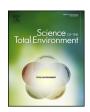
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Ivermectin residues disrupt dung beetle diversity, soil properties and ecosystem functioning: An interdisciplinary field study



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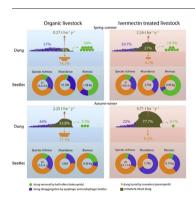
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HIGHLIGHTS

At the short term, ivermectin residues cause a strong decrease in dung relocation and dung spreading by dung beetles.

- Conventional use of ivermectin disrupts diversity by affecting species richness, abundance and biomass of dung beetles.
- Reduction in the functional efficiency of dung degradation resulted in the longterm accumulation of manure.
- Use of ivermectin causes lower quality in soil organic C and the increase of the in-situ mineral N and P production.
- The results of this study highlight that the effects of ivermectin must be investigated from a global perspective.
- The use of this veterinary medical product must be monitored and controlled following a precautionary principle.

GRAPHICAL ABSTRACT



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$A\ B\ S\ T\ R\ A\ C\ T$

Ivermectin is the most common endectocide used to control parasites affecting livestock. Short-term physiological and behavioural effects of ivermectin on dung beetles may have long-term consequences for beetle populations and ecosystem functioning. Long-term effects of the use of ivermectin can be estimated by comparing dung assemblages and ecosystem functions in areas with conventional ivermectin-treated livestock and environmentally similar areas in which livestock are not treated with veterinary medical products (organic farming). In this study, we investigated both short-term and long-term effects of the administration of ivermectin on the characteristics of dung beetle assemblages and the services they provided in a protected area (Doñana National Park, SW Spain). We examined short-term dung colonization, dwelling, relocation, and disaggregation rates and the associations

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between these processes and the key assemblage parameters of species richness, abundance, biomass and functional diversity. Furthermore, we analysed changes in soil physical-chemical properties and processes. Short-term differences were observed in the total amount of dung relocated by dung beetles at different colonization vs. emigration stages, suggesting that dung beetles in this area were affected by the recent treatments of livestock with ivermectin. Moreover, short-term effects could also be responsible for the significant differences in dung spreading rates between sites. Conventional use of ivermectin disrupted ecosystem functioning by affecting species richness, abundance and biomass. The decrease in diversity parameters was related to a reduction in the functional efficiency, which resulted in the long-term accumulation of dung on the ground and considerable changes in soil functionality.

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1. Introduction

Ivermectin is the most common endectocide substance used to control arthropods and nematodes affecting livestock (Campbell et al., 1983). Since the early 1980s, over 5 billion doses have been sold worldwide (Shoop and Soll, 2002). After application, the primary route of excretion is via faeces (Canga et al., 2009), which constitute a food source for very many invertebrate species (Holter, 2016), in addition to an important input of organic matter into soils (Irshad et al., 2013). Dung degradation is a complex process that involves different taxonomic groups, including dung beetles, which may be affected by ivermectin. Previous studies suggest that the massive treatment of entire flocks in a given area can lead to a significant reduction in the number and composition of coprophagous invertebrates, delaying dung degradation (Wall and Strong, 1987; Floate, 1998) and altering soil nutrient cycling (Madsen et al., 1990; Sommer and Bibby, 2002). Short-term effects of ivermectin on dung beetles include increased attractiveness of ivermectin-containing dung (Holter et al., 1993; Lumaret et al., 1993), loss of sensorial and mechanical activity (Verdú et al., 2015), disruption of reproductive physiology (Martínez et al., 2017), and mortality at both adult and larval stages (Wardhaugh and Rodriguez-Menendez, 1988; Krüger and Scholtz, 1997; Iwasa et al., 2007). According to recent evidence, even low doses of ivermectin can significantly decrease the olfactory and locomotor capacity of dung beetles (Verdú et al., 2015). Thus, ivermectin seriously affects basic biological activities of dung beetles such as food detection, intraspecific communication, locomotion and interaction with the environment.

These short-term physiological and behavioural effects of ivermectin on dung beetles may have long-term consequences for beetle populations and ecosystem function. For example, long-term disruptions caused by ivermectin residues could be responsible for increases in local extinctions and changes in guild structure, causing alterations in ecological functioning and ecosystem services (Lobo, 2001; Beynon et al., 2012; Nature-England, 2016). Despite the increasing number of studies investigating the effect of ivermectin on several ecosystem services, no studies have examined long-term effects (several years) of ivermectin residues. Long-term consequences of the use of ivermectin can be anticipated by comparing both dung assemblages and ecosystem functions in areas with conventional ivermectin-treated livestock and environmentally similar areas in which livestock is not treated with antiparasitic substances (organic farming). Unfortunately, these types of comparisons are becoming more difficult because intensive farming has been the dominant livestock system for decades; whereas organic livestock continues to remain scarce (Tilman et al., 2001; IAASTD, 2009). Furthermore, under current regulations, "organic farming" does not guarantee ivermectin-free livestock (Coffey and Baier, 2012). On the contrary, legislation in many countries requires organic livestock farmers to establish preventive health care practices that include the use of synthetic veterinary substances, such as ivermectin (Chander et al., 2011; Agricultural Marketing Service, USDA, 2017; European Union, 2007). In this study, we conducted an experiment in a protected area (Doñana National Park, SW Spain) with a stringent health management program that impeded the use of ivermectin in a core area belonging to a biological reserve (ICTS-DBR) for the last 30 years and a nearby site within the park in which ivermectin has been used to treat livestock. We investigated both short-term and long-term effects of the administration of this medication on the characteristics of dung beetle assemblages and the services they provide. To better understand the long-term effect of ivermectin use, we examined short-term dung colonization, dwelling, relocation, and disaggregation rates and the associations between these processes and the key assemblage parameters of species richness, abundance, biomass and functional diversity. Finally, we analysed changes in soil physical-chemical properties and functions that were potentially derived from the effects of ivermectin on dung beetle assemblages. Specifically, we focused on soil carbon (C), nitrogen (N), and phosphorous (P) pools and availability, in addition to C and N mineralization rates. These variables were all selected because of their importance for maintaining soil productivity and fertility and for the provision of key ecosystem services (Compton et al., 2011).

2. Materials and methods

2.1. Study area and livestock management

The Doñana region is located in SW Spain between the provinces of Huelva and Sevilla. This region hosts a nature reserve that includes both Doñana National Park and Doñana Natural Park. In Doñana National Park, livestock is composed of Andalusian endemic races of feral 'Mostrenca' cows and marsh horses. The climate is Mediterranean with some oceanic influence. Average annual rainfall is approximately 560 mm, and average annual temperature is approximately 17 °C.

The dung beetle assemblages of Doñana National Park have been rigorously studied (Lobo et al., 1997; Cárdenas and Hidalgo, 2006), because the area is one of the most diverse in the Palearctic region for species richness, abundance and guild diversity. We selected two field sites within Doñana National Park that differed in farming history and current use: one located within the Doñana Biological Reserve (DBR-ICTS) and the other, separated by 10 km, outside this reserve, called "Los Sotos." The DBR-ICTS site (traditional organic livestock, hereafter the ECO site) was located in an ivermectin-free area (6794 ha) and was selected because it constitutes a good example of 'organic farming'. The livestock density on the ECO site was 0.33 head of cattle per hectare (PAG-PNDoñana, 2000). In the ECO site, ivermectin (or similar compounds) has never been used. The Los Sotos site (ivermectin-treated livestock, hereafter the IVM site) was also an extensive landscape with similar climate, vegetation (Muñoz-Reinoso and García-Novo, 2005), soil type (Siljeström et al., 1994), livestock density (0.30 head per hectare) and area (6423 ha) to the ECO site but differed in farming history because livestock have been regularly treated with ivermectin and other veterinary medical products since the 1980s. According to a farmer survey conducted in 2014 (Figs. S1-S3 in Supporting Information), ivermectin is the VMP local farmers preferred (92% of VMPs used). Injectable and oral formulations of ivermectin are administrated to livestock in two different periods during the year. In July, horses (with oral gel application) and cows (with subcutaneous injection) are massively treated with ivermectin (generally in combination with praziquantel, for tapeworm control). In September-November, cows, and horses in

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