The actin cytoskeleton is a key component in the machinery of eukaryotic cells, and it self-assembles out of equilibrium into a wide variety of biologically crucial structures. Although the molecular mechanisms involved are well characterized, the physical principles governing the spatial arrangement of actin filaments are not understood. Here we propose that the dynamics of actin network assembly from growing filaments results from a competition between diffusion, bundling and steric hindrance, and is responsible for the range of observed morphologies. Our model and simulations thus predict an abrupt dynamical transition between homogeneous and strongly bundled networks as a function of the actin polymerization rate. This suggests that cells may effect dramatic changes to their internal architecture through minute modifications of their nonequilibrium dynamics. Our results are consistent with available experimental data.
he cytoskeleton of living cells is an extremely dynamic system, of which actin is a vital component. Actin monomers are continuously assembled during polymerization; at the same time, actin filaments are bundled together by crosslinkers to form a large variety of structures. Tight bundles thus appear in filopodia, in stress fibres and in the contractile ring involved in cell division, whereas homogeneous networks are found in the cell cortex and in the lamella. Understanding the fundamental mechanisms that determine the formation and the morphology of the actin cytoskeleton is thus necessary to explain how the cell regulates its own shape, internal structure and motility.

A tool of choice to characterize these structures is to isolate a few essential ingredients and study the result of their interactions. Bottom-up experiments thus put one type of crosslinker in solution together with purified actin, resulting in in vitro reconstituted actin networks. As filaments grow and become crosslinked into bundles, the morphology of the resulting networks strongly depends on their assembly kinetics: different protocols leading to the same filament number and lengths through different kinetic pathways thus result in different structures, demonstrating that the observed phases are out of equilibrium. The structure of keratin networks similarly results from the competition between filament elongation and bundle formation. Despite the highly dynamic nature of these experiments, theoretical attempts to describe such networks have largely relied on equilibrium physics, modelling morphological transitions as the result of the competition between crosslinker binding and thermal fluctuations. In many cases, however, the bundling of two or more filaments by hundreds of crosslinkers can involve energies of the order of thousands of $k_B T$, indicating that equilibrium thermal fluctuations alone cannot account for the presence of structural disorder in the final networks.

Here we propose a theoretical framework to account for the architecture of actin networks from the dynamics of their assembly. We study the simplest situation of actin structures assembling de novo from a fixed number of initially short filaments, mirroring existing in vitro experiments, as well as, for example, cytoskeletal reassembly in a newly formed bleb, and actin recovery following drug treatment. We consider a system of polymerizing and diffusing filaments that tend to bundle irreversibly when they come into contact (Fig. 1a), as observed experimentally. Bundling can however be sterically blocked by the presence of other filaments (Fig. 1b) that becomes increasingly likely as the filaments elongate. At early times, the filaments are very short and diffusion is fast. Bundling thus proceeds unimpeded by steric constraints, and the number of bundles in the solution decreases over time as thicker bundles are formed through the merging of thinner ones. As a result, blocking becomes less likely and further bundling events are facilitated in a positive feedback mechanism. As the filaments grow, diffusion slows down and bundles come into contact more rarely. As a consequence, blocking finally outpaces reaction and the system becomes kinetically arrested. This basic mechanism allows us to formulate simple, experimentally testable scaling predictions for the bundle size and concentration, including an abrupt change in system behaviour upon kinetic trapping. We numerically validate these predictions in simulations of rod-like bundles over five orders of magnitude in concentration and four orders of magnitude in filament growth velocity, a much broader range than is accessible to existing detailed simulations. We thus develop a robust, easily extendable framework to describe the nonequilibrium physics of cytoskeletal network assembly.

**Results**

**A model for kinetic arrest based on filament entanglement.** We model actin bundles as impenetrable, infinitely thin, rigid rods in a homogeneous solution of crosslinkers. These rods grow at a constant velocity $v$, and their diffusion coefficient is given by $D \sim k_B T / \eta L$, in the Rouse approximation, where $v t$ is the length of a filament and $\eta$ is the viscosity of the surrounding solution. When two rods come within a distance $b$ of the order of the size of a crosslinker, they react with a rate $k$ to merge into a rod-like bundle as in Fig. 1a. Note that the chemical rate constant $k$ is associated with the crosslinker binding rate and not the filament merging time. Indeed, the latter is much shorter than the typical filament reaction time, as further detailed in the discussion. Although nonmerged bundles may be connected by a few crosslinkers, such connections are short-lived ($\sim 1$ s for $\gamma$-actinin) and we neglect them over the timescale of minutes involved in network formation. As a result, kinetic trapping in our model arises from steric entanglement between densely packed rods. This scenario is consistent with experimental evidence that entanglement can induce kinetic trapping in actin networks even in the absence of crosslinkers.

**Filament interactions involve several dynamical regimes.** We first develop a mean-field approach considering a homogeneous solution of isotropically oriented rods of concentration $c$. This concentration accounts for bundles of any thickness, including single filaments, which we see as ‘one-filament bundles’, and evolves according to

$$\frac{dc}{dt} = -r(c, L)c,$$

where $L = vt$ and $r(c, L)$ is the rate with which one rod bundles with any other.

In dilute systems, where the average distance between two rods is much larger than their length ($cL^2 \ll 1$), $r(c, L)$ is effectively due to a two-body interaction. We denote it by $r^{(2)}$ and estimate it separately in the case of reaction-limited and diffusion-limited systems. In a reaction-limited system, two rods within interaction range $b$ bundle at a rate $k$. As the probability for a rod to be...
within interaction range of another is \( \sim cbL^2 \), the total two-body rate of bundling is:

\[
\dot{r}_{\text{react}}^{(2)} = A_t k c b L^2,
\]

where \( A_t \) is a dimensionless prefactor of order one. In the diffusion-limited case, the orientation of the rods can rotationally diffuse over the whole sphere in a much shorter time than it takes them to come into contact with one another. The rate with which one rod encounters any other through diffusion is thus given by \( \dot{r}_{\text{diff}}^{(2)} \approx c DL \) (ref. 20). Therefore:

\[
\dot{r}_{\text{diff}}^{(2)} = A_d \frac{k_b T}{4},
\]

where \( A_d \) is another dimensionless prefactor. As evidenced by the different \( L \)-dependencies of \( \dot{r}^{(2)} \) in equations (2 and 3), while the rods grow, diffusion slows down relative to reaction and the dynamics transition from reaction limited to diffusion limited. This happens at a critical length \( L \sim L_c = \sqrt{k_b T / \eta k b} \).

In concentrated systems \( (cL^3 \geq 1) \) bundling is affected by the presence of surrounding rods, yielding a rate:

\[
r(c, L) = \dot{r}_{\text{diff}}^{(2)} (c, L) \left[ 1 - P_b (c L^3) \right],
\]

where the rate \( r^{(2)} \) at which bundling is attempted is given by equations (2 and 3) and \( P_b \) is the probability for the attempt to be blocked as in Fig. 1b. To determine the blocking probability, we note that \( 1 - P_b = (1 - p_b)N^{-2} \), where \( p_b \) is the probability for an individual, randomly placed rod to block the attempt, and \( N \) is the total number of rods in the system. To estimate \( p_b \), we consider two rods with tangent vectors \( n \) and \( \tilde{n} \) coming into contact at their midpoints. Denoting with \( V \) the volume of the system, with \( \tilde{n} \) the orientation of the third, potentially blocking rod and letting \( x(\tilde{n}, \tilde{n}') \) be the area of the bundling path pictured in Fig. 1b, the probability that the third rod intersects this path reads:

\[
p_b = \int d^2 \tilde{n}' x(\tilde{n}, \tilde{n}') / V \sqrt{1 - \tilde{n} \cdot \tilde{n}'},
\]

In the thermodynamic limit \( (N, V \to \infty) \) this yields \( 1 - P_b (c L^3) = \int_0^\pi d \theta \sin \theta \exp (- \pi c L^3 / V) \), which we plot in Fig. 2b. The blocking probability \( p_b \) becomes large at low concentration, accounting for the experimental observation that although bundling speeds up with increasing \( c \) at low \( c \) (because of binary collisions), the opposite trend is observed at higher concentrations (when three or more body blocking becomes predominant).

**Mean-field dynamical scenarios and final morphologies.** We now use our model to predict the final structure of a system of filaments. As shown in Fig. 2a, four different scenarios can develop, depending on the initial bundle concentration \( c_0 \), on the reaction rate \( k \) and on the polymerization velocity \( v \). As \( L(=0) = 0 \), the system always starts off in the \( cL^2 \ll 1 \) reaction-limited regime, implying, through equations (1 and 2):

\[
c(t) = \frac{c_0}{1 + (t/t_c)^{2}}
\]

with \( t_c \sim 1/(c_0 k b N^2)^{1/3} \). This solution predicts a crossover from \( c = c_0 \) for \( t \ll t_c \) to \( c \propto t^{-3} \) for \( t \gg t_c \). Scenario (1) (Fig. 2a, topmost line) applies to slow-reacting filaments \( (kb < v) \), for which blocking happens before this first transition. The concentration \( c \) thus never departs from its initial value \( c_0 \): a homogeneous network of single filaments of concentration \( c_0 \) is formed.

For fast-reacting filaments \( (kb > v) \) blocking takes over at a time larger than \( t_c \). Bundles thus form, and three possible scenarios can develop depending on \( c_0 \). Scenario (2) (Fig. 2a, second line from the top) describes cases where substantial bundling takes place before the system transitions from reaction limited to diffusion limited, that is, \( t_c < t_r < L/v, \) or equivalently \( c_0 > c_f = (kb N^{-1})^{3/2} \). Equation (5) is thus valid for \( t < t_c \) and the \( c \propto t^{-3} \) decay is valid for \( t_c < t < t_r \). As a result \( cL^3 \) remains constant while the rods grow, thus staying off blocking as long as \( t < t_c \). At \( t_c \) the system becomes diffusion limited, and equations (1 and 3) imply that the concentration decays as \( c \sim \eta / k_b T \) as long as \( cL^3 < 1 \). Blocking then induces kinetic arrest for \( cL^3 \sim 1 \), implying \( L-L_0 = \sqrt{k_b T / \eta v} \), or equivalently \( t \sim t_c = \sqrt{k_b T / \eta v} \), yielding a final concentration \( c_f \sim c_0 = \eta / k_b T t_0 = (\eta v / k_b T)^{3/2} \) independent of the initial concentration \( c_0 \). Scenario (3)
bundle. Read off from the white-filled symbols in Fig. 4a. In this figure the cross-sectional area of each rod is proportional to the number of filaments within the bundle. To facilitate visualization despite order of magnitude changes in filament concentrations, each of the four panels is taken from a different simulation with a concentration lower by several orders of magnitude.

Our results regarding the final morphology of the networks are summarized in Fig. 2c: in low-concentration ($c_\text{f} \ll c_0$) and/or slow-reacting ($kb \lesssim \nu$) systems, no bundling takes place. The final state is a homogeneous network of single filaments of concentration $c_\text{f} \sim c_0$. In contrast, in fast-reacting, high-concentration systems ($c_\text{f} > c_0$ and $kb > \nu$), the system evolves to a network of bundles with concentration $c_\text{f} \sim c_0$, independent of $c_0$ and a characteristic number of filaments per bundle equal to $c_0/c_\text{f} \sim c_0/c_0$. For $c_\text{f} \geq c_0$, going from a slow- to a fast-reacting system produces an abrupt shift from a homogeneous network of filaments to a network of bundles with a concentration lower by several orders of magnitude.

Brownian dynamics simulations. To assess the validity of our homogeneous, isotropic, mean-field dynamical scenarios, we conduct numerical simulations of our model. We simulate a solution of initially very short, randomly oriented, impenetrable rods and implement their growth as well as their standard Brownian dynamics with diffusion coefficients $D_L = k_b T/\eta L$, $D_{\perp} = D_L/2$ for their longitudinal and transverse translation, respectively, and $D_t = 6D_L/\nu^2$ for their rotation. For each time step, the algorithm assesses the probability for each rod to react with its closest neighbour (the ‘target’ rod) that is assumed to be fixed (the fixed rod is itself moved in a separate step). To estimate this probability, we write the Fokker–Planck equation describing the stochastic dynamics of the distance $d$ between the two rods in the limit where $d \ll L$ (cases that violate this condition are benign in practice, as they yield a negligible reaction probability anyway):

$$\frac{\partial}{\partial t} P(d, t) + D_{\perp} \frac{\partial^2}{\partial x^2} P(d, t) - 2kb \delta(x) P(d, t) = D_L \frac{\partial}{\partial d} \left( \frac{\partial P(d, t)}{\partial d} \right).$$

Here $P(d, t)$ is the probability distribution of $d \in [0, +\infty)$, and the right-hand side includes a sink term representing reactions between the two rods in the limit of a very short interaction range $b$. Equation (6) assumes the convention $\int_{-\infty}^{\infty} \delta(x) \, dx = 1/2$. This yields a probability of reaction between the two rods initially separated by $d_0$ over one time step $dt$ of the simulation:

$$P_{\text{attempt}} = \text{erfc} \left( \frac{d_0}{2 \sqrt{D_L} \, dt} \right) - \text{erfc} \left( \frac{d_0 + 2kb \nu t}{2 \sqrt{D_L} \, dt} \right).$$

Note that equation (7) is and must be fully valid even in cases where $d_0$ is of the order of the typical diffusion and reaction length scales $\sqrt{D_t \, dt}$ and $kb \nu t$.

In the case where bundling is indeed attempted, the algorithm determines if any blocking rod is present in the bundling path (Fig. 1). If there is one, bundling is aborted and the attempting filament is moved in close proximity to the closest blocking rod. If bundling is successful, the attempting rod is deleted, representing its merging with the target rod. In the case where bundling is not attempted, diffusion proceeds as in normal Brownian dynamics, although with a reflecting boundary between rods to ensure their impenetrability. Note that a single blocking rod can never derail bundling if it is not itself entangled with the rest of the network. Indeed, if the blocking rod is free to move and bundle with the attempting rod, they will do so within a few diffusion steps and the bundling of the two first rods will then be allowed to proceed. Thus, the transition to kinetic arrest is a true many-body effect in our simulations.

Snapshots of the resulting dynamics are shown in Fig. 3 at times corresponding to the four successive regimes of scenario (2). The evolution of the concentration in our simulations confirms the four predicted scenarios for the evolution of the rod concentration (Fig. 4a). Consistent with Fig. 2c, they also show that the final morphologies are either homogeneous networks of single filaments or strongly bundled phases, with an abrupt transition from one to the other (Fig. 4b) as a high-concentration regime goes from slow reacting to fast reacting. The final structures do not display significant overall orientational order (Fig. 4c), consistent with both our model and in vitro observations.

The good agreement between our theory and simulations comes with one quantitative difference. Because of the more complex geometry of the simulations, kinetic arrest there sets in for a relatively large value of $c \sqrt{L}$ ($\sim 10$), allowing more time for bundling and thus driving the final concentration down. As seen in Fig. 4a, this delayed blocking reveals an additional dynamical regime with a slope steeper than $-1$. In this regime, the rod crosses over to a faster-than-diffusive exploration of space thanks to its ballistic polymerization, leading to a speed-up of bundling before blocking (see Methods and Fig. 6). Note however that this regime can never fully develop if blocking is present, and thus that the scaling scenarios described above remain valid in our simulations, although with modified prefactors.

Discussion

The cytoskeleton of living cells is fundamentally out of equilibrium, and is constantly shaped by two major active processes: the operation of embedded molecular motors, and the constant self-assembly of its components. Although the statistical

Figure 3 | Snapshots of our Brownian dynamics simulations. The state of the system in each of the four different regimes identified in our mathematical model are illustrated: (a) initial plateau $c \propto t^0$, (b) reaction-limited regime $c \propto t^{-3}$, (c) diffusion-limited regime $c \propto t^{-1}$ and (d) blocked regime $c \propto t^0$. To facilitate visualization despite order of magnitude changes in filament concentrations, each of the four panels is taken from a different simulation with a different box size, ensuring that between 100 and 1,000 rods are visible in each picture. The corresponding parameters and absolute concentrations can be read off from the white-filled symbols in Fig. 4a. In this figure the cross-sectional area of each rod is proportional to the number of filaments within the bundle.
mechanics of the former is the subject of a substantial experimental and theoretical literature\(^2\), our understanding of the collective dynamics induced by the latter is very limited. Inspired by recent experiments, we introduce a versatile theoretical framework to investigate this problem, based on rate equations supplemented with a mean-field, entanglement-induced kinetic trapping term. Brownian dynamics simulations validate our theoretical assumptions, and show that our results are robust to changes in the detailed interactions between bundles.

We analyse our model in a simple situation consistent with existing \textit{in vitro} experiments\(^4,5\,7\). Although quantitative comparisons are impeded by technical challenges in resolving single filaments and thin bundles in these specific studies, our main qualitative predictions are all paralleled by the data. Bundle densities thus vary over orders of magnitude upon changes in the initial filament concentration \(c_0\), and the timescale required for their formation decreases sharply upon an increase of \(c_0\) (ref. 4). This is reminiscent of our predicted transition from the slowly relaxing (\(c \propto t^{-1}\) at early times) scenario (3) at low \(c_0\) to the faster (\(c \propto t^{-3}\)) scenario (2) at larger \(c_0\). An increasing crosslinker concentration (analogous to an increase in \(k_b\) in the model) further induces a sharp transition from a homogeneous (scenario (1)) to a bundled network (scenario (2) or (3)). An additional 10-fold increase in crosslinker concentration however hardly modifies the mesh size of the network, strongly reminiscent of our abrupt transition from a slow-reacting to a fast-reacting system of fixed concentration \(c_b\) for \(c_0 > c_b\) (ref. 4). Our model also predicts that an increased reaction rate is equivalent to a decreased polymerization velocity through the dimensionless parameter \(k_b/\nu\). Consistent with this, in ref. 5 an increase in \(\nu\) through the use of the formin mDia1 causes the final bundle concentration to rapidly increase, then plateau out. More quantitatively, refs 4,5 use crosslinker \(\alpha\)-actinin at concentrations of the order of \(c_0 \approx 2 \mu M\). Given the \(\alpha\)-actinin–actin binding rate \(k_{on} = 5 \mu M^{-1} s^{-1}\) (ref. 23), we estimate that two actin filaments within an interaction distance \(b \approx 30\) nm (the size of an \(\alpha\)-actinin molecule) bind with a rate \(k = k_{on}c_0 = 10^{-1}\) s\(^{-1}\). For \(\nu = 10^{-2} \mu m s^{-1}\), this yields \(k_b/\nu \approx 30\) for the typical initial actin filament concentration \(c_0 \approx 0.1\) \(\mu M\). This is consistent with the formation of bundles observed under the aforementioned experimental conditions, and suggests that those \textit{in vitro} assays can indeed transition between scenarios (1), (2) and (3) as their parameters are varied. We moreover predict \(\tau_{c} \approx 370\) s and \(\tau_{b} \approx 2000\) s, comparable to the observed gelation time \(t \approx 600\) s.

These quantitative estimates further allow a discussion of the domain of validity of our model’s main assumptions. We first discuss our approximation that the merging between two bundles is instantaneous. In general, the time required to merge two filaments is the sum of the time for the two filaments to find each other and form their first crosslink, plus a time \(\tau_m\) required to complete their merging. Direct measurements\(^15\) indicate that the latter timescale is of the order of a few hundred milliseconds at most. (This number is measured in the presence of large beads that slow down the merging dynamics because of hydrodynamic friction; the merging timescale \(\tau_m\) is probably significantly smaller in the situation considered here, where such beads are not present.) This timescale is much shorter than the typical evolution timescales \(\tau_c\) and \(\tau_b\) evaluated above. More quantitatively, we estimate in Methods that the delay \(\tau_m\) to merging will have negligible effects on the final concen-

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**Figure 4** | Dynamical evolution of our Brownian dynamics simulations. (a) Concentration of solutions of \(N = 10^3\) rods as a function of their length for \(k_b/\nu = 0.1\) (light grey (green)) and \(k_b/\nu = 10^3\) (dark (blue) symbols). The initial concentration \((c_0)\) and filament length \((L_0)\) for each simulation can be read on the graph, and the white-filled symbols indicate the data points used for the snapshots of Fig. 3. The grey region materializes the condition \(cL > 10\), where blocking is observed. (b) Final bundle concentration as a function of \(c_0\) for \(N = 10^4\) rods of initial length \(L_0/L_b = 0.1\). Error bars give an estimate of the relative uncertainty on the number of remaining filaments at the end of the simulation \([\delta \ln(c_f) = \delta N_{final}/N_{initial} = 1/\sqrt{N_{initial}}]\). For the most highly concentrated, fast-reacting conditions investigated \((c_0/c_b = 10^2\) and \(k_b/\nu \geq 1\), marked with an asterisk), bundling is so strong that all rods in our simulations collapse into one, terminating the dynamics for reasons independent of blocking. (c) Scalar nematic order parameter \(S = (3(\hat{n}(1) \cdot \hat{n})^2 - 1)\) for the data of (b) following blocking. In the definition of \(S\) the index \(a\) refers to the \(a\)-th rod, and \(\hat{n}\) is the eigenvector corresponding to the largest eigenvalue of the tensorial order parameter \(Q_m = \sum_{\alpha\beta}(\hat{n}(\alpha) \cdot \hat{n}(\beta) - \delta_{\alpha\beta}/3)\) (ref. 32). Error bars show the s.e.m. associated with the determination of the average involved in the computation of \(S\), again indicating the error associated with small filament numbers. The nematic order parameter is not computed for final states where only one rod is present (namely, for the data points with \(c_0/c_b = 10^2\), \(k_b/\nu \geq 1\)).
cytoskeletal systems. Depending on the system considered, between filaments could be the major driver of kinetic arrest in experiments of refs 4,5 suggests that topological entanglement gaps as observed in refs 26,27.

compromise the mechanical integrity of these networks and favour a (that is, more crosslinked) state. This could collapse of entangled structures towards a more energetically described by our model. These deformations could facilitate the bending and deformation, and may therefore not be well those studied in some in vitro approach. In contrast, networks with larger mesh sizes, including

We consider bundles crosslinked by the very short crosslinker fascin29, although the importance of this mechanism is less clear in the α-actinin bundles used in refs 4,5. Other effects ignored here, for example, transient sticking between unbundled filaments or the effective increase in length incurred by a bundle upon coalescence with another, may thus not be essential to gain a first understanding of the resulting network structures. Such effects could however easily be included in our framework if warranted by more precise experimental comparisons, as will the physiologically important effects of spontaneous filament nucleation or the coexistence of several crosslinker types with different bundling behaviours30. Detailed simulations will also be useful in assessing the influence of the addition of these and other experimentally relevant features to our model. Although our current solution-like model does not explicitly describe the network’s mechanical properties, it does predict its typical mesh size and bundle thickness, whose relationship to the network’s mechanical response has been the subject of substantial modelling efforts31. Finally, further experimental and theoretical work is needed to elucidate the network structure in the biologically relevant presence of depolymerization/severing that could give rise to fundamentally nonequilibrium steady states.

Overall, our study provides a first theoretical account of the nonequilibrium mechanisms responsible for the actin structures observed in vivo and in vitro. It further illustrates that these dynamical processes can lead to sharp transitions between dramatically different network structures, hinting that cells need only harness relatively modest changes in their internal composition to generate the large variety of morphologies that characterize the cytoskeleton.

Methods

Speed-up of bundling before blocking. Here we rationalize the speed-up of bundling observed in our Brownian dynamics simulations just before the system
becomes blocked in Fig. 4a. This speed-up is a signature of the system crossing over to a new scaling regime for $r^{(2)}$ as $L$ becomes larger than $L_0$ that is, as the longitudinal growth of the rod becomes faster than its longitudinal diffusion.

In practice, this regime has little incidence on our model as bundling becomes hindered by blocking precisely at $t = 0$ and $H$ is the Heaviside step function. The final solution of this equation displays two regimes, depending on whether kinetic arrest takes over before or after the first bundling event is completed:

$$c(t) = \begin{cases} c_0 \left(1 + \frac{2\beta}{1 - \beta} t\right)^{1/3} & \text{if } c_0 0 > 1 \text{ and } t_m < c_0^{-1/3}.
$$

with $x = 1/c_0 - t_m$.

These final concentrations are plotted in Fig. 7, along with their relative deviation from the result at $t_m = 0$. In practice, our results are insensitive to the value of $t_m$ as long as $t_m < c_0^{-1/3} \Rightarrow t_m < c_0^{-1/3} - \ell$, as discussed in the Results section.

Data availability. The computer code used for this study as well as the data generated and analysed are available from the corresponding author on request.

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**Author contributions**

M.L. designed the research. G.F., N.L. and M.L. carried out the analytical calculations. G.F. carried out the numerical simulations. G.F. and M.L. wrote the paper.

**Additional information**

**Competing financial interests:** The authors declare no competing financial interests.

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