Archaeal community structure and underlying ecological processes in swine manure slurry

Priyanka Kumari^{1*} and Hong L. Choi¹

¹ Department of Agricultural Biotechnology, Research Institute for Agriculture and Life Science, Seoul National University, Gwanak, Seoul, 151-921, Republic of Korea

* Corresponding author

Email: priyanka.shahi1983@gmail.com

Abstract

The ecological processes underlying the observed patterns in community composition of archaea in swine manure slurry are poorly understood. We studied the archaeal communities from six swine manure slurry storage tanks using paired-end Illumina sequencing of the v3 hypervariable region of 16S rRNA gene. Across all samples, the archaeal community was dominated by methanogens related to *Thermoplasmata*, *Methanomicrobia*, and *Methanobacteria* classes. At the genus level, the archaeal community was dominated by a single uncultured lineage of archaea, *vadinCA11*, followed by methanogenic genera *Methanobrevibacter*, *Methanosarcina*, *Methanosphaera*, *Methanogenium*, *Methanocorpusculum*, *Methanoculleus*, and *Methanomicrococcus*. Significant phylogenetic signals were detected across relatively short phylogenetic distances, indicating that closely related archaeal operational taxonomic units (OTUs) tend to have similar niches. The standardized effect sizes of mean nearest taxon distance (SES.MNTD) showed that archaeal community was phylogenetically clustered, suggesting that environmental filtering deterministically influence the within-community composition of archaea. However, between-community analysis based on β -nearest taxon index (β NTI) revealed that both deterministic selection and stochastic processes operate simultaneously to govern the assembly of archaeal communities. These findings significantly enhance our understanding of archaeal community assembly and underlying ecological processes in swine manure slurry.

Keywords: archaea; Illumina sequencing; methanogens; phylogenetic signal; swine manure slurry

32 Introduction

33 Archaea constitute a small fraction of the microbial biomass on Earth, however they involved in
34 various essential ecological processes including nitrification, denitrification and methanogenesis
35 (Offre et al. 2013). Originally, it was believed that archaea inhabit only under extreme conditions,
36 however, now they are known to be present in wide variety of ecosystems including soil (Bintrim
37 et al. 1997; Bates et al. 2011; Tripathi et al. 2013), marine and fresh-water habitats (Lipp et al.
38 2008; Lliros et al. 2008; Teske and Sørensen 2008; Auguet et al. 2011), animal feces and stored
39 manure (Mao et al. 2011; Barret et al. 2015), and intestinal tracts of animals (Shin et al. 2004;
40 Pei et al. 2010). Culture-independent high-throughput sequencing methods have significantly
41 improved our understanding of the diversity and composition of archaea across various
42 ecosystems (Auguet et al. 2010). However, comparatively little is known about the ecological
43 processes that shape the assembly of archaeal communities.

44
45 The assembly of microbial communities is influenced by two types of ecological processes-
46 deterministic selection and stochastic processes (Stegen et al. 2012; Wang et al. 2013).
47 Traditionally, the assembly of microbial communities has been thought to be influenced
48 primarily by deterministic selection (Baas-Becking 1934; Torsvik et al. 2002), in which certain
49 abiotic and biotic factors select a particular assemblage of microbial species (Fierer and Jackson
50 2006; Lozupone and Knight 2007). However, some studies have reported that stochastic
51 processes (e.g., random birth, death, dispersal, and colonization) can also play a major role in
52 shaping assembly of microbial communities (Peay et al. 2010; Caruso et al. 2011). More recently,
53 it has been found that both deterministic and stochastic processes can act concurrently to

influence the community assembly of microbial communities (Chase and Myers 2011; Langenheder and Székely 2011; Stegen et al. 2012; Zhou et al. 2014).

Modern swine production facilities are more intensified and generate large quantities of manure slurry (Barker and Overcash 2007). The swine manure slurry is commonly stored in deep pits for months before being applied to agricultural lands as fertilizer. During storage the swine manure slurry decomposes, and emits a considerable quantity of methane (EPA 2013), which is exclusively generated by methanogenic archaea (Whitman et al. 2006). Over the past decade, several studies have characterized the archaeal community present in swine feces and stored manure slurry by using both cultivation-based (Miller et al. 1986) and culture-independent methods (Whitehead and Cotta 1999; Mao et al. 2011; Da Silva et al. 2014). However, it is still not clear what underlying ecological processes are important in structuring the community composition of archaea in swine manure slurry.

Therefore, this study was conducted to answer the following questions:

- 1) What are the dominant archaeal taxa present in swine manure slurry?
- 2) How does the community composition of archaea is governed by the underlying ecological processes (deterministic vs. stochastic) in both within- and between-community-level analyses?

We applied an ecological null modeling approach to understand the relative influence of deterministic selection and stochastic processes in structuring the archaeal communities (Webb et al. 2002; Stegen et al. 2012).

Material and Methods

Sample collection

Swine manure slurry samples were collected from six different commercial pig farms in South Korea in June 2013. For each sample, slurry was collected from the top 1 m of the storage tank at five different points, and finally one liter of slurry was collected after thorough mixing (for detailed information on sampling, see (Kumari et al. 2015). After collection the swine manure slurry samples were kept in an ice box and transported to the laboratory for DNA extraction.

DNA extraction, PCR amplification, and Illumina sequencing

DNA was extracted from the pellet of centrifuged (at 14,000 x g for 5 min) swine manure slurry samples (5-ml each) using a PowerSoil DNA isolation kit (MoBio Laboratories, USA). The v3 hypervariable region of archaeal 16S rRNA gene was amplified using primer pair S-D-Arch-0349-a-S-17 (5'GYGCASCAGKCGMGAAW3')/ S-D-Arch-0519-a-A-16 (5'TTACCGCGGCKGCTG3') (Klindworth et al. 2012). The resulting amplicons were sequenced at the Beijing Genome Institute (BGI) (Hong Kong, China) using Hiseq™ 2500 platform (Illumina, USA) to generate 150 bp paired-end reads. The raw sequence data were deposited into the NCBI short reads archive (SRA) database under accession number SRP117664.

Sequence processing

The paired-end sequences were assembled using PANDAseq (Masella et al. 2012), and subsequently processed in Mothur (Schloss et al. 2009). The assembled 16S rRNA gene sequences were aligned against SILVA reference alignment v123 (<http://www.arb-silva.de/>). The

alignment was screened for chimeric sequences using ‘chimera.uchime’ command implemented in mothur in de novo mode (Edgar et al. 2011). The resulting alignment was classified against Greengenes reference taxonomy database (release gg_13_8_99; August 2013). The sequences were grouped into operational taxonomic units (OTUs) at $\geq 97\%$ sequence similarity using the average neighbor clustering algorithm. Singleton OTUs were removed to avoid spurious results due to sequencing errors. Finally, the dataset was rarified to a depth of 10,291 sequences per sample using the ‘sub.sample’ command in mothur.

Phylogenetic and statistical analysis

A maximum-likelihood tree was constructed from the sequence alignment of representative OTUs using FastTree (Price et al. 2010). For evaluating phylogenetic signals, environmental-optima for all OTUs were calculated by following the procedure described by Stegen et al. (2012). Then, among-OTU differences in environmental optima were calculated as Euclidean distances. Mantel correlogram was used to assess the correlation coefficients between differences in environmental optima and phylogenetic distances with 999 permutations. We calculated mean nearest taxon distance (MNTD) to emphasize phylogenetic relationships between archaeal OTUs (Webb et al. 2002). To analyze the turnover in phylogenetic community composition, β MNTD was calculated using ‘comdistnt’ function (abundance.weighted = TRUE) in ‘picante’ R package (Kembel et al. 2010). Whereas, Bray-Curtis distance was calculated using ‘vegdist’ function in ‘vegan’ R package (Oksanen et al. 2013) to evaluate the turnover in taxonomic community composition (at the OTU level). Nonmetric multidimensional scaling (NMDS) plots were used to visualize the taxonomic and phylogenetic composition of archaeal community

across all samples. To assess congruence among taxonomic and phylogenetic NMDS ordinations, we used ‘procrustes’ function in vegan R package.

The standardized effect sizes of MNTD (SES.MNTD) was calculated using the null model ‘taxa.labels’ (999 randomization) in ‘picante’ R package to evaluate the phylogenetic community assembly. The mean value of SES.MNTD across all communities deviating significantly from zero indicates clustering (SES.MNTD<0) or over dispersion (SES.MNTD>0) (Webb et al. 2002; Kembel et al. 2010).

Furthermore, a null modeling approach was employed to disentangle the community assembly processes using R software (Stegen et al. 2012; Stegen et al. 2013; Wang et al. 2013). To infer this, the β -nearest taxon index (β NTI) was calculated which estimates the deviation of the observed β MNTD from the null distribution of β MNTD (using 999 randomizations). The $|\beta$ NTI| values > 2 indicate deterministic selection (β NTI < -2 : homogeneous selection; β NTI $> +2$: variable selection) is responsible for differences in community composition, whereas $|\beta$ NTI| values < 2 indicate that the observed difference in community composition is result of stochastic processes (Dini-Andreote et al. 2015).

Results and discussion

A total of 122,321 archaeal 16S rRNA gene sequences (with an average length of 191 bp) were obtained from six swine manure slurry samples, with coverage ranging from 10,896 to 26,661 reads per sample. Despite the high level of sequencing coverage, the rarefaction curves did not reach a plateau (Fig. 1), indicating that the actual archaeal diversity is much higher. Of the

122,321 sequences, around 98.8% sequences were classified up to phylum *Euryarchaeota*. *Euryarchaeota* are known to inhabit pig feces and mostly dominated by the methanogenic members of this phylum (Cardinali-Rezende et al. 2012; Da Silva et al. 2014).

Across all samples, *Thermoplasmata* was the most abundant euryarchaeal class (75,127 sequences, 61.4% of all sequences) followed by *Methanomicrobia* (22,432 sequences, 18.3% of all sequences) and *Methanobacteria* (16,226 sequences, 13.3% of all sequences) (Fig. 2). All these archaeal classes have been reported to dominate pig feces (Dridi et al. 2009; Mao et al. 2011). The candidate genus *vadinCA11* (Godon et al. 1997) predominated across all samples representing around 56% of the whole archaeal community (Fig.3). The dominance of *vadinCA11* is previously documented in swine manure and storage pits (Whitehead and Cotta 1999; Snell-Castro et al. 2005). So far, the metabolic activity of this uncultured archaeal lineage is remains unclear. The other most dominant archaeal genera detected across all samples were *Methanobrevibacter*, *Methanosarcina*, *Methanosphaera*, *Methanogenium*, *Methanocorpusculum*, *Methanoculleus*, and *Methanomicrococcus* (Fig.3). All these genera use hydrogenotrophic methanogenesis pathway except *Methanosarcina*, which can use both the acetoclastic and the hydrogenotrophic methanogenesis pathways (Phelps et al. 1985).

Testing phylogenetic signal in the ecological niches of species is a prerequisite for making ecological inferences using phylogenetic information (Cavender-Bares et al. 2009; Fine and Kembel 2011). Mantel correlogram showed significant phylogenetic signal across relatively short phylogenetic distances (Fig.4), which indicates that niche preferences of closely related archaeal OTUs are more similar to each other than to the niche preferences of distant relatives

(Losos 2008). Therefore we calculated MNTD to quantify the phylogenetic distances among close relatives (Webb et al. 2002). This result is consistent with the observation of our previous study on bacterial communities in swine slurries (Kumari et al. 2015), which also found significant phylogenetic signal across relatively short phylogenetic distances. Furthermore, similar results were also reported on archaeal communities in soil ecosystems (Tripathi et al. 2015).

Procrustes analysis comparing spatial fit between NMDS plots generated using Bray-Curtis (taxonomic) and β MNTD (phylogenetic) distances showed concordance (procrustes correlation, $R = 0.80$, $P = 0.04$; Fig. 5), indicating that archaeal communities in swine slurry samples have a strong evolutionary structural component. The SES.MNTD distribution mean was significantly less than zero across all samples (Fig. 6a; one-tailed t-test, $P < 0.05$), indicating that the each archaeal community was phylogenetically clustered largely due to environmental filtering. Environmental filtering play a key role in shaping species community assembly (Webb et al. 2002). This result is consistent with previous studies on microbial communities in different environments (Kumari et al. 2015; Ren et al. 2015; Yan et al. 2016), which showed that microbial communities had a tendency to be phylogenetically clustered.

To infer the relative influence of deterministic selection vs. stochastic processes that govern the phylogenetic turnover of archaeal communities in swine manure slurry, we employed between-community null modeling approach and calculated β NTI (Stegen et al. 2012; Stegen et al. 2013; Wang et al. 2013). The median of the β NTI distribution was significantly greater than the expected value of zero (median β NTI = +2.00, one-tailed t-test, $p < 0.0001$; Fig. 6b), indicating

that on average variable selection governs archaeal community dynamics. The physico-chemical properties of swine manure slurry varied widely across samples (Suresh and Choi 2011), and this heterogeneous condition among swine manure slurry samples could results in strong influence of variable selection. We also quantified the relative proportions of both ecological processes across all comparisons (Fig. 6b). The results showed that though the relative contribution of deterministic selection was higher (variable selection = 53%), stochastic processes (47%) also appeared to influence the turnover of archaeal communities. These results are consistent with the recent theoretical frameworks that both deterministic selection and stochastic processes govern the assembly of microbial communities (Chase and Myers 2011; Langenheder and Székely 2011; Stegen et al. 2012; Zhou et al. 2014).

Conclusions

In conclusion, we found that the archaeal community in swine manure slurry was dominated by methanogenic archaea. The phylogenetic signals detected across relatively short phylogenetic distances revealed that closely related archaeal OTUs have more similar niche preferences, hence they are ecologically similar. The archaeal community was phylogenetically clustered within-community analysis (SES.MNTD) and thus environmental filtering deterministically governs the assembly of each community in swine manure slurry. However, relative to within-community analysis, the turnover between archaeal communities (β NTI) was governed by both deterministic selection and stochastic processes. Overall, this study provides valuable insights into the community assembly and ecology of archaea in swine manure slurry.

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References

- Auguet JC, Nomokonova N, Camarero L, Casamayor EO (2011) Seasonal changes of freshwater ammonia-oxidizing archaeal assemblages and nitrogen species in oligotrophic alpine lakes. *Appl Environ Microbiol* 77:1937–1945
- Auguet JC, Barberan A, Casamayor EO (2010) Global ecological patterns in uncultured Archaea. *ISME J* 4:182-190
- Baas-Becking LGM (1934) *Geobiologie of inleiding tot de milieukunde*. Van Stockum WP & Zoon NV, The Hague
- Barker J, Overcash M (2007) Swine waste characterization: a review. *T ASABE* 50:651–657
- Barret M, Gagnon N, Morissette B, Kalmokoff ML, Stephen ET, Brooks PJ et al. (2015) Phylogenetic identification of methanogens assimilating acetate-derived carbon in dairy and swine manures. *Syst Appl Microbiol* 38:56-66
- Bates ST, Berg-Lyons D, Caporaso JG, Walters WA, Knight R, Fierer N (2011) Examining the global distribution of dominant archaeal populations in soil. *ISME J* 5:908-917
- Bintrim SB, Donohue TJ, Handelsman J, Roberts GP, Goodman RM (1997) Molecular phylogeny of Archaea from soil. *Proc Natl Acad Sci USA* 94:277-282
- Cardinali-Rezende J, Pereira ZL, Sanz JL, Chartone-Souza E, Nascimento AM (2012) Bacterial and archaeal phylogenetic diversity associated with swine sludge from an anaerobic treatment lagoon. *World J Microbiol Biotechnol* 28:3187-3195

- Caruso T, Chan Y, Lacap DC, Lau MC, McKay CP, Pointing SB (2011) Stochastic and deterministic processes interact in the assembly of desert microbial communities on a global scale. *ISME J* 5:1406-1413
- Cavender-Bares J, Kozak KH, Fine PV, Kembel SW (2009) The merging of community ecology and phylogenetic biology. *Ecol Lett* 12:693-715
- Chase JM, Myers JA (2011) Disentangling the importance of ecological niches from stochastic processes across scales. *Philos Trans R Soc Lond B Biol Sci* 366:2351-2363
- Da Silva MLB, Cantão ME, Mezzari MP, Ma J, Nossa CW (2014) Assessment of bacterial and archaeal community structure in swine wastewater treatment processes. *Microbial Ecol* 70:77–87
- Dini-Andreote F, Stegen JC, van Elsas JD, Salles JF (2015) Disentangling mechanisms that mediate the balance between stochastic and deterministic processes in microbial succession. *Proc Natl Acad Sci USA* 112:E1326-E1332
- Dridi B, Henry M, El Khechine A, Raoult D, Drancourt M (2009) High prevalence of *Methanobrevibacter smithii* and *Methanosphaera stadtmanae* detected in the human gut using an improved DNA detection protocol. *PLoS ONE* 4:e7063
- Edgar RC, Haas BJ, Clemente JC, Quince C, Knight R (2011) UCHIME improves sensitivity and speed of chimera detection. *Bioinformatics* 27:2194-2200
- US Environmental Protection Agency (2013) Inventory of US Greenhouse Gas Emissions and Sinks: 1990-2011. EPA Publication 430-R-13-001 (US Environmental Protection Agency, Washington, DC)
- Fierer N, Jackson RB (2006) The diversity and biogeography of soil bacterial communities. *Proc Natl Acad Sci USA* 103:626-631

- 260 Fine PV, Kembel SW (2011) Phylogenetic community structure and phylogenetic turnover
261 across space and edaphic gradients in western Amazonian tree communities. *Ecography*
262 34:552-565
- 263 Godon J-J, Zumstein E, Dabert P, Habouzit F, Moletta R (1997) Molecular microbial diversity of
264 an anaerobic digester as determined by small-subunit rDNA sequence analysis. *Appl*
265 *Environ Microbiol* 63:2802-2813
- 266 Kembel SW, Cowan PD, Helmus MR, Cornwell WK, Morlon H, Ackerly DD et al. (2010)
267 *Picante: R tools for integrating phylogenies and ecology. Bioinformatics* 26:1463-1464
- 268 Klindworth A, Pruesse E, Schweer T, Peplies J, Quast C, Horn M et al. (2012) Evaluation of
269 general 16S ribosomal RNA gene PCR primers for classical and next-generation
270 sequencing-based diversity studies. *Nucleic Acids Res* 41:e1
- 271 Kumari P, Choi HL, Sudiarto SI (2015) Assessment of bacterial community assembly patterns
272 and processes in pig manure slurry. *PLoS ONE* 10:e0139437
- 273 Langenheder S, Székely AJ (2011) Species sorting and neutral processes are both important
274 during the initial assembly of bacterial communities. *ISME J* 5:1086-1094
- 275 Lipp JS, Morono Y, Inagaki F, Hinrichs K-U (2008) Significant contribution of Archaea to
276 extant biomass in marine subsurface sediments. *Nature* 454:991-994
- 277 Lliros M, Casamayor EO, Borrego C (2008) High archaeal richness in the water column of a
278 freshwater sulfurous karstic lake along an interannual study. *FEMS Microbiol Ecol*
279 66:331-342
- 280 Losos JB (2008) Phylogenetic niche conservatism, phylogenetic signal and the relationship
281 between phylogenetic relatedness and ecological similarity among species. *Ecol Lett*
282 11:995-1003

- 1
- 2
- 3
- 4
- 5 283 Lozupone CA, Knight R (2007) Global patterns in bacterial diversity Proc Natl Acad Sci USA
- 6
- 7
- 8 284 104:11436-11440
- 9
- 10 285 Mao SY, Yang CF, Zhu WY (2011) Phylogenetic analysis of methanogens in the pig feces. Curr
- 11
- 12
- 13 286 Microbiol 62:1386-1389
- 14
- 15 287 Masella AP, Bartram AK, Truszkowski JM, Brown DG, Neufeld JD (2012) PANDAseq: paired-
- 16
- 17
- 18 288 end assembler for illumina sequences. BMC Bioinformatics 13:31
- 19
- 20 289 Miller TL, Wolin M, Kusel E (1986) Isolation and characterization of methanogens from animal
- 21
- 22
- 23 290 feces. Syst Appl Microbiol 8:234-238
- 24
- 25 291 Offre P, Spang A, Schleper C (2013) Archaea in biogeochemical cycles. Annu Rev Microbiol
- 26
- 27 292 67:437–457
- 28
- 29
- 30 293 Okasanen J, Blanchet FG, Kindt R, Legendre P, Minchin PR, O'Hara RB et al. (2013) vegan:
- 31
- 32 294 Community Ecology Package. R package version 2.0-7. Available at: [http://CRAN.R-](http://CRAN.R-project.org/package=vegan)
- 33
- 34 295 [project.org/package=vegan](http://CRAN.R-project.org/package=vegan)
- 35
- 36
- 37 296 Peay KG, Garbelotto M, Bruns TD (2010) Evidence of dispersal limitation in soil
- 38
- 39
- 40 297 microorganisms: isolation reduces species richness on mycorrhizal tree islands. Ecology
- 41
- 42 298 91:3631-3640
- 43
- 44 299 Pei CX, Mao SY, Cheng YF, Zhu WY (2010) Diversity, abundance and novel 16S rRNA gene
- 45
- 46
- 47 300 sequences of methanogens in rumen liquid, solid and epithelium fractions of Jinnan cattle.
- 48
- 49 301 Animal 4:20-29
- 50
- 51
- 52 302 Phelps T, Conrad R, Zeikus J (1985) Sulfate-dependent interspecies H₂ transfer between
- 53
- 54 303 *Methanosarcina barkeri* and *Desulfovibrio vulgaris* during coculture metabolism of
- 55
- 56 304 acetate or methanol. Appl Environ Microbiol 50:589-594
- 57
- 58
- 59
- 60
- 61
- 62
- 63
- 64
- 65

- Price MN, Dehal PS, Arkin AP (2010) FastTree 2-approximately maximum-likelihood trees for large alignments. PLoS ONE 5:e9490
- Ren L, Jeppesen E, He D, Wang J, Liboriussen L, Xing P et al. (2015) pH influences the importance of niche-related versus neutral processes in lacustrine bacterioplankton assembly. Appl Environ Microbiol 81:3104-3114
- Schloss PD, Westcott SL, Ryabin T, Hall JR, Hartmann M, Hollister EB et al. (2009) Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. Appl Environ Microbiol 75:7537-7541
- Shin EC, Choi BR, Lim WJ, Hong SY, An CL, Cho KM et al. (2004) Phylogenetic analysis of archaea in three fractions of cow rumen based on the 16S rDNA sequence. Anaerobe 10:313-319
- Snell-Castro R, Godon JJ, Delgenès JP, Dabert P (2005) Characterisation of the microbial diversity in a pig manure storage pit using small subunit rDNA sequence analysis. FEMS Microbiol Ecol 52:229-242
- Stegen JC, Lin X, Fredrickson JK, Chen X, Kennedy DW, Murray C et al. (2013) Quantifying community assembly processes and identifying features that impose them. ISME J 7:2069-2079
- Stegen JC, Lin X, Konopka AE, Fredrickson JK (2012) Stochastic and deterministic assembly processes in subsurface microbial communities. ISME J 6:1653-1664
- Suresh A, Choi HL (2011) Estimation of nutrients and organic matter in Korean swine slurry using multiple regression analysis of physical and chemical properties. Bioresource Technol 102:8848-8859

- 1
- 2
- 3
- 4
- 5 328 Teske A, Sørensen KB (2008) Uncultured archaea in deep marine subsurface sediments: have we
- 6
- 7
- 8 329 caught them all? ISME J 2:3-18
- 9
- 10 330 Torsvik V, Øvreås L, Thingstad TF (2002) Prokaryotic diversity--magnitude, dynamics, and
- 11
- 12
- 13 331 controlling factors. Science 296:1064-1066
- 14
- 15 332 Tripathi BM, Kim M, Lai-Hoe A, Shukor NAA, Rahim RA, Go R et al. (2013) pH dominates
- 16
- 17
- 18 333 variation in tropical soil archaeal diversity and community structure. FEMS Microbiol
- 19
- 20 334 Ecol 86:303–311
- 21
- 22 335 Tripathi BM, Kim M, Tateno R, Kim W, Wang JJ, Lai-Hoe A et al. (2015) Soil pH and biome
- 23
- 24
- 25 336 are both key determinants of soil archaeal community structure. Soil Biol Biochem 88:1-
- 26
- 27 337 8
- 28
- 29
- 30 338 Wang JJ, Shen J, Wu YC, Tu C, Soininen J, Stegen JC et al. (2013) Phylogenetic beta diversity
- 31
- 32 339 in bacterial assemblages across ecosystems: deterministic versus stochastic processes.
- 33
- 34
- 35 340 ISME J 7:1310-1321
- 36
- 37 341 Webb CO, Ackerly DD, McPeck MA, Donoghue MJ (2002) Phylogenies and community
- 38
- 39
- 40 342 ecology. Annu Rev Ecol Syst 33:475–505
- 41
- 42 343 Whitehead T, Cotta M (1999) Phylogenetic diversity of methanogenic archaea in swine waste
- 43
- 44 344 storage pits. FEMS Microbiol Lett 179:223-226
- 45
- 46
- 47 345 Whitman WB, Bowen TL, Boone DR (2006) The methanogenic bacteria. Prokaryotes 3:165–207
- 48
- 49 346 Yan Q, Li J, Yu Y, Wang J, He Z, Van Nostrand JD et al. (2016) Environmental filtering
- 50
- 51
- 52 347 decreases with fish development for the assembly of gut microbiota. Environ Microbiol
- 53
- 54 348 18:4739-4754
- 55
- 56
- 57
- 58
- 59
- 60
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- 62
- 63
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46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

349 Zhou J, Deng Y, Zhang P, Xue K, Liang Y, Nostrand JD et al. (2014) Stochasticity, succession,
350 and environmental perturbations in a fluidic ecosystem. Proc Natl Acad Sci 111:E836-
351 E845

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Figure legends

Fig. 1. Rarefaction curves of archaeal operational taxonomic units ($\geq 97\%$ sequence similarity) for each sample of swine manure slurry.

Fig. 2. The relative abundance of dominant archaeal taxa (at class level) present in each sample of swine manure slurry.

Fig. 3. Heat map showing the relative abundance (square root transformed) of the most dominant archaeal genera in each sample of swine manure slurry.

Fig. 4. Mantel correlogram between differences in environmental optima of OTUs and phylogenetic distances of archaea in swine manure slurry. Closed squares represent significant ($P < 0.05$) phylogenetic signals.

Fig. 5. NMDS ordinations showing the first two axes of (a) taxonomic community composition based on Bray-Curtis distance; (b) phylogenetic community composition based on β MNTD and (c) Procrustes analysis comparing taxonomic and phylogentic community composition of archaea. The arrows in Procrustes analysis point towards the target configuration (taxonomic community composition), and symbols represent the rotated configuration (phylogenetic community composition). Correlation and significance values were calculated using the ‘protest’ function in vegan R package.

Fig. 6. (a) Box plot showing variation in SES.MNTD values across all samples. The SES.MNTD distribution mean was significantly less than zero (one-tailed t-test, $P < 0.05$). (b) Frequency estimates for distributions of β NTI. The median of the β NTI distribution was significantly different from expected value of zero (one-tailed t-test, $P < 0.0001$).

Fig.1

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Fig.6