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Assessment of Soil Bacterial Community Structure Changes in Response to Hydrogen Gas Released by N_2-fixing Nodules

Xiang He[1], Yinan Zou[1], Bryan Flynn[1], Amber Golding[1], and Zhongmin Dong[1*]

[1]Department of Biology, Saint Mary's University, Halifax, Nova Scotia, B3H 3C3, Canada.

Authors' contributions

This study was carried out in collaboration between the authors. All authors managed the literature searches, read and approved the final manuscript.

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ABSTRACT

Nucleic acid based technologies are useful tools in the investigation of microbial community composition. This study used three such methods – T-RFLP/PAT, clone library and metatranscriptome analyses – to probe changes in soil bacterial populations exposed to hydrogen gas. Total DNA and RNA were collected from both lab and greenhouse soil samples exposed to H_2, with soil samples not treated with H_2 used as controls. All three methods showed the same general trends, with members of *Actinobacteria* being suppressed by H_2 exposure and populations of *Proteobacteria*, particularly β- and γ-*proteobacteria*, being stimulated by the presence of H_2. These results agree with previous studies and show that all three techniques, either alone or in combination, are reliable methods for investigating shifts in soil microbial community composition when exposed to a selective pressure such as H_2. This study also shows that H_2 has a marked effect on soil biology and microbial ecology. This soil bacterial community change explains the reported soil gas production switch and associated plant growth promotion.

Keywords: Bacterial community; hydrogen gas; metatranscriptome analyses; soil; T-RFLP.

**Corresponding author: E-mail: zhongmin.dong@smu.ca;*

1. INTRODUCTION

Hydrogen gas (H_2) is a major by-product of the biological nitrogen fixation (BNF) process. Root nodule symbioses in legumes produce H_2in amounts equivalent to 5-6% of the plant's net photosynthesis gain [1]. While some symbioses reuse the H_2 generated from BNF as an energy source (HUP^+), those symbioses involving nodules lacking an uptake hydrogenase enzyme (HUP^-) release large quantities of H_2 into the soil. Studies show that most, if not all, of the H_2 is oxidized in the surrounding soil of the plant root system, more specifically, the soil within a few centimeters' distance from root nodules [1,2]. Popelier*et al.*[3] found a highly positive correlation between the microbial biomass-C (carbon) of the soil and soil H_2 uptake rate, which suggested that H_2 uptake is a biological process. By measuring the H_2 uptake rate of soil samples that were treated with both an antibiotic and a fungicide, respectively, McLearn and Dong [4] were able to further determine the uptake of H_2 in soil is bacterial in nature. However, with several thousand bacterial species present in each gram of soil and over 95% of soilbacteria unknown, it has been very difficult to study H_2-induced soil bacterial community changes [5].

Molecular methods have been applied to study the diversity and ecology of microbial environments since the mid-1980s [6]. Fluorescence *in situ* hybridization (FISH) is one technique used to identify different bacterial groups/species and detect shifts in bacterial community structure by using group/species specific probes without the need of cultivation [7]. Fluorescently labeled oligonucleotide probes targeting the 16S rRNA have been used to determine total bacterial counts and shifts of soil microbial community in soil exposed to H_2 [8]. Although FISH analysis sheds light on studies of soil microbial community ecology, the ability of FISH analysis to determine soil microbial diversity and shifts in soil microbial community is limited by the small number of known soil bacterial species for probe design and lack of sensitivity for bacteria presenting as small populations within soil samples [9,10].

DNA fingerprinting is another way to study microbial diversity and community structure in an environmental sample. It is a drastically simplified representation of an organism's or a community's total DNA. With PCR- amplified DNA fragments, several techniques can be used for generating patterns reflecting microbial community structure. Terminal Restriction Fragment Length Polymorphism (T-RFLP) analysis is a fingerprinting technique for quickly obtaining the general profile of microbial diversity in a collected sample [11]. In a T-RFLP profile, DNA fragments produced by restriction enzyme digestion are grouped together as peaks according to their sizes and the peak height represents the quantity of DNA fragments of the same size. T-RFLP analysis has been reported to be capable of quantifying changes in microbial communities [12]. This technique has also been provento be a rapid and reproducible method for comparison of microbial communities and assessment of community dynamics [13]. With high-tech systems, T-RFLP has become even more reliable and reproducible. Generating TRF patterns within one week is now feasible with the rapid improvements in modern techniques and technology.

The Phylogenetic Assignment Tool (PAT) is a useful (and free) tool to analyze T-RFLP data and is available online to researchers. The PAT program uses a database containing different bacteria's DNA fragment sizes generated by different restriction enzymes to match T-RFLP profiles in order to determine the phylogeny of the organisms in the profiles [14]. When studying complex communities, PAT can analyze T-RFLP data very quickly and efficiently, especially for T-RFLP profiles produced by multiple restriction enzymes [10,14].

Although T-RFLP is becoming widely used in many laboratories, it still has several limitations. For example, biases from DNA extraction and PCR amplification can affect T-RFLP analyses. While able to analyze complex soil communities, Terminal Restriction Fragments (TRFs) cannot provide solid information about relative phylotype richness and evenness [15]. In addition, sometimes TRF results may not match any existing sequence in the database, if the target gene is highly specific [13]. Clone libraries can be applied to solve some of these problems by analyzing the sequences for identification and digital TRF profile generation [15,16,17]. Metatranscriptome analysis is another powerful molecular biology method that can be used for phylogenetic characterization and analysis of gene expression patterns of microbial communities. The aim of the present study is to use T-RFLP profiles, clone library sequence data, and RNA -based phylogeny generated by metatranscriptome analysis to detect soil microbial community changes induced by exposure to hydrogen gas. A flowchart of the methodology stream used in this study is shown in Fig. 1.

2. MATERIALS AND METHODS

2.1 Soil Treatment

Sandy loam soil collected from the Plumdale Facility, Agriculture Faculty, Dalhousie University, Truro, Nova Scotia was mixed with fine sand (2:1 v/v). The mixed soil was distributed into twelve 6-inch-diametric pots and four 60ml syringes.

Three different soil treatments (three replicates for each) were carried out in a greenhouse. Soybean (*Glycine max*, cv. RR Drako) seeds were surface sterilized with bleach and 70% ethanol. The seeds were then planted in a tray with Promix for germination. Soybean seedlings were inoculated with JH47 (HUP^-) (derived from the parent strain USDA DES 110 with a Tn5 mutation in the 33-kDa subunit of the hydrogenase gene) [18,19], and JH (HUP^+) (derived from USDA 110 and expresses hydrogenase activity) [20,21,22]. Non-inoculated soybean plants were used as a control group. The soybean seedlings were transplanted into separate pots in the greenhouse. Lab treated soils were treated at the H_2 exposure rate equivalent to a soybean plant [1] and ambient air in the lab. During the treatment, the H_2 uptake rates were monitored.

The lab and greenhouse treatments resulted infive different types of soil samples. After one month (laboratory treatment) and three months (greenhouse treatment), soil samples were collected and packed into 2.0ml sterile microcentrifuge tubes. The soil lumps from the greenhouse pots were opened very carefully. The soil layer within 5mm of the nodules was collected into microcentrifuge tubes. All samples collected were frozen in liquid nitrogen immediately and stored at -80^oC until use.

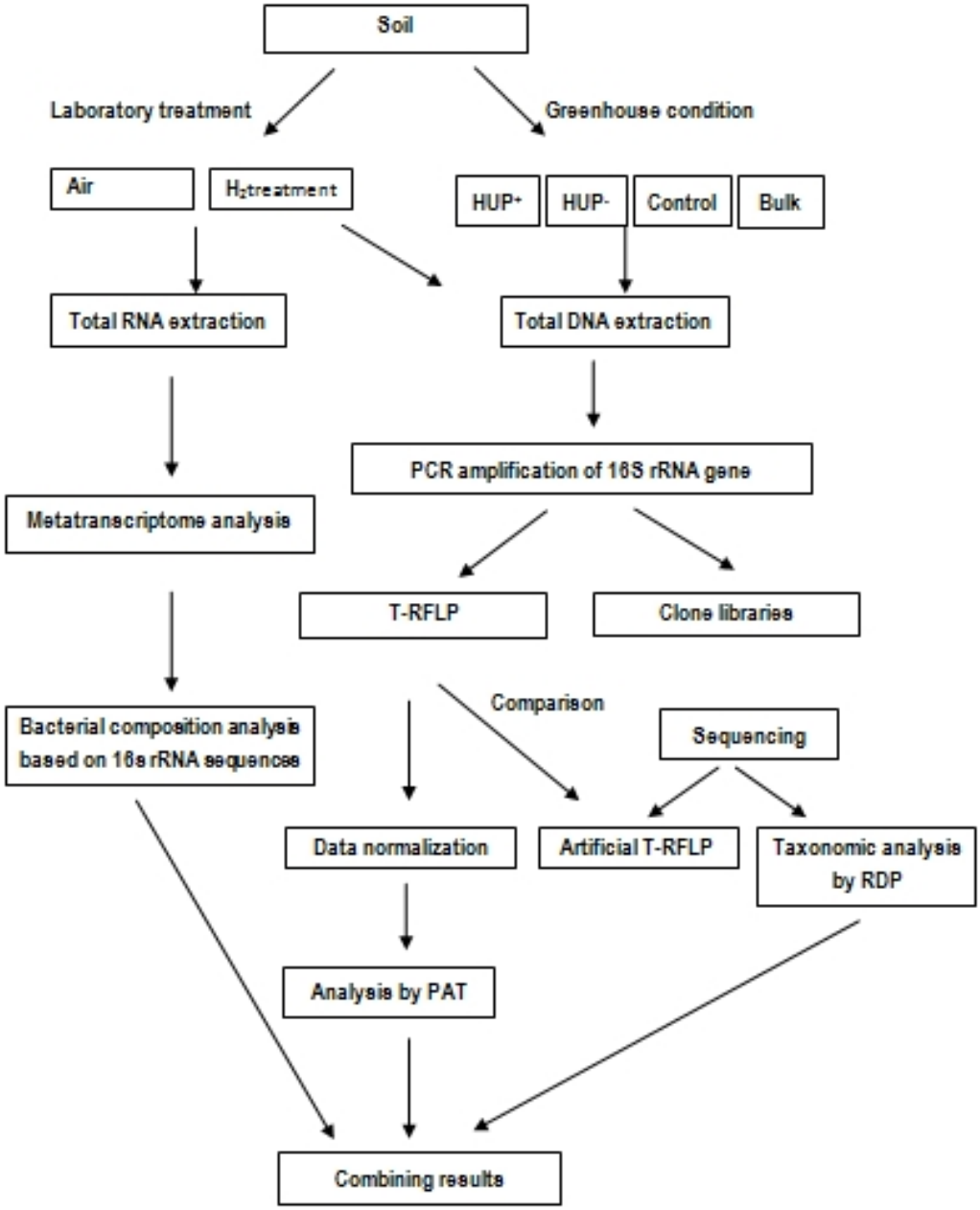

Fig. 1. Flow chart for investigation of soil bacterial community structure by combination of T-RFLP/PAT, clone library and metatranscriptome methods.

2.2 Extraction of Total Soil DNA and RNA

The total DNA of 0.5 grams of soil from each sample was extracted by using theFastDNA® SPIN kit for soil (MP Biomedicals, Solon, OH) following the manufacturer's protocols.

2.3 RNA Sample Preparation

Total RNA was collected from one gram soil samples (air- and H_2-treated) following four weeks of hydrogen treatment using a PowerSoil ® Total RNA Isolation Kit (Catalog No. 12866-25) following the manufacturer's protocol. Purified RNA samples were then dried out overnight in a vacuum chamber and stored as a powder in GenTegra RNA Cluster Tubes (Catalog No. GTR3122) to maintain RNA quality and prevent degradation. Dried samples were packaged at room temperature and shipped to the Beijing Genomics Institution (BGI) in Hong Kong *via* FedEx International shipment for metatranscriptome analysis using an Illumina's HiSeq™ 2000 sequencer.

2.4 PCR Conditions

Partial 16S rRNA genes (527bp) from all soil samples were amplified with a pair of bacterial universal primers: BSF8/20(5'-AGAGTTTGATCCTGGCTCAG-3') and BSR534/18 (5'-ATTACCGCGGCTGCTGGC-3'). The PCR reactions for T-RFLP and clone library were identical except that the forward primer for T-RFLP was fluorescently labeled with 6-FAM.

Amplification was carried out with a Thermal Cycler (Applied Biosystems, Foster City, CA). The reaction mixture of 25µl contained: 2µL of 10mMof each primer for 16S rRNA, 2.5µl of 10× Buffer, 2.5µl of 2mM dNTP, 2µl of $MgSO_4$, 0.2 µl of 5U/µl Taq enzyme (UBI Life Sciences Ltd, SK, Canada), 0.5µl of DNA template corresponding to 15 to 20 ng of total DNA and Sigma® water to complete the 25µl volume.

The conditions for the PCR were 3 minutes at 95°C for denaturation, 40 cycles of 30s at 95°C, 45s at 55°C for annealing, 45s at 72°C for extension, and a final cycle of 10 minutes at 72°C. Multiple PCR reactions were pooled together to minimize PCR random bias(3 for clone libraries and 10 for T-RFLP analysis). PCR products were purified with the QIAquick® PCR purification kit (QIAGEN Inc. Mississauga, ON).

2.5 TRF Profiles and PAT Analysis

Three restriction endonucleases, *Bst*UI, ,*Hae*III and *Msp*I (New England Biolabs, Ltd., Pickering, ON, CA) were used to obtain three separate TRF profiles for each sample. The digestion reaction of 50µl contained: 5µl 10× Buffer, 10µl purified PCR products, 20U of one of the three restriction enzymes, and Sigma water to complete the 50µl volume. All the reactions were performed at 37°C in a water bath for 4 hours except those for *Bst*UI, which were incubated at 60^{o}C. After digestion, the products were purified with QIAquick® nucleotide removal kit (QIAGEN Inc. Mississauga, ON, CA). Samples were sent to University Core DNA Services at the University of Calgary, Alberta. 6-FAM labeled terminal restriction fragments between 50 and 500bp were separated and recorded by model ABI3730 DNA sequencer.

TRF profiles were generated by the softwareGeneMarker V-1.4 (SoftGenetics LLC, USA). The information in the profiles included the following: the fragment length (represented by the apex position of each peak on a base pair scale relative to a DNA size ladder, GeneScan 500 LIZ Size Standard, Applied Biosystems, Foster City, CA, USA), the height of each peak, and the area under each peak.

All profiles were standardized by the application of the variable percentage threshold method [10,23]. The normalized TRF profiles were compiled into 3 files separated by restriction enzymes *Bst*UI, *Msp*Iand*Hae*III. The data sets were uploaded and automatically analyzed by PAT. An example of a normalized TRF profile is shown in Figure 2 (bottom) illustrating the TRF profile generated by the restriction enzyme *BstUI*.

2.6 16S rRNA Clone Libraries and Sequence Analysis

PCR products of 4 treatments (H_2, air, HUP- and HUP+) were inserted in top GEM®-T Easy Vectors following the protocols. The ligation products were transformed into *E. coli* cells (strain JM109). The positive colonies were selected by LB/ampicillin (100µg/ml)/IPTG/X-Gal plate.

Positive colonies, 350 for each treatment, were randomly picked and plasmid DNA was extracted by Gene JET™ Plasmid Miniprep Kit (Fermentas Inc., Burlington, ON, CA). All of the DNA plasmids were sequenced by Macrogen Inc. (South Korea) using an ABI 3700 sequencer. The sequences of four libraries were submitted to the RDP-II database for taxonomic analysis.

Artificial TRF profiles were generated manually by locating the first cutting site of each of the three restriction enzymes for each sequence in the clone libraries. The theoretical TRFs were used to generate artificial TRF profiles for each clone library and then compared with actual TRF profiles from soil total DNA analysis. An example of an artificial TRF profile generated from clone library data and also representing the *BstUI* restriction enzyme is shown in Fig. 2 (top).

2.7 Metatranscriptome Analysis

Image data were transformed into sequence data *via* base calling to give raw reads. Adapters, unknown and low quality bases were then removed to give clean reads.

Transcriptome *de novo* assembly was carried out using SOAPdenovo software. This created unigenes, whose sequence direction was then decided using BLASTX alignment (evalue<0.00001) between the unigenes and the protein databases nr, Swiss-Prot, KEGG and COG, in that order of priority in case of any conflicts between databases.

To estimate the community structure of each soil sample, all pair-ended reads aligned to the Ribosomal Database Project (RDP) were overlapped to create longer tags, with an overlap length of at least 10 bp, from both air- and H_2-treated samples. Approximately 130,000 tags were aligned against SILVA-106 using BLASTN (evalue $1e^{-}5$). Only the first 50 hits were used to determine taxonomy for each tag based on the Lowest Common Ancestor (LCA) principle.

3. RESULTS

3.1 T-RFLP Analysis

3.1.1 Lab samples (air- treated *versus* H_2-treated soil samples)

PAT analysis showed that the dominant bacterial groups in both air- and H_2-treated soil bacterial communities were *Proteobacteria*, *Actinobacteria* and *Firmicutes*(Table 1).Differences between the two communities consisted mainly in the richness of *Proteobacteria*, *Firmicutes* and *Bacteroidetes*. Following H_2 treatment, the relative abundance of *β-proteobacteria* and *γ-proteobacteria* increased significantly, while that of *α-proteobacteria* decreased. Among the *Firmicutes*, *Clostridia* and *Bacilli* dramatically increased and decreased, respectively. Members of *Bacteroidetes, Sphingo bacteria* and *Flavobacteria* decreased slightly after H_2 treatment.

3.1.2 Greenhouse samples (HUP^+ *versus* HUP^- soil samples)

Greenhouse results differ somewhat from those in the lab trials. The dominant groups in both HUP^+ and HUP^- soil samples were still found to be *Proteobacteria*, *Actinobacteria* and *Firmicutes* (see Table 1) but the composition of said groups changes in the greenhouse samples. Firstly, the relative abundance of *α-proteobacteria* increases dramatically in HUP^- samples, the complete opposite of the above, with the abundance of *β*-and *γ-proteobacteria*also increasing significantly in HUP^- soils, as with the lab samples (see Table 1). In the *Firmicutes*, *Clostridia* and *Bacilli* abundance both fall dramatically in HUP^- soils, whereas *Clostridia* increased significantly in abundance in H_2-treated lab samples (see Table 1). Members of the *Bacteroidetes*, *Flavobacteria* and *Sphingobacteria*, saw increases in relative abundance in H_2-treated lab samples but both showed decreases in abundance in HUP^- samples from the greenhouse trials (see Table 1).

Outside of the above differences, T-RFLP/PAT analysis showed no significant difference between lab and greenhouse samples as concerns species richness, with nine distinct phylogenetic groups being revealed in all samples.

3.2 Clone Library Analysis

Rarefaction curves are employed to estimate taxonomic diversity in ecological research [24]; they were used in this study to estimate the number of clone sequences that can accurately represent species richness in the plant-soil bacterial community (see Fig. 3).

Four clone libraries were assigned to thirteen distinct phylogenetic groups by RDP-II analysis. Most clones were affiliated with *Proteobacteria*, followed by *Bacteroidetes* and *Actinobacteria*. Clones related to *TM7*, *Nitrospira*, *Cyanobacteria*, *Chloroflexi*, *Verrucomicrobia* and *Planctomycetes* were rare.

Within the *Proteobacteria*, the *α*-, *β*- and γ- subdivisions made up the majority of the relative abundance of this phylum. FollowingH_2 treatment, the γ subdivision saw the most significant increase. Among the *Bacteroidetes*, *Sphingobacteria* increased dramatically following H_2treatment (see Table 1).

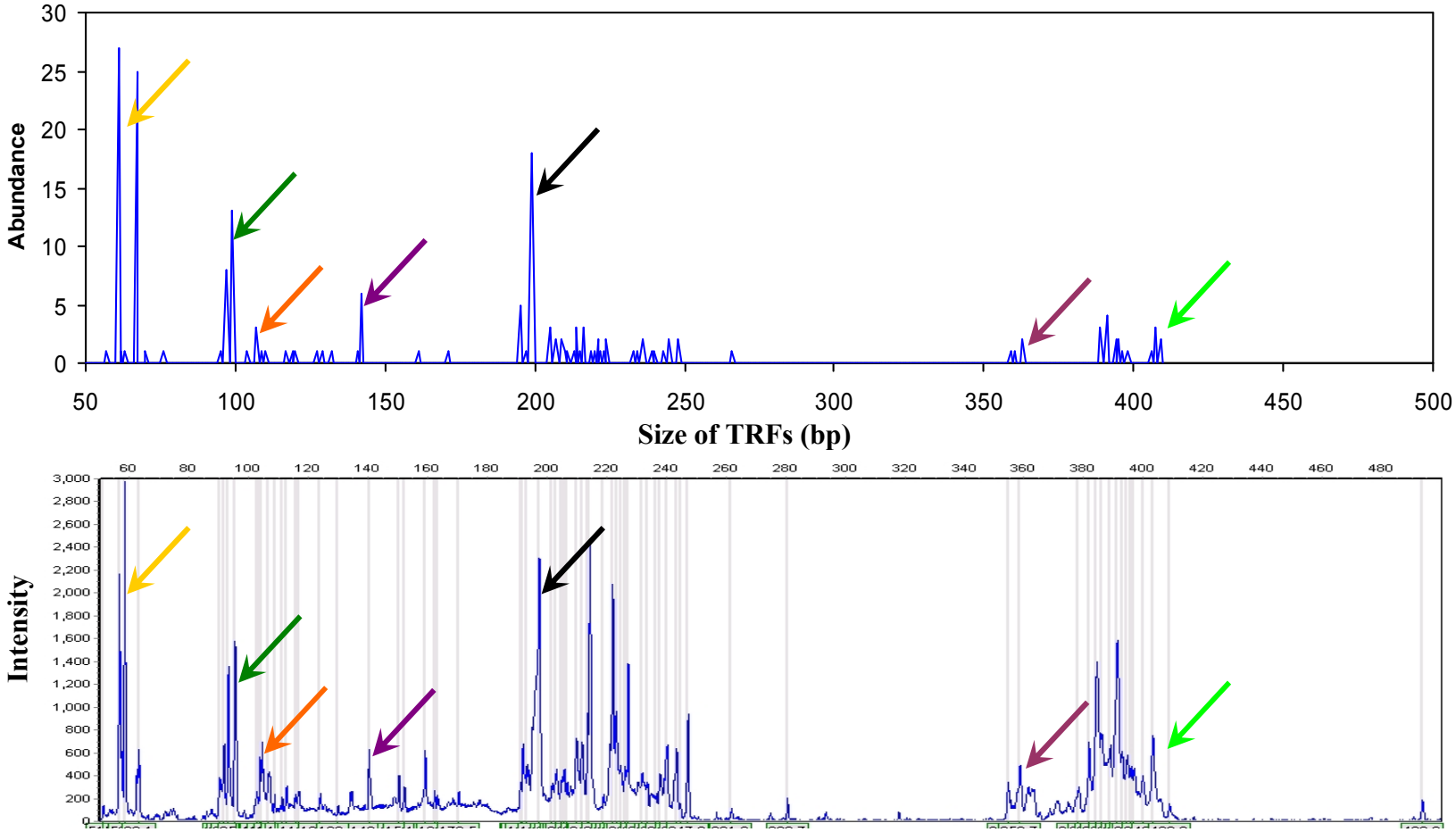

Fig. 2. Abundance of TRFs generated from air-treated soil clone library (above) and T-RFLP (below) by restriction enzyme *Bst*UI

Table 1. Phylogenetic composition of some major soil bacteria groups assessed by different methods (in % of total bacteria detected)

Bacterial Groups	PAT Analysis						Cloned Libraries				Transcriptome	
	Air	H_2	Bulk	Cont.	Hup+	Hup-	Air	H_2	Hup+	Hup-	Air	H_2
Actinobacteria	21.6	10.2	16.4	22.9	10.9	11.0	1.8	0.9	2.0	4.4	26.6	13.2
Firmicutes	18.0	0.9	16.4	22.9	10.9	11.0	-	-	-	-	2.69	2.79
Proteobacteria	19.3	31.4	24.5	16.1	24.4	32.6	29.9	27.6	28.6	53.8	26.0	45.4
α-proteobacteria	9.5	4.9	10.8	8.5	11.3	17.6	14.1	7.2	16.6	24.1	10.4	8.8
β-proteobacteria	2.3	13.3	3.8	1.7	5.2	6.5	9.1	6.9	5.1	11.5	4.9	17.8
γ-proteobacteria	7.5	13.2	9.9	5.9	7.9	8.5	1.2	7.8	1.4	13.5	2.4	1.7
δ-proteobacteria	-	-	-	-	-	-	0.0	0.3	0.3	0.0	7.9	16.7

The main difference between the HUP^- and HUP^+ nodule soil communities was that the *α*-, *β*- and γ-*proteobacteria* subdivisions were much more abundant inHUP^- nodule soil communities. *Sphingobacteria* and *Flavobacteria* also increased, though the latter was not significant at only 0.3% (see Table 1).

Comparing the laboratory treatments and greenhouse conditions, some differences can be seen. Three more phyla, *TM7*, *Nitrospira* and *Planctomycetes*, were detected in the laboratory treatment. Only one novel phylum, *Actinobacteria,* was found in greenhouse clone libraries. Among the *Proteobacteria*, the γ-subclass increased in both laboratory and greenhouse conditions, while the α- and β-subgroups increased only under greenhouse (HUP^-) conditions; α- and β-*proteobacteria* both decreased in relative abundance under laboratory H_2 treatment (see Table 1).

For other minor groups, it is difficult to deduce whether the data reveal the real situation. Clones selected from the community by chance may onlyrepresent parts of a group. For example*, TM7* clones picked from the H_2-treated soil community were confirmed to have exactly the same sequence by aligning them, and all of them were selected at one time during the experiment. A major limitation of clone libraries, especially for complex communities, appeared here: under-sampling. However, screening of clone libraries provided accurate sequence information and revealed the major contributors to the bacterial community structure after H_2 treatment and between HUP^+ and HUP^- nodule soil ecosystems.

3.3 Metatranscriptome Analysis

Like the T-RFLP/PAT analysis above, metatranscriptome analysis of total RNA from soil showed that for both air- and H_2-treated samples, the dominant bacterial phyla were the *Proteobacteria* and *Actinobacteria* (see Table 1). The data also show that under H_2 treatment, the relative abundance of the *Proteobacteria* increased whereas that of the *Actinobacteria* decreased. The most significant increases under H_2 treatment within the *Proteobacteria* were seen in the β–*proteobacteria* and the δ-*proteobacteria*, whereas there was a decrease in the relative abundance of α-*proteobacteria* under H_2 treatment (see Table 1). The most dramatic increased orders with hydrogen exposure were seen in the *Burkholderiales* (β–*proteobacteria*) and in the *Myxococcales* (δ-*proteobacteria*) (Fig. 4). The most significant decrease order seen under hydrogen exposure was in the *Actinobacteridae* (*Actinobacteria*) (see Table 1).

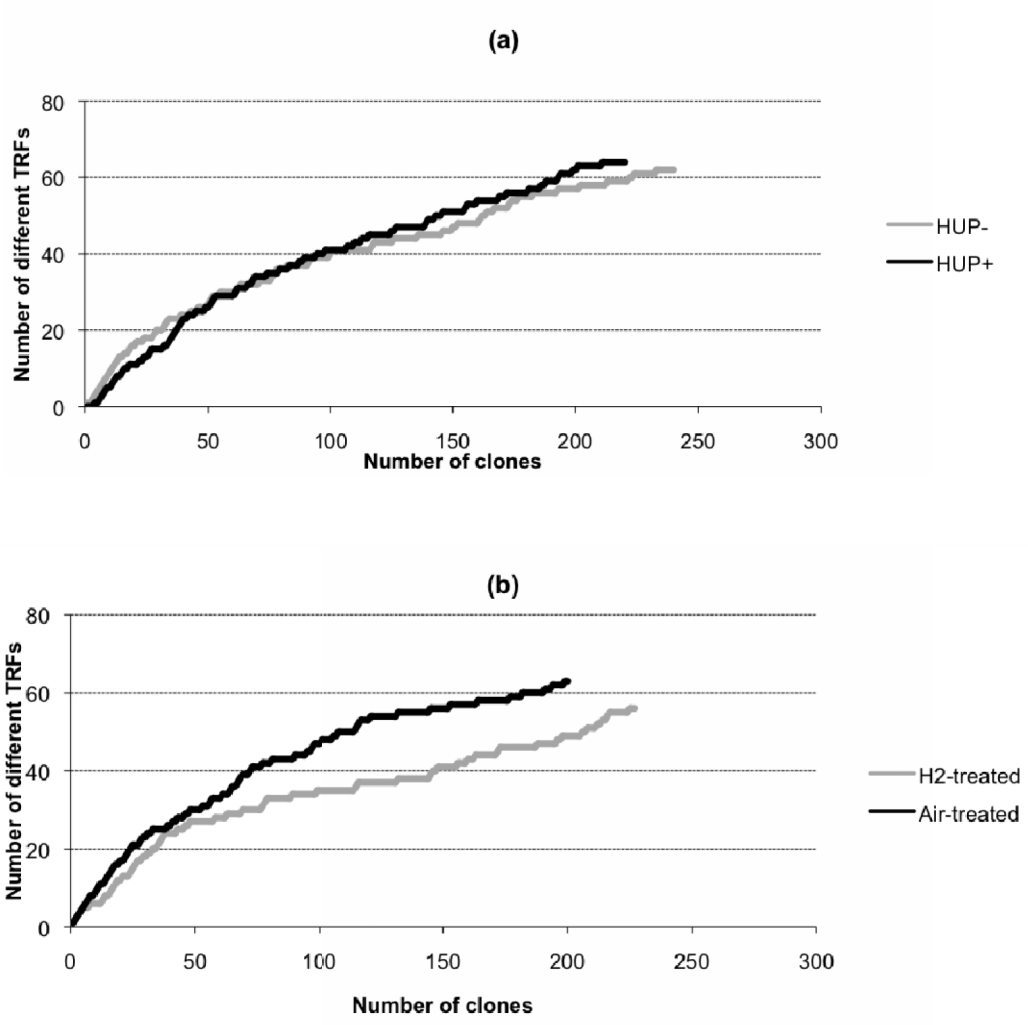

Fig. 3. Rarefaction curves for (a) HUP^+ and HUP^- libraries, and; (b) H_2-treated and air-treated libraries.

3.4 Comparison of T-RFLP, Clone Library and Metatranscriptome Analysis

The size and diversity of TRFs between the results of clone library and T-RFLP analysis are very similar. The number and location of peaks in the artificial profile are very similar to those in the full T-RFLP profile (Fig.2). Additionally, the heights of the peaks match between profiles. While some groups appear to be missing from the artificial profile, it can be explained by the limitation of sample size; T-RFLP profiles represent an entire community, which contains billions of individuals, while artificial profiles consisted of only a few hundred cells.

Results of the T-RFLP analysis confirmed the clone abundance observed in the clone libraries. The 16S rRNA genes of *Proteobacteria* have the highest abundance in 4 libraries and T-RFLP analysis under increased H_2 conditions (H_2-treated and HUP- soil). However,

T-RFLP analysis showed that *Firmicutes* and *Actinobacteria* are a major contributor to plant-soil bacterial diversity; this result was not supported by the RDP analysis from the clone libraries. In air- and H_2-treated soil clone libraries, *Firmicutes* were not present at all.

Metatranscriptome analysis confirmed the T-RFLP analysis and clone abundance for *Actinobacteria*, showing a decrease in this phylum in soils exposed to hydrogen (see Table 1). *Firmicutes* were present in both T-RFLP profiles and in the metatranscriptome analysis and possibly this can again be explained by these two methods representing communities comprised of billions of cells over clone libraries only representing hundreds. The phylum *Proteobacteria* showed an overall increase with hydrogen exposure across all three methodologies with some inconsistencies between methods when considering the classes within this phylum.

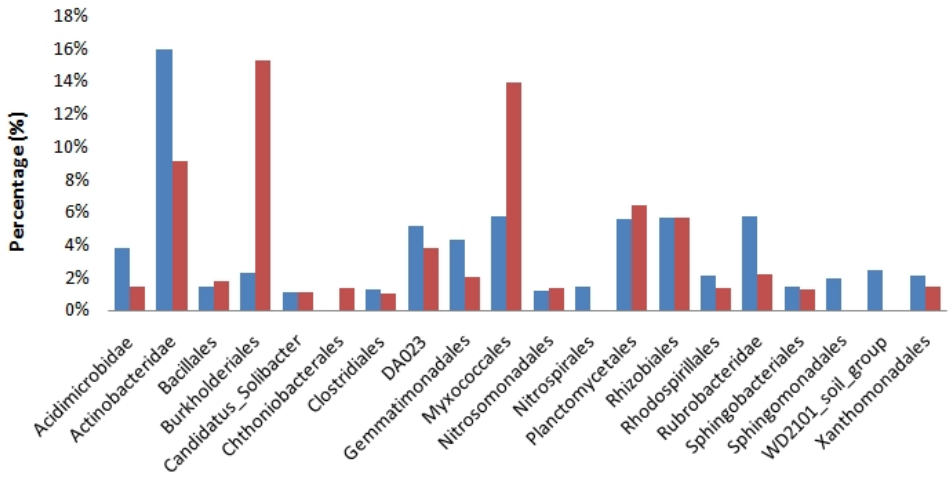

Fig. 4. Soil bacterial community change induced by H_2 treatment at order level.

4. DISCUSSION

This is the first attempt to measure the effect of H_2 on soil bacterial community structure using a combination of results from T-RFLP, clone library and metatranscriptome analytical methods. The richness and relative evenness of a microbial community reflect the selection pressures (such as the presence of H_2) present within those communities [15]. Acquisition of these values is very useful to assess treatment effects.

4.1 T-RFLP, Clone Library and Metatranscriptome Analysis

A comparison of T-RFLP and artificial T-RFLP profiles showed very similar information about how community structure varies with the presence of H_2. The profiles generated from both clone library (artificial profile) and T-RFLP (full DNA fingerprint) data revealed similar results (Fig 2): the DNA fragment size (TRF lengths) and the abundance (peak areas)in both profiles match well respectively. There are some peaks missing from the artificial profile, as compared to the full profiles provided by T-RFLP and transcriptome data, most likely caused by the limited sample size. Despite this, the data suggest that the 350 samples

gathered were sufficient to generally represent the entire microbial community. The similarities in the results from all three methods indicate that T-RFLP, clone library and metatranscriptome analysis are capable of detecting changes of soil microbial diversity *and* community structure; The agreement between T-RFLP analysis and clone library data shows the PAT tool can also be reliably used for phylogenetic grouping in this line of research and reveal the groups/species responsible for H_2-induced changes in microbial community.

The combination of T-RFLP, clone library and metatranscriptome analysis may not be able to accurately reflect the true microbial diversity and community structure because of the respective limitations of these techniques. However, it is a reliable method for examining changes in the community stimulated by certain environmental conditions.Multiple DNA extractions, PCR reactions and restriction enzymes used to generate T-RFLP minimized artifacts and biases that can undermine the integrity of PCR dataand the accuracy of grouping by PAT was maximized [23].

4.2 H_2 Induced Soil Bacterial Population Changes

According to the results of both PAT and RDP analysis, the dominant groups under increased H_2 conditions were *Proteobacteria* and *Bacteroidetes*. In particular, the γ-*proteobacteria* subgroup increased dramatically.

Both transcriptome analysis and DNA sequence analysis of cloned library showed that the Burkholderiales is the most significantly increased group after H_2 treatment. The 7 folds increase of Burkholderiales after H_2 treatment echoes the isolation of H_2 oxidizing *Burkholeria* and *Variovorax* from H_2 treated soil samples [25].Plant growth promoting effects of these isolates suggests that part of the rotation benefit with nitrogen fixing crops may be explained by some increased populations of Burkholderiales stimulated by H_2[25,26].

Results between laboratory treatments and greenhouse conditions were somewhat inconsistent. After H_2 treatment in the lab, γ-*proteobacteria* and *Sphingobacteria* were the bacteria that increased most significantly. However, between two communities of soil adjacent to HUP^- and HUP^+ nodules, α-,β-,γ-*proteobacteria, Sphingobacteria* and *Flavobacteria* (both of phylum *Bacteroidetes*) increased, although some only marginally (α–and β-*proteobacteria*). This difference can be attributed to the presence of plants in the greenhouse condition; Zhang *et al.*[10] found that the single biggest factor that influences soil microbial structure is the presence of plants followed by H_2 exposure. HUP+ nodules do leak some hydrogen resulting in a different environment than that found in the air-treated soil; this resulted in the less drastic changes seen between greenhouse conditions as compared to H_2 and air treatment in the lab.

Results from Zhang *et al.* [10] and Stein *et al*. [8] also support the results found in this study; although the soils were different, the prolific groups were similar. The populations of γ–*proteobacteria* and Bacteroidetesrose after H_2 treatment in both studies.

In conclusion, H_2 promotes populations of γ-*proteobacteria* and *Bacteroidetes* in soil bacterial communities, while suppressing *Actinobacteria* populations. Further, this study concludes that all three methods utilized herein, including T-RFLP/PAT analysis, although possibly unable to detect smaller changes, are thoroughly capable of revealing major shifts in soil microbial community composition due to a change in environmental conditions such

as H_2 exposure; the results of the metatranscriptome analysis support the results from both T-RFLP/PAT and clone library analysis.

Metatranscriptome analysis could be used not only to study soil microbial community composition changes, but also to reveal bacterial gene expression pattern in response to environmental changes or agricultural practice. It could shed further light on plant-microbial interactions. With decrease of cost this method could be used in a wide range of microbiological studies as a powerfully tool.

COMPETING INTERESTS

There exist no financial or non-financial competing interests.

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