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토양 박테리아 군집 조성에 관한 연구: 배양 환경에서의 영양분 농도와 시간에 따른 접근

Predictable communities of soil bacteria in relation to nutrient concentration and successional stage, in a laboratory culture experiment.

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송 우 진

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by Woojin Song

Supervisor: Dr. Jonathan Miles Adams, Ph.D.

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ABSTRACT

The best hope of understanding the processes that govern soil bacterial communities is to represent them as simpler microcosms, which may give hints to the ecology of natural systems. We set up a microcosm culturing experiment with soil bacteria, at a range of nutrient concentrations, and compared these over time. DNA from each replicate was analyzed using HiSeq2000 Illumina sequencing of the 16S rRNA gene. We found that each nutrient treatment, and each time point during the experiment, produces characteristic bacterial communities that occur predictably between replicates. It is clear that within the context of this experiment, many soil bacteria have distinct niches from one another, in terms of both nutrient concentration, and successional time point since a resource first became available. This fine niche differentiation may in part help to explain the coexistence of a diversity of bacteria in soils. In this experiment, we show that the unimodal relationship between nutrient concentration/time and species diversity often reported in communities of larger organisms, is also evident in microbial communities.

Key words: Bacteria, Succession, Microbial communities, Microbial ecology, Niche differentiation

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1. INTRODUCTION

Soil bacteria make up much of Earth's biodiversity. It has been estimated that there are over 10^9 bacterial 'species' in the world (Whitman et al., 1998) and there are a minimum of 10,000 different bacterial species per gram of soil (Schloss and Handelsman, 2006; Roesch et al., 2007). Bacteria in soil play a central role in the biogeochemical cycles of major elements (e.g. carbon, nitrogen, etc.) and trace elements (e.g. iron, nickel, etc.) (Sylvia et al., 2005). Also, bacteria are considered as key drivers of and responders to climate change (Singh et al., 2010). It is therefore necessary to study soil bacterial community structure and its diversity in order to better understand broader ecological processes.

The effect of nutrients on species diversity has long been of interest to ecologists, particularly in the case of plant and coral communities. Although, there is no consensus on general relationship between productivity and richness, the evidence, mostly derived from terrestrial plant communities, demonstrates a humped-back relationship between productivity and species richness with maximum species richness at

intermediate productivity (Huston, 1979; Tilman, 1981; Grace, 1999; Mittelbach et al., 2001; Keddy, 2005). However, other relationships have also been observed (Waide et al., 1999; Gillman and Wright, 2006), but several studies have shown that this is typical of studies that investigate a restricted biomass range, which suggests that many of these relationships were artefacts arising from the study of diversity over limited productivity ranges (Moore and Keddy, 1988; Guo and Berry, 1998; Keddy, 2005). Recently, a large-scale survey of herbaceous vegetation has described that relationships between productivity and richness are 'weak and variable'(Adler et al., 2011). However, the conclusion of Adler et al. study is still controversial (Fridley et al., 2012; Pierce, 2014), and the mechanisms behind observed richness changes in response to increased productivity remain obscure.

Despite the fact that bacteria make up much of Earth's biodiversity and play a crucial role in ecosystem functioning, we know relatively little about the relationship between bacterial diversity and productivity. Horner-Devine et al. (2003) found that different microbial taxa can differ in how their diversity responds to changes in productivity,

exhibiting both unimodal and *U*-shaped relationships. The results of several simplified microcosm experiments showed a unimodal relationship between bacterial diversity and resource availability (Kassen et al., 2000; Hall and Colegrave, 2007; Benmayor et al., 2008; Langenheder and Prosser, 2008; Bell et al., 2010). However, these studies used low-resolution molecular fingerprinting tools, which lacked the coverage and depth of high-throughput sequencing methods. To investigate soil bacterial community structure and diversity, microbial ecologists have traditionally used culturing techniques. However, the maximum value for the cultivation success of soil bacteria has been in the range of 1-10% of the species (Torsvik et al., 1990) . This limitation hinders microbial ecologists in their quest to understand the mechanisms behind soil bacterial diversity. Using lower nutrient concentration media (Button et al., 1993; Bussmann et al., 2001; Connon and Giovannoni, 2002) and longer-incubation time studies (Connon and Giovannoni, 2002; Janssen et al., 2002; Sait et al., 2002; Joseph et al., 2003) have, however, been found to yield distinct and often more diverse bacterial communities in culture.

Here, we set out to examine how differing nutrient concentrations and incubation time affects the diversity and composition of bacterial communities directly derived from soil, and whether there are parallels with the patterns found for plant and coral communities – which likewise consist of sedentary organisms. We used a high throughput sequencing method (HiSeq2000) which has the potential to cover almost the entire assemblage of bacteria growing on a particular medium. Our main objectives in this study were to answer the following questions:

1) *Is there a predictable and distinct bacterial community for each sampling time point and each nutrient concentration?* It is unclear to what extent bacterial niches in soil are divided, and what environmental axes they may divide along. Such questions may be a key to understanding how the vast bacterial diversity in soil is maintained – whether by niche differentiation, or by neutral processes. Here, we concentrated on testing for evidence of niche differentiation along just two axes, the starting nutrient concentration, and time. We predicted that if niche differentiation is important in soil bacteria, distinct communities would arise predictably for each nutrient concentration and time point of the experiment.

2) *Is there a humpback curve in bacterial diversity along a nutrient gradient, with the greatest diversity found at moderate nutrient concentrations?* Starting from low nutrient conditions, there is an initial increase in diversity that is thought to represent the greater survivability and ease of evolutionary adaptation to that environment (Puerto et al., 1990; Graham and Duda, 2011). However, beyond a certain concentration of nutrients, diversity declines. This is thought to be due to the effect of rapid growth promoting competition between individuals, where stronger competitors may exclude others which have overlapping niches. We predicted that, as is the case with other better-studied sedentary communities, bacterial diversity would show a humpbacked curve against nutrient concentrations.

3*) Is there a peak in bacterial diversity at earlier sampling time point in relatively high nutrient concentrations compared to relatively low nutrient concentrations?* We predicted that, in our culture experiment, there would be a phase of increased diversity as slower growing OTUs become more abundant and mix with the early-successional community of faster growing OTUs able to exploit high nutrient concentrations. As nutrients from the medium are absorbed into cells and sequestered or

metabolized, while biomass builds up, only slower-growing oliotrophic OTUs will become abundant as faster-growing copiotrphic OTUs are eliminated by competition. This should produce a humpbacked diversity curve as the successional process evolves forward in time. In our experimental system, we used six different initial nutrient concentrations. It is predicted that the potential rate of bacterial growth should be different according to the nutrient level, as described in the classic studies by Monod (Monod, 1949). Relatively high nutrient conditions (TSA 1/1 and TSA 1/10) may be predicted to show a peak in OTU diversity earlier in time compared to relatively low nutrient conditions (TSA 1/10000 and pure agar). Therefore, at some high nutrient concentrations one would be expect to detect the complete humpbacked curve during succession, while at other low nutrient concentrations one might detect only the initial phase of monotonically increasing species diversity.

2. MATERIALS AND METHODS

2.1 Sampling, experimental design and DNA extraction

The soil used in this study was collected from an overgrown weedy garden behind the Natural Science building of Seoul National University in June 2013. The upper 5 cm of soil was taken to the laboratory within 30 minutes of being dug from the ground. Using a standard soil pH meter, the fresh soil sample was measured as being slightly acidic, at pH 6.1. For the culturing experiment, 20 g of this soil was diluted in 190 ml of 0.85% NaCl. The sample was then blended for 10 minutes and incubated in a shaking incubator (250 rpm) at 24^{o}C for 30 minutes to release cells from soil particles. We diluted the soil elute sample to 10^{-2}, 10^{-3}, 10^{-4}, and 10^{-5} of original concentration for testing which one is the best dilution in this culturing experiment. Then finally, we inoculated 100 μl of 10^{-2} dilution on each plate. Remaining soil was stored at -80^{o}C for DNA extraction as a standard for initial conditions.

We applied two kinds of treatments to the culturing experiment: variation in initial nutrient level and variation in incubation time. To explore the effect of nutrient gradient on the diversity and composition

of the culturable bacterial community, five different nutrient levels were prepared. These were: undiluted Tryptic Soy Agar (Difco) (TSA 1/1), 10-fold diluted TSA (TSA 1/10), 100-fold diluted TSA (1/100 TSA), 1000-fold diluted TSA (1/1000 TSA), and 10000-fold diluted TSA (1/10000 TSA). The negative control was set as 'no added nutrient' pure agar medium without any TSA input. Incubation time before final sampling also varied between: Day 2, 7, 14, 28, 56 and 84. Each treatment was run as three replicates. Cycloheximide (100μg/ml) was added to inhibit fungal growth, and all culture media were incubated at 25ºC up to 84 days. Sterile cellophane was overlaid on each medium to facilitate the detachment of bacterial colonies from media. Plates were kept together in the incubator in sets of five. DNA from these five plates was pooled in one tube per one replicate (Fig. 1). The reason for this was that, in trial runs of this experiment we found that not enough DNA could be obtained reliably per one plate (in many low nutrient treatments, the bacterial biomass based on colony coverage and DNA yield per plate was very low, at least in the first two of weeks). It was necessary to pool plates to ensure we obtained enough material. So in total we used 15 plates for making a set of three replicates. Colonies were collected by plate washing and subsequent

vortexing to increase the colony recovery. Collected colonies were stored at -80^{o}C for later DNA extraction.

Total bacterial DNA was extracted from each collected culture sample using the Genomic DNA purification kit (Promega, Madison, Wisconsin, USA). Total soil DNA was also extracted from the soil sample using the Power Soil DNA extraction kit (MoBio Laboratories, Carlsbad, CA, USA) following manufacturer's instructions. A separate and specialized soil extraction methodology was used for the soil samples because soil humic acid and also the physical form of the soil particles will tend to impede the effective DNA yield. In total, 111 DNA samples were extracted and stored at -20^{o}C until further processing.

2.2 HiSeq2000 paired-end sequencing and data analysis

The bacteria-specific primers 338F (5'-XXXXXXXX-GTACTCCTACGGGAGGCAGCAG-3') and 533R (5'-TTACCGCGGCTGCTGGCAC-3') were used to amplify the V3 region of bacterial 16S rRNA ('X' denotes barcode sequence). PCR condition is as follows: 94^{o}C for 2 min (pre-denaturation), 25 cycles for 94^{o}C for

30 s (denaturation), 57°C for 30 s (annealing), and 72°C for 30 s (extension), followed by 5 min at 72°C (final extension). PCR products were visualized 1.5% agarose gel to verify product band size. SV Gel and PCR Clean-Up system ((Promega, Madison, Wisconsin, USA) was used to purify DNA amplicons and to remove remained reaction buffers and primers. After purification, DNA concentration was determined using a Quant-iT dsDNA Assay Kit (Invitrogen, Eugene, Oregon, USA). Identical amounts (500ng/μl) of 10-amplicons were pooled in a single tube (total 12 tubes), and the amplicons were sequenced at Celemics (Seoul, Korea) using HiSeq2000 paired-end of 2 x 150bp (Illumina). 2 x 150bp paired-end reads were assembled using PANDAseq (Masella et al., 2012), and assembled reads were processed following the MiSeq SOP in mothur v.1.32.1 (Schloss et al., 2009). All trimmed high-quality remaining sequences were classified based on EzTaxon-e database (Kim et al., 2012). Sequence data was deposited in SRA at NCBI with an accession number of SRP046038.

2.3 Statistical analysis

The number of reads was standardized at 4,959 per sample for cross-comparison between samples. Diversity indices such as Shannon index,

PD, and OTU richness were estimated on the basis of OTUs defined here at 97% sequence similarity of 16S rRNA gene in Mothur. A maximum likelihood (ML) tree was inferred from all aligned sequences using FastTree 2 with a default setting (Price et al., 2010). The resulting tree was used for calculating PD and weighted-Unifrac distance. To assess the best fitting model of correlations between time/nutrient and OTU diversity, linear, polynomial (quadratic) and power (cubic) models were tried out using SigmaPlot v 10.0. To see if there was any clustering pattern in bacterial communities between samples across the nutrient gradient and incubation times, a NMDS plot was generated using weighted-UniFrac distance. A PERMANOVA was performed with 999 permutations using the adonis function in vegan R package to test if culturable bacterial communities differed significantly by nutrient level and incubation time (Anderson, 2001). Indicator species analysis (Dufrêne and Legendre, 1997) was performed using the indval function of the labdsv R package (Roberts, 2007). We also analyzed our data after removal of singletons, but the result was same (data not shown).

3. RESULTS

A total of 2,731,383 sequences were obtained from 111 samples (range of 4,959 to 84,357 sequences per sample with a mean of 24,232) with 24,751 OTUs at 97% similarity. The most abundant culturable phyla across all samples were *Proteobacteria* (72.7%), followed by *Firmicutes* (11.1%), *Bacteroidetes* (10.6%) and to a lesser degree, *Acidobacteria* (1.9%), *Verrucomicrobia* (1.6%) and *Actinobacteria* (1.0%), while 0.3% of the sequences were unclassified (Fig. 2).

A Non-metric multidimensional scaling (NMDS) using weighted Unifrac showed that both time and nutrient concentration influenced bacterial community composition (Fig. 3 and Fig. 4; Table 1). A permutational multivariate analysis of variance (PERMANOVA) results showed that bacterial community composition was not only affected by time ($F=46.83$, $P<0.0001$; Table 2) and nutrient concentration ($F=41.89$, $P<0.0001$; Table 1), but also by the interaction between time and nutrient concentration ($F=4.507$, $P<0.0001$; Table 1). During the initial time point (Day 2) *Firmicutes* and *Proteobacteria* were consistently abundant across most nutrient concentrations (65%

and 35% of all bacterial sequences, respectively), and majority of the *Proteobacteria* were dominated by class *Gammaproteobacteria* (31.6% of all bacterial sequences). Amongst these, phylum *Firmicutes* and class *Gammaproteobacteria*, are generally known as copiotrophs (broadly definable as *r*-strategists) (Eilers et al., 2010; Jenkins et al., 2010). At the mid time point (Day 56) *Proteobacteria*, *Bacteroidetes*, and *Firmicutes* were the most abundant phyla (53.3%, 25% and 16.7% of all bacterial sequences, respectively). The labelling of bacterial groups (at any taxonomic level) as either oligotrophic or copiotrophic can be fraught with difficulty. Partly, this is due to the fact that members of the same phylum, class or even genus may have quite different nutrient requirements and growth characteristics. For instance, *Sphingomonas oligophenolica* of phylum *Proteobacteria* was the most dominant taxa across all nutrient concentrations except for TSA1/1, while in TSA 1/1 the family *Alcaligenaceae* of *Proteobacteria* was dominant. *Sphingomonas oligophenolica* is a known oligotroph (Ohta et al., 2004), whereas, *Alcaligenaceae* is known to have copiotrophic taxa (Goldfarb et al., 2011). At the late time point (Day 84) *Sphingomonas oligophenolica* of phylum *Proteobacteria* was also most dominant across all nutrient concentration with the exception of

TSA1/1 and agar. Interestingly, genus *Acinetobacter* and family *Xanthomonadaceae* of *Proteobacteria* were most dominant in TSA1/1 and agar respectively. , and these taxa are well known copiotrophs (Juteau et al., 1999; Bhadra et al., 2007).

Overall, except for the highest nutrient concentration (TSA 1/1), bacterial community composition at relatively low nutrient concentrations shifted over time from copiotrophic taxa to oligotrophic taxa (Table 3.). Furthermore, heatmap of 100 most abundant OTUs also shows bacterial community composition changing in relation to initial nutrient concentration and time (Fig. 5).

OTU richness was highest at intermediate nutrient concentration at initial time point (Fig. 6). However, after day 7 agar shows high OTU richness and Shannon diversity index similar to intermediate nutrient concentrations (Fig. 6A and Fig. 6B). Regression analysis with Faith's phylogenetic diversity (PD) also showed a humpback pattern in relation to initial nutrient concentration, for all time point except for day 7 (Fig. 6C). Furthermore, the most dominant phylum *Proteobacteria* also

showed similar diversity patterns with the whole bacterial community across all time and nutrient concentrations (data not shown).

We found that diversity (OTU richness, Shannon index and PD) peaked at day 56 across all nutrient concentrations (Fig. 7). Further analysis of indicator OTUs identified 65 OTUs specific to day 56 across all nutrient concentrations, most of which were; *Alphaproteobacteria* (33%) and *Bacteroidetes* (24%) (Table 3). However, by day 84 most indicator OTUs had changed to phylum *Verrucomicrobia* (45%) (Table 3). The decrease in *Alphaproteobacteria* and *Bacteroidetes* OTUs after DAY 56 was a large part of the reason why species diversity declined. Although, the indicator OTUs found are assemblages uniquely adapted to a certain set of conditions, some OTUs will be distinct between treatments purely by chance variation in the recruitment of OTUs in the samples.

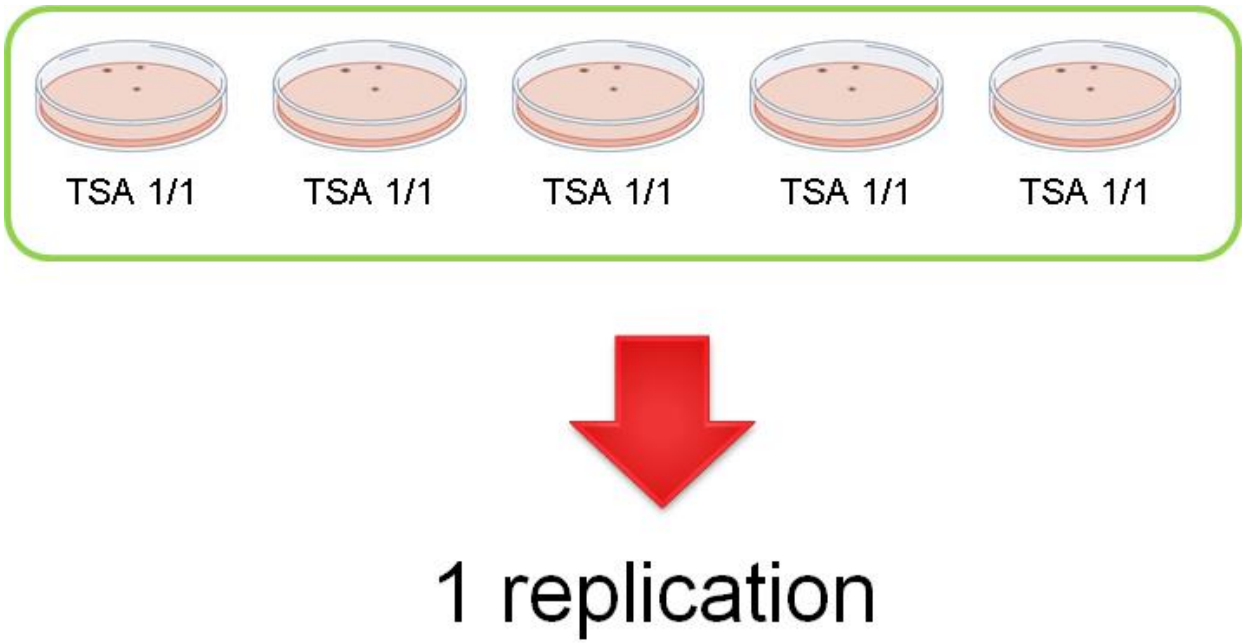

Fig. 1. Figure shows that each replicate is a pooled mass of five plates.

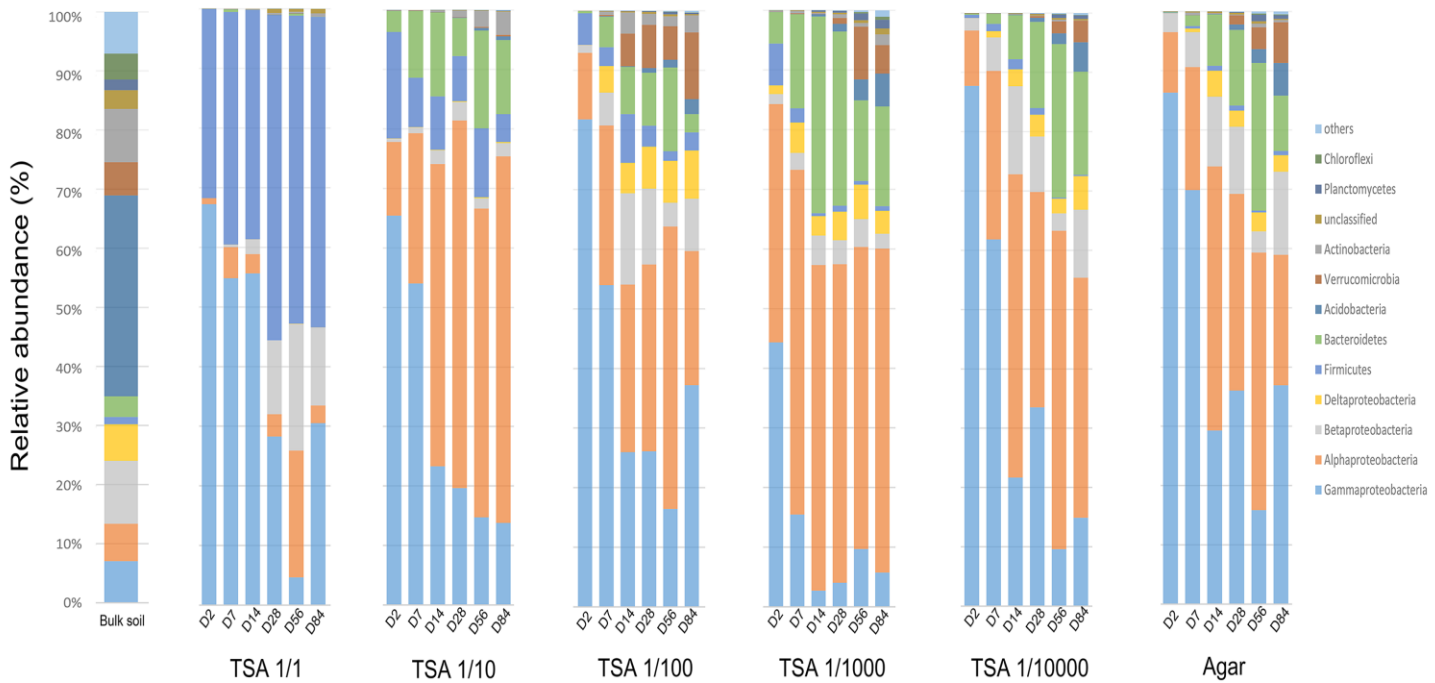

Fig. 2. Relative average abundances of dominant bacterial taxa across time point. (D2=2 days of incubation, D7=7 days of incubation, D14=14 days of incubation, D28=28 days of incubation, D56=56 days of incubation, D84=84 days of incubation, TSA 1/1=undiluted TSA, TSA 1/10=10-fold diluted TSA, TSA 1/100=100-fold diluted TSA, TSA 1/1000=1000-fold diluted TSA, TSA 1/10000=10000-fold diluted TSA, Agar=no nutrient)

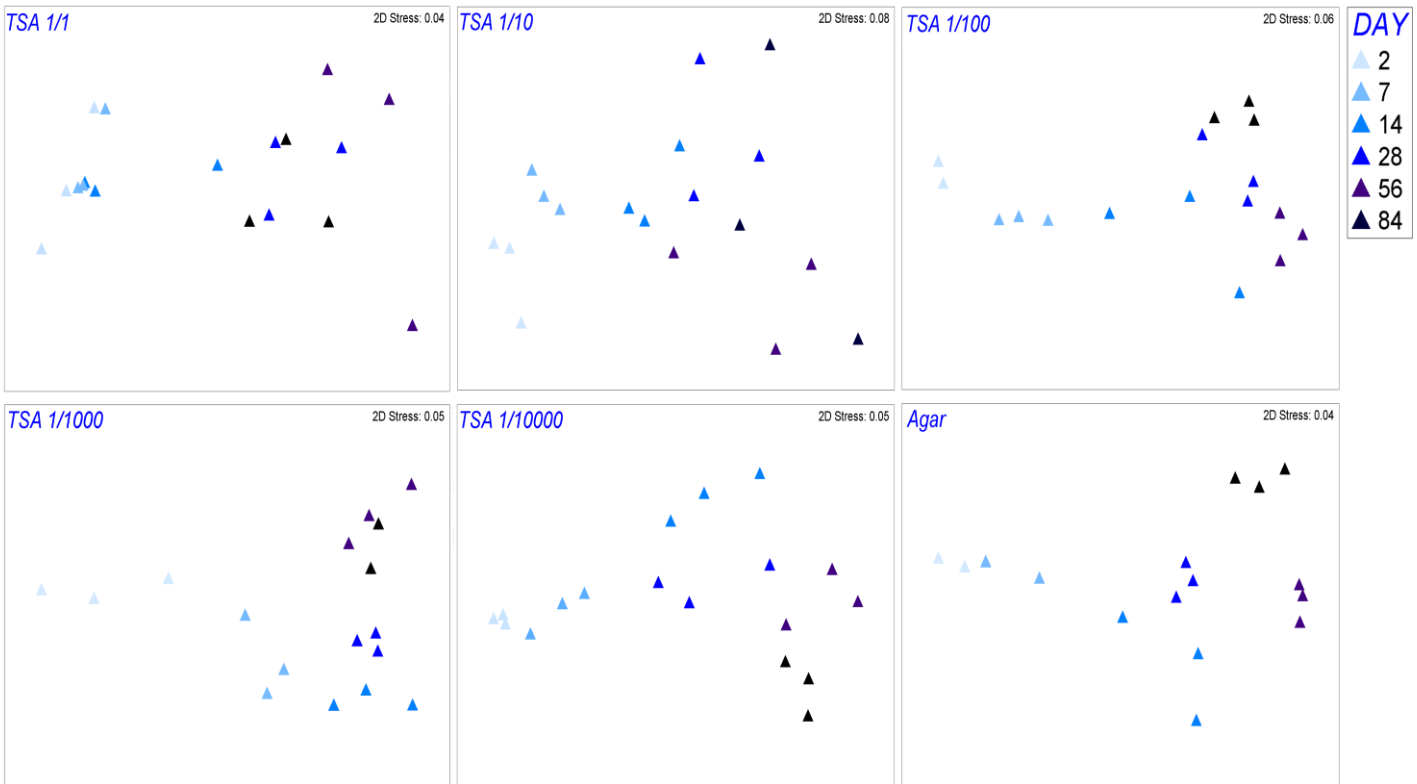

Fig. 3. Nonmetric multidimensional scaling plot of the bacterial community at each nutrient concentration using the Weighted-Unifrac distance with *symbols* coded by different sampling time.

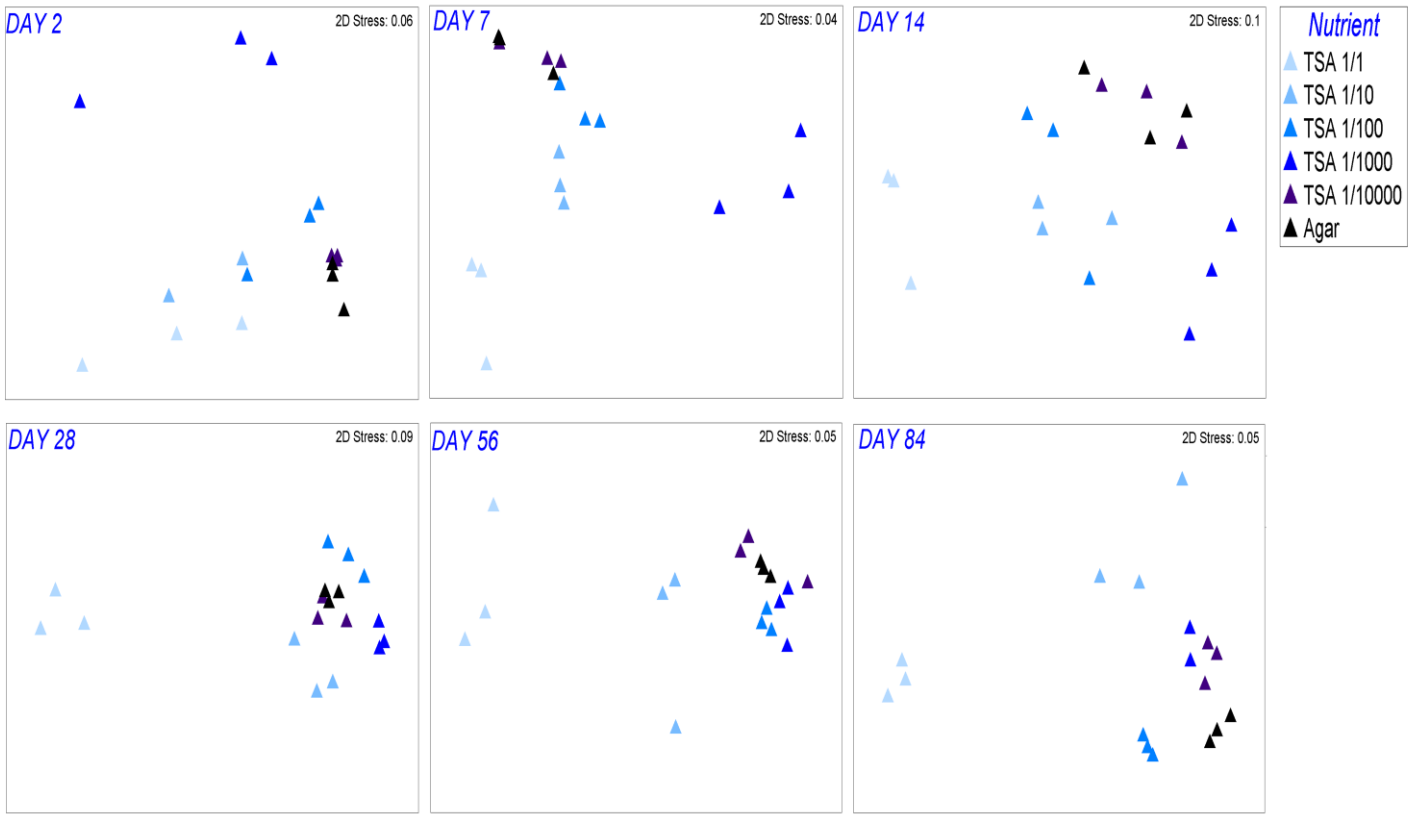

Fig. 4. Nonmetric multidimensional scaling plot of the bacterial community at each sampling day using the Weighted-Unifrac distance with *symbols* coded by different nutrient concentrations.

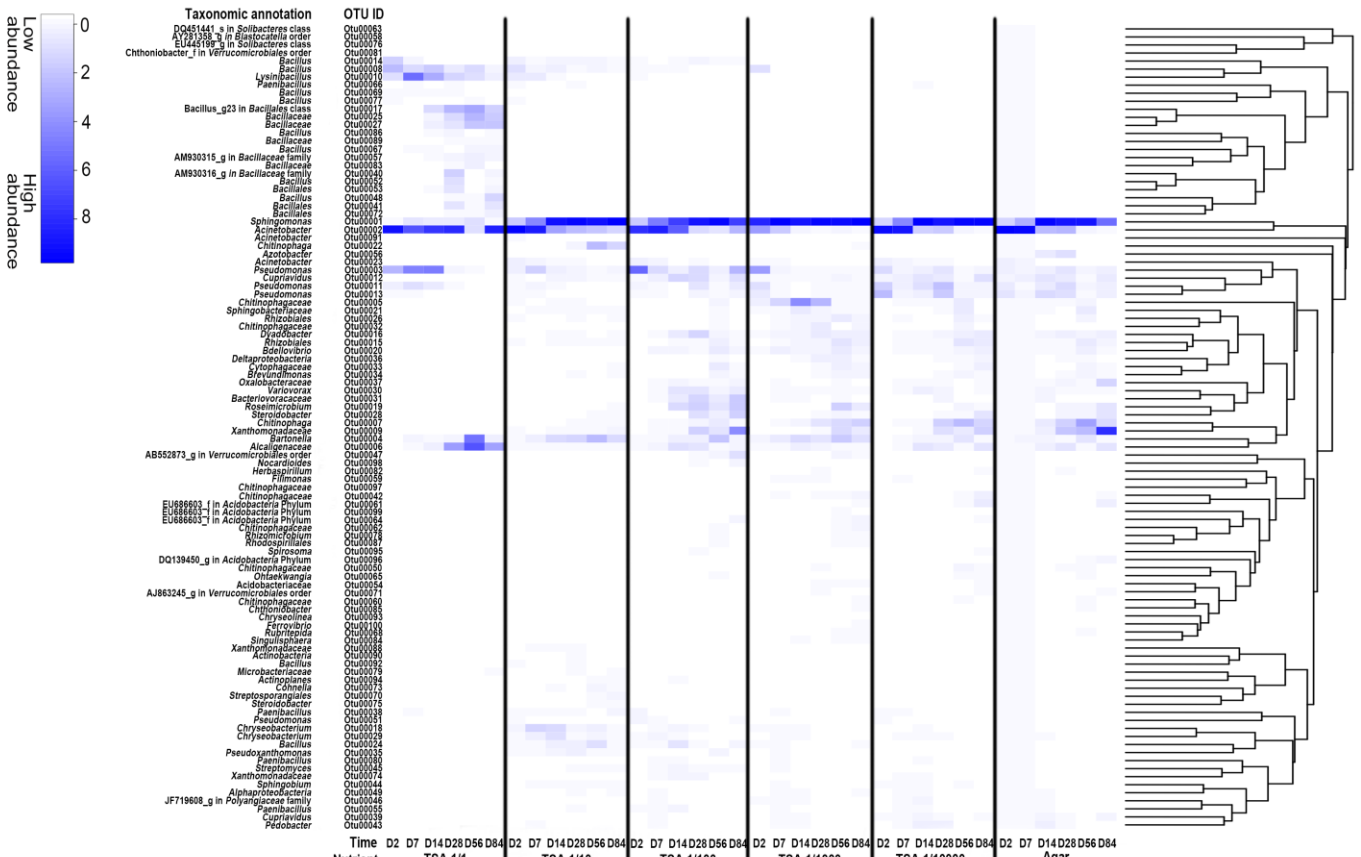
Low abundance
High abundance
0
2
4
6
8
Taxonomic annotation
OTU ID
Time
Nutrient
D2 D7 D14 D28 D56 D84
TSA 1/1
TSA 1/10
TSA 1/100
TSA 1/1000
TSA 1/10000
Agar

Fig. 5. Heat map showing the relative abundance of each bacterial operational taxonomic unit (OTU) (rows) at each sampling time (D2=2 days of incubation, D7=7 days of incubation, D14=14 days of incubation, D28=28 days of incubation, D56=56 days of incubation, D84=84 days of incubation, TSA 1/1=undiluted TSA, TSA 1/10=10-fold diluted TSA, TSA 1/100=100-fold diluted TSA, TSA 1/1000=1000-fold diluted TSA, TSA 1/10000=10000-fold diluted TSA, Agar=no nutrient).

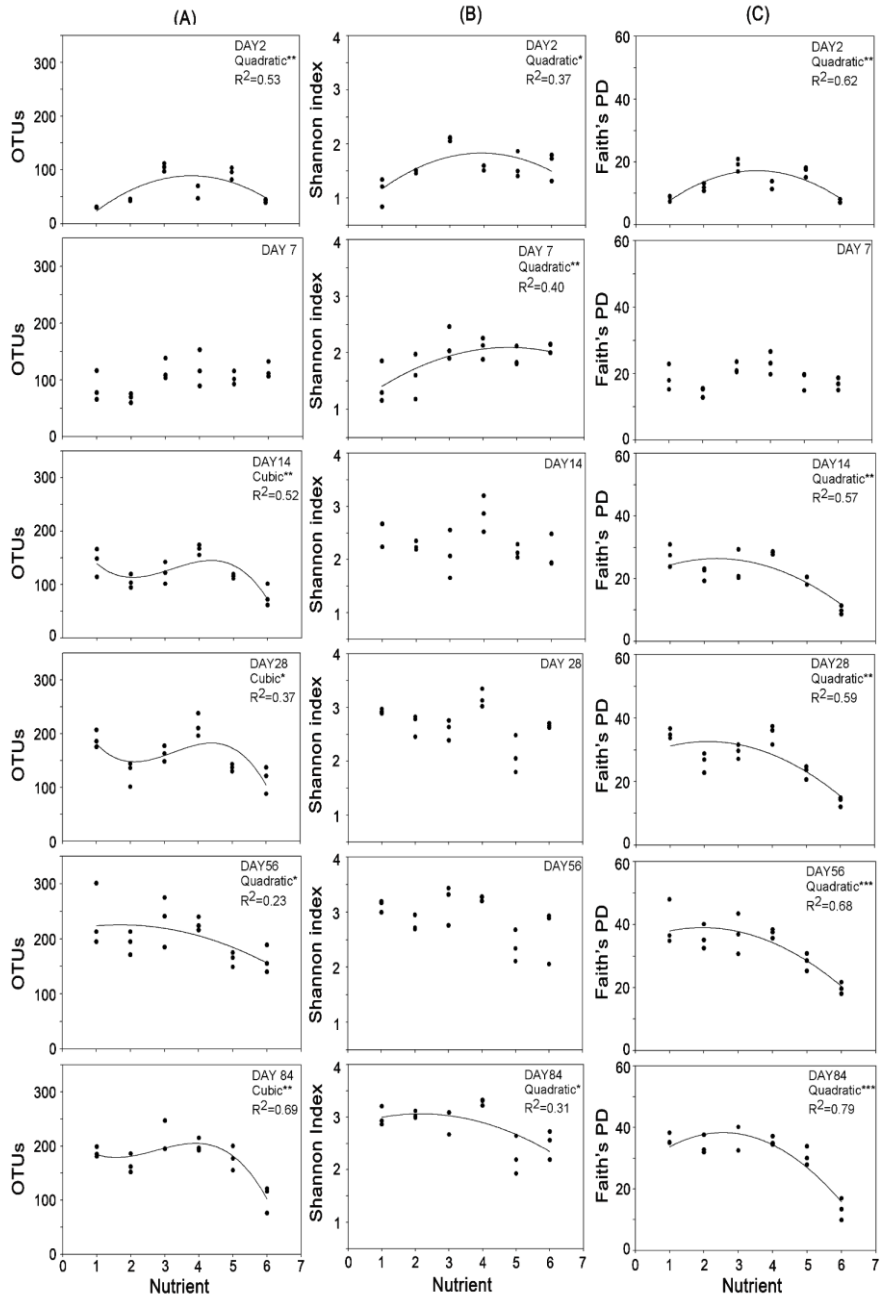
(A)
(B)
(C)
OTUs
Shannon index
Faith's PD
Nutrient
DAY2
Quadratic**
R2=0.53
DAY2
Quadratic*
R2=0.37
DAY2
Quadratic**
R2=0.62
DAY 7
DAY 7
Quadratic**
R2=0.40
DAY 7
DAY14
Cubic**
R2=0.52
DAY14
DAY14
Quadratic**
R2=0.57
DAY28
Cubic*
R2=0.37
DAY 28
DAY28
Quadratic**
R2=0.59
DAY56
Quadratic*
R2=0.23
DAY56
DAY56
Quadratic***
R2=0.68
DAY 84
Cubic**
R2=0.69
DAY84
Quadratic*
R2=0.31
DAY84
Quadratic***
R2=0.79

Fig. 6. Relationship between nutrient concentration and OTUs (*left*), Shannon index (*middle*), and PD (*right*) at each sampling time. We tested three models (linear, quadratic, and cubic) to describe the relationships and model selection was carried out based on adjusted R^2 with associated *P* value. Significance level was shown with ***$P \leq 0.001$, **$P \leq 0.01$, and * $P \leq 0.05$.

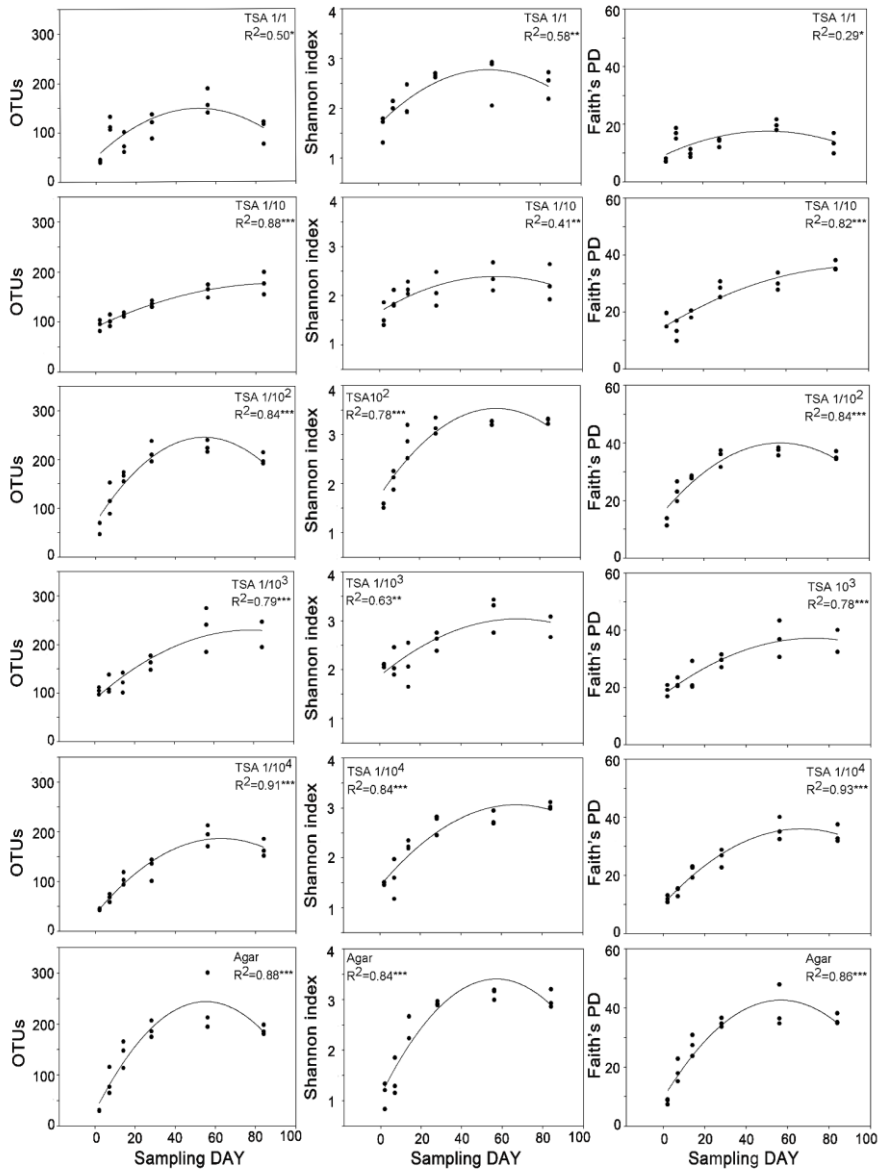

Fig. 7. Relationship between time and OTUs (*left*), Shannon index (*middle*), and Faith's PD (*right*) at each nutrient concentration. We tested three models (linear, quadratic, and cubic) to describe the relationships and all samples are fitted to a quadratic model. Model selection was carried out based on adjusted R^2 with associated *P* value. Significance level was shown with ***$P \leq 0.001$, **$P \leq 0.01$, and * $P \leq 0.05$.

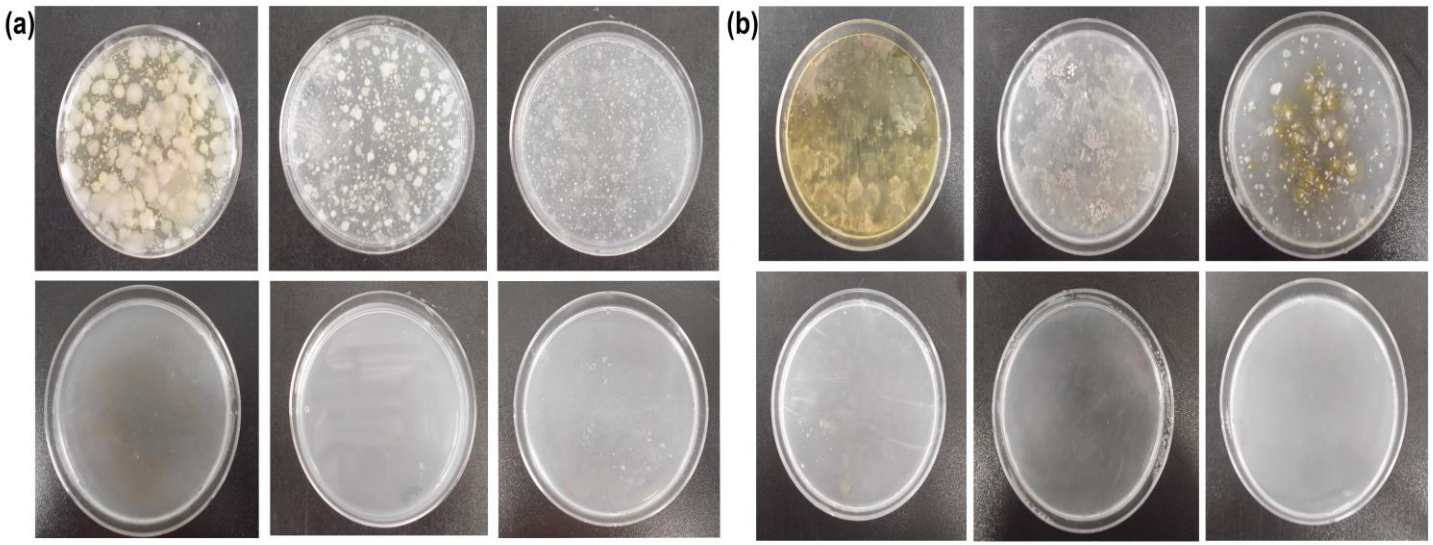

Fig. 8. Plate photographs of various nutrient concentrations at (a) day 2 and (b) day 84. Each picture: top left; TSA 1/1, top middle; TSA1/10, top right; TSA 1/100, bottom left; TSA 1/1000, bottom middle; TSA 1/10000, bottom right; Agar

Table 1. The results of ANOSIM *post-hoc* test on bacterial community composition.

ANOSIM Pairwise Test		
	R^2	**Significance Level %**
Groups		
TSA 1/10000, TSA 1/1000	0.155	1.1
TSA 1/10000, TSA 1/100	0.168	1.2
TSA 1/10000, TSA 1/10	0.277	0.1
TSA 1/10000, TSA 1/1	0.628	0.1
TSA 1/10000, Agar	-0.027	69.1
TSA 1/1000, TSA 1/100	0.325	0.1
TSA 1/1000, TSA 1/10	0.394	0.1
TSA 1/1000, TSA 1/1	0.808	0.1
TSA 1/1000, Agar	0.202	0.5
TSA 1/100, TSA 1/10	0.393	0.1
TSA 1/100, TSA 1/1	0.744	0.1
TSA 1/100, Agar	0.14	2.5
TSA 1/10, TSA 1/1	0.595	0.1
TSA 1/10, Agar	0.318	0.1
TSA 1/1, Agar	0.634	0.1

Groups		
DAY14, DAY28	0.021	26
DAY14, DAY2	0.582	0.1
DAY14, DAY56	0.277	0.1
DAY14, DAY7	0.278	0.1
DAY14, DAY84	0.266	0.2
DAY28, DAY2	0.726	0.1
DAY28, DAY56	0.124	0.7
DAY28, DAY7	0.431	0.1
DAY28, DAY84	0.064	7.5
DAY2, DAY56	0.902	0.1
DAY2, DAY7	0.132	0.5
DAY2, DAY84	0.807	0.1
DAY56, DAY7	0.706	0.1
DAY56, DAY84	0.069	4.8
DAY7, DAY84	0.607	0.1

Table 2. Results of multivariate PERMANOVA verify significant differences in bacterial community composition between treatment groups.

	Bacterial community composition				
	d.f	MS	F. model	R^2	*P*
DAY(Time)	5	1.23	46.83	0.37	<0.001
Nutrient concentration	5	1.10	41.89	0.33	<0.001
DAY (Time)* Nutrient concentration	25	0.11	4.507	0.18	<0.001
Residuals	70	0.026		0.11	
Total	105			1.00	

d.f., degree of freedom; MS, mean square; *P*<0.001, probability level

Table 3. Bacterial composition changes at the initial sampling time point (Day 2), mid sampling time point (Day 56), and late sampling time point (Day 84), Comparing the ten most abundant OTUs. (*; appearing at both initial and mid sampling time point, #; appearing at both mid and late sampling time point).

Initial time point (DAY 2)			**Mid time point (DAY 56)**			**Late time point (DAY 84)**		
Day2 TSA1/1	**Relative abundance (%)**	**Taxonomy description**	**Day56 TSA1/1**	**Relative abundance (%)**	**Taxonomy description**	**Day84 TSA1/1**	**Relative abundance (%)**	**Taxonomy description**
Otu00003	48.54	*Pseudomonas*	Otu00006 #	20.94	*Alcaligenaceae*	Otu00002 #	30.05	*Acinetobacter*
Otu00008 *	14.78	*Bacillus anthracis*	Otu00004 #	16.84	*Bartonella*	Otu00006 #	12.88	*Alcaligenaceae*
Otu00077	13.35	*Bacillus*	Otu00017 #	10.53	*Bacillus_g23*	Otu00027 #	7.91	*Bacillaceae*
Otu00281	9.45	*Bacillaceae*	Otu00025 #	9.07	*Bacillaceae*	Otu00025 #	6.72	*Bacillaceae*
Otu00013	5.56	*Pseudomonas*	Otu00027 #	6.73	*Bacillaceae*	Otu00017 #	6.53	*Bacillus_g23*
Otu00235	3.02	*Bacillus*	Otu00008 *	4.34	*Bacillus*	Otu00048	5.53	*Bacillus*
Otu00002	1.00	*Acinetobacter*	Otu00001	4.10	*Sphingomonas oligophenlica*	Otu00041	3.18	*Bacillales*

Otu02400	0.96	*Bacilli*	Otu00010 #	3.65	*Lysinibacillus sphaericus*	Otu00072	2.16	*Bacillales*
Otu00360	0.89	*Bacillaceae*	Otu00002 #	3.62	*Acinetobacter*	Otu00010 #	2.13	*Lysinibacillus sphaericus*
Otu00208	0.46	*Bacillales*	Otu00057	2.05	*AM930315_g*	Otu00004 #	1.97	*Bartonella*
Day2 TSA1/10			**Day56 TSA1/10**			**Day84 TSA 1/10**		
Otu00003	57.08	*Pseudomonas*	Otu00001 #	38.11	*Sphingomonas oligophenlica*	Otu00001 #	50.54	*Sphingomonas oligophenlica*
Otu00002 *	11.09	*Acinetobacter*	Otu00022 #	11.10	*Chitinophaga*	Otu00022 #	7.57	*Chitinophaga*
Otu00077	6.91	*Bacillus*	Otu00004 #	10.37	*Bartonella*	Otu00002 #	7.57	*Acinetobacter*
Otu00013	4.05	*Pseudomonas*	Otu00002 * #	8.93	*Acinetobacter*	Otu00004 #	7.18	*Bartonella*
Otu00281	3.56	*Bacillaceae*	Otu00024	4.63	*Bacillus*	Otu00009	1.83	*Xanthomonadaceae*
Otu00260	3.05	*Enterobacteriaceae*	Otu00018	2.01	*Chryseobacterium*	Otu00070	1.56	*Streptosporangiales*
Otu00008 *	2.99	*Bacillus anthracis*	Otu00014 *	1.76	*Bacillus*	Otu00003	1.31	*Pseudomonas*

Otu00236	2.35	*Serratia*	Otu00008 *	1.72	*Bacillus anthracis*	Otu00038	1.26	*Paenibacillus*
Otu00014 *	0.89	*Bacillus*	Otu00073	1.59	*Cohnella*	Otu00045	1.09	*Streptomyces aurantiogriseus*
Otu08409	0.81	*Bacillales*	Otu00029	1.18	*Chryseobacterium*	Otu00012	1.05	*Cupriavidus*
Day2 TSA1/100			**Day56 TSA1/100**			**Day84 TSA1/100**		
Otu00003 *	32.70	*Pseudomonas*	Otu00001 #	32.62	*Sphingomonas oligophenlica*	Otu00001 #	18.92	*Sphingomonas oligophenlica*
Otu00008	26.72	*Bacillus anthracis*	Otu00004	7.51	*Bartonella*	Otu00009 #	11.30	*Xanthomonadaceae*
Otu00002 *	10.03	*Acinetobacter*	Otu00002 * #	3.80	*Acinetobacter*	Otu00002 #	7.28	*Acinetobacter*
Otu02150	2.20	*Bacillales*	Otu00009 #	3.53	*Xanthomonadaceae*	Otu00003 #	7.25	*Pseudomonas*
Otu00235	2.01	*Bacillus*	Otu00019 #	3.45	*Roseimicrobium gellanilyticum*	Otu00019 #	5.20	*Roseimicrobium gellanilyticum*
Otu00260	1.96	*Enterobacteriaceae*	Otu00031 #	2.74	*Bacteriovoracaceae*	Otu00031 #	5.11	*Bacteriovoracaceae*
Otu00621	0.98	*Bacillaceae*	Otu00007	2.56	*Chitinophaga*	Otu00028	4.67	*Steroidobacter*

Otu00203	0.98	*Bacillus*	Otu00020	2.51	*Bdellovibrio*	Otu00047	3.29	AB552873_g
Otu02353	0.91	*Ralstonia_f*	Otu00033	2.48	*Cytophagaceae*	Otu00012	3.05	*Cupriavidus*
Otu00602	0.68	*Planococcaceae*	Otu00003 * #	2.42	*Pseudomonas*	Otu00011	2.31	*Pseudomonas parafulva*
Day2 TSA1/1000			**Day56 TSA1/1000**			**Day84 TSA1/1000**		
Otu00002	37.05	*Acinetobacter*	Otu00001 #	30.18	*Sphingomonas oligophenlica*	Otu00001 #	38.72	*Sphingomonas oligophenlica*
Otu00008	16.76	*Bacillus anthracis*	Otu00019 #	7.32	*Roseimicrobium gellanilyticum*	Otu00007 #	6.57	*Chitinophaga*
Otu00003	16.76	*Planococcaceae*	Otu00004 #	6.96	*Bartonella*	Otu00004 #	4.75	*Bartonella*
Otu00235	5.26	*Bacillus*	Otu00009 #	4.92	*Xanthomonadaceae*	Otu00009 #	2.72	*Xanthomonadaceae*
Otu00077	5.15	*Bacillus*	Otu00007 #	4.34	*Chitinophaga*	Otu00019 #	2.35	*Roseimicrobium gellanilyticum*
Otu00208	3.42	*Bacillales*	Otu00015 #	3.48	*Rhizobiales*	Otu00015 #	2.19	*Rhizobiales*
Otu00010	2.69	*Lysinibacillus sphaericus*	Otu00020 #	2.99	*Bdellovibrio*	Otu00020 #	2.12	*Bdellovibrio*

Otu00014	1.74	*Bacillus*	Otu00026 #	2.71	*Rhizobiales*	Otu00026 #	1.89	*Rhizobiales*
Otu00260	1.04	*Enterobacteriaceae*	Otu00036	2.44	*Deltaproteobacteria*	Otu00016 #	1.74	*Dyadobacter*
Otu00203	0.82	*Bacillus simplex*	Otu00016 #	2.21	*Dyadobacter*	Otu00034	1.54	*Brevundimonas nasdae*
Day2 TSA1/10000			**Day56 TSA1/10000**			**Day84 TSA1/10000**		
Otu00003	52.50	*Pseudomonas*	Otu00001 #	39.20	*Sphingomonas oligophenlica*	Otu00001 #	33.22	*Sphingomonas oligophenlica*
Otu00208	13.91	*Bacillales*	Otu00007 #	10.95	*Chitinophaga*	Otu00009 #	6.06	*Xanthomonadaceae*
Otu00235	10.06	*Bacillus*	Otu00015 #	3.94	*Rhizobiales*	Otu00007 #	5.90	*Chitinophaga*
Otu00002	9.03	*Acinetobacter*	Otu00004	3.78	*Bartonella*	Otu00012	4.37	*Cupriavidus*
Otu00008	7.11	*Bacillus anthracis*	Otu00005	3.64	*Chitinophagaceae*	Otu00006	3.73	*Alcaligenaceae*
Otu00260	3.09	*Enterobacteriaceae*	Otu00021	3.18	*Sphingobacteriaceae*	Otu00028	3.27	*Steroidobacter*
Otu00203	1.55	*Bacillus simplex*	Otu00009 #	3.17	*Xanthomonadaceae*	Otu00015 #	3.03	*Rhizobiales*

Otu02150	0.78	*Bacillales*	Otu00026	2.01	*Rhizobiales*	Otu00061	2.77	EU686603_f
Otu00565	0.28	*Paenibacillus*	Otu00016 #	1.86	*Dyadobacter*	Otu00016 #	2.75	*Dyadobacter*
Otu00621	0.24	*Bacillaceae*	Otu00032	1.70	*Chitinophagaceae*	Otu00033	2.40	*Cytophagaceae*
Day2 Agar			**Day56 Agar**			**Day84 Agar**		
Otu00003	67.26	*Pseudomonas*	Otu00001 #	31.17	*Sphingomonas oligophenlica*	Otu00009 #	25.60	*Xanthomonadaceae*
Otu00002	9.92	*Acinetobacter*	Otu00007 #	12.57	*Chitinophaga*	Otu00001 #	17.73	*Sphingomonas oligophenlica*
Otu00208	6.10	*Bacillales*	Otu00009 #	9.44	*Xanthomonadaceae*	Otu00037	5.40	*Oxalobacteraceae*
Otu00235	5.24	*Bacillus*	Otu00004	3.46	*Bartonella*	Otu00007 #	4.30	*Chitinophaga*
Otu00260	3.64	*Enterobacteriaceae*	Otu00015 *	2.91	*Rhizobiales*	Otu00019 #	3.59	*Roseimicrobium gellanilyticum*
Otu00008	3.57	*Bacillus anthracis*	Otu00021	2.81	*Sphingobacteriaceae*	Otu00006	3.12	*Alcaligenaceae*
Otu00203	2.49	*Bacillus simplex*	Otu00020	1.86	*Bdellovibrio*	Otu00028	2.98	*Steroidobacter*

Otu00565	0.49	*Paenibacillus*	Otu00019 #	1.74	*Roseimicrobium gellanilyticum*	Otu00012	2.34	*Cupriavidus*
Otu02150	0.21	*Bacillales*	Otu00026	1.70	*Rhizobiales*	Otu00003	2.30	*Pseudomonas*
Otu00015 *	0.12	*Rhizobiales*	Otu00033	1.47	*Cytophagaceae*	Otu00013	2.13	*Pseudomonas*

Table 4. The results of indicator OTUs at each sampling time point.

Time	OUT ID	Number of Sequencing	Taxomomy	Indicator Value
DAY2	Otu00404	126	Bacteria(100);Proteobacteria(100);Gammaproteobacteria(100); Pseudomonadales(100);Pseudomonadaceae(93);Pseudomonas(85);unclassified(62)	34.7
	Otu00051	4163	Bacteria(100);Proteobacteria(100);Gammaproteobacteria(100); Pseudomonadales(100);Pseudomonadaceae(100);Pseudomonas(97);unclassified(94)	43.7
	Otu00011	45598	Bacteria(100);Proteobacteria(100);Gammaproteobacteria(100); Pseudomonadales(100);Pseudomonadaceae(100);Pseudomonas(90);Pseudomonas_parafulva(87)	40
	Otu00003	132181	Bacteria(100);Proteobacteria(100);Gammaproteobacteria(100); Pseudomonadales(100);Pseudomonadaceae(100);Pseudomonas(100);unclassified(89)	43.9
	Otu00389	134	Bacteria(100);Proteobacteria(100);Gammaproteobacteria(100); Pseudomonadales(100);Moraxellaceae(98);Acinetobacter(96);unclassified(96)	51.2
DAY7	Otu01043	29	Bacteria(100);Firmicutes(100);Bacilli(100);Bacillales(100); Alicyclobacillaceae(100);Tumebacillus(100);unclassified(69)	36.8
	Otu00164	580	Bacteria(100);Firmicutes(100);Bacilli(100);Bacillales(100); Paenibacillaceae(100);Paenibacillus(100);unclassified(67)	38.9
	Otu00264	262	Bacteria(100);Bacteroidetes(100);Flavobacteria(100);Flavobacteriales(100); Flavobacteriaceae(100);Flavobacterium(100);unclassified(100)	33.5
DAY14	Otu00043	5044	Bacteria(100);Bacteroidetes(100);Sphingobacteria(100);Sphingobacteriales(100); Sphingobacteriaceae(100);Pedobacter(100);Pedobacter_ginsenosidimutans(100)	37.6

DAY28	Otu00139	757	Bacteria(100);Firmicutes(100);Bacilli(100);Bacillales(100); Paenibacillaceae(100);Paenibacillus(100);unclassified(99)	43.8
	Otu00183	482	Bacteria(100);Actinobacteria(100);Actinobacteria_c(100); unclassified(100);unclassified(100);unclassified(100);unclassified(100)	37.5
	Otu00019	22207	Bacteria(100);Verrucomicrobia(100);Verrucomicrobiae(100);Verrucomicrobiales(100); Verrucomicrobiaceae(100);Roseimicrobium(100);Roseimicrobium_gellanilyticum(100)	43.8
	Otu00369	148	Bacteria(100);Verrucomicrobia(100);Verrucomicrobiae(100);Verrucomicrobiales(100); Verrucomicrobiaceae(100);Roseimicrobium(100);Roseimicrobium_gellanilyticum(100)	33.3
	Otu00085	2001	Bacteria(100);Verrucomicrobia(100);Verrucomicrobiae(100); Verrucomicrobiales(100);Chthoniobacter_f(100);Chthoniobacter(60);unclassified(60)	46.6
DAY56	Otu00207	400	Bacteria(100);unclassified(100);unclassified(100);unclassified(100); unclassified(100);unclassified(100);unclassified(100)	39.4
	Otu00355	159	Bacteria(100);unclassified(100);unclassified(100);unclassified(100); unclassified(100);unclassified(100);unclassified(100)	38.2
	Otu00366	150	Bacteria(100);unclassified(100);unclassified(100);unclassified(100); unclassified(100);unclassified(100);unclassified(100)	31.2
	Otu00169	546	Bacteria(100);Proteobacteria(100);unclassified(100);unclassified(100); unclassified(100);unclassified(100);unclassified(100)	31.1
	Otu00220	365	Bacteria(100);Proteobacteria(100);unclassified(100);unclassified(100); unclassified(100);unclassified(100);unclassified(100)	34.3
	Otu00673	58	Bacteria(100);Proteobacteria(100);unclassified(100);unclassified(100); unclassified(100);unclassified(100);unclassified(100)	31.6

Otu00009	53050	Bacteria(100);Proteobacteria(100);Gammaproteobacteria(100); Xanthomonadales(100);Xanthomonadaceae(100);unclassified(100);unclassified(100)	38
Otu00116	980	Bacteria(100);Proteobacteria(100);Gammaproteobacteria(100) ;unclassified(100);unclassified(100);unclassified(100);unclassified(100)	31.6
Otu00075	2345	Bacteria(100);Proteobacteria(100);Gammaproteobacteria(100); Steroidobacter_o(100);Steroidobacter_f(100);Steroidobacter(100);FJ654261_s(94)	46.9
Otu00028	14480	Bacteria(100);Proteobacteria(100);Gammaproteobacteria(100);Steroidobacter_o(100); Steroidobacter_f(100);Steroidobacter(100);FJ654261_s(100)	34.9
Otu00422	117	Bacteria(100);Proteobacteria(100);Deltaproteobacteria(100);Myxococcales(100); Sandaracinaceae(100);Sandaracinus(100);Sandaracinus_amylolyticus(98)	37.2
Otu00020	22047	Bacteria(100);Proteobacteria(100);Deltaproteobacteria(100);Bdellovibrionales(100); Bdellovibrionaceae(100);Bdellovibrio(100);unclassified(99)	33.1
Otu00173	523	Bacteria(100);Proteobacteria(100);Deltaproteobacteria(100);Bdellovibrionales(100); Bacteriovoracaceae(100);Peredibacter(98);unclassified(98)	34.5
Otu00700	54	Bacteria(100);Proteobacteria(100);Betaproteobacteria(100);Burkholderiales(100); Alcaligenaceae(100);unclassified;unclassified	30.9
Otu00123	937	Bacteria(100);Proteobacteria(100);Alphaproteobacteria(100);unclassified(100); unclassified(100);unclassified(100);unclassified(100)	34.7
Otu00191	463	Bacteria(100);Proteobacteria(100);Alphaproteobacteria(100);unclassified(100); unclassified(100);unclassified(100);unclassified(100)	35.8
Otu00258	275	Bacteria(100);Proteobacteria(100);Alphaproteobacteria(100);unclassified(100); unclassified(100);unclassified(100);unclassified(100)	33.5

Otu00403	126	Bacteria(100);Proteobacteria(100);Alphaproteobacteria(100);unclassified(100); unclassified(100);unclassified(100);unclassified(100)	41.3
Otu00739	49	Bacteria(100);Proteobacteria(100);Alphaproteobacteria(100);unclassified(100); unclassified(100);unclassified(100);unclassified(100)	37.3
Otu00896	37	Bacteria(100);Proteobacteria(100);Alphaproteobacteria(100);unclassified(100); unclassified(100);unclassified(100);unclassified(100)	37.4
Otu00994	32	Bacteria(100);Proteobacteria(100);Alphaproteobacteria(100);unclassified(100); unclassified(100);unclassified(100);unclassified(100)	31
Otu00649	61	Bacteria(100);Proteobacteria(100);Alphaproteobacteria(100);Rhodospirillales(100); unclassified(100);unclassified(100);unclassified(100)	31.6
Otu00857	40	Bacteria(100);Proteobacteria(100);Alphaproteobacteria(100);Rhodospirillales(100); Rhodospirillaceae(100);EU589272_g(100);unclassified	38
Otu00100	1358	Bacteria(100);Proteobacteria(100);Alphaproteobacteria(100);Rhodospirillales(100); Ferrovibrio_f(100);Ferrovibrio(100);Ferrovibrio_denitrificans(100)	46.1
Otu00492	90	Bacteria(100);Proteobacteria(100);Alphaproteobacteria(100);Rhodospirillales(100); Acetobacteraceae(99);unclassified(63);unclassified(63)	33.2
Otu00282	231	Bacteria(100);Proteobacteria(100);Alphaproteobacteria(100);Rhodospirillales(100); Acetobacteraceae(100);unclassified;unclassified	38.4
Otu00068	2573	Bacteria(100);Proteobacteria(100);Alphaproteobacteria(100);Rhodospirillales(100); Acetobacteraceae(100);Rubritepida(100);Rubritepida_flocculans(100)	42.5
Otu00015	24377	Bacteria(100);Proteobacteria(100);Alphaproteobacteria(100);Rhizobiales(100); unclassified;unclassified;unclassified	42.1

Otu00303	208	Bacteria(100);Proteobacteria(100);Alphaproteobacteria(100);Rhizobiales(100); unclassified(84);unclassified(84);unclassified(84)	33.2
Otu00026	14938	Bacteria(100);Proteobacteria(100);Alphaproteobacteria(100);Rhizobiales(100); unclassified(62);unclassified(62);unclassified(62)	43.2
Otu00078	2194	Bacteria(100);Proteobacteria(100);Alphaproteobacteria(100);Rhizobiales(100); Rhizomicrobium_f(98);Rhizomicrobium(96);AB179498_s(95)	40.2
Otu00428	116	Bacteria(100);Proteobacteria(100);Alphaproteobacteria(100);Rhizobiales(100); Hyphomicrobiaceae(97);unclassified(85);unclassified(85)	42.6
Otu00172	525	Bacteria(100);Proteobacteria(100);Alphaproteobacteria(100);Rhizobiales(100); Hyphomicrobiaceae(95);Hyphomicrobium(93);Hyphomicrobium_vulgare(61)	31.3
Otu00215	385	Bacteria(100);Proteobacteria(100);Alphaproteobacteria(100);Rhizobiales(100); Bradyrhizobiaceae(99);DQ123621_g(95);EF520418_s(80)	37.3
Otu00200	431	Bacteria(100);Proteobacteria(100);Alphaproteobacteria(100);Rhizobiales(100); Bradyrhizobiaceae(90);DQ123621_g(90);EU223948_s(90)	37.2
Otu00004	100803	Bacteria(100);Proteobacteria(100);Alphaproteobacteria(100);Rhizobiales(100); Bartonellaceae(56);Bartonella(56);unclassified(56)	38.8
Otu00104	1227	Bacteria(100);Planctomycetes(100);Phycisphaerae(100);Phycisphaerales(100); AY673410_f(100);AY673410_g(100);unclassified(100)	45.3
Otu00293	218	Bacteria(100);Planctomycetes(100);Phycisphaerae(100);Phycisphaerales(100); AY673410_f(100);AY673410_g(100);unclassified(100)	35.2
Otu00357	157	Bacteria(100);Planctomycetes(100);Phycisphaerae(100);Phycisphaerales(100); AY673410_f(100);AY673410_g(100);unclassified(100)	33.5

Otu00392	133	Bacteria(100);Planctomycetes(100);Phycisphaerae(100);Phycisphaerales(100); AY673410_f(100);AY673410_g(100);unclassified(100)	34.3
Otu00339	176	Bacteria(100);Cyanobacteria(100);Vampirovibrio_c(100);AF544207_o(100); AY957901_f(100);unclassified(89);unclassified(89)	42.3
Otu00394	133	Bacteria(100);Chloroflexi(100);Thermomicrobia(100);DQ129389_o(100); DQ129389_f(100);DQ129389_g(100);FM209153_s(100)	37.8
Otu00062	3032	Bacteria(100);Bacteroidetes(100);Sphingobacteria(100);Sphingobacteriales(100); Chitinophagaceae(100);unclassified(93);unclassified(93)	36.6
Otu00165	564	Bacteria(100);Bacteroidetes(100);Sphingobacteria(100);Sphingobacteriales(100); Chitinophagaceae(100);unclassified(90);unclassified(90)	42.7
Otu00334	182	Bacteria(100);Bacteroidetes(100);Sphingobacteria(100);Sphingobacteriales(100); Chitinophagaceae(100);unclassified(73);unclassified(73)	32.3
Otu00060	3334	Bacteria(100);Bacteroidetes(100);Sphingobacteria(100);Sphingobacteriales(100); Chitinophagaceae(100);unclassified(55);unclassified(55)	49.9
Otu00050	4191	Bacteria(100);Bacteroidetes(100);Sphingobacteria(100);Sphingobacteriales(100); Chitinophagaceae(100);unclassified(100);unclassified(100)	44.9
Otu00097	1405	Bacteria(100);Bacteroidetes(100);Sphingobacteria(100);Sphingobacteriales(100); Chitinophagaceae(100);unclassified(100);unclassified(100)	37.8
Otu00157	617	Bacteria(100);Bacteroidetes(100);Sphingobacteria(100);Sphingobacteriales(100); Chitinophagaceae(100);Niastella(76);unclassified(76)	50.1
Otu00181	500	Bacteria(100);Bacteroidetes(100);Sphingobacteria(100);Sphingobacteriales(100); Chitinophagaceae(100);GQ263856_g(100);GQ263856_s(97)	38.6

Otu00358	157	Bacteria(100);Bacteroidetes(100);Sphingobacteria(100);Sphingobacteriales(100); Chitinophagaceae(100);FN428761_g(95);EF032763_s(89)	41.5
Otu00107	1193	Bacteria(100);Bacteroidetes(100);Sphingobacteria(100);Sphingobacteriales(100); Chitinophagaceae(100);Chitinophaga(97);Chitinophaga_ginsengisegetis(60)	37.1
Otu00007	55472	Bacteria(100);Bacteroidetes(100);Sphingobacteria(100);Sphingobacteriales(100); Chitinophagaceae(100);Chitinophaga(100);DQ279356_s(100)	50.8
Otu01695	14	Bacteria(100);Bacteroidetes(100);Sphingobacteria(100);Sphingobacteriales(100); Chitinophagaceae(100);Chitinophaga(100);DQ279356_s(100)	36
Otu00033	9813	Bacteria(100);Bacteroidetes(100);Cytophagia(100);Cytophagales(100); Cytophagaceae(100);unclassified(96);unclassified(96)	51.8
Otu00225	347	Bacteria(100);Bacteroidetes(100);Cytophagia(100);Cytophagales(100); Cytophagaceae(100);unclassified(100);unclassified(100)	42.2
Otu00065	2810	Bacteria(100);Bacteroidetes(100);Cytophagia(100);Cytophagales(100); Cytophagaceae(100);Ohtaekwangia(87);Ohtaekwangia_kribbensis(86)	48.7
Otu00093	1499	Bacteria(100);Bacteroidetes(100);Cytophagia(100);Cytophagales(100); Cytophagaceae(100);Chryseolinea(76);Chryseolinea_serpens(76)	43.7
Otu00101	1312	Bacteria(100);Acidobacteria(100);EU686603_c(100);EU686603_o(100); EU686603_f(100);DQ139450_g(100);unclassified(100)	33.8
Otu00141	754	Bacteria(100);Acidobacteria(100);EU686603_c(100);EU686603_o(100); EU686603_f(100);DQ139450_g(100);unclassified(100)	35.5
Otu00361	154	Bacteria(100);Acidobacteria(100);EU686603_c(100);EU686603_o(100); EU686603_f(100);DQ139450_g(100);DQ139450_s(100)	32

DAY84	Otu00109	1169	Bacteria(100);Verrucomicrobia(100);Verrucomicrobiae(100);Verrucomicrobiales(100); Chthoniobacter_f(100);unclassified(100);unclassified(100)	47.9
	Otu01104	27	Bacteria(100);Verrucomicrobia(100);Verrucomicrobiae(100);Verrucomicrobiales(100); Chthoniobacter_f(100);Chthoniobacter(89);unclassified(89)	30.9
	Otu00151	657	Bacteria(100);Verrucomicrobia(100);Verrucomicrobiae(100);Verrucomicrobiales(100); Chthoniobacter_f(100);Chthoniobacter(87);unclassified(87)	43.2
	Otu00228	657	Bacteria(100);Verrucomicrobia(100);Verrucomicrobiae(100);Verrucomicrobiales(100); Chthoniobacter_f(100);Chthoniobacter(100);unclassified(100)	40.5
	Otu00669	59	Bacteria(100);Verrucomicrobia(100);Verrucomicrobiae(100);unclassified(100); unclassified(100);unclassified(100);unclassified(100)	36.2
	Otu00271	247	Bacteria(100);Proteobacteria(100);unclassified(100);unclassified(100);unclassified(100); unclassified(100);unclassified(100)	30.5
	Otu00175	519	Bacteria(100);Proteobacteria(100);Betaproteobacteria(100);Burkholderiales(100); Burkholderiaceae(100);Burkholderia(100);unclassified(100)	35.5
	Otu00294	217	Bacteria(100);Planctomycetes(100);Phycisphaerae(100);Phycisphaerales(100); FJ936783_f(100);AF407701_g(93);AF407701_s(93)	30.1
	Otu00518	84	Bacteria(100);Bacteroidetes(100);Bacteroidia(100);Bacteroidales(100); unclassified(100);unclassified(100);unclassified(100)	32.6
	Otu00436	112	Bacteria(100);Acidobacteria(100);EU686603_c(100);EU686603_o(100); EU686603_f(100);unclassified(100);unclassified(100)	46.3
	Otu00443	110	Bacteria(100);Acidobacteria(100);EU686603_c(100);EU686603_o(100); EU686603_f(100);DQ139450_g(100);unclassified(74)	35.2

4. DISCUSSION

In this study, changes in bacterial community composition and diversity with time and nutrient concentration were examined using NGS methodologies. We observed strong and highly predictable changes in bacterial community composition and diversity.

4.1 Distinct bacterial community composition at each incubation sampling time point and nutrient concentration

At each sampling point there was a distinct bacterial community for a given initial nutrient concentration (Fig.2). At the initial time point, the culture medium provides relatively high concentrations of available resources, so copiotrophic bacteria can grow fast to exploit this environment. However, as time goes on and bacterial biomass builds up, nutrient concentrations will decrease, and the environment in the medium will become more favorable to oligotrophic bacteria. This predictable successional change in bacterial community composition with initial dominance of a copiotrophic subpopulation, followed by a gradual increase in a more specialized, oligotrophic subpopulation is an example of endogenous heterotrophic succession as classified by Fierer

et al. (2010). An additional aspect is that copiotrophic bacteria which grow rapidly and achieve high biomass early on, especially in higher nutrient treatments, may continue to show up later even if they are no longer active. Therefore, a more definitive answer would require analysis of rRNA sequencing rather than rRNA genes to understand which groups are truly active at each sampling time point (Jones and Lennon, 2010). Similarly, we found that each nutrient concentration tended to have its own distinct bacterial community at each sampling time point. Our results are consistent with previous studies showing that primary productivity or nutrient status can alter bacterial community composition (Schäfer et al., 2001; Claire Horner-Devine et al., 2003; Ø VREAS et al., 2003; Bell et al., 2010). At higher nutrient concentrations bacterial community was mainly dominated by copiotrophs, whereas at lower nutrient concentrations the community contained more oligotrophs. These results support the copiotroph/oligotroph functional classification model proposed by Fierer et al. (2007), which suggests that some phyla may be copiotrophic and relatively more abundant under high-C conditions, while others may be considered oligotrophic. The observed community pattern in this experiment, suggests that niche of soil bacteria may be

fairly specialized in relation to range of nutrient concentrations. This niche differentiation along the nutrient gradient offers one mechanism by which so many species of soil bacteria are able to coexist (Zhou et al., 2002). The experimental system studied here is simplified compared to soil, but its simplicity is in itself an advantage as it allows particular environmental gradients to be clearly defined, and also the lower diversity in this system allows bacterial communities to be analyzed more thoroughly. Although nutrient concentrations have shown to play an integral role in structuring bacterial community in this study, there are numerous other factors especially pH that have been shown to delimit soil bacterial community composition (Fierer and Jackson, 2006; Lauber et al., 2009). Regrettably, because of the solid nature of culture medium, we were unable to measure the pH.

4.2 Unimodal curve in bacterial diversity along the nutrient gradient

The 'expected' humpbacked curve in OTU richness across nutrient levels was to some extent found here (Fig 5). These results are largely consistent with the experimental and observational results for larger organisms (Mittelbach et al., 2001), as well as more simplified microcosm communities (Kassen et al., 2000; Hall and Colegrave,

2007; Benmayor et al., 2008; Langenheder and Prosser, 2008; Bell et al., 2010). The possible explanation for this unimodal relationship between nutrient concentration and diversity could be that both copiotrophic and oligotrophic bacterial taxa coexist at intermediate nutrient concentration, thereby resulting in a peak in diversity (Kassen et al., 2000). Alternatively, the model proposed for the humpbacked curve in plant communities (Grime and Pierce, 2012) could be operating: at high nutrient concentrations competitive exclusion reduces diversity; at low nutrient concentrations only extremely stress – tolerant (specialized) species could survive; the diversity 'peak' occurs at intermediate nutrient concentrations. The higher nutrient concentration of undiluted TSA and TSA 1/10 would be expected to show intense bacterial overgrowth and competitive exclusion early in the experiment, which would explain the generally lower OTU richness and PD for these treatments (Fig. 5). However, Shannon index and nutrient concentration showed no correlation at various sampling time point (from Day14 to Day56). Shannon index gives extra weight to rare OTUs, and the community obtained through NGS sequencing with high number of rare OTUs heavily biases the true value (Hill et al., 2003). This could be the reason why Shannon index does not show the

unimodal relationship similar to OTU richness and PD at most of the sampling time points.

4.3 Bacterial diversity peaks at same sampling time point independent of initial nutrient concentration

We had expected that high nutrient treatments would show maximum species diversity at relatively earlier sampling time points, because bacteria generally grow more slowly at lower nutrient concentrations (Monod, 1949), and most slower growing species are thus less likely to be detectable. However, the highest species diversity was exhibited on day 56 at all nutrient concentrations, apparently independent of initial nutrient concentration (Fig. 6). This is despite the fact that though colonies and total plate coverage by bacteria were evidently much smaller on the lower nutrient concentration plates (Fig. 8). We are unable to explain why the peak in diversity reached at about the same time for all nutrient concentrations, despite the predicted differences in growth rate and the very different bacterial community composition in different nutrient treatments. This paradox requires further theoretical and experimental investigation. However, one can speculate that the peak in diversity at day 56 may be a result of intermediate nutrient

concentrations allowing coexistence of both copiotrophic and oligotrophic taxa. We suggest that the decline in diversity after day 56 was a combination of strongly limiting resources and competition among bacterial taxa towards the end of the experiment. Given the practical constraints on nutrient analysis of small volumes of culture plate medium at each sampling point, this putative drop in nutrient concentrations must be regarded as a conjecture, which could be tested in follow-up studies using larger volumes of culture medium. However, it is unclear from a single study to discern how humpbacked bacterial diversity patterns would vary across nutrient concentrations along a temporal scale. Analogies might be sought with the 'mid-successional' diversity peak observed in plant communities (Huston, 1979), thought to exist because a mix of fast growing early successional species temporarily exists alongside slower growing later successional species, before competitive exclusion of the early fast growing species. A clearer conclusion would require a range of different studies in different systems, as have been done for the world of plant ecology.

5. CONCLUSIONS

The results of this study reveal several interesting conclusions that may provide insight into the ecology of soil bacterial communities in general. The strong predictability of bacterial community composition in relation to both nutrients and time provides evidence for subtle niche specialization, and the potential for an important role of niche structuring in producing soil bacterial communities. The predictability we find here may provide a clue to how the vast diversity of bacteria in soil are able to coexist. Thus while we do not exclude the strong role of stochastic and biotic processes in structuring soil bacterial communities, we view the results of this experiment as supporting the notion that initial nutrient concentration play an integral role in structuring communities. The results of this experiment provide evidence that the 'classic' unimodal/humpback curves which has been reported along productivity and time successional gradients in microbes and other organisms could also exist in diverse bacterial communities. Furthermore, in this experiment these unimodal patterns are observed empirically, without any detailed understanding of the mechanisms that produce them. Therefore, it is uncertain whether they are produced by

the same mechanisms as unimodal diversity peaks in other groups of organisms.

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국문초록

토양 박테리아 군집 조성의 변화과정을 이해하는 가장 좋은 방법은 이들의 자연 생태를 대표할 수 있는 간단한 microcosm 실험을 통해서 일 것이다. 이를 통해 우리는 자연계의 생태를 이해하는데 있어 큰 도움을 받을 수 일을 것이라 생각된다. 본 연구에서는 영양분의 농도 조절을 통한 토양 박테리아 군집의 변화를 시간의 흐름에 따른 상관관계를 알아보는 위한 배양실험을 수행하였다. 각 샘플의 DNA 는 16S rRNA 유전자의 증폭 후, Illumina Hiseq2000 을 통해 시퀀싱되었다.

본 실험에서 분석된 108 개의 배지에서 총 24,751 OTU(Operational Taxonomic Unit)가 검출되었는데 이는 비록 토양 박테리아의 다양성만큼 높지는 않았지만, 이전에 수행된 다른 배양실험들과 비교했을 때 매우 높은 다양성을 나타낸 것이다. 본 실험의 결과, 각 영양분의 농도와 배양시간에 따라 서로 구분되는 박테리아 군집이 존재한다는 것이 관찰되었다. 이를 통해 많은 토양 박테리아가 영양분의 농도와 시간에 따라 서로 다른 생태자리(niche)를 가지고 있는 것을 알 수 있다. 서로 다른 생태자리는 영양분과 시간에 따라 미세하게 다른 박테리아 군집이 같은 토양에 공존하는 것을 이해하는 데에 도움을 줄 것으로 사료된다.

이러한 분서(niche differentiation)는 더 높은 분류군에서 명확하게 관찰되었다. 일부 문(Phylum), 예를 들면, *Proteobacteria* 와 *Firmicutes* 는

배양 초기 단계에서 명확하게 관찰되었으나, 배양 시간이 지날수록 다른 문의 비중이 더 중요하게 나타났는데, 이는 K-strategists 의 역할에 관해서 암시하는 것으로 보인다.

본 실험에서 영양분이 아주 낮을 경우를 제외하고, 비교적 낮은 영양분에서 높은 다양성이 관찰되었는데, 이러한 결과는 대형동물 군집에서 종종 보고된 바 있다. 이는 영양분과 생물다양성 상관관계에 있어 단봉형 패턴(unimodal pattern) 으로 나타난다. 또한 시간과 세균 다양성 상관관계에서도 단봉형 패턴이 관찰되었는데, 배양 초기에는 다양성이 증가하다가 배양 후기로 갈수록 그 다양성이 줄어드는 경향성이 나타났다.