

## RESEARCH ARTICLE

# Influence of dormancy on microbial competition under intermittent substrate supply: insights from model simulations

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**One sentence summary:** The present study investigates the relevance of factors controlling the abundance and activity of individual bacterial species competing for an intermittently supplied substrate.

**Editor:** Tillmann Lueders

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## ABSTRACT

Most natural environments are characterized by frequent changes of their abiotic conditions. Microorganisms can respond to such changes by switching their physiological state between activity and dormancy allowing them to endure periods of unfavorable abiotic conditions. As a consequence, the competitiveness of microbial species is not simply determined by their growth performance under favorable conditions but also by their ability and readiness to respond to periods of unfavorable environmental conditions. The present study investigates the relevance of factors controlling the abundance and activity of individual bacterial species competing for an intermittently supplied substrate. For this purpose, numerical experiments were performed addressing the response of microbial systems to regularly applied feeding pulses. Simulation results show that community dynamics may exhibit a non-trivial link to the frequency of the external constraints and that for a certain combination of these environmental conditions coexistence of species is possible. The ecological implication of our results is that even non-dominant, neglected species can have a strong influence on realized species composition of dominant key species, due to their invisible presence enable the coexistence between important key species and by this affecting provided function of the system.

**Keywords:** dormancy; microbial starvation; microbial resuscitation; modeling; ecological niches

## INTRODUCTION

Many ecological relevant processes in various environments such as soils are catalyzed by the activity of microorganisms,

which includes remineralization processes and the degradation of organic compounds (Paul and Clark 1996; Dublier, McFall-Ngai and Zhao 2015). Although the dynamics of such degradation processes differ with the involved chemical compounds and

Received: 12 November 2015; Accepted: 1 April 2016

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environmental conditions, the general activity of the catalyzing microorganisms has a crucial impact on the overall rate of the process (Paul and Clark 1996). Therefore, a sound theoretical description and the prediction of such microbial rates require knowledge on community composition, species abundances, individual traits of the involved microorganisms and on the factors controlling these variables.

Experimental microbial studies in the laboratory are traditionally performed at constant, nutrient-enriched conditions, which rarely allow for coexistence or coactivity (i.e. simultaneous activity) of competing species (Codeço and Grover 2001). In contrast, natural microbial systems are normally oligotrophic, and are characterized by the continuous change of their environmental parameters, facilitating the coexistence of competing microbial species (Hibbing et al. 2010). Environmental changes can differ in their regularity and vary in both, their frequency and amplitude. In soils changes may include natural variations ranging from daily cycles (e.g. regular tidal fluctuations of groundwater levels or evapotranspiration processes triggered by daylight plant activity) to seasonal changes (e.g. submergences and droughts) as well as anthropogenic activities across different time scales (De Biase et al. 2011). Since microorganisms cannot physically evade local changes by migration, they respond by switching their physiological state from active to dormant (Lennon and Jones 2011). This allows them to endure long periods of unfavorable environmental conditions (Stevenson 1977; Kaprelyants, Gottschal and Kell 1993a). In many natural environments, actually, the largest fraction of the microbial population is found to be metabolically inactive (Lennon and Jones 2011). Thus, the competitiveness of microbial species and consequently also their abundance and activity is not only controlled by their growth performance under favorable conditions but to a large extent by their ability and readiness to respond to periods of unfavorable environmental conditions (Stolpovsky et al. 2011).

Composition and dominance patterns in microbial communities are generally shaped by competition for common resources, cooperation, predation and abiotic stress (Hibbing et al. 2010) creating ecological niches for a multitude of species to coexist in a given environment (Sillero 2011). Realized niches (the one a species occupies) often differs strongly from its potential niche (the determined physiological available range) since additional factors such as competition and predation often strongly constrains the latter (Peterson et al. 2011). Final distribution and overlaps of physiological niches and competition strength finally explains theoretical long-term coexistence under given environmental conditions (Gage 1996). Stolpovsky et al. (2011), moreover, showed for microorganisms that even in spatial uniform environments coexistence of competing species in time is possible by species switching into dormancy during periods of starvation. Simulation of microbial community dynamics in porous media suggested that in soils the intermittent substrate supply is also at the pore scale, the main driver for microbial competition (Stolpovsky, Gharasoo and Thullner 2012). However, up to now available results only demonstrate the relevance of dormancy for the dynamics of microbial communities during intermediate substrate supply but they do not allow for an analysis attributing the observed phenomena to either the magnitude or the variation of substrate concentrations. It is therefore unknown which characteristics of substrate supply or more generally of environmental changes are most relevant for the competition and coexistence of microbial species and to which extent phenomena observed in previous studies are representative for a given set of characteristics.

Although microbial communities represent the most diverse type of communities on the Earth (Dykhuizen 1998; Curtis, Sloan and Scannell 2002), the estimation of their biodiversity in natural communities and the factors controlling them is still a challenge. Established analyzing methods determining functional and structural compositions of natural communities (Forney, Zhou and Brown 2004) are hitherto only capable to detect the most dominating microbial groups (e.g. >1% of total microbial biodiversity) (Blackwood et al. 2007). Although low abundant groups usually remain undetected these groups may still play a significant role in microbial ecosystem functioning (Forney, Zhou and Brown 2004; Bent and Forney 2008). While new, recently developed fast sequencing methods allow for increased resolution and thus better insights into microbial biodiversity, abundances below a certain threshold are still ignored (Lynch and Neufeld 2015).

Computational modeling is a promising method to be applied in the field of microbiology and ecology and models are increasingly used to theoretically describe the behavior of organisms under different environmental conditions (Jamieson et al. 2004; Banitz et al. 2012; Ebrahimi and Or 2014; Gharasoo et al. 2014; Centler and Thullner 2015; Esser, Leveau and Meyer 2015). These modeling approaches enable not only predictions of microbial systems behavior but provide also the ability to give new insights into the underlying mechanisms for the observed biological phenomena. This includes dormancy effects and their relevance for the dynamics of microbial ecosystems (Stolpovsky et al. 2011; Wang et al. 2014; Hawkes and Keitt 2015).

The aim of the present study is to (i) systematically demonstrate that the characteristics of periodically changing environmental conditions (here changing substrate supply/feeding patterns) can affect the coexistence of competing microbial species, (ii) to link community composition and activity dynamics to such characteristics and (iii) to show that species with abundance and activity levels often considered as negligible might still have an influence on the ecological behavior of their competitors.

For this purpose, we use a computational model for understanding the principal behavior of dynamic dominance and coexistence patterns of competing species in a variable environment. An applied numerical simulation approach is used to study the coexistence and coactivity of competing microbial species within a systematically covered range of different intermittent feeding conditions. In particular, we investigate the variation of species dominance patterns in a system containing up to three competing bacterial species using different strategies in terms of resource use efficiency, growth rate and ability to switch between active and inactive (dormant) states.

## MODEL DESCRIPTION

### General concept

Simulations were performed for microbial systems containing different bacterial species competing for a common, intermittently supplied carbon substrate. Each active species is able to switch into a dormant state. This conceptual approach has been introduced by Stolpovsky et al. (2011) where a detailed description is provided. Thus, in this manuscript only a short overview of the underlying assumptions and used conditions is given. The model describing the dynamics of these two-state (active/dormant) microbial species considers (i) growth of active cells coupled to the consumption of an energy yielding carbon substrate, (ii) intrinsic mortality of active cells, (iii) intrinsic

mortality of inactive cells (considered to be significantly slower than for active cells), (iv) deactivation of active cells controlled by the energy supply of the cells and (v) reactivation of inactive cells controlled by the potential energy supply of the cells. Active cells are able to build up biomass by growth and/or cell division, to consume available substrate and are subject to mortality by cell death. Inactive (dormant) cells do not exhibit any growth and degradation activity while their death rate is highly reduced compared to their active state. Depending on the environmental conditions cells are assumed to switch between these two states using the potential supply of catabolic energy to the cells as control variable. Species' active and dormant biomass is included as state variables, and their growth is described by established approaches (Thullner, Regnier and Van Cappellen 2007; Stolpovsky et al. 2011). These approaches are combined with kinetic expressions describing the deactivation and reactivation of the simulated species. The kinetic expressions are functionally linked to the energy yields of the corresponding respiration pathways, as well as to the maintenance requirements of the organisms. We also take into consideration variations in the yield factor, depending on the energy budget and consider loss of biomass during reactivation.

The rate of degradation of dissolved organic carbon (DOC) is assumed to follow standard Michaelis-Menten- or Monod-type kinetics (e.g. Thullner, Regnier and Van Cappellen 2007 and references therein).

$$\frac{dC_{\text{DOC}}}{dt} = -\theta \cdot k_c \cdot B \cdot \frac{C_{\text{DOC}}}{K_{\text{DOC}} + C_{\text{DOC}}}, \quad (1)$$

where  $C$  stands for concentration,  $B$  is the concentration of active biomass (given in  $M$  of carbon and referred to later on as (biomass) 'concentration' of active cells),  $k_c$  is the maximum specific rate of degradation of DOC by the active cells,  $K_{\text{DOC}}$  is the half-saturation constants for DOC utilization.  $\theta$  represents a switching function possessing values between 0 and 1 (see Stolpovsky et al. 2011 for further details) and regulating the degradation activity of bacterial communities as well as the direction of transformation between different physiological states depending on an energy threshold level  $G_0$  and the current level of the catabolic energy supply which is given by the maximum rate of Gibbs energy release per unit biomass and calculated as

$$G = \Delta G \cdot \frac{dC_{\text{DOC}}}{dt} \Big|_{\theta=1, Y_{\text{eff}}=0} \cdot \frac{1}{B} = -\Delta G \cdot k_c \frac{C_{\text{DOC}}}{K_{\text{DOC}} + C_{\text{DOC}}}. \quad (2)$$

Values for  $G$  are computed with  $\Delta G$  as the Gibbs energy change of the oxidation of DOC by the given electron acceptor.

The change of concentration of active cells  $B$  is given by biomass growth (linked to the substrate consumption rate through the effective growth yield factor  $Y_{\text{eff}} = \max(Y_{\text{max}}(1 - \frac{G_0}{G}), 0)$ ,  $Y_{\text{max}}$  represents the highest possible cell yield for the given microbial group assuming a decrease of growth efficiency at less favorable conditions (Kleerebezem and Van Loosdrecht 2010)), by intrinsic cell decay and by transitions between active and inactive cells:

$$\begin{aligned} \frac{dB}{dt} = & \theta \cdot Y_{\text{eff}} \cdot k_c \cdot B \cdot \frac{C_{\text{DOC}}}{K_{\text{DOC}} + C_{\text{DOC}}} - m_{\text{dec}} \\ & - (1 - \theta) \cdot m_{\text{dec}} \cdot B + Y_{\text{reac}} \cdot \theta \cdot m_{\text{reac}} \cdot B^{\text{in}}, \end{aligned} \quad (3)$$

where  $m_{\text{dec}}$  stands for the specific cell decay rate,  $m_{\text{dec}}$  is the specific rate of deactivation,  $Y_{\text{reac}}$  is the reactivation cell yield,

$m_{\text{reac}}$  is the specific rate of reactivation. The change of concentration of inactive (dormant) cells  $B^{\text{in}}$  is given by

$$\frac{dB^{\text{in}}}{dt} = -m_{\text{dec}}^{\text{in}} \cdot B^{\text{in}} + (1 - \theta) \cdot m_{\text{dec}} \cdot B - \theta \cdot m_{\text{reac}} \cdot B^{\text{in}}, \quad (4)$$

where  $m_{\text{dec}}^{\text{in}}$  is the specific cell decay rate of the inactive cells.

The length of the starvation period slows down the reactivation and (residual) decay rate of the dormant cells (Kaprelyants, Gottschal and Kell 1993b) gradually entering a so-called deep dormant state (see Stolpovsky et al. 2011 for further details). Active and dormant biomasses are included in the model as state variables. These mathematical expressions describing mortality of active and (at significantly lower rate) inactive cells, deactivation of active and reactivation of dormant biomass are implemented into the numerical simulation software BRNS (Biogeochemical Reaction Network Simulator; Regnier et al. 2002; Aguilera et al. 2005) and the model is used to simulate the dynamics of microbial model systems for different time period lengths between substrate supply events.

### Simulated scenarios

Given the vast range of microbial species and their physiological potential, coverage of this range by model simulations is not feasible. The simulated scenarios shall thus represent a set of numerical experiments, which aim at representing reasonable yet generic conditions.

The simulated generic microbial systems is considered to contain one to three bacterial species: the first species (termed 'Growers') has a fast and effective metabolism, i.e. relatively high growth rate, yield factor and mortality rate; in contrast, the second species (termed 'Switchers') is characterized by an effective dormancy, i.e. parameter values for de-/reactivation and deep dormancy which allow to effectively use these abilities for maintenance and/or growth of their population; the third species (termed 'Intermediate') has no specific advantages, but represents an average between characteristics and performance of Growers and Switchers. All bacterial species are considered to compete for the same carbon substrate, the concentration of which is assumed as the only growth-limiting factor. Parameters values describing the behavior of these three bacterial strains (Table 1) are adapted from a batch experiment (Martínez-Lavanchy et al. 2009). To ensure a different behavior of the simulated bacterial species and to obtain an arbitrarily determined trade-off between growth and dormancy performance the experimental values were modified but remained within the range of values analyzed previously in Stolpovsky et al. (2011).

Simulations were performed for virtual batch type systems inhabited by bacteria and intermittently supplied with substrate pulses, which were for simplicity applied as individual spikes (i.e. singular events) of organic carbon that depletes due to bacterial decomposition. The concentration and frequency of the intermittent carbon supply scheme were constant in each scenario but differed between the different scenarios. Considered pulse frequencies were within the range of  $0.01$ – $1 \text{ h}^{-1}$ . The concentration applied as individual feeding pulse varied from  $10^{-6}$  to  $10^{-2} \text{ M}$  carbon and cover the wide range of substrate concentrations found in the subsurface (e.g. Fischer et al. 2007; Thullner, Dale and Regnier 2009). For each scenario the transient behavior of the microbial community was simulated until simulated concentrations reached stable average concentrations of all considered species (referred to as 'quasi-steady-state'). The obtained 'quasi-steady-state' results were analyzed

**Table 1.** Parameter values used to simulate the different competing microbial species.

Parameters	Symbols	Units	'Growers'	'Intermediates'	'Switchers'
Maximum reaction rate of degradation of DOC by microbial group	$k_C$	$\text{h}^{-1}$	0.2	0.12	0.05
Half-saturation constant for carbon substrate	$K_{\text{DOC}}$	$\mu\text{M}$	1.0	2.0	3.0
Growth yield factor (max)	$Y_{\text{max}}$	–	0.8	0.65	0.5
Mortality rate of active bacteria	$m_{\text{dec}}$	$\text{h}^{-1}$	0.02	0.015	0.01
Initial mortality rate of inactive bacteria	$m_{\text{dec},0}^{\text{in}}$	$\text{h}^{-1}$	$1.0\text{e}-4$	$1.0\text{e}-4$	$1.0\text{e}-4$
Thermodynamic threshold	$G_0$	$\text{kJ}\cdot\text{M}_{\text{biomass}}^{-1}\cdot\text{h}^{-1}$	25.0	18.0	12.0
Gibbs energy change of the oxidation of DOC with oxygen	$\Delta G$	$\text{kJ}\cdot\text{M}_{\text{biomass}}^{-1}$	–522	–522	–522
Reactivation yield	$Y_{\text{reac}}$	–	0.1	0.5	1.0
Reactivation rate parameter	$m_{\text{reac}}$	$\text{h}^{-1}$	0.1	1.0	2.0
Deactivation rate parameter	$m_{\text{deac}}$	$\text{h}^{-1}$	2.0	2.0	2.0
Dormancy rate parameter	$k_{\text{incr}}$	$\text{h}^{-1}$	0.05	0.25	0.5
Dormancy rate parameter	$k_{\text{decr}}$	$\text{h}^{-1}$	0.5	0.25	0.05

for the contribution of the single species to the total/active biomass. A detection threshold of 1% was used: species contributing to the total/active biomass less than this threshold value are considered in this study to be undetectable. Species with biomass contributions higher than this threshold are either considered to dominate the bacterial community as the only abundant species (exceeding the threshold) or to coexist in a community of multiple species (each of them exceeding the threshold). The temporal pattern of active biomass concentration changes was analyzed for each species with respect to the full period length (or frequency) of any repetitive patterns. A pattern was considered as repetitive with a period length  $t_p$  if the concentrations  $C(t)$  and  $C(t + t_p)$  differed for all times  $t$  by less than 1%. If no repetitive pattern was found for  $t_p \leq 5$  (times the period length of the carbon feeding), the temporal active biomass concentration changes were considered as irregular/chaotic.

## RESULTS

### Single-species system

As a reference case we use single-species systems populated with either Growers or Switchers. These systems exhibited amounts of total biomass generally following the average amount of carbon substrate supplied to the system, with the activity of the species showing strong fluctuations due to the intermittent substrate supply (data not shown). The most remarkable behavior of the system populated with Growers was the discrepancy between frequencies of the external constraint and the internal response i.e. between the substrate supply and changing of active and total biomass concentrations (Table 2). A match between these frequencies was only found for the highest substrate supply frequency or for a combination of high frequencies with low substrate concentrations. For all other pulse characteristics, the changes of the active biomass followed a more complex temporal pattern. For intermediate pulse frequencies (approximately between 0.1 and  $0.5 \text{ h}^{-1}$ ) biomass changes appeared rather irregular or chaotic (except for very low substrate concentrations), while at lower frequencies most scenarios exhibited regular patterns of the biomass changes but their period length was 2 to 4 times increased compared to the substrate supply. Examples representing the different types of frequency responses are given in Fig. 1. In contrast, systems inhabited by Switchers showed an exact match between frequencies of external constraint and internal response for the entire range of simulated conditions (data not shown).

**Table 2.** Factors linking period lengths of concentration changes of active biomass of Growers to period lengths of substrate supply for the single-species system. '>5' is considered as irregular concentration changes.

Freq. (hours <sup>-1</sup> ) → Amplitude (M) ↓	1	0.5	0.2	0.1	0.05	0.02	0.01
1.00E-06	1	1	1	1	4	4	4
2.00E-06	1	1	1	>5	>5	>5	2
5.00E-06	1	1	>5	5	3	3	2
1.00E-05	1	>5	>5	>5	>5	3	2
2.00E-05	1	>5	>5	>5	>5	3	2
5.00E-05	1	>5	>5	>5	4	3	2
1.00E-04	1	>5	>5	>5	2	3	2
2.00E-04	1	>5	>5	>5	2	3	2
5.00E-04	1	>5	>5	>5	2	4	2
1.00E-03	1	>5	>5	>5	2	4	2
2.00E-03	1	>5	>5	>5	2	4	2
5.00E-03	1	>5	>5	>5	2	4	2
1.00E-02	1	>5	>5	>5	2	4	2

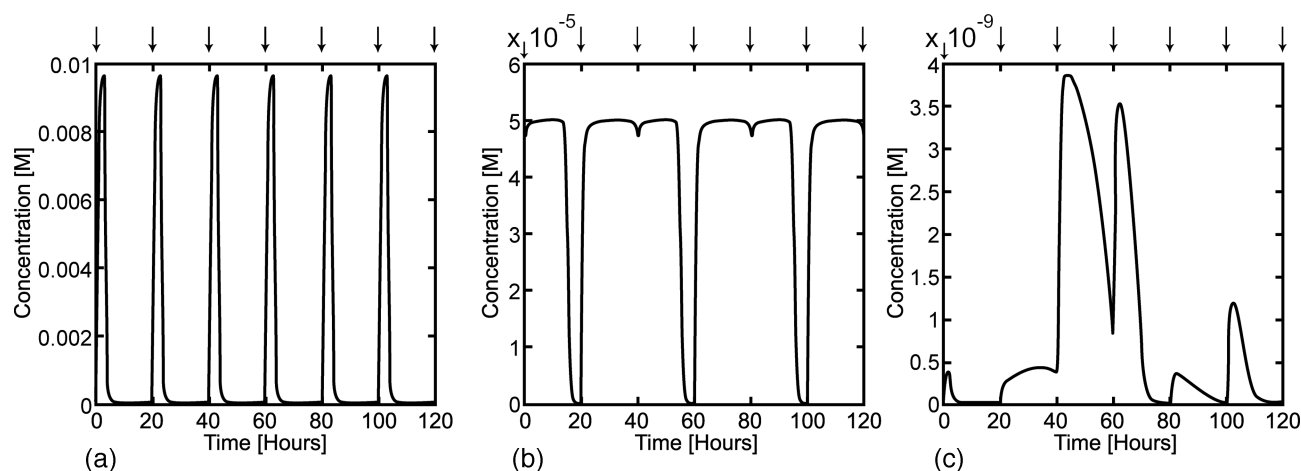
### Two-species system

Simulation results revealed that a long-term coactivity (coexistence of active fractions) of Growers and Switchers is found at substrate concentration pulse concentrations around  $10^{-4} \text{ M}$  (Table 3a). For lower concentrations of substrate supply Growers were dominating the activity of the community while higher concentrations led to a dominance of the Switchers. Little dependency of activity results was found on the pulse frequency showing only a slight decrease of the concentrations allowing for species coactivity when decreasing the pulse frequency from 1 to  $0.01 \text{ h}^{-1}$ . For frequencies of  $0.05 \text{ h}^{-1}$  and higher the active biomass fraction (active Growers plus active Switchers) represented 0.1 or more of the total biomass, while for lower frequencies active fractions were below 0.01 (data not shown).

Analysis of results obtained for total biomass revealed a more complex response (Table 3b). Here the pulse frequency and amplitude range of Growers presence and dominance expanded to some of the values ranges where Switchers were more active than Growers. On the other hand, pulse characteristics leading to Growers dominated activities occasionally showed a coexistence of Growers and Switchers (e.g. at lowest concentrations).

The frequency analysis of the active biomass concentration showed for the Switchers a discrepancy between the frequencies of the external carbon pulses and of the active biomass concentration changes (Table 4). For a frequency of  $1 \text{ h}^{-1}$  the frequency of the active biomass matched the frequency of the carbon supply. For decreasing pulse frequencies, active biomass concentrations became less regular exhibiting for most pulse amplitudes





**Figure 1.** Examples for different responses of active biomass concentration changes to the frequency of the spike-like feeding pulses (indicated by arrows) as periodical external stimulation. Feeding frequency is  $1/20 \text{ h}^{-1}$  for all shown examples. (a.) Regular response with same frequency as external stimulation of  $10^{-3} \text{ M}$ , (b.) regular response with period length increases by factor 2 compared to external stimulation of  $5 \cdot 10^{-5} \text{ M}$  and (c.) irregular response to external stimulation of  $2 \cdot 10^{-6} \text{ M}$ .

**Table 3.** Abundance of (a) active biomass fractions and (b) total biomass of Growers and Switchers for the tested feeding pulse periods and amplitudes. Threshold of existence—1% of active or total biomass. ‘G’—dominance of Growers, ‘S’—dominance of Switchers. ‘GS’—coactivity or coexistence of Growers and Switchers.

Freq. (hours <sup>-1</sup> ) → Amplitude (M) ↓	1	0.5	0.2	0.1	0.05	0.02	0.01
(a)							
1.00E-06	G	G	G	G	G	G	G
2.00E-06	G	G	G	G	G	G	G
5.00E-06	G	G	G	G	G	G	G
1.00E-05	G	G	G	G	GS	G	G
2.00E-05	G	G	GS	GS	G	GS	GS
5.00E-05	G	G	GS	GS	GS	GS	GS
1.00E-04	GS	GS	GS	GS	S	S	S
2.00E-04	GS	GS	S	S	S	S	S
5.00E-04	S	S	S	S	S	S	S
1.00E-03	S	S	S	S	S	S	S
2.00E-03	S	S	S	S	S	S	S
5.00E-03	S	S	S	S	S	S	S
1.00E-02	S	S	S	S	S	S	S
(b)							
1.00E-06	GS	GS	GS	GS	GS	G	GS
2.00E-06	GS	GS	GS	G	G	G	G
5.00E-06	G	G	G	G	G	G	G
1.00E-05	G	G	G	G	G	G	G
2.00E-05	G	G	GS	G	G	G	G
5.00E-05	G	G	GS	GS	GS	G	G
1.00E-04	GS	GS	GS	GS	S	G	G
2.00E-04	GS	GS	GS	S	S	GS	GS
5.00E-04	S	S	GS	S	S	GS	GS
1.00E-03	S	S	GS	GS	S	GS	GS
2.00E-03	S	GS	GS	GS	S	GS	GS
5.00E-03	GS	GS	S	S	GS	GS	GS
1.00E-02	S	S	S	S	S	GS	GS

first a rather irregular or chaotic temporal pattern, while further decrease led again to concentration patterns which were stable/repetitive but at period lengths two to four times larger than the period of each carbon supply cycle. These changes in pattern stability with decreasing carbon pulse frequency occurred first at pulse amplitudes in the order of  $10^{-5} \text{ M}$ . For concentrations of smaller or larger order of magnitude, pattern changes occurred at increasingly lower pulse frequencies. For the Growers a similar frequency behavior was observed (data not shown).

All obtained results were independent of the initial conditions. In particular, for all considered scenarios adding a seed concentration of Growers to a population of Switchers having

**Table 4.** Factors linking period lengths of concentration changes of active biomass of Switchers to period lengths of substrate supply for the two-species system. ‘>5’ is considered as irregular concentration changes.

Freq. (hours <sup>-1</sup> ) → Amplitude (M) ↓	1	0.5	0.2	0.1	0.05	0.02	0.01
1.00E-06	1	1	1	1	2	4	4
2.00E-06	1	1	1	>5	>5	>5	2
5.00E-06	1	1	1	5	3	3	2
1.00E-05	1	>5	>5	>5	>5	3	2
2.00E-05	1	>5	>5	>5	>5	>5	2
5.00E-05	1	>5	>5	4	2	3	2
1.00E-04	1	2	4	>5	3	3	2
2.00E-04	1	1	1	>5	>5	>5	>5
5.00E-04	1	1	1	1	2	>5	3
1.00E-03	1	1	1	1	1	>5	>5
2.00E-03	1	1	1	1	1	2	>5
5.00E-03	1	1	1	1	1	1	1
1.00E-02	1	1	1	1	1	1	1

reached a ‘quasi-steady-state’ led to the same results than obtained for adding a seed concentration of Switchers to a population of Growers. The relaxation time of the model, i.e. the time needed to reach ‘quasi-steady-state’ depended mainly on the frequency of feeding events and was usually in the range 1000 feeding cycles or below. The presented activity and abundance patterns (Tables 3a and b, 5a and b) did in general not depend on the used detection threshold (1% for the shown results), with lower threshold values (e.g. 0.1% suggested by Lynch and Neufeld 2015 to define the ‘rate biosphere’) showing only an increase in the range of pulse characteristics (amplitude, frequency) leading to coexistence (see Tables S.1 and S.2, Supporting Information) and higher threshold values having the opposite effect (data not shown).

### Three-species system

Simulating the response of an established two-species system to the introduction of the third intermediate species showed that the resulting community allowed for five out of seven possible combinations of species activity and abundances at ‘quasi-steady-state’ (Table 5a and b). Only coexistence or coactivity of

**Table 5.** Abundance of (a) active biomass fractions and (b) total biomass of Growers and Switchers for the tested feeding pulse periods and amplitudes. Threshold of existence —1% of active or total biomass, 'G'—dominance of Grower; 'S'—dominance of Switchers; 'GS'—coexistence of Growers and Switchers; 'GI'—coexistence of Intermediate species and Growers. 'GSI'—coexistence of all three microbial species.

Freq. (hours <sup>-1</sup> ) → Amplitude (M) ↓	1	0.5	0.2	0.1	0.05	0.02	0.01
<b>(a)</b>							
1.00E-06	G	G	G	G	G	GI	G
2.00E-06	G	G	G	G	G	GI	GI
5.00E-06	G	G	G	GI	GI	GI	GI
1.00E-05	G	G	G	GI	GI	GI	GI
2.00E-05	G	G	GI	GI	GI	GI	GI
5.00E-05	G	G	GS	GSI	GI	GSI	GSI
1.00E-04	GS	GS	GS	GS	S	S	S
2.00E-04	GS	GS	S	S	S	S	S
5.00E-04	S	S	S	S	S	S	S
1.00E-03	S	S	S	S	S	S	S
2.00E-03	S	S	S	S	S	S	S
5.00E-03	S	S	S	S	S	S	S
1.00E-02	S	S	S	S	S	S	S
<b>(b)</b>							
1.00E-06	GSI	GSI	GSI	GSI	GS	G	G
2.00E-06	GS	GS	GS	G	G	G	G
5.00E-06	G	G	G	GI	GI	G	G
1.00E-05	G	G	G	GI	GI	GI	GI
2.00E-05	G	G	GI	GI	GI	GI	GI
5.00E-05	G	G	GS	GSI	GI	GS	GS
1.00E-04	GS	GS	GS	GS	S	GS	GS
2.00E-04	GS	GS	GS	S	S	GS	GS
5.00E-04	S	S	GS	S	S	GS	GS
1.00E-03	S	S	GS	S	S	GS	GS
2.00E-03	S	GS	GS	S	GS	GS	GSI
5.00E-03	GS	GS	S	GS	GS	GS	GSI
1.00E-02	S	S	S	GS	GS	GS	GSI

Switchers and Intermediates, or a dominance of Intermediates was not observed.

As for the two-species system higher (medium to high) feeding pulse amplitudes led to activities dominated by Switchers, while lower amplitudes allowed the Growers to contribute to the activity either as single, dominant species (pulses with low amplitudes and high frequencies), or together with one of the other species (Intermediates at low amplitudes and low frequencies, or Switchers at medium amplitudes and high frequencies) (Table 5a). When combined with lower frequencies medium amplitude pulses also allowed for a few scenarios with all three species contributing to total activity. Compared to the activities of the two-species scenarios, most three-species scenarios show either the same dominance/coactivity of Growers and Switchers or exhibit an additional activity contribution of the Intermediates. Only for few scenarios (medium concentrations and lower frequencies) a coactivity of Growers and Switchers is (partially) replaced by a coactivity of Growers and Intermediates.

Similar to the two-species scenarios, contributions of species to total abundance (biomass) showed a more complex dependency on feeding pulse frequencies and amplitudes (Table 5b). For most pulse characteristics the same results were found for the three-species and for the two-species scenarios (i.e. the Intermediates were not abundant and did not affect the abundance of Growers and/or Switchers). In contrast, some scenarios allowed for an abundance of the Intermediates in addition to the Growers (low to medium concentrations and lower frequencies) or in addition to Growers and Switchers (low concentrations and higher frequencies, high concentrations and low frequencies or medium concentrations and medium frequencies). Less often a community of coexisting Growers and Switchers was replaced by a community of Growers and Intermediates (medium amplitudes and medium frequencies). On the other hand, for

some scenarios a dominance of Growers in a two-species system was replaced by a coexistence of Growers and Switchers (but no abundance of the Intermediates) in the corresponding three-species system (medium concentrations and low frequencies).

The frequency analysis of the active biomass concentration exhibited a behavior which was very similar to one observed for two-species systems (data not shown).

## DISCUSSION

### Factors regulating microbial community composition

Simulation results show that the characteristics of the intermittent substrate feeding control the competition between the considered microbial species. Depending on the specific ecological characteristics of the species, both, the amplitude and the frequency of feeding pulses have a considerable impact on the composition of abundance and activity of the microbial community, and by this regulating dominance and coexistence/coactivity of species. However, within the tested range of these two parameters, variations of pulse amplitudes has a much higher influence on the competitive activity of species in the system (Tables 3a and 5a). On the other hand, although the frequency of feeding is of lesser importance for the competitive behavior, an intermittent supply of substrate is the prerequisite for Switchers to coexist with or to dominate over Growers, while at any constant substrate supply Growers would dominate over Switchers due to their better growth performance (Table 1). These characteristics of the feeding pattern determine a time window during which a given species is able to grow. To which extent this species is able to use this opportunity depends on its activity level or ability to react, respectively, and on the ability of any competitors to consume parts of the available substrate. It is also worth noticing that the time averaged carbon supply rate (i.e. amplitude × frequency) is no useful predictor for the composition of the simulated microbial communities. The parameter set describing the growth, activity and dormancy behavior is not only controlling the competitiveness of the single species but also their ability to follow with their activity the frequency of the external feeding, which leads to the complex frequency response observed for the Growers' activity already in the single-species system. Such complex frequency response of the two species system is mainly restricted to conditions allowing for both: a complex frequency response already for the Growers' activity as single species and significant abundance of Growers (dominance or coexistence) in the multi-species system. Initial conditions (i.e. starting concentration of species) had no impact on the shown results indicating that for the long-term behavior of the system only external constraints (here the feeding pattern) are responsible and that the 'history' of the system is of minor/no importance if these constraints have constant characteristics for a sufficiently long time. The latter might be much longer than observed for natural environments which explains why for such environment the 'history' of the microbial community might be of relevance (e.g. Hawkes and Keitt 2015).

The presented simulation scenarios are designed to be a set of numerical experiments where the substrate supply regime was systematically varied while all parameters describing microbial dynamics remained unchanged. Certainly, a variation of the latter parameters would have affected the competitive behavior of the simulated bacterial species and might have resulted in different coexistence/dominance observations. In a previous study (Stolpovsky et al. 2011), the reactivation yield

( $Y_{\text{reac}}$ ) and the thermodynamic threshold for de-/reactivation ( $G_0$ ) were found to be most relevant for the amount of active biomass at all intermediate substrate regimes, while at low substrate supply frequencies also the deactivation rate parameter ( $m_{\text{deac}}$ ) was relevant. Given the number of simulated scenarios and the simulation time required to cover the relaxation of the multi-species systems towards a quasi-steady-state any systematic analysis of the sensitivity of the bacterial community composition towards variations of individual parameters (i.e. an exploration of the entire parameter space of more than 10 dimensions) was beyond the scope of this study. However, the present study shows that for realistic assumptions concerning the behavior of bacterial species a coexistence of competing species is possible and can be linked to the characteristics of the intermittent substrate supply. The observations presented in this study were derived from the simulation of three different bacterial species and these results show that different two-species combination may coexist (i.e. Growers and Switcher; and Growers and Intermediates) but obviously not all two-species combination would show such behavior. To which extent such observations can be done also for other species (i.e. other parameter combinations) and which trade-offs between growth and dormancy performance are most competitive at given environmental conditions has to be determined in future studies.

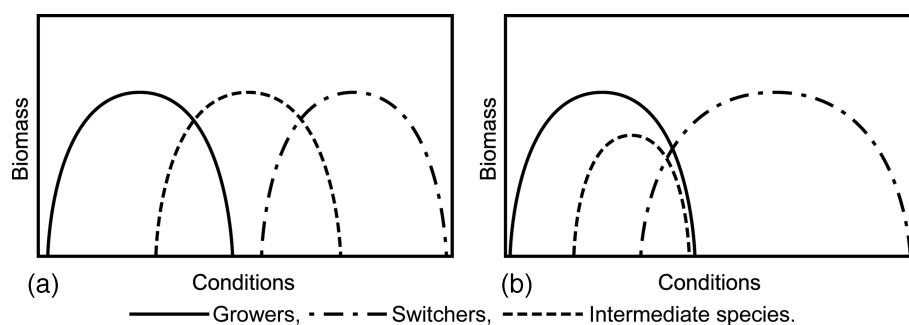
### Ecological implications

The coexistence of competing species is a known phenomenon in soils and similar systems allowing for a spatial differentiation of habitats and leading to the formation of different niches (Adler, HilleRisLambers and Levine 2007; Stolpovsky, Gharasoo and Thullner 2012). The presented simulation results are in line with ecological studies showing that recurrent resource pulses promote the coexistence of competing species (Chesson 1994; Holt 2003) and that temporal variability is an important control factor for maintaining biodiversity (Yi and Dean 2013). This also confirms previous modeling results (Stolpovsky et al. 2011) showing that intermittent feeding conditions with environmentally relevant frequencies can lead to a long term coexistence of competing microbial species even in the absence of spatial differentiation of habitats, i.e. in a well-mixed batch system. Simulations also confirm common observations that active biomass represents only a subfraction of the total microbial biomass (Lennon and Jones 2011), which also explains the difference observed for some scenarios in the composition of active and total biomass. Although there are more scenarios or feeding pulse characteristics allowing for coexistence than for a coactivity of competing species, there are quite a number of tested conditions allowing for more than one competing species to contribute to the total activity of the community. For the two-species system (and to lesser extent for the three-species system, too) the dependence of the activity patterns on pulse amplitudes confirm predictions from intermediate disturbance hypothesis (Wilkinson 1999) with moderate pulse amplitudes best suited to allow for a coactivity of species. These observations are in agreement with disturbance experiments exhibited in various natural macro- and microbial systems (Scholes, Warren and Beckerman 2005; Catford et al. 2012; Hall et al. 2012). Surprisingly, contrary to the amplitude, the frequency of the pulses has—for the tested range of values—less influence of the composition of the resulting communities. This is also in so far interesting since in lab experiments commonly disturbances frequencies rather than pulse amplitudes are manipu-

lated (Scholes, Warren and Beckerman 2005; Hall et al. 2012). It appears that in case of small pulses the ability of the Growers to grow faster and more efficiently allows them to consume most of the substrate and to compensate the delayed reactivation. For a larger pulse the faster and more effective reactivation and lower energy requirements allow Switchers to be active in sufficient amounts to suppress Growers, and effect similarly discussed for annual plant species responding to rain pulses (Chesson 1994). If these processes take place the same way during each single feeding cycle it would to some degree explain the limited influence of the pulse frequency. However, for many scenarios a rather irregular response of the microbial dynamics to the external feeding frequency has been observed which challenges such straightforward explanations of the observed activity patterns. This irregular frequency response also shows that individual species especially when within interacting communities can exhibit a temporal behavior which is controlled by external forcing dynamics but does not allow establishing a direct causality link. A phenomenon which might also be relevant in the analysis of temporal fluctuations observed in many existing natural ecosystems making it difficult to link behavior to disturbance regime (Fox 2013; Tonkin, Death and Barquín 2013). Such irregular frequency responses of the Switcher have been found also in cases where the abundance and activity of the Growers are considered as negligible although in single species systems the Switchers have shown regular frequency responses at all tested conditions. This emphasizes the need to assess as many as possible of the species and processes of an ecological system to understand its dynamics and functioning. The potential relevance of species with negligible abundance also becomes apparent in the comparison of the community composition of the two- and the three-species systems. While for most of the tested scenarios introduction of the Intermediate species does not change the composition patterns of active (71 out of 91 scenarios) or total biomass (60 out of 91 scenarios), those scenarios exhibiting a change do not only show the expectable results of Intermediate species coexisting with one or both of the other species: for some scenarios (frequency  $\leq 0.02 \text{ h}^{-1}$ ; concentration =  $5 \cdot 10^{-5}$  to  $1 \cdot 10^{-4} \text{ M}$ ) a community formerly dominated solely by Growers in the two-species system turns by introducing a subdominant and, thus, potentially undetectable species suddenly into exhibiting a coexistence of Growers and Switchers under the same environmental conditions. Similarly, for one scenario (frequency =  $0.01 \text{ h}^{-1}$ ; concentration =  $1 \cdot 10^{-6} \text{ M}$ ) the coexistence of Growers and Switchers for the two-species system turns into a Grower dominated community for the three-species system. For these scenarios the introduction of the Intermediates as an additional competitor has affected the relative advantage or disadvantage between Growers and Switchers in sense that the 'enemy of my enemy is my friend'. When regarding these results the other way round: the ecological consequences for our findings would give evidence to that the coexistence of important key species, and thus, the ecosystem services they provide, can completely fail in an ecosystem if some subdominant and apparently unimportant species become extinct by e.g. human activities.

An opposite situation is observed in simulations with a low DOC concentration (favorable conditions for Growers in two-species system) where we can observe either quantitative coexistence of Growers and Switchers or common quantitative coexistence of all three species (Table 5b).

Although in soils the competition between species is controlled by many other factors, too, the supply of suitable carbon substrate is one of the main drivers of the activity of



**Figure 2.** Distribution of niches. Here 'Conditions' is the schematic representation of a combination of factor determining microbial activity, i.e. amplitude and frequency of feed events. (a) Theoretical prediction (Loreau 2001), in this particular case this assumption is based on extrapolation of the results shown in Table 3 for three species, (b) niches as found in the simulated scenarios.

different microbial species and thus of their ability to compete with each other. Our results show that understanding such competitive behavior requires consideration of the temporal dynamics of the carbon supply and potentially of other environmental constraints and that time averaged values of these constraints may not provide sufficient information to understand the dynamics and competitive behavior of soil microorganisms. Analyzing microbially driven processes in soils does furthermore also require consideration of spatial heterogeneities in the distribution of the microorganisms (Harms and Bosma 1997; Dechesne et al. 2010; Hesse et al. 2010). While such spatial differentiation is not considered in this study, the presented results are still applicable to individual microbial habitats as long as at the scale of the habitats the abundance of microorganisms is determined by their activity in response to the local environmental conditions and not by spatial processes such as microbial transport or motility.

### Comparison of ecological niches

In accordance to the ecological niche theory, species may occupy only subunits of the habitat containing the environmental conditions that enable them to survive (potential versus realized niche). Depending on competition strength under the given environmental conditions they dominate over or coexist with other species (Sillero 2011). As already shown above, the two species system allows distinguishing between conditions (each defining a potential niche) leading to a dominance of either Growers or Switchers with coexistence/coactivity found mainly at the transition between the conditions allowing for single species dominance. This is in agreement with considering separated and only partially overlapping niches for each single species (Loreau 2000). When the third, Intermediate species is introduced it does not affect the dominance patterns of Growers and Switcher, i.e. their abundance is not affected by the presence of the Intermediates. The only exceptions—besides the few examples discussed above—are some scenarios where a coexistence/coactivity of Growers and Switchers is replaced by Growers and Intermediates, indicating a slight reduction in the realized niche size of the Switchers. In all other cases, the Intermediates only represent an additional contribution to the community not being able to outcompete the other species and to occupy their own niche—as suggested by niche theory (Gage 1996). As a coexistence of Intermediate and Switchers only is not observed, the Intermediates are obviously limited to intruding areas where Growers dominate (Fig. 2).

Dormancy is known to be one of the relevant bacterial strategies (Lennon and Jones 2011) and under different conditions it

could be replaced by or combined with any other advantage, for instance high efficiency of biomass building or bacterial 'educability'—their ability to evolve adapting to a new set of conditions. Considered in this context, the Intermediates represent a generalist species while Growers and Switchers represent end members of specialist species, the obtained simulation results suggest that when in competition with both end members a generalist strategy does not lead to any advantages in case of regularly occurring intermittent environmental conditions. Similar observations were presented in Legan and Owens (1988). Further investigations will have to show if irregular conditions (irregular changing amplitudes and/or frequencies of feeding pulses; e.g. Wardle 1995) might lead to different observations.

### SUMMARY AND CONCLUSION

In the present study we showed that intermittent environmental conditions, represented here by the feeding regime, have a high impact on the composition of microbial communities. Depending on the frequency and magnitude of the regularly applied feeding pulses, communities of only up to three different competing microbial species may exhibit rather complex dominance and coexistence patterns and may also exhibit dynamics different from the external forcing. The study also revealed that species abundances below the threshold value of 1% of the total community (molecular profiling method, Blackwood et al. 2007) may still have an important impact on the competitive performance of the dominating species.

The presented results are theoretical predictions and the discussed hypotheses eventually need to be tested experimentally. However, the shown observations do suggest that the characterization of as many species from a community as possible might be necessary to understand the behavior of the community as a whole, and the behavior of its key players responsible for any ecosystem services. This raises the question for the best theoretical approaches to describe the functional dynamics of a complex microbial system especially in highly dynamic systems like soils: What is the required number of microbial species we need to take into account to obtain sufficiently precise theoretical results? Future research must show which might be the best compromise between the accuracy of the results, the computational demands and the information needed for simulating the behavior of multi-species microbial communities.

### SUPPLEMENTARY DATA

Supplementary data are available at FEMSEC online.



## FUNDING

This study was supported by the Helmholtz Association grant VG-NG-338 (GReaT MoDE) and the program topic 'CITE-Chemicals in the Environment'.

**Conflict of interest.** None declared.

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