

GENOMICS TOOLS FOR A BETTER USE OF PESTICIDES IN AGRICULTURE

– POLICY BRIEF –

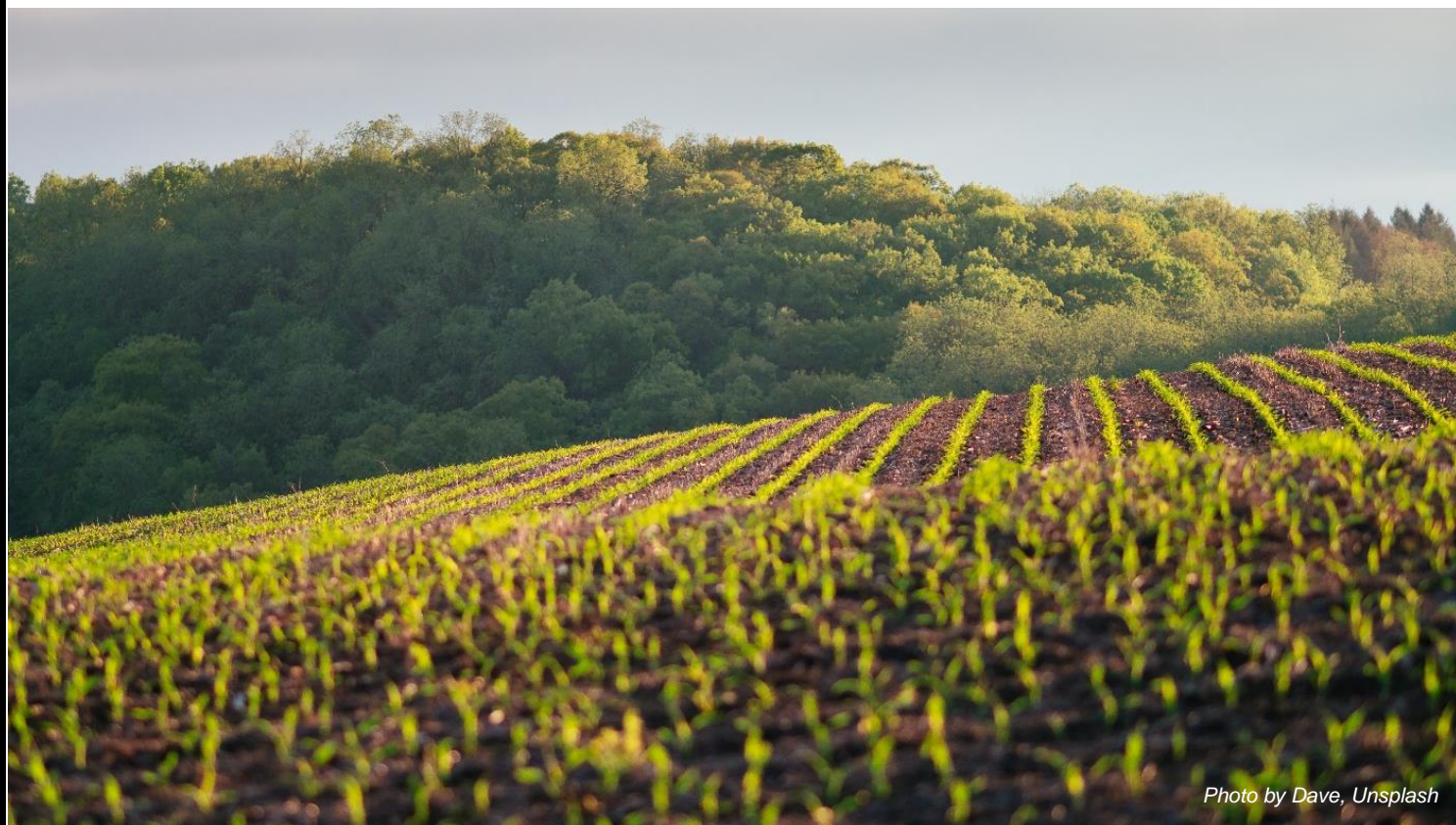


Photo by Dave, Unsplash

RAYNALD DUPRAS, ANN LÉVESQUE, JÉRÔME DUPRAS

QUEBEC CENTRE FOR BIODIVERSITY IN SCIENCE
UNIVERSITÉ DU QUÉBEC EN OUTAOUAIS (UQO)

MONTRÉAL – SEPTEMBER 2020

Génome Québec

Génome Québec is a private, non-profit organization whose mission is to catalyze the development and excellence of genomics research and promote its integration and democratization. It is a pillar of the Québec bioeconomy and contributes to Québec's influence and its social and sustainable development.

Quebec Centre for Biodiversity Science

The Quebec Centre for Biodiversity Science (QCBS) brings together more than 120 researchers working at the forefront of the field both nationally and internationally. The QCBS provides added value to biodiversity research.

It has a three-fold mission:

1. To foster and promote world-class research at all levels of academia (undergraduate, graduate, postgraduate and faculty) in biodiversity science.
2. To facilitate scientific cooperation within a cross-disciplinary group of Québec biodiversity researchers and promote Québec research nationally and internationally.
3. To support the development of public policies on biodiversity and contribute to the academic and public debate on biodiversity loss in Québec, Canada and around the world.

Objectives of the Task Assigned to QCBS by Génome Québec

To promote a better understanding of the issues related to pesticide use in agriculture, Génome Québec tasked the Quebec Centre for Biodiversity Science (QCBS) with producing a policy brief on how genomics tools can be applied in agriculture to improve the way pesticides are employed. This document is the result of a literature review and a discussion among four researchers in a focus group from the academic and institutional community and semi-structured interviews with three government agency professionals. The views expressed herein do not necessarily reflect those of Génome Québec.

TABLE OF CONTENTS

1. Executive summary	4
2. Background.....	6
2.1. The use of pesticides in agriculture and related socio-ecological issues.....	6
2.2. Current utilization of genomics tools to improve the use of pesticides in agriculture.....	7
3. Genomics providing solutions to phytosanitary challenges in agriculture	8
3.1. Genomics tools to support the study of agroecosystems.....	8
3.2. Genomics to better understand the ecological mechanisms involved in the breakdown of pesticides in the environment.....	10
3.3. Genomics tools to support decision making in integrated management strategies.....	12
3.4. Advances in genomics in terms of biocontrols.....	13
3.5. Genomics to breed and optimize cultivars for a reduced use of pesticides	15
4. Obstacles to the use of genomics tools in agriculture	16
5. Conclusion	17
6. Bibliography.....	18

1. Executive summary

Despite significant agricultural progress made in the integrated management of crop pests, the use of pesticides remains pervasive. Pesticides are generally exceptionally efficient at maintaining or enhancing agricultural productivity. However, they also have negative repercussions on wildlife, plants and human health. Consequently, their use represents a major challenge for the agricultural and public health sectors. Policymakers are faced with many difficulties resulting from the use of pesticides, including the loss of biodiversity, the contamination of ecosystems and food and the exposure of farm workers to these products.

The science of genomics can help us make better use of pesticides in many different ways. In addition to helping to improve cultivars and develop biopesticides, genomics tools can characterize entire microbial communities found in plants and in soils. This major advance leads to a better understanding of the relationships between plants and their microbiota, as well as the biotic and abiotic characteristics of an environment. This is useful since these microbial communities form an essential part of the biomass and make a major contribution to all biochemical activities going on in ecosystems. Genomics tools can also help with the detection of pathogens in agroecosystems, determine the response of these pathogens to chemicals and assess their level of resistance to various pest control products. This information can contribute to strategies aimed at reducing the use of pesticides.

Despite all the opportunities afforded by genomics science, there are still many obstacles impeding the integration of genomic tools in farming. For instance, major investments will be needed to set up experimental sites in agricultural environments. Moreover, some of the research, especially the work on soils, may take a number of years before valid and robust reference values can be established. Oversight mechanisms will also be required to ensure the effective use of databases as well as cooperation among the various stakeholders from the agricultural sector and the optimal transfer of knowledge to farms.

This report provides an overview of the issues associated with the use of pesticides in agriculture and presents the genomics tools available to respond to the challenges identified. It also provides policymakers with recommendations to help make use of these tools in agriculture and to position Québec as a global leader in this field.

Recommendations to Policymakers¹

1. Promote the use of next generation sequencing technologies to gain a complete and accurate picture of microbial populations found in soil (p. 9).
2. Position, as a first-line solution, the use of genomics tools, such as PCR and qPCR, to evaluate specific functions within agroecosystems (p. 10).
3. Institute field research programs to collect data over time (8 to 10 years) in order to establish valid reference values on soil quality (p. 10).
4. Using genetic engineering, study the role played by microorganisms in the degradation of pesticides focusing on the level of enzyme expression specific to the degradation (p. 11).
5. Implement a program to detect resistant genes in different crops in order to target and adapt the interventions carried out (p. 12).
6. Develop soil health indicators prioritizing the use of metagenomics to analyze samples in order to identify one or more microorganisms representative of a given state of soil health (p. 13).
7. Promote the use of genomics tools, such as PCR and qPCR, among stakeholders working on the ground and provide them with appropriate training (p. 13).
8. Offer interested agricultural producers training on the proper use of genomics tools and provide them with financial support as they integrate these tools into their business (p. 13).
9. Implement new biological control strategies through the identification or modification of certain microorganisms using metagenomics and genome editing technology (p. 15).
10. Using genomics-based tools, produce an independent seed guide featuring phylogenetic biodiversity well suited to the soil and climate conditions in different regions of Québec (p. 16).
11. Set up a network of public laboratories with the required instrumentation and expertise to offer a rapid results analysis service in support of sound decision-making (p. 16).
12. Promote cooperation among various groups of researchers who will implement standardized protocols on the use of genomics tools to be followed across laboratories (p. 16).
13. Facilitate collaboration among various research groups to implement public-access databases available to all stakeholders in agriculture (p. 17).

¹ The above recommendations are not listed in any particular order of priority.

2. Background

2.1. The use of pesticides in agriculture and related socio-ecological issues

Since the early 20th century, agricultural production has increased significantly in response to rapid demographic growth. According to the United Nations, the global population is, in fact, expected to reach 9.5 to 10 billion people by 2050 (UN, 2015). Having this many more mouths to feed has triggered a “green revolution,” which was made possible by the introduction of chemical fertilizers combined with higher yield crop varieties and the arrival of chemical pesticides (Carvalho, 2006). As a result, the production of these chemicals has gone from 0.2 million tons in 1950 to 5 million tons in 2000 (FAO, 2017). Since then, sales for all types of pesticides have risen, with herbicides ranking first, followed by insecticides and fungicides (Carvalho, 2017). Over time, pesticides have become an essential part of the world’s agricultural systems, paving the way for a notable increase in crop yields and, in turn, greater food production (Alexandratos and Bruinsma, 2012).

The growing demand for food has become even more important since the effects of climate change, such as more frequent droughts and floods, are now compromising commercial crops, thus reducing the availability of staple foods for a large proportion of humanity (Chapli-Kramer *et al.*, 2019). The rise in temperature and changes in precipitation patterns also impact crop protection strategies (Delcour *et al.*, 2015) and affect the fate of pesticides in soil, sediment and water systems. To remedy these problems and protect their businesses, agricultural producers already use a number of different approaches (e.g., shelterbelts, intercropping, buffer strips). Another harmful effect of climate change involves the arrival of new pest species or the expansion of the distribution area of existing pests (*ibid*). This situation can lead to increased pesticide use in the absence of other solutions.

The current approach to crop protection is based on circular reasoning that involves identifying a pest, developing a crop protection product in response, observing the collateral effects of its use and the rise of new problems, developing new products in response, and so on. This trial-and-error approach does deliver results, but it also comes with negative consequences for the environment and public health (Carvalho, 2017).

The use of pesticides is a major source of environmental contamination at the local level, but its impact can also be felt globally. Generally speaking, chemical residues present in the environment can have dramatic consequences for land and aquatic ecosystems as a whole. Over time, considerable efforts have been made to understand how pesticides behave in the environment, including their toxicity and the manner in which they break down. Contemporary pesticides have better degradability, but tend to build up in surface water, year after year, as is the case with glyphosate (McKnight *et al.*, 2015). Furthermore, the toxic effects of pesticides are not always limited to the harmful organism being targeted; they also spread to other similar organisms, compromising biodiversity, ecosystem health and, ultimately, human health. These products generally undergo many chemical transformations before ending up in ecosystems outside the region of applications, where their toxic effects are felt by organisms not originally targeted (Taylor *et al.*, 2003). Moreover, some pesticides contain volatile components, parts of which can be carried on air currents over long distances (Garbarino *et al.*, 2002).

Soil erosion, runoff water and rivers can transport large quantities of pesticides from one location to another. Some products, such as DDT and chlordane, which have been banned for many years now, can still be found in coastal environments. Even at very low levels, these toxic residues can destroy aquatic species, compromising biodiversity and ecosystem functioning (Rand, 1995). An extensive study of these ecosystems around the globe has shown that such residues are present

everywhere in the world and primarily concentrated in marine life (Jamieson *et al.*, 2017). Other studies have demonstrated that pesticides (mainly herbicides) also affect the symbiotic relationship of algae and destroy coral (Lewis *et al.*, 2009). Thankfully the gradual elimination of persistent organic pollutants in keeping with the Stockholm Convention of 2002 has contributed to reducing human exposure to these toxic materials. In recent decades, for example, studies conducted in several countries have shown that the concentration of DDT in human fatty tissue and milk is constantly going down (Carvalho, 2017).

Despite notable improvements in managing pesticide residues, their use remains an issue that raises concerns not only for the environment but also regarding human health and public opinion (Goddard *et al.*, 2018; Reeves *et al.*, 2019). In Canada and Québec, these concerns are taken into account by public officials, more specifically through the Pest Control Products Act (2002), the Pesticides Management Code (2003) and various strategies aiming to minimize the risks associated with pesticide use. A study conducted by the Institut national de santé publique du Québec argued that the health benefits of eating fruit and vegetables far outweigh the cancer risks associated with pesticide residues found on these foods (Valcke, 2017). The risk, however, is not negligible, and there is a level of uncertainty surrounding the safety of these chemicals for human health (*ibid*). In response, efforts should be made to reduce the public's exposure to such products.

2.2. Current utilization of genomics tools to improve the use of pesticides in agriculture

Pests (phytopathogens and others) and weeds limit crop yields and threaten sustainable production. Most of today's plant food production relies on chemicals to control these harmful species. To reduce the collateral effect of pesticides on the environment, other measures that can enhance crop resistance and control pests must be adopted. Examples of such strategies include the development of new cultivars, the creation of biopesticides and biostimulants, the application of integrated pest management principles on farms, the management of soil erosion and crop rotation.

Recent advances in molecular biology have prepared the way for the development of genomics tools, highly sophisticated in some cases, which can prove useful in many areas of agriculture. Here are a few examples of the ways in which genomics tools can play a major role:

1. Early detection of pathogens
2. Identification of pathogens during infestations
3. Detection of virulence genes associated with a pathogenic agent
4. Selection and modification of cultivars
5. Identification of microbial indicators to gauge the health of ecosystems
6. Development of biostimulants, biopesticides and other management methods.

The tools used vary based on the needs of the spheres of activities involved. Here is a description of some of these tools.

Molecular detection methods that use **PCR** (polymerase chain reaction) have significantly contributed to the accurate, sensitive, reliable identification and quantification of many species of pathogens (Sanzani *et al.*, 2014). However, most PCR-based tests (or tools) are specifically designed to detect one or a few species by targeting specific genes. This means they are not suitable for the broader detection of phytopathogens in agroecosystems (defined as cultivated ecosystems corresponding to the spatial unit of a farm and whose ecosystem functions are valued in the form of agricultural goods and services).

Metabarcoding is a tool capable of characterizing many species found in an ecosystem. It can play an important role, for instance, in terms of detecting and dispersing exotic phytopathogens, which have become a threat due to climate change and expanding world trade (Brasier, 2008). In their native environments, many of these pathogens cause minimal harm. This is why they may be less known and less likely to have been identified. Detecting all organisms in an ecosystem is important, yet remains a challenge. This is where metabarcoding comes in. It is a powerful tool that can be used to monitor and prevent invasions by exotic pathogens (Comtet *et al.*, 2015) in an ecosystem. This tool, combined with specific primers, can facilitate the description of host-pathogen interactions, as well as new phylotypes whose DNA sequence in a given segment resembles that of a known species. These phylotypes then need to be identified (Abdelfattah *et al.*, 2018). As a result, the data collected can play a pivotal role in the development of biological approaches (e.g., biopesticides and biofertilizers) to help manage pathogens (Massart *et al.*, 2015).

Genome editing technologies (GETs) are used to modify plant immunity in different ways and in many different crop species. There are several examples of targeted genes having been successfully altered to become more resistant to pathogens. Two of these, CRISPR-cas9 and TALEN, have both been used to act on wheat's locus of resistance to mildew, creating plants that are less likely to succumb to powdery mildew and white rot, two diseases that affect yields. Second, GETs have been used to develop plants that can resist bacterial blight in leaves. A third example is the introduction of new strains resistant to geminiviruses, plant pathogens that cause severe damage to many crops (Ji *et al.*, 2015). By modulating the resistance of plants in this manner, GETs help improve crop performance to make agriculture more sustainable. They allow, for instance, specific mutations to be introduced, reducing the pleiotropic effects of complete gene deletion (Andolfo *et al.*, 2016).

GETs holds great promise for the future, but public acceptance of them has yet to be achieved. In fact, the level of public support varies greatly from one person to another, depending on the way the technologies are used and on the information provided (Muringai, *et al.*, 2020). Further research is needed to better understand the conditions under which the general public would be willing to accept food derived from genome editing. Knowing the developers and regulations involved in these new technologies, as well as their impact on agricultural productivity, the environment and human health, are essential factors that can help build consensus around the issue (*ibid*). Relevant information from both pro- and anti-GMO groups may also help consumers form their own opinion on the matter (*ibid*).

3. Genomics providing solutions to phytosanitary challenges in agriculture

3.1. Genomics tools to support the study of agroecosystems

Soil microorganisms play a key role in agriculture, for instance in the biogeochemical cycling of carbon, nitrogen, sulphur, phosphorus and many other metals (Barnard *et al.*, 2005). Microorganisms, including bacteria, archaeobacteria, microeukaryotes or the viruses that infect them, are rarely found on their own soil; they form complex communities that change over time and space and give soils their level of fertility. Pesticides, however, can alter this microbial diversity and reduce its associated benefits. Microorganisms have a key role to play in soil ecosystems and, when measurable, their structural and functional characteristics can serve as excellent indicators of the effects of pesticide exposure (Gwenaël *et al.*, 2012).

To date, a great deal of research has focused on the effect of pesticides on soil microbial communities, but most of it is based on standard bacterial cultures. Traditional methods of analyzing microorganisms involve collecting samples in an ecosystem, followed by culturing individual strains in a lab. With this method, the entire range of microbial diversity cannot be studied (Berdy *et al.*, 2017), since the greater part of the bacteria are uncultivable in the lab. In fact, only 1% of microbial diversity can be identified through this method (Epstein, 2013; Rappé and Giovannoni, 2003; Chi-chu, 2010). Yet access to the entire microbial population is important given that all microorganisms play a key role in the biosphere (Epstein, 2013). The use of molecular techniques, such as metagenomics, must be prioritized since it provides all the information needed to identify the full bacterial population in a given environment.

Genomics tools offer enormous potential for analyzing variations in microbial communities. Structural changes can be associated with the expression of specific genes, charting the course for more in-depth studies on the relationship between biological diversity and ecosystem functioning and the influence of contaminants on this relationship (Johnston *et al.*, 2015). Molecular-based methods have huge potential to provide sensitive and efficient measurements suited to the evaluation of pesticide side effects on soil ecosystem functions and microbial community composition (Feld *et al.*, 2015). Depending on the tools used, the information on microbial communities can be structural or functional in nature. Some examples of these tools include denaturing gradient gel electrophoresis (DGGE), qPCR and DNA chips, which deliver information on targeted genes already known, and metagenomics, which can sequence the entire DNA of a community and provide data on its taxonomy and function (Feld *et al.*, 2015).

RECOMMENDATION 1

Promote the use of next generation sequencing technologies to gain a complete and accurate picture of microbial populations found in soil.

In the past, strategies to evaluate ecosystem health focused on a description of their chemical characteristics combined with toxicity test results obtained in laboratories (Scanes *et al.*, 2007). More recent approaches, however, recognize the importance of including biological monitoring in order to improve their predictive capacity (Dequiedt *et al.*, 2011). The need for a more realistic picture of ecotoxicology has led scientists to test whole communities rather than a single individual (Edge *et al.*, 2015). The response of an entire microbial community provides a more accurate and sensitive assessment of the effects of contaminants given biotic interactions and processes that naturally occur in a contaminated system (Birrer *et al.*, 2017).

Analyses that lay the groundwork for the study of the harmful effects of pesticides on microorganisms often involve the nitrogen and carbon transformation process. The nitrogen cycle is one example of soil function that can be assessed using genomics tools targeting known genes. This cycle involves two important biochemical processes: nitrification (oxidation of ammonium) and denitrification. Both of these phenomena are directly associated with soil fertility and its buffer effect, making them suitable biological indicators of soil quality (Wessen *et al.*, 2011). Communities involving these biochemical processes are used as models to better understand the importance of microbial diversity in soil response to various environmental stressors (Philippot *et al.*, 2005).

The PCR and qPCR molecular techniques are used to study the nitrogen cycle by targeting the genes coding for the enzymes responsible for nitrification (e.g., *amoA*) and denitrification (e.g., *nirK*) (Crouzet *et al.*, 2016). Traditional chemical measurements, it should be noted, can also be used to help interpret the results obtained from genomics tools. One example is the nitrogen cycle,

where the measurement of enzyme activity responsible for nitrification can complement the study of the *amoA* gene expression (associated with nitrification) in microbial communities. Other examples include measuring phosphorus metabolism, the breakdown of sugar and breathing. All these biochemical processes are often associated with the expression of genes of interest that can be targeted by genomics tools. Since molecular tools are so efficient and generally less and less expensive, they could easily be integrated for use in standard toxicity testing and biomonitoring programs.

RECOMMENDATION 2

Position, as a first-line solution, the use of genomics tools, such as PCR and qPCR to evaluate specific functions within agroecosystems.

In terms of soil balance, the use of genomics tools must be optimized with access to reference soils and microbiomes in order to better evaluate the state of disruption in a given environment. Establishing valid reference values for soils takes a long time, that is, 8 to 10 years. With agricultural seasons changing from year to year, funding programs that last only three years cannot provide the robust reference values needed. As a result, it is critically important to support the implementation of long-term experimental sites.

RECOMMENDATION 3

Institute field research programs to collect data over time (8 to 10 years) in order to establish valid reference values on soil quality.

3.2. Genomics to better understand the ecological mechanisms involved in the breakdown of pesticides in the environment

Metagenomics also has the potential to teach us more about the microbial dynamics in ecosystems, including the mechanisms involved in response to contaminants. Until now, traditional microbiological studies could only measure microbial diversity (Johnston *et al.*, 2015), without being able to simultaneously quantify the processes or functioning involved (Kinsella *et al.*, 2016). Yet structural changes within ecosystems do not necessarily reflect changes in their functioning – for example, if members of the microbial community are functionally redundant (Allison *et al.*, 2008). With molecular-based tools, however, microbes become sensitive, efficient bio-indicators. Due to their direct interaction with the environment, bacterial and archaeobacterial communities are highly sensitive to anthropogenic modifications at large spatial scales (Sun *et al.*, 2012). This high level of sensitivity to disruptions means that response is strong and/or swift, compared to resistant organisms; this makes possible early detection and detection when contaminant levels are still low.

We still know very little about the capacity of soil to break down pesticides and the influence that these chemicals have on microbial communities (Chi-Chu, 2010). It is difficult to establish the relationship between the chemical structure of a pesticide and its effect on various groups of soil microorganisms. The spectrum of bacterial response to pesticides in soil ecosystems is vast and involves changes to the composition and function of bacterial communities. Certain microbial groups are capable of using pesticides as a source of energy and nutrients, while these same pesticides can be toxic to other microorganisms (Johnsen *et al.*, 2001). Due to their toxicity,

pesticides can reduce microbial diversity while promoting the functional diversity of certain communities through the supply of energy and nutrients (Wang *et al.*, 2006). Pesticides can also inhibit or kill some groups of microorganisms, eliminating the competition and leading to the appearance of new groups (Hussain *et al.*, 2009; Feld *et al.*, 2015).

The composition of the microbial community and its activity play a key role in the breakdown of pesticides. Microbes that use pesticides as a source of nutrients end up breaking them down into small inorganic molecules, such as CO₂ and H₂O, through enzymatic reactions involved in a process known as “mineralization” (Tang, 2018). This prevents secondary pollution due to metabolites. Mineralization involves the decomposition of organic molecules into inorganic compounds through microbial activity. There are pesticides that are analogues of natural compounds, with some microorganisms having the enzymes needed to break them down. However, most existing contaminants are synthetic molecules that do not occur in nature, which, as a result, cannot degrade under the influence of microbes. Given that pesticides are used on a large scale and that the natural degradation process by microorganisms is very slow, the pace at which compounds are broken down is insufficient, meaning that pesticides cannot be completely eliminated. (Huang *et al.*, 2018).

To prevent the harmful effects of pesticides on the environment, bioprophylaxis can be a promising avenue. With this approach, indigenous and allochthonous microorganisms selected to break down a specific compound can be added to soil during spraying. In this way, the microbial complex is applied along with the pesticide to optimize its degradation and that of its metabolites, acting on pollutants when they are most available and reducing their dispersion into the environment. At the moment, however, it is difficult to determine with complete accuracy the exact group of mineralizing microorganisms to use. Other challenges include developing a galenic formulation that would allow the microorganisms to conserve their power to break down the pesticide, while also preserving the efficacy of the pesticide in the presence of the selected microorganisms (Carles, 2016). In addition, microorganisms can lose some of their power to break down the harmful compound during the application, or the success of the process could be compromised by the pesticide’s reduced bioavailability in the soil (Carles, 2016). Another solution is to combine microbial degradation with the capture of pesticides as they are drained from the agricultural fields. This could bypass the problem of microorganisms involved in biodegradation remaining in the soil.

The enzymatic degradation of pesticides has attracted a great deal of interest. In this regard, genomics tools have been used to identify the microbial strains that have improved degradation capacity. This is the case, in particular, for organophosphates, a group of pesticides that can naturally be broken down by a variety of microorganisms, but for which new strategies to promote their degradation are needed to prevent them from accumulating in the environment (Kumar *et al.*, 2018). To address this challenge, researchers have shed light on the *oph* gene, responsible for their enzymatic degradation. The use of this gene and its associated enzyme help scientists better understand the microbe-pesticide interaction involved in the process of biodegradation (Gao *et al.*, 2012). It is, therefore, important to implement genomics research programs that focus on the development of innovative technologies that can lead to improved pesticide degradation.

RECOMMENDATION 4

Using genetic engineering, study the role played by microorganisms in the degradation of pesticides focusing on the level of enzyme expression specific to the degradation.

3.3. Genomics tools to support decision making in integrated management strategies

Microbial activity in a given environment is an important factor to consider when developing integrated management strategies. Genomics tools can be used to support optimal decision-making in agriculture when it comes to the use of pesticides, with the ultimate goal of significantly reducing their utilization at the source. PCR and qPCR tools and Sanger sequencing are employed in diagnostic labs to help agricultural producers select their pesticide treatments.

Generally speaking, the use of genomics tools is showing strong growth, but in case of doubt, traditional methods of analysis remain a reliable resource for supporting genomics test results. Conversely, genomics tools can be used to complement results obtained with traditional methods. In entomology, for example, visual identification is the commonly used approach. In some cases though, it can only identify the genus. PCR, as it turns out, is the tool of choice for a more accurate taxonomic identification. It can be used to characterize a species at any stage of its development, thus avoiding the need to monitor its development to the adult stage. PCR can also be used to identify a resistance gene in an insect, which can help producers select the best pest control product.

In Québec, fungal diseases attack a number of fruits and vegetables. To detect these diseases as soon as they start, certain growers use spore “traps,” mainly for sensitive crops, such as lettuce, strawberries and onions. When spores are found, a PCR test can determine the presence and quantity of the fungal species. These results can serve as a starting point for making the right decision about treatment. In terms of reducing pesticide use, the treatment recommended will be proportional to the severity of the infestation. If it is low, no intervention will be needed; alternatively, biocontrol or chemical treatment measures may be required. Given that these technologies are relatively new, interpreting results and making the right decisions are often very challenging.

Captors are also an interesting option in weed science to identify resistance genes from pollen collected, leading to the selection of the most suitable treatments. This type of screening does, however, have certain limitations, for instance when several mutations account for the resistance. In such cases, recourse to traditional measures is needed to validate the data collected with the genomics tool, involving multiple tests and, in turn, higher costs.

RECOMMENDATION 5

Implement a program to detect resistant genes in different crops in order to target and adapt the interventions to be carried out.

In soil science, genomics tools can also support decision making in agricultural businesses. The microbial biomass is an important indicator that provides insight into the relationship between microorganism activity and the transformation of nutrients and other processes inherent in the functioning of ecosystems (Schultz *et al.*, 2008). First, metagenomics analysis can identify one or more microorganisms associated with an ecosystem trait (e.g., productive or suppressive soil or one containing toxic elements). The specific function of the microorganisms is not the most important factor, but what does matter is the impact of their presence in the ecosystem. When microorganisms representative of the state of an ecosystem are at a detection threshold, they serve as soil health indicators. The goal is not to understand all the interactions that account for soil functioning, but rather to find positive or negative correlations between communities that can

serve as indicators for agricultural functions. The assessment of soil health provides a comprehensive, holistic understanding of the impact of certain agricultural practices on agroecosystem balance. Ultimately, reaching this state of balance will lead to a more rationalized use of pesticides.

RECOMMENDATION 6

Develop soil health indicators prioritizing the use of metagenomics to analyze samples in order to identify one or more microorganisms representative of a given state of soil health.

Alongside the identification of microbial indicators derived from metagenomic analysis, the use of specific tools (PCR, qPCR) is essential to determining whether specific communities reach a certain threshold – or harmfulness level. These tools help to better interpret certain phenomena, in conjunction with other disciplines, such as biochemistry. By employing genomics tools associated with specific molecular markers, indicator microorganisms can be identified without recourse to metagenomic analysis each time, reducing cost and testing response time.

In Québec, an extensive network of unaffiliated agricultural consultants, the Réseau Agriconseils, offers independent consulting services tailored to the needs of agricultural businesses. Since the science of genomics has a special place in agricultural business, professionals need adequate training in the use of indispensable genomics tools. The Réseau d'avertissement phytosanitaire, whose role is to inform producers on the threats to their crops and on strategies to address them, could benefit from genomics tools to improve the information provided to producers.

In terms of disseminating knowledge to those on the ground, it would be interesting to offer agricultural producers training on the use of genomics tools to guide their choice of agro-environmental practices and plant protection strategies. Moreover, the option of providing financial support to integrate these tools into agricultural businesses could also be considered.

RECOMMENDATION 7

Promote the use of genomics tools, such as PCR and qPCR, among stakeholders working on the ground and provide them with appropriate training.

RECOMMENDATION 8

Offer interested agricultural producers training on the proper use of genomics tools and provide them with financial support as they integrate these tools into their business.

3.4. Advances in genomics in terms of biocontrol

Most pesticides used in agriculture are of synthetic origin. The repeated utilization of a few groups of pesticides against certain pests and weeds has led to the development of resistance in some, not to mention the accumulation of toxic residues in the environment (Nawaz *et al.*, 2016). To combat the pesticide-resistant pests recorded each year around the world, new pesticides need to be synthesized. However, it is becoming increasingly difficult to develop new products for many of the crops (Borel, 2017).

To address the problem, biopesticides represent an interesting solution. These pesticides are now part of several pest management programs. They are made from bacteria, fungi, algae, viruses, nematodes and certain metabolites produced by these microorganisms (Lenteren, 2012). There are currently some 3,000 microbes that can cause diseases in insects, 100 of which are bacteria, such as *Bacillus thuringiensis* (BT), which play an important role as a microbial agent (Nawaz *et al.*, 2016).

There are many advantages to using microbial pesticides. Bioactive agents are not toxic for the environment and most are not harmful to the organisms not targeted. In other words, they have no impact on useful entities, such as pollinating or predatory insects. Biopesticides can be employed with synthetic insecticide, which in most cases does not neutralize their compounds. At times, the microorganisms that make up the biopesticide end up settling in the ecosystem; there, they can promote the growth of plants through their beneficial action on soil microflora over several seasons (Jindal *et al.*, 2013). Biopesticides, however, are not a panacea, since some of them – for instance, Bti to manage mosquito larvae – is not selective, thus potentially having significant repercussions on the environment (Brühl *et al.* 2020).

Developing biopesticides using genomics tools primarily involves bacteria, viruses and fungi. Viral biopesticides, for example, those derived from baculoviruses, offer many advantages as pest control tools: they do not harm vertebrates and plants and lend themselves well to gene editing. Yet they do have one major drawback: they are very slow at destroying the targeted organisms. To address this issue, genomics tools have been employed to insert genes coding for the making of insect-specific enzymes, toxins and hormones (Gramkow *et al.*, 2010).

As is the case with bacteria and viruses, there is a wide variety of fungi recognized for their insecticidal effect. Unlike bacteria and viruses, which must be consumed by pests, entomopathogenic fungi (that is, fungus harmful to insects) are toxic when the insect's outer layer comes into contact with the conidia (fungal spore). The entire insect and foliage must be covered. Approximately 80% of entomopathogenic fungi are part of the *Metarhizium* and *Beauveria* genera (Butt *et al.*, 2016). They occur naturally in soil in many habitats around the world (Zimmerman, 2007). Genetic engineering combined with an enhanced understanding of fungi pathogenicity and ecology offers interesting opportunities to improve the efficacy and cost effectiveness of mycoinsecticides by strengthening their tolerance to environmental stress and their virulence (Lovett *et al.*, 2018).

A more holistic approach to preventing the degradation of agroecosystems involves the use of biostimulants to improve soil biodiversity. Biostimulants keep the soil environment rich in all sorts of macro and micro elements via nitrogen fixation, the solubilization and mineralization of phosphorus and potassium, the release of plant-growth hormones, the production of antibiotics and the degradation of organic matter (Sinha *et al.*, 2014). All these processes derive from interactions among the different microbial communities that surround roots. In this way, these microorganisms contribute to mechanisms that improve the bioavailability of nutrients, thus enhancing plant growth.

In some cases, biostimulants are composed of genetically engineered bacteria. Using various recombinant DNA technologies, one or several genes can be altered or introduced inside the bacteria. These modified bacteria can then either do things that their indigenous parental strains could not do or be more efficient at doing it (Garcia-Fraile *et al.*, 2015). Many of these strains work well in the lab, but once subjected to natural conditions in soils, they sometimes fail to play their role as boosters of plant productivity. This is because the genetically modified strains in biostimulants are unable to interact with indigenous soil microorganisms in order to obtain the

nutrients essential for their metabolism (Garcia-Fraile *et al.*, 2015). It could be possible, however, to introduce into these strains an isolated gene from another bacterium, which would facilitate iron metabolism (chelation) (Joshi *et al.*, 2008). Gene editing is a promising avenue for the development of stable biostimulants that would be compatible with a wide range of soils and plants (Garcia-Fraile *et al.*, 2015).

RECOMMENDATION 9

Implement new biological control strategies through the identification or modification of certain microorganisms using metagenomics and genome editing technology.

3.5. Genomics to breed and optimize cultivars for a reduced use of pesticides

Due to the growing number of pathogens affecting plants, strategies are needed to improve crop resistance. In recent decades, genomics tools have been helping to rapidly identify desirable and undesirable genes so that the plants most resistant to various pests could be bred. These strategies call for the integration of genomics into the different plant breeding programs. The genomics tools used will differ depending on whether the plant's resistance is controlled by one or more genes with a substantial impact on a phenotypic trait or by several genes with a mitigated impact on the same phenotypic trait, in this case, resistance to disease.

Major advances in molecular biology have prepared the way for the development of transgenic plants with new genetic properties. Their use has rapidly spread worldwide; corn, soy, potatoes, tomatoes and cotton are a few examples (Babu, 2003). A 2014 study has shown that the use of transgenic plants has led to a notable drop in pesticide use (37%) and a rise in yields (22%) worldwide (Klümper and Qaim, 2014). However, in addition to the ethical issues they raise, GMOs are not suitable for all types of farming, for instance, biological farming, an agricultural approach experiencing tremendous growth around the world. Moreover, the public's lack of understanding of these new technologies has resulted in mixed reviews in developed countries, including Canada (McFadden and Smyth, 2018).

According to experts consulted, in Québec very little is done by way of developing new varieties, including plants with better resistance. The process is not only very expensive, but also requires a long period of time. Every year, many new cultivars arrive on the market, but these are not necessarily more resistant to disease. They have rather been bred for better performance or faster growth, making them a more interesting economic option compared to resistant cultivars. Another drawback is that resistance can also be at the cost of other positive characteristics, such as flavour or other culinary properties, which are valued by consumers.

Genomics science can assist agricultural producers in selecting cultivars and strains. Choosing a cultivar suited to the specific farming conditions (e.g., growing method, soil type, past plant protection practices) is an important factor when attempting to reduce pesticide use. The genetic characterization of cultivars and strains well suited to Québec could help agricultural producers make the best choice for their specific farming needs. At the moment, seed guides are provided by the companies that produce them. Developing an independent guide could help to provide additional information by way of improving farming protocols. Such a document could feature a wider range of cultivars promoting the genetic biodiversity of crops and a reduced use of pesticides.

RECOMMENDATION 10

Using genomics-based tools, produce an independent seed guide featuring phylogenetic biodiversity well suited to the soil and climate conditions in different regions of Québec.

4. Obstacles to the use of genomics tools in agriculture

Microbial analysis has undeniable advantages when it comes to understanding ecosystem dynamics. However, the high cost of certain equipment, the expertise required to analyze and interpret the data, in addition to the lengthy wait time before receiving results, all make it difficult, at the moment, to implement an efficient network of laboratories. In addition, since genomics tools evolve very rapidly, it is often difficult to have the right in-house expertise for the advanced interpretation of results.

RECOMMENDATION 11

Set up a network of public laboratories with the required instrumentation and expertise to offer a rapid results analysis service in support of sound decision-making.

Another challenge involves the use of certain sophisticated tools and specific approaches needed for the analysis of different organisms. Employing traditional methods to validate some of the results of genomics is common practice; but this proliferation of testing leads to considerably higher costs. Some technologies related to specific and often complex tests require a major investment, and, for certain labs, the workforce is simply insufficient. All these limitations can have an impact on the implementation of genomics analyses in a laboratory.

RECOMMENDATION 12

Promote cooperation among various groups of researchers who will implement standardized protocols on the use of genomics tools to be followed across laboratories.

Despite the undeniable benefits of metagenomics, the discipline is often faced with a lack of experts to interpret results. This complex molecular tool can sequence the entire DNA in a community and define its components. But the variations observed in microbial communities following exposure to environmental stressor (e.g., a pesticide) are difficult to interpret in terms of understanding their relationship to ecosystem functioning. The accuracy of sequencing using metagenomics, combined with the need to interpret databases, means that analyzing the results requires advanced expertise, which is not always available. Together, these factors make the technique very expensive to use. To simplify matters, metagenomics could be used to provide a preliminary overview to identify, as a first step, the organisms that react to pesticides. Simpler tools, such as PCR, could subsequently be employed to target the desired genes previously identified.

Another challenge associated with this discipline is access to databases. Organizations and research centres often have relevant data, which are not, however, available to other groups. To establish accurate and sizeable databases, collaboration among the various stakeholders is essential. While raw genomics data from academic research are available in public directories, accessing them can prove burdensome. Despite the trend toward more and more open public access to databases, the sharing of certain information, particularly biological data, seems to remain a problem.

RECOMMENDATION 13

Facilitate collaboration among various research groups to implement public-access databases available to all stakeholders in agriculture.

5. Conclusion

Despite their current drawbacks, modern molecular techniques have led to a major breakthrough in our understanding of microbial dynamics in ecosystems. They help us, among other things, to better grasp the magnitude of the threats from human activity by measuring their impact on microbial communities (Birrer *et al.*, 2017). Molecular approaches have shed light on several facets of the functioning of various previously unknown microbe species, allowing us today to conduct more advanced research into the effects of contaminants on ecosystems (Gibson *et al.*, 2015).

Advances in molecular biology have paved the way for the development of genomics tools to support the agricultural industry as it faces phytosanitary challenges, particular in terms of making optimal use of pesticides. As outlined in this report, their applications are many. Genomics tools, which can be used to identify pathogens and their virulence genes or facilitate early screening as part of integrated pest management programs, can come alongside actions already taken to reduce pesticide use. Genomics science can also contribute to the development of more resistant cultivars or promote the creation of biostimulants and biopesticides using genetic engineering. Lastly, genomics tools are a strong ally in the study of agroecosystems. They can be employed to characterize microorganisms present in agricultural soils, to implement biological indicators for the assessment of soil quality or to aid our understanding of the mechanisms of pesticide degradation in the environment.

Their use, however, should not be considered a panacea. Genomics tools can only identify and monitor genes whose function is very specific. They do not always take into account the overall dynamics at play in all ecosystems. This means they must be part of a wider attempt at understanding ecosystems. Genomics tools, therefore, must be integrated into a comprehensive agricultural management strategy, which includes, among other things, crop rotation and monitoring, plant residue recycling, biocontrol of pathogens and insects and cultivar selection.

6. Bibliography

- Abdelfattah, A., Malacrino, A., Wisniewski, M. Santa O. Cacciola, S. O., Schena, L. (2018). Metabarcoding: A powerful tool to investigate microbial communities and shape future plant protection strategies. *Biological Control*, 120,1-10.
- Alexandratos, N., and J. Bruinsma. (2012). World agriculture towards 2030/2050: the 2012 revision. *ESA Working paper* No. 12-03. FAO, Rome.
- Allison, S. D., Martiny, J.B.H (2008). *Resistance, resilience, and redundancy in microbial communities. Proceedings of the National Academy of Sciences*,105,11512–11519.
- Andolfo, G., Lovieno, P., Frusciante, L. and Ercolano, M. R. (2016). Genome-Editing Technologies for Enhancing Plant Disease Resistance. *Front. Plant Sci.* doi: 10.3389/fpls.2016.01813
- Babu, R., Sajeena, M. A., Seetharaman, K., Reddy, M. S. (2003). Advances in genetically engineered (transgenic) plants in pest management—an overview. *Crop Protection*, 22, 1071-1086.
- Balint, M., et al. (2013). Host genotype shapes the foliar fungal microbiome of balsam poplar (*Populus balsamifera*). *PLoS ONE*, 8(1), 53987.
- Barnard, R., Leadley, P. W. and Hungate, B. A. (2005). Global change, nitrification, and denitrification: a review. *Global Biogeochemical Cycles*, 19.
- Berdy, B., Spoering, A. L., Ling, L. L. and Epstein, S. S. (2017). *In situ* cultivation of previously uncultivable microorganisms using the ichip, *Nature Protocols*, 12(10), 2232-2242.
- Birrer, S. C., Dafforn, K. A., and Johnston, E. L. (2017). Microbial community responses to contaminants and the use of molecular techniques, in Cravo-Laureau, C., Cagnon, C., Lauga, B. and Duran, R. (Eds.). *Microbial Ecotoxicology*, 165-183. Cham, Switzerland: Springer, Springer Nature. https://doi.org/10.1007/978-3-319-61795-4_8
- Brasier, C.M. (2008). The biosecurity threat to the UK and global environment from international trade in plants. *Plant. Pathol.* 57, 792-808.
- Brühl, C. A., Després, L., Frör, O., Patil, C. D., Poulin, B., Tetreau, G., Allgeier, S. (2020). Environmental and socioeconomic effects of mosquito control in Europe using the biocide *Bacillus thuringiensis* subsp. *israelensis* (Bti). *Science of The Total Environment*, 137800. doi:10.1016/j.scitotenv.2020.137800
- Borel, B. (2017). When the pesticides run out. *Nature*, 543, 302-304.
- Butt, T. M., Coates, C. J., Dubovskiy, I. Mand Ratcliffe, N. A. (2016). Entomopathogenic fungi: new insights into host-pathogen interactions, in *Advances in Genetics*, ed. by Lovett B, St. Leger R.J. Elsevier, London, pp. 307-364.
- Carles, L. (2016) Devenir de mélanges de pesticides : étude des voies de biodégradation et développement d'une méthode préventive de bioremédiation. Thèse de doctorat en sciences agricoles. Université Blaise Pascal-Clermont-Ferrand II, France.
- Carvalho, F. P. (2006). Agriculture, pesticides, food security and food safety. *Environ. Sci. Policy*, 9,685-692.
- Carvalho, F. P. (2017). Pesticides, environment, and food safety. *Food and Energy Security*, 6(2), 48-60.
- Chaplin-Kramer, R., Sharp, R.P., Weill, C., Bennett, E.M., Pascual, U., Arkema, K.K., Brauman, K.A., Bryant, B.P., Guerry, A.D., Haddad, N.M., Hamann, M., Hamel, P., Johnson, J.A., Mandle, L., Pereira, H.M., Polasky, S., Ruckelshaus, M., Shaw, M.R., Silver, J.M., Vogl, A.L., Daily, G.C. (2019). Global modeling of nature's contributions to people. *Science*, 366, 255-258.
- Chi-Chu, L. (2010), Effect of pesticides on soil microbial community. *Journal of Environmental Science and Health, Part B*, 45 (5), 348-359.
- Comtet, T., Sandionigi, A., Viard, F., Casiraghi, M. (2015). DNA (meta) barcoding of biological invasions: a powerful tool to elucidate invasion processes and help managing aliens. *Biol. Invasions*, 17, 905-922.

- Crouzet, O., Poly, F., Bonnemoy, F., Bru, D., Batisson, F., Bohatier, J., Philippot, L. and Mallet, L. (2016). Functional and structural responses of soil N-cycling microbial communities to the herbicide mesotrione: a dose-effect microcosm approach; *Environ Sci Pollut Res*, 23, 4207-4217.
- Delcour, I., Spanoghe, P., Uyttendaele, M. (2015). Literature review: impact of climate change on pesticide use. *Food Res. Int.*, 68, 7-15.
- Dequiedt, S., Saby, N. P. A., Lelievre, M. et al. (2011). Biogeographical patterns of soil molecular microbial biomass as influenced by soil characteristics and management. *Glob. Ecol. Biogeogr.* 20, 641-652.
- Edge, K. J., Dafforn, K. A., Simpson, S. L. et al. (2015). Resuspended contaminated sediments cause sublethal stress to oysters: a biomarker differentiates total suspended solids and contaminant effects. *Environ. Toxicol. Chem.*, 34, 1345-1353.
- Epstein, S. S. (2013). The phenomenon of microbial uncultivability. *Curr. Opin. Microbiol.* 16, 636-42.
- Feld, L., Hjelmsø, M. H., Nielsen, M. S., Jacobsen, A. D., Ronn, R., Ekelund, F., Krogh, P. H., Strobel, B. W., Jacobsen, C. S. (2015). Pesticide side effects in an agricultural soil ecosystem as measured by amoA expression quantification and bacterial diversity changes. *PLoS ONE*, 10, e0126080.
- Gao, Y., Chen, S. H., Hu, M. Y., Hu, Q. B., Luo, J. J., Li, Y. N. (2012). Purification and characterization of a novel chlorpyrifos hydrolase from *Cladosporium cladosporioides* Hu-01. *PLoS ONE*. 7, e38137.
- Garbarino, J. R., Snyder-Conn, E., Leiker, T. J. and Hoffman, G. L. (2002). Contaminants in arctic snow collected over northwest Alaskan sea ice. *Water Air Soil Pollut.* 139, 183–214.
- Garcia-Fraile, P., Menendez, E., Rivas, R. (2015). Role of bacterial biofertilizers in agriculture and forestry; *AIMS Bioengineering*. 2(3), 183-205.
- Gibson, J. F., Shokralla, S., Curry, C. et al. (2015) Large-scale biomonitoring of remote and threatened ecosystems via high-throughput sequencing. *PLoS ONE*, 10, e0138432.
- Goddard, E., Muringai, V., and Boaitay, A. (2018). Food Integrity and Food Technology Concerns in Canada: Evidence from Two Public Surveys. *Journal of Food Quality*, 2018, 1–12. doi:10.1155/2018/2163526
- Gramkow, A. W., Perecmanis, S., Sousa, R. L. B., Noronha, E. F., Felix, C. R., Nagata, T. et al. (2010). Insecticidal activity of two proteases against *Spodoptera frugiperda* larvae infected with recombinant baculoviruses. *Virology*, 29(7), 43.
- Huang Y., Xiao L., Wu Z. (2018). Microbial Degradation of Pesticide Residues and an Emphasis on the Degradation of Cypermethrin and 3-phenoxy Benzoic Acid: A Review. *Molecules*, 11(23), 9.
- Hussain, S., Siddique, T., Saleem, M., Arshad, M. and Khali, A. (2009). Impact of Pesticides on Soil Microbial, Diversity, Enzymes, and Biochemical Reactions. *Advances in Agronomy*, 102, 159-200.
- Imfeld, G., Vuilleumier, S. (2012). Measuring the effects of pesticides on bacterial communities in soil: A critical review. *European Journal of Soil Biology*. 49, 22-30.
- Lovett, B. and St. Leger, R. J., (2018). Genetically engineering better fungal biopesticides. *Pest Manag. Sci.*, 74, 781-789.
- Jamieson, A. J., T. Malkocs, S. B. Piertney, T. Fujii, and Z. Zhang. (2017). Bioaccumulation of persistent organic pollutants in the deepest ocean fauna. *Nat. Ecol. Evol.* 1, 0051.
- Ji, X., Zhang, H., Zhang, Y., Wang, Y., and Gao, C. (2015). Establishing a CRISPR/Cas-like immune system conferring DNA virus resistance in plants. *Nat. Plants*, 1, 15144. doi: 10.1038/nplants.2015.144.
- Jindal, V., Dhaliwal, G. S., Koul, O. (2013). Pest Management in 21st century: Roadmap for future. *Biopestic. Int.* 9 (1), 1-22.
- Johnsen, K., Jacobsen, C. S., and Torsvik, V. (2001). Pesticide effects on bacterial diversity in agricultural soils—A review. *Biol. Fertil. Soils* 33, 443-453.
- Johnston, E. L., Mayer-Pinto, M., Crowe, T. P. (2015). REVIEW: chemical contaminant effects on marine ecosystem functioning. *J. Appl. Ecol.*, 52, 140-149.

- Joshi F., Chaudhari A., Joglekar P., et al. (2008). Effect of expression of Bradyrhizobium japonicum 61A152 fegA gene in Mesorhizobium sp., on its competitive survival and nodule occupancy on Arachis hypogea. *Appl. Soil Ecol.*, 40, 338-347.
- Karpouzias, D. G., Tsiamis, G., Trevisan, M., Ferrari, F., Malandain, C., Sibourg, O., Martin-Laurent, F. (2016). Love to hate pesticides: felicity or curse for the soil microbial community? *Environ. Sci. Pollut. Res.*, 23, 18947-18951.
- Kinsella, C. M., Crowe, T. P. (2016). Separate and combined effects of copper and freshwater on the biodiversity and functioning of fouling assemblages. *Mar. Pollut. Bull.*, 107, 136-143.
- Klümper, W. and Qaim, M. (2014). A Meta-Analysis of the Impacts of Genetically Modified Crops. *PLoS ONE*, 9, e111629.
- Kumar S., Kaushik G., Dar M.A., Nimesh S., L'opez-Chuken U. J., Villarreal-Chiu J. F. (2018). Microbial Degradation of Organophosphate Pesticides: A Review. *Pedosphere.*, 28(2), 190-208.
- Lerch, T. Z., Dignac, M. F., Nunan, N., Barriuso, E., Mariotti, A. (2009). Ageing processes and soil microbial community effects on the biodegradation of soil 13C-2,4-D nonextractable residues. *Environ. Pollut.*, 157, 2985-2993.
- Lewis, S. E., Brodie, J. E., Bainbridge, Z. T., Rohde, K. W., Davis, A. M., Masters, B. L. et al. (2009). Herbicides: a new threat to the Great Barrier Reef. *Environ. Pollut.*, 157, 2470-2484.
- Massart, S., Martinez-Medina, M., Jijakli, M.H. (2015). Biological control in the microbiome era: challenges and opportunities. *Biol. Control*, c89, 98-108.
- McFadden, B. R. and Smyth, S. J. (2018). Perceptions of Genetically Engineered Technology in Developed Areas. *Trends in Biotechnology*. doi:10.1016/j.tibtech.2018.10.006
- McKnight, U. S., Rasmussen, J. J., Kronvang, B., Binning, P. J. and Bjerg, P. L. (2015). Sources, occurrence and predicted aquatic impact of legacy and contemporary pesticides in streams. *Environ. Pollut.*, 200, 64-76.
- Mendes, R., Garbeva, P., Raaijmakers, J. M. (2013). The rhizosphere microbiome: significance of plant beneficial, plant pathogenic, and human pathogenic microorganisms. *FEMS Microbiol Rev*, 37, 634-663.
- Muringai, V., Fan, X, Goddard, H. (2020). Canadian consumer acceptance of gene-edited versus genetically modified potatoes: A choice experiment approach. *Canadian Agricultural Economics Society*, 68, 47-63.
- Nawaz, M., Mabubu, J. I., Hua, H. (2016). Current status and advancement of biopesticides: Microbial and botanical pesticides, *Journal of Entomology and Zoology Studies*, 4(2), 241-246.
- Nøstbakken, O., Hove, H., Duinker, A., Lundebye, A., Berntssen, M., Hannisdal, R. et al. (2015). Contaminant levels in Norwegian farmed Atlantic salmon (*Salmo salar*) in the 13-year period from 1999 to 2011. *Environ. Int.* 74, 274-280.
- Philippot, L. and Hallin, S. (2005). Finding the missing link between diversity and activity using denitrifying bacteria as a model functional community. *Curr. Opin. Microbiol.* 8, 234-239.
- Puglisi, E., Vasileiadis, S., Demiris, C., Bassi, D., Karpouzias, D. G., Capri, E., et al. (2012). Impact of fungicides on the diversity and function of non-target ammonia-oxidizing microorganisms residing in a litter soil cover. *Microb. Ecol.*, 64, 692-701.
- Rand, G. (1995). Fundamentals of aquatic toxicology: effects, environmental fate and risk assessment. CRC Press, Boca Raton, Florida, USA.
- Rappé, M. S. and Giovannoni, S. J. (2003). The uncultured microbial majority. *Annu. Rev. Microbiol.*, 57, 369-94.
- Reeves, W. R., McGuire, M. K., Stokes, M., Vicini, J. L. (2019). Assessing the Safety of Pesticides in Food: How Current Regulations Protect Human Health. *Advances in Nutrition*. doi:10.1093/advances/nmy061
- Santos, V. B., Araujo, S. F., Leite, L. F., Nunes, L. A., Melo, J. W. (2012). Soil microbial biomass and organic matter fractions during transition from conventional to organic farming systems. *Geoderma*, 170, 227-231.

- Sanzani, S.M., Li Destri Nicosia, M.G., Faedda, R., Cacciola, S.O., Schena, L., (2014). Use of quantitative PCR detection methods to study biocontrol agents and phytopathogenic fungi and oomycetes in environmental samples. *J. Phytopathol.*, 162, 1-13.
- Scanes, P., Coade, G., Doherty, M., Hill, R. (2007). Evaluation of the utility of water quality based indicators of estuarine lagoon condition in NSW, Australia. *Estuar. Coast Shelf. Sci.*, 74, 306-319.
- Schultz, P., and Urban, N. R. (2008). Effects of bacterial dynamics on organic matter decomposition and nutrient release from sediments: A modeling study. *Ecol. Model.*, 210, 1-14.
- Schipper, L. A., Degens, B. P., Sparling, G. P., Duncan, L.C. (2001). Changes in microbial heterotrophic diversity along five plant successional sequences. *Soil Biol. Biochem.*, 33, 2093-2103.
- Sinha, R. K., Valani, D., Chauhan, K., Agarwal, S. (2014). Embarking on a second green revolution for sustainable agriculture by vermiculture biotechnology using earthworms: reviving the dreams of Sir Charles Darwin. *Int. J. Agric. Health Saf.*, 1, 50-64.
- Sun, M. Y., Dafforn, K. A., Brown, M. V., Johnston, E. L. (2012). Bacterial communities are sensitive indicators of contaminant stress. *Mar. Pollut. Bull.*, 6, 1029-10.
- Tang, W. (2018). Research Progress of Microbial Degradation of Organophosphorus Pesticides. *Prog. Appl. Microbiol.*, 29-35.
- Taylor, M. D., Klaine, S. J., Carvalho, F. P., Barcelo, D. and Everaarts, J. (Eds) (2003). Pesticide residues in coastal tropical ecosystems. Distribution, fate and effects. Taylor & Francis Publ., CRC Press, London. 576 pp.
- Torsvik, V., Goksoyr, J. and Daae, F. L. (1990). High diversity in DNA of soil bacteria. *Appl. Environ. Microbiol.*, 56, 782-787.
- UN (2015). United Nations, Department of Economic and Social Affairs, Population Division. World Population Prospects: The 2015 Revision, Key Findings and Advance Tables. Working Paper No. ESA/P/WP.241. United Nations, New York.
- Valcke, M., Bourgault, M.-H., Rochette, L., Normandin, L., Samuel, O., Belleville, D., Blanchet C, Phaneuf, D. (2017). Human health risk assessment on the consumption of fruits and vegetables containing residual pesticides: A cancer and non-cancer risk/benefit perspective. *Environment International*, 108, 63-74. doi:10.1016/j.envint.2017.07.023
- Wang, M. C., Gong, M., Zang, H. B., Hua, X. M., Yao, J., Pang, Y. J., and Yang, Y. H. (2006). Effect of methamidophos and urea application on microbial communities in soils as determined by microbial biomass and community level physiological profiles. *J. Environ. Sci. Health B*, 41, 399-413.
- Wessen, E. and Hallin, S. (2011). Abundance of archaeal and bacterial ammonia oxidizers—possible bioindicator for soil monitoring. *Ecol. Indic.*, 11, 1696-1698.
- Zimmerman, G. (2007). Review on safety of the entomopathogenic fungi *Beauveria bassiana* and *Beauveria brongniartii*. *Biocontrol Sci. Technol.*, 17 (6), 553-596.