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Transient dynamics of competitive exclusion in microbial communities

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Summary

Microbial metabolism drives our planet's biogeochemistry and plays a central role in industrial processes. Molecular profiling in bioreactors has revealed that microbial community composition can be highly variable while maintaining constant functional performance. Furthermore, following perturbation bioreactor performance typically recovers rapidly, while community composition slowly returns to its original state. Despite its practical relevance, we still lack an understanding of the mechanisms causing the discrepancy between functional and compositional stability of microbial communities. Using a mathematical model for microbial competition, as well as simulations of a model for a nitrifying bioreactor, we explain these observations on grounds of slow non-equilibrium dynamics eventually leading to competitive exclusion. In the presence of several competing strains, metabolic niches are rapidly occupied by opportunistic populations, while subsequent species turnover and the eventual dominance of top competitors proceeds at a much slower rate. Hence, functional redundancy causes a separation of the time scales characterizing the functional and compositional stabilization of microbial communities. This effect becomes stronger with increasing richness because greater similarities between top competitors lead to longer transient population dynamics.

Introduction

Microbial metabolism drives the biochemistry of virtually all ecosystems and plays a central role in industrial processes such as biofuel production and wastewater treatment (Falkowski *et al*., 2008; Antolli and Liu, 2012). Thus, understanding the mechanisms that shape the dynamics and metabolic performance of microbial communities is of great practical importance. Experiments with bioreactors have shown that bioreactor performance can be constant despite highly variable microbial communities (Wang *et al*., 2011). For example, following functional stabilization, methanogenic or nitrifying bioreactors can exhibit species turnover for several more years (Fernández *et al*., 1999; Wittebolle *et al*., 2008). In some cases non-convergent community trajectories have been reproduced across replicated experiments, suggesting that the underlying processes are deterministic (Ayarza *et al*., 2010; Vanwonterghem *et al*., 2014). Even when communities converge to a steady composition, recovery of community composition following perturbation can take several months. This is in contrast to metabolic throughput, which recovers within a few days (Sundh *et al*., 2003; Gentile *et al*., 2006). Fluctuations and the rate of stabilization of microbial communities are thus multifaceted properties that depend greatly on whether the focus is on metabolic function or taxonomic composition. An improved understanding of these properties in microbial communities is crucial for optimizing microbially driven industrial processes and interpreting the response of ecosystems to anthropogenic perturbations.

It has been hypothesized that functional redundancy and non-equilibrium population dynamics within each metabolic compartment could promote fast stabilization of performance with slow convergence of community composition (Briones and Raskin, 2003). Here we show that temporary population dynamics leading to an eventually steady community composition via competitive exclusion can indeed last much longer than the time required for the stabilization of overall metabolic performance. We first formalize our reasoning using a microbial community model, in which multiple strains compete for a common resource. The model illustrates in a simple way how taxonomic community composition can vary almost independently of the community's metabolic performance. We then construct a more realistic model of a nitrifying bioreactor and use simulations to demonstrate the validity of our arguments and their consistency with previous experimental observations.

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Results and discussion

Competition for a common resource

Microbial community richness can be disproportionally high compared with the metabolic complexity of bioreactors, whereas the coexistence of metabolically similar organisms has been contrasted to the competitive exclusion principle (Hardin, 1960; Fernández *et al*., 1999; Fernandez *et al*., 2000; Wittebolle *et al*., 2009). In the case of a single limiting resource, Tilman's (1982) competition theory predicts that at equilibrium the only persisting competitor will be the one that can survive at the lowest resource concentration. However ecosystems can be subject to long transient dynamics, i.e. temporary population dynamics far from equilibrium, and convergence to equilibrium might occur at much longer time scales than assumed (Hastings, 2004). For example, slow species turnover has been suggested to be responsible for the perplexingly high diversity seen in many microbial systems (Chesson, 2000) and, as we show here, can explain the discrepancy between functional and taxonomic stability in bioreactors.

To formalize our argument, we use a model for multiple populations competing for the same limiting resource. We focus on the transient dynamics eventually leading to steady state, where resource input is balanced by microbial consumption. The cell density of strain *i*, denoted by N_i, as well as the resource concentration, denoted by R, are described by the following differential equations:

$$\frac{dN_i}{dt} = N_i\left[\beta_i \Phi_i(R) - \lambda_i\right], \tag{1}$$

$$\frac{dR}{dt} = f_o - \sum_i N_i \Phi_i(R). \tag{2}$$

Here, f_o is the constant resource supply rate, λ_i is the decay rate of strain *i* in the absence of growth, $\Phi_i(R)$ is the rate at which cells take up the resource as a function of R, and β_i is a biomass yield factor. We assume that $\Phi_i(R)$ increases with R. For example, $\Phi_i(R)$ could be a Monod function that increases linearly with R at low concentrations but saturates at high concentrations, an assumption often made in geobiological and bioengineering models (Jin *et al*., 2013). The last term in Eq. 2 is a sum over all strains accounting for resource depletion by microbial metabolism.

The growth rate of strain *i* is positive whenever R is greater than the threshold concentration, R_i^o, defined by $\Phi_i(R_i^o) = \lambda_i/\beta_i$. In general, the equilibrium of Eqs. 1 and 2 is characterized by the extinction of all but one strain, namely the strain with the lowest survival threshold R_i^o. To elucidate the transient dynamics preceding this competitive exclusion, we consider the total cell density $N = \sum_i N_i$ and the relative cell densities $\eta_i = N_i/N$. Using the community-average growth kinetics (denoted $\overline{\Phi}$, $\overline{\beta\Phi}$ and $\overline{\lambda}$), one can derive the dynamics

$$\frac{d\eta_i}{dt} = \varepsilon_i \cdot \eta_i\left(\overline{\beta\Phi} - \overline{\lambda}\right) \tag{3}$$

for the relative cell densities,

$$\frac{dN}{dt} = N\left(\overline{\beta\Phi} - \overline{\lambda}\right) \tag{4}$$

for the total cell density N, and

$$\frac{dR}{dt} = f_o - N\overline{\Phi} \tag{5}$$

for the resource concentration R (details found in Supporting Information). Here, the ε_i account for deviations of strain *i* growth kinetics from the community average. For example, if N is growing and ε_i is positive, then strain *i* grows faster than average and thus increases in relative abundance.

As the resource is depleted, weaker competitors decay and the average growth kinetics are determined by a few remaining competitors of similar efficiency, for which the deviations ε_i from the average become very small ($\varepsilon_i << 1$). Hence, while the dynamics of N and R are determined by the community-average growth kinetics (Eqs. 4 and 5), the relative cell densities are slowed down by the factors ε_i. This means that while metabolic niches are quickly filled, establishing a high rate of resource uptake, some of the competing populations can coexist during prolonged transition periods until eventual competitive exclusion. In agreement with these predictions, Gentile and colleagues (2006) report a quick functional stabilization and long transient periods in community composition following mechanical shock, and Vanwonterghem and colleagues (2014) report a gradually decreasing richness in anaerobic digesters over the course of several months following inoculation.

The probability of similar strains being present in a random inoculum, or a microbial community in general, increases with the number of strains. In particular, the expected dissimilarity between top competitors decreases with increasing community richness. The underlying assumption is that growth kinetic parameters are bound within some natural finite range. Hence, one should expect longer transient dynamics of competitive exclusion and slower convergence to a steady community composition at higher inoculum richness.

It has been previously hypothesized that as richness increases, the variability of ecosystem functions decreases, whereas the variability of individual populations increases (Tilman, 1996; Lehman and Tilman, 2000; Loreau *et al*., 2001). The proposed mechanisms typically involve stochastic fluctuations of independent populations, so that the total community biomass and functional

performance become more stable when more populations contribute to them. This statistical inevitability (Doak *et al*., 1998), which has been criticized on grounds of interspecific interactions (Tilman *et al*., 1998), differs fundamentally from the deterministic mechanisms explored here. Namely, competition between strains leads to a slow decay of weaker competitors, which is compensated by the growth of other populations that stabilize overall functional performance.

Bioreactors as model systems

The above competition model explains how populations occupying a common metabolic niche can, in principle, undergo long transient periods of coexistence. The actual duration and nature of these transients depend on the similarity between competing strains, as well as their typical intrinsic growth kinetics. To test the relevance of our predictions to realistic microbial communities, we examined a separate numerical model for a nitrifying bioreactor (Wittebolle *et al*., 2008). Apart from their practical relevance to industrial processes such as sewage treatment and biofuel production (Antolli and Liu, 2012), bioreactors are also ideal model systems for understanding microbial ecology and processes shaping microbial community structure (Fernandez *et al*., 2000; Graham *et al*., 2007; Vanwonterghem *et al*., 2014). The bioreactor considered here is a flow-through chemostat continuously fed with ammonium (NH_4^+), which is aerobically oxidized to nitrate (NO_3^-) in a two-step process. Oxidation occurs in a microbial community that consists of chemoautotrophic ammonium-oxidizing bacteria (AOB), which transform ammonium to nitrite (NO_2^-), and chemoautotrophic nitrite-oxidizing bacteria (NOB), which transform nitrite to nitrate. Nitrate is exported from the bioreactor as part of a continuous outflow through a filter membrane that retains cells within the bioreactor. The substrate feed rate and the hydraulic dilution rate are kept constant and in line with previous bioreactor experiments (Wittebolle *et al*., 2008), allowing the establishment of a steady metabolic throughput following an initial start-up period.

The bioreactor's microbial community is modelled using differential equations for the cell population densities and the ambient ammonium, nitrite and nitrate concentrations. These metabolites are subject to microbial production and depletion, as well as physical addition and removal from the bioreactor. The metabolic activity of individual cells is determined using flux balance analysis (FBA), a widely used framework in cell-metabolic modelling (Orth *et al*., 2010). In FBA, the chemical state of cells is assumed to be steady, leading to stoichiometric constraints that need to be satisfied for any particular combination of intracellular reaction rates. These rates are assumed to be regulated by the cell in such a way that some objective function, commonly associated with biomass production, is maximized subject to additional constraints on substrate uptake rates (Feist and Palsson, 2010). In our case, the optimized biosynthesis rate is translated to a growth rate by dividing by the cell mass. Ammonium and nitrite uptake rates are limited by substrate concentrations in a Monod-like fashion, thus constraining the achievable growth rates depending on the bioreactor's chemical state (Mahadevan *et al*., 2002; Harcombe *et al*., 2014).

The assumption of cells maximizing biosynthesis, subject to environmental and physiological constraints, is rooted in the idea that evolution has shaped regulatory mechanisms to induce maximum growth whenever possible (Burgard and Maranas, 2003; Harcombe *et al*., 2013). This assumption is less valid for genetically engineered organisms or those exposed to environments that are radically different from the environments that shaped their evolution, and other objectives such as ATP production or metabolic efficiency have been proposed (Segrè *et al*., 2002; Gianchandani *et al*., 2008). Biosynthesis has been experimentally verified as an objective for, among others, *Saccharomyces cerevisiae*, *Escherichia coli* and *Nitrosomonas europaea* (Duarte *et al*., 2004; Feist and Palsson, 2010; Perez-Garcia *et al*., 2014). Despite its limitations, FBA with maximization of growth has greatly contributed to the understanding of several single-cell metabolic networks as well as metabolic interactions between cells (Klitgord and Segrè, 2010; Orth *et al*., 2010; Freilich *et al*., 2011; Chiu *et al*., 2014). One advantage of FBA models over full biochemical cell models is their independence of intracellular kinetics and gene regulation, which limits the number of required parameters to stoichiometric coefficients and uptake kinetics. Recent work has shown that FBA-based models with maximization of growth can accurately predict microbial community dynamics (Meadows *et al*., 2010; Chiu *et al*., 2014; Harcombe *et al*., 2014; Louca and Doebeli, 2015).

Our bioreactor model comprises multiple AOB and NOB strains, which are constructed by randomly choosing several cell parameters around those of two template AOB and NOB models. The AOB and NOB templates were calibrated and validated beforehand using data from previous bioreactor experiments (Fig. 1; see Experimental Procedures for details). Because metabolites can be depleted or produced by several cells, the environmental metabolite pool mediates the metabolic interactions between cells (Schink and Stams, 2006). For example, AOB deplete ammonium from their environment, rendering it a limiting resource that mediates competition between AOB strains. The excretion of nitrite as a by-product, in turn, enables the growth of nitrite-limited NOB. The metabolic optimization of individual cells striving for maximal growth, while modifying their environment,

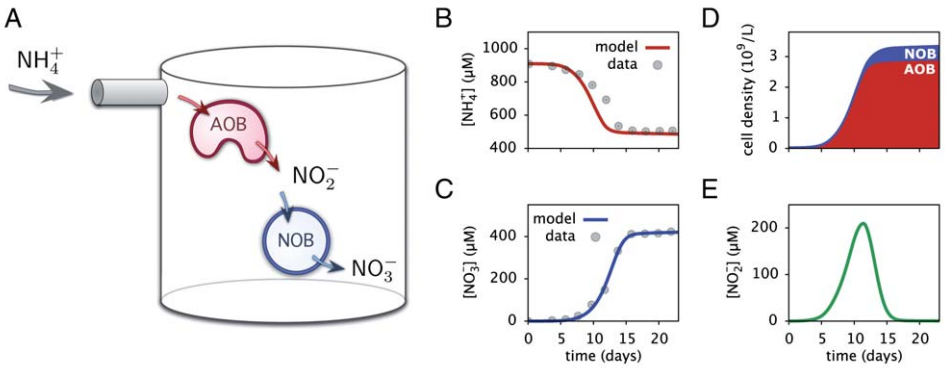

Fig. 1. Calibration and validation of the template AOB and NOB cell models using data from experiments with a nitrifying batch-fed bioreactor (A). Ammonium (NH_4^+) was added at the beginning of the experiment, and was sequentially oxidized to nitrite (NO_2^-) and nitrate (NO_3^-) by a growing nitrifier community. (B) and (C): Ammonium (B) and nitrate (C) concentration time series data (dots), compared with the calibrated model (continuous lines). (D): AOB and NOB cell densities over time, as predicted by the model. (E): Nitrite concentration over time, as predicted by the model. Experimental data from de Boer and Laanbroek (1989). Note that while the template cell models were calibrated using batch-bioreactor experiments, for our subsequent analysis we consider continuously fed flow-through bioreactors because these can support metabolically active microbial communities at steady state.

thus leads to non-trivial community dynamics that can include cooperation, competition and extinction.

Bioreactor community dynamics

Following inoculation of the bioreactor, two phases can generally be distinguished (Fig. 2): Initially, the concentration of inflowing ammonium increases until AOB populations have grown to sufficient densities to balance ammonium supply by ammonium consumption. The accumulation of nitrite as an AOB waste product, in turn, triggers the growth of NOB populations until nitrite production is eventually balanced by nitrite consumption. This initial startup phase is dominated by fast-growing opportunists

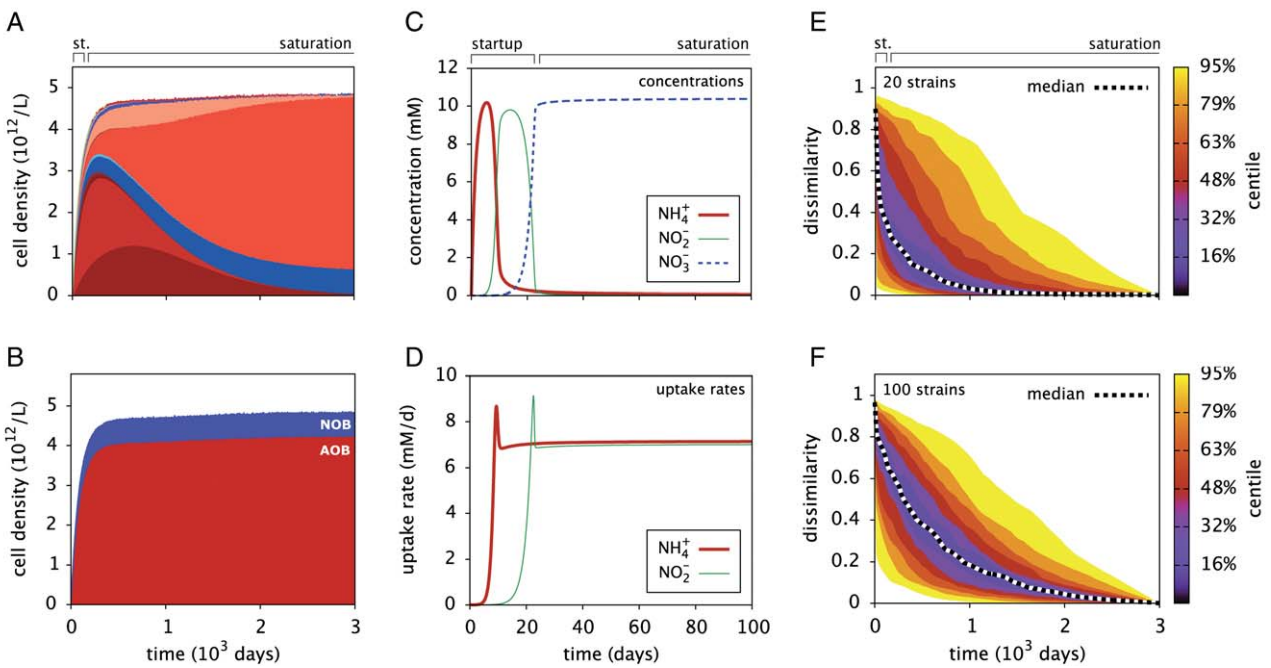

Fig. 2. (A) Simulated cell densities over time in the ammonium-fed nitrifying membrane bioreactor, inoculated with 100 random strains (AOB in variations of red, NOB in variations of blue). (B) Corresponding total cell densities per functional group (AOB or NOB). (C) Corresponding ammonium (NH_4^+), nitrite (NO_2^-) and nitrate (NO_3^-) concentrations. (D) Corresponding community-wide ammonium and nitrite uptake rates. (E, F): Bray–Curtis dissimilarity of the community to the long-term steady state, following inoculation with 20 (E) or 100 (F) random strains. Shown as a probability distribution over 100 random simulations (colours correspond to centiles). Notice the faster rate of convergence to steady state (i.e. resilience) in (E) compared to (F). The two intervals on the top of figures (A, C, E) indicate rough start-up and saturation phases respectively.

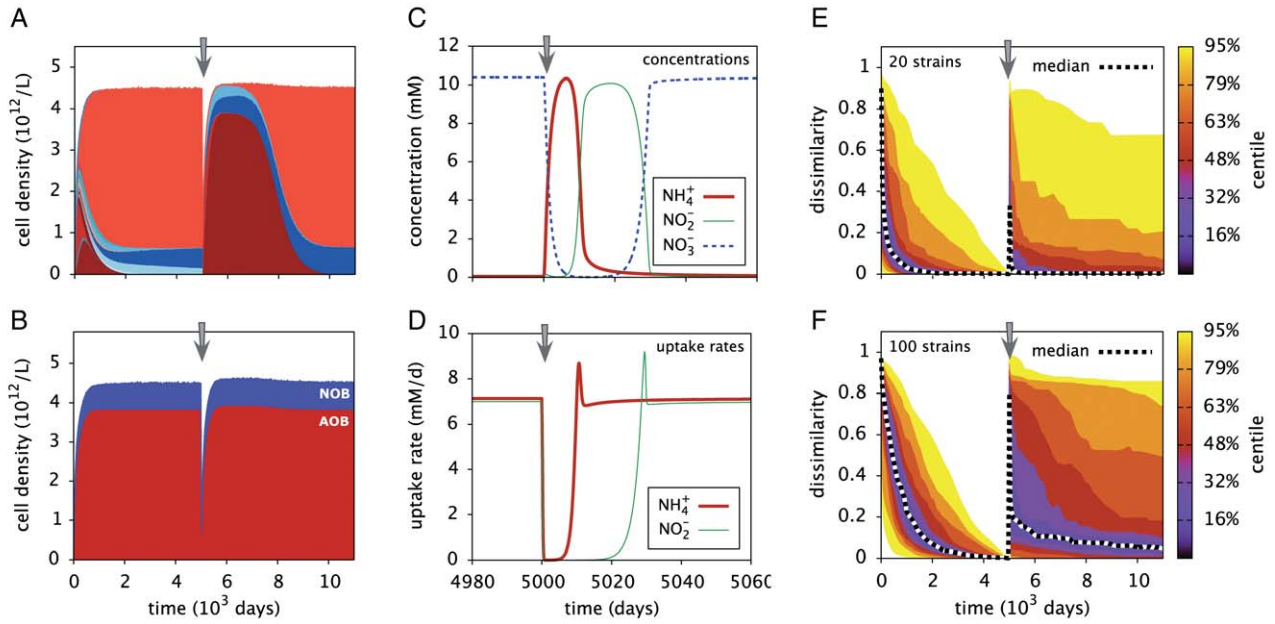

Fig. 3. (A) Simulated cell densities over time in the ammonium-fed nitrifying membrane bioreactor, inoculated with 100 random strains (AOB in variations of red, NOB in variations of blue). A strong perturbation during day 5000 (grey arrow) causes a temporary collapse of the microbial community. (B) Corresponding total cell densities per functional group (AOB or NOB). (C) Corresponding ammonium (NH_4^+), nitrite (NO_2^-) and nitrate (NO_3^-) concentrations, at times near the perturbation. (D) Corresponding community-wide ammonium and nitrite uptake rates, at times near the perturbation. (E, F) Bray–Curtis dissimilarity of the community to the state shortly prior to the perturbation, in bioreactors inoculated with 20 (E) or 100 (F) random strains. Shown as a probability distribution over 100 random simulations (colours correspond to centiles). Notice the greater resistance to perturbation and greater resilience in (E) compared to (F).

that benefit from an excess of substrates and little competition. The duration of this phase is mainly determined by the hydraulic renewal rate, ammonium supply rate and bacterial growth rates, and the duration predicted by our model (roughly 3 weeks) is in line with typical nitrifying bioreactor experiments (Dumont *et al*., 2009; Martens-Habbena *et al*., 2009).

As ammonium and nitrite consumption increase, their concentrations decrease to near or below the survival thresholds for an increasing number of strains (Fig. 2C). This second saturation phase is characterized by low and relatively stable substrate concentrations, stagnation of growth, a gradual extinction of less competitive strains and a long coexistence of similar top competitors (Fig. 2A). The microbial community slowly converges to a stable composition of decreased diversity in which each metabolic niche is occupied by a single strain, with transient periods occasionally lasting up to several thousands of days. A gradual decrease in diversity is expected under the competitive exclusion principle of equilibrium ecology (Hardin, 1960) and is consistent with similar observations in previous bioreactor experiments (Vanwonterghem *et al*., 2014). On the other hand, the total cell densities of metabolically similar strains (e.g. AOB) stabilize much faster and only vary weakly during the saturation phase (Fig. 2B). Hence, each of the two available metabolic niches is rapidly filled by several competing and temporarily coexisting strains, which are only slowly replaced by the top competitor.

These results show how transient dynamics of competitive exclusion can lead to a separation of time scales characterizing functional and compositional stabilization of communities. This separation of time scales is also expected to be reflected in the community's response to perturbations. Perturbations such as mechanical biofilm removal (Gentile *et al*., 2006) or nutrient shocks (Sundh *et al*., 2003) can alter the relative abundances of individual clades or lead to a temporary collapse of the community. Such a collapse would initiate a race for the (re-)occupation of metabolic niches and a subsequent saturation phase, analogous to the dynamics following inoculation. For example, Gentile and colleagues (2006) observed that after the shearing of biofilms inside a fluidized bed reactor community composition recovered much slower, i.e. it had lower resilience (Shade *et al*., 2012), than the bioreactor's performance.

Simulations of the bioreactor model including a strong pulse perturbation, applied simultaneously to the entire community, reproduced these observations (Fig. 3). The modelled perturbation corresponds to an increased mortality for 1 day, with a strength chosen randomly for each strain and resulting in a temporary collapse of the community by several orders of magnitude. Consistent with experimental observations, the bioreactor's performance

quickly recovers within a few days to weeks (Fig. 3C and D), whereas the community's recovery to its original composition typically takes several months to years (Fig. 3A,E,F). Metabolic niches are reoccupied rapidly and concurrently with the bioreactor's functional stabilization (Fig. 3B); however, metabolic niches can be temporarily shared by several coexisting strains. Non-equilibrium processes, particularly following perturbation, are frequently thought to maintain high diversity, for example in rain forests (Connell, 1978) or phytoplankton (Sommer, 1984). Furthermore, a meta-analysis by Shade and colleagues (2012) found more studies reporting the recovery of microbial community function than composition, following pulse perturbation.

As predicted above by our competition model, the discrepancy between functional and taxonomic stability should be stronger for communities with high richness because the likelihood of two similar top competitors increases, thus delaying competitive exclusion. Simulations of bioreactors inoculated with different numbers of random strains verify this prediction. For example, the time until compositional convergence following inoculation, i.e. reaching a 90% Bray–Curtis similarity to the steady state (Legendre and Legendre, 1998), ranges from roughly 600 days for 20 strains to 1300 days for 100 strains (median values, Fig. 2E and F). Moreover, richer communities are expected to be more prone to temporary changes in composition during perturbation because of a greater reservoir of opportunistic strains that could temporarily invade (Wittebolle *et al*., 2008). This is reflected in our simulations, where a greater number of strains correlates with a stronger change in community composition following the pulse perturbation (Fig. 3E and F). The insensitivity to disturbance is known in ecology as resistance and is, together with resilience, a common measure of community stability (Shade *et al*., 2012). Our work suggests that microbial communities with higher functional redundancy have lower resilience and lower resistance to pulse perturbation in terms of taxonomic composition.

Variable does not mean unstable

Previous bioreactor experiments have revealed variable community composition despite stable bioreactor performance over hundreds of days following inoculation (Fernández *et al*., 1999; Zumstein *et al*., 2000; Wittebolle *et al*., 2008; Vanwonterghem *et al*., 2014), whereas others have reported convergence to steady compositions within a few months (Xing *et al*., 1997; Gentile *et al*., 2006; McGuinness *et al*., 2006). Fluctuating community compositions are often interpreted as unstable, non-convergent or even chaotic. However, the observed dynamics may be mere transients of slowly converging communities. Typical richness in bioreactors can range from hundreds to thousands of operational taxonomic units (OTUs, a species analogue based on rDNA similarity) (Stackebrandt *et al*., 2002; Kim *et al*., 2013; Vanwonterghem *et al*., 2014). As shown here, at these richness scales transient dynamics of competitive exclusion can last several years. Much longer operation times might thus be needed to actually observe an eventual community convergence in typical bioreactors. However, at these time scales other destabilizing processes, such as the invasion of new strains introduced by contamination, could prevent community convergence.

Model limitations

The simple models considered in this paper focus on generic ingredients of microbial ecosystems, namely substrate-limited growth and competition, stoichiometric constraints on coexisting pathways, as well as physical substrate repletion and waste removal (e.g. in continuous-flow bioreactors). In particular, we have assumed that microbial growth increases with increasing substrate concentrations, thus ignoring the possibility of substrate inhibition. For example, substrate inhibition can occur during nitrification by excess ammonia and nitrous acid (Anthonisen *et al*., 1976), resulting in reduced bioreactor performance (Sheintuch *et al*., 1995). Similarly, growth may also be subject to product inhibition, e.g. when the partial pressure of accumulating waste products renders a pathway unfavourable (Kaspar and Wuhrmann, 1977). Accurately modelling specific industrial setups may thus require a consideration of more complicated kinetics, e.g. including substrate and product inhibition. Our main point is that long transient dynamics can emerge even in the simple cases considered here, acknowledging that more complex communities are likely subject to further destabilizing mechanisms (see below).

Alternative destabilizing factors

Transient dynamics of competitive exclusion provide a simple explanation for the discrepancy between functional and taxonomic stability of microbial communities, and our simulations underline the relevance of these processes at least to typical bioreactor setups. However, other mechanisms likely contribute to a long-term variability of community composition. For example, time lags associated with the degradation of organic matter, such as cellulose hydrolysis in anaerobic digesters (Vanwonterghem *et al*., 2014), can result in slow changes of the metabolic landscape and optimal electron flow, in turn driving adaptive changes in community composition (Fernández *et al*., 1999). More complicated non-sequential pathways, ubiquitous in organic carbon catabolism, could also lead to positive feedback loops that further destabilize community dynamics. Furthermore, in contrast to well-controlled

bioreactors, many natural ecosystems are subject to intense environmental variation that can drive adaptation and succession in microbial communities. For example, annual deep-water renewal in a seasonally anoxic fjord has been shown to cause significant changes in microbial community structure (Zaikova *et al*., 2010).

We emphasize that mechanisms that destabilize community composition need not necessarily destabilize community function. For example, in open systems such as wastewater treatment plants (Wang *et al*., 2011) occasional invasion by novel competitors could drive species turnover without significantly affecting ecosystem functioning; however, this scenario is unlikely in bioreactors with a sterile feed (Vanwonterghem *et al*., 2014). Similarly, repeated adaptation of bacteriophages to dominant hosts ('killing the winner' dynamics) has been shown to sustain bacterial diversity and drive continuous species turnover (Thingstad, 2000; Shapiro and Kushmaro, 2011). Collapsing populations could be replaced by less susceptible but functionally similar populations that ensure the overall stability of biochemical fluxes.

Reciprocally, negative feedback mechanisms stabilizing biochemical fluxes may only weakly affect community composition. For example, substrate built-up can promote the growth of functional groups benefiting from the underutilized resource, in turn counteracting the processes causing substrate built-up. This stabilizing mechanism, perhaps comparable with La Châtelier's principle of an 'opposing force' (Schneider, 2004), a priori acts on functional groups rather than taxonomy.

Towards a pathway-centric microbial ecology

Environmental rDNA marker gene profiling has become a standard approach in microbial ecology for describing community composition in terms of OTUs (Wittebolle *et al*., 2008; Gilbert *et al*., 2010). However, single prokaryotic OTUs can comprise several distinct ecotypes (Welch *et al*., 2002; Kashtan *et al*., 2014) and, reciprocally, ecologically important pathways such as nitrogen fixation can exhibit non-monophyletic distributions across distant clades (Raymond *et al*., 2004; Frigaard *et al*., 2006). In fact, environmental conditions and ecological function often show a stronger correlation to particular metabolic pathways or even individual genes, than to the presence or absence of particular clades (Ofiţeru *et al*., 2010; Burke *et al*., 2011; Raes *et al*., 2011). Consistent with this, as we have shown here, compositional stability can be independent from functional stability while key genes (for example ammonia monooxygenase, present in AOB) remain synchronized with the community's performance. Hence, prokaryotic OTUs should be questioned as an ecologically relevant unit altogether (Gevers *et al*., 2005; Ward *et al*., 2007; Doolittle and Zhaxybayeva, 2010). Microbial ecology and biogeography might be best understood using function-centric theories in which individual genes, operons or pathways are considered as basic reproductive and functional units, particularly under conditions where metabolic functions define the microbial niche space (Dumont *et al*., 2009; Reed *et al*., 2014). Accordingly, metagenomic, metatranscriptomic and metaproteomic profiling would be more suitable than marker genes for monitoring or predicting fluctuations of community function (Ward *et al*., 2007; Doolittle and Zhaxybayeva, 2010; Hawley *et al*., 2014).

Conclusions

Convergence of microbial community composition is a gradual process that can last much longer than typical bioreactor experiments and environmental surveys. Transient dynamics of competitive exclusion explain why microbial communities can remain variable long after inoculation or perturbation, while exhibiting high functional stability. The correct interpretation of observed community dynamics in bioreactors and natural ecosystems thus requires a proper consideration of the involved time scales. Previous work has highlighted the general mismatch between the duration of typical experiments and the time scales assumed by conventional steady-state ecological theories (Hastings, 2004), and our work demonstrates some of the implications of this mismatch. Fluctuations in natural and less controlled microbial communities likely result from several destabilizing processes; however, the effects of these processes could be augmented by transient dynamics of competitive exclusion.

Furthermore, less resilient and more flexible communities need not imply a compromised functional stability, and previous experiments have indeed indicated a positive correlation between flexible community structure and stable performance (Fernandez *et al*., 2000). Several competing strains can rapidly and concurrently fill a metabolic niche when opportunities arise, while slowly replacing each other and maintaining constant performance during saturation. The time required for convergence or recovery of community composition correlates positively with functional redundancy because more competitors are likely to have similar efficiencies under substrate limitation.

The extreme case in which each functional group consists of equal competitors is comparable with the so-called emergent group theory in ecology, according to which assembly within each group is subject to neutral dynamics (Hubbell, 2005; Hérault, 2007). In that limit, transient periods of competitive exclusion can be extremely long, whereas community composition appears dissociated from environmental conditions and driven by purely stochastic factors. Although exact neutrality is an extreme idealization, some natural communities may

indeed include functional groups consisting of almost-equal competitors. For example, previous work on a wastewater treatment plant found that fluctuations within the group of ammonia-oxidizing bacteria, as well as within the heterotrophic community, were predominantly explained by neutral processes rather than environmental factors (Ofiţeru *et al*., 2010). Similarly, a global study of desert microbial communities by Caruso and colleagues (2011) found that climatic effects were detectable at the whole community level, but became undetectable when restricted to variations within the photosynthetic or heterotrophic groups. Frossard and colleagues (2012) found that spatiotemporal variations of microbial community structure in stream catchments were best described by a neutral assembly model, whereas potential activities of several carbon-, nitrogen- and phosphorus-acquiring enzymes showed clear seasonal patterns. This discrepancy between potential enzyme activities and the composition of their host communities indicates high functional redundancy and a decoupling between community function and taxonomy. Similarly, Yin and colleagues (2000) showed significant functional redundancy in soil microbial communities by measuring population responses to enrichment with individual carbon sources. However, it is unclear whether all detected OTUs were active prior to the enrichment, and at any point in time a significant fraction of functionally similar clades may have only been present at low densities (Shade *et al*., 2014). Furthermore, subtle partitioning along additional non-functional axes such as moisture or pH may create micro-niches that enable the long-term coexistence of functionally similar populations, particularly in spatially or temporally heterogenous environments. For example, the coexistence of hundreds of sub-populations of the marine cyanobacterium *Prochlorococcus* is likely enabled by subtle niche differentiation such as adaptations to different nutrient availabilities (Kashtan *et al*., 2014). The role of neutrality in natural microbial communities and its proper reconciliation with niche theory remain controversial. Nevertheless, our work shows that approximate neutrality within ecological niches can explain several patterns of microbial community assembly in engineered environments and should also be considered when interpreting the dynamics of natural microbial communities.

Experimental procedures

Computational framework

Model calibration, simulations and statistical analysis were performed using MCM (Microbial Community Modeler), a mathematical and computational framework that we developed for modelling microbial ecosystems (Louca and Doebeli, 2015, in press). MCM combines FBA-based cell models with a dynamical environment that influences, and is influenced by, microbial metabolism. The combination of FBA with a varying environmental metabolite pool is known as dynamic flux balance analysis (DFBA) (Mahadevan *et al*., 2002; Chiu *et al*., 2014; Harcombe *et al*., 2014) and has been shown to be a promising approach to microbial ecological modelling (Meadows *et al*., 2010; Chiu *et al*., 2014; Harcombe *et al*., 2014; Louca and Doebeli, 2015, in press). MCM can accommodate microbial community models with arbitrary environmental variables and metabolite exchange kinetics. For example, environmental variables may be stochastic processes (e.g. representing climate fluctuations), or specified using measured data (e.g. pH in bioreactor experiments). Metabolite uptake and export rate limits can be arbitrary functions of metabolite concentrations or environmental variables. Similar interdependencies are possible for reaction rate limits, thus allowing the inclusion of inhibitory or regulatory mechanisms (Covert *et al*., 2008). Metabolite concentrations can be explicitly specified, e.g. using measured time series, or depend dynamically on microbial export and other external fluxes.

MCM keeps track of a multitude of output variables such as cell concentrations, reaction rates, metabolite concentrations and metabolite exchange rates. Model predictions can then be compared with time series from experiments or environmental surveys, such as rate measurements, chemical profiles or optical cell densities. Reciprocally, time series data can be used to automatically calibrate unknown model parameters, e.g. using least-squares fitting or maximum likelihood estimation (e.g. Fig. 1, see details below). Because MCM can calibrate unknown measurement units, raw uncalibrated data (e.g. optical cell densities with no calibration to colony-forming units) can also be used. MCM was recently validated using laboratory experiments with bacterial communities (Louca and Doebeli, 2015, in press). MCM is Open Source and available at http://www.zoology.ubc.ca/MCM.

Construction of the cell models

The metabolism of each cell was modelled using FBA with optimization of biomass synthesis (Orth *et al*., 2010). The cell-internal reaction networks of the AOB and NOB are based on the core metabolic models published by Poughon and colleagues (2001) and Perez-Garcia and colleagues (2014). More precisely, the biomass synthesis functions of both cell types are taken from Perez-Garcia and colleagues (2014), the energy metabolism of AOB is adopted from Perez-Garcia and colleagues (2014) and the energy metabolism of NOB is adopted from Poughon and colleagues (2001). Assimilatory nitrite reduction to ammonium, required for biomass synthesis, was added to NOB (Starkenburg *et al*., 2006). The constructed AOB and NOB models are comprised of 16 and 11 reactions respectively (see Supporting Information S2 for details). The nitrogen substrate half-saturation constants were set to 26 μM NH_3 for the AOB template according to Suzuki and colleagues (1974) and to 229 μM NO_2^- for the NOB template according to Remacle and De Leval (1978). Cell masses were set to 3×10^{-13} g dW cell^{-1} for the AOB and 4×10^{-13} g dW cell^{-1} for the NOB, according to Keen and Prosser (1987).

Calibration of the template cell models

The maximum cell-specific substrate uptake rates for the AOB and NOB templates (V_{max,NH_4^+} and V_{max,NO_3^-} respectively) were calibrated using time series from a previous experiment with an ammonium-batch-fed nitrifying bioreactor by de Boer and Laanbroek (1989), inoculated with strains of the AOB *Nitrosospira* and NOB *Nitrobacter* genera. For the calibration, our bioreactor model was adjusted to de Boer and Laanbroek's experiment (Fig. 1A): The initial ammonium concentration was set to 0.916 mM, nitrite and nitrate were initially absent, the pH was set to the reported profile and oxygen was assumed to be non-limiting.

We used the reported concentration profiles of the gradually depleted ammonium (Fig. 1B) and produced nitrate (Fig. 1C) to calibrate V_{max,NH_4^+} and V_{max,NO_3^-} via maximum likelihood estimation (Eliason, 1993). This approach estimates unknown parameters by maximizing the likelihood of observing the available data, given a particular candidate choice of parameter values. Maximum likelihood estimation is widely used in statistical inference such as multilinear regression, computational phylogenetics and modelling in physics (Lyons, 1986). In our case, the likelihood of the data was calculated on the basis of a mixed deterministic-stochastic structure, in which the deterministic part is given by the microbial community model, and errors are assumed to be normally distributed. The likelihood was maximized using a subspace-searching simplex algorithm (SBPLX; Johnson, 2014), which uses repeated simulations and gradual exploration of parameter space and is integrated into MCM (Louca and Doebeli, 2015, in press). To reduce the possibility of only reaching a local maximum, fitting was repeated 100 times using random initial parameter values and the best fit among all 100 runs was used. While some fitting runs reached alternative local maxima, the best overall fit was reached in most cases. This procedure yielded the fitted values $V_{max,NH_4^+} = 6.48\times10^{-13}\text{mol } NH_3/\text{cell/d}$ and $V_{max,NO_2^-} = 7.31\times10^{-13}\text{mol } NO_2^-/\text{cell/d}$, which are consistent with the literature (Prosser, 2005).

Nitrifying membrane bioreactor model

Using the calibrated AOB and NOB template cell models, we constructed a model of an ammonium-fed nitrifying membrane bioreactor similar to the one described by Wittebolle and colleagues (2008). The hydraulic turnover rate for all metabolites was 0.672 per day, ammonium input was 7.14 mM per day and pH was fixed to 7.4. The input medium was assumed to be sterile and to contain micronutrients in sufficient amounts for autotrophic growth via nitrification (Wittebolle *et al*., 2008; Dumont *et al*., 2009). The bioreactor medium was assumed to be well mixed. Microbial communities started with an equal number of AOB and NOB strains, each with an initial density of 10^7 cells L^{-1}. Cell death was modelled as exponential decay (see Supporting Information S2 for details).

Each strain was a random variation of the calibrated template cell models, with physiological parameters chosen as follows: Substrate uptake kinetic parameters, i.e. the maximum cell-specific nitrogen substrate uptake rates (V_{max}) and substrate affinities (α; Aksnes and Cao, 2011), were randomly and uniformly chosen within an interval ranging an order of magnitude above and an order of magnitude below the template values. To account for the typically assumed trade-off between V_{max} and α, these parameters were multiplied by a factor κ and $(1 - \kappa)$, respectively, where κ was chosen randomly between 0 and 1 for each strain (Smith *et al*., 2009). Cell life times were randomly chosen within 50–100 days for each strain according to typical nitrifier decay rates (Alleman and Preston, 1991).

Perturbations were modelled as a temporary increase in mortality rates, such that after 1 day each cell population declines by some random factor, chosen log-uniformly and independently for each strain within the interval $[1, 10^{12}]$.

Statistics of community convergence

The distance between two community compositions was expressed using the Bray–Curtis dissimilarity, which is well established in the ecological literature (Legendre and Legendre, 1998). The maximum dissimilarity between any two communities is 100%, whereas identical communities have a dissimilarity of 0%. The convergence of the bioreactor community was examined by calculating its dissimilarity to the steady composition established after a long time. This dissimilarity curve is typically decreasing in time because communities eventually converge to a steady composition in which each metabolic niche is occupied by a single strain. A steeper curve implies a faster convergence. Following inoculation, the dissimilarity curve depends on the particular strains present in the community, which are chosen randomly for each simulation. The resulting probability distribution of the dissimilarity curves (Figs 2E,F and 3E,F) was estimated using 100 repeated random simulations of the model.

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Supporting information

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Mathematical details of the competition model introduced at the beginning of this article are available as Supporting Information S1. Details on the bioreactor model, including the AOB and NOB reaction networks, can be found in Supporting Information S2. The model files for the batch-fed bioreactor along with the MCM script required for simulation and calibration are available as Supporting Information S3 or on the official MCM website, http://www.zoology.ubc.ca/MCM. The model files and simulation script for the continuous-flow bioreactor are available as Supporting Information S4 or on the official MCM website.

Supporting Information S1. Elaboration on the competition model.
Supporting Information S2. Details on the bioreactor model.
Supporting Information S3. Model files for the batch-fed bioreactor and MCM script for simulation and calibration.
Supporting Information S4. Model files and simulation script for the continuous-flow bioreactor.