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Title page

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Authors information

Dr. CARLO FRATARCANGELI (*) Department of Biology, University of Rome Tor Vergata
Via della Ricerca Scientifica, 1. 00133 Rome, Italy. 0039 3283613033
carlo.fratarcangeli@uniroma2.onmicrosoft.com

Dr. GIULIANO FANELLI Department of Biology, University of Rome Tor Vergata
Via della Ricerca Scientifica, 1. 00133 Rome, Italy.
giuliano.fanelli@uniroma2.it
Orcid: https://orcid.org/0000-0002-3143-1212

Dr. RICCARDO TESTOLIN BIOME Lab., Department of Biological, Geological and Environmental Sciences, Alma Mater Studiorum University of Bologna
Centro Interuniversitario per la Biodiversità Vegetale Big Data - PLANT DATA, Department of Biological, Geological and Environmental Sciences, Alma Mater Studiorum University of Bologna
Via Irnerio 42, 40126 Bologna, Italy.

LifeWatch Italy, Italy
riccardo.testolin@gmail.com
Orcid: https://orcid.org/0000-0002-8916-7231

Dr. FRANCESCA BUFFI Department of Environmental Biology, Sapienza University of Rome,
Piazzale Aldo Moro, 5. 00185 Rome, Italy.
francesca.buffi@uniroma1.it

Orcid: https://orcid.org/0000-0001-6420-764X

Dr. ALESSANDRO TRAVAGLINI Department of Biology, University of Rome Tor Vergata
Via della Ricerca Scientifica, 1. 00133 Rome, Italy.
alessandro.travaglini@uniroma2.it
Orcid: https://orcid.org/0000-0002-4373-0105

(*) = Corresponding author

Abstract

Cities are considered important areas for biodiversity and host a high plant species richness. However, many factors, such as urbanisation or changes in land use, can affect the presence of spontaneous flora and, consequently, represent a threat for biodiversity. How species respond to these factors of change in cities over time is a relevant and current issue and spatiotemporal analyses represent an essential step forward to better understand these dynamic systems and to fill gaps of knowledge.

In this paper we present a comparison between a floristic survey carried out in 1995 on a grid-cell for the city of Rome and a new survey, performed between 2015 and 2018, in order to verify if the species composition significantly changed over time and to which drivers this change was related to. For 76 grid-cells of the raster, each of which of 1.6 km^2, we recorded all spontaneous vascular species. We analysed the differences between the two surveys by means of statistical tests on species richness, by species turnover, by generalised linear models (GLMs) and by Ellenberg indicator values. The patterns of species richness are similar between the two surveys, although an increase in the number of species per grid-cell, on average, was observed. This increase regarded both native and alien richness, with significant differences only for aliens. Many species significantly reduced or increased their frequencies, comparing the two surveys. A set of environmental variables, among which the presence of protected areas, are relevant for explaining the pattern of species' frequencies and its change over time.

Our results suggest that the flora of the city, notwithstanding the steady human pressure and the increase in alien species, maintained a high level of heterogeneity.

Keywords

Urban flora; Biodiversity; Distribution maps; Time-space series; Species turnover

Declarations

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Not applicable

Authors' contributions

Carlo Fratarcangeli: Field samplings, data analysis, manuscript writing

Giuliano Fanelli: Field samplings, manuscript writing

Riccardo Testolin: English revision, data analysis, manuscript writing

Francesca Buffi: Field samplings, data analysis, manuscript writing

Alessandro Travaglini: Manuscript writing, supervision of research

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Text

FLORISTIC CHANGES OF VASCULAR FLORA IN THE CITY OF ROME THROUGH GRID-CELL CENSUS OVER 23 YEARS

Abstract

Cities are considered important areas for biodiversity and host a high plant species richness. However, many factors, such as urbanisation or changes in land use, can affect the presence of spontaneous flora and, consequently, represent a threat for biodiversity. How species respond to these factors of change in cities over time is a relevant and current issue and spatiotemporal analyses represent an essential step forward to better understand these dynamic systems and to fill gaps of knowledge.

In this paper we present a comparison between a floristic survey carried out in 1995 on a grid-cell for the city of Rome and a new survey, performed between 2015 and 2018, in order to verify if the species composition significantly changed over time and to which drivers this change was related to. For 76 grid-cells of the raster, each of which of 1.6 km^2, we recorded all spontaneous vascular species. We analysed the differences between the two surveys by means of statistical tests on species richness, by species turnover, by generalised linear models (GLMs) and by Ellenberg indicator values. The patterns of species richness are similar between the two surveys, although an increase in the number of species per grid-cell, on average, was observed. This increase regarded both native and alien richness, with significant differences only for aliens. Many species significantly reduced or increased their frequencies, comparing the two surveys. A set of environmental variables, among which the presence of protected areas, are relevant for explaining the pattern of species' frequencies and its change over time.

Our results suggest that the flora of the city, notwithstanding the steady human pressure and the increase in alien species, maintained a high level of heterogeneity.

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Introduction

Urban floras are ecologically rich and diverse (Schwartz et al. 2006): This huge diversity, usually greater than surrounding rural areas (Sukopp and Werner 1983; Kühn et al. 2004), can be attributed to several factors such as the position of cities in naturally diverse locations (Kühn et al. 2004) and the decreased competition in urban ecosystems (Kowarik 1995).

Among the main factors promoting this great diversity, there is also the high heterogeneity of the urban environment, resulting from a mixture of artificial, semi-natural or natural habitats (Deutschewitz et al. 2003; Kühn et al. 2003). On the one hand, urban ecosystems, due to the great availability of ecological niches, can host threatened and rare species (Ives et al. 2016; Planchuelo et al. 2019; Soanes and Lentini 2019). On the other hand, land use changes in urban contexts, by threating the integrity of semi-natural or natural fragments embedded in the urban fabric, usually favour ruderal and so-called 'urban specialist' species (Hill et al. 2002; Kalusová et al. 2017). Not surprisingly, urban floras are rich in alien species which, by realising the 'urban' niches sometimes better than native species (Kowarik 1995), find in cities their centres of arrival and expansion (Keller et al. 2011). Moreover, urbanisation and land use change can represent a serious threat for rare species, considering their adaptation to specialist habitats (van der Veken et al. 2004; Knapp et al. 2008; Dolan et al. 2011).

Since urbanisation is a rapid process, urban flora is highly dynamic and can quickly evolve (Sukopp 2002). Thus, spatio-temporal approaches are required to analyse such changing patterns. Urban floras have been frequently studied by means of grid-cells distribution maps (raster), which are specifically suitable for the study of species occurrence patterns and their relationships with environmental factors (Godefroid 2001; van der Veken et al. 2004). Nevertheless, studies comparing floristic censuses over time in cities are rare (Godefroid 2001; van der Veken et al. 2004), because of the great sampling efforts needed and an increasing lack of fundings for field research (Crisci et al. 2020).

For the city of Rome, the most recent flora distribution atlas dates back to the mid-90's (Celesti-Grapow 1995) and, although many studies have contributed to the knowledge of floristic and vegetation of the city (Celesti-Grapow et al. 2001, 2013; Fanelli 2002; Ceschin et al. 2006, 2010; Capotorti et al. 2013), no comprehensive re-assessment nor large scale analysis of changes, following similar protocols, have been carried out since 1995.

Here we present the results of a new floristic census, carried out from 2015 to 2018, on 76 of the original 190 grid-cells of the atlas in Celesti-Grapow (1995), focusing on the qualitative and quantitative changes occurred over time. We addressed the following questions:

1) Did the number of species change in the last 23 years?
2) Did the number of alien species increase?
3) Which species changed their frequencies?
4) Did the number and frequency of rare species change?
5) Which environmental variables are related to changes in native and alien richness over time?

Materials and Methods

Study Area

Rome covers an area of 1,286 km^2 and has 2,856,000 inhabitants (demo.istat.it 2019). The city and surrounding areas have rainy winters and dry summers, with average annual rainfall of 800 mm/y and average annual temperature of 15 °C. Whereby its climate is considered transitional between Mediterranean and temperate (Blasi 1994). The geology is various and mainly referred to Plio-Pleistocene. Many sedimentary rocks are present, such as sandy substrates, especially in the western sector, as well as clayey and slightly alkaline pyroclastic materials forming plateaus or hills. The heterogeneous landscape morphology is characterised by mild hills (from 50 to 139 metres a.s.l.), valleys, and two main rivers, Tiber and Aniene, with many small tributaries. The potential natural vegetation is referred to mixed oak forest dominated by *Quercus cerris* and *Quercus frainetto*, with forests of evergreen oak (*Quercus ilex*) and cork oak (*Quercus suber*) limited to the slopes (Celesti-Grapow and Fanelli 1993). The present vegetation is strongly affected by human impact and mostly represented by anthropogenic communities (Fanelli 2002), which are widely related to the most urbanised sectors of the city. Nevertheless, remnant of wood patches, grasslands, agricultural areas, fallows and riparian vegetation are still present within the urban matrix (Celesti-Grapow and Fanelli 1993).

Rome has faced different phases of urbanisation over its long history. Starting from the historical urban core, dating back to more than 2000 years ago, a rapid urban expansion began after the city became the capital of Italy (1870). This urbanisation process increased after the World War II, transforming the surrounding agricultural landscape in a complex urban texture (Salvati et al. 2016; Egidi et al. 2020). The resulting urban pattern is strongly irregular (Insolera 1993; Salvati 2015) and characterised by large open areas and heavy urbanised areas, located in the entire municipality in a discontinuous way. This development model has continued up to the last decades, so that the current framework of Rome municipality is still rich of fragments of open areas as well as patches of semi-natural woods. Nowadays, Rome's municipality presents a wide system of environmental protection areas, composed of 14 urban parks and semi-natural areas with over 14.000 hectares (RomaNatura 2021).

Study design

The starting point of this research was the *Atlas of the flora of Rome* (Celesti-Grapow 1995), a comprehensive survey of the flora of the city carried out in the late '80s and early '90s in the area enclosed within the Grande Raccordo Anulare ring-road (henceforth: GRA). The area was subdivided into 190 rectangular grid-cells of 1.6 km^2 each, amounting to about 300 km^2. In every grid-cell all vascular plant species were recorded.

We carried out a new floristic census in 76 of the original 190 grid-cells, selected according to a checkerboard pattern (Fig. 1b). In very few cases, we didn't receive the permission by some private estate or deliberately chose to investigate grid-cells with large urban parks, thus deviating from a perfect checkerboard. In order to minimise possible bias, we were careful in assuring that sampling method and sampling efforts were the same in both surveys (1995 and 2018) in terms of number of field excursions and coverage of the area. Every grid-cell has been investigated at least three times, from autumn 2015 to summer 2018, through investigations in early spring, late spring/early summer and autumn, in order to cover all blooming seasons. For more heterogenous or species-richer grid-cells, more than three field surveys were necessary. Field sampling has been carried out by one person, with the support of three researchers. On average, every field trip lasted half a day (about 4 hours).

All spontaneous vascular species (native and alien species) were recorded for every grid-cell, while cultivated and casual were not considered. This study is mainly based on field identification but about 500 specimens have been collected and deposited in Tor Vergata herbarium (RMTV). The determinations were carried out following Flora d'Italia (Pignatti 1982), Flora Europaea (Tutin et al. 1993) and the Portal of the flora of Rome (2015). The nomenclature follows Bartolucci et al. (2018) for native species and Galasso et al. (2018) for alien species.

In this article we refer to "1995 survey" or "1995" for the 1995 study and dataset and to "2018 survey" or "2018" for the 2018 study and dataset. Moreover, we refer to "total richness" for all species found (1995 or 2018), to "native richness" for all native species found (1995 or 2018) and to "alien richness" for all alien species found (1995 or 2018).

Space for FIGURE 1.

Caption figure 1: Study area. a) Study area in Italy. b) Grid-cells investigated in 2018 survey, in turquoise, inside the Grande Raccordo Anulare (GRA) ring road (white circle). Light blue segments are the main rivers: The broader is the Tiber river, the narrower is the Aniene river. c) Urbanisation development in last 150 years and current system of urban parks or protected areas.

Data analysis

The 2018 field campaign resulted in a presence/absence database consisting in a species list for all the investigated grid-cells. To compare the species occurrences between the two censuses and to analyse their changes, we created a *site x species* matrix of 152 rows (76 grid-cells of the 1995 survey + 76 grid-cells of the 2018 survey) and 1080 columns (species found during the two surveys in the grid-cells).

Maps

We produced the distribution maps for the species found in 2018 and compared them to their distribution in 1995 by means of presence-absence in each grid-cell (Supplementary Materials II). In the 1995 study, the species categorised as adventive were not reported with the associated distribution map over the city and simply listed in the species list. In 2018 survey, a few of them, which became meanwhile fully naturalised (Table 1 in Supplementary Materials I), are instead reported with the corresponding distribution map.

Similarly, we visualised the number of species for each grid-cell both in 1995 and 2018. We also produced the maps displaying the changes in the number of species per grid-cell between the two surveys, as well as their turnover calculated as the Jaccard distance (Legendre and Legendre 2012). All these maps have been produced using the R software (R Development Core Team 2020).

Temporal changes in species richness

We tested for significant differences in native richness between 1995 and 2018 using the Mann-Whitney *U* test, while differences in total richness and alien richness were tested using the Student's *t*-test. The choice to use two different univariate tests for the subsets of data was related to data distribution, which were previously checked with normal probability plots.

We tested for difference between the occurrences of the not common species (*not common*), comparing 1995 and 2018, using a Mann-Whitney *U* test. The *not common* category (Table 2 in Supplementary Materials I) comprises the Uncommon (PC), Rare (R), Very Rare (MR) and Less than very Rare (RR) species presented in Anzalone et al. (2010). These analyses have been carried out using Past software (Ryan et al. 2001).

We tested for differences between current and previous frequencies for each species individually, using the McNemar non-parametric test (McNemar 1947), with a Benjamini-Hochberg correction (Benjamini and Hochberg 1995). This test was applied to identify species that significantly changed their frequency.

Models of floristic changes

We modelled the species richness in natives and alien temporal changes as functions of a set of environmental and land use covariates (Table 1). In order to detect drivers of the floristic changes, we fitted generalised linear models using different sets of explanatory variables (*Land Use*, *Urban Structure*, *Geographical Location*) that have been calculated for every grid-cell with the QGis software (QGis Development Team 2021). All variables have been standardised by subtracting the mean and dividing by the standard deviation calculated across all grid-cells. All these variables are reported in Table 1.

All percentage covers have been computed on polygons previously drawn for every grid-cell on several thematic maps: IGM – Military Geographical Institute maps (Italian National Portal 2021) for historical covers, WMS Orthophoto Service of Italian National Portal (2021) for the 1995 covers and Google satellite for the 2018 covers.

As *Urban Structure* variables, we calculated the cover of *green*, *consolidated* urbanisation (urbanisation before 1951) and *recent* urbanisation (urbanisation after 1951). In addition, we added a nominal explanatory variable (*prevailing category*) by assigning to every grid-cell the category ("green", "consolidated", "recent") that covers more than 45% of its total surface. These categories are the minimal set of variables that allows to identify the land use of the cells. A few cells with a mixture of *recent* and *green* (40/50% each) have been assigned to the "mix" category.

For *Land Use* variables, we calculated the change in cover of agricultural areas (*change agricultural*), wooded areas (*change woods*), lawns (*change lawns*) and urbanisation (*change urbanisation*) between 1995 and 2018: The changes over time have been computed as *Land Use 2018-Land Use 1995* for every grid-cell. The agricultural areas include arable lands, pastures and grasslands; Woods include natural woods as well as regrowing thickets; Lawns include meadows, artificial greening and gardens; Urbanisation includes all the impervious surfaces (buildings, roads, etc.).

In addition, we computed the percentage cover of protected areas (*RomaNatura*) as a further independent variable.

Regarding the *Geographical location*, we considered 3 different variables (*centreness*, *southerness* and *easterness*), similarly to Celesti-Grapow et al. (2006), based on distances between grid-cell centroids and the city centre, identified as the centroid of the grid-cell H9. Such cell was not investigated in the present survey but belonged to the original grid (1995 survey). Distances have been approximated in order to obtain only integer values.

Values for *centreness* range from 1 to 10; the closer the grid-cells are to the city centre, the lower the value and vice versa. Values for *southerness*, a north-south gradient, assumed both negative and positive values, ranging from -6 (northern parts of the grid) to +7 (southern parts of the grid). Grid-cells in the same row of the above-cited H9 grid-cell have zero values for *southerness*. Values for *easterness*, a west-east gradient, assumed both negative and positive values, ranging from -5 (western parts of the grid) to +8 (eastern parts of the grid). Grid-cells in the same column of the above-cited H9 grid-cell have zero values for *easterness*.

The dependent variables of the two models were:

a) The number of native species in 2018 (*Natives 2018*).

b) The *proportional variation of aliens*, calculated with the following formula:

$$\frac{percentage\ of\ aliens\ 2018 - percentage\ of\ aliens\ 1995}{percentage\ of\ aliens\ 1995}$$

In the first model, we used the number of native species in 1995 (*Natives 1995*) as a covariate to control for changes in the number of species.

A Poisson generalised linear model for count data was used to model the *Natives 2018*, while a generalised linear model with Gaussian distribution was adopted to model the *proportional variation of aliens*. In both regressions, we performed a stepwise procedure (based on forward and backward approach) to obtain the minimal optimal model. Since spatial datasets can present spatial dependency, we checked for spatial autocorrelation of model residuals using the *lm.morantest* function from *spdep* R package (Bivand and Wong 2018).

These analyses have been performed using R software (R Development Core Team 2020).

Sets of explanatory variables	**Variables**	**Min value**	**Max value**	**Average value**	**Standard deviation**

Urban structure	*consolidated*	0	1.52	0.28	0.43
	recent	0	1.36	0.75	0.42
	green	0	1.52	0.56	0.41
	prevailing category	“consolidate”			
		“recent”			
		“greening”			
		“mix”			
Land use	*change woods*	-0.08	0.16	0.01	0.004
	change agricultural	-1.44	0	-0.19	0.26
	change lawns	-0.24	0.48	0.01	0.13
	change urbanisation	0	1.04	0.17	0.23
	RomaNatura	0	1.6	0.16	0.42
Geographical variables	*centreness*	1	10	6.24	2.18
	southerness	-6	7	0.26	3.66
	easterness	-5	8	0.80	3.61
Control variable[1]	*natives 1995*				

Table 1 Explanatory variables used for generalised linear models. Minimum, maximum, average and standard deviation values for continuous variables are given: Values for *Urban structure* are expressed in km^2 and the *prevailing category* variable refers to the main coverage of the grid-cell between consolidate urbanisation, recent urbanisation and green areas; *Land use* are expressed in km^2 and represent the changing values comparing 1995 and 2018 values; values for *Geographical variables* are expressed considering the own range of every variable (1 to 10 for centreness, -6 to +7 for southerness, -5 to +8 for easterness) compared to the distance from the H9 grid-cell, intended as centroid.

Ecological evaluation of changes

For the ecological interpretation of the comparison between species frequencies, we also rely on Ellenberg indicator values adapted for the Italian flora by Pignatti et al. (2005): These indicator values express, synthetically, the existing relationship between a species and a set of environmental parameters, expressed by values ranging from 1 to 9 for *Light* (L), *Temperature* (T), *Continentality* (C), *Soil moisture* (U), *Soil reaction* (R) and *Nutrients* (N). In this case, the indicator values, called by Pignatti et al. (2005) as “Bioindicators”, have been associated to every species found in 1995 and in 2018. Thus, we calculated the average values of every Ellenberg indicator for each grid-cell by taking their average across species.

We performed Student’s *t*-tests to evaluate which Ellenberg indicator value was significantly different between the two surveys, considering for the tests both the entire dataset (total richness in 1995 and total richness in 2018) and the dataset composed only of species with significant values returned by McNemar test.

Results

General results of 2018 survey

Within the 76 grid-cells sampled in the 2018 census, the number of species found was 922. 840 species were natives, while 82 species were aliens. The average number of species per grid-cell in 2018 was 259, the average number of native species per grid-cell was 234 and the average number of alien species was 25 (Table 2). On average, alien species represented about 10% of the total richness for each grid-cell.

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[1] Only for *proportional variation of aliens* model

All the species found in the 2018 survey are reported in the distribution maps in Supplementary Materials II, with the corresponding distribution in 1995 alongside.

Temporal changes in species richness

Comparing the data of the two surveys, in 1995, in the same 76 grid-cells, 935 species were found. The average number of species per grid-cell in 1995 was 241, 223 for natives and 18 for aliens (Table 2). Considering both surveys, a total of 1,080 species were recorded: The species shared by both amounted to 777, 158 taxa have not been found in 2018 and 145 taxa belonged only to the present survey. The patterns of species richness comparing the two surveys are quite similar, with an east-west and south-north gradient of increasing species richness. Some differences between the two surveys are mainly detected in semi-central grid-cells and in the southern area of the city where, generally, has been detected an increase in species number. A moderate decrease in species richness, on average, has been detected for grid-cells encompassing the main rivers and the outermost belt of the area investigated (Figure 2d).

Total richness, native richness and alien richness showed an increase which was, however, significant only for total richness and alien richness (Table 2 in the main text and Figure 1 in Supplementary Materials I).

The total turnover was 28%. The turnover values, reported in Figure 2e for every grid-cell and in Figure 2 in Supplementary Materials I for the whole area investigated, were quite high all over the city, ranging from 0.40 to 0.60. The areas with lower turnover values (around 0.40), are the grid-cells encompassing parks, large open areas or wooded areas. On the contrary, areas with a general higher turnover (peaks of over 0.55) were mainly located in the first suburban belt and in the outskirts.

			Student's		
Category	Average number	Standard deviation	t		*p*
Total richness 1995	241	± 59	56.9		0.041
Total richness 2018	259	± 48			
			Mann-Whitney		
			U	z	*p*
Native richness 1995	223	± 58	2408	1.76	0.07
Native richness 2018	234	± 48			
			Student's		
			t		*p*
Alien richness 1995	18	± 3.5	10.428		< 0.001
Alien richness 2018	25.5	± 5.2			

Table 2 Total average richness in 1995 and in 2018, average richness for natives in 2018 and 1995, average richness for aliens in 2018 and 1995. Student's *t* significant tests for total richness and alien richness comparing 1995 and 2018 data. Mann-Whitney *U* significant test for native richness comparing 1995 and 2018 data. 1995 data are taken from (Celesti-Grapow 1995).

The total number of *not common* species decreased from 116 species in 1995 to 95 species in 2018. Instead, the average number of *not common* species per grid-cell increased significantly from 3.22 in 1995 to 3.93 in 2018 (Mann-Whitney *U*-test Table 3 in Supplementary Materials I).

The McNemar test identified a number of species with significantly different frequency between 1995 and 2018 surveys (Table 4 in Supplementary Materials I): 75 species significantly decreased, while 124 species significantly increased.

Space for FIGURE 2

Caption figure 2: a) Number of species in every grid-cell comparing 1995 survey (blue lettering) and 2018 survey (red lettering). b) Species richness for 1995 survey; data are taken from (Celesti-Grapow 1995)**. c) Species richness for 2018 survey. d) Changes in species richness in every grid-cell comparing 1995 and 2018 surveys. e) Species turnover comparing 1995 and 2018 surveys.**

Models of floristic changes

We didn't find any evidence of spatial autocorrelation in both models (*Natives 2018* model: Moran I = 0.7618, p-value = 0.22; *Proportional variation of aliens* model: Moran I = -1.1607, p-value = 0.87).

- ***Natives 2018***

Since not significant changes occurred in the richness of natives over the investigated time frame (Table 2), we modelled their richness in 2018 (*Natives 2018*) against the environmental variables related to the current distribution.

The optimal model obtained for *Natives 2018* (Table 3) returned a strong correlation between the dependent variable and *natives 1995*, which acts as a covariate. *change urbanisation*, *change agricultural* and *change lawns* were negatively correlated to native richness. Except for the *consolidate* variable, the other *Land use* variables were positively correlated with native richness in 2018; *RomaNatura* variable had a strong positive relationship with native species richness of 2018; *southerness* was negatively related to number of native species (the north area is richer in native species compared to the south area), although without a significant p-value. The explained deviance is 0.77.

Coefficients:	Estimate	Std. Error	z value	Pr (>|z|)
(Intercept)	4.8141106	0.0586216	82.122	< 2e-16 ***
natives 1995	0.0022429	0.0001548	14.49	< 2e-16 ***
change urbanisation	-0.1355713	0.0432062	-3.138	0.001702 **
change agricultural	-0.1691339	0.0493045	-3.43	0.000603 ***
change lawns	-0.0941338	0.0317895	-2.961	0.003065 **
*prevailing category "*green"	0.1378032	0.0604257	2.281	0.022576 *
prevailing category "mix"	0.218282	0.0621909	3.51	0.000448 ***
prevailing category "recent"	0.1276543	0.0556596	2.293	0.021820 *
prevailing category "consolidated"	0.032217	0.0192115	1.677	0.093550 .
RomaNatura	0.0229724	0.0104106	2.207	0.027340 *
southerness	-0.0149406	0.0088618	-1.686	0.091806 .
AIC:	728.45			
Explained deviance:	0.77			

Table 3 Generalised linear model of the *Natives 2018*. Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1.

- ***Proportional variation of aliens***

In the optimal model obtained for *proportional variation of alien* species (Table 4), the variable *change woods* was positively related to a proportional increase of alien species. The *centreness* (which increases toward suburbs) shows a negative relationship (in the centre there is a higher proportional increase in alien species), as well as *RomaNatura* variable (a lower proportional increase of alien species in those areas with higher covers of *RomaNatura*). The South variable is positively related with the *proportional variation of aliens* (in the south area of the study there is a higher proportional increase of alien species). The explained deviance is 0.26.

Coefficients:	Estimate	Std. Error	t value	Pr(>|t|)
(Intercept)	0.023803	0.001995	11.93	< 2e-16 ***
change woods	0.004722	0.002216	2.131	0.03653 *
centreness	-0.00525	0.002085	-2.517	0.01409 *
RomaNatura	-0.00678	0.002225	-3.047	0.00325 **
southerness	0.005422	0.002034	2.666	0.00949 **
AIC:	-393.34			
Explained deviance:	0.26			

Table 4 Generalised linear model of the *proportional variation of aliens* between 1995 and 2018. Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1.

Change of Ellenberg indicator values

Taking into account the entire set of data (1995-2018), the Student's *t*-tests performed for Ellenberg indicators values (Table 5 - left side) showed no significant differences for *Light*, *Continentality* and *Soil Reaction*, while significant differences were detected for *Temperature*, *Humidity* and *Nutrients* with a significant increase for the first two Ellenberg indicator values and a significant decrease for the other.

The additional Student's *t*-tests performed only with McNemar's significant species test showed significant differences only for *Temperature* and *Nutrients* (Table 5 - right side). *Temperature* values showed, on average, an increase in 2018, while *Nutrients*, on average, a decrease.

<table>
<tr><th colspan="5"><i>Student's</i> t<i>-tests for the entire set of data</i></th><th colspan="5"><i>Student's</i> t<i>-tests for McNemar's species test</i></th></tr>
<tr><th colspan="2">Ellenberg indicator value</th><th>Average values in 1995 survey</th><th>Average values in 2018 survey</th><th><i>p</i></th><th colspan="2">Ellenberg indicator value</th><th>Average values in 1995 survey for McNemar's species test</th><th>Average values in 2018 survey for McNemar's species test</th><th><i>p</i></th></tr>
<tr><td>L</td><td>Light</td><td>7.8 ± 0.21</td><td>7.8 ± 0.19</td><td>0.960</td><td rowspan="2">T</td><td rowspan="2">Temperature</td><td rowspan="2">7.37±0.10</td><td rowspan="2">7.57±0.09</td><td rowspan="2"><0.001</td></tr>
<tr><td>T</td><td>Temperature</td><td>7.35 ± 0.12</td><td>7.46 ± 0.09</td><td><0.001</td></tr>
<tr><td>C</td><td>Continentality</td><td>4.89 ± 0.05</td><td>4.89 ± 0.06</td><td>0.735</td><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td>U</td><td>Humidity</td><td>3.84 ± 0.31</td><td>3.75 ± 0.22</td><td>0.036</td><td rowspan="2">N</td><td rowspan="2">Nutrients</td><td rowspan="2">4.47±0.29</td><td rowspan="2">4.32±0.18</td><td rowspan="2"><0.001</td></tr>
<tr><td>R</td><td>Reaction</td><td>5.62 ± 0.15</td><td>5.62 ± 0.13</td><td>0.964</td></tr>
<tr><td>N</td><td>Nutrients</td><td>4.62 ± 0.23</td><td>4.53 ± 0.24</td><td>0.029</td><td></td><td></td><td></td><td></td><td></td></tr>
</table>

Table 5 Student's t-test for the entire set of data (left side of the table) and Student's t-test for significant species returned by McNemar's test (right side of the table). For every year of survey, average indicator value and ± SD is given.

Discussion

General results of 2018 survey

Twenty-three years, the time span between the two censuses, is a very long time for a city where human impact leads to steady changes in plant species and communities (Kowarik 2011). After two decades, the flora of Rome in the area investigated in this study is still very rich, with 922 taxa in an area of 122 km^2 characterised by intensive human pressure and representing about 12% of the flora of Italy (Pignatti 2017). The 91% of the species found in 2018 survey are natives, while alien species represents only the 9%. These findings confirm, once again, how Mediterranean cities are mainly composed of native species (Celesti-Grapow and Blasi 1998), differing from Central European cities where aliens can represent as far as 50% (Pyšek 1989; Kowarik 1995).

The species richness patterns in 2018 follow the same patterns of 1995 (Celesti-Grapow 1995; Celesti-Grapow et al. 2013) (Figure 2b and 2c). Floristic richness is higher in the suburban belt and in the north-west sector, where the urban matrix is interrupted by several parks, fields and open areas. A few central grid-cells, characterised by the presence of urban parks or villas (e.g., grid-cells M11, L5, G9, F6), show a high richness notwithstanding their location embedded in the urban matrix. Similar patterns, with a high floristic diversity in the western sectors of the city and in a few central grid-cells, have also been detected by Ricotta et al. (2001). The greater species richness in highly structured areas, with the presence of semi-natural patches and high habitats heterogeneity, is well documented at local (Wania et al. 2006; Godefroid and Koedam 2007; Malkinson et al. 2018) as well as at large scales (Deutschewitz et al. 2003); for instance, Godefroid and Koedam (2007) found an inversely proportional correlation between built-up areas and species richness in Brussels.

Temporal changes in species richness

Urban floras are highly dynamic (Godefroid 2001; Chocholousková and Pyšek 2003; van der Veken et al. 2004; Knapp et al. 2010; Gregor et al. 2012) and a certain degree of fluctuations in species number is easily detectable (Klotz 1987; Landolt 2000; Pyšek et al. 2004; Salinitro et al. 2019).

In this study, there was a significant change in the total richness and a significant increase in the number of alien species (Table 2 in text and Fig. 1 in Supplementary Materials I). The success of aliens could simply depend on different environmental requirements compared to natives (Ricotta et al. 2010), but this subject deserves deeper investigations, particularly the study of the population dynamics. Our results are in contrast with observations derived from temporal analyses carried out in Central European cities (Godefroid 2001; Chocholousková and Pyšek 2003; Pyšek et al. 2004; van der Veken et al. 2004; Knapp et al. 2010; Gregor et al. 2012). These studies found a decrease in the number of native species, but similar trends have been detected in other urban studies, such as Knapp et al. (2017) and Wirth et al. (2020), where the number of species increased. Areas rich in native species can also host many aliens, as in the case of the city of Pécs, where Wirth et al. (2020) identified the increased numbers of neophytes as the main cause of increase of species richness.

Despite the increase of aliens, native species remain the dominant component of the flora of Rome. Their average increase per grid-cell, even if not statistically significant, suggests that also the native species contributes to the floristic change. Consistently with Thomas and Palmer (2015), who observed no net effect of aliens on native species in Great Britain, the increment of native species in Rome has been also observed for grid-cells where aliens grew. Stohlgren et al. (2003) and Wania et al. (2006) already highlighted that naturally rich areas can host many aliens: The reason of this coexistence is probably due to the great heterogeneity of Rome’s landscape, in terms of geographical features and land use (Blasi et al. 2005).

The increase in species number was not observed for every grid-cell: A decreasing trend was detected along the main rivers and the outermost belt of the area investigated. The decrease in species number is probably linked to several factors, such as the steady human pressure in the urban stretch of the rivers, which includes ruderalisation, pollution or eutrophication (Ceschin et al. 2010; Ceschin and Salerno 2021), the change in agricultural practices (a relevant interpretation for some grid-cells in north-west sector of the city characterised by agricultural areas), the general change in land use in many areas of the city, that switched from agricultural to built-up areas or from urban fabric to abandoned vacant lots (Frondoni et al. 2011; Salvati and Carlucci 2014).

The analysis of species with significant changes in frequencies added further qualitative information: The species increased in frequency are mainly related to open ruderal habitats and small niches of the urban fabric, like flowerbeds, managed parks, or wastelands (e.g. *Trifolium nigrescens* subsp. *nigrescens*, *Medicago lupulina*, *Beta vulgaris* subsp. *maritima*, *Allium neapolitanum*). Many of these species belong to a characteristic component of the flora of Rome, namely grasslands dominated by sub-ruderal therophytes, like *Dasypyrum villosum* or *Avena sterilis*, a habitat with a high species richness (Fanelli 1998). The spread of ruderal species within the flora of Rome has been already highlighted on minor scales (Bianco et al. 2003; Filibeck et al. 2015). At large scale, the increase of generalist species was found also in the city of Turnhout (van der Veken et al. 2004) as they benefit of urbanisation.

Most of the significantly decreased species are related to xeric, open and grazed areas (e.g., *Xanthium italicum*, *Centaurea solstitialis* subsp. *solstitialis, Anthemis arvensis* s.l., *Rapistrum rugosum*), which were rather common in the previous work. Moreover, also many species with previous low occurrences decreased their distribution or even disappeared. These species are related to traditional agricultural practises, microthermic woody habitat (*Mycelis muralis*) or wet areas (*Juncus effusus*, *Scutellaria galericulata*, *Persicaria hydropiper*, *Hydrocharis morsus-ranae*).

All these floristic results are consistent with the analyses of Ellenberg indicator values (see below in Par. ***Ecological evaluation of changes***). Although we did not relate the distribution of single species to land-use, the ecology of the significantly changed species is an indicator of a qualitative shift, from a landscape characterised by a mix of agricultural and urban patches to a metropolitan landscape.

Regarding the analyses of not common species, the most important information is the significant average increase considering every grid-cell. As already highlighted, in urban fabric, rare species can survive in small patches of favourable habitats and natural areas (Diamond and Heinen 2016), as well as in the hybrid ecosystems emerging in these contexts, which can act as stepping stones (Planchuelo et al. 2020).

Turnover was generally high, not surprisingly for a highly dynamic habitat as a city (Lososová et al. 2016). The period from 1990 to 2018 saw the setting of an important system of protected areas in Rome, the network *RomaNatura* (RomaNatura 2021). Despite the shift of many of these areas from pasture/agricultural areas to urban parks, the floristic pool has maintained its high diversity and is stable over time, suggesting that the system of protected areas of the city has preserved these areas.

Models of floristic changes

Natives 2018

The distribution of native species in 2018 (*Natives 2018*) is explained by several environmental variables. The “green”, “mix” and “recent” *prevailing category* are positively correlated with the dependent variable. Within the *prevailing category*, “mix” category shows the strongest effect (Table 3), meaning that more heterogeneous grid-cells are highly diverse compared to grid-cells where the urban fabric remained stable over the last 70 years (“consolidated”). The positive effect of habitat heterogeneity in the city has been already observed by Celesti-Grapow et al. (2006). Also recently urbanised areas (“recent”), probably due to their high dynamism and the presence of heterogeneous surfaces and green open sites (“green”) host a high diversity of natives.

Concerning the land use change variables (*Land use*), the model detected a significant effect of the disappearance of agricultural patches, which favours the increment of natives. The change of agricultural areas (*change agricultural*), that on average diminished per grid-cell (Table 1), is in fact the strongest driver among the other land use change variables. The correlation between *change lawns* (increased, on average) and native species diversity is apparently counterintuitive: Despite lawns naturally host a floristically rich vegetation (Fanelli 2002), their change over time in the city of Rome is negatively correlated with the current natives’ diversity. However, it seems necessary to highlight that the lawns category includes several kinds of vegetation, in particular managed lawns, that are floristically poor compared to more natural patches. Lawns, on average, increased all over the area (Table 1) but particularly in suburban areas (for instance in grid-cells N12, F15, M15, R10, C11, C13) where, probably due to the recent urbanisation, agricultural land have been replaced by urban fabric and managed meadows (such as backyards or urban greening). This is the case, for instance, of the grid-cell N12, where a portion of the agrarian landscape became a golf course. The suburban belt of the city (for instance, N16, P5, D12, C13, E14, G16, S9, Q12 grid-cells) is where most of urbanisation took place in the last two decades. This land use change (*change urbanisation*), along with *change lawns*, is negatively related to the species richness of natives. Despite urbanisation is an ongoing process in the area investigated (*change urbanisation* increased, on average, over time; Table 1), the native species maintain their high species number and, although not significantly, increased over time. Our results suggest that the native species benefit from the presence of the RomaNatura network. The important positive correlation with the variable *RomaNatura* is consistent with other results stressing the primary role of urban parks in preserving biodiversity and promoting species plant richness (Nielsen et al. 2014).

Proportional variation of aliens

The model of the *proportional variation of aliens* suggests that increasing in woods (*change woods*), closeness to the centre (*centreness*), *southerness* and the absence of protected areas (*RomaNatura*) are all related to the increase of alien species. The temporal increment of aliens in areas close to the centre (*centreness*), where the urban matrix is more compact, confirmed and added a dynamic dimension to the patterns already detected by Celesti-Grapow et al. (2006), who found a high representation of neophytes for the historical centre.

The climate might affect aliens' increment in the southern area of the city (*southerness*) as this sector is warmer and hosts a more Mediterranean vegetation compared to the north (Fanelli 2002). Southern areas probably are more sensitive than northern areas, consistently with the results of Ellenberg Indicator values that showed an increase in Temperature values. The important role of the geographical gradients in explaining the distribution of species richness in the city has already been recognised by Celesti-Grapow et al. (2006), who found a decrease of richness along a north-south gradient. The increase of aliens in warm habitats has already been reported in the literature (Walther et al. 2009). Yet, to make pertinent comparison with the 1995-2018 data, further analyses with bioclimatic parameters should be performed.

Differently from our expectations, *change urbanisation* has no significant relationship with the increase of alien species. This is in contrast with previous findings by Kühn et al. (2017) for Germany, where urbanised areas have an important effect in explaining neophyte richness patterns.

Unexpectedly, the proportional increase of alien species is related to the increase of wood percentage cover over the years. Notwithstanding the important role of little remnants of seminatural woody areas in maintaining native species richness in urban ecosystems (Yang et al. 2021), our results report that the increase in cover of these woody areas is not to be consider solely a regrowth of natural potential woods but probably an increase of thickets of woody neophytes, such as *Robinia pseudoacacia* and *Ailanthus altissima* thickets which can host a great number of allochthonous species (Fanelli 2002; Vítková et al. 2020). Especially understory communities of this thickets seem to be particularly vulnerable and prone to colonisation by alien plants (Trammell et al. 2020). For the city of Rome, at least for black locust canopies, such regrowth does not necessarily produce homogenisation of understorey communities (Sitzia et al. 2021) and, generally for urban areas, these regrowth are considered shared habitats of native and alien species (Kowarik et al. 2019).

Despite the significant spread of aliens all over the city, particularly in the historical centre and southern areas, it's interesting to note how the presence of the parks network has prevented the increase of aliens, acting as a filter. The *RomaNatura* variable is in fact negatively correlated with the increase of alien species.

Ecological evaluation of changes

The analyses of the Ellenberg indicator values reported some significant changes. The significant increase in *Temperature* values could be correlated to global climate change and the urban heath island phenomenon (Bechtel and Schmidt 2011). The increase of this Ellenberg indicator value was found also by in Godefroid (2001) for Brussels, although only as a trend for total species number. The preference of neophytes for warmer habitats, as reported in Knapp et al. (2010) for the city of Halle, could partially explain the increase in aliens in Rome found in this study. Moreover, the most favourable conditions in urban habitats for thermophilous species is well documented in literature (Williams et al. 2015) and our case confirms these findings.

The significant decrease in *Nutrient* values is harder to explain. Rome’s species richness decreases in more disturbed areas (Celesti-Grapow et al. 2006) and, even if in Ranta et al. (2013) an increase in *Nutrient* values has been observed, many studies stress the relation existing between high nutrient tolerant species and urban rich soils (Pyšek 1995; Godefroid 2001; Hill et al. 2002). A possible explanation is related to the structure of the inner urban texture: The Municipality of Rome has stabilised over the last 20 years, while urban expansion is still in act mainly outside the GRA highway (ISPRA 2020), where none of the grid-cells is located. This fact could have influenced the distributions of species related to shuffled soils, e.g., *Dysphania ambrosioides*.

The analysis carried out on the subset of species that significantly changed over time returned strongly significant values for *Temperature* and *Nutrients*, highlighting how these trends are more pronounced for those species with major variations, which are the main agents of the change.

Limitations of the study

Floristic data research based on large-size grid-cells, especially if compared with data from different authors, may presente some weaknesses. One of the main drawbacks encountered during the field samplings is the large size of every grid-cell and the inner high heterogeneity: Rome, given its patchy urban texture, hosts a wide array of habitats even in small areas and these habitats are rich in species. At the scale of our research, it was hard to investigate all this heterogeneity. We are aware that a margin of uncertainty exists between the two surveys, due to above mentioned issues. Secondly, bias due to sampler/s is an important drawback to take into account that could lead to misinterpretations, although we were careful in assuring that sampling method and sampling efforts were the same in both surveys (1995 and 2018).

However, we are confident that these weaknesses do not invalidate the interpretation of our results due to the range and size of study area, which allow to make pertinent ecological interpretations.

Finally, our study concerns the frequency of species but not their abundance and this can mask local patterns such as patterns of establishment.

Conclusions

Our study added the temporal dimension to the important existing works about the flora of Rome. The results showed that the main changes in the flora of the city are represented by an increase in the total number of species and by an increase in alien species, resulting in a high turnover. Many species rather rare at regional level are still present in Rome, notwithstanding the moderate decrease in the number of *not common* species.

Current changes are not limited to the inner area of the GRA highway: Thus, the urban sprawl is mainly regarding the outside areas in the last years and it would be interesting to study change in this outer suburban belt, although data in this format are not available for the past nor for current years.

If it is true that we are undertaking the way of the end of botany (Crisci et al. 2020), field research is becoming less and less, with a direct consequence of information loss, misinterpretation of current issues and inappropriate management plans. The conservation of urban biodiversity, above all in a period where more than half of the human population live in urban contexts, necessarily finds its foundation in the collection, analysis and interpretation of field data. Our approach, which is transferable to other cities, would allow useful comparisons in understanding patterns and processes of biodiversity in urban contexts.

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