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Influential sources of uncertainty in glyphosate biochemical degradation in soil

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Abstract

Reactive transport models are important numerical tools to support decision making in many fields, such as herbicide use regulation. Though, models may be affected by multiple sources of uncertainty. Therefore, uncertainty and sensitivity analyses should become the practice to assess the confidence of such models. Here, the uncertainty in steady-state concentrations of glyphosate (GLP) and its metabolite aminomethylphosphonic acid (AMPA) was assessed using a reaction network that accounts for GLP and AMPA biotic and abiotic degradation pathways in soil including biological oxidation or hydrolysis in aerobic conditions via metabolic or cometabolic reactions. The mathematical framework is based on Michealis-Menten-Monod kinetic equations, which allow to account for microbial strategies to biodegrade contaminants. Chemical oxidation is assumed to occur independently from environmental conditions and resulted in a reduction of GLP concentration up to 15% when it was accounted for. The wide spectrum of interconnected catabolic reactions, each occurring at a different rate, as well as uncertainties in kinetic parameters estimation, suggest variability in modelling outcomes, which were addressed by means of a sensitivity analysis. In particular, the tested reaction network was mainly driven by GLP oxidation to AMPA; increasing the corresponding rate constant or decreasing the half-saturation constant resulted in a substantial decrease of GLP concentration but to an increase in AMPA concentration. Identification of the conditions responsible for GLP degradation to non-toxic metabolites, as well as for AMPA production and degradation, can allow to forecast unexpected consequences of GLP use and to design optimal land management and bioremediation plans.

Keywords:

Glyphosate, AMPA, Uncertainty, Sensitivity, birnessite, biodegradation

1. Introduction

The environment is being more and more exposed to new synthetic molecules developed to achieve specific purposes [46]. Those molecules may have unforeseen effects to human health [46] and ecosystem services (e.g., Rose *et al.* [73] reviewed the consequences of herbicide pollution). Prediction of the dynamics of those molecules in the environment together with an estimation of the associated uncertainty can allow to quantitatively regulate the use of those molecules and to evaluate adoption of

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precautionary measures for health protection and pollution control. Regulations report maximum levels of known contaminants in water resources [29, 23], air [24, 28], and food [84, 71], while, surprisingly, no safety limits exist for soil residues. Indeed, a recent report by the United Nations Food and Agriculture Organization [72] brings to light the hidden reality of soil pollution. Agricultural lands are stressed by applications of agrochemicals, and in particular by herbicides [55] to address farmer needs and to overcome weeds resistance. Multiple classes of stakeholders are involved in the process of herbicide formulation, approval, use, and monitoring. Modellers can potentially collaborate with stakeholders in each process to build robust and accurate mechanistic representations of herbicide dynamics in the environment [56, 27]; these models can be used as decisional tools for each class of stakeholders given that model outcomes can be tailored to answer specific inquiries. Given the important role numerical models can play, their confidence should be consistently evaluated [9]. Nowadays, the interplaying processes affecting herbicides degradation have been comprehensively integrated in models [43, 45, 85]. For example, the same herbicide can be biodegraded along different pathways depending on the species of microorganisms involved and their current metabolic requirements. *In situ* conditions such as availability of additional carbon (C) sources or the presence of inhibitors, can cause switches in degradation pathways and affect catalytic rates. Soil organic and inorganic matter may possess catalytic sites, which enhance herbicides degradation, or adsorption sites, which impair it by reducing herbicides availability. Reactive models have been developed to account for the spectrum of metabolites liberated during degradation of the parent compound. Each molecule may undergo biotic and abiotic degradation and may have different susceptibility to be transported by soil water. More importantly, under the perspectives of health protection and pollution control, toxic metabolites contribute to dietary risk assessment [25] as well as surface water quality risk assessment [61]. Recent developments in coupling reaction networks with ecohydrological processes have improved the capability to predict herbicide dynamics in spite of the increased complexity in model structure (e.g., PRZM [17], MACRO [39], SWAT [4], HYDRUS [47], MODFLOW-RT3D [40], TOUGHREACT [87], and BRTSim [54]). These types of simulations need to be endowed with uncertainty and sensitivity analyses to account for errors in data collection, parameter value estimation, and model structure, which usually result in nonlinear model responses and unforeseen outcomes [20, 70, 76, 88]. Other benefits from using this approach regard improvements in model structure resulting in a more robust or simpler model than the one previously conceived, a better understanding of the modelled system, and the capability to design effective land management plans and bioremediation strategies [20, 56, 62, 70, 88]. It is evident that such objectives can be successfully achieved only if an interdisciplinary approach is taken; that is, when experts in different fields collaborate by sharing their technical knowledge to develop comprehensive numerical models. Note that, the

capability to communicate effectively and comprehensibly is fundamental.

This paper introduces to good modelling practices in modelling herbicide biochemical reactions in soil. We provide a practical and simplified example of uncertainty and sensitivity analyses of a glyphosate (GLP) biochemical reaction network. In a concurrent work, we are testing the presented GLP reaction network under the effects of varying eco-hydrological boundary conditions using the sensitivity indices AMA [22], which are mentioned in this manuscript together with other indices.

2. Methods

A modelling study may involve several phases and multiple classes of stakeholders. The following sections will provide an overview of how and why stakeholders may use modelling to make decisions, the available numerical models, the steps to develop, integrate, and manage reaction network in models, and the importance of clear communication of results.

2.1. Stakeholders

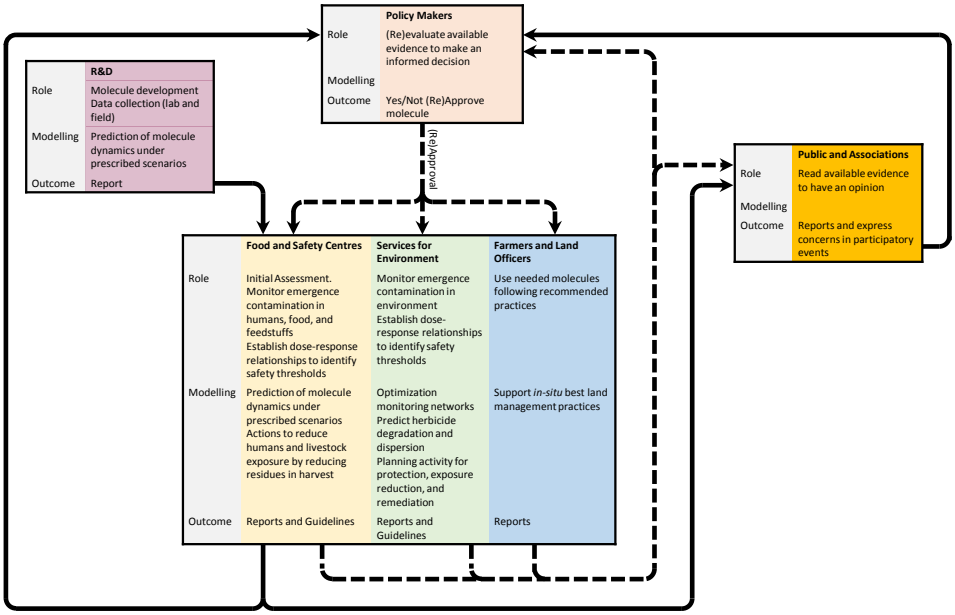

Figure 1: Solid black lines represent the possible interactions amongst stakeholders during the process for herbicides approval, while dashed black lines indicate interactions after approval. Scheme assuming adoption of precautionary principle, that is, preliminary information concerning herbicides safety must be available. Names, roles, and actions of some identified stakeholders are indicated, as well as the corresponding potential use of numerical modelling to support actions.

Herbicides (re)approval process can be specific for each country. Generally, the process is multi-step, sometimes iterative, and involves Research and Development (R&D) centers, designated experts of concerned countries, safety authorities, public audience, and policy makers (Figure 1). For example, European countries adhere to the precautionary principle, meaning that in the absence of scientific

evidence about safety, one situation can pose a risk. This approach is fundamental when policy makers decide whether to (re)approve a herbicide. The process is described under the Regulation (EC) No 1107/2009 of the European Parliament and of the Council of 21 October 2009 concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC (`http://data.europa.eu/eli/reg/2009/1107/2014-06-30`). R&D laboratories formulate new herbicides and collect preliminary data about their biogeochemical characteristics under controlled conditions in the laboratory and in the field (Figure 1). Next, modelling of molecule dynamics under prescribed scenarios is carried out. The results are documented and submitted to risk assessors, who evaluate the completeness of the data provided, carry out their own risk assessments, and, in cooperation with other stakeholders, produce a peer reviewed report to be submitted to regulatory bodies. General public can access available documents on herbicide and have an opinion, which may have a role in questioning the licensing of herbicides (Figure 1). Yet, all organizations and authorities, at national and international level, may contribute by providing an additional portfolio of evidence with regard to the herbicide under assessment. Once a herbicide is approved by regulatory bodies, farmers may want to apply it at the recommended rates, which may be adjusted based on *in situ* conditions (Figure 1). Governmental, private, or university laboratories may carry out independent studies to characterize herbicide properties in the environment. Based on those findings, modellers use numerical solvers with the aim to reproduce the data observed about herbicide levels. Those models can be site specific; therefore, they can be used to design and optimize land managements plans by officers. Environmental protection agencies (EPAs) are particularly involved in this step because they have an active role in monitoring contaminants concentration in the environment to safeguard human health and ecosystems and possibly to elaborate documentation concerning soil pollution by herbicides. Food authorities and research centers monitor herbicide levels in the workplace, in food, and in feedstuffs. In case the residues exceed safety thresholds they may deliberate more stringent thresholds and suggest management practices aiming at reducing the residues in the workplace and along the food chain.

2.2. Modelling process

Many processes can affect herbicide activity and persistence in soil, such as land management operations, biogeochemical reactions, and variability in meteorological events. The development of a robust model can be complex. Moreover, each stakeholder may have different understanding about any complex system and may seek for different answers from the modeller. For example, farmers may be interested in practical advice on what herbicide to apply, when, and at what rate to guarantee crop protection while avoiding to exceed maximum herbicide concentration in food. Land management officers may be interested in comparing different crop management plans to find the most advantageous one considering

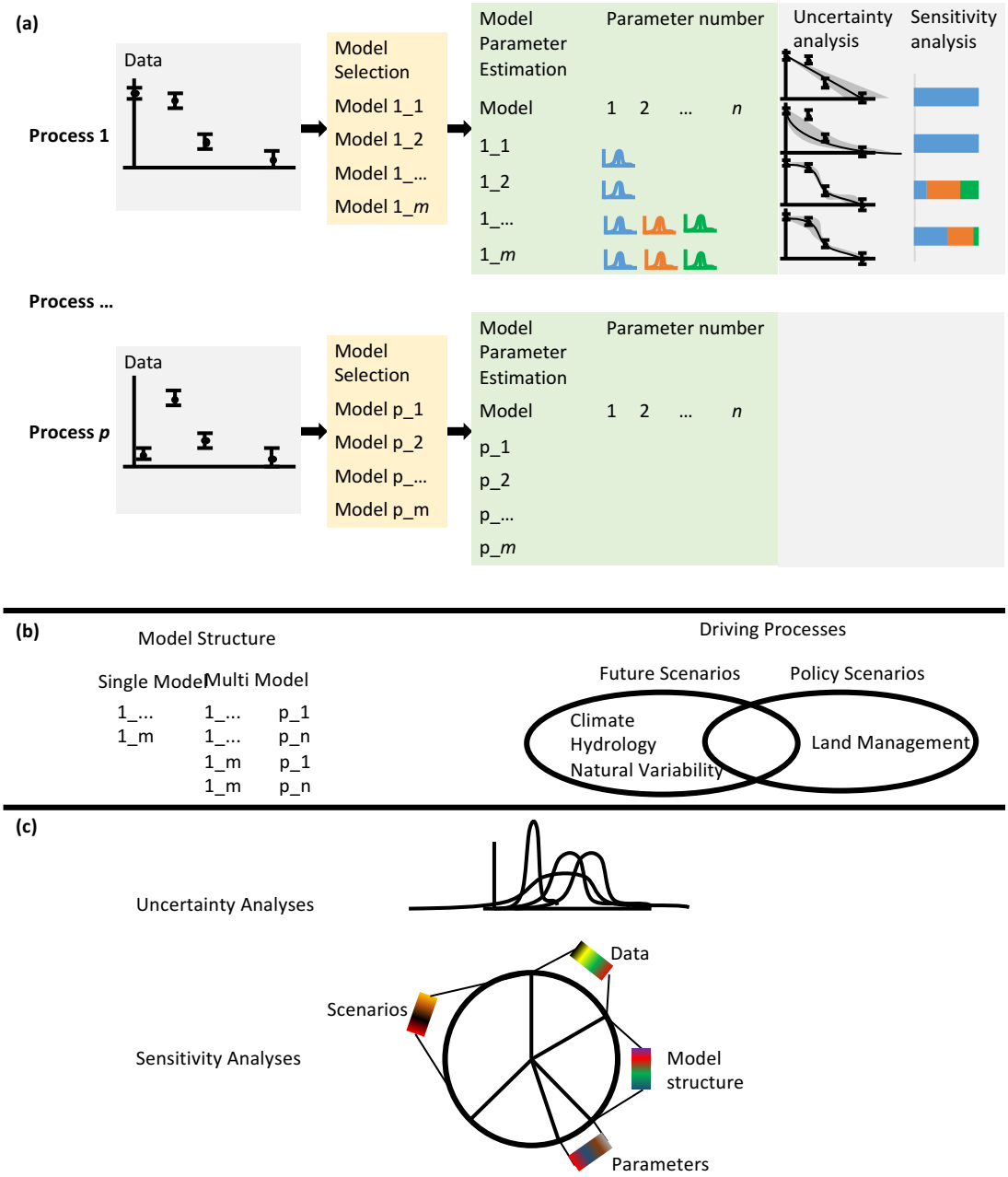

Figure 2: (a) Sketches of observed data for different processes over time (left gray boxes). Multiple numerical models may be used to describe the same process (yellow boxes). Models may contain a different number of parameters that should be estimated. Parameter uncertainty analyses carried out for each model assess the confidence of the model in reproducing the data (green boxes). Parameter sensitivity analyses assess the contribution of each parameter variability to the model output (right gray boxes). (b) The most appropriate models selected to describe each process are coupled together to develop a multi-model. Modelling scenarios include other driving processes; simulations are run. (c) Uncertainty analyses show the probability density functions of the likely outcome due to variability in parameters. Sensitivity analyses allocate the sources of uncertainty amongst the components of the model structure.

multiple objectives including health, sustainability, and environmental protection. EPAs may be interested in building an efficient monitoring network and may use modelling to localize the most sensitive and informative sites where to collect data on herbicide environmental levels; these sites may provide an early warning in case of contamination.

Herbicide dynamics are affected by a high level of interacting processes in agricultural soils; therefore, robust predictions of herbicide fate in the environment are particularly difficult to make. The mod-

eller should gather all the possible knowledge about herbicide interactions in the *rhizosphere* such as: sorption, biochemical degradation, toxicological effect to micro- and macroorganisms, effects on nutrients cycle, and plant responses. However, those interactions may vary spatially and temporally, while additional processes contributing to herbicide dynamics may only be brought to light at a future time. Depending on the scenario being investigated, the modeller may need to account for additional processes other than biogeochemical ones, such as water dynamics. The modeller may find that one process can be described by a suite of solvers (Figure 2a, yellow panel). Selection of the most suitable solver may be achieved based on expert judgment [13, 64], who assesses the mathematical modelling, and by carrying out preliminary analyses to test model robustness against available observations, as well as to rank parameters contribution to outcome variability. Later, the selected models, each describing a single process, are coupled with each other. This task results in the development of a multi-model and it allows to solve biogeochemical reactions coupled with hydrological forcing, at the requested detail over space and time, for example. It is common that several models can adequately describe one single process in hydrology[10] and in biogeochemistry[83]. Then, each single hydrological and biogeochemical model could be differently combined to frame several multi-models, and each multi-model will be separately validated. Also, each single model should be independently characterized using specific observations to quantitatively allocate the contribution of single processes to a target outcome, such as herbicide concentration (Figure 2). This would allow to increase confidence in the formulated multi-model.

For reliability, models require to be calibrated and validated and different approaches can be used. For example, the available dataset (e.g., herbicide concentration at some locations and over time) is separated in two or more sets: the calibration set is used to calibrate the parameters and to assess the correctness of the model structure, while the calibrated model will be used to predict the observations contained in the validation set. For this, field surveys to measure herbicide levels and soil characteristics are necessary. However, these studies can be expensive and time-consuming; because of the lack of resources to carry out extensive monitoring campaigns, field data are usually poor in spatial and temporal resolution. Thus, modellers usually apply biochemical reaction developed under controlled conditions and couple them with hydrological boundary conditions, which are more likely to have been measured and validated. Modelled herbicide concentrations may then be compared with values reported at sites with similar meteorological and hydrological conditions.

After model validation, simulations are run to meet the objectives and requirements from the stakeholders. Typical outputs show the concentrations of the herbicide and its metabolites over space and time, partitioning of the molecules in their aqueous, adsorbed, and gaseous phases, and the molecules degradation potential.

At this stage, a good modelling practice is to carry out both uncertainty and sensitivity analyses (Figure 2). The former quantifies the model confidence in terms of output variability and can be represented using probability density functions (*pdf*). The latter ranks the contribution of each parameter input to the output variability, thus identifying and ranking dominant processes in the model system. These analyses will provide a spectrum of possible outcomes, which may be used to predict herbicide activity in soil under varying conditions, to design bioremediation strategies, and to inform policy makers about herbicide (re)approval. Debates about herbicide (re)approval and use can be very intense because herbicides allow to maintain good crop yields but may have significant impacts on human health and ecosystem services. Clear, explicit, and unbiased studies are crucial for making informed decisions in relatively short time.

2.3. History of numerical solvers

Numerical models can be used to (re)approve herbicide use, assess consequences of policy and herbicide alternatives [56] or changes in environmental conditions [80], raise awareness in potential contamination over the long term, determine areas of intervention, and suggest mitigation strategies. Better technologies allow to measure more mechanisms with higher accuracy. For instance, degradation experiments coupled with mass spectrometry allow to identify metabolites produced during degradation of the parent compound. Each theoretical and technological step forward also bring an additional level of complexity in the modelling phase. Before the development of numerical approaches, analytical solutions for herbicide disappearance were derived, such as first-order kinetic equations. These solutions are still applied nowadays [33, 78] because they can show good agreement between predicted and observed *in situ* herbicide concentrations. However, they cannot distinguish nor quantify the importance of each process involved in herbicide disappearance. To overcome these limitations, a suite of numerical solvers have been developed. Nolan *et al.,*[63] screened 20 available numerical models and reviewed in further detail 7 of them based on their capabilities for predicting environmental concentrations of agricultural chemicals. In their review, they concluded that informative mathematical models should account for water movement, sorption, biogeochemical transformation and degradation, and metabolite production. Numerical models are continuously developed in order to easily implement comprehensive reaction networks with the aim to accurately described herbicide dynamics and their feedbacks on the network itself. Indeed, herbicides toxicity to specific microbial populations may cause detrimental effects at the ecosystem level (e.g., sulfonylurea herbicides substantially impair the soil nitrogen cycle [73]). Numerical solvers have also become more user-friendly as a result of the close collaboration between software developers and end-users. Typical improvements may regard:

- Ease of change inputs, boundary conditions, settings, and parameters;

- Ease of integrate additional processes affecting the reaction network, which can be achieved by developing an open source software or by providing technical support;
- Availability of clear software documentation to provide insights on mathematical modelling, hence allowing to understand the confidence and the validity range of the model
- Presentation of model outputs in informative manner both textually and graphically to address users inquiries.

2.4. Uncertainties: sources and their management

In the context of biochemistry, uncertainty may refer to error in laboratory procedures, parameter estimation in the calibration phase, and lack of knowledge of all the possible biogeochemical processes and their spatial and temporal variability in the modelling phase. Examples regard the lack of detailed quantitative description of microbial processes (e.g., microbial dynamics affected by varying environmental conditions, exposure to exogenous and endogenous stressors such as toxic molecules, or varying amounts of nutrients, etc.) or the relationships within microbial communities that may affect microbial activity towards other relevant processes. Uncertainties will result in variability of modelling outcome and deviation from expectation may be large. To account for laboratory uncertainties, experiments are usually carried out in triplicates, and results are reported with their standard deviation. For example, the output of a herbicide biodegradation experiment is sketched in Figure 2a as Process 1, where concentrations are monitored over time. In this case, the modeller would choose some kinetic model to describe the observed average concentrations to estimate the model parameters. The reported variability in measured concentrations may be taken into account in the estimation procedure. The typical approach for parameter estimation is by inverse problem solution, where parameter values are fine-tuned by minimizing the error between observations and predicted values. This numerical procedure allows to calculate some calibration statics, such as parameter uncertainties and cross correlation amongst parameters. These statistics already provide an indication about the robustness of the model. Single reactions integrated in biochemical networks or each parameter part of the equation may contribute to output uncertainty to different extents. Each parameter is inherently associated with a probability distribution, which may be assumed based on expert judgments or on the statistics generated after parameter estimation. In the literature, it has been assumed that kinetic parameters can follow several distributions including Gaussian [44] and uniform [68]. Gaussian distributions may represent well laboratory studies where bacteria achieve similar kinetic performances, while uniform ones may encompass environmental variability. However, no explicit studies have investigated parameter variability in real agricultural conditions [18]. Assumptions made by experts of natural systems can assist to overcome that lack of knowledge. For instance,

microorganisms may adopt different strategies depending on environmental conditions to make the most of the bioavailable resources. Some bacteria may enhance the rate at which they consume a herbicide, while others may enhance their affinity toward the herbicide [65], hence affecting herbicide concentration in the environment. Those strategies cannot be captured by first-order kinetic approaches, but they can within the Michaelis-Menten-Monod kinetic framework, which is explained in Section 3.1.2.

When coupling biogeochemical processes with others, boundary conditions may strongly affect the expected outcome. These driving forces include, but are not limited to, meteorological and hydrological conditions, changes in land use and land management, and spatial variability in soil characteristics. As more processes are deemed fundamental to accurately describe some natural system, so the uncertainty associated with the model structure increases, which should be thoroughly investigated.

2.5. Model uncertainty assessment: methods and insights

The practice of model uncertainty assessment is becoming more important over time [31]. Many model uncertainty assessment techniques exist and are reviewed in [70] and [69]). Those techniques can be applied to assess reactive transport processes too. Once the biogeochemical model is calibrated and validated, a distribution is assigned to input parameters, from which parameter values are extracted using some technique. Random sampling is one option, but advanced sampling methods [15, 16] allow to adequately sample the input parameter space, thus allowing to reduce the number of simulations needed to obtain a robust outcome in terms of a defined model target output(s) (e.g., concentration of herbicide or microbial biomass). Note that, cross-correlation amongst parameters should be specified and accounted for in the sampling.

Different approaches to perform sensitivity analyses should be followed depending on the model output space. Monotone spaces may be assessed using differential analysis. With this technique, the modeller calculates multiple model outcomes from a small neighborhood of the input parameters (Local sensitivity analysis). Input values are generally varied one at a time so that the partial derivative of the output with respect to the input can be calculated. However, as demonstrate in Saltelli *et al.,*[75], this approach is not suitable for nonlinear models and complex output spaces, for which Global sensitivity analyses are preferred. Usually, this technique explore perturbations of input parameters using a Monte Carlo analysis followed by variance-based methods to identify the most influential parameters. The influence of one or more parameters on the variance of the output can be quantified through Sobol's indices [79], the family of AMA indices [22], the Fourier Amplitude Sensitivity Test [19], and others.

Biochemical reaction networks may contain many uncertain parameters. To decrease computational time, it could therefore be convenient to carry out two-steps sensitivity analyses. In the first phase, referred to as parameter screening phase, modellers identify and neglect those parameters with low in-

fluence to the output. It is common to resort to a differential analysis because this technique is less time-consuming and generates less data than a Monte Carlo analysis. The second step consists of the realization of the variance based global sensitivity analysis on the predominant parameters.

In most real life modelling applications, the modeller assembles multiple single models to develop a multimodel system. For example, when biochemical reaction networks are coupled with hydrological models to predict herbicide persistence and dispersion in the environment. Global sensitivity analyses allow to rank each hydrological and biogeochemical process to outcome variability and to assess the correctness of model structure. We remind the reader that a suite of solvers may be used to describe the same process. In this case, uncertainty analyses are useful to identify the most appropriate solver for each process. The correctness of model structure can be assessed by means of the process sensitivity index proposed by Dai *et al.* [20] and of the Framework for Understanding Structural Errors applied in Borgonovo *et al.* [12]. Finally, sensitivity analyses may allow to determine the set of parameters that if optimized would minimize herbicide concentration at a specific location.

The sensitivity of the model output with respect to particularly important parameters may require further work. In case parameter variability results in a wide range of possible outcomes, then the modeller may want to carry out additional investigations with the aim to reduce the parameter uncertainty. On the contrary, a narrow range of possible outcomes may induce the modeller to simplify the model. Model simplification can be achieved by reducing the number of redundant or negligible parameters or by creating a surrogate model. A surrogate model is a simple mathematical function, typically a polynomial, that approximate the response of the numerical model given the input, within a prescribed tolerance. Both methods would result in a simpler system, less computationally demanding and time-consuming.

2.6. Data management and update

Biochemical reaction networks continuously evolve as new processes are found. Also kinetic parameters should be updated as microorganisms may adapt to a herbicide and enhance their activity towards herbicide degradation [3]. As a result, biochemical reaction networks should be kept up-to-date. Interested parties can be universities, research centers, and private consultants because all pursue interest in discovering new biochemical mechanisms and developing new strategies to optimize some desired process [86]. Outputs can then be calculated to provide farmers, policy makers, and the public audience with current comprehensive information. A greater sharing of data on kinetic reactions, and therefore data availability in the literature (e.g., [42, 43, 45, 50]), is key to the success of continuous development of mathematical models.

2.7. Model framework and output communication

Stakeholders such as farmers, policy makers, and the public audience are the end-users of reactive transport simulations carried out under different scenarios. The modeller should aim to tailor the communication of model formulation and output to address stakeholders knowledge and inquiries. Information about herbicide concentration in the environment and their effect to human health and ecosystem services is vast. Policy makers may therefore be interested in clear, explicit, simple, concise, informative, and comprehensive documents to describe modelling assumptions and scenarios, and to report the consequent predicted concentrations. Comparative numerical analyses may be included to capture the implications of policy alternatives.

3. The glyphosate case study

In the following section, we will assess the uncertainties of a GLP biochemical reaction network. Despite the rather simplistic analysis carried out in this study, the contribution of kinetic parameter uncertainty to predicted soil concentrations of GLP and AMPA was quantified, the most significant biotic process regulating mass fluxes from GLP to AMPA was determined, and the importance of chemical processes for GLP and AMPA removal was shown. The effects of other environmental conditions including pH, O_2 levels, and varying availability of an additional carbon source and birnessite mineral to model outcomes were investigated in a comprehensive in-silico analysis in la Cecilia *et al.* [45].

3.1. Methods

3.1.1. Glyphosate reaction network

The GLP reaction network was developed in la Cecilia *et al.* [45] using biological and chemical catabolic pathways reported in the literature and following the validation by construct approach proposed by McCarl and Apland [58] (References in Table 1). The kinetic parameters corresponding to each reaction were estimated using laboratory observations contained in the sourced references. The biochemical reactions and the later developed GLP reaction network were published on peer-reviewed scientific journals, which can give confidence in the formulated biochemical system.

Soil bacteria can degrade GLP along two pathways: one produces aminomethylphosphonic acid (AMPA, P1R1 and P1R1s, Figure 3) and the other produces sarcosine (SRC, P2R1s, Figure 3) (References in Table 1). Sarcosine does not raise health issues, and therefore, its predicted concentrations will be neglected in this analysis, as it is also neglected in the glyphosate registration process [2, 26]. In contrast, AMPA is toxic and it has been shown to persist longer than GLP in the environment [34, 77, 78, 82], Therefore, the conditions leading to AMPA production and its fate in the environment

has to be understood. Some bacterial strains can biodegrade it to non-toxic metabolites (P1R2s, Figure 3) but this process occurs at a slow rate. In fact, also AMPA has been found in the environment [81, 66]. Li *et al.,* [51] and Paudel *et al.,* [67] have shown GLP and AMPA chemical degradation (P2R1c and P1R2c respectively, Figure 3) catalysed by Mn^{3+} and Mn^{4+} ions contained in birnessite mineral ($(Na_{0.3}Ca_{0.1}K_{0.1})(Mn^{3+}, Mn^{4+})_2O_4 \cdot 1.5H_2O$).

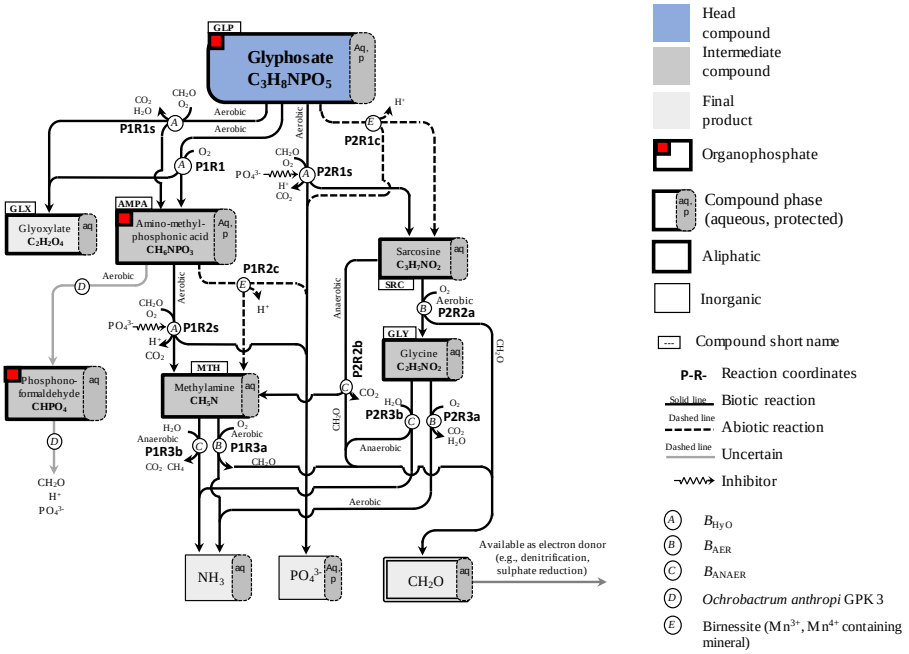

Figure 3: GLP biochemical degradation reaction network in soil from [45]. Extended biochemical reactions and the corresponding kinetic parameters are reported in Table 1.

3.1.2. Numerical solver

BRTSim-v2.2 ([based on 52]) is a 1-D general-purpose multiphase and multicomponent bioreaction transport solver for variably saturated soil systems. The soil moisture dynamics are dealt with a finite volume scheme that solves the Richards equation along the vertical direction. BRTSim can account for any number of chemical and biological species. Equilibrium reactions can be defined for aqueous complexation, ion exchange, gas dissolution, and mineral adsorption and are calculated in BRTSim using the mass-action law. Transport of chemical species is accounted for by the Darcy's advection and Fick's diffusion. Note that advection of gas species in the gas phase was neglected given the time scales of interest in this work. Chemical and biochemical reactions involving primary species and microbial functional groups, described in this solver as primary species, are accounted for in BRTSim by means of the Michaelis-Menten-Monod (MMM) kinetic equations [7, 8, 53, 59], which can be written in their generic

Equation	Pathway	Biochemical aqueous reaction	Kinetic Parameters					Functional group	Specific[(n)] biomass affinity
			μ (s^{-1})	K (M)	K_I (M)	Y (g-C-Biomass g-C-Substrate^{-1})	Y (mg-wet-Biomass mol-Substrate^{-1})		Φ' s^{-1}
EQ1(a)	P1R1s	$\underset{GLP}{C_3H_8NPO_5} + CH_2O + 2\,O_2 \rightarrow \underset{AMPA}{CH_6NPO_3} + \underset{GLX}{C_2H_2O_4} + CO_2(aq) + H_2O(aq)$	3.17×10^{-5}	1.04×10^{-3}	2.53×10^{-4}			B_{HyO}	1.24×10^{-6}
				1.26×10^{-4}		1.03×10^{-1}	2.46×10^{4}		1.02×10^{-5}
EQ2(b)	P2R1s	$\underset{GLP}{C_3H_8NPO_5} + CH_2O(aq) + O_2(aq) \rightarrow \underset{SRC}{C_3H_7NO_2} + 3\,H^+ + {PO_4}^{3-} + CO_2(aq)$	3.34×10^{-5}	1.09×10^{-4}				B_{HyO}	8.39×10^{-6}
				2.12×10^{-4}		1.52×10^{-1}	3.64×10^{4}		4.32×10^{-6}
EQ3(c)	P1R1	$\underset{GLP}{C_3H_8NPO_5} + O_2(aq) \rightarrow \underset{AMPA}{CH_6NPO_3} + \underset{GLX}{C_2H_2O_4}$	3.35×10^{-5}	4.05×10^{-3}	2.53×10^{-4}	3.86×10^{-2}	2.78×10^{4}	B_{HyO}	2.97×10^{-7}
EQ4(d)	P1R2s	$\underset{AMPA}{CH_6NPO_3} + CH_2O(aq) + O_2(aq) \rightarrow \underset{MTH}{CH_5N} + 3\,H^+ + {PO_4}^{3-} + CO_2(aq)$	5.04×10^{-6}	2.08×10^{-3}	2.53×10^{-4}			B_{HyO}	5.86×10^{-7}
				1.38×10^{-4}		1.73×10^{-2}	4.14×10^{3}		8.80×10^{-6}
EQ5(e)	P1R3a	$\underset{MTH}{CH_5N} + \frac{1}{2}\,O_2(aq) \rightarrow CH_2O(aq) + NH_3(aq)$	1.39×10^{-4}	2.15×10^{-4}		2.66×10^{-3}	6.39×10^{2}	B_{AER}	1.01×10^{-3}
EQ6(f)	P1R3b	$\underset{MTH}{CH_5N} + \frac{1}{2}\,H_2O(aq) \rightarrow CH_4(aq) + CO_2(aq) + NH_3(aq)$	1.17×10^{-4}	5.38×10^{-1}		1.29×10^{-3}	3.09×10^{2}	B_{ANAER}	7.05×10^{-7}
EQ7(g)	P2R2a	$\underset{SRC}{C_3H_7NO_2} + \frac{1}{2}\,O_2(aq) \rightarrow \underset{GLY}{C_2H_5NO_2} + CH_2O(aq)$	4.08×10^{-3}	3.37×10^{-5}		2.50×10^{-3}	1.80×10^{3}	B_{AER}	6.74×10^{-2}
EQ8(h)	P2R2b	$\underset{SRC}{C_3H_7NO_2} + CH_2O(aq) + H_2O(aq) \rightarrow \underset{MTH}{CH_5N} + 2\,CH_2O(aq) + CO_2 + 2\,H^+$	5.36×10^{-5}	2.95×10^{-4}		6.87×10^{-2}	4.95×10^{4}	B_{ANAER}	5.04×10^{-6}
				4.39×10^{-3}					2.46×10^{-7}
EQ9(g)	P2R3a	$\underset{GLY}{C_2H_5NO_2} + \frac{3}{2}\,O_2(aq) \rightarrow 2\,CO_2(aq) + NH_3(aq) + H_2O(aq)$	1.22×10^{-4}	1.06×10^{-4}		5.21×10^{-4}	2.50×10^{2}		4.57×10^{-3}
EQ10(i)	P2R3b	$\underset{GLY}{C_2H_5NO_2} + \frac{1}{2}\,H_2O(aq) \rightarrow \frac{3}{2}\,CH_2O(aq) + CO_2(aq) + NH_3(aq)$	2.20×10^{-4}	2.94×10^{-1}		9.25×10^{-5}	4.44×10^{1}	B_{ANAER}	1.69×10^{-5}
EQ11(l)	R4	$CH_2O + O_2 \rightarrow CO_2(aq) + H_2O(aq)$	2.55×10^{-5}	1.55×10^{-4}		9.36×10^{-2}	2.25×10^{4}	B_{HyO}	7.33×10^{-6}
			Reaction rate (M s^{-1})	Adsorption rate (M s^{-1})		Desorption rate (M s^{-1})	Desorption rate (M s^{-1})		
EQ12(m)	R1	$\underset{GLP(aq)}{C_3H_8NPO_5} \rightleftharpoons \underset{GLP(p)}{C_3H_8NPO_5}$	-	2.08×10^{-2}		1.03×10^{-2}	1.17×10^{-2}	Birnessite	
EQ13(m)	P2R1c	$\underset{GLP(p)}{C_3H_8NPO_5} + \frac{1}{2}\,O_2 \rightarrow \underset{SRC}{C_3H_7NO_2} + {PO_4}^{3-} + H^+$	2.67×10^{-3}					Birnessite	
EQ14(m)	R2	$\underset{AMPA(aq)}{CH_6NPO_3} \rightleftharpoons \underset{AMPA(p)}{CH_6NPO_3}$	-	2.63×10^{-1}		1.59×10^{-4}	1.47×10^{-2}	Birnessite	
EQ15(m)	P1R2c	$\underset{AMPA(p)}{CH_6NPO_3} + \frac{1}{2}\,O_2 \rightarrow \underset{MTH}{CH_5N} + {PO_4}^{3-} + H^+$	1.52×10^{-5}					Birnessite	
EQ16(m)	R3	${PO_4}^{3-}(aq) \rightleftharpoons {PO_4}^{3-}(p)$	-	1.09×10^{-2}		1.50×10^{-2}	7.72×10^{-4}	Birnessite	

Table 1: Biochemical reactions implemented in the numerical solver together with their corresponding kinetic parameters as estimated in [45] against laboratory observations published in (a) [5]; [38]; (b) [60]; (c) [57]; (d) [5]; (e) [49]; (f) [36]; (g) [1]; (h) [37]; (i) [21]; (l) B_{HyO} was assumed to grow on CH_2O as an independent reaction, with MMM kinetic parameters averaged from estimations against experiments in [5]; [38]; [60]. (n) Specific biomass affinity $\Phi' = \mu B K^{-1} Y^{-1}$, with B=1 mg L^{-1} and Y in mg-wet-Biomass mol-Substrate^{-1} [42]. B_{HyO} encompasses *Achromobacter* Group V D, *Agrobacterium radiobacter*, *Arthrobacter* sp. GLP-1, *Flavobacterium* sp. GD1, *Pseudomonas* sp. LBr, and *Pseudomonas* PG2982; B_{AER} encompasses *Arthrobacter* P1 and *Pseudomonas Ovalis*; B_{ANAER} encompasses *Clostridium purinolyticum*, *Methanosarcina barkeri* and *Eubacterium acidaminophilum*. SRC (sarcosine); GLX (glyoxylate); GLY (glycine); MTH (methylamine). The order of K values follows the order of C-containing compounds in the corresponding biological reaction. B_{HyO} encompasses *Achromobacter* Group V D, *Agrobacterium radiobacter*, *Arthrobacter* sp. GLP-1, *Flavobacterium* sp. GD1, *Pseudomonas* sp. LBr, and *Pseudomonas* PG298; B_{AER} encompasses *Arthrobacter* P1 and *Pseudomonas Ovalis*; B_{ANAER} encompasses *Clostridium purinolyticum*, *Methanosarcina barkeri* and *Eubacterium acidaminophilum*.

form as:

$$\frac{1}{x_k}\frac{\mathrm{d}X_k(t)}{\mathrm{d}t} = \mu_k \prod_{n_O} X_{n_O}^{x_{n_O}}(t) \cdot \prod_{n_M M} \frac{X_{n_{MM}}(t)}{X_{n_{MM}}(t) + K_n\left(1 + \sum_{n_{COM}} \frac{X_{n_{COM}}(t)}{K_{n_{COM}}}\right)} \prod_{n_I} \frac{X_{n_I}(t)}{X_{n_I} + K_{n_I}} \tag{1}$$

where x is the stoichiometric number relative to molecule k with concentration X (mol L^{-1} or M), t is time (s), μ is the reaction rate (s^{-1}), n_O is the number of biological-mineral-chemical species contributing to the reaction, n_{MM} is the number of Michalis-Menten (MM) terms with the corresponding half-saturation constant K_n (M), n_{COM} is the number of competition terms with the corresponding constant $K_{n_{COM}}$ (M), n_I is the number of inhibition terms with the corresponding constant K_{n_I} (M). In case a molecule k is transformed by any microbial biomass i with concentration B (mg L^{-1}) and biomass yield constant Y

(mg-wet-biomass mol-substrate^{-1}), then X_{n_O} takes the form of $\frac{B_i}{Y_i}$ and biomass dynamics can be written as:

$$\frac{\mathrm{d}B_i(t)}{\mathrm{d}t} = \frac{1}{x_k}\frac{\mathrm{d}X_k(t)}{\mathrm{d}t} \cdot Y_i - \delta_i B_i(t) \tag{2}$$

where δ is the microbial mortality rate constant (s^{-1}).

3.1.3. Scenario

In a previous research [45], the sensitivity of the GLP reaction network used in this work was assessed with respect to abiotic factors, such as dissolved oxygen content, dissolved carbon content, birnessite concentration, and pH. To make use of this knowledge, we numerically investigate GLP and AMPA dynamics under identical conditions, which could represent slow GLP leaching through a contaminated agricultural soil. Hence, in a 1 L bioreactor, GLP at 0.003 M concentration and an additional carbon source (CH_2O) at 0.001 M concentration were released at a Q = 0.0036 L h^{-1} flow rate in an aqueous solution without and with birnessite mineral at 1.20 g $kg^{-1}_{\text{dry-soil}}$ concentration, with constant pH = 7 and O_2 levels equal to 3 mg L^{-1}. GLP and AMPA concentrations were modelled over time as a function of both biological and chemical processes. Output concentrations represent steady-state conditions. Chemical degradation occurred only after GLP or AMPA absorbed onto birnessite [51]; adsorption was described by means of Langmuir kinetics [48], while degradation was described by means of MM kinetics. Using two separate experiments in the same laboratory conditions, Li *et al.,* [51] showed GLP and AMPA chemical degradation and measured the concentration of PO_4^{3-} liberated by these two reactions. The release of PO_4^{3-} was very quick with GLP, while it was 1 order of magnitude slower with AMPA. Although birnessite mineral can break GLP down to both AMPA and SRC, the very high rate at which PO_4^{3-} concentration increased following GLP degradation might suggest that GLP was preferentially degraded to SRC (P2R1c, Figure 3). Therefore, it was assumed that GLP could only be degraded to SRC, and not to AMPA. The microbial functional group B_{HyO} can grow on GLP and AMPA. The bacteria mortality rate δ (s^{-1}) was assumed to be constant and equal to 10^{-6} s^{-1} after [32]. Phosphate (PO_4^{3-}) inhibitory effect on GLP and AMPA biodegradation along P1R1 and P1R2, respectively, was accounted for using an inhibition value $K_I = 2.53 \times 10^{-4}$ M estimated against observations in [5]. Substrate competition was not included in this work due to the limited variety of substrates available. O_2 consumption in aerobic reactions was accounted for using a MM value $K = 1.40 \times 10^{-5}$ M after [14], while an inhibition value $K_I = 3.125 \times 10^{-6}$ M was used for O_2 inhibition on anaerobic processes (adapted from [41]). The pH effect on biological activity was accounted for by using a $K = 10^{-9}$ M for high pH and an inhibition value $K_I = 10^{-5}$ M for low pH, respectively, after [11].

3.1.4. Uncertainty and sensitivity analyses

Microorganisms may evolve different strategies for scavenging nutrients and energy from anthropogenic molecules depending on the surrounding environmental conditions. High substrate concentration may select for fast GLP biodegraders (high μ), while low substrate concentration may favor GLP biodegraders with a high affinity for GLP (low K). A suite of sensitivity analyses were run to assess the uncertainty to GLP and AMPA equilibrium concentrations resulting from a specific group of MMM kinetic parameters (i.e., μ, K, or Y) or a specific biological reaction (i.e. EQs 1 to 4). To this aim, the MMM kinetic parameters relative to one group and to EQs 1 to 4, were randomly chosen from a Gaussian distribution with mean equal to the corresponding experimentally retrieved parameter and standard deviation (σ) equal to 5, 10, 15, 20, 25, and 30% of that value, per each analysis. For each generated parameter space, we referred to "low" values as those smaller than the 33^{th} quantile, "middle" values as those between the 33^{th} and 66^{th} quantiles, and to "high" values as those greater than the 66^{th} quantile. For the stochastic sensitivity analysis, 2000 simulations were run for each group of parameters and for each σ. Simulations were repeated with and without accounting for the effect of chemical degradation after [6] observed that ions may inhibit GLP and AMPA degradation by Mn-oxides. The difference between GLP equilibrium concentration predicted in each model run ($GLP_{c,sto}$ and GLP_{sto}, with and without birnessite respectively) and the concentration predicted using experimentally retrieved parameter values ($GLP_{c,ref}$ and GLP_{ref}, with and without birnessite respectively) was used as the sensitivity measure ($SM_{c,GLP}$ = $GLP_{c,sto}$ - $GLP_{c,ref}$ and SM_{GLP} = GLP_{sto} - GLP_{ref}). The same approach was repeated for AMPA; therefore, the difference between AMPA equilibrium concentration predicted in each model run ($AMPA_{c,sto}$ and $AMPA_{sto}$, with and without birnessite respectively) and the concentration predicted using average parameter values ($AMPA_{c,ref}$ and $AMPA_{ref}$) was calculated as $SM_{c,AMPA}$ = $AMPA_{c,sto}$ - $AMPA_{c,ref}$ and SM_{AMPA} = $AMPA_{sto}$ - $AMPA_{ref}$.

3.2. Results

3.2.1. Uncertainty analysis: GLP and AMPA concentrations

GLP and AMPA equilibrium concentrations were reached within 100 simulated days. GLP and AMPA concentrations showed unimodal distributions (Figure 4). When abiotic catalytic reactions were not accounted for, output distributions were more skewed, GLP and AMPA concentrations were higher, and output ranges were larger. $GLP_{c,ref}$ was nearly 7.4×10^{-4} g $kg^{-1}_{dry\text{-}soil}$ (thin dashed black line in Figures 4a, c, and e), value in line with field data in Silva *et al.* [77]. $AMPA_{c,ref}$ was nearly 1.5×10^{-3} g $kg^{-1}_{dry\text{-}soil}$ (thin dashed gray line in Figures 4b, d, and f), value in line with field data in Silva *et al.* [77]. These concentrations are higher than those modelled for GLP, highlighting that produced AMPA was slowly biodegraded and suggesting that AMPA can persist in soil longer than GLP; consequently, AMPA may be

regarded as more concerning than GLP in the perspective of environmental protection. GLP and AMPA distribution skewness was opposed, meaning that GLP biodegradation to AMPA rather than SRC was the preferential pathway in the reaction network because the more GLP was degraded the more AMPA was produced.

3.2.2. Sensitivity analysis: contribution of kinetic parameters

The parameter space corresponding to the 4 input variables was assumed to be adequately sampled by 2000 simulations, and increasing variability for each parameter group revealed interesting results (Figure 5). Chemical and biological processes collaborated to fast degrade GLP. Lower μ resulted in slower biodegradation rates, which were flanked by the catalytic action of birnessite mineral. The lowest μ values caused the mineral surface to become saturated; in this case, GLP concentration increased. In the lack of birnessite, the increasing variability in μ resulted in a nonlinear increase in GLP concentration. Biotic processes alone could fast degrade GLP; low μ resulted in a substantial increase in GLP concentration, while high μ did not substantially decrease it. Increasing variability in K resulted in lower GLP concentration both with and without birnessite. This is because GLP application concentration was similar to K; low K substantially increased the biodegradation rate, while high K did not decrease it likewise. Similarly, increasing variability in Y resulted in lower GLP concentration. In the presence of birnessite, bacteria consumed small amounts of substrate; therefore, varying Y did not substantially affect GLP. In the lack of birnessite, high Y resulted in an trade off between a slower degradation rate but a higher biomass concentration; conversely, low Y resulted in faster rates but lower biomass concentration. Therefore, GLP concentration did not change in average.

3.2.3. Sensitivity analysis: contribution of biochemical processes

Boxplots in Figure 6 represent the variability in SM values resulting from uncertainty in kinetic parameter values for the scenario $\sigma = 10\%$. The predicted SM values were grouped as "low", "middle", or "high" according to the values taken by the corresponding stochastic kinetic parameter. The two most important features in Figure 6 are: (1) the deviation of the mean SM from 0 g $kg^{-1}_{\text{dry-soil}}$, which means there was no difference between reference and uncertain scenarios on average, and (2) the range of each boxplot. Reaction P1R1 (Table 1, EQ3) mostly drove the GLP reaction network because the average of $SM_{c,GLP}$ and SM_{GLP} substantially changed as the parameter values relative to EQ3 changed (red horizontal lines in Figure 6a and c, boxplots in 3rd, 7th, and 11th column); P1R1s contributed little to the reaction network, while P2R1s and P1R2s did not affect the reaction network (Figure 6a, boxplots in 1st, 2nd, and 4th column, respectively). Results from EQ3 showed that higher $GLP_{c,sto}$ (therefore greater positive $SM_{c,GLP}$) resulted from lower μ values (or high K or Y values, Figure 6a)

and corroborated that Y did not affect GLP_{sto}, that is when there was no birnessite mineral (Figure 6c, 9th to 12th column). EQ3 also decreased the model output variability as indicated by the smaller $SM_{c,GLP}$ and SM_{GLP} range for EQ3 compared to those relative to EQs 1, 2, and 4 (Figure 6a and c). EQ2 did not influence the reaction network to a great extent given that this is a cometabolic reaction, and therefore, the overall reaction rate is a function of CH_2O concentration; CH_2O was consumed by GLP biodegraders for growth in the competing reaction R4 (Table 1). Yet, sarcosine produced along EQ2 and its further metabolites were assumed to not contribute to the C sources available to GLP biodegraders for their growth. EQ4 influenced the least the reaction network. In fact, this reaction involves AMPA biodegradation, which poorly contributes to GLP biodegraders growth (i.e., Y relative to AMPA is 1 order of magnitude lower than Y relative to GLP as reported in Table 1) and occurs at a slow rate (Figure 6b and d). GLP biodegradation to AMPA described by EQ3 was found to be the most important regulatory process on the reaction network; therefore, it was expected that EQ3 influenced $SM_{c,AMPA}$ and SM_{AMPA} as well. A faster AMPA production was not followed by the same increase in AMPA degradation rate, thus it accumulated. In the event that microorganisms degrade GLP to AMPA, then AMPA would pose an even more serious risk to the environment.

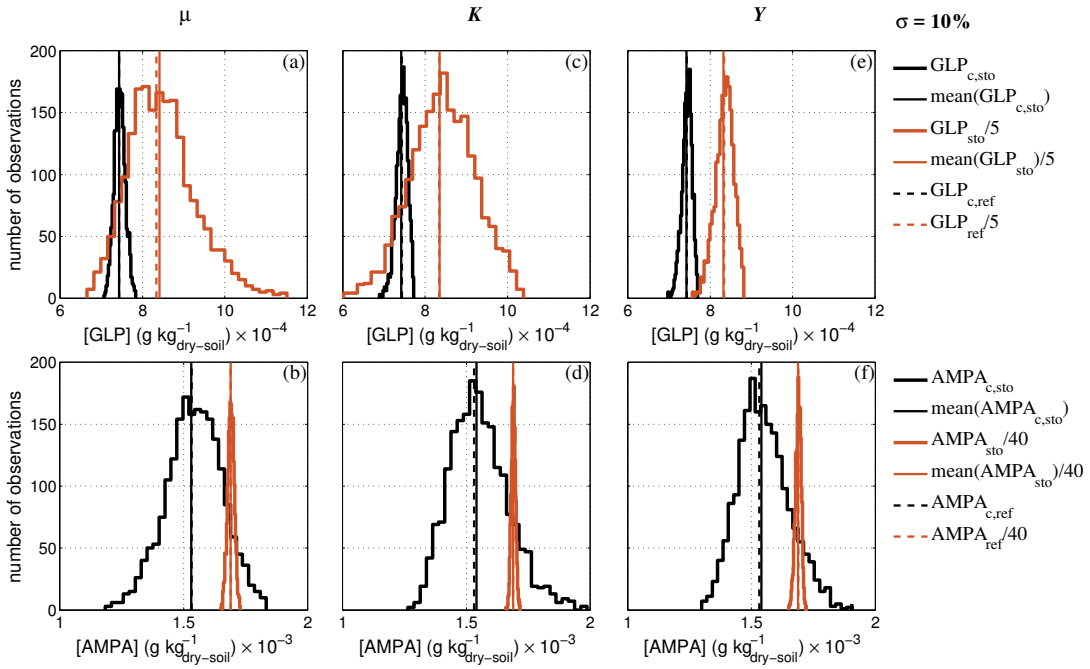

Figure 4: Distribution of $GLP_{c,sto}$ and GLP_{sto} around $GLP_{c,ref}$ and GLP_{ref}, respectively, in (a), (c), and (e) and $AMPA_{c,sto}$ and $AMPA_{sto}$ around $AMPA_{c,ref}$ and $AMPA_{c,ref}$, respectively, in (b), (d), and (f). σ = 10%. Number of bins were chosen according to Freedman-Diaconis rule.

4. Conclusions

Reactive transport solvers are a great tool to support decision-making for farmers, regulatory bodies, and EPAs to sustainably manage and protect the environment. Overall, uncertainty analyses of herbicide

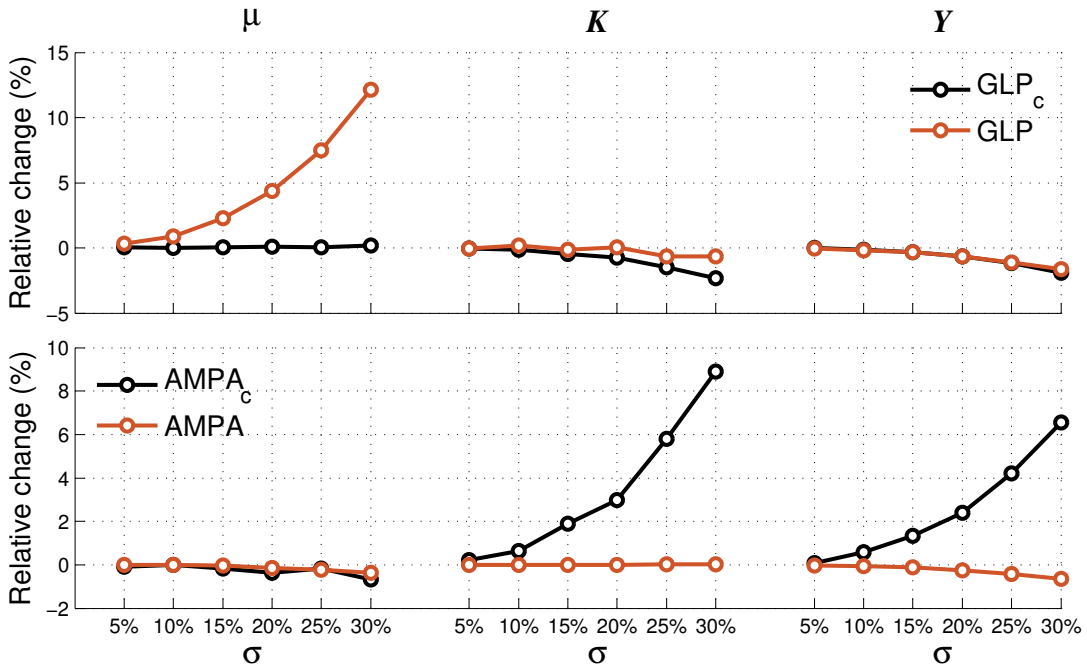

Figure 5: Relative change in $GLP_{c,sto}$ and GLP_{sto} with respect to $GLP_{c,ref}$ and GLP_{ref}, respectively, as a function of σ in the upper panel; relative change in $AMPA_{c,sto}$ and $AMPA_{sto}$ with respect to $AMPA_{c,ref}$ and $AMPA_{ref}$, respectively, as a function of σ in the lower panel.

degradation networks can provide policy makers and stakeholders with a quantitative decisional tool to explore possible outcome variability resulting from a range of modelling assumptions, thus supporting herbicides approval. Yet, sensitivity analyses allow to rank the processes or parameters contributing to outcome variability, hence providing a wider insight into sustainable land management planning. In this study, we found that chemical degradation of glyphosate (GLP) and its metabolite AMPA in soil by manganese-containing oxides would result in a reduction of GLP and AMPA concentrations by nearly 25%. While we accounted for competition for catalytic sites on the oxide by GLP, AMPA, and orthophosphate, we did not account for other likely competing cations abundantly available in soil [6]. When only biological reactions were accounted for, we found that GLP oxidation to AMPA was the main process driving GLP degradation. This could have been expected as the other three cometabolic biological reactions are functions of CH_2O concentration; in fact, la Cecilia & Maggi [45] showed that more GLP was converted into sarcosine along one of the cometabolic reaction at increasing CH_2O bioavailability. A relatively small uncertainty of the reaction rate constant μ and the half-saturation constant K resulted in minimum predicted GLP concentrations that were nearly half of the maximum concentrations. These two parameters have been shown to describe the strategies used by microorganisms to transform a substrate (e.g., pesticides) [65]. Of utmost importance, Porta *et al.,* [68], showed that uncertainties in kinetic parameters of the reaction network for the herbicide atrazine coupled with the nitrogen cycle in soil can result in ecological imbalances, which might be detrimental to soil quality and functioning. As a

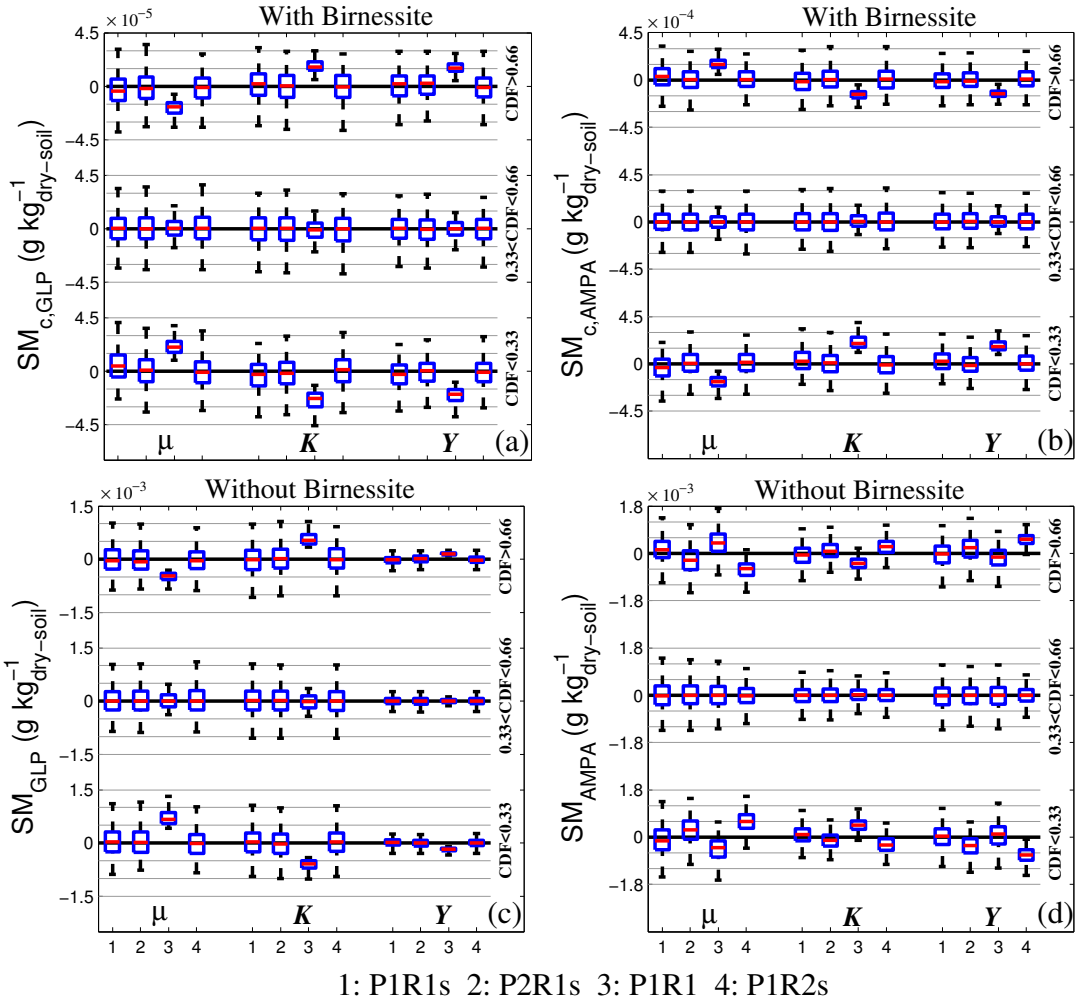

Figure 6: Boxplot showing the outcome variability in $SM_{c,GLP}$ (a), SM_{GLP} (c), $SM_{c,AMPA}$ (b), and SM_{AMPA} (d), grouped horizontally by parameter quantiles (i.e., Q1 = 33th and Q2 = 66th) and vertically by MMM kinetic parameter, and organized by equation number. Black horizontal lines indicate $SM_{c,GLP}$, SM_{GLP}, $SM_{c,AMPA}$, and SM_{AMPA} equal to 0. σ = 10%.

second important remark, Greskowiak *et al.,* [35] showed that the variability in first-order degradation constants estimated within and across laboratory and field conditions ranged over 3 orders of magnitude for 82 compounds. Similarly, Charnay *et al.* [18] concluded that pesticides degradation rates vary spatially possibly due to the dynamics of peculiar biodegraders. Such uncertainty can therefore be relevant in environmental risk assessment studies, where the practice is to average the available information and predict one time-series of environmental concentrations [26, 30]. The reduction of uncertainty in pesticide biodegradation kinetic values might be achieved through studies aiming at better understanding what are the factors the favor or limit microbial communities in removing pesticides at the global scale; at the European scale, a similar investigation was carried out by Pierre *et al.,*[74] in the context of chloroethene-contaminated aquifers.

To sum up, our analyses suggested that:

- All kinetic parameters (i.e., μ, K, and th biomass grwoth yield Y) are important descriptors of biological processes within a complex reaction network, and their variability may cause different

responses;

- At background concentrations of O_2 and an additional carbon source, GLP is preferentially biodegraded to AMPA;
- The metabolite AMPA is suggested to be an emerging contaminant in the environment;
- Effort should be put into AMPA monitoring campaigns to collect data on its level of contamination for consideration in future regulation initiatives;
- Birnessite mineral addition into the soil and soil biostimulation by adding an additional carbon source may be successful strategies for cleaning up soils contaminated with GLP and AMPA.

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