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Deep sequencing of soil transconjugal pools reveals unexpected phylogenetic diversity of bacteria receiving broad host range plasmids

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The extent by which conjugal plasmids encoding antibiotic resistance genes transfer in a microbial community is of acute relevance in this age of massive antibiotic usage. When a plasmid enters a new bacterial community, the plasmid host range is a key parameter that controls the ecology and fate of the plasmid. Its transfer and maintenance in the community is determined by specific traits of the plasmid as well as donor and recipient strains. Evaluation of conjugal plasmid host range has traditionally involved few individual strains as recipients, a situation that contrasts the fact that most bacteria exist within complex communities made of hundreds to thousands of species. Furthermore, even for broad host range plasmids, the diverse strains making up such a community are not equally permissive towards plasmid receipt, limiting the predictability of *in situ* transfer events. Efforts to explore this *in situ* host range of plasmids in complex communities are so far limited to checking a few hundred transconjugants at best, and may not provide a complete image of the diversity of transconjugal pools. A deeper insight into the *in situ* permissiveness is needed to better predict the fate of plasmids introduced to a complex community.

Therefore, we aim to explore the host range of broad host range plasmids introduced in complex communities in an exhaustive manner. By using multiple plasmids and donor strains, we intend to evaluate the importance of the phylogenetic distance between recipient and donor on the occurrence of transfer.

Hence, three mCherry-tagged donor strains (*P. putida*, *E. coli* & *Kluyvera* spp.) carrying one of three gfp-tagged broad host range plasmids (RP4, pKJK5, pIPO2Tet) were mixed with a soil bacterial community in a filter mating assay mimicking natural nutrient conditions, with maximized cell-to-cell contact. Plasmid transfer was observed and quantified by detecting green fluorescent transconjugant microcolonies using confocal laser scanning microscopy. Transconjugants were isolated using fluorescent activated cell sorting with triple gating for bacterial size, gfp-based green fluorescence and exclusion of mCherry-based red fluorescence. Sorted transconjugants were subsequently phylotyped by 16S rRNA gene amplicon pyrosequencing.

Results reveal that a surprisingly diverse fraction of the soil microbial community was permissive towards broad-host-range plasmids, with transconjugants belonging to 11 different phyla distributed over 200 observed taxonomic units. These included diverse gram-positives, such as Firmicutes, and potentially pathogenic strains such as *Staphylococcus*.

We show that plasmid transfer in a complex community is independent of the phylogenetic distance between donor and recipient strain and identified a core super-permissive fraction of strains that are able to take up diverse broad host range plasmids from diverse donor strains at high frequencies. This fraction, which included several rare members of the recipient soil community, might play a previously unrecognized role in the mobilome dynamics of bacterial communities.

Quantifying the roles of immigration and regrowth during secondary succession

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Little work has focused on the mechanisms that contribute to diversity maintenance, community assembly and food-web transitions when microbial ecosystems are subjected to long-term disturbance events (i.e., press-disturbances). Here, the diversity of sewage-derived microbiota is monitored through time as the sole immigration source into a full-scale activated sludge bioreactor undergoing secondary succession. The operational parameter sludge retention time, which selects for microorganisms along a life-history continuum, was chosen to reduce microbial diversity (i.e., disturbance) and simulate secondary succession. We hypothesized that sewage-derived microbiota would be significantly enriched in the system during the maximum disturbance state, when resource availability is greatest and constraints on density-dependent growth are lowest. Next, we quantified which mechanism, immigration (i.e., colonization) or regrowth, played a larger role in shaping community structure by estimating the standardized effect size of each community's mean phylogenetic distance (MPD_{SES}). MPD_{SES} is a measure of phylogenetic relatedness that can be used to investigate contemporary ecological processes that structure community composition.

Sewage-derived and activated sludge samples were collected in parallel over a 313-day time series. DNA was extracted with MoBio PowerBiofilm DNA extraction kit per manufacturer's protocol. Barcoded small sub-unit (SSU) rRNA gene primers 515F-907R were incorporated with adapters for the GSFLX-Titanium 454 DNA sequencing platform. SSU rRNA gene amplicons generated from pyrosequencing were binned by barcode and quality filtered using the 'split_libraries.py' script in QIIME v1.8-dev. Demultiplexed sequences were denoised with Acacia and processed in Mothur (Schloss SOP, version date 15/2/2014) with the Silva seed alignment and Greengenes reference taxonomy (13_5-release).

610,311 high quality reads (singletons removed) were obtained from both sewage-derived and sludge libraries and clustered into OTUs_{0.03} (2606 total: average-neighbor method). The proportion of OTUs shared between sewage-derived communities and bioreactor treatment categories (pre-disturbance, max-disturbance, recovery, and post-disturbance) were 0.24, 0.25, 0.24, and 0.19, respectively. The phylogenetic distance (weighted UniFrac) between sewage-derived communities and bioreactor treatments was lowest during the max-disturbance (0.68 ± 0.02), with pre- and post-disturbance distances of 0.83 ± 0.01 and 0.80 ± 0.01 , respectively. As the disturbance progressed, mean MPD_{SES} values increased significantly: 0.89 ± 0.62 (pre-disturbance) to 2.82 ± 0.57 during the max-disturbance and recovery period then returned to 1.50 ± 0.80 (post-disturbance).

The largest proportion of shared OTUs between sewage-derived and sludge communities were during the max-disturbance. This is explained by increased resource and niche availability during the max-disturbance, as indicated by Food:Microorganism ratio (0.33 kg COD/kg MLVSS) and biomass concentration (0.5 g/L), respectively. Our assumptions of phylogenetic niche conservatism help explain these results because phylogenetic dispersion increases significantly ($MPD_{SES} = 2.81$, $p < 0.005$) during the max disturbance, indicating that co-occurring species are significantly less related than expected (i.e., competitive exclusion). Conversely, pre- and post-disturbance communities are randomly spread across the phylogenetic tree (MPD_{SES} values approaching zero), which indicates randomly assembled communities relative to the three null models tested. We suggest that neutral mechanisms affect community assembly as resources become more limited. As succession progresses, facilitative and competitive interactions give way to neutral assembly. Thus, colonization is

greatest during the max-disturbance while local growth excludes colonizers as succession proceeds towards a climax community.

Continental-scale biogeographical patterns of bacteria and fungi found in the near-surface atmosphere

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Microorganisms enter the atmosphere as aerosol particles and, due to their size, they may remain in the atmosphere for many days until they are removed by precipitation or direct deposition on surfaces. Despite their potential importance to atmospheric processes, human health, and the health of other plants and animals, we know little about the composition, diversity and geographical patterns of the microbial communities living in the near-surface atmosphere. Here, we used door trims as passive aerosol collectors of bacterial and fungal cells across ~700 houses in the continental USA. Fungal communities showed a stronger geographical signal than bacterial communities. For both microbial groups, this geographical signal was more relevant at small scales (i.e. scales <100 km). We further explored which variables might explain the observed geographical patterns, and the spatial distribution of human pathogens and indicator taxa from different environments (i.e., soil, marine, plants, humans). We found that the observed biogeographical patterns were largely predictable by considering the relative importance of different microbial source environments and we present the first maps of microbial diversity across the continental U.S.