As sequencing capacity increases, microbial community analysis is undergoing a revolution in throughput. Where it was once a monumental task just to analyze microbial communities in a handful of samples, we can now process thousands of samples in a single sequencing run (Caporaso et al., 2010; Gonzalez et al., 2007; Caporaso et al., 2011; DeLong 2009; Gilbert et al., 2010; Gonzalez et al., 2011).

However, the power of these analyses is often limited by our ability to visualize the data. When analyzing biogeographical data, researchers would like to know where specific microbes live, how abundant they are and whether there are any notable patterns relating microbial taxa to each other or to environmental conditions. Traditional ordination methods for visualizing large numbers of microbial communities such as Principal Coordinates Analysis (PCoA) (Gower and Legendre, 1986) are extremely useful for reducing the dimensionality of vast multivariate datasets, but the patterns are often unclear, especially when the results do not map easily onto the sampling structure. For example, it may not be clear that all the points that cluster together at one end of an axis are on the surface of a complex shape without closely examining the sample identifiers (Fig. 1C1).

To address this key barrier to microbial community research, we developed SitePainter that provides compelling visualizations that allow the researcher to better explore spatial patterns in microbial communities. SitePainter allows the user to draw or import representative images for biogeographical sites [in the example, we show a human hand (Fig. 1B–C), although any picture from micro- to landscape-scale is possible] and regions within that site (e.g. a finger, palm, finger tips, etc.). Once the biogeographical image is drawn, the user can import tab-delimited text files, such as those produced by Quantitative Insights Into Microbial Community (QIIME) (Caporaso et al., 2010) to map onto their image. For example, it is often useful to plot the α-diversity (the number of species present in each sample), taxonomy summaries (the abundance of particular kinds of species in the sample) and/or β-diversity (the overall similarities and differences among samples).

SitePainter is a software package with an interactive user interface, implemented with HTML and Javascript, which accepts Scalable Vector Graphics (SVG) images and annotates them with per sample data defined by the user. As an example of usage, we show the biogeography of the human hand and assign bacterial information based on QIIME-formatted files. The main work areas of SitePainter are: (i) file image manipulation that allows the user to load and save SVG images; (ii) the metadata loading and processing menu, where the user can select the metadata (tab-delimited format) to be displayed in combination with the images; (iii) the coloring scheme, where the user can select how to represent low and high values, reset the image color or show/hide the display of lines and absent paths, to show more compelling images; and (iv) the interactive menu that allows the user to work with previous selections to find patterns that may not have been apparent when using other visualization tools and techniques (Fig. 1A).

To illustrate the effectiveness of SitePainter, we show the results of a study in which different regions from a subject’s hand were sampled for microbes. The subject’s hand was outlined, and each area was colored according to the diversity of microbial communities present. SitePainter thus provides a means for researchers to explore the spatial diversity of microbial communities and understand how different regions of the body may support unique microbial communities.
region divided into smaller areas that were then sampled using cotton swabs dipped in saline solution. Using SitePainter, we were able to rapidly deduce which bacteria were in different regions of the hand, along with a graphical representation of microbial abundances by processing the sequence data with QIIME (Caporaso et al., 2010) and mapping the abundance of each taxon onto the hand image (Fig. 1B). Using this display, we can immediately see that Gammaproteobacteria is present in the palm, Bacilli are more visible toward the fingertips and Actinomycetales is present in only one area of the hand. This feature thus allows the user to easily visualize the distribution and abundance of microbes across sites.

Going beyond the abundances of individual microbial taxa, SitePainter also allows the user to view similarities and differences at the whole-community level by loading the PCoA axis of the microbial data to reveal patterns that are not obvious in the PCoA plot but are immediately obvious when displayed in the context of the site itself (Fig. 1C: compare left panel to the right two panels. In this case, we can see a clear gradient from the thumb and index fingers to the left-bottom corner of the hand; additionally we can see that the distal phalanges are similar, maybe due to constant contact).

In addition to analyses of microbial taxa, as shown here, SitePainter can also be used to interpret the abundance of genes or pathways across sites for metabolic studies. Several further examples can be found in the Human Microbiome Project website: http://hmpdacc.org/sp/. These examples analyze the entire Human Microbiome Project (Peterson et al., 2009; Turnbaugh et al., 2007) dataset at several taxonomic levels, and at several different levels of functional classification for shotgun metagenomic reads. We expect that this will be a useful community resource for those trying to interpret the complexities of the human microbiome.

3 CONCLUSIONS
SitePainter provides a user-friendly tool that allows rapid, clear visualization of microbial data on arbitrary user-supplied images. Additionally, it contains several user interface features, such as rapid switching between taxa/coordinates and animations of e.g., diversity overtime. These features facilitate fast exploratory analyses of spatial data. We believe that SitePainter will have a large impact on the field, especially in the case of diseases with complex spatial structure such as posttasis and ulcerative colitis, and also in large-scale environmental sampling projects such as the Earth Microbiome Project (Gilbert et al., 2010).

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