ORIGINAL RESEARCH

Mining exploration infrastructure affects biophysical habitat characteristics and ground-dwelling arthropod communities

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Abstract

Monitoring of environmental impacts of mining activities typically focuses on the main operation footprint, neglecting exploration infrastructure like tracks, roads, and drill pads. These areas are cleared of native vegetation and impacts on the surrounding environment can be both cumulative and enigmatic. Here, we study the impacts of mining exploration infrastructure on habitat characteristics and ground-dwelling arthropod communities in the Midwest region of Western Australia. The study was conducted at three mine sites, each with three infrastructure types: maintained tracks, unmaintained tracks, and drill pads along transects extending 100 m away from the disturbance into remnant vegetation. Habitat characteristics were measured, and arthropods collected from pitfall traps along these transects and identified using COI metabarcoding. The overall arthropod community and two indicator groups, ants (Formicidae) and springtails (Collembola) - were used to measure arthropod responses to changes in response to habitat disturbance. Whilst changes in habitat were only visible to 10 m from the disturbance, impacts on arthropod communities could be detected up to 100 m into the remnant vegetation, and these responses were more complex. In general, we found similar patterns expressed in the compositional changes for arthropods overall and between our chosen indicator groups, but they were not the same across all sites and infrastructure types. Our results demonstrate the utility of bulk arthropod metabarcoding and different arthropod indicator groups for documenting the effects of fine-scale habitat destruction, degradation, or disturbance. They also highlight the need to monitor the negative impacts of mineral exploration on the environment.

Keywords Mining · Exploration infrastructure · DNA metabarcoding · Arthropod communities · Habitat characteristics · Indicator species · Environmental impacts

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Communicated by Akihiro Nakamura.

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Introduction

The main drivers of species extinction across the globe are human activities that cause the destruction, transformation and fragmentation of landscapes (Ellis et al. 2010). Although mining operations only occupy 0.3% of land globally (Hooke et al. 2012), they can have more severe impacts on natural habitats than other visible and wide-ranging activities such as agriculture and urbanisation (Redondo-Vega et al. 2017). This is because mining activities often target geologically unique landscape formations sur-rounded by pristine and endemic ecosystems (Guijón et al. 2011; Redondo-Vega et al. 2017; Tibbett 2015).

The ecological impacts of habitat disturbance caused by anthropogenic activities including mining are complex and pervasive, affecting population dynamics and community structure of both flora and fauna (Bolger et al. 2000; Fahrig 2003). For example, fragmentation increases the extent of edges around habitats. These edges naturally occur between two habitats with varying light, humidity, and temperature levels, potentially impacting vegetation community structure and the species present (Ries and Sisk 2004; Santos et al. 2008). These biotic and abiotic changes between the contrasting ecotones are known as 'edge effects' (Ewers and Didham 2006; Murcia 1995). With the creation of more edges in anthropogenically-altered landscapes, the resulting changes in microclimate can also allow the invasion of weeds and other alien species, leading to changes in overall community structure, and potentially, the functionality of ecosystems (Goosem 2012; Pohlman et al. 2009). The extent to which the impacts of the edge effect can penetrate adjoining habitats can extend from a few metres to several hundreds of metres into remnant habitat (Goosem 2007; Laurance et al. 2009).

In the context of mining, habitat fragmentation and the resulting edge effects are further complicated by the associated mining exploration and linear infrastructure that is constructed. Mining exploration and linear infrastructure encompass haul roads, unpaved tracks, exploration (or seismic) lines, drill pads, power lines, pipelines, railways, and fence lines (Raiter et al. 2014). While the impacts of individual roads and tracks may only require a small area to be cleared, the overall impacts can be significant (Raiter et al. 2014). These impacts are known as 'cumulative' or 'enigmatic impacts' (Canter and Ross 2010; Raiter et al. 2017; Therivel and Ross 2007). As these impacts are rarely accounted for in impact assessment protocols and regulatory frameworks (McKenney and Kiesecker 2010), it is important to understand the extent of their impact on species and communities and the biophysical drivers behind them to improve how mining operations are managed (Cross et al. 2021).

Changes in biophysical habitat characteristics, such as vegetation cover and soil composition, can serve as indicators of edge effects and habitat quality (Bolger et al. 2000; Fahrig 2003), and this approach is often combined with surveys of ubiquitous organisms that are responsive to disturbance, such as arthropods (Hodkinson and Jackson 2005; Kwok et al. 2016; McGeoch 1998; Moir et al. 2005; Roy et al. 2003). Arthropods are widely targeted for monitoring in a mining context, for example, tracking restoration trajectories after mining activities (Casimiro et al. 2019; Fernandes et al. 2019; Majer and Nichols 1998; van der Heyde et al. 2022a; Heyde et al. 2022b). Traditionally, arthropod biomonitoring is undertaken by trapping, followed by morphological identification of selected taxa from impacted and reference areas (Yu et al.

2012). However, this methodology is resource-intensive, relying on considerable time and taxonomic expertise to identify large volumes of taxa, especially in biodiverse areas or over large spatial scales (Ji et al. 2013), meaning that only a few selected taxa can be assessed (Pik et al. 1999).

DNA metabarcoding is a useful molecular tool that can address some problems associated with traditional morphology-based monitoring techniques (Ji et al. 2013; Yu et al. 2012). With metabarcoding, it is possible to identify species across multiple taxonomic groups simultaneously and with a fraction of the person-hours of morphology-based techniques (Ji et al. 2013). Thus, DNA metabarcoding of invertebrates could be a beneficial addition to mine site monitoring programs and provide valuable insights into environmental impacts across the whole community of arthropods inhabiting these areas. In recent years, DNA metabarcoding has been used in various systems, including a mine site monitoring context, to help understand and document the impact of environmental changes on invertebrates (for example, using pitfall traps in Fernandes et al. (2019) and van der Heyde et al. (2022a), and using malaise traps in Lynggaard et al. (2020)).

In traditional biomonitoring studies, indicator taxa are often used instead of whole arthropod communities to measure the impacts of disturbance (Majer and Nichols 1998; Heino 2014; Kerr et al. 2000). Indicator taxa are focal groups of organisms with well-known taxonomy and ecology that can be used as representatives of overall changes in the ecosystem. The most common arthropod groups used as indicators in terrestrial ecosystems include beetles, ants, spiders, and butterflies (Oliver and Beattie 1996; Pik et al. 1999; Terlizzi et al. 2003). Ants (Formicidae) are favoured as an indicator taxon due to the consistency of their response to disturbance (Andersen 2019; Hoffmann and Andersen 2003). Another group, springtails (Collembola), has also been recommended as an indicator in Australian systems as they are highly sensitive to disturbance in habitat structure (Greenslade 2007). However, unlike more well-studied focal groups, such as ants, there is limited knowledge on springtail taxonomy, and many species are yet to be described (Jordana and Greenslade 2020). Further, focusing only on certain invertebrate taxa may not be reflective of whole ecosystem dynamics as some groups may not interact with each other spatially or have comparable taxonomic richness (Khosravi and Hemami 2019; Prendergast et al. 1993; van Jaarsveld et al. 1998), and thus they may not fully encapsulate the changes and impacts on the diversity of multiple taxonomic groups and total arthropod assemblages (Siddig et al. 2016; Silva et al. 2017). Therefore, a direct comparison of patterns detected by all arthropods present in samples, along with particular groups such as ants and springtails, is valuable for validating the use of indicator groups.

In this study, we used invertebrate DNA metabarcoding to explore the impacts of mining exploration infrastructure on ground-dwelling arthropod communities associated with banded ironstone formations (BIFs) in the Midwest region of Western Australia. Here we aimed to identify (1) the response of biophysical habitat characteristics and arthropod community diversity and composition to mining exploration disturbances, and (2) to identify whether ants and springtails could function as indicator groups by reflecting patterns in the overall arthropod community.

Materials and methods

Experimental design and study sites

This study was conducted across three iron-ore mines: Koolanooka Hills (KO), Karara (KA), and Mount Gibson (MG) (Fig. 1A) within the Midwest region of Western Australia. The Midwest region is characterised by a dry-warm Mediterranean climate, with mild, wet winters and hot, dry summers, receiving an average rainfall of 300–400 mm, mostly occurring during the winter months (Meissner and Caruso 2008). The average summer and winter daily maximum temperatures are 36.3 °C and 19.1 °C, respectively (Meissner and Caruso 2008). Vegetation communities differed between the three sites, but all were Acacia-dominated shrublands. Vegetation communities in the KO site remnants exhibited considerable disturbance, mainly from feral goats grazing on the natural vegetation but the other two sites were less disturbed.

At each site, three exploration infrastructure types were chosen: maintained tracks (MT); unmaintained tracks (UT); and drill pads (Pad). MT was identified as a frequently used and disturbed track with no evidence of vegetation regrowth. By contrast, UT would display vegetation growth, typically along the centre of the track or in old tyre tracks, and it may have vehicular access blocked off. Pads were areas where land had been cleared of all vegetation to drill into the BIF from which iron-ore is extracted. These were small, cleared areas that, once disturbed, had been left relatively untouched, with many having some degree of vegetation regrowth on them. Disturbance age varied across all three sites. The age of the Pads at each site was estimated as follows: KA Pads were constructed after 2012; KO Pads were constructed between 2005 and 2012; and MG Pads were constructed before 2000 (Stephens 2018). The age of the UT and MT is unknown, although MT were still used at the sampling time.

Sampling was carried out along a transect from the interior of each chosen disturbance to around 100 m into the adjacent remnant shrublands along the natural contour of the terrain so as to avoid any potential effects of elevation. For each infrastructure type at each site, four transects were used. Sampling points were located along a transect at six different quadrat locations: (i) disturbance interior; (ii) disturbance adjacent to edge; (iii) edge; (iv) remnant adjacent to edge; (v) 10 m from the natural edge into remnant vegetation; and (vi) remnant interior (Fig. 1B). There were 72 sampling points per site and 216 in total (3 sites x 12 transects per site x 6 sample points per transect). Transects varied slightly in length. This was necessitated because exploration disturbance features vary in shape and size, so the interior of a disturbance and its edges are unique distances apart. Moreover, the edge of exploration land uses is not always clearcut, so soil disturbance and movement were used as an indication of edge locations. Lastly, due to the fragmented landscape mosaic across these sites, the remnant interior sampling point was also at varying distances from the actual edge. For comparability, the criteria for these sampling points were as follows: at least 100 m away along the same contour from its paired edge sampling point and, if possible, at least 100 m away from any other disturbance edge (Swart et al. 2018).

Sample Collection

Samples were collected between the 30th of October and the 23rd of November 2018. Each sampling point along the transect comprised a 1 m^2 quadrat. Within each quadrat, three pitfall traps with a diameter of 4 cm and a capacity of 120 mL, containing ~40 mL of ethylene glycol (undiluted commercial engine coolant, "Nulon Premium Long Life Coolant 100% Concentrate") were dug into the ground, with the rim flush with the soil surface. The pitfall traps were left open for approximately nine days (minimum of eight, maximum of ten days), and once collected, the three pitfall traps were pooled into one sample.

Habitat characteristics

A selection of habitat characteristics was measured and recorded within each 1 m^2 quadrat. Slope aspect was measured using a compass and slope angle (degrees) measured using a Suunto PM-5 clinometer. A modified Braun-Blanquet scale (1, < 1% cover; 2, 1–5%; 3, 5–10%; 4, 10–25%; 5, 25–50%; 6, 50–75%; 7, > 75%) was used to approximate total vegetation cover, bare ground cover, rock cover and litter cover within the quadrat. Soil depth (soil compaction) was measured at the centre of each quadrat using a soil probe. Rugosity was measured as the ratio between the length of the contoured surface and a 1 m chain along the 1 m horizontal and 1 m vertical transect, which crossed the centre of the quadrat. All fine woody debris (FWD; 0 < diameter < 0.5 cm) touching the 1 m horizontal transect within the quadrat was counted. All medium woody debris (MWD; 0.5 ≤ diameter < 2.5 cm) was counted along both 1 m horizontal and vertical transects, and all coarse woody debris (CWD; 2.5 ≤ diameter) present in the quadrat had its diameter measured and recorded.

Metabarcoding methodology

Arthropod samples were visually size sorted; any organisms larger than the size of a honey bee were removed and two legs were removed and added to the overall sample (as per Ji et al. 2013; Meier et al. 2016). Samples were homogenised and then processed using a modified DNEasy Blood and Tissue kit (Qiagen, Netherlands), with a 400 μL starting digest volume and a 100 μL double elution in EB buffer, resulting in 200 μL of the eluate. All extractions were carried out on an automated Qiacube (Qiagen). DNA extraction controls were included in every 23 samples.

A qPCR assay was run using an invertebrate mitochondrial COI primer set: fwhF2 (5'-GGDACWGGWTGAACWGTWTAYCCHCC-3') and fwhR2n (5'-GTRATW-GCHCCDGCTARWACWGG-3') targeting a 205 bp region (Vamos et al. 2017). Samples were amplified using 'fusion primers', gene-specific primers labelled on both the forward and reverse with 6–8 bp molecular identification (MID) tags and Illumina sequencing adaptors (Bohmann et al. 2022). Samples were amplified in duplicate, using the same tag combinations for each sample, including negative controls. Samples were pooled in approximate equimolar concentrations. The library pool was sequenced on an Illumina MiSeq (Illumina, USA) using a single-end 300-cycle V2 kit per the manufacturer's directions. For detailed methods, see Appendix 1.

Bioinformatic processing

Bioinformatic processing was conducted using the 'eDNAFlow' pipeline (Mousavi-Derazmahalleh et al. 2021). Briefly, raw sequences were demultiplexed to their sample using MID-tag combinations and length filtered using 'OBITools' (Boyer et al. 2016) with a minimum length of 50 base pairs. Sequences were then quality filtered with errors and chimeras removed using USEARCH (Edgar 2010) with a minimum Phred quality score of 20 and a minimum abundance of 8 sequences for zero-distance operational taxonomic unit (zOTU) creation. A zOTU approach was used because it is a highly sensitive method to characterise biodiversity, without the need for reliance on complete reference databases. zOTUs were curated using 'LULU' at default parameters (Frøslev et al. 2017) on R v 3.6.1 (R Core Team 2019). Appropriate sample depth per sample was assessed using rarefaction curves with the 'rarecurve' function in vegan (Oksanen et al. 2019) and samples with less than 2,000 sequences were removed. A 0.05% minimum abundance filtering threshold was used to combat false, low abundance zOTUS (Prodan et al. 2020). Low occurrence zOTUs with less than five sequences and occurring in only one sample were also removed. zOTUs were matched to the GenBank Reference database (www.ncbi.nlm.nih.gov/genbank/) and the Barcode of Life Database (Ratnasingham and Hebert 2007) using the Basic Local Alignment Search Tool (BLAST) for taxonomic assignment. BLASTn results returned the top ten hits with a minimum query coverage of 95% and a minimum percentage identity of 85% to account for low representation of Australian arthropod species in reference databases. zOTUs present in the extraction and PCR controls (Fungal zOTUs) were removed from the dataset. The dataset was divided into three categories; firstly, all zOTUs that were able to be identified to the phylum Arthropoda (overall arthropods), secondly all zOTUs belonging to the family Formicidae (ants), and lastly all zOTUs belonging to the class Collembola (springtails).

Statistical analysis

Statistical analysis was performed using PRIMER v.7 (Clarke and Gorley 2015) with the PERMANOVA+add-on (Anderson et al. 2008). Firstly, to illustrate the scale of environmental disturbance the environmental variables from all samples were normalised and Draftsman's plots were generated to check for correlation between every habitat characteristic. Those that were correlated were not both included, and a representative of the correlating pair was instead chosen for the analysis. A principal component analysis (PCA) was performed with Euclidean distance on the centroids of quadrat number located at each treatment and each site to illustrate the main differences in patterns. A Pearson's correlation test was conducted comparing the normalised habitat characteristic values to the quadrat number. A permutational ANOVA (PERMANOVA) was then conducted on the Euclidian distance matrix with 9999 permutations. The factors were as follows: Site (Fixed; KA, KO, MG); Infrastructure type (Fixed; MT, Pad, UT); Transect (Random; Nested in Site and Infrastructure type); and Quadrat location (Fixed; i – vi). The terms of interest to assess the impacts of linear infrastructure on arthropod communities were: the interaction between site, infrastructure type, and quadrat location; the interaction between infrastructure type and quadrat location; plus the interaction between site and infrastructure type. This was to account for the geographic separation between the different sites, so patterns of inter-

est were from within each individual site. For the overall arthropod community data, the ant community, and the springtail community, the number of zOTUs (zOTU richness) was calculated and tested using univariate PERMANOVA with Euclidean distance and 9999 permutations using the same design as above. Arthropod, ant, and springtail communities were then transformed to presence-absence. For ease of interpretation, community composition was illustrated using nonmetric multidimensional scaling (nMDS) on Jaccard similarity on centroids of quadrat number located at each treatment and each site with a k value of 2. A PERMANOVA was then conducted with the design as above using Jaccard similarity and 9999 permutations.

Results

Habitat characteristics

Of the terms of interest that may indicate an impact of infrastructure on the habitat characteristics measured, a significant interaction was detected between the infrastructure type and quadrat (Table 1). We found no significant interaction among site, infrastructure type, and quadrat, nor between site and infrastructure type. The factors of site, infrastructure type, and quadrat were all significantly different. While all three infrastructure types were different to each other on the disturbance interior (Quadrat 1), the MT habitat characteristics retained differences from the other two infrastructure types until the remnant interior (Quadrat 6). These habitat characteristics were higher bare ground cover, lower leaf litter, lower woody debris levels, lower elevation, and lower soil depth at the MT sites (Supp Fig. S1). In comparison, for both the Pads and the UT, there were no significant differences in habitat characteristics from the disturbance edge (Quadrat 2) onwards into the remnant interior (Appendix Table 1). The patterns of change with quadrat position among infrastructure types (interaction) were reflected in the PCA plots (Fig. 2) and in the correlation tests (Table 2). There was a trajectory of change as the quadrats moved further from the disturbance interior (Quadrat 1) into the remnant bushland interior (Quadrat 6). This directional change was characterised by less bare ground cover and increased woody debris and soil depth moving away from the disturbance (Table 2).

DNA metabarcoding results

A total of 15,507,193 quality-filtered sequences were generated across the 217 samples. A total of 212 samples had the minimum sequencing depth required of 2,000 sequences per sample and 208 samples passed the minimum required quality filtering thresholds. Only 5 zOTUs were detected in the negative control samples (extraction negatives and the PCR negatives); however, none of these zOTUs were from the phylum Arthropoda and thus were removed from the data set. In total 2,250 zOTUs were generated. However, there were only 1,101 zOTUs that met filtering criteria, and these were used in this study. These zOTUs represented 4 classes, 19 orders and 84 families of arthropods (simplified in Appendix Table 2, for full taxonomic information of all zOTUs and the zOTU tables, see the spreadsheet provided as Appendix 2). Ants made up 21% of the total zOTUs from this data set (238 zOTUs) and springtails made up 14% (170 zOTUs).

zOTU richness

Significant differences in the zOTU richness of the overall arthropod, ant and springtail communities between sites were evident (Table 3), with KO having lower richness compared to the other sites (Fig. 3). Overall arthropod richness differed significantly with infrastructure type, while ant and springtail richness did not. For the terms of interest indicating a potential disturbance effect, a significant interaction among site, infrastructure, and quadrat was observed for the ant community (Table 3 A), but not for the overall arthropod community or the springtail community. This might be influenced by the KA site, which showed more variation in richness across the quadrats at the different treatments. No significant difference in zOTU richness was found across the infrastructure type and quadrat interaction for ants or springtails, but there was for the overall arthropod community. The overall arthropod community showed some trends towards higher richness at Quadrat 6 compared to Quadrat 1 for the KO site and in the MT and Pads for the MG site. However, this trend was reversed for the MG UT infrastructure. Significant interactions were observed at the site and infrastructure level for all three arthropod communities. These were predominantly driven by a higher zOTU richness at the MG site UT and MT infrastructure types compared to the other sites and infrastructure types (e.g., the MG Pads) (Fig. 3).

Community composition

The site and infrastructure type interaction were influential in differentiating the communities of overall arthropods, ants, and springtails (Fig. 4, Table 3B), and this was evident int the distinct separation of arthropod communities when examining them at a site-by-site level (Fig. 5). However, unlike the habitat characteristics, no significant interaction was detected between the infrastructure type and the quadrat location (Table 3B), and for all the arthropod communities, there was no consistent directional trend observed between the quadrat locations (Fig. 5). For the overall arthropod communities, at the KA site there was a distinct trajectory observed from the quadrats on the disturbance (1) to the quadrats in the
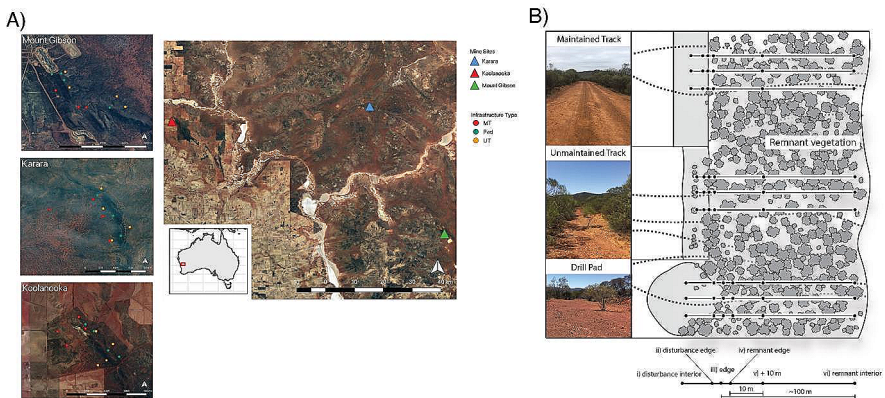

Fig. 1 A) Study site showing location of three mine sites and the locations of the different infrastructure types at each site, where MT is Maintained Track, Pad is Drill Pad, and UT is Unmaintained track B) Stylised image of experimental design and pitfall trap placement along each transect across the ecotone between the disturbance (maintained track, unmaintained track, or drill pad) and remnant vegetation. The grey dashed lines indicate prospective topographic lines
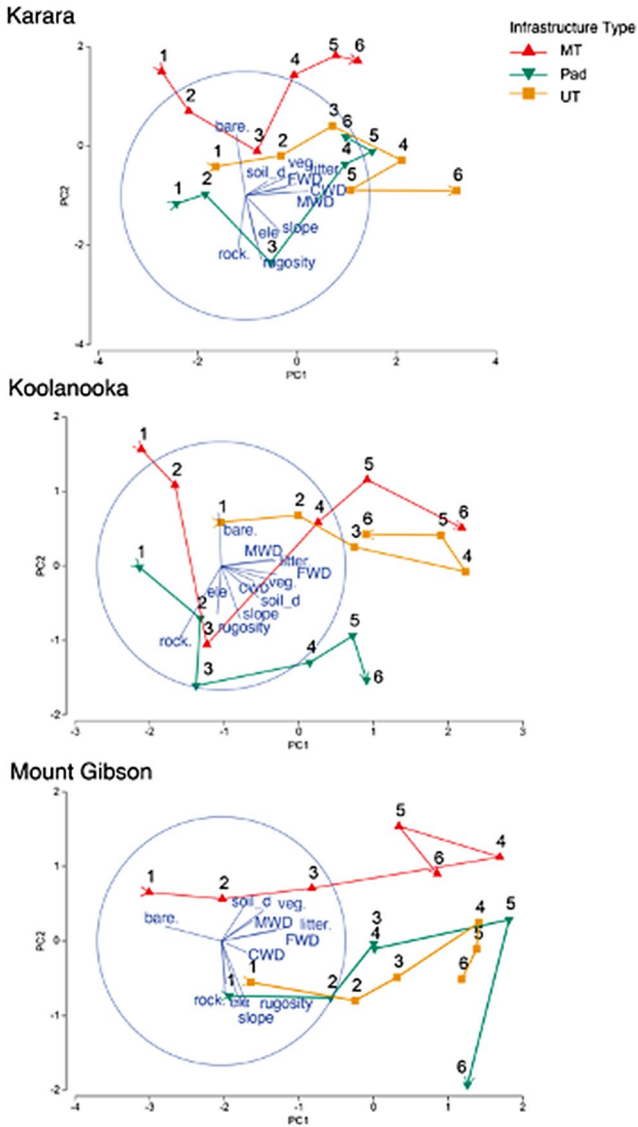
Fig. 2 Principal component analysis (PCA) plot of the habitat characteristics at each site showing the three different infrastructure types where MT is Maintained Track, Pad is Drill Pad, and UT is Unmaintained track. Trajectories are overlaid starting from Quadrat i (1) on the disturbance to Quadrat vi (6) approximately 100 m into the remnant vegetation. Vectors overlaid of the correlation between each habitat characteristic and the principal component axes. The circle is a unit circle (radius=1.0), used to illustrate the nature and strength of the correlation

Fig. 3 zOTU richness at the different quadrat locations (Quadrat Number i-vi) at the different sites and infrastructure types for the (A) overall arthropod communities, (B) Formicidae communities, and (C) Collembola communities. Sites are notated as KA for Karara, KO for Koolanooka, and MG for Mount Gibson. Different infrastructure types and sites are highlighted where MT is Maintained Track, Pad is Drill Pad, and UT is Unmaintained track
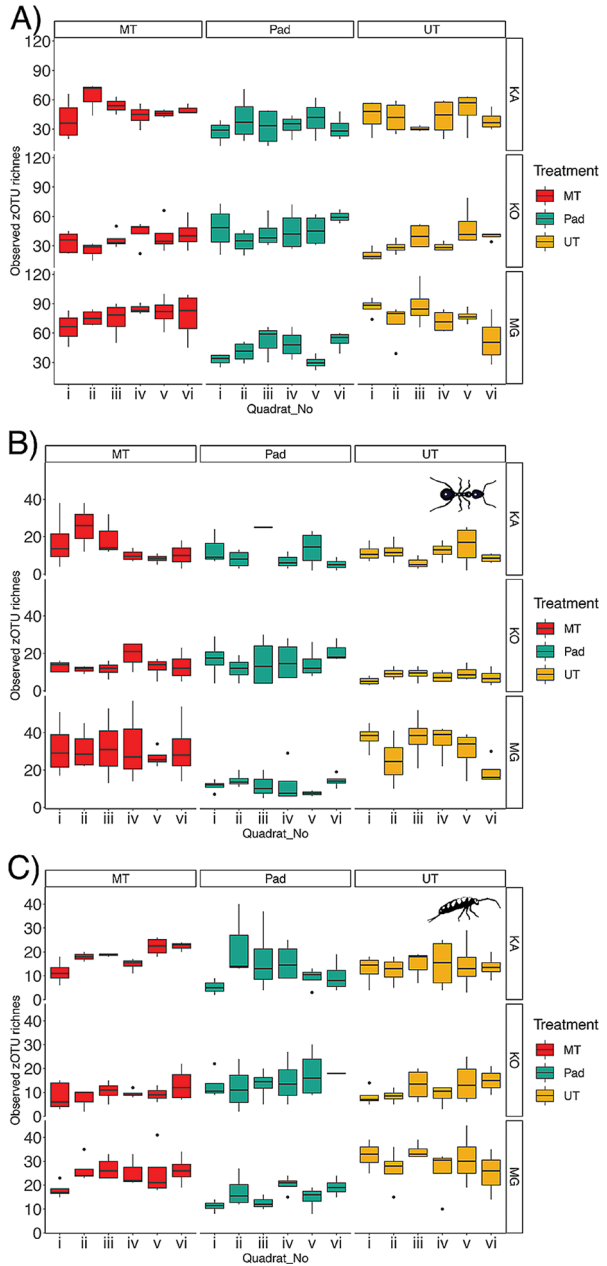
remnant vegetation (6) for both the MT and Pad infrastructure type (Fig. 5A). However, at the other sites, this pattern was less clear, particularly at MG, suggesting a homogenization of the overall arthropod communities across the transect from which quadrats were sampled (100 m).

For the springtail communities, only the site and infrastructure type interaction were found to be significantly different (Table 3B), and this was seen through the strong cluster-

Fig. 4 Non-metric multidimensional scaling (NMDS) plots showing (A) overall arthropod communities, (B) Formicidae communities, and (C) Collembola communities at the three sites. Sites are notated as KA for Karara, KO for Koolanooka, and MG for Mount Gibson. Different infrastructure types and sites are highlighted where MT is Maintained Track, Pad is Drill Pad, and UT is Unmaintained track
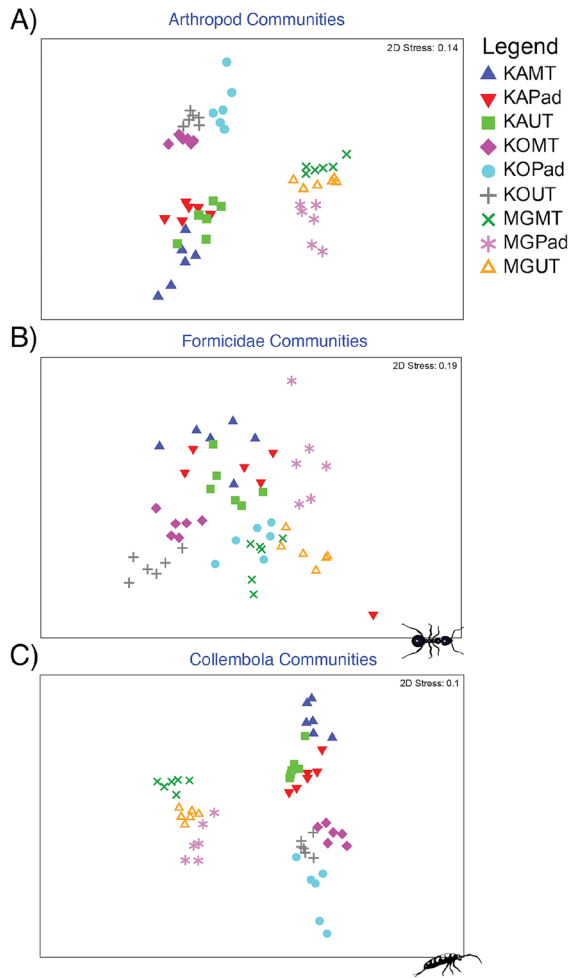
ing of points on the NMDS (Fig. 4C), but the finer patterns observed in the overall communities were not observed when sites and infrastructure types were separated as all quadrat locations clustered closer together on the NMDS (Fig. 5C). For the ant communities at KA, the distinction between the infrastructure types was not as clear as for the overall arthropod community (Fig. 5B). However, at the other sites (KO and MG), there was a separation of the ant communities by infrastructure type (Fig. 5B). But this distinction between the quadrats did not show the same directionality as the overall arthropod community, i.e., a clear path from Quadrat 1 to Quadrat 6.

Discussion

Mining has a large global environmental footprint, but knowledge and understanding of its impact is still limited (Tang and Werner 2023), and this is particularly the case for exploration infrastructure (Raiter et al. 2017). In this we study, we investigated the effect of tracks,
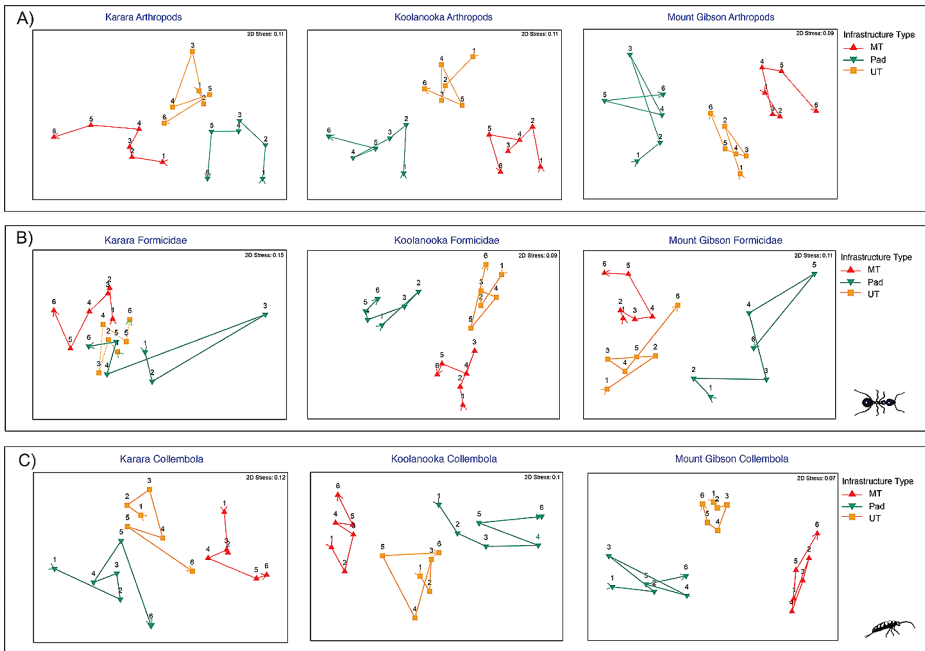
Fig. 5 Non-metric multidimensional scaling (NMDS) plots showing the (A) arthropod communities, (B) Formicidae communities, and (C) Collembola communities at the three sites. Different infrastructure types are colour coded where MT is Maintained Track, Pad is Drill Pad, and UT is Unmaintained track. Trajectories are overlaid starting from Quadrat i (1) to Quadrat vi (6)

Table 1 PERMANOVA results from the habitat characteristics compared at each site, infrastructure type, quadrat, transect and their interactions. Significance codes are as follows: '***'0.001, '**'0.01

Factor	df	Pseudo-F	P value
Site	2	3.957	<0.001***
Infrastructure Type	2	4.159	<0.001***
Quadrat	5	14.409	<0.001***
Site x Infrastructure Type	4	0.712	0.823
Site x Quadrat	10	1.118	0.239
Infrastructure Type x Quadrat	10	1.537	0.004**
Transect(Site x Infrastructure Type)	27	3.704	<0.001***
Site x Infrastructure Type x Quadrat	20	0.974	0.572

roads and drill pads on biophysical habitat characteristics and ground-dwelling arthropod communities. While changes in habitat were only visible to 10 m from the disturbance, impacts to arthropod communities could be detected up to 100 m into the remnant vegetation, and these responses were more complex. In general, we found similar patterns expressed in the compositional changes for arthropods overall and between our chosen indicator groups (ants and springtails), but they were not the same across all sites and infrastructure types (MT, UT, and Pads).

Table 2 Spearman's correlation test results across habitat characteristics in relation to quadrat locations (1–6). Significance codes are as follows: '***'0.001, '**'0.01, '*'0.5

Habitat characteristics	Correlation coefficient	Test statistic	P value
Elevation	0.012	25,916	0.930
Slope	0.37	16,510	0.006**
Soil depth	0.61	10,163	<0.001***
Rugosity	0.17	21,799	0.222
Vegetation cover	0.74	6896	<0.001***
Bare ground cover	-0.32	34,745	0.017*
Rock cover	-0.21	31,852	0.120
Leaf litter cover	0.69	8037	<0.001***
Fine woody debris	0.79	5408	<0.001***
Medium woody debris	0.7700	6095	<0.001***
Course woody debris	0.7700	5995	<0.001***

Table 3 PERMANOVA results from the overall arthropod, Formicidae and Collembola zOTU richness (A) and zOTU composition (B) compared at each site, infrastructure type, quadrat, transect and their interactions. Significance codes are as follows: '***'0.001, '**'0.01, '*'0.05, ' '1

A) zOTU richness

Factor	df	Overall Arthropods Pseudo-F	P value	Formicidae Pseudo-F	P value	Collembola Pseudo-F	P value
Site	2	30.512	<0.001***	15.184	<0.001***	21.064	<0.001***
Infrastructure Type	2	6.366	0.006**	2.922	0.066	1.930	0.164
Quadrat	5	1.055	0.393	1.531	0.189	2.297	0.053
Site x Infrastructure Type	4	7.844	<0.001***	5.804	0.002**	4.634	0.006**
Site x Quadrat	10	1.919	0.046*	1.355	0.214	0.753	0.668
Infrastructure Type x Quadrat	10	1.208	0.292	1.112	0.357	1.425	0.177
Transect (Site x Infrastructure Type)	27	2.563	<0.001***	4.861	<0.001***	3.445	<0.001***
Site x Infrastructure Type x Quadrat	20	1.529	0.085	1.716	0.042*	1.210	0.254

B) zOTU Composition

Factor	df	Overall Arthropods Pseudo-F	P value	Formicidae Pseudo-F	P value	Collembola Pseudo-F	P value
Site	2	6.837	<0.001***	4.434	<0.001***	10.571	<0.001***
Infrastructure Type	2	1.917	<0.001***	2.005	<0.001***	2.516	<0.001***
Quadrat	5	1.231	<0.001***	1.245	0.012*	1.052	0.283
Site x Infrastructure Type	4	1.596	<0.001***	1.793	<0.001***	1.690	<0.001***
Site x Quadrat	10	1.108	0.006**	1.155	0.017*	1.071	0.149
Infrastructure Type x Quadrat	10	1.026	0.254	1.019	0.374	1.015	0.404
Transect (Site x Infrastructure Type)	27	2.476	<0.001***	2.412	<0.001***	4.247	<0.001***
Site x Infrastructure Type x Quadrat	20	1.067	0.012*	1.140	0.004**	1.063	0.107

Habitat disturbance around linear infrastructure and impacts on arthropod communities

Habitat characteristics were altered by anthropogenic disturbance similarly across the different infrastructure types. This change was broadly characterised by decreased woody debris and shallower soil depth (i.e., increased compaction) closer to the actual disturbance. This pattern, consistent across sites and infrastructure types, aligns with previous research on linear infrastructure impacts on natural vegetation (Dániel-Ferreira et al. 2020; Goosem 2012). For some of our sites, this change in habitat characteristics extended out to 10 m from the disturbance, suggesting edge effects of at least this spatial scale. However, zOTU richness of arthropods could not capture this pattern, possibly because richness (number of zOTUs) does not necessarily respond to disturbance like other metrics such as community composition do (Fernandes et al. 2019). In general, a habitat can often only support a certain number of species. As the habitat changes over time or because of disturbances, the composition of species rather than species richness changes (Lengyel et al. 2016). Other traditional univariate diversity measures that consider abundance alongside the number of species (e.g., Shannon's, (Maurer and McGill 2011)) may be more useful for measuring these differences; however, it is not possible to obtain robust species abundance data using metabarcoding techniques to calculate these metrics. Therefore, looking at both zOTU richness and composition together, alongside changes in habitat characteristics, is more effective at assessing community responses to change (Fernandes et al. 2019; van der Heyde et al. 2022a).

Using zOTU composition, we were able to detect finer scale effects between quadrats that indicated disturbance. For some site-infrastructure combinations, such as the MT at KA, there was a pattern of change in zOTU composition among quadrats from communities on the infrastructure through to those in remnant bushland, which indicated an edge effect extending 100 m into the remnant bushland. However, this pattern was inconsistent across all the MT at the three sites. This is not unexpected, as a previous study found that physical changes in the landscape caused by seismic lines were very visible, but they did not have a great impact on ant communities beyond one year post-disturbance (Watts et al. 2002). This was attributed to changes in vegetation within 10–100 m of the infrastructure resulting in only minor reorganisation of foraging patterns for ants, but not the overall community structure (Watts et al. 2002).

The overall habitat characteristics of quadrats on the Pads and UT remained the same as for MT. Even years after the initial disturbance, the infrastructure areas we studied were relatively clear, and the surrounding vegetation community structure had not been restored. This has been attributed to the specialisation of vegetation communities to particular soil chemical and physical characteristics, which remain altered following disturbance and limits the return of vegetation (Gibson et al. 2012; Yates et al. 2011). Rehabilitation of such sites around exploration infrastructure needs to be considered and planned for, just as other aspects of mining impacts are in the development of mine rehabilitation plans (Manero et al. 2020). At our study sites, exploration disturbances were either currently maintained (MT) or left to revegetate passively (UT and Pads), without any intervention methods such as recontouring or seeding (Meli et al. 2017). Tracks and roads are considered as part of mine infrastructure, and mine closure requirements now consider rehabilitation of tracks as part of the overall environmental rehabilitation and closure obligations (DMIRS, 2021;

Young et al. 2019). However, Australia has an extensive legacy of unrestored exploration infrastructure (Mills 2022).

Effectiveness of indicator groups to assess changes in arthropod communities

One of our study aims was to examine whether two indicator groups of arthropods, ants and springtails, reflected overall patterns in arthropods at our sites in response to exploration disturbances. While we observed similar patterns between the three sites and infrastructure types in terms of ant and springtail communities, the patterns were not identical across both groups. Furthermore, the effect of site, infrastructure type, and quadrat was not significant for the springtail community composition but was significant for the whole arthropod communities and for ants. Overall, the ant communities were more reflective of the differences in the total arthropod communities than were springtails, presumably reflecting the consistent responses of ants to disturbance (Hoffmann and Andersen 2003). This implies that while springtail communities may be useful in examining broader disturbances in the environment (Greenslade and Majer 1993), to examine the edge effects at a fine scale, ant communities are more informative.

Choosing an indicator taxon group can be an important consideration when designing metabarcoding studies. Ants and springtails are valuable indicator taxa in their own right (Greenslade 2007; Tiede et al. 2017). For example, ants are reported to have consistent responses to disturbance (Hoffmann and Andersen 2003) and hence make good indicators. Springtails can be highly spatially aggregated in the soil (Greenslade 2007), and are very sensitive to subtle changes in soil conditions (e.g., pH) (Sławski and Sławska 2000). So, they theoretically could be useful for understanding the fine-scale impacts of disturbance. However, springtail species composition is impacted by weather events or seasonal conditions (Yin et al. 2019), and patterns of disturbance may be confounded by these variables. From this, we conclude that comparing the performance of different taxa (whole arthropods, ants, and springtails) is important to determine the utility of these groups as indicators. It is also important to build reference libraries, improve the taxonomic resolution of assays, and improve the understanding of specific species ecology when focusing only on select indicator taxon groups in order to choose the appropriate indicator group. In comparison, capturing whole species assemblages in a tree of life fashion (Stat et al. 2017) may give more holistic understanding of changes in the environment, but would require the development of barcodes and functional understanding of species groups across a larger scale, at a much higher cost.

DNA metabarcoding as a tool to assess arthropod communities

In this study, we demonstrated the utility of DNA metabarcoding for assessing arthropod community responses to environmental changes at a fine scale. Our analysis revealed over 84 families of arthropods and identified differences between sites, infrastructure types, quadrats, and indicator groups. However, like any survey method, the effectiveness of DNA metabarcoding is influenced by the technological limitations, study design, and execution (van der Heyde et al. 2020). A significant challenge in DNA metabarcoding studies is the reliance on reference databases, particularly in understudied arthropod systems like ours (van der Heyde et al. 2020; van der Heyde et al. 2022a, b). While

we detected 2,250 zOTUs, only 1,101 could be identified as being from the phylum Arthropoda (~ 49%), which is consistent with findings from other studies lacking custom reference databases (for example, Klimova et al. 2023; van der Heyde et al. 2022a, b; Wildermuth et al. 2023). Improving database representation is crucial for enhancing the resolution of zOTU identifications, which, coupled with a deeper understanding of species ecology, could facilitate the assignment of ecological function to taxonomic groups (Pawlowski et al. 2018).

Enhancing the representation of understudied taxonomic groups, such as Collembola, would reduce the number of sequences that are discarded because of unassignable identities (van der Heyde et al. 2022a). With less than 50% of our generated data usable, we may be only capturing half the disturbance impacts at these sites. However, the dynamic nature of global barcode databases, including initiatives like the International Barcode of Life project (www.ibol.org), offers hope for future analysis providing a more comprehensive understanding of disturbance effects on arthropod biodiversity. Despite relying on an incomplete arthropod barcode database, our findings align with those of previous studies using morphological identification to study arthropod responses to disturbance (Majer et al. 2020; Yu et al. 2006). Therefore, we have confidence that our observed trends reflect real patterns in arthropod communities at our study sites.

Visual observations of our samples strongly suggested that there were differences in the abundance of some taxa in samples collected from the disturbances and within the remnant vegetation. However, because reliable abundance is difficult to obtain from metabarcoding data (Elbrecht and Leese 2015), we were only able to make assessments based on the presence/absence of our arthropod zOTUs and were not able to examine abundance-related differences. However, abundance data is critical for calculating different diversity metrics and may allow for a more nuanced interpretation of community responses to disturbance at our sites. Approaches to obtain biomass or abundance estimates will be a valuable addition to metabarcoding surveys and there is scope for development in this space using various techniques; recent papers have shown success with using modelling frameworks (Shelton et al. 2022) and DNA spike-ins (Luo et al., 2023) to deduce relative abundance of species within samples.

Seasonal variation impacts the composition of arthropod (Santorufo et al. 2014) and plant communities and their interactions (CaraDonna et al. 2017). Therefore, the resolution of our findings may have been improved by surveying arthropod communities over multiple time points. Thus, repeated surveys and other monitoring methodologies (e.g., indicator species studies) may be required to determine if the observed patterns are one-off occurrences or a result of broader ecological trends.

Furthermore, metabarcoding of arthropod communities in this way is subject to the same considerations as traditional methods. Study design may have also played a role in the patterns observed. Here, we used multiple 100 m transects across infrastructure types at one site, and we detected differences in arthropod communities at this scale. An interesting comparison would be to expand these transects out to greater distances into remnant bushland in order to determine broader landscape scale differences that might result from habitat modification within the same site. Increasing the replicates within a quadrat may also be beneficial in adding support to these results.

Conclusions

Mining exploration infrastructure significantly influenced both the physical habitat and arthropod communities. These disturbances resulted in similarly altered physical habitat characteristics across infrastructure types at all sites. There were detectable edge effects on the habitat characteristics, but these impacts did not extend far into remnant bushland (< 10 m). For arthropod communities, however, the effect of disturbance extended into the remnant bushland for 100 m, showing similar patterns for whole communities, and for ants, but not for springtails. Our study has highlighted the utility of DNA metabarcoding as a tool to identify changes in arthropod communities impacted by mining and showcased the differences between whole arthropod communities versus common indicator species groups. We recommend continued monitoring of arthropod communities in our study system, especially over different spatial scales, seasons, and time points, to develop a more holistic understanding of their responses to disturbance. Our data emphasise the need to consider different types of disturbances associated with mining activity when assessing the impact of new mining projects, including exploration infrastructure, as they may all have the potential to impact surrounding faunal communities and associated ecosystem processes to a significant degree.

Author contributions SC-I, and PN conceptualised the study. SC-I collected the samples and processed them. KF conducted the experimentation and analysis. BJS assisted with statistical analysis. KF wrote the original draft of the manuscript. All authors contributed to review and editing of the manuscript.

Data availability Sample information, zOTU Tables, taxonomy information and de-convoluted unique sequences are available at: https://doi.org/10.5281/zenodo.6395458.

Declarations

Competing interests The authors have no relevant financial or non-financial interests to disclose.

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