The Inverted Pyramid:

Guidelines for Strategically Formatting a Scientific Paper

Think of the parts of your paper as an inverted pyramid. The accuracy of your findings and interpretation rest heavily on your data, your code, and your reading of the literature. But that is not the order in which most readers will approach your paper. Instead, many, many people will read your title in online search results, or in citations by other papers. Many fewer will click read the abstract. Fewer still will download and skim the paper. Those who do might read from snout-to-tail, but will more likely skim first, looking at the result section subheadings and at the figures. If these look interesting, casual readers will invest the time to read your paper once. Only an even more interested subgroup will reread several times carefully, delving into your supplemental results, code, and even raw data.

What does this imply? Clearly most *research* time should be spent designing experiments and analyses, acquiring and cleaning data, testing code, preparing raw supplemental data tables of analytical results, etc. All these pieces must be in place before writing a scientific paper can commence in earnest. Once they are, most *writing* and polishing effort should go into final summaries like the title, abstract, figures, figure legends, etc. It makes sense to hone the presentation of the parts of your paper that most readers will encounter. You want to excite interest in the full text that you spent so long preparing, while also accurately representing its contents so that a casual skim will ideally not produce any misapprehensions about your main conclusions. You may also find that trying to generate concise, impactful titles, abstracts, and figures suggests other experiments, controls, or analyses that are needed.

Key points

1. The title of your paper is important (see below). It should attract interest, while also representing the most key aspect of your work. Ideally, you want a single, clear, intriguing conclusion that will draw readers in. If you can't summarize ideas in a single statement about what you found, at least try to make clear your methods. ***NOTE****: if you can't summarize your findings briefly in a single impactful title, do you need to do more analysis so you can?*
2. The abstract should be a short, intriguing, self-contained representation of why someone should your paper. It should have sentences that say: why someone should care about your topic in general (e.g. evolution of echolocation, horizontal gene transfer detection, predicting antigens, etc), how your paper/project fits into that general topic, any gaps in the field that your paper fills (even if it's just exploring a new gene/species/environment/pathway), your methods, your results, and how your results relate back to the overall field. You only have 250-350 words to do all this! Usually you have about 1 sentence background; 1 sentence past work and it's limits ('in bats, , echolocation is dependent on the xyz gene, but the genetic basis for echolocation in whales in unknown', or whatever); 1 sentence starting 'In this paper, we …' that describes both your methods and the main finding. 1-2 other sentences of findings and 1 short conclusion sentence relating out to the broader field.

3. Because many readers read figures first, figure legends are important. You want to spend time on figures and make them as self-contained as possible. ***Each figure should have a legend underneath it, that can be pretty long (****up to maybe 400 words, though exact length varies by journal) .*

Whole document formatting

- **Turn on line numbers and page numbers.** This helps reviewers tell you what line/page each of their (often many) comments applies to. If you don't they'll be vague and on revision you'll have to hunt around or guess what they wanted you to change, which wastes a ton of time.
- **Use standard, consistent fonts and margins**. Most reviewers expect a standard, size 12 font (typically Times New Roman or Calibri)**.**

Sections of a scientific paper

Most journals will ask for the following sections:

Title
Running Title
Authors
Author Affiliations
Abstract
Keywords
Introduction
Methods*
Results†
Discussion†

* High impact journals may ask that methods go after results

† Some journals allow results and discussion to be combined into a single section. This has the advantage of allowing you to interpret each finding and place it in the context of the broader literature as you present it, which can make papers a lot easier to read. The main argument against combined results and discussion sections is that the reader doesn't get a chance to read a 'straight' or 'uninterpreted' version of the results and make up their own interpretation before being influenced by yours.

The First Page: **Title, Running Title, Authors, Author Affiliations, Abstract, Keywords**

A first page describes a bunch of logistical information about the paper, including the title, abstract, authors, author affiliations, and keywords for indexing on the journal's cite. An example (from a perspective piece) will be on the next page. Feel free to copy/modify.

I added in one example title page and abstract for a review paper (since I figured it might come up in your future work or classes) and one for a research paper (relevant for Project 2). I also added some notes on the word count breakdown of the abstract.

Title:
The title of your paper is super-important. It should attract interest, while also representing the most key aspect of your work. A second running title or short title can be used on internal pages of the paper.

Title Templates

Some formats for paper titles that I use are below. The **[brackets]** mean you should fill in something of that type, but reflecting the focus of your paper.

[main biological finding]
Examples:

Coral microbiomes reflect host phylogeny and disease susceptibility
Ribosomal RNA diversity predicts genome diversity in gut bacteria and their relatives
Chronic Nutrient Enrichment Increases Prevalence and Severity of Coral Disease and Bleaching

[method/study system] reveals [neat finding]

Examples:
Comparative metagenomic, phylogenetic, and physiological approaches reveal how long-term nitrogen fertilization impacts soil microbial communities.

Natural volcanic CO2 seeps reveal future trajectories for host-microbial associations in corals and sponges.

A closely related title style is:
[Method] and its implications for **[topic]**
Examples:

Phage-bacteria network analysis and its implications for understanding coral disease.
Note: *can you see how this is a mealy-mouthed version of the above? 'Implications for…', while sometimes useful to collect diffuse results is a lot less clear than a direct statement of what you've shown. Readers will immediately question whether you found anything that you could defend, or if the results were inconclusive due to poor experimental design/analysis etc.*

For software papers usually the algorithm and/or data sources are highlighted
(sometimes the results of application can be too). Some example formats:

[research task] using [datasource]

Examples:
Predictive functional profiling of microbial communities using 16s rRNA marker gene sequences

RNASTAR: an RNA STructural Alignment Repository that provides insight into the evolution of natural and artificial RNAs

[Software package name] allows [capability]
QIIME allows analysis of high-throughput community sequencing data

[cute phrase/joke/question/general topic]: [full scientific explanation of what your paper is about]
Are all horizontal gene transfers created equal? Prospects for mechanism-based studies of HGT patterns. (Review paper)

The Abstract: your paper in miniature

The abstract should be a short, intriguing, self-contained representation of why someone should your paper. It should have sentences that say:

1. why someone should care about your topic in general (e.g. evolution of echolocation, horizontal gene transfer detection, predicting antigens, etc)
2. how your paper/project fits into that general topic
3. any gaps in the field that your paper fills (even if it's just exploring a new gene or whatever)
4. your methods
5. Each of your main, summarized biological results
6. How your results relate back to the overall field.

You only have 250-350 words to do all this! Usually you have about 1 sentence background; 1 sentence past work and it's limits ('in bats, , echolocation depends on the xyz gene, but the genetic basis for echolocation in whales in unknown', or whatever); 1 sentence starting 'In this paper, we …' that describes both your methods and the main finding. 2-3 other sentences of findings and 1 short conclusion sentence relating out to the broader field.

Perspective piece example (Nature Microbiology)

Title:

Stress and Stability: applying the Anna Karenina Principle to animal microbiomes

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Abstract

All animals studied to date are associated with symbiotic communities of microorganisms. These animal microbiotas often play important roles in normal physiological function and susceptibility to disease; predicting their responses to perturbation represents an essential challenge for microbiology. Most studies of microbiome dynamics test for patterns in which perturbation shifts animal microbiomes from a healthy to a dysbiotic stable state. Here we consider a complementary alternative: that the microbiological changes induced by many perturbations are stochastic, and therefore lead to transitions from stable to unstable community states. The result is an 'Anna Karenina Principle' for animal microbiomes, in which dysbiotic individuals vary more in microbial community composition than healthy individuals - paralleling Tolstoy's dictum that 'all happy families look alike; each unhappy family is unhappy in its own way'. We argue that Anna Karenina effects are a common and important response of animal microbiomes to stressors that reduce the ability of the host or its microbiome to regulate community composition. Patterns consistent with Anna Karenina effects have been found in systems ranging from the surface of threatened corals exposed to above-average temperatures to the lungs of patients suffering from HIV/AIDs. However, despite their apparent ubiquity, these patterns are easily missed or discarded by some common workflows, and therefore likely underreported. Now that a substantial body of research has established the existence of these patterns in diverse systems, rigorous testing, intensive time-series datasets, and improved stochastic modeling will help to explore their importance for topics ranging from personalized medicine to theories of the evolution of host-microbe symbioses.

Keywords: alternative stable states, β-diversity, dispersion, instability, 16S, Anna Karenina Principle, HIV, SIV, coral, immunity, microbiome

Research Paper example (Nature Communications)

Title: Overfishing and nutrient pollution interact with temperature to disrupt coral reefs down to microbial scales

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Research Paper example (Nature Communications)

Abstract

Losses of corals worldwide emphasize the need to understand what drives reef decline. Stressors such as overfishing and nutrient pollution may reduce resilience of coral reefs by increasing coral-algal competition and reducing coral recruitment, growth, and survivorship. Such effects may themselves develop via several mechanisms, including disruption of coral microbiomes. Here we report the results of a 3-year field experiment simulating overfishing and nutrient pollution. Both stressors increase turf and macroalgal cover, destabilize microbiomes, elevate putative pathogen loads, increase disease >2-fold, and increase mortality >8-fold. Above-average temperatures exacerbate these effects, further disrupting microbiomes of unhealthy corals and concentrating 80% of coral mortality in the warmest seasons. Surprisingly, nutrients also increase bacterial opportunism and mortality in corals bitten by parrotfish, turning otherwise mild trophic interactions deadly for corals. Thus, overfishing and nutrient pollution impact reefs down to microbial scales, killing corals by sensitizing them to predation, above-average temperatures, and bacterial opportunism.

Notes and Analysis:

We had to use a very short (150 word format for this abstract, which took a ton of revision to get OK. 250 word abstracts are *much* easier and more forgiving. We also couldn't address gaps in literature in this short of a space, as 150 words is wildly short. With more room it would have improved the abstract.

Let's break it down.

Frame General problem: 13 words/ 1 sentence

Losses of corals worldwide emphasize the need to understand what drives reef decline.

Necessary background to understand our research question: 37 words/ 2 sentences

Stressors such as overfishing and nutrient pollution may reduce resilience of coral reefs by increasing coral-algal competition and reducing coral recruitment, growth, and survivorship. Such effects may themselves develop via several mechanisms, including disruption of coral microbiomes.

"Here we report" sentence of what you'll hear about if you read our paper: 1 sentence 15 words

Here we report the results of a 3-year field experiment simulating overfishing and nutrient pollution

Results: 62 words/ 3 sentences / 3 specific "quote at a cocktail party" numbers

Both stressors increase turf and macroalgal cover, destabilize microbiomes, elevate putative pathogen loads, increase disease >2-fold, and increase mortality >8-fold. Above-average temperatures exacerbate these effects, further disrupting microbiomes of unhealthy corals and concentrating 80% of coral mortality in the warmest seasons. Surprisingly, nutrients also increase bacterial opportunism and mortality in corals bitten by parrotfish, turning otherwise mild trophic interactions deadly for corals.

Conclusion 1 sentence / 24 words

Thus, overfishing and nutrient pollution impact reefs down to microbial scales, killing corals by sensitizing them to predation, above-average temperatures, and bacterial opportunism.

Figures:

Here are some general guidelines for figures:

Be sure to include a figure legend. Each figure should have a figure legend (~150-400 words). Often, several panels representing related graphs, illustrations, or even mini-tables are combined into a single figure and labelled as subpanels (usually a-g). The first phrase is typically a very short summary of what the figure is about. There may be some general information first, and then a description of each panel should follow its letter (see examples). Note that some readers will read figure legends before methods/results so it's good to include a little bit of information about motivation, methods, and conclusions – though you won't have room for as full of an explanation as in the text. I usually aim mine to a somewhat more specialist reader who has probably seen many of the methods already, but wants to know what we specifically found.

Each figure must be cited in the text. If the figure has subpanels, and you want the reader to look at a particular one to support your claim, be sure to cite the relevant panel.

Example: "Maximum Likelihood phylogenetic tree inference (**Fig. 1a**) shows that the unidentified microbial eukaryote is most closely related to *Paramecium*. We obtained a similar result using Bayesian Methods (**Supplemental Fig. 17**)"

Mark up your figure with significance values. Overall p values for everything on a chart can be listed in the upper right. P-values for comparisons are typically represented in bar charts, box plots and the like by drawing a line above the compared categories, and writing the p-value on the line. Some folks will use one or more asterisks *** for significance. Each stands for a certain level of significance, which must be spelled out in the legend. Typical values are *, $p < 0.05$, **, $p < 0.01$, *** $p < 0.001$. Not significant results can be abbreviated 'n.s.' This asterisk notation is very clean, and can be useful for complex figures with dozens of comparisons. However, it has a major drawback: it does not report the actual p-values. Often readers will want to know these, as a not significant result at $p=0.06$ is very different from one at $p=0.95$. Similarly, if the reader disagrees with the amount of multiple comparisons correction you applied, they can mentally apply a Bonferroni correction (multiplying p by the number of comparisons, capped at 1.0) if they have a raw p-value, but not with an asterisk. One compromise is to use both the asterisk (for quick visual assessment) and the raw p-value. This could be considered a form of micro/macro view. It can work, but does end up taking up a lot of room, and so isn't suitable for a figure with many comparisons.

Remove any 'overhead' chart titles. While in much graphing software, titles are added on top of your chart, for publication this is handled by your figure legend. Therefore, please remove the chart titles from above your figures.

Include workflow, conceptual, and experimental design figures. Not every figure panel or every full figure must be pure results. Some can be used creatively to frame the problem, and especially to clearly communicate experimental designs or conceptual models. I've included examples of conceptual and workflow figures.

Use relatively large fonts. Figures are shrunk down greatly when embedded in a journal article. You often need to set font sizes to at least 16, and often larger when working on a figure as a full-page document or excel chart in order to have them be very legible when shrunk down for the page. Ideally, if you know the final target size in your journal, you can make a document that size and fit your figure to it. In practice, frequent changes to figures and resubmission to more than one journal can make this challenging and it can be easier to error on the side of legibility.

Most journals expect figure text in Arial or Helvetica. For better or worse, this is the current standard. I recommend picking one and using it consistently.

Remove 'chartjunk'. Try to remove as many borders, lines, markings, shadows, etc that use pixels/ink but don't convey specific information about your data. For a more extended and amusing description please read Edward Tufte's classic book, "The Visual Display of Quantitative Information". Excel is particularly notorious for adding a bunch of garbage to new figures, and failing to remove those extra uninformative graphical elements gives figures an unprofessional look. To fix, be sure to remove chart borders, drop shadows, generic gradients (replace with carefully considered colors), unnecessarily colored backgrounds, etc.

Pick consistent color schemes, and write them down. Say you have four treatments, A, B, C,D. I recommend early in your paper development process picking colors for those four treatments, showing them to readers and your group and getting agreement. You can then make a data file that lists each treatment and the RGB and/or hex value for the color to represent it. (the same strategy works for symbols or line styles) Then whenever anyone in the group makes a figure in their code or analysis, they can refer to that central document to make it consistent in style from the get go. Since you want to settle on colors pretty early, they are worth considering carefully. There are many considerations that go into picking color schemes, but here are a few:

1. **If possible, use shape or line style as well as color to represent categories.** This will make things easier for readers that can't easily distinguish colors and readers that printed your paper out in black-and-white. Note too that while heatmaps can be beautiful and effective, humans are pretty bad at inferring a quantitative value from color information.
2. **Select colors that can be distinguished by readers with the most common types of color blindness.** If understanding a key result depends on distinguishing red and green or blue and green, it will be inaccessible to ~10% of readers.
3. **Pick colors that are evocative of what you want to represent.** This is less important than avoiding hard-to-distinguish colors, but if you are plotting temperature, it will likely confuse many readers if blue is hot. There are often opportunities to get creative with color choices (while being aware of color differentiation) so that your colors are intuitive representations of their categories.
4. **Within a given figure, colors must be distinguishable**. This sounds obvious, but it is actually extremely challenging to distinguish more than ~12 data series by color.

Carefully consider how many categories you need to represent, and consult online resources to find *tested* color schemes that can represent the categories you need while also being distinguishable.

Where relevant, include error bars and say what they represent. Anytime you show a mean quantity, it is often very important to also show some measure of variability in that quantity. Typically, standard deviation (Std. Dev.) is used to show variability of *specific observations* around the population mean. Standard error of the mean (SEM or s.e.m) speaks to variability of the *population means themselves.* It can be obtained by dividing standard deviation by the square root of the number of observations. Multiplying standard error by 1.96 gives a 95% confidence interval for the population mean, which is arguably more useful than the standard error. In phylogenetics, bootstrap and Bayesian posterior probability scores for each tree node play a similar role. The general principle, applicable to any kind of data, is to *quantify uncertainty,* and report how you did so.

Micro/Macro Views: where possible, show raw data, BUT ALSO make the main trend apparent. Ideally, a reader should be able to see both the underlying data, and the main summary or most important trend in that data. Specific examples:

1. **Consider using swarm or violin plots in place of bar charts.** These show the positions of each measured point as well as the means and error bars. This makes it easier to visually assess the influence of outliers, clumps in the data etc.

2. **Use opacity to your advantage!** Viewers first impression will be formed by what parts of a figure have the most *visual weight*. You can use this to enable micro/macro views. For example, if plotting a complex regression or model prediction line, you could represent the regression line in a **bold, heavyweight line** and each data point in 20-50% opacity. Can you see how this lets the reader take in the main trend first without being overwhelmed, and then look in more detail at the underlying data?

Example multipart results figure legend.

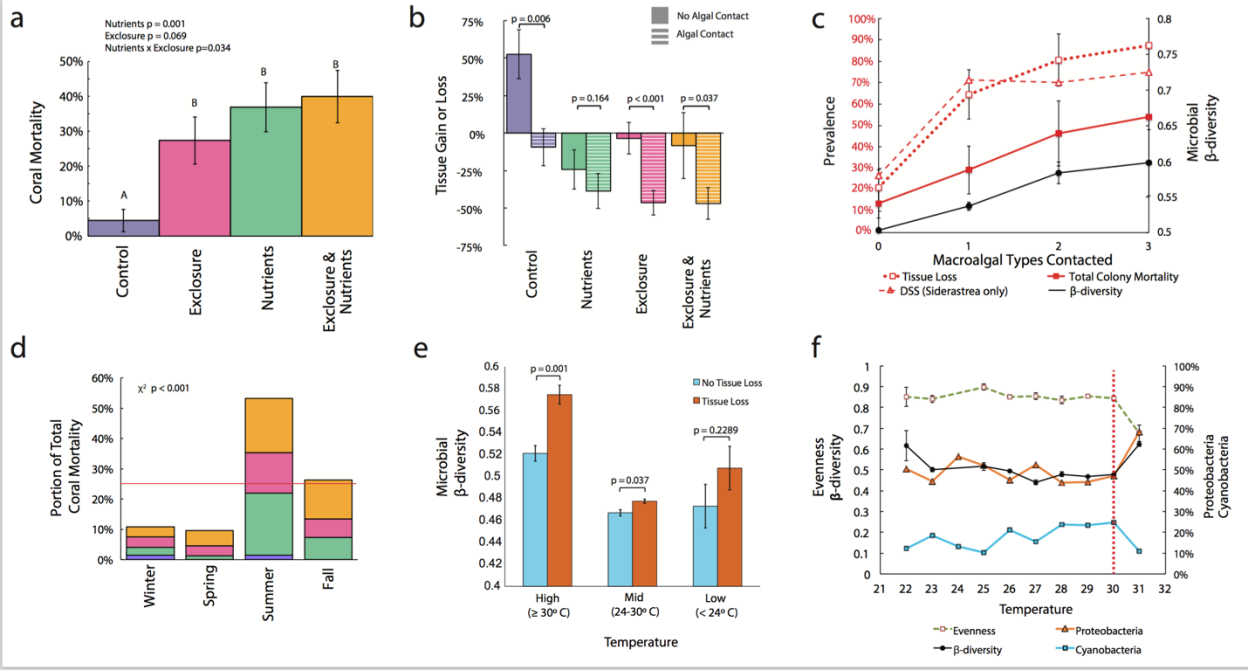

Fig. 3. Multiple stressors disrupt coral microbial communities and produce coral mortality. **a,** Cumulative coral mortality at end of experiment. P-values are from mixed effect models, letters over bars show differences in Tukey's post-hoc tests. Herbivore removal significantly increased coral mortality relative to controls (Tukey's post-hoc test $p < 0.05$), but not relative to nutrient pollution alone (post-hoc test and mixed effects model $p > 0.05$). **b,** Effects of algal contact on coral tissue area, across treatments. P-values from ANOVAs test the effect of algal contact within each treatment. **c,** Number of algal taxa contacting corals *vs.* microbiome β-diversity (Weighted UniFrac distance), and the prevalence of coral tissue loss, mortality, and *Siderastrea* Dark Spot Syndrome (DSS). **d**, Seasonal distribution of coral mortality, colored by treatment (as in panel a). Red line marks null expectation of equal mortality across seasons. P-value is from a $\chi 2$ test. **e,** Microbial community β-diversity for corals with or without tissue loss, split by temperature. P-values reflect non-parametric t-tests of distances. **f,** Temperature effects on coral microbial variability, evenness, and relative abundance of *Proteobacteria* or *Cyanobacteria*. Evenness and β-diversity data are means ± SEM. Microbial and coral health data are averaged within each 1 degree Celsius interval on the x-axis. The vertical red line at 30 ºC indicates the point nearest to the MMM +1 ºC value for our site (30.26 ºC); temperatures beyond this result in accumulation of degree heating weeks of coral thermal stress (see Methods)."

Example of using a figure to illustrate data expected under different hypotheses (from a review).

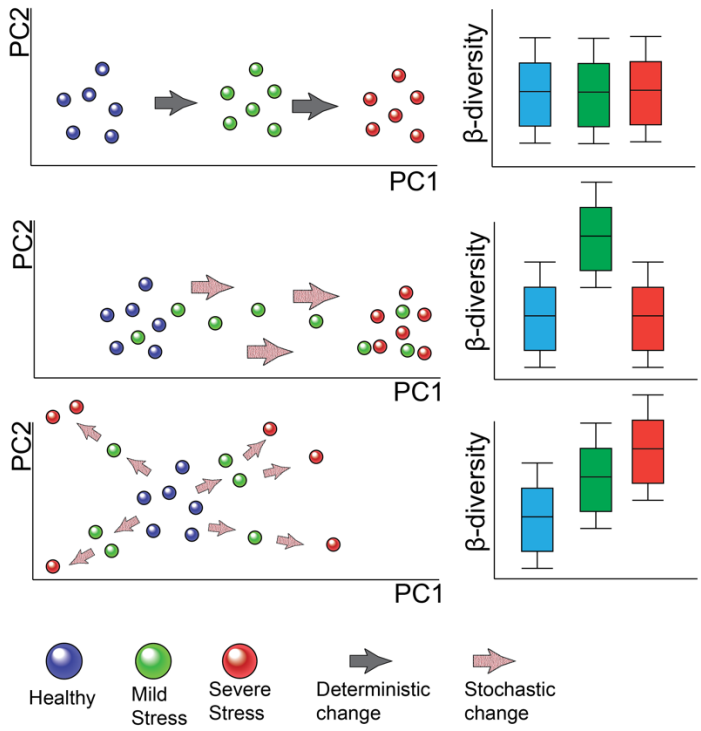

Figure 2. Stochasticity produces contrasting effects of mild and severe perturbations under different models of microbiome dynamics. *Top panel:* The top panel depicts hypothetical ordination results for a perturbation that alters microbiomes by driving them towards a new deterministic configuration (solid gray arrows). For example, an environmental variable might linearly increase the relative abundance of certain taxa. Such deterministic changes produce new clusters of 'stressed' samples (green and red spheres) with similar dispersion as healthy controls. Under this scenario, the within-category β-diversity of healthy, mildly stressed, or severely stressed hosts is identical (top box plot). *Middle panel:* In the middle panel, we consider the case where the way in which a perturbation alters the microbiome is deterministic (i.e. all arrows point right), but the extent of alteration is stochastic based on the severity of the stressor. As above, this produces clusters of healthy (blue spheres) and severely stressed (red spheres) samples. Under mild levels of stress, however, only a subset of samples are shifted to the stressed cluster. This produces elevated β-diversity in samples from moderately stressed hosts, but low β-diversity in both healthy and severely stressed hosts. *Bottom panel:* The bottom panel explores a stressor that alters the microbiome in unpredictable ways. For example, a stressor that suppresses host immunity may allow invasion by myriad opportunists, with the specific outcome determined in part by chance effects like exposure. Under those circumstances, increasing levels of the stressor produce greater dispersion around the healthy centroid. Thus, severe stressed hosts have greater microbiome β-diversity than healthy hosts, while mildly stressed hosts have microbiomes with an intermediate level of dispersion.

Example of a workflow figure

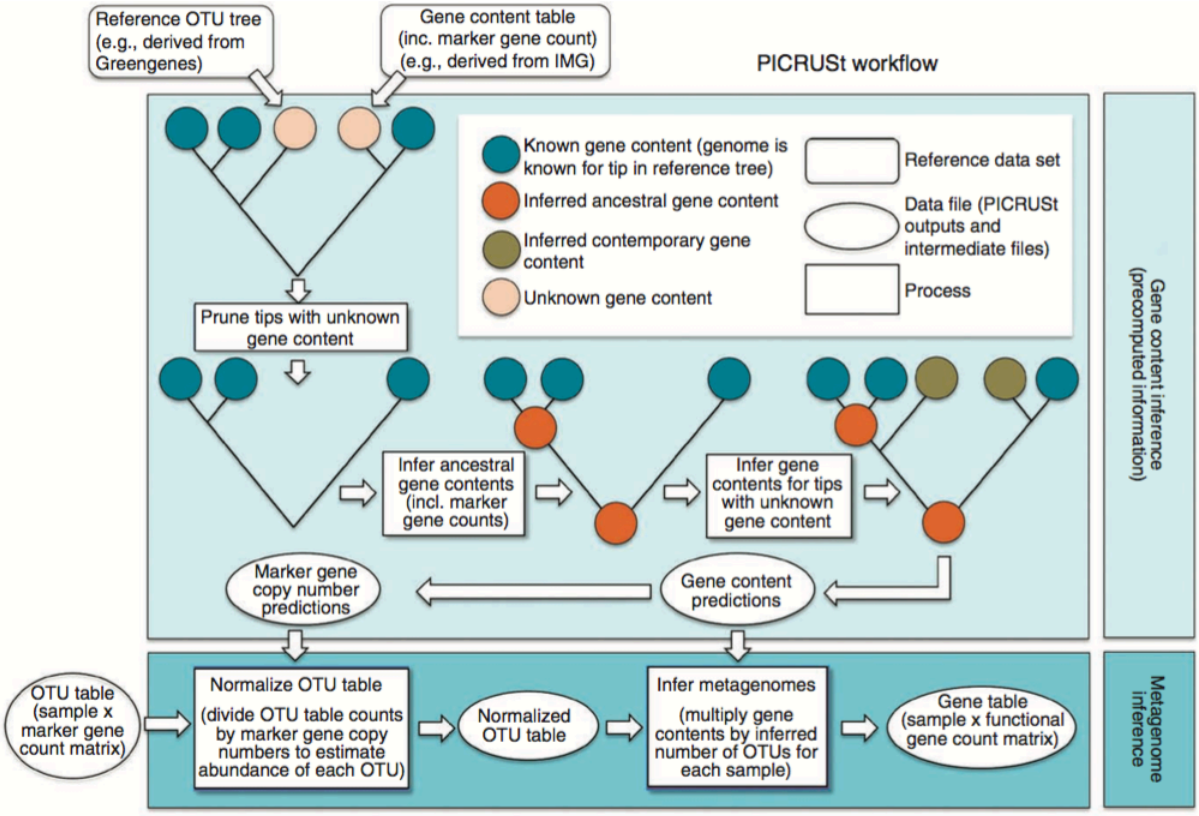

Figure 1 The PICRUSt workflow. PICRUSt is composed of two high-level workflows: gene content inference (top box) and metagenome inference (bottom box). Beginning with a reference OTU tree and a gene content table (i.e., counts of genes for reference OTUs with known gene content), the gene content inference workflow predicts gene content for each OTU with unknown gene content, including predictions of marker gene copy number. This information is precomputed for 16S based on Greengenes[29] and IMG[26], but all functionality is accessible in PICRUSt for use with other marker genes and reference genomes. The metagenome inference workflow takes an OTU table (i.e., counts of OTUs on a per sample basis), where OTU identifiers correspond to tips in the reference OTU tree, as well as the copy number of the marker gene in each OTU and the gene content of each OTU (as generated by the gene content inference workflow), and outputs a metagenome table (i.e., counts of gene families on a per-sample basis).

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Final Items to Cross-check:

1. Are all comments resolved? For example, are any (cite) comments outstanding?
2. All figures, tables, and supplemental datasets *must be referenced at least once in the text*
3. Code should be uploaded to a repository like GitHub
4. Large datasets should (for publication) be uploaded to a resource like Dryad (trees/phylogenetic data), the Sequence Read Archive (SRA), European Bioinformatics Initiative archive (EBI), etc.
5. If possible, also include a copy of raw data with your paper.
6. Are all non-obvious statements of fact cited? What about methods and software tools?