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Invasive Species May Disrupt Protected Area Networks: Insights from the Pine Wood Nematode Spread in Portugal

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Abstract: The expansion of invasive alien species is considered a major threat to forest ecosystems and biodiversity. Their potential impacts range from local changes in species composition to wider-scale effects on forest habitat and landscape functioning, although the latter has been relatively little explored in the literature. Here, we assessed the impact of an invasive forest pest, the pine wood nematode (PWN), in the Natura 2000 network of protected areas (PAs) in Portugal, the first European country in which PWN was reported. We considered the impacts of the pest's spread (up to 2016) on individual PAs, in terms of the fraction of their coniferous forest infected, and on the corridors between PAs, which were mapped and prioritized through least-cost path modelling, geographic information system analysis, and the graph-based probability of connectivity metric. We found that PWN by 2016 had spread into 49% of the Portuguese Natura 2000 coniferous forest habitat, while it had invaded 68% of the coniferous forests that form the priority corridors between the PAs. These impacts are likely to be aggravated in the next years, given the pace of PWN expansion and the predicted rates of natural spread to new areas in Portugal and, increasingly likely, in Spain. Our results suggest that the connectivity of PA systems may be significantly disrupted by alien species, and that spatially prioritized control measures can help mitigate the impacts of invasive species on the coherence and functionality of protected area networks such as Natura 2000.

Keywords: pine wood nematode; pine wilt disease; invasive species; forest pests; environmental impacts of invasions; Natura 2000 network; priority forest habitats; protected areas; corridors

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1. Introduction

Globalization of trade is increasingly leading to the introduction of species outside their native distribution ranges. These alien species, once introduced and successfully established, may become invasive and expand their ranges, severely affecting ecosystems and economies [1–5]. Several authors have analyzed the economic impacts of invasive forest pests or other invaders [1–5], but there are comparatively few assessments of the potential ecological effects of biological invasions at the forest landscape scale. Some studies have evaluated the effects of invasive forest pests on local forest species composition, structure, or functioning [6–8]. There is, however, a significant lack of knowledge on the wider-scale impacts that invasive forest pests may have on the performance, conservation goals, or functioning of protected area (PA) networks at a regional or national level.

As invasive species cause some of the greatest conservation problems in PAs worldwide [9], studies that can guide the related management and control efforts are urgently needed. In networks of PAs, biological invasions may disrupt the functional corridors and connectivity between PAs that serve native species of conservation concern. Poor PA connectivity for native flora and fauna may eventually reduce native genetic and species diversity in forest ecosystems due to the reductions in gene flow, in metapopulation dynamics, and in the ability of species to shift their ranges as a response to climate change, among other factors [10,11]. For these reasons, the United Nations Convention on Biological Diversity (CBD) adopted a Strategic Plan for Biodiversity for the 2011–2020 period, in which the international community agreed, under Aichi Target 11, to make efforts towards well-connected systems of PAs [12]. Recent assessments have shown, however, that most of the countries lag behind the PA connectivity target for the year 2020, and that considerable efforts are needed to improve the connectivity of PA systems globally [13].

The presence of the pine wood nematode (PWN; *Bursaphelenchus xylophilus* Steiner & Buhrer, 1934 (Nickle, 1970)) in Europe for almost two decades has caused a considerable ecological, social, and economic impact [14]. The PWN, which is native to North America, can cause severe tree wilt and ultimately kill coniferous trees in a matter of weeks [15,16] under suitable climatic conditions that trigger the pine wilt disease. European and Asian coniferous tree species, which have not evolved alongside the parasite, are particularly vulnerable to it [17]. After causing massive mortality in Asia [18], PWN was first detected in Europe in 1999, in the Setubal area of Portugal, near Lisbon [19]. Since then, it has quickly expanded, aided by the native insect *Monochamus galloprovincialis* Olivier [20], its only known vector in Europe, and by the transport of infected wood [21]. Currently, the PWN outbreak encompasses more than 30% of mainland Portugal. Beyond the obvious potential economic impacts due to the loss of products with market value [2], the epidemic of this invasive pest may cause the greatest damage due to the loss of non-market ecosystem services [22]. Important and fragile Mediterranean coniferous forests may be altered, and their biodiversity and connectivity impacted, due to the expansion of the PWN.

Here, we assess the impact of the spread of the invasive pine wood nematode (PWN) on the Natura 2000 network of protected areas (PAs) in mainland Portugal, considering both the individual PAs and the corridors between PAs. The Natura 2000 network of PAs in the European Union (EU) is one of the largest coordinated international actions for biodiversity conservation. The aim of this network is to ensure the long-term persistence of Europe's most valuable and threatened species and habitats, listed under both the Birds Directive (79/409/EEC, amended as 2009/147/EC) and the Habitats Directive (92/43/EEC), many of which are forest habitats and forest-dwelling species. Currently, the Natura 2000 network consists of more than 27,000 sites covering 18.1% of the EU territory and 20.6% of Portugal's land area [23]. The Natura 2000 sites in Portugal include forest habitats that have been declared as priority habitats for conservation in the EU legislation, such as coastal and inner wooded dunes with *Pinus pinaster* and/or *Pinus pinea* (habitat 2270 in Annex I of Directive 92/43/EEC).

Our assessment relies on a combination of recently developed methods for connectivity modelling in heterogeneous landscapes. Using these methods, we map the corridors between Natura 2000 forest sites, characterize the width of the permeable land strips along these corridors, prioritize the key corridors in which to concentrate conservation management efforts, and identify the bottlenecks (weak sectors) along these priority connectors. Then, using data on the observed PWN range for 2016, we examine the extent to which the coniferous forest habitat patches and connectivity providers in this network of PAs are being compromised by the pine wilt disease. In doing so, we provide both an assessment of these important landscape-level ecological impacts of an invasive forest pest, and highlight particular areas in which concentrated control could mitigate the PWN impacts on key native forest habitats and corridors. More generally, our study proposes and demonstrates a methodological approach that can be used to assess these impacts and priority control areas for other invasive forest pests.

2. Materials and Methods

We evaluated the impacts of the PWN on the Natura 2000 network in two main ways. First, we quantified the proportion of coniferous forest infected by the PWN in each of the Natura 2000 sites as of 2016. Second, we mapped, characterized, and prioritized the corridors between Natura 2000 forest sites, and evaluated the impact of the PWN on these corridors.

2.1. Natura 2000 Sites and Their Rate of Infection by the PWN

We downloaded the geodatabase of the Natura 2000 sites in mainland Portugal from the European Environment Agency in the version reported by the member states for 2016. For each of the Natura 2000 sites, we determined the areas covered by purely coniferous forest using the Carta de Uso e Ocupaçao do Solo de Portugal Continental (COS) for the year 2007. With a minimum mapping unit of 1 ha, COS is the most detailed land cover map available in Portugal. All European pine species appear to be, to some degree, susceptible to PWN [17], and pine trees make up 95% of all coniferous forest in Portugal [24]. For this reason, we focused our analysis on the coniferous forest as mapped in COS. COS differentiates purely coniferous, purely broadleaved, and mixed forests, with the latter defined as those in which both broadleaved and coniferous trees occupy more than 25% of the forest. Since the mixed forests are likely to be much less impacted and threatened by the PWN given the significant abundance of broadleaved trees, which are not susceptible to the disease, we restricted the assessment of the PWN impacts to the purely coniferous forests only.

Observed data on the PWN range in 2016 were gathered and provided by the Portuguese authorities (Instituto da Conservação da Natureza e das Florestas: http://www2.icnf.pt/portal/florestas/prag-doe/ag-bn/nmp). The data mapped the presence of PWN, in October 2016, at the level of local administrative units called parishes (close to the municipality level). The data are the result of the PWN spread over an almost 20-year period, from 1999, when it was first detected in Portugal, to 2016. The data show the spatial distribution of areas where the PWN has been detected, but do not include information on tree decline or mortality within the infected areas. The latter is not available for the study area (mainland Portugal) and was, therefore, not considered in our study.

We overlaid the PWN range data with the Natura 2000 and COS layers to determine the area of coniferous forest that was infected by the PWN in each Natura 2000 site and in the entire Natura 2000 network in mainland Portugal. The same procedure was used to determine the area of infected coniferous forest in all of mainland Portugal and in the inter-PA corridors and their bottlenecks, as identified through the connectivity analyses described in the next section. In all cases, we considered coniferous forest as infected if located in a parish reported as infected in the observed PWN range data, and as uninfected otherwise.

2.2. Mapping Corridors between Natura 2000 Forest Sites

We mapped the corridors between Natura 2000 forest sites in mainland Portugal following the PA connectivity modelling approach recently described in [25], with some adaptations for the purposes of this study. We followed five steps: First, we identified forest locations within the Natura 2000 sites to serve as the focal points for the connectivity analyses. Second, we used a resistance surface to capture the difficulty that forest animals experience when moving through different land cover types. Third, we applied least-cost path modelling to identify favorable inter-PA pathways (corridors) across heterogeneous landscapes and to characterize the width of these corridors. Fourth, we identified the priority corridors between Natura 2000 sites based on their contribution to sustaining the connectivity of the forest PA network. Fifth, we mapped the bottlenecks (weak sections) along the priority corridors. These steps are further detailed in the next sections.

Although the aim of this study was to assess the impacts of the PWN on the Natura 2000 network, and the pine wilt disease only affects coniferous forest, for the connectivity analyses we considered all forest cover (whether coniferous or broadleaved) to identify the focal Natura 2000 sites and the forest corridors between PAs. We did so because we assumed that forest-associated wildlife species may use any type of forest cover, either coniferous or broadleaved, to move between their forest habitat areas. Indeed, all forest cover may provide shelter and suitable pathways for dispersal movements, in accordance with the dispersal plasticity reported for forest wildlife [26,27]. Therefore, the loss of a particular corridor composed of purely coniferous forest can impact not only the movement possibilities of conifer-affiliated species, but also of species that have a specific habitat preference for broadleaved forest as residents, as well as those that have forest cover in general as their habitat.

2.2.1. Focal Locations within Natura 2000 Forest Sites

The PAs we considered were the Natura 2000 sites covered by forests in mainland Portugal, i.e., excluding sites located on Portuguese islands. The same portion of land could be covered by more than one Natura 2000 site; this happened in those areas that were designated both as a Special Area of Conservation (SAC) under the Habitats Directive and as a Special Protection Area (SPA) under the Birds Directive. To avoid double counting these overlaps, we dissolved the Natura 2000 geospatial layer, which resulted in a set of Natura 2000 polygons corresponding to sites covered by either SACs or SPAs, or by both at the same time. For brevity, hereafter, we refer to these Natura 2000 polygons simply as Natura 2000 sites.

We used a graph-theory approach to represent the network as a set of nodes corresponding to forest locations within Natura 2000 and links corresponding to the possibility of movement between the Natura 2000 forest locations. In order to identify forest habitat locations within Natura 2000, we overlaid a set of 2000 random points on the Natura 2000 network of mainland Portugal and retained those (n = 383) that fell within forest. This number of locations (nodes) was considered sufficient for the purpose of this study because: (i) it is more than three times larger than the approximately 100 terrestrial Natura 2000 sites in mainland Portugal (http://www2.icnf.pt/portal/pn/biodiversidade/cart/ap-rn-ramsar-pt); and (ii) it gives a much more dense and detailed representation of the forest sites in mainland Portugal than in a similar connectivity study focused on the Natura 2000 forest sites in Spain [25], which used, as nodes for the connectivity analysis, about 500 forest locations to represent a much larger study area and Natura 2000 network (note that the number of Natura 2000 sites in Spain is more than 10 times larger than in Portugal [23]). The links were determined, using least-cost path modelling, as the corridors that, starting and ending in the Natura 2000 forest locations, best allowed traversing the heterogeneous landscapes between these locations, as described next.

2.2.2. Landscape Resistance to Movement

We used a resistance surface (in the form of a geospatial raster layer) to characterize the difficulty of forest species movement through different land cover types. The concept of resistance to movement is related to species mortality risk, behavioral aversion, and energy expenditure, among other factors influencing the likelihood of successful movement through different land cover types [28]. In particular, we used a resistance surface with a spatial resolution of 100 m that was parameterized with resistance values for forest mammals and that has been used in previous studies [25,29–31]. This resistance surface was built based on expert knowledge [32] and validated with landscape genetic data for the European pine marten [33]. The values in the cells of the resistance surface are the lowest (equal to 1) for forests, and increase when movement has to occur outside forests (e.g., value 8 for shrublands, 50 for agro-forestry areas, and 150 for arable lands), up to a value of 1000 for urban areas and transport infrastructure.

In Portugal, the connectivity of the PA system depends more on transnational linkages than in almost any other country in the world [13]. In order to incorporate patterns of transnational connectivity, our resistance surface and connectivity analysis considered, besides all of mainland Portugal, also Spanish lands within 200 km from the border. For both Portugal and Spain, we assigned resistance values based on the land cover types differentiated in the Corine Land Cover map with a spatial resolution of 100 m for the year 2012, similarly to [25] and [29], because this map provided a consistent and comparable land cover characterization for the two countries.

2.2.3. Corridors between PAs

We used least cost path modelling to calculate the effective distance between the focal Natura 2000 forest locations and to identify the corridors between these locations. The effective distance, or accumulated cost along a least-cost path between habitat locations, can be defined as a distance measure that is weighted by the cumulative resistance values of all cells that need to be traversed to move between these locations [28,34]. The least cost path is then the combination of cells that minimizes the effective distance between two locations [35] and is used to determine the location of the optimal or favorable linkages between the focal areas [36]. In this way, we identified least cost paths between all the adjacent Natura 2000 forest locations from the resistance surface described in Section 2.2.2, using Linkage Mapper 1.0.9 [37] in ArcGIS 10 software (ESRI, Redlands, CA, USA). Linkage Mapper identified the adjacent locations by allocating the cells to the nearest forest location as measured either in Euclidean or effective (cost-weighted) distances [37]. If the pathway from one forest location to another must not pass through the allocation zone of a third location, and this happens either for the allocation zones determined using Euclidean or effective distances, then the two locations are considered adjacent [37]. Besides identifying the line (central axis) of the least cost path, we also considered the width of the corridors, i.e., the width of the permeable land swaths around that line. This was assessed, using Linkage Mapper, through the increased difficulty of movement (effective distance) when the movement needs to happen at a certain distance away from the central axis of the corridor. This result allowed us to evaluate which areas of the connectors are immediately surrounded by hostile conditions for movement (see Section 2.2.5), and which ones offer a wide band of land with similar permeability characteristics as the least cost path line. Although, as noted above, the mapped corridors started and ended in the forest locations in the Natura 2000 sites (hence inside those sites), we only considered, for characterizing the PWN infection of corridors and their bottlenecks, the part of the corridors located outside Natura 2000, given that the situation inside the Natura 2000 sites was already considered (see Section 2.1).

2.2.4. Priority Corridors for Conservation

Not all corridors are equally important in maintaining the connectivity of the Natura 2000 forest network; a subset of corridors can have a disproportionate role in sustaining the connectivity of the protected area system. For this reason, we ranked the connectors by evaluating the degree to which a degradation of their current condition (and ultimately a complete blocking of their connecting capacity) would more negatively affect the connectivity of the entire Natura 2000 forest network in mainland Portugal. The importance of each corridor was quantified as the percent decrease in the Probability of Connectivity index [38–40] for the protected area network that the loss of the corridor would cause. The Probability of Connectivity index (PC) quantifies the reachable habitat resources in a study area (here Natura 2000 forest in mainland Portugal), considering either direct or indirect (i.e., stepping stone) connections. The corridors with an importance value (decrease in PC) exceeding the 75th percentile of all corridors were considered as the priority corridors.

We evaluated the PC index and the importance value of each corridor for the connectivity of the Natura 2000 forest network for a mean dispersal distance of 10 km. This distance was recently selected as the reference for a global assessment of PA connectivity [13,41]. Similar analyses have also shown that the results for other dispersal distances are highly correlated with those obtained for 10 km [25] and hence this distance should describe the connectivity patterns and priorities well at the scale of the PA network. See [25] for further details on the procedure for corridor prioritization.

All calculations for corridor prioritization were performed using the Conefor software [42] version 2.6, available at www.conefor.org.

2.2.5. Bottlenecks (Weak Points) along Priority Corridors

We identified bottlenecks as the narrow parts of the priority corridors surrounded by hostile landscapes for the movement of forest species, i.e., as the narrowest sections of the priority corridors identified in the previous step. These bottlenecks are the sections in which corridor functionality could be easily compromised, especially for species with larger spatial requirements, as well as the sections where additional small modifications to the forest cover could translate into a more drastic reduction of corridor functionality, ultimately blocking it. We identified the bottlenecks in sections in the priority corridors where the corridor width was below its 10th percentile [25].

3. Results

3.1. Impacts of PWN on the Natura 2000 Sites

The terrestrial Natura 2000 network covers about 21% of mainland Portugal (≈1.9 Mha, Figure 1). Purely coniferous forests cover 6.9% of the Natura 2000 network in mainland Portugal (Figure 1), while mixed forests and purely broadleaved forests cover 3.9% and 16.3% of this PA system, respectively. However, these figures vary considerably between PAs (Figure 1); in particular, there are thirteen Natura 2000 sites where purely coniferous forests cover more than 25% of the area (Figure 2).

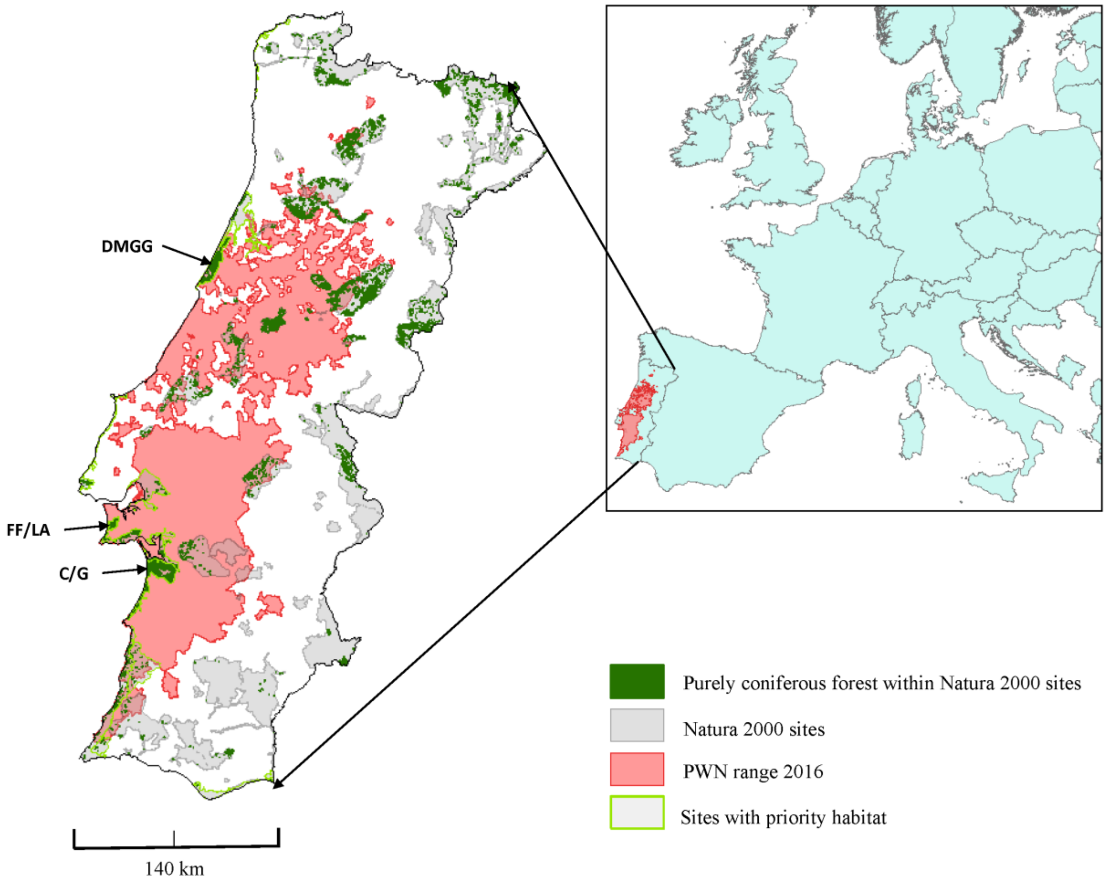

Figure 1. Natura 2000 sites, purely coniferous forest within the Natura 2000 sites, Natura 2000 sites designated to protect priority habitat of wooded dunes with *Pinus pinaster* and/or *Pinus pinea*, and pine wood nematode infected range in mainland Portugal as of October 2016. The location of the Natura 2000 sites Fernão Ferro/Lagoa Albufeira (FF/LA), Dunas de Mira, Gandara e Gafanhas (DMGG), and Comporta/Galé (C/G) is highlighted. The location of the study area (mainland Portugal) is shown in the inset map of Europe.

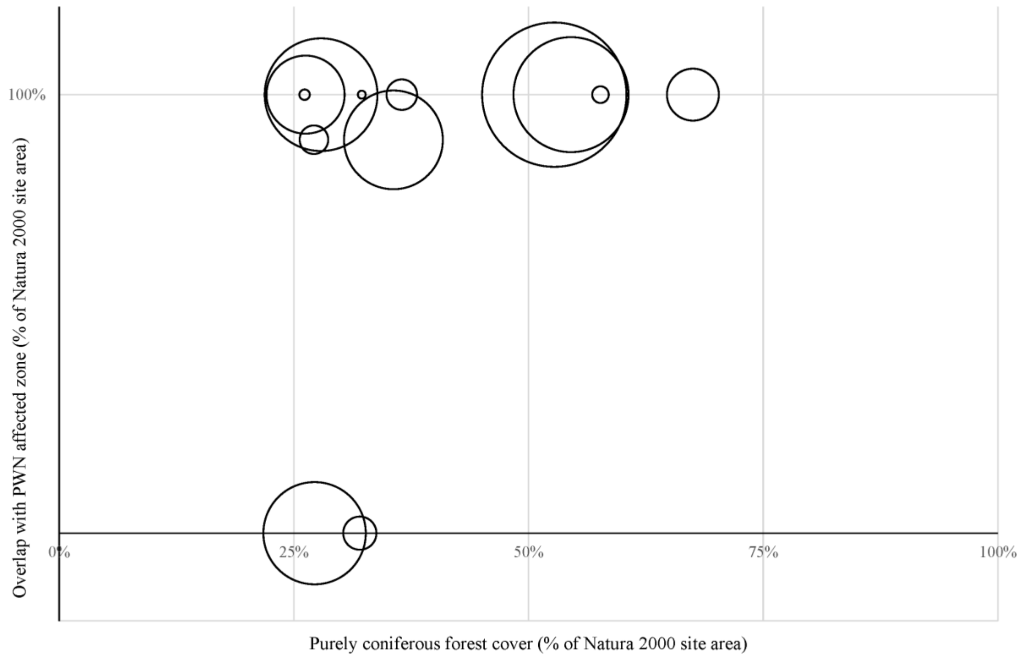

Figure 2. Areal overlap between Natura 2000 sites and the pine wood nematode affected zone (vertical axis) versus the purely coniferous forest cover in these sites (horizontal axis). Data are shown for the 13 Natura 2000 sites of mainland Portugal with at least 25% of their area covered by purely coniferous forests. The area of the circles is proportional to the total area of each Natura 2000 site (the maximum circle size corresponds to the 32,049 ha of the largest of these 13 Natura 2000 sites).

The current range of the PWN covers around 62,000 ha of purely coniferous forest in the Natura 2000 sites, which means that about half (49%) of the Natura 2000 purely coniferous forest is affected by the PWN. PWN is already present in most or all of the coniferous forest in eleven of the thirteen Natura 2000 sites with the largest proportion of purely coniferous forest (>25%) in Portugal (Figure 2); of these thirteen sites, only Serra da Malcata and Litoral Norte are not currently (as of 2016) affected by the PWN.

Particularly important in the Natura 2000 sites are the pine forests declared as priority habitats for conservation by the Habitats Directive 92/43/EEC, especially the wooded dunes with *Pinus pinaster* and/or *Pinus pinea* (habitat type 2270 of Annex I of the Directive; Figure 1). According to the European Nature Information System of the European Environment Agency (http://eunis.eea.europa.eu/habitats), there are 20 Portuguese Natura 2000 sites designated to protect this priority habitat type 2270. Of these 20 sites, 15 are already within the PWN affected zone, either entirely (11 sites) or partially (four sites) (Figure 1). The Natura 2000 sites of Fernão Ferro/Lagoa Albufeira, of Dunas de Mira, Gandara e Gafanhas, and of Comporta/Galé are the three sites currently most threatened by PWN (Figure 1), given that they are entirely inside the PWN-affected zone, are designated to protect this priority wooded dune habitat type, and have most (50–70%) of their area covered by purely coniferous forests according to the COS map of Portugal.

3.2. Corridors between Natura 2000 Forest Sites

We identified an extensive network of corridors between the Natura 2000 forest sites in mainland Portugal (Figure 3a). There were notable differences in the spatial distribution of the corridors, from areas with many and wide corridors (primarily in southwestern and central Portugal), to areas with very few corridors (as in certain parts of the Southeast and some relatively large coastal areas, Figure 3a). Some of the corridors spanned over wide stretches of land, so that movement could be distributed over relatively large areas in quite an unrestricted manner; i.e., with little increase in the difficulty of movement (accumulated cost) even at relatively large distances from the central axis of the corridor (least cost path line). These wide corridors are those shown as 'corridors through a wide area with low resistance' in Figure 3a. On the contrary, some corridors were constricted to a very narrow band, covering little or no more land than the least cost path, and with large permeability drop-offs in the lands immediately adjacent to this central axis of the corridor. These narrow corridors traversed landscapes largely dominated by high resistance areas and funneled through the relatively sparse strips with suitable vegetation, since these were the only available movement pathways to reach certain Natura 2000 forest sites. These narrow connectors are those shown as 'corridors through a narrow band in a high-resistance area' in Figure 3a. Some low-resistance areas were not traversed by any connector, either because they were peripheral areas that did not give access to any Natura 2000 forest site, or because they were surrounded by other areas of high resistance. Some of the connectors between the Portugal Natura 2000 forest sites, including some priority connectors, traversed land outside Portugal, mainly through the north of Spain (Figure 3).

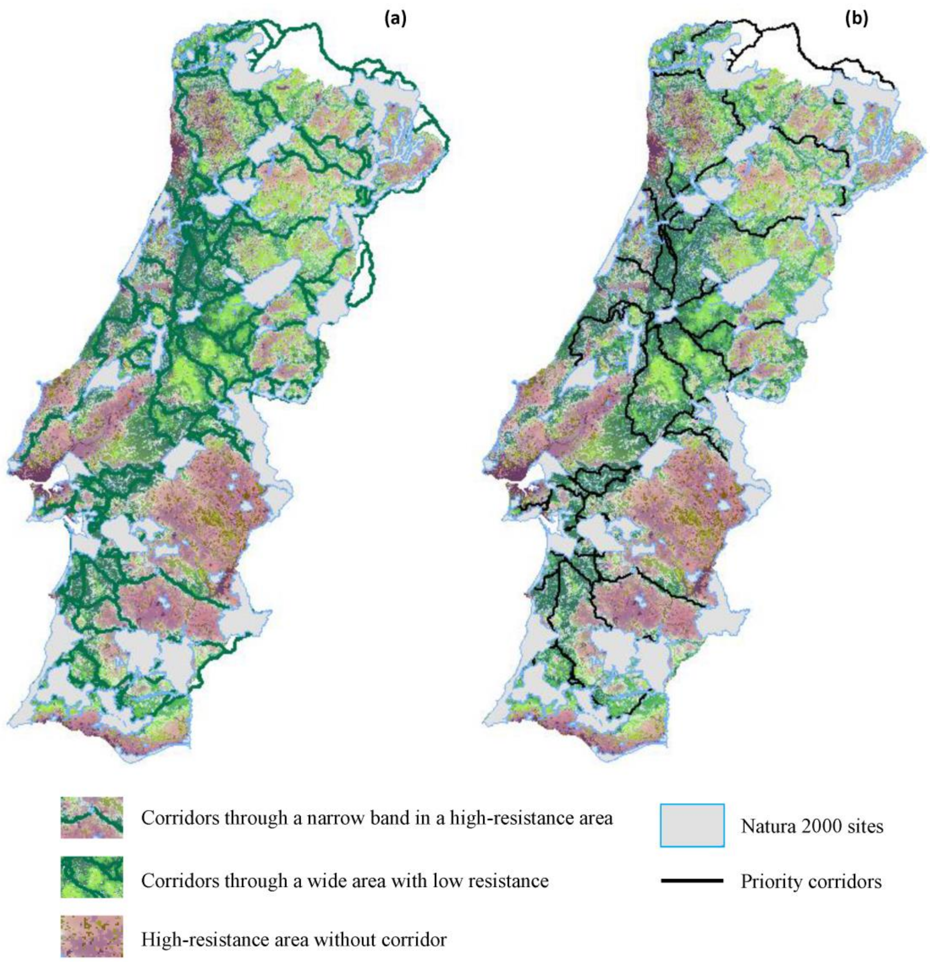

Figure 3. Corridors of variable width between the Natura 2000 forest sites (**a**) and central axes of the priority corridors for maintaining the connectivity of the Natura 2000 forest network (**b**) in mainland Portugal. Land suitability for corridors is shown ranging from high (dark green) to low (violet).

About 19% of the identified corridors were covered by purely coniferous forests in Portugal. Of these coniferous forest corridors, 66% were already inside the PWN affected zone, which is a larger fraction than for the purely coniferous forests in the Natura 2000 sites (49%). Furthermore, purely coniferous forest cover in the corridors falling within the PWN range is 12.7%, which is four times greater than in the Natura 2000 sites falling within the PWN range (3.3%).

The priority corridors, i.e., those that contribute most to the connectivity of the Natura 2000 forest network, followed a predominant south-north axis with some ramifications to the East and West to connect some of the key Natura 2000 forest sites near the coast and Spanish border (Figure 3b). Purely coniferous forests covered about 20% of the priority corridors, and 68% of the purely coniferous forest in the priority corridors is already in the PWN range.

The bottlenecks in the priority corridors often fell in relatively small discontinuities in the forest-dominated areas in the North of Portugal, as well as in longer corridor sectors through non-forested landscapes in the Southeast (Figure 4). About 16% of the bottlenecks were covered by purely coniferous forest (Figure 4b); these were the fragile corridors sectors in which narrow strips of coniferous forest were the only vegetation supporting the remaining corridor functionality. Of these conifer bottlenecks, 60% were already within areas currently affected by the PWN. In many of these bottlenecks, the PWN threatens the linkages of priority habitats in coastal PAs with other inner Natura 2000 sites (Figure 4b). This is the case of the coastal Natura 2000 sites of Fernão Ferro/Lagoa de Albufeira, of Arrábida/Espichel, of Dunas de Mira, Gandara e Gafanhas, and of Ría de Aveiro (Figure 4b). These sites are those currently at greatest risk of becoming isolated given that the PWN has already expanded to these areas and may disrupt the weak connections that were still maintained, in these bottlenecks, by the remaining coniferous forest cover. Apart from these sites and linkages, the corridor linking Serras d'Aire e Candeeiros with Serra da Lousã is also highly threatened (Figure 4b); the movement of species between these important PAs could be significantly hampered by the PWN. The Natura 2000 site of Litoral Norte (Figure 4b), and the bottleneck through which this site is linked to other Natura 2000 sites (Figure 4b), may also become impacted by the PWN in the next eight to 10 years, according to the spread predictions by [43], if PWN expansion range continues unabated to the north.

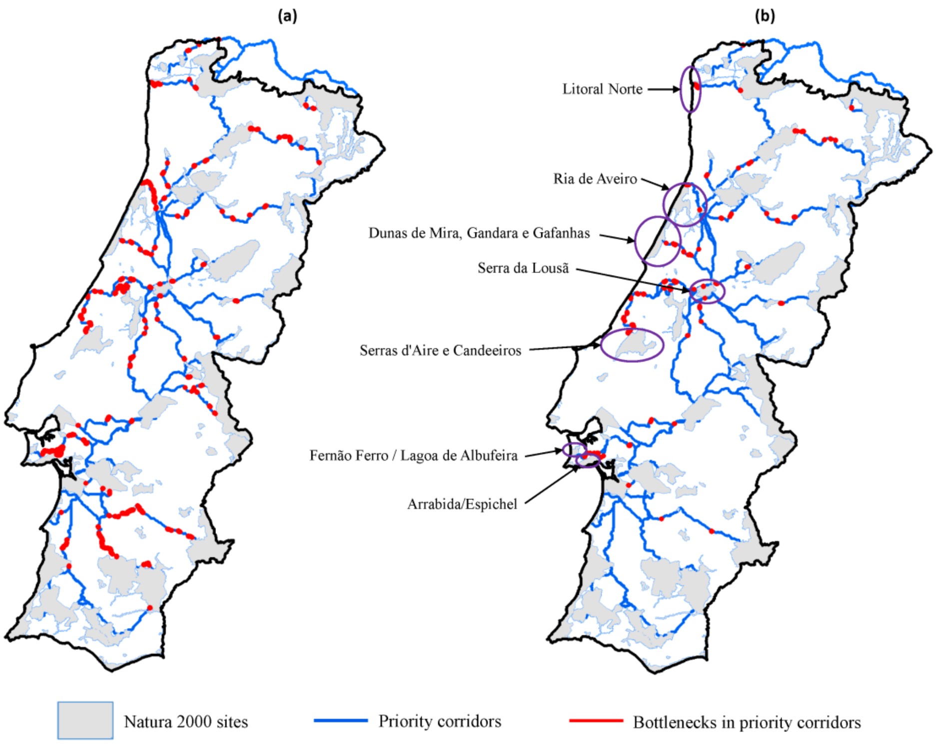

Figure 4. Bottlenecks inside priority corridors (**a**) and bottlenecks covered by purely coniferous forest, and hence more sensitive to the impacts of the pine wood nematode (PWN), in the priority corridors (**b**). The map in (**b**) specifies the name and location of some Natura 2000 sites at risk of increased isolation by PWN impacts. These sites are namely connected to other sites through linkages with bottlenecks in which purely coniferous forest exclusively ensures the remaining corridor role.

4. Discussion

Previous studies about the impacts of the pine wood nematode (PWN) have mainly focused on economic aspects [2,44,45]. In particular, the economic impact of the PWN has been estimated at 1.0 billion €/year in Europe [2] considering wood and wood production losses. Other important PWN effects are not easily quantified in economic terms and include changes in soil properties and pine forest communities [6], in landscape structure and forest succession [8], and in regeneration [16]. These effects, however, have been mostly studied outside Europe. The consequences of changes in the structure, function, and composition of the PWN invaded ecosystems and landscapes are still largely unknown in Europe. This is particularly the case for the potential impacts of the PWN, or of other damaging invasive forest pests, on the functionality of protected area networks.

With about 21% of mainland Portugal under Natura 2000 protection and a PWN outbreak that has already expanded, after almost two decades, to more than 30% of the country, the forest habitats in the Natura 2000 network might be facing new, and poorly understood, challenges. Given that the PWN is able to kill susceptible coniferous trees in a matter of weeks [15,16] and that we have found that, as of 2016, half of all Natura 2000 coniferous forests are already within the areas infected by the PWN, the impact and pressures of the PWN might become more relevant to the conservation and management of coniferous forest ecosystems and priority habitats in Portugal. High mortality rates have been reported or are expected in PWN-infected coniferous forest stands in Asia and Europe [46,47]. The oldest and biggest pines seem to be more affected by the PWN-caused mortality, as reported in the Tróia peninsula, near the city of Setúbal, in Portugal [14]. These old large trees are also those that may have a higher conservation and biodiversity value [48,49], as well as a particularly important role in the sequestration of new carbon in the forest [50]. The PWN-caused mortality in coniferous forest may, therefore, result in large shifts in forest community composition, canopy structure, and related ecological functions and processes. These shifts may be particularly significant in unique Mediterranean habitats such as the coastal and inner wooded dunes with *Pinus pinaster* and/or *Pinus pinea*, which have been included in the European lists of priority habitats for conservation (Annex I of Directive 92/43/EEC), and which we have here shown to be largely distributed in areas under the impact of PWN spread.

In certain areas, the PWN may disincentivize the planting or maintenance of tree species that are susceptible to it, which can have an additional knock-on effect on biodiversity if it indirectly promotes the planting of non-native tree species, specifically Eucalyptus. In fact, according to the results of the 6th Portugal National Forest Inventory, the area covered by Eucalyptus species in Portugal has increased by 13% between the years 1995 and 2010, in contrast with the 27% reduction in the area occupied by maritime pine (*Pinus pinaster* Ait.) in the country [24]. In 63% of the area lost by *Pinus pinaster*, no other tree species have been introduced to replace that niche, while the remaining 27% has been planted with Eucalyptus. Although these trends may be driven by multiple incentives [51] and a complex set of causes, including market demands and species productivity in each particular location, the expansion of PWN is likely to aggravate, through direct and indirect mechanisms, the decline of some pine tree species and forest habitats in Portugal.

The ability of wildlife species, and of the genes and propagules they carry, to move across heterogeneous landscapes is critical for maintaining regional population persistence and to allow native biota to shift their ranges in response to climate change [52]. The connectivity of the PA system in Portugal strongly depends on the permeability of the unprotected landscapes [13]. Here, we have shown that coniferous forests cover a large portion of the corridors that best allow forest species to traverse these unprotected landscapes in their movements between Natura 2000 sites. Furthermore, these coniferous corridors are strongly affected by the PWN range expansion, even more so than the coniferous forests within the protected Natura 2000 sites. Our findings suggest that the PWN expansion may significantly weaken the functionality of the network of Natura 2000 forest sites in Portugal, by having a particularly large effect on the more fragile and narrow parts of the priority corridors (their bottlenecks), in which the decline of pine forest cover may be most damaging to the connectivity of the

PA system. The fact that the coniferous forest in these key corridors is significantly more affected by the PWN spread than the forest within the Natura 2000 sites may be a consequence of the modalities and patterns of PWN spread in Portugal. These modalities are, on the one hand, the natural spread of the nematode by the dispersal flights of its only known vector in Europe, the longhorn beetle *Monochamus galloprovincialis* [20], and, on the other hand, the human-mediated dispersal of the disease through the accidental transportation of infected beetles in timber trade vehicles [21]. While vector beetle dispersal may operate similarly, and with the same intensity, in protected or unprotected forests, the human-mediated dispersal is strongly linked to human population and road density and to the presence of the wood processing industry [53,54]. All these anthropogenic factors may be considerably lower in the Natura 2000 sites than in the coniferous forest in the corridors outside the PA system, which may be embedded in, or closer to, landscapes with a more intense human footprint.

The magnitude of the potential impacts of PWN on the priority forest habitats, corridors, and Natura 2000 sites in Portugal indicates that mitigating their effects on the conservation status of the coniferous forest ecosystems could benefit from spatially-targeted management measures to control the pest's spread. These measures could include the intensive surveillance of coniferous forests for the early detection and removal of infected trees (before PWN-infected beetles emerge and further infect other trees in the stand), clear-cutting in localized areas to break the continuity of pine forest cover and prevent further PWN spread, mass trapping of the vector beetle, and control of the origin and sanitation of pine wood for transportation [14,55], among others. According to recent results [43], these measures may need to be applied in combination, as part of integrated containment strategies, to increase their effectiveness in halting or slowing down PWN spread. Other longer-term measures that could contribute to enhancing the resilience of the coniferous forest stands to the arrival of the disease are silvicultural treatments, such as thinning, aimed at increasing tree vigor or tree species diversity [56–58], or promoting the establishment of pine half-sib families with higher genetic tolerance to PWN [59,60]. These measures are of interest because, in more diverse natural areas, the loss or dramatic reduction in density of one species may be more easily compensated by other suitable species filling the new gaps. However, [61] noted that the changes in canopy structure and stand composition caused by forest pests may trigger subsequent impacts to the community of flora and fauna that inhabit those sites. Such impacts may not be easy to compensate even with dedicated management efforts. A compensatory response by other tree species that grow to occupy the space created by pest disturbances can reestablish some forest services such as carbon sequestration or water regulation or purification, while others could remain permanently disrupted, such as the specific biodiversity supported by the diseased tree species [62].

Some of the above-mentioned measures for controlling PWN spread, such as the felling of infected trees and, particularly, clear-cuts to break the continuity of the forest cover, may be in conflict with the aim of maintaining or promoting the connectivity of forest habitats and PAs for native biota. This may be particularly the case when the areas that might be key to containing the spread of the PWN [43] intersect with important corridors providing connectivity between PAs. The tension between these two potentially conflicting objectives may require further research to elucidate two related interactions: first, the degree to which the spread of the PWN, or of other invasive pests, may be enhanced by the corridor conservation or restoration measures aimed to promote PA connectivity; and second, the degree to which the invasion control efforts may negatively impact, or may be adapted to minimize the impacts on, the connectivity of native species of conservation concern.

Here, we have considered the potential impacts of current PWN spread on Natura 2000 sites and their connectivity, up to 2016. However, predictions of future PWN spread indicate that, in the absence of efficient control measures, the PWN range will continue to expand naturally, through the dispersal flights of the vector beetle, towards the rest of Portugal, and eventually spread into Spain in about five years [43]. In less than 10 years, it may reach the major forest and climatic corridors that provide a gateway for subsequent expansion towards the rest of the Iberian Peninsula and, in the longer term, towards other European countries [43]. Therefore, the impacts here reported on the coniferous forest Natura 2000 sites and corridors are likely to be aggravated in the next years.

Our approach and assessment could be refined, either in Portugal or in other areas that may be affected by the PWN in the future, by considering the different susceptibility to PWN infection of individual European pine tree species as reported by recent studies [63,64]. The stone pine (*Pinus pinea* L.), for example, is considerably less susceptible to PWN infection than *Pinus pinaster* [63,64]. In the Portuguese purely coniferous forests analyzed here, *Pinus pinaster* is, however, much more abundant than *Pinus pinea*: 89.5% and 9.5% cover, respectively, according to the COS map of Portugal. The magnitude and patterns of the PWN impacts on the Portuguese Natura 2000 network reported here are therefore unlikely to significantly change by a more detailed characterization of tree species susceptibility. However, between-species differences might be more relevant in specific smaller study areas. Finally, we emphasize that the data on the PWN range that we used describe the areas that are infected by PWN but not tree decline or mortality rates in each site. The latter may be addressed in further studies if such tree-level infection and mortality information becomes available at the national scale or, most likely, in some smaller areas through intense forest surveillance and inventory based on high-resolution remote sensing and/or field data.

5. Conclusions

The Natura 2000 network aims to conserve the priority species and habitats in the European Union, and the connectivity of this system of protected areas is a key objective of the biodiversity policies in Europe. Our study shows that these conservation objectives may be significantly jeopardized by the spread of an invasive tree pest, the pine wood nematode, whose range is likely to expand in the future, not only within Portugal, but also into Spain. We show that the spread of this invasive pest is impacting priority coniferous forest habitats, with poorly understood, but potentially significant, consequences for their long-term conservation status. Pine wood nematode prevalence appears particularly high in the coniferous forest corridors between the protected areas, thereby potentially disrupting the connectivity of the Natura 2000 network. Our assessment highlights the relevance of considering the impacts of the pine wood nematode and other invasive pests in the management of the Natura 2000 sites and the landscapes in which they are embedded. This would require an integrated strategy that combines actions both within and outside the protected areas. Our approach is able to highlight the specific protected areas, corridors, and portions of these corridors that are under the greatest threat from the expansion of invasive forest pests. Whether for the pine wood nematode or in other settings, these insights and approach can help to inform the measures for the eradication or control of invasive pests. Such measures could contribute to better defend the forest areas that are most vulnerable and have the highest conservation value either as priority habitats or as key connectivity providers for protected area systems.

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