THE 1000 PROJECT: A WORLD-FIRST MICROBIAL BIOINVENTORY OF NEW ZEALAND'S GEOTHERMAL ECOSYSTEMS

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ABSTRACT

Geothermal environments are a striking feature of New Zealand's natural landscape. They have a strong place in our cultural, recreational and economic psyche. However, the current knowledge of microbial diversity in geothermal environments, arguably New Zealand's greatest untapped resource, is inadequate for a thorough assessment of ecosystem uniqueness, biotechnology potential and/or ecosystem health, function, and resilience.

GNS Science and The University of Waikato have recently been awarded a two-year grant from the Ministry of Business, Innovation and Employment (MBIE) to collate physical, chemical, and microbial biodiversity information from 1,000 geothermal ecosystems ("The 1000 Project") from the Taupo Volcanic Zone (TVZ). "The 1000 Project" will generate a genetic archive, microbial diversity database and will see the development 'uniqueness' metric tool as a means to define and assess conservation, matauranga Māori, recreation, biotechnology potential and resource development value of these iconic New Zealand environments. This ambitious goal utilises state-of-the-art molecular, bioinformatics and archiving technologies, which will be unprecedented for microbial ecology research, resource evaluation, conservation and biodiscovery, underpinning future scientific endeavour, and informing future decision making and policy development.

A publicly-accessible online database and archive will ensure that stakeholders have access to the data generated. The 'uniqueness' metric developed as part of the project will provide a means for end- users to interpret this vast data source for their individual purposes. The knowledge generated pertains to natural resources that (i) Landowners and/or Māori may wish to sustainably develop and/or protect, (ii) companies might wish to utilise (e.g. energy, power, biotechnology, tourism), (iii) central and regional government agencies are charged with managing and conserving (e.g. DOC, EPA, MPI, regional councils), and (iv) the scientific community seeks to understand.

This paper will present an overview of the historical and current knowledge of geothermal microbial ecosystems and biodiversity in New Zealand, and discuss "The 1000 Project" and it's relevance to the New Zealand geothermal community.

1. THE MICROBIAL KNOWLEDGE GAP

New Zealand is renowned for its unique flora and fauna of which the visible indigenous "macro" species are comparably well studied. However, consistent with the global trend (Curtis, 2006; Griffith, 2012; Keller and Zengler, 2004; Parker, 2010), our knowledge of microbial diversity is lacking.

Microorganisms (*Bacteria* and *Archaea*) make up more than half of the entire global biomass (~350-550 Pg of C) (Whitman et al., 1998), but their 'micro' size makes them an 'unseen', and therefore unconsidered component of ecosystems. Despite their incredibly massive numbers, microorganisms and their interactions with the environment, are often overlooked despite their critical roles in health, look and function in practically all global ecosystems (Coleman et al., 2004).

Global biodiversity is utterly dominated by the microscopic world (Table 1). The numbers are truly staggering; for example current estimates suggest that there are up to one billion microbial cells per gram of soil (Editorial, 2011) and almost 10 trillion bacteria in the stomach of the average human (Hooper and Gordon, 2001). Yet, despite the best efforts of microbiologists, knowledge of microbial diversity is still in its infancy.

Table 1. The number of estimated species globally.

		Number of Species
	Eukarya[1]: all plants, animals, insects, fungi, algae etc. discovered	~1,300,000 [2]
	Estimated numbers of *Eukarya*[1]	8,700,000 [2]
Microorganisms	*Bacteria*[1] & *Archaea* [1]: all cultivated bacterial and archaeal strains	10,440 [3]
	Microorganisms that have been detected, but have not yet been cultivated	2,578,902 [4]
	The microbial majority that has not been detected but is known to exist	~10^{9} [(5)]

[1] *Bacteria*, *Archaea* and *Eukarya* are the three domains of life and encompass all living organisms. [2](Mora et al., 2011); [3](Anonymous, 2011) ; [4] (Anonymous, 2012), [5] (Schloss and Handelsman, 2004)

While the numbers of cultivated and described microorganisms is comparatively small compared to that of the Eukaryotes, the advent of cultivation independent (molecular) detection of microorganisms has facilitated the assessment of the microbial community make-up without the requirement of laboratory-based culturing. This technology has highlighted what microbiologist's have suspected for years; that most microbial species, including the majority of microbial phyla or divisions, remain uncultivated, and thus

poorly understood (Rappe and Giovannoni, 2003; Schloss and Handelsman, 2004).

To highlight this, a recent estimation (Figure 1) showed that more than 75 % of the known ~100 phylogenetic divisions (the proceeding taxonomic classification after domain/kingdom) of *Bacteria* contain exclusively uncultured representatives. Of the 29 bacterial divisions with cultivated representatives (Euzéby, 2011), five phyla (*Actinobacteria*, *Bacteroidetes*, *Cyanobacteria*, *Proteobacteria* and *Firmicutes*) represented 95% of all the cultivated strains (Keller and Zengler, 2004), further highlighting global infancy in understanding microbial diversity. Despite the growing scientific and industrial effort to characterise these uncultivated bacteria, over 70 entire divisions (known as candidate divisions) have so far evaded all attempts at cultivation.

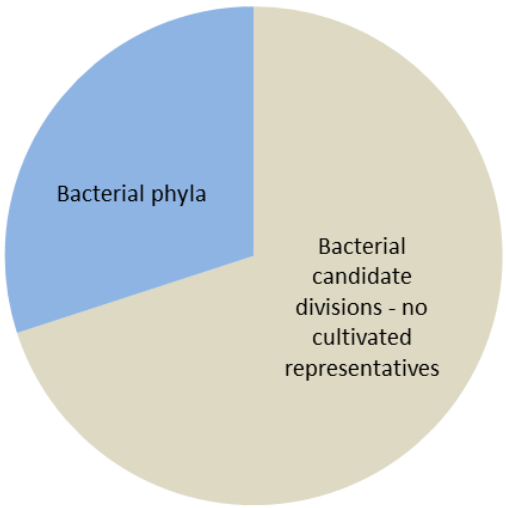


Figure 1. An approximate representation of spread of bacterial phylum-level divisions with and without cultivated representatives.

2. GEOTHERMAL MICROBIOLOGY

New Zealand is renowned for its unique biodiversity, yet we know comparatively little of the microbial diversity and ecological functions. This holds true also for the NZ geothermal ecosystems, while we understand that microbial communities flourish in geothermal environments, we have only a rudimentary understanding of diversity and almost no understanding of community function or ecology.

2.1 Geothermal environments

Extremophiles (extremophilic microorganisms) are a generic term used to describe microorganisms that thrive in non-anthropogenic conditions. This can include temperatures as high as 121˚C, pH ranges from 0.5-11, high levels of radiation, salts and/or heavy metals (Canganella and Wiegel, 2011).

New Zealand geothermal systems are without exception 'extreme'. The nation's location on the collision boundary between two tectonic plates has created a chain of undersea and terrestrial volcanoes and geothermal systems. This is ideal for the formation of environments conducive to extremophile colonisation. These habitats include hot and warm springs, bubbling mud pools, crater lakes, and undersea volcanoes.

New Zealand's most recognised geothermal area is the Taupō Volcanic Zone, a 100 km wide by 350 km long volcanic region of the central North Island, abundant with geothermal activity. However, warm and hot waters (20°C - 100°C) are also discharging from springs throughout the country (Figure 2), including Ngawha and Northland, East Cape, the Hauraki and Auckland regions, around Tauranga and along the Alpine Fault in the South Island. Geothermal ecosystems are also found in New Zealand's Antarctic territory e.g. on Mt Erubus and in offshore islands and seamounts in the Kermadec Arc.

2.2 Novel geothermal microorganisms and diversity

While non-anthropogenic or extreme ecosystems seem unlikely environments to encounter high levels of species diversity, in fact, two of the most genetically diverse environments in the world are dominated by extremophilic microorganisms. A hypersaline microbial mat from a saltern at Guerrero Negro (Ley et al., 2008) and Obsidian Pool, a geothermal spring in Yellowstone National Park (Hugenholtz et al., 1998) both support in excess of 36 and 43 bacterial divisions respectively. To put this in perspective, the entire domain *Eukarya* has a total 40 recognised phyla.

Novel microorganisms and unique microbial habitats have been detected in New Zealand's geothermal environments. Pioneering extremophile research by Hugh Morgan and Roy Daniel highlighted the diversity of the geothermal microbiota in New Zealand (Hetzer et al., 2008; Niederberger et al., 2006; Olsson et al., 2003; Patel et al., 1985; Ronimus et al., 1997). Other successes also include the isolation and characterisation of new bacterial phylum (Dunfield et al., 2012; Lee et al., 2011; Stott et al., 2008) and the first description of acidophilic methanotrophy strains (Dunfield et al., 2007), and ecosystems (Childs et al., 2008; Niederberger et al., 2008; Soo et al., 2009; Stott et al., 2008).

2.3 Biotechnology potential

Geothermal environments are chemically and physically unusual, as well as globally rare. The broad diversity of microorganisms in these extreme habitats have developed novel mechanisms to survive and flourish. These rare metabolisms and capabilities represent significant opportunities for new biotechnological applications and improvement of existing processes. We have listed some of the biotechnologies developed from geothermal microorganisms below. However, it is worth highlighting what is considered the extremophile (and in fact microbiological) gold standard biotechnology developments; the Polymerase Chain Reaction (PCR). PCR was developed by Kary Mullis using a thermostable enzyme known as DNA Taq polymerase from a thermophilic bacterium *Thermus aquaticus*. The technique, which Kary won the Nobel Prize for Chemistry, allows for repeat and exponential amplification of target DNA *in situ*. This technique revolutionised molecular biology, diagnostic medicine and research and development. Until the 2009 when the patent for Taq polymerase expired, the use of this enzyme earned approximately $2 billion USD (Fore et al., 2006).

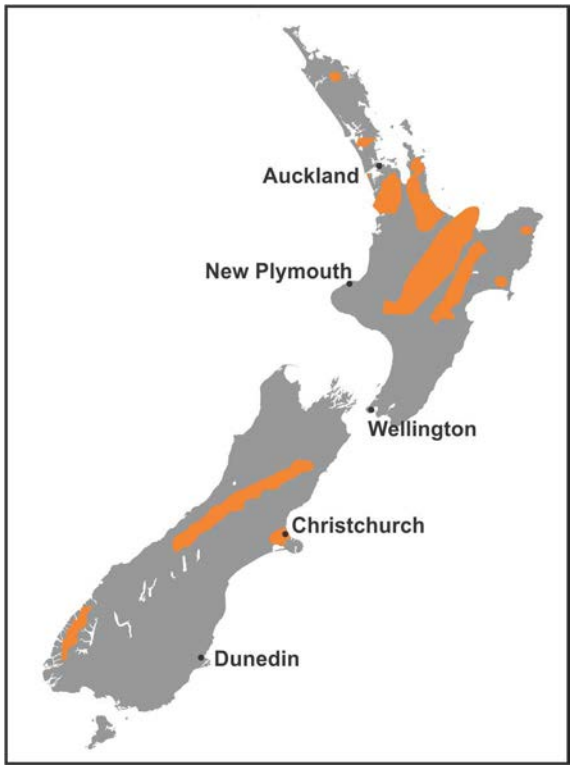


Figure 2. Areas of warm and hot waters discharging from geothermal features in terrestrial New Zealand.

Without a doubt, microorganisms represent the lion's share of genetic diversity (Wu et al., 2009) and therefore hold an overwhelming potential for novel biotechnologies. The major future biotechnology and medical advances will no doubt take advantage of the novel genetic diversity housed by microorganisms (Turner et al., 2007).

Some highlights of novel biotechnologies developed from in microorganisms cultivated from New Zealand's geothermal ecosystems include:

- Novel bioactives for medicines and disease treatment
- Reagents for forensics and livestock breeding programmes
- Thermophilic cellulases for application in biofuels
- Reagents for industrial processes

2.4 Managing geothermal ecosystems

Considering the current investment (~$4b) in greenfield geothermal developments and the expansion of existing geothermal energy production in the next five years (Harvey et al., 2010), research is needed to characterise New Zealand's unique microbial diversity.

There is ample evidence that geothermal power generation can modify the terrestrial geothermal expression and the surrounding landscape (Daysh et al., 1998; Energy, 2001; Limited, 2005; Trust, 1997). As microbial population composition is extremely sensitive to perturbation (Banning et al., 2011; Lesaulnier et al., 2008; Lewis et al., 2010), it is fundamental that the microbial diversity of these unique ecosystems is quantified to determine i) ecosystems of high or low conservation values and ii) any effect (or lack of) from development.

It is imperative that regulators and power companies have robust information available to assess all environmental impacts, otherwise these 'unseen' biota with unknown conservation value and overwhelming biotechnological potential (see below), could conceivably become an 'unseen' loss.

The current process of management, including resource consent applications, of the microbial component of geothermal systems is well-intended, but lacks the quantifiable microbial diversity and associated uniqueness data. The current microbial diversity information for geothermal environments is inadequate for an assessment of ecosystem uniqueness, biotechnology potential and/or ecosystem health, function, and resilience.

Presently, there is no specific framework for assigning value to a microbial ecosystem or a microbial strain by the Department of Conservation (DOC) (Cody, 2007), Bay of Plenty Regional Council (BOPRC) (EBOP, 2010), or Waikato Regional Council (WRC) (EW, 2008). At a similarly embryonic state is the policy development for microbial biodiscovery (Bioprospecting) in New Zealand (MED, 2007; MFE, 2009) and its application to Vision Mātauranga. Additionally this is still subject to a governmental response to the Wai 262 treaty claim.

3. THE 1000 PROJECT

GNS Science and The University of Waikato have recently been awarded a 2-year grant from the Ministry of Business, Innovation and Employment (MBIE) to develop a bioinventory of geothermal ecosystems in the TVZ. This project will determine the microbial diversity of 1,000 geothermal springs and collate these data with the physical, and geochemical parameters measured during sampling.

The project goal is the development of a comprehensive bioinventory and environmental indicator ('uniqueness' metric) of the microbial diversity in New Zealand's geothermal ecosystems. Currently, the lack of microbial diversity knowledge in these environments highlights that the lack of even a base level knowledge or inventory of the microbial populations in these environments let alone an understanding of how populations may or may not be effected by geothermal development.

This bioinventory targets terrestrial geothermal features in the TVZ for sampling, analysis and archiving (Figure 3). The TVZ spans from the Mt. Ruapehu Crater Lake to White Island (Whakaari) and ranges from hot water occurrences (e.g. Matata) to large geothermal fields, such as Wairakei or Waiotapu. There are at least 38 known geothermal systems in the TVZ (DoC 2007) representing (conservatively) 7,000-10,000 features. We aim to sample a representative 10% of these sites.

Outside the TVZ, a further ~90 terrestrial systems (Houghton et al., 1989; Mongillo and Clelland, 1984) (not including marine or freshwater hydrothermal vents, offshore islands or territories) represent an additional ~3,000 features. These additional areas are not included in the project due to the sampling costs and limited timeframe. However, these features could be the subject of future research grants or end-user funded studies.

3.1 Methodology

The project has three research aims. Figure 4 depicts the planned flow diagram of the project activities, outputs and outcomes:

i) *Microbial and physicochemical diversity of geothermal features assessment* - The microbial diversity, and associated physicochemical conditions will be determined for 1,000 geothermal features sampled in the TVZ. The total genetic content of each sampled microbial ecosystem will be extracted.

ii) *Archiving microbial diversity and genetic potential* - DNA from 1,000 geothermal ecosystems will be archived for long-term access.

iii) *Environmental indicator: 'uniqueness' metric prediction* – A schema for ranking the uniqueness of a microbial geothermal ecosystem will be developed based on the microbial and physicochemical diversity data collected during this project.

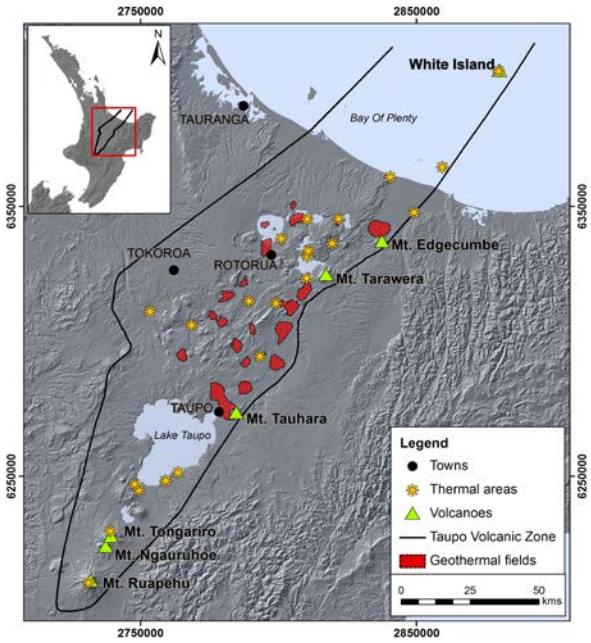


Figure 3: Map of geothermal systems in the Taupō Volcanic Zone.

3.1.1 Sample collection

Samples will be collected from 1,000 geothermal ecosystems. The features targeted will be selected via input from end-users to encompass current cultural, recreational and conservation values for the geothermal features. Additionally they will span the known pH and temperature ranges within the TVZ (pH 0.0-9.0 and 20-99°C respectively (Cody, 2007) and any known unusual geochemical or geophysical profiles (e.g. Champagne Pool (Childs et al., 2008); Inferno Crater (Glover et al., 1994)).

3.1.2 Microbial biodiversity

The total environmental DNA (eDNA) will be extracted for each sample and fractioned for microbial biodiversity assessment and archiving. The DNA extracts will be processed via tag-based amplicon sequencing of the SSU rRNA gene (both the archaeal and bacterial components will be sequenced in each geothermal feature). All samples will be screened using an Ion Torrent™ sequencer, and results managed using an established bioinformatics pipeline configured for environmental microbial ecosystems. We anticipate that ~2,000 bacterial and archaeal strains will be detected per sample.

3.1.3 Physicochemical characterisation

Samples will be collected for physicochemical analyses from the 1000 geothermal features investigated. These will be analysed for dissolved gases (where applicable) via gas chromatography (H_2, H_2S, CO_2, CO, CH_4, O_2, N_2, Ar and He), soluble cations and anions (HCO_3^-, NO_3^-, NH_4^+, NO_2^-, SO_4^{2-}, $B(OH)_4^-$, F^-, Cl^-, Br^-, Li^+, Na^+, K^+, Ca^{2+}, Mg^{2+}, PO_4^{3-}, Fe, As, Hg and Al) via ion chromatography, spectrophotometry, and inductively coupled plasma – mass spectrometery. Physical field measurements will include temperature, pH, Eh (redox), conductivity and dissolved oxygen.

The chemical and physical parameters within an environment define the ecosystem functional response and structure (Amend and Shock, 2001). By sampling a diverse range of features and accurately defining the microbial diversity and physicochemistry, there is an opportunity to observe trends between the physicochemistry and microbial population structures that will allow us to predict the microbial diversity from environmental parameters in future studies. Physicochemical data analysis will also assist in tailoring extraction protocols, vetting sequencing results with known physiological and metabolic capabilities of characterised microorganisms, and identifying changes in environmental parameters associated with ecosystem perturbation in future temporal studies.

3.1.4 Genetic archive

A portion of the total eDNA extracted will be archived using Biomātrica™ technology (Wan et al., 2010), to archive both the microbial diversity and genetic potential of each sample. This technique permits nucleic acid storage at room temperature, which avoids sample degradation via sheering during freeze-thaw cycles, and is also not reliant on high cost maintenance of cryogenic temperatures. Genomic DNA samples can be stored for more than 30 years using this methodology and be resampled repeatedly, making this technology ideal for this project.

3.1.5 Environmental "uniqueness" indicator

A critical component and output of this project will be the development of a 'uniqueness' metric that can be applied to these ecosystems. This metric can be used as a tool by end-users for assigning value to geothermal ecosystems as (i) an environmental indicator for sustainable management and/or (ii) a biodiscovery tool to guide biotechnology opportunities.

The 'uniqueness' metric will provide a numerical measure of the site, physical, chemical, microbial species and community uniqueness, developed using phylogenetic dissimilarity and abundance criteria (Caporaso et al., 2011), and incorporating the ecosystem's physicochemical diversity. The uniqueness metric could be applied to both individual species/phylotypes and/or the microbial communities. However, how this indicator is incorporated into policy or development would be dependent on the end-user's objectives and aspirations, and would need to be

established on an individual basis. The end-users application of value to this metric is outside of the scope of this project.

3.1.6 Data access

The abundance of high quality genetic and physicochemical data generated through this project will be stored in purpose-built databases, and accessible via a web-enabled interface. The physicochemical and microbial diversity data collected in this project will be publically accessible and will provide researchers, regulatory authorities, landowners and the general public.

As an extension of this database, a Smart Phone App will be developed to facilitate public access to rudimentary data, site descriptions, photos and maps of sites. This outreach plan will be especially useful for geothermal tourist operations where this App is envisaged to add to the recreational experience and value.

This project fulfils the need for accessible and useable information for end-users to establish a valuation of the microbial component of geothermal ecosystems. The knowledge generated pertains to natural resources that (i) Māori / landowners / tourism operations may wish to sustainably develop and/or protect, (ii) companies might wish to utilise (e.g. energy, power, biotechnology, tourism), (iii) central and regional government agencies are charged with managing and conserving (e.g. DoC, EPA, regional councils), and (iv) the scientific community seek to understand. The 'uniqueness' metric developed will provide a means for end- users to interpret this vast data source for their individual purposes.

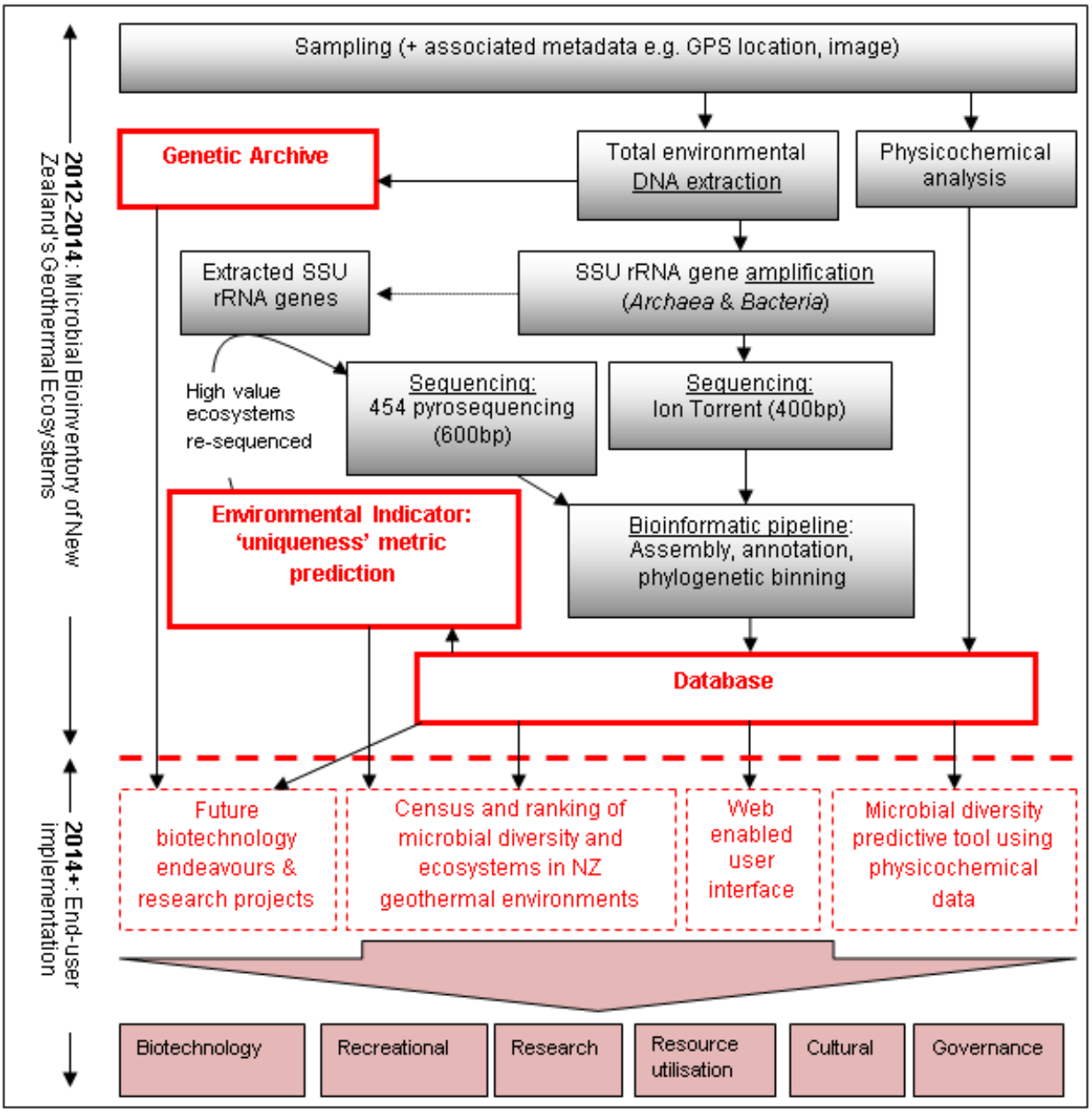


Figure 4. Flow diagram of the research project. Bolded red boxes denote direct outputs from the project. Hatched lines indicate future outputs or application of data to ecosystem valuation. Pink fill indicates the areas where valuation data could be applied.

3.2 Outcome Benefits

The 1000 Project addresses the need to first define and understand these ecosystems before the biotechnological potential and intangible qualities such as microbial uniqueness and mātauranga Māori significance, can be accurately valued and therefore effectively managed. This project will provide a means to assess the value of the microbial component of geothermal ecosystems, for a range of purposes (Figure 4).

Additionally, the project will fulfil a need to identify and address gaps in (i.e. microbial diversity data), and develop fit-for-purpose methodologies for defining environmental criteria. In turn this will inform policy and planning, enhance sustainable management, and promote biodiscovery opportunities.

Benefits include:

- improved understanding of resources;
- robust resource management, planning and utilisation including mātauranga Māori;
- better biosecurity policies;
- competitive advantage for tourism ventures; and
- a springboard for scientific endeavour and biotechnology/industrial opportunities.

Considering the current investment and expansion of geothermal power generation (including greenfield projects), this project is time bound and prudent for thorough resource and impact assessment.

4. CONCLUSION

The 1000 Project position New Zealand for the future by recognising 'extremophile' microorganisms and their associated ecological function and biotechnological potential as a priority, and therefore geothermal ecosystems as a valued natural capital.

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