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The most efficient microbial community dominates during community coalescence

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ABSTRACT

Immigration has major impacts on both the structure and function of microbial communities(*1*, *2*) and evolutionary dynamics of populations(*3*). While most work on immigration in microbial ecology deals with relatively low numbers and diversity of immigrants, this does not capture the natural context, which frequently involves the coalescence of entire communities(*4*, *5*). The consequences, if any, of such community coalescence are unclear, although existing theoretical(*6–9*) and empirical(*10–13*) studies suggest coalescence can lead to single communities dominating resulting communities. A recent extension(*9*) of classical ecological theory(*14*, *15*) may provide a simple explanation for such dominance: communities that exploit niches more fully and efficiently prevent species from other communities invading. Here, we test this prediction using complex anaerobic microbial communities, for which methane production is a measure of resource use efficiency at the community scale(*16*). We found that the communities, which were most efficient methane producers when grown in isolation, dominated when multiple distinct communities were coalesced. As expected from this dominance effect, the total methane production increased with increased number of distinct communities mixed and was linked with the methane

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production of the most productive community. These results are likely to be relevant to the ecological dynamics of natural microbial communities, as well as demonstrating a simple method to predictably enhance microbial community function in biotechnology, health(*17*) **and agriculture**(*18*)**.**

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Anaerobic digestion is a multi-stage process carried out by highly diverse bacterial and archaeal communities. Methanogenesis is the final stage of the process and results from the conversion of H_2, CO_2 and short chain fatty acids produced by hydrolysis and fermentation of more complex organic material(*16*). It is carried out exclusively by methanogenic Archaea and is the only thermodynamically feasible way of actively removing inhibitory end-metabolites under many conditions where anaerobic digestion occurs(*16*). Methane production can therefore be a useful proxy of the ability of an anaerobic community to fully exploit available resources, and should correlate with community-level productivity. As such, methanogenic communities provide an ideal system to investigate the interplay between community productivity and community coalescence.

An emerging hypothesis(*9*) from classical ecological theory(*14*, *15*) is that community coalescence can result in the mixed system being dominated by the single community that was the most productive prior to coalescence. We tested this hypothesis by determining the methane production and composition of two natural methanogenic communities grown in isolation or as a mixture in laboratory scale Anaerobic Digestors (ADs) over 5 weeks. To remove any confounding effects caused by differences in starting density of tested communities, we standardized microbial density based on qPCR-estimated counts of 16S rDNA copies. We found that the methane production of the mixed community was initially intermediate between the two individual communities, but soon started to produce gas at a rate indistinguishable from the more productive of the individual communities (Figure 1A). The composition of the mixed community was initially a hybrid of the two communities propagated in isolation but at the end of the experiment it most closely resembled the best performing individual community (Figure 1B). These results suggest that the most productive community dominates the mixed community, thus enhancing productivity beyond the average of its individual community components. Note that this conclusion is based on considering community composition based solely on the presence and absence of specific taxa: when the proportional weight of the community members was considered, the composition of the mixture was more intermediate between the endpoint individual communities.

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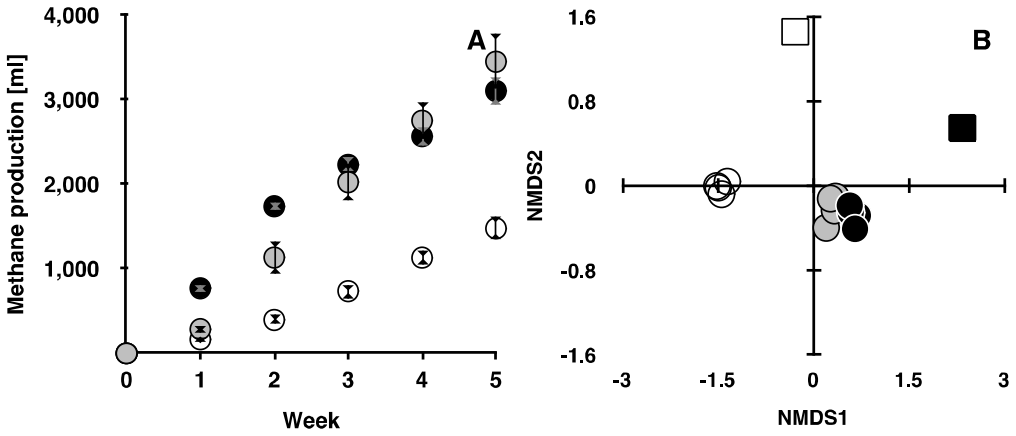

Figure 1: Methane production predicts the composition when two communities coalesce. A) Cumulative methane production in ml (±SEM) over time of: community P01 (white circles), community P05 (black circles) and their mixes (grey circles). Cumulative methane production differed between treatments ($F_{2,9}$ = 23.2, P < 0.001), but did not differ between the mixed community and P05 (Tukey-Kramer HSD: P = 0.5). P01 was lower than both other treatments (P < 0.001 in both cases). B) NMDS plot of unweighted UniFrac of communities P01 (white), P05 (black) and their mixes (grey). Ancestral samples are represented by squares with samples from the endpoint of the experiment by circles. At the endpoint, P05 was compositionally more similar to the mixtures than P01, based on both unweighted (mean distance to each mixture for each replicate single community: t_6 = 8.3, P < 0.001) and weighted (t_6 = 2.3, P = 0.03) UniFrac distances.

To see if the results from coalescing two communities can be generalized, we next investigated the effect of mixing ten distinct communities. Methane production in mixed communities was higher than the average of the individual communities, but did not differ from the best performing single community (Figure 2A). The community composition in ten

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replicated mixtures varied little and most closely resembled the highest performing community (Figure 2B). More generally, the higher the methane production of an individual community, the more similar was its composition to the mixtures (Figure 2C).

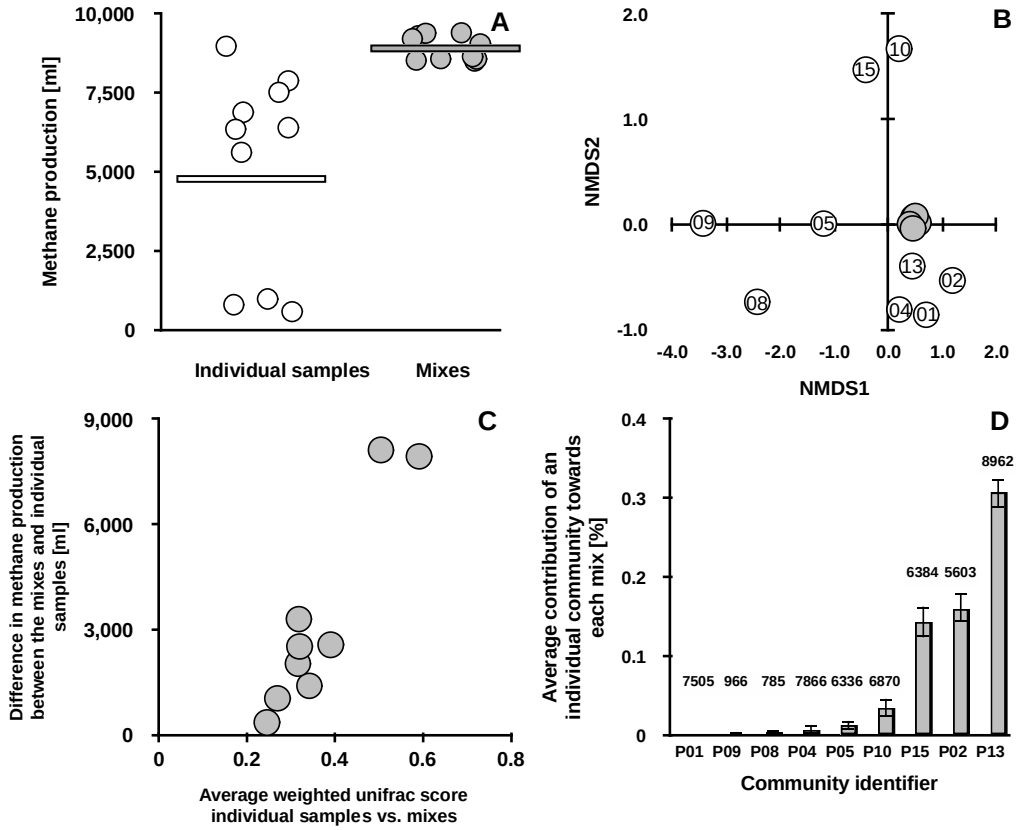

Figure 2: Methane production predicts the greatest contributor to coalesced community composition. A) Cumulated methane production of Mixed (grey) and Individual communities (white). The average performance is shown as a horizontal line. Mean cumulative methane production was greater for mixtures than for most individual communities (*t*-test: $P < 0.001$ in 9 cases), but did not differ from community P13, which was the best performing community. B) NMDS plot of unweighted unifrac of 10 mixed (grey) and 9 individual communities (white). Numbers in circles refer to individual community identifiers (Table 1). Community 13, the highest methane producer shown in A, was significantly closer in composition to the 10 mixed communities than all other communities based on both weighted and unweighted UniFrac distances (Paired *t*-tests; $P < 0.001$, in all cases). Note that we failed to extract the DNA for sequencing from the community P06 (lowest methane producer) so it is not included in this

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and following graphs. C) Relationship between an individual community's similarity to the mixed communities in terms of methane production (here shown as a difference between the average of the mixes and individual sample) and community composition (weighted UniFrac; Spearman $\rho = 0.86$, $P < 0.001$)). Note that same correlation stands for unweighted UniFrac (Spearman $\rho = 0.75$, $P < 0.02$). D) Estimated percentage contribution of each individual community towards the 10 coalesced communities (±SEM). Values over the bars indicate methane production of each individual community over the course of the experiment [ml].

We used a non-negative least squares (NNLS) approach to estimate the actual contribution of each community to the mixtures, rather than just the degree of similarity between mixtures and communities. This confirmed that the most productive community contributed the most to the composition of the mixture (Figure 2D). However, other productive communities (notably communities P01 and P04) contributed very little to the final composition. One possible explanation for this is that these communities were very similar in composition to the most productive community and hence could not occupy the same ecological niches in the mixed community (Figure 2D). In summary, even when mixing multiple communities together, the community that is the most productive in isolation makes the greatest contribution to both the composition and community level phenotype (i.e., methane production) of the mixture.

There are two non-mutually exclusive explanations for the link between methane production and contribution to coalesced communities. First, competitive interactions could override any other ecological drivers and individual species in more productive communities are on average more competitive and more productive than their ecological equivalents in other communities(*8*). Second, community level properties, such as the efficiency of niche packing(*14*), mutualistic interactions(*19*, *20*) and interactions with consumers, such as viruses(*21*) determine a community's contribution in a mixture and best performing communities are simply dominating on the basis of these community level properties. Note that the importance of community-level characteristics does not invoke any "higher order selection" resulting in community-level adaptation(*22*), but simply that communities whose members have evolved to exploit ecological niches more completely or more efficiently are

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both more productive and can be less readily invaded(*9*, *23*, *24*). Quantifying the respective roles of these mechanisms would require testing competitiveness of all individual community members, which is unfeasible in complex anaerobic communities such as these. However, both community- and individual species-level properties are likely to be important. First, mutualistic interactions are commonplace in methanogenic communities(*15*, *19*, *20*), and hence the fitness of one community member is intimately linked to that of co-occurring mutualistic partners. Second, both within-community diversity and microbial biomass positively correlated with methane production – a finding consistent with greater niche packing(*14*) (Figure 3). Third, the density of the organisms directly responsible for methane production, the methanogenic Archaea, did not correlate with methane production (Figure 3A), further emphasizing the importance of interactions between taxa in explaining our results.

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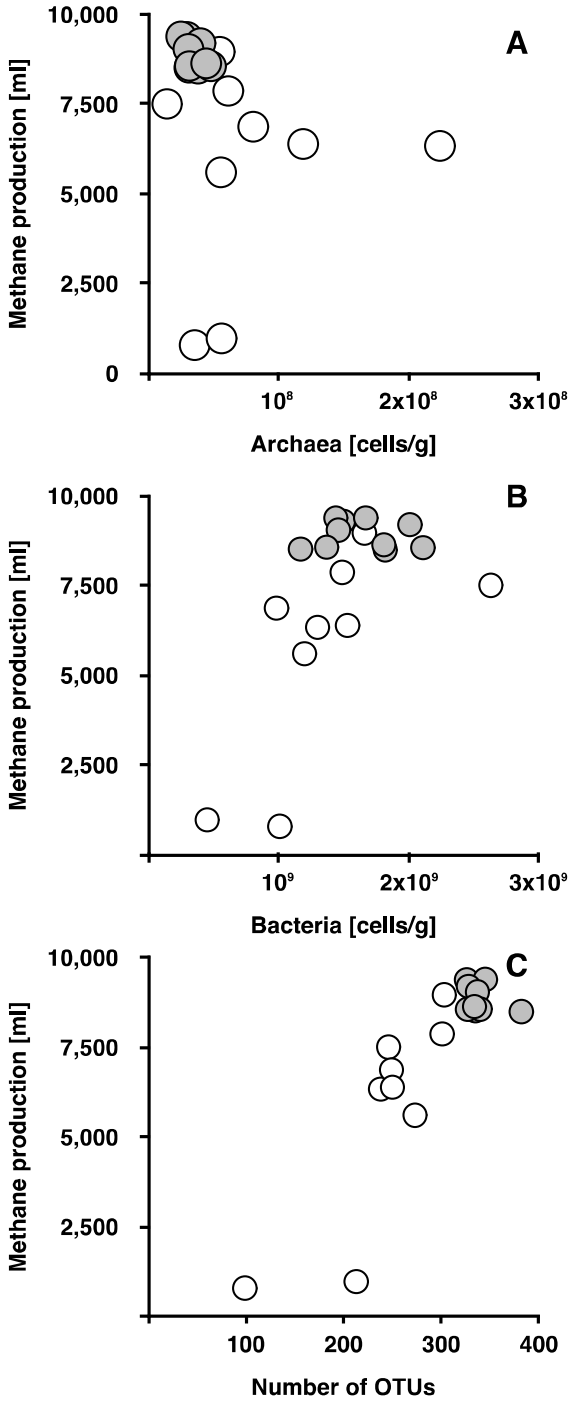

Figure 3: Within-community predictors of methane production. Relationships between A) Archaeal densities [cells/g] ($F_{1,15} = 0.32$, $P > 0.2$) B) Bacterial densities [cells/g] ($F_{1,16} = 16.5$, $P < 0.001$) and C) number of OTUs ($F_{1,16} = 51.6$, $P < 0.001$)

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and methane production [ml]. Note that qualitatively the same results apply when mixed communities are excluded from analyses.

The finding that coalescence can result in the most productive individual community to dominate the resulting mixed community has direct implications for biotechnological uses of microbial communities. Given that the best performing community in isolation largely determined both the composition and performance of mixtures of communities, we hypothesized that methane production in such biotechnological applications would increase with increasing number of communities in a mixture. We therefore inoculated laboratory-scale anaerobic digesters with 1, 2, 3, 4, 6 or 12 communities, ensuing that each of the 12 starting communities was only used once at each diversity level (see Extended Data Table 1). We found that cumulative methane production over a five-week period increased with increasing number of communities used as an inoculum (Figure 4). The positive correlation between community function and the number of inoculating communities is analogous to the commonly observed finding that community productivity increases with increasing species or genotype diversity in plant and microbial communities(*24–26*).

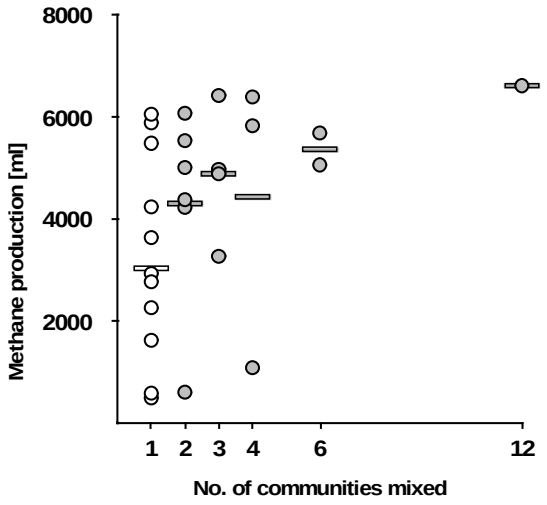

Figure 4: Cumulative methane production over time increases with number of inoculated communities. No single community was represented more than once at each diversity level, and there was a monotonic increase in methane production with number of communities used

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($F_{1,26}$ = 5.4, P = 0.03). Individual communities (white circles) and their average methane production (white line) are compared with mixes of communities (grey circles) and their averages (grey line) at different numbers of communities used.

Here, we have shown that coalescence of microbial communities results in domination by a single community, and that the winning community can be predicted from its original productivity. These results have important implications for understanding the dynamics of microbial communities in biotechnological applications, in which communities are frequently mixed together, as well as microbiome-associated human health(*17*) and agriculture(*18*) related processes. Moreover, we have identified a way to significantly improve methane yield during anaerobic digestion, which is different from standard practice: inoculate digesters with a broad range of microbial communities. This is likely to ensure that the mixed inoculum contains a community reasonably closely adapted to the specific reactor conditions. This community will subsequently come to dominate and result and enhance methane production. This approach could be applied to a range of biotechnological processes driven by microbial communities, as well as to manipulate microbiomes in clinical(*17*) and agricultural(*18*) contexts.

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Author Contributions

AB, JH, KM, FB, OSS and PS conceived the study; FB and PS conducted the experiments PS, AB, MA, SB, DS analysed the data AB and PS wrote the manuscript and all authors contributed revisions.