Tansley insight

From synthetic communities to synthetic ecosystems: exploring causalities in plant–microbe–environment interactions

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Contents

Summary

The plant microbiota research field has rapidly shifted from efforts aimed at gaining a descriptive understanding of microbiota composition to a focus on acquiring mechanistic insights into microbiota functions and assembly rules. This evolution was driven by our ability to establish comprehensive collections of plant-associated microbes and to reconstruct meaningful microbial synthetic communities (SynComs). We argue that this powerful deconstruction–reconstruction strategy can be used to reconstitute increasingly complex synthetic ecosystems (SynEcos) and mechanistically understand high-level biological organization. The transitioning from simple to more advanced, fully tractable and programmable gnotobiotic SynEcos is ongoing and aims at rationally simplifying natural ecosystems by engineering them. Such reconstitution ecology approaches represent an untapped strategy for bridging the gap between ecology and functional biology and for unraveling plant–microbiota–environment mechanisms that modulate ecosystem health, assembly, and functioning.

I. Introduction

Roots and leaves of healthy plants are colonized by a staggering diversity of microbes that evolved in different kingdoms of life (Bai *et al.*, 2015; Berg & Koskella, 2018; Durán *et al.*, 2018; Hassani *et al.*, 2018) and are primarily acquired horizontally from the surrounding environment (Vorholt, 2012; Bulgarelli *et al.*, 2013;

Simonin *et al.*, 2022). A substantial fraction of these microbes is reproducibly detected in plant tissues across time and space or show high heritability, suggesting that plants and (at least some of) its microbiota members have engaged in stable associations (Hacquard *et al.*, 2015; Yeoh *et al.*, 2017; Thiergart *et al.*, 2020; Chesneau *et al.*, 2022; Durán *et al.*, 2022b; He *et al.*, 2024). The microbiota provides key functions to the host that promote

pathogen resistance, nutrient acquisition, or abiotic stress tolerance (Vandenkoornhuyse *et al.*, 2015; Fitzpatrick *et al.*, 2020; Trivedi *et al.*, 2020). Obtaining a deeper mechanistic understanding of microbiota assembly rules and beneficial functions is currently an active research line in the plant microbiota research field. A major breakthrough was that a substantial fraction of these plant-associated microbes is culturable (i.e. > 50% at ASV level-resolution (Bai *et al.*, 2015; Armanhi *et al.*, 2018; Wippel *et al.*, 2021; Zhang *et al.*, 2021)), opening the way to assemble and reconstitute synthetic microbial communities (SynComs) that are representative of naturally occurring plant microbiomes (Durán *et al.*, 2018; Zhou *et al.*, 2022).

SynCom reductionist experiments in simple gnotobiotic systems have been critical for establishing causality (Vorholt *et al.*, 2017) and particularly for validating the importance of keystone species, priority effects, and organ-to-organ microbial transmission during plant microbiota establishment (Bai *et al.*, 2015; Agler *et al.*, 2016; Carlström *et al.*, 2019; Arnault *et al.*, 2024). They have also been used to validate the relevance of microbial primary and specialized metabolites for shaping microbiota composition (Helfrich *et al.*, 2018; Getzke *et al.*, 2023, 2024; Schäfer *et al.*, 2023), to identify microbial strains/genes/molecules that modulate host development (Garrido-Oter *et al.*, 2018; Finkel *et al.*, 2020; Gonin *et al.*, 2023), immune responses (Ma *et al.*, 2021; Teixeira *et al.*, 2021), nutrient acquisition (Harbort *et al.*, 2020; Zhang *et al.*, 2022), resistance to biotic and abiotic stresses (Castrillo *et al.*, 2017; Durán *et al.*, 2018; Vogel *et al.*, 2021; Wolinska *et al.*, 2021; Hou *et al.*, 2021a; Emmenegger *et al.*, 2023), or host colonization (Vannier *et al.*, 2023). This strategy was also instrumental for characterizing host genes that prevent microbial dysbiosis (Chen *et al.*, 2020; Pfeilmeier *et al.*, 2021; Entila *et al.*, 2024; Su *et al.*, 2024) or selectively shape composition and function of plant-associated microbes (Zhang *et al.*, 2019; Loo *et al.*, 2024).

Nowadays, it is becoming realistic to apply this powerful reconstruction approach at ecosystem-level resolution to better understand the reciprocal interplay between the host and the microbiota with its environment as well as the cascading consequences on ecosystem health and functioning. This evolution is also critical to place microbial assembly processes and beneficial functions in an eco-evolutionary framework (Bergelson *et al.*, 2021). Here, we discuss current approaches and future roadmaps for the reconstruction of plant SynEcos, define SynEco's prospects, advantages, and constraints, and provide a conceptual framework to leverage the power of reconstitution ecology to address ecological questions.

II. SynEco as a tool for reconstitution ecology

SynEcos are artificially designed and constructed ecological systems that aim at replicating the functions of natural ecosystems in a controlled environment (Fig. 1). There are already multiple existing SynEcos; however, they vary significantly in terms of their complexity. For instance, a Petri dish with a defined growth medium, a plant, and a bacterial strain incubated in a growth chamber represents the simplest plant SynEco. By contrast,

a fully tractable soil-based plant system inoculated with a multi-kingdom synthetic community in a fully programmable gnotobiotic growth chamber represents evolution toward more advanced and complex SynEcos, referred to here as next-generation SynEcos. In our view, a SynEco ought to combine (1) an environmental/climatic sphere, (2) a nutritional/chemical sphere, and (3) an organismal sphere that allows for inter- and intraspecific interactions. The core of next-generation SynEco lies in the concept we term here 'reconstitution ecology', which seeks to recapitulate ecosystem complexity while following strict reductionist principles. To achieve this, next-generation SynEcos must focus on the rational design of ecosystems, balancing ecological complexity with system control (Tecon *et al.*, 2019) in order to bridge the gap between laboratory observations and natural ecosystem processes (Fig. 1).

We anticipate that next-generation SynEcos and associated reconstitution ecology principles will represent an important research line in the near future to recapitulate meaningful ecological events outside their natural contexts, to mechanistically understand ecosystem assembly, and to make use of reductionist approaches for addressing key ecological questions. With reconstitution ecology, we seek to define a potentially novel and exciting research strategy for researchers that aim at linking functional biology and ecology and to subject ecological hypotheses to experimental testing with a high turnover. Next-generation SynEcos will be key to bridging holistic-reductionist divides in ecology-based research fields (Tecon *et al.*, 2019) and might serve as potential missing links to cross the divide between controllability and complexity (Fig. 1).

III. From SynComs to SynEcos

Similar to SynCom reductionist strategies (Vorholt *et al.*, 2017), SynEco reconstitution encompasses the disassembly and rational re-assembly of diverse ecosystem components consisting of both biotic (plant, micro-/macrobiomes) and abiotic (climatic and edaphic factors) variables (Fig. 1). Reconstitution ecology using next-generation SynEcos will rely on deep descriptive knowledge of soil physical and chemical composition, organismal diversity and composition in various plant organs, and temporal dynamics of climatic conditions of a given ecosystem. This descriptive, temporal understanding of ecosystem behavior is a key prerequisite for informing the design and reconstruction of simpler yet representative and meaningful synthetic versions that are tractable and gnotobiotic (Fig. 1). Building next-generation SynEcos requires resources such as microbial culture collections and genomic information for the organisms involved, as well as engineering technologies to mimic ecosystem parameters, monitor them, and assess phenotypic traits.

SynCom reconstruction experiments with germ-free plants have been extensively reported in relatively simple SynEcos (Agar systems, perlite, sand, and FlowPots) (Gao *et al.*, 2018; Kremer *et al.*, 2021; Ma *et al.*, 2022) and in some cases include abiotic stresses (light, salt, drought, and nutrient) that are used as environmental perturbations (Finkel *et al.*, 2019; Hou *et al.*, 2021a; Getzke *et al.*, 2024; Novak *et al.*, 2024). Recently, the development of a multitude of more advanced plant SynEcos that aimed at better integrating plant–microbiota interactions in a
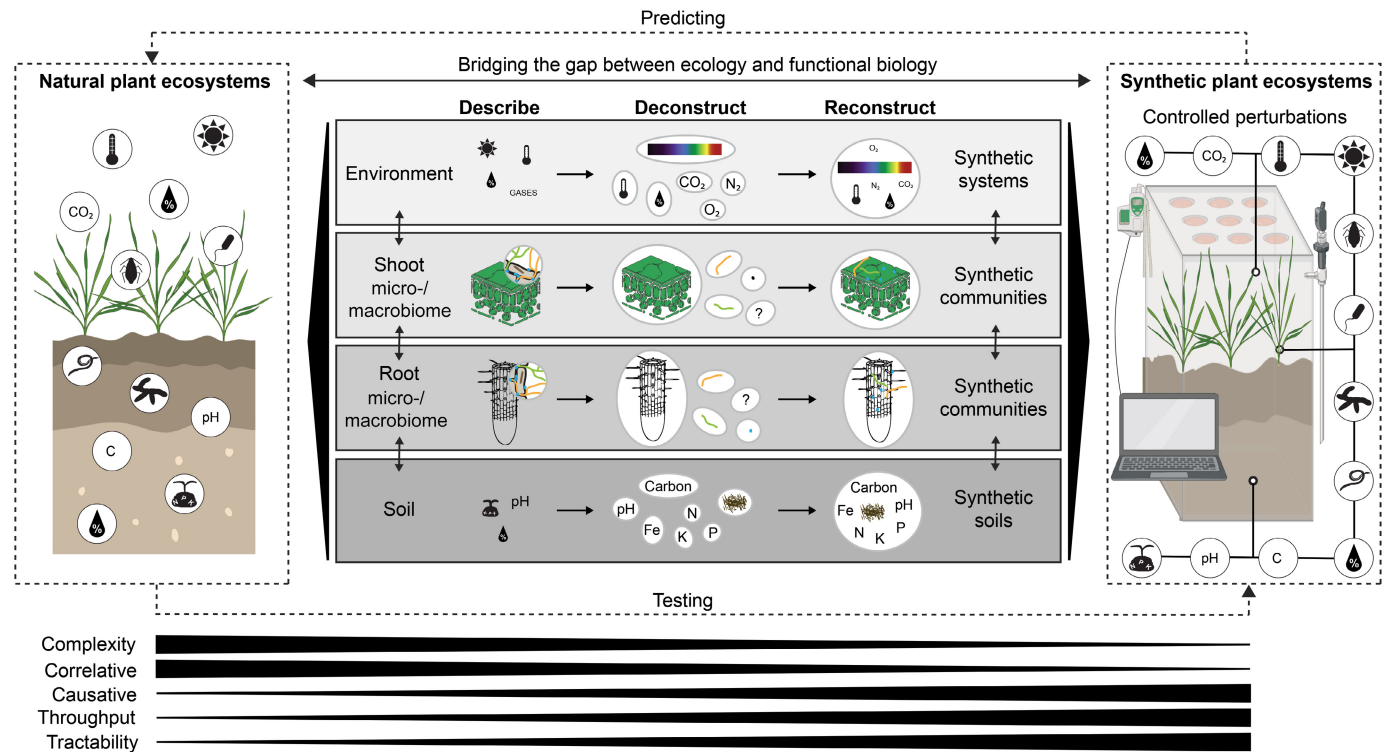
Fig. 1 Synthetic plant ecosystems to bridge the gap between ecology and functional biology. Framework illustrating the transition from natural plant ecosystems to synthetic plant systems (SynEco). This encompasses description, deconstruction, and reconstruction of climatic conditions, organismal diversity in plant shoot and roots, and soil physical and chemical properties of a given natural ecosystem. Recapitulating natural ecosystem conditions with next-generation SynEcos will allow causal understanding of ecosystem processes and will inform prediction of natural ecosystem behavior. C, carbon; Fe; iron; K, potassium; N, nitrogen; P, phosphorus.

precise environmental context have emerged. This includes the development of (1) gnotobiotic systems that monitor the metabolic interplay between plant, microbes, and their environment (EcoFabs) (Zengler *et al.*, 2019; Finkbeiner *et al.*, 2021; Novak *et al.*, 2024), (2) climatic chambers mimicking seasonal changes in temperature, day length, and light intensity of a given site (Durán *et al.*, 2022a), (3) low-cost, programmable, and miniaturized growth chambers that can continuously measure ecosystem functions at the soil–plant–microbiome-atmosphere continuum (Padmanabha & Streif, 2019; Salvatori *et al.*, 2021), and (4) sophisticated and more expensive ecotron-like chambers (iDiv ecotron, EcoPOD) (Schmidt *et al.*, 2021; Yee *et al.*, 2021). Today, we are in a transitory phase in which various SynEco approaches emerge and provide valuable insight into ecosystem functioning. As we continue to explore these innovative methods, reducing the cost of these advanced systems will be key to elevating the relevance of next-generation SynEcos and revolutionizing the way we can modulate, reconstitute, and understand high-level biological organization with unprecedented speed.

IV. The value of SynEcos

SynEcos do not only enable research at various ecological scales ranging from chemical processes to complex multikingdom communities but also allow controlled manipulation of environmental variables (Fig. 2). Nowadays, holistic ecosystem-level research is primarily conducted in natural environments. Despite important breakthroughs, this remains a challenging task due to the inherent stochasticity of ecosystems behavior and the difficulty to precisely control their biotic and abiotic components.

In SynEcos, targeted experiments can be conducted in the absence of confounding factors, which enables precise assessment of causal relationships and associated mechanisms. SynEcos enhance reproducibility by maintaining consistent environmental settings over time. Furthermore, it also facilitates experimentation turnover by allowing continuous studies throughout the year and/or by running multiple SynEco setups in parallel, each addressing a different ecological question. An inherent feature of a SynEco is the ability to monitor ecosystem variables in real time and, therefore, provides researchers with invaluable insights into dynamic ecosystem processes. This can be facilitated by the implementation of advanced sensors (gas, temperature, humidity, …) as well as cameras and tools that enable nondestructive and continuous observation of ecosystem traits, which leads to a comprehensive understanding of plant–microbe-environment dynamics over time (Buckley *et al.*, 2020; Ang & Lew, 2022; Chen *et al.*, 2023; Kolhar & Jagtap, 2023).

In next-generation SynEcos, various perturbations will have to be applied either individually or in combination in order to explore a wide range of research questions (Fig. 2a). Similar to SynComs, which allow for modularity within microbial composition and abundance, SynEco reconstitution extends this flexibility to key
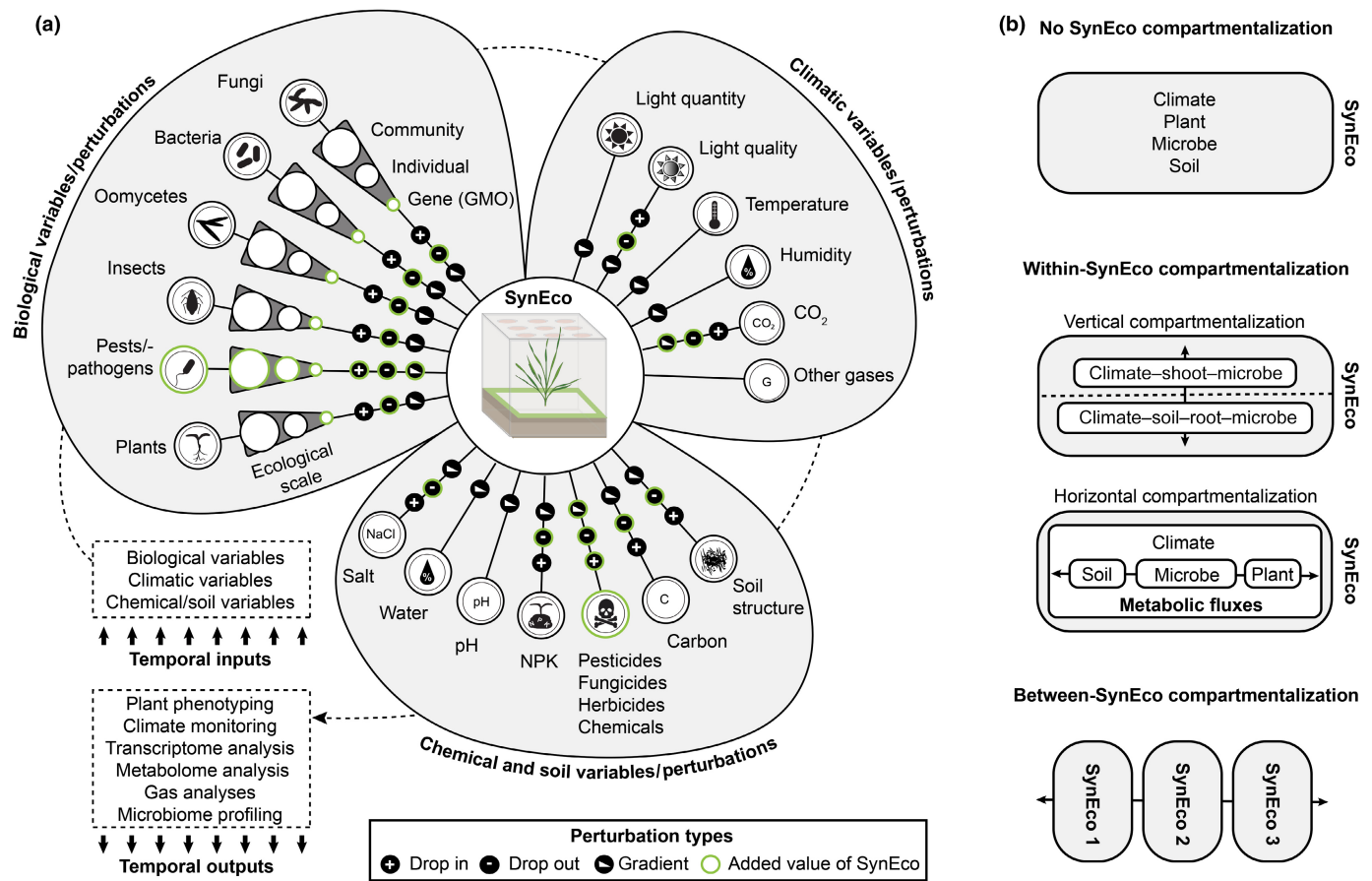
Fig. 2 Next-generation synthetic ecosystems (SynEcos) as modular and controllable platforms to study causality in plant–microbe-environment interactions. (a) Representation of possible variable grouped into biological, chemical, and climatic categories that can be reconstituted in SynEco. These variables/perturbations can be applied (+, drop in), removed (−, drop out) or gradually adjusted (>, gradient), either individually or together. Objects highlighted in green represent perturbations difficult to apply in natural ecosystems (added value of SynEco). Several environmental and biological outputs can be monitored to assess ecosystem characteristics and dynamics. (b) Modular compartmentalization within and between SynEco(s), opening new avenues to study ecosystem functioning. C, carbon; K, potassium; N, nitrogen; P, phosphorus.

edaphic and climatic factors (e.g. CO_2, light, temperature, water, pH, and nutrients) and chemical conditions (e.g. amendments, pesticides, and fertilizers), which gives researchers full control over ecosystem heterogeneity. Importantly, SynEcos permit the use of treatments whose application is typically restricted in natural environments (Fig. 2a). These include, for example, chemicals such as heavy metals, fungicides, pesticides, and hormones. SynEcos benefit from a lack of limitations on concentration or frequency of application and no accompanying negative environmental implications. This also includes invasion experiments with pests, pathogens, or exotic plants. Another distinct advantage lies in the utilization of genetic perturbations such as the use of genetically modified organisms. Indeed, SynEcos afford researchers the freedom to introduce engineered organisms (including plants, microbes, and insects) customized to specific research objectives and to test the broad effect of mutations at ecosystem-level resolution (Barbour *et al.*, 2022). This capability opens up avenues for generating mechanistic understanding of natural ecosystems and the exploration of novel interactions within them that are, otherwise, difficult to uncover. With SynEco, it is also possible to

remove or deplete specific biotic variables (dropout experiments) that can range from entire communities to individuals and genes as well as abiotic variables encompassing, for instance, specific soil nutrients, water content, or light spectra (Fig. 2).

SynEcos allows researchers to generate large amounts of data that can be used in predictive models to forecast plant behavior under various conditions or to predict ecosystem response to perturbations or global change stressors. These data allow for preventive management of agricultural crops. Reductionist synthetic ecosystems will, however, never fully recapitulate the complexity of natural environments and, therefore, confirming observations in natural settings remains imperative to ensure the robustness and broader applicability of research findings derived from SynEcos.

V. Unlocking new ecological questions using next-generation SynEcos

Next-generation SynEcos and reconstitution ecology offer a range of opportunities to test research hypotheses and ecological concepts that remain difficult to tackle in natural settings.

Assessing the impact of global change on plant ecosystem behavior is a topic of great interest. Although the effects of different climate conditions such as elevated atmospheric CO_2 or temperatures on plants or on soil microbial communities have been extensively studied in natural ecosystems (Ben Keane *et al.*, 2023), it remains difficult to resolve complex feedback loops that govern abiotic–biotic connections along the climate–plant–microbe–soil axis. Indeed, plant microbiota composition and activity likely heavily influence plant processes such as respiration and photosynthesis and, in turn, photosynthesis perturbations caused by environmental change will heavily impact soil microbial communities via changes in root exudates. SynEcos provide opportunities to study these complex feedback loops connecting host C-fixation/sequestration with plant microbiota functions and soil respiration. Considering the plant, the microbiota, and the climate as a dynamic signaling network, in which all constituent parts can be individually manipulated will bring invaluable knowledge informing climate models and projections.

In relation to the abovementioned climate–plant–microbe–soil axis, it is also important to consider plant roots and shoots as separated yet interconnected organs that integrate different signals belowground and aboveground, respectively. These organs interact with their own respective microbial community and environment and extensively influence each other through long-distance-signaling pathways within the plant. SynEco approaches that focus on proper separation of root and shoot systems represent a unique opportunity to better understand root–shoot–environment connectivity and, particularly, how perception of biotic and abiotic cues by roots and shoot translates into long-distance transfer of information that shape structure, function, and evolution of plant–microbe interactions (Hou *et al.*, 2021b). Through step-by-step reconstitution and manipulation of microbial community members and environmental parameters it will become possible to investigate the intricate link connecting root-microbe-soil and shoot-microbe-climate modules (Fig. 2b). This will be important to understand how they influence each other and how they evolved in response to each other.

Because SynEco-based reconstitution ecology will encompass strategies to compartmentalize ecosystems (i.e. by physically separating biotic components whilst allowing metabolic exchanges between them), it will open novel opportunities to study metabolic fluxes between organisms and communities that are connected (Fig. 2b). Understanding metabolic fluxes between constituent parts of an ecosystem is key to understanding the reciprocal interplay between host and microbial metabolites and the influence of the environment in driving high-level biological organization through production of key exometabolites. An exciting possibility of compartmentalized SynEcos is, for example, the prospect of performing experimental evolution experiments of plant-associated microbes in response to host exudates or upon metabolic perturbations.

Reconstitution ecology also opens the exciting opportunity to connect multiple SynEcos in series to understand ecosystem–ecosystem interplays and dynamics (Fig. 2b). Findings here could inform the design of more efficient systems that are built based on circular economy principles in order to minimize waste and maximize production between ecosystems. This particularly applies not only to the one health concept, food webs, but also to future food production systems that will be designed based on closed energy and efficient material flow principles.

Finally, the simplification of ecosystems will make it possible to identify a minimal set of components that are necessary and sufficient to modulate ecosystem functioning and to define whether emergent property and simplicity drive higher order biological assembly or core properties of certain ecosystems. Similarly, it can lead to identification of key perturbations and conditions driving ecosystem dysbiosis. This can guide future experimentation and standardization of experimental settings across institutions and disciplines.

VI. Conclusions

Although development of next-generation SynEcos will remain a challenging task, a better approximation of soil–plant–microbe-climate interactions is urgently needed to gain mechanistic and predictive insights into how complex ecosystems react and adjust in response to perturbations. Reconstitution ecology will be instrumental for the establishment of causal relationships to observed phenomena by providing a means to functionally validate mechanisms. Tractable ecosystem parameters together with gnotobiotic system compartmentalization will help to elucidate the roles of both individual microbial community members and SynComs together with plant growth conditions and an array of environmental conditions that shape these communities and interactions. SynEco approaches can be extended to many other research fields and disciplines. We predict that model next-generation SynEco systems could be shared across laboratories, similar to model strains or plants. We envision that ecological data will be standardized, recorded, and deposited in public repositories alongside phenotypes and sequencing data to increase reproducibility of experiments and to harness the power of the microbiota under ever changing environmental conditions.

Competing interests

None declared.

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