



Perspective

Overhauling the assessment of agrochemical-driven interferences with microbial communities for improved global ecosystem integrity

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ABSTRACT

Recent studies have shown that various agrochemicals can substantially affect microbial communities; especially those that are associated with cultivated plants. Under certain circumstances, up to 50% of the naturally occurring microorganisms can be negatively affected by common agricultural practices such as seed coating with fungicide-based matrices. Nevertheless, the off-target effects of commonly applied agrochemicals are still understudied in terms of their interferences with microbial communities. At the same time, agrochemical inputs are steadily increasing due to the intensification of agriculture and the increasing pathogen pressure that is currently observed worldwide. In this article, we briefly reflect on the current knowledge related to pesticide interference with microbial communities and discuss negative implications for the plant holobiont as well as such that are spanning beyond local system borders. Cumulative effects of pesticide inputs that cause alterations in microbial functioning likely have unforeseen implications on geochemical cycles that should be addressed with a high priority in ongoing research. A holistic assessment of such implications will allow us to objectively select the most suitable means for food production under the scenario of a growing global population and aggravating climatic conditions. We present three hypothetical solutions that might facilitate a more sustainable and less damaging application of pesticides in the future.

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1. Introduction

Global agrochemical inputs have steadily increased since the green revolution in the 60s and are currently the most reliable solution to ensure food supply for a growing population [1]. Furthermore, the use of agrochemicals is predicted to further increase within the next decades and follow a non-linear rise caused by a growing human population and simultaneously aggravating cultivation conditions due to global warming [2]. Fertilizers and various pesticides safeguard high crop yields, yet the changes they induce in terrestrial, aquatic, and especially in host-associated microbial populations remain mostly unexplored on a large scale. With growing chemical inputs in agroecosystems, it can be expected that detrimental effects will progressively accumulate and therefore increasingly affect human, animal and plant health. Traditionally, off-target effects of exogenic compounds are mainly

studied in terms of their implications on higher eukaryotes, e.g. mammals, insects, and aquatic organisms [40,42]. However, recent findings indicate that plant-associated microbial communities harbor hidden responders that were mostly neglected so far by ongoing research [4]. Microbes have coevolved with their host plants and the resulting structures are often termed as holobionts [5]. Plant-associated microbial communities are not only species-specific but also often distinguishable between different genotypes within one plant species even if they are grown under the same conditions [6,7]. A functional network of bacteria, fungi, and archaea, which is commonly termed as the plant microbiota (when addressing living microorganism) or the plant microbiome (when addressing the genetic repertoire accessible with modern sequencing techniques), contributes to host health and productivity. The microbiota is subjected to temporal and spatial dynamics that are mainly connected to the host organism's life stages as well as to external factors in the local environment. Recent studies have confirmed the implications of varying degrees in terms of agrochemical interferences with the structure and functioning of plant-

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associated microbial communities [8,41]. Pesticides unevenly affect different members of natural microbial communities; this is most likely due to differences in the susceptibility of distinct species [4,9]. Large fractions of microbial communities in above-ground tissues of pesticide-exposed plants often show high resilience towards distinct chemicals; however, responsive microorganisms within the same communities can either substantially decrease or increase in their abundance [8,41]. In the close surroundings of plant roots (rhizosphere), distinct pesticides were shown to significantly reduce the prevalence of microbial genes that encode various enzymes that are essential for nitrogen fixation and cycling [10]. Herbicides, insecticides, and fungicides account together for over 90% of the global agrochemicals market [11] and should thus be addressed with the highest priority in terms of their interference with native microbial communities. It was only recently shown that broad-spectrum fungicides can have detrimental effects on around 50% of the naturally occurring bacterial species in crop plants [12]. Remarkably, proteobacteria with nitrogen-fixing potential and other beneficial traits were identified as the most susceptible responders. Reduction or removal of certain members in the plant holobiont is closely related to reduced host fitness and increased disease susceptibility [13,14]. Moreover, microbes that are depleted by agrochemicals cannot contribute to local ecosystem services as well as to geochemical cycles of global importance. These implications remain hypothetical based on the current state of knowledge and it will require decades of research to assess the full extent of pesticide-depleted microbes in the environment. While pioneering studies have clearly shown that off-target effects modulate microbial communities associated with plants, all of the observations were obtained in small-scale experimental systems [4,8,41]. Once far-reaching implications are uncovered, it will likely evoke countermeasures to preserve the integrity of global ecosystems. It is of utmost importance to intensify ongoing research and to implement the first risk assessments in order to allow consequence predictions on a global scale.

In this perspective article, we highlight that the current knowledge base indicates i) that agrochemicals shape plant-associated microbial communities in an unprecedented way, ii) that they interfere with microbe-host functioning with various consequences, and iii) that most of the implications of such interference remain to be uncovered. In addition, we want to point out that large-scale changes in microbial communities have already taken place and will continue to take place in the foreseen future. There is a high necessity to further expand our knowledge related to the interference of agrochemicals in naturally evolved host-microbe interactions to preserve plant, human as well as animal health. Knowing the implications will provide the basis for sustainable developments that will likely require bioengineering approaches in agroecosystems to mitigate detrimental effects in the future. We have elaborated three hypothetical solutions that are partially based on ecotechnology and might find application in the future to alleviate detrimental effects of agrochemicals on microbial communities. In the final part, we discuss which society-level health consequences are likely connected with plant microbiota alterations and what to expect in the future if current trends are continued.

2. Understanding global implications of agrochemical interference with microbes

Microorganisms are the main drivers of various biogeochemical element cycles [15]. They account for the major proportion of biological fluxes for five of the six major building blocks; H, C, N, O, and S are mainly recycled by specific molecular machineries of microbes [15]. Soils are naturally the largest reservoirs of living

biomass, which is mainly composed of a yet unexplored diversity of microorganisms. In total, around 38% of Earth's terrestrial surface is used for agriculture [16] and thus to a certain extent affected by growing inputs of agrochemicals. It is important to point out that not only microbial soil populations are affected due to exposure to the applied chemicals, but also those microbes that are directly associated with plants. It can be assumed that ongoing technological progress and formulation developments will reduce the runoff from agrochemical use into the surrounding environments [17,18] and thus make plants, as well as their belowground root-soil interface, the remaining accumulation hotspots for the applied substances. In this context, plant-associated communities that are affected by pesticides will increasingly become important in the next years. The plant's rhizosphere, this is a specific term for the surrounding area of roots influenced by exudates, is one of the most densely colonized naturally occurring microenvironments and comparable to the human gut in many aspects [19]. Microbial densities are commonly 10 to 100 times higher in these host-influenced microenvironments compared to bulk soil [20]; however, the extent of biomass enrichment in the rhizosphere highly depends on soil types and plant species. A high proportion of rhizosphere colonizers is involved in nutrient provision, pathogen defense, as well as in stress protection and thus an essential component of the plant microbiota [21]. The plant's phyllosphere, synonymous with microhabitats in the aerial parts of plants, is the largest biological surface on Earth and accounts for approximately twice the surface of the total land area [22]. Members of the phyllosphere microbiota are mostly different from those found in the rhizosphere, because they require other traits to survive in a microenvironment that is naturally exposed to high fluctuations in temperature, humidity, and UV radiation. Due to the size of this habitat, the present microbes contribute to a substantial proportion of biogeochemical element cycles. Plant endophytes account for the lowest biomass and are present at relatively lower densities when compared to the aforementioned microbial hotspots; however, they are involved in the most intimate interactions with their hosts. Endophytic microbes are characterized by their ability to colonize inner host tissues without causing disease or any damage [23]. They are involved in similar functions to rhizosphere inhabitants, but can also be transferred across plant generations via seeds [14,24]. Currently, there is robust, multi-source evidence that agrochemical inputs affect microbial communities in the soil, rhizosphere, phyllosphere, as well as endophytes located inside plant tissues [8,9,12,25]. Recent studies have shown that naturally positive as well as negative responders occur within the plant microbiota [12]. Positive responders are those that accumulate upon pesticide exposure, while the opposite is the case for negative responders. Previous studies have indicated that the bacterial genus *Acinetobacter* (Gammaproteobacteria) is a common positive responder to fungicide use, while various members of Alphaproteobacteria, which are known to be intrinsically associated with plants, are commonly found as negative responders [4,9,12]. As of now, studies addressing such implications were mainly descriptive; however, broader implications can be inferred from the known interactions of microbes with their hosts (Fig. 1). Furthermore, the plant microbiota accounts for a substantial proportion of microbial biomass in terrestrial habitats, especially in the aforementioned phyllosphere and rhizosphere habitats, and thus exogenically induced shifts in its composition will simultaneously affect distinct element fluxes in biogeochemical cycles with a potential to cause imbalances of global importance.

The design of future applications in agriculture will likely have to take these implications into consideration in order to maintain global ecosystem integrity. Off-target effects exerted on soil

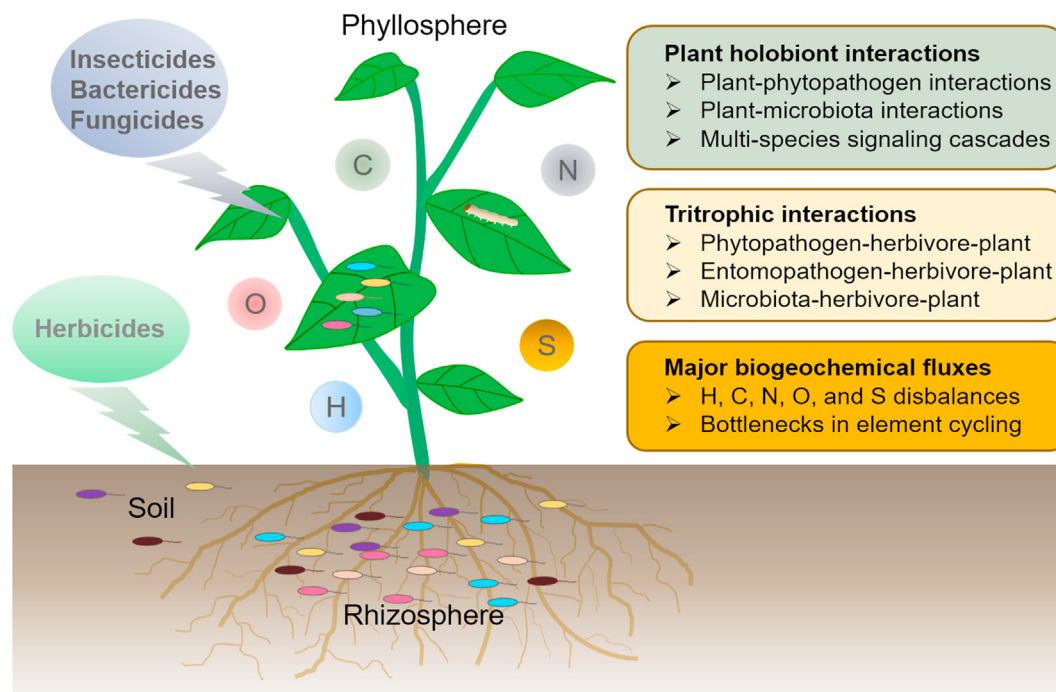


Fig. 1. Interference of agrochemicals with interactions in the plant holobiont and further implications beyond local system borders. The schematic visualization includes an overview of implications that agrochemical-driven modulation effects (e.g. Ref. [4,9,12] likely have on the complex interaction networks within the holobiont. In addition, potential consequences of microbiota modulations are presented from the perspective of a larger model that includes tritrophic interplay as recently discussed by Shikano and colleagues [29]. On a global scale, microbiota alterations are connected with potential disbalances in biogeochemical element fluxes.

microbial communities can be substantially reduced by various technological advancements, including high-precision pesticide application by artificial intelligence-guided robots and automated systems [26]. However, these high-precision applications will leave the intrinsically connected plant microbiota exposed to unwanted off-target effects, because plant tissues and the microbes therein will still be in contact with the applied substances, while the run-off to surrounding soil will be reduced. Here, bioengineering of the plant microbiota, design of microbiota-inert chemicals, or a complete replacement of conventional chemicals can provide the required means to mitigate detrimental effects in the future (Fig. 2). We propose three potential strategies that will all rely on a better understanding of the underlying mechanisms of agrochemical interference with microbial communities. The first strategy will make use of ‘community stabilizers’ within a bioengineered microbiota that will guard indigenous microbes from exposure to exogenic compounds that are deposited in plant tissues or transmitted from their original application sites. Current technology developments allow the introduction of microbes into plant seeds that will be present in various tissues of the plant upon germination [27]. Systematic bioengineering approaches can ensure that implications related to the introduction of ‘community stabilizers’ will be kept at a minimum and that the introduced microbes will maintain natural functioning; *i.e.* plant-microbe interactions and biogeochemical fluxes will not be affected due to functional redundancy of the introduced microorganisms. Another strategy will focus on the implementation of microbiota-inert agrochemicals that preserve the integrity of the native microbiota. To design such agrochemicals, other evaluation criteria than the traditional efficacy assessment and classical off-target identification will be required. During the evaluation of new candidate agrochemicals in terms of their environmental safety, potentially adverse effects on the plant and environmental

microbiota, caused by exposure after application, must also be taken into consideration in the future. The applicable spectrum of candidate agrochemicals should be re-defined according to their interference with native microbiota in addition to their harmlessness towards host plants, mammals, aquatic organisms, and beneficial insects.

The third proposed strategy will move away from classical concepts and employ synthetic compounds, semi-synthetic compounds or defined mixtures of natural and synthetic compounds that will indirectly lead to the desired effects by activating natural processes. These compounds might include such that trigger immune responses of the plant, or such that elicit ‘immune system-like’ responses of the local microbiota by simulating the presence of natural enemies for pathogen and pest defense. The required compounds might be based on enzymes due to their specificity, versatility, and controllable degradability [28]. All three proposed strategies aim at preserving native microbial populations that are associated with plants in order to maintain their *in situ* functioning as well as far-reaching implications beyond local system borders. The main goal of pesticide development in the near future should be to keep microbiota responses to plant protectants as low as possible, in addition to the already established criteria.

From the current perspective, it does not seem feasible that agrochemicals can be entirely replaced by purely biological effectors. Therefore, targeted assessments of bioactive agents will be required in the future to evaluate the risk-free applicability of widespread compounds in order to preserve evolutionary old plant-microbe associations. Nevertheless, we want to point out that there is potential for partial replacements of chemicals by certain biological effectors; however, their impact on the native microbiota remains to be separately assessed, because they might be equally engaged in the modulation of the native microbiota [8,10,29].

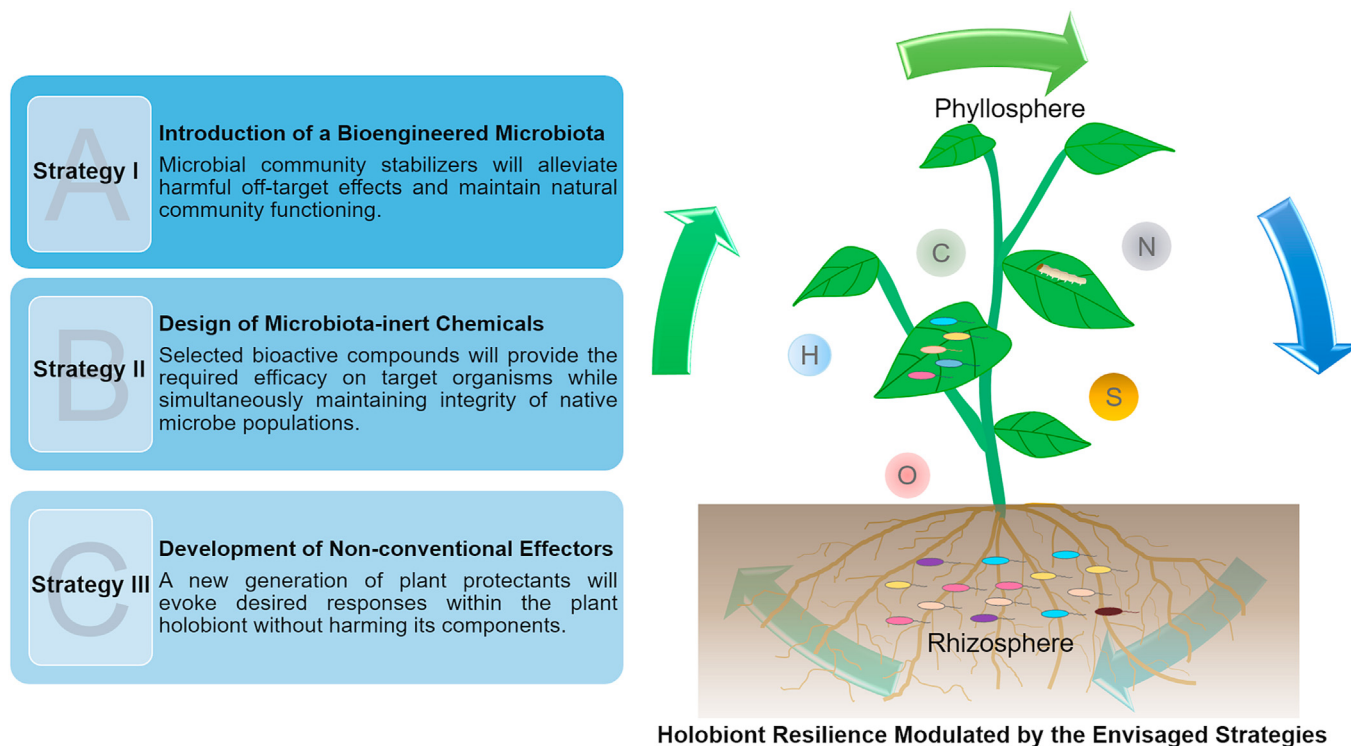


Fig. 2. Envisaged strategies to improve resilience of native microbial populations under agrochemical-caused stress. Three general strategies are conceivable from the current perspective in order to mitigate detrimental effects of agrochemicals. (A) Microbiota bioengineering will introduce community stabilizers that will alleviate harmful off-target effects and maintain natural community functioning. (B) Development of microbiota-inert chemicals will provide the required efficacy while maintaining integrity of native microbe populations. (C) Non-conventional plant protectants will evoke desired responses by the holobiont (e.g. triggering of immune system-like responses of the microbiota) rather than directly exerting target and off-target effects. The development of all proposed strategies requires a better understanding of the full extent of agrochemical interference with the native microbiota of plants.

3. Drawing first conclusions related to plant and human health

From the *One Health* perspective, changes in the environment and food production affect all subsequent trophic levels and thus are important disease drivers that can be predicted with sufficient data availability [30]. Agrochemical-induced alterations in microbial communities have a yet unexplored potential to trigger cascades that affect plant health, but also to evoke health consequences at higher trophic levels. This invisible link connecting agrochemical inputs, microbial ecology, and plant as well as human and animal health needs to be further elaborated. It will require targeted approaches in the future to unveil the full extent of the so-far elusive implications. This knowledge will provide the basis to define the requirements for countermeasures that will reduce disease incidence.

Irrespective of potential solutions in the future to mitigate detrimental effects of agrochemicals, it is very likely that they have substantially influenced plant and human health during the last decades by altering the native microbiota at large scales. There are strong indications that depletion of distinct microbes that are intrinsically connected with their host plants can be causative for their susceptibility to certain diseases [13]; currently applied agrochemicals have the potential to cause such depletions. Moreover, alterations in the composition of microbial communities associated with agricultural practices can have direct as well as indirect implications on human health. Direct implications are more evident as they mainly correlate with the elimination or introduction of either plant- or human-pathogenic microbes. When agrochemicals are applied, they can result in a so-called ‘vacuum effect’ which allows

non-native microbes from the surrounding environment to settle in unoccupied niches. On the other hand, the indirect effects of altered microbiome composition in plants affect humans and animals via the food chain as elaborated in the *One Health* concept. It is likely that crops with an altered microbiota will evoke changes in their consumer’s gut microbial communities. Such changes are connected to various physiological changes as well as disease development [39]. With growing agrochemical inputs, it can be assumed that chronic disease incidences linked to the microbiota will further rise in the human population. Diseases that were so far linked to the microbiota include allergies, obesity, type 2 diabetes and Crohn’s disease among many other [30–33]. Other unwanted, negative off-target effects can be more imminent. It was already shown that certain fungicides can effectively inhibit a target pathogen, but reversely stimulate mycotoxin production in non-target, toxigenic microbes, which poses high health risks to both humans and animals [34]. The holistic impact of agrochemical-inducible off-target effects on microbial biotoxin production remains to be uncovered systematically in future. This is mainly due to the complexity that arises from the number of agrochemicals that are currently applied in combination with the natural biodiversity that is associated with the different target plants.

The plant microbiota is also a natural reservoir of antimicrobial resistance genes (ARGs) [35,36]. Due to the nature of cross-resistance formation [37], it can be assumed that agrochemicals can trigger certain resistances in the vast resistance pools. Although there is no evidence so far, there is a certain likelihood that agrochemical inputs have evoked new opportunistic human pathogens. Typical plant colonizers have many features in common with opportunistic pathogens and it has been already hypothesized that

the plant microbiota is a potential source of them [38]. We, therefore, propose that in the future risk assessments for novel agrochemicals should also include an evaluation of their potential to trigger ARGs with clinical implications. Finally, we also want to emphasize for the sake of completeness that from the human perspective, microbiota interference caused by agrochemicals can have negative as well as positive implications on plant health. Examples include the displacement of pathogens caused by direct off-target effects or the enrichment of certain beneficial microbes that subsequently antagonize them.

Due to the many uncertainties that were mentioned in this perspective, there is a particular urgency to better understand implications of agrochemicals on microbial communities and to provide the basis for ecotechnological and other solutions that will improve global ecosystem integrity and interconnected health aspects.

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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List of abbreviations

ARG	antimicrobial resistance gene
UV	ultra-violet

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