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Metabolic modelling: Insights into the machine room of plant metabolism

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ABSTRACT

Plant growth, development, and environmental interactions is enabled through the coordinated activity of numerous biochemical reactions that constitute plant metabolic networks. The inherent complexity and interconnectivity within these networks underscore the importance of investigating plant metabolism from a network perspective. Metabolic modelling provides a holistic *in silico* representation of plant metabolism, enabling mechanistic insights into network-level processes. In this review, we consolidate recent trends in plant metabolic modelling, highlighting how these approaches can be exploited to study metabolism from subcellular to community and ecosystem levels. We discuss how the scope of plant metabolic modelling has broadened to represent diverse plant species, genotype- and context-specific metabolism as well as specialized metabolic pathways, and to capture spatiotemporal resolution and plant-microbe interactions. Moreover, we review machine learning and deep learning frameworks that assist model reconstruction, parameterization, and analysis, explore hybrid strategies that enhance mechanistic models, and address current challenges and future directions in the field of plant metabolic modelling.

1. Introduction

Plant metabolic networks have evolved to sustain growth and optimize resource allocation through adaptive responses to dynamic environmental conditions. This involves interactions with both the biotic and abiotic environment, which are often mediated by a wide range of specialized metabolites. Furthermore, as for all multicellular organisms, plant metabolism exhibits a complex spatial organization from subcellular compartments to diverse specialized cell types, tissues, and functionally distinct organs, as well as temporal dynamics in response to fluctuating environmental conditions, over the diel cycle, and across different developmental stages. Mathematical modelling helps dissecting these complex spatiotemporal interactions, and provides quantitative and mechanistic insights to decode the "machine room" of plants.

While there is a vast repertoire of mathematical modelling approaches, kinetic and constraint-based modelling are most commonly used to analyze plant metabolic behavior. Kinetic modelling deploys rate laws to represent metabolic reactions and predicts reaction rates and metabolite concentrations over time. While kinetic models can quantitatively capture the dynamic nature of metabolism, their predictive power relies on the availability of kinetic parameters for the involved enzymes and is thus typically the approach of choice to model individual pathways (Islam et al., 2021).

In contrast to this, constraint-based modelling relies only on the reaction stoichiometry of the metabolic network and is thus suitable to study metabolism at the network level (Islam et al., 2025). These typically large-to genome-scale metabolic network models encompass known metabolic reactions encoded by the organism's genome (the reason they are called genome-scale metabolic models) and can be reconstructed using various databases and additional information from the literature. Flux balance analysis (FBA), a constraint-based modelling framework, predicts steady-state flux distributions within these metabolic networks by assuming metabolism has evolved to optimize specific criteria, which is mathematically defined as a so-called objective function. The choice of the objective function plays an important role in predicting flux distributions (Orth et al., 2010). While the maximization of biomass is a commonly used objective for microbial systems, defining a suitable objective function for plant systems can be less intuitive. Depending on the cell-type, organ as well as the environmental context and the plant's developmental stage, cellular objectives can be very different and not straightforward to define (Islam et al., 2025). This is even more apparent when modelling plant-microbe interactions, where plants engage in mutualistic, commensal and pathogenic interactions; each requiring a cautious consideration of the systems or subsystems objective. Depending on the system, several objectives such as maximizing the export of photoassimilates from the leaf to the phloem,

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maximizing ATP yield, minimizing resource usage or minimizing the discrepancy with experimental data can offer realistic alternatives. Constraint-based modelling frameworks require minimal parameterization due to the steady-state assumption—the assumption that intracellular metabolite concentrations remain constant over short time periods. This approach enables predicting metabolic behavior without requiring detailed kinetic parameters. Instead, it relies on biological and thermodynamic constraints such as exchange rates and reaction reversibility, making it well-suited for modelling metabolic networks at the genome scale—the focus of this review.

The first application of constraint-based modelling in plants goes back to 2009, when Grafahrend-Belau et al. developed a medium-scale model of the primary metabolism of barley (*Hordeum vulgare*) seeds (Grafahrend-Belau et al., 2009). Shortly after, Poolman et al. presented the first genome-scale metabolic model of *Arabidopsis thaliana* (Poolman et al., 2009). This model was still an uncompartmentalized representation of the metabolism of a heterotrophic cell suspension and, as such, reminiscent of its prokaryotic counterparts from the microbial community (Edwards and Palsson, 1999). Since these seminal papers, the field has advanced significantly through improvements in genome sequencing (Sun et al., 2022), annotation (Bolger et al., 2018), and tools facilitating high-quality network reconstruction and contextualization (Seaver et al., 2021). Thriving from these advancements, plant metabolic models have now expanded to encompass diverse plant species. Their evolution extends beyond static representations of cellular metabolism to more complex multi-tissue and whole-plant models and modelling approaches that capture spatiotemporal dynamics as well as plant-microbe metabolic interactions. Constraint-based modelling integrated with kinetic modelling approaches can tackle limitations of the steady-state assumption, capture nonlinear metabolic behavior and regulatory effects. Machine learning recognizes patterns in high-dimensional heterogeneous omics data and facilitates model input extraction and output analysis. It can further serve as surrogates for constraint-based metabolic models to efficiently solve dynamic control problems for metabolic systems (Espinel-Ríos and Avalos, 2024).

In this review, we highlight recent progress in the field of plant metabolic modelling. Beginning with advancements in modelling cellular metabolism, we explore how the development of spatiotemporal and community-level models have enabled simulating more sophisticated metabolic interactions as illustrated in Fig. 1. We further discuss the role of machine learning and deep learning in advancing metabolic modelling approaches and assess how hybrid and multi-scale modelling

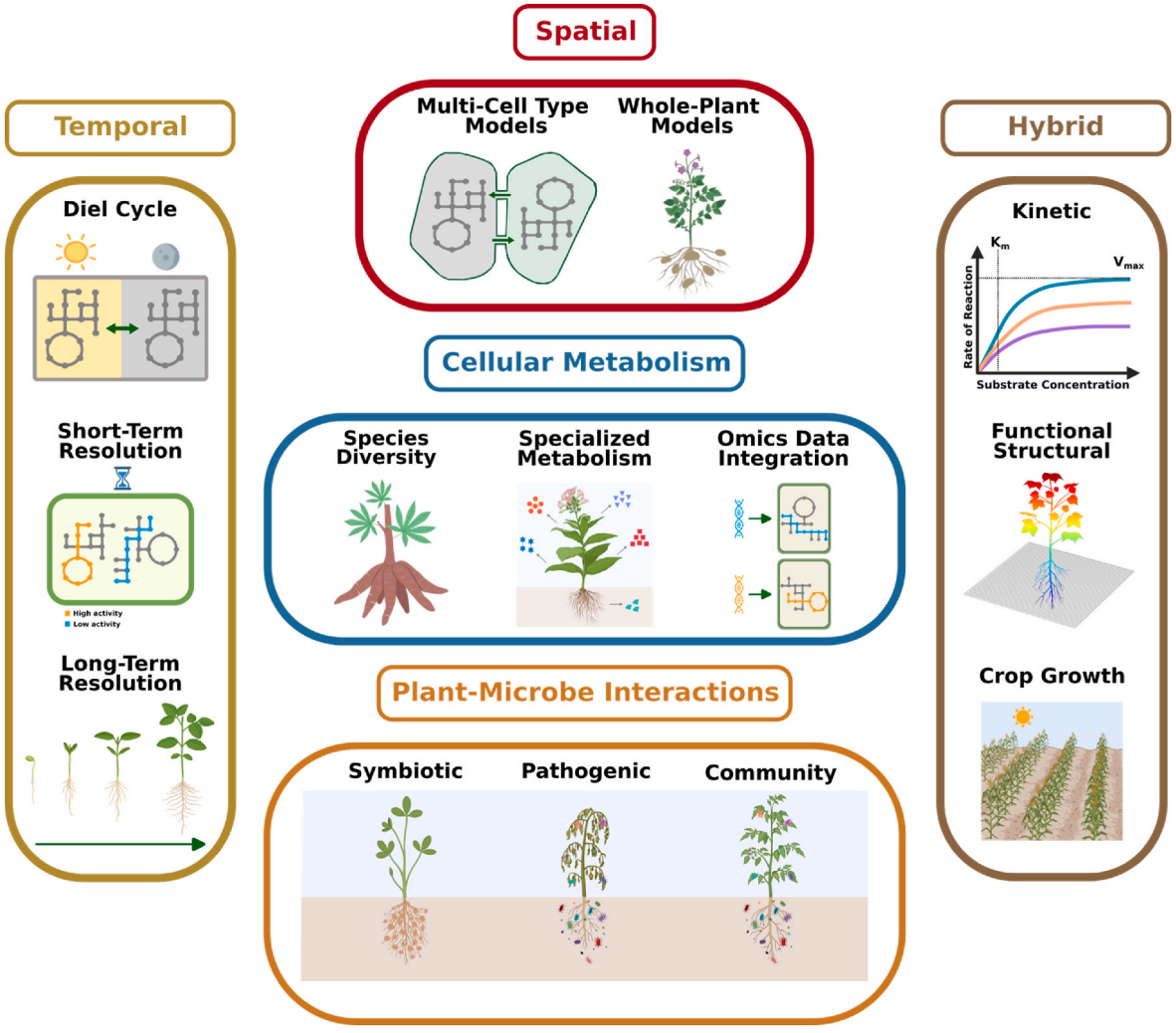

Fig. 1. Frameworks for modelling plant metabolic systems across various scales. Recent advances in plant constraint-based metabolic modelling categorized by their methodological focus: (blue) models of cellular metabolism, including *in silico* representations of diverse plant species, specialized metabolic pathways, and condition- or genotype-specific metabolism facilitated by omics data integration; (red) spatially resolved models–from multi-cell type models to whole-plant systems; (yellow) temporally resolved models – from diel cycles and short-to long-term metabolic dynamics; (orange) plant-microbe interaction modelling for symbiotic, pathogenic, and community systems; and (brown) hybrid approaches that integrate constraint-based frameworks with kinetic, structural-functional, and crop growth models (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article).

frameworks have expanded the biological scope of constraint-based metabolic models. We conclude by denoting current limitations and future research directions for plant constraint-based modelling.

2. Models of cellular plant metabolism provide basic knowledge and have applications in biotechnology and agriculture

Early works using constraint-based models explored the cellular metabolism of Arabidopsis to study energy metabolism and metabolic responses to environmental changes (Poolman et al., 2009; Williams et al., 2010). Further efforts were made to improve model resolution by incorporating subcellular compartmentalization, specialized metabolism, and tissue specificity (Dal'Molin et al., 2010a; Mintz-Oron et al., 2012). In parallel, large-to genome-scale models of important crops, such as barley (*Hordeum vulgare*) (Grafahrend-Belau et al., 2009; Rolletschek et al., 2011), maize (*Zea mays*) (Dal'Molin et al., 2010b; Chowdhury et al., 2023), sorghum (*Sorghum bicolor*) (Dal'Molin et al. 2010a; Clark and Schwender, 2022), sugarcane (*Saccharum officinarum*) (Dal'Molin et al., 2010b), rapeseed (*Brassica napus*) (Pilalis et al., 2011; Hay et al., 2014), rice (*Oryza sativa*) (Lakshmanan et al., 2013; Chowdhury et al., 2025), tomato (*Solanum lycopersicum*) (Yuan et al., 2016; Gerlin et al., 2022), potato (*Solanum tuberosum*) (Botero et al., 2018) and soybean (*Glycine max*) (Moreira et al., 2019; Vinson et al., 2025), here cited as the first and latest published models, were reconstructed and analyzed for systematic characterization of metabolism and finding engineering strategies to enhance crop productivity and resilience to environmental stresses.

Applications of models of cellular metabolism have expanded beyond Arabidopsis and model crops to encompass broader phylogenetic and functional diversity. Recent studies have increasingly focused on diverse species, including cassava (*Manihot esculenta*) (Chiewchankaset et al., 2019), alternative oilseed crops (*Jatropha curcas*) (Correa et al., 2020), bionergy grasses (*Setaria italica* and *Setaria viridis*) (Dal'Molin et al., 2016; Maiti et al., 2023), and fruit species (Shameer et al., 2020; Colombié et al., 2023). For example, Chiewchankaset et al. measured the sucrose uptake, growth rates and biomass compositions of high- and low-yielding cassava cultivars to construct cultivar-specific models. Using these models, the authors identified targets for improved carbon allocation to the roots, which could ultimately lead to enhanced yield (Chiewchankaset et al., 2019). This study highlights metabolic models as tools for rapidly generating flux solutions for specific genetic scenarios (Sonnewald et al., 2020). Moreover, many of these reconstructions capture not only pathways of primary metabolism but also incorporate specialized metabolic pathways, such as nicotine and steroidal glycoalkaloid biosynthesis in tobacco (*Nicotiana tabacum*) (Yu et al., 2023b), detailed lipid biosynthesis in *Jatropha curcas* (Correa et al., 2020), isoprenoid biosynthesis in poplar (*Populus trichocarpa*) (Sarkar and Maranas, 2020), antioxidant biosynthesis in rice (Wanichthanarak et al., 2020), and cannabinoids, terpenes, flavonoids, and alkaloids in Cannabis (*Cannabis sativa)* (Camargo et al., 2023). In the later study the authors used metabolomics data collected from cannabis leaf extracts to curate the model. Constraint-based modelling efforts to capture specialized metabolism have been reviewed in further detail in (Beilsmith et al., 2022). Overall, these comprehensive and high-quality models enable *in silico* exploration of traits linked to defense chemistry, nutritional quality, or pharmaceutical potentials.

Plant metabolism changes over developmental stages and in response to environmental stimuli through transcriptional and post-transcriptional changes, it differs between cell types, and it can vary between accessions of the same species. To account for these changes and to capture context-specific differences, a suite of omics-data integration methods have been developed, such as iMAT (Zur et al., 2010), E-Flux (Colijn et al., 2009), GIMME (Becker and Palsson, 2008) and tINIT (Agren et al., 2012) and more extensively reviewed in (Blazier and Papin, 2012; Robaina Estévez and Nikoloski, 2014; Moškon and Režen, 2023; Sen and Orešič, 2023). For instance, transcriptomics data integration revealed metabolic pathways underlying light and temperature acclimation in Arabidopsis (Töpfer et al., 2013), and reprogramming of rice leaf (Wanichthanarak et al., 2020) and Arabidopsis root cell metabolism (Esterhuizen et al., 2025) in response to salinity stress. Similarly, transcriptomics data were used to explore metabolic differences between soil-grown and *in vitro*-grown tobacco plants (Yu et al., 2023b). Additionally, integration of proteomics and metabolomics data enabled the study of temporal acclimation to cold stress in Arabidopsis (Herrmann et al., 2021). Furthermore, omics data-contextualized models have been used to capture genetic variation in cultivar- and accession-specific metabolic differences. Kamsen et al. integrated cassava transcriptomics data from different cultivars with a cassava root model. Their analysis revealed cytosolic carbon channeling and nitrogen-linked shuttles potentially linked to increased yield (Kamsen et al., 2021). Sarkar and Maranas integrated single nucleotide polymorphisms (SNPs) and metabolomics data with metabolic models of Arabidopsis and poplar and were thus able to associate genomic variations with shifts in metabolic fluxes (Sarkar and Maranas, 2020). Tong et al. used SNPs and metabolomics data to generate 67 condition- and accession-specific models of Arabidopsis. They investigated metabolic plasticity of these genotypes and found photorespiration and central carbon metabolism to be critical for adaptive flux plasticity under nitrogen and carbon limitation (Tong et al., 2020, 2023).

Besides omics data, isotope labelling data have also been used to contextualize and validate plant metabolic models. Hay et al. integrated transcriptomics and isotope labelling data of high-oil and -starch rapeseed genotypes with a model of rapeseed seed metabolism to identify key pathways for oil content optimization (Hay et al., 2014). More recently, Kaste and Shachar-Hill used isotope labelling data to evaluate how the integration of transcriptomics and proteomics data with an Arabidopsis model improved their flux predictions (Kaste and Shachar-Hill, 2024). Lastly, thermodynamic information can be integrated with constraint-based models. By incorporating changes of Gibbs free energy (ΔG) as constraints that ensure that all predicted fluxes are thermodynamically feasible under realistic metabolite concentrations, Thermodynamics-based Flux Analysis (TFA) can improve metabolic modelling accuracy (Henry et al., 2007; Salvy et al., 2019). Applications to plant systems, however, are challenged by the complexity of plant subcellular compartmentation with distinct physicochemical conditions and limited availability of compartment-resolved metabolomics data (Allen et al., 2009; Sweetlove and Ratcliffe, 2011; Fürtauer et al., 2019).

Overall, models of cellular metabolism have progressed toward a higher diversity of modelled species, consideration of specialized metabolism, and omics-data integration, with recent developments in genomic data integration. They have significantly contributed to our understanding of plant metabolic networks and find application in agriculture and biotechnology research. In addition, the availability of plant pan-genome data (Hirsch et al., 2014; Li et al., 2014; Schatz et al., 2014) for a variety of model and crop species provides an opportunity to investigate intraspecies genetic diversity and scale plant metabolic modelling to the pan-genome level (Danilevicz et al., 2020; Ruperao et al., 2025). These advances pave the way to describe genotype-to-metabolism interactions in response to environmental stimuli and improve our understanding of metabolic diversity at the intra-species level.

3. Spatiotemporal modelling reveals how plant metabolism varies across time and space

As multicellular organisms, plants orchestrate their metabolic processes across diverse cell types, tissues and organs, each exhibiting distinct metabolic characteristics. Moreover, plant metabolism presents distinct patterns over the course of the diel cycle, throughout developmental stages, and in response to environmental perturbations. To capture the spatiotemporal complexity and cross-talk of metabolic

systems, the community has developed a range of spatially and temporally-resolved models of metabolism. These models have enabled us to simulate metabolism at unprecedented resolution.

3.1. Spatially-resolved constraint-based models: from two-cell systems to whole-plant networks

Spatially-resolved models can be constructed by creating copies of an initial base model, customised with distinct parameters, such as known exchanges with the environment, omics data, biomass compositions or growth rates, to represent metabolism within specific cell types, tissues or organs. Subsequently, to capture metabolic interactions, these sub-models are coupled through a set of transfer reactions enabling exchange of metabolites across cells, tissues or organs.

Dal'Molin et al. used a cell-type specific model, with one submodel representing mesophyll and the other bundle sheath metabolism, to simulate C_4 photosynthesis and to study metabolic interactions between the two different cell types (Dal'Molin et al., 2010b). In subsequent years, the two-cell modelling framework was extended to represent more complex multi-cell, -tissue and -organ metabolic interactions as reviewed by (Shaw and Cheung, 2020). Applications of this approach have provided valuable insights into metabolic phenotypes of C_4 crops and various aspects of C_4 photosynthesis including system-level metabolic reprogramming under genetic and environmental perturbations (Wang et al., 2012; Simons et al., 2014), evolutionary trajectories of C_4 photosynthesis (Mallmann et al., 2014; Blätke and Bräutigam, 2019), metabolic coordination between mesophyll and bundle sheath cells (Robaina-Estévez and Nikoloski, 2016), metabolic variation among genetically diverse maize lines (Cañas et al., 2017), potential engineering targets for triacylglycerol overproduction (Clark and Schwender, 2022), metabolic organization in the less studied C_4+ Crassulacean Acid Metabolism (CAM) photosynthetic type (Moreno-Villena et al., 2022), and the source-sink relationships within a single tissue (Bogart and Myers, 2016; Maiti et al., 2023). Moreover, Hunt et al. developed a multi-cell metabolic model of mesophyll, companion cell and sieve elements to investigate the metabolism and energetics underlying phloem loading in Arabidopsis (Hunt et al., 2023a). The same authors also investigated metabolic interactions across the root biogeography which is further discussed in the hybrid and multi-scale modelling section. Studies of root systems particularly benefit from multi-cell type or -tissue modelling approaches since roots are highly heterogeneous (with various cell types across the radial and different developmental zones across the longitudinal axis). Scheunemann et al. used omics data sets collected from various cell types, tissues, root zones, and developmental stages to develop a multi-tissue model of Arabidopsis root metabolism that predicted the developmental stage-dependent flow of phytohormones across root tissues (Scheunemann et al., 2018).

More recently, the reconstruction of multi-tissue or whole plant models progressed from model plants like Arabidopsis, soybean, maize, tomato, barley, and rice to non-model and long-lived species such as green millet (Shaw and Cheung, 2019), cork oak (*Quercus suber)* (Cunha et al., 2023b), poplar (Simas Coutinho Barbosa et al., 2024) and grapevine *(Vitis vinifera*) (Sampaio et al., 2024). The development of multi-tissue or -organ models for commercially important species unlocks new avenues to systematically investigate genetic and metabolic modifications aimed at improving fruit taste or production of valuable bioproducts.

3.2. Temporally-resolved constraint-based models: capturing metabolic changes across temporal scales

Alongside models that account for the spatial organization of metabolism, time-resolved models that capture the dynamics of metabolic systems have been developed using a similar modelling paradigm. Temporal resolution is achieved by assigning copies of the initial base model to specific time intervals. Each interval operates in a pseudo-steady-state and metabolites known to accumulate in the plastid or vacuole, such as transient starch, amino acids, and carboxylic acids, can be transferred between consecutive intervals, thus enabling to capture time interval-specific flux modes. While exchanges in spatially-resolved systems are typically bidirectional, in temporally-resolved systems, they are unidirectional to account for the arrow of time.

Time-resolved models have been used to study metabolic distinctions across the diel cycle. Cheung et al. pioneered the first diel metabolic model of Arabidopsis by constructing light and dark phase models by permitting photon uptake only in the light phase. The resulting model captured interactions between light and dark metabolism in C_3 and CAM leaves (Cheung et al., 2014). Recent studies employing diel models have provided significant insights into CAM productivity and energetics (Shameer et al., 2018), characterized C_3-CAM evolutionary metabolic transitions (Tay et al., 2021), and explored water-saving potential (Töpfer et al., 2020). The study of Töpfer et al. identified vacuolar storage capacity as a main determinant of the extent of CAM and thus provided a link between leaf metabolism and anatomy. Additionally, investigations into CAM pH homeostasis have highlighted the important role of the mitochondrial phosphate carrier and cytosolic glyceraldehyde 3-phosphate dehydrogenase enzyme in supporting CAM functioning and proton homeostasis (Daems et al., 2024). These findings hold potential applications for engineering CAM into C_3 species–a promising strategy for developing climate-resilient plants.

Diel models have also advanced our understanding of guard cell metabolism, which undergoes fluctuations in osmolyte concentrations and water fluxes that regulate stomatal conductance. For instance, Tan and Cheung constructed a diel model of guard cell metabolism to systematically investigate the network-level metabolic contribution to the turnover of osmolytes across the diel cycle (Tan and Cheung, 2020). Sprent et al. used a time-resolved model of the integrated metabolism of guard and mesophyll cells and investigated metabolic fluxes in both blue and white light-mediated stomatal opening. Their model elucidated distinct roles of sugars and carboxylic acids as well as unexpected carbon fluxes in stomatal opening (Sprent et al., 2025).

Recently, Martins et al. developed a dedicated package for integrating diel cycles into plant metabolic models to meet automation needs (Martins et al., 2025). It shall be noted that selecting meaningful time-intervals in which the assumption of a pseudo-steady state is justified is crucial for these models to capture realistic metabolic behaviors. This can be evaluated by comparing modelling results obtained from different temporal resolutions (Töpfer et al., 2020). The definition of biologically meaningful storage metabolites can be guided by metabolomics data.

Furthermore, data-contextualized models can capture metabolic snapshots across environmental stresses and developmental stages. This approach has, for instance, revealed metabolic reprogramming during 13-day progressive drought in Arabidopsis (Siriwach et al., 2020) and varying water stress intensity in different intervals of the soybean grain filling stage (Vinson et al., 2025), and enhanced our understanding of drought adaptations and temporally distinct network-level stress responses. Furthermore, Colombié et al. generated 96 fruit- and developmental stage-specific models for eight fruit species from a generic model by integrating metabolomics and biomass data. This allowed them to comparatively investigate the species- and developmental stage-specific metabolic flux patterns (Colombié et al., 2023).

In conclusion, incorporating spatial and temporal resolutions enables modelling metabolism from two-cell systems to the whole-plant level as well as capturing the dynamics of the diel cycle, responses to environmental stimuli, and different developmental stages across the plant life cycle to more accurately reflect the complex nature of plant metabolism.

4. Metabolic modelling provides mechanistic insights into plant-microbe interactions

Plants engage in various mutualistic, pathogenic, and commensal interactions with their surrounding microorganisms and shape these interactions through metabolic exchanges. Integrated constraint-based metabolic models of plant-symbiont interactions have been developed for two important legume species, i.e., barrel clover (*Medicago truncatula*) and soybean, to investigate the effect of symbiotic nitrogen fixation on plant metabolism, energetics, growth and yield (diCenzo et al., 2020; Holland et al., 2023; Pfau et al., 2018). Furthermore, models of host-pathogen interactions have been developed through model coupling or model contextualization and have advanced our understanding of various aspects of pathogen infection, including pathogenic metabolic dependencies (Rodenburg et al., 2019), pathogen-mediated growth repression (Botero et al., 2018), the effect of host immunity and pathogen metabolic events on virulence (Peyraud et al., 2019; Tubergen et al., 2023), and more recently, the complex effect of environmental fluctuations on plant-microbe interactions (Lacrampe et al., 2024). Models of plant-microbe interactions are not limited to bipartite interactions. Advances in microbial genomics, automated model reconstruction tools and the availability of microbiota culture collections for Arabidopsis and Lotus (*Lotus japonicus*) (Bai et al., 2015; Wippel et al., 2021) have facilitated the application of constraint-based modelling to develop synthetic microbial communities (SynComs) for plant microbiome research. Studies employing community-level models have provided valuable insights into how nutrient availability and niche overlap influence metabolic dependencies and interactions within microbial communities (Mataigne et al., 2022; Schäfer et al., 2023), have enabled systematic investigations of rhizosphere bacteria hierarchical trophic interactions (Ginatt et al., 2024) and designing SynComs that promote crop yield (Gonçalves et al., 2023; Ruan et al., 2024). Applications and challenges of constraint-based metabolic modelling to study plant microbe-interactions have been recently reviewed by (Blonde et al., 2025; Feierabend and Töpfer, 2025).

With the accelerating development of multi-cell type and -tissue plant models, we anticipate also more sophisticated community models to emerge. These models will enable us to comprehensively investigate higher-order interactions as well as metabolic and compositional diversity across phyllospheric and rhizospheric microbiota.

5. Machine learning approaches enhance both network reconstruction and simulation accuracy

Progress in artificial intelligence (AI) has revolutionized computational biology. AI and particularly machine learning (ML) applications have advanced metabolic network reconstruction and flux simulations.

With respect to reconstruction, ML can address challenges such as incomplete genome annotations, missing enzyme commission (EC) numbers, absent transport reactions, unknown enzyme localisation and network gaps (Fig. 2). More specifically, deep learning, language models and hybrid ML-homology models can be used to improve genomic sequence annotation beyond standard homology methods. These techniques can address gene structure prediction, cross-species generalizability, *de novo* annotations in orphan genomes, and regulatory element mapping, thereby enabling high-throughput, accurate annotation in systems with limited experimental resources (Brůna et al., 2021; Stiehler et al., 2021; Gabriel et al., 2024). In addition, several ML tools automate the assignment of EC numbers based on genome and protein sequences. This creates common links across diverse databases and facilitates enzyme annotation (Dalkiran et al., 2018; Ryu et al. 2019, ; Buton et al., 2023; Shi et al., 2023). The required data for these approaches can be obtained through comprehensive, high-quality genomic datasets available for diverse plant species (Kress et al., 2022; Ahmad et al., 2025). Furthermore, ML algorithms assist in identifying and incorporating transport reactions across organelles and cell membranes from DNA sequences which are crucial for reconstructing high-quality compartmentalized models (Chen et al., 2023b; Cunha et al., 2023a; Elbourne et al., 2023; Kroll et al., 2024). Accurate prediction of enzyme localisation is equally important for precise metabolic flux predictions. Here, recent deep learning strategies circumvent expensive alignment or homology searches, making them suitable for high-throughput annotation of enzymes across eukaryotic organisms, including plants (Sperschneider et al., 2017; Stärk et al., 2021; Jiang et al., 2023; Ødum et al., 2024; Zhang et al., 2025). Additionally, ML approaches aid metabolic network reconstruction by predicting missing reactions, which is particularly important for incomplete genomes (Chen et al., 2023a; Boer et al., 2024). While current gap-filling methods predominantly target microbial models, the growing availability of annotated eukaryotic and particularly plant genomes enables us to adapt these methods for plants (Bernal-Gallardo and de Folter, 2024).

Advances in flux simulations through ML can be classified into two categories: ML for generating model inputs and ML for analyzing FBA outputs (Fig. 2) (Sahu et al., 2021; Sampaio et al., 2022). On the one

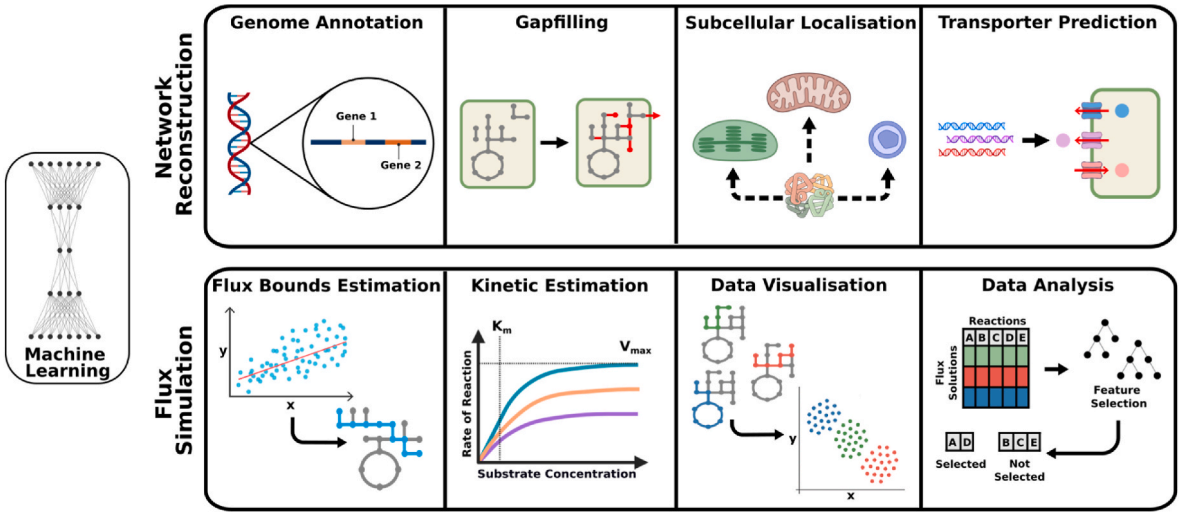

Fig. 2. —Machine learning methods for assisting constraint-based model reconstruction and flux simulation. Recent advances in ML guide plant constraint-based modelling in two ways: Top, network reconstruction; Bottom, applications guiding flux simulation and interpretation.

hand, ML models can be trained on omics or phenotypic data and then used to extract features that can constrain the model and improve its flux predictions. For example, Tong et al. trained a regression ML model on genome-wide SNP markers that predicts flux values, which were then used as constraints in FBA to predict growth rates for a specific Arabidopsis genotype (Tong et al., 2020). Furthermore, deep learning approaches like DLKcat (Li et al., 2022), TurNuP (Kroll et al., 2023), CatPred (Boorla and Maranas, 2025), and CataPro (Wang et al., 2025) can predict enzyme kinetic parameters such as Michaelis (K_M) and catalytic (k_{cat}) constants. More recent extensions of these tools, such as PreTKcat (Cai et al., 2025), DLTKcat (Qiu et al., 2024), MPEK (Wang et al., 2024), and UniKP (Yu et al., 2023a) even predict temperature- and/or pH-specific kinetic parameters. Incorporating ML-predicted k_{cat} values can benefit the construction of enzyme-constrained models. A recent study used enzyme structural data to adapt k_{cat} values and examined the relationship between the concentration and saturation of maize bottleneck enzymes under temperature stress (Chowdhury et al., 2023). Additionally, Wendering et al. integrated temperature-adjusted k_{cat} values, protein content and photosynthetic capacity to account for the effect of temperature on plant metabolism and identified thermal adaptive genetic and metabolic targets (Wendering et al., 2025). Current ML approaches can complement such studies by both estimating k_{cat} values of uncharacterized enzymes and providing temperature-specific kinetic estimates.

On the other hand, ML can be used to analyze flux predictions and effectively address the increasing complexity of metabolic modelling approaches. Especially, models that incorporate temporal and spatial aspects or studies which analyze metabolism across diverse environmental and genetic conditions generate large fluxomics datasets, which create challenges for data interpretation and visualization. For example, Sampaio et al. used a diel multi-tissue grapevine model to analyze metabolic flux differences and to identify ripening biomarkers between green and mature grape developmental stages. They visualized their flux data through dimensionality reduction (t-SNE) and used ML models to identify key reactions distinguishing between developmental stages (Sampaio et al., 2024).

In conclusion, ML approaches can significantly advance plant metabolic modelling by improving metabolic reconstructions and model interpretability, thus facilitating more complex modelling approaches that deepen our understanding of plant metabolism.

6. Hybrid and multi-scale modelling extends the biological scope of constraint-based metabolic models

Integrating constraint-based models with other mechanistic modelling approaches enables us to overcome some of the limitations of standalone constraint-based modelling as well as to expand their biological scope. These integrated, hybrid or multi-scale frameworks can capture nonlinear metabolic processes (e.g., the relationship between substrate concentrations and enzyme activity), and allow us to consider plant physiology at multiple scales by incorporating plant anatomical and architectural traits, and to better simulate metabolic phenotypes under field conditions.

Hybrid kinetic-constraint-based models can be constructed by incorporating the kinetic model outputs as constraints to the constraint-based model and vice versa. These models have helped capturing source-sink dynamics (Grafahrend-Belau et al., 2013), progressive metabolic shifts across leaf developmental gradients (Bogart and Myers, 2016), cellular resource allocation in Arabidopsis (Goelzer et al., 2024), dynamic metabolic response to varying resource availability (Shaw and Cheung, 2018; Shameer et al., 2022), and simulating hour-wise metabolic transitions across Arabidopsis's life cycle (Schroeder and Saha, 2020a).

A key limitation of using kinetic parameters to estimate reaction rates is that they are typically measured under controlled conditions, which may not reflect the actual plant physiological conditions. To address this limitation, Küken et al. combined condition-specific protein abundance data and fluxes predicted by a contextualized model of Arabidopsis to estimate the condition-specific maximal enzyme catalytic rates (Küken et al., 2020). However, the broader applicability of this method to larger networks or non-model species depends on the proteomics data availability.

Functional-structural models are computational frameworks that integrate plant physiological processes (functional aspects) with three-dimensional plant architecture and morphology (structural aspects) to simulate how form and function interact in plant growth and development (Vos et al., 2010). Hunt et al. recently demonstrated how these two complementary modelling approaches–functional structural plant models and constraint-based models–can be integrated. The authors deployed a root functional-structural model to constrain a multi-cell model of maize root metabolism, with each cell-specific metabolic model coupled in a way that reflected their anatomical order within the root cross-section. Using this integrative approach, the ring-specific difference in cell size was translated into distinct cell-specific metabolic and energetic demands. The model captured intercellular metabolic cross-talks and revealed the role of root fermentation processes under aerobic conditions (Hunt et al., 2023b).

Finally, by integrating constraint-based and crop growth models, we can extend the scale of metabolic modelling to the field level. Crop growth models can simulate growth and development based on environmental conditions, genetic traits and management practices across various temporal stages (Benes et al., 2020). Shaw and Cheung used this approach and constrained a multi-organ rice metabolic model with growth rates derived from a crop growth model to characterize drought-induced metabolic reprogramming across vegetative and reproductive developmental stages on a daily interval. Moreover, the model effectively captured the metabolic processes underlying early senescence induction under drought stress conditions (Shaw and Cheung, 2021). Holland et al. used predictions from a nodulated soybean whole-plant model as input for a crop growth model to evaluate how symbiotic nitrogen fixation affects plant metabolism and yield during the growing season (Holland et al., 2023). Worth mentioning here is a study that coupled an ocean biogeochemical model with algal metabolic models and investigated how phytoplankton metabolism acclimates to different ocean environments (Régimbeau et al., 2025). We anticipate such multi-scale approaches to also emerge for plant systems.

7. Discussion

In this review, we highlight how constraint-based modelling approaches have facilitated genome-scale exploration of metabolic networks. Recent advances include expanding metabolic modelling to diverse species and specialized metabolism, integrating multi-omics data for context-specific models, developing multi-cell, -tissue, and whole-plant models as well as models with temporal resolution at different scales, and investigations of plant-microbe interactions. ML has been successfully incorporated with constraint-based modelling for improved network reconstruction and flux simulations. Hybrid frameworks integrating kinetic, functional-structural, and crop growth models extend the biological scope and predictive capability of plant metabolic models. These developments enable us to address increasingly complex questions about plant metabolism under various environmental and genetic conditions.

7.1. Increasing species diversity in plant metabolic modelling

Reconstructions of diverse species will illuminate metabolic diversity and enable us to study evolutionary and ecological adaptations. More specifically, these models hold the potential to reveal stress adaptations under extreme environmental conditions and to elucidate enzyme functionalities absent in crops and model plants. For example, weedy species of crop plants, such as weedy rice (*Oryza sativa f. spontanea*)

display greater tolerance to both abiotic and biotic stresses, and reconstructing the metabolic networks of these species could help us understanding their tolerance mechanisms (Fogliatto et al., 2020). Moreover, these new models can enhance our understanding of how metabolic networks have diversified across plant lineages and elucidate the roles of secondary metabolism in plant-insect and plant-microbe interactions.

7.2. Scaling plant constraint-based modelling to the pan-genome level

Several pan-genomes for photosynthetic species are now available and provide the foundation for reconstructing pan-genome-scale metabolic models, which are already well-established in the microbial community (Fang et al., 2018; Lu et al., 2019; Blázquez et al., 2023; Mirhakkak et al., 2023). These models will bridge the divide between genetic variation and metabolic flux and present opportunities to capture metabolic differences between plant accessions, including SNPs and structural variations (Arend et al., 2025). This will enable screens of existing germplasm collections for metabolic traits of agronomic importance and elucidate the connections between genetic and metabolic variation. Furthermore, these models could be equipped with ML-derived accession-specific kinetic parameters to further enhance their predictive capabilities.

7.3. Genome-scale kinetic models

Hybrid kinetic-constraint-based metabolic models have improved our understanding of plant metabolic responses (Shaw and Cheung, 2018; Schroeder and Saha, 2020a; Shameer et al., 2022; Goelzer et al., 2024). Available repositories for kinetic parameters, together with novel ML-based parameter estimation pipelines facilitates the development of kinetic and kinetic-constraint-based metabolic models at a large to genome-scale (Wittig et al., 2018; The UniProt Consortium, 2019; Chang et al., 2021; Domenzain et al., 2022; Haddadi et al., 2022). Genome-scale kinetic models are not constrained to steady-state flux solutions and enable time-resolved simulations of perturbations, feedback loops and cross-pathway regulation. However, the accuracy and predictability of such models is challenged by the need to evaluate these time-resolved flux predictions. Time-dependent isotope labeling data could provide some degree of validation (Cheah and Young, 2018; Koley et al., 2024). Advances in genome-scale kinetic models have been recently reviewed by (Toumpe et al., 2025).

7.4. Current challenges in plant metabolic network reconstruction and modelling

Plant specialized metabolism contains numerous unresolved pathways, which necessitates extensive gap-filling. Moreover, especially enzymes involved in specialized metabolism can exhibit promiscuity, which makes metabolic network reconstruction difficult. Novel rule-based methods improve annotation of enzyme promiscuity and can enable the prediction of new reactions and pathways (Duigou et al., 2019; Koch et al., 2020; Balzerani et al., 2024). Moreover, while ML methods address challenges with respect to genome annotation, network gap-filling and prediction of transporters and subcellular enzyme locations, they require a broad range of high-quality plant-specific annotated datasets to achieve good accuracy and generalization. This data can be difficult to obtain across genomically complex and highly compartmentalized plant cells, particularly for pathways of specialized metabolism (Dönnes and Höglund, 2004; Mudge and Harrow, 2016). Additionally, current automated reconstruction pipelines for plant metabolic models, such as PlantSEED, are predominantly limited to primary metabolism (Seaver et al., 2014, 2018). In contrast, sophisticated tools exist for microbial reconstructions (Henry et al., 2010; Aite et al., 2018; Arkin et al., 2018; Machado et al., 2018) and we expect to see analogous tool and pipeline developments for the plant community. Towards this goal, Python-based CobraMod (Camborda et al., 2022) and optimization methods such as OptFill (Schroeder and Saha, 2020b) and BioISO (Cruz et al., 2024) expedite gap-filling refinement, while databases such as SUBA4 (Hooper et al., 2017) thus facilitate comprehensive retrieval of protein subcellular localisation data. Furthermore, efficiently solving large compartmentalized kinetic or hybrid kinetic-constraint based models requires algorithms that make full usage of available high-performance computing (Arabzadeh et al., 2020).

While approaches that include kinetic information hold great promise to better capture species-, accession- and environment-specific metabolism, their large-scale application critically depends on the availability of proteomics and other omics datasets to meaningfully constrain these models. In reality these input datasets are often obtained through combining and integrating heterogeneous data, which often come from different experimental setups and measurement times. We expect to see the emergence of ML tools that integrate these datasets to generate sensible model parameters (Choudhury et al., 2022, 2024). Moreover, temporally resolved models generate large amounts of flux data which can be difficult to analyze. Here again, ML approaches can aid this process by extracting meaningful biological insights from numerous flux solutions (Sampaio et al., 2024).

Lastly, the mechanistic integration of environmental constraints with models of plant metabolism remains challenging. Previous approaches have integrated environmental data with leaf metabolic models through the integration with biophysical (Töpfer et al., 2020) and biochemical (Wendering et al., 2025) models. Further advances in measurements of gas diffusion and intra- and intercellular gas concentrations and temperatures, combined with advanced biophysical modelling and integration strategies, could provide important mechanistic insights into leaf functioning.

7.5. FAIR data and good practices

Additionally, metabolic modelling requires rigorous data management practices to ensure model accessibility and reproducibility. A recent investigation revealed that approximately half of a subset of models from the BioModels database were not directly reproducible (Tiwari et al., 2021). To address these issues, models must adhere to community standard formats, such as SBML (Keating et al., 2020). Tools such as MEMOTE enable quality control of SBML format reconstructed networks by generating reports based on the quality, consistency, and reproducibility of the input model (Lieven et al., 2020). Furthermore, metadata annotation, containerized workflows using Docker and version control systems such as Git need to become standard (Krantz et al., 2021). Both the model, metadata, and code pipelines ought to adhere to FAIR (Findable, Accessible, Interoperable, and Reusable) principles. Platforms such as DataPLANT enable scalable and centralized implementation of Research Data Management (RDM) for scientific projects in accordance with FAIR principles for plant scientists (Weil et al., 2023; Zhou et al., 2023).

8. Conclusion

Metabolic modelling of plant systems has made remarkable progress in recent years. The plant systems biology community now has access to a range of constraint-based, hybrid, multi-scale, and pan-genome metabolic modelling frameworks. Rapid developments in AI and ML approaches for metabolic reconstruction and flux analysis, combined with open and FAIR workflows, will improve our understanding of metabolic diversity at the inter- and intraspecies levels, enabling us to explore the drivers of metabolic plasticity and metabolic evolutionary and ecological adaptations. Furthermore, it will allow us to investigate traits associated with acclimation and defence, chemical composition, nutritional value and pharmaceutical applications, accelerating the identification of metabolic traits for metabolic engineering and crop improvement.

CRediT authorship contribution statement

Tiago M. Machado: Writing – review & editing, Writing – original draft, Visualization, Conceptualization. **Nadine Töpfer:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization. **Fatemeh Soltani:** Writing – review & editing, Writing – original draft, Visualization, Supervision, Conceptualization.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

No data was used for the research described in the article.

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