

Article

Invasion at the Edge: The Case of *Rosa rugosa* (Rosaceae) in Italy

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Abstract: The Japanese Rose (*Rosa rugosa*) is a perennial shrub belonging to the family Rosaceae. It was introduced in Europe from East Asia as an ornamental plant in the XIX century and is now considered an invasive species, especially in northern Europe, colonising the Atlantic and Baltic coastal dune habitats and threatening local biodiversity. However, little is known about its presence and invasion patterns in the Mediterranean area. In Italy, *R. rugosa* has been classified as naturalised and just a few observations have been recorded in dune habitats in the North Adriatic coast. Here, we review the published data on *R. rugosa* in Europe and present preliminary data on the invasive pattern of *R. rugosa* on the Italian North Adriatic coast. We surveyed the coastline in two locations (i.e., Brussa and Bibione, Italy) where we characterised the dimension and structure (i.e., number of ramets and stem height) of the *R. rugosa* populations and listed the associated floristic composition. No occurrence of *R. rugosa* was recorded in Bibione, probably due to the success of the restoration project carried out on that site. In contrast, several stands of *R. rugosa* were found in Brussa, where many other alien species were also found (accounting for 15.28% of the sampled species). Given the strong invasiveness of *R. rugosa*, it is important to keep data on its distribution up-to-date and investigate its ecology and physiology to promote appropriate management strategies to control its spread and anticipate its future potential distribution.

Keywords: genetic diversity; invasive alien species; Japanese Rose population; psammophytes; priority habitat; sand dunes



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1. Introduction

Biological invasions are among the major drivers of global changes and are commonly recognised as a threat to biodiversity [1]. Anthropogenic activities, which include any social, economic or institutional action that deliberately or accidentally triggers the introduction of invasive alien species (IAS [2]), are considered the main driver of invasion processes. In recent years, the globalisation of trade and travel has drastically increased the spread of IAS [3], and climate change further influences the transport, establishment and spread of alien species [4]. Alien species may have negative impacts spanning from native resident communities up to habitats, landscape, ecosystem production, naturalistic value and human health. The ecological impacts of alien plants may concern the characteristics of the community and ecosystem processes [5]. Invasive alien plant species are often characterised by peculiar functional strategies and traits, such as a fast growth rate and deep roots [6,7], which aid in the invasion of new ecosystems and out-compete native resident species [8]. Since species traits may influence ecosystem processes [9,10], IAS may alter soil properties and biogeochemical cycles [11] and other abiotic conditions that typically impact the growth of both native species and IAS in invaded areas.

Coastal habitats are among the most invaded regions worldwide [12]: here, high propagule pressure, along with local abiotic conditions and anthropogenic factors, are the main factors leading to high levels of invasion [13–15]. Increasing urban sprawl and littoralisation on coastal areas squeeze dune ecosystems between coastal erosion and the expanding human pressure. In the last 50 years, those habitats have been increasingly disturbed and threatened by several hazards, depleting biodiversity and transforming the structure of resident communities [16–19]. This situation brings questions regarding the appropriate management to preserve the remnants of coastal dune ecosystems [20] and the need to focus on the role of IAS, considering that recent European regulations (EU Regulation 1143/2014 on Invasive Alien Species) provide legal instruments for IAS control and eradication. Especially in sand dune ecosystems, IAS alter the structure of native plant communities in different ways, such as reducing functional, phylogenetic and taxonomic diversities and excluding closely related and ecologically similar taxa [14,15,21–23]. Based on a recent study by Giulio et al. [24], alien species constitute about 7% of the flora of European coastal dunes and many of them are naturalised at the country level. The vast majority of the neophytes (i.e., species introduced after 1500 CE) on European dunes are generalist and ruderal species, originating from North America and the Mediterranean-Turanian region. *Erigeron canadensis*, *Xanthium orientale*, *Oenothera biennis* and *Oenothera ookesiana* are the most common aliens across the European sand coasts. Some aliens are of particular concern for all European-introduced flora due to the recognised transformation they induce in the habitat structure (i.e., transformers). For instance, *Baccharis halimifolia* is currently listed in the Consolidated list of IAS of Union Concern (EU Regulation 1262/2019), due to its dense thickets that suppress the native dune vegetation of Atlantic coastal dunes. Similarly, *Carpobrotus edulis* and *Carpobrotus aciniformis* threaten native plants and the species diversity of the Mediterranean basin.

Another spotlighted species is *Rosa rugosa*, a transformer forming dense stands with a high impact on native vegetation [25,26]. The effect of *R. rugosa* on the Atlantic and Baltic coastal dune grasslands is well known, whereas little has been reported about its presence and invasion pattern in the Mediterranean basin to date. Thus, we (1) review and summarise the existing literature and data on the invasion process of *R. rugosa* in Europe and (2) present preliminary data on its invasive pattern in North-East Italy, where the species has now been established, and its expansion is concerning.

2. Materials and Methods

2.1. Survey Method for the Literature Review

The published literature on *R. rugosa* as an invasive plant was sourced from scientific databases such as Google Scholar, Web of Science and Scopus (accessed on 10 October 2021). The following queries were created, using a combination of keywords and logic operators (e.g., AND/OR) as follows, together with the species name *Rosa rugosa*: alien, invasive, exotic, introduced, species, plant, biological, invasion, introduction, distribution, range, Europe, Italy, ecological, impacts, sand dunes, coastal area, dune ecosystem, genetics, DNA, mycorrhizae, fungi. The results of the international literature search were integrated using grey literature (e.g., local books and magazines of the local museums of natural history) to obtain exhaustive information about the distribution of *R. rugosa* in Italy.

2.2. Collection of New Data on *R. rugosa* in Italy: Study Area

R. rugosa was censused on sand dunes in two sampling sites: Brussa (Caorle, 45°37′07″ N, 12°56′10″ E; Data collection) and Bibione (45°37′36″ N, 12°59′30″ E), both located in north-east Italy. These sites host some of the few remnants of natural sand dune vegetation in N. Adriatic coastline [23], belonging to two Natura 2000 Special Protection Areas (“Valle Vecchia-Zumelle—Valli di Bibione”, IT 3250041; “Foce Tagliamento”, IT3250040) given the presence of species of conservation concern. From 2017 to 2021, this area underwent the actions of a European LIFE project named “LIFE REDUNE”, aiming to restore and maintain the ecological integrity of dune habitats and *Stipa veneta* populations in Natura 2000 sites

present along the Adriatic coast (LIFE16 NAT/IT/000589, www.liferedune.it (accessed on 1 September 2021)). The habitat restoration process was facilitated by the planting of native plants and the removal of invasive alien species, such as *Oenothera stucchii* and *R. rugosa*.

The climate of the area is temperate-subcontinental [27], mean annual temperature ranges between 10 and 14 °C, without a dry phase, and mean annual precipitation is about 828 mm. The Brussa site consists of a sandy shoreline, east-to-west oriented, located between the Adriatic Sea and the Caorle lagoon behind. It includes a large non-urbanised area, allowing for the preservation of natural patches with plant species of great conservation value. The alteration of dune vegetation is now a matter of concern, especially through treading and the expansion of tourist and residential settlements. The site hosts several animal and plant species included in the Annex I of the Birds Directive (Directive 2009/147/EC) and in the Annex II of the Habitat Directive (Directive 92/43/EEC). The Bibione site is completely surrounded by urbanised and touristic areas. This place is famous for its sandy beaches and many outdoor activities and aquatic sports that are possible to practice (e.g., walking, cycling, windsurfing, and kayaking). Here, trampling and human activities associated with beach management may be the primary cause of the reduction in or loss of specific habitat features. The sampling area of Bibione is delimited on the east side by the Tagliamento River and the west side by the town of Bibione.

2.3. Data Collection and Sampling Design

We performed a detailed field assessment of *R. rugosa* populations in the Italian invasion range, aiming to provide useful information to anticipate/prevent the potential spread of the species.

In July 2021, the entire 5 km coastline of the Brussa and 3 km of the coastline from the lagoon to the Bibione Lighthouse were surveyed. The census was run by walking along the whole beach width and inspecting the vegetation from the yellow dune, across the grey dune, to the brown dune. Three expert surveyors were involved, for many days, in inspecting the areas. After spotting *R. rugosa* stands, we georeferenced them using a GPS and, if the stand included more than 10 ramets, we sampled it using a 10 m × 10 m plot. The plot was always North-oriented and placed in such a way to include the largest number of individuals. In the 10 × 10 plot, coordinates were recorded on the South-East corner. From that corner, we also measured the distance to the sea (m) and the width of vegetation belt (m) in between the plot and the sea. We further divided the 10 m × 10 m plot in 25 squared subplots (2 m × 2 m) in which we counted the number of ramets of *R. rugosa* and measured the height of each stem. The percentage of *R. rugosa* coverage was visually estimated for each subplot. At the 10 m × 10 m plot scale, all plant species were recorded, along with their percentage of coverage. We also evaluated the total cover of trees, shrubs, herbaceous plants, litter, and bare soil at the plot scale. When plant identification was not possible in the field, we collected one specimen and identified it later using an identification key [28,29]. Nomenclature was standardised according to Pignatti et al. [29]. Plants were classified as native or alien species according to Celesti-Grappo et al. [30] and Galasso et al. [31].

2.4. Data Analysis

Based on the dune zone and the microtopography, surveyed plots were classified into three categories: (1) dune slack, (2) grey dune and transition to woodland, (3) yellow dune. In addition, the number of natives, aliens, *R. rugosa* ramets, cover and mean height were graphically compared using boxplots and significant differences among dune zones were evaluated using the Kruskal–Wallis test.

The relationships between *R. rugosa* population and other features of the sampling area were calculated using non-parametric correlation analysis (Spearman's ρ). Coefficients were tested for significance using the "corrplot" package [32] and R version 4.0 [33].

Finally, to characterise plant communities invaded by *R. rugosa*, plot-to-plot compositional dissimilarity was calculated using the Bray–Curtis index, and the resulting

dissimilarity matrix was analysed using non-metric multidimensional scaling (NMDS). We chose an NMDS because it retains as much information as possible about floristic relationships among sites, including nonlinear patterns [34]. The ordination diagram was arranged in relation to the distance from the sea using the *ordisurf* function in the “vegan” R package [35].

3. Results

3.1. From the Far East to West: The Establishment of *Rosa rugosa* in Europe

Rosa rugosa Thunb. (Figure 1a), also known as Japanese Rose or Rugosa Rose, is a perennial sprouting shrub belonging to the Rosaceae family, native to temperate and cold coastal areas of East Asia [36]. The species native range encompasses northern Japan (Hokkaido and Honshu), the Korean Peninsula, north-east China, and the Russian Far East (Kamchatka, Sakhalin, Kuriles, Khabarovsk, and Primorye region [37]). Its native range is characterised by a temperate humid climate with hot/warm summer [27]. *R. rugosa* can be found in stabilised dunes, as well as rocky shores and species-rich meadows, always near the coast and at low elevations [37]. It often occurs on sandy or gravelly soils, while it seems to avoid highly acid soils: in Japan, natural stands of *R. rugosa* grow in topsoil with a pH varying from 5.1 to 7.6, whereas the deeper layers (45–50 cm below the surface) range between 5.4 and 8.5 [37]. *R. rugosa* often forms dense stands due to its rhizomes from which suckers arise, probably originating from several coalescent clones. Thickets of *R. rugosa* are predominantly found in dune scrub, dune grassland communities and shingle beaches, and less frequently in tall-herb meadows [37]. In its native range, instead, the species is only one component of a species-rich shrub zone between open dune grassland and landward dune forest [36]. It is even listed as an endangered plant in China, due to the rapid decline in populations in the past three decades [37]. Similarly, in Japan the species is classified as “vulnerable” in the “Red data book” of Tottori Prefecture [38]. This is mainly due to anthropogenic activities in the area, such as increasing industrial development, tourism, aquaculture, which are compromising the endemic habitats of the *R. rugosa*, resulting in a decreased availability of suitable habitats.

Due to its ornamental importance, *R. rugosa* has been traded and imported in many countries around the world and, where the conditions were favourable, it was established in nature. To date, the species has become naturalised in New Zealand [39] and in North America, in the latter the species is widely distributed especially in Nova Scotia, Canada [40].

In Europe, *R. rugosa* is reported as an invasive garden-escape around the Baltic Sea and the North Sea, particularly in Belgium, Denmark, Estonia, Finland, Germany, Latvia, Lithuania, the Netherlands, the United Kingdom, Norway, and Sweden [37,41,42]. In addition to the above-mentioned countries, *R. rugosa* is known to be present in Austria, Belarus, Bulgaria, Czech Republic, France, Ireland, Moldova, Poland, Russia (European), Slovakia, Slovenia, and Ukraine [37,42,43]. The first introduction event from Japan to Europe dates back to 1796 (the record is published in Lægaard [44] in Danish), but the country of introduction is unknown. After 1845, the species became more abundant due to a growing interest in its ornamental use [36], and the first records of naturalised *R. rugosa* were from Germany in 1845 [45] and Denmark in 1875 [46,47]. Other naturalisation events took place at the same time as introduction; for instance, in Norway, the species arrived spontaneously along the coast of inhabited islets, probably as a result of long-distance dispersal by sea currents [37].

In the native distribution area, *R. rugosa* occurs in coastal scrub communities. For example, in Japanese coastal dunes, *R. rugosa* grows in a narrow zone between the first dune ridge and older dunes, together with other dwarf shrubs such as *Juniperus conferta*. In Japan the position of *R. rugosa* shrubland in the dune series is probably comparable with the *Hippophae rhamnoides* shrubland in NW Europe [48]. Regarding both vegetation structure and taxonomic composition, the Japanese dune scrub is highly similar to European dune scrub communities [37].



Figure 1. *Rosa rugosa* flower and leaves on an adult individual (a); large fleshy rose hip bitten by animals (small mammals probably) (b); characteristic *R. rugosa* dense prickles on a juvenile ramet (c). *R. rugosa* root stolon from where a ramet generates, developed just below the leaf litter of the Pine (d); observed invasion stage in Brussa (g) in comparison to different invasion stages from North Germany, characterised by different aged individuals, on coastal sand dunes (e,f,h). (a–d,g) photos were taken in the Brussa sampling site in summer 2021; (e,f,h) photos were taken in Rostock, Baltic Sea, North Germany, in summer 2020. Photos were taken by the authors G.B. and L.M.

As described by Bruun [37], *R. rugosa*, in its new range, builds dominant stands in various plant communities (e.g., Figure 1e–h): on the coasts of the North Sea, *R. rugosa* is predominantly found in yellow dune vegetation dominated by *Ammophila arenaria*, and, to a lesser extent, by *Elymus arenarius*. *R. rugosa* is mostly confined to dunes with moderate sand accumulation, where *Carex repens* and *Corynephorus canescens* also occur. More specifically, in Germany, *R. rugosa* occurs from yellow dunes with *Ammophila arenaria*, across short grasslands of grey dunes (e.g., with *Phleum arenarium* and *Corynephorus canescens*) and shrublands of *Hippophae rhamnoides* to brown dunes with heathlands of *Empetrum nigrum* and *Calluna vulgaris* [48]. On the coasts of the Baltic Sea, *R. rugosa* is found in similar communities, i.e., dune vegetation dominated by *Ammophila arenaria*, *Ammocalamagrostis baltica*, *Elymus arenarius* and *Carex repens*, and with subordinate species such as *Artemisia campestris*, *Corynephorus canescens*, *Eryngium maritimum*, *Festuca polesica*, *Festuca rubra* ssp. *arenaria*, *Hieracium umbellatum*, *Lathyrus japonicus* ssp. *maritimus* and *Phleum arenarium* [37].

3.2. Functional Characteristics of *Rosa rugosa* in Invaded and Native Ranges

Plant functional traits are usually related, to a certain extent, to species niches and it is assumed that strong invasiveness can occur if the invader has large functional trait differences (niche differences hypothesis, [15,49]). In the native range, trait (niche) overlap might be higher than in the invaded range because, in the native range, the invader and the co-occurring species evolved under similar environmental constraints. This might reduce the invader's fitness through a stronger size-symmetric competition in the native range if compared to the invaded one. This is expected to be particularly common for invaders originating from resource-poor environments, where more severe environmental filtering might increase trait overlap among co-occurring species. Concerning *R. rugosa*, Zhang et al. [50] showed that the invasive population in Germany outperformed the native population in China in terms of reproductive traits (i.e., seed production) and growth traits (i.e., shrub size), but some introduced populations appeared to be more successful invaders than others. The study by Helsen et al. [51] also pointed out that *R. rugosa* had limited trait overlap with the invaded plant community where it is invasive: on average, it contributed to the functional space of the community introducing highly conservative leaf economic traits and strong light-competition related traits. A more recent study by Helsen et al. [52] provided a comparison based on five functional traits related to the leaf economics spectrum (specific leaf area (SLA), leaf dry matter content (LDMC), leaf nitrogen content (N), phosphorous content (P), potassium content (K)) and two traits related to light-competition ability (plant height and leaf area) between individuals of *R. rugosa* collected in Belgium (invaded range) and those surveyed in Japan (native range). It was observed that the species in Belgium had a higher plant height and lower leaf P and K than those growing in Japan (all other traits showed no differences between Belgium and Japan). Furthermore, if compared to the hosting native plant communities, *R. rugosa* in Japan had higher leaf area and LDMC, lower SLA and leaf K, and it had lower leaf N than the *Rosa* plot community in Belgium, where it had higher leaf P than the *Rosa* plot community in Japan. Thus, *R. rugosa* showed traits conferring a higher competitive ability in its invaded range than its native range, such as a higher plant height [53] and more resource-conserving leaf traits (leaf P and K) [54]. In synthesis, many observations support the idea that *R. rugosa* has much lower functional overlap with the co-occurring species in the invaded range, and this pattern very likely enhances its invasiveness [52].

3.3. Genetic Characterisation of *R. rugosa*

The ability of *R. rugosa* to adapt to many environmental conditions (e.g., salinity and alkaline soils, shade, low temperatures, drought, and high humidity) and to produce a wide spectrum of secondary metabolites that can be industrially exploited makes it an ideal organism for gene mining and molecular breeding to create novel varieties [55]. Indeed, a high-quality genome sequence for *R. rugosa* both enables comparative genomic studies of *Rosa* species and reveals the mechanisms underlying its ornamental traits, such as floral biology and its unique ecology and distribution.

3.3.1. The Genome of *R. rugosa*

The chloroplast and the mitochondrial genomes of *R. rugosa* were sequenced and characterised by Jiang et al. [56], Kim et al. [57] and Park et al. [58], while the whole chromosome-level genome (whole genome sequencing, WGS) was assembled and published by Chen et al. only very recently [55]. The high-quality genome sequence that was obtained was produced thanks to the combination of sequencing technologies (HiFi sequencing and Hi-C scaffolding), while the genomic structural differences could be compared with the genome data already available for the sister species *R. chinensis* [55]. The genome size predicted for *R. rugosa* is 454.78 Mb, and genome contigs were assembled in seven chromosomes. This genome is longer than that of *R. chinensis* but the GC content does not significantly differ between the two species (39.30% GC for *R. rugosa* and 38.84% for *R. chinensis*). The *R. rugosa* genome was composed of 50.27% repetitive sequences; most

of these (26.75%) are long terminal repeats (LTRs), with a slightly lower proportion in *R. rugosa* than in *R. chinensis* (28.3%; [59]), suggesting a rapid evolution of LTRs in Rosaceae plants. The *R. rugosa* genome encoded 39,704 protein-coding genes, close to the number in *R. chinensis* [55,59]. A total of 53 terpene synthases (TPSs), which are key genes responsible for terpene biosynthesis, were annotated and, by pathway enrichment of all *R. rugosa* genes, 850 genes were identified to be involved in environmental adaptation, including those related to salt stress and water stress tolerance [55]. Furthermore, the WGS revealed the genetics responsible for floral biology, and flowers were used to investigate the regulation of gene expression by small RNA, such as miRNA and siRNA [60]. The availability of a chromosome-level resolution genome allows access to high-resolution analyses to study the genomic association of phenotypic traits related to the invasiveness of this species.

3.3.2. Genetic Diversity of *R. rugosa* from Native to Invaded Areas

The genetic diversity of *R. rugosa* was studied at the population level using microsatellite markers to uncover the patterns underlying its expansion in Europe [26]. The authors aimed to understand whether the non-native European populations of *R. rugosa* genetically depauperated relative to the native East Asian populations (as expected after a recent founder event), and became geographically structured, and if specific East Asian sources of the European populations could be identified. The chloroplast DNA trnL-F region was selected because it is known to show intraspecific haplotypic variation in several species of Rosaceae [61,62]. Leaf material was sampled from a total of 32 populations, 16 from Europe and 16 from East Asia, with an average sample size of 18 and 19 individuals, respectively. The study revealed that *R. rugosa*, in its exotic range, had similar within-population genetic variation as populations in the native range. The between-population differentiation was smaller because the species has spread in Europe from multiple original introductions from native sources in East Asia [26]. At least three independent colonisation events, one of which was particularly successful, were supposed to be at the origin of the current *R. rugosa* populations in Europe. Kelager et al. [26] found that the northern region of Japan was the origin of three spatially disconnected European populations, while they could not unambiguously identify the origin of the remaining analysed exotic populations, likely due to insufficient sampling in the native range [26]. The results suggested that high propagule pressure at the primary establishment phase explained the low genetic diversity that was found. Long-distance seed dispersal, coastal habitat connectivity and an outcrossing breeding system seemed to be the key elements that enabled populations of *R. rugosa* to avoid the detrimental effects of genetic bottlenecks and will further increase the species' range size and abundance in Europe [26].

Interestingly, *R. rugosa*'s secondary non-admixing colonisation was facilitated by human dispersal, with this providing a further example of invasive species (as particularly reported for plants), where successful establishment in the introduced range is increased by human transportation [26,63,64].

3.4. Mycorrhizal Association in *R. rugosa*

Plant–fungal associations, i.e., mycorrhiza symbioses, are key drivers of the establishment and success of plants in their environment [65]. Mycorrhizal fungi are ubiquitous symbionts on plant roots, with ectomycorrhizal (ECM) fungi being the dominant group in temperate forest ecosystems, and arbuscular mycorrhizal fungi (AMF) being the most widespread and host-specific group [66]. Mycorrhizal fungi are known to increase carbohydrate flow, nutrient cycling, water uptake, contribute to the aggregation and stabilisation of soil particles, and enhance plant tolerance to salinity, herbivory, root pathogens and toxic heavy metals [66]. In alien plants, research has shown that mycorrhiza symbioses play an important role in the colonisation of exotic habitats, but it is still unclear whether they represent key factors explaining plant invasiveness [67]. Plant IAS usually lack mycorrhizal symbionts upon arrival to a new area. This may lead to a failure in establishment due to mutualist limitation; however, this can be avoided by reduced dependence on the fungal

symbiont(s) and the establishment of a novel mycorrhizal association [67]. In the latter case, the plant has a low specificity for the available fungi in the habitat and can easily find a compatible fungal symbiont. The type of new plant–fungus interaction will be decisive in the colonisation success of the alien species. In the case of low specificity towards the mycorrhizal fungus, the alien species will associate with different fungi according to their availability in the exotic habitats [67,68]. This will lead to between-habitat differences in the mycorrhizal fungal communities associated with that alien plant species (context-dependency). Though, diverse mycorrhizal association with the same alien species may lead to differences in the successful establishment and spread of the alien plant depending on the new habitat context [68].

Moyano et al. [69] tested the relationship between plant naturalisation success and their mycorrhizal associations, considering arbuscular, ectomycorrhizal, ericoid and orchid mycorrhizal plants. They analysed a database composed of mycorrhizal status and type for 1981 species, covering 155 families and 822 genera of plants from Europe and Asia, and matched it with the most comprehensive database of naturalised alien species across the world (GloNAF [70]). The authors found that the number of naturalised regions was the highest for facultative mycorrhizal (i.e., plants with low mycorrhiza specificity), followed by obligate mycorrhizal and the lowest for non-mycorrhizal plants, providing evidence that the ability to form mycorrhizas is an advantage for the introduced plants. Furthermore, their analysis showed that the naturalisation success (based on the number of naturalised regions) was highest for arbuscular mycorrhizal and dual mycorrhizal plants (associated with both an ECM and AMF).

In sand dune habitats, plant species' ability to successfully colonise these sites seems to depend on the presence of AMF in the soil [71]. Gemma and Koske [71] showed that AMF were routinely present in the planting materials prior to outplanting in the stock of several species of plants used to vegetate barren sand dunes and dune flats of the eastern seaboard of the U.S.A. The authors reported AMF occurring in the planting stock of seven varieties of *Ammophila breviligulata*, *Prunus maritima*, *Rosa rugosa*, and *Spartina patens*, and the early presence of AMF in dune soil allowed a network of ECM fungal hyphae to develop sooner [71]. The fungal network, once formed, functioned as a trigger for seedlings of the invading species *P. maritima* and *R. rugosa*, that later appeared to be dependent on mycorrhizae. These observations led to speculation that plant succession may be regulated by the original presence or absence of AMF at a site [71].

Knowledge of the mycorrhizal associations for *R. rugosa* in Europe is still poor and only one study characterised the fungal communities associated with its fine living roots in plants grown on the frontal dunes of Curonian Spit at the Baltic Sea coast in Lithuania [72]. The roots of *R. rugosa* were sampled at five sites and the sequencing of fungal ITS rRNA resulted in 134 high-quality sequences, representing 31 fungal taxa, among which saprotrophs and endophytes were identified. The most common fungi were *Mycena* sp., *Tumularia* sp., *Penicillium spinulosum* and *Cadophora malorum*. The AMF *Entrophospora baltica* and *Rhizophagus irregularis*, while potentially root-pathogenic fungi, such as *Ceratobasidium* sp., *Fusarium oxysporum*, *F. culmorum* and *Ilyonectria crassa* were detected at low proportions. Interestingly *Entrophospora baltica* was previously described in Poland in the soil below European beachgrass (*Ammophila arenaria*) [73], which is a species commonly present in the habitat where *R. rugosa* grows. These results provide further support to the previously mentioned importance of AMF in the successful colonisation and naturalisation of an IAS, such as *R. rugosa*. The detection of AMF in *R. rugosa* roots indicated that these might be important for the mineral nutrition of the plant established on dry and poor fertility coastal dunes. Instead, the detection of potentially pathogenic fungi is explained by their potential facultative parasitic and/or endophytic lifestyle ([72] and references therein). It is likely that, in association with *R. rugosa*, their pathogenic behaviour changes, as was reported for *Fusarium culmorum* associating with the coastal dune grass (*Leymus mollis*), where it was a non-pathogenic symbiont, which likely increased both the salt- and drought-tolerance of the host [74].

3.5. Impacts on Ecosystem Services

3.5.1. Habitat and Native Resident Communities

R. rugosa invasions are related to considerable changes in the soil environment of coastal dunes, resulting in increased concentration of nutrients such as C, N and P and modified microbial community structure [52,75–77]. The increase in concentrations of some elements in soils under *R. rugosa* is presumed to be associated with differences in functional traits between the invader and native vegetation [53]. Soils with low organic matter content and sparse vegetation cover (i.e., coastal sand dunes), which are invaded by plants with a high biomass, such as *R. rugosa*, may accumulate C through higher litter returns and the increased N stock in the topsoil can directly result from the higher C inputs into soil, which provide more carbohydrates for N fixation by soil microbes. An increased concentration of P may occur through nutrient uplift, given that the *R. rugosa* rooting depth is greater than that of the sand dune's native vegetation [78]. Despite the higher nutrient concentrations in the soil underneath *R. rugosa*, total and bacterial biomass seems to decrease (especially saprotrophic fungi biomass), which might result from high concentrations of phenolics in the senescing tissues of the invader. Indeed, phenolic compounds are secondary metabolites exhibiting significant antimicrobial activity against saprotrophic or phytopathogenic fungi [77].

Another major alteration caused by *R. rugosa* regards the light availability beneath its dense thickets. In general, relative irradiance decreases with an increasing cover of *R. rugosa* in the invaded habitat because broad-leaved shrubs, such as *R. rugosa*, offer more shade than other native herbaceous species, resulting in a decline in light transmission below the shrub [75,76]. This is particularly noticeable for those light-demanding species, such as herbaceous and typical grassland species [25]. In addition, *R. rugosa* builds up a large dominant shrubland in which only a few gaps are available for the recruitment and germination of any species. Most of the species which manage to grow in the shrubland dominated by *R. rugosa* are neophyte. This is probably related to the higher nutrient content, which supports the growth of other neophytes, resulting in a self-preventing system of positive feedback circles [25].

Together, the alterations in soil nutrient concentrations, microbial community, and light availability seem to have severe consequences for the structure and functioning of coastal dune ecosystems, leading to a negative effect on both species' composition and species richness in the invaded range.

Different levels of impact of *R. rugosa* invasion have been described on arthropod fauna. Specifically, invaded dunes in Danish North Sea coasts displayed a shift in community composition with respect to the native dune vegetation [79], especially in relation to the abundance of distinct functional groups. Specifically, flower-visiting insects (*Hymenoptera* (excluding ants), *Diptera* families), generalist herbivores (*Lepidoptera* larvae), and some detritivores (*Diptera* families) increased in abundance, and one species-rich predatory group (spiders) showed a reduced species richness due to a decreased abundance of diurnal xerotherm species. In addition, Toft [80] showed that, after *R. rugosa* removal, habitat specialist spiders, and particularly those species with a narrower habitat occurrence and stronger preference for the openness of the habitat, as well as the proportion of xerothermic individuals, consequently increased in richness. No specific changes in richness, abundance and composition were observed for large detritivores, (i.e., terrestrial isopods and millipedes) [79], and for ants [81] between invaded vs. not-invaded *R. rugosa* stands.

3.5.2. Commercial Implication in Relation to Cultural Ecosystem Services

In Asia, *R. rugosa* is economically important in many fields: petals are a source of rose essential oils, which are used in perfumes, cosmetics, aromatherapy and spices; petals and flower buds are commonly consumed for the production of jams, teas, pies, juices and beverages; hips and roots have been widely applied in China, Japan and Korea as an agent for the treatment of many diseases and research into many biologically active compounds, mainly secondary metabolites, pointed out their potential as natural antiradical, antitumor

and antioxidative properties [82]. In Europe, *R. rugosa* economic values mainly rely on its ornamental use due to its large and attractive flowers. On the other hand, the species may have adverse effects on outdoor recreation and seaside tourism due to its thorny and impenetrable shrubs, making it difficult to access the beach and seashore [36].

3.5.3. Human Health

No negative effects on human health have been recorded, except for allergies towards the pollen.

3.6. Management and Control

3.6.1. Prevention

To prevent the expansion of *R. rugosa* on the coastal area, it is important to raise awareness of the danger caused by its introduction into natural habitats [36]. The Danish Forest and Natura Agency has produced a folder on the invasive *R. rugosa* and warns against planting this rose along with the coastal site, suggesting a range of indigenous species as an alternative [83]. In Norway, the Directorate for Nature Management in 2008 issued a general information letter recommending suspending the cultivation, import, sale, and further use of a number of selected invasive alien plants, *R. rugosa* included [36].

3.6.2. Eradication

The techniques used to control *R. rugosa* have been digging, cutting, grazing, or using herbicides, but once the control measure has been initiated, there is a need for continued dedication to ensuring successful eradication. The Invasive Alien Species Fact Sheet of *R. rugosa* Weidema [36] listed several eradication methods. The most efficient way of removing the species is to dig it up, but all rhizomes and roots must be removed to avoid re-suckering. However, complete removal of the plant from large dune areas may lead to sand drift, since no other vegetation will be left to cover the sand. Digging up the plants can also be combined with the application of herbicides (such as Glyphosate) if local conditions and legislation permit this approach. The herbicide should be applied specifically and only to *R. rugosa*. This can be achieved with some kind of “weeper” (a device with one or more wicks). The important issue is to avoid affecting other plant species; therefore, education of the technical staff is necessary. Grazing may be preventive during the seed establishment phase, but a well-established stand of *R. rugosa* cannot be eradicated by grazing animals, which can also cause other flora elements to be lost, adversely altering the plant community. It has also been shown that many insects and fungal species present in both its native and introduced range could be used as biological control agents [84], but this practice must be used with caution to avoid unintended negative consequences [85].

3.7. *Rosa rugosa* Establishment in Italy: Quantitative Characterisation of Its Populations on North-Eastern Italian Coasts

In Italy, *R. rugosa* has been classified as naturalised by Galasso et al. [31], and occurs only in few regions, as described by the Portal to the Flora of Italy [86]: Emilia-Romagna (casual), Lombardy (casual), Sicily (casual), Trentino-Alto Adige (casual), Veneto (naturalised). In Emilia-Romagna, no exact localisation for its occurrence is available, although it was described as present by Celesti-Grapow et al. [87]. In Lombardy, it has been observed in the province of Milan, along the Scolmatore Canal [88]. Two distinct records are from Trentino Alto Adige, one in Ziano di Fiemme (TN) and the other in Braies (BZ; Wikiplantbase Italia [89]).

Many of the above-mentioned records were found in landlocked regions, probably in urbanised area, adding little information about the invasiveness of the species.

Focusing on the Italian coast (Figure 2b), *R. rugosa* has been spotted in Veneto, more specifically on the dunes of Valle Vecchia near the city of Caorle (VE), on the dunes of Ca' Savio (VE) and on the dunes of Scano Cavallari (RO; [90]) and in Sicily on Pantelleria island (TP; [91]). To date, no other published data revealed the presence of *R. rugosa* in

Italy, where patches of coastal dunes can still be found, and none of the available records offer details of the observed populations, their status and extension. This knowledge gap clearly needs to be filled to plan the correct management and monitoring actions to control the spread of *R. rugosa* in the Mediterranean basin.

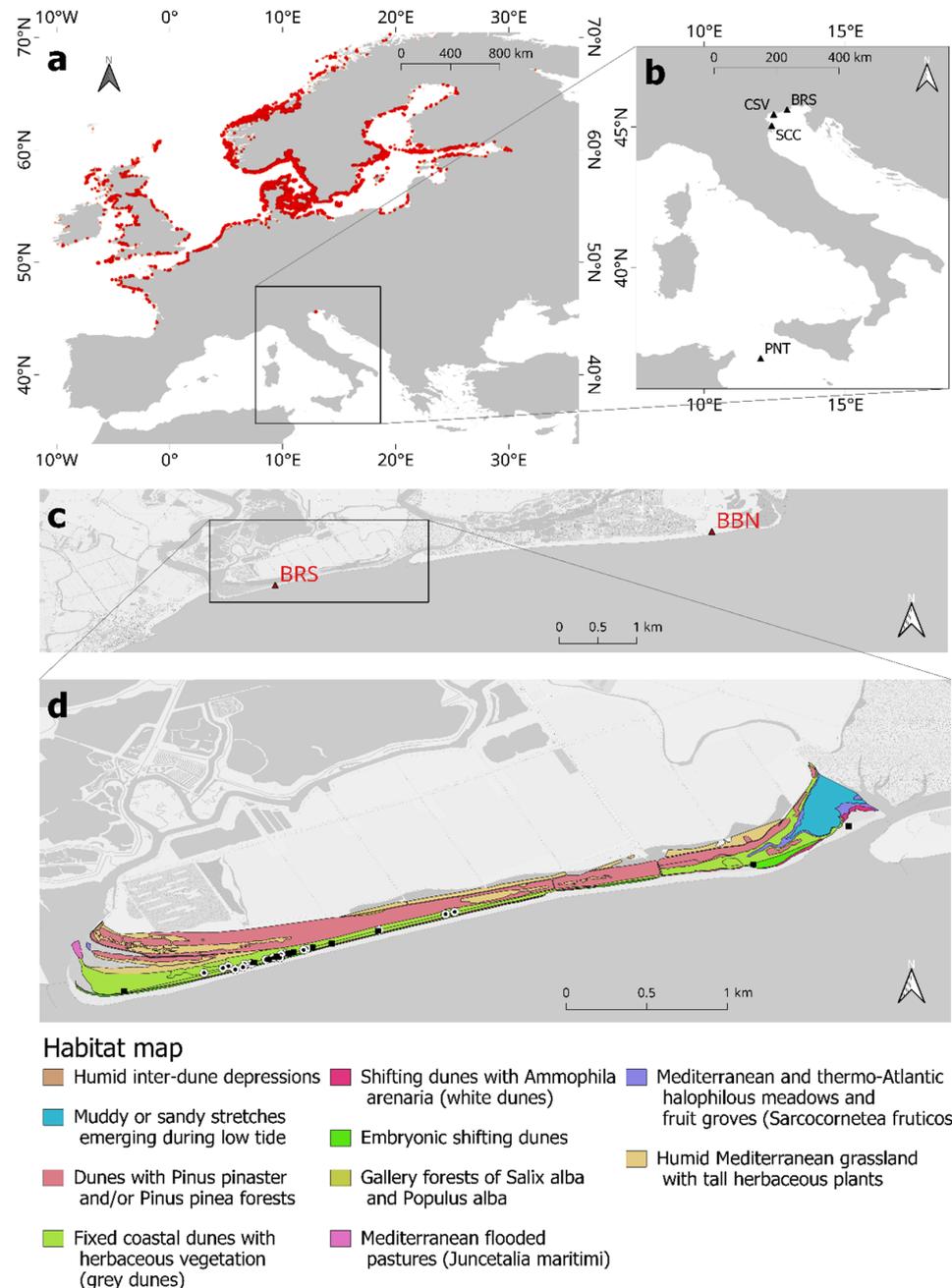


Figure 2. Distribution of *Rosa rugosa* on European coastlines (a), with details of occurrence in Italy (b) and in the sampling sites (c,d). In (a), the heatmap represents the distribution of *R. rugosa* on European coastline obtained from GBIF [42] occurrences, which were filtered using a buffer area of 1 km from the coastline, dark red colours indicate high abundance of *R. rugosa* records. (b) Black triangles represent *R. rugosa* observations on Italian coastal areas: PNT = Pantelleria island; CSV = dunes of Ca’ Savio; SCC = dunes of Scano Cavallari; BRS = dunes of Brussa. In (c), the two sampling sites BRS = Brussa and BBN = Bibione. In (d) habitat map of BRS, sampled plots with populations (squares) and scattered individuals (black-and-white circles) of *R. rugosa*.

R. rugosa was observed in its north-eastern Mediterranean range on sand dunes on the Adriatic coast near Caorle (Veneto Region). No other occurrences have been retrieved for the Italian coastline based on the international and national literature and database search. The species seems to also be absent in the Slovenian and Croatian (Istrian) coasts (Barbara Sladonja, pers. comm.), suggesting that it was not yet able to overpass the Caorle Lagoon separating Brussa from Bibione (Figure 2c). In the Bibione site, we found no evidence of *R. rugosa*, whereas, in the Brussa site, we sampled 11 stands of *R. rugosa* (i.e., plots) and several other scattered individuals across the area.

A total of 72 species were found in the 11 sampled plots (Table S1). A total of 61 species (84.72% of the total) were classified as natives and 11 as aliens (15.28%). The most frequent families of native species were Poaceae (19.67% of sampled species), Asteraceae (11.48%), Caryophyllaceae and Rosaceae (both Families with 11.48%), while the most frequent native species were *Fumana procumbens*, *Phleum arenarium*, and *Elymus farctus*. Regarding alien species, the most frequent families belong to Asteraceae, Rosaceae (both 27.27% of sampled species), and Poaceae (18.18%), while the most frequent alien species were *Amorpha fruticosa*, *Oenothera biennis* and *Sporobolus pumilus* (all present in 100% of the plots). *R. rugosa* was always present.

Native and alien species richness were distributed as shown in Figure 3a,b, depending on the different dune zones, but significant differences were only found for native species richness. The distributions of *R. rugosa* cover, number of ramets, and mean height among dune zones are represented in Figure 3c–e. However, no significant differences were observed between groups.

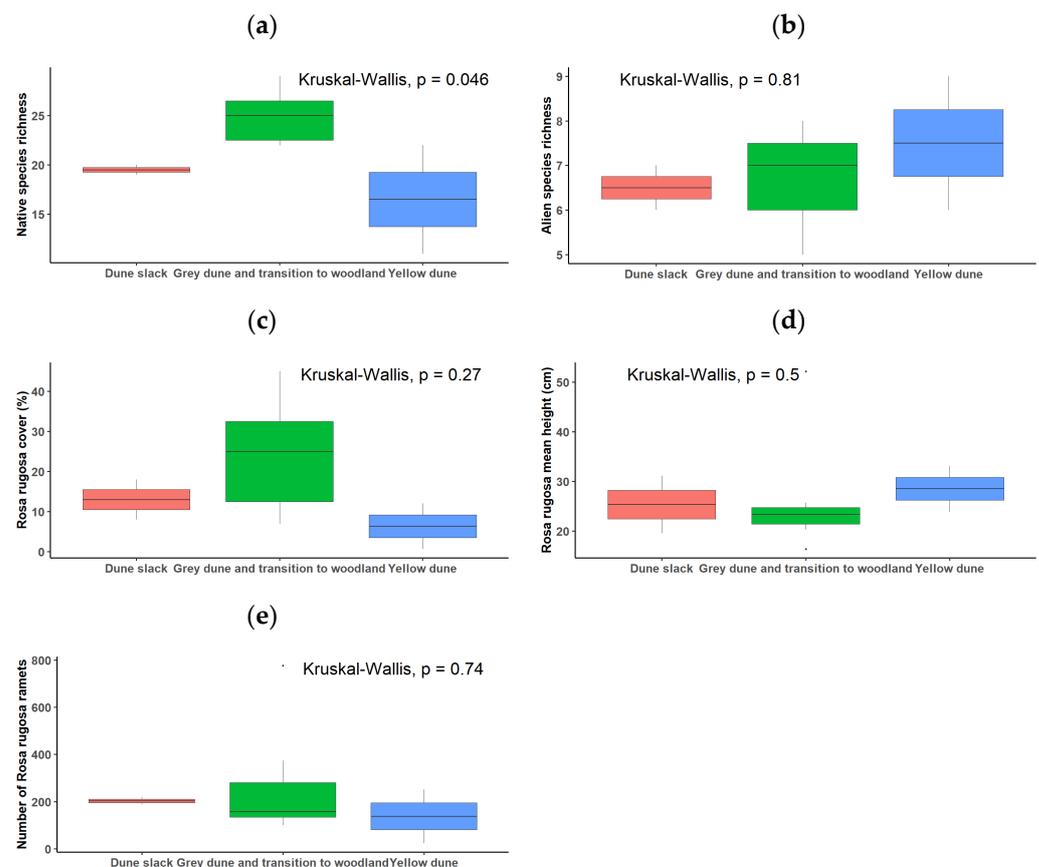


Figure 3. Distribution of native number of species (a), alien number of species (b), *Rosa rugosa* cover (c), *R. rugosa* mean height in cm (d), and number of *R. rugosa* ramets (e) between the three dune zones.

The results of the correlation analysis are represented in Figure 4 and Table S2. The cover of *R. rugosa* was significantly positively related to the amount of bare soil in the plot,

herbaceous cover, and number of ramets. Ramets, in turn, were also positively related to bare soil and herbaceous cover, and negatively related to the number of species, the number of alien species, the number of herbaceous species, the number of native species, plot distance to the sea, vegetated belt width, and *R. rugosa* mean height. Its mean height was positively related to the number of alien species, the number of tree species, *Pinus pinea* cover and vegetated belt width.

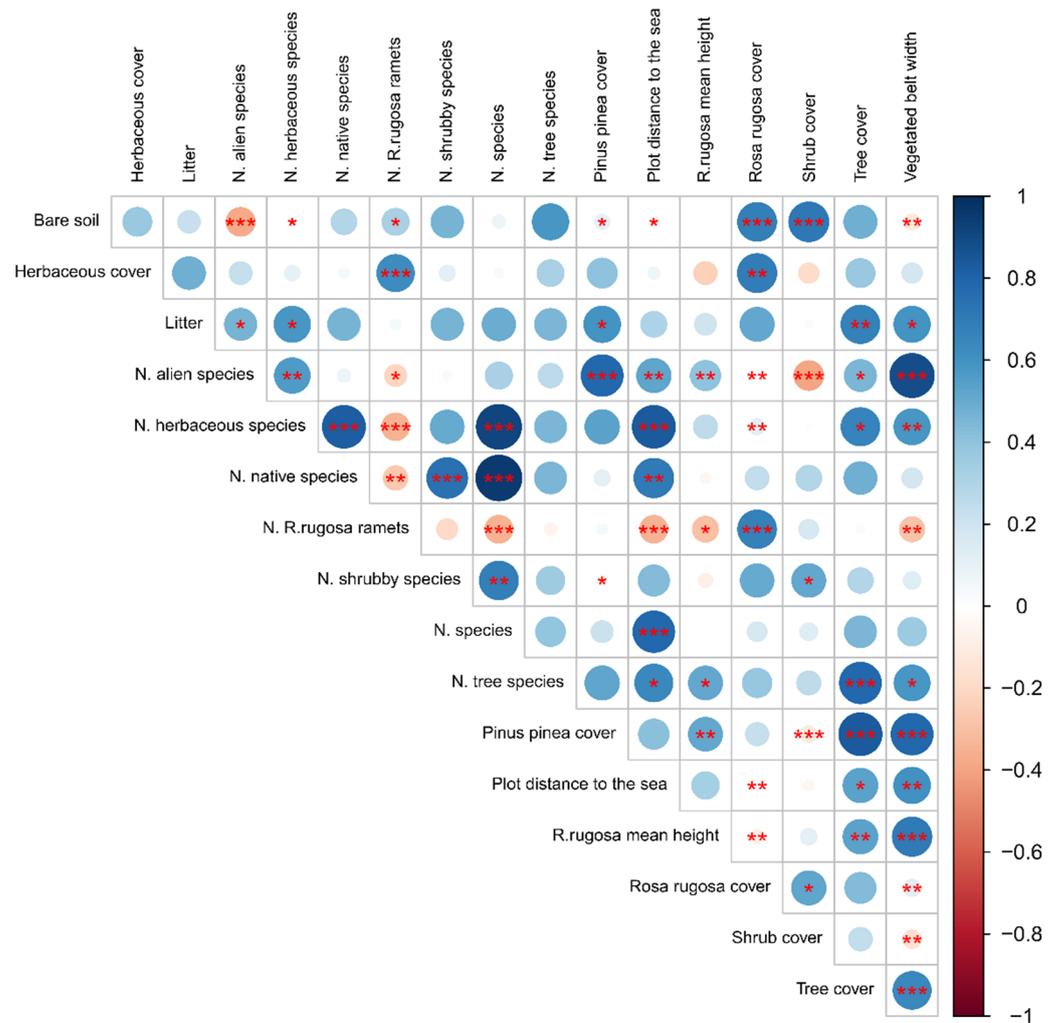


Figure 4. Correlation analysis results. Circle diameters are proportional to the level of correlation. Asterisks represent the significance of the correlation: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

NMDS analysis (Figure 5) provides a clear gradient of plant communities along the sea–inland gradient (from left to right). Starting from the shifting dune zone on the right, it is possible to observe *Ammophila arenaria* that, in natural conditions, would dominate this zone, along with a few other dune-building perennial rhizomatous grasses. In the centre, it is possible to recognise species typical of fixed dune plant communities, such as *Fumana procumbens*, *Silene conica*, *Lagurus ovatus*, and *Lomelosia argentea*. Finally, on the right, we find back dune and woodland species (e.g., *Pinus pinea*, *Quercus ilex*). At about 70 m from the sea, all the alien species that we found most often are distributed, i.e., *R. rugosa*, *Oenothera biennis* and *Sporobolus pumilus*. The position of *R. rugosa* along the gradient fits into the part of the coast between shifting and fixed dunes.

Furthermore, several planting campaigns of indigenous species were organised to restore the dune habitat, thus making the establishment of *R. rugosa* more arduous. In Brussa, the percentage of alien plants (15.28%) is slightly lower than the Italian percentage (16.6%, [92]), but much higher than the percentage of alien plants on European dunes (7%, [24]). In Germany, it was also shown that the invaded plot with *R. rugosa* favoured other alien plants [25]. This might also be the case in Italy, given the high number of alien plants that were observed. These results raise great concern considering the high conservation value of this habitat, which hosts an elevated proportion of endangered and exclusive plant taxa [93].

We observed that the majority of *R. rugosa* populations stabilised in the ecotonal area between grey dunes and Pine Forest (Figure 3c). Our observations slightly differ from the distributional pattern described in northern Europe, where *R. rugosa* forms dense thickets from the yellow dune, across the grassland of grey dunes, to brown dunes [25]. Furthermore, many single individuals with a greater stem height, were situated in the proximity of pine trees (Figure 4), suggesting that, at these latitudes, *R. rugosa* may benefit from the shade of the trees during the hottest hours of the day. Again, this result appears to be in contrast with what was observed in northern Europe, where *R. rugosa* prefers open sites, which are more exposed to direct daylight [25].

With regard to its correlation with other native and alien species, the *R. rugosa* dense thickets (i.e., those with a greater number of ramets) may cause a general reduction in the number of species and, more specifically, a decrease in herbaceous plants (Figure 4). Indeed, by growing in dense thickets and by having broad and, thus, shadier leaves, *R. rugosa* causes a strong reduction in light intensity beneath the shrub and, therefore, a decrease in many light-demanding species, such as typical grassland and herbaceous species [25,48,75,76]. Moreover, *R. rugosa* builds up large dominant shrubland, in which only a few gaps are available for the recruitment and germination of any species [25]. Most of the species that manage to grow in the shrubland dominated by *R. rugosa* are neophytes, which are likely related to the higher nutrient contents that support their growth. In this way, the growth of other non-native species is facilitated, and this strategy may result in a self-preventing system of positive feedback circles [48].

The positive correlation between *R. rugosa* cover and bare soil (i.e., patches of exposed sand) is of further interest. The increased cover of *R. rugosa* on bare soil may be due to its greater success in terms of seed emergence and establishment on disturbed soil (i.e., soil not stabilised by other plant roots, and thus subjected to wind erosion and trampling), which promotes its growth and survival [94]. Finally, all the records were found close to the many tracks leading to the beach, raising questions about the role of human disturbance and transport in the spread of *R. rugosa* in this area [95].

R. rugosa is a widespread invasive plant in Europe. Its strong invasiveness makes it hard to eradicate and control. Therefore, it is imperative to monitor its distribution to better understand its ecology and physiology, and to promote appropriate management strategies. A recent study by Khapugin et al. [96] demonstrated that even small efforts at data collection (i.e., observation data and herbarium data aggregation) may result in numerous new locations of *Rosa* species being found, including *R. rugosa*. This information will also contribute to a better prediction of *R. rugosa* distribution, coupled with future climate change. From this perspective, our collected data and investigations already provide helpful information to develop Species Distribution Models (SDM) based on climate change forecasts and used to predict the probability of occurrence under present and future conditions [97]. Two opposing scenarios can be expected: given the origin of the species from cold-temperate areas, global warming could impede the spread of *R. rugosa* in its introduced range; on the other hand, its recently studied phenotypic plasticity could facilitate the adaptation of the species to new climate conditions. Further investigations are also needed to understand how *R. rugosa* manages to grow and reproduce in Italy and discover whether other Mediterranean coastal areas could host or are already colonised by *R. rugosa* populations.

Supplementary Materials: The following are available online at <https://www.mdpi.com/article/10.3390/d13120645/s1>: Table S1: List of collected plant species. Species names, average percent cover within the vegetation layer (H = Herbaceous, S = Shrub, and T = Tree), and frequencies over the 11 sampled plots, Table S2: Results of correlation analysis. Numbers in bold represent significant correlations ($p < 0.05$).

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