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Computing the Structural Influence Matrix for Biological Systems

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Abstract We consider the problem of identifying structural influences of external inputs on steady–state outputs in a biological network model. We speak of a structural influence if, upon a perturbation due to a constant input, the ensuing variation of the steady–state output value has the same sign as the input (positive influence), the opposite sign (negative influence), or is zero (perfect adaptation), for any feasible choice of the model parameters. All these signs and zeros can constitute a structural *influence matrix*, whose (i, j) entry indicates the sign of steady–state influence of the jth system variable on the ith variable (the output caused by an external persistent input applied to the jth variable). Each entry is structurally determinate if the sign does not depend on the choice of the parameters, but is indeterminate otherwise. In principle, determining the influence matrix requires exhaustive testing of the system steady–state behaviour in the widest range of parameter values. Here we show that, in a broad class of biological networks, the influence matrix can be evaluated with an algorithm that tests the system steady–state behaviour only at a finite number of points. This algorithm also allows us to assess the structural effect of any perturbation, such as variations of relevant parameters. Our method is applied to nontrivial models of biochemical reaction networks and population dynamics drawn from the literature, providing a parameter–free insight into the system dynamics.

Keywords Structural analysis · Steady–state variation · Influence matrix · Vertex algorithm · Perfect adaptation

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1 Introduction

Predictive mathematical models are crucial for highlighting the fundamental features of biological systems, in order to perform both the study of natural systems and the design of synthetic biochemical circuits (Kitano (2002); Alon (2006)). Yet, a significant challenge arises from the lack of exact quantitative knowledge and from the variability of parameters. Interestingly, despite the large variability and intrinsic parametric uncertainty, living organisms are able to perform robust regulatory tasks (Kitano (2004); Alon (2007); Steuer et al (2011)); hence the need to understand the inherent roots of such an extraordinary robustness (Gorban and Radulescu (2007); Kitano (2007)). Theoretic and computational tools are thus required to assess *parameter–independent* (within a feasible domain), *structural* properties (Nikolov et al (2007); Shinar and Feinberg (2010); Blanchini and Franco (2011); Franco and Blanchini (2013)).

Extensive numerical simulations exploring the parameter space are often employed to investigate robustness of biological systems (Ma and Iglesias (2002); Kwon and Cho (2008)). However, many analytical tools are available, based for instance on the renowned deficiency theory (Feinberg (1987, 1995a,b); Shinar et al (2007)), the theory of monotone systems (Smith (2008); Sontag (2007); De Lenheer et al (2007)), algebraic geometry and graph theory (Craciun and Feinberg (2005, 2006); Mincheva and Craciun (2008); Angeli and Sontag (2009); Angeli et al (2010); Mincheva (2011); Domijan and Pécou (2011); Blanchini et al (2014)). To robustly (or structurally) assess properties in a biological context, control theoretic models have also been employed (El-Samad et al (2006)), providing criteria for robustness based on the analysis of the Jacobian matrix (Blanchini et al (2012)) and on Lyapunov theory and set–invariance (Blanchini and Miani (2015); Abate et al (2007); Chesi and Hung (2008); Blanchini and Franco (2011); Blanchini and Giordano (2014)).

Among the properties of interest, *adaptation* is widely observed in biological systems, *e.g.*, in bacterial chemotaxis (Barkai and Leibler (1997); Spiro et al (1997); Alon et al (1999)), eukaryotic gradient sensing (Levchenko and Iglesias (2002)) and yeast osmoregulation (Muzzey et al (2009)). A variable embedded in a system is adaptive if, in the presence of a persistent input, after a transient it reverts to its pre–perturbation value; adaptation is perfect if the pre–perturbation value is exactly recovered at steady state. Perfect adaptation obeys the internal model principle (Sontag (2003)) and is equivalent to the presence of integral feedback (Yi et al (2000)) and of zeros at the origin in the system transfer function (Drengstig et al (2008)). Efforts have been made to determine motifs that can achieve perfect adaptation (Ma et al (2009)) and to design biomolecular network modifications that enable perfect adaptation (Waldherr et al (2012)).

In this paper, we consider the general concept of *steady–state influence*, which includes perfect adaptation as a special case. When a persistent input is applied to the system, the steady–state variation of a variable (regarded as an output) may have the same or the opposite sign of the applied input, or may be zero (perfect

adaptation). *A structural influence* is present if the sign of the variation does not depend on the value of the system parameters. In particular, we are interested in the effect of an external input *applied to one of the system variables* on the steady–state variation of each of the variables: in this case, the steady–state interactions can be represented by the structural *influence matrix*.

The problem of determining structural influences is well known in the field of ecology, and is related to the notion of community matrix (Levins (1968)), which is the Jacobian matrix of the system of growth equations and thus describes interactions among and within species in a community near equilibrium. The community matrix, expressing direct effects only, was first qualitatively analysed by Levins (1974, 1975) in terms of signed entries, graphs and loops. The net steady–state effect, combining all direct and indirect effects, is expressed by the adjoint matrix of the negative of the community matrix (Levins (1974, 1975); Dambacher et al (2002, 2003a,b, 2005)). When the sign of some entries of the adjoint matrix is indeterminate, the net response predicted by the model is uncertain: to quantify this uncertainty, a weighted–predictions matrix was introduced (Dambacher et al (2002); Dambacher and Ramos Jiliberto (2007)) that assigns a probability to the predicted sign of the response. Recently, a theoretical approach and an algorithm to determine the sign of changes in steady states upon parameter perturbations in biochemical reaction networks have been proposed by Sontag (2014a,b). Mochizuki and Fiedler (2015) have studied the structural variation in species concentrations/fluxes when a reaction in the network is altered, under qualitative assumptions (positivity, smoothness, monotonicity of rate functions). This type of investigation is focused on the *steady–state* response to external stimuli, in contrast with other approaches that consider the *dynamic* interactions between variables (Hernandez (2009)).

The main contribution of this paper is a rigorous and efficient computational approach to *structurally* assess the steady–state input–output influences. The structural influence is *determinate* if, for any feasible values of the system parameters, the steady–state variation in the output value is zero ('0', perfect adaptation), or its sign is concordant ('+') or discordant ('−') with the sign of the external stimulus. In contrast, if the sign of the variation depends on the magnitude of the parameters, the influence is *indeterminate* ('?'). A particular type of structural steady–state influences can be represented by means of the *influence matrix*: the (i, j) entry of this matrix represents the influence on variable i (output) of an additive input persistently applied to variable j. In ecological models (at least, in their Lotka–Volterra approximations), the structural influence matrix corresponds to the sign pattern of the adjoint of the negative of the community matrix. We propose an efficient algorithm to compute structural steady–state variations due to external stimuli (Section 4), and in particular the structural influence matrix (Section 5). The algorithm exploits the structure of a wide class of biological systems (Blanchini et al (2012); Blanchini and Giordano (2014)) whose model can be written in terms of a "stoichiometry" matrix and a vector of "reaction rates". For these systems, the

Jacobian matrix can always be expressed as the product of three matrices that depend on the stoichiometry matrix and on the partial derivatives of the reaction rate functions (which we call BDC–decomposition, described in Section 3). Based on this decomposition, the algorithm allows us to determine the structural sign of the input–output influence, for *any* feasible choice of the parameters, by checking just a *finite* number of points in the parameter space corresponding to the vertices of the unit hypercube. This is possible because, for systems admitting a BDC–decomposition, the expression of the input–output influence turns out to be a multi–affine function defined on a hypercube, hence it reaches its extreme values on the vertices of the hypercube.

An important feature of our algorithm is that it can handle parametric dependencies between the entries of the Jacobian matrix; thus, the algorithm can assess structural sign determinacy of the influence even in cases in which the Jacobian matrix is not sign–definite. This is a significant advantage relative to the existing literature that considers solely matrices with independent entries. For systems with independent Jacobian entries, we describe a tree–like recursive algorithm to enhance computational efficiency (Section 7). We also consider the case of neutrally stable systems, whose trajectories evolve in the stoichiometric compatibility class associated with the initial conditions. For these systems, any persistent input might cause divergence of the trajectories in time; however, a structural input–output influence analysis can be carried out by considering impulsive, rather than persistent, inputs (see Section 6).

The paper is organised as follows. Background and motivation are provided in Section 2 by analysing meaningful case studies. Section 3 illustrates the BDC–decomposition and the concept of structural analysis. Our vertex algorithm for an efficient computation of structural steady–state influences is described in Section 4 and applied in Section 5 to the computation of the structural influence matrix. In Section 6, the analysis is carried out for neutrally stable systems in their stoichiometric compatibility class, while Section 7 describes the tree–like algorithm that computes the influence matrix for systems with independent Jacobian entries. In Section 8, to show the effectiveness of our algorithm in explaining the intrinsic behaviour of the system in terms of steady–state response to external stimuli, we analyse several models of biochemical and ecological systems proposed in the literature. Some future extensions and links with related problems (partially) explored in the literature are finally discussed in the concluding Section 9.

2 Background and motivating examples

We introduce the concept of influence matrix with two illustrative examples. Consider the following Lotka–Volterra prey–predator model (May (1974))

$$\begin{aligned} \dot{H}(t) &= H(t)[r(1 - H(t)/K) - aP(t)] + u_1, \\ \dot{P}(t) &= P(t)(-b + cH(t)) + u_2, \end{aligned} \tag{1}$$

where $H(t)$ are the prey and $P(t)$ the predators; a, b, c, r and K are positive parameters; u_1 and u_2 are persistent external inputs. The birth rate of the prey population is modelled with the Verhulst–Pearl logistic term $r(1 - H(t)/K)$. When $u_1 = u_2 = 0$, the stable equilibrium is E_0: $\bar{H}_0 = \frac{b}{c}$, $\bar{P}_0 = \frac{r}{a}\left(1 - \frac{b}{cK}\right)$ (other equilibria are E_1: $\bar{H}_1 = K$, $\bar{P}_1 = 0$ and E_2: $\bar{H}_2 = 0$, $\bar{P}_2 = 0$). Consider equilibrium E_0 and suppose now that there is a constant injection or removal of prey or predators, *i.e.*, inputs u_1 or u_2 become non–zero and constant. How does this perturbation affect the equilibria? We assume that the perturbation is sufficiently small that the equilibrium remains stable and non–zero, and does not disappear (for instance, for large negative values of u_i the equilibrium may not exist).

Assume there is an injection or removal of prey, *i.e.*, input $u_1 \neq 0$, and denote as $\bar{H}$ and $\bar{P}$ the new coordinates of the equilibrium E_0. From the second equation we have

$$\bar{P}(-b + c\bar{H}) = 0,$$

so the steady–state value of the prey will be unchanged: $\bar{H} = b/c$. From the first equation

$$\bar{H}[r(1 - \bar{H}/K) - a\bar{P}] + u_1 = 0,$$

being $\bar{H}$ unchanged, we see that $\bar{P}$ is an increasing function of u_1.

The conclusion is that, no matter how the parameters are taken within the domain where E_0 exists, after a transient the predator population variation $\bar{P} - \bar{P}_0$ has the same sign of u_1: $\bar{P} > \bar{P}_0$ if $u_1 > 0$, $\bar{P} < \bar{P}_0$ if $u_1 < 0$. Hence, we say that the influence of u_1 on P is *structurally positive*. Conversely, since $\bar{H} = \bar{H}_0$ is unchanged, the influence of u_1 on H is structurally zero. This phenomenon is known as *perfect adaptation*: a positive (negative) u_1 causes an initial increase (decrease) of the prey, but at steady–state the prey population converges to the unperturbed value $\bar{H}_0$, due to a compensating effect associated with the variation in the predator population.

With similar computations we can argue that, if we consider a predator injection (input $u_2 \neq 0$), the structural influence on H is negative and on P is positive: if $u_2 > 0$ ($u_2 < 0$ respectively), $\bar{H} < \bar{H}_0$ and $\bar{P} > \bar{P}_0$ ($\bar{H} > \bar{H}_0$ and $\bar{P} < \bar{P}_0$).

Since the steady–state values $\bar{H}$ and $\bar{P}$ are functions of u_1 and u_2, $\bar{H}(u_1, u_2)$ and $\bar{P}(u_1, u_2)$, implicitly defined by the equilibrium conditions, we can equivalently compute and inspect the signs of the corresponding partial derivatives. In this case we have that *structurally* $\partial\bar{H}/\partial u_1 = 0$, $\partial\bar{P}/\partial u_1 > 0$, $\partial\bar{H}/\partial u_2 < 0$, $\partial\bar{P}/\partial u_2 > 0$.

To visualise at once the steady–state interactions between inputs applied to each system variable and outputs taken as a single system variable, we can build a structural steady–state *influence matrix*, which in the following will be denoted as matrix M. We say that the (i, j) entry of matrix M is '+', '−', or '0' if, for any feasible choice of the model parameters, an input u_j (representing a persistent external injection applied to the jth population) causes a change of the same sign, opposite sign, or no change in the steady state of the ith population (seen as an output). Based on our calculations, the influence matrix M for the prey–predator system (1) is:

$$M = \begin{bmatrix} 0 & - \\ + & + \end{bmatrix}.$$

The prey–predator model is a sufficiently simple case where the influence matrix can be computed analytically. However, building matrix M in large and complex networks requires a more sophisticated and computationally efficient approach. Two issues are worth pointing out.

(a) An equilibrium (steady–state) may not exist, or it may be unstable. However, in many cases of practical interest in biology and ecology, the existence of a stable equilibrium is possible or even guaranteed. In the following, we assume that an equilibrium exists and is stable. In the concluding section, we discuss parameter–independent criteria for the existence of an equilibrium and its stability.

(b) Especially in complex systems, some entries of M may not be structurally determinate, namely the sign may depend on the choice of the system parameters. The structural influence matrix M has a '?' entry in the position (i, j) whenever the sign of the variation in the steady state of the ith variable, due to an additive input persistently applied to the jth variable, is not structurally determinate.

As shown by Dambacher et al (2002, 2003a), the influence matrix M corresponds to the sign pattern of $\mathrm{adj}(-J)$, where J is the system Jacobian matrix computed at the equilibrium. This highlights the relationship between the system Jacobian and the structural influence matrix M. However, sign–definiteness of the Jacobian entries does not directly relate to the existence or absence of structural steady–state influences.

Consider a synthetic gene network designed to regulate the transcription rate of RNA species, which bind to form output products (Franco et al (2008); Franco and Murray (2008); Giordano et al (2013); Franco et al (2014)). Two RNA species, transcribed by two separate genes, bind to form a product; the presence of self–repression loops causes their transcription rates to be equated at steady state. The system is described by the following equations (Franco et al (2008, 2014)):

$$\begin{cases} \dot{g}_i = \alpha_i(g_i^{tot} - g_i) - \delta_i g_i r_i \\ \dot{r}_i = \beta_i g_i - \delta_i g_i r_i - k r_i r_j \end{cases}, \qquad i,j \in \{1,2\}, \quad i \neq j,$$

where g_i are active gene template concentrations (active and inactive gene templates are present in a total amount $g_i^{tot} = g_i + g_i^*$), transcribed to produce RNA species (with concentrations r_i), which in turn bind to form a product; α_i are gene activation rates, β_i are transcription rates, δ_i is the self–repression coefficient for subsystem g_i–r_i, while k is the product generation rate. Besides its rate regulatory task, we expect this network to exhibit *tracking properties* when the total concentration of a gene template varies. In fact, if g_i^{tot} increases (this is equivalent to an input u_i acting on g_i), then, at steady state, g_i increases and so does r_i. Due to the increase in r_i, more binding sites are created, hence r_j decreases (the RNA species is more required). Stoichiometric self–inhibition is reduced and g_j increases. Of course, the concentration increases *provided that it can*: since the reagents bind according to a given stoichiometry, the expected increase can only occur if the reagent with the lowest concentration is augmented until it is no more a bottleneck for the output production. Still, the behaviour of r_j is not clearly predictable: the increase in r_i should increase its consumption, while the increase in g_j should increase its production. Does one of the two opposite effects structurally dominate?

The Jacobian matrix for this network is

$$J = \begin{bmatrix} -(\alpha_1 + \delta_1 \bar{r}_1) & -\delta_1 \bar{g}_1 & 0 & 0 \\ \beta_1 - \delta_1 \bar{r}_1 & -(\delta_1 \bar{g}_1 + k\bar{r}_2) & 0 & -k\bar{r}_1 \\ 0 & 0 & -(\alpha_2 + \delta_2 \bar{r}_2) & -\delta_2 \bar{g}_2 \\ 0 & -k\bar{r}_2 & \beta_2 - \delta_2 \bar{r}_2 & -(\delta_2 \bar{g}_2 + k\bar{r}_1) \end{bmatrix}.$$

Computation of the steady–state influence shows that, for any feasible choice of the parameters, when g_1^{tot} increases, g_1 increases too and this leads to an increase in r_1 and in g_2, while r_2 decreases. Analogously, when g_2^{tot} decreases, g_2 decreases as well and this leads to a reduction in r_2 and in g_1, while r_1 increases. Hence, the rate regulatory system exhibits tracking properties at steady state for any feasible choices of the

parameters. The influence matrix M, which can be computed as the sign pattern of $\mathrm{adj}(-J)$, is

$$\begin{array}{c} \\ \text{effect on variable} \end{array} \begin{array}{c} \text{input applied to variable} \\ \begin{array}{cccc} \overbrace{}^{g_1} & \overbrace{}^{r_1} & \overbrace{}^{g_2} & \overbrace{}^{r_2} \end{array} \\ \begin{array}{c} g_1 \{ \\ r_1 \{ \\ g_2 \{ \\ r_2 \{ \end{array} \left[\begin{array}{cccc} + & - & + & + \\ + & + & - & - \\ + & + & + & - \\ - & - & + & + \end{array} \right] \end{array} .$$

Independent of the system parameters, an external input affecting r_i decreases both g_i and r_j, while increases g_j and r_i itself. The effect of an external input affecting g_i is the same we obtain when incrementing g_i^{tot}. We have thus gained a powerful parameter–free insight into the system behaviour, in terms of sign of the change in the steady states of the variables due to an external stimulus.

Note that the entries J_{21} and J_{43} of the Jacobian are structurally positive, in view of the equilibrium conditions $(\beta_i - \delta_i \bar{r}_i)\bar{g}_i = k\bar{r}_i\bar{r}_j$.

The size and complexity of this second example are still tractable: the analytical computation of the influence matrix is tedious but possible. However, for large and complex systems, algebraic calculations are not scalable and a computational approach is needed. In this paper, we describe our systematic and efficient numerical method to calculate steady–state influences.

Remark 1 As we will show, with respect to the work by Dambacher et al (2002, 2003a,b, 2005), the algorithm we propose can deal with the existence of constraints due to parameters repeated in more than one entry (*e.g.*, in this second example, $\delta_1\bar{r}_1$ appearing in both J_{11} and J_{21}).

3 *BDC*–decomposition and structural analysis

In this section we introduce the key mathematical tool that will be employed in the algorithm for computing structural steady–state influences: the *BDC*–decomposition of the system Jacobian matrix. Since this decomposition depends on the Jacobian only, in this section we temporarily neglect, for simplicity, the presence of an input (and an output) in the system; we will comment on this at the end of the section.

Consider the generic nonlinear system

$$\dot{x}(t) = f_x(x(t)), \tag{2}$$

where $f_x(\cdot)$ is continuously differentiable, $x \in \mathbb{R}^n$ and $\bar{x}$ is an equilibrium point, $f_x(\bar{x}) = 0$.

Definition 1 System (2) admits a *BDC–decomposition* iff its Jacobian matrix evaluated at $\bar{x}$, $J = (\partial f_x/\partial x)_{x=\bar{x}}$, can be written as the positive linear combination of rank–one matrices, namely

$$J = \sum_{h=1}^{q} R_h D_h = \sum_{h=1}^{q} B_h D_h C_h^\top, \tag{3}$$

where $R_h = [B_h C_h^\top]$ are rank–one matrices[1] and D_h, $h = 1, \ldots, q$, are positive scalars.

In a compact form, we can write

$$J = BDC,$$

where D is a diagonal matrix with positive diagonal entries D_h, B is the matrix formed by the columns B_h and C is the matrix formed by the rows $C_h^\top$.

For a wide class of models, including (bio)chemical reaction networks, we show that a BDC–decomposition (having a graph interpretation that will be described later) always exists, and can be easily and systematically computed. Consider the system

$$\dot{x}(t) = Sf(x(t)) + f_0, \tag{4}$$

where the state $x(t) \in \mathbb{R}^n_+$ represents species concentrations evolving over time, $f(x(t)) \in \mathbb{R}^m$ is a vector of reaction rate functions and $f_0 \in \mathbb{R}^n$ is a vector of constant influxes ($f_0 \geq 0$ componentwise); $S \in \mathbb{Z}^{n\times m}$ is the stoichiometric (flow) matrix of the system, whose entries $[S]_{ij}$ represent the net amount of the ith species produced or consumed by the jth reaction, excluding the contribution of constant influxes. This class of models includes any chemical reaction network, or any phenomenological biomolecular model (*e.g.*, gene regulatory models, signalling networks, *etc.*) that can be written as an equivalent chemical reaction network. Also some models typically used in ecology and population dynamics (such as Lotka–Volterra systems) can be rewritten as in (4), where $x(t)$ and $f(x(t))$ represent population density and growth rate functions, although not all ecological models fall in the category of systems we consider.

Assumption 1 *Each function $f_j(\cdot)$, for $j = 1, \ldots, m$, is nonnegative and continuously differentiable, with sign–definite (*i.e.*, always positive, always negative or always zero) partial derivatives in the interior of the positive orthant.*

Assumption 2 *Each function $f_j(\cdot)$, for $j = 1, \ldots, m$, is zero if and only if at least one of its arguments is zero. Furthermore, if $[S]_{ij} < 0$, then $f_j(\cdot)$ must have $x_i(t)$ as an argument.*

The latter assumption ensures that (4) is a positive system, since for $x_i = 0$ we have $\dot{x}_i \geq 0$.

The following result guarantees that systems of the form (4) always admit a BDC–decomposition.

[1] A rank–one matrix R_h can always be written as the product of a column vector B_h and a row vector $C_h^\top$.

Proposition 1 *Any system falling in the class* (4) *admits a BDC–decomposition:* $J = BDC$. *Matrices* B *and* C *can be built systematically, based on the stoichiometric matrix* S *and on qualitative information about* $f(\cdot)$.

Proof: To prove the statement constructively, we rewrite equation (4) as

$$\dot{x} = \sum_{j=1}^{s} S_j \; f_j(x) + f_0,$$

where S_j is the jth column of matrix S. The corresponding Jacobian is

$$J = \sum_{j=1}^{s} S_j \; \begin{bmatrix} \frac{\partial f_j}{\partial x_1} & \frac{\partial f_j}{\partial x_2} & \dots & \frac{\partial f_j}{\partial x_n} \end{bmatrix}.$$

Denoting by $D_1, D_2, \dots, D_q$ the absolute values of all the non–zero partial derivatives we can write

$$J = \sum_{h=1}^{q} B_h D_h C_h^\top,$$

where

- $D_h = \left| \frac{\partial f_j}{\partial x_i} \right|$ for some i and j;
- $B_h = S_j$, the column of S associated with f_j;
- $C_h^\top$ has a single non–zero entry in the ith position, equal to the sign of $\frac{\partial f_j}{\partial x_i}$.

□

We substantiate the claim in Proposition 1 by describing more in detail how to construct the decomposition matrices. First of all, we identify all the distinct, non–zero partial derivatives $\frac{\partial f_j}{\partial x_i}$ and we take their absolute values, which we denote as D_h; second, we assign an order to elements D_h and we build the diagonal matrix $D = \text{diag}\{D_1, \dots, D_q\} \in \mathbb{R}^{q \times q}$. Then, in correspondence to each element $D_h = \left| \frac{\partial f_j}{\partial x_i} \right|$, matrix $B \in \mathbb{Z}^{n \times q}$ includes the column S_j of S associated with f_j. Since each function f_j may depend on p_j different variables, the corresponding column S_j will be repeated p_j times in matrix B. Finally, in correspondence to each element $D_h = \left| \frac{\partial f_j}{\partial x_i} \right|$, matrix $C \in \mathbb{Z}^{q \times n}$ includes a row that has a $+1$ or -1 entry in the i–th position (corresponding to the variable x_i with respect to which the derivative in D_h is taken), while the other entries are zero; the sign of the non–zero entry depends on the sign of the corresponding derivative.

We build the BDC–decomposition step by step in the following example.

Example 1 We consider the metabolic network proposed by Chen et al (2005), p. 106., defined by reactions $\emptyset \xrightarrow{a_0} A$, $A + B \xrightarrow{f_{ab}} C + D$, $D \xrightarrow{f_d} B$, $C \xrightarrow{f_c} \emptyset$. We denote chemical species with uppercase letters and their concentrations with the corresponding lowercase letters. Note that species B and D, involved in the

second and third reactions, are linked by a mass conservation constraint: $b + d = K$ is constant. Hence, $\dot{d} = -\dot{b}$ and variable d can be neglected, so as to obtain the *reduced–order* system

$$\begin{cases} \dot{a} = a_0 - f_{ab}(a, b) \\ \dot{b} = -f_{ab}(a, b) + f_d(K - b) \\ \dot{c} = f_{ab}(a, b) - f_c(c). \end{cases}$$

This system can be rewritten as in model (4) defining

$$x = \begin{bmatrix} a \\ b \\ c \end{bmatrix}, \quad S = \begin{bmatrix} -1 & 0 & 0 \\ -1 & 1 & 0 \\ 1 & 0 & -1 \end{bmatrix}, \quad f(x) = \begin{bmatrix} f_{ab}(a, b) \\ f_d(K - b) \\ f_c(c) \end{bmatrix}, \quad f_0 = \begin{bmatrix} a_0 \\ 0 \\ 0 \end{bmatrix}.$$

The Jacobian matrix and its BDC–decomposition are

$$J = \begin{bmatrix} -\alpha & -\beta & 0 \\ -\alpha & -(\beta + \delta) & 0 \\ \alpha & \beta & -\gamma \end{bmatrix} = \underbrace{\begin{bmatrix} -1 & -1 & 0 & 0 \\ -1 & -1 & 0 & 1 \\ 1 & 1 & -1 & 0 \end{bmatrix}}_{=B} \underbrace{\mathrm{diag}\{\alpha, \beta, \gamma, \delta\}}_{=D} \underbrace{\begin{bmatrix} 1 & 0 & 0 \\ 0 & 1 & 0 \\ 0 & 0 & 1 \\ 0 & -1 & 0 \end{bmatrix}}_{=C},$$

where the Greek letters denote partial derivatives, in absolute value: $\alpha = \partial f_{ab}/\partial a > 0$, $\beta = \partial f_{ab}/\partial b > 0$, $\gamma = \partial f_c/\partial c > 0$, $\delta = |\partial f_d/\partial b|$. To compute the BDC–decomposition, we choose an order for the non–zero partial derivatives: 1) α, 2) β, 3) γ and 4) δ. Then:

1) the first column of B corresponds to S_1, associated with the reaction rate function $f_{ab}(\cdot, \cdot)$, and the first row of C has a 1 entry in the first position, corresponding to variable a;
2) the second column of B corresponds to S_1, associated with $f_{ab}(\cdot, \cdot)$, and the second row of C has a 1 entry in the second position, corresponding to b;
3) the third column of B corresponds to S_3, associated with $f_c(\cdot)$, and the third row of C has a 1 entry in the third position, corresponding to c;
4) the fourth column of B corresponds to S_2, associated with $f_d(\cdot)$, and the fourth row of C has a -1 entry in the second position, corresponding to b (since δ is the opposite of $\partial f_d/\partial b < 0$).

Note that column S_1 is repeated twice in B because $f_{ab}(\cdot, \cdot)$ has two arguments. ◇

Remark 2 The BDC–decomposition is not unique. Even when an order for the diagonal entries of D is assigned, we can change the sign of both B_k and $C_k^\top$, obtaining matrices $\hat{B}$ and $\hat{C}$, and still have that $J = \hat{B}D\hat{C}$. The only requirement is that matrix D, containing the free positive parameters, is diagonal.

The BDC–decomposition can be extended to non–positive systems and also to the case of dependencies between partial derivatives. For instance, the system

$$\begin{cases} \dot{a} = a_0 - f_{ab}(a-b) \\ \dot{b} = f_{ab}(a-b) - f_b(b) \end{cases} \quad \text{with} \quad S = \begin{bmatrix} -1 & 0 \\ 1 & -1 \end{bmatrix}, \quad f = \begin{bmatrix} f_{ab}(a-b) \\ f_b(b) \end{bmatrix}, \quad f_0 = \begin{bmatrix} a_0 \\ 0 \end{bmatrix},$$

would have the BDC–decomposition

$$J = \begin{bmatrix} -\alpha & \beta \\ \alpha & -(\beta+\gamma) \end{bmatrix} = \underbrace{\begin{bmatrix} -1 & -1 & 0 \\ 1 & 1 & -1 \end{bmatrix}}_{=B} \underbrace{\mathrm{diag}\{\alpha,\beta,\gamma\}}_{=D} \underbrace{\begin{bmatrix} 1 & 0 \\ 0 & -1 \\ 0 & 1 \end{bmatrix}}_{=C}$$

if we considered the three parameters $\alpha = \partial f_{ab}/\partial a > 0$, $\beta = |\partial f_{ab}/\partial b|$ and $\gamma = \partial f_b/\partial b > 0$. However, since $\beta = \alpha$, we need two parameters only, α and γ. Hence:

$$J = \begin{bmatrix} -\alpha & \alpha \\ \alpha & -(\alpha+\gamma) \end{bmatrix} = \underbrace{\begin{bmatrix} -1 & 0 \\ 1 & -1 \end{bmatrix}}_{=B} \underbrace{\mathrm{diag}\{\alpha,\gamma\}}_{=D} \underbrace{\begin{bmatrix} 1 & -1 \\ 0 & 1 \end{bmatrix}}_{=C}.$$

Also when the Jacobian of system (4) has independent (hence sign–definite, due to Assumption 1) entries, the system always admits a BDC–decomposition. For instance, for the prey–predator model (1), at the equilibrium E_0, we have

$$J = \begin{bmatrix} -r\bar{H}/K & -a\bar{H} \\ c\bar{P} & 0 \end{bmatrix} = \begin{bmatrix} -\alpha & -\beta \\ \gamma & 0 \end{bmatrix} = \underbrace{\begin{bmatrix} 1 & 1 & 0 \\ 0 & 0 & 1 \end{bmatrix}}_{=B} \underbrace{\mathrm{diag}\{\alpha,\beta,\gamma\}}_{=D} \underbrace{\begin{bmatrix} -1 & 0 \\ 0 & -1 \\ 1 & 0 \end{bmatrix}}_{=C}. \tag{5}$$

Remark 3 In system (1), an external input is present. We underline that a system can admit a BDC–decomposition also when an external input u is introduced, affecting the system, or when one of the system parameters is considered as an external input u,

$$\dot{x}(t) = f_x(x(t), u(t)),$$

as long as $f_x(\cdot,\cdot)$ is continuously differentiable and the system Jacobian can be written as the positive linear combination of rank–one matrices (3).

The BDC–decomposition plays a key role in our main problem of assessing the *structural* steady–state influence between the input and the output of a system. The parameter–independent results we are pursuing must be valid for *families* of systems having the same *structure*. In the case of systems admitting a BDC–decomposition, we provide the following definition.

Definition 2 The *structure* of a family of systems admitting a BDC–decomposition is given by the matrices B and C. A *realisation* of the structure is given by a choice of the positive diagonal entries of D, $D_k > 0$, $k = 1, \ldots, q$.

The BDC–decomposition is a structural representation associated with the Jacobian matrix of the system, which can be equivalently represented by a graph whose nodes and arcs denote, respectively, species and interactions among them. Examples are provided by the graphs in Fig. 1. Each arc is associated with positive parameters that are in the diagonal of D; if the arc has k tails, it is associated with k parameters. The arc corresponds to k identical columns of matrix B (having a negative entry in the positions associated with the nodes from which the arc tails start, a positive entry in the positions associated with the nodes reached by the arc arrows, zero entries elsewhere) and to k rows of matrix C, each having a single non–zero entry in one of the positions associated with the nodes from which the arc tails start.

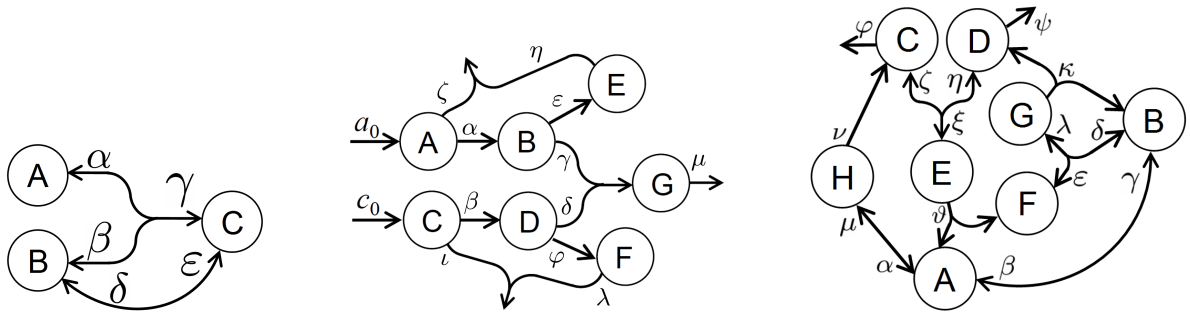
Example 2 Consider the system

$$\begin{cases} \dot{a} = -f_{ab}(a,b) + f_c(c) \\ \dot{b} = -f_{ab}(a,b) - f_b(b) + f_c(c) + f_c^*(c) \\ \dot{c} = f_{ab}(a,b) + f_b(b) - f_c(c) - f_c^*(c) \end{cases} \quad \text{with} \quad S = \begin{bmatrix} -1 & 1 & 0 & 0 \\ -1 & 1 & -1 & 1 \\ 1 & -1 & 1 & -1 \end{bmatrix}, \quad f = \begin{bmatrix} f_{ab}(a,b) \\ f_c(c) \\ f_b(b) \\ f_c^*(c) \end{bmatrix}. \tag{6}$$

Denoting by $\alpha = \partial f_{ab}/\partial a$, $\beta = \partial f_{ab}/\partial b$, $\gamma = \partial f_c/\partial c$, $\delta = \partial f_b/\partial b$ and $\varepsilon = \partial f_c^*/\partial c$ the positive partial derivatives, the system Jacobian matrix, along with its BDC–decomposition,

$$J = \begin{bmatrix} -\alpha & -\beta & \gamma \\ -\alpha & -(\beta+\delta) & \gamma+\varepsilon \\ \alpha & \beta+\delta & -(\gamma+\varepsilon) \end{bmatrix} = \underbrace{\begin{bmatrix} -1 & -1 & 1 & 0 & 0 \\ -1 & -1 & 1 & -1 & 1 \\ 1 & 1 & -1 & 1 & -1 \end{bmatrix}}_{=B} \underbrace{\mathrm{diag}\{\alpha,\beta,\gamma,\delta,\varepsilon\}}_{=D} \underbrace{\begin{bmatrix} 1 & 0 & 0 \\ 0 & 1 & 0 \\ 0 & 0 & 1 \\ 0 & 1 & 0 \\ 0 & 0 & 1 \end{bmatrix}}_{=C} \tag{7}$$

corresponds to the graph in Fig. 1 (a).



(a) System in Example 2. (b) System in Example 5. (c) System in Section 8.1.

Fig. 1: Graphs corresponding to the Jacobian structure of three examples in this paper.

In the next section, we show how *parameter–independent steady–state influences* between the input and the output of any system admitting a BDC–decomposition can be structurally assessed based on matrices B and C.

4 A vertex algorithm to identify structural steady–state influences

In this section we describe an efficient algorithm to assess structural steady–state input–output influences for any system admitting a BDC–decomposition. The algorithm is based on the evaluation of the sign of the determinant of a matrix at a finite number of points. We begin by specifying the assumptions required for evaluating a steady–state input–output influence.

Consider a general nonlinear system

$$\dot{x}(t) = f(x(t), u(t)), \tag{8}$$

$$y(t) = g(x(t)), \tag{9}$$

where $f(\cdot,\cdot)$ and $g(\cdot)$ are continuously differentiable, $x \in \mathbb{R}^n$, $u \in \mathbb{R}$ is an input and $y \in \mathbb{R}$ is an output. Assume that there exists an equilibrium point $\bar{x} > 0$ (componentwise), corresponding to $\bar{u}$, such that $f(\bar{x}, \bar{u}) = 0$, and consider the corresponding output steady–state value $\bar{y} = g(\bar{x})$. So both the steady–state values $\bar{x}(u)$ and $\bar{y}(u)$ are functions of u. We work under the following assumptions.

B1 The considered equilibrium $\bar{x}$ is asymptotically stable.

B2 The input perturbation u is small enough to ensure that the stability of $\bar{x}(u)$ is preserved.[2]

[2] We remind that the eigenvalues of the Jacobian matrix are continuous functions of its entries, which, in turn, are continuous functions of u.

The influence is determined by the derivative of the steady–state map that relates a given input u to a specific output y. For system (8)–(9), the implicit function theorem provides an analytical expression for the derivative of the steady–state input–output map:

$$\frac{\partial \bar{y}}{\partial \bar{u}} = \left.\frac{\partial g}{\partial x}\right|_{\bar{x}} \left(-\left.\frac{\partial f}{\partial x}\right|_{(\bar{x},\bar{u})} \right)^{-1} \left.\frac{\partial f}{\partial u}\right|_{(\bar{x},\bar{u})}. \tag{10}$$

If we consider the linear approximation of the nonlinear system in a neighborhood of the equilibrium $\bar{x}$, denoting by $z(t) = x(t) - \bar{x}$, $v(t) = u(t) - \bar{u}$, $w(t) = y(t) - \bar{y}$, we obtain the linearised system:

$$\begin{aligned} \dot{z}(t) &= Jz(t) + Ev(t), \\ w(t) &= Hz(t), \end{aligned}$$

where $[J]_{ij} = \left.\frac{\partial f_i}{\partial x_j}\right|_{(\bar{x},\bar{u})}$, $[E]_i = \left.\frac{\partial f_i}{\partial u}\right|_{(\bar{x},\bar{u})}$ and $[H]_j = \left.\frac{dg}{dx_j}\right|_{\bar{x}}$. J is the Jacobian matrix, while E and H are a column and a row vector representing, respectively, how the input acts on the system state and how the output depends on the system state in the linearised system.

Proposition 2 *For system* (8)–(9),

$$\frac{\partial \bar{y}}{\partial \bar{u}} = H(-J)^{-1}E = \frac{1}{\det(-J)} \det \begin{bmatrix} -J & -E \\ H & 0 \end{bmatrix}. \tag{11}$$

Proof: The first equality is immediate in view of (10). As for the second equality, since

$$\begin{bmatrix} -J & -E \\ H & 0 \end{bmatrix} = \begin{bmatrix} I_n & 0 \\ -HJ^{-1} & 1 \end{bmatrix} \begin{bmatrix} -J & -E \\ 0 & H(-J)^{-1}E \end{bmatrix},$$

where the matrices in the product are block–triangular and $H(-J)^{-1}E$ is a scalar, we have that

$$\det \begin{bmatrix} -J & -E \\ H & 0 \end{bmatrix} = 1 \cdot \det(-J) \left[H(-J)^{-1}E \right].$$

□

Since the equilibrium is assumed stable, $\det(-J)$ is always positive. For any system whose Jacobian J admits a BDC–decomposition, to evaluate the sign of $\frac{\partial \bar{y}}{\partial \bar{u}}$ we just need to consider the sign of

$$r(D) \doteq \det \begin{bmatrix} -J & -E \\ H & 0 \end{bmatrix} = \det \begin{bmatrix} -BDC & -E \\ H & 0 \end{bmatrix}. \tag{12}$$

To assess the *structural* influence on the output y due to the input u, we need to verify whether function $r(D)$ is sign–definite, namely it has the same sign *for all* the possible choices of D. The following result states that the *structural* sign of $r(D)$ in (12) can be determined by checking a *finite* number of points, namely the vertices of the unit hypercube

$$\mathcal{C}_D = \{D_k : 0 \leq D_k \leq 1,\ k = 1, \ldots, q\}.$$

Theorem 1 *Denote by $D^{(v)}$ the matrices corresponding to the vertices of the hypercube $\mathcal{C}_D$, $v = 1, \ldots, 2^q$. Then:*

a) $r(D) > 0$ structurally if and only if $r(D^{(v)}) \geq 0$ for all v and $r(D) > 0$ for $D = I$;

b) $r(D) < 0$ structurally if and only if $r(D^{(v)}) \leq 0$ for all v and $r(D) < 0$ for $D = I$;

c) $r(D) = 0$ structurally if and only if $r(D^{(v)}) = 0$ for all v.

Proof: First of all, note that the sign of $r(D)$, with $D_i > 0$, does not change if we scale D with a positive factor: $\text{sign}[r(D)] = \text{sign}[r(\varphi D)]$ for any positive φ. Indeed, consider $\varphi J = B(\varphi D)C$. Then

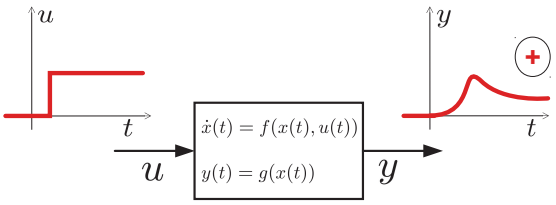
$$\det \begin{bmatrix} -\varphi J & -E \\ H & 0 \end{bmatrix} = \det \begin{bmatrix} \varphi I_n & 0 \\ 0 & 1 \end{bmatrix} \det \begin{bmatrix} -J & -E \\ H & 0 \end{bmatrix} \det \begin{bmatrix} I_n & 0 \\ 0 & 1/\varphi \end{bmatrix} = \varphi^{n-1} \det \begin{bmatrix} -J & -E \\ H & 0 \end{bmatrix}.$$

Therefore, $r(D)$ is positive (negative, zero) for all $D_i > 0$ if and only if it is positive (negative, zero) in all points $D_i > 0$ in the unite cube.

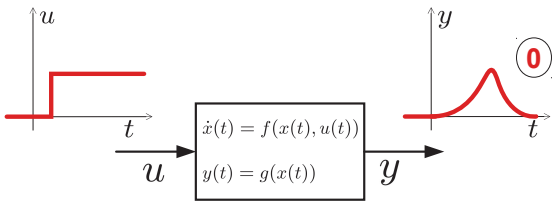
We just prove the first claim, since the others can be proved similarly. Necessity is immediately based on continuity arguments. To prove sufficiency by contradiction, we recall that a multi–affine function defined on a hypercube reaches its minimum (and maximum) value on a vertex of the hypercube, thus $r(D_1, D_2, \ldots, D_q) \geq 0$ in the whole cube. Assume there is an internal point $D^* > 0$ of the hypercube such that $r(D_1^*, D_2^*, \ldots, D_q^*) = 0$. Consider variations along the direction of $0 \leq D_1 \leq 1$. The restricted function is linear and nonnegative; since it is zero in one point, it must be zero in the extrema: $r(0, D_2^*, \ldots, D_q^*) = 0$, $r(1, D_2^*, \ldots, D_q^*) = 0$. Fix $D_1 = 1$, corresponding to the second condition: the new point $(1, D_2^*, \ldots, D_q^*)$ is in the relative interior of the $(q-1)$–dimensional cube where $D_1 = 1$. Then we can repeat the same argument along the direction of D_2, to conclude that $r(1, 1, D_3^*, \ldots, D_q^*) = 0$. Proceeding in the same way for all the directions, we finally get $r(1, 1, 1, \ldots, 1) = 0$, in contradiction with $r(D) > 0$ for $D = I$. □

Based on Theorem 1, for any system (8) *admitting a BDC–decomposition*, the structural steady–state influence of *any* input provided to the system on *any* output of the form (9) can be tested as follows.[3]

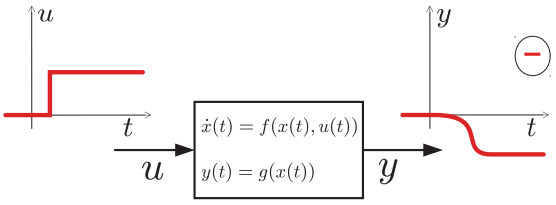
[3] A Matlab implementation of our algorithm is available at: https://users.dimi.uniud.it/~franco.blanchini/influence.zip.



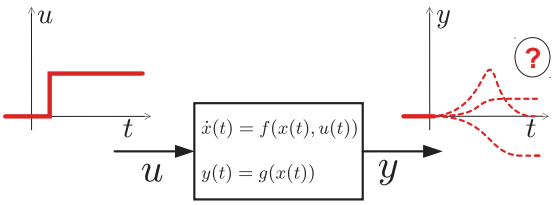
(a) Increase: the structural influence is '+'.



(b) Perfect adaptation: the structural influence is '0'.



(c) Decrease: the structural influence is '−'.



(d) Indeterminate: the structural influence is '?'.

Fig. 2: Illustration of the structural input–output influence. The structural influence is determinate if a step in the input causes a positive (a), zero (b), or negative (c) change in the output steady state, for any feasible choice of the system parameters. The structural influence is indeterminate when the output variation can be positive, zero, or negative, depending on the system parameters (d).

Procedure 1 ***Vertex algorithm.***

Input: *Matrices B and C of the BDC–decomposition and matrices E and H appearing in* (12).

Output: *The steady–state structural influence sign* $\sigma \in \{+, -, 0, ?\}$.

1. *Let* $\omega_{max} = \omega_{min} := 0$.
2. *For* $k = 0, 1, \ldots, 2^q - 1$, *consider its binary representation* $k_{bin} := [D_1, D_2, \ldots, D_q] \in \{0, 1\}^q$.
   (a) *Let* $D = diag\{D_1, D_2, \ldots, D_q\}$.
   (b) *Let* $\omega_{min} := \min\{\omega_{min}, sign[r(D)]\}$ *and* $\omega_{max} := \min\{\omega_{max}, sign[r(D)]\}$;
3. *IF* $\omega_{min} = 0$ *and* $\omega_{max} = 1$, *then* $\sigma := +$;
4. *IF* $\omega_{min} = -1$ *and* $\omega_{max} = 0$, *then* $\sigma := -$;
5. *IF* $\omega_{min} = 0$ *and* $\omega_{max} = 0$, *then* $\sigma := 0$;
6. *IF* $\omega_{min} = -1$ *and* $\omega_{max} = 1$, *then* $\sigma :=?$.

The outcome of the procedure is one of the four cases represented in Fig. 2. Figures 2 (a)–(c) illustrate the cases where the sign of the output variation, when the input varies as a step function, is structurally determinate; Fig. 2 (d) depicts the case where no structural influence can be identified, because the output could either increase, decrease or not vary, depending on the specific choice of the parameters.

Remark 4 The algorithm has exponential complexity: if the diagonal matrix D has dimension q, the sign of $r(D)$ must be explored on the 2^q vertices of the hypercube.

We stress that our result exploits the fact that J is the positive linear combination of rank–one matrices, hence $r(D)$ is a multi–affine function defined on a hypercube (or a hyper–box) and reaches its extrema on the vertices, which is crucial in the proof of Theorem 1. In the general case of a convex combination of matrices that do not have rank one, such a vertex result is not assured. For instance,

$$\det \begin{bmatrix} a & 0 \\ 0 & a-b \end{bmatrix} = a(a-b)$$

is not multi–affine in the parameters. If we consider the square $0 \leq a, b \leq 1$, this determinant is never negative on the four vertices. However, for $b = 1$ and $a = 1/2$ the determinant is $-1/4 < 0$. This happens because the matrix associated with a (which is the identity $I_2 \in \mathbb{R}^{2\times 2}$) has rank two.

Remark 5 The vectors E and H are in general functions of the parameters, namely of the partial derivatives (with respect to x and to u): $[E]_i = \frac{\partial f_i}{\partial u}\Big|_{(\bar{x},\bar{u})}$ and $[H]_j = \frac{dg}{dx_j}\Big|_{\bar{x}}$. This not an issue, because $r(D)$ is still a multi–affine function of the parameters.

The method presented in this section will be used to compute the structural steady–state influence matrix M in Section 5.

4.1 Structural influence of system parameters on the system outputs

Our approach can be used to establish the existence of a structural influence of *any input* on a given output. Since uncertain parameters (for example, binding rates, dissociation constants, or Hill coefficients) can be considered as inputs subject to variations, our method can be applied to identify the structural influence of a parameter on system outputs. We illustrate this case with an example.

Example 3 Consider the system

$$\begin{cases} \dot{x}_1 = -pf_1(x_1, x_2) + f_3(x_3) + x_{1,0} \\ \dot{x}_2 = -pf_1(x_1, x_2) - f_2(x_2) + x_{2,0} \\ \dot{x}_3 = pf_1(x_1, x_2) - f_3(x_3) - f_4(x_3) \end{cases}, \qquad J = \begin{bmatrix} -\tilde{\alpha} & -\tilde{\beta} & \gamma \\ -\tilde{\alpha} & -(\tilde{\beta}+\delta) & 0 \\ \tilde{\alpha} & \tilde{\beta} & -(\gamma+\varepsilon) \end{bmatrix}, \qquad \tilde{\alpha} = p\alpha, \quad \tilde{\beta} = p\beta,$$

where p is a positive parameter that we consider as input, $u = p$, and where the Greek letters denote the absolute value of the partial derivatives $|\partial f_j/\partial x_i|$. The BDC–decomposition and vector E are

$$J = \underbrace{\begin{bmatrix} -1 & -1 & 1 & 0 & 0 \\ -1 & -1 & 0 & -1 & 0 \\ 1 & 1 & -1 & 0 & -1 \end{bmatrix}}_{=B} \underbrace{\mathrm{diag}\{\tilde{\alpha}, \tilde{\beta}, \gamma, \delta, \varepsilon\}}_{=D} \underbrace{\begin{bmatrix} 1 & 0 & 0 \\ 0 & 1 & 0 \\ 0 & 0 & 1 \\ 0 & 1 & 0 \\ 0 & 0 & 1 \end{bmatrix}}_{=C}, \qquad E = \begin{bmatrix} -\zeta \\ -\zeta \\ \zeta \end{bmatrix},$$

where $\zeta = |\partial \dot{x}_i/\partial p| = f_1$ (see Remark 5). If we are interested in considering as an output the variable x_i, then $H = H_i$ will have a 1 in the ith position and zero elsewhere. By means of the proposed vertex algorithm, we can derive the following steady–state influence of p on the variables x_1, x_2 and x_3: [4]

$$\begin{matrix} & \overbrace{}^{p} \\ x_1\,\{ & \\ x_2\,\{ & \\ x_3\,\{ & \end{matrix} \begin{bmatrix} - \\ 0 \\ 0 \end{bmatrix}.$$

We see that both x_2 and x_3 are subject to a perfect adaptation with respect to variations of p. This fact, although surprising, can be explained by considering the system steady state. We can see that $\bar{x}_3$ and $\bar{x}_2$ depend on the constant influx only (since by adding the first and third equation at steady state we have $f_4(\bar{x}_3) = x_{1,0}$, and by subtracting the second to the first we have $f_3(\bar{x}_3) + f_2(\bar{x}_2) = x_{2,0} - x_{1,0}$), while $\bar{x}_1$ depends on p and can thus compensate any variation in p, preventing it from affecting $\bar{x}_2$ and $\bar{x}_3$. ◇

The observation in Example 3 can be generalised.

Proposition 3 *Consider a system of the form (4) and assume that the jth reaction has rate $f_j(\cdot) = p\tilde{f}_j(\cdot)$, with p a positive parameter, and that (at least) one of the species, having concentration x_i, is involved as a reagent in the jth reaction only and in no other reactions (hence the corresponding column J_i of the Jacobian matrix contains one coefficient only). Then all the variables of the system are insensitive to variations in p, except for x_i itself.*

Proof: Since E and J_i are linearly dependent columns, the determinant in (12) is zero as long as h_i is zero.

[4] In this simple case, the reader can easily check the results by direct computation of $\det \begin{bmatrix} -J & -E \\ H_i & 0 \end{bmatrix}$, by considering H_i associated with the considered variable (*e.g.*, for x_1, $H_1 = [\,1\ 0\ 0\,]$).

5 Structural influence matrix

To assess the structural influence on the ith variable of a persistent, additive input applied to the jth variable, we consider a system (8)–(9) having the form

$$\dot{x}(t) = f(x(t)) + Eu(t), \tag{13}$$

$$y(t) = Hx(t), \tag{14}$$

and we take vectors $E = E_j$ and $H = H_i$ with a single non–zero entry equal to one

$$E_j = [0 \ \dots \ 0 \ \underbrace{1}_{\text{position } j} \ 0 \ \dots \ 0]^\top, \qquad H_i = [0 \ \dots \ 0 \ \underbrace{1}_{\text{position } i} \ 0 \ \dots \ 0].$$

If the system admits a BDC–decomposition, then we can generate each entry $[M]_{ij}$ of the influence matrix $M \in \mathbb{R}^{n\times n}$ by means of the numerical vertex algorithm described in Procedure 1, choosing the corresponding E_j and H_i. $[M]_{ij}$ is:

- '+' if the influence is positive for any realisation of the structure;
- '0' if there is perfect adaptation for any realisation of the structure;
- '−' if the influence is negative for any realisation of the structure;
- '?' if the influence is not structurally sign–definite.

As illustrated in Fig. 2, each entry of the influence matrix (or, more in general, any structural steady–state derivative) can be interpreted as the response of the system output to a step input.

Remark 6 Consistently with the results by Dambacher et al (2002, 2003a,b, 2005); Dambacher and Ramos Jiliberto (2007), computing the influence matrix is equivalent to determining the sign pattern of the adjoint matrix $\mathrm{adj}(-J)$ of $-J$, namely the matrix such that $(-J)^{-1} = \mathrm{adj}(-J)/\det(-J)$.

Example 4 (Metabolic network: influence matrix). Reconsider the system proposed in Example 1: $\det(-J)$ is structurally positive and the vertex algorithm provides the influence matrix

$$M = \begin{bmatrix} + & - & 0 \\ - & + & 0 \\ + & 0 & + \end{bmatrix}.$$

◇

Example 5 The system in Fig. 1 (b) corresponds to the Jacobian matrix

$$J = \begin{bmatrix} -(\alpha+\zeta) & 0 & 0 & 0 & -\eta & 0 & 0 \\ \alpha & -(\gamma+\varepsilon) & 0 & -\delta & 0 & 0 & 0 \\ 0 & 0 & -(\beta+\iota) & 0 & 0 & -\lambda & 0 \\ 0 & -\gamma & \beta & -(\delta+\varphi) & 0 & 0 & 0 \\ -\zeta & \varepsilon & 0 & 0 & -\eta & 0 & 0 \\ 0 & 0 & -\iota & \varphi & 0 & -\lambda & 0 \\ 0 & \gamma & 0 & \delta & 0 & 0 & -\mu \end{bmatrix}.$$

By means of the proposed algorithm, we find that $\det(-J) > 0$ structurally and

$$M = \begin{bmatrix} + & - & + & + & - & - & 0 \\ + & + & - & - & - & + & 0 \\ + & + & + & - & - & - & 0 \\ - & - & + & + & + & - & 0 \\ ? & + & - & - & + & + & 0 \\ - & - & ? & + & + & + & 0 \\ + & + & + & + & - & - & + \end{bmatrix}.$$

◇

We conclude the section with some remarks on the influence matrix in two particular classes of systems.

We begin with monotone systems. A system of the form $\dot{x}(t) = f(x(t))$, with f differentiable, is *monotone* (Smith (2008); Sontag (2007)) iff its Jacobian $J(x)$ is a *Metzler matrix* for any x, *i.e.*, its off–diagonal entries are nonnegative. We have the following.

Proposition 4 *Given a monotone system, denote by J its Jacobian evaluated at a stable equilibrium. Then its influence matrix is such that $[M]_{ij} \in \{+, 0\}$ for all (i, j). If furthermore J is irreducible, $[M]_{ij} = +$ for all (i, j).*

Proof: If J is a stable Metzler matrix (hence its diagonal entries are negative, see Farina and Rinaldi (2000)), then $(-J)^{-1} \geq 0$, where the inequality has to be intended componentwise; if, moreover, the matrix is irreducible, the inequality is strict: $(-J)^{-1} > 0$ (Hale et al (2014)). Since the equilibrium is stable, $\det(-J) > 0$. Then $\mathrm{adj}(-J) = (-J)^{-1} \det(-J) \geq 0$ (> 0 in the irreducible case), which proves the thesis. □

Yet monotonicity is not necessary to have a positive influence matrix. In fact, there are stable matrices which are not Metzler, but for which all of the entries of $\mathrm{adj}(-J)$ are positive.

Another remarkable case is that in which some diagonal entries of the influence matrix are zero, as in the prey–predator case in Section 2, or even negative. Consider the system

$$J = \begin{bmatrix} -\alpha & \beta \\ -\gamma & \delta \end{bmatrix}, \quad \text{for which} \quad M = \begin{bmatrix} - & + \\ - & + \end{bmatrix}.$$

Assuming stability, if we apply a constant additive input on the first state variable, we have that its own steady–state value decreases. This situation may arise because this is a non minimum–phase system; such systems have been recently studied in a biological context (Motee et al (2010); Yeung et al (2013)).

6 Analysis within the stoichiometric compatibility class

In some chemical reaction networks, the system represented by (4) is neutrally stable, and its state is forced to stay inside the *stoichiometric compatibility class* associated with the initial conditions:

$$x(t) \in \mathcal{C}(x(0)) = \{x(0) + \mathrm{Ra}[S]\} \cap \mathbb{R}^n_+.$$

This happens, for instance, in the presence of mass conservation constraints. Therefore, the rank of the stoichiometric matrix S (hence of B) corresponds to the dimension of the stoichiometric compatibility class. If matrix B is not full row rank, the system trajectories evolve inside a subspace having dimension smaller than n and, of course, BDC is structurally singular. We assume however that 0 is the only eigenvalue of the system having nonnegative real part.

In this case, to restrict our analysis to the stoichiometric compatibility class, we can consider a transformation that provides a full row rank $\tilde{B}$. Let $z(t) = x(t) - \bar{x}$, $v(t) = u(t) - \bar{u}$, $w(t) = y(t) - \bar{y}$ and consider the linearised system

$$\dot{z}(t) = BDCz(t) + Ev(t), \tag{15}$$

$$w(t) = Hz(t). \tag{16}$$

Consider the state transformation

$$\begin{bmatrix} M \\ N \end{bmatrix} z = \begin{bmatrix} z_M \\ z_N \end{bmatrix}, \qquad z = \begin{bmatrix} P & Q \end{bmatrix} \begin{bmatrix} z_M \\ z_N \end{bmatrix},$$

where $M^\top$ is a basis of $\ker[B^\top]$ (ker denotes the kernel of a matrix; *i.e.*, $M^\top$ is such that $MB = 0$) and N is a basis of $\mathrm{Ra}[B]$. The transformed system is

$$\begin{bmatrix} \dot{z}_M \\ \dot{z}_N \end{bmatrix} = \begin{bmatrix} 0 & 0 \\ NBDCP & NBDCQ \end{bmatrix} \begin{bmatrix} z_M \\ z_N \end{bmatrix} + \begin{bmatrix} ME \\ NE \end{bmatrix} v \doteq \begin{bmatrix} 0 & 0 \\ B_N DC_P & B_N DC_Q \end{bmatrix} \begin{bmatrix} z_M \\ z_N \end{bmatrix} + \begin{bmatrix} E_M \\ E_N \end{bmatrix} v,$$

$$y = \begin{bmatrix} HP & HQ \end{bmatrix} \begin{bmatrix} z_M \\ z_N \end{bmatrix} \doteq \begin{bmatrix} H_P & H_Q \end{bmatrix} \begin{bmatrix} z_M \\ z_N \end{bmatrix}.$$

If $E_M = 0$, we can compute the *step response* of the system: $z_M(t) \equiv z_M(0) = 0 \; \forall t$ and the z_N–subsystem is asymptotically stable by assumption. Then we can consider $\det(-B_N DC_N)$ and the sign of

$$\det \begin{bmatrix} -B_N DC_Q & -E_N \\ H_Q & 0 \end{bmatrix}$$

and our analysis can be carried out without changes.

If instead $E_M \neq 0$, the equation for z_M would be $\dot{z}_M = E_M u$, so that $z_M(t) = z_M(0) + E_M ut$ would diverge. The analysis can be carried out by considering the *impulse response* of the system, namely by assuming u to be the Dirac delta function. In this case we have that $z_M(t) \equiv z_M(0^+) = E_M$ is constant and can be considered as the input for the z_N–subsystem, which is asymptotically stable (therefore its impulse response converges to zero) so that the sign of the steady–state derivative is given by the sign of

$$\det \begin{bmatrix} -B_N DC_Q & -B_N DC_P E_M \\ H_Q & H_P E_M \end{bmatrix}.$$

In the previous cases, we have assumed that $B_N DC_Q$ is non–singular. If $B_N DC_Q$ is singular because C does not have full column rank, we can consider an additional state transformation that provides a full column rank $\tilde{C}$, as previously done for B. However, even when both B and C have full rank, matrix BDC can be structurally singular. In this case, as long as zero is a simple eigenvalue, the (i, j) entry of the structural influence matrix can represent the sign of the steady–state variation of the i–th variable due to an impulsive additive input applied to the j–th variable. Otherwise, unfortunately, it is useless to resort to further transformations.

Example 6 Consider the system introduced in Example 2 and depicted in Fig. 1 (a). Its Jacobian matrix, provided in (7) along with its BDC–decomposition, is structurally singular. Note that a mass conservation constraint is present, because $\dot{b} + \dot{c} = 0$ in system (6), hence $b + c$ is constant. Since zero is a simple root of

the characteristic polynomial $p(s) = s^3 + (\alpha+\beta+\gamma+\delta+\varepsilon)s^2 + \alpha(\delta+\varepsilon)s$, the influence matrix can be used to describe structural steady–state variations induced by impulsive inputs:

$$M = \begin{bmatrix} 0 & ? & ? \\ 0 & + & + \\ 0 & + & + \end{bmatrix}. \tag{17}$$

Note that, while C has full column rank, $\mathrm{rank}(B) = 2$. Therefore, we can restrict our analysis to the stoichiometric compatibility class. A basis of $\ker[B^\top]$ is given by the vector $[0 \;\; 1 \;\; 1]^\top$. Completing with a basis of $\mathrm{Ra}[B]$, we achieve the following state transformation:

$$T^{-1} = \begin{bmatrix} 0 & 1 & 1 \\ 0 & 1 & 0 \\ 1 & 0 & 0 \end{bmatrix}, \qquad T = \begin{bmatrix} 0 & 0 & 1 \\ 0 & 1 & 0 \\ 1 & -1 & 0 \end{bmatrix}.$$

To account for all the possible variable selections, we take $E = I_3$ and $H = I_3$ as the identity matrices, so that the influence (i, j) is achieved by selecting the jth column of E and the ith row of H. The transformed system becomes

$$T^{-1}JT = \left[\begin{array}{c|cc} 0 & 0 & 0 \\ \hline \gamma+\varepsilon & -(\beta+\gamma+\delta+\varepsilon) & -\alpha \\ \gamma & -(\beta+\gamma) & -\alpha \end{array}\right] = \begin{bmatrix} 0 & 0 \\ B_N D C_P & B_N D C_Q \end{bmatrix},$$

$$T^{-1}E = \left[\begin{array}{ccc} 0 & 1 & 1 \\ \hline 0 & 1 & 0 \\ 1 & 0 & 0 \end{array}\right] = \begin{bmatrix} E_M \\ E_N \end{bmatrix}, \quad HT = \left[\begin{array}{c|cc} 0 & 0 & 1 \\ 0 & 1 & 0 \\ 1 & -1 & 0 \end{array}\right] = \begin{bmatrix} H_P & H_Q \end{bmatrix}.$$

We denote by $E_M^{(j)}$ the jth column of E_M and by $H_Q^{(i)}$ the ith row of H_Q. Now $\det(-B_N D C_Q)$ is structurally positive and, by considering the sign of

$$\det \begin{bmatrix} -B_N D C_Q & -B_N D C_P E_M^{(j)} \\ H_Q^{(i)} & H_P^{(i)} E_M^{(j)} \end{bmatrix},$$

we can compute the (i, j) entry of the influence matrix associated with the impulse response:

$$M_{(\mathrm{scc})} = \begin{bmatrix} 0 & ? & ? \\ 0 & + & + \\ 0 & + & + \end{bmatrix}. \tag{18}$$

As expected, matrix (18) is equal to (17), which we had been previously able to compute without any state transformation just because $\dim(\ker[B^\top]) = 1$. If the kernel had a higher dimension, a state transformation would be necessary to compute the influence matrix in the stoichiometric compatibility class. ◇

Example 1, with mass conservation constraints, was previously analysed in a reduced–order form, neglecting the variable $d = K - b$. Yet, it is also possible to consider *all of the four variables*: in this case, the influence matrix in the stoichiometric compatibility class can be computed as illustrated in this section.

7 Tree–like algorithm for the influence matrix of systems with independent Jacobian entries

Most of our analysis in Section 5 focuses on flow–governed systems, in which a simultaneous production and consumption occur: with a given reaction rate, one or more species are converted into other species, leading to Jacobian matrices with coefficients appearing repeatedly on the same column (possibly with different sign). Yet, in several cases all the Jacobian entries are independent (hence, due to Assumption 1, J is a sign–definite matrix), as in the prey–predator system (1), having Jacobian (5).

When the entries are independent, the problem can still be solved by adopting the proposed vertex algorithm. However, this can involve a very large number of parameters. For instance, in Example 1 we have 4 parameters, thus 16 vertices; if all the non–zero entries were independent, we would have 128 vertices. If all the non–zero entries of Example 5 were independent, the number of vertices would be $2^{19} = 524288$.

Example 7 (Snowshoe hare population dynamics). To describe the interactions among vegetation, snowshoe hare and predators in boreal forests, a simple model is proposed by Dambacher and Ramos Jiliberto (2007), corresponding to the sign–definite community matrix

$$J = \begin{bmatrix} -\alpha & -\beta & 0 \\ \gamma & -\delta & -\varepsilon \\ \varphi & \eta & -\psi \end{bmatrix}, \qquad \text{having sign pattern} \qquad \Sigma_J = \begin{bmatrix} - & - & 0 \\ + & - & - \\ + & + & - \end{bmatrix}. \tag{19}$$

The influence matrix

$$M = \begin{bmatrix} + & - & + \\ ? & + & - \\ + & ? & + \end{bmatrix} \tag{20}$$

can be found by simply computing the adjoint, as well as by means of the proposed vertex algorithm, which involves 8 parameters, hence 256 vertices. ◇

For systems with very high dimension, complexity could render the vertex algorithm unfeasible. Yet, complexity can be reduced by adopting a tree–like algorithm that takes advantage of the sparsity of the Jacobian (namely, of the presence of several zero entries). To determine the influence matrix, we have to structurally assess the sign of the determinant (12) for all input–output pairs, or, equivalently, to establish the sign of the entries of $\mathrm{adj}(-J)$, which are just determinants of sub–matrices.

Procedure 2 ***Tree Recursive Algorithm for Determinant Sign: TRADS(Σ_n).***

Input: *$\Sigma_n \in \mathbb{R}^{n\times n}$, the sign matrix which forms the root of the tree.*

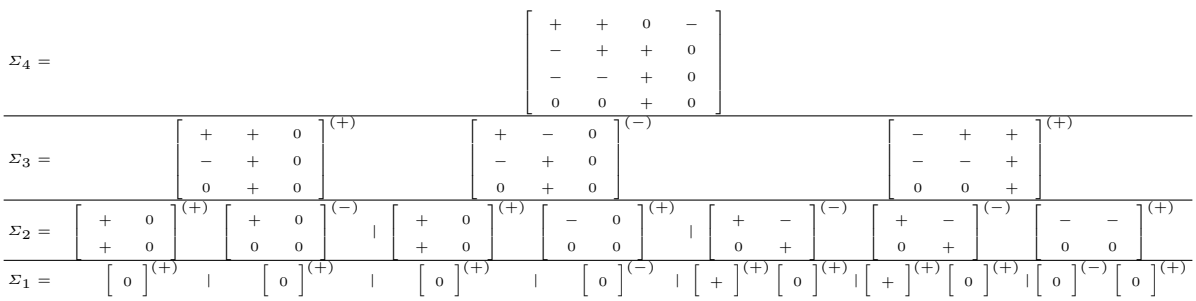
Output: *The structural sign of the determinant of Σ_n, $\sigma \in \{+,-,0,?\}$.*

1. *Consider the first row of matrix Σ_n.*
2. *For each non zero entry $[\Sigma_n]_{1i}$, create a link to a new node, marked by '$-$' or by '$+$' depending on the sign of $[\Sigma_n]_{1i}\,(-1)^{i+1}$, and associate with this node the sign matrix Σ_{n-1} (of dimension $n-1$), which is the complementary matrix of the considered entry.*
3. *For each new node connected to the original node with a '$-$' arc, change sign to the first row of the corresponding matrix Σ_{n-1}.*
4. *Apply the procedure TRADS(Σ_{n-1}) to all the new nodes.*

The procedure stops when all of the sign matrices have dimension 1. *Then $\sigma = +$ if all the matrices are $[+]$, or $[0]$ with at least a $[+]$ matrix; $\sigma = -$ if all the matrices are $[-]$, or $[0]$ with at least a $[-]$ matrix; $\sigma = 0$ if all the matrices are $[0]$; $\sigma = ?$ otherwise.*

Obviously, the outcome of the procedure is the same if we alter the matrix by transposing it or by permuting rows and/or columns, with appropriate sign changes. This can be done at each iteration, preferably choosing the row or column having most zero entries.

Example 8 (Snowshoe hare population dynamics). Consider the signed Jacobian matrix (19), in Example 7. We want to find the sign of the structural influence on the third variable of an additive input persistently applied to the first variable. The algorithm (applied without transposing or permuting) would produce



Indices $[]^{(-)}/[]^{(+)}$ mean that the sign of the first row is changed/unchanged. Since the final matrices are all $[0]$ with at least a $[+]$, the determinant is structurally positive, consistently with $[M]_{31} = +$ in (20). Of

course, the number of computations dramatically reduces if we notice that the most convenient choice at the beginning is the last row, or the last column, having a single non–zero entry. ◇

Remark 7 In the worst case in which all the entries are non–zero, the tree algorithm would have a complexity of $n!$, where n is the matrix dimension. This complexity is negligible if compared to that of the vertex algorithm directly applied, which would be 2^{n^2}. For instance, for a 5×5 matrix we have $5! = 120$ instead of 2^{25} (roughly $32 \cdot 10^6$). In general, meaningful matrices are sparse; therefore, the operation count would be at most $\nu_1 \times \nu_2 \times \ldots \times \nu_n$, where ν_i is the number of non–zero entries of the ith row. Note also that sparsity is typical in several cases of practical interest.

Example 9 (Population dynamics in Danish shallow lakes). Two models are analysed by Dambacher and Ramos Jiliberto (2007) to describe the interactions among 10 different species in Danish shallow lakes: model "I" considers modified or non–linear interactions in a model of eutrophic shallow lakes, while model "J" considers only linear trophic interactions in a model of mesotrophic lakes. In both cases, starting from a sign–definite community matrix A, a qualitative adjoint matrix $\mathrm{adj}(-A)$ is computed. The qualitative adjoint matrices of the two models are then compared with experimental responses measured in field studies: model J is considered less reliable, because the resulting signs are largely inconsistent with the observed response. By means of the proposed algorithm, we can compute the structural influence matrices. The entries are all indeterminate for model "I". Also for model "J" most entries are indeterminate, however there are notable exceptions: $[M]_{34} = +$, $[M]_{43} = -$, $[M]_{44} = +$, $[M]_{99} = +$ have sign consistent with that reported by Dambacher and Ramos Jiliberto (2007) and, interestingly, are not among the entries reported to be inconsistent with field observations. ◇

8 Examples

8.1 *Escherichia coli* EnvZ-OmpR osmoregulation system

The proposed vertex algorithm can be applied to the biochemical network in Fig. 1 (c), representing the complex *E. coli* EnvZ-OmpR osmoregulation model studied in Shinar and Feinberg (2010), with additional auto–degradation reactions for species C and D. For this system, steady–state effects are not easy to foresee

based on qualitative considerations. The system has Jacobian matrix

$$J = \begin{bmatrix} -(\alpha+\beta) & \gamma & 0 & 0 & \vartheta & 0 & 0 & \mu \\ \beta & -(\gamma+\delta) & 0 & 0 & 0 & -\varepsilon & \kappa+\lambda & 0 \\ 0 & 0 & -(\zeta+\varphi) & -\eta & \xi & 0 & 0 & \nu \\ 0 & 0 & -\zeta & -(\eta+\psi) & \xi & 0 & \kappa & 0 \\ 0 & 0 & \zeta & \eta & -(\xi+\vartheta) & 0 & 0 & 0 \\ 0 & -\delta & 0 & 0 & \vartheta & -\varepsilon & \lambda & 0 \\ 0 & \delta & 0 & 0 & 0 & \varepsilon & -(\kappa+\lambda) & 0 \\ \alpha & 0 & 0 & 0 & 0 & 0 & 0 & -(\mu+\nu) \end{bmatrix}.$$

By means of the vertex algorithm, we can see that $\det(-J)$ is structurally positive and the influence matrix is

$$M = \begin{bmatrix} + & + & + & + & + & + & + & + \\ + & + & + & + & + & + & + & + \\ + & + & + & 0 & + & 0 & + & + \\ 0 & 0 & 0 & + & + & + & + & 0 \\ + & + & + & + & + & + & + & + \\ ? & ? & ? & ? & ? & ? & ? & ? \\ + & + & + & + & + & + & + & + \\ + & + & + & + & + & + & + & + \end{bmatrix}.$$

8.2 A case study: biofuel production

In this section, we *structurally* compare two biofuel production models and we show that their peculiar properties can be inferred independent of parameter values. An important issue of microbial biofuel production methods, based on engineered bacteria, is that production cannot be unrestrictedly increased because biofuel is toxic to the cell that is producing it. Efflux pumps have been shown to be effective at increasing tolerance to biofuel, but an over–expression of efflux pumps hinders the growth of the cell population, hence decreases biofuel production: the toxicity of biofuel and of pump over–expression must then be properly balanced to maximise the biofuel output. We consider the two models for cell growth and biofuel production provided by Harrison and Dunlop (2012): in the former, efflux pumps are expressed at a constant level; in the latter, a synthetic feedback loop is implemented, using a biosensor to control efflux pump expression.

The feedback system is described by the equations

$$\begin{cases} \dot{n} = n\left[\alpha_n\left(1-\frac{n}{n_{max}}\right) - \delta_n b_i - \frac{\alpha_n p}{p+\gamma_p}\right] \\ \dot{R} = \alpha_R + k_R\left(\frac{I}{I+\gamma_I}\right) - \beta_R R \\ \dot{p} = \alpha_p + k_p \frac{1}{\frac{R}{1+k_b b_i}+\gamma_R} - \beta_p p \\ \dot{b}_i = \alpha_b n - \delta_b p b_i \end{cases},$$

where n is the cell density, R is the concentration of repressor proteins, p is the concentration of pumps, b_i is the concentration of intracellular biofuel; n_{max} is the maximum population size. Both biofuel toxicity and pump toxicity can prevent population growth. Repressor activation by the inducer is modelled as $I/(I+\gamma_I)$, where γ_I indicates the inducer value that corresponds to half maximal activation of the repressor and I can be considered as a constant input. Since R reaches its steady state independently of all other state variables, it can be considered just as an external inflow.

Note that n has two possible equilibrium values: the trivial $\bar{n} = 0$ (all the cells are dead) and the nontrivial $\bar{n}$ we are interested in, such that $\left[\alpha_n\left(1-\frac{\bar{n}}{n_{max}}\right) - \delta_n \bar{b}_i - \frac{\alpha_n \bar{p}}{\bar{p}+\gamma_p}\right] = 0$. Thus $[J]_{11}$, *i.e.*, the partial derivative computed at the equilibrium point, is simply $-\frac{\alpha_n n}{n_{max}}$. Denote by φ_p the positive partial derivative of $\frac{p}{p+\gamma_p}$ with respect to p and by ψ_{b_i} the positive partial derivative of $k_p \frac{1}{\frac{R}{1+k_b b_i}+\gamma_R}$ with respect to b_i. The Jacobian matrix of the reduced system, including only the variables n, p, b_i, is then:

$$J_{FL} = \begin{bmatrix} -\frac{\alpha_n \bar{n}}{n_{max}} & -\alpha_n \bar{n}\varphi_p & -\delta_n \bar{n} \\ 0 & -\beta_p & \psi_{b_i} \\ \alpha_b & -\delta_b \bar{b}_i & -\delta_b \bar{p} \end{bmatrix}.$$

It can be seen that $\det(-J_{FL})$ is structurally positive and the influence matrix is

$$M_{FL} = \begin{bmatrix} + & ? & - \\ + & + & + \\ + & - & + \end{bmatrix}.$$

As expected, b_i is reduced by a positive input applied to p and augmented by a positive input applied to n; a positive input applied to b_i reduces n, due to the biofuel toxicity for the cells. The effect on n of an input applied to p is not structurally determinate, which is consistent with the considerations about the trade–off regarding efflux pumps expression: a significant level of p helps increasing cell tolerance to biofuel (enhancing population growth), but, when over–expressed, pumps burden cells (hindering population growth). We finally point out the effect of the feedback, which allows modulating the expression of efflux

pumps. In fact, an input applied to n increases p, to adjust it to the needs of a grown population, and an input applied to b_i also increases p, because more efflux pumps are needed to protect the cells from an increased level of toxicity.

We now consider the system in which efflux pumps are expressed at a constant level. The repressor equation is no longer present in the system, since an inducer is now directly used to control pump expression, while the other equations remain unchanged:

$$\begin{cases} \dot{n} = n\left[\alpha_n\left(1-\frac{n}{n_{max}}\right) - \delta_n b_i - \frac{\alpha_n p}{p+\gamma_p}\right] \\ \dot{p} = \alpha_p + k_p\left(\frac{I}{I+\gamma_I}\right) - \beta_p p \\ \dot{b}_i = \alpha_b n - \delta_b p b_i \end{cases}, \qquad J_C = \begin{bmatrix} -\frac{\alpha_n \bar{n}}{n_{max}} & -\alpha_n \bar{n}\varphi_p & -\delta_n \bar{n} \\ 0 & -\beta_p & 0 \\ \alpha_b & -\delta_b \bar{b}_i & -\delta_b \bar{p} \end{bmatrix}.$$

It can be seen that $\det(-J_C)$ is structurally positive. The influence matrix is:

$$M_C = \begin{bmatrix} + & ? & - \\ 0 & + & 0 \\ + & - & + \end{bmatrix}.$$

The comparison between M_C and M_{FL} immediately highlights the effect of the feedback loop: all the structural influences are the same but those on p due to a persistent input applied to n and to b_i. In the absence of feedback, p is completely insensitive to both inputs applied to n and to b_i; feedback allows to tune the expression of efflux pumps, increasing it when a positive input is applied to n or to b_i. Thanks to the feedback regulation, pumps can be produced at the exact amount needed for increasing cell tolerance to toxic biofuel, without excessively burdening cells.

According to the sensitivity analysis performed by Harrison and Dunlop (2012), the feedback model is almost insensitive to many system parameters, while a few key parameters ($\alpha_n, \delta_n, \gamma_p, n_{max}, \alpha_b, \delta_b$) strongly influence growth and production. To assess if the sign of such an influence is independent of the other parameter values, we can perform a *structural* analysis by computing the determinant in (12) for each parameter κ, where $E_\kappa = [\partial\dot{n}/\partial\kappa \;\; \partial\dot{p}/\partial\kappa \;\; \partial\dot{b}_i/\partial\kappa]^\top$ and H selects the desired output among n, p and b_i. The influence of *parameter variations* on the steady–state of the state variables can be summarised as follows:

$$\begin{array}{c} \\ n\,\{ \\ p\,\{ \\ b_i\,\{ \end{array} \begin{array}{c} \overbrace{}^{\alpha_n}\;\overbrace{}^{\delta_n}\;\overbrace{}^{\gamma_p}\;\overbrace{}^{n_{max}}\;\overbrace{}^{\alpha_b}\;\overbrace{}^{\delta_b}\;\overbrace{}^{\alpha_p}\;\overbrace{}^{k_p}\;\overbrace{}^{\beta_p}\;\overbrace{}^{k_b}\;\overbrace{}^{\gamma_R} \\ \begin{bmatrix} + & - & + & + & - & + & ? & ? & ? & ? & ? \\ + & - & + & + & + & - & + & + & - & + & - \\ + & - & + & + & + & - & - & - & + & - & + \end{bmatrix} \end{array}.$$

The obtained structural influences are fully consistent with numerical results, but now we have an advantage: we can guarantee that the same trends are achieved independently of the other system parameters.

9 Discussions and future work

We have considered the problem of *structurally* assessing the steady–state influence of a stimulus, regarded as a persistent input provided to a system, on a system variable, regarded as an output. Based on theoretical results that exploit the particular structure of a broad class of biological systems, we have proposed an efficient algorithm to compute the structural steady–state input–output influence by checking the determinant of a matrix only at a finite number of points (the vertices of the hypercube defined by the space of parameters). Under asymptotic stability assumptions, the steady–state influence can be used to describe the system response to a step input. In the case of neutrally stable systems, it can be used instead to describe the system response to an impulsive input in the *stoichiometric compatibility class*, after a suitable state transformation. Such a vertex algorithm has exponential complexity: to enhance computational efficiency, we have proposed a tree–like algorithm for systems whose Jacobian has independent entries. The outcome of our investigation is the influence matrix, which summarises the steady–state net influence on each variable due to an additive input persistently applied to a single variable. In some cases, entries with indeterminate sign are found in the influence matrix. Interestingly, we have noticed that several complex systems proposed in the literature have a surprisingly small fraction of indeterminate entries, so revealing an intrinsic robustness aspect in the system steady–state interactions.

An interesting side result of our approach is its ability to structurally identify *perfect adaptation*, a property widely observed and studied in the literature (Yi et al (2000); Drengstig et al (2008); Ma et al (2009); Waldherr et al (2012)). In our framework, a 0 influence represents a structural perfect adaptation. This phenomenon may occur in two different cases: the first is the trivial case in which there is no path leading from the input to the output variable in the system graph (this corresponds to a reducible Jacobian); the second is the case in which a path between the input and the output variables exists and has a zero complement, resulting in zero complementary feedback (Puccia and Levins (1985); Dambacher et al (2002)).

Another promising future direction concerns robust stability analysis, which may be a preliminary step for the application of the proposed algorithm. We have assumed that the equilibrium exists and is locally stable. However, we may need to check if this assumption holds. The existence of an equilibrium is assured if the system solutions are bounded. Some structural boundedness criteria have been suggested by Blanchini and Giordano (2014), along with stability criteria based on Lyapunov theory. Stability investigation can be also carried out with the methodologies presented in the book by Barmish (1993). For instance, the

robust stability test for a system admitting a BDC–decomposition can be achieved by noticing that the corresponding characteristic polynomial

$$\det(\lambda I - BDC) = p_0(D) + p_1(D)\lambda + p_2(D)\lambda^2 + \cdots + \lambda^n$$

has coefficients $p_i(D)$ which are *multi–affine functions* of the coefficients D_k. So, if reasonable bounds $0 < D_k^- \leq D_k \leq D_k^+$ can be assumed for each coefficient D_k, then the graphical test based on the Mapping Theorem (Barmish (1993), Sections 14.6–14.10) provides a nice sufficient condition for stability.

Finally, we note that an important extension of our approach (and algorithm) would be the inclusion of *a priori* information to determine structural influences. Consider for instance the community matrix associated with a stable three–dimensional system involving two species in competition, x_1 and x_2, preyed upon by a common predator x_3 (Levins (1975), Figure 6b):

$$J = \begin{bmatrix} -a_{11} & -a_{12} & -a_{13} \\ -a_{21} & -a_{22} & -a_{23} \\ a_{31} & a_{32} & 0 \end{bmatrix}, \qquad M = \begin{bmatrix} + & - & ? \\ - & + & ? \\ ? & ? & (-) \end{bmatrix}.$$

Without any additional information, the entry M_{33} of the influence matrix would be '?'. However, if we assume that the system involving only the prey x_1 and x_2 is unstable, so that $a_{11}a_{22} < a_{12}a_{21}$ (thus the overall system is stabilised by the presence of the predator), then we have $M_{33} = -$. Hence, a positive input to the predator population results in a steady–state decline in the abundance of the predator itself, due to the instability of the competing–prey subsystem.

References

Abate, A., Tiwari, A., Sastry, S. (2007). Box invariance for biologically-inspired dynamical systems. In: Proceedings of the IEEE Conference on Decision and Control, pp 5162–5167

Alon, U. (2006). An Introduction to Systems Biology: Design Principles of Biological Circuits. Chapman & Hall/CRC

Alon, U. (2007). Network motifs: theory and experimental approaches. Nature Reviews Genetics 8(6):450–461

Alon, U., Surette, M.G., Barkai, N., Leibler, S. (1999). Robustness in bacterial chemotaxis. Nature 397(6715):168–171

Angeli, D., De Leenheer, P., Sontag, E.D. (2010). Graph–theoretic characterizations of monotonicity of chemical networks in reaction coordinates. Journal of Mathematical Biology 61(4):581–616

Angeli, D., Sontag, E.D. (2009). Graphs and the dynamics of biochemical networks. In: Ingalls, B., Iglesias, P. (eds) Control Theory in Systems Biology, vol Volume 371, MIT Press, pp 125–142

Barmish, B.R. (1994). New Tools for Robustness of Linear Systems. Macmillan

Barkai, N., Leibler, S. (1997). Robustness in simple biochemical networks. Nature 387(6636):913–917

Blanchini, F., Franco, E. (2011). Structurally robust biological networks. BMC Systems Biology 5(1):74

Blanchini, F., Giordano, G. (2014). Piecewise–linear Lyapunov functions for structural stability of biochemical networks. Automatica 50(10):2482–2493

Blanchini, F., Miani, S. (2015). Set-theoretic methods in control. Systems & Control: Foundations & Applications. 2nd edn. Birkhäuser, Basel

Blanchini, F., Franco, E., Giordano, G. (2012). Determining the structural properties of a class of biological models. In: Proceedings of the IEEE Conference on Decision and Control, pp 5505–5510

Blanchini, F., Franco, E., Giordano, G. (2014). A structural classification of candidate oscillatory and multistationary biochemical systems. Bulletin of Mathematical Biology 76(10):2542–2569

Chen, L., Wang, R., Li, C., Aihara, K. (2005). Modeling Biomolecular Networks in Cells. Springer

Chesi, G., Hung, Y. (2008). Stability analysis of uncertain genetic sum regulatory networks. Automatica 44(9):2298–2305

Craciun, G., Feinberg, M. (2005). Multiple equilibria in complex chemical reaction networks: I. the injectivity property. SIAM Journal on Applied Mathematics 65(5) pp 1526–1546

Craciun, G., Feinberg, M. (2006). Multiple equilibria in complex chemical reaction networks: II. the species-reaction graph. SIAM Journal on Applied Mathematics 66(4):1321–1338

Dambacher, J., Li, H., Rossignol, P. (2002). Relevance of community structure in assessing indeterminacy of ecological predictions. Ecology 83(5):1372–1385

Dambacher, J., Li, H., Rossignol, P. (2003a). Qualitative predictions in model ecosystems. Ecological Modelling 161(1-2):79–93

Dambacher, J., Levins, R., Rossignol, P. (2005). Life expectancy change in perturbed communities: Derivation and qualitative analysis. Mathematical Biosciences 197(1):1–14

Dambacher, J.M., Ramos Jiliberto, R. (2007). Understanding and predicting effects of modified interactions through a qualitative analysis of community structure. The Quarterly review of biology 82(3)

Dambacher, J.M., Luh, H.K., Li, H.W., Rossignol, P.A. (2003b). Qualitative stability and ambiguity in model ecosystems. The American naturalist 161(6)

Dambacher, J.M., Gaughan, D.J., Rochet, M.J., Rossignol, P.A., Trenkel, V.M. (2009). Qualitative modelling and indicators of exploited ecosystems. Fish and fisheries 10(3):305–322

De Lenheer, P., Angeli, D., Sontag, E.D. (2007). Monotone chemical reaction networks. Journal of Mathematical Chemistry 41(3):295–314

Domijan, M., Pécou, E. (2011). The interaction graph structure of mass-action reaction networks. Journal of Mathematical Biology 51(8):1–28

Drengstig, T., Ueda, H.R., Ruoff, P. (2008). Predicting perfect adaptation motifs in reaction kinetic networks. The Journal of Physical Chemistry B 112(51):16,752–16,758

El-Samad, H., Prajna, S., Papachristodoulou, A., Doyle, J., Khammash, M. (2006). Advanced methods and algorithms for biological networks analysis. Proceedings of the IEEE 94(4):832–853

Farina, L., Rinaldi, S. (2000). Positive Linear Systems; Theory and Applications. John Wiley, Hoboken

Feinberg, M. (1987). Chemical reaction network structure and the stability of complex isothermal reactors I. the deficiency zero and deficiency one theorems. Chemical Engineering Science 42(10):2229–2268

Feinberg, M. (1995a). The existence and uniqueness of steady states for a class of chemical reaction networks. Archive for Rational Mechanics and Analysis 132(4):311–370

Feinberg, M. (1995b). Multiple steady states for chemical reaction networks of deficiency one. Archive for Rational Mechanics and Analysis 132(4):371–406

Franco, E., Blanchini, F. (2013). Structural properties of the MAPK pathway topologies in PC12 cells. Journal of Mathematical Biology 67:1633–1668

Franco, E., Murray, R.M. (2008). Design and performance of *in vitro* transcription rate regulatory circuits. In: Proceedings of the IEEE Conference on Decision and Control

Franco, E., Forsberg, P.O., Murray, R.M. (2008). Design, modeling and synthesis of an *in vitro* transcription rate regulatory circuit. In: Proceedings of the American Control Conference

Franco, E., Giordano, G., Forsberg, P.O., Murray, R.M. (2014). Negative autoregulation matches production and demand in synthetic transcriptional networks. ACS Synthetic Biology 3(8):589–599

Giordano, G., Franco, E., Murray, R.M. (2013). Feedback architectures to regulate flux of components in artificial gene networks. In: Proceedings of the American Control Conference, pp 4747–4752

Gorban, A., Radulescu, O. (2007). Dynamical robustness of biological networks with hierarchical distribution of time scales. IET Systems Biology 1(4):238–246

Hale, D., Lady, G., Maybee, J., Quirk, J. (2014). Nonparametric Comparative Statics and Stability. Princeton University Press

Harrison, M.E., Dunlop, M.J. (2012). Synthetic feedback loop model for increasing microbial biofuel production using a biosensor. Frontiers in Microbiology 3(360)

Hernandez, M.-J. (2009). Disentangling nature, strength and stability issues in the characterization of population interactions. Journal of Theoretical Biology, 261: 107–119.

Kitano, H. (2002). Systems biology: A brief overview. Science 295(5560):1662–1664

Kitano, H. (2004). Biological robustness. Nature Reviews Genetics 5(11):826–837

Kitano, H. (2007). Towards a theory of biological robustness. Molecular Systems Biology 3(137):1–7

Kwon, Y.K., Cho, K.H. (2008). Quantitative analysis of robustness and fragility in biological networks based on feedback dynamics. Bioinformatics 24(7):987–994

Levchenko, A., Iglesias, P.A. (2002). Models of eukaryotic gradient sensing: application to chemotaxis of amoebae and neutrophils. Biophysical Journal 82:50–63

Levins, R. (1968). Evolution in changing environments: some theoretical explorations. Princeton University Press

Levins, R. (1974). The qualitative analysis of partially specified systems. Annals of the New York Academy of Science 231:123–138

Levins, R. (1975). Evolution in communities near equilibrium. In: Cody, M., Diamond, J.M. (eds) Ecology and evolution of communities, Harvard University Press, pp 16–50

Ma, L., Iglesias, P. (2002). Quantifying robustness of biochemical network models. BMC Bioinformatics 3(1)

Ma, W., Trusina, A., El-Samad, H., Lim, W.A., Tang, C. (2009). Defining network topologies that can achieve biochemical adaptation. Cell 138(4):760–773

Marzloff, M.P., Dambacher, J.M., Johnson, C.R., Little, L.R., Frusher, S.D. (2011). Exploring alternative states in ecological systems with a qualitative analysis of community feedback. Ecological Modelling 222(15):2651–2662

May, R.M., 1974. Stability and Complexity in Model Ecosystems, 2nd ed. Princeton University Press, Princeton, NJ.

Mincheva, M. (2011). Oscillations in biochemical reaction networks arising from pairs of subnetworks. Bulletin of Mathematical Biology 73:2277–2304

Mincheva, M., Craciun, G. (2008). Multigraph conditions for multistability, oscillations and pattern formation in biochemical reaction networks. Proceedings of the IEEE 96(8):1281–1291

Mochizuki, A., Fiedler, B. (2015). Sensitivity of chemical reaction networks: A structural approach. 1. Examples and the carbon metabolic network. Journal of Theoretical Biology 367(2):189–202

Motee, N., Chandra, F., Bamieh, B., Khammash, M., Doyle, J.C. (2010). Performance limitations in autocatalytic networks in biology. In: Proceedings of the IEEE Conference on Decision and Control, pp

4715–4720

Muzzey, D., Gómez-Uribe, C.A., Mettetal, J.T., van Oudenaarden, A. (2009). A systems-level analysis of perfect adaptation in yeast osmoregulation. Cell 138(1):160–171

Nikolov, S., Yankulova, E., Wolkenhauer, O., Petrov, V. (2007). Principal difference between stability and structural stability (robustness) as used in systems biology. Nonlinear Dynamics, Psychology, and Life Sciences 11(4):413–33

Puccia, C.J., Levins, R. (1985). Qualitative Modeling of Complex Systems. Harvard University Press, Cambridge, MA

Shinar, G., Feinberg, M. (2010). Structural sources of robustness in biochemical reaction networks. Science 327(5971):1389–1391

Shinar, G., Milo, R., Rodrìguez Martìnez, M., Alon, U. (2007). Input-output robustness in simple bacterial signaling systems. Proceedings of the National Academy of Sciences of the USA 104:19,931–19,935

Smith, H.L. (2008). Monotone Dynamical Systems: An Introduction to the Theory of Competitive and Cooperative Systems. American Mathematical Society

Sontag, E.D. (2003). Adaptation and regulation with signal detection implies internal model. Systems & Control Letters 50(2):119–126

Sontag, E.D. (2007). Monotone and near-monotone biochemical networks. Systems and Synthetic Biology 1:59–87

Sontag, E.D. (2014a). A technique for determining the signs of sensitivities of steady states in chemical reaction networks. IET Systems Biology 8:251–267

Sontag, E.D. (2014b). Quantifying the effect of interconnections on the steady states of biomolecular networks. In: Proceedings of the IEEE Conference on Decision and Control, pp 5419–5424

Spiro, P.A., Parkinson, J.S., Othmer, H.G. (1997). A model of excitation and adaptation in bacterial chemotaxis. Proceedings of the National Academy of Sciences of the USA 94(4):7263–7268

Steuer, R., Waldherr, S., Sourjik, V., Kollmann, M. (2011). Robust signal processing in living cells. PLoS Computational Biology 7(11)

Waldherr, S., Streif, S., Allgöwer, F. (2012). Design of biomolecular network modifications to achieve adaptation. IET Systems Biology 6(6):223–231

Yeung, E., Kim, J., Murray, R.M. (2013). Resource competition as a source of non-minimum phase behavior in transcription-translation systems. In: Proceedings of the IEEE Conference on Decision and Control, pp 4060–4067

Yi, T.M., Huang, Y., Simon, M.I., Doyle, J. (2000). Robust perfect adaptation in bacterial chemotaxis through integral feedback control. Proceedings of the National Academy of Sciences of the USA 97(9):4649–4653