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The unseen effect of pesticides: The impact on phytobiota structure and functions

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In the last years, the diffusion and implementation of next-generation sequencing and the reduction of costs raised the interest in phytyobiome studies allowing to dissect the ecological interactions regulating the holobiont. Indeed, crop plants are associated with a wide diversity of microorganisms in all their parts. Crop microbiota influences plant phenotype, growth, yield and quality by contributing to plant resistance toward diseases, plant adaptation to abiotic stresses, and plant nutrition. The association between terrestrial plants and microbes developed at least 460 million years ago, as suggested by the fossil evidence of the earliest land plants, indicating the essential role of microbes for plants. Recent studies indicate that plants actively recruit beneficial microorganisms to facilitate their adaptation to environmental conditions. Cultivation methods and disease control measures can influence plant microbiome structure and functions. Both pesticide and biological control agent applications may alter the biodiversity inside the phytobiota and suppress beneficial functions. Nonetheless, to date, the effects of disease control measures on phytobiota and their possible side consequences on plant growth, crop productivity and quality remain a neglected field of study. The present work summarizes the known effects on phytobiota providing evidence about the role of plant microbial community in determining the overall efficacy of the applied control measure and suggests that future studies on plant disease control consider also the microbe-mediated effects on plant fitness.

Introduction

Role of phytobiome in plant health

Plants live in close association with a dynamic phytobiota, consisting of a macrobiota as well as a microbiota, in particular the microbes that inhabit the soil in which plants grow. The importance of soil microbial communities is well recognized for their role in plant performance, including the improvement of plant growth, vigor and fitness traits (Marschner and Rumberger, 2004; Lau and Lennon, 2011; Chaney and Baucom, 2020), as well as nutrient acquisition (Chaparro et al., 2012; Reverchon et al., 2015), associated with the presence of rare taxa (Hol et al., 2010), and the attraction of pollinators, predators and parasites of herbivores (Wagner et al., 2014; Berg et al., 2016; Etemadi et al., 2018). Plants modulate rhizosphere microbiota (Berendsen et al., 2012; Kwak et al., 2018) and selectively recruit beneficial microbes (Park and Ryu, 2021) thanks to signals, including root exudates, volatile compounds (VOCs) and phytohormones, that help to shape the phytobiota by recruiting, repelling and coordinating the interactions (Dicke, 2016). Plant-associated microorganisms form complex networks with other members of the same species as well as with different species, genera, families and even domains of life (Wassermann et al., 2019a). The cooperation between plants and microorganisms, as well as the intra-microbiome interplay, require an intense communication that regulates community assembly (Agler et al., 2016; Venturi and Keel, 2016).

The homeostatic balance between both microbe-microbe and host-microbe interactions is critical for plant health. The perturbation of this balance is often referred to as microbial dysbiosis and it may represent an important mechanism of disease (Martins dos Santos et al., 2010; Sekirov et al., 2010; Kemen, 2014). For example, under high humidity conditions, several *Arabidopsis* immuno-compromised mutants display leaf-tissue damages related to an altered composition of the phyllosphere microbiota, somewhat similar to the dysbiosis occurring in human inflammatory bowel disease (Chen et al., 2020). Importantly, experiments of bacterial community transplantation showed that the application of a dysbiotic leaf bacterial community to healthy plants results in tissue damage, demonstrating that, in this case, dysbiosis is the cause of the observed symptomatology (Chen et al., 2020). Nevertheless, it is worth mentioning that stress-induced deviation from eubiosis is not always associated with reduced plant performance (Paasch and He, 2021).

The phytobiota protects crops from diseases through different modes of action: i) indirect effects mediated by the activation of plant induced systemic resistance (ISR), a primed resistance against pathogen infections transmitted from roots to plant tissues (Pieterse et al., 2014; Conrath et al., 2015), ii) niche exclusion of pathogens by competition for nutrients and space (Spadaro and Droby, 2016), and/or iii) direct interactions with pathogens through hyperparasitism (invasion and killing of mycelium, spores and resting structures) or antibiosis (production of antimicrobial secondary metabolites) (Raaijmakers and Mazzola, 2012; Ghorbanpour et al., 2018). Finally, some fungal viruses are used to induce hypovirulence (Milgroom and Cortesi, 2004; Double et al., 2018) and microbial antagonists can act *via* the inactivation of enzymes involved in pathogen infections (Elad, 2000) or the enzymatic degradation of pathogen structures (Köhl et al., 2019).

One easily overlooked, but nonetheless intrinsically important, aspect to consider when studying phytobiota is that pathogenic species are themselves part of all microbiota (Kamada et al., 2013). This is actually a core aspect of epidemiology since the presence of a pathogen does not necessarily lead to infection and disease is caused only under specific inducive conditions (Scholthof, 2007). In the context of dysbiosis, the pathobiota concept, which integrates pathogenic agents within biotic environments, was established and applied to several pathosystems (Baltrus, 2017). The analyses of pathobiota often revealed that pathogens do not operate independently, and multiple pathogens might be involved in severe dysbiosis (Singer, 2010; Lamichhane and Venturi, 2015; Berg et al., 2021). This is the case of Fusarium Head Blight, a highly dynamic disease caused by a complex of different *Fusarium* species, whose composition is geographically dependent, and each of them can respond differently to the changing climate (Yli-Mattila, 2010; Vaughan et al., 2016). Even if predominant, the highly virulent *F. graminearum* is only one member of the species complex (Ioos et al., 2005; Xu et al., 2005) and it was recently established that control strategies against *F. graminearum* are hampered by the presence of the weakly pathogenic *F. poae*, showing the complexity of developing control strategies against plant diseases caused by multiple pathogens (Tan et al., 2021). A similar scenario has been observed for phytopathogenic bacteria. *Pseudomonas syringae* pv. *actinidiae* is always present in syndemic association with *Pseudomonas syringae* pv. *syringae* and *Pseudomonas viridiflava*, two other kiwifruit pathogens, suggesting the establishment of a pathogenic consortium leading to a higher pathogenesis capacity (Purahong et al., 2018). For these reasons, the novel disease control strategies should target the whole phytobiota and the possible pathogenic consortia therein rather than single pathogens to develop suppressive microbial communities able to restrict several pathogens at the same time.

Our current knowledge of communications within the phytobiota is still largely based on interactions involving two or three components, and is frequently measured in controlled conditions. However, a further level of complexity in studying phytobiota relates to eukaryotic organisms that interact with plants, each of them harboring its own internal and external microbiota, influencing the interactions between plants and their microbial communities (Cusano et al., 2011; Khaitov et al., 2015). The intense communication among plant holobiont members and the observations that signals can be co-opted, modified, or even destroyed, by another member of the
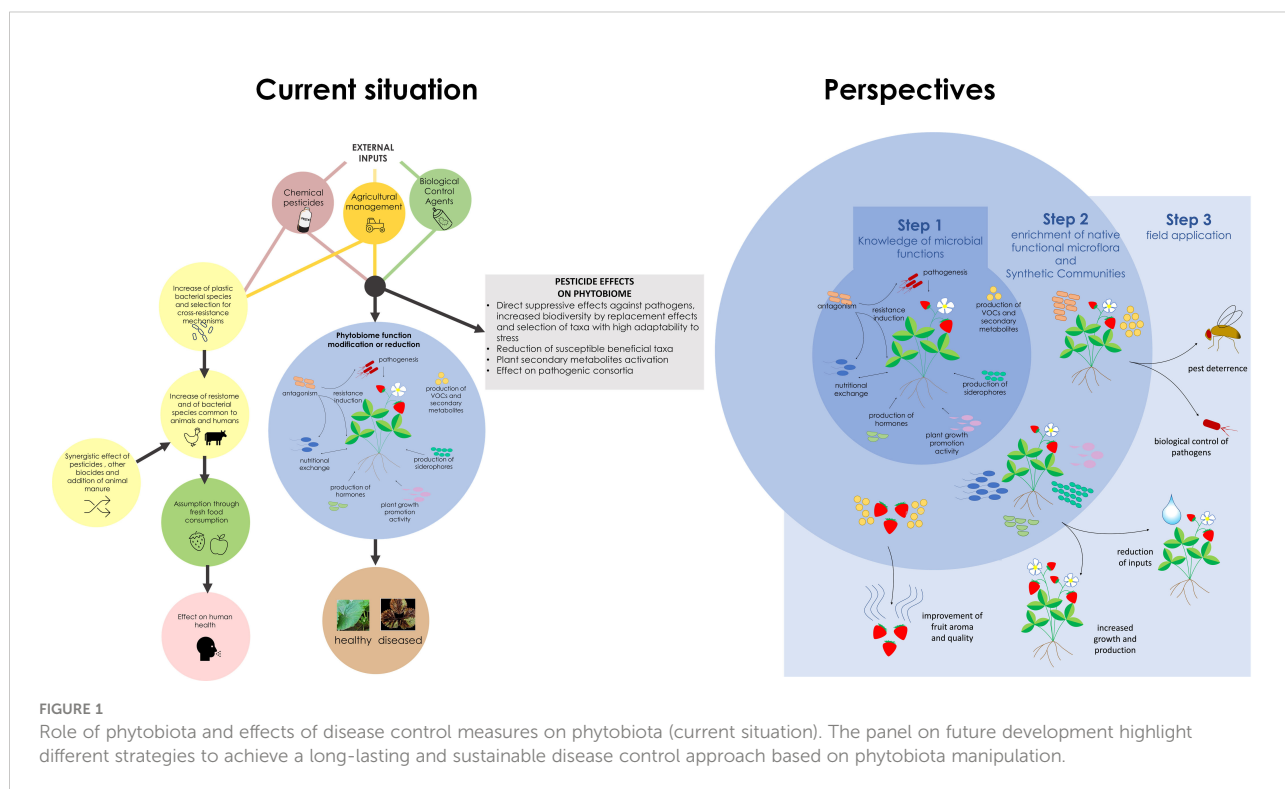
community reinforce the need for a system-level analysis of communication mechanisms to exploit phytobiota manipulation for crop improvement (Figure 1).

Effects of chemical pesticide on phytobiota

Chemical pesticides are extensively used in intensive agriculture and they are still the most effective, reliable and economic tool to minimize losses caused by pests and diseases (Huang et al., 2021a). In this view, pesticides and fertilizers are essential to ensure food security for the ever-growing world population. At global level, more than 2 million tons of pesticides are utilized annually (Sharma et al., 2019). In Europe, the awareness of the risks of pesticides led to the development of policies for their sustainable use (Directive 2009/128/EC) and actions, such as the Farm2ForK Strategy, aiming at a reduction of 50% of pesticide use by 2030. Indeed, in the last decade, pesticide use in the EU was reduced by 10.2% with the lowest total volume (333,500 tones) of sales recorded in 2019. Fungicides, bactericides and herbicides are still the major groups of pesticides currently used (EUROSTAT- European Statistics, 2021). The rise of concern on the effect of pesticides on human and environmental health fostered the research on sustainable alternatives, as well as the extensive study of the direct and indirect effects of their use on non-target organisms and on the ecosystem services they provide (Ramakrishna et al., 2019). Soil microbiota is crucial for ecosystem

functions (Karas et al., 2018). Since synthetic fertilizers and pesticides have a long persistence in soil, in the last decade, several studies investigated the effect of pesticides and fertilizers on soil microflora and microbial fertility, which have a direct impact on environmental quality as well as plant health and productivity (Hartmann et al., 2014; Zhu et al., 2016; Huang et al., 2021b). This led the European Food Safety Authority (EFSA) to the inclusion of soil microorganisms in the environmental risk assessment of pesticides (EFSA Panel on Plant Protection Products and their Residues (PPR), 2010; Karas et al., 2018). However, pesticide toxicology assessment is based only on their impact on the N mineralization test (OECD, 2000) and, thus, it does not provide information about key functions of soil microbiota (Karas et al., 2018). Despite the growing body of knowledge on the impact of pesticides on soil microorganisms, standardized methods have not been developed yet and the majority of studies have been performed at a lab-scale or using exposure levels higher than the recommended application doses (Medo et al., 2015; Wang et al., 2016). Soil microbial communities respond to organic and conventional fertilizer inputs by specific community adaptations (Hartmann et al., 2014). This perturbation often leads to an increase in community richness partially related to the higher proportions of microbial groups associated with the degradation of the introduced pesticide (Ramakrishna et al., 2019; Cernava et al., 2019a; Huang et al., 2021a; Huang et al., 2021b).

Concerning the plant-associated microbiota, the effect of xenobiotic pesticides is a neglected field of study, with scarce information about their impact on the structure and dynamic of

FIGURE 1

Role of phytobiota and effects of disease control measures on phytobiota (current situation). The panel on future development highlight different strategies to achieve a long-lasting and sustainable disease control approach based on phytobiota manipulation.

epiphytic, endophytic and rhizosphere microbial communities (Table 1). Furthermore, no study has been conducted on the interplay of plant-associated microbiota on the uptake, translocation and degradation of pesticides (Vryzas, 2016). Root tip exudates mediated by rhizobacteria could modify the uptake of specific pesticides, while bacterial ligands and enzymes can affect the metabolism and fate of pesticides within crop plants (Vryzas, 2016). Finally, the impact on the phytobiota of transformation products, which may have a higher toxicity than

the original pesticide (Wu et al., 2014), has not been elucidated yet. The effect on the phytobiota depends on the pesticide application strategy (i.e. seed coating, pre- or post-emergent crop) and the affinity to systematic translocation. Pre-emergent pesticides are generally applied to the soil. Soil is also the major accumulation site of pesticides from seed coating. In addition to the direct effect on shaping soil microbiota, these practices also define the species abundance and diversity of available beneficial microorganisms that can be recruited by crop plants (Berg, 2009;

TABLE 1 List of research works focusing on the influence of chemical and biological pesticide or agricultural management strategies on phytobiota.

Pesticide type	Crop	Compartment	Reference
Chemical pesticide vs Biocontrol (*Piriformospora indica*)	Tea	Phyllosphere	Cernava et al., 2019a
Fungicide application in different cultivation system (no till and conventional)	Maize and soybean		Noel et al., 2022
Chemical fungicide (penconazole)	Grapevine		Perazzolli et al., 2014
Variety of fungicides application	Apple		Glenn et al., 2015
Chemical fungicide (dimethachlon)	Tobacco		Chen et al., 2021
Chemical herbicide (S-metolachlor)	Wheat		Xu et al., 2020
Pesticide (clothianidin)+ Fertilizer	Sugarcane	Rhizosphere	Huang et al., 2021a; Huang et al., 2021b
Chemical herbicide (diclofop-methyl)	Rice		Qian et al., 2018
Chemical fungicides (fludioxonil, metalaxyl-M, captan and ziram)	Maize		Kusstatscher et al., 2020
Cultural Strategy			
Diversified vs Conventional	Maize	Root	Wattenburger et al., 2019
Biodynamic vs Conventional	Grapevine	Bark and Grape	Vitulo et al., 2019
Organic vs Conventional	Raspberry	Fruit	Sangiorgio et al., 2021
	Wheat	Root	Hartman et al., 2018
	Apple	Fruit	Wassermann et al., 2019
	Several Fruit and Vegetables	Consumable part of fruit or vegetables	Leff and Fierer, 2013
	Wheat	Phyllosphere	Karlsson et al., 2017
	Apple	Fruit	Granado et al., 2008
	Strawberry	Fruit	Jensen et al., 2013
	Apple	Fruit	Ottesen et al., 2009
	Sugarcane	Phyllosphere	Khoiri et al., 2021
	Apple	Phyllosphere	Glenn et al., 2015
Copper application (Organic management)	Grapevine	Fruit	Martins et al., 2014
Organic vs Intensive vs Traditional	Coffee	Rhizosphere	Caldwell et al., 2015
Low Input	Apple	Flower Phyllosphere	Gschwend et al., 2021
Biological control		**Disease**	**Compartment**	
Pseudomonas, Pantoea, Enterobacter, Curtobacterium, and their combinations	Apple	Fire Blight (*Erwinia amylovora*)	Flower Phyllosphere	Cui et al., 2021
Pseudomonas fluorescens	Kiwifruit	Kiwifruit bacterial canker (*Pseudomonas syringae* pv. *actinidiae*)	Phyllosphere	Purahong et al., 2018
Metschnikowia fructicola (pre-harvest)	Strawberry	Rot (*Botrytis cinerea*)	Fruit	Zhimo et al., 2021
Streptomyces	Wheat	Rot (*Rhizoctonia solani*)	Phyllosphere and Rhizosphere	Araujo et al., 2019

(Continued)

TABLE 1 Continued

Pesticide type	Crop	Compartment		Reference
Pseudomonas fluorescens	Potato	scab of potato (*Streptomyces scabiae*)	Rhizosphere and Geocaulosphere	Roquigny et al., 2018
Mixed communites	Tobacco	Wilfire disease (*Pseudomonas syringae* pv. *tabaci*)	Phyllosphere	Qin et al., 2019
Aureobasidium pullulans	Strawberry	- (interaction with native microbiome)	Phyllosphere	Sylla et al., 2013a
Bacillus amyloliquefaciens, Trichoderma harzianum and *Beauveria bassiana*	Strawberry	Fruit rot (*Botrytis cinerea*)	Phyllosphere	Sylla et al., 2013b
Lysobacter capsici	Grapevine	Downy mildew (*Plasmopora viticola*)	Phyllosphere	Perazzolli et al., 2014
Bacillus subtilis	Strawberry	- Microbiome composition	Phyllosphere	Wei et al., 2016
Bacillus velezensis and Pseudomonas fluorescens	Tomato	Bacterial wilt (*Ralsonia solanacearum*)	Rhizosphere	Elsayed et al., 2020
Stenotrophomonas rhizophila	Maize	- Microbiome composition	Rhizosphere	Kusstatscher et al., 2020

Despite the plant microbiota is a continuum from soil to above-ground organs, studies on soil microbiota have been explicitly excluded from the table.

Cernava et al., 2019a). Karas and colleagues investigated the effect of TCP, 3,5,6-trichloro-2-pyridynol (Chlorpyrifos (CHL), isoproturon (IPU) and tebuconazole (TBZ), which are three pesticides heavily used in Europe and represent the three major pesticide classes (insecticide, herbicide, and fungicide) (Karas et al., 2018). The findings highlighted that functional microbial guilds involved in N and S cycling are the most sensitive endpoints to pesticide exposure. Ammonia-oxidizing microorganisms are the most sensitive and show a consistent response to pesticide exposure, while, in contrast, denitrifying bacteria are stimulated upon exposure to all pesticides. Interestingly, the combined application of pesticides along with biofertilizers may mitigate their negative effects (Huang et al., 2021a; Huang et al., 2021b). In a study conducted on sugar cane, the combination pesticide/biofertilizer enhanced bacterial community diversity compared with control treatments and led to an increase of the expression of the nitrification-related genes *pmoA-amoA* and the denitrification-related genes *nirK* and *norB* (Huang et al., 2021b). However, the type of fertilizer may also have different specific effects. Organic fertilizers generally enhance microbial beneficial functions (Cai et al., 2017; Xiong et al., 2017), whereas synthetic and high nitrogen fertilizers tend to induce dominance effects by stimulating the multiplication of fast-growing microbes (Esperschütz et al., 2007) and to impair soil C/N cycling (Paungfoo-Lonhienne et al., 2015). In this view, to provide a comprehensive elucidation of the effects of a specific pesticide on phytobiota, not only the chemical properties of the pesticide should be considered, but also its interactions with the crop and the other agricultural inputs, including the type of fertilizer and the time of application.

In post-emergent application, pesticide sprays target the above-ground plant organs to selectively reduce pathogenic microorganisms in complex communities. In addition to the effects on pathogen epiphytic growth, pesticides also impact the phyllosphere microbial biocoenosis that hosts a variety of microorganisms, thus influencing plant susceptibility to diseases (Purahong et al., 2018), plant secondary metabolism (Sangiorgio et al., 2021) as well as fruit quality and productivity (Sangiorgio et al., 2022a; Sangiorgio et al., 2022b). The phyllosphere is an oligotrophic and dynamic environment subjected to both circadian and seasonal changes of abiotic (e.g. RH, temperature, light, nitrogen deposition) and biotic (e.g. plant exudates and secondary metabolism) conditions. Thus, epiphytic microorganisms evolved several adaptation mechanisms to this ever-changing environment (Bringel and Couée, 2015; Etemadi et al., 2018). Pesticides elicit a distinct shift in rare phyllosphere microbiota (Cernava et al., 2019a; Xu et al., 2020; Chen et al., 2021), with minor or no effect on the core microbiota, generally leading to increased microbial diversity in treated plants when compared to untreated samples (Cernava et al., 2019a; Xu et al., 2020). However, the increase in biodiversity can be detrimental to its functions. Indeed, beneficial bacteria, known for their intrinsic association with plants, are among the most sensitive responders to the employed fungicide (Chen et al., 2021). Moreover, the pesticide-increased biodiversity is associated with replacement effects, where the niche created by the biocidal activity against pathogen is occupied by microbial competitors (Cernava et al., 2019a; Chen et al., 2021) and linked with community expansion related to the selection of those taxa with distinct stress response systems (Cernava et al., 2019a; Xu et al., 2020). For example, in a study conducted on tea leaves, the most abundant bacterial genera in lime sulfur pesticide -specific signatures were assigned to *Brevundimonas*, *Arsenophonus* and candidatus *Portiera*, which also includes species adapted to resist harsh conditions (Cernava et al., 2019a). Similarly, in tobacco plants, the application of the broad-spectrum fungicide N-(3,5-dichlorophenyl) succinimide induces an increase in

Gammaproteobacteria over *Alphaproteobacteria* (Chen et al., 2021). *Gammaproteobacteria* are common colonizers of plants and are characterized by a fast growth when nutrients are not a limiting factor. To confirm the shift toward taxa with higher adaptability to stress, genes related to the pathways associated with stress responses, biofilm formation and efflux pumps, are increased by pesticide treatment. Both in the soil and the phyllosphere, pesticide-induced changes may also increase the occurrence of potential human pathogens, such as members of the Enterobacteriaceae family, which are common residents of crop phyllosphere and excellent degraders of organic compounds (Brandl, 2006; Teplitski et al., 2011; Erlacher et al., 2015; Cernava et al., 2019b). Due to the commonalities between the adaptation mechanisms to pesticides and the resistance to antimicrobial compounds, such as antibiotics, the pesticide-induced changes in phytobiota can facilitate the development of adaptive mechanisms to multiple antimicrobial resistance, especially in microbes able to degrade the pesticides, which may also be transferred to the human pathogens present in the phytobiota (Kurenbach et al., 2015; Cernava et al., 2019a; Cernava et al., 2019b). For example, glyphosate (N-(phosphonomethyl) glycine) can induce the cross-resistance to antibiotics in *Escherichia coli* and *Salmonella enterica* serovar *typhimurium* (Kurenbach et al., 2015). Methyl salicylate, along with its derivatives sodium salicylate, acetylsalicylate and salicyl alcohol, may facilitate the development of antibiotic resistance through the activation of the *mar* operon in *Escherichia coli* (Cohen et al., 1993). Thus, pesticides can increase and diversify the plant resistome, representing an important reservoir of antibiotic resistance genes, especially in fresh consumed vegetables and fruit, therefore posing potential issues to human health (Blair et al., 2015; Cernava et al., 2019b). Finally, the increase in the phyllosphere of taxa able to metabolize pesticides may result in the accumulation in/on fruit and vegetables of intermediate products, which may exert a higher toxicity than the original pesticide (Cycoń and Piotrowska-Seget, 2015).

Short- and long-term effects of biological control on phytobiota

Although many studies demonstrated that rhizosphere or phyllosphere microbiota, in field conditions, are not greatly affected by the introduced biological control agents (BCAs) (Perazzolli et al., 2014; Wei et al., 2016; Roquigny et al., 2018), other researches, conversely, reported that the application of BCAs significantly change the autochthonous microbial community in terms of both diversity and composition (Schmidt et al., 2014; Qin et al., 2019), which can be neutral or even beneficial for the host plant (Pieterse and Dicke, 2007; Méndez-Bravo et al., 2018). Indeed, microbiota alteration may represent an added value for the plants, thanks to a more

diversified community enrichment (Araujo et al., 2019; Cernava, 2021), or through the decrease in the abundance of pathogenic strains, allowing for instance the suppression of decay development during fruit storage (Zhimo et al., 2021). Nevertheless, it cannot be ruled out that the modification of the structure and functions of the microbial community by BCA application can also negatively impact plant fitness and health. Indeed, individual members of a community contribute to the overall stability or instability of the system and, consequently, BCAs, as new community members, must be viewed as potential internal drivers of microbial community assemblage (Hacquard et al., 2015). Moreover, BCAs must present specific features, such as a high adaptability and stress tolerance, a rapid growth and a high competitiveness, providing an advantage to BCAs and leading to a shift in the microbiota related to dominance effects (Viera-Arroyo et al., 2020). Because of the ecological and economic impacts of invasive species on natural sustainability and societal development (Cullen et al., 2008), there is also a concern that BCAs may eventually become a dominating species in crop microbiota, causing permanent damage to local ecosystems, as documented outside the field of plant pathology (Brown and Mathews, 2007; Gaba et al., 2016). From an ecological point of view, biological control may lead to the exclusion of other species in addition to pathogens and, in extreme cases, threaten phytobiota ecological function and resilience (Sanders et al., 2015), although there is no clear evidence of such phenomenon in plant pathology (Barbosa et al., 2017). Moreover, BCAs may become emerging pathogens of local crops or even humans due to host-jumping or other eco-evolutionary events. In this view a crucial role is played by horizontal gene transfer which is facilitated by mobile genetic elements (Frost et al., 2005). For example, *Delftia tsuruhatensis* strains have been used for biological control and plant growth promotion. However, recently *D. tsuruhatensis* has also became an emergent human pathogen (Yin et al., 2022). Interestingly, the pan-genome of *D. tsuruhatensis* shows extensive genetic diversity a high degree of genetic plasticity characterized by diverse mobile genetic elements, massive genomic rearrangement, and horizontal genes suggesting that horizontal gene transfer and purifying selection are important forces in *D. tsuruhatensis* genetic evolution and adaptation to new hosts (Yin et al., 2022).

BCAs may also contribute to the induction of virulence in autochthonous microbial species, by interfering with the communication system of the pathogens. In a microbial consortium, bacteria may respond to self-like autoinducers from other species, as reported for the olive knot pathogen *Pseudomonas savastanoi* pv. *savastanoi* and the harmless endophyte cooperator *Erwinia toletana* that form a stable community in the olive knot, where they share quorum-sensing signals and cooperate, resulting in a more aggressive disease (Caballo-Ponce et al., 2018). Going further in this concept, *Pseudomonas chlororaphis* isolates stimulate plant growth through direct pathogen antagonism as well as the

induction of systemic resistance in the plant by an array of metabolites under the control of a global regulatory system, the Gac/Rsm regulon (Anderson et al., 2017). Since, in Pseudomonads, this pathway governs the expression of a wide range of genes involved in bacterial fitness, motility, tolerance to stress, biofilm formation and virulence (Latour, 2020), it cannot be excluded that these beneficial bacteria may activate such signaling pathway in pathogenic species present in the phytobiota, leading to an induction of their virulence.

Effect of cultural strategies on microbiome

Unraveling the effect of cultivation management on plant native microbiota and the ecological services that they provide is pivotal for the evaluation of the sustainability of the different agricultural practices (Wattenburger et al., 2019). Indeed, the exploitation of plant-associated microorganisms in agricultural systems is one of the main steps toward the implementation of sustainable management strategies (Compant et al., 2019). Generally, cultivation management can be distinguished between organic and conventional. Although the first consists of the exclusion of synthetic pesticides and fertilizers and low or no-tillage, organic farming may differ from conventional management in several ways (Leff and Fierer, 2013), restricting the comparability among different researches (Lupatini et al., 2017). The cultivation method, affecting the nutrient availability and altering the ecological niches, might also alter the composition and structure of the plant microbiota in several agricultural systems (Caldwell et al., 2015; Hartmann et al., 2014; Sangiorgio et al., 2021). However, the observed effects might be complex and time-dependent (Jonason et al., 2011). Additionally, the comparison among different agricultural management strategies can be compromised by the fact that the different practices are often implemented on farms located in similar, but not identical, areas, thus showing diverse edaphic and environmental conditions. Here, we suggest future experiments to aim at field condition homogeneity to better highlight the contribution of the cultivation management in shaping microbial communities. The influence of management practices on the microbial composition and functions of the below-ground microbiota (Ishaq et al., 2017; Karlsson et al., 2017) has been extensively investigated. Most of these studies focus on the effect of fertilizer management, soil management practices (low/no-tillage vs conventional tillage agriculture) and/or the application of soil health treatments on microbiomes (Hartmann et al., 2014; Bill et al., 2021; Ishaq et al., 2017; Lupatini et al., 2017). Additionally, several efforts have been put into the understanding of belowground microbial communities associated with cover crops, which are widely applied as soil improvement and conservation techniques, although most of the studies focus on annual rather than perennial crops (Castellano-Hinojosa and Strauss, 2020). Cover crops may influence soil characteristics, such as pH and temperature, and thus impact soil microbiota composition.

Although organic farming aims at limiting the negative environmental impacts of agriculture (i.e. water eutrophication, biodiversity loss, toxic chemical accumulation), its implementation does not straightforwardly lead to positive consequences, such as the increase of species richness (Karlsson et al., 2017). For example, in organic agriculture, it is a common practice to combine the application of manure and pesticides to the soils, which might increase the content of antibiotic-resistant genes in soil microbiota, potentially leading to the generation of multiple antimicrobial resistance phenotypes (Ramakrishna et al., 2019). Similarly, the application of copper compounds, widely used in organic agriculture for the control of bacterial and fungal diseases, has been found to be negatively correlated with population size, species richness and evenness of grape yeasts and yeast-like fungi (Martins et al., 2014).

Even though the phyllosphere-associated microbiome gained increasing attention in recent years (Remus-Emsermann and Schlechter, 2018), the study of the influence of the cultivation management on the above-ground microbiota is still in its infancy. Different investigations led to very diverse results (Leff and Fierer, 2013), which might depend on the variety of cultivation and disease control practices implemented, on the rates and frequency of treatments and on the target plant of the study. For example, organically-grown apples display a higher bacterial evenness and diversity (Wassermann et al., 2019b), whereas, in raspberry, conventional cultivation promotes bacterial biodiversity on fruit (Sangiorgio et al., 2021). This might be due to the fact that in low-input agriculture, microbial communities are dominated by highly adapted bacterial species able to outcompete generalist colonizers (Sangiorgio et al., 2021).

As we already underlined, besides the description of the taxonomical variations of microbiotas, we suggest to prioritize the comparative analysis of the functionalities, actually or potentially expressed by the microbial communities, respectively, through metatranscriptomic approaches (Sharuddin et al., 2022) or by means of tools such as Tax4fun (Asshauer et al., 2015), Picrust (Douglas et al., 2020) and FAPROTAX (Louca et al., 2016). For example, this approach highlighted that conventional management lowered the abundance of phyllosphere bacterial genes associated with cell motility and energy metabolism, potentially explaining the lower microbial diversity observed in this condition (Khoiri et al., 2021).

Conclusions

Our knowledge of the impact of xenobiotic pesticides on plant phytobiota is still very limited and no information is currently available regarding the effect of the pesticides-induced shift in phytobiota on crop physiological processes and resistance. In the light of the 'One Health' paradigm, the pesticide-induced changes

in the structure and functions of phytobiota may pose risks to human health, by influencing human microbiota upon consumption of fresh produces, increasing the spread of cross-resistance against different antimicrobials and leading to the accumulation in fruit and vegetables of residues of intermediate products of microbially-metabolized pesticides (Figures 1, 2). Despite the use of BCAs is generally regarded as a sustainable disease control measure, information is still lacking about their effects on phytobiota ecology and their use may pose unexpected threats to plant health (Figures 1, 2). Future BCAs should be selected from the host native phytobiota by dissecting their ecological relations within the microbial community. Moreover, long-term studies should be performed to figure out their impact on plant, environmental and human microbiota. These studies will allow to understand their influence in a new environment, in particular the biological, ecological and evolutionary outcomes

that may unbalance the disease triangle in favor of pathogens. Thus, plant microbiota composition and functions should be precisely monitored following BCA application and this may be achieved thanks to Next Generation Sequencing (NGS) methods, such as metagenomics and metatranscriptomics, powerful techniques that should be accompanied by the knowledge of BCA characteristics and mode of action, allowing human intervention to convert a good candidate into a highly effective BCA, as for *Rhizobium rhizogenes* K84 (Ellis J.G. 2017). Moreover, it is crucial to decipher the microbial communication mechanisms to minimize the risk of possible BCA conversion and to finely tune the ecological interactions within the phytobiota. Overall, a holistic in-depth understanding on BCA-phytobiota interactions will support better timing, formulation and application of BCAs and prevent failures. In that sense, microbiota studies can enhance the efficiency of BCA selection,
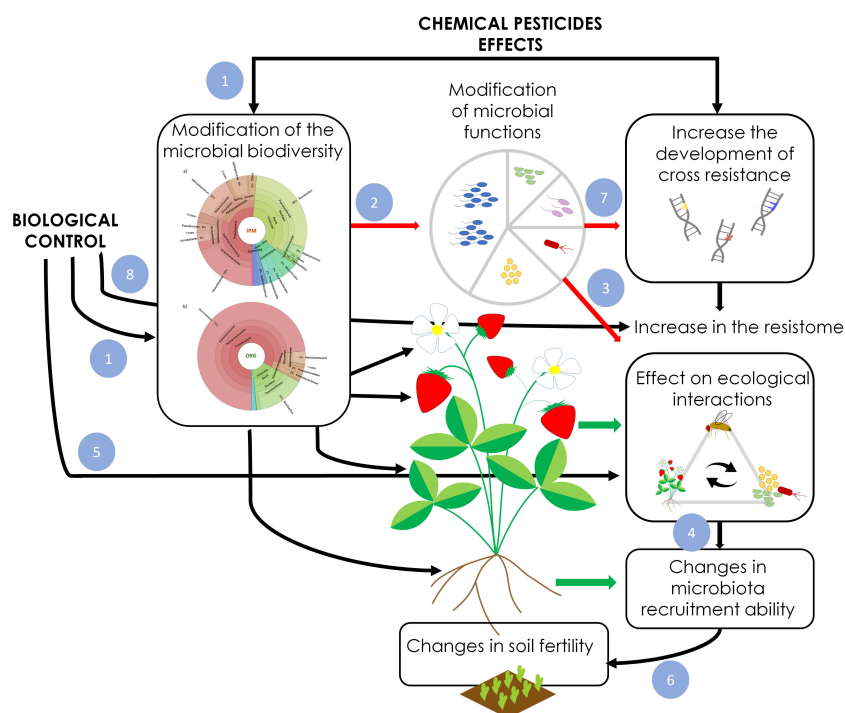

FIGURE 2
Effects of pesticides and biological control application on the phytobiota. Black and red arrows indicate direct and indirect effects, respectively. The green arrow highlights plant-mediated effects. Both pesticides and biological control agents directly modify (1) the structure of the plant-associated microbial communities of flower, fruit, leaves, root and endosphere. The modifications of phytobiota structure and composition leads to changes (2) in microbial functions which directly affect plant fitness, resistance and phenotype. Moreover, the shifts in microbial functions influence plant ecological interactions (3), including the interplay with pests and pollinators (Liu et al., 2019). Since phytobiota composition and functionality is influenced by the exchange of microbes vectored by other organisms [e.g. pollinators (Keller et al., 2021) and pests (Humphrey and Whiteman, 2020)], pesticides use and biological control influence the plant ability to recruit beneficial microbes (4). These effects are also mediated by the plant which responds to the changes in the phytobioma by producing different signaling molecules (e.g. VOCs, root exudates) (Park and Ryu, 2021). The application of biological control agents directly influences these mechanisms (5). The changes in the phytobiota, the ecological interactions and the recruitment of beneficial microbes affect soil fertility (6), which may further amplify the long-term effects on phytobiota, plant fitness, production and fruit quality. Finally, the pesticide and biological control application may facilitate the selection of broad resistance against antimicrobial compounds (7) which may result in an expansion of the microbial resistome with detrimental effects on human health. Finally, the application of microbial biological control agents, introducing alien microorganisms, may facilitate the horizontal transfer of new genes with the native microbiome directly influencing the expansion of the resistome (8).

but also of "helper" strains, which support BCAs in establishment, survival and antagonistic activity *in situ*. The identification, selection and characterization of native BCAs could overcome the risk related to phytobiota structure modification due to the introduction of exotic microbial strains. These advances will allow to finely manipulate plant and environmental microbiota to develop strategies going beyond both xenobiotic pesticides and traditional biological control applications (Figure 1).

Author contributions

Authors equally contributed to the manuscript. EV drafted paragraphs 1, 3 and 5. FS drafted paragraphs 2 and 5. DS paragraph 3 and 4. All authors contributed to the article and approved the submitted version.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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