

REVIEW ARTICLE

Genomics to assist mine reclamation: a review

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Mine reclamation succeeds when healthy, self-sustaining ecosystems develop on previously mined lands. Regulations require reclamation of ecosystem services; however, there are few specified targets, and those that are presented are vague. Sequencing genomic DNA and transcribed RNA from environmental samples may provide critical supportive information for attempts to recreate ecosystem functions from the ground up on disturbed lands. In this review, we highlight the use of genomics to meet mine closure goals, to enhance ecosystem development, and to optimize ecosystem services inherent in self-sustaining reclaimed ecosystems. We address the development of environmental genomics—sequencing and analysis of environmentally derived DNA—to characterize microbial communities on mine sites. We then provide four areas where genomics has proven instrumental for informing management and assisting in reclamation of mine sites in the form of bioreactors, passive treatment systems, novel gene discovery, and DNA barcoding. Finally, we describe how recently developed techniques have transferable value to mine reclamation and provide evidence for future applications of genomics and the necessary steps to integrate these data into comprehensive management of mined sites.

Key words: bioreactors, metagenomics, microbial community, mine drainage, passive remediation

Implications for Practice

- Sequencing and polymerase chain reaction (PCR)-based methods of quantifying environmentally derived DNA have seen a growing prominence in field research aimed at understanding the processes underlying restoration, and will likely become common practice for monitoring biological capital and restoration progress on mine sites.
- Sustainable and reliable containment of contaminants from mine sites will require periodic monitoring of microbial community composition and activity, and in the case of active treatment systems, incorporation into feedback models for continued operation.
- There is a drastic need for education and technical training in environmental genomics for reclamation practitioners in the mining industry, and for tailored bioinformatics workflows that facilitate rapid evaluation of genomic data from mine sites specifically.

Introduction

The science of mine reclamation exists as the proof of an industry-wide promise of mine-site transience. In an ideal system, mines operate with minimal environmental impact outside of the operational footprint. After the period of profitable extraction is reached, a desirable condition is recreated, be it forest, pasture, or suburban neighborhood, so that the biota that develops on previously mined substrate (soil microbes, vegetation, wildlife, and concerned citizens alike) can function within a natural, self-sustaining ecosystem. In reality, mine sites often leave a legacy, including perpetually altered plant communities (Holl

2002), elevated contaminants in surface and groundwater (Cidu et al. 2001), thin, compact soils (Skousen et al. 2009), altered soil function (Mummey et al. 2002), and magnification of contaminants within the food chain (Allan 1995; Muscatello & Janz 2009).

Government regulations on mining operations set the guidelines for reclamation (where such regulations exist), and as a result, direct the fate of many mine sites. Champigny (1991) performed a comparison of regulations on mine rehabilitation for 25 jurisdictions worldwide. Although the author identified variations in the level of responsibility levied on mine operators to reclaim mine sites, he concluded that greater than 90% of governments within the study mandated mine site rehabilitation beyond minimum human health and safety standards (Champigny 1991). In British Columbia, Canada, the Mines Act, informally called the “Code,” specifies that reclamation must satisfy the requirements of the Chief Inspector (B.C. MEMPR 2008). Because the legislation is vague and subject to interpretation (by both the mining companies and the inspectors), there has been considerable variability in defined goals

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and measures of success. Historically, the goals were set for plant productivity because it was easy to measure. The current “Code” (10.7.5) expresses a desire to attain an equivalent land capability, which is a vague and challenging target to achieve. Partly in response to this ambiguous terminology, there has been a recent trend to consider mine reclamation in terms of the whole ecosystem, with all of its functions and services. Ecosystem services can be defined as “... the aspects of ecosystems utilized (actively or passively) to produce human well-being” (Fisher et al. 2009). The ecosystem services targeted for reclaimed mined lands include forage for cattle and habitat for wildlife, fish, timber, clean air and water, carbon sequestration, biodiversity, and culturally significant natural products related to traditional practices and medicines. Regulations exist to protect these services, or ensure their continuation, during and after mining activities take place. Despite pervasive efforts to meet and improve upon regulatory guidelines for ecosystem services at previously mined sites, there is a large degree of uncertainty in mine land restoration. Consistent application of revegetation, soil amendments, and regrading treatments can lead to very different results, even on the same site (Martínez-Ruiz & Marrs 2007). Why such inconsistency? Many authors attribute it to variability in starting conditions (the prevailing mineralogical substrate), slope, aspect, and a myriad of unmeasured factors (Martínez-Ruiz & Marrs 2007), with one emerging frequently, the soil microbiota (Harris 2003).

Microorganisms are catalysts for soil formation. They are responsible for creating complex microenvironments that lead to nutrient uptake in plants, semihomeostatic water and chemical regulations, and overall resilience to erosion, contamination, and invasion by exotic plant species. New soils that form on exposed bedrock often follow a predictable successional sequence. Microbial communities form on weathered inorganic substrates, fix atmospheric nitrogen and CO₂, and contribute the key elements of organic life. Multi-cellular plants and animals are relative latecomers to the developing community, arriving when soil formation processes have provided sufficient organic resources to sustain them. See Frouz et al. (2008) for a discussion of this process on previously mined sites. Chronologically, this natural approach can be considered “bottom-up,” with soil organisms and processes establishing and “preparing” the substrate before larger plants and animals arrive. The science of restoration has not developed a mechanism for recreating (much less accelerating) this “bottom-up” process. Rather, practitioners often attempt to recreate the desired ecosystem irrespective of the natural chronological sequence by superimposing soil amendments and seeding or planting vascular plants (Tordoff et al. 2000). This practice is not out of ignorance of the role that soil microbial community’s play, but because the ability to observe, quantify, and manipulate these communities is not within the typical organization’s toolkit.

Until recently, techniques for observing and quantifying microbiota have been extremely restrictive (Ficetola et al. 2008). A vast majority of microorganisms are deemed unculturable in a laboratory setting with current technologies (Hugenholtz et al. 1998; Harris 2009; Pham & Kim 2012), though substantial progress has been made in culturing new

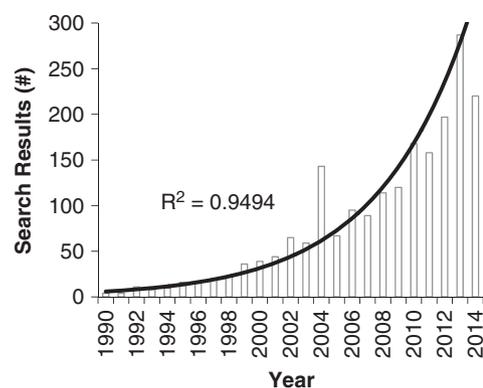


Figure 1. Frequency of articles published containing “genome” and “mine reclamation.” Search results are from Google Scholar for the period 1990–2014. All included articles contain the terms “mine,” “reclamation,” and “genome,” and at least one keyword that distinguishes the publication as relating to mineral extraction (i.e. “ore,” “mineral,” and common mined metals including “gold,” “copper,” and “zinc”). Publications from the Journal Horticultural Science were excluded for a large number of unrelated records. The solid black line represents an exponential fit to the observed records.

groups of species through co-culture and improved controls of the laboratory environment, for a review, see Stewart (2012). As one alternative, microbiologists have developed techniques for quantifying the mass-action of microbes in soils including respiration and production rates of metabolic products. Environmental genomics offers an opportunity to observe the complexity of microbial communities as they form on mined sites, and to apply ecological theory to soil community formation and structure in a way that has—until very recently—been impossible. Beginning in 1990, publications addressing aspects of mine reclamation in combination with genomic analysis have grown exponentially, reaching 224 articles or book chapters in 2014 (Figure 1). The majority of these publications apply environmental genomics to describe microbial community composition and function on mined sites, but these techniques have yet to be incorporated into a comprehensive monitoring and troubleshooting paradigm for mine closure and mine site reclamation. Below we provide a context for genomics in the mining community and highlight specific applications of genomics and metagenomics to mine closure.

Metagenomics for Characterizing Complex Microbial Communities at Mine Sites

Microbial communities comprised of many individuals can be found in diverse environments including oceans (DeLong 2009), soils (van der Lelie et al. 2012), and places far from visible life forms deep beneath the earth’s surface (Rastogi et al. 2010). In all of these environments, metagenomics has been an essential tool for characterizing their microbial community structure and function. Microbial communities have been observed even in the hostile environments found at mine sites, where water is very acidic and metal concentrations are well above levels known to be toxic to aquatic life (Tyson et al. 2004).

It is well known that microorganisms actively contribute to geochemical reactions that include elemental, metal, and mineral transformations (Gadd 2010). Their existence in mine environments results from their ability to couple geochemical transformations to promote growth or mediate metal transformations as part of an adaptive response to tolerate the toxicity of these environments. Microbial mediated metal transformations may be deleterious such as in acid mine drainage (Johnson & Hallberg 2003), or beneficial as is the case of bioremediation (Johnson & Hallberg 2005). In most instances it has not been possible to grow microorganisms from these extreme environments outside of their natural habitats, which has impeded the accumulation of knowledge about what types of microorganisms are found in mine environments and their functions. One of the first significant advances in characterizing microbial communities in extremely acidic mine environments was achieved through the application of high-throughput sequencing of whole DNA from a biofilm obtained from within the Richmond Mine, California (Tyson et al. 2004). As this community comprised only a few individual species, their entire genomes could be reconstructed from the metagenome, which revealed information about the unique metabolic processes that they employ to survive in such an extreme environment. This highlighted the importance of microbes in environmental impacts from mining and opened the door to the use of metagenomics for characterizing mine microbial communities. Further metagenomic analyses of metal-contaminated ecosystems have revealed greater diversity than expected including many novel uncharacterized species with the capabilities to transform metals and promote biomineralization, two processes necessary for successful containment and remediation of mine wastes (Bertin et al. 2011).

Bioreactors for Water Treatment

Microorganisms obtained from acidic mine environments (Moya-Beltrán et al. 2014; Sánchez-Andrea et al. 2015) have been used to develop new biotechnologies for remediating mine environments and extracting valuable metals from mine wastes (Johnson 2014). For example, acidophilic iron-oxidizing and sulfate-reducing bacteria were used in a modular bioreactor system to precipitate zinc, iron, and arsenic from acidic mine wastewater (Hedrich & Johnson 2014). Microbe-mediated industrial processes termed “bioreactors” offer an alternative to reagent-based water treatment technologies traditionally applied on mine sites. Dissolved metals can be ubiquitous on mine sites, where metals such as selenium, copper, molybdenum, zinc, and arsenic, often along with sulfate and nitrate, are found in seepage from mine tailings storage facilities, waste rock piles, open pits, underground workings, and excess process water (McDonald & Stroscher 1998; Wang & Mulligan 2006). High-density sludge treatment is considered an industry standard for treatment and used at many sites to remove these constituents from mine-influenced water before it can be released into the environment. This is a chemical process that consumes large quantities of reagents and produces high volumes of toxic sludge requiring long-term safe storage (Zinck & Griffith 2013).

Many pilot-scale bioreactors have been developed as alternatives to chemical treatment. These systems typically take advantage of microbial metabolic processes to reduce sulfate to produce sulfide, which chemically binds with metal ions causing them to precipitate out of solution (Barnes et al. 1994). Bioreactors have also been developed to remove selenium and arsenic from effluent waters (Morita et al. 2007), to harvest valuable metals for refinement (Zinck & Griffith 2013), and to remove nitrate residues left from the use of explosives (Koren et al. 2000).

Despite their success, bioreactors have not been employed extensively to date. This may result from a lack of predictability owing to our limited understanding of the microbial processes involved. Bioreactors contain consortia of microorganisms and treatment performance depends on both the types of microorganisms present and their responses to shifting influent parameters (Dar et al. 2008). Shifts in microbial community composition can lead to incomplete treatment, putting the receiving environment at risk (Mirjafari & Baldwin 2011). To overcome this limitation, it is necessary to continually monitor microbial community composition in bioreactors and derive correlations between this and operational settings and performance metrics. To date, genomics technologies provide the only reasonable means to monitor the composition and function of these consortia in a time frame that is relevant for responding the changes in reactor conditions.

Important functional groups for metal removal in bioreactors (e.g. members of genera known to be involved in sulfate reduction including *Desulfobulbus*, *Desulfovibrio*, and *Desulfosporosinus*) are often rare, even though their action produces effective treatment (Rezadehbashi et al. 2012). Other microbes may facilitate the activity of desired groups, or some might compete for nutrients and hinder their activity. Nutrient consumption is one of the major costs in running a bioreactor, and when species that do not facilitate metal removal compete successfully for most of the nutrients, the desired organisms decline in number and bioreactor performance attenuates. Genomics technologies provide researchers with the capability to study population dynamics within these systems.

Given the affordability of high-throughput sequencing, use of metagenomics and transcriptomics for bioreactor monitoring will likely increase. As these datasets are new, interrelationships between microbial community composition, metagenomics, and metatranscriptomics with bioreactor operation and performance are under development. As the informatics improves, these metagenomics tools show promise for future process control of bioreactors.

Passive Treatment Systems for Mine Water

The benefit of the above bioreactors for mine remediation is that they can be controlled using tanks, pumps, valves, instrumentation, and defined nutrients to achieve and maintain rapid kinetics for treating large flow rates. However, their capital and operating costs are great. Plus, although highly automated, operators are needed. The vast array of natural processes for biogeochemical

cycling of metals and nutrients can be harnessed in so-called passive or semi-passive treatment systems (Ziemkiewicz et al. 2003). Passive treatments are distinct from active treatments (e.g. bioreactors) in that they require little to no maintenance or management once established. These typically take the form of constructed wetlands, either subsurface flow anaerobic, surface flow aerobic, or most commonly, combinations thereof (Younger et al. 2002). Instead of defined nutrients, mixtures of waste organic materials such as wood debris, hay, compost, pulp, and paper mill biosolids are used. These complex organic materials are decomposed into smaller carbon compounds that are needed for sulfate and metal-reducing bacteria. If successful, passive treatment can remove metals at seeps disseminated across the mine site for a fraction of the cost of active bioreactor treatment. The metal residuals are captured and secured inside the organic matrix, most often as marginally soluble metal sulfides. The ecosystem of a passive remediation system is as complicated as that of soils. Like bioreactors, they are consortia of interacting species that shift in composition with geochemical gradients, seasons, and the age of the system (van der Lelie et al. 2012). Their performance may decline as the organic material decomposes evolving toward microbial communities with completely different metabolic potentials than at the start. Preliminary studies of the microbial communities in these systems reveal that the metabolic potential for metal removal in them is much wider than previously thought (Baldwin et al. 2015). Successful metal removal occurs even in places where sulfate-reducing bacteria (SRB) are extremely rare, either meaning that many other groups of organisms that we do not know about are capable of sulfate reduction and/or metal precipitation, or that only a few sulfate reducers are needed for successful treatment. Charting of microbial communities in passive remediation bioreactors has revealed that they are not static, but fluctuate cyclically.

Bioremediation of radionuclides at mine sites is a classic example of the successful application of in situ passive remediation (Wu et al. 2006). Radioactive wastes from, for example, uranium extraction cannot be safely transported. Biostimulation with nutrients has been used to activate microorganisms (e.g. *Desulfobacteriales*) that can transform uranium species to less soluble and therefore less toxic forms (Sitte et al. 2010). Because in situ remediation takes place in a poorly controlled environment, microbial communities within the site are subjected to changeable environmental conditions. These are heterogeneous environments and dynamical systems not at any one steady-state. Metagenomics has been instrumental in charting shifts in microbial community structure and function over time and enabling correlation of effective remediation with environmental variables (Vrionis et al. 2005; Hwang et al. 2009). This has allowed key controlling factors to be identified that can be used to improve rates and the effectiveness of bioremediation (Barlett et al. 2012).

Novel Gene Discovery

Many of the biological processes necessary to reclaim previously mined substrates are present in mine wastes at the time

of closure. Successful restoration may be achieved, at least in part, through the discovery of genes unique to the mined substrate, and adapted to processing and sequestering leachable contaminants. Sulfate-reducing bacteria have demonstrated the capacity to over-produce extracellular polymeric materials that bind up copper ions, thereby creating nucleation sites for precipitation (Jalali & Baldwin 2000). *Thermoactinomyces* sp. strains are capable of sequestering the highly toxic metal tellurium via intracellular mineralization (Amoozegar & Khoshnoodi 2012). *Methanocorpusculum labreanum* metabolic processes lead to the methylation and volatilization of arsenic (Khoshnoodi et al. 2012). Enzymes involved in metal cycling can be used in future biotechnologies to improve bioremediation, and even to develop methods for in situ mining. The field of functional metagenomics involves cloning large pieces of DNA taken from microbial communities into a host (e.g. *Escherichia coli*) that can be easily and quickly grown in the laboratory (Mewis et al. 2011). Functional metagenomics applications are being realized in an array of fields including forestry, agriculture, animal husbandry, human health, and food, beverage and fuel production (Ekkers et al. 2012). Large fragments of environmental DNA obtained from metal-rich sites may contain novel genes for metal cycling, and using selective media in the laboratory, host clones are screened for metal resistance. This growing avenue of research may lead to the ability to construct biochemical pathways for metal removal using simple and easy to grow organisms that can serve as a preferable alternative to costly and potentially damaging chemical treatment of mine wastes.

DNA Barcoding: A Tool for Rapid Species Inventory

Given their much larger, more complex genomes, the molecular tools for identifying plant and animal species have developed more slowly than for microorganisms. Unlike the 16S rRNA gene that is found in all Bacteria and Archaea, the corresponding 18S rRNA gene in Eukaryotes is not as reliable as a species identifier. As such, much effort is being put into finding signature genes, called “DNA barcodes” for different groups of plants, invertebrates, and vertebrates (Hebert et al. 2003; Ratnasingham & Hebert 2007). Although a majority of barcoding to date has utilized the mitochondrial cytochrome C oxidase subunit I (COI-5P) or 16S rRNA sequences as a basis for resolving species, a number of other regions are also being used including the internal transcribed spacer (ITS) region located between the small and large ribosomal subunits. The Barcode of Life project database currently lists 1,291 published primers used for barcoding, targeting 58 separate genomic regions (Ratnasingham & Hebert 2007). Each species collected can be rapidly and cost-effectively identified, and with the proper sampling protocols, quantified in terms of relative abundance and diversity by site (Ratnasingham & Hebert 2013). New Gold’s New Afton Mine near Kamloops, British Columbia began operation in 2012. Although the mine is in an early stage of operation, managers have taken a proactive approach to future mine closure plans by implementing a partnership with the Biodiversity Institute of Ontario to perform DNA barcoding for environmental impact assessments, which is in line with New Afton’s

Biodiversity Conservation Management Plan. The pilot program involved four sites: two grassland sites (disturbed and undisturbed) and two wetland sites (disturbed and undisturbed). Invertebrate samples from these four sites were collected in the summer of 2013, and DNA barcode analyses were completed in August 2014. Between 294 and 5,560 individual invertebrates were captured in Malaise traps each week, and 3,956 species were identified representing 51,264 specimens (B.I.O. 2015). The sites were dominated by Diptera (64% by relative abundance), with greater than 50% overlap in species composition between sites with the greatest overlap and exhibiting similar habitats (wetlands vs. grassland). Wetlands contained approximately 25% more species than grasslands, and the natural grassland had approximately 50% more species than the disturbed grassland. Sequence data for this project are available within the BOLD systems database (www.boldsystems.org) under project code “NGNAA,” whereas a species inventory and additional data for the project are available on the Biodiversity Institute of Ontario’s BIObus website (www.biobus.ca). The volume of taxonomic and functional data collected for arthropods at the New Afton site is unprecedented for the mining industry, owing to the successful application of DNA barcoding. New Afton managers intend to continue monitoring with this approach on a 4–5 year time scale. Such baseline data will provide a comprehensive understanding of the trajectory arthropod communities take as this mine site develops and is reclaimed.

Beyond Genomics: Meta-Omics Approaches to Elucidate Microbial Functions

With the exception of DNA barcoding (above), we have described the process of using environmentally derived DNA to quantify and describe properties of primarily microbial communities. This approach is often termed “metagenomics” (Ellis et al. 2012). A short number of years ago, many high quality publications were based solely on 16S rRNA gene surveys of microbial communities, while currently these surveys are considered one of many standard analytical tools for scanning microbial landscapes. Although metagenomics will continue to be a powerful set of tools, the genomic record does not universally reflect the metabolic potential and biochemical activities of individual microorganisms, let alone complex microbial communities. To elucidate these mechanisms further, additional “meta” tools have been developed to qualify and quantify the mRNA and proteins expressed by microbial communities (metatranscriptomics and metaproteomics) and to relate these data to biochemical fluxes (metabolomics) and, ultimately, ecosystem functions (Krause et al. 2014).

Currently, there are well-established and growing genomic databases that include genomes for pure microbial (bacterial, archaeal, and fungal) cultures grown under laboratory conditions hosted by organizations such as the National Centre for Biotechnology Information (GenBank, RefSeq), the National Institute of Genetics (DNA Data Bank of Japan), and the European Bioinformatics Institute (EMBL). Generating drafts of microbial genomes can now be performed in days rather

than years, by individuals rather than teams, for hundreds not millions of dollars. This is a major shift from less than a decade ago (Kyrpides et al. 2014), and access to the so-called “uncultured microbial majority” (Rappé & Giovannoni 2003; Giovannoni et al. 2014) is increasing as it is now possible to physically capture and prepare de novo genome sequences for single microbial cells from the environment (Woyke et al. 2010; Lasken & McLean 2014). High-throughput next generation sequencing-based transcriptomics (Luo et al. 2014) and liquid chromatography-tandem mass spectrometry-based proteomics tools have come on-line and are being increasingly used for pure culturable microorganisms as well as mixed microbial communities (Otto et al. 2014). Although applying these tools to characterize natural microbial communities is possible, analyzing data from even a few samples in a meaningful way still requires the use of supercomputers or powerful computer clusters, and is based on imperfect databases established in the early days of genomics (Howe et al. 2014). Major breakthroughs are currently being made to advance our understanding of the dominant roles that microorganisms play in the metabolism and lifestyles of all macroorganisms such that plants and animals are no longer viewed “as autonomous entities but rather as biomolecular networks composed of the host plus its associated microbes” (Bordenstein & Theis 2015). Furthermore, biotechnological applications are being developed in an array of fields including forestry (e.g. biocatalysts for lignocellulose conversion; Strachan et al. 2014), agriculture and animal husbandry (e.g. genomics-based animal breeding; Hill 2014), human health (e.g. personalized medicine, acceptance of fecal transplantation for *Clostridium difficile* infections; Sharon et al. 2014; Fischer et al. 2015), and food, beverage (e.g. novel yeast hybrids for wine production; Carrau et al. 2015), and fuel production (e.g. selection of yeast strains for improved bioethanol production; Wohlbach et al. 2014).

Given the rich literature on metal–microbe interactions (Gadd 2010), there is tremendous scope for applying meta-omics to mine reclamation, particularly given the small but solid foundation of genomic work being done in phytoremediation (Bai et al. 2014), metal–plant interactions (Hanikenne & Nouet 2011; Bhargava et al. 2012), and soil ecology (Howe et al. 2014) on which to build. Using metagenomics and metatranscriptomics, considerable progress has been made to understand the succession of microbial constituents that takes place during acid generation and resulting acid rock drainage. Microbial communities have been characterized within tailings containing high concentrations of metal sulfides, and along gradients of acid generation revealing high community turnover from neutral (dominated by *Proteobacteria*) to highly acidic substrates (dominated by *Euryarchaeota* and increasing prevalence of *Nitrospira* and *Ferroplasma*) (Chen et al. 2013). Expression of the active microbiota via metatranscriptomics has revealed complex metabolic responses to acid generation, providing evidence for mechanisms governing how microbes both catalyze and respond to shifts in the chemical environment (Chen et al. 2014). In addition, it appears that not all critical biochemical transformations in these substrates are performed

by numerically prevalent taxa, where nitrogen fixation and sulfate oxidation are performed by rare members of the microbial community (Hua et al. 2015). Looking to the future, the gene, transcript, protein, and metabolite signatures from plants and other macroorganisms must be integrated in holistic models in order to better appreciate the holobionts (macroorganisms and their associated microbial and viral communities) that are essential to healthy ecosystems.

Understanding and Improving Ecosystem Trajectories

The mining industry has recognized for some time that planning for mine closure begins even before overburden is removed from the site (Thirgood 1986). Until recently, this has not included a significant consideration for the pre-impact community of plants, animals, and microorganisms residing in the unaltered substrate. In the future, such assessments should include both evaluations of unimpacted overburden soil communities and of stockpiles of such material for eventual recovering of the site. Microbial genomics may play a key role in developing our understanding of the processes underlying mine reclamation, by providing information on soil community dynamics in overburden stockpiles, allowing managers to “pre-store” these communities in a way that expedites recovery once these substrates are used to recover the mined site. Microbial genomics may also serve as an additional indicator for success in these systems, providing evidence of soil community formation and ecosystem trajectory in addition to traditional measures of reclamation success (i.e. plant and animal diversity or water quality). To be able to set objectives for effective mine reclamation and evaluate what is successful, the meaning of “equivalent land capability” needs to be defined for every mine site, where currently, this “capability” refers to the capacity of a site to be used for some anthropogenic purpose that is of equal value to its uses before mining activities commenced. Barcoding and metagenomics can be incorporated into methods for evaluating the degree to which ecosystem services, those qualities of the post-mine landscape that bring about human well-being have been successfully restored. Microbial community analysis provides valuable information about nutrient (carbon, nitrogen, and sulfur) cycling, greenhouse gas emissions, and metal transformations that can be fed into determining pre-mining land capability assessments. This will allow targets to be specified for post-mine reclamation, and the same tools used for evaluating whether or not remediation strategies are working.

Integration into Mine Site Management

The great questions for applying environmental genomics to mine reclamation can be put quite simply. (1) What do we sequence? and (2) How do we integrate these data into a comprehensive mine site management paradigm focused on containment and reclamation? Research and development (R&D) resources (both academic and industrial) should be

leveraged to develop sampling and data processing workflows that address the sorts of questions of interest at mine sites related to containment (e.g. “What sorts of microbial communities promote successful passive remediation?” and “What species/metabolites indicate an imminent release of contaminants and decline in water quality?”) and site reclamation (e.g. “How do soil microbial communities form on mined substrates?” and “What soil conditions promote recovery of desirable macrophyte communities?”).

Bioinformatics pipelines exist (e.g. MOTHR and QIIME) (Schloss et al. 2009; Caporaso et al. 2010), which provide for rapid processing and interpretation of sequencing runs. These pipelines can be tailored to industry-specific questions, and rapid assessment procedures developed to determine the presence and abundance of indicator groups of microorganisms. For example, *Acidithiobacillus ferrooxidans* is a well-studied microorganism that promotes the dissolution of copper from pyrite, and has been employed for industrial bioleaching to recover copper from mine wastes (Valdés et al. 2008). Mielke et al. (2003) discovered that in addition to being prevalent in substrates generating acid mine drainage, *A. ferrooxidans* is a useful predictor of future acid mine drainage formation in pH-neutral substrates. Quantitative polymerase chain reaction (PCR) protocols have been developed for this and other species involved in bioleaching, and these can be easily tailored to evaluate both their relative abundance and consortium gene expression from environmental samples (Schippers 2007). Surveying *A. ferrooxidans* prevalence in substrates and effluents affected by mining may provide an efficient early warning indicator of chemical changes in mine wastes and coincident declines in water quality. This example highlights the potential for genomics tools to drastically improve our ability to predict and proactively respond to the potential degradation of mined substrates.

Conclusions

Mining is a key global industry for development and for quality of life. The integration of environmental genomics into mine reclamation may drastically reduce the impact individual mines have on the environment by improving the reliability of biologically mediated treatment systems and reducing the need for costly chemical treatments. Biologically mediated treatment systems require the use of sequencing or PCR-based methods for rapidly quantifying microbial consortia. As these technologies are in development, there is some risk to the mining industry and the receiving environment due to uncertainty related to their long-term performance and maintenance. We believe that it is this element of uncertainty that has stalled their incorporation into industry-wide standards to date.

Management Recommendations

Environmental genomics should be incorporated into all processes at mine sites for which an understanding of microbial community composition/function is desired, and a potential

contributor to rapid/accurate biological surveys. Reclamation practitioners should consider environmental DNA and associated data products as a component of a thorough monitoring program for soils and discharge waters, and a contributor to understanding/troubleshooting ecosystem recovery. In conducting this review, we identified two key constraints on the widespread application of genomics for mine closure. First, industry-wide standard operating protocols need to be developed for mine closure, including sampling procedures designed for representativeness and comparability (spatial extent, replication, and temporal frequency). Second, sequencing and data interpretation pipelines should be established in parallel with the development of these standards, allowing mine managers to more easily respond to shifts in microbial communities in treatment systems as well as the receiving environment and generate supportive evidence for reclamation success.

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