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## The Ecobiomics project: Advancing metagenomics assessment of soil health and freshwater quality in Canada

Thomas A. Edge<sup>a</sup>, Donald J. Baird<sup>b,\*</sup>, Guillaume Bilodeau<sup>c</sup>, Nellie Gagné<sup>d</sup>, Charles Greer<sup>e</sup>, David Konkin<sup>f</sup>, Glen Newton<sup>g</sup>, Armand Séguin<sup>h</sup>, Lee Beaudette<sup>i</sup>, Satpal Bilkhu<sup>g</sup>, Alexander Bush<sup>b</sup>, Wen Chen<sup>g</sup>, Jérôme Comte<sup>a,p</sup>, Janet Condie<sup>f</sup>, Sophie Crevecoeur<sup>a</sup>, Nazir El-Kayssi<sup>g</sup>, Erik J.S. Emilson<sup>j</sup>, Donna-Lee Fancy<sup>f</sup>, Iyad Kandalaf<sup>g</sup>, Izhar U.H. Khan<sup>g</sup>, Ian King<sup>c</sup>, David Kreutzweiser<sup>j</sup>, David Lapen<sup>g</sup>, John Lawrence<sup>k</sup>, Christine Lowe<sup>g</sup>, Oliver Lung<sup>l</sup>, Christine Martineau<sup>h</sup>, Matthew Meier<sup>i</sup>, Nicholas Ogden<sup>m</sup>, David Paré<sup>i</sup>, Lori Phillips<sup>n</sup>, Teresita M. Porter<sup>j,q</sup>, Joel Sachs<sup>g</sup>, Zachery Staley<sup>a</sup>, Royce Steeves<sup>d</sup>, Lisa Venier<sup>j</sup>, Teodor Veres<sup>o</sup>, Cynthia Watson<sup>a</sup>, Susan Watson<sup>a</sup>, James Macklin<sup>g</sup>

<sup>a</sup> Environment and Climate Change Canada, Burlington, Ontario, Canada

<sup>b</sup> Environment and Climate Change Canada @ Canadian Rivers Institute, Department of Biology, University of New Brunswick, Fredericton, New Brunswick, Canada

<sup>c</sup> Canadian Food Inspection Agency, Ottawa, Ontario, Canada

<sup>d</sup> Fisheries and Oceans Canada, Moncton, New Brunswick, Canada

<sup>e</sup> National Research Council Canada, Montreal, Quebec, Canada

<sup>f</sup> National Research Council Canada, Saskatoon, Saskatchewan, Canada

<sup>g</sup> Agriculture and Agri-Food Canada, Ottawa, Ontario, Canada

<sup>h</sup> Natural Resources Canada, Quebec, Quebec, Canada

<sup>i</sup> Environment and Climate Change Canada, Ottawa, Ontario, Canada

<sup>j</sup> Natural Resources Canada, Great Lakes Forestry Centre, Sault Ste. Marie, Ontario, Canada

<sup>k</sup> Environment and Climate Change Canada, Saskatoon, Saskatchewan, Canada

<sup>l</sup> Canadian Food Inspection Agency, Winnipeg, Manitoba, Canada

<sup>m</sup> Public Health Agency of Canada, St. Hyacinthe, Quebec, Canada

<sup>n</sup> Agriculture and Agri-Food Canada, Harrow, Ontario, Canada

<sup>o</sup> National Research Council Canada, Ottawa, Ontario, Canada

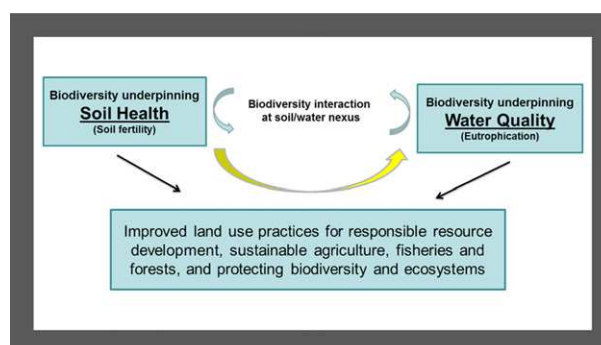
<sup>p</sup> Institut National de la Recherche Scientifique, Québec, Québec, Canada

<sup>q</sup> Biodiversity Institute of Ontario, University of Guelph, Ontario, Canada

### HIGHLIGHTS

- Ecobiomics Project applies metagenomics to assess soil health and water quality.
- Integrates metagenomic analysis of microbial and invertebrate communities
- Centralized sequencing and bioinformatics across seven departments and agencies
- Soil and aquatic microbiome, invert zoobiome projects and genomic observatories.
- Government-wide platform established to harmonize metagenomics of soil and water.

### GRAPHICAL ABSTRACT



\* Corresponding author.  
E-mail address: [djbaird@unb.ca](mailto:djbaird@unb.ca) (D.J. Baird).

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## ABSTRACT

Transformative advances in metagenomics are providing an unprecedented ability to characterize the enormous diversity of microorganisms and invertebrates sustaining soil health and water quality. These advances are enabling a better recognition of the ecological linkages between soil and water, and the biodiversity exchanges between these two reservoirs. They are also providing new perspectives for understanding microorganisms and invertebrates as part of interacting communities (i.e. microbiomes and zoobiomes), and considering plants, animals, and humans as holobionts comprised of their own cells as well as diverse microorganisms and invertebrates often acquired from soil and water. The Government of Canada's Genomics Research and Development Initiative (GRDI) launched the Ecobiomics Project to coordinate metagenomics capacity building across federal departments, and to apply metagenomics to better characterize microbial and invertebrate biodiversity for advancing environmental assessment, monitoring, and remediation activities. The Project has adopted standard methods for soil, water, and invertebrate sampling, collection and provenance of metadata, and nucleic acid extraction. High-throughput sequencing is located at a centralized sequencing facility. A centralized Bioinformatics Platform was established to enable a novel government-wide approach to harmonize metagenomics data collection, storage and bioinformatics analyses. Sixteen research projects were initiated under Soil Microbiome, Aquatic Microbiome, and Invertebrate Zoobiome Themes. Genomic observatories were established at long-term environmental monitoring sites for providing more comprehensive biodiversity reference points to assess environmental change.

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## 1. Introduction

Unprecedented environmental changes and impacts on biodiversity are occurring within natural and managed ecosystems around the world. These impacts are most visible for larger vertebrate species, whose ongoing loss has been described as a mass extinction event (Ceballos et al., 2017). However, much less is understood about the changes and impacts occurring at lower trophic levels such as the less visible level of microorganisms and invertebrates.

Microbial and invertebrate biodiversity support the maintenance of ecosystem functions which underpin soil health and water quality. This in turn supports the sustainable management of terrestrial and aquatic ecosystems, and thus important economic sectors ranging from agriculture to forestry and fisheries. Recent technological advances have made it possible to more comprehensively assess changes to the enormous biodiversity at lower trophic levels. Advances in genomics are now providing opportunities to better characterize the extent of biodiversity in soil and water, and detect changes based on a more comprehensive assessment of entire communities of microorganisms and invertebrates. It is important to understand the significance of changes and adverse impacts on these communities as they can have important implications for ecosystem services that sustain plant, animal, and human health, the health of ecosystems, and the local economies dependent on soil and water.

The Ecobiomics Project outlined here summarizes the model for how the Government of Canada has begun coordinating metagenomics research across departments and agencies to advance the integrated assessment, monitoring and remediation of soil and water resources. The Project was established in 2016 and is comprised of 64 scientists working across a range of government departments and agencies. We will use the term metagenomics as a composite term to include taxonomic applications of DNA metabarcoding, taxonomic and functional gene applications of shotgun metagenomics, and gene-expression applications of metatranscriptomics. We describe the Ecobiomics Project and outline our roadmap for incorporating metagenomics into soil health and water quality programs.

## 1.1. Soil health and water quality

Soil plays an essential role in sustaining diverse ecosystems from boreal forests to prairie grasslands, and diverse economic sectors from agriculture to forestry. However, current land management practices often result in an unsustainable loss of soil over time: one centimeter of soil can take many years to form, yet be eroded in days (Anon, 2017). Human pressures on soil resources are reaching critical limits,

resulting in ongoing environmental land-use conflicts and impacts on aquatic ecosystems (Valle Junior et al., 2015). Current estimates suggest that as much as a third of global soils are degraded and 24 billion tons of soil is lost each year (FAO, 2015; UNCCD, 2017). In Canada, it has been estimated that 68% of cropland in Ontario has a risk of erosion above the annual rate of soil regeneration, and that 82% of farmland in Ontario is losing organic matter important for soil health (Canada-Ontario, 2018).

Communities of microorganisms and invertebrates are an integral part of soil formation and retention, and they are a key determinant of soil health (Kvas et al., 2017). In fact, the enormous diversity of bacteria, fungi, protozoa, viruses, as well as invertebrates such as nematodes, collembolla, springtails, mites, insects, and earthworms in soil constitute a large portion of the world's biodiversity (Bardgett and van der Putten, 2014). This biodiversity contributes to soil health by physical structuring of soils through aggregation and aeration activities, nutrient cycling, carbon storage and transformations, as well as improving water retention and preventing soil erosion. Bacteria and fungi secrete polymeric substances that serve as binding agents for agglomerating soil particles, fungal hyphae contribute to holding aggregates together, and invertebrates such as earthworms can grind and remold ingested particles into new aggregates and create soil pores to enhance aeration and water infiltration (Tecon and Or, 2017; Lehmann et al., 2017). The microorganisms in soil are major drivers of soil fertility and plant nutrition, while also having the potential to provide disease suppressive properties that are important for protecting plant health and preventing productivity losses (Schlatter et al., 2017). They also contribute to elemental cycling, delivery of diverse ecosystem services, and soil security (McBratney et al., 2014).

Healthy aquatic ecosystems provide clean drinking water, sustain aquatic and terrestrial biodiversity and provide ecosystem services that support the fisheries and aquaculture sectors. Freshwater ecosystems have been considered more degraded than many other ecosystems, and in a state of crisis (Vörösmarty et al., 2010; UNWater, 2011; Garcia-Moreno et al., 2014). Changes to microbial communities in water can affect carbon, nutrient and contaminant cycling, lead to boil water advisories, drinking water outbreaks, closure of beaches and shellfish harvesting areas, harmful algal blooms, and adverse impacts on aquatic plant and animal health, fisheries and aquatic ecosystems. Changes to invertebrate communities can lead to adverse impacts on the food webs that sustain fish habitat and aquatic ecosystems. In Canada, a Threats Assessment conducted in 2001 identified 13 threats to sources of drinking water and aquatic ecosystem health (Environment Canada, 2001). Many of these threats to water quality such as algal blooms are still prominent today. For instance, it was

recently estimated that future harmful and nuisance algal blooms could cost the Canadian Lake Erie basin economy \$272 million annually (Canada-Ontario, 2018).

### 1.2. Limitations of current assessment methods

Much of the current assessment and monitoring of aquatic and soil ecosystem health is based on measuring standard physical and chemical parameters such as temperature, pH and concentrations of carbon, nitrogen and phosphorus. While these parameters are important, they do not allow for characterization of the living properties of soil and water, nor do they support a comprehensive integrated approach to land & water management. Biological parameters such as culture-based enumeration of fecal indicator bacteria, as well as microscope-based identification of algae and invertebrates have been important advances in characterizing the living properties of soil and water. However, many of these methods are decades-old, and they can have significant limitations for characterizing the complex biodiversity found in communities of microorganisms and invertebrates.

The limitations of culture-based methods have been recognized for many decades in microbiology, where standard laboratory culture conditions have been estimated to permit detection of less than 1% of microbes found in the environment (Hugenholtz, 2002). Dormancy in microbial communities is thought to be particularly common in soil environments, with estimates suggesting 80% of soil bacteria could be dormant, yet important as resilient microbial “seed banks” for sustaining soil functions (Tecon and Or, 2017). Commonly used culture-based assays for fecal indicator bacteria like *E. coli* are also recognized as often only weakly correlated to occurrence of other microorganisms such as waterborne protozoan and virus pathogens with different survival characteristics (Wu et al., 2011a; Edge et al., 2013). There have been numerous drinking water outbreaks where culture-based assays for fecal indicator bacteria did not adequately indicate human health risks (Rizak and Hruday, 2007). As well, existing microscope-based methods are laborious, difficult to reproduce at scale, and place limits on large-scale assessments of, for example, diatom diversity (Rimet et al., 2018) and harmful algal/cyanobacterial blooms (Steffen et al., 2014).

The limitations of existing methods are also apparent for assessing invertebrate biodiversity (Baird and Hajibabaei, 2012; Oliverio et al., 2018; Pawlowski et al., 2018). Important advances have been made to incorporate microscope-based characterizations of invertebrate assemblages in some areas such as stream biomonitoring programs using standardized kicknet sampling approaches (CABIN, 2018). However, microscope-based methods still have significant limitations as morphological identification of invertebrates can be hampered by the availability of taxonomic expertise, the variability between experts in taxonomic assignments, and taxonomic assignments that are typically focused on a relatively restricted set of better-known groups of invertebrates. Microscope-based methods may also not provide the taxonomic resolution needed for some invertebrate groups, and they may be incapable of identifying early life history stages or partially fragmented specimens in complex samples. The relatively high labour costs, and long delays for results, particularly for large-scale sampling efforts, are also limitations.

The limitations of many current methods for assessing microbial and invertebrate biodiversity mean that only the tip of the biodiversity “iceberg” may be seen in soil and water samples. This has important implications for environmental assessment, monitoring, and remediation programs. Are there significant differences between what is seen to be happening or changing at the tip of the biodiversity iceberg compared to the whole iceberg of microorganisms and invertebrates? How reliable are environmental impact assessments, soil health and water quality monitoring trends, and evaluations of soil and water remediation practices based on existing methods for microbial and invertebrate biodiversity assessment? It will be important to apply the best available tools for comprehensive assessment of changes occurring within complex microbial and invertebrate communities in soil and water.

## 2. A new metagenomics perspective

### 2.1. Advances in DNA sequencing and bioinformatics

The advent of second generation ‘massively parallel’ sequencing around 2005 opened the door to more widespread and cost-effective applications of genomics tools for assessing microbial and invertebrate biodiversity. Second generation sequencers such as the Illumina MiSeq and HiSeq platforms are capable of massively parallel sequencing of short DNA fragments with high sequencing accuracy. In addition, emerging third generation sequencing such as Oxford Nanopore and Pacific Biosciences sequencing platforms are providing new opportunities to generate much longer sequence lengths. These sequencing advances are placing genomics in the realm of “Big data” science, with future needs for data management likely to be as large as, or greater than for sectors such as astronomy, YouTube, and Twitter (Stephens et al., 2015). In parallel with improvements in sequencing, synergistic advances in high performance computing, software tools, and Internet resources have been needed for bioinformatics capabilities to meet data challenges. Continued advances in bioinformatics, including data storage, analysis and visualization, will be needed in the future as the ability to extract meaningful information from growing DNA sequence datasets is recognized as a bottleneck for advancing metagenomics applications (Thompson et al., 2017).

Metagenomics technologies offer a potential solution to the challenge of comprehensive characterization of the complex microbial and invertebrate biodiversity found in soil and water. It is now feasible to extract nucleic acids from an entire microbial community in a soil or water sample and sequence the DNA or RNA as part of metabarcoding, metagenomics or metatranscriptomics characterization of the sample. Similarly, nucleic acids can be extracted from complex invertebrate communities once the organisms are separated from soil or water samples. More broadly, profiling of environmental DNA (eDNA) from soil or water samples through metabarcoding applications can be applied to detect the DNA of invertebrate and vertebrate species directly from soil or water DNA extracts without having to first separate organisms or tissues from their environmental matrix (Taberlet et al., 2012; Deiner et al., 2017).

While there have been significant advances in sequencing and bioinformatics in recent years, there are still challenges to address. For example, metagenomics techniques are still unable to provide complete characterization of all microbial and invertebrate biodiversity in soil and water samples (Elbrecht and Leese, 2015; Nesme et al., 2016; Rodriguez et al., 2018; Oliverio et al., 2018; Pawlowski et al., 2018). There are challenges for how to address relic DNA in soil (Lennon et al., 2018), and to ensure low numbers of microbes in the rare biosphere are not the result of sequencing errors (Lynch and Neufeld, 2015). In addition, many DNA sequences from environmental samples still cannot be taxonomically identified. Thompson et al. (2017) found less than 50% of 16S rRNA gene sequences from soil, water and other environmental samples from the Earth Microbiome Project were a good match to known bacterial taxa in the Greengenes or SILVA reference 16S rRNA gene databases.

Despite challenges such as these, advances in metagenomics have provided a significant leap forward in providing new knowledge and perspectives for more scientifically-defensible assessments of biodiversity to protect soil health and water quality. Metagenomics analyses will also benefit in the future from parallel barcoding initiatives like the International Barcode of Life Project (<http://ibol.org/>) and whole genome sequencing initiatives to expand reference databases for microorganisms (e.g., Kyprides et al., 2014) and invertebrates (e.g., GIGA, 2014). The Earth BioGenome Project, launched in 2017, has estimated that sequencing the genomes of ~1.5 million known eukaryote species (including fungi, protists and invertebrates), in addition to 100,000 new eukaryote species, could cost less than the costs (in today's dollars) for creating the first draft human genome sequence (Lewin et al., 2018). Lewin et al. suggested that investments in the Human Genome Project alone have



created an entire industry, with an estimated workforce of more than 47,000 people generating \$1 trillion in economic activity. It is still uncertain what additional benefits might arise from further investments in metagenomics to advance soil health and water quality decision-making.

## 2.2. Emerging perspectives for soil health and water quality

One of the first new perspectives from metagenomics has been a better understanding of the entirety of microbial communities, and the extent of microbial biodiversity in soil and water. For example, early shotgun metagenomics research led to the discovery of an enormous diversity of microbial species and genes within seemingly barren areas like the mid-ocean surface waters of the Sargasso Sea (Venter et al., 2004). Metabarcoding studies have more clearly shown the significance of soil as a reservoir and source for seeding microorganisms into stream, river, and lake ecosystems (Crump et al., 2012; Ruiz-Gonzales et al., 2015). This soil microorganism reservoir perspective raises the need to better understand how different land use and soil management practices change the soil microorganisms running off the land to impact aquatic ecosystems. Advances in metagenomics have also contributed to a growing recognition of the importance of microbial communities in the area immediately surrounding larger phytoplankton cells called the phycosphere, and which can be considered the aquatic analogue to the rhizosphere for a terrestrial plant (Seymour et al., 2017). Some of the phytoplankton-bacteria community interactions in the phycosphere can be considered as symbioses, with important implications for better understanding and predicting aspects such as primary production, biogeochemical cycling, and cyanobacterial toxin production. These advances from applications of metagenomics methods have enabled a better recognition that microorganisms occur in interacting communities, now increasingly referred to as microbiomes.

Microbiomes can be considered as the entire microbial community of bacteria, archaea, fungi, algae, protozoans, and viruses, including their genes and habitat, in a particular place. This perspective has been most advanced through the Human Microbiome Project (HMPC, 2012), where investigations of bacterial communities living on, and in, the human body are leading to transformations in the practice of medicine (Gilbert et al., 2018). However, a microbiome perspective also provides a new lens to look at the health of animals (Barhndorff et al., 2016) and plants (Vandenkoornhuys et al., 2015), as well as how we manage built environments (Gilbert and Stephens, 2018) and look more broadly at the ecology of health (Trinh et al., 2018). The ability to better characterize microbiomes in soil and water means we can now better recognize their importance in soil capital and water resource evaluations, and in assessments of soil health and water quality. It also means we can better investigate the significance of the acquisition of microorganisms from soil and water for plant, animal, or human health in a new perspective that sees plants, animals, and humans as holobionts, comprised of the host and its associated microorganisms. From this perspective, a plant, animal, or human is no longer seen as an autonomous entity, with an extrinsic microbiome. Instead, the host and its associated microorganisms can be seen as a unit of biological organization upon which ecology and evolution can act (Bordenstein and Theis, 2015). The implications of this perspective for medicine, the plant, animal, and environmental sciences, and our understanding of evolution is only starting to emerge.

Advances in metabarcoding have also enabled a new perspective for understanding the significance of the enormous biodiversity of invertebrate assemblages found in soil and water (Hajibabaei et al., 2011; Wu et al., 2011b). Next generation sequencing methods can now provide a more comprehensive assessment of invertebrate biodiversity, with potential for greater taxonomic breadth beyond coverage of well-studied groups, and greater taxonomic resolution down to the population level at times. The increased biodiversity information from metagenomics characterization of invertebrate samples has been described as a step change in capabilities from traditional biomonitoring methods, leading to a new paradigm of Biomonitoring 2.0 for ecosystem assessment

(Baird and Hajibabaei, 2012), which anticipates the availability of consistently-observed high-resolution biodiversity information to match with new earth observation methods, supporting more nuanced environmental diagnostics for complex global change situations. Dafforn et al. (2014), Gibson et al. (2015) and most recently, Bush et al. (2019) have provided strong evidence that biodiversity information generated through metabarcoding not only provides a step-change in the numbers of taxa observed, but also permits improved diagnosis of environmental degradation with enhanced statistical power (Bush et al., 2019 PREPRINT). In addition to providing a better understanding of the diversity and changes in invertebrate communities, advances in metagenomics provide an opportunity to scale-up and expand existing biomonitoring programs (Porter and Hajibabaei, 2018). In the Ecobiomics Project, we have used the term “zoobiome” to refer to all the individual invertebrates, including their genes and habitat, occurring in a particular place such as the benthos from a particular stream area.

## 2.3. The Canadian Genomics Research and Development Initiative

The Government of Canada's Genomics Research and Development Initiative (GRDI) coordinates genomics research and development activities across federal government departments and agencies in order to build genomics capacity and apply genomics research to support public policy objectives (GRDI, 2018). It does this in collaboration with other national and international genomics research providers, in particular with Genome Canada, a not-for-profit organization also funded by the Government of Canada. Each year since 2011, GRDI funds have been allocated to support two large and highly integrated shared priority projects that extend over five years. A governing interdepartmental Assistant Deputy Minister (ADM) Coordinating Committee selects these shared priority projects through a consultation process with an interdepartmental Working Group of policy advisors, and scientists nominated from departments and agencies. In 2015, the governing ADM Committee selected “Preventing the Spread of Antimicrobial Resistance” and “Protecting and Conserving Canada's Biodiversity and Ecosystems” as shared priority project directions to elaborate. Project Leads were identified, and scientists were nominated from departments and agencies to attend workshops and work towards elaborating each shared priority project. To focus the biodiversity and ecosystems priority project, scientists prepared short expressions of interest reflecting existing research interests and capacity. The Project Leads and scientists then proceeded to negotiate and elaborate a cohesive interdepartmental project that focused on applications of metagenomics to better characterize microbial and invertebrate communities for advancing the assessment of soil health and water quality: the Ecobiomics Project (short for ecosystem biomonitoring using metagenomics). For practical reasons, the Project had to limit its focus to water quality research on freshwater rather than marine ecosystems, and surface water rather than groundwater ecosystems.

## 3. The ecobiomics project

The Ecobiomics Project is a 5-year metagenomics project launched in April 2016. It is comprised of 64 scientists, biologists, postdoctoral fellows, technologists, and bioinformatics specialists, from seven federal departments and agencies ((Agriculture and Agri-Food Canada (AAFC), Canadian Food Inspection Agency (CFIA), Environment and Climate Change Canada (ECCC), Fisheries and Oceans Canada (DFO), National Research Council Canada (NRC), Natural Resources Canada (NRCan), and the Public Health Agency of Canada (PHAC)). The Project established an organizational structure and work Themes that are outlined in Fig. 1. A centralized DNA sequencing facility was used at the National Research Council in Saskatoon, Saskatchewan, and a centralized Bioinformatics Platform was established in a high-performance computing environment of Shared Services Canada in Dorval, Quebec. This established the ground floor of a harmonized approach to metagenomics data sharing and data analyses across the federal government. There are significant benefits of such

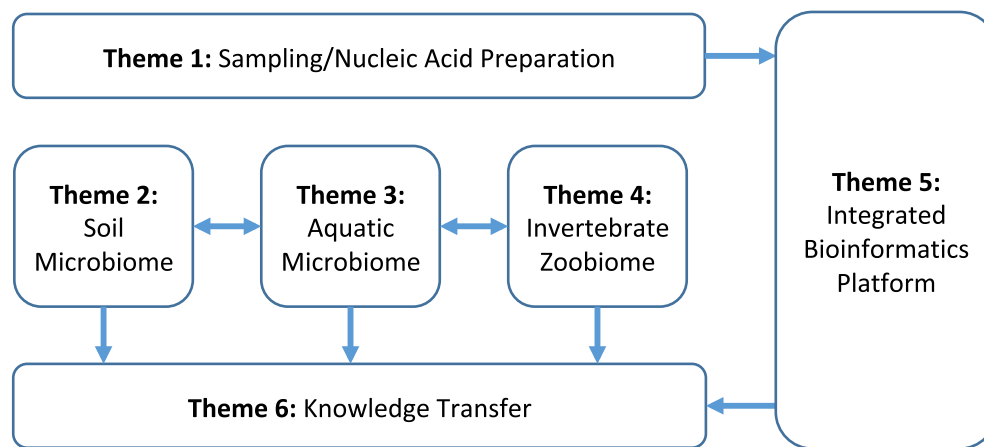


Fig. 1. The organizational structure of the Ecobiomics Project.

collaboration, where departments and agencies avoid the cost and complications of establishing separate bioinformatics platform silos. The Project has facilitated interdepartmental discussions about balancing the need for standardization with the need to avoid being too prescriptive for the evolving state of the science of metagenomics. It has also provided opportunities for multidisciplinary collaboration between soil and water scientists, and between microbiologists and invertebrate zoologists working at the soil-water interface. A Scientific Advisory Board (SAB) comprised of five members from Canada, the United States and the European Union is providing scientific advice.

### 3.1. Objectives

The Ecobiomics Project has three overarching objectives:

- Develop standard approaches and methods for collection of soil, water, and invertebrate samples, nucleic acid extraction, high-throughput DNA sequencing, bioinformatics analyses, and a Bioinformatics Platform for harmonizing metagenomics studies across federal departments and agencies;
- Pilot genomic observatories for establishing comprehensive metagenomics baselines to assess future changes to water and soil biodiversity at long-term environmental monitoring sites in Canada; and
- Generate new knowledge by applying high-throughput DNA sequencing to better characterize aquatic microbiomes, soil microbiomes, and invertebrate zoobiomes, and test hypotheses for improving environmental monitoring, assessment and remediation activities for water quality and soil health.

### 3.2. Sampling to sequencing

Meetings at the outset of the Project were important for reviewing sampling approaches for soil microbiomes, aquatic microbiomes, and invertebrate zoobiomes. These discussions led to early decisions on standardizing aspects like using 0.2 µm filters for filtering water samples for bacteria, archaea, and eukaryotes in aquatic microbiome studies, and collecting streambed invertebrate zoobiome samples using kicknet protocols already established by the Canada-wide CABIN network (CABIN, 2018). DNA extraction methods for soil and aquatic microbiomes were based on using the Qiagen DNEasy PowerSoil DNA isolation kits (formerly MoBio PowerSoil), consistent with the Earth Microbiome Project (2018). DNA extraction for invertebrate zoobiomes requires preliminary steps (i.e. homogenisation of a maximum of ~500 mL of semi-solid material, aliquoting, ethanol removal and extraction with the Qiagen DNEasy PowerSoil DNA kit). Additional research was initiated in parallel to: investigate a microfluidics approach for extracting DNA from larger soil samples; apply mock microbial communities and

invertebrate spiking to evaluate DNA extraction methods; and compare other DNA extraction methods for assessing biodiversity representativeness and potential method biases.

An Ecobiomics Project workflow was established (Fig. 2), and each Principal Investigator (PI) was responsible for sample collection and DNA extraction according to their project's experimental design. PIs forwarded DNA extracts to Theme Lead labs who were responsible for library preparation. This provided an additional level of workflow standardization across departments and agencies, and enabled the Theme Leads to coordinate cost-effective batching of samples to send for sequencing. For amplicon metabarcoding library preparation, PCR primers were selected for bacteria and archaea (16S v4–5 region with 515F–Y and 926R primers) consistent with the Earth Microbiome Project (<http://www.earthmicrobiome.org/protocols-and-standards/16s/>). Primers for fungi (ITS2 region with ITS9F and ITS4R primers) and other eukaryotes like algae (18S V4 region with primers 565F and 948R) were consistent with those used by the Joint Genome Institute (JGI, 2016). For invertebrate library preparation, a pooled primer approach was selected using primers F230 and F230R\_modN, and Befrag-B-F and Befrag-R5-R, consistent with Folmer et al. (1994) and Gibson et al. (2014), and Hajibabaei et al. (2011) respectively. Amplicon and shotgun metagenomics library preparations were based on protocols following Illumina manufacturer's instructions for MiSeq and HiSeq.

The centralized approach to sequencing was established by using a dedicated sequencing centre at NRC-Saskatoon to perform Ecobiomics Project sequencing. Amplicon metabarcoding sequencing is being conducted on the Illumina MiSeq platform. Shotgun metagenomics sequencing is being conducted on the Illumina HiSeq platform. We have focused on using the Illumina MiSeq and HiSeq sequencing platforms as they have emerged as the most widely used platforms to support metagenomics analyses (Caporaso et al., 2012; Tan et al., 2015; Ionescu et al., 2016) and they have served as the basis for large international initiatives such as the Earth Microbiome Project (2018). DNA sequence data from the sequencing centre are transferred to our Bioinformatics Platform where PIs are responsible for logging on to access their data, assessing needs for sequencing reruns, and conducting the analyses needed to interpret and visualize results.

### 3.3. Bioinformatics platform

The Bioinformatics Platform was established in the first year of the Ecobiomics Project to serve as a government-wide platform to harmonize collection, storage, and analysis of metagenomics data. It was developed at the General Purpose Science Cluster (GPSC) supercomputing facility in Dorval, Quebec, with installation of 1500 cores, 35 TB of RAM, and over 500 TB of data storage. The need for network upgrades to access and use the resources on the GPSC was assessed for all Project

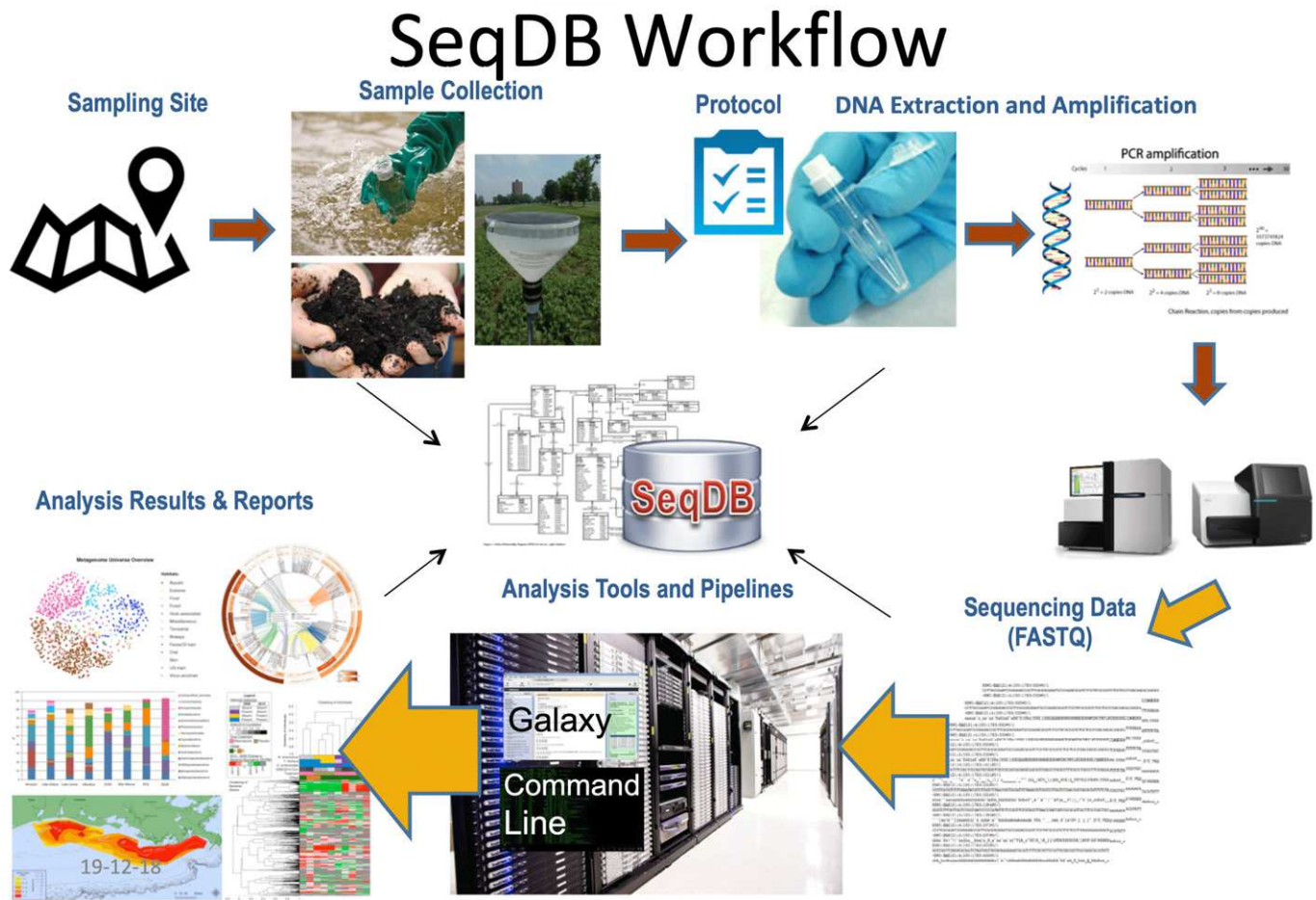


Fig. 2. The Ecobiomics Project workflow centered around the SeqDB information management system.

participants, and upgrades (e.g. increased internet bandwidth) were made for some participating locations. The resulting Platform has provided for a standardized Project-wide workflow from sampling, to sequencing, to raw and analyzed data management, while adhering to information management and information technology standards and security requirements on systems architecture. This standardization and centralization of data management within the Bioinformatics Platform enabled cost-savings, and unprecedented collaboration, accessibility and harmonization of data sharing across federal departments and agencies.

The centralized SeqDB data repository was deployed to house environmental sampling metadata, high-throughput sequencing data, reference data, and results of analyses. Ecobiomics participants are able to login to an access-controlled web interface that facilitates data capture, retrieval, and exploration. The repository's web interface enables the establishment of relationships between environmental samples, sequence extraction methods, raw sequence data, and a subset of analysis results such as operational taxonomic unit tables, taxonomic abundance tables, and functional profiles. Through this integrated data management approach, participants can follow the provenance chain from sample to sequence to taxonomic or functional information.

At the outset of the Project, Ecobiomics participants discussed similarities in sampling workflows, required database fields, and development of a controlled vocabulary to be used across the Project. Minimum metadata requirements prior to sequencing were established, and metadata needs from across all Project Themes were evaluated to ensure consistency with existing standards such as the Darwin Core (Wieczorek et al., 2012) and Minimal Information for X Sequence (Yilmaz et al., 2011). An assessment identified 170

bioinformatics tools (e.g. QIIME, Mothur), various interpreters and compilers that are standard within the community, and 230 libraries that were required by the bioinformatics tools or are widely used in the genomics community that needed to be migrated to the Platform. Selected reference databases were also transferred to the Platform and were scheduled to execute recurrently using the Hcron system. These databases included locally available copies of NCBI's non-redundant database, NCBI's nucleotide databases, various taxonomic subsets of these databases, NCBI's Taxonomy database, and the Unite fungal database. The central SeqDB repository will continue to evolve to comply and contribute to the community accepted standards for biodiversity ([www.tdwg.org](http://www.tdwg.org)) and genomics data ([www.gensc.org](http://www.gensc.org)), as well as Government of Canada initiatives such as OpenData and OpenScience. As the Ecobiomics Project progressed, a bioinformatics working group was formed to discuss data analyses, share bioinformatics pipelines, and look for opportunities to standardize bioinformatics analyses.

### 3.4. Microbiome and zoobiome projects

The Ecobiomics Project has sixteen soil microbiome, aquatic microbiome and invertebrate zoobiome research projects across Canada. These are exploratory and hypothesis-driven projects addressing specific departmental/agency policy objectives, with a number of projects co-located to enable interdepartmental collaboration and interdisciplinary research at the soil-water interface. The projects are establishing biodiversity baselines for monitoring soil health, water quality and environmental changes in the Great Lakes, Oil Sands region, and other areas across Canada. They are also enhancing an inventory of



the microbial and invertebrate biodiversity wealth across Canada, perhaps with similarities to the Geological Survey of Canada that began investigating Canada's mineral wealth in the 1800s and provided a foundation for subsequent development of mineral and energy resources. To date, the Ecobiomics Project has collected 7263 soil, water, and invertebrate samples from across Canada.

Soil microbiome projects are using metabarcoding and metagenomics tools to investigate the impacts of anthropogenic disturbances on soil microbial communities. This is to understand optimal soil microbial succession trajectories for remediation of disturbed and managed soils, and to provide stable baselines for long-term ecosystem monitoring of soil health. Soil microbiome projects are characterizing microbial communities following the remediation and re-forestation of disturbed soils in the Athabasca oil sands region (Stefani et al., 2018), and the recovery of boreal forest soils affected by disturbances such as wildfire, pest infestation, and biomass removal practices. Studies of agricultural soils in Ontario are investigating the impact of agricultural management practices (e.g. tillage, crop rotation, drainage management) on soil microbial communities and the need to consider microbial community changes in managing soil carbon, nitrogen and phosphorus. Another soil microbiome project is applying metagenomics tools to assess changes to microbial communities and soil health from disturbances such as nano-silver contaminants in order to improve environmental risk assessment and regulatory decision-making for toxic chemicals.

Aquatic microbiome projects are using metabarcoding and metagenomics tools to investigate the temporal and spatial impacts of land use disturbances and nutrient inputs (agriculture, forestry, mining, urban sprawl) on the structure and function of aquatic microbiomes. Studies on harmful algal blooms (HABs) in Lake Champlain, Quebec for example, are applying metabarcoding and metagenomics to investigate bacterial and algal community dynamics in the lake in order to better predict cyanobacterial blooms and reduce environmental and health risks associated with cyanobacterial toxins (Fortin et al., 2015; Tomas et al., 2017). Aquatic microbiome projects are also investigating harmful algal and cyanobacterial blooms in Lake Winnipeg, Lake Erie, Lake St. Clair (and its tributary the Thames River). These projects are collaborating with federal and provincial water quality monitoring programs to add a microbiome perspective onto conventional water quality monitoring programs for physical and chemical parameters such as phosphorus concentrations. The studies are also investigating the significance of soil microbial reservoirs for seeding microorganisms into tributaries draining into the lakes, as well as new fecal pollution source tracking approaches to identify non-point sources of nutrients. In addition, studies are also integrating metabarcoding and metagenomics tools to investigate aquatic biofilms in headwater streams of managed forests, where biofilm communities play important roles in regulating ecosystem functions along the river continuum. Other aquatic microbiome studies are investigating river microbial community responses to changes in agricultural land use practices such as stream/ditch management and tree cover loss in agroecosystems (Chen et al., 2018), and changes from oil sands activities and associated soil remediation activities.

Invertebrate zoobiome projects are using metabarcoding tools to better characterize communities of invertebrates in aquatic ecosystems. These communities have traditionally had a prominent role in monitoring environmental health, particularly of rivers and lakes. They are relatively simple to collect and identify, and their diversity is in part driven by their sensitivity to environmental factors. Perhaps most importantly, the spatial and temporal scale of invertebrate ecology is appropriate to guiding local land-use decisions and providing feedback to management authorities. Invertebrates are also a crucial link in the food chain of economically important species like fish. Invertebrate zoobiome projects are being conducted in collaboration with the Canadian Aquatic Biomonitoring Network (CABIN), a national program that provides a standardized methodology to evaluate the condition of rivers and streams across Canada relative to reference conditions (CABIN, 2018). One Ecobiomics project in Atlantic Canada is evaluating whether DNA metabarcoding is a more

sensitive method to determine the effects of anthropogenic disturbance, and whether this data can be produced more efficiently (i.e. faster and more cheaply) than traditional morphological-based invertebrate detection methods. This project is also comparing invertebrate communities in streams with those in Atlantic salmon guts as a new way to assess salmonid habitat suitability. Another project is building upon previous Joint Oil Sands Monitoring Program work to test whether metabarcoding would be more sensitive for detecting invertebrate community changes in wetlands in the Peace-Athabasca Delta (PAD) due to potential downstream contamination from Alberta Oil Sands activities (Gibson et al., 2015). One other project is applying a multidisciplinary approach to integrate invertebrate zoobiome, soil microbiome, and aquatic microbiome research in the South Nation watershed near Ottawa, Ontario. The experimental disturbance of agricultural streams in this watershed through catastrophic removal of riparian habitats has provided an opportunity to test responses to a controlled, yet realistic disturbance event. Invertebrate community responses are being measured through composition, but also on the basis of proxies for ecological function such as litter decomposition and the flux of energy from emerging insects to riparian wildlife. Metabarcoding applications are also investigating disturbance impacts on soil and aquatic microbiomes in this watershed, as well as impacts on mosquito communities as part of West Nile virus public health research.

#### 4. Future directions

The Ecobiomics Project has provided a turning point for applying new metagenomics approaches in environmental assessment, monitoring, and remediation programs for soil health and water quality across the Canadian federal government. We anticipate that metagenomic tools will be able to provide more comprehensive approaches for biomonitoring of soil and aquatic ecosystems, and for discovering more robust and reliable multi-species indicators and early warning tools for soil health and water quality. We also anticipate that in the future, the Project's Bioinformatics Platform will be able to support a wide range of new metagenomics research opportunities across federal government programs beyond the Ecobiomics Project. These include support for including microbiome perspectives for risk assessment of toxic chemicals (Adamovsky et al., 2018); improvement of the productivity of agricultural crops (Gopal and Gupta, 2016; Busby et al., 2017), trees (Mercado-Blanco et al., 2018), livestock (Mizrahi and Jami, 2018), and aquaculture species (Limborg et al., 2018), as well as to enhance food quality and safety (De Filippis et al., 2018), and advance public health surveillance (Miller et al., 2013). These also include applications of eDNA metabarcoding to scale-up and expand biomonitoring programs (Porter and Hajibabaei, 2018); improve freshwater fisheries assessment (Evans and Lamberti, 2018), advance wildlife conservation (Bohmann et al., 2014), protect endangered species (Balasingham et al., 2018; Doi et al., 2018; West et al., 2019), and address invasive pests (Valentin et al., 2018) and alien species (Kowalski et al., 2015; Klymus et al., 2017).

The Ecobiomics Project has selected some of its sampling sites as genomic observatories to coincide with sites that are part of long-term environmental monitoring programs. These include water sampling stations in the middle of the west, central, and east basins of Lake Erie that are part of Environment and Climate Change Canada's long-term water quality monitoring program for the Great Lakes. The characterization of bacterial, archaeal, algae, and fungal communities at these stations will provide added value to the conventional physical and chemical water quality parameters that are currently used to monitor and predict algal blooms and other environmental changes occurring in this lake. Similarly, genomic observatories have been established for soil microbiome studies in the Montmorency Forest in Quebec that are part of long term monitoring programs for this boreal forest (<http://www.foretmontmorency.ca/en/>). Genomic observatories have also been established for microbiome and invertebrate zoobiome sites in the South Nation watershed, Ontario as part of studies of agro-



ecosystem changes, and at Peace-Athabasca Delta sites downstream of Alberta oil sands activities. It is anticipated that Ecobiomics observatories will provide legacy DNA sequence datasets to be mined into the future as part of temporal analyses of biodiversity trends at long term monitoring sites, and spatial analyses as part of global efforts to integrate DNA sequence data into broader Earth Observing systems (Davies et al., 2012).

While applications of metagenomics have been pervasive in many fields of science, there remain challenges for advancing metagenomic approaches beyond the research stage into environmental assessment, monitoring, and remediation programs for soil health and water quality. These include the need to better understand the biases from DNA extraction methods, PCR amplicon strategies, bioinformatics pipelines, and reference databases that can affect the representativeness of microbial and invertebrate biodiversity detected in soil and aquatic samples (Elbrecht and Leese, 2015; Tan et al., 2015; Nesme et al., 2016; Oliverio et al., 2018; Pawlowski et al., 2018; Hering et al., 2018). They include the need to evaluate new metrics, thresholds, and metagenomics endpoints for environmental decision-making. They also include government policy and regulatory constraints that may influence readiness for uptake regardless of the benefits of new metagenomics approaches (Henrich et al., 2016). In addition, while there have been notable advances from initiatives like the Earth Microbiome Project (Gilbert et al., 2014), the science of metagenomics is still evolving, which presents a challenge for developing widely recognized standards to enable comparison of results between studies.

The Ecobiomics Project has focused much of its initial end-user engagement efforts on invertebrate zoobiome collaborations, particularly with Canada's CABIN network. There is less of an existing framework for soil and water microbial community biomonitoring (other than for diatoms) to guide comparative evaluation and incorporation of metagenomics approaches. On the other hand, CABIN provides an

existing framework for assessing the health of aquatic ecosystems based on a national standardized approach for enumerating benthic macroinvertebrates, generally visible to the naked eye (CABIN, 2018). Ecobiomics researchers are comparing invertebrate zoobiome metabarcoding datasets with conventional macroinvertebrate taxonomic identifications obtained using microscopes to demonstrate advantages for incorporating metagenomics data into the CABIN biomonitoring programs. These collaborations are leading towards capabilities for CABIN to provide faster, less expensive, and more comprehensive characterizations of benthic macroinvertebrate communities responding to environmental stressors at sites across Canada (Fig. 3).

While we are not aware of another government-wide metagenomics initiative for microorganisms and invertebrates comparable to the Ecobiomics Project, there are a growing number of initiatives around the world that are advancing metagenomics applications to better characterize microbial and invertebrate communities for soil and freshwater management programs. These include environmental microbiome initiatives in Australia (Bissett et al., 2016), Brazil (Pylro et al., 2014), China (CSMI, 2014), France (Terrat et al., 2017), the United States (Stulberg et al., 2016), and Africa (Wild, 2016). There are also large international microbiome collaborations such as the Earth Microbiome Project (Gilbert et al., 2014) and the TerraGenome Project (Vogel et al., 2009), as well as calls for a more globally integrated initiative for the study and sharing of data on microbiomes (Alivisatos et al., 2015; Dubilier et al., 2015; Blaser et al., 2016). Similarly, there are metabarcoding initiatives for better characterizing invertebrate communities such as DNAqua-Net in Europe (Leese et al., 2016). With the pervasive expansion of metagenomics in the soil and aquatic sciences, there are also companies establishing metabarcoding services for analyzing soil and water samples. In order to continue to advance metagenomics applications, it will be important to continue progress on developing best practices and standards for microbiome and

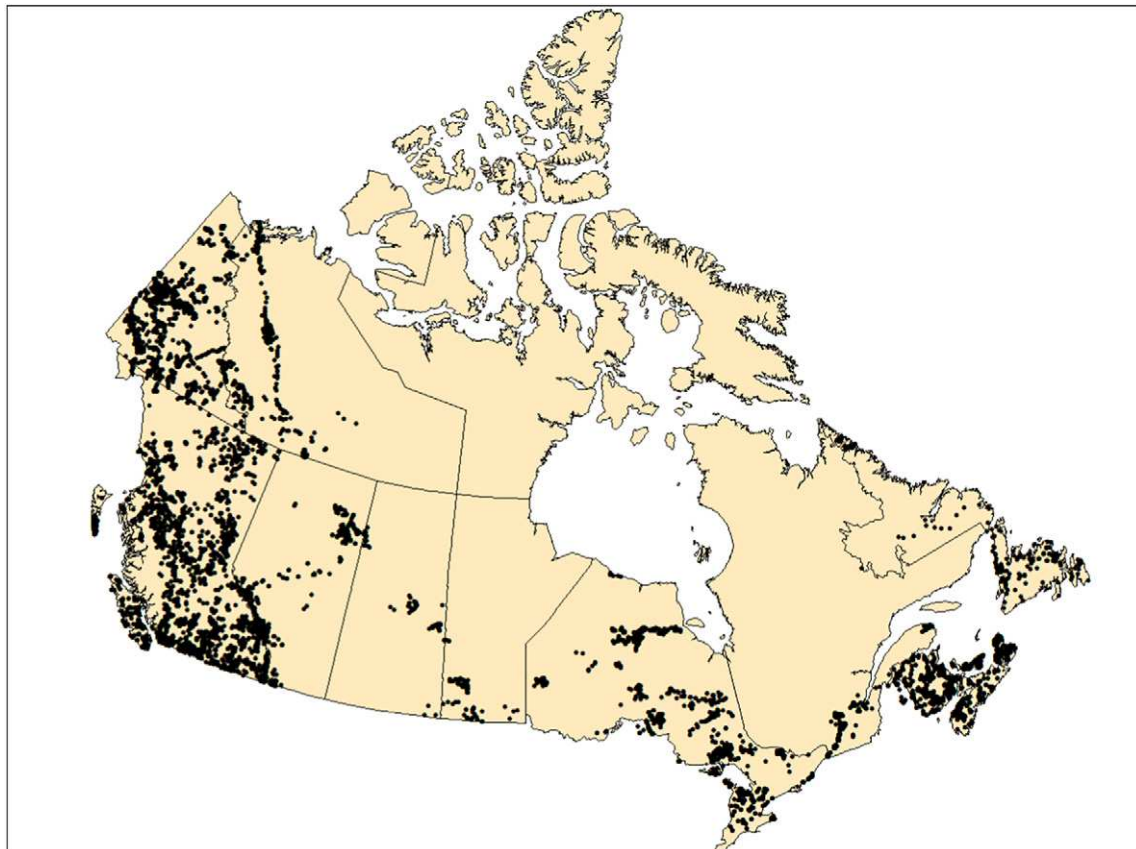


Fig. 3. Map of Canada showing Canadian Aquatic Biomonitoring Network (CABIN) monitoring sites.

invertebrate zoobiome studies around the world (e.g., Yilmaz et al., 2011; Knight et al., 2012; Ionescu et al., 2016; Lear et al., 2018; Knight et al., 2018). This will be essential to ensure metagenomics data are collected, analyzed, and interpreted based upon the best available science, and that results are comparable as widely as possible. In addition, agencies involved in assessment, monitoring, and remediation of soil health and water quality will increasingly need to consider capacity building and education/training requirements for gaining familiarity with metagenomics in their soil and water research, policy, and program management environments.

## 5. Conclusions

The Ecobiomics Project has provided a model for beginning to incorporate metagenomics into environmental assessment, monitoring and remediation programs for soil health and water quality. The Project has been able to highlight a number of conclusions and advances to date:

- Advances in metagenomics are enabling more comprehensive characterization of the biodiversity found in microbial and invertebrate communities at lower trophic levels in soil and water ecosystems.
- The results from metagenomics studies are providing new perspectives on the importance of microbiome and invertebrate zoobiome communities for soil health and water quality, and for providing early warning indicators of environmental changes.
- The Government of Canada has advanced the Ecobiomics Project to apply metagenomics to improve environmental assessment, monitoring, and remediation of soil health and water quality.
- A centralized sequencing facility and Bioinformatics Platform were established across seven departments and agencies for collection, storage and analysis of metagenomics data.
- Sixteen research projects were initiated, and genomics observatories were established with environmental assessment and monitoring end user communities under Soil Microbiome, Aquatic Microbiome, and Invertebrate Zoobiome Themes.
- The ground floor of a novel government-wide platform was established for harmonizing metagenomics characterization of biodiversity in terrestrial and aquatic ecosystems.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal.

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