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DNA Metabarcoding - a new approach to fauna monitoring in mine site restoration.

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Abstract

Ecological restoration of landscapes is an integral part of the mining process. However, restoration is often constrained by a lack of consistent monitoring approaches. For example, the need for specialist techniques and trapping approaches often limits monitoring of fauna recovery. Application of molecular tools has made important contributions to understanding factors influencing restoration success. Here we outline advances in next-generation sequencing (NGS) methods, especially metabarcoding of environmental DNA (eDNA). These have potential to revolutionize the practical contribution of genetics to the monitoring of fauna in a restoration context. DNA metabarcoding involves the simultaneous characterization of biota using DNA barcodes. It is a powerful method to assess the biodiversity contained within environmental samples (e.g. scats, bulk arthropods, soil, water and sediment). This review outlines the challenges associated with current approaches to monitoring faunal biodiversity throughout ecological restoration. We demonstrate how DNA metabarcoding can contribute to improving ecological restoration outcomes through improving fauna monitoring capacity.

Key words: Ecological restoration; Environmental DNA; Fauna; Metabarcoding; Mine Site; Monitoring

Conceptual Implications

- Following mining, there is a lack of monitoring of fauna in ecological restoration, and a reliance on vegetation monitoring to indicate whole ecosystem recovery.
- DNA metabarcoding offers an approach to address this through making fauna biodiversity surveys easier to conduct by using DNA extracted from environmental

45 samples (e.g. scats, bulk arthropods, soil or water) and sequenced using next-
46 generation platforms.
47 - Current challenges associated with implementation of DNA metabarcoding for
48 ecological restoration monitoring include DNA persistence, barcode choice,
49 taxonomic reference databases, inability to survey abundance and vagrant DNA.
50 - As the DNA metabarcoding methodology develops and the challenges are addressed
51 through further research, this technique has potential to become a key component
52 of best-practice fauna monitoring as well as broader biodiversity monitoring to
53 improve ecological restoration outcomes.
54

55 **Mine Site Restoration**

56 Mining is the basis of many global economies. In the last 60 years this importance has
57 grown as demand and production of metals has increased (Cooke & Johnson 2002). In 2016,
58 a global analysis of the top 40 mining companies estimated the value of mining to be $US
59 748 billion, with profits of $US 20 billion (PWC 2017). However, this high economic value is
60 accompanied by a resource footprint in the range of 1000's km^2 per company operating
61 globally (Environmental Protection Authority 2014; Stevens & Dixon 2017). Environmental
62 considerations have now become a key legislative requirement of mining projects in most
63 developed countries (Mudd 2007). Therefore, ecological restoration is increasingly an
64 integral part of the mining process (Mchaina 2001; Bridge 2004; Cross et al 2018). However,
65 mine closure and ecological restoration is increasingly expensive (Costanza et al. 2014). For
66 example, it is estimated that in one mining domain in Australia, the total rehabilitation,

restoration and closure costs for all mines will be $US 3-4.5 billion ($A 4-6 billion) (Gorey et al. 2016).

Common goals set for mine sites following closure include restoration of structure, diversity, and function of the disturbed ecosystems (Miller et al. 2017; Majer & Nichols 1998; Cristescu et al. 2012). In most cases, returning lost biodiversity is focused on restoring plant communities (Bisevac & Majer 1999; Cristescu et al. 2012; Longcore 2003; Majer & Nichols 1998; Miller et al. 2017). However, the restoration of other elements of biodiversity, including fauna, is relatively poorly understood. Monitoring of other than vascular plants is often neglected in the reporting of restoration outcomes. In part, this assumes that elements such as fauna will migrate and naturally colonize restored sites (Thompson & Thompson 2004; Cristescu et al. 2012). Thus several studies have demonstrated the natural regeneration of mobile fauna (Majer & Nichols 1998; Majer et al. 2007; Andersen et al. 2002). However, many animals such as short-range endemics have narrow distributions and poor colonisation capacity (Harvey 2002). Relatively high proportions of such taxa occur in some environments (Rix et al. 2015; Mason et al. 2016), particularly in old stable landscapes (Mucina & Wardell-Johnson 2011; Wardell-Johnson et al. 2016). This limits the generality of findings from studies on highly mobile organisms, or those from more recent landscapes.

The lack of understanding of faunal recovery represents a significant gap in mine site restoration practices. Ultimately, in the evaluation of the trajectory of mine site restoration, methods to assess faunal biodiversity are equally as important as those provided by floristic assessment. Importantly, a clear basis for defining restorative activity is now available

through the International Standards for the Practice of Ecological Restoration (McDonald et al. 2016). Thus, key regulatory objectives in many countries now demand establishment of a self-sustaining ecosystem. Currently the focus remains on the return of a sufficient representation of plant biodiversity, and a lack of weeds and pest species (Miller et al. 2017). However, incorporation of fauna is essential, not just because fauna is important in their own right. Rather, fauna plays a critical role in ecosystem structure and processes including pollination, nutrient cycling, soil aeration, plant composition, seed dispersal, and pest control (Andersen et al. 2002; Ruiz-Jaen & Mitchell Aide 2005; Haddad et al. 2009; Cristescu et al. 2012). Therefore, a more holistic model of mine restoration monitoring is overdue. Such a model would enable assessment of whether post-mining restoration is achieving the return of a suite of biodiversity from microbes to mammals.

DNA metabarcoding is the process of characterising a set of genetic markers to capture a broad snapshot of the biodiversity chronicled within environmental samples. This approach has been used to develop detailed audits of terrestrial faunal biodiversity in a range of environments (Table 1). Here we outline the approaches available, demonstrate the effectiveness of DNA metabarcoding and show the benefits of the approach for more effective interpretation of ecological restoration outcomes. We discuss the challenges associated with methodologies currently used for monitoring biodiversity in mine site restoration, and outline the benefits of utilising this emerging DNA 'toolkit'.

Monitoring in a Mine Site Restoration Context

We use examples from Australia where mining is pervasive, and causes impacts in a wide

variety of biodiverse ecosystems. Until recently, the focus of mine restoration programs in Australia was to establish adequate plant diversity, density and cover. This has sometimes been for purely aesthetic purposes (Longcore 2003; Thompson & Thompson 2004). However, ecological restoration programs now aim for resilient, self-sustaining, functional ecosystems that require minimal ongoing management inputs (Environmental Protection Authority 2010; ANZMEC 2000; Lacy et al. 2016). Nevertheless, the concentration of monitoring effort on plant communities persists. A substantial component of "ecosystem function" relies upon interactions between several elements of the biosphere. This includes soil microbial interactions with plants and animals to facilitate nutrient cycling (Wardle et al. 2004; Lavelle et al. 2006), and provision of pollination and seed dispersal to plants through animal vectors (Brown et al. 1997; Dixon 2009; Traveset et al. 2007). Therefore, a reliance on plants in the monitoring of restoration may lead to under-achievement in restoration outcomes (Miller et al. 2017).

A survey of Australian mine sites in 2004 found that 64% of mines conducted consistent flora monitoring, while only 8% of mines monitored vertebrate fauna systematically. Further, each had a different monitoring protocol (Thompson & Thompson 2004), despite the availability of standardized techniques for the survey of all major faunal groups (Environmental Protection Authority and Department of Environment and Conservation 2010; Catterall et al. 2004; De Bondi et al. 2010). The monitoring process also usually overlooks invertebrates due to their disproportionate crypsis, high levels of diversity, and paucity of knowledge (particularly outside Europe and North America) (Oliver & Beattie 1996). While there may be reliable benchmarks against which to monitor the restoration of

vertebrate fauna, there is rarely anything against which to measure invertebrate return. Fauna of recognized conservation significance tend to be specifically monitored throughout all stages of mineral extraction, including restoration. However, many conservation critical invertebrate taxa are understudied, difficult to detect, and have poorly understood drivers of population decline or responses to management (Mason et al. 2016).

To overcome the constraints imposed by the difficulties of monitoring invertebrate restoration, some groups (e.g. ants, Andersen & Majer 2004) have been recommended as proxies for biodiversity and ecosystem functionality (bio-indicators). However, their validity as measures of total biodiversity has been disputed (Yu et al. 2012; Lindenmayer et al. 2002). If the goal of restoring diversity is focused on returning ecosystems to baseline levels, then broader representation of biodiversity must be accounted for in site monitoring (Proctor et al. 2003).

DNA Metabarcoding for Monitoring Fauna in Ecological Restoration

It is clear that there is a need to investigate new methodologies to overcome shortcomings associated with restoration monitoring practices (Williams et al. 2014). DNA metabarcoding is an approach that can rapidly assess biodiversity using genetic material found in bulk organic samples (e.g. arthropods; Ji et al. 2013; Yu et al. 2012) or from degraded DNA in environmental samples (e.g. soil; Taberlet, Coissac, Pompanon, et al. 2012; Taberlet, Coissac, Hajibabaei, et al. 2012). The rationale behind metabarcoding is elegantly simple. DNA is extracted from an environmental sample, and a genetic marker (barcode) is then selectively amplified with a metabarcoding primer assay using a Polymerase Chain Reaction

159 (PCR). Multiplex Identifier (MID) tags attached to each barcode during the PCR stage links
160 each sequence to the sample it came from. The amplified DNA is then sequenced using
161 Next-Generation Sequencing (NGS) technologies. The resulting sequences are identified
162 taxonomically using reference databases or, in a taxonomically independent approach,
163 grouped into operational taxonomic units (OTUs) (Figure 1). NGS allows for the rapid
164 sequencing of millions of barcodes at a much faster and cheaper rate than previous
165 technologies. This makes DNA sequencing more accessible. DNA metabarcoding workflows
166 can rapidly identify taxa, establish baselines (pre-disturbance or reference ecosystems) and
167 compare these to restoration chronosequences. Metabarcoding data can be used on its own
168 or in conjunction with data collected by traditional methods. (Figure 1).
169
170 The source of DNA (sample material) for metabarcoding greatly influences what organisms
171 can be detected. Water, soil, air and faeces contain traces of eDNA that can be extracted,
172 sequenced and processed to inform biological monitoring (Bohmann et al. 2014). Bulk
173 samples of invertebrates can also be sequenced rather than identified morphologically (Yu
174 et al. 2012). For targeted monitoring (e.g. predator diet) sample choice is relatively simple
175 (i.e. predator feces). However, for broad biodiversity assessment sampling is more complex
176 and there is greater potential to sample vagrant DNA (i.e. species not resident in a survey
177 site; Thomsen et al. 2012).
178
179 As with all sampling and analysis technologies, metabarcoding clearly must be deployed
180 with expertise and awareness of its associated complexities. Nevertheless, there are distinct
181 advantages to this technology over traditional biodiversity assessment approaches (Table 2).

182 These advantages have led to rapid acceptance in some fields, for example, aquatic
183 biosecurity (Hosler 2017; Pochon et al. 2017). The biggest benefit of metabarcoding is that
184 the data can be gathered quickly (Ji et al. 2013) and can be analysed and verified by
185 independent parties (Yu et al. 2012). The latter is significant in mine rehabilitation as it adds
186 accountability to restoration activities. Monitoring can occur without verified taxonomic
187 identification using only Operational Taxonomic Units (OTUs). Based on sequence similarity,
188 researchers can monitor the trajectory of restored communities and/or assess whether they
189 are becoming more similar to reference communities over time. This is done by comparing
190 the OTU community composition of the restoration to that of reference or pre-disturbance
191 sites (e.g. Gellie et al. 2017; Ji et al. 2013). From this analysis it is also possible to detect
192 indicator OTUs that could be used to characterise stages in a restoration trajectory. Once
193 indicator OTUs are identified, classical morphological taxonomic approaches could also be
194 applied in a more targeted fashion (Figure 1). Similar to traditional approaches to
195 monitoring (Cooke & Johnson, 2002), there is no overarching sampling strategy that will
196 work for each project, target taxa or questions asked. Pilot studies are often required to
197 make informed a priori decisions about the approach (Dickie et al. 2018). However,
198 metabarcoding technology, if appropriately deployed and accurately analysed, can
199 overcome the detectability and sampling bias of traditional sampling, in an efficient and cost
200 effective and manner (Calvignac-Spencer et al. 2013; Ji et al. 2013; Biggs et al. 2015;
201 Thomsen & Willerslev 2015).
202
203 These benefits support the use of metabarcoding in fauna biodiversity monitoring. If
204 metabarcoding is undertaken with an appropriate sampling design that is sensitive to the

biology and ecology of the target fauna, then it increases the opportunity for detecting reclusive fauna (Radulovici et al. 2010). Such taxa may be of conservation priority or important to monitor for other reasons (e.g. indictors of restoration trajectory). This approach may be of particular interest where traditional trapping approaches are resource intensive. For example, Si, Kays, & Ding (2014) found that 8700 camera trapping days were required as a minimum to detect all the known species at a site. This increase in detectability in metabarcoding does, however, require careful interpretation as it also increases the chances of PCR contamination by vagrant DNA from the field or the laboratory, but can also occur from other sources such as prey DNA or from transient individuals (Wilcox et al. 2013; Lahoz-Monfort et al. 2016).

Metabarcoding also has substantial potential to minimize the challenges of morphological approaches in some faunal groups. Ji et al. (2013) compared the morphological and metabarcoding methodology for species identification for 55,813 bird and arthropod specimens. Morphological identification required 2,505 person hours of taxonomic expertize. By contrast the metabarcoding approach equated to 645 person hours and 520 hours of computing time. Both approaches achieved statistically similar alpha and beta-biodiversity levels and came to the same policy conclusions for conservation management. Gibson et al. (2014) found that the metabarcoding methodology could isolate an additional four orders, 21 families, 40 genera, and 19 species than individually identified and Sanger sequenced (single organism sequencing) specimens in a sample of tropical arthropods. This is due to the metabarcoding approach allowing for non-target species within the habitat to

be identified, with traces of organisms found via gut contents of parasitic or predatory animals (Rougerie et al. 2011; Leray et al. 2012).

Until recently, most studies applying metabarcoding for biodiversity management have investigated aquatic systems (reviewed in Thomsen & Willerslev (2015)). For aquatic systems, metabarcoding studies have examined the number and size of samples required (Mächler et al. 2016; Rees et al. 2014), different methods for capturing and extracting eDNA (Deiner et al. 2015; Eichmiller et al. 2016; Minamoto et al. 2016), persistence of eDNA (Barnes et al. 2014; Díaz-Ferguson & Moyer 2014; Rees et al. 2014), choice of molecular makers (Freeland 2017), and more (see Freeland 2017). Terrestrial studies have examined the assays required for adequate sampling depth (Drummond et al. 2015) in comparison with traditional biomonitoring methodologies for eukaryotes. In a restoration context, metabarcoding has been applied to characterise soil microbe communities (e.g. Gellie et al. 2017; Yan et al. 2017) and terrestrial microhabitats (Creer et al. 2011; Porazinska et al. 2010) (for more examples, see Table 1). Metabarcoding data can be gathered using the same trapping and sampling design methodologies for invertebrates as traditional approaches. These yield similar land management outcomes to those reached through classical taxonomically-based approaches (Ji et al. 2013). However, as a field, DNA metabarcoding of terrestrial eukaryotes is still in the process of development for landscape management. Further research focusing on the development of metabarcoding assays, sample design, sensitivity analysis and reference databases is required.

Current Limitations and Future Directions

A key assumption of DNA metabarcoding as a monitoring tool is that the DNA presence indicates the local presence of the living organism. While this is logical for bulk organic samples that include parts of the actual organism, it is not so obvious for eDNA. Soil for example can preserve DNA for thousands of years under specific conditions (Epp et al. 2012; Willerslev et al. 2003). In temperate soils, DNA has been shown to remain detectable up to 77 days (Widmer et al. 1997), six years (Andersen et al. 2012) and ~3300 years (Haile et al. 2007) after the organisms' removal. Temperature, soil chemistry and texture can all influence the distribution and persistence of eDNA (Levy-Booth et al. 2007; Andersen et al. 2012). These issues represent a challenge for mine site monitoring, where the aim is to determine the biodiversity present at any point in time. Restricting sampling to the soil surface may reduce the risk of detecting past diversity, as DNA is leached (Andersen et al. 2012) or degraded (Lindahl 1993) over time. However, more research is needed on DNA persistence in terrestrial systems. Alternatively, RNA metabarcoding approaches have been suggested to capture biota that are actively transcribing their genes and may therefore be a better proxy for viable organisms (Cenciarini-Borde et al. 2009). Whilst RNA can provide a better proxy for understanding more immediate effects on organisms, DNA is more reliable for assessing effects on community composition (Laroche et al. 2017). Thus, if funding, expertise, and time permits conducting both DNA and RNA metabarcoding biomonitoring surveys may be recommended.

Even with an understanding of the persistence of terrestrial DNA, determining absolute population abundance from DNA metabarcoding is still improbable. First, the amplification step in metabarcoding skews sequence abundances such that relative sequence abundance

of a species is an unreliable predictor of abundance in the sample material (Clarke et al. 2014; Elbrecht & Leese 2015). If sequencing without PCR becomes a viable possibility (Taberlet, Coissac, Pompanon, et al. 2012) this may not be an issue in future. However, the microbial content within samples may make this problematic (Stat et al. 2017). Second, the amount of DNA in a sample is affected by the organisms' biomass more than by population density (Elbrecht & Leese 2015; Andersen et al. 2012). This is not resolved by eliminating the dependency on PCR, but can be remedied by using parts of individuals in a sample to reduce their biomass (i.e. Beng et al. 2016, Ji et al. 2013). In addition, other variables such as seasonal spawning (de Souza et al. 2016) or even different DNA shedding rates between organisms can reduce the ability to estimate population abundances. Murray et al. (2011) were able to use relative sequence abundance for dietary analysis. However, this does not necessarily reflect absolute abundances of each prey species. Elbrecht and Leese (2015) recommend using presence/absence data in spatially separated sampling locations rather than relative sequence abundance. Ultimately, using the number of positive samples as a coarse proxy of species abundance is achievable. However, but it is unlikely that metabarcoding will ever deliver accurate direct estimates of multispecies population densities. Nevertheless, this is not a problem unique to genetic methods. Rather it is an issue with all relative sampling methods (e.g. Topping & Sunderland 1992; Santos et al. 2007).

A further challenge in DNA metabarcoding is choosing the appropriate barcode and then developing robust assays to detect the target taxa. There is no universal barcode (i.e. one gene region for all biota) that provides powerful enough resolution to identify all DNA in a

296 sample. Primers are chosen, depending on the target organisms, to amplify barcodes on a
297 particular gene region. These are theoretically similar within a species but contain enough
298 variation to separate different species/lineages. Where researchers are interested in
299 detecting particular taxa they can design PCR assays that are specific to the target.
300 Researchers interested in broader assessment of biota in a sample may use a series of
301 genetic markers that are shared by a range of organisms. For example, Lahaye et al. (2008)
302 recommend matK as a universal barcode for plants, while Fahner et al. (2016) recommend
303 using rbcL and ITS2, partly because of existing databases for taxonomic identification. The
304 P6 loop of the trnL intron is suggested for plants because while it has low resolution, it is
305 short (10-143bp). It is more likely to be found in degraded environmental DNA than longer
306 barcodes (Taberlet et al. 2007).
307
308 In selecting genetic regions, there are trade-offs between the size, breadth, and resolution
309 of barcodes. Larger barcodes provide greater taxonomic resolution, but are less likely to be
310 found in degraded environmental DNA and may be too long to be sequenced on current
311 NGS platforms. There are also certain taxa that cannot be reliably detected because of
312 amplification biases (Clarke et al. 2014; Deagle et al. 2014). For mine site monitoring, it is
313 likely that multiple barcodes and metabarcoding assays will be necessary. Thus, a recent
314 study found the best (18S rRNA) metabarcoding assay recovered only 44% of the taxa (at
315 family level) when compared to a multi-assay approach (Stat et al. 2017).
316
317 Perhaps the most important challenge in the use of metabarcoding is the current state of
318 reference sequence databases particularly for biodiversity hotspots where species richness

and endemism are high. Taxonomic reference databases contain sequence data from taxonomically identified specimens, and are key to identifying the metabarcoding sequences. Initiatives such as the Barcode of Life Data Systems (BOLD) are aimed at producing high quality reference libraries, improving on databases like GenBank by having permanent voucher specimens, minimum sequence length of 500 base pairs, and limits to certain barcoding regions (Ratnasingham & Hebert 2007). At present, BOLD accepts only the cytochrome c oxidase subunit I (COI) gene for fauna barcoding. However, the lack of conserved regions makes this gene unsuitable for most amplicon based metabarcoding workflows (Deagle et al. 2014). Without conserved regions within the gene, creating assays to amplify smaller sections is unreliable and leads to biases in the amplified taxa that in turn affect biodiversity estimates (Sefc et al. 2007; Yu et al. 2012; Deagle et al. 2014).

At present, there is no best practice bioinformatics workflow able to be undertaken and multiple methodologies are used. This can contribute to discrepancies between studies (Fonseca et al. 2010; Hao et al. 2011; Gibson et al. 2014; Yu et al. 2012). Also, in many regions, much of the invertebrate fauna remains undescribed, so species level identification is not within the capacity of morphological identification (Austin et al. 2004). No doubt reference databases will continue to grow. In addition, more barcoding regions will be developed that rely on 'morpho-species' in the absence of formal nomenclature.

The greatest challenge for any kind of monitoring in ecological restoration is the question of how to ascertain ecological functionality (Ruiz-Jaen & Mitchell Aide 2005; Hobbs & Cramer 2008), which is increasingly the standard required (McDonald et al. 2016). However,

metabarcoding may be able to detect markers indicative of insect pollinators or decomposers that have key roles in reference ecosystems.

Conclusion

Better tools are needed to monitor ecological restoration. DNA metabarcoding offers an enhanced capacity to monitor not only fauna but holistic biodiversity. Whereas traditional taxonomic approaches may be most appropriate in some situations (i.e. when population abundance data is needed), a combination of both traditional and molecular methods to measure biodiversity can increase the breadth and richness of monitoring data. Indeed, metabarcoding workflows may offer a technique for faunal diversity studies that is faster, more accessible and less invasive than standard approaches.

How current limitations to fauna survey apply to the new technology must be carefully considered in applying a metabarcoding approach to fauna monitoring in ecological restoration. Further, what developments are required of the technology to meet these challenges? Over time, some of the limitations and lack of knowledge currently intrinsic to metabarcoding methodologies will be overcome with DNA sequencing technologies. These include the limitations of complete genetic databases, and standardized assays/workflows.

Research and development into new techniques to enhance mine site monitoring will be paramount to the mining sector's continuation to meet environmental objectives. Using metabarcoding to establish baselines, monitor fauna during operational phases and then to track restoration chronosequences trajectories, will likely become a key component of the

364 'toolkit' employed in the mining sector. In the future, DNA metabarcoding will need to
365 appear on the radar of regulatory bodies charged with setting up the legal framework for
366 what constitutes best-practice in mining restoration.

367

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373

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Table 1. Examples of recent studies in terrestrial ecosystems where DNA metabarcoding has been used in biodiversity audits of fauna.

Application/Study Description	Sample Type	Target Taxon	Reference
Restoration and systematic conservation planning of several sites determined through the metabarcoding methodology.	Arthropod and Bird Specimens	Arthropods and Birds	(Yu et al., 2012; Ji et al., 2013)
Dietary analysis to determine if introduced species are competition for endangered native.	Faeces	Plants, Invertebrates	(Brown et al., 2014a; Gebremedhin et al., 2016)
Determining patterns of litter arthropod diversity and composition among natural and human impacted sites.	Arthropods	Arthropods	(Beng et al., 2016)
Diet analysis of an endangered land snail to facilitate the ecological restoration and relocation of this species.	Faeces	Earthworms	(Boyer et al., 2013; Waterhouse et al., 2014)
Identify the biological impacts of logging and oil palm plantations and develop cost effective biodiversity protection methods.	Arthropods	Arthropods	(Edwards et al., 2014)
Tracing environmental contaminants and bioaccumulation of heavy metals through diet analysis of beetles.	Faeces	Invertebrates	(Šerić Jelaska et al., 2014)
Vulnerability assessment of threatened species to determine niche occupation and flexibility within an ecosystem.	Faeces	Vertebrates and Invertebrates	(Brown et al., 2014b)
Testing the utility of soil as a non-invasive and fast way of profiling vertebrate diversity in an environment.	Soil	Vertebrates	(Andersen et al., 2012)
The utility of soil and leaf-litter samples to rapidly sample and assess diversity in the environment.	Leaf litter, Soil, Arthropods	Arthropods	(Yang et al., 2014)
Determining vertebrate diversity in an environment using the gut contents of parasites and predators.	Carrion flies, Scat and Leeches	Vertebrates	(Schnell et al., 2012; Shehzad et al., 2012; Calvignac-Spencer et al., 2013)
Drinking water as a source of environmental DNA for the detection of terrestrial wildlife species	Water	Mammals	(Rodgers & Mock 2015; Ushio et al. 2017; Klymus et al. 2017)

Determining the diversity of important species useful in ecotoxicology or pharmacology using DNA metabarcoding.	Dung	Dung insects	(Blanckenhorn et al. 2016)
Detecting mammalian biodiversity from natural salt-licks.	Water	Mammals	(Ishige et al. 2017)
Identifying mammalian predators of at-risk species to inform management plans.	Saliva	Vertebrates	(Hopken et al. 2016)
Establishing trophic linkages and food webs	Faeces	Invertebrates	(Kaunisto et al. 2017; Roslin & Majaneva 2016)
Enhancing standard monitoring protocols for characterising community composition and potential conservation implications for threatened fauna.	Water	Amphibians	(Sasso et al. 2017)
Detecting soil fauna diversity using a metabarcoding approach.	Soil	Coleoptera	(Andújar et al. 2015)

Table 2: Comparison of Traditional Survey and DNA Metabarcoding monitoring methodologies

	Traditional Survey	eDNA Metabarcoding
Impact on species	Risk of harming or disturbing fauna with trapping and observation methods.	Non-invasive sampling methodology. Minimal disturbance at most.
Resource effort required	High fieldwork effort required. Costly in remote areas and requires experienced/trained personnel for fieldwork components and taxonomic expertise for specimen identification.	Potential time and cost benefits over traditional survey methods. Requires trained professionals for laboratory work.
Reliability of method	Not affected by false-positive detection, but could be affected by false-negatives for cryptic/smaller species.	Can be affected by false-positives or false-negatives due to contamination or PCR errors. Biodiversity estimates are highly dependent on the resolution of markers used and could be impacted by differences in organism biomass
Standardization	Mine-site specific/poor monitoring procedures due to cost/personnel limitations restricts data comparisons across time and space and across multiple mining operations.	High degree of standardization possible Auditable by third parties.
Biomonitoring information	Information can be gathered on distribution, abundance, population structure, and demography of species.	Generation of presence/absence data with the need for subsequent field verification for target species locations. Issues with the generation and maintenance of barcode databases to link to sequences generated to species. Can detect difficult to trap species.

Figure 1. Seven step process for the integration of the DNA metabarcoding methodology to create a best practice frame work for monitoring in ecological restoration.