Modelling the survival of bacteria in drylands: the advantage of being dormant

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We introduce a simple mathematical model for the description of 'dormancy', a survival strategy used by some bacterial populations that are intermittently exposed to external stress. We focus on the case of the cyanobacterial crust in drylands, exposed to severe water shortage, and compare the fate of ideal populations that are, respectively, capable or incapable of becoming dormant. The results of the simple model introduced here indicate that under a constant, even though low, supply of water the dormant strategy does not provide any benefit and it can, instead, decrease the chances of survival of the population. The situation is reversed for highly intermittent external stress, due to the presence of prolonged periods of dry conditions intermingled with short periods of intense precipitation. In this case, dormancy allows for the survival of the population during the dry periods. In contrast, bacteria that are incapable of turning into a dormant state cannot overcome the difficult times. The model also rationalizes why dormant bacteria, such as those composing the cyanobacterial crust in the desert, are extremely sensitive to other disturbances, such as trampling cattle.

Keywords: cyanobacteria; dormancy; mathematical modelling

1. INTRODUCTION

Bacterial populations in natural settings are exposed to stresses of various kinds. The stress may result from lack of nutrients, exposure to extreme temperatures, water scarcity, or fluctuations in other environmental parameters. Various survival strategies are then employed by bacteria to tolerate stress. Cooperative behaviour, resulting in pattern formation, is one strategy that has attracted considerable recent interest (Ben-Jacob *et al.* 2000). Another strategy, currently under investigation, consists of the transformation of bacteria from an active to a dormant state (Stevenson 1978; Roszak & Colwell 1987). Bacteria in the dormant state function at very low metabolic rates and do not undergo cell division. When the stress is released the bacteria resume cell division; the transformation to a dormant state considered here involves adaptive mechanisms but not morphological differentiation such as sporulation (Roszak & Colwell 1987; Barer & Harwood 1999). Bacteria following this strategy are sometimes referred to as viable but non-culturable (VBNC) (Oliver 1993; Kell *et al.* 1998; Barer & Harwood 1999; Colwell & Grimes 2000). They have been observed in fresh water, in marine environments and in the soil (Roszak & Colwell 1987). Temperature fluctuations may also lead to dormancy and resuscitation, as in the case of *Vibrio vulnificus* (Oliver 1995).

A different example of dormancy is provided by cyanobacteria (blue-green algae) in drylands. In this case, the transformation to a dormant state occurs as a result of

water stress, possibly resulting in desiccation of bacteria (Potts 1994). Cyanobacteria constitute a major component of microphytic soil crusts, and play an important role in immobilizing sand dunes and in sustaining dryland ecosystems (Danin *et al.* 1989; West 1990; Lange *et al.* 1992; Verrecchia *et al.* 1995).

We develop a simplified mathematical model to describe the survival strategy of dormancy-capable bacteria considering, in particular, the effect of a time-dependent stress. We therefore construct a set of ordinary differential equations modelling the temporal variability of a population of active and dormant bacteria whose dynamics are coupled to the soil humidity field. Analogous models can be built for different types of stress fields, such as nutrient density, or temperature.

2. A MODEL FOR DORMANCY

To study the performance of a survival strategy based on dormancy, we compare the dynamics of two different types of bacterial populations. One population is assumed to be capable of becoming dormant and is described by two state densities, $A(t)$ and $D(t)$, of active and dormant bacteria, respectively. The other population is assumed to be genetically incapable of becoming dormant, and the bacterial density is defined by a single state, $B(t)$. For the sake of brevity, and with a small abuse of terminology, we shall call the latter bacteria 'sleepless'.

(a) *Dynamics of dormancy-capable bacteria*

The active and dormant bacterial state densities $A(t)$ and $D(t)$ are assumed to be coupled to a stress field, $W(t)$,

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that represents soil humidity close to the surface. The density of active bacteria grows logistically at a rate $\mathscr{G}(W)$, whereas the density of dormant bacteria does not grow (as the latter do not undergo cell division). We further assume that for high stress (i.e. low soil humidity, W), active bacteria tend to become dormant. Conversely, for low stress (i.e. large W), dormant bacteria tend to transform back into active bacteria.

We quantify this behaviour by introducing a function, $\mathscr{T}(W - W_{AD})$, that is proportional to the probability for dormant bacteria to become active. The probability for active bacteria to become dormant is assumed to be $1 - \mathscr{T}(W - W_{AD})$. Here, we have introduced a critical value of the stress field, W_{AD}, which separates two types of behaviour. Below W_{AD}, active bacteria tend to become dormant. Above W_{AD}, dormant bacteria tend to transform into active ones. In principle, the transformation from active to dormant ($A \rightarrow D$) and vice versa ($D \rightarrow A$) could take place at different threshold values of the stress field. In the present case, however, consideration of different thresholds does not introduce any new qualitative behaviour. We therefore introduce a single threshold W_{AD}.

We assign mortality rates to the two bacterial states. Because dormant bacteria do not undergo cell division, a dormant strategy can be potentially advantageous only if the mortality of dormant bacteria, α_D, is lower than that of active bacteria, α_A. This choice is consistent with the fact that dormant bacteria exhaust energy reserves very slowly, by maintaining low levels of metabolic activity (Roszak & Colwell 1987).

Our model for dormancy-capable bacteria (model I) thus reads:

$$\frac{dA}{dt} = \mathscr{G}(W)A(1 - A - D) - \alpha_A A - \varepsilon\{[1 - \mathscr{T}(W - W_{AD})]A - \mathscr{T}(W - W_{AD})D\}, \tag{2.1}$$

$$\frac{dD}{dt} = -\alpha_D D + \varepsilon\{[1 - \mathscr{T}(W - W_{AD})]A - \mathscr{T}(W - W_{AD})D\}, \tag{2.2}$$

where ε measures the transformation rate from active to dormant and vice versa.

Next, we need to specify the forms of $\mathscr{G}$ and $\mathscr{T}$. The explicit form of $\mathscr{G}(W)$ can of course be complicated, and it may vary from one type of stress field to another. In the context of dryland crusts, we may simply assume that the growth rate of active bacteria is proportional to the soil water density,

$$\mathscr{G}(W) = W. \tag{2.3}$$

For the function $\mathscr{T}$, we choose an antisymmetric form such that $1 - \mathscr{T}(W - W_{AD}) = \mathscr{T}(W_{AD} - W)$, and we use either a simple step function, $\mathscr{T}(W - W_{AD}) = \theta(W - W_{AD})$, or the smooth form

$$\mathscr{T}(W - W_{AD}) = \frac{1}{2}\left[\tanh\frac{W - W_{AD}}{s}\right], \tag{2.4}$$

where $1/s$ measures the steepness of the transformation function $\mathscr{T}$. In the limit $s \rightarrow 0$, the form (equation (2.4)) of $\mathscr{T}$ reduces to the step function.

When the stress field W is externally imposed, and there is no feedback of either A or D on W, the set of equations

(2.1)–(2.4) is closed. In most cases, however, the bacterial population feeds back on the stress field, e.g. by consuming water or nutrients. In this case, equations (2.1)–(2.4) should be supplemented by an evolution equation for the stress field. Motivated by the behaviour of microphytic crusts in drylands, we write

$$\frac{dW}{dt} = P(t) - W[1 - \mu(A + D)] - \gamma WA. \tag{2.5}$$

Here, $P(t)$ is a source term representing precipitation. The second term on the right-hand side describes losses due to evaporation or infiltration in the soil. The last term on the right-hand side describes consumption of water by the active bacteria. The parameter γ is the rate of water consumption per unitary bacterial density, and μ measures the fractional decrease in water losses due to the presence of bacteria. Note that, in equations (2.1), (2.2) and (2.5), the variables A, D and W and the time coordinate, have been scaled to dimensionless quantities.

A crucial assumption of this simple model is that water losses are reduced by the presence of bacteria. This is motivated by the observation that the presence of a microphytic crust tends to increase soil humidity, due to the swelling properties of the crust itself.

(b) Sleepless bacteria

At this point, we introduce a second model, model II, representing bacteria that do not have the dormant-state strategy to survive stress. The model is simpler and reads

$$\frac{dB}{dt} = \mathscr{G}(W)B(1 - B) - \alpha_B B, \tag{2.6}$$

$$\frac{dW}{dt} = P(t) - W(1 - \mu B) - \gamma WB, \tag{2.7}$$

where α_B is the mortality of the sleepless bacteria. In the following, we use $\alpha_B = \alpha_A$ and the same form of $\mathscr{G}(W)$ defined in equation (2.3). As mentioned above, we will use this model as a reference point in assessing the performance of the survival strategy described by model I.

3. STEADY-STATE BEHAVIOUR UNDER CONSTANT STRESS

It is instructive to consider first a constant precipitation, P, and to evaluate the steady states of model I and model II and their linear stability. Consider first model I in the limit $s \rightarrow 0$, where the transformation function $\mathscr{T}$ becomes a step function. At very low precipitation values, $P < P_1 = \alpha_A + \varepsilon$, bacteria die and the only stable steady state is $(A_0, D_0, W_0) = (0, 0, P)$. Above the threshold, P_1, bacterial life becomes possible, as reflected by the appearance of a new stable steady state given by (A_1, D_1, W_1) when $W = W_1 < W_{AD}$ and by $(A_2, 0, W_2)$ when $W = W_2 > W_{AD}$, where

$$A_1 = \frac{P - \alpha_A - \varepsilon}{P(1 + \varepsilon/\alpha_D) - (\alpha_A + \varepsilon)[\mu(1 + \varepsilon/\alpha_D) - \gamma]}, \tag{3.1}$$

$$D_1 = \frac{\varepsilon}{\alpha_D}A_1, \tag{3.2}$$

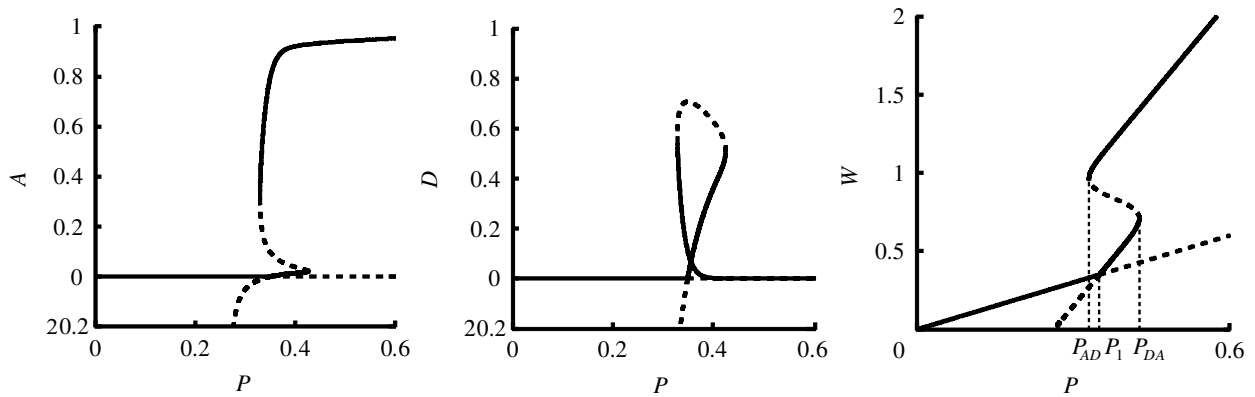$$W_1 = \frac{P}{1 - [\mu(1 + \varepsilon/\alpha_D) - \gamma]A_1}, \tag{3.3}$$

Figure 1. Steady-state solutions of model I (equations (2.1)–(2.5)) as a function of the imposed precipitation P. Solid and dashed lines indicate stable and unstable states, respectively. The values of the parameters are given in the text.

$$A_2 = \frac{P - \alpha_A}{P - \alpha_A(\mu - \gamma)}, \tag{3.4}$$

$$W_2 = \frac{P}{1 - (\mu - \gamma)A_2}. \tag{3.5}$$

The first fixed point represents a population of mostly dormant bacteria, whereas the second fixed point reflects a state where water is abundant and all bacteria are active and potentially replicate. The stability of these steady states is obtained by computing the eigenvalues of the Jacobian for the respective steady states. We therefore revert to a case where $\mathscr{T}$ has the smooth form (equation (2.4)). The steady states and their stability are then computed by using the software package Auto97 (Doedel *et al.* 1997).

Figure 1 shows the steady-state densities A and D and the soil humidity W as a function of the precipitation P, for $\alpha_A = 0.1$, $\alpha_D = 0.01$, $\varepsilon = 0.25$, $\gamma = 0$, $\mu = 0.75$, $W_{AD} = 1$, and $s = 0.1$. For these parameter values, we observe both saddle-node bifurcations (such as those leading to the S-shaped curve for W) and transcritical bifurcations. The crossing of the two stable branches in the plot for D is an effect of projection onto the two-dimensional (P,D) plane, as A has different values along the two branches. The behaviour for other values of the parameters (in particular, for $\gamma \neq 0$) is similar.

These plots show a bistable range, which can lead to hysteretic behaviour even in the simple model adopted here. Assume that we start with a large precipitation P on the active branch $(A_2,0,W_2)$ and gradually decrease P. When W_2 decreases to below the threshold W_{AD} at $P = P_{AD}$, the system jumps to the other branch, and a large fraction of the bacterial population becomes dormant $(D_1 \gg A_1)$. If P increases again, the system remains on the branch $(A_1,0,W_1)$ until W_1 reaches W_{AD} at $P = P_{DA} > P_{AD}$, where it jumps back to the upper branch and all the bacteria adopt an active state. This hysteretic behaviour reduces the sensitivity of the bacterial population to minor fluctuations in precipitation. Only major changes in soil humidity trigger a change from dormancy to activity, and vice versa. Note that different precipitation thresholds, P_{AD} and P_{DA}, are obtained from a unique internal stress threshold W_{AD}.

Model II does not possess a bistable region for any parameter range and hysteretic behaviour is not possible.

The equilibria of model II are considerably simpler, and they are shown in figure 2 for the same parameter values used above. The system again possesses a zero-life branch $(B_0,W_0) = (0,P)$, which is stable at low P. As P exceeds $P_2 = \alpha_B$ this branch loses stability to the stable branch (B_2,W_2) where

$$B_2 = \frac{P - \alpha_B}{P - \alpha_B(\mu - \gamma)}, \tag{3.6}$$

$$W_2 = \frac{P}{1 - (\mu - \gamma)B_2}. \tag{3.7}$$

Model II does not possess a bistable region for any parameter range and hysteretic behaviour is not possible.

If there are no fast variations in P, the dormant-state strategy is clearly inferior to the sleepless strategy. Given the same mortality $\alpha_A = \alpha_B$, the total bacterial density $A + D$ in the first case is always smaller (when $P_2 < P < P_{DA}$) or equal to the sleepless bacterial density B for all values of P.

4. DYNAMIC RESPONSE TO INTERMITTENT STRESS

Dormancy introduces one advantage and one disadvantage with respect to sleepless bacteria. The advantage is that the mortality factor for dormant bacteria, α_D, is lower than that for active bacteria, α_A. If this were not the case, then there would be no advantage in developing dormancy as a strategy for survival. Conversely, dormant bacteria do not undergo cell division, and this is clearly a disadvantage with respect to sleepless bacteria. Thus, the relative survival efficiency of the two strategies depends on a balance between these two factors. The main parameters controlling these effects are the ratios of mortality to growth rates, i.e. α_A and α_D in our non-dimensional description, and the critical value of soil humidity, W_{AD}.

For a low but constant precipitation rate, the analysis reported in the previous section shows that sleepless bacteria are favoured. For the parameter values used in figure 1, we see that when the precipitation rate is between about $P_2 = 0.1$ and $P_1 = 0.35$ (in our non-dimensional units), sleepless bacteria achieve a small but finite density, while bacteria that turn dormant are not able to become active again (due to the small precipitation rate) and are
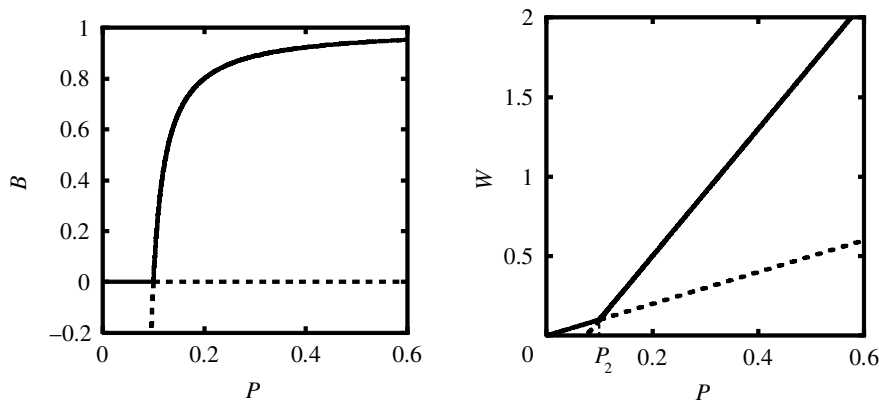
Figure 2. Steady-state solutions of model II (equations (2.3)–(2.7)) as a function of the imposed precipitation P. Same details as in figure 1.
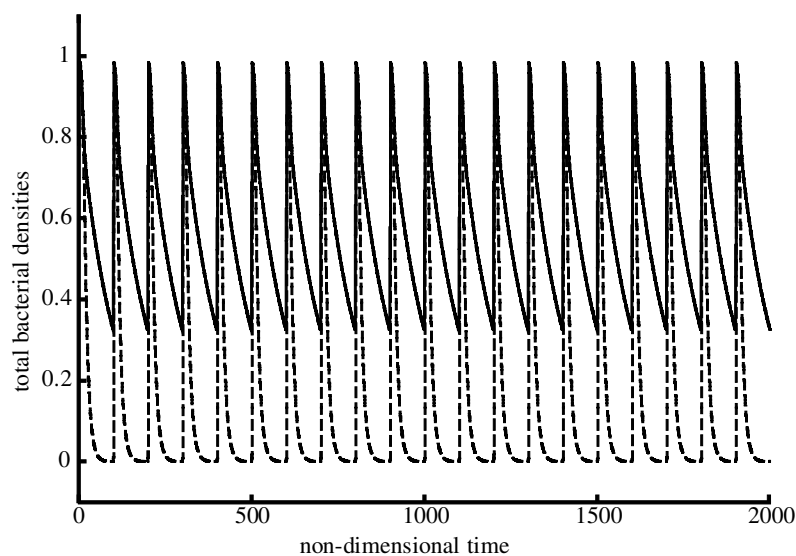

Figure 3. Time evolution of total bacterial densities $A + D$ (solid line) and B (dashed line) for intermittent precipitation with an average precipitation rate $P = 0.12$. Other parameter values are described in the text.

doomed to die out (in our simple model, we do not take into consideration spatial dependence and dispersal). However, for $P < 0.1$, both types of bacteria cannot survive. Thus, in the case of constant external conditions, the dormant-state strategy apparently does not introduce any real advantage.

In contrast, the capability of alternating between activity and dormancy becomes crucial for survival when external conditions have a significant temporal variability. This is a typical situation in deserts where rain comes only in intense and sporadic events, in tidal and estuarine environments where large salinity fluctuations can occur, and in many other environments where dormant bacteria have been observed. In such a situation, bacteria that become dormant can overcome the difficult times, and the strategy of dormancy displays all its survival power. In the following, we consider the temporal variability of the two types of bacteria for the same parameter values used above, for a case where precipitation is different from zero only for a fraction $f = 0.01$ of the time. That is, the precipitation has a finite value P_r for one time unit, and it is zero for the subsequent 99 time units. To compare with the

constant precipitation case, we shall refer to the average precipitation rate $P = fP_r$. For simplicity, in the following numerical integrations we use a step-function form ($s \rightarrow 0$) for the transformation function $\mathcal{T}(W - W_{AD})$.

Figure 3 shows the time evolution of the total bacterial densities (i.e. active plus dormant bacteria, $A + D$, and sleepless bacteria, B) for an average precipitation rate $P = 0.12$. Recall that, for a constant precipitation rate, this value of P leads to the disappearance of the dormant bacteria and to survival of sleepless bacteria. For intermittent precipitation, however, the bacteria that are capable of becoming dormant survive better than sleepless bacteria, and their average total density becomes larger. In addition, the density of dormant bacteria remains relatively large during dry periods, whereas the density of sleepless bacteria becomes so low that the population is prone to local extinction due to environmental stochasticity (e.g. Halley 1996).

Even more strikingly, the strategy of dormancy allows for survival of bacterial populations below the critical precipitation threshold P_2. Figure 4a shows the total bacterial densities for an average precipitation rate $P = 0.08$. For a
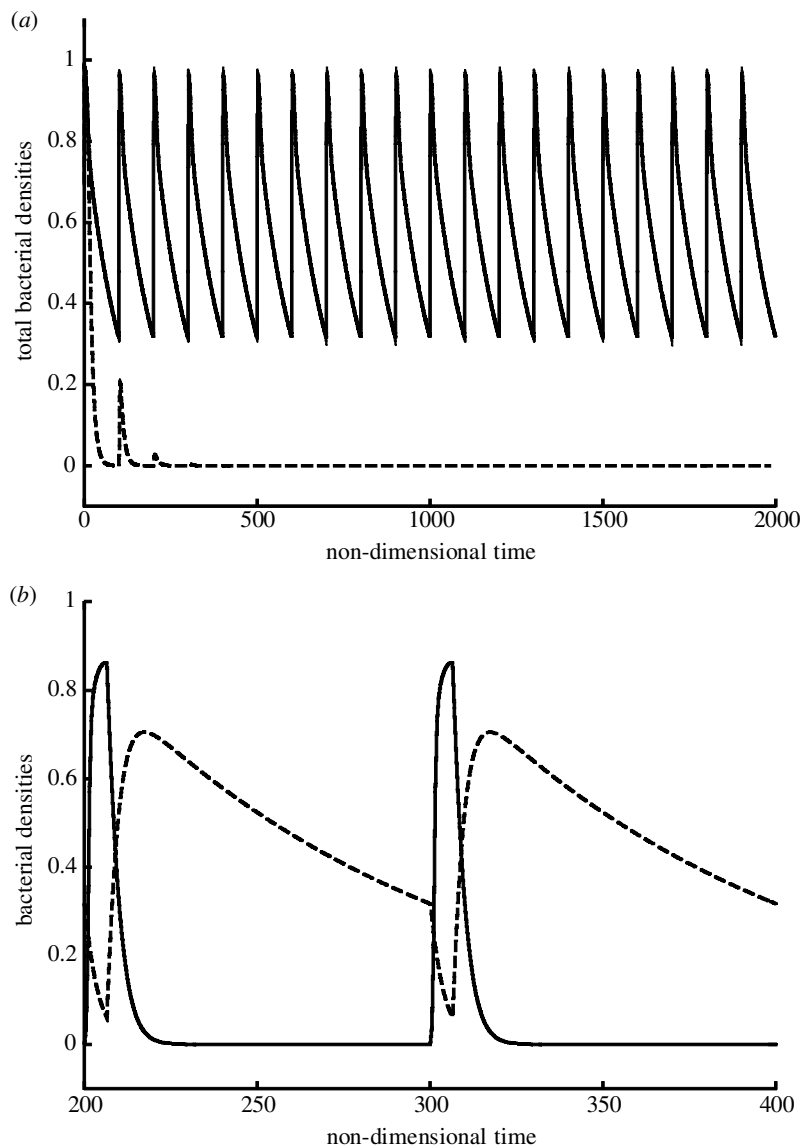
Figure 4. Time evolution of bacterial densities for intermittent precipitation with average precipitation rate $P = 0.08$. (a) Total bacterial density for model I ($A + D$, solid line) and bacterial density for model II (B, dashed line). (b) Active (A, solid line) and dormant (D, dashed line) bacterial densities in model I. Other parameter values are described in the text.

constant precipitation rate of this entity, neither sleepless nor dormant bacteria could survive (see figure 1). For intermittent precipitation, sleepless bacteria still decay and disappear, whereas bacteria that become dormant can survive the dry periods and maintain a stable, albeit oscillating, population. Figure 4b illustrates the two components of the population, i.e. active bacteria, A, and dormant bacteria, D. During the periods of intense rain, dormant bacteria turn into active ones and start replicating, generating a bacterial bloom. When the rain ceases, bacteria revert to the dormant state, and undergo a slow decay (determined by the value of α_D) until the next rainfall episode. This dynamics is associated with a hysteretic cycle in parameter space: the precipitation P oscillates around the critical value and it forces the system to jump between different stable branches in the bifurcation diagram. For the same parameter values, sleepless bacteria cannot resist the prolonged period of dry conditions, and disappear.

5. DISCUSSION AND CONCLUSIONS

We have developed a simple model to rationalize the survival strategy of the microphytic crust in drylands. The model is based on an alternation between active and dormant bacterial states. Although almost metaphorical, this minimal model has the merit that it expresses, in simple mathematical terms, the dynamics of bacteria that share a dormant-state strategy for survival, and it hopefully permits a deeper understanding of this strategy. Although derived for bacteria in drylands, we believe that appropriate modifications of this model can be used to study dormancy in other types of bacteria exposed to external stress.

The main conclusion of this work is that the dormant-state strategy becomes crucially important when the bacteria are exposed to wildly varying environmental conditions. When rain comes only in intense episodes, spanning a small fraction of time, as often happens in desert areas, the advantage of becoming dormant mani-

fests all its survival power. In this case, even if the average precipitation rate is below the critical threshold for survival of active bacteria, a dormant population can overcome the hard times as Sleeping Beauty waiting to be awoken.

We have focused on a particular set of parameters but the same qualitative behaviour can be expected to hold under more general conditions. In general, the exploration of parameter space shows that dormancy enhances survival when the mortality of dormant bacteria, α_D, is significantly lower than that of active bacteria, α_A. It is also important that the transition from dormant to active, and vice versa, takes place rapidly enough. Thus, we can say that in our simple model dormancy is a good strategy only when dormant bacteria are less prone to mortality than active ones, and when they can promptly respond to changes in environmental conditions.

Finally, a comment on the vulnerability of microphytic crusts to external disturbances. Trampling, for example, may lead to rapid destruction of cyanobacterial crusts (Karnieli & Tsoar 1995). In our model, the effect of external disturbances can be mimicked by an additional mortality factor, affecting both the active and the dormant bacteria. The net effect of trampling is thus to increase both α_D and α_A. Given the sensitivity of the system to the value of α_D, and the lack of replication in the dormant state, it is clear that any external disturbance capable of significantly increasing the mortality of the dormant state can lead to a catastrophic response of the system, and to the rapid disappearance of the bacterial populations.

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