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# Rapid assessment of ant assemblages in public pine forests of the central Iberian Peninsula

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#### ABSTRACT

Ants are good indicators of ecosystem health and therefore a good choice for rapid terrestrial bioassessments (RBAs) in land management. However, the application of these rapid protocols is unfeasible until efficient sampling methodologies adapted to management goals have been developed. Taking into account the need to improve tools to synthesize broad-scale RBA, the aim of this study is to search for a simple and efficient ant sampling protocol suitable to assess changes in ant assemblages taking, as an example, the specific case of the management of selective logging activities in public pine forests of the central Iberian Peninsula. Ants were sampled at eighteen sites. Each one corresponded to a tranzón, a quadrangular unit of management. In our case all the tranzones had similar areas (25-30 ha). Ants were sampled at each site using two methods: pitfall traps and hand collecting. Ant species richness (number of species) and the Shannon diversity index (H) were compared both for sampling method and for sample point. The Shannon diversity index was not considered as a biodiversity index, but a measure of entropy (uncertainty or information content) like in its original proposed sense. In both comparisons, hand collecting was the method that achieved a greater ant species collection and greater diversity indexes, and therefore, a higher information content, suggesting that it is the best choice to use for ant RBA protocols in the particular case of temperate forest habitats. This is an important issue for land managers of these forests in order to detect changes in ant assemblages between logging events. Moreover, the relatively large ant sizes reported in the Mohago pine forests in comparison with ant species across the whole Iberian Peninsula make the process of ant collection and recognition easier, simplifying forest management through the elaboration of simple protocols for rapid ant bioassessments (RBAs). Overall, this information greatly improves the current application of rapid ant assessment protocols for monitoring the recovery status of these temperate forests.

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### 1. Introduction

Ants play an important role in monitoring ecosystem health for various reasons (Wike et al., 2010; Underwood and Fisher, 2006). They are abundant and present in almost all terrestrial environments (Andersen, 1990). This particularity is mainly achieved by a wide range of tolerance to conditions (Wike et al., 2010). All these traits give them the ability to be sensitive and rapid responders to environmental variables (Underwood and Fisher, 2006), which make these invertebrates a good choice when it comes to initiating rapid terrestrial bioassessments (RBAs).

Underwood and Fisher (2006) outlined five areas where ants can provide valuable information for management-based monitoring: detecting the presence of invasive species; detecting trends among threatened and endangered or keystone species; evaluating

land management actions and assessing long-term ecosystem changes. All these areas are perfect circumstances for the application of RBA. One example of this is the use of ants as bioindicators of ecosystem recovery in land management actions (Andersen, 1991a; Andersen et al., 2002; Bestelmeyer and Wiens, 1996; Castracani and Mori, 2006; Gunawardene et al., 2010; Majer, 1983; Nakamura et al., 2007; Ottonetti et al., 2006; Palladini et al., 2007; Read and Andersen, 2000; Stephens and Wagner, 2006; Vasconcelos et al., 2000; Watt et al., 2002). For instance, the evaluation of the impact of logging on recovering ecosystems can be easily assessed by analyzing ant community responses, as the reduction of vegetation structure can cause changes in the ant community (Gómez and Abril, 2011; Puntilla et al., 1991; Uhl and Vieira, 1989; Vasconcelos et al., 2000).

Notwithstanding the previously mentioned benefits of the use of ants as bioindicators for management-based monitoring, using these terrestrial invertebrates will not be widely adopted until efficient RBA protocols have been developed (Andersen et al., 2002). The main issue for a successful ant RBA is to determine the

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sampling method most appropriate for the management goal (Underwood and Fisher, 2006). A good RBA should provide the maximum information at a lower cost (in terms of time and difficulties to carry out the sampling). However, these issues can greatly differ depending on the method. For example, the Winkler method can be a good estimator of the ant community in the leaf litter (Fisher, 1999; Ivanov et al., 2010), but it is only appropriate in forests and scrublands; pitfall traps do not catch the ants randomly, the large-bodied species can be biased, and the number and composition of the collected ants can be affected by the pitfall contents (i.e. water or ethylene glycol) (Calixto et al., 2007). Additionally, this sampling method undersamples arboreal species (Majer, 1983) and is not a good technique for ant sampling in areas with a well-developed litter layer, rocky places or human-disturbed habitats (King and Porter, 2005; Gotelli et al., 2011). On the other hand, results provided by resource baits may be biased to behaviorally dominant species that monopolize these resources. Overall, each method has its particular advantages and disadvantages, for this reason, several studies suggest the combination of at least two methods to report ant diversity (Ellison et al., 2007; King and Porter, 2005; Véle et al., 2009), or at least only the pitfall trap as the unique method for comparing assemblages among habitats (Oliveira et al., 2009; Steiner et al., 2005). One effective way to choose a standard ant sampling method is using its efficiency at accumulating species (Gotelli et al., 2011). Several studies have established that, in terms of sampling efficiency, Winkler sacks or leaf litter traps are the most efficient methods for ant sampling, followed by pitfall traps, and finally by bait traps (Andersen, 1991b; Fisher, 1999; Bestelmeyer et al., 2000; Delabie et al., 2000; Wang et al., 2001). However, we must also take into account that the cost and logistics of an ant monitoring program are vital considerations for land managers and conservationists (Underwood and Fisher, 2006). Therefore, the use of methodologies such as Winkle sacks or pitfall traps for sampling ant diversity, particularly when the goal is the rapid assessment of species number, may not be worth the effort in relatively low ant diversity ecosystems as they are extremely time-consuming and labor-intensive (Agosti et al., 2000: King and Porter, 2005: Underwood and Fisher, 2006).

On the contrary, hand sampling is a little used method in ant surveys, even though it offers important advantages over other ant sampling methods, in particular the most commonly used one, the pitfall trap. Firstly, the hand collecting method is much less laborious and time-consuming. It can also be used in waterlogged, steep or rocky places and areas with high human disturbance without losing its efficiency. It also avoids the capture of non-target insects and the negative effects of temperature on ant activity (nests can be detected even without ant foraging activity outside) (Gotelli et al., 2011). However, the main issue with hand collecting is that results may vary depending on the expertise of the collector (Gotelli et al., 2011; Longino et al., 2002; Sorensen et al., 2002). This can be an important factor for land managers and conservation practitioners involved in ant diversity RBA monitoring programs, as their level of expertise in both the collection and the subsequent identification of ant samples can be very problematic. One way to make the searching process by non-professionals easier is to search for specific functional groups according to their responses to environmental stress and disturbance, and from these, to search for the larger body sized species belonging to these groups.

Against this background, and taking into account that methods for collecting invertebrates in the field have remained practically the same for over a century, and thus, incompatible with the application of broad-scale ant RBA protocols in terms of time and effort (Gotelli et al., 2011), the aim of this study is to search for a simple and efficient ant sampling protocol suitable to assess changes in ant assemblages of temperate forests. For this reason, we want

(1) to compare the efficiency and time cost of pitfall traps (as the most common ant collecting method used), versus hand collecting (as an easier alternative to pitfall traps) to report ant diversity in public pine forests of the central Iberian Peninsula, and (2) to examine the body size of the ant community in the area of study, in order to evaluate how easy the searching process by non-professionals could be in the case of hand collecting. Testing the application of these two kinds of ant collecting methods, in the specific case of the management of selective logging activities in this area, could improve the current knowledge of ant sampling as regards its use in ant RBA.

#### 2. Methods

#### 2.1. Study site

The study was carried out in Tierra de Pinares (Pine Land) situated on the northern plateau of the Iberian Peninsula (Castilian Plateau, Castile and León, Central Spain) (Fig. 1). These forests have a long history of use, exploitation and management (Aránzazu et al., 1997). The Mohago pine forest is located near the village of Olmedo  $(41^{\circ}17'N, 4^{\circ}41'W, elevation 750-780 m)$ . This is a zone with large pine forests surrounded by a matrix of arable and grazing areas, and pine forest fragments. The forests under study are public use forests (timber and pine nut exploitation), and the Environment Service of the Government of Castile and León in Valladolid is the management authority. The climate is continental Mediterranean, with 452 mm of annual rainfall. Mean monthly temperatures reach a high of 20.1 °C in July and a low of 2.2 °C in January. Soils are quaternary diluvial sands. Mohago pine forests are dominated by Pinus pinea L. and Pinus pinaster Ait., with a poor underwood (Romero, 1990).

#### 2.2. Ant sampling

Ants were sampled at eighteen sites. Each one corresponding to a tranzón, a quadrangular unit of management. In our case all the tranzones had similar areas (25-30 ha). Sampling was conducted in the warmer season, during June and July 2010, when ant activity is high in Mediterranean ecosystems (Cros et al., 1997). Ants were sampled using two methods at each site: pitfall traps and hand collecting. Firstly, a 100 m long transect was established through the center of each of the eighteen sites. Along each transect, we established 10 sample points and placed a pitfall trap consisting of a 6 cm diameter plastic cup buried flush with the substrate surface. Traps were partly filled with methylene glycol as a preservative. Secondly, we searched for and hand-collected ants for one person-hour in the morning and one person-hour in the afternoon throughout each sample site. We established four 15 min time units per person-hour of sampling. That is, as many species of ants as possible were collected in 15 min by visual search of the ground surface, looking under rocks, on low-growing vegetation, around the base and the first 1.75 m of tree trunks. Not more than three individuals were captured from a single nest. At each site eight time units of sampling were made.

A total of 180 traps were placed in the pine forest, and 36 h (144 time units of 15 min) of hand collecting took place.

The entire ant sampling was conducted by a non-expert ant collector or professional myrmecologist in order to simulate a potential situation of an RBA protocol carried out by non-professional myrmecologists, such as technical employees from the environmental service with no specific ant knowledge, whereas the processing and identification of samples were conducted by an expert (Dr. C. Gómez).

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