Challenging the term symbiosis in plant-microbe associations to create an understanding across sciences

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Abstract: Scientific progress relies on clear and consistent definitions for effective communication and collaboration. The term "symbiosis" in the context of plant-microbe associations suffers from diverse interpretations, leading to ambiguity in classification of these associations. This review elaborates on the issue, proposing an inclusive definition as well as a keyword.

DOI: https://doi.org/10.1111/jipb.13588

JIPB Journal of Integrative Plant Biology

Commentary
https://doi.org/10.1111/jipb.13588

Challenging the term symbiosis in plant–microbe associations to create an understanding across sciences[FA]

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THE IMPORTANCE OF WELL DEFINED TERMINOLOGY

Effective communication across fields and disciplines relies on well defined terminology. Standardized use of accurate definitions has been advocated by scientists across various disciplines (PySek, 1995; Rabin and Brownson, 2017; Tipton et al., 2019; Dubrovsky, 2022; Rillig, 2023). Divergent interpretations or the application of differing terminology can negatively impact interdisciplinary exchange, leading to missed information, hampered methodology transfer, and ultimately impeding progress (Sapp, 2010; Tipton et al., 2019). An impressive example of the use of multiple terms with the same interpretation is the definition of non-native or invasive plants encompassing at least 14 different terms (PySek, 1995). An abundance of definitions for the same term can be equally confusing and is thoroughly discussed in a recent review on plant root terminology. This study highlights that new interpretations of established terms may result in misunderstandings (Dubrovsky, 2022). The issue of ambiguous terminology is further exacerbated by diverging perspectives among scientists that result in the prevalence of a narrow and biased definition. This can lead to the underreporting or omission of relevant information, especially if the subject lies just beyond the restricted definition. A recent post by ecology professor Matthis Rilling criticized the restrictive use of the term "common mycorrhizal network" (Rillig, 2023). This example shows how specific cases can dominate a field and subsequently restrict the definition of a term. A similar issue can be observed in studies on beneficial diazotrophic bacteria, the *Rhizobia*. Here, the dogma that *Rhizobia* only colonizes plants in nodules prevails, despite the documentation of free-living species and non-nodule endophytic *Rhizobia* that are beneficial to their plant hosts (Khan et al., 2012). The examples above highlight that words can carry hidden biases that hinder research progress.

Furthermore, inconsistent definitions and diverse terms hamper the translation of scientific knowledge into practical use and decision-making (Rabin and Brownson, 2017), particularly when technical terms with differing meanings are commonly used in everyday language. Hence, the application of precise and well defined terms is crucial for mutual understanding across science, policy, and the general public. Although the standardization of terminology is crucial, it is important to acknowledge that terminology is not static, and definitions should be allowed to evolve over time. To avoid confusion, new interpretations or terms should ideally be reported. In reality, changes in definitions often occur subtly over time and are thus rarely published. Therefore, impulses for discussions such as this viewpoint are crucial to avoid inconsistent terminology.

A prime example of an ever-evolving definition in dire need of revision is the term "symbiosis" in plant–microbe associations. The request for a universal understanding of the term symbiosis may currently appear as a niche concern. However, while plant-symbiosis studies have been focused on a few prominent examples, microbiome studies and the general progress of technology have generated increased interest in this topic which requires better interdisciplinary collaboration (Khatabi et al., 2019). This has led to a notable increase of studies resulting in a dual challenge: On the one hand, the risk of further complicating the already diverse landscape of definitions; on the other hand, the opportunity to leverage molecular mechanisms underlying plant–microbe symbiosis to establish clearer categorizations of these interactions. For this reason, we aim to review the general history and complexities of the concept of symbiosis. Ultimately, we propose a unifying framework to prevent confusion and miscommunication, improving collaboration and scientific progress in this expanding field.

THE OBSTACLES ACCOMPANYING THE UMBRELLA-TERM SYMBIOSIS

Generally, symbiosis or symbiotic is used to describe mutually advantageous relationships between individuals or entities. In plant sciences, the definition of symbiosis can become more restricted based on various criteria and has been an ongoing problem since the word was coined. When screening the present literature on plant–microbe interactions, various definitions of the term symbiosis can be encountered (Martin and Schwab, 2012). Therefore, we will briefly outline the history of the term symbiosis. The term symbiosis was first introduced to the botanical society by lichenologists in the 19th century when in 1877 Albert Bernhard Frank proposed using the term *symbiotismus* to describe two species living on or in one another, regardless of their interaction (Frank, 1877). Two years later, in 1879, Heinrich Anton de Bary defined symbiosis as "the living together of unlike organisms" and further described it as a close and long-term biological interaction between two different organisms (de Bary, 1879). Since then, the definition has evolved and diverged alongside the increasing complexity of natural sciences. An analysis of general biology and ecology textbooks in the USA revealed that the term symbiosis is predominantly employed when referring to mutualistic, commensal, or parasitic associations (Martin and Schwab, 2012). Notably, general biology textbooks in the USA align with de Bary's original definition of symbiosis, whereas only 40% of ecology textbooks do so (Martin and Schwab, 2012). In contrast, a recent review states that in the field of ecology the term symbiosis largely kept its original meaning ("interactions among species") regardless of the duration of the interaction, while being used discordantly in microbiome studies (Tipton et al., 2019). This interpretation of ecology is contrasted by the definition in the *Oxford Dictionary of Plant Sciences*. Here, it was stated that the term symbiosis has undergone a shift in usage over time in which the modern interpretation is becoming increasingly confined to mutually beneficial interactions, as opposed to encompassing a broad range of mutualistic and parasitic interactions (Allaby, 2019).

Besides the diverse interpretations of the term symbiosis, the requirements to be classified as a mutually beneficial association vary greatly among scientists. In scientific articles, authors hardly ever include a definition of their concept of symbiosis, leaving room for speculation and contributing to confusing information. In the field of molecular plant sciences, some researchers argue that evidence of a mutual exchange of chemicals or symbiogenesis of specific organs is required. However, there are examples of well defined plant–microbe symbiotic interactions that do not require the development of specific organs in the plant (Khan et al., 2012; Alvarenga and Rousk, 2022; Álvarez et al., 2022). In the same way, there is evidence of symbiotic interactions in which clear benefits to both partners cannot be ascertained and, thus, their categorization as mutualistic is debated. One example is the *Oryza*–*Nostoc* association, in which the chemical exchange is not yet directly proven, despite documentation of endophytic colonization and an increase in plant yield in co-cultivation (Álvarez et al., 2022). Hence, often neither symbiosis nor mutualism are applied to describe rice–cyanobacteria associations, resulting in a potential disregard of these interactions. Other scientists classify mutualistic symbioses more broadly based on hypothesized benefits for one partner. Plant scientists mostly focus on the chemical benefits of the host plant. This explains why physical properties, such as the sheltered habitat for the symbiont in moss–*Nostoc* associations, are not considered and, thus, the word symbiosis is avoided (Rousk, 2022). Another non-chemical benefit, the dispersing of sperm by other organisms, is also often not considered to be symbiotic despite contributing to the species' fitness as shown by the increase in reproductive success of moss when microarthropods disperse their sperm (Shortlidge et al., 2021). Controversially, these efforts to classify plant–microbe associations assume that the interaction can be categorized in a binary format (beneficial for one or for both parties), ignoring the reality of plant–microbe interactions that often occur on a continuum depending on the availability of nutrients (Rousk, 2022).

While the examples provided thus far have primarily focused on plant–microbe associations, it is essential to acknowledge that plant symbioses encompass a broader array of interactions, including plant–plant and plant–animal interactions. Establishing a unified definition of symbiosis within the context of plant science poses a formidable challenge due to the diverse nature of these interactions. Consequently, this article specifically examines plant–microbe associations, spanning multiple disciplines such as molecular plant sciences, microbiology, soil sciences, agricultural sciences, ecology, and paleobotany. However, we encourage readers from various fields beyond plant–microbe interactions to use this appeal as a framework for engaging in discussions with their peers regarding their definition of symbiosis and other potentially ambiguous terminologies.

EXPLORING THE COMPLEXITY OF SYMBIOTIC PLANT–MICROBE ASSOCIATIONS

To find a definition that applies to all disciplines studying plant–microbe associations, it is necessary to delve even deeper into the literature to examine related terminology. Before the introduction of the term symbiosis in the 19th century, interactions between organisms were divided into three subcategories: Mutualism or beneficial to both partners, commensalism or beneficial to one while neutral to the other, and parasitism or beneficial to one but detrimental to the other (Beneden, 1876). Nowadays, various other subcategories exist, including competitive (detrimental to both), neutral, and agonistic (predatory or parasitic) associations (Martin and Schwab, 2012), although the criteria underlying each category can vary. To complicate matters even further, biological reality is rarely black and white, which is illustrated, for example, by facultative associations that shift from commensal to mutualistic under certain conditions (Margulis et al., 1991; Rousk, 2022). To describe their observations, scientists have introduced a plethora of terms to refer to the areas colonized, the composition of colonizing organisms, and their relationships (Table 1). Microbes that are attached to the surface of their host are referred to as ectosymbionts, even in the case of the colonization of internal organs or linings. Conversely, symbionts inside the plant tissue are referred to as endosymbionts. Often this term is restricted to intracellular symbioses, but can also include symbioses in which the intercellular space in the plant tissue is colonized. The space created within a host cell that is usually outlined by a symbiotic interface, often a membrane, is called

Table 1. Plant–microbe symbiosis-associated terminology

Term	Definition
Ectosymbiosis/ ectosymbiont	An ectosymbiosis describes an association in which the microbe is located on the host's surface, including internal cavities and linings. The term is more commonly used in microbe–animal than microbe–plant associations. The symbionts are referred to as ectosymbionts. To further specify ectosymbionts, they can be called epibionts if located outside the plant.
Endosymbiosis/ endosymbiont	Often only symbionts that are penetrating the intracellular space of the plant host are classified as endosymbionts. Nevertheless, the term can also include intercellularly located symbionts.
Endocytobiosis	This term describes plant–microbe associations in which the microbe is hosted inside the plant cell.
Exocytobiosis	In exocytobioses, the microbe colonizes the intercellular space of the host plant tissue. This term was frequently used in the last decade of the 20th century but does not appear in current literature.
Microbiome	The microbiome includes all abiotic and biotic factors concerning the plant host. However, the microbiome is often used synonymously with microbiota. The latter is defined as the multitude of microorganisms and their genomes, including virus-like particles, bacteria, fungi, and oomycetes that surround the plant.
Superorganism	The entirety of the host and symbiont represents a collective survival enterprise.
Symbiogenesis	The process that describes the creation of specialized host structures is called symbiogenesis. More precisely, the term describes the evolutionary origin of new morphologies or physiologies shaped by the selection for beneficial symbiosis.
Symbiome	Similarly to the microbiome, the symbiome includes beneficial chromosomal and organellar genes, as well as symbiotic bacteria and viruses.
Symbiosome	The compartment within the host cells comprising the microbe or arbuscular fungi complex is referred to as the symbiosome. The symbiont can penetrate the host cell partially or completely but needs to share a symbiotic interface with the plant cell.
Symbiotrophy	The uptake of nutrients through symbiosis is called symbiotrophy.
SynCom	A synthetic community composed of beneficial microbes is abbreviated as SynCom. This artificial microbiome is hoped to promote plant robustness and health.

the symbiosome. This specialized symbiotic host structure evolved via a process described as symbiogenesis. All microbes colonizing a plant and sometimes even abiotic factors as well as plant-associated archaea and arthropods are referred to as the plant host's microbiome. Microbes and their plant host can together be referred to as a superorganism.

Due to the complex nature of plant–microbe associations and the vaguely defined classification, certain associations seem to be systematically disregarded (Figure 1). Attempts have been made to avoid overlooking less prominent plant–microbe associations and to provide a comprehensive classification of interactions. In 2019, Wein and colleagues 2019 proposed three elements that are essential in symbiotic interactions (which they define as commensal or mutually beneficial): (i) currency, (ii) symbiosis-specific molecular adaptation, and (iii) symbiont inheritance. This study is a great start to discussing the classification of plant–microbe associations, particularly in an evolutionary context. However, it remains unclear if non-chemical currencies, such as shelter, are also included. The molecular adaptation in one or both partners to exchange currencies is thought to be under selective pressure and is therefore an indicator of a stable interaction. This specialization does make sense for their conceptual framework, but it is time intensive to prove and might lead to the exclusion of associations that are not well studied. The final element mentioned is inheritance. Microbes can be transmitted to a new host plant vertically or horizontally. While horizontal colonization seems to be predominant, vertical transmission can be observed, for example, in the bacteria-coated spores of the peat moss *Sphagnum*. A third option to acquire a symbiont is a pathogen-first scenario in which the interaction changes from harmful to beneficial. In addition to the elements proposed by Wein et al., 2019, a fourth and less ambiguous measure to classify plant–microbe associations can be the location of the microbe on or within the host plant. Epiphytic microbes occur on the plant's surface, either aboveground in the phyllosphere or belowground in the rhizosphere. Endophytes colonize the interior space of the plant body and can be further distinguished by their presence in specialized organs (extracellular), between plant cells (intercellular), or within host cells (intracellular).

WHAT DEFINITION SHOULD BE USED IN PLANT–MICROBE ASSOCIATIONS?

Researchers and scientific writers seem to choose the terminology most suitable for their research project. This is not an ideal situation. The use of precise and unambiguous terminology is essential for clear communication in science. Thus, we argue to define plant–microbe symbiosis as:

> Plant–microbe associations encompassing all "living together" associations that are mutually advantageous for the

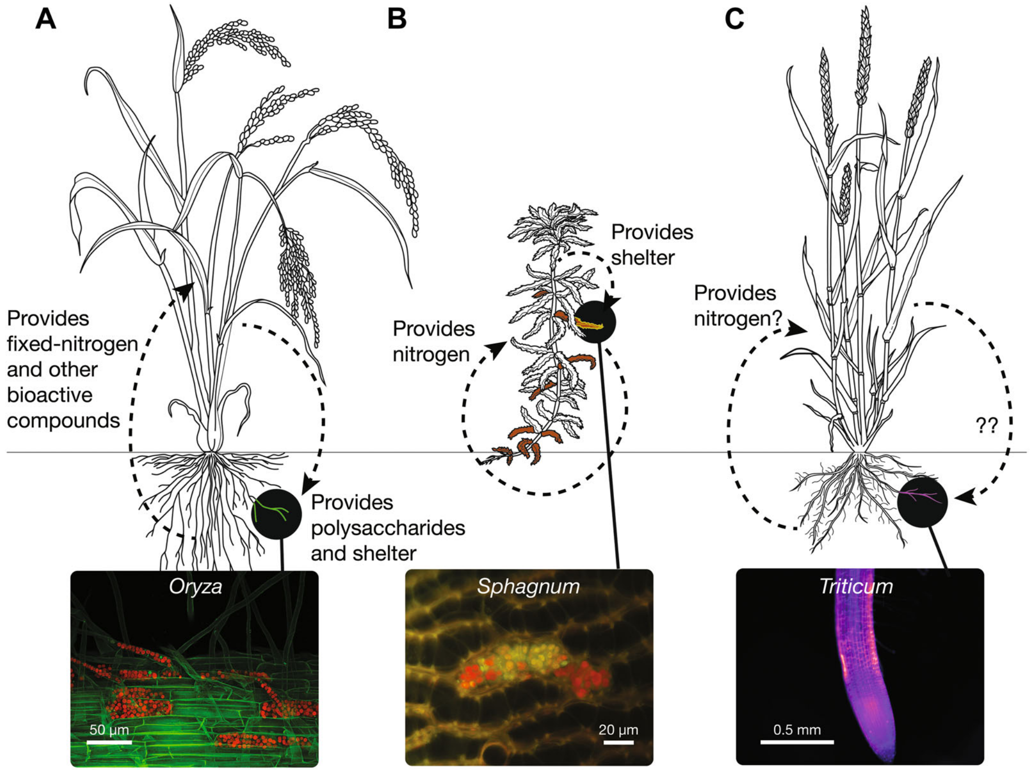

Figure 1. Three cases of debated or overlooked associations of land plants with cyanobacteria
(A) While the role of cyanobacterial nitrogen fixation and its role in improving the fertility of rice (*Oryza*) fields has been studied for almost a century, the endophytic association of *Nostoc punctiforme* and rice has only recently been recreated in vitro (Álvarez et al., 2022). The exchange of photosynthetic polysaccharides and fixed nitrogen between rice and cyanobacteria is depicted schematically. The corresponding microscopy image shows the endophytic colonization of a rice root (green: autofluorescence of the suberin in the plant cell wall) by *N. punctiforme* (red: autofluorescence of chlorophyll *a* in the vegetative cells). **(B)** Ecosystems dominated by mosses such as boreal forests rely on nitrogen fixation by cyanobacteria. However, the abundance, structural and physiological diversity of moss–cyanobacteria associations are poorly known but fixed nitrogen is likely figure as a key currency (Alvarenga and Rousk, 2022; Rousk, 2022). The microscopy image portrays the colonization of water-filled *Sphagnum* sp. hyalocysts (yellow autofluorescence of neighboring chlorophyllose cells and the cell walls) by filamentous bacteria (red and yellowish autofluorescence of chlorophyll α in their vegetative cells). **(C)** Even less understood are cyanobacteria as part of the general microbiome of crop plants. It has been shown that modern breeds of *Triticum* sp. are susceptible to *Nostoc* spp. but details of the association remain unclear (Tkacz et al., 2020). The illustration shows a wheat plant exemplarily for all unknown crop hosts susceptible to cyanobacteria symbiosis. The microscopy image shows a *Triticum* root without cyanobacteria (autofluorescence of the cell walls in purple false color).

members of the community. This advantage should not only be measured in nutrient exchange between partners but also as mutual protection and survival.

Most importantly, given the various definitions of symbiosis, we propose a fixed keyword. Keywords are crucial for search engines and review papers (Gross et al., 2015; Bramer et al., 2018). We propose to apply "plant–microbe symbiosis" to all studies that are concerned with plant–microbe associations, dismissing any classification of the interaction.

Our definition represents a unified and permissive interpretation rather than a rigid one because we believe that language is a powerful and flexible tool and unnecessary restrictions on thoughts and ideas must be avoided. The number of studies dealing with plant microbiome interactions is expected to increase in the future with the advancements of omics-based technologies, allowing a holistic understanding of the underlying mechanisms (Khatabi et al., 2019). This will naturally require more interdisciplinary collaborations. Therefore, we are convinced that now is the time to adapt the language and, thus, the definitions to prepare for the necessary interdisciplinary exchange. This review highlights the importance of applying terminology with care and encourages researchers to do so across all fields of science.

ACKNOWLEDGEMENTS

The authors thank Fabian Haas and Stuart McDaniel for their constructive comments on the argument. We thank Danillo O. Alvarenga and Kathrin Rousk for providing the *Sphagnum* microscopy image. We also thank Nicholas James Desnoyer for taking the time to take a *Triticum* root image and Beat Keller for providing the plant.

This project was carried out supported by funding as follows: MAdLand (https://madland.science) DFG priority program 2237 (PS-1111/1), Swiss National Science Foundation grant nos. 160004, 184826 and 212509 to P.S. UZH GRC

Travel Grant and Foundation of German Business (sdw) Scholarship to A.N. University Research Priority Program ‘Evolution in Action’ of the University of Zurich Project Funding, and Georges and Antoine Claraz Foundation to A.N. and P.S.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

A.N. wrote the initial manuscript, inspired by an exchange with V.M., which was later edited by A.N., D.A.-M., V.M., and P.S. The figure was designed by A.N. and D.A.-M. and later produced by D.A.-M. with the *Oryza sativa* microscopy image provided by V.M. The revisions of the manuscript were done by A.N. and were read and approved by all authors.

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Edited by: Ertao Wang, Center for Excellence in Molecular Plant Sciences, Institute of Plant Physiology and Ecology, CAS, China

Received Jul. 30, 2023; **Accepted** Nov. 28, 2023; **Published** Dec. 1, 2023

FA: Free Access

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