CONTRASTING PATTERNS OF NATIVE AND NON-NATIVE PLANTS IN A NETWORK OF PROTECTED AREAS ACROSS SPATIAL SCALES

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Abstract: Networks of protected areas are fundamental for biodiversity conservation, but many factors determine their conservation efficiency. In particular, on top of other human-driven disturbances, invasions by non-native species can cause habitat and biodiversity loss. Jointly understanding what drives patterns of plant diversity and of non-native species in protected areas is therefore a priority. We tested whether the richness and composition of native and non-native plant species within a network of protected areas follow similar patterns across spatial scales. Specifically, we addressed three questions: *a*) what is the degree of congruence in species richness between native and non-native species? *b*) do changes in the composition of non-native species across ecological gradients reflect a similar turnover of native species along the same gradients ? *c*) what are the main environmental and human disturbance drivers controlling species richness in these two groups of species?

Species richness and composition of native and non-native plant species were compared at two spatial scales: the plot scale (10 m x 10 m) and the Protected Area scale (PA). In addition, we fit Generalized Linear Models to identify the most important drivers of native and non-native species richness at each scale, focusing on environmental conditions (climate, topography) and on the main sources of human disturbance in the area (land use and roads). We found a significant positive correlation between the turnover of native and non-native species composition at both plot and PA scales, whereas their species richness was only correlated at the larger PA scale. The lack of congruence between the richness of native and non-native species at the plot scale was likely driven by differential responses to fine scale environmental factors, with non-natives favoring drier climates and milder slopes (climate and slope). In addition, more non-native species were found closer to road-ways in the reserve network. In contrast, the congruence in the richness of native and non-native species at the broader PA scale was mainly driven by the common influence of PA area, but also by similar responses of the two groups of species to climatic heterogeneity. Thus, our study highlights the strong spatial dependence of the relationship between native and non-native species richness and of their responses to environmental variation. Taken together, our results suggest that within the study region the introduction and establishment of non-native species would be more likely in warmer and dryer areas, with high native species richness at large spatial scale but intermediate levels of anthropogenic disturbances and mild slope inclinations and elevation at fine scale. Such an exhaustive understanding of the factors that influence

the spread of non-native species, especially in networks of protected areas is crucial to inform conservation managers on how to control or curb non-native species.

Keywords: plant species richness, Habitat Directive, habitat heterogeneity, Natura 2000, scale dependence, protected areas, human disturbance.

INTRODUCTION

The introduction and spread of non-native species (also known as alien or exotic species) is considered a major cause of habitat transformation and biodiversity erosion, especially in the Mediterranean biome (Sala et al. 2000, Lucy et al. 2016). Rather than only focusing on controlling species that are already established or naturalized in a territory, proactive management of biological invasions should aim at curbing the arrival or establishment of new non-native species (Spear et al. 2013) or controlling their populations during early-stages of the invasion *continuum* (*sensu* Richardson and Pyšek 2006). Identifying the features that make certain areas more likely to harbor many non-native species (which are also known to correlate with areas harboring many invasive species, Williamson & Fitter, 1996) is an important information for controlling non-native species spread. In addition, by understanding whether the same features also drive the distribution of native species it might be possible to highlight potential hotspots for biodiversity management. For example, if native species tend to be associated with similar conditions as non-native species, valuable areas of high biodiversity will inevitably overlap with hotspots of invasions (e.g. Stadler et al. 2000), potentially leading to greater risks of biodiversity loss as a consequence of non-native species impacts (Simberloff et al. 2013). These might thus represent joint priorities for conservation and non-native species control efforts.

An enormous body of literature has tried to identify general features or ecosystem properties facilitating the establishment of non-native species (*e.g*., Ashton and Mitchell 1989; Faliński 1998; Sukopp 1998; Pyšek and Richardson 2006, Bjarnason et al. 2017), and to understand whether the same features also promote native species richness and high overall biodiversity (*e.g*., Shea and Chesson 2002; Deutschevitz et al. 2003; Carboni et al. 2010; Tordoni et al. 2017). In general, environmental factors which influence patterns of species richness at regional scales include climate, landscape heterogeneity, and geomorphological processes, all of which typically affect native as well as non-native species (Davies et al. 2005; Moser et al. 2005; Carboni et al. 2010). But in addition, in the current Anthropocene era (Crutzen 2006), human disturbance and management practices are a major agent of change of species richness and diversity patterns across spatial scales (Maestre 2004; Gaston 2005). For example, human disturbance may generate environmental heterogeneity, which may increase extinction risk of native species, but also allow for resource partitioning by creating new niche opportunities (Shochat et al. 2006). These factors may thus facilitate the arrival of non-native species pre-adapted to such altered conditions (Callaway 2007). Indeed, while natural or near-natural ecosystems often display a certain ecological resistance against biological invasion (*e.g.,* Faliński 1998; Simberloff et al. 2013), densely populated areas or areas subject to strong human disturbance are typically found to be prone to higher levels of non-native species establishment and invasion success (*e.g.*, Pyšek et al. 1998; Sukopp 1998; McKinney 2002; Chytrý et al. 2008; Tordoni et al. 2017).

The scale dependence of biodiversity patterns is a well-known issue in ecology (*e.g.*, Huston 1999; Richardson and Pyšek 2006). In particular the relationship between native and non-native species richness seems to change across spatial scales, which led scientists to even coin the term "invasion paradox" (Shea and Chesson 2002; Fridley et al. 2007). Specifically, a negative relationship is usually observed at small spatial scales, at which species typically interact (*e.g.,* Cornell and Karlson 1997; Levine 2000; Tilman 1997), but this relationship tends to become positive when increasing the grain of the sampling units or the extent of the study area (Planty-Tabacchi et al. 1996; Lonsdale 1999; Levine 2000; Stadler et al. 2000; McKinney 2002; Sax 2002; Kumar et al. 2006; Stohlgren et al. 2006). Although there has been a heated debate to try to explain these contrasting patterns, this conundrum of invasion ecology is still far from being resolved. Further studies are thus needed in order to understand the relationships between native and non-native species patterns and the environmental and anthropogenic features which foster biological invasions across spatial scales.

In the Convention on Biological Diversity (art. 8 *In-situ Conservation*, https://www.cbd.int/), prevention measures, control, or eradication of problematic non-native species are called for. Identifying pathways for non-native species establishment and management priorities are possible actions in this direction. In particular, protected areas are a key component of the global response to environmental change and degradation (*e.g.*, Hannah et al. 2007; Gaston et al. 2008; Foxcroft et al. 2017), and can be part of a framework to devise effective invasion control measures. Nevertheless, they face many challenges, such as the effectiveness of reserve design, governance (Pressey et al. 2015), and anthropogenic change (Foxcroft et al. 2017), with generally few restrictions currently in place for preventing the introduction of non-native species (Pyšek et al. 2003). Most protected areas in Europe are in a mosaic of land use types that can form a network of potential sources for non-native species introductions (*e.g.*, Foxcroft et al. 2007; Meiners and Pickett 2013). In addition, recent evidence shows that there is almost no difference in the patterns of non-native and invasive species inside and outside protected areas, suggesting that currently habitat protection has little or no effect on non-native species richness (e.g. Moustakas et al. 2018). Studying which features are linked to higher invasion levels can thus help identify the main pathways that need regulation and which areas are most at risk, to guide future conservation planning within protected area networks.

In this study, we investigated the importance of biotic (represented by native species richness and composition), environmental (climate) and anthropogenic (road network and land uses) factors in driving non-native plant species spatial patterns within a network of protected areas in central Italy. We aimed at providing insights on the ecological mechanisms useful for the effective control of non-native species establishments, which is extremely important in the context of the management of reserve networks. Ideally, reserve networks strive to maximize the protection of biodiversity features, while non-native species clearly represent a potential threat for nature reserves and their management (Pyšek et al. 2003). If native and non-native species follow similar patterns within the network, an overlap of high native biodiversity and invasion hotspots is likely to emerge. Identifying such areas at high risk of biodiversity loss and the ecological features that may promote invasion or otherwise hamper biodiversity protection is therefore essential to improve control and management actions. We aim at testing, at different spatial scales, whether species richness levels of native and non-native vascular plants are correlated and whether similar factors control the turnover in species composition of native and non-native species along ecological gradients. Specifically, our research questions are: *a*) what is the degree of congruence in species richness between native and non-native plant species, and does this relationship vary across spatial scales? *b*) do changes in the composition of non-native species across environmental gradients reflect native species turnover along the same gradients? *c*) what are the main environmental and anthropogenic drivers controlling species richness in each of these two groups of species?

METHODS

Study area

This study was performed in the local network of protected areas (PAs) of the province of Siena (Italy), including four Nature Reserves (designated under national or regional regulations) and 17 Special Areas of Conservation of Natura 2000 network (SACs, designated under the EU Habitat Directive 93/43/EEC) (Figure 1). The size of the single PAs within the network ranges from 268 ha to 13747 ha, while their elevation ranges from 122 m to 1660 m a.s.l. (Chiarucci et al. 2012) with a cumulative area of 593 km^2 (15.6% of the Province).

The study area is characterized by a Mediterranean macro-climate, even though there is a strong variation across sampling sites due to differences in morphology and local elevations (Castrignanò et al. 2006). Long term mean annual precipitation ranges from 630 to 1275 mm (Barazzuoli et al. 1993). The highest precipitations (above 1000 mm on average) and lowest mean annual temperature values (lower than 12 °C) are found at higher elevations (Monte Amiata). A relatively arid and warm zone (mean annual temperature of about 14 °C and average precipitations of ca. 600 mm) is localized in the South-East of the province of Siena (Orcia river valley; Barazzuoli et al. 1993).

The geology is rather varied and complex (including, *inter alia*, limestone, clay, marl, metamorphic and volcanic bedrock), resulting in highly heterogeneous morphology and a great variety of landscapes. The main land-cover types include evergreen coppice woods (dominated by *Quercus ilex*) and deciduous coppice woods or forests (thermophilous types dominated by *Quercus pubescens* or *Q. cerris* at lower elevations, and mesophilous types dominated by *Castanea sativa* or *Fagus sylvatica* at higher elevations); evergreen Mediterranean shrublands (characterized *e.g*. by *Erica arborea*, *E. scoparia*, *Phillyrea latifolia*, *Pistacia lentiscus*, *Arbutus unedo*, *Cistus salvifolius*, *Juniperus communis* and *J. oxycedrus*); croplands (mainly wheat and horticultural crops), vineyards and olive groves. Other relevant land cover types include pastures, meadows, garigues (on calcareous and ultramafic substrates), conifer plantations and wetlands.

Sampling design

Plants were sampled by using an operational approach of plant communities (Chiarucci 2007), defined by a fixed grain and uniform sample density within each protected area (PA). Sampling design was based on a grid of 1 km × 1 km cells, covering the whole study area, with a sampling point randomly selected within each cell (Chiarucci et al. 2008, 2012). A sampling unit represented by a square plot of 10 m x 10 m was centered at each sampling point. This was further divided in 16 contiguous squared 2.5 m x 2.5 m subplots (Figure S1 of Supplementary material), on which the occurrence of all species of vascular plants was recorded.

The field data collection was performed from April to June, during the years 2005-2009. The total number of sampled plots was 604.

Native and non-native species richness and composition

All the vascular plants recorded within each plot were identified at the species or subspecies level with standard floras (Pignatti 1982; Tutin et al. 1964–1980, 1993) or monographs (Grunanger 2001; Weber 1995). Nomenclature was standardized according to Conti et al. (2005). Plants were classified as native or non-native, depending on their distributional status as given by Celesti-Grapow et al. (2011).

Composition and species richness for both native and non-native species were then separately assessed at plot and PA scale, i.e. two data matrices were prepared (species by plots and species by PAs). At plot scale, frequency of each species, calculated as the sum of occurrences within the 16 subplots, was used as a coarse measure of abundance. At PA scale, relative frequency of each species, measured as the ration between the number of occupied plots *versus* the number of recorded plots, was used as measure of abundance.

The PA-scale species lists were obtained by pooling the data from the plots included within each PA.

Environmental and human disturbance predictors

At plot scale, four groups of predictor variables for evaluating the environmental and human-mediated and spatial factors affecting species richness were considered: 1) climatic, 2) topographic, and 3) human disturbance (Table 1):

1) Climatic variables: we derived one synthetic variable named *climate.* We obtained this variable from 36 climatic variables related mainly to monthly temperatures and precipitations (e.g. maximum annual temperature, minimum annual temperature, annual rainfall) for each plot, from the LaMMa consortium (Laboratorio di Monitoraggio e Modellistica Ambientale per lo sviluppo sostenibile; http://www.lamma.rete.toscana.it/en). Specifically the LaMMa data consisted of local interpolated climatic grids with a resolution of 250 m x 250 m. Since these variables were highly inter-correlated, a Principal Component Analysis (PCA) was performed to reduce their multicollinearity (Taylor et al. 2002). Given that the first PCA factor explained more than 90% of variance (Figure S2 of Supplementary material), this was used as a single variable (hereafter called *climate*). This was found to be negatively correlated with all the variables related to total and summer rainfall (Pearson correlation coefficients from -1to -0.85), positively correlated with the minimum temperatures (Pearson correlation coefficients from 0.77 to 0.83) and maximum ones (Pearson correlation coefficients from 0.84 to 0.90). This compound *climate* variable corresponds therefore to a gradient from wet and cold to warm and (summer-) dry conditions, that is from Temperate to Mediterranean meso-climate, moving from the negative to the positive extreme of the axis.

2) Topographic variables: *elevation*, *slope*, and potential *solar radiation* values (the latter was obtained from slope, aspect and latitude, following McCune and Keon 2002). The resolution used to develop these variables was 30 m.

3) Human disturbance variables: *distance to the nearest road* (paved or unpaved) and *land-use type* variables. We used the distance to the nearest road as a proxy of human disturbance as the road network is a well-known introduction pathway for non-native species (e.g. Pauchard and Alaback 2004; Arévalo et al. 2010). Road layer have been derived from GEOscopio Geoportal, Tuscany Region - Territorial and Environmental Information System (http: // www.Geografia.toscana.it/)

In addition, *landscape diversity* (H_{plot}) and *landscape evenness* (E_{Plot}) within a buffer area of 250 m radius around the plot were assessed based on the Shannon Index of diversity and the Pielou Index of evenness applied on the Corine Land Cover (CLC) map. At plot scale, the human disturbance variables linked to land use have not been considered due to the resolution of the CLC map which did not allow to detect artificial land use types in the plots or in related buffer areas. So we used artificial land use types as proxy of human disturbance only at protected area scale.

At PA scale, the same groups of predictor variables as at the plot scale were considered, with some unavoidable differences in the definition of the single predictors due to different spatial resolution (Table 2):

1) Climatic variables: the variability of climate across plots was accounted for by calculating two derived climatic variables at PA scale: *mean climate* (calculated as the centroid, along the first axis in the climate PCA, of the plots belonging to each PA) and *climate range* (calculated as the range of the same points along the PCA axis).

2) Topographic variables: *elevation range* and *mean elevation* within each PA.

3) Human disturbance variables: total *road density* and *land-use type* variables. We obtained the total road density as the linear extension of paved and unpaved roads per km^2 within each PA and was considered as a proxy of human disturbance and potential propagule pressure of non-native species.

Moreover, as human disturbance, we obtained 4 land-use type variables reflecting the different artificial land-use types found in each PA based on CLC map (I level) of the area extracted from the Copernicus database (https://land.copernicus.eu/pan-european/corine-land-cover). Land-use types variables, artificial, semi-natural and natural, were expressed as percentages of relative Corine land-use classes (human, agriculture, natural, wetland). The variables *landscape diversity* (H_{PA}) and *landscape evenness* (E_{PA}) were calculated using the Shannon Index of diversity and the Pielou Index of evenness on the III level CLC map. The calculation of the landscape diversity was done at the III level of CLC because this allowed for a finer classification of landscape units, that was not necessary for detecting the dominant land use type.

4) Geographical variable: *area* (expressed in km^2) of each PA area.

STATISTICAL ANALYSES

Separate analyses were performed at the plot and PA scales. At each scale, we assessed: 1) the correlation between native and non-native species richness, 2) whether specific sets of non-native species tend to be associated with specific native species assemblages, and 3) which predictors best explained native and non-native species richness, respectively.

Relationships between native and non-native species

First, we assessed the correlation between native and non-native species richness by computing the Spearman correlation coefficient ρ at both spatial scales. This was done to assess whether native and non-native species richness within the reserve network followed 1) similar trends, suggesting that factors which favor high richness of native species, also increase opportunities for non-native species (Thuiller et al. 2010), leading to a positive native-non-native richness relationship (Shea and Chesson 2002), or 2) opposite trends suggesting higher biotic resistance of more diverse native communities, as interpreted by several authors (Cornell and Karlson 1997; Stohlgren et al. 1999; Levine 2000; Tilman 1997; Brown and Peet 2003; Davies et al. 2005; Souza et al. 2011).

Second, we verified if turnover in native species composition is associated also to a turnover in non-native species and thus if specific sets of non-native species tend to always be associated with the same natives. To do so, we first obtained plot-to-plot (and PA-to-PA) dissimilarities in species composition, separately for non-native and for native species, using Bray Curtis pairwise dissimilarities on log(x+1) transformed species frequencies. Then, we tested whether the pairwise dissimilarity matrices (or distance matrices) of native and non-native species were linearly independent by performing a Mantel test (Spearman correlation, 999 permutations, McCune and Keon 2002). For this we considered only the plots or PAs that had at least one non-native species.

Determinants of species richness across spatial scales

To assess the relationship between vascular plant richness (separately for native and non-native species) and the environmental and human disturbance predictors, we used two different approaches depending on the scale of investigation (plot or PA scale). In both cases, we first evaluated the presence of spatial autocorrelation in the response variables by calculating Moran's I coefficient using the R package 'ape' (Paradis and Schliep 2018), based on the geographical coordinates of the plots and of the centroid of the PA, respectively. Also, multicollinearity among explanatory variables at each scale was tested by computing Spearman's (correlation matrix available in Table S1 of the Supplementary material) and ensuring that there were no pairs of variables in the models where $|\rho| > 0.7$ (Dormann et al. 2013).

At plot scale, we fit Generalized Linear Mixed Models (GLMM) using R package "lme4" (Bates et al. 2015) and assuming Poisson family errors. PA was considered as random effect to control for the spatial dependence of plots within PA. At PA scale, Generalized Linear Models (GLM) were used using Poisson family errors; in case of overdispersion in the data a quasipoisson family was used instead. A Minimum Adequate Model (MAM) and a set of models with good support were thus obtained by performing a stepwise variable selection procedure through AICc minimization using R package "MuMIn" (Barton 2019). Only the models with ΔAICc ≤ 2 compared to the best model were considered to have good support (Burnham and Anderson 2002). In case of more models with similar AICc values, the one which retained a lower number of predictors was considered as minimum adequate model (MAM) according to Occam's razor. Note that, since results were qualitatively similar across the set of models with good support (Table S2 of Supplementary material), we present and discuss only the MAMs in the main text. In case of overdispersion in the data, it was not possible to use an AICc-based selection approach, then the amount of deviance accounted for by the GLM adjusted by the number of predictors was used instead ($D^2_{adjusted}$; Guisan and Zimmermann 2000; Barbosa et al. 2014). In addition, R^2 statistics (marginal effect) were derived for GLMMs using the "r2glmm" R package (Jaeger 2017). Prior to analysis, quantitative variables were standardized in order to have mean zero and unit variance. Any residual spatial autocorrelation after modeling was assessed by means of spline-correlograms using the R package "ncf" (Bjørnstad 2019). Specifically, 95% pointwise bootstrap confidence intervals were calculated from 5000 bootstrap samples of Pearson residuals after accounting for the level of spatial autocorrelation explained by the explanatory variables in each model.

All the statistical analyses were conducted using R 3.6.1 (R Core Team 2019).

RESULTS

Species patterns at plot scale

In total, 993 native species and 48 non-native species were recorded in 604 plots. Plant species richness ranged from 0 to 117 (mean 31.0) for native species and from 0 to 9 (mean 0.6) for non-native species. Native and non-native species richness exhibited a slight positive correlation, though not significant (Spearman $\rho = 0.06$, $p = 0.11$). In contrast, distance matrices based on plot-to-plot compositional dissimilarity showed a significant positive correlation (Mantel test, Spearman $\rho = 0.24$, $p = 0.001$).

Table S2 reports the full list of candidate models for species richness predictors derived from the model selection procedure. Table 3 describes the best set of predictors for species richness in the GLMM MAM. Specifically, minimum adequate models showed, on the one hand, that native species richness was positively associated with *landscape diversity* (H_{Plot}) and negatively with the first axis of PCA derived from climatic variables (*climate*) and slope. On the other hand, non-native species richness was negatively related to *road distance* and *slope*, and positively associated with *climate*. However both models, in particular the native-species model, are characterized by a weak goodness of fit (R^2 = 0.02 and R^2 = 0.11 for native and non-native species, respectively).

Species patterns at protected area scale

Plant species richness at PA scale ranged between 22 and 547 (mean 219.8 species) for native species and 0-24 (mean 6.6 species) for non-native species. Species richness values of natives and non-native species (Figure 2) were positively and significantly correlated (Spearman $\rho = 0.75$, $p < 0.001$). The relative proportion of non-native species (ratio of non-native to native richness) in PAs was positively correlated with the richness of native species ($R^2 = 0.32$, $p < 0.01$), increasing up to a limit of about 5% (Figure 2). Similarly, the Mantel correlation between native and non-native species dissimilarity matrices was significant (Spearman $\rho = 0.33$ with $p = 0.003$). Further, according to the MAMs, species richness at PA scale was positively correlated with *area* and *climate range* for both species groups (Table 3). In addition, native species richness showed a positive relationship with *mean climate* and a negative, but not significant, relationship with *% wetland*. In contrast, non-native species richness was negatively related with *mean elevation*. In both species groups, the deviance accounted for by the model was relatively high ($D^2_{adjusted} = 0.76$ and $D^2_{adjusted} = 0.71$ for native and non-native species model, respectively).

All four spline correlograms (Figure S3 of Supplementary material) failed to reveal any evidence of spatial autocorrelation in the residuals, thereby allowing us to exclude its influence on model parameter estimates.

DISCUSSION

The Italian flora is currently estimated to include 7634 *taxa* (species and subspecies), 13.4% of which (1023 taxa) are considered to be non-native (Celesti-Grapow et al. 2011). In our dataset, collected using a probabilistic sampling strategy within the protected areas of the Siena province only, we recorded a rich flora (1041 species were recorded by this survey) and a relatively low proportion of non-native species (4.9% of the whole sample). However, even if the province of Siena is characterized, overall, by a well preserved landscape of traditional land uses (Geri et al 2010), only protected areas were included in this study and thus most of the sampled sites are on average less disturbed than the remainder of the landscape at province (or country) scale. Even though the proportion of non-native species in the investigated system is generally low, we found evidence of scale dependency of the relationship between native and non-native species richness. While there was a strong positive relationship at the PA scale, native and non-native species richness were not correlated at the plot scale. This was the consequence of different responses of the two groups of species to environmental and human factors at the fine scale, while similar factors (chiefly reserve area) explained both native and non-native species richness at large scale.

Species patterns at the plot scale

At the plot scale, we did not observe a relation between native and non-native species richness but we observed a relationship between the compositional gradients of native and non-native compositionspecies. This suggests that non-native species tend to be associated with specific sets of native species (*i.e.,* native plant communities), either because they have similar environmental requirements or because of more direct biotic interactions (*e.g.,* facilitation or competitive exclusion). However, the lack of significant correlation between native and non-native species richness instead suggests a limited role for biotic interactions and for biotic resistance through competitive exclusion in these plant communities.

Indeed, our results suggested that different factors affected the species richness of the two groups of species at plot scale. Specifically, native species richness was only very weakly related to the variables we measured at this scale, and our best model only explained a very small proportion of variability ($R^2 = 0.02$). Nevertheless, we found evidence that native richness was positively associated with *landscape diversity* around the plot (250 m radius) and negatively associated with the *climatic* gradient ranging from Temperate to Mediterranean meso-climatic conditions. The first result is in agreement with previous studies suggesting that greater *landscape diversity* may be related to a higher number of available niches, potentially hosting species with different ecological requirements (Deutschewitz et al. 2003; Kumar et al. 2006). In our study area, higher values of small-scale *landscape diversity* might specifically indicate fragmentation and the survival of more natural ecosystems within an otherwise homogeneous agricultural landscape. The negative relationship between native species and the temperate-mediterranean gradient indicates that more native species were found in the cooler and wetter, rather than in the drier, meso-climatic conditions. This is likely to be connected to the regional context, in which higher temperatures at plot scale might represent a significant ecological constrain in summer, while lower temperatures are not likely to be a limiting factor in winter.

In contrast, more variability could be explained for non-native species richness. Non-native species were negatively related with *road distance,* and *slope* inclination and positively related to the temperate-mediterranean climatic gradient.

As expected, non-native species richness was higher in plots in close proximity to roadways. This is in accordance with the well-documented notion that roads, and roadside habitats, are a major source from which non-native species colonize natural areas (Parendes and Jones 2000; Pauchard and Alaback 2004; Bacaro et al. 2015; Ullmann and Heindl 1989; Ullmann et al. 1995; Arévalo et al. 2010). Roads may facilitate the dispersal of non-native species inducing habitat fragmentation and altering (micro-) environmental conditions, facilitating the human-mediated dispersal of propagules (via air movement associated with vehicle traffic, and via the seeds attached to the vehicles themselves) and facilitating the colonisation by non-native species by suppressing the growth or removing stands of native species (Trombulak and Frissel 2000; Bacaro et al. 2015). Therefore, limitation to the construction of new roads within or close to protected areas is important for preserving local biodiversity both directly, but also indirectly via the reduction of non-native introductions.

In terms of environmental factors, non-native species richness at plot scale was positively associated with warmer and drier Mediterranean meso-climatic conditions and negatively correlated with slope inclination. This can partly be a consequence of the well-known conservative role of steep slopes, because of soil- and microclimatic- limiting factors: for instance, Bennie et al. (2006) found that in Britain, grassland swards on steep slopes were more resistant to invasion by competitive grass species than those on flatter sites, due to phosphorus limitation in shallow minerogenic soils, and to increased drought events. Filibeck et al. (2016) showed that, in grasslands in a protected area at the periphery of Rome, the removal or leveling of some steep debris heaps led to an increase in non-native and cosmopolite species. However, our dataset is not restricted to grasslands and involves a huge variety of land-cover types, so it is also possible that the observed effect is mediated by disturbance levels and land-use: in the study region, a high slope steepness usually allows only land-use types that feature an inherently low human disturbance (*e.g*., woods or extensive rangelands), while flat morphologies are usually exploited with intensive crops or urban land-use, typically favouring non-native taxa invasions.

Species patterns at the protected area scale

We found that the turnover in non-native species composition was correlated with native species composition also at the scale of protected area. At this spatial grain, however, we also detected a strong congruence in the richness of the two groups of species. These findings, highlight that the protected areas that are most biodiverse are also the ones that are most easily colonized by alien species (and thus, potentially, most at risk of invasion). More generally, these findings support the "biotic acceptance hypothesis" or "the rich get richer" hypothesis, according to which sites with high native species richness are the most readily invaded by non-native species (*e.g*., Stohlgren et al. 1999, 2006; Fridley et al. 2007; Pyšek and Richardson 2006; Sandel and Corbin 2010, Bartomeus et al. 2012). This would be explained because habitats that are generally 'good' for native species would also be 'good' for non-native ones (*e.g*., McKinney 2002; Souza et al. 2011). This hypothesis can also be linked to Grime's theory (Grime 1973), according to which all species (meaning both native and non-native species) respond, to some degree, in a similar way to stress, competition and disturbance (Tomasetto et al. 2013).

We therefore tested whether the similar compositional and richness patterns emerged as a consequence of similar responses to environmental factors and human disturbances. Indeed, we found that two main variables were retained in both the models for native and non-native species richness at the PA scale: *area* of the protected area, and *climate range* (*i.e*. spatial heterogeneity of climate within the PA). Both these variables showed positive effects, even if with different weights for native and non-native species richness. Thus, our research shows a positive native-non-native relationship related to the same response of native and non-native species to the available area in the PA and to the main gradients at large scale. The positive effect of *area* on both native and non-native species richness is likely dependent on the higher availability of niches in larger protected areas for both groups of species. The congruence of native and non-native species richness is thus largely the net result of concordant well-known species-area relationships, that show similar patterns for both groups of species. Indeed a congruence in species-area relationships for native and non-native species had already been reported in this network of protected areas (Chiarucci et al. 2012), as well as in other reserve networks (Pyšek et al. 2002a) or insular systems (*e.g*., the islands of the Tuscan archipelago, Chiarucci et al. 2017). In addition, the positive effect on richness of the climatic variables at the scale of the protected area (*climate range*) indicates that more climatically heterogeneous areas enhance regional native richness but also promote non-native species establishment. Indeed, environments with greater spatial heterogeneity (*e.g*., higher habitat diversity or spatial variability in resources or conditions; Davies et al. 2005) have been shown to support higher numbers of both native and non-native species at broad spatial scales in numerous other systems. Thus, overall we conclude that in our reserve network "rich protected areas get richer" because of more available area (as expected) but also because of more favorable conditions in terms of available niches and of climates.

In addition to the drivers that were common to both natives and non-natives, the model for native species at PA scale also included a significant positive effect of mean climate, which suggests that reserves with warmer and drier average climates tend to support greater species richness. The model for non-native species included a significant effect of mean elevation, that was negatively related to non-native species richness indicating that fewer non-native species were found at higher altitudes. This finding is in agreement with previous studies that showed a negative relationship between non-native plant species richness and elevation in various systems (*e.g.,* Pyšek et al. 2002b; Stevens 1992; Pausas 1994; Rey-Benayas 1995; Marini et al. 2009; Siniscalco et al. 2011; Barni et al. 2012; Bacaro et al. 2015). Nevertheless it is important to caution that upward movements of non native species are increasingly being detected in many montane ecosystems (Kalwij et al. 2015), and are also predicted to accelerate in the future (Carboni et al. 2018). Hence, even if our results show that currently higher elevations are of lesser concern for non-native plants, potential future invasion risks should not be discarded.

Conclusions and cross-scale comparisons

Overall, our results support the idea that the relationship between the number of native and non-native species is altered when changing the scale of analysis. Indeed, it is widely acknowledged that the strength and form of this relationship (in many studies known as NERR – native exotic richness relationship – *e.g.,* Souza et al. 2011; Symonds and Pither 2012) is scale-dependent. Here, we found evidence that the lack of congruence at plot scale was likely driven by differential responses to fine scale environmental factors (*climate* and *landscape diversity*) and human disturbances (*road distance*), while at the larger PA scale native and non-native species largely responded in a similar way to available *area* and to *climate* variability (climate range). While at coarse scales the species richness of native species (and non-native species) was generally higher in warmer Mediterranean climates, at the fine plot scale the native species richness was even mildly associated with cooler meso-climatic conditions. Overall richness of non-native species in the protected area network increased under moderate anthropogenic disturbances at at fine scale, coupled with high levels of habitat and climatic heterogeneity at large scale. Thus, our data suggest that within the study region the introduction and establishment of non-native species would be more likely in warmer and dryer areas, with high native species richness at large spatial scale but intermediate levels of anthropogenic disturbances and mild slope inclinations and elevation. We also found potential conservation hotspots, especially because the very biodiverse protected areas potentially feature a higher risk of invasion, due to higher establishment success of non-native species in general. These areas should thus be prioritized for invasion monitoring. Finally, the results also highlight that both 1) the measure of the proportion of non-native species and 2) the relationship with potential predictors should be studied at the appropriate spatial scale in order to be comparable among different regions and informative for conservation purposes.

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All the authors contributed to the interpretation of results and writing.

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CONTRASTING PATTERNS OF NATIVE AND NON-NATIVE PLANTS IN A NETWORK OF PROTECTED AREAS ACROSS SPATIAL SCALES

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Abstract: Networks of protected areas are fundamental for biodiversity conservation, but many factors determine their conservation efficiency. In particular, on top of other human-driven disturbances, invasions by non-native species can cause habitat and biodiversity loss. Jointly understanding what drives patterns of plant diversity and of ~~potential invasions~~non-native species in protected areas is therefore a priority. We tested whether the richness and composition of native and non-native plant species ~~(not necessarily invasive species)~~ within a network of protected areas follow similar patterns across spatial scales. Specifically, we addressed ~~two~~ three questions: *a*) what is the degree of congruence in species richness b~~between species richness and composition of~~etween native and non-native ~~plants~~species? *b*) ~~what is the degree of congruence, between~~do changes in the composition of ~~native and~~ non-native species across ecological gradients reflect a similar turnover of native species along the same gradients ~~,~~ ~~in floristic assemblage variation environmental gradients~~? *c*) what are the main environmental and human disturbance drivers controlling ~~for~~ species richness in these two groups of species?

Species richness and composition of native and non-native plant species were compared at two spatial scales~~,~~ ~~namely at~~: the plot scale (10 m x 10 m) and the Protected Area scale (PA). In addition, we fit Generalized Linear ~~Mixed~~ Models ~~(GLMMs) and Generalized Linear Models (GLMs) were applied on native and non-native species richness~~ to identify the most important drivers of ~~species~~ native and non-native species richness at ~~plot and PA~~each scale, focusing on environmental conditions (climate, topography) and on the main sources of human disturbance in the area (land use and roads). We found a significant positive correlation ~~was observed~~ between ~~the~~the ~~responses~~turnover of native and non-native species ~~composition~~composition at both plot and PA scales, whereas their species richness was only correlated at the larger PA scale. The lack of congruence between the richness of native and non-native species at ~~fine~~ the plot scale was likely driven by differential responses to fine scale envi-

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ronmental factors, with non-natives favoring drier climates and milder slopes ~~and human disturbances~~(climate and slope). In addition, more non-native species were found closer to road-ways in the reserve network. In contrast, the congruence in the richness of native ~~, while at the larger PA~~ and non-native species at the broader PA scale ~~native and non-native species largely~~ was mainly driven by the common influence of PA area, but also by similar responses of the two groups of species to ~~responded in a similar way to resource~~climatic ~~availability and variability~~heterogeneity.~~Across spatial scales, richness of non-native species increased under moderate anthropogenic disturbances.~~ Thus, our study highlights the strong spatial dependence of the relationship between native and non-native species richness and of their responses to environmental variation. Taken together, our results suggest that within the study region the introduction and establishment of non-native species would be more likely in warmer and dryer areas, with high native species richness at large spatial scale but intermediate levels of anthropogenic disturbances and mild slope inclinations and elevation at fine scale~~In particular, non-native species seem to respond to different drivers at fine scale, highlighting the primary role of local abiotic conditions (such as climatic and slope) and habitat heterogeneity. At PA scale, native and non-native species richness was more affected by regional-scale factors such as climatic variablese and anthropogenic disturbances~~. Such an exhaustive understanding of the factors that influence the spread of non-native species~~(some of which are invasive species such as Robinia pseudoacacia, or Amaranthus retroflexus)drivers of invasion~~, especially in networks of protected areas ~~such as Natura 2000 sites~~ is ~~may be~~ crucial to inform conservation managers on how to control or curb ~~of those that could be or become problems of biological invasions~~non-native species ~~especially in the light of ongoing global changes~~.

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Keywords: plant species richness, Habitat Directive, habitat heterogeneity, Natura 2000, scale dependence, protected areas, human disturbance.

INTRODUCTION

The introduction and spread of non-native species (also known as alien or exotic species) is considered a major cause of habitat transformation and biodiversity erosion, especially in the Mediterranean biome (Sala et al. 2000, Lucy et al. 2016). Rather than only focusing on controlling species that are already established or naturalized in a territory, proactive management of biological invasions should aim at curbing the arrival or establishment of new non-native species (Spear et al. 2013) or ~~to~~ controlling their populations during early-stages of the invasion *continuum* (*sensu* Richardson and Pyšek 2006). ~~Thus, i~~Identifying the features that make certain areas more likely to harbor many non-native species (which are also known to correlate with areas harboring many invasive species, Williamson & Fitter, 1996) ~~along with the driving factors of previously successful invasions~~is an important ~~baseline~~ information ~~to be used for a proper management of~~controlling non-native species spread ~~and conservation goals~~~~traartegies~~. In addition, ~~Understanding~~ by understanding whether the same features also drive the distribution of native species ~~can then help~~it might be possible to highlight potential ~~conflicts or~~ hotspots for biodiversity management. For example, if native species tend to be associated with similar conditions as non-native species, valuable areas of high biodiversity will inevitably overlap with hotspots of invasions (e.g. Stadler et al. 2000), potentially leading to greater risks of biodiversity loss as a consequence of non-native species impacts (Simberloff et al. 2013). These might thus represent joint priorities for conservation and non-native species control efforts.~~, also especially within reserve networks or specific areas with conservation interest protected area networks, such as Natura 200000 sites~~

An enormous ~~large~~ body of literature has tried to identify general features or ecosystem properties facilitating the establishment of non-native species (*e.g.*, Ashton and Mitchell 1989; Faliński 1998; Sukopp 1998; Pyšek and Richardson 2006, Bjarnason et al. 2017), and to understand whether the same features also promote native species richness and high overall biodiversity (*e.g.*, Shea and Chesson 2002; Deutschevitz et al. 2003; Carboni et al. 2010; Tordoni et al. 2017). In general, environmental factors which influence patterns of species richness at regional scales include climate, landscape heterogeneity, ~~spatial patterns,~~ and geomorphological processes ~~and level of protection~~, all of which typically affect native as well as non-native species (Davies et al. 2005; Moser et al. 2005; Carboni et al. 2010). But in addition, in the current Anthropocene era (Crutzen 2006), human disturbance and management practices are ~~is~~ a major agent of change of species richness and diversity patterns across spatial scales (Maestre 2004; Gaston 2005). For example, human disturbance may generate environmental heterogeneity, which may increase extinction risk of native species, but also allow for resource partitioning by creating new niche opportunities (Shochat et al. 2006). These factors may thus facilitate the arrival of non-native species pre-adapted to such altered conditions (Callaway 2007). Indeed, while natural or near-natural ecosystems often display a certain ecological resistance against biological invasion (*e.g.*, Faliński 1998; Simberloff et al. 2013), densely populated areas or areas subject to strong human disturbance are typically found to be prone to higher levels of non-native species establishment and invasion success (*e.g.*, Pyšek et al. 1998; Sukopp 1998; McKinney 2002; Chytrý et al. 2008; Tordoni et al. 2017).

The scale dependence of biodiversity patterns is a well-known issue in ecology (*e.g.*, Huston 1999; Richardson and Pyšek 2006). In particular the relationship between native and non-native species richness seems to change across spatial scales, which led scientists to even coin the term "invasion paradox" (Shea and Chesson 2002; Fridley et al. 2007). Specifically, a negative relationship is usually observed at ~~a small~~ ~~finer~~small spatial scales, ~~that are those in~~at which species typically interact (*e.g.*, Cornell and Karlson 1997; Levine 2000; Tilman 1997), but this relationship tends to become positive when increasing the grain of the sampling units or the extent of the study area (Planty-Tabacchi et al. 1996; Lonsdale 1999; Levine 2000; Stadler et al. 2000; McKinney 2002; Sax 2002; Kumar et al. 2006; Stohlgren et al. 2006). Although there has been a heated debate to try to explain these contrasting patterns, this conundrum of invasion ecology is still far from being resolved. Further studies are thus needed in order to understand the relationships between native and non-native species patterns and the environmental and anthropogenic features which foster biological invasions across spatial scales.

In the Convention on Biological Diversity (art. 8 *In-situ Conservation*, https://www.cbd.int/), prevention measures, control, or eradication of problematic non-native species are called for. Identifying pathways for non-native species establishment and management priorities are possible actions in this direction. In particular, protected areas are a key component of the global response to environmental change and degradation (*e.g.*, Hannah et al. 2007; Gaston et al. 2008; Foxcroft et al. 2017), and can ~~offer~~ be part of a framework to devise effective invasion control measures. Nevertheless, they face many challenges, such as the effectiveness of reserve design, governance (Pressey et al. 2015), and anthropogenic change (Foxcroft et al. 2017), with generally ~~little~~ few restrictions currently in place for preventing the introduction of non-native species (Pyšek et al. 2003). Most protected areas in Europe are in a mosaic of land use types that can form a network of potential sources for non-native species introductions (*e.g.*, Foxcroft et al. 2007; Meiners and Pickett 2013). In addition, ~~there is no~~recent evidence shows that there is almost no difference in the patterns of non-native and invasive species inside and outside protected areas,

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~~i.e.~~suggesting that currently habitat protection has little or no effect on non-native species richness (e.g. Moustakas et al. 2018). ~~SStudying~~Studying which features are linked to higher invasion levels can thus help identify the main pathways that need regulation and which areas are most at risk, to guide future conservation planning within protected area networks.

In this study, we investigated the importance of biotic (represented by native species richness and composition), environmental (climate) ~~, landscape diversity)~~ and anthropogenic (road network and land uses) factors in driving non-native plant species spatial patterns within a network of protected areas in central Italy. We aimed at providing insights on the ecological mechanisms useful for the effective control of ~~biological invasions~~non-native species establishments, which is extremely important in the context of the management of reserve networks. Ideally, reserve networks strive to maximize the protection of biodiversity features, while non-native species clearly represent a potential threat for nature reserves and their management (Pyšek et al. 2003). If native and non-native species follow similar patterns within the network, ~~local conflicts between~~an overlap of high native biodiversity and invasion hotspots ~~can~~ is likely to ~~potentially~~ emerge. Identifying such ~~potential conflicts between natives and non-native species~~areas at high risk of biodiversity loss and the ecological features that may promote invasion or otherwise hamper ~~high~~ biodiversity protection is therefore essential to improve control and management actions.

~~Specifically, w~~We aim at testing, at different spatial scales, whether ~~composition and~~ species richness ~~patterns~~ levels of native and non-native ~~plants~~ vascular plants ~~species follow the same patterns~~are correlated ~~of native species at different spatial scales~~ and whether similar factors control the turnover in species composition of native and non-native species along ecological gradients. Specifically, ~~by answering the following specific~~our research questions are: *a*) what is the degree of congruence in species richness between native and non-native plant species, and does this relationship vary across spatial scales? *b*) do changes in the composition of non-native species across environmental gradients reflect native species turnover along the same gradients? ~~what is the degree of congruence, between native and non-native species, in floristic assemblage variation across environmental gradients?~~ *c*) what are the main ~~biotic~~environmental and ~~abiotic~~anthropogenic drivers controlling species richness in each of these two groups of species?

~~*a*) is there a relationship between composition and richness of native and non-native species and does this relation vary across spatial scales? *b*) is native species richness affected by the same abiotic and human-mediated factors affecting non-native species across spatial scales?~~

METHODS

Study area

This study was performed in the local network of protected areas (PAs) of the province of Siena (Italy), including four Nature Reserves (designated under national or regional regulations) and 17 Special Areas of Conservation (SACs, designated under the EU Habitat Directive 93/43/EEC) (Figure 1). The size of the single PAs within the network ranges from 268 ha to 13747 ha, while their elevation ranges from 122 m to 1660 m a.s.l. (Chiarucci et al. 2012) with a cumulative area of 593 km^2 (15.6% of the Province).

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The study area is characterized by a Mediterranean macro-climate, even though there is a strong variation across sampling sites due to differences in morphology and local elevations (Castrignanò et al. 2006). Long term mean annual precipitation ranges from 630 to 1275 mm (Barazzuoli et al. 1993). The highest precipitations (above 1000 mm on average) and lowest mean annual temperature values (lower than 12 °C) are found at higher elevations (Monte Amiata). A relatively arid and warm zone (mean annual temperature of about 14 °C and average precipitations of ca. 600 mm) is localized in the South-East of the province of Siena (Orcia river valley; Barazzuoli et al. 1993).

The geology is rather varied and complex (including, *inter alia*, limestone, clay, marl, metamorphic and volcanic bedrock), resulting in highly heterogeneous morphology and a great variety of landscapes. The main land-cover types include evergreen coppice woods (dominated by *Quercus ilex*) and deciduous coppice woods or forests (thermophilous types dominated by *Quercus pubescens* or *Q. cerris* at lower elevations, and mesophilous types dominated by *Castanea sativa* or *Fagus sylvatica* at higher elevations); evergreen Mediterranean shrublands (characterized *e.g.* by *Erica arborea*, *E. scoparia*, *Phillyrea latifolia*, *Pistacia lentiscus*, *Arbutus unedo*, *Cistus salvifolius*, *Juniperus communis* and *J. oxycedrus*); croplands (mainly wheat and horticultural crops), vineyards and olive groves. Other relevant land cover types include pastures, meadows, garigues (on calcareous and ultramafic substrates), conifer plantations and wetlands.

Sampling design

Plants were sampled by using an operational approach of plant communities (Chiarucci 2007), defined by a fixed grain and uniform sample density within each protected area (PA). Sampling design was based on a grid of 1 km × 1 km cells, covering the whole study area, with a sampling point randomly selected within each cell (Chiarucci et al. 2008, 2012). A sampling unit represented by a square plot of 10 m x 10 m ~~(plot)~~ was centered at each sampling point. This was further divided in 16 contiguous squared 2.5 m x 2.5 m subplots (Figure S1 of Supplementary material), on which the ~~presence~~occurrence of all species ~~(or subspecies)~~ of vascular plants was recorded.

~~At plot scale, frequency of each species, calculated as the sum of occurrences within the 16 subplots, was used as a coarse measure of abundance~~The field data collection was performed from April to June, during the years 2005-2009. The total number of sampled plots was 604.

Native and non-native species richness and composition

All the vascular plants recorded within each plot were identified at the species or subspecies level with standard floras (Pignatti 1982; Tutin et al. 1964–1980, 1993) or monographs (Grunanger 2001; Weber 1995). Nomenclature was standardized according to Conti et al. (2005). Plants were classified as native or non-native, depending on their distributional status as given by Celesti-Grapow et al. (2011).

Composition and species richness for both native and non-native species were then separately assessed at plot and PA scale, i.e. two data matrices were prepared ~~as separate community matrices~~ (species by plots and species by PAs). At plot scale, frequency of each species, calculated as the sum of occurrences within the 16 subplots, was used as a coarse measure of abundance. At PA scale, relative frequency of each species, measured as the ration between the number of occupied plots *versus* the number of recorded plots, was used as measure of abundance.

~~Species composition and richness values at t~~The PA-scale species lists were obtained by pooling the data from the plots included within each PA.

Environmental and human disturbance predictors

At plot scale, four ~~categories~~ groups of predictor variables for evaluating the environmental ~~(first two),~~and human-mediated ~~(last two)~~ and spatial factors affecting ~~native and non-native~~ species richness were considered: 1) climatic, 2) topographic, and 3) ~~land use, and~~ human disturbance ~~variables , and 4) geographical variables~~ (~~Quantum GIS Development Team 2016, version 2014;~~ Table 1):

1) Climatic variables: we derived one synthetic variable named *climate.* We obtained this variable ~~was derived based on~~from 36 climatic variables related mainly to ~~annual and~~ monthly temperatures and precipitations ~~, including~~(e.g. ~~mean annual temperature,~~ maximum annual temperature, minimum annual temperature, ~~absolute maximum temperature, absolute minimum temperature, and total~~ annual rainfall) ~~mean monthly data for total rainfall, minima and maxima temperatures obtained~~ for each plot, ~~the~~ ~~Data source was~~from the LaMMa consortium (Laboratorio di Monitoraggio e Modellistica Ambientale per lo sviluppo sostenibile; http://www.lamma.rete.toscana.it/en). ~~which~~ Specifically the LaMMa data consisted of ~~has available provides~~local interpolated climatic ~~data for on a~~ grids with a resolution of 250 m x 250 m ~~for the area~~. Since these variables were highly inter-correlated, a Principal Component Analysis (PCA) was performed to reduce their multicollinearity (Taylor et al. 2002). Given that the first PCA factor explained more than 90% of variance (Figure S2 of Supplementary material), this was used as a single variable (hereafter called *climate*). This was found to be negatively correlated with all the variables related to total and summer rainfall (Pearson correlation coefficients between -1 and -0.85), positively correlated with the minimum temperatures (Pearson correlation coefficients between 0.77 and 0.83) and maximum ones (Pearson correlation coefficients between 0.84 and 0.90). This compound *climate* variable corresponds therefore to a gradient from wet and cold to warm and (summer-) dry conditions, that is from Temperate to Mediterranean ~~conditions~~meso-climate, moving from the negative to the positive extreme of the axis.

~~iables: *latitude* and *longitude* of each center~~

2) Topographic variables: *elevation*, *slope*, and potential *solar radiation* values (the latter was obtained from slope, aspect and latitude, following McCune and Keon 2002). The resolution used to develop these variables was 30 m.

3) Human disturbance variables: distance to the nearest road (paved or unpaved) and land-use type variables. We used the distance to the nearest road as a proxy of human disturbance as the road network is a well-known introduction pathway for non-native species (e.g. Pauchard and Alaback 2004; Arévalo et al. 2010). Road layer have been derived from GEOscopio Geoportal, Tuscany Region - Territorial and Environmental Information System (http: // www. Geografia.toscana.it/)

~~We obtained 9~~ land-use type variables~~we obtained 9 dummy variables reflecting the different land use types found in the area by recording the II level of the CORINE Land Cover (CLC) of each plot based on a Corine Land Cover map~~ (II level) ~~(III level) of the area from the Copernicus database (https://land.copernicus.eu/pan-european/corine-land-cover).~~ In addition, ~~we calculated~~ *landscape diversity* (H_{plot}) and *landscape evenness* (E_{Plot}) within a buffer area of 250 m radius around the plot were assessed~~. For this we used the~~ based on the Shannon Index of diversity and the Pielou Index of evenness applied on the CLC map. At plot scale, the human disturbance variables linked to

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land use have not been considered due to the resolution of the clc map which did not allow to detect artificial land use types in the plots or in related buffer areas. So we used artificial land use types as proxy of human disturbance only at protected area scale.

~~(III level).~~

5 ~~Human disturbance variablehe distance to the nearest road (paved or unpaved) was used to calculate the disturbance related variable *road distance*.as a proxy of human disturbance as the road network is a well-known introduction pathway for non-native species (e.g. Pauchard and Alaback 2004; Arévalo et al. 2010).~~At PA scale, ~~we considered~~ the same groups of predictor variables as at the plot scale were considered, with some unavoidable differences in the definition of the single predictors due to different spatial resolution (~~Quantum GIS Development Team 2016, version 2014;~~ Table 2):

1) Climatic variables: ~~ts: the variables per PA were based on estimated values on the two of climatic variables calculated per each plot, as described above. In particular, data for each plot as previously reported. Then,ince each PA contains more than one plot,~~ the variability of climate across plots was accounted for by calculating two derived climatic variables at PA scale: ~~two climatic variables per PA were obtained as the follow: from the PCA described above:~~ *mean climate* (calculated as the centroid, along the first axis in the climate PCA, ~~axis~~ of the ~~cloud of points~~ plots belonging to each PA) and *climate range* (calculated as the range of the same ~~cloud of~~ points along the PCA axis). ~~The gradient remains the same described above for the *climate* variable at plot scale.~~

~~2Planar surfaceGeographical variables: *area* of each PA expressed in km^2.~~

~~3~~) Topographic variables: *elevation range* and *mean elevation* within each PA.

34) Human disturbance variables: total *road density* and land use type variables. We obtained the total road density as the linear extension of paved and unpaved roads per km^2 within each PA and was considered as a proxy of human disturbance and potential propagule pressure of non-native species.

Moreover, as human disturbance, we obtained 4 land-use type variables reflecting the different artificial land use types found in each PA based on Corine Land Cover map (I level) of the area extracted from the Copernicus database (https://land.copernicus.eu/pan-european/corine-land-cover). Land use types variables, artificial, semi-natural and natural, were expressed as percentages of relative Corine land use classes (human, agriculture, natural, wetland). The variables *landscape diversity* (H_{PA}) and *landscape evenness* (E_{PA}) were calculated using the Shannon Index of diversity and the Pielou Index of evenness on the III level CLC map. The calculation of the landscape diversity was done at the III level of CLC because this allowed for a finer classification of landscape units, that was not necessary for detecting the dominant land use type.

~~Moreover, as human disturbance, we obtained 4 land-use type variables reflecting the different land use types found in each PA based on a Corine Land Cover map (I level) of the area from the Copernicus database (https://land.copernicus.eu/pan-european/corine-land-cover). Land-use cover type variables were as percentages of land use classes (human, agriculture, natural, wetland, and water body) were calculated from the CLC map (I-~~

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~~level) to obtain 5 variables for the land cover type. Then, the~~The ~~variables *landscape diversity* (H_{PA}) and *landscape evenness* (E_{PA}) were calculated using the Shannon Index of diversity and the Pielou Index of evenness on the III level CLC map. The calculation of the landscape diversity was done at the III level of CLC because this allowed for a finer classification of landscape units, that was not necessary for detecting the dominant land use type.~~

~~5) Human disturbance variable: total *road density* was measured as the linear extension of for paved and unpaved roads per km^2 within each PA and was considered as a proxy of human disturbance and potential propagule pressure of non-native species.~~5) Geographical variables: ~~*latitude* and *longitude* of the centroid, and~~ *area* (expressed in km^2) of each PA area.

STATISTICAL ANALYSES

Separate analyses were performed at the plot and PA scales. At each scale, we assessed: 1) the correlation between native and non-native species richness, 2) whether specific sets of non-native species tend to be associated with specific native species assemblages, ~~2) the correlation between native and non-native species richness~~, and 3) which predictors best explained native and non-native species richness, respectively ~~by using Minimum Adequate Model selection procedures on Generalized Linear Mixed-effects Models (GLMMs, plot scale) and Generalized Linear Models (GLMs, PA scale)~~. ~~Multicollinearity among explanatory variables at each scale was tested computing Spearman's ρ (correlation matrix available in Table S1 of the Supplementary material) and ensuring that in the minimum adequate models there were not variables where |ρ| > 0.7 (Dormann et al. 2013).~~

Relationships between native and non-native species

First, we assessed the correlation between native and non-native species richness by computing the Spearman correlation coefficient ρ at both spatial scales. This was done to assess whether native and non-native species richness within the reserve network followed 1) similar trends, suggesting that factors which favor high richness of native species, also increase opportunities for non-native species (Thuiller et al. 2010), leading to a positive native-non-native richness relationship (Shea and Chesson 2002), or 2) opposite trends suggesting higher biotic resistance of more diverse native communities, as interpreted by several authors (Cornell and Karlson 1997; Stohlgren et al. 1999; Levine 2000; Tilman 1997; Brown and Peet 2003; Davies et al. 2005; Souza et al. 2011).

~~First~~Second, ~~in order to~~we ~~verified whether the same environmental and anthropogenic turnover in native species composition was associated also to turnover in non-native species and thus if specific sets of non-native species tend to always be associated with the same set of natives~~ ~~verified~~to verify if turnover in native species composition is associated also to a turnover in non-native species and thus if specific sets of non-native species tend to always be associated with the same natives ~~resulted in concordant shifts effects on multivariate dissimilarities, across ecological gradients, for native and non-native species groups in both native and non- native plant communities~~ ~~if species composition of native and non-native plants follow the same gradients and if specific sets of non-native species tend to always be associated with the same native species~~. we comparedTo do so, ~~a comparison~~ we first obtained plot-to-plot (and PA-to-PA) ~~compositional~~ dissimilarities in species composition, separately for non-native and for native species, using Bray Curtis pairwise dissimilarities on log(x+1) transformed species frequencies. ~~was~~

~~performed~~. ~~To do so~~Then, we tested whether the pairwise dissimilarity matrices (or distance matrices) of ~~distances among plots in~~ native and non-native ~~sub-communities~~species were linearly independent by performing a Mantel test (Spearman correlation, 999 permutations, McCune and Keon 2002). For this we considered only the plots or PAs that had at least one non-native species. ~~cond, we assessed the correlation between native and non-native species richness by computing the Spearman correlation coefficient ρ at both spatial scales available. This was done to assess whether native and non-native species richness within the reserve network followed 1) similar trends, suggesting that factors which favor high richness of native species, also increase opportunities for non-native species (Thuiller et al. 2010), leading to a positive native-non-native richness relationship (Shea and Chesson 2002), or 2) opposite trends suggesting higher biotic resistance of more diverse native communities, as interpreted by several authors (Cornell and Karlson 1997; Stohlgren et al. 1999; Levine 2000; Tilman 1997; Brown and Peet 2003; Davies et al. 2005; Souza et al. 2011). Also this relationship was tested at the two spatial scales.~~

Determinants of species richness across spatial scales

To assess the relationship between vascular plant richness (separately for native and non-native species) ~~vs, response variables)~~ and the environmental and human disturbance predictors, we used two different approaches depending on the scale of investigation (plot or PA scale). In both cases, we first evaluated the presence of spatial autocorrelation in the response variables by calculating Moran's I coefficient using the R package 'ape' (Paradis and Schliep 2018), based on the geographical coordinates of the plots and of the centroid of the PA, respectively. Also, multicollinearity among explanatory variables at each scale was tested by computing Spearman's ρ (correlation matrix available in Table S1 of the Supplementary material) and ensuring that there were no pairs of variables in the models where |ρ| > 0.7 (Dormann et al. 2013).

At plot scale, we fit Generalized Linear Mixed Models (GLMM) using R package "lme4" (Bates et al. 2015) and assuming Poisson family errors. ~~Furthermore,~~ PA was considered as random effect to control for the spatial dependence of plots within PA. At PA scale, Generalized Linear Models (GLM) ~~approach was~~were used using Poisson family errors; in case of overdispersion in the data a quasipoisson family was used instead. A Minimum Adequate Model (MAM) and a set of models with good support were thus obtained by performing a stepwise variable selection procedure through AICc minimization using R package "MuMIn" (Barton 2019). Only the models with ΔAICc ≤ 2 compared to the best model were considered to have good support (Burnham and Anderson 2002). In case of more models with similar AICc values, the one which retained a lower number of predictors was considered as minimum adequate model (MAM) according to Occam's razor. Note that, since results were qualitatively similar across the set of models with good support (Table S2 of Supplementary material), we present and discuss only the MAMs in the main text. In case of overdispersion in the data, it was not possible to use an AICc-based selection approach, then the amount of deviance accounted for by the GLM adjusted by the number of predictors was ~~considered~~used instead ($D^2_{adjusted}$; Guisan and Zimmermann 2000; Barbosa et al. 2014). In addition, R^2 statistics (marginal effect) were derived for GLMMs using the "r2glmm" R package (Jaeger 2017). Prior to analysis, quantitative variables were standardized in order to have mean zero and unit variance. Any residual spatial autocorrelation after modeling was assessed by means of spline-correlograms using the R package "ncf" (Bjørnstad 2019). Specifically, 95% pointwise bootstrap confidence intervals were calculated from 5000 bootstrap samples of Pearson residuals after accounting for the level of spatial autocorrelation explained by the explanatory variables in each model.

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~~sampling units were strongly spatially autocorrelated (natives: Moran's I = 0.05; *p* < 0.001; non-natives: Moran's I = 0.03, *p* < 0.001). For this reason, for further analyses we used GLMMs via Penalized Quasi-Likelihood ("GLMMPQL"), using R package "MASS" (Venables and Ripley 2002) and assuming a Poisson family error. Spatial autocorrelation was taken into account by adding a matrix describing the correlation structure of the data (Gaussian correlation). Furthermore, PA was considered as random effect to control for the spatial dependence of plots within PA. Prior to analysis, quantitative variables were standardized in order to have mean zero and unit variance. A Minimum Adequate Model (MAM) was thus obtained performing a manual backward selection from the full dataset and considering potential unimodal relationships taking care to treat independently the two most correlated variables (*landscape diversity*, H_{plot} and *landscape evenness*, E_{Plot}). Goodness of fit was evaluated comparing R^2 statistics derived using "r2glmm" R package (Jaeger 2017).~~

~~Unlike at the plot scale, no signals of spatial autocorrelation were detected at the PA scale for either native or non-native species richness (natives: Moran's I = -0.05; *p* = 0.95; non-natives: Moran's I = - 0.04, *p* = 0.80). For this reason, classical Generalized Linear Models (GLMs) with a Poisson family error were used, and MAMs were obtained using a stepwise procedure aiming at AICc minimization by means of package "GLMULTI" R package (Calcagno 2013) plus a backward selection. As a measure of goodness of fit, the amount of deviance accounted for by each GLM (adjusted by the number of predictors) was computed ($D^2_{adjusted}$; Guisan and Zimmermann 2000; Barbosa et al. 2014).~~

All the statistical analyses were conducted using R 3.6~~5~~.1 (R Core Team 2019~~8~~).

RESULTS

Species patterns at plot scale

In total, 993 native species and 48 non-native species were recorded in 604 plots. ~~At the plot scale, p~~Plant species richness ranged from 0 to 117 (mean 31.0) for native species ~~(one plot was found to harbor no species at all)~~ and from 0 to 9 (mean 0.6) for non-native species. Native and non-native species richness exhibited a slight positive correlation, though not significant (Spearman ρ = 0.06, *p* = 0.11). In contrast, distance matrices based on plot-to-plot compositional dissimilarity showed a significant positive correlation (Mantel test, Spearman ρ = 0.24, *p* = 0.001).

Table S2~~3~~ reports the full list of candidate models for species richness predictors derived from the model selection procedure. Table 3~~4~~ describes ~~describes the~~the best set of predictors for species richness in the GLMM MAM. Specifically, minimum adequate models showed, on the one hand, that native species richness ~~were~~ was positively associated with *landscape diversity* (H_{Plot}) and negatively with the first axis of PCA derived from climatic variables (*climate*) and slope. On the other hand, non-native species richness was~~were~~ negatively related to ~~*elevation,*~~ *road distance* and *slope*, and positively associated with *climate*. However both models, ~~however~~~~and~~ in particular the native-species model, are characterized by a weak goodness of fit (R^2 = 0.02~~1~~ and R^2 = 0.11~~6~~ for native and non-native species, respectively).

Species patterns at protected area scale

Plant species richness ~~within~~ at PA scale ranged between 22 and 547 (mean 219.8 species) for native species and 0-24 (mean 6.6 species) for non-native species. Species richness values of natives and non-native species (Figure 2)

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were positively and significantly correlated (Spearman ρ = 0.75, p < 0.001). ~~Similarly, the Mantel correlation between native and non-native species dissimilarity matrices was significant (Spearman ρ = 0.33 with *p* = 0.003). The r elationship between the ratio of non-native species richness to native species richness and the native species richness proportion of non-native species per PA significantly increased with the size of the native flora~~The relative proportion of non-native species (ratio of non-native to native richness) in PAs was positively correlated with the richness of native species (R^2 = 0.32, p <0.01), increasing up to a limit of about 5% (Figure 2). Similarly, the Mantel correlation between native and non-native species dissimilarity matrices was significant (Spearman ρ = 0.33 with p = 0.003). Further, according to the MAMs, species richness at PA scale was positively correlated with *area* and *climate range* for both species groups (Table 3~~4~~)~~Table 4xx2 shows a~~A ~~positive relationship with *areaa, mean climate* and *climate range* was observed in both species' groups~~. In addition, native species richness showed a positive ~~relathionship~~relationship with *mean climate* and a negative~~ and not~~, ~~althought~~but not significant, relationship with *% wetland*. In contrast, non-native species richness was negatively related with *mean elevation*. ~~showed a unimodal relationship with *road density* and a negative relationship with *% agricultural area* (Table 3).~~ In both ~~cases~~species groups, the deviance accounted for by the model was relatively high ($D^2_{adjusted}$ = 0.~~69~~76 and $D^2_{adjusted}$ = 0.7~~8~~1 for native and non-native species model, respectively).

All four spline correlograms (Figure S3~~2~~ of Supplementary material) failed to reveal any evidence of spatial autocorrelation in the residuals, thereby allowing us to exclude its influence on model parameter estimates.

DISCUSSION

The Italian flora is currently estimated to include 7634 *taxa* (species and subspecies), 13.4% of which (1023 taxa) are considered to be non-native (Celesti-Grapow et al. 2011). In our dataset, collected using a probabilistic sampling strategy within the protected areas of the Siena province only, ~~the protected areas of the Siena province host a relatively~~we recorded a rich flora (1041 species were recorded by this survey) and a relatively low proportion of non-native species (4.9% of the whole sample ~~and an average of 3.9% at the plot scale~~ ~~and 4.9% at the whole sample scale, respectively~~). However, even if the province of Siena is characterized, overall, by ~~well~~ a well preserved landscape ~~which is experiencing a process of abandonment~~ of traditional land uses (Geri et al 2010), ~~we should consider the fact that~~ only protected areas ~~have been~~were included ~~into the~~in this study and thus ~~only better preserved~~ most of the ~~habitat are likely to have been~~ sampled sites are on average less disturbed than the remainder of the landscape at province (or country) scale~~.~~. Even though the proportion of non-native species in the investigated system is generally low, we found evidence of scale dependen~~t effects~~cy of the relationship between native and non-native species richness. ~~proportion of~~ ~~relationship between native and non-native species richness that increase from the plot to the PA scale and the non-native richness from not being correlated at the plot scale to haigh~~ ~~and a strong~~While there was a strong positive relationship at the PA scale, native and non-native species richness were not correlated at the plot scale. This was the consequence of different responses of ~~non-native species~~ the two groups of species to environmental and anthropogenic factors at the fine scale, while similar factors (chiefly reserve area) explained both native and non-native species richness at large scale~~, in primis a similar scale dependence (Chiarucci et al. 2012)~~.

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Species patterns at the plot scale

At the plot scale, we did not observe a relation between native and non-native species richness but we observed a relationship between the compositional gradients of native ~~species composition~~ and non-native composition~~species~~. This suggests that non-native species tend to be associated with specific sets of native species (*i.e.*, native plant communities), either because they have similar environmental requirements or because of more direct biotic interactions (*e.g.*, facilitation or competitive exclusion). However, ~~we also observed no~~the lack of significant correlation between native and non-native species richness~~which~~ instead suggests a limited role for biotic interactions and for biotic resistance through competitive exclusion in these plant communities.

~~Further~~Indeed, our results suggested that different factors affected the species richness of the two groups of species at plot scale. Specifically, native species richness was only very weakly related to the variables we measured at this scale, and our best model only explained a very small proportion of variability ($R^2 = 0.02$). Nevertheless, we found evidence that native richness was positively associated with *landscape diversity* around the plot (250 m radius) and negatively associated with the *climatic* gradient ranging from Temperate to Mediterranean ~~micromacro-~~meso-climatic conditions. The first result is in agreement with previous studies suggesting that greater *landscape diversity* may be related to a higher number of available niches, potentially hosting species with different ecological requirements (Deutschewitz et al. 2003; Kumar et al. 2006). In our study area, higher values of ~~fine~~small-scale *landscape diversity* might specifically indicate fragmentation and the survival of more natural ecosystems within an otherwise homogeneous agricultural landscape. The negative relationship between native species and the temperate-mediterranean gradient indicates that more native species were found in the cooler and wetter, rather than in the drier, ~~micro~~meso-climatic conditions. This is likely to be connected to the regional context, in which higher temperatures at plot scale might represent a significant ecological constrain in summer, while lower temperatures are not likely to be a limiting factor in winter.

In contrast, more variability could be explained for non-native species richness. Non-native species were negatively related with ~~*elevation*,~~ *road distance*, and *slope* ~~acclivity~~inclination and positively related to the temperate-mediterranean climatic gradient. ~~Elevation was important in this model, in agreement with previous studies that showed a negative relationship between non-native plant species richness and elevation in various systems (*e.g.*, Pyšek et al. 2002b; Stevens 1992; Pausas 1994; Rey-Benayas 1995; Marini et al. 2009; Siniscalco et al. 2011; Barni et al. 2012; Bacaro et al. 2015). However, upward movements of non-native species are increasingly being detected in montane ecosystems (Kalwij et al. 2015), probably due to the short residence time of the species or to niche unfilling, and are also predicted to accelerate under future global change scenarios (Carboni et al. 2018). This suggests that, even though higher elevations are currently less invaded in this protected area network, caution is needed and potential future invasion risks should not be discarded.~~

~~Further, a~~As expected, non-native species richness was higher in plots in close proximity to roadways. This is in accordance with ~~many studies reporting that~~the well-documented notion that roads, and roadside habitats, are a major source from which non-native species ~~invade~~ colonize natural areas (Parendes and Jones 2000; Pauchard and Alaback 2004; Bacaro et al. 2015; Ullmann and Heindl 1989; Ullmann et al. 1995; Arévalo et al. 2010). ~~Roads are one of the main anthropogenic features that affect the distribution of non-native species and represent a pathway for their spread (*e.g.*, Pauchard and Alaback 2004).~~ Roads may facilitate the dispersal of non-native species inducing habitat fragmentation and altering (micro-) environmental conditions, facilitating the human-mediated

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dispersal of propagules (via air movement associated with vehicle traffic, and via the seeds attached to the vehicles themselves) and facilitating the colonisation by non-native species by suppressing the growth or removing stands of native species (Trombulak and Frissel 2000; Bacaro et al. 2015). Therefore, limitation to the construction of new roads within or close to protected areas is important for preserving local biodiversity both directly, but also indirectly via the reduction of non-native introductions.

~~Finally~~In terms of environmental factors, non-native species richness at plot scale was positively associated with warmer and drier Mediterranean meso-climatic conditions and ~~also~~ negatively correlated with slope inclination. This can partly be a consequence of the well-known conservative role of steep slopes, because of soil- and micro-climatic- limiting factors: for instance, Bennie et al. (2006) found that in Britain, grassland swards on steep slopes were more resistant to invasion by competitive grass species than those on flatter sites, due to phosphorus limitation in shallow minerogenic soils, and to increased drought events. Filibeck et al. (2016) showed that, in grasslands in a protected area at the periphery of Rome, the removal or leveling ~~by the management~~ of some steep debris heaps led to an increase in non-native and cosmopolite species. However, our dataset is not restricted to grasslands and involves a huge variety of land-cover types, so it is also possible that the observed effect is mediated by disturbance levels and land-use: in the study region, a high slope steepness usually allows only land-use types that feature an inherently low human disturbance (*e.g.*, woods or extensive rangelands), while flat morphologies are usually exploited with intensive crops or urban land-use, typically favouring non-native taxa invasions.

Species patterns at the protected area scale

~~Based on the Mantel test,~~We found that the turnover in non-native species composition was correlated with native species composition also at the scale of protected area. ~~In this~~At this spatial grain, ~~case~~ however, we also detected a strong congruence in the richness of the two groups of species~~ across protected areas~~. These findings, ~~first of all,~~ highlight ~~potential conservation hotspots, since~~that the protected areas that are most biodiverse are also the ones that are most ~~at risk of~~easily colonized by alien species (and thus, potentially, most at risk of invasion). ~~Secondly~~More generally, these findings support the ~~hypothesis that has been sometimes termed~~ “biotic acceptance hypothesis” or “the rich get richer” hypothesis, according to which sites with high native species richness are the most readily invaded by non-native species (*e.g.*, Stohlgren et al. 1999, 2006; Fridley et al. 2007; Pyšek and Richardson 2006; Sandel and Corbin 2010, Bartomeus et al. 2012). This would ~~result~~ be explained because habitats that are generally ‘good’ for native species would also be ‘good’ for non-native ones (*e.g.*, McKinney 2002; Souza et al. 2011). This hypothesis can also be linked to Grime’s theory (Grime 1973), according to which all species (meaning both native and non-native species) respond, to some degree, in a similar way to stress, competition and disturbance (Tomasetto et al. 2013).

We therefore tested whether the similar compositional and richness patterns emerged as a consequence of similar responses to environmental factors and human disturbances. Indeed, we found that ~~three~~ two main variables were retained in both the models for native and non-native species richness at the PA scale: *area* of the protected area, ~~*mean climate*~~ and *climate range* (*i.e*. spatial heterogeneity of climate within the PA). ~~All~~ Both these variables showed positive effects, even if with different weights for native and non-native species richness. Thus, our research shows a positive native-non-native relationship related to the same response of native and non-native species to the available area in the PA and to the main gradients ~~(*area* and *mean climate*~~ at large scale. The positive effect of *area* on both native and non-native species richness is likely dependent on the higher availability of niches in larger protected areas for both groups of species. The congruence of native and non-native species richness is thus largely the net result of concordant well-known species-area relationships, that show similar patterns for both groups of species. Indeed ~~similar~~ a congruence in species-area relationships for native and non-native species had already been reported in this network of protected areas (Chiarucci et al. 2012), as well as in other reserve networks (Pyšek et al. 2002a) or insular systems (*e.g.*, the islands of the Tuscan archipelago, Chiarucci et al. 2017). In addition, the positive effect on richness of the climatic variables at the scale of the protected area (~~*mean climate* and~~ *climate range*) indicates~~, on the one hand, that warmer and drier average climates support greater species richness for both groups of native and non-native species (Barni et al. 2012) and, on the other hand,~~ that more climatically heterogeneous areas enhance regional native richness but also promote non-native species establishment. Indeed, environments with ~~favorable (mean) abiotic conditions (*e.g.*, higher soil fertility or optimal climate conditions; Levine and D'Antonio 1999; Stohlgren et al. 1999) and~~ greater spatial heterogeneity (*e.g.*, higher habitat diversity or spatial variability in resources or conditions; Davies et al. 2005) have been shown to support higher numbers of both native and non-native species at broad spatial scales in numerous other systems. Thus, overall we conclude that in our reserve network “rich protected areas get richer” because of more available area (as expected) but also because of more favorable conditions in terms of available ~~area and~~ niches and of climates.

In ~~additio~~addition to the drivers that were common to both natives and non-natives,~~ to the drivers that were in common with native species~~, the model for native species at PA scale also included a significant positive effect of mean climate, which suggests that reserves with warmer and drier average climates tend to support greater species richness ~~confirms what expressed above in particular for native species. Instead, non-native species also included aa non significant effect of _ *road density*, which was similar to the effect of roads found at the plot scale, and the percentage of *agricultural area* within the protected area. A positive effect of *road density* is a typical finding in studies explaining the number of non-native species at broad scales, because roads serve as introduction pathways as outlined above. Here we found instead a unimodal relationship with non-native species richness, suggesting that, non-native species were most abundant at intermediate levels of anthropogenic disturbance (while their spread was potentially hampered by very high levels of fragmentation at higher road densities). Perhaps, the high degree of naturalness of the protected areas studied here, and the small number of intensively used roads could have driven this unimodal relationship. In contrast, the percentage of *agricultural land wetland* was negatively related to non-native richness, which counters what has been observed in many other agricultural landscapes. Chytrý et al. (2009), for example, showed that the highest levels of non-native invasion among the CORINE land-cover classes in Europe are predicted not only in urban and industrial areas, but also on arable lands. In our case the negative correlation between non-native species and agricultural land wetlands use might be due to the specific local agricultural practices. They are represented by the lakes of Chiusi (CHU) and Montepulciano (MPU), where the anthropic disturbance is extremely high. In fact these lakes are surrounded by agricultural fields and vineyards and their water is used for irrigation....~~Relatedly, the model for non-native species, included a significant effect of mean elevation, that was negatively related to non-native species richness indicating that fewer non-native species were found at higher altitudes. ~~Mean Eelevation and in general elevation, was important in this model,~~This finding is in agreement with previous studies that showed a negative relationship between non-native plant species richness and elevation in various systems (*e.g.*, Pyšek et al. 2002b; Stevens 1992; Pausas 1994; Rey-Benayas 1995; Marini et al. 2009; Siniscalco et al. 2011; Barni et al. 2012; Bacaro et al. 2015). Nevertheless it is important to caution that upward movements of non native species are increasingly being detected in many montane ecosystems (Kalwij et al.

2015), ~~probably due to the short residence time of the species or to niche unfilling,~~ and are also predicted to accelerate ~~under~~in the future ~~global change scenarios~~ (Carboni et al. 2018). Hence, even if our results show that currently higher elevations are of lesser concern for non-native plants, ~~is suggests that, caution is needed and~~ potential future invasion risks should not be discarded.

~~, that are still largely done according to traditional techniques, on small-sized fields interspersed with many hedges, remnants of natural forests (Amici et al. 2015) and grasslands. The traditional agriculture present in many parts of the province of Siena might thus promote resistance to non-native species invasion, rather than facilitating invasions. Alternatively, this result might reflect the lues of negative correlation of largely agricultural protected areas with their apercentage of urban land covers, w PA, that resulthich might indicate less potential introduction sources of non-native species.~~

Conclusions and cross-scale comparisons

~~In conclusion, o~~~~Our overall~~Overall, our results ~~suggest that native and non-native species richness are only weakly related at a fine scale, but share similar patterns at the PA scale. This~~ support the idea that the relationship between the number of native and non-native species is altered when changing the scale of analysis. Indeed, it is widely acknowledged that the strength and form of this relationship (in many studies known as NERR – native exotic richness relationship – *e.g.*, Souza et al. 2011; Symonds and Pither 2012) is scale-dependent. Here, we found evidence that the lack of congruence at ~~finesmall~~plot scale was likely driven by differential responses to fine scale environmental factors (*climate* and *landscape diversity*) and human disturbances (*road distance*), while at the larger PA scale native and non-native species largely responded in a similar way to ~~variability of~~ available *area* and to *climate* variability (climate range). ~~Interestingly, this pattern also resulted from the fact that the effect of climatic variables (climate range and mean climate) on native species changed when moving from the coarse to the fine scale.~~ While at coarse scales the species richness of native species (and non-native species) was generally ~~more abundant~~ higher in warmer Mediterranean climates, at the fine plot scale the native species richness ~~were~~ was even mildly ~~more~~ associated with cooler ~~micro~~meso-climatic conditions. Overall richness of non-native species in the protected area network increased under moderate anthropogenic disturbances at at fine ~~both~~ scale, coupled with high levels of habitat and ~~structural~~ climatic heterogeneity at large scale. Thus, our data suggest that within the study region the introduction and establishment of non-native species would be more likely in warmer and dryer areas, with high native species richness at large spatial scale but intermediate levels of anthropogenic disturbances~~,~~ ~~but with limited fine-scale heterogeneity~~ and mild slope inclinations and elevation. We also found potential conservation ~~conflicts and~~ hotspots, especially because the very biodiverse protected areas ~~are~~ potentially ~~most at~~ feature a higher risk of invasion, due to ~~the presence of invasive~~higher establishment success of non-native species in general. These areas should thus be prioritized for invasion monitoring. Finally, the results also highlight that both 1) the measure of the proportion of non-native species and 2) the relationship with potential predictors should be studied at the appropriate spatial scale in order to be comparable among different regions and informative for conservation purposes.

~~Although biological invasions represent a potential threat for the biodiversity in Europe, few studies have attempted to propose model-based methodologies for preventing the expansion of invasive species in Natura 2000 sites (Dimitrakopoulos et al. 2017; Bazzichetto et al. 2018). job~~~~work we do not consider invasive~~ species~~, but rather focus on non-native species~~~~but only the alien ones, we think that these results could increase the knowledge about~~

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~~the presence of non-native species and help to prevent spread of biological invasions. In fact, biological invasions represent a potential threat for the biodiversity in Europe and few studies have attempted to propose model-based methodologies for preventing the expansion of invasive species in Natura 2000 sites (Dimitrakopoulos et al. 2017; Bazzichetto et al. 2018).~~

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Author Contributions:

All the authors contributed to the interpretation of results and writing.

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TABLES

Table 1.

Measurement units and variability (Min=minimum, Max=maximum and Mean values) of the predictor variables used to model native and non-native species richness at the plot scale.

Predictors	Measurement Unit	Min	Max	Mean
Climatic variable				
Climate (Scores on 1st PCA axis)		-7.64	2.61	0.00
Topographic variables				
Elevation	m	122.00	1660.00	406.63
Slope	radiants	0.00	26.82	7.67
Solar radiation	$MJ*cm^{-2}*year^{-1}$	4555.86	6975.20	5639.80
Human disturbance variables				
Road distance	m	0.00	3492.85	775.57
Landscape diversity (Shannon's H) - H_{Plot}		0.00	1.22	0.42
Landscape Eveness (Pielou's E) - E_{Plot}		0.00	0.10	0.03

Table 2. Measurement units and variability (Min=minimum, Max=maximum and Mean values) of the predictor variables used to model native and non-native species richness at the PA scale.

Predictors	**Measurement Unit**	**Min**	**Max**	**Mean**
Climatic variables				
Mean climate (scores on 1st PCA axis)		-5.23	2.03	-0.26
Climate range (scores on 1st PCA axis)		-1.47	4.04	1.66
Topographic variable				
Elevation range	m	22.00	989.00	390.76
Mean elevation	m	215.70	1242.06	454.87
Human disturbance variables				
Road density	m/km^2	0.00	1.23	0.58
% anthropogenic area	%	0.00	0.28	0.04
% agricultural area	%	0.00	92.90	25.19
% natural area	%	0.00	99.72	67.97
% wetlands	%	0.00	50.94	4.16
Landscape diversity (Shannon's H) - H_{PA}		0.00	1.55	0.96
Landscape evenness (Pielou's E) - E_{PA}		0.00	0.09	0.06
Geographical variables				
Area	km^2	2.68	137.47	28.24

Table 3

Summary output of the minimum adequate model for native and non-native species across spatial scales. Please note that for GLMM, Wald confidence (CI) intervals were computed.

	Native species		***Non-native species***	
	Estimate	*CI (2.5%, 97.5%)*	*Estimate*	*CI (2.5%, 97.5%)*
Plot scale (GLMMs)				
(Intercept)	2.77***	2.45, 3.09	3.10***	1.40, 4.80
Climate	-18.64***	-23.82, -13.45	107.59***	63.73, 151.45
H_{plot}	6.48***	3.81, 9.15	-	-
Slope	-0.06***	-0.08, -0.05	-0.27**	-0.44, -0.09
Road distance	-	-	-0.22**	-0.38, -0.06
PA scale (GLMs)				
(Intercept)	4.97***	4.70,5.22	0.68***	0.26, 1.03
Area	6.53E-05*	1.33E-05, 1.14E-04	1.01E-04**	3.91E-05,1.60E-04
% Wetland	-0.37	-0.82, -0.05	-	-
Mean climate	0.36*	0.12, 0.61	-	-
Climate range	0.43*	0.14, 0.73	0.62**	0.23, 1.03
Mean elevation	-	-	-0.90***	-1.44, -0.45

***$p < 0.001$; ** $p < 0.01$; * $p < 0.05$.

FIGURES

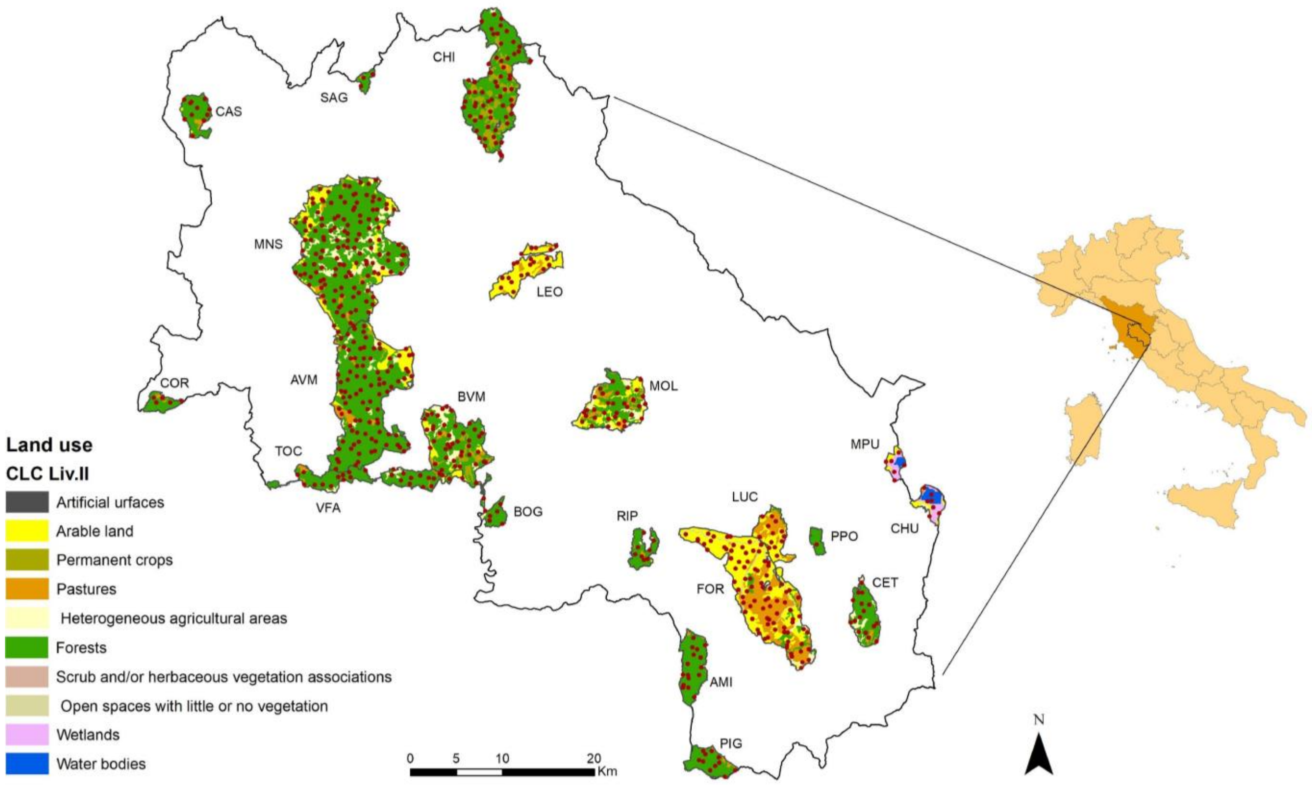

Figure 1. Survey area and the II level of CORINE Land Cover (CLC) of plots on each PA (Protected Areas, including 17 SACs and 4 Nature Reserves) in Siena province. The sampling plots within each PA are shown in black. AMI: Cono Vulcanico del Monte Amiata; AVM: Alta Val di Merse; BOG: Bogatto; BVM: Basso Merse; CAS: Castelvecchio; CET: Monte Cetona; CHN: Monti del Chianti; CHU: Lago di Chiusi; COR: Cornate e Fosini; FOR: Crete dell'Orcia e del Formone; LEO: Crete di Camposodo e Crete di Leonina; LUC: Lucciolabella; MNS: Montagnola Senese; MOL: Monte Oliveto Maggiore e Crete di Asciano; MPU: Lago di Montepulciano; PIG: Foreste del Siele e del Pigelleto di Piancastagnaio; PPO: Pietraporciana; RIP: Ripa d'Orcia, SAG: Bosco di Sant'Agnese; TOC: Tocchi; VFA: Val di Farma.

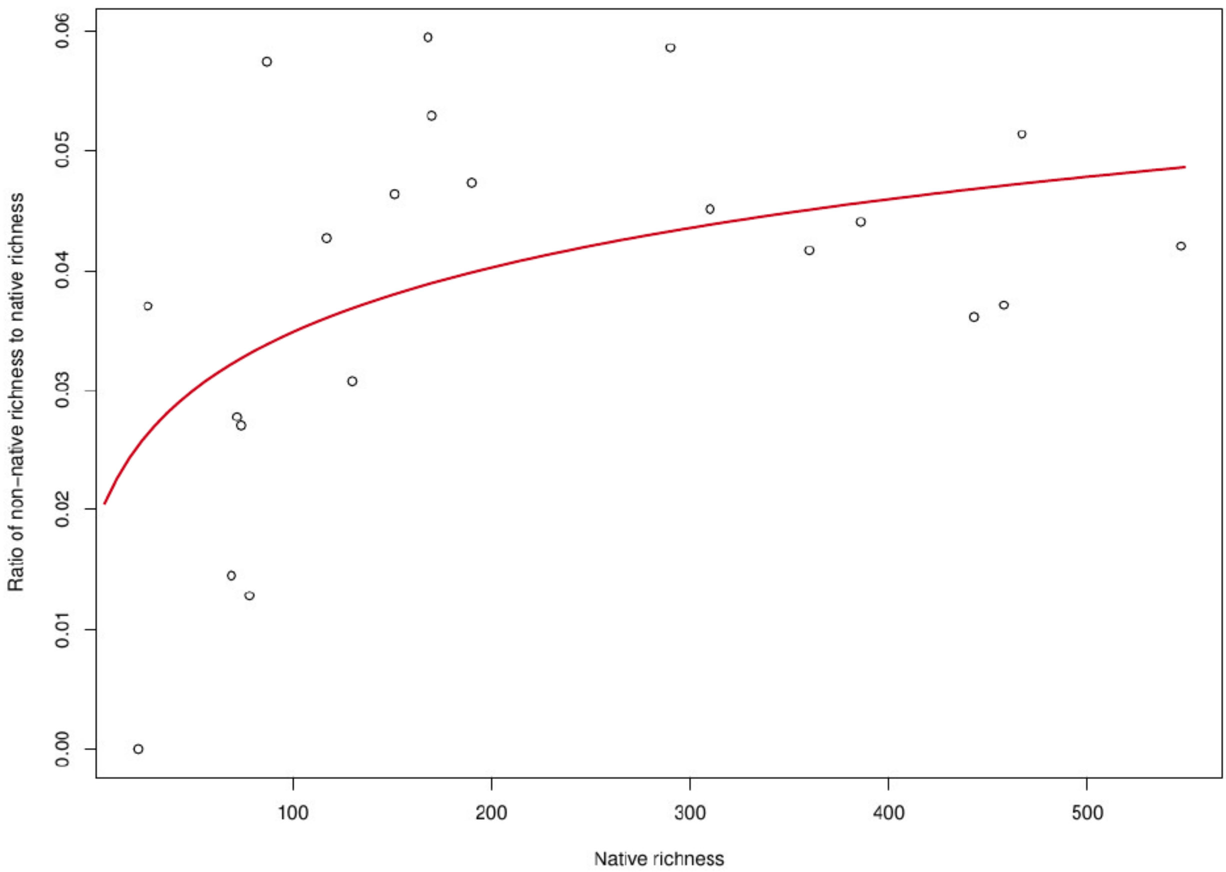

Figure 2. Relationship between the ratio of non-native species richness to native species richness and the native species richness.