

More assembly

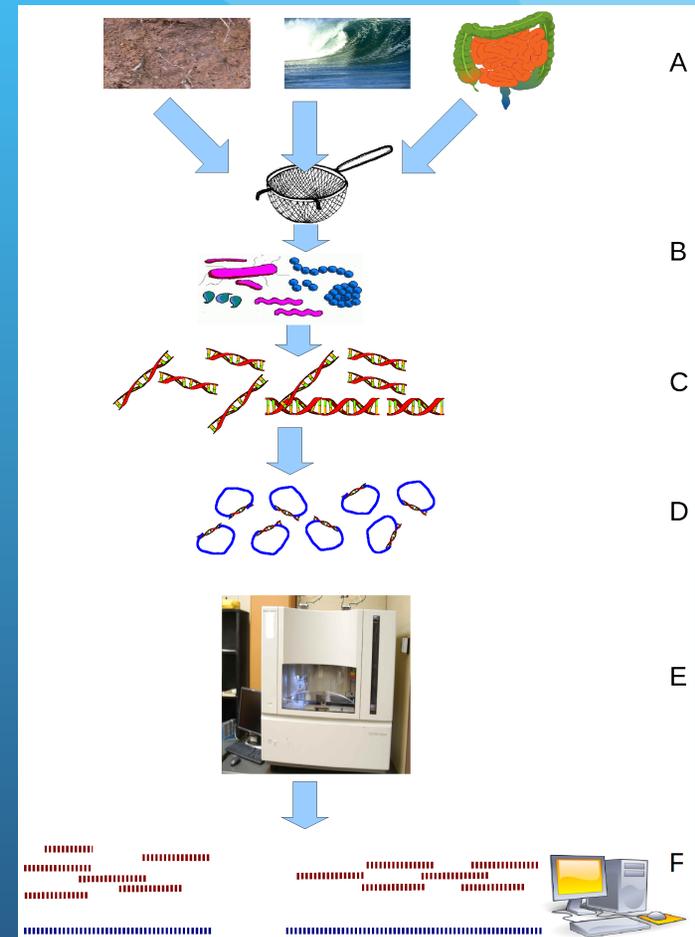
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Shotgun metagenomics

- Collect samples;
- Extract DNA;
- Feed into sequencer;
- Computationally analyze.



Wikipedia: Environmental shotgun sequencing.png

Annotating individual reads

- Works really well when you have EITHER
 - (a) evolutionarily close references
 - (b) rather long sequences

(This is obvious, right?)

Annotating individual reads #2

- We have found that this does not work well with Illumina samples from unexplored environments (e.g. soil).
- *Sensitivity* is fine (correct match is usually there)
- *Specificity* is bad (correct match may be drowned out by incorrect matches)

Recommendation:

For reads < 200-300 bp,

- Annotate individual reads for human-associated samples, or exploration of well-studied systems.
- For everything else, look to assembly.

So, why assemble?

- Increase your ability to assign homology/ orthology correctly!!

Essentially all functional annotation systems depend on sequence similarity to assign homology. This is why you want to assemble your data.

Why else would you want to assemble?

- Assemble new “reference”.
- Look for large-scale variation from reference - pathogenicity islands, etc.
- Discriminate between different members of gene families.
- Discover operon assemblages & annotate on co-incidence of genes.
- Reduce size of data!!

Why *don't* you want to assemble??

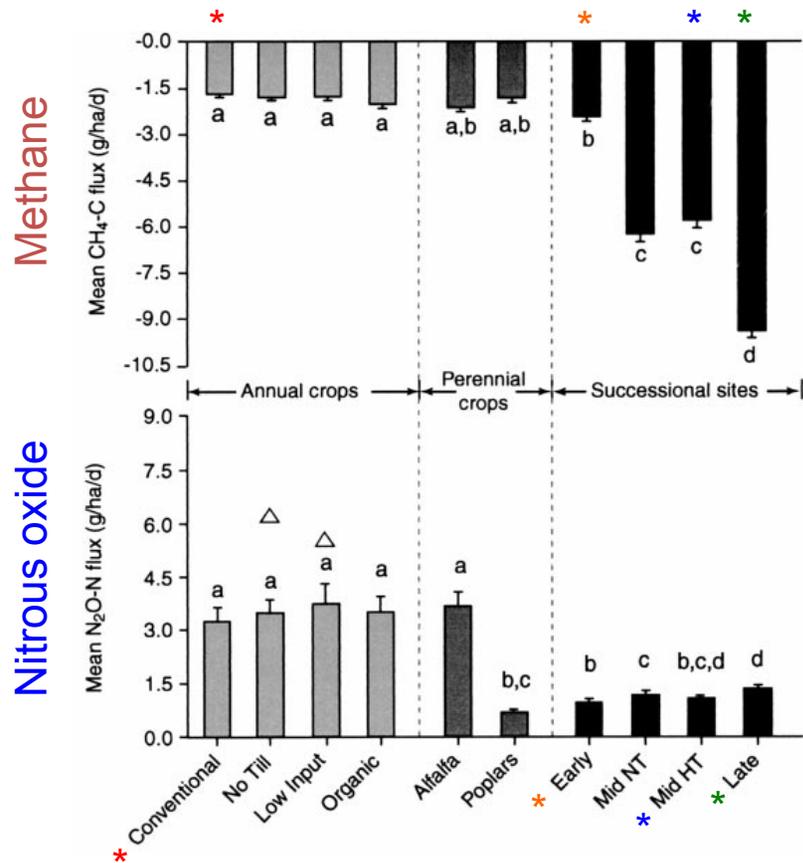
- Abundance threshold - low-coverage filter.
- Strain variation
- Chimerism

A story: looking at land management with 454 shotgun

- Tracy Teal, Vicente Gomez-Alvarado, & Tom Schmidt
- Ask detailed questions of @tracykteal on Twitter, please :)

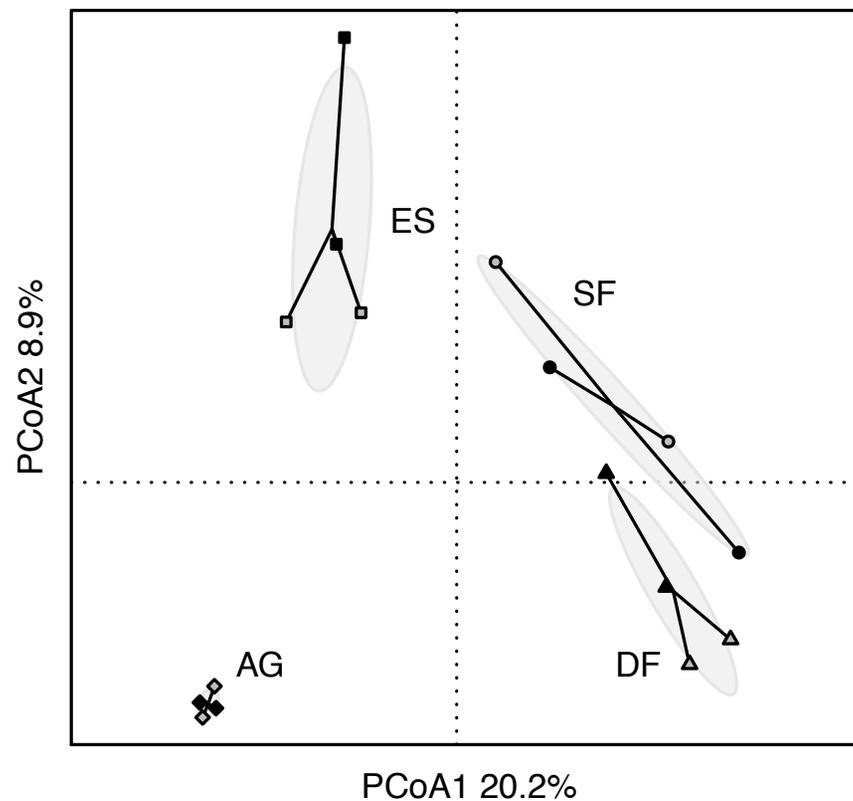
How do microbial communities change with land management?

Kellogg Biological Station LTER



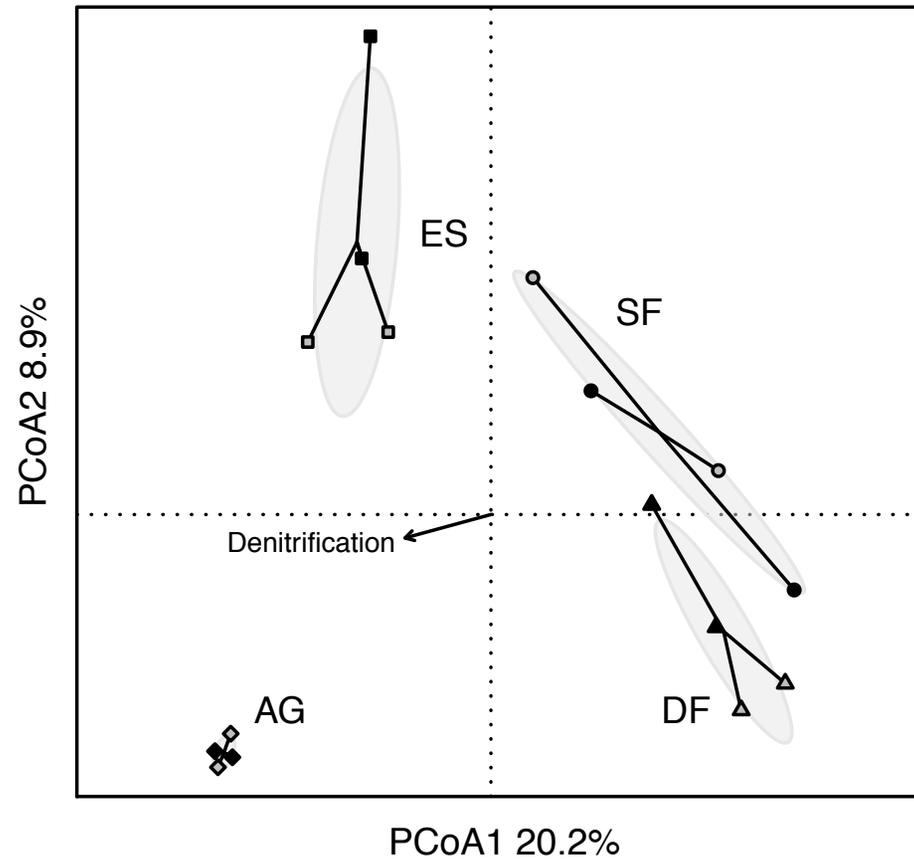
- AG Conventional Agriculture
- ES Early Successional
- SF Successional Forest
- DF Deciduous Forest

Functional potential changes with land management



454 shotgun metagenomes annotated with MG-RAST
Analysis uses a matrix of the 7058 genes annotated

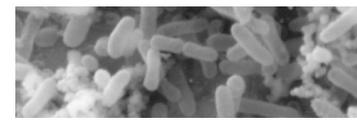
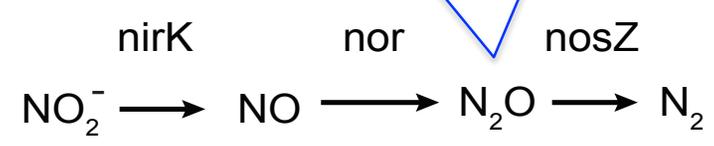
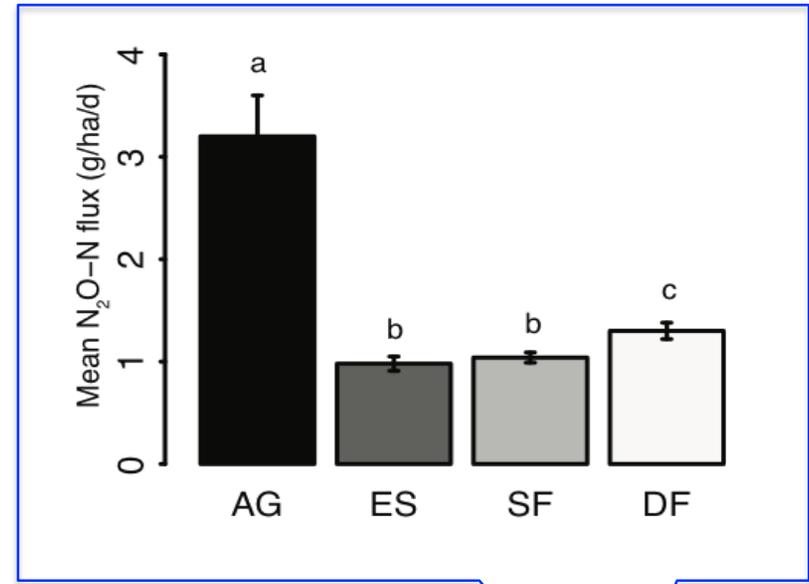
Nitrogen metabolism contributes to the differentiation of communities



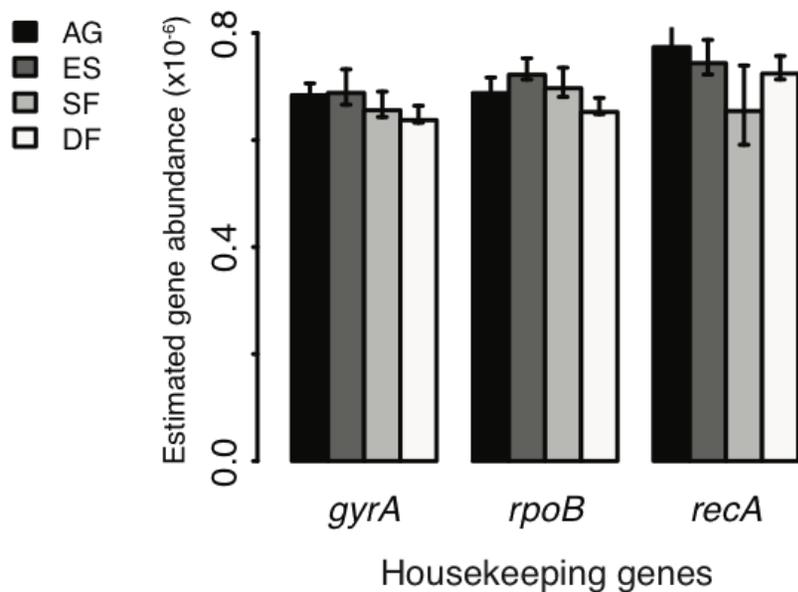
Teal TK, Gomez-Alvarez V, Schmidt TM

Denitrifying microbes

Nitrous oxide production differs with land management



Assessing gene abundance



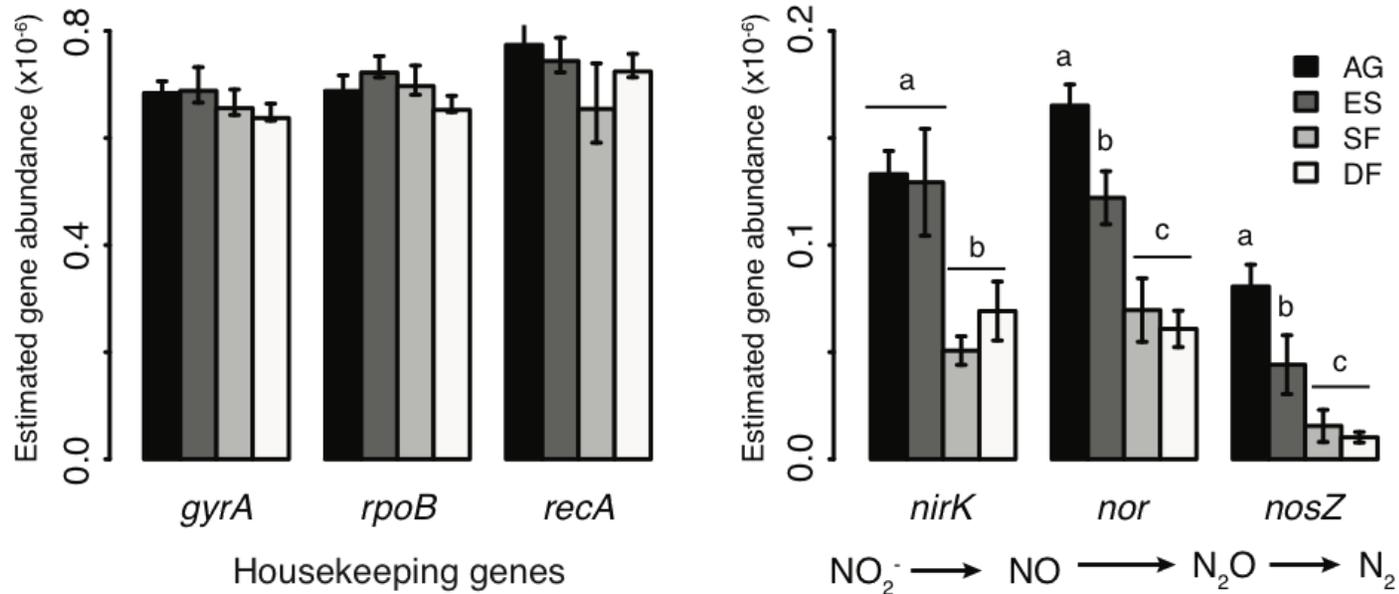
Abundance of housekeeping genes in the libraries consistent across samples and different genes

$$Z = \frac{\# \text{ of matches to gene } X \text{ in library } Y}{\# \text{ of annotated reads in library } Y} * \frac{len_a}{len_x}$$

Where len_a is average gene length (924bp) and len_x is the average length of gene X.

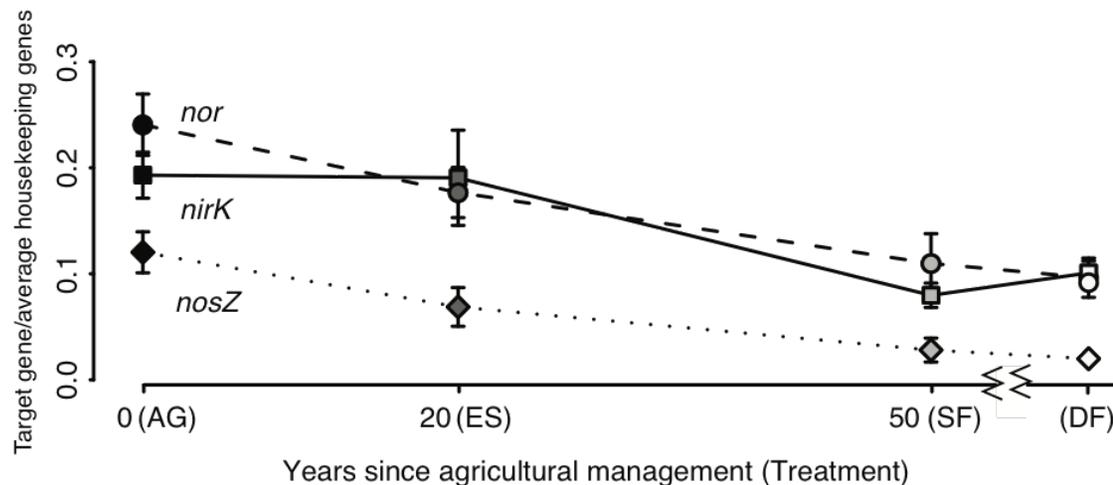
of annotated reads is used for normalization because it incorporates both library size & quality.

Assessing denitrification potential (presence of denitrification genes)



Increased denitrification potential in AG and ES sites
Consistent across denitrification pathway

Proportion of the community that are denitrifiers changes with land management

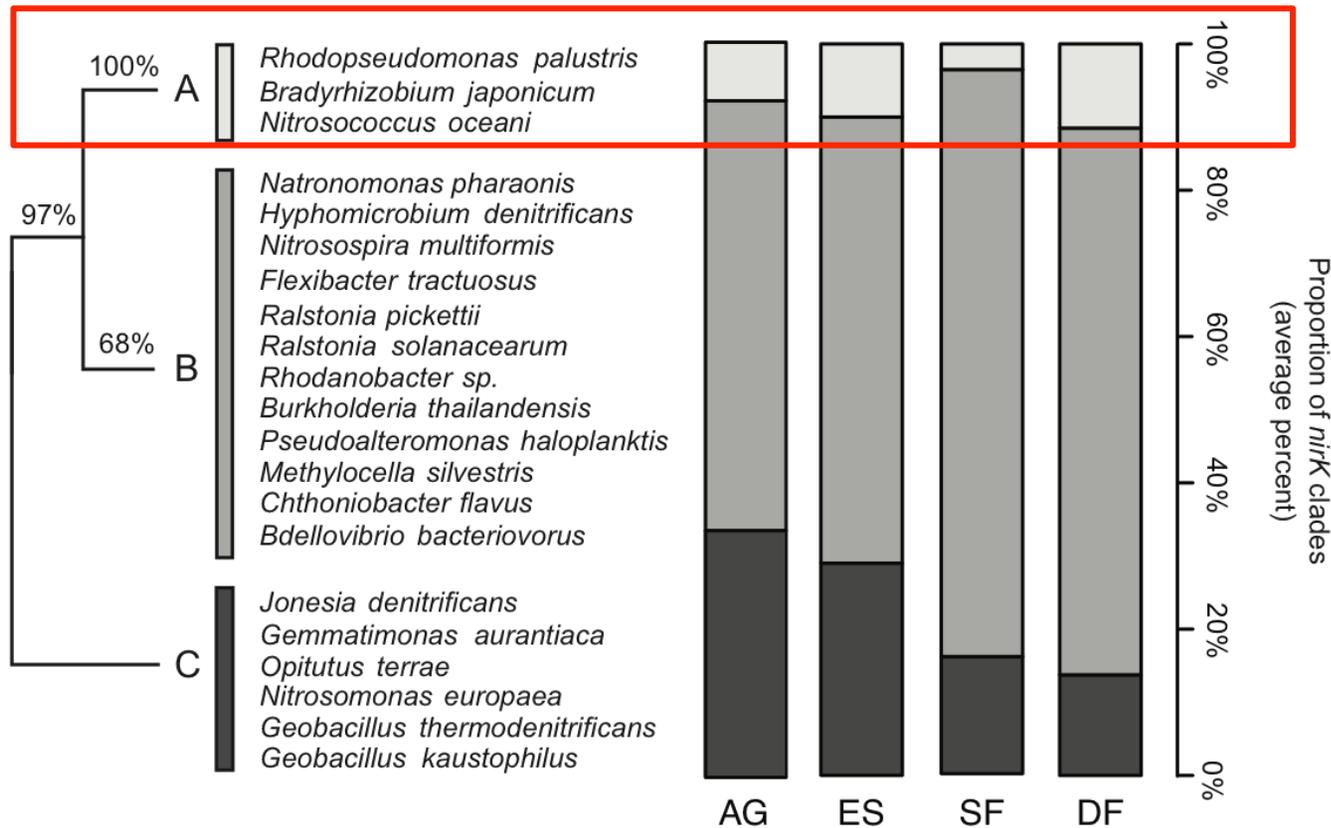


Denitrification gene abundance is normalized to average housekeeping gene abundance. This is used as an approximation of the proportion of the community that has that gene (assuming single copy housekeeping and target genes).

e.g. It can be seen here that in AG ~20% of the community has the *nirK* gene versus ~12% in DF

High denitrification diversity

Only Clade A captured in standard PCR surveys



Reads annotated as *nirK* blasted against reference database of diverse *nirK*s and each read assigned to one of three clades. Phylogeny of *nirK*s is challenging and BLAST matches, especially since we're using varying *nirK* regions is inexact, so analysis limited to this clade level.

Annotating soil reads - thoughts

- Possible to find well-known genes using long (454) reads.
- Normalize for organism abundance!
- **Primer independence can be important!**
- Note, replicates give you error bars...

Osedax symbionts

Table S1 | Specimens, and collection sites, used in this study

Site	Dive ¹	Date	Time Zone (months)	# of specimens	<i>Osedax frankpressi</i> with Rs1 symbiont
2890m	T486	Oct 2002	8	2	
	T610	Aug 2003	18	3	
	T1069	Jan 2007	59	2	
	DR010	Mar 2009	85	3	
	DR098	Nov 2009	93	4	
	DR204	Oct 2010	104	1	
	DR234	Jun 2011	112	2	
1820m	T1048	Oct 2006	7	3	
	T1071	Jan 2007	10	3	
	DR012	Mar 2009	36	4	
	DR236 ²	Jun 2011	63	2 →	

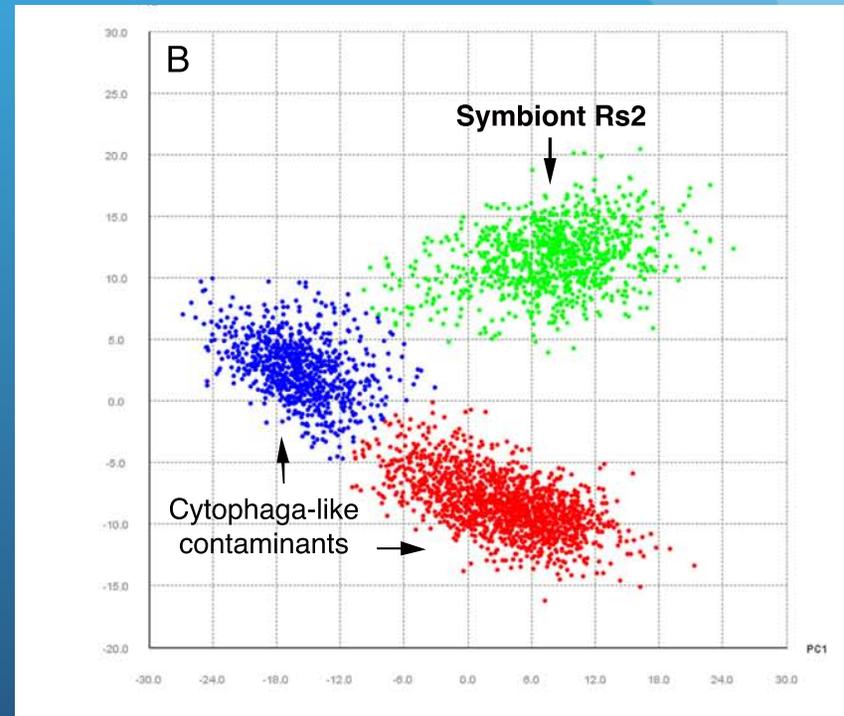
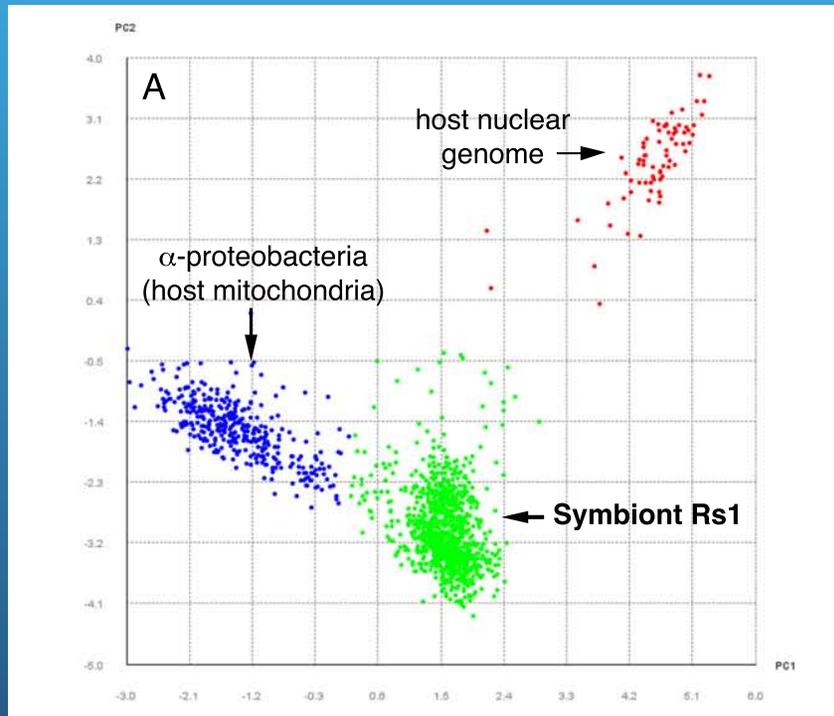


O. frankpressi from DR236

¹Dive numbers begin with the remotely operated vehicle name; T= *Tiburon*, DR = *Doc Ricketts* (both owned and operated by the Monterey Bay Aquarium Research Institute).

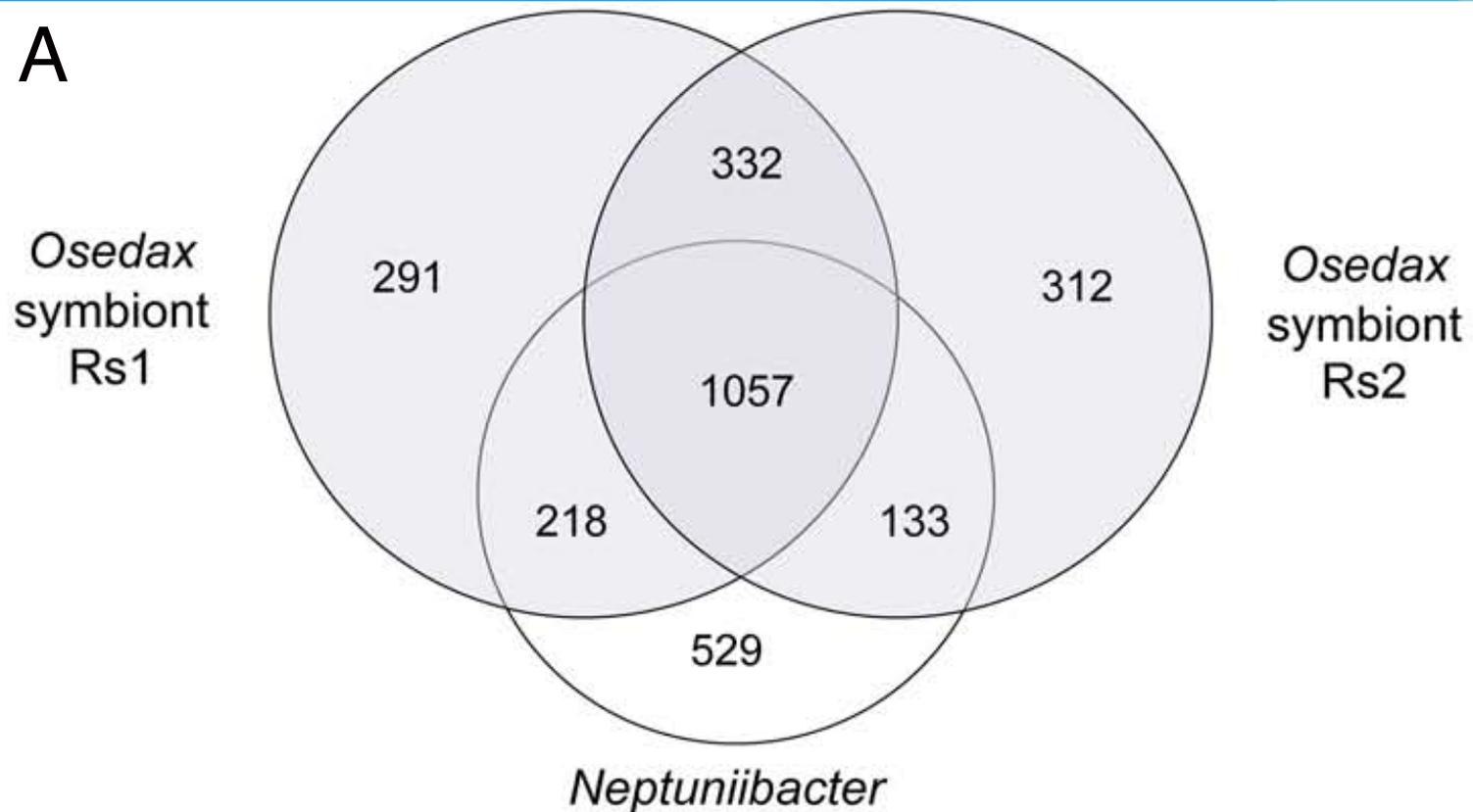
²Samples used for genomic analysis were collected during dive DR236.

Metagenomic assembly followed by binning enabled isolation of fairly complete genomes



Assembly allowed genomic content comparisons to nearest cultured relative

A



Osedax assembly story

- Low diversity metagenome!
- Physical isolation => MDA => sequencing => diginorm => binning =>
 - 94% complete Rs1
 - 66-89% complete Rs2
- Note: many interesting critters are hard to isolate => so, basically, metagenomes.

Human-associated communities

- “Time series community genomics analysis reveals rapid shifts in bacterial species, strains, and phage during infant gut colonization.” Sharon et al. (Banfield lab); *Genome Res.* 2013 23: 111-120

Setup

- Collected 11 fecal samples from premature female, days 15-24.
- 260m 100-bp paired-end Illumina HiSeq reads; 400 and 900 base fragments.
- Assembled > 96% of reads into contigs > 500bp; 8 complete genomes; reconstructed genes down to 0.05% of population abundance.

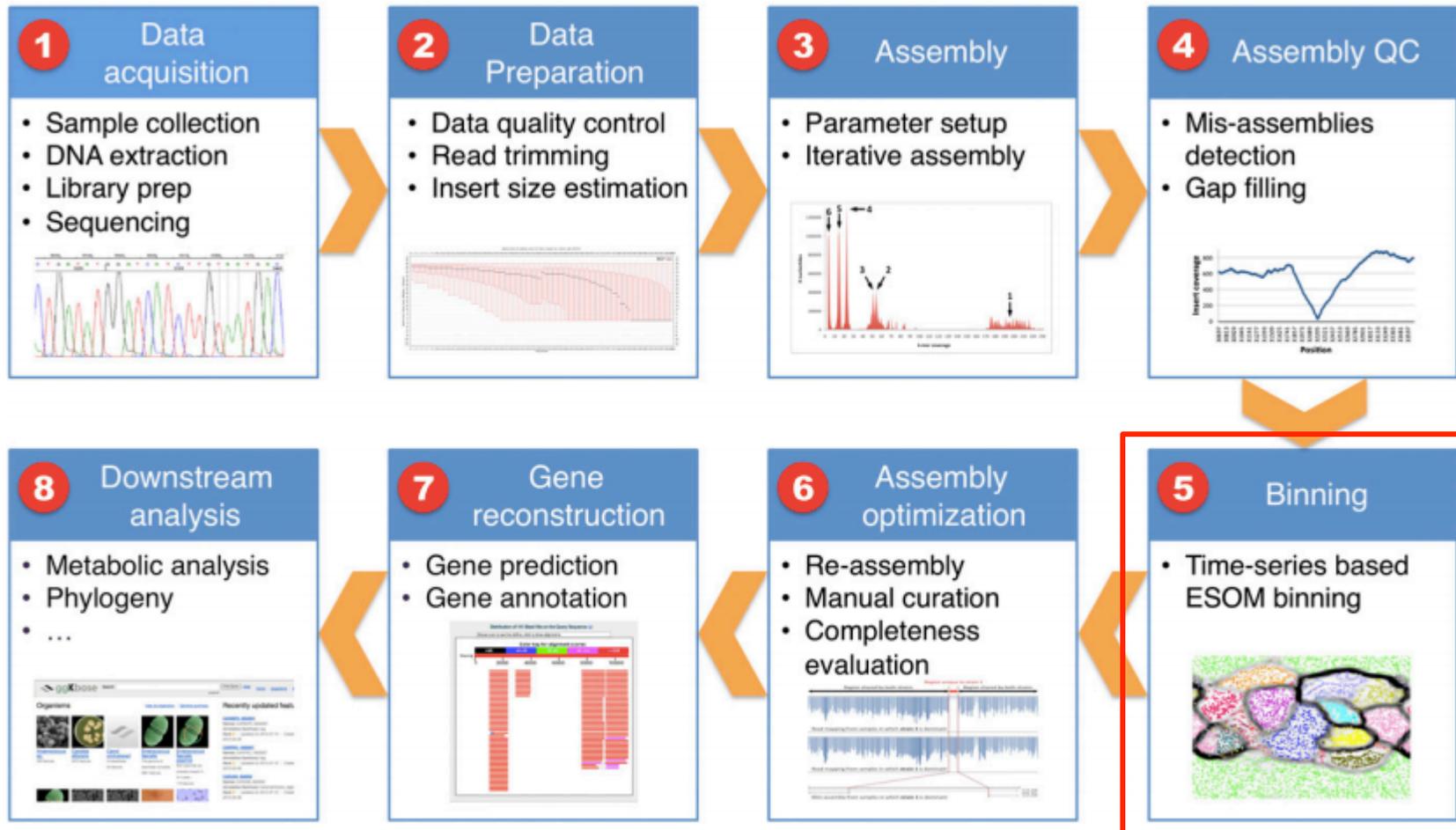
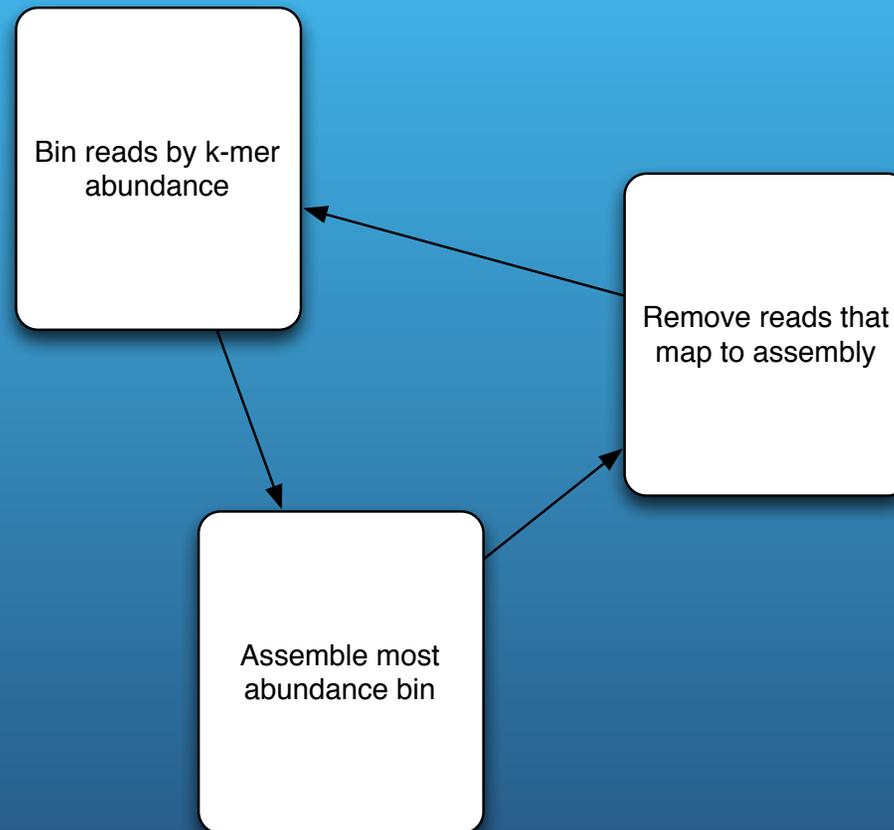
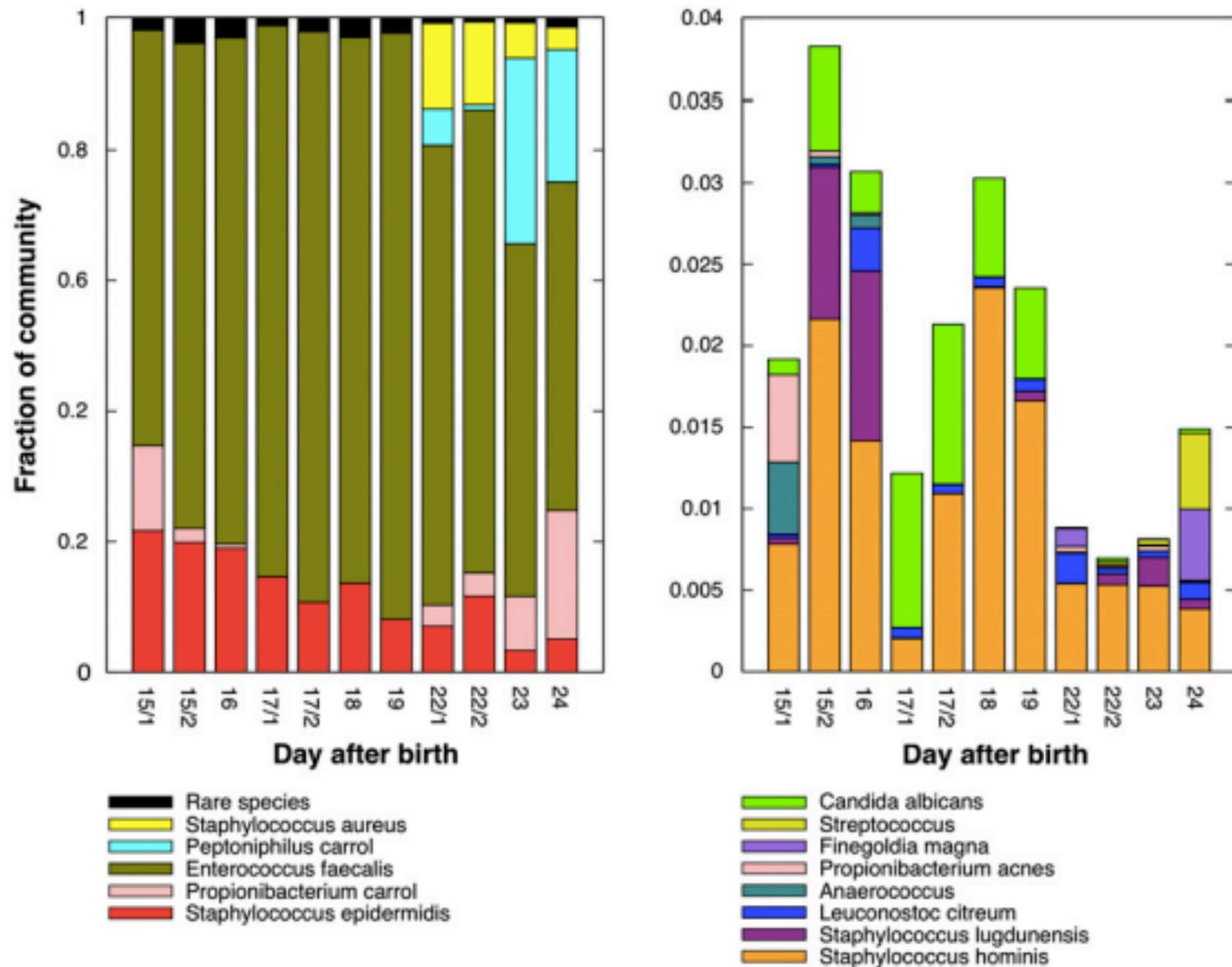


Figure 6. Analysis pipeline for metagenomic data employed in this study. Refer to the Methods section and the supporting online material for more details.

Key strategy: abundance binning



Sharon et al., 2013; pmid 22936250



Tracking abundance

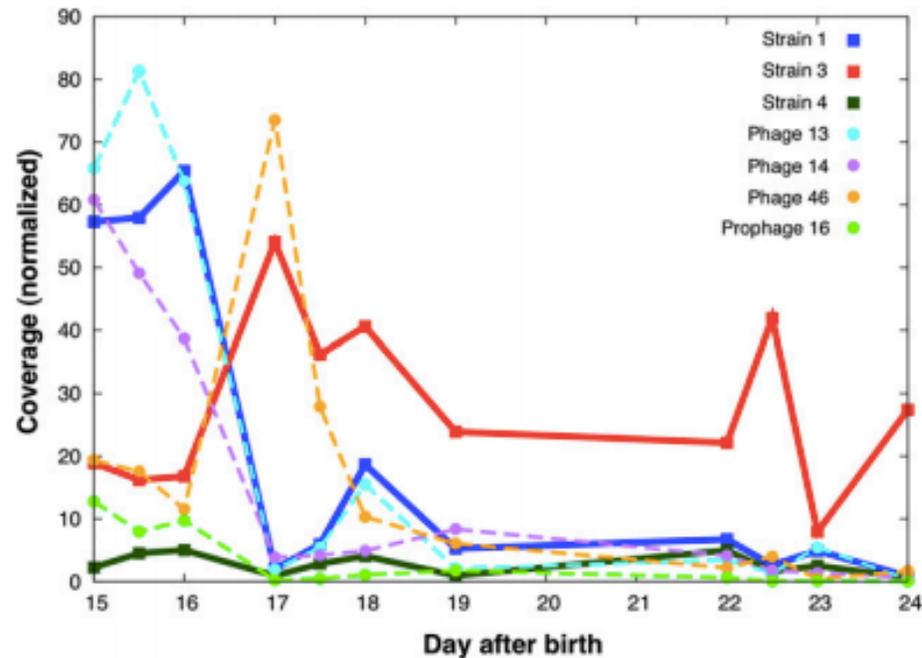


Figure 3. Abundance patterns of the *Staphylococcus epidermidis* strains (thick lines) and their infecting phage (thin dashed lines). All three phages exist primarily as prophages but also were identified as free-existing phages. Insertion positions are in regions that are shared by both abundant strains (and probably also by strain 4), but abundance patterns suggest that phage 13 and 14 infect strain 1, while phage 46 infects strain 3.

Conclusions

- Recovered strain variation, phage variation, abundance variation, lateral gene transfer.
- Claim that “recovered genomes are superior to draft genomes generated in most isolate genome sequencing projects.”

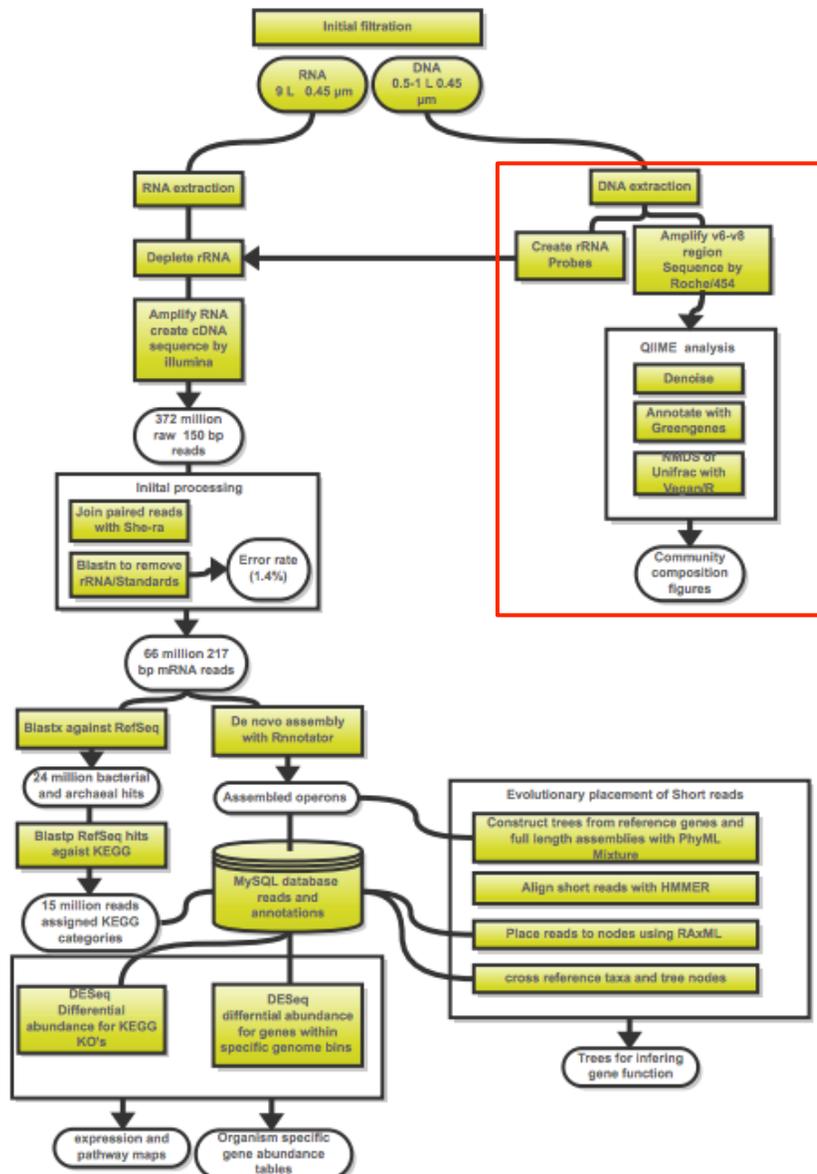
Environmental metagenomics: Deepwater Horizon spill

- “Transcriptional response of bathypelagic marine bacterioplankton to the Deepwater Horizon oil spill.” Rivers et al., 2013, Moran lab. Pmid 23902988.

Sequencing strategy

Table 1 Characteristics of metatranscriptome samples

	<i>P16</i>	<i>P52</i>	<i>IP16</i>	<i>NP52</i>
<i>Physical</i>				
Depth (m)	1116	1198	1240	1286
Hydrocarbon plume	Yes	Yes	No	No
Oxygen anomaly	Yes	Yes	Yes	No
<i>Chemical^a</i>				
CDOM (relative fluorescence units)	2.14	47.44	0.44	0.44
Methane (nmol l ⁻¹)	210 171	235 980	4517	106
Nitrate (μmol l ⁻¹)	21.26	15.14	26.21	27.08
Nitrite (μmol l ⁻¹)	0.28	0.89	0.13	0.05
Phosphate (μmol l ⁻¹)	1.46	0.96	1.78	1.72
<i>Biological</i>				
Cell density (cells ml ⁻¹)	4.95E + 05	1.39E + 05	3.30E + 04	1.74E + 04
Total RNA (μg per l seawater)	1.90E - 04	4.20E - 05	7.12E - 07	3.37E - 07
<i>Sequencing</i>				
Median read length (bases)	217	208	207	233
Total joined reads	28 599 525	23 871 295	22 614 967	29 171 472
rRNA reads (%)	1 780 537 (6%)	9 664 134 (43%)	8 538 576 (36%)	6 104 284 (21%)
Internal standard reads (%)	NA	3 619 342 (16%)	3 918 756 (16%)	5 007 057 (17%)
Possible proteins (%)	26 792 869 (94%)	9 331 491 (39%)	11 413 963 (50%)	18 060 131 (62%)
Archaeal and bacterial hits	10 054 034 (35%)	6 075 577 (25%)	5 611 064 (35%)	1 945 004 (35%)
RefSeq genes	341 983	421 562	428 614	242 768
RefSeq taxa	3627	3628	3742	3485
KEGG assignments	6 141 041	3 781 411	3 680 128	1 097 381



Supplementary Figure S2. Schematic representation of sampling and data analysis.

Protocol for messenger RNA extraction, assembly, downstream analysis, including differential expression.

Note: used overlapping paired-end reads.

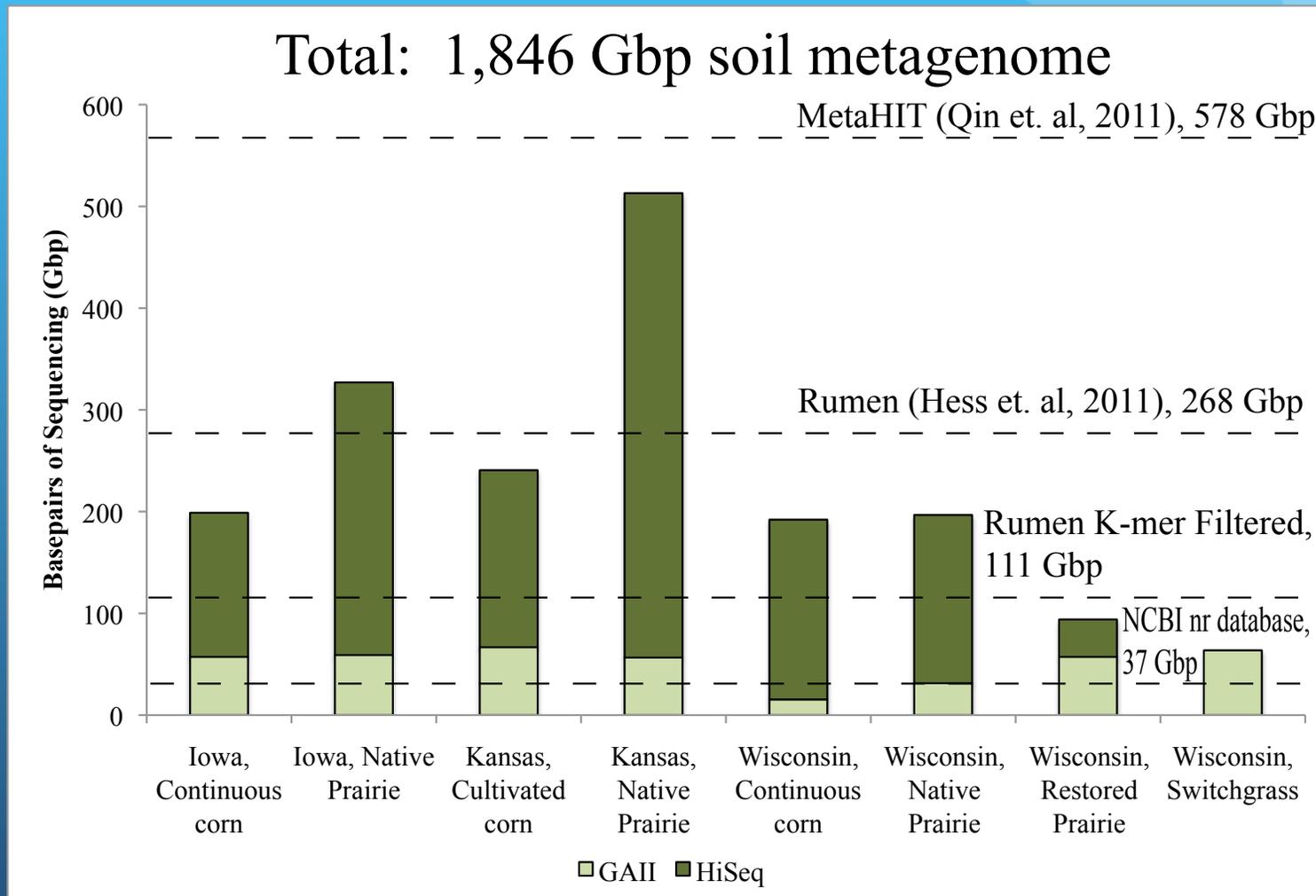
Great Prairie Grand Challenge - *soil*

- Together with Janet Jansson, Jim Tiedje, et al.
- “Can we make sense of soil with deep Illumina sequencing?”

Great Prairie Grand Challenge - *soil*

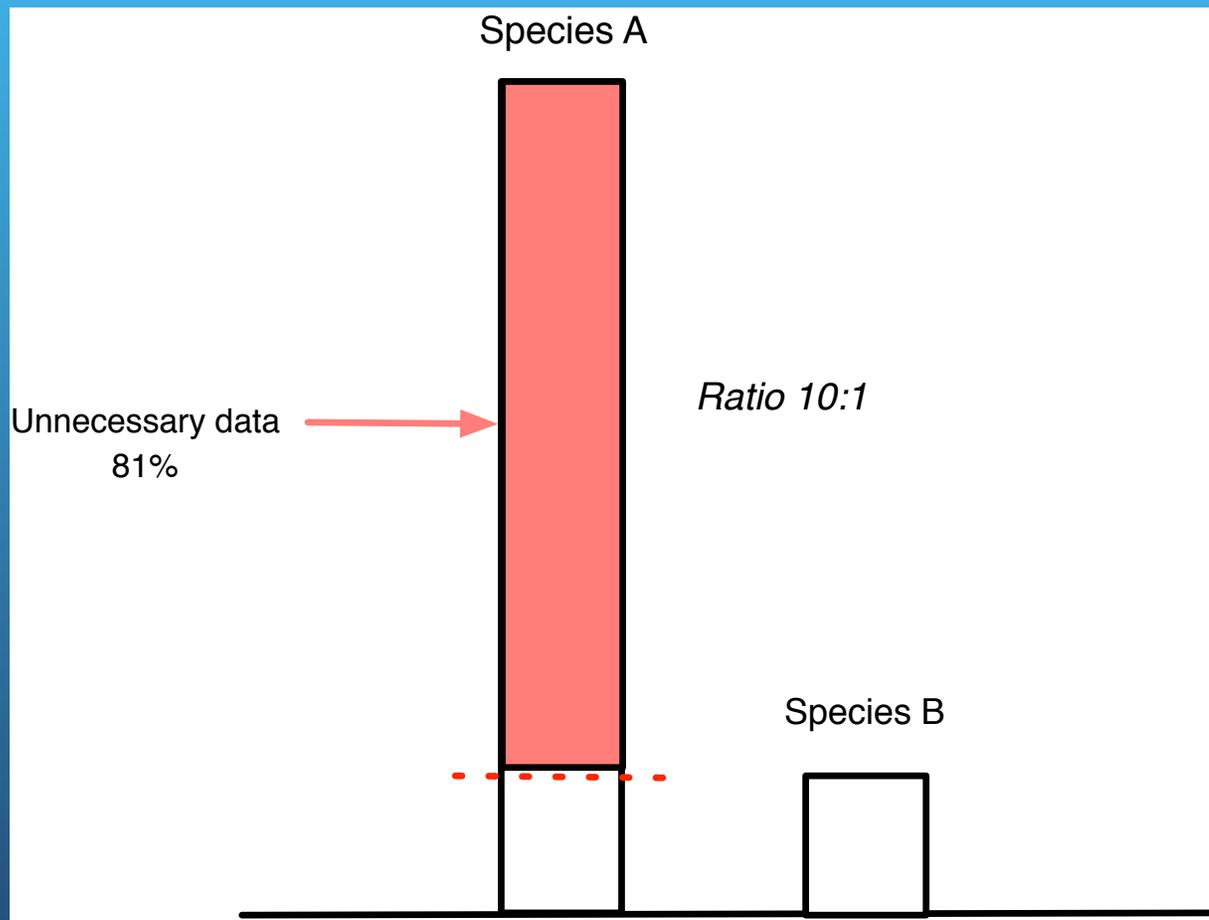
- What ecosystem level functions are present, and how do microbes do them?
- How does agricultural soil differ from native soil?
- How does soil respond to climate perturbation?
- Questions that are not easy to answer without shotgun sequencing:
 - What kind of strain-level heterogeneity is present in the population?
 - What does the phage and viral population look like?
 - What species are where?

A “Grand Challenge” dataset (DOE/JGI)



Approach 1: Digital normalization

(a computational version of library normalization)



Suppose you have a dilution factor of A (10) to B(1). To get 10x of B you need to get 100x of A! Overkill!!

This 100x will consume disk space and, because of errors, **memory**.

We can discard it for you...

Approach 2: Data partitioning

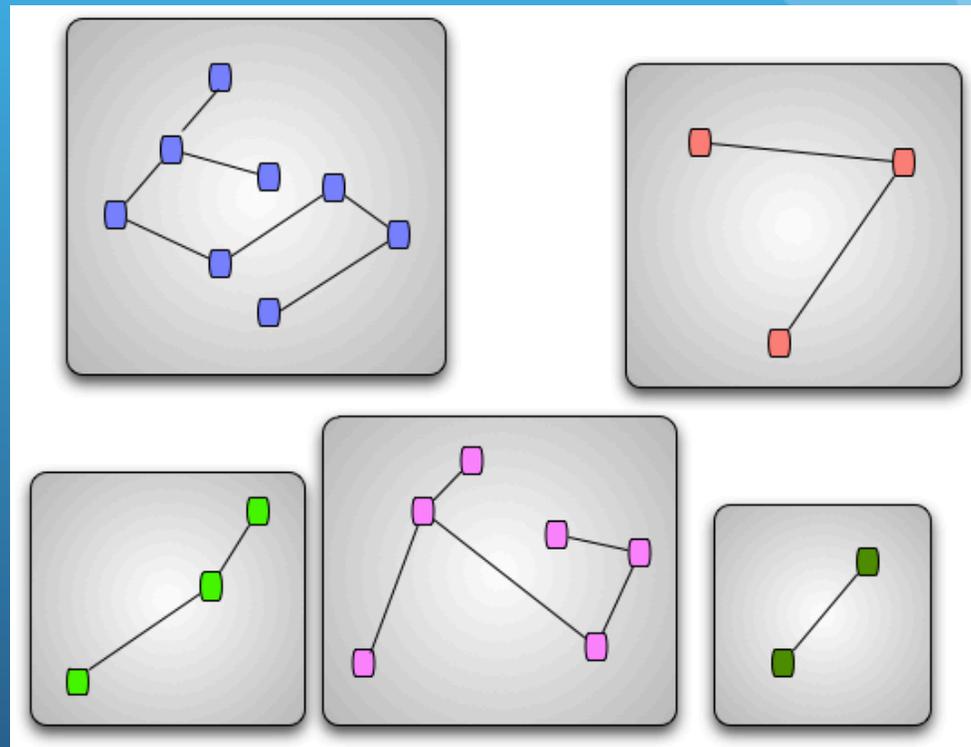
(a computational version of cell sorting)

Split reads into “bins”
belonging to different
source species.

Can do this based almost
entirely on *connectivity*
of sequences.

“Divide and conquer”

Memory-efficient
implementation helps
to scale assembly.



Assembly results for Iowa corn and prairie (2x ~300 Gbp soil metagenomes)

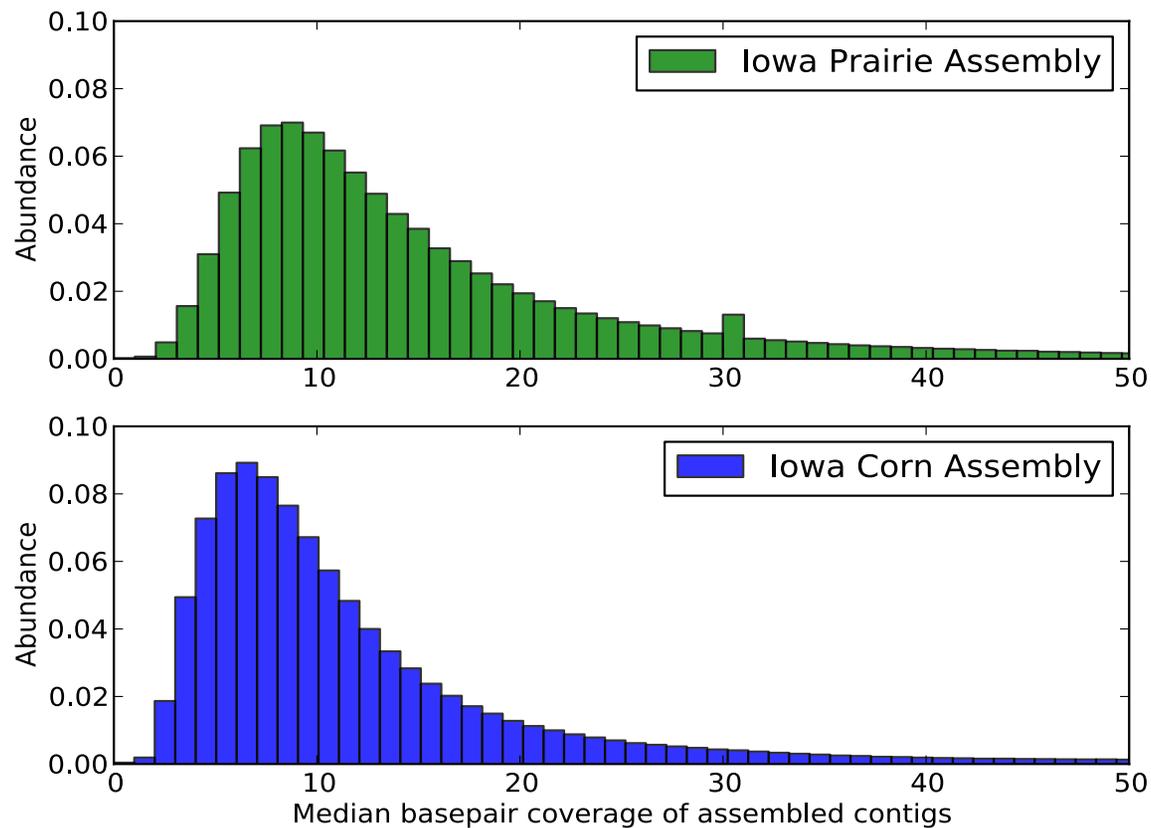


Total Assembly	Total Contigs (> 300 bp)	% Reads Assembled	Predicted protein coding
2.5 bill	4.5 mill	19%	5.3 mill
3.5 bill	5.9 mill	22%	6.8 mill

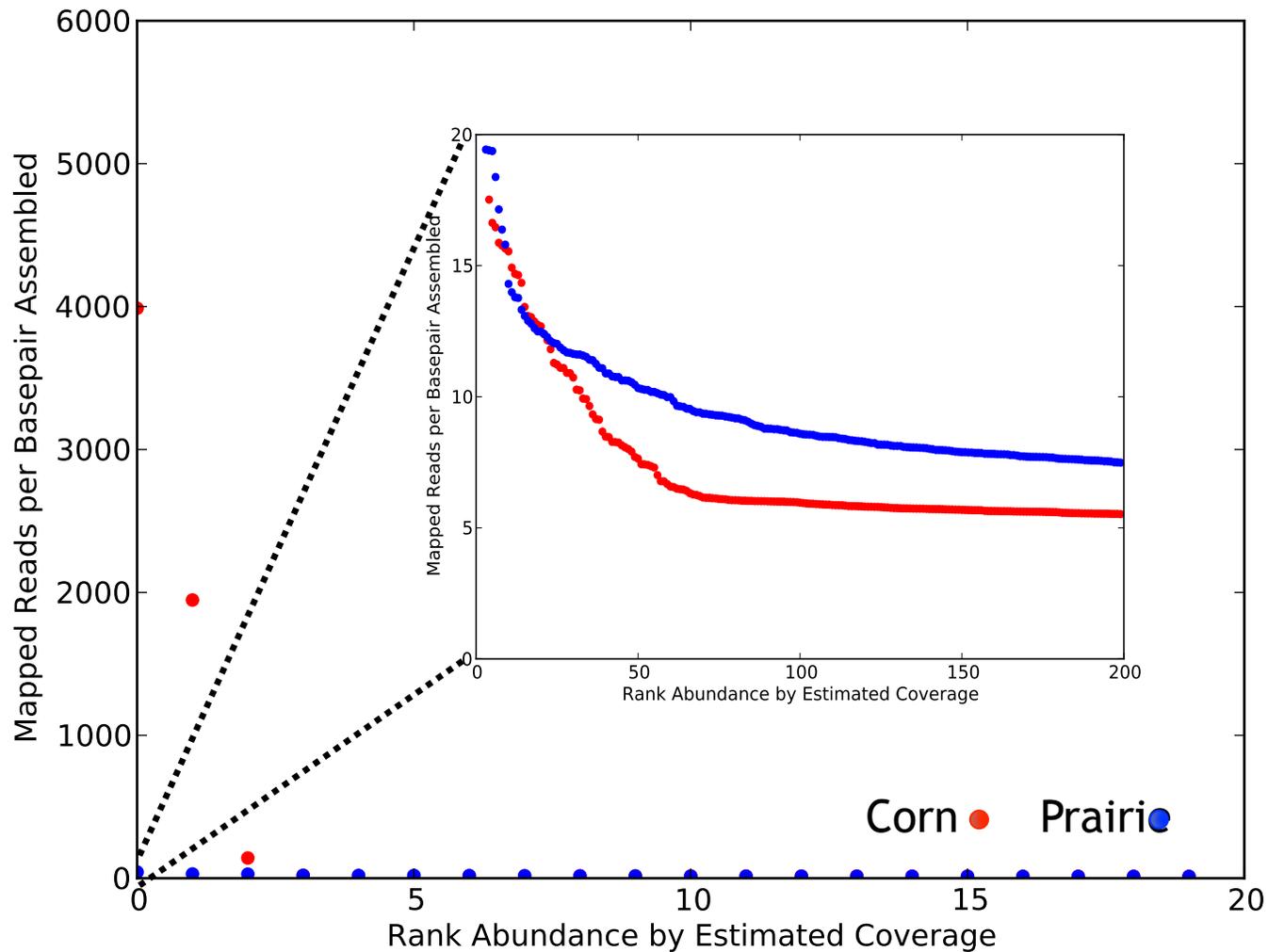
Putting it in perspective:
Total equivalent of ~1200 bacterial genomes
Human genome ~3 billion bp

Adina Howe

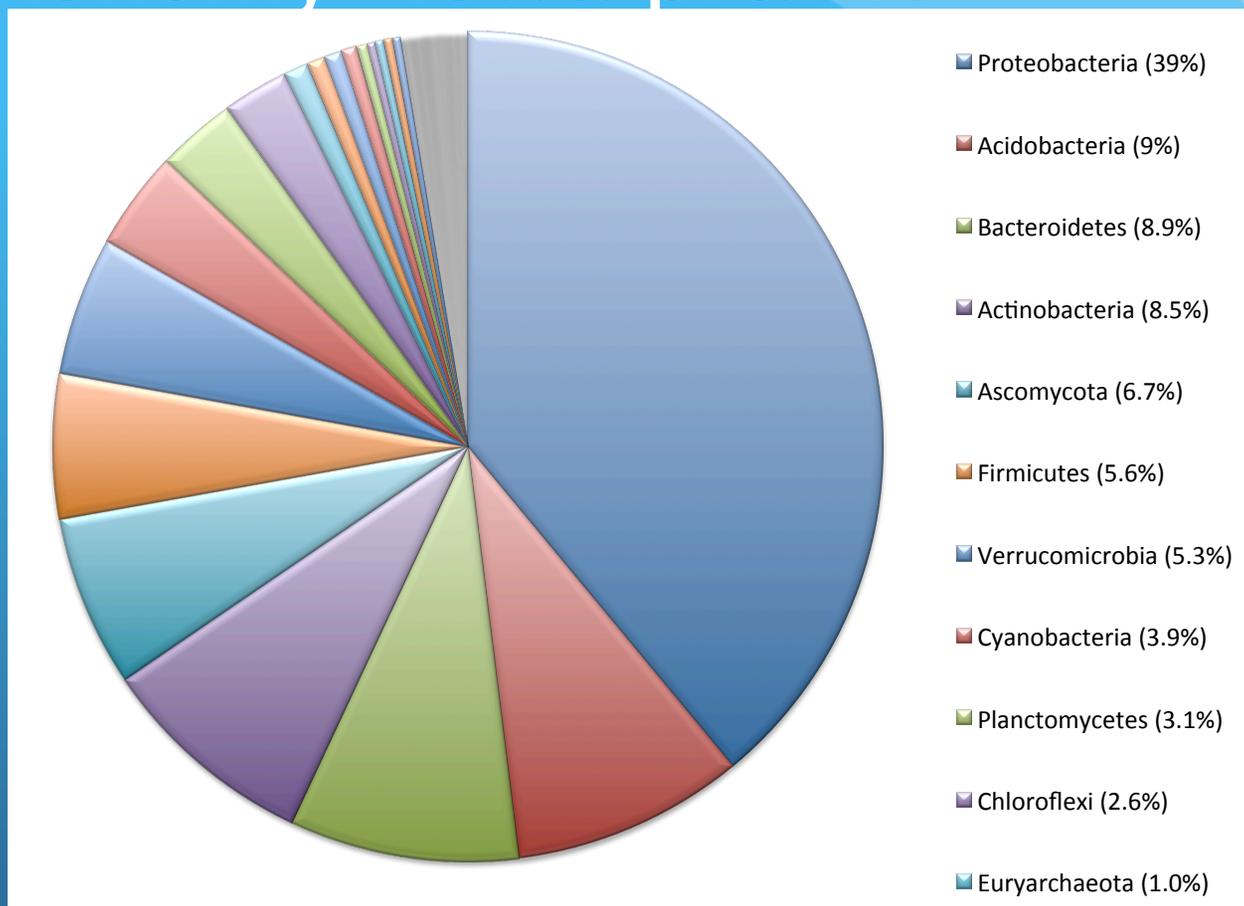
Resulting contigs are low coverage.



Iowa prairie & corn - very even.

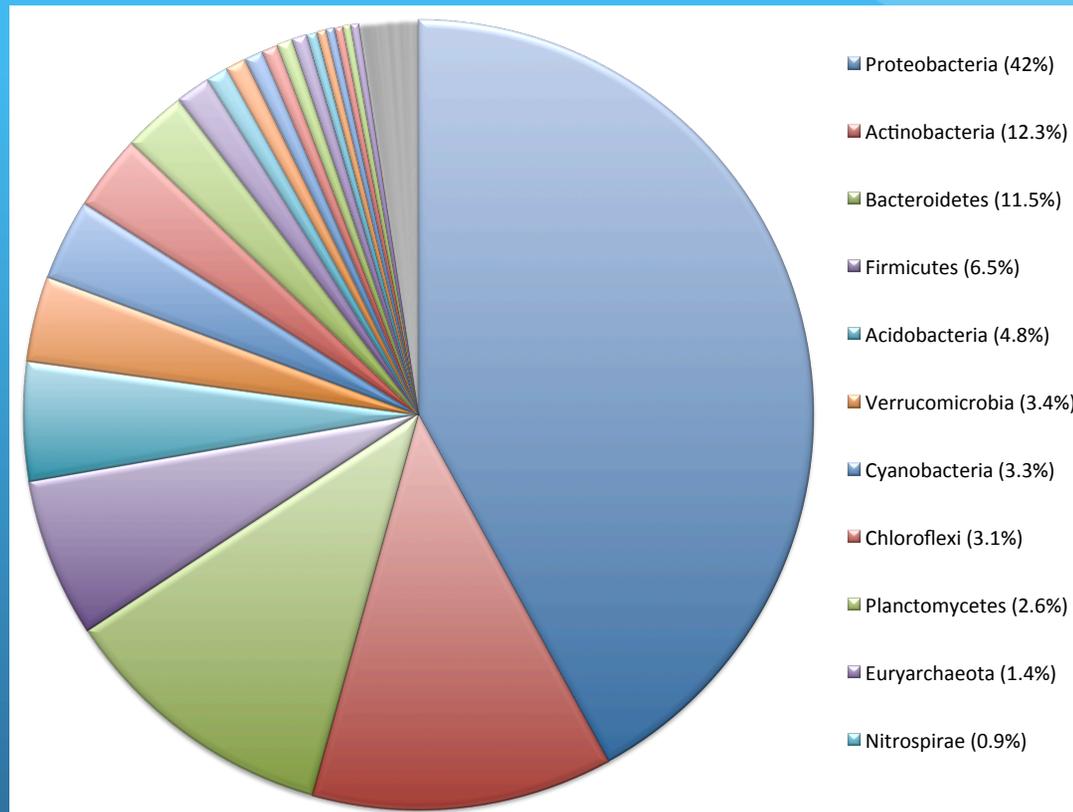


Taxonomy- Iowa prairie



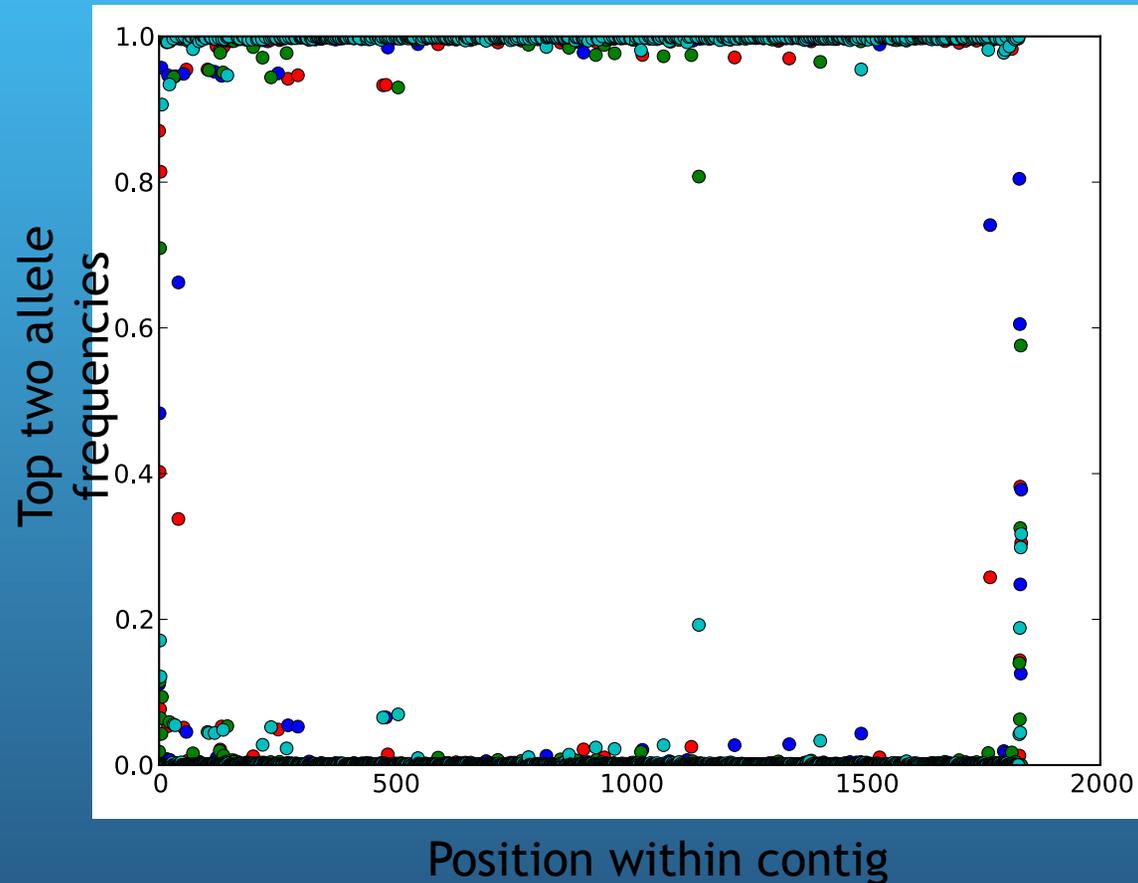
Note: this is predicted taxonomy of contigs w/o considering abundance or length. (MG-RAST)

Taxonomy - Iowa corn



Note: this is predicted taxonomy of contigs w/o considering abundance or length. (MG-RAST)

Strain variation?



Can measure by
read mapping.

Of 5000 most
abundant
contigs, only 1
has a
polymorphism
rate > 5%

Concluding thoughts on assembly (I)

- There's no standard approach yet; almost every paper uses a specialized pipeline of some sort.
 - More like genome assembly
 - But unlike transcriptomics...

Concluding thoughts on assembly (II)

- Anecdotally, everyone worries about strain variation.
 - Some groups (e.g. Banfield, us) have found that this is not a problem in their system so far.
 - Others (viral metagenomes! HMP!) have found this to be a big concern.

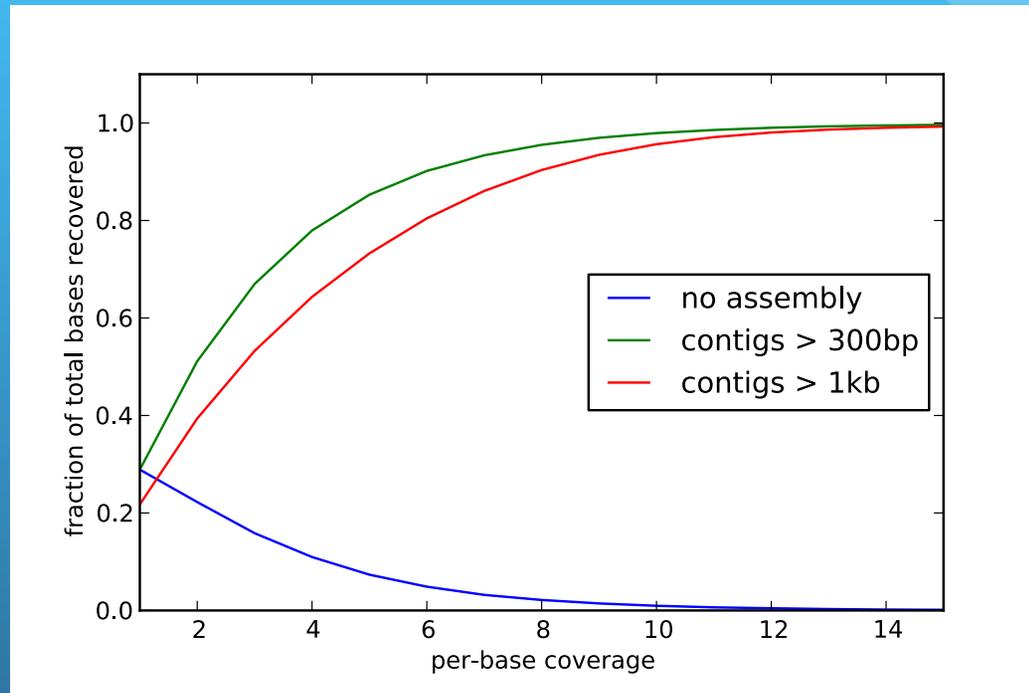
Concluding thoughts on assembly (III)

- Some groups have found metagenome assembly to be very useful.
- Others (us! soil!) have not yet proven its utility.

Questions that will be addressed *tomorrow* morning.

- How much sequencing should I do?
- How do I evaluate metagenome assemblies?
- Which assembler is best?

High coverage is critical.



Low coverage is *the dominant problem* blocking assembly of metagenomes