrobia then verrucomicrobial rRNA abundance should be similar in the CT and HCS fields. Observations of verrucomicrobial rRNA relative abundance in CT, HCS, and NCS fields at four times do not provide convincing evidence that either plant community composition or soil management history have a significant influence on the rRNA abundance of Verrucomicrobia in the soil. As this analysis was restricted to three replicate fields from each treatment it is possible that subtle differences in verrucomicrobial rRNA relative abundance may occur between the treatments at different times and that these differences were not detected due to the magnitude of temporal variability. For example, when the numbers of treatments sampled and the numbers of fields sampled per treatment were increased and the analyses were limited to samples in July 1998 it was possible to detect differences in verrucomicrobial rRNA abundance between fields that had differences in past management history (Fig. 5B). This result most likely indicates that the rRNA abundance of the Verrucomicrobia is influenced by changes in the soil caused by past management history, but that these differences in abundance may be small in comparison to those caused by temporal variability and only apparent at certain times of the year. In contrast no evidence was found to indicate that plant community composition has an influence on the abundance of verrucomicrobial rRNA in the soil.

Samples were taken at different times to determine the degree to which treatment effects were influenced by temporal variability. Though the data do not provide strong evidence that verrucomicrobial rRNA abundance varies between the fields examined there is evidence of significant temporal variability in verrucomicrobial rRNA abundance in the soil (Fig. 3B). Changes in verrucomicrobial rRNA relative abundance could result from repeatable seasonal variations or from isolated meteorological phenomena that occurred prior to the time of sampling. The data are sufficient to show that verrucomicrobial rRNA abundance can change significantly at time scales relevant to seasonal or meteorological events though they are not sufficient to distinguish the specific causes of the temporal variability.

Soil moisture content varies considerably with time and has been observed to influence both the activity of soil microorganisms [26–28], and the structure of soil microbial communities [29,30]. To examine whether soil moisture has an influence on the abundance of the Verrucomicrobia in soil, the moisture content was determined for soil samples from July 1998. Though only a weak positive correlation was observed between the abundance of verrucomicrobial rRNA and soil moisture content, the use of soil moisture as a covariant in ANCOVA revealed that a statistically significant amount of the variability in verrucomicrobial rRNA abundance can be explained by soil moisture content. It is interesting to note that the few species of Verrucomicrobia that have currently been isolated have originated from either aquatic environments or saturated soils [31,32]. Though these data provide evidence that the relative abundance of verrucomicrobial rRNA is affected by soil moisture content, the low correlation coefficient between soil moisture content and verrucomicrobial rRNA abundance indicates that soil moisture is not the only variable influencing the abundance of Verrucomicrobia in the soil.

Changes in soil moisture can have substantial impacts on microbial activity in the soil [26,33,34]. Increases in soil moisture have been associated with the enhanced diffusion of nutrients [35], decreased oxygen tension [36,37], and increased activity of microbial predators in soils [38]. Any of these changes in the soil could be associated with changes in microbial community structure. For example, if the Verrucomicrobia in soil are anaerobes then increases in the abundance of anoxic microenvironments, associated with increases in soil moisture content, should favor the growth of Verrucomicrobia. Likewise, the Verrucomicrobia in soil may have extremely small cell dimensions permitting them access to small soil pores and thereby making them inaccessible to microbial predators [39]. In such a case, refuge from the increased predation rates that accompany increases in soil moisture content may cause increases in verrucomicrobial relative abundance. Either of these explanations are possible as the few Verrucomicrobia that have been isolated from saturated soils have both fermentative metabolisms and extremely small cell dimensions [31]. However, the small number of verrucomicrobial isolates currently characterized and the limited ecological data available for this microbial group make any hypotheses at this time purely speculative. In addition, changes in

soil moisture content are commonly related to changes in soil organic matter content and soil structure [40]. As a result it is possible that changes in verrucomicrobial rRNA abundance are not caused by soil moisture content, but rather reflect the response of these organisms to some soil characteristic that is itself correlated with soil moisture content.

The most striking difference in verrucomicrobial rRNA abundance occurs with depth in the soil. Verrucomicrobial rRNA was significantly more abundant in 5-cm deep soil cores than in 10-cm deep soil cores. Because the 0–5-cm portion of the soil is included in the 0–10-cm soil cores our measurements actually underestimate the difference in verrucomicrobial rRNA relative abundance that occurs with depth. Soil characteristics such as total organic carbon, total nitrogen, and soil moisture all decrease with depth in the soil [41].

Though rarely isolated from the soil, Verrucomicrobia are commonly detected in this environment through the use of cultivation-independent analyses of soil microbial communities [2–9,11]. At the KBS-LTER site Verrucomicrobia were observed to account for as much as 9.8% of the 16S rRNA present in the soil. The abundance of verrucomicrobial rRNA in soil was strongly influenced by sampling time and depth in the soil. In addition, there was evidence that verrucomicrobial rRNA relative abundance may be influenced by changes in the soil caused by past cultivation history. If the Verrucomicrobia respond to changes in the soil environment that are associated with changes in soil moisture content as suggested by data from July 1998 than this relationship may help to explain the variability in verrucomicrobial rRNA abundance observed in relation to sampling time, soil depth, and soil management history. To understand if there is a relationship between verrucomicrobial rRNA relative abundance and soil moisture content and how such a relationship could influence the behavior of Verrucomicrobia in the soil will require experimentation on verrucomicrobial isolates from the soil in addition to further observation of environmental samples. The probe described in this study can be used to measure the abundance of Verrucomicrobia in environmental samples and can also be used to verify the successful enrichment and isolation of Verrucomicrobia from the soil.

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