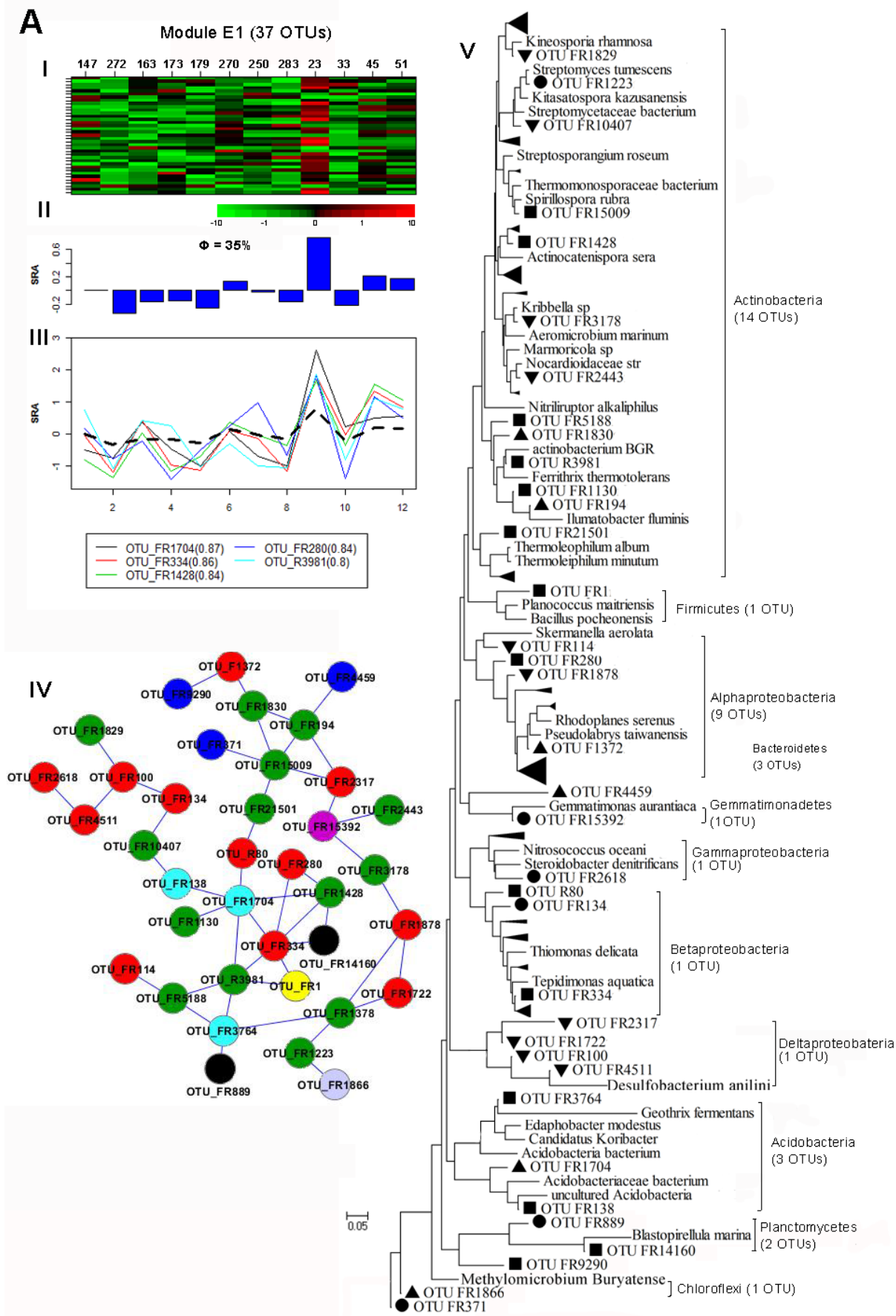


# Supplemental text for “Phylogenetic Molecular Ecological Network of Soil Microbial Communities in Response to Elevated CO<sub>2</sub>”

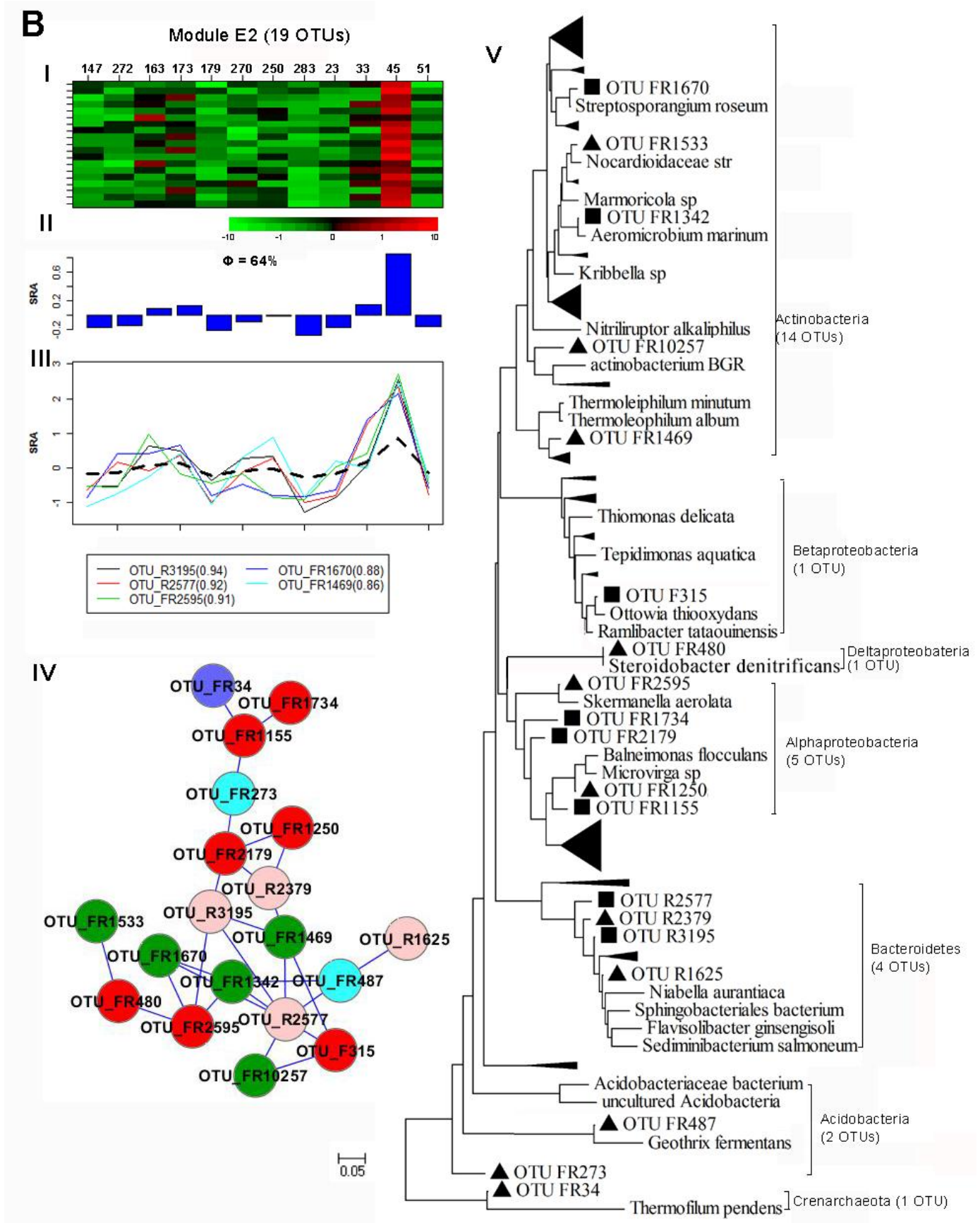
Module eigengene analyses on eCO<sub>2</sub> and aCO<sub>2</sub> phylogenetic molecular ecological network analysis

## 1) Supplemental Figures of module eigengene analyses

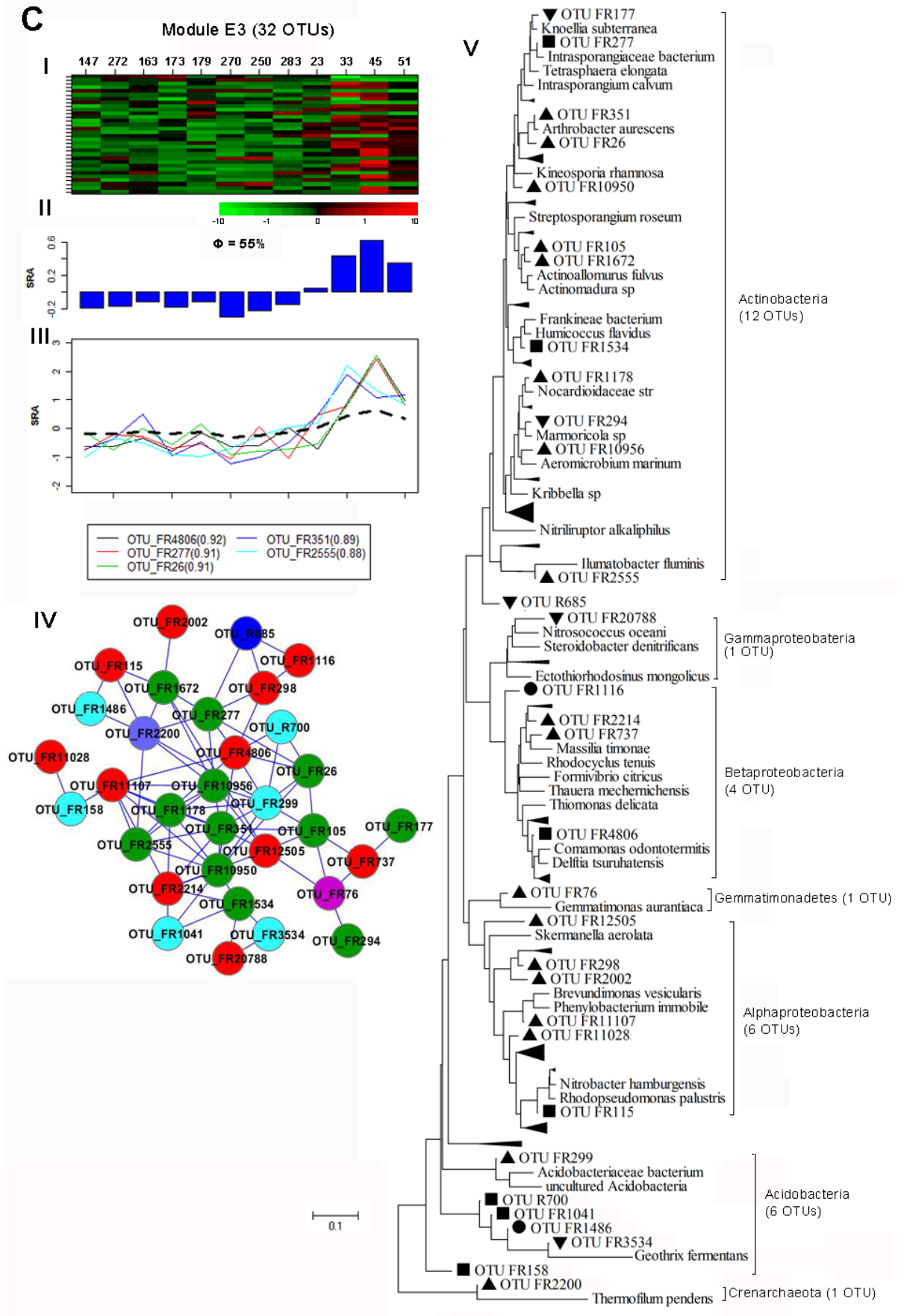
1. **Online Fig. 1.** Eigengene network analysis of individual modules (A-J) under eCO<sub>2</sub>. Only the modules with  $\geq 5$  nodes were displayed here. (I) Heatmap of the standardized relative abundance (SRA) of OTUs across different samples. Rows correspond to individual OTUs in the module whereas columns are the samples. The number above each column is the experimental plot number in the BioCon experiment. Red color corresponds to the OTUs whose SRAs are  $> 0$  and green color signifies those are  $< 0$ . (II). Eigengene. SRA of the corresponding eigengene (y-axis) across the samples (x-axis). The parameter ( $\Phi$ ) indicates the percentage of the total variance explained by the eigengene. (III) Module memberships identifies groups of OTUs that consistently co-exist in these microbial communities. Only 5 OTUs with significant module memberships were shown in this figure where y-axis is SRA and x-axis is individual samples. The numbers in parenthesis indicate the values of module memberships. (IV) Module visualization to show the interactions among different OTUs within the module. Blue line, positive interactive (positive correlation), red line, negative interaction (negative correlation). The different colors of the shading of nodes represent different phylogenetic groups (see Fig. S5 for their meanings). (V) Phylogenetic tree to show the relationships of the OTUs observed in the corresponding module. The tree was constructed with Neighbor-Joining approach with 1,000 bootstrap. Due to space limitation, bootstrap percentages were not shown on the tree. The symbols before individual OTUs signified different features of nodes in the module. ▲ indicates that the OTU exists in both aCO<sub>2</sub> and eCO<sub>2</sub> networks with significant module memberships; ■ indicates that the OTU has significant module membership but not shared in the corresponding network under aCO<sub>2</sub>; ● indicates that the OTU shared but without significant module membership; while ▼ indicates that the OTU neither shared nor had significant module membership.



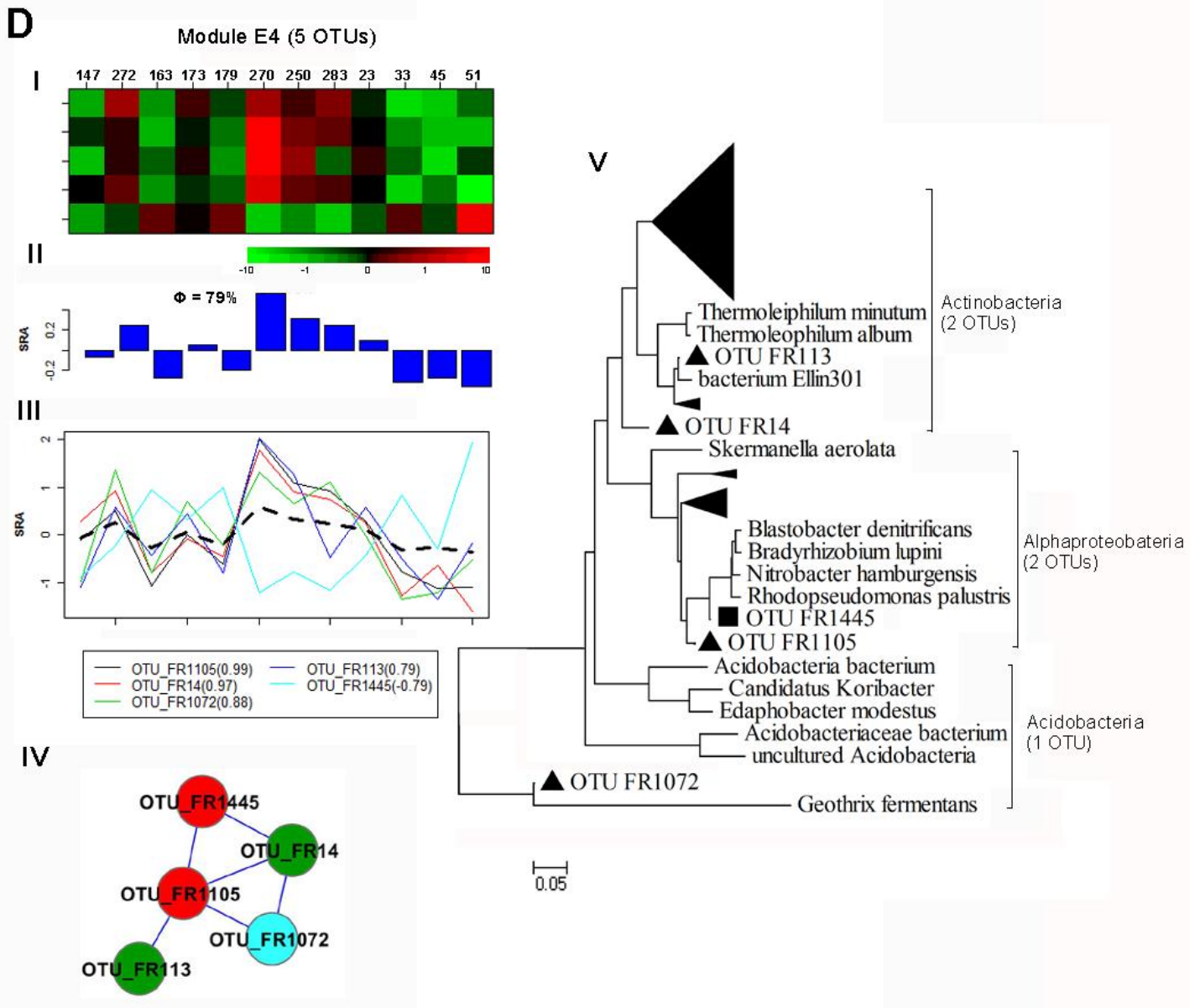
Online Fig. 1.



Online Fig. 1.

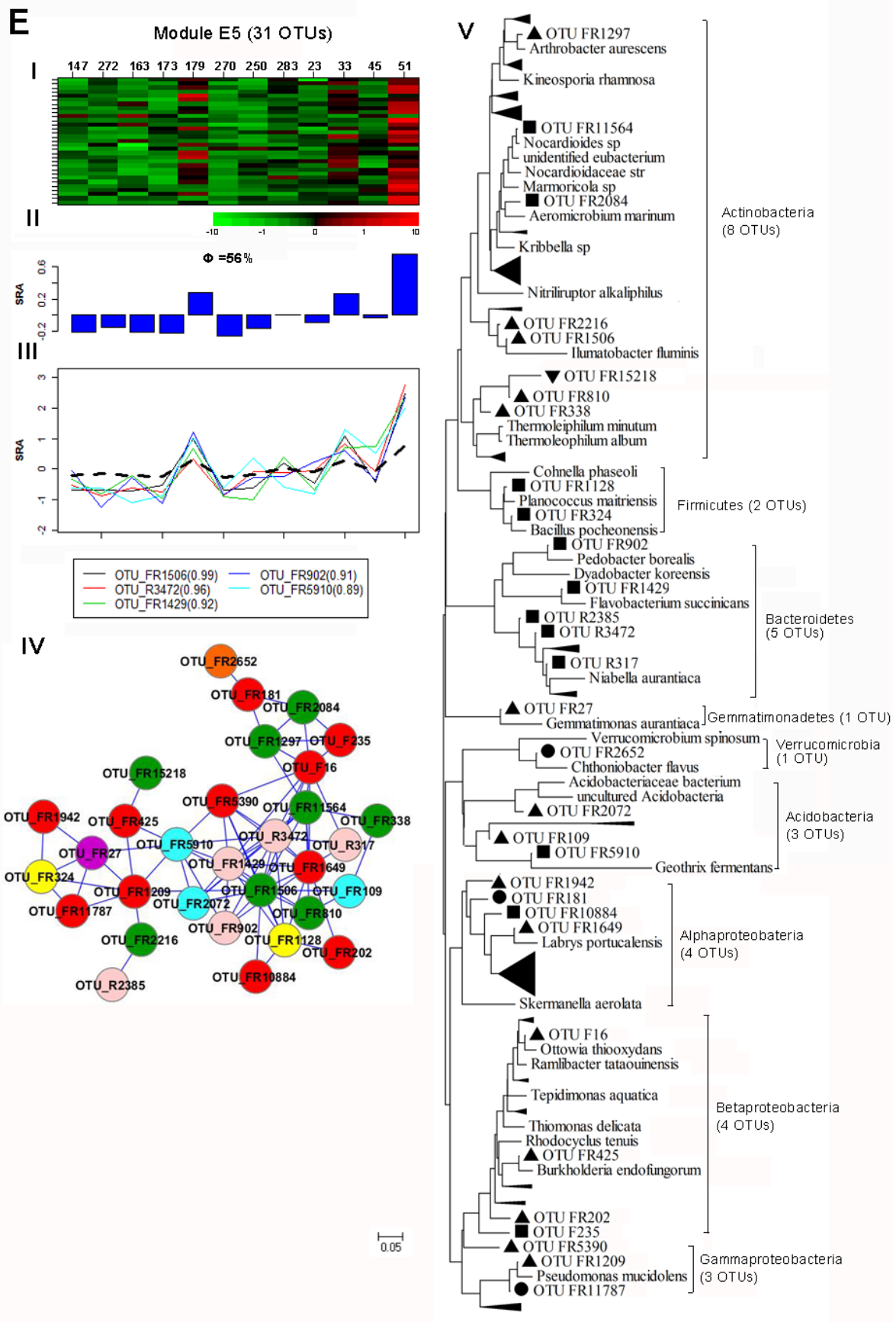


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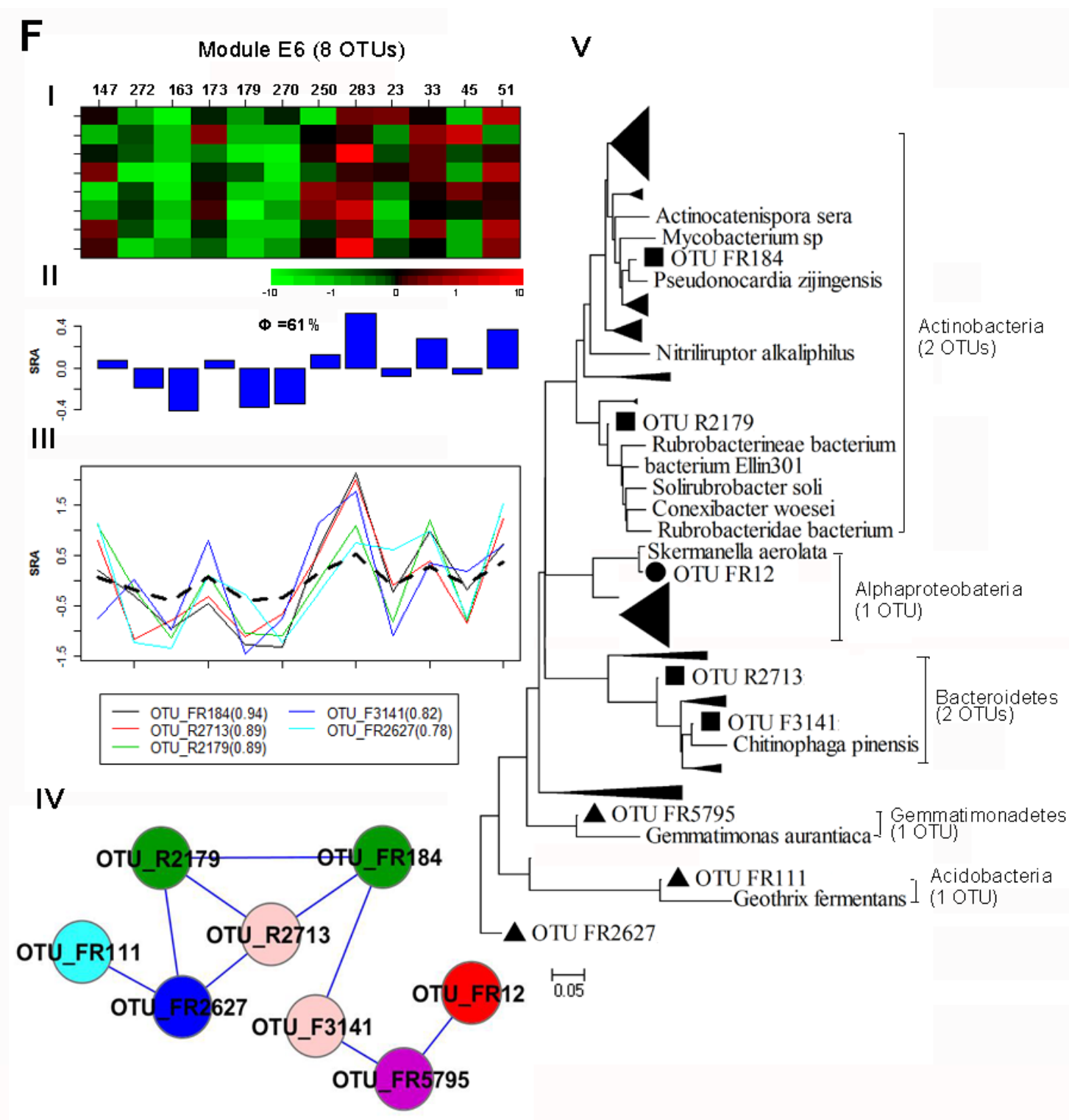


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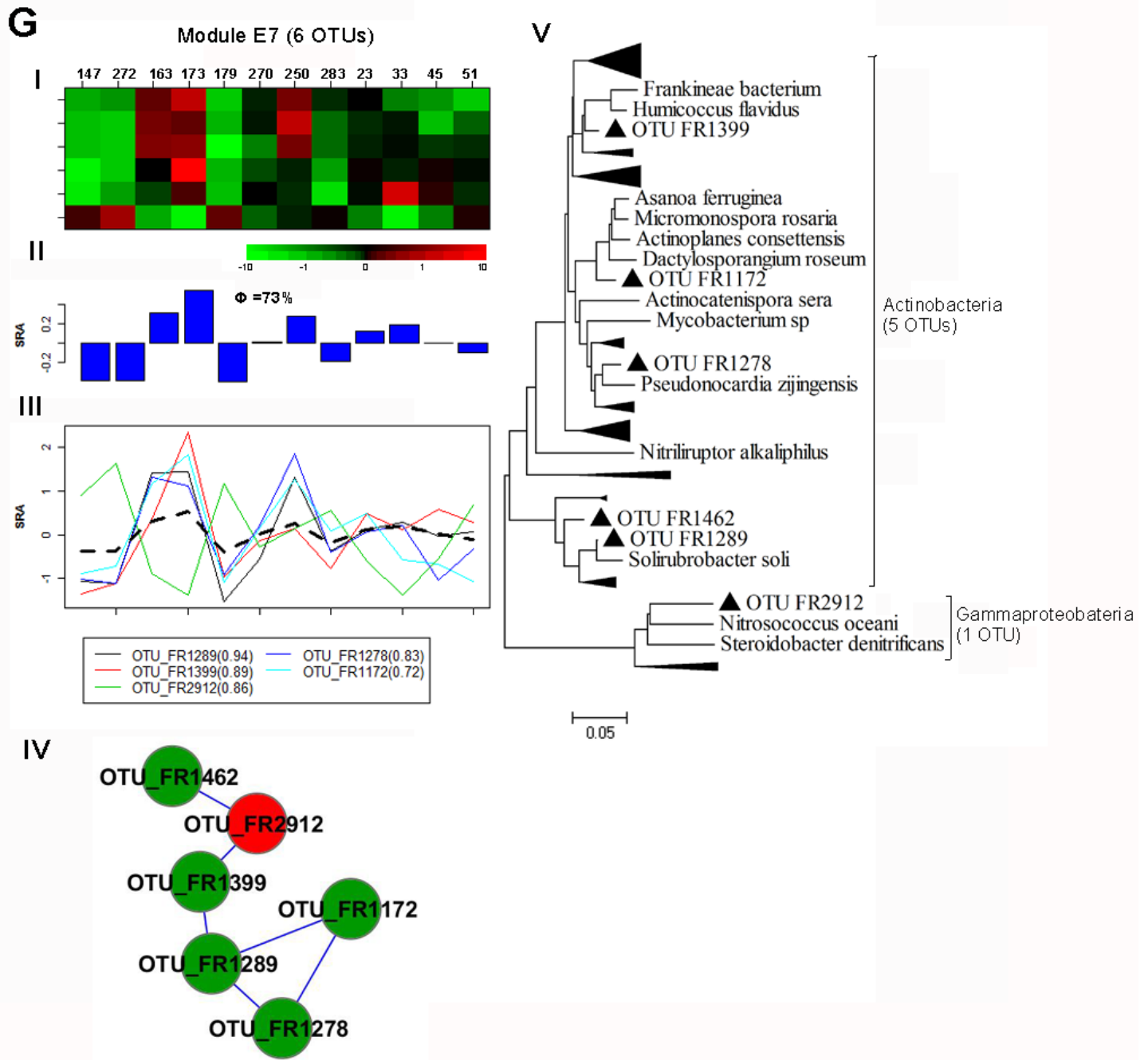




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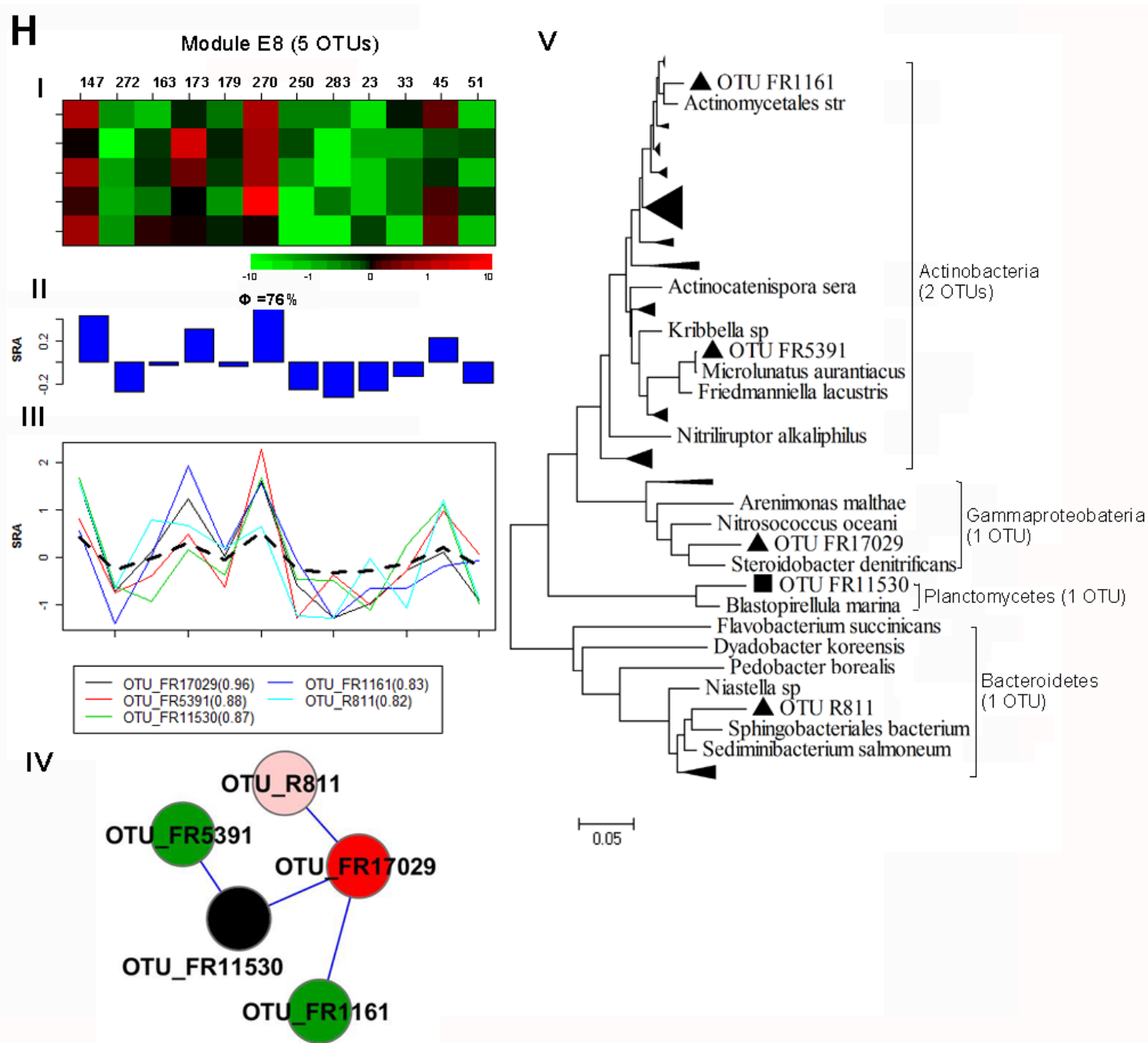


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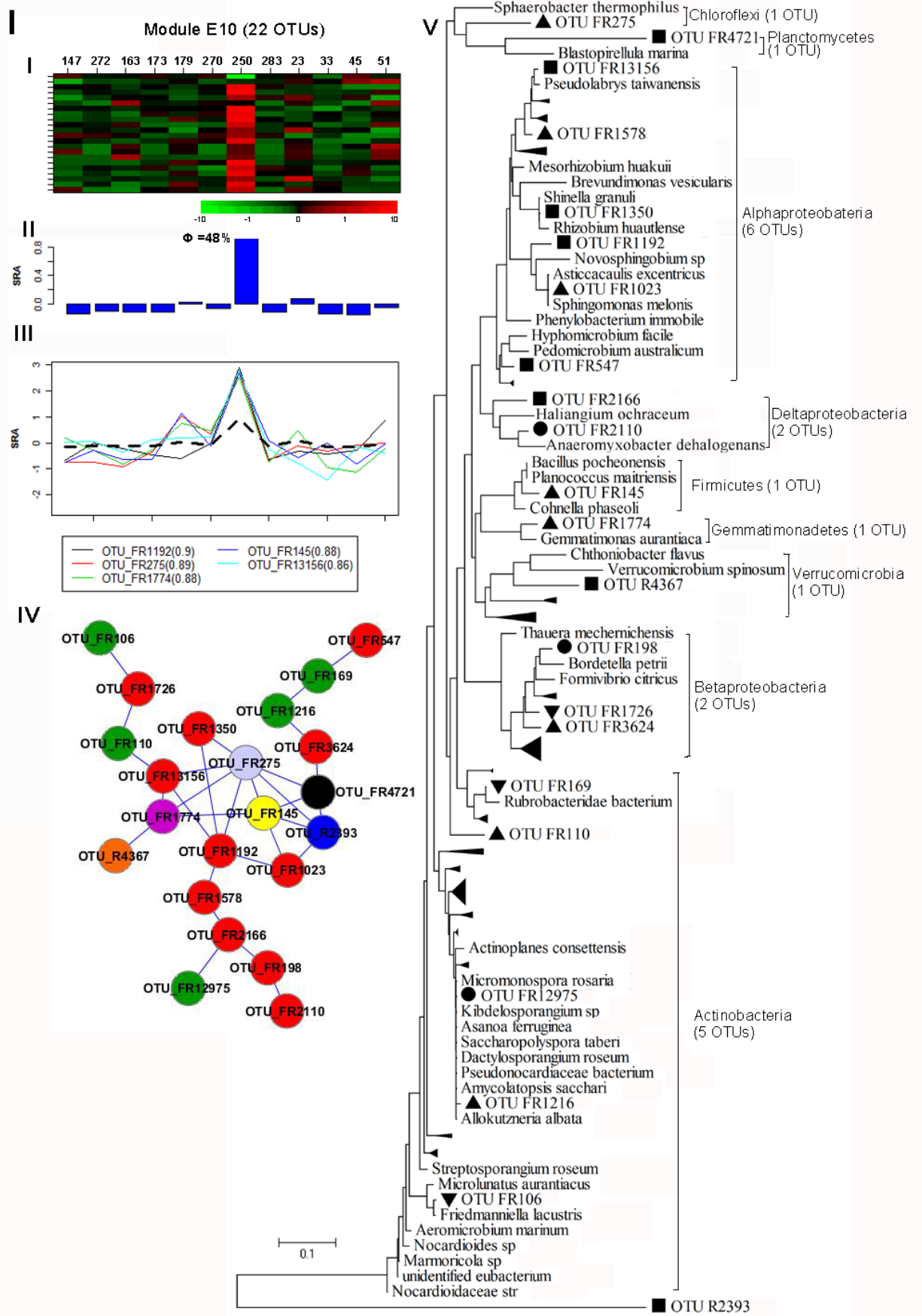


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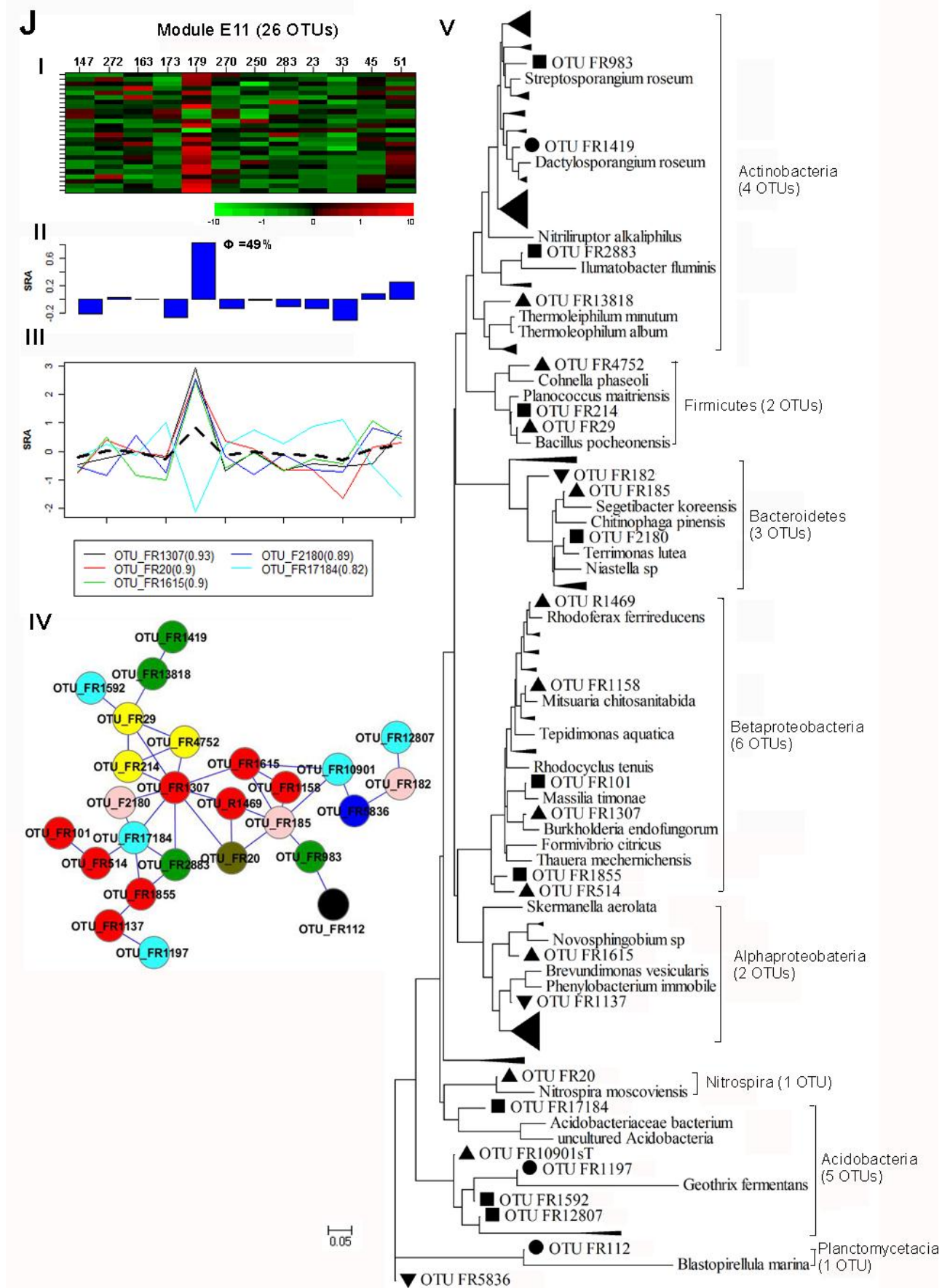




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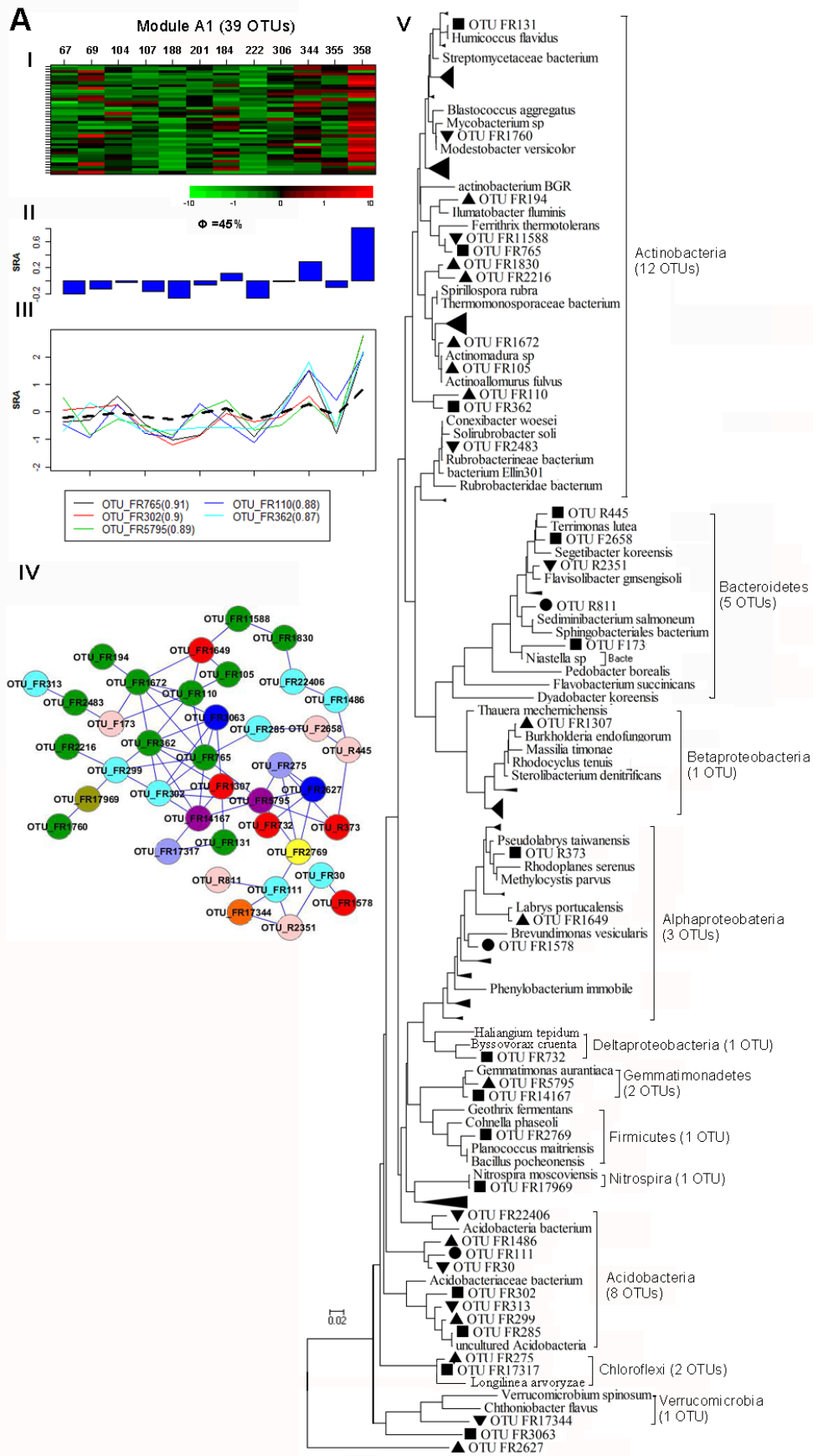


Online Fig. 1.



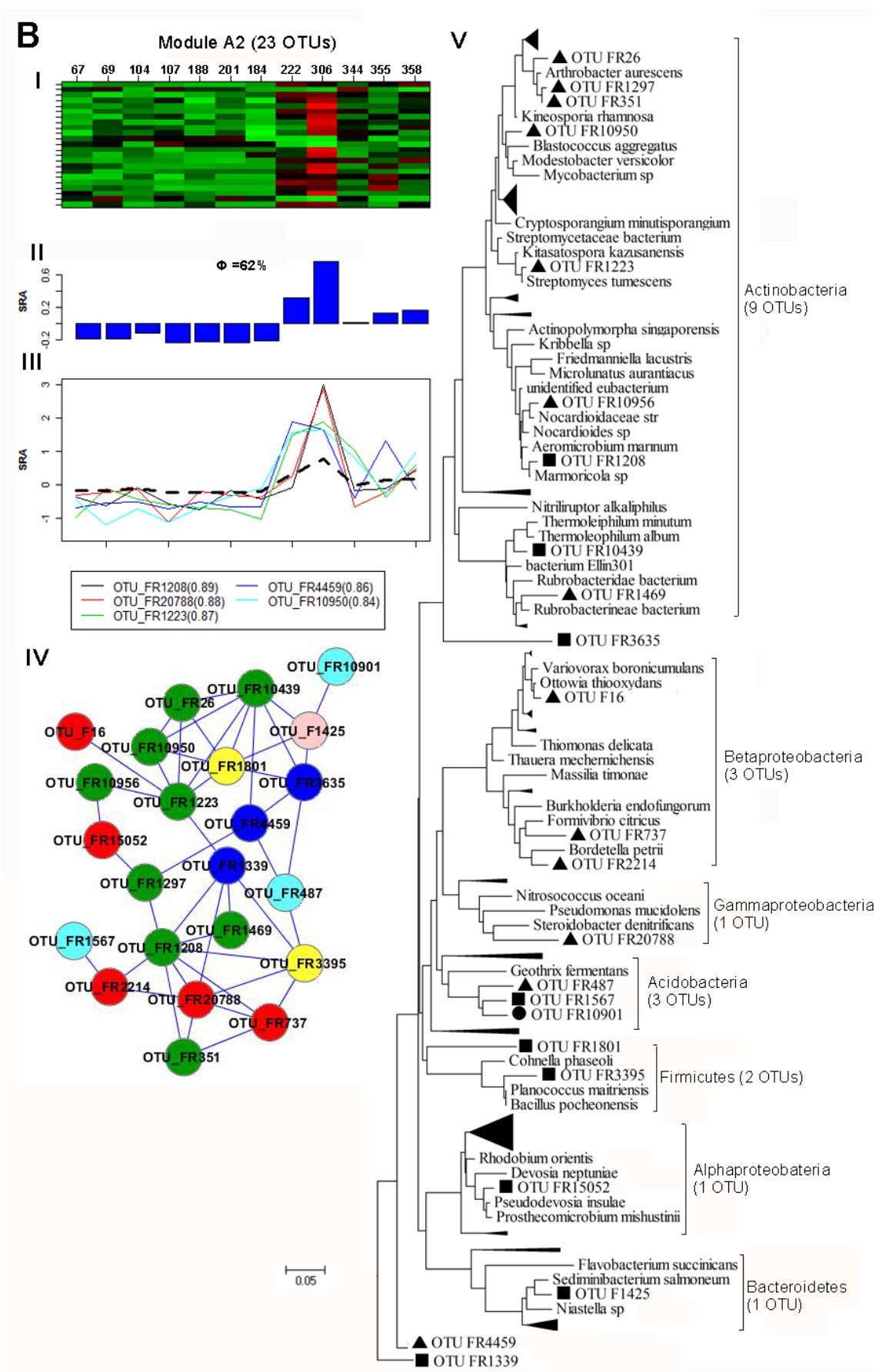
Online Fig. 1.

**2. Online Fig. 2.** Eigengene network analysis of individual modules (A-N) under aCO<sub>2</sub>. Only the modules with  $\geq 5$  nodes were displayed here. See above legend for details.

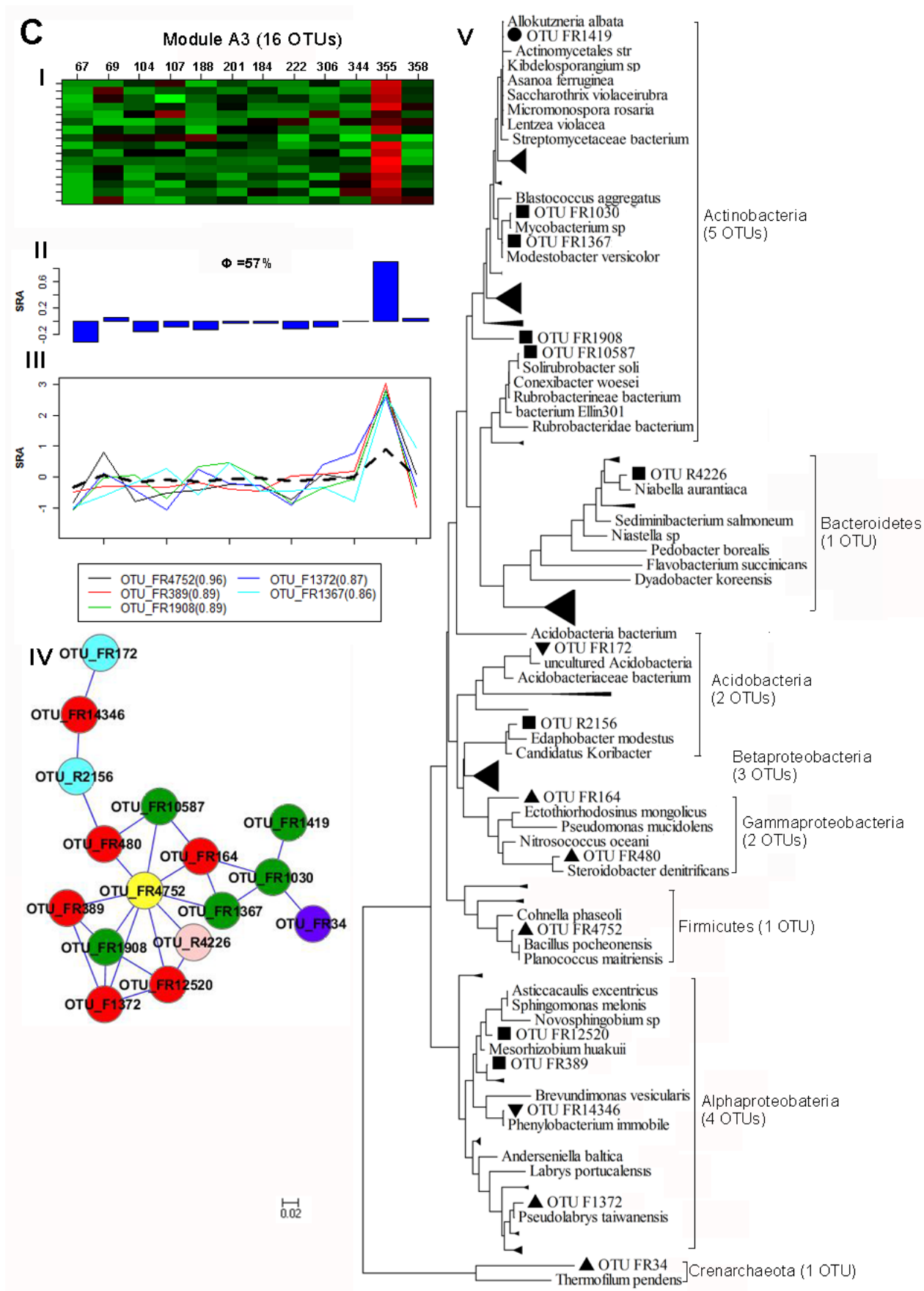


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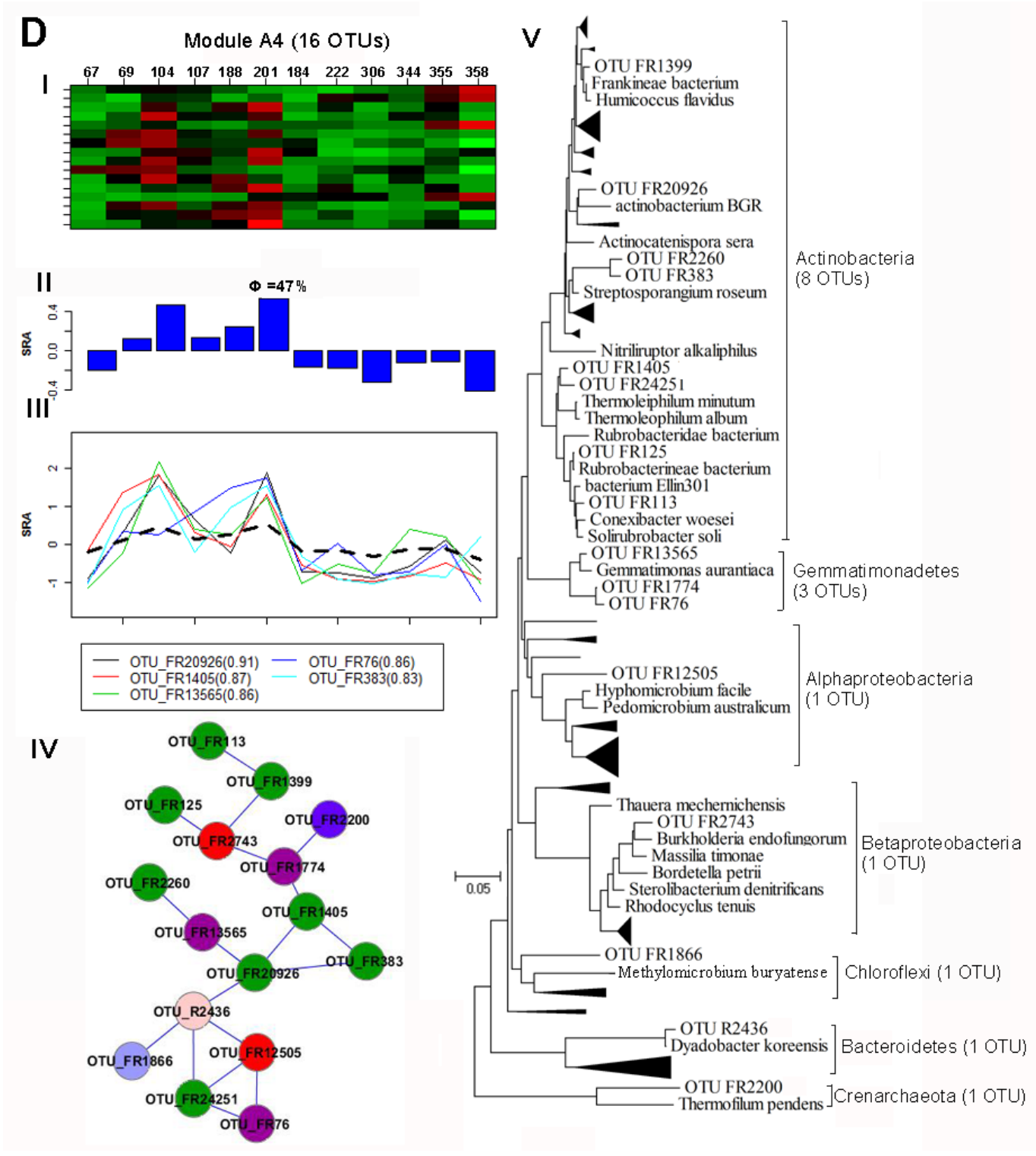




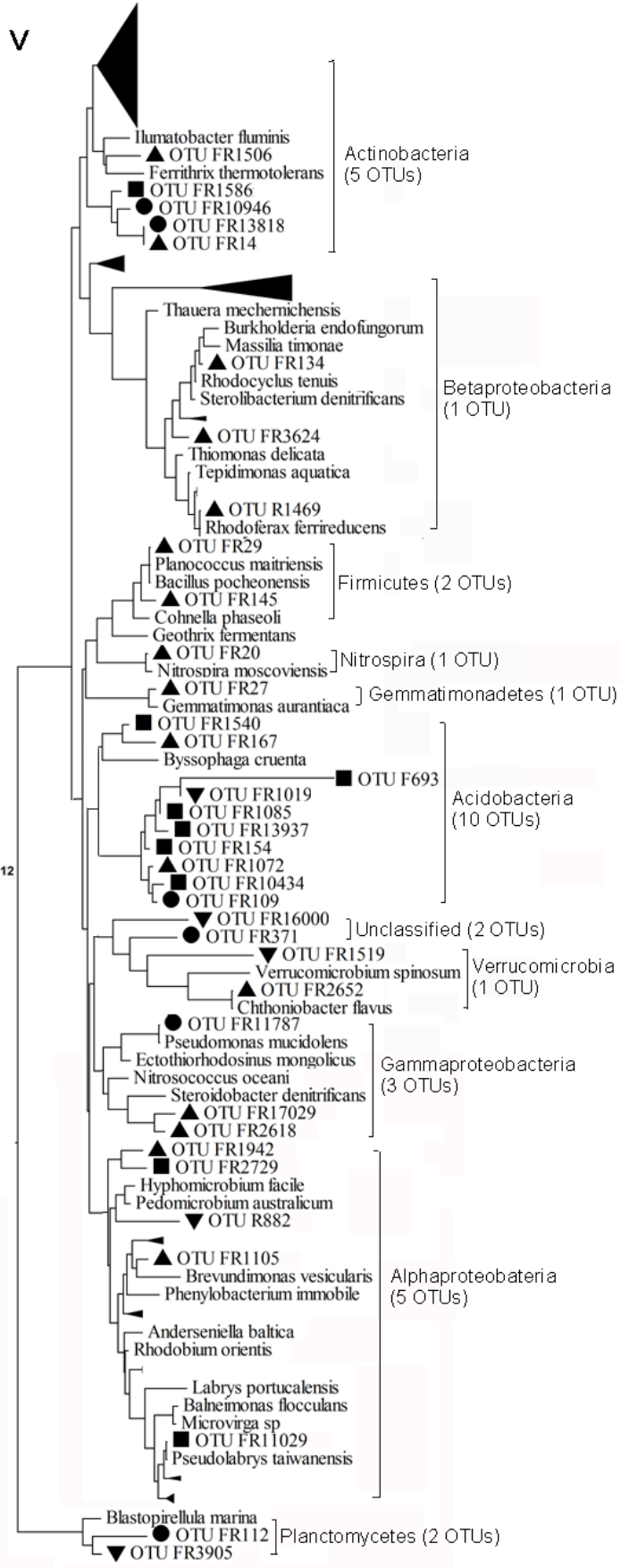
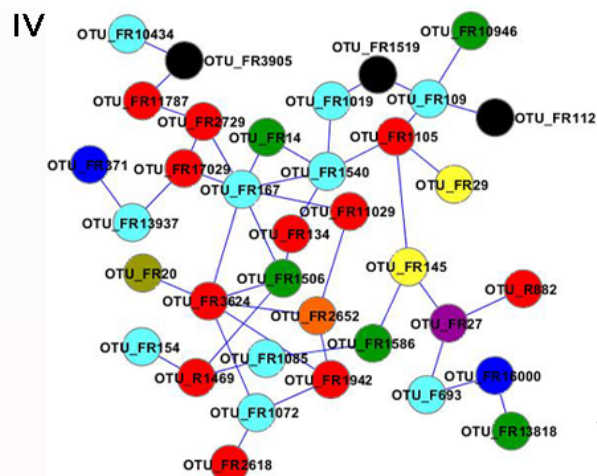
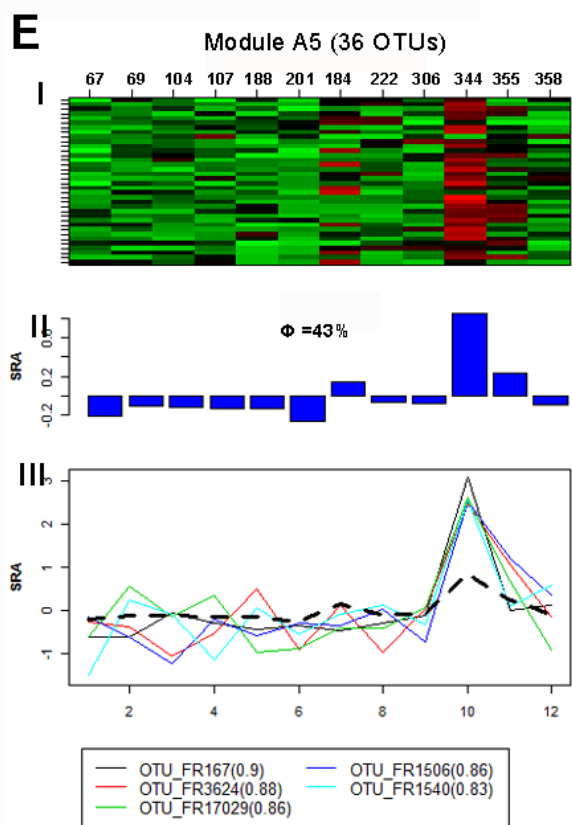
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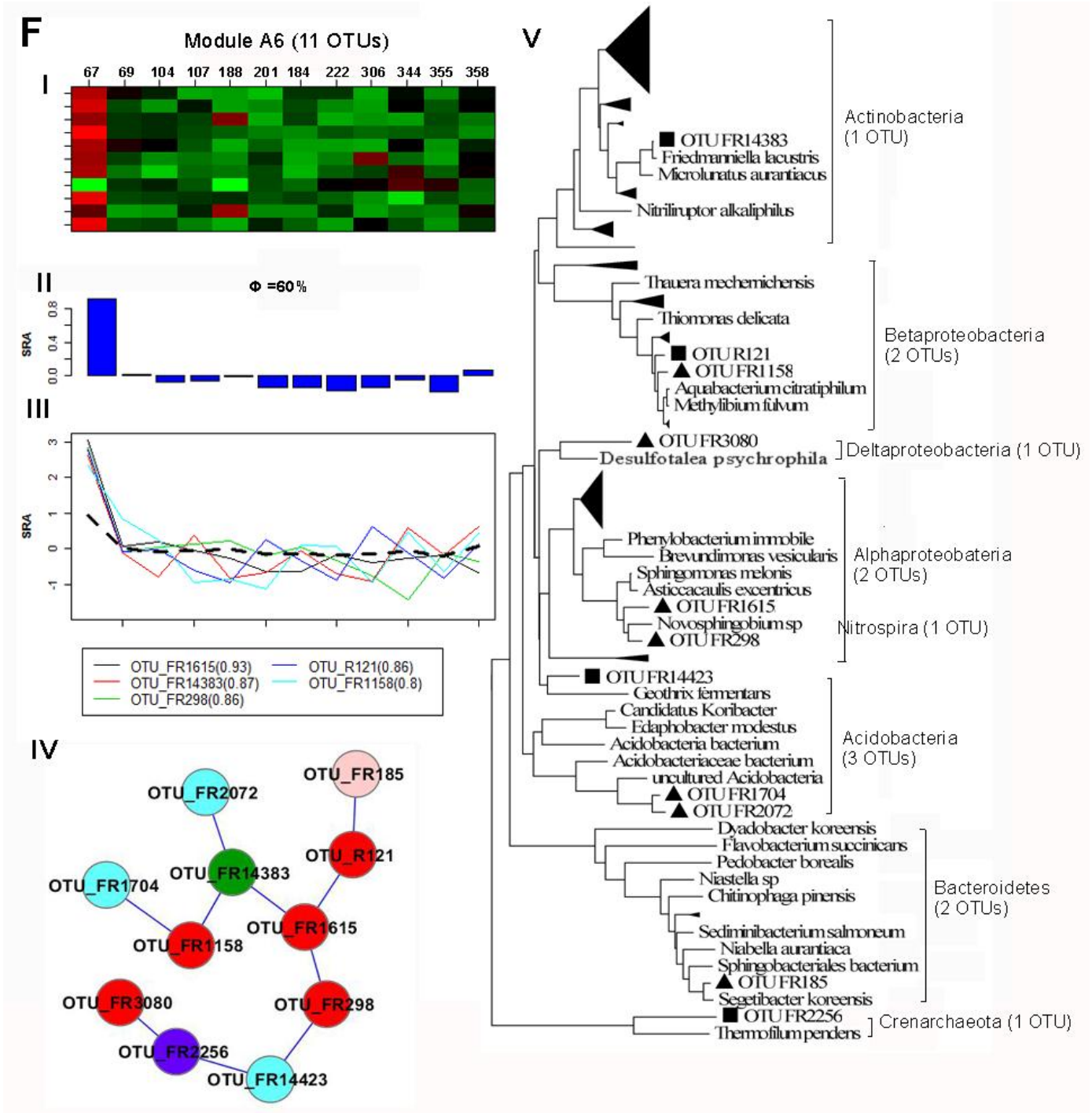
Online Fig. 2.



Online Fig. 2.

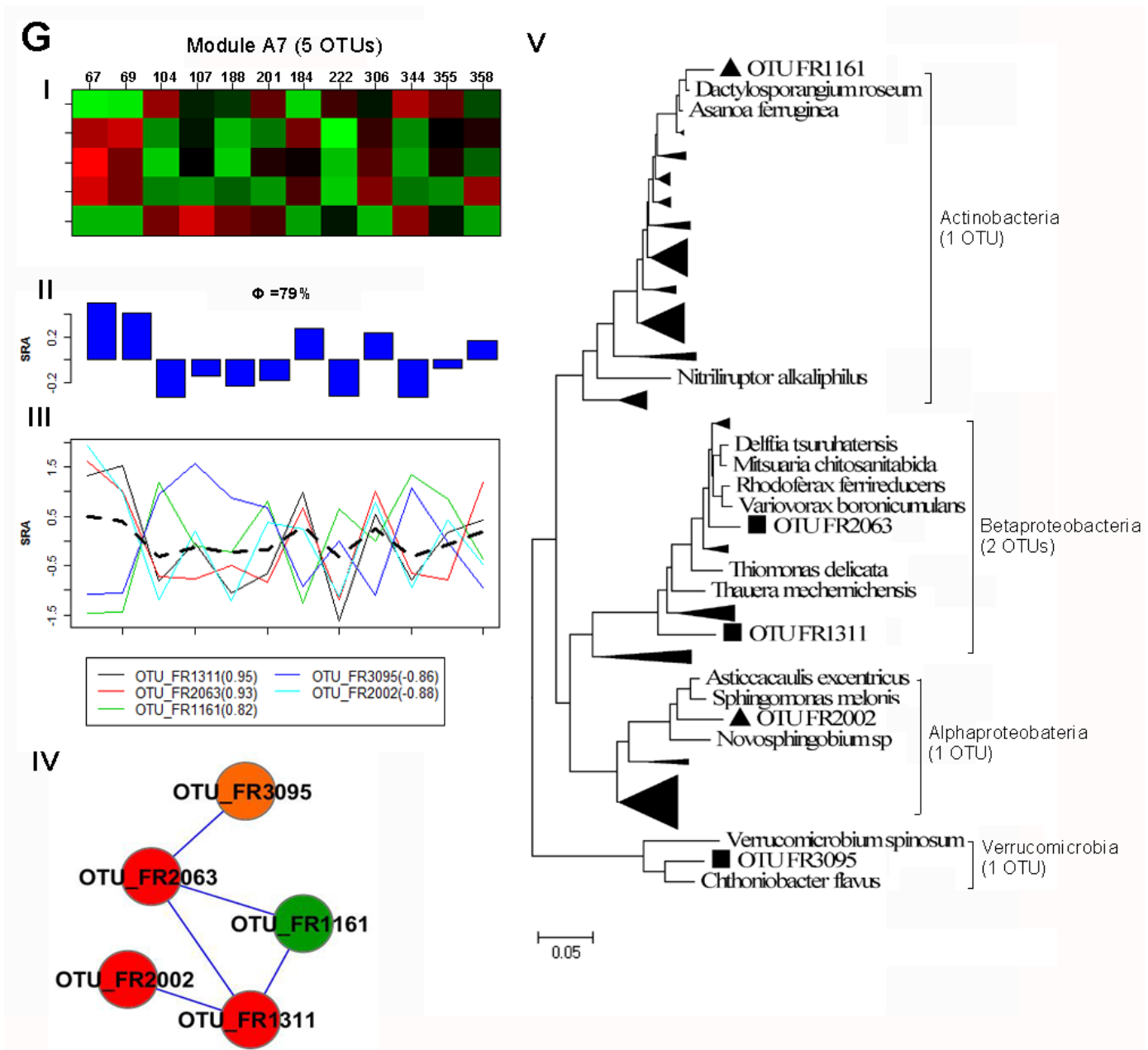


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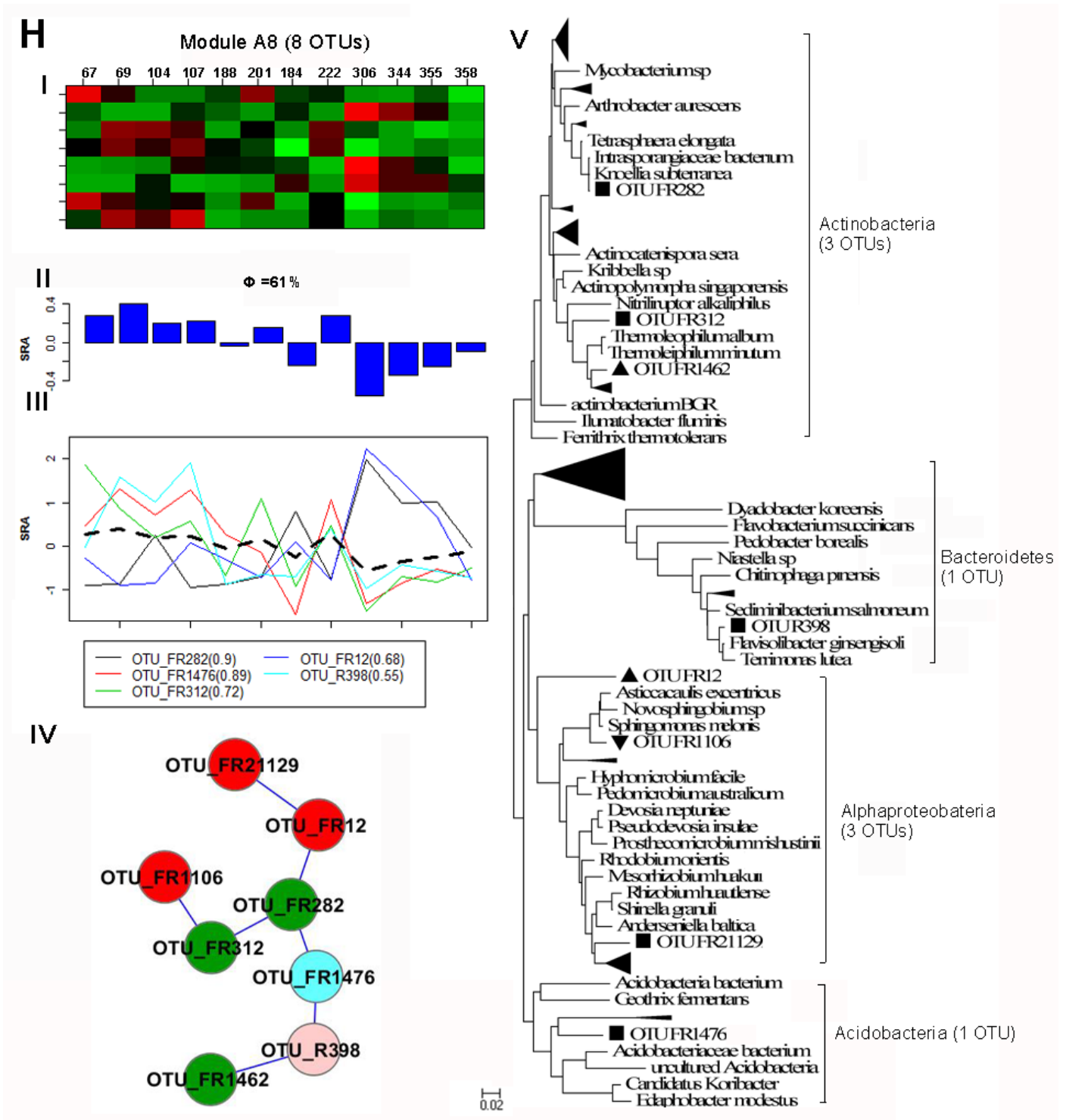


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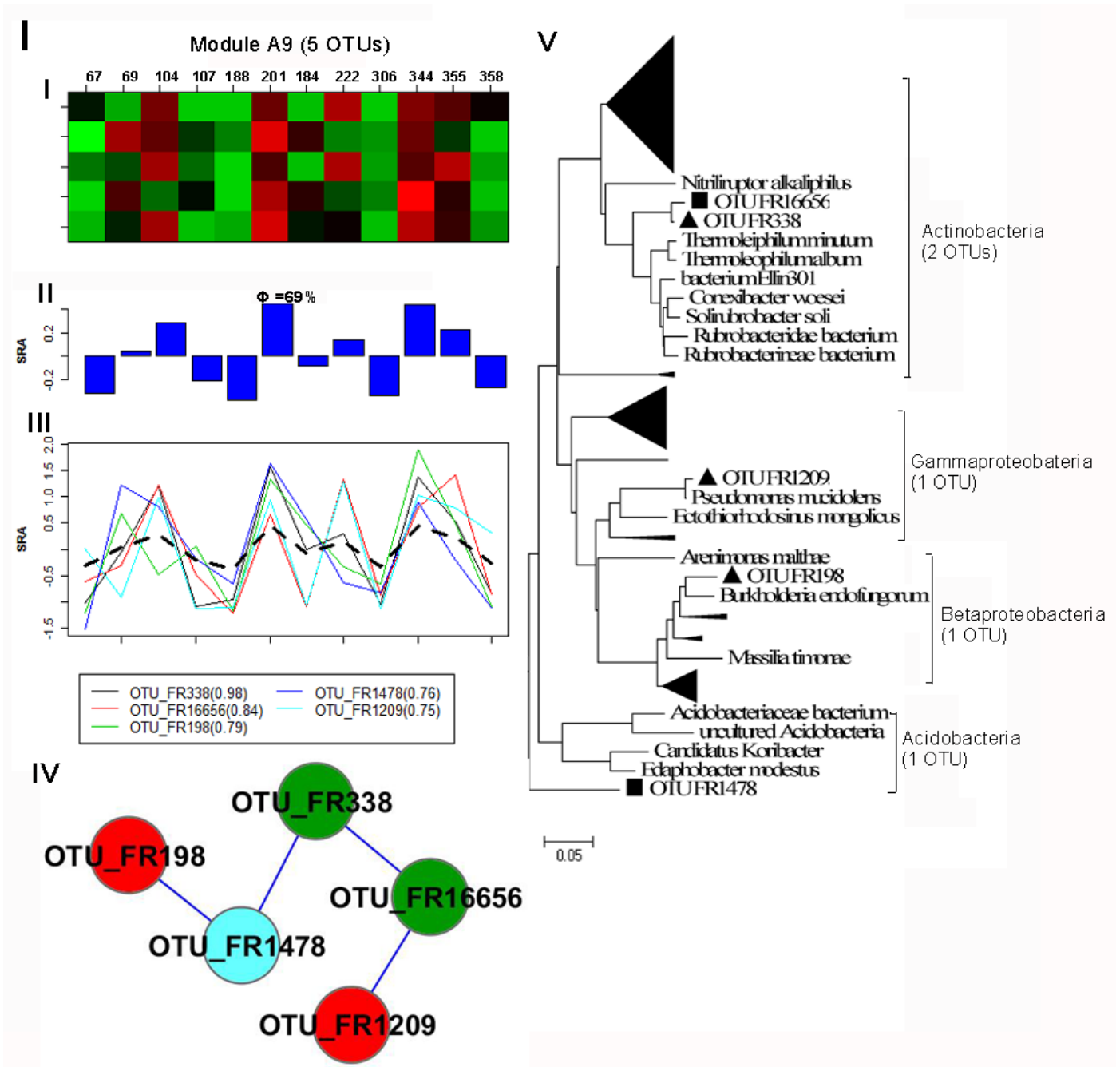




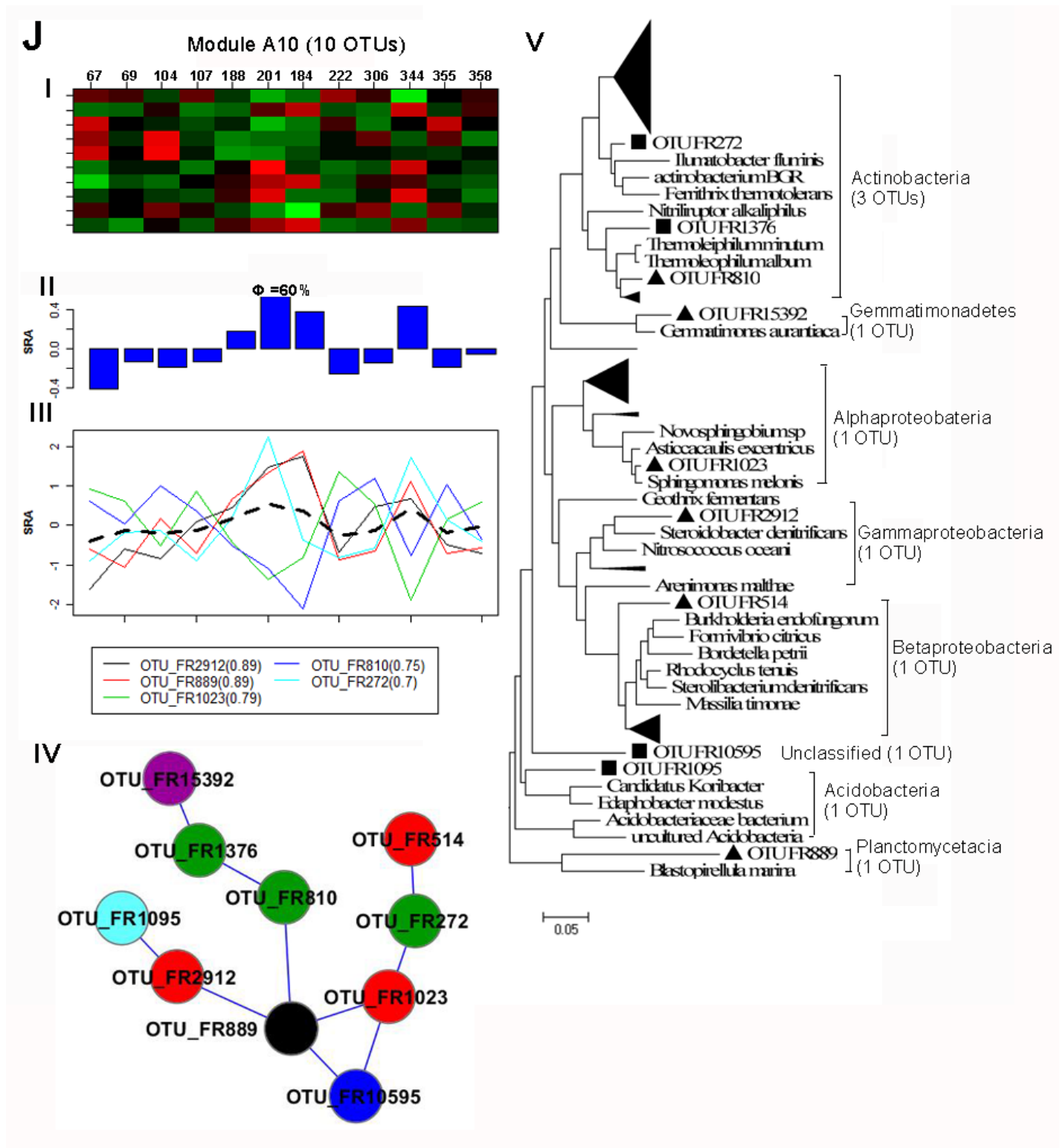
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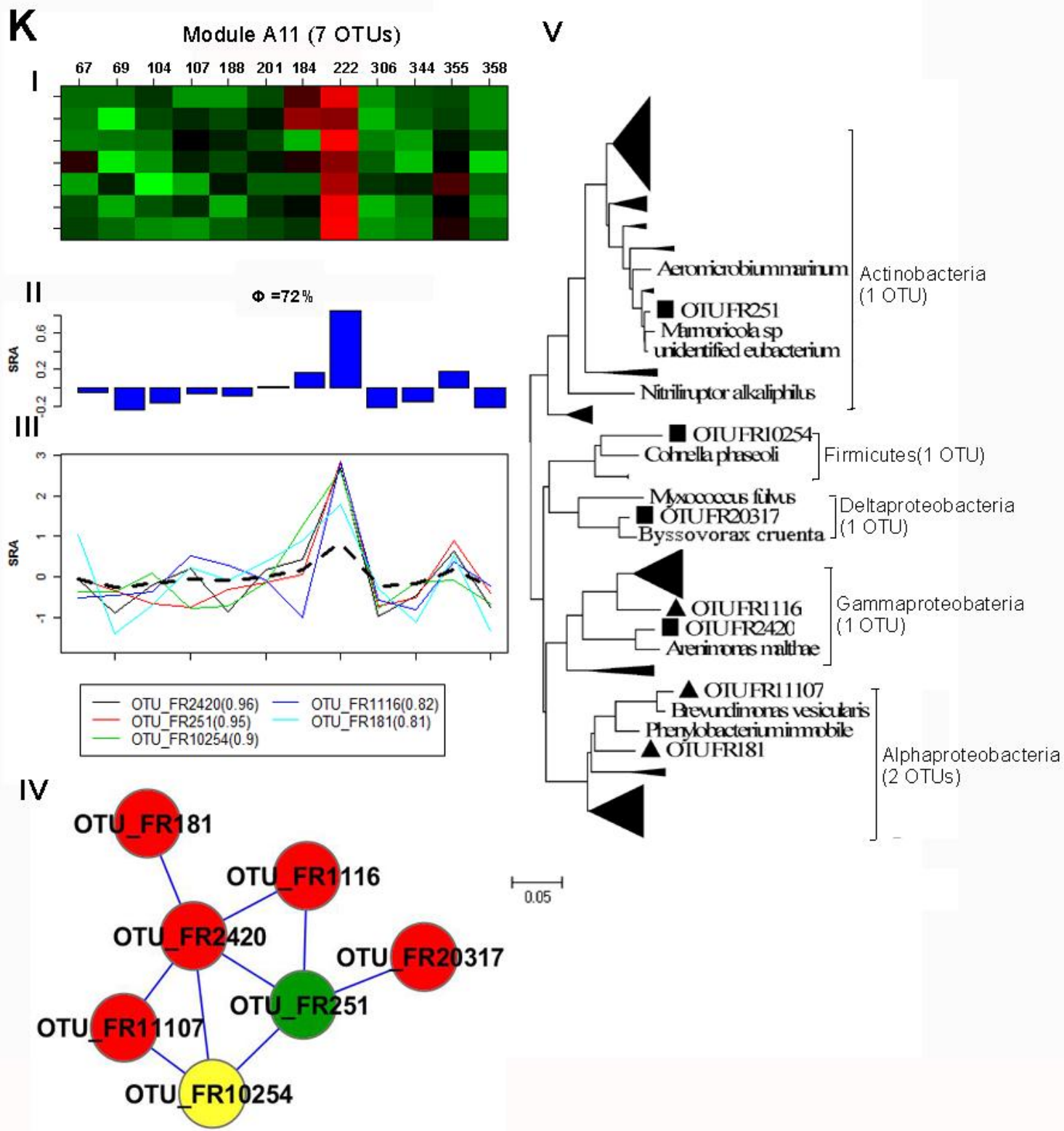
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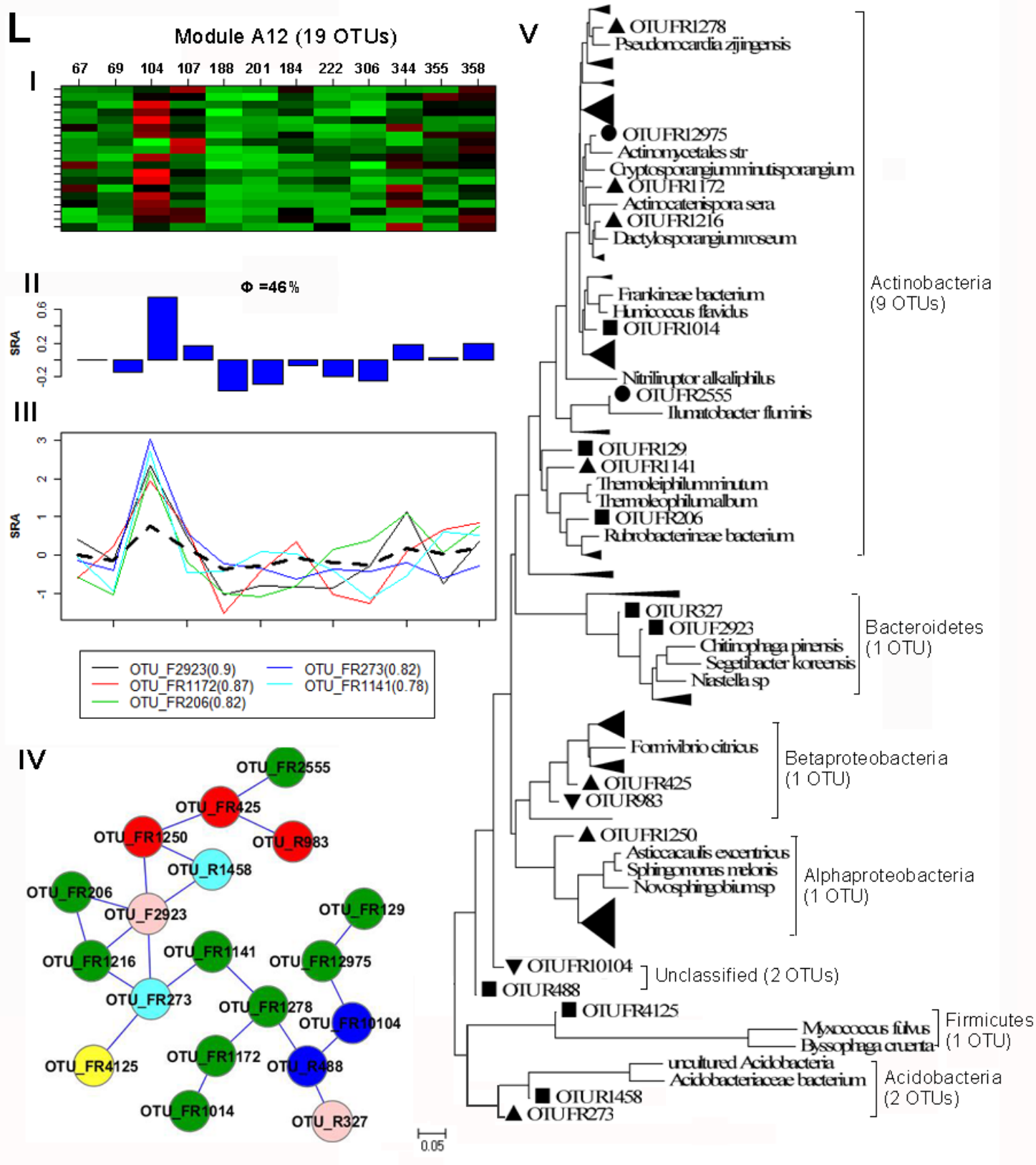


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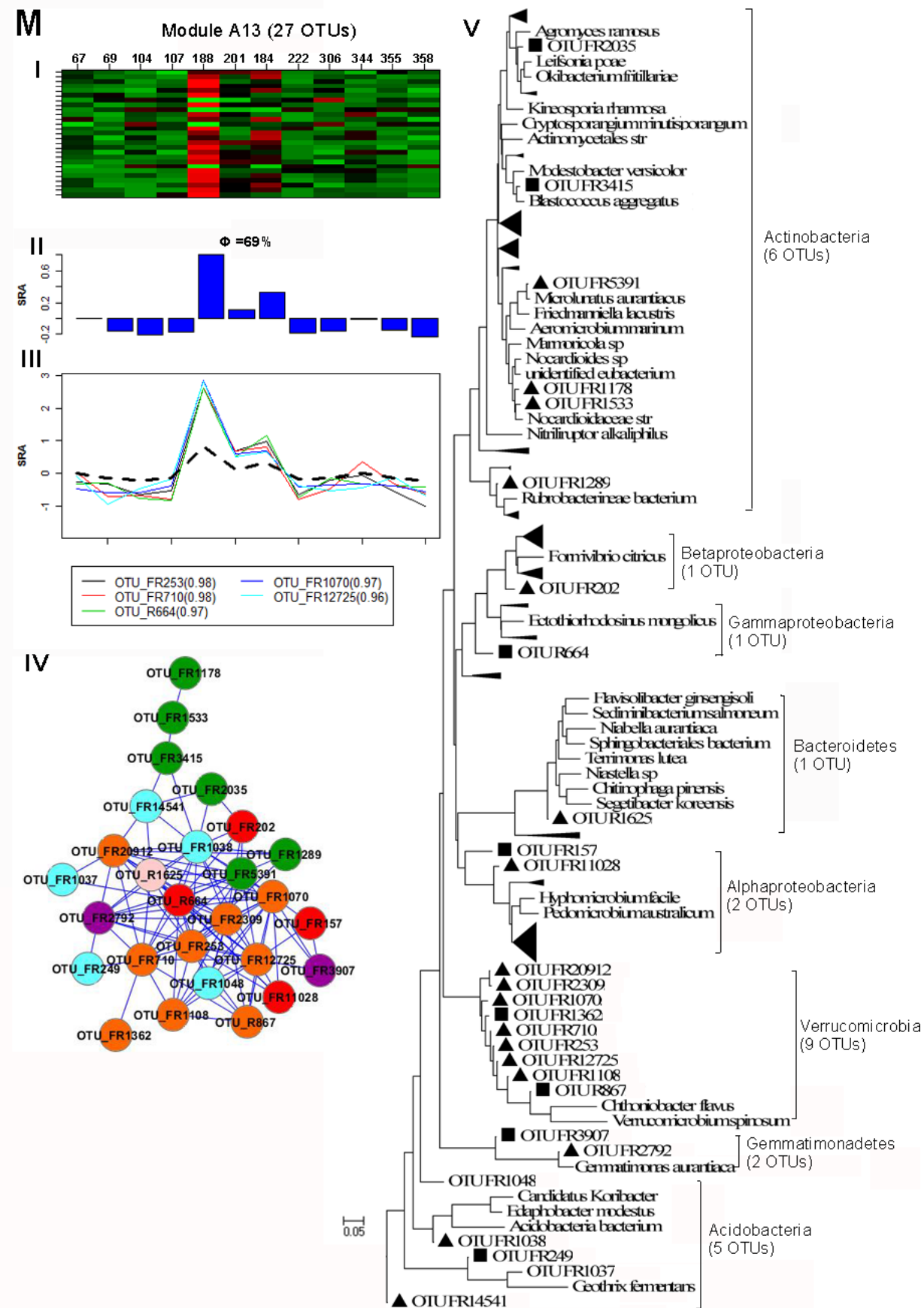


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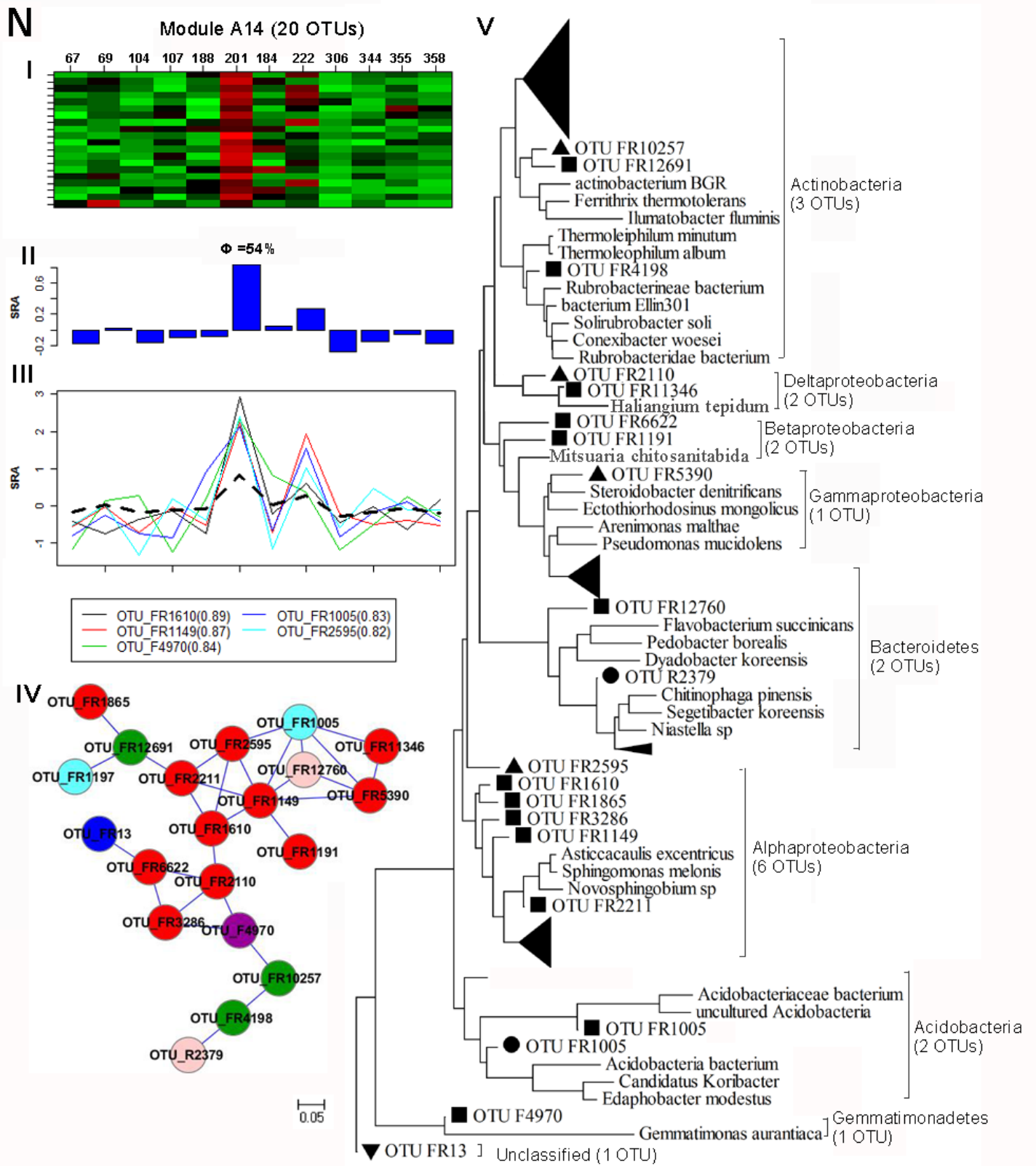




Online Fig. 2.



Online Fig. 2.



Online Fig. 2.

## 2) Supplemental Text for module eigengene analyses

### Detailed description of individual modules

Since the pair-wise correlations of OTU abundance across different samples are used to define the adjacency matrix, the pMENs constructed here in fact describe the co-occurrence of OTUs in microbial communities. Thus a module in a pMEN could indicate that the microbial populations within a module may have similar ecological niches. Detailed examination of the module memberships of individual OTUs are rich sources of new hypotheses for understanding the potential interactions and ecological roles of various microbial species/populations in microbial communities, detailed analyses of individual modules are described as below.

#### *a. Modules under elevated CO<sub>2</sub>*

**Module E1.** This module contained 37 members, making it the largest module in eCO<sub>2</sub> network. The change pattern of the eigengene across all 12 samples was more or less consistent with those of individual OTUs (Fig. S6A-I, II). The eigengene explained 35% of the total OTU abundance variance for this module (Fig. S6A-II). About 54% of the OTUs had significant module memberships with their eigengene of this module (Fig. S6A-III). This module was enriched with *Actinobacteria* (38%) and *Proteobacteria* (32%). As a group, *Actinobacteria* include some of the most common soil, freshwater and marine bacteria. They play an important role in the decomposition of organic materials, such as cellulose and chitin, playing a vital part in organic matter turnover and carbon cycling, replenishing the supply of nutrients in the soil and is an important part of humus formation. OTU FR21501 in *Actinobacteria* is closely related to *Thermoleophilum album* and *Thermoleophilum minutum* (Fig. S6A-V), obligately aerobic thermophilic bacteria restricted to growth on a narrow range of *n*-alkane substrates from C13 to C20, who have been isolated from mud samples obtained from both thermal and nonthermal environments (1).

**Module E2.** This module contained 19 members. The change pattern of the eigengene across all 12 samples was more or less consistent with those of individual OTUs (Fig. S6B-I, II). The eigengene explained 64% of total OTU abundance variance for this module (Fig. S6B-II). All OTUs had significant module memberships with their eigengene of this module and more than half of them (10) were shared with aCO<sub>2</sub> network (Fig. S6B-V). Although this module was composed of diverse groups, 4 of the 15 *Bacteroidetes* from the network were enriched in this module and one (OTU R2577) was a module hub (Fig. 3). Members of the *Bacteroidetes* phylum are found in a wide variety of environments, including soil (2), aquatic habitats (3), insect symbionts (4), and are also dominant in the human gut as beneficial bacteria (5).

**Module E3.** This module had 32 members. The change pattern of the eigengene across all 12 samples was more or less consistent with those of individual OTUs (Fig. S6C-I, II). The eigengene explained 55% of total OTU abundance variance in this module (Fig. S6C-II). About 78% of the OTUs had significant module memberships with their eigengene of this module. This module was primarily enriched with *Actinobacteria* (38%),  *$\alpha$ -Proteobacteria* (19%) and *Acidobacteria* (19%) (Fig. S6C-V). OTU FR351 had the highest connectivity within the module and is closely related to *Arthrobacter aurescens* (Fig. S6C-V), which was originally isolated from soil at an atrazine spill site (6). *Arthrobacter* species are among the most frequently isolated, indigenous, aerobic bacteria found in soils, are metabolically and ecologically diverse, and have the ability to survive harsh environmental conditions (e.g., starvation, radiation, oxygen radicals, and toxic chemicals) for extended periods of time (6).

**Module E4.** This small module contained only 5 members. The change pattern of the eigengene across all 12 samples was very consistent with those of individual OTUs (Fig. S6D-I, II). The eigengene explained 79% of total OTU abundance variance of in this module (Fig. S6D-II). All of the OTUs had significant module memberships with their eigengene of this module. This module was composed of two *Actinobacteria*, two  *$\alpha$ -Proteobacteria* and one *Acidobacteria*, but most of them were a little bit far away from the known isolates except OTU FR113 close to bacterium Ellin301 (Fig. S6D-V) (7, 8).

**Module E5.** This module contained 31 members. The change pattern of the eigengene across all 12 samples was more or less consistent with those of individual OTUs (Fig. S6D-I, II). The eigengene explained 56% of total the OTU abundance variance for this module (Fig. S6D-II). About 87% of the OTUs had significant module memberships with their eigengene of this module. Similar to module E2, 5 of 15 Bacteroidetes were enriched in this module (Fig. S6E-V). OTU FR1506, most significantly correlated with the eigengene, was detected as the module hub as well. Its closest known relative is *Ilumatobacter fluminis*, a Gram-positive, , aerobic *Actinobacterium* estuary sediment isolate (9).

**Module E6.** This is a small module with 8 members. The change patterns of the eigengene across all 12 samples were consistent with those of individual OTUs (Fig. S6F-I, II). The eigengene explained 61% of total variance of the OTU abundance in this module (Fig. S6F-II). All except one OTU had significant module memberships with their eigengene of this module. This module was composed by diverse groups (Fig. S6F-V), and OTU FR184, most significantly correlated with the eigengene, is closely related to *Pseudonocardia zijingensis* that was isolated on an enrichment culture (10).



**Module E7.** This module had 6 members. The change pattern of the eigengene across all 12 samples was very consistent with those of individual OTUs (Fig. S6G-I, II). The eigengene explained 73% of total OTU abundance variance in this module (Fig. S6G-II). All OTUs had significant module memberships with their eigengene of this module and shared with aCO<sub>2</sub> network. Most of the OTUs belonged to *Actinobacteria* (83%) (Fig. S6G-V). OTU FR1399 is closely related to *Humicoccus flavidus* a strictly aerobic soil isolate that grows under conditions of 4~32°C, pH 5.0-8.5 with less than 5% of NaCl. It can use cellobiose, trehalose or arabinose as a sole carbon and energy source, but not benzoate, citrate, or formate (11).

**Module E8.** This is a small module with 5 members. The change pattern of the eigengene across all 12 samples was highly consistent with those of individual OTUs (Fig. S6H-I, II). The eigengene explained 76% of total OTU abundance variance in this module (Fig. S6H-II). All OTUs had significant module memberships with their eigengene of this module. This module was composed of two *Actinobacteria*, a *Planctomycetes*, a *Bacteroidetes* and a  $\gamma$ -*Proteobacteria* (Fig. S6H-V). OTU FR17029, most significantly correlated with the eigengene, was close to *Steroidobacter denitrificans* that is a steroidal hormone-degrading  $\gamma$ -proteobacterium (12).

**Module E9.** This is an intermediate module with 20 members. The change pattern of the eigengene across all 12 samples was very consistent with those of individual OTUs (Fig. 2-I, II). The eigengene explained 63% of total variance of the OTU abundance in this module (Fig. 2-II). All OTUs except one had significant module memberships with their eigengene of this module. This module was dominated by *Verrucomicrobia* (35%) that were closely related to *Chthoniobacter flavus* and *Verrucomicrobium spinosum*, which were enteric bacterium found in aquatic and soil habitats and in the gastrointestinal tract of humans. These organisms can ferment sugars without generating gas as end product (7). *Verrucomicrobium* species can make up to 3% of soil microbiota (7, 8). All OTUs were significantly correlated with the module eigengene and all were shared in both eCO<sub>2</sub> and aCO<sub>2</sub> networks.

**Module E10.** This module had 22 members. The change pattern of the eigengene across all 12 samples was more or less consistent with those of individual OTUs (Fig. S6I-I, II). The eigengene explained 48% of total variance of the OTU abundance in this module (Fig. S6I-II). Most OTUs (68%) had significant module memberships with their eigengene of this module. Although this module was dominated by *Actinobacteria* (23%) and  $\alpha$ -*Proteobacteria* (28%), OTU FR275 from the *Chloroflexi* was found to serve as the module hub (Fig. 3; Fig. S6I-V). As yet this OTU does not have any closely related isolates. OTU FR106 was identified as a

module connector, which was the only one in eCO<sub>2</sub> network. It was closely related to *Friedmanniella lacustris*, a bacterium isolated from a water sample of the hyper-saline Ekho Lake, Antarctica water sample (13).

**Module E11.** This module contained 26 members. The change pattern of the eigengene across all 12 samples was consistent with those of individual OTUs (Fig. S6J-I, II). The eigengene explained 49% of total variance of the OTU abundance in this module (Fig. S6J-II). About 69% of the OTUs had significant module memberships with their eigengene of this module. This module was enriched with  $\beta$ -*Proteobacteria* (23%) and *Acidobacteria* (19%) (Fig. S6J-V). OTU FR1307 which had highest connectivity within the module and mostly correlated with the module eigengene, was closely related with *Burkholderia endofungorum*. The members of genus *Burkholderia* are very abundant and widely spread, including soil and hospital environments (14). Many strains have been found as plant-associated bacteria (14), including both obligate endosymbionts and phytopathogens (14).

#### ***b. Modules under ambient CO<sub>2</sub>***

**Module A1:** With 39 members, A1 was the largest module in the aCO<sub>2</sub> network. The change pattern of the eigengene across all 12 samples was consistent with those of individual OTUs (Fig. S7A-I, II). The eigengene explained 45% of total variance of the OTU abundance in this module (Fig. S7A-II). This module was enriched with *Actinobacteria* (31%), *Acidobacteria* (21%). About 69% of the OTUs had significant module memberships with their eigengene of this module (Fig. S7A-III). OTU FR765 acted as a module hub and OTU FR11588 acted as a connector in this module (Fig. 3). Both are closely related to *Ferrithrix thermotolerans*, an extremely acidophilic, iron-oxidizing *Actinobacteria* isolated from a geothermal site in Yellowstone National Park, Wyoming, USA (15). Under anaerobic conditions it catalyzes the dissimilatory reduction of ferric iron in oxygen-free medium with glycerol as the electron donor (15).

**Module A2.** This module had 23 members. The change pattern of the eigengene across all 12 samples was quite consistent with those of individual OTUs (Fig. S7B-I, II). The eigengene explained 62% of total variance of the OTU abundance in this module (Fig. S7B-II). All OTUs except one had significant module memberships with their eigengene of this module (Fig. S7B-III). This module was still primarily enriched with *Actinobacteria* (39%). OTU FR1208 had highest connectivity within modules and most correlated with the module eigengene and it was close to *Marmoricola sp.*. The members of *Marmoricola* genus were widely isolated from quite different habitats, such as marine sediment (16), soil (17), volcanic ash (18) and even marble statue (19). They were aerobic and Gram-positive coccoid *Actinobacterium*.

**Module A3.** This module had 16 members. The change pattern of the eigengene across all 12 samples was well consistent with those of individual OTUs (Fig. S7C-I, II). The eigengene explained 57% of total variance of the OTU abundance in this module (Fig. S7C-II). About 81% of the OTUs had significant module memberships with their eigengene of this module (Fig. S7C-III). This module was composed of diverse groups, with the module hub OTU FR4752 that was closely related to *Bacillus pocheonensis*. *B. pocheonensis* is an aerobic, halotolerant bacterium originally isolated from a ginseng field soil (20). OTU FR4752 was identified as a connector in this module and it was closely related to *Edaphobacter modestus* in *Firmicutes* phylum. *E. modestus* was isolated from alpine and forest soils and adapted to low carbon concentrations and to neutral to slightly acidic conditions (21).

**Module A4.** This module contained 16 members. The change pattern of the eigengene across all 12 samples was more or less consistent with those of individual OTUs (Fig. S7D-I, II). The eigengene explained 47% of total variance of the OTU abundance in this module (Fig. S7D-II). Majority of the OTUs (69%) in this module had significant module memberships with their eigengene (Fig. S7D-III). This module still was enriched by *Actinobacteria* (50%) (Fig. S7D-V). OTU FR383 and OTU FR125 were both identified as connectors in this module, which were close to *Streptosporangium roseum* and *Rubrobacterineae bacterium* in *Actinobacteria*, respectively. OTU FR113 is closely related to *Conexibacter woesei*, another member of the common soil *Actinobacteria*, that is able to use a variety of substrates and reduce nitrate to nitrite (22).

**Module A5.** This module had 36 members, which was the second largest module in this network. The change pattern of the eigengene across all 12 samples was not so consistent with those of individual OTUs (Fig. S7E-I, II). The eigengene explained 43% of total OTU abundance variance in this module (Fig. S7E-II) and 69% of the OTUs had significant module memberships with their eigengene of this module. This module was composed of a diverse group of bacteria with *Acidobacteria* (28%) as most abundant (Fig. S9E-V). Among them, OTU FR167, as a module hub, was closely related to *Byssophaga cruenta* that degraded crystalline cellulose completely and could be cultivated on filter paper as the only carbon source with inorganic nitrogen source (23) (Fig S7E-V). Besides, The OTU R882 in  $\alpha$ -*Proteobacteria* was closely related to *Rhodopseudomonas palustris* and *Hyphomicrobium facile* (Fig. S7E-V). *Rhodopseudomonas* bacteria are purple nonsulfur phototrophic organisms that can be found in many types of marine environments and soils (24).

**Module A6.** This module had 11 members. The change pattern of the eigengene across all 12 samples was highly consistent with those of individual OTUs (Fig. S7F-I, II). The eigengene explained 60% of total OTU abundance variance in this module (Fig. S7F-II). All of the OTUs had significant module memberships with

their eigengene of this module. This module was dominated by *Proteobacteria* (64%) and one, OTU FR1615, has highest correlation with the module eigengene. This OTU is close to *Novosphingobium* species, which are capable of degradation of a variety of organic contaminants, such as naphthalene (25) and carbofuran, a broad-spectrum pesticide (26).

**Module A7.** This small module had 5 members only. The change pattern of the eigengene across all 12 samples was very consistent with those of individual OTUs (Fig. S7G-I, II). The eigengene explained 79% of total OTU abundance variance in this module (Fig. S7G-II). All the OTUs had significant module memberships with their eigengene but only two of them were shared with eCO<sub>2</sub> network (Fig. S7G-III). This module was dominated by *Proteobacteria* (60%), one  $\alpha$ -*Proteobacteria* and two  $\beta$ -*Proteobacteria*. OTU FR1311 had the most connectivity and highest correlation with the module eigengene, but it was not close to any isolates within the  $\beta$ -*Proteobacteria*.

**Module A8.** This module had 8 members. The change patterns of the eigengene across all 12 samples were quite consistent with those of individual OTUs (Fig. S7H-I, II). The eigengene explained 61% of total variance of the OTU abundance in this module (Fig. S7H-II). The majority (88%) of the OTUs had significant module memberships with their eigengene of this module (Fig. S7H-III). This module was primarily enriched with *Actinobacteria* (38%), and  $\alpha$ -*Protoebacteria* (38%) (Fig. S7H-V). The central node OTU FR282 was significantly negative correlated the module eigengene. This OTU was closely related with *Intrasporangiaceae bacterium* and *Knoellia subterranea* of the *Actinobacteria*. *K. subterranea* was isolated from a soil sample in the Reed Flute Cave near Guilin, China (27) and is an aerobic to microaerophilic bacterium, capable of growth on acetate, formate, malate or succinate as a carbon source, but not aconitate, benzoate, citrate or tartrate (27).

**Module A9.** This is a small module with 5 members. The change patterns of the eigengene across all 12 samples were quite consistent with those of individual OTUs (Fig. S7I-I, II). The eigengene explained 69% of total variance of the OTU abundance in this module (Fig. S7I-II) and all of the OTUs had significant module memberships with their eigengene of this module. This module was composed by *Actinobacteria*,  $\gamma$ -*Proteobacteria*,  $\beta$ -*Proteobacteria* and *Acidobacteria* (Fig S9I-V). The top two significant OTUs correlated to their eigengene were both from *Actinobacteria* and clustered together but do not have any closely related isolates.

**Module A10.** This module had 10 members. The change pattern of the eigengene across all 12 samples was well consistent with those of individual OTUs (Fig. S7J-I, II). The eigengene explained 60% of total OTU

abundance variance in this module (Fig. S7J-II) and all had significant (positively or negatively) module memberships with their eigengene of this module (Fig. S7J-III). Although this module was relatively small it was composed of several bacterial groups (Fig. S7J-IV, V). OTU FR889 had the highest connectivity and most significant correlation with the module eigengene, but its taxonomy is unclear. The nearest relative was *Blastopirellula marina*, a strictly aerobic *Planctomycetacia* (28).

**Module A11.** This module had 7 members. The change pattern of the eigengene across all 12 samples was quite consistent with those of individual OTUs (Fig. S7K-I, II). The eigengene explained 72% of total OTU abundance variance in this module (Fig. S7K-II) and all of the OTUs had significant module memberships with their eigengene of this module (Fig. S7K-III). This module was dominated by *Proteobacteria* (71%), including two  $\alpha$ -, one  $\delta$ - and two  $\gamma$ -*Proteobacteria* (Fig. S7K-IV, V). One of the  $\gamma$ -*Proteobacteria*, OTU FR2420, had most connectivity and highest correlation with the module eigengene. It is closely related to *Arenimonas malthae*, an isolate from oil-contaminated soil from Taiwan, China (29).

**Module A12.** This module had 19 members. The change pattern of the eigengene across all 12 samples was more or less consistent with those of individual OTUs (Fig. S7L-I, II). The eigengene explained 46% of total OTU abundance variance in this module (Fig. S7L-II) and 76% of the OTUs had significant module memberships with their eigengene of this module. This module was dominated by *Actinobacteria* (47%) however the identified module hub belonged to a *Bacteroidetes*, OTU F2923 (Fig. 3), which had no known close relatives (Fig. S7L-V).

**Module A13.** This module had 27 members. The change pattern of the eigengene across all 12 samples was well consistent with those of individual OTUs (Fig. S7M-I, II). The eigengene explained 69% of total variance of the OTU abundance in this module (Fig. S7M-II) and all of the OTUs had significant module memberships with their eigengene of this module. Most of this network's *Verrucomicrobia* OTUs (9 of 12) were concentrated in this module (accounting for 33% of the members). All these nine OTUs had significant module memberships with their eigengene (Fig. S7M-V). Although most of them (78%) were shared in both eCO<sub>2</sub> and aCO<sub>2</sub> networks, they had many more connections among themselves and neighbors under the aCO<sub>2</sub> network than eCO<sub>2</sub> network (Fig. S4). Similar to the OTUs in the module E9 (Fig. 2), these OTUs were closely related to *Chthoniobacter flavus* and *Verrucomicrobium spinosum*, which were enteric bacterium found in aquatic and soil habitats and in the gastrointestinal tract of humans. They can ferment sugars without making gas as a product (7).



**Module A14.** This module had 20 members. The change pattern of the eigengene across all 12 samples was consistent with those of individual OTUs (Fig. S7N-I, II). The eigengene explained 54% of total variance of the OTU abundance in this module (Fig. S7N-II) and 85% of the OTUs had significant module memberships with their eigengene of this module. This module was composed of diverse bacterial groups but the major one was Proteobacteria, including six  $\alpha$ -, two  $\beta$ -, two  $\delta$ - and one  $\gamma$ -Proteobacteria. OTU FR1149 was identified as module hub (Fig. 3) but OTU FR1610 was most correlated with the module eigengene. Both of them belonged to  $\alpha$ -Proteobacteria (Fig. S7N-V).

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