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Global sensitivity analysis used to interpret biological experimental results

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Abstract Modeling host/pathogen interactions provides insight into immune defects that allow bacteria to overwhelm the host, mechanisms that allow vaccine strategies to be successful, and illusive interactions between immune components that govern the immune response to a challenge. However, even simplified models require a fairly high dimensional parameter space to be explored. Here we use global sensitivity analysis for parameters in a simple model for biofilm infections in mice. The results indicate which parameters are insignificant and are 'frozen' to yield a reduced model. The reduced model replicates the full model with high accuracy, using approximately half of the parameter space. We used the sensitivity to investigate the results of the combined biological and mathematical experiments for osteomyelitis. We are able to identify parts of the compartmentalized immune system that were responsible for each of the experimental outcomes. This model is one example for a technique that can be used generally.

Keywords Global sensitivity analysis · MRSA · IRM · Immune system

Mathematics Subject Classification 92B05 · 92C50 · 2910 · 49Q12

1 Introduction

Incorporating biological observations into mathematical models often requires high dimensional parameter space, even if the mathematical model is merely a caricature of the biological complexity. Typically there are three major approaches to explore a

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biological question: experimentation, theoretical design, and computational analysis. In general, experimental results inspire mathematical models, which are subsequently solved and analyzed. However, each step in any investigation contributes a certain level of uncertainty in results and predictions due to approximations, assumptions, error, lack of information, etc.

One biological issue that has gained recent notoriety for its impact on the medical community is *Staphylococcus aureus* (*S. aureus*) which is presenting as MRSA (methicillin-resistant *S. aureus*) with more regularity. *Staphylococcus aureus* infections and the subsequent immune responses have diverse and complex interactions. To understand this type of infection, experiments are often very specific and require many parameter estimates in order to capture the behavior in a mathematical model. However, many of these parameters cannot be identified either because of the cost of gathering data or because of the difficulty in experimental design. For example, mice models are often used to study the immune response's reaction to *S. aureus* challenge, and gathering data requires the sacrifice of multiple hosts for each data point. There are also various types of assays that can be used to characterize immune components which can lead to conflicting data. This information gathered for mouse experiments and parameters do not necessarily give insights into human parameters.

There are many models that describe the immune system's response to different diseases and therapies. These models are all built with different levels of complexity and detail. Detailed models either require a substantial number of variables and assumptions, many of which cannot yet be completely characterized, or only focus on the major players for the specific biological problem, ignoring/eliminating other subtle interactions (Bianca and Pennisi 2012; Chow et al. 2005; Gammack et al. 2005; Marino and Kirschner 2004; Marino et al. 2004; Wigginton and Kirschner 2001). Many modelers choose to handle the complexity of the immune system by taking a mechanistic approach combining parts of the immune response into generalized compartments (Day et al. 2006; Herald 2010; Jarrett et al. 2014; Kumar et al. 2004; Reynolds et al. 2006). Additionally, for some diseases, incorporation of a time-delay is necessary to accurately describe the biology such as the time needed for the regeneration or recruitment of cells (Buric et al. 2001; Culshaw and Ruan 2000; Perelson and Nelson 1999) but can introduce much more complexity to a model.

A very useful tool to help understand and ultimately deal with uncertainty in a model's results is sensitivity analysis (SA). SA is used to identify parameters that have effects on the outputs of the system when they are varied. We discuss the usefulness of this information in detail below, but SA is primarily used to identify parameters to reduce the parameter space of a model as well as parameter targets for experimental exploration. This analysis is computational, which is far less cumbersome from regular trial and error exploration methods. Previously, different types of SA have been used for biology motivated mathematical puzzles, but they are mostly local and limited to first order interactions or regression methods (Arino et al. 2008; Bailey and Duppenthaler 1980; Banks and Bortz 2005; Lee et al. 2013; Neilan et al. 2010).

Here we present a more recently developed form of global SA (Liu 2013). We present its results for a simple ordinary differential equation (ODE) model previously created to understand the immune response to an *S. aureus* infection in mice (Jarrett

et al. 2014). We also provide an interpretation of the results of the global SA and the biological implications that the outcomes suggest.

This paper is an example of a collaboration that links mathematical analysis directly with experimental results. This coupling allowed us to focus on parameters of possible biological importance which could have been easily passed over. Specifically, our focus was to use global SA to characterize parameters previously linked to biological experiments to possibly simplify/reduce the model and better understand the dominant parameters for each experiment. Exploring the sensitivity and uncertainty of parameters in a model is an exercise that requires both experimental and mathematical results. Without biological evidence, understanding the meaning of the sensitivity of parameters is impossible. Likewise, without having a mechanism to identify parameters that are significant, or, on the other hand, unimportant, model design and model reduction can only proceed with intuition.

2 Biological problem

Strains of resistant infectious bacteria are becoming more prevalent in medical facilities every year. These strains are putting a costly burden on the health care system due to the fact that their resistance often requires the complete removal of any surface harboring the bacteria when sanitation protocols fail including medical equipment/plumbing from the facility or even the medical implants themselves from patients (Gould et al. 2012). The most prevalent nosocomial infection for indwelling medical devices is MRSA (Brady et al. 2006).

Standard treatment protocols often fail at preventing and removing resistant infections (Shirtliff et al. 2001). MRSA is the cause for infections of the skin, soft tissue, pneumonia, musculoskeletal infections, and also the resulting infections of indwelling medical devices such as intravenous catheters and prosthetic implants. MRSA has many resistance mechanisms including enzymes that degrade, deactivate, or change antibiotics.

Bacteria are able to attach to a surface and embed themselves in an extracellular hydrated slime matrix (derived from both the microbes themselves and the host) to form what is called a biofilm. MRSA forms a protective biofilm structure, which becomes a source of infection that resists clearance by the host immune response and antimicrobial agents (Shirtliff et al. 2002). The biofilm structure provides protection for the microbes from antibiotics in many ways, including reduced antibiotic penetration, low metabolic rate, and specialized phenotypic expression (Gilbert et al. 1990; Proctor et al. 1998; Stewart 2003; Stewart and Costerton 2001; Thien-Fah and O'Toole 2001). There is also evidence that the protective biofilm increases the spread of phenotypes that result in drug resistance (Cogan 2006). Due to these resistance mechanisms, biofilm infections cannot usually be eliminated using only antibiotic treatment. Additionally, only surgical removal of the biofilm can eliminate the infection if a mature biofilm is formed. This in itself causes significant morbidity, mortality, and complications for the patient (Prabhakara et al. 2011b). This biological problem has led to a massive research effort focusing on not only MRSA but also biofilms in general. These efforts include, but are not limited to, antibiotic dosing strategies,

pretreatment of surgical implants with antimicrobial agents, immunomodulation therapies, and vaccines. Studies focusing on immunomodulation therapies and vaccine development for MRSA are important due to the antibiotic resistance and ability to form a biofilm. However, the nature of *S. aureus* presents major challenges to current vaccine strategies (Harro et al. 2010).

The Shirtliff group at the University of Maryland has completed numerous experiments to characterize this type of infection by identifying antigens associated with its biofilm as well as documenting the host immune response in mice. These experiments consist of creating a *S. aureus* biofilm infection on a medical pin, which is then implanted in the tibia of different mouse strains and treated with several types of immunomodulation therapies. At several time points, post infection, various cytokine levels, amount of infection, and the morbidity of the infection were documented (Brady et al. 2011; Prabhakara et al. 2011a, b; Shirtliff et al. 2012).

Eight experiments were designed to compare different immune defects and vaccine strategies. Three strains of mice with differing immune potentials were used and treated with several immunomodulation therapies representing different immune-compromised states and a normal/healthy immune response. These mice strains have biased immune responses where one type has a dominant pro-inflammatory response and another strain has a stronger anti-inflammatory response. Inflammation, in the form of blood flow, and pro-inflammatory leukocytes move into areas of infection as the primary component of the body to remove invading pathogens. However, if not specific or overly activated, host tissue damage can result from uncontrolled inflammation, whereas the anti-inflammatory responses reduce inflammation with chemical signaling and specialized cells (Delves and Roitt 2000a).

The first of a series of experiments compared the immune responses of the different strains of mice, and they found that the infection was less severe in the anti-inflammatory dominant strain versus the pro-inflammatory dominant strain (experiments 1 and 2 below). They also compared the damage to the bone at the pin implant site of these mouse strains caused by pro-inflammatory cells moving into the infected area. They observed significant damage to the pro-inflammatory dominant mice, and very little damage to the anti-inflammatory dominant mice (Prabhakara et al. 2011a).

After these initial experiments they performed several more experiments to determine the effects of particular parts of both the pro- and anti-inflammatory responses to elucidate whether the anti-inflammatory response is a protective mechanism or if an over active pro-inflammatory response simply can exacerbate infections (experiments 3–6 below) (Prabhakara et al. 2011b).

The Shirtliff group was also able to develop different vaccines for MRSA caused osteomyelitis in mice. Without vaccination, the pro-inflammatory dominant mice would not have an anamnestic immune response to these antigens and would not be able to prevent a biofilm matrix from forming. The first vaccine they created provided partial protection against a *S. aureus* biofilm infection. The vaccine only expressed biofilm-specific antigens, so adjunctive antibiotic therapy was required to clear planktonic populations of the bacteria. A later vaccine consisted of the original four-components of the earlier vaccine with one additional antigen. The planktonic bacteria express the additional antigen in vivo during the infection. The five-component vaccine provided complete protection and elimination of *S. aureus* populations in this

Table 1 Comparison of mouse strains: their biological elements, basic results of experiments, and experiment numbers used here and citations

Mouse strain	Biological elements	Basic results	Experiments
BALB/c	*Th2* and *Treg* (anti-inflammatory) dominant immune response	Able to clear infection, but when treated with antibodies against *Treg* cells the mice lose their ability to overcome the infection	1, 4 (Prabhakara et al. 2011b)
C57BL/6	*Th17* and *Th1* (pro-inflammatory) dominant immune response	Unable to clear infection unless treated with antibodies that block both the *Th17* and *Th1* responses or with a vaccine	2, 5–8 (Brady et al. 2011; Prabhakara et al. 2011b; Shirtliff et al. 2012)
STAT6 KO BALB/c	BALB/c mice with *Th2* response removed	Unable to clear the infection	3 (Prabhakara et al. 2011b)

particular mouse model (experiments 7 and 8 below) (Brady et al. 2011; Shirtliff et al. 2012).

Specific and detailed data for these mouse experiments can be found in the papers mentioned above, but we have included Table 1 for a brief description the mouse strains, their biological elements, and the basic results of the experiments.

These experiments and their results inspired a mathematical model to predict other experimental outcomes and possibly elucidate targets for immunotherapy and other experiments which we briefly describe in the next section (Jarrett et al. 2014).

3 Mathematical model

This model consists of four nonlinear ODEs represented by the following equations:

$$\begin{aligned}
\frac{dP}{dt} &= (\alpha_1 I + \rho_1 B)(1-P) - \left[\beta_1 A + \mu_1\left(1 - \frac{B}{K_B}\right)\right] P \\
\frac{dA}{dt} &= \alpha_2 P - \left[\beta_2 I + \mu_2\left(1 - \frac{B}{K_B}\right)\right] A \\
\frac{dI}{dt} &= \alpha_3 P + \rho_2 B - (\beta_3 A + \mu_3) I \\
\frac{dB}{dt} &= \left[g\left(1 - \frac{B}{K_B}\right) + \alpha_4 I - \beta_4 P\right] B + e^{-\gamma t}
\end{aligned} \tag{1}$$

Three of the components represent parts of the immune system, and the fourth component represents the infection (B). The immune system components are the pro-inflammatory response (P), anti-inflammatory response (A), and inflammation/damage (I). This style of model has been developed previously, although in a more generic form (Reynolds et al. 2006; Day et al. 2006). Specifically, previous

models neglected the interaction between the bacterial dynamics and the inflammation and focused on general outcomes (e.g. infection clearance). Whereas the components of this system have been linked to the findings of the Shirtliff group pertaining to the immune response to this type of biofilm infection as well as recent biological evidence.

The bacteria component (B) is treated as a growing population that benefits from inflammation with rate α_4 and is reduced by the pro-inflammatory response with rate β_4. Logistic-type growth is used since nutrient that the bacteria harvest from the body is not accounted for, where g is the growth rate. A source term representing the initial source of the biofilm infection was incorporated to better represent the slowly decaying infection from the pin implant in the experiments.

The pro-inflammatory response is the combined efforts of the *Th1* and *Th17* responses of mice described by the Shirtliff group. This response depends on both inflammation and the bacteria with rate α_1 and ρ_1 respectively. However, this recruitment is not exponential but has a maximal, active capacity that depends on the amount of the pro-inflammatory response present. The pro-inflammatory response is down regulated by the anti-inflammatory response with rate β_1, and it decays at a rate μ_1. In addition, the natural decay rate decreases when bacteria are present in the system, agreeing with recent biological evidence (Coxon et al. 1999).

In the model, the anti-inflammatory response represents the effort of the *Treg* cells and is recruited by the pro-inflammatory response at rate α_2. This is a simplification since the anti-inflammatory response is actually recruited by the inflammation. The anti-inflammatory response should not be effective against the inflammation until macrophages (part of the pro-inflammatory response) are activated, so this is a reasonable simplifying assumption. This separate activation is the "reprogramming" of already recruited macrophages. The anti-inflammatory response is decreased by inflammation caused by platelet blockage with rate β_2 (Moura and Tjwa 2010), which is an interaction novel to this model. It also decreases by its natural decay rate μ_2- again, it was assumed that the natural decay rate also depends on the magnitude of the infection (Coxon et al. 1999).

The inflammation component (I) reflects the damage caused the pro-inflammatory response and bacteria as well as increased blood flow bringing cells and platelets to the area. It is reduced by the anti-inflammatory response and natural decay. The pro-inflammatory response and bacteria cause the inflammation to increase with rate α_3 and ρ_2 respectively. The inflammation is reduced by the immune system's anti-inflammatory response with rate β_3 and by its natural decay rate represented by μ_3. The coupling between the pathogen and damage had not been incorporated in previous models and plays a key role in the model's results.

We note that the *Th2* response is represented in the combination of both pro-inflammatory and anti-inflammatory components due to the fact that these cells recruit pro-inflammatory cells to attack the biofilm, and *Th2* cells also produces cytokines that down-regulate the pro-inflammatory *Th1* and *Th17* responses.

This simple model is capable of representing the qualitative results for all eight of the major experimental results gathered by the Shirtliff lab, and it also includes a healthy state represented by an all-positive equilibrium for the immune response components and the infection component equal to zero. This represents the basal level of the host immune response (Delves and Roitt 2000b), not previously seen by simple,

compartmental modeling efforts for the immune system responding to infection. See Table 2 for a brief comparison of each of the experiments to the outcomes of the model including steady-states, eigenvalues, and the biological references. For further details about this model and comparison to the biological data and all experimental outcomes see (Jarrett et al. 2014).

4 Uncertainty and sensitivity analysis

Each stage of mathematical modeling introduces uncertainty that can be categorized into non-reducible and reducible uncertainty. Non-reducible uncertainty stems from parameters and conditions for the system of equations being analyzed. This type of uncertainty implies that the biological process has variability that affects the model predictions. Reducible uncertainty stems from a lack of information about a particular aspect of the system. By collaborating with experimentalists data can be gathered specific to reducing the uncertainty of parameters and the interaction of variables.

SA refers to a broad group of methods that ranks parameters by their effect on output variables, which has several roles to play in investigations. One role is to describe the effects of both non-reducible and reducible uncertainty. A second role that SA plays is in model reduction. Model results can depend heavily on particular parameters, but other parameters may be essentially irrelevant to the overall results. Identifying and 'freezing' these parameters can reveal simpler models for the same complex biological system.

Uncertainty and sensitivity are terms that are sometimes used interchangeably, especially when referring to parameter analysis. However, uncertainty analysis almost always refers to a lack of knowledge regarding the value of the parameter, whereas sensitivity refers to how much the outcome depends on variations in the parameters. There may be parameters that the model is not sensitive to, but are highly uncertain, that have negligible effect on the predictions. Likewise, a model that has a highly sensitive parameter requires some level of certainty in order to make robust predictions.

There are many methods used to understand sensitivity and uncertainty such as differential SA, sampling methods, and segmented input distribution. These tools include those that investigate the parameters one at a time; those that sample all of parameter space; and those that partition parameter space, based on output analysis.

5 Global sensitivity analysis

SA is generally classified into two types: local SA and global SA. Local SA, usually described by the partial derivatives or gradients of the output response with respect to input parameters, only considers the impact on the output of the variation of a given input variable around a certain value while the other inputs are kept constant at their nominal values. Global SA, on the other hand, considers variations of all input parameters simultaneously over the whole space. As a result, interactions among different inputs can be detected. Another advantage of global SA is that type II errors (failure to identify a significant parameter) can be avoided with a higher probability (Saltelli 2002).

Table 2 Summary of the model results compared to experimental evidence (Jarrett et al. 2014)

Experiment description	Model results	Corresponding biological results
1. BALB/c tibia implanted with *S. aureus* coated pin	Clearance of infection and return to basal/healthy equilibrium. The stable equilibrium (basal level) is $(\bar{P}, \bar{A}, \bar{I}, \bar{B}) \approx (0.82, 0.20, 2.10, 0)$ with eigenvalues $\lambda \approx -0.06, -0.17, -0.44, -0.95$	After 21 days, 41.67 % of mice infected and after 49 days, 25 % infected with decreasing CFU amounts; no biofilm formation; lack of neutrophil infiltration to bone (Prabhakara et al. 2011b)
2. C57BL/6 tibia implanted with *S. aureus* coated pin	Infection persists and bacteria positive equilibrium is stable which has a higher inflammation/damage value. The stable equilibrium is $(\bar{P}, \bar{A}, \bar{I}, \bar{B}) \approx (0.94, 0.22, 2.69, 0.54)$ with eigenvalues $\lambda \approx -0.07 + 0.33i, -1.04 + 0.33i, -1.04 - 0.33i, -0.07 - 0.33i$	At all time points 100 % of mice infected; definite biofilm formation; large numbers of neutrophil infiltration to bone (Prabhakara et al. 2011b)
3. STAT6 KO BALB/c (no *Th2* response) tibia implanted with *S. aureus* coated pin	Infection persists and bacteria positive equilibrium is stable. However, it has a lower inflammation/damage value. The stable equilibrium is $(\bar{P}, \bar{A}, \bar{I}, \bar{B}) \approx (0.91, 0.38, 1.56, 0.57)$ with eigenvalues $\lambda \approx -0.08 + 0.39i, -0.64, -1.33, -0.08 - 0.39i$	After 21 days, 100 % of mice still infected but with CFU amounts comparable to BALB/c mice still infected at 21 days (Prabhakara et al. 2011b)
4. BALB/c tibia implanted with *S. aureus* coated pin and treated with *Treg* antibodies (anti-CD25)	Infection persists and bacteria positive equilibrium is stable. The stable equilibrium is $(\bar{P}, \bar{A}, \bar{I}, \bar{B}) \approx (0.96, 0.28, 3.03, 0.70)$ with eigenvalues $\lambda \approx -0.06 + 0.36i, -1.16 + 0.43i, -1.16 - 0.43i, -0.06 - 0.36i$	After 21 days, infected mice increased to 87.5 % (Prabhakara et al. 2011b)
5. C57BL/6 tibia implanted with *S. aureus* coated pin and treated with *Th17* antibodies (anti-IL-6)	Infection persists and bacteria positive equilibrium is stable but with a lower level of infection. The stable equilibrium is $(\bar{P}, \bar{A}, \bar{I}, \bar{B}) \approx (0.84, 0.18, 2.17, 0.18)$ with eigenvalues $\lambda \approx -0.09 + 0.18i, -0.62, -0.80, -0.09 - 0.18i$	After 21 days, infected mice decreased slightly to 85.7 % (Prabhakara et al. 2011b)
6. C57BL/6 tibia implanted with *S. aureus* coated pin and treated with *Th17* and *Th1* antibodies (anti-IL-6, anti-IL-12p40)	Small changes in the specific parameters for experiments 5 and 6 result in either persistence of infection or clearance. The stable equilibrium (healthy) is $((\bar{P}, \bar{A}, \bar{I}, \bar{B}) \approx (0.68, 0.16, 1.46, 0)$ with eigenvalues $\lambda \approx -0.16, -0.19 + 0.08i, -0.72, -0.19 - 0.08i$	After 21 days, infected mice decreased to 62.5 % (Prabhakara et al. 2011b)

Table 2 continued

Experiment description	Model results	Corresponding biological results
7. C57BL/6 tibia implanted with *S. aureus* coated pin and treated with quadrivalent vaccine and antibiotics	Infection persists and bacteria positive equilibrium is stable unless antibiotic treatment is incorporated which gives stability to the healthy/basal equilibrium. The stable equilibrium (without antibiotics) is $(\bar{P}, \bar{A}, \bar{I}, \bar{B}) \approx (0.91, 0.15, 3.08, 0.07)$ with eigenvalues $\lambda \approx -0.07 + 0.07i, -0.78, -1.25, -0.07 - 0.07i$	After previous vaccination, 14 days after implantation of infection 50 % of mice remained infected (Shirtliff et al. 2012) and in rabbits 66 % remained infected (Brady et al. 2011), but combined with antibiotics there was a 99.9 % reduction in bacterial population for rabbits (Shirtliff et al. 2012)
8. C57BL/6 tibia implanted with *S. aureus* coated pin and treated with pentavalent vaccine	Clearance of infection and return to basal/healthy equilibrium. The stable equilibrium is $(\bar{P}, \bar{A}, \bar{I}, \bar{B}) \approx (0.90, 0.15, 3.06, 0)$ with eigenvalues $\lambda \approx -0.82, -0.11, -0.68, -1.30$	After 21 days, there was 100 % clearance in all mice (Shirtliff et al. 2012)

One simple global method is calculating the partial rank correlation coefficient (PRCC) while utilizing Latin Hypercube Sampling (LHS) which has been applied to many biological models (Bianca et al. 2012; Blower and Dowlatabadi 1994; Jarrett et al. 2014; Marino et al. 2008). Sobol' sensitivity measures (Sobol' 1993, 2001; Saltelli 2002; Liu and Owen 2006) that utilize the analysis of variance (ANOVA) of the model output are among the most widely used global SA methods. There are alternative methods to Sobol' sensitivity indices such as Fourier amplitude sensitivity test (FAST) method (Cukier et al. 1973) and its extended version (eFAST) which are variance-based methods. A clear comparison of these methods is provided by Saltelli and Bolado (Saltelli and Bolado 1998). Currently we are focusing on improving the efficiency of computing the high-dimensional integrals in the Sobol' method (Liu 2013).

Consider a mathematical model represented by a square integrable function $f(\mathbf{x})$, where $f(\mathbf{x})$ can be a system of algebraic, integral or differential equations, and $\mathbf{x} = (x_1, x_2, \ldots, x_d)$ are d uncertain input parameters of the model. In the present work, $f(\mathbf{x})$ denotes the ODE system (1), and $\mathbf{x}$ denote the set of input parameters associated with it. Without loss of generality, the model $f(\mathbf{x})$ is defined on the d-dimensional unit hypercube. The ANOVA decomposition of $f(\mathbf{x})$ is defined as

$$f(\mathbf{x}) = f_{\emptyset} + \sum_{i} f_{\{i\}}(x_i) + \sum_{i<j} f_{\{i,j\}}(x_i, x_j) + \sum_{i_1<\cdots<i_r} f_{\{i_1,\ldots,i_r\}}(x_{i_1}, \ldots, x_{i_r}) + f_{\{1,2,\ldots,d\}}(x_1, x_2, \ldots, x_d) \quad (2)$$

where $f_{\{i_1,\ldots,i_r\}}(x_{i_1}, \ldots, x_{i_r})$ is a function that only depends on r variables $x_{i_1}, \ldots, x_{i_r}$, and $f_{\emptyset}$ is a constant independent of all $\mathbf{x} = (x_1, \ldots, x_d) \in [0, 1]^d$. Let $u \subseteq \{1, \ldots, d\}$

be an index set and $\mathbf{x}^u$ denote the $|u|$-dimensional vector with elements x_j for $j \in u$. Then Eq. (2) can be rewritten as

$$f(\mathbf{x}) = \sum_{u \subseteq \{1,\ldots,d\}} f_u(\mathbf{x}^u). \tag{3}$$

The functions on the right hand side (RHS) of Eq. (3) are obtained recursively by

$$f_\emptyset = \int_{[0,1]^d} f(\mathbf{x})\, \mathrm{d}\mathbf{x}$$

and

$$f_u(\mathbf{x}^u) = \int_{[0,1]^{|-u|}} f(\mathbf{x})\, \mathrm{d}\mathbf{x}^{-u} - \sum_{v \subsetneq u} f_v(\mathbf{x}^v)$$

where $-u$ denotes the complement set of u. The following orthogonality is obvious:

$$\int_{[0,1]^d} f_u(\mathbf{x}^u) f_v(\mathbf{x}^v)\, \mathrm{d}\mathbf{x} = 0, \quad \text{for} \quad u \neq v. \tag{4}$$

Variances are then defined as

$$\sigma_u^2 = \int_{[0,1]^{|u|}} f_u(\mathbf{x}^u)^2\, \mathrm{d}\mathbf{x}^u, \quad \sigma^2 = \int_{[0,1]^d} f(\mathbf{x})^2\, \mathrm{d}\mathbf{x} - f_\emptyset^2.$$

ANOVA decomposition (3) and the orthogonality property (4) imply

$$\sigma^2 = \sum_{u \subseteq \{1,\ldots,d\}} \sigma_u^2.$$

Sobol' (1993) introduced two types of global sensitivity indices (GSI)

$$\underline{S}_u = \frac{1}{\sigma^2} \sum_{v \subseteq u} \sigma_v^2, \quad \overline{S}_u = \frac{1}{\sigma^2} \sum_{v \cap u \neq \emptyset} \sigma_v^2.$$

$\underline{S}_u$ sums all the normalized variances whose index sets are subsets of u, and $\overline{S}_u$ sums all those whose index sets have non-empty intersections with u. It is obvious that $\underline{S}_u \leq \overline{S}_u$, and hence they can be used as the lower and upper bounds, respectively, of the sensitivity measures on the parameters $\mathbf{x}^u$. Sobol' (1993) first proposed Monte Carlo algorithms to compute GSI and Saltelli (2002) further improved the efficiency of the algorithms. In the literature, typically the indices with respect to a single parameter x_i, $\underline{S}_{\{i\}}$ (first order indices or main effects) and $\overline{S}_{\{i\}}$ (total indices or total effects) for $i \in \{1, \ldots, d\}$, are computed. If $\overline{S}_{\{i\}}$ is relatively small, then the corresponding parameter can be frozen at its nominal value without losing much uncertainty in the model output.

6 Results

The mean values of the sixteen model parameters are given in Table 3. We assume each parameter satisfies a uniform distribution with a coefficient of variation (CV) 10 %. The CV can be different for different parameters. The CV could be 100 %, but one should ensure sampling does not produce a negative value for the relevant parameter. In practice, if the sample value of the parameter is negative, it is ignored. However, the assumption of uniform distribution, particularly with a large variance, could sometimes lead to non-intuitive results.

The evolution of the model outputs at the mean parameter values is shown in Fig. 1. The model solutions are integrated to $t = 500$ h (about 21 days) and reach steady states. The steady state solutions are used as the model outputs. All the ODEs were solved using implementations of MATLABs ODE45; Sobol's algorithm was executed in Fortan 90.

Figures 2 and 3 plot the main effects and total effects respectively. A direct comparison of the main and total effects indicates that noticeable secondary interactions exist among parameters for outputs A and B, while for outputs P and I the higher interactions are very weak. Based on the total sensitivity indices $\bar{S}_i$, we have the following observations:

Table 3 Parameter mean values for the immune response model for the healthy state (BALB/c mouse)

Parameters	Values	References
α_1	0.27	Estimated
ρ_1	0.2	Estimated (Reynolds et al. 2006)
β_1	0.01	Estimated
μ_1	0.12	Coxon et al. (1999)
α_2	0.11	Estimated
β_2	0.1	Moura and Tjwa (2010)
μ_2	0.25	Huhn et al. (1997), Coxon et al. (1999)
α_3	1.05	Estimated
ρ_2	0.45	Estimated
β_3	2	Brandwood et al. (1992), Edelson et al. (1975), Matsui and Ito (1983)
μ_3	0.0174	Reynolds et al. (2006)
g	0.9	Spector (1956)
K_B	1	Assumed
α_4	1.5	Estimated
β_4	5	Brandwood et al. (1992), Edelson et al. (1975), Matsui and Ito (1983)
γ	0.01	Estimated

All parameters have units h^{-1} except for the following: β_1, β_2, β_3, β_4, and α_4 have units of $(amount \times h)^{-1}$; K_B has units of relative amount

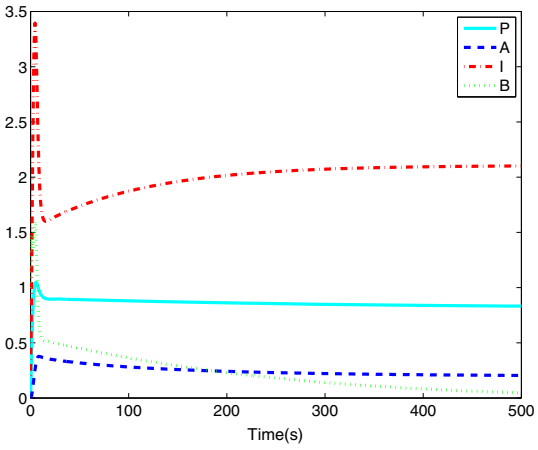
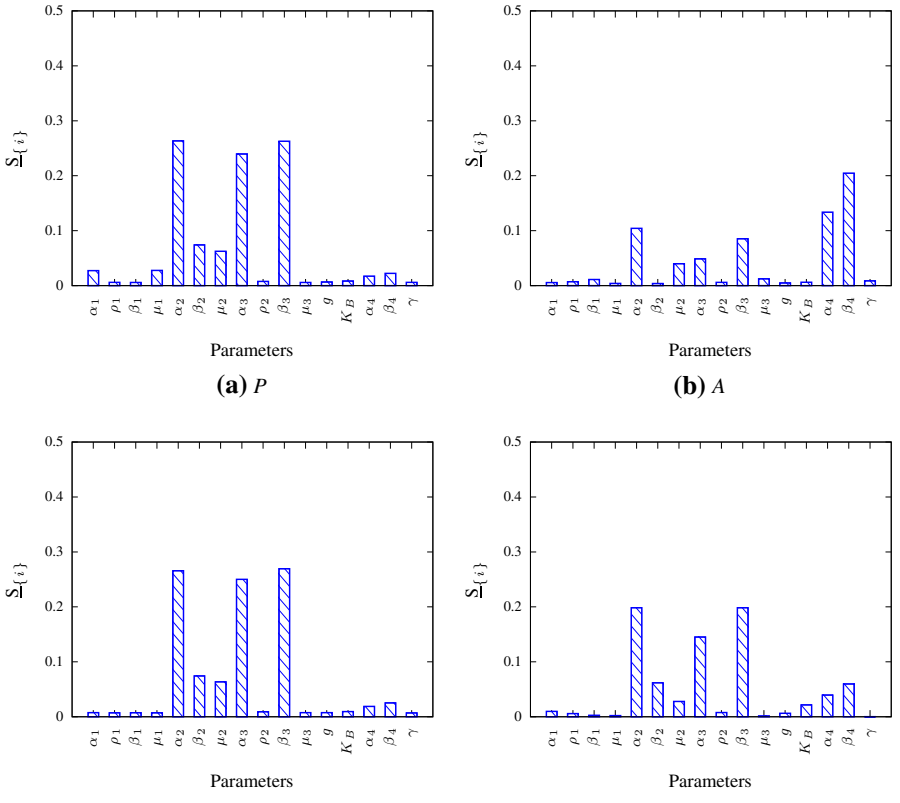


Fig. 1 Evolution of model outputs at parameter mean values representing the healthy state removal of infection (BALB/c mouse). The final time $t = 500$ h



(a) P **(b)** A **(c)** I **(d)** B

Fig. 2 Sensitivity analysis on the IRM model-**main effects**. Sensitivity analysis is run under the assumption that all parameters are uniformly distributed with $CoV = 10\,\%$. The sample size for MC simulations is 50,000. The four subplots correspond to the four outputs, P, A, I and B, with final time set to 500 h

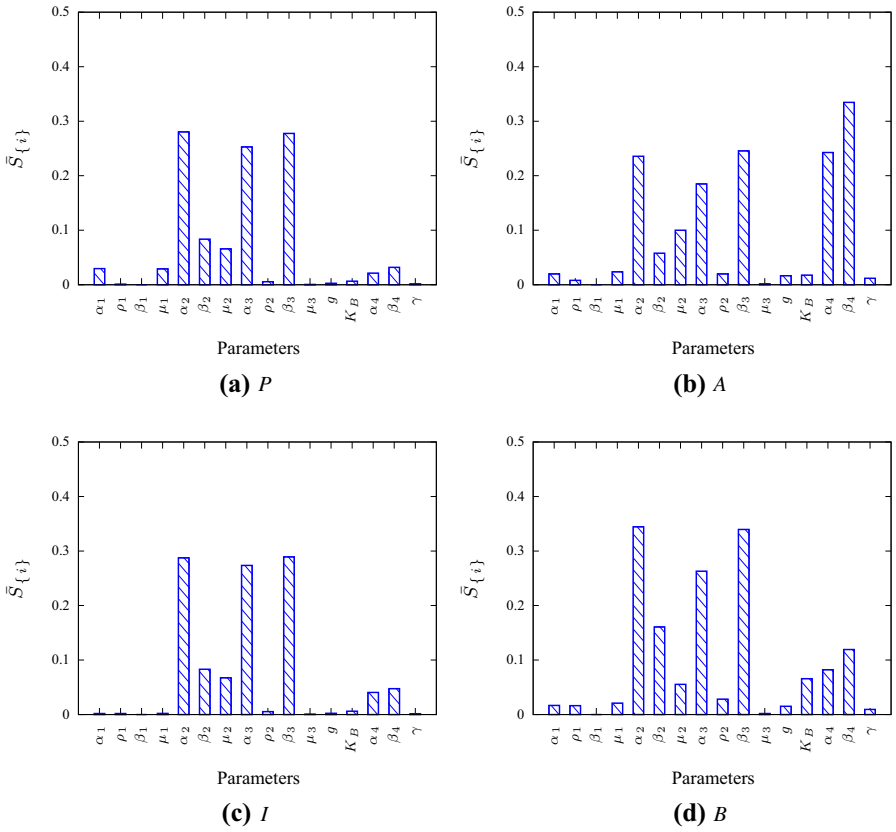


Fig. 3 Sensitivity analysis on IRM model-**total effects**. Sensitivity analysis is run under the assumption that all parameters are uniformly distributed with $CoV = 10\,\%$. The sample size for MC simulations is 50,000. The four subplots corresponds to the four outputs, P, A, I and B, with final time set to 500 h

- For output P, parameters α_1, ρ_1, β_1, μ_1, ρ_2, μ_3, g, K_B, α_4, β_4, γ can be labeled as insignificant (here parameters with total index values less than 0.03 are considered insignificant).
- For output A, parameters α_1, ρ_1, β_1, μ_1, β_2, ρ_2, μ_3, g, K_B, γ, can be labeled as insignificant.
- For output I, parameters α_1, ρ_1, β_1, μ_1, ρ_2, μ_3, g, K_B, γ can be labeled as insignificant.
- For output B, parameters α_1, ρ_1, β_1, μ_1, ρ_2, μ_3, g, γ can be labeled as insignificant.

The threshold of the total index values is chosen subjectively. In our simulation, we have used relatively large samples to compute those sensitivity indices to ensure that the indices are relatively accurate. Additionally, the total indices converge much faster than the first order indices, which can be negative when their true values are close to 0.

As a whole, we can see that α_1, ρ_1, β_1, μ_1, ρ_2, μ_3, g and γ are insignificant for all output variables, while α_2, β_2, μ_2, α_3, β_3, K_B, α_4 and β_4 are significant for at least one variable. Therefore, we will keep the eight insignificant parameters fixed at their mean values. The resulting model has only eight uncertain parameters, and we call it the "reduced model".

Table 4 displays the first and second moments for all outputs estimated with Monte Carlo sampling. The moments estimated for the two models agree very well. The estimated first moments of the reduced model have two-digit accuracy compared to the estimates of the full model. The estimated second moments of the reduced model are 94.1–99.5 % of those of the full model.

Figure 4 compares the histograms of the full and reduced models. For each output, the histogram of the reduced model is in good agreement with that of the full model, indicating that the uncertainty in the reduced model is preserved. Note that the dimension of the stochastic space of the full model is only half of that of the full model. The 95 % confidence intervals for the Monte Carlo estimates of the first moments with sample size 32,000 are given in Table 5.

7 Discussion and conclusion

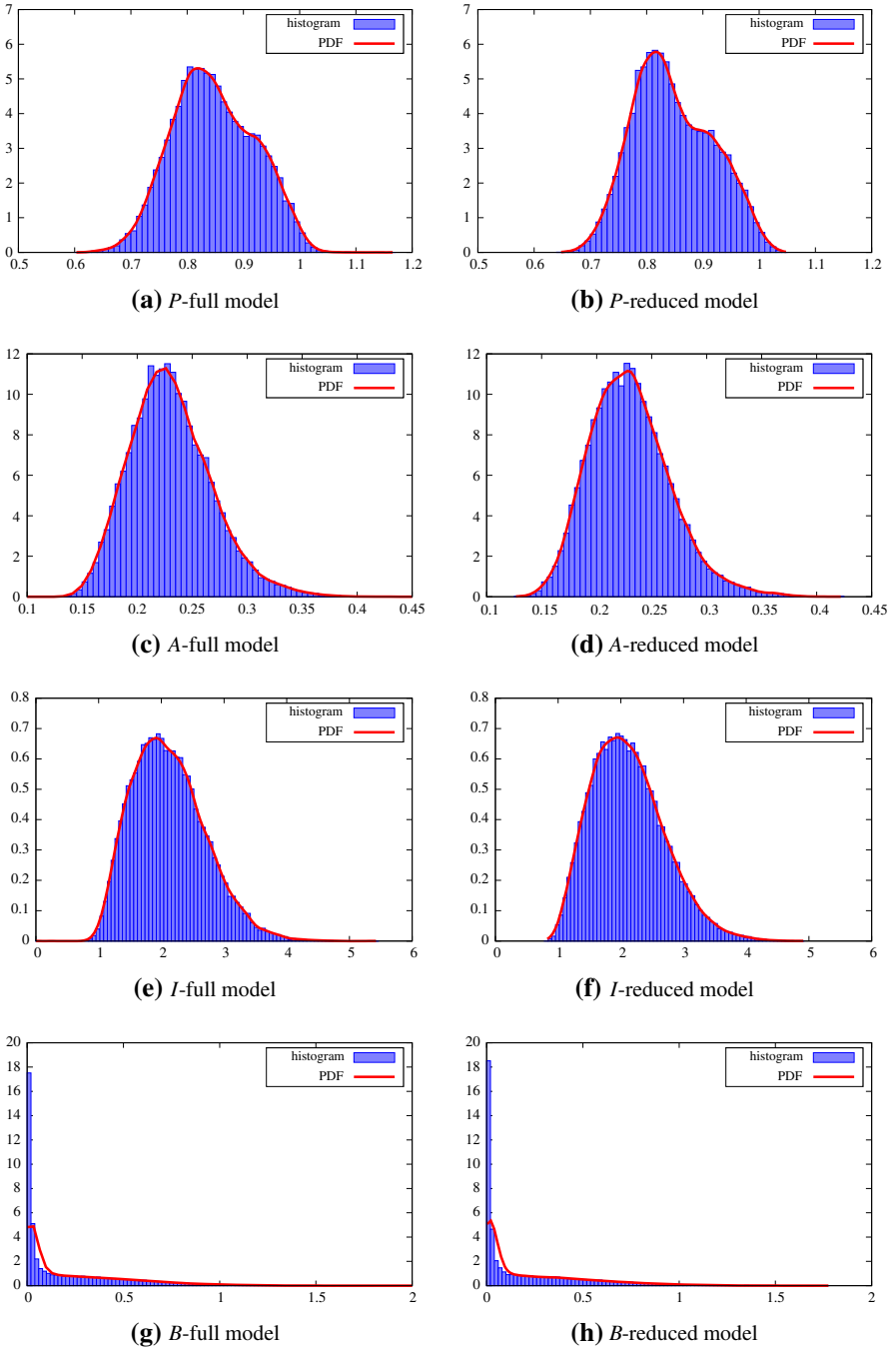
This work identified several parameters as significant for different outputs. For the pro-inflammatory component significant parameters are: α_2, α_3, β_2, β_3, and μ_2. For the anti-inflammatory component significant parameters are: α_2, α_3, α_4, β_3, β_4, and μ_2. For the inflammation component significant parameters are: α_2, α_3, α_4, β_2, β_3, β_4, and μ_2. Finally, for the infection component of the model significant parameters are: α_2, α_3, α_4, β_2, β_3, β_4, μ_2, and K_B. This is summarized in Table 6.

The parameters that are significant for all four components are: α_2, α_3, β_3, and μ_2. α_2 is the anti-inflammatory recruitment rate from the pro-inflammatory response; α_3 is the inflammation production rate from the pro-inflammatory response; β_3 is the rate the anti-inflammatory response reduces inflammation, and μ_2 is the natural decay rate of the anti-inflammatory response. Table 7 lists the significant parameters by the number of outputs they affect. There are three other parameters that were significant

Table 4 First and second moments for the (original) model and the reduced model

Moment		Full model	Reduced model
$\mathbb{E}[\mathbf{f}]$	P	0.8465	0.8469
	I	0.2304	0.2300
	B	2.1133	2.1181
	A	0.2090	0.2071
$\mathbb{V}[\mathbf{f}]$	P	5.2144E−3	4.9074E−3
	I	1.3810E−3	1.3517E−3
	B	0.3345	0.3334
	A	7.4324E−2	7.3938E−2

The moments are estimated using Monte Carlo method with sample size 32,000



(a) *P*-full model **(b)** *P*-reduced model

(c) *A*-full model **(d)** *A*-reduced model

(e) *I*-full model **(f)** *I*-reduced model

(g) *B*-full model **(h)** *B*-reduced model

Fig. 4 Comparison of full model and reduced model

Table 5 95 % confidence intervals for the Monte Carlo estimates of the first moments

Model output	Full model	Reduced model
P	[0.8457 0.8473]	[0.8461 0.8476]
I	[0.2300 0.2308]	[0.2295 0.2303]
B	[2.1070 2.1196]	[2.1118 2.1244]
A	[0.2060 0.2120]	[0.2041 0.2100]

The sample size is 32,000

for three components (α_4, β_2, and β_4) while only one parameter was significant for one component (K_B). The parameters α_4, β_2, μ_2, and K_B were not changed for any of the experiments. Therefore we chose to ignore them for this particular exploration.

After we identified these parameters, we compared them to the different parameter sets used for the biological experiments described in (Jarrett et al. 2014). By calculating the local stability of the healthy versus unhealthy states of the model system, we were able to identify the specific parameters that controlled the outcome of each experiment (even though for each experiment several parameters were changed to fully capture the immunomodulation implemented).

The most interesting cases involve α_3 and β_4. The α_3 parameter was changed for experiments involving immunomodulation of the pro-inflammatory dominant mouse strain. These mice were given antibodies against different pro-inflammatory cells to determine if the over active pro-inflammatory response was only making the infection worse due to host tissue damage. In the biological experiments, as the pro-inflammatory response was reduced, more of the mice were able to clear the infection and become healthy. For this particular parameter set, two parameters considered sensitive (α_2 and β_4) were at values that would normally force the healthy state of the system to be unstable. However, the parameter α_3 dominated this particular parameter set, allowing the healthy state to become stable (all negative eigenvalues). Note that parameter β_3 was not changed for these particular experiments, but its value was considered an addition to stability for the healthy state.

Of the parameters changed for the vaccination experiments, β_4 is significant. For these parameter sets, α_2 was at a value that normally would make the healthy state unstable. Recall that α_2 was considered significant for all four components. The parameter β_4 was significant for only three components, but this parameter was increased enough to create stability for the healthy state (all negative eigenvalues). Again, β_3 was not changed for these particular experiments, but its value was considered an addition to stability for the healthy state.

These results have several possible biological implications. The fact that α_3 is the rate inflammation is produced by the pro-inflammatory response and the fact that it is able to overcome two other significant parameters for experiments involving the reduction of the pro-inflammatory response, suggest that a serious effect on the system is damage caused by the pro-inflammatory response on the host tissue. This effect should be vigorously explored for the treatment of biofilm infections.

We mentioned above that β_4 is able to overcome a parameter that is sensitive for all four components whereas this parameter is only sensitive for three, the anti-inflammatory, inflammation, and infection components. β_4 is the rate the pro-inflammatory response removes the infection, so it is perhaps obvious this rate would

Table 6 Outputs paired with their respective sensitive parameters

Model output	Sensitive parameters
P	$\alpha_2, \alpha_3, \beta_2, \beta_3, \mu_2$
A	$\alpha_2, \alpha_3, \alpha_4, \beta_3, \beta_4, \mu_2$
I	$\alpha_2, \alpha_3, \alpha_4, \beta_2, \beta_3, \beta_4, \mu_2$
B	$\alpha_2, \alpha_3, \alpha_4, \beta_2, \beta_3, \beta_4, \mu_2, K_B$

Table 7 Sensitive parameters organized by the number of output variables they affect

Number of outputs affected	Corresponding sensitive parameters
4	$\alpha_2, \alpha_3, \beta_3, \mu_2$
3	$\alpha_4, \beta_2, \beta_4$
1	K_B

be significant to the bacteria and even the inflammation components of the model. However, it is mysterious how this also affects the anti-inflammatory response. In what way is effectiveness of the pro-inflammatory response against infection changing the anti-inflammatory response's behavior? This is also the only parameter implicated in the success for vaccination experiments.

Finally, the parameters α_4, β_2, μ_2, and K_B were ignored here because they did not necessarily apply to the parameter sets for the eight experiments explored. It is worth mentioning, however, that α_4 and β_2 are significant for three out of the four components like β_4 mentioned above, and they could lead to significant changes in the system if manipulated. Further experiments need to be carried out to characterize the effects of these four parameters especially α_4 and β_2 (the rate the bacteria benefits from inflammation and the rate the anti-inflammatory response is blocked by the inflammation/damage) which are interactions not seen before this particular model. These additional interactions can now be considered necessary and significant for this particular system. See Table 8 for a summary of the experiments and their significant parameters.

Here we presented a global sensitivity analysis for a simple ODE model used to describe specific experiments for characterizing osteomyelitis in mice. The results of this sensitivity analysis identified eight important parameters and eight insignificant parameters. The analysis also indicated that there are no overall noticeable secondary interactions between parameters, further validating the model structure.

By freezing the eight unimportant parameters we have a reduced model. This reduced model was shown to agree with the full model. The reduced model can be used with very good accuracy to estimate the outputs compared to the full model. With only half the parameters of the original, the reduced model can still capture the necessary behaviors to describe these biological experiments. This narrows the scope of analysis for the system simplifying the mathematical work significantly. It is also clear which parameters are of particular importance for good estimations.

Finally, we used the sensitivity results to investigate combined biological and mathematical experiments. This knowledge of sensitive parameters was used to identify parts of the system responsible for the experimental outcomes. We were able to iden-

Table 8 List of parameters, their values, and outcomes for each specific experiment

Experiment	Sensitive parameters	Value	Effective change to healthy equilibrium	Healthy state stability
1	α_2	0.11	Stable as α_2 increases	Stable
	α_3	1.05	Stable as α_3 decreases	
	α_4	1.5	Remains unchanged	
	β_2	0.1	Remains unchanged	
	β_3	2	Stable as β_3 increases	
	β_4	5	Stable as β_4 increases	
	μ_2	0.25	Remains unchanged	
	K_B	1	Remains unchanged	
2	α_2	0.9	Instability	Unstable
	β_4	4.75	Instability	
3	β_4	3	Instability	Unstable
4	β_3	1.5	Instability	Unstable
5 and 6	α_2	0.09	Instability	Stable
	α_3	0.7	Stability	
	β_4	4.75	Instability	
7 and 8	α_2	0.09	Instability	Stable
	β_4	7	Stability	

For all the experiments after the first, we only list the sensitive parameters that change for the experimental set. The first set can be considered a reference or basis set

tify the dominant parameters for each experiment based upon the parameters varied, the sensitive parameters identified, and stability of the healthy steady-state. This information is of great importance to the biological investigations—especially with regard to the successful vaccination experiments. We may not be able to identify the actual mechanism for the change in stability, but we can identify the particular element of the system that is necessary for the desired result. It is important to note that none of these conclusions were by intuition or by process of elimination but came directly from mathematical results and logic—a repeatable process.

Our future endeavors are to improve the mathematical model synergistically involving biological experiments and sensitivity analysis, and also to improve sensitivity analysis tools by making them more efficient, thereby enabling them to be applied to complex and computation-intensive models.

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