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Ants as Indicators of Terrestrial Ecosystem Rehabilitation Processes

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Abstract

Habitat transformation is one of the main drivers of the ecosystem degradation on earth that is ameliorated by restoring some of the degraded ecosystems by regaining their natural ecological functions with all their biotic and abiotic components. The biotic and abiotic components of the ecosystem under restoration can be used to assess the response of the ecosystem to the restoration. Ideal variable to use as the indicator should be able respond positively to the diminishing elements that we causing the degradation and interact positively to some of the biotic and abiotic components expected to prevail when the ecosystem is fully restored. One of such variable is ants. We here provide the information about the eligibility of using ants as indicators of terrestrial ecosystems undergoing restoration and sampling and basic analytical methods to apply when implanting ants at assessing ecosystem undergoing restoration.

Keywords: habitat degradation, restoration, indicator, ant sampling techniques, ant species estimation, ant species richness, ant abundance

1. Introduction

The ecological integrities of many ecosystems are presently at risk of degradation due to habitat transformation caused by several drivers including climate change [1], introductions of invasive species [2, 3], desertification, mining and heavy grazing [4, 5]. Such disturbances decrease species diversity and cause subsequent declines in ecological function and resilience in the affected ecosystems [6, 7]. Therefore, these anthropogenic disturbances or degradations should be mitigated and ecological processes restored to reduce impacts on the biological integrity of ecosystems.

The ecosystem degradation referred here is the anthropogenic disturbance or unnatural change in an ecosystem that are mainly caused by humans [8–11]. Ecosystem degradations have affected about 25% of the earth's land [9, 11], that include almost 33% of the land within Protected Areas [12, 13]. Considering that the long-term objective of the Protected Areas is to conserve nature and its associated ecosystem services and cultural values [14], such degradations within and beyond the Protected Areas threaten the achievement of this objective. The ecosystem degradation further delays the achievement of some of the Aichi Biodiversity Targets that aim to reduce and reverse the biodiversity loss on earth [15].

Ecosystem degradations can be ameliorated by implementing ecological restorations which are approaches/processes that stop and reverse the degradation to regain

the ecological functionality of the affected ecosystems – for the benefit of both nature and humans [8, 10, 16–18]. Ecosystem restoration is becoming central to conserving biodiversity and stabilizing the climate, and has started to feature prominently in global and national policy frameworks [18, 19]. It has also been recognised that ecosystem restoration will assist to achieve the 15th goal of the UNs Sustainable Development Goals that intends to protect, preserve and sustainably use the terrestrial ecosystems [20, 21], and further assists in achieving three of the Aichi Biodiversity Target in Protected areas [10] which aim to restore about 350 million hectares of degraded land globally by 2030 [22]. Furthermore the years 2021 to 2030 have been declared as decade for ecosystem restoration by the United Nations General Assembly [23].

The success rate of the ecological restoration is influenced by various factors that include the intensity and the type of the degrading agents in the system, the conditions of supporting variables such as temperature and nutrients, and the distance of the degraded system from sources that will augment its biodiversity and ecosystem services. Ecosystems that recover slow often have experienced intense degradations, or have low supporting variables, or are remote from places that can augment them with biodiversity or ecosystem services [9, 23]. These turn to cause the restoration of such ecosystems to be partially restored relative to their natural states [8, 18].

Basic measures that should be taken to restore the degraded ecosystems include the reduction or removal of pressures causing the degradation [9, 18], and then allow the natural recovery of the system (also known as passive restoration), or take further interventions (also known as active restoration) such as reintroducing/augmenting the affected species [9, 10, 24]. The restoration should include research and monitoring activities that will provide the baseline and long-term information about the progress of the restoration in achieving its goals [10, 18]. These include baseline data and information about variables related to biodiversity, trophic structures and biophysical features of the degraded area (relative to undisturbed ecosystems) that will indicate/describe the ecological integrity of the degraded area and the progress of the restoration [10, 18]. If the pre-degradation data/information is available, it can be used as the reference information to determine the extent of the degradation and the progress of restoration [18].

2. Indicators of ecosystem restoration

The ultimate aim of restoring the ecosystem is to regain its natural ecological functions where the roles of all biotic and abiotic components exist and interact naturally – without human assistance [8]. The presence of ecological variables that are facilitating these ecological functions in the recovering systems are considered enough to demonstrate that the ecosystem is being restored, or undergoing restoration [24, 25]. Their presence in an ecosystem firstly demonstrate that the factors that were degrading the system are no longer present, or are at the levels that are no longer impeding the ecological functions of the system. Secondly they demonstrate the potential of the ecological functions to occur in that ecosystem [26] for example the presence of pollination agents. In contrast, the absence of such indicators implies that the system is still not hospitable for such functions (e.g. the humus-feeding termites that were not present at the rehabilitated sites because the trees which are their primary food have not yet grown [27]), or the degrading factors are still impeding the processes of such functions to occur (e.g. deposited nitrogen hindering butterflies to colonise areas that high concentrations of it [28]).

These variables are selected to diagnose the condition or the status of the ecosystem studied. They normally comprise organisms of the ecosystem under study (mostly vegetation and animals), characteristics of the landscapes (such as the patchiness

of the vegetation) and properties of the physical factors of the ecosystem (such as soil) [25]. These indicators can be directly measured (e.g. by analysing the organisms constituting the diversity in that ecosystem or measuring the size of the bare-ground) or indirectly measured where the agents associated with the concerned ecological function are measured (e.g. the concentration of some chemical elements of the soil are used to measure the quality of such soil during rehabilitation) [8, 24, 29].

3. Ants as indicators of restoration

The significant increase in the number of environmental disturbances has given rise to a need for further research to quantify the ecological effects of environmental change. Due to complexity of most ecological systems, individual species or functionally similar species groups are often used as bio-indicators of environmental processes [30]. Surveys of such indicator species help guide land managers and decision makers to identify environmental disturbances and subsequently to take actions in time to reduce damages, mitigate consequences and restore ecosystems.

One group of organisms that have been recognised as good indicators of terrestrial ecosystems are ants. Ants started been used as indicators of ecological systems in Australia in the mid 1970's assessing impacts of mining [31] and are currently used internationally assessing different degradations in many ecosystems. They were used in a five year study on a spillage pollution at the riparian of Guadiamar River, Spain, to determine their response on the areas undergoing restoration [32]. The study discovered that ants clearly respond to the restoration with species richness significantly increasing throughout this five year period, and a progressive variation in the species composition of ant communities at different riparian habitats. Another study included ants in investigating the rehabilitated sites from coal mining in Colombia and discovered two ant species (viz. *Ectatomma ruidum* and *Pheidole fallax*) could be contributing in seed dispersal and re-establishment of vegetation in these areas [33]. Another study on sites with different ages of rehabilitation from coal mining found that the nest density of ant *Pheidole fallax* ranged increases with the rehabilitation age of the sites [34]. A study on clear cut logging of timer trees in USA found that ant species assemblages respond to alteration of habitats where the populations of the invasive ant *Solenopsis invicta* and *Pheidole* species increased while the populations of the native ant species are significantly fell [35]. Another study done by Andersen and Sparling [36] found that ant species richness from site undergoing rehabilitation in Kakadu Australia, was positively correlating with the below-ground soil microbial biomass which further demonstrates that the aboveground ant activities at restoration habitats can even indicate the conditions of the organisms associated with decomposition processes that are underground of these habitats.

The interactions of ants with both abiotic and biotic factors of their ecosystems make ant one of the most suitable components of the ecosystem to include when studying the impact of the degradation to the ecosystem and response of that ecosystem when undergoing/undergone restoration. One of the variables that ant can be used for as indicators, is their nests. Ants build their nests on and in variety of components within their ecosystems ranging from the soil to a specific plant species [37] which make their nest alone quite accessible to use as indicators. Some species such as *Atta bisphaerica* build their nests in the soil [38, 39], while others build theirs on or inside the plants, notably the trees like *Colobopsis nipponicus* [40, 41]. Other ant species like *Polyrhachis* species of Malaysia, build theirs on variety of objects ranging from soil to constructing them with dead vegetative and soil particles [42]. The presence or absence of the ant nests, in their habitats, could indicate the conditions of their habitats. For example Díaz [43] found no nests of ants *Messor capitatus*,

M. barbarous, *M. bouvieri* and *M. structor* built in the soil of the ploughed fields and attributed this mainly to the frequent tilling that impedes the ants to build their nests especially for winter survivorship. On other hand Sorvari and Hakkarainen [44] found high nest abandonments by ant *Formica aquilonia* at forest habitats with clear-cut logging relative to the intact habitats that they attributed to changed abiotic conditions, resource limitation, and the disturbance of the ant reproduction in clear-cut sites. These demonstrate that nests of ants can assist in indicating the conditions of the ecosystem in their habitats.



Ant species at their nest at Mokala National Park, South Africa.

The symbioses some ants have with other organisms also make them suitable as indicators of restoration. The relationship some ant species have with other organisms include hosting these organisms in their nests in exchange of some benefit from these organisms. Ant species such as *Pheidole pallidula* hosts beetle *Paussus favieri* in its nest in exchange of consuming the secretion from the beetle [45]. On other hand ant *Azteca pittieri* defends the Spanish elm (*Cordia alliodora*) against browsers in exchange of getting nesting place and honeydew from the tree [46]. Some ant species even assist other organisms to suppress the organisms deemed undesirable to these ants. For instance, ants genera from *Trachymyrmex* and *Acromyrmex* transport filamentous bacteria (actinomycetes) that suppress fungus *Escovopsis weberi* (the parasite of the fungi the ants cultivate) [47]. Others influence the reproduction behaviour of other organisms. For example, butterfly *Jalmenus evagoras* prefer laying her eggs on plants that have *Iridomyrmex* ant [48]. These demonstrate that apart from indicating the habitat conditions for their own, ants could indirectly indicate the conditions of other organisms they have symbiosis with. Although Hazra [49] found that ant *Pseudomyrmex ferrugineus* did not occupy *Vachellia cornigera*, for their mutualism relationship to occur, at the restored tropical forest of Mexico, the occupation of *P. ferrugineus* at tallest *V. cornigera* could be indicating that the interaction between these would commence

Author, Year & Publication	Title	Summary of the study & findings	Ant Role Studies or Discovered
Del Toro, I., Ribbons, R.R. & Pelini, S.L. 2012. <i>Myrmecological News</i> 12 pp. 133–146.	The little things that run the world revisited: a review of ant-mediated ecosystem services and disservices (Hymenoptera: Formicidae)	<ul style="list-style-type: none"> Summarizes the information about ecosystem services provided by ants. Present negative roles of ants on human and environment. 	Ecosystem services
Lester, P.J., Baring, C.W., Longson, C.G. & Hartley, S. 2003. <i>New Zealand Entomologist</i> 26: pp. 79–89.	Argentine and other ants (Hymenoptera: Formicidae) in New Zealand horticultural ecosystems: distribution, hemipteran hosts, and review	<ul style="list-style-type: none"> Fifteen hemipteran species of horticultural crops in New Zealand are tended by invasive ant <i>Linepithema humile</i> and other six ant species. 	Symbiosis
Canner, J.E., Dunn, R.R., Giladi, I. & Gross, K. 2012. <i>Acta Oecologica</i> 40 pp. 31–39	Redispersal of seeds by a keystone ant augments the spread of common wildflowers	<ul style="list-style-type: none"> Ant <i>Aphaenogaster rudis</i> re-dispersed >90% of the seeds it took into its nest. 	Seed dispersal
Chan, K.H., & Guénard, B. 2020. <i>Urban Ecosyst</i> 23 pp. 1–12	Ecological and socio-economic impacts of the red import fire ant, <i>Solenopsis invicta</i> (Hymenoptera: Formicidae), on urban agricultural ecosystems	<ul style="list-style-type: none"> Invasive ant <i>Solenopsis invicta</i> decreased ant species richness and evenness in Hong Kong and impacted about 80% of agricultural production. 	Invasive ant impacts
Clarke, K.M., Fisher, B.L. & LeBuhn, G. 2008. <i>Urban Ecosyst</i> 11 pp. 317–334	The influence of urban park characteristics on ant (Hymenoptera, Formicidae) communities	<ul style="list-style-type: none"> Urban forests of San Francisco reduced ant richness and abundance. Invasive ant <i>Linepithema humile</i> had little or no impact to native ants of San Francisco. 	Habitat change & invasive ant impacts
Fiedler, K. 2006. <i>Myrmecologische Nachrichten</i> 9 pp. 77–87.	Ant-associates of Palaearctic lycaenid butterfly larvae (Hymenoptera: Formicidae; Lepidoptera: Lycaenidae) – a review	<ul style="list-style-type: none"> The number of Palaearctic lycaenid butterfly species correlates significantly with species richness of that ant genus attending it 	Symbiosis
Andersen, A. 2008. <i>Journal of Biogeography</i> 24 pp. 399–539.	Functional groups and patterns of organization in North American ant communities: a comparison with Australia	<ul style="list-style-type: none"> Eight functional groups of ant communities of North America 	Interactions of ants at food resources
Roth, D.S., Perfecto, I. & Rathcke, B. 1994. <i>Ecological Applications</i> 4 pp. 423–436.	The Effects of Management Systems on Ground-Foraging Ant Diversity in Costa Rica	Ant diversity at forest and abandoned cacao sites of Costa Rica was significantly more than from productive cacao and banana plantations	Impact of land use to ant Diversity.
Frouz, J, Holec, M. & Kalčík, J. 2003. <i>Pedobiologia</i> 47 pp. 205–212.	The effect of <i>Lasius niger</i> (Hymenoptera, Formicidae) ant nest on selected soil chemical properties	Most chemical elements and other parameters of the soil from the nests of ant <i>Lasius niger</i> were significantly higher than the surrounding soil.	Soil property Influence

Author, Year & Publication	Title	Summary of the study & findings	Ant Role Studies or Discovered
Dostál, P., Březnová, M., Kozlíčková, V., Herben, T. & Kovář, P. 2005. <i>Pedobiologia</i> 49 pp. 127–337.	Ant-induced soil modification and its effect on plant below-ground biomass	Ant <i>Lasius flavus</i> changes physical properties and distribute nutrients vertically from plant to access in their nests.	Soil property Influence
Gonthier, D.J., Ennis, K.K., Philpott, S.M., Vandermeer, J. & Perfecto, I. 2013. <i>BioControl</i> 58 pp. 815–820.	Ants defend coffee from berry borer colonization	Diverse ant species limit coffee pest beetle <i>Hypothenemus hampei</i> from colonizing coffee berries.	Agricultural biocontrol

Table 1.
List of some of publications related to the role of ants in the ecosystems.

when trees have grown big enough to accommodate the ant as the restoration age progresses. Relatively not much ant studies – on restoration – have been done investigate the conditions of ant species and the symbioses with other organisms. This further increase the untapped roles ants could be playing as indicators of restored ecosystems. (See **Table 1** for additional information about the roles of ants in ecosystems).

The relatively easy way to identify ants to genus, then to morphospecies level, further makes ants suitable to be added to the lists of indicators of restoration. The training one needs is to correctly identify the specimen using the external morphology of the specimen and properly following the identification keys provided by different ant taxonomic books and internet [50–54]. Which relatively does not take much time especially to someone with entomological background.

Identification of ant specimen also do not need relatively expansive equipment. A dissecting/stereo microscope with eye pieces with 10x magnification level and zooming ranges of 1x to 6.3x is enough to observe morphological features of the specimen when identifying them. Such microscopes satisfactory and clearly show ant body parts (such as antennal segments, waist segments, gaster segment etc.) that are commonly used to identify the specimen. They even easily allow one to increase or reduce the magnifications to accommodate the different parts of a specimen under observation, or change magnifications to accommodate specimen with different sizes. Following the identification keys and using a correct microscope are sufficient for one to correctly identify most specimen to genus or morphospecies levels [55].

The use of ant workers, instead of the males or the queens, as representatives when identifying their species, further makes ants more convenient to be used as indicators of restoration. Unlike their queens, that are rarely outside of the nests and very few relative to the workers, nor their male counterparts that are available temporarily, ant workers are most abundant and frequently outside the nests for easy sampling [37].

The flexibility of using different biodiversity indices from ant data also make ants suitable for indicating the restoration condition of the ecosystem. Biodiversity indices such as species richness and abundance are often used. Palladini et al. [56] used them to report the response of ant diversity to timber harvesting where they found that the high species richness and abundance of ants in sites with new harvest, relative to the sites with older harvests, indicate that ant community in those new harvested sites will take about hundred years after the disturbance to resemble the ones in mature forests. Carvalho & Vasconcelos [57] also used the species richness

index to analyse the conditions of ants from fragmented forest relative to the continuous ones. The arranging ant data in functional groups of the sampled ants is also used to assess the ant communities from areas undergoing restoration. KING et al. [58] used it to assess the condition ant communities from the degraded forest sites where they found ant with opportunist functional group common at the disturbed sites relative to the reference sites, and almost all the tropical climate specialists and specialist predators were absent from the disturbed sites. Functional group was also used by Stephens and Wagner [59] to report that different functional groups dominate sites with different disturbances differently with the dominating groups at 'their' site suppressing or excluding other groups that less suited to the disturbance in that side. Ottonetti et al. [60] used both species richness and functional group plus diversity index to assess the response of ants to the rehabilitation done in their habitats where they found that richness and diversity index were not yet significantly different among the habitats but the functional groups were starting to change.

4. Sampling epigeal ants

There are four common methods used to sample the epigeal ants videlicet: hand collection, pitfall trapping, baiting and passive extraction¹. Hand collection is one of the earliest methods used to collect ants where the specimen seen are directly picked up, from their nests or at the areas they are frequenting. Usually a pair of forceps or/ and aspirators are used to pick up these specimen [54, 62–66] (see **Figure 1**).

Hand collection is efficient as it its data can produce ant species richness and more exclusive species [67]. It is often implemented in transects (of an appropriate lengths) in the study areas where ants seen are often collected at interval places of such transects [63, 65, 68, 69], for certain durations [69–71]. Sometimes hand collection is applied to the specific target area within the study site such as collecting specimen at the nest entrance or a specific microhabitat of interest [64, 67, 72, 73]. Cuautle et al. [54] used hand collection to sample the specimen of ants that were interacting with plant species at their sites to achieve the objective of their study investigates ant species that are consuming resources from the plants. Hand collection is often applied diurnally (usually from 7 h00 to 16 h00) when the collector can see ants and the weather conditions of the area are permitting and it assists in providing the information about ant species that are active at the specific time [54, 63, 64, 67].

Another common sampling technique for ground dwelling ants is pitfall trapping [74]. Pitfall trapping passively collects ants by capturing them after the ants fell into it when they are frequenting their habitats. Usually handle-less containers of different volumes are used as a pitfall traps [68, 69, 75–78] (See **Figure 2**). A set of pitfall traps are often spatially set at distance from each other [76, 79] for a certain durations ranging from 24 hours to two weeks [70, 80, 81].

It important to determine the efforts needed to set pitfall traps (such as the number of traps and the duration of trapping) that will produce the maximum ant information from the site/habitat/ecosystem at minimum resources before setting them. Gomes et al. [82]) found that only two days instead of 14 of pitfall trapping is needed to monitor ants of Ducke Reserve, Brazil which saved much needed time and funds. Andersen et al. [83] developed a further simplified pitfall trapping method that produced all the key findings of the intensive survey but with less than 10% of the intensive survey effort and made ant sampling achievable to anyone with limited ant training, time or resources.

¹ Extraction is more suitable for sampling areas with litter [61] and is hardly used by the authors.



Figure 1.
 Examples of a pair of forceps (pictures above) and an aspirator (pictures above) and their collection demonstrations that were used to collect ants from Mokala National Park, South Africa.

Pitfall traps are buried in a ground, with their rims at level to the ground surface (see **Figure 3**) where a half to two-thirds of their capacity is often filled with a solution of water- propylene glycol (car antifreeze/coolant) to impound, kill and preserve the ants [83, 84]. Sometimes a soapy water is also used [68, 69]. Pitfall traps catch ants continuously on their own for a relative long time, at different times, without human presence. It therefore provide a wider information about ant diversity of the habitat as a whole and is one of the effective sampling methods to estimate ant species richness in a concerned study site [85].

Another most common technique to sample ants is baiting. Similar to hand collection, baiting is also often implemented in transects (of an appropriate lengths) in the study areas where ants seen. Baiting stations are often placed at interval places of such transects where foods such as tuna, sardines, biscuits, water-sugar or water-honey solutions are placed on material such as petri dishes, vials or pieces of paper [86–89] (see **Figure 4**). Baiting is left operating for certain durations [90–92]. Sometimes baiting can be placed at a specific target area depending of the objective of the study. For instance Tonhasca [93] placed baiting on the trail of ant *Atta sexdens rubropilosa* as they were investigating the behaviour of *Neodohrniphora declinata*, a parasitic fly of the ant, roosts along ants' foraging trails. Baiting is often



Figure 2.
Containers that are commonly used as pitfall traps to sample ground dwelling ants in South Africa.



Figure 3.
A pitfall trap set in the ground to catch ants from a site that was heavily degraded by livestock grazing in Tankwa Karoo National Park, South Africa.

applied diurnally (usually from 8 h20 to 18 h00) when the collector can see ants and their interactions [94] and it can be done when weather conditions of the area are permitting [95].

Baiting enable a researcher to record the interactions amongst ant species/colonies and provide the mechanisms the different species employ to access resources such as food [96, 97]. Peral et al. [98] used it to investigate if the dominating ant species could be restrictively influencing the traits of the subordinate ones in

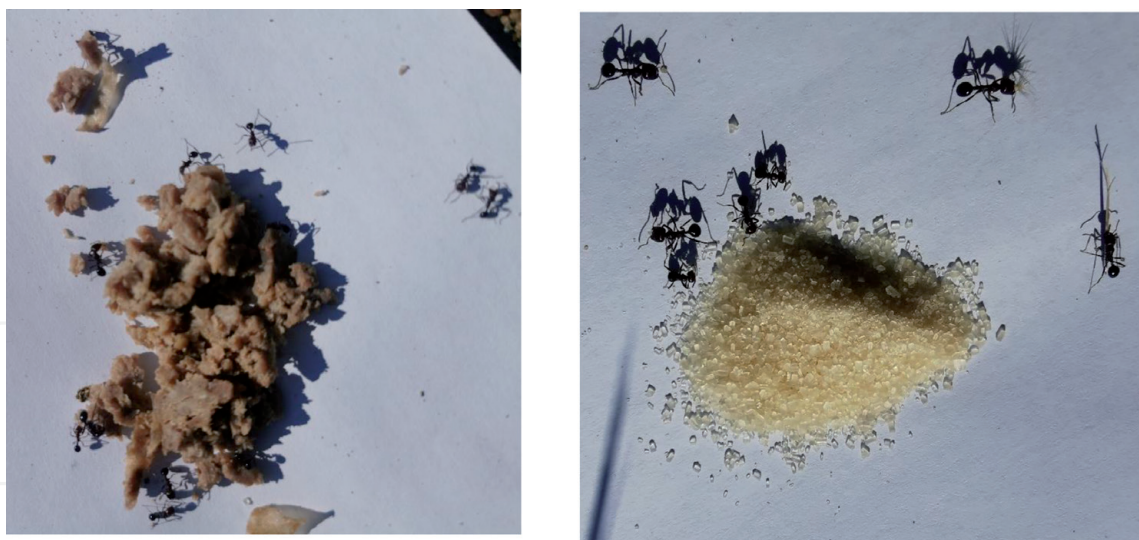


Figure 4. Example of baits (viz. tuna on left and sugar on right) on a white paper sheet (aiding to easy see ants), with some ant species consuming them, that were used to sample ants from Mountain Zebra National, South Africa.

habitat where they discovered that the presence of dominant ant species limited the abundance and occurrence of subordinates. It can also be used to investigate the interactions amongst individuals of the same ant species from different colonies. Garnas et al. [99] also used baiting to study the magnitude of intercolony aggression amongst the invasive *Myrmica rubra* ant colonies in Maine, USA, where they discovered that *M. rubra* foragers that originate from the same colony, that has however fragmented, still have the high levels of intraspecific tolerance and has intercolony aggression to foragers from neighbouring colonies. Baiting also assists in studying or identifying the mechanisms some ant species employ to be competitive/dominant to other species. Holway [100] used it to learn that the invasive *Linepithema humile* in California, uses the faster resource discovery and faster member recruitments than the native species as one of the mechanisms it dominate the ecosystem.

Baiting also assist in studying the activities of different ant species at different climatic conditions, and the influence such abiotic factors have to them [101–103]. For instance, Cerdá et al. [104] used it to learn that the foraging activities of the sub-dominant ant species of the open grassland habitat of the southeastern Spain is influenced more by the temperature of their habitat than the competition with their dominant counterparts.

For all these sampling methods, a pair of forceps and an aspirator (**Figure 1**), are used to transfer the specimen into containers such as vials that mostly contain a 75%, or more, alcohol solution to preserve the specimen [105, 106]. A magnifying glass (**Figure 5**), can also be used in the field to enlarge the ant specimen, and to make the observations of their interactions clearer.

In most cases the specimen are transported to the laboratories where they are identified to the genus and morphospecies levels following the appropriate taxonomic books and internet websites [107].

The efficiency of these methods in producing the highest biodiversity information of ants can sometimes differ. It is therefore prudent to assess the most efficient one for the habitats/ecosystem one is going to sample. Although some sampling methods are more efficient in getting more species, applying the combination of different methods is often the most effective way of sampling ants. King and Porter [108] reported that the combination of baiting, pitfall trapping, extraction and hand collecting was more efficient in sampling more ant species than using only one method as these methods complement each other on species that were not collected



Figure 5.
Example of a magnifying glass, and its collection demonstration that were used to sample ant specimen from Mokala National Park, South Africa.

by other methods. Antoniazzi et al. [67] also reported that although hand collection sampled more ant species in the Mexican rainforest than baiting, the combination of these methods yielded more ant species than just one method. These methods can be implemented simultaneously in the habitat undergoing restoration to accommodate wider variables - and get bigger picture - related to ant conditions. Pitfall trapping, which is a passive sampling method, will catches ants continuously on its own for a relative long time, at different times – diurnal to nocturnal and provide ant specimen for diversity information even when human is not around. It can however can be complemented by baiting and hand collection that will record the interactions amongst ant species/colonies in the habitat and provide the mechanisms the different species employ to access resources such as food [96, 97].

The efficiency of sampling methods can also be assessed by using estimators (such as incidence-based coverage estimator (ICE), Jackknife estimator (Jack2) etc.). Lopes and Vasconcelos [68] and de Souza et al. [85] used estimators to evaluate the efficiency of the three methods (viz. sardine baiting, pitfall trapping and extractor) at the savanna and forest habitats of Brazil.

5. Analysing the sampled ant data

There are many calculations that can be used to analyse and compare the collected ant data. One of the first analyses to do is to determine if the species of the sampled habitat/site have been adequately sampled, or determine the sampling efforts needed to represent the adequate ant species of the habitat/site/ecosystem. This is often achieved by calculating the estimated species that should be at the habitats/ecosystems relative to the ones sampled from the data collect, and then determines if the number of the sampled species is sufficient relative to the expected ones [63, 67, 68, 71, 78, 109–111]. For instance, Urrutia-Escobar and Armbrrecht [112] used the averages of the three estimator (ICE, Chao2) and Jack1) to determine that they have sampled about 85% of the expected ant species of their study sites while Cerdá et al. [113] used Chao1 estimator to decide that the number

of ant species they have sampled was similar to the expected ones for the habitat. ICE, Jack2 and Michaelis–Menten richness estimator (MMM_{Mean}) was used by Calcaterra et al. [77] to find that ant species they sampled were below the expected of the habitats they studied.

The expected ant species and the amount of sampling time required for a site/habitat/ecosystem can be calculated by using species accumulation curves as they can identify the size of the sampling site and the sampling period needed to get the maximum ant species in a habitat/ecosystem. Wang et al. [61] used them to assess that to capture the maximum ant species, using pitfall traps at George Washington and Monongahela National Forests of USA, one needs to sample eight plots for eight weeks (see **Figure 6**).

One of the basic and common indices that are used to analyse the diversity of ants in sampled habitats/sites is species richness. The ant species richness is the total number of ant species that has been sampled from the study sites [114]. Finding the species richness can be done by just counting these species from the sampled data of the concerned site/habitat/ecosystem or period. Species richness can be used to assess the conditions of the habitats under study like King et al. [58] and Graham et al. [84] did to evaluate the impact of habitat disturbances and found low species richness on sites of the disturbed areas relative to their undisturbed counterparts. Porter and Savignano [115] used it to find that the invasive *Solenopsis invicta* ant has drastically reduced the native ant community of Texas, USA.

Another basic index that is used to analyse the diversity of ants at a particular habitat/ecosystem/site is abundance. Abundance is the total number of ant individuals that have been sampled [116], and can be found by just counting the number of the ant individuals sampled. Just like any comparative analysis indices, abundance can be used to evaluate and compare the ecological conditions amongst sites/habitats/ecosystems or of interest. Morrison [117] used it to assess the short and long term impact of the ant *Solenopsis invicta* invasion to the native ant species of Texas, USA, and discovered that its impact is more severe at the beginning of the invasion and subsides as time goes on. Rivas-Arancibia et al. [118] also used the abundance from ant data collected from in Puebla, Mexico to learn that the most abundant ant species, at both the disturbed and undisturbed sites, are more active in mornings than in the evenings.

Abundance can also be used to determine the ant species that are numerically dominating the habitats. Sarty et al. [119] and Parr [120] both used it to investigate

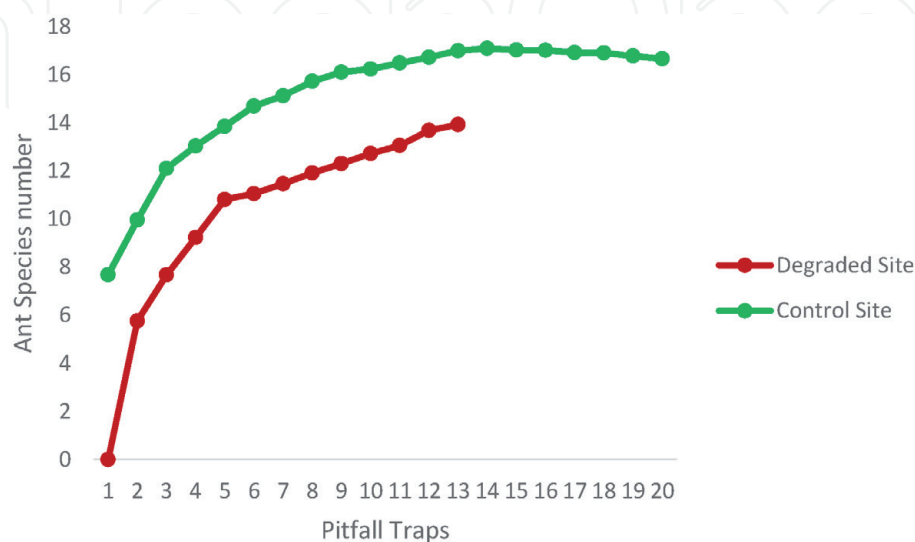


Figure 6.

An example of species accumulative curves that shows ant species from a site undergoing restoration relative to the control one.

the dominating ant species at different habitats of Tokelau, New Zealand and Kruger National Park, South Africa, respectively. The information about the abundance also assists to investigate the association of different ant species to different habitats in at different ecosystems. Calcaterra et al. [77] used it to learn that the cosmopolitan invasive ant, *Solenopsis invicta*, dominates both the natural and the modified habitats in its native range of Corrientes, Argentina. Calcaterra et al. [121] also used it to study the competitive mechanisms of the other cosmopolitan invasive ant *Linepithema humile*, in its native range, and learned that it recruits its members quickly to the food resources.

The abundance can also be used to identify ant species that could be the indicators of the conditions of their habitats. Herrera-Rangel et al. [81] learned that ant *Gnamptogenys bisulca* could be an indicator species of a healthy forests (that have thick leaf litter profiles and high relative humidity values) of Central Mountain Range, in Colombia more abundant in such habitats.

The calculations of both species richness and abundance of ants can be done by many programmes available of different platforms. Abundance is one of the basic components of other analyses that also investigate the biodiversity of ants of a particular habitat or ecosystem such as Indicator Value Analysis index (IndVal) that identifies indicator species of the habitat/ecosystem under study [122, 123]. With the inclusion of the abundance in IndVal, Sanabria et al. [124] identified 14 ant species that can indicate the ecosystem service condition of the soils from five different land uses.

6. Basic gradual progress to use ant data for rehabilitation processes

1. Choose sampling sites.

- a. Choose a rehabilitated site. This is a place/s where the rehabilitation is taking place from degradation (see **Figure 7**).



Figure 7. An example of places where rehabilitation site (forefront of the red line in the picture) and the reference site (behind the red line in the picture) were set to sample ants to assess the ant diversity conditions of the rehabilitated site relative to their natural conditions [125].

- i. Choose a reference site (in cases where available) (see **Figure 7**). This is a place/s that has not experienced the concerned degradation. Ant diversity from the rehabilitation site/s will be compared to the ant diversity from the reference site to determine how different ant diversity of rehabilitation site from their natural diversity.
 - b. If possible, replicate sampling sites.
2. Select an appropriate ant sampling method and durations related to your objectives (see section four).
3. If possible do a pilot sampling for each side to determine sampling efforts that will be needed in each site [61].
4. Execute the sampling methods accordingly.
5. Ensure that step 1 to 4 are carefully considered as they are the most expensive (in both monetary- and manpower-wise) to rectify in case they were miscalculated.
6. Prepare specimen for identification – under the microscope.
 - a. For pitfall trapping remove ant specimen from none-ant at each trap.
 - i. Use basic characteristics of ants to distinguish them from none-ants of each trap (viz. a pair of antenna (consisting funiculus and a scape) that is usually jack-knifed and a petiole/wait (consisting of either a single or two segments) (see **Figure 8**).
 - b. Group the specimen according to the similarity of their morphology such as their sizes and colours.
7. Identify specimen to the taxonomical rank appropriate to your objectives.
8. Determine if the species of the sampled site have been adequately sampled by using appropriate species accumulation curves.
9. Determine the diversity of each side and interpret the results.

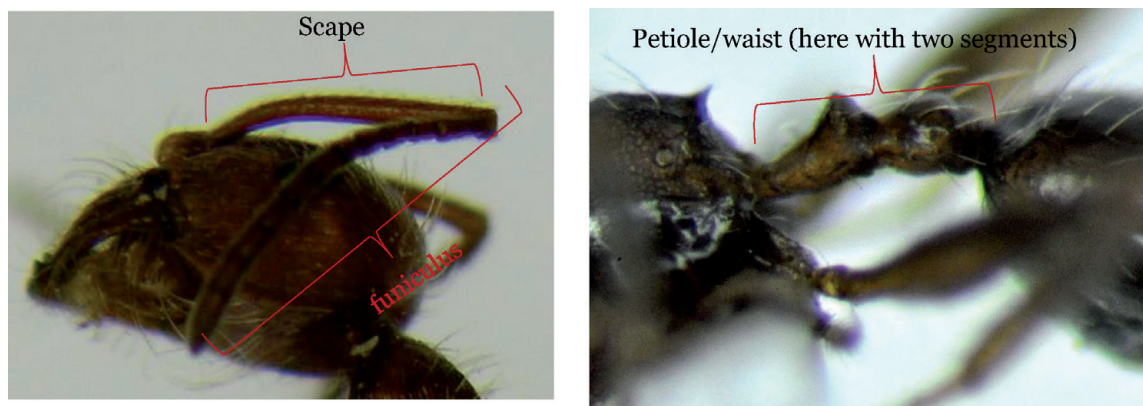


Figure 8.
A close view of antennal and waist parts of ants use distinguish ants from non-ant insects.

7. Ant biodiversity indices that can be used indicators of restoration

Biodiversity indices can show species that are associated with or indicate the conditions of their habitats/sites – therefore be the indicators of those habitats/sites. For example abundance reduction of ants can indicate the condition of their habitats as it did on land transformed into urbanisation in Indiana, U.S.A [126]. Ant abundance also indicates the condition of the degraded habitats relative to the un-degraded ones like it was significantly lower on habitats turned into oil plantation relative to the intact forest in Sabah, Malaysia [127]. Ant species also can indicate the conditions of their habitats like *Pachycondyla impressa* indicated the influence of shading to its habitat as it was found on shaded coffee plantation that accommodated its natural habitat requirements of the forest floor, than at unshaded ones [112].

The other ant indicator of the habitats/sites can be the ant community composition as it can also indicate the conditions of the habitats/sites. For instance, the ant community compositions of habitats undergoing forest restoration in Colombia were still having different ant community compositions to the pristine forests [81].

The presence of ant species can also be used as indicator of the state of the habitats. The presence of some species such as *Crematogaster cerasi* and *Prenolepis imparis*, can indicate that status of the degradation at the site/habitat/ecosystem undergoing restoration as such species disappeared at the presence of degradation like they did after the residential development in Indiana, U.S.A [126]. Some such as tramp ant species (which are alien and invasive species) can also indicate the conditions of habitats/sites that have been degraded as they did at habitats that have been turned into rubber and oil plantations relative to forest in Sumatra, Indonesia [128].

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